SYNTHESIS, CHARACTERIZATION, CYTOTOXIC STUDIES AND ANTI-MICROBIAL EVALUATION OF METAL COMPLEXES WITH A DERIVATIVE OF 4-AMINOANTIPYRINE

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Abstract

Metal(II) chelates of Schiff bases derived from the condensation of 4-aminoantipyrine with 2- amino 3-pyridine and characterized by ^{1}H NMR, IR, UV-Vis and EPR spectral studies. The complexes are of the type $M(X\text{-}4AAP)_2$ [where M = Cu(II), Co(II)), Ru(II), & VO (IV)] . The metal atom is coordinated to nitrogen and oxygen atoms of the Schiff base ligand. The free ligand and its metal complexes were screened for their antibacterial activity. The results indicate that the copper complexes are having ligand significant activity than its ligands against the some microorganisms under identical experimental conditions.They also exhibit enzymatic properties. Anticancer activity of ligands and their metal complexes and evaluated in human breast cancer cells. The preliminary bioassay indicates that the Schiff base and its complexes exhibit good inhibitory activity against the human breast adenocarcinoma cancer cell lines.

Key words: *4 –Aminiantipyrine*, 2- amino 3- pyridine , *Antimicrobial studies*, *Cytotoxic Studies*

1. INTRODUCTION

Schiff bases are significant class of compounds which can be used in a variety of studies, such as organic synthesis, catalyst and drug design [1–3] and models for active sites of metalloenzymes [4]. They are the most versatile group of chelators for facile preparation of metal–organic hybrid materials [5–10], single molecule based magnets [11–16], highly porous materials [17,18], optoelectronic devices [19–21], and sensors [22–24]. The synthesis of 4-aminoantipyrine derivatives have attracted the attention of several research groups due to their potential biological activities [25]. In this context, broad spectra of bioactive 4-aminoantipyrine derivatives and their metal complexes have been investigated and diversities of bioactivities such as analgesic [26-27], antiinflammatory, antimicrobial [28-29], and anticancer activity [30] have been reported.

The increasing microbial resistance to antibiotics is a very important necessity which inculcates the search for new compounds with potential effects against pathogenic bacteria and fungi [31,32]. The most spectacular advances in medicinal chemistry have been made when heterocyclic compounds played an important role in regulating biological activities. Heterocyclic moieties can be found in a large number of compounds which display biological

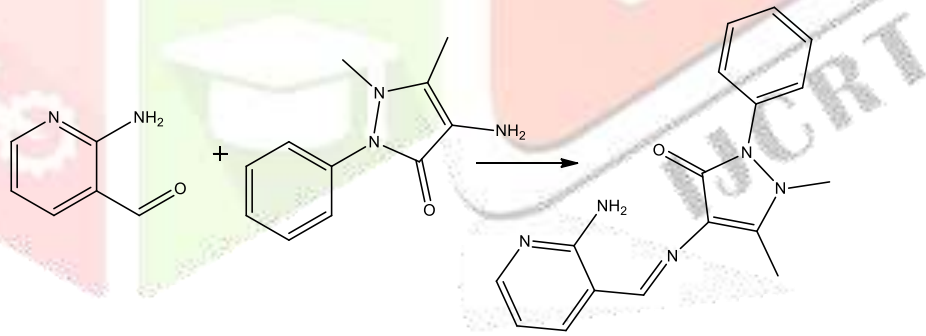
activity [33]. Antipyrine (N-heterocyclic compound) and its derivatives exhibit a wide range of biological activities and applications. Because of their interesting structural features as well as the biological activity, a wide range of metal complexes derived from antipyrine derivatives have been reported [34,35]. Pyrazolone-based ligands display variable complexing behavior and a variety of coordination possibilities to metal centers.
In view of biological importance of Schiff base derived from the condensation of 4-aminoantipyrine derived with various subsitution its applications in various fields, in the present investigation it is thought to synthesize the metal complexes with transition metal ions such as Co(II), VO(IV), Cu(II) and Ru(II). It is therefore of interest to carryout investigations to understand how a ligand environment and its metal complexes show the activity in bacterial and fungal acitivity and the spectral properties show the interest in cytotoxic studies of the inorganic complex.

2. EXPERIMENTAL & Methods

In Chapter II, details of the instruments used for various physical measurements (**IR, NMR, UV-Vis, EPR**) and biological studies (Antimicrobial, Anticancer activities) are explained.

2.1 Synthesis of ligand [(E)-4-((2-hydroxy-5-methylbenzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one]

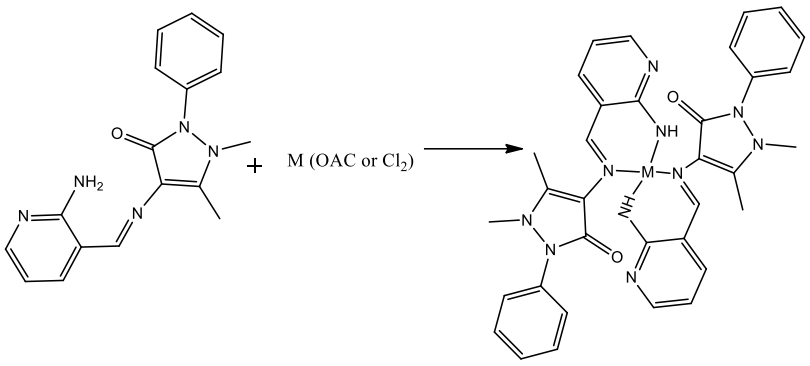
A solution of 1-phenyl-2,3-dimethyl-4-amino-3-pyrazolin-5-one (0.203 g, 1 mmol) in ethanol (5 ml) was added to a solution of 2- Amino 3- pyridine carboxaldehyde (0.193 g, 1 mmol) in ethanol (5 ml). The reaction mixture was stirred for 2 h at room temperature then heated to reflux for 2 h and kept at 273 K for 4h. The characteristic pale-green precipitate obtained was filtered and recrystallised by dissolving in methanol (m.p. 438 K). Yield: 83 %.



Scheme 1: Synthesis of ligand

2.2 Synthesis of metal complexes

An ethanol solution of Metal (II) acetate (1 mmol, 15 mL aqueous ethanol) was added dropwise to a stirred ethanol solution of the Schiff base ligand. The resulting solution was gently heated for 5 h with constant stirring. The precipitate solid was filtered, washed with hot water, and then ethanol followed by ether and dried in vacuo. Yield: 70%; M.p. >250ºC. The complex is soluble in DMF and DMSO, and is partially soluble in chloroform and methanol.



Scheme – 2 Synthesis of Metal Complexes

2.3 Determination of antimicrobial activity

The in-vitro biological activity of the Schiff base and its metal complexes in DMSO were tested against the bacteria and fungi by disc diffusion method using nutrient agar as medium and Amikacin and ketakonazole as control. The inhibition zone was developed at which the concentration was noted. The antimicrobial activity was estimated based on the size of inhibition zone in the disc[13-16].From the results, the activity index was calculated using the following formula.

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

2.4 In vitro assay for Cytotoxicity activity (MTT assay).

The anticancer activity of samples on MCF7 cells was determined by the MTT assay (*Mosmann et al*.,1983). Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO_2 incubator for 72 hours. Then, add various concentrations of the samples in 0.1% DMSO for 24hrs at 5 % CO_2 incubator. After removal of the sample solution and 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the samples on the proliferation of MCF7 cells was expressed as the % cell viability, using the following formula:

Calculation

% cell viability = A540 of treated cells / A540 of control cells × 100%

Graphs were plotted using the % of Cell Viability in Y-axis and concentration of the sample in X-axis.Cell control and sample control were included in each assay to compare the full cell viability in cytotoxicity and anticancer activity assessments

2.5 FITC Assay method

(Ethidium Bromide(EB) / Acridine Orange (AO) method)

Suspend 1×10^6 cells/ml in 100µl warm medium in 96 titer plate. Add treated compound/drugs 24 hrs Incubation.Add 50 µl/ml of EB/AO mixed solution.Incubate cells at 37ºc at 5% Co_2 for 30 mins.Wash cells once by adding 200 µl of warm PBS.View cells under florescence microscopy using blue filter (510-590 nm).

3. Results and discussion

Analytical, colour and magnetic susceptibility data of all metal complexes are given in Table 1 and are in good agreement with proposed composition.

Table 1: Colour, Analytical data and Magnetic Moment of complexes

Compound	Empirical formula	Color	Elemental Analysis (Found)			Magnetic moment (μ_B)
			C	H	N	
Ligand	$C_{17}H_{18}N_2O_2$	Yellow	72.32 (69.75)	6.43 (6.82)	9.92 (9.54)	-
Cu(II)	$C_{34}H_{34}CuN_4O_4$	Brown	65.21 (65.55)	5.47 (5.65)	8.95 (8.72)	1.89
Co(II)	$C_{34}H_{34}CoN_4O_4$	Red	65.70 (65.43)	5.51 (5.89)	9.01 (8.85)	4.62
Ru(II)	$C_{34}H_{34}N_4O_5V$	Green	64.86 (64.55)	5.44 (5.75)	8.90 (8.61)	1.92
Vo(IV)	$C_{34}H_{34}N_4O_4Zn$	Dark yellow	65.02 (64.82)	5.46 (5.34)	8.92 (8.75)	-

3.1 NMR Spectra

The ^{1}NMR spectrum of the ligand were recorded at room temperature in $CDCL_3$.The ^{1}NMR spectrum shows the peaks at 6.2-7.2 δ shows the phenyl multiplet of Schiff base ligand (Fig.3.1) (with condensation of 4- aminoantipyrine and 2-amino 3- pyridine carboxaldehyde). The ligand also shows the following signals: C-CH_3 2.1–2.8; CH=N 9.3 δ (s) and 7.72, 7.94 and 7.983.

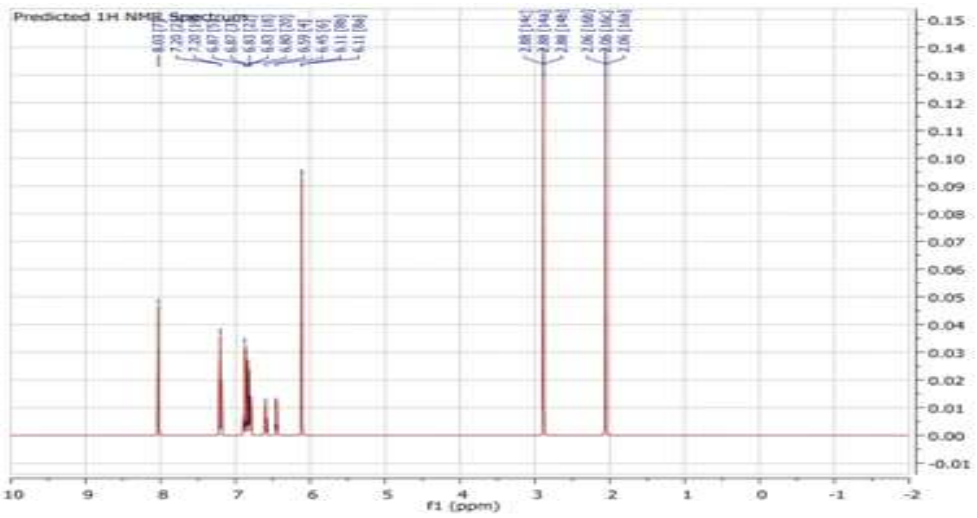


Figure 1 - ^{1}NMR spectrum of the ligand

3.2 Infrared spectra of the ligands and their metal complexes

The coordination sites of the ligand to the metal ions were investigated by comparing the infrared spectra of the free ligand with their metal complexes. The IR spectra of the Schiff base ligand (L_1) show a band at 1671 cm^{-1} which is assigned to azomethine ν (CH=N) linkage. These bands are shifted towards lower frequencies in the spectra of their metal complexes (1608–1606 cm^{-1}). The comparison of the IR spectra of the complexes with the above Schiff bases indicates the involvement of the azomethine nitrogen in chelation with the metal ion. The coordination of nitrogen to the metal ion could be expected to reduce the electron density of the azomethine link and thus causes a shift in the ν(CH=N) group Conclusive evidence of the bonding is also shown by the observation that new bands in the spectra of all metal complexes appearing in the low frequency regions at 768-763 cm^{-1} and 681-603 cm^{-1} characteristic to ν(M-O) and ν(M-N) stretching vibrations respectively, that are not observed in the spectra of free ligands.

Table 2: IR Spectrum of ligand and its metal complexes (in cm^{-1})

Compound	υ(C=N)	υ(C-O)	υ(M-N)	υ(M-O)
Ligand	1630	1315	-	-
Cu	1608	1216	768	603
Co	1621	1262	760	681
Ru	1611	1287	763	617
Vo	1606	1306	767	676

Figure 1 – FT-IR spectrum of Schiff base ligand

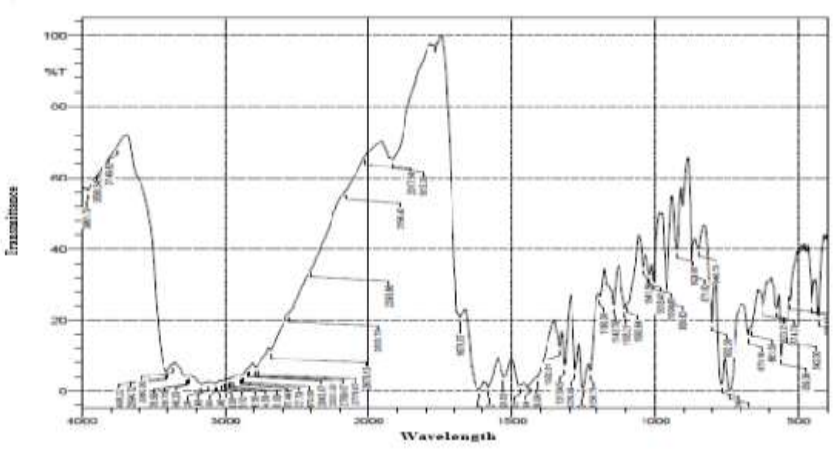


Figure 2 - FT-IR spectrum of copper

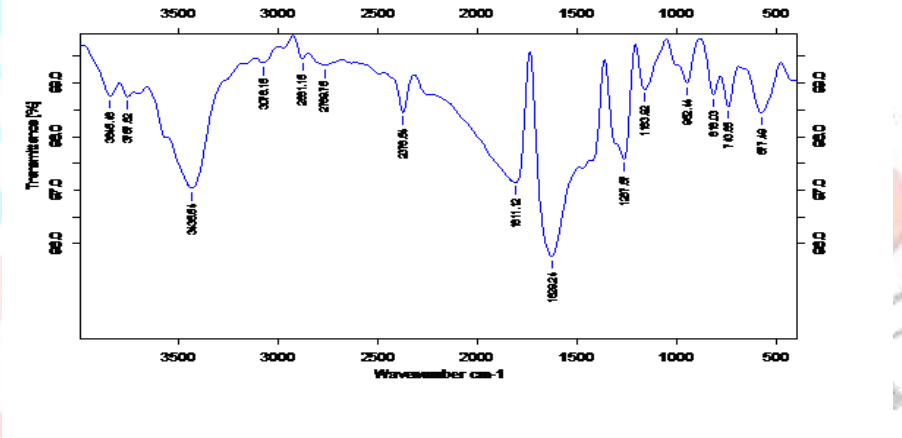


Figure 3 - FT-IR spectrum of vanadium

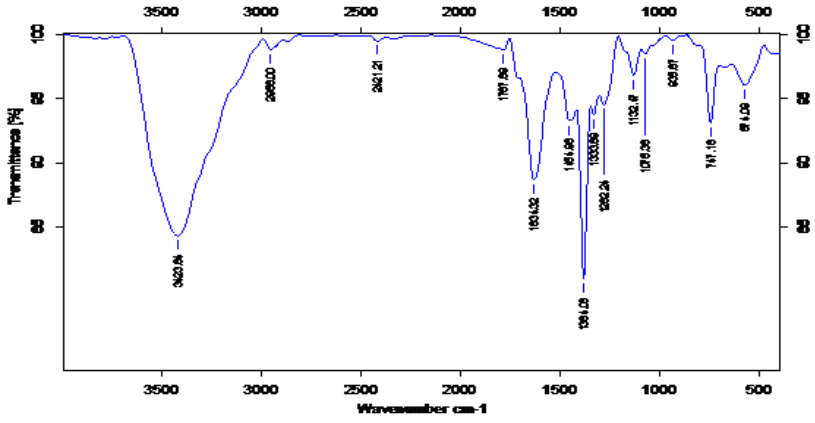


Figure 4 - FT-IR spectrum of Ruthenium

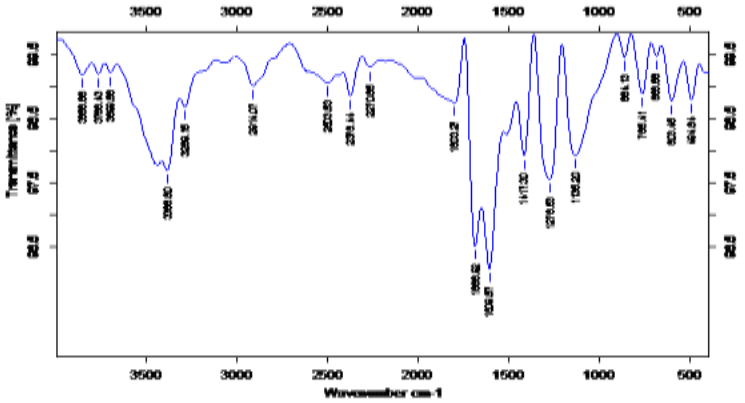


Figure 5 - FT-IR spectrum of Cobalt

3.3 Electronic spectra of ligand and its metal complexes

The UV-Visible spectra are recorded in $CHCl_3$ in the range of 726 nm of the Schiff base.The UV–Vis. spectrum of the ligand exhibits an absorption band at 355 nm, which can be attributed to the n-π* transition of azomethine Chromophore. The molar absorbtivities ligand at 355nm may be assigned to an transition between the lone-pair electrons of the p orbitals of the N atoms in the azomethine (HC=N) groups and the π bonds of the aromatic rings [36, 37]. The peaks at 276 nm are assigned to the π-π* transitions of the Schiff base. In the present case, The absorption band in the 420 nm range, which is assigned to a metal ligand charge transfer band. The electronic spectrum of the Cu (II) complex shows a broad band at 515 nm assignable to transition [38] which is characteristic of octahedral environment.

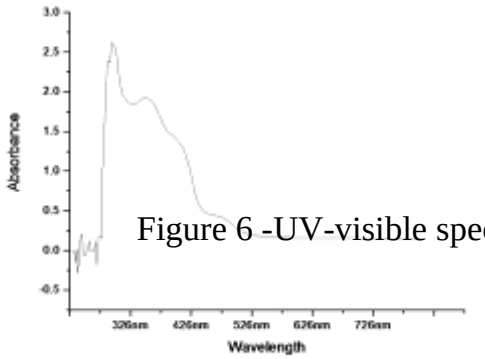


Figure 6 -UV-visible spectrum of ligand

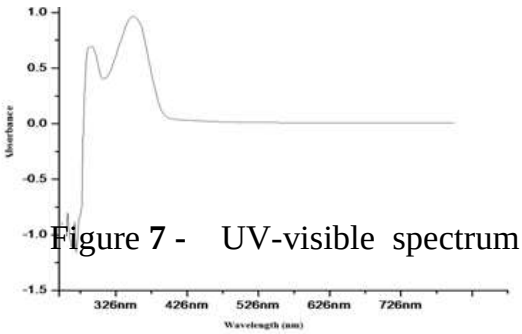


Figure **7 -** UV-visible spectrum of copper

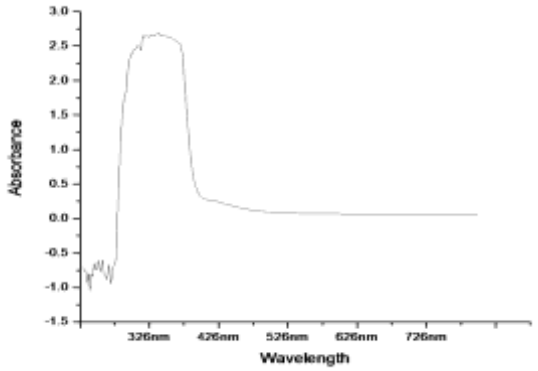


Figure 8 - UV-visible spectrum of vanadium

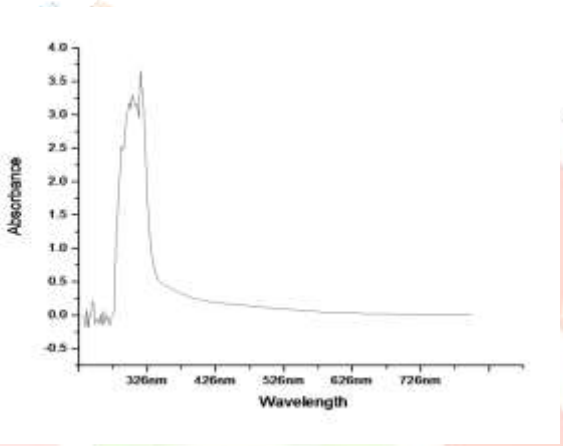


Figure 9 - UV-visible spectrum of Ruthenium

3.4 EPR Spectra

The EPR spectrum pattern of the solid copper(II) complex at room temperature exhibits g values($g_{//}$ =2.132 and $g_{\perp}$ =2.033) > 2.003 indicate that the complex is axially symmetric and Copper site has a dx^2-dy^2 ground state characteristic of octahedral geometry[30]

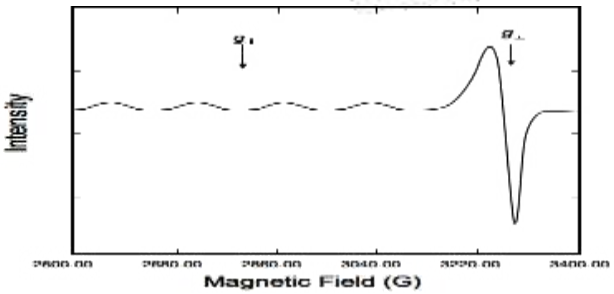


Figure 10 : EPR Spectrum for Copper

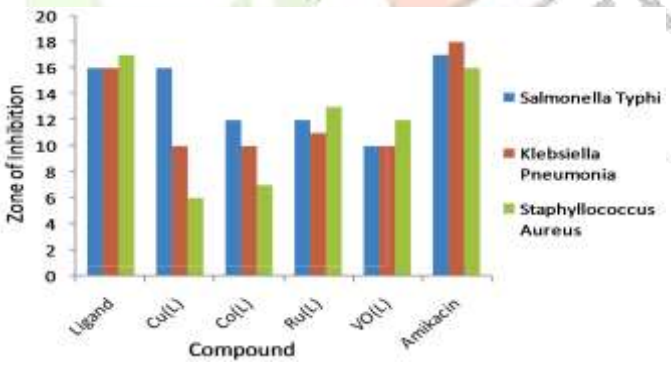
3.5 Antibacterial activity

The results of the antibacterial and antifungal activity are tabulated in Table 1. The ligand and metal complexes show greater antimicrobial activity than those of the control drug; this indicates that the complexation with metal enhances the activity of the ligand. This is explained on the basis of Overtone's concept and chelation theory [39]. The synthesized compounds exhibit moderate to strong antimicrobial activity. The Cu(II) complex exhibits a higher activity than the other metal complexes towards bacterial species. The Co(II) shows equal activity against *S.Typhi, K.Pneumonia, S.Aureus* bacteria compared to the standard and moderate activity was found against other bacterial species. Co(II) and VO(II) complexes are having low activity compared to the standard. Ru(II) complex displays moderate activity against the bacteria.

Table 3: Minimum inhibition Concentration (MIC) data of the synthesized ligand and metal complexes against growth of bacteria

Compound	Salmonellatyphi	Klebsiella Pneumonia,	Staphylococci aureus
Standard(Amikacin)	**17**	**18**	**16**
Ligand	**16**	**16**	**17**
$[Cu(HL)_2]Cl_2$	**16**	**10**	**6**
$[Co(HL)_2]Cl_2$	**12**	**10**	**7**
$[Ru(HL)_2]Cl_2$	**12**	**11**	**13**
$[VO(HL)_2]Cl_2$	**10**	**10**	**12**

Figure 11 : Zone of Inhibition for Synthesized compounds against various pathogenic bacteria



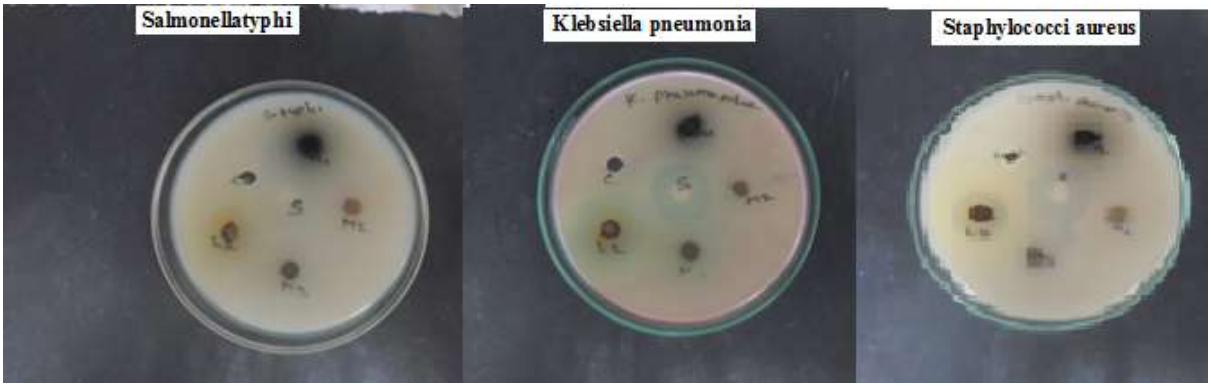


Figure 12 : Image Zone of Inhibition of Synthesized compounds against various pathogenic bacteria

3.6 Antifungal activity

To provide a medicinal scope in the field of bioinorganic chemistry, consequently, the metal complexes synthesized have been evaluated for their antifungal actions. The antifungal tests were carried out using the disc diffusion method. The Schiff base ligands and their metal complexes were screened in vitro in order to find out the antifungal activity against Aspergillus niger, Candidatrophicala and Candida albicans. The results of the antifungal studies are which reveal that the metal complexes are low than the free ligands against the same organisms..

Table 4: Minimum inhibition Concentration (MIC) data of the synthesized ligand and metal complexes against growth of Fungi

Compound	Candidiatrophicalis	Aspergillus	Candidaalbicans
Standard(Ketakonazole)	17	18	16
Ligand	16	16	17
$[Cu(HL)_2]Cl_2$	16	10	13
$[Co(HL)_2]Cl_2$	10	8	10
$[Ru(HL)_2]Cl_2$	12	11	13
$[VO(HL)_2]Cl_2$	10	10	12

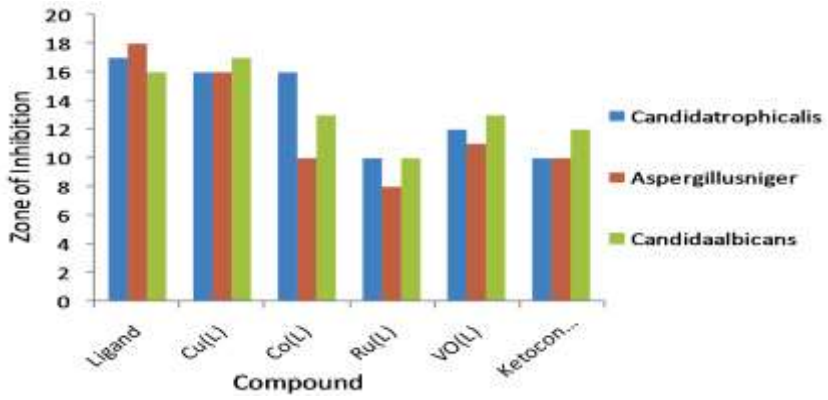


Figure 13 : Zone of Inhibition for synthesized compounds against various pathogenic fungal

Figure 14: Images Zone of Inhibition of synthesized compounds against various pathogenic Fungi

3.7 Cytotoxic activity

Hep-2 cell lines was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 g/ml) in a humidified atmosphere of 50 g/ml CO2 at 37 °C. Viable cells were determined by the absorbance. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks. Cytotoxicity of Schiff base and its metal complexes are shown in **Figure 15** The effect of the ligand and its metal complexes on the proliferation of Hep-2 was expressed as the % cell viability. The affected Hep-2 cell line at different concentrations and a graphical representation of the ligand and its metal complex's effect on cancer cells by % cell viability is shown in **Figure 16.** IC50 of the ligand and its metal complexes were determined and are shown in **Table 5 .** The cytotoxic assay shows that the ligand and its metal complexes are toxic to the Hep-2 cell and 40 to 60% of these cells were killed after incubation for two days with the extract.

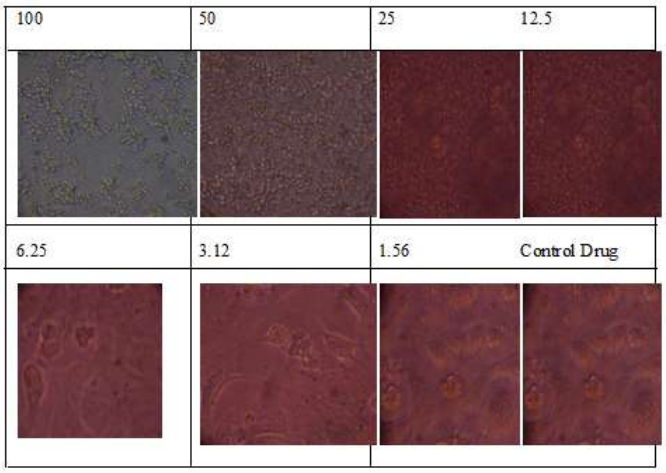


Figure 15 **:** Images Percentage of cell viability and death analysis in duplicate study model for their metal complex.

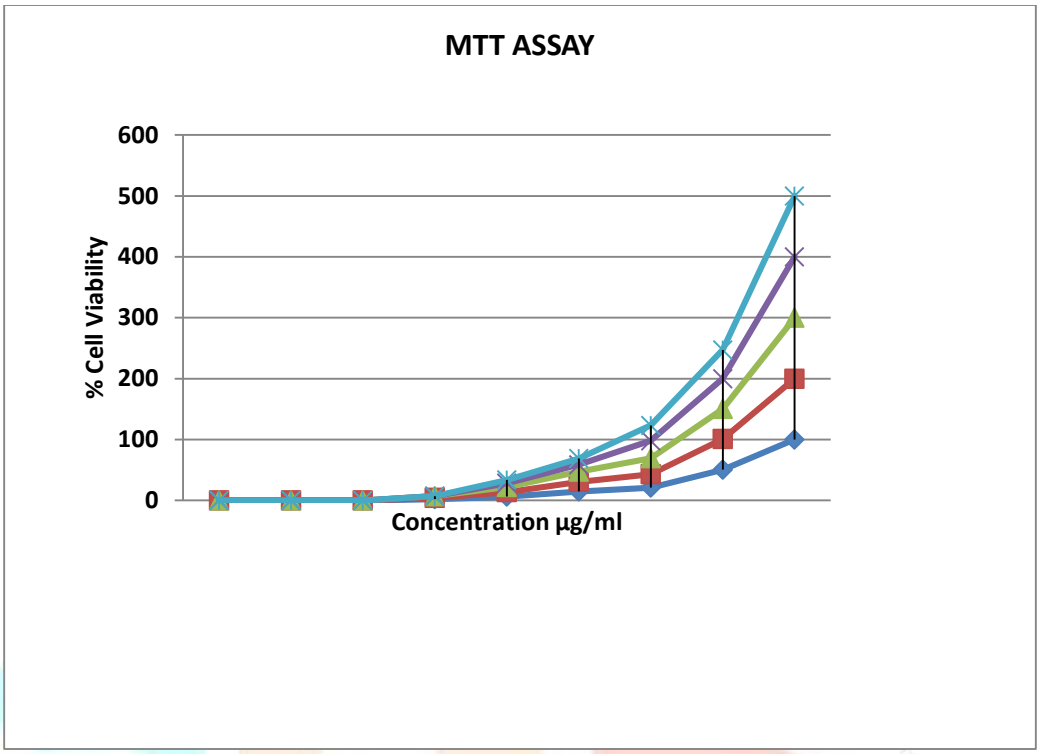


Figure 16 : Graphical representation of Schiff base and metal complexes on Hep-2 cancer cell line

Table 5: Percentage of cell viability and death analysis in duplicate study model

Concentration µg/ml	% cell Viability	% cell Viability	% cell Viability	% cell Viability	% cell Viability
100	0	0	0	0	0
50	0	0	0	0	0
25	2	2.1	3.1	0	0
12.5	6.3	7.3	8.4	6.3	5.3
6.25	14.7	15.7	16.8	11.5	10.2
3.12	21.1	22.1	26.3	28.4	25.3
1.56	50.5	50.5	49.4	49.4	47.5
control	100	100	100	100	100

4. Conclusion.

The present study describes the synthesis of new Schiff bases derived from 4-aminoantipyrine and 2-amino 3- pyridine. The EPR study confirms the structure of newly synthesiszed Schiff bases. The spectral data show that the Schiff bases act as monobasic bidentate NO chelating agents coordinating the metal ion *via* the azomethine nitrogen atom and the phenolic oxygen atom. Further, the promising results have been observed for the antimicrobial screening especially for the metal complexes against both the fungi and bacteria and the

results are attributed to the fact that the metal complexes are potentially active against bacterial cells than fungi cells. The free ligand and their metal complexes show anticancer activity against **HepG2** cells. In view of the biological activity, copper complexes have shown a higher activity.

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