



Green Synthesis of Gold Nanoparticles: Catalytic Reactivity towards Degradation of p-Nitrophenol

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ABSTRACT

In this work we have developed various gold nanoparticles out of different natural leaves using *Mimosa pedica* and *Anacardium occidentale*. These synthesized nanoparticles are characterized using UV visible spectroscopy and SEM. To compare the catalytic activity of these nanoparticles two different gold nanoparticles are synthesized by using sodium borohydride and trisodium citrate. The catalytic activity of these nanoparticles is examined by the reduction of p-nitrophenol. It was observed that the nanoparticles having lower particle size have more catalytic activity towards the reduction. As the particle size increases the reactivity of these nanoparticles decreases.

INTRODUCTION

Nanomaterials in general, in which a single particle is measured between 1 and 100 nanometres (10^{-9} meters) at least in one dimension. Nano-scale materials often have special optical, electronic, or mechanical properties, based on their form and size. Among the nanoparticles, Gold Nanoparticles (AuNPs) are the most attractive for their well-established synthetic procedure with various size and shape. In addition, AuNPs can be synthesized both in organic or aqueous media with high monodispersity and stability. The AuNPs are the most organized and stable metal nanoparticles that can be easily synthesized by chemical reduction method with high mono dispersity. The unique behaviour of the nanoparticles toward mechanical, magnetic, and optical functions makes them applicable in various fields of science and technology, catalysis or biology. Different methods for the preparations of gold colloids were documented and analysed in the 20th century [1]. The issue has been so intensively studied particularly after the breakthroughs reported by Schmid [1] and Brust et al. [2,3] due to the fundamental and applicable aspects of the quantum effect [4]. The shape and size of nanoparticles during synthesis mainly depends upon the strength/concentration of reducing agent, concentration of stabiliser/capping agent, concentration of metal precursor, temperature, speed of stirring etc. For the synthesis of metal nanoparticles, phytochemicals with a broad spectrum of reducing properties (mild or strong) present in different plants may be used. The reduction in capping/stabilizing properties of plant phytochemicals make the nanoparticles of metal environmentally safe, functional, and



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biocompatible. Taking these considerations into account, numerous study groups used various plant extracts as a substrate for the synthesis of nanoparticle with various metal salts (gold, silver, copper etc.) [5]. A sol or colloidal suspension of nanoparticles of gold in a fluid, typically water, is AuNPs or Colloidal gold. Usually, the colloid is either an extreme red colour (less than 100 nm for particles) or blue/purple (for larger particles). Gold nanoparticles are the focus of extensive study, with several possible or promised uses and material science owing to their optical, electrical, and molecular-recognition properties. The properties of nanoparticles of colloidal gold, and therefore their uses, strongly rely on their size and morphology.

Owing to the nanoparticle's interactions with visible light, colloidal gold has been utilized by artists for decades. With remarkable efficiency, gold nanoparticles absorb and scatter light. Colloidal gold has the potential to show a broad variety of colours based on particle size, form local refractive index, and aggregation condition, varying from bright reds and blue to black and eventually to transparent and colourless. These colours arise due to a process called localized surface plasmon resonance (LSPR), in which conduction electrons oscillate in resonance with incident light on the surface of the nanoparticle. In general, because of rising nano particle diameter, the wavelength of light absorbed increases. Pseudo-spherical gold nanoparticles with diameters of ~ 30 nm, for example, have a peak absorption of LSPR at ~ 530 nm. The optical properties of the particle alter as gold nano particles accumulate, owing to the change in effective particle size, form, and dielectric state. Modification of the nanoparticles by suitable monolayers provides greater stability and generate additional properties which may be useful for sensing, molecular recognition, imaging, drug delivery, and catalysis.

MATERIALS AND METHOD

The chemicals required for the experiment are HAuCl_4 , NaBH_4 , Sodium citrate. Collect fresh *Mimosa pudica* (Lazabati) Leaves and *Anacardium occidentale* (Cashew) Leaves. Clean all the glassware with freshly prepared aquaregia.

Preparation of *Mimosa pudica* and *Anacardium occidentale* leaf extract

10 g of fresh leaf of *Mimosa pudica* was taken and washed with ethanol followed by thorough wash with triple distilled water. The clean leaves were soaked in 50 mL of triple distilled water and boiled in a microwave oven at 800 W for 1 minute. This process was repeated for 6 times in 1 h in 10 min interval. It was cooled at room temperature and filtered. The filtrate was used as stock solution. The same procedure was followed for the preparation of *Anacardium occidentale* Leaf extract.

Synthesis of AuNP using the leaf extract

0.25 mM aqueous solution of HAuCl_4 in 20 mL DI water was prepared and stirred for 30 minutes at room temperature. To this solution, 500 μL of *Mimosa pudica* Leaf extract was added with vigorous stirring. The colour of the solution gradually changed from yellow to violet indicating the formation of AuNPs. Same procedure was followed for the synthesis of gold nanoparticles by using *Anacardium occidentale* leaf extract (200 μL).

Synthesis of gold nanoparticles using tri-sodium citrate

0.25 mM aqueous solution of HAuCl_4 in 20 mL DI water was prepared and refluxed it at 100 °C. After 15 min 2.5 mM of sodium citrate was added to the solution and refluxed. After 15 min the colour of the solution changed to pink indicating the formation of gold nanoparticles.

Synthesis of gold nanoparticles using tri-sodium citrate and NaBH_4 as reducing agent.

0.25 mM aqueous solution of HAuCl_4 in 20 mL DI water was prepared and 2.5 mM tri-sodium citrate was added as stabiliser. The solution was stirred for 15 min under ice bath. To this solution 100 μL of 2.5 mM of NaBH_4 was added. The solution suddenly turned to red indicating formation of gold nanoparticles.





RESULTS AND DISCUSSION

To confirm the formation of the AuNPs UV-Vis spectroscopy was utilized by measuring the characteristic SPR band of AuNP at 520-560 nm (Figure 3). The UV-Visible peaks in the range of 520-560 nm confirms the synthesis of the gold nanoparticle with various size or diameter. The graph indicates that the gold nanoparticles formed by the *Mimosa pudica* has higher diameter in sizes whereas the nanoparticles synthesized by using sodium borohydride have smallest diameter.

Catalytic activity

Studies of catalytic activity of synthesised AuNPs towards degradation of p-Nitrophenol by NaBH₄ using UV-Vis spectrophotometer: The time course spectra of degradation of p-nitrophenol is conducted to compare the catalytic activity of synthesised nanoparticles. Take 790 µL of distilled water in 1 mL quartz cuvette. To this, add 100 µL of AuNPs solution and 100 µL of 100 mM NaBH₄ solution. After 5 min add 10µL of 10 mM p-Nitrophenol to the solution and record the time course spectra at 403 nm in 1 min time interval. Follow the same procedure to study the catalytic activity of other AuNPs.

CONCLUSION

Gold nanoparticles having various particle size was developed using various plant extracts and reducing agents. The nanoparticles were characterized by UV-Vis Spectroscopy and SEM images. These nanoparticles were examined towards of p-Nitrophenol to explore the catalytic activity of these nanoparticles. The nanoparticles synthesized from the NaBH₄ as found to be less in size and higher rate towards the catalytic reduction of PNP. Whereas the nanoparticles synthesized from the leaf extract of *Mimosa pudica* has highest particle size and least reactivity towards the reduction reaction.

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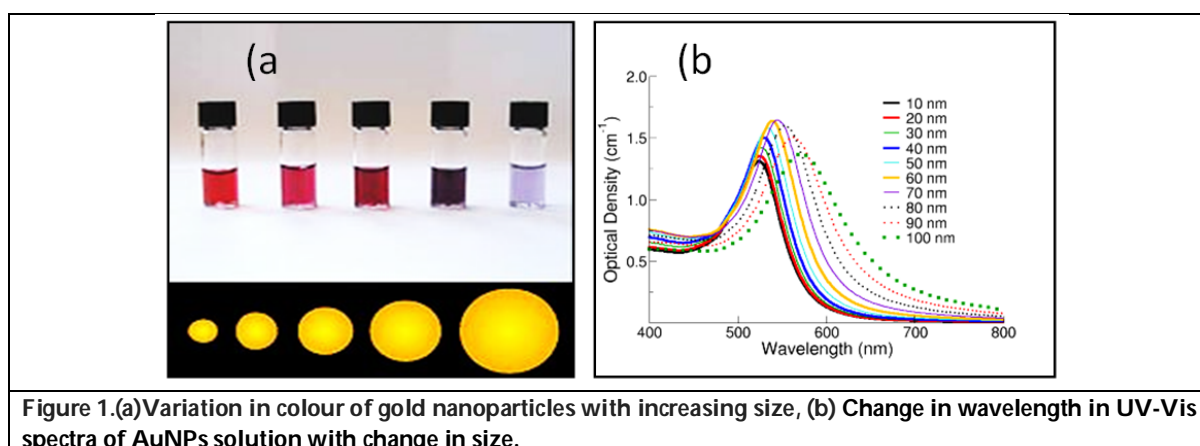


Figure 1.(a)Variation in colour of gold nanoparticles with increasing size, (b) Change in wavelength in UV-Vis spectra of AuNPs solution with change in size.





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Anacardium occidentale (Cashew)

Mimosa pudica (Lazzabati)

Figure 2. Leaves of *Anacardium occidentale* (Cashew) and *Mimosa pudica* (Lazzabati).

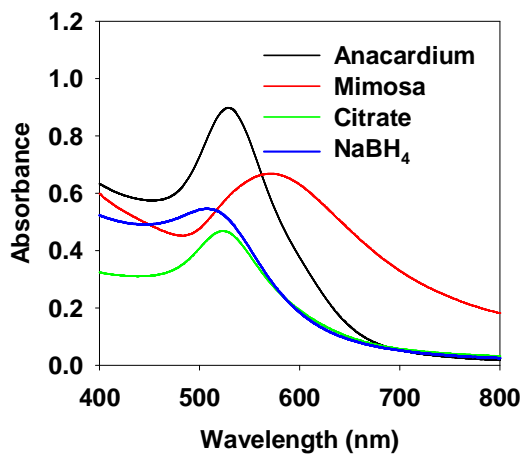


Figure 3. UV-Vis Spectra of synthesized nanoparticles.

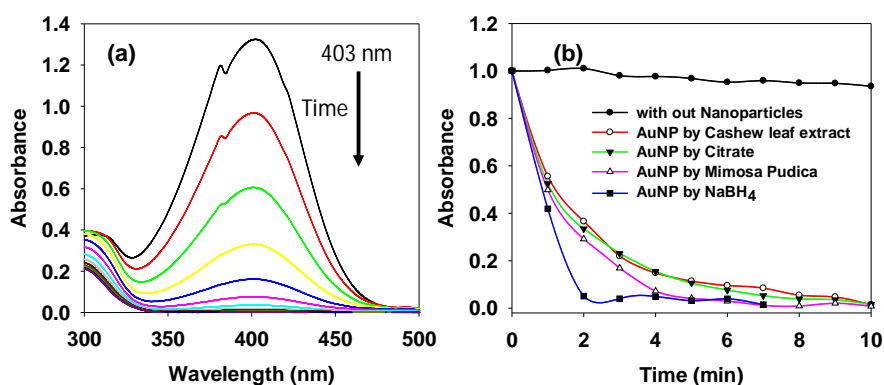


Figure 4. (a) UV-Vis spectra of p-nitrophenol with time, (b) Time course spectra of degradation of p-nitrophenol by NaBH₄ in presence of AuNPs.





Phytochemical Screening and *In vitro* Larvicidal Activity of Thorn Extracts of Plants on *Drosophila melanogaster*

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ABSTRACT

The aim of our study is screening of phytochemicals and larvicidal activity of methanolic extract of selected thorns of medicinal plants namely *Acacia catechu* wild, *Acacia ferruginea* DC, *Acacia nilotica* (L.) *Acacia senegalensis*, *Aegle marmelos* (L.) Corr, *Gymnosporia senegalensis* (L), and *Limonia acidissima*. The larvicidal activity was carried out using late third instar larvae of *Drosophila melanogaster* by direct contact method of methanolic extract of thorns of plants in graded concentration with 24 hr and 48 hr time interval at graded concentration from 50mg/L to 400mg/L. High mortality rate was observed at 400mg/L after 48 hrs. It was observed that *Acacia ferruginea* showed highest percentage of mortality compared to other plants with significant LD50 activity against *Drosophila melanogaster* larva. The qualitative analysis of methanolic extract of thorns of plants were investigated for flavonoids, terpenoids, glycosides, tannins, alkaloids, reducing sugars, phenols and saponins. *Acacia catechu* and *Aegle marmelos* revealed the presence of all the phytochemicals investigated. Where as in *A. ferruginea* all the phytochemicals were present except glycosides, *Acacia nilotica* showed the presence of all phytochemicals except alkaloids. In *Acacia senegella* there was absence of flavonoids, glycosoids and alkaloids. In *Gymnosporia senegalensis* L. and *Limonia acidissima* L. except tannins all the phytochemicals were present. The results of the present study clearly indicate that possible secondary metabolites of the extract of thorns can be isolated and be developed as eco-friendly larvicidal agent. As *A. ferruginea* showed potential larvicidal activity compared to other plants and hence can be used as insecticidal providing a better and alternate agent for the control of harmful insects.

Keywords: Phytochemical analysis, Larvicidal activity, *Drosophila melanogaster*, Medicinal plants.



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INTRODUCTION

Majority of the medicinal plants form a very large group considering as commercial and economically important plants. Insecticidal activities of medicinal plants against wide range of harmful insects have been reported by many researchers [1]. The plant metabolites has detrimental effect on insects growth and development, which can be manifested in several manners including sterility, morbidity, toxicity, mortality, growth inhibitor, anti-feedant, reduction of fecundity, CNS depressants, fertility and regulation of reproductive activities. Seeds and floral extracts of various medicinal plants have been reported to have toxicity, inhibiting the activities and potent in growth reduction of insects and other animal models [2-4]. Various studies have reported for the insecticidal activity depends upon the parts of the plants distributed the specific metabolites and exhibit such specific activity to the lethal in different concentration s of the active principles may vary from plants to plants [5,6].

The hazardous environment associated with the use of synthetic insecticides has led to an urgent need to explore suitable, cost effective and safer alternative agents for pest management and control. There is an emerging trend in recent years in research and development to support the bioactivities of medicinal plants [7]. The scientific reports indicates that plant derived molecules serve as potential sources of novel antioxidant, antimicrobial, anti-inflammatory, anticancer, cytotoxic, etc. There is significant evidence that the many of the plants and their product posses' insecticidal activity and it has been confirmed by investigations by various research groups in different parts of the world. Most of the higher plants are used as insecticidal agents [8]. Tobacco (*Nicotiana tabacum*) was one of the first finding to exhibit insecticidal property in a plants. Tobacco leaves were used to kill aphids which led to the isolation of their active molecule alkaloid called as nicotine. The chemical investigation of plant, *Rhododendron hortense* showed the presence of an active component called rotenone, with considerable insecticidal activity. The Chrysanthemum plants are the source of insecticidal molecules, pyrethrum and the active constituents called pyrethrins ct as potential biocontrol agent. The plants produce polyphenols called tannins which confer bitter taste and consequently herbivores stay away from eating such plants [9]. As a result, the use of insecticides has contributed to the increase in agricultural productivity, richness of biodiversity and improvement in human health, particularly the eradication of diseases. In this context, current study planned to screen for the 7 selected medicinal plants to identify their active ingredients as phytochemicals and *in vivo* larvicidal activity on *Drosophila melanogaster*.

MATERIAL AND METHODS

Plant Material

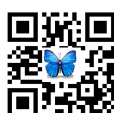
Medicinal plant thorns of *Acacia catechu*, *Acacia ferruginea*, *Acacia nilotica*, *Acacia senegalensis*, *Aegel marmelos*, *Gymnosporia senegalensis* (L) and *Limonia aciddisima* (L) of were collected from in and around Bengaluru, Karantaka, India. The plant samples were authenticated by from the Department of Botany, St. Joseph Autonomous College, Bangalore, India.

Preparation of Plant Extracts

The thorns of *Acacia catechu*, *Acacia ferruginea*, *Acacia nilotica*, *Acacia senegalensis*, *Aegel marmelos*, *Gymnosporia senegalensis* L. and *Limonia aciddisima* L. were collected, washed cleanly in distilled water and shade dried for complete removal of moisture. Around 20 g of thorny plant material was weighed, chopped, powdered and dissolved with 125 ml of methanol, kept for 4h extraction on water bath at 50°C, filtered using Whatman filter paper No.1. For the dryness of methanol, Buchi's rotary vacuum evaporator was used to concentrate the extract and refrigerated for the use planned experiments [10].

Qualitative Phytochemical Screening

Qualitative phytochemical screening of the extract of the 7 medicinal plants were carried out in order to know the class of organic molecules present in the methanol extract of the thorns selected for the study, which further





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facilitates for the identification of active ingredients and their characterization. The methanol extract of selected 7 medicinal plants thorns were subjected to standard chemical tests as described by Sharangouda and Patil, [11], Harnborne, [12] and Fransworth, [13] to determine the presence or absence of flavonoids, terpenoids, glycosides, tannins, alkaloids, reducing sugars, phenols and saponins. Preparation of reagents for phytochemical assay followed standard protocol of Harnborne [14]. To make the concentration to obtain the proper solution methanol extract was re-dissolved in double distilled water and filtered. The obtained filtrates were used for the each assay three times to get current value.

Larvicidal Activities of Thorn Extracts

Culture Medium

Adult *Drosophila melanogaster* were collected and reared on the artificial diet at 25 °C in the culture bottle. Artificial diet contained: brewers' yeast (60 g), glucose (80 g), agar (12 g) and propionic acid (8 mL) in 1000 mL double distilled water.

Larvicidal Screening

The refrigerated crude methanolic thorn extracts of 7 plants were used for the larvicidal screening. The extract graded concentrations 50, 100, 200, and 400 mg/L were used for the test. Seven late third instar larvae of *Drosophila melanogaster* were used for the each set of treatment for the experiment. Five numbers of glass beakers of 250 mL capacity were taken and labelled for different concentrations of thorn extracts and in addition one was maintained for control and four for the treatments. In case of control, distilled water and for the extract graded concentrations of seven plants were maintained respectively. Larvae were dipped into the solution for two minutes and then transferred back in the rearing medium (composition mentioned above). Each experiment was conducted in triplicates along with the control group. Mortality of larvae followed by the exposure was recorded after 24 hours up to 48 hours. The mortality due to treatment of 3rd instar larvae with different concentrations of the thorn extracts were recorded after 24 and 48 hours [15].

The LC₅₀ value was determined as following:

$$LC_{50} = \frac{LC_{100} - \text{Mean death} \times \text{Concentration difference}}{\text{No. of organism per group}}$$

Statistical Analysis

All the experiments were carried out in triplicates and the results were expressed as mean ± standard error of the mean. The data were statistically analyzed using Microsoft Office Excel 2007.

RESULT AND DISCUSSION

Phytochemicals Screening for Methanolic Thorn Extracts

The qualitative analysis of phytochemicals of methanol extract of 7 medicinal plants of thorns showed result maximum positive only. In *Acacia ferruginea* extract showed positive for flavonoids, terpenoids, tannins, alkaloids, reducing sugars, phenols and saponins. In *Gymnosporia senegalensis* L. & *Limonia acidissia* L. extracts showed positive for flavonoids, terpenoids, cardiac glycosides, alkaloids, reducing sugars, phenols and saponins. In *Acacia nilotica* extract showed positive for flavonoids, terpenoids, cardiac glycosides, tannins, reducing sugars, phenols and saponins. In *Acacia catechu* & *Aegle marmelos* extracts showed positive for flavonoids, terpenoids, cardiac glycosides, tannins, alkaloids, reducing sugars, phenols and saponins. In *Acacia senegal* extract showed positive for terpenoids, tannins, reducing sugars, phenols and saponins (Table1). Similar reports were revealed in *Euphorbia mili* thorns of ethanolic extracts and exhibited potential antioxidant property in *in vitro* studies [16].



**Malathi H and Thamizhseran N****Larvicidal Screening of Methanol Extract of 7 Medicinal Plant Thorns**

The larvae of *Drosophila melanogaster* (twenty five in each set) were administered with graded concentrations of methanolic thorn extracts for the duration 24 and 48 hours. The control treated with distilled water to know the comparative result of the studies. The effect of all the thorn extracts on larval mortality exhibited shown in figures 1 to 9. Out of the 7 plants, *A. ferruginea* extract exhibited larvicidal activity by their mortality rate 71.43% at 24 hour and 100% at 48 hour in a 400mg/L and determined their LC₅₀ at 200mg/L concentration. It is considered as best mortality rate at 200 mg/L concentration potential compare to others in very minimal concentration effect and confirmed it by purifying through column chromatography fraction (Figure 9), further studied for larvicidal activity and found significant LC₅₀ in *D. melanogaster* larva after 48 hours. Whereas other 6 plants in *G. senegalensis* extract exhibited larvicidal activity by their mortality rate 28.57% at 24 hour and 71.43% at 48 hour in a 400mg/L and determined their LC₅₀ at 400mg/L concentration extract, in *A. mermelos* extract exhibited larvicidal activity by their mortality rate 42.85% at 24 hour and 71.43% at 48 hour in a 400mg/L and determined their LC₅₀ at 400mg/L concentration extract, in *A. senegal* extract exhibited larvicidal activity by their mortality rate 57.14% at 24 hour and 71.43% at 48 hour in a 400mg/L and determined their LC₅₀ at 400mg/L concentration extract, in *L. acidissia* extract exhibited larvicidal activity by their mortality rate 28.57% at 24 hour and 71.43% at 48 hour in a 400mg/L and determined their LC₅₀ at 400mg/L concentration extract, in *A. catechu* extract exhibited larvicidal activity by their mortality rate 0% at 24 hour and 71.43% at 48 hour in a 400mg/L and determined their LC₅₀ at 400mg/L concentration extract and in *A. nilotica* extract exhibited larvicidal activity by their mortality rate 14.28% at 24 hour and 71.43% at 48 hour in a 400mg/L and determined their LC₅₀ at 400mg/L concentration extract, all these extracts did not exhibit any significant larvicidal activity and found its LC₅₀ value at the 300 to 400mg/L concentration and considered poor larvicidal activity. The results of the present study clearly indicate that possible metabolites of the extract can be developed as eco-friendly larvicidal agent and in that *A. ferruginea* thorn was also quite effective as insecticidal for providing a better and excellent alternate for the control of harmful insects.

Screening of medicinal plant extracts for inhibiting effect on insects activity is one of the novel application in the search of biocontrol agents as insecticides or larvicides. The application of chemically synthesized compounds to pest control (insect) is observed many adverse effects which include lower efficacy rate, resistance, human and ecological toxicity, contamination of air, water and soil to induce toxicity to a non target species. Hence, there is a need of hour to innovate safer, suitable, ecofriendly and cost effective. Plant metabolites are considered to be highly potential, non-toxic, non-pollutant and easy to biodegrade in any environment. The reports of Nair and Kavrekar [6] of larvicidal activity of methanol extracts of *Artocarpus heterophyllus*, *Artocarpus altilis* and *Piper betle* were as studied against *D. mealnogastor* larva and found significant LC₅₀ at 250 ppm concentration. In another study on larvicidal activity on some indigenous weed plant extracts in *D. melanogaster* on mortality rate at 10, 20, and 30% concentrations after 24 and 48 hours was found comparatively low. *Euphorbia prostrata* caused high mortality (51.64%) at 30% concentration and was found more toxic (LC₅₀ 27.76) after 72 hours. *Azadirachta indica* showed high LC₅₀ value compared to other plants. The synergistic effect of *E. prostrata* and Bti exhibited high rate of mortality (100%; LC₅₀ 12.49) after 72 hours [17].

To troubleshoot the increasing issues associated with the use of hazardous chemicals there has been an alarming time to find alternative development as safer, bio-protectants such as natural insecticides, biofeedants and bio-repellents. The larvicidal activity of a large number of plant metabolites like polyphenolics, alkaloids, glycosides, essential oils, aromatic compounds and other plant molecules has been successfully assessed against various agro-horticultural pests. The findings of the current study exhibit the larvicidal property out of the 7 plants found maximum and potential only *Acacia ferruginea* thorn extract and proved with their chromatographic fraction for the same, which may be due to the presence of compounds in the extract may possessing the activity. The results emphasized from the current study clearly revealed that *Acacia ferruginea* thorn extracts could be the best solution as potential candidate for developing biocontrol agent as insecticide for insect pest control.



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CONCLUSION

Biological products from plants have gained researchers interest as a modern trend as potent natural sources of pesticides. The application of medicinal plants by the native origin of different parts of the country as insecticidal agent has been well known. In this scenario, current findings explored to add few more plants potential as larvicidal property. *Acacia ferruginea* thorn extract found maximum among the screened plants and further studies with biochemical, molecular and spectroscopic analysis will help to clear the concept and to consider as best product for the biocontrol of pests.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1: Phytochemical investigations of the methanol extract of 7 medicinal plant thorns

Qualitative phytochemical analysis							
Tests	AF	GS	AN	LI	AC	AM	AS
Flavanoids	+	+	+	+	+	+	-
Terpenoids	+	+	+	+	+	+	+
Cardiac glycosides	-	+	+	+	+	+	-
Tannins	+	-	+	-	+	+	+
Alkaloids	+	+	-	+	+	+	-
Reducing sugars	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+

+ = Positive; - = Negative; **AF**: *Acacia ferruginea*; **GS**: *Gymnosporia senegalensis*; **AN**: *Acacia nilotica*; **LI**: *Limonia acidissima*; **AC**: *Acacia catechu*; **AM**: *Aegle marmelos*; **AS**: *Acacia Senegal*

Table 2: Mortality ratio of larvae for the determination of LC₅₀ of *Acacia ferruginea* thorn extract

Group	Number of larva/group	Methanolic extract of <i>Acacia ferruginea</i> thorn		
		Number of dead larva	Mortality (%)	LC ₅₀
24hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	0	0	
400mg/L	7	5	71.43	
48hrs				
Control	7	0	0	200mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	4	57.14	
400mg/L	7	7	100	

Table 3: Mortality ratio of larvae for the determination of LC₅₀ of *Gymnosporia senegalensis* thorn extract

Group	Number of larva/group	Methanolic extract of <i>Gymnosporia senegalensis</i> thorn		
		Number of dead larva	Mortality (%)	LC ₅₀
24hrs				
Control	7	0	0	-
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	0	0	
400mg/L	7	2	28.57	
48hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	1	14.28	
400mg/L	7	5	71.43	





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Table 4: Mortality ratio of larvae for the determination of LC₅₀ of *Aegel marmelos* thorn extract

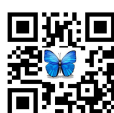
Group	Number of larva/group	Methanolic extract of <i>Aegel marmelos</i> thorn		
		Number of dead larva	Mortality ratio (%)	LC ₅₀
24hrs				
Control	7	0	0	-
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	0	0	
400mg/L	7	3	42.85	
48hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	1	14.28	
400mg/L	7	5	71.43	

Table 5: Mortality ratio of larvae for the determination of LC₅₀ of *Acacia senegal* thorn extract

Group	Number of larva/group	Methanolic extract of <i>Acacia senegal</i> thorn		
		Number of dead larva	Mortality ratio (%)	LC ₅₀
24hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	0	0	
400mg/L	7	4	57.14	
48hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	1	14.28	
400mg/L	7	5	71.43	

Table 6: Mortality ratio of larvae for the determination of LC₅₀ of *Limonia acidissima* thorn extract

Group	Number of larva/group	Methanolic extract of <i>Limonia acidissima</i> thorn		
		Number of dead larva	Mortality ratio (%)	LC ₅₀
24hrs				
Control	7	0	0	-
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	0	0	
400mg/L	7	2	28.57	
48hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	2	28.57	
400mg/L	7	5	71.43	





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Table 7: Mortality ratio of larvae for the determination of LC₅₀ of *Acacia catechu* thorn extract

Group	Number of larva/group	Methanolic extract of <i>Acacia catechu</i> thorn		
		Number of dead larva	Mortality ratio (%)	LC ₅₀
24hrs				
Control	7	0	0	-
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	0	0	
400mg/L	7	0	0	
48hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	1	14.28	
400mg/L	7	5	71.43	

Table 8: Mortality ratio of larvae for the determination of LC₅₀ of *Acacia nilotica* thorn extract

Group	Number of larva/group	Methanolic extract of <i>Acacia nilotica</i> thorn		
		Number of dead larva	Mortality ratio (%)	LC ₅₀
24hrs				
Control	7	0	0	-
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	0	0	
400mg/L	7	1	14.28	
48hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	1	14.28	
400mg/L	7	5	71.43	

Table 9: Mortality ratio of larvae for the determination of LC₅₀ of purified chromatographic fraction *Acaica feruginnea* thorn extract

Group	Number of larva/group	Chromatographic fraction <i>Acaica feruginnea</i> thorn extract		
		Number of dead larva	Mortality ratio (%)	LC ₅₀
24hrs				
Control	7	0	0	400mg/L
50mg/L	7	0	0	
100mg/L	7	0	0	
200mg/L	7	0	0	
400mg/L	7	7	100	
48hrs				
Control	7	0	0	200mg/L
50mg/L	7	0	0	
100mg/L	7	2	28.5	
200mg/L	7	5	71.43	
400mg/L	7	100	100	





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Figure 1: Control

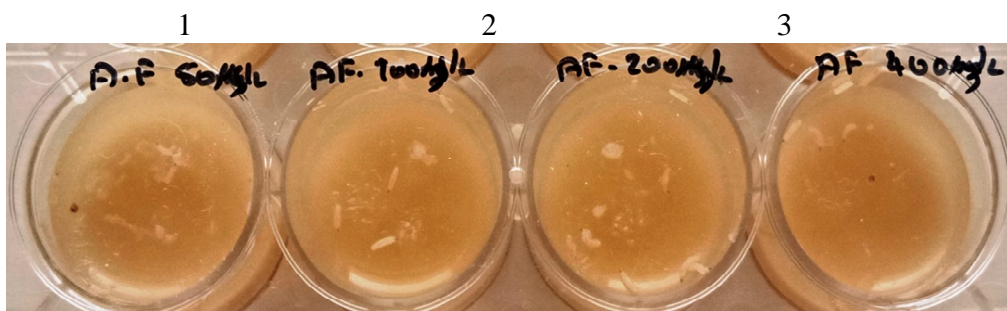


Figure 2.

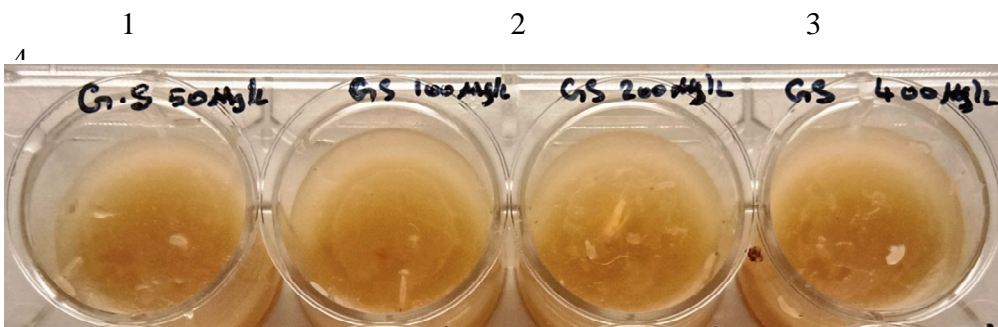


Figure 3.

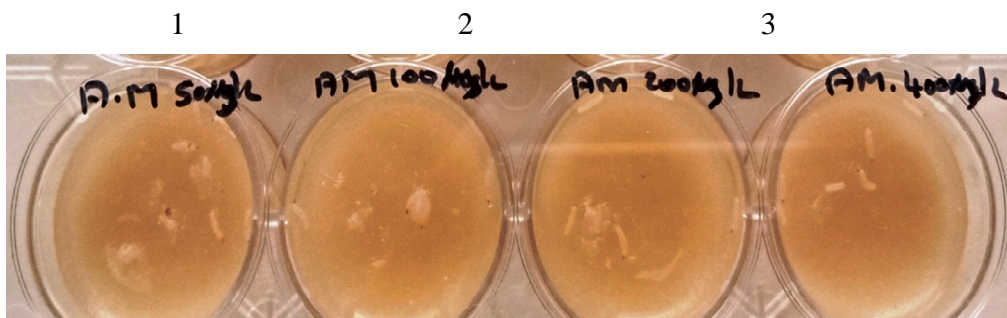


Figure 4.





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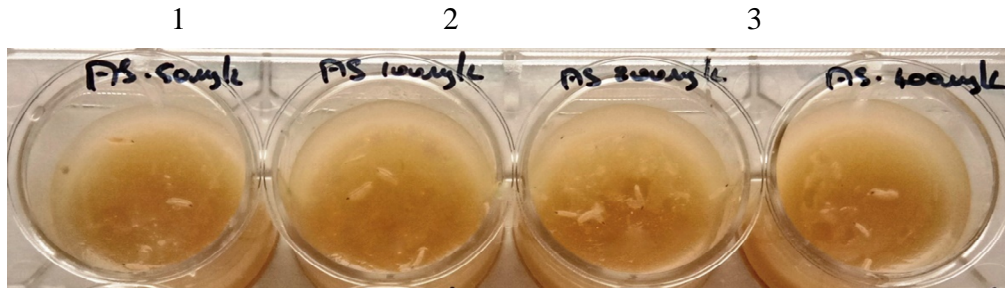


Figure 5.

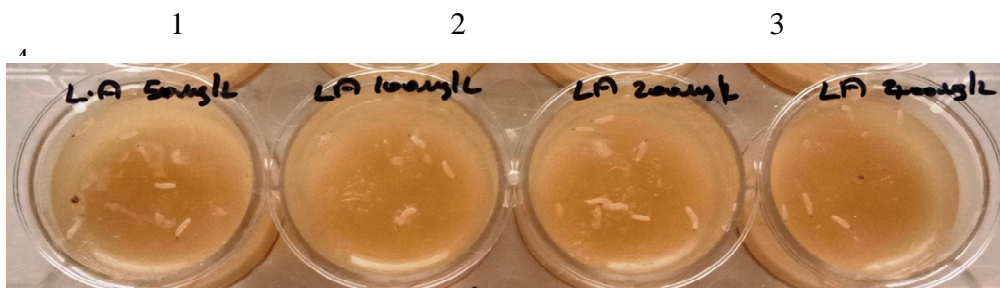


Figure 6.

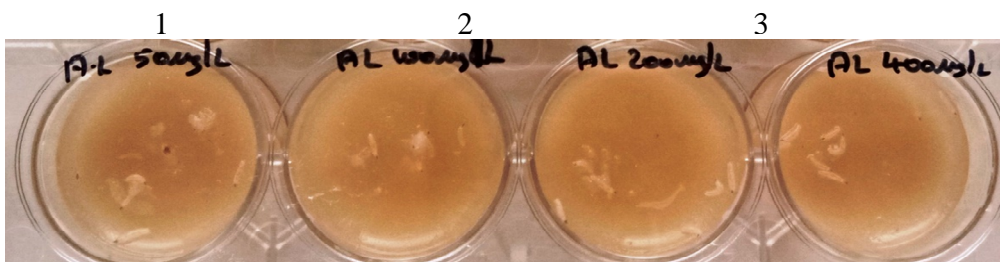


Figure 7.



Figure 8.

AF: *Acacia ferruginea*; GS: *Gymnosporia senegalensis*; AM: *Aegle marmelos*; AS: *Acacia Senegal*; LA: *Limonia acidissima L.*; AC: *Acacia catechu*; AN: *Acacia nilotica*

Figure 2-8: showed the mortality of methanol extract of 7 thorn plants at lane wise concentration, lane 1: 50µ/L; lane 2: 100 µ/L; lane 3: 200 µ/L and lane 4: 400 µ/L





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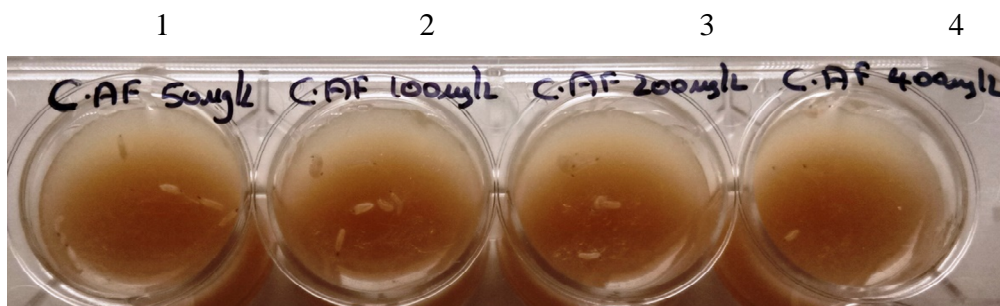


Figure 9: showed the mortality of purified chromatographic fraction of methanol extract of *Acacia ferruginea* thorns at lane 1-50 μ/L , lane 2- 100 μ/L , lane 3-200 μ/L , lane 4-400 μ/L





A Critical Review on Performance Characteristics of Compression Ignition Engine using Algal Biodiesel and its Blends as a Fuel

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ABSTRACT

In the current volatile environment, it is a need of time to search a promising alternative to petroleum diesel. Thin pursuit many researchers find out various biodiesel from edible and non-edible oil resources. Among this Microalgae and its blends found more promising and better alternative to petroleum diesel.

As per as the experimentation is taken into consideration, the performance parameters of Internal combustion engine play a vital role in its selection. The performance parameters mainly focused were brake power, brake specific fuel consumption and brake thermal efficiency. The performance is mainly depends upon the physical and chemical properties of fuel. In case of biodiesel, viscosity is major concern, which can reduced by transesterification. In this paper, review of biodiesel uses on year 2019 and various biodiesel feed stock litre yield per acre was platted on graphical way. From review clearly indicated that, microalgae have given more litre per yield in comparison with other biodiesel feedstock. the more Also an all-inclusive review of performance characteristics of Compression Ignition engine using algal biodiesel and its blends as a fuel carried out from available articles. This comprehensive review reveals that performance characteristics of compression ignition using algal biodiesel as a fuel are quite compatible in comparison with petro-diesel.

Keywords: Comprehensive Review, Algal Biodiesel, Performance Characteristics, Compression Ignition Engine.



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INTRODUCTION

The current situation of petroleum products and especially petro diesel is little bit upsetting. The currently available and rapidly diminishing sources along with daily increasing demands added more concern in this issue. It is well known fact that the petroleum product availability and demand somehow influence country's economy. At the same time the emissions from engine using petro diesel is a big concern problem for environmental friends. Considering these facts, the researchers throughout the world searching and actually propose some environmental friendly alternatives to petro diesel. The alternative suggested by researcher community is oils derived from vegetable or green plants, which were compatible with petro-diesel and environment friendly named as biodiesel. Biodiesel obtained from various sources and its properties transformed chemically by Transesterification normally.

As per as the feasibility of these oils are concern, the performance parameters of engine such as brake power, specific fuel consumption and brake thermal efficiency will be the measuring parameters, which found to be in close to petro-diesel.

Feedstock for Biodiesel

Biodiesel produced from several feedstock. For better understanding, they are categorised as follows.

- Edible Oils
- Non-Edible Oils
- Waste or Recycled oil and
- Animal fats

In addition, they classified as-

- First generation biodiesel
- Second generation biodiesel and
- Third generation biodiesel

Biodiesel producer industries are getting good response but the raw material (feedstock) supply retards its pace at the same time strain on margin of biodiesel production. As per the review, there are 89 biodiesel plants from which biodiesel produced. The percentage use of such biodiesel feedstock in year 2029 given in following.

Soybean oil feed stock is the most widely used for biodiesel production in industries. Canola oil, corn oil, yellow and white grease, and lastly tallow are the other feed stocks who are in top. From this it is very clear that the other feedstock are still on experimental or research level and not used commercially. This will be the inspiration for finding another competent and commercially feasible feedstock for biodiesel production. It is not possible to convert edible oil in to biodiesel always since it may produce oil scarcity. Due to this reason, the attention focused on non-edible oil sources such as jatropha, karanja, and canola as well as used cooking oil. At the same time, 'Algae Oil' is the new entry in this feedstock list.

Algae

Algae is the microscopic aquatic plants, which consumes carbon-di-oxides, sunlight and water to convert it into biomass, Lipids and oxygen. It is the fast growing in comparison with other green plants. Algae does not require fresh water as well as no separate land requirement as in case of other feed stocks. Production of lipids from algae is a promising for biodiesel industries. Another significant distinguishing feature of algae source is oil content and per hectare yield. Above points made algae as a promising alternative in comparison with other feedstock as a fuel for compression ignition engine.





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Engine

Engine converts chemical energy of fuel into mechanical work. That means any type of engine converts mechanical work into rotational work at shaft with specific revolution per minute (RPM). So it is very essential to understand the engine geometry because it is directly proportional to volume of cylinder and hence for power. The number of cylinders, bore diameter, stroke length, and clearance volume are some important terms which should be considered while investigating performance of engine.

Performance Characteristics

By continuous entire fuel burning, how much mechanical work engine produces at specific torque and revolution is the performance of engine. This will be evaluated in terms of following parameters (known as performance parameters).

Torque

The torque produced by engine is normally measured by using a dynamometer (eddy current, hydraulic or rope brake). The engine shaft is connected to the dynamometer. The rotor is coupled with stator. Torque exerted on the stator is measured. The torque developed by engine is calculated as-

$$\text{Torque (T)} = F \times L$$

Power

Product of angular speed and torque is the power developed by an engine. It is calculated as –

$$\text{Power (P)} = 2\pi NT$$

There are mainly three types of power which should be calculated for getting performance curves.

- i. Indicated power (IP)
- ii. Brake power (BP)
- iii. Frictional power (FP)

The relation between these three is-

$$IP = BP + FP$$

Specific Fuel Consumption

The amount of fuel consumed by engine to develop unit power is termed as specific fuel consumption. It is calculated as-

$$SFC = \frac{\text{Mass of Fuel}}{\text{Brake Power}}$$

It is a very essential parameter since it clearly indicates the engine capacity to burn fuel for power production. It is always desirable that value of specific fuel consumption will be low.

DISCUSSION

For the better understanding the reviews were putted in tabular form. This will help to compare the engine type along with performance parameters at a glance. From above table following conclusions can be drawn.

1. The oil yield (L/acre) was highest most from algae (19000-57000 L/acre), which make him promising feedstock for biodiesel extraction.
2. Most of the experimentations was carried out on four stroke engine and mainly on Kirloskar engine in India.
3. The performance parameters were found closer with petroleum diesel and compatible.
4. All the literature cited shows that the brake power was found to be increase (1% -20 %) and within acceptable range.
5. Increase in brake specific fuel consumption (0.9%-13.90%) was also noted in literature reviews.

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6. The brake thermal efficiency was found to be increase from 1 % to 27.65% which is a wide range.

This review reveals that the algae and its blends characteristics were compatible with petroleum diesel and other biodiesel resources. Also no adverse remark was found in entire literature review regarding use of algae biodiesel and its blends as fuel for diesel engine. So it can be concluded that, algae and its blends can be used as fuel for diesel engine without any major modification in existing diesel engines.

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Table 1. Review of Performance Characteristics

Ref. No.	Author	Engine	Brake thermal Efficiency	Brake Specific Fuel Consumption	Brake Power
01	Shiv Kumar Sonkar et.al.	NM	Decrease	Increase	NM
02	Sara Tayari et.al.	3 LD 510 Lombardini	Decrease (15-20 %)	Increase (12-15%)	Decrease (15-20 %)
03	Gongping Mao et.al.	186F diesel engine	NM	Increase (7-12%)	Increase (2.43%)
04	Murat Kadir Yesilyurt, et.al.	Katana Km 178 Fe	Decrease	Increase (7.19-13.90%)	NM
05	Upendra Rajak, et.al.	1/4, DI engine	Decrease (3.3%-8 %)	Increase (up to 3.3 %)	Decrease (3 % -8 %)
06	S. Karthikeyan, et.al.	NM	NM	Decrease (0.1-0.21 %)	NM
07	Nabam Hina Papu et.al.	VCR- Kirlosar TV 1	Decrease (1.4-4.3 %)	Increase (2.6-15.38 %)	Decrease
08	Arangarajan M., et.al.	VCR- Kirlosar TV 1	Decrease (1.2.%)	NM	NM
09	R Sam Sukumar et.al.	Computerized VCR engine	Increase (32.54%)	Increase	NM
10	Sunil Kumar et.al.	Kirloskar, AA35	Decrease	Increase	NM
11	B. Rajendra Prasad Reddy, et.al.	Kirloskar AV1	Decrease (1-2%)	Increase	Decrease
12	Sanjay Singh, et.al.	Kirloskar TV1	Decrease	Increase	Decrease (0.4-1.16%)
13	Mohankumar Subramaniam, et.al.	Kirloskar engines	NM	Increase	Decrease
14	Amit Kumar Sharma et.al.	DICE	Decrease	Increase (9.3%)	NM
15	A P Jagadevkumar et.al.	Kirloskar, TV1	Decrease (3.15 %)	Increase (15.96 %)	Decrease (2.89 %)
16	Piyushi Nautiyal et.al.	NM	Decrease	NM	NM
17	Xinyu Weia, et.al.	Ford Duratorq	Decrease	Increase	Decrease





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18	Avinash Kumar et.al.	Kirloskar TV1	Decrease	Decrease	Decrease
19	Mahesh Prakash Joshi et.al.	K TA-1	Increase (27.65%)	Decrease (3%)	NM
20	V. Hariram, J. et.al.	K 240PE	NM	NM	NM
21	Supramani Saravanan, et.al.	Kirloskar, TAF1	Increase	Decrease	Increase
22	Hariram Venkatesan, et.al.	Kirloskar 240 PE,	Increase (16.67%)	Decrease (6%)	Increase
23	M. P. JOSHI et.al.	NM	Increase	Increase	Increase
24	Chang Chun Xu et.al.	NM	Increase	Increase	Increase
25	Nambaya Charyulu Tatikonda, et.al.	Kirloskar TV1	Increase	Increase	NM
26	M. Vijay Kumar et.al.	NM	Increase (1-2 %)	Increase (1-2 %)	Increase (1-2 %)
27	Babban Yadav et.al.	Perkins 4.236	NM	Increase (4.72%)	Decrease (3.56 %)
28	Rajendra Pawar et.al.	NM	Increase	Increase	NM
29	Muragesh Bellad et.al.	NM	Increase	Increase	NM
30	V.Naresh et.al.	Kirloskar	Decrease	Increase	NM
31	V. Naresh et.al.	Kirloskar	Decrease	Increase	NM
32	M. Saraswat et.al.	Kirloskar	Decrease	Increase	decrease
33	V Naresh et.al.	AV-I	Decrease	Increase	NM
34	A. Prabhu, et.al.	HS 4S vertical, air cooled type.	Decrease	Increase	Vol. Eff. Decrease at higher load
35	Upendra Rajak, et.al.	Legion Brothers	Decrease	Increase	Decrease
36	Fevzi Yasar et.al.	4DW81-23D	Slight Decrease	Slight Increase	Decrease
37	Senthil Ramalingam et.al.	NM	Slight Decrease	Slight Increase	Decrease
38	Katam Ganesh Babu et.al.	Kirloskar - APEX (240)	NM	Increase	NM
39	V Hariram et.al.	Kirloskar 240	High (16.67%)	lower (6%)	Increase
40	C.Syed Aalam et.al.	Kirloskar AV1,	Minor Increment	High	NM
41	A. Prabu et.al.	Kirloskar	Increase	Decrease	NM
42	Hariram V et.al.	Kirloskar AV1	Increase	Decrease	Increase
43	Saddam H. Al-lwayzy et.al.	Yanmar L48N6	Minor Increment	High	NM
44	Florian Pradelle et.al.	NM	Decrease	Increase	less
45	Ahmed I. El-Seesy et.al.	HATZ-1B30-2	Increase (16%)	Decrease (15 %)	Increase
46	Kuberan J. et.al.	Kirloskar	Increase	Increases	NM
47	S. Naveeth Ahamed et.al.	NM	Increase	Increase	Increase
48	Ahmad Taghizadeh-Alisaraei et.al.	Lombardini Model 3LD510,	Increase (10.63 %)	Decrease (14.66%)	Increase (5.36%)





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49	Muhammad Aminul Islama et.al.	NM	Increase	Increase	Increase
50	J. Jayaprabakar, et.al.	Kirlosker,	Decrease	Increase	Decrease

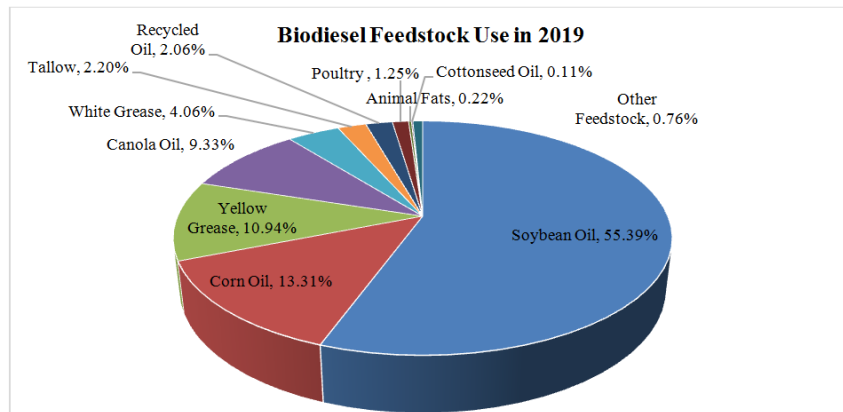


Figure 1. Biodiesel feedstock use in 2019

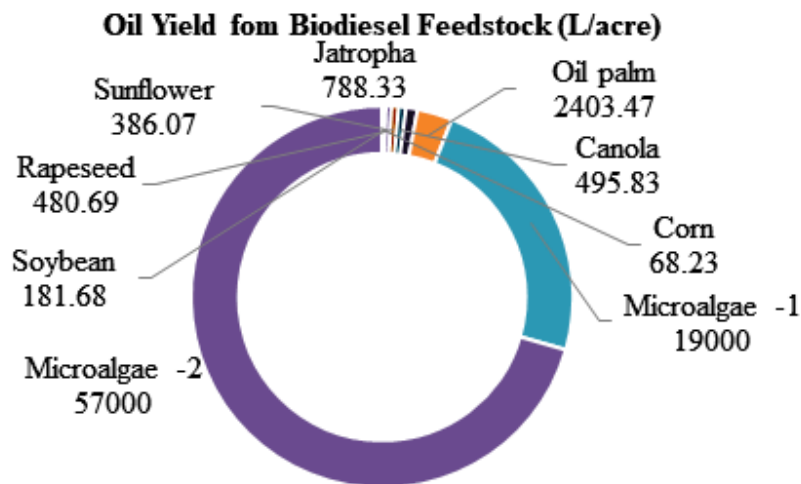


Figure 2. Oil yield from various biodiesel feedstocks (L/acre)





Food Culture of Sangam Tamil Civilization - in Purananuru

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ABSTRACT

Literature is a powerful tool to apprehend the culture and activities of all species on earth. Tamil nadu is one among the ancient places of human evolution. Purananuru is an ancient Tamil anthology, which is one among the eight anthologies of Sangam Tamil Literature. It consists of 400 songs on different themes. It states on war, wisdom, the rulers and socio culture of common people in those days. The poems in puram provides abundant food references about the rich Tamil cuisine during Sangam period. The knowledge of traditional food according to the geographic location is indeed essential. This helps to recollect the food culture of ancient Tamil's. According to a Tamil saying "Unave Marundhu" Food is always considered as medicine among Tamil community in ancient days. Hence the present review was done to Explore food culture of Tamil Nadu. and bring back the typical nutritional delicacies of Tamil Nadu. The Padal's (Poems) in Puranauru was reviewed to find the food culture of Sangam Tamil civilization. Different varieties of food sources and preparations were evidenced. Several Ethnic foods of Tamil Nadu were identified from the padals of purananuru. The dishes were categorized as main courses (31), accompaniments (19), desserts and snacks (15), Non- alcoholic beverages (8) and alcoholic beverages (12).

Keywords : Literature, Purananuru, Tamil Civilization, Sangam period, Ethnic Foods





INTRODUCTION

Literature is a powerful tool to apprehend the culture and activities of all species on earth. Tamilnadu is one among the ancient places of human evolution. Tamil can be claimed as one of the longest unbroken literary traditions of any of the world's living languages. The ancient Tamil literature is termed as sangam literature. It has ceased to be a living literature and became part of an "extinct" classical heritage sometime in the 6th-8th century. A. D (Zvelebil, 1974). The earliest corpus of Tamil literary texts may be dated roughly between 100 BC and 250 AD. The term 'sangam' literally means 'confluence' or 'assembly' The sangam age is considered as classic or golden age (Rangarajan, 2018). The historical resources such as the classic literature, epigraphs, leaf scripts, archaeological identifications serve as evidences and offers detailed descriptions on Tamil culture. Sangam literature is broadly classified as agam (internal character or love) and puram (external or heroism, kings etc). *Tolkappiyam* is one of the most ancient work in Tamil sangam literature. After *Tolkappiyam* the sangam as literary work is classified into two different anthologies *padhinenmelkanakku noolgal* and *padhinenkeelkanakkunoolgal*, that is commonly termed as *Thogai Noolgal*. The *pathinenmelkanakkunoolgal* is classified into two as *ettuthogai* (eight anthologies) and *pathupaattu* (ten idylls). These consist collection of poems by different poets irrespective of a common theme (Pillai KK, 19 5 9).

Purananuru is an ancient Tamil anthology, which is one among the eight anthologies of Sangam Tamil Literature. It consists of 400 songs on different themes. It states on war, wisdom, the rulers and socio culture of common people in those days. This anthology has been translated in English by G. U.Pop . The knowledge of traditional food according to the geographic location is indeed essential. Purananuru is a world known anthology which highlights the perspectives of food in different poems . This helps to recollect the food culture of ancient Tamil's. The poems in puram provides abundant food references about the rich Tamil cuisine during Sangam period. In puram the food perspectives have been mentioned in different ways. Its either mentioned as a compound meal or a single dish, as a parable, staples of a particular region, the agricultural cultivations, work time foods, war time foods, food of a wife after her husband's condolence, or as different food resources in respect with the type of land (*Ainthinai*).

The food culture of Tamil's has a very long history since antiquity. The Food consumption is majorly influenced on the geographic location and occupation of men. The ancient Tamil's classified their geographic into five different landscapes which is termed in tamil as *Ainthinai*. The Kurinji, Mullai, Marudham, Neidhal and Paalai (Rajayyan 2005). Each land was named after a characteristic flower bloomed in the particular region. The people's culture, occupation, god, foods, flora, fauna, musical instruments, equipment differs in respect to the *thinai*. Food holds a dignified position among Sangam Tamils. A phrase in purananuru highlights the preciousness of offering food . "உண்டுகொடுத்தோர் உயிர்கொடுத்தோரே" (Purananuru). Which means offering food is considered as offering life. The ingredients were fresh, healthy and cultivated in the same region and cooked food is free from other added synthetics. According to a Tamil saying UnaveMarundhu Food is always considered as medicine among Tamil community in ancient days. Hence the present review was done to Explore food culture of TamilNadu. and bring back the typical nutritional delicacies of Tamil Nadu.

METHODOLOGY

The Padal's (Poems) in Puranauru was reviewed to find the food culture of Sangam Tamil civilization. Different varieties of food sources and preparations were evidenced.

RESULTS AND DISCUSSION

The Food Sources

Agriculture was the primary occupation of sangamtamils. All the foods were cultivated in their own region. Hence were fresh and healthy. Their flavors were unique with the use of regional ingredients. The plethora of naturally





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cultivated sources was used in daily cookery. The importance of agriculture in SangamTamilagam is highlighted in the Tamil Epic Thirukkural.

உழுதுண்டு வாழ்வாரே வாழ்வார்மற் றெல்லாம்
தொழுதுண்டு பின்செல் பவர்

- Tirukural 1033

A padal in puram has also stated the Agricultural beauty of a city in Sangam Tamilagam.

ஏர்பரந்த வயல் நீர்பரந்த செறுவின்
நெல்மலிந்த மனைப் பொன்மலிந்த மறுகின்

-Purananuru 338

Tamil civilization in Sangam era had a highly nutritious food frequency. Tamarind, curd and butter milk were the primary ingredients to offer tanginess or sour taste in their dishes. Pepper is the common spice added to dishes to offer spiciness. Honey is the primary sweetener and also extracts of sugarcane. The use of salt is evident. The paste of sesame seeds were used in curry preparations. It is surprising that, the so called primary bases of all curries of India or Tamil Nadu of present day, the onion and tomato is actually not evident in purananuru, as its not originated in Tamilnadu. Tomato was introduced by the portugese to the world (Mehta, 2017). Though the use of onion i n India is evident during the 6 BC in a famous early medicine treatise charaka Samhita (Mehta, 2017), its use is not evident in purananuru. Coconuts and palmyra are the most common. The flesh and water of coconut is consumed. The palyra flesh, fruit and sprouts are consumed like a snack .

Food Sources Evident in Purananuru

Table 1 to 4

Cooking Techniques - Evident In Purananuru

Table 5

Food Preparations & Nutritional Perspectives

Table 6

Rice in Sangam Tamil

Rice holds special mention in sangam literature. It has several mentions in the padal's of purananuru. The following phrases stat es the heritage of rice and rice cultivation in ancient Tamilagam.

நெல்உகுத்துப் பரவும் கடவுளும் இலவே - Purananuru 335: 12

கதிர், நெல்லின் செம்மல் மூதூர் - Purananuru 97: 18

நெல்லரி தொழுவர்- Purananuru 379: 3

The importance of rice in Sangam Tamil Diet is strongly stated in the 335th song of purananuru. The poet states that there are no foods other than the following four: Varagu Thinai , Horse Gram and Broad bean. Hence its evident that a meal is not complete without rice

இருங்கதிர்த் தினையே
சிறுகொடிக் கொள்ளே, பொறிகிளர் அவரையொடு
இந்நான் கல்லது உணவும் இல்லை

-Purananuru 335: 4-6





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Rice was consumed in different forms. Its mentioned in different names according to the combination made. For example *Neiyudaiadisil* (Rice with Ghee), *Paalsoru* (Rice with milk), *Neersoru* (Rice with water), *Oon Soru* (Rice with meat), *Vensoru* (White rice). Foxtail millet (*Thinai*), Kodo millet (*Vargu*), Red rice, White rice and Bamboo rice were the rice varieties used for food preparation. The grains were unmilled, unpolished, rich in fiber and micronutrients, with zero synthetics. The Cooking techniques were also slow and mild. The rice preparations were mostly consumed as main courses. Rice was termed as a *disil*, *amalai*, *attu*, *soru*.

Meat in Sangam Tamil

Puranauru tags several kinds of meat consumption, which is not in existence today. Several varieties of fishes were freshly caught from different water sources and the slush in the agricultural fields. It's evident that the sangamtamils were predominant meat eaters. The rabbit meat was mostly dominant in their platter.

Beverages

Both alcoholic and non-alcoholic beverages were consumed among sangam Tamils.

Alcoholic beverage consumption was predominant among the Sangam Tamils. They named those beverages with different terms such as *mattu*, *madhu*, *theral*, *vengal*, *naravu*, *kal* according to the source or type (Rajamanickam, 1970). Most of the Beverages were prepared from juices of coconut and palmyra trees. *Mattu* was prepared from honey. The toddy in which rice is cooked is called *naravu*. A padal in agham has stated that toddy is also derived from the millet called *Thinai*. Toddy was considered as food. The following phrase in purana nuru states the importance of toddy in sangam period. “ கள்ளும் குறைபடல் ஒம்புக ! ”, Which means have toddy in abundance so that there is no shortage (Purananuru).

The Meal and Food Culture

The portrayal of the meal in tamil literature is elucidated in different regions at different situations. A feast is not only on a festival day, tamil people make each day a feast, with available food commodities. They enjoy their meal as feast, while gazing cattles, doing agriculture. The foods were highly nutritious foods. Mullai is a landscape surrounded with forest. It is considered as a land of renewal. This is named after the flower mullai (jasmine). This land is often used for grazing livestock. According to the below mentioned *padal*, this particular area in mullai zone is a dry land, where there is no cultivation of rice. Hence millets like Kodo (*varagu*) and Foxtail (*Thinai*) are consumed. Here the meal is served with curd, a fruit called *thodaripazham*, sour toddy, well cooked meat pieces (*vaadunKozhungurai*) and white rice cooked with ghee (Purananuru 328).

Marudham is the region of agricultural fields. It is named after the flower named marudham, which is commonly known as arjuna tree these days. The meal in the marudham landscape is beautifully explained in the 395th verse of purananuru. The farmers of the marudham landscape let their cattle graze in the mullai land and relish their meal in the meantime. They wore the mullai flowers and sway off the birds which flock to eat the cultivated paddy in the field. This meal consists of a variety of dishes. They consume cooked meat of rabbits, aviyaal made with big sword fishes (*neduvaalai pal uviyaal*), previous day; s rice gruel (*pazhanjotrupugavu*), rice with highly tempered thuvaial (*Kozhunthuvaiaadisil*) along with *avizhnellinariyaal*, a liquor made from the strained water of cooked rice. Another meal is also described in the same padal. The cooked rice which was in the shape of a crane's nail is eaten along with porikari and hot meat (Puram 395).

Parambunadu is a region in ancient Tamil Nadu which is called pranamalai today in Sivagangai District. In this area *MattinTheral* is an aged alcoholic beverage fermented underground. This is served along with rice and Naru *Neikkadalai* (nuts roasted in aromatic ghee) (Purananuru 120:12). The 34th song of puram belongs to chola dynasty. Here *parpeipunagam* is made with *varagu* rice, milk and sweetened with honey. This dish is served along with hot meat of small rabbits. It is stated that the *parpeipunagam* is consumed both morning and evening (Purananuru 34:11). Ollaiyur in Pandiyanadu is a region which belongs to the pandiya dynasty and later





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captured by the chola ruler. In this area a simple meal of a widow after the death of her husband is stated in the 246th song of puram. She hasn't use the aromatic ghee these days. The cooked rice which is soaked in the water is squeezed, placed on the leaf & eaten along with a paste of white sesame and a sour curry made with tamarind and velai leaves (Purananuru 246) .

It is evident that the flowers of this velai is also used in recipes. The following meal was stated in a paadal sung by koperuncholan a history marking chola king. Cooked and strained rice of varagu along with *ambulimidhavai* is eaten more by the harvesters of broad beans in the field of pandiyanadu. For the preparation of *ambuli midhavai* the white flowers of velai is cooked with salt and nicely mixed with curd. It is tempered with pepper powder and tamarind paste. At present in Tamil Nadu the paddy rice used for regular cooking is mostly the old or aged one. The new paddy rice is significantly cooked in a Tamil festival Thai thirunaal / Pongal. The harvested rice is cooked and offered to god. In *puram a paadal* reveals a meal with new paddy rice. The fatty pieces of vaalai fish is eaten with white rice cooked from new paddy. There is also large coconuts (*thengupaduvian pazham*) in this region youngsters who hate this, pluck the fruits of palmyra palms.

Palmyra is considered as a typical tree of Tamil Nadu. In puram three edible offerings of palmyra is mentioned. The sweet flesh of palm seeds, the flesh of the fruits and hot *panankilangu* (Palm sprouts) cooked on flame. Sangamtamils were predominant meat eaters and most of their accompaniments were dominant with meat and fish. The green leaves curry or *keerai* curry is stated in some *padal* of purananuru.

வளைக்கை விறலியர் படப்பைக் கொய்த
அடகின் கண்ணுறை ஆக யாம் சில
அரிசி வேண்டினெம் ஆகத், தான் பிற.

- Purananuru 140: 5

கொய்யடகு வாடத், தருவிறகு உணங்க,
மயில்அம் சாயல் மாஅ யோளொடு
பசித்தன்று அம்ம, பெருந்தகை ஊரே

- Purananuru 318

The above *padal* states that greens curry was consumed with rice. Even though greens are nutritious the *padals* mention the consumption mostly among poor people or widow women. The 159th song of purananuru states the simple meal of a women after her husband's death. She prepares a simple curry with boiled kuppaikeerai, without salt and butter milk and consumes with rice.

குப்பைக் கீரைக் கொய்கண் அகைத்த
முற்றா இளந்தளிர் கொய்துகொண்டு, உப்பின்று,
நீர்உலை யாக ஏற்றி, மோரின்று,
அவிழ்பதம் மறந்து, பாசடகு மிசைந்து

- Purananuru 159: 9

A delicious meal called *Avizhpuzhukkal* is stated in the 399th song of Purananuru. The harvested white paddy is pounded ,dehusked and rice is used for cooking . A meal is cooked with the pounded rice, fermented gruel, fatty pieces of two varieties of fish, vallarai leaves, tamarind and sweet mangoes. Ethnic foods are foods originating from a heritage and culture of an ethnic group who use their knowledge of local ingredients of plants and / or animal sources. TamilNadu is a state of rich cultural heritage since antiquity. Hence the ancient anthology Puranauru was reviewed to find the ethnic foods of Tamil Nadu.





CONCLUSION

Food and Culture are interlinked and has a strong impact on people's health. The awareness on different perspectives of food culture creates a good impact on people's knowledge to make ethnic food choices. Hence the literary evidences collected in this study acts as a reference to determine the foods of our ancestors.

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Table 1. Cereals, Millets and Pulses

Food Source	Padal / Verse	Source
Foxtail millet	சாரல் புனத் தினன அயிலும்	143: 5
Kodo millet	வாலிதின் வினைந்த புது வரகு அரியத்	120: 9
Paddy	அவிழ் நெல்லின் அரியல் ஆருந்து	395-8
Red rice	அலங்கு நசந்நெல் கதிர் வவய்ந்த	22:14
White rice	களிக்நகாள் நவண்வசாறு உண்டு	328: 11-12
Bamboo rice	ஒன்வே சிறியினல நவதிரின் நெல் வினையும்வே	109: 4
Javanam (Wild mountain rice variety)	ஐவனம் வித்தி மையுறக் கவினி	159: 17
Pullarisi (Seeds of grass)	அல்லிப்படுஉம் புல் ஆயினவவ	248: 5
Broad Bean	அவரைக் கொழுங்கொடி	120: 10-11
Horse Gram	கொள் உழு வியன் புலத்துழை கால் ஆக	105: 5





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Table 2. Vegetables, Fruits and Nuts

Food Source	Padal / Verse	Source
Ridge gourd	பீரை நாறிய சுரை இவர் மருங்கின்	116: 6
Bottle gourd	பீரை நாறிய சுரை இவர் மருங்கின்	116: 6
Bitter gourd	சிறு கொடிப் பாகல்	399: 6
Broad bean	அவரைக் கொழுங்கொடி விளர்க்காய் கோள் பதம்	120: 10 - 11
Cucumber	அணில் வரிக் கொடுங்காய்	246: 4
KuppaiKeerai	குப்பைக்கீரைக் கொய்கண் அகைத்த முற்றா இளந்தளிர் கொய்து	159: 9
Munj aikeerai	குறு நறு முஞ்சைக் கொழுங்கண் குற்றடகு	197: 11-12
Keerai	கொய் அடகு வாட	318: 1
Velai leaves	வேளை வெந்தை வல்சி ஆகப்,	246: 7-8
Velai flowers	வேளை வெண்பூ வெண்தயிர்க் கொளீஇ, ஆய் மகள் அட்ட அம் புளி மிதவை	215: 3-4
Vallarai Greens	செறுவின் வள்ளை	399: 6
Jackfruit	தீஞ் சுளைப் பலவின் மாமலைக் கிழவன்	129: 4
Mango	ஓங்கு சினை மாவின் தீங்கனி	399: 4
Gooseberry	சிறியிலை நெல்லித் தீங்கனி குறியாது	91: 9
Banana	செழுங் கோள் வாழை அ	168: 13
Bread Fruit	ஆசினிக் கவினிய	158: 22
Palm Fruit	இடையோர் பழத்தின் பைங்கனி மாந்தக்	225: 2
Coconut	தெங்குபடு வியன் பழம் முனையின்	61: 8-9 / 37
Tender coconut	தெங்கின் இளநீர் உதிர்க்கும்	29:15 - 16
Kalapazham	புளிச் சுவை வேட்ட செங்கண் ஆடவர் தீம் புளிக் களாவொடு துடரி முனையின், மட்டு அறல் நல்யாற்று எக்கர் ஏறிக்,	177: 8-11
Naval fruits	கருங்கனி நாவல் இருந்து கொய்து உண்ணும்	177: 8-11
Groundnuts	நறு நெய்க் கடலை விசைப்பச்	120:14
Palm sprout	கடையோர் விடுவாய்ப் பிசிரொடு சுடு கிழங்கு நுகர	225: 3
Kolungkilangu (a tuber variety)	கொழுங்கிழங்கு மிளிரக் கிண்டி	168: 3
Vallikizhangu (a tuber variety)	மூன்றே கொழுங்கொடி வள்ளிக் கிழங்கு விழக்கும்மே,	109: 6

Table 3. Milk and Meat

Food Group	Padal / Verse	Source
Milk	பால் புரை பிறை நுதல் பொலிந்த சென்னி	91: 5
Curd	ஆய் மகள் தயிர் கொடு வந்தத சம்பும்	33: 2-3
Butter Milk	மோர் இன்று, அவிழ் பதம் மறந்து	159: 11
Butter	இழுது மறப்பச்	62: 2
Ghee	நெய்யுடை அடிசில் மெய்ப்பட விதிர்த்தும்	188: 5
Deer Milk	மரையான் கறந்த நூரை கொள் தீம்பால்	168: 8
Fish	ஊர்தொறும் மீன் சுடு புகையின் புலவு நாறு	
Varalmeen	மோட்டு இருவரா அல்	399: 5
Kottumeen	கோட்டு மீன் கொழுங்குறை	399: 5





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Valai fish	பழன் வாளைப் பருஉக்கண் துணியல்	61: 5
Kelirufsh	குருஉக் கெடிற்ற குண்டு அகழி	18:10
Goat meat	மை ஊன் மொசித்த ஒக்கலொடு	96: 7
Deer meat	மான் தடி புழுக்கிய புலவு நாறு குழிசி	168: 9
Rabbit meat	குறு முயல் கொழுஞ் சூடு கிழித்த ஒக்கலொடு	34-11
Porcupine meat	எயினர் தந்த எய்ம்மான் எறிதசைப் பைஞ்ஞினம் பெருத்த பசு வெள் அமலை, வருநர்க்கு வரையாது தருவனர் சொரிய,	177: 15
Monitor lizard meat	உடும்பு உரித்து அன்ன என்பு எழு மருங்கின்	68: 1
Winged Termites	செம்பற்று ஈயலின் இன் அளைப் புளித்து	119: 3

Table 4 Others: (Sugars, Spices, Condiments)

Food Group	Padal / Verse	Source
Honey	நெடுங்குன்றம் தேன் சொரியும்மே	109: 8
Sugarcane	பூங்கரும்பின் தீஞ்சாறும்	24:13
White Sesame	வெள் எள் சாந்தொடு புளிப்பெய்து அட்ட	246: 7-8
Pepper	கறி வளர் அடுக்கத்து மனைக் குவைஇய கறி மூடையால்	168: 1-8 343: 3
Tamarind	நறும் புளி மோட்டு	399: 4-5
Salt	இளந்தளிர் கொய்து கொண்டு உப்பு இன்று	159: 10

COOKING TECHNIQUES - EVIDENT IN PURANANURU

Table 5. Evident in Purananuru

S. No.	Techniques	Phrase	Source	Description
1	Boiling	நீர் உலையாக ஏற்றி	159: 11	Tender shoots of <i>kuppaikerai</i> is cooked with water
2	Tempering	குய் குரல் மலிந்த கொழுந்துவை அடிசில்	250: 1	The term <i>kukikural</i> refers to the sound of tempering
3	Fermentation	நிலம்புதைப் பமுனிய மட்டின் தேறல்	120: 12	An alcoholic beverage named <i>theral</i> is buried underground for fermentation.
4	Roasting	ஒழி மடல் விறகின் கழிமீன்க ட்டு	29: 14	Fish is roasted on Fire
5	Smoking	பூ நாற்றத்த புகை கொளீஇ, ஊன் துவை கறிசோறு உண்டு	14: 13-14	Smoked Meat with smoky aroma consumed with rice.
6	Grinding	வெள் எள் சாந்தொடு	360: 5	White sesame is ground into paste and added in a curry.
7	Pounding	தொடி மாண் உலக்கைப் பருஉக் குற்று அரிசி	399: 2	Rice is Pounded for culinary use
8	Flaking	பாசவல் முக்கித்தண் புனல் பாயும்	63:13	Rice flakes made from paddy





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Table 6 Ethnic Food Preparations found in Purananuru

S.no	Course	Numbers (n)
1	Main courses	31
2	Accompaniments	19
3	Desserts & Snacks	15
4	Beverages	20

Table 7 Rice Preparations Evident in Purananuru

S. No	Dishes	Phrase	Source	Description
1	Neiyudaiadisil	குய்கொள் கொழுந்துவை நெய்யுடை அடிசில்	160: 7	Rice with Ghee
2	Paarpeipunagam	பால்பெய் புன்கம் தேனொடு மயக்கிக்	34:10	Sweet rice dish made with honey
3	Pazhanjoru	பழஞ்சோற்றுப் புகவு அருந்திப்	395: 5	Old Rice
4	Kozhunthuvaiadisil	குய்குரல் மலிந்த கொழுந்துவை அடிசில்	250: 1	Rice with fatty thuvaial
5	Oon Soru Amalai	ஊன்சோற்று அமலை பாண்கடும்பு அருத்தும்	33:14	Rice cooked with meat
6	Amalaikozhunsoru	அமலைக் கொழுஞ்சோறு ஆர்ந்த பாணர்க்கு	34:14	Big bolus of rice
7	Oonthuvaikarisoru	பூ நாற்றத்த புகைகொளீஇ, ஊன்துவை கறிசோறுஉண்டு	14: 13-14	Cooked Rice with meat curry
8	Kalikulvenjsoru	துடுப்பொடு சிவணிய களிக்கொள் வெண்ணோறு உண்டு இனிதிருந்தபின்	328: 11-12	Cooked White Rice
9	Kozhunthuvaioonsoru	அட்டு ஆன்று ஆனாக் கொழுந் துவை ஊன் சோறும்	113:2	Rice with Fatty meat Curry
10	Adisil for Kamazl	கமழ்குய்யஅடிசில்	10: 7	Aromatic Rice preparation
11	Kari soru	கறிசோறு உண்டுவருந்து தொழில்அல்லது	14:14	Rice with Meat
12	Soru	சோறு படுக்கும் தீயோடு ஏற்றுக உலையே! ஆக்குக சோறே!	20 172: 1	Cooked Rice
14	Nelludai k avalamodu neimidhi	நெல்லுடைக் கவளமொடு நெய்யம்மிதி பெறாஅ	44: 2	Paddy Rice with Ghee
15	Pudhunelvensoru	புது நெல் வெண் சோற்று	61: 5	Rice cooked from newly harvested paddy
16	Adai	மெழுகு மெல் அடையின் கொழு நிணம் பெருப்ப	103: 10	A preparation made with rice
17	Kozhunthuvaioonsoru	மைவிடை வீழ்ப்பவும், அட்டு ஆன்று ஆனாக் கொழுந்துவை ஊன்சோறும்	113: 1-2	Meat Cooked with rice consumed with a fatty accompaniment
18	Thinai	சாரல் புனத்தினை அயிலும்	143: 5	Foxtail Millet consumption
19	KuikolKozhunthuvai Neiyudaiadisil	குய்கொள் கொழுந்துவை நெய்யுடை அடிசில்	160: 7	Ghee rice consumed with a fatty accompaniment
20	Kool and Soru	கூழும் சோறும் கடைஇ, ஊழின்	160:20	A fermented porridge
21	Rice with sweet deer Milk	மரையான் கறந்த நுரை கொள் தீம்பால் வான்கேழ் இரும்புடைகழாஅது, ஏற்றிச், சாந்தவிறகின் உவித்த புன்கம்	168: 10-13	Rice with sweet deer Milk





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22	Red Rice	செந்நெல் உண்ட பைந்தோட்டு மஞ்சை	344: 1	Red variety of rice was also consumed by sangam Tamils
23	MuluAvizhPuzhukkal	முழு அவிழ்ப் புழுக்கல்	399: 9	Well cooked Rice

Table 8 Meat Preparations Evident in Purananuru

S.NO	Meat	Phrase	Source	Description
1	Fish	இழுதின் அன்ன வால்நிணக் நகாமுங்குளேதான்நைலிதீயின் வினரவனன் சுட்டு,	150: 5-13	Flame Roasted Fish
2	Deer meat	தான் உயிர் செகுத்த மான் நிணப் புழுக்கோடு ஆன் உருக்கு அன்ன வேரியை நல்கி	152: 26-27	Boiled deer meat
3	Porcupine meat	எயினர் தந்த எயம்மான் எறிதசைப் பைஞ்சிணம் பெருத்த பசுவெள்அமலை ,	177: 13-14	Porcupine meat consumed with white rice
4	Nedu Vaalai Fish	நெடுவாளைப் பல்உவியல்	395-4	A preparation termed uviyal done with vaalai fish
5	Young Rabbit meat	குறுமுயல் கொழுஞ்சுடு கிழித்த ஒக்கலொடு	34-11	Hot fatty pieces of rabbit
6	Fatty Fish	கொழு மீன் விளைந்த	13:13	Fatty Fish
7	Meat	பூ நாற்றத்த புகைகொளிஇ, ஊன்துவை	14:13	Aromatic meat Curry
8	Vaalai fish	பழன வாளைப் பருஉக்கண் துணியல்	61: 5	A variety of curry prepared with Vaalai fish
9	Monitor lizard meat	உடும்பு உரித்து அன்னஎன்பு எழுமருங்கின்	68: 1	Monitor lizard meat
10	Goat meat	மைஊன் மொசித்த ஒக்கலொடு	96: 7	Goat meat preparation
11	Termites	செம்புற் றீயலி னின்னளைப் புளித்து மென்றினை யாணர்த்து நந்துங் கொல்லோ	119: 3 -4	A Curry preparation with
12	Boar	குறுந்தாள் ஏற்றைக் கொழுங்கண் அவ்விளர்	379: 8	Fatty boar meat
13	Big Fatty Pieces of meat	நிணம் தயங்கு கொழுங்குறை	125: 2	Fatty meat pieces
14	Porcupine meat	எயினர் தந்த எயம்மான் எறிதசைப்	177: 13-17	Porcupine meat preparation





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Table 9. Desserts & Snacks

S.No.	Dishes	Phrase	Source	Description
1	ThengupaduvianP azham	தெங்குபடுவியன்பழம் முனையின்	61: 8	Coconut
2	Sudukizhangu	கடையோர் விடுவாய்ப்பிசிரோடு சுடுகிழங்குநுகர	225: 3	Hot Smoked Tubers
3	NoonkinTheensoru	தலையோர் நுங்கின் தீங்சோறு மிசைய	225-1	Sweet flesh of palmyra
4	Neikadalai	நறு நெய்க் கடலை விசைப்பச்	120:14	Groundnuts
5	Pennaipazham	செழுங்கோட் பெண்ணைப் பழந்தொட முயலும்	61: 11	Coconut
	Nellitheengani	சிறியிலை நெல்லித் தீங்கனி குறியாது	91: 9	Gooseberry
6	Pala	தீஞ்சுளைப்பலவின் மாமலைக் கிழவன்	129: 4	Jackfruit
7	Kala	புளிச்சுவை வேட்டசெங்கண் ஆடவர் தீம்புளிக்களாவொடு துடரிமுனையின், மட்டு அறல்நலயாற்று எக்கர்ஏறிக்,	177: 8-11	Kala Fruit
8	Naval	கருங்கனிநாவல் இருந்துகொய்து உண்ணும்	177: 8-11	Naval fruit
9	Paingani	இடையோர் பழத்தின் பைங்கனிமாந்தக்	225: 2	Palm fruit
10	KuiyudaiAdisil	சுவைக்கு இனிதாகிய குய்யுடைஅடிகில்	127: 7	Sweet Rice preparation
11	Pasaval	பாசவல் முக்கித்தண்புனல் பாயும்	63:13	Flaked Rice

Table 10 Non - Alcoholic Beverages

S.No.	Item	Phrase	Source	Description
1	Panaiyinkurumbaineer	இரும் பனையின் குரும்பை நீரும்	24: 12	Fluid from palmyra
2	Poongkarumbintheenjaru	பூங்கரும்பின் தீஞ்சாறும்	24: 13	Sugarcane juice
3	Thaazaineer	ஓங்கு மணல் குவவுத் தாழைத் தீ நீரோடு	24: 14-1 5	Coconut water
4	Munneer	உடன் விராஅய் முந்நீர் உண்டு	24: 16	Mixture of palmyra fluid, sugarcane extracts and coconut water
5	Ela neer	தெங்கின் இள நீர் உதிர்க்கும்	29: 15 - 16	Tender coconut water
6	Purkai	என் ஐ புற்கை உண்டும்	84: 1	Porridge
7	Maraiyan Theme paal	மரையான் கறந்த நுரை கொள் தீம் பால்	168: 8	Deer Milk
8	Kaadi	காடி வெள்உலைக் கொளிஇ	399: 4	A fermented beverage





Uma Mageshwari and Tinu

Table 11 Alcoholic beverages

S.No.	Dishes	Phrase	Source
1	MattinTheral	நிலம் புதைப் பழுனிய மட்டின் தேறல்	120: 12
2	Nellin Ariyal	அவிழ் நெல்லின் அரியல் ஆருந்து	395: 8
3	VengalTheral	நார் பிழிக் கொண்ட வெங்கள் தேறல்	170: 12
4	Mattu	மகிழ்தரல் மரபின் மட்டே அன்றியும்	390: 16
5	Vengal	வெங்கள் தொலைச்சியும்	29: 15
6	Theral	சூரும்பு ஆர் தேறல் சுற்றம் மறப்ப	65: 3
7	Kadungal	கடுங்கள் பருகுநர் நடுங்கு கை உகுத்த	68:15
8	Naravu	நீ நயந்து உண்ணும் நறவே	125: 8
9	Kal	இவற்கு ஈத்து உண் மதி கள்ளே	290: 1
10	PazhunyaTheral	பழுனிய தேறல் மகிழ்ந்து	129: 2
11	Veri	ஆன் உருக்கு அன்ன வேரியை நல்கித்	152: 27
12	Adai Arial	அகல் அடை அரியல் மாந்தி	209: 4

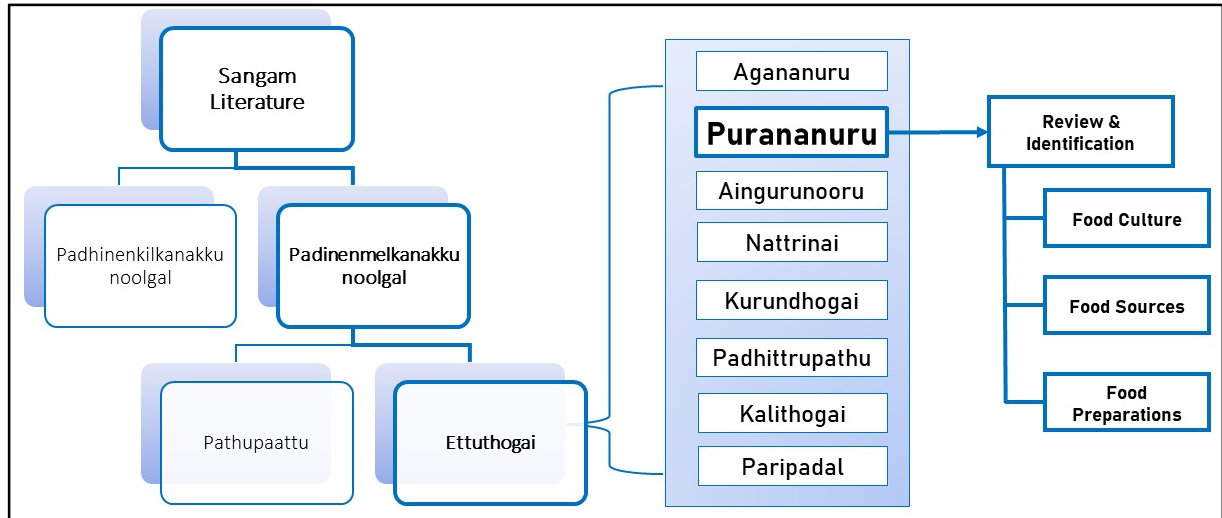


Figure.1 : Methodology





RESEARCH ARTICLE

The Analysis of Macro-Environmental Factors Influencing Marketing Practices of Solar Energy Industry in Pune

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ABSTRACT

This study focuses on the macro-environment factors and their impact on solar energy, marketing practices, and its focus is on the assessment of the current state of the solar industry, especially in the geographical area of Pune. The goal is to show that the changes in the solar industry and its impact on the solar energy marketing practices. The influence of the solar industry, and a variety of features, such as how it is from, decisions, and strategies that form at the same time. It is very dynamic and is constantly changing. Some of the micro-economic factors can be influenced by factors that are beyond the company's control. Subsidies for solar energy, play a very important role in this field. They are available in a variety of forms. In India, the subsidies are the two divisions of the Central Government, as well as government-node setup (SNAs) of people to install solar photovoltaic systems on the roofs of houses. This article focuses on the six major consumers, the quality factors associated with the care of the environment, and motivate purchase behavior is acceptable in residential solar technology. Therefore, the study took into account a range of factors, from environmental issues, based on the previous literature review by scholars. To obtain the results, the developed questionnaire was drawn up, and the details of the 69 residents have been collected with the help of the survey. The results showed that the environmental factors, and issues such as the social impact, the environment, union, environmental, knowledge, responsibility, and initiative of the government, it will have a positive effect on customers' intention to invest in residential solar technology. The environmental factors of the problem, it has little effect on the intention to adopt. Thus, the results of the study yielded a number of valuable insights for policy-makers, providers, and government agencies. It is also useful in the development of the solar energy market by means of a variety of advertising programs and strategies.

Keywords: photovoltaic system, design, approval, customers, and the environment.





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INTRODUCTION

The aim of this paper is to present a review of the current state of the solar industry, particularly in the geographical area of Pune. The goal is to show that the changes in the solar industry and its impact on the solar energy marketing practices. The influence of the solar industry, and a variety of features, such as how it is from, decisions, and strategies that form at the same time. It is very dynamic and is constantly changing. Some of the micro-economic factors can be influenced by factors that are beyond the company's control.

Factors

Economic factors

While some of the economic factors that play a significant role in the field of solar energy production, the economy and can have a positive impact on two of the most important elements of a firm's level of production, and the customer's decision-making process. Economic factors, such as reduce bills, subsidies provided by the central and state governments for the installation of solar panels, or Help in an emergency from the food, to the creation of new jobs.

Demographic forces

The market will be influenced by demographic factors such as gender, age, level of education, and the education level of the head of the family, cultural identity, country, and region, and way of life, etc., etc.

Technical factors

Technical know-how and skills that will be used in production, as well as all of the materials and technologies that are needed to create a certain product. The success of the solar-energy company is dependent on common technological factors, such as Automation, Internet connectivity, 3D, Computer speed and performance of your engine, and the performance of the cryptographic

Natural and Physical Strength

Natural and physical resources, is also an important factor that will play a very important role in the solar industry. Forest and agricultural products, coal, minerals, oil, and a strong association with the production. So, in the natural and physical forces, climate change, pollution, the weather, the renewable and non-renewable resources, the environment, etc., etc.

Political and Legal forces

Political and legal forces can have a very significant impact on the solar industry, and not only in the solar industry but also in all sectors of the economy. The growth of the market will develop in line with the political and legal situation in the various fields. Each of the companies should be aware of all of these forces are the world, in order to be able to make the right decisions. Some of them are the norms of the labor law, copyright law, and the Law of the Fraud, the act of non-discrimination Act, the Occupational Safety and Health Act, the Imports and Exports

The social and cultural forces

The product is available in a society has a big impact on society. in the production process to ensure human health and safety, and to eliminate any practices that are harmful to the environment. There is also a wide variety of social and cultural factors, such as habits, level of education, religion, and belief, to raise awareness of the health issues, the size of the population, emigration and immigration rates, and different ways of life. The sun is the main source of solar energy production. Solar energy is a form of renewable energy. Abundant solar energy is available in India due to its great location makes it a land of tropical sunshine. In addition, the country and each year see more than 300 days of clear skies. Thus, in India, it receives a large amount of solar radiation throughout the year. Solar energy is



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free and clean energy and reduces reliance on natural resources, including water and carbon. The government aims to install solar power in the home for any period of time, the roof of each house, and will be, the generation of solar power to its own power. Solar power has been facing major changes, at the global level. The solar industry is heavily dependent on government grants. In India, the state and the central government offered the gifts of the people, for the installation of solar panels on the roof. There are three types of actors involved in the process, Customers, Partners, and Discomfort.

Benefits For The Customer

Solar energy subsidies, and are only available for residential purposes, not commercial and industrial sectors. The grant was issued, via the Solar grid system. The inclusion of a solar system is a major investment in financial aid, which encourages people to invest in it and illuminates some of the burdens of their lives. Also given up to 3 kW, 40% subsidy for 4-kW to 10-kW and 20% of revenue, too greater than 10 hp. Homeowners will be able to install a solar power system and use it using the drive mode. They will be in the customer information areas and run to the nearest software partner.

A solar installation company, which offers a five-year warranty, was provided with grants from the facility. This is a gift and is not available to commercial and industrial customers, as they will be able to exercise other rights, such as rapid price reductions, tax benefits, jobs, exemptions if any.

Benefits of channel partners

Partners will use customer information and good business. It is very difficult for the average person to understand the process of how to get a grant, even though they really wanted it. Now the partner channel you have to bring to the stage. Try to work with customers and the government, and speed up the process of getting a customer subsidy.

Benefits of Discoms

The requirements for the use of basic energy in the housing sector, and can be applied to commercial activities. We know that business travelers will have more power than residential buildings. The customer will be happy to receive financial support from the channel partner, and is happy to be in the order of the new customer made, and the government is also happy to be one step closer to the solar-powered plant.

Disadvantages of subsidies

Let's look at some of the disadvantages of grant laws:

Customer Needs: I think, there are 2 major shortcomings when we install a solar system with a government subsidy system.

Sub-Warranty: If you install a solar system with a government solar subsidy program, then you will receive a full warranty of the program for five years. You will replace the inverter after 5 - 7 years.

There is no Top Brands option - If you install a solar system with this system, you will not be able to force solar panels and inverter brands, such as Loom Solar, Luminous, Microtek, Exide, Enphase, Solar Edge, etc.

Channel Partner Industries: In consultation with industry experts, they address the following issues:

- Great Investment Uncertainty of a Refund Grant from DISCOM after providing a grant amount
- System service and warranty for the next five years
- Solar Cost Estimation of the Panel

Both central and government institutions provide subsidized programs to the people by installing solar panels. The central government pays a 30% subsidy for these programs to say in the ordinary categories. In special provinces such as Uttarakhand, Sikkim, Himachal Pradesh, Jammu & Kashmir, and Lakshadweep, up to 70% of subsidies are provided by the central government.



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For funding, the average cost of installing a PV roof system without funding should be Rs 60,000 - 70,000. In order to get generation-based incentives, the customer must produce 1100 kWh - 1500 kWh per year.

Solar energy refers to the energy available from the sun, which is a renewable energy source. More solar power is available in India because of its ideal location that allows the country to experience the hot sun. In addition, the earth is witnessing more than 300 days in a clear sky each year. Therefore, India receives a large amount of solar radiation throughout the year.

Objective:

- To perform a macro-environmental analysis of the Solar energy industry
- To study the factors impacting marketing practices of the Solar energy industry
- To find out the ability to meet growing market needs is being challenged by macro-environmental factors beyond its control.

Hypothesis

H1: there is a correlation between macro-environmental factors and marketing practices of the solar energy industry.

H2: the factors of macro environment impact marketing practices of the solar energy industry.

Research Methodology

a) **Primary Data**-Primary data is information that is collected specifically for the purpose of research purpose.

b) **Secondary Data**-Secondary data refers to data that was collected by someone other than the user.

The primary and secondary data described above are provided by:

Solar energy users

This is an empirical survey, based on primary data. For this study, Pune district of Maharashtra is taken as the geographical area

Sample Size

Sample size of 69 Respondents

Population

The population is 208 and the Random Sampling Method is used to collect data, The current study is largely based on primary data. Data were collected through structured questions. The formal questionnaire is designed for consumers. The respondent's questionnaire consists of six sections that include population variation, awareness of renewable energy products, market spread among consumers in solar energy technologies as another source of energy, consumer perspective, consumer attitudes, prudent purchasing decisions, and consumer acceptance factors. Apart from demographics and consumer information items are analyzed on an ordinal scale, such as consumer awareness is measured with the help Not at all aware, slightly aware, somewhat aware, moderately aware and Extremely aware. Market pervasiveness, consumer perception, and consumer acceptance towards the solar power technology as an alternative power source was also measured with the help of 5 point scale such as strongly disagree, disagree, neither agree nor disagree, agree and strongly agree and cognitive buying decisions and consumer acceptance is measured by means of 5 point scale. From the above table it is observe that the female respondents are 62.31% and male respondents are 43% female consumers are more than the male consumers . From the above table it is observed that the 37.68 % respondents are from the age group of 25-45 years ,26.08% respondents are from 45-65 age groups , 19% of respondents are above the age of 65 years .

Reliability and Validity of Data

Reliability test is conducted on the following data which indicate the Cronbach's Alpha .869 and more than .5 So, it is confirmed that the data are highly reliable and valid for analysis. The following table mention of and acceptance of solar renewable energy .



**Swapnil A. Shah and Subhash J. Jadhav****Case Processing Summary**

a. Listwise deletion based on all variables in the procedure.

Reliability Statistics

there is no correlation between macro environmental factors and marketing practices of solar energy industry

The significance value of is 0.088 indicates that the null hypothesis is rejected there is no correlation between macro environment and marketing practices. The significance value of is 0.515 indicates that the null hypothesis is rejected There is no significant relationship between the mean score of socio-demographic profile of consumer and awareness towards solar renewable energy as alternative source of power.

Suggestions and recommendations

In India, the world's demand for energy is growing out of power capacity. In order to reduce supply and demand for alternative energy sources need to be actively explored. An energy-efficient solution that will need to be able to solve the problems in the long run by the use of alternative and renewable sources of energy. the solar energy is certainly a very promising solution, as it is widely available. The use of solar energy and can lead to benefits for the environment and the economy of the developing countries.

Explanation

In this research paper, it is clearly presented to you. In accordance with the tasks, hypotheses can be generated and will be used for the analysis. This article will provide you with a clear understanding of the methods by which the study was carried out in order to achieve the objectives, and the sampling procedure. The tools that are used to test the hypothesis, the analysis, and provides a brief description of Solar energy industry, macro environmental factors and its impact. In 2020-21, the population of the district in Pune, which is about 1,00,89,916, and the power consumption is 20,330 MW out of which 3,500 MW of renewable energy, 628 MW of solar power, which can be further increased.

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Table 1. Gender wise distribution of solar energy consumption

Gender	Frequency	Percent
Male	26	37.681
Female	43	62.319

Table 2. Age

Age					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Below 25	12	17.4	17.4	17.4
	25-45	26	37.7	37.7	55.1
	45-65	18	26.1	26.1	81.2
	above 65	13	18.8	18.8	100.0
	Total	69	100.0	100.0	

Table 3. Case Processing Summary

		N	%
Cases	Valid	69	100.0
	Excluded ^a	0	.0
	Total	69	100.0

Table 4. Reliability Statistics

Cronbach's Alpha	N of Items
.869	19

Table 5. macro environment and marketing practices

Correlations			
		macro_fact	Prom
macro_fact	Pearson Correlation	1	-.207
	Sig. (2-tailed)		.088
	N	69	69
prom	Pearson Correlation	-.207	1
	Sig. (2-tailed)	.088	
	N	69	69

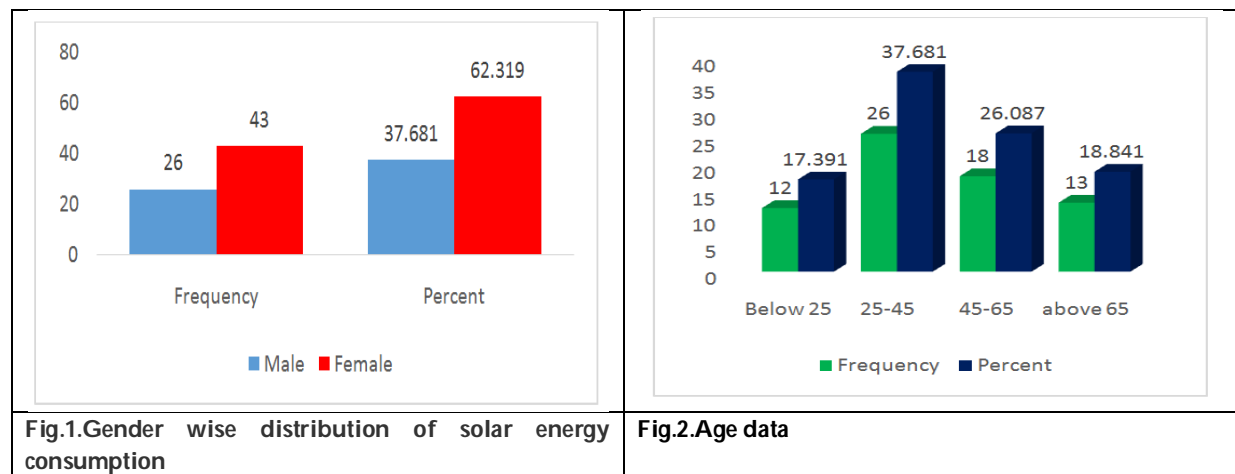




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Table 6. score of socio-demographic profile

Correlations			
		aware	demo
aware	Pearson Correlation	1	-.080
	Sig. (2-tailed)		.515
	N	69	69
demo	Pearson Correlation	-.080	1
	Sig. (2-tailed)	.515	
	N	69	69





12- Deoxyphorbol 13- (9, 10- Methylene) Undecanoate as a Drug Candidate for Tuberculosis

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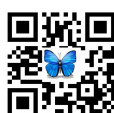
ABSTRACT

Tuberculosis is an age old disease. Though DOTS is the practiced therapy for the disease, however because of increase in number of multi and total drug resistant cases there is an urgent appeal to look for alternative therapy. Rhamnose, is unique to *Mycobacterium tuberculosis* as it is present in the pathogen and is absent in the host. The present study is an attempt to find out possible plant based inhibitor for rhamnose biosynthetic enzymes RmlC. Twenty plant derived anti cancerous compounds were retrieved from the NPACT database. They were then evaluated for their binding affinity with RmlC by molecular docking procedure. 12- deoxyphorbol 13- (9, 10- methylene) undecanoate was found to be the best candidate inhibitor for RmlC. Because, 12- deoxyphorbol 13- (9, 10- methylene) undecanoate works to bind RmlC efficiently, therefore it can work as a potential drug for Tuberculosis.

Keywords : Tuberculosis, RmlC, *Mycobacterium tuberculosis*, DOTS, NPACT..

INTRODUCTION

Tuberculosis (TB) is an age old disease caused by *Mycobacterium tuberculosis* (*Mtb*) [1-2]. As per global statistics recorded by World Health Organisation almost 10 million people are infected with the menace in the year 2018 [1]. The infected person spreads the disease through air by coughing, sneezing or spitting [3]. The disease is on the rise because of immunosuppressive disease like AIDS [4]. Though WHO has prescribed DOTS as the effective therapy for Tuberculosis however, increase in the number of multi drug and total drug resistant cases appeals to look for new drugs for Tuberculosis [1]. *Mycobacterium tuberculosis* cell wall is a validated target for tuberculosis drug [5]. A disaccharide linker α -L-rhamnosyl-(1 \rightarrow 3)- α -D-N-acetyl-glucosaminosyl-1-phosphate is present in the cell wall [6]. This linker connects the outer mycolyl arabinogalactan layer to the peptidoglycan layer [6]. Thus, it is crucial in maintaining the structural integrity of the cell wall [6]. Of these disaccharide linker, the L-rhamnosyl residue is



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absent in human host, thus enzymes catalyzing the synthesis of this residue can be possible drug targets [6]. The L-rhamnosyl residue is being synthesized by a reaction cascades catalyzed by four enzymes i.e. RmlA (glucose-1-phosphate thymidyl transferase), RmlB (dTDP-D-glucose 4, 6-dehydratase), RmlC (dTDP-6-deoxy-D-xylo-4-hexulose 3, 5-epimerase) and RmlD (dTDP-6-deoxy-D-xylo-4-hexulose reductase) [6]. However, availability of crystal structure and high substrate specificity makes RmlC as the target of interest [7]. As searching altogether new drug is more laborious, cumbersome and time consuming. Therefore, we thought of drug repositioning approach i.e. investigation of existing drugs for new therapeutic purposes. In this study some known plant derived anti cancerous agents were evaluated for their role in inhibiting RmlC activity. Structure based screening of NPACT compounds were performed to findout suitable inhibitors of RmlC [8].

MATERIALS AND METHODS**Software used**

We have used ArgusLab 4.0.1. [9] for structure based screening or molecular docking of compounds against RmlC.

Method

The enzyme, RmlC (PDB id – 2IXC) was retrieved from the PDB database (www.rcsb.org/pdb) with its substrate analogue dTDP-rhamnose. The active site was defined as all residues lying within 5 Å radius of the bound substrate analogue. The substrate analogue and water molecules were then removed. As the protein is a functional dimer therefore two chains of the proteins were retained removing the other. Twenty ligand molecules were then retrieved from the breast cancer subset of the NPACT (Naturally occurring Plant based Anti-cancerous Compounds-Activity-Target) database. The ligand molecules were then docked to the active site of the enzyme one by one. The highest binding energy was then recorded. Top five molecules having high binding energy was then listed.

RESULTS AND DISCUSSION

Most of the compound observed to bind to the active site of RmlC properly. However, 12- deoxyphorbol 13- (9, 10-methylene) with NPACT id NPACT00036 binds to RmlC most efficiently. The resultant binding energy was recorded to be -11.17 Kcal/mol.

CONCLUSION

From this study 12- deoxyphorbol 13- (9, 10- methylene) undecanoate emerges as the possible inhibitor for RmlC enzyme and thus can work as potential drug candidate against tuberculosis. However, wet lab study will confirm the result more profoundly.

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Table 1. Twenty NPACT Molecules that were Docked to RmIC.

SI. No.	NPACT ID	Name of the molecule
1	NPACT00006	(+)Galocatechin
2	NPACT00009	(2, 4- cis and trans)- gigantecicone
3	NPACT00010	(2, 4- cis and trans)- squamoxinone
4	NPACT00020	(4R, 6R)- dihydroxy- 4- [10 (Z)- heptadecetyl]- 2- cyclohexanone
5	NPACT00021	Camdenatenin A
6	NPACT00022	1, 2, 4- trihydroxyheptadec- 16- ene
7	NPACT00023	1, 2, 4- trihydroxyheptadec- 16- yne
8	NPACT00024	1, 2, 4- trihydroxynonadecane
9	NPACT00025	1, 3, 5- trihydroxy- 4- methoxyxanthane
10	NPACT00026	1, 3-diacetylvilasinin
11	NPACT00027	1, 6- Dihydroxy- 7- methoxy- 8- (3- methylbut- 2-enyl) 6', 6'- dimethyl- pyrano (2', 3', 3, 2) xenthane
12	NPACT00028	1- [3- (4- hydroxyphenyl)- 2- propenoate]- beta- D- glucopyranoside
13	NPACT00029	10- Epi- olguine
14	NPACT00031	10- Hydroxyasimicin
15	NPACT00032	10- Hydroxyglaucanetin
16	NPACT00033	10- Hydroxytrilobacin
17	NPACT00034	11- hydroxy- 1- isomangostin
18	NPACT00035	12, 15- cis- squamostatin- A
19	NPACT00036	12- deoxyphorbol 13- (9, 10- methylene) undecanoate
20	NPACT00037	12- Deoxyphorbol 20- acetate 13- angelate

Table 2. Top five NPACT molecules with high binding energy

SI. No.	NPACT ID	Name of the molecule	Binding energy
1	NPACT00036	12- deoxyphorbol 13- (9, 10- methylene) undecanoate	-11.17 kcal/mol
2	NPACT00027	1, 6- Dihydroxy- 7- methoxy- 8- (3- methylbut- 2-enyl) 6', 6'- dimethyl- pyrano (2', 3', 3, 2) xenthane	-10.71 kcal/mol
3	NPACT00037	12- Deoxyphorbol 20-acetate 13- angelate	-10.54 kcal/mol
4	NPACT00032	10- Hydroxyglaucanetin	-9.86 kcal/mol
5	NPACT00026	1, 3- diacetylvilasinin	-9.73 kcal/mol





Subhankar Dash and Jyoti Prakash Rath



Figure 1. NPACT00036 (12- Deoxyphorbol 13- (9, 10- Methylene) Undecanoate) with Highest Binding Energy Docked to the Active Site of RmlC.

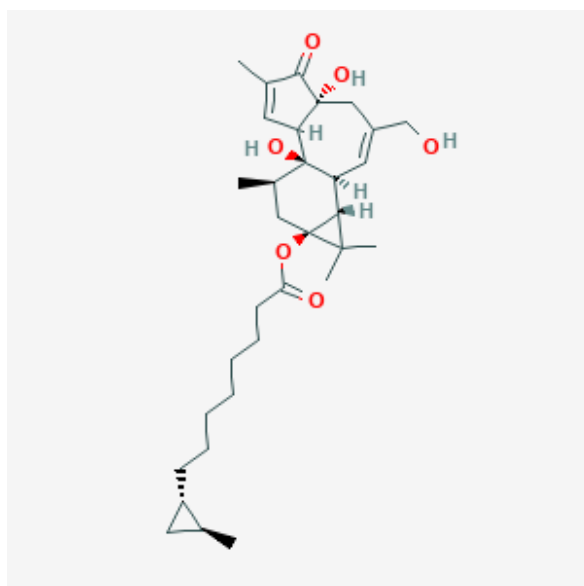


Figure 2. Structure of NPACT00036 (12- Deoxyphorbol 13- (9, 10- Methylene) Undecanoate).





Reassessment of Wireless ZigBee Mesh Network Data Retardation

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ABSTRACT

The aim of the article is to evaluate the area of research, simulations and replicas of accessible wireless communication mesh networks enabling guidance and strategic efficiency in key situations. This network is a robust conceptual function that provides useful Communications and media information, database, online applications, etc. A multi-layer network architecture using integrated Arduino circuitry and X-Bee Series 2, which enables processes and wireless networks of the ZigBee security protocol. With its multi-layer aspect, the data seclusion between individual sub networks, modules and users may be efficiently assembled. At the assumption of a vague intruder system of embedded systems, observational data analysis and control system of intruder current actions are used. Studies were conducted in order to duplicate and identify situations in which the wireless network simulator was really implemented.

Keywords: X-Bee, wireless communication network, security instances, simulation and replicas.

INTRODUCTION

The study is centered on the issue of effective, strategic plan dependency inside wireless communications networks between interdependent embedded systems and sensors. The effectiveness of crisis response tactics is classified as follows:

1. Involving many crisis reaction services, including quality assurance of work.
2. The emergence of interconnected, uniquely operational systems, such as sensors, actuators, remote communicators, digital information scanners, etc.
3. The random presence and the progressive receipt, refinement and storage of information concerning crises and the consequent constant change in the life of data gathering, refining and storage at the policy level are combined with the requirement for quick changes to the set of parameters.



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4. The possibilities of an integrated system operating technique with bidirectional communication being limited.
5. Enhanced error detection and security needs.

This requires a realistic approach in the operation of such networks with the ability to connect moving devices and to swap settings and topology without cutting off vital functions. The features detected the planned development and absence of repercussions of virtual-physical safety concerns for the targeted networks impact the significance of linked problems to the simulation and analysis of safety concerns in these networking networks. The implementation of multi-level mesh networks focusing on the Arduino and Digi X - Bee circuits is proposed. The evaluation should be made of potentially intrusive actions. Simulations on the design and external use of a network system for the security concerns were performed.

Related Work

In order to enhance subsystem performance, simulations and evaluation of security concerns have been employed by forecasting abnormal behavioral patterns, taking into account various kinds of security problems and statistical criteria for safety activities [1,2]. There have also been published several research on the review of mesh-based strategic planning [3, 4, 5, 6, 7]. Data collected throughout this article are distinguished by taking into account the generic paradigm of the integrated computer attacker in the assessment and evaluation of safety occurrences and the overall character of incidents, including cognitive effects on system entities and aspects of devices/data.

Wireless Network Model

A portable strategy communications network is a network that is quickly created to prevent and remove event consequences. The network must establish a uniform framework of communication for data transfer from devices to service workers, e.g. radiation security, medical services, fire departments, defense, mental aid, etc. Data services deliver sensor data dissemination, system controls and interactive data. The base is a multifunctional and movable mesh network, based on the position, volume and saturation of network points that may be reassembled during operation. Network hub can be established or be personal mobile end-user units on special service trucks in the strategic hub of the project. Network devices in particular may have varied levels of functional ownership, dependent on power consumption characteristics and wireless and wireless connections.

The models of the network include the following:

A Wireless network node

The system integration device is put in the gateway to manage digital communication and transmit network configuration with other network nodes.

A Wireless network gateway

The system connects two system nodes and merges their children's nodes.

Client device

Other applications involved directly in network integration systems include user interfaces, smartphones, private communicators, handheld devices, system attribute sensors, section elements for human health metrics tracking, sensors diverse, RFID scanners, surveillance cameras and speakers, QR- and biometric scanners.

Network Elements

Additional components such as Wi-Fi for the use of TCP/IP interface to communicate with existing data centers and mobile apps.

System Architecture

The system offers a series of Digi XBee Series 2, each of which is part of a domain system controller (see Table I).



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The Digi X -Bee s2 node with the control position is installed in level 1, as the fundamental device for determining mobile network interactions and slowdowns. Generally speaking, this location is not the site of convergence for all network traffic flows [7, 9]. The Arduino Mega 2560 integrated circuits, each incorporating the Level 1 X - Bee router (X - Bee 1) and Level 2 X - Bee coordinator, are used for precursor systems installed on Level 1 and 2. Data streams from multiple sub-networks were transferred from the device, data streams were separated from different subnetworks, and encrypted data was cryptic. A module for protective event analysis was also built based on the specific case association rules principle. The following form exists for these rules: I, where J_p and J_i are both exterior and cognitive events occurring under structural and informative conditions and A_p and A_i premises, combined with an appraisal of the event I. (J_p, A_p, J_i, A_i). Activities from many sources such as remote control systems deployed on specified network areas, sensor read spectrum analyzers, DBMS question files are to be taken into account when the type of operation and occurrence of a security event is acquired and collectively reviewed.

Incident Survey Location

Various frameworks were also considered prepared to examine communications. The study shows that even with a very small data framework, the SVM-based model may obtain good results. However, binaries cannot be sufficient to measure a more realistic profile. The conviction assessment parameter was low for all kinds of hyper parameter measures in addition to the ANN model. This might be because the dataset is not in place.

CONCLUSION

This article describes the approach for implementing the analysis of sensitivity to devices. The development of a system for analyzing a range of user attributes based on text data collected from the profile page of VK.com Social Media. Several approaches have also been presented for evaluating text content on social networking sites. More work in this domain might be important in order to enhance the data supply needed for the procedure. Moreover, the concept of raising the issue of descent to distinction one must be examined. The most important developments in the model framework are the implementation of polynomial designation procedures. In a single system too, a composite of numerous other ways can be applied.

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TABLE I: TWO LEVELS OF SYSTEM ARCHITECTURE

Level	Subnets	Devices
Level 1	Network management	Level 1 X - Bee Management; Gateways
Level 2	Central Networking	Level 2 X - Bee Management; End devices; Gateways for sending and receiving outsource data's.

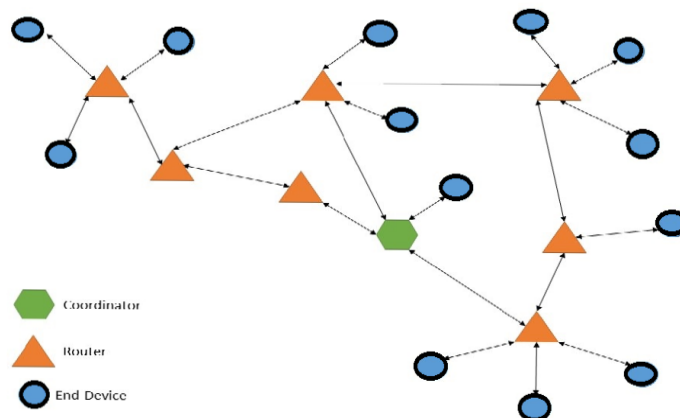


Figure 1. Wireless Network Model





A Homomorphic Encryption for Preserving the Privacy of Data: Wearable IoT Devices

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ABSTRACT

Now, more than ever, it is so important to look after our health. In the 21st century, people are diverted towards maintaining balanced health due to the Covid-19 pandemic. To monitor their health conditions, usage of wearable IoT devices like Fitness Tracker Bands, GPS Tracking Band, Biosensors, Smartwatch Monitors, etc. has increased exponentially. These devices store information like heartbeat rate, walking routine, stress levels, BP, BMI levels, distance walked, the weight of the person and generate a report of the health conditions and their average activity per week or per month. This sensitive information is stored and maintained in a centralized server of the device which is less secure. This information becomes lethal in the hands of people who want to target them. So, there is a need for a secure mechanism that doesn't allow an illegitimate/third-party user to access these details. This paper focuses on the Homomorphic Encryption strategy to save the data in an encrypted format on the server, which also allows the server to perform operations on the encrypted data.

Keywords: IoT, Wearable Devices, Privacy, Homomorphic Encryption

INTRODUCTION

The Internet of Things (IoT) is changing the way we live. It allows us to gain a better understanding of how things work around us. The Internet of Things is a network of interconnected devices that use the internet to send and receive data. With the help of the Gateway, IoT devices are connected to the network. These gateways, also known as processing nodes, process data acquired from sensors and send it to the cloud, acting as both a storage and processing unit. All actions taken on the obtained data are used for further learning and interference. The best example of IoT is a smartphone. Current Internet of Things (IoT) research is mostly focused on enabling general things to see, hear, and smell the physical environment for themselves, as well as connecting them to share their insights. In this way, monitoring and decision-making can be delegated from humans to machines [7]. Many sci-fi TV

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episodes, cartoons, and movies have used the idea of IoT, and though it may appear tough to achieve, it is a truth that many aspects of IoT will be realized in the near future [9]. Security is critical in the IoT since a successful attack might bring down the entire manufacturing, transportation, healthcare, and other sectors. The Internet of Things (IoT) is made up of a variety of devices, network protocols, and technologies, all of which have their own vulnerabilities, increasing the attack surface throughout the entire network [10]. For various reasons, preserving privacy in the IoT is difficult. For starters, the CPU in IoT devices is limited and incapable of performing sophisticated commands. Second, because most IoT devices use batteries, the security algorithm's power consumption must be extremely low. Third, in order to cover as many devices as possible, the cost of implementing the security algorithm should be minimal [11].

IoT Architecture

There are numerous layers of IoT that are built on the capabilities and performance of IoT parts and provide the best solution for businesses and end-users. The IoT architecture is a key technique to design the many aspects of IoT in order for it to deliver services through networks and meet future needs. IoT platforms are made up of a large number of linked objects all across the world. It connects cloud services and applications to the edge of devices, gateways, and data networks. The objects may be surrounding or separated by large distances in diverse contexts, but they are all managed by the centralized administration that serves as the IoT platform's processing unit [8]. The important components of IoT Architecture technologies are sensors, Networks & gateway, cloud.

Sensors

Sensors are devices that can send, receive, and process data. there are various types of sensors and they can be connected in different ways. the different types of applications that use sensors are RFID, GPS, gyroscope, and other technologies are included.

Networks and Gateways

High-speed gateways and networks are used to transmit the significant amounts of data produced by the sensors. The gateways can be of various types such as LAN and WAN. Before the data is transmitted to the cloud in IoT architecture it is preprocessed and evaluated using Edge IT. if the data read from the sensor is the same as the previous value then data is not transferred to the cloud.

Cloud is part of Management Services, which processes data through analytics, device management, and security controls. The cloud aside from sending the data to security and device management also sends it to the end user applications including the retail sector, healthcare services, emergency, environment, and others. According to Cisco Annual Internet Report [1], the number of devices connected to the internet will be 29.3 billion by 2023. The data generated by these IoT devices are mostly of unstructured format and is stored in the public cloud by a third-party service provider, centralized architecture, which is more prone to security threats and also a single point of failure. Based on [2] IoT statistics, the IoT devices are more easily prone to security threats which are tabulated in Table 1. Hence, there is a need to encrypt the data before storing that in the servers.

Homomorphic Encryption:

Homomorphic Encryption is a variant of encryption methods that allows computations on the encrypted data without decrypting it. In this method, after performing an operation on the encrypted data, the result after decrypting will be the same as the operation performed on the original data [4]. This allows the third party to perform analysis of the data without compromising the confidentiality of the data [5]. Homomorphic encryption algorithms are categorized into three types which are depicted in Figure 2. They are:

- Partially Homomorphic Encryption (PHE)
- Somewhat Homomorphic Encryption (SWHE)
- Fully Homomorphic Encryption (FHE)



**Krishna Sowjanya K and Bindu Madavi K P****Partially Homomorphic Encryption**

This allows the user to perform one operation i.e. either addition or multiplication but not both [6].

Somewhat Homomorphic Encryption

This allows the user to perform more than one operation but limited operations on the encrypted data. The operations are limited only to addition and multiplication [6].

Fully Homomorphic Encryption

This allows the user to perform unlimited operations like addition and subtraction for unlimited times on the encrypted data.

The concatenation of two words is performed by homomorphic encryption is showed in the above figure 3. The Plaintexts HELLO and WORLD are converted into ciphertext and the operation of concatenation showed in this example.

Proposed Method

The proposed method to preserve the privacy of the IoT data implements a Partial Homomorphic Encryption algorithm called Paillier's Algorithm. Data generated from IoT devices first hashed with the public key and is uploaded to the servers. The analysis is performed on the encrypted data and the reports are generated and it is sent to the user. The user will decrypt with a private key and view the report of his activities. The overall procedure is illustrated in figure 4.

The step-by-step procedure of the algorithm is illustrated as follows:

Step1: Encrypts the data using a public key by generating a hash value for metrics like age, heart rate, BMI, weight, distance traveled, etc.

Step 2: The encrypted data is loaded into the servers.

Step 3: The server performs various operations to generate the weekly report of the activity, using Homomorphic encryption.

Step 4: The encrypted report is sent to the user.

Step 5: The report is decrypted using a private key of the user.

Process of Key Generation

In the proposed method, two keys are used. The public key to encrypt the data and the private key to decrypt the data. To do so, the first two large prime numbers are chosen such that the gcd of these two prime numbers should be one. Later the values of n, g, λ, μ are calculated using the formulae represented in Table 2.

Process of Encryption

In this process of encrypting the data, the user chooses the generated public key (n, g) and cipher text is generate using the formulae represented in table 3. This encrypted data is sent to the servers for storage purposes, where the third-party people use this encrypted to analyze and make a report and is shared with the user.

Process of Decryption

The user receives the encrypted report generated by the third-party admins and decrypts the data with the private key (λ, μ) using the formula represented in Table 4. And the final report can be decrypted and checked by the user to check his report of activities with various details.



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RESULTS AND DISCUSSIONS

To generate the results, the day-to-day data generated from the IoT devices like Step count, BMI, Age, Weight, Heart rate, Distance traveled in Kms, Stress Levels (0-No stress, 5- Medium stress, 10- High stress). After the data is generated from the device, it is encrypted with the public key (n, g) and a hash value is generated as represented in the table follows:

In addition to this, the day-to-day step count is taken and encrypted, and stored in the server, their analysis is performed and a cumulative step count is given after 7 days with a total hash value. The user decrypts it with the private key and checks the actual data. It is represented in the table below. The cumulative hash and Total cumulative hash is calculated as follows:

1. A hash value (97244) is generated for Day1 data. Since it is only the first-day value, the cumulative hash and the Total Cumulative hash will be set to 0.
2. A hash value (36672) is generated for Day2 data. The Cumulative hash (62419) is generated by performing the addition operation of Day1 and Day2 hashes.
3. Total cumulative hash (26222) is calculated by adding the cumulative hash (62419) with the Hash value of Day3 (43474) and so on. The values are calculated cumulatively for 7 days as the weekly report considered.
4. Finally, the total cumulative hash value (9580) is taken and decrypted with the private key yielding the result, in total 60K steps.

CONCLUSION

Without a doubt, IoT is going to drive the future generation in the form of smart home appliances, smart vehicles, and smart personal gadgets. All the data generated from these devices must be maintained confidentially to preserve the privacy of the device user. Results have shown that the data can be encrypted and stored in the server and they can be decrypted at the user's end without violating privacy when third-party servers are using this data for analysis purposes. This paper focused on performing the encryption and decryption of numerical data such as weight, step count, heart rate, BMI, age, etc., using partial homomorphic encryption. As a future direction, a model can be developed to encrypt and preserve privacy for non-numerical data such as images, GPS data, etc., generated by these devices.

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Table 1. IoT Security Statistics [2]

IoT Security Statistics	
5 mins	Time is taken to attach an IoT device
48%	Businesses are not able to detect the IoT Security Breach.
75%	Cyberattacks are happened by hacking the routers.
74%	User's worry about losing their personal data because of IoT

Table 2. Process of Key Generation

Key Generation
Select two random large prime numbers <i>prime1</i> and <i>prime2</i>
calculate $n = prime1 * prime2$
calculate $\lambda = lcm(prime1-1 * prime2-1)$
Select an integer <i>g</i> such that, <i>g</i> is a modular multiplicative inverse of <i>n</i>
calculate $\mu = (L(g^\lambda \text{ mod } n^2) - 1) \text{ mod } n$
where $L(x) = (x-1)/n$
Public Key is (<i>n</i> , <i>g</i>)
Private Key is (λ , μ)

Table 3. Process of Encryption

Encryption of the data
Let <i>mes</i> be the data that is to be encrypted
Select a random integer <i>r</i> , such that $0 < r < n$ and $gcd(n, r) = 1$
ciphertext is computed as $cipher = g^{mes * r} \text{ mod } n^2$





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Table 4. Process of Decryption

Decryption of the data
Let cipher be the encrypted data.
The plain text from the encrypted data is calculated as: $mes = L(cipher^{\lambda} \text{ mod } n^2). \mu \text{ mod } n$

Table 5. Hashed values of day-to-day data

S.No	Data	Original Value	Encrypted Value
1	Step Count	1 K	14993
2	BMI	23.1	63595
3	Distance Travelled in km	5	97244
4	Stress Levels	2	63082
5	Heart Rate	75	70796
6	Age	36	63595
7	Weight	76	48490

Table 6. Cumulative hash and decrypted values for the report

Day	Step Count	Encrypted Value	Cumulative Hash value day-to-day	Cumulative Hash day-to-day	Decrypted Value
Day 1	6K	97244			
Day 2	5K	36672	62419	26222	
Day 3	10K	43474	76574	68497	
Day 4	13K	29984	97683	60821	
Day 5	6K	36672	82350	94850	
Day 6	8K	66426	77790	30117	
Day 7	12K	83697	9580	9580	60K

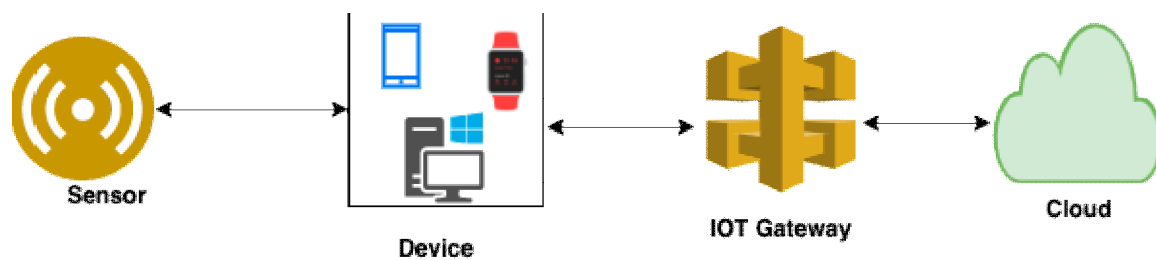


Figure 1. Stages of IoT architecture





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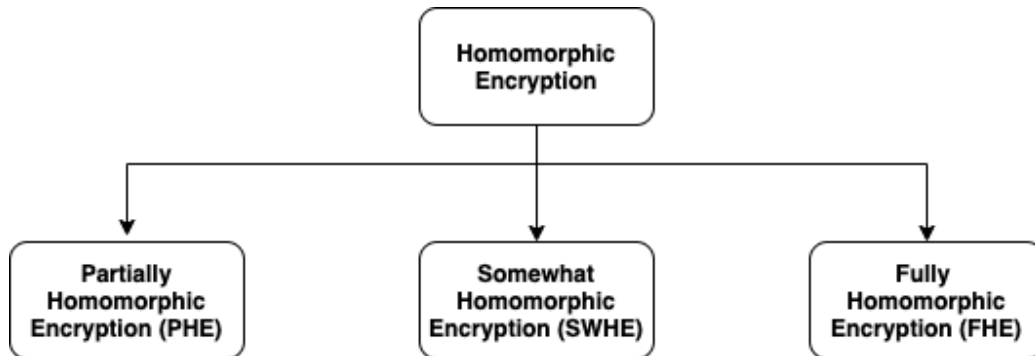


Figure 2. Categories of Homomorphic Encryption

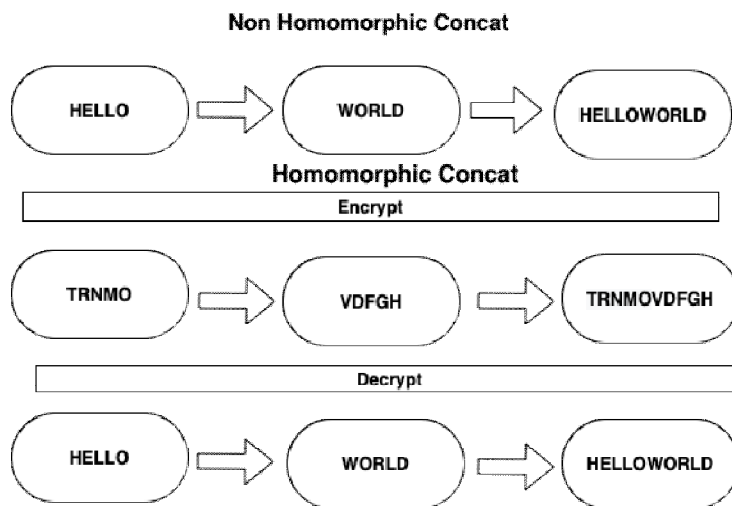


Figure 3. Homomorphic Encryption for concatenating two words

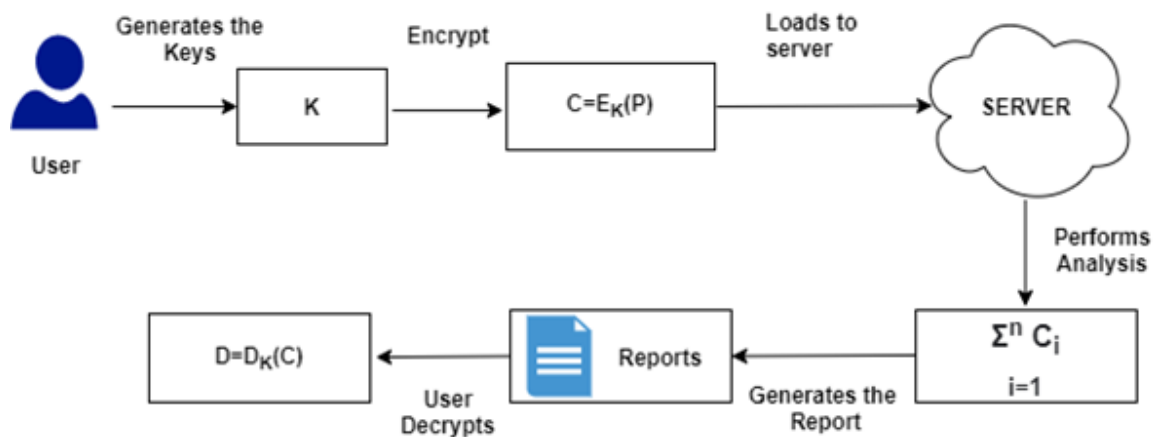


Figure 4. Proposed Method Data flow diagram





Changing Audience Practices and Consumption Patterns Online: A Study on the Popularity of OTT Platforms during COVID-19 Pandemic

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ABSTRACT

Nationwide lockdowns and social distancing norms have led to an inevitable surge in the use of digital technologies during the pandemic. (De et al., 2020). Improved smartphone utility benefit, mobile data rate, and a growing number of users are said to contributors to mobile-heavy internet connectivity in India (Thakker,2021). Changes in online consumption habits may be due to a plethora range of choices in the genre, titles, customization, flexibility, and localization option for digital content (Thakkur, 2021). This exploratory study attempts to provide a critical analysis of the relevance of convergence to the field of media audience during the COVID-19 pandemic. The paper argues the probable causes for embracing web 2.0 practices and changing consumption patterns among audiences, its impact on the entertainment media industry. Furthermore, this paper seeks to draw closure and theorize on the early entrance of de-globalization (Ántras,2020) due to the rise of regionalization of content and individual fragmentation of content by OTT platforms leading to heterogeneous culture and identities.

Keywords: COVID-19, Convergence, Audience Consumption, Web 2.0, Entertainment Media industry.

INTRODUCTION

Lockdown implementation and activity shutdowns that facilitate human interaction have resulted in audiences shift to internet services to communicate and interact (De et. al, 2020). Web 2.0 platforms are critical in the democratization of knowledge for the audience. The audience practice to seek traditional media outlets for entertainment has changed during pandemic. Access to content in digital form across multiple devices has allowed the users and audience to easily control their own media environment online. The increased usage of web2.0 platforms during the pandemic played a pivotal role to hold the government accountable for actions taken to control COVID-19 and to create social awareness via social media. The convergent media has caused a change that has



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realigned the roles of audience and producer role in novel ways (Bird, 2011). It has transformed traditional audience who aren't producers (Bruns, 2006) to use multi-media platforms for interacting with celebrities, influencers and OTT services for entertainment (Bird, 2011). The sudden popularity of web 2.0 platforms leaped India forward in digital innovations. Audiences' use of OTT platform directly impacted the sales of SMART TV and smartphones. Four trends are identified with increased usage of web 2.0 for entertainment. First, the popularity of OTT platforms. Second, technology integration that is SMART TV. Third, redefined terms of engagement online and lastly, language is no longer a barrier to human communication. Despite lifting lockdowns and operationalizing offices, malls, cinema halls, audience continue to use web 2.0 and OTT platforms.

OTT services popularity

OTT is a term defined as the delivery of service, or content on the Internet without network operators' involvement (Jirakasem and Mitomo, 2020). OTT business model is a two-sided market, between viewers and advertisers, wherein charge from one side and reduce the price from other (Krämer & Wohlfarth, 2015). Presently, there are 40 OTT platform providers in India (Nijhawan & Dahiya, 2020). Several reasons may be attributed to the popularity and usage of the OTT platform. First, a stable internet connection is a primary need for OTT consumption. The FICCI-EY 2020 report found there are 723 million total internet subscribers in 2019. Presently, Reliance Jio has been instrumental in increasing internet penetration and support for OTT Platforms. Jio telecom network serves 55% of Indian OTT traffic and 65% smartphone use (Keshavdev, 2019). Thus, lowered data costs are a crucial factor to increase internet usage among rural populations. A similar approach is adopted by Vodaphone and airtel too (Jirakasem and Mitomo, 2019).

Second, to consume OTT content, the audience must have access to internet-enabled devices such as laptops, cell-phones, or SMART TVs. Convergence allowed the users to connect and interact with the broadcaster through content. Hence, SMART TVs grew their base by a million homes (FICCI-EY, 2020). However, Smartphones are most preferred for video streaming devices in India. Smartphone penetration is expected to reach 859 million by 2022 as India is said to be a promising country by Original Equipment Manufacturers (OEMs) market Gevers (2019). Third, the pricing of OTT services is a critical attribute for the audience. For the first time in 2020, paid subscriptions to OTT platforms crossed 50 million. Ad-based Video Demand (AVoD) services such as Disney plus Hotstar, Alt Balaji, Zee 5 allow consumers to access the library for free and attain revenue through advertisements.

Lastly, availability of localized content. HIS Markit Report revealed 76% of individuals prefer the availability of localized content and 74% revealed the quality of dubbing and subtitles of international content as for decision-making attributes (Begum, 2018). Data analytics gather information on users' wants and technology development enabled OTT players to attain insight on understanding user's viewing patterns. No stone is left unturned by players to catch the attention of consumers, grow the user base and retain each user for a longer duration. (Lee et. al. 2018).

Content consumption

Nijhawan & Dahiya (2020) quoted a BCG report, 'Entertainment goes Online' that classified OTT consumers into three categories, One, traditionalist (who consume on other than OTT platform), two OTT Experimenter (consume both conventional and OTT Platforms), and lastly early adopter, whose consumption occurs on OTT platforms. The adoption and consumption of OTT have increased manifold since the pandemic began. Cricket and Bollywood movies are major consumption content on OTT platforms. Late Sushant Singh Rajput's Dil Bechara received the massive opening on Disney plus Hotstar, which paved the way for several other Bollywood banners such as Akshay Kumar starred Laxmi Bomb, Ayushmann Khurrana starred Gulabo Sitabo, etc. Netflix revealed 80% of its members in India watched a film every week. A survey by YouGov in May-June 2020 found women are most likely to consume VoD (Video on demand). Several films with female leads such as Bhumi Pednekar's in Durgamati-The Myth (on amazon prime), Jhanvi Kapoor in Gunjan Saxena: The Kargil girl, and Parineeti Chopra's A girl on the train on Netflix were released in late 2020 and early 2021.





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Another avenue is the gradual increase of web series consumption. Web series attracted audiences due to their originality in content and cast (Kumari, 2020). For the past two decades, there was no change in television content, thus, a freshness in content appealed to maximum audiences to adopt OTT services. The total amount of fresh original content created in 2019 is 1600 hours, this has led to the growth of content production (Consultancy. in,2020). To meet the quality standards of OTT and international consumers the cost of content is 2 to 10 times higher than TV. Demand for a diversity of content has pushed storytellers to explore the stories that connect with both native and global audiences. Hence, series such as inside edge, sacred games, breathe have gained popularity and carved a new genre and attention among audiences. One cannot overlook the fact that OTT platforms are willing to give a chance to indie and regional filmmakers, which resulted in significant growth in the output of alternative and edgy genres. Amazon has invested one-fourth of its Rs.2,000 crore budget to collaborate with Indian production houses and digital content creators including several filmmakers. (ET Brand Equity, 2020)

Audience Behaviour

Studies and theories have been reviewed by several scholars to understand audience behavioural patterns and practices with regard to OTT adoption. In order to recognize the reasons for audience acceptance of new technology, several authors have created models and have attempted to correlate with the adoption of Television, OTT media, and others. A few of these include the Technology acceptance model (Davis,1989), Diffusion of Innovation Theory (Rogers, 1995), Expectation Confirmation Theory (Oliver,1980). Technology Acceptance Model and Expectation Confirmation theory model are the two most used theories in respect to study OTT platforms. There are several reasons that can be attributed to this change. Social mobility restrictions, restrictions on outdoor activities like sports, films, etc have made audiences adopt digital consumption via the screen. Lack of routine, availability of content is leading to addiction behaviour among audiences.

Survey highlighted 87.3% have noticed an increase in screen time during COVID. Furthermore, 75% have OTT preference over television (Nijhawan & Dahiya, 2020) Thus, posing as a tough competition for traditional digital and non-digital outlets such i.e. DTH. Despite portability, ad-free, user friendly, and diversity of content on the OTT platform. Reports suggest distraction in form of content overload, the need for high-speed internet, and lack of censorship as some of the downsides of these OTT platforms. Audience believe complete viewer discretion and content overload may have a psychological impact on impressionable young audiences (Nijhawan & Dahiya, 2020). Perceived pleasure, customization, quality of content and user experience have a positive impact on the acceptance of OTT adoption. They are. Publicis report 'Reboot to a New Normal', gave an account on purchase behaviour and media usage in India in face of the Covid-19 pandemic. The report concluded that the consumers' behavioural changes are to be long-term and not transient in nature.

Films & TV Entertainment

The entertainment sector has been disrupted by OTT popularity. Its market revenue is eclipsing the box office revenue. The audience became accustomed to watching films and series on OTT platforms. Closure of cinema hall and complexes faced the brunt for the next normal. For instance, Fox Star Studios and Universal pictures have shut studios and ceased operations in India. The film industry faced a loss of 30 billion rupees. Past 8 months, 90 films have been released digitally, hence, cinema owners have limited new offerings of movies (Shahidi, 2020). Release dates of films with big investments such as '83, Sooryavanshi, or even Laal Chaddha are being delayed due to massive investments. Suspension of television shoots, lack of original content on television, cheap subscription fee, and unlimited video library has led to a decline in the TV industry by 13%. There was a 21% fall in advertising revenue in 2020 (FICCI.IN, 2021). Despite the downside, an opportunity for technology integration cannot be ignored. SMART TV sets crossed the 5 million marks and ushered into a new era of convergence. Production houses can take advantage of convergence. For instance, the simultaneous release of films on OTT and DTH can capture billion of viewers. Production houses could earn money by capitalizing on online advertisements on the first weekend, threat to piracy may be eliminated (due to mass availability of content) and by taking a minimum guarantee deal for cost covered in production, monetary risk may be minimized. Production houses' collections may



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be found on YouTube but they may reach out to OTT platforms for showing their content. Television series and films can attain more profit by releasing simultaneously on films and VoD.

Regional language

India is an amalgamation of multiple markets each with its own characteristics. Availability of regional languages in web 2.0 platforms, makes the transition easier for an audience. It allows the audience to interact with each other on a hassle freeway. Services such as Netflix and amazon prime are investing more to produce content in regional language. Its inclusion has paved way for mass-market adoption and its consumption on OTT platforms will cross 50% of time spent by 2025 (FICCI, 2021). It isn't surprising that Telegu the most popular language followed by Punjabi, Bengali, Marathi, Tamil, and others (Sundaravel & Elangovan 2020). Several regional language series and films have dominated internet trends and forums. For instance, Telegu movie, Ala Vaikunthapurramuloo trended for an entire day on Twitter pre-release.

CONCLUSION

Content personalization and recommendations by OTT providers make the experience rewarding to the common man. Production house should take this as an opportunity that the audience wants to binge-watch and see the latest movies at home, no longer is COVID-19 a reason for consuming them. Web 2.0 sites allow the audience to have freedom of choice and flexibility to create and consume any type of content. The report suggests that we have peaked globalization and in reversed-phase (Antres, 2020). De-globalisation is countries going back to economic and trade policies that put the national interest first. The popularity of content in regional language in TIER II and Tier III are transiting to the creation of a Heterogenous environment. Individual fragmentation depends on audience wants' and recommendations for same this may further contribute to the creation of own identities and absence of universalization of culture. The embrace of web 2.0 tools during pandemic have facilitated even traditional audiences to use multimedia platforms. In India, we still haven't fully embraced the potential of web 2.0 tools, but, fragments of de-globalization can be found.

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Perfect Matching of an Undirected Complete Graph Based on Algebraic Multiplicity

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ABSTRACT

One of the important concepts of Graph Theory is Matching Theory. Several Concepts on Matching Theory has been dealt in [1, 2, 3]. The technique of maximum matching on directed graphs have been studied in [1]. In this paper, a new approach for finding maximum matching of an undirected complete graph based on largest Algebraic multiplicity of Eigen values using exact controllability network is studied. This concept has been used for a complete graph with vertices $N = 4, 6, 8, \dots, 2V_n$ for $V_n \geq 2$ and it is proved that the maximum matching obtained is a perfect matching. It will be interest to further study on their properties.

Keywords: Algebraic Multiplicity, Complete graph, Graph Theory, Matching, Maximum Matching.
AMS Classification Key: 05C, 05C70, 911368, 15A18.

INTRODUCTION

One of the important concepts of Graph Theory is Matching Theory. Under this topic we discuss the method to find maximum matching of an undirected complete graph using Largest Algebraic Multiplicity of Eigen values. Maximum Matching nodes can be obtained using the largest Algebraic multiplicity through the transpose of adjacency matrix. The maximum matching of an undirected complete graph is discussed under this topic. The basic





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idea of this method is obtained from the Exact Controllability for general networks. Maximum Matching based on largest algebraic multiplicity of an undirected complete graph are illustrated by a theorem.

Notation: $G_u = (V_n, E_n)$, where G_u is the undirected graph, V_n be the Nodes (non empty finite set of elements) and E_n is the Edges (finite set of ordered pairs of different nodes).

Preliminaries

Definition 2.1. (Size): The size of undirected graph is the number of nodes in the graph G_u and it is denoted by $|G_u| = N$.

Definition2.2. (Algebraic Multiplicity (Complete graph)): The maximum multiplicity of each Eigen values of a diagonalizable undirected graph is known as Algebraic multiplicity.

Definition2.3. (Matching of an undirected graph): The independent set of edges where no two share a node is Matching M of an undirected graph.

Definition2.4. (Matching Node): If a node is incident to an edge then it is matched, otherwise it is unmatched.

Definition2.5. (Complete graph): A graph is called a complete graph if every pair of distinct vertices is connected to a unique edge in an undirected graph. All complete graphs are maximally connected. It has $\frac{n(n-1)}{2}$ edges and of degree $(n-1)$

Note: An Undirected graph can have at most $\frac{n(n-1)}{2}$ edges.

Definition2.6. (Maximum Matching of an undirected graph): The Matching of Maximum cardinality among all matching is known as Maximum Matching M^* If all the nodes are matched then the Maximum Matching M^* is a perfect matching. The edges corresponding to the matched nodes are the matched edges.

Largest Algebraic Multiplicity

The minimum number of driver's node N_D of an undirected graph is obtained by the largest Algebraic multiplicity $\delta(\lambda_j)$ of the Eigen value λ_j .

$$N_D = \max \{ \delta(\lambda_j) \}.$$

Maximum Matching of an Undirected Graph Based on Largest Algebraic Multiplicity

If $\delta(\lambda_j)$ is the Algebraic multiplicity of the Eigen values λ_j of A which is the maximum number of identical Eigen values and the sum of Algebraic multiplicity $\delta(\lambda_j)$ of all identical Eigen values is N (i.e.) $\sum_{j=1}^l \delta(\lambda_j) = N$

Maximum Matching of an Undirected Complete Graph Based on Largest Algebraic Multiplicity

The Adjacency matrix of a complete graph with unit weight in the absence Self-loops is of the form

$$A = \begin{bmatrix} 0 & 1 & \dots & \dots & 1 \\ 1 & 0 & \dots & \dots & 1 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 1 & 1 & \dots & \dots & 0 \end{bmatrix}$$

The characteristic polynomial is

$$|\lambda I_N - A| = [\lambda - (N - 1)](\lambda + 1)^{N-1}$$

The Eigen values and the corresponding Algebraic multiplicities are,

If $\lambda_1 = N - 1$ then $\delta(\lambda_1) = 1$ and if $\lambda_2 = -1$ then $\delta(\lambda_2) = N - 1$





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$$\therefore \sum_{j=1}^l \delta(\lambda_j) = \delta(\lambda_1) + \delta(\lambda_2) = 1 + N - 1 = N$$

Also if A is diagonalizable then the diagonal elements which give the Eigen values will be identical then $\delta(\lambda_j) = N$

Theorem: .3.1.Maximum matching of an undirected complete graph $K_n = G_u(V_n, E_n)$ without self-loops will be a Perfect matching if the size of the graph consists of even number of vertices $N = 2V_n, V_n \geq 2$

Proof: Consider an undirected Complete graph of any size $N = 2V_n, V_n \geq 2$ without self-loops. Then the expected Eigen values $\lambda_n (n=1, 2, 3... N)$ of the linearly independent rows is found by reducing the matrix to an upper or lower triangular matrix using fundamental column transformation.

The diagonal elements of the reduced matrix gives the Eigen value as $(N-1), -1, -1... (N-1)$ times

(i.e). $\lambda_1 = N - 1, \lambda_2 = -1, \lambda_3 = -1, \lambda_4 = -1, \dots, \lambda_{N-1} = -1$

According to (3) the maximum matching of an undirected complete graph based on Algebraic multiplicity is the sum of the Algebraic multiplicity $\delta(\lambda_N)$

$$\delta(\lambda_1) = 1, \delta(\lambda_{N-1}) = N - 1$$

$$\therefore \sum_{j=1}^l \delta(\lambda_N) = \delta(\lambda_1) + \delta(\lambda_{N-1}) = 1 + N - 1 = N$$

Thus, all the nodes (N) are matched nodes, since according to maximum matching of an undirected graph the largest Algebraic multiplicity $\delta(\lambda_N)$ is related to matched nodes.

In general, the maximum matching of an undirected complete graph of size N (nodes) and $E_n = \frac{N(N-1)}{2}$ edges without self-loops based on its Largest Algebraic multiplicity will have a perfect matching if the size of the graph consists of even number of vertices with maximum number of matched edges $\frac{N}{2}$ and $(N - 1)!!$ different perfectly matched graphs.

The above proof is illustrated by an undirected complete graph for N vertices.

Case (i) consider an undirected complete graph with 4 vertices and 6 edges

Matrix Form: The Adjacency matrix is, $A \in R_{N \times N}$ of a graph $G_u(V_n, E_n)$ with vertices $V_n = \{v_1, v_2, \dots, v_N\}$ is given by

$$a_{ij} = \begin{cases} 1, & \text{if } (v_i, v_j) \in E \\ 0, & \text{otherwise} \end{cases}$$

$$A = \begin{bmatrix} 0 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 \\ 1 & 1 & 0 & 1 \\ 1 & 1 & 1 & 0 \end{bmatrix}$$

By performing fundamental column transformation for A gives

$$A' = \begin{bmatrix} 3 & 1 & 1 & 1 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix}$$

Eigen Value of the Matrix A':

$$|\lambda I - A'| = \begin{vmatrix} \lambda - 3 & 1 & 1 & 1 \\ 0 & \lambda + 1 & 0 & 0 \\ 0 & 0 & \lambda + 1 & 0 \\ 0 & 0 & 0 & \lambda + 1 \end{vmatrix} = 0$$





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$$[\lambda - 3](\lambda + 1)^3 = 0$$

$\lambda = 3, -1, -1, -1$ are the Eigen values of the matrix A

If $\lambda_1 = 3$ then the Algebraic multiplicity $\delta(\lambda_1) = 1$

If $\lambda_2 = -1$ then the Algebraic multiplicity $\delta(\lambda_2) = 3$

$$\sum_{N=1}^l \delta(\lambda_N) = \delta(\lambda_1) + \delta(\lambda_2) = 1 + 3 = 4$$

Therefore, all the nodes are perfectly matched

Perfectly Matched Edges

The Perfectly Matched Edges for the 4 matched nodes are 2 given in red lines. It is given in a way that no two edges share a node. Fig 2 shows that there are 3 different perfectly matched graphs for an undirected complete graph with 4 nodes and 6 edges.

Case (ii) Consider an undirected complete graph with 6 vertices and 15 edges

Matrix Form: The Adjacency matrix is, $A \in R_{N \times N}$ of a graph $G_u(V_n, E_n)$ with vertices $V_n = \{v_1, v_2, \dots, v_N\}$ is given by

$$a_{ij} = \begin{cases} 1, & \text{if } (v_i, v_j) \in E \\ 0, & \text{otherwise} \end{cases}$$

$$A = \begin{bmatrix} 0 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 & 1 & 1 \\ 1 & 1 & 0 & 1 & 1 & 1 \\ 1 & 1 & 1 & 0 & 1 & 1 \\ 1 & 1 & 1 & 1 & 0 & 1 \\ 1 & 1 & 1 & 1 & 1 & 0 \end{bmatrix}$$

By performing fundamental column transformation for A gives

$$A' = \begin{bmatrix} 5 & 1 & 1 & 1 & 1 & 1 \\ 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 \end{bmatrix}$$

Eigen Value of the Matrix A' :

$$|\lambda I - A'| = \begin{vmatrix} \lambda - 5 & 1 & 1 & 1 & 1 & 1 \\ 0 & \lambda + 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & \lambda + 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & \lambda + 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & \lambda + 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & \lambda + 1 \end{vmatrix} = 0$$

$$[\lambda - 5](\lambda + 1)^5 = 0$$

$\lambda = 5, -1, -1, -1, -1, -1$ are the Eigen values of the matrix A

If $\lambda_1 = 5$ then the Algebraic multiplicity $\delta(\lambda_1) = 1$

If $\lambda_2 = -1$ then the Algebraic multiplicity $\delta(\lambda_2) = 5$





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$$\sum_{N=1}^l \delta(\lambda_N) = \delta(\lambda_1) + \delta(\lambda_2) = 1 + 5 = 6$$

Therefore, all the nodes are perfectly matched

Perfectly Matched Edges

The Perfectly Matched Edges for the 6 matched nodes are 3 given in red lines. It is given in a way that no two edges share a node. Fig 4 shows that there are 15 different perfectly matched graphs for an undirected complete graph with 6 nodes and 15 edges.

Case(iii) In general consider an undirected complete graph with N vertices and E_n edges (Table 1)

Matrix Form: The Adjacency matrix is, $A \in R_{N \times N}$ of a graph $G_u(V_n, E_n)$ with vertices $V_n = \{v_1, v_2, \dots, v_N\}$ is given by

$$a_{ij} = \begin{cases} 1, & \text{if } (v_i, v_j) \in E \\ 0, & \text{otherwise} \end{cases}$$

$$A = \begin{bmatrix} 0 & \dots & 1 \\ \vdots & \ddots & \vdots \\ 1 & \dots & 0 \end{bmatrix}$$

By performing fundamental column transformation for A gives

$$A' = \begin{bmatrix} N - 1 & \dots & 1 \\ \vdots & \ddots & \vdots \\ 0 & \dots & -1 \end{bmatrix}$$

Eigen Value of the Matrix A' :

$$|\lambda I - A'| = \begin{vmatrix} \lambda - (N - 1) & \dots & 1 \\ \vdots & \ddots & \vdots \\ 0 & \dots & \lambda + 1 \end{vmatrix} = 0$$

$$[\lambda - (N - 1)](\lambda + 1)^{N-1} = 0$$

$\lambda = N - 1, -1, -1, -1 \dots (N - 1)$ times are the Eigen values of the matrix A

If $\lambda_1 = N - 1$ then the Algebraic multiplicity $\delta(\lambda_1) = 1$

If $\lambda_2 = -1$ then the Algebraic multiplicity $\delta(\lambda_2) = N - 1$

$$\sum_{N=1}^l \delta(\lambda_N) = \delta(\lambda_1) + \delta(\lambda_2) = 1 + N - 1 = N$$

Therefore, all the nodes are perfectly matched

Perfectly Matched Edges

The Perfectly Matched Edges for the N matched nodes are $\frac{N}{2}$. It is given in a way that no two edges share a node. There are $(N - 1)!!$ different perfectly matched graphs for an undirected complete graph with $N = 2V_n$ and $E_n = \frac{N(N-1)}{2}$ Edges





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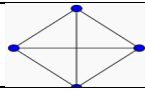
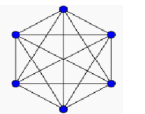
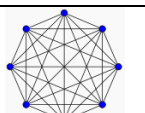
CONCLUSION

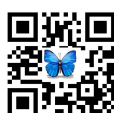
In this topic the new method for finding maximum matched nodes of an undirected complete graph based on Algebraic multiplicity of Eigen values which has a relation with exact controllability network is proved through they adjacency matrix. For Complete graph matched nodes are got through some fundamental column transformation. The maximum matching obtained is a perfect matching if the vertices are even and this proved in this paper as a theorem with an undirected complete graph for vertices 4,6,8,10,12 ,..., N and the corresponding edges , matched edges, Number of different perfectly matched graphs are given in a tabular form. The corresponding maximum edges for matched nodes are also found through the new method.

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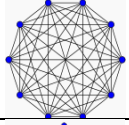
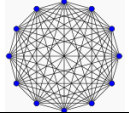
Table 1. In general consider an undirected complete graph with N vertices and E_n edges

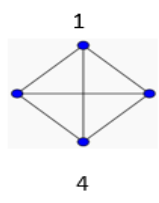
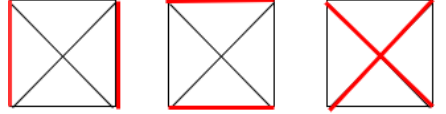
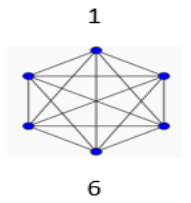
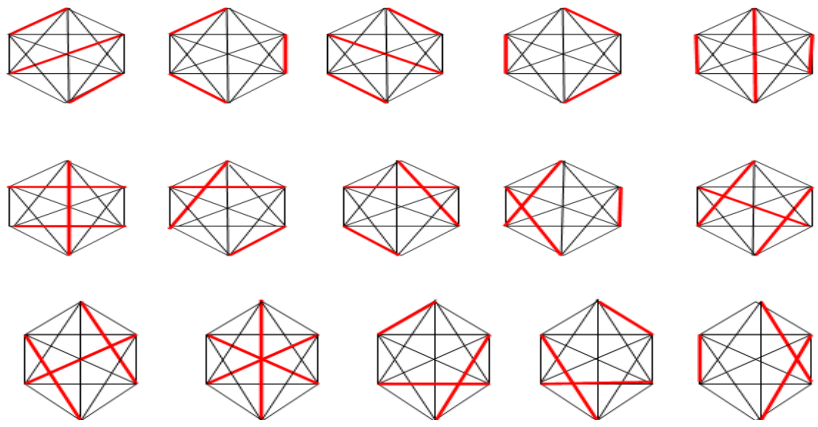
Complete graph	No. of vertices	No. of edges	No. of matched edges	No. of different perfectly matched graph
	4	6	2	3
	6	15	3	15
	8	28	4	105

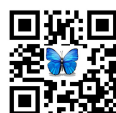




Jini J and Hemalatha S

	10	45	5	945
	12	66	6	10,395

		
Fig 1 Undirected graph with 4 vertices and 6 Edges	Fig 2 Perfectly matched edges	Fig 3 Undirected graph with 6 vertices and 15 Edges
		
Fig 4. Perfectly matched edges		





A Novel Lightweight Cloud Storage Examinee with De-Duplication Authorizing Secure Protection with Hybrid Encryption

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ABSTRACT

With the rapid advancement of cloud computing, cloud storage has been widely acknowledged as it can be accessed, processed and maintained by anyone, at any time from any part of the world. In light of this trend, it is very critical to develop a cloud storage examinee scheme that allows users to check the data integrity, as data can be compromised due to errors or hardware/software failure. All the existing cloud storage examinee with deduplication system is vulnerable to several attacks like brute-force, dictionary attack when the file is predictable or comes from a small space. In this paper, we propose a novel lightweight cloud storage examinee with deduplication authorizing secure protection with hybrid encryption scheme to overcome the limitations of the existing systems. The basic goal of this system is to address the issue of user's privacy leakage in the cloud storage. A hybrid encryption technique has been proposed for providing high level of security to the user's confidential data with the combined security of symmetric and asymmetric encryption. To increase the storage efficiency of the cloud, secure block-level deduplication is performed directly on encrypted data as well as secure cloud storage auditing, which allows the user to check the integrity of cloud data without downloading the entire file. So, the proposed scheme can securely verify the integrity of the data while keeping only a single copy of the duplicated file and provides greater security to the user data.

Keywords: Cloud Computing, RSA and AES Algorithm, MD5 Algorithm, LBA Technique, De-duplication.



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INTRODUCTION

The advancement of the present technology has risen to the level that individuals and enterprises are not at all satisfied with the classic data storage method. The fast advancement of distributed computing, distributed storage for its benefits of general access, ease and request administration has been a gigantic advantage for the clients. Clients can re-appropriate complex calculations to the cloud to decrease their computational weight. Moreover, people likewise can re-appropriate their immense scope information to the cloud to free their neighborhood stockpiling trouble. In such circumstances, guaranteeing the consistency of information stockpiling administrations for shoppers and the cloud gets basic. In light of the unavoidable activity blunders or programming/equipment disappointments in the cloud, the reevaluated information could be undermined or lost. Accordingly, improving distributed storage examining is significant, as it empowers clients to confirm the precision of cloud information without downloading the whole document from the cloud. Then again, loads of information put away in the cloud are copied. To improve the capacity effectiveness of the cloud, it is important to perform information deduplication, where the cloud keeps just a solitary duplicate of the copied file and makes a connection to the file for the clients. To tackle the issue of client security spillage a distributed storage reviewing plan with deduplication supporting solid protection insurance is proposed.

The administration and support issues are addressed for the clients by the cloud worker. The greatest contrast of distributed storage from customary in-house stockpiling is that the information is moved through the Internet and put away in an unsure space, not heavily influenced by the customers by any means, which definitely raises customer's extraordinary worries about the uprightness of their information. These worries start from the way that distributed storage is vulnerable to security dangers from both outside and within the cloud, and the uncontrolled cloud workers may inactively ensure their uprightness and cover certain information misfortune cases from their clients. What's more awful, cloud workers can purposefully and intentionally dispose of seldom got to information records having a place with conventional customers to set aside cash and space. Given the immense size of the re-appropriated information records and the restricted asset assets of the customers.

LITERATURE SURVEY

Jiaojiao Wu et al. [5] proposed CDPA: A privileged-shield de-duplication cloud storage with public cloud auditing wherein they recommend a confidentiality-retaining de-duplication cloud storage with common cloud auditing (CPDA). CPDA course of action realizes the de-duplication on an encrypted report with the aid of using the usage of fulfilled-understand convergent encryption method which produces the same cipher textual content report from identical plaintext report. The benefit of this system turned into numerical survey and assessment outcome exhibit that the scheme is efficient through evaluating with the contemporary schemes on communication, computation and storage expenses however this approach is forgeable by a few malicious attacks.

Shangping Wang et al.[4] proposed Blockchain based fair payment Protocol for de-duplication cloud system. They propose a replacement decentralized fair payment protocol for cloud de-duplication storage systems by utilizing Ethereum blockchain technology. The new protocol takes advantage of the decentralization of blockchain technology, permitting direct transactions without the participation of trusty third parties and within the new protocol, if a malicious scenario occurs, the system will guarantee fair payment by pre-storing penalty money in the sensible contract and feasible. The disadvantage of this technique was The cloud storage server might come back with incorrect results or return no results to avoid wasting resources. Shengwan Fan et al.[2] proposed A hybrid chaotic encryption scheme for wireless body area networks. This algorithmic program uses a hybrid chaotic system which might choose the effective preciseness consistent with the importance. The advantage was it's a low-power and high security wireless communication protocol particularly utilized in medical environments. However the experimental analysis and performance comparison show that the projected encoding algorithm doesn't have ample





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security to resist common attacks. Vishruti Kakkad et al. [3] proposed a Biometric authentication and image encryption for image security in cloud framework, This presents the concept of authentication of image in two basic steps of image compression using discrete wavelet transform methodology followed by image encoding using hybrid method of SHA and Blowfish. Image stored into the database of the cloud and accessed whenever the user requests it. The advantage is Fingerprint alongside multifactor authentication is additionally one amongst the wide used systems however the print scanner doesn't contemplate any physical changes and It uses metrics to human characteristics resembling palm veins, face recognition, DNA, palm print, retina. The disadvantage of this technique was if any characteristics are taken the actual user cannot replace it with a replacement one. Once taken the characteristics as a result of it's useless for the user.

Alexandro s bakas et al. [1] proposed The cloud we share: Access control on symmetrical encrypted data in untrusted clouds. The objective is to identify both the benefits and drawbacks of SSE and ABE, they propose a solution that uses the most effective out of both techniques. They propose a reversible hybrid encryption scheme combining ABE and SSE. In our construction, the ABE scheme is employed as a tool that enables economical sharing of the SSE key between legitimate users. The advantage was the key is decrypted on condition that the decryptor satisfies the policy specified by the ABE policy .However the planned solutions are supported by the properties of the underlying ABE scheme and hence, the revocation costs grow beside the complexities of the policies. All of the preceding schemes cannot attain the de-duplication authorizing secure protection for encrypted information in cloud storage auditing. Thus, a way to understand de-duplication authorizing secure protection with hybrid encryption in cloud auditing is extremely important.

PROPOSED WORK

In this paper, we are diving the file into blocks using LBA technique and then generating the hash code, encrypting the block using a hybrid encryption algorithm and uploading blocks to the cloud, if the hash code is already there in the database then increase the instance of the block. While downloading, get the block details using LBA technique and decrypt the block, check the hash code, if it's matching from the hash code which is stored in the database then merge all the blocks and download the file to the client system.

LBA technique Algorithm:

Step 1: Start

Step 2: Set the packet size (packet_size=500), no_block=0, rem=0, no_padding=0

Step 3: Get the number of blocks; no_block=(content.length())/ packet_size;

Step 4: Get the remainder, rem= (content.length())% packet_size;

Step 5: If rem is not equal to 0 no_padding= packet_size - rem; no_block = no_block + 1;

i = 0 till i < no_padding , increment i temp = temp+"**"; content=content+temp;

Step 6: Initialize a variable Pointer = 0;

Step 7: Packet Formation

i=0 till i<no_block, increment i Packet[i] = content.substring(Pointer, (Pointer + packet_size));

Pointer = Pointer + packet_size;

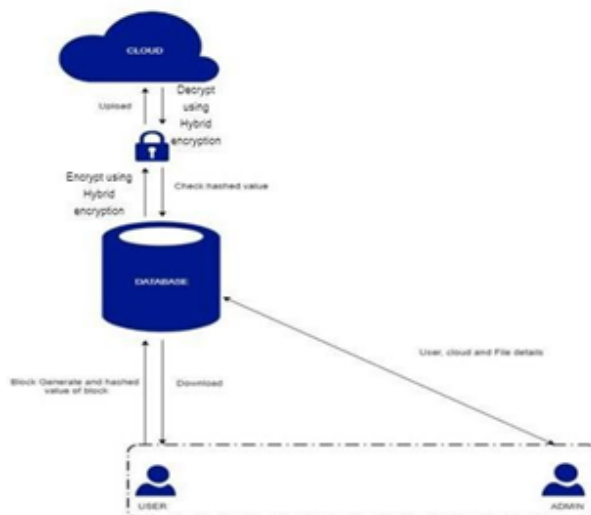
Step 8: Stop

SYSTEM ARCHITECTURE

The system architecture consists of three entities: as shown in a Fig.

- (1) The Cloud: The cloud has an excessive storage, and to provide the uploading and downloading services for users. The cloud performs the de-duplication, to improve the storage efficiency.
- (2) The User: The user is categorized into two , one is the User and another is Admin. Admin is responsible for all the user details and file details, whereas, the User can upload or download the files.
- (3) The database: the database keeps record of admin, user, files, blocks and cloud





If a user wish to upload a file F1, The file will be divided into N blocks using LBA technique, take the first block i.e. $i=1$ till N block and generate the hash code for the i^{th} block, check for the deduplication if the block is available then increase the instance of the block ,increment the file block i.e. $i=i+1$ till N and continue until condition fails i.e. $i>N$, if the hash code doesn't exist, encrypt the block and increment the file block i.e. $i=i+1$ till N and continue until condition fails i.e. $i>N$. Insert the LBA details to the database and upload the blocks to cloud storage. If a user wishes to download a file F1, get the blocks LBA numbers using LBA technique, retrieve all the blocks from the cloud. Let N1 be the number of blocks, start from $i=1$, read the i^{th} block and decrypted then generate a hash code, check for the integrity(whether the old and new hash code is the same or not), if the integrity passes, then increment the block size and repeat the procedure till the condition fails($i>N1$). When the condition fails, merge all the blocks and download the file to the client system.

ALGORITHM

This paper presents a novel lightweight cloud storage examined with de-duplication assisting secure protection using hybrid encryption algorithm. A hybrid AES, RSA and MD5 is used for cryptography where AES and RSA are hybrid encryption and MD5 is a hash function scheme.

AES Algorithm

AES relies upon an arrangement standard suggested as a substitution change that arranges the mix of each change and organizes and is smart in each item system and gear. Earlier days DES algorithm we used which was published in the year of 1977 but this algorithm supersedes. AES algorithm makes use of the same key for encipher and decipher of particular information. Rather than its forerunner network AES could be an alternative to Rijndael with a fast chunks dimension of 128bits and essential expanse of 128,192 or 256bits. Against this, the Rijndael attributes all things considered is apparent with block and key size which will be many 32bits with a least of 128bits and vast majority of 256bits. AES run on 4×4 section important structure of bytes. AES counts have consumed a unique limited field. The key size utilized for quantity of redundancies of charge adjust that converts the information and mention in a plaintext into a final which is known as ciphertext.

RSA Algorithm

In RSA a communal solution of cryptanalysis is the cipher key is common and well defined from the perceptible key from the resolution key which is kept confidential. The safety of RSA depends on the experimental problems of





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factoring the outcome of a massive prime number that is “factoring problem”. From this algorithm four trades can be implied i.e., key-production, key-dispensation, encrypt, decrypt. From RSA algorithm we observe that there is three sizeable productive integer x, y, and b such that there is modular exponentiation for all integer m i.e., $0 \leq a \leq b$ where $(ax) y \equiv (\text{mod } a)$. From the given equation we know that x and b or even a, its arduous to observe y, whereas (\equiv) triple bar indicates modular congruence. From the RSA algorithm it implies a common solution and particular solution. The common solution can be familiar by one and all and is used for encryption messages. The purpose of that message is in the form of a public key which helps to decrypt in a logical aggregate of time using the particular explanation. Common solution denoted by the integer b and x and particular solution by the integer y.

step 1: - Choose m and n

Where m and n are both prime numbers.

step 2: - Compute $a=m*n$

We have to multiply both the prime number

step 3: - Evaluate $\phi(a) = (m-1)*(n-1)$

step 4: - Determine integer x i.e., $\text{gcd}(\phi(a),x)=1; 1 < x < \phi(a)$

step 5: - Evaluate d

Public Solution: - $KU=x,a$

Private Solution: - $KR=y,a$

MD5 Algorithm

In MD5 (Message digest algorithm), the info message is different into a piece of 512bits block (each with sixteen i.e., 32bit sub blocks). After an advancement of performance MD5 assembles a 128 bit message process with four connected 32bit obstruct for the integrity of a document.

RESULT

SNo	FileId	UserId	File_Name	Date	Time	Day
1	1	1	test.txt	19-04-2021	09:14:43	Monday

file_no	file_name	file_size1	file_size2	padding	no_blocks	hash_blk_no	user_id	date	time	day
1	test.txt	343	500	157	1	1	1	19-04-2021	09:14:43	Monday

hash_unique_code	hash_code	blk_name	no_instno	uploaded_status
1	1ec99a703b49810ca9233864230c10fd9	blk.txt	1	yes





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CONCLUSION

In this Paper, we review on how to resolve the problem of security in cloud examinee with de-duplication. We design a novel lightweight cloud storage examinee scheme with de-duplication authorizing secure protection, in which the file will be divided into blocks to reduce the burden of cloud and checking the integrity of blocks. The cloud storage examinee with de-duplication is able to review the integrity of blocks stored in the cloud and provides greater security to the user data with hybrid encryption. The results show that proposed work accomplish greater storage ability and adequate in data security and examinee and provides greater storage ability.

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A Study of Bollywood Film Trailers: A Multimodal Approach

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ABSTRACT

Movie trailers have been one of the most important marketing tools for any film over a long period of time. Earlier trailers were only released during screening of a film. Today, due to advancement of digital technology, trailers of films are released on Television as well as interactive multimodal platform like YouTube. The narrative of the trailers has become more structured in order to keep pace with the changing times. Now we see a mix play of visual, audio, text, voiceover, graphics, animations to lure the attention of people. The researcher conducted a content analysis of Hindi film trailers released between 2015 and 2020 and identified the various elements of a film trailer including visuals, sound effects, background music, interstitials, text, symbols and narration. Also, a quantitative research was conducted to investigate the perception of college students regarding the trailers of Hindi films (2015-2020) in order to understand what elements of the trailer did they like.

Keywords: visual, visual language, film, movie trailers, narrative, multimodality.

INTRODUCTION

Trailers are unique form of advertisement that have an integrated narrative. They have a brief text that display images from a specific feature film for the purpose of projecting in theatres and promoting for a theatrical release (Kernan, 2004) They utilize codes of voice over narration, sound, sound overlapping, music, graphics, editing and montage. Trailer is a condensed form of film that is lesser than 2 minutes 30 seconds. They generally try best to give a glimpse of the film story by using various dimensions of visuals, music score, sound effects, voiceover and editing to lure the spectators to watch the film. Trailers help in differentiating the film within a conventional genre and also help in building up expectation of a film extravaganza. They have evolved over a period of time. Earlier, studio makers would withhold the elements of the story as they considered story to be a product, and the entire trailer would build up questions in such a dramatic manner, that only watching the film will provide the answers to them.

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According to film researcher, Vinzez Hediger, trailers have shifted to two- thirds formula wherein two thirds of story arc is revealed in the trailer due to the marketing requirements (Kernan, 2004). Trailers have condensed layers of storytelling. Story is the principal appeal of the trailer. They are persuasive in nature and they depict high action with minimal dialogues and they touch upon references regarding the premise of the film with the help of narration. Also, most of the times when films are adapted from a best seller book, they try to reemphasize it to make the audience familiar.

REVIEW OF LITERATURE

Today, language is no longer a carrier of all meaning. In every text there are two modes of communication, language as writing and image. It is no longer possible to understand language and its uses without understanding the effect of all modes of communication that are present along with text. While image is founded on the logic of display in space, writing and speech is founded on the logic of succession in time. Image is spatial, nonsequential while speech and writing are temporal and sequential (Kress, 2000). Multimodal methods deal with communicative language in different modes and other devices. Multimodal computer mediated communication includes interactive multimodal platforms that support modes of text, audio, video and graphics for user-to-user communication. (Herring, 2015). In audio we have different modalities in terms of music, speech, and sound. In visual we have still images and video (motion). While in text, we have printed and handwritten text.

Film Trailers are meta or para text which is a part of large textual film. They are designed and marketed as commercial texts in order to present the upcoming film in an attractive manner that lures people to watch the film after its theatrical release. Trailer is a model of a film that establishes relationship between shots, sound, mise-en-scene. The creators of a trailer guide the reception process of its viewers (Oja, 2019). Trailers create filters, frames suggesting viewers what to think about the film's genre, tone and themes. According to (Gray, 2017) character names offer guidance on how to read a text, ways of filming, generic codes, and setup. Overall, the mode of acting, use of color, dialogue helps us make sense of the film trailer as we try to comprehend and make sense of it by our past viewing experience.

(Maier, 2009) mentioned that the trailers are set out to persuade, entertain as well inform their viewers of the product: the film to be released. The information in a film trailer have two different contexts. The diegetic context that includes, visual and verbal information regarding the characters, events, and sounds belonging to the film's narrative while the non-diegetic context includes visual and verbal information regarding the names of the film, director, the film companies, actors, date of release, website addresses, voice- over narration.

RESEARCH QUESTION

Which Hindi film trailers were effective with its visuals, audio, sound effects and narration among college students between 2015-2020?

Objectives of Research

- To understand overall which Hindi film trailer released between 2015-2020 was most impactful in its communication.
- To identify whether audio or visual is more effective in communicating the film trailer among the college students.

RESEARCH METHODOLOGY

The researcher has conducted qualitative research of multimodal approaches used in film trailers of Hindi cinema ranging between years 2015-2020. Top 4 grossing films of each year have been taken into consideration. Sample size - 24 trailers.



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Also, a quantitative research was conducted among media students (BMM and MAEMA) between the age group of 17-25 years. The students were shown all 24 trailers online through Microsoft Teams platform and they had to watch each trailer and simultaneously answer the Google form questionnaire that was created for all 24 trailers. Sample size-103 students

HYPOTHESIS

Ho- Star studded Hindi film trailers have got higher mean score than content driven films

Ha- Star studded Hindi film trailers and content driven films have got a mean score that is high.

CONTENT ANALYSIS

Top grossing four Hindi film trailers per year ranging from 2015-2020 were chosen as a sample. Overall trailers were judged on basis of its visual appeal, sound effects, background music, narration, symbols and interstitials used. It was found apart from a formal mode of interstitials that emphasized the name of producer, director, writer, production house, studio, date of release, and the festival release; many a times text was used in order to help navigate with the narration. Trailers tried to establish the canvas, the mise-en-scene, set up of the story along with the pivotal interesting plot points that could interest the audience. Vocals, background music, theme film music emphasizing title of the film was used effectively for the trailer. Also, snappy dialogue intercuts were also used in the trailers.

FINDINGS

It's interesting to note, the researcher found that among college students no Salman Khan films have scored the same ratings. Eg, Bajrangi Bhaijaan, Prem Ratan Dhan Payo, Sultan and Tiger Zinda Hai. It shows that while watching the film trailers, students had genuinely judged the various parameters of individual films by their own merit. There was no fan adulation among the students. In 2015 we had big banner star studded films like Bajrangi Bhaijaan, Prem Ratan Dhan Payo, Dilwale and Bajirao Mastani being the top 4 top grossing films. But as we progressed into 2016, apart from big banner films like Dangal, Sultan and Ae Dil Hai Mushkil we also had MS Dhoni: The Untold Story which got a thumping response from the college students. In 2017, apart from big banner film star studded like Tiger Zinda Hai, Secret Superstar that had only Aamir Khan in a cameo role, along with content driven films like Hindi medium and Toilet Ek Prem Katha, students preferred Hindi medium trailer over all other trailers that starred Irrfan Khan. In 2018, we had big banner star studded films like Sanju, Padmaavat, Simmba along with content driven film like Andhadhun. students liked Padmaavat and Andhadhun over the other two films. In 2019, Uri film that didn't have a mega star in the film got immense response from students. Films like Ae Dil Hai Mushkil, War, Saaho that was basically made targeting youngsters didn't get a mean score of more than 4 suggesting that students have liked trailers that are intriguing, bringing in new concepts and a visual treat and overall good narration.

Out of 24 trailers, 12 film trailers are star studded films of big production houses, while remaining 12 film trailers are content driven films or films that don't have mega star cast. We can safely say that alternate hypothesis has got proven correct. Both star studded film trailers as well as content driven films have got a mean score more than 4 out of 5 depending on the parameters of visuals, music, sound effects and narration.

LIMITATION

The research has only taken into account top 4 grossing films of Hindi cinema between 2015-2020 which is a limitation. Also, quantitative research has been conducted with 103 students which is not a true sample of the universe as there are moviegoers belonging to various age groups.

SCOPE

Research can be done on film trailers released during the pandemic era. Quantitative research can be undertaken focusing on different film genres and their target audience. User engagement of film trailers can be studied on





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various new media platforms. Study can also be undertaken for new upcoming film trailers and identifying their trends.

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Table 1. In the year 2015, Bajirao Mastani scored mean of more than 4 out of 5 in all categories including visuals, music, sound effects and narration.

2015

Statistics					
		What did you feel about Bajrangi Bhaijaan film trailer? [Visually effective?]	What did you feel about Bajrangi Bhaijaan film trailer? [Music effective?]	What did you feel about Bajrangi Bhaijaan film trailer? [Sound effects impactful?]	What did you feel about Bajrangi Bhaijaan film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.85	4.06	3.90	3.69
Statistics					
		What did you feel about Prem Ratan Dhan Payo film trailer? [Visually effective]	What did you feel about Prem Ratan Dhan Payo film trailer? [Music effective]	What did you feel about Prem Ratan Dhan Payo film trailer? [Sound effects impactful]	What did you feel about Prem Ratan Dhan Payo film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.05	3.26	3.10	2.62





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Statistics					
		What do you feel about Dilwale film trailer? [Visually effective]	What do you feel about Dilwale film trailer? [Music effective]	What do you feel about Dilwale film trailer? [Sound effects impactful]	What do you feel about Dilwale film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.68	3.61	3.50	3.08
Statistics					
		What do you feel about Bajirao Mastani film trailer? [Visually effective]	What do you feel about Bajirao Mastani film trailer? [Music effective]	What do you feel about Bajirao Mastani film trailer? [Sound effects impactful]	What do you feel about Bajirao Mastani film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		4.55	4.47	4.48	4.33

Table 2. In 2016, two films namely Dangal and MS Dhoni scored a mean of 4 out of 5 in all categories including visuals, music, sound effects and narration.

2016

Statistics					
		What do you feel about Dangal film trailer? [Visually effective]	What do you feel about Dangal film trailer? [Music effective]	What do you feel about Dangal film trailer? [Sound effects impactful]	What do you feel about Dangal film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		4.03	4.10	4.11	4.15
Statistics					
		What do you feel about Sultan film trailer? [Visually effective]	What do you feel about Sultan film trailer? [Music effective]	What do you feel about Sultan film trailer? [Sound effects impactful]	What do you feel about Sultan film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.49	3.61	3.49	3.56





Geeta Sharma

Statistics					
		What do you feel about Ae Dil Hai Mushkil film trailer? [Visually effective]	What do you feel about Ae Dil Hai Mushkil film trailer? [Music effective]	What do you feel about Ae Dil Hai Mushkil film trailer? [Sound effects impactful]	What do you feel about Ae Dil Hai Mushkil film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.71	4.25	3.90	3.65
Statistics					
		What do you feel about MS Dhoni: The Untold Story film trailer? [Visually effective]	What do you feel about MS Dhoni: The Untold Story film trailer? [Music effective]	What do you feel about MS Dhoni: The Untold Story film trailer? [Sound effects impactful]	What do you feel about MS Dhoni: The Untold Story film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		4.04	4.11	4.13	4.04

In 2017, no film got a mean score of 4 in all parameters including visuals, music, sound effects and narration. Overall Hindi medium scored mean close between 3.88 to 4.05

2017

Statistics					
		What do you feel about Secret Superstar film trailer? [Visually effective]	What do you feel about Secret Superstar film trailer? [Music effective]	What do you feel about Secret Superstar film trailer? [Sound effects impactful]	What do you feel about Secret Superstar film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.57	3.94	3.69	3.47
Statistics					
		What do you feel about Tiger Zinda Hai film trailer? [Visually effective]	What do you feel about Tiger Zinda Hai film trailer? [Music effective]	What do you feel about Tiger Zinda Hai film trailer? [Sound effects impactful]	What do you feel about Tiger Zinda Hai film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.61	3.36	3.50	3.28





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Statistics					
		What do you feel about Hindi medium film trailer? [Visually effective]	What do you feel about Hindi medium film trailer? [Music effective]	What do you feel about Hindi medium film trailer? [Sound effects impactful]	What do you feel about Hindi medium film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.93	4.05	3.88	3.99
Statistics					
		What do you feel about Toilet Ek Prem Katha film trailer? [Visually effective]	What do you feel about Toilet Ek Prem Katha film trailer? [Music effective]	What do you feel about Toilet Ek Prem Katha film trailer? [Sound effects impactful]	What do you feel about Toilet Ek Prem Katha film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.43	3.37	3.43	3.77

In 2018, we had two films namely Padmaavat and Andhadhun scoring a mean of more than 4 out of 5 in all parameters of visuals, music, sound effects and narration.

2018

Statistics					
		What do you feel about Sanju film trailer? [Visually effective]	What do you feel about Sanju film trailer? [Music effective]	What do you feel about Sanju film trailer? [Sound effects impactful]	What do you feel about Sanju film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		4.14	3.83	3.90	4.10
Statistics					
		What do you feel about Padmavaat film trailer? [Visually effective]	What do you feel about Padmavaat film trailer? [Music effective]	What do you feel about Padmavaat film trailer? [Sound effects impactful]	What do you feel about Padmavaat film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		4.54	4.43	4.43	4.32





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Statistics					
		What do you feel about Andhadhun film trailer? [Visually effective]	What do you feel about Andhadhun film trailer? [Music effective]	What do you feel about Andhadhun film trailer? [Sound effects impactful]	What do you feel about Andhadhun film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		4.11	4.25	4.16	4.12
Statistics					
		What do you feel about Simmba film trailer? [Visually effective]	What do you feel about Simmba film trailer? [Music effective]	What do you feel about Simmba film trailer? [Sound effects impactful]	What do you feel about Simmba film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.57	3.74	3.63	3.34

Table 3. In 2019, Uri The Surgical Strike got a mean score of close to 4.5 for all categories of visuals, music, sound effects and narration.

2019

Statistics					
		What do you feel about War film trailer? [Visually effective]	What do you feel about War film trailer? [Music effective]	What do you feel about War film trailer? [Sound effects impactful]	What do you feel about War film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.84	3.82	3.70	3.32
Statistics					
		What do you feel about Saaho film trailer? [Visually effective]	What do you feel about Saaho film trailer? [Music effective]	What do you feel about Saaho film trailer? [Sound effects impactful]	What do you feel about Saaho film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.39	3.22	3.22	3.05





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Statistics					
		What do you feel about Kabir Singh film trailer? [Visually effective]	What do you feel about Kabir Singh film trailer? [Music effective]	What do you feel about Kabir Singh film trailer? [Sound effects impactful]	What do you feel about Kabir Singh film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.70	4.26	3.95	3.54
Statistics					
		What do you feel about Uri: The Surgical Strike film trailer? [Visually effective]	What do you feel about Uri: The Surgical Strike film trailer? [Music effective]	What do you feel about Uri: The Surgical Strike film trailer? [Sound effects impactful]	What do you feel about Uri: The Surgical Strike film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		4.53	4.50	4.50	4.52

Table 4. In 2020- There was no film trailer that got a mean score of more than 4 for all parameters of visual, music sound effects and narration. Only film Tanaji scored decently between a mean range of 3.76 to 4.09 for various categories.

2020

Statistics					
		What do you feel about Tanhaji film trailer? [Visually effective]	What do you feel about Tanhaji film trailer? [Music effective]	What do you feel about Tanhaji film trailer? [Sound effects impactful]	What do you feel about Tanhaji film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		4.09	3.94	3.86	3.76
Statistics					
		What do you feel about Baaghi 3 film trailer? [Visually effective]	What do you feel about Baaghi 3 film trailer? [Music effective]	What do you feel about Baaghi 3 film trailer? [Sound effects impactful]	What do you feel about Baaghi 3 film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.18	2.97	3.02	2.81





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Statistics					
		What do you feel about Street Dancer 3D film trailer? [Visually effective]	What do you feel about Street Dancer 3D film trailer? [Music effective]	What do you feel about Street Dancer 3D film trailer? [Sound effects impactful]	What do you feel about Street Dancer 3D film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.50	3.78	3.52	3.12
Statistics					
		What do you feel about Shubh Mangal Zyada Savdhan film trailer? [Visually effective]	What do you feel about Shubh Mangal Zyada Savdhan film trailer? [Music effective]	What do you feel about Shubh Mangal Zyada Savdhan film trailer? [Sound effects impactful]	What do you feel about Shubh Mangal Zyada Savdhan film trailer? [Narration]
N	Valid	103	103	103	103
	Missing	0	0	0	0
Mean		3.73	3.67	3.44	3.66

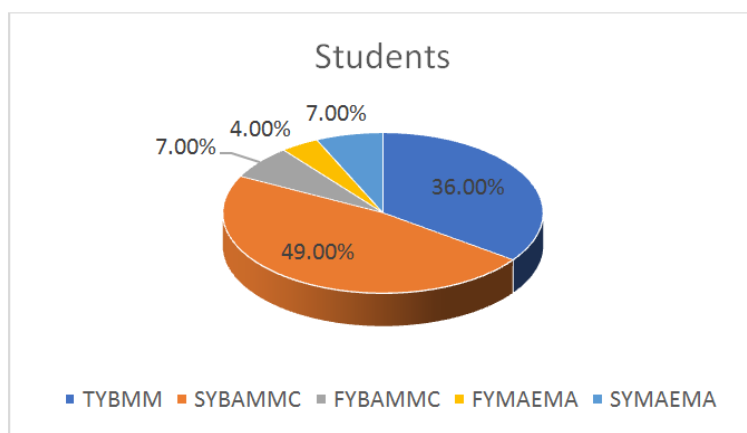


Fig.1. Content Analysis





Social Media and Copyright Infringement: An Incessant Debate

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ABSTRACT

Copyright statutes almost everywhere in the world were devised without anticipating the rise of Internet. As it evolves, the work capable of copyright protection has found new avenues to be displayed and shared in this borderless world. This paper attempts to understand the state of copyrighted work as it exists on social media. For this purpose, doctrinal method of research is employed. The paper has been divided into three parts. The first part discusses the broad nature of the “Terms of Use” being used by websites as a tool for obtaining consent from its users to indulge in otherwise infringing behavior. The second part compares the legal steps taken by two countries - USA and India- and suggests changes to curb the issue of online infringement. The third part deals with the steps which can be taken by both website proprietors and copyright holders to prevent infringement as an interim solution.

Keywords: Copyright, Copyright infringement, Creative Commons, Digital Millennium Copyright Act, Facebook, Information Technology Act, Instagram, Internet, social media, terms of use.

INTRODUCTION

It is true that law has always struggled to catch up with technology. The emergence of new technology has compelled the law to constantly reinvent itself to better protect it and its misuse. According to statistics, 3.6 billion people in the world use a social network site at least once per month. That is roughly 46% of the total world population. This means that a significant number of people choose to either create, view or share content on the internet which maybe literary, musical, artistic or even dramatic works in nature (Zimmerman, 2011).

Since it is still a very recent phenomenon, the implications of concepts like copyright over social media has largely remained untouched, both by the legislature as well as the judiciary. However, although recent, the phenomenon has grown at a groundbreaking rate. It is considered that the advent of the Internet is perceived to have put the problem of copyright infringement beyond the scope of the copyright law as it exists now (Zimmerman, 2011). The entire



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model of social networking sites is based on providing its users a platform to share their work publicly. However, signing up on these websites mean that the users accept certain terms and conditions which often results in considerable loss of control over one's own work (Gatignol, 2016). It is true that exploitation of work requires letting go of some control through licensing or assignment of the author's rights to those who would be able to do the needful like publishers, recording studios, production houses etc. However, in that case, it is also true that the copyright holder is always aware of the extent of rights that they are giving away. On social networking sides, on the other hand, a user tends to give away rights that they probably would not wish to otherwise.

In this paper we aim to understand the terms of use of social media websites in all its complexity and ambiguity. We make an attempt to understand how the law deals with copyright issues in the USA by looking at their statutes and judicial precedents and compare the same with those in India. In the end we try to give suggestions as to what can be done till there is no effective legislation to help resolve the issue in the long term.

"TERMS OF USE" AND ITS IMPLICATIONS

Every social networking website contains its own terms of use. They are used to regulate the relationship between the website and its users and govern a user's access of the particular platform (Patton, 2019). These terms contain copyright licenses that provide important information about the ownership of content created by its users also known as 'user-created content'. These terms essentially lay down which rights the user is agreeing to give to the website when they choose to upload their content on that platform right from when they open an account with them. While it is natural that anyone would care about what happens to the content that they decide to share online, it is also equally true that a very small percentage of people read the Terms of Use of these websites while even fewer understand them.

While these sites do not require an assignment of the copyright, they do require one to license their work to these platforms so that the website may be allowed to display the works of its users to others. However, sometimes these licenses tend to comprise a much broader aspects to include terms like non-exclusive, worldwide, revocable, irrevocable, royalty free, transferable, sub-licensable etc. According to a research done on the reality of such terms of services when it comes to copyright for online content creation, it was found that only five of the thirty websites assessed wrote their terms of services in a simple language which can be understood by somebody who does not have a legal background (Bruckman et al., 2020). Some more shocking observations revealed that only 11% of the 410 participants chosen for the study had actually read the terms of services- which the authors fear could be inflated due to a self-reporting bias (Bruckman et al., 2020). More importantly, the study was regarding the perceptions that users have about these terms as compared to what these terms actually entail (Bruckman et al., 2020). The study revealed that the users could predict with much less frequency that terms like '*the websites shall have the right to transfer their license to a third party*' or that '*the parties cannot take back the license once they have granted it to them*' existed (Bruckman et al., 2020). In other words, the users did not know that they were giving permission to transfer or irrevocably modify their work. This is indicative of the high level of trust with regards to the safety of their content that the users place on these websites, however misplaced (Bruckman et al., 2020).

The article also discusses the implication of this by taking an example of LinkedIn's terms of use which talk about assignable, sub licensable, irrevocable licenses along with the right to create derivative works (Bruckman et al., 2020). According to the authors, this basically gave the website owners the right to create a book or a film from any blog posted on their website as the users have already granted the proprietors permission to do so (Bruckman et al., 2020). Taking another example, it is found that YouTube, a popular video-based website, very carefully licenses to itself all the 6-copyright owner's exclusive rights granted by the US Copyright Act along with the right to sublicense all of them (Alm, 2014). In fact, some websites use very vague and ambitious language which states that the user is granting a license to the website to use their work but fails to specify how (Alm, 2014). Even the most popular websites like Facebook had failed to provide its users the option to terminate these licenses when they decided to delete their accounts from the website as the work would most certainly have been shared by another user (Alm,



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2014). This is just another of the many examples of how users lose control of their work and it's important that we all understand the implication of it. Another experimental study revealed that when users were asked to read terms of use of a fictional website, they read it for merely 51 seconds and failed to take note of clauses which required them to share their data with the National Security Agency and provide their first-born child as payment for access (Patton, 2019). Although extreme, this study revealed that Terms of Use can have serious consequences if they aren't read and understood properly (Patton, 2019). Terms of Service even binds users to arbitration and include forum selection clauses when it comes to resolution of legal disputes, depriving them of their own choice of legal recourse.

It is worth noting that when the entire premise of social media websites is based on a sharing culture and on the idea that a user can make their work accessible to the entire world at the click of a button, rights like copyright are bound to take a backseat. Friends and even strangers will share artistic pictures that they liked or a song they resonated with or a poetry they really appreciated. And most creative users will not have an issue with it as it increases the reach of their work and their audience, giving them traction. For most users gaining traction on social media has become more viable than preventing infringement. While this may be fine, it cannot be ignored that there is nothing stopping websites to gain monetarily from the works that the users post without them receiving anything for it. Since websites like Instagram license the works to themselves, their terms allow them to give access of their users' works to third parties. In fact, companies like Olapic and Candid use these works provided by Instagram to sell it to their consumer for the purpose of advertising or other promotional campaigns (Patton, 2019). These websites consider that the companies have 'earned' this content from their consumers and thus can use it to further market their own products and the authenticity of their brand (Patton, 2019). This is a direct consequence of the overly broad licenses which grant websites a great number of rights with respect to a user's work which can and is being exploited. It can also be argued that due to these automatic licenses, the practice of asking for permission is reducing, adding to the misconception that anything on the internet is free for the public to use. Surely if these practices are so unfair, the law must have something to say about it. In this next section, we try to compare the legal position of USA and India in order to understand where they stand on the whole issue.

COMPARATIVE ANALYSIS**United States of America**

According to the 1976 Copyright Act, a work needs to be "original" and "fixed" in order for it to be copyrightable in the USA (*Copyright Act, 1976 s. 102*). "Original" is further described as a work that is of an "independent creation" which exhibits a "modicum of creativity" (*Feist Publ'ns v. Rural Tel. Serv. Co., 499 U.S. 340, 345 (1991)*). Applying this to the world of social media we find that user-generated content fulfills this criterion and if a user decides to put up a picture clicked by them or a video made by them to their social media account be it as a display picture on Facebook or as an Instagram post, the users will be deemed as copyright owners who would have automatically licensed some of their rights to these websites. However, it is true that social media goes beyond pictures and videos and it includes 'tweets', status updates, comments etc. Applying the aforementioned criteria to these may result in specific answers especially with respect to whether or not they are 'creative'. The US courts in a lot of cases have already identified that copyright-ability has nothing to do with the length of a written work. Creativity can exist even in the arrangement of words (Alm, 2014). Furthermore, the courts have also elaborately laid down what 'tangible' entails and has clarified that a work needs to be "perceived, reproduced, or otherwise communicated for a period of more than transitory duration" (*Copyright Act, 1976 s. 101*). This is thus, again a fact specific question. We know that anything put up on the social media stays there for a considerable amount of time (Alm, 2014). Therefore, as far as copyright-ability is concerned, based on the facts of a case, US courts would find works on social media copyrightable. This means that a social media user would thus originally be enabled to all the 6 statutory rights.

Digital Millennium Copyright Act, 1998

The USA passed a federal copyright statute called the Digital Millennium Copyright Act in 1998 in order to update the domestic copyright law so that it was more in tune with the digital age (*Universal City Studios, Inc. v Corley, 273 F.3d 429, 440 (2d Cir.2001)*). The Act was put in place so as to make it possible for the owners of copyright to prevent



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infringement of their work without dragging the court in the issue (Zimmerman, 2011). Within this statute we find the “Online Copyright Infringement Liability Limitation Act” (OCILLA) which was specifically designed so as to provide Online Service Providers (OSPs) who are ‘a provider of online services or network access, or the operator of facilities...’, protection from liability in terms of copyright infringement that may arise out of the infringing conduct of their users (*Copyright Act, 1976* s. 512(i)(1)(A)). The statute mentions that an OSP is required to fulfill a certain criterion for it to be declared as one. To receive the protection of the Act, the OSP must then (*Copyright Act, 1976* s. 512(i)(1-2)(2015)):

- a. Implement a policy that provides a procedure to terminate repeat infringing users
- b. Itself not “interfere with standard technical measures” that are used to protect copyright holders.

The statute also contains ‘a red flag’ provision whereby the ISP must act on its own if it has actual knowledge of the presence of infringing content or is aware of the facts and circumstances from which infringing activity is apparent (*Copyright Act, 1976* s. 512(c)(1)(A)(ii)). Thus, essentially under the Act, OSP needs to be provided with some signature/authorization of the copyright holder, identification of the copyrighted work, contact information of the copyright holder, and a statement demonstrating good faith that their work is being used in an unauthorized manner. Thus, upon the receipt of a proper notice, the ISP has to act promptly and take the same down. This process however is extremely expensive for both sides (Zimmerman, 2011). Google alone receives 6 million take down notices per week where many of them are filed by businesses against their competitors with invalid claims (Zimmerman, 2011). If the OSP takes proper action after receiving the information, only then they will fall under the safe harbor provision and will not be held liable (*Copyright Act, 1976* s. 512(c)(1)(C)(2015)).

However, this basically means that despite infringing activity being allowed to take place on their website, there is a way for the website proprietors to be absolved of all liability if they are able to prove that their conduct attracts the safe harbor provisions. While this is definitely effective if other users have infringed your work, it fails to be useful when the social media website itself is the infringer. This also means that if a third party decided to copy a user’s image from Instagram and shared it on a platform other than Instagram, Instagram shall not be held liable in any way whatsoever. This is exactly what happened when Shereen Way posted a picture of her daughter wearing a ‘Crocs’ product. As discussed above, Crocs is Olapic’s customer which was able to have access to this image because Instagram had licensed it to them (Patton, 2019). This is again where the terms of use come into the picture to absolve the proprietors of all liability (Patton, 2019).

The reason why this point is being stressed upon time and again is because between the copyright holders/users and the website proprietors, it will always be the proprietors who would be in a better position to be able to prevent this infringement. This is evident as most of the websites follow a certain algorithm which shows content most relevant to that particular user or that which is more popular (Curtis, 2016). However, the courts have always been reluctant to place the burden on website proprietors (Zimmerman, 2011). This is evident in the fact that the general awareness by the proprietors of infringing content on their website is not considered enough to meet the red flag test (*Viacom Int’l Inc. v YouTube Inc* 940 F Supp 2d 110 (SDNY) (2013)). Another obstacle in the statute is that it does not require the service providers to affirmatively monitor the infringement (*Copyright Act, 1976* s. 512(m)(1-2)(2015)). Nothing in the Act requires any additional safeguards to be put in place by these websites which only allows and encourages these platforms to put broad and ambiguous terms of use in place (Curtis, 2016).

Doctrine of FAIR USE

Under the US Copyright statute, people can be protected when they make use of copyrighted work on the basis of four factors (*Copyright Act, 1976* s. 107 (2015)):

1. The purpose and character of the use
2. The nature of copyrighted work



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3. The amount and substantiality of the portion used in relation to the copyrighted work as a whole
4. The effect of the use upon the potential market for or value of the copyrighted work

It is a fact that the more a work is disseminated and made available, the less will be its market value (Curtis, 2016). This is the downside of the Internet and as more and more artists, especially photographers, have gone digital to showcase or sell their works, the more infringement they have had to face (Curtis, 2016). A lot of people have made money off of 'user-found' content. Some respite may be provided to such people through the fair use provision if the work that is taken is 'transformative' enough, when something new is added "with a different purpose or different character, altering the first (use) with new expression, meaning or message..." (*Campbell v Acuff-Rose Music, Inc.*, 510 US 569, 579 (1994)). This however, is unlikely to save most users who tend to either copy and paste the work to their own profiles or 'share' it just as it is. This essentially means that the users of social media are opening themselves up to more and more liability as they sign on to these websites which are based on a sharing model and provide open access to copyrighted works of other users while the website proprietors themselves are absolved of all liability by law.

With respect to the amount and substantiality factor we find that the courts look at the question of whether the quantity of the material used was reasonable when we talk about the purpose for which it was used with respect to copying (*Campbell v Acuff-Rose Music, Inc.*, 510 US 569, 579 (1994)). In the case of *North Jersey Media Group Inc. v. Pirro* 74 F Supp 3d 605, 609 (SDNY 2015) the court considered the fact of a news agency juxtaposing a picture taken by the defendant of 3 firefighters raising an American flag near the ruins of the World Trade Center. The picture was juxtaposed with a similar photo of first responders raising a flag on Iwo Jima and the news agency posted the same on their Facebook page. The court was neutral as they felt that it was 'unclear' whether any less use would have had an effect on the recognition of the photograph by audience or not. The defendant was denied a summary judgment. This is a problematic judgment for copyright holders. The photographer in this case had a history of licensing their image (Curtis, 2016). It reinforces the fact that just because a picture is available on the internet, those like news media agencies can pick it up and use it without permission and with minimal transformation and no regard for copyright.

It is the last factor regarding potential market harm that is the most important when it comes to fair use (*Harper & Row, Publishers, Inc. v Nation Enterprises, Inc.*, 471 U.S. 539, 566 (1985)). Thankfully, applying this to the *North Jersey Media Group Inc.* judgment discussed above the court said that the use by Fox of this image without paying license fee to the photographer would set an extremely dangerous precedent in the future. Taking this further, it can be argued that whilst certain users may say that just sharing an image on the social media might seem harmless, what it does is not only reduce the value of the image by making it widely available but also blurs the identity of the real owner (Curtis, 2016). Thus, the users will then resort to infringing conduct comparatively more (Curtis, 2016).

VISUAL ARTISTS RIGHTS ACT, 1990

The personality theory has given a lot of weight to a particular kind of rights called "moral rights" (Fisher, 2001). While this theory has mostly been popular in the Europe, it has very recently caught the interest of American lawmakers and led to the creation of the Visual Artists Rights Act after joining the Berne Convention for the Protection of Literary and Artistic Works in 1988 (Fisher, 2001). Moral rights are based on the connection that is established between the artist as well as their creations, protecting the personal and reputational rights of the artist themselves (Curtis, 2016). Creations are considered an extension of the personality of the creator himself/herself. It aims to protect "works of visual arts" which would include sculptures, paintings, still photographs etc. produced for exhibition however limited to single copies of photographs or a limited number of 200 or fewer photos which are numbered and signed (*Copyright Act, 1976 s. 101*). These rights cannot be transferred but can be waived off (*Copyright Act, 1976 s. 101*).



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The Act was passed before the social media age and thus the Congress could never predict the impact digital photographs were to have and this is why protection was only extended to those which were going to be displayed at exhibitions (Curtis, 2016). The need now is definitely to expand the law to encompass the idea that the definition of exhibition has changed as well as the fact that with the internet, it does not make sense to provide protection to only a limited number of copies (Curtis, 2016). Systems need to be put in place whereby the social networking sites incorporate attribution of the creator along with the images either via linking or watermarking or by preventing reposting until image of the source is provided (Curtis, 2016). Moral rights are extremely valuable and are particularly important in the social media age which greatly focusses on aspects like popularity through number of likes, subscribers or follower count. Narrowing the power of these rights to protect only a particular type of work and a limited number of copies is to hinder its power to provide better protection. A social media user today would probably not have a problem with more and more people sharing his/her work but would have a problem if that work is not credited to them or is mutilated as this directly affects their reputation.

This is reflected in *Agence France Press v Morel* 934 F. Supp 2d 547 (SDNY 2013) which is one of the first cases which determined how users' photos can be protected from commercial use by third parties. The facts of the case were that the defendant, a professional photojournalist, took pictures of a 2010 earthquake which took place in Haiti and posted them to the website Twitter. The plaintiff re-posted those pictures and asked the defendant whether they had any more? Before he could reply, the pictures had already been posted on the online photo database and licensed to Getty images, an image licensing company from whom it was licensed to a number of other parties (Patton, 2019). The credits were given to other people which did not include Morel. The judgment laid down that although Morel did give away his rights while posting on twitter, it did not give the plaintiff the right to sell it off to a third party. On the negative side, this meant that such an action done by the website would have not been considered infringing owing to their terms of use (Patton, 2019). Even though the judgment may have its downsides, it did open up the debate about the implications of social media on copyright law.

It can be said that the US has tried two approaches to protect its copyright holders in the digital age a.k.a. the content providers (Zimmerman, 2011):

They have tried to pass more domestic laws which have unfortunately brought in strict enforcement tools. These included a statute through which ISPs were made to cut off access to sites that harbored content that was infringing primarily to stop online piracy and online threats to economic creativity and intellectual property via the Stop Online Piracy Act and Preventing Real Online Threats to Economic Creativity and Theft of Intellectual Property Act (Zimmerman, 2011). After a lot of backlash, the same was dropped.

They have tried to negotiate trade agreements which once they are ratified could enable the Congress to bring about statutory changes so that laws can be made more universal in nature. Efforts have been made to promote Digital Millennium Copyright Act, 1998 (DMCA) at an international level, but without much success. An effort at the Trans-Pacific Partnership Agreement failed after several countries failed to implement it. The Anti-Counterfeiting Trade Agreement (ACTA) has failed to be of much success as well despite going a step further and providing enforcement measures which included "expeditious remedies to prevent infringement and remedies which constitute a deterrent to further infringement" along with criminal sanction for infringement at a "commercial scale" along with the creation of a committee with representatives from all signatories to oversee enforcement (Zimmerman, 2011). ACTA failed as the European Parliament refused to ratify it. It is, thus, suggested that private agreements may be the best interim way to tackle the problem of a digital copyright reform owing to the jurisdictional issues due to the universal nature of the internet as well as factors like the cost of litigation (Zimmerman, 2011). Some of these efforts are:

Copyright Alert System: This protocol was adopted for infringement which took place through peer-to-peer networks for which the ISP may be serving as a medium rather than as a host (Zimmerman, 2011). This system was born at a time when copyright infringement had become a major problem in the US. It was realized that individual



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lawsuits were no more proving to be useful in battling the issue of infringement. The Copyright Alert System was developed by those involved in the content creation business which involved Universal Music Group and EMI Music, Walt Disney Studios and Paramount Pictures, the Independent Film and Television Alliance, the American Association of Independent Music along with US Internet Service Providers which includes Verizon, Comcast, Time Warner Cable, AT&T and Cable Vision (Ward-Bailey, 2015). This was an entirely voluntary system and under the guidance of the governor of New York, Andrew Cuomo, which grew as a system for alerting subscribers of copyright infringement. This consortium eventually led to the creation of Center for Copyright Information in 2011.

Content ID: This is a digital fingerprinting system developed by Google to filter out content on the basis of copyrighted work and limit it from being played or viewed by flagging it or keeping it from loading in some way (Zimmerman, 2011). Essentially this is done by creating digital fingerprints of the original work so that if somebody else tries to create a file, then the fingerprint database would detect to see if there are any matches between the two (Zimmerman, 2011). Post this, the content owner decides what action is to be taken which may involve demonetizing it or directing the money to themselves, muting the copyrighted part or getting it taken down, etc. (Zimmerman, 2011). This system is most used by YouTube. Although very useful, automated systems come with their own set of problems. It tends to overlook the law, especially fair use (Zimmerman, 2011). This was evident in the absurd pursuit of Liberation Music which sought to take down a lecture by Larry Lessig dealing with 'fair use in copyright' itself because a particular song was used by the professor in the video to demonstrate fair use. This incident indicated how a lot of content creators are being suppressed by copyright holders by disallowing the free and fair use of the content. Similarly, Michelle Obama was served with a takedown notice for her address at the Democratic National Convention for using a music clip, when in actual fact, her team had already obtained a license for the use of that music (Zimmerman, 2011). A lot of online gamers have faced the same fate through developers when they critically review their design of the games (Zimmerman, 2011). YouTube had created an appeals process, however the "judicial body" which looks at them is far from neutral as it includes the alleged copyright holders themselves (Zimmerman, 2011). But because it involves private parties, it escapes constitutional scrutiny as well as due process (Zimmerman, 2011). Essentially this system is serving the interests of the major players that exist in the copyright industry who make claims to either suppress competition or negative reviews (Zimmerman, 2011). While the ultimate goal is to ensure that copyright is protected on the Internet, it cannot be done by taking away the rights of other content creators especially because it has now started having an adverse impact on free speech (Zimmerman, 2011). This system is simply incapable of maintaining the delicate balance between the rights of the public and the copyright holders (Zimmerman, 2011). However, according to the American view- the number of mistakes made by such systems are too small to render them incapable (Zimmerman, 2011). This shows how the US government has decided to take a backseat when it comes to maintaining the delicate balance. Thus, while private agreements can always be used to smoothen out the edges, it must never be considered as the permanent overarching solution.

India

In India, copyright exists in an original literary, dramatic, musical, artistic work and cinematographic films and sound recordings (*Copyright (India) Act, 1957*, s. 13). Unlike the USA, the only criteria required for copyright in the Indian copyright law is the aspect of originality along with the fact that the same needs to be expressed in some form. Creativity is not a criterion in the Indian statute. India has acceded to the WIPO Copyright Treaty as well as the WIPO Performances and Phonograms Treaty. As digitization brought with it problems of piracy, the signatories were required to provide protection via technological measures and rights management information. As a signatory to the TRIPS Agreement as well as a WTO member, India has introduced several relevant provisions under its copyright statute through the Copyright (Amendment) Act, 2012 as well as the Information Technology Act, 2000 (IT Act).

Legislations- Information Technology Act, 2000 and Copyright Act, 1957

The IT Act, 2000 was passed in order to legally recognize transactions which take place through electronic data exchange as well as other means of electronic communication in accordance with the Model Law on Electronic



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Commerce adopted by the United Nations. Internet services providers (ISPs) would come under the purview of the 'intermediary' under the IT Act (*Information Technology (India) Act, 2008 s. 2(1)(w)*). While Section 79 of the IT Act gives these intermediaries a safe harbor against copyright infringement liability much like the American DMCA, Section 81 of the IT Act says that nothing will prevent anybody from exercising their rights provided under the Copyright Act 1957. In accordance with the Intermediary Guidelines Rules, 2011, the intermediary is required to observe due diligence while overseeing that there is no infringement of IPR (*Intermediary Guidelines (India) Rules, 2011 r. 3(d)*). This is reflected in Rule 3(2)(d) of the Intermediary Guidelines Rules, whereby an intermediary has to publish rules and regulations governing the use of the medium by its users and ensure that they are made aware that no such material shall be shared by them which infringes patent, trademark, copyright or other proprietary rights. While we can see an attempt by the law to oversee that fair terms of use are drafted by the websites, they seem premature as the law is silent when it comes to imposing liability on the intermediaries in the event of breach.

Under Rule 75(3) of the Copyright Rules, 2013, the ISP is required to act within 36 hours of receiving the takedown notice which does not necessarily mean that the ISP is required to takedown the relevant content immediately. This only applies when a competent court has issued the order as the hosting company has no right to make this judgment call about whether any post is defamatory or infringing or not. The same was reiterated in the case of *Kent RO Systems Ltd. & Anr. V. Amit Kotak & Ors. CS(COMM) 1655/2016* What the host is required to do is take measures to refrain from facilitating access for a period of 21 days from the date of receipt of the complaint (*Copyright (India) Rules, 2013 r. 75(3)*). This is problematic primarily because content moves around at lightning speed on the internet. Things become viral and start trending within a few minutes making the content accessible all over the globe. In such a case, if the court order is not expedited, the damage may have already been done making the takedown of the content a redundant activity.

Under the Indian law, liability is attracted when there is an 'intention of infringing rights' (Ashok, 2012). Along with that we find that where it can be established that the intermediary had 'knowledge' then even though they may not have 'desired' a particular situation, they can still be held liable on the basis of this knowledge that the outcome might have been a consequence of that particular action (Ashok, 2012). Comparing the law with that of DMCA, we find that no direct link exists between circumvention and infringement in USA (Ashok, 2012). Thus, the remedy is granted even when there has been no copyright infringement as proving circumvention is enough. In the Indian law we find that circumvention has to be connected with infringement of copyright so that it can be ensured that the same is not being used to counter market competition (Ashok, 2012).

We see these reflections in various cases as well. These include *Myspace Inc. v. Super Cassettes Industries Ltd* 2016 SCC OnLine Del 6382 wherein it was held that upon receiving "actual knowledge" or obtaining it from the affected person in writing or through email, the intermediary has to act within 36 hours or more and disable such infringing. If they do not take down the content, then the safe harbor is not provided. That being said, the copyright owners have to specify the details and the locations of the works and cannot be vague and ambiguous about it. In the case of *Eros International Media Limited & Anr. V Bharat Sanchar Nigam Limited and Others* 2016 SCC OnLine Bom 10459 the courts protected the rights of the movie from being uploaded online by directing the ISPs from blocking access to the 134 URLs/links which showed this infringing content. In fact, the court even gave the Plaintiff full police assistance if they so required along with the fact that they will not have to move to the court again if they face a similar conduct in the future to immediately take action. A ray of hope is found for the copyright holders in India with respect to moral rights. According to Section 57 of the Indian Copyright Act, 1957, an author retains their right to 'restrain or claim damages in respect of any distortion, mutilation, modification....' if the same is harming their reputation or honor. Unlike the laws in the USA, we find that Indian laws keep the power of moral rights alive and in case either the website proprietor themselves or other social media users commit any of the abovementioned acts, the author may rely upon this section for protection.



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The Indian Copyright Act is well equipped to incorporate content posted on social media within the meaning of copyright but inadequate when it comes to protecting it. Unlike the USA, we find no individual statute dedicated to the protection of content online. That being said, the provisions within the Information Technology Act, 2000 as well as the Copyright Act, 1957 do help mitigate the situation to an extent by taking into account the occasion where an intermediary themselves have conspired to infringe or with respect to protecting moral rights. However, they are moot when it comes to dealing with their arbitrary 'terms of use'. The involvement of court orders creates unnecessary delay which is fatal when it comes to the Internet. The provisions are also not strict enough and work more in favour of absolving the intermediary in most occasions much like the laws in the US. In such a case, it is the copyright holder who is at a loss of an adequate remedy.

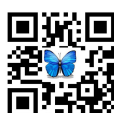
CONCLUSION AND SUGGESTIONS

When there was a rise in peer-to-peer file sharing technology in consequence with the advent of the digital age, the USA took various steps to curb it. However, a study in Canada had shown that this technology did not have an adverse impact on the sale of music (Zimmerman, 2011). In fact, it did the opposite. Banking on this we saw the rise of companies like Spotify, Apple Music etc. which creatively synthesized this system with the law and revolutionized digital music wherein music was provided for free but with advertisements. This system was able to get the balance right. It has thus become very important to note that going back to the pre-Internet world is not an option anymore and the copyright industry needs to understand that.

Since majority of the problems exists within the terms of use themselves, the website proprietors must be coaxed to try to resolve them at their level, at least till the law catches up. The fact that the website proprietors can sub-license the work creates a lot of issues and is clearly present to benefit the website makers (Patton, 2019). Technological measures are ill-equipped to deal with that. A simple solution for the same would be a plea to remove the sub-licensable aspect altogether (Patton, 2019). Along with that, there is a need to rewrite the terms of services so that a layman may be able to understand them (Patton, 2019). This will also enable the website to regain the trust of their consumers making social media a safer and more cordial place that can actually stimulate creativity rather than hinder it. Another initiative by the social media websites themselves can be to email and explain any change in terms of services as and when they take place and users need to lobby for the same (Patton, 2019). This is possible and has happened when Facebook decided to change their terms which now gave them the right to retain the license in the content of its users even after their accounts had been deleted. Only after much public outcry and backlash from all its users the website was compelled to issue a statement saying that the user will be allowed to end their license at any time by deleting their account (Patton, 2019).

In fact, Instagram did something similar when in 2012 they changed their terms of services to allow it to sell images uploaded by its users which would be used for interesting paid or sponsored content and promotions without compensating the user. After a lot of backlash, Instagram decided to drop this extremely controversial change. Simply making the terms of use simpler to read and thus to understand, avoiding legal jargon or finding a way to incorporate legal language as well as explaining what has been written in simpler terms and emailing changes or updates made to them to the users can go a long way in mitigating the problems if not solving them. A change that the website proprietors can make to ensure more users read these terms are to display them before a user makes their account on the website instead of placing them at the bottom of the screen which can easily go unnoticed by users. Digital watermarks come to the rescue of photographers and their images which allows an invisible layer to be placed on an image containing metadata which cannot be removed and can be read by a software (Patton, 2019). This will even enable tracking down the real owner of the image (Patton, 2019).

Even users can take steps from their end by taking efforts to read and understand the terms of use on the websites they are releasing their content on. It will give them an option to make an informed choice about joining the platform in question. Those who wish to ensure that their works are protected should consider filing for copyright



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registrations and put a copyright notice while uploading their work (Patton, 2019). Some people like to see their work being shared by others which can be classified as another consequence of the digital age. In that case, a creative commons license comes to the rescue. Creative Commons is a non-profit organization which collects works which are copyrighted from the creators themselves and licenses them to the public legally (Patton, 2019). It allows the creators to “retain their copyright while allowing others to copy and distribute” their work for both commercial and non-commercial purposes. The license allows the creator to keep the copyright along with other benefits like ensuring that the licensee credits the licensor and puts up the copyright notice on all copies and links etc. This initiative maintains the balance as both the public as well as the creator are able to reap the requisite benefits in a legal fashion. In addition to that, the creator is able to retain more control over their work comparatively. The websites must be persuaded to facilitate this system on their platforms.

The European Parliament in order to bring about an updated copyright law for the internet age passed a new directive on April 15, 2019 which would require the OSP to play a more hands on role to ensure there is no copyright infringement by bringing about an “upload filter” that will scan the data before it is uploaded on this site, preventing others to upload it. While it has received a lot of backlash in the light of it being another form of censorship, it definitely shifts the entire dynamic by shifting the responsibility to the shoulders of the social media websites. These are changes which do not require a bill to be drafted, debated and then passed in the Parliament. The process only involves a small effort from the companies’ and users’ end themselves to ensure that correct information is being transmitted to the users especially in relation to their work and what will happen to it once they join the platform.

It is safe to assume that a re-furbishing of copyright law and social media is the need of the hour. It has become fundamental to synergise the two so that neither leads to the disruption of the other. An increase in the spread and impact of social media is inevitable. In its current state it is also inevitable that more and more users will end up unwillingly licensing their rights away as a result of broad and ambiguous terms of use. Through this paper we have attempted to outline how this has taken away control from the hands of the original creator to the website owners and eventually to third party users. The paper has attempted to decode these terms particularly because this is where ‘the myth that anything on social media is considered to be in the public domain’ stems from. To solve the same, it needs to be nipped in the bud. And in order to do that, it is primarily important to comprehend it and acknowledge why it is a problem in the first place. Non-profit organizations and private players have been able to significantly achieve this target to some extent. Organizations like the Creative Commons have especially been able to get closer to synthesizing the model of social media and the purpose of copyright law, making the internet a safer and a more creative space. It is need of the hour that the legislature of the different nation states follows along and together with these entities try and create a space better equipped to harmonize the current digital and legal sphere.

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Policies to Handle Retail Disruptions Internationally due to COVID-19

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ABSTRACT

With changing economic conditions there is a histrionic change in the retail sector. In 2019 the economy slowed down with real GDP growth declining from 3.1% to 1.9%, where investment growth has diluted and personal consumption growth has been constant. Further disruptions were made by the Covid-19 pandemic situations which made a breakdown on retail sector. Considering the situation, the retail scenario-its trends, growth, impact of Covid-19, suggested measures and future prospects globally. Through this paper the researcher has made a small attempt on the basis of a secondary data to evaluate the covid crisis in the retail sector. The scope of the report on the global retail industry provides an extensive assessment of the market, with an investigation of market segments. The report also targets the in-depth movements in import/export, production, and consumption data of the product, cost, and manufacturing processes.

Keywords: global retail scenario, impact of Covid-19, policy measures, future prospects.

INTRODUCTION

An overview of the market

If we look globally the retail market is full-blown and extremely competitive in the developed countries like Europe and North America. Whereas, the developing countries like the Middle East, Latin America, and Asia-Pacific have played a small contributory role in the impellent growth of the market. Other countries of the world such as Thailand, Singapore and Malaysia remain crowd-pleasing for shopping with tourists visiting the Asia-Pacific region contributing by and large to the retail sectors in the respective markets. There was a hyping demand for products related to fashion, apparel, and electronics due to visitors.





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IMPACT OF COVID ON RETAIL SECTOR GLOBALLY

Over the past few months due to coronavirus (COVID-19) pandemic situations, the demand, supply, and daily operations in the retail sector have decreased to a great extent. There is a slower upcome of this sector even after the slow-down of the effect of the coronavirus situation. This effect on the retail sector has a huge overall impact on the economy at a large. Mentioned below are a few factors. The retail sector is an economic major league: if we take an average across the globe, about 1 in 10 employees are employed in retail, and the retail sector amounts to almost 5% of GDP. To add to it the retail sector gives a lot of emphasis on labour which leads to unreasonable employment, which weakens social growth and thus creating a crisis in the sector. It occupies an important position in value chains both as a provider to households and as an outlet for upstream sectors as it mainly serves final demand. To add to it another area of retail i.e., tourism has also hit the activities.

The retail sector has witnessed more than $\frac{2}{3}$ rd of the GDP worldwide due to aggressive consumer spending, which indicates the good health of this sector. To add to it there is an increase in the belief in online shopping even during the crisis of covid. Even though there is a big challenge to retail growth due to variations in the pricing of online and offline shops. Setback in financial markets globally as an impact of the coronavirus pandemic and further the lockdown can be very well seen. Even though there is no clear picture of the root impact and damage it has made in businesses and industrial sectors all over the world. Many analysts and industry body have made their assessments on the impact of covid on the retail sector. Some major concerns on which the world has to pay attention are further highlighted.

Earnings as a significant loss

Coronavirus levied a serious impact on people, business and thus the economy of countries. To reduce this impact as responsible employers, the retail players are considering needed preventive measures and adopting different policies to assure the safety of their employees and customers. A similar touch effect we could feel at the time of demonetisation but not as much as this covid crisis. It has significantly hit the consumer income by pay cuts of employees and daily wagers. Consumer demand as such was slowing in 2019 which further has decreased, further reducing the growth.

Change in demand for essential goods over non-essential retail goods

Survey checks upon a drastic drop in sales of the retail sectors, more than 50% affecting severely the companies during the lockdown. Whereas the daily basic purchases of staples are comparatively less affected and will even recover, but the impact on non-staples will be continuing beyond the lockdown. This resulted in the temporary closure of non-essential goods. Staple items or essential items include the categories as grocery, food items, medical items, electronics, etc, whereas non-essential items include home furnishings, appliances, gifts, luggage, traveling, luxury goods, etc. This can be viewed in the following graph.

Temporary Closure of Non-essential Stores

The first lockdown laid by the government forced the non-essential retail stores to completely shut down, though temporarily just to avoid person-to-person contact. It was done for the safety measures of retailers, customers, and employees but affected the retail business a lot where still the survival is not sufficed.

Unemployment in retail reached a new height: Due to covid, the lockdown of retail stores has hit the businesses and employees in most of the part of the world causing recession. Entrepreneurs had to file for unemployment as no choice left for their income due to the closure of their shops. In 2020, there were many cases of bankruptcy in the retail sector as compared to any other year. On the other hand, retailers of essential goods were open with their services: The retail sector consisting of essentials like medicines, household necessities, food items, etc., were open for the customers throughout the pandemic. Despite the fact that they were to keep stores open, a lot of care was taken to protect the employees as well as their customers.



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To overcome the above problems and reduce the drastic impact of the covid situation on the retail sector a five policy measures have been suggested. Suggested are the five policy measures for the safeguard of retailers, employees, and customers that countries need to follow to protect themselves from the crisis and strengthen the retail sector.

Policy measures are recommended for the survival and growth of the retail sector to overcome the shocks of low productivity demand and supply

The applied policy measures if adopted can be useful for all sectors throughout the globe but in particular for the resilience of the retail sector.

First and foremost, the retail sector should be made available with quick and ready liquidity availability to sustain. Here the role of government is very important as proper assistance can be provided to retailers so as to keep them resilient. The government has already started working on it by offering huge loans, even exceeding the necessary limits in an urgent situation. This should be continued with new guidelines and new variations to keep the retail market upfront. Such support should be unbiased to all the retail firms barring their size and area. But with such provisions, they should take care of providing liquidity support for sustaining only to a particular limit of emergency. Beyond that, it will be harmful as the prices will increase and harm the consumers. With that a record of providing this measure to the needed business only.

The second measure is about the labour supply to the retail sector. Covid has created a huge loss of employees creating a shortage of labourers. Not only the non-essential retail sector but also the essential goods retail sector is suffering from this crisis. Essential retail firms are facing a problem of an increase in demand for goods and decreasing labour supply. Uneven of demand and supply may further create a problem. For eg: a report represents that in UK, in a particular week there was an increase in essential goods demand by 100%, and in the very same week it reports to have 20% and above during the lockdown period.

For this problem, there is a four-point policy measure suggested to support the retail sector.

- 1) financial assistance should be increased for retail employees
- 2) continuous matching of demand and supply for retail jobs
- 3) training is given to employees at retail stores to maintain health and safety measures
- 4) liberalising retail regulations in the labour market for the time being

Thirdly there should be proper provision and guidelines by the government to the retail sector for social distancing to be maintained. Time and again government support should be available to implement measures of social distancing. It may include flexible hours of opening and closing of retail stores. For eg many countries even in India, retail stores with essential commodities were allowed to keep the stores open even from 1 am to 5 am as per flexible hours. This should be considered under the 24/7 opening of stores to maintain the social distancing rule.

Such a government rule can help the retailer to come up from the revenue shock of lockdown.

The fourth policy measure is recommended for a close check on the competition. Competition among the retailers should persist sufficiently to overcome the crisis. This policy will give a lot of weight on the shoulders of the government, as the retail sector has a competitive difference between offline and online stores sales, which as a big drift between the two. Further, there should be a policy wherein a world of crisis the competitors should try to help each there to face the same. With the same beholding the competition it should take care that the customers should not have a negative impact.

Lastly, the fifth policy is the assurance to the retail sector that in the long run they will be benefited from the various efforts that they are making. The efforts should be regularly increased by the retail sector with each other's support to increase its resilience to shocks. The small retail stores should also adopt multiple channel distribution of their goods to bear the jerk. With the support of the government can make cartels of such stores and reduce the cost. They



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can also make an entry in online sales to be prominent with the new trends of retailing. Over and above financial support the government can also help the retailers to reduce the regulatory barriers for their exports. A mutual understanding between a group of countries and a new relaxed framed policy can bring the sector. Some regulatory hindrances and barriers are to be loosened for the retailers to float (e.g. permitting and zoning rules). Another area of concern is the supply of food and agricultural commodities, where the government can open the route channels of various supplies of commodities so that the supply chain is uninterrupted. The above-mentioned policy measures are suggested to be focused on assistance to retail firms. To support the above measure's government can take the initiative to announce a tax-free bonus so that firms can by providing bonus motivate their employees.

This will lead the retail firms to hire new recruits to meet consumer demand. Different countries government can join hands and can reduce the transaction costs for the free flow of goods from one to another country. This will help in reducing prices of essential goods, availability and increase in sales of retail, which help the sector for a comeback. If these policies are carefully undertaken by the government of different countries then there is a sure gateway open to new horizons of the retail sector. To be very precise there will be a total change in the scenario of world retail sales form the past few years as compared to the coming year.

TOTAL RETAIL SALES WORLD WIDE FROM 2018 TO 2022

Further to add to the prevailing situation a small secondary research as an attempt has been made to analyse the post covid situation of retail sector. For this analysis a random market sizing of five major countries and their currencies have been undertaken:

1. USD (UNITED STATES DOLLARS)- USA
2. EURO (EURO) – (ITALY, BELGIUM)
3. GBP (GERMAN POND) - GERMANY
4. JPY (JAPENESE YEN) - JAPAN
5. AUD (AUSTRALIAN DOLLAR) – AUSTRALIA

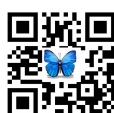
The above market statistics report provides a sizeable market in near future and a better forecast of retail sectors. This will help retail industry leaders to make sustainable decision.

CONCLUSION

Presently every thing is unclear but then too the situation should not be left on the mercy of God. Every stone should be unturned till the success comes. Covid 19 has shown us the ground realities that no matter how big your business is, it can anytime have a break down. So, to meet such consequences one should always be grounded. Planning needs to be altered for today and an unpredictable tomorrow. Lot of confusions and unhappening's showed us blind fold towards the future. The retail sectors could not track any schedule or any transactions, with confusion in maintain records of tasks, its progress and no transparency. This delayed in resolving different issues at the retail sector and the list continues. The journey of retail sector even though with lot of difficulties and hurdles has a satisfactory report. Which proves that global retail sector can prove themselves and do much better even with the un imaginable situation of Covid-19 pandemic.

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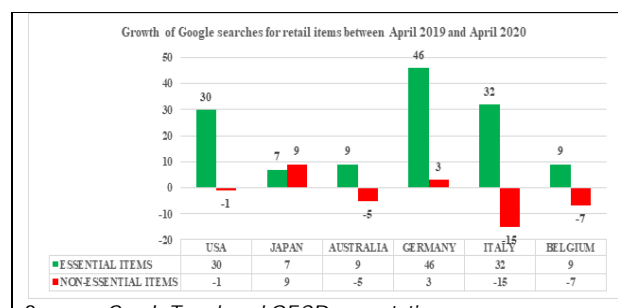


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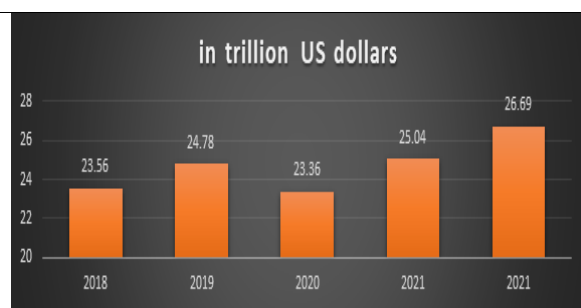
Table 1. Forecast of retail sector across five major countries and currencies between 2020-25

NAME OF COUNTRIES/CURRENCIES	2020	2025
US Dollars	4,545.28	10,064.45
EURO (ITALY, BELGIUM etc)	3,985.39	8,824.70
GERMANY POUND	3,543.02	7,845.18
JAPAN YEN	4,85,097.20	10,74,132.08
AUSTRALLIAN DOLLARS	6,600.35	14,614.92



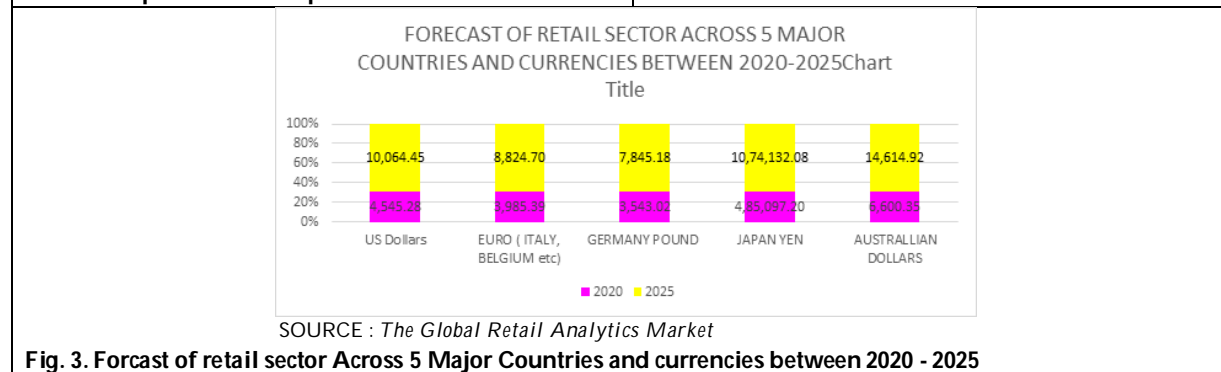
Sources: Google Trends and OECD computations.

Fig.1. Growth of Google searches for retail item between April 2019 and April 2020.



SOURCE: Statista 2021

Fig.2. Total Retail Sales World Wide From 2018 To 2021



SOURCE : The Global Retail Analytics Market

Fig. 3. Forecast of retail sector Across 5 Major Countries and currencies between 2020 - 2025





RESEARCH ARTICLE

A Study of Working Capital Management and Profitability of Two and Three Wheeler Listed Automobile Manufacturing Industry in India

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ABSTRACT

This research study aims to examine working capital management practice carried out by five two and three wheeler automobile industry listed on BSE/NSE. The study tries to undertake the data evaluation of selected variables for the tenure of ten years i.e. 2010-2019. The aim of this study is get to know association between dependent and independent component. Dependent Variable is a return on equity and Independent Variables is a Fixed Assets Turnover Ratio, Long Term Debt Equity Ratio and Current Ratio. Matrix of Co-efficient of Correlations, Descriptive and Regression has been used for analysis. The result shows that there is positive association between Fixed Assets Turnover Ratio, and Current Ratio and return on equity. The result shows that only one variable has negatively significant relationship between long term debt equity ratio and return on equity and rest of the two variables has positively insignificant relation among Fixed Assets Turnover Ratio, current assets and current ratio.

Key words: working capital management, automobile companies, profitability

INTRODUCTION

Business administrator must comprehend and permit his period to every single daytime internal operation, relating to current assets and current liabilities of the enterprises. Financial administrators have to take care to get the

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supreme income because its vast percentage of their valued time is devoted to working capital complications. One of the most essential things signifies by investment in current assets i.e. the total investment in asset. Financial supervisor ought to compensation extraordinary attention to the organization of current assets on a consistent base. Working capital management surfaces precise acute situation for all corporations mostly for minor businesses. Usually small companies invest in current and may not much invest in fixed assets. In India small companies faces serious problem of collecting their debts. Working capital management in the role paly of current liabilities in financing current assets is far more important in case of small enterprises. As Dissimilar for huge firms, they aspect difficulties in aggregate long term assets. There is straight relationship amongst a corporation's development and its necessity of working capital management. Prerequisite of the firm's for sales progress to finance more in borrowers and stock. The sales raise often then condition develop very wild and standard. The commercial supervisor should be prepared of such necessities and finance them quickly. Systematic development in sales requirements additional investment in fixed assets. The finance executive ought to pay precise courteousness to the stages of current assets and the financing of current assets.

LITRATURE REVIEW

Shikha Bhatia, Aman Srivastava (2016) examined 179 companies over the time period of 13 years viz. 2002 to 2014. They are considered gross operating profit and Tobin's Q as dependent variable and cash conversion cycle accounts receivable days, inventory days and accounts payable days as independent variable and size, sales growth, leverage, fixed financial assets, variability of net operating income and growth in GDP as control variables. Descriptive, regression, correlation analysis, panel data analysis have been used for this study. Result indicates that independent and control have significantly effect on dependant.

Dr. Benjamin Owuor Ombok, Judith Auma Omanga and Grace Ivy A. Odongo (2017) analysed 4 sugar enterprises for the period of ten years i.e. 2005 to 2014. They are considered return on assets as dependent variable and Average collection period (ACP); Inventory turnover in days (ITO); Average payment period (APP) and Cash conversion cycle as independent variables. Regression analysis and penal data methodology have been used in this study. It was notice that all independent elements had a more effect on profitability.

Dr. Johnson Abiodun Oladimeji & Dr. Olufemi Aladejebi (2020) examine 30 SMEs over the time period of 5 years i.e. 2014 to 2018. They are considered return on assets as dependent variable and Account Collection Period, Inventory Conversion Period, Average Payment Period, Cash Conversion Cycle, Debt Ratio, Current Ratio and Quick Ratio as independent variables. They were used descriptive statistics, correlation and regression analysis. It was point out that there is not relevant association among independent variable and dependent variable. Found that there is negative relationship with Account Collection Period, Average Payment Period and dependent variable.

Zbigniew Gołaś (2020) analysed 76 police milk industry for the period of decade with the span of 2008 to 2017. He is considered return on assets as dependent variable and Days Sales of Inventory, Days Sales Outstanding, Days Payable Outstanding and the Cash Conversion Cycle as independent variables. Descriptive, matrix correlation and regression analysis have been used in this study. Results indicate that there is negative association among dependent variable and working capital cycle.

Phadindra Kumar Poudel and Pujan Maharjan (2020) examine the performance of 10 non -financial companies over a period of five years i.e. 2071-72 to 2075-76. The variable measure return on assets as dependent variable and days sales outstanding, days inventory outstanding, cash conversion cycle, current ratio as independent variable. Descriptive, correlation, regression analysis have been used in this study. It was notice that there is significantly positive relationship among current ratio and profitability. Found that there is significantly negative association among Days' sales outstanding and financial performance of the company.





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Dr. Namita Yash (2021) analysed the dabur India Ltd. company for the period of 15 years i.e. 2005-06 to 2019-20. She had taken current assets, current liability, net working capital, inventory turnover ratio, receivable turnover ratio, cash turnover ratio, working capital turnover ratio, gross working capital, total assets, working capital leverage return on net worth ratio and liquidity ratio as variable. She used ratio analysis, rank test, averages, standard deviation, co-efficient variations, and correlation coefficient. It was notice that there is negative relationship with dependent variable and liquidity.

RESEARCH METHODOLOGY

This study has measured only five two and three wheeler automobile industry over a time period of ten years i.e. 2010 to 2019. The evidence was acquired from Capitaline database. The aim of this study is get to know association between dependent and independent component. Dependent Variable is a Return on equity and Independent Variables is a Fixed Assets Turnover Ratio, Long Term Debt Equity Ratio and Current Ratio. Matrix of Co-efficients of Correlations, descriptive and regression has been used for analysis.

OBJECTIVE

The objective of this study is to know and assess the extent of influence and the relationship among dependent and independent variable of the business enterprise in the two and three wheeler automobile industry.

HYPOTHESIS

- H0: Fixed Assets Turnover Ratio has no influence on Return on Equity
- H1: Fixed Assets Turnover Ratio has influence on Return on Equity
- H0: Long Term Debt Equity Ratio has no influence on Return on Equity
- H1: Long Term Debt Equity Ratio has influence on Return on Equity
- H0: Current Ratio has no influence on Return on Equity
- H1: Current Ratio has influence on Return on Equity

INTERPRITATION

1. Table – A obtain the regression coefficient. It is specifying that direction; standardized Beta, significance level, values and VIF are remark in Table – A. Table – A displays that Fixed Assets Turnover Ratio, Long Term Debt Equity Ratio and current ratio as independent variables has association with Return on equity. The β of Fixed Assets Turnover Ratio as presented in Table – A, stands at +0.053 viz. indicate that Fixed Assets Turnover Ratio has positive relationship with Return on equity. The value of significance level is 0.674. It is notice that the significance coefficient beta (β) is statistically insignificance. Therefore null hypothesis (FAR) is accepted and H1 be rejected.
2. Table – A measured the cost of Long Term Debt Equity Ratio i.e. -0.505 it is revel that β has negative relationship with Return on equity. The value of significance level is 0.000 it means β is statistically clearly significant. Thus null hypothesis (LTDER) be accepted and H1 be rejected.
3. The β of Current Ratio as presented in Table – A, stands at +0.103 viz. point out that Current Ratio has positive relationship with Return on equity. The value of significance level is 0.417 It means that the significance coefficient beta (β) is statistically not insignificance. Therefore null hypothesis (CR) be rejected and H1 be accepted.
4. Table – B indicate that the matrix of co-efficient of correlations between independent variables i.e. FAR, LTDER and CR have been considered by karl-person coefficient of correlation. It is precisely displays that all component of co-efficient of correlations' value has not more than +0.1
5. Table – C obtainable the element of descriptive statistics. The outcomes presented this studied will be more suitable to the organisations whose data sets resemble the descriptive method indicate in Table – C.





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FINDINGS

1. It is finding that there is insignificantly positive association among Fixed Assets Turnover Ratio and Return on equity and the significance coefficient beta (β) is statistically not relevant.
2. Further find that Long Term Debt Equity Ratio has negatively association with Return on equity and significant co-efficient beta is statistically significant.
3. The Current Ratio has positive association with profitability and significance coefficient beta is statistically insignificant.

CONCLUSION

The research disclosed relationship between Fixed Assets Turnover Ratio, Long Term Debt Equity Ratio and current ratio and return on equity that there is positive association between Fixed Assets Turnover Ratio, and Current Ratio and return on equity. The result shows that only one variable has negatively significant relationship between long term debt equity ratio and return on equity and rest of the two variables has positively insignificant relation among Fixed Assets Turnover Ratio, current assets and current ratio.

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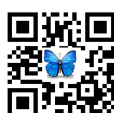
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Table 1. Regression Co-efficients

	Regression Co-efficients		t	Significance Level
	Direction	Value		
Constant		2.449		
FAR	+	0.053	0.423	0.674
LTDER	-	0.505	-4.016	0.000
CR	+	0.103	0.819	0.417

Dependent variable: ROE

Independent variables: FAR, LTDER, CR





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Table 2. Matrix of Co-efficients of Correlations

	FAR	LTDER	CR
FAR	1.000	-0.047	0.068
LTDER	-0.047	1.000	-0.111
CR	0.068	-0.111	1.000

Table 3. Descriptive statistics

Variables→	ROE	FAR	LTDER	CR
Mean	-2.39	5.98	0.51	1.24
Std. Deviation	10.18	15.26	2.90	0.78





Post Covid-19 Recovery of Indian Economy – Textile Industry A Forceful Engine

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ABSTRACT

India, like the whole world, is reeling under the impact of COVID-19 and the unparalleled impression it has left on economies, industries, businesses and individuals. Textiles was the starter sector in the road to industrialization early-on in the world and now this situation will give rise to new opportunities in this sector. India should aim to be the textile capital of the world. We are an aspirational country with majority demography favoring the working age group. Harnessing the potential of this age group and the inherent capacity of India to adapt and bounce back from calamities should be capitalised fully. This will help us to recover from COVID-19 and its impact. This paper is devoted to understanding and recommending actions for bolstering Indian economy through textile sector as an engine. Swift response of policy makers is a must in this scenario where unpredicted changes are the norm. Enhancing image of a country as a fashion destination in global markets will aid a sustainable thriving textiles and apparel industry.

Keywords: Exports, T&A Exports, Policy response, Textile sector.

INTRODUCTION

India, like the whole world, is reeling under the impact of COVID-19 and the unparalleled impression it has left on economies, industries, businesses and individuals. Textiles was the starter sector in the road to industrialization early-on in the world and now this situation will give rise to new opportunities in this sector. The textile and apparel industry will be a key that will spur the global and country economies to the path of recovery and growth. The retail textiles and apparel (T&A) market was 1.9 trillion dollars globally as per a research of Boston Consulting Group (BCG) in 2019 and is projected to reach 3.3 trillion in 2030 with a 3.5% compounded annual growth rate. These projections were done before COVID, but are an indicator of the direction that needs to be follow in future. All



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countries will try to rebuild the economies and textiles industry will be the engine that will drive the economies to recovery. It will be the starter sector again when the world is looking for an out of the box solution to this unprecedented pandemic led economic crisis. Typically developing countries can use T&A industry as a base to increase the economic activities. This industry creates employment opportunities, this plays a significant role because of the special concerns facing the economy during the pandemic. The T&A industry is typically a labour-intensive industry and contributes to the workforce of the manufacturing industry. Any recovery will have to address employment issues and this industry will be the boon for all economies of the world. India has a special advantage here because we are already positioned ourselves strategically well in the global textile trade market over the years.

RESEARCH METHODOLOGY

This paper highlights this advantage India can cash on to overcome tough times the world will face in the days to come. The paper is based on secondary data collected from reports of world associations, Indian government reports and private agency studies that have undertaken extensive research during recent days to unravel aspects of economies and sectors. Statistical tools are used to study understand and recommend. Use of secondary data was a conscious decision taken so that reports which are detailing up-to-date details are used to provide practical and relevant suggestions.

OBJECTIVES

- To study role of textiles sector in global trade
- To establish importance of textiles sector in recoveries of economies
- To establish role of textile sector exports in Indian exports
- To suggest measures for textile sector in India that will spearhead COVID-19 recovery

DATA ANALYSIS

India should aim to be the textile capital of the world. We are an aspirational country with majority demography favoring the working age group. Harnessing the potential of this age group and the inherent capacity of India to adapt and bounce back from calamities should be capitalised fully. This will help us to recover from COVID-19 and its impact. The further parts of this paper are devoted to understanding and recommending actions for bolstering Indian economy. From the above Figure – I, it is seen that there is decline in global trade in all major sectors that support economies. In the first quarter of 2020 there was a sharp fall in all, this gradually has changed in the month of April. This change is skewed and manifests differently for all sectors, showing that all have recovered or not recovered in a different manner. The significant pointers that can be seen in this mapping of sectors are:

Global trade in automobiles sector fell from (-8% to -49%), energy sector fell from (+5% to -39%). Global trade in communication equipment (-6% to -4%), office machinery (-8% to +8%) and textiles and apparel have shown signs of recovery (-11% to -6%).

- This is a clear indication that of all the other industries textiles have already started showing a good trend which can be capitalised by economies.

As can be seen in Figure – II, China is ranked first all other countries second and India is ranked third in share in global exports of fashion goods according to the UNCTAD data released to study the impact of COVID on different sectors of economies. In effect it indicates that India is the second largest export contributor to fashion goods industry. As can be seen from the figure above global textile exports from these countries are the maximum from China and India. Fashion goods industry mainly consists of textiles and apparels.

As shown in the Figure – III above, where the share of exports of total exports is concerned India ranks 4th in the world, after Bangladesh, Pakistan, Cambodia. The first three countries are also not contributing much to the overall



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share in global exports. Fashion goods industry mainly consists of textiles and apparels. The two figures and the deductions from this is clear that India is a major contributor to global fashion goods exports and at the same time the share of T&A exports is 4th in the world for Indian exports.

- The two facts that the data throws to light is India ranks second in the world trade and that contribution of textiles in Indian overall exports is also high. Thus it is a clear indicator that Indian exporters have the pulse of global markets and promoting T&A industry in India will go a long way to boost recovery of economy. The strategy will be having the maximum bang for the buck as Indian textile products have earned a position an image well appreciated in the global markets.

From the two data above Table-I and Figure-IV, it is seen that overall Indian exports are showing an increasing trend in three years. At the same time there is also seen that there is an increase in T&A exports. This is a trend which can be interpreted as a fact that T&A sector is a good and significant contributor to Indian exports. This can be put to further test and analysis by a Correlation test which is seen in Table-II and Table-III.

A correlation of -1.0 shows a perfect negative correlation, while a correlation of 1.0 shows a perfect positive correlation. Correlation coefficient values less than +0.8 or greater than -0.8 are not considered significant. Therefore, the correlation of total exports and T&A exports is showing a significant positive relationship.

- Thus proven that targeting T&A exports will strategically be of great advantage to Indian economy. Handicrafts are a green area, which show a lot of promise though they do not have a significant impact on total exports they are a niche kind of product and so targeting handicrafts and charging levying a premium price for these products especially in developed countries could show positive results on Indian exports.

Recommendations

Even before the pandemic people were thinking of sustainable means of clothing and minimalistic living. Methods of disposal of used clothing was questioned, textile and apparel industry were under pressure to develop environment friendly ways for this. Developing production techniques that would reduce footprints was essential. Fast fashion changes and subsequent disposal of clothes was a concern,

Government level

Create opportunities for new 'smart' textiles that can be used in construction, medical or automobile industries. Blockchain technology has been propelled into the forefront this pandemic. New opportunities in logistics chains can be explored in the policies. Time is ripe to embrace blockchain rather than to resist it. Encourage factories to adopt in a cyber-physical environment should be started to venture into industry 4.0. Ensure a sound cyber-law to protect the employees and factories which use industry 4.0 and modern technology. Form a group which will focus on sales at institutional levels, organise trade fairs and exhibitions that promote handicrafts.

Industry level

Collaborations with peer companies, associations will strengthen sharing of knowledge and technology transfer. Joint projects forged with universities will bring in fresh ground breaking ideas into the sector.

Company level

Use of transformative methods of technology that builds on AI for product designs, machine learning for upscaling production, 3D printing for reducing production time. Extensive utilisation of blockchain technology has already been initiated during COVID which has enabled traceability and transparency in the logistics chain. Factories which work in a cyber-physical environment using Internet of Things (IoT) should be started to venture into industry 4.0. The list of first tier suppliers should be ready so that demands can be supplied immediately without any delay.





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Introduce customised production for high value high return categories. Conduct training programs that will enable skill development to use new technology like IoT, Block chain, cyber-physical machines, real-time monitoring of production.

CONCLUSION

Swift response of policy makers is a must in this scenario where unpredicted changes are the norm. Strategic decisions rather than knee-jerk reactions are needed during this crisis. Restructuring, restarting, improving productivity, creating wealth parities is the future path for countries. These shifts will require tactical changes, broad basing capabilities, strengthening infrastructure. Enhancing image of a country as a fashion destination in global markets will aid a sustainable thriving textiles and apparel industry. It will propel India into post COVID-19 recovery. India's geographical location will support its textile industry as post COVID manufacturers will look for efficient lead times and a marked shift towards near shoring raw material to produce cost effectively, ensuring competitive advantage of producers to manufacture quality products.

Abbreviations used in the paper

TEU - Twenty-foot Equivalent Unit

UNCTAD- United Nations Conference on Trade and Development

T&A - Textile and Apparel

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Table 1. Export performance of India

Values in Million US Dollars	2016-17	2017-18	2018-19	CAGR	2018-19 (Apr-Nov)	2019-20 (Apr-Nov)	% Change
India T & A	35,472	35,723	36,558	1.50%	23,483	21,518	-8.40%
Handicrafts	3,639	3,573	3,804	2.20%	2,419	2,460	1.70%
Total T&A including Handicrafts	39,110	39,296	40,362	1.60%	25,902	23,977	-7.40%
India's overall exports	2,75,852	3,03,376	3,29,536	9.30%	2,17,092	2,11,691	-2%
%Textile and Clothing Exports of overall exports	14%	13%	12%	12%	11%		





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Table 2. Indian Exports

Years	Total Exports (x)	Handicrafts Exports (y)	Textile & Apparel Exports (z)
2016-17	2,75,852	3,639	35,472
2017-18	3,03,376	3,573	35,723
2018-19	3,29,536	3,804	36,558

Source: Extracted by researcher from Annual report: Ministry of Textiles

Table 3. Correlation Analysis

Correlation between
Total Exports and Handicrafts exports (x and y)
0.682731448
NOT SIGNIFICANT RELATIONSHIP
Correlation between
Total exports and T&A exports (x and z)
0.950577899
SIGNIFICANT RELATIONSHIP

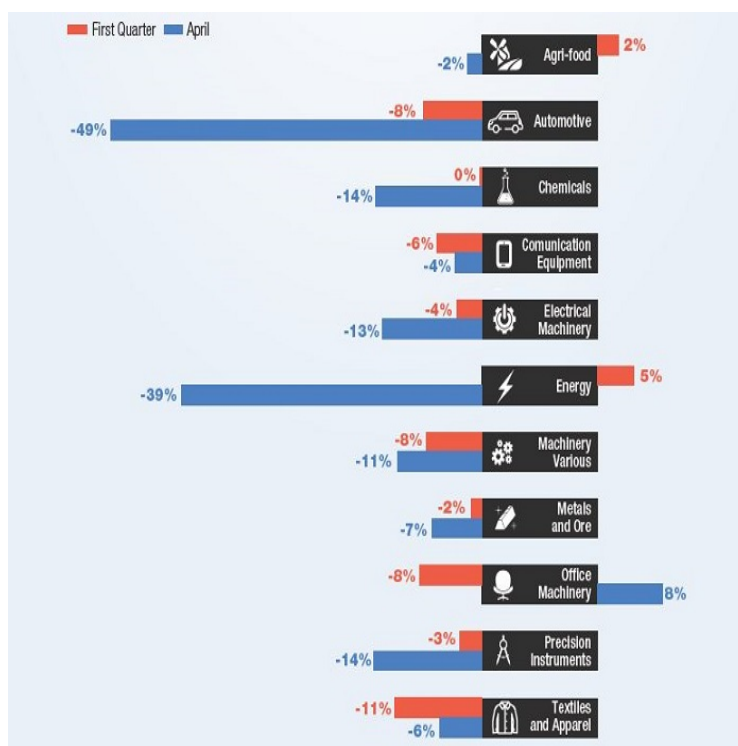
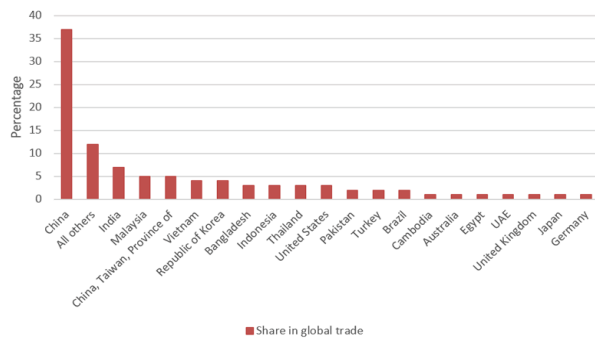


Figure 1. Fall in Sector-wise Global Trade in 2020

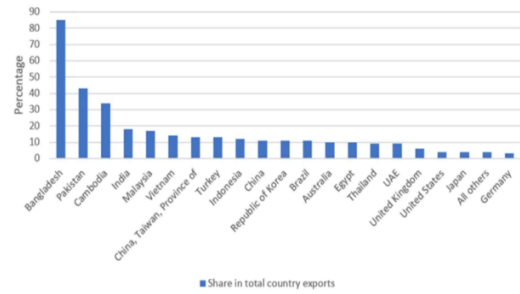




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Source: <https://www.mdst.co.uk/>
(Share in global exports), estimated TEU 2019



Source: <https://www.mdst.co.uk/>
(Share in total countries exports), estimated TEU 2019

Figure 2. Top 20 exporting countries of fashion goods

Figure 3. Top 20 exporting countries of fashion goods

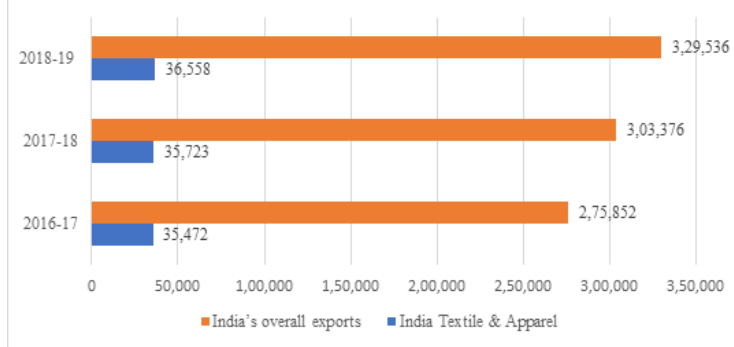


Figure 4. India's Exports (Million US Dollars)





Synthesis and Morphological Characterization of Neodymium Oxide-SBA-16 Nanocomposites

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ABSTRACT

In this research work, Neodymium oxide /SiO₂ nanocomposites prepared by chemical method. The surface morphology and order of arrangement of prepared nano-composite was characterized by Scanning Electron Microscopy (SEM) and High Resolution Transmission Electron Microscope (HRTEM). Fourier transformation infrared (FTIR) analysis represents clear peaks of different chemical bonds. Neodymium oxide nano crystallites is obtained with average size ~40.80nm at temperature 600°C calcined for 8h.

Keywords: chemical, FTIR, HRTEM, oxide, Microscope.

INTRODUCTION

Synthesis of the bulk nanocrystalline of rare-earth oxides under manageable dimension and characteristics is vastly required for several applications such as photonic devices, catalytic systems and photoluminescence [1]. Furthermore, Neodymium oxide nanoparticle have many applications of in such as transparent and anti-reflecting coatings[2], gas-insulators [3] and shielding coatings [4]. Silica is a non-toxic material and its wall protects the doped materials from the external environment and also enables sustainable release due to the formation of silanol bonds [5], [6, p. 16]. In this work we have revealed that at high temperature, calcination time shows vital part in the evolving the crystal-like phase of the neodymium silicate and Neodymium oxide. The average size of neodymium oxide nano crystallites in a silica matrix was ~40.80nm. The SEM, HRTEM and Fourier transformation infrared spectroscopy (FTIR) data are presented of Neodymium oxide -SBA-16.



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EXPERIMENT

The synthesis of metal oxide nanocomposite was performed by following the previously given method mentioned by Duhan et al [7]. Neodymia– silica (Binary-Oxide) nanoparticles were synthesized by sol-gel method. In a typical synthesis method 2.5wt% Neodymium oxide was hosted in the previously hydrolyzed solution in the condition of nitrate under heating, pH of the prepared mixture was up to 1.5. After approximately 3 days, gelation is observed. After the gelation for 17, 27 and 40 days the samples were still kept inside the oven for aging. Shrinkage and stiffening of the gel process allowed after the aging. The percentage of shrinkage of the samples was very low before 17 days. To this end, it was noticed that the silica samples were transparent and colorless and doped samples colored was obtained glassy violet-purple due to the presence of neodymia. The powder form of the pure silica samples was obtained by agate and pastel. The obtained samples were calcined in muffle furnace (KSL 1600X, MTI) in air at different heating rates i.e. from room temperature up to 300°C at 2°C/h and after that 4°C/h from 300°C to 600°C.

CHARACTERIZATION

The HRTEM images in this work were collected using a TECNAI G20 at 200 kV. To prepare an example for HRTEM analysis, the powder content is dispersed in an ethanol arrangement and then sonicated for 1 hour to ensure an evenly scattered arrangement [8]. This configuration is lowered onto a 400 work copper lattice coated with a holey carbon film and dried in air for 20 minutes to remove the ethanol from the carbon matrix. SEM image obtained by using the instrument FEI QUANTA 200F. SEM processing is non-destructive since no sample volume loss occurs, resulting in the analysis of the same samples being repeated. SEM employs a directed beam of high-energy electrons, ranging from a few KeV to 50KeV, to produce a spectrum of signals at the surface of solid specimens [9], [10]. As these signals communicate with the sample, they disclose details about the sample such as its color, crystalline shape, orientation, and chemical composition of the materials that make up the sample. Fourier Transform Infrared Spectra (FTIR) is used to find out different bands present in the organic as well as in the inorganic materials. for the preparation of sample potassium bromide, KBr were used to form the pellet to get the FTIR spectra. Perkin Elmer Frontier FTIR Spectrophotometer was used to record spectra between 4000 - 400 cm⁻¹ range.

RESULTS AND DISCUSSION

SEM

The heat treatment at 600°C(8h) leads to a well-crystallized hexagonal phase of Neodymium oxide. The average particle size of Neodymium oxide is affected by the calcination temperature. When calcination temperature is increases, the particle size of Neodymium oxide is also increases. The Scanning Electron Microscopy (SEM) produces an image through scanning a directed electron beam above a molecular structure. The electrons in the ray network with the surface, creating a range of pointers that can be used to examine the outward topographic features and morphology. The SEM picture of the sample reveals that its morphology is not altered during Neodymium oxide integration into the SBA-16 framework. There was no accumulation detected, which may indicate that the Neodymium oxide was evenly dispersed or that no extra structure nickel oxide was present.

HRTEM

The materials' HRTEM images confirmed the existence of well-ordered cubic pores, as predicted for the SBA-16. Transmission electron microscopy was used to investigate the homogeneity of the Neodymium oxide distribution and the ordering of the cubic array of mesoporous. The HRTEM picture reveals dark spots between pores, which are Neodymium oxide nanoparticles found inside the mesopores. These nanoparticles are smaller than the diameter of the mesopores. The number of nanoparticles on the surface is greater than the size of particles within the nano-pores.



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FTIR

Figure 3 reveals different band structures which assign the presence of different metals. Amorphous SiO₂ have the characteristics bands at 800, 970 and 1090cm⁻¹ at temperature 300°C. However, several discrete bands appeared between 640 to 970cm⁻¹, when the sample was sintered at 600°C for 8h. The characteristics band of neodymium silicates appeared around 905cm⁻¹ and this band confirmed the presence of Neodymium oxide in the silica.

CONCLUSIONS

The Neodymium oxide nano crystallized was successfully synthesized by chemical method using neodymium nitrate. SEM and HRTEM was used to characterize morphological and topographical property respectively and FTIR was used to find out the presence of Neodymium oxide in SBA-16. The size of Neodymium oxide nanoparticles increase with increasing calcining temperatures.

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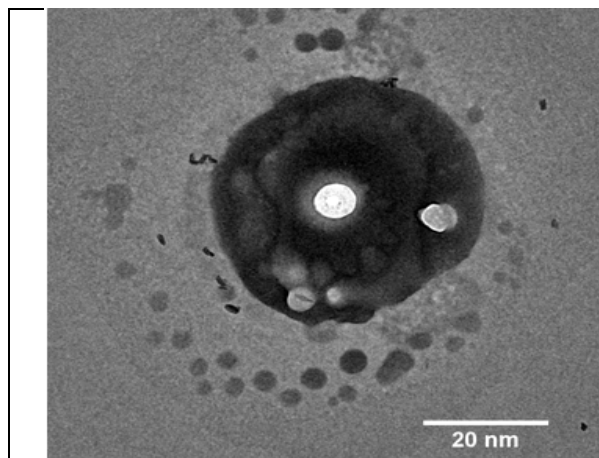


Figure 1. SEM image of the Neodymium oxide -SBA-16

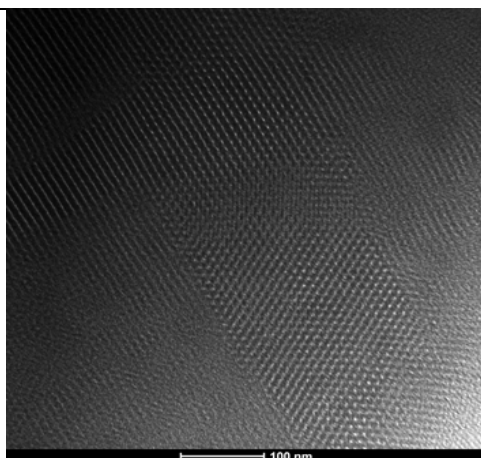


Figure 2. HRTEM image of the Neodymium oxide -SBA-16

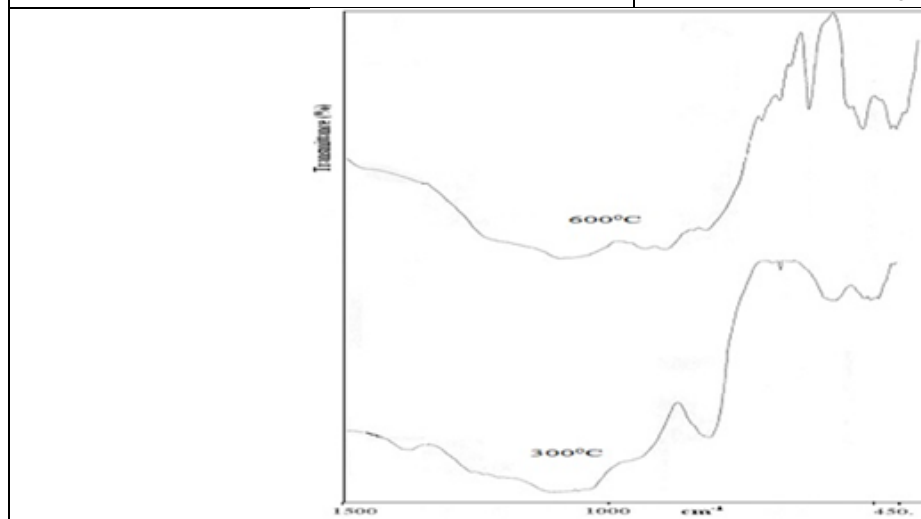


Figure 3. FTIR spectra of Neodymium oxide –SBA-16





Feasibility Analysis of a Microstrip Patch Antenna being used as a Conformal Antenna

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ABSTRACT

Conformal antennas have an advantage of being non obstructive and provide an ease of integration with irregular geometrical shapes. These are preferred where space is a huge constraint and any kind of protruding of antenna is avoidable. In this paper a feasibility analysis will be given for a conformal antenna using a Microstrip patch Antenna. This analysis can aid in making a right design choice for a conformal structure, specific to the application. For this analysis, a Microstrip Patch antenna operating at 2.4GHz (ISM band) is taken as the reference. The analysis will include resonant frequency variation with respect to structural variation and the parameters that dictate the design criteria of a conformal antenna.

Keywords: Conformal Antenna, Planar Antenna, Microstrip Patch Antenna, Finite Element Analysis Method, Omni Directional Pattern, Radiation Pattern.

INTRODUCTION

Conformal structures are possible with Microstrip Patch antennas as it is easy to form curved surfaces with them as long as the curve is in one direction only. Conformal Antennas are used for various applications in the field of Communications, Automobiles, Satellites, Missiles, Aircrafts, Ships where they can be made conformal to an existing structure. The geometrical structures which are generally explored are Cylindrical, Conical and spherical. Cylindrical antenna is the most commonly utilized non planar geometry which is used in the applications requiring Omnidirectional radiation coverage. Non planar structure cylinder is taken as a reference here to achieve conformity with a Microstrip Patch Antenna.



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The Microstrip Patch antenna was designed for 2.4GHz frequency of operation. The length, width and thickness of the patch were calculated from the standard Microstrip patch antenna design equations taking the centre resonant frequency as 2.4GHz.

Microstrip Patch Antenna with Inset Feed

Microstrip line feeding is one of the easier methods to fabricate, as it is just the conducting strip connecting to the patch and therefore can be considered as an extension of the patch. The modelling is done in HFSS and is matched to the patch by controlling the inset position. This arrangement is one of the preferred methods in feeding a patch antenna as both the transmission line and the patch are made on the same substrate as shown in Figure 1.

Frequency of Operation

The frequency of operation of a Microstrip patch antenna is dependent on the length of the patch and the dielectric constant of the substrate being used. The relation between the frequency of operation and these parameters is

$$f_c = \frac{c}{2L\sqrt{\epsilon_r}}$$

Increasing the dielectric constant of the substrate decreases the frequency of operation and increase in the length of the patch decreases the frequency of operation. Hence if L is increased, ϵ_r has to be decreased to maintain a constant frequency of operation.

Simulation of Microstrip Patch Antenna at 2.4GHz

A microstrip patch antenna was designed in HFSS with $\epsilon_r= 4.4$ mm, $h=1.5$ mm and frequency of operation $f_c=2.4$ GHz. The length and width of the patch were calculated for these parameters using the standard Microstrip design equations and the values obtained were $L=29.47$ mm and $W= 38.03$ mm. The layout is as shown in figure 2. For the designed layout, the port was defined as lumped type at the edge of the inset feed to analyse the electric and magnetic field conditions. The simulated result for the return loss is as shown in figure 3. The return loss can be observed to centered around 2.38GHz which is a bit offset from the desired center frequency. By tuning the parameters, it can be centered and optimized to 2.4GHz.

Conformal Antennas

This patch antenna was wrapped onto a cylindrical structure as shown in Figure 4. The radius of curvature is the main parameter which will be affecting the frequency of operation. This was simulated to observe the variation in frequency of operation by varying the radius of curvatures as represented in figure 5. By observing the simulated results it was noticed that the deviation in return loss was a maximum of 2dB variation for the different curvatures considered. The deviation in centre frequency for the designed conformal patch antenna was also minimal as observed from the simulated results.

CONCLUSION

A conformal antenna is a more feasible solution if an application requires conformal structures as the performance of both planar structure and cylindrical conformal structure seems to be on par with respect to its performance as observed through the simulated results in HFSS.

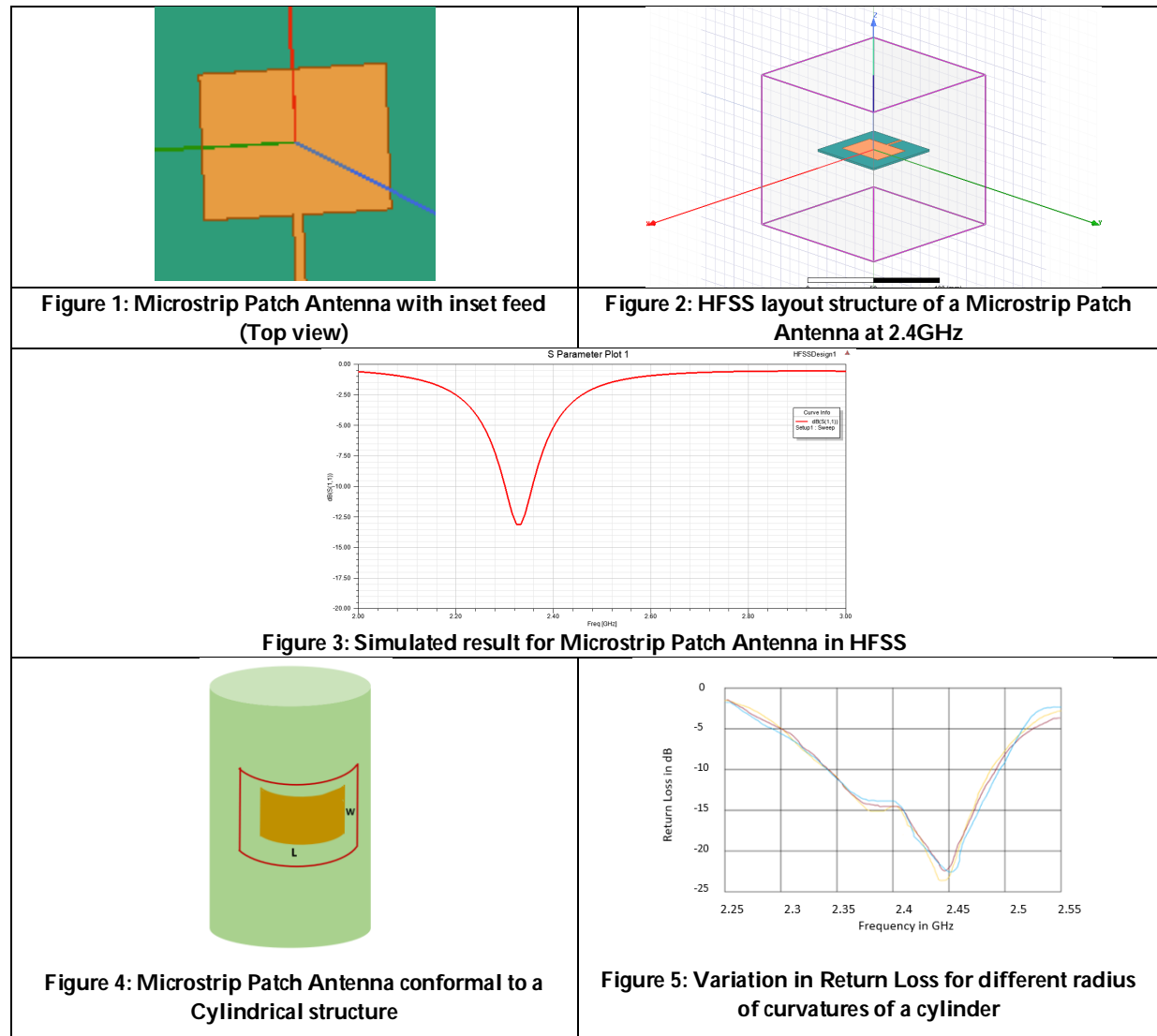




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Analysis of Rainfall Trends in the Semi-Arid District of Latur, Maharashtra

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ABSTRACT

The variability of rainfall pattern and their occurrences directly influences groundwater, stream flow, soil moisture content, fresh water and drought vulnerability. Rainfall is the crucial parameter in any hydrological process, and a slight change in rainfall has a significant impact on the water cycle process. Besides, in a country like India, whose agriculture depends on rainfed farming, planning for water resources management has a detrimental influence on food security and economic development. Therefore, a study of trend analysis of rainfall was conducted at a drought-prone area in Latur, Maharashtra, for 102 years from 1901- 2002. In the study area, agriculture plays a vital role. The non-parametric Mann-Kendall statistical test for trend and Sen's slope method for magnitude was used to identify monthly and annual rainfall. Furthermore, the individual study of months revealed that January, March, May, June, July, August, October, November showed a rising trend of rainfall, whereas February, April, September, and December show negative value. On the other hand, the annual trend analysis shows a rising trend using Sen's slope. According to results, the highest rainfall has occurred in the year





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1916 was 1299 (mm), and a minimum of 394 (mm) was observed in 1972. Thus, the inferences from the data can be used to plan agricultural and water management for the non-monsoon season.

Keywords: Trend Analysis, Climate change, Mann-Kendall, Rainfall, Sen's Slope method.

INTRODUCTION

Water is the fundamental element for plants, animals, human life, and the earth's environment. Globally, water availability is determined by the frequency and uniformity of rainfall, which is a crucial element of the hydrological cycle. Agriculture consumes around 70% of total freshwater for production. The report of the Indian water index has stated that approximately 6% loss in the Gross domestic product (GDP) owing to depleting water level by 2050. Moreover, scarcity and variability of rainfall may lead to extreme events like drought and flood. The southwest monsoon significantly impacts Indian agriculture, with the monsoon season contributing to almost 80% of the total rainfall. There is an imperative need to identify rainfall trends and variability to reduce its impact on sustainable development. As a result, planning for water resource management is the primary concern for long-term sustainability and alleviating agricultural failures and drought risk.

The Trend and variation in rainfall data on various spatial and temporal scales are important aspects of studies related to future climate change scenarios. Trend analysis is used to analyse time-series data, which includes comparing numerous parameters over a considerably long time to identify the general arrangement of relationships between involving factors and projecting these patterns to future relevance and scope. However, the trend is an important aspect of the time series, and it is usually significant in the global environmental change issues. As a consequence, the World Meteorological Organization recommends non-parametric methods, such as Mann-Kendall (WMO), are available to identify the trends of hydrometeorological parameters using time series data. Many researchers use the Mann-Kendall test to analyse temperature, evapotranspiration, and rainfall trends for a monthly and annual period. Hence, understanding the rainfall trend is critical since the rainfall pattern and variability are relevant to water resources' long-term development.

The variability and erratic rainfall impact the distribution of rainfall, soil moisture, groundwater, and agricultural droughts, according to (Kumar et al., 2010). The southwest monsoon influences Maharashtra, an area of northwestern peninsular India further, water shortages are seen almost every year (Guhathakurta & Saji, 2013). Besides (Singh et al., 2020) reported a tremendous decline in the pre-monsoon and non-monsoon season rainfall trends in districts of Maharashtra. Further, (Dhawale & Paul, 2018) observed that the Latur district was facing agricultural drought, which was exacerbating across the area, and drought conditions were severe to moderate. Hence, most farmers are dependent on rainfall for agriculture, so analysis of rainfall trend is crucial for climate change impact. In addition to the previously mentioned concerns, a study was conducted to identify rainfall trend analysis over 102 years (1901-2002) in Latur, Maharashtra. The average annual rainfall for the area is almost non-existent from November to April, indicating that rainfall is less than monsoon season.

MATERIALS AND METHODS

Study Area and Data Used

The present analysis was carried in the Latur district of Maharashtra's Marathwada state, India. Geographically the study area enclosed with an area of 7,157 square kilometres and latitudes between 17°53'N and 18°50'N and 76°18'E and 79°12'E longitudes in the Deccan plateau. It has approximately 631 meters above mean sea level (MSL), and the temperature varies from 15° C to 40° C. The study area relies on agriculture, the primary source of revenue for the farmers and known for the biggest grain market in India, along with major crops growing: cereals, oilseeds, pulses, and grapes. Moreover, the average annual rainfall is 754mm, dependent on the southwest monsoon for agriculture,

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whereas the non-monsoon season is insignificant and affecting farmers' economy. Further, for trend analysis, the rainfall data of 102 years from 1901-2002 were obtained from the Indian Institute Of Tropical Meteorology, Maharashtra.

Trend Analysis

A trend is a major shift in a random variable over time that may be determined using parametric and non-parametric statistical procedures. It is time-series data that is compared over a long time to identify the relationship between the variables concerned and forecast the future direction of this pattern. Trend analysis allows for predicting data over time for a long-running survey, labelled as long-term variations in a time series. According to Spatial and temporal changes, it may result in increasing or decreasing trend.

Mann-Kendall Test

A statistical test Mann-Kendall (MK) (Mann, 1945), widely used to analyse rainfall trends with various hydrological data, was used in this study. The adoption of this test provides multiple advantages. Firstly, it is a non-parametric test without dependence upon normally distributed data. Second, the test is resistant to unexpected interruptions induced by non-uniform time series data. This statistical test compares the null hypothesis H_0 , which presumes no trend, against the alternative hypothesis H_1 , implying a trend. The Mann-Kendall test is a robust statistical method for identifying stations with substantial magnitude change and hence evaluating these results. (Hirsch et al., 1982). Additionally, it is a significant non-parametric test for determining the importance of a monotonic rising or declining trend in time series data. It is widely used in climate change, meteorology, and hydrological studies.

The Mann-Kendall statistics (S) is given in equation (i).

$$S = \sum_{i=1}^{N-1} \sum_{j=i+1}^N \text{sgn}(x_j - x_i) \quad (i)$$

Where,

x_j and x_i are the annual values at different time j and i , respectively and 'N' is the dataset length.

$$\text{sgn}(x_j - x_i) = \begin{cases} 1 & ; \text{if } x_j - x_i > 0 \\ 0 & ; \text{if } x_j - x_i = 0 \\ -1 & ; \text{if } x_j - x_i < 0 \end{cases} \quad (ii)$$

If the datasets show a positive value of S, then the trend will be increasing, whereas the negative values show the decreasing trend. The larger value indicates the Trend is more reliable in its direction, and the smaller values indicate that the Trend is less reliable.

Under the assumptions that data are dependent or independent, the variance of the S statistics is provided in equation (iii)

$$\text{var}(S) = \frac{n(n-1)(2n+5) - \sum_{i=1}^n t_i(t_i-1)(2t_i+5)}{18} \quad (iii)$$

Where n represents the total number of tied rank groups and t_i is the number of data points in the i^{th} tied group, and Σ shows the summation over all tied groups.

After obtaining the data variance, the standard normal distribution Z-Statistics is calculated using equation (iv)

$$Z = \begin{cases} \frac{S-1}{\sqrt{\text{var}(S)}} & ; \text{if } S > 0 \\ 0 & ; \text{if } S = 0 \\ \frac{S+1}{\sqrt{\text{var}(S)}} & ; \text{if } S < 0 \end{cases} \quad (iv)$$

Further, with a two-tailed significance level at $\alpha = 0.05$, the calculated standard Z value is compared to the standard normal distribution table. If the calculated Z is greater than $z_{\alpha/2}$, the null hypothesis (H_0) of no trend is rejected, and





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the trend is statistically significant; otherwise, the H_0 hypothesis is accepted, which indicates that the trend is not statistically significant (Mehmet, 2003). In this study, the null hypothesis was tested at a 0.05 significance level. Thus, a positive calculated value of Z indicates a growing trend, whereas a negative number indicates a decreasing trend.

Sen's slope method

(Sen, 1968) provided a method to calculate the magnitude of the slope of the trend in hydrometeorological data series. It is the non-parametric, linear slope method that performs effectively on monotonic data. Despite linear regression, it is unaffected by missing data, outliers, or large data errors. So, the linear slopes T_i and all data sets are determined by using equation (v).

$$T_i = \frac{x_j - x_k}{j - k} \text{ for } i = 1, 2, 3 \dots n, j > k \quad (v)$$

Where x_j and x_k are data values at time j and k ($j > k$). On the other hand, the median of these N values of T_i implies to the slope of trend, which is calculated using equation (vi)

$$Q_i = \begin{cases} \frac{T_{N+1}}{2} & \text{if } N \text{ is odd} \\ \frac{1}{2} \left(T_{\frac{N}{2}} + T_{\frac{N+2}{2}} \right) & \text{if } N \text{ is even} \end{cases} \quad (vi)$$

An increase in trend is depicted if Q_i show positive value; conversely, a negative value shows that the trend in the time series is declining.

RESULTS AND DISCUSSION

In the current study, the monthly and annual rainfall trend analysis of the Latur district for the data series of the 102 years. A non-parametric MK method was performed to determine trends for consecutive years. In addition, the magnitude of the trend is measured using Sen's slope method. Hence, the variation of monthly data was calculated and represented in Fig. 2. The Z statistics values for all months were calculated which are 0.273, -0.111, 0.274, -0.141, 0.975, 0.292, 0.346, 2.68, -1.491, 3.114, 0.498 and -1.037. The months February, March, September, and December show a decrease in trends and May, August, and October show an exponential increase in trend. These results have depicted that non-monsoon and winter season shows no trend along with decreasing trend.

Annual Trend Analysis

The trend analysis of annual rainfall is depicted in fig.4 with a rising trend. It shows that the highest rainfall has occurred is 1299mm in 1916 and a minimum of 394mm in 1972. Hence, on an annual basis the positive values of Man-Kendall (Z) and Sen's Slope (Q_i) shows a rising trend. The maximum rainfall occurred during the monsoon season, and there was no trend and decreasing trend during non-monsoon seasons. Table 1 depicts that Kendall has negative Z values for months February, April, September, December. Similarly, Sen's Slope shows a negative value and no trend for respective months.

CONCLUSION

In the present era, climate change and environmental issues are rising and affecting our agriculture system with less rainfall. Therefore, trend and variation in rainfall data are essential aspects for assessing the climate change impact on sustainable development. Trend analysis for the Latur district was carried out over 102 years (1901-2002). For the analysis of the trend, a nonparametric Mann-Kendall test was performed, and subsequently, the Sen's Slope method was utilised to estimate the size of the trend. It reveals that January, March, May, June, July, August, October, November depicts an increasing trend of rainfall, whereas February, April, September, and December show negative





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value from Z Statistics. Similarly, the Sen's Slope Q_i Showed positive values for May, June, July, August, October, and no trend and negative values were observed for the rest of the months. This analysis revealed that the Rabi season would affect agriculture, livestock, forestry. Further, these results can be helpful for improved water resources planning, cropping pattern for higher crop production and monitoring drought risk assessment for economic development.

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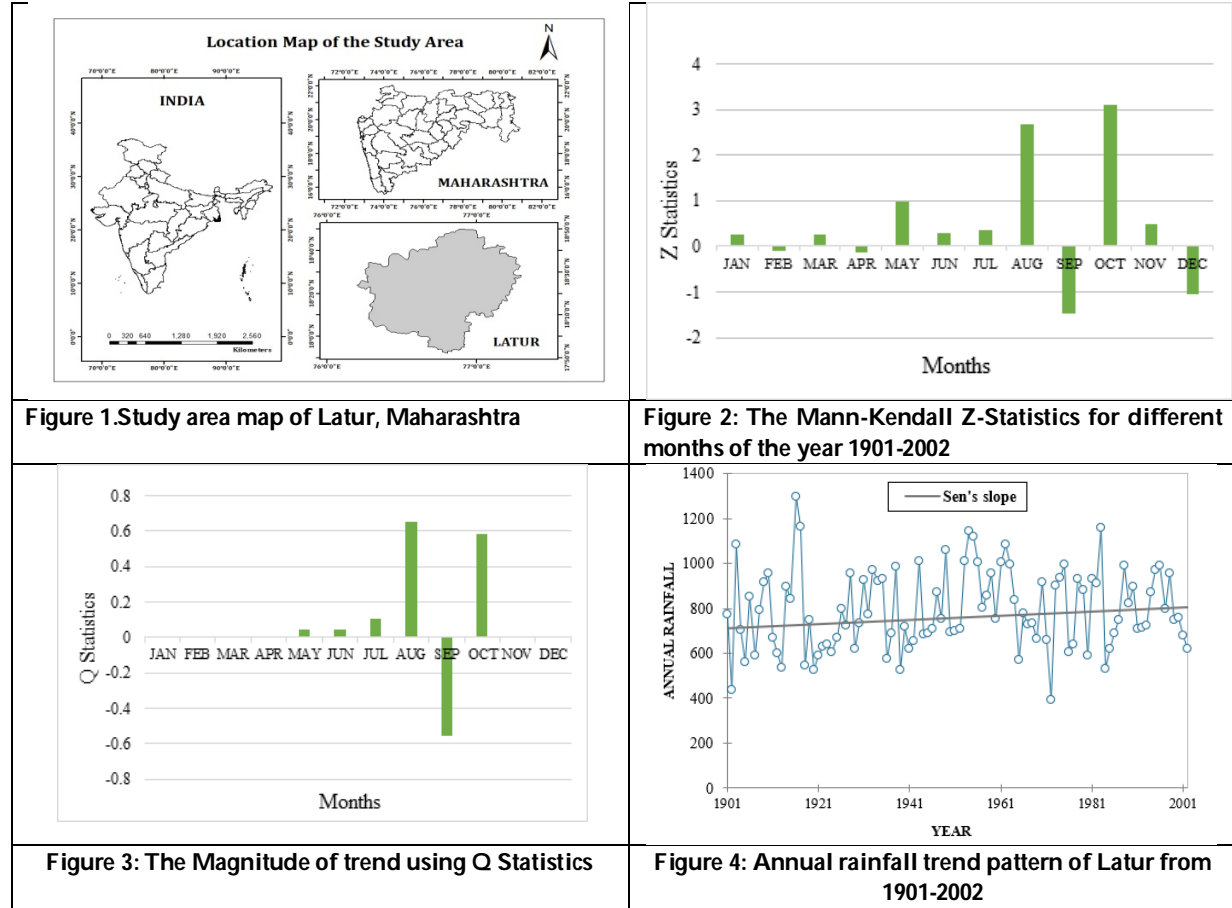
Table 1: Values of Man-Kendall (Z) statistics and Sen's Slope (Q_i) for different months

Months	Mean	Standard Deviation	Sen's Slope	Kendall's test statistics(Z)
Jan	3.824	7.693	0.000	0.273
Feb	4.873	9.859	0.000	-0.111
Mar	8.196	10.710	0.000	0.274
Apr	15.382	14.597	0.000	-0.141
May	27.588	29.681	0.043	0.975
Jun	124.686	52.732	0.045	0.292
Jul	148.461	65.032	0.103	0.346
Aug	143.245	76.599	0.654	2.68
Sep	209.784	105.295	-0.553	-1.491
Oct	75.725	63.815	0.583	3.114
Nov	25.186	34.143	0.000	0.498
Dec	6.529	21.900	0.000	-1.037
Annual	793.480	177.979	0.926	1.43





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Changing Trends in Hindi Songs and Its Impact on Development of Self-Image in Youth

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ABSTRACT

Songs which are a combination of Music and Lyrics are widely used in films and are a very popular means of entertainment in India. Purpose of writing this paper is to understand the changing trends in Hindi Filmy and non-filmy songs and their impact on the Youth between age group of 18 to 30. The main focus will be on the effects that visuals, Lyrics and the character portrayal has on the below mentioned area of youth psychology and self-image- Creating beauty standards, Relationship and love standards, Machismo, objectification of humans Fashion, Consent and sexual innuendos, The conclusions of this paper will help in understanding the idea of gender and self being created among the youngsters today also, how symbolisms, changing trends and digitization of media may create huge impact on the social image of a young individual.

Keywords: Hindi songs, changing trends and digitization of songs, impact on Youth, creating stereotypes, Hindi songs and self-image and objectification.

INTRODUCTION

India has one of the biggest film industries of the world in terms of tickets sales. The West says that a movie is successful with a good story and script, but for Indian films the scenario has been completely different for over almost a century now. Songs and Movie's success have been taken as synonyms in the Indian film industry. The Trend began with the first talkie film of India "Alam Ara". This film had seven songs and back then the actors themselves sang the songs while it was being shot. The songs became very popular for mainly two reasons, first it helped in replacing the boring dialogues used back then in films and second the audience now had an easy sing-able version of the difficult classical music. From being beautifully written and composed in soulful melodies the music industry today has songs that mock the rich artistic heritage of Indian music.





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According to Dagnaud and Feigelson (2007) India ranks 1st in media production specially cinema. While films and film stars are a big influence on the way the audience think and behave, songs are no less in having a great impact on the mind of the viewers. In fact, in a country where stars are worshipped and filmy songs have managed to replace the traditional folk songs, it is not wrong to think that songs have a huge impact on the young minds and their personality. There was once a time when consumers were dependent on the TV or Radio to listen to songs but with the Internet explosion and media using any and every way of reaching their audience, songs are easily available on various applications and can be viewed as many times as desired which increases the effect by means of bombarding and repetition.

Hindi movie makers realised that songs are an inseparable part of cinema, use of women in skimpy clothes, loud music and suggestive dance became a formula to attract audiences to the cinema hall. According to Barrett Grant (2006), 'An item number or an item song, in Indian cinema, is a musical performance that holds little or no relevance at all to the film in which it features. It appears in a film to present beautiful dancing women in revealing and exposing clothes.' These songs have little to do with the plot of the movie. The role of the song would be to make the movie popular and attract audience to the movie. This research is also important from the view that India as country is still struggling with a lot of social issues like violence against females, breaking stereotypes, lack of positive representation given to Youth due to norms where only elder are taken seriously the popular Hindi songs are doing no good in improving the situation. Some of the biggest issues are-

Stereotyping

There are so many stereotypes propagated in the songs to name a few of them, Perfect model like body(all item numbers), Fair skin (chitiya Kalayan, kilichundan jasa ye badan), Weird fashion trends, Unrealistic Love standard(Tujhe Bitha Ke Rakha Tha, Rani maine palko pe, hum tere bin ab reh nahi sakte tere bina kya wajood mera), Alpha Males(Huachokara jawan re, Ram Jiki Chal Dekho)Etc.

These stereotypes are hampering the way youngsters feel about themselves and also in setting up social standards for love, affection and relationships.

Objectification of human

The word "ITEM" signifies objectification of women's image as a thing used for sex. Poets have forever made use of symbolism to beautify the hidden feelings and emotions. Songs being made today have made some of the most absurd comparisons of humans to things. A few examples like in the recent Song- "haagarmi" from the film Street dancer, the dancer is compared to a red velvet cake, "baby doll" from the film Ragini MMSis compared to a golden doll, "Fevicol se" from Dabaang where the actress calls herself Tandoori murgi (tandoori chicken), "saadike Fall sa" from Rajkumar, "Makhana" by Honey Singh call a women a diamond, a craker, and dish to be eaten off etc. the sad part is that all these songs are on top of the chart busters.

Consent and sexual innuendos

Showcasing females as Sexual objects has always been the popular way of gaining attention from the audience but portraying Youth to be only sex craving beings and not taking consent to be cool is a very wrong idea being promoted in the songs. Also, usage of ambiguous and sexual suggesting lyrics has subsequented in young minds to think that being disrespectful to the opposite gender with usage of such Innuendos is completely alright. Few examples of the same are- "Dilkebanduk" from GoliyonkirassleelaRamleela, "abkarungateresaathGandibaat" from the film Rajkumar, "tuhaankaryanakartuhaimerikiran" from Daar, "Kundi mat kadkao Raja" from the Film Gabbar."Mai tera boyfriend tumeri girlfriend vomainukhendi Na NaNa!" from Rabta, "Sochahaiyekitumhe Rasta Bhulaye".





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Visuals of the songs

Not only are the lyrics of the songs but the visuals equally disturbing. Most of the shorts either focus on the buttocks or the cleavage of women. Men are either shirtless or wearing clothes that show off their perfectly shaped body. The dance moves performed are mostly sexually oriented. All the party popular songs have the actor and actress dancing around in bare minimum clothing and alcohol flowing freely.

Research Methodology

Research Method is the systematic, theoretical analysis of the methods applied to a field of study. The research type which has been used for this survey is descriptive research method. We have created a survey form to understand whether these songs have any influence on their behaviour pattern or any social problem caused due to these songs. This research method will help us get perspective about what the common people think about the popular songs and their effect on the youngsters.

Universe

The universe selected was people between the age group of 18-30 years but we have also accepted responses from people above the age of 30 so that we know what the higher age groups, who is or will be raising the next generation thinks about the songs in Indian Films. We have accepted responses from all genders and have tried to reach out to people from different educational and economic backgrounds.

Sample

We have total of 120 respondents for the survey. The respondents were grouped in 3 age criteria's. 1 (18-25 years), 2(26-30 years) and 3 (30 years and above). The responses we received in percentage were as follows.

- 1 18-25 years – 49.6%
- 2 25-30 years – 28.1%
- 3 30 years and above – 23.3%

If we see the responses Maximum percentage of respondents are from group 1 next is group 2 and followed by group 3. These groups represent different ages and so have difference in perception, mind-set and capability of processing information. Even the effect these songs have on the age group can be different.

Data Analysis

There were in total 13 questions asked. Each question covered the aspect that we tried to uncover through our research here. The topic that we have selected required Audio and visual support hence in the survey form we had also put a short video and Audio clip to get deeper and prompt responses. In the last year (2019) total of 104 big starter films released in India, on an average if we consider that each film has 4 songs then in the year 2019 India produced minimum of 416 Hindi songs just in Films. India also has a big market for independent album maker like Honey Singh and Badshah to name a few, these artists also produced at least 2-3 albums per year. So the number easily reaches around 500-550 songs just in Hindi language per year. This is a huge number and has the potential to affect millions of youngsters in the country. Based on this fact we designed our question.

Do you regularly watch/ Listen to Hindi songs?

This question was asked so that we can establish the fact that the younger audience is still listening to Hindi music as opposed to the popular notion that they are completely hooked to western media and entertainment only.

Response analysis

Regular Listener 71.9%
Sometimes 20.7%



**Yashashree Mhatre****Do you think a movie can be successful without a popular song or an Item number?**

This question was asked to understand if film audience thinks that popularity of a Movie is determined by the popular songs. Also if this could be one of the reasons why Script oriented films get less attention and hence the film makers are forced to make use of sleazy songs in their films. Example film like Gabbar, a movie with good story and performances also had song “kundi mat khadkao raja”.

Response analysis

Yes 62%

Maybe 24%

No 14%

What aspect of the songs do you enjoy the most?

Through this question we understand that what elements of a song attracts major attention and has the maximum effect on its audience. Each individual has different sensory preferences and hence perceives various elements like music, words, dance etc. in variations.

Response analysis

Lyrics 81.3%

Visuals 21.1%

Dance 35%

Melody 56.9%

Fashion and Attire 8.1%

The highest percentage of people said that they listen and enjoy the lyrics of the songs. Most of the songs have ambiguities in term of sexual words or unrealistic expectation in terms of love and relationships. The second highest is Melody and yes some songs do have beautifully woven melodies.

Did you find anything inappropriate in the video above?

In order to give visual Aid to the respondents, a small video 1.5 minute was created which comprised of sections from 3 songs namely “aarepritampyare”, “Rocket in my pocket”, “GandiBaat” The songs we picked are easily available on YouTube without any age restriction and also are played on the music channels on Television. The respondents were asked if they felt anything inappropriate in the visual.

Response analysis

Yes 77.2%

No 22.8%

Majority opinion was yes there is objectionable material in the songs. These songs that the respondents may have seen so many times before causally. Only 22.8% people felt there was no problem in the songs.

What exactly did you feel was inappropriate?

Just knowing the superficial view is not enough, to analyse in details we had to find out what exactly is tagged as inappropriate in the songs so we can determine the though process of our respondents. This will also help us in knowing the factors that impact the most on the viewers.

Response analysis:

Lyrics 69.1%

Sexual innuendos 57.7%

Visuals 31.7%

Attire of the dancers 24.4%



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In this question we had given the respondents the freedom to choose multiple options amongst the Elements of the song. Given the fact not even one element has been on zero. That means all the things in the songs had inappropriate content. The highest level is given to Lyrics of the songs. Next was the sexual innuendos used in the songs, now here for long time people have been debating that why stop innuendos because its indirect anyways but this survey shows that people very well understand these ambiguities and do feel uncomfortable. The Visuals and attire of the dance are also not very soothing to the eyes most of the time and same with the video in the survey.

What do you think is inappropriate in this song?

To establish the fact that not only are visuals disturbing but even a songs that has absolutely no Visuals and an amazing poetic melody has a message that may propagate so many inappropriate standards for its listeners. We shared an audio along with the survey that had the song “Afreen” by Nusratfateali khan performed at the coke studio. In this question also we had kept the option of choosing multiple choses open.

Response analysis

Mention of only the Physical features of the women for appreciation 57%

Dependence of happiness on others validity 38%

Female dependence on male validity 28.9%

Idea of Love 24.8%

The reason to select this song is that it is considered as a melodious song by all generations older and younger and its poetic lyrics are also savoured. When we look at the responses, it is clear people do feel that the whole song only focuses on the physical attributes of female body. The lyrics sung by female singer clearly presents that thought of, how women even today place so much importance on the validity from men regarding beauty, love and self-image. This song also promotes high standards for what love means and how one should look at love.

Do you feel that these songs have any effect on the behaviours of the youth today?

With the above questions, we tried to establish that songs today do have a lot of objectionable content and that even the audience acknowledged; but content and its impact are two different factors. Hence to understand, if people thought weather such songs have the potential to affect the minds of youth the above question was asked in the survey.

Response analysis

Yes 48.1%

Maybe 38.8

No 13.2%

We can clearly see, that the majority feels that these songs do have great impact on the behaviour of youngsters. Second majority people were not sure if the songs did or did not have any effect on the audiences. And only a small section of respondents felt there was absolutely no impact.

Are these songs propagating the Idea of an unrealistic perfect body?

From this question on, I have tried to understand some specific influences that Hindi songs might have on the young audience. This question is focusing on, how when all the songs and films have actors and dancers flashing slim and spotless bodies; It may result in the young audience to feel that such bodies are socially accepted standards. In recent times, we have seen many young boys spending more time in the gym building body than in the class studying and the girls are involved in diets.





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Response analysis

Yes 48.1%

To some extent 38.8%

No 13.2%

Through the responses we can analyse that total of 86.9% people felt there was surely some impact on the impression of an ideal body. Out of which 48.1 % felt there is a great impact on the how younger people look at their physical appearances. Only 13.2 % felt that youngster's do not associate their self-image with the songs.

Are these songs responsible for unrealistic expectation from Romance and relationship?

Another area where, I thought that the songs create a big impact, is the idea of Love and relationship. Comparing affection, love and relationship expectation with utter dedication and ultimate sacrifice leads to youngsters feel that's the way any relationship is supposed to be. The above question tried to understand if people feel that's songs and specially the lyrics add to rising such expectations.

Response analysis

Yes 56.6%

Maybe 27.1%

No 16.3%

Respondents feel that the picture standards that are shown in these songs are unrealistic and have no connection to what real relationships are. A big majority felt that such songs may impact on the expectations that youth have from their partners and when these expectations are not fulfilled it may lead to many problems such as higher divorce rates, depression and in worst cases things like acid attack.

Do you think such kind of songs are propagating the idea that Love and Lust are the same things?

Even if we keep the sexual innuendos aside, there are so many songs that portray sexual fantasies and sexual desires as love or fantasising about some sexually is equal to loving them. The above question is trying to understand that weather the audience understands the difference between what Love and lust.

Response analysis

Yes 46.5%

Maybe 25.6

No 27.9

Although a big section of the respondents choose yes as their answer, the other majority still feel that, songs may not have much impact on how they differentiate between love and lust.

Have you ever been eve teased or cat called using Bollywood references?

This question was included in the survey to understand if the songs have any impacts beyond just self-image and image of love in minds of the target group. Cat calling or eve teasing is a big problem in our country. Various studies have also proved that Eve teasing is one of the most commonly used harassment by men. Songs that have various sexual innuendos are used so casually to harass women.

Response analysis

No 60.5% (16% men and 84% women)

Yes 39.5% (92% women and 8% men)



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Initially as we look at the responses it looks like songs are not to be blamed for social problems like Eve teasing, but if we analyse deeper it is very clear that the in the responded who said no were majorly men and the once who said yes were majorly women. It is quite clear that most of the women have faced the problem of eve teasing through songs.

What aspects of Man does Bollywood idolize?

It is not only women who face the effects of these derogatory songs and the unrealistic standards. Even young boys who are still in the phase of finding their identities also face dilemma of perfect image, expectation of high performing careers among many other things. This question is focusing on these misconceptions surrounding Men in the songs. The respondents had an option of selecting

Response analysis

Muscular body 107 votes

Popular and extrovert 78 votes

Money 62 votes

Aggressive 63 votes

Bad boy 60 votes

Fair 52 votes

On the basis of the responses, we can assess that these songs are propagating the idea of Muscular body as trend, these songs also Make unhealthy assumption that all men/boys have to be extroverts or popular in order to gain importance. Respondents have also expressed that in their votes that aggression and Bad boy image is being portrayed in Positive light.

Do you think these songs showcase the youth of our country in the correct light?

This question being the last in the survey, was asked to know what opinion people had regarding the portrayal of so called icons of youngsters in these songs. Weather they felt that, all that's shown in the songs is correct or not.

Response analysis

Yes 6.2%

Sort off 24%

No 69.8%

It is very clear from the responses that majority feels that youth is not anywhere close to what is shown in these songs. Second majority was unsure whether these songs represent the youth or not and very small number of people feel they youth is fully represented through the songs.

CONCLUSION

This study is restricted to very small number of respondents, but the society at large has also taken note of the obvious vulgarity in songs like "Cholikepeechekyahai", "Sexy, sexy, sexy" etc and many films have been asked to make cuts and changes in songs and film scenes. The biggest matter of concern is that individuals in their adolescence subliminally absorb all the messages in the songs on do not realise the effects it has on them. Most of the songs made today have unnecessary sexual innuendos in them, which have made such open sexual behaviours very causal. Also the portal of love in form of stalkers and the fact that its ok to keep trying until the desired partner agrees being portrayed as cool, has had a made it absolutely normal for people to abuse and invade the grave matter of consent.





Yashashree Mhatre

INFERENCE

This paper was started off with certain speculations and to go deeper the research was narrowed down to certain specific areas mentioned below:

1. Hindi filmy and non-filmy songs have a great impact on the youth's self-assessment, self-image and body standards.
2. The sexual innuendos used in the songs are clearly understood and in turn affect the young minds.
3. The assumption that the opposite gender is always ready for sexual advances presented in the songs, have a grave impact on the issue of consent and rise in sexual harassment as social problems.

The findings determine that Hindi filmy and non-filmy have a great potential to influence the minds of its young audience. Most of the respondents did feel that there is problem with the way songs are being made today, but did not know how to voice it or how to keep the youth away from it. In fact, most of these sleazy songs are the party anthems and most of us enjoy dancing to these numbers without understanding the impact it has subconsciously on us.

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3. A Systematic Review on Effect of Electronic Media on Diet, Exercise, and Sexual Activity among Adolescents
4. Sexual knowledge, attitude, behaviors and sources of influences in Urban college youth: A study from India





A Study on Attitude of Students towards use of Smart Board by Teachers and Professors in the Education Industry

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ABSTRACT

Smartboards are used by educators across the world. Unlike whiteboards, smart boards are computer-based screens which help in giving education to students at an altogether different level. Smart boards have been implemented to improve the quality of education imparted to the students across the world. The exploratory study carried out in this research paper is based on a survey designed by Mr. Suleman Sad published in his paper "An attitude scale for smart board use in education: Validity and reliability studies" published in the year 2011. This research was carried out in Mumbai suburbs. Various students of undergraduate programs affiliated to University of Mumbai had participated. The study was carried out to find out a general attitude of college students over the use of smart boards. For this purpose, the students of the undergraduate program were taken as a part of the study. 215 students responded to this survey. An exploratory research was carried out to find out what do undergraduate students think about smart boards. Data was collected using Google forms and processed using SPSS. Since the data is of nominal type Descriptive statistics were used to report the findings. From the findings it can be concluded that students do like smart boards when they are in the classrooms. from the study it was concluded that the students of undergraduate program affiliated to University of Mumbai have a positive attitude towards the use of Smart boards in a classroom environment.

Keywords: Smart boards, attitude scale, undergraduate teaching, computer-based education, computer-based learning environment, information and communication technology.



**Abhijeet Mohite and Deepak Raverkar**

INTRODUCTION

It was somewhere in the year of 1991 that smart technologies introduced interactive whiteboards to the education industry. (Overly, 2016). Later in the year of 2003 smart technologies went ahead with patenting the digital vision touch technology. (Business Knowledge on Demand Smart Digital Vision Touch technology, 2003). The other word for smart boards is interactive whiteboards. interactive whiteboards are basically very large computer monitors with the application of touch. Smart board is basically a very large computer screen the size of a regular whiteboard which has an inbuilt computer with the latest Windows operating system. this smart board can work like a television, a standalone computer or even a projector where laptop could be connected and images, PowerPoint or any multimedia content can be directly displayed.

One of the studies carried out in London indicates that there was no significant impact of the education carried out on interactive whiteboards as compared to the regular whiteboards. The study suggested that there was only an impact on subjects like English well smart boards proved to be an effective method. Whereas subjects like maths it was seen that there was a negative impact on the students when smart board was used. (Dr. Gemma Moss, 2007). Another study carried out in London showed that there was evidence to show an improved performance in the understanding of students when interactive whiteboards were being used. This particular project was known as the DfES primary schools white board expansion project where it was found out that there was a significant amount of learning gain while using these smart board devices. According to the research it showed that the longer the teachers were using smart boards it became embedded in the pedagogy as a mediating artifact for interacting with students. Also, the research indicated that emerging technology in the classroom has led to increase in the impact of the second cohorts. (Bridget Somekh, 2007).

Advantages of smart board: Many of the advantages of smart board include enhancing students learning experience with the use of multimedia technology. Smartboards are highly interactive in nature. Smart boards are highly interactive in nature. These devices create a hands-on learning experience for students thanks to its touch screen capabilities. Also, one of the Other important features of smart boards is that they are very low on maintenance. In fact, smart boards have access to online resources which I can help in searching UpToDate contents on the world wide web. Smart boards are also eco-friendly as they do not need paper. With the use of Smart boards there are various other Technologies that can be integrated together to enhance the learning experience. Smart boards have been approved successful so far in most of the cases. (Cox, 2019).

Disadvantages of smart boards: There are 2 primary reasons why smart boards are not really preferred. one of the major reasons or disadvantages of smart boards is the time requirement. in short, the time that is required to prepare the course Material by teachers takes a long amount as compared to traditional methods of teaching where the material can be directly presented via the white board. It is also observed that due to lack of training teachers are not able to use smart boards as well as it is supposed to be. Hence training plays an important role for smart board uses especially the teachers. There is a greater limitation on the size provided by the smart boards as they are large televisions and monitors with the size of 70 inches. whereas your white boards have much larger surface area and can cover lots of subject matter in detail. seems to be that projection of light affects the way that information is displayed on the smart board. this could lead to poor visibility E for or content not clearly visible from far off distances. in fact, it can cause strain on eyes for constantly watching on the big screen. The cost of these is much higher as compared to regular white boards. (Hutt, 2017).

LITERATURE REVIEW

In this paper "An attitude scale for smart board use in education: Validity and reliability studies" by Suleyman Nihat Sad, it was reported that students do have a positive attitude towards smart board but that is a very little research being carried out on the effect of smart boards on student's attainment level. In order to find out what were the





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various factors that affect the attitude of the students towards the smart board the research had carried out the validity and reliability study in which he had found 24 factors on the basis of which the questionnaire was prepared and distributed among students the research carried out exploratory factor analysis to find out the underlying factors and use confirmatory factor analysis to check their validity. (Sad S. N., 2011). In one of the articles written by Leigh Bryant, it is seen that smart boards have tried to be the next evolution in teaching but have not been able to deliver at par with what teachers have expected. The author writes about the various problems that students are facing due to the use of Smart boards, she writes in her article that students are not able to get the touch and feel for the learning that you have at a nursery or kindergarten level. also, the teachers face difficulty in use of this technology as smart boards would have its own disadvantages of failure where it can stop in the middle of the session and create disruption in the class. The author believes that although smart boards have you see impact in meetings but not the correct equipment's to be used in a classroom environment. (Bryant, 2017)

RESEARCH DESIGN

As it has been observed from literature review that there are mixed opinions of teachers and students with regards to the use of smart boards. In order to understand this better a survey was conducted using a tested questionnaire designed by Mr. Suleyman Nihat Sad for the faculty of education, Inonu University published in the year of 2011. (Sad, 2011). This survey was conducted among the students to find out their general attitude towards the use of smartboards in a learning environment.

SAMPLE SIZE

The survey was conducted among the various courses run across in Usha Pravin Gandhi College of Arts, Science and Commerce. SVKM has 26 institutes under its brand and one of them being this college. It was recently that smart boards were implemented across all of the 26 institutes replacing the traditional white boards. There are approximately 1800 students currently studying in the campus. to understand their general attitude towards the new implementation of smart boards this survey was carried out. A sampling formula was used to calculate the desired sample for which the questions would be asked.

The formula used is

$$SS = \frac{Z^2 * (p) * (1-p)}{c^2}$$

Where:

Z = Z value (e.g., 1.96 for 95% confidence level)

p = percentage picking a choice, expressed as decimal (.5 used for sample size needed)

c = confidence interval, expressed as decimal

(e.g., .04 = ±4) (Sample Size Formulas for our Sample Size Calculator, n.d.)

Based on the population size of 1800 students, at 99% level of confidence and a confidence interval of 9, the sample population what was 184 students.

Findings

As from the data shown 99 of the 215 students accounted to male whereas 116 students counted to females. Since most of our data is based on Likert scale, which is a form of a nominal data, it was very difficult to carry out statistical methods which could have been carried out on other types of data like ordinal or ratio. Based on the questionnaire carried out why are online Google forms, the data consisted of question based on gender, Class in which the student is studying and 24 questions designed on the Likert scale to understand the attitude of students while using a smart board.



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Multiple modes exist. The smallest value is shown

Since the data was on Likert scale the only statistical method that has been implemented is the mode. From the survey carried out it can be seen that question # 4,6, 7, 8, 9, 11, 12, 13, 14, 15, 17, 19, 23, 24, 25, 26 were in favour of using smart boards whereas the rest of the questions excluding the first 3 we're not in favour of using the smartboards. As it can be clearly understood from the moral values drawn most of the students have be positive impression of smart boards weather used in their learning environment. Most of the students have the disagreed to the fact do the teaching and learning environment. In certain cases, students have a neutral opinion about smart boards and whether these smartboards have helped in making students study hard.

DATA ANALYSIS

In order to understand whether there is a same level of understanding among both the genders a hypothesis was formulated for all the Likert Scale questions among the distribution of Gender.

H₀: The distribution is same across the categories of gender

H_a: The distribution is not the same across the categories of the gender.

Based on the data collected the Mann Whitney U test was performed to find out whether the whether the distribution is same across the genders. A Mann Whitney U test is used to compare the differences between two independent groups when the dependent variable is either ordinal or continuous. (Laerd, n.d.). As from Table no 4 it can be seen that for Q 15: I feel nervous using smart board the null hypothesis had to be rejected as there is a difference between the groups.

CONCLUSION

In general, it is safe to assume that the undergraduate students do enjoy learning with the smart board. Unlike earlier methods where whiteboards had limited capabilities which were limited to how a professor uses it. Thanks to modern computer systems and touch screen capabilities smartboards can now discuss topics with much detail using various media forms like images, videos, audios or even power points. Although, smartboards cost much more as compared to regular white boards the way that smartboards function in bringing rich learning environment to the students would never be achieved by using regular white boards.

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Table 1. Gender wise distribution of responses

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	99	46.0	46.0	46.0
	Female	116	54.0	54.0	100.0
	Total	215	100.0	100.0	

Table 2. Mode values of responses taken with standard deviation

		Gender	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12
N	Valid	215	215	215	215	215	215	215	215	215	215	215	215	215
	Missing	0	0	0	0	0	0	0	0	0	0	0	0	0
Mode		2	4	2	4	4	3	4	2	4	3	4	4	4
Std. Deviation		.500	.825	.851	.819	.810	.931	.876	.814	.755	.913	.888	.834	.801

		Q13	Q14	Q15	Q16	Q17	Q18	Q19	Q20	Q21	Q22	Q23	Q24
N	Valid	215	215	215	215	215	215	215	215	215	215	215	215
	Missing	0	0	0	0	0	0	0	0	0	0	0	0
Mode		2	4	2	3 ^a	2	2	2	4	3	4	4	2
Std. Deviation		.837	.954	.956	.915	.831	.705	.977	.783	.874	.783	.800	.950





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Table 3. Frequencies and Percentage response of questions

	Strongly Disagree		Disagree		Neutral		Agree		Strongly Agree	
	Row N %	Cou nt	Row N %	Cou nt	Row N %	Cou nt	Row N %	Cou nt	Row N %	Cou nt
I enjoy learning with a smart board	1.9%	4	0.9%	2	15.3 %	33	48.8 %	105	33.0 %	71
I am fed up with teachers teaching with a Smart Board	27.0 %	58	52.6 %	113	15.3 %	33	3.3%	7	1.9%	4
When lessons are taught with a Smart Board, I learn better.	1.4%	3	2.8%	6	23.7 %	51	51.2 %	110	20.9 %	45
I can concentrate better when lessons are taught with a Smart Board.	1.4%	3	3.3%	7	28.8 %	62	50.2 %	108	16.3 %	35
I study harder thanks to Smart Board.	4.7%	10	12.1 %	26	49.8 %	107	24.7 %	53	8.8%	19
I learn many new things thanks to Smart Board.	2.8%	6	3.7%	8	24.7 %	53	51.6 %	111	17.2 %	37
I cannot understand anything when a Smart Board is used.	31.6 %	68	52.6 %	113	11.6 %	25	2.8%	6	1.4%	3
I enjoy lessons taught with a Smart Board.	1.4%	3	1.4%	3	19.5 %	42	58.1 %	125	19.5 %	42
As the Smart Board is used in lessons, I come to school more willingly	6.0%	13	9.3%	20	49.3 %	106	29.3 %	63	6.0%	13
It is very important for me to learn how to use a Smart Board.	1.9%	4	7.4%	16	32.1 %	69	44.2 %	95	14.4 %	31
I feel comfortable while using the Smart Board.	0.9%	2	4.7%	10	28.4 %	61	47.4 %	102	18.6 %	40





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I enjoy doing things on the Smart Board	0.9%	2	2.8%	6	24.2 %	52	50.7 %	109	21.4 %	46
Using a Smart Board in lessons causes waste of time.	18.1 %	39	51.6 %	111	24.2 %	52	4.7%	10	1.4%	3
I am not afraid of using the Smart Board	3.7%	8	5.1%	11	22.3 %	48	48.8 %	105	20.0 %	43
I feel nervous when using the Smart Board.	24.7 %	53	36.3 %	78	31.2 %	67	6.0%	13	1.9%	4
Smart Board can be used easily without any problems	2.8%	6	9.8%	21	38.1 %	82	38.1 %	82	11.2 %	24
Actually, there is no need to use a Smart Board in lessons.	18.1 %	39	51.2 %	110	25.1 %	54	4.2%	9	1.4%	3
It is very difficult to use a Smart Board.	19.5 %	42	54.0 %	116	25.1 %	54	1.4%	3	0.0%	0
I think Smart Board is not different from normal board in terms of learning.	15.8 %	34	43.3 %	93	27.0 %	58	11.2 %	24	2.8%	6
Writings, pictures and shapes are more understandable on the Smart Board.	1.4%	3	1.9%	4	20.0 %	43	55.3 %	119	21.4 %	46
I raise my hand more often during lessons thanks to Smart Board	5.1%	11	14.4 %	31	57.2 %	123	17.2 %	37	6.0%	13
The Smart Board enables learning lesson topics from different sources	0.9%	2	2.8%	6	26.0 %	56	52.1 %	112	18.1 %	39
It is easier to follow lessons taught with a Smart Board.	1.4%	3	2.8%	6	32.6 %	70	48.4 %	104	14.9 %	32
I prefer a normal black/whiteboard to a Smart Board.	20.5 %	44	40.5 %	87	30.7 %	66	5.6%	12	2.8%	6





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Table 4. Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
15	The distribution of I feel nervous when using the Smart Board. is the same across categories of Gender.	Independent-Samples Mann-Whitney U Test	.000	Reject the null hypothesis.
Asymptotic significances are displayed. The significance level is .050.				





Feature Learning from Big Data by using Deep Learning Technique

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ABSTRACT

The big data are created and gathered together from several fields and on IoT systems, by using the object abstraction layer the gathered data sets are transmitted from the object layer to service management layer. The upper layers will get some services like decision support and prediction from the service management layer. It provides such services by analysing the data received from the object layer. For clients, the upper layers will offer interface, it provides an intelligent service for big data. The bygone learning method was not able to manage huge amount of heterogeneous information available on distributed domains. It needs high speed and storage devices to handle these maximum numbers of varied information. Usually, the Big Data Analysis (BDA) denotes a method of information gathering, move in the centralized cloud data centers, pre-processing, assessment, and visualization.

Keywords: Big Data, Feature Learning, Deep Learning.

INTRODUCTION

The huge size data is represented as Big Data. The collection of huge size data that are growing exponentially with respect to time is referred as Big Data. In short, such information is so gigantic and complex so this information cannot be effectively stored or processed by the conventional data administration apparatuses. Hadoop was planned particularly for the analysis of huge data indexes to construct versatile, appropriated applications. To manage sizably voluminous data, Hadoop actualizes the worldview called Map Reduce characterized by Google as per which the applications are isolated into minute pieces of programming. Every one of which can be kept running on an unmistakable hub of each one of the individuals who make up the framework. The large volume of data gathered with various distributed devices refers to Big Data. Based on the specific types of datasets, the big data which contain unstructured data are classified. The big data include three features they are, variety, velocity and volume. Here, various data gathering sources are considered as varieties, the speed of analysing and processing is termed as velocity likewise the volume indicates the storage size of the big data.

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The classical deep learning methods like deep belief networks, DCNNs, and stacked auto encoders learn the features of some file types like image, text, and audio. It was understood as a single type feature learning (Vamsi Krishna et al.) [1]. For feature learning, various multi-modal feature learning methods are introduced recently and some popular multi-modal methods are multi-modal deep neural networks and deep Boltzmann machines. Initially, these multi-modal learning methods learn the features of every assorted information example and learned characteristics are integrated as a solitary vector to provide the combined illustration of heterogeneous data model. With the help of unlabelled data, the multi-modal methods will learn the representation of single modal (M Dunja) [2].

Deep learning models for big data feature learning

The following four characteristics are normally applied to define big data they are, velocity, variety, volume, and veracity. In big data, for performing feature learning process, the deep learning approach was reviewed from four features, which include, deep learning for low-quality data, heterogeneous data, huge quantity of data, and also for real-time data.

Deep learning for large volume of data

Initially, in deep learning process the data in large volume is identified as a major challenging issue. Frequently, a large number of samples with wide range of attributes are included in big dataset. Till now, a large number of deep learning approaches were developed to learn features and also to represent this huge data. The deep learning model contains some hidden layers, each with enormous neurons, leading to millions of parameters. The training process performed in deep learning model is found to be difficult. Recently, a large number of algorithms are developed to train these models, and these developed techniques were grouped under three categories they are implementation based on GPU, optimized deep learning, and parallel deep learning methods. The parallel deep learning method normally referred as deep stacking network suggested by (L. Deng et al.) [3] is identified as the most representative model. Some modules are included in this deep stacking network.

With three modules, a deep stacking network is given as an example in Fig. 1. The modules obtained in this stacking network are considered as a neural network with two sets of weight and also with a hidden layer as exhibited in Fig. 1. The three layers of lowest module are obtained in an ascending order. The lowermost layer is linear in which the original data is employed as input. The second layer is non-linear which contains hidden neurons and so called as hidden layer. The topmost layer is also identified as linear layer; it contains C output neurons to represent the classification target. Like all other deep learning methods, for mapping the input layer with hidden layer along with a bias vector and weight matrix, a sigmoid function is applied in the deep stacking network. The concatenated vector is defined by joining the previous output layer(s) with the original data. Above the lowest module, in each module the concatenated vector is utilized as an input. Consider an example, if an n-dimensional vector is applied to represent original data object and it contains 'D' dimension, class type 'C' of the input vector of the i^{th} counting from the lower to upper layer. Then the dimension is evaluated as $D = n + c \times (m - 1)$.

This deep stacking network model is paralleled so this method can be effectively applied to perform training process. The training efficiency is furthermore enhanced by introducing a tensor deep stacking network moreover on the CPU clusters. DistBelief a recent framework of software which was introduced in various machines in parallel order to perform the training process in large-scale deep learning approaches. DistBelief includes combination of both model parallelism and data parallelism to perform the training process with huge amount of data and free parameters. The model parallelism is achieved by partitioning the deep learning process into some sub-blocks, whereas this each sub-block is provided with computer to perform the training process.

A solitary sample for this DistBelief with four blocks is presented in Fig. 2 (X. Chen) [4]. To perform the training process in deep learning approach, DistBelief have to transfer the data between the computers. This data transfer improves the communication particularly, for fully connected networks like deep belief network and stacked auto-encoder. In (J. Dean et al.) [5], the deep model was partitioned into 144 blocks, for enhancing the efficiency of training



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of DistBelief. Two optimization strategies like Sandblaster and Downpour are implemented by this DistBelief to achieve the data parallelism process. The sandblaster is widely applied for batch optimization, whereas the Downpour is for online optimization. A high speed is achieved by this DistBelief process to perform the training process in the deep learning approach which is extensive. Consider an example; it achieves 12 times speedup in CNN along with 16 million images and 1.7 billion parameters on 81 machines instead of applying single machine. Besides, a significant improvement was achieved in training efficiency for an alternative deep learning framework having 14 million number of images, everyone with 16 CPU cores, 200 × 200 pixels size on 1000 machines (X. Chen and X. Lin) [6]. Therefore, this DistBelief is identified as a significant process to learn the features in deep learning as it has the capacity to scale-up enormous computers. This is identified as an important advantage of DistBelief.

The training efficiency of both DistBelief and Deep stacking network is improved by employing the multiple CPU cores for the deep learning methods which are extensive. Multiple CPU cores are applied to scale up the deep belief networks and some details regarding SSE2 instructions and data layout implementation were discussed in (V. Vanhoucke) [7]. Recently, some GPUs (graphic processors units) based large-scale deep learning architectures were explored. GPUs are perfectly appropriate for the parallel computing process of extensive deep learning methods, because they are equipped with huge memory bandwidth and maximum computing power. The advanced merits of this GPU based large-scale deep learning frameworks is demonstrated by few experiments. The best suitable example is, a deep learning architecture was developed by (R Raina) [8] to accomplish parallel training process based on GPUs in both sparse coding and deep belief networks by applying millions of training objects and more than 100 million parameters. The efficiency of parallelizing process performed in linear model was improved by applying some specialized schemes in the learning framework developed by (R Raina) [8]. For example, some parameters and training objects are included within the global memory for minimizing the data transfer.

Moreover, the sampling parameters $p(x | h)$ and $p(h | x)$ are produced to implement the parallel Gibbs sampling of both visible and hidden neurons. The speed of DBN developed by multiple restricted Boltzmann machines along with this architecture each having 1 million training objects and 45 million free parameters is increased by a factor of 70x.

Deep learning for heterogeneous data

In big data, the variety is identified as an individual characteristic, which inferred that big data is gathered from enormous sources in different layouts like data of unstructured and structured, also in the semi-structured form. Mostly the multi-model objects are found in the big datasets. For instance, both the text and image are contained instantaneously in the webpage. The multimedia objects like video clips containing text, audio, and still images are also identified as an example for multi-model approach. In multi-modal objects, diverse characteristics are included in each modality, which produce complexity in heterogeneous data. So, this heterogeneous data is identified as another challenge in the deep learning approach. Some multi-model deep learning approaches were developed to learn the representation of heterogeneous data. For instance, a multi-modal deep learning approach was advanced by (J Ngiam et al.) [9] to perform the feature learning process in audio-video objects. The framework for this multimodal deep learning model is depicted in Fig. 3. The representation and feature of both video and audio are learned separately by (J Ngiam et al.)⁹ applying the restricted Boltzmann machines. After that, for several multi-modal objects the learned features are then fused to form joint representation. Finally, the recognition or classification process of deep learning process or logical regression layer is performed by providing this obtained joint representation as an input.

Another one model for multi-model deep learning approach commonly referred as bi-modal deep Boltzmann machine was introduced by the authors Srivastava and Salakhutdinov, for performing the feature learning process in the objects of both the text and image as depicted in Fig. 4 (J. Xue et al.) [10]. Two deep Boltzmann machines are developed in this approach to execute the feature learning process for each modality of image as well as text respectively. In the same way, the joint representation is developed by concatenating the learned features of both



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image and text into a vector. The SVM classifier is trained along with the joint input representation to achieve the classification process.

(W Ouyang et al.) [11] delivered a multi-modal deep learning approach commonly referred as multi- source deep learning technique to estimate the human pose. Apart from the above said multi-model method, the primary objective of this multisource deep learning method is to learn the non-linear representation to estimate the human pose from various data sources like human body clothing and articulation. In this technique, the features are separately extracted by applying the several data sources as input for the deep learning approaches having two hidden layers. To obtain the joint representation the extracted features are fused. The heterogeneous DNN along with conditional random fields are then included in the multi-modal deep learning approaches to recognise the Chinese dialogue act and also the multimodal DNN is provided with sparse group lasso to perform the heterogeneous feature selection process (L. Zhao) [12] and so on (A. Wang et al.) [13] (N. Neverova et al.) [14] (S. Rastegar et al.) [15]. Even though they have diverse architectures, but their concepts are similar. Initially, the features are learned by multi-modal deep learning technique to accomplish the single modality, after that, for several multi-modal object the learned features are then fused to form joint representation. Finally, the recognition or classification process of deep learning process or logical regression layer is performed by providing this obtained joint representation as an input.

Deep learning for real-time data

The most important characteristics obtained in big data are high velocity, so in real time, it is identified as an essential characteristic to perform the analysis big data. In big data, the rapid speed of data gathering is identified as an important challenging issue in real-time processing. Unfortunately, a high computational complexity is obtained in various deep learning techniques particularly in large-scale DNN, so a large number of parameters are comprised in this method to perform the feature learning process in big data. So, in real-time the feature learning and big data representation process are identified as a difficult process in big data of classical deep learning approaches. The feature learning process performed with high velocity is developed for various incremental learning approaches. An object arrived by stabilising the network structure is very much valuable for the online learning approach is known as the sorts of incremental learning technique for apprising the parameters. The limited weight adjustment was applied by (L.M Fu et al.) [16] for implementing an incremental learning approach. The information regarding the present network is covered over the newly arrived objects, in which for preventing the over-training the weights are not changed. This method is very much sensitive for the former knowledge, so defining the bound beforehand is identified as a difficult task. The bounded weight modification process is suitable only for the NNs that having only two layers, but this process is found to be a difficult approach for an incremental deep learning approach.

Deep learning for low-quality data

For the feature learning process of big data, one more challenge is emerged from its veracity. In big data, only the data having low-quality is obtained particularly due to the presence of noise, imprecise objects, redundant objects, huge quantity of incomplete data, and inaccurate objects. The development of data with low quality are need to the different reason exist in here. There are different causes for the development of data with low quality. For instance, an enormous amount of information is obtained from sensors, whereas some incomplete objects are collected due to the presence of broken sensors. In big data, the transmission fault that obtained in the network also introduces some noise. The data having low-quality is not taken into account by various deep learning approaches. Otherwise it can be said in another way, i.e., methods of the deep learning are developed only to study the features of data having high quality. (P Vincent et al.) [17] presented a stacked denoising auto-encoder approach to perform the feature learning process in noise corrupted data. The original data is reconstructed from the corrupted data by the denoising auto-encoder approach to train the parameters as showed in Fig.5

The denoising auto-encoders objective function is eqn. (4.1) for the reconstruction of original input





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$$J = \sum_t E_{q(\bar{x}|x^{(t)})} [L(x^{(t)}, g_{\theta}(f_{\theta}(x)))] \dots\dots\dots (4.1)$$

Where, the corrupted cases of $x(t)$ is represented as x on the basis of corruption $q(x|x^{(t)})$ and the averages that are obtained over the instances x are represented as $E_{q(x|x^{(t)})}$.

The gradient descent methodology is applied to train the parameters of objective function. The isotropic pepper noise or Gaussian noise is added to obtain x . (P Vincent et al.)¹⁸ offered a stacked denoising auto-encoder approach to perform the feature learning process in noise corrupted data. The features of incomplete object were learned as shown in Fig. 6, by the imputation auto-encoder model developed by (Q Zhang et al.)¹⁹. The small part of the attribute values that are obtained from the object x to 0, is located to generate the simulated incomplete object x' .

The imputation auto-encoder approach output the reconstructed object z by considering an incomplete object x' as an input

$$Z = g_{\theta}(f_{\theta}(x)) \dots\dots\dots (4.2)$$

The subsequent objective function is minimized to train the parameter θ :

$$J = L(x, z) \dots\dots\dots (4.3)$$

In Fig. 7, each imputation auto-encoders are stacked within the developed deep imputation network to learn the features of incomplete objects. Recently, a non-local auto-encoder was introduced by Wang and Tao to perform the reliable feature learning in corrupted data. The neurological observation motivates their work, where the human brain is stimulated by similar input to obtain the same response. Therefore, the similar hidden patterns are provided by this neural network for parallel input objects. In detail, suppose that h_1, h_2 and h_3 are the learned representations of x_1, x_2 and x_3 , respectively. If $\|x_1 - x_2\| < \|x_1 - x_3\|$, $\|h_1 - h_2\|$ should be

smaller than $\|h_1 - h_3\|$. However, the relationship $\|h_1 - h_2\| < \|h_1 - h_3\|$ was not always guaranteed by the neural network, because the Sigmoid function i.e, non-linear function is employed in the neural networks as a function for activate the process.

A regularization term is presented by Wang and Tao by including the regularization term within the objective function to learn the reliable representations. Then, the training sample x is provided, so that a corrupted sample x_i is obtained by including a noise within x . The hidden representations for both x and x_i are represented as h_i and h respectively. Then, regularization is represented as in eqn. (4.4),

$$\sum_i \omega_i \|h - h_{i,p}\| \dots\dots\dots (4.4)$$

Where the weight of the i^{th} corrupted sample is represented as ω_i . The regularization term is applied to obtain the non-local auto-encoder method's objective function.





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$$J = \left\| x - g_{\theta}(f_{\delta}(x)) \right\|_2^2 + \lambda \sum_i \omega_i \|h - h_{i,p}\| \quad (4.5)$$

This experiment demonstrates that superior performance is achieved in both image restoration and denoising process by the non-local auto-encoder approach.

CONCLUSION

The Deep Learning has the benefit of possibly delivering a solution for addressing the data assessment and learning difficulties discovered in the huge volume of input data as resisted to feature manufacturing algorithms and additional conventional machine learning. Specifically, it supports owing to segregating the representation of complex data from the extensive volume of unsupervised data. For the BDA, this creates it as an essential tool that contains information research from the huge gatherings of raw data which is commonly unsupervised and unclassified. Deep learning method proposed in this model was used for the feature learning of big data.

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<p>Fig. 1 Deep stacking network with three modules (L. Deng et al.) [3]</p>	<p>Fig..2 Example of DistBelief with four blocks (J. Dean et al.) [5]</p>
<p>Fig. 3. Architecture of the multi-model deep learning model (J Ngiam et al.) [9]</p>	<p>Fig. 4. Architecture of the bi-modal deep Boltzmann machine (W Ouyang et al.) [11]</p>





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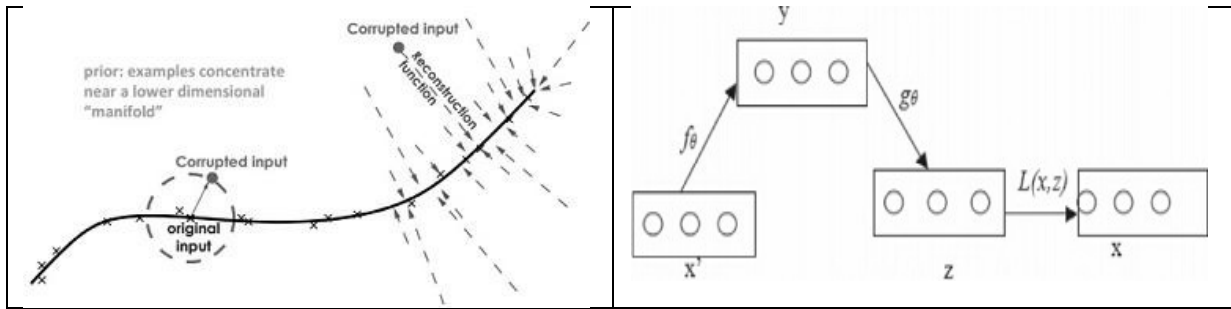


Fig. 5. Denoising auto-encoder (P Vincent et al.) [18]

Fig. 6. Imputation auto-encoder model (Q Zhang et al.) [19]

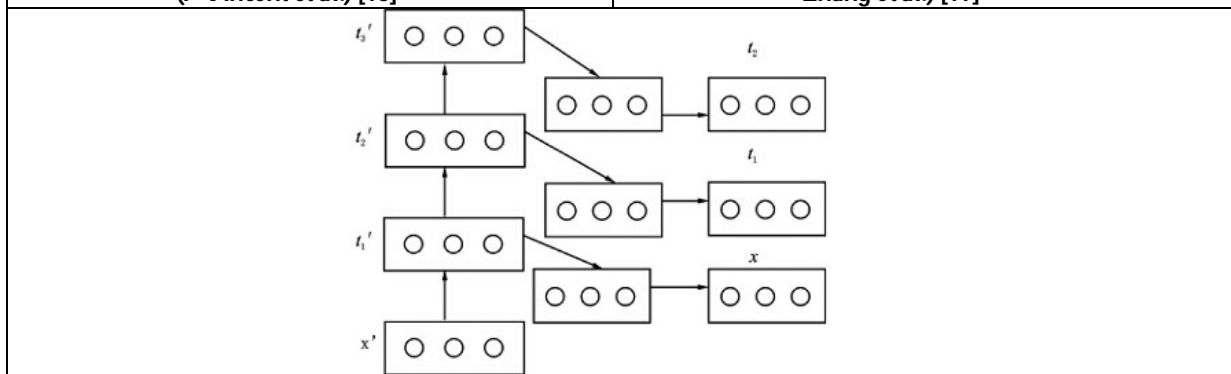


Fig. 7. Deep imputation network (Q Zhang et al.) [19].





Development of Optimal Information System to Prevent Deterioration of Digestive Function after Gallbladder Surgery

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ABSTRACT

This study is to develop an optimal information system to prevent deterioration of digestive function after gallbladder surgery. The survey was conducted on 104 people who visited the general hospital in Chungcheongnam-do from October 7 to December 8, 2020. The general characteristics of study subjects are analyzed by χ^2 -test. Experimental comparison of digestive conditions before and after application of the information system was performed with t-test. The main findings are as follows. Firstly, the group that consumed a lot of fatty food was significantly higher than the control group ($\chi^2=1.95$, $p<.05$). Secondly, bad cholesterol levels decreased significantly after application than before information systems were applied ($t=4.73$, $p<.01$). Therefore, it has been confirmed that the application of this information system is effective in relieving digestive symptoms and complication in patients with gallbladder resection. This information system is expected to contribute to the treatment of other cancer patients.

Keywords: Gallbladder resection, Information system, Complications, Digestive function, Deterioration

INTRODUCTION

Gallbladder cancer is a cancer that occurs in gallbladder. Adenocarcinoma, which occurs in gallbladder cells, accounts for almost all of them[1]. The main causes of gallbladder cancer are polyps and gallstones. Chronic inflammation is the cause of gallbladder cancer, and the main cause of this is gallstones, which are the most important risk factors for gallbladder cancer[2],[3]. Gallbladder cancer accounted for 3% of the total cancer cases, with 6,682 new cases occurring in 2018. The sex ratio of gallbladder cancer is 1.1:1, which is more common in men. Gallbladder cancer has a fairly high incidence among cancers that occur in Korea. The symptoms of gallbladder cancer are silent and are one of the diseases with a low five-year survival rate. Even if gallbladder cancer is found, its survival rate is not high, so early detection is very important. The survival rate of gallbladder cancer is about 29% and the prognosis is not good. There are important organs around the gallbladder, such as liver, small intestine,

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colon, pancreas, stomach, etc., so if it spreads to this side, the prognosis is not good. Complications and metastasis are high after cholecystectomy. So, management is needed for this purpose. Regular examinations and preventive measures are needed to prevent gallbladder cancer. However, there is no choice but to avoid and prevent what is pointed out as a risk factor in daily life. Digestion may deteriorate after cholecystectomy[4],[5] To this end, the information system needs to be applied. In prior studies, most of the studies are on survival rates after gallbladder resection. So far, no studies have applied information systems to prevent deterioration of digestive function after cholecystectomy [6],[7]. Therefore, this study is to develop an optimal information system to prevent deterioration of digestive function after gallbladder surgery. Through this, this is to prevent digestive problems and complications after cholecystectomy and to verify the efficiency of the information system.

MATERIAL AND METHODS

New Strategic Execution of information Systems

Figure 1 shows the new execution effect of the information system. 1) Connectivity of the information system 2) Gallbladder cancer information 3) Efficiency of information systems 4) Reliability of health information 5) Patient management of the information system This information system is designed to optimize patient care through a new information system in Figure 2

Materials

The survey was conducted on 104 people who visited the general hospital in Chungcheongnam-do from October 7 to December 8, 2020. The digestive function was compared with 5, 10, 15 and 20 days before and after the information system was applied.

Methods

The general characteristics of study subjects are analyzed by X^2 -test. Experimental comparison of digestive conditions before and after application of the information system was performed with t-test.

RESULTS

General Characteristics of Study Subjects

Table 1 presents general characteristics of study subjects. The experimental group sitting for a long time every day was higher than the control group. The group that consumed a lot of fatty food was significantly higher than the control group ($X^2=1.95$, $p<.05$).

Experimental Comparison of Digestive Conditions

Table 2 shows comparisons between before and after experiments of digestive function states. Bad cholesterol levels decreased significantly after application than before information systems were applied ($t=4.73$, $p<.01$).

The Process of Change in Digestive Symptoms

Figure 3 shows the process of change over time of digestive symptoms. The experimental group continued to improve its digestive function after the information system was applied. However, the group showed a tendency to decline again after the 16th. The control group was the highest on the 10th, followed by a slight decrease.

Changes in Health Practice after Cholecystectomy

Figure 4 shows changes in health practice over time after cholecystectomy. Chiropractic pressure increased continuously after the system than before information system application. However, it tended to decline after 15 days.



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DISCUSSION

This study measures the new experimental effectiveness of the information system to prevent digestive functional degradation after gallstone resection. According to this study, bad cholesterol levels were significantly lower after application than before information system application. It was similar to prior studies that alleviated insomnia through various functional applications in breast patients[8],[9]. There is evidence that chiropractic stimulates the body's blood points, which is effective in improving health after gallbladder cancer surgery. Physical strength is significantly improved after system application than before information system. The results of this study are similar to the results of strengthening immunity of stomach cancer patients in previous studies[10],[11]. Physical strength is the strength of the mind and body that can work with the human body. To improve physical strength, patients need to strengthen their immunity after cholecystectomy. Healthy habits build up every day to strengthen the body's immune system. Since this study is a limited regional survey, it is expected that it will be necessary to expand the sample size in the future. However, this study has been confirmed that the application of this information system is effective in relieving symptoms in patients with gallbladder resection. The significance of this study is expected to contribute to the treatment of other cancer diseases based on the results of this study.

CONCLUSION

This study is to develop an optimal information system to prevent deterioration of digestive function after gallbladder surgery. The main findings are as follows. Firstly, the group that consumed a lot of fatty food was significantly higher than the control group ($X^2=1.95$, $p<.05$). Secondly, bad cholesterol levels decreased significantly after application than before information systems were applied ($t=4.73$, $p<.01$). Therefore, it has been confirmed that the application of this information system is effective in relieving digestive symptoms and complication in patients with gallbladder resection. This information system is expected to contribute to the treatment of other cancer patients.

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Table 1. General Characteristics of Study Subjects

Variables	Expei group	Cont. group	X ²
	N(%)	N(%)	
Age			
≤49	8(15.4)	10(19.2)	11.85*
50-59	15(28.8)	13(25.0)	
60-69	11(21.2)	17(32.7)	
≥70	18(34.6)	12(23.1)	
Sitting time(hrs.)/daily			
<5	12(23.1)	15(28.8)	7.29
5-9	19(36.5)	21(40.4)	
≥10	21(40.4)	16(30.8)	
Gender			
Men	24(46.2)	27(51.9)	3.17
Women	28(53.8)	25(48.1)	
Greasy food			
A lot of	33(63.5)	20(38.5)	1.95*
Little	19(36.5)	32(61.5)	
Drinking alcohol			
Yes	35(67.3)	21(40.4)	4.22*
No	17(32.7)	31(59.6)	
	52(100.0)	52(100.0)	

*p<.05





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Table 2. Experimental Comparison of Digestive Conditions

Variables	Before	After	t
	Mean±S.D.	Mean±S.D.	
Abdominal pain	42.18±5.29	30.64±3.42	2.95*
Displeasure	45.62±0.73	39.52±0.68	5.34
Fever	39.28±1.46	25.17±1.93	1.82*
Jaundice	51.04±3.81	40.92±3.85	3.59
Nausea	29.35±1.47	21.58±5.44	-2.6 5.10
Hypertension	55.71±0.53	41.92±0.67	1.76*
Bad cholesterol	53.14±2.76	39.17±2.54	4.73**
Greasy food	51.39±4.18	35.64±3.92	2.81**
Regular exercise	24.54±0.62	39.17±0.38	-3.27*
Physical strength	30.26±3.19	43.53±3.52	-1.64*
Diabetes mellitus	47.91±1.82	31.26±1.84	5.42*

*p<.05 ** p<.01

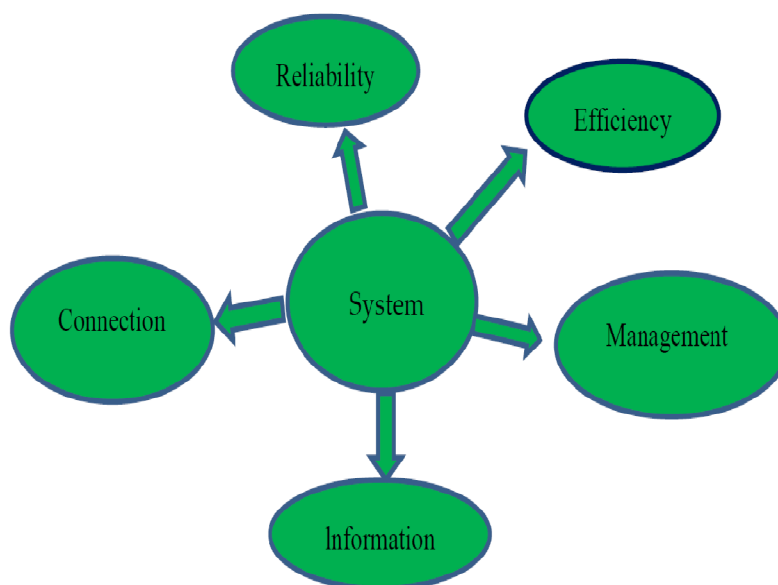


Figure 1 Successful Execution of the information System





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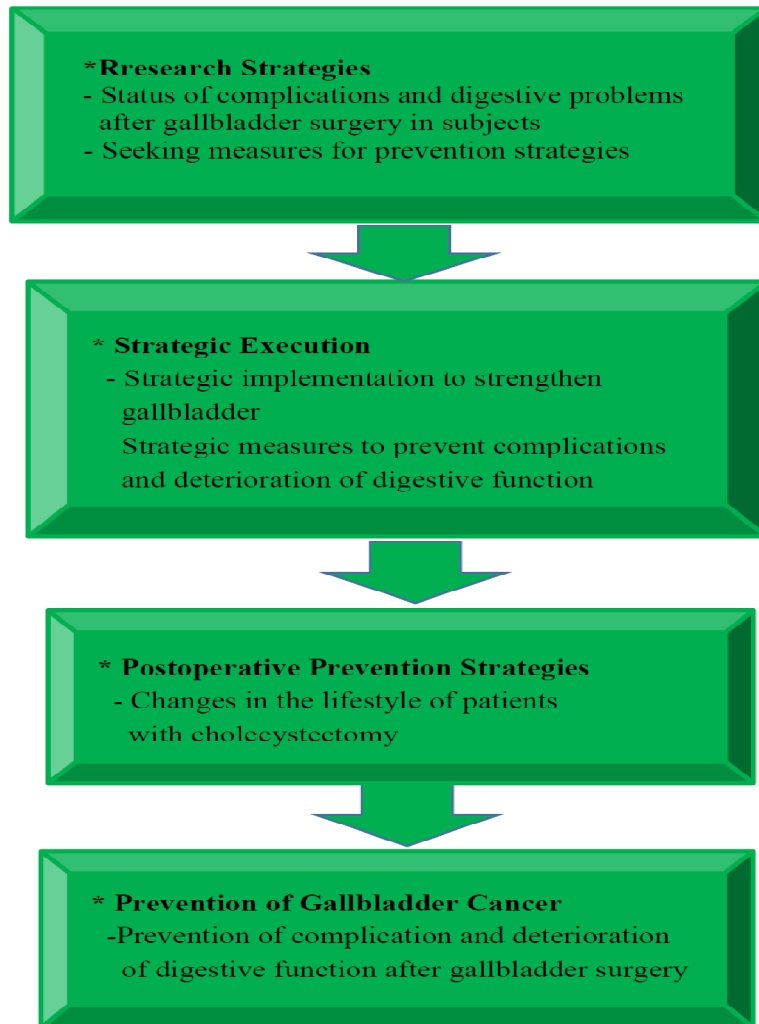


Figure 2. Design of Patient Management through Information System

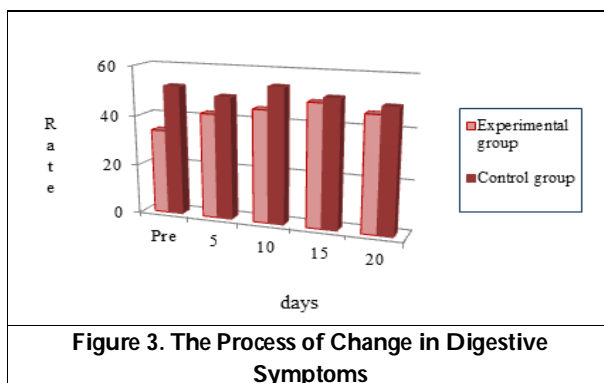


Figure 3. The Process of Change in Digestive Symptoms

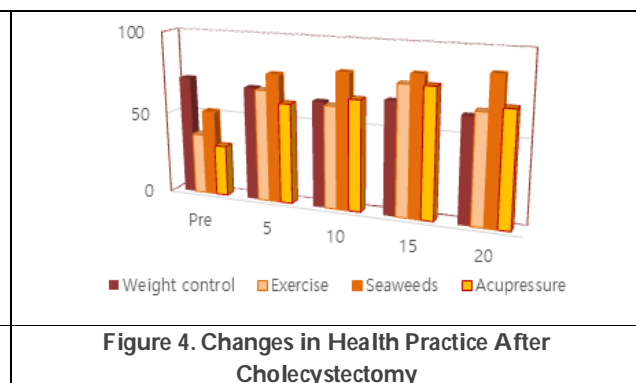


Figure 4. Changes in Health Practice After Cholecystectomy





***In silico* Analysis of Gas Permeability Properties of Polyvinyl Acetate and Poly - 1, 2 - α - D – Glucose Composite**

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ABSTRACT

A blend is formed from the combination of two or more components. The change in properties of polyvinyl acetate and poly-1, 2- α -D-glucose was studied to form a miscible blend using Biovia Materials Studio. The permeability properties of composition of the blend were studied so that it can be used in different potential applications. The molar volume and density of the blend was observed to be decreased with increase in vinyl acetate fraction. The permeability properties of the composite were observed based on permeability of oxygen, nitrogen, and carbon dioxide. From the results, it was noticed that the permeability for all the gases such as oxygen, nitrogen and carbon dioxide are increased with increase in mass fraction of vinyl acetate. This technique will be useful to determine pairs without performing laboratory experiments saving materials, money, and time.

Keywords: Blend; Biovia Material Studio; Gas permeability properties; Polyvinyl acetate; In silico analysis

INTRODUCTION

Blends or composites are formed with combination of more than one component where components do retain their identity in the mixture. As it is incredibly tough to seek out multiple properties from a single material, it is suggested to mix totally different component thereby enhancing the properties of the material. Development of one material with the required property involves important analysis and time. A blend uses the upsides of various materials, blend them to get the ideal property. In this manner a blend spares time to build up a new material along with decreasing the cost of advancement of products with wanted properties. Polymer blends can be made of two or more polymers, or fibers and polymer, or particles and polymer. Nano material modified polymers paved the way to multi-functional materials. Polymers combined with carbon based (graphene, carbon nanotube) nano-materials [1] have drawn attention. Biodegradable polymers- natural fiber composites [2] have been accounted for to upgrade mechanical properties and water resistance. Researchers have given attention on fire retardant/fireproof materials

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[3]. There are reports of inorganic additives in polypropylene; that can improve fire retardancy without expanding the weight [4]. Scientists have emphasized on synthesis and production of lightweight composite materials having high quality significant for upgrading fuel efficiency in the field of transportation [5]. There are uses of composites in structural Engineering because of high strength to weight proportion and resistance to corrosion. Consequently, glass fiber incorporated polymers, latex polymer cementations composites [6] were created for development of construction of bridges, light rail transit, mining and tunneling, holding dividers and other waterside buildings. All the previously mentioned models depended on research center analyses.

Usually, blends are prepared by trial and error method. Thus, it involves wastage of materials, time and money. Thus, researchers have focused on the use of in silico approach to develop new blends. Software (Materials Studio [7]) has been used to identify compatible pairs. Poly (vinyl acetate) (PVAc), a biocompatible biodegradable (due to side-chain hydrolysable groups), polymer was often used in biomedical applications, including medicinal and in tissue engineering among the numerous polymers [9, 10]. In contact with blood, hydrogels such as PVAc containing functional groups such as COOH typically demonstrate strong biocompatibility [11]. PVAc is an inert polymer with the quality that living tissue does not cause an adverse reaction. PVAc has also been shown to remain inert in blood vessels, suggesting that this polymer is non-toxic [12]. Researchers have used D-glucose in combination with other materials to different applications [13]. Polysaccharides has been reported to be used in Curtius reaction [14]. This study is intended to find out the change of gas permeability value of blend of polyvinyl acetate (PVAc) and poly-1, 2- α -D-glucose with different proportions.

MATERIALS AND METHODS

Software used: Materials studio module of Biovia software (Dassault Systems of France) was used for analysis. The software utilizes machine learning techniques and standard algorithms to predict the level of interaction.

Methodology: The structures of polyvinyl alcohol and poly-1, 2- α -D-glucose were fed to the synthia menu of Materials Studio. It was then run for different weight fractions of the components. Different properties of the composite were displayed in a tabular form. The values were used to plot graphs to identify the effect of weight fraction of polyvinyl alcohol on the mechanical properties of the composite.

RESULTS AND DISCUSSION

In this work the use of polyvinyl alcohol and poly-1, 2- α -D-glucose as potential components of a composite was analyzed using Biovia Materials Studio. Synthia module of Biovia Material studio uses pre-defined correlations (advanced quantitative structure-property relationships) to compute a wide range of polymeric properties. To predict the characteristics of polymers group additive methods were used for many years as these methods are particularly fast and easy to use. They are therefore incredibly useful when a simple calculation of a property is appropriate without a thorough knowledge of the atomist interactions that lead to it. The biggest drawback of these approaches though is their reliance on a group contribution database. Therefore, if a polymer includes a group by which the contribution of the group cannot be determined, the property of that polymer cannot be measured. To address this constraint, Synthia's approach uses topological polymer knowledge in predictive correlations. The graph theory connectivity indices are used. No selection contribution database is required, and the properties for any polymer consisting of a mixture of nine components can be predicted: hydrogen, carbon, oxygen, nitrogen, sulfur, silicon, chlorine, bromine, fluorine.

Molar volume: It is the volume occupied by one mole of a substance. Figure 1 shows that the molar volume of the composite decreases with increase in mass fraction of vinyl acetate.



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Density: Increase in density indicates decrease in porosity. A higher porosity will enhance the surface area making it suitable for absorption/adsorption applications. Figure 2 shows that the density of the composite decreases linearly with increase in mass fraction of vinyl acetate.

Permeability of gas: Permeability is the rate at which the gas can pass through the polymer membrane after the gas has come to equilibrium. Lower permeability indicates longer time lag for the gas to pass through the membrane. Figure 3 and 4 display the permeability of oxygen and Nitrogen at 298 K with increasing mass fraction of vinyl acetate with respect to 1, 2- α -D-glucose, respectively. It indicates that the permeability increases exponentially with increase in mass fraction of mass fraction of vinyl acetate. Figure 5 shows the permeability of carbon dioxide at 298 K with increasing mass fraction of vinyl acetate with 1, 2- α -D-glucose. The composite permeability increases exponentially with increase in mass fraction of vinyl acetate. Thus, the results indicated that an increase in vinyl alcohol fraction reduces the permeability of different gases. The rate of permeability might be influenced by the molecular weight of the gases.

CONCLUSIONS

The possibility of use of polyvinyl alcohol and poly-1, 2- α -D-galactose to form a homogeneous blend was explored using Biovia Materials Studio. The molar volume, density, and permeability of different gases such as oxygen, nitrogen and carbon dioxide were demonstrated using Biovia Materials Studio. From the results it was found that the molar volume and density decreased with increase in vinyl alcohol fraction. The permeability properties of the composites were studied based on permeability of oxygen, nitrogen, and carbon dioxide. The results indicated that the permeability for all the gases increased with increase in mass fraction of vinyl acetate. Due to increase in gas permeability value, the blend can be used in different practical applications such as medicine, biocompatible coating, packaging, etc. Usually, components for a blend are identified experimentally. This *in silico* study will help to determine components of a blend without doing experiments in laboratory.

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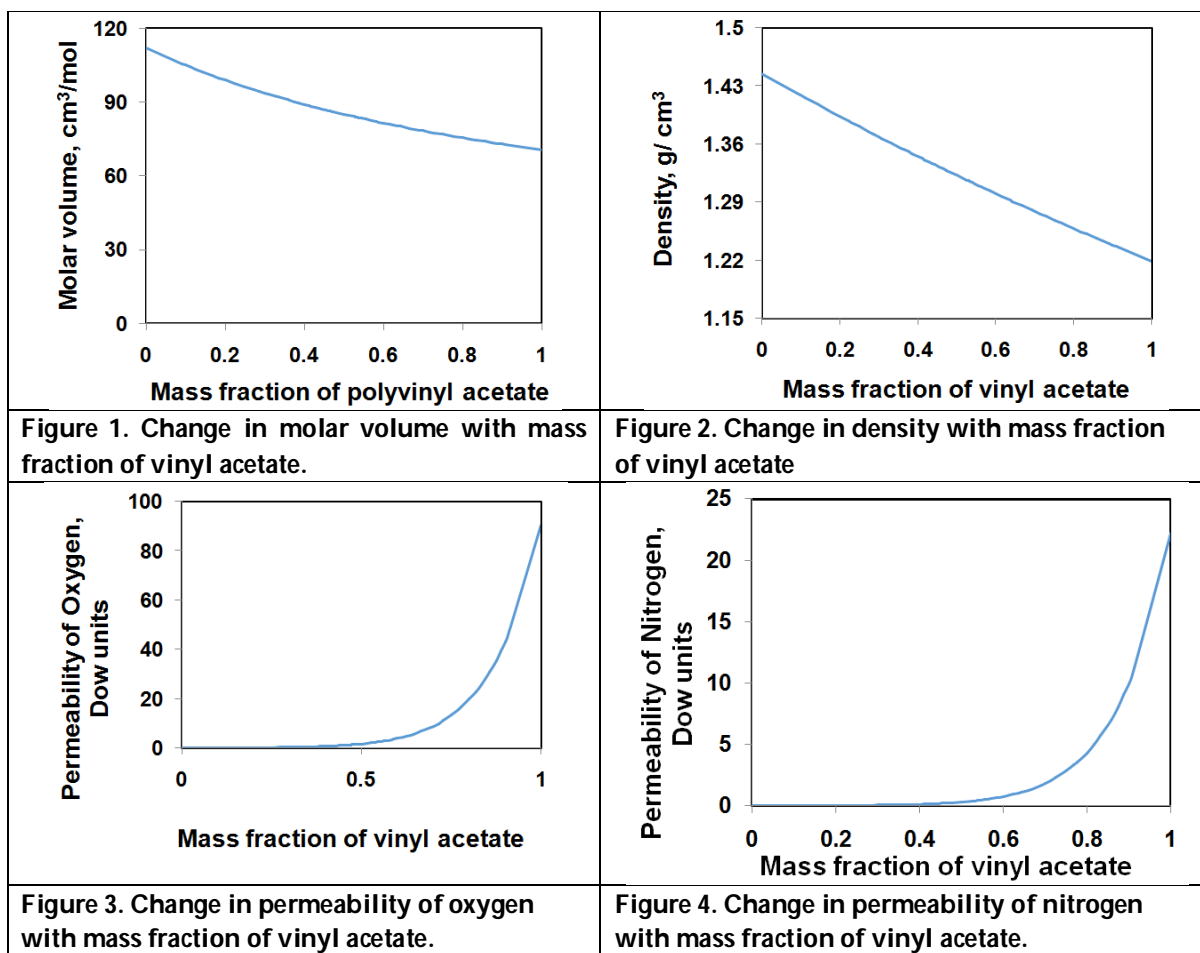
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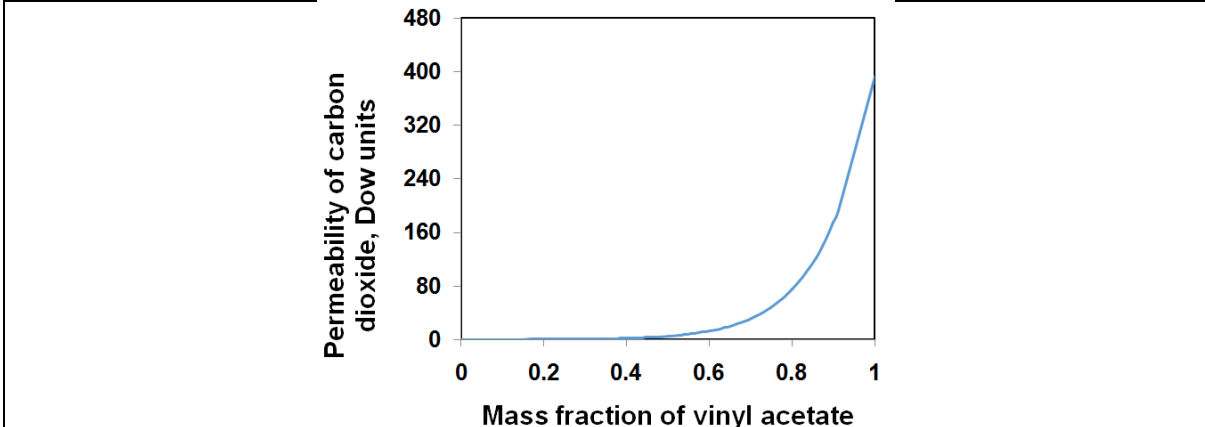


Figure 5. Change in permeability of carbon dioxide with mass fraction of vinyl acetate.





Video QOE Prediction using Regularization Based Regression with Network QoS Parameters

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ABSTRACT

With the growing Multimedia data traffic on the Internet, it is estimated that Video streaming service will account for almost 73 to 75% of the total data traffic. An ever-increasing demand for video delivery services has offered an exponential growth in multimedia business but along with high profits has raised the service expectation by many folds. Since the services are moving from technology centric to user centric, entire focus is on user's satisfaction of the service. In case of streaming video service which is the intent of study in this paper, Quality of Experience (QOE) is the grade of service given by customers to indicate the level of service acceptance. In this paper video QOE is predicted which is in line with the subjective evaluation of the videos using regularization technique to take into account various video quality influencing factors, their inter dependencies and redundancies.

Keywords: Quality of Service (QoS), Quality of Experience (QoE), regularization, linear regression, network impairments, Mean Opinion score (MOS).

INTRODUCTION

For over 10 years, network local area is zeroing in on checking the exhibition of services and investigating various strategies to improve the equivalent. QoS is above all characterized as the assistance given by a service supplier or application provider. QoE is characterized as a rating given by a, a great many clients encounters sound or video or both. It is amazingly mind-boggling errand to build up a connection amongst QoE and QoS and different potential



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models are referenced in [1]. At first QoS used to be the solitary factor which used to consider, and it tends to be determined utilizing various strategies. It is fundamental to know the experience of a customer after the assistance has been given. As per an overview, directed by Infosys, nearly everybody would have encountered a great deal of issues with the services at a point or other and liked to change to some other video service provider rather to settle the issue. In this way, it is significant for a video service provider to investigate and improve their services too to keep up their norms to remain with the rivals in the business.

Elements which may affect client's experience can be terminal sorts, mental elements, video boundaries and service boundaries [4]. In this paper we are zeroing in on Wi-Fi services, as this paper is particularly about network impairments. As the utilization of advanced cells, tablets and workstations are expanding step by step, use of Video related applications are likewise expanding [2]. Primary worry for the video service provider to the specialist co-ops is to fulfil the client with their degree of satisfaction. Maintain that connection between the help given and the client fulfilment. QoS and QoE are profoundly related and there should be an appropriate equilibrium looked after [1]. Machine Learning (ML) has its own importance in all the domains. Main aim of this paper is to discuss how ML algorithms are applied to improve the prediction of video QoE service provided which helps to reach the user expectation.

RELATED WORK

Study of network is the main concept of the paper and as there are various types of networks available, it is necessary to specify the study of network. As the usage of Wi-Fi seems high, it is needed to study various Wi-Fi standards. Nature of involvement directs the achievement or disappointment of an application or service by the end clients. Specialist video service provider need to have nonstop assessment of the services given to clients; video service provider should offer superior video services to simply ensure client encounters the best constantly. To meet the assumptions for a client, it is imperative to break down the administrations completely and discover the boundaries affecting client satisfaction. The most powerful factors can be network boundaries which firmly affects client satisfaction.

Fig.1 Nature of video service management is fundamentally reliant on to two distinct layers of OSI model, application layer and network layer. Various boundaries that are considered in application layer are video and sound codec type, frame rate, resolution, colour and so forth. The boundaries of network layer are delay, jitter, packet loss, throughput and so forth Wi-Fi, network layer and application layer have an extraordinary effect together on client experience as demonstrated in Fig 1.

Customer Experience**Service Provider**

QoS and QoE are between reliant as demonstrated in Fig 2. Client subsequent to encountering the QoS will rate the assistance which is QoE. In light of the rating given by the client, Specialist co-op thusly should improve the assistance according to the client necessity which will be a ceaseless cycle. QoE is the term which fundamentally gives the last appraising from client however that to accomplish, we need to zero in on different boundaries like application boundaries, network boundaries, sort of client gadgets, mental elements and so forth as demonstrated in Fig3. Machine Learning calculations have likewise been actualized to discover the connection among QoE and QoS [1]. As per an overview led, supervised, and semi supervised are the best fit in finding the best model for the relationship [1]. It is difficult to build up a nonexclusive ML model to figure a QoE as it is being affected by various boundaries. As there are a great deal of papers with the execution of ML models and their correctness. As indicated by [7], Random Neural Networks (RNN) shows the significant exactness. [4] clarifies that they executed six diverse ML algorithms among which Random Forests (RF) performs better among them.





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Proposed Algorithm

In this paper all the standards of Wi-Fi like 802.11 a/b/g/n/ac are studied. Various factors influencing the QoE, there might be an alternate solution to remaining factors, but the network parameters are the important ones to be considered as the remaining factors depends on user and might vary from time or conditions which are complex to study. Network parameters solely depend only on network or service provider. It is important to study the network parameters and provide the network provider the detailed report of the parameters affecting. MOS is considered subjectively. NETSIM is used to study the various conditions of video as the network parameters. In this paper, continuous normal VBR which uses normal distribution for the generation of bits per pixel are studied. It is a simple model where consecutive packet sizes are not dependent on one another. Fig 4. gives the information how delay impacts the MOS. Plot indicates the inverse proportionality of delay and MOS. Fig 5 indicates the inverse relation between Jitter and MOS. Most of the samples indicate more the jitter value less the MOS

Fig6. implies the more packet loss, MOS value reduces. It clearly explains the packet loss the most important parameter which influences the QoE. For all the lower values of packet loss, MOS is clearly poor. In this article we present an AI model for Nature of Involvement expectation, using the higher dimensional element space as contribution for the Nature of Involvement forecast work out. Accordingly, discriminative Nature of Involvement portrayal by catching complex Nature of Involvement Impact Elements and the hidden illustrative transaction were learned. Video Nature of Involvement depends on numerous frequently interrelated components, from framework boundaries incorporates goal and edge rate for segment data incorporates sex and age. The variables frequently portrayed as impact factors, fall into three different ways: framework Uncertainties, setting Uncertainties, and human Uncertainties. Since the mind-boggling dynamic cooperation between different Uncertainties were inconvenience for measuring, past work just spotlights on highlights that were given and, in this way, gives almost no knowledge on effect of the exchange among Uncertainties on Nature of Involvement forecast. Data driven approach is the method which requires less knowledge in up-front and need more back-end statistics and computations. Data driven approach uses estimation methods like split, k-fold cross validation, boosting etc. Cross validation is a technique which tests and trains each part of dataset in equal number of times.

Fig 8. explains the cross-validation technique with k-fold = 5, k iterations are followed, each fold gets a chance to act as both train set and test set. Cross validation provides an unbiased estimation on the models but the model itself uses randomness. Albeit, different video Nature of Involvement forecast undertakings, paying little mind to type of arrangement or relapse, regularly share critical similitudes in element pre-handling approaches or calculation of moderate results. Albeit many displaying structures reject the likenesses and complete excess designing errands. To address the issues, we have objective to plan the Nature of Involvement forecast conspire with underneath plan rules and objectives. At first demonstrating may fuse earlier space information, instead of depends just on data given by the dataset. In this way, estimation, displaying, and expectation of video Nature of Involvement stay hazardous errands. We propose Lasso regularization based QOE, the portrayal learning structure of video Nature of Involvement expectation in a start to finish way as given in Figure.9. Lasso technique of regularization minimizes the number of squared residuals using the L1 criteria, accordingly, to limit the size of coefficients in the regression model. Assuming that the dataset has n observations and p predictors, then $a_i = (a_{i1}, a_{i2}, a_{i3} \dots a_{ij})$ where $i=1, 2 \dots n$ and $j = 1, 2 \dots p$

$b = (b_1, b_2 \dots b_n)$ is the response variable
and $\beta = (\beta_1, \beta_2, \beta_3 \dots \beta_p)$ is the coefficient set.

In a standard multiple linear regression
 $y = 1\beta_0 + X\beta + e$, assuming $e \sim N(0, \sigma^2)$, & Eq. (1) is used to minimize the residual sum of squares to determine β :

$$\hat{\beta} = \text{arg}_{\beta} \min \sum_{i=1}^n (b_i - \beta_0 - \sum_{j=1}^p \beta_j A_{ij})^2 \text{-----} (1)$$





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ordinary least squares (OLS) regression method is not very efficient due to low bias and high variance, which leads to low prediction accuracy. Lasso regression, is used to improve OLS estimates. Lasso, on the other hand, is used for automatic variable selection and continuous shrinkage for the coefficients of predictors. Lasso regression uses L1 norm penalized least squares

$$\hat{\beta} = \text{arg}_{\beta} \min \sum_{i=1}^n (b_i - \beta_o - \sum_{j=1}^p \beta_j A_{ij})^2 + \lambda \sum_{j=1}^p |\beta_j| \text{-----} (3)$$

Where lambda (λ) is the penalty parameter

NETWORK CONFIGURATION

NETSIM (Network Simulator) is a tool which helps us to study the behaviour as well the performance of a network by virtually creating the same network. Fig 10 shows how a network can be established virtually using a NETSIM. A required model can be built using devices, applications, links etc. NETSIM has played an important role in studying the networks under different conditions. Dataset used in this paper has been developed using NETSIM. The different conditions we applied to network helped us to build a reliable dataset. NETSIM provides that flexibility to adjust the variables as per the range and let us understand the study of network and shows its metrics as shown in Fig11. Study of different networks is done in this paper with different conditions like varying in number of nodes in a network, varying the distance between the nodes, varying the frame rate, varying bit rate, varying bandwidth etc.

NETSIM provides various metrics as shown in Fig12. Various metrics like link, Queue, TCP, IP, UDP, IEEE 802.11, Application are studied. As we are mostly focusing on network parameters in this paper, focus will be on IEEE 802.11 and application metrics. As we are studying IEEE 802.11 network, detailed metrics about the network are clearly explained in Fig13. Applications metrics is even studied as it provides the metrics related to some of network parameters like Packet information, Delay, Jitter, etc as shown in Fig14. All the data of network parameters has been collected using NETSIM. MOS has been considered using subjective analysis. As the various parameters of dataset have different units, it is important to normalise the dataset. Fig 15. shows the samples of the dataset after the normalisation procedure is done. This is given to Lasso regression model and technique like CV is followed to tune the parameters and find the best parameters.

RESULTS

Fig 16 shows the important features obtained using Lasso which reveals Packet loss is the most important feature and the throughput is the less important feature. Accuracy is measured using Spearman Rank Order Correlation Coefficient (SROCC): SROCC which measures the direction and strength of the monotonic relationship between two variables (e.g., predicted MOS and true MOS) and Pearson Linear Correlation Coefficients (LCC): Unlike SROCC, LCC that measures the linear correlation between two variables and presents the degree of simplicity of a trained machine learning model. For regression tasks of video QoE prediction, we use the Spearman Rank Order Correlation Coefficient (SROCC) and Linear Correlation Coefficient (LCC) as metrics. Both are widely used in comparing regression results in many QoE related papers.

DISCUSSION AND CONCLUSION

QoE and QoS correlation is the toughest task to measure and it is because the number of factors impacting it. This paper thoroughly explains the different network parameters and its impact on dataset developed using NETSIM using data driven approach. Lasso regression gives better results than any ML algorithm alone and it has been proved in this paper that accuracy for Lasso regression is high. Methods like logistic regression, SVM, RF, GBM have been trained and tested on our dataset where Lasso regression achieved higher accuracy using CV. To improve the





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more generic model in calculation of QoE, it is important to consider more influencing factors into account and study them.

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Fig 1: Factors affecting the end user experience

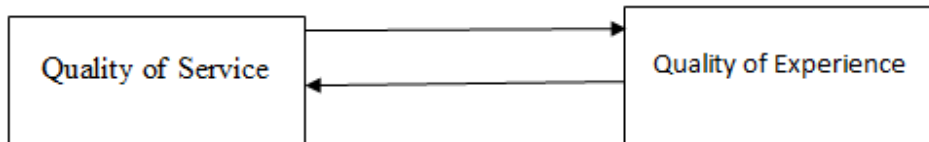


Fig 2: Relation between QoS and QoE

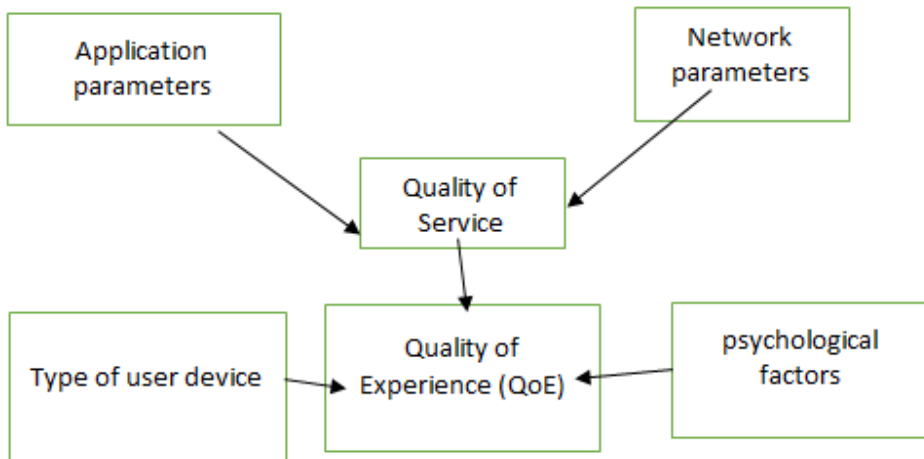


Fig 3: Different parameters affecting QoE

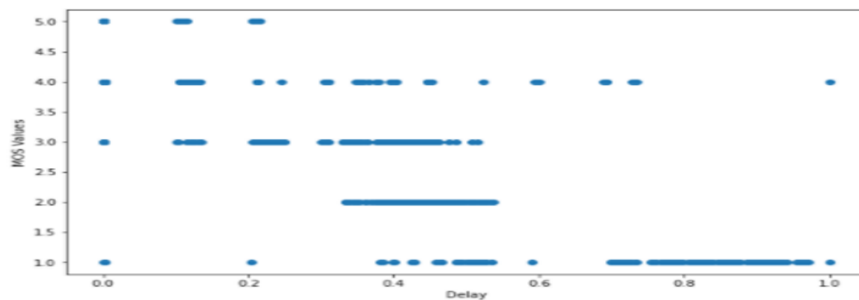


Fig 4: Plot between Delay and MOS





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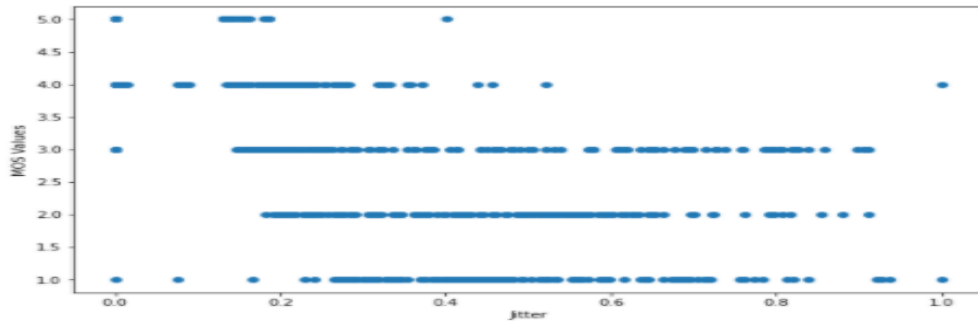


Fig 5: Plot between Jitter and MOS

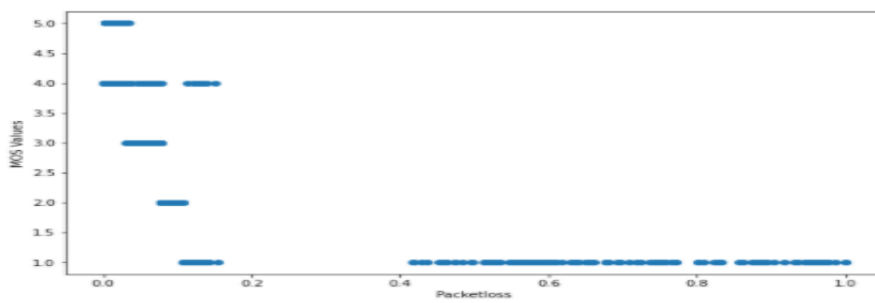


Fig 6: Plot between packet loss and MOS

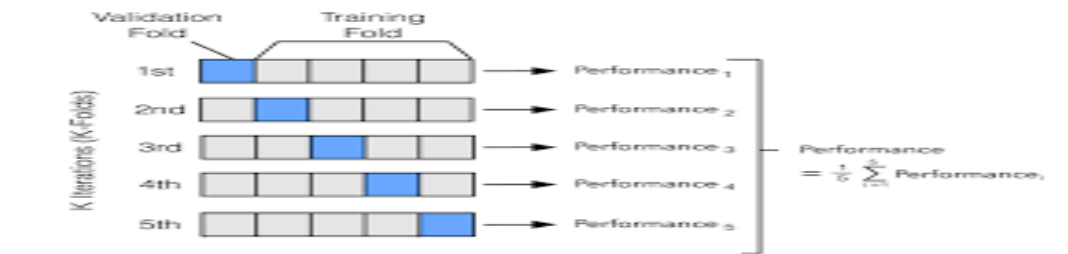


Fig 8. K-Fold Cross Validation

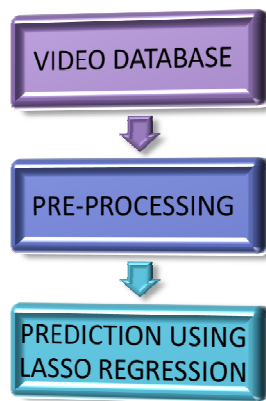


Fig 8. Proposed Method





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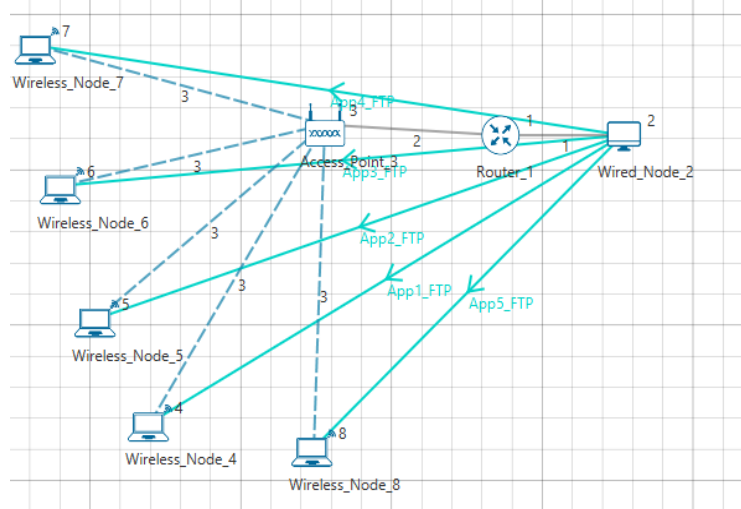


Fig 10: Network establishment in NETSIM

MODEL

Model_Type	CONTINUOUS_NORMAL_VBR
Frame_Per_Sec	10
Pixel_Per_Frame	10000
Mu	0.52
Sigma	0.23

Fig 11: Parameters which can be adjusted as required for a video type

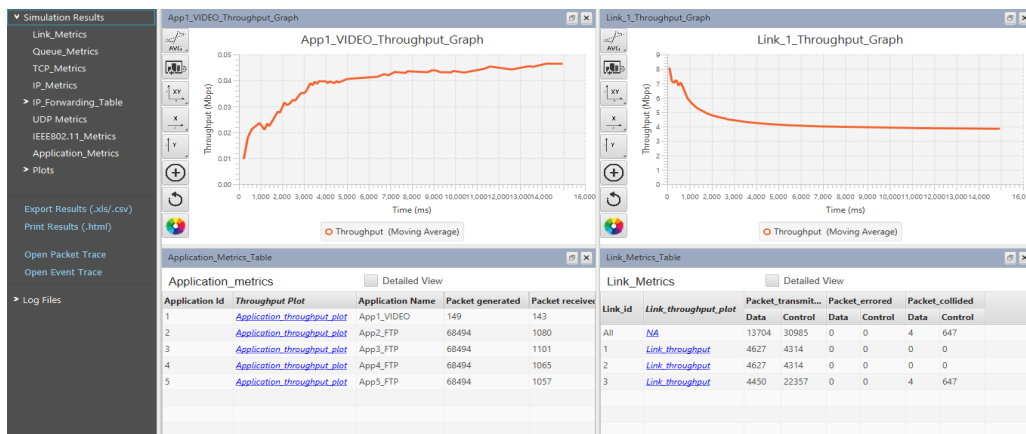


Fig 12: Various metrics of a network





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IEEE802.11_Metrics Detailed View

DeviceId	InterfaceId	Frame Sent	Frame Received	RTS Sent	RTS Received	CTS Sent	CTS Received	Successful Backoff	Failed Backoff
3	1	4460	4306	4318	0	0	4318	4719	4258
4	1	0	0	0	0	0	0	0	0
5	1	1188	1225	0	1094	1094	0	1188	380
6	1	1193	1103	0	1101	1101	0	1193	403
7	1	1164	1067	0	1066	1066	0	1164	384
8	1	1148	1059	0	1057	1057	0	1148	380

Fig13: Detailed view of application metrics

Application_metrics Detailed View

Application Id	Throughput Plot	Application Name	Source Id	Destination Id	Packet generated	Packet received	Payload generated (bytes)	Payload received (bytes)	Throughput (Mbps)	Delay(microsec)	Jitter(microsec)
1	Application throughput plot	App1_VIDEO	2	5	149	143	89655	86581	0.046177	596716.831049	8255.274718
2	Application throughput plot	App2_FTP	2	5	68494	1080	100000000	1576800	0.840960	7453849.956380	13871.020593
3	Application throughput plot	App3_FTP	2	6	68494	1101	100000000	1607460	0.857312	7447270.377838	13601.789655
4	Application throughput plot	App4_FTP	2	7	68494	1065	100000000	1554900	0.829280	7465093.754366	14065.729511
5	Application throughput plot	App5_FTP	2	8	68494	1057	100000000	1543220	0.823051	7594173.164494	14012.657898

Fig14: Detailed view of application metrics

PacketsLoss	Bitrate	Framerate	Throughput	Delay	Jitter	MOS_new
0.0440408973	0.002293577982	0	0	8.42E-07	9.20E-07	3
0.02190926729	0.008027522936	0.1111111111	7.57E-08	2.58E-05	1	4
0.02180026596	0.01376146789	0.2222222222	1.50E-07	2.60E-05	9.86E-07	4
0.01092193325	0.01949541284	0.3333333333	2.24E-07	2.61E-05	9.73E-07	4
0.008737546598	0.02412667608	0.4444444444	2.97E-07	2.63E-05	9.65E-07	5
0.01090013298	0.03096330275	0.5555555556	3.68E-07	2.63E-05	9.50E-07	5
0.009330513832	0.03669724771	0.6666666667	4.35E-07	2.63E-05	9.63E-07	5
0.005456606571	0.04243119266	0.7777777778	5.02E-07	2.61E-05	9.71E-07	5
0.004849687166	0.04816513761	0.8888888889	5.79E-07	2.63E-05	9.73E-07	5

Fig 15. samples of the dataset





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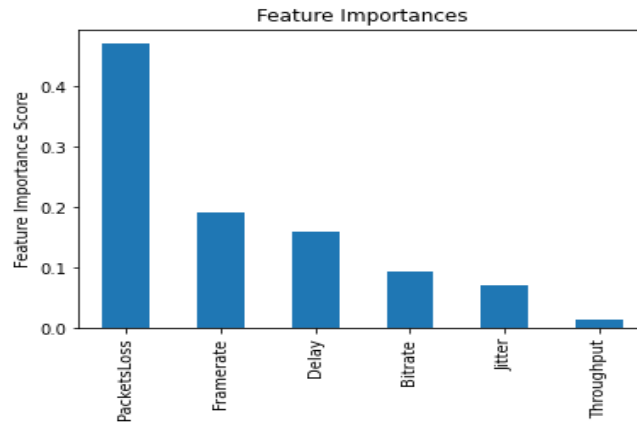


Fig 16: Important features predicted

SUMMARY OF FEATURES	
TYPES	FEATURES
QoE Parameters	Packet Loss
	Frame Rate
	Delay
	Jitter
	Resolution
	Video bit rate
	Throughput

Fig 17: Features predicted

FEATURE	SROCC	PLCC
Packet Loss	0.967	0.932
Delay	0.942	0.9348
Total Frames	0.915	0.933
Jitter	0.920	0.95
Video bitrate (kbps)	0.921	0.932
Throughput	0.951	0.90

Fig 18: Important features Correlation

S.No.	year	Proposed method	Accuracy
1	2018	<i>K-means + LR (proposed)</i>	96.94%
2	2015	<i>FIS based</i>	92%
3	2010	<i>RNN-based</i>	90%
4		<i>Lasso regression based QoE prediction</i>	98.00%

Fig 19: Comparison with other Methods





Assessing Search and Rescue Optimization Based DNN Model for Stream Flow Data Prediction

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ABSTRACT

For many activities related to water resource management, such as flood and drought control, reservoir service, water supply planning and hydroelectric power generation, accurate stream flow prediction is important. While both short- and long-term forecasts are important, reservoir activities are usually planned on the basis of monthly periods; monthly stream flow forecasts therefore play a major role in the management of water resources. Therefore, there is need to propose an efficient approach for prediction of stream flow to improve the system efficiency. Hence, in this paper we have developed an adaptive model based on Search and rescue optimization based DNN for prediction of monthly stream flow. The analysis shows that the adaptive model outperforms existing models such as ANN, SVM and OANN. This AI based learning model shows that this model can able to handle huge number of data for prediction of monthly inflow.

Keywords: Data prediction, Deep Neural Network, Stream flow, Optimization, and monthly inflow

INTRODUCTION

Streamflow is an essential part of the water cycle of the Earth and has a critical role in a wide range of applications. The prediction of streamflow has several advantages and it would help to provide reliable, useful and important information in large areas such as water resource management. It has been shown over the years that streamflow can be predicted and predicted using Artificial Intelligence (AI)-based models at different timescales [1]. Streamflow forecasting is one of the most important issues in hydrology and is a key measure in the development and planning





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of water resources. The forecasting of the river flow alerts the impending stages during the floods and helps to regulate the outflow of reservoirs during low river flows for the management of water resources. Accurate streamflow prediction is essential for the proper management. The proper streamflow forecasting may also help in providing information for city planning, hydroelectric projects, efficient management plans preparation, proactive mitigation programs and real-time operation of water resources projects that decreases the climatic events impact on the environment. Thus, the river flow or streamflow forecasting is very important [2].

The process of streamflow is difficult and not easily predictable. This streamflow process is affected by the huge number of parameters like temperature, evapo-transpiration, land use, precipitation and is described by the non-linear link among the streamflow and the watershed. Streamflow prediction can be categorised as physical models and data-driven models. Physically based models are data-intensive and require a wide range of parameters based on intensity and distribution; rainfall levels, land use; watershed physiographic characteristics; and human activities. Data-driven models mathematically present linear or non-linear relationships between streamflow and its parameters [3]. The streamflow prediction is predicted by means of daily stream basis, monthly basis, annual streamflow, seasonal streamflow prediction, etc. [4].

Monthly streamflow prediction with high and stable performance is of great importance and application value in originating the rational allocation and best water resources management and enhancing the depth and breadth of hydrological forecasting integrated services. Meteorological forecasts coupled with hydrological models, and data-driven methods are two main approaches for monthly streamflow forecasting. Monthly meteorological forecasts such as precipitation and evaporation to drive the hydrological models to achieve the monthly streamflow forecast. Data-driven models based on various machine learning algorithms directly build the relationship between predictors and predictand [5-8]. Hence in this paper we are implementing DNN and S-ROA for improving the efficiency by tuning the parameters of DNN. The paper is organized in the manner such as the section 2 reviews the existing models used in streamflow prediction. The section 3 shows the proposed S-ROA based DNN for streamflow data prediction. The section 4 shows the results and discussion followed by conclusion in the section 5.

RELATED WORKS

In this section the table 1 reviews the existing methods used for streamflow prediction, study area, performance measures, and drawbacks.

PROPOSED S-ROA BASED DNN FOR STREAMFLOW PREDICTION:

The main objective of this paper is to develop an efficient adaptive model for monthly streamflow prediction using search and Rescue optimization Algorithm (S-ROA) based Deep Neural Network (DNN). The proposed model is compared with the existing techniques such as Support vector Machine (SVM) , Artificial Neural Network (ANN) and Optimal Artificial Neural Network (OANN).The data have been collected more than 100 years (1871 to 2000)[17] Aswan High Dam, Egypt from which we have evaluated the performance metrics such as Root Mean Squared Error (RMSE), Mean Absolute Error (MAE), Nash-Sutcliffe coefficient (NSE) and Correlation Coefficient (CC). Here 60% of the data is used for training and remaining 40% of the data is used for testing the proposed model.

Prediction using Optimal Deep Neural Network:

At first, initializing the inputs based on input layer weight α_j and the hidden layer weights β_{ij} . Where, Input I_i represent the database. The input layer consists of neurons. The dataset is used for training network which is denoted as i_1, i_2, \dots, i_n and inputs are represented as W_1, W_2, \dots, W_n . The basis function is calculated using the below equation

$$I_b = \sum_{b=1}^y W_i \times \omega_{ab} \quad (1)$$





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Where, ω denotes the weight, W represents the input value and 'l' represent the bias function. This layer consists of number of neurons as h_1, h_2, \dots, h_n the hidden layers are connected to the output layer by using the neurons. The connection between input parameter d , and the hidden layer, h_1 , is represented as

$$h_1 = A(w_1 d + bias_1) \tag{2}$$

Where, w_1 represent the the weight and $bias_1$ represent the bias. The connection among the ' m^{th} ' hidden layer, h_m and ' $(m-1)^{th}$ ' hidden layer, h_{m-1} , is afforded as follows,

$$h_m = A(w_m h_{m-1} + bias_m) \tag{3}$$

$$\tilde{o} = R(h_M) \tag{4}$$

At the output layer, the estimation of the network output is obtained as,

$$\tilde{o} = \xi^* = \arg \min_{\xi} \{P(o, \tilde{o}; d, \xi) + \kappa \cdot \gamma(w) + \chi \cdot \phi(s)\} \tag{5}$$

$$\gamma(w) = \sum_m \|W_m\|_F^2 \tag{6}$$

The activation parameter is defined as

$$A_f = \sum_{b=1}^{\square} \alpha_b * \left(\frac{1}{1 + \exp(-\sum_{a=1}^N M_a \omega_{ab})} \right) \tag{7}$$

$$O_i = \sum_{i=1}^n \alpha_i \sigma(F_{i(optimal)}) \tag{8}$$

$$E_i = \sqrt{\frac{\sum_{i=1}^{ND} (D_i - P_i)^2}{ND}} \tag{9}$$

Where, ND is the total number of data used. D is the original range and P is the expected range of output. Thus, to improve the efficiency of DNN we proposing search and rescue optimization for updating those weight.

Search and rescue optimization algorithm (S-ROA)

The group members collect clues of information during the search operation. A few of these clues remain a group to determine more significant clues but the searching operation is improved via their information. The matrix N dimension is similar to matrix Y . The number of group member N with the problem dimension d is denoted as $N \times d$ matrices. Based on the clue's matrix, each new solution in social and individual stages are generated.





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$$c = \begin{bmatrix} Y \\ N \end{bmatrix} = \begin{bmatrix} Y_{11} & \dots & Y_{1d} \\ \vdots & \ddots & \vdots \\ Y_{M1} & \dots & Y_{Md} \\ N_{11} & \dots & N_{1d} \\ \vdots & \dots & \vdots \\ N_{M1} & \dots & N_{Md} \end{bmatrix} \quad (10)$$

$$Sd_j = (Y_j - CL_i) \quad i \neq j \quad (11)$$

Here, the j^{th} human position and k^{th} clue positions are denoted as Y_j and CL_i . The search direction of j^{th} human is Sd_j .

$$Y_{j,k}^{\wedge} = \begin{cases} \left\{ \begin{array}{ll} CL_{i,k} + R_1 \times (Y_{i,k} - CL_{i,k}) & \text{if } F(CL_i) > F(Y_j) \\ Y_{j,k} + R_2 \times (Y_{j,k} - CL_{i,k}) & \text{Otherwise} \end{array} \right. & \text{if } R_2 < AE \text{ or } k = k_{random}, k = 1, 2, \dots, d \\ Y_{j,k} & \text{Otherwise} \end{cases} \quad (12)$$

For j^{th} human, the k^{th} dimension with its new position is $Y_{j,k}^{\wedge}$. For i^{th} clue, the k^{th} dimension with its position is $CL_{i,k}$. The objective function values of CL_i and Y_j are expressed as $F(CL_i)$ and $F(Y_j)$. The random number R_1 randomly distributed to $[-1, 1]$ interval. Similarly, the random number R_2 randomly distributed to $[0, 1]$ interval.

The third stage is the individual stage where the humans search their current position in the individual stage.

$$Y_j^{\wedge} = Y_j + R_3 \times (CL_i - CL_n), \quad j \neq i \neq n \quad (13)$$

Hence, the random integer i and n were distributed to the interval $[1, 2M]$. The i and n are selected in such a way that $j \neq i \neq n$ to prevent movement along with other clues. The random integer R_3 randomly tends to the interval $[0, 1]$. The individual and social stage located in the solution space obtain the solutions.

$$Y_{j,k}^{\wedge} = \begin{cases} (Y_{j,k} + Y_k^{\max})/2 & \text{if } Y_{j,k}^{\wedge} > Y_k^{\max} \\ (Y_{j,k} + Y_k^{\min})/2 & \text{if } Y_{j,k}^{\wedge} < Y_k^{\min} \end{cases} \quad k = 1, \dots, d \quad (14)$$

For the k^{th} dimension, the maximum and minimum threshold values are Y_k^{\max} and Y_k^{\min} . After each stage, the group member will search in each iteration based on these two stages.

$$N_m = \begin{cases} Y_j & \text{if } F(Y_j^{\wedge}) > F(Y_j) \\ N_m & \text{Otherwise} \end{cases} \quad (15)$$





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$$Y_j = \begin{cases} \hat{Y}_j & \text{if } F(\hat{Y}_j) > F(Y_j) \\ Y_j & \text{Otherwise} \end{cases} \quad (16)$$

$$U_j = \begin{cases} \hat{U}_j + 1 & \text{if } F(\hat{Y}_j) < F(Y_j) \\ 0 & \text{Otherwise} \end{cases} \quad (17)$$

$$Y_{j,k} = Y_k^{\min} + R_4 \times (Y_k^{\max} - Y_k^{\min}), \quad k = 1, \dots, d \quad (18)$$

Hence, the random number R_4 is distributed to [0, 1] interval and it is varied for all dimensions. In the possible regions, the population is converged in the local optimum. The similarities between them are excessive and the entire population is possible. The solution is possible when the constraint violation degree with a standard deviation is less than to predefined value (β). The restart mechanism is used thereby randomly generates the matrices such as human and memory. Finally, the optimal solution is updated to improve the prediction accuracy of proposed DNN.

RESULTS AND DISCUSSION

In this paper we have compared our proposed S-ROA based DNN with other existing methods like SVM, ANN and Optimal ANN to show the monthly inflow prediction. The proposed model is implemented using dataset collected from Egypt [17]. We have used 60% of the data for training and remaining 40% of the data for testing the proposed model. To evaluate the performance of the proposed system metrics such as RMSE, MAE, NSE and correlation coefficient are used.

Correlation Coefficient

$$C_c = \frac{\sum_{x=1}^M (K_{S,x} - K'_s)(K_{T,x} - K^-_T)}{\sqrt{\sum_{x=1}^M (K_{S,x} - K'_s)^2 \sum_{x=1}^M (K_{T,x} - K^-_T)^2}} \quad (-1 < C_c < 1)$$

Root Mean square Error:

$$RMSE = \sqrt{\frac{1}{M} \sum_{x=1}^M (K_{S,x} - K_{T,x})^2} \quad (0 < RMSE < \infty)$$

Nash Sutcliffe Coefficient

$$NSE = \left[\frac{\sum_{x=1}^M (K_{S,x} - K_{T,x})^2}{\sum_{x=1}^M (K_{S,x} - K'_s)^2} \right] \quad (-\infty < NSE < 1)$$

Mean Absolute Error

$$MAE = \frac{1}{M} \sum_{x=1}^M |K_{T,x} - K_{S,x}| \quad (0 < MAE < \infty)$$

Where, $K_{S,x}$ is the observed streamflow range. $K_{T,x}$ is the forecasted value. K'_s is the average observed range. K^-_T is the average range of forecasting and M is the total number of data.



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The table 2 to table 5 shows the comparison of performance evaluation of RMSE, CC, MAE and NSE for S-ROA based DNN, OANN, ANN and SVM respectively. The collected data were trained and tested using presented model for all set of combinations. The SROA based DNN outperforms all the existing models in terms of accuracy for both training and testing stage which is followed by OANN, ANN and SVM. The figure 1 (a-d) shows the graphical representation of those performance evaluation metrics and the improvements over the presented model. The performance of the S-ROA based DNN and the existing models were summarized in the table. Also, these existing methods providing better accuracy when there is a smaller number of data as the input and our method outperforms those models by showing better accuracy in terms of RMSE, MAE, NSE and CC.

CONCLUSION

The proposed S-ROA based DNN was implemented to predict the monthly streamflow. S-ROA model is used to optimize the DNN parameters for improving the prediction result and then compared with OANN, SVM and ANN. The outcome shows that the proposed S-ROA based DNN outperforms all the models in terms of efficiency for the evaluated parameters such as RMSE, MAE, NSE and correlation coefficient. Moreover, search and rescue optimization prove to better algorithm for optimizing the parameters which improves the prediction accuracy. In future research a hybrid model should be presented to improve the efficiency for the prediction of any number of data. In addition, a real-time forecasting model should be implemented by evaluating the time complexity and accuracy of data prediction model.

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Table 1: Review of Literature

References	Method	Study area	Performance measures	Drawbacks/ Future scope
Khatibi R et al. (2017) [9]	MLP with the Levenberg–Marquardt and MLP integrated with the Fire-Fly Algorithm	Bear River, U.S.A	Taylor diagram and Correlation coefficient	In the future, modelling the formation of science and minimising haphazard modelling practises will be developed.
Jaeger KL et al. (2019) [11]	PROSPER model	Pacific Northwest, US	Accuracy, Error rate	Predictor models shall be included in the future for better capturing of local processes and characterize the streamflow permanence
Keteklahijani VK et al. (2019) [12]	Two Global climate model (GCMs), four downscaling methods (DSMs), and four representative concentration pathways (RCPs)	Karaj dam reservoir, Iran	RMSE	Assembly of various hydrological models to boost accuracy in the future
Chu H et al. (2020) [13]	Fuzzy C-means (FCM) and Deep Belief Networks (DBN) called LASSO-FCM-DBN model	Tennessee River, USA	RMSE correlation coefficient and MAE	An efficient parameter optimization in the future
Tikhamarine Y et al. (2019) [15]	Hybrid wavelet support vector regression based on grey wolf optimizer	Hydrometric station, Algeria	Nash–Sutcliffe efficiency (NSE), correlation coefficient, RMSE, and MAE	<ul style="list-style-type: none"> • Modified GWO for tuning SVR parameters in future





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Table 2: Performance Evaluation of RMSE, CC, MAE and NSE for proposed S-ROA based DNN

Models	S-ROA based DNN			
	RMSE	CC	MAE	NSE
S1	2.8103	0.8821	1.8652	0.7751
S2	2.7269	0.9123	1.1452	0.8693
S3	2.0125	0.9412	1.2128	0.8829
S4	2.2498	0.9389	1.2954	0.8656
S5	2.1681	0.9275	1.3352	0.8748

Table 3: Performance Evaluation of RMSE, CC, MAE and NSE for Existing optimal ANN model

Models	Optimal ANN			
	RMSE	CC	MAE	NSE
S1	3.2124	0.8425	2.6742	0.7159
S2	2.2315	0.9364	1.3125	0.8563
S3	2.1589	0.9174	1.3000	0.8505
S4	2.4298	0.9253	1.4012	0.8428
S5	2.3965	0.9197	1.3498	0.8142

Table 4: Performance Evaluation of RMSE, CC, MAE and NSE for Existing ANN model

Models	ANN			
	RMSE	CC	MAE	NSE
S1	3.1289	0.8695	2.2956	0.6719
S2	2.5128	0.9022	1.4331	0.8821
S3	2.4396	0.9138	1.7153	0.8382
S4	2.5878	0.9100	1.6891	0.7085
S5	3.3368	0.9178	1.6729	0.7278

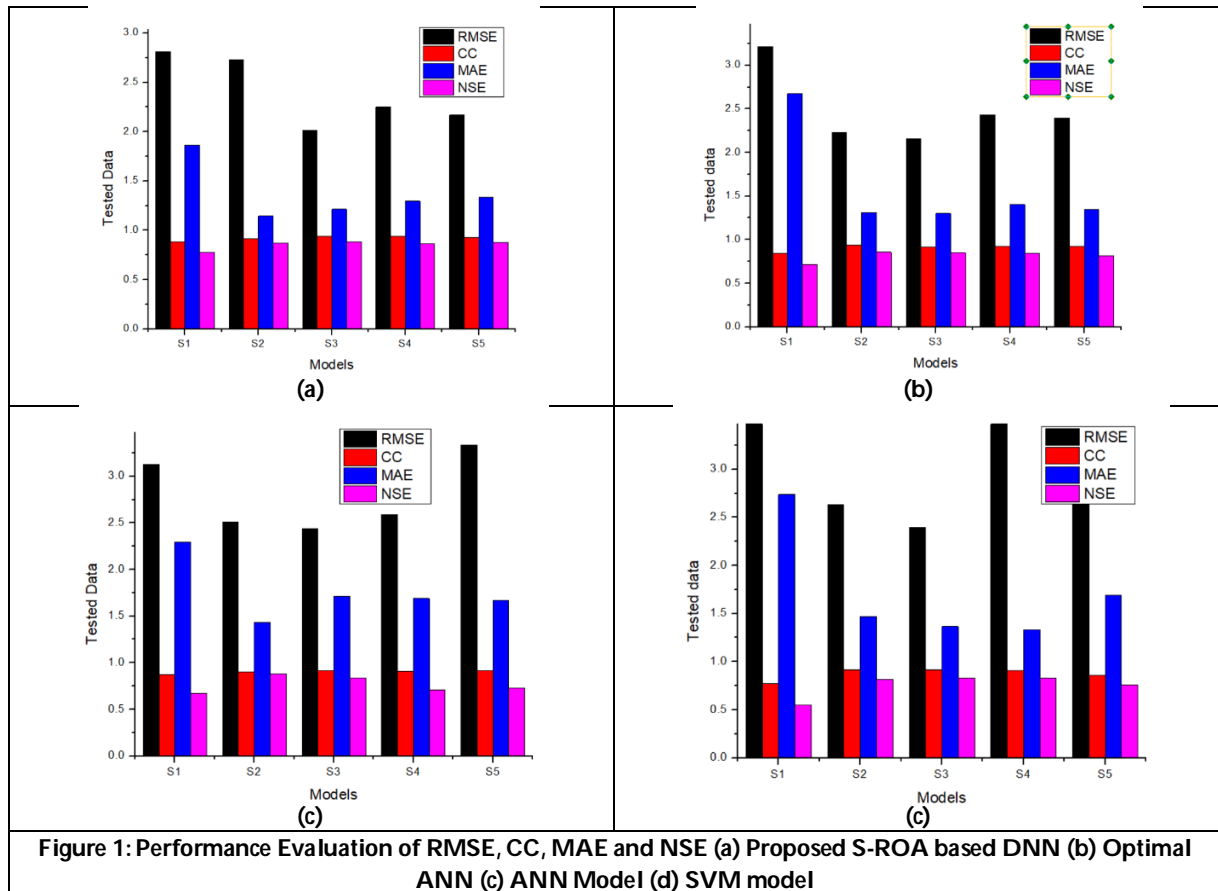
Table 5: Performance Evaluation of RMSE, CC, MAE and NSE for Existing SVM model

Models	SVM			
	RMSE	CC	MAE	NSE
S1	3.8145	0.7699	2.7352	0.5489
S2	2.6289	0.9178	1.4689	0.8162
S3	2.3947	0.9145	1.3645	0.8311
S4	2.5825	0.9054	1.3329	0.8277
S5	2.9417	0.8612	1.6895	0.7596





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Emotional Intelligence among Academicians Working in Higher Education Institutions

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ABSTRACT

This study seeks to quantify and analyse Academicians' Emotional Intelligence (EI) in order to generate information for future research. The thesis would conduct a comprehensive analysis of academicians' roles in chosen educational institutions. A questionnaire is being used to collect data from respondents who work for institutions of higher education. Finally, the views of 150 respondents were analysed using methodological methods. To measure emotional intelligence, a variety of research instruments have been created, each with a unique content and methodology. Additionally, it demonstrates a clear disparity between observational studies on determinants and research on Emotional Intelligence results. Employee behaviours are among the most often cited causes of Emotional Intelligence. Self-awareness, social awareness, self-control, and relationship management are among the more observed outcomes. Manufacturing firms conduct the majority of studies, led by hospitals and other public health organisations, as well as banks and insurance agencies. This comprehensive study of the literature is the first of its type to examine the triggers and implications of academicians' EI. This encompasses a broad body of literature which includes many determinants and effects of results.

Keywords: Emotional Intelligence, Academicians, Performance, Relationship

INTRODUCTION

Emotional Intelligence (EI) must integrate the cognition and power of two of the three mental states, namely intelligence and emotion. Emotional intelligence is the capacity to perceive, control, and quantify feelings. According to some scholars, emotional intelligence may be acquired and reinforced, although others assert that it is an innate

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characteristic. To measure emotional intelligence, a variety of research instruments have been created, each with a unique content and methodology. If an Academician has a high level of emotional intelligence, he or she is more likely to be able to express his or her emotions effectively and to understand the feelings of others for whom they interact, thus strengthening working relationships and success. It is not a matter of being soft! Intuitive Emotional Intelligence Developing the opportunity to utilise one's emotions to aid throughout decision-making in the moment and to exert more influence of one's self and its impact on others is a new form of becoming wise. Emotional Intelligence enables individuals to think more creatively and overcome challenges with the use of their emotions. Emotional intelligence almost always overlaps with general intelligence. The emotionally intelligent person is skilled in four areas: emotion recognition, emotion use, emotion knowledge, and emotion management.

EMOTIONAL INTELLIGENCE CONCEPTUAL BACKGROUND

Mayer, Carsuo, and Salovey (1999) suggested that "feelings are the product of a person's internal and external environment, perception, and capacity for understanding." Though Peter Salovey and John Mayer coined the word Emotional Intelligence and were also the first to create an EI scale, the term gained widespread recognition in the business environment and in 1995 with the publication of Daniel Goleman's book Emotional Intelligence.

Goleman suggested a paradigm that encompasses four primary constructs of emotional intelligence: self-knowledge, self-control, social awareness, and relationship management.

- Self-awareness is described as the "ability to analyse one's feelings and recognise their consequences through the use of gut feelings as a driving force."
- Self-management is a term that applies to "directing sentiment in response to circumstances."
- Social cognition encompasses the "ability to comprehend and react to the feelings of others in a social context."
- Relationship management: "the capacity to inspire and influence others by empathy while still handling tension" (Goleman, 2002).

Emotional intelligence may be described in a number of ways. While there are some similarities between the meanings, there are still some distinctions. It is a subset of social intelligence that relates to the capacity to manage and manage one's own and others' feelings, as well as the ability to distinguish between them (Salovey & Mayer, 1990). Other personality traits, such as self-confidence, conscientiousness, and accomplishment motivation, were later introduced to the concept. Mayer and Salovey (1997) proposed an emotional intelligence paradigm that includes four abilities: perceiving emotions, utilising emotions, comprehending emotions, and controlling emotions. Mayer, Caruso, and Salovey (1999) go on to describe emotional intelligence as a collection of mental skills. Furthermore, Bar-On and Parker (2000) broaden the definition of emotional intelligence to include emotional, intimate, and social abilities that have a significant effect on an individual's overall ability.

It enables individuals to handle challenges and problems in their social and personal lives more effectively. Emotional intelligence, according to Zeidner, Roberts, and Matthews (2002), consists of the assessment, speech, utilisation, and control of one's own and others' emotions. Emotional intelligence, according to Mayer, Salovey, and Caruso (2004), is the capacity to think and to use emotion to aid thinking. Other researchers distinguished between social intelligence and emotional intelligence. Emotional intelligence, according to Cherniss (2010), is the capacity to recognise and control emotions, while social maturity is the ability to understand and regulate emotions. In a nutshell, emotional intelligence was regarded as a talent, a capability, a competency, and a personality attribute (Wicks, Nakisher and Grimm, 2018).

REVIEW OF LITERATURE

Emotional abilities, according to Wicks, Nakisher, and Grimm (2018), will increase academic performance by 11 percentile points and reduce violence and behaviour issues by 9%. In order to attain academic and job excellence, students must learn to regulate their feelings and develop their emotional intelligence competencies. Students must understand how to deal with acceptable feelings at particular times and in specific locations.



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S. Jain, V. Jain, and S. Das (2018) suggested that service efficiency and emotional intelligence are related. As a result, if emotional intelligence is combined with self-management, self-awareness, and social skills/networking, service efficiency would suffer. These are the aspects of emotional intelligence that determine job achievement and guide individuals toward professional advancement. Professional growth relates to having expertise, experience, and ability in a particular area, and in today's inconsistency, individuals must be willing to acquire new techniques if needed and unlearn old habits in order to be professionally established. In his study, Bar-On stressed the value of emotional intelligence, equating emotional intelligence to logical intelligence. Emotional intelligence has a wide range of applications that include not just professional advancement but also job development. Donald Super considers it to be a lifetime method of learning.

Mayer, Caruso, and Salovey (2016) demonstrated that feelings and cognitions have a connection. It also discusses the current and potential of the notion of emotional intelligence as a mental capacity. Both related EI ideas contribute to a better understanding of how people interpret and use EI to help them be more successful in their personal and professional lives. They had written extensively about the ways in which EI affected organisational leadership and referred to EI as a kind of intellectual inkblot, an unstructured term subject to a wide range of interpretations. Elhaj (2015), who proposes a system for school-based emotional intelligence growth. It concentrates on four main fields. The first is relational maturity as a subject of the classroom. An emphasis on aural, visual, physical mathematics room, and interpersonal intelligences should be used to meet students' emotional needs. Emotional maturity, as well as the school and classroom setting, are the second and third areas to include. The classroom environment can support students' emotional control as well as their ability to make rational choices. Emotional maturity and social wellbeing are the third and final areas to consider. Teachers must use therapeutic approaches that both inspire and promote their students' learning. The relationship between the school and the family is the fourth sector. Schools should engage with families and teach them how to be better parents and to exchange information about their children's concerns and success.

The history of the concept of "emotional intelligence" is discussed by Mehta and Singh (2013). The term "social intelligence" was coined in the 1930s by Edward Thorndike, who described "social intelligence" as "the ability to understand and interact with others." "As well as the ability to communicate effectively with others. Abraham Maslow, a humanistic psychologist, concentrated on the field of mental power creation in the 1950s. In his book "Multiple Intelligences," published in 1975, Howard Gardner coined the phrase "multiple intelligences." "Linguistic intelligences, logical-mathematical intelligence, musical intelligence, bodily-kinesthetic intelligence, intrapersonal intelligence, and interpersonal intelligence are among the six categories of intelligences described in "The Shattered Mind." Keith Beasley coined the word "emotional quotient" in 1987 and was the first to use it in print. Three years later, in 1990, Peter Salovey and John Mayer wrote an essay titled "Emotional Intelligence." Finally, in 1995, Daniel Goleman published "Emotional Intelligence: Why it should Matter More Than IQ," and the idea of emotional intelligence has gained widespread acceptance since then.

"Emotional intelligence competencies in the team and team leader: A multi-level analysis of the effect of emotional intelligence on team performance," by E. S. Koman and S. B. Wolff, published in 2008. The association between team leader EI competencies and team success is investigated in this research. A total of 349 aircrew and maintenance military team members from 81 aircrew and maintenance teams took part in the report. The prevalence of emotionally competent community norms (ECGN) on the teams that team leaders lead is strongly related to their EI, and ECGN are connected to team success, according to the findings. Three recommendations are made by the reviewers as well. To begin with, employee leaders who have improved their EI skills improve not just their own personal success but also the performance of the teams they head. Second, through training or recruiting managers who are emotionally capable. Finally, companies can cultivate emotionally capable executive leaders in addition to emotionally competent first line leaders, since each member of the executive management team has an impact on the growth of ECGNs on the teams he or she oversees.





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STATEMENT OF THE PROBLEM

Employees put forward their utmost efforts at work, but growth is not strictly based on output outcomes. Emotional intelligence (EI), for example, is one of the reasons that leads to job advancement. The aim of this research is to classify various EI traits among employees and to investigate the function of EI traits in career promotion and performance. In educational programmes, the academic and memory abilities of students receive the most consideration. The interaction between feelings and learning receives less consideration. When coping with the domain of language acquisition, emotional competence must be considered. Affective characteristics including possessing a strong level of emotional maturity may help students succeed in studying a second or foreign language. Fahimand Pishghadam is a character in the film Fahimand Pishghadam (2007). Several studies have recently begun to look at the relationship between emotional intelligence and academic performance in students, but they have yielded inconsistent findings, indicating the need for further research. As a result, the aim of this research was to examine the emotional intelligence level of teaching faculty in higher education institutions, as well as its effect on their performance emotions. Knowing how to support emotionally intelligent behaviour when it is needed will be beneficial. In order to increase the standard of their schooling, it is therefore essential to investigate the impact of Academicians' Emotional Intelligence on their academic success.

OBJECTIVES OF THE STUDY

The aim of this analysis is to evaluate the emotional intelligence level of Academicians, as well as the impact of emotional intelligence on their performance.

The below are the study's objectives:

- To determine the degree of emotional intelligence among academicians in higher education institutions.
- To see if their relational intelligence affects their output.

HYPOTHESIS OF PRESENT STUDY

Hypothesis 1: Ho: Emotional Intelligence has a significant impact on organizational performance.

Hypothesis 2: Ho: Self-awareness has a significant relationship on Social awareness

METHODOLOGY OF STUDY

The study is descriptive of nature, and related data has been gathered from both primary and secondary sources of information. A random sample methodology was used to collect data from 150 respondents who worked as academicians in higher education institutions. The data was gathered through a structured questionnaire split into two parts in order to reveal their personal identifiers, such as age, education, salary, marital status, and so on, in the first part of the questionnaire. The second part of the questionnaire was used to determine Goelman's emotional intelligence rating among his staff. Primary data was collected from a variety of sources, including papers, blogs, and academic studies.

RESULTS AND DISCUSSION

In order to collect data for this study, I used a survey methodology. The questionnaire is well-structured, and feedback from researchers employed in higher education organisations is solicited. It employs a straightforward random sampling technique. After the data was analysed, mistakes such as typos and missing values were sorted out. Following the study, the final comments and recommendations are made. The SPSS programme was used to do the study study. The questionnaire was double-checked for Cronbach's alpha reliability test. Cronbach's alpha is the most commonly employed metric for assessing the questionnaire's internal precision ('reliability'). It is the most commonly employed method because we have a number of Likert questions in the survey. The questionnaire has been shown to be trustworthy. From the model summary table the Exogenous variables are showing 89% of the variance on endogenous variables. And also observed from the coefficient tables that the two independent variables (Self-awareness, Social awareness) consider in the study are having significant impact on dependent variable i.e.,





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performance. The statistical tool requires predictor and response variables; the independent variables / predictors are denoted by X1 and X2. I noticed that Self Awareness (X1) is one independent variable, and social awareness (X2) is other independent variable, The Performance (Self-management & Relationship management) is a dependent variable denoted by 'Y'.

The table is portraying that the statements of Self-awareness has 89% effect on dependent variable i.e. Performance. The R² (80.8%) and Adjusted R²(0.804) are also explaining the effect of independent is high on dependent variable. The F value (204.654) is significant at 5% level and also denoting model as fit. The above table is showing that majority of the elements of independent are showing significant impact of dependent variable. The factor with high β value shows high impact on dependent variable. In table 5, X2 is the most influencing factor with highest β value (0.837) with $p < 0.01$. X1 ($\beta = 0.078$, $p < 0.01$) also have significant impact on dependent variable.

$$Y = 8.889 + 0.142X_1 + 1.418X_2$$

Hence, Null Hypothesis (H_{02a}) is rejected.

LIMITATIONS OF STUDY

As per the spectrum of access of scholars, the study is limited, leading to an interpretation that cannot be extended to the whole planet. Therefore, the higher volume of data may have given this analysis the numerous outcomes, which serves here as the constraint for this study. With the aid of authentic and neutral data sources to explain the relationship between emotional intelligence and academic success, more specific data can then be retrieved over a longer period of time.

RECOMMENDATIONS OF THE STUDY

The majority of respondents in the sample have just average emotional skills, so it is suggested that the training and learning staff keep regular programmes at work to increase the degree of emotional maturity so that superior performance at work can be achieved.

- Management can include adequate recreational amenities for employees, which would aid in the interaction with teaching faculty and, most importantly, reduce work-related stress.
- The company should be sure that its human resources are being utilised effectively.
- Every week, as a regular occurrence inside each organisation, open sessions can be conducted to learn and appreciate the perspectives of the employees. It can be used to solve challenges at work and can even be used to generate new productive ideas.
- Educational institutions must continue hiring emotionally mature people for a better learning atmosphere and culture, as well as developing the emotional maturity of existing employees, enabling them to face and overcome tremendous challenges at work.
- While emotional intelligence has grown in prominence, it is still necessary to increase the level of sensitivity for all teaching personnel.
- Emotional intelligence can be promoted by incorporating 'Emotional Integrity' educational programmes for academics on a regular basis. The organization's leaders must develop personal wellbeing in order to ensure their own physical and mental health, as well as that of the service.

CONCLUSION

Our results suggest that EI improves four aspects of education: self-esteem, self-control, social awareness, and relationship management. Still, a more systematic approach to identifying and evaluating EI is required, in addition to rigorously planned empirical and experimental research to assess the long-term and causal effect of EI on teaching staff and the educational environment. Furthermore, EI can be integrated into the preparation of teaching and potential leaders to increase the standard of higher education teaching faculty. Future research should focus on the





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impact of EI on employee conduct, not only in terms of job satisfaction and corporate commitment, but also in terms of other variables including stress intention and attrition. EI-related industry, revenue, and profit creation, as well as EI knowledge, are critical constructs for the growth of companies and individuals, and can be investigated in a variety of fields.

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Table 1. Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.899 ^a	.808	.804	3.71716

a. Predictors: (Constant), SOCIAL_AWARENESS, SELF_AWARENESS

Table 2. ANOVA^a

Model	Sum of Squares	df	Mean Square	F	Sig.	
1	Regression	5655.514	2	2827.757	204.654	.000 ^b
	Residual	1340.276	97	13.817		
	Total	6995.790	99			

a. Dependent Variable: Performance_C
 b. Predictors: (Constant), SOCIAL_AWARENESS, SELF_AWARENESS

Table 3. Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	8.889	1.601		5.553	.000		
	SELF_AWARENESS (X1)	.142	.129	.078	9.101	.002	.393	2.546
	SOCIAL_AWARENESS (X2)	1.418	.120	.837	11.804	.000	.393	2.546

a. Dependent Variable: Performance





RESEARCH ARTICLE

Perceived Social Support and Depression, Anxiety and Stress among Infertile Couple with IVF in Gujarat-India

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ABSTRACT

There is a high rate of infertility among couples in the world. This problem is perceived differently in each socio-cultural context in which it is experienced. "Infertility" is a couple's failure in conceive after 12 months of unprotected sexual intercourse and pregnancy attempts. According to the statistics, globally 15% of couples are suffering from infertility. Treatment of IVF has been used safely and effectively for more than two decennium for excellent treatment of infertility. In-vitro-fertilization (IVF) describe both the egg and the sperm are extracted and allowed to fertilize under the lab in petri dish and later these embryos are inserted into the uterus. Infertility has a negative impact on women's psychological well-being. Social support and coping styles are external and internal resources respectively that researcher described their relation with depressive and anxiety symptoms. The present research is to study the relationship between infertile couple with IVF and perceived social support and depression, anxiety and stress. To evaluate infertile couple with marital status (Love or Arrange) and family type (Joint and Nuclear). The tool used is multidimensional Scale of Perceived Social Support by Zimet, Dahlem, Zimet & Farley (1988), which has 12 statement and second tool is depression anxiety and stress scale (DASS) Lovibond, S.H. and Lovibond, P.F.(1995), which has 42 statement. Sample size comprised of 30 infertile couples with IVF treatment. The present study suggested that treatment of In-vitro-fertilization affect psychological well-being but perceived social support negatively affect level of depression, anxiety and stress of infertile couples. We determined that the infertile couples who were married by arranged and who were living in joint family had higher perceived social support and lower the level of depression, anxiety, and stress.

Keywords: Infertility, In-vitro-fertilization, Perceived Social Support, Depression.



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INTRODUCTION

Having a child is one of the life beauties. Individuals wishing to have children not only enjoy but moreover have a valuable memory of themselves. Infertility represents as the emotional and mental changes that may occur to a couple after being having infertility. Even after a 12 month or more of regular sexual intercourse without use of contraceptives it is failure to achieve a pregnancy. In many cultures, childless ladies are suffer from stigma, face discrimination and ostracism. A female who's unable to conceived pregnancy will be diagnosed as a primary infertility and who's previously conceived but was unable to conceive later will be diagnosed with secondary infertility. Infertility is not only female problem. In reality, male and female both are similarly likely to have issue of infertility. Worldwide 60 to 80 million couples are suffer from infertility problem, approximately 8-10% couples in developed nations and 15-20% of developing countries have infertility (2018). According to WHO one in every four couples in developing nations is impacted by infertility (2019). Indian Society of Assisted reproduction describe that about 10 to 14% of the Indian population are suffer from infertility (2019). The 2017 revision report showed that India's fertility rate gradually down from 4.97 to 2.3, moreover it will be reduced to 2.1 during 2025 to 30, 1.86 from 2045 to 50, and 1.78 from 2095 to 100. When a married couple fails to reproduction they feel like not fulfilling the role of "being family". By negatively affecting marriage life, sexual life, future plans, body image, life quality of couples. Infertile couple suffer from stress, anxiety, depression, feeling of guilt, loss of social status, fear, social labelling and despondence.

***In vitro* fertilization**

In vitro fertilization (IVF) is intricate procedure used to help to prevent genetic issues and assist with conceiving pregnancy. IVF is one of the highly efficient form of assisted reproductive technology. IVF has been utilizing successfully for more than two decades to help blocked fallopian tubes and hereditary. From the ovaries mature embryo are retrieved and fertilized by semen in a medically controlled laboratory under artificial conditions. One full cycle of IVF treatment takes almost three weeks. Medical advances described that most of cases are treatable of infertility. Success rate of IVF is most importantly according to the patient's age. For female who under 30 years of age, the rate of success is almost 60%, for those between 30 to 35 years, the ratio is about 50%, for those between 35 to 40 years of age, it is approaching 40% and over 40 years of age, it is about 33% (Okafor NI, Ikechebelu NN, Ikechebelu JI. 2017). Normal people recognition the use of IVF treatment by infertile couples has raised from 60%(2008) to 76%(2015). The acceptance statistics of using IVF for heterosexual couples is 60%, compared to 44% for an survivor. The ratio of acceptance of IVF is reportedly more higher for families, individuals (61%) and for same sex couples (64%) (Fauser et al., 2019).

Perceived Social Support and Depression, Anxiety, and Stress

In Recent years the effect of infertility on the mental well being of couples included has been the object of expanding attention. It can not be denied that childlessness is a profoundly cruel experience for numerous couples. Social support is the source of coping and is of major importance in protecting physical and psychological well-being for women without children. Social support seen relates to how individuals regard friends, family members and others as sources ready to help them in case of need. Perceived social support has been constantly connected to well-being, as the perceived levels of support, care and love can provide positive experiences (Siedlecki et al., 2014). Study described that high level of perceived social support is connected to good physical and psychological health outcomes as well (Uchino et al.,2013). According to WHO depression is a common mental disorder, more than 264 million people are suffer from worldwide. It is feeling of sadness, lack of interest, sleep and appetite disturbance, tiredness etc. Anxiety and stress both are similar, but they are not the same. Stress is a reflection of daily pressures and difficult situation of life, while anxiety is a counter action to the stress (2017).



**Soni Mittal and Neeta Sinha****Correlation between perceived social support and Depression, Anxiety, and Stress**

Infertile couples suffer from fear, hostility, distrust, anxiety and sorrow (Csemiczky et al., 2000) and experienced inability to achieve pregnancy are related to increased depression level (Slade et al., 1997). Response of male and female are different for infertility related stress (Benyamini et al., 2004, 2009). Both the partner use different techniques to deal with stress related with infertility (Peterson et al., 2006). Study suggested that negative psychological effect of being childlessness, the very process of ART and in special IVF treatment moreover affect people (Eugster and Vingerhoets, 1999). Different stages of IVF procedure [blood testing, daily injection, providing sperm samples, ultrasonography examination etc.] may affect the psychosocial functioning of both partner and as individual. Moreover stress are related with the negative social evaluation of infertility treatment, becoming as a parent is an important segment of adult social role and identity (Möller and Fällström, 1991; Malina et al., 2016). Diverse viewpoint of social appraisal of IVF method can heightening the feeling of loss, disgrace and social mismatch that regularly accompanies infertility (Whiteford and Gonzales, 1995; Monga et al., 2004; Pawelec and Pabian, 2012). Research shows that social support reduces the level of stress, which related to childlessness (Cobb, 1976; McNaughton-Cassill et al., 2000; Dudek and Koniarek, 2003; Koss et al., 2014; Ying et al., 2015). One of the characteristics of childlessness is the inability to utilize normal social support resources because of the low level of disclosure of infertility (Holas et al., 2002; Hoff et al., 2009).

Hence, processes of social support offer an first step to help infertile couples dealing with infertility (Dembinska , 2012). Although there are many studies showing that psychological support can improve the subjective well-being of infertile couples, few studies have explored social support and its effect on stress hormones level (Boivin, 2003). The support of partners has always been an important factor for coping with infertility (Ying et al., 2015a). Research explain that Partner support may be an important intervention goal to improve pregnancy outcomes. Studies have shown that pregnant women with poor partner support are more likely to suffer from prenatal anxiety, depression and smoking (Cheng et al., 2016). Some studies suggest using psychological support to reduce stress through relaxation training or behavioral therapy and increase pregnancy rates (Eugster and Vingerhoets, 1999). Supportive social interaction is essential for managing stressful conditions associated with fertility treatment. On the other hand, insufficient psychological support, failure of treatment interventions, low socioeconomic status, poor foreign citizenship, and poor partner support are all related to an increased risk of depression (Atherton F, Howel D. 1995). An important finding suggests that negative response of spouse and relatives are associated with higher prevalence of depression and anxiety (Sezgin H, Hocaoglu C, GuvendagGuven ES. 2016). It has been reported that long-term infertility and unsuccessful but expensive treatment attempts are associated with higher levels of depression and anxiety (Berg, B. J., & Wilson, J. F. 1991). A different study indicated that anxiety and depression are more common after 4 to 6 years of infertility, further depression is particularly severe during 7 to 9 years of infertility (Ramezanzadeh, F., Aghssa, M.M., Abedinia, N. *et al.* 2004). The research found that MPSS sub variables significant other, family and friends support was negatively associated with DASS sub variables depression, anxiety and stress (Khalid, A., Dawood. 2020).

METHODOLOGY**Aim**

The aim of the present research is to study the relationship between infertile couple with IVF treatment and perceived social support and depression, anxiety and stress.

METHODS AND MATERIALS

Through purposive sampling data was collected from 30 infertile couples with IVF (30 males; 30 females) from Gujarat. The criterion for selection is married couples who had been processing IVF treatment after diagnosed with



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infertility. The participants had different marital status from love marriage (25%) and arrange marriage (75%) and different family type from Joint family (25%) and Nuclear family (75%).

Tools**Multidimensional Scale of Perceived Social Support (MSPSS)**

MSPSS developed by Zimet et al. in 1988. It is a 12 item scale to subjectively assess the social support was used in this study. The reliability and validity study of the scale was conducted by Eker and Arkar in 1995. The MSPSS include 12 items, 7-point, Likert-scaled, which comprise three sub-variables: family, friends, and significant other. Each sub variable contains 4 items. In the MSPSS, item no. 3, 4, 8, and 11 measure the family support; items 6, 7, 9, and 12 measure the friend support; and items 1, 2, 5, and 10 measure the support of significant others. The higher score obtained from the scale signifies a higher level of perceived social support.

Depression Anxiety and Stress Scale (DASS)

DASS developed by Lovibond, S.H. & Lovibond, P.f. in 1995. It is a 42-item scale, which includes three self-reported scales to measure the negative emotional states of depression, anxiety and stress and each scale contain 14 items. The depression scale evaluates dysphoria, devaluation of life, hopelessness, lack of interest, self-deprecation, anhedonia, and inertia. The anxiety scale evaluates autonomic arousal, situational anxiety, skeletal muscle effects and subjective experience of anxious affect. The stress scale is evaluated nervous arousal, difficulty relaxing, irritable/over-reactive, impatience and being easily upset. DASS is a 4-point severity/frequency scale, 28+ score describe extremely severe depression, the 20+ score describe extremely severe anxiety, and the 34+ score describe extremely severe stress level.

DATA ANALYSIS

Responses obtained on various measure and data subjected to various analyses such as Mean, Standard Deviation and t-ratio. Obtained results was interpreted and discussed in the light of available literature.

RESULTS

In the present research work data analysis has been done by statistical package for social sciences version 23 (SPSS-23). Results as per hypothesis are follows as:

Hypothesis 1 & 2

There is a significant difference in the MPSS between Marital status.

There is a significant difference in the MPSS between Family type.

Results showed that both the hypothesis are rejected as p-value is grater than 0.05($p>0.05$). This shows that there is no significant difference between love and arrange marriage and joint and nuclear family. Figure 1: Graph depicting means of MPSS among marital status and family type of infertile couples with IVF. There are no higher difference seen between means score of love and arranged marriage & joint and nuclear family, it was nearly similar. Based on the results, a significant difference was observed in the relationship between demographic variables and MPSS, means of marital status ($p>0.05$) and family type ($p>0.05$).

Hypothesis 3 & 4

There is a significant difference in the DASS between Marital status. There is a significant difference in the DASS between Family type. Results showed that both the hypothesis are rejected as p-value is grater than 0.05($p>0.05$). This shows that there is no significant difference between love (8 couples) and arrange marriage (22 couples) and joint (8 couples) and nuclear family (22 couples).



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Figure 2: Graph depicting means of DASS among marital status and family type of infertile couples with IVF. There are not much difference seen between means score of love and arranged marriage & joint and nuclear family, it was nearly same. Based on the results, a significant difference was observed in the relationship between demographic variables and DASS, means of marital status ($p > 0.05$) and family type ($p > 0.05$). Results described that the mean score of different domains of MPSS (Family, Friends, and Significant Others). The p-value is less than 0.01 ($p < 0.01$). This implies that there is a highly significant difference in the MPSS domains. Results described that the mean score of different domains of DASS (Depression, Anxiety, and Stress). The p-value is less than 0.01 ($p < 0.01$). This implies that there is a highly significant difference in the DASS domains. Results described that the t-value of MPSS and DASS is 6.301. The p-value is less than 0.01 ($p < 0.01$). This implies that there is a highly significant difference in the MPSS and DASS.

DISCUSSION

The results of t-test showed that no significant difference seen in MPSS among marital status love and arrange marriage and family type joint and nuclear family. The present result has been supported by Erdem K and Ejder Apay S(2014) on a sectional study: the relationship between perceived social support and depression in Turkish infertile women. Examining the DASS mean scores also showed that no significant difference between marital status (love and arrange marriage) and family type(joint and nuclear family). The mean score of MPSS sub-variables "family, friends, and significant others" was found to be statistically highly significant ($p < 0.01$). There is the level of social support is higher among significant others as compared to family and friends. The supportive study conducted by Ying et al.(2015) on Gender differences in experiences with and adjustments to infertility: a literature review. The result of DASS was found to be there is a high significant difference between depression, anxiety, and stress ($p < 0.01$). The study has been supported by Ramezanzadeh, F. et al.(2004) on a survey of the relationship between anxiety, depression and duration of infertility. In the consequence of this study, it is observed that as the couples perceived social support score is lower, the level of depression, anxiety, and stress automatically higher. The present study has been supported by Erdem, K. & Ejder Apay, S.(2014) on a sectional study: the relationship between perceived social support and depression in turkish infertile women. Another research study was supported by Khalid, A. and Dawood, S.(2020) on social support, self-efficacy, cognitive coping and psychological distress in infertile women.

CONCLUSION

We specified that the study results evidenced the research objectives the marital status and family type of infertile couples affect their perceived social support and depression, anxiety, and stress. We observed that infertility with IVF negatively affected both perceived social support and depression, anxiety, and stress for the couple. We determined that the infertile couples who were married by arrange had higher perceived social support than love marriage and those who were living in the joint family had higher perceived support and lower the level of depression, anxiety, and stress.

IMPLICATION AND RECOMONDATION

The finding of the present study focuses the light on the psychological problem faced by couples who suffer from infertility treatment like IVF. The finding can also be helpful for researchers and mental health professionals to understand the problem of couples, suffers from infertility. perceived social support emerged out as one of the important variable that can make individuals less vulnerable for infertility treatment. Thus, future studies can utilize self-esteem, quality of life, self-efficacy as an effective intervention for infertility treatment. Further research in this area is needed with a larger sample size is taken and a comparison of different metropolitan cities different states is done the research would be more meaningful.



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Table-1: Showing t-test was used to compare the mean score of Multidimensional Scale of Perceived Social Support (MPSS) obtained from marital status and family type.

	Variables		N	Mean	SD	t-value	p-value
MPSS	Marital Status	Love	8 couples	61.13	7.31	-.63	.53
		Arrange	22 couples	62.41	6.83		
	Family Type	Joint	8 couples	63.00	6.57	.63	.53
		Nuclear	22 couples	61.73	7.08		

Table-2: Showing t-test was used to compare the mean score of Depression, anxiety, and stress scale (DASS) obtained from marital status and family type.

	Variables		N	Mean	SD	t-value	p-value
DASS	Marital Status	Love	8 couples	34.25	28.64	-.874	.39
		Arrange	22 couples	42.14	31.65		
	Family Type	Joint	8 couples	38.63	30.17	-.212	.83
		Nuclear	22 couples	40.55	31.41		





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Table-3: Showing the t-test was used to compare the mean score of Multidimensional Scale of Perceived Social Support (MPSS) obtained from their different domain.

Variable		Mean	SD	t-value
MPSS	Family	19.30	5.96	25.09
	Friends	20.38	1.85	85.29
	Significant other	22.38	2.39	72.42

Table-4: Showing the t-test was used to compare the mean score of Depression, Anxiety, and Stress Scale (DASS) obtained from their different domain.

Variable		Mean	SD	t-value
DASS	Depression	15.40	9.39	12.7
	Anxiety	11.72	10.8	8.40
	Stress	12.92	11.37	8.80

Table-5: Showing the t-test was used to compare the mean score of Multidimensional Scale of Perceived Social Support (MPSS) and Depression, Anxiety, and Stress Scale (DASS).

Variable	N	Mean	SD	t-value
MPSS	30 couples	40.03	30.84	6.301
DASS	30 couples	62.07	6.92	

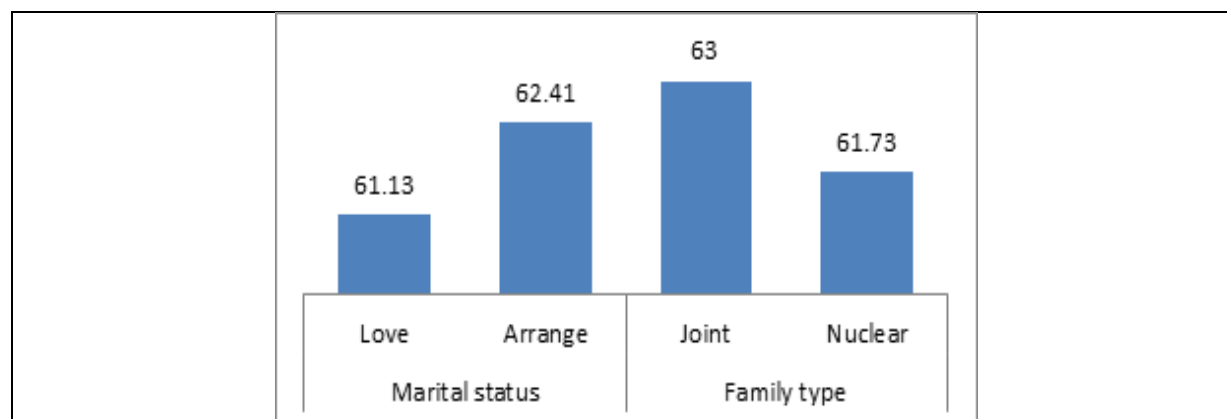


Figure 1: Graph depicting means of MPSS among marital status and family type of infertile couples with IVF. There are no higher difference seen between means score of love and arranged marriage & joint and nuclear family, it was nearly similar. Based on the results, a significant difference was observed in the relationship between demographic variables and MPSS, means of marital status ($p>0.05$) and family type ($p>0.05$).





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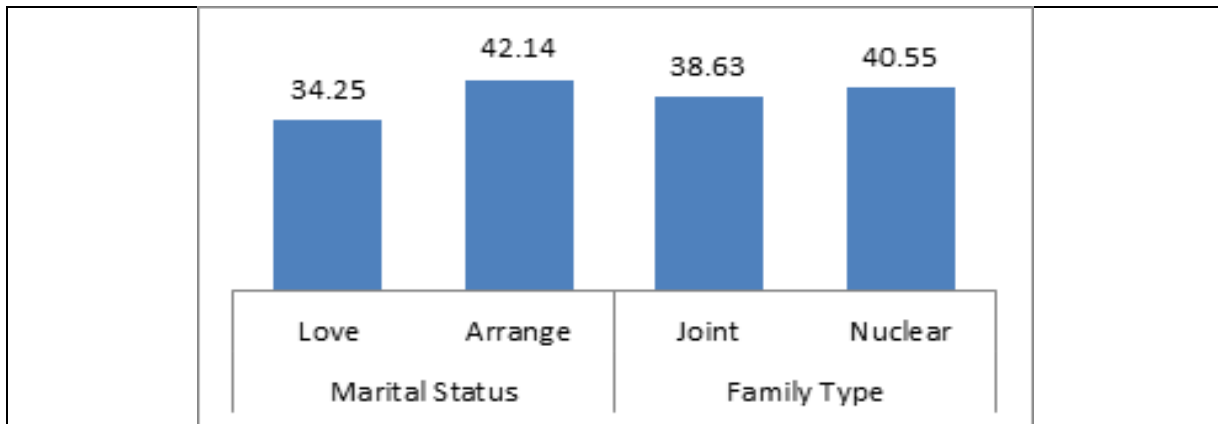


Figure 2: Graph depicting means of DASS among marital status and family type of infertile couples with IVF. There are not much difference seen between means score of love and arranged marriage & joint and nuclear family, it was nearly same. Based on the results, a significant difference was observed in the relationship between demographic variables and DASS, means of marital status ($p>0.05$) and family type ($p>0.05$).

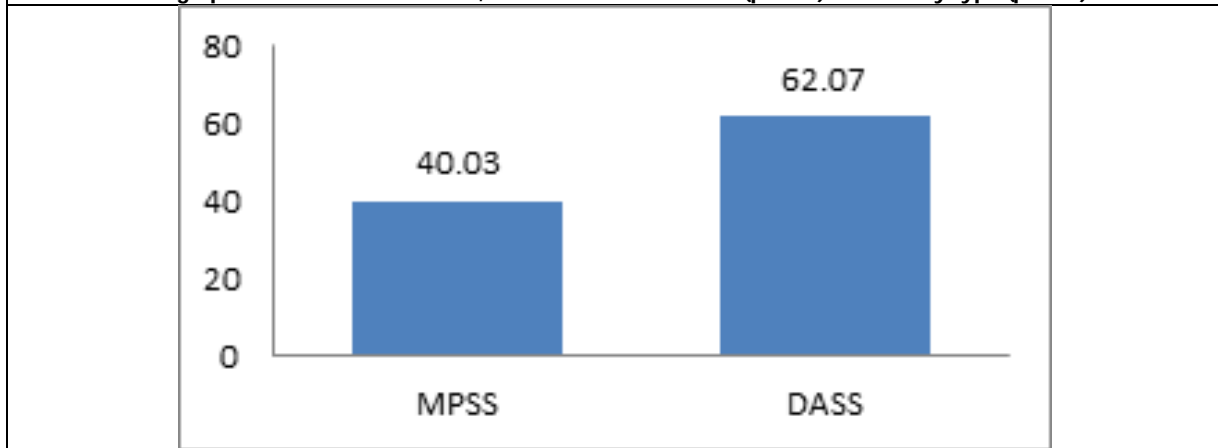


Figure 3: Graph depicting means of MPSS and DASS among infertile couples with IVF.





On Learning Upgraded Response of Successful Image Restoration and Enhancement with Fuzzy System

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ABSTRACT

Image reclamation and improvement is one of the primary assessment regions in the field of advanced picture taking care of. This part deals with the fundamental pieces of image reclamation and upgrade, and besides discusses the utilization of fragile preparing strategy, for instance, Fuzzy Logic in dealing with rebuilding and improvement issues. Image rebuilding attempts to reproduce or recover a image that has been adulterated by using from the previous data on the degradation wonder. On the other hand, image improvement insinuates accentuation or sharpening of picture features, for instance, edges, cutoff points or unpredictability to make a sensible exhibit continuously accommodating to look good and assessment. Picture rebuilding and upgrade techniques are for the most part used in the field of PC vision, video perception, clinical and satellite picture planning, etc.

Keywords: Image, Restoration, Fuzzy Logic, Satellite, Image Processing

INTRODUCTION

Images are regularly corrupted by arbitrary noise which can happen during image securing, transmission or handling. The corruptions may happen because of sensor noise, relative article camera movement, irregular environmental choppiness, etc. Noise might be either reliant or autonomous of image content, and is typically portrayed by its probabilistic qualities. During image transmission, noise which is typically free of the image signal happens. Gaussian noise is an awesome guess of noise that happens in numerous reasonable cases [Gonzalez and Woods, 2008]. Image noise decrease has come to explicitly mean a procedure of smoothing noise that has by one way or another defiled the image. Image restoration is worried about sifting the watched image to limit the impact of





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corruptions, where earlier data of the debasement structure is required [Jain, 1989]. The objective of image restoration is to recuperate an image that takes after the first image as intently as conceivable by lessening the noise. The viability of the image restoration channels relies upon the degree and the exactness of the information on the corruption procedure just as on the channel structure rule. An every now and again utilized measure is the mean square mistake. Other models, for example, weighted mean square and most extreme entropy are additionally utilized, albeit less as often as possible [Jain, 1989].

One of the major issues of image handling is to successfully diminish noise from a digital image while keeping its highlights flawless (e.g., edges, shading segment separations, and so forth.). Three primary kinds of noise exist: drive noise, added substance noise, and multiplicative noise. Motivation noise is typically portrayed by some segment of image pixels that are defiled, leaving the rest of the pixels unaltered. Instances of motivation noise are fixed-esteemed drive noise and arbitrarily esteemed motivation noise. In added substance noise, an incentive from a specific dissemination is added to each image pixel, for instance, a Gaussian conveyance. Then again, in multiplicative noise, the quality of the noise shifts with the sign power (e.g., spot noise) and thus, such kinds of noise are commonly more hard to expel from images than added substance noise [Schulte et al., 2007].

Restoration strategies are situated toward displaying the corruption and applying the opposite procedure so as to recoup the first image. This methodology as a rule includes figuring a basis of goodness that will yield an ideal gauge of the ideal outcome. Image restoration varies from image enhancement in that the last is concerned more with complement or extraction of image includes instead of restoration of significant data that has been debased. Image restoration issues can be evaluated absolutely, though enhancement models are hard to speak to scientifically. Therefore, restoration strategies frequently rely just upon the class or outfit properties of an informational index, though image enhancement methods are considerably more image subordinate [Gonzalez and Woods, 2008].

RESTORATION PROCESS MODEL

The corruption cycle is typically demonstrated as a debasement work (as demonstrated in fig 2.1) that, along with an added substance commotion term, works on an information picture $f(x, y)$ to deliver a debased picture $g(x, y)$. Given $g(x, y)$, some information about the corruption work H , and some information about the added substance clamor term $\eta(x, y)$, the goal of rebuilding is to acquire a gauge (x, y) of the first picture. The gauge should be pretty much as close as conceivable to the first information picture and, by and large, the more about H and η is known, the nearer (x, y) will be to $f(x, y)$. In the event that H is a direct, move invariant cycle, at that point the debased picture is given in the spatial space by

$$g(x, y) = h(x, y) * f(x, y) + \eta(x, y)$$

where $h(x, y)$ is the spatial portrayal of the debasement work and the image "*" demonstrates convolution [Gonzalez and Woods, 2008]. Clamor might be multiplicative, where commotion is a component of sign size.

$$f = g + gv = g(1 + v) \approx gv$$

where f is the noisy image signal, g is the original image signal and v is the noise which is the function of the signal magnitude. Noise may be impulse noise, which is usually characterized by some portion of image pixels that are corrupted, leaving the remaining pixels unchanged. The most common example of impulse noise is the salt-and-pepper noise [Jain, 1989]. Noise decrease procedures are theoretically comparative paying little mind to the sign being handled. In any case, from the earlier information about the qualities of noise, tainting the first sign, can mean the usage of these strategies shift significantly relying upon the sort of sign. All account gadgets, both simple and digital, have attributes which make them defenseless to noise. Noise presented during securing or transmission can be either arbitrary or repetitive sound reasonable or no cognizant noise [<http://css.engineering.uiowa.edu/~dip/address/ImageProperties3.html>].





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IMAGE ENHANCEMENT

Image enhancement incorporates honing, differentiate control, separating, introduction and amplification, pseudocoloring, etc. The best trouble in image enhancement is evaluating the basis for enhancement. Consequently, countless image enhancement strategies are exact and require intuitive methods to acquire good outcomes. Be that as it may, image enhancement stays significant on account of its value in for all intents and purposes all image handling applications. Shading image enhancement may require improvement of shading parity or shading contrast in a shading image. Enhancement of shading images turns into a progressively troublesome errand not just in view of the additional component of the information yet additionally because of the additional unpredictability of shading observation [Gonzalez and Woods, 2008].

Image enhancement methods are utilized to improve the presence of the image or to separate the better subtleties in the corrupted images. The foremost goal of image enhancement is to process an image with the goal that the outcome is more reasonable than the first image for a particular application. A strategy that is very valuable for improving one classification of images may not be essentially be the best methodology for upgrading other class of images. Shading image enhancement utilizing RGB shading space is seen as wrong as it pulverizes the shading arrangement in the first image. Because of this explanation, the vast majority of the image enhancement strategies, particularly differentiate enhancement methods, use HSV shading space [Hanmandlu and Jha, 2006]. Image enhancement strategies might be ordered into two wide classes: change space techniques and spatial area strategies. The methods in the principal class depend on changing the recurrence change of an image, where as strategies in the subsequent classification straightforwardly work on the pixels. Be that as it may, figuring a two dimensional (2-D) change for an enormous exhibit (image) is a very tedious undertaking even with quick change procedures and isn't reasonable for constant preparing.

At the point when an image is prepared for visual translation, the watcher is a definitive appointed authority of how well a specific strategy functions [Chen et al., 2006a] [Chen et al., 2006b]. Visual assessment of image quality is an exceptionally emotional procedure, hence making the meaning of a decent image a slippery standard by which to think about calculation execution. At the point when the issue is one of preparing images for machine observation, the assessment task is to some degree simpler. Be that as it may, even in circumstances when a reasonable - cut basis of execution can be forced on the issue, a specific measure of experimentation is generally required before a specific image enhancement approach is chosen. The term spatial space alludes to the total of pixels making an image. Spatial space techniques are methods that work straightforwardly on these pixels. Spatial space process is indicated by the articulation:

$$g(x, y) = T[f(x, y)]$$

where $f(x, y)$ is the information image, $g(x, y)$ is the prepared image, and T is the administrator on f , characterized over some area of (x, y) . The chief methodology in characterizing an area about a point (x, y) is to utilize a square or rectangular sub-image territory focused at (x, y) . The sub-image/neighborhood territory is moved from pixel to pixel beginning at the upper left corner. The administrator T is applied at every area (x, y) to yield the yield g , at that area. The procedure uses just the pixels in the territory of the image crossed by the area [Gonzalez and Woods, 2008] [Jain, 1989]. The least complex type of T is the point at which the area is of size 1×1 . For this situation, g relies just upon the estimation of f at (x, y) , and T turns into a force or planning change elements of the structure

$$s = T(r)$$

where, r and s are factors signifying, individually, the dark degree of $f(x, y)$ and $g(x, y)$ anytime (x, y) . Since enhancement anytime in an image relies just upon the dark level by then, strategies in this class are frequently alluded to as point handling.



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Difference enhancement which is a subset of image enhancement looks to improve the clear visual nature of an image, and to underscore explicit image highlights. The procedures for differentiate enhancement incorporate dark level change based strategies (viz., logarithm change, piecewise-direct change, and so forth.) and histogram preparing methods (viz., histogram adjustment (HE), histogram determination, and so on.) [Kim, 1997] [Jin et al., 2001].

Given the wide assortment of sensors accessible (and their comparing attributes) for imaging, the differentiation enhancement in images requires explicit calculations for specifically improving certain highlights of an image. Expectedly, the difference enhancement is typically performed utilizing spatial area techniques, as there is commonly a need to choose explicit boundaries for enhancement. Hence, traditional complexity enhancement procedures have a characteristic failure for mechanization and furthermore can't be applied for expansive assortment of images. In addition, if the images are initially of low differentiation - like those relating to satellite images, flying images and clinical images of organs and tissues - at that point extra confinements which emerge out of utilizing the regular difference enhancement methods incorporate the cleaned out impact, intensification of foundation noise, abstract manual control, non-conservation of brilliance and the powerlessness to observe restricted force changes [Kim et al., 2001] [Lakshmanan et al., 2010] [Nair et al., 2011]. So as to adequately deal with the above issues progressively advanced calculations are required.

DELICATE COMPUTING AND IMAGE PROCESSING

The point of image restoration and enhancement is to improve the interpretability or impression of data in images for human watchers, or to give better contribution to other mechanized image handling strategies. The intensity of delicate processing methods is that they can deal with the unclearness and uncertainty productively. The vulnerabilities inside image preparing assignments are not generally because of arbitrariness however frequently because of dubiousness and uncertainty. Delicate processing procedures are joined to deal with such vulnerabilities emerging from inadequacies of data because of fragmented, uncertain and ambiguous information [Ross, 2005]. Among the delicate registering procedures, fuzzy rationale [Zadeh, 1965] assumes a significant job in overseeing issues related with the vulnerabilities in the image preparing undertakings, for example, restoration and enhancement, adequately. Fuzzy rationale gives a scientific system to portrayal and handling of master information. It is an incredible asset to speak to and process human information as fuzzy on the off chance that rules [Ross, 2005]. The control of these ideas prompts hypothesis of surmised thinking utilizing fuzzy frameworks in image preparing. Lately, numerous analysts have applied the fuzzy rationale to grow new image handling calculations. The fuzzy image preparing is one of the significant application regions of fuzzy rationale [Gonzalez and Woods, 2008].

FUZZY IMAGE PROCESSING

Fuzzy set hypothesis is valuable in taking care of different vulnerabilities in PC vision and image preparing applications. Fuzzy image preparing is an assortment of various fuzzy ways to deal with image handling that can comprehend, speak to and process the image [Gonzalez and Woods, 2008]. The portrayal and preparing rely upon the chose fuzzy procedure and the issue to be explained. It has three fundamental stages, specifically, image fuzzification, alteration of participation work esteems and defuzzification. Fig.2. shows the general advances associated with fuzzy-based image handling calculations.

The coding of the image information (fuzzification) and translating of results (defuzzification) are steps that make conceivable to process images with fuzzy methods. The principle intensity of fuzzy image preparing is in the center advance, that is, the capacity to adjust or use the participation esteems as indicated by the requirements of the issue to be understood. After the image information are changed from the spatial space to fuzzy area, suitable fuzzy strategies alter or use the enrollment esteems. This can be fuzzy principle based methodology, fuzzy grouping, fuzzy mix approach, etc [Ross, 2005]. Delicate figuring calculations proposed in this postulation for image restoration and enhancement utilizes fuzzy guideline based methodology for taking care of the participation esteems.





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Fuzzy thinking, otherwise called surmised thinking, is an induction method whose result is a decision for a lot of fuzzy on the off chance that rules. The means of fuzzy thinking can be given as follows:

- Input factors are contrasted and the participation work on the reason part to get the enrollment estimations of each etymological mark (fuzzification).
- The participation esteems on the reason part are joined through explicit fuzzy set activities to get terminating quality (weight) of each standard.
- Qualified subsequent (either fuzzy or fresh) is created relying upon the terminating quality.
- Qualified consequents are collected to create fresh yield as per the characterized strategies (defuzzification).

Fuzzy frameworks are made of an information base and thinking instrument called fuzzy derivation framework. A fuzzy deduction framework (FIS) comprises of four useful segments, for example, Fuzzification: Transforms the fresh contributions to degrees of match with phonetic qualities. Information Base: Consists of a standard base and a database. A standard base comprises of various fuzzy on the off chance that rules. A database characterizes the enrollment capacity of the fuzzy sets utilized in the fuzzy guidelines.

Fuzzy Inference Engine: Performs the derivation procedure on the guidelines.

Defuzzification: Conversion of fuzzy set to single fresh esteem.

There are two fundamental sorts of fuzzy models: Mamdani and Tagaki-Sugeno [Ross,2005]. In the Mamdani type, both standard predecessor and resulting are as enrollment capacities. The model yield is communicated in etymological terms: it is an enrollment evaluation to a semantic trademark. Now and again, an etymological yield is attractive, along these lines, defuzzification can be maintained a strategic distance from. In Mamdani models, defuzzification becomes important just when a numerical yield is required. In Tagaki-Sugeno models, the standard decision is as of now a fresh worth, and consequently, this model kind doesn't require an unequivocal defuzzification system. Fuzzy-based delicate processing calculations proposed in this proposition were created dependent on Tagaki-Sugeno fuzzy model sort.

A portion of the significant reasons that roused us to utilize fuzzy-based delicate registering strategy for tackling image restoration and enhancement issues are as per the following:

- Fuzzy rationale is a useful asset for speaking to and handling human information as fuzzy on the off chance that rules.
- Fuzzy procedures can deal with the equivocalness and unclearness proficiently.
- It is adaptable. With some random framework, it is anything but difficult to oversee it or layer greater usefulness on head of it.
- It is thoughtfully straightforward. The numerical ideas driving fuzzy thinking are basic.
- It is open minded toward loose information and fuzzy thinking incorporates this comprehension with the procedure.
- It depends on characteristic language. The reason for fuzzy rationale is the reason for human correspondence. This perception supports huge numbers of different explanations about fuzzy rationale.

FUZZY-BASED DECISION ALGORITHM FOR HIGH DENSITY IMPULSE NOISE REMOVAL

Digital image could be polluted by drive noise during image obtaining or transmission. Two regular kinds of drive noise are the salt-and-pepper noise and the irregular esteemed noise. For images adulterated by salt-and-pepper noise (separately, arbitrary esteemed noise), the boisterous pixels can take just the most extreme and the base qualities (individually, any irregular worth) in the dynamic range. Therefore, it could seriously debase the image quality and cause some loss of data. Different techniques have been proposed for the restoration of images ruined by drive noise, and it is notable that direct channels could deliver genuine image obscuring even in low noise thickness [Gonzalez and Woods, 2008].



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Therefore, nonlinear channels have been broadly abused because of their significantly better sifting exhibition as far as motivation noise weakening. The middle channel was before the most mainstream nonlinear channel for expelling motivation noise due to its great denoising power and computational effectiveness [Bovik, 2000] [Huang et al., 1979]. The middle channel replaces the focal estimation of a M-by-N neighborhood with its middle worth. One of the most well known and strong nonlinear channels is the standard middle channel (SMF) [Gonzalez and Woods, 2008] [Jain, 1989], which abuses the rank-request data of pixel forces inside a separating window and replaces the inside pixel with the middle worth. Because of its viability in noise concealment and straightforwardness in usage, different alterations of the SMF have been presented, for example, the weighted middle (WM) [Bovik, 2000] channel and the inside weighted middle (CWM) channel [Ko and Lee, 1991]. The significant downside of the SMF is that, the channel is powerful just for low noise densities, and moreover, shows obscuring if the window size is enormous. This prompts lacking noise concealment if the window size is little [Pomalaza-Racz and Macgille, 1984].

Ordinary middle separating [Gonzalez and Woods, 2008] [Bovik, 2000] [Ko and Lee, 1991] methodologies apply the middle activity to every pixel genuinely, that is, without thinking about whether it is uncorrupted or adulterated. Thus, the image subtleties contributed from the adulterated pixels causes image quality debasement. A natural answer for conquer this issue is to actualize a motivation noise recognition component before sifting. For this, exchanging middle channels can be utilized [Wang and Zhang, 1999] [Zhang and Karim, 2002] [Eng and Ma, 2001] [Ng and Ma, 2006], which gives huge execution improvement contrasted with existing propelled strategies for drive noise evacuation. In exchanging middle channels a noise location system has been joined with the goal that lone those pixels recognized as "defiled" would experience the sifting procedure, while those distinguished as "uncorrupted" would stay unblemished.

LITERATURE SURVEY

Non-direct channels, for example, Adaptive Median Filter (AMF) [Hwang and Haddad, 1995] can be utilized for segregating debased and uncorrupted pixels, and afterward apply the sifting procedure. Loud pixels will be supplanted by the middle worth and uncorrupted pixels will be left unaltered. AMF performs well at low noise densities since the undermined pixels which are supplanted by the middle qualities are not very many. At higher noise densities, window size must be expanded to show signs of improvement noise expulsion, which prompts less relationship between's adulterated pixel esteems and supplanted middle pixel esteems. In choice based or exchanging middle channel the choice depends on a pre-characterized edge esteem. The significant downside of this strategy is that characterizing a strong choice measure is troublesome. These channels won't consider the nearby highlights, because of which, edge subtleties may not be recuperated agreeably, particularly when the noise is high. [Chan et al., 2005] proposed a calculation to defeat the above issue, which comprises of two phases. The main stage is to arrange the debased and uncorrupted pixels by utilizing AMF and in the subsequent stage, regularization technique is applied to the defiled pixels to save edges and stifle noise. The downside of this strategy is that for high drive noise, it requires enormous window size of 39×39, and also requires complex hardware for the execution and assurance of smoothing factor β to get great outcomes.

The Alpha-Trimmed Mean Filter (ATMF) [Astola and Kuosmanen, 1997] and Alpha cut midpoint channel (ATMP) [Srinivasan and Ebenezer, 2000] are another sort of non-straight channels examined in the writing. These channels are additionally used to expel the motivation noise in which the boundary α , called the cutting element, controls the quantity of qualities that are cut. It tends to be considered that to be the estimation of the cutting variable α builds, the capacity of the channel to expel motivation noise is additionally expanded. Be that as it may, when the noise thickness is as high as half or more, there is lacking noise-evacuation and loss of image edge subtleties. The explanation behind this misfortune in image edge subtleties is because of the way that these channels trim the outrageous qualities regardless of whether they are not drive esteems. Likewise the mean activity of the channel smoothen the image which will make the edge data be lost.



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[Srinivasan and Ebenezer, 2007] proposed an effective choice based calculation (EDBA) in which the ruined pixels are supplanted by either the middle pixel or neighborhood pixel by utilizing a fixed window size of 3×3 bringing about lower handling time and great edge safeguarding. In spite of the fact that EDBA channel indicated promising outcomes, a smooth change between the pixels is lost prompting debasement in the visual nature of the image as line antiquities, since it just thinks about the left neighborhood from the last prepared worth. To beat this issue an improved choice based calculation (IDBA) has been proposed [Nair et al., 2008a] [Nair et al., 2008b], in which, debased pixels can either be supplanted by the middle pixel or, by the mean of prepared pixels in the area, bringing about a smooth progress between the pixels with edge conservation and better visual quality. The downside of this technique is that on account of high thickness drive noise, the fixed window size of 3×3 will bring about image quality debasement because of the nearness of defiled pixels in the area.

To address high noise thickness, a noise versatile delicate exchanging middle (NASM) channel was proposed in [Eng and Ma, 2001], which comprises of a three-level progressive delicate exchanging noise location process. The NASM accomplishes a genuinely strong exhibition in expelling drive noise, while protecting sign subtleties over a wide scope of noise densities. Anyway the nature of the recouped image turns out to be essentially debased when noise thickness is more noteworthy than half. To conquer execution corruption at higher noise thickness another proficient technique called BDND [Ng and Ma, 2006] has been presented and it has demonstrated better outcomes. Be that as it may, at high noise thickness BDND shows higher misdetection and bogus caution rate (indiscriminately noise). Thus, it couldn't protect the edge subtleties of the recouped image and the nature of the reestablished image is diminished. The fundamental downside of exchanging middle channels like BDND is that on account of high thickness motivation noises, there is as yet an opportunity for good portrayal of the adulterated pixels in the chose window to participate in the separating procedure, which may prompt the corruption of image quality. Another downside of BDND exchanging channel is that it first uses a window size of 21×21 to distinguish whether a pixel is tainted or not and again a subsequent cycle is performed on a decreased window size of 3×3 with same arrangement of steps to lessen the misclassification of pixels. Real separating process starts after the two degrees of cycles. Therefore, BDND calculation devours a great deal of time.

So as to beat the downsides of the above channels, we propose another fuzzy-based choice calculation (FBDA) for expelling motivation noise at a wide scope of noise densities, particularly for high drive noise. FBDA is an improved fuzzy-based exchanging middle channel in which the sifting is applied distinctly to ruined pixels in the image while the uncorrupted pixels are left unaltered. What makes FBDA not the same as other exchanging middle channels, for example, BDND is that during the hour of separating process FBDA chooses just uncorrupted pixels in the chose window dependent on a fuzzy separation participation esteem. Consequently the benefit of FBDA is that it has both the noise discovery power just as the intensity of taking out undermined pixels during the separating procedure. Another preferred position of FBDA is that it utilizes just one degree of emphasis to recognize whether a pixel is tainted or not and it utilizes a fixed window size of 3×3 or 5×5 (in light of the noise thickness) for both the noise location and the separating procedure. The initial phase in FBDA is to group a pixel as either adulterated or uncorrupted. For a pixel distinguished as a defiled pixel, FBDA chooses a window which comprises of neighborhood pixels. It at that point figures the distinction measure for every pixel in that chose window dependent on the focal pixel (the tainted pixel) and afterward ascertains the enrollment esteem for each dependent on the most noteworthy contrast. FBDA wipes out those pixels from the window with high (near 1) and exceptionally low (near 0) enrollment esteems, since they may speak to the motivation noises. Middle channel is then applied to the rest of the pixels in the window to get the new pixel esteem for the current pixel position.

Proposed FBDA technique is an exchanging middle channel which safeguards the edge subtleties on account of high thickness drive noise circumstance contrasted with non - exchanging ATMF. Rather than ATMF or ATMP, FBDA disposes of the tainted pixels in the window from the separating procedure utilizing the fuzzy separation enrollment esteem determined from the focal pixel, which subsequently prompts better outcomes. FBDA is along these lines increasingly versatile and proficient contrasted with ATMF or ATMP.





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The proficiency of the proposed calculation is tried utilizing standard images in the wake of applying four distinctive noise models, which are clarified in segment 3.3. From test examination, it has been discovered that SMF, AMF, EDDBA, IDDBA and BDND techniques are not giving persuading results for the diverse noise models. Be that as it may, FBDA gives better visual outcomes for all the diverse noise models contrasted with above channels. It has additionally been demonstrated through exploratory investigation that FBDA produce better outcomes as far as quantitative just as subjective measures, contrasted with different channels.

CONCLUSION

Images are regularly debased by arbitrary noise. Noise can happen during image catch, transmission or preparing, and might be subject to or free of image content. Noise is typically depicted by its probabilistic attributes. Gaussian noise is a generally excellent guess of noise that happens in numerous down to earth cases [Jain, 1989]. A definitive objective of restoration procedures is to improve an image in some pre-characterized sense. Restoration endeavors to reproduce or recoup an image that has been corrupted by utilizing from the earlier information on the debasement marvel [Gonzalez and Woods, 2008]. In this manner restoration strategies are arranged toward displaying the debasement and applying the converse procedure so as to recuperate the first image. During image transmission, noise which is typically autonomous of the image signal happens. The added substance noise model, where noise v and image signal g are free, can be spoken to as:

$$f(x, y) = g(x, y) + v(x, y)$$

where $f(x, y)$ is the boisterous image signal, $g(x, y)$ is the first image signal and $v(x, y)$ is the noise signal [Gonzalo, 2006]. The added substance noise image v models a bothersome, eccentric defilement of g . The image v is known as a two-dimensional arbitrary procedure or an irregular field. The objective of restoration is to recoup an image h that takes after g as intently as conceivable by decreasing v . One technique to evacuate noise is to utilize straight channels by convolving the first image with a veil [Bovik and Acton, 2006]. The Gaussian cover includes components dictated by a Gaussian capacity. It gives the image an obscured appearance if the standard deviation of the veil is high, and has the impact of spreading out the estimation of a solitary pixel over a zone of the image. Averaging sets every pixel to the normal estimation of itself and its close by neighbors. Averaging will in general haze an image, since pixel force esteems which are altogether higher or lower than the encompassing neighborhood would spread over the territory. Traditionalist smoothing is another noise decrease method that is unequivocally intended to expel noise spikes (for example salt and pepper noise) and is, thusly, less successful at expelling added substance noise from an image [<http://homepages.inf.ed.ac.uk/rbf/HIPR2/csmooth.htm>]. Another technique is to utilize regular non-straight channel, for example, Standard Median Filter (SMF) to expel the noise. Despite the fact that it is useful for expelling motivation noise, it isn't productive in evacuating added substance noise [<http://homepages.inf.ed.ac.uk/rbf/HIPR2/csmooth.htm>]. Wiener channel [Gonzalez and Woods, 2008] is a decent channel to expel added substance noise, however the visual nature of the outcome acquired isn't sufficient contrasted with different channels.

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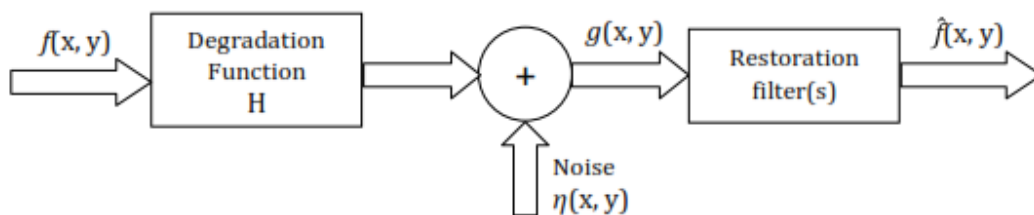


Fig. 1. Corruption cycle is typically demonstrated as a debasement work

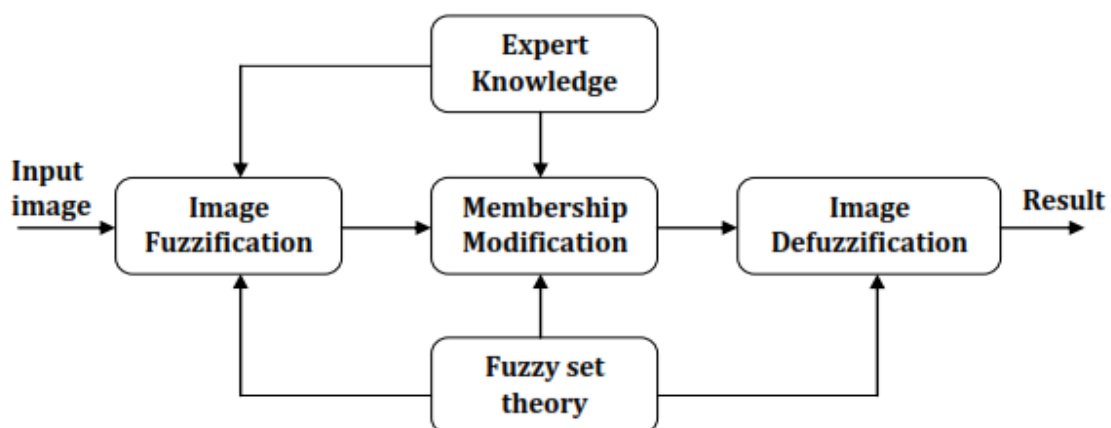
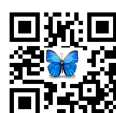


Fig.2. Fuzzy image processing.





A Study on Customer Satisfaction towards Digital Marketing

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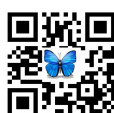
ABSTRACT

In This Modern World the usage of internet and online based components are more. Digital marketing plays an important role in this modern society to reach the customers based on the marketing of products or services through this technology. They attract the customers by giving advertisements in the online and mobile apps to sell their products, in this study it reveals that the digital marketing are given much important in this society. Digital marketing is important because more and more customers are now using internet. It is an immediate way to reach them. It is a new way of approaching customer and a new way of understanding the customer satisfaction in modern marketing and digital marketing.

Keywords: Digital, Components, Modern, Marketing, Online, Traditional.

INTRODUCTION

The digital marketing target a specific customers based on the interactive in online google search. Digital marketing is like a direct marketing which connect costumers with sellers digitally using interactive technologies like emails, websites, online forums and newsgroups, interactive television, mobile communications , (Kotler and Armstrong, 2009). It mainly uses the social media marketing, website marketing, search engine marketing, pay per click, mobile marketing (google apps and apple store), email marketing, online banner marketing, and video marketing. They mainly use this source to market the business products or services to the customers. The customer satisfaction is main profit for the business; it includes the wants and needs of the customer. There are different types of media, they are owned media, paid media and earned media.





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STATEMENT OF THE PROBLEM

The digital media plays an important role now a day. The marketing has diversified because of this digital marketing. The traditional method of marketing has changed. The customers are aware of this source and from the place itself they come to know about new products. The traditional marketing has vanished from the customers mind.

OBJECTIVE

To know the satisfaction level of the customers in digital marketing
To find the level of achievement in digital marketing

LIMITATION OF THE STUDY

The study is only done in Coimbatore city. It deals with the digital marketing only. It shows the customer interest in this type of marketing.

STATISTICAL TOOLS USED

Percentage analysis
Rank analysis

REVIEW OF LITERATURE

G. Pasupathi, IJMSS, (2018) In this digital era, the Digital avenues available onsite through the electronic media have enhanced the possibility and success of Digital Marketing all over the world. The main aim of Marketing is to offer standard products at the right place, at the right time and at the right price. The advent of Digital Marketing enables to accomplish this basic objective by providing an arena for the display of variety of goods and services at varied prices and standards, thus facilitating the consumers to choose the right product / service at the right time from the supplier at the cost and also at their own convenience. Kowsalya KR, International Journal of Science and Research, (2018) The study significantly reveals the concept of digital marketing that is marketing through various digital platforms like mobile phone application and other similar forms. It also creates on the impact by the same with regard to the consumers buying decision.

ANALYSIS AND INTERPRETATION

Interpretation

From the table 1 it is clear that 32% of the respondents are in the age group of 31 to 40 years, 30% of the respondents are in the age group of 41 to 50 years, 15% of the respondents are in the age group of above 61 years, 14% of the respondents are in the age group of 51 to 60 years and only 9 % of the respondents are in the age group of 21 to 30 years. Majority of the respondents are between the age group of 31 to 40 years.

Interpretation

From the table 2 it is clear that 45% of the respondents are says that they use mobile phone, 30% of the respondents are says that lap tops, 20% of the respondents are use to watch television and 5% of the respondents are use other electronic items to watch the advertisement. Majority of the respondents are using mobile phones to watch the advertisements.

Interpretation

From the table 3 it is clear that 72% of the respondents are satisfied with digital marketing, and only 18% of the respondents are not satisfied with digital marketing. Majority of the respondents are satisfied with digital marketing.





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Rank Analysis

Interpretation

From the table 4 it is understood that 72% of the respondents are fully aware of the enhanced user friendly facility and ranked as 1, 52% of the respondents are not aware of the secured facility and ranked as 2, 50% of the respondents are fully aware of the privacy facility and ranked as 3, 56% of the respondents are partially aware of the reliability facility and ranked as 4, 28% of the respondents are partially aware of the cost facility and ranked as 5. Majority of the respondents are fully aware of the user friendly services and ranked as 1.

CONCLUSION

The strategies to sell may vary from product to product in the markets, So nowadays they are follow new method to attract the customers, mainly they using digital marketing to promote their products. Other than traditional marketing it plays a vital role in digital marketing in the markets to attract the customers on the online portals. They can visit more virtual shop in this digital marketing platform and can select the product by just a click. Majority of the customers are more satisfied. It reduce the time of the customers. They provide more options to buy and sell the products in the online platform, so everyone is found of using digital marketing.

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Table 1: Targeted Age group of the customers

S.No	Particulars	Number of Respondent	Percentage
1	21 to 30	9	9
2	31 to 40	32	32
3	41 to 50	30	30
4	51 to 60	14	14
5	Above 61	15	15
	Total	100	100

Table 2: The electronic usage of the customers to watch advertisements.

S.No	Particulars	Number of Respondent	Percentage
1	Televisions	20	20
2	Lap tops	30	30
3	Mobiles	45	45
4	others	05	05
	Total	100	100





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Table 3: Do you satisfied with digital marketing?

S.No	Particulars	Number of Respondent	Percentage
1	Yes	72	72
2	No	18	18
	Total	100	100

Table 4: Rank the following usage of digital marketing

S.no	Factors	Fully aware		Partially aware		Not aware		Rank
		No	%	No	%	No	%	
1	Use friendly	72	72	38	38	-	-	1
2	Secured	18	18	52	52	30	30	2
3	Privacy	50	50	40	40	10	10	3
4	cost	28	28			-	-	5
5	reliability	32	32	56	56	12	12	4

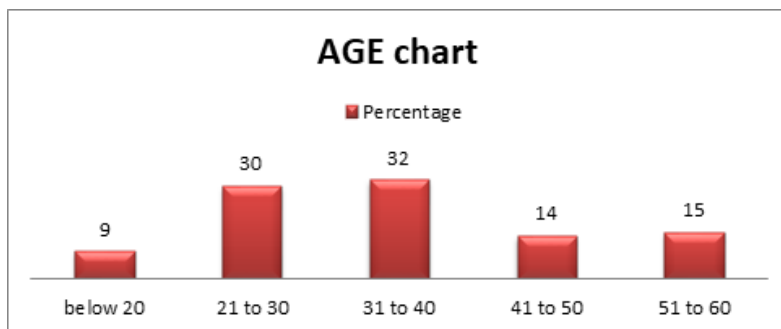


Fig. 1: Age Chart

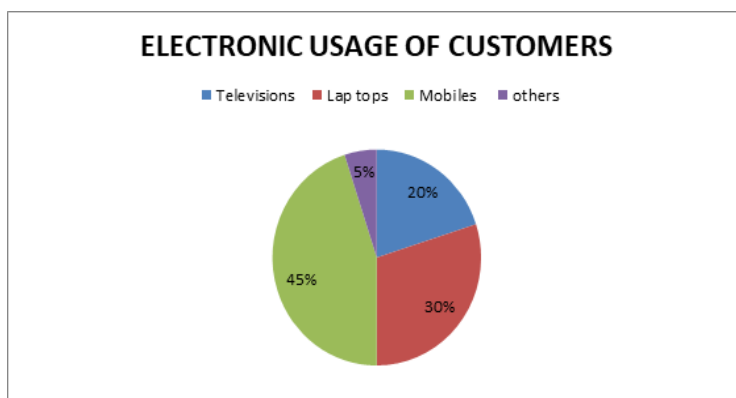


Fig. 2. Electronic usage of customers





Simulation of FT-IR and FT-Raman Spectra Based on quantum chemical Calculations, Vibrational Assignments, Hyperpolarizability, and Homo-Lumo Analysis of 5(4 methyl phenyl)tetrazole (5MPTZ)

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ABSTRACT

The spectra of 5(4 methyl phenyl)tetrazole (5MPTZ) have been recorded in the regions 4000–400 cm^{-1} for FT-IR and 3500–100 cm^{-1} for FT-Raman. The geometry optimization, vibrational frequencies were obtained by the density functional theory (DFT) using B3LYP method with 6-31G and 6-311+G basis sets. The complete assignments were performed on the basis of the potential energy distribution (PED) of the vibrational modes, calculated and the scaled values were compared with experimental FT-IR and FT-Raman spectra. The HOMO and LUMO energy gap reveals that the energy gap reflects the chemical activity of the molecule. The dipole moment (μ), polarizability (α), anisotropy polarizability ($\Delta\alpha$) and first hyperpolarizability (β_{tot}) of the molecule have been reported. Information about the size, shape, charge density distribution and site of chemical reactivity of the molecule has been obtained by molecular electrostatic potential (MEP).

Keywords: 5(4 methyl phenyl)tetrazole (5MPTZ) , DFT, FT-IR,FT-Raman, MEP.

INTRODUCTION

Tetrazole-related molecules have attracted considerable attention due to their biological activities. The synthesis of new members of this family of ligands is an important direction in the development of modern coordination chemistry [1,2]. Tetrazole compounds have a wide range of applications in coordination chemistry, medicinal

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chemistry and material science[3,4] . Tetrazole derivatives are used as antibiotics and optically active tetrazole-containing antifungal preparations of azole type was reported. There is always a need for new and effective antifungal and antibacterial agents with broad-spectrum activities. It was decided to develop this interest by ascertaining the molecules features essential for activity and utilizing them to develop a new class of potential drugs [5]. The detailed investigations of the 5(4 methyl phenyl) tetrazole (5MPTZ) were carried out with Gaussian 09 software package [6] using the Beeke-3-Lee-Yang-Parr (B3LYP) functional[7,8] supplemented with the standard 6-31G and 6-311+G basis set.

Experimental Details

The fine sample of 5(4 methyl phenyl) tetrazole (5MPTZ) was obtained from Lancaster Chemical Company.UK, with a stated purity of 99% and it was used as such without further purification. The FT-Raman Spectrum of 5MPTZ was recorded using 1064 nm line of ND: YAG laser for excitation wavelength in the region 3500-100 cm^{-1} on Thermo Electron Corporation model Nexus spectrometer equipped with FT-Raman module accessory. The FT-IR Spectrum of the title compound was recorded in the region 4000-400 cm^{-1} on Perking Elmer Spectrophotometer in KBr pellet.

Computational Details

The combination of Vibration spectra with quantum chemical calculation is effective for understanding the fundamental mode of vibration of the compound. The structural characteristic, stability and energy of the compound under investigation are determined by DFT with the three-parameter hybrid functional (B₃) for the exchange part and the Becke Three Lee Yong-Pare (LYP) and 6-31G ,6-311+G basis sets with Gaussian 09 Program Package. The Cartesian representation of the theoretical force constants has been compound at the fully optimized geometry by assuming the molecule belongs to C₁ point group symmetry. The Transformation of force field from Cartesian to internal local symmetry coordinates, the scaling, and the subsequent normal coordinate analysis (NCA) Calculation of potential energy distributions (PED) has been done on a PC with the VEDA program.

Geometrical parameter

The molecular structure along with numbering of atoms of 5(4 methyl phenyl)tetrazole (5MPTZ) was as shown in the Fig.1 . The Global minimum energies of the title molecule calculated by Density Functional Theory structure optimization for different basis sets such as B3LYP/6-31+ G(d,p) ,B3LYP/6-31++ G(d,p) , B3LYP/6-311+ G(d,p) and B3LYP/6-311++ G(d,p) are given in Table 1. Geometry optimization is the procedure that attempts to find the configuration of minimum energy of the molecule. The procedure calculates the wave function and the energy at a starting geometry and then proceeds to search a new geometry of a lower energy. This is repeated until the lowest energy geometry is found. The procedure calculates the force on each atom by evaluating the gradient or the first derivative of the energy with respect to atomic positions. Sophisticated algorithms are then used at each step to select a new geometry, aiming for rapid convergence to the geometry of the lowest energy. In the final, minimum energy geometry the force on each atom is zero. The optimized geometric parameters like bond length, bond angles of 5(4 methyl phenyl) tetrazole (5MPTZ) were calculated and given in Table II .

The title compound is a tetrazole ligand with a toluene substituent in position 5 (Fig. 1). In the solid state structure the molecule possesses crystallographic mirror symmetry, with four C atoms lying on the reflecting plane, which bisects the phenyl and tetrazole rings [9].The C-C bond lengths in the benzene ring obtained from B3LYP ranges from 1.41 to 1.40 Å and C-H bond length ranges from 1.09 to 1.08 Å. The C-N bond lengths in the tetrazole ring have calculated as 1.36 and 1.35 Å. the N1-N2 and N3-N4 bond length of the tetrazole ring has longer than the N2-N3 bond length in the same ring,the C-H bond lengths of the methyl group were 1.10-1.09 Å .The bond angle of the phenyl ring does not have equal value .it varied from 118° to 121°.All the values were compared with experimental values[9],those are good agreement with each other.



**Rajeswari et al.****Electronic properties**

When we are dealing with interacting molecular orbitals, the two that interact are generally The Highest energy Occupied Molecular Orbital (HOMO) of one molecule, The Lowest energy Unoccupied Molecular Orbital (LUMO) of the other molecule. These orbitals are the pair that lie closest in energy of any pair of orbitals in the two molecules, which allows them to interact most strongly. These orbitals are sometimes called the frontier orbitals, because they lie at the outermost boundaries of the electrons of the molecules. The energy gap between the HOMOs and LUMOs called as energy gap. It is a critical parameter in determining molecular electrical transport properties because it is a measure of electron conductivity [10]. The HOMO energy characterizes the ability of electron giving, the LUMO characterizes the ability of electron accepting, and the gap between HOMO and LUMO characterizes the molecular chemical stability [11]. Surfaces for the frontier orbital's were drawn to understand the bonding scheme of present compound. The features of these Molecular Orbitals can be seen in Figure 2.

This electronic absorption corresponds to the transition from the ground state to the first excited state and is mainly described by one electron excitation from HOMO to LUMO. While the energy of the HOMO is directly related to the ionization potential, LUMO energy is directly related to the electron affinity. There are lots of applications available for the use of HOMO and LUMO energy gap as a quantum chemical descriptor. It establishes correlation in various chemical and bio-chemical systems [12]. The HOMO–LUMO energy gap is an important value for stability index. A large HOMO–LUMO gap implies high stability for the molecule in the sense of its lower reactivity in chemical reactions [13]. According to B3LYP calculation, EHOMO, ELUMO and the energy band gap (translation from HOMO to LUMO) of the title molecule in electron Volt are presented in Table 2. Considering the chemical hardness, large HOMO-LUMO gap represent a hard molecule and small HOMO-LUMO gap represent a soft molecule. From the Table 2, it is clear that the molecule under investigation is very soft since it has a small HOMO-LUMO gap and also having a high value for softness.

NLO properties

The NLO activity provide the key functions for frequency shifting, optical modulation, optical switching and optical logic for the developing technologies in areas such as communication, signal processing and optical interconnections [14]. The first static hyperpolarizability (β_{tot}) and its related properties (β , α and $\Delta\alpha$) have been calculated using B3LYP/6-31G and 6-311+G level based on finite field approach. In the presence of an applied electric field, the energy of a system is a function of the electric field and the first hyperpolarizability is a third rank tensor that can be described by a $3 \times 3 \times 3$ matrix. The 27 components of the 3D matrix can be reduced to 10 components because of the Kleinman symmetry [15]. The matrix can be given in the lower tetrahedral format. It is obvious that the lower part of the $3 \times 3 \times 3$ matrices is a tetrahedral.

The values of the polarizabilities (α) and first hyperpolarizability (β_{tot}) of the Gaussian 09 output are reported in atomic units (a.u.). All the calculated values then have been converted into electrostatic units (esu). (For α : 1a.u. = 0.1482×10^{-24} esu; For β : 1a.u. = 8.639×10^{-33} esu). The total molecular dipole moment and first order hyperpolarizability are 7.0628 and 7.2331 Debye and 1.1830×10^{-30} and 1.2410×10^{-30} esu, respectively and are depicted in Table 4. Total dipole moment of title molecule is greater than that of urea and first order hyperpolarizability is very much greater than that of urea (μ and β of urea are 1.3732 Debye and 0.3728×10^{-30} esu) obtained by B3LYP/6-31G and 6-311+G method. This result indicates the nonlinearity of the title molecule.

Mulliken Atomic charge

Atomic charges has been used to describe the process of electronegativity equalization and charge transfer in chemical reactions [16,17]. Mulliken atomic charge calculation has an important role in the application of quantum chemical calculation to molecular system because atomic charges affect dipole moment, molecular polarizability, electronic structure and a lot of properties of electronic systems. The Mulliken atomic charges are calculated at B3LYP/6-31G and 6-311+G level by determining the electron population of each atom as defined by the basis function and collected in Table 3.



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A Graph of Mulliken atomic charge on individual atom of 5MPTZ was drawn and given in Figure 8. It is worthy to mention that N3, N4 and C8, C9, C11 and C12, C17 atoms of the title molecule exhibit negative charge where all hydrogen atoms exhibit positive charges.

Molecular Electrostatic Potential

The molecular electrostatic potential is the potential that a unit positive charge would experience at any point surrounding the molecule due to the electron density distribution in the molecule. The electrostatic potential generated in space by charge distribution is helpful to understand the electrophilic and nucleophilic regions in the title molecule. Electrostatic potential maps, also known as electrostatic potential energy maps, or molecular electrical potential surfaces, illustrate the charge distributions of molecules three dimensionally. Knowledge of the charge distributions can be used to determine how molecules interact with one another. Molecular electrostatic potential (MEP) mapping is very useful in the investigation of the molecular structure with its physicochemical property relationships [18-21]. In the electrostatic potential map, the semispherical blue shapes that emerge from the edges of the above electrostatic potential map are hydrogen atoms.

The molecular electrostatic potential surface (MESP) which is a 3D plot of electrostatic potential mapped onto the iso electron density surface simultaneously displays molecular shape, size and electrostatic potential values. The Electrostatic potential surface of 5MPTZ is shown in Figure 3. The colour scheme for the MESP surface is red - electron rich or partially negative charge; blue - electron deficient or partially positive charge; light blue - slightly electron deficient region; yellow - slightly electron rich region, respectively. Areas of low potential, red, are characterised by an abundance of electrons. Areas of high potential, blue, are characterised by a relative absence of electrons. That is negative potential sites are on the electronegative atoms like nitrogen while the positive potential sites are around the hydrogen and carbon atoms. Green area covers parts of the molecule where electrostatic potentials are nearly equal to zero. This is a region of zero potential enveloping the π systems of aromatic ring leaving a more electrophilic region in the plane of hydrogen atom. Nitrogen has a higher electronegativity value and would consequently have a higher electron density around them. Thus the spherical region that corresponds to nitrogen atom would have a red portion on it. The MESP of 5MPTZ clearly indicates the electron rich centres of nitrogen atom.

Vibrational Assignment

Vibrational spectroscopy has been shown to be effective in the identification of functional groups of organic compounds as well as in studies on molecular conformations and reaction kinetics [22]. The symmetry possessed by the title molecule helps to determine and classify the actual number of fundamental vibrations of the system. The observed spectrum is explained on the basis of C₁ point group symmetry. The title molecule consists of 20 atoms, which undergo 36 normal modes of vibrations. The total number of 54 fundamental vibrations ($3N-6$, where N is the number of atoms) are distributed as $\Gamma_{\text{vib}} = 37 A'$ (In plane vibrations; $2N-3$) $17 A''$ (out of plane vibrations; $N-3$). All vibrations are active both in Raman and infrared absorption. The detailed vibrational assignment of fundamental modes of 5MPTZ along with the calculated IR and Raman frequencies, normal mode descriptions using PED (Potential Energy Distribution) are reported in Table III. The calculated frequencies are usually higher than the corresponding experimental quantities, due to the combination of electron correlation effects and basis set deficiencies.

C-H vibrations: The presence of C-H stretching vibrations in the region 3000 - 3200 is common for heteroaromatic structure. In the present study the C-H stretching vibrations of the title compound are observed at 3082 and 3045 cm^{-1} in the FT-IR spectrum and 3050 cm^{-1} in the FT-Raman spectrum. The calculated wave numbers at 3097, 3067, 3058, 3044 cm^{-1} are assigned to C-H stretching vibrations. The C-H out of plane bending vibrations are occurring in the region 900-667 cm^{-1} [23]. In the present investigation the computed wave numbers at 1073, 1009, 1008 are assigned to C-H out of plane vibrations and the scaled values are in good agreement with the experimental values. The assignments of other in-plane and out-of-plane C-H bending vibrations are as shown in Table III.



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Ring vibrations: There are six equivalent C-C bonds in benzene and consequently there will be six C-C stretching vibrations. In addition, there are several C-C-C in-plane and out-of-plane bending vibrations of the ring carbons. However, due to high symmetry of benzene, many modes of vibrations are infrared inactive. In general, the bands around 1400 to 1650 cm^{-1} in benzene derivatives are assigned to skeletal stretching C-C bands[24]. In the case of title compound the carbon stretching vibrations have been observed at 1613,1580,1505 cm^{-1} in the FT-IR spectrum and 1630.1595 cm^{-1} in FT-Raman spectrum were assigned to C-C stretching vibration which show good agreement with scaled frequencies. The measured wavenumbers at 1603,1558,1533 cm^{-1} are assigned to C-C stretching vibrations. It is clear from the above values that the difference between observed and scaled frequencies is very small. In general, the C-C-C out-of-plane and in-plane-bending vibrational wavenumber observed in FT-IR spectrum and FT-Raman spectrum shows good agreement with theoretically computed wavenumber.

C-N Vibrations: The mixing of several bands are possible in this region. The C-N stretching frequency is a rather difficult task. In the present study the band observed at 1285,1116 cm^{-1} in FT-IR spectrum is attributed to C-N bending vibration. The theoretically calculated value of corresponding C-N bending vibration is predicted at 1298,1131 cm^{-1} . B3LYP computation gives mode arising from the N-N stretching at 1158,1147 cm^{-1} corresponding to the peak at 1187,1163 cm^{-1} in IR spectrum. The methyl group CH stretching vibrational frequency band is found in 5MPTZ at 2924, 2910 cm^{-1} respectively, in the FT-Raman, FT-IR spectrum. The CH stretching mode for methyl is in the zone of 3000-2800 cm^{-1} [25,26].theoretically found at 2994,2965,2906 cm^{-1} .

CONCLUSION

The present investigation thoroughly analyzed the HOMO-LUMO, and vibration spectra, both infrared and Raman of 5MPTZ molecules with B3LYP method with standard 6-31G and 6-311+G basis sets. All the vibration bands are observed in the FT-IR and FT-Raman spectra of the compound are assigned to various modes of vibration and most of the modes have wave numbers in the expected range. The complete vibration assignments of wave numbers are made on the basis of potential energy distribution (PED).The electrostatic potential surfaces (MEP) together with complete analysis of the vibration spectra, both IR and Raman and help to identify the structure and symmetry. The excellent agreement of the calculated and observed vibration spectra reveals the advantages over the other method. Finally, calculated HOMO-LUMO energies show that the charge transfer occurs in the molecule, which are responsible for the bioactive property of the biomedical compound 5MPTZ.

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Table 1: Geometry Optimization Parameter of 5(4 methyl phenyl)tetrazole (5MPTZ) based on B3LYP/6-31G and B3LYP/6-311+G method and basis set

Bond Length(Å)			Bond Angle(°)		
Atom	6-31G	6-311+G	Atom	6-31G	6-311+G
C10-C17	1.51	1.51	C5-N1-H6	131.076	131.348
C5-C7	1.46	1.46	N1-C5-C7	126.995	126.910
C7-C8	1.41	1.41	N4-C5-C7	125.406	125.550
C9-C10	1.41	1.41	C5-C7-C12	122.300	122.211
C7-C12	1.41	1.40	C8-C9-C10	121.259	121.342
C10-C11	1.40	1.40	C11-C10-C17	121.104	121.250
C11-C12	1.40	1.39	C10-C11-C12	121.068	121.120
N3-N4	1.39	1.39	C9-C8-H13	120.957	120.821
C8-C9	1.39	1.39	C9-C10-C17	120.797	120.776
N1-N2	1.39	1.39	C7-C12-H16	120.709	120.758
N1-C5	1.36	1.36	C7-C12-C11	120.464	120.543
N4-C5	1.35	1.34	C7-C8-C9	120.287	120.345
N2-N3	1.32	1.32	N2-N1-H6	119.550	119.503
C17-H18	1.10	1.09	C10-C11-H15	119.469	119.410
C17-H20	1.10	1.09	C12-C11-H15	119.463	119.377
C17-H19	1.09	1.09	C10-C9-H14	119.396	119.248
C12-H16	1.09	1.08	C8-C9-H14	119.345	119.130
C9-H14	1.09	1.08	C5-C7-C8	118.874	119.110





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C11-H15	1.09	1.08	C11-C12-H16	118.826	118.897
C8-H13	1.08	1.08	C8-C7-C12	118.826	118.678
N1-H6	1.01	1.00	C7-C8-H13	118.756	118.637
			C9-C10-C11	118.095	117.972
			C10-C17-H19	111.510	111.460
			C10-C17-H20	111.392	111.377
			C10-C17-H18	111.099	111.138
			N2-N3-N4	110.866	110.610
			N2-N1-C5	109.374	109.523
			H19-C17-H20	108.043	107.892
			N1-C5-N4	107.599	107.584
			H18-C17-H19	107.485	107.541
			H18-C17-H20	107.106	107.188
			N3-N4-C5	106.612	106.712
			N1-N2-N3	105.549	105.614

Table 2: HOMO-LUMO energy (eV) and other related properties of 5(4 methyl phenyl)tetrazole (5MPTZ) based on B3LYP/6-31G and B3LYP/6-311+G method and basis set

Parameters	6-31G (eV)	6-311+G (eV)
Homo(I)	-6.8600	-7.1484
Lumo(A)	-1.6599	-2.0000
Energy gap(ΔE)	5.2001	5.1484
Electronegativity	4.2599	4.5742
Global hardness	2.6000	2.5742
Global softness(eV^{-1})	0.3846	0.3885
Chemical potential	-4.2599	-4.5742
Electrophilicity	3.4898	4.0641

Table 3: Mulliken charge (charge/e) of 5(4 methyl phenyl)tetrazole (5MPTZ) based on B3LYP/6-31G and B3LYP/6-311+G method and basis set.

Atom	6-31G	6-311+G
	Charge/e	
N1	0.6088	0.5648
N2	0.0148	0.1788
N3	-0.0822	-0.1907
N4	-0.3317	-0.3710
C5	0.4033	1.0604
H6	0.3623	0.4352
C7	0.1626	1.7626
C8	-0.1369	-0.2400
C9	-0.1635	-0.5421
C10	0.1262	1.1972
C11	-0.1665	-1.1517





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C12	-0.1386	-0.5323
H13	0.1839	0.2560
H14	0.1362	0.2139
H15	0.1321	0.2051
H16	0.1165	0.1870
C17	-0.4836	-1.3766
H18	0.1660	0.2596
H19	0.1497	0.2312
H20	0.1582	0.2416

Table 4: Dipole moment(μ), Polarizability(α), Anisotropy polarizability($\Delta\alpha$) and First order polarizability(β_{tot}) of 5(4 methyl phenyl)tetrazole (5MPTZ) based on B3LYP/6-31G and B3LYP/6-311+G method and basis set

Parameters	6-31G	6-311+G
μ_x	6.0797	6.2633
μ_y	3.5941	3.6175
μ_z	0.0489	0.0429
α_{xx}	-82.6947	-84.9573
α_{yy}	-63.8621	-65.4107
α_{zz}	-71.5172	-73.5735
α_{xy}	-9.0345	-9.1645
α_{xz}	0.1748	0.0651
α_{yz}	-0.0015	-0.0182
β_{xxx}	130.0165	135.7744
β_{yyy}	20.1929	21.203
β_{zzz}	0.5809	0.4321
β_{xyy}	7.982	9.1765
β_{xxy}	17.6595	17.3876
β_{xxz}	0.708	0.462
β_{xzz}	-6.36	-6.5217
β_{vzz}	-0.1262	-0.2017
β_{vyz}	-0.3797	-0.2368
β_{xvz}	-0.0077	-0.0152
$\mu(\text{debye})$	7.0628	7.2331
$\alpha(\text{esu})$	-10.7583×10^{-24}	-11.0478×10^{-24}
$\Delta\alpha(\text{esu})$	144.1678×10^{-24}	148.1296×10^{-24}
$\beta_{tot}(\text{esu})$	1.1830×10^{-30}	1.2410×10^{-30}





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Table 5: Observed Frequency (cm⁻¹), Theoretical Frequency (cm⁻¹) and Vibrational assignment with PED(%) of 5(4 methyl phenyl)tetrazole (5MP TZ) based on B3LYP/6-31G (0.9555, 0.9826) and B3LYP/6-311+G (0.9642, 0.9860) method and basis set

Species	Observed Frequency (cm ⁻¹)		Theoretical Frequency (cm ⁻¹)				Vibrational assignment (PED %)
	FT-IR	FT-Raman	6-31G		6-311+G		
			Calc	scaled	Calc	Scaled	
A	3407	-	3710	3524	3687	3502	v NH(100)
A	3082	-	3241	3097	3206	3046	v CH(95)
A	-	-	3210	3067	3176	3018	v CH(73)
A	-	3050	3201	3058	3165	3007	v CH(93)
A	3045	-	3186	3044	3153	2995	v CH(75)
A	-	-	3134	2994	3097	2942	v CH(69)
A	2924	-	3103	2965	3069	2915	v CH(47)
A	-	2910	3041	2906	3013	2862	v CH(58)
A	1613	1630	1678	1603	1656	1574	v CC(29)
A	1580	1595	1631	1558	1611	1530	v CC(27)
A	1505	-	1605	1533	1587	1508	v CC(18)
A	1458	-	1539	1471	1528	1452	β HNN(10)+β HCC(11)
A	-	-	1536	1468	1527	1451	β HCH(28)
A	-	-	1533	1464	1524	1448	β HCH(39)+τ HCCC(14)
A	-	-	1477	1451	1460	1435	v CC(17)+β HCC(10)
A	1404	1400	1464	1438	1454	1429	β HCH(43)
A	1377	-	1396	1372	1384	1360	β HCC(21)
A	-	-	1378	1354	1365	1341	β HNN(19)+v CC(14)
A	-	1310	1370	1346	1348	1325	β HNN(35)
A	1285	-	1321	1298	1302	1279	v NC(33)+v CC(16)
A	1258	1250	1258	1237	1247	1225	v CC(39)+β HCC(12)
A	-	1217	1247	1226	1237	1215	β HCC(27)+β HCC(11)
A	1187	1196	1178	1158	1172	1151	β HNN(19)+β HCC(12) + v NN(23)
A	1163	-	1167	1147	1159	1139	v CC(14)+v CC(10)+ v NN(49)
A	1116	-	1151	1131	1141	1121	v NC(22)
A	-	1070	1101	1082	1092	1073	τ HCCC(30)+β HCH(14)
A	1054	-	1059	1041	1050	1032	β CCC(37)
A	-	1049	1049	1031	1041	1023	β NNN(65)
A	1027	-	1037	1019	1027	1009	τ HCCC(34)+v CC(10)
A	1013	-	1030	1012	1026	1008	τ HCCC(51)
A	992	980	1022	1004	1017	999	β NCN(26)
A	-	-	984	967	982	965	τ HCCC(37)
A	-	-	955	938	974	957	v NN(64)
A	-	-	918	902	923	907	β NCN(27)+β NNN(17)
A	-	-	885	870	878	863	τ HCCC(51)
A	823	-	858	843	850	835	τ CCC(44)
A	-	800	824	809	818	803	v CC(24)
A	744	740	768	755	737	725	τ NNCN(15)+δ CNNC(17)
A	698	700	734	721	720	708	τ CCCC(26)+τ CCCC(26)





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A	-	-	696	683	687	675	τ NNNC(71)
A	-	-	672	660	669	657	τ HNNN(64)
A	-	650	671	659	645	634	β CCC(30)
A	615	-	630	619	626	615	τ CCCC(23)
A	506	-	531	522	520	511	β CCC(31)
A	-	-	467	458	465	457	τ CCCC(37)
A	-	-	422	415	427	420	δ CCCC(27)+ τ CCCC(26)
A	-	360	367	361	342	336	τ CCCC(22)
A	-	350	337	331	336	330	β CCC(68)
A	-	-	325	319	323	317	ν CC(31)
A	-	-	213	209	207	204	δ CNNC(34)
A	-	-	139	136	136	133	β CCC(49)
A	-	-	83	81	80	79	τ CCCC(55)
A	-	-	44	44	42	41	τ HCCC(26)
A	-	-	39	39	32	31	τ CCCN(75)

ν -Stretching; β -Bending; δ -out-of-plane bending; τ -Torsion

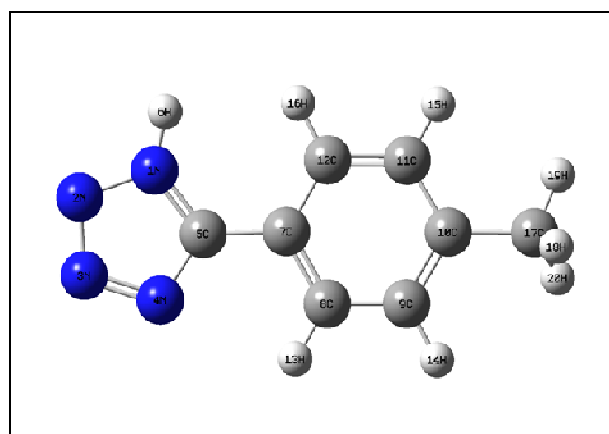


Fig. 1. The theoretical geometry structure and atomic numbering scheme of 5(4 methyl phenyl)tetrazole (5MPTZ)

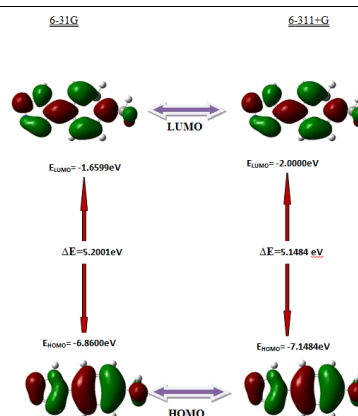


Fig 2: The atomic orbital compositions of the frontier molecular orbital for 5(4 methyl phenyl)tetrazole (5MPTZ).

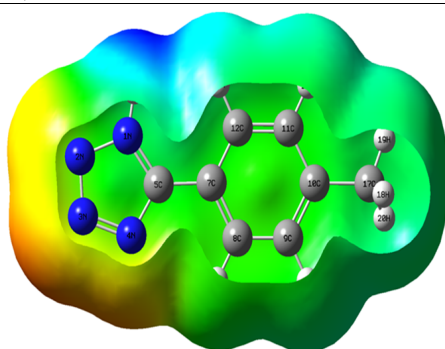


Fig. 3. The total electron density surface mapped with of 5(4 methyl phenyl)tetrazole (5MPTZ)

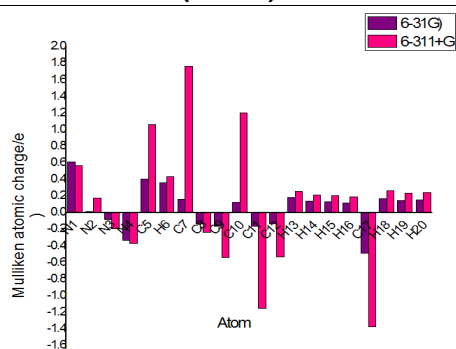
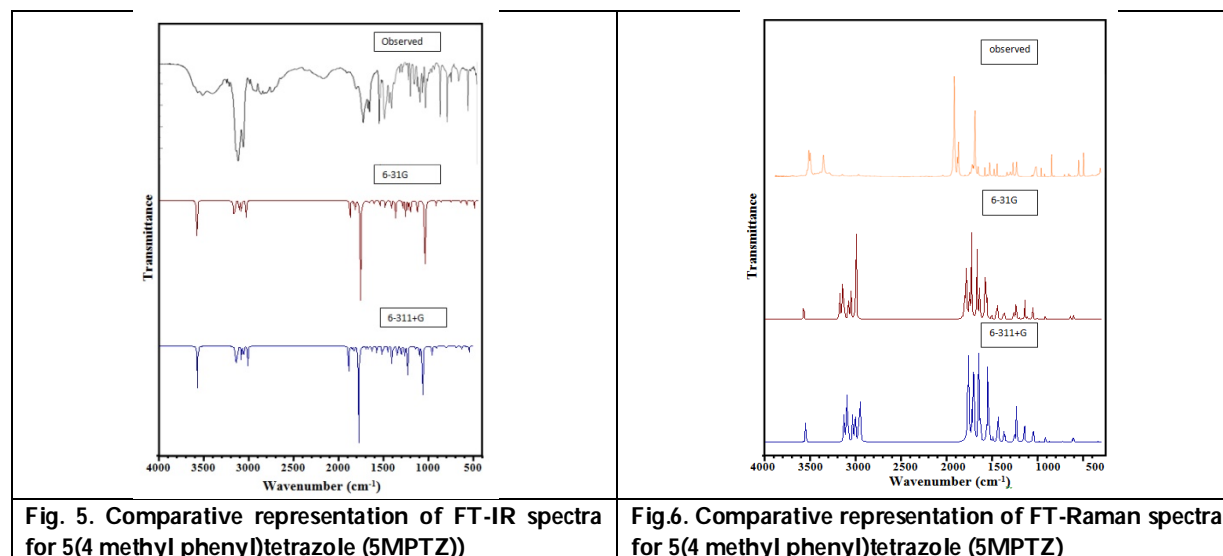


Fig. 4 Bar diagram representing the Mulliken atomic charge distribution of 5(4 methyl phenyl)tetrazole (5MPTZ)





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Socio-Cultural Impact of Globalization and Health Practice among Tribal Women in Thiruvarur District

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ABSTRACT

The objective of the study is to measure the impact of modernization on the religious customs beliefs in health practices among tribal women of Thiruvarur district, which is one of the most backward areas of Tamil Nadu. Tribals who reside in different settlements located in Nanilam taluk. The research tries to find out how the health practices among women in terms of menstruation and hygiene practices has been changed due to the impact of modernization lifestyle entering into their community overcoming the traditional beliefs of using hygiene products during menstruation. The study also tries to enquire how education for women in this region have impacted and aided the tribals from exploitation and provide them better-living conditions, including education and health care.

Keywords: Tribal women, health, menstruation, hygiene practices, education & Modernization.

INTRODUCTION

Thiruvarur was one of the five conventional capitals of the Chola Empire. Thiruvarur locale is prominently known as "The Granary of South India." Lying in the delta area of the popular stream Cauvery, this region is wealthy in paddy fields, tall coconut forests, and other verdant vegetation. The stream Cauvery prevalently called as "Mother Cauvery" makes this land prolific with her tributaries.



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Thiruvavur has mentions in the Saiva standard work, Tevaram by Thirugnana Sambanthar, Tirunavukkarasar and Sundarar, the principal Saivite holy people of the seventh, eighth century and named Padalpetrastalam. Tirunavukkarasar specifies a few conventions of the sanctuary like Marghazhi Aathirai Vizha, Panguni Uttirai Perunaal, and VeedhivitananinVeedhi Panni. The stone structure of the temple was first developed by Aditya Chola I in the ninth century and redid during the rule of Rajaraja Chola I. The temple was redesigned and modified with stone by Rajendra Chola I. The sanctuary has engravings from both the rulers, later Cholas and Pandyas. Engravings from the temple demonstrate Thiruvavur as the capital of Kulothunga Chola I, during which the region rose a focal point of Saivism. After the fall of Cholas during the reign of Rajendra Chola II in the thirteenth century, the region was gotten under a power battle among Pandyas and Hoysalas. The imperial support proceeded, and the locale thrived as a social focus during the standard of the Nayaks, Vijayanagar rulers and Marathas. During the time of the Marathas, the area turned into an impermanent home to the Nataraja of Chidambaram sanctuary. The locale was quickly caught by French troops led by Lally in 1759. The Thyagarajar sanctuary was scoured in a bombed endeavor to find concealed fortune. During the endeavor, six Brahmins of the sanctuary, associated with being spies with the British, were slaughtered in an experience. After autonomy, Thiruvavur kept on being a piece of the Thanjavur area and Nagapattinam region till 1991 and 1997 separately.

Indeed, Thiruvavur has been a focal point of prominent individuals in religion, expressions, and science. Well-known chronicled sanctuaries are situated in Thiruveezhimalai, Thirupamparam, Thirumeichur, Shrivanchiyam, Thillaivilagam, and Thirukkannamangai. At Jambavanodai close Muthuppetai, there is an old and celebrated dargah, a mosque. The triumvirate of Carnatic music, Shri Thiyagaraja Brahmam, Shri Muthuswamy Dheekshathar, and Shyma Shastri were brought into the world here, and this includes profound respect, poise, and greatness to this locale. Horticulture is the main occupation in Thiruvavur region. Over 70% of the total work power is needy upon farming. Paddy is the main harvest of the area. This area structures some portion of the rice bowl of Tamil Nadu. The area has involved a predominant place in farming part because of its alluvial soil favored by "Mother Cauvery" and her various branches, which fill in as a primary wellspring of the water system.

Globalization

There is more and more agreement on the fact that globalization is an extremely complex phenomenon; it is the interactive co-evolution of multiple technological, cultural, economic, institutional, social and environmental trends at all conceivable spatiotemporal scales." (Huynen, Martens, & Hindeink, 2005). It has an impact on almost all aspects of society. With the advent of globalization, the lifestyle of people is changing very fast. It has its implications on health practices also. As our people are more exposed to the overall world practices, they tend to adopt healthy practices. In the case of women's health practices, also we can see a sudden shift. Before globalization and after globalization. The shift is evident in health practices, hygiene practices, and menstrual practices, and so on. People tend to avoid traditional health practices and indulge in western health practices. The change is also obvious in menstrual practices, hygiene practices in women.

Globalization and Modernization

The processes of globalisation in the twenty-first century have wide implications. Hence, globalisation has transformed the way people see themselves in the world. It is also evident that the immediate effect of globalization is modernization that is the process by which a society achieves modernity. Modernity means using new techniques, practices that make our lives easier. As we can say globalisation has its effect in the whole society modernization also occurs in all parts of society. Every person in the world in one way or another influenced by modernization and adapted to modernisation techniques. In traditional societies like India it takes time to modernise but in some radical societies its very easy to adopt modernisation. This modernisation has also influenced tribal people, tribal women, and children. It is also influencing the health practices, hygiene practices of tribal women. For the tribal community globalization has entered their life through means of mass media such as radio, television, internet and mobiles which has been the carrier of the social changes and create more awareness about their rights.



**Shamala et al.****Scheduled Tribes in Thiruvavur**

According to census 2011; the total population of Thiruvavur district is 10,06,482 among them 4,99,954 males and 5,06,528 females. The total scheduled tribe population in Thiruvavur district is 3,034, among them 1,466 males and 1,568 females. The scheduled tribe literacy rate is meager compared to the other weaker sections of the society. In Tamilnadu Scheduled Tribe community population have been notified with six area restrictions. In Thiruvavur, the Scheduled Tribe community people are mostly agricultural laborers and other marginalized workers. The district has the highest Scheduled Tribe sex ratio of 1070 among the districts. The study area we choose is nannilam block in which the total tribal women are 30 out of which 17 women are literate (basic reading), 07 pursuing education and 06 women are illiterate. They belong to a tribe called “Kaatanaykar”.

Menstrual health among tribal women.

The tribals are usually living in remote areas under diverse ecological conditions. Few tribes in India have been into conventional health care system those who have migrated to urban and semi-urban life. The tribes live in remote and rural areas are yet to accept the new medicine areas. Modern medicine is not acceptable in most tribal areas, where magico-religious health care systems prevail, and they still believe in traditional forms of health practices. As per a report from government Health conditions in tribal areas have been described as deficient insanitary conditions, personal hygiene, and health education. The common factor among Indian tribes are poor health and sanitation conditions. The tribal health problems among women need special attention in tribal communities of India. Few studies tipped out that the tribal population has peculiar health-related problems due to their living conditions and habitat, tough conditions to live. The health, nutrition and medico-genetic problems of diverse tribal groups have been found to be unique and present a formidable challenge for which appropriate solutions have to be found out by planning and evolving relevant research studies” Basu S(2000).

The tribal women have been exploited to various living conditions due to this their health and child mortality rate is in diminishing point to be addressed with proper implementation of health policy. The tribal “Women are more vulnerable in terms of health care and hygiene. Jose and Navaneetham (2010)” study examined the association between social infrastructure and women’s health in India. The study result states that poor working conditions and low economic status has led to minimal sanitation facilities. The women in the tribal community lack access to proper sanitation facilities which leads to the vulnerability of women’s health, and it might cause infections during menstruation. Most of them are affected by communicable and non-communicable diseases. The most concerning matters are of women’s health is their reproductive health and is directly associated with sanitation hygiene. Regular periods in menstruation is equally important for the reproductive health of a woman. The tribals pay less attention to their irregularity in the menstrual cycle. Most women are found to be attributed to delayed or a missed period to pregnancy because of the lack of awareness of other causes related to it and they are more ignorant. Therefore, the study focuses more on the impact of sanitation hygiene on women’s menstrual health. Education plays an important role in creating awareness and to learn the use of proper sanitation facilities. With the intervention of modernization and technology into the tribal community have impacted on tribes who have migrated to urban areas for work or education, have been able to challenge religious belief and taboos attached to menstruation among few tribal women.

Review of literature

R.K. Kar (1993) stated that the tribals believe in traditional methods of curing the ailments. Firstly they approach the traditional healers for treatment and then to other medical care. Further, he has concluded that though the traditional customs, rituals, beliefs, and habits, as well as diagnosis and treatment of diseases, are changing fast under the impact of an ongoing process of modernization and it revealed that in most of the tribal society’s traditional health-seeking behavior dominate over the modern medicine system. Sujata Rao (1998) in her study of in Andhra Pradesh observed that poverty along with several factors like lack of access to the right food, iron, protein and micronutrients deficiency are the primary cause of poor health of the tribal people. Maternal Mortality Rate (MMR) is eight per 1000 as against four per 1000 for the state. Infant Mortality Rate (IMR) 120- 150 per 1000 compared to 72 per 1000 for the



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state. Lack of accommodation, poor infrastructure, extensive scale absenteeism and vacancies, poorly trained and unskilled workforce, is thus the reason for indigent health care services, which in turn leading to the poor health of the tribals in the region. Salil Basu (2000) observed that tribal is the firm believers of the supernatural therapy for the cure of diseases. The unsanitary conditions, lack of personal hygiene, and health education are the leading causes of ill health. The high rate of maternal and child mortality is due to the primitive practices of parturition. The consumption of iron, vitamins, calcium during pregnancy is inadequate, which leads to poor health and even sometimes the death of the woman and child both. Vaccination and immunization of infants and children have been inadequate among the tribal groups. Besides, extremes in magico religious beliefs and taboos tend to deteriorate the problems. Genetic disorders, particularly sickle-cell diseases and sexually transmitted diseases, are quite prevalent among the majority of tribal groups.

Mallikharjuna Rao et al. (2006) explain the dietary habits of the Sahariya Tribes of Rajasthan, the dietary habits which include cereals, millets, milk, pulses, jaggery, etc. are being consumed in less amount compared with recommended dietary intakes (RDI). The intake of protein, iron, calcium, and thiamin are comparable to the recommended level, while other nutrients such as vitamin A, Riboflavin, fat, vitamin C, and folic acid are below the average intake. It was revealed that the nutritional intake of the Sahariya tribe comparatively good to the other drought-affected areas. Though the Study presents a good picture of the nutritional status of the Sahariya, on the other side, some nutritional deficiency disorders can be found among them. About 4 percent of the infant was found to be emaciated, while an equal proportion had conjunctival xerosis. The Study furthers reveals that the major cause of neonatal death prematurity, while the death of adults is mainly due to infectious disease. Arlappa et al. (2008) study states about the diet and nutritional status of elderly tribes in India. The Tribal particularly elderly are, in general, the most disadvantaged groups. The dietary intake of the elderly has been found below the recommended dietary intake (RDI). The mean consumption of all foods other than cereals and millet were low among males, and green leafy vegetables among the women were below the recommended intake. Regarding the nutritional status, this study reveals that the prevalence of Chronic Energy Deficiency (CED) among the females was higher (65.4 percent) compared to males (61.8 percent) and it also varies with the socio-economic condition of the dwellers. Similarly, overweight/obesity was relatively high among females compared with males. It has also been observed the prevalence of severe anemia, riboflavin deficiency, and dental caries among the elderly.

Sanjoy Deka (2011), in his article, describes the health and nutritional status of tribes in Tripura. The author reported a higher rate of mortality among children in Tripura state in the region of Dhalai and South district. The health conditions of the tribes in the region are still unsatisfactory. The maternal mortality rate is also high among the tribals groups. Manikanta (2013) focuses on the health status of tribal elderly of Andhra. It shows that the 80+ age category was found to be having more health complication (61.2 percent) followed by 70-79 age category (52.4 percent) and 60-69 age category (39.4 percent) respectively. The Study further reveals that the main illness related to aged people was knee pain (39 percent) cold (0.3 percent), Blood Pressure (9.7 percent), Asthma (1.7 percent), Sugar/Diabetes (1.3 percent) and Digestion (1.3 percent). The poor health of elderly is due to the lack of caretakers, lack of sufficient health care facilities, migration of their children to urban areas, poor economic conditions and the beliefs that the failing of health is a normal occurrence of life. Sarkar (2016) during the Study of health status and nutritional aspect of tribal women in Godam Line Village of Darjeeling District, West Bengal finds the presence of high rate of illness and diseases among the respondents. The most common being diarrhea (50%), cough and cold (50%) and dysentery (50%). The disease like hypertension, vision problems, and arthritis are also commonly found among them. The author also mentions the problems of lack of health care facilities in the region resulting in the number of cases goes unattended and remains untreated. It is also found that during pregnancy, women consume some fruits and health drinks.



**Shamala et al.****Objectives of the Study**

1. To study the socio-demographic profile of the respondents.
2. To measure the impact of modernization on the religious customs & beliefs in Menstrual health practices among tribal women of Thiruvavur district.
3. To study the association of age and educational status with regard to the menstrual hygienic practices of the respondents.
4. To draw interventions based on the study.

Aim of the study

To study the Socio-cultural impact of Globalization and menstrual health practice among Tribal women in Thiruvavur district.

Significance of the study

Tribal people, especially women in Thiruvavur district are in a subjugated state as their socio-economic conditions are very poor. The significance of the study lies in finding the problems faced by scheduled tribe women regarding health and menstrual hygiene practices and studying the influence of modernization among tribal women in developing healthy practices. This will in turn help in advocating the most suitable way to the tribal women. This will contribute to the dissemination of progressive ideas among tribal women in terms of health and hygiene.

METHODOLOGY OF THE STUDY

Mixed method research is applied for the present study through the application of self-prepared interview schedule in order to collect the socio-demographic profile of the respondent combined with Focus Group Discussion to assess their practices in reality. A total of 60 respondents were included for the study from the area. The focus group discussion included 30 tribal women who belong to Malai karuvar (kuruvar means tribes in Tamil), kaatur naykar, and illur (snake catchers) caste shelters in thiruvavur district. Malai Koruvara and Kaatur Nayakar Community are marginal labours mainly sweeping and garbage cleaning work. the language they speak Telugu mixed tamil. Irula tribes are difficult to find them settled in Vedararnyam forest in Thiruthuripondi taluk of Thiruvavur district, there traditional work is been said as snake catchers. The discussion is about the health practices among women in terms of menstruation and hygiene practices, religious practices, beliefs and the impact of modernization on these practices. At the outset of the present study four parameters for this study were found-Modernization, religious customs, beliefs in practices, menstrual health. The linkage between these parameters were passed to draw a conclusion on the impact of globalization and health practice among these tribal women.

Hypotheses testing

H1: There is a no significant association between the age of the respondents and their menstrual health practices.

A Chi-square test is performed and the result indicated that there exists no relationship between the two variables. Hence, the hypothesis that age is not associated with the menstrual health practices. ($\chi^2=2.380$, $p=0.497$). Null hypothesis is accepted and research hypothesis is rejected and it is concluded that, there is no significant association between the age of the respondents and their menstrual health practices.

H2: To test the relationship between education and menstrual health practices,

A Chi-square test is performed and the results indicated that there is no significant relationship between the two variables. Hence, the hypothesis that education is not associated with the choice of menstrual health practices ($\chi^2=2.950$, $p=0.399$). Null hypothesis is accepted and research hypothesis is rejected and it is concluded that, there is a significant no association between the educational status of the respondents and their menstrual health practices.





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Discussing about Education is associated with awareness level of menstrual health practices

To test this hypothesis, a Pearsons product-moment correlation was run and the correlation test results showed negative correlations between prevalence of education and awareness level of menstruation ($r = -0.144, p=0.272$).

Focus Group Discussion

It was conducted with 30 women from the Women belonging to the scheduled tribes with the FGD guide focusing on four major dimensions namely modernization, Religious Customs, Beliefs in practices and Menstrual health aspects. Major observations of the focussed group discussions are recorded below.

Major observation- Focus Group Discussion

Modernization

Education, Media (TV) as source of information, Advertisements on sanitary napkins, Employment, Mobile phones, internet through mobile.(Whatsapp).

Religious Customs

Untouchable, Abandoning religious places, Not permitted to touch sacred items.

Beliefs in practices

Taking bath, Staying outside the house, Use of neem leaves, Washing clothes by themselves, Burning the clothes, Believes that disposing may led to curse, Does not wear flowers to hair and kumkum, Using the separate vessels, Should avoid touching and watering plants, Should avoid venturing out after dark, Have high chances of prying by evil spirits, Should not enter kitchen

Menstrual health

Use of cloths, Not using napkins, Stomach pain, Not consulting doctor

Focus Group Discussions (few responses)

Participant 1: Age 28, House wife, Education- 10th std

In this village, people treat a woman during her periods as untouchable, and women cannot enter holy places. She did not discuss menstruation with others. Nobody should touch a menstruating woman. Should not touch the trees and should not watering plants. Should not touch the sacred items.

Participant 2: Age 15, Student, Education- 10th std

A 15-year-old girl says that she hated the days when she was isolated in her own home. She has asked her mother about this issue. Her mother's response was that 'we are from an orthodox family, and this isolation was a part of our tradition.' She is also suffering from irregular periods and stomach pain. However, she did not consult the doctor. During menstruation, she wears a neem twig while going to school.

Participant 3:Age 35, Farmer, Education-10th std

She says that menstruation is impure. Menstruating women should use a separate mat and be washed every day. She believes that women should sleep alone. She preferred using cloth during menstruation. She never seeks help from anyone. She used to burn their clean clothes as she believed that if it is disposed of in other ways it can cause a curse.

Participant 4: Age 24, Housewife, Education-10th

She does not want to follow the myths and misconceptions, but she was forced to follow because of her elders. She has the necessary knowledge to manage menstruation hygienically, and her preference is sanitary material during menstruation. She washes her absorbents with hot water. She did not enter the temple and did not water the plants during menstruation. She said TV was the primary source of information about sanitary napkins before she saw these advertisements. She is having much openness about this topic. Now TV breaks the silence about menstruation.



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She is well educated but still believes and follows the menstruation myths. She did not wear flowers during menstruation and never discussed menstruation with others. She did not participate in the religious rituals. She is also suffering from stomach pain during menstruation.

Participant 6: Age 34, Employed, Education- 8th std

In her village, every home has a toilet facility. She preferred to use cloth because sanitary napkins irritate the skin mainly in summer. She does not have a belief about menstruation myths. Because in her working area, everyone talks frankly about their menstruation, but her parents will not allow it.

Participant 7: Age 30, Housewife, Education- 10th std

She has a strong belief about menstruation myths, and she shared this with her daughter. Now her daughter is also following the myths. She did not enter her home for five days during menstruation. She uses separate vessels during menstruation. Moreover, her clothes are seen as untouchable. She cannot touch the sacred things

Participant 8: Age 45, agrilabour, Education- Nil

She does not believe in modern systems, and says the cloth is comfortable. She is ignorant about her menstrual health and expressed that she does not remember that she had any menstrual problems. Her daughter tried to convince her to use sanitary napkins but in vain. They follow all the religious beliefs and customs that are practised during menstruation time. They also fears something will happen if they do not follow it.

Findings of the study

The outcome of our study revealed that menstruation practices among tribal women is more associated with traditional beliefs and cultural practices that tribal women are following since ages. But our study findings revealed that globalization is being impacted through modernization, which also have a positive effect in upholding the healthy, scientific menstruation practices among the tribal women. Globalization have entered these communities in the form of mass media-radio, television and technological advancement like mobile phones, internet and social media (only among educated and migrated women for working to urban areas) There by influencing in the socio-cultural context.

Another important finding of our study is Education is very vital in following hygienic menstrual practices and shattering the myths that are related to menstruation. Educated tribal women are willing to rebuff the traditional beliefs and cultural practices that exists among tribal women regarding menstruation and sanitization. Tribal women who gets good education even dare to question the taboos and myths regarding menstruation in their society. (use of toilets is common in young and educated women where as older age women still use open defecation). Another major finding of our study acknowledges the impact of advertisements of sanitary napkins on changing the traditional menstrual practices. From the focus group discussion we find out that it is through the advertisements they started to use sanitary napkins which are more scientific and healthier than cloths and neem leaves. Our study also displays the role of employment in using healthy menstruation practices. Those who are employed uses more healthy practices than unemployed. The women who are employed uses sanitary napkins and those people didn't believe in social taboos that is already existed regarding menstruation in the society. They have been the result of modernization.

Another important finding of our study is that older people tend to be more religious and they are backing up the myths and taboos regarding menstruation while the young generation who have their basic schooling tend to be less religious and they are not supporting the myths and taboos regarding menstrual health practices. Through the focus group discussions we also affirms that people especially mothers and older age women are communicating the taboos and myths to their children and through that way the taboos are spreading in to the next generation.



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One of the other important findings of our study is tribal people especially girls and their parents, are not open to having a discussion about the hygienic practices during menstruation. But girls and women are comfortable with discussing these issues with their peer groups. Through the focus group discussions we found out that television has a significant role in changing the mindset of people. Tribal people, especially women are a regular consumer of television programs and they tend to imitate what they see on television. So television can be used wisely to give awareness to tribal people.

CONCLUSION

The tribal community people selected for the study are agricultural laborers, marginal workers, homeless unemployed people in the south street of Thiruvarur. Its a closed community with less socialization with other community. Their menstrual health practices are of both orthodox and traditionally backed up. Very few have constructed toilets under state and central government schemes. The public health department provides Sanitary Napkins free every month in order to support them to have hygienic menstruation. But due to myths and taboos many tribal women are not using this facility. Modernization, education, employment, and media can be considered as the influencing agent among the tribal women in changing their health practices as the interference education and use of mobile and TV have influenced their approach towards modernization. The local governance must facilitate these to tribal women and try to uphold their health and sanitary conditions. Proper awareness should be given to girls and their parents regarding menstruation and about the myths and taboos that exist in the society. Governments must include health, sanitation and hygiene in their school curriculum so that they can be aware of the scientific aspect of menstruation which in turn helps them to choose the best health practices. The tribal communities have been adopting modern life slowly with the present generation.

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Investigation on Phytoconstituents and Antihyperlipidemic Activity of *Solanum xanthocarpum* Linn.

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ABSTRACT

Solanum xanthocarpum (Solanaceae) is a perennial herb with prickly prostrate available in India. It is found in waste places, road side and open spaces. *Solanum xanthocarpum* (Linn.) has astringent, stimulant, diuretic, pungent, bitter, carminative, digestive, expectorant, febrifuge, and laxative. In Siddha, it is used in treatment of fever, cough, asthma, bronchitis, influenza, enteric fever and allergic conditions. The study suggested that the Pet. ether extract of the plant *Solanum xanthocarpum* (Linn) was found to produce significant anti-hyperlipidemic property in dose dependant manner (100 and 200 mg/kg, p.o.). The treatment with test drug 100mg/kg and 200mg/kg significantly lowered the elevated levels of TC, TG, VLDL when compared with the statins currently used in the management of hyperlipidemic condition. The present study indicates that the observed significant anti-hyperlipidemic activity of *Solanum xanthocarpum* (Linn) may be contributed to the phytoconstituents present in it. Further work is in progress to identify the possible mechanisms of action and to identify the lead molecules responsible for anti-hyperlipidemic activity.

Keywords: *Solanum xanthocarpum* (Linn), Anti-hyperlipidemic activity, Petroleum ether extract, Statins.

INTRODUCTION

Our world is fulfilled by various medicinal plants which are widely have been used in treatment of various diseases since ancient time. Herbal preparations are effectively used for their medicinal properties and have become increasingly popular worldwide. Herbal medicines generally have fewer side effects than synthetic compounds and



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their effectiveness can be improved by modern pharmacological methods(1). Hyperlipidemia, also known as Hyperlipoproteinemia, is abnormally elevated levels of any or all lipids or lipoproteins in the blood. It is the most common form of dyslipidemia (which includes any abnormal lipid levels). Hyperlipidemias are classified according to which types of lipids are elevated, that is Hypercholesterolemia (high blood cholesterol levels), Hypertriglyceridemia (high blood triglycerides levels) or both as Combined Hyperlipidemia (known as multiple-type hyperlipoproteinemia) is a commonly occurring form of hypercholesterolemia characterized by increased LDL and triglyceride concentrations accompanied by decreased HDL levels(2).

Hyperlipidemias may basically be classified as either,

I. **Familial** (also called Primary) (2) caused by specific genetic abnormalities.

II. **Acquired** (also called Secondary)(2) when resulting from another underlying disorder that leads to alterations in plasma lipid and lipoprotein metabolism.

I. Familial hyperlipidemias are further classified, based on the pattern of lipoproteins and their levels affected(2), as

- Type I: Characterized by increased levels of Chylomicrons
- Type II: Characterized by increased levels of Low-density lipoprotein (LDL)
- Type III: Characterized by increased levels of Very-low-density lipoprotein (VLDL) and LDL
- Type IV: Characterized by increased levels of Intermediate-density lipoproteins (IDLs)
- Type V: Characterized by increased levels of Chylomicrons and VLDL

II. Acquired hyperlipidemias may result in increased risk of premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to pancreatitis and other complications of the chylomicronemia syndrome. The most common causes of acquired hyperlipidemia are Diabetes mellitus, Hypothyroidism, Kidney failure, Nephrotic syndrome, Alcohol consumption, endocrine disorders, metabolic disorders and use of drugs such as thiazide diuretics, beta blockers, estrogens(3). Hyperlipidemia is caused by lifestyle habits or treatable medical conditions. Lifestyle habits include obesity, sedentary life without exercise, smoking. Medical diseases that may result in Hyperlipidemia are diabetes, kidney disorders, pregnancy, and an under active thyroid gland. Common secondary causes of hypercholesterolemia are hypothyroidism, pregnancy, and Kidney failure. Common secondary causes of hypertriglyceridemia are diabetes, excess alcohol intake, obesity, and certain prescription medications (4).

S. xanthocarpum, a folklore medicinal plant, shows the presence of a range of active pharmacological agents including alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols etc., and used against disorders such as migraine, asthma, swollen and painful arthritis, low appetite, helminthic infection, diabetes. Hence, the present study was attempted to investigate antihyperlipidemic activity of the plant *S. xanthocarpum*linn.

MATERIALS AND METHODS

Plant Collection and Extraction

Leaves of *S. xanthocarpum* linn. were collected from local areas of Yercaud foothills, Salem (Dist.), Tamil Nadu, India. The plant was botanically identified and authenticated by **ABS Botanical Conservation, Research & Training centre, Kaaripatti, Salem (Dist.), Tamil Nadu, India and a specimen voucher received.**

The plant leaves were shade dried at room temperature (32 °C) and then grounded into fine powder using mechanical grinder and stored in an airtight container until the time of use. The dried and coarsed powder of *S. Xanthocarpum* linn. leaves were packed appropriately into the Soxhlet apparatus and extracted by successive solvent method using Petroleum ether (1000 mL), Ethanol (1000 mL) and Chloroform (1000 mL) for 72 Hrs each. The resulting semisolid mass was obtained by vacuum drying using vacuum desiccator. The percentage yield of ethanolic extract of *S. Xanthocarpum*linn.were found to be 4.6%.

Preliminary Phytochemical Screening

The extract was screened for the presence of various chemical constituents such as alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids and saponins using standard procedures.**Animals:**Albino rats(150-200g) of either sex



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at the laboratory animal. All the animals were housed in standard cages under laboratory condition in the Department of Pharmacology and toxicology. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals.

Evaluation of Anti-Hyperlipidemic Activity

The antihyperlipidemic activity was performed in Cholesterol rich diet induced (High Fat Diet, HFD) hyperlipidemia rat model(5). Feeding the animals with Cholesterol rich diet induces hyperlipidemia, especially hypercholesterolemia and hypertriglyceridemia. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances. (6),(7). The animals were divided into 5 groups of 6 animals in each group. Group I animals were given Normal diet once daily for 28 days. The groups II to V were fed with HFD diet once a day for 28 days to induce hyperlipidemic condition. Group II fed on HFD once a day for 28 days is administered with normal saline orally. Group III was given HFD once a day for 28 days and treatment with standard antihyperlipidemic agent, Atorvastatin at dose of 10mg/kg. Group IV and V on HFD were treated with SXPE (Pet. Ether extract of *Solanum xanthocarpum*) at dose of 100mg/kg and 200mg/kg (Orally as suspension in 5% gum acacia solution) respectively. The treatment was given for 20 days. In between mean body weight of the animals was checked time to time. On 21st day the blood samples were withdrawn from the arterial damage. All the lipid profile parameters Total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), very low density lipoproteins (VLDL), low density lipoproteins (LDL) were determined and analysed from serum. Statistical analyses of the data were performed and the results were expressed as mean + SEM using one way ANOVA test.

RESULTS AND DISCUSSION**Pharmacological Studies**

Phytoconstituents like Flavonoids, Tri-terpenoids, and Sterols are well known for their hypolipidemic effects. Thus, the Anti-hyperlipidemic activity of the *S. xanthocarpum* linn. extract evaluated may be due to the presence of these phytoconstituents especially the Sterols. Evaluation of the Anti-hyperlipidemic action of the Pet. Ether extract of leaves of *S. xanthocarpum* Linn.in cholesterol rich diet induced hyperlipidemia model, revealed an improvement in the levels of study parameters towards normality (Table No.: 1).The results were comparable to that of treatment with statin drug currently used in management of hyperlipidemic condition.

CONCLUSION

In conclusion, the study results suggest that SXPE (Pet. Ether extract of *S. Xanthocarpum*linn.) markedly lowered the hyperlipidemic condition in cholesterol rich diet induced hyperlipidemic rat. Thus, the hypolipidemic effect may be due to the presence of phytochemicals such as Flavonoids, Tri-terpenoids, Saponins, Glycosides and Sterols in the leaf extract which is in line with several authors [80]. The present investigation also indicates the possibility of further research in the development of phytomedicine for Anti-hyperlipidemic action from *S. xanthocarpum*linn. leaves.

Future Aspects

As per the overall study conducted we can conclude that the use of the plant *Solanum xanthocarpum* Linn.is much more beneficial for anti-hyperlipidemia. In future it may be a good anti-hyperlipidemiccuring. so it is necessary to have a detailed study on the same.





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Table 1. Effect of *S. xanthocarpum* linn. leaf extract on Plasma Lipid Profile in of normal and cholesterol rich diet induced hyperlipidemic rats (means±SD)

S N	GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/md)	VLDL (mg/dl)	LDL (mg/dl)
1	Control (Group I)	64.89±2.280	53.90±1.666	36.15±1.125	11.76±0.3387	16.00±2.656
2	Positive Control (Group II)	177.20±2.698**	149.13±2.165***	20.71±1.221***	29.23±0.4326***	116.26±3.507***
3	Standard (Group III)	100.22±0.9657*	93.95±1.205*	32.51±0.7098***	18.78±0.2407*	48.89±0.7986*
4	SXPE (100mg/kg) (Group IV)	149.54±1.231*	129.23±3.205*	23.22±0.412	29.52±0.6128*	104.21±0.231*
5	SXPE (200mg/kg) (Group V)	125.21±1.895*	106.45±2.906*	32.97±0.3054	20.68±0.5916*	75.55±1.561*

Values are mean±SEM, n=6, ** p<0.01, when compared with Control group; * p<0.01, when compared with Toxic group

Table 2: Effect of *S. xanthocarpum* linn. leaf extract on mean Body Weight changes (in Gm) in of normal and cholesterol rich diet induced hyperlipidemic rats (means±SD)

S N	GROUPS	Mean Body Weight Change (in gms)				
		0 th Day	5 th Day	10 th Day	15 th Day	20 th Day
1	Control (Group I)	141	144	149	152	156
2	Positive Control (Group II)	143	150	168	176	199
3	Standard (Group III)	144	147	154	161	171
4	SXPE (100mg/kg) (Group IV)	142	153	159	176	197
5	SXPE (200mg/kg) (Group V)	142	145	152	158	169

The treatment with test drug 100mg/kg and 200mg/kg significantly lowered the elevated levels of TC, TG, VLDL. There was also increase in the levels of HDL-C after the treatment of 200mg/kg and 400 mg/kg in the models.





Evaluation of Thousand Kernel Weight Performance, Its Variability and Stability in Promising Winter Wheat (*Triticum aestivum* L.) Breeding Lines

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ABSTRACT

The aim of the research was to reveal the peculiarities of thousand kernel weight performance, its relation with yield, as well as to identify promising winter wheat breeding lines with high and stable level of the trait manifestation in weather-contrasted years under conditions of the Ukrainian Forest-Steppe. It was established that the variability of the thousand kernel weight is most due to the conditions of the year (48.45%). The genotype by environment interaction and genotype determined the variability of the trait at 28.48% and 22.62%, respectively. According to the genotype adaptability rating calculated on the basis of adaptability and stability parameters (b_i , S^2_{di} , Hom_i , Sc_i , GAA_i , $\sigma^2 SAA_i$, Sg_i , GSV_i), winter wheat breeding lines 44 KS, 42 KS, and 29 KS with the optimal combination of thousand kernel weight performance and its stability were identified. It was established with correlation analysis direct moderate and significant relationship between the thousand kernel weight and grain yield which indicates its





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importance for evaluation and selection of high-yielding and stable breeding material of winter bread wheat.

Keywords: *Triticum aestivum* L., plasticity, adaptability, genotype adaptability rating, correlation.

INTRODUCTION

The rapid increase in the world's population requires the need to increase the production of major crops [12]. According to experts, world agricultural production should increase on 100-110% by 2050 [54]. At the same time, the dynamics of its current increase does not meet the necessary pace to ensure parity of needs [48]. The problem is significantly complicated by global climate change [42, 52].

Winter wheat (*Triticum aestivum* L.) is the main grain crop in the world, providing about 20% of humanity's need for calories and protein [53, 56]. Therefore, increasing the production of this crop is one of the important components of human food security [14]. The role of plant breeding in increasing productivity and improving the quality of wheat products has been convincingly proven [31, 44]. Today, one of the most important breeding tasks is to genetically increase the productivity potential of varieties with appropriate product quality and increase resistance (tolerance) to abiotic and biotic stresses [1, 11, 38, 58]. That is why the evaluation of the stability of winter wheat genotypes is the subject of large number of studies in different countries [3, 9, 13, 15, 40, 45, 46, 59]. Moreover, yield is a complex trait. It is a result of combination a number of quantitative yield-related traits – its components. Thereby yield increasing depends on genetic improvement and the optimal combination of these individual traits [49]. That is why the level of manifestation and interrelation between yield and its components has received considerable attention in breeding, genetic and technological studies of wheat [22, 25, 39, 50]. The thousand kernel weight (TKW) is one of the most important traits related to yield [4, 5, 19, 43, 63, 64]. Many authors suggest that the selection of genotypes with high TKW could be a successful strategy for increasing yield in breeding programs [21, 32, 33, 60, 61]. The TKW is also very important qualitative trait [18, 28, 30, 34].

The significance of the TKW is confirmed by numerous studies on its genetic determination. Many yield-related quantitative trait loci (QTLs) have been revealed in wheat recently [23, 35, 62, 65, 66]. For example, ten QTLs for TKW were detected on wheat chromosomes 1A, 1D, 2B, 2D, 4B, 5B, and 6B, whereas six QTLs for kernel length were detected on 1A, 2B, 2D, 5A, 5B, and 5D, nine QTLs for kernel width were detected on chromosomes 1D, 2B, 2D, 4B, 5B, and 5D [47]. Major quantitative trait loci for TKW were identified on chromosomes 1B, 2A, 2D, and 4D, and their locations coincided with major QTL for kernel size traits, supporting the common belief that TKW is a function of other kernel traits [41]. The most significant QTL was identified on chromosome 4B. Major loci were also identified on the homeologous regions of Group 5 chromosomes and in the regions of *TaGW2* (6A) and *TaGASR7* (7A) genes [29]. Eleven QTLs associated with kernel traits were identified on chromosomes 1A, 2B, 2D, 3D, 4A, 6A, and 7A, respectively. Among them, three major QTLs QKL.sicau-2D, QKW.sicau-2D and QTKW.sicau-2D were detected [37]. Different three QTLs for TKW (Qtgw.ahau-1B.1, Qtgw.ahau-4B.1, and Qtgw.ahau-4B.2) were detected [6]. The loci and significant SNP markers identified can be used for pyramiding favourable alleles in developing high-yielding varieties [36]. At the same time, the TKW like another quantitative trait is significantly modified by environmental conditions. Therefore, it was assessed for the level of manifestation and stability by many researchers in different countries [2, 20, 51, 55].

Given the above, the aim of our research was to identify the peculiarities of variation in the TKW, its relation to yield, as well as the selection of promising winter wheat breeding lines with high and stable level of trait manifestation under contrasting weather conditions in the Ukrainian Forest-Steppe.



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MATERIALS AND METHODS

The study was conducted at the Bila Tserkva Research and Breeding Station during 2011–2013. The material for the research was 11 promising winter wheat breeding lines which individual selected within hybrid populations from crossing parent components of different ecotype (Table 1). The standards were the varieties of soft winter wheat Bilotserkivska napivkarlykova, Perlyna Lisostepu and Podolyanka. The trial was laid out with randomized complete block design in three replications. The net plot size was 10 m². The predecessor is pea. Agricultural techniques are common for the Forest-Steppe of Ukraine zone.

The parameters of adaptability for TKW were calculated according to generally accepted methods. The regression coefficient (b_i) and the standard deviation (S^2_{ai}) were determined by Finlay & Wilkinson [10], Eberhart & Russell [8], the homeostatic index (H_{omi}) and the selection value (S_c) according to Khangildin & Litvinenko [26], general adaptive ability (GAA_i), variance of specific adaptive ability (σ^2SAA_i), relative stability of genotype (S_{g_i}), genotype selection value (G_{SV_i}) according to Kilchevskiy & Khotylova [27]. At the generalized estimation of genotypes adaptive potential, ranking and calculations of the genotype adaptability rating (GAR) according to Vlasenko [57] were applied. The results of the experimental data were processed using Excel 2010 and Statistica 6.0.

RESULTS AND DISCUSSIONS

Hydrothermal conditions in the years of research (2011–2013) were characterized by contrasting indicators, which significantly affected the time of spring vegetation restoration, growth and development of winter wheat plants during ontogenesis (Table 2). This significantly affected the formation of the TKW. Thus, in 2011 the mean TKW in the trial was the lowest (36.4 g) with variation from 28.5 g to 41.9 g. The breeding lines with significant predominance over standard G1 (41.1 g) were not identified (Table 3). The highest TKW for prevalent part of genotypes was formed in 2012 with variation from 37.2 g in breeding line G8 to 52 g in breeding line G4. 2012 was more favorable for the formation of the TKW. Note. Mean is for the mean value of the trait, Min is for the minimum value of the trait, Max is for the maximum value of the trait, $R_{(Max-Min)}$ – measure of variation, S^2 – dispersion, V – coefficient of variation.

The breeding lines G4 and G14 had a significant higher TKW over G1 (48.1 g). In 2013, the TKW varied from 36.6 g to 47.8 g. Predominance over the best standard G1 (39.3 g) was observed in nine of the eleven breeding lines. For genotypes G8, G10, G11, and G13, the weather conditions in 2013 were more favorable for the formation of TKW. On average, in 2011–2013, the G1 standard by the TKW (42.8 g) significantly exceeded the genotypes G4 (+1.7 g), G11 (+1.2 g), G14 (+1.1 g), and G7 (+0.9 g). In most breeding lines, the range of variability of the TKW in the years of the research ranged from 7.9 g in G4 to 14.8 g in G14 with an average value of the coefficient of variation ($V = 10.9$ – 18.6%). In the G2 standard, the coefficient of variation was significant (20.5%) with a range of the trait of 28.5 to 43.3 g. The breeding lines G6, G11, and G9 were characterized with low trait variability ($V = 5.6$ – 9.8%), while in G6 and G11 the TKW exceeded the standards (G1, G2, G3).

Analysis of variance revealed a significant effect of the studied factors on the formation of the TKW. The conditions of the year determined the variability of the TKW at the level of 48.45%. The genotype and genotype by environment interaction modified the trait by 22.62% and 28.48%, respectively (Fig. 1). Genotype by environment interaction is a part of phenotypic variation that occurs due to a mismatch of genetic and non-genetic effects. That is, when studying genotypes in different conditions, there is a change in the rank of the trait performance in connection with their reaction to environmental conditions. Therefore, the selection of genotypes in some conditions may not provide their advantage in other conditions [16]. Many statistical methods have been developed to analyze data to gain a better understanding and interpretation of observed genotype by environment interaction patterns, with the aim of identifying genotypes with high stability in crop breeding programs. In particular, the approach of Finlay &



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Wilkinson [10] involves the calculation of the regression coefficient (b_i) on environmental conditions as an indicator of adaptability. The coefficient b_i can be more or less than 1.0, or be equal to 1.0. The higher the value of the coefficient $b_i > 1.0$, the more sensitive the genotype is to the improvement of growing conditions. A value $b_i < 1.0$ may indicate both higher stability of the genotype and its lower ability to respond by increasing yields to improved growing conditions. When approaching b_i to 1.0, there is a correspondence between the change in genotype yield and the change in growing conditions. There are preferred genotypes with high mean TKW and coefficient b_i close to 1.0. The data obtained in our study indicate that the most sensitive to favorable growing conditions were genotypes G14 ($b_i = 1.74$), G2 ($b_i = 1.62$), G13 ($b_i = 1.47$), and G3 ($b_i = 1.28$) (Table 4). The breeding line G14 combined a significantly high TKW and specific adaptability to favorable conditions. The breeding line G7 had a coefficient 1.14 and a significantly higher TKW than the best standard. The highest TKW (44.5 g) and the coefficient b_i close to 1.0 ($b_i = 0.99$) was set in G4. Genotypes G11 ($b_i = 0.17$), G8 ($b_i = 0.63$), and G1 ($b_i = 0.64$) had low variability. The lowest value of the coefficient b_i (0.17) and one of the highest values of the TKW (44.0 g) were in G11.

Eberhart & Russell [8] developed a method for assessing the adaptability done by previous researchers, supplementing it with the parameter of standard deviation of the actual values of the trait from the theoretically expected (S^2_{di}) as an indicator of stability. Genotypes with a smaller numerical value σ^2_{di} will be more stable. G12 ($S^2_{di} = 0.11$) and G7 ($S^2_{di} = 1.30$) had lower values of S^2_{di} than the standard G2 ($S^2_{di} = 1.64$). The method of estimating the homeostaticity by Khangildin & Litvinenko [26] is practical and quite simple in calculations. According to it, the homeostaticity index (Hom_i) is calculated which characterizes the stability of the variety when tested under different conditions, and the selection value (Sc_i) which reflects the value of the trait transformed by stability. The breeding lines G6 ($Hom_i = 780.26$), G11 ($Hom_i = 596.75$), and G9 ($Hom_i = 409.23$) were superior the G1 standard ($Hom_i = 394.64$). These genotypes also had higher Sc_i values than the G1 standard ($Sc_i = 35.00$).

Kilchevskiy & Khotyleva [27] developed quite a thorough method of adaptability evaluation, which allows to assess the general (GAA) and specific (SAA) adaptive ability. Under the adaptive capability the authors mean the property of the genotype to maintain its characteristic value of the phenotypic trait manifestation. In this case, GAA_i characterizes the average value of the trait under different conditions, SAA does deviation from GAA in a particular environment. As an indicator of genotype stability, according to this method, there is a variance of specific adaptive capability ($\sigma^2 SAA_i$). In our study only the breeding line G4 ($GAA_i = 65.53$) exceeded by GAA standard Perlyna Lisostepu ($GAA_i = 60.97$). Compared with G1 ($\sigma^2 SAA_i = 21.57$), the breeding lines G6 ($\sigma^2 SAA_i = 5.82$), G11 ($\sigma^2 SAA_i = 10.45$), G9 ($\sigma^2 SAA_i = 15.71$), and G5 ($\sigma^2 SAA_i = 20.00$) were characterized by lower value of SAA variance; a significant excess over the standard by TKW was observed only in breeding line G11. Smaller indicators of relative stability of the genotype (Sg_i) and significantly higher TKW as compared with the best standard Perlyna Lisostepu were noted in breeding line G11 ($Sg_i = 7.35$).

For evaluation and selection by GAA, taking into account stability, a criterion characterizing the combination of yield and stability in the genotype is required. Kilchevskiy & Khotyleva [27] indicate that the selection value index (Sc_i) takes into account the average, maximum, and minimum values of the trait and is limited by two backgrounds – the worst and the best. Therefore, they offer a slightly different approach to calculating the selection value of the genotype (GSV_i). For GSV_i high values were observed in G6 (GSV_i = 34.49) and G11 (GSV_i = 31.94), for the corresponding values in the standards G1... G3 (GSV_i = 8.57... 25.55)

The considered indicators of plasticity and stability characterize various aspects of such a complex phenomenon as the adaptive potential of the variety. Therefore, for a generalized assessment of the adaptability of a particular genotype, it is necessary to calculate a weighted average that would take into account as fully as possible the values of various parameters. For this purpose, one can use grouping by means of non-parametric statistics, which allows determining the ranks of individual indicators and calculating the average rank by their sum. However, as noted by Vlasenko [57], the productivity potential will be taken into account only in part, as one equivalent among other characteristics. Therefore, he considered it appropriate to normalize the average value of the sum of ranks, dividing



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it by the average yield. As a result, the productivity potential becomes decisive in the integrated parameter, which is characterized by the term "genotype adaptability rating" (GAR). To rank the genotypes, in addition to the mean yield (Mean), plasticity and stability parameters, we also included the minimum (Min) and maximum (Max) yield for the period studied (Table 5).

These values inform about the extreme limits of the realized productivity potential, and therefore are indicators of the rate of genotype response for these gradations of limiting factors. Higher places in the rank series, according to the parameters of adaptability were assigned to the parameters X, Max, Min, GAA_i , GSV_i , Hom_i , and Sc_i for their more numerical value, parameters σ^2SAA_i , Sg_i , and $S^2_{d_i}$ for less. According to the coefficient b_i , the highest rank was given to samples with $b_i = 1.0$, with a decrease in a distance from 1.0, in both direction of increase and decrease. According to GAR, the first place was taken with the breeding line G10 with an average rank of three. The second and third places were taken by G6 and G7, respectively, and the fourth by G1 standard.

When selecting desired genotypes for breeding programs, it is very important establish the relationship of each trait with the yield and its direct and indirect effects on it [17]. Knowledge of the relationship between grain yield and its components is significant for efficient breeding for yield, considering that it is the most important agronomic trait and has a very complex mode of inheritance [24]. There is a direct moderate and significant correlation between the TKW and the grain yield ($r = 0.485 \dots 0.640$) (Table 6). This coincides with the data obtained before [7]. It was found that TKW and number of kernels per spike had a direct positive effect on grain yield.

Our results show that the formation of the TKW of winter wheat was associated with the aboveground weight of the plant, weight of the main stem, spike weight, straw weight, grain weight of the main spike, stem length, and upper internode; the length of the main spike. There is a relation between the TKW and the weight of the main stem which varied from moderate to significant. A stable correlation was determined between the TKW and the weight of the spike, the length of the stem, the length of the spike-bearing internode ($0.5 < r < 0.7$), the length of the main spike ($0.3 < r < 0.5$). Unstable positive relationships were between the TKW and vegetative weight of the plant ($r = 0.192 \dots r = 0.766$), TKW and straw weight ($r = 0.496 \dots r = 0.755$), TKW and the length of the second upper internode ($r = 0.331 \dots r = 0.548$).

CONCLUSIONS

Peculiarities of thousand kernel weight variation for promising winter wheat breeding lines under contrasting weather conditions are revealed. We found that the variability of the 1000 kernel weight was most influenced by the conditions of the year (48.45%) and the genotype by environment interaction (28.48%). The part of genotype in the total variance was 22.62%. According to the genotype adaptability rating, the breeding lines 44 KS, 42 KS, and 29 KS with the optimal combination of thousand kernel weight performances and its stability were selected. These genotypes are involved in practical breeding process to develop a highly productive and adapted to the conditions of the Ukrainian Forest-Steppe initial source material and varieties of winter bread wheat. We established with correlation analysis a reliable relationship between the thousand kernel weight and grain yield which indicates its importance for the evaluation and selection of highly productive and stable breeding material of winter bread wheat under conditions of the Ukrainian Forest-Steppe.

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Table 1. Characteristics of the studied winter wheat genotypes by origin

Code	Variety, breeding line	Pedigree
G1	Perlyna Lisostepu	Bezosta 1 / Bilotserkivska 198 / Bilotserkivska 21 // Myronivska 27
G2	Bilotserkivska napivkarlykova	Donskyi napivkarlyk / Bilotserkivska 47 // Donskyi napivkarlyk
G3	Podolyanka	Seed treatment of Donetska 48 variety with N-nitroso-dimethyl-urea-0.001%
G4	7 KS	Donetska48 / Veselka
G5	8 KS	Donetska48 / Bilotserkivska intensive
G6	42 KS	Povaga / Perlyna Lisostepu
G7	29 KS	Luganchanka / Bilotserkivska 71/03
G8	26 KS	Rostavytsia / Driada 1
G9	24 KS	Bilotserkivska 47 (squarehead) / Odeska 162
G10	12 KS	Elegia / Perlyna Lisostepu
G11	44 KS	Kyivska 8 / Rostavytsia
G12	54 KS	Veselka / Myronivska 65
G13	22 KS	Donetskabezosta / Century
G14	17 KS	Napivkarlyk3 / Century





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Table 2. Meteorological conditions for period from date of spring vegetation resumption to ripening of winter wheat

Month	Decade	The amount of precipitation, mm				Air temperature, °C			
		2011	2012	2013	Average long-term	2011	2012	2013	Average long-term
April	II	2.5	40.4	8.4	17	7.2	10.8	10.4	7.8
	III	2.3	6.2	0.0	16	13.8	17.5	15.9	10.4
May	I	33.9	5.8	0.0	16	11.2	19.5	18.0	13.5
	II	8.2	0.5	50.9	12	16.4	17.2	19.8	15.3
	III	9.5	0.5	28.6	18	19.3	18.0	17.6	15.8
June	I	0.0	35.3	39.3	23	22.3	18.5	19.0	17.3
	II	38.9	0.0	19.0	27	20.6	17.4	21.2	17.4
	III	96.1	4.5	40.3	23	17.4	18.7	21.8	18.7
July	I	27.8	4.6	7.7	35	18.4	18.5	20.2	18.5
	II	-	-	2.0	24	-	-	18.9	19.4

Table 3. Performance and variability of the TKW in the winter wheat genotypes

Code	TKW, g							S ²	V, %
	2011	2012	2013	Mean	Min	Max	R _(Max-Min)		
G1	41.1	48.1	39.3	42.8	39.3	48.1	88	21.61	10.9
G2	285	43.3	36.6	36.1	28.5	43.3	14.8	54.92	20.5
G3	35.5	48.1	37.8	40.5	35.5	48.1	12.6	45.02	16.6
G4	41.5	52.0	40.1	44.5	40.1	52.0	11.9	42.30	14.6
G5	38.8	46.4	38.5	41.2	38.5	46.4	7.9	20.04	10.9
G6	40.7	44.5	45.2	43.5	40.7	45.2	4.5	5.86	5.6
G7	37.8	47.7	45.7	43.7	37.8	47.7	9.9	27.40	12.0
G8	33.1	37.2	43.4	37.9	33.1	43.4	10.3	26.89	13.7
G9	37.3	44.8	38.8	40.3	37.3	44.8	7.5	15.75	9.8
G10	33.0	42.1	44.9	40.0	33.0	44.9	11.9	38.71	15.6
G11	41.9	42.3	47.7	44.0	41.9	47.7	5.8	10.49	7.4
G12	36.3	47.3	43.1	42.2	36.3	47.3	11.0	30.81	13.2
G13	29.6	41.7	42.7	38.0	29.6	42.7	13.1	53.17	19.2
G14	34.5	49.3	47.8	43.9	34.5	49.3	14.8	66.36	18.6
SD ₀₅	0.87	0.80	0.49	0.72	-	-	-	-	-





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Table 4. Parameters of adaptive ability, homeostaticity and stability for TKW in winter wheat genotypes, 2011–2013.

Code	b_i	S^2_{di}	Hom_i	Sc_i	GAA_i	σ^2SAA_i	Sg_i	GSV_i
G1	0.64	26.33	394.64	35.00	60.97	21.57	10.84	25.55
G2	1.62	1.64	176.17	23.78	42.67	54.88	20.50	8.57
G3	1.28	22.89	244.05	29.87	54.87	44.98	16.57	15.52
G4	0.99	44.26	304.92	34.34	65.53	42.26	14.60	20.35
G5	0.73	17.99	379.76	34.21	56.70	20.00	10.85	24.60
G6	0.47	2.61	780.26	39.14	58.93	5.82	5.55	34.49
G7	1.14	1.30	365.36	34.66	59.40	27.36	11.96	24.27
G8	0.63	37.46	277.00	28.91	43.43	26.85	13.67	18.62
G9	0.76	7.52	409.23	33.55	53.70	15.71	9.83	25.56
G10	1.15	22.79	257.16	29.40	48.73	38.67	15.55	16.87
G11	0.17	19.81	596.75	38.62	58.77	10.45	7.35	31.94
G12	1.22	0.11	321.32	32.41	56.63	30.77	13.13	21.60
G13	1.47	17.30	198.03	26.34	44.20	53.13	19.18	10.88
G14	1.74	8.40	236.21	30.70	58.40	66.32	18.56	13.57

Note. G1...G14 – genotype code according to Table 1, b_i is regression coefficient, S^2_{di} is standard deviation from regression line, Hom_i is homeostaticity, Sc_i is selection value, GAA_i is general adaptive ability, σ^2SAA_i is variance of specific adaptive ability, Sg_i is relative stability, GSV_i is genotype selection value

Table 5. Winter wheat genotypes ranking according to TKW performance and parameters of adaptability, 2011–2013.

Code	Ranks												Mean/ X	GAR
	Mean	Min	Max	b_i	S^2_{di}	Hom_i	Sc_i	GAA_i	σ^2SAA_i	Sg_i	GSV_i	X		
G11	2	1	5	4	9	2	2	5	2	2	2	3	13.45	1
G6	5	2	9	12	4	1	1	4	1	1	1	4	11.65	2
G7	4	6	6	2	2	6	4	3	7	6	6	5	9.25	3
G1	6	4	3	9	12	4	3	2	5	4	4	5	8.41	4
G4	1	3	1	1	14	8	5	1	10	9	8	6	8.02	5
G5	8	5	8	7	8	5	6	7	4	5	5	6	6.67	6
G9	10	7	11	6	5	3	7	10	3	3	3	6	6.52	7
G12	7	8	7	5	1	7	8	8	8	7	7	7	6.36	8
G14	3	10	2	14	6	12	9	6	14	12	12	9	4.83	9
G3	9	9	4	8	11	11	11	9	11	11	11	10	4.24	10
G10	11	12	10	3	10	10	12	11	9	10	10	10	4.07	11
G8	13	11	12	10	13	9	10	13	6	8	9	10	3.66	12
G13	12	14	14	11	7	13	13	12	12	13	13	12	3.12	13
G2	14	13	13	13	3	14	14	14	13	14	14	13	2.86	14

Note. G1...G14 is genotype code according to Table 1. Ranks: Mean is the mean value of the trait, Min is the minimum value of the trait, Max is the maximum value of the trait, b_i is for regression coefficient, S^2_{di} is standard deviation from the regression line, Hom_i is homeostaticity, Sc_i is selection value, GAA_i is general adaptive capability, σ^2SAA_i is variance of specific adaptive capability, Sg_i is relative stability, GSV_i is genotype selection value, X is average rank. Mean / X is the ratio of average yield to average rank. GAR is genotype adaptability rating





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Table 6. Correlation of the TKW with other quantitative characteristics

Quantitative traits	Coefficient of correlation		
	2011	2012	2013
Grain yield	r=0.640	r=0.485	r=0.524
Weight of the plant vegetative part	r=0.664	r=0.192	r=0.766
Weight of the main stem	r=0.718	r=0.629	r=0.536
Weight of the main spike	r=0.650	r=0.598	r=0.546
Weight of straw of the main stem	r=0.755	r=0.496	r=0.496
Stem length	r=0.609	r=0.501	r=0.582
Length of the spike-bearing internode	r=0.528	r=0.587	r=0.591
Length of the second upper internode	r=0.548	r=0.502	r=0.331
Length of the main spike	r=0.333	r=0.358	r=0.442
Weight grain per main spike	r=0.672	r=0.674	r=0.382

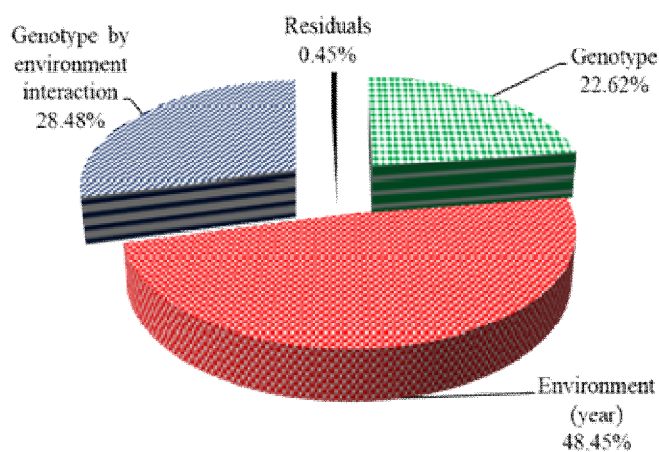


Figure 1. Part of sum square in the total variation for the TKW in the winter wheat genotypes, 2011–2013





Antibacterial and Acute Toxicity Studies of Silver Nanoparticles of *Cassia marginata* Roxb.

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ABSTRACT

In recent times, nanotechnology received much attention in the field of science and biotechnology. In the present study, silver nanoparticles were biosynthesized using aqueous extracts of leaves of *Cassia marginata* Roxb and was confirmed by spectral studies. The main aim of the present study is to investigate the bio synthesized silver nanoparticles for its antibacterial activity and to evaluate its acute oral toxicity study as per OECD guidelines. Green synthesised silver nanoparticles showed strong inhibiting activity against some of the gram positive and gram negative bacteria. In the acute toxicity tests, single oral administration of 5,50,300 & 2000 mg/kg doses of silver nanoparticles of *Cassia marginata* did not showed 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 green synthesised silver nanoparticles possess strong antibacterial activity and the acute toxicity studies also revealed that the silver nanoparticles were safer and non -toxic to rats.

Keywords: *Cassia marginata*; Silver nanoparticles; Antibacterial activity; Acute toxicity study

INTRODUCTION

Silver is widely used in Nano medicines and is used for several biomedical applications. In comparison to other metallic nanoparticles, silver nanoparticles have superior medicinal and nonmedical qualities and applications [1,2]. The green synthesis of nanoparticles has low or no toxicity, and such synthesis involves of a number of plants and plant extracts [3]. Plant extracts contain number of secondary metabolites that play a vital role in the formation of





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nanoparticles, as a reducing or capping agent [4]. Many studies have demonstrated that Silver nanoparticles, are very durable and bacterial, fungal and viral hazardous.

Cassia marginata Roxb is an annual or perennial plant belongs to the family of Fabaceae. It is commonly called a "Rainbow shower" tree, Pink tree, and Red or Rose Cassia tree, because of the appearance of the flowers and also called as Ceylon senna [5]. It is widely distributed in South India, Srilanka, and the West Peninsula. In India, it is found as an ornamental plant in the eastern region. Traditionally, the plant is used in the treatment of gastric problems, nausea, vomiting, muscle spasms, infections, diarrhea, blotches, cold and stomach spasms, and diabetics by tribal people of Andhra Pradesh and Tamilnadu. Cassia species were already reported in the ayurvedic literature for its wide spectrum of activities. They are mainly used against several skin diseases such as ringworm, eczema, and scabies [6]. Although limited pharmacological studies have been carried out with this plant, there is no experimental evidence on its nanosynthesis, phytochemical and toxicity studies. In the present study, we planned to synthesize silver nanoparticles of *Cassia marginata* and to evaluate its acute toxicity study as per OECD guidelines.

MATERIAL AND METHODS

Plant Materials Collection And Authentication

The fresh leaves of *Cassia marginata* was collected from Tanjore(Dt), Tamilnadu in October 2020. The collected plants (leaves) were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu.

Chemicals and Reagents

All chemicals used in this study were high quality analytical grade and used without further purification. Silver nitrate (AgNO_3 , 99.9 %) purchased from Sigma-Aldrich, Bengaluru.

Preparation of Plant Leaf Extracts

The fresh leaves of *Cassia marginata* (20g) were crushed with the help of pestle mortar, mixed in 200 ml of deionized water, boiled in a water bath for 30 min, and allowed to cool. The extracts were filtered using Whatman filter paper after filtration equal amount of ethanol is added to precipitate the mucilage present in the leaf extract; further, the extract was centrifuged at 7000 rpm for 10 min to make it mucilage free. The supernatant was collected and kept at 4°C until used.

Green synthesis of Silver Nanoparticles [7]

0.1 M of aqueous solution of silver nitrate (AgNO_3) was prepared and used for the synthesis of silver nanoparticles. 5 mL of leaf extract of *Cassia marginata* was added to 45 mL of 0.1 M AgNO_3 solution for bioreduction process at room temperature. The reaction mixture was observed for color change depending on parameter studied such as time, silver nitrate, and extract concentration at 80°C. The green synthesised silver nanoparticles of *Cassia marginata* (CM-AgNPs) was confirmed by various spectral studies.

Antibacterial Activity of Biosynthesized AgNPs

The synthesized AgNPs obtained from the leaf extract of *Cassia marginata* was tested for its antibacterial potent against Gram-positive and Gram-negative bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The antibacterial activities of the sample were determined by agar plate well diffusion method [8].

Animals

Healthy female Swiss albino mice weighing about 30-35g were used for the study. They were housed in



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polypropylene cages and fed with standard chow diet and water ad libitum. The animals were exposed to alternative cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 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[9]. 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 green synthesised silver nanoparticles of *Cassia marginata* 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 silver nanoparticles of *Cassia marginata* 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**Green Synthesis of Silver Nanoparticles**

Silver nanoparticles have been used widely for different biomedical applications and were reported for its easy permeability in tissues. The formation of silver nanoparticles using leaf extract of *Cassia marginata* was viewed by the colour change from greenish to blackish brown. it was [10] reported that silver nanoparticles exhibited colour change due to excitation of surface plasmon vibrations in silver nanoparticles. The leaf extract of cassia marginata contains several bioactive components and high level of phenolics and flavonoids. Biosynthesis of silver nanoparticles may be influenced directly or indirectly by the presence of phytochemicals. Further, the shape and size of nanoparticle was confirmed by various spectral studies.

Antibacterial activity of synthesized CM-AgNPs

AgNPs have found wide usage in various industries, and they are known to inhibit a number of microorganisms. AgNPs are extensively used in the pharmaceutical and medical industries as they have shown inhibitory activities against various microorganisms. In the present study, Green synthesized AgNPs were evaluated for their antibacterial activity against different pathogenic bacteria: Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. They were highly sensitive to AgNPs, whereas less sensitive to AgNO₃. Green synthesised AgNPs showed strong activity against these pathogenic organisms, while gentamicin showed significant antibacterial activity. The zone of inhibition and the MIC calculated was shown in table no.1and fig.no.1.

Acute Oral Toxicity Study

To determine the safety of AgNPs 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 green synthesised silver nanoparticles of *Cassia marginata* at different doses of 5,50,300& 2000mg/kg did not showed 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.



**Arul and Kothai****CONCLUSION**

In conclusion, it was observed that the silver nanoparticles were successfully obtained from the bio-reduction of AgNO₃ using *Cassia marginata* leaf extract. The CM-AgNPs were appropriately characterized and confirmed using different spectral methods. Green synthesized CM- AgNPs showed strong inhibiting activity against some of the gram positive and gram negative bacteria. Acute toxicity studies also revealed the safe level of the CM-AgNPs. This forms the basis for scientific evidence to conduct further studies and to investigate its various pharmacological activities.

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Table.no.1. Antibacterial activity of silver nanoparticles of *Cassia marginata*

Organisms	Zone of inhibition(mm)				MIC (µg/mL)
	AgNPs	AgNO ₃	Gentamicin	Plant extract	AgNPs
<i>Escherichia coli</i>	17	11	20	-	8.1
<i>Pseudomonas aeruginosa</i>	19	8	23	-	8.5
<i>Bacillus subtilis</i> ,	16	13	21	-	9.5
<i>Staphylococcus aureus</i>	20	14	25	-	6.9

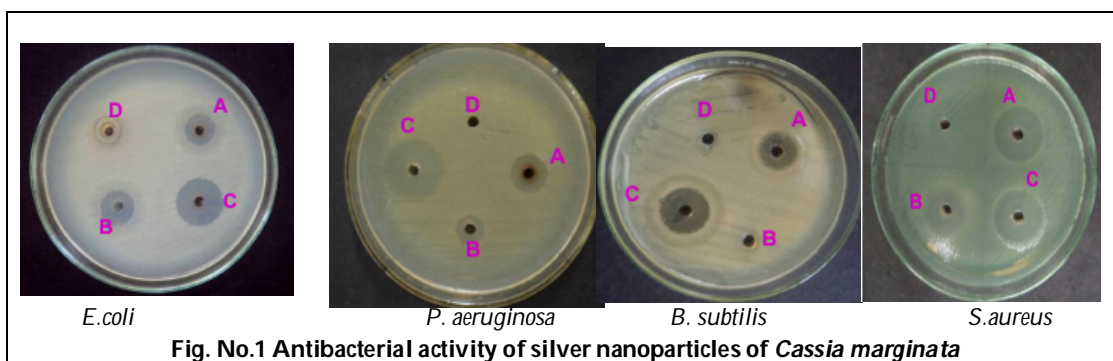


Fig. No.1 Antibacterial activity of silver nanoparticles of *Cassia marginata*





The GC MS Analysis of Ethyl Acetate Extract of *Merremia emerginata* Burm. F (*Ipomoea reniformis*)

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ABSTRACT

This study deals with the GC MS analysis of one herbal medicinal plant *Merremia emerginata* which is used ethnobotanically for the treatment of cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever etc. The plant was collected at Chengalpattu, Tamil Nadu, India and the ethyl acetate extract of the whole plant was prepared. The extract was subjected to GC MS analysis after processing it as per protocol. It was observed that some very important molecules were present in the GC MS profile such as Methyl 4-O-methyl-.beta.-D-xylopyranoside, D-Mannitol, Caryophyllene oxide, 11-Dodecanoic acid, 10-hydroxy-, methyl ester, Pentadecanoic acid, Phytol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, Octadecanoic acid, Methyl stearidonate, Eicosanoic acid, Gamolenic Acid, Docosanoic acid, .beta.-Tocopherol, O-methyl-, Stigmasterol, .beta.-Sitosterol, dl-.alpha.-Tocopherol, p-Coumaric acid, 1-Heptatriacotanol etc. These molecules indicate functions similar to the roles of this plant towards curing the ailments.

Keywords: GC MS, Herbal, *Merremia emerginata*, D-Mannitol, Methyl stearidonate, Eicosanoic acid, Gamolenic Acid, Docosanoic acid, .beta.-Tocopherol.



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INTRODUCTION

The present study is to probe into the medicinal role of one herbal medicinal plant, *Merremia emerginata*, by GC MS analysis. This knowledge could help in understanding the function of the molecules present in the plant. There are many reports on the GC MS studies of almost all herbal medicinal plants, still lot more need be done [1-14]. Traditionally *M. emerginata* is used as diuretic, for cough, headache, neuralgia and rheumatism. In the Indigenous system of Medicine, *M. emerginata* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, and fever due to enlargement of liver and also in kidney diseases. Powder of leaves is used as a snuff during epileptic seizures, Juice acts as purgative and the root is having diuretic, laxative, and applied in the disease of the eyes and gums. Babu *et al*, 2009 have studied the antioxidant activities of various extracts of *M. emerginata* [15]. Prabhu *et al*, 2012 have reported the phytochemical analysis, anti-inflammation, anti-arthritic analgesic and anticancer role of this plant [16-18]. Baskar *et al*, 2012 have shown the antioxidant, anti-inflammation, anti-arthritic analgesic and anti-proliferative role of *M. emerginata* [19]. Thakare *et al*, 2014 have reviewed the various medicinal aspects of this plant [20]. Angappan *et al*, 2018 have shown the diuretic role of this plant [21]. Elumalai *et al*, 2001 have reported the antibacterial role of this plant [22].

MATERIALS AND METHODS

The plant *Merremia emerginata* was collected from the nearby hills at Chengalpattu, Tamil Nadu. The plant was identified by a qualified botanist at Chennai. The ethyl acetate extract of the shade dried whole plant was collected after 48 h of soaking. The extract was evaporated and the dried powder was used for GC-MS analysis by standard procedures.

GC-MS Procedure

Instrument: GC (Agilent: GC: (G3440A) 7890A. MS/MS: 7000 Triple Quad GCMS) was equipped with MS detector. Sample Preparation. About 100 ml sample was dissolved in 1 ml of suitable solvents. The solution was stirred vigorously using vortex stirrer for 10 s. The clear extract was determined using GC for analysis.

GC-MS Protocol

Column DB5 MS (30 mm × 0.25 mm ID × 0.25 μm, composed of 5% phenyl 95% methylpolysiloxane), electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min injector temperature 280°C; auxiliary temperature: 290°C ion-source temperature 280°C. The oven temperature was programmed from 50°C (isothermal for 1.0 min), with an increase of 40°C/min, to 170°C C (isothermal for 4.0 min), then 10°C/min to 310°C (isothermal for 10 min) fragments from 45 to 450 Da. Total GC running time is 32.02 min. The compounds are identified by GC-MS Library (NIST and WILEY).

RESULTS

The results of the GC-MS analysis of the whole plant ethyl acetate extract along with the possible medicinal role of each molecule of *Merremia emerginata* extract are tabulated in Table 1. Figure 1 represents the GC-MS profile of ethyl acetate extract of the whole plant of *Merremia emerginata*. The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS along with the possible pharmaceutical roles of each bio molecule as per Dr. Duke's Phytochemical and ethnobotanical data base (National Agriculture Library, USA) and others as shown in Table 1 [23].





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DISCUSSION

The GC MS profile of *Merremia emerginata* indicated the presence of the some phytochemicals such as Methyl 4-O-methyl-.beta.-D-xylopyranoside, D-Mannitol, Caryophyllene oxide, 11-Dodecenoic acid, 10-hydroxy-, methyl ester, Pentadecanoic acid, Phytol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, Octadecanoic acid, Methyl stearidonate, Eicosanoic acid, Gamolenic Acid, Docosanoic acid, .beta.-Tocopherol, O-methyl-, Stigmasterol, .beta.-Sitosterol, dl-.alpha.-Tocopherol, p-Coumaric acid, 1-Heptatriacotanol etc. the medicinal roles of which are indicated in Table 1. From the table it is clear that these molecules do have some relationship with the medicinal roles of this plant. It is concluded that the ethnomedical uses of this plant is supported by the roles of the molecules present in this plant as shown by the GC MS profile.

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Conflict of interest

The authors declare that no conflict of interest exists among them

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Table1. Indicates the retentions values, types of possible compound, their molecular formulae, molecular mass, peak area and their medicinal roles of each compound as shown in the GC MS profile of *Merremia emarginata*

Sl. No	Retention Time	Compound Name	Mol. Formula	Mol. Weight	% Peak Area	Possible medical Role
1	4.71	Methyl 4-O-methyl-.beta.-D-xylopyranoside	C7H14O5	178.1	1.59	Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor, 17-beta-hydroxysteroid dehydrogenase-Inhibitor, Decrease glutamate oxaloacetate transaminase, decrease oxalate excretion, Anti-amyloid-Beta, Down regulate nuclear and cytosol androgen reuptake, Inhibits destruction of Glycosaminoglycans, Ornithine decarboxylase





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						inhibitor, AntiTGF-beta, Beta-2-Receptor-Agonist, Beta-Adrenergic Receptor Blocker, Beta-Andrenergic-Agent, Beta-Blocker, Beta-Galactosidase-Inhibitor, Beta-Glucuronidase-Inhibitor, ER-Beta-Binder, Aldehyde-Oxidase-Inhibitor, Anticancer, Antidote
2	6.52	D-Mannitol	C ₆ H ₁₄ O ₆	182.1	1.17	Smart drug, 17 beta hydroxysteroid dehydrogenase inhibitor, Alcohol dehydrogenase inhibitor, anticancer, antidote, antileukotrine D-4, circulatory depressant, CNS depressant, coronary dilator, Cyclin-D1-Inhibitor, Decalcifier, Decarboxylase-Inhibitor, Decongestant, Decrease C-Teleopeptide Excretion, Decrease Deoxypyridinoline Excretion, Decrease Endothilial Leukocyte Adhesion, Decrease Endothilial Platelet Adhesion
3	8.75	Silane, [(1,1-dimethyl-2-propenyl)oxy]dimethyl -	C ₇ H ₁₆ OSi	144.1	1.39	Not known
4	10.42	Caryophyllene oxide	C ₁₅ H ₂₄ O	220.2	0.84	Nitric oxide synthase inhibitor
5	12.56	11-Dodecenoic acid, 10-hydroxy-, methyl ester	C ₁₃ H ₂₄ O ₃	228.2	1.34	17 beta hydroxysteroid dehydrogenase inhibitor, Aryl hydrocarbon hydroxylase inhibitor, Testosterone hydroxylase inducer, Catechol-O- methyl transferase inhibitor, Methyl donor, Methyl guanidine inhibitor, Acidifier, Arachidonic acid inhibitor, Increase aromatic Amino acid decarboxylase activity
6	13.29	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.3	1.55	Oligosaccharide provider
7	13.45	Benzene, 1-isocyanato-2-methoxy-	C ₈ H ₇ NO ₂	149	0.73	Not known





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8	13.54	Pentadecanoic acid	C15H30O2	242.2	0.93	Acidifier, acidulant, Arachidonic acid inhibitor, Increase aromatic amino acid decarboxylase activity, inhibits production of Uric acid,
9	14.34	Scopoletin	C10H8O4	192	1.65	Not known
10	15.54	Tetradecanoic acid	C14H28O2	228.2	1.70	Acidifier, acidulant, Arachidonic acid inhibitor, Increase aromatic amino acid decarboxylase activity, inhibits production of Uric acid,
11	15.90	Phytol	C20H40O	296.3	3.50	Antimicrobial, anti-inflammatory, antioxidant, diuretic
12	16.28	Methyl 5,11,14-eicosatrienoate	C21H36O2	320.3	10.73	Catechol-O-methyltransferase inhibitor, methyl donor, methyl guanidine inhibitor
13	16.35	Pyrazine-2-carbohydrazide, N2-(2,4-dichlorobenzylideno)-	C12H8Cl2N4O	294	5.39	Not known
14	16.42	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2	278.2	2.35	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, insectifuge antihistaminic
15	16.59	Octadecanoic acid	C18H36O2	284.3	4.46	Acidifier, acidulant, Arachidonic acid inhibitor, Increase aromatic amino acid decarboxylase activity, inhibits production of Uric acid,
16	16.83	Phytol, acetate	C22H42O	338.3	1.66	Not known
17	17.96	Methyl stearidonate	C19H30O2	290.2	0.95	Catechol-O-methyltransferase inhibitor, methyl donor, methyl guanidine inhibitor
18	18.08	Eicosanoic acid	C20H40O2	312.3	1.59	Arachidonic acid Inhibitor, Increase Aromatic Amino acid decarboxylase activity, Inhibit production of uric acid, Urine acidifier,
19	18.17	Gamolenic Acid	C18H30O2	278.2	1.48	Acidifier, acidulant, Arachidonic acid inhibitor, Increase aromatic amino acid





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						decarboxylase activity, inhibits production of Uric acid,
20	19.07	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C38H68O8	652.5	1.52	Not known
21	19.59	Docosanoic acid	C22H44O2	340.3	0.81	Acidifier, acidulant, Arachidonic acid inhibitor, Increase aromatic amino acid decarboxylase activity, inhibits production of Uric acid,
22	20.35	Butyl 9,12-octadecadienoate	C22H40O2	336.3	2.01	Not known
23	20.40	Butyl 9,12-octadecadienoate	C22H38O2	334.3	1.53	Not known
24	20.58	Octadecanoic acid, 2,3-dihydroxypropyl ester	C21H42O4	358.3	0.99	Acidifier, acidulant, Arachidonic acid inhibitor, Increase aromatic amino acid decarboxylase activity, inhibits production of Uric acid
25	21.09	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4	352.3	1.25	Not known
26	22.83	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane	C33H56	452.4	3.08	Not known
27	23.30	.beta.-Tocopherol, O-methyl-	C29H50O2	430.4	1.29	Tocopherol-Synergist, Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor, 17-beta-hydroxysteroid dehydrogenase-Inhibitor, Anti amyloid-Beta, AntiTGF-beta, Beta-2-Receptor-Agonist, Beta-Adrenergic Receptor Blocker, Beta-Adrenergic-Agent, Beta-Blocker, Beta-Galactosidase-Inhibitor, Beta-Glucuronidase-Inhibitor, ER-Beta-Binder, Aldehyde-Oxidase-Inhibitor, Anticancer, Antidote, Antiretinitic





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28	23.37	Docosanoic anhydride	C44H86O3	662.7	0.92	Not known
29	23.79	Campesterol	C28H48O	400.4	7.80	Not known
30	24.04	Stigmasterol	C29H48O	412.4	14.52	Precursor of progesterone act as intermediate in the biosynthesis of androgens, and estrogens Antiosteoarthritic, antihypercholesterolemic, cytotoxic, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory, Analgesic
31	24.34	.beta.-Sitosterol	C29H50O	414.4	6.32	17 beta dehydrogenase inhibitor, androgen blocker, anti-amyloid beta, anticancer, Anti TGF beta, Beta 2-receptor, beta blocker, beta-galactosidase inhibitor, beta-glucuronidase inhibitor
32	24.44	Dodecane, 1-fluoro-	C12H25F	188.2	0.78	Not known
33	24.53	Phytonadione	C31H46O2	450.4	1.66	Not known
34	24.61	Cholesterol margarate	C44H78O2	638.6	1.24	Cholestolytic
35	24.73	4,22-Stigmastadiene-3-one	C29H46O	410.4	1.08	Not known
36	24.79	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.,22Z)-	C31H50O2	454.4	0.95	Not known
37	25.12	dl-.alpha.-Tocopherol	C29H50O2	430.4	1.09	Tocopherol synergist, 5 alpha reductase inhibitor, Alpha agonist, Alpha amylase inhibitor, Alpha glucosidase inhibitor, HIF-1 alpha inhibitor, Ikappa B-alpha phosphorylation inhibitor, Increase alpha mannosidase activity, Interleukin 1-alpha inhibitor, Testosterone-5-Alpha-Reductase-Inhibitor, TNF- alpha inhibitor
38	25.56	p-Coumaric acid	C9H8O3	164	3.83	Inhibit Production of Uric Acid, Acidifier, Acidulant, Arachidonic acid-Inhibitor, Arachidonic-Acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity, Urinary-Acidulant, Urine-Acidifier, Adrenalin-Pressor, Algogenic (pain-causing),





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						Anesthetic-potentiator, ANS-Paralytic, Anti-cAMP-Phosphodiesterase, Anticancer, Antidote
39	25.62	1-Heptatriacotanol	C37H76O	536.6	0.99	Antibacterial Lupeol: Anticancer, antiprotozoal, chemopreventive and anti-inflammatory properties, Antimalarial Antiflu, Antiviral, antiprotozoal, Antioxidant, Antiperoxidant, Antitumor, anticancer, Enzyme inhibitor, anti-hypercholesterolemic effects
40	28.13	11,13-Dimethyl-12-tetradecen-1-ol acetate	C18H34O2	282.3	1.35	Oligosaccharide provider

Qualitative Compound Report

Data File 220620093.D **Sample Name** Merremia emarginata
Sample Type **Position** 106
Acq Method GC Screening Method.M **Acquired Time** 03-07-2020 PM 09:56:18
Comment

User Chromatogram

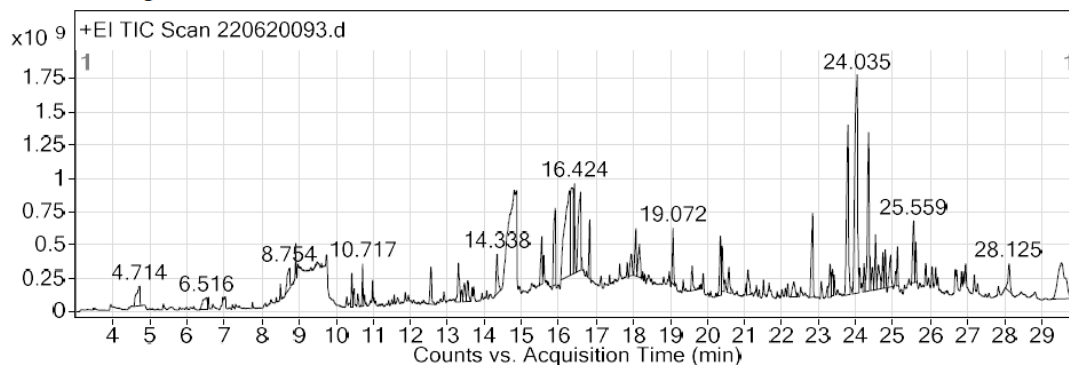


Figure 1. Indicates the GC MS profile of Merremia emarginata





Evaluation of Phytochemical Constituents and Anti-Ulcer Activity of *Solanum xanthocarpum* Linn.

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ABSTRACT

Solanum xanthocarpum (*solanaceae*) is a perennial herb with prickly prostrate available in India. It is found in waste places, road side and open spaces. *Solanum xanthocarpum* (*linn.*) has astringent, stimulant, diuretic, pungent, bitter, carminative, digestive, expectorant, febrifuge, and laxative. In Siddha, it is used in treatment of fever, cough, asthma, bronchitis, influenza, enteric fever and allergic conditions. A decoction of plant is used in gonorrhoea treatment. It promotes conception in females. It is used in the management of rheumatism. The whole plant is used traditionally for curing various ailments. The study suggested that the ethanolic extract of the plant *Solanum xanthocarpum* (*linn*) was found to produce significant anti-ulcer property in dose dependant manner (100 and 200 mg/kg, p.o.). These activities were comparable with the standard drug (Ranitidine 150mg). The present study indicates that the observed significant anti-ulcer activity of *Solanum xanthocarpum* (*linn*) may be contributed to the phytoconstituents present in it. Further work is in progress to identify the possible mechanisms of action and to identify the lead molecules responsible for anti-ulcer activity.

Keywords: *Solanum xanthocarpum* (*linn*), Anti-ulcer activity, Ethanolic extract, Ranitidine

INTRODUCTION

Our world is fulfilled by various medicinal plants which are widely have been used in treatment of various diseases since ancient time. Herbal preparations are effectively used for their medicinal properties and have become increasingly popular worldwide. Herbal medicines generally have fewer side effects than synthetic compounds and

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their effectiveness can be improved by modern pharmacological methods(1). Gastric ulcers occur approximately ten years later in life than duodenal ulcer. Peak incidence is usually at the range of 55 – 65 years. Like duodenal ulcer, gastric ulcers occurs almost equally in both males and females. Gastric ulcer is usually deep, and the lesion is surrounded by inflammation. The usually occur in the funds of the stomach and are accompanied by antral gastritis due to the colonization by helicobacter pylori. Gastric ulcer affects the lesser curvature of the stomach, and appears as a single lesion. It is not uncommon for patients to develop both duodenal ulcer and gastric ulcers, simultaneously (2). There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer (3). India has rich history of using plants for medicinal purposes. *Solanum xanthocarpum* is a medicinal plant extensively used traditionally. The objective of this study was therefore, to evaluate the anti-ulcer activity of *Solanum xanthocarpum*. The plant is traditionally used for anti-ulcer. The leaves are eaten as a vegetable or famine food, despite their laxative action. It has a caustic effect upon the skin and mucous membrane. It has the strong purgative effects. Root decoction is used as febrifuge, diuretic, and expectorant(4). *Solanum xanthocarpum* is specified for cough and asthma. Lincture prepared from the stamens of flowers is prescribed for chronic cough in children(5). The whole plant is used traditionally for curing various ailments. Decoction of the plant is used in gonorrhoea; paste of leaves is applied to relieve pains; seeds act as expectorant in cough and asthma; roots are expectorant and diuretic, useful in the treatment of catarrhal fever, cough, asthma and chest pain(6).

MATERIALS AND METHODS**Plant Collection And Extraction**

The aerial parts of the plant *Solanum xanthocarpum*, belongs to the family Solanaceae was collected and authenticated by the ABS botanical conservation Research and training centre, Salem. The plant was collected from Yercaud hills, Salem district, Tamilnadu, India. The plant is largely available in the rainfall areas. The leaves are picked with care to avoid mixing of some other parts. The aerial parts of the plant materials were dried in shade and extract with ethanol. The extracts were filtered and concentration to dryness in vacuum at 40°C.

Preliminary Phytochemical Screening

The extract was screened for the presence of various chemical constituents such as alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids and saponins using standard procedures.

Animals: Albino rats (150-200g) of either sex were housed in standard cages under laboratory condition in the Department of Pharmacology and toxicology and were fed with pellet feed and water *ad-libitum*. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals.

Pylorus-Ligated (PL) - Induced Ulcer

Gastric ulcers were produced in rats by the following method. Briefly the rats were fasted for 24hrs before pylorus-ligation but water was allowed *ad libitum*. At the end of 24hr starvation, rats were anaesthetized with chloroform. Abdomen was opened by a midline incision and a ligature was placed at the pyloric end of the stomach taking care not to exclude any blood vessels. The abdomen was then closed in two layer and rats were left in a cage with a false bottom of wide mesh wire gauze to prevent caprophagy. Water was withheld from one hour before pylorus ligation and till the end of 4 hour period when the rats are sacrificed by overdosing with chloroform. Immediately afterwards abdomen was again opened, cardiac end of the stomach was ligated and the stomach was taken out. The stomach was then cut and open along the greater curvature and the mucosa was washed under slow running tap water(7). The ulcer index was calculated by adding the total number of ulcers per stomach. The total severity of ulcers was determined by recording the severity of each ulcer after histological confirmation as follows:



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+ - **Pin Point ulcer** and histological changes limited to superficial layers of mucosa and no congestion,

++ - **Ulcer size less than 1mm** and half of the mucosal thickness showed necrotic changes,

+++ - **Ulcer size 1-2 mm** with more than two third of mucosal thickness destroyed with marked necrosis and congestion, muscular is remaining unaffected,

+++ ulcer either more than 2mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscular is still remaining unaffected(8).

Histopathological Studies

Stomach slices fixed for 12 hrs in 10% formaldehyde solution were processed for paraffin embedding following standard micro technique. Histopathological changes i.e., normal, damaged and recovered stomach were studied as shown in Fig. No. 1A – 1E

RESULTS AND DISCUSSION

The ayurvedic system of medicine includes number of plants and minerals which should be investigated to determine the hidden potential by using the modern methodology. The goal should be searching for drugs of plant origin with minimum side effects and maximum benefits. The plant *Solanum xanthocarpum* linn., is an indigenous herb which was chosen for this study. The plant belongs to the family Solanaceae. The scanty availability of information on this plant facilitates the study on it. Since ages various parts of this plant are being used for their medicinal use. The attempt is made to study the features, phytoconstituents and pharmacological activities of the leaves of the plant.

Preliminary Phytochemical Screening

The phytoconstituents were identified by chemical tests which showed the presence of various phytoconstituents which are expressed in table no: 2.

Pharmacological Studies

Ethanollic extract from the plant possessed Anti ulcer activity at the good extent. The extract 200mg was found more potent anti-ulcer activity on comparison to the extract 100mg.

CONCLUSION

Based on the traditional uses, the plant was selected. The phytochemical and pharmacological studies were done on leaves of *Solanum xanthocarpum* linn. The leaves of the plant were separated, dried under shade and subjected for Extraction process. The phytochemical constituents were extracted by successive solvent extraction. The photochemical constituents were identified by chemical tests and these tests showed the presence of numerous active compounds like phenolic compounds and flavonoids. It may showed the good pharmacological activities. Ethanolicextracts of leaves of *Solanum xanthocarpum* Linn shows the presence of many phytoconstituents such as carbohydrate, glycosides, phytosterols, fixed oils and fats, saponins, flavonoids, gums and mucilage and also of sufficient quantity necessary for the studies. In the pharmacological studies ethanolic extracts of leaves of *Solanum xanthocarpum* Linn showed significant antiulcer activity. The antiulcer activity was evaluated by using Pylorus ligated induced ulcer models in rat. It shows that flavonoids present in these extracts may be responsible for Antiulcer activity.

The Histopathological results are shown in fig no 1.1 to 1.5. In case of histopathological analysis the recovery of the ulcer shown by the Ethanolic Extract 200 mg was much better than 100 mg ethanolic extract. It was nearly equal to the standard.





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Future Aspects

As per the overall study conducted we can conclude that the use of the plant *Solanum xanthocarpum* Linn. is much more beneficial for ulcer curing. In future it may be a good anti-ulcer curing. In future it may be a good Antiulcer treatment which can cure various types of ulcer completely, so it is necessary to have a detailed study on the same.

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Table No. 1: Effect of *S. Xanthocarpum* extract (twice daily for five days) on pylorus ligation induced gastric ulcers.

Groups	Treatment	Dose (mg/kg)	Ulcer index(mm ² /rat)	Percent Protection(%)
I	Normal	10 ml/kg	19.6±2.2	-
II	Negative control	-	12.8±1.81	34.74%
III	<i>S. Xanthocarpum</i>	100 mg	8.1±2.08	58.49%
IV	<i>S. Xanthocarpum</i>	200 mg	4.8±0.94*	76.43%
V	Ranitidine	50 mg	3.5±0.84	82.19%

Table No. 2: Preliminary phytochemical screening of plant constituents

S. NO.	COMPOUND	PET ETHER	CHLOROFORM	ETHANOL
1	Alkaloids	-ve	+ve	+ve
2	Glycosides	-ve	-ve	-ve
3	Carbohydrates	-ve	-ve	-ve
4	Tannins	-ve	+ve	+ve
5	Flavonoids	-ve	+ve	+ve
6	Sterols	+ve	+ve	+ve
7	Tri-terpenoids	-ve	-ve	+ve
8	Proteins	-ve	-ve	+ve
9	Amino acids	-ve	-ve	-ve
10	Fixed oils	-ve	-ve	-ve
11	Fats	+ve	-ve	-ve
12	Mucilage	-ve	-ve	-ve
13	Saponins	+ve	+ve	-ve





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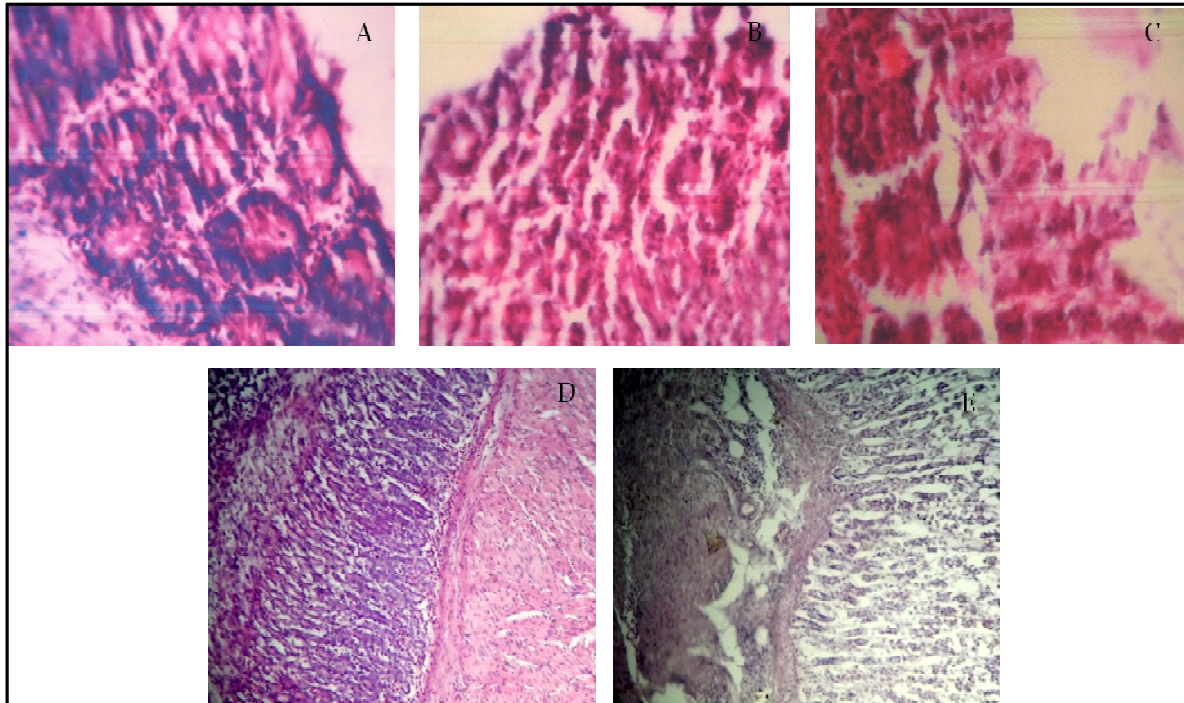


Fig. No. 1: Histopathological evaluation as an indication of anti-ulcer activity in various animal treatment groups studied (A , B , C , D , and E .)





Sense of Equality in Society: The Fact of Gender Discrimination in Indian Contemporary Era

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ABSTRACT

To have a harmonious society, regardless of gender, every human should have equal access to fundamental rights. It is discussed and acknowledged worldwide, including developed and developing nations during the 21st century. Although it is identified as necessary for the growth and development of society, we cannot deny that the concept does not exist worldwide and has been mistreated in a few places. Additionally, one cannot refuse that gender equality hardly exists in some areas. It is exciting to understand the thriving demand for equality among both sexes to ascertain nature's harmonious balance. It's an undeniable fact that both genders require human existence. However, one of the current discussion issues, i.e. gender discrimination, has been highlighted the issues and challenges still existing during the 21st century. Therefore, this article would attempt to unfold how contemporary novelists voice themselves and emphasise the quest for the meaning of life. This paper explores autobiographical work by Khushwant Singh and Shobha De regarding gender equality among the plethora of literature on feminism. These eminent figures cast light on people from various socio-cultural backgrounds and presented delicately even the compassionate social aspects of human life. The writers reflect the in-depth understanding of the human psyche from both rural and urban environments. They meaningfully organised the written moral orthodoxy of the Indian diaspora while searching the context of their own identity throughout.

Keywords: Experience, humanity, Journey, Literature, Emotions, Autobiography, Gender Equality.



**Renu Kumari and Richa Arora**

INTRODUCTION

A Life story constitutes a mixed event of personal existence, situations faced and experiences gained during an entire lifetime. Life events substantially impact one's life and begin as early as birth and continue affecting until death. Life's journey cannot remain unaffected by their surrounding familial circumstances either; for instance, where a person lives, his family situation and nearby society would undoubtedly impact individual behaviour. Autobiographer assigns narrative milestones to his life journey while writing the context of a story, reflecting the influence of time, events, place, people and particular beliefs systems. Human life can be depicted cumulatively as a pilgrimage odyssey in a spiritual sense and progression towards senescence physically. Some people may describe their life as ordinary, while others may commit to possession of an extraordinary life. The meaning of ordinary and extraordinary is just a perspective of the autobiographer; mere survival may be remarkable, or achieving the new heights in his vocation or thoughts may be exceptional. It's upon readers judgment to interpret whether he relates to the perspective presented by the autobiographer. It implies to everyone, including both genders, equally. Gender identity can be self-recognised in nearly all dimensions; it combines biological and surrounding circumstances and reflects an individual's overall development. One's identity could be defined or influenced through various social means, & quot; upbringing of an individual or an effect of society & quot; to an extent or capacity which can affect the character. Whereas societal gender identification is an observation, a particular feature like behaviour and appearance could help it identify. Society is a process rather than a thing or a motion and structure where members share a common cultural and social background. Even in the present society, only two genders might be misleading as society is rapidly adopting the more wide definition of the terms. Some individuals could not identify that they belong to the two genders mentioned above and reflect upon their bias over history. They deserve equal rights to live and work and should be recognised as vital members of society despite their sexual orientation and physical appearance.

The notion of gender identity occurs through phases and experiences of one's life. Life is a compilation of emotions or behaviour, impacting physical development, familial dynamics, cultural and social factors; therefore, it plays a cumulative part while self-identification. Widely accepted is that life lived with achievements and a successful career has witnessed various obstacles, hindrances, and challenges. Nevertheless, an undeniable fact is that achievements such as glory, satisfaction, and perseverance could play an important role. Khushwant Singh and Shobha De's Autobiographies and literature present vastly varied critical life events regarding how they handled the various circumstances and how they overcame the challenges. Mainly how their gender affected their work and success in society can either eliminate or reward, motivate, and inspire the readers. Here, the literature represents gender equally and concludes that nature comprises both the male and female as similarly essential components of society and vitally complement each other. Thus, this article consists of various issues of gender equality and is taken up from the work of two Indian contemporary writers of different genders, i.e., Khushwant Singh and Shobha De. Khushwant Singh was born on 02/02/1915 in a well- established and renowned family who followed Sikhism as a religion and principles. He spent his childhood in a rural village Hadali (Now in Pakistan), Delhi, India, and later moved to various places like Canada, United Kingdom, and France for professional reasons and educational purposes. Khushwant had a vast professional carrier and achieved different credentials, such as Indian author, Lawyer, Diplomat, Journalist, and politician. However, in his initial 40 years of life, he mainly spent wandering searching for his genuine interest, vocation. First, he tried his carrier as a professional lawyer to justify his education as an LLB, joined the Indian foreign services, and then worked as a journalist in all Indian radio in India. Afterwards, he moved to Paris, settled down working in mass communication for a while, and began writing thereon. That turns out to be his primary profession and passion till his deathbed.

Along with his professional carrier, he has lived both urban and rural LIFE while exploring worldwide. Khushwant Singh's writing covers varied-minded people broadly; while he portrays the accurate picture of human relationships, he also focuses sensibly on females's strength. For instance, he genuinely expressed that females are the gender with



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courage and power, provided a positive and encouraging environment. Later during his life, he eventually started Indian journalism as an editor and developed an inclination towards novel writing. Writing was one of the best interests he explored in himself, and after that, he continued writing, and it was his Novel writings that only made him renowned across the world. One of his famous pieces of literature expresses the purposeful character Bharati & quot;Burial at Sea & quot; and witnessed women empowering. This novel narrated how a female prognostic character managed to break the deep- rooted patriarchy and stood firm, self-independent, and obstinate. Here, the girl's father encourages her to dream big and think beyond; literature provided a new explanation for women's empowerment and discourages gender inequality. He has written all sorts of possible and purposeful topics and life events. He was a sensitive artist, used realism to present his humanistic eyesight, and explored life's realities. His priority as a person focuses on witnessing the truth throughout. He has also successfully depicted or portrayed the accurate picture of modern-day women and demonstrated that the individuals; social, political, and spiritual behaviour could be meaningful if nurtured well. Khushwant Singh's writing comprises various subjects, including religion politics, Nature, Man- Woman relationships, jokes, and tragedy of riots, famous Indian personalities, etc. He was consistently honest and comfortable sharing and presented all the aspects of life meaningfully. Here the writings were much more enriching in autobiographical content, his personal views and precise details of his life. His work explored the entire plethora of social issues, religion, riots, politics, and gender equality.

On the other hand, Shobha de also an elite writer. She is a former model, a Copywriter, a journalist, a socialite, a scriptwriter, and a novelist; most of her writing focused on urban India's various aspects. She is very well known for speaking my mental attitude; she graduated in psychology from St. Xavier College Mumbai, pursued a career in modelling, and made quite a fame for herself. She then got inclined towards writing later and gained significant experience in print media. She headed a few top magazines like stardust, society, and celebrity. Her major writings focus on politics, economics, culture, whereas relationship matters frequently exist in her writing. She concentrates mainly on the lives of the contemporary urban world, especially women. Her character as a woman has a strong sense of individuality and confidence. She commonly brings forth the social settings in which the women protagonists act and fight for their self-identity and assertion. She also expresses the inner psyche of women in her novels and presents their fundamental problems, unique experiences, journeys, and learning through them. This notion of her writing is quite apparent in the novel's character, Karuna, where the name breaks the shackles of a conservative community.

Karuna, one of the social figures from Bombay, mentioned how she escaped to the past while narrating the present. She happened to see the worst of unhappy divorce and began to write memoirs about suffering. It is evident in that work and expressed details about how a middle-class girl shined through her writing. Karuna, a famous Mumbai socialite, tried to escape the present's pressures and reality by diverging into the past. Although the unhappy married life ended in divorce, and a series of sordid affairs also made her upset, she began to write memoirs about the sufferings. The biography of her life takes the form of words and explains how even a simple middle-class girl can transform into the glamorous star that she became. It was the transcendence of her journey through all the hardships and opportunities she could excel through her confidence and experiences.

CONCLUSION

Published autobiographical literature provides a broader platform for readers to experience the narrator's life, soul-fulfilling experiences and gains a perspective of a meaningful life. It imparts intellectual development through continuous improvement in principles of writing, which means it has helped in sharpening the observation of life among the writers and the readers equally and imparted a psychological depth of equality among both genders. It has helped harmonise and release the tension from the early oppressors of history and provided a purpose to imagine a life where opportunities are equal for everyone, which further cultivated the strength in vision and courage to nurture harmony and a sense of success fulfilment in society. It would offer the freedom to live and





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express and contribute to building a prosperous society without an additional hurdle of bias to be cleared and gave a more walkable path for this dream to be achieved. Ultimately a sustainable future could be a significant outcome to expect. Autobiographies are promising to play a vital role in maintaining this balance of genders in the community. Also, such literature reflects society's current state and inspires the readers and the writers to imagine a social fabric standing on men's and women's pillars equally, well strengthened and prospered. Therefore, the literature thus holds power and responsibility to reflect a gender-neutral society and provide a landscape for humanity to imaging a diverse community where everyone has equal opportunity, whether men or women. India has seen many prominent women English writers on its account in this contemporary world and loved their writings. And with the changing environment, there will be a new paper from both genders, which will further enhance this harmony. Additionally, there are many great writings of male writers that reflect women's perspectives incredibly positively.

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Population and Diversity of Planktons in A Freshwater Pond, Ananthamangalam, Nagappattinam District, Tamilnadu, India

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ABSTRACT

The productivity of an aquatic environment is directly correlated with the population density of planktons. The present study was focused on the population and diversity of planktons as ecological indicator for identifying the ecological quality of freshwater pond located at Ananthamangalam, Nagappattinam district, Tamil Nadu, India. A total of 51 taxa of different classes of planktons were identified from the water sample collected from the study area. The planktons comprises phytoplanktons, Cyanobacteria, algae, diatoms, protista and protozoa. The phytoplankton was found being most abundant taxa. Species richness and diversity of planktons was found to be more during monsoon season. Fluctuations in the plankton count were observed among the seasons. Large number of planktons were identified during post monsoon season and less numbers of planktons were identified during Pre Monsoon season. From this study it is concluded that the freshwater pond located at Ananthamangalam showed the seasonal variations in plankton population, high diversity and more richness of species.

Key words: Freshwater pond; Plankton; Phytoplankton; Zooplankton; Ananthamangalam, Tamilnadu.

INTRODUCTION

Plankton is very small and sensitive aquatic organisms that are non-motile and weak to swim against water current, that existing drifting in state. Plankton encountered in the water body reflects existed ecological characteristics and

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therefore, plankton organisms may be used as indicators of water quality [1,2]. It is also one of the biological parameters that is influenced by other parameters and is a very important link in supporting the life of other organisms [3]. Therefore, the present study was aimed to determine the population and diversity of plankton community to know the condition of pond water quality at the Ananthamangalam, Nagappattinam District, Tamil Nadu, India.

MATERIALS AND METHODS

Study Area

Ananthamangalam (Latitude 11.04°N and Longitude 79.83°E) is a holy Village located at Tranquebar taluk and Nagappattinam District of Tamil Nadu, India (Fig. 1). It is situated in the deltaic region of the famous river Cauvery. The Cauvery delta system is the most ancient of all irrigation schemes and major water source for all freshwater ponds in this district.

Population estimation of planktons

Water samples for plankton analysis were collected by standard methodology [4] from predetermined sampling sites from the point of effluent outfall along with the downstream water stretch, arbitrarily designed two stations from January 2018 to December 2018. The filtered samples were fixed and preserved in 4% formalin with a few drops of Lugol's iodine solution. The collected samples were fixed in 3-4% formalin and brought to the laboratory for plankton analysis. Numerical plankton analysis was carried out using an inverted microscope and photographed with digital camera. Planktons were identified and enumerated by using the methods described by Hosamani and Bharathi [5]. Shannon Wiener diversity index (H') was calculated using the following formula:

$$\text{Shannon - Wiener Index (H)} = \sum \frac{ni}{N} * \ln * \frac{ni}{N}$$

Where, H = Shannon – Wiener index of diversity; ni = total numbers of individuals of species, N = total number of individual of all species. Values are represented as mean \pm Standard error. To know the seasonal variation in the plankton populations was analysed using one way ANOVA and SNK post hoc test. All statistical analyses were performed by SPSS windows based software.

RESULTS AND DISCUSSION

Planktons are very sensitive to change as their species replacement with the altering of environmental conditions in aquatic ecosystem and exhibited the present status of ecological and biological characteristics of aquatic ecosystem. The present study focused on the seasonal variation in the population of planktonic taxa to determine the quality of pond water. Since the local people use this water for irrigation and maintenance of domestic animals, the results of the study will be helpful to the local society. Seasonal abundance and population fluctuation of planktonic taxa are presented in table 1 and their images are presented in figure 2. The planktonic study has shown the presence of 51 taxa of 6 varieties of planktons viz., phytoplankton, cyanobacteria, algae, diatoms, protista and protozoa. One way analysis of variance showed a significant ($p > 0.05$) difference in the planktonic population among the seasons. During the winter season, total planktonic population was significantly more than other seasons. However, it was more or less similar during the seasons of monsoon, pre monsoon and post monsoon (SNK Test; table 3).

The high value of phytoplankton in winter might be due to relatively low temperature, availability of nutrients, and high amount of dissolved oxygen [6]. Low biomass of plankton during monsoon season could be attributed to cloudy weather, rain fall and dilution in the concentration of some salts [7]. Phytoplankton constituted dominant



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component which includes 17 taxa (Species richness) of the total planktons and exhibited two peaks one maximum at winter and the other at post monsoon season. It showed low biomass during the monsoon season (Tables 1 and 2). Shannon-wiener diversity index calculated for Planktonic taxa in different seasons (Table 4). High diversity (H) of phytoplanktons was observed during pre monsoon season with a species richness of 15. *Oscillatoria sp* was dominant species during winter (species dominance 250), and Premonsoon seasons (species dominance 244) while, *Phytoconis sp* was the dominant species during monsoon (191) and post monsoon seasons (255).

The phytoplankton serves as the producers in the food chain in the aquatic ecosystem and the productivity depends upon the quality of water, limnological feature of a pond in different seasons are related to hydrological condition which reflects in the physico-chemical characters and the plankton community of water [8]. Cyanobacteria, previously known as blue-green algae, are photosynthetic microorganisms that are abundant in nature. Cyanobacteria, the first oxygen-evolving group of photosynthetic Gram-negative prokaryotes, are unique among microbial world and grow in diverse habitats [9]. Cyanobacteria was a second dominant component of total planktons in the study area which includes 8 taxa (Table 2). Its population reached two heights one at post monsoon and another at winter season (Table 1). It exhibited high diversity ($H = 1.91$) during post monsoon season and low at monsoon (1.53) with species richness of 8 and 7, respectively. *Chlorogleoca sp* (166), *Aphanocece sp* (122), *Arthrospira sp* (100) and *Anabaena variabilis* (144) were recorded as dominant species during winter, pre monsoon, monsoon and post monsoon season, respectively (Table 4).

Cyanobacteria tend to become overpopulate at certain temperatures. Otherwise, the growth rate is inhibited and the population size remains low. In this way, climate plays an important role in early period of cyanobacteria growth [10]. Algae were recorded as third dominant component of total planktons. It comprised 13 species (Table 2). Algal population showed an elevation at winter and lowered at post monsoon season (Table 1). Algal diversity index ('h') values for winter, pre monsoon, monsoon and post monsoon seasons were calculated as 2.0, 1.84, 2.24 and 4.0, respectively. High species richness (9) was recorded winter season. *Spirogyra sp* was found to be a dominant species in all seasons.

Diatoms are unicellular they occur either as solitary cells or in colonies, which can take the shape of ribbons, fans, zigzags, or stars. Individual cells ranged in size from 2 to 200 micrometers [11]. Diatoms showed fourth dominant planktonic taxa in the study area. It included 10 species. It found thorough out the study period with a peak of 582/liter during the winter season. However, the diversity and species richness were found to be more or less similar in all season. *Amphora sp.*, *Synedra ulna*, *Syndra sp* and *Cocconeis sp* were observed as dominant species with a dominance of 183, 89, 80 and 67 respectively during winter, pre monsoon, monsoon and post monsoon seasons. Protista and protozoa (*Prorodon sp*) were also observed in seasons.

The planktons have been intimately connected directly or indirectly with human beings as a source of food, fodder, manure and many other types of uses e.g. algae as medicines and antibiotics, water purification, water pollution control, land reclamation, deleterious effect, industrial uses and indicators of water quality [12]. Planktons are limits to uppermost layers of the water where intensity of light is essential for photosynthesis. Indented light at different depths depends on the number of factors, like absorption of light by water, wave length of light, water transparency, reflection of water and reflection of suspended particles.

CONCLUSION

The Ananthamangalam pond showed seasonal variations in Planktonic population, diversity and richness. It included 51 Planktonic taxa of 6 varieties with dominant population of phytoplakton and cyanobacteria. From the result, it is concluded that the fluctuation of planktonic taxa occurs seasonally and indicated that pond of





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Ananthamangalam is similar like other tropical fresh water wetlands. Thus, good quality of water is maintained in this pond.

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Table 1. Total biomass of Planktons in the study area in different seasons

S.No	planktons	Winter Season	Pre Monsoon	Monsoon	Post Monsoon	Grand Total
1	Phytoplanktons	1,917	1,312	1,125	1,644	5999
2	<i>Cyanobacteria</i>	799	456	283	844	2383
3	Algae	783	544	700	322	2,350
4	Diatoms	582	211	150	199	1,144
5	Protista	266	211	250	255	983
6	Protozoa	50	22	-	22	94





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Table 2. Mean population of Planktons in the study area in different seasons.

Planktons	Mean Population (n/L) of at different Season			
	Winter Mean \pm SE	Pre Monsoon Mean \pm SE	Monsoon Mean \pm SE	Post Monsoon Mean \pm SE
Phytoplanktons				
<i>Phytoconis sp</i>	250.00 \pm 56.27	66.67 \pm 33.33	191.67 \pm 32.39	255.56 \pm 55.56
<i>Nitzschia sp</i>	84.00 \pm 27.73	77.78 \pm 27.78	0.00 \pm 0.00	55.56 \pm 14.22
<i>Oscillatoria sp</i>	250.00 \pm 50.00	244.44 \pm 33.79	150.00 \pm 39.89	200.00 \pm 52.52
<i>Kirchneriella sp</i>	116.67 \pm 30.73	0.00 \pm 0.00	0.00 \pm 0.00	22.22 \pm 12.65
<i>Tetraedron caudatum</i>	83.33 \pm 32.14	33.33 \pm 13.34	33.33 \pm 12.47	0.00 \pm 0.00
<i>Closterium sp</i>	150.00 \pm 34.16	55.56 \pm 33.79	16.67 \pm 6.24	111.11 \pm 26.06
<i>Selenastrum sp</i>	66.67 \pm 33.33	11.11 \pm 4.21	33.33 \pm 12.80	188.87 \pm 35.14
<i>Phormidium sp</i>	166.67 \pm 51.12	122.22 \pm 36.43	133.33 \pm 51.61	77.78 \pm 27.78
<i>Phacus sp</i>	66.67 \pm 33.33	55.56 \pm 24.28	116.67 \pm 20.72	44.44 \pm 17.57
<i>Chlorella sp</i>	200.00 \pm 51.64	122.22 \pm 26.20	133.33 \pm 25.53	155.56 \pm 37.68
<i>Merismopedia sp</i>	200.00 \pm 36.51	77.78 \pm 26.43	41.67 \pm 14.87	88.89 \pm 28.89
<i>Scenedesmus sp</i>	50.00 \pm 14.16	155.56 \pm 44.44	41.67 \pm 22.76	155.56 \pm 33.79
<i>Stephanodiscus sp</i>	0.00 \pm 0.00	66.67 \pm 18.87	8.33 \pm 1.67	0.00 \pm 0.00
<i>Ankistrodesmus sp</i>	0.00 \pm 0.00	77.78 \pm 33.39	66.67 \pm 25.52	11.11 \pm 2.21
<i>Chlamydomonas sp</i>	233.33 \pm 49.44	88.89 \pm 22.31	116.67 \pm 40.51	244.44 \pm 33.79
<i>Cosmarium sp</i>	0.00 \pm 0.00	56.66 \pm 23.76	0.00 \pm 0.00	0.00 \pm 0.00
<i>Navicula sp</i>	0.00 \pm 0.00	0.00 \pm 0.00	41.67 \pm 14.86	33.33 \pm 11.67
Cyanobacteria				
<i>Arthrospira sp</i>	100.00 \pm 51.64	33.33 \pm 13.57	100.0 \pm 30.157	144.44 \pm 29.39
<i>Anabaenopsis sp</i>	50.00 \pm 34.16	0.00 \pm 0.00	8.33 \pm 2.33	133.33 \pm 23.57
<i>Aphanocoece sp</i>	133.33 \pm 62.16	122.22 \pm 46.48	8.33 \pm 1.67	66.67 \pm 23.57
<i>Cylindrospermopsis sp</i>	116.67 \pm 60.09	56.55 \pm 23.78	8.33 \pm 1.67	133.33 \pm 26.67
<i>Anabaena variabilis</i>	83.00 \pm 17.44	55.56 \pm 24.22	25.00 \pm 7.67	144.44 \pm 17.57
<i>Chlorogleoca sp</i>	166.67 \pm 33.33	33.33 \pm 13.57	41.67 \pm 21.99	22.22 \pm 10.69
<i>Monoraphidium sp</i>	0.00 \pm 0.00	100.00 \pm 33.33	0.00 \pm 0.00	22.22 \pm 10.14
<i>Arthrospira platensis</i>	150.00 \pm 34.16	55.56 \pm 27.68	91.67 \pm 32.86	177.78 \pm 27.78
Algae				
<i>Pithophora sp</i>	33.33 \pm 10.42	0.00 \pm 0.00	8.33 \pm 2.41	44.44 \pm 13.79
<i>Micrasterias radiosa</i>	66.67 \pm 33.33	0.00 \pm 0.00	0.00 \pm 0.00	33.33 \pm 12.33
<i>Desmodesmus sp</i>	116.67 \pm 24.26	44.44 \pm 24.22	41.67 \pm 13.62	0.00 \pm 0.00
<i>Pandorina sp</i>	133.35 \pm 33.33	33.33 \pm 20.57	100.00 \pm 30.15	0.00 \pm 0.00
<i>Spirogyra sp</i>	200.00 \pm 57.74	177.78 \pm 57.19	116.67 \pm 42.34	144.44 \pm 24.22
<i>Chlorococcum sp</i>	66.67 \pm 49.441	111.11 \pm 26.38	91.67 \pm 18.76	88.89 \pm 25.47
<i>Pediastrum sp</i>	16.66 \pm 4.12	22.22 \pm 10.22	58.33 \pm 22.89	11.11 \pm 2.21
<i>Dinoflagellate sp</i>	0.00 \pm 0.00	0.00 \pm 0.00	75.00 \pm 27.87	0.00 \pm 0.00
<i>Sphaerocystis sp</i>	0.00 \pm 0.00	0.00 \pm 0.00	100.00 \pm 28.93	0.00 \pm 0.00
<i>Cryptomonas sp</i>	0.00 \pm 0.00	0.00 \pm 0.00	66.67 \pm 28.43	0.00 \pm 0.00
<i>Micractinium sp</i>	100.00 \pm 24.64	77.78 \pm 26.43	8.33 \pm 1.67	0.00 \pm 0.00
<i>Pinnularia opulenta</i>	0.00 \pm 0.00	33.35 \pm 13.57	16.67 \pm 6.67	0.00 \pm 0.00
<i>Treubaria sp</i>	50.00 \pm 14.14	44.44 \pm 13.79	16.67 \pm 10.24	0.00 \pm 0.00





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Diatoms				
<i>Cyclotella sp</i>	100.00 ± 29.44	0.00 ± 0.00	8.33 ± 1.67	33.33 ± 12.57
<i>Bacillriophyceae sp</i>	0.00 ± 0.00	0.00 ± 0.00	8.33 ± 1.67	44.44 ± 20.38
<i>Amphora sp</i>	183.33 ± 30.73	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Gyrosigma sp</i>	50.00 ± 14.32	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Achanthidium sp</i>	33.33 ± 11.12	0.00 ± 0.00	8.33 ± 1.67	0.00 ± 0.00
<i>Synedra ulna</i>	100.00 ± 41.64	88.89 ± 25.48	12.33 ± 2.61	44.44 ± 14.21
<i>Craticula cuspidate</i>	0.00 ± 0.00	11.11 ± 6.11	33.33 ± 11.21	0.00 ± 0.00
<i>Pleurosigma sp</i>	0.00 ± 0.00	66.67 ± 23.57	0.00 ± 0.00	11.11 ± 2.21
<i>Cocconeis sp</i>	0.00 ± 0.00	22.22 ± 14.69	0.00 ± 0.00	66.67 ± 44.06
<i>Syndra sp</i>	116.00 ± 30.73	22.22 ± 11.68	80.00 ± 19.46	0.00 ± 0.00
Protista				
<i>Euglena sp</i>	266.67 ± 54.26	211.11 ± 65.49	250.00 ± 37.94	255.56 ± 50.31
Protozoa				
<i>Prorodon sp</i>	50.00 ± 14.16	22.2 ± 6.78	0.00 ± 0.00	22.22 ± 10.69

Table 3. One way ANOVA and SNK test show the impact of season on the population of planktons.

Parameter	One way ANOVA (p>0.005); Student-Newman-Keuls Post hoc test			
	Number of planktons among the Season (subset for alpha = 0.005)			
Season	48.53 Monsoon	54.03 Premonsoon	67.10 Post monsoon	85.29 Winter

Mean values are arranged in ascending order. Horizontal lines connect similar mean.

Table 4. Shannon-Wiener Diversity index, species Dominance and Species Richness of Planktons in different seasons in the study area.

S. No	Season	Winter			Pre monsoon			Monsoon			Post Monsoon		
		SR	SD	SWI (H)	SR	SD	SWI (H)	SR	SD	SWI (H)	SR	SD	SWI (H)
1	Phytoplanktons	13	250	2.44	15	244	3.00	14	192	2.38	14	256	2.00
2	Cyanobacteria	7	167	1.88	7	128	1.88	7	100	1.53	8	177	1.91
3	Algae	9	200	2.00	8	178	1.84	12	117	2.24	5	144	4.00
4	Diatoms	6	183	1.66	5	89	1.35	6	80	1.35	5	67	1.49

SR = Species Richness; SD = Species Dominance; SWI= Shannon-Wiener Index.





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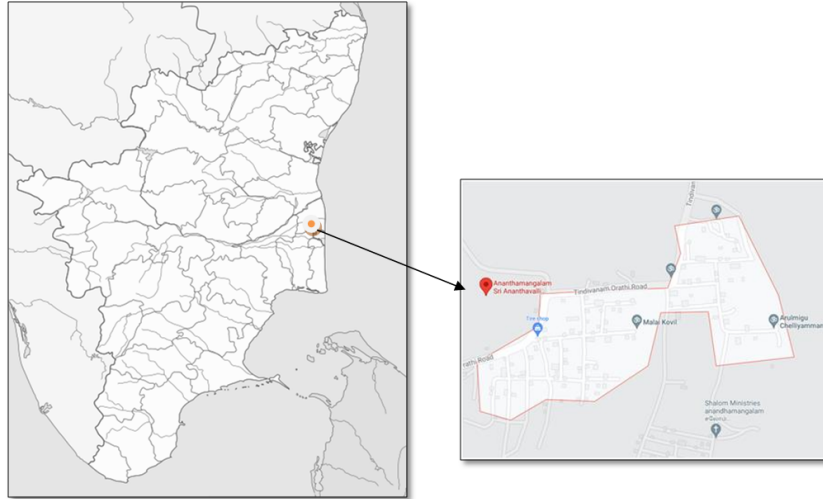











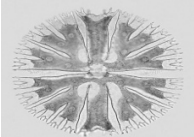


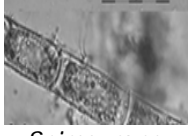
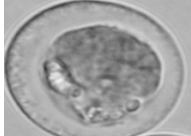






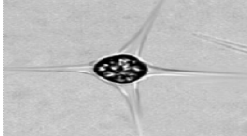
Fig. 1. Study Area of Ananthamangalam, Nagappattinam District

Phytoplankton			
 <i>Phytoconis sp</i>	 <i>Nitzschia sp</i>	 <i>Oscillatoria sp</i>	 <i>Kirchneriella sp</i>
 <i>Tetradron caudatum</i>	 <i>Closterium sp</i>	 <i>Selenastrum sp</i>	 <i>Phormidium sp</i>
 <i>Phacus sp</i>	 <i>Chlorella sp</i>	 <i>Merismopedia sp</i>	 <i>Scenedesmus sp</i>
 <i>Stephanodiscus sp</i>	 <i>Ankistrodesmus sp</i>	 <i>Chlamydomonas sp</i>	 <i>Cosmarium sp</i>
 <i>Coelastrum sp</i>		 <i>Navicula sp</i>	





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Cyanobacteria			
 <i>Arthrospira sp</i>	 <i>Anabaenopsis sp</i>	 <i>Aphanocyce sp</i>	 <i>Cylandrospermopsis sp</i>
 <i>Anabaena variabilis</i>	 <i>Chlorogleoca sp</i>	 <i>Monoraphidim sp</i>	 <i>Arthrospira platensis</i>
Green algae			
 <i>Pithophora sp</i>	 <i>Micrasterias radiosa</i>	 <i>Desmodesmus sp</i>	 <i>Pandorina sp</i>
 <i>Spirogyra sp</i>	 <i>Chlorococcum sp</i>	 <i>Pediastrum sp</i>	
 <i>Dinoflagellate sp</i>	 <i>Sphaerocystis sp</i>	 <i>Cryptomonas sp</i>	 <i>Micractinium sp</i>
 <i>Pinnularia opulent</i>		 <i>Treubaria sp</i>	





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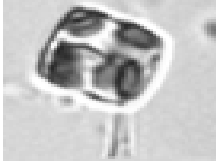



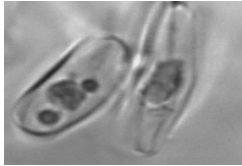

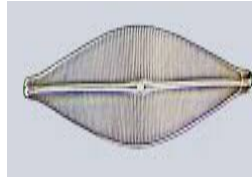

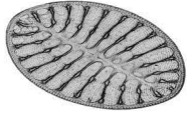
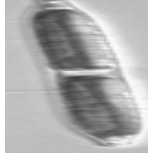
Diatoms			
 <p><i>Cyclotella</i> sp</p>	 <p><i>Bacillriophyceae</i> s</p>	 <p><i>Amphora</i> sp</p>	 <p><i>Gyrosigma</i> sp</p>
 <p><i>Acanthidium</i> <i>minutissimum</i></p>	 <p><i>Synedra</i> <i>ulna</i></p>	 <p><i>Craticula</i> <i>cuspidat</i></p>	 <p><i>Pleurosigma</i> sp</p>
 <p><i>Cocconeis</i> sp</p>		 <p><i>Syndra</i> sp</p>	

Figure 2: Images of different Planktons collected from freshwater pond at Anathamagalam





A Pharmacological Investigation of Cr³⁺, Co²⁺ and Ni²⁺ Complexes with Mixed Ligands

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ABSTRACT

Three new metal complexes of 4-Methylaminopyridine (MAP) and azide (**N₃-**) ion with Cr³⁺, Co²⁺ and Ni²⁺ have been prepared by using microwave irradiation. The DNA-binding properties of the free ligand 4-Methylaminopyridine and its Cr³⁺, Co²⁺ and Ni²⁺ complexes have been investigated by fluorescence measurements. The results suggest that MAP, Cr³⁺, Co²⁺ AND Ni²⁺ complexes both bind to DNA *via* an intercalative binding mode and the affinity for DNA is stronger in case of Cr³⁺, Co²⁺ and Ni²⁺ complexes when compared with MAP. The intrinsic binding constants (*K_b*) of the Cr³⁺, Co²⁺ and Ni²⁺ complexes and ligand with DNA were $2.19 \times 10^4 \text{ M}^{-1}$, $2.34 \times 10^4 \text{ M}^{-1}$, $3.80 \times 10^4 \text{ M}^{-1}$ and $1.34 \times 10^4 \text{ M}^{-1}$, respectively. Ni²⁺ complex is strongly bound to DNA compared to other complexes and the ligand. Furthermore, the free radical scavenging activity of the free ligand and their complexes has been determined by measuring their interaction with the stable free radical DPPH. The complexes have larger antioxidant activity as compared to the ligands.

Keywords: 4-Methylaminopyridine, azide ion, , DNA-binding property.

INTRODUCTION

In recent years, DNA binding studies of transition metal complexes have become very important in the expansion of DNA molecule probes and chemotherapeutics [1]. DNA is the pharmacological target of many of the drugs that are at present in clinical use or in advanced clinical trials. Targeting DNA to control cell functions by modulating





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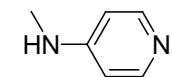
transcription or by intrusive with imitation seems logical, instinctively appealing and conceptually straightforward. Small ligand molecules bound to DNA artificially alter and/or inhibit the functioning of DNA. These small ligand molecules act as drug when alteration or inhibition of DNA function is required to cure or control a disease [2]. The study of interaction of drug with DNA is very thrilling and important not only for understanding the mechanism of interaction, but also for the design of new drugs. The studies have shown that metal complexes can interact with DNA in different binding fashions and exhibit effective nuclease activities [3,4].

On the other hand, the transition metal complexes are becoming a spectacular area of inorganic research due to the demand for new metal based antibacterial and antifungal compounds [5]. The staid medical trouble [6] of bacterial and fungal resistance and the rate at which it develops has led to the escalating levels of resistance to conventional antibiotics. The recognition and growth of antibacterial and antifungal drugs with inspired mechanism of action have become a critical task for communicable diseases research programs [7, 8]. In general, the biological activities of the metal complexes differ from those of either the ligand or the metal ion itself, and increased and/or decreased biological activities are reported for various metal complexes [9]. In the present study aims at synthesis and DNA-binding investigation of Cr³⁺, Co²⁺ and Ni²⁺ complexes with 4-Methylaminopyridine (MAP) and azide (N₃⁻) ion as ligands.

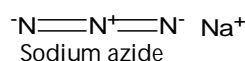
EXPERIMENTAL METHOD

Materials

4-Methylaminopyridine, sodium azide and chromium nitrate, cobalt nitrate and nickel nitrate were purchased from Alfa Aaser Company and used as such. The organic solvents DMSO, DMF, methanol, ethanol were of AnalaR grade and used as such without further purification.



4-Methylaminopyridine



Sodium azide

Synthesis of Cr(III) complex

4-Methylaminopyridine 0.83g (7.53 mmol) in ~10 ml methanol and sodium azide 0.49g (7.53 mmol) in ~10 ml ethanol were added to the chromium nitrate 1.00g (2.50mmol) in ~10 ml methanol followed by microwave irradiation for a few seconds after each addition by using IFB 25 BG-1S model microwave oven. The consequential precipitate was filtered off, washed with 1:1 ethanol: water mixture and desiccated under vacuum. An ash yellow colored complex was obtained with 14.17% yield.

Synthesis of Co(II) complex

Sodium azide 0.45g (6.90 mmol) in ~10 ml ethanol and 4-Methylaminopyridine 1.52g (13.82 mmol) in ~10 ml methanol were added to the cobalt nitrate 1.00g (3.40 mmol) in ~10 ml methanol followed by microwave irradiation for a few seconds after each addition by using IFB 25 BG-1S model microwave oven. The consequential precipitate was filtered off, washed with 1:1 ethanol: water mixture and desiccated under vacuum. A pink colored complex was obtained with 30.88% yield.

Synthesis of Ni(II) complex

1.00g (3.40 mmol) nickel nitrate in ~10 ml methanol and 4-Methylaminopyridine 1.52g (13.82 mmol) in ~10 ml methanol were added to the Sodium azide 0.45g (6.90 mmol) in ~10 ml ethanol followed by microwave irradiation for a few seconds after each addition by using IFB 25 BG-1S model microwave oven. The consequential precipitate was filtered off, washed with 1:1 ethanol: water mixture and desiccated under vacuum. A pale blue colored complex was obtained with 49.01% yield.





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DNA binding studies

The DNA binding experiments involving interaction of the Cr³⁺, Co²⁺ and Ni²⁺ complexes and the ligand with calf thymus CT-DNA were conducted in Tris buffer containing HCl (0.01 M) adjusted to pH 7.2 with hydrochloric acid. The CT-DNA was dissolved in Tris-HCl buffer and was dialyzed against the same buffer overnight. Solutions of CT-DNA gave the ratios of UV absorbance at 260 and 280 nm above 1.8, demonstrating that the DNA was adequately free of protein. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient 6600dm³mol⁻¹cm⁻¹ at 260 nm. The stock solutions were stored at 4°C and used within 4 days. For fluorescence-quenching experiments, DNA was pre-treated with ethidium bromide (EtBr) for 30 minutes. The MAP, Cr³⁺, Co²⁺ and Ni²⁺ complexes then added to this mixture respectively and their effect on the emission intensity was measured. Samples were excited at 450 nm and emission was observed between 500 nm and 800 nm [10].

Antioxidant activity

Evaluation of antioxidant activity stock solution (1 mg/ml) was diluted to final concentrations of 10–500 µg/ml. Ethanolic DPPH solution (1 ml, 0.3 mmol) was added to the sample solutions in DMSO (3 ml) at different concentrations (10–500 µg/ml) [11]. The mixture was shaken energetically and acceptable to stand at room temperature for 30 min. The absorbance was then measured at 517 nm in a UV-Vis Spectrophotometer. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ethanol was used as the solvent and ascorbic acid as the standard. The DPPH radical scavenging activity is designed by the following equation:

$$\text{DPPH Scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A₀ is the absorbance of the control reaction and A₁ is the absorbance in the presence of the samples or standard.

RESULTS AND DISCUSSION

DNA Binding properties

The binding of MAP, Cr³⁺, Co²⁺ and Ni²⁺ complexes to CT-DNA can be studied by competitive binding experiments. Ethidium bromide (EB) is known to show fluorescence when bound to DNA, due to its strong intercalation between the adjacent DNA base pair. The fluorescent light is quenched by the addition of a second molecule [12,13]. The quenching extent of fluorescence of ethidium bromide binding to DNA is used to determine the extent of binding between the second molecule and DNA. The addition of the complex to DNA pretreated with ethidium bromide causes appreciable reduction in the emission intensity, indicating the replacement of the ethidium bromide fluorophore by the complex results in a decrease of the binding constant of the ethidium to the DNA (Fig 1-4).

According to the classical Stern-Volmer equation: $I_0/I = 1 + K_{sv}r$, where I₀ and I are the fluorescence intensities in the absence and the presence of complex respectively. K_{sv} is a linear Stern-Volmer quenching constant, r is the ratio of the total concentration of complex to that of DNA. The quenching plots illustrate that the quenching of ethidium bromide bound to DNA by the complex are in good agreement with the linear Stern-Volmer equation, which also indicates that the complex binds to DNA. In the plot of I₀/I versus C_{Complex}/C_{DNA}, K is given by the ratio of the slope to intercept. The K_b values for Cr³⁺, Co²⁺ and Ni²⁺ complexes and ligand MAP with DNA were 2.19 × 10⁴ M⁻¹, 2.34 × 10⁴ M⁻¹, 3.80 × 10⁴ M⁻¹ and 1.34 × 10⁴ M⁻¹, respectively. Furthermore, Ni²⁺ complex is complex strongly bind to DNA compared to other complexes and the free ligand. The binding constant (K_b) values indicate that interaction of the complexes with DNA is intercalative mode [14].





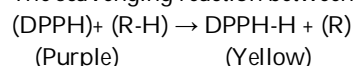
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Antioxidant activity

DPPH Radical scavenging assay

The scavenging activity of a chemical/or compound on the DPPH radical as a fast and reliable parameter to measure the *in vitro* antioxidant activity of such sample has been used by diverse researchers [15]. This assay is based on the measurement of the decrease in the molar absorptivity of DPPH at 517 nm after reaction with the test compound. The effect of antioxidants on DPPH radical scavenging is due to the hydrogen donating ability or radical scavenging activity of the samples [16].

The scavenging reaction between (DPPH) and an antioxidant (R-H) can be written as:



Antioxidants react with DPPH, a stable free radical that is reduced and as a result, the absorbance decreases due to the formation of the DPPH-H from the DPPH radical. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or samples in terms of hydrogen donating ability [17]. The graph was plotted with the percentage of scavenging effects on the y-axis and concentration ($\mu\text{g/ml}$) on the x-axis. The scavenging ability of the Cr^{3+} , Co^{2+} and Ni^{2+} complexes and ligand MAP were compared with ascorbic acid as standard [18]. The complexes showed enhance activity as a radical scavenger compared with ascorbic acid, these results were in good agreement with previous metal complexes studies where the ligand has the antioxidant activity and it is expected that the metal moiety will increase its activity [19-22].

The Cr^{3+} , Co^{2+} and Ni^{2+} complexes were found to have better antioxidant activity than the free ligand. At the lowest concentration (125 $\mu\text{g/ml}$) the antioxidant activity of the free ligand was found to be 10% but, upon complexation, it increased significantly to the range of 25.02% –65.22% in all the complexes.

CONCLUSION

In the present study, our efforts were to synthesize the Cr^{3+} , Co^{2+} and Ni^{2+} complexes with 4-Methylaminopyridine and azide ion as ligands. The new complexes were synthesized using microwave irradiation. The synthesized metal complexes were characterized by DNA binding properties and antioxidant activities. The metal complexes have significant antioxidant activity as compared to the free ligands. The effectiveness of the DNA binding property of the complexes is being confirmed by means of change in intensity of emission in the case of emission spectral studies.

ACKNOWLEDGEMENT

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Table 1. DNA-binding constant (Kb) of ligand and its complexes

S. No.	Ligands / Complexes	Binding constant (Kb)
1	4-Methylaminopyridine	$1.34 \times 10^4 \text{ M}^{-1}$
2	Cr ³⁺ complex	$2.19 \times 10^4 \text{ M}^{-1}$
3	Co ²⁺ complex	$2.34 \times 10^4 \text{ M}^{-1}$
4	Ni ²⁺ complex	$3.80 \times 10^4 \text{ M}^{-1}$

Table 2. Antioxidant activity of ligands and their complexes

S. No.	Ligands / Complexes	IC ₅₀ values (µg/ml)
1	4-Methylaminopyridine	>1000
2	Cr ³⁺ complex	344.22
3	Co ²⁺ complex	476.55
4	Ni ²⁺ complex	318.39

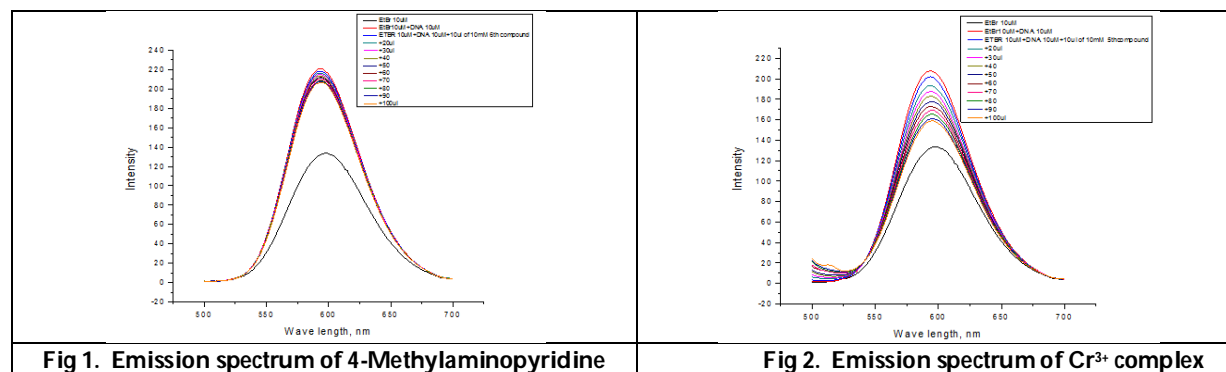


Fig 1. Emission spectrum of 4-Methylaminopyridine

Fig 2. Emission spectrum of Cr³⁺ complex



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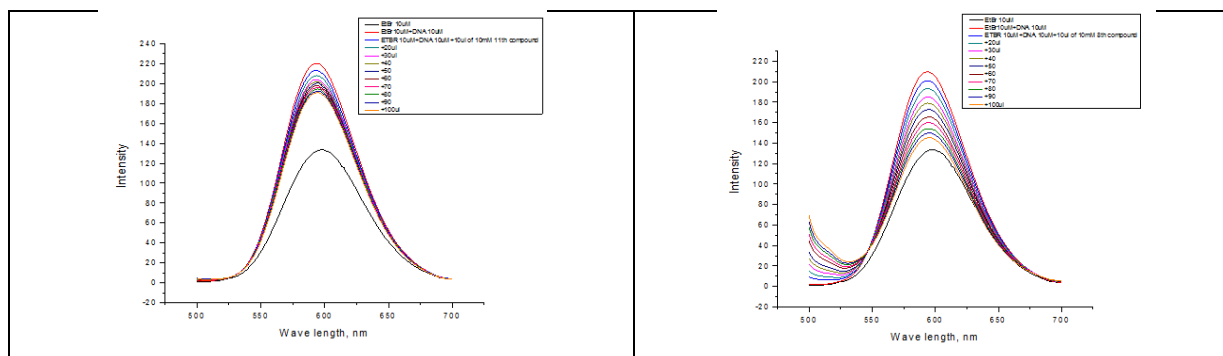


Fig 3. Emission spectrum of Co²⁺ complex

Fig 4. Emission spectrum of Ni²⁺ complex

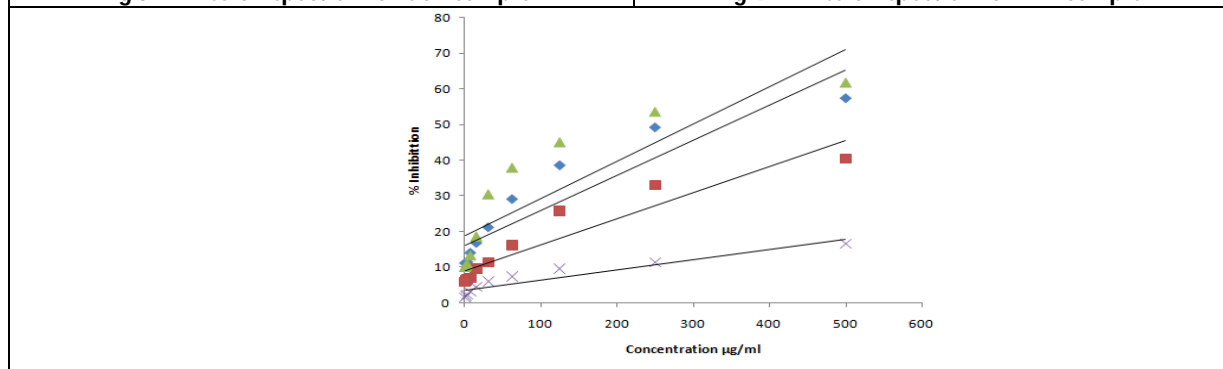


Fig 5. Antioxidant activity of Cr³⁺, Co²⁺ and Ni²⁺ complexes and ligand MAP





The GC MS Study of One Ayurvedic Formulation, Chandanasavam

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ABSTRACT

Objective: The present work deals with the GC MS analysis of one Ayurvedic medicine, Chanadansavam, which is prescribed for the treatment of various kidney and urinary ailments. **Methods:** The medicine, Chanadanasavam was procured from standard Ayurvedic vendor at Chennai and was subjected to GC MS analysis after processing as per standard protocol. **Results:** Important biomolecules such as Phenylethyl Alcohol, Undecanoic acid, 10-methyl-, methyl ester, Methyl tetradecanoate, Hexadecanoic acid, methyl ester, 11,14-Octadecadienoic acid, methyl ester, 11-Octadecenoic acid, methyl ester and Squalene were shown in the GC MS profile with far reaching medicinal roles. **Conclusion:** These molecules indicate that they could play an important role in the cure of kidney and urinary tract diseases. It will be of interest to probe the roles of such molecules whose roles are not known yet.

Keywords: Chanadanasavam, Ayurvedic, GC MS, Kidney, Urinary, Phenylethyl Alcohol, Squalene





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INTRODUCTION

The standardization of Ayurvedic and other forms of alternative medicines requires thorough testing with latest pharmacological and other modern procedures. This knowledge can go a long way in establishing their real medicinal efficacy. Some reports in regard is available and much more to follow [1-21]. The present work in one step in this direction which embarks upon the GC MS analysis of one Ayurvedic medicine, Chandanasavam mainly used for urinary disorders and kidney diseases. It is an effective ayurvedic solution for burning micturition, urinary tract infections (UTI), pyuria, dysuria, hyperuricemia, kidney stones (renal calculi), cystitis, chronic kidney failure, pyospermia (leukocytospermia) and gonorrhoea. It is prepared in the following method: 4.8 kg of sugar candy and 2.4 kg of jiggery are dissolved in 24.576 lit of water and filtered. To the filtrate, 960 g of grapes (*Vitis vinifera*), 760 g of flowers of *Woodfordia fruticosa* and 48 g of coarse powders of each of the following ingredients are added. White Sandal wood (*Santalum album* heart wood), Netrabala (*Pavonia odorata* root), Nagarmotha (*Cyperus rotundus* Rhizome), Gambhari or Kasmari (*Gmelina arborea* stem bark and root), Neel Kamal (*Nymphaea stellate* flowers), Priyangu (*Callicarpa macrophylla* flowers), Padmaka (*Prunusserasoides* stem), Lodhra (*Symplocos racemosus* stem bark), Manjistha (*Rubia cordifolia* root), Red sandal (*Pterocarpus santalinus* heart wood), Patha (*Cyclea peltata* root/ whole plant), Kiratatikta (*Swertia chiraita* whole plant), Long pepper (*Piper longum* fruits), Rasna (*Pluchea lanceolata* root/ whole plant), Patola leaf (*Luffa acutangula* leaf), Kanchanara (*Bauhinia variegata* stem bark), Bargad or Banyan (*Ficus bengalensis* stem bark), Mango tree stem bark (*Mangifera indica*), Salmali Nirryasa (*Salmalia malabarica* exudate). This mixture is kept in special pots known as asava / arishta vessels, closed tightly and allowed to ferment for one month, removed, filtered and stored in air tight bottles to be used as medicine.

MATERIALS AND METHODS

Chandanasavam was obtained from standard Ayurvedic vendor at Chennai and was subjected to GC MS analysis by standard procedure.

Instrument

Gas chromatography (Agilent: GC: (G3440A) 7890A. MS MS: 7000 Triple Quad GCMS,) was equipped with Mass spectrometry detector.

Sample Preparation

100 micro lit sample Dissolved in 1 ml of suitable solvents. The solution stirred vigorously using vortex stirrer for 10 seconds. The clear extract was determined using gas-chromatography for analysis.

GC-MS protocol

The GC MS Column consisted of DB5 MS (30mm×0.25mm ID ×0.25 μm , composed of 5% phenyl 95% methyl poly siloxane), Electron impact mode at 70 eV; Helium (99.999%) was used as carrier gas at a Constant flow of 1ml/min Injector temperature 280 °C; Auxiliary Temperature : 290°C Ion-source temperature 280 °C. The oven Temperature was programmed from 50 °C (isothermal for 1.0 min), with an increase of 40°C/min, to 170°C C (isothermal for 4.0 min), then 10°C/min to 310°C (isothermal for 10min) fragments from 45 to 450 Da. Total GC running time is 32.02 min. The compounds are identified by GC-MS Library (NIST & WILEY).

RESULTS AND DISCUSSION

The GC MS profile of Chandanasavam is represented in Figure 1. Table1 indicates the retention time, types of possible compound, their molecular formulae, molecular mass, percentage peak area and their medicinal roles as observed in the GC MS profile of Chandanasavam. The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the

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computer software (version 1.10 beta, Shimadzu) of the GC-MS along with the possible pharmaceutical roles of each bio molecule as per Dr. Duke's Phytochemical and ethnobotanical data base (National Agriculture Library, USA) and others as shown in Table 1.^[22] Table 1 indicates the presence of some important molecules having far reaching medicinal roles such as Phenylethyl Alcohol (antibacterial), Undecanoic acid, 10-methyl-, methyl ester, Methyl tetradecanoate, Hexadecanoic acid, methyl ester, 11,14-Octadecadienoic acid, methyl ester, 11-Octadecenoic acid, methyl ester and Squalene (control steroid metabolism). These molecules must be playing a medicinal role in curing the disease for which this medicine is used.

CONCLUSION

The presence of so many important molecules which control the internal metabolic processes and could in turn must be helping to cure the disease. Some molecules whose medicinal roles and not known require further work.

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Table1. Indicates the retentions values, types of possible compound, their molecular formulae, molecular mass, percentage peak area and their medicinal roles of each compound as shown in the GC MS profile of Chandanasavam

Sl. No	Retention Time	Compound Name	Mol. Formula	Mol. Wt.	% Peak Area	Possible medical Role
1	4.64	Phenylethyl Alcohol	C8H10O	122.1	27.54	Antibacterial
2	6.53	Coumarin, 3,4-dihydro-4,4,7-trimethyl-	C12H14O2	190.1	0.91	Not known
3	8.60	Benzene, 1,1'-(1-methylethylidene)bis[4-methoxy-	C17H20O2	256.1	0.87	Not known
4	9.64	Tetradecane, 2,6,10-trimethyl-	C17H36	240.3	0.95	Not known
5	9.95	Undecanoic acid, 10-methyl-, methyl ester	C13H26O2	214.2	1.74	Catechol-O-methyl-Transferase Inhibitor, methyl Donar, Methyl Guanidine Inhibitor, Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
6	12.18	Methyl tetradecanoate	C15H30O2	242.2	1.82	Catechol-O-methyl-Transferase Inhibitor, methyl Donar, Methyl Guanidine Inhibitor, Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
7	13.58	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, .alpha.,.alpha.,6,8-tetramethyl-, stereoisomer	C15H24O	220.2	4.68	Not known





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8	13.79	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	C12H20O	180.2	0.59	Not known
9	14.20	Hexadecanoic acid, methyl ester	C17H34O2	270.3	6.27	Catechol-O-methyl-Transferase Inhibitor, methyl Donar, Methyl Guanidine Inhibitor, Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
10	14.46	Phthalic acid, isobutyl tridec-2-yn-1-yl ester	C25H36O4	400.3	0.56	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
11	15.74	11,14-Octadecadienoic acid, methyl ester	C19H34O2	294.3	3.51	Catechol-O-methyl-Transferase Inhibitor, methyl Donar, Methyl Guanidine Inhibitor, Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
12	15.81	11-Octadecenoic acid, methyl ester	C19H36O2	296.3	10.84	Catechol-O-methyl-Transferase Inhibitor, methyl Donar, Methyl Guanidine Inhibitor, Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
13	16.07	Methyl stearate	C18H34O2	282.3	0.59	Catechol-O-methyl-Transferase Inhibitor, methyl Donar, Methyl Guanidine Inhibitor
14	18.32	Oleic Acid	C18H34O2	282.3	0.59	Acidifier, arachidonic acid inhibitor, Increase aromatic amino acid decarboxylase activity
15	18.80	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	C16H50O7Si8	578.2	1.67	Not known
16	19.15	Tert-Hexadecanethiol	C16H34S	258.2	0.65	Not known
17	19.36	Methyl 16-hydroxy-hexadecanoate	C17H34O3	286.3	0.76	Catechol-O-methyl-Transferase Inhibitor, methyl Donar, Methyl Guanidine Inhibitor,
18	19.42	Bis(2-ethylhexyl) phthalate	C24H38O4	390.3	2.58	Not known
19	19.91	Heptasiloxane, hexadecamethyl-	C16H48O6Si7	532.2	19.32	Not known
20	20.63	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366.4	0.65	Not known
21	20.89	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C24H38O4	390.3	0.85	Acidifier, arachidonic acid inhibitor, Increase aromatic amino acid decarboxylase activity





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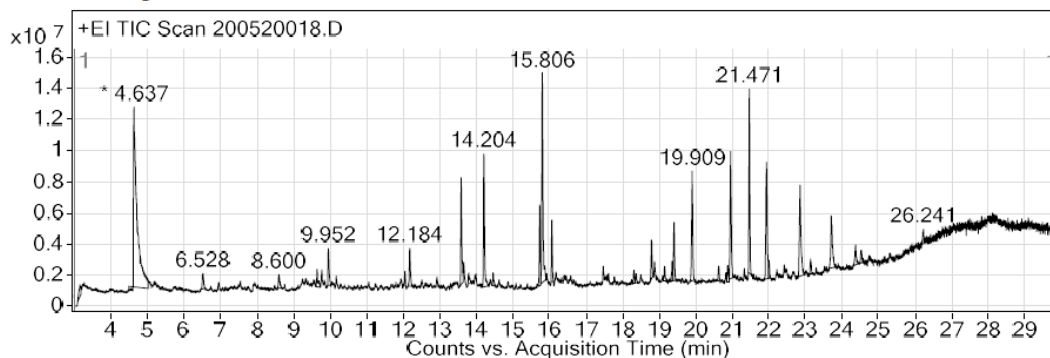
22	21.47	Squalene	C30H50	410.4	7.03	Monoxygenase inhibitor, biochemical precursor in the preparation of steroids, natural moisturizer, used in cosmetics
23	22.00	Dasycarpidan-1-methanol, acetate (ester)	C20H26N2O2	326.2	0.80	Not known
24	23.15	Cholesterol	C27H46O	386.4	0.68	It is the precursor for all steroids hormones.
25	24.38	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	C26H36O8	476.2	1.19	Not known
26	26.24	Tris(tert-butyl)dimethylsilyloxy)arsane	C18H45AsO3Si3	468.2	0.65	Not known

Figure 1. Indicates the GC MS profile of Chandanasavam

Qualitative Compound Report

Data File 200520018.D **Sample Name** Chandanasavam
Sample Type **Position** 30
Acq Method GC Screening Method.M **Acquired Time** 22-05-2020 AM 07:38:36
Comment

User Chromatogram





Evaluation of *In vitro* Antioxidant Potential of *Acacia eburnea* willd.

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ABSTRACT

Free radical plays an importance role in major health problems such as Cancer, Cardiovascular disorders, Rheumatoid arthritis, Cataract, Alzheimer, Parkinsonism and degenerative disease associated with ageing. Antioxidants, protecting the system from disease and disorders, are components neutralizing free radicals actions before they attack cells and preventing damage to cell macromolecules. Natural antioxidants constitutes broad range of compounds including phenolic, nitrogenous compounds, vitamins and more, exhibiting free-radical scavenging action. Phytochemicals such as flavonoids, phenols, phytosterols, proanthocyanin, tannin, terpenes etc., have been identified to be present in the leaves and roots of *Acacia eburnea* willd. rendering the possibility of potential anti-oxidant activity for this plant. *In vitro* antioxidant action of the ethanolic extract of *Acacia eburnea* willd. leaves was studied using DPPH (1,1-diphenyl-2-picryl hydrazyl) method. The ethanolic solution of the DPPH radical system, exhibiting absorption peak at 517 nm, was used in testing the free radical scavenging potential of the extract of the leaves of *Acacia eburnea* willd. The prominent decrease in absorbance intensity of the DPPH radicals observed indicated *in vitro* anti-oxidant potential of the ethanolic extract of the leaves of *Acacia eburnea* willd.

Keywords: *Acacia eburnea* willd., Anti- oxidant activity, Ethanolic extract, DPPH method

INTRODUCTION

All through human history there has been a noticeable concern for healthcare and curing of diseases/disorders though the concepts took a long time to develop into a system of knowledge. Logical approach of studying the drugs



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and their activities is the recognition of basic principles involved in the biochemical events leading to drug activity. Increasing amount of insight into the behaviour of drugs at the macromolecular level are being developed and direct and/or indirect evidences supporting these biochemical postulations for any drug action are documented. Electron acceptors, such as molecular oxygen reacts free radicals to become themselves as a radicals known as Reactive Oxygen Species (ROS) which includes radicals with unpaired electrons such as superoxide anions (O_2^-), hydroxyl radicals ($OH\cdot$) and non-radical hydrogen peroxide (H_2O_2). ROS, essential cellular components, generated in aerobic living organisms, plays an important role in different physiological processes and pathological conditions. Accumulation of excessive ROS, mainly due to external influences such as radiation, ultraviolet light, cigarette smoke, pathogens, drugs, etc., can inflict damage upon cellular macromolecules such as DNA, proteins and lipids. Insufficient levels of endogenous antioxidants leading to increased oxidative stress circumstances ending up with increased levels of ROS. This concept of increased oxidative stress circumstances and ROS levels may be associated with nearly 200 diseases such as cardiovascular diseases, cancer, atherosclerosis, hypertension, ischemia, diabetes mellitus, rheumatoid arthritis, neurodegenerative disorders (alzheimer and parkinsonism) and aging [1].

Antioxidants (naturally or synthetically derived) delays or prevents the oxidation of cellular oxidizable substrates by either preventing the generation of ROS or by activating detoxifying proteins thereby scavenging ROS[2].Antioxidant agents prevents the damage to the body caused by the free radicals and retard the progress of many chronic diseases. Antioxidants are substances that when present in low concentrations, compared to those of an oxidizable substrate significantly delay or prevent oxidation of that substance. Antioxidants have been shown to be effective in the treatment of various health problems, including neurodegenerative, systemic and infectious diseases. Apart from the role as health benefactors, antioxidants are added to food for preventing oxidation, normally initiated by free radicals formed during the food's exposure to environmental factors such as air, light and temperature. Synthetic antioxidants, such as Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT) and Tert-Butyl Hydroquinone (TBHQ) are commercially available and are currently in use. Unfortunately, new data indicating that their use is now restricted due to their side effects [3].

Antioxidants are substances that can protect your body's cells from the harmful effects of free radicals. This can happen as a result of exposure to certain substances, smoking, pollution, and radiation, among other things as a function of natural metabolic processes Selenium, vitamin A, and other associated antioxidants are found in the diet, carotenoids, vitamin C, and vitamin E, as well as phytochemicals like lycopene, lutein, and zeaxanthin,quercetin is a form of quercetin [4]. Antioxidants benefits are Support kidney function, Improve re-productive function, Support the immune system and improve defense power of the body, Maintain healthy vision, Improve quality of sleep, Reduce obesity, improve nervous system functioning, Product good dental health, Support respiratory system[5].

Many studies have supported that antioxidant nutrients or/and medicines play a protective role in human role in human health [6,7]. Antioxidants principles from herbal resources are multifaceted in their effects and provide enormous scope in correcting the imbalance through regular intake of a proper diet. It has been assumed that nutritional intervention to increase intake of Phyto-antioxidants may reduce treat of free radicals[8,9]. Plants play a significant role in maintaining the human health and improving the quality of human life. The WHO estimated that less than 80% of the earth's inhabitants reply on traditional medicine for therapy which involves the use of plant extracts and active components [10]. The balance between antioxidation and oxidation is believed to be critical concept for maintaining healthy biological system. Current understanding on the role of oxidative stress and free radicals in pathogenesis of various disease/disorders and for advancements made in developing antioxidant-based therapeutics and also discussed the opportunities to develop therapeutics from traditional medicinal practice. Based on ethno-pharmacological survey, leaves of *Acacia eburnea* willd. has been selected to prove scientifically having antioxidant activity. Hence, the present study is an attempt to investigate antioxidant activity of the plant *Acacia eburnea* willd.





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MATERIALS AND METHOD

Selection and Collection of the Plant

Medicinal properties of plants have been investigated, in recent scientific developments throughout the world, due to their advantage of having potent pharmacological activities, low toxicity and economic viability. A search was undertaken on folk medicinal plants utilized in particular area and discussions with a tribal medical practitioner for the identification, selection and collection of a medicinal plant for which potential possibility of antioxidant activity has been reported but have not yet been experimentally evaluated for the same. *Acacia eburnean* Willd., one such folklore medicinal plant has been reportedly used in the treatment of disorders such as eczema and Skin disorder, genital disorders Leucorrhoea, conjunctivitis, epiphora, erectile dysfunction, night fall etc. The plant has also been reported to have potential to be used as antidiuretic, antidiabetic and anti-inflammatory and antioxidant agent. Hence, the present study was made as an attempt to investigate the antioxidant activity of the extracts of *Acacia eburnean* Willd. The leaves of *Acacia eburnean* were collected from the foothills of Kodaikkanal, Tirunelveli district in the month of November 2017. The plant was taxonomically identified and authenticated by the botanist Mr. Chelladurai.

Preparation of Extract of the Plant

Acacia eburnean Willd. leaves were gathered, air dried in the shade and then grounded into fine powder using mechanical grinder and stored in an airtight container until the time of use after the dried coarse powder of the leaves were sieved through mesh no. 40. 1000 gms of powdered material was packed appropriately into the Soxhlet apparatus and extracted by continuous hot solvent percolation method using Petroleum ether (1000 mL), Chloroform (1000 mL), Ethanol (99% v/v, 1000 mL), Distilled water (1000 mL), for 72 Hrs each. The resulting semisolid mass obtained is then vacuum dried using vacuum desiccator. The percentage yield of various extracts of *Acacia eburnean* Willd. were determined.

Preliminary Phytochemical Studies [11,12]

The extracts of Pet. ether, Chloroform, Ethanol and Aqueous obtained were subjected to the following Phytochemical studies. The extract were screened for the presence of various chemical constituents such as alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids and saponins using standard procedures.

Pharmacological Screening for *In vitro* Antioxidant Activity [13]

Various *in-vitro* models are available for the determining antioxidant potential in reducing the induced disproportionate radical generation, among which DPPH free radical scavenging assay model has been utilized in this study.

DPPH Scavenging Assay

The antioxidant activity of ethanolic extract of leaves of *Acacia eburnean* Willd. was determined by the radical scavenging activity model using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [14]. Briefly, about 0.05 mL aliquot of methanolic solutions of the leaf extract at different concentrations of 20, 40, 60, 80 and 100 µg/mL were added to methanolic DPPH solution (100 M, 2.95 ml) and mixed well. The absorbance of the reaction mixture was measured spectrophotometrically at 517 nm at regular intervals of 30 seconds for 5 min. 0.05 mL methanolic solution of Ascorbic acid was used as the standard for comparison. The percentage level of discoloration of the DPPH solution was used as the indication of the effectiveness of free radical scavenging of the test and standard. The absorbance of methanolic DPPH solution alone at 517 nm was used as the control value.

The percentage radical scavenging behaviour, evaluated as the decrease in DPPH absorbance was calculated as follows, Scavenging activity (in %) = $[(Abs_0 - Abs_t)/Abs_0] \times 100$



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Where,

Abs₀ = absorbance of the Control

Abs₁ = absorbance of the Test/Standard sample

RESULTS AND DISSUCTION

Preparation of Extract of the Plant

Acacia eburnean willd. leaves were gathered, air dried in the shade, grounded into fine powder using mechanical grinder and extracted by continuous hot solvent percolation method using Petroleum ether, Chloroform, Ethanol (99% v/v), Distilled water for 72 Hrs. The percentage yield of various extracts of *Acacia eburnean* willd. were found to be as given in Table No. 01

Preliminary Phytochemical Studies

The extracts of Pet. ether, Chloroform, Ethanol and Aqueous obtained were subjected to phytochemical studies. The extracts were screened for the presence of various chemical constituents. The phytochemical evaluation showed the presence of Flavonoids, Phenolic compounds, Glycosides, Saponins and Carbohydrates in the Ethanolic and Aqueous extracts of the plant (Table No. 02). In the above stated extracts, chloroform, methanol and aqueous extracts showed the presence of same type of constituents. Hence, the ethanolic extract which has the polarity in between was selected for further pharmacological evaluation.

Pharmacological Screening for *In vitro* Antioxidant Activity

The ethanolic solution of DPPH, a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule due to its odd electron, shows a strong absorption band at 517 nm. The radicals formed react with suitable reducing agents and the electrons become paired and the solution loses color stoichiometrically with the number of electrons taken up[15]. Such reactivity has been widely used to test the ability of compounds/extracts acting as free radical scavengers. Antioxidant property of ethanolic extract of *Acacia eburnean* willd. leaves examined using radical scavenging activity of DPPH radical. The tested extract showed potent DPPH radical scavenging activity and the free radical scavenging activity was evident at all tested concentrations of the ethanolic extract. The percentage scavenging activity was observed to be increased with increasing concentrations of extract and the DPPH scavenging potential of the extracts at varying concentration measured were as depicted in Table No. 3 and Fig No. 01. Ethanolic extract of leaves of *Acacia eburnean* willd. exhibited potent *in vitro* anti-oxidant potential studied using DPPH method

CONCLUSION

It is becoming increasingly apparent that the majority of today's diseases are caused by a change in the body's pro-oxidant and antioxidant homeostatic balance. Pro-oxidant conditions predominate either as a result of increased free radical development, excessive oxidative stress in modern life, or inadequate scavenging/quenching in the body as a result of dietary anti-oxidant depletion. As a result, anti-oxidants have become the most commonly prescribed medication today.

Future Aspects

As per the overall study conducted we can conclude that the use of the plant *Acacia eburnean* willd. is much more beneficial for acts synergistic action in prevention of ageing and related degenerative disorders.





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Table No. 01: Extractive Values of Leaves of *Acacia eburnea* willd.

S. No.	Different Type of Fraction	Extractive Yield (in %)
1.	Petroleum ether fraction	5.80
2.	Chloroform fraction	3.80
3.	Ethanol fraction	11.0
4.	Aqueous fraction	6.50

Table No. 02: Phytochemical Evaluation of Extracts of *Acacia eburnea* willd. Leaves

S. N	Constituents	Test	Pet. Ether	Ethanolic	Aqueous
1.	Alkaloids	Mayer's test	-Ve	+Ve	-Ve
		Dragondraff's test	-Ve	+Ve	-Ve
		Hager's test	-Ve	+Ve	-Ve
		Wagner's test	-Ve	+Ve	-Ve
2.	Sterols	Libermann'sburchard Test	-Ve	+Ve	+Ve
		Salkowski's test	-Ve	+Ve	+Ve





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3.	Carbohydrates	Molisch's test	-Ve	+Ve	+Ve
		Fehling's test	-Ve	+Ve	+Ve
		Benedict's test	-Ve	+Ve	+Ve
		Anthrone test	-Ve	+Ve	+Ve
4.	Fixed Oils and Fats	Spot test	-Ve	-Ve	-Ve
5.	Phenolic compound	FeCl ₃	+Ve	+Ve	+Ve
		Gelatin test	-Ve	+Ve	+Ve
		Lead acetate test	-Ve	+Ve	+Ve
6.	Saponins	Foam test	-Ve	-Ve	+Ve
7.	Tannins	Gelatin test	-Ve	-Ve	-Ve
		FeCl ₃ tests	-Ve	+Ve	-Ve
8.	Gums and mucilage	Ppt. with 95% Alcohol	+Ve	-Ve	-Ve
9.	Flavonoids	Shinoda's test	-Ve	+Ve	+Ve
		Conc.H ₂ SO ₄	-Ve	+Ve	+Ve
10.	Glycosides	Molisch's test	-Ve	+Ve	+Ve
11.	Protiens and Amino acids	Biuret test	-Ve	+Ve	+Ve
		Ninhydrin test	-Ve	+Ve	+Ve
		Millon's test	-Ve	+Ve	+Ve

Table No. 03: Free radical scavenging activity of ethanolic extract of AE leaves by DPPH method

S. N	Treatment	Scavenging Activity (in %)				
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
1	Ethanolic extract of AE	67.01 ± 0.35	70.36 ± 0.37	77.13 ± 0.15	89.40 ± 0.23	94.05 ± 0.29
2	Ascorbic Acid	71.50 ± 0.50	76.33 ± 0.33	81.50 ± 0.43	91.17 ± 0.40	95.34 ± 0.20

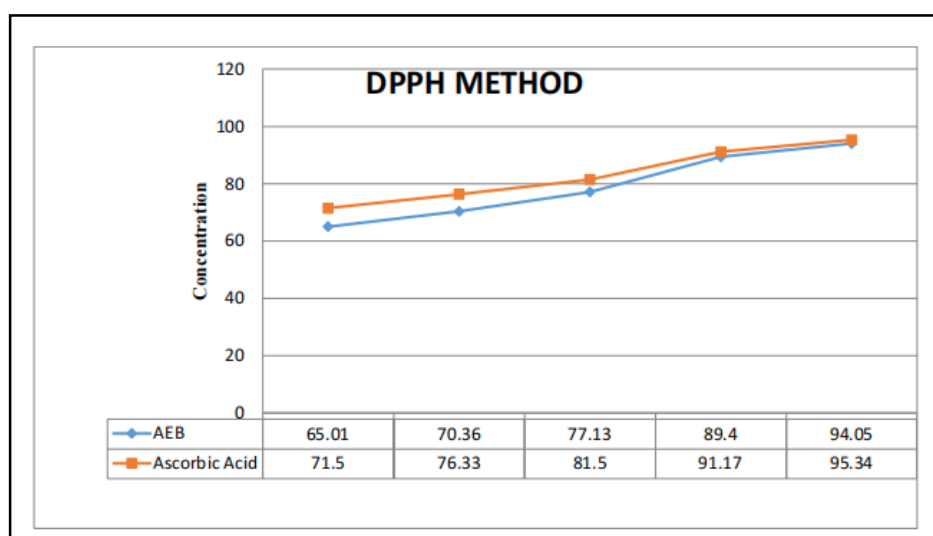


Fig. No. 01: Free radical scavenging activity of ethanolic extract of AE leaves by DPPH method





Ethno Medicinal Exploration of Selected Medicinal Plants of Baijnath Region, Himachal Pradesh

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ABSTRACT

The dependence on traditional medicines in the long run has helped in preserving the knowledge in the form of founded traditional health care systems like Ayurveda, Tibetan, Chinese, and Unanni. Extensive field visits were undertaken to various localities and areas of the Baijnath to collect desired information on ethno-medicinal aspects. In present study total 20 medicinal plants were documented having 18 families and 20 genus and species. Observation shows that 09 plant parts i.e., Root, Leaf, Seed, Aerial part, Fruit, Latex, Whole plant, tuber and Rhizome were used by local inhabitants while total 07 mode of use i.e., decoction, Direct, fluid, juice, powder, paste and secretion were recorded. With the help of extensive survey and through interview documented 12 major health issues i.e., whooping cough, toothache, stomach ache, eye problem, kidney stone, urinary infection, joint pain, mouth ulcer, liver problem, wound healing, skin problem and diabetes for which local inhabitants are using these medicinal plants.

Keywords: Ethnomedicinal plants, Baijnath , Traditional knowledge, Documented

INTRODUCTION

The Himalayan traditional knowledge is one of the oldest, distinguished, sinuous, well maintained depositories which have played a major role in healing diseases prevalent among the Himalayan folks (Palni and Rawal, 2010). The dependence on traditional medicines in the long run has helped in preserving the knowledge in the form of founded traditional health care systems like Ayurveda, Tibetan, Chinese, and Unani (Bhattarai, 2010). Still, 70% of the health care needs are fulfilled through village based health traditions carried out by housewives, birth attendants and traditional healers (e.g., vaidyas, hakims, and amchis) (Pei *et al.*, 1987). With time, the traditional medicines have gained importance not only at local level, but with the onset of many incurable diseases, medicinal plants and their



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healing properties are being used in modern medicines and are considered as a crucial source in many formulations (Wadkar *et al.*, 2008). Various research were also recorded by Samant *et al.*, 2006, Upreti *et al.*, 2020a, 2020b, About 80% of medicinal plants derived compounds show positive correlation between modern remedies and the traditional uses (Fabricant and Farnsworth, 2001).

METHODOLOGY

Description of Study Site

The area selected for the study was Baijnath region lying in the lap of Dhawaladhar Range of District Kangra of Himachal Pradesh (Western Himalaya), ranges widely from 550m to 5000m. The study area nestles between 31°57'21"N to 32°27'21"N Latitude & 76°34'11"E to 77°80'23"E longitude & surrounded by Chamba, Lahul-Spiti, Kullu & Mandi Districts of Himachal Pradesh.

Data Collection

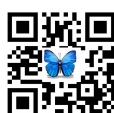
Extensive field visits were undertaken to various localities and areas of the Baijnath. Desired information on ethno-medicinal aspects was collected through interviews from knowledgeable people as per method outlined by Jain (1987, 1989, 1991).

RESULTS

For the present study total 20 medicinal plants were documented having 18 families and 20 genus and species. Observation shows that 09 plant parts i.e., Root (31.8%), Leaf (31.8%), Seed (4.5%), Aerial part (4.5%), Fruit (4.5%), Latex (4.5%), Whole plant (4.5%), tuber (9 %) and Rhizome (4.5 %) were used by local inhabitants (Fig 1) while total 07 mode of use i.e., decoction (25%), Direct (5%), fluid (5%), juice (5%), powder (30%), paste (25%) and secretion (5%) were recorded (Fig 2). With the help of extensive survey and through interview documented 12 major health issues i.e., whooping cough, toothache, stomach ache, eye problem, kidney stone, urinary infection, joint pain, mouth ulcer, liver problem, wound healing, skin problem and diabetes for which local inhabitants are using these medicinal plants. (Table 1)

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Table 1: Ethno-Medicinal Description						
S. No	Botanical name	Local Name	Family	Part used	Mode of utilization	Ethnomedicinal uses
1	<i>Abrus precatorius</i> L.	Chadannu, ratti, rattak	Leguminosae	Roots. Leaves. Seed.	Powder, paste, decoction	½ teaspoon of powdered roots given with honey once daily for 5 days to cure whooping cough
2	<i>Achillea millefolium</i> L.	Birni	Compositae	Leaves		Leaves are chewed to get relief from toothache
3	<i>Achyranthes aspera</i> L.	Puthkanda	Amaranthaceae	Leaves, Root, Seeds	Juice, powder	Gargles of its juice good for throat infection
4	<i>Aconitum heterophyllum</i> Wall. Ex	Patish	Ranunculaceae	Root	powder	Powered dried roots are given to cure fever & stomach ache early morning empty stomach
5	<i>Berberis aristata</i> DC.	Kashmal	Berberidaceae	Fruits	Decoction	a traditional preparation made to cure eye problems; it is prepared by reducing the decoction of sun-dried roots of the plant
6	<i>Bergenia ciliata</i> (Haw.) Sternb.	Patharchat, pakhanbhed	Saxifragaceae	Roots & Leaves	Decoction	Juice & powder of the plant is given empty stomach early to cure kidney stones and urinary infection
7	<i>Boerhaavia diffusa</i> L.	Eat-seat	Nyctaginaceae	Root, Stem Leaves, Whole plant	Paste	Paste of the plant prepared in 'til' oil (<i>Sesamum indicum</i> Linn) which is good for joint pains
8	<i>Calotropis procera</i> (Aiton) Dryand.	Aak, ak	apocynaceae	Latex	Secretion	Latex is used in very small quantity to cure the infection of teeth
9	<i>Centella asiatica</i> (L.) Urb.	Meenki	Umbelliferae	Leaves	Direct	Leaves are consumed empty stomach to boost memory
10	<i>Clematis gouriana</i> Roxb. ex DC.	Baaker bel	Ranunculaceae	Leaves	Fluid	Mucilagenous fluid used as gum and also for curing mouth ulcers





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11	<i>Cuscuta reflexa</i> Roxb.	Amarbel, gasbel, akashbel	Convolvaceae	Aerial plant parts	decoction	5-10ml decoction of the aerial plant (twice daily for 3 days) checks dysentery
12	<i>Dactylorhiza hatagirea</i> (D.Don) Soó	Salampanja	Orchidaceae	Tuber	Powder	Tubers are grind with milk and consumed to increase strength and stamina
13	<i>Micromeria biflora</i> (Buch.-Ham. ex D.Don) Benth.	Jungle- ajwain	Lamiaceae	Leaves	decoction	Decoction is made to cure cough & cold
14	<i>Picrorhiza kurroa</i> Royle ex Benth.	Kutki	Plantaginaceae	Roots, Rhizome	Decoction	Decoction is prepared with chirayata, ajwain and fennel seeds, taken early morning empty stomach to cure liver problems & detoxification
15	<i>Plumbago zeylanica</i> L.	Chitra	Plumbaginaceae	Leaves, Root	Paste	Extr. of leaves and root mixed with mustard oil applied for relieving rheumatic pain but care should be taken as excessive use can cause white marks on skin
16	<i>Rheum australe</i> D. Don.	Chukri	Polygonaceae	Roots, Leaves	paste	Roots are dried and preserved, paste of root with other herbs applied on cut and wounds, that heals fast
17	<i>Rhododendron campanulatum</i> D.Don.	Kashmiri patha	Ericaceae	Leaves	Powder	Leaves powder is taken in small quantity to treat cold and headache
18	<i>Rumex nepalensis</i> Spreng.	Jangli palak	Polygonaceae	Leaves	paste	Applied over boils or over wounds to treat it
19	<i>Stephania glabra</i> (Roxb.) Miers	Bis- khapar	Menispermaceae	Tubers	paste	Paste of tuber is mixed with fodder given to cattle to treat skin problems
20	<i>Syzygium cumini</i> (L.) Skeel	Jamun	Myrtaceae	Leaves, Fruit, Stem	powder	Dried seeds powder is used to treat diabetes





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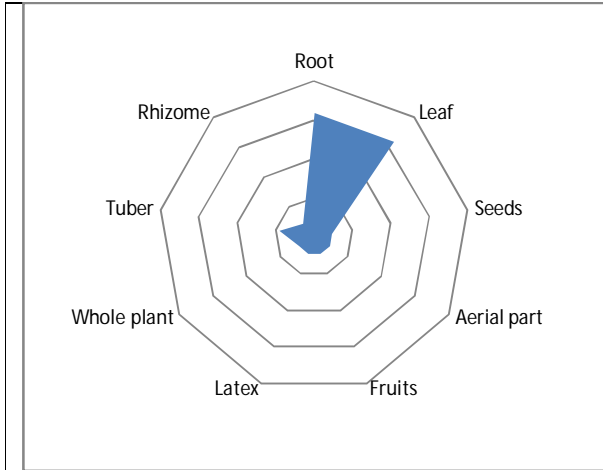


Fig 1: Graphical presentation of Plant part used for medicinal purpose.

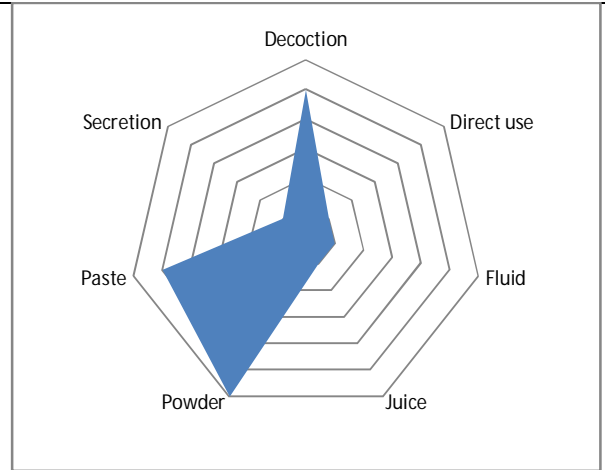


Fig 2: Graphical presentation of mode of utilization for medicinal purpose.





Extraction of Iron Ore Deposits from Kavuthimalai and Vedyappanmalai in Tiruvannamalai District using Landsat ETM+ Satellite Image

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ABSTRACT

The Archean granulite-gneissic terrain of south India mainly composed of Banded Iron formations. In this study, spectral remote sensing and digital image processing of Landsat ETM+ satellite image, technique is used to identify the iron ore deposits of Kavuthimalai and Vedyappanmalai located in Tiruvannamalai district. Banded iron formations were extracted by various advanced image processing techniques such as color composites, band ratios (2/1, 3/1, and 4/1), and PCA (principal component analysis) was used to highlight the areas of iron ore deposits. Hence, from the mapping results, the image processing techniques can be used to explore and characterize the iron ore deposits of study areas with limited field checks.

Keywords: Spectral remote sensing, Landsat ETM+, Iron ore deposits, Kavuthimalai and Vedyappanmalai

INTRODUCTION

In India, iron formations were designated as banded Magnetite and Hematite. The banded iron formations occur in high grade granulite terrain and greenstone terrain of south India in the states of Tamil Nadu, Kerala, Karnataka and Andhra Pradesh. In Tiruvannamalai district, banded magnetite quartzite with hematite is found in Kavuthimalai and Vedyappanmalai. The banded Iron formations are associated with charnockite, granitic gneiss and pyroxene granulites. The Iron ore band is being investigated by the State Geology Department with the aid of the U.N.D.P (Madras mineral project). The bands of iron ore occur in three detached synclinal basins on either side of Tiruvannamalai-Kanchipuram road with converging dips of 65° to 80°. According to the state geology department of Tamil Nadu, the inferred reserves are about 60 million tonnes. In recent, remote sensing techniques used to identify and mapping surface minerals. Abulghasem et al. (2011) have studied the integrated data of remote sensing and geophysical data for Iron ore exploration. There are numerous studies of the application of spectral remote



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sensing techniques in Iron ore exploration. Spectral domain of short wave infrared (SWIR) part of the electromagnetic wavelength for detection of iron oxide alteration is one of the most important usages of geological remote sensing (Abrams et al. 1983; Sabins 1999). In recent years, the iron ore minerals and its alterations can be detected by remote sensing (Rutz-Armenta and Prol-Ledesma 1998; Tangestani and Moore 2001; Hewson et al. 2001). The iron ore exploration and investigations using the geospatial technologies were attempted by Azizi and Saibi (2015) and Mogren (2017). The most reliable mineral detection including iron ore grade estimation was obtained from Landsat ETM+, ASTER and EO-1 Hyperion images (Magendran and Sanjeevi 2014). Several geoscientists have used multispectral, hyper-spectral, and in-situ data to detect the various minerals present in the earth surface through image enhancement, principal component analysis (PCA), spectral analysis and band ratio technique (Gabr et al. 2010; Pour and Hashim 2012). The main aim and objective of the paper is to utilizing ETM+ satellite image for identification of iron deposits and prediction and proposition the Iron-rich areas and prospects in kavuthimalai and Vedyappanmalai. We performed some algorithms for image enhancements such as color composite, band ratios and PCA analysis were used to determination iron alteration zones using ENVI 4.7 image processing software.

About the study area

The study area falls in Toposheet no. 57L/15, P/3 and bounded by the latitude of 12°16' – 12°19' North latitudes and 78°57' – 79°02' East longitudes and shown in Fig 1. The total aerial extent is about 28.48 sq.km. The study area is a part of the high grade granulite terrain of south India in Tiruvannamalai district. The banded magnetite quartzite deposit of Kavuthimalai, Vedyappanmalai hills near Tiruvannamalai is one of the potential deposits. The red loamy and sandy soils are predominantly found here. The annual rainfall of the region is around 1033mm.

Geological setting of the study area

In the study area pyroxene granulites, charnockites and banded iron formations (BIFs) are present according to their order of superposition and shown in Fig. 2. The pyroxene granulites and charnockites show sharp contacts with one another. The charnockites have xenoliths of pyroxene granulites, which indicate the former as younger formation. The BIFs deposited after the fault zone was taken place in NE-SW direction and later metamorphosed. The banded iron formations in the western portion of the study area seen to be steeply dipping on the top as basin-like structure. The rock shows distinct banding, due to the occurrence of thin discontinuous ribbons and laminae of quartz alternating with those rich in dark iron minerals (Prasad et al. 2012).

Spectral characteristics of Iron minerals

The reflectance spectrum of a rock depends on the mineralogical composition of its surface, which is usually a mixture of the whole rock mineralogy and weathering minerals. Electronic and vibrational processes in minerals affect the absorption of bands in the visible and short wavelength infrared. The presence of ferrous iron (Fe²⁺) in weathered surface produced absorptions centered at about 0.45 μm, 1.0-1.1 μm, 1.8-1.9 μm, and 2.2-2.3 μm. The ferric iron (Fe³⁺) produces absorptions at about 0.65 μm and 0.87 μm (Abrams et al. 1988; Rajendran et al. 2011). Goethite has particularly absorption features in the VNIR wavelength region due to electronic transitions of ferric iron (Clark 1999)(Fig 3).

MATERIALS AND METHODS

The distribution of minerals on the Earth surface can be highlighted using different image-processing techniques, which combine Landsat spectral bands, such as band ratios, color composites, principal component analysis, intensity hue saturation, and de-correlation processing (Al Rawashdeh et al. 2006; Madani 2009; Shalaby et al. 2010; Dehnavi et al. 2010).



**Kasilingam et al.****Data used**

Spectral properties are used to detect iron oxide mineral in image data. In general, Landsat TM/ETM+ data have been widely used for mapping Iron-rich deposits because of free download image that can be used for mineral exploration. The Landsat ETM+ with path/row of 143/51 data which is used in this study area was acquired on Feb 12, 2012 with 0% cloud cover. Landsat 7 ETM+ images consists of eight spectral bands with a spatial resolution of 30 meters for band 1 to 7. The panchromatic band 8 has a resolution of 15 meters. All bands can collect one of two gain settings (high or low) for increased radiometric sensitivity and dynamic range, while band 6 collects both high and low gain for all scenes (Table 1).

Iron extraction by band ratio

This method includes dividing two bands on each other. The analyst chooses one wavelength band in which the material is highly reflective (appears bright), and another in which the material is strongly absorbing (appears dark). The band with the most reflection places in the numerator and another band with the most reflection places in the denominator. The resulting values in a ratio image the black and white extremes of the grey scale represent pixels having the greatest difference in reflectivity between the two spectral bands. Band ratios have been used extensively in mineral exploration and to map vegetation condition. Landsat band ratios like 3/1, 3/5, 3/7, 5/1, 5/4, 3/7, and 5/7 are very useful for the discrimination of lithologies and minerals (Drury 1993).

Iron extraction by color composite

Another basic image enhancement technique is represented by the false color composites (FCC) of ETM+ bands, which are often used to discriminate lithologies (Alessandro et al. 1997; Shalaby et al. 2010). Three selected images are combined as FCCs using primary colors, red, green and blue (RGB) to enhance the differences among areas underlain by different lithologies, or to detect possibly altered rocks (Dehnavi et al. 2010). The production of color composite images is based on known spectral properties of rocks and alteration minerals in relation to the selected spectral bands. For instance, Landsat ETM band 7 is used primarily for mineral and rock discrimination, whereas bands 3 and 4 are primarily used for vegetation monitoring. Spectral analysis exploits spectral properties of rocks in order to interpret lithological variations or rock alterations that are expressed as variations in color intensity values within color composite images. R-G-B FCCs can be created by combining three ratio images (Sabins 1999; Elsayed Zeinelabdein and Albiely 2008).

Iron extraction by principal component analysis (PCA)

Principal Component Analysis (PCA) is often used as a method of image compression. It allows redundant data to be compacted into fewer bands that is; the dimensionality of the data is reduced. The principal component analysis is a multivariate statistical technique that selects uncorrelated linear combinations (eigenvector loadings) of variables. The principal component analysis is widely used for alteration mapping in metallogenic provinces. Through the analysis of the eigenvector values, it allows identification of the principal components that contain spectra information about specific minerals, as well as the contribution of each of the original bands to the components. This technique indicates whether the materials are represented by bright or dark pixels in the principal components according to the magnitude and sign of the eigenvectors loadings. The technique can be applied on ETM and ASTER data (Crosta et al. 2003). FCCs derived from PCA are considered effective for detecting areas containing by iron deposits (Loughlin 1991). Bands 1, 3, 4, and 5 were chosen to map iron ore deposits using the selective PCA because they are the most suitable to detect iron oxides, which exhibit strong absorption in ETM+ Landsat band 1 and high reflectance in bands 3 and 4 (Loughlin 1991).

Data analysis and Results

For iron minerals the reflectance in LANDSAT ETM band 1 (0.45-0.52 μm) is weaker in comparison to band 2 (0.52 – 0.60 μm), band 3 (0.63-0.69 μm), and band 4 (0.77 – 0.90 μm) which is stronger, so these combinations of band ratios are most suitable for discriminating iron oxides. Therefore, the spectral properties of iron alteration minerals in the index, usually, are these band ratios 2/1, 3/1,4/1 (iron oxides), and 5/7 (hydroxyl bearing minerals) are used (Rowan



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et al. 2003; Zumsprekel and Prinz 2000) resulted in the most suitable images to delineate the extent of iron deposits, which appear as bright pixels and shown in Fig 4. The most important color combination for obviating the alteration of iron is RGB (531), which display iron alteration in brown-reddish, brown-red, and orange (Fig 5). Among the possible band combinations, the FCC formed by ratios 3/5, 4/1, and 5/7 in R-G-B resulted in the most useful image to highlight the occurrence of iron deposits (Andrea Ciampalini et al. 2013). Principal component analysis can be used to reduce the dimensionality of the data while minimizing loss of information. The approach for the computation of the principal components analysis comprises the calculation of (i) Covariance (unstandardised PCA) or correlation ((standardized PCA) matrix (ii) Eigenvalues, vectors (iii) PCs. The eigenvector matrix used to calculate PCA for each subset was examined and identified PC contained for the target (mineral) information. The iron oxide has high reflectance values in Landsat ETM image band 1 and 3, and ferrous mineral has high reflectance values in band 4 and 5 in Kavuthimalai and Vedyappanmalai.

PCA analysis is applied for Landsat ETM+ data (Table 2). The figure 6 shows principal component analysis images (PC1 to PC6). PC1 represents albedo and topographic shadowing. PC4 enhances the iron oxide bearing areas (Fig 7) as this PC has higher loadings of band 1 and 3. PC3 enhances the ferrous mineral bearing areas as this PC has higher loadings of band 4 and 5 (Fig 7). The iron oxide can enhance between 400nm-600nm. Principal component analysis is done using six input bands (Table 3). The first principal component does not contain spectral features relevant in this analysis, as it is a combination of all bands. This component contains 98.28% of the variance of six bands. This PC1 gives information mainly on albedo and topography. Analysis of PC4 shows that the most important contributions come from band 1 (0.4171) and band 3 (0.1851). Based on spectral characteristics of iron oxide, it follows that iron oxide will be mapped by bright pixels. Iron oxide image is obtained by using eigenvector loading of PC4. The similar analysis of PC3 shows that the most important contributions come from band 4 (0.4145) and band 5 (0.4380). Ferrous mineral image is obtained by using eigenvector loadings of PC3.

CONCLUSION

The spectral remote sensing and digital image processing of Landsat ETM+ image provided useful information for the extraction of Iron ores in the study area. In this study various image enhancement techniques (i.e., band ratios, color composite (FCC), and principal component analysis) were used to detect and map iron deposits hosted in Archean granulite-gneissic terrain of south India. Band rationing technique with different band combinations were useful for identification of the abundance of iron oxide content present in the study area. Band ratio 4/1 resulted in the most effective image, especially if depicted with the related density slicing as shown for the same. The color composites of band 5, 3, 1 and 3/5, 4/1, and 5/7 in red, green, and blue respectively gives most satisfactory results for identifying iron alterations. Principal component analysis is used to enhance or distinguish lithological differences. Spectral differences between rock types may be more apparent in principal component images than in single bands. This analysis suggests that the PC3 is the most informative PC image because it contains the highest contrast between band 1 and band 4. The eigenvectors value loading confirms that the PC image, containing information regarding the maximum contrast between band 4 and band 3, is the most suitable bands for detecting iron and ferrous oxides is formed in the study area.

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Table 1. Spectral bands of Landsat 7 (ETM+)

	Band number	Spectral range (µm)	Spatial resolution (m)
Visible	1	0.45 – 0.52	30
	2	0.52 – 0.60	
	3	0.63 – 0.69	
NIR	4	0.77 – 0.90	60
SWIR	5	1.55 – 1.75	
TIR	6	10.40 – 12.50	30
MIR	7	2.08 – 2.35	15
PAN	8	0.52 – 0.90	

Table 2. Landsat ETM+ image Principal Component Analysis statistics

Eigenvalues and associated percentages			
Axis	Eigenvalues	Percentages	Cumulative
1	235433904.8485	98.2817	98.2817
2	3217632.7263	1.3432	99.6249
3	710358.9903	0.2965	99.9214
4	95384.7437	0.0398	99.9613
5	66428.6239	0.0277	99.9890
6	26316.3154	0.0110	100.0000

Table 3. Factors scores (eigenvectors) and factor loadings (degree of correlation) of each component from the matrix, Landsat image band 1, 2, 3, 4, 5, 7 of ETM+ data.

----- Eigenvectors -----						
Axis	Band 1	Band 2	Band 3	Band 4	Band 5	Band 7
1	0.4240	0.5274	0.4849	0.3443	0.3239	0.2885
2	0.4606	0.4896	-0.6682	0.0179	-0.1943	-0.2519
3	0.0694	-0.3744	-0.5199	0.4145	0.4380	0.4699
4	0.4171	-0.4595	0.1851	0.4075	0.0625	-0.6407
5	-0.6550	0.3543	-0.0940	0.5322	0.1697	-0.3527
6	-0.0086	-0.0715	0.0689	0.5097	-0.7954	0.3122





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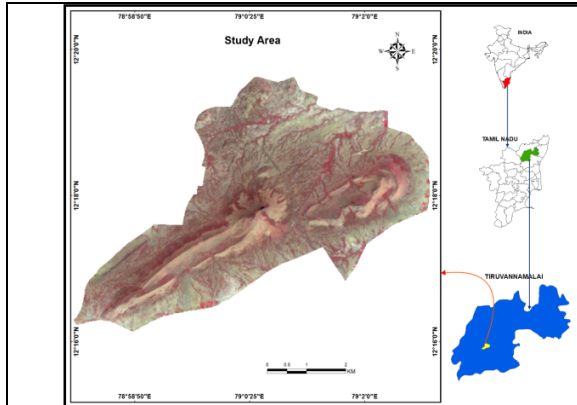


Figure 1 Location map of the study area (Landsat ETM+ image)

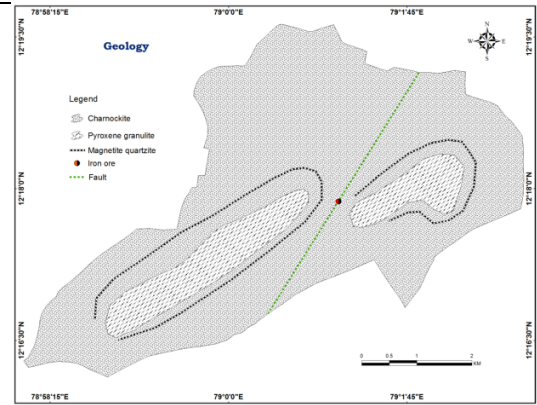


Figure 2 Geological map of the study area

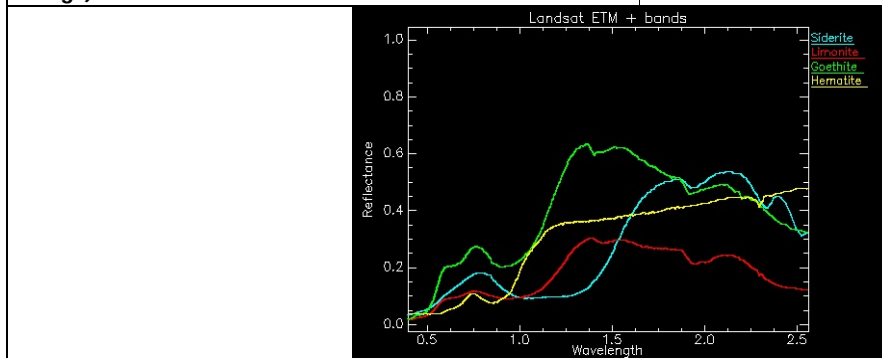


Fig 3 Spectral profile showing the absorptions of Iron ore minerals (Spectra from USGS spectral library)

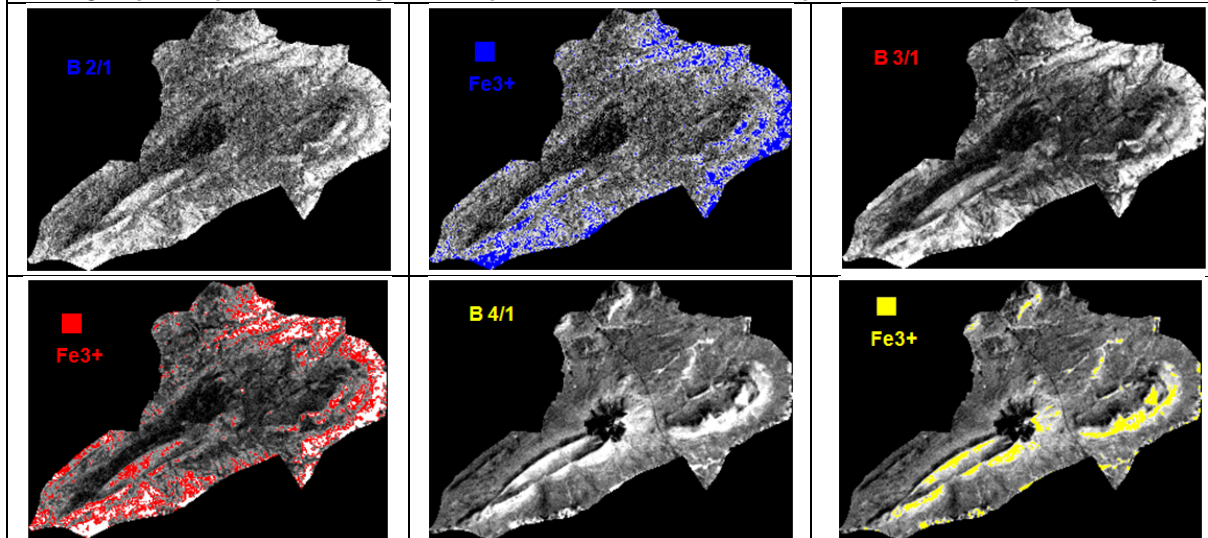


Figure 4. Ratio image of ETM+ bands 2/1, 3/1, 4/1. The presence of iron oxides is depicted in brightest pixels in these images





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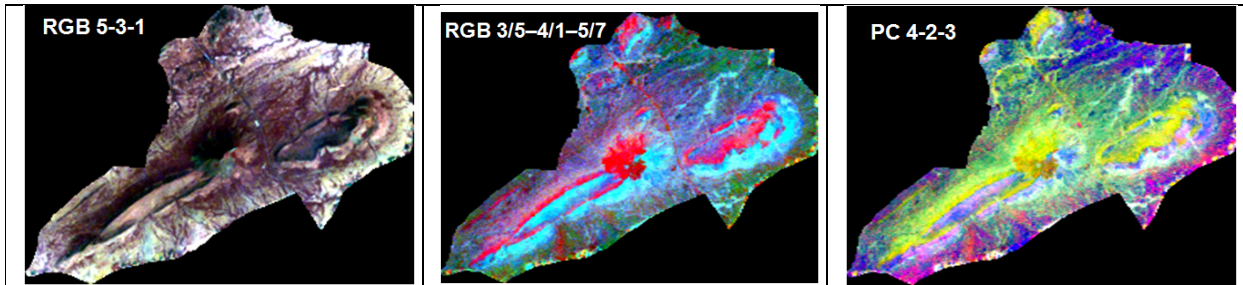


Figure 5. Color composite image of RGB: 531(Iron alterations brown and reddish brown and reddish orange), RGB: 3/5, 4/1, 5/7 (Iron alterations are appear bluish in color) and PC 4-2-3 (Iron alterations appear violet in color).

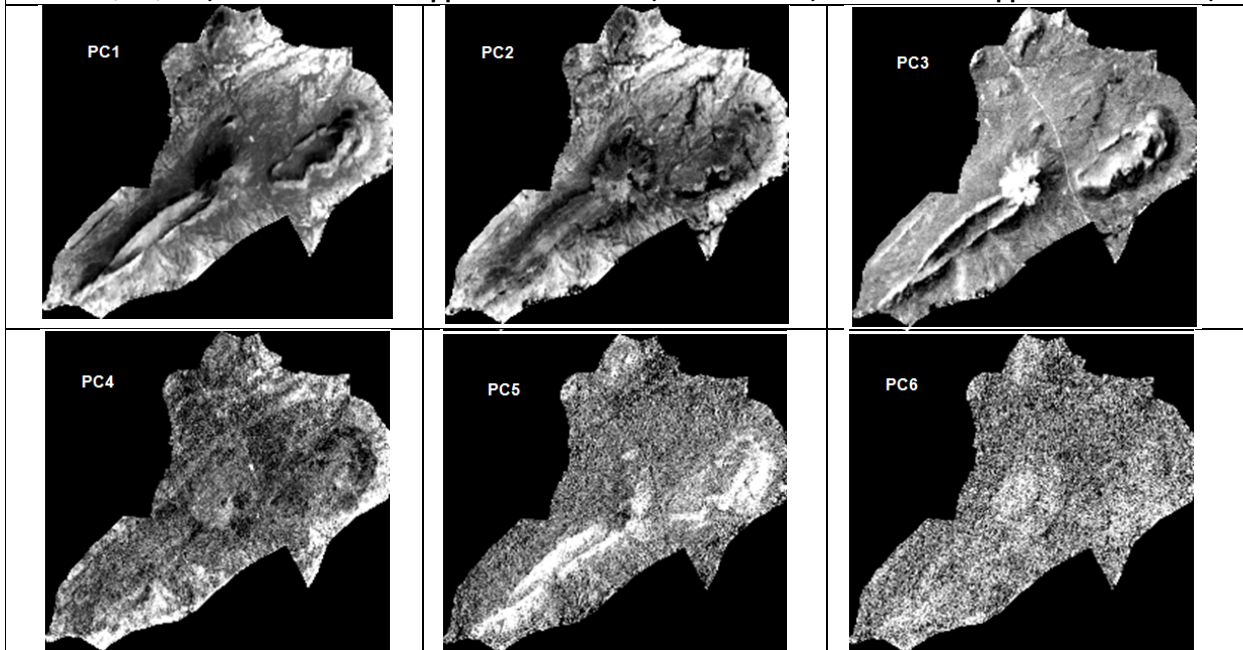


Figure 6. Principal Component Analysis images (PC1 to PC6) of Kavuthimalai and Vedyappanmalai

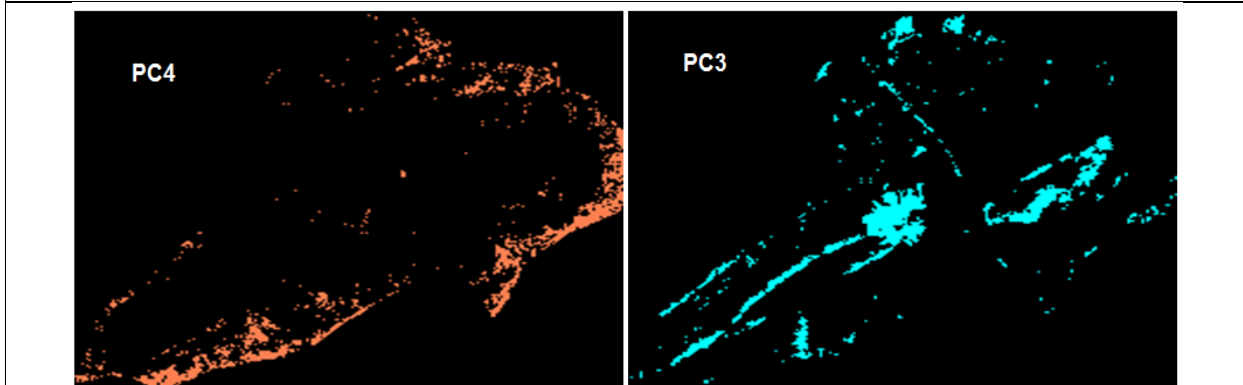


Figure 7. Iron oxide detection on PC4 and ferrous oxide detection on PC3 of Landsat ETM+ image





Thermodynamic, Adsorption and Electrochemical Studies of Mild Steel in 0.5M HCl Solution by using Green Inhibitor

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ABSTRACT

Green inhibitor which is the plant extract of *Abelmoschus esculentus* is used as corrosion inhibitor in controlling the corrosion of mild steel in aqueous medium of 0.5M HCl. The corrosion inhibition efficiency and corrosion rates have been systematically examined by weight loss method, potentiodynamic polarisation technique and electrochemical impedance spectroscopy (EIS). Maximum reached inhibition efficiency is 95.7% for 10 % *Abelmoschus esculentus* plant extract is determined by weight loss method. The inhibition efficiency of *Abelmoschus esculentus* on corrosion of mild steel in 0.5M HCl solution increases on increasing in its concentration and decreases with rise in temperature. This plant extract act as cathodic type inhibitor which has been confirmed by Potentio dynamic Polarization measurement. The increase in activation energies of corrosion process in presence of the extract indicates that *Abelmoschus esculentus* extract retarded the rate of corrosion of mild steel in 0.5M HCl solution. The nature of adsorption of the extract on mild steel surface was in conformity with Langmuir isotherm, temkin isotherm and Arrhenius parameters. The surface protective film is formed on the surface of mild steel is confirmed by Electrochemical studies of EIS. The surface film has been characterized by FTIR. Smoothness and roughness of the film have been analyzed by Scanning electron microscopy (SEM) and Atomic force microscopy (AFM).

Keywords: Acid Corrosion, Acid inhibition, AFM, EIS, Weight loss method, Mild steel and Plant extract.





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INTRODUCTION

Corrosion is defined as devastation of materials by its chemical or electrochemical reaction with their environments. The use of corrosion inhibitors to control the corrosion is one of the most important technique. The study of corrosion inhibition using inhibitor in acidic media is one of the most stimulating areas in the present research, due to its potential applications in industries such as acid pickling, industrial cleaning, acid descaling, oil-well acid in oil recovery and petrochemical processes [1-4]. The ability of a compound to serve as inhibitor is dependent on its ability to form a compact barrier film and/or nature of adsorption on metal surface. The common well-known inhibitors are organic compounds containing heteroatoms, such as O, N, S and multiple bonds [5-7]. Although many synthetic compounds show good anticorrosive properties, most of them are highly toxic to both human beings and environments while working and off [8].

The identified hazardous effect of most synthetic organic inhibitors and restrictive environmental regulations have now made researchers to focus on the need to develop cheap, non-toxic and environmental friendly inhibitors like natural products. The natural product extracts are viewed as an incredibly rich source of naturally synthesized chemical compounds that can be extracted by simple procedures with low cost, and are biodegradable in nature [9]. This area of research is of much importance because in addition to being environmentally friendly and ecologically acceptable, plant products are inexpensive, readily available and renewable source of materials. *Abelmoschus esculentus* is used in the treatment of certain diseases such as catarrhal infections, dysuria and gonorrhoea. The seeds are antispasmodic, cordial and stimulant. An infusion of the roasted seeds has sudorific properties[10]. But its use in the field of corrosion is new and it is used to inhibit the corrosion of mild steel immersed in acid. So Plant extracts have been used as green inhibitors which control the corrosion reaction.

The present study is to evaluate the inhibitive influence of *Abelmoschus esculentus* leaves extract on the mild steel corrosion in 0.5M HCl using weight loss, and electrochemical techniques (OCP, potentiodynamic polarization, EIS). The protective film is formed on the mild steel surface is characterized by FTIR spectra. The obtained results will be confirmed by surface morphology analysis of the surface layer of the mild steel coupons using SEM and AFM instruments.

MATERIALS AND METHODS

Mild steel specimens; (27.36 % O, 8.23 % C and the rest iron) of dimensions 1.0 cm × 4.0 × 0.2 cm were polished to mirrors finish and degreased with acetone and used for weight loss method. The solution (0.5M HCl) was prepared by dilution of an analytical grade hydrochloric acid with double distilled water.

Inhibitor Preparation

Double distilled water and analytical reagents-grade (E Merk, India, AR Grade) were used for preparing solutions. *Abelmoschus esculentus* was dried for 6 hours in an oven at 70°C and grinding to powdery form and 10 grams of the powder of *Abelmoschus esculentus* was refluxed in 100 ml double distilled water for 1 hour. The extract of the plant was prepared by evaporating the filtrate. The required concentrations of solution were prepared by using the residues in aqueous solution of 0.5M HCl[11-14].

Phyto -Constituents of *Abelmoschus esculentus*

Phytochemicals which are secondary metabolites of plants are: tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenols which are accountable for their biological aspects given by such as antimicrobial, antidiabetic, anti-inflammatory, antibacterial, antioxidant activity. Previous phytochemical studies indicated that the main components that were isolated from the flower of *A. esculentus* are flavonoids. The flavonoids that are isolated from the flower of *A. esculentus* include quercetin-3'-O-glucoside, hibifolin, isoquercetin, quercetin-3-O-





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robinobioside, hibifolin, myricetin, and quercetin [15-16]. Okra plays an important role in the human diet by supplying carbohydrate, minerals and vitamins. K, Na, Mg and Ca were found to be the principle elements, with Fe, Zn, Mn and Ni also present [17]. Okra seeds could serve as alternate rich sources of protein, fat, fiber and sugar. The natural phenolic content of okra seeds has been reported [18].

Weight Loss Method

Among many experimental methods available to determine the percentage inhibition efficiency and corrosion rate, weight loss method is the simplest and frequently used method. In this study, the experiments were carried out by varying the concentrations of the inhibitor. The study was also carried out at different temperatures and the immersion period was fixed as 3 hours. The weight loss calculated, in grams, is the difference between the weight of metal coupon before and after immersion in corroding solution. The results obtained are discussed to know the effect of concentration

$$IE = 100[1-(W_2-W_1)] \% \longrightarrow (1)$$

Where W_1 is the mass loss in the absence of inhibitor and W_2 is mass loss in the presence of inhibitor and temperature [19]. The inhibition efficiency increases due to the inhibitor molecules present in plant extract getting adsorbed on the metal surface [20]. The maximum inhibition efficiency and the lower corrosion rate is found at high concentration 10% v/v for all inhibitors. With further increase in inhibitor concentration above 10%, the inhibition efficiency and corrosion rate almost remained constant. So the 10% concentration is fixed as the concentration for maximum inhibition for inhibitors. This concentration corresponds to the attainment of a saturation value in surface coverage of metal [21]. The comparison of performance of inhibitors show that the maximum efficiency and have low corrosion

Effect of Temperature

Temperature effect would be high on mild steel corrosion in acid medium. Inhibitor efficiency depends on temperature was used to determine activation energy (E_a) of corrosion of the mild steel in the presence and in the absence of the inhibitors. It was used to identify the mechanism of the action of inhibitor on mild steel corrosion. The temperature factor is most important parameter in corrosion studies and also the effect of the temperature on the inhibitive nature is examined by weight loss method. The study of temperature effect on corrosion concentrations of the inhibitor in the temperatures 303K for the immersion period of 3 hours have been adopted [22].

Adsorption Isotherm

Phyto-constituents present in the extracts are possibly adsorbed on the mild steel surface and the degree of adsorption depends on, the temperature and the electrochemical potential at the metal solution interface [23]. Adsorption isotherms provides details about the interaction among the adsorbed molecules among themselves and also their interactions on the metal surface. The degree of surface coverage (θ) with respect to different concentrations of the inhibitor and different temperatures have been determined to deduce the fittest isotherm. The experimental data were fitted to the following two isotherms.

Langmuir isotherm – Plot of $\log(C/\theta)$ Vs. $\log C$

Temkin isotherm – Plot of θ Vs. $\log C$

Electrochemical Methods

Potentiodynamic Polarization Study

Polarization experiments were performed in Princeton Applied Research versa CHI 660 A , an electro-chemical workstation impedance analyzer, using three cell assembly systems. The working electrode was a zinc alloy, with the remaining part shielded with red lacquer on one side of the electrode exposed to 1cm². The reference electrode was a saturated calomel electrode (SCE) and the counter electrode was used with a rectangular platinum foil. Compared to





the operating electrode, the field of the counter electrode was considerably greater. The counter electrode should have a uniform potential field. In the absence and presence of an inhibitor, the working electrode and the electrode platinum is immersed in 0.5 M Hydrochloric acid [24-26]. A salt bridge was used to link saturated calomel electrode to the test solution. Plots (I) have been reported for possible (E) Vs log current plots. E Vs Log I plots have determined the outcomes, such as the propensity for corrosion (E_{corr}) and I_{corr} , Tafel Paths b_a , b_c .

AC Impedance Measurements

Studies of AC impedance is performed at the Princeton Advanced Research versa CHI 660 A electro-chemical workstation impedance analyser model. The set-up of the cell was similar to that used to calculate polarisation. For that device to reach a continuous open circuit capacity a time interval of 5 to 10 minutes was given. Then an A.C regarding the stable potential of the economy, super posed capacity of 10 mV. The actual part (z') and the picture part (z'') of cell impedance were calculated in ohmics for different frequencies from a frequency of 100 KHz up to 100 MHz. Calculation has been made of the importance R_t (charge transfer resistance) and C_{dl} (double capacities). The following relationship was used to measure C_{dl} values [27-28].

$$C_{dl} = \frac{1}{2 \times 3.14 \times R_t \times f_{max}}$$

Surface Characterization

FT-IR spectral Analysis

Perkin Elmer FT –IR spectrophotometer was used to record the FT-IR spectrum from 4000 to 400 cm^{-1} . The adsorbed plant leaf extract inhibitor on metal surface have been analyzed by FT-IR spectra [29]. After it was scratched from the mild steel surface, which was immersed in 0.5M HCl in the presence of the studied plant leaf extract inhibitors for 3 hours at room temperature.

Scanning Electron Microscope (SEM)

It was used to examine the difference in the nature of metal surface before and after the metal surface is in direct contact with the corrosive solution and to observe the effect of the addition of the inhibitor [30]. Thus SEM was used to analyze the topography of the mild steel surface after corroding in presence and absence of the inhibitor. The SEM image was taken by the SEM instrument, JEOL MODEL JSM 6390.

Atomic Force Microscopy (AFM)

Surface morphology was also characterized by using an atomic force microscope. After the inhibition test, the mild steel specimens were placed in vacuum desiccators, mounted on sample holder under the objective of the atomic force microscope and the 3D -images were taken from the 100 × magnified surface through operating program on computer. The surface of mild steel specimens after immersed in 0.5 M H_2SO_4 solution in the absence and presence of aqueous leaf extract of *Abelmoschus esculentus* for two hours were evaluated by atomic force microscopy analysis [31]. From the AFM cross sectional 3D –image, the line and surface roughness parameters of the specimens such as R_a , R_q and peak to valley value were obtained for the mild steel after immersion in 0.5M HCl in the absence and presence of the inhibitor.

RESULTS AND DISCUSSION

The corrosion rates (CR) and inhibition efficiencies (IE) of mild steel immersed in a 0.5M HCl in the absence and presence of the extract of the extract of *Abelmoschus esculentus* inhibitor obtained by weight loss method. The corrosion parameters values are given in Table.1. It is observed that 10% of the extract of the extract of *Abelmoschus esculentus* offers 95.3% of inhibition efficiency in an aqueous solution of 0.5M HCl. It is observed from Table 1 that as





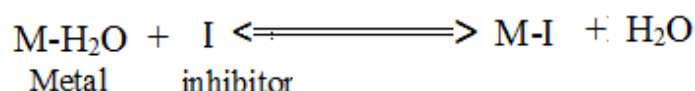
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the concentration of the extract of *Abelmoschus esculentus* increases, the IE increases. This is due to an increase of surface coverage at higher concentration of the *Abelmoschus esculentus* which retards dissolution of mild steel [32]. The possibility of interaction between the hetero atoms present in the plant leaf extract and metal ion from the metal surface can be attributed for higher inhibition efficiencies. The presence of many phytochemical constituents in the plant extracts are responsible for the inhibition of mild steel corrosion. There may be the reasons for the anti-corrosive actions of plant extracts. This surveillance is in good agreement with the results reported by many researchers [33].

Adsorption Isotherm

An adsorption isotherm gives the direct relationship between the corrosion inhibition efficiency with the degree of surface coverage at constant temperature for different concentrations of inhibitor solutions. The adsorption isotherm provide the basic information about the nature of interaction between the mild steel surface and inhibitor molecular constituents [34].

Adsorption of the corrosion inhibitor molecules occurs on the mild steel surface by the displacement of molecule of water adsorbed on metal surface.



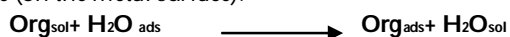
Also, the adsorption depends on the temperature, chemical composition and concentration of inhibitor and the electrochemical potential at the metal – solution interface. There are several isotherms proposed to account for the adsorption of the corrosion inhibitor molecules on the surface of metal. From the isotherm, the linear relationship between θ and concentration of inhibitor can be found. In this study, the changes in the θ and thereby the change in the efficiency of inhibitor is determined by using different isotherms model [35]. Adsorption parameters obtained from Langmuir and Temkin adsorption isotherms for the corrosion inhibitive effect of aqueous leaf extracts of *Abelmoschus esculentus* on the corrosion of mild steel in 0.5 HCl are given in table 2 & 3 and graphs are shown in figure 1 & 2 respectively.

Temkin Adsorption Isotherm

The Temkin adsorption isotherm is based on the assumption of uniform distribution of the inhibitor (monolayer) on the metal surface. The adsorption energy linearly decreases with the increase of surface coverage values (θ). The values of surface coverage θ , at various concentrations of each inhibitor in 0.5M HCl solution, obtained from mass loss measurements, were fitted to Temkin adsorption isotherm shown below [36].

$$\begin{aligned} \exp(-2a\theta) &= K_{\text{ads}} \cdot C \\ \theta &= -2.303 \log K_{\text{ads}} / 2a - 2.303 \log C / 2a \end{aligned}$$

Where, “a” denotes the lateral molecular interaction parameter. The adsorption of organic molecules at a metal-solution interface is a quasi-substitutional process between the inhibitor molecule (in aqueous solution) and water molecule (on the metal surface).



Where, X is the size parameter and it represents the number of adsorbed water molecules replaced by the given adsorbate (inhibitor molecule). The plot of θ vs. $\log C$ for the mild steel in 0.5M HCl in the presence and in the absence of plant extract inhibitors *Abelmoschus esculentus* are given in the [Fig 2] for various temperatures.

Activation parameter for corrosion inhibition process

Energy of activation (E_a)

The energy of activation for the corrosion of mild steel in 0.5M HCl was determined using the Arrhenius type of plot using the following equation [37]

$$\log CR = K \exp(-E_a/RT)$$

where,

CR = corrosion rate,





R = universal gas constant,

E_a= energy of activation,

T=absolute temperature

And K= Arrhenius pre-exponential constant. The values of E_a, is calculated for mild steel in the presence and in the absence of all studied inhibitors *Abelmoschus esculentus* from the slope value of **logCRVs.1/T** plots [Fig. 4, 5] and the E_a values are shown [Table -4].

Electrochemical Analysis

The electrochemical measurements extend a way for calculating the rate of corrosion of a mild steel. It permits quick evaluation of the performance of inhibitor, durability of surface film and also the rate of corrosion [38]. The following techniques were used for mild steel corrosion in 0.5M HCl in the absence and in the presence of the studied inhibitors, *Abelmoschus esculentus* to know whether they are cathodic or anodic or mixed type inhibitor and also to formulate an appropriate mechanism for their inhibition action on corrosion.

Potentiodynamic Polarization Method

Polarization study has been used to confirm the formation of protective film on the mild steel surface during corrosion inhibition process. If a protective film is formed on the mild steel surface, the linear polarization resistance values (LPR) increases and the corrosion current value (I_{corr}) decreases and corrosion potential increases (E_{corr}) [39]. The potentiodynamic polarization curves of mild steel immersed in an aqueous solution 0.5 M H₂SO₄ and in the absence and presence of *Abelmoschus esculentus* are shown in Fig 2. The corrosion parameters are given in Table 2. When mild steel was immersed in an aqueous solution of 0.5M HCl the corrosion potential was -0.454 mV Vs SCE. When *Abelmoschus esculentus* 10% was added to the above system, the corrosion potential shifted to the positive side - 0.512 mV Vs SCE. This indicates that the protective film is formed on the anodic sites of the mild steel surface. This film controls the anodic reaction of mild steel dissolution by forming complex on the anodic sites of the mild steel surface. Further, the LPR value in an aqueous solution of 0.5 M 1.847 × 10⁻⁴ A/cm² to 1.771 × 10⁻⁶ A/cm². Thus polarization study confirms the formation of a protective film on the mild steel surface. Corrosion parameters of mild steel in an aqueous solution of 0.5M HCl and in the absence and presence *Abelmoschus esculentus* system obtained by potentiodynamic polarization method. The Tafel plots, obtained from the potentiodynamic polarization study for the inhibition of corrosion of mild steel in 0.5M HCl by the addition of (10% v/v) of the studied inhibitors *Abelmoschus esculentus* are shown [Fig.6].

Analysis and Electrochemical Impedance Spectroscopy

Nyquist Plots

AC impedance spectra have been used to confirm the formation of protective film on the mild steel surface. If a protective film is formed on the mild steel surface, charge transfer resistance (R_t) increases; double layer capacitance value (C_{dl}) decreases and the impedance log (z/ohm) value increases [40]. The AC impedance spectra of mild steel immersed in an aqueous solution 0.5M HCl in the absence and presence of *Abelmoschus esculentus* are shown in Figures 7 (a & b) (Nyquist plots) and Figures 8 (a & b) (Bode plot). The AC impedance parameters namely charge transfer resistance (R_t) and double layer capacitance (C_{dl}) derived from Nyquist plots are given in Table 6. The impedance log (z/ohm) values derived from Bode plots are also given in Table 6. It is observed that when the inhibitor of *Abelmoschus esculentus* is added, in the medium of 0.5M HCl the charge transfer resistance (R_t) increases from 13.88 Ω cm² to 41.05 Ω cm². The C_{dl} value decreases from 1.1859 × 10⁻⁷ F cm² to 4.012 × 10⁻⁸ F cm². The impedance value [log (z/ohm)] increases from 1.17248 to 1.60376. These results lead to the conclusion that a protective film is formed on the mild steel surface.

Analysis of FT-IR spectral studies

FT-IR analysis helps to identify the absorption bands for the functional groups and the alignment of inhibitor molecules on the metal surface. Many of the researchers have found that FT-IR studies are a major tool that can be



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used to predict the nature of bonding of phytochemical components of the inhibitor on the metal surface [41]. The FT-IR spectrum of *Abelmoschus esculentus* [Figure 9a] and the FT-IR spectral pattern of the adsorbed layer [Figure 9b], scratched from the surface of mild steel after immersion in 0.5M HCl for 3 hours in the presence of higher (10% v/v) concentration of the inhibitor (AELE) at room temperature have been compared. The absorption bands of the functional groups present in corresponding systems are listed in [Table 7].

The FT-IR spectral pattern of crude AELE is shown (Fig.9a) and the protective layer scratched from the surface of mild steel immersed in 0.5M H₂ SO₄ with (10% v/v) inhibitor concentration is shown (Fig.9b). The corresponding frequencies of FT-IR peaks of crude *Abelmoschus esculentus* and of barrier layer are enlisted (Table - 8). The shift at 3466.13 cm⁻¹ to 3416.05 cm⁻¹ can be attributed to the presence O-H stretching. The peaks shifts from 2074.41 cm⁻¹ to 2924.463 cm⁻¹ indicate the presence of C-H bond in both systems [42]. The peaks shifts from 1634.38 to 1636.96cm⁻¹ corresponds to C=O group. The shift at 1383.99cm⁻¹ can be attributed to the presence O-H bending. The peaks at 1355.45cm⁻¹ are assigned to the presence C-N group. The peaks at 1120.86 cm⁻¹ is assigned to the presence C-O group. The peaks at 620.72 are attributed to CH "oop". The new adsorption band frequencies are observed at 473.84 cm⁻¹ in case of adsorbed layer. It may be due to the formation of Fe-complex [43].

Analysis of mild steel surface by Scanning Electron Microscope (SEM)

To understand the surface condition of the studied mild steel specimens, its surface morphology has been examined by scanning electron microscope [44]. In the present evaluation, the SEM micrograph of the polished mild steel specimen in the protected condition has been recorded and it is presented in [Fig. 10a]. In the SEM images, surface of the mild steel specimens is smooth and there is no corrosion product on the surface of the metal. The SEM micrograph of the mild steel specimen, after immersion in the 0.5 HCl for 3 hours at room temperature, is taken and the image is shown in [Fig. 10b].

It reveals the complete destruction or deterioration of the smoothness of the metal surface and formation of corrosion spots on it. Similar reports have appeared in [45]. The image shows that due to the corrosion, the metal surface is highly damaged. The SEM micrographs of the mild steel specimen after immersion period for 3 hours at room temperature in 0.5MHCl solution in presence of inhibitors *Abelmoschus esculentus* leaf extracts at higher concentration (10%) are shown in [Fig. 10c]. It can be said that the inhibitor was absorbed into the mild steel to produce a protective layer to prevent the corrosion process on the surface.

Analysis of Atomic Force Microscopy (AFM)

It is a most important tool to evaluate the morphology of surface for different samples at nano-micro scales. It is used in our study to investigate the effect of corrosion inhibitors on the surface of mild steel in acid medium. The useful parameters observed from the 3D images of AFM analysis, are average roughness R_a, root mean square roughness R_q and peak-to-valley value are noted. Among all the parameters, the average roughness R_a calculated from the average deviation of roughness, for all points from a mean line over the evaluation length, plays an important role in giving an idea about the nature of the protective adsorbed layer on the surface of the metal [46]. The 3D AFM morphologies and the AFM cross sectional profiles of polished mild steel and mild steel after immersion in 0.5M HCl for 3 hours at room temperature, are shown in (Fig.11a and 11b).

The AFM cross sectional images of the mild steel coupon after being immersed for 3 hours at room temperature in 0.5M HCl in the presence of the high concentrations of leaf extracts of *Abelmoschus esculentus* are shown in [Fig-11c]. The different parameters R_q, R_a and R_y from the AFM images of metal surfaces are given [Table – 8] for the polished mild steel and that after immersion in the absence and presence 10% of the inhibitor *Abelmoschus esculentus*. The analysis of values from [Table – 8], indicates that the average roughness R_a parameter is reached very high for the blank. The small average roughness R_a is noticed for the polished mild steel. It is found that the R_a values, after the mild steel is immersed in 0.5M HCl in the presence of inhibitor at high concentration, is in between the blank and polished mild steel. It is lower than that of the blank and higher than that of the polished metal surface and it can be



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inferred that a protective film is formed on the surface of metal [47]. The root mean square roughness, R_q , is the average of the measured height deviations measured from the mean line [48]. For the blank system [Fig. 11a], a few pits in the corroded metal surface is observed and the slight roughness is noted on the polished steel surface [Fig. 11b]. It indicates that the roughness is greater in blank steel coupon compared to the polished metal surface. The measured R_q values of the AELE inhibitor systems *Abelmoschus esculentus* are lower than that of blank and higher than that of polished system. The decrease in the R_q and R_a values reflects that the inhibitor molecules from the plant leaf extract, are adsorbed on the mild steel surface, reducing the rate of corrosion and thereby increasing the efficiency of inhibition.

The values of the maximum peak-to-valley highest (P-V) is actually a largest single -peak-to-valley height in five adjoining sampling heights. The (P-V) height for the mild steel surface corroded in 0.5M HCl is greater than that for the polished mild steel. The surface topography of the metal surfaces, in the presence of high concentrations of inhibitor, show that the (P-V) height is greater for the inhibited system compared to the polished system and less than that of the blank system [49]. Thus the greater surface roughness is observed for the steel coupon immersed in 0.5M HCl, without inhibitor than that of the polished metal coupon. It indicates the mild steel surface becomes rougher in acid medium, without the inhibitor. The roughness probably decreases, due to the protective barrier layer formation on metal surface on the addition of inhibitor and the surface becoming smoother. The parameters derived from the three dimensional AFM morphologies and AFM cross sectional profiles, show that the R_q , R_a and R_y values for inhibitors decreases compared to those of blank studies.

This observations prove that the surface is smoother, in presence of inhibitor due to the layer formation and also the protective film is in nanometer scale of all the inhibitors *Abelmoschus esculentus* in 0.5M HCl. It also proves *Abelmoschus esculentus* has the maximum inhibition efficiency among the five inhibitors. The same trend is observed in weight loss as well as polarization and impedance analyses.

CONCLUSION

The present study leads to the following conclusion based on the above results *Abelmoschus esculentus* plant extract can be used as green corrosion inhibitor in controlling the corrosion of mild steel immersed in 0.5M HCl solution. The higher concentration of inhibitor is the lower corrosion rate and the higher corrosion inhibition efficiency which minimize the corrosion of mild steel. Weight loss experiment shows that maximum inhibition efficiency of the high concentration of inhibitor is 95.25%. Electrochemical studies confirms the formation of a protective film over the mild steel surface. Polarization studies shows the plant extracts *Abelmoschus esculentus* act as anodic inhibitor which control the anodic reaction predominantly. Polarization experiment also shows similar results to that of mass loss experiment. The surface analysis like FTIR spectra have been carried out to confirm the formation of protective film over the mild steel surface. SEM and AFM have been used to observe the microstructure of mild steel in the absence and presence of inhibitor. It showed that the *Abelmoschus esculentus* extract have effectiveness in preventing corrosion of mild steel in 0.5M HCl solution.

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Table -1: Inhibition efficiency of aqueous leaves extract of *Abelmoschus esculentus* on the corrosion of mild steel in 0.5M HCl at room temperature (303K).

Concentration of AELE Inhibitor (ml)	Corrosion rate (mmd)	Inhibition Efficiency (%)
Blank	225.1	-
2	99.9	55.62
4	67.80	69.89
6	32.1	85.74
8	25.0	88.91
10	10.7	95.25

Table-2: Adsorption parameters obtained from Langmuir adsorption isotherm for the corrosion inhibitive effect of aqueous leaf extracts of *Abelmoschus esculentus* on the corrosion of mild steel in 0.5 HCl

Inhibitor system	Temperature, (K)	R ²	Slope	Intercept	K _{ads}	ΔG°_{ads} KJ mol ⁻¹
AE	303	0.9955	0.855	2.030	0.4926	8.335
	313	0.985	0.890	2.480	0.4030	8.088
	323	0.9664	0.920	3.140	0.3185	7.714
	333	0.9889	0.940	4.06	0.2463	7.241





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Table-3: Adsorption parameters obtained from Temkin adsorption isotherm for the corrosion inhibitive effect of aqueous leaf extracts of *Abelmoschus esculentus* on the corrosion of mild steel in 0.5M HCl.

Inhibitor system	Temperature, (K)	R ²	Slope	Intercept	-a	K _{ads}	-ΔG ^o _{ads} KJ mol ⁻¹
Abelmoschus esculentus	303	0.9835	0.5791	0.3757	1.988	4.453	13.882
	313	0.9596	0.5756	0.3106	2.000	3.463	13.686
	323	0.9334	0.5642	0.2516	2.041	2.792	13.545
	333	0.986	0.5695	0.1716	2.022	2.001	13.042

Table -4: Average activation energy for corrosion of mild steel in 0.5M HCl in the absence and in the presence of *Abelmoschus esculentus* leaf aqueous extract(10% v/v)

Inhibitor System	E _a (KJ/mol)
Blank	19.2
AELE	72.7

Table -5: Potentiodynamic Polarization parameters for the corrosion of mild steel in 0.5M H₂SO₄ without and with various concentration of *Abelmoschus esculentus* leaf aqueous extract

Concentration of the aqueous leaf of AE (%V/V)	-E _{corr} , Mv/SCE	Tafel slope		I _{corr} A/cm ²	LPR Ω/cm ²
		ba, Mv/dec	bc, Mv/dec		
0	-0.454	11.577	8.897	1.847 × 10 ⁻⁴	115.0
10	-0.512	12.699	8.040	1.771 × 10 ⁻⁶	118.4

Table -6: Electrochemical impedance parameters from Nyquist plots for the corrosion of mild steel without and with the various concentrations of *Abelmoschus esculentus* leaf aqueous extract in 0.5M HCl

Concentration of the aqueous leaf extract of AE (%v/v)	Nyquist plot		Impedance Log (z/ohm)
	R _{ct} , Ω/cm ²	C _{dl} (μF/cm ²)	
Blank	4.248	6.44793.72 × 10 ⁻⁵	0.8201
10.00	41.05	4.012 × 10 ⁻⁸	1.60376

Table 7: FT-IR spectral data for the aqueous leaf extract of AE and the scratched film from mild steel surface after immersion in 0.5M H₂ SO₄ with 10% *Abelmoschus esculentus*

IR bands of crude plant extract	IR bands of film from mild steel surface	Frequency assignment to functional groups
3435.7	3416.05	-OH
2074.41	29246.48	C-H
1634.38	1636.96	C=O in organic ring
-	1383.99	O-H bending, S=O stretching
-	1355.45	C-N in amides
-	1120.86	C-O
666.39	-	N-H
-	620.72	CH "oop"
-	473.84	Y-Fe ₂ O ₃





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Table - 8: AFM surface and line roughness data for AELE systems

System	Surface roughness			Line roughness		
	Average roughness (Ra), nm	Root mean square roughness (Rq), nm	Maximum peak to valley (P-V) height, nm	Average roughness (Ra), nm	Root mean square roughness (Rq), nm	Maximum peak to valley (P-V) height, nm
Polished mild steel	180.64	222.09	1558.8	178.81	219.84	861.06
Mild steel +0.5 M H ₂ SO ₄	473.91	589.46	4569.7	415.15	562.3	2272.8
Mild steel + 0.5 M H ₂ SO ₄ with 10 % of AELE	143.41	197.60	2766.6	113.92	576.21	276.92

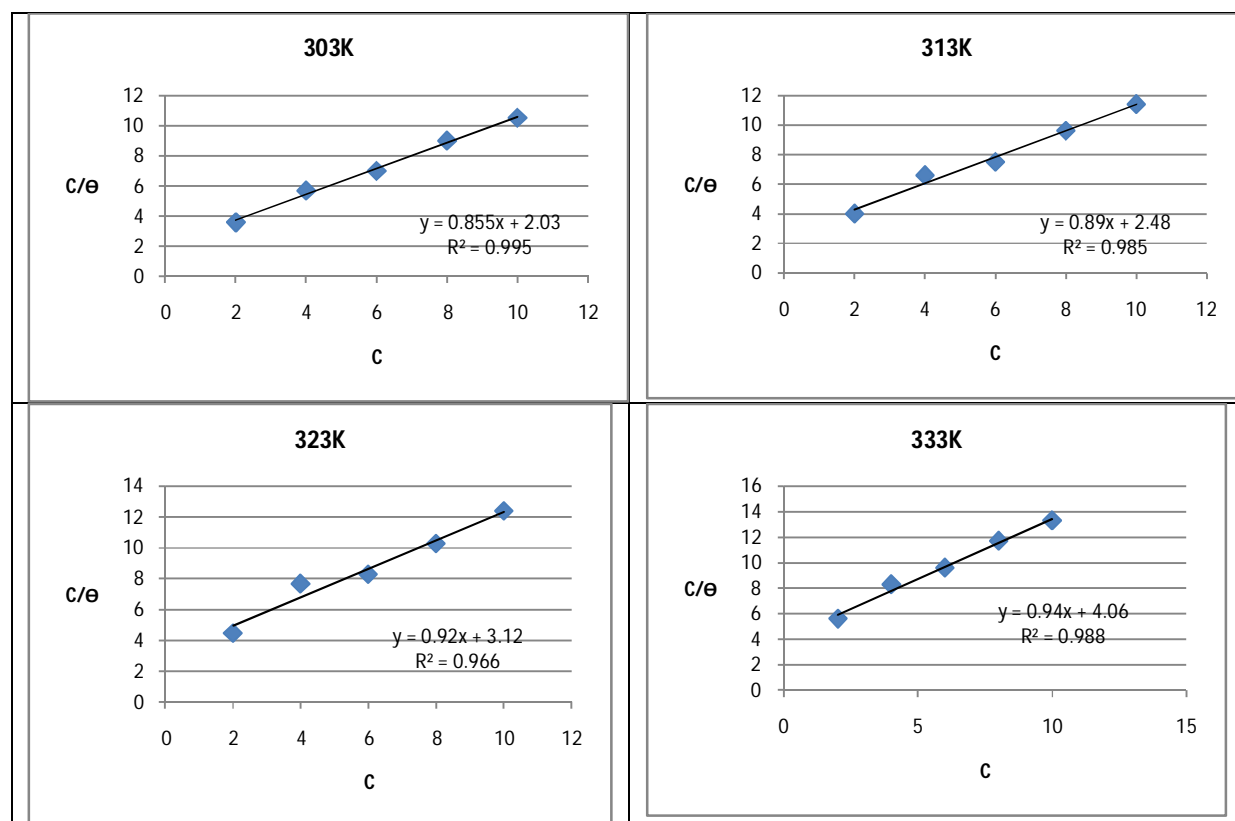
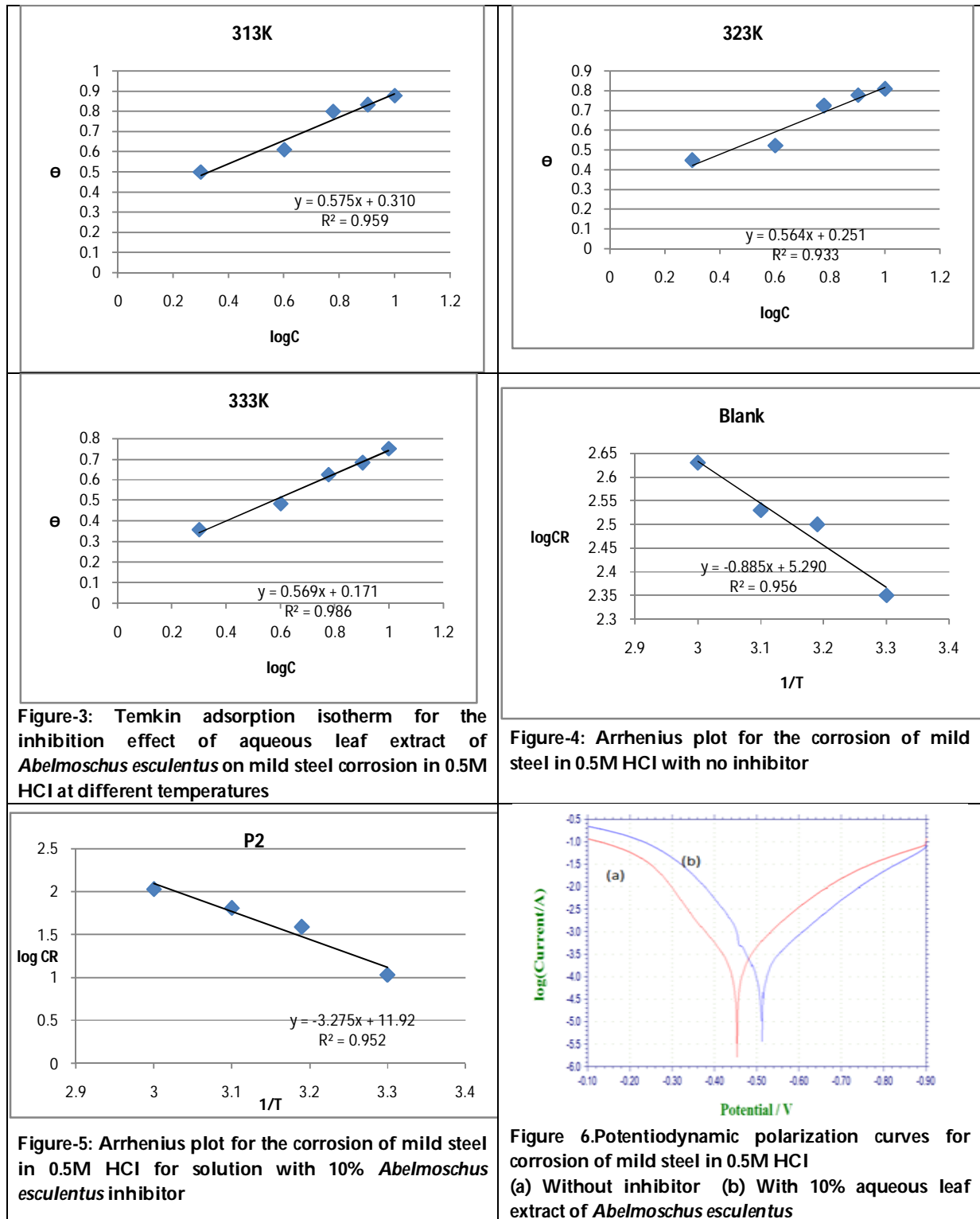


Figure 1. Langmuir adsorption isotherm for the inhibition effect of aqueous leaf extract of *Abelmoschus esculentus* on mild steel corrosion in 0.5M HCl at different temperatures.





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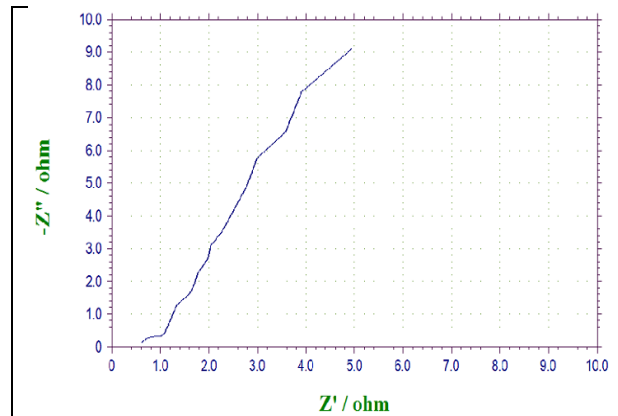


Figure -7a: Nyquist plot for the corrosion of mild steel in 0.5M HCl without inhibitor

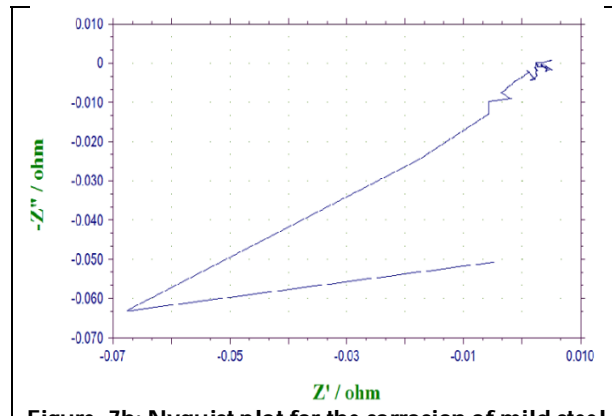


Figure -7b: Nyquist plot for the corrosion of mild steel in 0.5M HCl with 10% aqueous leaf extract of *Abelmoschus esculentus*

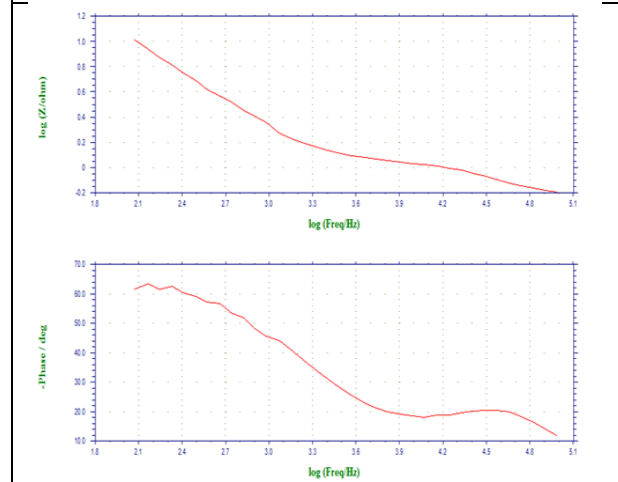


Figure- 8a. Bode plots for the corrosion of mild steel in 0.5M HCl without inhibitor

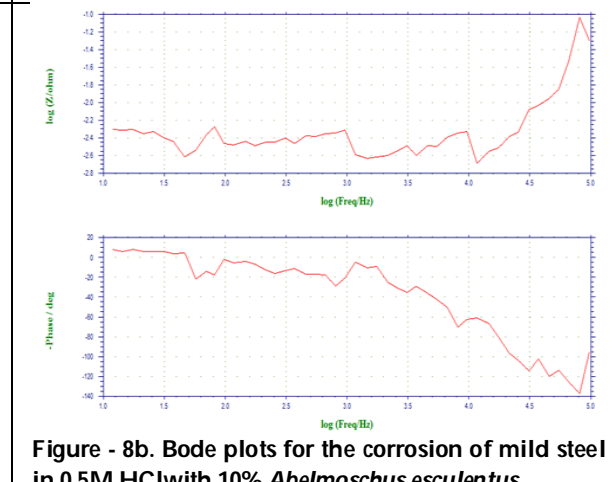


Figure - 8b. Bode plots for the corrosion of mild steel in 0.5M HCl with 10% *Abelmoschus esculentus* Surface Characterization

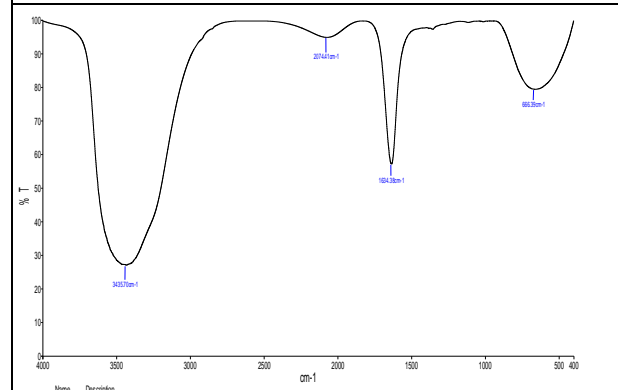


Figure-9a: FT-IR Spectrum of aqueous leaf extract of AE

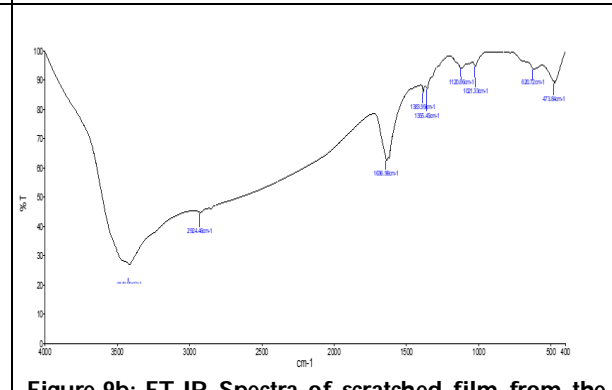


Figure-9b: FT-IR Spectra of scratched film from the mild steel surface after immersion in 0.5 HCl with 10% aqueous leaf extract of *Abelmoschus esculentus*





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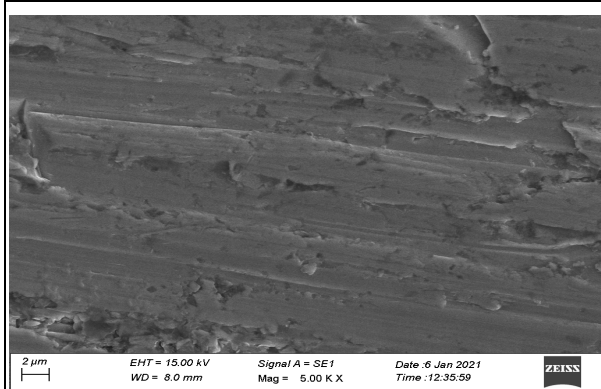


Figure – 10a: SEM image of polished mild steel coupon before immersion in 0.5 HCl

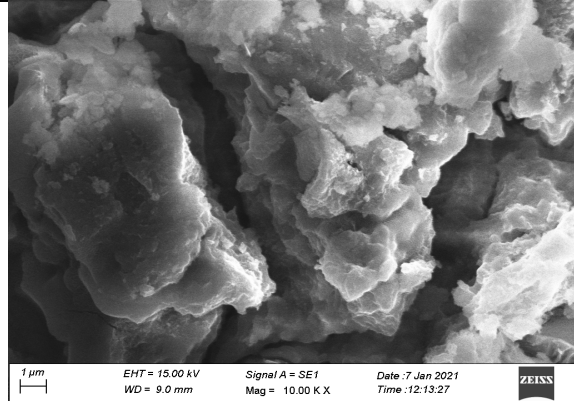


Figure – 10b: SEM image of mild steel coupon after immersion in 0.5M HCl

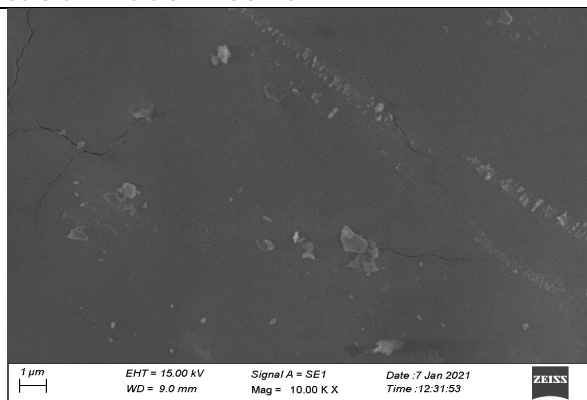


Figure – 10c: SEM image of polished mild steel coupon after immersion in 0.5M HCl in the presence of 10% Aqueous Leaf Extract of *Abelmoschus esculentus*

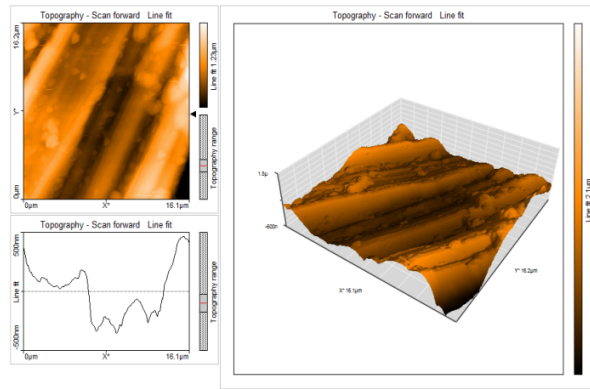


Figure -11a: AFM cross sectional images of the polished mild steel surface

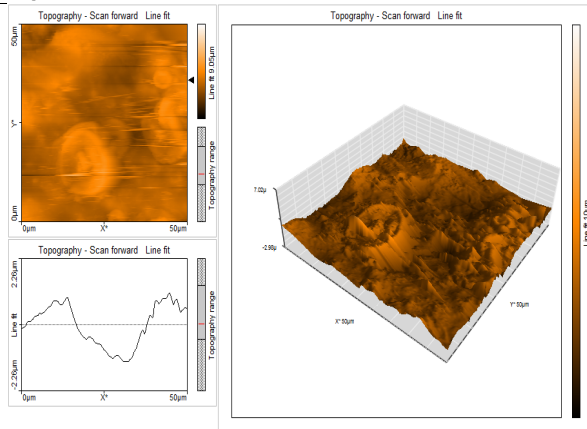


Figure – 11b. AFM cross sectional images of the mild steel surface after immersion in 0.5M HCl

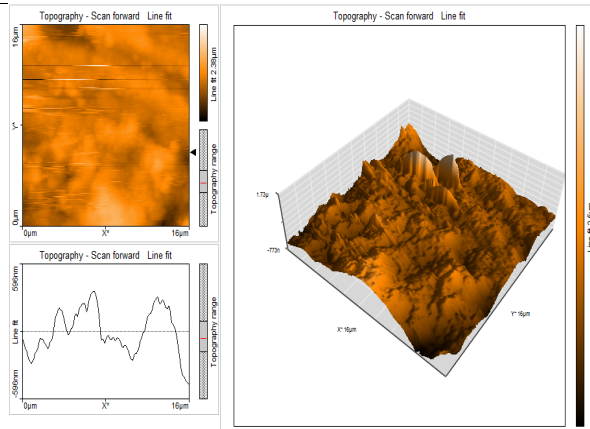


Figure – 11c. AFM cross sectional image for the mild steel surface after immersion in 0.5M HCl with 10% aqueous Leaf Extract of *Abelmoschus esculentus*





GC MS Analysis of One Ayurvedic Medicine, Mustakarisham

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ABSTRACT

The present study deals with the GC MS analysis of one Ayurvedic medicine Mushtakarisham used for weak digestion, vomiting, diarrhea, indigestion, dysentery, irritable bowel syndrome etc. Mushtakarisham was bought from a standard Ayurvedic vendor at Chennai and subjected to GC MS analysis by standard procedures. The medicinal roles of the biomolecules indicated in the GC MS profile were screened for their various medicinal roles using Dr. Duke's Phytochemical and ethno-botanical data and other data. The GC MS profile indicated the presence of important biomolecules such as Carbamic acid, N-(2-carbamoyloxyethyl)-, 4-methoxyphenyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, .alpha.-Terpineol, Thymol, Eugenol and Piperine which have a direct bearing on the role of Mushtakarisham. The GC MS profile of Mushtakarisham indicates some very important molecules such Carbamic acid, N-(2-carbamoyloxyethyl)-, 4-methoxyphenyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, .alpha.-Terpineol, Thymol, Eugenol and Piperine which have anti-inflammatory, antioxidant, antibacterial and digestive properties which could help in the medicine in treatment of diseases such as a diarrhea, dysentery etc. Further work is needed to understand these mechanisms better.



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Keywords: GC MS, Mushtakarishtham, Carbamic acid, N-(2-carbamoyloxyethyl)-, 4-methoxyphenyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, .alpha.-Terpineol, Thymol, Eugenol and Piperine.

INTRODUCTION

The establishment of traditional medicines to the forefront of treatment is urgent need of the hour due to the various pitfalls associated with modern molecular medicines. The Ayurvedic and Sidhha medicines should be thoroughly studied in the modern efficacy parameters to achieve this. There are some report pouring in in this direction which is a welcome sign [1-23]. The present study is one more step in this direction in which, one Ayurvedic medicine, Mushtakarishtham, is subjected to GC MS analysis and the molecules indicated therein are discussed for their medicinal values vis a vis the medicine's proclaimed role of treatment. Mustakarishtham or Mushtarishtama is a digestive tonic used to treat weak digestion, vomiting, diarrhea, indigestion, dysentery, irritable bowel syndrome etc. It increases bile (*Pitha*) secretion which helps in better digestion. The medicine is prepared as per the Ayurvedic treatise, Bhaishajya Ratnavali, Agnimansya Rogadhikara. The medicine is prepared by the following method:

- Mushtaka (*Cyperus rotundus*- roots) -2.4 Kg powder and boiled with 12.280 liters water to get a final volume of 3.072 liters decoction and cooled.
- The coarse powder of the following is made and mixed with the above mentioned decoction, kept in a suitable vessel and allowed to ferment for 30 days. After 30 days the mixture is filtered and bottled for use as medicine. Jaggery, 3.6 kg and 24 gms each of *Trachyspermum ammi* seeds; *Zingiber officinale* roots; *Piper nigrum* fruits, *Syzygium aromaticum*; *Trigonella foenum graecum* seeds; *Plumbago zeylanica* root; *Cuminum cyminum* seeds; *Woodfordia fruticosa* flowers. The medicine is taken at the dose of 12 to 24 ml twice daily after food or as advised by the physician.

METHODS

Mushtarishtam or Mushtakarishtha was obtained from standard Ayurvedic vendor at Chennai and was subjected to GC MS analysis by standard procedure. Gas chromatography (Agilent: GC: (G3440A) was equipped with Mass spectrometry detector. One hundred micro lit of mustakarishtham extract was charged for GC MS analysis.

RESULTS AND DISCUSSION

The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library (NIST and WILEY) stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS along with the possible pharmaceutical roles of each bio molecule as per Dr. Duke's Phytochemical and ethnobotanical data base (National Agriculture Library, USA) and others as shown in Table 1 [24]. Figure 1 indicates the GC MS graph representing the various peaks corresponding to each molecule present in Mushtakarishtham. There are very few scientific reports on the medicinal efficacy of Mustakarishtham. Phadtare *et al*, 2012 have reported the comparison among laboratory prepared and market samples of Mustakarishtham by TLC and HPTLC studies [25]. Kadam *et al*, 2012 have tested various tradition methods for the preparation of Mustakarishtham [26].

CONCLUSION

The GC MS profile indicates some very important molecules such Carbamic acid, N-(2-carbamoyloxyethyl)-, 4-methoxyphenyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, .alpha.-Terpineol, Thymol, Eugenol and Piperine which have anti-inflammatory, antioxidant, antibacterial and digestive properties which could help in



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the medicine in treatment of diseases such a diarrhea, dysentery etc. Further work is warranted to understand the roles of each molecule shown in the GC MS profile.

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Table 1. Indicating the GC MS profile of Mushtakarishtham showing Retention time, Name of the compound, peak area, peak height, molecular mass and possible medicinal roles of each molecule.

Sl. No	Retention Time	Name of Molecule	Area	Height	Mass	Possible medicinal roles
1	4.48	Diglycerol	13507373	3245839	166.1	Not Known
2	5.75	Butanoic acid, 3-hydroxy-, ethyl ester	18596210	5504575	132.1	This molecule functions as acidifier, inhibits 17 beta hydroxysteroid dehydrogenase and Aryl hydrocarbon Hydroxylase enzymes, Arachidonic acid activity, Increases aromatic amino acid decarboxylase activity, inhibits Uric acid production, and induces Testosterone Hydroxylase activity.
3	6.39	Carbamic acid, N-(2-carbamoyloxyethyl) - , 4-methoxyphenyl ester	6937715	1793898	254.1	This molecule is anacidifier, inhibit Arachidonic acid activity, Increases aromatic amino acid decarboxylase activity, inhibits production of Uric acid, anticancer, GABA-nergic, Increases NK cell activity, Inhibits tumor necrosis factor activity, myo-neuro stimulant and decrease norepinephrine production.
4	6.86	Phenylethyl Alcohol	81099165	26913265	122.1	Inhibits alcohol dehydrogenase production, inhibitor, alcohol detoxicant
5	7.47	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	13806629	4676229	144	This molecule inhibits Catechol O methyl transferease, 11B HSD, 17 Beta hydroxysteroid dehydrogenaser, 5HETE, Aryl hydrocarbon hydroxylase and HDL genic
6	8.48	Ethyl hydrogen succinate	13109680	3623589	146.1	This is an inhibitor of hydrogen Peroxide and Succinate dehydrogenase enzymes
7	8.56	.alpha.-Terpineol	4836711	2475840	154.1	This molecule inhibits 5-Alpha-Reductase, HIF-1-alpha, Alpha Glucosidase, testosterone-5-Reductase, IKappaB-Alpha





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						Phosphorylation, Increases alpha mannosidase activity
8	8.89	Catechol	11734028	4757528	110	This molecule is known to inhibit Catechol O methyl transferase enzyme
9	9.25	Benzofuran, 2,3-dihydro-	18610421	9638886	120.1	Not Known
10	9.35	5-Hydroxymethylfurfural	9426799	4146252	126	It is reported to stop neuron apoptosis
11	9.56	Propanal, 2-methyl-3-phenyl-	12374415	3785260	148.1	Inhibits Catechol O methyl transferase enzyme
12	10.72	p-Cymen-7-ol	5699802	2838681	150.1	This molecule has functions such as anticancer, oligosaccharide provider, inhibits CAMP phosphodiesterase and works as antidote
13	10.85	Thymol	74193140	32896102	150.1	Thymol has hair growth potential. Thymol derivatives have antioxidant, antibacterial and anti-inflammatory activities. ^[27]
14	11.18	4-Hydroxy-2-methylacetophenone	19198502	8879355	150.1	This molecule inhibits 17 beta hydroxysteroid dehydrogenase, Aryl hydrocarbon Hydroxylase and induces Testosterone Hydroxylase activity
15	11.99	Phenol, 2,6-dimethoxy-	6068992	2997475	154.1	Not Know
16	12.16	Eugenol	448733910	158476662	164.1	Eugenol is reported to have antifungal, antioxidant and anticonvulsant properties. It also acts as a local anesthetic, antistress, bacteriostatic, bactericidal, anticarcinogenic. It is known to depress the activity of central nervous system, functions as anti-radiation and antiviral. It also induces apoptosis in melanoma cells and HL-60 leukemia cells. ^[28, 29]
17	12.62	1,2,3-Benzenetriol	102680680	21549783	126	Not Known
18	13.40	Bisphenol C	23428055	6586921	256.1	This molecule functions as Acetyl choline antagonist, inhibits Acetyl Co A





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						carboxylase, as anticancer and as antitumor
19	13.57	Benzeneethanol, 4-hydroxy-	17792944	6954431	138.1	This molecule inhibits 17 beta hydroxysteroid dehydrogenase and Aryl hydrocarbon Hydroxylase and induces Testosterone Hydroxylase enzyme
20	13.70	Phenol, 3,4-dimethoxy-	13343613	5009993	154.1	Not Known
21	14.35	2,6-Dimethyl-3,5,7-octatriene-2-ol, ,E,E-				This molecule has functions such as anticancer, inhibitor of Cytochrome P450 2E1, decreases endothelial leucocyte adhesion, decreases epinephrine production
22	15.87	2,5-Dimethoxythiophenol	7586736	2121285	152.1	Not Known
23	16.28	2',4'-Dimethoxyacetophenone	9793624	3059875	170	Not Known
24	17.34	Galacto-heptulose	6365388	1718317	210.1	This molecule is a beta galactosidase inhibitor
25	17.76	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	6141068	2382846	190.1	Not Known
26	19.95	3-Buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-	12266269	3148765	224.2	Not Known
27	20.52	cis-Z- alpha- Bisabolene epoxide	9854562	3813454	220.2	It is a methyl donor, Makes zinc available to the body, Inhibit tumor necrosis factor, inhibits 5 alpha reductase, NADH oxidase inhibitor, HIF 1 Alpha and Aryl hydrocarbon Hydroxylase, alpha agonist, Increases alpha Mannosidase activity, inhibits TNF alpha and Catechol O methyl transferase
28	20.80	2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde	4892421	2227386	234.2	Inhibits 5 alpha reductase enzyme, functions as Acetylcholin antagonist and as an acidifier





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29	20.91	Ethanone, 1-[2-(5-hydroxy-1,1-dimethylhexyl)-3-methyl-2-cyclopropen-1-yl]-	18180682	5619047	224.2	Not Known
30	21.31	Corymbolone	8196940	2414031	236.2	Not Known
31	21.56	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	12659181	5477812	238.2	Not Known
32	23.85	Hexadecanoic acid, ethyl ester	7487097	3696493	284.3	Functions as Acidifier, Arachidonic acid inhibitor, increases aromatic amino acid decarboxylase activity and inhibitsuric acid production
33	24.30	Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl	6940062	2584447	254.2	Not Known
34	24.43	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one	7851043	2623629	236.1	Not Known
35	26.32	Linoleic acid ethyl ester	7282891	3058156	308.3	Functions as Acidifier, Arachidonic acid inhibitor, increases aromatic amino acid decarboxylase activity and inhibitsuric acid production
36	26.42	cis-Vaccenic acid	6618740	2527560	282.3	Functions as Acidifier, Arachidonic acid inhibitor, increases aromatic amino acid decarboxylase activity and inhibitsuric acid production
37	26.84	Octadecanoic acid, ethyl ester	5832140	2388053	312.3	Functions as Acidifier, Arachidonic acid inhibitor, increases aromatic amino acid decarboxylase activity and inhibitsuric acid production
38	34.41	Piperine	12783895	5748753	285.1	Piperine and Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxopentyl]- are related compounds having diverse biological and supportive therapeutic activities such as radioprotective, immunomodulatory, anti-tumor, antidepressant, anticonvulsant, anti-





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						nociceptive and anti-arthritic. ^[30] It helps in absorption of Selenium, vitamin B, Beta carotene and other nutrients. Piperine depresses the activity of drug metabolizing enzymes
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Qualitative Compound Report

Data File	190619029.D	Sample Name	Mustaishtam
Sample Type		Position	44
Acq Method	Compound Screening Method.M	Acquired Time	21-06-2019 PM 08:13:00
Comment			

User Chromatogram

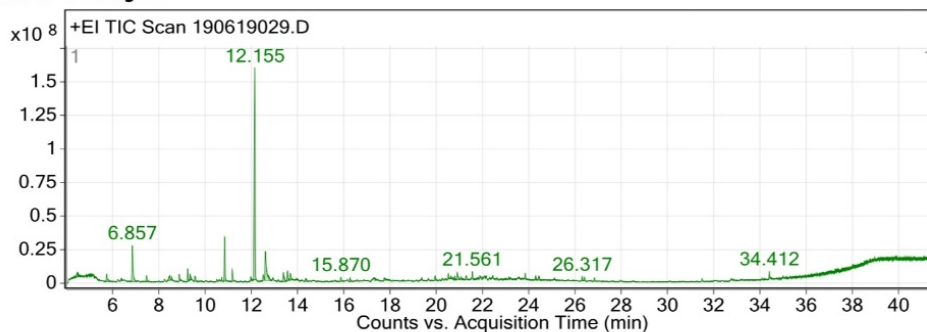


Figure 1. indicates the GC MS graph with various important peaks of Mustakarishtam





The Effect of Transparency and Disclosure in Accounting Information on Financial Reports and Their Appropriateness in Reducing Corruption (in Saudi Public Shareholding Companies)

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ABSTRACT

The study aimed to explore the impact of the transparency of accounting information and its suitability in reducing corruption in the Saudi shareholder's companies. It also aimed to explore the role of accounting information transparency in meeting the investors' needs. It should be noted that the absence of transparency shall negatively affect the growth and development this companies. The present study is considered significant because it sheds a light on a significant issue. The latter issue is represented in the impact of accounting information transparency financial reports and their appropriateness in reducing corruption in the Saudi Public Shareholding Companies. The researcher adopted a descriptive analytical approach. In addition, an inductive approach was adopted too. The latter approach was adopted when reviewing the relevant studies and references. Questionnaire forms were distributed to several financial managers and managers of internal review department. The response rate is 82 % which is a high percentage. It has been found that having transparent accounting information will help managers prepare financial reports to become relevant and reduce corruption. It was also found that transparent accounting information will reduce investment-related risks.

Keywords: Transparency, investment, disclosure, financial statements

INTRODUCTION

Providing the financial departments of companies with reliable financial information is very important. To assist in making financial and investment decisions. There is a positive relationship between the level of transparency and



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accounting disclosure, the rate of investment flow and the distance from corruption. In other words, transparency plays an important role in attracting investors, so the more the accounting information is transparent and there is full disclosure of all the information, the look at the financial statements becomes a complete and clean look away from all suspicions. In order for investors in the financial markets to make wise investment decisions, they must be provided with reliable information on financial statements that is relevant to them in making their decisions. After witnessing the stock market crash of 2008, investors became very interested in conducting transactions with companies that provide reliable information. They have become interested in conducting transactions with companies that reveal sufficient accounting information through their documented and approved financial statements and reports from accounting firms with good reputations in the profession.

Due to the growing awareness of investors' rights, corporations should focus their efforts to elevate their T&D and overall level of corporate governance standards for the benefits of all their stakeholders [1]. Transparency as the extent to which investors have access to the financial information required of the company, in which they want to invest their money [2]. The last term can also be defined as the flow of information from companies to investors. It should be noted that transparency is not a substitute for disclosure. In fact, transparency is an ideal way to disclose information [3]. financial decision makers must be provided with sufficient information about the company in which they want to financial decision and making the financial reports. This information should be very reliable. This is because recent information can greatly enable the aforementioned stakeholder to predict the market value of stocks. If the information provided is accurate and sufficient, then financial decision makers will be able to predict the price of shares in the financial market)and the can to financial decision [4].

Search objective

The aim of the research is to identify:

1. Identify the meaning of transparency in the financial statements and their suitability to reduce corruption. As well define the meaning and types of disclosure.
2. Identify the impact of transparency and disclosure and their suitability to reduce corruption. Explore the relationship between disclosure and transparency and their role in curbing corruption.

Research Importance

this research comes through the importance of disclosure and transparency of accounting when preparing financial reports, which works on the preservation of corporate funds and the reduction of corruption. Because the process of concealing information, whether intentionally or unintentionally, exposes joint stock companies to great losses, and this means that stakeholders are unable to Make rational financial and investment decisions. In addition, the current research is important because it aims to explore another important issue. It is represented in knowing the effect of accounting information on the process of reducing corruption within these companies.

The hypothesis**The first hypothesis**

There is a statistically significant relationship between transparency and reducing corruption in Saudi public shareholding companies

The second hypothesis

There is a relationship of statistical significance between the transparency of the public shareholding companies in the presentation of accounting information through the financial statements

The third hypothesis

There is a statistically significant relationship between the disclosure of accounting information and the reduction of corruption.



**Khaled Adnan Oweis****Study Population and Sample**

The sample consists of public shareholder companies listed on the Saudi Stock Exchange during the year 2020 [5]. The questionnaire forms were distributed: Financial directors and internal audit managers). A questionnaire was retrieved. All of them are valid for statistical analysis.

Study tool

A questionnaire designed by the researcher for this research was used to collect primary relevant data. It is designed based on previous studies and theoretical framework consisting of two parts. Through the first part, the researcher sought to collect data about the demographic characteristics of the sample. The second part consists of three sections.

section One:

It includes eight phrases related to the first hypothesis:
1: There is a statistically significant relationship between transparency and reducing corruption in Saudi public shareholding companies.

Section Two:

It includes eight phrases related to the second hypothesis:

2: There is a statistically significant relationship between transparency in the companies contributing to the presentation of accounting information through the financial statements and enabling investors to meet their needs

Section Three:

It includes eight phrases related to the third hypothesis:

3: There is a statistically significant relationship between the disclosure of accounting information and the reduction of corruption.

Scientific methodology of the research proposal

The research follows the descriptive and analytical method for describing the research topic and presenting the research literature and its theoretical framework. Defining the research problem, analyzing the questionnaire and the findings of the research.

Data collection sources

Information was collected through the following:

Books and references in Arabic and English, Previous research and studies, Scientific periodicals and magazines, University theses, Official reports. and the questionnaire.

Previous studies:

[6] This study aimed to define the principles underlying corporate governance, which lead to the reduction of fraudulent accounting practices. For this, the most important regulations issued by the Saudi Capital Market Authority, related to disclosure and transparency of financial information, were studied, to highlight the effective role of this authority in reducing accounting practices. It was concluded that the quality of information in the financial markets is related to the credibility of the financial information contained in the financial reports, and this will be of benefit to users; To achieve this, it must be free from distortion and misleading, especially since ensuring the quality of these reports guarantees the quality of the information disclosed in their core. Therefore, the study recommends the application of various principles and procedures by the listed companies to provide the market with good and reliable information, especially the principles of disclosure and transparency, which are the most important to achieving that.

[7] This study seeks to highlight the impact of the application of accounting disclosure on the information efficiency of the stock exchanges of a group of developing countries. To this end, it was used Two methods of research tools, where the first is a test study to identify the importance of accounting information by interpreting the relationship between the accounting information contained in the financial reports for the year 2018 as independent variables and the market value of the stock exchanges under study as a dependent variable and the extent of the impact of that information on the market value. To use the multiple regression model, as for the second method, the event study



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methodology was used to identify the efficiency of the financial markets under study, and to find out whether there was an effect of disclosing the financial statements on stock prices on the day of the announcement of these statements, by calculating the abnormal average return. For the shares of a sample of 80 companies listed on the Saudi Stock Exchange, Jordan and Egypt, , in addition to all companies listed on the Algeria Stock Exchange in 2018, for a period of five days before the date of disclosure and five days after the date of disclosing the financial statements. The study found, based on the results of the test study, that the most important information of interest to the Saudi investor is represented in profitability and the ratio of fixed assets to total assets, while the Jordanian investor attaches importance to information related to stock dividends and the ratio of equity to total assets, while the Egyptian investor gives importance to information related to Indebtedness and the ratio of equity to total assets. The results of the event study also showed that there was no statistically significant effect on the extraordinary returns of the stock exchanges under study on the day of the financial statements announcement, which confirms the inefficiency of the stock exchanges under study at the semi-strong level.

[8] This study the researcher examines the effects of proprietary information on corporate transparency and voluntary disclosure. To do so, he develops and validate two measures of firms' reliance on trade secrecy: one based on 10-K disclosures and one based on subsequent litigation outcomes. And he complements these measures by using the staggered passage of the Uniform Trade Secrets Act as a shock to trade secrecy. In finale he finds that firms that begin to rely more heavily on trade secrecy substitute increased voluntary disclosure of nonproprietary information for decreased disclosure of proprietary information. The total effect of trade secrecy is a decrease in corporate transparency.

[9] Disclosures are expected to foster financial transparency and improve the quality of information available to investors. The research has examined the role of non-financial disclosures in achieving this goal. Corporate social responsibility (CSR) disclosure has been widely employed as representative of non-financial disclosure. Recent legislation in some countries mandating non-financial disclosure makes this debate even more pertinent. They investigate the role of CSR disclosure in financial transparency in India, where mandatory CSR disclosure is required for firms to meet the thresholds set by the Companies Act 2013. Their investigation straddles mandatory disclosure regime and considers different classes of investors. The final findings suggest that CSR disclosure improves financial transparency during mandatory disclosure regime. ownership by the retail investors strengthens the association between CSR disclosure and financial transparency. There is no significant effects of ownership by the institutional investors on the association between CSR disclosure and financial transparency. [10] The purpose of this study is to examine the effect of accounting information transparency on decision making effectiveness via mediating influences, which include financial report quality and information advantage. The author improves novel components of accounting information transparency: disclosure, accuracy, and clarity. Data was collected from 238 Thai firms, divided into two categories: financial institutions and insurance companies. The result indicates that accounting information transparency has significantly positive influence on financial report quality and two of three dimensions of accounting information transparency have significantly positive influence on information advantage. Moreover, financial report quality and information advantage have significantly positive influence on decision making effectiveness.

[11]. The study focuses on the relationship between accounting information disclosure and investment decisions in Nigerian listed companies. To achieve this, three variants of accounting information disclosures (IFRS disclosures, Regulatory-induced-disclosures and Voluntary disclosures) and two variants of investment decisions (volume of shares traded and market value of shares) were considered through descriptive, content and regression analysis. Data were collected from audited financial reports of 52 selected firms (out of 174 firms listed on the Nigerian Stock Exchange as at December 2016) for 10 years period from 2006 to 2015, using purposive judgment sampling technique. Pre-estimation and post estimation tests were conducted on the series and the final regression estimate reveals accounting information disclosure indicators jointly having significant effects on both the market value of shares and volumes of shares traded on Nigerian stock exchange.



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The study therefore favors the full disclosure of all accounting information to assist the investors in making wise decision on their investments in Nigerian listed companies, as this is the only way by which corporate failures could be reduced to the barest minimum if not totally eliminated. It was therefore recommended that financial statement preparers should ensure full disclosure practices to support meaningful investment decisions in order to improve the market value of the company.

The importance of transparency

Transparency and disclosure are even more important if companies have more credibility in presenting financial information [12]. The latter importance increases when the company specifies the accounting methods used to prepare the financial statements. It increases when the company determines the policies adopted for this preparation. It should be noted that companies must disclose reliable financial data. This is to enable investors and financial decision-makers in interpret these data and take the right decision at the right time. This data should be easily accessed by all people. It should be clear, reliable, and easy to understand. In order for the financial decision-makers in companies to benefit from the accounting information, this information must be distinguished with certain features [13]. These features include: convenience, comparison, reliability and comprehension. The term (financial transparency) refers to the process of disclosing the full and actual financial position of a company.

[14] Disclosure and transparency are among the most important principles of corporate governance in order to enable shareholders to obtain the required information with transparency and fairness. Therefore, companies listed on the financial market are required to put in place disclosure policies, procedures and supervisory systems in writing. The companies should also include with their financial statements a report issued by the Board of Directors that includes a review of the company's operations during the past financial year, and the factors affecting its business that help the Financial decision preparer to evaluate the company's assets, liabilities and financial position, in addition to including the Board of Directors' report of the provisions of the Market Authority. Apply and what has not been implemented, along with the reasons for not applying [15]. according to al Majnabi the elements of transparency is [16] Based on international accounting standards, companies must adhere to the following standards to obtain a high level of transparency.

1. Firms should develop strategies and regulations that consist of both written and published legal rules
2. Companies must set clear rules governing the process of publishing and disclosing information. These rules should clarify the nature of the information that must be included.
3. Companies must disclose sufficient information that enables people to understand the nature of their business. This disclosure must be done in a way that facilitates controlling its performance
4. Companies must disclose the information that individuals and private companies need to develop future plans and assess the level of risks.
5. Everyone should have easy access to procedures, regulations, instructions and rules
6. Companies must disclose information related to their financial situation. It should be done in a regular, accurate and fast manner.
7. Companies should disclose information related to their economic performance in a quick and timely manner
8. Companies should present and conduct studies and references on things that could affect people's lives directly.

The accounting disclosure is done to provide the recipients of the financial statements with highly accurate financial and non-financial information. Financial decision preparer the financial data as the most reliable source of information. This is because the financial statements are prepared on the basis of international accounting standards and generally accepted accounting principles. Accounting standards specify minimum disclosures. However, it does not specify the maximum level of disclosure [17]. The people who benefit from the financial statements are prudent people who are knowledgeable and knowledgeable and specialized in reading and analyzing financial statements and reports. Therefore, in order to assist these persons who making investment decisions, companies are obligated to disclose sufficient financial information of the same quality and high quality in accordance with accounting



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standards. [18] Accounting disclosure and transparency are critical factors for investors. This is because investors rely on disclosed information to make their decisions, including investment decisions. They rely on this information because they consider it reliable. It should be noted that the absence of transparency will negatively affect the growth and development of financial markets. It will also discourage potential investors from investing in the market. Today, financial markets require all listed companies to disclose financial information in accordance with international accounting standards, international auditing standards and reporting standards. In the Kingdom of Saudi Arabia, the Saudi Stock Exchange adopted international auditing standards and international accounting standards on 1/1/2017 [19]. Since that date, all companies listed in the recent market must comply with these standards. Companies have become obligated to show a high level of transparency in disclosing their financial and non-financial reports that may indicate their financial position at the present time or in the future. This disclosure must be done in accordance with the market system and regulations. This should also be done in accordance with Articles 48 and 40 of the Disclosure Section of Chapter Seven of the Market Law (VII) [20]. The disclosure of accounting information should provide investors with information about the efficiency of the company's management. It should enlighten them about the management's ability to protect their money and manage the company's capital.

Accounting Disclosure

The importance of disclosure increases with the increasing need of joint-stock companies to finance. Disclosure is a prerequisite for establishing and managing efficient capital markets, and these markets are often supervised by professional or quasi-governmental bodies that oblige transacting companies to follow the basic rules defined by the profession (GAAP) and follow the instructions issued by supervisory bodies, in order to gain disclosure and reports [21]. The published financials are credible to users. Added to that, the checker. Accounts must certify the fairness of the financial statements and accounting information that were disclosed to confirm the reliability of that disclosure [22]. Disclosure is given several characteristics to determine the appropriate amount of information that must be disclosed: (1) full disclosure, (2) adequate disclosure (3) fair disclosure (4) differential disclosure, there is a general consensus in accounting that disclosure of accounting information should be complete (Also called inclusive), appropriate and fair [23]. Full disclosure requires that the financial statements addressed to the general users be designed and prepared in a way that accurately reflects all the material financial events and facts that affected the enterprise during the period, meaning that full disclosure indicates the extent of the comprehensiveness of the financial reports and the importance of their coverage of any information that has a tangible impact on the decisions of the user of those reports, and so on. Applying the concept of materiality to the purification of events and processes and focusing only on what is essential and relatively important is essential in accounting disclosure, as the accountant and auditor relies on it in explaining and applying the scope of comprehensive disclosure, while adequate disclosure is the presentation of the minimums required of financial data and information. Determining the minimums varies according to needs and interests, and this type of disclosure is the most used by professional organization [24].

The financial statements and reports must include sufficient information to make these lists useful and not misleading to the ordinary investor. More clearly, any essential or beneficial information should not be deleted or concealed for this ordinary investor [25]. Fair or honest disclosure is an ethical requirement that the auditor used to adopt upon expressing a clean opinion or not. Finally, it should be noted that there is no fundamental conflict between the three concepts of the scope of disclosure. Comprehensive disclosure must be sufficient and fair, and these three concepts represent concepts generally accepted by the accounting profession.

Differential disclosure [26]

In addition to the three previous concepts (comprehensive, adequate and fair) that are generally accepted and which represent a complementary case between them, some accounting literature presents a fourth concept of disclosure, which is "differential disclosure" and the same name discloses the content where the focus in the financial statements is in a summary and concise manner on differentiation [27] Or the discrepancy between the items by making



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comparisons to clarify the fundamental changes and determine the general direction of all the changes or differentials. The differential disclosure approach depends on the brief annual reports (summarized

Precautionary or conventional disclosure

The preventive or traditional disclosure adopts the concept of comprehensive disclosure, as it is currently applied in the contemporary accounting model, and the category of shareholders, lenders or creditors - external investors in general - is the primary focus for determining the content and tools of this preventive disclosure.

Informative or educational disclosure [28]

This disclosure assumes a prudent investor who is well-informed and knowledgeable, and has the ability to analyze, make comparisons and make forecasts in a professional manner. This disclosure is generally characterized by an increasing trend towards expansion and diversification of disclosure in its fields and focusing not only on financial accounting information but also includes quantitative and descriptive non-financial information, such as Quantitative information on the production capacity of the accounting unit and on the adequacy and qualifications of workers and the development of their efficiency and productivity.

Methods of data analysis

For the purposes of statistical treatment of data, descriptive and analytical statistical methods were used using the SPSS statistical treatment system, and the researcher used the arithmetic mean, standard deviation, T - Test, significance level 0.05, and T-test for double samples (T-test paired samples)

Questionnaire analysis

The present section provides analysis of demographic variables and hypothesis testing: Demographic variables, .By analyzing the research sample of 122 valid questionnaires, I found that 42% of the sample is less than 35 years old. And 58% of the sample hold a bachelor's degree in accounting.

Questionnaire analysis

The mean and standard deviation are used to describe attitudes towards the following questions:

Questionnaire analysis

The mean and standard deviation were used to describe the attitudes towards the questions of the first paragraph of the questionnaire, which relate to transparency, as 8 special paragraphs were placed in the transparencies and the ratios .The second table describes attitudes towards study variables and questions; It was found that there are positive attitudes towards the above questions because the mean was found above the critical value (3).This high average also reflects positive attitudes towards all variables. Which indicates that all categories of the sample support transparency and disclosure of financial information, which leads to giving confidence in the companies' data by stakeholders. Cronbach's alpha test was used to ascertain tool consistency. The value was = 0.949 for the questionnaire. Thus all values are accepted because they are above 60%.

The first hypothesis test

There is a statistically significant relationship between transparency and reducing corruption in Saudi public shareholding companies Multiple regression was used to test the first hypothesis at the 0.05 level and it was found that there is an effect at 121.569as shown in table (2). The question (lack of transparency negatively affects the preparation of financial statements according to accounting standards) got the highest answer from the target sample. As for the paragraph that got the lowest arithmetic average (the availability of transparent accounting information enhances feelings of confidence among decision makers). by 3.5985. The researcher sees it means that transparency has a significant impact on the process of reducing corruption and financial decision-making, and disclosure has a significant impact on the process of financial decision-making.



**Khaled Adnan Oweis****Second hypothesis test**

There is a relationship of statistical significance between the transparency of the public shareholding companies, in the presentation of accounting information through the financial statements. Simple regression was used to test the second hypothesis; found that the value of (t value = 10.224). Calculated at the level (0.05). This means that there is a significant statistical effect. Transparency on financial decisions in Saudi public shareholding companies has a moderate level of correlation of 0.641, as shown in table (4). Simple regression was used to test the second hypothesis, found that the value of (t value = 10.224). Calculated at the level (0.05). This means that there is a significant statistical effect. Transparency on financial decisions in Saudi public shareholding companies has a moderate level of correlation of 0.641, as shown in table (4). It has been found that the presentation of accounting information through the financial statements enables the owners of the money to make financial and investment decisions or to be reassured that the financial information is honestly and fair, which gives great satisfaction to the beneficiaries of the data.

The third hypothesis test

There is a statistically significant relationship between the disclosure of accounting information and the reduction of corruption. Simple regression was used to test the third hypothesis; found that the value of (t value = 15.017) is calculated at the (0.05) level. As shown in table (9). This means that there is a statistically significant effect between the disclosure of accounting information and the reduction of corruption contributing to a high level of 0.775. The researcher believes that honest representation and confidence in accounting information helps to gain the confidence of stakeholders in the presented financial statements. The impact of transparency and disclosure on financial statements and reports aims to reduce corruption as well as reduce obstacles in preparing these lists.

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RECOMMENDATIONS

The researcher recommends the following:

1. Enhancing knowledge among financial managers of companies listed in the financial markets about the role of transparency in raising shareholders' confidence in companies and increasing investment opportunities in the market.
2. Encouraging companies to raise levels of disclosure. It should include different types of disclosure.
3. Requiring companies to comply with financial market regulations. This would raise the quality of financial decisions.
4. Holding more training courses by the Capital Market Authority in the Kingdom of Saudi Arabia. On the methods used in disclosing financial and non-financial information and data on the basis of international accounting standards.
5. Focus on auditors' reports in full and comprehensive disclosure of all corporate policies and exceptional effects.
6. Companies should follow up on changes to international accounting standards.

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Table 1. Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.787	.620	.615	.51433

Table 2. ANOVA

Model	Sum of Squares	df	Mean Square	F	Sig.	
1	Regression	73.783	1	73.783	110.683	.000
	Residual	30.74	121	0.260		
	Total	104.521	122			

Table 3. Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.733	.204		3.598	.000
	transparency	.190	.069	.195	2.765	.006
	disclosure	.599	.066	.639	9.060	.000

Table 4. Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.641	.411	.407	.63839





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Table 5. ANOVA

	Model	Sum of Squares	df	Mean Square	F	Sig.
1	Regression	32.874	1	32.874	104.539	.000
	Residual	71.65	121	0.60		
	Total	104.521	122			

Table 6. Coefficients

	Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.306	.240		5.438	.000
	transparency	.626	.061	.641	10.224	.000

Table 7. Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.775	.601	.598	.52560

Table 8. ANOVA

	Model	Sum of Squares	df	Mean Square	F	Sig.
1	Regression	52.897	1	52.897	225.504	.000
	Residual	51.630	121	0.430		
	Total	104.521	122			

Table 9. Coefficients

	Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.985	.186		5.292	.000
	Disclosure	.727	.048	.775	15.017	.000





Evaluation of Hypolipidemic Activity in Rat Fed with Atherogenic Diet using Ethanolic Extract from the Whole Plant of *Ziziphus xylopyrus* (Retz.)

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ABSTRACT

The present study was designed to investigate the hypolipidemic effect of ethanolic extract from whole plant of *Ziziphus xylopyrus* (Retz.) (Family: Graminae) in rats fed with atherogenic diet. An acute toxicity study was showed that the ethanolic extract are safe up to 2000mg/kg body weight, thus one tenth of this dose was consider as evaluation dose. Ethanolic extract of *Ziziphus xylopyrus* (Retz.) was administered in doses of 250 and 500mg/kg body weight/day to rats fed with atherogenic diet to assess its possible lipid-lowering potential. A recognized raise in the body weight is recorded in AD fed group ($p < 0.001$), which was reduced by the administration of ethanolic extract of *Ziziphus xylopyrus* (Retz.) (400mg/kg body weight). The elevated levels of total cholesterol, triglycerides, phospholipids, LDL-C and VLDL-C and decrease the plasma HDL-C were observed in rats fed with atherogenic diet (group II). After treatment of ethanolic extract of *Ziziphus xylopyrus* (Retz.) (400mg/kg body weight/day) showed a significant ($p < 0.001$) decrement in body weight, plasma and tissue total cholesterol, triglycerides, phospholipids, plasma LDL-C and VLDL-C all along with an raise in plasma HDL-C when compared to AD rats (group II). The ethanolic extract of *Ziziphus xylopyrus*(Retz.) could protect against atherosclerosis and decrease the atherogenic index. This finding provides some biochemical basis for the use of ethanolic extract of whole plant of *Ziziphus xylopyrus* (Retz.) as hypolipidemic agent having preventive and curative effect against hyperlipidemia.

Key words: Atherogenic diet; Hypolipidemia; Rats; *Ziziphus xylopyrus* (Retz.)





INTRODUCTION

Every year about 17 million deaths caused by hyperlipidemia in world wide. Coronary Artery Disease (CAD) has been accounted for as the most widely recognized reason for death in grew and in addition creating nations [1-3]. Atherosclerosis (AS), the most important pathologic process leading to cardio and cerebrovascular diseases, is suggested to be mediated b the increase in the serum lipid, thrombosis, and injuries of the endothelial cells [4]. *Ziziphus xylopyrus* (Retz.) is a huge, sprawling shrub, having 6-10m tall, young shoots rusty tomentose armed with spines. The leaves are chewed for 15 days as well as fruit is used in urinary troubles [5]. the roasted seed powder paste is applied over the chest for relieving pain after cough and colds [6] The major chemical composition of *Z. xylopyrus* are Quercetin, Kempferol-4'-methylether and Kempferol, Cyclopeptide alkaloids AmphibineH and Nummularine-K [7-8]. The leaves contains quercetin and quecitrin. The bark contains tannins, 7, 3, 4-trihydroxy flavan-3, 4-diol and oleanic acid [9]. Fruit and bark is used as antimicrobial, bark is used as an anti inflammatory [10].

MATERIAL AND METHODS

Collection and identification of plant materials

The whole plant of *Ziziphus xylopyrus* were collected from kakinada, Andhra Pradesh, India. Botanical Survey of Department of Botany and microbiology, pithapur Rajah Government college, kakinada AP, India. has helped with the taxonomic identification of the plant. The whole plant of *Ziziphus xylopyrus* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Extract preparation

The plant powder after the pulverization is carried out for extraction using solvents. The extraction is then performed using ethanol by hot continuous percolation method with the aid of Soxhlet apparatus [11] the process is carried out for a period of 24hrs. The extract obtained was then concentrated using a rotary evaporator and successively subjected to freeze drying in a lyophilizer. A dry powder was obtained at the end. The collected extract was suspended in 2% Tween 80 [12].

Animals and treatment

Male Wistar rats of 16-19 weeks age, weighing 152-170g were procured from the Central Animal House, Acharya & BM Reddy College of Pharmacy, Bengaluru. Two animals are housed in each cages. All other standard guidelines are followed wile maintain the animal before during and post experiment i.e., 12:12 hr light and dark cycle at 25^o±2^oC is maintained. The animals were added their respective diets and water *ad libitum*. Clearance from Animal Ethical Committee was obtained to carry out the experiment. After the design of the protocol the animals were divided into following 5 groups with 6 animals in each group:

Group I (Control) : Standard chow diet

Group II : High Fat Diet

Group III : High fat diet + ethanol extract of *Ziziphus xylopyrus* (250mg/kg Body weight)

Group IV : High fat diet + ethanol extract of *Ziziphus xylopyrus* (500mg/kg Body weight)

Group V : High fat diet + standard drug Atorvastatin (1.2 mg/kg Body weight)

Animal diet

Compositions of the diets fed to experimental animals are as follows [13].

Control diet

Wheat flour, roasted Bengal gram powder, skimmed milk powder, casein, refined oil, salt mixture with starch and vitamin & choline mixture. 22.5%, 60%, 5%, 4%, 4%, 4%, 0.5% respectively.



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High fat diet

Wheat flour, roasted Bengal gram, skimmed milk powder, casein, refined oil, coconut oil, salt mixture with starch and vitamin & choline mixture, cholesterol. 20.5%, 52.6%, 5%, 4%, 4%, 9%, 4%, 0.5%, 0.4% respectively. Rats of group III were orally fed with the ethanolic extract of *Ziziphus xylopyrus* (250mg/kg body weight), rats of group IV were fed with the ethanolic extract of *Ziziphus xylopyrus* (500mg/kg body weight) and rats of group V were fed with the standard drug Atorvastatin (1.2 mg/kg body weight). Both the ethanolic extract of *Ziziphus xylopyrus* and Atorvastatin were suspended in 2% tween 80 separately and fed to the respective rats by oral intubation. At the end of 9 weeks all the animals were sacrificed by cervical decapitation after overnight fasting. Liver, heart and aorta were cleared of adhering fat, weighed accurately and used for the preparation of homogenate. Animal Ethical Committee's recommendations are followed while taking care of the experimental animals.

Biochemical estimation

Plasma samples collected from the experimental animals were analyzed for total cholesterol, High Density Lipopolsaccharide (HDL)-cholesterol and triglycerides were estimated using Erba Smart Lab analyzer USA and Boehringer Mannheim kits. Low Density Lipopolsaccharide (LDL)-cholesterol and Very low Density Lipopolsaccharide (VLDL)-cholesterol were calculated by using Friedwald method [14]. Digitonin is used to analysed the Ester cholesterol [15] and free cholesterol [15]. Portions collected from liver, heart and aorta tissues were blotted, weighed and homogenized with methanol (3 volumes) and by using the method of Folch *et al* [16] lipid extracts were obtained. The extracts were then used for the estimation of ester cholesterol and free cholesterol, triglycerides [17], and phospholipids [18]. Plasma total cholesterol: HDL-cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio was also calculated to access the atherogenic risk.

Statistical analysis

The results of this experimental study were expressed as mean \pm SE of 6 animals in each group. One way analysis of variance (ANOVA) test was used to determine the statistical significance. Significance level was fixed at 0.05.

RESULT AND DISCUSSION

Effect of Ethanolic extract of *Ziziphus xylopyrus* on Body weight changes of rats

The body weight of all the 6 rats in each group have increased after giving HDF. The average body weight in control and experimental animals in each Group was determined. The body weight of Group II animals increased significantly ($p < 0.001$) in comparison with normal control Group I animals. Cholesterol feeding has significantly elevated the levels of serum and hepatic lipid levels. These results produced are consistent when compared with many earlier records [19]. The increment in the body weights has reduced more significantly ($p < 0.001$) with the administration of *Ziziphus xylopyrus* ethanolic extract (doses 250 mg/kg and 500mg/kg body weight) as well as Atorvastatin 1.2mg/kg body weight in comparison with Group II animals.

Group I : Standard chow diet. (Control)

Group II : High Fat Diet.

Group III : High fat diet + Ethanolic extract of *Ziziphus xylopyrus* (250mg/kg body weight)

Group IV : High fat diet + Ethanolic extract of *Ziziphus xylopyrus* (500mg/kg body weight)

Group V : High fat diet + standard drug Atorvastatin (1.2 mg/kg body weight)

Effect of Ethanolic extract of *Ziziphus xylopyrus* on Plasma Lipid in HFD rats

There was a significant ($p < 0.001$) increase in the levels of plasma lipid profile in the experimental Group II rats fed with high fat diet in comparison with the normal untreated control rats (Group I). Studies conducted till date has revealed significant elevation of lipid parameters in plasma when fed with atherogenic diet or high fat diet [20,21]. Treatment of the experimental rats with ethanolic extract of *Ziziphus xylopyrus* at the dose of 250mg/kg & 500mg/kg



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(Group II and Group IV) body weight which were fed with HFD significantly decreased the concentration of total cholesterol when compared to HFD rats (Group II). However, the administration of ethanolic extract of *Ziziphus xylopyrus* (500 mg/kg body weight) along with HFD showed that the plasma cholesterol was restored to near normal as that of Atorvastatin (Group V). Free and ester cholesterol levels in the plasma were significantly ($P < 0.001$) increased in rats fed with high fat diet (Group II). One of initial event in the aetiology of atherosclerosis is nothing but the high cholesterol concentration in circulation which may damage the endothelial cells lining the large arteries and aorta [22]. High cholesterol concentrations i.e., both free and ester cholesterol levels has been reduced remarkably on treating the HFD fed rats with ethanolic extract of *Ziziphus xylopyrus* (Group III and Group IV).

The concentration of plasma triglyceride and phospholipids were measured in rats fed with high fat diet (Group II) and control rats (Group I), there is an significant elevation in the concentrations of plasma triglyceride and phospholipids levels in Group II when compared with Group I. Serum triglyceride levels elevation is considered as a major risk factor in case of cardiovascular disease [23]. Therefore ethanolic extract of *Ziziphus xylopyrus* (250mg/kg & 500mg/kg) has shown significant reduction in both plasma triglyceride levels in Group III and Group IV similar to standard drug Atorvastatin when compared with HFD rats (Group II). Henceforth the current study is totally focused on the findings related to reduction in these serum TG levels. Previous data available has shown that the triglyceride levels or the concentrations are indirectly related to coronary heart diseases [24]. Atherogenic Index (AI) was calculated in rats fed with HFD (Group II) and the rats with Non HDF diet (Group I), increased atherogenic index is recorded in Group II over Group I animals. Administration of ethanolic extract of *Ziziphus xylopyrus* to Group III & IV (doses 250 mg/kg and 500mg/kg) has significantly reduced the atherogenic index when compared to HFD fed rats (Group II).

Effect of Ethanolic extract of *Ziziphus xylopyrus* on Plasma lipoprotein in HFD fed rats

Good cholesterol HDL-C is a beneficial factor that is entangled with decrease in the Coronary artery diseases according to earlier study data [25]. The HDL-cholesterol measured has shown Low HDL levels in Group II when compared with control rats (Group I). Further treatment of Group III & IV with ethanolic extract of *Ziziphus xylopyrus* (dose at 250mg/kg & 500mg/kg) produced a significant and steady increase in the beneficial HDL-cholesterol concentration. HFD fed rats (Group II) had elevated levels of LDL and VLDL-cholesterol when compared with the control rats (Group I) LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries. A rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence is bad for health and a direct risk factor for coronary heart disease [26]. High levels of LDL and VLDL- cholesterol are major risk factor for coronary heart disease areas [27]. Administration of ethanolic extract of *Ziziphus xylopyrus* significantly reduced the levels of LDL and VLDL-cholesterol in plasma when compared with HFD rats (Group II).

Effect of Ethanolic extract of *Ziziphus xylopyrus* on tissue Lipid content in HFD fed rats

There was a significant ($p < 0.001$) increase in level of plasma lipid profile in the Group II rats fed with high fat diet in comparison with the normal untreated control rats (Group I). Treatment with ethanolic extract of *Ziziphus xylopyrus* at the dose of 250mg/kg & 500mg/kg body weight to rat fed with HFD significantly decreased the total cholesterol as compared level to HFD rats (Group II). However, the administration of ethanolic extract of *Ziziphus xylopyrus* treated rats (500 mg/kg) with HFD showed that the plasma cholesterol was restored to near normal as that of Atorvastatin (Group V). Significant ($P < 0.001$) increase in levels of both free and ester cholesterol were observed in plasma of rats fed with high fat diet (Group II). Increased intake of saturated fatty acid results in an increase cholesterol production by liver. Both free and ester cholesterol reduced remarkably on treating the HFD rats with ethanolic extract of *Ziziphus xylopyrus* (Group III and Group IV). The concentration of tissue triglyceride and phospholipids were elevated in rats fed with high fat diet (Group II) as compared to control rats (Group I). High blood triacyl glycerols have been linked to atherosclerosis, and by extension the risk of heart disease and stroke [28]. Both plasma triglyceride levels were significantly reduced in rats treated with ethanolic extract of *Ziziphus xylopyrus* (250mg/kg & 500mg/kg) and standard drug Atorvastatin along with high fat diet in comparison with HFD rats (Group II). The decrease of serum TG level is an important finding the present study. The results suggest that the plants could

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reduce hepatic triacylglycerols biosynthesis and favour the redistribution of cholesterol among the lipoprotein molecules [29].

CONCLUSION

Hyperlipidemia is one of the prime factors that cause atherosclerosis and coronary heart disease. By 2030, the annual death rate due to CVD is estimated to be 23.3 million people according to WHO. Atherosclerosis is a condition where the patient receives declined levels of blood supply portraying arteries blockade due to accumulation of lipid particles along the walls of the blood vessels. Therefore the current study aims to demonstrate the hypolipidemic activity of the *Ziziphus xylopyrus* (Retz.) ethanolic extract; which can help to lower the risk of atherosclerosis (CVD). The ethanolic extract has resulted in reduced plasmalipids, free cholesterols, ester cholesterols, triglycerides, phospholipids and lipoprotein profile thereby reducing the cardiac risks. There is also reduction in the body weights administered with HFD. Furthermore it was concluded that the extract has similar therapeutic profile as that of Atorvastatin. *Ziziphus xylopyrus* ethanol extract can be used in the treatment of the cardiovascular and hyperlipidemic diseases. Further clinical research is needed to provide the particular pathway or mechanism of action.

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Table 1. Effect of Ethanolic extract of *Ziziphus xylopyrus* (Linn) on Body weight changes of rats[Values are mean \pm SE of 6 rats]

Groups	Initial Weight (g)	Final Weight (g)	Average Body weight gain (g)
Group I	120.60 \pm 0.67 b ^{NS}	167.80 \pm 1.06 b ^{**}	47.2 \pm 1.15 b ^{**}
Group II	124.56 \pm 0.65 a ^{NS}	251.60 \pm 2.13 a ^{**}	127 \pm 2.19 a ^{**}
Group III	133.60 \pm 1.21 a ^{NS} , b ^{NS}	222.2 \pm 2.13 a ^{NS} , b ^{**}	88.6 \pm 1.86 a ^{NS} , b ^{**}
Group IV	141.20 \pm 1.15 a ^{NS} , b ^{NS}	188.6 \pm 1.20 a ^{NS} , b ^{**}	47.4 \pm 0.60 a ^{NS} , b ^{**}
Group V	143.80 \pm 2.31 a ^{NS} , b ^{NS}	180.8 \pm 1.24 a ^{NS} , b ^{**}	37.2 \pm 3.09 a ^{NS} , b ^{**}

P values : * $<$ 0.001, ** $<$ 0.05

NS : Non significant

a \rightarrow group I compared with groups II, III, IV, Vb \rightarrow group II compared with groups III, IV, V**Table 2- Effect of Ethanolic extract of *Ziziphus xylopyrus* on plasma lipid level HFD rats**[Values are mean \pm SE of 6 rats]

Groups	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglyceride (mg/dl)	Athrogenic index
Group I	107.90 \pm 0.42 b [*]	23.80 \pm 0.61 b [*]	85.94 \pm 0.47 b [*]	86.15 \pm 0.31 b [*]	55.97 \pm 0.45 b [*]	1.80 \pm 0.01 b [*]
Group II	169.50 \pm 0.26 a [*]	47.70 \pm 0.52 a [*]	121.80 \pm 0.50 a [*]	106.10 \pm 0.30 a [*]	69.98 \pm 0.32 a [*]	3.59 \pm 0.03 a [*]
Group III	121.21 \pm 0.33 a [*] , b [*]	28.58 \pm 0.78 a [*] , b [*]	92.63 \pm 0.45 a [*] , b [*]	92.67 \pm 0.51 b [*]	63.08 \pm 0.33 a [*] , b [*]	2.44 \pm 0.04 a [*] , b [*]
Group IV	98.12 \pm 0.33 a [*] , b [*]	23.01 \pm 0.30 a [*] , b [*]	75.11 \pm 0.31 b [*]	82.60 \pm 0.40 a [*] , b [*]	52.21 \pm 0.40 a [*] , b [*]	1.72 \pm 0.01 b [*]
Group V	99.14 \pm 0.32 a [*] , b [*]	21.71 \pm 0.46 a [*] , b [*]	83.23 \pm 0.31 a [*] , b [*]	83.43 \pm 0.46 a [*] , b [*]	55.30 \pm 0.48 a [*] , b [*]	1.78 \pm 0.01 a [*] , b [*]

P values : * $<$ 0.001, ** $<$ 0.05

NS : Non Significant

a \rightarrow group I compared with groups II, III, IV, Vb \rightarrow group II compared with groups III, IV, V

Details of group I-V are same as in Table 1.



Prabhu *et al.***Table 3- Effect of Ethanolic extract of *Ziziphus xylopyrus* on plasma lipoprotein in HFD rats**[Values are mean \pm SE of 6 rats]

Groups	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)
Group I	59.92 \pm 0.36 b [*]	36.79 \pm 0.51 b [*]	10.83 \pm 0.06 b [*]
Group II	47.13 \pm 0.35 a [*]	108.30 \pm 0.58 a [*]	13.97 \pm 0.07 a [*]
Group III	49.96 \pm 0.29 a [*] , b [*]	58.67 \pm 0.89 a [*] , b [*]	12.61 \pm 0.06 a [*] , b [*]
Group IV	56.93 \pm 0.36 a [*] , b [*]	30.74 \pm 0.13 a [*] , b [*]	10.44 \pm 0.08 a [*] , b [*]
Group V	55.57 \pm 0.41 a [*] , b [*]	32.50 \pm 0.66 a [*] , b [*]	11.05 \pm 0.09 a [*] , b [*]

P values : * $<$ 0.001, ** $<$ 0.05

NS : Non Significant

a \rightarrow group I compared with groups II, III, IV, Vb \rightarrow group II compared with groups III, IV, V

Details of group I-V are same as in Table 1.

Table 4- Effect of Ethanolic extract of *Ziziphus xylopyrus* on tissues free cholesterol level in HFD rats[Values are mean \pm SE of 6 rats]

Groups	Free cholesterol (mg/g tissue)		
	Liver	Heart	Aorta
Group I	0.81 \pm 0.01 b [*]	0.72 \pm 0.01 b [*]	0.46 \pm 0.01 b [*]
Group II	1.23 \pm 0.01 a [*]	1.02 \pm 0.01 a [*]	2.52 \pm 0.01 a [*]
Group III	1.11 \pm 0.01 a [*] , b [*]	0.88 \pm 0.05 a ^{**} , b ^{**}	1.71 \pm 0.01 a [*] , b ^{**}
Group IV	0.83 \pm 0.07 a ^{**}	0.65 \pm 0.01 a [*] , b [*]	0.67 \pm 0.05 a [*] , b ^{**}
Group V	0.86 \pm 0.08 a ^{**}	0.68 \pm 0.05 a [*]	0.82 \pm 0.05 a [*]

P values : * $<$ 0.001, ** $<$ 0.05

NS : Non Significant

a \rightarrow group I compared with groups II, III, IV, Vb \rightarrow group II compared with groups III, IV, V

Details of group I-V are same as in Table 1.

Table 5- Effect of Ethanolic extract of *Ziziphus xylopyrus* on tissues ester cholesterol level in HFD rats[Values are mean \pm SE of 6 rats]

Groups	Ester cholesterol (mg/g tissue)		
	Liver	Heart	Aorta
Group I	3.41 \pm 0.09 b [*]	2.90 \pm 0.01 b [*]	2.78 \pm 0.08 b [*]
Group II	7.22 \pm 0.01a [*]	4.56 \pm 0.02 a [*]	8.38 \pm 0.07 a [*]
Group III	5.20 \pm 0.01 a [*]	3.86 \pm 0.01 a [*] , b ^{**}	6.55 \pm 0.12 a [*] , b ^{**}
Group IV	3.88 \pm 0.01 a [*] , b [*]	2.98 \pm 0.01 a [*] , b [*]	3.71 \pm 0.01 a ^{**}
Group V	4.24 \pm 0.01 a [*] , b [*]	3.16 \pm 0.08 a [*] b [*]	3.82 \pm 0.01 a [*]

P values : * $<$ 0.001, ** $<$ 0.05

NS : Non Significant

a \rightarrow group I compared with groups II, III, IV, Vb \rightarrow group II compared with groups III, IV, V

Details of group I- V are same as in Table 1.



Prabhu *et al.***Table 6- Effect of Ethanolic extract of *Ziziphus xylopyrus* on tissues Triglyceride level in HFD rats**[Values are mean \pm SE of 6 rats]

Groups	Triglyceride (mg/g tissue)		
	Liver	Heart	Aorta
Group I	7.12 \pm 0.08 b [*]	9.77 \pm 0.03 b [*]	9.41 \pm 0.02 b [*]
Group II	27.87 \pm 0.09 a [*]	45.62 \pm 0.02 a [*]	23.11 \pm 0.02 a [*]
Group III	18.72 \pm 0.01 a ^{**} , b [*]	33.43 \pm 0.03 a [*] , b ^{**}	18.22 \pm 0.01 a ^{**} , b [*]
Group IV	10.87 \pm 0.01 a [*] , b [*]	17.22 \pm 0.02 a [*] , b [*]	11.34 \pm 0.16 a ^{**} , b [*]
Group V	12.43 \pm 0.02 a [*] b [*]	21.17 \pm 0.04 a [*] b [*]	11.65 \pm 0.02 a [*] b [*]

P values : * < 0.001, ** < 0.05

NS : Non Significant

a \rightarrow group I compared with groups II, III, IV, Vb \rightarrow group II compared with groups III, IV, V

Details of group I-V are same as in Table 1.

Table 7- Effect of Ethanolic extract of *Ziziphus xylopyrus* on tissues Phospholipids level in HFD rats[Values are mean \pm SE of 6 rats]

Groups	Phospholipids (mg/g tissue)		
	Liver	Heart	Aorta
Group I	20.39 \pm 0.01 b [*]	25.77 \pm 0.05 b [*]	9.06 \pm 0.02 b [*]
Group II	31.70 \pm 0.02 a [*]	37.27 \pm 0.02 a [*]	18.29 \pm 0.02 a [*]
Group III	27.42 \pm 0.02 a [*] , b [*]	36.82 \pm 0.01 a [*] , b [*]	12.68 \pm 0.02 a [*] , b [*]
Group IV	22.92 \pm 0.02 a ^{**} , b [*]	26.41 \pm 0.01 a [*] , b [*]	10.19 \pm 0.01 a [*] , b [*]
Group V	23.54 \pm 0.03 a [*] b [*]	27.13 \pm 0.02 a [*] , b [*]	11.31 \pm 0.02 a [*] , b [*]

P values : * < 0.001, ** < 0.05

NS : Non Significant

a \rightarrow group I compared with groups II, III, IV, Vb \rightarrow group II compared with groups III, IV, V

Details of group I-V are same as in Table 1.





Preliminary Phytochemical Screening and Acute Toxicity Studies of *Costus comosus* Linn.

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ABSTRACT

Costus comosus Linn is a rhizome commonly called red tower ginger. It is a favourite plant in tropical gardens worldwide due to its source of brilliant colour. Traditionally, the leaves and rhizomes have been used to treat fever, rash, asthma, bronchitis, and intestinal worms, diabetes, and liver diseases. The main aim of the present study is to investigate the phytochemical analysis of leaves of *Costus comosus* 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 *Costus comosus* 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 *Costus comosus* are more than 2000mg/kg and found to be safer and non -toxic to rats and further chronic studies are required to confirm its therapeutic efficacy in animals and humans.

Keywords: *Costus comosus*; Phytochemical analysis; Acute toxicity;

INTRODUCTION

Herbal medications are commonly used by around 70-80% of the world's population, primarily in developing nations, for healthcare services because of their simple availability and minimal cost. They have existed for centuries because of their widespread availability, safety, cheap cost-efficacy, cultural tolerance, and lower incidence of

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unwanted effects. Because the phytochemical elements present in herbal plants, play a crucial role in the physiological functions and are well tolerated by the human body. Many studies reported that numerous active molecules were derived from plant products[1]. Due to their diverse medicinal properties, it has created the belief that all plant products are safe [2]. Hence, a systematic study of medicinal plants for their phyto constituents and their potential toxicity is a necessary step for the evaluation of their therapeutic effect. In this connection, many toxicological studies were conducted to evaluate the toxicity of medicinal plants and their products.

Costus comosus is a perennial plant that belongs to the family of costaceae. It is commonly distributed in tropical and subtropical regions of Asia, Africa, and the Americas. One of the best ornamental ginger. Its large size and showy flowers make it a great addition to any garden. It flowers nearly year-round, both from the tops of the stems and from basal stems. *Costus* species is often characterized and distinguished from relative species such as *Zingiber* (true ginger) by its spiraling stems and thus commonly called red tower ginger. Its dark green leaves have a light green velvety underside, are elliptic with entire margins, up to 30cm long and 10cm across. Its yellow flowers emerge from red bracts and appear terminally was shown in fig.no.1. Its flowers will only appear if the plant is not exposed to freezing conditions during the previous winter months. Its roots contain rhizomes which aid its spread. Traditionally, the leaves and rhizomes have been used to treat fever, rash, asthma, bronchitis, and intestinal worms, diabetes, and liver diseases[3]. Despite these studies and the widespread use of this plant in traditional medicine, no works on the phytochemical and toxicological profile of extract from leaves have been reported. Thus, this study aims to evaluate the phytochemical analysis of leaves of *Costus comosus* and to evaluate its acute toxicity study as per OECD guidelines.

MATERIALS AND METHODS

Plant Material

The leaves of the plant, *Costus comosus* was collected from the Shervaroy hills, Salem, and from the tribal medical shops. in the month of October 2020. The collected plants (leaves) were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu. The herbarium specimen of the plant (KVCC-1) was maintained in the college 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 leaves 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 [4,5]. 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.

Phytochemical Screening of the Crude Drug

Qualitative phytochemical analysis of the Pet ether, Chloroform, acetone, ethanol, and aqueous plant extracts was carried out to test the presence of phytochemicals such as alkaloids, flavonoids, terpenoids, sterols, tannins, glycosides, etc. The following tests were done for the preliminary phytochemical screening [6].

Test for Flavonoids (Shinoda Test)

2mL of the extracts were mixed in the methanol, to this a minor part of magnesium ribbon was added and 1 mL of concentrated hydrochloric acid was added from the sides of the test tube. A magenta pink color designates the presence of flavonoids.



**Venkateshwara Rao et al.****Test for Saponins**

To 5mL of the 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 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 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 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 in 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 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 [7]. 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



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nm using a Shimadzu 1601 UV spectrophotometer. All the readings were repeated three times for precision and values were expressed in mean \pm 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 [8]. 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 \pm standard deviation in terms of flavonoid content (Quercetin equivalent, QE) per g of dry weight.

Animals

Healthy adult female Swiss albino mice (25-30gm) were used for the acute and subacute toxicity studies respectively. 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 12h 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 Animal

Healthy animals were randomly selected for the study and kept in their cages for at least one week prior to dosing to allow for acclimatization to the laboratory conditions. Before each test, the animals were fasted for at least 12h; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee 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.

Toxicity Studies

Acute and subacute toxicity studies were performed as per OECD (Organisation for Economic Co-operation and Development) – Guidelines 423 [9].

Acute Oral Toxicity Studies

The acute toxicity studies were performed as per OECD guidelines 423. A total of 12 mice weighing between 25-30g were randomly divided into four groups of 3mice each. Animals were fasted prior to dosing (food but not water was withheld overnight). Following the period of fasting, the bodyweight of the animals was measured and the ethanolic extract of leaves of *Costus comosus* was administered orally to each group at single doses of 5, 50, 300, and 2000 mg/kg, respectively. The control groups received the same volume of distilled water. All the animals were individually observed periodically during the first 24h after administering the extracts and then once a day for 14 days. All the animals were then allowed free access to food and water and observed for signs of acute toxicity. It includes changes in body weight, food and water intake, skin and fur, eyes and mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems, somatomotor activity, and behavior patterns. The number of deaths within this period was recorded. The urine analysis was performed to investigate any abnormalities in the excretion pattern after exposure to the test drug for 14 days.





RESULTS AND DISCUSSION

The purpose of this research is to give scientific validation to the plants by collect and extract plant materials and then screening them for potential phytochemical and Toxicological aspects. Till date, there is no phytochemical and toxicity studies have been reported with the leaves of this plant. Hence, the current research work focused on the evaluation of phytochemical and toxic effects of ethanolic extracts of leaves of *Costus comosus*.

Phytochemical Screening

The leaves of the plant *Costus comosus* were collected from the Shervaroy hills, Salem. The collected plants were identified and authenticated by a botanist. The leaves were shade dried at room temperature and coarsely powdered. The active principles present in the leaves were extracted by a continuous hot percolation method using various solvents and aqueous extract by the cold maceration method. 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 *Costus comosus*. 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 acetone extracts were sticky semisolid, ethanol, and aqueous extracts were powder in their consistency. The extractive values and percentage yield of leaves of *Costus comosus* were shown in Table.No.1. The qualitative phytochemical screening of Pet ether, chloroform, acetone, ethanol, and aqueous extracts of leaves of *Costus comosus* 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 amino acids, terpenoids and saponins, tannins, Gums and mucilage, and flavonoids.

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 *Costus comosus* 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., (58.92±0.19) mg/g GAE followed by (40.82±0.14) 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 20.24±0.24 and 16.12±0.14 mg of quercetin equivalent/g for EECC and AECC 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 were shown in Table No.3.

Acute Toxicity Studies

Acute toxicity studies are performed to determine the short-term adverse effects of the drug when administered in a single dose orally. It also indicates the safety of the drug *in-vivo*. Acute toxicity study is generally carried out for the determination of LD50 value in experimental animals. The LD50 determination was performed in mice as per OECD guidelines 423 and LD50 of the ethanolic extracts of leaves of *Costus comosus* was found to be non-toxic upto 2000 mg/kg and the ED50 values were 200 mg/kg, respectively.

CONCLUSION

Natural products play a major role in medicine because of their minimal side effects. Despite these, there is still a lack of scientific validation regarding the toxicological aspects of natural compounds. Hence, Scientific knowledge of toxicity studies is much needed. This will help us to identify the safe dose levels of the drug and also the therapeutic index of drugs [10]. In conclusion, it was observed that the plant *Costus comosus* Linn contains several bioactive components and a high level of total phenolics and flavonoids content. In acute toxicity studies, the animals showed





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no significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses, or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any of the four groups. Hence, the plant was considered as an enriched source of different phyto compounds. 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.

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Table:1 Colour, Extractive values and Percentage yield of various extracts of leaves of *Costus comosus*

Plant name	Part used	Method of extraction	Solvent	Colour of extract	Nature of extract	% yield of extract
<i>Costus comosus</i>	Leaves	Continuous hot percolation	Chloroform	Green	Semisolid	4.76
			Acetone	Greenish brown	Greasy solid	12.22
			Ethanol	Green	powder	14.24
			Aqueous	Green	powder	10.72





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Table:2 Preliminary phytochemical screening of the Different extracts of leaves of *Costus comosus*

S.No	Constituents	Tests	Chloroform	Acetone	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 *Costus comosus*.

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	8.23±0.14	1.30±0.20
2		Chloroform	19.28±0.54	6.28±0.35
3		Acetone	29.14±0.14	9.32±0.16
4		Ethanol	58.92±0.19	46.64±0.14
5		Aqueous	40.82±0.14	16.24±0.14

Values are expressed as mean ± SD, n=3





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Fig.1. Leaves and flowers of *Costos comosus* Linn





***In silico* Screening of *Myristica fragrans* Extracts Licarin A, Licarin B, Licarin C, Malabaricone B and Malalabaricone C for their Activity towards A Methionine Aminopeptidase Type II**

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ABSTRACT

Five selected compounds found in nutmeg extract were screened against Methionine aminopeptidase (MetAP) computationally and the results are discussed in the current study. The 3D structures of the phytochemicals/ligands were obtained from chemical structure database PubChem and the protein structure complexed with D-methionine was acquired from Protein Data Bank. Ligands were prepared using AutoDock tools and docked against the target protein. Binding affinity of the phytochemicals towards the protein was predicted. Among the studied phytochemicals, Malalabaricone B and Malalabaricone C exhibited highest binding affinity.

Keywords

Keywords: Methionine aminopeptidase, angiogenesis, nutmeg, licarin A, Licarin B, Licarin C, Malabaricone B, Malalabaricone C

INTRODUCTION

Growth of tumors are aided by new blood vessel formation in a process called angiogenesis. Angiogenesis is responsible for growth of cancer cells, diabetic retinopathy, arthritis, psoriasis etc. Anti-angiogenic compounds are known to target human methionine aminopeptidase type 2 (hMetAP-2) (1). Active phytoconstituents found in medicinal plants possess lower side effects and can be persuaded to be therapeutic leads to target various diseases (1). Apart from being a popular spice, Nutmeg (*Myristica fragrans*) seeds have been used to treat various diseases in traditional medicine (2). Nutmeg essential oil also claimed to possess important medicinal properties (2). The components of the extracts obtained by different extraction methods and different solvents tend to be different (1). In continual interest to determine different activities of the components present in Nutmeg, an *in silico* study was



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conducted to find out binding affinity of a selected range of compounds with human methionine aminopeptidase type 2 (hMetAP-2) and find out their potency as probable anti-angiogenic agents.

RESULT AND DISCUSSION

3D structures of licarin A, Licarin B, Licarin C, Malabaricone B and Malabaricone C were taken from chemical structure database PubChem. The structures were obtained as .sdf format which were converted into .pdb format using PyMol software. Ligands were prepared in AutoDock and the corresponding files were saved in .pdbqt format. The compounds chosen for the current study are shown in figure 1. Structure of the target protein was obtained from Protein Data Bank (<https://www.rcsb.org>). Water molecules were removed and the protein was prepared. Docking was performed using AutoDockVina (3). The computational calculations produced nine best docking poses for each ligand and protein pair. Ligand-protein interactions were visualized in PyMol. Malabaricone B and C (4) and (5) exhibited maximum binding affinity among the phytochemicals those were studied. The binding energy for both the ligands were -7.6 kcal/mol. Malabaricone B had 2 hydrogen bonds with the protein whereas Malabaricone C showed 3 hydrogen bonding. Among the analogous structures of licarins, licarin B (2) exhibited highest binding affinity with the protein target. The binding energy for licarin B was found to be -7.5 kcal/mol with three hydrogen bond interactions with the protein (Table 1).

CONCLUSION

Among the phytochemicals those were studied, malabaricone B and C demonstrated highest binding affinity to the human methionine aminopeptidase type 2 (hMetAP-2). However, the highest number of hydrogen bonding was shown by licarin A. The outcome of the above *in silico* study can help to develop lead molecules as anti-cancer agents.

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Table 1: Binding energy (BE) in Kcal/mol and number of hydrogen bonds (NHB) of ligand protein interaction
The first pose with minimum binding energy of each molecule which were visualized in PyMol are presented in the figures given below.

PDB ID	Binding energy and number of hydrogen bond of protein 1BHS with each ligand									
	Licarin A		Licarin B		Licarin C		Malabaricone B		Malabaricone C	
	BE	NHB	BE	NHB	BE	NHB	BE	NHB	BE	NHB
1KQ0(4).	-6.4	4	-7.5	3	-6.6	0	-7.6	2	-7.6	3

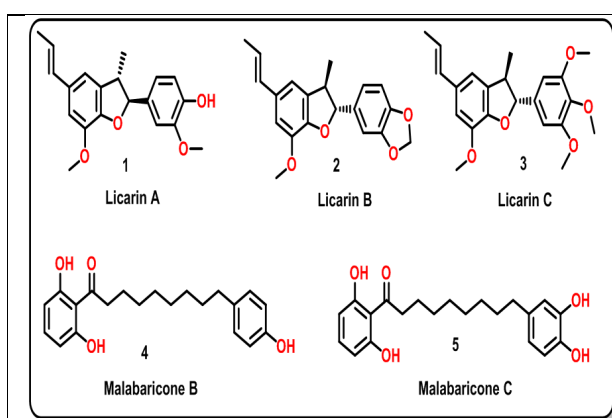


Figure 1: Chemical structures of the compounds Licarin A (1), Licarin B (2), Licarin C (3), Malabaricone B (4) and Malabaricone C (5)

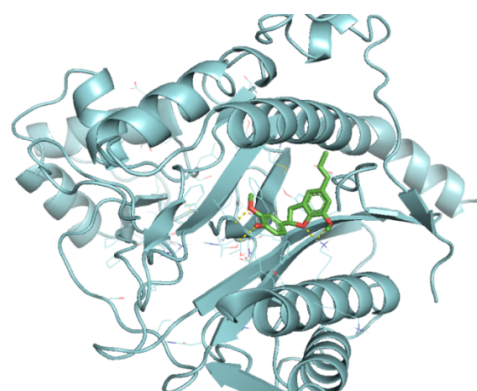


Figure 2: Ligand licarin A in the binding pocket of the protein 1KQ0

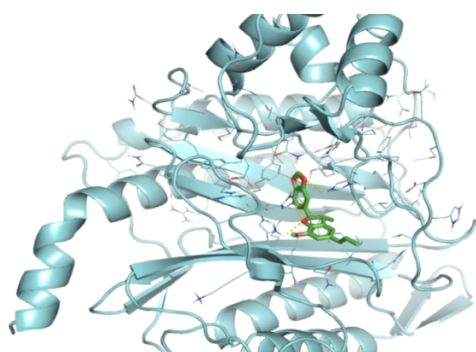


Figure 3: Ligand licarin B in the binding pocket of the protein 1KQ0

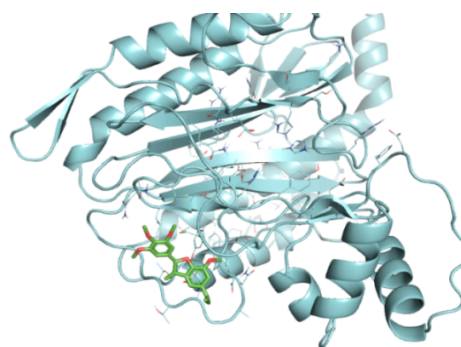
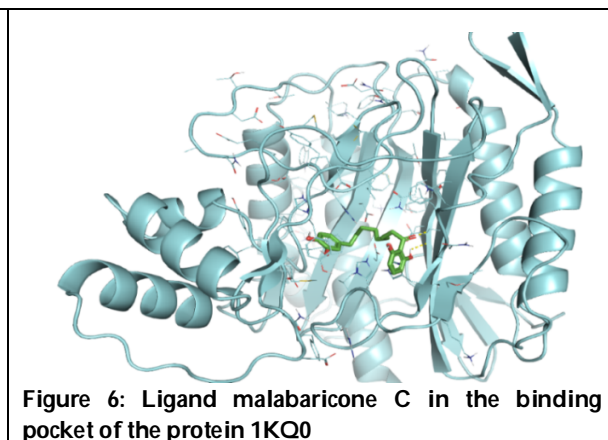
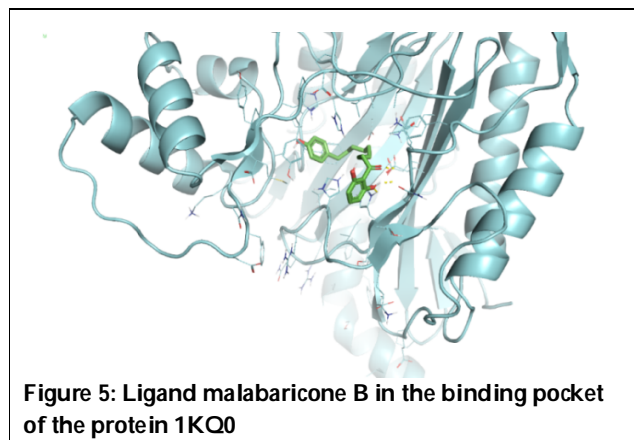


Figure 4: Ligand licarin C in the binding pocket of the protein 1KQ0





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The GC MS Study of One Ayurvedic Formulation, Rajanyadi Churnam

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ABSTRACT

The present study embarks upon the GC MS analysis of one Ayurvedic medicine, Rajanyadichurnam, which is prescribed for a variety of complaints such as diarrhoea, fever, jaundice, anaemia, cough and cold in children. The medicine was procured from standard Ayurvedic vendor at Chennai and was processed by standard protocols before subjecting it to GC MS analysis. It was observed that 27 compounds were present among which 4-Hydroxy-2-methylacetophenone, Ar-tumerone, i-Propyl 5,8,11,14,17-eicosapentaenoate, Abietic acid, .gamma.-Sitosterol, .beta.-Amyrin, Lanosta-8,24-dien-3-ol, acetate, (3.beta.)- etc. do have medicinal role which correspond well with that of Rajanyadichurnam. The GC MS profile indicates the presence of some biomolecules which support the function of Rajanyadichurnam as a potent medicine.



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Keywords: RajanyadiChurnam, GC MS, Ayurvedic, Ar-tumerone, Abietic acid, .gamma.-Sitosterol, .beta.-Amyrin.

INTRODUCTION

There is an urgent need to understand the mechanism of action of Ayurvedic and other alternative forms of medicine. This need has become all the more relevant after the advent of Covid-19, since the whole mankind has been kept guessing about its cure. Ayurveda and Sidhha medicines have been extensively used in India as well as abroad with fair amount of success. If this importance on these forms of medicine is to continue on tackling diseases it is imperative to understand their molecular roles as medicines. Of late some work in this regard is going on and much more need be done [1-21]. The present study is one more step in this direction in which the GC MS study of one Ayurvedic medicine, Rajanyadichurnam was undertaken. This powder is used to treat diarrhoea, fever, jaundice, anaemia, cough and cold in children. It is also used as a general tonic in children. It contains eight ingredient plants which are separately powdered and mixed in equal quantity.

The ingredients are: Rajani – Turmeric – *Curcuma longa*, Daru – *Cedrus deodara*, Sarala – *Pinus roxburghi*, Shreyasi – *Piper chaba* Brihati – *Solanum indicum*, Kantakari – *Solanum xanthocarpum*, Prishnaparni – *Uraria picta* and shatahva – *Anethum sowa*. It finds its reference in Ayurvedic treatise, Astangahridayam. The dose is 1 to 3 g along with water, honey or milk before or after food or as directed by the physician.

MATERIALS AND METHODS

Rajanyadichurnam was obtained from standard Ayurvedic vendor at Chennai and was subjected to GC MS analysis by standard procedure using Agilent (G3440A) 7890A with Mass spectrometry detector.

RESULTS AND DISCUSSION

The GC MS profile of Rajanyadichurnam is represented in Figure 1. Table 1 indicates the retention time, types of possible compound, their molecular formulae, molecular mass, percentage peak area and their medicinal roles, as shown in the GC MS profile of Rajanyadichurnam. The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS along with the possible pharmaceutical roles of each bio molecule as per Dr. Duke's Phytochemical and ethnobotanical data base (National Agriculture Library, USA) and others as shown in Table 1 [22].

Table 1 indicated the presence of some important biomolecules such as 4-Hydroxy-2-methylacetophenone, Ar-tumerone, Ar-tumerone, i-Propyl 5,8,11,14,17-eicosapentaenoate, Abietic acid, .gamma.-Sitosterol, .beta.-Amyrin, Lanosta-8,24-dien-3-ol, acetate, (3.beta.-). The Table also depicts that these biomolecules have functions such as anti-inflammatory, antimicrobial and steroid metabolism controlling roles. These roles could contribute to the medicinal role of Rajanyadichurnam. There are some molecules for which the medicinal roles are not known. Further probe in this regard could throw some more light on the mechanism of action of this medicine.

CONCLUSION

It is concluded that the medicinal roles of the molecules present in Rajanyadichurnam indicate their contributory role in the medicine.





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Table1. Indicates the retentions time, types of possible compound, their molecular formulae, molecular mass, percentage peak area and their medicinal roles of each compound as shown in the GC MS profile of Rajanyadi Churnam

Sl. No	Retention Time	Compound Name	Mol. Formula	Mol. Weight	% Peak Area	Possible medical Role
1	7.30	4-Hydroxy-2-methylacetophenone	C ₉ H ₁₀ O ₂	150.1	0.71	This molecule is inhibitor of 17 beta hydroxysteroid dehydrogenase, Aryl Hydrocarbon hydroxylase and Testosteronehydroxylase enzymes
2	8.39	Longifolene	C ₁₅ H ₂₄	204.2	1.87	Not known
3	10.98	Apiol	C ₁₂ H ₁₄ O ₄	222.1	3.09	Not known
4	11.43	Ar-tumerone	C ₁₅ H ₂₀ O	216.2	5.35	Antimicrobial.
5	11.49	Tumerone	C ₁₅ H ₂₂ O	218.2	4.74	Antimicrobial.
6	11.83	Curione	C ₁₅ H ₂₂ O	218.2	3.96	Antimicrobial.
7	12.60	1-[3-(2,6,6-Trimethylcyclohex-2-enyl)-4,5-dihydro-3H-pyrazol-4-yl]-ethanone	C ₁₄ H ₂₂ N ₂ O	234.2	0.64	Not known
8	14.20	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.3	1.31	It is known to be as Acidifier, Arachidonic acid inhibitor and to Increase Aromatic Amino acid Decarboxylase activity
9	15.81	6-Octadecenoic acid, methyl ester, (Z)-	C ₁₉ H ₃₆ O ₂	296.3	2.67	It is known to be as Acidifier, Arachidonic acid inhibitor and to Increase Aromatic Amino acid Decarboxylase activity
10	16.18	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536.6	6.37	This molecule has many roles such as antibacterial, Anticancer, antiprotozoal, chemo-preventive, anti-inflammatory, Antimalarial Anti-flu, Antiviral, antiprotozoal, Antioxidant, Antiperoxidant, Antitumor, anti-hypercholesterolemic etc.
11	17.65	Methenolone	C ₂₀ H ₃₀ O ₂	302.2	3.26	Not known
12	18.15	i-Propyl 5,8,11,14,17-eicosapentaenoate	C ₂₃ H ₃₆ O ₂	344.3	8.20	This molecule functions as Ionotropic, inhibitor of 11B-





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						HSD, 5 alpha reductase, HIF1 alpha, alpha amylase, Ikappa B-alpha phosphorylation, Interlukine- 1 alpha, Testosterone 5 alpha reductase, 12 Lipoxygenase, 17 beta hydroxysteroid dehydrogenase, 5 HETE, 5 HT, 8 HETE inhibitor, ACE, and Acetyl CoA carboxylase enzymes
13	18.48	Dehydroabietic acid	C ₂₀ H ₂₈ O ₂	300.2	2.02	It is known to be as Acidifier, Arachidonic acid inhibitor and to Increase Aromatic Amino acid Decarboxylase activity
14	18.82	Abietic acid	C ₂₀ H ₃₀ O ₂	302.2	6.56	It is known to be as Acidifier, Arachidonic acid inhibitor and to Increase Aromatic Amino acid Decarboxylase activity
16	19.41	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390.3	1.49	It is known to be as Acidifier, Arachidonic acid inhibitor and to Increase Aromatic Amino acid Decarboxylase activity
18	21.33	Decanedioic acid, bis(2-ethylhexyl) ester	C ₂₆ H ₅₀ O ₄	426.4	0.81	It is known to be as Acidifier, Arachidonic acid inhibitor and to Increase Aromatic Amino acid Decarboxylase activity
19	21.47	Squalene	C ₃₀ H ₅₀	410.4	1.20	Monoxygenase inhibitor, biochemical precursor in the preparation of steroids, natural moisturizer, used in cosmetics
20	23.39	3-Methoxytyrosine	C ₁₀ H ₁₃ N O ₄	211.1	2.21	Not known
21	24.38	.gamma.-Sitosterol	C ₂₉ H ₅₀ O	414.4	1.45	This molecule is a PPAR-gamma antagonist
22	24.49	.beta.-Amyrin	C ₃₀ H ₅₀ O	426.4	0.79	The molecule has activities such as 17 beta hydroxysteroid dehydrogenase inhibitor, Anti-amyloid beta, Anti TGF beta, Beta receptor agonist, Beta-adrenergic receptor blocker, beta blocker, beta galactosidase inhibitor, beta glucuronidase inhibitor, ER beta binder
23	24.77	Betulin	C ₃₀ H ₅₀ O ₂	442.4	1.20	Not known
24	25.21	Lanosta-8,24-dien-3-	C ₃₂ H ₅₂ O ₂	468.4	6.90	Anti-amyloid beta, Anti TGF





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		ol, acetate, (3.beta.)-				beta, Beta receptor agonist, Betaadrenergic receptor blocker, beta blocker, beta galactosidase inhibitor, beta glucuronidase inhibitor, ER beta binder
25	25.43	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	C32H52O2	468.4	30.86	Not known
26	26.20	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl-, [1ar-(1a.alpha.,1b.beta.,1c.alpha.,2a.alpha.,3a.beta.,6a.alpha.,6b.alpha.,7.alpha.,8.beta.,8a.alpha.)]-	C22H30O8	422.2	0.65	Not known
27	26.23	Benzeneethanol, 4-fluoro-	C8H9FO	140.1	1.70	Not known

Qualitative Compound Report

Data File 200520012.D **Sample Name** Rajanyadi Chumam
Sample Type **Position** 24
Acq Method GC Screening Method.M **Acquired Time** 22-05-2020 AM 04:02:10
Comment

User Chromatogram

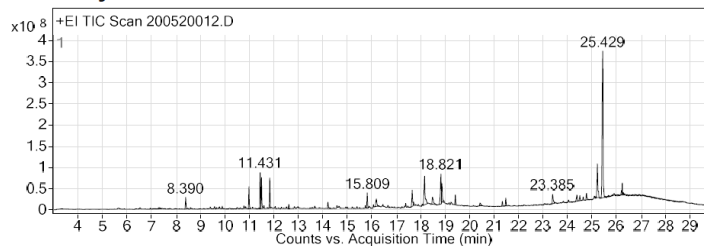


Figure 1. Depicts the GC MS profile of Rajanyadichumam.





Synthesis, Characterization and Anti-Cancer Studies of Transition Metal Complexes of Tetrazole Derivative

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ABSTRACT

Tetrazole derivative have played a vital part in the development of heterocyclic compounds. During the last two decades, the study of the biological activities of tetrazole derivative has been the aim of many researchers. Based on these findings, a series of benzoic acid substituted tetrazole were synthesized and their metal complexes were synthesized by condensation of tetrazole and different transition metal chloride salt of Zn(II) and Cu(II) their characterization are done by different analytical techniques, such as elemental analysis, FT-IR, UV, ¹H-NMR, ¹³C-NMR, and ES-Mass. All the compounds were tested for their anticancer activity against MCF-7 lung cancer cell line with MTT assay. Docking studies of the synthesized compounds was done with the help of HEX 6.1 software using GRIP batch docking method to study their observed activity. Docking study was done and the compounds were found to fit well with the target PDB ID: 1NOW.

Keywords: Anticancer activity, Synthesis, Docking, MCF-7 cell line.



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INTRODUCTION

The chemistry of heterocyclic compounds has been an attracting field of study of long time. The synthesis of novel tetrazole derivatives and investigation of their chemical and biological behaviour has gained more importance in recent decades for biological and pharmaceutical reasons (Sankari Kanakaraju *et al.*, 2013). 1, 2, 3, 4-tetrazole represent an important class of heterocyclic compounds. Tetrazoles are class of synthetic organic heterocyclic compounds consisting of five-member ring of four nitrogen and one carbon atom (plus hydrogen). The simplest is tetrazole itself CN_4H_2 . It is white to the pale yellow crystalline solid with the weak characteristic odour, soluble in water and alcohol. It is acidic in nature due to presence of four nitrogen atoms. Numbering of tetrazoles is as shown below. Usually, tetrazole is explosives. Nature of tetrazoles was unknown. It is used as gas generating agent for air bags (Mohite P.B and Bhaskar V.H, *et al.*, 2011). A number of tetrazoles are used as pharmaceutical agents. They undergo electrophilic as well as nucleophilic substitution reactions. Tetrazoles can act as pharmacophore for the carboxylate group, raising their utility. Angiotensin II blocker often contain tetrazoles, as Losartan and candesartan. A well-known tetrazole is MTT, which is dimethyl thiazolyl diphenyl tetrazolium salt. This tetrazole is used in MTT assay to quantify the respiratory activity of live cells in cell culture, although it kills cells in the process (Dhayanithi Varadaraji *et al.*, 2010) (Diana Pintos *et al.*, 2007). Tetrazole and their derivatives possess broad spectrum of biological activity in both medicinal and pharmaceutical, such as anti-inflammatory (V. H. Bhaskar, P. B. Mohite *et al.*, 2011), antibacterial (Helen P Kavitha *et al.*, 2000), antifungal (Ismail Y, Ikay O *et al.*, 2000), antituberculous (Heffeter P., Jakupec M. A *et al.*, 2006) antiviral (Lyakhov, S. Alexander *et al.*, 2001), and anticancer activities (J.H. Toney, *et al.*, 1996)

MATERIALS AND METHODS

All the chemicals and solvents used were of AR-grade obtained from Sigma- Aldrich, Sisco Research Laboratories, Qualingens, Hi-media, nice chemicals, Spectrochem and were used without further purification. All melting points were taken in open capillaries and are uncorrected. Elemental analysis was performed on a Perkin-Elmer analyzer. IR spectral [12] were recorded in KBr on Shimadzu spectrometer, 1H -NMR and ^{13}C -NMR in DMSO- d_6 on a Bruker AC-400 spectrometer using TMS as an internal standard. The microorganisms were obtained from National Chemical Laboratory, Pune. Thin-layer chromatography (TLC) was performed on pre-coated aluminium plates (silica gel 60F254, Merck). Plates were visualized by UV light and iodine vapor.

Synthesis of Thiocyanate (TC1)

The substituted/unsubstituted benzoic acid (0.5 mole) (Maruthamuthu *et al.*, 2016) was dissolved in acetic acid (125 ml) and the solution was added to the solution of ammonium thiocyanate (1.05mol, 80 g) in glacial acetic acid (250 ml). This solution was cooled to 10-20 °C. To this well stirred solution, a solution of bromine (0.5 mol, 25.7 ml) in acetic acid (250 ml) was added drop wise for thirty minutes and the temperature was maintained below 20°C. After the addition of bromine, it was kept at room temperature for ten minutes and then it was diluted with an equal amount of water. The solid material was filtered, washed, dried and recrystallized from ethanol.

Compound TC-1 2-acetoxy-4-thiocyanatobenzoicacid

Anal. Calcd. For $C_{10}H_7SNO_4$: C, 45.98; H, 2.81; N, 2.97; O 26.98; Found: C, 45.05; H, 2.94; N, 5.97; O, 27.05; Yield %(72), ES (+) 237.23 (M+H); M.p.: 242–243 °C; IR KBr (cm^{-1}): $\nu_{C=N}$: 2251.5 cm^{-1} .

Synthesis of Tetrazole (TT1)

A mixture of thiocyanate TC1 (0.01 mol), sodium azide (0.01 mol) and NH_4Cl , in DMF (10 mL) (Helen P Kavitha *et al.*, 2000) was heated for 6 hours at 160°C. The solvent was removed under reduced pressure and the residue was dissolved in (50mL) water and acidified with dil. HCl to pH. The solution was cooled in ice bath to give a precipitate which was recrystallized from aqueous ethanol.



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Anal. Calcd. For $C_{10}H_8SN_4O_4$: C, 52.16; H, 2.98; N, 08.16; O 11.50; Found: C, 52.25; H, 3.03; N, 08.21; O, 11.57; Yield % (79), ES (+) 280 (M+H); M.p.: 231–235 °C; 1H NMR [DMSO- d_6 , ppm]: δ 7.90 (Ar-H, multiplet), δ 12.5 (Ar-OH, singlet)ppm; ^{13}C -NMR [DMSO- d_6 , ppm]: δ 169 (OH), δ 155 (C=N)ppm; IR KBr (cm^{-1}): 1607.19 (C=Nstr), 1412.38 (N=Nstr), 3513.80 (OH str).

Synthesis of metal complexes (1-5)

4-((H-tetrazol-5-yl) thio) 2-acetoxybenzoic acid (0.01M) was dissolved in ethanol than solid $M \cdot Cl_2 \cdot 6H_2O$ (0.02M) (where M= Zn, Cu) was added to reaction mixture. The resulting reaction mixture was refluxed for 24hours in the presence catalytic amount of NH_3 with continuous stirring. After completion of the reaction, the resulting solid was filtered and washed with cold methanol and dried at room temperature.

Zinc complex (3) [Zn (L) $_2$]

Anal. Calcd. For $C_{20}H_{16}N_8ZnS_2O_8$: C, 47.02; H, 2.31; N, 07.66; O, 12.54; Found: C, 47.17; H, 2.39; N, 07.70 ;O, 12.61; Yield % (68), ES (+) 625.83 (M+H); 1H NMR (DMSO- d_6) δ 7.2(Ar-H, multiplet), δ 12.5.0 (Ar-OH, singlet), ^{13}C -NMR: δ 178 (OH), δ 150.7(C=N), IR KBr (cm^{-1}): 1629.01(C=Nstr), 1547.28 (C=Cstr), 2919.18 (OH str) 3010.23 (C-Hstr) 505.72(N-Zn).

Copper Complex (4) [Cu (L) $_2$]

Anal. Calcd. For $C_{20}H_{16}N_8CuS_2O_8$: C, 44.02; H, 2.11; N, 03.67; O, 12.57; Found: C, 45.12; H, 2.48; N, 07.73 ;O, 12.49; Yield % (82), ES (+) 624.99 (M+H); 1H NMR (DMSO- d_6) δ 7.4 (Ar-H, multiplet), δ 12.7 (Ar-OH, singlet), ^{13}C -NMR: δ 168 (OH), δ 152.3(C=N), IR KBr (cm^{-1}): 1692.99 (C=Nstr), 1554.21 (C=Cstr), 2919.78 (OH str) 3012.89 (C-Hstr) 450.84(N-Cu).

BIOLOGICAL EVALUATION**ANTICANCER ACTIVITY**

MTT (3-4, 5 dimethylthiazol-2-yl-2, 5-diphenyl tetrazolium bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilisation of cells by the addition of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created. The color can be quantified using a multi-well plate reader. DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plate and wash beaker were from Tarson (India).

Cell culture

A549 (human lung carcinoma) cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 μ g/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37°C.

MTT Assay

The SAMPLE 6 was tested for in vitro cytotoxicity, (V. H. Bhaskar, P. B. Mohite et al, 2011) using A549 cells by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured A549 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 μ L) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the SAMPLE 6 sample in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (20 μ L of 5 mg/ml)

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was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 μ L) were aspirated off the wells and washed with 1X PBS (200 μ l). Furthermore, to dissolve formazan crystals, DMSO (100 μ L) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC₅₀ value was calculated using GraphPad Prism 6.0 software (USA).

$$\% \text{ Cell viability} = [\text{A}] \text{ Test} / [\text{A}] \text{ control} \times 100$$

Molecular Docking

Docking studies (Software Details)

Docking studies (R.K. Gundampati et al., 2012) of ligand (L) and metal complexes have been performed using HEX 6.1 software which is an interactive molecular graphics program for the drug – protein binding interaction. The structure of the complexes were sketched by CHEMSKETCH (<http://www.acdlabs.com>). The crystal structure of EGFR kinase domain (PDB ID: CYP3A4 lung cancer) in complex with an irreversible inhibitors was obtained from the protein data bank. (<http://www.rcsb.org.pdb>). Visualization of the docked poses has been done by using PyMOL software.

Reaction Scheme

Scheme 1 Schematic route for the synthesis of ligand (L) and its metal complexes

RESULTS AND DISCUSSION

In the present work, novel seven Schiff bases and their metal complexes were synthesized as outlined in the Scheme 1. The substituted tetrazole and 4-((H-tetrazol-5-yl) thio) 2-acetoxybenzoic acid (L) and their metal complexes (2) were synthesized by reported procedures (S. Voitekhovich et al, 2006). Schiff bases and their metal complexes were obtained by condensation of both the moieties. The formation of compounds (L) and their metal complexes was evidenced by appearance of a band between 1631-1602 cm^{-1} for C=N, the infrared spectra of the tetrazole derivative TT1 reveal the presence of bands at 1450 cm^{-1} respectively, indicating the presence of N=N bond in all these tetrazole derivative. Similarly, the appearance of bands at 1272 cm^{-1} in the tetrazole derivative, TT 1 respectively reveals the presence of the cumulative bond, N=N=N, in all theses tetrazole compound in the IR spectra, presence of a peak in ¹³C NMR spectra with a δ value between 164.4-141.1 for two carbons of C=N. The appearance of a band between 1735-1658 cm^{-1} for C-O of (L) and their metal complexes in the IR spectra; a peak in ¹³C NMR spectra with a δ value between 148.5-131.0 for carbonyl carbon of (L) and their metal complexes. The presence of the OH group of (L) and their metal complexes was indicated at 3292-3057 cm^{-1} in the IR spectra; presence of a singlet in the ¹H NMR spectra at δ value 12.5-11.4. The OH of (L) and their metal complexes which undergoes tautomerisam was indicated by a band at 3178-2868 cm^{-1} and by a singlet peak at δ value 11.0-9.44 in ¹H NMR spectra. The presence of tautomeric form was also confirmed by a sharp band of C-S around 700-600 cm^{-1} and a peak at 172.2-180 in ¹³C NMR spectra. Mass spectra of the ligand and its metal complex show molecular ion peaks, which are in good agreement with the expected values. The mass spectrum of ligand L gives a peak at 222.20 m/Z, which is assigned for [L+H] peak. Zn(II) > Cu(II) complexes gives molecular ion peak at 625.04 and 624.23 m/Z respectively and are assigned as [M+1] peak.

ANTI-CANCER ACTIVITY

All the synthesized compounds (1-3) were evaluated for their *in vitro* inhibitory activities against four Lung cancer cell lines (MCF-7) using MTT assay. The results as IC₅₀ are mentioned in the Table - 1. The following table (Table - 1) shows the anti-cancer activity of the synthesized compounds (Ligand). All the synthesized compounds were screened for cytotoxicity on lung cancer cell line by MTT method. Cytotoxicity was checked at 24 hours and 48 hours duration. It was found that the activity of the compounds was increased after 48 hours as compared to 24 hours.



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Among the tested compounds ligand and Cu-ligand showed potent activity and their % growth inhibition was 97.477 and 76.7878 at 100 $\mu\text{M/ml}$. Compounds ligand and Cu-ligand were showed IC_{50} 50 $\mu\text{M/ml}$ and 47.1114 $\mu\text{M/ml}$ (Table-1).

Docking Study

The interaction residues and energy values of the synthesised compounds with the target.

Molecular docking technique allows us to understand the interaction between a drug and protein at the molecular level (Schrodinger Release et al., 2017). In the present study, molecular docking of the compounds (ligand, Zn-ligand and Cu-ligand) with protein the crystal structure of EGFR kinase domain (PDB ID: 1NOW) was performed in order to rationalize the mode of protein and most favourable binding conformations of the molecules. (Figure.3) shows the minimum energy docked pose of the compounds (ligand, Zn-ligand and Cu-ligand) from the results it is clear that, the compounds (ligand, Zn-ligand and Cu-ligand) interact with protein via intercalation mode of binding. This could be explained by the fact that, stacking interaction of compounds (ligand, Zn-ligand and Cu-ligand) with oxygen atom of the phosphate backbone leads to the formation of stable complex as reported in literature. The resulting relative binding energy of compounds (ligand, Zn-ligand and Cu-ligand) with protein were found to be -243.78, -273.1 and -281.12 KJ mol^{-1} , respectively. The results of the docking view revealed the fact that the complexes bind with protein via intercalation and that the complexes stabilize the protein by van der Waal's and hydrophobic interaction. It is also to be noted that the complexes exhibit more binding affinity than compounds (ligand, Zn-ligand and Cu-ligand). The binding energy of the complexes follows the order Cu-ligand > Zn-ligand > ligand which is in good agreement with the binding constants obtained from absorption and emission spectral study (Table – 2).

CONCLUSION

The present investigation is focused on the synthesis, characterization and biological activities of a series of tetrazole compounds from 2-acetoxy-4-thiocyanatobenzoic acid and metal complex. The findings are furnished below:

- Compounds ligand, Zn-ligand and Cu-ligand are prepared using scheme 1.
- IR, Spectra are taken. The results are in good agreement with the reported results.
- The ^1H NMR and ^{13}C NMR spectra of all the three compounds provide the expected signals.
- The mass spectra of all the one compounds were recorded.
- The Anti-cancer activity is studied and the compound ligand has maximum activity.
- Docking study is done and the compound Cu-Ligand are found to fit well with the target protein.

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Table - 1: % Growth of inhibition of Ligand synthesized against MCF 7 Cell line

Compound	% Growth inhibition				
	0.25 μ M	2.5 μ M	25 μ M	50 μ M	100 μ M
Ligand	0.5210	2.1774	20.2214	37.125	97.477
Zn-Ligand	0.4270	3.1400	25.2587	21.2570	65.1898
Cu-Ligand	0.1450	1.2047	18.8520	47.1147	76.7878

Table - 2: Energy values of the synthesized compound

S.No	Compound	Energy
1	Ligand	-243.78
2	Zn-Ligand	-273.10
3	Cu-Ligand	-281.11

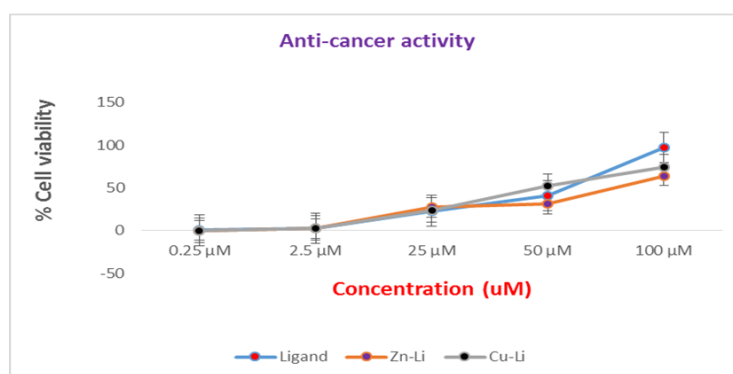


Fig.1 Anti-cancer activity of the synthesized compounds





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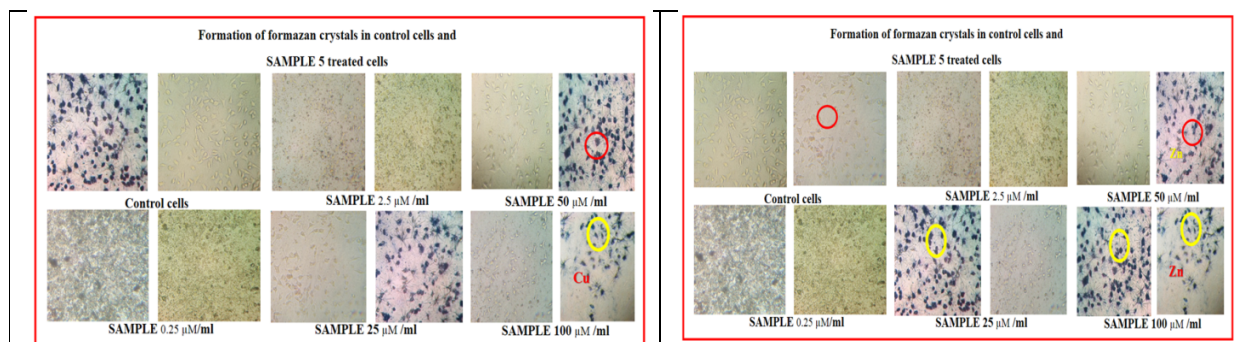


Fig.2. Formation of formazan crystals in control cells and sample treated cells

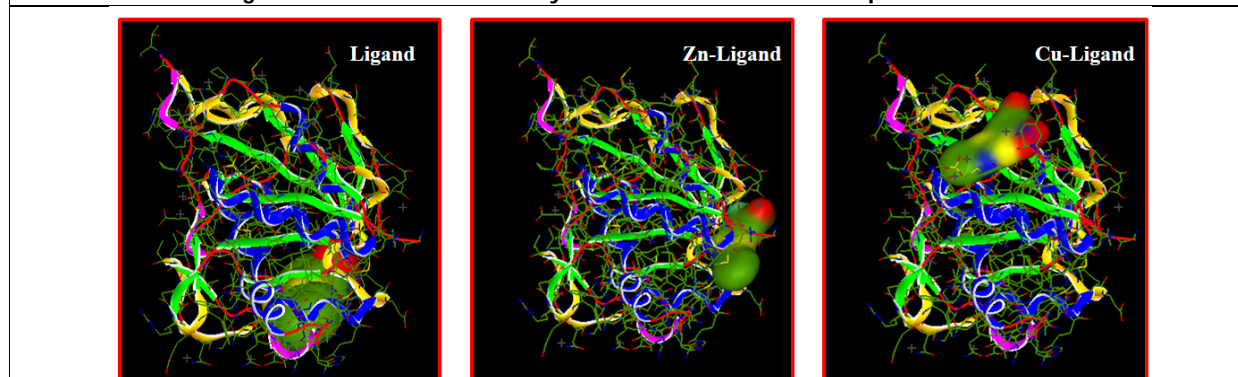


Fig .3 Molecular docked model of compounds (Ligand, Zn-Ligand and Cu-Ligand)





Immunostimulation of Dietary Prebiotic Mushroom and Disease Resistant of *Oreochromis mossambicus* Against Septicemia

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ABSTRACT

The effect of diet supplemented with *Pleurotus* fed for 30 days was investigated in tilapia challenged with *A. hydrophila* & *E. tarda* infected and treated fish had a significantly higher percent weight gain, feed efficiency in fed with enriched diet and challenged with *A. hydrophila* & *E. tarda*. The mortality rate was continued up to 14 days. Relative percent survival of *O. mossambicus* after challenging *E. tarda* and *A. hydrophila* in differential experimental groups. The highest survival was recorded in T3 group followed by T2 group and lowest survival was observed in T4 and followed by control group. The mortality rate declined with a consequent rise in survival rate than with other pathogen on the other hand in groups fed with *pleurotus* enriched diet and challenged with *A. hydrophila* & *E. tarda* the both (cellular and humoral) immune response such as neutrophil activity, lysozyme activity, significantly higher than in the control group. The result reveal tilapia fed for 30 days with *Pleurotus* enriched diet had higher cellular & humoral immune response, disease protection from *A. hydrophila*, than the group fed on basal diet with the protection linked to stimulation of immune system were statistically significant ($p < 0.05$). The maximum influence on immune response occurred in tilapia fed with 2% mushroom extract, as a result of the challenge test, the survival rate was found to be the highest in the 2% *Pleurotus* supplemented feeding group. The results suggested that fish fed with *Pleurotus* mushroom extract supplemented diet enhanced the immune response of fish and decreased the mortality rate in tilapia against *A. hydrophila* and *E. tarda*.

Keywords: Immunity, *Aeromonas hydrophila*, *Edwardsiella tarda*, Tilapia, AgNPs.



**Shyamala and UmaMaheswari****INTRODUCTION**

Nanotechnology is the use of science to control substance at the molecular level and it involve a wide range of technology such as material science to biotechnology where it is useful in the field of diagnostics and new delivery systems. It searches for particles in the dimension smaller than 100 nm and works on design, synthesis and manipulation of their structures and applications. The potential benefits of nanomaterials for human health and environment are reported by several researchers (Bhatt., 2003; James and Browning, 1999). There is a investigate for nanoparticles produced by microorganisms and isolation, characterization, uptake and subcellular distribution of several nano materials have been brought to light. However, only a few nanoproducts are currently in use for medical purposes such as disease diagnosis and drug delivery. The development of better experimental procedures for the synthesis of nanoparticles of different chemical composition, sizes, shapes and controlled polydispersity is vital in this field (Sanjeeb and Vinod., 2003; Goodsell, 2004).

The tilapia, *Oreochromis mossambicus* is an eminently commercial fish species in aquaculture. The *O.mossambicus* culture in fresh water is a popular aquaculture activity worldwide including turkey. However, the diseases caused by bacterial pathogens in their culture are becoming severe and result in significant morbidity and mortality (Dalsgaard and Madsen, 2000). They usually suffer the bacterial infections, particularly the occurrence caused by *A. hydrophila*, *E. tarda*, which results in heavy losses and cause economic loss to fish farmers. In order to control the proliferation of these bacteria, antibiotics are used widely in intensive aquaculture. Immunostimulants increase resistance to infectious disease ,not only by stimulating the acquired immune response ,but also by enhancing innate immune mechanisms(Galindo-villegas and Hosokawa,2004)up to now, many immunostimulants have been shown to be effective in fish (Award and Austin,2010, Bilen et al.,2011,Binaii et al.,2014.,Tang et al.,2014.,Wang et al.,2015).

Pleurotus ostreatus is a commercially important edible mushroom, which is also known as the oyster mushroom. The various medicinal effects of *P. ostreatus*, such as anticancer, immunomodulatory, antiviral, antibiotic anti-inflammatory and cholesterol-lowering activities are known worldwide (Bhat et al., 2011; Jagadeesh et al., 2015; Mustafa et al., 2015). The antifungal activity of silver nanoparticules synthesised using the culture supernatant of *P. ostreatus* have been reported (Shanmtyan, et al., 2004). However, there are no reports on the green synthesis of AgNPs using the basidiocarps of *P. ostreatus*. Hence, a study was carried out to explore the effects due to administration for shorter duration on immune mechanism to *E. tarda* and *A. hydrophila*.

MATERIALS AND METHODS

Silver nitrate ($\geq 99.0\%$, AgNO_3) and all the other chemicals were purchased from Sigma Aldrich. Mushroom was purchased commercially from ICAR, Aduthurai, Kumbakonam.

Experimental animal and their maintenance

Healthy tilapia wet weight: 125 ± 2.10 g; length: 12.3 ± 0.5 cm were bought from a nearby fish farm, Kumbakonam, Tamilnadu, India and refined from dechlorinated water. Before the treatment all experimental fish were acclimatized at $\text{pH } 7.0 \pm 0.2$ at a constant temperature of $22 \pm 1^\circ\text{C}$ and aphoto-period of 16 h: 8 h (light: dark) for two weeks and fed once every a day with control feed. .

Mushroom sample and strains

Pleurotus ostreatus fresh basidiocarps were collected from ICAR, Aduthurai, Kumbakonam, TamilNadu, India. The basidiocarps were sliced, oven-dried at $50 \pm 2^\circ\text{C}$ for 24 hours and ground to fine powder (Harikrishnan, et al., 2011). Ten grams of the *P. ostreatus* powder was soaked in distilled water in a ratio of 1:10 (w/v) and boiled for 30 min at $60 \pm 2^\circ\text{C}$. The boiled mushroom powder was left covered in room temperature for 30 min and filtered. Suspended



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residues were removed by centrifuging the filtrate (10,000 x g for 30 min at 4°C) and the supernatant collected was filtered through Whatman No.1 filter paper. The filtrate was freeze-dried (Christ, model Alpha 2-4 lyophilizer, The Netherlands) at $-53 \pm 2^\circ\text{C}$ for 48 h. The freeze-dried powder was used as the aqueous extract and stored at 4°C prior to use (Harikrishnan, et al., 2011; 2012).

Biosynthesis of silver nanoparticles (AgNPs)

Different concentrations (1-6 mg/mL) of the aqueous extract of *P. ostreatus* was added to 5 mL of 1 mM aqueous silver nitrate (AgNO_3 ; Sigma Aldrich) solution and kept at $28 \pm 2^\circ\text{C}$ in dark incubation for the bio reduction of Ag^+ ions to Ag^0 . The mixed solution was continuously stirred and incubated for 6, 12, 18, 24, 30, 36 and 40 hours. The color change of AgNO_3 solution was monitored. The fully reduced solution was centrifuged at 20,000 x g for 30 min. The residue was retained after discarding the supernatant. The residue was washed in sterile distilled water and dried for further studies (Sastry, et al., 2003; Arun, et al., 2014).

Diet Preparation

A basal diet was obtained commercially which formulated to contain 13% moisture, 47% protein, 12% oil, 16% crude ash and 3,500 kcal/g energy for tilapia. The basal diet served as the control diet and no mushroom extract was added to the control diet. The experimental diet was prepared using the basal diet supplemented with 0.1%, 0.25%, 0.5% and 1% *P. ostratus* AgNPs. The commercial feed was blended first, then combined with the mushroom *P. ostratus* AgNPs with water (100 mL of water/kg of diet) to form a paste, which passed through a meat grinder and pelleted again to produce 2.0-mm pellets. The feed was air dried at room temperature (25°C) for at least 48 h and it was stored at 4°C until further use (Sattanathan et al., 2020).

Experimental design

Experiments were performing in rectangular plastic tubs (95 × 70 × 60 cm, 180-L) with lids and the water use for rearing was drawn from dechlorinated water. Tilapia fishes ($n=375$) were distributed into 15 tanks with each tank containing 25 fish were maintained triplicate. The fish were given a basal diet without supplementation of mushroom AgNPs as a control diet (C-basal diet; T1 (0.1 %); T2 (0.25%); T3 (0.5%); T4 (1.0%) for a duration of 28 days. The fishes were fed with the investigational diet at the rate of 4% of body weight twice per day, at 09.00 and 17.00 h. for 30 days. Six fish from each experimental group were sample and blood was drawn on 7, 14, 21 and 28 days for various immunological parameters. left over fish were challenged with virulent *A. hydrophila* and *E. tarda* 30 days after post feeding and the relative percentage survival (%) was recorded over 14 days post treatment.

Blood collection

Blood and serum samples were collected from fish on 7, 14, 21 and 28 days post treatment. Blood was drawn from the caudal vein of each fish using a 1.0 ml hypo-dermal syringe and 24-gauge needles, which was rinsed with 2.7% EDTA solution before use. The collected blood was immediately transferred to a test tube coated with a thin layer of EDTA (as an anticoagulant) and shaken to prevent hemolysis and blood clotting. Serum was collected without using anticoagulant and was separated from the remaining blood by keeping the tubes in a slanted position for about 2 h and then centrifuged at 3500 rpm for 15 min at 4°C for further analysis (Baba, et al., 2015).

Immunological parameters**Neutrophil Activity**

The modified Stasiack and Bauman method (1996) for the NBT (Nitro Bluetetrazolium) assay was followed. To facilitate the adhesion of the cells, 100 μl of blood was placed into the wells of flat bottom micro plates and incubated at 37°C for 1 hour. The supernatant was removed and the loaded wells were washed three times using phosphate buffer saline (PBS, pH 7.5). After washing, 100 washing I of 0.2% NBT was added and the plate was further incubated for 1 hour. The cells were mixed with 100% methanol for 2-3 minutes and then washed three times with 70% methanol. The plates were then air dried. 120 μl 2N KOH and 140 μl DMSO (dimethyl sulfoxide) were added to





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each well to form a pharماسone blue precipitate. The OD of the solution was then read into a microplate reader (Systronics, India) at 620 nm.

Serum Lyzyme Activity

Lysozyme activity was measured by the turbidimetric assay of Parry et al., (1965) with the microplate optimization of Hutchinson and Manning (1996). In this technique, 0.05% lyophilized *Micrococcus luetus* in 0.05 mM sodium phosphate buffer (pH 6.2) was used as the substrate. Ten microliters of fish serum were added to a 250 µl bacterial suspension in a microplate and a reduction in absorbance was determined at 490 nm after 0.5 and 4.5 minutes of incubation at 22 ° C using a microplate reader. One unit of lysozyme activity was defined as a decrease in absorbance of 0.001 per minute.

Myeloperoxidase Activity

The total myeloperoxidase activity of the serum was described by Quade and Roth (1997) and partially modified by Sahu et al. (2005). Briefly, 10 mL serum was diluted with 90 mL of HBSS with Ca²⁺ + or Mg²⁺ + in a 96-well microtitre plate containing 25µl 20 mM 3,3',5,5'-tetra methyl benzene hydrochloride (TMB) (Genie, India) and 5 m H₂O₂ with H₂O₂ added at 1:20 dilution. After 2 minutes of incubation, 50µl of 4 M sulfuric acid (H₂SO₄) was added to stop the reaction. The optical density was read at 450 nm in a microtitre plate reader (Cyper Lab).

Serum Antiprotease Activity

The serum antiprotease assay was performed as described by Rao and Chakraborty, (2004). Briefly, 10 µl serum incubation was performed with 100 mL trypsin (type 1 from bovine pancreas) for 30 min at 25 ° C. Two blank (110 µl PBS) and three references (10 mL PBS / 100 mL trypsin) were also taken. Each of the reaction mixtures was added with 1 µl casein for 15 min. The reaction was stopped by adding 500 addingl of 10% trichloroacetic acid (TCA) to all. The mixture was centrifuged at 5,551 xg for 5 min and the optical density of the supernatant at 280 nm was taken. Percent blocking was calculated using the following formula:

$$\text{Percent Prohibition (\%)} = \frac{\text{OD reference} \times \text{OD sample}}{\text{OD reference}} \times 100$$

Preparation of viable leukocytes from peripheral blood

For the dissociation of peripheral blood leukocytes, fish should be fed 5–5 ml syringe-filled 2 ml blood collecting medium (RPMI-1640 supplemented with 50,000 IU-1 sodium heparin, 1,00,000 IU-1 penicillin and 100 mg L-1 streptomycin). Diluted blood was carefully visualized on the same volume of lymphocyte dissociation medium (Lymphosep, ICN Biomedicals, Inc., USA) and cells were reduced to 800 g for 20 min. Leukocytes were collected at the interface and washed twice with medium (RPMI-1640 supplemented with 10,000 IU-1 sodium heparin, 1,00,000 IU-1 penicillin, and 100 mg L-1 streptomycin) and RSMI through the culture medium. Resuspended in 1640 supplemented with 3% (v / v) of tilapia serum, 1,00,000 IU-1 penicillin, 100 mg L-1 streptomycin and 4 mM L-glutamine, Biochrome AG, Germany). The number of viable cells was enumerated using the trypan blue exclusion method and adjusted to 4 x 10⁷ cells through ml-1 use culture medium.

Production of reactive oxygen species

Intracellular respiratory burst activity was measured by the method of Secombes (1990) with minor modifications. Peripheral blood leukocytes (1x10⁶ cells / well) were incubated with 25 µl of 25 µM nitroblue tetrazolium (NBT, 1 g L-1) in 175 forl culture medium at 28 ° C for 2 hours. The supernatant was carefully removed and the cells were mixed in 100% (v / v) methanol for 5 min. Each well was washed twice with 125 µl of 70% (v / v) methanol. The fixed cells were allowed to air-dry overnight. Low NBT (as formazan) was dissolved using 125 N 2 L potassium hydroxide





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(KOH) and dimethyl sulfoxide (DMSO) per 150 DLT per well. The optical density was measured in a microplate reader at 650 nm.

Production of reactive nitrogen species

Nitric oxide (NO) released by peripheral blood leukocytes in the medium was measured using the Griess reagent (Palacit al., 2005). Leukocytes produce nitric oxide which is rapidly converted to more stable nitrate. The nitrite present in the culture supernatant can be measured colorically by adding Grimase reagent and converting it to pink. Peripheral blood leukocytes were cultured in a chamber in 175 h culture medium with 96% 1% copper sulfate solution at 28 ° C. Fifty microliter supernatants of the culture were collected and transferred to a separate microtitre plate. For each well containing the culture supernatant, 50 g of grease reagent (1% sulfalamide, 0.1% N-naphthul-ethylenediamine, 2.5% phosphoric acid) was added. After 10 minutes of incubation, molar concentrations of NO₂ were read from a standard curve generated before a hierarchical series of NaNO₂ concentration in the culture medium.

Post challenge

After the immunstimulation trial for 28 days, 50 fish in duplicate tanks under each treatment group were pooled together. Then the fish were distributed in four tanks under two subgroups for challenge study each containing 12 fish. Fish under first and second group were injected with the pathogens obtained from IMTECH, Chandigarh, India at a level of 0.1 mL suspension of *A. hydrophila* (1.7x10⁸ cfu/per fish, 10 times of the LD₅₀ concentration) in PBS and 0.1 mL suspension of *E. tarda* (1.3x10⁸ cfu/per fish, 10 times of the LD₅₀ concentration) in PBS, respectively. The cumulative mortality and relative percent survival (RPS) of challenged fish was observed up to the 14 days after challenge.

$$\text{Relative percentage survival (RPS)} = \frac{\text{Number of surviving fish after challenge}}{\text{Number of fish infected with bacteria}} \times 100$$

Statistical analysis

The data be statistically analyzed using the statistical package SPSS version 21 in which data were subjected to one-way ANOVA; Duncan's multiple range test (DMRT) was used to determine the significant differences between the means (S.E.M). The level of significance was set at p<0.05

RESULTS

The neutrophil activity (NBT activity at OD 620 nm) of the treatment groups were found to have a different values that are statistically significant (p<0.05), in comparison with the control groups and the highest value has been observed in T2 group on the 14 and 21st day gradually decreased (Fig 1; Table 1). Though neutrophil activity was always higher in treated group than control group, the difference was significant on 14 and 21. The lysozyme activity of the probiotic mushroom treated fish began to rise significantly (p<0.05) from the day 7 to day 21 of administration. Lysozyme levels higher in the treated group than the control group on 14 and 21 and there was no significant difference between other treated groups (Fig 2; Table 1) T2 (0.25%). The maximum level of myeloperoxidase activity was found in T3 treated groups when compared to control and significant higher (p<0.05). The minium myeloperoxidase activity was noted in control. The highest antiprotease level was found on 14 and 21 days of post treatment (Fig 3; Table 1). Similarly, results showed that highest level of antiprotease activity such as trypsin inhibition (p<0.05) in T2 group at 14th day of experiment than other groups, however decreased on 21 and 28th day of experiment (Fig. 4; Table 1) T3 (0.5%) 1.39 ± 0.09.

There was a significant increase in ROS and RNS level from 7 to 14. The increased level was maintained up to day 21, then decreased by day 28, which treated 0.5% probiotic mushroom treated group (Fig. 5 and 6; Table 1). However there was no significant difference of ROS and RNS level between the treated groups and control group T3 (0.5%) 0.0061 ± 1.4 and T3(0.5%) 0.029 ± 0.04.



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Mortality was observed from 2nd day of the post challenge which was confirmed due to the challenge infection with *E. tarda* and *A. hydrophila*. The mortality rate was continued up to 14 days. Relative percent survival of *O. mossambicus* after challenging *E. tarda* and *A. hydrophila* in differential experimental groups (Fig. 7). The highest survival was recorded in T3 group followed by T2 group and lowest survival was observed in T4 and followed by control group.

DISCUSSION

In recent years there is an increasing demand for the nanoparticles in the field of metal industries, and biomedical science etc. Nowadays the nanoparticles are even used in the household appliances. Different health benefits have been attributed to *Pleurotus* spp. They are an important source of bioactive compounds that have anticancer, antihypertensive, anti-inflammatory, antimicrobial, antinociceptive, antioxidant, antiviral, hypolipidemic, immunomodulatory, cytoprotective, neuritogenic, antiaging and anticataractogenic effects. Among the mycochemicals related with these bioactivities are polysaccharides, peptides, proteins, triterpenes, polyketides, nucleotides and an array of PC (Wang et al., 2000). In the present study *P. osteratus* synthesized AgNPs as supplement feed on immunity in *O. mossambicus* against *E. tarda* and *A. hydrophila*.

Mushrooms immunomodulators exhibit stimulating activities for both innate and adaptive immune systems. They proliferate and activate innate immune system components such as natural killer (NK) cells, neutrophils, and macrophages, and stimulate cytokines expression and secretion. These cytokines in turn activate adaptive immunity through the promotion of B cells for antibodies production and stimulation of T cell differentiation to T helper (Th) 1 and Th2 cells, which mediate cell and humoral immunities, respectively (Ramy et al., 2014). Based on their high molecular weight, mushroom polysaccharides are not able to penetrate the immune cells to activate immune cells directly. Thus, the stimulation mechanism of polysaccharides involves different cell receptors such as dectin-1, Complement receptor 3 (CR3), Lactosylceramide (LacCer), and Toll-like receptor (TLR). In such cases, the effectiveness of polysaccharides is governed by their binding affinity to immune cell receptors (Sastry et al., 1997).

Superoxide anions are important antimicrobial effectors. In this study, NBT reduction activity showed an increase in T2 groups. Similar result was observed by Bilen et al. (2011) when rainbow trout was treated with dietary tetra leaves. Powdered ginger (*Zingiber officinale*) showed an elevated superoxide anion production in rainbow trout (Haghighi and Rohan, 2013). Similarly, water soluble extraction of *Nyctanthes arbortristis* leaves in *Oreochromis mossambicus* showed an enhancement of NBT (Devasree et al., 2012). In the present study the different dosages of *P. osteratus* AgNPs significantly increased neutrophil activity when compared to control groups. Lysozyme activity is another component in the first line of barrier in defense system. Lysozyme has bactericidal effect by hydrolyzing β (1 \rightarrow 4) linkages of bacterial cell wall peptidoglycans resulting in bacteriolysis (Magnadottir, 2006). Immunostimulant compounds are considered to increase serum lysozyme activity (Kumar et al., 2013) following the application that demonstrated in a number of fish species (Sattanathan et al., 2019; Awad et al., 2013). In the present study, fish fed diets supplemented with different levels of mushroom extract AgNPs showed significantly higher lysozyme activities when compared to the control group. The increased activity of lysozyme in the serum supported the role of mushroom extract supplemented diet in enhancing the non-specific immune response in tilapia.

Myeloperoxidase (MPO) is another important enzyme which plays a role in the killing of microorganisms (Abdel-Tawwab et al., 2010). In this study, MPO activity of serum in the experimental groups showed an increase compared to the control, especially after feeding with 0.5% mushroom AgNPs supplemented diet. Similarly, MPO activity increased significantly in *O. mossambicus* fed with diets supplemented with different levels of extract of *Camellia sinensis* (Alexander and Ingram 1992). Many authors reported an enhancement of MPO activity after using immunostimulant compounds in fish (Sattanathan et al., 2019). ROS-mediated oxidative damage to macromolecules namely lipids, proteins and DNA have been implicated in the pathogenicity of major diseases. The oxidative stress



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implicated in the pathology of a number of disorders, such as atherosclerosis, ischemia–reperfusion injury, cancer, malaria, diabetes, inflammatory joint disease, asthma, cardiovascular diseases, cataracts, immune system decline, play a role in neurodegenerative diseases and aging processes (El-Boshy et al., 2010). Furthermore, nanoparticles are also shown to cause toxicity by increasing concentration of intracellular ROS and decrease in antioxidant level (Kathatun et al. 2007; Sattanathan et al., 2019). The increase in ROS level is also an important indication of predominant mechanism of acute toxicity (Nya, and Austin 2009). Activated macrophages express NOS and then increases NO synthesis. NO plays a dual role in the inflammatory process and has been shown to be involved in numerous regulatory functions, ranging from altering the cardiovascular system to modulating neuronal functions. SOD reacts with O₂ at rates limited only by diffusion and enhanced by electrostatic guidance (Bilen et al., 2014), providing a highly effective means of removing O₂. Our results indicates that the NOS activities had significantly increased in the 0.5% AgNPs treated group compared to the control (P < 0.05), but 1% AgNPs treated group was almost similar to the control (P > 0.05).

Mushroom extracts have shown varied antimicrobial activities against Gram-positive and Gram-negative bacteria. This observation agrees with findings of a study on antimicrobial activities and mineral compositions of shiitake mushrooms cultivated on agricultural wastes (Düğenci et al., 2002). Survival rate in *A. hydrophila* and *E. tarda* challenged tilapia was affected by dietary intake of mushroom AgNPs of *P. astratus*. No differences were observed in AgNPs groups compared to control. These findings indicated the effectiveness of 0.5% AgNPS against *A. hydrophila* and *E. tarda* infection. Similarly, Bilen et al. (2014) found an increased survival rate when rainbow trout treated with methanolic extracts of tetra in koi fish against *A. hydrophila*. In line with our result, an increased survival rate during *A. hydrophila* infection was observed in *O. mossambicus* treated with Chinese herbal mixture composed of astragalus, angelica, hawthorn, Licorice root and honeysuckle (Tang et al., 2014) dietary *Saccharomyces cerevisiae* (El-Boshy et al., 2010) and green tea (*Camellia sinensis*) (Abdel-Tawwab et al., 2010) and in *O. mossambicus* treated with hot-water extract of *Toona sinensis* (Wu et al., 2010). According to several studies (Rattanachaikunsopon and Phumkhachorn, 2009; Chang et al., 2013, Chang and Cheng, 2014) immunostimulants can enhance resistance of fish to several bacterial pathogens including *L. garvieae*. Wu et al. (2010) reported that a dietary hot-water extract of *Eichhornia crassipes* leaves administration at 2.0 and 3.0 g kg⁻¹ for 12 days significantly increased survival rates of tilapia against *E. tarda*. Similarly, the different products of *E. crassipes* administration increased survival rates of *O. mossambicus* against *E. tarda* (Abdel-Tawwab et al., 2010). In some other studies were reported in the tilapia, the decreased mortality on *A. hydrophila* and *E. trada* challenge was reported in *O. mossambicus* fed *Zingiber officinale* (Nya and Austin, 2009), *Lupinus perennis*, *Mangifera indica* and *Urtica dioica* (Awad and Austin, 2010), *Allium sativum* (Nya and Austin, 2011). They did not record any mortality after challenge. It might be the pathogen concentration, water temperature value or fish species which play a critical role in challenge trial. The present study demonstrated that feeding the fish with mushroom extract AgNPS supplemented diet remarkably increased the relative percentage of survival rate of *O. mossambicus* against *A. hydrophila* and *E. tarda* pathogen (Sattanathan et al., 2019; Sattanathan et al., 2020).

Challenge test results showed no difference in survival rate in control and treatment groups. Similarly, oyster mushroom did not cause any difference in survival of rainbow trout 297 after challenged with the *A. hydrophila* (Sattanathan et al., 2019). Therefore, our results suggest that administration of dietary black cumin extract to rainbow trout did not elevate fish resistance to *A. hydrophila*. In conclusion, it was clear from the results that the supplementation of mushroom AgNPs at certain levels in the diet of fish significantly decreased the mortality of *O. mossaambicus* experimentally infected with *A. hdyrophila* and *E. tarda* and could enhance the non-specific immunity. Mushrooms have been used as a dietary supplement or medicinal food in Korea, Japan, and China for over 2000 years; edible mushrooms are of great interest because they contain a large number of biologically active compounds, such as polysaccharides, glycoproteins, triterpenes, antibiotics and carbohydrates act as prebiotic. Prebiotics are reported to be particularly suitable to enhance the growth and activities of probiotics, bifidobacteria, and lactobacilli and suppress the growth of clostridia and bacteroides. There are great advantages of incorporating the mushroom extracts in diet as its polysaccharides exhibit immunomodulating properties. A number of studies



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suggested that the mushrooms polysaccharides possess antibacterial, antifungal, immunomodulating properties, including the enhancement of lymphocyte proliferation and antibody production. They did not record any mortality after challenge. It might be the pathogen concentration, fish species which play a critical role in challenge trial. The present study demonstrated that feeding the fish with mushroom extract supplemented diet remarkably increased the relative percentage of survival rate of *O. mossambicus* against *A. hydrophila* & *E. tarda* pathogen.

CONCLUSION

Prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of one (or) a limited number of bacteria in the intestine and thus improve host health. The main advantage of prebiotics over the probiotics is that they are natural feed ingredient their incorporation in the diet does not require particular precautions on their authorizations as feed additives may be more easily obtained in spite of some concerns about their safety and efficacy. In recent years there has been considered increase in the prebiotics using in aquaculture. The use of probiotics or beneficial bacteria, which control pathogens through a variety of mechanisms. Hence the study highlights that synthesis of silver nanoparticles from mushroom extract protects *Oreochromis mossambicus* from infection by *A. hydrophila* and *E. tarda*. This protection may be mediated by both specific and non-specific immune mechanisms, as demonstrated by the increased activity of several non-specific immunostimulatory parameters, such as increased levels of neutrophil, lysozyme, ceruloplasmin, and serum antiprotease activity, as well as ROS and RNS. Increased levels of non-specific immune response parameters and high survival suggest that *P. ostreatus* doses of 50mg/kg body weight are appropriate high survival rates. According to the findings of this report, synthesis of silver nanoparticles of mushroom extract administered as an IP treatment to fish could potentially substitute antibiotics. The immunostimulants contained in *P. ostreatus* extract improve fish health and, as a result, lower aquaculture costs.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Table: 1 Effect of dietary *P. osteratus* synthesized AgNPs on the immune response in tilapia, *Oreochromis mossambicus*. Values are expressed as mean \pm SE.

Parameters	Treatment groups	Treatment Days			
		7 th day	14 th day	21 st day	28 th day
Neutrophil activity	Control (0%)	0.017 \pm 0.02	0.021 \pm 0.02	0.024 \pm 0.02	0.018 \pm 0.03
	T1 (0.1%)	0.021 \pm 0.02	0.028 \pm 0.01	0.032 \pm 0.02	0.021 \pm 0.04
	T2 (0.25%)	0.022 \pm 0.01	0.027 \pm 0.06	0.032 \pm 0.03	0.03 \pm 0.05
	T3 (0.5%)	0.025 \pm 0.05	0.031 \pm 0.02	0.038 \pm 0.04	0.02 \pm 0.03
	T4 (1%)	0.023 \pm 0.06	0.03 \pm 0.01	0.029 \pm 0.02	0.013 \pm 0.02
Lysozyme activity	Control (0%)	180.43 \pm 33	315.33 \pm 22	216.32 \pm 21	130.76 \pm 22
	T1 (0.1%)	210.32 \pm 23	390.54 \pm 47	285.33 \pm 22	160.33 \pm 23
	T2 (0.25%)	225.34 \pm 12	416.43 \pm 25	312.43 \pm 25	215.33 \pm 42





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	T3 (0.5%)	205.22±23	372.54±22	240.32±25	210.53±25
	T4 (1%)	212.43±22	395.26±24	234.35±20	198.54±19
Myeloperoxidase activity	Control (0%)	175.5±54	310.43±38	200.43±28	180.34±23
	T1 (0.1%)	1.12±0.05	1.15±0.07	1.2±0.04	1.14±0.04
	T2 (0.25%)	1.25±0.04	1.27±0.03	1.32±0.05	1.03±0.02
	T3 (0.5%)	1.34±0.04	1.39±0.09	1.35±0.05	1.25±.009
	T4 (1%)	1.2±0.04	1.1±0.03	1.12±0.06	1.03±0.06
Antiprotease activity	Control (0%)	0.65±0.03	0.72±0.05	0.85±0.03	0.75±0.05
	T1 (0.1%)	0.67±0.02	0.79±0.04	0.89±0.05	0.98±0.04
	T2 (0.25%)	0.7±0.05	0.89±0.05	0.98±0.05	0.85±0.02
	T3 (0.5%)	0.74±0.02	0.91±0.06	1.08±0.02	0.99±0.06
	T4 (1%)	0.72±0.05	0.86±0.09	0.9±0.02	0.87±0.07
Reactive oxygen speices	Control (0%)	0.0023±1.3	0.0032±2.5	0.0043±1.44	0.0038±2.2
	T1 (0.1%)	0.0024±4.2	0.0035±3.4	0.0045±1.97	0.0039±2.31
	T2 (0.25%)	0.0032±2.5	0.0045±1.86	0.0053±2.7	0.0038±3.6
	T3 (0.5%)	0.0034±2.4	0.0051±2.6	0.0061±1.4	0.004±1.2
	T4 (1%)	0.0025±3.2	0.0048±3.1	0.0056±2.8	0.0038±3.8
Reactive Nitrogen species	Control (0%)	0.012±0.2	0.014±0.05	0.017±0.02	0.014±0.02
	T1 (0.1%)	0.013±0.04	0.017±0.2	0.019±0.04	0.016±0.05
	T2 (0.25%)	0.014±0.06	0.019±0.3	0.023±0.06	0.018±0.09
	T3 (0.5%)	0.015±0.8	0.021±0.4	0.029±0.04	0.019±0.04
	T4 (1%)	0.014±0.07	0.019±0.6	0.025±0.04	0.018±0.05

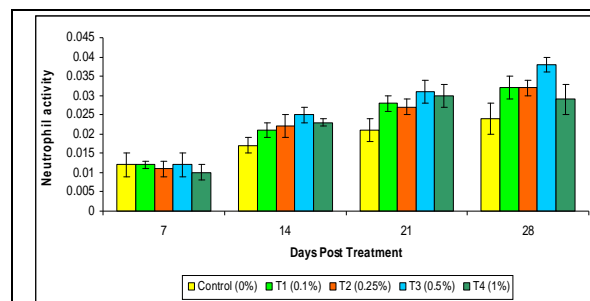


Fig. 1: Effect of dietary *P. osteratus* synthesized AgNPs on the neutrophil activity of tilapia, *Oreochromis mossambicus*. Values are expressed as mean ± SE.

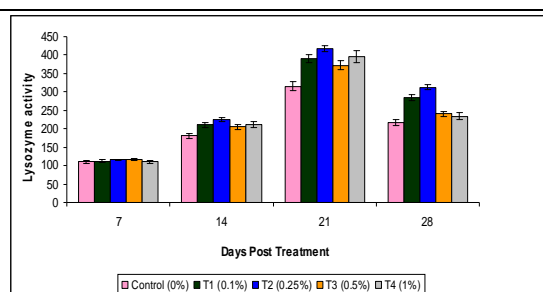


Fig. 2: Effect of dietary *P. osteratus* synthesized AgNPs on the lysozyme activity of tilapia, *Oreochromis mossambicus*. Values are expressed as mean ± SE.





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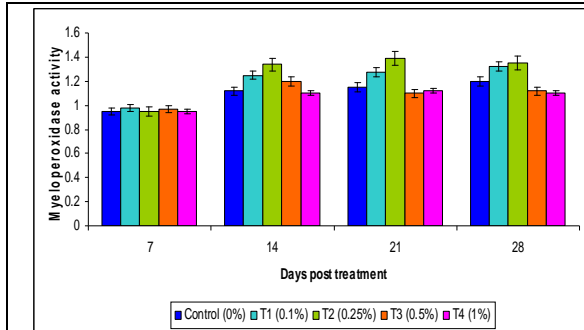


Fig. 3: Effect of dietary *P. osteratus* synthesized AgNPs on the myeloperoxidase activity of tilapia, *Oreochromis mossambicus*. Values are expressed as mean ± SE.

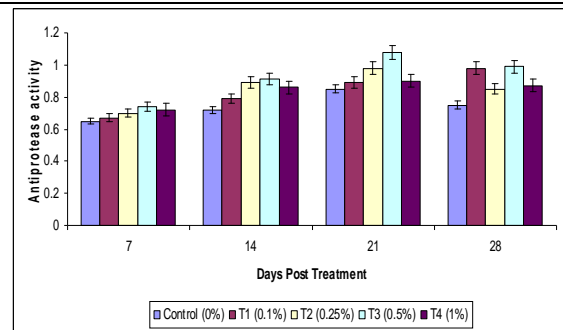


Fig. 4: Effect of dietary *P. osteratus* synthesized AgNPs on the antiprotease activity of tilapia, *Oreochromis mossambicus*. Values are expressed as mean ± SE.

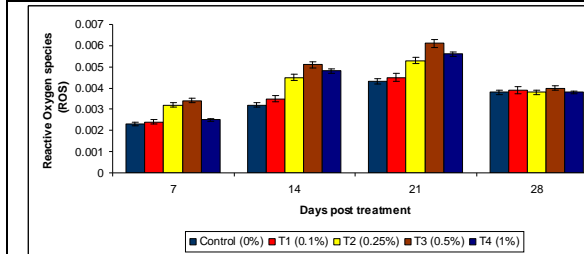


Fig. 5: Effect of dietary *P. osteratus* synthesized AgNPs on the Reactive oxygen species in tilapia, *Oreochromis mossambicus*. Values are expressed as mean ± SE.

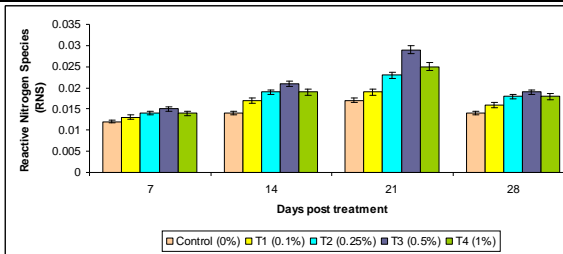


Fig. 6: Effect of dietary *P. osteratus* synthesized AgNPs on the Reactive nitrogen species in tilapia, *Oreochromis mossambicus*. Values are expressed as mean ± SE.

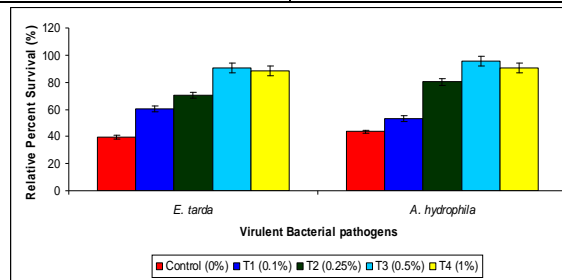


Fig. 7: Effect of dietary *P. osteratus* synthesized AgNPs on the relative percent survival (%) in tilapia, *Oreochromis mossambicus*. Values are expressed as mean ± SE.





Application, Spectral Studies and Antimicrobial Activities of Transition Metal Zn (II) with Substituted Arylaldehyde Thiosemicarbazone

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ABSTRACT

Complexes of Zn have been prepared by reacting metal chloride with p- fluoro benzaldehyde thiosemicarbazone, p-chloro benzaldehyde thiosemicarbazone, and p- hydroxybenzaldehyde thiosemicarbazone as ligands and their complexes were screened for their IR, NMR and Antifungal, Antibacterial studies. Schiff bases and their coordination complexes have acquired great significance in the field of inorganic research mainly because of their biological Activity. Metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry.

Keywords: Synthesis, Spectral Studies, Antibacterial and Antifungal activity, Analytical and Physical data, Zinc Metal Complexes.

INTRODUCTION

Schiff bases and their coordination complexes have acquired great significance in the field of inorganic research mainly because of their biological Activity [1-5]. Metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry. A metal surrounded by a cluster of ions or molecules is used for preparation of complexes compounds named as Schiff bases [6]. The Schiff's base and their metal chelates is effective anticancer, antitumor, anti tuberculosis, antipyretic agent as well as anti fertility [7-10]. Schiff's base possess industrial application as catalysts, dyes, fiber, perfumes an aesthetic, plant growth inhibitors cosmetics corrosion inhibitors, oxygen absorbents, polymers, lubricating agents, for removing metal impurities of oil and drying accelerators [11-14]. Thiosemicarbazone ligands and their Zn (II) complexes have been screened for antimicrobial activity against some gram (+ve) and gram (-ve) bacterial and fungal species. The metal complexes are found to have higher activity than the ligands [15]. The Fe II Cu II and Zn II complexes of Schiff bases are also biologically active [16] and they exhibit enhanced activities as compared to their parent ligands. The chemistry of chalcone thiosemicarbazone and their allied derivative has great interest due to theirdeverse pharmaceutical activities. Their

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use as antifungal, [17] antibacterial, [18] antituberculosis [19] antimicrobial, [20] agents. Metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry. The Schiff base and their metal chelate are effective anticancer, antitumor, antituberculosis, antipyretic, agent as well as antiperfumes, polymers, lubricating agent [21-23].

MATERIALS AND METHODS**Synthesis of substituted arylaldehydethiosemicarbazones (1a-d)****Synthesis of 4-Fluorobenzaldehydethiosemicarbazone**

4-Fluorobenzaldehydethiosemicarbazone was prepared by the method of Compaigne and Archer [24]. 4-Fluorobenzaldehyde (2ml, 20mmol) was dissolved in ethanol (25ml) in a 250 ml round bottomed flask heated on a water bath for 5-15 minutes. This hot solution was treated with a hot ethanolic solution (25ml) of thiosemicarbazide (1.82g, 20mmol) and refluxed for 5-6 hrs on a water bath using reflux water condenser. The change in colour of solution indicates the formation of desired 4-Fluorobenzaldehydethiosemicarbazone. On cooling the reaction mixture the brown colored product crystallized out which was filtered off under water suction and further recrystallized from absolute ethanol and finally dried over anhydrous CaCl_2 in a vacuum desiccators. All the other substituted arylaldehydethiosemicarbazones were synthesized similarly and analytical, physical data of these compounds are listed in Table 1.

Syntheses of substituted arylaldehydethiosemicarbazones metal complexes**Bis [4-fluorobenzaldehydethiosemicarbazones] Zinc (II) (2a-d)**

4-Fluorobenzaldehydethiosemicarbazone (0.398g, 2mmol) was dissolved in (20 ml) ethanol and heated on water bath. This solution was slowly treated with an aqueous solution of metal salt $\text{ZnCl}_2 \cdot 6\text{H}_2\text{O}$ (0.136g, 1mmol) was slowly added in 2:1 molar ratio with stirring. Reaction mixture was refluxed on water bath for 2-3 hrs and left overnight to yield the cream crystalline complex which was filtered off under suction and washed with water till filtrate became colorless. The product was finally washed with dilute ethanol and dried over fused calcium chloride in vacuum desiccators. All the other substituted arylaldehydethiosemicarbazones metal complexes were synthesized similarly and analytical, physical data of these compounds are listed in Table 2.

RESULT AND DISCUSSION**Bis [4-fluorobenzaldehydethiosemicarbazones] Zinc (II) (2a-d)**

The substituted arylaldehydethiosemicarbazones (1a-d) was prepared by the method of Compaigne and Archer [25]. This hot solution was treated with an aqueous solution of metal salt in 2:1 molar ratio with stirring give final product (2a-d). The product was finally washed with dilute ethanol and dried over fused calcium chloride in a vacuum desiccator. All the synthesized compounds gave single spot on TLC. The names and m. p. of all synthesized compounds (1a-d) and (2a-d) are recorded in Table-3 and 4, respectively.

The synthetic steps are illustrated in the following Scheme.

Substituted arylaldehyde – In the IR spectra of substituted arylaldehyde a sharp absorption band at $1720\text{--}1650\text{cm}^{-1}$ is observed due to aldehydic >C=O and at 1610cm^{-1} due to >C=C< stretching vibration. In the $^1\text{H NMR}$ spectra of substituted arylaldehyde a sharp singlet at $\delta 7.9$ ppm due to methine proton of formyl group and multiplet from $\delta 6.5\text{--}7.7$ ppm due to aromatic protons is observed. The IR and $^1\text{H NMR}$ data of substituted arylaldehyde thiosemicarbazones and their metal complexes are summarized in Table-5 and 6.

Antibacterial and Antifungal Activities- Representative Substituted arylaldehydethiosemicarbazones ligands and their metal complexes were screened for their antibacterial activity and antifungal activity at 60, 80, and 100 ppm concentrations.





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Table: 1 Analytical and physical data of substituted arylaldehyde-thiosemicarbazones

Comp.	Y	Mol. formula	color	Mol.Wt.	m.p.	yield	Elemental analysis% (calcd.)			
							C	H	N	S
1a	H	C ₈ H ₉ N ₃ S	Green	179.24	170	82	53.61	5.06	23.44	17.89
1b	4-F	C ₈ H ₈ FN ₃ S	Cream	197.23	168	75	48.72	4.09	21.30	16.26
1c	4-Cl	C ₈ H ₈ ClN ₃ S	Brown	213.69	125	82	44.97	3.77	19.66	15.01
1d	4-OH	C ₈ H ₉ ON ₃ S	Cream	195.24	185	78	49.21	4.65	21.52	16.42





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Table: 2 Analytical and physical data of substituted arylaldehyde-thiosemicarbazones metal complexes.

Comp.	Y	Mol. Formula	color	Mol.Wt.	m.p.	yield	Elemental analysis% (calcd.)			
							C	H	N	S
2a	H	C ₁₆ H ₁₆ N ₆ S ₂ Zn	Green	421.86	195	74	45.55	3.82	19.92	15.20
2b	4-F	C ₁₆ H ₁₄ F ₂ N ₆ S ₂ Zn	Cream	457.84	210	75	41.72	3.09	18.36	14.01
2c	4-Cl	C ₁₆ H ₁₄ Cl ₂ N ₆ S ₂ Zn	Brown	490.15	215	78	39.16	2.88	17.12	13.07
2d	4-OH	C ₁₆ H ₁₆ O ₂ N ₆ S ₂ Zn	Cream	453.86	195	78	42.34	3.58	18.52	14.13

Table: 3 Substituted arylaldehydethiosemicarbazonescc (1a-d)

Comp No.	Name	M. P. (°C)
1a	Benzaldehydethiosemicarbazone	170
1b	4-Fluorobenzaldehydethiosemicarbazone	168
1c	4-Chlorobenzaldehydethiosemicarbazone	125
1d	4-Hydroxybenzaldehydethiosemicarbazone	185

Table: 4 Bis [Substituted benzaldehydethiosemicarbazones] Zinc (II) (2a-d)

Comp No.	Name	M. P. (°C)
2a	Bis[Phenylcarbaldehydethiosemicarbazone] Zinc (II)	195
2b	Bis[4-Fluorophenylcarbaldehydethiosemicarbazone] Zinc (II)	210
2c	Bis[4-Chlorophenyl carbaldehydethiosemi carbazone] Zinc (II)	215
2d	Bis[4-Hydroxyphenyl carbaldehydethio semicarbazone] Zinc (II)	195

Table: 5 IR data of substituted arylaldehyde thiosemicarbazones and their metal complexes

Comp.No.	IR (KBr : ν max cm ⁻¹)								
	>NH ₂	>NH	Ar-H	>C=N	>C=C<	M-N	>C=S	C-S	-N=N-
1a	3390	3150	3010	1650	1605	-	1150	-	850
1b	3410	3090	3020	1640	1610	-	1090	-	810
1c	3410	3110	3250	1610	1605	-	1110	-	820
1d	3420	3150	3021	1630	1605	-	1120	-	830
2a	3440	-	3010	-	1610	420	-	1120	865
2b	3350	-	3050	1580	1550	430	-	1090	840
2c	3400	-	3065	1585	1540	430	-	1156	845
2d	3450	-	3085	1650	1480	420	-	1150	846

Table: 6 ¹H NMR data of substituted arylaldehyde thiosemicarbazones and their metal complexes

Compd. No.	Mol. Formula	¹ H NMR spectral data(δ ,ppm)				
		Ar-H (m)	NH(s,1H)	-NH ₂	CH=CH-	-OH
1a	C ₈ H ₉ N ₃ S	7.3-7.6	2.0	2.0	8.1	-
1b	C ₈ H ₈ FN ₃ S	7.0-7.6	2.0	2.0	8.0	-
1c	C ₈ H ₈ ClN ₃ S	7.3-7.6	2.0	2.0	8.1	-
1d	C ₈ H ₉ ON ₃ S	6.8-7.4	2.0	2.0	8.0	5.0
2a	C ₁₆ H ₁₆ N ₆ S ₂ Zn	7.3-7.6	-	2.0	8.1	-
2b	C ₁₆ H ₁₄ F ₂ N ₆ S ₂ Zn	7.0-7.6	-	2.0	8.1	-
2c	C ₁₆ H ₁₄ Cl ₂ N ₆ S ₂ Zn	7.3-7.6	-	2.0	8.1	-
2d	C ₁₆ H ₁₆ O ₂ N ₆ S ₂ Zn	6.8-7.4	-	2.0	8.1	5.0





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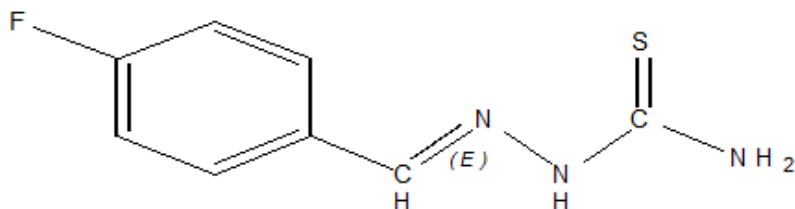


Fig.1. 4-Fuorobenzaldehydethiosemicarbazone

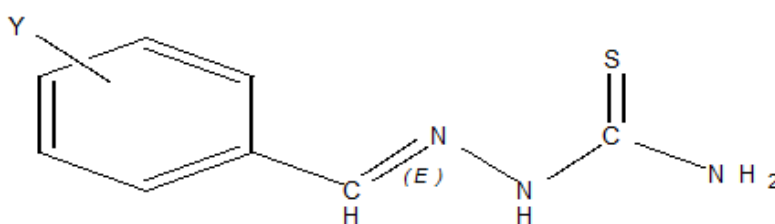


Fig.2. Substituted arylaldehyde-thiosemicarbazones.

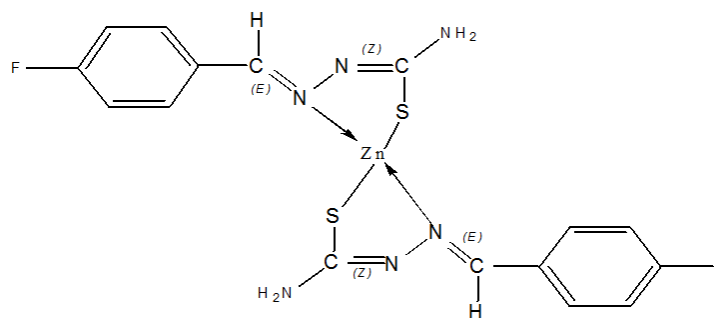


Fig.3. Bis [4-fluorobenzaldehydethiosemicarbazones] Zinc (II)

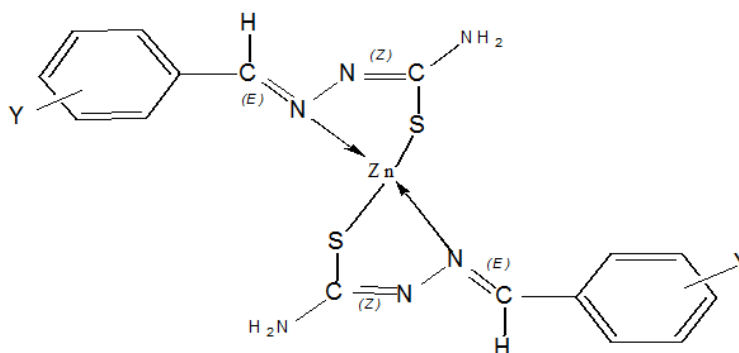


Fig.4. Substituted arylaldehyde-thiosemicarbazones metal complexes.





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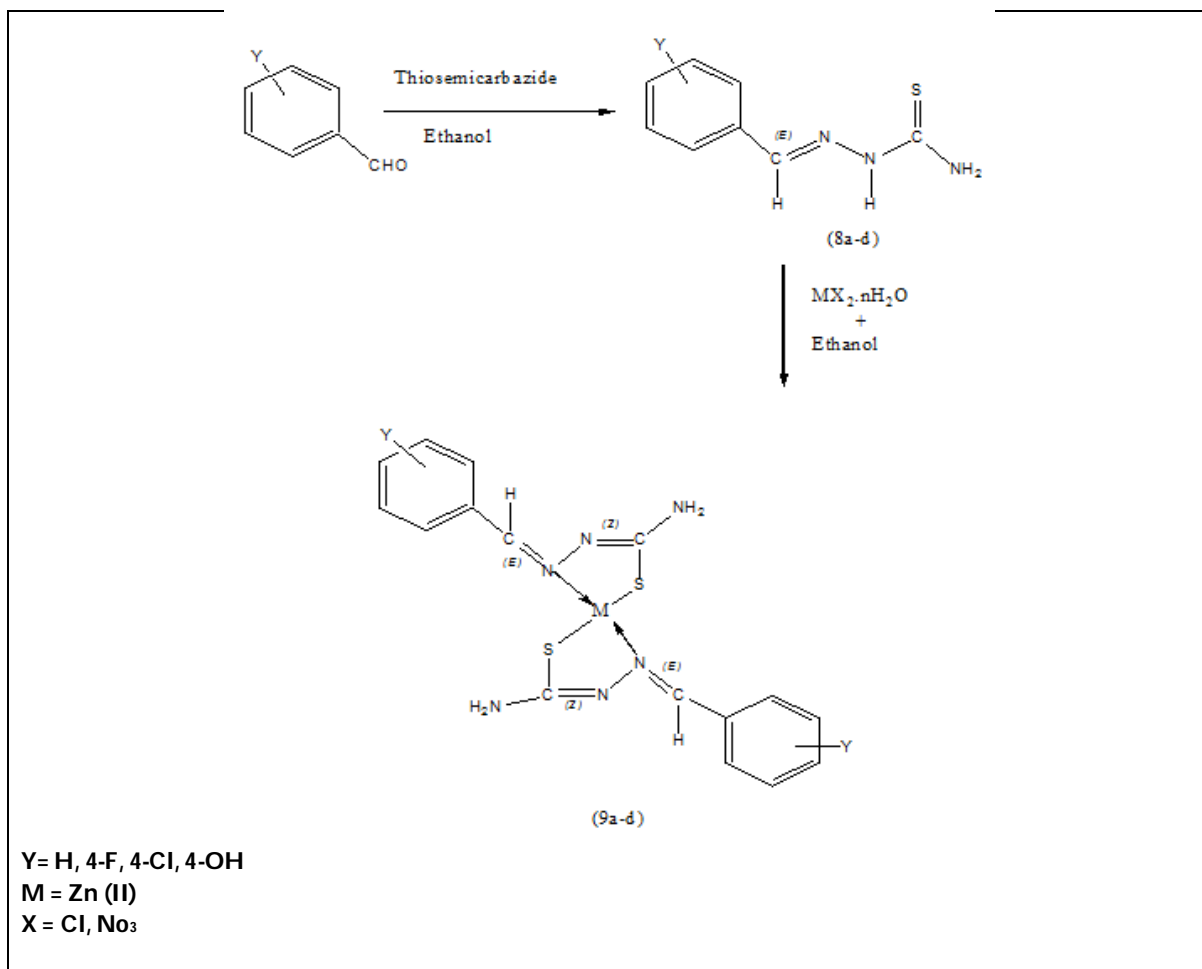
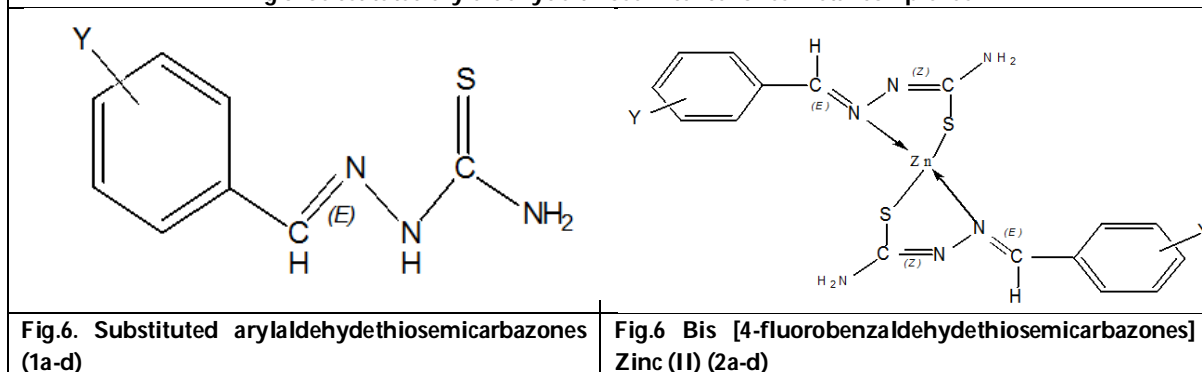
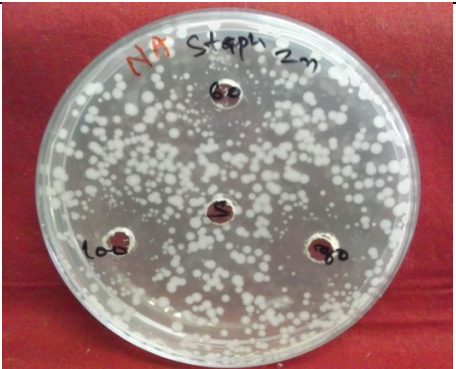



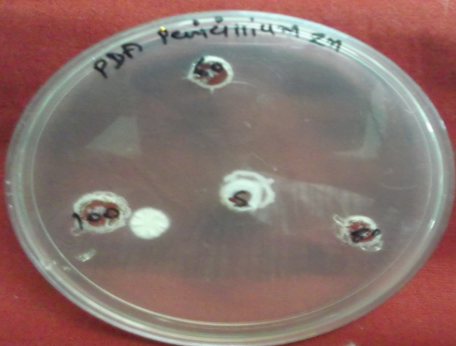

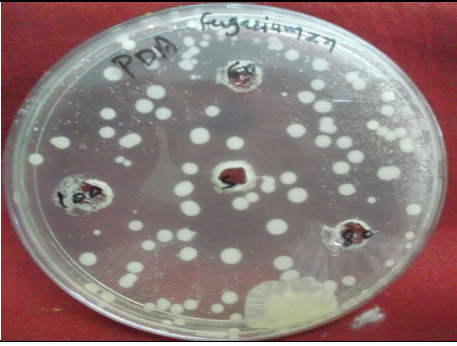


Fig.5. Substituted arylaldehyde-thiosemicarbazones metal complexes





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Antibacterial species - <i>Streptococcus aureus</i>	Antibacterial species - <i>Bacillus cereus</i>
	
Antibacterial species - <i>Streptomyces</i>	Antibacterial species - <i>E. coli</i>
	
Antifungal species - <i>Penicillium</i>	Antifungal species - <i>A. niger</i>
	
Antifungal species - <i>Fusarium</i>	





Binding Affinity Comparison of Selected Phytocompounds from Nutmeg with Human Hypocretin-1/Orexin-A.

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ABSTRACT

Five compounds among the phytocomponents present in nutmeg extracts were selected for the study of their binding affinity with orexin-A, a hypothalamic neuropeptide responsible for regulating sleep and satiety. The computational results of the study are discussed in this communication. The online sources for the chemical structures of the selected compounds and the protein are PubChem database and Protein Data Bank respectively. AutoDock tools was used for protein as well as ligand preparation and Molecular docking software AutoDockVina for docking with the protein.

Keywords: Neuropeptide, Orexin-A, nutmeg, obesity

INTRODUCTION

Orexin-A, otherwise known as hypocretin-1 is a novel neuropeptide secreted in hypothalamus. This neuropeptide is known to regulate sleeping behaviour and hunger. Lack of orexin-A causes sleepiness and narcolepsy, whereas its administration has been found to delay the feeling of satiety causing over consumption of food(1). Hence this protein has been linked to obesity (1,2). Various research groups have reported synthetic antagonists of orexin-1 receptor which reduce food intake in rat subjects (1,2). Due to their lower side effects, bioactive components present in traditional or folk medicinal plants have the potential to be explored as drug candidates targeting various diseases(1). One such natural source of valuable bioactive compounds is *Myristicafragrans* or nutmeg. Nutmeg seed and its outer web like covering (mace) are used as spices in various cuisines worldwide. Nutmeg in its various forms, such as oil, powder or other extracts has been used in traditional medicinal practice to treat different ailments (1). Several medicinal properties have been attributed to the nutmeg essential oil and its extracts (1). Determining activities of different phytochemicals present in nutmeg has captured our curiosity. Binding affinities of a few selected compounds found in nutmeg with orexin-A were computationally determined to evaluate their probability of being anti-obesity drug candidates.





MATERIALS AND METHODS

Five phytochemicals which were selected for this study are licarin A, Licarin B, Licarin C, Malabaricone B and Malabaricone C. Their 3D structures were collected from PubChem database. The correct format for docking (.pdbqt) was obtained for each of the structure first by using PyMol software where the .sdf format was converted into .pdb format followed by ligand preparation in Autodock tools and saving the corresponding file in .pdbqt format. The 3D protein structure of orexin-A was retrieved from Protein Data Bank in .pdb format. Protein preparation was conducted by water molecule removal followed by addition of polar hydrogens and Kollman charges. Docking was carried out using AutoDockVina from the protein structure (1). Nine best poses were produced by the computational calculations for each ligand and protein pair. PyMol software was used to visualize ligand-protein interactions. The results for individual ligands are discussed here.

RESULT AND DISCUSSION

Interaction with licarin-A

Licarin-A (figure 1) showed binding affinity with the protein with binding energy of -5.5 kcal/mol. There were three hydrogen bonding interactions with amino acid residues His-26 and Ala-23. The hydrogen bonding interaction and corresponding bond lengths are shown in figure 2.

Interaction with licarin B

Licarin B formed a complex with the protein with binding energy -6.1 kcal/mol. It had one H-bond interaction with His-21 residue of the protein (figure 4).

Interaction with licarin C

In case of licarin C the 1st and 2nd poses had binding energy of -5.5 kcal/mol each. These poses did not show any hydrogen bonding with the protein. But the 3rd pose had one H-bonding interaction with the His-21 residue and a binding energy of -5.4 kcal/mol (figure 6).

Interaction with Malabaricone B

Malabaricone B (4) and Malabaricone C (5) exhibited binding energy of -5.1 kcal/mol each. First pose of Malabaricone B had no hydrogen bonds with the protein. However, the 3rd pose had H-bonding interaction with His-21 residue (figure 8).

Interaction with Malabaricone C

Malabaricone C showed similar binding affinity as malabaricone B in its 1st pose. It did not show any H-bonding as well. But from the surface view of the 1st pose (figure 10 b), it can be seen that the ligand fits into the cavity of the protein very well which might be the reason of lower binding energy of the pose. The binding energy of the 1st pose for all the ligands with orexin-A along with the number of hydrogen bond interactions are listed in Table 1.

CONCLUSION

Among the various phytochemicals present in nutmeg, five were selected for *in silico* study. Computational calculation was carried out for their activity towards human hypothalamic neuropeptide orexin-A. Highest binding affinity was found for licarin B whereas highest number of H-bond interactions were noticed for licarin A.





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Table 1: Binding energy (BE) in Kcal/mol and number of hydrogen bonds (NHB) of ligand protein interaction

PDB ID	Binding energy and number of hydrogen bond of protein 1BHS with each ligand									
	Licarin A		Licarin B		Licarin C		Malabaricone B		Malabaricone C	
	BE	NHB	BE	NHB	BE	NHB	BE	NHB	BE	NHB
1R02(2).	-5.5	3	-6.1	1	-5.5	0	-5.1	0	-5.1	0

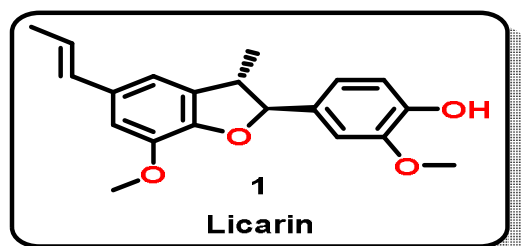


Figure 1: Chemical structure of Licarin A (1)

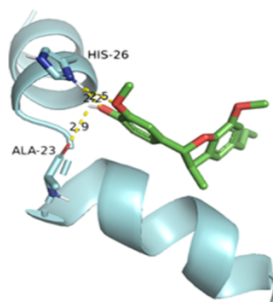


Figure 2: Interaction of licarin A and the protein 1R02





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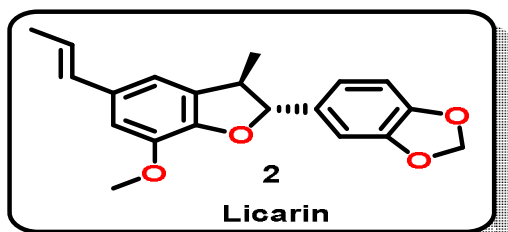


Figure 3: Chemical structure of licarin B (2)

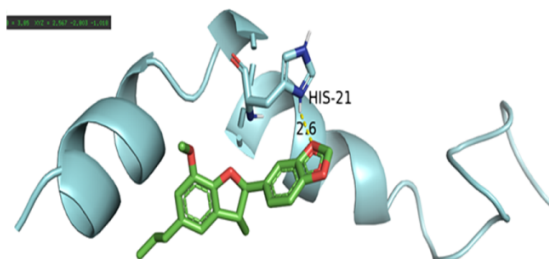


Figure 4: Interaction of licarin B and the protein 1R02

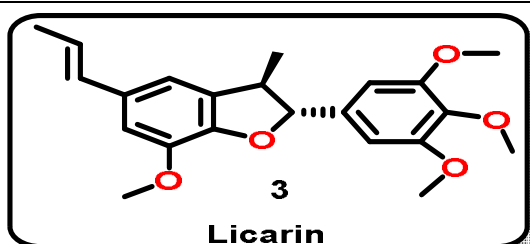


Figure 5: Chemical structure of licarin C (3)

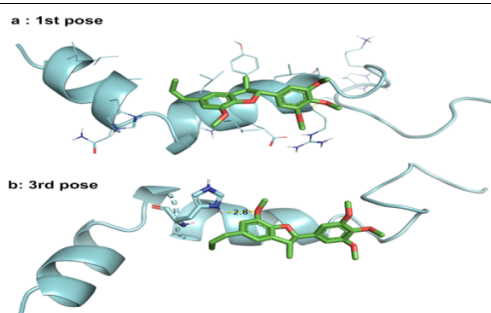


Figure 6: Interactions of licarin C and the protein 1R02

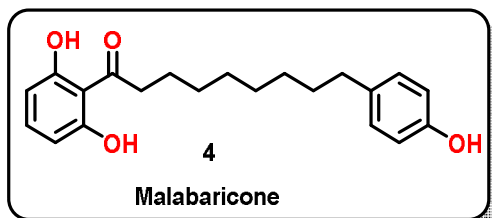


Figure 7: Chemical structure of malabaricone B (4)

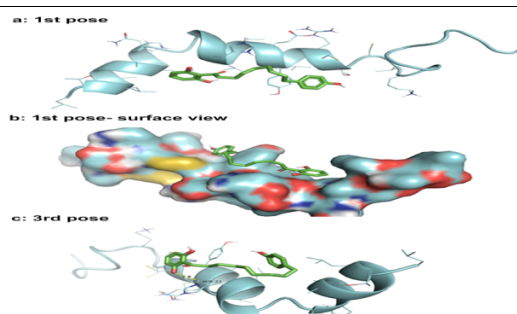


Figure 8. Interactions of Malabaricone B and the protein 1R02

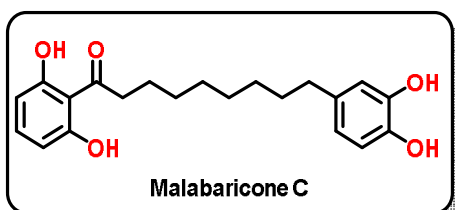


Figure 9: Chemical structure of Malabaricone C (5)

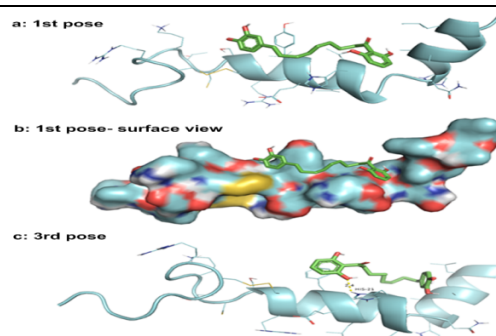


Figure 10. Interactions of Malabaricone C and the protein 1R02





200 KeV Nitrogen Irradiation Effects on Cadmium-Selenide (CdSe)

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ABSTRACT

This manuscript includes a Monte-carlo simulation by bombarding 200 KeV nitrogen ions on the cadmium selenide target by using SRIM package. We explore the variation in surface modifications of this compound with effect of the radiation. Where we discuss the distribution of displaced and recoil atoms after collision and also explore different energy recoils with respect to the effect of radiation

Keywords: Monte-carlo simulation, SRIM, cadmium selenide

INTRODUCTION

Cadmium Selenide (CdSe) is well known compound for its efficient applications in current time. This exist in different crystalline forms like wurtzite, sphalerite and rock-salt etc. However the rock-salt structure is retained at high pressure and the sphalerite structures are little unstable [1,2]. The nanostructures of CdSe is found to be quite interesting with vast industrial applications. After quantum confinement, this exist as quantum dot of about 10nm size. This energy discretization leads to the enhanced electronic and optical properties. As per the literature, trioctylphosphine oxide (TOPO), a neutral ligand derived from a common precursor, is used in the synthesis of CdSe dots [3-7]. Thus this caps the surface of CdSe quantum dots. However, the current results is little contradiction to the model. CdSe dots have excess cadmium cations on the surface that can form bonds with anionic species such as carboxylate chains. This can be verified from NMR results, which reveals the non-stoichiometric behavior of CdSe quantum dot. Lot of research work exist on the synthesis techniques of CdSe and the well known methods are photochemical synthesis, chemical injection, microwave radiation etc. The semiconducting wurtzite structure of CdSe makes its implementation in solar cell devices [8-10]. In the present context, we try to study the surface modifications of CdSe by irradiating the 200 KeV Nitrogen ion. The enhanced properties are explored by using the SRIM calculations. There exist literature, where the change in optical properties of CdSe is studied after radiation with gold and swift heavy ions [11-13].

We perform a Monte-carlo simulation using SRIM [14-16] for irradiating low energy alkali ions on CdSe surface to predict the change in behavior and properties [17]. Where, the atomic distribution and energy recoils are discussed in detail.

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RESULTS AND DISCUSSION

We perform a SRIM calculation of N-ion irradiation of energy 200 KeV on the targetted CdSe surface, where, we observed the back scattered and transmitted atoms. The simulation of those atoms are displayed in Fig. 1. This figure represents the path distribution of recoiled Cd and Se atoms along with the Nitrogen ion and the vacancy produced after the N-ion is irradiated to the CdSe target. The blue dots represent the recoiled atoms and the red line represents the path followed by those atoms and ions after collision. The N-ion range and distribution is illustrated in Fig. 2. When the N-ion with energy 200 KeV is projected to CdSe surface it penetrates to the surface up to a micrometer length and displace the atoms by creating the vacancy. Few Cd and Se atoms recoils after the collision, which leads to the surface modification of this compound. The atomic distribution of Cd and Se is represented in the left panel of Fig. 2, where the cyan and orange color signify the Se and Cd atoms respectively. The lateral distribution is shown in the right panel of the same Fig. Where the radial and projected displacement is clearly significant. The radial and projected straggled atoms are displayed in red and blue colors respectively. Fig. 3 justify the collision events and impact on target atoms. Here, the target displacement, target vacancies and collision replacement are shown in red, blue and green respectively. We can easily interpret that the replacement collision is very less as compared to the displacement and vacancies. This may be due to the low energy of Nitrogen ions. In the left panel of Fig. 4, the energy recoils of Cd and Se atoms are represented in orange and cyan color respectively. The right panel shows the energy loss after collision. Where the blue and red colors stand for the energy loss of recoiled atoms and the bombarded ions respectively after ionization. The ionization is more in the N ions as compared to the recoils. The right panel shows that the phonon vibrations are more in the recoils as compared to the ions. In Fig.5, the energy loss due to ionization is given, where the blue color represents the recoils of target atoms and the red color displays the ions.

CONCLUSION

We studied the surface modifications of CdSe compound by irradiating with nitrogen atoms of 200 KeV energy. We discuss the displacement and recoils of surface atoms in the target compound. We found the energy of the bombarded ion is sufficient to penetrate upto a micrometer depth in the target surface. The SRIM calculation provided a significant inference of energy distributions and ionization data and tempt the options to open for further studies.

ACKNOWLEDGEMENT

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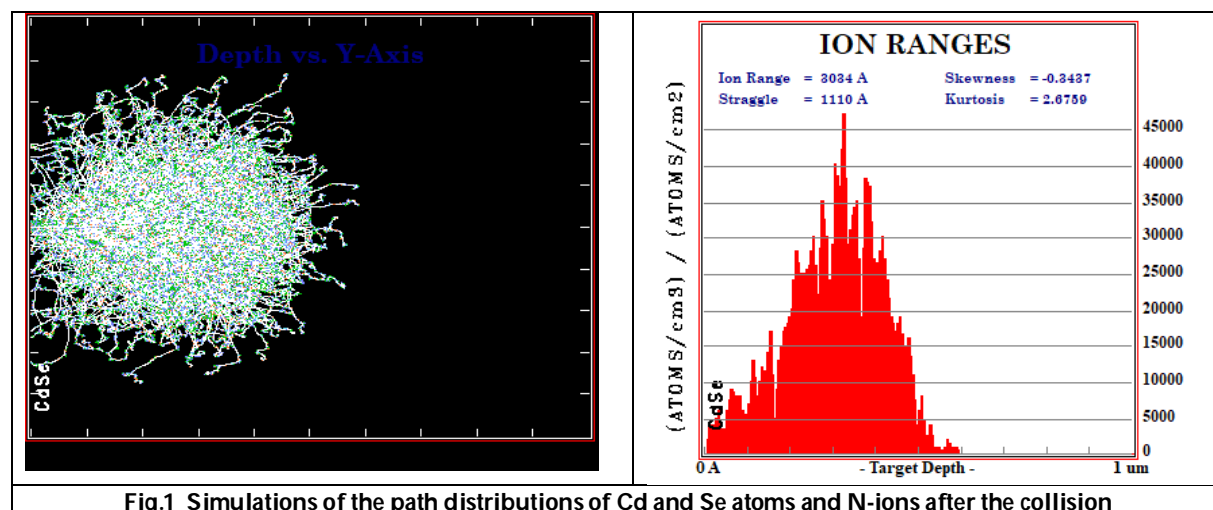
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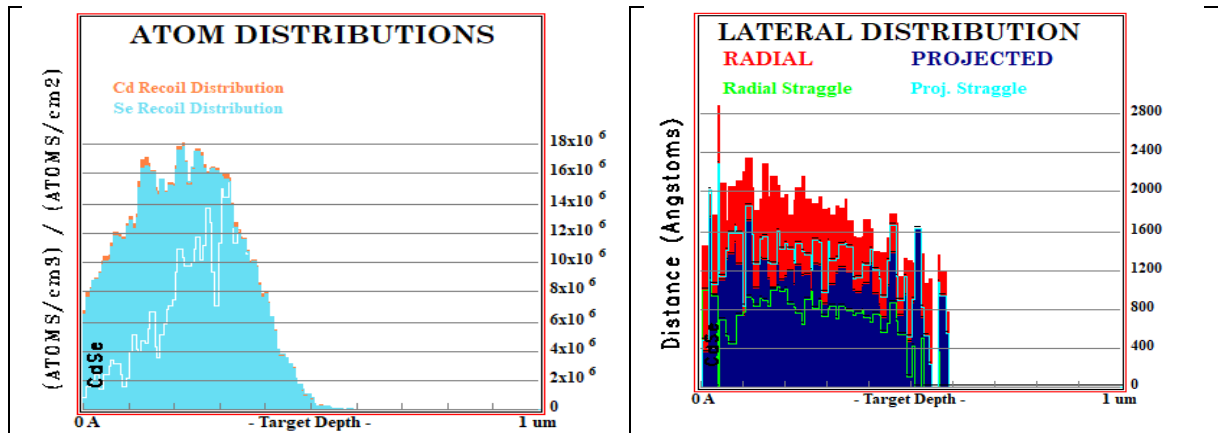


Fig.2 Atomic distribution of the Cd and Se atoms after the collision. Right panel displays the lateral and radial distributions.

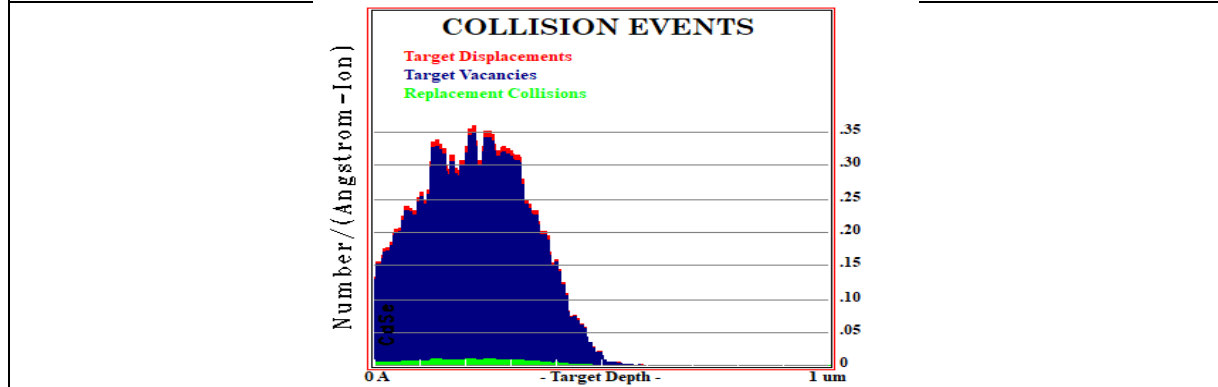


Fig. 3 Collision events of the N-ion irradiation on CdSe target

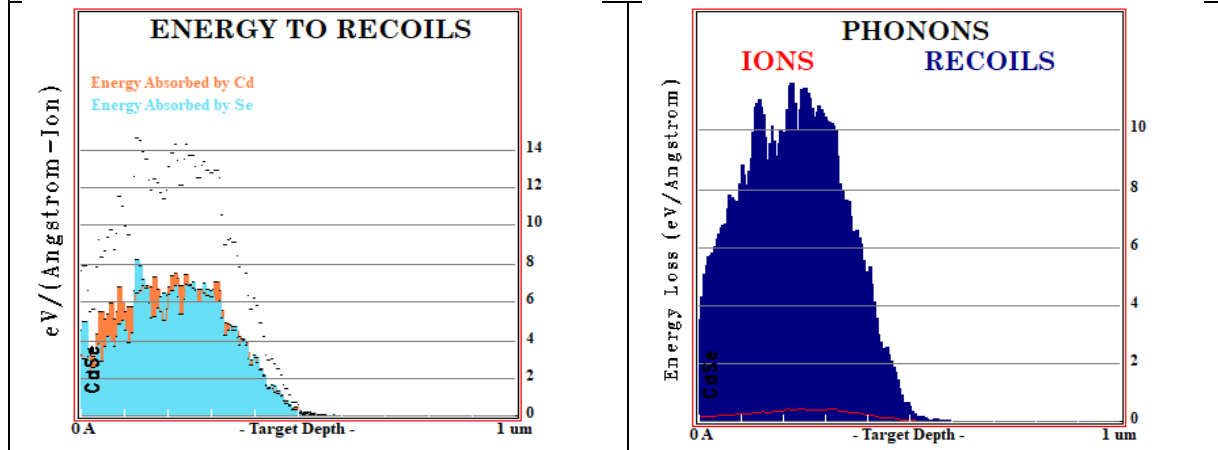
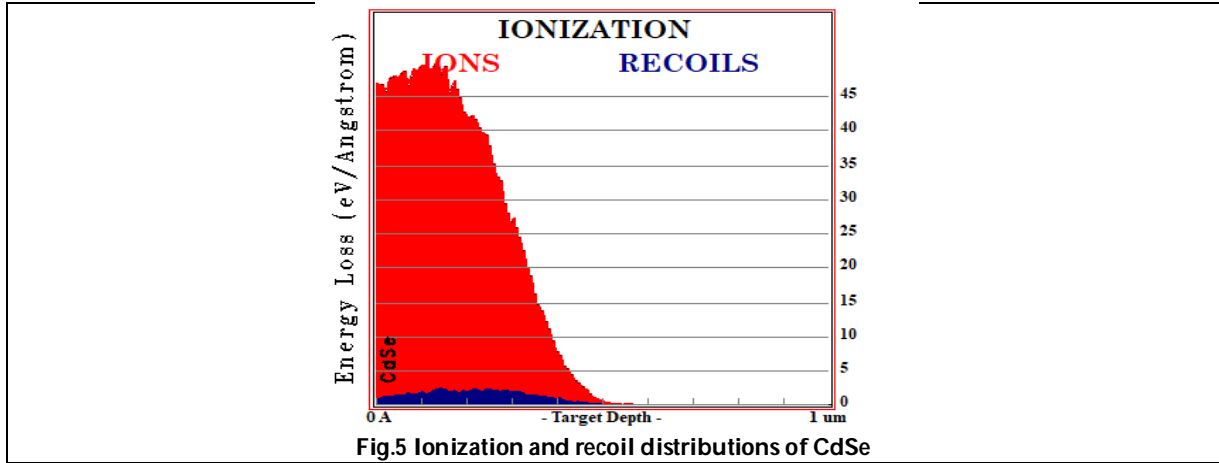


Fig.4 Energy recoils and phonon distributions after collision





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Perioperative and Anesthetic Death Due to Lidocaine Overdose: Forensic Analysis on Biological Samples (Blood and Viscera)

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ABSTRACT

Lidocaine is used as anesthetic drug past decades, the medical negligence is still seen by many medical practitioners. In this paper, the perioperative death is investigated and forensic analysis was performed on the biological samples i.e. Blood and Viscera (liver) where highly, specific, accurate and rapid instrument; High Performance Liquid Chromatography (HPLC) was performed. The extraction of lidocaine is done by liquid – liquid method. In the following study, a new method is developed in HPLC under modified chromatographic conditions of the mobile phase consisting disodium hydrogen phosphate: methanol: triethylamine in the ratio of 50:50:0.1 (v/v/v) where the flow rate has been set at 1.2ml/min. The assay exhibited peculiar linearity range ($r^2 > 0.997$) via graph interpretation of area v/s concentration whose value ranges from 10-40ppm. The HPLC-UV method is one of the most reliable and analytical method in order to determine and to form calibration curve from linearity as well as to determine accuracy, precision, % RSD, robustness and detection of LOD & LOQ.

Keywords: High - Performance Liquid Chromatography, anesthetic drug, lignocaine, forensic samples, toxicology

INTRODUCTION

Lidocaine is commonly known as lignocaine, and chemically referred as 2-(diethyl amino)-N-(2,6-dimethylphenyl) acetamide (Figure 1). It is most commonly used by the medical personnels at the time of oral surgery as it is a local anesthesia. The major impact of Lignocaine is on central and peripheral nervous system, it causes the blockage of sodium channels that further leads to depolarization of electrical rate in the nerve impulse. Thus, due to an aesthetic drug – Lignocaine a painless effect is induced, depolarization effect of electrical rate is generated in the nerve

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impulse by the blocking the sodium conduction channels [1]. The most developed analytical method used is the High Performance Liquid Chromatography along with Ultraviolet Detector predicts reliable method in detection of lidocaine from the forensic samples mainly blood and viscera (liver) by modifying the HPLC conditions [2].

Experimental**Reagents**

Lidocaine, disodium hydrogen phosphate, methanol, triethylamine were obtained from Central Drug House (P) Ltd., Daryaganj, New Delhi, India

Chromatographic Conditions

The modified analytical method of HPLC contains a HPLC pump model with sample programmed at 0.02ml of injection volume. Chromatographic conditions were set up on a C18 column with dimensions (4.6mm X 150mm X 5 µm). The mobile phase was modified by mixing disodium hydrogen phosphate: methanol: triethylamine in the ratio of 50:50:0.1 (v/v/v) and using for isocratic elution. The flow rate was set at 1.2ml/min, column temperature kept at 25°C and ultraviolet wavelength detection was set at 220nm

Sample Preparation

To 1mL blood in a 10mL of test tube, 0.5 NaOH was added (1M) and 3mL extracted solvent (disodium hydrogen phosphate: methanol: triethylamine) was added. Mix it thoroughly and centrifuged the solvent at 2000g for approximately 5minutes. Upper layer which was formed on the top of the test tube extracted with 100µl of orthophosphoric acid. The organic part was aspirated and rest of the residue injected into the column.

Calibration Procedure

In order to make lidocaine standard concentrations, to 1mL of blood of blood, 100µL of lidocaine standard solution at concentrations 10,20,30 and 40µg/mL was added. Further, all the calibration samples were taken for extraction purpose and the calibration curve was made using peak ratio of lidocaine (Internal standard) versus lidocaine interactions. Then the final sample concentrations calculations were determined in order to obtain peak area ratio of lidocaine related to internal standard and comparing the ratio with the standard curve which is obtained after the analysis of calibration samples [3].

Linearity and Range

The value of linearity is generally estimated via mathematical calculation of plots which serves as a function of analyte concentration. In order to determine the linearity, the calibration curves are plotted over a concentration range 10-40ppm v/s peak area respectively. The amount of 0.02ml of injection volume is injected in the HPLC to obtain the graph under our modified conditions. Thus, in order to draw a plot of calibration curve the peak area of the solute or analyte is drawn against corresponding concentration of lidocaine drug [5]. The graph obtained given in (Figure 2) after the linear regression shows a good and linear relationship and the linear regression equation was $Y=24047X+24844$ ($r^2 = 0.997$) for lidocaine drug.

Limitation of Detection and Quantitation (LOD & LOQ)

The values of Limit of Detection [LOD] & [LOQ] were calculated with the help of standard deviation [σ] of the peak area and the slope [s] of the corresponding calibration curve [6]. Therefore, the calculated values for LOD & LOQ under specific and reliable conditions in HPLC were found as **0.520 µg/ml** & **1.560 µg/ml** respectively.

Accuracy

Accuracy was assessed by means of the percentage recovery data from spike analysis. Accuracy can be defined as the nearest measured value to an accepted reference value or known value. In order to validate the degree of accuracy, recovery has been performed or studied with the known quantities at the two different levels, namely, 25% and 50%.



**Aldon Fernandes et al.****Precision**

The precision of method was evaluated in terms of repeatability, intermediate precision and reproducibility parameters. The precision is one of the specific parameter of the HPLC, an analytical method, that was assessed for intraday which means for two days and interday i.e. 6 times in a day by analyzing the sample in repeatability [7]. The intraday precision known as Relative Standard Deviation (RSD%) was done by analyzing standard solutions of drug along with the calibration range, six times each day. Interday precision % RSD is calculated by analyzing solutions of drug along with the calibration range on two different days in the period of same week.

Robustness

The validated parameter, Robustness, can be analyzed by deliberately altering the chromatographic conditions and evaluating the consequences occurred by changing the conditions.[8] This is a modified analytical step in order to generate new data or setting up chromatographic conditions. There are several factors that were taken into consideration which are mobile phase, flow rate and column temperature. This method was found to be unaltered by precise changes in the % RSD.

Forensic Analysis on Biological Samples**Blood**

The same protocol is followed in the forensic sample – blood as followed in the standard drug (Lidocaine) when the sample run under the newly modified parameters of HPLC. The standard drug is spiked in the blood sample and further, the drug is extracted from the sample by liquid – liquid extraction. As a consequence, the above depicted graph (Figure 3) shows that the peak area value calculated as 4085438.2 at RT 4.44

Viscera (Liver)

The same protocol is followed in the forensic sample – viscera as followed in the standard drug (Lignocaine) when the sample run under the newly modified parameters of HPLC. The standard drug is spiked in the viscera sample and further, the drug is extracted from the sample by liquid – liquid extraction. As a consequence, the above depicted graph (Figure 4) shows that the peak area value calculated as 5307094.8 at RT 4.43

CONCLUSION

Perioperative and its evaluation for medico-legal purposes are of great interest for forensic examiners. As such fatal events with regard to medical negligence, implement a strategy for forensic analysts to detect the overdose of anesthetic drugs from the biological samples. The analytical method which was modified and developed in HPLC for the identification and analysis of anesthetic – Lidocaine drug in the forensic samples – blood and viscera (liver) reveals its presence even if it is present in low concentration in the blood and accumulated in the viscera. The values of LOD & LOQ are estimated from the standard anesthetic drug – lidocaine are about 0.52 and 1.56. In addition, when the standard drug is spiked in blood and viscera the peak is obtained (area v/s concentration) and RT value is found at 4.44 and 4.43 respectively. The forensic analysts can be used this method for identification of anesthetic drugs in such medical negligence cases.

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Table 1: Different concentration of Lidocaine drug with their peak area

Conc (µg/mL)	10	20	30	40	Slope	LOD	0.520
Area	1463335	2578681	4085438	5307094	130380.3	LOQ	1.560

Table 2: Accuracy for the proposed analytical method HPLC

ACCURACY	DRUG AREA OF STANDARD DRUG	AREA OF TEST DRUG	RECOVERY
	5332082.9	5332082.9	
At 50% [Trial 1]	5332082.9	5333161.4	98.45%
[Trial 2]	5332082.9	5335240.8	98.20%
At 25% [Trial 1]	5332082.9	4081689.3	92.6%
[Trial 2]	5332082.9	4085709.9	92.4%

Table 3: Precision study for the proposed analytical method HPLC

Day 1		Day 2	
	5374369		5502193
	5374200		5550887
	5338863		5510225
	5399690		5453612
	5372180		5414820
	5339887		5455625
Avg.	5366532	Avg.	5481227
Std. Dev	21319.39	Std. Dev	44648.46589
RSD %	0.40	RSD %	0.81

Table 4: Robustness for the proposed analytical method HPLC

Chromatographic conditions	R.T. (mins)	% RSD
Flow Rate	12.26	0.40
1.2mL	12.24	0.81





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1.0mL		
Mobile Phase Composition (%v/v)		
0.05M Phosphoric acid & ACN (35%/65%)		
0.05M disodium hydrogen phosphate – methanol – triethylamine (50 – 50 – 0.1)	12.26	0.40
	12.24	0.81
Column Temperature 1 [240°C]	12.26	0.40
Column Temperature 2 [220°C]	12.24	0.81

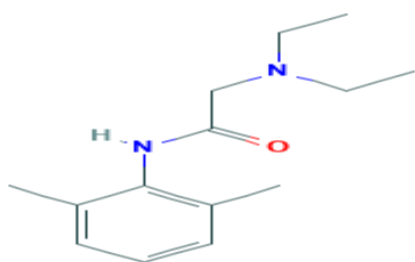


Figure 1: Chemical Structure of Lidocaine

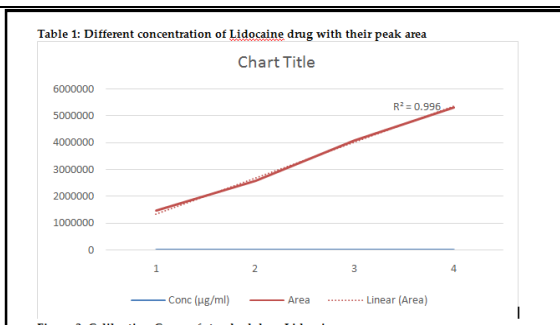


Figure 2: Calibration Curve of standard drug Lidocaine

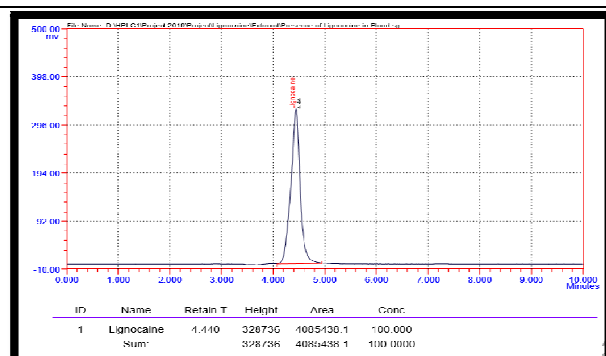


Figure 3 – HPLC Extraction of Lidocaine drug from blood sample HPLC chromatogram

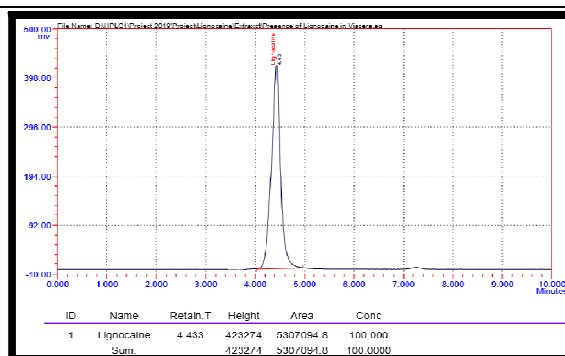


Figure 4 – Extraction of viscera (liver) from HPLC chromatogram



Fig.5 : HPLC Instrumentation





Characterization and Antioxidant Application of Postbiotics of *Leuconostoc spp.* from Chicken Intestine through *in vitro* and *in silico* Analysis

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ABSTRACT

Probiotics have many health benefits by modifying gut microbiome; however, techno-functional hindrance such as viability controls have hampered their full potential applications within the food and pharmaceutical sectors. Therefore, the focal point is progressively shifting from viable probiotic bacteria towards non-viable paraprobiotics and/or probiotics derived biomolecules, named as Postbiotics. Paraprobiotics and Postbiotics are the new evolving concepts in the functional food industry because they reveal an array of health-nurturing properties. Hence, postbiotic are accepted as health boosters for humans and have been appreciated for their medical attributes in adjuvant therapies. In addition to the beneficial effects mentioned on top of, in recent decades, several findings have shed new light on the understanding of the inhibitor capability of such postbiotics. LAB strains were screened for therapeutic potential and their metabolites were characterized. The expansion patterns were analyzed through a growth curve. This study strategizes on the identification and molecular identification of the various probiotic bacteria from the chicken gut. The fatty acids from the probiotic microorganism were analyzed by a Gas Chromatography – mass spectrometry analysis (GC – MS). This investigation confirms the therapeutic effectiveness of *Leuconostoc mesenteroides* and establishes the therapeutic properties of its vital lipids. GCMS was performed to detect the presence and range of compounds within the lipids isolated. 16s rRNA sequencing confirmed the isolate to be *Leuconostoc mesenteroides*. Molecular docking of the filtered ligands with the target site of the protein was performed with the Autodock Tools 1.5.6. to unravel the mechanism of action of the ligands.

Keywords: Probiotics, Antibiotics, Short chain fatty acids, GCMS, Gene sequencing, *Leuconostoc mesenteroides*.



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INTRODUCTION

The exploration in finding the metabolites of the probiotic bacteria as alternatives to the use of drugs and evaluating its therapeutic potential has created huge attention due to the emergence of side effects and antimicrobial resistance. Recently, Postbiotics, which are the secondary metabolites of probiotic bacteria, have been reported as great potential to substitute the use of antibiotics in animals and humans.

Postbiotics are described as “non-viable bacterial products or metabolic products from microorganisms that have biological activity in the host. The various postbiotic molecules include metabolic byproducts of live probiotic bacteria such as cell-free supernatant, vitamins, organic acids, short-chain fatty acids, secreted proteins/peptides, bacteriocins, neurotransmitters, secreted biosurfactants, amino acids, flavonoids derived postbiotics (desaminotyrosine, equol daidzein, daidzein, norathyriol), terpenoids derived postbiotics (genipin, paeoniflorin, paeoni lactone glycosides, paeonimetalin I, II, III), phenolic-derived postbiotics (equol, urolithins, valerolactones, enterolactone, enterodiol, 8-prenylnaringenin) etc. (Cavallari et al., 2017, Cortes-Martin et al., 2020, Wang et al., 2019). Mostly live bacteria (such as probiotic or non-probiotic) additionally secreted some soluble factors (products or metabolic by-products) are known as Postbiotics. These postbiotics discharged when microorganism lysis, which will be helpful to the host. (Aguilar-Toalá et al., 2018). The mechanisms involved in most bioactivities of postbiotics don't seem to be totally understood; scientific proof supports that postbiotics possess numerous functional/bioactive properties like antimicrobial, antioxidant and immunomodulatory activities via direct (interaction with the enteric microbiota or immune cells) or indirect (outside the Gastro intestinal tract, within the system and different organs) pathways. (Aguilar-Toalá et al., 2018, Caroline et al., 2016). Antioxidant additives exploitation substances that delay or forestall the oxidization of cellular substrates have incontestable capability to safeguard the human body against oxidative damage. Although many synthetic antioxidants, including butylated hydroxyanisole and butylated hydroxytoluene are widely employed in retarding lipid oxidization, their safety has recently been questioned because of liver damage and carcinogenicity.

Free radicals destructively react with biomolecules, inducing diseases like cancer, aging, and coronary-artery disease (Yang Wang et al., 2017). Antioxidants could forestall lipid oxidization in the plasma membrane through inhibition of the attack of free radicals, leading to accrued ability to defend against cellular damage (Reuter et al., 2010). Thus, taking supplements containing antioxidants is an alternate manner of reducing oxidative stress. Since natural associate antioxidants have an inherent safety advantage, there is increasing interest regarding the utilization of natural antioxidants to interchange artificial antioxidants. Therefore, in recent years, finding safer and natural antioxidants from bio-resources to interchange synthetic antioxidants has received an excellent deal of attention. In order to counter the oxidative damage gaining the attention is given to postbiotics as Probiotic-based additives with relevance to the capacity to exhibit antioxidant potential with a large form of antioxidant enzyme activities. It's been proven by many studies that the probiotics from lactic acid-producing bacteria exhibit high antioxidant enzymes activity (Tang et al., 2018, Kullisaar et al., 2002, Shimamura et al., 1992). Among the several metabolites of the probiotic bacteria, fatty acids have gained significance, due to the array of activities attributed to them. Hence, the extraction and identification of fatty acids were focussed, which rely on solvent extraction and HPLC techniques. Nevertheless, the use of GC-MS is more analytical preparative technique. However, GC-MS provides overall insights on fatty acids within the mixture (Nagpal et al., 2018, Ribeiro et al., 2018). Recently, there has been emerging evidence on the therapeutic approaches of short-chain fatty acids within the management of IBD and colorectal cancer because of their potentiality to beat and overcome the inflammation and proliferation of cancerous cells respectively (Venegas et al., 2019). Lactic acid bacteria (LAB) are the most common bacteria found in foods and also make up the microbiota found in the intestines of humans and animals. We hypothesized that dietary postbiotics from *Leuconostoc* spp. modulates antioxidant activity and has also been analyzed *in vitro* and further confirmed by *in silico* analysis.



**Jasmine et al.****AIM**

The study's primary goal was to extract lactic acid bacteria (LAB) from the non-broiler chicken's gastrointestinal tract. To find out whether pathogenic bacterial strains have antagonistic activity. Physiological and biochemical characterization of isolated LAB strains in order to evaluate their potential as a probiotic supplement based on tolerance in conditions similar to those found in the intestinal tract (Carnevalli et.al., 2010).

Lactobacillus strains were isolated from chicken intestines, classified, and characterized for their probiotic properties in order to choose suitable probiotic strains as possible probiotic candidates for chickens. The *Lactobacillus* strains were identified to species level using phenotypic and molecular characteristics, and the primary probiotic properties (recommended by FAO/WHO, 2001) studied (*in vitro*) were those that would allow the strains to survive and colonize the GIT, such as the ability to tolerate acid, bile and pancreatic enzymes, and the ability to adhere to intestinal epithelial cells (Antonia et al., 2019). In gastrointestinal health, the gut microbiota is very important. Probiotics will replace the gut microbiome composition and introduce useful functions to gut microbial communities, resulting in improvement or hindrance of inflammation of the gut and different enteric diseases. Chickens are used as models to analyse the potential of gut microbiota. Hence, the current study was undertaken to isolate the probiotic bacteria from the chicken intestine and to evaluate their therapeutic potential.

MATERIALS AND METHODS**Isolation of Lactobacillus strains from Chicken Gastro-intestinal tract**

The source of LAB was the chicken gastrointestinal tract. The gastrointestinal tract was aseptically excised from non-broiler chicken. Approximately 22 to 25g of each section of the intestine tract was immersed in 250ml of normal saline (0.9 percent NaCl) for 5 minutes to remove any contaminants. The gut was finely cut and ground aseptically using mortar and pestle and the contents were serially diluted and plated on sterile MRS agar plates (Himedia, India) supplemented with 0.02 percent bromobically incubated for nearly 24 hours at 37°C. Isolated colonies were chosen from the highest dilutions of each MRS agar plate based on morphological variations between them and other colonies. Subculturing each colony 2-4 times on MRS agar was used to purify the colonies (Han.J et al., 2016).

Initial identification and Preliminary Screening of Isolates

Colony morphological analysis, Gram staining and catalase tests were initially performed to identify *Lactobacillus* sp.

Lipid Isolation

The pellet of bacterial cells was washed twice with 50ml of sample and centrifuged at 6000rpm for 10 minutes after being centrifuged for 100 minutes at 6000rpm. As a result, 50ml of distilled water was used to wash the bacterial cell pellet. Following centrifugation, 10ml 4M HCl was added and incubated at 60°C for 1 to 2 hours. At room temperature, the acid-hydrolyzed mass was stirred for 2-3 hours with a 20ml chloroform/methanol (1:1) mixture. To distinguish the aqueous upper phase and the organic lower phase, centrifugation was performed at 3000rpm for 10 minutes at room temperature. The lower phase containing the lipids was weighed and used for various bioassays (Mishra et al., 2020)

Antibacterial Activity

Disc diffusion assay was used to assess antimicrobial activity. The diameter of the zones was measured after incubation of the plates (Bauer, 1964)



**Jasmine et al.****Antioxidant Activity**

The antioxidant activity was determined by the DPPH radical scavenging activity using the method of Son and Lewis. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity (Pandey & Mishra, 2010). DPPH radical solution (0.004%, w/v, Sigma Aldrich) in 95% ethanol was prepared. A volume of 2 ml of DPPH in ethanol was added to 2 ml of various concentrations of SMH (0.5 mg/ml to 3 mg/ml), well vortexed and incubated for 30 min in dark room at room temperature. Absorbance of each sample at 517 nm was measured using UV-Visible spectrophotometer (Varian Carry 50 Conc). Ethanol was used as a blank, while DPPH solution in ethanol served as control. BHT (Sigma, Germany), ascorbic acid (Sigma, Germany) and Trolox (Acros, USA) at a concentration of 0.02 mg/ml was used for comparison. The test was carried out in triplicate. The antioxidant activity was expressed as percentage of DPPH activity using the following formula: DPPH activity (%) = $\frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$

The IC₅₀ value for antioxidant activity was defined as the concentration of sample (mg/ mL) required to scavenging DPPH radicals by 50%.

Probiotic characterization of the isolated bacterial strains**pH tolerance**

The isolated bacterial cultures were inoculated into sterile MRS of different pH as 4, 6, and aerobically incubated for 24 to 48 hours at 37°C. MRS broth cultures were tested for turbidity after an incubation time. (Nagl & Schatzmayr, 2015).

Temperature tolerance

MRS broth was inoculated with a colony of fresh overnight LAB culture and incubated at 25, 37, and 45 degrees Celsius for 24 hours to determine growth at different temperatures. Spreading on MRS agar and monitoring their growth were used to evaluate the growth. Triplicates of the test were carried out. (Nagl & Schatzmayr, 2015)

NaCl tolerance

MRS broth was adjusted with varying concentrations of NaCl to determine NaCl tolerance of isolated LAB strains (2 percent, 8 percent, and 10 percent). Each test tube was sterilized before being inoculated with a fresh overnight culture of bacterial isolates and incubated for nearly 24 hours at 37°C. The turbidity of each test tube was measured after the incubation period to determine growth. Maximum growth was defined by a double positive sign (++), average growth by a single positive sign (+), and no growth by a negative sign (-). Our findings are close to those of Elizete and Carlos, who found that lactobacilli from swine gastrointestinal tracts could tolerate 4–8% N. (Nagl & Schatzmayr, 2015)

Gas Chromatography Mass Spectroscopy

Chromatography by Gas Mass Spectroscopy is an analytical technique for identifying various substances inside a test sample that incorporates the features of gas chromatography. Since it is used to conduct a 100 percent specific test that positively identifies the existence of a specific drug, GCMS has been recognized as the gold standard for forensic substance detection.

Molecular characterization by 16s rRNA Amplification

The selected culture was inoculated in to 50 ml of culture medium and incubated until it reached the OD₆₀₀ of 1 to 2. The cells were harvested by centrifuging at 1250 g at ambient temperature. The cell pellet was resuspended in 467 µl TE buffer and further incubated with 33 µl of lysing buffer (30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K) for 1 hr at 37 ° C. The lysate was extracted with an equal volume of 25:24:1 phenol: chloroform: isoamyl alcohol. The aqueous phase was transferred to a 1.5 micro centrifuge tube and added with 1/10 volume of 3M sodium acetate and incubated at -20°C for 30 min. After the incubation 0.6 volumes of isopropanol was added and mixed gently and centrifuged at 14000 g for 20 min at 4° C. The DNA pellet was washed with 1 ml of 70% ethanol twice by spinning at

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14000 g for 20 min at 4° C. The DNA pellet was resuspended in 100 µl TE buffer and stored at 4°, overnight. The quality and quantity of the DNA was estimated by OD ratio of 260/280, 260/230 nm through 8 port Nanodrop (Thermo Fisher Scientifics, USA) and 1% agarose gel respectively.

Protein and ligand preparation

The three dimensional structure of the proteins FimH(4XOA) and cytochrome P450(1OG5) was retrieved from Protein Data Bank (PDB). The obtained structure was prepared by the addition of hydrogen atoms and Kollman united partial atomic charges were added for docking study. The removal heterogeneous molecules including water was done by using the using Accelrys Discovery Studio Visualizer 2.5. The chemical structure was downloaded in the SDF format from the Pubchem database and then converted to PDB format using OPEN BABEL 2.2.1 and used for docking studies(Werner et al.,2006). The druggability and pharmacokinetic assessment of the chosen compounds were done using Medchem designer 3.0 software (Douglas et al., 2015). Molecules violating more than one of Lipinski rule of 5 may have problems with bioavailability. Drug likeliness and bioavailability of the ligand is inspected using the Molinspiration tool (www.molinspiration.com)

Molecular Docking

Molecular docking protocols are widely used for predicting the binding affinities for several ligands. We aimed to examine the possibility of an existing relationship between the experimental bioactivities of the inhibitors under study and the docking scores in the current work. To get accurate results, all the docking experiments were performed with the default parameters. Molecular docking of the filtered ligands with the target site of the protein was performed with the Autodock Tools 1.5.6. The grid was fixed at the target site of the protein. AutoGrid was helped with the preparation of the grid map using a grid box. The grid size was set to 70 × 70 × 70 XYZ points with a grid spacing of 0.385 Å. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers was considered. (Morris et al., 1998). Finally, the confirmations were clustered and the poses with the lowest minimization energy were chosen. The best output poses were analyzed for interactions between the receptor and ligand. The 2D poses of the best hits of each of the compounds were generated using Accelrys Discovery Studio Visualiser 2.5.

RESULTS AND DISCUSSION**Isolation of Lactobacillus strains from Gastrointestinal Tract**

The colonies isolated from MRS agar plates on anaerobic incubation were considered as *Lactobacillus species* and were more than 300CFU/ml by spread plate at the dilutions of 10⁻⁵ and 10⁻⁶ (Fig 1).

Gram Staining

Isolated bacteria were gram-positive ovoid cocci and few rods in chains and in pairs were shown in Fig 2.

Catalase Test

The catalase test is the most effective test for confirming the members of *Lactobacillus*. No bubble was observed in the catalase test refer Fig 3. It is indicating that all of the isolated bacterial strains were catalase negative and this differentiated the species from *Staphylococcus sp.*

Lipid isolation

Lipids were isolated from 72hrs grown isolate in MRS broth. The total amount of lipid content was determined by taking the weight of dry lipid and deducting the weight of the centrifuge tube. It was found to be (7.332-6.8) 0.532g. Fig 4 is indicating Lipid layer formed in methanol and chloroform.





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Antioxidant activity

The results mentioned below Table 1 demonstrated that the radical scavenging activity of the metabolites of *Leuconostoc mesenteroides* was promising and the IC₅₀ value was 600 µg/ml. The lower IC₅₀ indicates higher free radical scavenging ability. It can be concluded that the present study indicates that the metabolites of *Leuconostoc sp.* demonstrated better DPPH activity with low IC₅₀ shown in Fig 5.

GCMS interpretation

The Gas Chromatography Mass Spectroscopy results were shown in Fig 6. This graph shown the amount of compounds present in the isolated lipids.

Gene Sequencing

GenBank

Leuconostoc mesenteroides strain BA 16S ribosomal RNA gene, partial sequence

GenBank: MW766999.1

[FASTA](#) [Graphics](#)

[Go to:](#)

LOCUS MW766999 1215 bp DNA linear BCT 23-MAR-2021
 DEFINITION Leuconostoc mesenteroides strain BA 16S ribosomal RNA gene, partial sequence.
 ACCESSION MW766999
 VERSION MW766999.1
 KEYWORDS .
 SOURCE Leuconostoc mesenteroides
 ORGANISM [Leuconostoc mesenteroides](#)
 Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Leuconostoc.
 REFERENCE 1 (bases 1 to 1215)
 AUTHORS Jasmine,R., Varsha,S., Lekhasri,R., Kaviyarasu,M. and Seralathan,M.V.
 TITLE Evaluating the therapeutic potential of Lactobacillus from chicken gut
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 1215)
 AUTHORS Jasmine,R., Varsha,S., Lekhasri,R., Kaviyarasu,M. and Seralathan,M.V.
 TITLE Direct Submission
 JOURNAL Submitted (18-MAR-2021) Department of Biotechnology, Bishop Heber College, Bishop Heber College, Trichy, Tamilnadu 620017, India
 COMMENT Sequences were screened for chimeras by the submitter using Ugene 1.3.

##Assembly-Data-START##
 Sequencing Technology :: Sanger dideoxy sequencing
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 121 ggcccaaat cctacgggag gctgcagtag ggaatcttc acaatgggag aaagcctgat
 181 ggagcaacgc cgcgtgtgtg atgaaggctt tgggctcgt aagcactgtt gtaggggaag
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 1201 accatggag tttgt

//

<https://www.ncbi.nlm.nih.gov/nucleotide/MW766999>

16srRNA sequencing confirms the isolated gram positive cocci to be *Leuconostoc mesenteroides*. Then gene sequencing was done to identify the organism. The lipid layer was isolated for metabolite extraction and the antioxidant activity

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was high with a low IC₅₀ value of 600 µg/ml, which is significant to decrease the risk of accumulation of ROS. The pH tolerance of the isolate was analysed and found to tolerate pH 2.0 - 4.0 but grow better between pH 3 and 4. The lipids demonstrated high antioxidant activity and the compounds responsible for the activity were identified through GCMS. Please refer Table 2.

Regulation of the Enzymes Producing ROS by *In silico* study

Oxidative stress is derived either from an increase in ROS production or decreased levels of ROS-scavenging proteins. Thus, recently many studies have investigated the effects of probiotics on ROS production. ROS are generated by several enzymatic reactions and chemical processes (Lin et al., 2015). NADPH oxidase (NOX) complex is considered to be a major source of ROS generation (Rahman et al., 2006, Lin et al., 2012, Lee et al., 2013). Several chronic diseases such as diabetes, neurodegenerative and cardiovascular diseases, and cancer can be caused by increased oxidative stress (White et al., 2014). This occurs from the activation of various transcription factors, which can express hundreds of different genes, such as growth factor promoters and inflammatory cytokines, which lead to the activation of inflammatory pathways transforming a normal cell into a cancerous one (Reuter et al., 2010).

Thus, maintenance of redox homeostasis and reduction of oxidative stress depend on the efficiency of antioxidant present in the cell, since the first and second defence barriers (antioxidant enzymes and proteolytic and lipolytic enzymes, respectively) have already been overcome (Cadenas E, 1997). Few enzymes, such as cytochrome P450 (CP450), lipoxygenase (LO), myeloperoxidase (MP), NADPH oxidase (NO) and xanthine oxidase (XO) that are known to generate ROS during the metabolism of arachidonic acid and their inhibitions break the ROS production cycle with the consequent reduction of the oxidative stress and maintenance of redox homeostasis (Dharmaraja, 2017). The increase of the oxidative stress mediated by ROS may lead to the appearance of several diseases, including cancer. Therefore, the search of agents that maintains the balance of redox homeostasis (antioxidants) has an important role in the discovery of molecules that prevent and halt the growth of cancer cells via reduction of the oxidative stress (Carnevali et al., 2010). The literature shows that molecular docking has been an important tool for studies of receptor-ligand interaction in the inhibition of enzymes related to antioxidant activity. This technique has clarified doubts and pointed out clarifications about the possible region of the receptor where the activity occurs, what amino acid residues are involved in the interactions and what atoms are directly interacting with the ligand (Gupta et al., 2018).

A structure-based druggability assessment was carried out using different standalone (MedChem Designer 3.0) . From the total availability of compounds, only 4 compounds show drug likeliness, which is obtained from Medchem Designer 3.0, were shown in Table 3. Molinspiration tools were used to calculate the molecular properties and drug-likeness score of the selected compounds were depicted in Table 4 respectively. A molecule having a bioactivity score of more than 0.00 is most likely to possess considerable biological activities, while values -0.50 to 0.00 are expected to be moderately active and if the score is less than -0.50 it is presumed to be inactive (Verma, 2012). The selected compounds have a good acceptable range of Kinase inhibitor, GPCR, Enzymelink, Nuclear receptor ligand, ion channel modulator. The harmfulness and toxicity evaluation of the ligands which is determined by Osiris property pioneer checks that every one of the chosen compounds is protected as a medication material with no risks. The predominant compound identified (based on peak area) through GC MS was Octadecenoic acid, that is additionally referred to as monounsaturated fatty acid is that the most vital and bumper compound present within the lipid layer. (34.63%) It has approved a health claim on reduced risk of coronary heart condition for top oleic oils. Hexadecanoic acid was the second most significant and abounding compound within the lipid macromolecule layer (14.02%). 1,1,3,5,7,9,9,9 nonamethylpentasiloxane was the third most significant and luxuriant compound within the macromolecule layer. (12.46%) Methyl stearate was found to be the fourth most significant and long compound within the lipid layer. (10.79%). A carboxylic acid methyl organic compound obtained by formal condensation of the carboxy group of octosecanoic (stearic) acid with the radical group of wood spirit. The compounds found in the lipids from *Leuconstoc sp.* was found to possess several biological activities. The significance of the compounds was determined by *insilico* analysis mentioned below Fig 7 . With the help of the Auto dock program, molecular docking



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was accomplished for the chosen ligands which have drug likeliness alongside the receptor protein, cytochrome P450(1OG5). The receptor has a good binding affinity towards the selected ligands as shown in Fig 8. Results of this docking study showed that the two chosen compounds binds effectively to the active site of cytochrome P450(1OG5) and it likewise gives good interaction with least binding energy and 2 hydrogen bond interactions with THR 301, ALA 297, and Hydrophobic communication ALA 106, VAL113, PHE 114, LEU 208, LEU 233, ALA 297, LEU 366. The top potential binding affinities were observed between both the compounds with cytochrome P450(1OG5). The docking connection between the ligands and the macromolecule appears in Figure 8 and their comparing binding affinity were shown in Table 5 respectively.

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Conflict of Interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article

Author's Contributions

All the contributing authors have participated in the preparation of the study

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Table 1: Antioxidant activity of *Leuconostoc mesenteroides* by percentage of inhibition

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (%)
1	Control	100
2	1000 µg/ml	76.54
3	750 µg/ml	62.58
4	500 µg/ml	44.62
5	250 µg/ml	39.40
6	100 µg/ml	34.71
7	50 µg/ml	32.56
8	10 µg/ml	15.74
9	Ascorbic acid	89.19

Table 2: The compounds screened through GC-MS analysis of the lipids from *L. mesenteroides*

Compound Name/No	M.formula	Mol.wt (g/mol)	RT (min)	Peak Area (%)	Reported Bioactivity
2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206.32	18.409	7.81	Anti-oxidant activity, Anti-inflammatory activity, Cytotoxicities Insecticidal and nematocidal activities Anti-bacterial &Antifungal activities
Hexadecanoic acid,methyl ester	C ₁₇ H ₃₄ O ₂	270.45	26.059	14.02	Antifungal, Antioxidant activity Anti- inflammatory &Antimicrobial activity
9-Octodecenoic acid,methyl ester	C ₁₉ H ₃₆ O ₂	296.5	28.832	34.63	Antifungal, Anti- inflammatory activity Anticancer & Antioxidant activity
Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5	29.24	10.79	Antifungal, Antibacterial activity
3-Nitropyridine,2-(4-methylphenyl)-	C ₁₂ H ₁₀ N ₂ O ₄	278.29	37.81	5.59	Antimicrobial activity
11-bromo-1,1-Dideuteroundecanol, O-Trimethylsilyl			37.84	5.78	





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BistrimethylN-Acetylenospinga-4,11-Diene			37.884	8.92	
1,1,1,3,5,7,9,9,9-Nonamethylpentasiloxane	C ₉ H ₃₀ O ₄ Si ₅	342.760	38.375	12.46	

Table 3. Druggability score prediction results (MedChem Designer 3.0)

Compound Name	SMILES	S+logP	S+logD	Rule of 5	MWt	T_PSA (Å ²)	HBDH	Structure
Hexadecanoic Acid, Methyl Ester	CCCCCCC(C(CCCCCC(=O)OC)SC)SC	7.670	7.670	1	362.641	26.300	0	
Octadecenoic Acid	CCCCCCCCC=CCCCCCCC(=O)O	7.208	4.928	1	282.470	37.300	1	

Notes: S+logP. LogP calculated using Simulations Plus' highly accurate internal model. S+logD. at user-specified pH (default 7.4), based on S+logP. HBDH. A number of Hydrogen bond donor protons. T_PSA. The topological polar surface area in square angstroms. Rule of Five. Lipinski's Rule of Five: a score indicating the number of potential problems a structure might have with passive oral absorption. Rule of Five Code. Lipinski's Rule of Five codes: LP=logP; Hb=number of Hydrogen bond donor protons; Mw=molecular weight; NO=number of Nitrogen- and Oxygen-based Hydrogen bond acceptors.

The presence of a code means that the corresponding Lipinski rule was violated. ($\log P \leq 5$, Mol.Wt ≤ 500 , number of hydrogen bond acceptors ≤ 10 , number of hydrogen bond donors ≤ 5).

Table 4: Bioactivity score of the compounds from Molinspiration

Compound Name	Ion channel modulator	Nuclear receptor ligand	Enzyme inhibitor	Protease inhibitor	Kinase inhibitor	Gpcr ligand	Toxicity and risk
9,10-Di(methylthio) hexadecanoic acid, methyl ester	-0.21	0.06	0.07	0.14	-0.40	-0.08	0
9-Octadecenoic acid	0.17	0.23	0.27	0.07	-0.22	0.17	0





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Table 5: Molecular Docking Analysis

Compound	Protein	Binding Affinity	Hydrogen Bond Interaction	Hydrophobic Interaction
9,10-Di(methylthio)hexadecanoic acid, methyl ester	cytochrome P450(1OG5)	-6.3	THR 301,ALA 297	ALA 106,VAL113,PHE 114,LEU 208,LEU 233, ALA 297,LEU 366
9-Octadecenoic acid	cytochrome P450(1OG5)	-6.3	THR 301,ALA 297	ALA 103,ALA 106,VAL 113,PHE 114,ASN 204,LEU 233, ALA 297,LEU 366

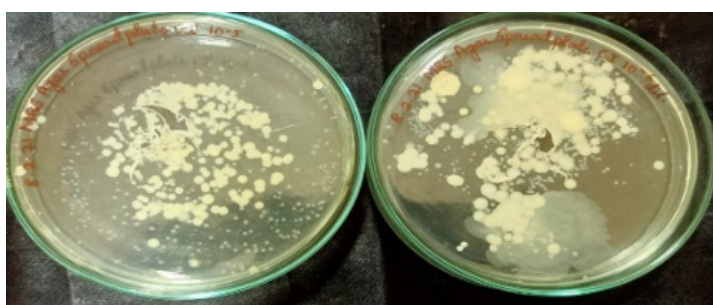


Fig.1: Isolated LAB colonies in MRS agar plate

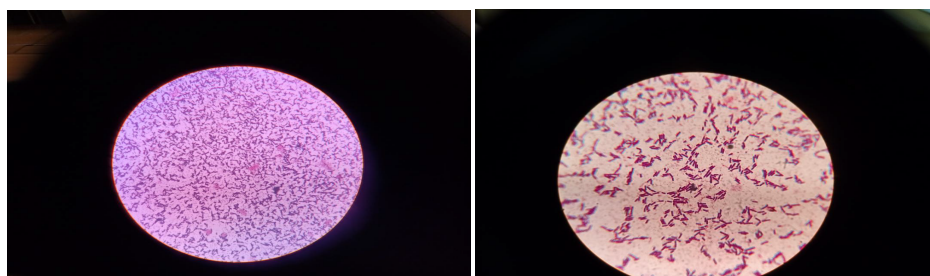


Fig.2: Gram positive Cocci (40x and 100x).



Fig.3: Absence of bubbles during catalase test

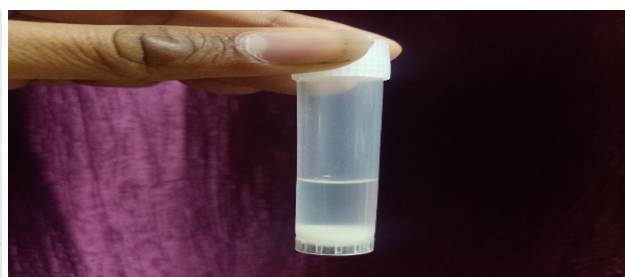
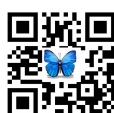


Fig.4: Lipid layer formed in methanol and chloroform





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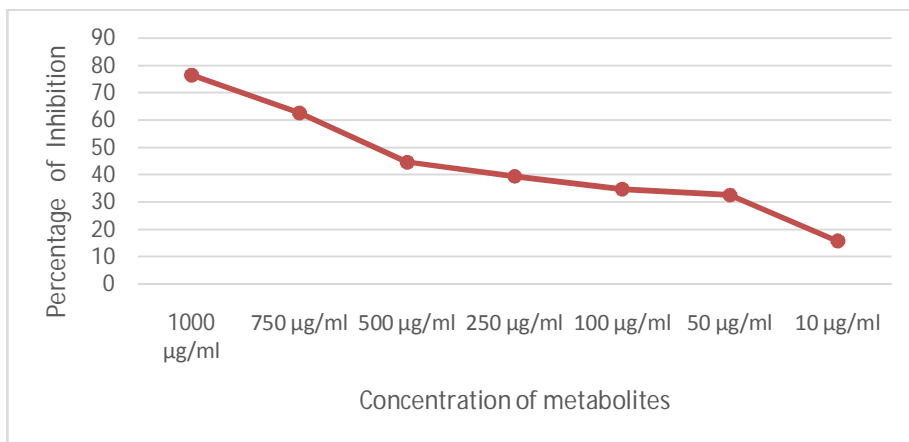


Fig. 5. Graph depicting the dose dependent antioxidant activity

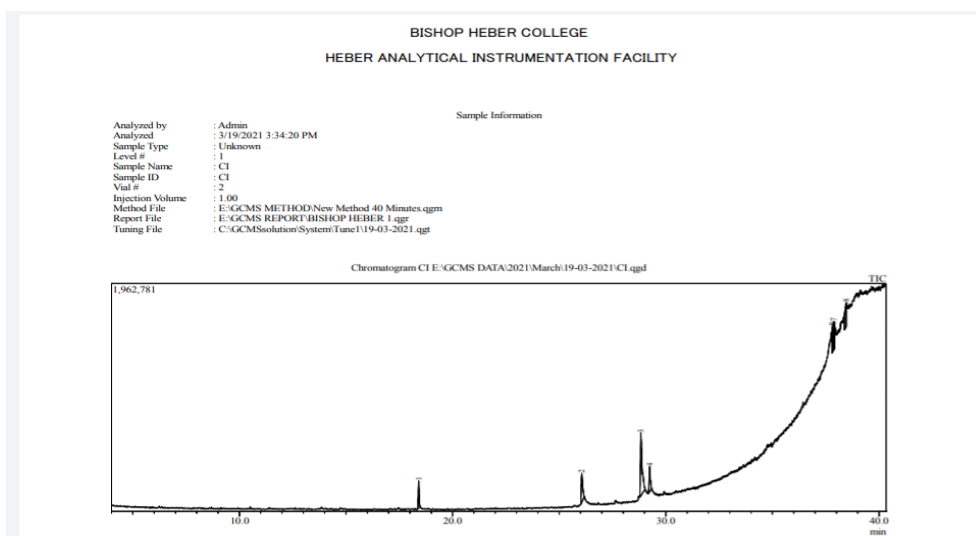


Fig.6: Graph for the amount of compounds present in lipid layer

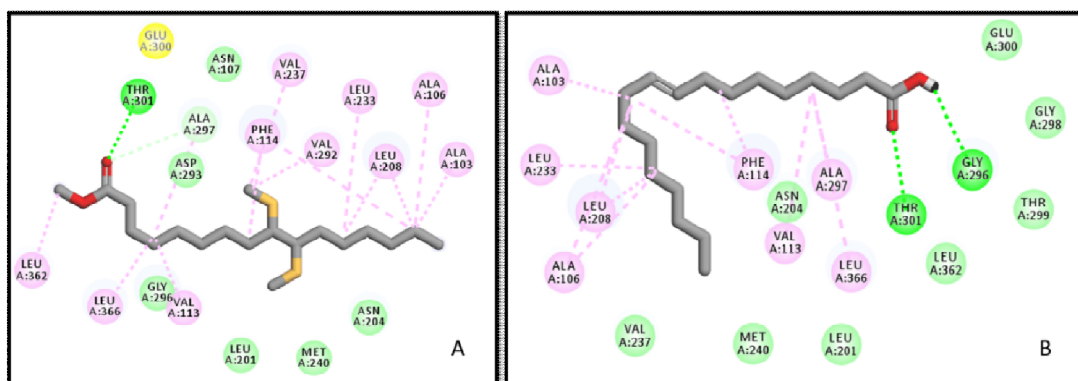


Fig.7: Ligands as Hexadecanoic Acid, Methyl Ester and Octadecenoic Acid docked with CP450





***In vitro* Propagation of *Smilax glabra* Roxb., a Highly Demanded Endangered Plant of Southern Assam, India**

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ABSTRACT

Smilax glabra Roxb. is a highly demanded endangered medicinal plant of Southern Assam. It is a non timber forest product. In the present work, a micropropagation protocol was developed for the conservation of this plant species. Four different types of media, i.e. MS full strength, MS half strength, GM5 and MSM were used for the *in vitro* propagation of *Smilax glabra* with the addition of IAA, Kin, NAA, IBA and 2, 4-D in varied concentration. The different explants nodal segments had shown good response for the regeneration of this plant species in MS and half strength MS media. The rooting of the regenerated plant was observed in MS half strength medium supplemented with 0.1mg/l IAA.

Keywords: Endangered, Explants, Medium, Micropropagation, *Smilax glabra*.

INTRODUCTION

Southern Assam is a biodiversity rich region. This region is popularly known as Barak Valley. The region is one of the richest zone in the floristic diversity in NE India harbouring host of medicinal as well as economically important plant species. Most of the medicinal plants are found to be in the wild condition. *Smilax glabra* Roxb. is a highly demanded less known wild medicinal plant species of Southern Assam. It is popularly known as green brier or cat brier and locally known as shuksini. It is a deciduous climber growing up to 3 m to 4m tall. It is mainly found in the hilly forest areas of Southern Assam. The main important part of the plant species is the rhizome. The plant can be identified due to two identifiable characters i.e. absence of spine and white coloured powdery layer present in the dorsal side of the leaf. This plant species differs from the other species of *Smilax* with these typical characters.





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It is a highly demanded medicinal plant among the top 20 medicinal plants found in India. The main useful part of this plant is rhizome. The rhizomes are used as medicine in curing different ailments, i.e. Cancer, mercury poisoning, acute bacterial dysentery, rheumatoid arthritis etc, (Duke and Ayensu, 1985). It is also used for the treatment of cough, hypertension, wound, sores and burns, sexual impotency, rheumatism, skin ailments, and as a general tonic for physical weakness etc.

Once upon a time the plant species was available in plenty in this region. One of the interesting finding of the present work is that only the aged people, (i.e. people above 50 years) know the importance of this plant species. Now *Smilax glabra* is becoming endangered due to some anthropogenic activities such as over exploitation, habitat destruction, illegal extraction, deforestation etc. According to the report of ENVIS Centre on Conservation of Medicinal Plants, FRLHT, Bangalore, the plant *Smilax glabra* Roxb. is identified as a Critically Endangered (CR) plant species of Assam (22 September 2010) specially in Barak Valley. It has also been reported to be a Critically Endangered (CR) plant species by the BSI (Mao et al., 2008) and by the Ministry of Environment and Forest (Press Information Bureau, Government of India; 9 February 2012) (Anon, 2012).

The percentage of seed germination in this plant is very less. Therefore, some conservation initiatives must be taken for its sustainable regeneration and use by the future generation. Vegetative propagation and micropropagation seem to be the only solution for the conservation of this plant species and therefore has been attempted in the present work. Development of conservation technology will help in promoting mass cultivation as well as it will reduce the pressure on the wild stock.

MATERIALS AND METHODS

The plant species was collected from the wild habitat of Southern Assam and cultivated in the nursery and green house of the Department of Ecology and Environmental Science, Assam University, Silchar. These plants were further used as the source of explants.

Explant

Different parts of *Smilax glabra* i.e. young stem, young leaf, axillary bud, node, internode, young tendrils were used as a source of explants for *in vitro* propagation of this plant. These plant parts were collected from the nursery of the Department of Ecology and Environmental Science, Assam University, Silchar, Assam.

Sterilization

Freshly collected explants, i.e. young leaf, young stem, axillary bud, node, internodes, and tendrils were thoroughly washed under the running tap water. Subsequently they were again washed with the double distilled water and then taken inside the Bio-safety cabinet and further washed with sterile double distilled water. Explants were then treated with few drops of tween 20 by constant stirring for 5 minutes, followed by 4-5 times washing with the sterile distilled water. Subsequently the explants were disinfected with 5% calcium hypochloride for 5, 10 and 15 minutes, respectively, followed by continuous washing with sterile distilled water.

Culture media

Murashige and Skoog, Murashige and Skoog modified medium and Gamborg B5 medium were used for the *in vitro* propagation of *Smilax glabra* from different explants. Different concentration of IAA (Indole-3-acetic acid), 2,4-D (2,4-dichloro-phenoxy-acetic acid), KIN (Kinetin), NAA (alpha-naphthalene acetic acid), IBA (Indole-3-butyric acid) were used as a source for plant growth hormone for the shooting and rooting of the propagated explants. The above mentioned culture media were readymade dehydrated plant tissue culture media (HIMEDIA). The pH of the medium was adjusted between 5.6 and 5.8. Agar was dissolved by boiling the mixture and about 20-50 ml media was dispensed into each culture tube and vessel. Subsequently, the culture media were autoclaved at 121°C for 20





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minutes at 15lb/sq. inch pressure. The medium were allowed to cool and kept under Bio safety cabinet for 42 hours. If no contamination was observed in them then the replicate media were considered for inoculation.

Inoculation and culture

The sterilized explants were cut into 0.5 cm pieces by the sterilized scalpel blade. The node, internodes, young stem explants were inoculated vertically on the surface of the different culture medium. On the other hand, the young leaf explants were inoculated both by its dorsal and ventral side on the surface of the culture medium.

Acclimatization

The regenerated plantlets were taken out from the culture vessels and tubes and were washed thoroughly with sterile distilled water. These were then treated with 0.2% Bavistin solution. The plantlets were potted into a medium with sand, water hyacinth root, coconut husk, wood and brick pieces alone and in combination. These were then kept in the culture room for 15-20 days and sprayed regularly with sterile nutrient liquid media.

Transplanting

After 15 to 20 days these plants were transferred into the pots containing different potting medium.

Growth parameters

The following growth parameters were recorded during the regeneration experimentation:

- 1) Number of days for shoot initiation,
- 2) Number of leaves produced
- 3) Average length of the shoot
- 4) Number of days for root initiation
- 5) Number of root
- 6) Average root length

RESULTS AND DISCUSSION

Shoot multiplication

In the present work, micropropagation of *Smilax glabra* was successfully established from the nodal segments of the explants, while others did not show any response, i.e. young stem, young leaf, axillary bud, internode, young tendril. Shoots were induced and proliferated from the nodal segments on the MS full strength and MS half strength basal medium. After 13 days culture on MS basal medium with the combination of 2 mg/l IAA and 3 mg/l Kin, the shoots were proliferated from the nodal segments. On the other hand the proliferation of shoot was observed on the 8th and 9th day after the culture of the nodal segments on the MS half strength basal medium supplemented with 0.5 mg/l of BAP and 1.0 mg/l of BAP. The number of leaves and the shoot length in the MS half strength basal medium supplemented with BAP as 0.5mg/l was recorded to be higher compared to Ms basal medium supplemented with the combination of IAA and Kin (2mg/l IAA and 3 mg/l KIN).

These results indicate that the micropropagation of *Smilax glabra* can be established from the nodal segments. Studies on the other species of *Smilax*, *Smilax corbularia*, *Smilax zeylanica*, have also reported that the shoot initiation and proliferation was obtained from the nodal segments only (Jirakiattikul *et al.*, 2013; Thirugnanasampandan *et al.*, 2009). The half strength MS media was found to be more effective compared to MS, GB₅ and MSM media in the rapid micropropagation of *Smilax glabra*. Similar results were also observed in the rapid micropropagation of *Smilax china* (Song *et al.*, 2010). MS basal medium contains very high dosages of nitrogen compared to other three culture medium and there are also differences in the micronutrient composition among the MS, MSM & GB₅ medium (Song *et al.*, 2010).



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The above mentioned results also supported that addition of BAP was the most effective in bud breaking and shoot proliferation in the explants (Mukherjee *et al.*, 2010). The results indicated that the initiation and proliferation of shoot, number of leaves and shoot length was significantly affected by the Cytokinin. BAP is found to be more effective than Kinetin in low concentration when used singly. The significant effect of BAP was also reported in the micropropagation of other species of *Smilax*, i.e., *Smilax oldhami* (Tazawa *et al.*, 1996), *Smilax zeylanica* (Thirugnanasampandan *et al.*, 2009), *Smilax discotis* (Liu *et al.*, 2006) and *Smilax china* (Song *et al.*, 2010).

Root initiation

Initiation and development of root in the plantlets of *Smilax glabra* was observed only in half strength MS medium supplemented with 0.1mg/l IAA. Rooting occurred in this basal medium after the 7th days following transfer into the rooting medium. It was also observed that the plants grown in the medium containing plant growth regulators (i.e. IBA, IAA and NAA) in different concentration did not response to root initiation. No rooting was observed in MS full strength basal medium supplemented with different concentration of IBA, IAA and NAA respectively. These results are in conformist with the micropropagation of *Smilax zeylanica*. In this species MS half strength basal medium was found to be more effective for the rooting of the microshoots (Thirugnanasampandan *et al.*, 2009). It has been reported earlier that half strength MS medium produced higher significant result compared to full strength MS medium on the rooting of *Smilax corbularia* under micropropagation (Jirakiattikul *et al.*, 2013).

Acclimatization

The plantlets with roots were successfully transferred to pot following primary hardening. The survival percentage of the *in vitro* propagated plants was found to be 70-75% in the pot containing the mixture of soil and sand.

Zeng *et al.* (2005) developed a protocol for the *in vitro* propagation of *Smilax glabra* Roxb. The results indicated that sterilized explants cultured in the MS + 6-BA 1.0 mg/L + NAA 0.1 mg/L could lead to faster budding, the medium MS + 6-BA 1.0 mg/L + NAA 0.1 mg/L + 15% CM was found to be suitable for the proliferation, while 3/2MS + 6-BA 0.05 mg/L + 4% Sugar was reported to be suitable for the production of strong buds. Treatment on the medium H (modified) + 0.5 mg/L NAA was found to be best for rooting. The methodology on the present study of the micropropagation of *Smilax glabra* is totally different from the above mentioned earlier studies.

CONCLUSION

Generally the woody plants are difficult to grow under *in vitro* propagation. Especially *in vitro* propagation of vines is more difficult (Song *et al.*, 2010). The present protocol has been established as a rapid *in vitro* propagation for *Smilax glabra*, which is a monocot and woody climber. These results may be used to develop an *in vitro* conservation strategy for the large scale production of the plantlets of *Smilax glabra* for their dissemination and commercialization/conservation.

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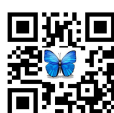
Table 1: Response of explants on the propagation of *Smilax glabra in vitro*.

Serial Number	Explants	Response
1	Young stem	-
2	Young leaf	-
3	Axillary bud	-
4	Node	+
5	Internode	-
6	Young tendril	-

-No response, + Response

Table 2: Response of different tissue culture media with different concentration of plant growth regulators on the propagation of *Smilax glabra* Roxb *in vitro*.

Media	PGR (mg/l)	Response
MS full strength	0.5 IAA	-
	1 IAA	-
	1.5 IAA	-
	2 IAA	-
	1 2,4-D	-
	2 2,4-D	-
	3 2,4-D	-
	4 2,4-D	-
	1 IAA + 1 2,4-D	-
	1.5 IAA + 2 2,4-D	-
	2 IAA + 3 2,4-D	-
	0.5 IAA + 1 KIN	-
	1.5 IAA + 2 KIN	-
2 IAA + 3 KIN	+	
MS half strength	0.5 IAA	-
	1 IAA	-





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	0.5 NAA	-
	1 NAA	-
	0.5 KIN	-
	1 KIN	-
	0.5 BAP	+
	1 BAP	+
	0.5 IAA+ 1 BAP	-
	1 IAA + 2 BAP	-
	1.5 IAA+ 3 BAP	-
	2 IAA + 4 BAP	-
GB5	0.5 IAA+ 0.5 BAP	-
	1 IAA + 1 BAP	-
MSM	0.5 IAA	-
	1 IAA	-
	1.5 IAA	-
	2 IAA	-

-No response, + Positive Response

Table 3: Effect of various media and hormonal concentrations on the shoot initiation of the nodal explants of *Smilax glabra* Roxb *in vitro*.

Media	Hormonal concentration			Number of Days for shoot initiation	Number of leaves	Average length (cm)
	IAA	KIN	BAP			
Ms (Full strength)	2.0	3.0	0.0	13.00±1.00	7.00±1.00	8.63±0.91
Ms (Half strength)	0.0	0.0	0.5	8.00±0.00	10.00±0.00	12.67±0.57
	0.0	0.0	1.0	9.30±0.58	9.00±1.00	10.20±0.60

Average, ± -SD

Table 4: Effect of various media and hormonal concentration on the root initiation/ growth of the microshoots of *Smilax glabra* Roxb *in vitro*.

Media	PGR	Response
MS full strength	0.5 IBA	-
	1 IBA	-
	0.1 IAA	-
	0.2 IAA	-
	0.5 IAA	-
	1 IAA	-
	0.5 NAA	-
	1 NAA	-
	0.5 IBA + 0.5 IAA	-
	1 IBA + 0.5 IAA	-
	0.5 IBA +0.5 NAA	-
	1 IBA + 0.5 NAA	-
	MS half strength	0.5 IBA
1 IBA		-
0.1 IAA		+





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	0.2 IAA	-
	0.5 IAA	-
	1 IAA	-
	0.5 NAA	-
	1 NAA	-
	0.5 IBA + 0.5 IAA	-
	1 IBA + 0.5 IAA	-
	0.5 IBA +0.5 NAA	-
	1 IBA + 0.5 NAA	-

-No response, + Positive Response

Table 5: Effect of half strength MS medium supplemented with hormonal concentration (0.1mg/l IAA) on the rooting of the microshoots of *Smilax glabra* Roxb *in vitro*.

Media	IAA	Number of Days for root initiation	Number of roots	Average root length(cm)
MS(Half strength)	0.1	7.33±1.53	8.67±1.53	3.2±0.46

Average, ± -SD

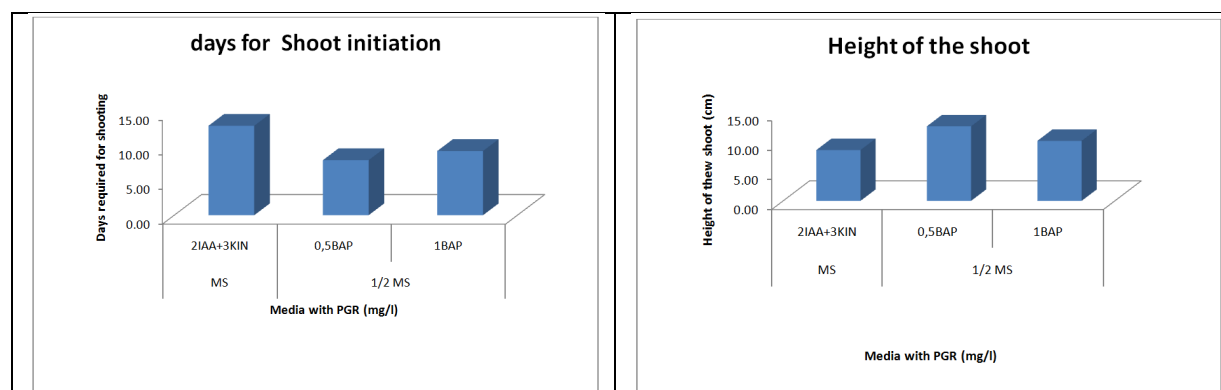


Fig 1: Number of Days required for shoot initiation of *Smilax glabra* Roxb. *in vitro*

Fig 2: Height of the microshoots in various media and hormonal concentrations of *Smilax glabra* Roxb. *in vitro*

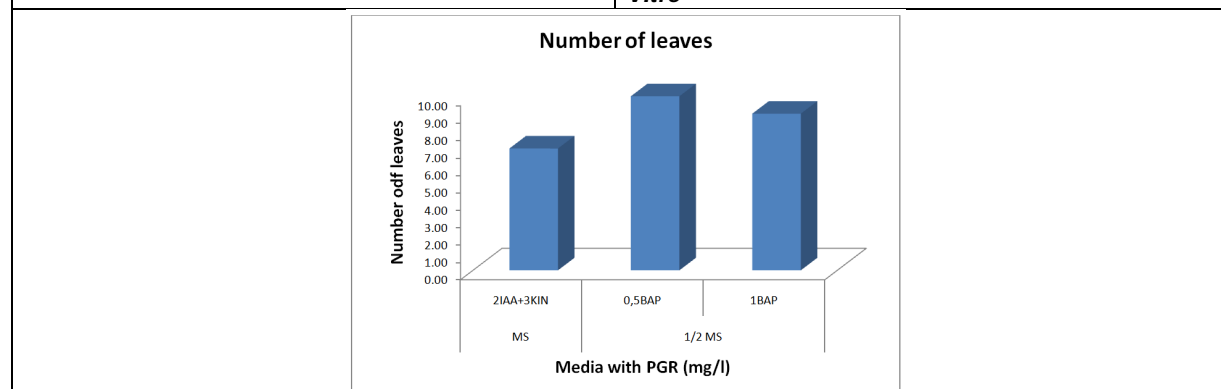


Fig3: Number of leaves of the microshoots of *Smilax glabra* Roxb. *in vitro* in various media supplemented with varied hormonal concentrations





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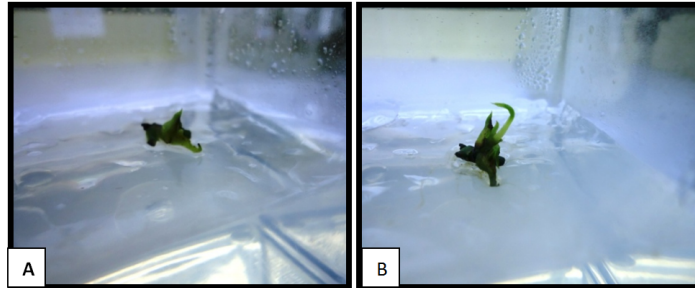


Plate 1: Proliferation of bud in *Smilax glabra* Roxb. in Full strength MS medium with the combination of 2mg/l IAA + 3mg/l Kin

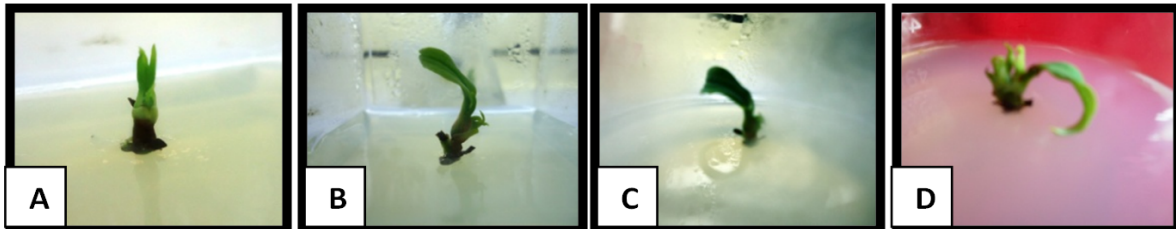


Plate 2: Initiation of leaf of *Smilax glabra* Roxb. in different media *in vitro*: A) MS medium with 2mg/l IAA + 3mg/l Kin, B) Half Strength of MS medium with 0.5 mg/l BAP, C) Half Strength MS medium with 1 mg/l BAP, D) Half Strength MS medium with 1.5mg/l IAA+ 3 mg/l BAP

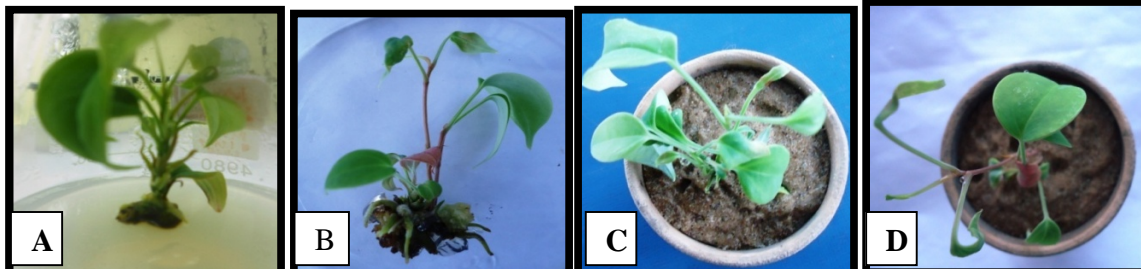


Plate 3: A), B) Plant body with Rooting of *Smilax glabra* Roxb. in Half Strength MS medium with 0.1mg/l IAA *in vitro*. C), D) Hardening of *Smilax glabra* Roxb. in pot culture (Lab to land culture)





Effect of Lead Nitrate on Greengram (*Vigna radiata*(L.) Wilczek

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ABSTRACT

A present research work was carried out to determine the effect of lead nitrate on seed germination and seedling growth of greengram (*Vigna radiata* L.). The seeds were treated under control (without treatment) 2.5, 5, 7.5, 10 and 12.5 g of lead nitrate $Pb(NO_3)_2$ concentration solutions individually. Each treatment was replicated thrice in a randomized block design. Observations were complete on root and shoot length, fresh and dry weight of seedling, vigour index of greengram (*Vigna radiata* L.) at 15, 30, 45, 60 and 75 days of intervals. Among the results gradual increase in lead nitrate $Pb(NO_3)_2$ concentration under different treatments significantly leads to inhibition of seed germination and other growth parameters. Percentage of phytotoxicity showed an increasing trend with gradual increase in lead concentration for the greengram seedlings. Maximum inhibition in all growth parameters and morphological parameters were recorded.

Key words: Lead nitrate $Pb(NO_3)_2$, Greengram, , Vigour index, Morphological parameters.

INTRODUCTION

The dictionary explains pollution as the presence in our environment of a substance which has harmful or poisonous effects. Environmental pollution occurs when pollutants contaminate the surroundings which brings about changes that affect our normal life style adversely. Pollutants are the key elements or components of pollution which are generally waste materials at different forms, pollution disturbs our ecosystem and the balance disturbs our ecosystem and the balance in the environment with modernization and development in our lives pollution has reached its peak, giving rise to global warming human illness. Environmental pollution occurs in different forms, air, water, soil, radioactive noise, heat / thermal and light every form of pollution has two sources of occurrence the point and the non-point sources. The point sources are easy to identify, monitor and control whereas the non-point sources are hard to control.

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pollutants contaminate the surroundings which brings about changes that affect our normal life style adversely. pollutants are the key elements or components of pollution which are generally waste materials at different forms, pollution disturbs our ecosystem and the balance disturbs our ecosystem and the balance in the environment with modernization and development in our lives pollution has reached its peak giving rise to global warming human illness. Rapid industrialization and other developmental activities with geochemical alterations posed a major threat to our environment. Among these myriad of environmental pollutants, chromium, cadmium, lead and mercury, merit a special attention due to their potential health hazard on human as well as wild life. Leather industry is one of the major industries that discharges many toxic pollutants like chromium, sulphide, phenolic compounds and other minerals salts, dyes, solvents etc., chromium contributes a major share to the hazardous nature of tannery effluents. lead is released in the environmental from chemical fertilizers, animals wastes, sewage sludge and by different industrial processes, such as electroplating, leather, tanning, paint, textile and wood preservation

MATERIALS AND METHOD

The seeds greengram (Variety CO 4 was obtained from Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu. India. The uniform seeds are selected for the experimental purpose. Source of lead nitrate Pb (No. 3) stock solution prepared by dissolving the molecular weight of (lead nitrate) and different concentrations viz., (Control, 2.5, 5, 7.5, 10, 12.5 and 15 g) of (Pb) the solution were prepared freshly at the time of experiments. The pods were filled with 5kg of garden soil, greengram seeds were sown in the pods and one set of pod irrigated with normal tap water was maintained as the control. Germination study was conducted with greengram seeds treated with lead nitrate. The seeds of greengram were surface sterilized with 0.2 per cent of HgCl₂ for two minutes and they were thoroughly washed with tap water. The seeds arranged in plastic cup filled with garden soil and they were treated with different concentrations of lead nitrate. The control set was maintained by using tap water. Three replicates were maintained for each treatment. On the 15, 30, 45, 60, 75, DAS the germination percentage, shoot length, root length, total leaf area, seedling fresh weight, and seedling dry weight were taken. From these data, the following values of vigour index, and percentage of phytotoxicity were calculated.

Germination percentage

The number of seeds germinated in each concentration was counted on the 15th day and the germination percentage was calculated by using the following formula.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Shoot and root length (cm/seedling)

Twenty seedlings were taken from each treatment and their shoot length and root length were measured by using a cm scale and the values were recorded.

Total leaf area

The total leaf area was calculated by measuring the length and width of the leaf as described by Yoshida *et al.* (1972).

Where

$$\text{Leaf area (cm}^2\text{)} = K \times \text{Length} \times \text{Breadth}$$

K = Kemp's constant (for dicot leaves 0.66)

Fresh weight (g/seedling)

Ten seedlings were collected from each treatment and their fresh weights were measured with the help of an electrical single pan balance.



**Thiyagarajan et al.****Dry weight (g/seedling)**

The same seedlings used for fresh weight were kept in hot air oven at 80°C for 24 h. Then, the seedlings were taken from the oven and kept in desiccators for some time. Their dry weights were taken by using an electrical single pan balance.

Vigour index

Vigour index of the seedlings was calculated by using the formula proposed by Abdul-Baki and Anderson (1973).

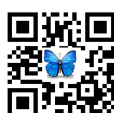
Vigour index = Germination percentage × Length of seedling by using the formula proposed by Chou *et al.*(1978).

RESULTS AND DISCUSSION

The seed vigour index of greengram were recorded on 15,30,45,60 and 75 DAS after seed sowing which are given in Table1. The vigour index of green gram with effect of lead nitrate was observed in 2.5g.As compare to others all treatment concentrations. The lowest vigourindex was observed in 12.5g of lead nitrate concentration. Shoot length of green gram plants was presented in Table2. Among green gram plants the highest shoot length was recorded in 2.5g of lead nitrate concentration. With comparison of other concentration of lead nitrate. The lowest shoot length was recorded in 12.5g of lead nitrate concentration. The effect of lead on the root length green gram plants was shown in Table3. The maximum root length was observed in 2.5g of lead nitrate concentration compare to other concentration solution. The minimum root length was observed in 2.5g of lead nitrate concentration. The effect of lead on the fresh weight and dry weight of green gram plants is shown in Tables4,5. The green gram plants were shown in better result in all analyzed parameters compare to other concentration.

The present study the morphological parameter of green gram, seedling growth and fresh weight, dry weight, root length ,shoot length and vigour index of greengram seedlings increased in with treatment of lead nitrate, and then it decreased at level of chromium and cadmium treatment. Reduction in seed germination percentage and growth at higher concentrations of metals might be due to the higher amount of toxicity, which caused changes in the osmotic relationship of the seed and water. Heavy metal contamination of soil and water is a serious problem for ecosystem which poses strong negative effects on plant growth and development (Kabata, 2001). Many authors reported inhibition Seed germination by heavy metals where as at 100% of effluent concentration decrease in length of root and shoot was recorded at 10, 15, 20, 25 and 30 days. Inhibition of seed germination may be due to high level of dissolved solids, which enrich the salinity and conductivity of the absorbed solute by seed before germination (Tantrey and Agnihotri, 2010; Heidari and Sarani, 2011; Gubrelay *et al.*, 2013). The study reported Rout *et al.* (2000) that the seed germination was reduced 25% with the treatment of 200 mM Cr concentration. The heavy metal stress could be assigned to the accelerated breakdown of stored nutrients in seeds and alteration of selection permeability properties of cell membrane.

However, Wu *et al.* (2008) mentioned that the seedlings of *Citrus tangerine* and *Poncirus trifoliata* importantly higher shoot and root dry weights, plant height, leaf area, leaf number per plant, and stem diameter with the influence of mycorrhizal (AM). Further, studies reported inhibition of seed germination by the heavy metal (Neogy *et al.*, 2002; Cavusoglu and Yalcin, 2010).Further study (Amna *et al.*, 2015) mentioned that fresh and dry biomass of plant reduced when the application of heavy metals with comparison of control plant. During the heavy metals treatment, biomass of plant was reduced. It might be changes in biochemical processes taking place at cellular and molecular level (Shanker *et al.*, 2005). Many authors reported inhibition seed germination by heavy metals (Farooqi *et al.*, 2009; Tantrey and Agnihotri, 2012; Heidari and Sarani, 2011; Gubrelay *et al.*, 2013). The study reported Rout *et al.* (2000) that the seed germination was reduced 25% with the treatment of 200 mM Cr concentration. Moreover, mycorrhiza can facilitate Cr toxicity and influence plant growth in Cr polluted soil (Davies *et al.*, 2001).Shafiq *et al.*(2008) during the seed germination, the heavy metal stress could be assigned to the accelerated breakdown of stored nutrients in seeds and alteration of selection permeability properties of cell membrane.



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Similar results were observed that the reduction of biomass in several crops were detected by Singh *et al.* (2013); Hosseini *et al.* (2007); Kamel (2008) and Farooqi *et al.* (2009). Kumari *et al.* (2011) observed that the plant fresh weight were reduced with the influence of cadmium treatment in *Vignaradiata*. Hatata and Abdel-Aal (2008) result revealed that the reduction in the fresh and dry weights of root and shoot, leaf area, and leaf are among the most sensitive responses to Cd exposure and are the indices for stress responses like other physiological reactions.

Muhammad *et al.* (2008) announced that *L. leucocephala* seedlings demonstrated a slow reduction in dry weight with increment in treatment of cadmium, which was clear in the poor development of roots and elevated parts. Fresh and dry weights of plant were reduced with increasing concentration of cadmium. Many researchers (Balestrasse *et al.*, 2003; Dell'Amico *et al.*, 2008) experimentally revealed that the with treatment of cadmium. Cheng and Huang (2006); Kumari *et al.* (2011) and Hirve and Bafna (2013) observed that the decrease of plant fresh weight under cadmium treatment in *Vigna radiata*. Similar result was also observed in *L. leucocephala* (Muhammad *et al.*, 2008). In addition to the plant growth reduction might be the Cd toxicity affects on plant roots and also it inhibit the plant photosynthesis under heavy metals stress (Zhang *et al.*, 2018). Heavy metal contamination is one of essential elements, which impact the germination conduct of any plant (Lalitha *et al.*, 1999). It has been accounted for that *Catharanthus roseus* with 500 μM CdCl₂ delivered hindered development with diminished leaf zone, biomass, chlorophyll add up to number of leaves and sterility (Pandey *et al.*, 2007). Seed germination is the main physiological process influenced by Cr treatment, the capacity of a seed to sprout in a medium containing Cr would be demonstrative of its level of resistance to this metal (Peralta *et al.*, 2001).

Similar results were observed that the reduction of biomass in several crops were detected by Singh *et al.* (2013); Kumari *et al.* (2011) observed that the plant fresh weight were reduced with the influence of cadmium treatment in *Vigna radiata*. Hatata and Abdel-Aal (2008) result revealed that the reduction in the fresh and dry weights of root and shoot, leaf area, and leaf are among the most sensitive responses to Cd exposure and are the indices for stress responses like other physiological reactions.

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Many authors reported inhibition Seed germination by heavy metals (Farooq *et al.*, 2009; Tantrey and Agnihotri, 2012; Heidari and Sarani, 2011; Gubrelay *et al.*, 2013). The study reported Rout *et al.* (2000) that the seed germination was reduced 25% with the treatment of 200 mM Cr concentration. Moreover, mycorrhiza can facilitate Cr toxicity and influence plant growth in Cr polluted soil (Davies *et al.*, 2001). Shafiq *et al.* (2008) during the seed germination, the heavy metal stress could be assigned to the accelerated breakdown of stored nutrients in seeds and alteration of selection permeability properties of cell membrane. However, Wu *et al.* (2008) mentioned that the seedlings of *Citrus tangerine* and *Poncirus trifoliata* importantly higher shoot and root dry weights, plant height, leaf area, leaf number per plant, and stem diameter with the influence of mycorrhizal (AM). Further, studies reported inhibition of seed germination by the heavy metal (Neogy *et al.*, 2002; Cavusoglu and Yalcin, 2010).





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CONCLUSION

The present investigation has been carried out to find out the effect of lead nitrate seed germination, seedling growth, morphological parameters, of green gram plants. The green gram seeds were obtained from the Tamil Nadu agricultural university Coimbatore. The lead nitrate salts were used for the treatment purpose. The germination percentage, seedling growth and fresh weight, dry weight, root length, shoot length of green gram seedlings increased in with lead nitrate concentration and then it decreased at high level of lead nitrate treatment. Reduction in seed germination percentage and growth at higher concentrations of metals might be due to the higher amount of toxicity, which caused changes in the osmotic relationship of the seed and water.

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Table 1. Effect of lead on vigour index of different varieties of greengram *Vigna radiata*

Lead treatment	Vigour index				
	15	30	45	60	75
Control	636.4±19.09	817.8±24.53	648±19.44	900±27.0	619.2±18.57
2.5mg kg ⁻¹	891.8±26.75	1041±31.24	874±26.22	1173±35.21	902±27.07
5mg kg ⁻¹	494±14.82	564.4±16.93	391±11.73	722.4±21.67	445.2±13.35
7.5mg kg ⁻¹	426.4±12.79	652.5±19.57	764.4±22.93	217.6±6.528	163.8±4.914
10mg kg ⁻¹	252±7.56	319.5±9.585	201.6±6.048	384.8±11.54	255.6±7.668
12mg kg ⁻¹	144±4.32	198.4±5.95	153.6±4.068	252±7.56	208±6.24

Table 2. Effect of lead nitrate shoot length of greengram *Vigna radiata*

Lead treatment	Shoot length(cm plant ⁻¹)				
	15	30	45	60	75
Control	7.4±0.22	9.4±0.28	7.2±0.22	10.0±0.30	7.2±0.22
2.5mg kg ⁻¹	9.8±0.29	11.2±0.34	9.5±0.29	12.1±0.36	9.6±0.26
5mg kg ⁻¹	6.1±0.18	6.8±0.20	4.6±0.14	8.4±0.25	5.3±0.16
7.5mg kg ⁻¹	8.4±0.25	8.4±0.25	8.4±0.25	8.4±0.25	8.4±0.25
10mg kg ⁻¹	3.6±0.11	4.5±0.13	2.8±0.08	5.2±0.16	3.6±0.11
12mg kg ⁻¹	2.4±0.07	3.2±0.10	2.4±0.0	3.6±0.11	3.2±0.10





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Table 3. Effect of lead nitrate on root length of greengram *Vigna radiata*

Lead treatment	Root length(cm plant ⁻¹)				
	15	30	45	60	75
Control	3.4±0.10	3.4±0.10	3.4±0.10	3.4±0.10	3.4±0.10
2.5mg kg ⁻¹	3.4±0.09	3.4±0.11	3.6±0.11	5.2±0.16	3.7±0.11
5mg kg ⁻¹	3±0.09	2.4±0.07	3.0±0.09	4.0±0.12	3.0±0.09
7.5mg kg ⁻¹	3±0.09	2±0.06	2.7±0.08	3.4±0.10	2.6±0.08
10mg kg ⁻¹	2.5±0.09	1.6±0.05	2.3±0.07	2.8±0.08	2.0±0.06
12mg kg ⁻¹	2.1±0.06	1.2±0.04	2.0±0.06	2.0±0.06	1.7±0.05

Table4. Effect of lead nitrate on fresh weight of greengram *Vigna radiata*

Lead treatment	Fresh weight (mg g ⁻¹ fr.wt)				
	15	30	45	60	75
Control	5.7±0.17	5.1±0.15	5.7±0.17	8.1±0.24	6.8±0.20
2.5mg kg ⁻¹	7.2±0.22	7.2±0.22	7.2±0.22	7.2±0.22	7.2±0.22
5mg kg ⁻¹	4.2±0.13	4.0±0.12	4.8±0.14	7.2±0.22	5.1±0.15
7.5mg kg ⁻¹	3.0±0.09	4.1±0.12	4.1±0.12	5.4±0.16	4.2±0.13
10mg kg ⁻¹	2.4±0.07	3.4±0.10	3.0±0.09	3.4±0.100	3.0±0.09
12mg kg ⁻¹	0.8±0.02	2.0±0.06	2.0±0.06	2.8±0.08	1.5±0.04

Table 5. Effect of lead on dry weight of different varieties of greengram *Vigna radiata*

Lead treatment	Dry weight (mg g ⁻¹ dr.wt)				
	15	30	45	60	75
Control	1.9±0.057	1.7±0.051	1.9±0.057	2.7±0.081	2.26±0.068
2.5mg kg ⁻¹	2.4±0.072	2.4±0.072	2.4±0.072	2.4±0.072	2.4±0.072
5mg kg ⁻¹	1.0±0.03	0.83±0.025	1.36±0.041	1.8±0.054	12.6±0.378
7.5mg kg ⁻¹	1.4±0.042	1.33±0.04	14.4±0.432	2.4±0.072	1.7±0.051
10mg kg ⁻¹	0.8±0.02	0.56±0.017	1.13±0.034	1.00±0.03	1.00±0.03
12mg kg ⁻¹	0.26±0.008	0.2±0.006	0.66±0.02	0.93±0.028	0.5±0.015





Review on Chemotherapeutic Nanoformulations

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ABSTRACT

Cancer is one of the major causes of death in the human population. Cancer is the unconstrained growth of abnormal cells in a body. Various strategies have been implemented to treat this dead-causing disease. Radiation therapy, chemotherapy, immunotherapy, hormone therapy, and surgery are the various cancer treatment strategies. This review deals with the various chemotherapeutic drugs and their nano formulations used for cancer treatment. There are several classes of drugs used as chemotherapeutic agents. These agents had several drawbacks which can be overcome by their nanoformulation. Drug Docetaxel is approved and used for breast cancer. But the drug is associated with systemic toxicities and to overcome this drawback the drug is encapsulated in Poly lactic-co-glycolic acid nanoparticles. Docetaxel-loaded solid self-nano emulsifying drug delivery system also shows enhanced antitumor efficacy. Drug Paclitaxel is a naturally occurring taxane and is a widely used anticancer agent. The nanoformulation of the drug shows enhanced stability and prolonged blood circulation time. Nanoformulation of the drug doxorubicin had reduced toxicity and increased therapeutic efficacy. Drug Teriflunomide is a potent anticancer agent with a high risk of hepatotoxicity, and the microemulsion of the drug is capable of overcoming this problem. The drug Rapamycin-loaded polymeric Poly lactic-co-glycolic acid nanoparticles shows high efficacy in breast cancer therapy. Nanoformulation of drugs Irinotecan and Methotrexate also show enhanced anticancer activity. Many molecules and formulations are developing to conquer this dead-causing disease and with the help of new technologies, we can overcome this disease and save human population.

Keywords; micelle, fullerenes, Glioblastoma multiforme, microemulsion.



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INTRODUCTION

In the world, cancer remains the most dominant cause of death [1]. Cancer is believed to be one of the most daring diseases, which cause the death of millions of people each year [2]. The use of anticancer medicaments is suboptimal because of their low efficacy and higher prevalence of adverse reactions. Following systemic administration, only a small amount reaches the solid tumor and even a little fraction reaches the target cell [3]. Now a day multi-targeted chemotherapeutic combinations have gained substantial recognition in solid tumor chemotherapy [4]. Chemotherapy is the most effective way of cancer treatment by chemicals or small molecule antineoplastic drugs. However, this is a misunderstanding [5]. Researchers are carried out for improving chemotherapy using various approaches based on polymer chemistry and nanotechnology [6]. This review deals with chemotherapeutic drug formulations developed based on Nanotechnology and polymer chemistry

Chemotherapeutic Agents and Formulations

The serious drawback of chemotherapy includes rapid metabolism of the drug, small ductile exposure, limited bioavailability, increased elimination rate, undeserved side effects and the high dose of the drug. Several advanced formulations are developed to overcome these drawbacks [7].

Nano formulations of drug Docetaxel

Docetaxel is a cytotoxic anti-microtubule agent. The drug had been approved and used for the treatment of breast cancer. But the drug has dose-limiting toxicities associated with systemic drug delivery [8]. Severe systemic toxicities including bone marrow suppression, cardiac toxicity, neutropenia, anemia, febrile neutropenia, hypersensitivity, thrombocytopenia and neuropathy are the major obstructions to successful treatment [9]. So to overcome this drawback the drug is encapsulated in Poly lactic-co-glycolic acid (PLGA) nanoparticles (NPs) via the Particle replication in nonwetting template (PRINT) process. The PRINT process is a platform that simplifies fabrication and particle design with the ability to control the size and shape of the particle [8]. This technique permits the design and synthesis of promptly defined micro and nanoparticles (NPs). PRINT permit researchers to have unparalleled control over the chemical composition, modulus, cargo, and surface properties of NPs [10]. The PRINT process allows high encapsulation of drug and controlled drug release. The docetaxel molecules released from the PLGA particle are delivered to the desired cellular location. 10% and 20% drug-loaded PLGA NPs via PRINT technique shows less toxicity when compared with marketed docetaxel formulation [8].

Docetaxel is effective against breast cancer, lung cancer, and ovarian cancer. Due to its systemic toxicity efforts are focused on the development of oral route as a viable route in drug delivery. But the major drawback of oral delivery is the poor bioavailability due to its high hepatic metabolism. Several formulations are developed to overcome this drawback. Docetaxel-loaded solid self-nano emulsifying drug delivery system (SNEDDS) shows improved bioavailability and enhanced anti-tumor efficacy. SNEDDS improve the bioavailability by circumventing the hepatic portal route and protect the drug from degradation in the harsh gastrointestinal tract. It also facilitates lymphatic transport of the drug [11].

Micelles are the nanocarriers mostly used because of their promising performance and technical simplicity. Micelles are colloids of amphiphilic molecules. They form spontaneously above their critical concentration with hydrophilic fragments forming the shell and hydrophobic fragments forming the core. Polyethylene glycol-b-PLGA (PEG-b-PLGA) copolymer micelles with the combination of chloroquine as an autophagy inhibitor and docetaxel as an anti-cancer drug show significant enhancement in the therapeutic effect of the drug. Micelles have a small particle size which is suitable to achieve high cellular uptake and facilitate passive targeting and thus provide enhanced permeability and retention effect. Docetaxel-loaded PEG-b-PLGA copolymer micelles are developed by membrane dialysis method. The micelle had 7.1% drug loading and 72.8% drug encapsulation efficiency. The co-administration of the micellar formulation with chloroquine as an autophagy inhibitor shows 12 fold more efficient treatment [12].



**Ammu Soman and Dhanish Joseph****Nano formulations of drug Paclitaxel**

Paclitaxel (PTX) is a naturally occurring taxane and is widely used for treating metastatic breast cancer ovarian cancer and several other malignancies [13]. Anticancer agent paclitaxel-loaded PLGA NPs were formulated by solvent evaporation technique with vitamin E d- α -tocopheryl polyethylene glycol (ETPGS) as an emulsifier. Here nanoparticles of biodegradable polymers are used and thus toxic adjuvant is avoided. And this formulation shows high drug encapsulation efficiency, high NPs uptake, and sustained drug release [14]. For lung cancer therapy, liposome aerosol delivery has been prosperously used. But this delivery route has certain drawbacks such as rapid clearance of the drug from lungs after cessation of aerosol delivery. Fullerenes have the potential to overcome these drawbacks. Fullerenes are biologically stable three-dimensional scaffolding for covalent attachment of multiple drugs to create a single dose. Fullerenes loaded with PTX could produce such an ideal lipophilic slow-release system because it is biologically stable [15].

Photothermal therapy (PTT) using near-infrared (NIR) light-absorbing nano agents to kill cancer cells has a great role in chemotherapy. The formulation composed of paclitaxel, human serum albumin, and indocyanine green which is an FDA-approved NIR dye, is used for the treatment of breast cancer and lung cancer. This formulation shows enhanced stability and prolonged blood circulation time [16]. Worm-like Filo Micelles loaded with anti-cancer drug PTX show that worm-like micelles load and solubilize twice as much drug as spherical micelles. These formulations are far less toxic and show fivefold higher anticancer efficacy on human lung cancer cells. Worm-like micelles loaded with the drug had a long retention time in blood and the formulation is much less toxic compared to Cremophor EL [17].

Nano formulation of drug Doxorubicin

Doxorubicin (DOX) is a drug approved by the FDA for the treatment of ovarian cancer and multiple myeloma. Doxil is the liposomal formulation of DOX, which is designed to retain the drug in circulation, minimizes the clearance of the drug and its uptake by healthy tissues. PEGylation of liposomes results in a long-circulating half-life, very small distribution volume, low clearance rate, and high area under the curve. And also Doxil could load 10,000-15,000 drug molecules [18]. DOX is associated with various side effects such as cumulative and irreversible cardiotoxicity. To overcome these drawbacks a drug delivery system with improved pharmacological properties was formulated. PLGA drug delivery system with simultaneous incorporation of chemotherapeutic agent DOX and thermo-optical agents showed biphasic drug release pattern and high drug entrapment efficiency. Also, it was a biodegradable and biocompatible polymer. Studies show that incorporation of DOX into PLGA NPs reduces cytotoxicity and decreases the undesirable side effects such as impaired cardiac function [19].

This drug had severe gastrointestinal toxicity and cardiac toxicity. So the drug is encapsulated in carbon nanotubes. Carbon nanotubes are used as novel delivery vehicles. Carbon nanotubes have great mechanical, optical, and chemical properties. Incorporation of doxorubicin on carbon nanotubes shows significantly enhanced therapeutic efficacy and great reduction in toxicity when compared with normal formulation [20]. NPs loaded with chemotherapeutic agents such as liposomes, polymer NPs and lipid NPs can overcome drug resistance, solubility, and stability problems which are the biggest challenges in chemotherapy. The size range of NPs is defined as 10-100nm. They had a small particle size and vast surface area and have unique mechanical, electronic, photonic, and magnetic properties. Targeted drug delivery to a tumor cell is another advantage of drug-loaded NPs. Doxil is the first nano-drug used to treat metastatic ovarian cancer. The formulation had high therapeutic efficacy. It allows the drug to stay longer in the bloodstream so that more of the drug reaches the cancer cells [21]. Iron oxide NPs loaded with Doxorubicin hydrochloride are a good drug delivery vehicle for targeting brain tumors. The accretion of iron oxide NPs in gliosarcomas is substantially increased by magnetic targeting and successfully measured by magnetic resonance imaging (MRI). And for glioma-targeted drug delivery, these NPs seem to be a better drug delivery vehicle. The formulation demonstrated sustained intracellular retention and dose-dependent anti-proliferative activity. [22,23].



**Ammu Soman and Dhanish Joseph****Nano formulation of drug Teriflunomide**

Glioblastoma multiforme (GBM) is regarded to be the highest degree lethal primary brain tumor. Teriflunomide (TFM) is a potent tyrosine kinase and dihydroorotate dehydrogenase kinase inhibitor. It is one of the potent anticancer agents in the treatment of GBM. But this drug has a high risk of hepatotoxicity, so the drug is advocated to deliver directly to the site of action. For the delivery of therapeutic agents directly to the CNS, the Nose-to brain pathway is considered the safest and effective way. Intranasal teriflunomide microemulsion is an improved chemotherapeutic approach in GBM [24]. A microemulsion is a lipid-based nanocarrier which is a promising approach for improving solubility and permeability of poorly soluble drugs [25]. This formulation displays high drug entrapment efficiency and direct brain transport of drugs [24].

Nano formulation of drug Rapamycin

Rapamycin is an effective drug that uses an alternative mechanism to inhibit the growth of breast cancer cells. But the drug had no solubility in water, no tumor tissue specificity, and low bioavailability. Rapamycin-loaded polymeric PLGA nanoparticles show high efficacy in breast cancer therapy. These NPs are surface conjugated with antibodies to epidermal growth factor receptor (EGFR) using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride. EGFR is expressed about 14-91% in breast cancer. By using tumor-specific antigen or antibody as a targeting moiety, chemotherapeutic agents are selectively delivered to cancer cells. The formulation has high drug loading capacity and high intercellular uptake and is selectively delivered to tumor cells [26].

Nano formulations of drug Methotrexate

Methotrexate is a hydrophilic anti-cancer agent used to treat various tumors. But this drug is highly cytotoxic. It administers the cytotoxic activity not only in cancerous cells but also in normal cells. So for targeted drug delivery, an effective carrier system was required. Thus Methotrexate-loaded solid lipid NPs were formulated and the formulation displays major drug accumulation in neoplastic tissues when compared with the drug solution alone [27]. Magnetic NPs conjugates were formulated as an enhancement agent in MRI and as a drug-carrying vehicle in controlled drug delivery. The conjugate was made of iron oxide NPs covalently bound with Methotrexate. The drug binds covalently to NPs is highly stable. Methotrexate incorporated super paramagnetic NPs can be used to target many cancer cells. Drug- NPs conjugate was formulated by grafting the drug into the nanoparticle surface. Hereby covalently modified the surface of iron oxide NPs via a peptide bond, cleavage of the bond occurs only under conditions present in lysosomal compartment, a typical environment inside the target cell. So these NPs provide controlled and targeted drug release to cancer cells [28].

Nanoformulation of drug irinotecan

Lung cancer is one of the main causes of death in the world. A great invention in cancer therapy is the development of magnetic NPs formulation of chemotherapeutic irinotecan to lungs. The major drawbacks of cytotoxic agents are the limited entry into the lung selectively, thus resulting in collateral damage to other tissues. This problem can be over helmed by the development of new magnetic irinotecan containing NPs which targets the lung over other tissues by over 5-fold. Selective targeting into lungs is attained by incorporating a facilitated transport mechanism into NPs. By using an external magnet the drug can be retained in the lungs. Studies display that this method of treatment is a cost-effective and efficacious therapy for lung cancer [29].

Nanoformulation of drug Sulforaphane

Targeted drug delivery by using biodegradable microspheres is a promising approach in cancer therapy. A magnetic targeted delivery system is an approach that can deliver therapeutic agents to a targeted site using an external magnetic field. Using an effective external magnet it is possible to target the microspheres injected in blood circulation to the disease site. Also, these particles are nontoxic and biocompatible. Sulforaphane, a histone deacetylase inhibitor loaded with targeted magnetic microspheres is developed by a spray-drying method. This formulation shows high efficacy in cancer therapy. Microspheres deliver a high concentration of drugs in the target site when compared with free drugs [30].



**Ammu Soman and Dhanish Joseph****Nano formulations containing a combination of multiple chemotherapeutic agents**

A synergistic combination of multiple chemotherapeutic agents in high-capacity poly (2-oxazoline) micelles is another promising step in cancer treatment. Paclitaxel, docetaxel, 17-allylamino-17-demethoxygeldanamycin, and etoposide were used. The micelles show high drug loading capacity. And multi-drug loaded poly (2-oxazoline) micelles displayed improved stability in comparison with single-drug loaded micelles [31]. A rational design of drug synergy-based design is required to increase chemotherapeutic effect while decreasing the toxicity effect of anti-cancer agents. Novel doxorubicin-mitomycin C co-encapsulated NPs formulation discloses anti-cancer synergistic effect in multidrug-resistant human breast cancer cells. This formulation display improved drug efficacy with reduced systemic toxicity [32]. Chemotherapeutic drugs PTX and Rapamycin had non-specificity and potential side effects to the healthy tissues. To overcome these problems, drug-loaded in multifunctional magnetic NPs are formulated. The development of glycol monooleate coated magnetic NPs loaded with PTX and Rapamycin shows high biocompatibility and high drug entrapment efficiency. Sustained drug release to target cells is also achieved. And also glycol monooleate coated magnetic NPs are devoid of any surfactants and are capable of carrying high drug load [33]. N-(2-hydroxy propyl) methacrylamide (HPMA) copolymers had been formulated as targeted drug carriers during recent years. HPMA is a synthetic soluble polymer and is internalized by cells by pinocytosis. They provide controlled intracellular delivery of anti-cancer agents. Anticancer agents like Daunomycin and Puromycin coupled to this copolymer provide controlled drug delivery [34].

CONCLUSION

Cancer is a leading death-causing disease in the world. Now a day many inventions are taking place in cancer therapy. Many molecules with anticancer properties were invented and various drug formulations were developed to conquer this dead-causing disease. Many new molecules with anticancer properties and new formulations are developing for saving human population from this death-causing disease. The nanoformulations of chemotherapeutic agents improve the therapeutic efficiency. Nanoformulations provide high drug entrapment efficiency and improved stability. They also provide a reduction in the toxicity level. These formulations facilitate a high concentration of drug delivery in the target site and controlled drug release when compared with free drugs. The major concern of antineoplastic agents is that they produce a cytotoxic effect not only in cancerous cells but also in normal healthy cells. The drugs encapsulated in nanoparticles facilitate targeted drug delivery and provide protection to healthy cells. Studies proved that nanoformulations allow the drug to stay longer in the bloodstream. Like this, the major drawbacks of anticancer agents can be overcome by developing their nanoformulations and the therapeutic efficacy also gets improved. Of course with the help of new technologies, we can overcome this dangerous disease and save our humanity.

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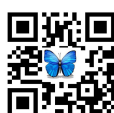
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Study of Structural Parameters of Light Weight Concrete

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ABSTRACT

Lightweight cements can either be lightweight total cement, frothed concrete or autoclaved circulated air through concrete (AAC). Lightweight substantial squares are regularly utilized in house development. Experiments has been conducted for determining the compressive strength, density, Water Absorption, self weight of the concrete, Void Capacity of light weight concrete with different percentages of Water – Cement ratio, Sand ratio, Foam percentage. The overall findings in this research paper will help us to know about the Structural Parameters and their upto which dimension we can use for the construction purpose.

Keywords: Lightweight, percentage, Cement, construction, concrete.

INTRODUCTION

Lightweight cement is a blend made with light weight coarse totals like shale, earth, or record, which give it its trademark low thickness. Underlying lightweight cement has a set up thickness of 90 to 115 lb/ft³, while the thickness of ordinary weight substantial reaches from 140 to 150 lb/ft³. This makes lightweight substantial ideal for building current constructions that require insignificant cross segments in the establishment. It is by and large progressively used to assemble smooth establishments, and has arisen as a practical option in contrast to standard cement.



**Lightweight Aggregate concrete**

Lightweight Aggregate concrete can be delivered utilizing an assortment of lightweight totals. Lightweight totals start from all things considered:

- Natural materials, as volcanic pumice.
- The warm treatment of normal crude materials like earth, record or shale for example Leca.
- Manufacture from modern side-effects like fly debris, for example Lytag.
- Processing of modern results, for example, pelletised extended section, for example Pellite.

The necessary properties of the lightweight substantial will have a course on the best sort of lightweight total to utilize. Assuming minimal underlying necessity, yet high warm protection properties, are required a light, feeble total can be utilized. This will bring about moderately low strength concrete.

Foamed concrete

Foamed concrete is an exceptionally serviceable, low-thickness material which can join up to 75 percent entrained air. It is by and large self-evening out, self-compacting and might be siphoned. Frothed concrete is ideal for making up for excess shortcomings, for example, neglected gas tanks, sewer frameworks, pipelines, and courses - especially where access is troublesome. It is a perceived mechanism for the reestablishment of brief street channels. Great warm protection properties make frothed concrete additionally reasonable for sub-tirades, making up for under-floor shortfalls and protection on level substantial rooftops.

Lightweight structural concrete

Lightweight structural concrete can be utilized for underlying applications, with qualities identical to ordinary weight concrete. The advantages of utilizing lightweight total cement include:

- Reduction in dead loads making investment funds in establishments and support.
- Improved warm properties.
- Improved imperviousness to fire.
- Savings in moving and taking care of precast units on location.
- Reduction in formwork and setting.

The flexible modulus of lightweight cements is lower than the same strength typical weight concrete, however while thinking about the redirection of a piece or shaft, this is checked by the decreased self-weight.

The lighter the substantial, the more prominent are the distinctions to be represented in the properties of the substantial. The elasticity, extreme strains and shear qualities are all lower than an ordinary weight concrete with similar chamber strength. Lightweight cements are additionally less hardened than the same ordinary strength concrete. Nonetheless, this is alleviated by the decrease in self-weight to be conveyed, so the general impact will in general be a slight decrease in the profundity of a shaft or section. Creep and shrinkage for lightweight cements are higher than that for the same ordinary weight cement, and this ought to be considered when planning the construction.

Autoclaved aerated concrete (AAC)

AAC was first financially delivered in 1923 in Sweden. From that point forward, AAC development frameworks like brick work units, supported floor/rooftop and divider boards and lintels have been utilized on all mainlands and each climatic condition. AAC can likewise be sawn by hand, etched and infiltrated by nails, screws and trimmings.

METHODOLOGY

To consider the conduct of lightweight concrete, ordinary substantial testing was done to decide the material and



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underlying properties of each kind of lightweight cement and how might these properties contrast as indicated by an alternate sort of blend and its arrangement.

COMPRESSIVE STRENGTH

Compressive strength is the essential actual property of cement (others are by and large characterized from it), and is the one generally utilized in plan. It is one of the central properties utilized for quality control for lightweight cement. Compressive strength might be characterized as the deliberate most extreme obstruction of a substantial example to pivotal stacking. It is found by estimating the most noteworthy pressure that a test chamber or 3D shape will uphold. There are three kind of test that can be use to decide compressive strength; 3D square, chamber, or crystal test. The 'substantial 3D square test' is the most recognizable test and is utilized as the standard strategy for estimating compressive strength for quality control purposes (Neville, 1994).

WATER ABSORPTION

These properties are especially significant in concrete, just as being significant for solidness. (J.H Bungey, 1996). It tends to be utilized to anticipate substantial sturdiness to oppose consumption. Assimilation limit is a proportion of the porosity of a totals; it is likewise utilized as a relationship factor in assurance of free dampness by stove drying strategy (G.E Troxell, 1956). Retention limit can be decide utilizing BS ingestion test. The test is proposed as a toughness quality control check and the predefined age is 28-32 days (S.G Millard). Test strategy as been portray by BS 1881: Part 122.

DENSITY

The thickness of both new and solidified cement is important to the gatherings required for various reasons remembering its impact for sturdiness, strength and protection from penetrability. Solidified substantial thickness is resolved either by straightforward dimensional checks, trailed by gauging and computation or by weight in air/water lightness strategies (ELE International, 1993).

RESULTS AND DISCUSSION

STRENGTH AND DENSITY COMPARISON

The motivation behind this test is to distinguish the exhibition of circulated air through lightweight cement in term of thickness and compressive strength. The outcome are introduced in Table 1 and showed in Figure 1. In view of Figure 1, it very well may be seen that compressive strength for circulated air through lightweight cement are low for lower thickness combination. The addition of voids all through the example brought about by the froth in the combination will bring down the thickness. Subsequently, compressive strength will likewise diminish with the addition of those voids. The necessary compressive strength of lightweight cement is 3.45 MPa at 28 days as a non burden bearing divider. The compressive qualities got from these blends completed are higher than 3.45 MPa and accordingly it is adequate to be delivered as non-load bearing construction.

Nonetheless, the compressive strength for the combination with thickness of 2050 kg/m³ is marginally low contrasted and thickness of 2040 kg/m³. This is because of the compaction issue during blending measure. The last blend is very dry and since compaction isn't totally done, examples are not all around compacted. This has come about the compressive solidarity to be lower than the combination with lower thickness.

COMPRESSIVE STRENGTH: As been examined previously, experimentation strategy was utilized in deciding the most appropriate combination in getting ready exploration tests. Fourteen (14) preliminary blends have been set up during the exploration and from the outcomes, the combination with the most noteworthy compressive strength with low thickness will be utilized for additional examination. Compressive strength of circulated air through lightweight cement is resolved on the 7, 14, 21 and 28 days for each example. There were three examples for each test





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and the outcomes would be taken as the normal of these three. Less factors had been set for various combination, this variable would be changed in like manner while the others were fixed to estimate their impact on the blend. Level of froth, froth specialist and water, concrete and sand proportion were the factors made during the blending interaction. For instance, three blends were set up to decide the impact of various froth specialist and water, concrete and sand proportion. The level of froth applied is fixed for three combinations and the distinction in the outcomes would happen in view of the froth specialist and water proportion. Every one of the outcomes depended on the 75% froth infused in the combination.

The thickness (Density) of 25%, half, 75%, and 100% of froth is 2040 kg/m³, 1820 kg/m³, 1810 kg/m³, and 1470 kg/m³ respectively. The thickness of half and 75% of froth blend is equivalent to been appeared in Figure 2, compressive strength for this two combination didn't vary a lot. Yet, it very well may be seen that there is a contrast between 25% of froth blend and 100% of froth combination. The thickness of 25% of froth blend is 27% higher when contrasted with 100% of froth combination and found in Table 2, the compressive strength is 85.4% higher at 28 days. For a 25% combination the compressive strength is 17.27 MPa and for 100% blend is 2.52 MPa. It is seen that the decrease in thickness or the expansion of voids in cement would impact on the strength of the substantial. The base compressive strength is 3.45 MPa of non-load bearing design ought to be achieve with the correct extent of froth. The subsequent variable set up is the froth specialist and water proportion. As per Pan Pacific Engineering Pty Ltd, one liter of froth specialist ought to be weakened with 40 liter of clean water and it makes the proportion of 1:40 however as indicated by Alex Liew on his paper of work of Lightweight Concrete Method, LCM the proportion ought to be 1:30. Subsequently, we have arranged three examples with froth specialist and water proportion of 1:40, 1:30, and 1:25 to see the distinctions. The outcomes were analyzed and classified Table 3 and Figure 3.0.

In view of Figure 3, it very well may be seen that, the froth specialist and the water proportion of 1:40 gives the most noteworthy compressive strength followed by 1:30 and 1:25. Compressive strength at 28 days for 1:40, subsequently it very well may be inferred that of the proportion somewhere in the range of 1:30 and 1:40 can be applied to the froth specialist and water. Be that as it may, concerning the 1:25 proportion, the compressive strength is somewhat lower to the past two combinations with compressive strength of 5.5 MPa. This is on the grounds that, during the blending interaction, it tends to be seen that the froth are not impeccably delivered. It isn't completely extended as the other combination with 1:40 and 1:30 proportion. 1:25 proportion ought not be suggested for future readiness since that the water was lacking to weaken the froth specialist effectively.

As indicated by Table 4, compressive strength at 28 days of combination with 1:2 concrete sand proportion is 22.99 MPa, for 1:3 is 13.12 MPa, and for 1:4 is 10.34 MPa. This shows that the compressive strength of blend with 1:2 proportion is 42.9% higher than combination with 1:3 proportion and the thickness is 12.15% higher. In spite of the fact that the compressive strength is marginally higher, yet combination of 1:2 proportions isn't financial and is viewed as more extravagant blend. Albeit the combinations of 1:3 and 1:4 concrete sand proportion gives lower compressive strength of 13.12 MPa and 10.34 MPa however it is adequate for non-load bearing design also.

Alluding to Table 5, the compressive strength of combination of 1: 0.35 water concrete proportion is 16.73 MPa, 1: 0.45 is 13.12 MPa, and for 1: 0.25 its 12.18 MPa. It very well may be seen that in spite of higher compressive strength combination with 1: 0.35, it has low thickness also of 1920 kg/m³ as contrasted with the blend proportion of 1: 0.25 which has the thickness is 2040 kg/m³. Along these lines, it very well may be presumed that the water concrete proportion of 1: 0.35 is reasonable for other combination of circulated air through lightweight cement.

WATER ABSORPTION

Water retention is a significant factor because of the permeable design of the circulated air through lightweight cement. The water ingestion test is finished utilizing the examples arranged at 28 years old days utilizing the technique has been portraying in system part. Other than that, distinctive froth specialist and water proportion will likewise influence the water retention proportion. This can be seen through Figure 7 which shows that froth specialist and water proportion of 1:40 gives the higher water ingestion contrasted and 1:30 and 1:25 proportion. As indicated by Table 7, water ingestion of 1:40 proportion is 4.46%, 1:30 is 3.31% and 1:25 is 2.6%. The differential between the water assimilation of 1:40 and 1:30 proportion is 25.78%. This shows that despite the fact that the compressive strength between 1:40 and 1:30 proportions doesn't vary that much; 0.68% however the water ingestion is especially contrast. It very well may be reason that, froth specialist and water proportion of 1:30 is more





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appropriate since the water ingestion is a lot lesser to contrast and 1:40. The high water assimilation of the substantial will likewise influence the thickness and compressive strength of the substantial. As indicated by Short (1978), lightweight cement utilized in water must be secured by reasonable material to stay away from or might be lessen water retention of the substantial.

SUPPLEMENTARY TEST

Moisture test and comparison between hardened and wet concrete is another supplementary test in this research. Figure 8 show that different percentage of foam will give different percentage of moisture content as well. It can be seen that moisture content is increased when percentage of foam is increased too. 100% of foam gives the highest moisture content followed by 75%, 50%, and 25% of foam. The explanation for this differential will be the same with the water absorption case where the increasing of voids that caused by the increment of percentage of foam will caused the moisture content to increased accordingly. According to Table 8, moisture content for 100% of foam mixture is 15.3%, while 75%, 50%, and 25% is 10.36%, 9.82%, and 8.93% respectively.

CONCLUSIONS

The underlying discoveries have shown that the lightweight cement has an alluring solidarity to be an elective development material for the industrialized structure framework. The strength of circulated air through lightweight cement are low for lower thickness blend. This brought about the addition of voids all through the example brought about by the froth. In this way the reduction in the compressive strength of the substantial. The frothed lightweight cement isn't appropriate to be utilized as non-load bearing divider as the compressive strength is 27% not exactly suggested. By and by the compressive strength is acknowledged to be created as non-load bearing design.

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Table 1: Density and Compressive Strength of Hardened Concrete at 28 days

Density (kg/m ³)	Compressive Strength (kn/m ²)
1480	2.50
1710	5.4
1760	10.33





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1790	9.12
1800	13.10
1820	11.90
1830	13.21
1840	16.70
1910	16.71
1980	16.5
2030	17.4
2040	12.20
2060	9.50
2070	22.9

Table 2: Variation of Different Percentage of Foam with No. of Days of Curing for finding Compressive Strength

Days	Compressive Strength (kN/m ²)			
	25% Foam	50% Foam	75% Foam	100% Foam
7	13.3	9.5	8.2	1.4
14	14.7	8.9	11.1	2.5
21	16.3	14.5	11.9	2.2
28	17.3	11.9	13.2	2.5
32	19.6	14.2	12.9	2.6

Table 3: Variation of Compressive Strength at Different water Ratio

Days	Compressive Strength (kN/m ²)		
	1:25	1:30	1:40
7	4.10	8.10	11.20
14	4.9	11.10	12.01
21	5.5	11.9	13.7
28	5.6	13.23	13.9
Density (kg/m³)	1710	1820	1840

Table 4: Variation of Compressive Strength at Different Cement and Sand Ratio

Days	Compressive Strength (kn/m ²)		
	1:2	1:3	1:4
7	19.5	8.2	7.6
14	18.12	11.22	7.2
21	21.56	12.20	7.62
28	23.2	13.2	11.22
Density (kg/m³)	2060	1810	1770





Table 5: Variation of Compressive Strength at Different Cement and Water Ratio

Days	Compressive Strength (kn/m ²)		
	1 : 0.25	1 : 0.30	1 : 0.45
7	10.31	13.95	8.2
14	10.15	13.60	11.12
21	11.50	16.20	11.98
28	12.3	16.86	13.12
Density (kg/m³)	2040	1910	1800

Table 6: Determination of Percentage of Water Absorption at Different Percentage of Foam

% Of Foam	Water Absorption (%)
25	1.5
50	2.52
75	3.42
100	7.42

Table 7: Determination of Percentage of Water Absorption at Different Foam Agent and Water Ratio

Foam Agent : Water	Water Absorption (%)
1:25	2.63
1:30	3.35
1:40	4.54

Table 8: Determination of Percentage of Moisture Content at Different Percentage of Foam

% Of Foam	Moisture Content (%)
25	8.94
50	9.84
75	10.45
100	15.65

Table 9: Density values of Hardened and Wet Concrete at Different Percentage of Foam

Percentage of Foam (%)	Density (kg/m ³)	
	Hardened Concrete	Wet Concrete
25	2030	2000
50	1810	1760
75	1800	1780
100	1460	1450





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Table 10: Properties of Lightweight Concrete

Cement: Water	Cement : Sand	Foam Agent : Water	Foam (%)	Density (kg/m ³)	Strength (N/mm ²)				Moisture Content (%)	Water Absorption (%)
					7 days	14 days	21 days	28 days		
1:0.45	1:3	1:30	100	1470	1.43	2.44	2.23	2.52	15.3	7.21
			75	1810	8.12	11.02	11.96	13.12	10.36	3.31
			50	1820	9.45	8.88	12.42	11.87	9.82	2.46
			25	2040	13.2	14.68	16.41	17.27	8.93	1.4
		1:25	50	1990	13.72	12.7	15.29	16.58	7.18	1.73
			75	1720	4.09	4.86	5.45	5.5	10.17	2.6
	1:40	50	1780	6.38	7.56	8.72	9.19	11.81	2.97	
		75	1840	11.15	11.95	13.72	13.21	8.79	4.46	
	1:4	1:30	50	2050	8.1	9.49	10.19	9.35	7.85	
			75	1770	7.57	7.16	7.44	10.34	7.98	
1:2	50		1840	10.9	12.55	15.84	16.78			
	75		2060	19.44	17.58	21.28	22.99			
0:0.35			75	1920	13.91	13.45	16.14	16.73		
0:0.25	1:3		75	2040	10.29	10.12	11.34	12.18		

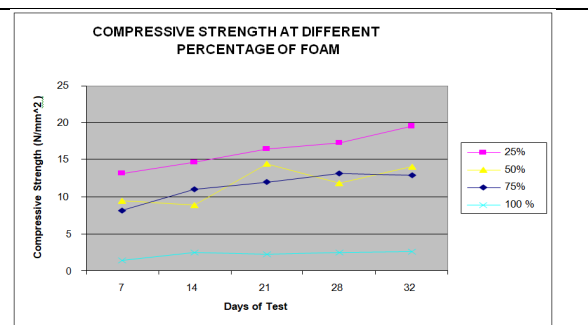
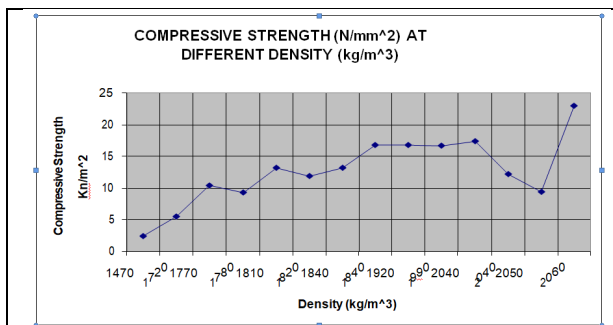


Figure 1: Determination of Compressive Strength at Different Density of Hardened Concrete

Figure 2: Determination of Compressive Strength at Different Percentage of Foam

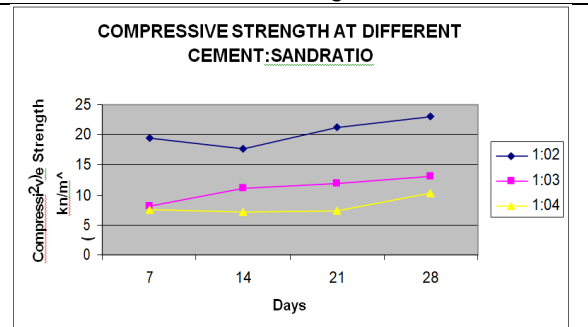
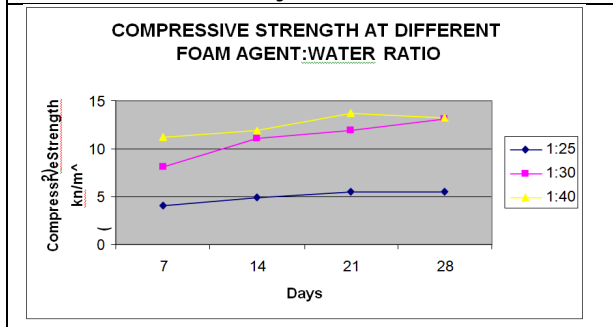


Figure 3: Determination of Compressive Strength at Different Foam Agent And Water Ratio

Figure 4: Determination of Compressive Strength at Different Cement And Sand Ratio





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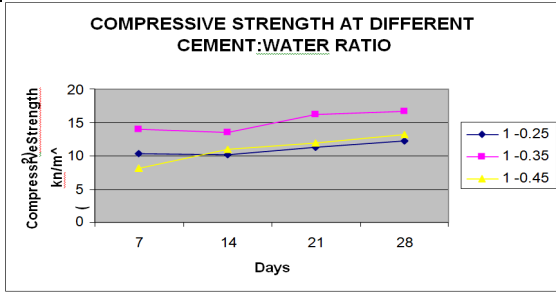


Figure 5: Determination of Compressive Strength at Different Cement And Water Ratio

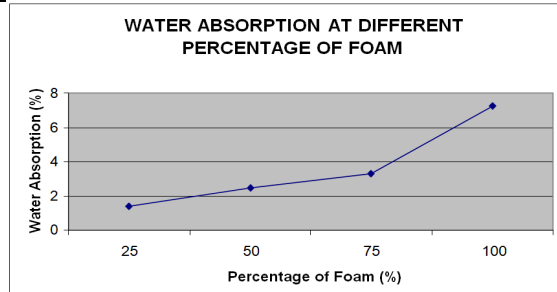


Figure 6: Determination of Percentage of Water Absorption at Different Percentage of Foam

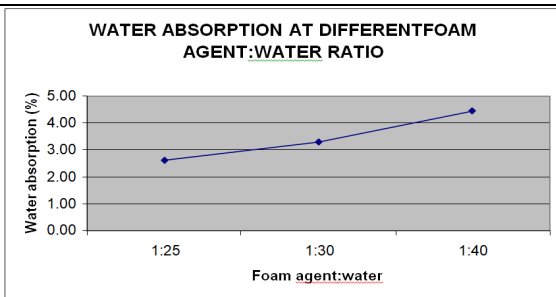


Figure 7: Determination of Percentage of Water Absorption at Different Foam Agent And Water Ratio

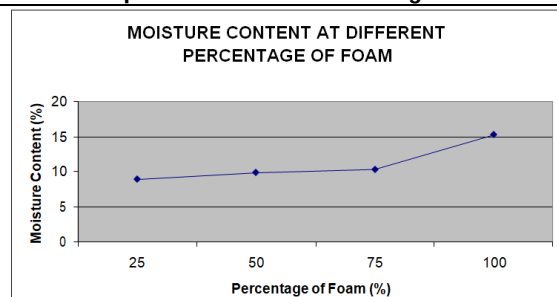


Figure 8: Determination of Percentage of Moisture Content at Different Percentage of Foam

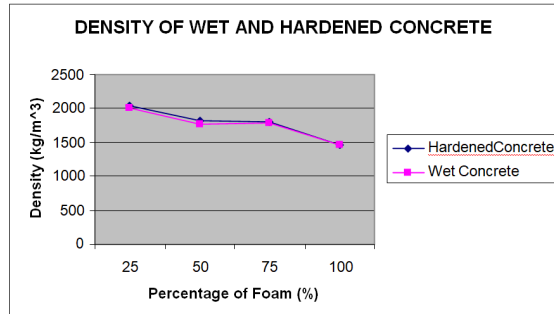


Figure 9: Determination of Density for Wet and Hardened Concrete





Synthesis, Characterization and Anticancer Activities of Metal(II) Complexes Resulting From 2, 4-Dichloro-6-(P-Tolylimino-Methyl)-Phenol (DICST) Schiff Bases

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ABSTRACT

Schiff bases and their metal complexes are one of the most important chemical classes of compounds having a common integral feature of a variety structural diversity and of active medicinal agents. Due to their preparative accessibility and structural variety, Schiff bases and their metal complexes are considered to be models of biological systems. Metal complexes of two general formulae $[M(L)_2(H_2O)_2]$ [$M = Mn(II), Co(II),$ and $Cu(II)$] and $[M(L)(Cl)(H_2O)]$ [$M = Zn(II)$] with 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (HL) were synthesized and characterized by thermal, spectroscopic (IR, 1H NMR, ^{13}C NMR & ESR) studies. Based on the physico-chemical studies octahedral geometry around $Mn(II)$, $Co(II)$, and $Cu(II)$ ions whereas square planar to $Zn(II)$ ion were suggested. The anticancer activity of the ligand and its complexes were screened against Hela cells (Human cervical cancer cells).

Keywords- Schiff base; metal(II) complexes; spectroscopy; anticancer activity.

INTRODUCTION

Hugo Schiff was the first to describe Schiff bases, which are the condensation products of primary amines with carbonyl compounds. These Schiff base compounds carrying imine or azomethine ($-HC=N-$) functional group. An important class of the most widely used inorganic compounds is formed by Schiff bases and have a wide variety of

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applications in many fields analytical, catalytical, biological and inorganic chemistry [2-5]. Schiff bases have gained popularity in the medical and pharmaceutical fields due to a wide range of biological activities such as anti-inflammatory [6-8], analgesic, antimicrobial [9-12], anticancer [13], antioxidant [14]. Nucleophilic attack on the carbonyl carbon atom of the aldehyde or ketone forming carbinol amine, which further undergoes dehydration in presence of mild acidic conditions or dehydrating agent forming imine or Schiff base, is shown by the nitrogen atom of amine or aniline. Powerful acidic and basic conditions are not recommended for the formation of Schiff bases, so carbinol amine does not undergo further dehydration to imine or the reaction can be stopped at carbinol amine. In a strong acidic environment, amino or aniline becomes protonated and no longer shows nucleophilic attack on the carbonyl group of an aldehyde or ketone. Many metabolic processes include Schiff bases of primary amines and carbonyl compounds.

In co-ordination chemistry [15] the preparation and flexibility in the varying chemical environment above $-HC=N-$ groups make it interesting ligand. In main group and transition metal coordination chemistry, Schiff base complexes of salicylaldehyde (vanillin) are considered to be among the most important stereo chemical models due to their preparative accessibility and structural variety. Being used as more or less successful models of biological compounds, considerable number of Schiff base complexes has potential biological interest. Not only have they played a seminal role in the development of modern coordination chemistry, but also they can also be found at key points in the development of bioinorganic chemistry, catalysis and optical materials. To produce a highly efficient Schiff base of 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) and its metal complexes, study the chlorine substitution effect through analytical, spectral characterization and biological activities, the present research is needed.

MATERIALS AND METHOD

Without using further purification, the chemical and solvents used are of inorganic and bio-application grade. At room temperature all the experiments are carried out, unless otherwise stated. All the metal(II) salts are used as chlorides. The spectroscopic analysis information were obtained from the subsequent studies; UV-Visible spectra by Perkin Elmer UV-Visible photometer model lambda twenty five within the vary of 200-800 nm. 1H and ^{13}C nuclear magnetic resonance spectra of Schiff base (DICST) and its Zn(II) advanced were recorded victimization nuclear magnetic resonance mass spectrometer model Bruker Avance (II) (400MHz, d6-DMSO-solvent). The TGA study was done below inert H_2 atmosphere, on STH6000 Perkin Elmer instrument. EPR spectra of Cu(II) complex were recorded in Varian E-112 machine at 77K victimization TCNE (Tetracyanoethylene) because the g-maker.

Schiff Base (DICST) Synthesis

According to the literature method [16,17], the Schiff base of 2, 4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) was synthesized. To a solution of 3,5-dichlorosalicylaldehyde (0.05 mol), the solution of p-toluidine (0.05 mol) dissolved in absolute ethanol was added in an 1:1 equimolar ratio and the reaction mixture was stirred for 1-2 hours at 35-40 °C. From absolute ethyl alcohol, the solid product was filtered, washed and crystallized.

Metal Complexes Synthesis

A warm ethanolic solution of 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) (0.05 mol) was added to warm ethanolic solution (0.05 mol) of metal salts Mn(II), Co(II), Cu(II) and Zn(II). The resulting mixture was heated under reflux with continuous shaking for 5-6 hrs in a mantle with water condenser. Product was collected as precipitate which was cooled, washed with ethanol and dried using hot air oven, after concentration to one half of the initial volume,





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Anticancer Activities

Cell Culture

In liquid medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/ml antibiotic penicillin and 100 µg/ml streptomycin antibiotic and maintained underneath an atmosphere of 5% CO₂ at 37 °C, Hela cells (Human cervical cancer cells) were refined.

MTT Assay

Using Hela cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyltetrazolium bromide (MTT) assay, the 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) and complexes Mn(II), Co(II), Cu(II) and Zn(II) were tested for *in vitro* cytotoxicity. Briefly, the classy Hela cells were harvested by trypsinization, pooled in a very 15 metric capacity unit tube. The cells were plated then at a density of 1x10⁵ cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10% FBS and 1% chronicles antibiotic solution for 24-48 hour at 37 °C. With varied concentrations of the ligand and complexes in a very blood serum free DMEM medium, the wells were washed with sterile PBS and treated. Every DICST and complexes was replicated thrice and therefore the cells were incubated at 37 °C in a very humidified 5% CO₂ incubator for 24 hrs. MTT (20 µL of 5 mg/ml) was added into each well and the cells were incubated for another 2-4 hrs until purple precipitates were clearly visible under an inverted microscope, after the incubation period. Finally, the medium together with MTT (220 L) was aspirated off the wells and washed with 1X PBS (200 L). Moreover, to dissolve form azan crystals, DMSO (100 L) was added and the plate was shaken for 5 mints. Using a microplate reader (Thermo Fisher Scientific, USA), the absorbance for each well was measured at 570 nm and the percentage cell viability and IC₅₀ value was calculated using Graph Pad Prism 6.0 software (USA).

RESULTS AND DISCUSSION

The Schiff base of 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) and Mn(II), Co(II), Cu(II) and Zn(II) metal complexes were prepared and characterized using spectroscopic techniques like, FT-IR, ¹H & ¹³C NMR, UV-Visible, ESR, TGA and also discuss with Anticancer activities.

FT-IR Spectra

Valuable information is provided by the FTIR spectra regarding the nature of Schiff base ligand and functional group attached to the metal atom. In table I, the important FTIR frequencies exhibited by the 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) and their corresponding mixed Schiff base complexes of Mn(II), Co(II), Cu(II) and Zn(II) are tabulated. At around 3084 cm⁻¹ due to the phenolic hydroxyl group in free Schiff base, the Schiff base shows broad band, which disappear in complexes indicating the coordination of phenolic oxygen to metal atom [18]. The band appeared in the range 1658 cm⁻¹ assigned due to ν(HC=N) which has been shifted towards lower region at around 1620, 1620, 1613, 1612 cm⁻¹ in the complexes indicating the participation of the azomethine group in the complexes formation, [19] this shift is also due to the variation in double bond character of carbon-nitrogen bond of azomethine group. A medium intensity band is shown at around 1295 cm⁻¹, the Schiff base of 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) due to phenolic oxygen ν(C-O) is shifted to lower region at 1275, 1286, 1285, 1245 cm⁻¹ in the complexes indicating the coordination through the phenolic oxygen atom and also due to the conversion of hydrogen bonded structure into a covalent metal bonded structure. Through nitrogen of azomethine and oxygen, the coordination of ν(C-O) of Schiff base are further evidenced by the appearance of bands in the complexes of non-Schiff base bands around 430-493 cm⁻¹ and 543-563 cm⁻¹ are due to M-O and M-N bonds respectively [20-22].

UV Spectra

UV-Visible electronic spectrum of the 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) and metal complexes in ethanol solution are given Table I. In metal complexes of Mn(II), Co(II), Cu(II) and Zn(II), the bands at 477, 459, 414, 463 nm where attributed to LMCT bands due to the shift of charge density from MO of Schiff base to the vacant orbital of metals [23,24] the absence of these charge transfer bands in the spectra of Schiff base confirms the



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complexation of metal atoms. As an extra information of coordination of metal complex, the Co(II) complexes also showed electronic transition of metal d orbital (d-d electronic transition) observed at 676 nm in the visible region.

NMR Spectra

The ^1H and ^{13}C NMR spectra of the 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) and its Zn(II) complex were studied with DMSO- d_6 as solvent.

 ^1H NMR Spectra

^1H NMR spectra of the Schiff base shows that, the OH signal appeared in the spectrum of 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) at 14.46 ppm is completely disappeared in the spectrum of Zn(II) complex indicating that the OH proton is removed by complexation with the metal ion [25]. The singlet peaks at 8.56 ppm characteristic to the azomethine [26] is down fielded to 8.64 ppm in Zn(II) complex supporting well binding of the azomethine groups of Schiff base to metal ions. As multiplet at 7.20-7.40 ppm are down fielded to 7.10-7.30 ppm in the spectra of metal complexes, the signals for aromatic protons of Schiff base were obtained.

 ^{13}C NMR Spectra

The signal appeared at 156.19 ppm were assigned to azomethine carbon atoms (HC=N), in ^{13}C NMR spectra of the 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST). Similarly with shift in intensity confirming the coordination of metal ion, the spectra of Zn(II) complex showed a signal of azomethine carbon at 148.52 ppm relatively up fielded to the 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST). The phenolic carbon showed that the signal at 159.19 ppm (Ph-C-O, 1C) in the ligand and the spectra of Zn(II) complex showed a signal of phenolic carbon at 161.57 ppm. The signals showed at 120.2-144.1 and 114.63-148.52 ppm [27] are assigned to aromatic carbons of 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST).

Analysis of Thermo Gravimetric

Thermo gravimetric (TGA) analysis of metal complexes is used to

- Determine if the water molecules are within or outside the central metal ion's inner co-ordination sphere.
- Gather information on new complexes' thermal stability, and
- Propose a general scheme for chelate thermal decomposition.

In the current study, heating rates were suitably regulated at $10\text{ }^\circ\text{C min}^{-1}$ under nitrogen atmosphere, and weight loss was measured from ambient temperature to $1000\text{ }^\circ\text{C}$. The TGA curve of the Zn(II) complex revealed a rapid first phase decomposition with 3.64 percent mass loss about $180\text{-}190\text{ }^\circ\text{C}$ (calculated 3.75 percent) indicating the loss of water molecules that are in a coordinated state. This confirms the existence of a coordinated water molecule in the Zn(II) complex. The second stage decomposition at $290\text{-}440\text{ }^\circ\text{C}$ suggests that the organic moiety has been eliminated, with a residue of 20.28 percent. The final decomposition pattern about $500\text{-}550\text{ }^\circ\text{C}$ corresponds to the formation of stable Zinc oxide [28].

ESR spectra

At room temperature, the X-band EPR spectra of Cu(II) complex was recorded. To calculate the geometry and oxidation state, the EPR spectrum is only observed complex containing an unpaired of electron. The spectra of the metal complex showed single isotropic resonance with g values of 2.3. The octahedral Cu(II) systems and are typical of axially symmetrical d^9 Cu(II) complex this parameter is in good agreement with.

Anticancer Activity**Cytotoxic Activity Evaluation by MTT Assay**

The Schiff base of 2,4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) and metal complexes of Mn(II), Co(II), Cu(II), and Zn(II) were evaluated for cytotoxicity in Hela cells (Human cervical cancer cells) using the MTT assay process. To determine the behaviour of the solvent, compounds were dissolved in DMSO and blank samples containing the



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same amount of DMSO were used as controls. Cis-platin was used as a standard to determine the cytotoxicity of the test compounds. The findings were analysed using cell inhibition represented as IC_{50} values, as shown in Table II. The compounds are cytotoxic at concentrations of 1 $\mu\text{g/ml}$ and higher. As the concentration of complexes was increased from 1 to 500 $\mu\text{g/ml}$, the percent cell inhibition increased as well. Schiff foundation, Mn(II), Co(II), Cu(II), and Zn(II) remove 67.03 percent, 74 percent, 92.41 percent, 76.63 percent, and 74 percent of the cell population, respectively, at 500 $\mu\text{g/ml}$. The IC_{50} values for compounds against Hela cells were determined to be 185.3 $\mu\text{g/ml}$ for Schiff base and 53.19 $\mu\text{g/ml}$, 26.51 $\mu\text{g/ml}$, 51.36 $\mu\text{g/ml}$, and 52.99 $\mu\text{g/ml}$ for complexes Mn(II), Co(II), Cu(II), and Zn(II), respectively. The IC_{50} values of synthesized complexes are slightly lower than those of standard Cis-platin. The anticancer activity of the tested compounds against the HeLa cell line follows the order Co(II) > Cu(II) > Zn(II) > Mn(II) > Schiff base. Surprisingly, other complexes with lower cytotoxic activity than the Co(II) complex displayed higher cytotoxic activity.

CONCLUSION

Mn(II), Co(II), Cu(II) and Zn(II) complexes of the Schiff base ligand derived from 3,5-dichlorosalicylaldehyde (0.05 mol) and p-toluidine were synthesized and characterized by elemental analysis and spectral studies. The results clearly demonstrate that the Schiff base ligand is bidentate, Mn(II), Co(II) and Cu(II) complexes have octahedral geometry, while the Zn(II) complex has square planar geometry. Anticancer activity of the free ligand and its complexes showed good to high activity against Hela cells. The anticancer activity of Co(II) complex showed higher activity than other complexes and ligand.

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Table 1. FT-IR and UV data of, 4-dichloro-6-(p-tolylimino-methyl)-phenol (DICST) and its metal complexes

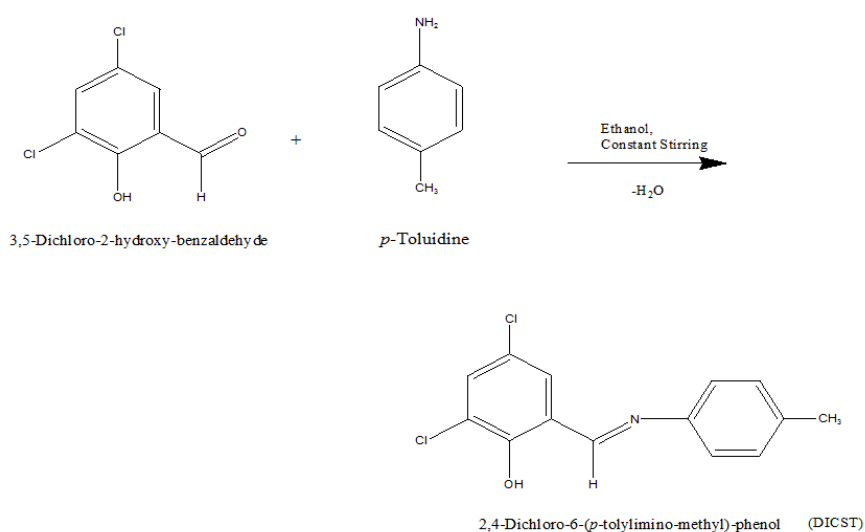
Compound (cm ⁻¹)	(HC=N)	(C-O)	(M-N)	(M-O)	(O-H)	UV λ_{\max} (L→M), nm
DICST	1658	1295	-----	-----	3436	-----
[Mn(DICST) ₂ (H ₂ O) ₂]	1620	1275	542	493	3394	477
[Co(DICST) ₂ (H ₂ O) ₂]	1620	1286	543	493	3400	459, 676
[Cu(DICST) ₂ (H ₂ O) ₂]	1613	1285	543	446	3428	414
[Zn(DICST)Cl(H ₂ O)]	1612	1245	523	436	3406	463



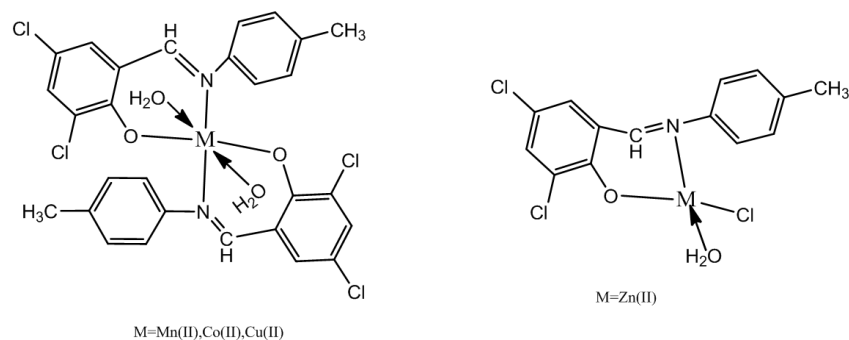
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Table 2. IC₅₀ (g/ml) value of 2,4-dichloro-6-(*p*-tolylimino-methyl)-phenol (DICST) and metal complexes and cisplatin against Hela cells.

Compounds	IC ₅₀ (g/ml) ^a
DICST	185.3
[Mn(DICST) ₂ (H ₂ O) ₂]	53.19
[Co(DICST) ₂ (H ₂ O) ₂]	26.51
[Cu(DICST) ₂ (H ₂ O) ₂]	51.36
[Zn(DICST) ₂ (H ₂ O) ₂]	52.99
Cisplatin	13.00



Scheme I. Formation of Schiff base (DICST)



Scheme II. Structures of metal complexes





A Comprehensive Study of a PID controller for Speed Control of DC Motor

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ABSTRACT

High performance DC motors are used for various actuation process in industries. Precision control of these motors are of utmost importance To obtain this precision in industrial drives various control systems are deployed where PID is one of the most widely used. In this paper we propose a simplified method of control using potentiometer as the feed-back unit to control the position of the DC-motors. The model is deployed in MATLAB and Simulink for stability verification. The tuning of PID control depends on the gain parameters such as K_p , K_i , K_d once tuned these result into a robust system and the same is implemented over embedded board for testing it for real-time application. This papers also describes the characteristics of the PID controller for the proposed system.

Keywords: DC Motor, Speed Control, PID controller.

INTRODUCTION

DC motors play an important role in many industrial applications like cars, trucks, aircraft, robot manipulators and home appliances because of their greater reliability, more flexibility and cheaper in cost. DC motors are popularly used since they have a wide range of speed control, high starting torque, high transient response and are very compact. In order to obtain better controllability and high performance the speed control of DC motors is highly essential. Proportional-Integral-Derivative (PID) controllers are the most commonly used controllers for controlling the speed and position of DC motors since they have very simple control algorithms and lesser cost. [1]. PID controllers help to minimize the error between measured speed and actual desired speed. The dc motors acquire the





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desired speed very smoothly and within a certain period of time when PID controllers are used [2]. In this paper we have examined the various response of the PID controller on the dc motor speed control by varying each of the proportional, integral and derivative parameters one by one and noted their effects in MATLAB .

MODELLING OF DC MOTOR

The working of direct current (DC) motors includes the conversion of electrical energy into mechanical energy by the interaction of two magnetic fields. One of these magnetic fields is produced by the magnetic poles whereas the other field is produced by the electromagnets when current passes through the motor windings. . The torque which causes the rotation of rotor is a result of these two fields. It is clearly indicated from the speed equation that the speed of a DC motor is directly proportional to the applied voltage whereas the torque equation shows that the torque is proportional to the motor current. Armature circuit of a dc motor is shown below which consists of an armature resistance indicated as (Ra) ,connected in series with inductance (La), and voltage (Vemf) representing the back emf produced in armature due to rotation. [3].

The relation between the motor Torque T_m and armature current, i_a , is given as;

$$T_m = K_t i_a \quad (1)$$

Where K_t is a torque constant

The back emf, V_{emf} , is related to angular velocity by;

$$V_{emf} = k_b \omega = k_b \frac{d\theta}{dt} \quad (2)$$

From fig.1 we can write the following equations based on the Newton's law combined with the Kirchhoff's law:

$$V = i_a R_a + L_a \frac{di_a}{dt} + V_{emf} \quad (3)$$

$$L_a \frac{di_a}{dt} + R_a i_a = V - k_b \frac{d\theta}{dt} \quad (4)$$

$$J m \frac{d^2\theta}{dt^2} + B m \frac{d\theta}{dt} = K_t i_a \quad (5)$$

PID Controllers

PID is an acronym which stands for Proportional Integral Derivative. A PID controller is a part of a closed loop feedback system that uses Proportional, Integral, and Derivative to drive elements for the control process [4]. In this system the error signal is generated when the feedback variable is compared with the fixed point and this error signal changes the system output. This process is repeated unless the error attains a zero value otherwise the feedback variable will become equivalent to a fixed point. In figure a closed loop system is shown in which the error signal produced by taking the difference of the desired input value from the actual output, is sent to the PID controller. The derivative and the integral of the error signal is obtained by the controller. The control signal to the dc motor is equal to the proportional gain times the magnitude of the error plus the integral gain times the integral of the error plus the derivative gain times the derivative of the error. This control signal is then sent to the dc motor from which we get a new output . This new output is then fed back and compared with the reference value to obtain the new error signal. The above process is repeated for this new error value and the process continues in an infinite loop.

Model consists of a 5 HP, 240 V, 1750 rpm DC Motor whose field winding is excited by 150V DC voltage source and the armature winding is excited by a controlled voltage source which is fed from a PID controller. In this model the actual speed of the dc motor is compared with a reference speed of 1750 rpm and the corresponding error signal obtained is fed to the PID controller. By varying different values of P,I,D different simulation results are obtained.





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RESULT ANALYSIS

Parameter Variation of Only P Controller

P- controller gives an output which is proportional to the current error $e(t)$. Here the desired value is compared with the actual value of the system which results in an error value. This error value is then multiplied with a proportional constant (K_c) to get the desired output. For zero error value there will be no controller output. With the increase in K_c , there will be an increase in the speed of the response.

From the above observations it is concluded that with the increase in the value of K_p , maximum overshoot (M_p) is increased, steady state error is gradually reduced and system response becomes faster (Rise time increases).

Parameter Variation of Only I Controller

In order to eliminate the limitation of P-controller an introduction of new controller comes into picture which is I-Controller that helps in reducing the steady state error of the system. Here the error is integrated with respect to time until its value becomes zero where the value to the final control device remains hold. Due to I control the speed of response is limited and the stability of the system gets affected. In other words with the decrease in integral gain, K_i there will be increase in the speed of the response.

From the above observations it is concluded that with the increase in the value of K_i , maximum overshoot (M_p) is increased slightly, steady state error is gradually reduced to zero and system response becomes slower (Rise time decreases).

Parameter Variation of Only D Controller

Since I-controller gives normal reaction when the set point is changed, the future behaviour of error can't be predicted by using I-Controller. This difficulty is overcome by D-controller which predicts the future behaviour of the error. D-controller basically measures the rate of change of error with respect to time which kick starts the output for which system response is increased.

From the above observations it is concluded that with the increase in the value of K_d , the system's stability is increased, the maximum overshoot is decreased, and the transient response of the system is improved.

Parameter Variation of PI Controller

When the outputs of proportional and integral controllers are merged together they produce the output of PI controller. This results in a decrement of the steady state error whereas there is no affect on the stability of the control system.

From the above observations it is concluded that with the increase in the value of K_p & K_i , steady state error approaches towards zero, maximum overshoot slightly increased, and the slower transient response of the system (Rise time is increased).

Parameter Variation of PD Controller

When the outputs of proportional and derivative controllers are merged together they produce the output of PD controller. This results in improvement of the control system stability whereas there is no affect on the steady state error.

From the above observations it is concluded that with the increase in the value of K_p & K_d , steady state error is zero, maximum overshoot slightly increased, and the faster transient response of the system (Rise time is increased).





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Tuning of PID Controller

When the outputs of proportional, integral and derivative controllers are merged together they produce the output of a PID controller due to which the stability of the control system is improved and the corresponding steady state error is reduced.

CONCLUSION

From the above observations Authors observed the significance of parameter variation by using different types of controllers like P, I, D, PI, PD controllers. Moreover authors observed tuned response of PID controllers and found out various parameters like Rise time, Settling time, Overshoot, Peak, Gain Margin, Phase Margin and stability of the System. All these parameters are found out by keeping the reference Speed as 1750 rpm and Load Torque as 10 N/m². Similar type of observations can also be found out by varying the reference speed or the load torque which is the further scope of this paper.

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Parameter	Tuned	Block
P	0.2146	1
I	5.2665	1
D	0.0021659	1
N	5488.4368	5488.4368

Parameter	Tuned	Block
Rise time	0.034 seconds	0.000255 seconds
Settling time	0.153 seconds	0.00133 seconds
Overshoot	5.41%	31.4%
Peak	1.05	1.31
Gain Margin	Inf dB	Inf dB
Phase Margin	64.5 deg	37.6 deg
Closed loop stability	Stable	Stable





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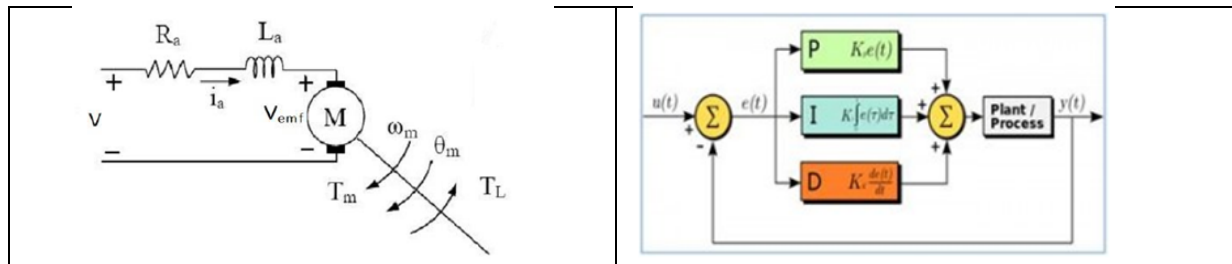


Fig.1.DC Motor

Fig.2.Block diagram of PID Controller

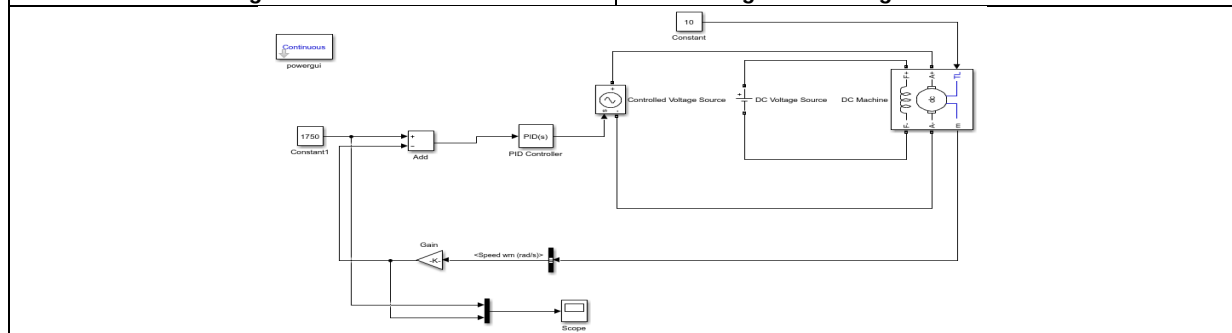


Fig.3.Different values of P,I,D

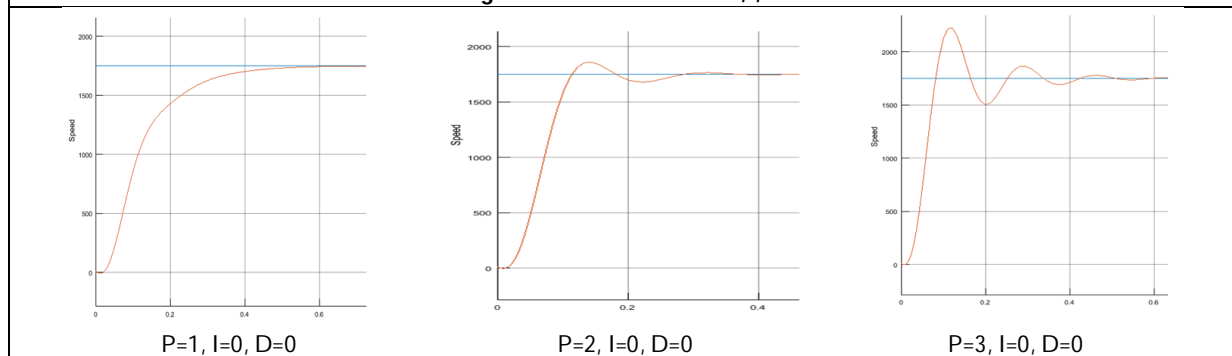


Fig.4.Parameter Variation of Only P Controller

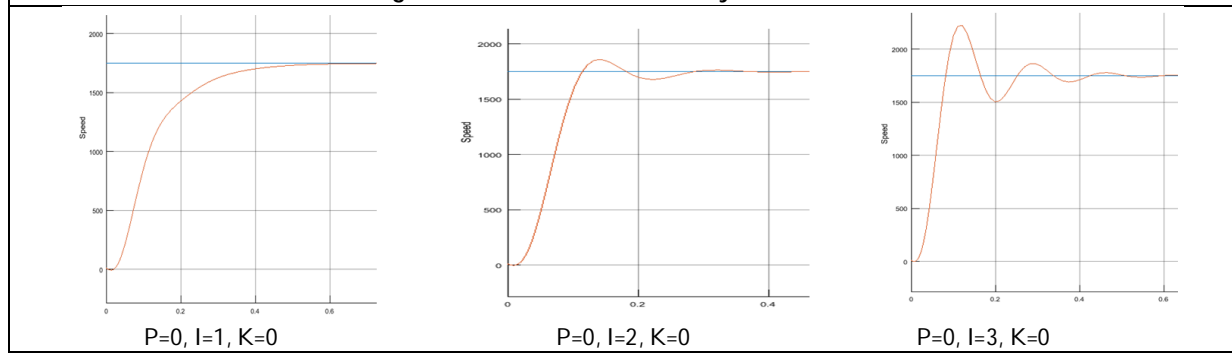
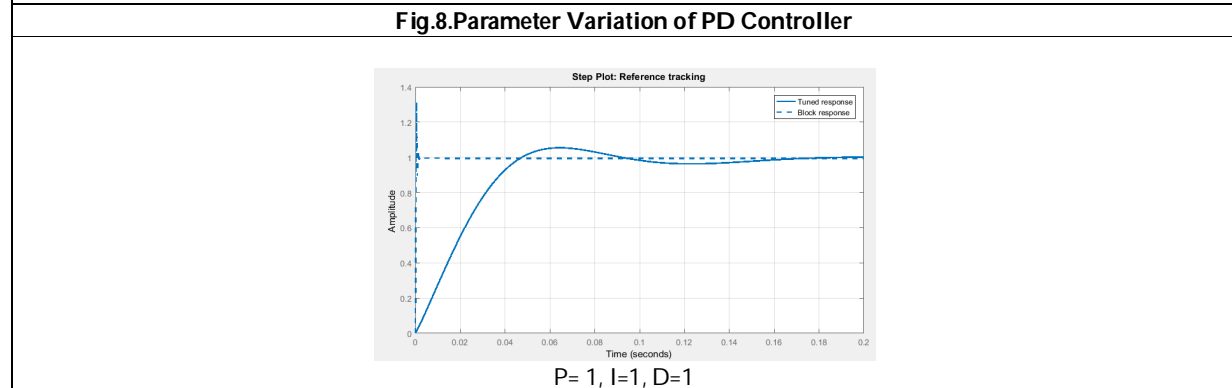
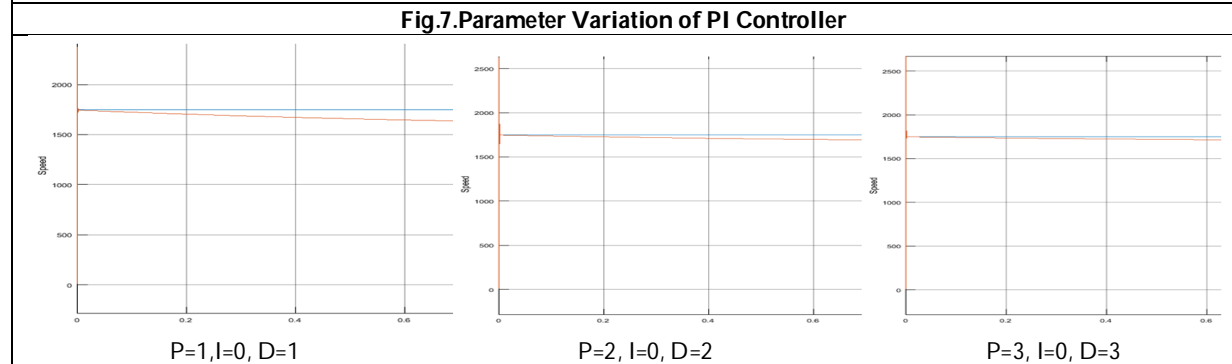
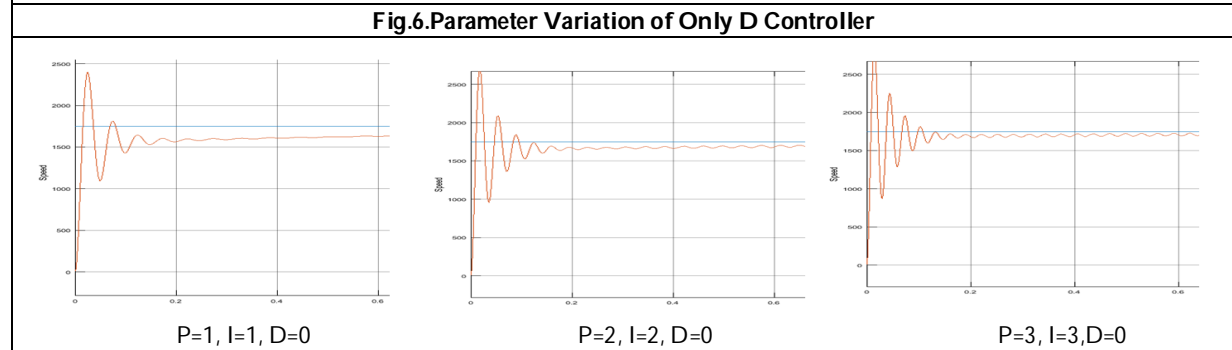
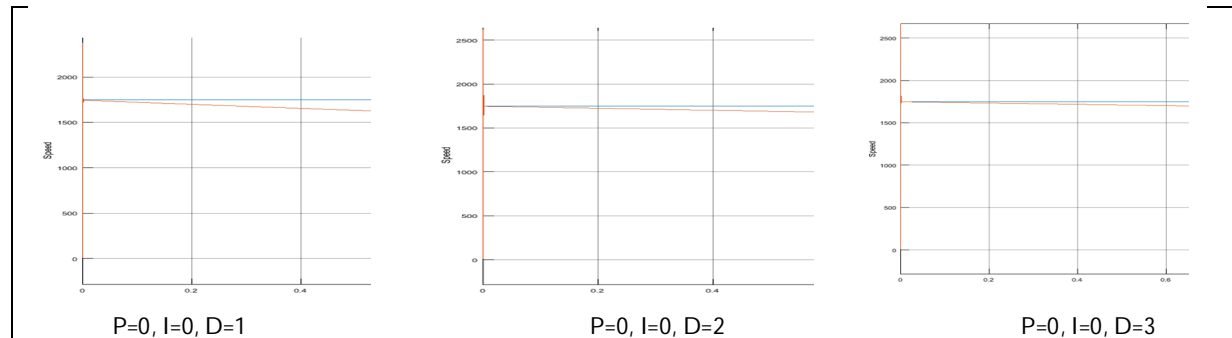


Fig.5.Parameter Variation of Only I Controller





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Pattern Analysis in Oral Cancer Images using Fractal Dimension

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ABSTRACT

Cancer begins with the development of cells in part of the body. Often it can also grow or spread to the mouth and the tissues around you. The fractal dimensions of the growth pattern were calculated with the process of counting boxes and the sausage procedure in this article. The population growth model to analysis the stages of cancer cell growth. Percolation models that depend on the time of complexity will model cell growth for this cancer. The shape-based characteristics of all regular to irregular cell images were determined numerically. The fractal method leads to very promising results, improving stage dedication and analysis.

Keywords: Oral Cancer, Fractals, Logistics Growth model, Compactness, Percolation.

INTRODUCTION

ORAL CANCER

The World heads for different varieties are also known as new epidemics of non-communicable diseases. The cancer second most frequent cause of death in developing countries is among these new epidemics. Oral cancer is one of the ten most common death causes in developed countries. India has one of the world's highest levels of oral cancer; this number is already one-third, and this statistic is sadly growing [1]. 40% of oral cancers diagnosed worldwide are in India, Pakistan, Bangladesh and Srilanka, according to the World Health Organization. Oral cancer cell spread the entire part of the mouth figure 1.





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FRACTAL

A *fractal* is a shape consisting of pieces that are identical in any way to the whole to view self-similarity properties, fractal object and process are described above[12]. Fractal geometry was developed by Benoit Mandelbrot and is a recent discovery in mathematics. It supports the exact analysis of the structural properties of natural objects, including specimens of histopathology. These objects are very fluid in their composition and yet distinguished by their own identity[2]. The "fractal objects" are identified. Measurements called "fractal dimensions" are measured by assessing and evaluating the degree of complexity and irregularity of these pieces. Many structures in the human body, such as retina veins, lung air channels and arterial tree, have been found to be considered fractal structures. It has been discovered [3].

MATHEMATICAL MODEL

Modeling is the practice of human beings who reflect, manipulate and communicate everyday things in the real world [4]. Increasingly, biomedical data interpretation is using mathematical models. Mathematical models of biologists vary in ways that range from experimental architecture to the mapping of complex biological systems. Model gives biologists the opportunity to discuss how molecules move into and out of cells, as tissues shuttle bacteria [5]. Hope Murphy, Hana Jaafari and Hana M. Dobrovolny analysis the Differences in predictions of ODE models of tumor growth some example [13]. Camila Oliveira Rodini, Nathália Martins Lopes, Vanessa Soares Lara, and Ian Campbell Mackenzie analysis the oral cancer stem cells properties[14]. In section II Calculating cell images using Fractal dimension and models. In section III the results are explained.

METHODS

Here we consider to find the Oral cancer analysis of Pattern of cells. HarFA fractal analysis its show the dimension of cells in various scaling. Radial Measure to measure the radius of the cells. Mathematical model have been broadly use to show the Growth of cancer cells.

NOTATION

n_{BW}	Number of black and white boxes
D_{BW}	Dimension of black and white boxes
r	Radius
A	Area
$d(i)$	Radial distance
D_s	Dimension of Sausage method
D_b	Dimension of Box counting method
β	Population size at time t
α	Maximum size of population
r	Growth rate
t	time

HarFA FRACTAL ANALYSIS

The most important tool for the assessment of the fractal dimension is the estimated box counting method. The box counting technique was used to assess the fractal characteristics of oral cancer at various times. The used programme was HarFa established in Brno, Technical University in the Czech Republic at the Physical and Applied Chemistry Institute. A box counting method that allows fractal dimension assessment is included in the programme. Analysis of black and white images using Harfa tools. A fractal cover pattern, raster of boxes A, B and C, is used in the box counting method and a fractal covering raster is required. The fact that differing values of the fractal parameters are obtained for the same images for different mask intensity values from up to 255 pixels is significant. This calculation





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would result in the logarithmic feature of box size r and number of boxes $n(r)$ that are needed to fully cover fractals when repeated with different sizes of boxes r . The slope of the linear function.

$$\ln n_{BW}(r) = \ln(k_{BW}) + D_{BW} \ln(r) \quad (1)$$

$$\ln n_{BBW}(r) = \ln(k_{BBW}) + D_{BBW} \ln(r) \quad (2)$$

$$\ln n_{WBW}(r) = \ln(k_{WBW}) + D_{WBW} \ln(r) \quad (3)$$

Give the fractal dimensions to D_{BW} , D_{BBW} and D_{WBW} . D_{BW} characterizes the characteristics of boundary of fractal form, D_{BBW} characterizes the white background fractal pattern, and D_{WBW} characterizes the black background fractal pattern [6]. For all digital images of the different stages of the Cells under study, this technique was used this procedure.

RADIAL MEASURE

The perimeter of a cell is a significant function. Contour-based characteristics that neglect the form of the interior depends on the cell perimeter or limit points. For parametric boundary representation, this perimeter is used[7].

$$T = \int (\sqrt{x^2(t) + y^2(t)}) dt \quad (4)$$

By sausage method or the process of boundary dilation very closely linked to minkowski dimension. The picture was dilated with circles of varying sizes. We once again used boxes with pixel sizes of 1×1 , 3×3 , 5×5 , ..., 17×17 . As an estimate for a circle. Estimated the equivalent approximated radius r in pixels.

$$r = \left(\frac{A}{\pi}\right)^{1/2} \quad (5)$$

Where A is denoted with the pixel field. The slope of the double logarithmic plot regression line give the counted pixels as regards the radii give

$$D_s = 2 - k_s \quad (6)$$

The approximate dimensions of fractal capacity are D_s . The cell diameter can be measured with this approach. Quantitative parameters can be found using the sausage method such as Area, perimeter, form factor and Invaslog.

$$Form\ factor = 4\pi \frac{area}{perimeter^2} \quad (7)$$

$$Invaslog = -\log (Form\ Factor) \quad (8)$$

A good quantitative measure of invasiveness of oral cancer was particularly proved to be the invaslog value. The distance from the center to perimeter is the radial distance (x_i, y_i) . Thus known as the radial distance

$$d(i) = \sqrt{(x_i - \bar{x})^2 + (y_i - \bar{y})^2}, i = 0,1,2,... \quad (9)$$

Here $d(i)$ is a vector measured from the boundary pixels by the distance. By dividing $d(i)$ by the maximum value, a normalized vector $r(i)$ is achieved.

LOGISTIC GROWTH MODEL

A statistical function that can be seen in many contexts is the logistic growth model. Rapidly increasing growth in the beginning but decreasing growth later, as it is closer to the limit, are characteristic for logistical growth. The





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justification for using rational growth in the simulation process is that the outbreak of the virus has been studied by epidemiologists and that the first epidemic has exponential growth and can be formed as a logistic growth model for the whole time [8].

The Logistic equation it can be in the form:

$$\frac{d\beta}{dt} = r \cdot \beta \left(1 - \frac{\beta}{\alpha}\right) \quad (10)$$

It form of differential equation and Integrate as

$$\int \frac{d\beta}{\beta \left(1 - \frac{\beta}{\alpha}\right)} = \int r dt \quad (11)$$

Then the equation (11) becomes

$$\ln|\beta| - \ln|\alpha - \beta| = rt + \gamma \quad (12)$$

$$\ln \left| \frac{\alpha - \beta}{\beta} \right| = -rt - \gamma \quad (13)$$

$$e^{\ln \left| \frac{\alpha - \beta}{\beta} \right|} = e^{-rt - \gamma} \quad (14)$$

$$\left| \frac{\alpha - \beta}{\beta} \right| = e^{-rt} \cdot e^{-\gamma} \quad (15)$$

Where $A = \pm e^{-\gamma}$ solve equation (15), we get

$$\frac{\alpha - \beta}{\beta} = A \cdot e^{-rt} \quad (16)$$

$$\frac{\alpha}{\beta} = 1 + A \cdot e^{-rt} \quad (17)$$

Thus the solution of the Logistic Growth model equation

$$\beta = \frac{\alpha}{1 + A \cdot e^{-rt}} \quad \text{where } A = \frac{\alpha - \beta_0}{\beta_0} \quad (18)$$

PERCOLATION AND CLUSTER MODEL

PERCOLATION MODEL

Percolation deals with the number and properties of the each sites in a lattice be occupied at random with probability p, that is, each site is occupied or empty independent of the status of any of the other sites in the lattice.

CLUSTER

A Cluster is a group of neighboring occupied sites.

CLUSTER DENSITY IN PERCOLATION MODEL

In the way that cancer spreads through the mouth, a percolation cluster is formed. Each site in a square grid represents a human with probability infection and probability immunity. The individual in the center of the lattice is





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infected at initial time $t=0$. We now conclude that the closest non-immune areas are infected in one unit of time. These infected areas and so on in the second unit of time [9]. All l th square grid non-immune sites around the cells are then infected after t time step. Formula is the number of small cluster densities in two-dimensional percolation models

$$n(s, p) = \sum_{t=1}^{\infty} g(s, t)(1 - p)^t p^s \quad (19)$$

$g(s,t)$ denote the number of all cluster with sizes s and perimeter t .

CONTRACTION

CONTRACTION MAPPING

A point x is a fixed point of a function iff $f(x)=x$. A function $f: s \rightarrow s$ is a contraction iff there is a constant $r < 1$ such that $\rho(f(x), f(y)) \leq r\rho(x, y)$ for all $x, y \in s$. A contraction is easily seen to be continuous [11].

CONTRACTION MAPPING THEOREM

Let $f: X \rightarrow X$ be contractive transformation on a complete metric space (X,d) . Then the transformation f possesses exactly one fixed point $x \in X$. Moreover for any $x \in X$ the sequence

$$x, f(x), f^2(x) = f(f(x)), \dots, f^k(x) = f(f^{k-1}(x)), \dots$$

Converges to the fixed point a .

$$\lim_{k \rightarrow \infty} f^k(x) = a$$

By the theorem is used to model the development of oral cancer cells. (x_n) is the cell sequence in this case. A cluster point exists in a cell sequence (x) [15]. As a result, each cell in the series has an analytic feature that can be represented mathematical model.

SHAPE BASED MODEL

Compactness is an irregular area of dimensional quantity and structure. It is a basic metric which is used as a representation of the area containing the average of the minimum and maximum levels and the number of cells above and below the average. Tissue cancer cells can be characterized by standardization in terms of land mass, population number etc., respectively [10]. The area of concern can be invariant from compactness and the irregular boundary form can also be seen.

$$compactness = \frac{perimeter^2}{4\pi \times area} \quad (20)$$

RESULT

Evaluating the Fractal dimension analysis D with two different methods for 14 samples Images. Figure 2 represent the microscopic image of oral cancer it's was collected from the oral cancer foundation in California. The result indicate the growth pattern analysis oral cancer cells. Box counting methods and Sausage method to differentiate the Oral cancer cell images. The Fractal dimension is a measurement of the roughness or irregularity degree of the surface. Randomly select the two images to convert the black and white image. To find the area it's denoted by B_w , perimeter its denoted by B and total area denoted by $B+B_w$ of the cells by using the box counting method with the help of HarFA Fractal software. In equation 5 analysis the radius of cell. The amount of invasiveness of the cancer cells in the oral cancer are find out by form factor in equation 7 and invaslog in equation 8. Table 1 and Table 2 show the data produced by way of the box counting and sausage methods. Similarly we done its other images also to find the value of D_b, k_s, D_s it show in the Table 3. The Logistic growth model it can be implement in Table 4 to takes



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random time to find size of cancer cell and analysis the oral cancer cell growth of different stage. Apply equation 18 to find the size of cancer cell it reach 2.6 micrometer to enter the advanced stage of cancer. Human to human depends on immunity range the growth rate will be differ.

The shape of cell can be analysis by the Contraction mapping theorem. The cluster point x shoes that the cancer cells .The cell sequence can be analysis by contraction.To calculate the density of cluster following equation 19. Each lattice point occupied independently with probability p and probability $1-p$ not occupied. Its work out the exact solution of the cluster number density for all cluster size. The numerical approach to obtain the general form of cluster size.Compactness is quantifies the cancer cell is the smooth shape of irregular cells. To measure the irregular cells in equation 20 the values reach maximum boundaries its high compactness and value reach minimum boundaries is a low compactness.

CONCLUSION

In this paper we have proposed Box-counting method and Sausage method will give accurate results. The radial distance measure and cell compactness of the irregularity border for the cancer cell which based on cell potential. The Logistic growth model to predict the measure of Cancer cell growth. It will be very useful for pathologist to diagnosing the cancer and give proper treatment.

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Table 5. Data Analysis of Oral Cancer cell in Fractal dimension and Sausage method

Image 5									
scaling	Area	Perimeter	Total area	Form Factor	Invaslog	Radius	D _b	k _s	D _s
3	1531.3	289.7	1821.11	0.229	0.640	22.08	1.84	0.26	1.74
5	474.0	181.4	655.4	0.1809	0.742	12.28			
7	210	129	339	0.1585	0.799	8.17			
9	109.6	96.01	205.6	0.1485	0.828	5.87			
11	52.3	84.32	136.68	0.0925	1.033	4.06			
13	35.3	62.2	97.5	0.1143	0.93	3.33			
15	16	58.04	74.04	0.059	1.229	2.25			
17	7.17	50.47	57.64	0.035	1.455	1.4			
20	3	38	41.65	0.026	1.585	0.69			

Table 2. Data Analysis of Oral Cancer cell in Fractal dimension and Sausage method

Image 10									
scaling	Area	Perimeter	Total area	Form Factor	Invaslog	Radius	D _b	k _s	D _s
3	10973.4	1645.6	12617.01	0.509	0.293	59.1	1.62	0.31	1.69
5	3749.1	805.8	4554.9	0.072	1.142	34.5			
7	1830.8	503.4	2334.2	0.090	1.045	24.1			
9	1064.6	350.2	1414.8	0.109	0.962	18.4			
11	686.6	259.9	946.5	0.128	0.892	14.7			
13	472.6	207.9	680.5	0.385	0.414	12.2			
15	341.4	171.2	512.6	0.146	0.835	10.4			
17	262.0	136.6	398.6	0.177	0.752	9.1			
20	174.9	113.3	288.2	0.17	0.769	7.46			

Table 3. Data Analysis of Oral Cancer cell in Fractal dimension and Sausage method

Images	D _b	k _s	D _s
l ₁	0.876	1.091	0.909
l ₂	1.273	0.838	1.162
l ₃	1.948	0.069	1.931
l ₄	1.531	0.556	1.444
l ₆	1.116	0.91	1.090
l ₇	1.784	0.274	1.726
l ₈	1.529	0.471	1.529
l ₉	1644	0.378	1.622
l ₁₁	1.851	0.237	1.763
l ₁₂	1.706	0.301	1.699
l ₁₃	1.476	0.600	1.400
l ₁₄	1.601	0.425	1.575

Table 4. Mathematical model of cancer cell growth in random time

Time(years)	Growth model $\beta(t)$
-------------	-------------------------





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0.5	0.74
1	1.023
1.5	1.524
2.5	1.85
3	3.16
5	4.386



Figure 1. Image of Oral Cancer

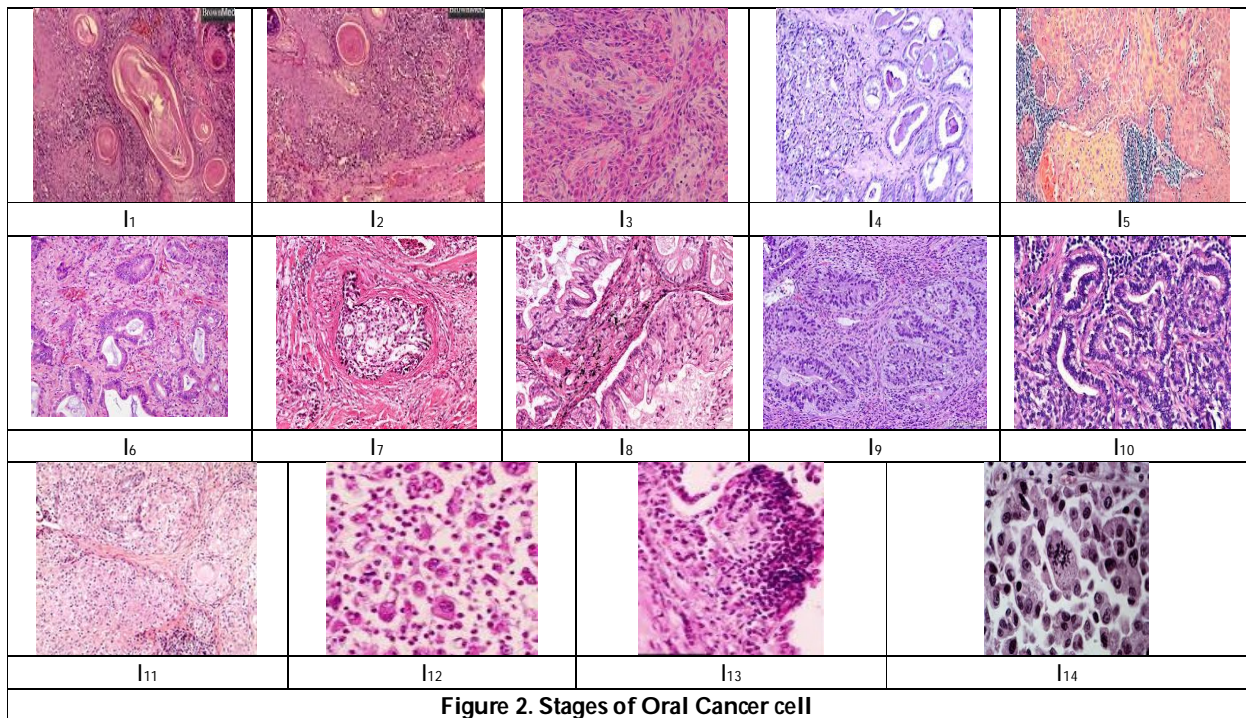


Figure 2. Stages of Oral Cancer cell

