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CONTENTS

AN EFFICIENT SYNTHESIS OF 3,4-DIHYDROPYRIMIDINONEDERIVATIVESBY THREE COMPONENT REACTIONAND ITS BIOLOGICAL EVALUATION

Mr. SANTHOSHKUMAR. V [1] & Mr. AYYANAR. P [2]

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ABSTRACT

A simple three component reaction in efficient approach has been developed for the synthesis of 3,4-dihydropyrimidinonefrom Benalydehyde, Ethylacetoacete& urea by using phosphorus pent-oxide as catalyst in a single step process. The synthesized compound was characterized using spectroscopic methods viz., UV, IR and NMR spectra and its biological activity was analyzed, which showed significant antibacterial activities.

KEYWORDS: Dihydropyrimidinone, UV, IR and NMR spectra.

INTRODUCTION

3,4-dihydropyrimidinonone derivatives have recently received great attention because of their wide range of therapeutic and pharmacological properties, such as antiviral antitumor, antibacterial and antifungal, anti-inflammatory, antihypertensive agents, and neuropeptide antagonists. Furthermore, these compounds have emerged as the integral backbones of several calcium-channel blockers. Also, several alkaloids containing the dihydropyrimidone were isolated from marine sources, we would like to propose a new naturally and very cheap catalysts used for the synthesis of 3,4-dihydropyrimidinones and 3,4-dihydropyrimidenthiones, using one-pot-Biginelli protocol, in refluxing ethanol.

The synthesis of dihydropyrimidones and their thio-analogues have become popular in the world of synthetic organic chemistry due to their activities such as antibacterial, anti-viral, anti- inflammatory, anti-hypertensive and anti tumor. They have been reported to serve as calcium channel blockers, as α-1-a antagonists and neuropeptide antagonists.

MATERIALS AND METHODS

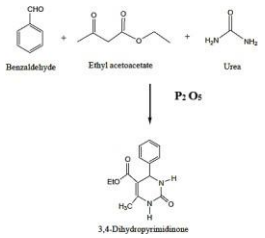
Benzaldehyde, Urea, Ethyl acetoacetate, Phosphorous pentoxide and Ethanol were the products of Aldrich (Coimbatore, India). Ethanol was purchased from Sigma Aldrich, India.

Instruments

A double beam UV–Visible spectrophotometer, Jasco–V 630 is used for absorption measurements using 1cm path length cells. FT IR (KBr,cm-1) spectra were obtained on Shimadzu-8201 spectrophotometer. 1H NMR spectra were recorded on Bruker AMX-400 (400 MHz) spectrometer using TMS as an internal reference (Chemical shifts in δ, ppm).

Synthesis of 3,4-dihydropyrimidinone

The mixture of reaction mixture was cooled and poured on crushed ice. The separated solid was filtered, washed with pet ether. Then it was dried. Finally the crude sample was recrystallized by using ethanol.



To synthesize dihydropyrimidinone the reaction afforded 1.609mg (87%) as of white solid was obtained.

ANTIBACTERIAL ACTIVITY:

Antibacterial activity of all the synthesized compounds was determined by disc diffusion method. All human pathogenic bacteria viz E-coli, Bacillus cereus. The agar well diffusion method was adopted using Muller Hinton Agar. 18-24 hours old nutrient Broth culture of test organism were loaded in well. The plates were incubated at 37° C for 24 hours.

The test organism were classified as sensitive (or) resistant to the compounds in each well based on the presence or absence of clear zone of inhibition.

RESULT AND DISSCUSSION

The UV-Visible absorption spectra of 3,4-dihydropyrimidinone was

measured in diluted solution in ethanol is shown in figure1. The absorption spectra of 3,4-dihydropyrimidinone exhibits in absorption peak at 320mn. The absorption band at 320 nm corresponds to π-π* electronic transition of the 3,4-dihydropyrimidinone.

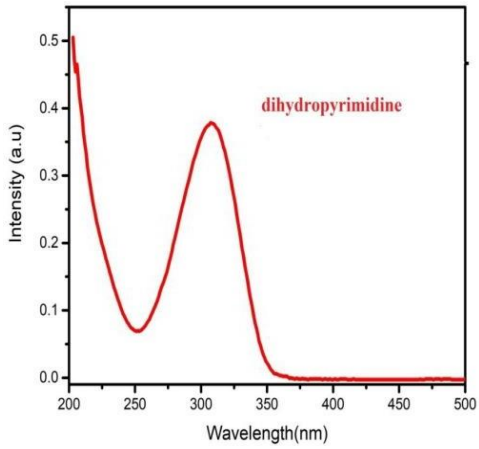


Fig.1 UV- Absorption spectra of 3,4-dihydropyrimidinonone in ethanol solvent

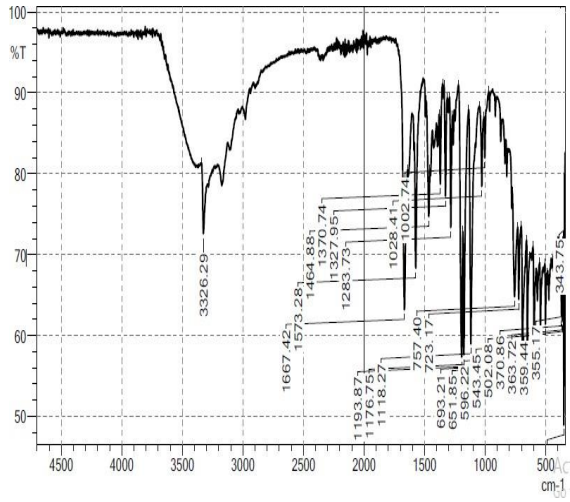


Fig. 2 3,4- FT-IR spectrum of 3,4-dihydropyrimidinone

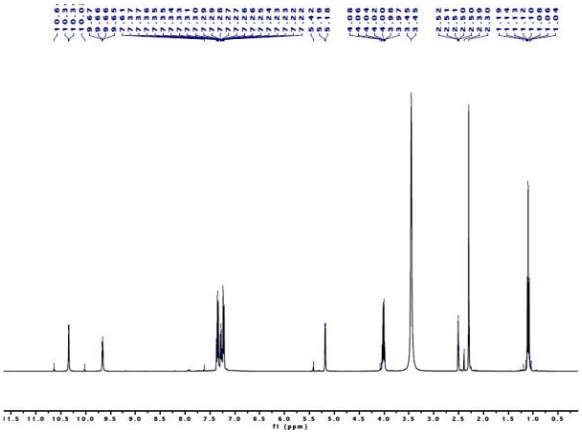


Fig. 31H NMR of 3,4-dihydropyrimidinone in DMSO-6:

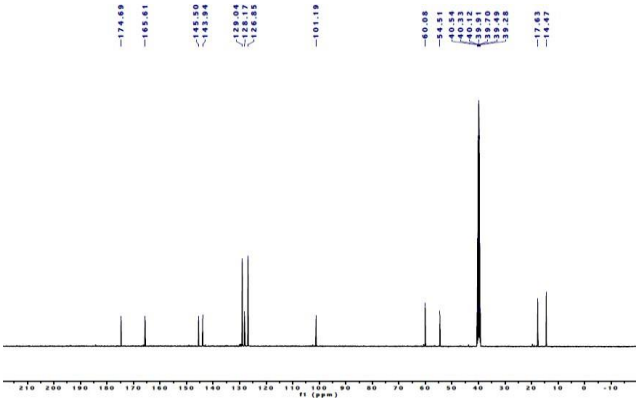
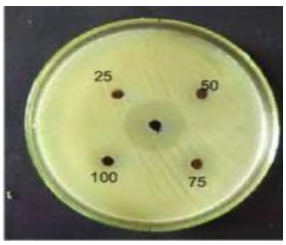


Fig. 4 ^{1}C 3,4- NMR of 3,4-dihydropyrimidinone in DMSO-6

The synthesized 3,4-dihydropyrimidinonerevealed that the presence of characteristic stretching vibrations due to NH2, and amidic CO at 3326V and 1667 cm−1 regions, respectively, in the IR spectrum. Also, the 1H-NMR spectrum of the isolated product shows signals at -CH2 -5.25,-NH2-5.45, Ar 7.20–7.56, and 10.3 NH ppm. The 13C-NMR spectrum of the isolated product shows signals at (CH aliphatic), 60.32 (CH2 aliphatic), 166.3 (C triazine ring), 175.4 (C=O amidic), and 140–160 (Benzenerings).

ANTIBACTERIAL ACTIVITY:



E-Coli

Bacillus cereus

Fig.5.Antibacterial activity of 4 Dihydropyrimi dinone

The synthesized 3,4-dihydropyrimidinone shows significant antibacterial activitieagainst human pathogenic bacteria viz E-coli, Bacillus cereus species which was almost similar to standard antibiotic drug and also observed appreciable inhibition activity against ,E- Coli Bacillus cereus.

CONCLUSION:

In summary, work aimed to synthesis 3,4-dihydropyrimidinone in a simple process. The synthesized compound was characterized using spectroscopic methods viz., UV, IR, 1HNMR and 13 CNMR spectra.The synthesized 3,4-dihydropyrimidinone showed over all significant antibacterial activities. More over the 3,4-dihydropyrimidinone derivative was more active against and human pathogenic bacteria viz E-coli, Bacillus cereus species which was almost similar to standard antibiotic drug and also observed appreciable inhibition activity against E-

Coli, Bacillus cereus.From this work we could found its use in a wide variety of biological applications due to its significant bioactivities.

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Table 1 ANTIBACTERIAL ACTIVITES OF 3,4-DIHYDROPYRIMIDINONE

S.No	Name of the Bacteria	Antibacterial activity of dihydropyrimidinone			
		Zone of the Inhibition (MM)			
		25mL	50mL	75mL	100mL
1	**E- Coli**	10	10	19	30
2	**Bacillus cereus**	_	_	10	10

SYNTHESIS, METAL ION DETECTION OF CURCUMIN DERIVATIVE AS RECEPTORS AND ITS BIOLOGICAL APPLICATIONS

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[*] Department of Chemistry, Muthayammal College of arts and Science, Rasipuarm.

ABSTRACT

In this paper we have extracted curcumin from turmeric followed by aimed to synthesis phenylhydrazone of curcumin in a simple two step process both conventional and microwave method. The synthesized compound was characterized using spectroscopic methods viz., UV, IR and NMR spectra. To analyzing the metal ion sensing ability of PHC in aqueous medium. The compound showed Al^{3+} ion selectivity based on fluorescence enhancement. The fluorescence enhancement was attributed to the photo-induced electron transfer mechanism involved in the compound. The binding constant of the complex and the lower detection limit of complex was identified. And also we could found the biological applications against Antibacterial and Antifungal due to its significant bioactivities.

KEYWORDS : Curcumin,UV, IR and NMR spectra.

INTRODUCTION

Supramolecular Chemistry

Supramolecular chemistry is the science of non-covalent, intermolecular interactions. The forces responsible for the spatial organization may vary from weak intermolecular forces, electrostatic or H-bonding to strong covalent bonding. Some important concepts demonstrated by supramolecular chemistry are molecular self-assembly, molecular recognition, host-guest chemistry, mechanically-interlocked molecular architectures etc. (Lehn J.M et.al., 1993)

In supramolecular chemistry, *host–guest chemistry*describes complexes that are composed of two or more molecules or ions that are held together in unique structural relationships by forces other than those of full covalent bonds. Host guest chemistry encompasses the idea of molecular recognition and interactions through noncovalent bonding.

Fluorescent chemosensors

Metal sensing is important in a variety of applications ranging from metal tracking in living cells to detection of toxic metal ions in the environment. Considerable effort has been dedicated to the development of small-molecule metal sensors; however, such sensors are often expensive, difficult to design, and not always compatible with living organisms. (Domaille DW et al., 2008) On the other hand, protein-based sensors are inherently biocompatible and biodegradable and can be potentially expressed as a fusion tag, offering new opportunities to track metals in vivo. Available sensors are based on inhibitory properties of metal ions, or fluorescence resonance energy transfer.(Doi N, Yanagawa H et al., 1999) These systems are either very bulky or suffer from low sensitivity and selectivity. The concept of catalytically amplified sensing, where an allosteric binding event is linked to catalysis, has emerged as a powerful tool to overcome these limitations. (Miyawaki A et al., 1997) The few reported examples of this method.(Ostermeier M et al., 2009)are not easily generalizable and rely mostly on small-molecule ligands for metal binding.

The uses of chemical reagents for the determination of heavy metal ions based on complexation reaction have been widely used for quite a long time. A large number of metal indicators containing various groups for binding exist naturally or be synthesised.(Yoon HJ et al., 2010) A chemical reagent is chosen to react sensitively and specifically to the heavy metal ion and the resultant change in its optical properties (e.g., fluorescence or absorption) is a direct measure of the concentration of heavy metal ions. Dyestuffs have been employed for the analysis of metal ions as these reagents form strong complexes with a large number of metal ions and the complexes can be determined spectrophotometrically. These indicators have been utilized in the titration processes, separation and pre-concentration processes of heavy metal ions for a long time. Edmund et al. have reported detailed information on these indicators for metal ions. (Bishop E et al., 1972) They are normally Lewis bases that attached to the metal ions in a complex. These groups are capable of donating a pair of electrons. If a metal forms a complexation, the maximum number of ligands that can be bound to the metal is known as the coordination number. Metal ions can have more than one characteristic coordination number, depending on the valence of the central atom and the coordination lignads. The strongest ligands are those multidentate and form the particularly stable five-numbered rings. Effective multidentate chelating ligands of

this type are those contain oxygen, sulphur and nitrogen which are electron donating atoms.

MATERIALS AND METHODS

Instuments

The UV – Visible absorption spectra were measured on a Jasco V-630 Spectrophotometer using 1cm path length quartz cuvettes. Fluorescence Measurements were done keeping the corresponding reference solution without the fluorophore. Fluorescence spectra were recorded on a Jasco FP – 8300 spectro fluorometer. The FT-IR spectrum was recorded on Perkin-Elmer spectrum RS-1 &^{1}H NMR Spectra were recorded on a Bruker 400 MHz (USA) spectrometer with tetramethyl silane as the ethanol was used as the solvent.

Extraction of Curcumin from turmeric

The turmeric sample with rich curcumin has been collected from Agricultural field. The purification was carried out by the boiling process of this turmeric with water and finally dried and powdered. The solvent used for extraction process 95% Methanol and The process is described as follows briefly . 5.0 g of turmeric dried powder weighed and taken in soxhlet apparatus with 250 ml of methanol .The extraction process carried out for 21 / 2 hrs . Pure curcumin powder was obtained from separation process.

Fig.2. Extraction of curcumin from turmeric

Synthesis of Curcumin receptors

The synthesized pure curcumin (10 mmol) was dissolved in ethanol and stirred well at room temperature. The phenyl hydrazine hydrochloride (10 mmol) was added to the prepared curcumin solution. The obtained orange coloured mixture was stirred and refluxed at 50^{o}C for 6 hours in mild acidic condition. After cooling the resulting reddish-brown fine precipitate of curcumino-iminophenylhydrazone was filtered and washed with double distilled water. The obtained reddish-brown powdered curcumino-iminophenylhydrazone was recrystallised by chloroform. The synthesis process was followed according to the scheme represented in Scheme.2.

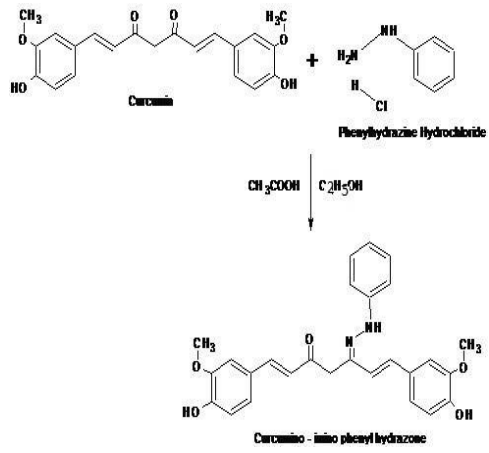

Fig.3. Synthesis of curcumino-iminophenylhydrazone

Physical Nature: Reddish-brown

Yield: 89%;

Molecular weight: 458.52

Preparation of solution of receptors.

Preparation of solution of receptor

The test solutions (1×10^{-5} mol dm^{-3}) were prepared using twice distilled water. The stock solutions was prepared in ethanol and after the addition of Compound PHC, the test solutions contain 1% ethanol. 100 µL of the chemosensor were diluted in 10 ml of water. The solutions were prepared just before the recording of UV–visible and fluorescence spectra at room temperature. The competitive binding of metal ions to PHC was studied by mixing 100 µL of each metal ion solution (1×10^{-3} mol dm^{-3}) and 100 µL of solution of Al^{3+} ions (1×10^{-3} mol dm^{-3}) and diluting to 10 mL with water. The effect of pH was studied by preparing solutions of various pH by mixing appropriate amounts of sodium hydroxide and phosphoric acid (1×10^{-3} mol dm^{-3}).

Preparation of solution of UV – Visible and fluorescence measurements:

The UV – Visible and Fluorescence was recorded just after the addition of metal salts in ethanol solution, while keeping the ligand concentration constant ($1x10^{-5}$M). The solution of metal ions were prepared from the sulphates salts of Zn^{2+}, Fe^{2+}, Al^{3+}, Ca^{2+}, Cu^{2+}, Cd^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Ba^{2+}, Hg^{2+}, K^{+}, Na^{+} and Co^{2+}. The blank solution was prepared without any added metal ion.

Job's Plot measurements:

The binding stoichiometry of sensor – metal complex determined by the continous variation method. From a stock solutions. Various concentrations of Al^{3+} Viz., $1x10^{-4}$, $8x10^{-6}$, $4x10^{-6}$, $6x10^{-5}$, $4x10^{-5}$, $2x10^{-6}$, $8x10^{-5}$, and $1x10^{-5}$ were prepared and added to the solution of compound PHC. Each test solution had a total volume of 10ml. After mixing for a few seconds, the fluorescence spectra were taken at 25^{0}C.

Antibacterial activity

The disc diffusion method (Bauer et al., 1966) was used to screen the antimicrobial activity . Stock cultures were maintained at

4oC on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37°C and 25°C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×106 colony forming units (CFU/ml) for bacteria. The Muller Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and allowed to dry for 5 minutes. The concentration of sample at 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Antifungal activity

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 10 5 CFU/ml. The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with the sample and solvent blanks (hydro alcohol, and hexane).

Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 h. The diameters of zone of inhibition observed were measured. it transparent ruler in millimeter.

RESULT AND DISCUSSION

Curcumino-iminophenylhydrazone was synthesized conventional as well as microwave assisted method as depicted in scheme 2. The synthesised compound was Characterized by FT-IR and H^1 NMR spectra are shown in Fig.18 and 19 respectively.

IR SPECTRAL STUDIES

IR-Spectra provide valuable information about the nature of the binding mode and functional group present in the complexes.An IR spectrum was recorded on Perkin-Elmer spectrum RS-1. The data obtained phenolic –OH shows that broad band in the range of 2946.10 cm^{-1}.

The strong peak for (C=C) aromatic ring was confirmed by the observed peak in the region

1444 cm^{-1}. The (C-O) band presence was assigned by the peak found at 1029.88 cm^{-1}.the band 1026.88cm^{-1}shows –C-O-C- for –OMe group and enolic –C-O- at 961.37 cm^{-1}. The peak due to the carboxyl group (C=O) was observed in both 1589.25 cm^{-1}. A sharp broad band at 1510.54 cm^{-1} shows that formation of azomethine (C=N) stretching vibrations, the aliphatic –C=CH stretch 692.19,765.26 and 817.22 cm^{-1}

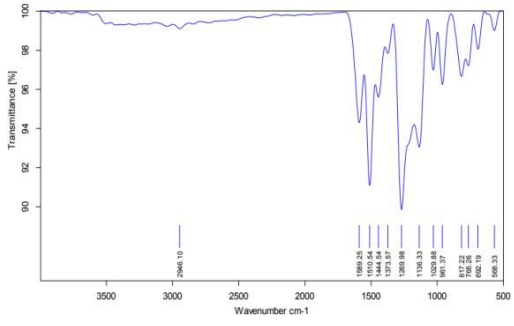

Fig.4.FT-IR Spectrum of Curcumino-imino Phenylhydrazone

NMR SPECTRAL STUDIES

The synthesized of Curcumino-imino Phenylhydrazonecharacterised by the presence of multiplets around 6.8 to 7.6 ppm .the $–OCH_3$ merged singlet 3.9 ppm, -NH group 5.7 to 5.8 ppm, phenolic –OH group singlet at 7.4ppm, active –CH group singlet 6.4 ppm and aliphatic –C=CH on enolic side double doublet observed 6.5 to 6.7 ppm, the benzene ring of phenylhydrazene (merged doublet,quartet,quartet) shows 7.42,7.5 & 7.64.the azomethine ,the benzene ring in curcumin moiety merged singlet, double doublet shows 6.96, 7.01 & 7.05 ppm

Study of Metal ion sensing in Curcumin derivative

In order to investigate the application of PHC as a chemosensor, the metal ion binding characteristics were investigated towards metal cations which are relevant to biology and present in human body, Although the absorption spectra of PHC in the presence of various added metal ions were not significantly different (Fig.20)

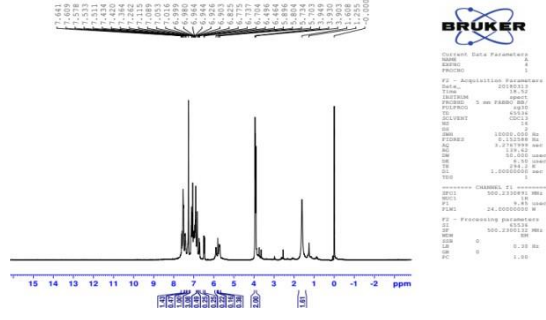

Fig.5. H^1 NMR Spectrum of Curcumino-imino Phenylhydrazone

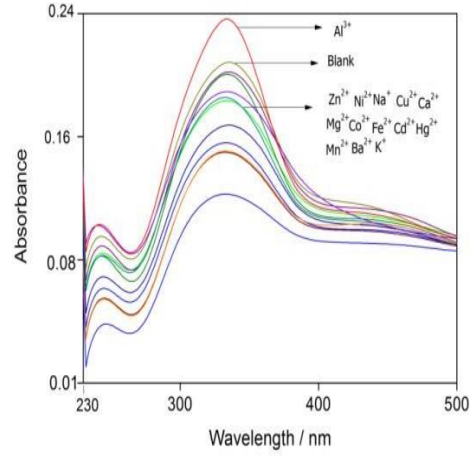

Fig.6. UV–visible absorption spectra of PHC in the presence of various metal ions

The fluorescence spectra shown by showed interesting result revealing the selectivity PHC. The changes of the fluorescence of PHC on metal ion addition were more pronounced than that of the absorption spectrum. The fluorescence spectrum showed a quenching behavior when each of the metal ion from the pool of different metals was added. However, at the addition of Al^{3+}, the fluorescence of PHC got enhanced. In particular, addition of Al^{3+} made the spectral band at 550 nm. enhance in intensity. The fluorescence spectrum of PHC showed two prominent bands, viz 520 nm and 550 nm. The shorter wavelength band this may be due to the selective binding of the compound with Al^{3+} ions as given in the (Fig. 21) and The color and fluorescence changes of PHCupon addition of various metal ions in solution.(Fig. 22) .

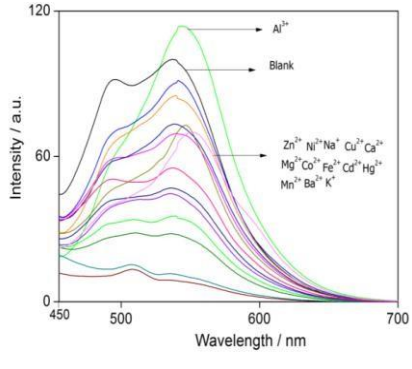

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Fig.8. The color and fluorescence changes of PHCupon addition of various metal ions in solution.

The selectivity of PHC for Al^{3+} was analyzed by measuring the fluorescence intensity changes at the addition of Al^{3+} in the presence of various metal ions.

The intensity changes are shown in (Fig.9.) Also, the competitive binding of Al^{3+} ion to PHC in the presence of other metal ions was studied. Compared to the fluorescence intensity changes observed at the addition of just any one of the metal ion of the chosen pool to PHC as shown in (Fig.10), the addition of Al^{3+} results in significant enhancement of fluorescence in the presence of various other metal ions.

Dramatic fluorescence enhancements arise due to the relief of quenched fluorescence drived by the electron hopping from lone pairs of atoms remote from the fluorophore unit.The photo induced electron transfer (PET) is affected by the metal ion binding involving the lone pairs of electrons on atoms like nitrogen of Compound PHC. Thus, the Al^{3+} metal ion sensing occurs through PET.

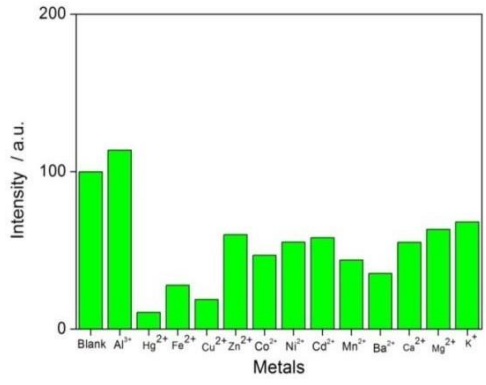


Fig.9. Fluorescence response of compound PHC upon addition of various metal ions

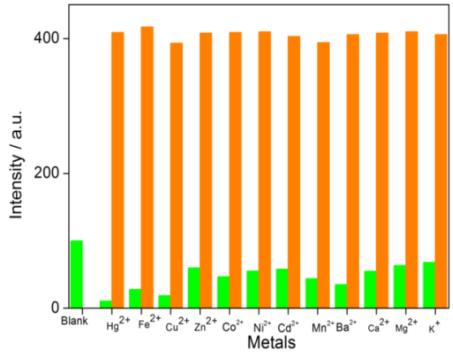


Fig.10. Fluorescence response of compound PHC addition of Al^{3+} in the presence of other metal ions.

where ■ Addition of various metal ions to PHC

& ■ Addition of Al^{3+} in the presence of other metal ions to PHC

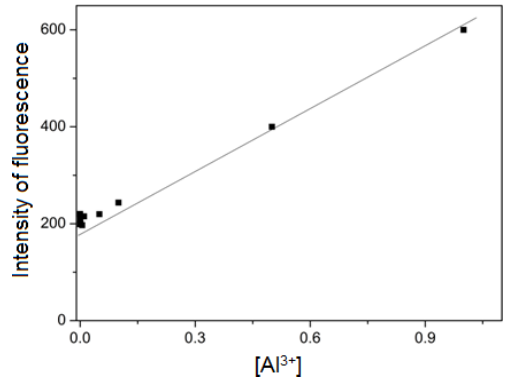


Fig. 11. The Al^{3+} detection limit of PHC**.**

The Al^{3+} detection limit of PHC was calculated by doing a binding titration in the concentration range of PHC from 1×10^{-3} to 1×10^{-5} mol dm^{-3}. The plot showing the titration is depicted in (Fig.11.) The lower detection limit of Al^{3+} by PHCis 3×10^{-9} mol dm^{-3}. The binding constant of the PHC–Al^{3+} complex was calculated by plotting the intensity data following the Benesi–Hildebrand equation [101-102].

$$\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{(I' - I_0)K[\mathrm{Al3+}]} \quad (1)$$

where I_0 is the intensity of fluorescence of the free Compound PHC, I is the intensity at the addition of Al^{3+} ions, and K is the binding constant. The linear Benesi–Hildebrand plot (Fig. 12) gave the binding constant value, K= 27347.97 of the PHC–Al^{3+} binding.

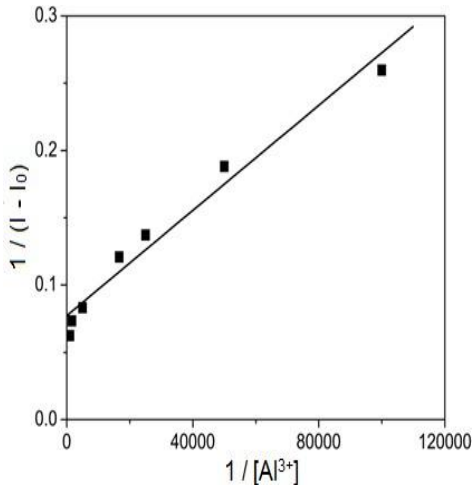


Fig. 12. Benesi–Hildebrand plot for complex of PHC with Al^{3+}.

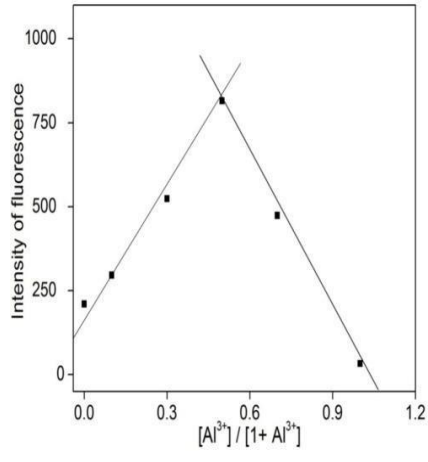


Fig.13. The stoichiometry of the PHC–Al^{3+} complex is 1:1 as inferred from the plot.

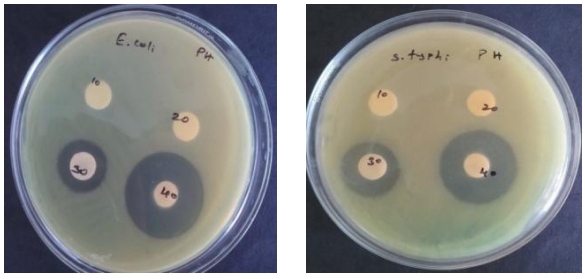
The stoichiometry of the PHC-Al^{3+} complex was obtained from the job's plots made using the data using fluorescence spectral data and the mole fraction is calculated as [Al^{3+}] / [PHC+ Al^{3+}] wherePHC refers to the Compound PHC are shown in (Fig 13.) The stoichiometry of the PHC–Al^{3+} complex is 1:1 as inferred from the plot.

Antibacterial activity

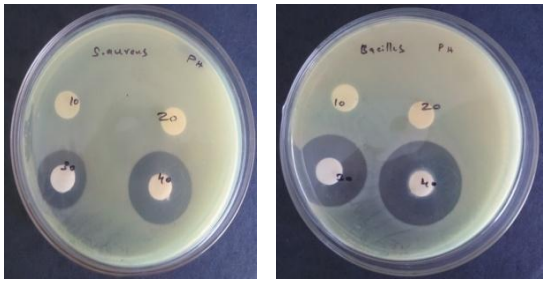
The antibacterial activities of curcumin derivative against two gram-positive (Staphylococcus aureus and Bacillus cereus) and two gram-negative bacteria (Escherichia coli andSalmonella typhi) were evaluated and their activity was compared to a well-known commercial antibiotic Chloramphenicol. The results are reported in table 1. From these results, curcumin derivative were found to be more active against all the bacteria tested.

Moreover, the zone of inhibition observed for those synthesized products active against E.coli showed the moderate antibacterial action when compared to the results obtained against other bacterial species. Therefore the activity exhibited by the curcumin derivative ligand were significantly appreciable. The results compared with standard drug have been indicated that the synthesized

compounds were active but activity was lower than the standard drug and also showed nearly similar activity to the standard drug. Results of antibacterial evaluation is summarized in Fig.(14).



EscherichiaColi Salmonella typhi



BacilluscereusStaphylococcus aureus

Fig.14. Antibacterial activities of curcumino-iminophenylhydrazone

Table.1. Antibacterial activities Zone of inhibition of curcumino-iminophenylhydrazone

Antibacterial activity (Zone of inhibition of Curcumino-iminophenylhydrazone)					
Zone of inhibition in mm					
Concentration in µg/ml					
S.No	**Bacterial species**	**10 mg**	**20 mg**	**30 mg**	**40 mg**
1	Salmonella typhi	12	14	16	18
2	Escherichia Coli	11	12	13	14
3	Bacillus cereus	11	12	13	15
4	Staphylococcus aureus	10	11	13	14

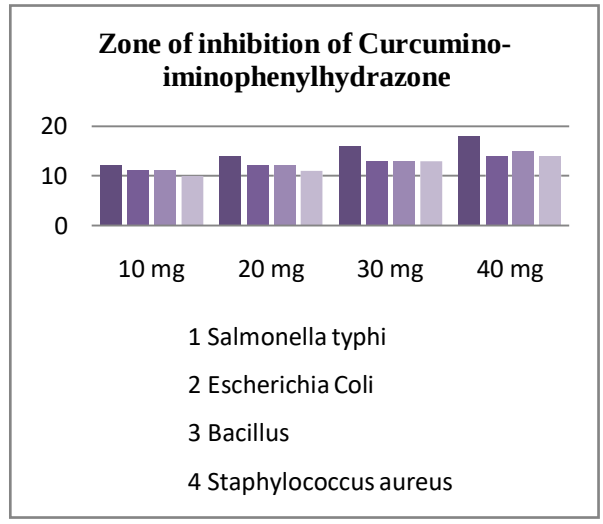


Fig.15. Antibacterial activity data of curcumino-iminophenylhydrazone

Antifungal activity

Curcumin derivative were determined for their antifungal activity against three fungal strains Candida albicans, Curvularia lunata and Aspergillus niger similar and their activity was compared with standard antifungal drug fluconazole. The results were shown in table.2. From the results, it can be concluded that the activity of the curcumin derivative were showed better inhibition .when tested against C.albicans, C.lunata and A.niger fungal species. However the activity of curcumin derivative was almost similar to the standard antibiotic (Fluconazole) but interestingly, it showed more activity than curcumin. Results of antifungal evaluation are summarized in (Fig. 16).

Candida albicans Curvularia lunata

As

Fig.16. Antifungal activities of curcumino-iminophenylhydrazone

Table.2. Antifungal activity Zone of inhibition of curcumino-iminophenylhydrazone

Antifungal activity (Zone of inhibition of curcuimino-iminophenylhydrazone)				
Zone of inhibition in mm				
Concentration in µg/ml				
S. No.	**Fungal species**	**10 mg**	**20 mg**	**30 mg**
1	Candida albicans	11	12	11
2	Curvularia lunata	13	14	13
3	Aspergillus niger	12	11	13

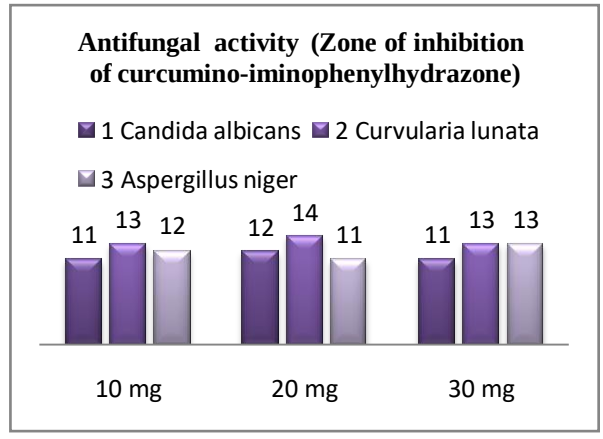


Fig.17. Antifungal activity data of curcumino-iminophenylhydrazone

CONCLUSION

In the present work aimed to synthesis phenylhydrazone of curcumin in a simple two step process. The synthesized compound was characterized using spectroscopic methods viz., UV, IR and NMR spectra. The compound was synthesized both conventional and microwave method, in microwave synthesis the yield of product was squat when compare to conventional synthesis. To analyzing the metal ion sensing ability of PHC in aqueous medium. the compound showed a Al^{3+} ion selectivity based on fluorescence enhancement. The fluorescence enhancement was attributed to the photo-induced electron transfer mechanism involved in the compound. The binding constant of the complex of the compound

with Al^{3+} ions was 27347.97 M^{-1} and the lower detection limit of Al^{3+} ions by the compound was 3×10^{-9} mol $dm^{-3.}$ The curcumin derivative was more active against Candida albicans, Curvularia lunata, Aspergillus niger species which was almost similar to standard antibiotic drug and also observed appreciable inhibition activity against Staphylococcus aureus, Bacillus cereus.

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ANTIBACTERIAL ACTIVITY, PHYTOCHEMICAL STUDIES AND GC-MS ANALYSIS OF INDIAN MEDICINAL PLANT EXTRACTS AGAINSTNOSOCOMIAL PATHOGENS.

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ABSTRACT

There is continuous discover new antimicrobial compounds with diverse chemical structures and novel mechanisms if action because there has been an alarming increase in the incidence of new and reemerging infectious disease. In recent years, drug resistance to human pathogenic bacteria has from all over the world. Plant-based therapies are an excellent alternative for antibiotics began to be offered in all the countries, the extracts of medicinal plants still used in traditional medicine. Plants have been the predominant source of medicine with eminence over millennia. Phytochemicals are the chemicals extracted from plants. Depends on the role in the plant metabolism the organic chemicals was classified as primary and secondary constituents. Phytochemical and GC-MS analysis act as an interesting tool in the evaluation of active principles in the extract obtained from the herbs utilized in the treatment against various clinical pathogens. The antibacterial activity of *Coleus aromaticus*, plant extract in different solvents such as ethanol, methanol, chloroform and petroleum ether of plant extracts against the *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*and *Klebsiella pneumonia* nosocomial pathogens.

KEYWORDS: *Coleus aromaticus*, Phytochemical studies, GC-MS analysis, Antibacterial activity, Nosocomial pathogens.

INTRODUCTION

Plants are emerging as a potential source of new drugs. Natural products derived from plants utilized as clinically effective potent and powerful drugs in the developing nations [1-2]. Plants gained more attention by the researcher due to the development of antimicrobial resistance nature of commonly used antibiotics. Over thousands of year's plant and plant extracts have been used in the treatment of skin disorders as antiseptics and antimicrobial agents [3-4]. Globally Four billion peoples of the world population still rely on herbal medicines as their primary source of health care in their treatment practice especially in economically developing nations [5]. A wide range of medicinal plants with its extract possessing various medicinal properties used as raw drugs. The raw drug was used by the local communities and folk healers in small quantity and it was traded commercially in larger quantities by many herbal industries [6-7].

Current research on natural molecules and its derivatives was focused on the plants with high ethno-medicinal values [8], the traditional medicine use plants that contain a wide range of substances

used in the treatment of chronic infection as well as infectious diseases [9-10]. Clinical microbiologists paid more interest in the discovery of new therapeutics by screening medicinal plants. The main property of a natural drug extracted from plants is their secondary metabolites. The antimicrobial activity of plant is determined by different components includes aldehyde and phenolic compounds [11-13]. Abuse and over usage of the antibiotics develop drug resistance in human pathogens has necessitated a search of new source includes plants or its products that possess antimicrobial nature. New compounds extracted from medicinal plants was Screened for antimicrobial activities is essential for therapeutic use [14].

A key source of indigenous pharmaceutical systems was Medicinal plants. WHO states, about 65–80% of the world's population in developing countries, due to the poverty and lack of access to modern medicine, depend essentially on plants for their primary healthcare [15]. The natural products are often selected for biological screening supported ethno-medicinal use of plants, because many infectious diseases are known to possess been treated with herbal remedies throughout the history of mankind. Even today, in many developing countries natural plant products pursue a lead role in primary early care as therapeutic remedies [16-17].

The most important herbs used in the Indian Auryedic medicine *Coleus aromaticus* which recently called as Plectranthusamboinicus (Lour) belongs to Lamiaceae family is a folkloric medicinal plant. The extract of leaf and stem exhibit phytochemical properties like antioxidant activity, antiosteoporosis activities, antidiabeticimmunostimulatory, antitumor, anti-inflammatory and antimicrobial activity [18] possess Antimicrobial activity, Antifungal activity , Anti-inflammatory activity, Antibacterial activity, Antidiabetic activity, Anxiolytic activity, Diuretic activity, Antineoplastic activity, Respiratory disorder), Wound healing activity, Analgesic activity, Antiurolithiatic activity, Antiplatelet aggregation activity, Antibiofilm efficacy and It is used to treat chronic cough, asthma, epilepsy, bronchitis, helminthiasis, colic, convulsions, dyspepsia, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, bronchitis, malarial fever, hepatopathy, renal and Rheumatoid arthritis [19-23].

'Nosocomial' or 'hospital-acquired infection'(HAI) or 'healthcare associated infections' (HCAI) appear in a patient under medical care in the hospital or other health care facility which was absent at the time of admission and emerges after 48-72 hours or 3 days during the hospital stay. Bacteria, viruses and fungal parasites are the main cause of Nosocomial infections. WHO states, approximately 15% of all patients under hospitalization acquired HAI [24]. The most common microbial agents of HAI are *Staphylococcus aureus* [25], *Pseudomonas aeruginosa, Enterobacteriaceae* [26], *E. coli* [27], *B. cereus, M. tuberculi, Streptococcus spp, Acinetobacterspp, Legionell* [28], *Candida* [29], *Aspergillus* [30], *Fusarium, Trichosporon and Malassezia* [31]. HAI infections show variations in Epidemiological and etiological characteristics among various countries [32-33].

Nosocomial infections are the major causes of death and increased morbidity and mortality. Nosocomial infections can cause severe pneumonia and the major site of infection is surgical site, gastrointestinal tract, urinary tract, bloodstream and other parts of the body [34]. These types of infections are difficult to treat with previous generation antibiotics due to the transfer of antibiotic resistance to Gram negative bacteria and can infect people outside the hospital [35-36]. Hospital waste serves as a potential source of pathogens and about 20%-25% of hospital waste is termed as hazardous [28].

The most common etiological agent of nosocomial infection is bacteria like *S. aureus, Streptococcus pneumoniae, Escherichia coli, P. aeruginosa, Haemophilusinfluenzae, Klebsiellapneumoniae, Acinetobacter, Proteus mirabilis, and Enterococc*i and antibiotic resistant bacteria which is outstandingly present in hospitals like MRSA and VRSA [37-39]. The aim of the present research work was to study the Phytochemical, GC-MS analysis and antibacterial activity of *Coleus aromaticus* against common nosocomial pathogens.

MATERIALS AND METHODS

Collection and Preparation of Plant Extract

The selected medicinal plant *Coleus aromaticus* were collected from different locations of Kollihills, Namakkal, Tamilnadu, India. The collected plants were authenticated by Botanical Survey of India (BSI-Southern Circle) - Government of India, Coimbatore, Tamilnadu. The plant Authentication reference letter No: BSI/SRC/5/23/2019-Tech/3135. The plant authenticated report and specimen was deposited in Microbiology Department, Muthayammal College of Arts & Science, Rasipuram, Tamil Nadu , India.

Processing and extraction

Garbling process

Garbling done by manually to separate a particular portion of a plant dried from other parts of the plant and other extraneous matter.

Drying process

After harvesting, all leaves had moisture content of 60 – 80 % and could not be stored without drying to avoid break down of important compounds and contamination by microorganisms. In room temperature the leaves collected from the plant was shadow dried. By processing proper drying the moisture content present in the plant product was reduced to 14%.

Grinding process

All the collected dried leaves were ground into fine powder by Mechanical shearing and sieving.

Extraction of Active Components

About 20g of the finely ground leaf powder was weighed and mixed with 100ml of ethanol, methanol, petroleum ether and chloroform in separate conical flasks and kept overnight. The filtrate was obtained by filtering these contents twice using No. 1 Whatman filter paper. The clear filtrate was condensed using rotary vacuum evaporator at 50°C for about 15 minutes.

Phytochemical Analysis of Plant extracts

The leaf extract that exhibited the maximum antimicrobial activity was assessed for the presence of phytochemicals. A preliminary phytochemical study was performed to seek out the presence of phytocompouds, like alkaloids, flavonoids, saponins, carbohydrates, proteins, phenols, steroids, glycosides, and tannins. The phytochemical analysis was performed supported color reactions with plant extracts, using the subsequent procedure described by Odebiyi and Sofowora (1999) and Jamil *et al.* (2012).

Alkaloids (Meyor's test)

To 1 ml of the acidic aqueous extract, few drops of Meyor's reagent were added. Presence of alkaloids was confirmed by the formation of white or pale yellow precipitate.

Flavonoids

In a test tube containing 0.5ml of aqueous extract, 5-10 ml of diluted HCl and a small amount of zinc or magnesium powder was added and the solution was boiled for few minutes. Presence of flavonoids was confirmed by the formation of reddish pink or dirty brown colour.

Saponins

In a test tube containing about 5ml of aqueous extract, a drop of sodium bicarbonate solution was added. The mixture was vigorously shaked for 3 minutes. Presence of saponins was confirmed by the formation of honey comb like froth appearance.

Carbohydrates (Fehling's test)

To a test tube containing 5 ml of aqueous extract about 1 ml of Fehling's solution was added, the contents were then boiled for few minutes. Presence of carbohydrate was confirmed by the formation of red or brick red precipitate.

Proteins (Biuret's test)

To 1ml of hot aqueous extract of the leaves, 5-8 drops of 10% sodium hydroxide solution, followed by 1 or 2 drops of 5% copper sulphate were added. Presence of proteins was confirmed by the formation of red or violet colour.

Phenols (Ferric chloride test)

To 1ml of alcoholic extract, 2ml of distilled water followed by few drops of 10% aqueous ferric chloride solution was added. Presence of phenols was confirmed by formation of blue or green colour.

Steroids (Solkowshy's test)

To the sides of the test tube add 2ml of chloroform extract and 1 ml of concentrated sulphuric acid. Formation of red colour in the chloroform layer confirms the presence of steroids.

Glycosides

To a small amount of aqueous extract, a few drops of aqueous NaOH solution was added. Presence of yellow colour confirmed glycosides.

Resins

To 2 ml of chloroform or alcoholic extract, about 5-10 ml of acetic anhydride was added and dissolved by gentle heating. The solution was cooled and about 0.5 ml of concentrated H_2SO_4 was added. Appearance of bright purple colour which immediately changed into

deep violet colour confirmed the presence of resins.

Tannins (Ferric chloride test)

To 1-2ml of an alcoholic extract, few drops of 5% aqueous ferric chloride solution were added. A bluish black colour, which disappeared on addition of few ml of dilute sulphuric acid, was followed by the formation of a yellowish brown precipitate which indicated the presence of tannins.

Test organisms

The four test bacteria used in the study such as, *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia,* and *Pseudomonas aeroginosa* were isolated from nosocomial sample. The clinical Nosocomial isolates obtained from the Microbiology Laboratory of at Namakkal. Processing of the specimen was started immediately once it reaches the laboratory of microbiology. 50 ml of sterile nutrient broth was prepared, the samples containing cotton swab was inoculated into the broth and incubated at 37˚ C for 24 hours. After incubation the samples were inoculated on the nutrient agar medium and incubated at 37˚ C for 24 hours. The organisms *Klebsiellapneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa,* and *Serratiamarcescens*fresh subcultures were made before use

Antibacterial activity of Coleus aromaticus against isolated Nosocomial pathogens

The antimicrobial activity of the leaf extracts was evaluated by agar disc diffusion method test organisms, *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia,* and *Pseudomonas aeroginosa* with 0.5 McFarland standards were spread over on the Muller Hinton agar plates by using separate sterile cotton swabs. The sterile disc was prepared and coated with 30 µg concentration of plant extracts. The prepared extracted discs were placed on medium with standard Tetracycline disc with 30 µg concentration. At 37˚C the inoculated plates was kept for 24 hours incubation. The zone of inhibition of bacterial growth was measured and compared with standard antibacterial agents (Das *et al.*2010,Ashraf A.Mostafa *et al.*2018)

Gas Chromatography-Mass Spectroscopy (GC-MS)

Identification of the main component was carried out by the comparison of both the GC retention times and MS data against those of the reference standards. A Hewlett Packard GC-MS system were used for research work, a model 5890A gas chromatograph, a model 5970B mass selective detector, a HP 5970C MS chemstation, and a HP 7946 disc drive. The fused-silica capillary column coated with HP-5 cross-linked with 5% phenylmethylsilicone was used. The GC temperature program was as follows: initial temperature was 100 ℃, held for 1 min, increased to 130 ℃ at a rate of 2 ℃/min, then to 200 ℃ at a rate of 3 ℃/min, and finally to 280 ℃ at a rate of 6 ℃/min and held for 10 min. The split ratio was 1:12, injection temperature was 250 ℃, transfer line temperature was 270 ℃, and ion source temperature was 200 ℃. The mass spectrometer was operated at 70 eV in the electron impact mode with SCAN or SIM. In the present research , the bulk herbal extract were analyzed for their chemical constituents by GC-MS (Ukwubile *et al.*

2019,KaruppasamyBalamuruganet *al.*2012)

RESULTS AND DISCUSSION

Phytochemical analysis of Coleus aromaticus

Phytochemical analysis of *Coleus aromaticus* was carried out by the following solvents Methanol, Ethanol, Petroleum ether and Chloroform. In Methanol extractof *Coleus aromaticus* contain all the components, CHO, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present. Whereas the Ethanol extract of *Coleus aromaticus* contain all the constituents except CHO. The presence of Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are noted.In Chloroform extract of *Coleus aromaticus* contain CHO, Alkaloids, flavonoids, terpenoids, glycosides, and steroids are present. Tannin and phenolic compounds are absent. Petroleum ether extract of *Coleus aromaticus* contains Steroids, flavonoids, tannin and Sugars are present. The predominant components present in all the extracts of *Coleus aromaticus* are flavonoids, Terpenoids and steroids (Table I)

The phytocompounds found in the *Coleus aromaticus* are carvacrol, thymol, α- humuleneundecanal,P-cymene, caryophyllene oxide, α-terpineol and β- selinene(Rout*et al.*2010, Arumugam*et al.*2016, Singh *et al.*2002). Another analysis obtained thymol, carvacrol, 1,8-cineole, eugenol, caryophyllene, trepinolene, α- pinene, β-pinene, methyleugenol, and β–phellandrence. The variations can be attributed to the methodology used in the extraction process, seasonal variations, soil type, climate, genetic and geographical variations of the plants (Lopes *et al.*2017). It also contain Xanthophylls(Kumaran andKarunakaran2006).

Antibacterial activity of Coleus aromaticus

The antibacterial effects of Plant extract of *Coleus aromaticus* had been investigated against isolated *Escherichia coli, Staphylococcus aureus, Klebsiellapneumoniae,* and *Pseudomonas aeroginosa.* The disc prepared with 30µg concentration of the plant extract was placed on Muller-Hinton agar plates with standard antibiotic tetracycline disc. The standard antibiotic tetracycline disc (30µg) impregnated on the Muller-Hinton agar plate exhibit the antibacterial activity with the zone of inhibition (22mm), against *Staphylococcus aureus.* The antibacterial activity of the ethanol extract of *Coleus aromaticus* , at the 30µg concentration was high (15mm and 11mm) against*S.aureus*and *Klebsiella pneumonia* respectively. Minimum activity was recorded (10mm, 10mm) against *Escherichia coli, Pseudomonas aeroginosa.* The antibacterial activity of choloroform extracts of *Coleus aromaticus* , at 30µg concentration the inhibition zone was high (11mm) against *Pseudomonas aeroginosa*. Whereas minimum activity (08mm) against *Staphylococcus aureus.* The antibacterial activity of methanol extracts of *Coleus aromaticus*, at 30µg concentration the inhibition zone was high (10mm) against *S.aureus*. Whereas minimum activity (2mm) against *Pseudomonas aeroginosa.*In petroleum ether extracts of *Coleus aromaticus*, not inhibit the growth of bacteria.(Table II). The results plainly reveal that the growth of the bacteria was inhibited by the ethanol and

chloroform extracts of *Coleusaromaticus*.

The leaf extracts of *Coleus aromaticus* have shown to have antibacterial activity (VasaviDathar and Afrojahan. 2017). Methanolic extract of *Coleus amboinicus*leaves shows antibacterial activity against Gram negative pathogens includes *Escherichia coli, Klebsiellapneumoniae, Citrobacterdivergens, Shigellaflexneri, Salmonella paratyphi*A, *Salmonella paratyphi*B, *Proteus mirabilis* and *Pseudomonas aeruginosa*and Gram positive *Staphylococcus aureus,* Methicillin resistant *Staphylococcus aureus*and Enterococci and (Vasavi*et al.*2015).

Muhammad Muzaffar Ali Khan Khattak*et al.* (2013) have reported antibacterial activity of aqueous methanol extract of *Coleus amboinicus*against the *Bacillus subtilis*and *Staphylococcus aureus*and the *Escherichia coli* and *Pseudomonas aeruginosa*. The plant extracts as well as the leaf oil of *Coleus amboinicus*exhibited antibacterial activity against clinical isolates of *Proteus mirabilis* and *Pseudomonas aeruginosa.* The *Coleus aromaticus* leaf extract inhibited human pathogenic microorganisms, like *B.subtilis*, *E.coli*, Methicillin Resistant *Staphylococcus aureus*(MRSA), *P.vulgaris*, *S.marcescens*, *S.aureus*and *S.epidermidis*(Ashwini*et al.*2013, da Costa *et al.* 2010).

*Plectranthusamboinicus*essential oil alters the membrane permeability and anti bacterial effect on its double concentration of MIC against *Klebsiellapneumoniae*. In addition *Plectranthusamboinicus* leaf oil potentialized the antibiotic activity against *Escherichia coli, Staphylococcus aureus, Proteus vulgaris* and *Bacillus cereus* with standard antibiotics like amikacin, kanamycin and gentamicin. Methanol extract using 1µml using antibiotic activity for this 5 bacteria's, and 1mg of Amikacin drug is used (Girish*et al.*2016, Shubhaand Bhatt 2016, Muniandy*et al.*2014).

GC – MS Analysis of Coleus aromaticus

The multifaceted applications of Plant-derived substances make them as an expertise one. From the ancient time the use of herbal medicine for the treatment of diseases and infections is in practice.The selected plant *Coleus aromaticus* was selected on the basis of ethanobotanical information collected from folk medicine and was subjected to GC-MS analysis (Figure II).

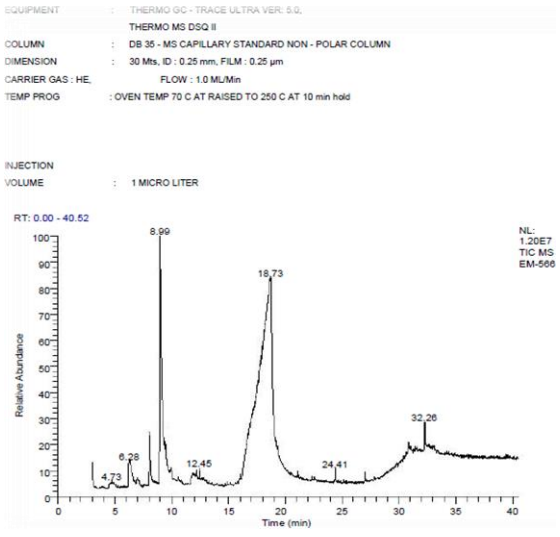

Figure II : Analysis of active ingredient peak of *Coleus aromaticus* byGC-MS

The present research work, the GC-MS analysis indicated that the *Coleus aromaticus* extract consist the following compounds, Monobenzylidene-d-glucose, Prosta-5,13-dien-1-oic acid, 9,11,15 tris[(trimethylsilyl)oxy]-, trimethylsilyl ester, 5-Carbethoxysuccinylacetone, (5Z,9à,11à,13E,15S), Quinine, trimethylsilyl ether, Estra-1,3,5(10)-trien-6-one, 3,16,17-tris(acetyloxy), O,O,O-tris(trimethylsilyl), 6-(O-methyloxime), (16à,17á)-, Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13 ,13,15,15-hexadecamethyl, Dipyridamole, Aspidospermidine-1-ethanol, 17-hydroxy-16-methoxy-à-methyl, 9,10-Anthracenedione,1-(methylamino)-4-[(4-methylphenyl)amino], 7-Chloro-3-[3,4-dichlorophenyl]-1-[[3-[dimethylamino]propyl]imino]-10-hydroxy-2-methyl-1,2,3,4,9,10-hexahydro-9-acridinone, Stearic acid, 5-(hexadecyloxy)-3-(octadecyloxy)propyl ester, Prost-13-en-1-oic acid, 1,2,8-Trihydroxy-6-methoxy-3-methylanthraquinone, 3,9á;14,15-Diepoxypregn-16-en-20-one, 3,11á,18-triacetoxy, 5-Carbethoxysuccinylacetone, Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis, Hydromorphonepfp, 2-pentadecyl, (5á)Pregnane-3,20á-diol,14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-,Diacetate, 9-(methoxyimino)-11,15-bis[(trimethylsilyl)oxy]-, 2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene, 2á,4a-Epoxymethylphenanthrene-7-methanol,1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2ylidene)methyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-, acetate, trimethylsilylester, (8.xi.,12.xi.), Acetic acid, 9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate, 1-Monolinoleoylglycerol trimethylsilyl ether, trans, 1,1,2,2-Tetrakis(adamant-1-yl)ethane, 3-Bromopiperidin-2-one, 3,9á;14,15-Diepoxypregn-16-en-20-one, 3,11á,18-triacetoxy, 1,3-Dioxane, O,O',O''-tris(trimethylsilyl), (Table III & IV).

Previous study was reported by Mamani and Alhaji (2019),the presence of following compounds 1,2-benzenedicarboxylic acid, diethyl ester, phytol, octadecenal, dibutyl phthalate, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl, hexadecanoic acid, methyl ester, oleic acid, 9,12,15-octadecatrienoic acid, (z,z,z), 9,12,15-octadecatrienoic acid, ethyl ester, (z,z,z) and solanesol. Table 2 represents the active phyto-components identified in the methanolic extracts of the Coleus aromaticus leaves by GC-MS.

The GC-MS study was carried out by Roja *et al.* (2006), to identify the chemical compounds of *P. amboinicus* . The results revealed that the presence of similar volatile constituents, though the parent plants and root cultures contained 21 compounds in comparison to only 15 compounds noticed in the *Coleus aromaticus.*

***Conclusion**:*

The present research concluded that the methanol extract of traditionally using medicinal plants showed the maximum antibacterial activity against the selected nosocomial pathogens. The results of this study showed that different plants belonging to various families have the ability to inhibit the growth of some nosocomial pathogens. This work may

furnish the necessary information in the selection of plants and its extraction for the isolation of constituents possesses antibacterial effect against the selected species. The present study may also provide a scientific basis on the use of crude plant extracts and oil on herbal medicine. The investigation culminate that methanol with its stronger extraction capacity produce more active constituents that own many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants used to treat many incurable diseases.

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Table I -Preliminary Phytochemical screening of various extracts of the leaves of *Coleus aromatic*

Organisms	Concentration of extract and zone of inhibition (mm)				
	Tetracycline	**Methanol extract**	**Ethanol extract**	**Chloroform extract**	**Petroleum ether extract**
Staphylococcus aureus	22	10	15	08	-
Klebsiellapneumoniae	-	-	11	10	-
E.coli	-	06	10	10	-
Pseudomonas aeroginosa	-	02	10	11	-

Table II -Antibacterial activity of *Coleus aromaticus* extracts against isolated organisms

Constituents	Ethanol	Methanol	Petroleum ether	Chloroform
Alkaloids	+++	+	-	+
Flavonoids	+++	++	+	+
Tannin	+	++	+	-
Carbohydrate	-	+	-	+
Terpenoids	++	+	++	+
Glycosides	+++	+	-	+
Steroids	++	+	+	++
Phenols	+	+	-	-

+++: Abundantly present, ++: Moderately present, +: Present, -: Absent

Table III -Analysis of active ingredient of *Coleus aromaticus* extract by GC-MS

S.no	R.time	Compound name	Molecular Formula	Molecular Weight	Area %
1	7.10	Monobenzylidene-d-glucose	$C_{13}H_{16}O_6$	268	1.76
2	30.28	Prosta-5,13-dien-1-oic acid, 9,11,15-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester, (5Z,9à,11à,13E,15S)-	$C_{32}H_{66}O_5Si_4$	642	2.58
3	30.69	Quinine, trimethylsilyl ether	$C_{23}H_{32}N_2O_2Si$	396	1.37
4	31.03	Estra-1,3,5(10)-trien-6-one, 3,16,17-tris(acetyloxy)-, 6-(O-methyloxime), (16à,17á)-	$C_{25}H_{31}NO_7$	457	4.92
5	31.43	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	578	3.94
6	31.64	Dipyridamole	$C_{24}H_{40}N_8O_4$	504	2.19
7	32.22	Aspidospermidine-1-ethanol, 17-hydroxy-16-methoxy-à-methyl-	$C_{23}H_{34}N_2O_3$	386	6.18
8	33.05	7-Chloro-3-[3,4-dichlorophenyl]-1-[[3-[dimethylamino]propyl]imino]-10-hydroxy-2-methyl-1,2,3,4,9,10-hexahydro-9-acridinone	$C_{25}H_{26}C_{l3}N_3O_2$	505	1.54
9	33.32	Stearic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{78}O_3$	594	2.30
10	33.60	1,2,8-Trihydroxy-6-methoxy-3-methylanthraquinone, O,O',O''-tris(trimethylsilyl)	$C_{25}H3_6O_6Si_3$	516	2.31
11	33.78	3,9á;14,15-Diepoxypregn-16-en-20-one, 3,11á,18-triacetoxy-	$C_{27}H_{34}O_9$	502	2.38
12	34.41	Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	$C_{39}H_{80}O_2$	580	5.78
13	34.98	Hydromorphonepfp	$C_{20}H_{18F5}NO_4$	431	4.56
14	35.17	(5á)Pregnane-3,20á-diol,14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-,Diacetate	$C_{28}H_{43}NO_6$	489	1.35
15	35.69	2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	$C_{17}H_{13}NO_6$	327	2.72
16	36.11	2á,4a-Epoxymethylphenanthrene-7-methanol,1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2ylidene)methyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-, acetate Acetic acid,	$C_{27}H_{38}O_4S_2$	490	1.83
17	36.19	9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate	$C_{28}H_{39}ClO_9$	554	3.40

18	36.76	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	3.12
19	37.43	3-Bromopiperidin-2-one	C_5H_8BrNO	177	23.42
20	38.13	3,9á;14,15-Diepoxypregn-16-en-20-one, 3,11á,18-triacetoxy-	$C_{27}H_{34}O_9$	502	3.41
21	38.47	1,3-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-, trans-	$C_{35}H_{70}O_3$	538	2.59
22	38.82	1,1,2,2-Tetrakis(adamant-1-yl)ethane	$C_{42}H_{62}$	566	3.27
23	39.20	Prost-13-en-1-oic acid,9-(methoxyimino)-11,15-bis[(trimethylsilyl)oxy]-, trimethylsilylester, (8.xi.,12.xi.)-	$C_{30}H_{61}NO_5Si_3$	599	1.88
24	39.43	9,10-Anthracenedione,1-(methylamino)-4-[(4-methylphenyl)amino]-	$C22H18N_2O_2$	342	4.36
25	39.83	5-Carbethoxysuccinylacetone, O,O,O-tris(trimethylsilyl)-	$C_{19}H_{38}O_6Si_3$	446	4.32
26	40.04	4-(9-Acridinyl)-N,N-dimethylbenzenamine	$C_{21}H_{18}N_2$	298	2.52

Table IV- Activity of phyto-components identified in the extracts of the Coleus aromaticus leaves by GC-MS.

S no	Name of the compound	Compound	Biological activity
1	4-(9-Acridinyl)-N,N-dimethylbenzenamine	Acridine compound	Antimicrobial activity. (Ramesh Kumar *et al.*, 2014)
2	9,10-Anthracenedione,1-(methylamino)-4-[(4-methylphenyl)amino]-	*Anthraquinone*	Antimicrobial activity (Dukes. 2013)
3	Prost-13-en-1-oicacid,9-(methoxyimino)-11,15-bis[(trimethylsilyl)oxy]-, trimethylsilylester	Alcoholic Compound	Anti ulcer agent and antimicrobial activates (Dukes. 2013)
4	1,3-Dioxane,5-(hexadecyloxy)-2-pentadecyl-, trans	Phenolic compounds	Antimicrobial, Anti-Inflammatory and Cytotoxic Activities (Jegadeeswari*et al.*, 2012)
5	3-Bromopiperidin-2-one	Terpene alcohol	Antibacterial, bactericidal, fungicidal and antinflammatory activities. (Rajashekar Reddy *et al.*, 2014)
6	1-Monolinoleoylglycerol trimethylsilyl ether	Steroid	Antimicrobial, Antioxidant Activities (Sheela and Uthayakumari, 2013).
7	Monobenzylidene-d-glucose	Acylated derivatives	Antibacterial and antifungal activities (Abul*et al.*, 2005).
8	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	Volatile organic compounds	Anti microbial activity (Venkatesh*et al.*, 2014)
9	Dipyridamole	Diterpene compound	*Antibacterial property* (Sarkar *et al.*, 2003).

EFFICIENT MARKET HYPOTHESIS AND STOCK MARKET ANOMALIES

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ABSTRACT

Evolution of Efficient Market theory had ushered a significant change in pricing capital asset. Before the development of efficient market theory by Fama in 1970s, there was no comprehensive theory on pricing of capital assets. Fama (1970) had come out with three different forms of Efficient market hypothesis i.e., Weak form, Semi-strong form and Strong form of efficient markets. Weak form of efficient markets asserts that all the published information must be reflected in stock prices, semi-strong form holds that all the available information must be reflected into the stock prices, whereas strong form of efficient markets contents that all the published and unpublished information must be reflected into the stock prices. Though, the weak form and strong form of efficient markets do not have much practical relevance, semi-strong form of efficient markets has its implications on the real life world of financial markets. Semi-strong form of efficient markets postulates that all the investors in the market will discount the published information at the similar level. But real life conditions are quite different, different investors have different levels of understanding of the available information and it leads to estimation of different levels of stock prices by different investors. Anomalies in stock market are the imperfections in discounting the available information by the market participants. In the present study, a modest attempt has been made to examine the anomalies every investors to know for getting extra profits. Market anomalies can be great opportunities for investors. Anomalies should influence but not dictate a trading decision. Proper research of a company's financials is more important for long-term growth.

KEYWORDS : Market Anomalies, Efficient Market Hypothesis, Market Participants, Stock Market, Stock price.

INTRODUCTION

The stock market broadly refers to the collection of exchanges and other venues where the buying, selling, and issuance of shares of publicly held companies take place. Such financial activities are conducted through institutionalized formal exchanges (whether physical or electronic) or via over-the-counter (OTC) marketplaces that operate under a defined set of regulations.

While both the terms "stock market" and "stock exchange" are often used interchangeably, the latter term generally comprises a subset of the former. If one trades in the stock market, it means that they buy or sell shares on one (or more) of the stock exchange(s) that are part of the overall stock market. A given country or region may have one or more exchanges comprising their stock market. The leading U.S. stock exchanges include the New York Stock Exchange (NYSE) and the Nasdaq. These leading national exchanges, along with several other exchanges operating in the country, form the stock market of the United States.

This study examined the Efficient Market Hypothesis and Indian stock market anomalies that every investor to know for taking investment decisions and making extra profit. The paper has the structure of: objectives the study. The second paragraph is a short literature review. In the next part it shows the theory of Efficient Market Hypothesis and the forms of efficiency. It follows topic of stock market anomalies and how to deal the anomalies. Final part includes the conclusion.

OBJECTIVES OF THE STUDY

1. The main objective of the study is to examine the Efficient Market Hypothesis and its form.
2. The other objective of the study is to assists the investors to take investment decisions and earn extra profit by knowing and dealing of the Indian stock market anomalies.

LITRATURE REVIEW

Nageshwari and Selvam (2011) for the period 1st April 2000 to 31 March 2010 found that the day-of-the-week effect and monthly effect pattern did not appear to exist for the BSE Sensex during the period understudy. Research by Pandey and Prachetas (2012) is limited to those stocks whose derivatives are traded on the National Stock Exchange (NSE) as they are traded in large volumes. Their analysis concluded that the high risk-high return paradigm is a fallacy in the capital market and higher

average monthly rate of return for low volatility stocks. The finding negates the popularly held assumption of high risk-high return in the capital market and presence of the risk - return anomaly confirms the existence of inefficient or imperfect capital markets.

Mylonakis and Tserkezos (2008) examine the Athens Stock market (ASE) in the 1985-2001. They find that the mean returns during January are higher than in other months. The Baltic Stock Market seasonalities was studied by Norvaisiene, Stankeviciene and Lakstutiene (2015)They examine the "the daily log return indexes of Nasdaq OMX Tallinn, Nasdaq OMX Riga, and Nasdaq OMX Vilnius in Baltic stock exchange were analyzed for the period of 2003 –2014" (Norvaisiene et al., 2015: 468). Their findings show that: "The research of the month effect in Baltic stock markets evidenced that January effect and October effect occurred in Estonia.

The most successful months for investors in Lithuanian market were January, August and November as the stock return then were higher than in other months. Together with January, August and November effects, the October effect was established in Lithuania as seasonal trends of stock price decreases were observed in October" (Norvaisiene et al., 2015: 472-473).

Osborne (1962) documented this CAs in the U.S. stock market. Later the weekdays effect was analysed by numerous researchers: Cross, 1973; French, 1980; Gibbons and Hess, 1981; Lakonishok and Levi, 1982; Keim and Stambaugh, 1984; Jaffe and Westerfield, 1985;Jaffe, Westerfield and Ma, 1989; Wang, Li and Erickson, 1997; Bildik, 2004.

Ajmi et al. (2014) examine the links between the Islamic and global conventional stock markets, and between the Islamic stock market and several global economic and financial shocks. Their findings reveal evidence of significant linear and nonlinear causality between the Islamic and conventional stock markets but more strongly from the Islamic stock market to the other markets.

EFFICIENCT MARKET HYPOTHESIS

The famous (EMH) was introduced by Fama (1965) which claims that in an efficient market stock prices always fully reflect available information. If the stock markets are efficient, stock prices are supposed to follow random walk. The random walk hypothesis states that future prices are not predictable on the basis of past

prices, that is, stock price changes are unpredictable. The information contained in the past prices is fully and instantaneously reflected in current prices in an efficient market as argued by Fama (1965). Subsequent to the study by Fama (1965) a good number of researches have been conducted to examine the randomness of stock price behavior to conclude about the efficiency of a capital market. More recently one of the popular areas of research in finance literature is finding out a particular seasonality or pattern in stock returns which demonstrate the inefficiency of the market. Since the introduction of EMH by Fama (1965) which states that the expected return on a financial asset should be uniformly distributed across different units of time, researchers have documented several calendar anomalies in the stock returns such as January effect, Turn of the month effect and Day of the week effect or Monday effect, Holiday effect and so on. Stock market sometimes documents the presence of the excess returns. These patterns are known as Calendar Anomalies.

The documentation of anomalies in finance literature violates the weak form of market efficiency because equity prices are no longer follow random trend and can be predicted based on past behaviour. This in turn, facilitates market participants to prepare the trading strategies which could help them to earn abnormal returns on the basis of past performance of the stock market. Day-of-the-Week anomaly states that investors may devise a trading strategy of selling securities on Fridays and buying on Mondays in order to make excess profits. Considering economic issues, Prime Minister Mr. Narendra Modi launched "Make in India" movement on 25th September, 2014 to invite large business houses from around the world to invest and manufacture in India. It is expected that it will result in efficient utilization in a maximum extent of Indian natural resources, labour, money, technological and machinery across the country will be possible. Consequently, this step, will create big opportunities for the new generation, produce products and services of good quality. As a result, it will help to transform India as a self relient country.

FORMS OF STOCK MARKET EFFICIENCY:

Relevant information includes past information, publicly available information and private information. On the basis of relevant information efficient market is divided into three stages, weak form, semi strong form and strong form. In weak form of EMH, all the past information including past prices and returns is already reflected in

the current prices of stocks (Bodie et al. 2007).The assumption of weak form is consistent with random walk hypothesis i.e. stock prices move randomly, and price changes are independent of each other. So if the weak form holds, no one can predict the future on the basis of past information. And no one can beat the market by earning abnormal returns. Therefore, the technical analysis, in which analysts make the chart of past price movements of stocks to accurately predict future price changes, is of no use (Bodie et al. 2007). However, one can beat the market and get abnormal returns on the basis of fundamental analysis or on the basis of insider information. In the semi strong form, current stock prices reflect all publicly available information as well as past information. So no one can make extra profit on the basis of fundamental analysis (Bodie et al. 2007). However, one can beat the market by insider trading. In the strong form of market efficiency, all relevant information including past, public and private information is reflected in the current stock prices. So if the strong form persists, then no one can beat the market in any way, not even by insider trading (Brealey et al. 1999).

STOCK MARKET ANOMALIES

Semi-strong form of efficient market hypothesis contends that all the publicly available information is reflected in stock prices and hence there is no scope for abnormal returns to an investor. The only way to get more returns is to bear more risk, as there is positive relationship between return and risk. Put it otherwise, an investor cannot expect more return than which can be expected for the given level of risk.

However, the empirical findings provide more controversial results of the "semi-strong" form of market hypothesis. The fundamental question which need to be answered is what cause the real life market situation quite dynamic than what is originally provided in the theory. The researches in this direction have identified many imperfections in the market which can distort the investor in discounting the right information at right time. Such imperfections prevailing in the market are named after as "stock market anomalies". The empirical studies discovered many anomalies in the market like size effect, January effect (or tax selling hypothesis),day of the week effect, P/E ratio effect etc., Semi-strong form of efficient market hypothesis contends that all the publicly available information is reflected in stock prices and hence there is no scope for abnormal returns to an investor. The only way to get more returns is to bear more risk, as there is positive relationship between return and risk. Put it otherwise, an investor

cannot expect more return than which can be expected for the given level of risk.

In financial markets, any opportunity to earn excess profits undermines the assumptions of market efficiency, which states that prices already reflect all relevant information and so cannot be arbitraged.

JANUARY EFFECT

The January effect is a rather well-known anomaly. According to the January effect, stocks that underperformed in the fourth quarter of the prior year tend to outperform the markets in January. The reason for the January effect is so logical that it is almost hard to call it an anomaly. Investors will often look to jettison underperforming stocks late in the year so that they can use their losses to offset capital gains taxes (or to take the small deduction that the IRS allows if there is a net capital loss for the year). Many people call this event tax-loss harvesting.

As selling pressure is sometimes independent of the company's actual fundamentals or valuation, this "tax selling" can push these stocks to levels where they become attractive to buyers in January. Likewise, investors will often avoid buying underperforming stocks in the fourth quarter and wait until January to avoid getting caught up in the tax-loss selling. As a result, there is excess selling pressure before January and excess buying pressure after Jan. 1, leading to this effect.

Commonly referred to as "January Effect", "Turn-Of-The-Year" effect is the tendency of the stock market to rise between the last day of the last financial month and the end of the first week of the first month of the next financial year. In most of the developed countries financial year starts in January and ends in December which is the reason behind the common nomenclature of this anomaly as the January Effect. Returns are high in small firm stocks which have been pulled-down in the immediate past and these small stocks tend to outperform large stocks during the course of the first month of the year (Wachtel, 1942; Rozeff and Kenny, 1976; Keim, 1983; Banch and Chang, 1990).This is the reason why this effect is predominantly noticeable in stock indices having higher proportion of small firm listings."Other January Effect" is the assumption that the trend set in the first month of the year of ten indicates the future performance of the market in the following 11 months. A „positive January returns" means the market is on the road for a gainful year, and a „negative January returns" indicate weakness in the market through the following December (Cooper et.al.,2006).

September Effect

refers to historically weak stock market returns for the month of September. There is a statistical case for the September effect depending on the period analyzed, but much of the theory is anecdotal. It is generally believed that investors return from summer vacation in September ready to lock in gains as well as tax losses before the end of the year.

There is also a belief that individual investors liquidate stocks going into September to offset schooling costs for children. As with many other calendar effects, the September effect is considered a historical quirk in the data rather than an effect with any causal relationship.

Days of the Week Anomalies

Efficient market supporters hate the "Days of the Week" anomaly because it not only appears to be true, but it also makes no sense. Research has shown that stocks tend to move more on Fridays than Mondays and that there is a bias toward positive market performance on Fridays. It is not a huge discrepancy, but it is a persistent one.

The Monday effect is a theory which states that returns on the stock market on Mondays will follow the prevailing trend from the previous Friday. Therefore, if the market was up on Friday, it should continue through the weekend and, come Monday, resume its rise. The Monday effect is also known as the "weekend effect."

On a fundamental level, there is no particular reason that this should be true. Some psychological factors could be at work. Perhaps an end-of-week optimism permeates the market as traders and investors look forward to the weekend. Alternatively, perhaps the weekend gives investors a chance to catch up on their reading, stew and fret about the market, and develop pessimism going into Monday.

Day of the week anomaly means regular pattern in stock market return across all week days (Islam and Watanapalachaikul 2005). Day of the week anomaly indicates that average returns and volatility of all days are different from each other. The presence of day of the week anamoly provides abnormal return on a particular day of the week. The term “day of the week effect” in stock market was documented in early nineties (Fields 1931; Kelly 1930).

Superstitious Indicators:

Aside from calendar anomalies, there are some non-market signals that some people believe will accurately indicate the direction of the market. Here is a short list of superstitious market indicators:

- **The Super Bowl Indicator**:

When a team from the old American Football League wins the game, the market will close lower for the year. When an old National Football League team wins, the market will end the year higher. Silly as it may seem, the Super Bowl indicator was correct almost three-quarters of the time over a 53-year period ending in 2021. However, the indicator has one limitation: It contains no allowance for an expansion-team victory!

- **The Hemline Indicator**:

The market rises and falls with the length of skirts. Sometimes this indicator is referred to as the "bare knees, bull market" theory. To its merit, the hemline indicator was accurate in 1987, when designers switched from miniskirts to floor-length skirts just before the market crashed. A similar change also took place in 1929, but many argue as to which came first, the crash or the hemline shifts.

- **The Aspirin Indicator**:

Stock prices and aspirin production are inversely related. This indicator suggests that when the market is rising, fewer people need aspirin to heal market-induced headaches. Lower aspirin sales should indicate a rising market.

How the Anomalies Should Be Dealt

The anomalies large enough to cause the hindrance in the normal research should be resolved and if its not that larger, then it could be left (Ball 1978) and Kleidon (1987) says that there is the need of the change of disciplinary foundation for the explanation of the anomalies. Kuhn (1977) perceives anomalies as beneficial for the finance itself and says that though most of the times the anomalies do not result in the discovery of something new but they do

break the existing paradigm thus causing in the emergence of the new theories. Another important aspect discussed by the Kuhn (1970) is about the replacement of the paradigm. In science you need to have another paradigm to replace the existing one and if you don‟t have then rejecting the existing paradigm is rejecting the science itself. There are hundreds of the anomalies existing but we don‟t regard them until we have a better one to replace EMH/CAPM (Lakatos 1970). In short we can code the Fama (1998) argument that until and unless behavioral finance do not prove itself as a better theory from the EMH/CAPM, the presence of anomalies can‟t shake the pillar

of efficient market hypothesis , no matter how many of them are being discovered.

CONCLUSION

This paper has brought to light the anomalies recorded in the Indian stock market and has also explained the different types of anomalies in the market. This study also explained that how to deal with the stock market anomalies. Having known the anomalies in the stock market the investors could now be aware of them and recognize those patterns which deviate from rationality. The anomalies are due to the emotions and anxiety expressed by the players of the market. Trading decisions should be influenced by anomalies, not dictated by them. Long-term growth necessitates thorough examination of a company's financials.

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CONVENTIONAL, MICROWAVE COMPARATIVE STUDY OF SCHIFF BASES AND ITS APPLICATIONS

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ABSTRACT :

In the present study, a new Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine and Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine were synthesised by Mannich condensation reaction by conevtional as well as microwave method. The synthesised compound was characterised by UV, FT-IR and H^1-NMR spectral studies. Compounds were evaluated for antibacterial and antifungal activity.

Keywords: Microwave, UV , FT-IR H-NMR spectra.

INTRODUCTION

Microwave assisted synthesis

Microwave synthesis is the major breakthrough in the synthetic organic chemistry whereas the conventional heating is the inefficient and time-consuming. Microwave synthesis is the new lead which is being used as the source of heating in the organic synthetic reaction. The present article will give an idea about microwave assisted synthesis. Microwave Synthesis – an introduction The great invention of burner was done in organic chemistry 1899 by Robert Bunsen. This invention was so useful that it lead to provide heat in a much focused manner required to carry out any chemical synthesis (Anant Prakash etal.,1896) . But this Bunsen burner was later superseded by microwave energy. Since the first published reports on the use of microwave irradiation to carry out organic chemical transformations by the groups of Gedye and Giguere/Majetich in 1986. Microwave heating has been shown to dramatically reduce reaction times, increase product yields and enhance product purities by reducing unwanted side reactions compared to conventional heating methods.

Advantages

- Uniform heating occurs throughout the material
- Process speed is increased
- High efficiency of heating
- Reduction in unwanted side reaction
- Purity in final product
- Improve reproducibility

- Environmental heat loss can be avoided

Disadvantages

- Heat force control is difficult.
- Closed container is difficult because it could burst
- In-situ monitoring
- Expensive setup

Microwave heating will result in instantaneous localized heating of the reaction mixture. This is due to the mechanism that microwave directly couple up with the molecules which are present in the reaction mixture.(P.Mishra.,etal.2005) This process of heating is not dependent on the thermal conductivity of the reaction vessel.

MATERIALS AND METHODS

Instruments

Melting points (mp) were determined using Boetieus micro heating table and are uncorrected. A double beam UV–Visible spectrophotometer, Jasco–V 630 is used for absorption measurements using 1cm path length cells. FT IR (KBr,cm-1) spectra were obtained on Shimadzu-8201 spectrophotometer. 1H NMR spectra were recorded on Bruker AMX-400 (400 MHz) spectrometer using TMS as an internal reference (Chemical shifts in δ, ppm).

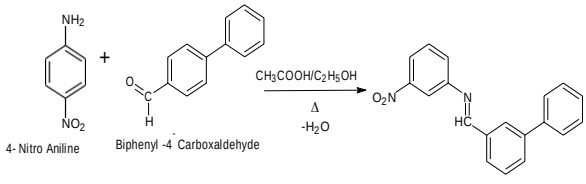
Synthesis of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine

Conventional method

A mixture of respective anilines and 10 mmol biphenyl -4-carboxaldehyde was taken in a 250ml round-bottomed flask mild acidic condition and mixed well. It was refluxed for 2-3 hours, After completion of the reaction, the reaction mixture was poured into ice water then the precipitate was filtered off, the crude was dried in air, recrystallized by ethanol.

Microwave method

A mixture of respective anilines and 10 mmol biphenyl -4-carboxaldehyde was taken in a 50 mL beaker mild acidic condition and mixed well. The mixture was irradiated in a microwave oven at a power of 160 W for the specified time . The reaction was monitored by thin layer chromatography (TLC) and spots were visualized in uv chamber. After completion of the reaction, the reaction mixture was poured into ice water. The pale green solid obtained was filtered, washed, dried and recrystalised from ethanol.



Scheme.1.Synthesis of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine

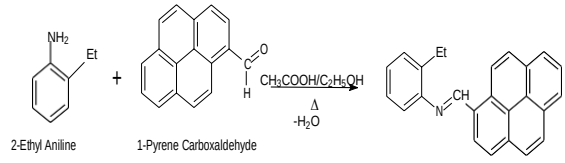
Synthesis of Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine

Conventional method

A mixture of respective anilines and 10 mmol pyrene -4-carboxaldehyde was taken in a 250ml round-bottomed flask mild acidic condition and mixed well. It was refluxed for 2-3 hours, After completion of the reaction, the reaction mixture was poured into ice water then the precipitate was filtered off, the crude was dried in air, recrystallized by ethanol.

Microwave method

A mixture of respective anilines and 10 mmol Pyrene -4-carboxaldehyde was taken in a 50 mL beaker mild acidic condition and mixed well. The mixture was irradiated in a microwave oven at a power of 160 W for the specified time . The reaction was monitored by thin layer chromatography (TLC) and spots were visualized in uv chamber. After completion of the reaction, the reaction mixture was poured into ice water. The yellow solid obtained was filtered, washed, dried and recrystalised from ethanol.



Scheme.2.Synthesis of Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine

Antibacterial activity

The disc diffusion method (Bauer et al., 1966) was used to screen the antimicrobial activity. Stock cultures were maintained at 4ºC on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37 ^{o} C and 25 ^{o} C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×106 colony forming units (CFU/ml) for bacteria. The Muller Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and allowed to dry for 5 minutes. The concentration of sample at 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5

minutes and the plates were kept for incubation at 37 º C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Antifungal activity

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 10 5 CFU/ml. The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with the sample and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37 º C for 72 h. The diameters of zone of inhibition observed were measured.it transparent ruler in millimeter.

RESULT AND DISCUSSION

Synthesis of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine and Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine were confirmed by UV, IR and H^1 NMR spectral analysis.

THIN LAYER CHROMATOGRAPHY

The progress of reaction monitoring with TLC method showed that target compound **A_1**&**A_2**has yellow & pale green spot when observed without UV lights and dark colour with Uv lights 254nm. This spot are certainly have a difference with spot of reactants that colours were observed without Uv lamp.

UV SPECTRAL STUDIES

A double beam UV–Visible spectrophotometer, Jasco–V 630 is used for absorption measurements using 1cm path length cells.

UV-VIS spectra of newly prepared compounds were recorded in ethanol. Both the compounds contain C=N group and the possible transitions are n-π* and π-π*.

CONCLUSION

In this article, we are reporting a new microwave procedure for the rapid and efficient synthesis of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine and Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine has been devoleped. The microwave heating effectively reduced reaction time from 2-3 hours to 5-10 minutes. By using microwave irradiation for heating, all the compounds were prepared in yields that

were appreciably morethan the conventional methods. From the result, it was observed that all the compounds promising Antibacterial activity against Gram positive and Gram negative bacterias, the synthesised compounds are active against all the pathogens to posses an important role in the antifungal activity of the compounds. The synthesised compound was characterised by UV , FT-IR H-NMR spectrum. The experiment could be conducted in much shorter duration by new microwave methods. Hence application of microwave technique for synthesis of title compounds with objective to reduce reaction time and increase the yield was explored.

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Table:1 Physical character of synthesized product

Physical character	**Compound A_1**	**Compound A_2**
Physical form	Solid	Solid
Colour	Pale Green	Yellow
Reaction time (Conventional / MW)	2 Hours	2 Hours
	5-10 minutes	5-10 minutes
Percentage of yield (Conventional / MW)	45%	50%
	85%	93%
Melting point	178°C	102°C
Solubility	$CHCl_3$,DMSO, C2H5OH	$CHCl_3$,DMSO, C_2H_5OH

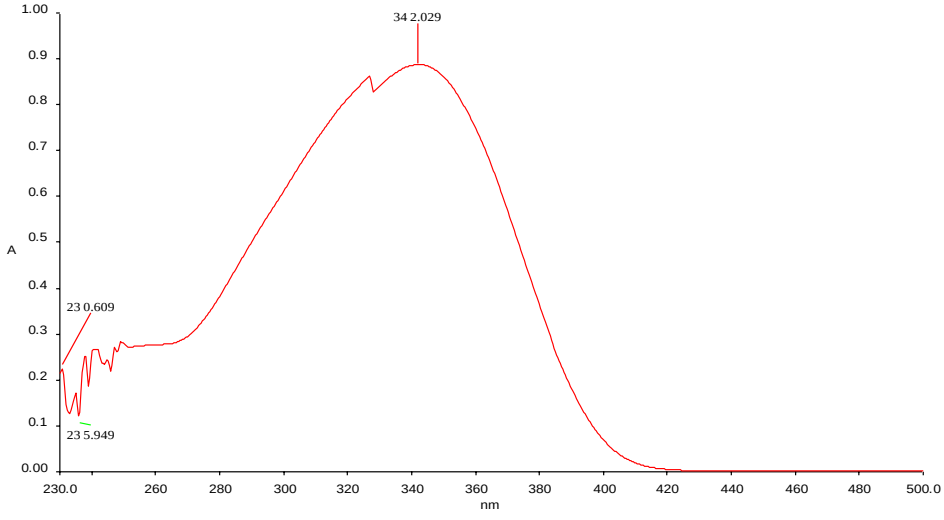

Fig.1.UV Spectrum of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine

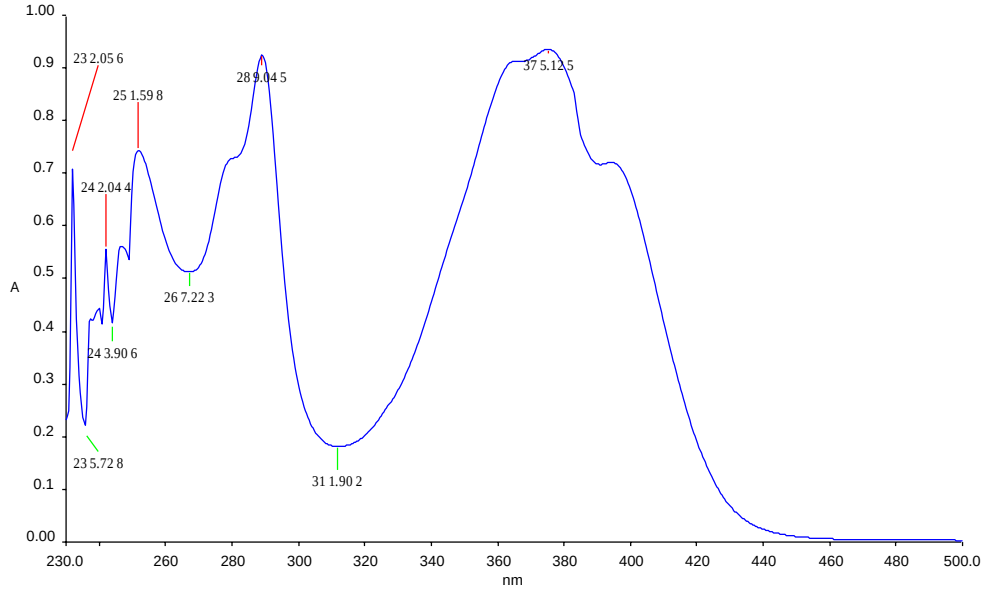

Fig.2.UV Spectrum of Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine.

Λ_{max} of the synthesized compounds are given below:

Compound	π-π* (nm)	n-π* (nm)
Compound- A_1	**230.609nm**	**342.029nm**
Compound-A_2	**232.056nm**	**375.125nm**

IR SPECTRAL STUDIES

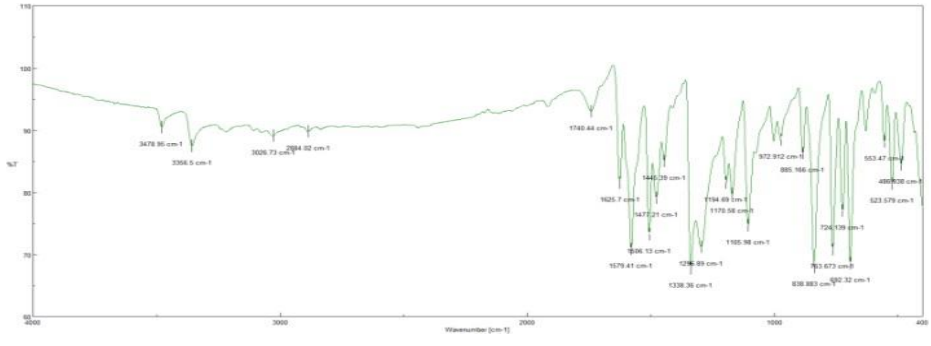

Fig.3. IR Spectrum of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine

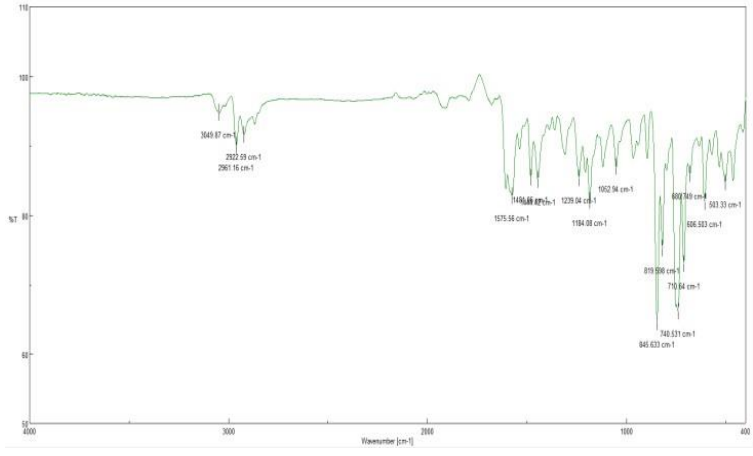

Fig.4. IR Spectrum of Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine

IR Spectra functional group frequency are given below

Compond	(C=C) aromatic	(C-H) aromatic	C=H(-CH_2) aliphatic asym	υ (C=N)
Compond- A_1	1625.7cm^{-1}	2884.02cm^{-1}	3026.73cm^{-1}	1579.41cm^{-1}
Compond- A_2	1680.74cm^{-1}	2961.16cm^{-1}	3049.87cm^{-1}	1575.56cm^{-1}

NMR SPECTRAL STUDIES

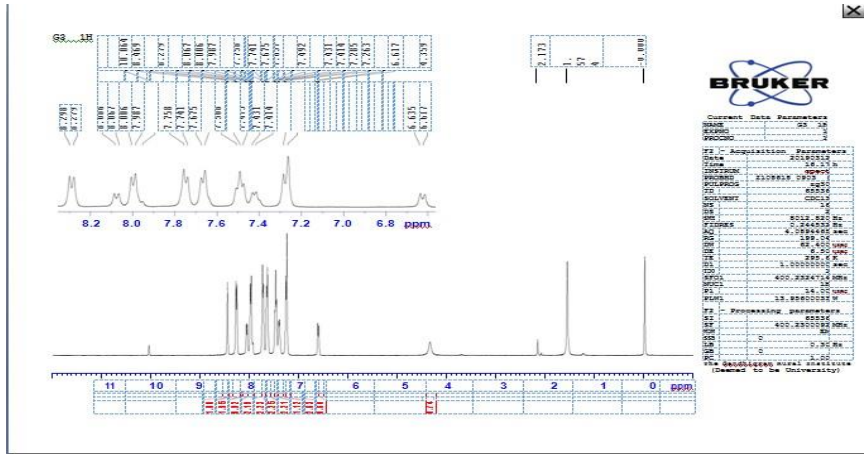

Fig.5. NMR Spectrum of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine

The synthesized Biphenyl-4-ylmethylene-(-4-nitro phenyl)-amine characterized by the presence of multiplets around 6.8 to 8.2 ppm. The siglet at 2.173 ppm is due to the N-H proton and the triplet at 7.414 ppm is due to C-H proton.

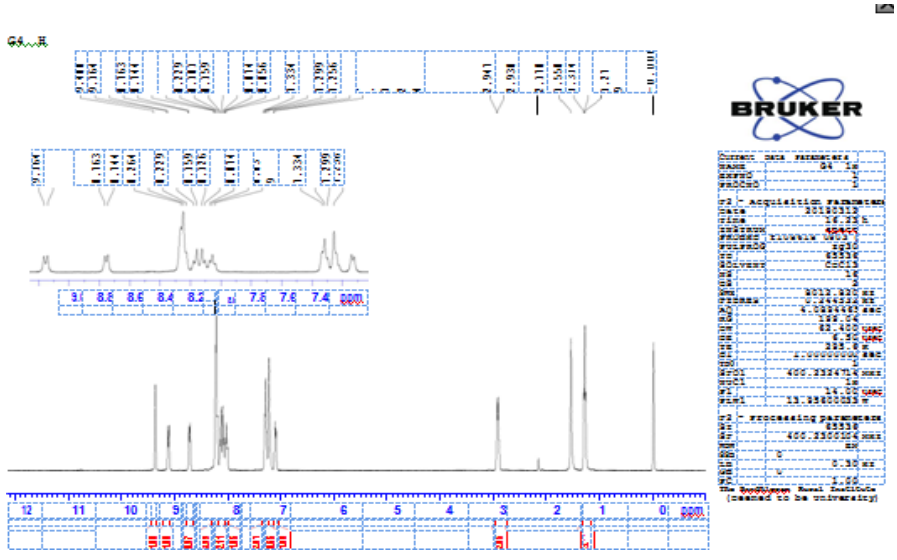

Fig.6. NMR Spectrum of Pyrene-4 ylmethylene-(2-ethyl-phenyl)-amine

The synthesized of Pyrene-4-ylmethylene-(-2- ethyl- phenyl)-amine characterised by the presence of multiplets around 7.4 to 9.0 ppm. The siglet at 1.296 ppm is due to N-H proton. The triplet at 8.104 ppm is due to C-H proton.

Antibacterial activity

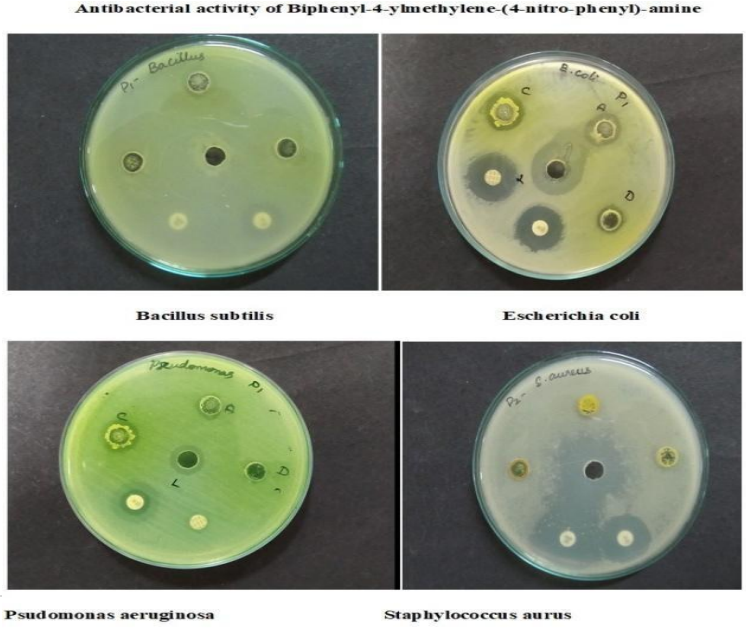

Fig.7.Antibacterial activity of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine

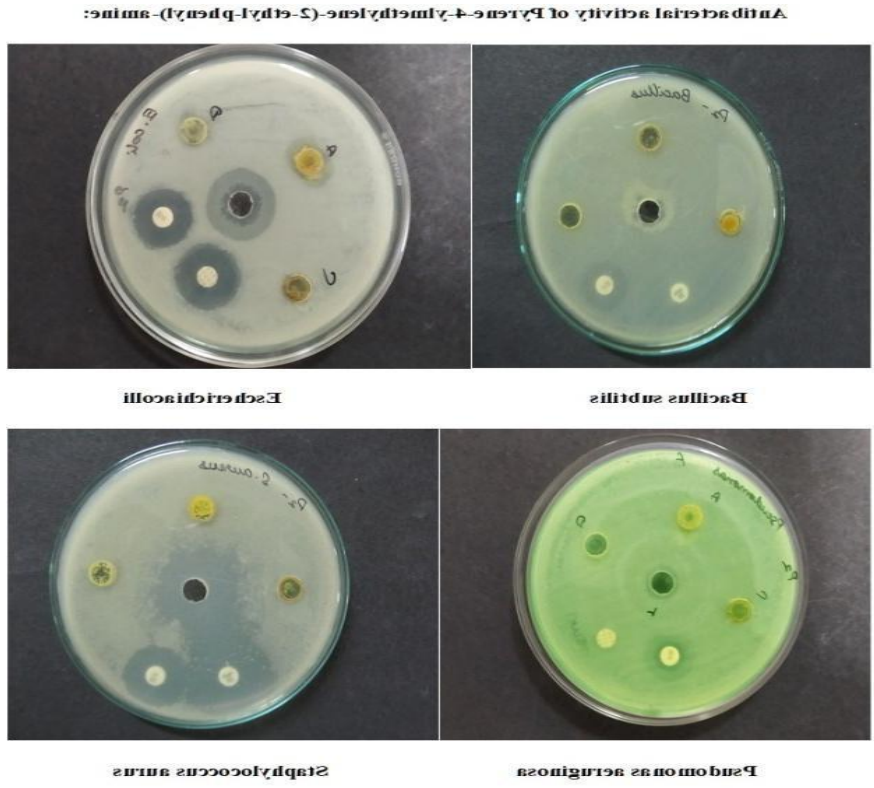

Fig.8. Antibacterial activity of Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine

Anti fungal activity

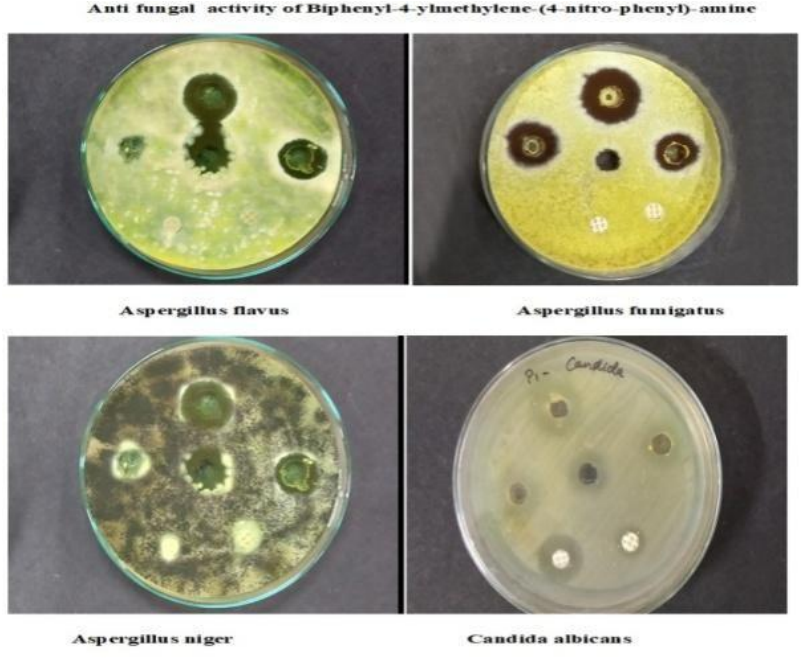

fig.9.Anti fungal activity of Biphenyl-4-ylmethylene-(4-nitro-phenyl)-amine

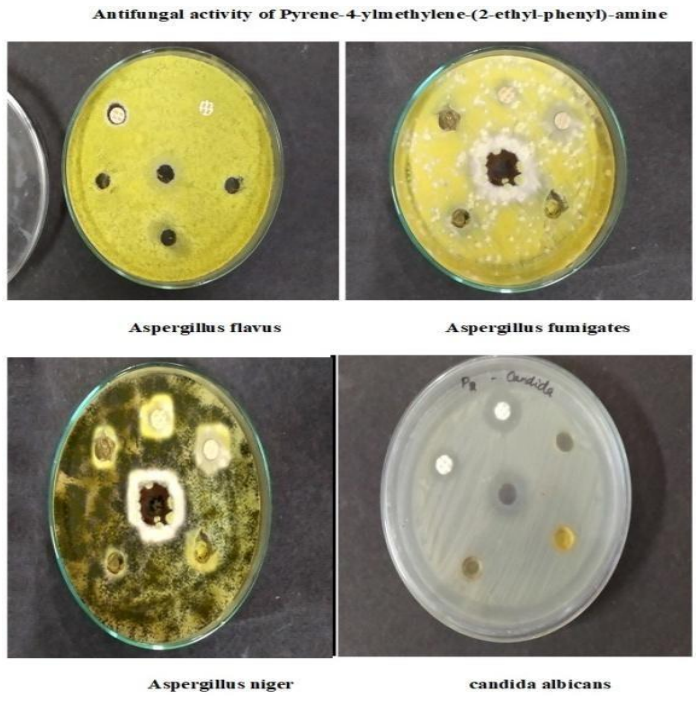

Fig.10.Antifungal activity of Pyrene-4-ylmethylene-(2-ethyl-phenyl)-amine

EVALUATION OF *STREPTOMYCES* PROBIOTIC ACTINOBACTERIAL ISOLATE FROM THESOIL WITH PRODUCTION OF BACTERIOCIN

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ABSTRACT

A probiotic actinobacterial isolate from the soil was isolated and screened for antibacterial activity by cross streak method and spot agar test and by well diffusion assay, bacteriocin activity for the actinobacterial isolates having antibacterial activity. Characterization and identification of the potent bacteriocin producing actinobacterial isolate AB3 were evaluated.

KEYWORDS:

Probiotic, Antimicrobial compound, Bacteriocin, Actinobacteria

INTRODUCTION

Novel antimicrobial compounds are increasingly important in the food, agriculture and medical fields due to decreasing efficacies of current antimicrobial treatments. Bacteriocins are ribosomally-synthesised antimicrobial peptides produced by bacteria which can target another bacterium of the same species (narrow spectrum) or bacteria of other species/genera (broad spectrum) (Hegarty *et al.*, 2016). Bacteriocin producers are self-protected through the production of specific immunity proteins, and as bacteriocins are gene encoded, they can be genetically modified.

Bacteriocins produced by Gram positive bacteria have been grouped according to their primary structure into class I (post-translationally modified bacteriocins) and class II (unmodified or cyclic bacteriocins) (Cotter *et al.*, 2005). Class II is split into several subgroups, including the class IId bacteriocins, which are a heterogenous group of linear, unmodified, non-pediocin like peptides (Iwatani *et al.,* 2011).

Defensins are antimicrobial peptides ubiquitous among eukaryotes which play a role in innate immunity but have also been found to act as signalling peptides, toxins, enzyme inhibitors, abiotic stress responders, and to have anti-cancer properties.

Defensins are small (<10 kDa), cysteine rich (forming three to six disulphide bonds) peptides with low amino acid identity and the two superfamilies are thought to have evolved convergently (Shafee *et al.,* 2016). Only two expressed defensin-like bacteriocins have been described; the laterosporulins have been previously identified among prokaryotes and contain disulphide bonds in positions homologous to eukaryotic defensins (Singh *et al.,* 2002; Baindara *et al.,* 2016). Other disulphide bond-containing bacteriocins, such as bactofencin have been compared with eukaryotic defensins due to their highly cationic 61nature (O'Shea *et al.,* 2013; O' Connor *et al.,* 2018).

Laterosporulin, and its homolog Laterosporulin10 are class IId bacteriocins produced by *Brevibacillus* spp. which have been described as broad-spectrum antimicrobials against both Gram negative and Gram positive bacteria. The two peptides are 5.6 kDa and 6.0 kDa and share only 57.6 % amino acid sequence identity but have conserved cysteines which are characteristic of eukaryotic defensins (Baindara *et al.,* 2016).

The phylum Actinobacteria is one of the major phyla of the domain Bacteria and contains Gram-positive filamentous bacteria with a high G+C DNA content and different morphological, physiological and metabolic characteristics (Barka *et al.,* 2016; Gao and Gupta, 2012). Non spore-forming facultative or obligate anaerobes that belong to the *Actinomycetaceae* family within the phylum Actinobacteria (Bergey *et al.,* 2012).

Most bacteria belonging to this phylum are free-living micro-organisms that are ubiquitously found in both aquatic and terrestrial ecosystems and include the following: (i) several pathogens, such as species of *Corynebacterium, Mycobacterium, Nocardia, Propionibacterium* and *Tropheryma*; (ii) soil inhabitants, such as *Micromonospora* spp. and

Streptomyces spp.; (iii) plant commensals, such as *Frankia* spp.; and (iv) astrointestinal commensals, such as *Bifidobacterium* spp. (Barka *et al.,* 2016).

Their diversity ensures one of the main features of this group: versatility in the production of biologically active compounds, including antimicrobial substances (Nett *et al.,* 2009). Until 2010, about 34 000 microbial bioactive compounds had already been reported and close to 40% of them are produced by Actinobacteria, especially *Streptomyces* spp., which produce nearly 80% of the actinobacterial compounds.

Approximately 10000 actinobacterial substances exhibit antimicrobial and/or antitumour activity (Berdy, 2012). Moreover, most of the antimicrobials already used in human and veterinary medicine or agriculture are either natural or semisynthetic derivatives of actinobacterial and fungal products (Baltz, 2007). These data highlight the importance and the potential practical application of the actinobacterial antimicrobial substances, notably to control pathogenic microorganisms. The actinobacterial antimicrobials belong to many different groups, including conventional antibiotics and bacteriocins (Barka *et al.,* 2016). In humans, a number of species are known colonisers of hard surfaces in the oral cavity where they play a key role in plaque biofilm formation (Segata *et al.,* 2012; Mager *et al.,* 2003).

They have been identified as core members of the oral bacteriome, present in moderate abundance (>0.1 - >2.0%) among geographically-diverse populations (Takeshita *et al.*, 2016; Li *et al.,* 2014; Peterson *et al.,* 2013; Ribeiro *et al.,* 2017). *Actinomyces* spp. have been implicated in oral health as being associated in greater abundance in individuals with dental caries, one of the most prevalent chronic oral diseases worldwide (Ribeiro *et al.,* 2017). Most characterized strains are clinical isolates of human origin, while some opportunistically pathogenic species such as *Actinomyces israelii* and *Actinomycesgerecseriae* are known to cause the uncommon infectious disease actinomycosis (Boyanova *et al.,* 2015). Though *Actinomyces* spp. are abundant in the oral cavity, little is known about their presence in the gut, probably due to their low abundance (<0.1%) (Segata *et al.,* 2012). Many *Actinomyces* spp. have been isolated from faecal material and from the gastrointestinal tract of different animals, indicating a propensity for gastric transit survival and their presence has also been noted in the urogenital tract (Meng *et al.,* 2018).

MATERIALS AND METHODS

Isolation of Actinobacteria from soil

Collection and preparation of soil sample

Top 4 cm soil is considered a good source of microorganisms as most activities take place in this region. Soil Samples were collected from the garden area. Soil sample (approx. 10g) were collected using clean, dry and sterile polythene bags along with sterile spatula and were marked properly. Varied soil with regards to moisture, texture and content was selected. Samples were stored at 40°C until pretreatment. Microorganisms other than actinomycetes are degraded because of pretreatment (Ardhi *et al.,* 2019).

Isolation of Actinomycetes

The pretreated soil suspensions were spread over starch casein agar followed by incubation at 35^0c up to 5 days. Dilution 10-7 gave well isolated white powdery growth on this agar surface a characteristic feature of actinomycetes and content was selected. Samples were stored at 40°C until pretreatment. Microorganisms other than actinomycetes are degraded because of pretreatment

Antibiogram - Primary screening

Cross streak method

Actinomycete isolates were screened for antagonistic activity against bacterial pathogens by cross streak method. In cross streak method, Actinomycete isolates were inoculated into modified nutrient glucose agar (MNGA) as a straight line. After incubation at 28^0C for 5 days, 18 hours old test bacterial pathogens were inoculated at right angle to the original streak of the actinobacterial strain TK2. Inhibition of bacterial pathogens was observed after incubation at 37^0C for 24 hours. (Baniya *et al.,* 2018)

Production of Bacteriocin

The isolated strain was propagated in MRS broth (1000 ml) seeded with 10% inoculum (10 CFU/ml) of overnight culture and incubated for 48 h at 150 rpm at 37°C. After incubation, the whole broth was centrifuged at 10,000×g for 15 min and the cell-free supernatant was used as crude bacteriocin (Li, 2018).

Partial purification of bacteriocin

The cell-free culture supernatant (crude bacteriocin) was saturated with 70% ammonium sulfate and stored at 4°C to precipitate out the proteins. The pellet was collected after centrifugation at 10,000×g at 4°C for 30 min. The pellet was dissolved in a phosphate buffer (0.1M, pH 7.0) and dialyzed against the same buffer at 4°C overnight. The dialyzed protein was applied to a Sephadex G-100 column (1.6 × 36 cm) pre- equilibrated with phosphate buffer (pH 7.0). The flow rate was adjusted to 24 ml/h and fractions (1 ml each) were collected. The fractions showing high bacteriocin activity were pooled and concentrated in a lyophilizer. (Li, 2018).

Determination of antibacterial activity of crude and partially purified bacteriocin

To determine the antimicrobial potential of the crude and partially purified bacteriocin, an agar well diffusion assay (AWDA) was performed. All indicator test strains (30 strains) used in this assay and the collection numbers of these strains are shown Table 1. Indicator test strains were first cultivated in appropriate broth media). Final cell density of each indicator strain was adjusted to 10^6 CFU/mL using McFarland standard no. 1 (Merck). Then, 100 µL of these cultures was inoculated into molten soft nutrient agar tubes (nutrient broth+agar (0.7% w/v)). After homogenizing, the inoculated soft agar was rapidly poured onto pre-prepared plates containing nutrient agar.

After solidification, the soft agar plates were maintained at 4°C for 1 h, and then, wells with 8-mm diameters were made with a sterile cork borer. Then, crude and partially purified bacteriocin was added into each well (10, 20 and 30 µL/well), and the plates were incubated for 24 h at 37℃ for *Escherichia coli*, *Klebsiella*, *Staphylococcus enterica*, *Staphylococcus aureus*, *Enterococcus* and *Clostridium sps*. Then diameter of the inhibition zone around the well was measured (including well diameter). In this study, all antimicrobial activity measurements were made in duplicate and repeated at least twice.(Abanoz & Kunduhoglu., 2018)

Molecular weight determination of protein by SDS-PAGE

The molecular weight of the bacteriocin was determined by glycine SDS-PAGE with 5% stacking gel and 12% separating gel using the molecular weight standard BSA (Merck). The gel was stained with Coomassie blue R-250 and washed at room temperature with a solution of acetic acid 5% to remove excess stains.

Characterization and Identification

Biochemical characterization of actinomycetes

Identification of isolates was based on cultural, morphological and biochemical characteristics. Motility has been performed according to the hanging drop method. The standard biochemical tests such as catalase, oxidase and fermentation of various sugars, methyl red reaction, Voges Proskauer test and citrate utilization on Simmon's citrate agar was performed. Further the enzymatic studies of these isolates have been studied (Mulyawati *et al.,* 2019).

Physiological characterization- Enzymatic study of isolates

The screened isolates were studied for the production of amylase enzymes.

Amylase Production Test

Starch Agar Medium containing soluble starch as carbon source was prepared and by the method of (Al-Dhabi *et al.,* 2019). The isolated actinomycetes were screened for the production of amylase. For amylase screening, 1% soluble starch was incorporated with minimal medium and incubated at 28 °C for 5 days. Then Gram's iodine was flooded and a clear distinct zone around actinomycetes indicates amylase production.

Lipase activity of the screened isolates

In this assay, 10 µL of actinomycetes culture isolates were plated onto tributyrin agar medium supplemented with coconut oil in the concentration of 5% v/v and the plates were incubated at 37°C for 48 h. Then, the zones of lysis around the colonies were observed.

Cellulase activity of the screened isolates

The medium used for the screening ofcellulase activity is carboxymethylcellulose (CMC). The actinomycetes strains were streaked for the Congo red test. Inoculation was carried out by using a platinum/ Nichrome needle to transfer the actinomycetes culture to the center of the plates containing the CMC medium. The inoculated plates were incubated for 96 h at 30°C and the growth of the microorganism was measured by the diameter of the colony. A 10 mL aliquot of Congo red dye (2.5 $g \cdot L^{-1}$) was then added to each plate. After 15 min, the solution was discarded and the cultures were washed with 10 mL of 1 $mol \cdot L^{-1}$ NaCl. Cellulase production was indicated by the appearance of a pale halo with orange edges, indicative of areas of hydrolysis.

Protease activity of the screened isolates

The actinomycetes strains were spread onto skimmed milk agar plates containing beef extract (3 g/L), peptone (10 g/L), NaCl (10 g/L), skimmed milk powder (10 g/L), and 18 g/L agar, with pH adjusted to 7. Plates were incubated 24 h at 37°C. It was confirmed that the screened bacteria could produce protease when the clear proteolytic zone was found around the colonies (Cui *et al.*, 2015).

RESULTS AND DISCUSSION

Antibiogram of Test isolates

The test isolates used in the study were clinical pathogens such as *E. coli, K. pneumonia, Salmonella enterica, Staphylococcus aureus, Clostridium sp, Enterococcus* which have clinical importance. The antibiogram study of the test isolates showed that all the test isolates were resistant to Tetracycline except *Salmonella enterica. Salmonella enterica* showed10 mm against Tetracycline.For Erythromycin, *Escherichia coli* and *Clostridium sp*, showed resistance whereas *Klebsiella pneumoniae* showed 10mm, *Salmonella enterica*, showed 21 mm, *Staphylococcus aureus* showed 20 mm,*Enterococcus* showed 9mm. For Nitrofurantoin, *Salmonella enterica* showed resistance whereas *Escherichia coli* showed 13 mm, *Klebsiella pneumoniae* showed 14 mm, *Staphylococcus aureus* showed 8 mm, *Clostridium sp* showed 12 mm, and *Enterococcus* showed 14 mm. For Cifroflaxacin, *Klebsiella pneumoniae, Salmonella enterica* and *Clostridium sp* showed resistance whereas *Escherichia coli* showed 8 mm, *Staphylococcus aureus* showed 9 mm and *Enterococcus* showed 10mm.

Table 1. Antibiogram of Test isolates

S. no	**Antibiotic discs**	***E. coli***	***K. pneumonia***	***Salmonella enterica***	***Staphylococcus aureus***	***Clostridium sp***	***Enterococcus***
1	Tetracycline	-	-	10	-	-	-
2	Erythromycin	-	10	21	20	-	9
3	Nitrofurantion	13	14	-	8	12	14
4	Cifroflaxacin	8	-	-	9	-	10

Antibacterial activity of actinobacterial isolates by Cross streak method

The isolated actinomycetes cultures were examined for the antibacterial activity by cross streak method. In that analysis, Isolate AB1 showed 12 mm for *Escherichia coli*, showed 14 mm for *Klebsiella pneumonia*, showed 05 mm for *Salmonella enterica*, showed 09 mm for *Staphylococcus aureus*, showed 11 mm for *Clostridium sp, and* showed 10 mm for *Enterococcus.*

Isolate AB2 showed 15 mm for *Clostridium sp, and* showed resistance for the remaining test isolates.

Isolate AB3 showed 28 mm for*Escherichia coli*, showed 25 mm for *Klebsiella pneumonia*, showed 12 mm for *Salmonella enterica*, showed 15 mm for *Staphylococcus aureus*, showed 22 mm for *Clostridium sp, and* showed 18 mm for *Enterococcus.*

Isolate AB4 showed 12 mm for *E. coli*, showed 14 mm for *Klebsiella pneumonia*, showed 07 mm for *Salmonella enterica*, showed 10 mm for *Staphylococcus aureus*, showed 11 mm for *Clostridium sp, and* showed 12 mm for *Enterococcus.*

Table 2. Antibacterial activity of actinobacterial isolates by Cross streak method

S. No.	Isolate Code	Zone of inhibition (mm)					
		Escherichia coli	***Klebsiella***	***Salmonella***	***Staphylococcus aureus***	***Clostridium***	***Enterococcus***
1.	AB1	12	14	5	9	11	10
2.	AB2	-	-	-	-	15	-
3.	AB3	28	25	12	15	22	18
4.	AB4	12	14	7	10	11	12

Antibacterial activity of actinobacterial isolates by Spot agar assay

In Spot agar test,the isolated actinomycetes cultures were examined for the antibacterial activity. In that analysis, Isolate AB1 showed 31 mm for *E. coli*, showed 34 mm for *K. pneumonia*, showed 27 mm for *Salmonella enterica*, showed 29 mm for *Staphylococcus aureus*,.

Isolate AB2 showed 31 mm for *E. coli*, 32 mm for *K. pneumonia*, 26 mm for *Salmonella enterica*, 27 mm for *Staphylococcus aureus.*

Isolate AB3 showed 36 mm for *E. coli*, 41 mm for *K. pneumonia*, 24 mm for *Salmonella enterica*, 34 mm for *Staphylococcus aureus.*

Isolate AB4 showed 35 mm for *E. coli*, 33 mm for *K. pneumonia*, 25 mm for *Salmonella enterica*, 28 mm for *Staphylococcus aureus.*

Table 3. Antibacterial activity of Actinobacterial isolates by Spot agar assay

S. No.	Isolate Code	Zone of inhibition (mm)			
		Escherichia coli	***Salmonella***	***Staphylococcus aureus***	***Klebsiella***
1.	AB1	31	27	29	34
2.	AB2	31	26	27	32
3	AB3	36	24	34	41
4	AB4	35	25	28	33

Production and partial purification of bacteriocin by Ammonium sulfate precipitation

In purification, bacteriocin from culture supernatant was concentrated by ammonium sulfate precipitation followed by column chromatography. The bacteriocin from actinobacterial culture broth isolate AB 3 was purified to 4.74 fold with 55.38 U/mg of specific activity with the yield of 28.92%.

Weight determination of protein by SDS-PAGE

The molecular weight of the purified bacteriocin of isolate AB3 was estimated as 21 kDa (**Figure 1**) by SDS-PAGE gel electrophoresis. Single protein band was observed when stained with Coomassie brilliant blue and it clearly indicated the purity of the protein.

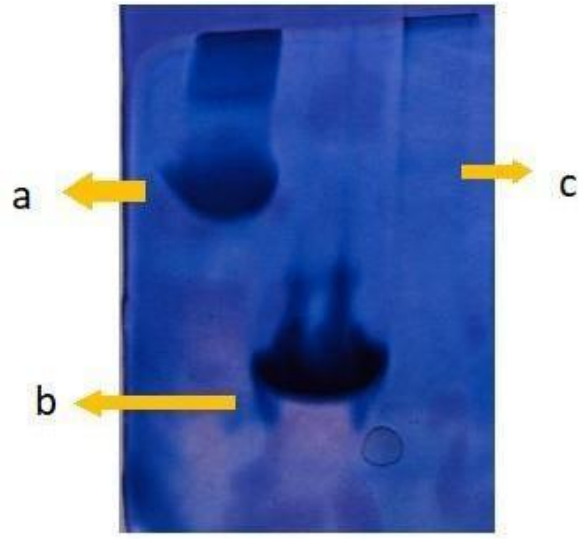

Fig 1. Molecular weight determination of protein by SDS-PAGE *a. Bovine serum albumin, b. Lysozyme, c. Partially purified bacteriocin of Actinobacterial Isolate AB3*

Antibacterial activity of crude bacteriocin of Isolate AB3

The antibacterial activity of the crude bacteriocin of Isolate AB 3 was evaluated. The AB 3 Isolate showed no zone of inhibition on 10 µl concentration for all the test isolates but showed 7 mm for *Escherichia coli*, 11 mm for *Staphylococcus enterica*, 8 mm for *Staphylococcus aureus* and *Enterococcus* at 20 µl. But in 30 µl concentration, *Staphylococcus aureus* showed 13 mm zone of inhibition, and lowest zone of inhibition of 8 mm for *Escherichia coli* and *Clostridium sps. Staphylococcus enterica* showed 12 mm, *Klebsiella* showed 10 mmand *Enterococcus* showed 10 mm.

Table 4. Antibacterial activity of crude bacteriocin of Isolate AB3

S. No.	Name of the Clinical pathogen	CRUDE BACTERIOCIN OF ISOLATEAB3		
		10 µl	20 µl	30 µl
1.	*Escherichia coli*	-	7	8
2	*Klebsiella*	-	-	10
2	*Staphylococcus*	-	11	12

	enteric			
3	*Staphylococcus aureus*	-	8	13
5	*Enterococcus*	-	8	9
6	*Clostridium sps*	-	-	8

Antibacterial activity of partially purified bacteriocin of Isolate AB3

The antibacterial activity of the crude bacteriocin of Isolate AB 3 was evaluated. The AB 3 Isolate showed a maximum zone of inhibition of 14 mm for *Klebsiella*,whereas for *Escherichia coli*, it showed a minimum zone of inhibition of 10 mm on 10 µl concentration whereas on 20 µl concentration, showed 11 mm for *Escherichia coli*, 14 mm for *Staphylococcus aureus* and *Klebsiella* sp. Moreover, in 30 µl concentration, *Staphylococcus aureus* and *Klebsiella* sp showed a highest of 16 mm zone of inhibition, and lowest zone of inhibition of 12 mm for *Escherichia coli*. *Staphylococcus enterica* showed a 15 mmzone of inhibition.

Table 5. Antibacterial activity of partially purified bacteriocin of Isolate AB3

S. No.	**Name of the pathogen**	**Zone of inhibition (mm)**		
		10 µl	**l20 µ**	**30 µl**
1.	*E. coli*	10	11	12
2	*S. enterica*	-	-	15
3	*S. aureus*	13	14	16
4	*Klebsiella*	14	14	16
5	*Enterococcus*	13	13	15
6	*Clostridium sps*	-	-	14

Characterization and Identification of Isolate AB3

The isolate AB3 grew well on all the media and the growth of the isolates was excellent in ISP5. The Gram staining was positive and Spore chain morphology was spiral. The colonies

of developed isolate AB3 were observed as smooth with aerial and substrate mycelia of varying colors usually with entire margins and pale yellow pigment was produced in any media. The strain isolate AB3 can tolerate a salt concentration up to 10%; the optimum temperature for the isolate ranged between 10 and 28 °C and the optimum growth was observed at pH range of 9. The strain isolate AB3 varied in terms of utilization of carbon sources such as fructose, rhamnose, sucrose, and glucose. In the presence of inhibitor Phenol (0.1%) and Crystal violet (0.0001%), growth was present but no growth was observed in the presence of Sodium azide (both 0.01 and 0.02%) (**Table 6**).The morphological, physiological and biochemical characteristics of the isolate AB3 showed that it belong to the genus *Streptomyces* sps.

Table 6. Characterization and Identification of Isolate AB3

S. No.	Name of the test	Isolate AB3
1.	Gram stain	+
2.	Catalase	+
3.	Spore chain morphology	Spiral
4.	Melanin pigment production	+
5.	IMViC	- - - +
6.	Urease	+
7.	Triple sugar iron agar	K/K
8.	H_2S production	-
9.	Gas Production	-
10.	Oxidase	+
11.	**Degradation activity (% w/v)**	
	Tyrosine (0.5%)	+
12.	**Growth in the presence of inhibitors (% w/v)**	
	Phenol (0.1)	+
	Crystal violet (0.0001)	+
	Sodium azide (0.01, 0.02)	-
13.	**Effect of pH**	
	5	+

	6	+
	7	++
	8	+++
	9	++++
	10	++++
	11	++++
14.	**Effect of NaCl concentration**	
	2%	++++
	4%	+++
	6%	++
	8%	+
	10%	+

Enzyme production of Isolate AB3

The results were expressed in units (U), where U is defined as the amount of enzyme required to liberate 1µM of Amylase, Lipase, Protease and cell-wall degrading Cellulase enzymes per min. The results obtained suggest that Actinobacterial isolate AB 3 is a good producer of Amylase, Lipase, Protease and cell-wall degrading Cellulase enzymes which can be valorized by submerged culture.

Table 7. Enzyme production of Isolate AB3

S. No	Enzyme	AB3
1	Amylase	+
2	Lipase	+
3	Protease	+
4	Cellulase	+

SUMMARY

- The antibiogram study revealed that the pathogens selected for the present were multi drug resistant in nature.
- The 4 isolated actinobacterial isolates showed significant inhibitory activity against clinical pathogens in cross streak and spot agar test.

- Among the 4 isolates, almost all the isolates showed considerable inhibition in well diffusion assay.
- The isolate AB3 showed bacteriocin producing ability against poultry and clinical pathogens.
- The antibacterial activity of the crude and partially purified bacteriocin showed that the inhibition was solely due to the bacteriocin.
- The morphological, physiological and biochemical characteristics of the isolate AB3 showed that it belong to the genus *Streptomyces*.

CONCLUSION

The abundance and diversity of bacteriocins make them ideal candidates for use in the treatment and prevention of infections. In addition to high natural diversity, the bio-engineering of bacteriocins and improvements in production processes allow us to tailor bacteriocins so that they fit specific needs. Non-antimicrobial properties of bacteriocins, such as immune modulation, also introduce new applications. The most pertinent question to answer is to what extent these bacteriocins are able to cross the GBB to achieve these beneficial effects systemically. Approval by medical control councils for using a newly developed drug is a slow and tedious process and involves a number of safety tests and clinical trials. Natural antimicrobial peptides and bacteriocins adhere to the same rules and regulations. In particular, bacteriocins could fill a gap in medical and food industry applications by playing a role as a "natural" and "safe" antimicrobial agent in the near future. They can regulate competitive interactions in the microbial community. Their narrow-target activity, surprising specificity, high stability and low toxicity make them an alternative or complement to current antibiotics. They could play a key role in antibiotic resistance and could become a useful approach in the treatment of infectious diseases.

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INVESTIGATION OF ALGINATE FLUROAPITATE NANOCOMPOSITES FOR BIOMEDICAL APPLLICATIONS

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ABSTRACT

The aim of this study is to develop dental nanocomposites of fluorapatite-alginate bysimple wet chemical method. Here, dental enamel like composites were prepared by in situ method with three different alginate concentration. As-prepared samples were characterized by simple physico chemical techniques and the results were discussed elaborately. Increasing alginate content, substituted fluorine ion concentration is decreased which prevents excess fluorine release in the host area. Presence of alginate in the compositescouldbe a favourto enhance and regulate the biomolecules or antibiotic release in the required cases.

KEYWORDS: Alginate; Fluorapatite; Biocomposites; Bone graft

INTRODUCTION

Cavities are strongly damaged area on the hard surface of our teeth that grow into small openings or holes(Deepa et al., 2019; Hujoel et al., 2017). Dental caries continues to pose amajor public health problem globally and untreated caries can lead to a severe toothache, infection and tooth loss(Abed et al., 2020; Hujoel et al., 2017; MacHiulskiene et al., 2020). The World Health Organization mentionthat the disease affects 60-90% of school going children, mostof adults and that dental caries contributes to an extensive loss of natural teeth in older people(P.E. Petersen et al., 2009). Enamel is the thin hardest outer covering of the tooth. It consists of inorganic fraction of cement (65 wt.%), is mainly a biological apatite, which is characterized by a much lower degree of crystallinity. This creates it more prone to dissolution, but also to the adsorption of external ions on the surface.A decline in the occurrence and the severity of dental

caries is particularly observed in countries having established public health program using fluoride for dental caries prevention, coupled with changing living conditions, healthier lifestyles(Poul Erik Petersen et al., 2016; Shaymieva et al., 2021; Weaver, 1948).

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) has been investigated as a bone implant materialin dental, periodontal, oral/maxillofacial surgical procedures due to itsexcellent bioactivity, bioresorption and biocompatibility(Carvalho et al., 2020; Habibah et al., 2020; Sangeetha et al., 2019). HA is also one of the fewmaterials that form strong chemical bond with bone *in vivo*.However, its bioactivity and stability should be tailored to more specific in particular applications. To improve the biostability of HA, fluorine additionto HA ceramics to form fluorapatite (FA) is a solution(Bossù et al., 2019; Pajor et al., 2019).It has been recently reported that F^- ionsmight stimulate extracellular matrix formation in vitro andenhance bone union in vivo, promoting osteoblastic activities interms of cell proliferation and differentiation. In order to enhance the drug release behaviourof FA, natural polymer was incorporated with FA during the fabrication. Alginate is a hydrophilic polysaccharide found in the cell walls of brown algae and forms a viscous gum when hydrated(Sangeetha et al., 2017).

Roeder et al. showed that composites reinforced with whisker like HA have improved mechanical properties over those reinforced with spherical HA(Conrad et al., 2020). Hollow structured HA or FA particles are more proper as drug carriers compared to solid counterparts(Chenthamara et al., 2019). Thus, tailoring the shape and morphology of HA or FA crystals is critical in the manufacture of materials with the desired properties.However, its poor thermostability and subsequent high dissolution rate ina biological environment has, to some extent, limited its applications. On the other hand, fluorine is known to be veryimportant in influencing the physical and biological properties of HA and in the treatment of osteoporosis.We have fabricated fluorapatite and alginate- fluorapatite nanocomposites by facile wet precipitation method using calcium and phosphate precursors and alginate natural polymer.

2. MATERIALS AND METHODS

Calcium nitrate tetrahydrate, diammonium hydrogen phosphate,

ammonium fluoride, and ammonia solution were purchased from Merck, India andsodium alginate was purchased from Loba, India. All the chemicals were used without further purification. Double distilled water was used as solvent.

PREPARATION OF FLUORAPATITE-ALGINATE NANOCOMPOSITE

1 M of calcium nitrate was dissolved in 100ml of distilled water and its pH adjusted to 10.5 by using ammonia solution. Similarly, 0.6 M of di-ammonium hydrogen phosphate and 0.2 M sodium fluoride were dissolved into 100 ml of distilled water and the pH was adjusted to 10.5. Then the solution of di-ammonium hydrogen phosphate was added dropwise into calcium solution by titration. After titration, the mixture was stirred further for 30 min, then it allowed for 24 h aging. The obtained precipitates were separated by centrifuging with distilled water and dried by hot air oven at 110 ℃ for 6 h. The resulted dried sample was named as F.

PREPARATION OF FLUORAPATITE ALGINATE COMPOSITE

1 M of calcium nitrate was dissolved in 100ml of distilled water and its pH adjusted to 10.5 by using ammonia solution. Similarly, 0.6 M of di-ammonium hydrogen phosphate, 0.2 M sodium fluoride and 1 wt % of sodium alginate were dissolved in 100 ml of distilled water and the pH was adjusted to 10.5. Then the phosphate mixture was added dropwise into calcium solution by titration. After titration, the mixture was stirred further for 30 min, then it allowed for 24 h aging. The obtained precipitates were separated by centrifuging with distilled water and dried by hot air oven at 110 ℃ for 6 h. The resulted dried sample was named as corresponding to alginate concentration.Three different concentration of sodium alginate (1 wt %, 1.5 wt % and 2 wt %) were taken to prepare the fluorapatite-alginate composites and named as F1A, F2A and F3A.

2.4. CHARACTERIZATION

The phase purity and crystal structure of the obtained sampleswere examined by X-ray diffraction (XRD) using D8 Advance X-raydiffraction (Bruker axs company, Germany) equipped with Cu-KRradiation (k) 1.5406 (Å), employing a scanning rate of 0.02 s1 inthe 2h range from 10° to 80°. The morphology of the FA microparticles were examined by scanning electron micrograph (SEM)

usinga field emission SEM (FESEM) instrument (Hitachi S-4800 II, Japan)equipped with energy dispersive X-ray spectroscopy (EDXS).

3. RESULTS AND DISCUSSIONS

FTIR

The FTIR spectra of F, A1F, A2F and A3F are shown in Fig. 3.1. The characteristic ν_4 phosphate bands (ν_2& ν_3) of FA occurs as doublet bands around 569 and 604 cm^{-1} along with other PO_4 broad band around 1040 cm^{-1}(Carvalho et al., 2020; Kis et al., 2022).The bands at 1617 and 1417 cm^{-1} observed in the spectrum of A1F, A2F and A3F are assigned to asymmetric and symmetric stretching peaks of carboxylate salt of alginate and the weak signal at ~2926 cm-1 is due to C-H stretching vibrations of alginate(Sangeetha et al., 2017). The broad band centered at ~3418 cm^{-1} in all the spectra is assigned to hydrogen bonded O-H stretching vibrations. Presence of both ceramic (Fluorapatite) and polymer (alginate) in samples are confirmed by the FTIR spectrum of A1F, A2F and A3F samples.

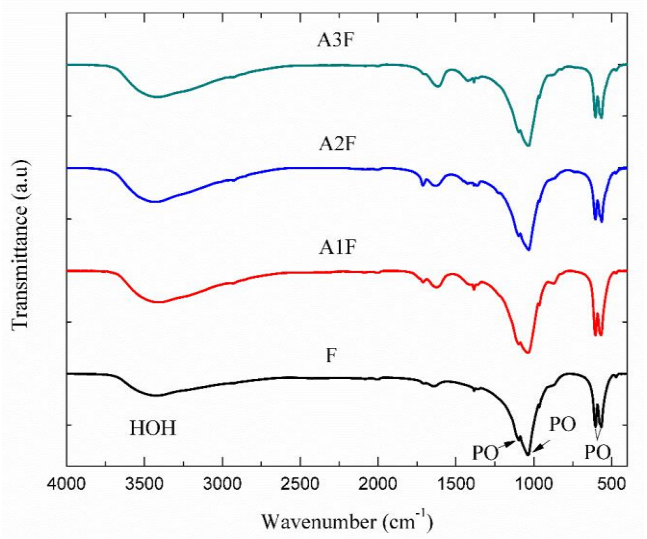


Fig.3.1. FTIR spectra of the samples

XRD

The crystalline nature of the samples is identified by the XRD patterns. All the four samples diffraction patterns (Fig.3.2) ascribed to the phase pure fluorapatite without any impurity peaks (JCPDS card no. 15-0876). Pristine sample F showed the sharp peaks of apatite phase with high intensity. It indicated that the apatite phase is high crystalline in nature.Whereas an increasing the concentration of alginate to the composites, the peaks (211), (300) and (202) gets merged the formed a broad peak and the broadness gets increased from A1F to A3F gradually. It suggested that addition of alginate suppressed the crystalline nature of fluorapatite. Also, alginate enhance the (002) orientation of

fluorapatite in some extent than the pristine samples.

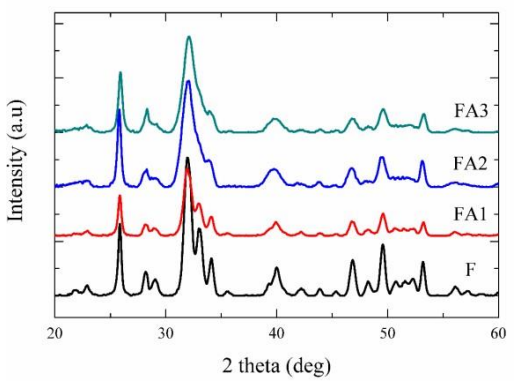
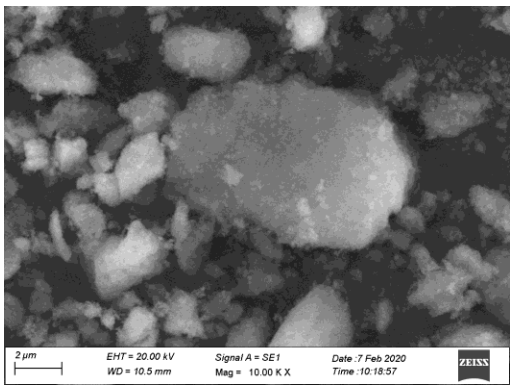


Fig. 3.2. XRD pattern of the sample F

SEM and EDAX

The morphology of the pristine fluorapatite (F) and alginate-fluorapatite composite (A3F) are examined by Scanning electron microscopy. The images of the sample F (Fig. 3.3) showed that the apatite formed as nano sized particles and may be identified as spherical in shape. The clear habit of the particles could not find out by the SEM clearly. The morphology of the composite material (A3F, Fig. 3.4) showed that alginate formed as a matrix and fluorapatite could embedded within the matrix. Apatite and alginate blended completely, and we could not distinguish ceramic and polymer separately in the A3F composites.

The elemental composition of pristine fluorapatite and fluorapatite-alginate composite materials are given in Table. 3.1. Both the spectrum and tabulated values clearly showed the presence of fluorine along with calcium and phosphate.



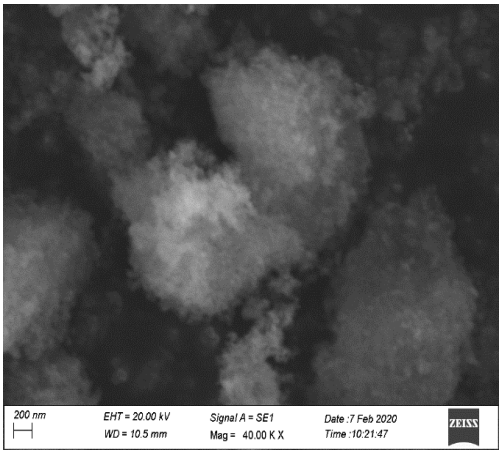
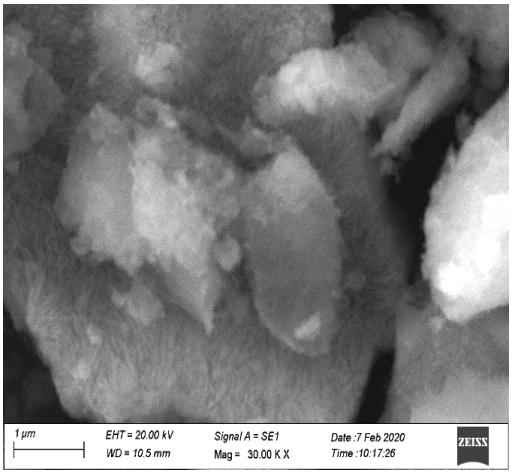


Fig.3.3. SEM images of the sample F



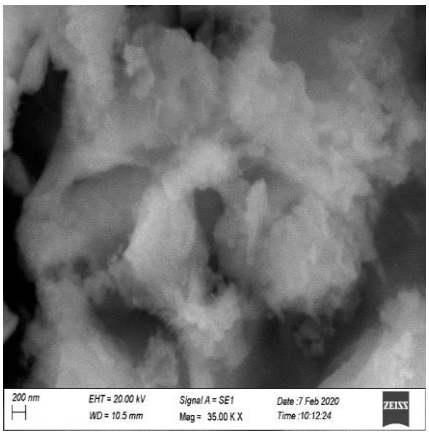


Fig.3.4. SEM images of the composite A3F

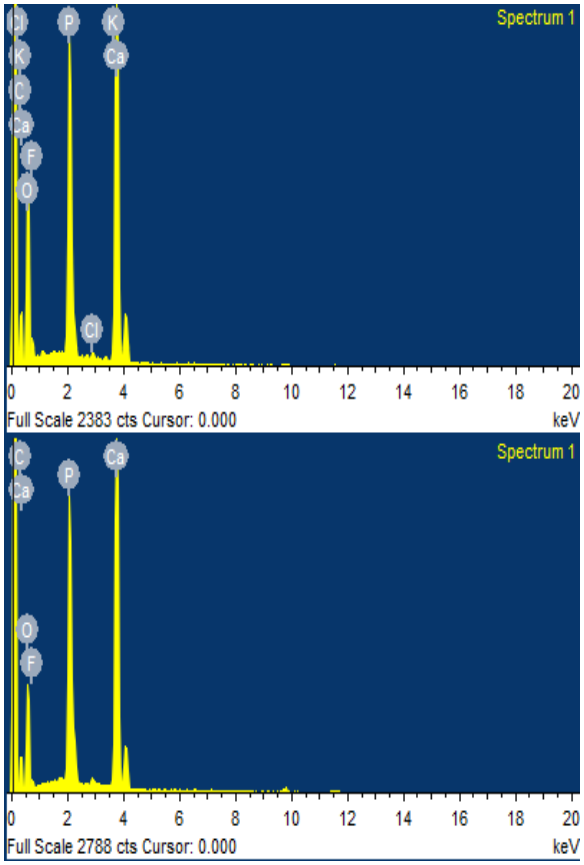


Fig. 3.5. EDAX spectrum of pristine fluorapatite (F) andcomposite (A3F)

Table. 3.1. Elemental composition of samples

Sample/ Element	F		A3F	
	Weight %	Atomic %	Weight %	Atomic %
Ca	22.06	10.58	26.30	12.67
P	12.09	7.50	12.82	7.99
F	8.16	8.26	4.61	4.68
O	45.60	54.61	39.41	47.56
C	12.08	19.05	16.86	27.10

4. CONCLUSION

Fluorapatite nanocrystals was fabricated by facile wet chemicdfal precipitation method using calcium, phosphate and flurine as the starting materials. In addition to this, natural polymer, alginate with different concentartion was added to the precursors of apatite for making in-situ ceramic - polymer nanocomposites. Fabricated pristine fluorapatite and alginate-fluorapatite nanocomposites were investigated using FTIR, XRD, Sem with EDAX. From the results obtained, we confirmed the formation of fluorapatite

phase in all the samples in nanocrystalline nature. Also, the formation of composites were confirmed by the presence of characteristics bands of apatite and alginate in FTIR spectra. Blending of apatite with alginate was observed by the morphological study. Also, the presence of fluorine was identified along with phosphate and calcium by the EDAX spectrum.

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PHYTOCHEMICAL ANALYSIS, *IN VITRO* ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF METHANOL EXTRACT OF *MARANTA ARUNDINACEA* RHIZOME

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ABSTRACT

The present study was aimed to evaluate the phytochemical analysis, *in vitro* antioxidant and antidiabetic activity of methanol extract of *Maranta arundinacea* rhizome. The preliminary phytochemical screening of methanol extract of *Maranta arundinacea* (R) was carried out using Harbone method. Various concentrations (25, 50, 100 and 200µg/ml) of methanol extract of *Maranta arundinacea* (R) was taken for *in vitro* antioxidant activity. The methanol extract showed the presence of alkaloids, flavonoids, glycosides, terpenoids, saponins, phenols, carbohydrates and proteins.

The methanol extract of *Maranta arundinacea* (R) exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical and hydroxyl radical scavenging activity. From our results, *Maranta arundinacea* (R) had shown better antidiabetic activity. The results of the present study revealed that strong antioxidant potentials and antidiabetic activity of methanol extract of *Maranta arundinacea* in dose dependent manner.

KEYWORDS: *Maranta arundinacea*, Rhizome, Phytochemical, Antioxidant, Antidiabetic.

INTRODUCTION

Diabetes mellitus is a serious complex multifactorial disorder that affects the metabolism of carbohydrate, fat and protein. It is characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells of the body do not respond properly to the insulin produced (Banerjee *et al*., 2017). Diabetes is characterized by hyperglycemia, altered lipids, carbohydrates, and proteins metabolism which affect the patient quality of life in terms of social, psychological well-being as well as physical ill health (Subramaniam *et al*., 2013). The effects of diabetes mellitus include long-term complications include heart disease, stroke, dysfunction and failure of various organs (Keerthana *et al*., 2013)

Diabetes mellitus is epidemic in India as a result of societal influence and changing lifestyles. Diabetes has been known in India for centuries as ‘a disease of rich man’ but now spread among all peoples (Narkhede *et al*., 2011). According to International Diabetes Federation (IDF), the number of individuals with diabetes and its complication in 2019 crossed 366 million, with an estimated 4.6 million deaths every year (Sundar Rajan *et al.*, 2017)

In India use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human cultivation. Plants and plant based medicine are the basis of many of the modern pharmaceutical (Ahirwar *et al*., 2015). The world health organization (WHO) reported that 80% of the World population is used to indigenous medicine and that the majority of traditional therapies involve the use of plant extracts or of their constituents. There are about 800 plants which have been reported to show antidiabetic potential. Numerous natural bioactive compounds from plant have established their role for possible use in the treatment of diabetes. (Sundar Rajan *et al*., 2017).

The present study was therefore undertaken to investigate *in vitro* antioxidant and *in vitro* antidiabetic activity of methanol extract of *Maranta arundinacea (R.)*

MATERIALS AND METHODS:

Collection and preparation of plant extract

The rhizome of *Maranta arundinacea* was collected from Salem district. The rhizome were dried in shade, coarsely powdered and was extracted with methanol by using soxhlet apparatus. The residue was filtered and the solvent were evaporated under reduced pressure and the resulted extracts were concentrated using a rotary evaporator with a water bath set at 40°C. The concentrated crude extracts used for further analysis.

Preliminary phytochemical analysis

The qualitative phytochemical analysis of methanol extract were carried out for the presence of alkaloids, flovonoids, steroids, saponins, tannins, phenols, terpenoids, glycosides, proteins, carbohydrate using the method adopted in similar surveys (Harbone 1973).

ANTIOXIDANT ACTIVITY

DPPH RADICAL SCAVENGING ACTIVITY

DPPH radical scavenging activity was carried out by the method of Molyneux (2004). To 1ml of 100 µm DPPH solution in methanol, equal volume of the test sample in methanol of different concentrations were added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1 ml of methanol instead of test sample was added to the control tube. Different concentrations of ascorbic acid were used as reference compound. Percentage of inhibition was calculated from the equation:

[(Absorbance of control - Absorbance of test)/ Absorbance of control)] X 100.

HYDROXYL RADICAL SCAVENING ACTIVITY

The hydroxyl radical scavenging activity of the test sample was estimated according to the method of Halliwel *et al.*, (1992). The hydroxyl radical was generated by a fenton - type reaction. The reaction mixture contained the 0.2ml sample in varied concentration to which 0.1 ml EDTA (1 mM) , $FeCl_3$ (10MmmM) mixture , 0.1 ml H_2O_2 (10mM) , 0.36ml deoxyribose (10mM) , 0.33 ml phosphate buffer (50 mM , PH 7.4) and 0.1 ml of ascorbic acid (1mM) was added in sequence .

The mixture was incubated at 37° C for 1 hour. To this mixture was added

1.0 ml each of TCA (10 %) and TBA (0.67 %) and kept in boiling water bath for 20 minutes. The colour develop was read at 532 nm. The control tube contains phosphate buffer, instead of sample.

IN VITRO ANTI-DIABETIC STUDIES

Assay of Alpha Amylase Inhibition (Narkhede *et al*., 2011)

A total of 500 µl of test samples and standard drug (25 – 125µg/ml) were added to 500 µl of 0.02 M phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and incubated at 25°C for 10 min. 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 - dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted by adding 10 ml distilled water and absorbance was measured at 540 nm.

RESULTS:

Preliminary phytochemical screening

The results of phytochemical screening of methanol extract of *Maranta arundinacea* (R) were presented in Table 1. The methanol extract showed the presence of alkaloids, flavonoids, glycosides, terpenoids, saponins, phenols, carbohydrates and proteins.

Table:1 Preliminary phytochemical analysis of methanol extract of *Maranta arundinacea (R)*

S.No	Phytoconstituents	Methanol extract
1	Carbohydrate	+
2	Protein	+
3	Amino acids	+
4	Flavanoids	+
5	Alkaloids	+
6	Saponin	+
7	Steriods	-
8	Terpenoids	+
9	Phenols	+
10	Glycosides	+
11	Fixed oils & fats	-

ANTIOXIDANT ACTIVITY

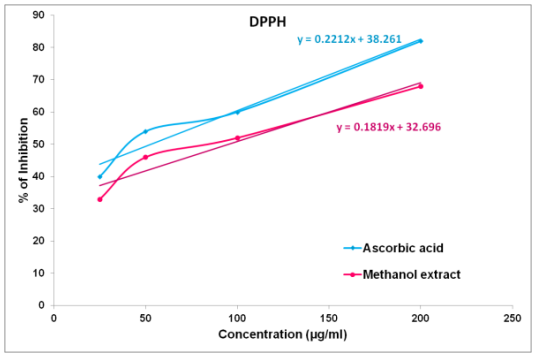
Different concentrations ranging from 25-200 µg/ml of the methanol extract of rhizome of *Maranta arundinacea* were tested for their antioxidant activity.

DPPH RADICAL SCAVENGING ACTIVITY

The activity of DPPH radical scavenging of the methanol extract of

Maranta arundinacea (R) was presented in figure 1. The percentage of inhibition in DPPH in different concentration like 25, 50, 100, 200 µg/ml were observed as 33, 46, 52, 68 respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200 µg/ml were found to be 40, 54, 60, 82 respectively. The IC 50 values for DPPH scavenging activity for methanol extract of *Maranta arundinacea (R)* and ascorbic acid were 95µg/ml and 53µg/ml respectively.

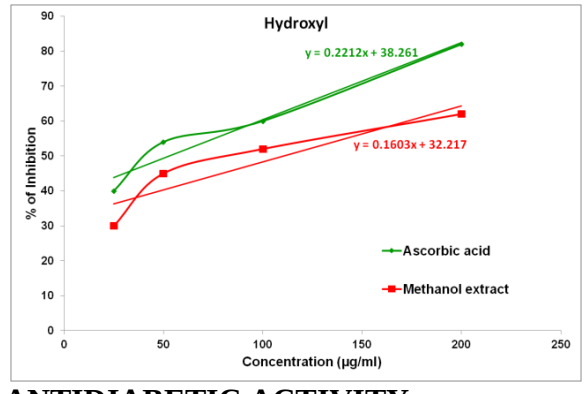
Figure 1: DPPH radical scavenging activity of methanol extract of Maranta arundinacea (R)



HYDROXYL RADICAL SCAVENGING ACTIVITY

The hydroxyl radical scavenging activity of methanol extract of *Maranta arundinacea (R)* was presented in Figure 2. Hydroxyl radicals were scavenging in different concentration like 25, 50, 100, 200µg/ml were observed as 30, 47, 56, 69 respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200 µg/ml were found to be 40, 54, 60, and 82 respectively. The IC50 values for hydroxyl radical scavenging activity for methanol extract of *Maranta arundinacea* and ascorbic acid were 111µg/ml and 53µg/ml respectively.

Figure 2: Hydroxyl radical scavenging activity of methanol extract of *Maranta arundinacea (R)*

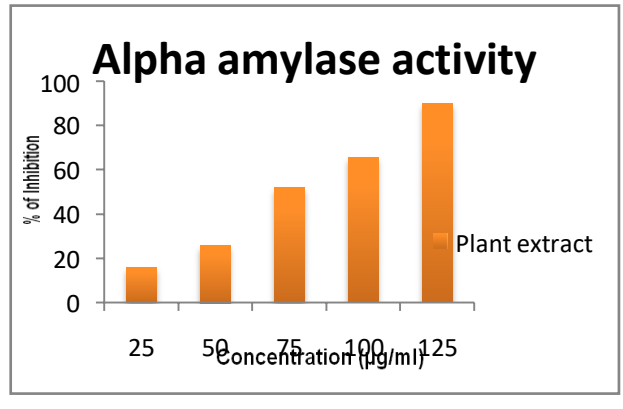


ANTIDIABETIC ACTIVITY

ALPHA-AMYLASE INHIBITORY ACTIVITY

In this study, the *in vitro* α–amylase inhibitory activities of methanol extract of *Maranta arundinacea (R)* was investigated and presented in Figure 3. Different concentration of methanol extract of *Maranta arundinacea (R)* like 25, 50, 75, 100, 125 µg/ml were observed with percentage inhibition of 16.04, 25.75, 52.15, 65.7, 90.5 respectively. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against α -amylase enzyme.

Figure 3: Effect of methanol extract of *Maranta arundinacea (R)* on α–amylase inhibitory activity



DISCUSSION:

The phytochemical screening of methanol extracts of *Maranta arundinacea* showed that the rhizome are rich in carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, saponins, phenols, terpenoids and glycosides. Flavonoids are most commonly known for their antioxidant activity. Secondary metabolites like alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc. are the natural compounds from plant sources, which are being utilized from ancient era to heal various alignments (Arutselvi *et al* ., 2012 ; Ancy *et al* ., 2017).

The antioxidant activity of phenol compounds is mainly due to their oxidation reduction properties, which can play an important role in adsorbing and neutralizing free radicals, reducing singlet and triplet oxygen, or decomposing peroxides. Oxidative injury now appears as the fundamental mechanism causing a number of human neurologic and other disorders such as autoimmune pathologies, inflammation, viral infections and digestive system disorders including gastrointestinal inflammation and ulcer (Satpathy *et al.*, 2011). The study clearly indicated that the methanol extract of *Maranta arundinacea* act as a potent material to scavenge free radicals. This might be due to the high content of phenol and flavonoids (Bajpai VK *et al.*, 2015).

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism (Rajiv Gandhi and Sasikumar, 2012). It was proposed that inhibition of the activity of such alpha-amylase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation (Rhabaso Lhoret and Chiasson, 2004).

Alpha–amylase, as a key enzyme in the digestive system, is involved in the breakdown of starch into disaccharides and oligosaccharides and finally liberating glucose which is later absorbed into the blood circulation. It was proposed that suppression of the activity of α–amylase would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation.

The results obtained revealed that there was a dose-dependent increase in percentage inhibitory activity of methanol extract of *Maranta arundinacea (R)* against α-amylase enzyme. The plant extract might be used as starch blockers since it prevents or slows the absorption of starch in to the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose and other simple sugars (Keerthana *et al.*, 2013) The reaction mechanisms involved in inhibition of α-amylase enzyme by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein flavonoids might cause conformational changes in structure. *Maranta arundinacea* have been reported to contain flavonoids.

CONCLUSION:

The observations from this study indicate that the methanol extract of *Maranta arundinacea (R)* possesses various

phytochemicals and have significant free radical scavenging activity and inhibitory activity against α-amylase, thus justifying the use of the plant by traditional medicine practitioners for the treatment of diabetes mellitus.

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SCREENING AND IDENTIFICATION OF DIVERSE DIATOMS ISOLATES

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ABSTRACT

Diatoms are hefty and eukaryotic algae of the varied group, that is the Bacillariophyceae family. Habitually microscopic, they are recognized to be plentiful in rice fields. They are the prime faction of earth's biomass producers; which are one of the chief contributors to universal carbon fixation and total oxygen production (25%) on earth. Diatoms are extremely booming organisms as judged by their distribution, adaptableness, biomass, and relative antiquity. The present research work was executed with the screening and identification of diverse diatoms isolates from different rice fields of Rasipuram, Namakkal, Tamilnadu. A total number of 10 samples were collected from rice fields of Rasipuram. Microscopically identifies 17 isolates of 10 diatom genera. We have to maintain the earth's biodiversity and take appropriate measures to protect habitats and species.

KEYWORDS: Diatoms, genera, rice fields

INTRODUCTION

Diatoms are a large and assorted collection of eukaryotic algae. They are the members of Bacillariophyceae, dispersed all through the globe from brackish and freshwater habitats. Habitually microscopic, some diatom species can attain up to the length of 2 mm. Diatoms are one of the mainly significant food resources in the freshwater and marine environment. They are generally unicellular, while some form simple colonies or chains or in the shape of ribbons or filaments and the cells are golden brown in colour since the existence of elevated levels of fucoxanthin, which is an accessory pigment of photosynthetic. There are numerous thousands of taxa with varied ecological necessities; their cell wall of siliceous is called the frustules ruins are used widely as indicators of the environment in climate change studies, quality of water, and acidic precipitation (Stoermer and Smol, 1999).

Several other Xanthophylls are present at lower levels, as well as β-carotene, chlorophyll a, and Chlorophyll C. There are more than 200 genera of living diatoms, and it is estimated that more than 100,000 species exist. Several recognized diatoms are 10000 – 12000 (Norton *et al.*, 1996). Diatoms are traditionally divided into two orders: Centric diatoms (Centrales), which are radially symmetric. Pennate diatoms (pennales) are bilaterally symmetric. Diatoms generally range in size from 2-200μm. The main storage compounds of a diatom are lipids (TAGS) and a β-1-3-

Linked carbohydrate known as chrysolaminarin (Sheehan *et al.*, 1998). Diatoms are considered as one of the fundamental players in the physical and biochemical processes that characterized the ecosystem, therefore, play a significant role in the earth's biochemistry (Graham and Wilcox, 2000).

Desikachary's contributions to the diatoms of India deserve a special mention (Desikachary, 1959). Previous studies and taxonomic lists of diatoms for the Chesapeake Bay have focused primarily on the phytoplankton component of the diatom flora (Wolfe *et al.*, 1926; Morse, 1947; Griffith, 1961; Mulford, 1962; Patten *et al*., 1963; Marshall, 1984, 1986). More recently, examined the distribution patterns of both planktonic and benthic diatoms in the Severn River (a Chesapeake Bay tributary). In a series of papers, Cooper has documented the diatom community structure changes about land-use changes over approximately the past 2,000 years (Cooper and Brush, 1991 and 1993).

Diatoms are enormously successful organisms as judged by their adaptability, distribution, biomass, and relative antiquity. They account for up to 25% of the total oxygen production on earth. They are the largest group of biomass producers on earth and they are one of the predominant contributors to global carbon fixation (Norton *et al.*, 1996). A careful examination shows that many of the attached or enclosed forms also occur in a free state; and that there are frequently very slight differences between species. These circumstances, together with the necessity of using the most excellent and powerful lenses, make the study of the diatoms a difficult one. But that study is of considerable importance. For the complete knowledge of these diatoms, it would have been desirable to study them in the living state. The present study was aimed at the prevalence and identification of diverse isolates of diatoms of the Rasipuram area, Namakkal District, Tamilnadu, India.

MATERIALS AND METHODS

Sample collection

Ten water and soil samples were collected from different rice fields in and around Rasipuram, Namakkal District, Tamilnadu, India. The samples were collected in thoroughly washed and autoclaved polypropylene sample bottles (Tarson, India), and sterile polythene bags, and then samples were transported into the laboratory (Bhardwaj and Tiwari, 2010; Nashima and Palanisamy, (2016); Maghimaa M, Palanisam, 2019; Pandeeswari *et al.*, 2021; Mathanmohun *et al*., 2021).

MICROSCOPIC OBSERVATION AND IDENTIFICATION

The microscopic observations of diatom isolates were carried out at 40x and 100x magnification using a compound microscope (Olympus). The diatom flora was identified based on the taxonomic criteria as described by Cramer, (1984), Jensen, (1985), Krammer and Lange-Bertalot, (1988), and Benson, (1998), Nashima and Palanisamy, (2012, 2016).

RESULTS AND DISCUSSION

The present investigation was carried out on the prevalence of diatoms from different paddy fields in and around Rasipuram location, Namakkal District. Ten samples were collected from different rice fields in and around the Rasipuram area, Namakkal District, Tamilnadu.

MICROSCOPIC OBSERVATION AND IDENTIFICATION

The samples collected were analyzed for the prevalence of diatoms and identified based on microscopic observation. A total of 17 isolates of 10 diatom genera were recorded (Table-1 and Fig 1). Our study closely matches the findings of Hamed, 2008 in different water habitats near Cairo and Suez town. Similar observations were found in Fujita and Ohtsuka, 2005: Fujita and Nakahara, (2006) and Bhattacharya et *al.*, (2011). These results suggest that the diatom is predominant in paddy soil. Therefore domination of species specifies to definite location, time, and change year to year. High population density due to high nutrient concentration.

Table- 1 Prevalence of Diatom taxa found in the paddy fields of Rasipuram

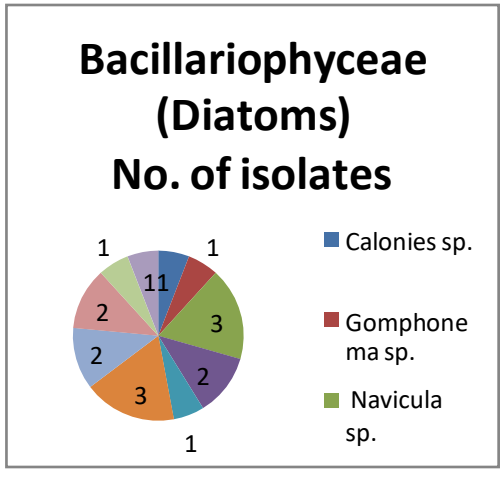

Fig. 1 Prevalence of Diatom taxa found in the paddy fields of Rasipuram

Diatoms are well known to be abundant in paddy fields (Ohtsuka and Fujita, 2001). A large population of algal as well as diatom flora is available from Kolkata, West Bengal. Several authors have reported diatom flora from various regions of India especially from south and west since long back (Venkataraman 1939; Gandhi, 1967; Nair, 1960; Ramamoorthy 1965; Gopalakrishnan 1972; Sarode and Kamat 1979; Somashekar 1984; Bongale 1985; Prasad and Jaitly 1985; Kannan and Vasantha 1992; Garg and Bhaskar, 2000). Though most of the diatoms reported in this region are marine and estuarine, there are very few reports of freshwater diatoms from west Bengal by Jena *et al.*, 2006 and Bhattacharya *et al.*, 2011.

Ohtsuka and Fujita (2001) reported 92 diatom taxa belonging to 28 genera in a Japanese paddy field. Gore and Sanap, (2009) found 8 genera. Low temperature, water and light deficiency, high light intensities, may act as limiting factors. The rice field ecosystem provides a favorable environment for the growth of algae since there is an adequate supply of light, water, heat, and nutrients (Vidyavati, 2012). *Calonies sp.* Gomphonema sp. *Cocconeis sp. Diadesmis sp. and Amphipleura sp.* were the lowest number. This was correlated with Leelahakriengkrai and Peerapornpisal, 2008. The temperature and nature of the climate conditions are the reason for the difference between the ratios of the diatom. In rice fields, temperature, light intensity, and oxygen concentration are the key factors that control the distribution and abundance of various zooplankton (Payne, 1986).

CONCLUSION

Diatoms are enormously successful organisms as judged by their adaptability, distribution, biomass, and relative antiquity. They account for up to 25% of the total

oxygen production on earth. We must create economic policies to maintain the earth's biodiversity and take appropriate measures to protect habitats and species.

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Synthesis, characterization of Schiff base metal complexes

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Abstract In the present investigation some new Schiff bases derived from Amoxicillin trihydrate with Cinnamaldehyde and p-Chlorobenzaldehyde and their complexes with bivalent transition metal ions viz. Co (II), Zn (II), Ni (II), and Mn (II), have been synthesized. The ligand and their metal complexes were characterized on the basis of elemental analysis and micro analytical datas. Shift in the characteristic spectral frequency of the metal complexes, confirms the coordination through metal ion with azomethine group. They were screened for antibacterial activity against several bacterial strains namely *E. coli* (-), *S. aureus* (+) *M. luteus* (+) and *B. lichenformis* (+) (ATCC), the metal complexes showed enhanced antibacterial activity compared to uncomplexed ligand.

Key words: Antibacterial activity, Amoxicillin trihydrate

Introduction

Compound containing imines bases have not only found extensive application in organic synthesis, [1-2] but several of these molecules display significant biological activity. In the last decade Schiff base ligands [3-6] have received more attention mainly because of their wide application in the field of catalysis and due to their antimicrobia, [11-12] anti-tuberculosis[7] and anti-tumor activity. They easily form stable complexes with most transition metal ion.[13-14] The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species.[8] Co- ordination compound have been reported to act as enzyme inhibitor[9] and are useful due to their pharmacological application.[10] In view of the importance of such imines, we describe here the synthesis and characterization of Co(II), Zn(II), Ni(II) and Mn(II) complexes of cinnemaldehyde and p-chlorobenzaldehyde withamoxicillintrihydrate.

Materials and Methods: All chemicals and solvent used were of analytical grade. All metal (II) salts were used as chloride.UV-VIS spectra were obtained on digital spectrophotometer in the range 300-900nm in DMF.

IR spectra were recorded using KBR disc on a FT-IR spectrophotometer, Shimadzu 8201PC in the range of 4000-400cm^{-1}. ^{1}HNMR spectra were recorded in MeOD at room temperature. Elemental analysis was carried out on Elementar Vario ELIII. Conductance measurement of 10^{-3} M solution of the complexes in DMF was carried out on an Equiptronic model no. Eq-660A. Melting point of the ligands and their metal complexes

were determined by open capillary method using sunsim electric melting point apparatus and are uncorrected. Molecular weight of ligands and their metal complexes were determined by Rast camphor method.

Synthesis of the organic ligand (CmA, PbA)

The aldehyde cinnamaldehyde (1 mol, 1.321gm), *p*-chlorobenzaldehyde (1 mol, 1.521gm.) were dissolved in methanol (10 ml) and added to the amoxicillin trihydrate (1 mol, 4.194 gm) dissolved in methanol (10 ml). To this KOH (0.1 % in methanol) was added to adjust the pH of the solution between 7-8 and then the mixture respectively was refluxed for 4 hrs. After complete refluxation Schiff base was separated out on removal of the solvent at room temp. A light yellowish and brown colored crystalline solid obtained and then dried over anhydrous $CaCl_2$ in vacuum.

Synthesis of metal complex (CmAM, PbAM)

Amoxicillin trihydrate (0.2mol 0.838 gm.) cinnamaldehyde(0.2 mol, 0.264 gm.),*p*-Chlorobenzaldehyde (0.2 mol, 0.838 gm) and (0.1 mol.) metal M= Zn (II) (0.199 gm.), Ni (II) (0.136 gm.), Mn (II) (0.197 gm), and Co (II) (0.237gm), were dissolved in methanol (10 ml)

Physical Properties

These complexes are air and moisture stable, intensely colored amorphous solid which decomposes above 200^0C. They are insoluble in common organic solvent like chloroform, acetone, ether, and ethanol and carbon tetra chloride but soluble in DMF and DMSO. Molecular weight determined by Rast Camphor method and were found in accordance with calculated value the range of ligands (533-542) and metal Complexes (1142-1176) confirming the monomeric nature of the compounds. The yield of compounds was found in the range of (60- 80 %)

The molar conductance of all the compounds using DMF as a solvent, have been found to be in the range 0.12 to 0.45 $ohm^{-1}cm^2mol^{-1}$ indicating their non electrolyte nature. Values are also shown in **table 1.**

Table-1

S .No	Nam of com und	C% Fou (Cal	H% Fou (Cal	O% Fou (Cal	N% Fou (Cal	Con tivit	M.P (°C)	M.W foun (cal	Colo	Yiel %
1	CmA	62.55	5.21	16.68	8.75	0.30	210	479.61	yello w	68
		62.23	5.20	16.50	8.65			479.25		
2	CmA - Zn^{+2}	54.91	4.39	14.64	7.68	0.20	320	1092.5	black	72
		54.62	4.31	14.56	7.60			1085.3		
3	CmA $-Co^{+2}$	55.25	4.42	14.73	7.73	0.19	340	1085.8	black	75
		55.20	4.40	14.60	7.58			1069.3		
4	CmA - Mn^{+2}	55.50	4.44	14.80	7.77	0.12	308	1080.9	brown	74
		55.48	4.32	14.72	7.62			1074.2		
5	CmA $-Ni^{+2}$	55.26	4.42	14.73	7.73	0.20	342	1085.6	brown	69
		55.18	4.30	14.62	7.63			1077.2		
6	PbA	59.03	4.50	16.39	8.60	0.45	240	487.82	light	78
		59.01	4.35	16.30	8.55			479.9	brown	
7	PbA - Co^{+2}	52.20	3.80	14.50	7.61	0.28	304	1103.44	brown	72
		52.12	3.74	14.38	7.59			1099.6		
8	PbA - Zn^{+2}	51.89	3.78	14.41	7.56	0.26	308	1110.04	black	74
		51.80	3.72	14.34	7.50			1103.2		
9	PbA - Mn^{+2}	52.43	3.82	14.56	7.64	0.30	338	1098.54	brown	69
		52.43	3.80	14.50	7.52			1080.3		
	PbA - Ni^{+2}	52.20	3.80	14.50	7.61	0.35	342	1103.24	yello w	76
10		52.16	3.74	14.40	7.50			1100.6		

Micro analytical datas of ligand and their metal complexes

All the spectral data was consistent with the assigned structure of the compounds. In The band IR spectrum, the (Ar-OH) observed at 3428, 3389 cm^{-1} in the ligands and disappeared in metal complexes showing the participation of the O-M group in coordination. The ligands show strong band in the regin1650-1661 cm-1 due to C=N which is assignable to the Schiff bases, which appeared in both synthesized ligands. This band gets shifted to lower frequency in the complexes, indicating the coordination through azomethine nitrogen. It is found from the IR spectra of the complexes that there are wide bonding and 460-490 cm^{-1} for (M-O) which is assigned to metal stretching vibration. The ^{1}HNMR spectral data of ligands (CmA) and **(**PbA) shows signal between δ7.45-7.60 and δ7.48-7.57 respectively due to aromatic ring which gets shifted downfield in their metal complexes. The VU-VIS spectra of ligands (CMA and PbA) showed two bands between 300-350 nm and 310-365 nm. The first band may be due to Π – Π* transition within the aromatic ring. The second band would be due to n- Π * transition within –C=N group. Shown in **table 2.**

Strong band at 564 – 670cm for (M-N)

Characteristic IR and ^{1}HNMR spectral datas of the ligands and their metal complexes

		IR spectra cm^{-1}				^{1}HNMR	U.V Spectra ppm			
No.	**Name Com**	**(M-O)**	**(N-M**	**(C=N**	**(ArOH**	**δ(CH)**	**δ(Ar-H)**	**N=H**	**C=C**	**C=N**
1	CmA	-	-	1659	3389	4.91	6.77-7.2	7.49	300	350
2	CmA - Zn^{+2}	462	670	1603	--	4.39	6.15-7.1	7.29	300	320
3	CmA - Co^{+2}	468	617	1635	--	4.87	6.44-7.1	7.32	300	318
4	CmA - Mn^{+2}	488	616	1605	--	4.85	6.16-6.83	7.36	300	340
5	CmA - Ni^{+2}	482	613	1604	--	4.87	6.30-7.02	7.24	300	320
6	PbA	-	-	1661	3428	5.19	6.79-7.18	7.53	310	365
7	PbA - Co^{+2}	472	669	1639	--	5.10	6.45-7.02	7.40	340	355
8	PbA - Zn^{+2}	468	670	1630	--	4.86	6.23-7.16	7.38	320	346
9	PbA - Mn^{+2}	469	564	1644	--	4.20	6.77-6.79	7.35	320	326
10	PbA - Ni^{+2}	474	570	1640	--	4.40	6.42-7.28	7.28	320	342

Antibacterial Studies

Evaluation of antimicrobial activity of all compounds *in vitro* was carried out by paper disc method against bacteria including *E. coli, S. aureus, M. luteus, and B. lichenformis.* Streptomycin was additionally tested as positive. Control. Significance level of all compounds (P<.001), (*P<.01). The datas represent the values of three replicates and are evaluated as mean ± SEM values were determined and are shown in **table 3,** also their MIC values in the **table 4**.

Table-3 Antimicrobial activity ligands and their metal Complexes

	***E. Coli* (-)**			***S. aureus* (+)**		
	100ppm	500ppm	1000ppm	100ppm	500ppm	1000ppm
CmA	19(±.528)	27(±.578)	36(±.305)	19(±.728)*	27(±.420)	35(±.378)
CmA-Zn^{+2}	22(±.305)	34(±.305)	37(±.503)	23(±.435)	22(±.264)	38(±.305)
CmA-Co^{+2}	21(±.416)	31(±.586)	37(±.493)	21(±.152)	32(±.676)	40(±.305)
CmA-Ni^{+2}	21(±.305)	32(±.297)	38(±.378)	22(±.493)	31(±.350)	37(±1.07)
CmA-Mn^{+2}	22(±.200)	32(±.305)	35(±.755)	20(±.251)	32(±.586)	39(±.152)
Pba	18(±.200)	27(±.551)	36(±.321)	18(±.952)*	28(±.379)	36(±.264)
Pba-Zn^{+2}	20(±.305)	29(±.264)	37(±.200)	21(±.400)	30(±.400)	37(±.152)
Pba-Ni^{+2}	21(±.231)	30(±.200)	38(±.462)	20(±.091)	30(±.208)	38(±.173)
Pba-Co^{+2}	20(±.115)	30(±.231)	35(±.208)	20(±.057)	30(±.379)	40(±.208)
Pba-Mn^{+2}	19(±.416)	29(±.346)	36(±.397)	21(±.993)*	29(±.208)	38(±.264)
Streptomycin	24±.235	29±.513	33±.350	25±.598	30±.265	34±.365
***M. luteus* (+)**	100 ppm	500ppm	1000ppm	***B. lichenformis* (+) 100 ppm**	500 ppm	1000ppm
CmA	18(±.557)	26(±.096)	30(±.305)	17(±.503)	25(±.305)	28(±.152)
CmA-Zn^{+2}	22(±.305)	31(±.305)	36(±.099)	20(±.264)	28(±.557)	38(±.305)
CmA-Co^{+2}	21(±.611)	29(±.379)	34(±.493)	20(±.305)	28(±.465	36(±.305)
CmA-Ni^{+2}	19(±.208)	29(±.712)	36(±.200)	19(±.305)	28(±.152)	37(±.305)
CmA-Mn^{+2}	20(±.400)	29(±.712)	30(±.057)	17(±.100)	26(±.305)	35(±.200)
Pba	18(±.231)	30(±.416)	32(±.152)	18(±.305)	25(±.305)	28(±.264)
Pba-	19(±.586)	26(±.251)	38(±.208)	19(±.231)	25(±.305)	36(±.305)

Zn^{+2}						
Pba-Ni^{+2}	21(±.493)	29(±.416)	37(±.503)	18(±.231)	27(±.200)	34(±.379)
Pba-Co^{+2}	21(±.551)	30(±.416)	34(±.264)	19(±.436)	26(±.100)	36(±.469)
Pba-Mn^{+2}	24±.256	30(±.208)	32±.542	20±.254	27(±.493)	34(±.611)

Table 4.

MIC of the ligand and their metal complexes

Name of Compound	***E. Coli (-)***	***S. Aureus (+)***	***M. Luteus(+)***	***B. Lichenformis(+)***
	mg/ml	mg/ml	mg/ml	mg/ml
CmA	0.48	0.50	0.50	0.48
CmA -Zn^{+2}	0.21	0.21	0.24	0.26
CmA -Co^{+2}	0.28	0.38	0.31	0.26
CmA -Mn^{+2}	0.24	0.35	0.32	0.39
CmA -Ni^{+2}	0.26	0.28	0.37	0.34
PbA	0.48	0.45	0.50	0.48
PbA -Co^{+2}	0.36	0.36	0.30	0.36
PbA -Zn^{+2}	0.37	0.34	0.37	0.36
PbA -Mn^{+2}	0.39	0.38	0.31	0.34
PbA - Ni^{+2}	0.30	0.36	0.32	0.39

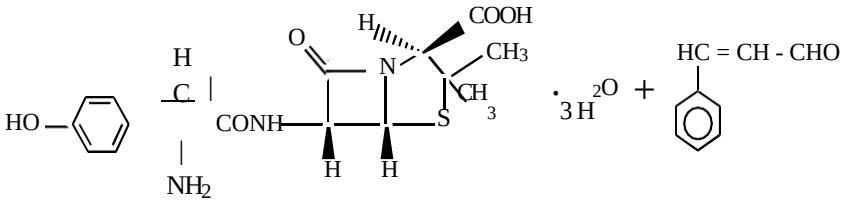
Conclusion

The result of this investigation supports the suggested structure of the metal complexes. A square planner structure was suggested for all the complexes, the Schiff base ligands were found to be biologically active and their metal complexes display enhanced antimicrobial activity against one or more strains, chelation.

Acknowledgements

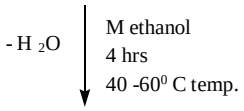
We express our sense of gratitude to Secretary and Principal of Muthayammal College of Arts and Science and Department of Chemistry, Karunya University and Coimbatore for spectroscopic and anti-bacterial studies.

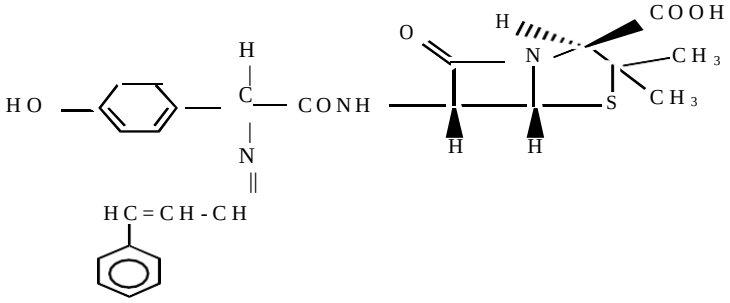
Figure of ligands and their metal Complexes



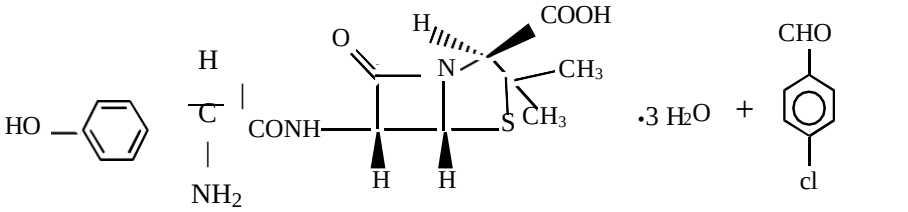
Amoxycillintrihydrate

Cinnamaldehyde



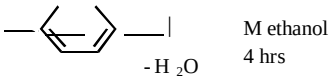


Scheme I of Synthesis of ligand CmA

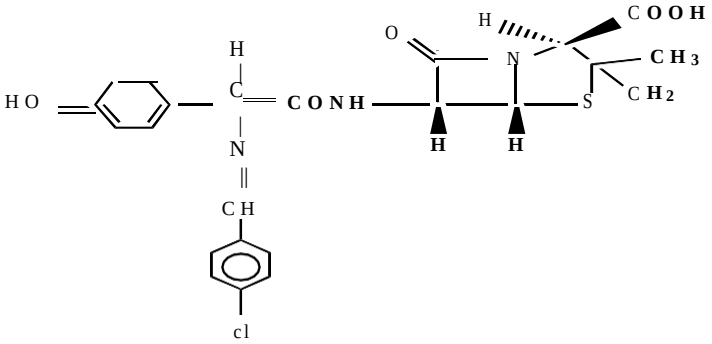


Amoxicillin trihydrate .

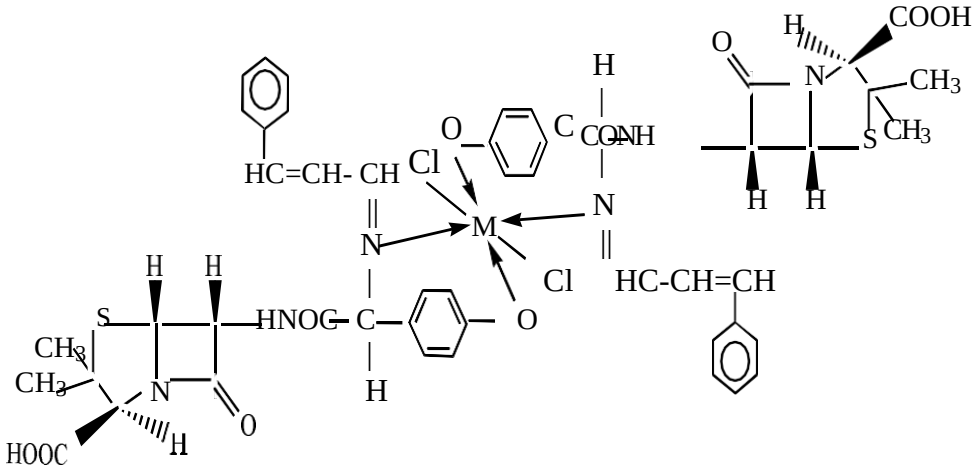
p- chlorobenzaldehyde



40 -60^0 C temp.



Scheme II of Synthesis of ligand PbA



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NOVEL RED EMITTING Eu^{3+} ACTIVATED $NaBaB_9O_{15}$ PHOSPHOR FOR WHITE LED APPLICATIONS: A STUDY ON PHOTOLUMINESCENCE PROPERTIES

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ABSTRACT

The aim of the present work is to study the photoluminescence properties of Eu^{3+} doped $NaBaB_9O_{15}$ phosphors prepared by high temperature solid state reaction method. The PL spectra showed five emission bands around 590, 600, 611, 651 and 700 nm corresponding to the transitions from ground state ($^5D_0$) to various excited states, such as $^7F_0$, $^7F_1$, $^7F_2$, $^7F_3$ and $^7F_4$, respectively. The θ value was calculated to be 4.09, which indicated that concentration quenching occurred due to interaction between nearby Eu^{3+} ions. CIE 1931 color chromaticity system was employed to find the dominant emission color. All these optical properties have been studied as a function of Eu^{3+} ion concentration.

1. INTRODUCTION

In recent years, white light emitting diodes (W-LED) have been used widely as a potential candidate for solid state lighting owing to their benefits like less energy consumption, high stability, longer lifetime, high brightness and environmental friendliness. W-LEDs were usually made by coating YAG:Ce^{3+} yellow phosphor on the surface of InGaN blue LED chip. But, they have demerits like poor CRI and high CCT (>4500K), which constrain their versatility **[3].** Hence, there is a demand for red emitting phosphor to fabricate WLEDs with high CRI value. In order to overcome this difficulty, WLEDs can alternatively made either by combining near-UV LED chip and RGB (red, green, blue) phosphors or by coating blue LED chip with green and red phosphors. Therefore, finding novel efficient red emitting phosphor has received great importance in the field of solid state lighting technology.

Among all the trivalent rare earth ions (RE^{3+}), Eu^{3+} ions doped phosphor materials can act as a potential red component in the WLEDs fabrication due to their red emissions corresponding to $^5D_0 \rightarrow {}^7F_J$ (J=0,1,2,3 and 4) transitions.

Among the different phosphor hosts, borates have superior properties, such as low synthetic temperature, high luminescent brightness, large band gap and high chemical and physical stability. In the present work, the photoluminescence (PL) properties of $NaBaB_9O_{15}:xEu^{3+}$ phosphors were investigated as a function of Eu^{3+} concentration.

2. EXPERIMENTAL

A series of samples with general formula $NaBa_{1-x}B_9O_{15}:xEu^{3+}$ (x = 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 and 0.4) were synthesized by high temperature solid-state reaction. Hereafter, $NaBa_{1-x}B_9O_{15}:xEu^{3+}$ phosphors will be named as NBB:xEu^{3+}. The high purity chemicals like $NaCO_3$ (99.9%), $BaCO_3$ (99.99%), H_3BO_3 (99.9%) and Eu_2O_3 (99.99%) were used as starting materials. The chemicals mixed thoroughly in an agate mortar and the mixture was taken into an alumina crucible and sintered at 800 °C for 4 h. The final products were reground into fine powders for further optical measurements. The excitation and emission spectra were measured by using Edinburgh FS5 spectrophotometer, where 150 W Xenon lamp was used as an excitation source. The internal quantum efficiency (IQE) of the studied phosphor was measured by the same Edinburgh FS5 spectrophotometer equipped with an integrating sphere coated with $BaSO_4$.

3. RESULTS AND DISCUSSION

PHOTOLUMINESCENCE (PL) PROPERTIES OF NBB:xEu^{3+} PHOSPHORS

The crystal structure of $NaBaB_9O_{15}$ phosphor was clearly investigated by Zhuo et al. [2]. For the present work, the X-ray diffraction has been made seperatly for one sample and XRD spectrum was coincide well with the report by Zhuo et al. The XRD spectrum was given in supplementary for reference. Fig.1 shows PL and PL excitation (PLE) spectra of NBB:0.25Eu^{3+} phosphor recorded by kept excitation at 395 nm and emission at 613 nm, respectively.

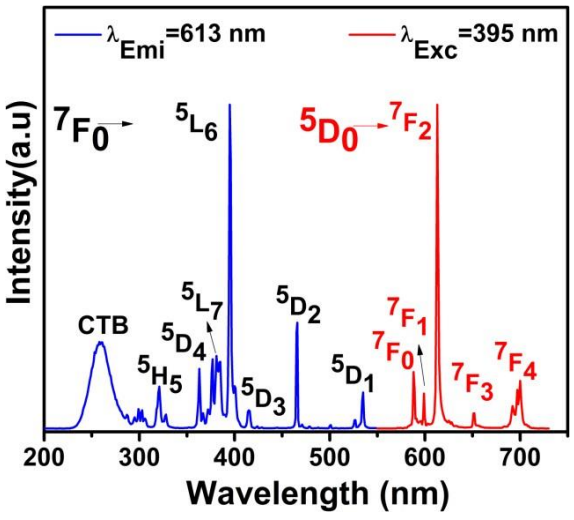

Fig.1: *Excitation and emission spectra of NBB:0.25Eu^{3+} phosphor*

The PLE spectrum shows broad band peaked at 260 nm, which is assigned to the CTB band due to charge transfer from 2*p* orbital of O^{2-} to empty 4*f* orbital of Eu^{3+} ions. Also, it shows sharp peaks centered around 325, 362, 380, 395, 413, 462 and 540 nm corresponding to the transitions from ground state ($^7F_0$) to various excited states, such as $^5H_3$, $^5D_4$, $^5L_7$, $^5L_6$, $^5D_3$, $^5D_2$ and $^5D_2$ states, respectively. The excitation band at 395 nm due to $^7F_0 \rightarrow {}^5L_6$ transition was found to be higher in intensity when compared to other transitions. The same peak position (395 nm) was used as an excitation wavelength to record PL spectra for NBB:*x*Eu^{3+} phosphors. The PL spectrum exhibits five sharp emission peaks around at 590, 600, 611, 651 and 700 nm attributed to the $^5D_0 \rightarrow {}^7F_0$, $^5D_0 \rightarrow {}^7F_1$, $^5D_0 \rightarrow {}^7F_2$, $^5D_0 \rightarrow {}^7F_3$ and $^5D_0 \rightarrow {}^7F_4$ transitions, respectively. The electric dipole (ED) transition $^5D_0 \rightarrow {}^7F_2$ peaked at 611 nm is sensitive to the ligand field and it is said to be a hypersensitive transition. Whereas, the $^5D_0 \rightarrow {}^7F_1$ transition found at 600nm is said to be magnetic dipole (MD) transition. Fig.2(a) represents the concentration dependent PL spectra of NBB:xEu^{3+} phosphors recorded under 395 nm excitation. The PL spectra showed that the emission intensity increases with the increase of Eu3+ ion concentration upto 25 mol% and falls down for higher concentration due to concentration quenching effect as shown in Fig.2(b).

Therefore, it is explored that 25 mol% is the optimal Eu^{3+} ion concentration. The luminescence quenching will occur due to non-radiative energy transfer between RE^{3+} either by exchange interaction or by multipole-multipole interactions. According to Blasses's theory, the critical distance (R_c) between sensitizer and activator Eu^{3+} ions can be expressed as follows [14, 15],

$$R_C \approx 2\left[\frac{3V}{4\pi x_c N}\right]^{1/3} \qquad (1)$$

where, *V* refers to the unit cell volume, *N* is the number of Eu^{3+} sites in the unit cell and X_c is the critical concentration. The *V*= 1857.28 and *N*= 6 values were taken from literature reported by Zhuo et al. [2]. The X_c value for the studied NBB:*x*Eu^{3+} phosphors was found to be 0.25. Then, the R_c value was calculated to be 13.3 Å, which is larger than 5 Å.

Hence, the exchange interaction did not work present case. This shows that multipole-multipole interaction may be responsible for energy transfer and the same was further lead to concentration quenching effect. Dexter theory [16, 17] gave the

relation between PL intensity (*I*) and activator concentration (*x*) as follows,

$$I/x = k[1 + \beta\ x^{\frac{\theta}{3}}]^{-1} \qquad (2)$$

where, *k* and *β* were constants. I is the emission intensity and x is the substituted mole fraction of Eu^{3+} ion.

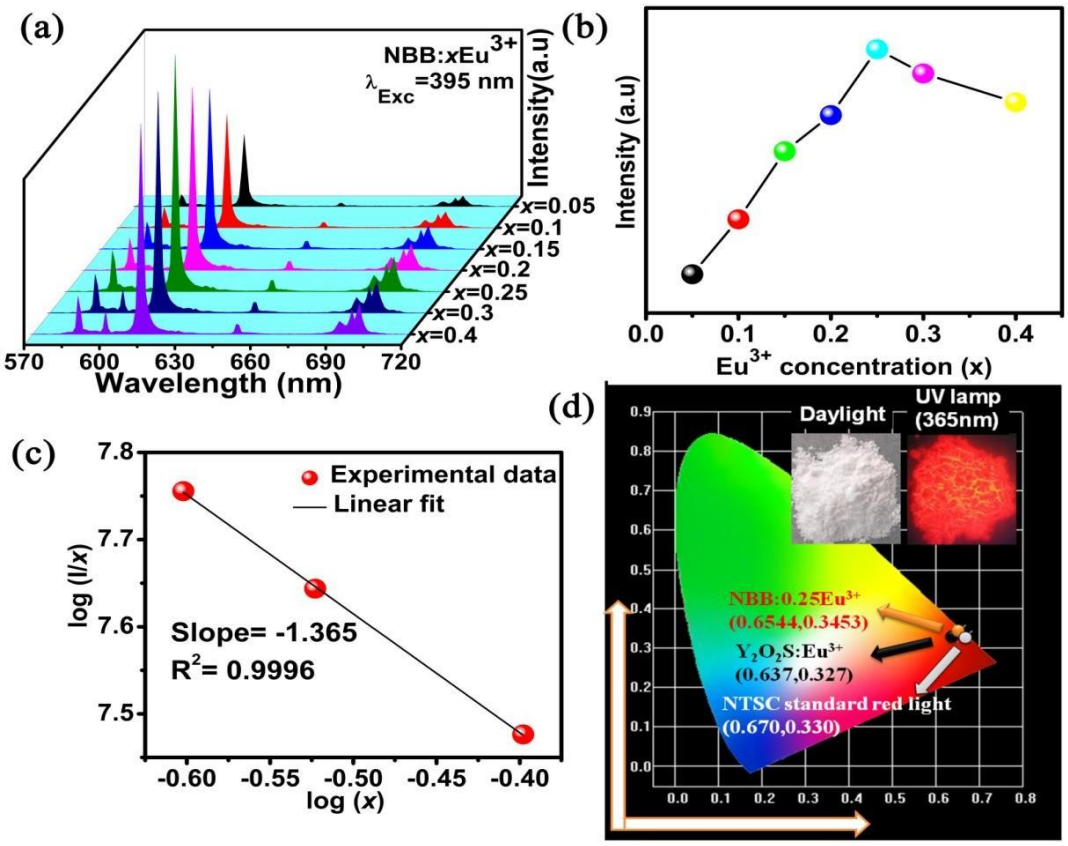

Fig. 2: *Concentration dependent PL spectra of* NBB:*x*Eu^{3+} phosphors *(a); The dependency of emission intensity on* Eu^{3+} *ion concentration (b); Linear fitting of log(I/x) vs. log(x) for NBB:xEu*$^{3+}$ *phosphors (c); CIE 1931 color diagram of NBB:0.25Eu*$^{3+}$ *phosphor (Insert shows the digital photograph taken in day light and UV lamp (d)*

The *θ* can have the values as 3, 6, 8 and 10 depending upon the exchange interaction, dipole-dipole, dipole-quadrupole and quadrupole-quadrupole interactions, respectively. The plot between *log(I/x)* and *log(x)* values was shown in Fig.2(c) and the slope value was estimated to be -1.635. Hence, the *θ* value was calculated to be 4.09, which is very close to the theoretical value 3. This result indicated that the exchange interaction is mainly contributes to the energy transfer mechanism.

CIE 1931 DIAGRAM

The dominant emission color of the studied phosphors can be obtained by examining the PL spectra by using CIE 1931 color chromaticity diagram. The x. y color coordinates were estimated from the PL spectra of NBB:*x*Eu^{3+} phosphors and the same was given in Fig.2(d) for 25 mol% as a representative case. The x, y color coordinates of NBB:025Eu^{3+} are located in the red region of CIE diagram and also found to very close to that of $Y_2O_2S:Eu^{3+}$ and National Television Standard Committee (NTSC) values for red phosphor. The studied NBB:0.25Eu^{3+} phosphor can be used as a potential candidate for near-UV LED based W-LEDs.

INTERNAL QUANTUM EFFICIENCY

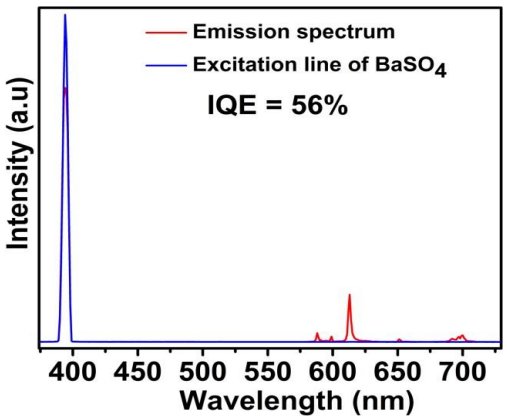

Fig. 3: *IQE spectra of* NBB:*0.25*Eu^{3+} phosphor

The internal quantum efficiency (IQE) was measured for optimal concentration NBB:0.25Eu^{3+} phosphor by using integrating sphere method. The IQE spectra of NBB:0.25Eu^{3+} phosphor were shown in Fig.3. From the spectra, the IQE value can be calculated from the equation given in the reported literature [20] and the value is found to be 56% for 25 mol% of Eu^{3+} doped NBB phosphor.

4. CONCLUSION

The NBB:*x*Eu^{3+} phosphors were successfully prepared by solid-state reaction method. From the photoluminescence studies, the optimum doping concentration of Eu^{3+} ions was found to be 25 mol%. Under the excitation of 395 nm, the IQE value of NBB:0.25Eu^{3+} phosphor was estimated as 56%. The (*x, y)* coordinates (0.6544,0.3453) of the NBB:0.25Eu^{3+} phosphor were passed through the red region of CIE diagram. These results suggested the fact that the studied NBB:0.25Eu^{3+} phosphor can be used as a efficient red component in the near-UV

based W-LEDs for solid state lighting applications.

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DEPICTION OF WOMEN CHARACTERS IN THE NOVELS OF CHETAN BHAGAT

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ABSTRACT

Chetan Bhagat is one of the most famous youth icons among the Indian society. This research paper tries to elucidate the feministic view in Chetan Bhagat's novel and the condition of women in his novels. He expresses his more importance for women rather than men. Chetan Bhagat tries to bring out the rights for women and exposes the thoughts of equality to men. He portrays most of the women characters as a modern girl. The novels of Chetan Bhagat are greatly influenced by the western world cultures like food habits, dresses and costumes etc. He brings out the emotions of feminine sex very lively and emotionally. In Chetan Bhagat's novel there is no discrimination between men and women by considering the masculine sex as superior and the feminine sex as inferior. In the global environment, the women are given their space, freedom and equality. This paper also depicts about the ideology of modernity through the female characters. Thus, Chetan Bhagat as a contemporary writer, he reflects the thoughts of the contemporary society. Chetan Bhagat through his novels attempts to exposes his idea for feminism by bringing the equality for education and financial freedom for women.

KEY WORDS

Freedom, Equality, Individuality, Women, Human Relationship

INTRODUCTION

The Word "Feminism" has its root from the Latin word "Femina" meaning "woman". The word feminism is introduced to achieve the goals of a woman for their social, political, cultural, and economic and individuality for women. It is to prove that both men and women are equal. The feminism has reaches its popularity from the 20th century. In most of the novels of Chetan Bhagat, he praises the female characters than the male characters.

Chetan Bhagat is a modern Indian writer who insists the patterns of gender equality, colour and language. He has been widely accepted as a writer for youth icon and most of the novel themes stick around a message for youths of an Indian society. He also depicts about the joint family system and nuclear family system in his novels. In joint family he lists out the broken relationship and also about the traditional values and system. Chetan Bhagat's novels are Revolution 2020 (2011), The Story of My Marriage: Two States (2009), One Night @ Call Center (2005), The Three Mistake of My Life (2008), Five Point Someone (2004), Half Girlfriend (2014), One Indian Girl (2016), What Young India Wants (2012) and Making India Awesome (2015).

Women Characters in the novels of Chetan Bhagat

The significance of the women characters are mainly implied in his novel "One Night @ Call Center". In the novel the feminine sex are given equal importance to the masculine sex. Even the feminine sex prefers to work in a call canter at night shift. It seems very common in the 20th century modern women. The girls can make their own residence depending upon their choice and career. In the novel Esha makes a reference that she bids a good bye to her family to achieve her burning desire to become a model. On the other hand Priyanka in the same novel proves that the women have their individuality to choose their own life partner without bothering about their family's wish. The modern girls surviving in the 20th century doesn't care about their parent's expectation. The same ideology is revealed through the characters Ananya and Krish who fights and convince their parents to accept the inter cultural marriage in the novel The Story of My Marriage: Two States. This makes evidence that Chetan Bhagat pays more importance to heroines that heroes like Shakespeare.

The concept of love, sex, marriage and break up are quite ordinary in the novels of all

age especially in Chetan Bhagat's novels. But only the people approaching method have changed.

> **"Love marriages around the world are simple: Boy loves girl. Girl loves boy. They get married. In India, there are a few more steps:**
> **Boy loves girl. Girl loves boy.**
>
> **Girl's family has to love boy. Boy's family has to love girl.**
> **Girl's family has to love boy's family. Boy's family has to love girl's family.**
> **Girl and boy still love each other.**
> **They get married". (Bhagat, Chetan. 2 States.)**

For example Vroom in One Night @ Call Center loved Esha deeply and proposed her. But he is continuously ignored by Esha. Though she rejects the proposal, they with holds a good and healthy relationship between them. The love between Shyam and Priyanka is also not a smooth relationship. Priyanka's mother is not happy with her daughters love. Because Shyam is working in a call center and it does not helps him to earn enough money. This shows the parental mind set of the 20th century. So, Priyanka is forced to break up with Shyam and suggested to marry Mr.Gupta, an NRI boy who earns surplus money to lead a luxurious life. But at the end Priyanka fights with her mother and marries Shyam. This proves that the women have their rights to decide their life partner.

The novels of Chetan Bhagat engage in displaying the broken family relationship in the contemporary and modern Indian society. In the novel One Night @ Call Center, Radhika is working in a call center at night shift and she is not happy with her mother in law. She proves to be a good wife and daughter in law but she is always criticised by her mother in law. She is very skilful and brilliant in handling the kitchen and managing the office also. She loves her husband very blindly but she is completely cheated by her husband. He has an illegal relationship with another girl. It proves to be a best illustration for the broken family

relationship. In the same way Ananya is also not cordial with Krish's mother before the marriage. Once, Ananya visited Krish family to attend a family function. Krish's family is a follower of Punjabi culture where the women must be an expert in cooking. Krish's mother asked Ananya to make a dinner for the whole family but she does not know. She struggles a lot and Krish has decided to help Ananya in the kitchen without making aware of his mother. Unfortunately it is noticed by Krish's mother and she shouted at them for making some nonsense in the kitchen. The kitchen scenario is explained below

We went to the kitchen. I took out the atta in a bowl.

"I have no clue how to knead this", she said.

It's ok. I've seen my mother do it. Let me try," I said and poured water into the bowl.And you fry the onions in… this?

Ananya pulled out a kadhai from the utensil shelf.

"Yes, please," I said and switched on the gas. I opened the box of spices. She didn't know how to use them……. A pungent smoke raise in the kitchen. Both of us had a coughing fit. What did you do? I said."I... don't… know" Ananya coughed uncontrollably.

My mother came into the kitchen. What are you doing? She ran to the stove and lowered the flame. "Who cooks on such a high flame? See, the spices have burnt. Ananya backed off from the stove. (Bhagat, Chetan. 2 States)

The novels of Chetan Bhagat imply that sex is the method of confessing their love in the modern century before the marriage. It becomes a trend among the modern Indian society. The lovers imagine that sex is the most required thing in love. They don't bother about whether they get married or not. But they have to make a sex before entering into the marriage life. By considering these events Chetan Bhagat poses a big question on women's virginity and chastity. Bhagat interrupts that this kind of activities affects the traditional values and customs. He

strongly depicts that it doesn't stand favourable to feminism. He also expresses his idea that involving in physical relationship before marriage is considered as a sin in Indian society. But he defines that he has to present the real incidents even that may create negative opinion about his novels. He strongly believes in his principle that the message should not be hidden from the readers. So, he presented the novel as it is.

Chetan Bhagat tries to explain that the girls should not be treated as a sex toy and says that the women too have some emotions and feelings in their heart. He insists that the girls should stop portraying as a eye-catching objects. Most of the protagonist in Chetan Bhagat's novel is portrayed as enjoying the pre-marital sex. The best illustration is taken from the novel Revolution 2020 and it is described below

> **I leaned over to kiss Aarti. She looked up at me in surprise. However, she did not protest. Just started. I kissed her again, more insistently. Nothing for two minutes and she was kissing me back. We kissed again and again. I kissed her lips,her cheeks, her forehead, her nose, her ears and her lips again. I switched off the lights. (Bhagat, Chetan. Revolution 2020)**

Bhagat is the first writer among his contemporaries to write about the pre-marital sex. The feminine sex has a lot of problems and they have to struggle a lot to achieve their dreams and sometimes they may even lead to sacrifice their family. Bhagat is a realist and so he pens down what he really mean in his mind. Chetan Bhagat is an Indian born author. He is a witness of family violence. He himself agrees that he has beaten his father in order to make him accept his marriage proposal with Ananya and he explained in his autobiographical novel The story of My Marriage: Two States. Chetan Bhagat is portrayed as a creator of new society where the youths can freely live their life and especially they can have pre-marital sex. He is criticised that he won't write about moral values and the readers cannot even pick a single good thing from his writings. Thus, Chetan Bhagat's women characters completely believe in "an absolute, a perfect, a pure and a noble freedom". Only the women take involvement in courtship. They change the usual system that men are the chasers and insists

that women are the chasers.

CONCLUSION

Chetan Bhagat through his novels attempts to exposes his idea for feminism by bringing the equality for education and financial freedom for women.

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