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Synthesis and Characterization of Novel Schiff base Cu(II) Complexes: Antimicrobial and Molecular Docking Studies

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Abstract N_2O_2 type complexes of Cu^{+2}ion have been synthesized by the reaction of Salicylaldehyde / 3,4-diaminobenzophenone / acetyl acetoneand glutaric anhydride. The ligands and respective metal complexes was established through spectroscopic data (FT-IR, UV-Vis,^{1}H NMR and ^{13}C NMR). They are non-electrolytic in nature as their molar conductivities (Λ_M) in DMSO of 10^{-3} M solution from the EPR study the complexes proposed to be octahedral geometry. All the metal complexes have been screened for their antibacterial

activity andthe predicted binding affinity using molecular docking studies.

Keywords: EPR, 3,4-diaminobenzophenone, Antimicrobial Activity, Molecular Docking.

1. INTRODUCTION

Schiff base metal complexes have a huge number of synthetic uses in organic chemistry. Acylation of Schiff bases by acid anhydrides, acid chlorides and acyl cyanides is initiated by attack at the nitrogen atom and leads to net addition of the acylating agent to the C=N bond. Reactions of this type have been used for good purpose in natural product synthesis [1]. Copper is well known for bioessential element. Itscomplexes have proven to be an excellent for biological importance due to their binding ability and positive redox potential [2-5] Cu(II) metal complex exhibits the fastest water exchange rate for any transition metal hydro complexes. In recent years, many research had been carried out to study the uses of copper containing coordination complexes in various fields like medicinal, bioinorganic, catalytic and analytical chemistry

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[6-7].This paper concentrates on the synthesis and biological activity of Schiff baseligandsand theircopper (II) complexes.

2. EXPERIMENTAL

All the chemicals used were of analytic grade, and were purchased from Sigma-Aldrich. Metal salt waspurchased from E. Merck and was used as received. All solvents used were of standard/spectroscopic grade.

2.1 Synthesis of Ligand L_1/L_2

Schiff base ligandwas synthesized by a hot ethanolic solution of Acetyl acetone / Glutaric anhydride, Salicylaldehyde. They were mixed slowly with constant stirring. To the above mixture was added an ethanolic solution of 3,4-diaminobenzophenone. Their molar ratio is 1:1:1 temperature was maintained at 70^0 C for 2.30 hrs. in the presence of Concentrated Hydrochloric acid. On cooling the substance 24 hrs. at 0^0C crystalline compound was separated out [8–10] This was filtered, washed with ethanol and thendried.

2.2 Synthesis of metal complexes (ML_1/ ML_2)

Hot ethanolic solution of Schiff base ligand $L_1/L_2$and copper nitrate hexahydrate in 1:1 molar ratio were mixed together. The reaction mixture was refluxed at 70^0 C for 2.30 hrs. The quantity of the reaction mixture was decreased to around 20-25%. The precipitate that formed was filtered off and washed with ethanol and dried using anhydrous $CaCl_2$.[8-10]Solubility's of metal complexes were checked with various solvents these are insoluble in H_2O, $CHCl_3$, CCl_4, CH_3CN and partially soluble in ether, alcohol but freely soluble in DMF and DMSO.

2.3 Characterization Techniques

The IR spectrum was recorded using KBr pellets in the range of 4400-400 cm^{-1}. UV-Visible spectra were recorded on Perkin Elmer Lambda 3B UV-Visible Spectrophotometer in the range 200-900 nm. The molar conductance were measured using 10^{-3}M solution of DMSO at 25^0C using an Elico CM-180 Conductivity meter and Elico type CC-03 Conductivity cell of cell constant 1.05 cm^{-1}.The ^1H&^{13}C NMR spectra of the ligand was recorded in Joel 500 MHz NMR spectrometer using $(CD_3)_2SO$. The mass spectra of the complexes were recorded by JEOL GC mate Mass Spectrophotometer. Magnetic susceptibility was measured at room temperature on a Gouy balance using $CuSO_4.5H_2O$ as a callibrant. The EPR spectra of the complex were recorded in DMF at room temperature on JEX-X3 Series of a system using the DPPH as the g-marker.

Antimicrobial activity were tested by using agar well diffusion method and molecular docking studies were recorded using AutoDockVinaPyRx software.

3. RESULT AND DISCUSSION

Salicylaldehyde based metal complexes have a lot of uses including biological and analytical chemistry. The synthetic routes of the ligands and complexes are presented in scheme 1.

Scheme 1

Step 1:

Schiff base ligands

1. Salicylaldehyde + 3,4-diamino benzophenone + acetylacetone→ L_1
2. Salicylaldehyde + 3,4-diamino benzophenone + glutaric anhydride → L_2

Step 2:

Metal Complexes

$$Cu(NO_3)_2.6H_2O + L_1/L_2 \rightarrow ML_1/ML_2$$

ESI Mass spectra

The purity of the ligand (L_1 / L_2) 98.62% has been verified by HPLC. The ESI-mass spectrum (Fig. 1&2) of the ligand (L_1 / L_2) shows a parental ion

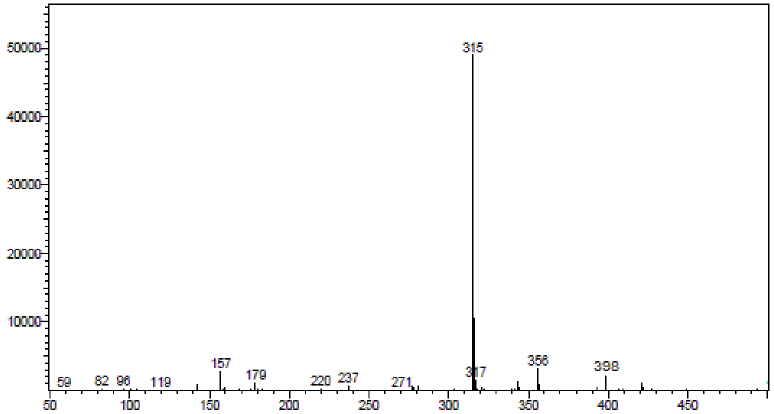


Figure 1: ESI Mass spectrum of Ligand (L_1).

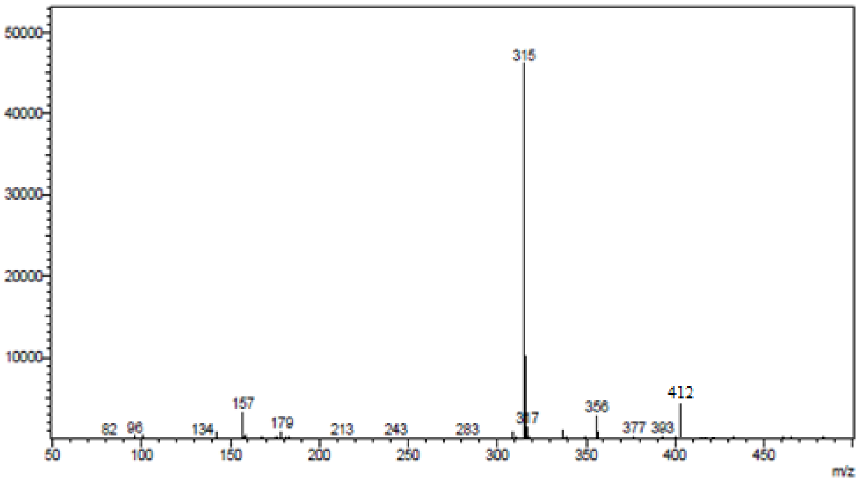


Figure 2: ESI Mass spectrum of Ligand (L_2).

peaks (M^+) *m/z* = 398 and 412 respectively. A base peak is present at *m/z* = 315 (85%) for both the ligands. This peak is corresponds to cationic species with three aromatic rings.

IR spectra

The IR spectra of Schiff base ligand and its metal complexes Table 1 (Fig. 3-6) shows that ν(C-O) and ν(C=N) modes appear at 1287-1327cm^{-1} and 1607-1618cm^{-1} respectively. The shifting of (C-O) to higher frequency as compared to the ligand (1287cm^{-1}) is owing to the conversion of hydrogen bonded structure into a covalent metal bonded structure[11]. 2924-3070 cm^{-1} are corresponding to C-H stretching of aromatic ring [12].(M-L) bond is further confirmed by the appearance of a medium intensity band in the range 474-478 and 522-536cm^{-1} in the spectra of the complexes allotted to stretching

Table 1: Molar conductance and Electronic Spectroscopic Data of the Schiff Base Ligands and its metal complexes.

Compound	Molar conductance Ω-1**cm2mol**-1	Colour	M.P (°C)	Yield (%)	Λmax (nm)
Ligand(L_1)	–	Off white	237	68	252,345
Ligand(L_2)	–	Off white	221	62	267,350
$[Cu(L_1)(NO_3)_2].xH_2O$	10.54	Dark brown	294	50	367,431,660,730
$[Cu(L_2)(NO_3)_2].xH_2O$	12.23	Dark brown	>300	48	310,415,540,645

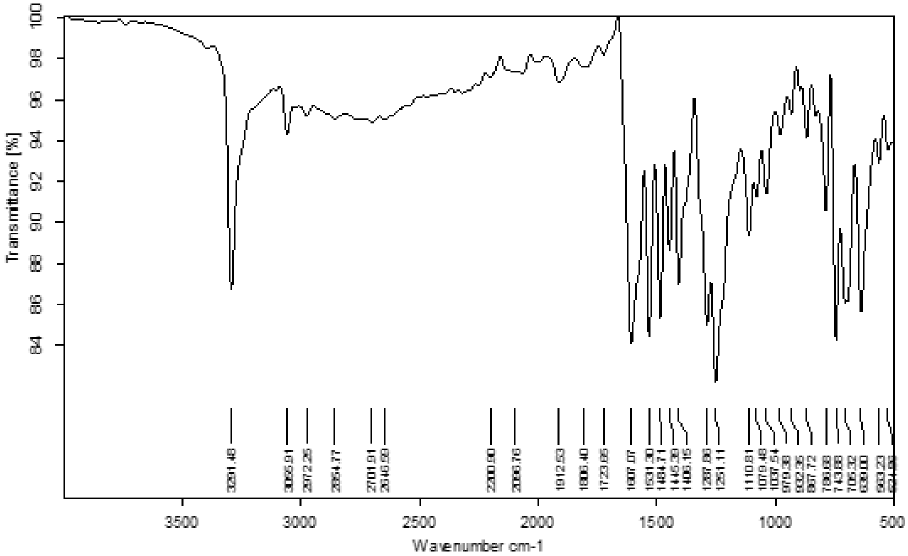


Figure 3: IR spectrum of L_1 Schiff base.

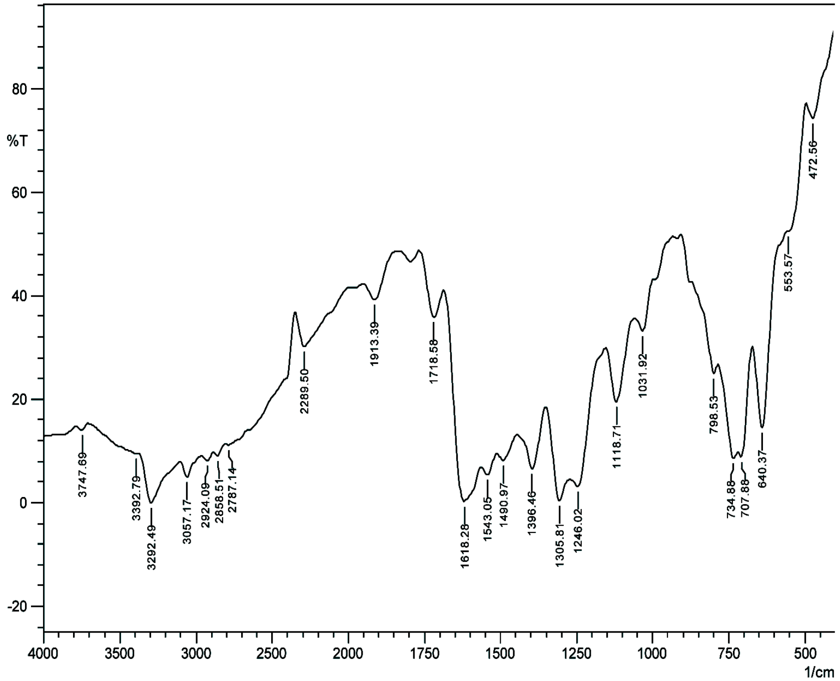


Figure 4: IR spectrum of L_2 Schiff base.

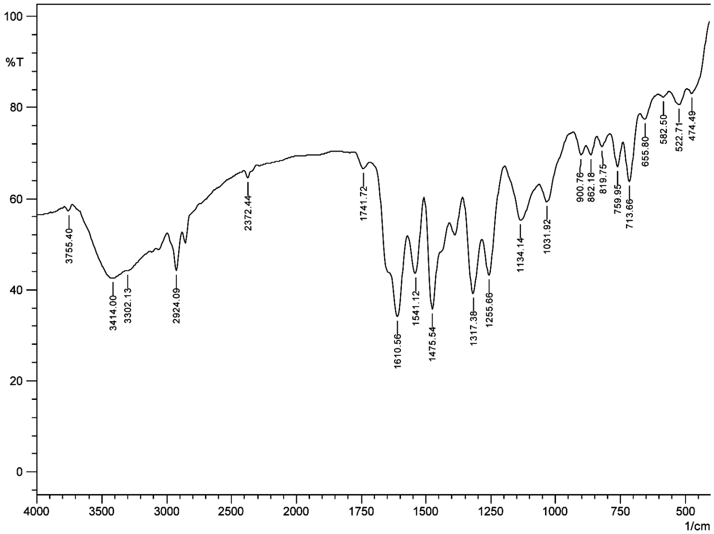


Figure 5: IR spectrum of $[Cu(L_1)(NO_3)_2].xH_2O$.

frequencies of (M-N) bond and (M-O) bond formation respectively [13]IR spectra of the nitrato complexes, display three medium intensity bands due to (N-O) stretching in the region ~1382–1384 cm^{-1}, suggesting that both the nitrate groups are coordinated to the central metal ion [14]

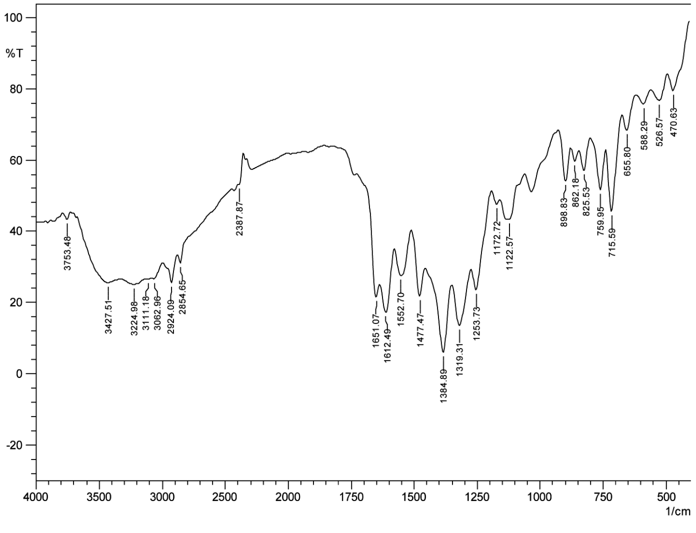


Figure 6: IR spectrum of $[Cu(L_2)(NO_3)_2].xH_2O$.

Electronic Spectra

The electronic spectrum of the Cu(II) complexes (Fig. 7-8) shows two absorption bands at 252, 345 nm and 267,350 nm for $L_1$and L_2 respectively. The first band arise from π-π* transition with the azomethine chromospheres [11].The second band is due to the n-π* transition. From the complex formation the absorption bands undergo a redshift compared to the free ligand as a result of coordination trough the nitrogen atoms of the C=N group. Electronic spectral data of ligand and metal complexes are shown in table 2.

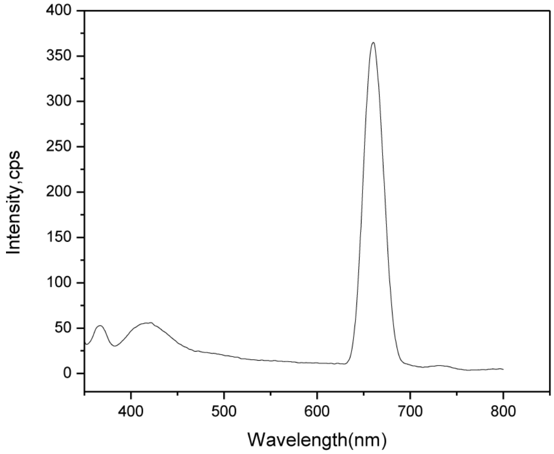


Figure 7: Electronic spectrum of $[Cu(L_1)(NO_3)_2].xH_2O$.

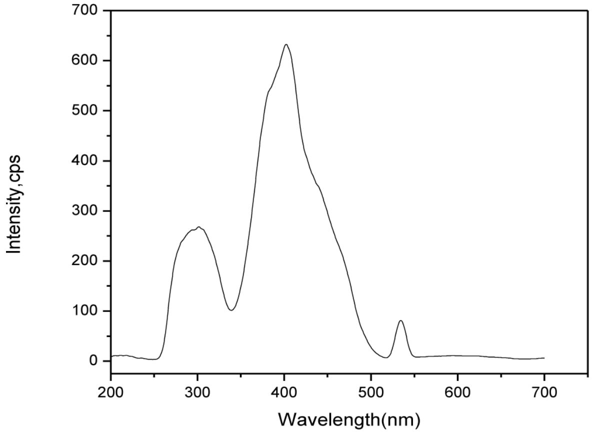


Figure 8: Electronic spectrum of $[Cu(L_2)(NO_3)_2].xH_2O$.

Table 2: Infrared Spectroscopic Data of the Schiff Base Ligand and its metal complex.

Compound	ν (C=N)	ν (C-O)	ν (M-N)	ν (M-O)	Ionic nitrate
Ligand(L_1)	1607	1287	–	–	–
Ligand(L_2)	1618	1305	–	–	–
$[Cu(L_1)(NO_3)_2].xH_2O$	1610	1317	474	522	1384
$[Cu(L_2)(NO_3)_2].xH_2O$	1612	1319	470	526	1384

NMR Spectra

^{1}H NMR spectrum of ligand (L_1 and L_2)(Fig. 9-10) shows signals at 8.10 ppm due to Ar-CH=N [15]A sharp multiplet signals7.03 – 7.81 ppm due to Ar-H. A singlet corresponding to one proton observed at 12.80-13.20 ppm is due to Ar-OH. As well as in the ^{13}C NMR spectrum of ligands (Fig. 11-12) indicated new resonance are 19.05(C-CH_3), 56 (C-CH_2-CO), 113-138(C=C), 158.45(C=N), 195.95(Ph-CO-Ph)[16].

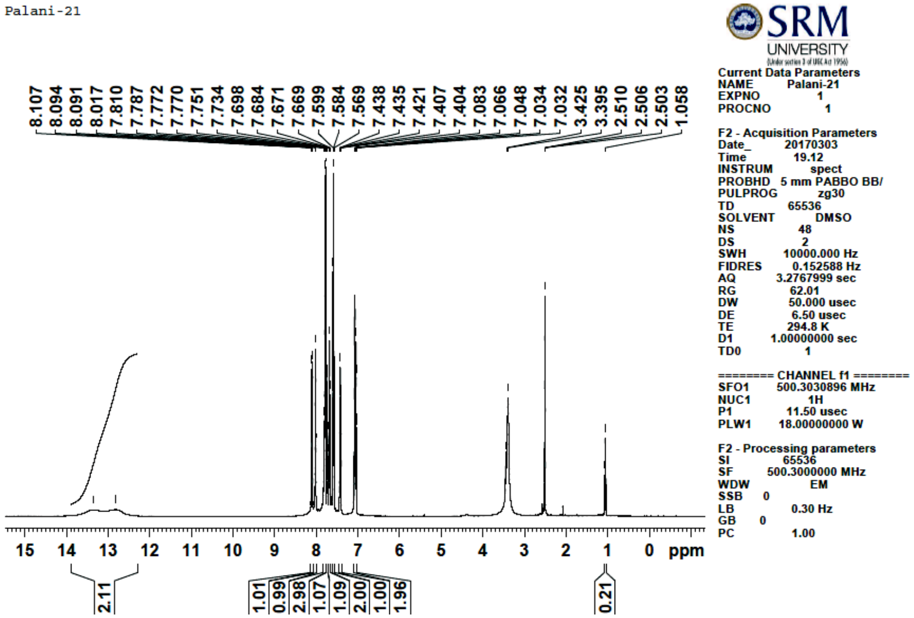
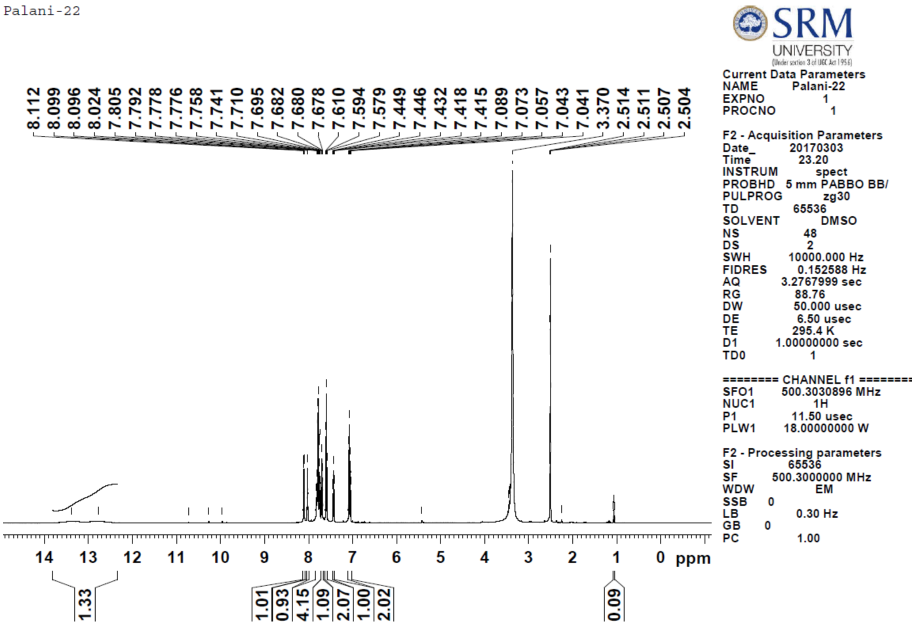


Figure 9: ^{1}H NMR spectrum of ligand (L_1).



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FIDRES     0.152588 Hz
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RG             88.76
DW            50.000 usec
DE              6.50 usec
TE             295.4 K
D1        1.00000000 sec
TD0                1

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P1             11.50 usec
PLW1     18.00000000 W

F2 - Processing parameters
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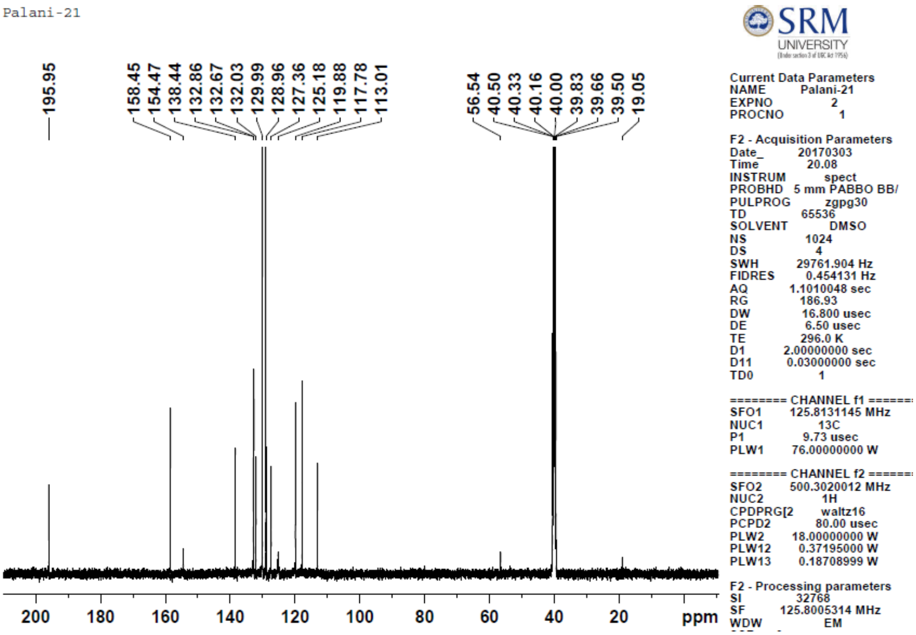
GB     0
PC              1.00
```

Figure 10: ^{1}H NMR spectrum of ligand (L_2).

Molar conductance and EPR Study

On the basis of molar conductance measurements (Table 1) of the copper (II) complexes in DMSO corresponds to be non-electrolytic in nature of the complexes. So the complexes may be formulated as $[M(L_1)(NO_3)^{-}_2].xH_2O$and $[Cu(L_2)(NO_3)^{-}_2].xH_2O$where L_1 is (E)-4-((5-benzoyl-2-((E)-(2-hydroxy benzylidine) amino) phenyl)imino)pentan-2-one and L_2 is(E)-6-((4-benzoyl-2-((E)-(2-hydroxyl benzylidine)amino)phenyl)imino) tetrahydro-2H-pyran-2-one.

The magnetic moment measurement ofboth the complexes at room temperature lie in the range of 1.83 –1.85 B.M. [17,18] Electronic spectrum (Fig.7-8) of six coordinated copper complex display bands at 730,660 and 431nm for $L_1$and 645,540 and 415 for L_2corresponding to the following transitions $^2B_1g \rightarrow {}^2B_2g$; $^2B_1g \rightarrow {}^2Eg$ and$^2B_1g \rightarrow {}^2A_1g$. The EPR spectral study (Fig.13-14) provides information of the metal ion environment. The spectrum of the complexes showed bandsg|| >g ⊥ >ge, indicating that unpaired electron is localized in the dx dx^2-y^2 orbital [19].In the Cu(II) complex G=(g||-2)/(g ⊥-2), which is more than 4 suggesting that there is no interaction between the copper centers [20] Thus theabove results suggest that Cu(II) complexes possesses distorted octahedral structure.



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TD              65536
SOLVENT          DMSO
NS               1024
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SWH       29761.904 Hz
FIDRES     0.454131 Hz
AQ        1.1010048 sec
RG             186.93
DW          16.800 usec
DE            6.50 usec
TE            296.0 K
D1       2.00000000 sec
D11      0.03000000 sec
TD0                 1

======== CHANNEL f1 ======
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NUC1              13C
P1            9.73 usec
PLW1      76.00000000 W

======== CHANNEL f2 ======
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NUC2               1H
CPDPRG[2      waltz16
PCPD2         80.00 usec
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PLW12      0.37195000 W
PLW13      0.18708999 W

F2 - Processing parameters
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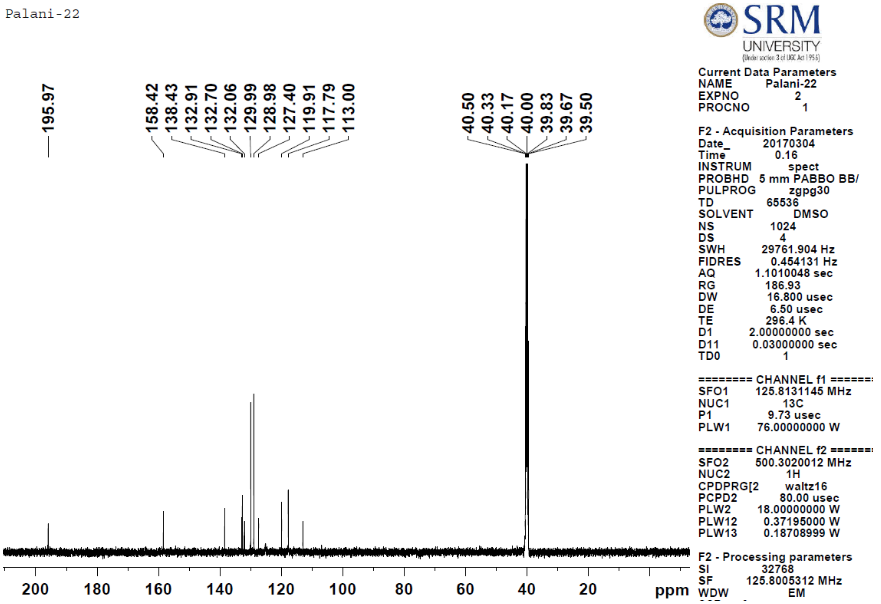
Figure 11: ^{13}C NMR spectrum of ligand (L_1).



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SOLVENT          DMSO
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DS                  4
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FIDRES     0.454131 Hz
AQ        1.1010048 sec
RG             186.93
DW          16.800 usec
DE            6.50 usec
TE            296.4 K
D1       2.00000000 sec
D11      0.03000000 sec
TD0                 1

======== CHANNEL f1 ======
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NUC1              13C
P1            9.73 usec
PLW1      76.00000000 W

======== CHANNEL f2 ======
SFO2     500.3020012 MHz
NUC2               1H
CPDPRG[2      waltz16
PCPD2         80.00 usec
PLW2      18.00000000 W
PLW12      0.37195000 W
PLW13      0.18708999 W

F2 - Processing parameters
SI              32768
SF       125.8005312 MHz
WDW                EM
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Figure 12: ^{13}C NMR spectrum of ligand (L_2).

Figure 13: EPR spectrum of $[Cu(L_1)(NO_3)_2].xH_2O$.

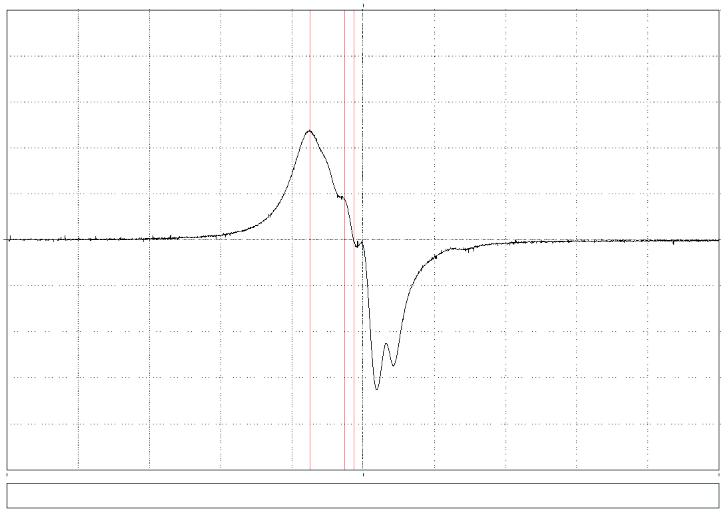

Figure 14: EPR spectrum of $[Cu(L_2)(NO_3)_2].xH_2O$.

4. ANTIMICROBIAL ACTIVITY

Antimicrobial activity was calculated as described by [21] Surface of the Mueller Hinton Agar (MHA) plates. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested the zone of inhibition was measured with a measuring scale. The antimicrobial activity of the ligand and itsCu(II) metal complexes were assayed against Gram-positive and Gram-negative bacteria. The result of antimicrobial activity are summarized in Table 3 and Fig. 15. From the data it is clear that the metal complex are effective against bacteria.

Table 3: Diameter of zone inhibition (mm) for the Schiff base ligand and its complexes.

Microorganism	Escherichia coli			*Staphylococcus*			*Enterococci*			*Pseudomonas*		
	25	50	100	25	50	100	25	50	100	25	50	100
Ligand(L_1)	–	5mm	9mm	8mm	10mm	11mm	4mm	7mm	13mm	–	–	–
$[Cu(L_1)(NO_3)_2].xH_2O$	–	–	–	–	–	–	–	–	5 mm	–	–	4mm
Ligand(L_2)	–	5mm	7mm	–	–	–	–	–	–	–	–	6mm
$[Cu(L_2)(NO_3)_2].xH_2O$	–	5mm	9mm	–	–	–	–	4mm	6mm	–	–	3mm

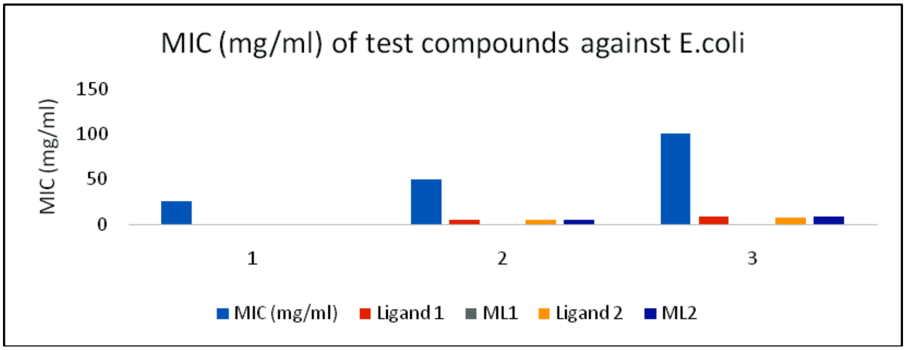

Figure 15: Comparison of MIC (mg/ml) of macrocyclic ligand and its Cu(II) Complexes against E.Coli.

5. MOLECULAR DOCKING STUDY

The biological importance of the ligands are assessed by performing docking studies using AutoDockVinaPyRx software[22]The retrieved pdb file (4s1y) is given as input in AutoDockVina and assigned as macromolecule that adds charges and hydrogen bonds to the atoms thus preparing the protein. Ligand preparation including the generation of various tautomers, assigning bond orders, ring conformations and stereo chemistries of the ligand were carried out. All the conformations generated were further used for docking study. A receptor grid was generated around the protein active site by selecting the active residues (His 288, Met 298, Met 329, Met 548) and Run autogrid option. The docking calculations were performed using Run Vina and the Binding affinity was used to determine the best docked structure from the output. The predicted binding affinity is in kcal/mol. Thepdb structure 4s1y [23]of human serum albumin is used for docking studies which plays a key role in increasing the growth and productivity of cells and increases overall cell health. The best docked complex selected has a binding score of -10.7 for Cu(II) complex of ligand 2 which predicts a good inhibition. The pdbstructure 4sy1 of human serum albumin is used for the docking studies with the Copper complexes. The following table shows the binding affinity of ligandwith 4sy1.

Docking score using Autodock Vina with the macromolecule 4s1y

Ligands /Complexes	Binding Affinity (kcal/mol)
Ligand(L_1)	−7.9
Copper (II) complex of L_1	−9.0
Ligand (L_1)	−9.2
Copper (II) complex of L_2	−10.7

The docked ligand (L_1) interacts with the protein by forming three H bonds with the residues Ser192 and Glu292 with bond distances 3.33Å and 3.53Å respectively (Fig. 16). Similarly Copper (II) complex(ML_1) also forms 3 H-bonds with the protein in residues Glu292, Gln196, Lys 199 with bond distances 3.43 Å, 3.09Å and 3.21Å respectively (Fig. 17).

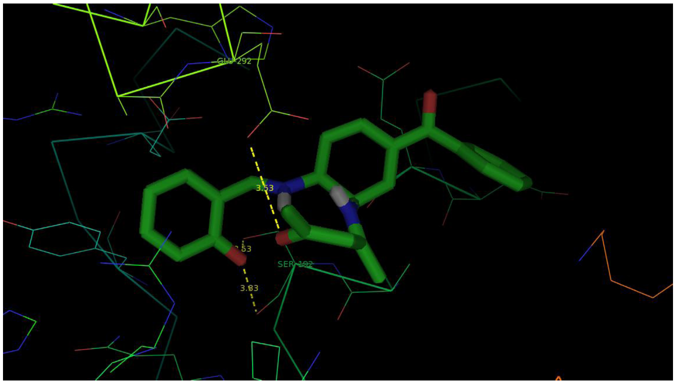

Figure 16: Ligand (L_1) docked with 4s1y showing formation of hydrogen bond and distances.

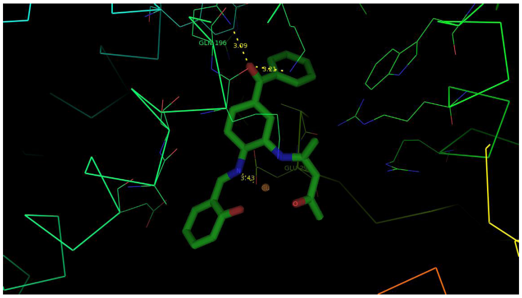

Figure 17: Copper (II) complex(ML_1) docked with 4s1y showing formation of hydrogen bond and distances.

The docked ligand (L_2) interacts with the protein by forming four H bonds with the residues Lys195 Å, Asp451 Å, Arg222 Å and Lys 92 Å with bond distances 3.12 Å, 3.49 Å, 3.21 Å and 3.36 Å respectively (Fig. 18). Copper (II) complex (ML_2) forms 3 H-bonds with Asp108, Asn429 and His146 residues with bond distances 3.22 Å, 3.11 Å and 3.22 Å respectively. (Fig. 19).

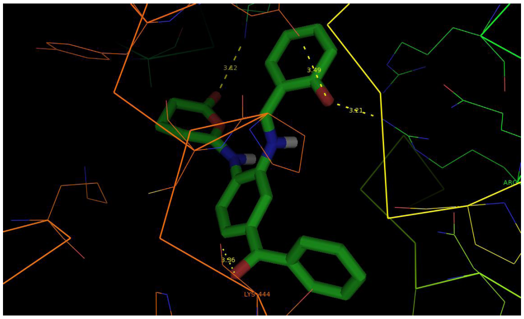

Figure 18: Ligand (L_2) docked with 4s1y showing formation of hydrogen bond and distances.

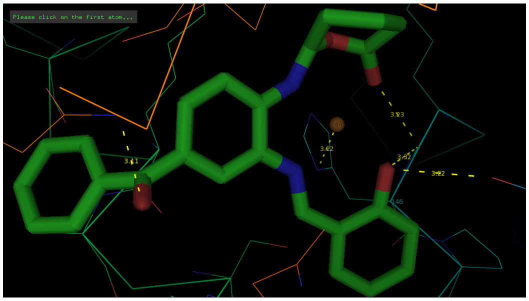

Figure 19: Copper (II) complex(ML_2) docked with 4s1y showing formation of formation of hydrogen bond and distances.

CONCLUSIONS

The formation of Copper (II) complexes are thermally stable. The complexes were characterized by spectral and analytical data. Based on the spectral data anCu(II) complexes assigned to thedistorted octahedral geometry, based on the biological study the complexes aregood antimicrobial agent. Binding affinity and inhibition level were determined using molecular docking study.

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