



SYNTHESIS AND CHARACTERISATION OF CU (II) SCHIFF BASE COMPLEX DERIVED FROM AMOXICILLIN TRIHYDRATE AND VANILLIN FOR ANTIMICROBIAL APPLICATION

AVILA JOSEPHINE.B¹, MARY TERESITA.V¹, ASHWINI ANANTHARAMAN¹,
RAJASEKAR.T², JERRINE JOESPH^{2*}

¹Department of chemistry, Stella Maris College, Chennai

²Col. Dr. Jeppiaar Research Park, Centre for Drug Discovery and Development,
Sathyabama University, Rajiv Gandhi Road, Chennai.

ABSTRACT

A new metal (II) complex of Schiff base were synthesized from the novel ligand 6-(2-(4-hydroxy-3-methoxybenzyl)benzylamino)-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-aza bicyclo[3.2.0]heptane-2-carboxylic acid (BAAMOTACA) derived from amoxicillin trihydrate and vanillin and the Schiff base is orange colour solid and having sharp melting point. The metal complex was investigated using spectroscopic techniques IR, ¹H NMR and magnetic measurements. Antibacterial and Antifungal activity of the Cu (II) Schiff base was done using Agar well diffusion methods. The IR spectra illustrated a bidentate ligand which coordinates through phenolic oxygen atom and imino nitrogen atom from azomethine bond. In ¹H NMR the azomethine proton signals in the spectrum of the Cu-BAAMOTACA (Cu-B) complex is shifted downfield. The molecular weight of the ligand was confirmed by GC-MS analysis and the retention time of the complex was found to be 17.27. The electron paramagnetic resonance spectral lines exhibited tetragonal distortion from axial symmetry, with $g_{\parallel} > g_{\perp}$ in the copper (II) complexes with diamagnetic behaviour. It may find a strong position in development of new pharmaceuticals especially as antibacterial and antifungal agents.

KEYWORDS: Antibacterial, Antifungal, Amoxicillin trihydrate, Vanillin, Novel ligand.



JERRINE JOESPH*

Col. Dr. Jeppiaar Research Park, Centre for Drug Discovery and Development,
Sathyabama University, Rajiv Gandhi Road, Chennai.

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INTRODUCTION

Metal Schiff base complexes are known since the mid nineteenth century¹ and Schiff base is a class of compounds derived by the chemical reaction (condensation) of primary amines with aldehydes or ketone to give a product known as imines which contains C=N bond that coordinate to metal ion and have been studied extensively. These imines are called Schiff bases since their synthesis was first carried out by a German chemist Hugo Schiff (1834-1915). Schiff base exhibit a range of biological activities, such as antibacterial, antifungal, antimicrobial, anticonvulsant, anti parkinsonian, anti HIV, anti inflammatory and antitumor activities². It forms a stable complex with different transition metal ions. In particular, the transition metal complexes have been the subject for thorough investigation because of their extensive application in wide ranging areas from material science to biological sciences. The pharmacological activity have been found to be highly dependent on the nature of the metal ion and the donor sequence of the ligands, different ligand shows different biological properties, though they may vary only slightly in their molecular structure³. Schiff bases and their metal complexes have paramount applications in the field of food and dyes industry, agriculture, analytical chemistry, catalysis, and polymer science⁴. In copper(II) complexes the d⁹ configuration makes the structure subject to distortions in octahedral and tetrahedral symmetries (Jahn-Teller effect). The distortion is usually seen as axial elongation, consistent with the lability and geometric flexibility of the complex. The complexes of copper with Schiff bases have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal; agrochemical, anti-inflammatory activity, antiradical activities and biological activities⁵. Amoxicillin(2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-(4-hydroxyphenyl)-acetyl] amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-24-carboxylic acid) is a member of penicillin group which are a very important class of β -lactamic antibiotics used to treat bacterial infections caused by microorganisms⁶. Amoxicillin requires stability for better antibacterial function which is achieved by complexation with metal ions. Such metal complexes are widely used as a model antibacterial agent due to their growth inhibiting activity towards bacteria and fungi⁷. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla which is one of the most widely used and important flavouring materials worldwide⁸. Vanillin displays antioxidant and antimicrobial properties and it

may be used as a food preservative. It is a tracer component in many dairy products, such as butter, ice cream and pastry products. Moreover, vanillin is used for other purposes, such as a constituent in cosmetics and drugs preparations⁹. Recently coordination compounds have been known to be useful in constructing molecular information processing systems, particularly by biological self-organizing processes¹⁰⁻¹¹. In this present to focus on synthesis and characterisation of cu (ii) schiff base complex derived from amoxicillin trihydrate and vanillin for antibacterial and antifungal activity.

MATERIALS AND METHODS

Vanillin and amoxicillin trihydrate were used to prepare the Schiff base that was mixed with Cu (II) Sulphate in order to synthesize their complex. All reagents and chemicals were of analytical grade, commercially available, and were used without further purification. The IR spectra were recorded on a Perkin Elmer spectrophotometer (FT/IR-RX1) instrument in anhydrous KBr pellets in the range 500-4000 cm⁻¹. ¹H NMR was recorded on Bruker Advance III 400MHz in DMSO solvent and TMS as the internal standard. The GC-MS analysis was performed at SAIF, IIT Madras, by means of a JEOL GCMATE II GC-MS high resolution, double focusing instrument and the Electron Impact method was followed. The ESR spectrum was recorded with Bruker EMX EPR spectrometer instrument.

Synthesis of the Schiff base ligand

6-(2-(4-hydroxy-3-methoxybenzylideneamino)-2-(4-hydroxyphenyl) acetamido)-3, 3-dimethyl-7-oxo-4-thia-1-aza-bicyclo [3.2.0] heptane-2-carboxylic acid.

Baamotaca

Firstly, the Schiff base was prepared from 80 mL methanol containing 1.64g (1mM) Amoxicillin trihydrate and 20mL methanol where has been dissolved in advance 2.44 g (1mM) Vanillin. In order to obtain the pH value between 7 and 8, 1.0 M NaOH solution was added and the mixture was refluxed a time of 5 hours. The volume of the solution was reduced to one half by evaporation when an orange precipitate was formed. It was filtered, washed with methanol and dried in vacuum at room temperature under anhydrous CaCl₂. Recrystallization from a mixture of ethanol – water (50:50) gave the Schiff base. Melting point-182°C (Figure 1).

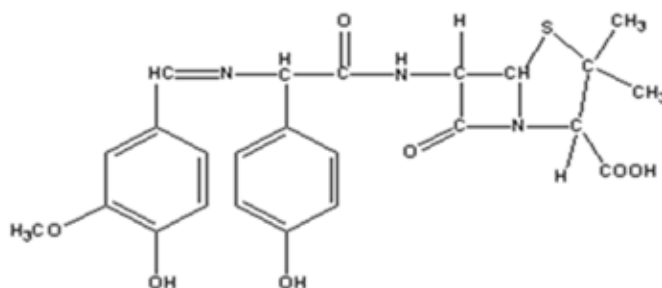


Figure 1
Structure of the Schiff base Ligand (BAAMOTACA)

Synthesis of the metal complex (Cu-BAAMOTACA)

The metal complex were prepared by mixing 0.400g (2.0mM) Schiff base with 25 mL methanol containing $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0mM). The mixtures pH value was adjusted to 7-8 by adding 1.0M NaOH solution and then they were refluxed a time of 2 hours. Finally, the volume of solutions was reduced to one half by evaporation, and

in both cases, an occurrence of colour precipitate was observed this being assigned to Cu (II) complex respectively. It was filtered, washed with methanol and dried in vacuum (Figure 2). Recrystallization from hot methanol gave the metal complexes. Melting point-198°C

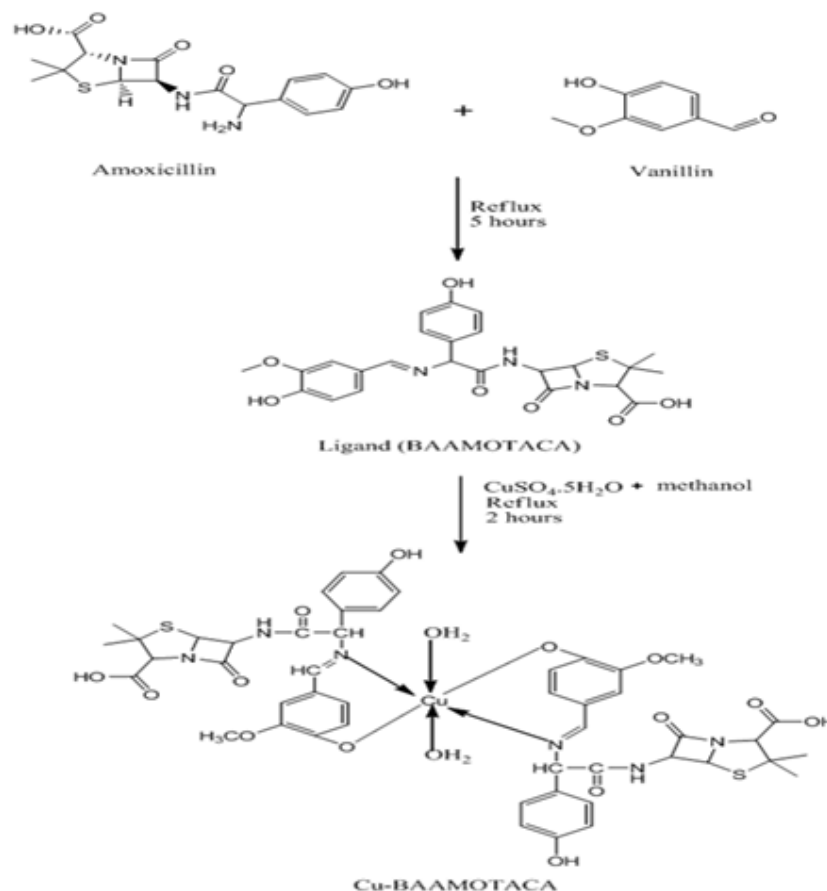


Figure 2
Reaction for the formation of Copper complex (Cu-BAAMOTACA)

Test organisms

The antibacterial activity of synthetic compounds was assessed against five bacteria species: *Bacillus cereus* (MTCC 1306), *Klebsiella pneumonia* (MTCC 530), *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC1304), *Staphylococcus aureus* (MTCC 1430) maintained in nutrient agar plate and two fungal pathogens: *Candida albicans* (MTCC 854) and *Cryptococcus sp* (MTCC 7075) maintained in Sabouraud dextrose agar (SDS). Overnight cultures were kept for 24h at 37°C±1°C bacteria and 28°C for 24 hours fungal pathogens. After 24 h of incubation, bacterial and fungal suspension (inoculums-10⁸ CFU/mL turbidity = McFarland barium sulfate standard (0.5) was diluted with sterile physiological solution for the well diffusion tests.

Agar well-diffusion method for determine the antimicrobial activity and antifungal activity

Muller-Hinton agar (MHA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and fungal pathogens are *Candida albicans* and

Cryptococcus neoformans plated on Sabouraud dextrose agar (SDS). Three Wells (5mm diameter) were made in each plates using sterile cork borer. Stock solution of each synthetic compound was prepared at a concentration of 5 mg/ml. About 50 µl synthetic extracts were added into the wells and allowed to diffuse at room temperature for 2hrs. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and and 28°C for 48 hours fungal pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

RESULTS AND DISCUSSION

The Schiff base ligand was prepared by refluxing the appropriate amount of amoxicillin with vanillin in methanol and its structure was established by IR spectrum and ¹H NMR (Figure 1). The metal complex of Schiff base ligand were prepared by Cu(II) Sulphate with the ligand in a molar ratio of 1 : 2. The complex were obtained as air-stable amorphous solids which are insoluble in water, partially soluble in methanol and ethanol, totally soluble in *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO).

IR Spectral Studies

The FT-IR spectrum of ligand (BAAMOTACA) is shown in Fig. 3. The spectrum shows an intense band at 3183cm^{-1} and 1511cm^{-1} which corresponds to the stretching and bending vibrations of (N-H) secondary amine group present in the ligand. The ligand also shows an absorption frequency at 1591cm^{-1} is due to C=N stretching vibrations. The absorption peaks at 1668cm^{-1} , 1157cm^{-1} and 588cm^{-1} corresponds to the stretching vibrations of C=O, C-N and C-S bonds present in the ligand. The aromatic C=C stretching vibrations are observed at 1459cm^{-1} . The ligand also shows a characteristic peak at 1266cm^{-1} due to alkyl aryl ether group. The FT-IR spectrum of [Cu (BAAMOTACA)] complex is shown in Fig.4 and the corresponding absorption band frequencies of the complex is given in Table 1. The complex shows the main bands at 3339 , 1596 and 1165cm^{-1} of the free Schiff base, they are attributed to $\nu(\text{OH})$, $\nu(\text{C}=\text{N})$ azomethine and $\nu(\text{CO})$ phenolic, respectively. On comparing the FT-IR spectrum of the ligand and the

complex (Fig. 3 and 4), the ligand shows a band at 3183cm^{-1} whereas it is not therein the spectrum of the complex, this confirms that deprotonation has taken place on the phenolic groups. This is further supported by the shift of the band 1165cm^{-1} by 35cm^{-1} in comparison with the ligand which corresponds to $\nu(\text{CO})$. As a result of deprotonation of both-OH groups present on the phenyl ring the ligand is coordinated to copper through the phenolic oxygen group. The spectra of metal complexes exhibited a broad band around 3339cm^{-1} , which is assigned to water molecules, $\nu(\text{H}_2\text{O})$, associated with the complexes. Coordinated water molecules exhibited $\rho(\text{H}_2\text{O})$ rocking near 818cm^{-1} and $\rho(\text{H}_2\text{O})$ wagging near 563cm^{-1} . IR spectra of the ligand shows a band at 1591cm^{-1} which is attributed to $\nu(\text{HC}=\text{N})$ azomethine group, which is shifted to a higher value in the complex at 1596cm^{-1} this confirms that the ligand is also coordinated to the metal ion through nitrogen atom from azomethine group. Thus it confirms that the ligand is coordinated to copper through nitrogen and oxygen.

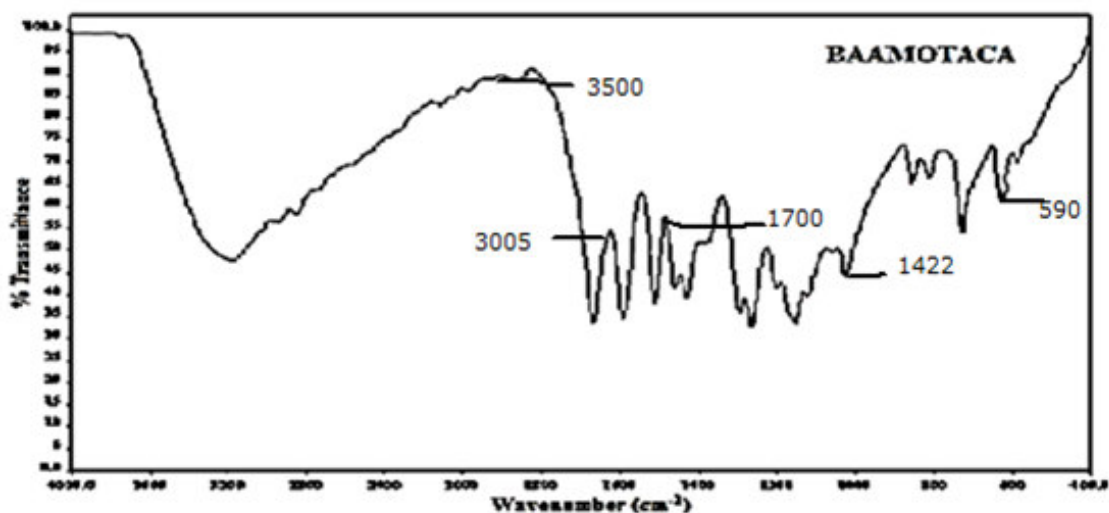


Figure 3
FTIR spectrum of Ligand (BAAMOTACA)

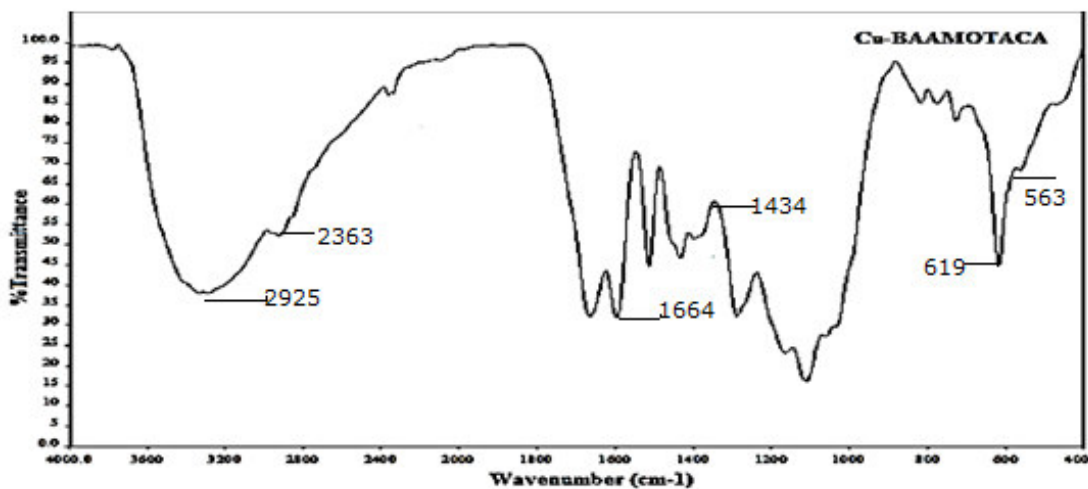


Figure 4
FTIR Spectrum of Cu- BAAMOTACA

Table 1
FTIR absorption band frequencies of [Cu (BAAMOTACA)] complex

Complex	Frequencies(cm ⁻¹)
C=N stretching	1664
C-H stretching	2925
C=C stretching	1434
C-H stretching (CH ₃)	2363
C-H bending (CH ₃)	1434
Cu-O stretching	563
Cu-N stretching	619

¹H NMR Spectral studies

The ¹H NMR spectrum of the Ligand (BAAMOTACA) is shown in Fig. 5 It shows that the protons are present at

different chemical and magnetic environments in the ligand. The ligand shows a singlet at 9.7 δ which is due to the presence of -OH proton.

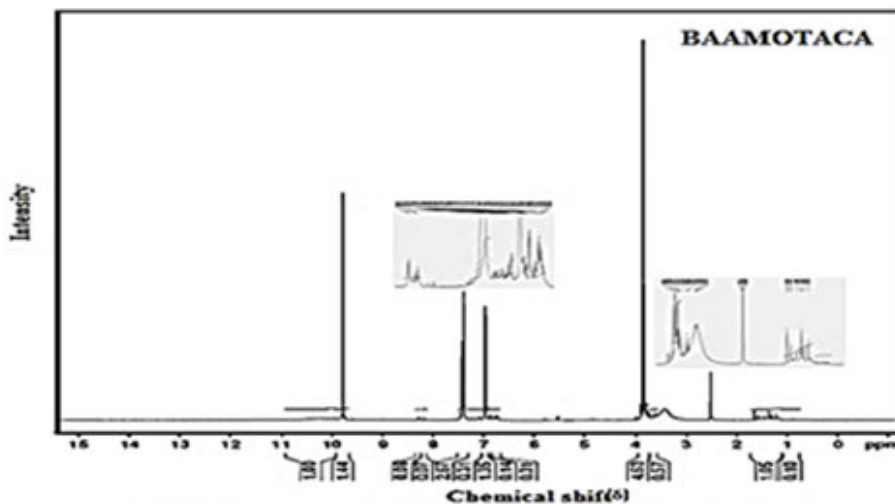


Figure 5
¹H NMR Spectrum of Ligand (BAAMOTACA)

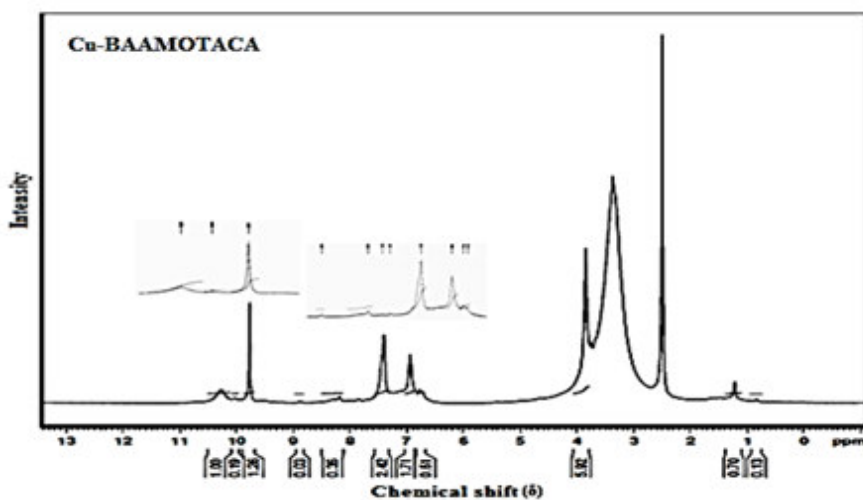


Figure 6
¹H NMR Spectrum of Cu-BAAMOTACA

Table 2
¹H NMR Chemical shift values of [Cu(BAAMOTACA)] complex

Complex	Chemical Shift (δ ppm)
C-OH	9.7ppm
-C=N	8.8ppm
Ar-H	6.9ppm
S-CH	3.8ppm
CH ₂	2.4ppm

A doublet peak at 8.29 δ is due to the presence of proton on C=NH group in the compound. The spectrum also shows a doublet at 6.9 δ is due to the presence of aromatic protons of the phenyl ring in the ligand. The spectrum at 3.8 δ correspond S-CH proton of dihydrothiazine ring and the peak at 2.5 δ correspond to the protons of the methyl group in the ligand. ^1H NMR spectrum of [Cu(BAAMOTACA)] complex is shown in Fig. 6. The chemical shift values of the complex obtained from ^1H NMR spectrum is given in Table 2. The spectrum was recorded in DMSO solution which shows a singlet at 2.4 δ due to the methyl protons. The ^1H NMR Spectra of the Schiff base complex exhibits signals at 8.8 δ and 7.3 δ which corresponds to CH=N- and -NH protons respectively. The azomethine proton signals in the spectrum of the corresponding complex is shifted downfield compared to the free ligand; this is due to the deshielding effect of the azomethine group which is

coordinated to the metal ion. There is no appreciable change in all other signals of the complex.

GC-MS Analysis

The GC curve obtained as a result of the analysis of the ligand was found to contain peaks with two different retention times. The peak that corresponds to the retention time, 18.95, was found to be that of the ligands, while the structure of the compound whose peak corresponds to the retention time, 17.27, could be a factor of impurity (Figure.7). The mass spectrum of the ligand showed a peak at m/z 499.54, which corresponds to the mass of the molecular ion (Figure.8). The base peak was observed at m/z 52.9, which corresponds to the mass of a cyclobutadiene fragment. The mass spectrum of the compound with the retention time, 17.27, showed the molecular ion peak at m/z 307.3, while the base peak was observed at m/z 73.62 (Figure.9).

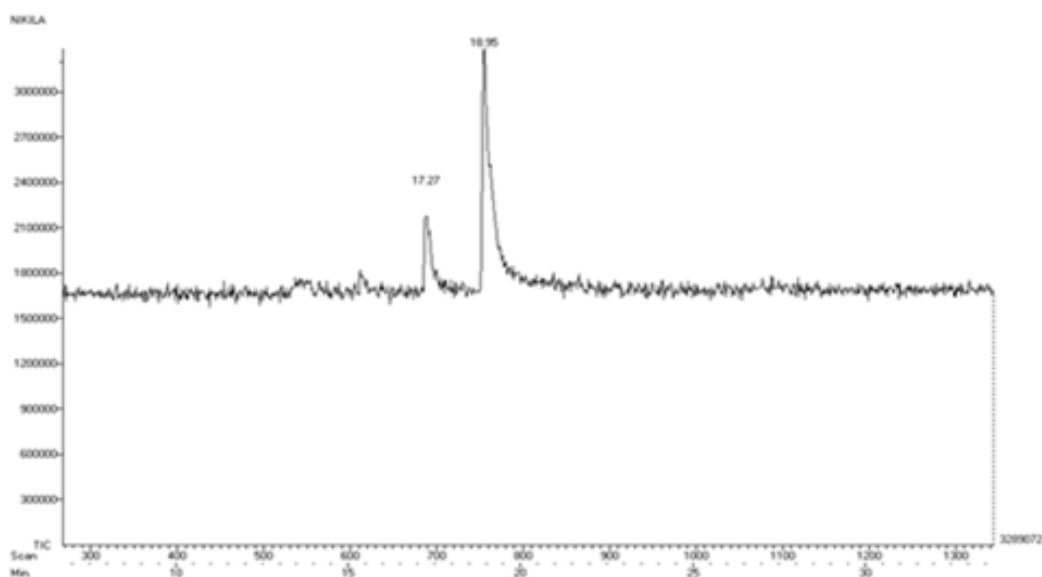


Figure 7
Gas Chromatogram of the Ligand (BAAMOTACA)

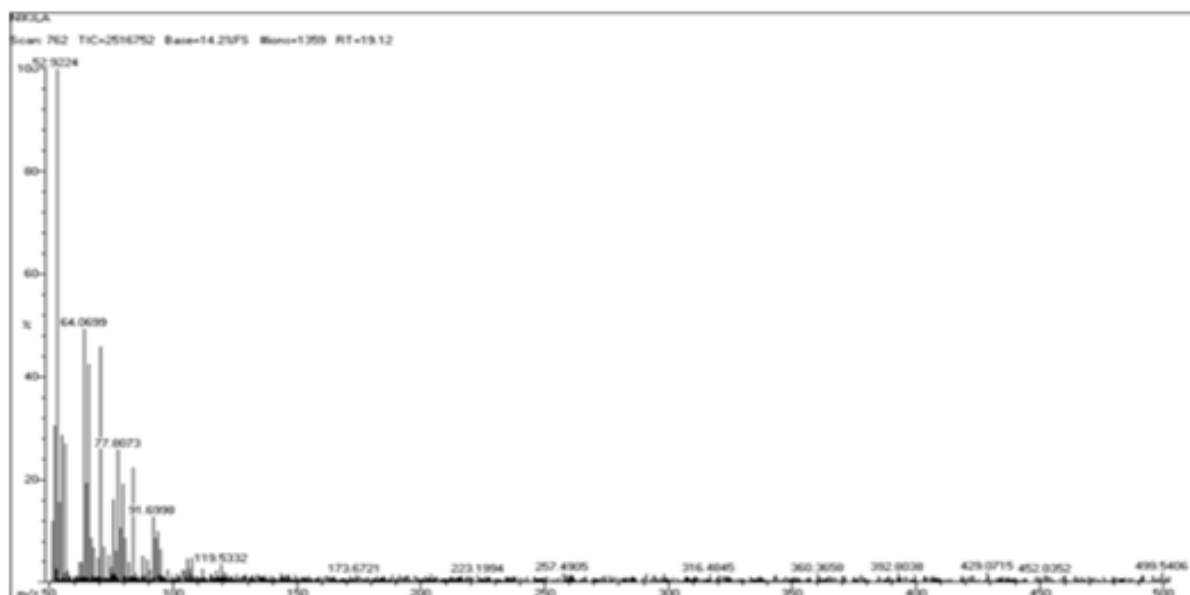


Figure 8
Mass Spectrum of the Ligand (BAAMOTACA)

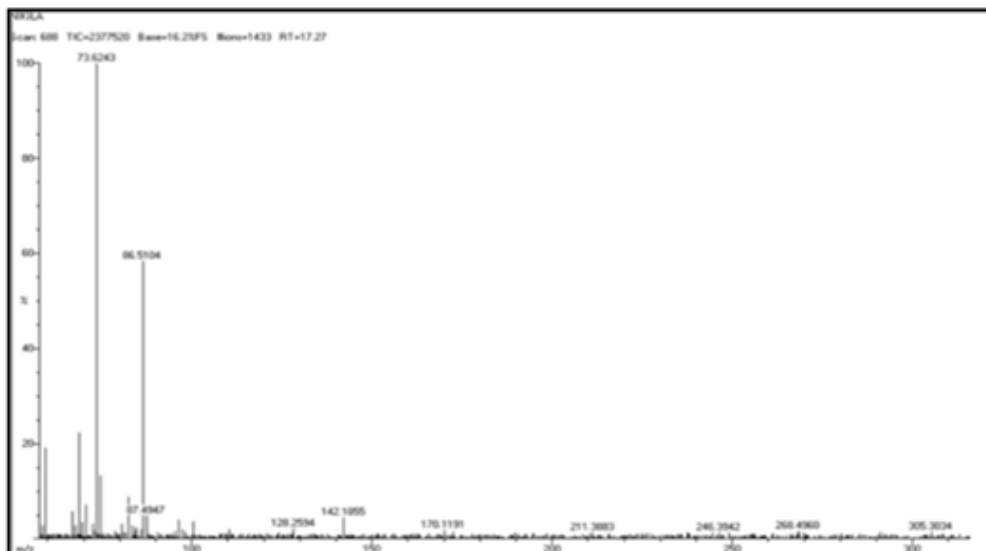


Figure 9
Mass Spectrum of the complex [Cu(BAAMOTACA)]with the retention time

Electron spin resonance spectral studies

The complex [Cu(BAAMOTACA)] was characterised by solid state Electron Spin Resonance (ESR) studies. This is used to study the paramagnetic nature of the metal ion present in the complex. The ESR spectrum of the complex was recorded at 9450.508MHz which is shown in Figure.10. The spectrum of the complex exhibited an intense broad signal with a shoulder peak and it was also found to be an anisotropic in nature. The center field of the broad signal is found to be at 282.244 mT and for shoulder peak the center field is 316.883mT. This

gives two characteristic g values g_{\parallel} and g_{\perp} . The g_{\parallel} is found to be at 2.3755 and g_{\perp} is found to be at 2.1308 for the complex. The g_{\parallel} value indicates that the unpaired electron is localized in dx^2-y^2 orbital of the Cu (II) ion and a g_{\perp} value for the broad peak passing through the abscissa with distorted signals are observed. Since the g_{\parallel} and g_{\perp} matches with the reported values the structure of [Cu(BAAMOTACA)] complex is found to be tetragonally elongated geometry is confirmed.

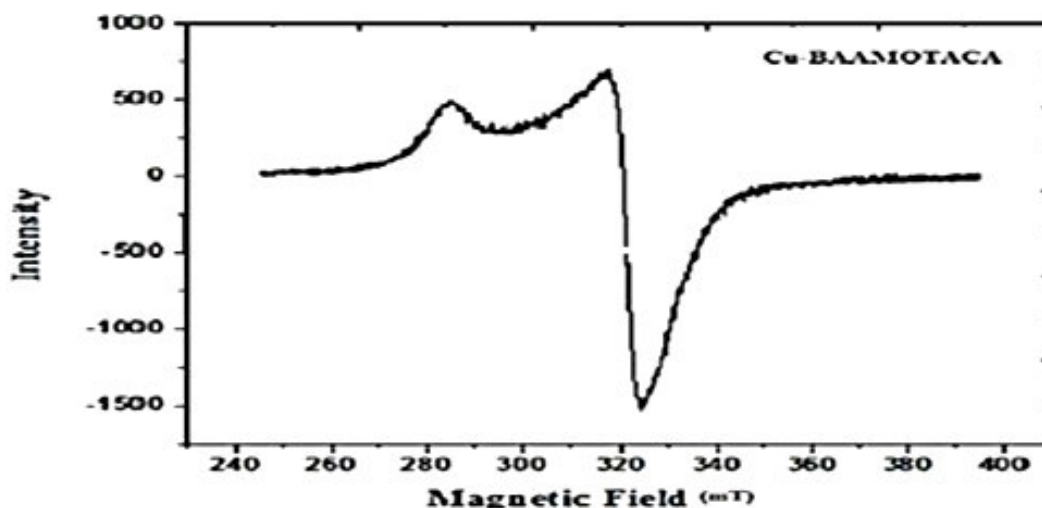


Figure 8
EPR Spectrum of the copper complex [Cu (BAAMOTACA)]

Antibacterial and antifungal activity Cu-BAAMOTACA

The antibacterial activity of synthetic compounds were observed using Agar well diffusion method by measuring the diameter of the growth inhibition zone. The results are showed in Table 3. Cu-B showed

antibacterial activity against *P. aeruginosa* (2.2cm), *B. Cereus* (2cm), *S. aureus* (1.8cm), and *E. coli* (2cm). Not showed antibacterial activity against *K.pneumonia*. Cu-B showed an activity against both *Candida albicans* (CA) and CN-*Cryptococcus neoformans*.

Table 3
Antibacterial and Antifungal activity of Cu-BAAMOTACA

S.No	Bacteria	Diameters (in cm) of the Zone of inhibition of the bacterial and fungal strains (\pm SD) Zone of inhibition in cm
1	<i>Pseudomonasaeruginosa</i> MTCC 1688	2.2 \pm 0.05
2	<i>Klebsiellapneumoniae</i> MTCC 530	ND
3	<i>Bacillus cereus</i> MTCC 1306	2 \pm 0.08
4	<i>Staphylococcus aureus</i> MTCC 1430	1.8 \pm 0.05
5	<i>Escherichia coli</i> MTCC1304	2 \pm 0.01
6	<i>Candida albicans</i> MTCC 854	2.8 \pm 0.01
7	<i>Cryptococcus sp</i> MTCC 7075	2 \pm 0.05

Note: ND: Not detected.

CONCLUSION

Metal complexes play a significant role in catalysis, biochemistry, environmental applications, and molecular sensors and have innumerable scope for the future research. In this present work, Cu-BAAMOTACA complex was prepared with different ratios of the metal ligand in 1:2 respectively. The ligand was prepared and characterized by FT-IR and ^1H NMR. The presence of the functional group and the nature of the proton bonded to carbon, nitrogen and oxygen was confirmed from FT-IR and ^1H NMR spectrum. The formation of the complexes involves the nucleophilic condensation of both the ligands amoxicillin and vanillin to form Schiff base which then coordinate to the copper metal. The synthesized complexes involves were characterized using different technique like FT-IR, ^1H NMR and ESR. FT-IR gives information about the functional group and metal nitrogen and metal oxygen bond present in the complexes. In ^1H NMR the azomethine proton signals in the spectrum of the Cu-BAAMOTACA

complex is shifted downfield compared to the free ligand; this confirms the azomethine group is coordinated to the metal ion. The molecular weight of the ligand was confirmed by GC-MS analysis. The ESR spectrum confirms paramagnetic nature of the complex and the g_{\parallel} is found to be at 2.3755 and g_{\perp} is found to be at 2.1308 for the complex and the $g_{\parallel} > g_{\perp}$ values confirms the structure of $[\text{Cu}(\text{BAAMOTACA})]$ complex is tetragonally elongated geometry. Copper (II) Schiff bases complex showed a good an antibacterial and antifungal activity. In this complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms. It may find a strong position in development of new pharmaceuticals especially as antibacterial and antifungal agents.

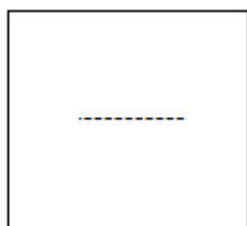
CONFLICT OF INTEREST

Conflict of interest declared none.

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