

A New Schiff Base Coordinated Copper (II) Complex Induces Apoptosis and Cytotoxicity in Human Adrenocarcinoma Breast Cancer Cells

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Abstract: A new class of metal complexes of type M(X)₂ [where M = Cu(II) X=1-(3-ethoxy-2-hydroxybenzylidene-4-phenylsemicarbazide)] have been characterized by spectral techniques. In the visible range, the complexes exhibit a strong Metal to Ligand Charge transfer (MLCT) transition. The bonding through imine nitrogen and deprotonate phenolic oxygen is demonstrated by the ligands' reduced frequency shift ($\nu(\text{C}=\text{N})$) and absence of $\nu(\text{O}-\text{H})$ in the IR spectral measurements upon complexation with the metal atom. In their complexes, the Schiff bases exhibit the properties of monobasic tridentate ligands. The spectrum data show that the nitrogen atoms in azomethine and the phenolic oxygen serve as the ligand's points of coordination.

The observed $A||$ values in the Cu (II) complexes indicate a tetrahedrally distorted square planar structure. The metal complexes are screened for biopotency. The Ligand (L1) and their complexes of Cu (II) show cytotoxicity in human adrenocarcinoma breast cancer cells giving IC_{50} values for Cu (II) complexes 55.52, 58.96 μg , respectively. The results demonstrate that Cu (II) complexes, with potent antiproliferative activity, is able to induce caspase-dependent apoptosis in human cancer cells. Cytotoxicity of the complexes is further confirmed by the MCF-7 cancer cells.

Keywords: Schiff base, Cu (II) Complex, biopotency, apoptosis.

1. INTRODUCTION

Most of the tropical and subtropical regions of the world are suffering from parasitic diseases. The intestinal causative agent of amoebiasis is protozoan parasite *Entamoeba histolytica*, which is affecting millions of people throughout the world. Millions of people die every year because of these diseases [1]. Major side effects such as neurological complications and the possible selection of a resistant *E.histolytica* strain have been reported by using metronidazole [2]. Therefore, it has become a topic of interest for the researchers to find some new amoebicidal drug and the treatment of infectious diseases still remains an important and challenging problem. Numerous compounds have been

synthesized to treat such infectious diseases, but their medical use has been limited by their relatively high toxicity, bacterial resistance, and pharmacokinetic deficiencies. It is found that transition metals and their complexes have varying utility and interesting chemistry. Metal complexes with suitable ligands are said to be chemically more significant and specific than the metal ions [3]. Preparation of transition metal complexes with thiosemicarbazone ligands has been paid considerable attention due to the pharmacological properties of both ligands and complexes [4,5].

Semicarbazone/Thiosemicarbazone derivatives form an important class of inorganic compounds due to their structural chemistry and biological activities. Heterocyclic nitrogen, oxygen and sulphur containing

semicarbazone/thiosemicarbazones have been the subjects of extensive investigation because of their use for the biological applications [6, 7]. Semicarbazones and thiosemicarbazones have received great interest because of their bonding modes, biological implications, structural diversity, and ion-sensing ability [8]. The biological application and properties of metal complexes differ from those of either ligands or the metal ions, and increased and/or decreased biological activities of transition metal complexes like Cu(II) are reported in the literature [9–11].

Heterocyclic Schiff bases are basically important because of their raised biological activity, and also they make possible development of novel materials with unique properties. This Schiff base has N,O donor site and good coordination ability for the metal ions. The novel Schiff base and its metal complexes were evaluated for their antimicrobial properties against bacteria and fungi. Cytotoxic effect of the

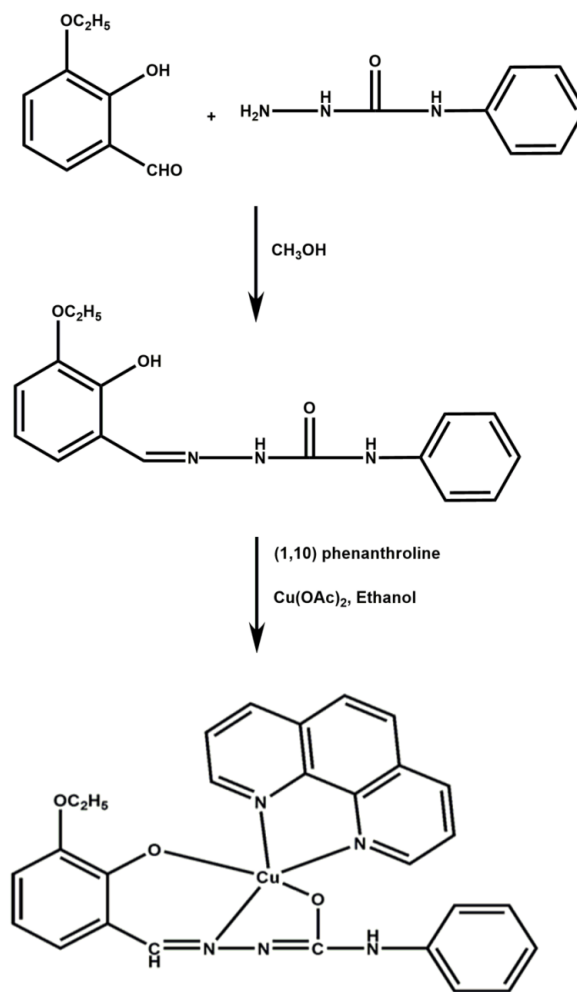
Copper(II), Nickel(II), and Zinc(II) complexes on human breast carcinoma cells (MCF-7) was measured using the MTT assay.

2. EXPERIMENTAL

2.1. Synthesis of Copper (II) Complexes

2.1.1 Synthesis of Copper (II) (1,10- phenanthroline) complex 1

About 40mg of the copper acetate was dissolved in ethanol and the ligand L1 60mg was dissolved in ethanol. Then the contents are taken in the round bottomed flask and stirring continued for half an hour. After half an hour about 40mg of (1,10) phenanthroline was dissolved in ethanol and added in RB flask. Then stirring continued for 3 hours. The mixture was refluxed for 2-3 hours at 70–80°C on water bath. On cooling, green colored solid metal complexes were precipitated out (Scheme.1). The products were filtered, washed with cold methanol, and dried under vacuum over P₄O₁₀.

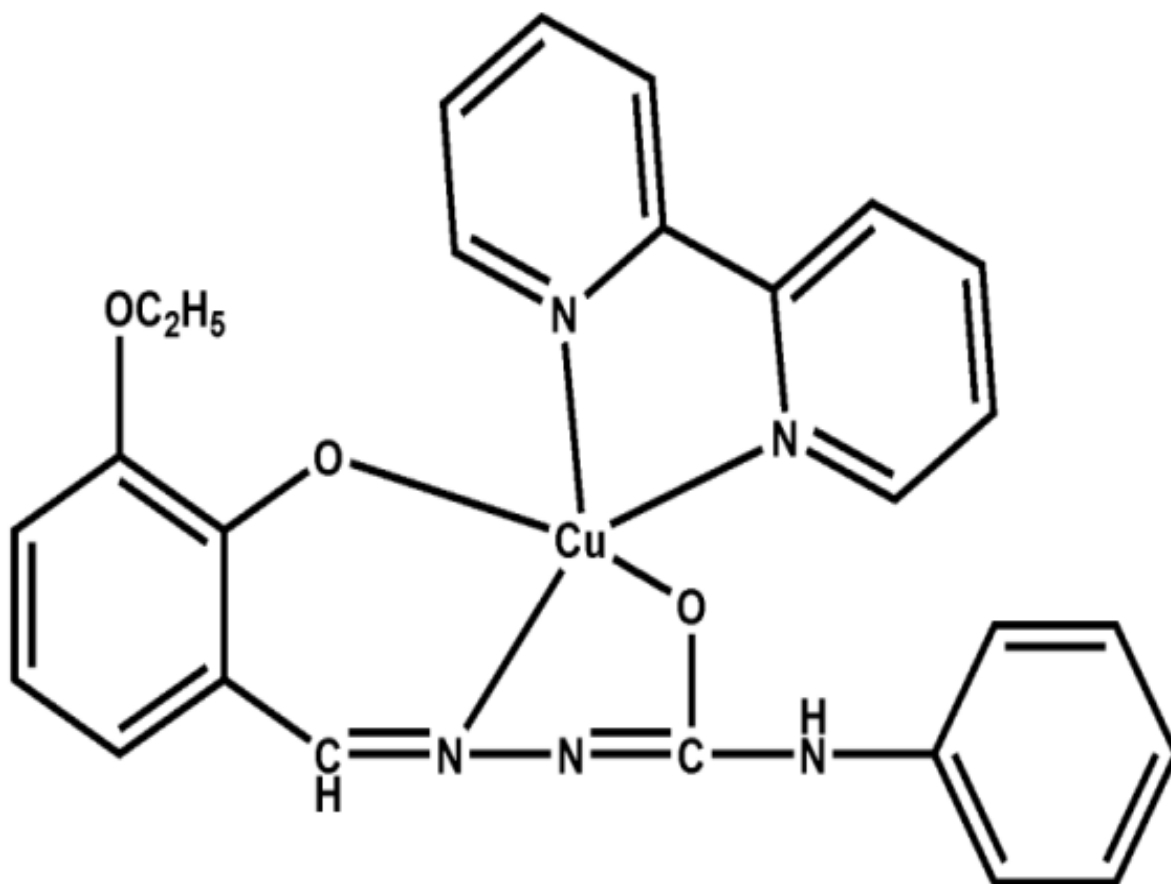


Scheme: 1 proposed structure of complex 1

2.1.2. Synthesis of Copper (II) (bipyridine) complex 2

About 40mg of the copper acetate was dissolved in ethanol and the ligand L1 60mg was dissolved in ethanol. Then the contents are taken in the round bottomed flask and stirring continued for half an hour. After half an hour about 31mg of

Bipyridine was dissolved in methanol and added in RB flask. Then stirring continued for 3 hours. The mixture was refluxed for 2-3 hours at 70–80°C on water bath. On cooling, green colored solid metal complexes were precipitated out (Scheme.2). The products were filtered, washed with cold methanol, and dried under vacuum over P₄O₁₀.



Scheme:2 Proposed Structure of complex 2

3. RESULTS AND DISCUSSION

3.1 ¹H NMR Spectra

The ¹H NMR spectra of the ligand L1 and the complexes are recorded in DMSO-*d*₆-solvent. Representative spectra are given in Fig. (1). Singlet peaks observed in the ¹H NMR spectra of the ligand L1 at ~10.67 and 8.87 ppm are due to phenolic (-OH) and azomethine(-CH=N) protons of ligands respectively. The peak due to the phenolic proton is absent,

while the peak due to the azomethine proton is shifted to 8.28 ppm in the spectrum of the complexes. This observation clearly supports the involvement of the -OH chromophore and azomethine nitrogen in coordination. The aromatic ring protons are observed as multiplets in the region 6.76-7.31 ppm in the spectrum of ligand. These peaks are shifted slightly in the spectrum of the complexes. Multiplets observed in 3.19-3.22 ppm and 3.79-3.89 ppm ranges are due to aliphatic -CH₂ protons.

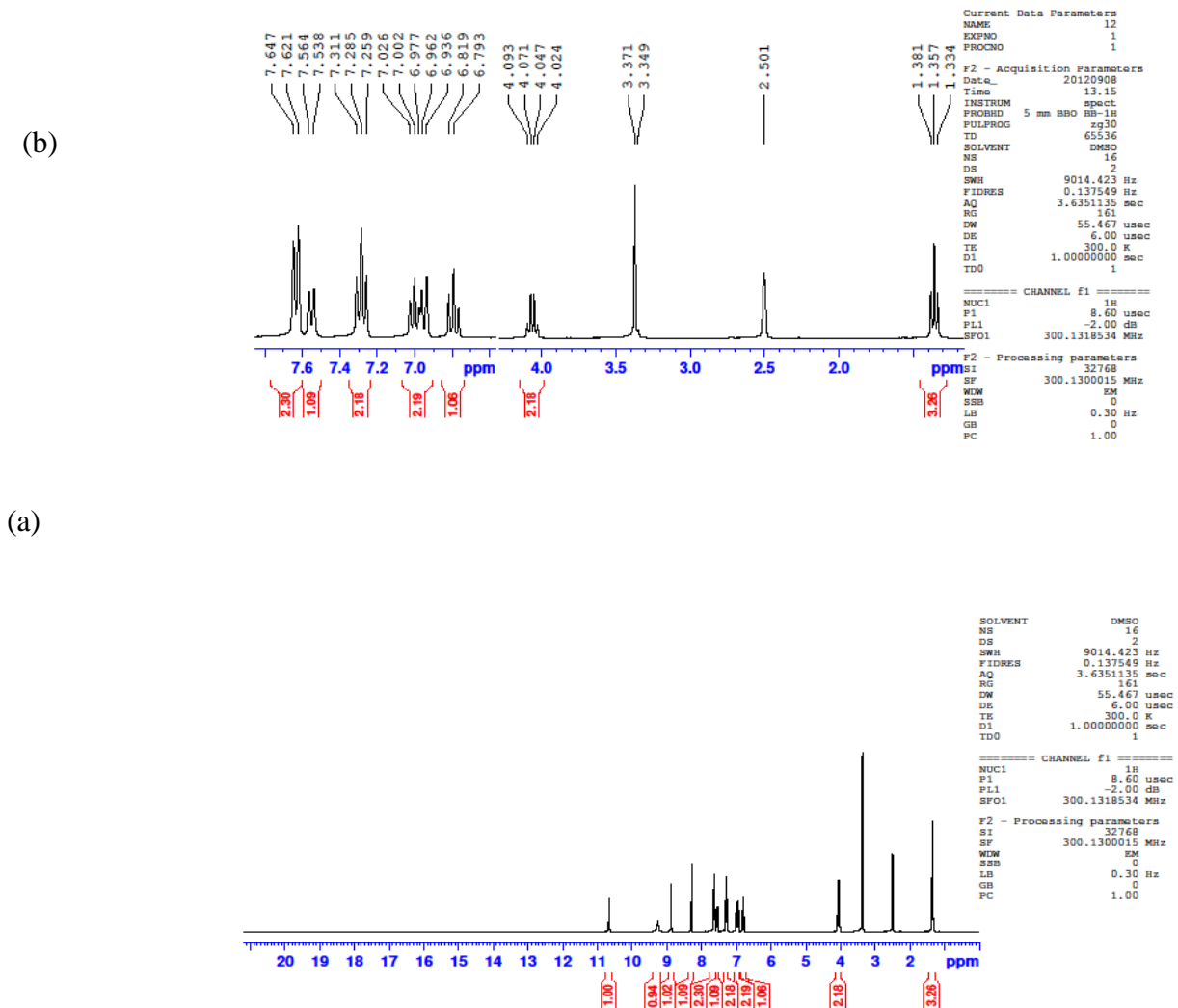


Fig.1. ¹HNMR spectrum of synthesized ligand (L1) (a) and expanded form (b) in DMSO *d*₆

4. EPR SPECTRA

The powdered X-band EPR spectrum of Cu(II) complex has been recorded at room temperature is shown in Fig.2. The spectrum of Cu(II) complex reveals three sets of resonances at low, mid and Cu(II) with semicarbazone high fields corresponding to g^1 , g^2 and g^3 respectively. From the peak positions g -values evaluated are $g^1 = 2.26$, $g^2 = 2.16$, and $g^3 = 2.07$. Hyperfine structure is not resolved. The calculated g values provide valuable information on the electronic ground

state of the ion [12]. Anisotropy of the g -tensor is due to the Jahn-Teller effect, which reduces the symmetry from octahedral to distorted octahedral. For g values $g^1 > g^2 > g^3$, the quantity $R = g^2 - g^3 / g^1 - g^2$. If R is greater than unity, then the ground state is $2A_1(dz^2)$ and if R is less than unity, then the ground state is $2A_1(dx^2-y^2)$. In the present study, the calculated R value is less than unity ($R = 0.8$) and hence the ground state is $2A_1(dx^2-y^2)$. From the results and analysis of EPR spectra, the site symmetry of Cu(II) ion in Cu(II) complex is ascertained to be a rhombically distorted octahedron with $2A_1(dx^2-y^2)$ as the ground state [13].

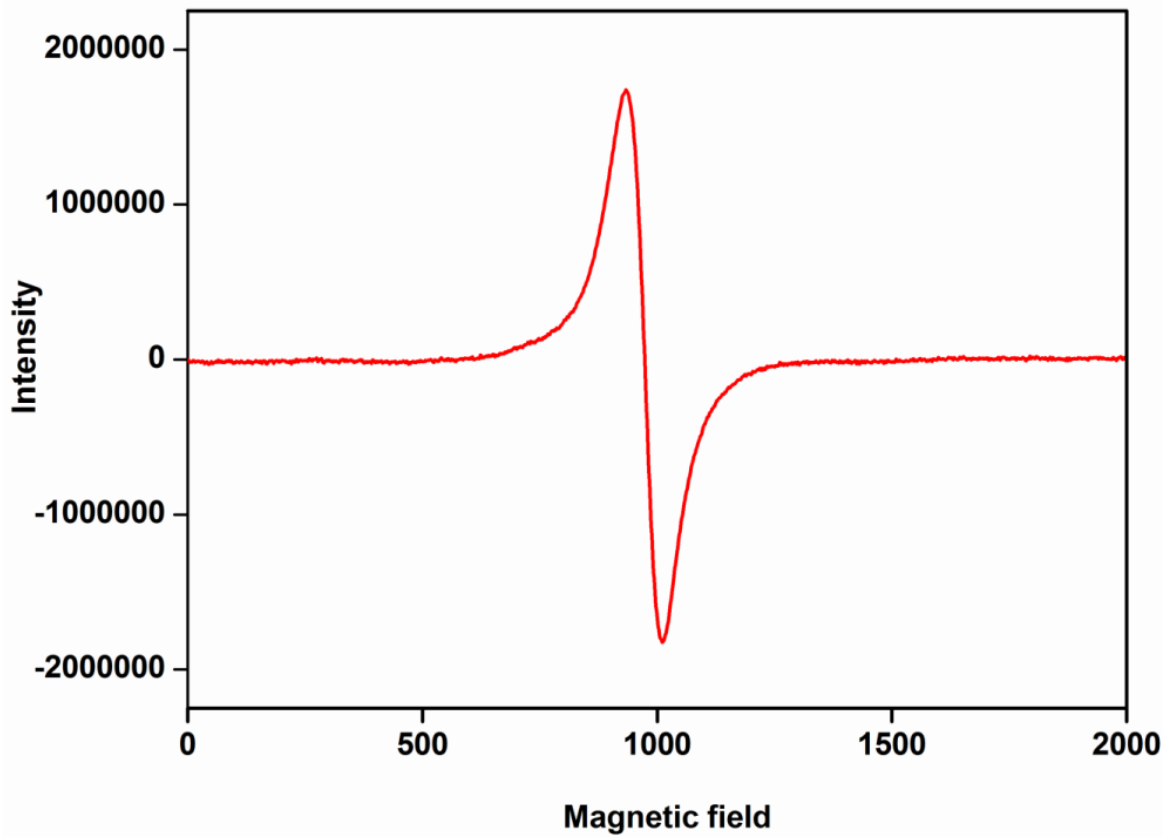


Fig.2. Powder X-band EPR spectrum of complex 1 at room temperature

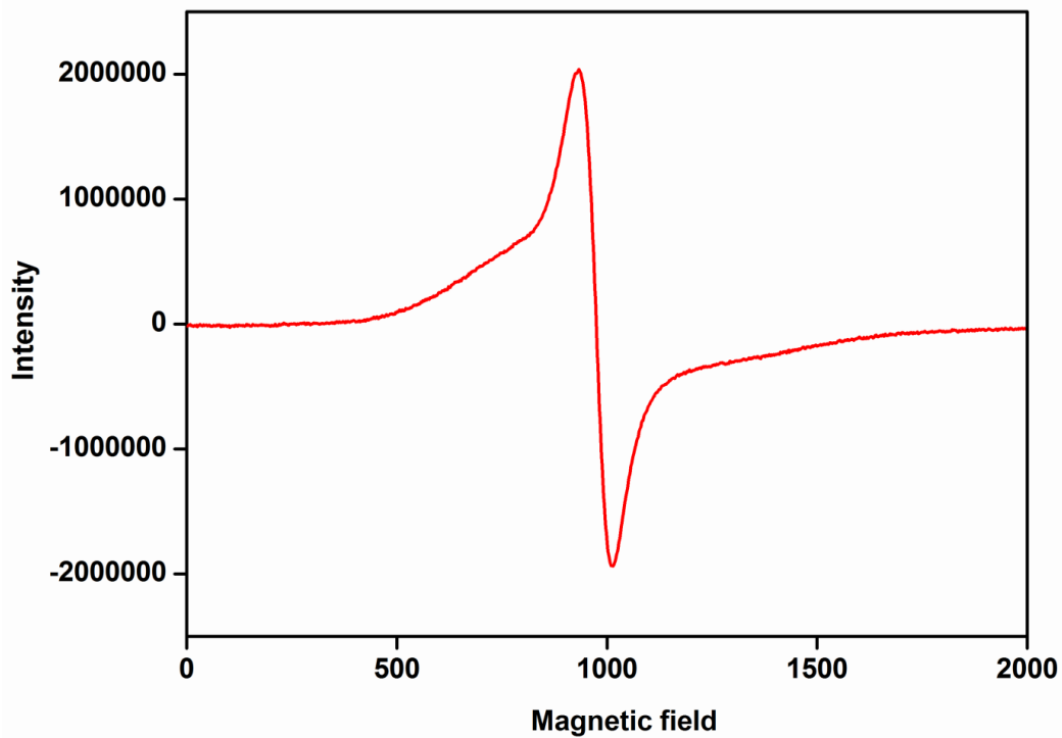


Fig.3. Powder X-band EPR spectrum of complex 2 at room temperature

5. BIOLOGICAL STUDIES

5.1. Antibacterial Activity

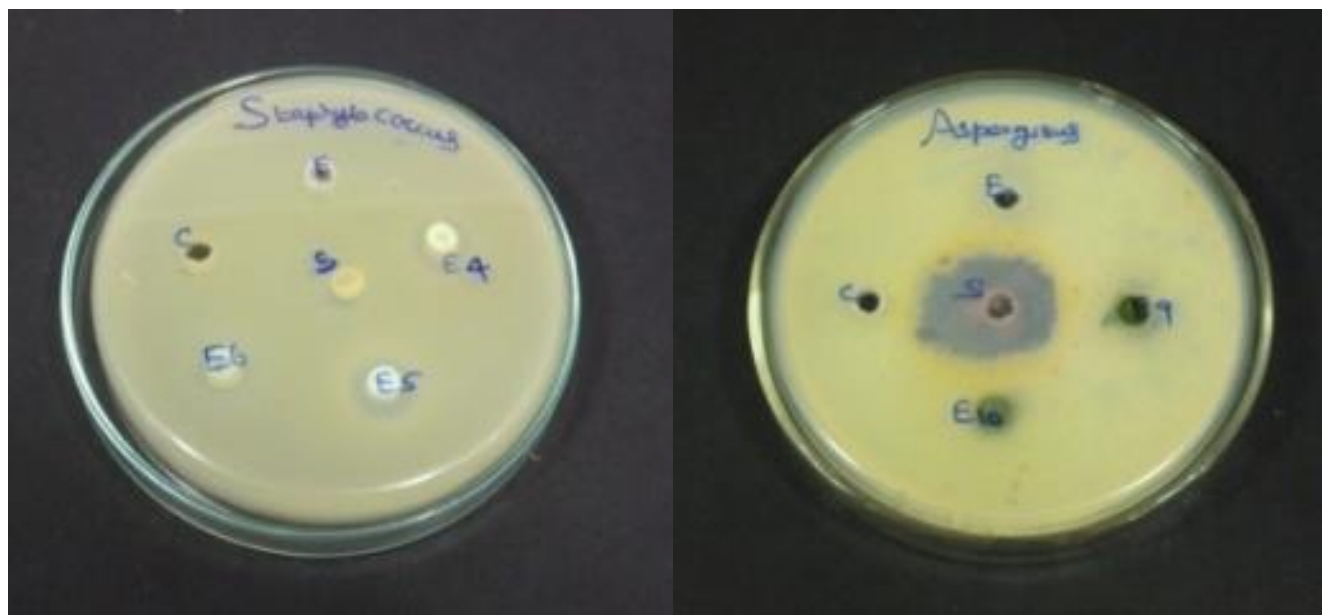
The invitro anti-bacterial activity [14] of the ligand and its complexes have been carried out against the gram negative bacteria *Escherichia.Coli* and gram positive bacteria *staphylococcus aureus* and *Pseudomonas aeruginosa* and standard amikacin drug using disc diffusion method by taking DMSO as solvent.

A comparative study of the growth inhibition zone values of schiff base and its complexes indicate that metal complexes exhibit higher antibacterial activity than the free ligand and the same is indicated from the results given in the Table. This is probably due the greater lipophilic nature of the complexes. Such increased activity of the metal chelates can be explained on the basis of Overtone's concept and Tweedy's chelation theory [15]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is considered to be an important factor that controls the anti microbial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups [16, 17] Further, it increases the delocalization of the π electrons over the whole chelate ring and enhances the lipophilicity of the complex. This

increased lipophilicity enhances the penetration of the complexes into lipid membrane and thus blocks the metal binding sites on enzymes of microorganisms [18]. These metal complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism [19]. The variation in the activity of different complexes against different organisms depend either on the impermeability of the cells of the microbes or difference in ribosomes of microbial cells. The results indicate that copper (II) complexes shows highest activity than other complexes and ligand 1.

5.2. Antifungal Activity

The antifungal activities [20] of the ligand and its metal complexes were tested against seven-day old cultures of *Aspergillus niger*, *candidida albians*, *macrophonia phaseolina* and standard *ketokonazole* using paper disc method. The results show that the metal complexes were more active than the free ligand. The activity of the free ligand is resistant to the fungal species. The mode of action of the compounds may involve formation of hydrogen bond through the azomethine group with the active centers of cell constituents, resulting in an interference with normal cell process [21]. The antifungal activity data indicate that the complexes show an appreciable activity against *Aspergillus niger*, *Candidida albians* and *Macrophonia phaseolina* at 1 mg/ml concentration.



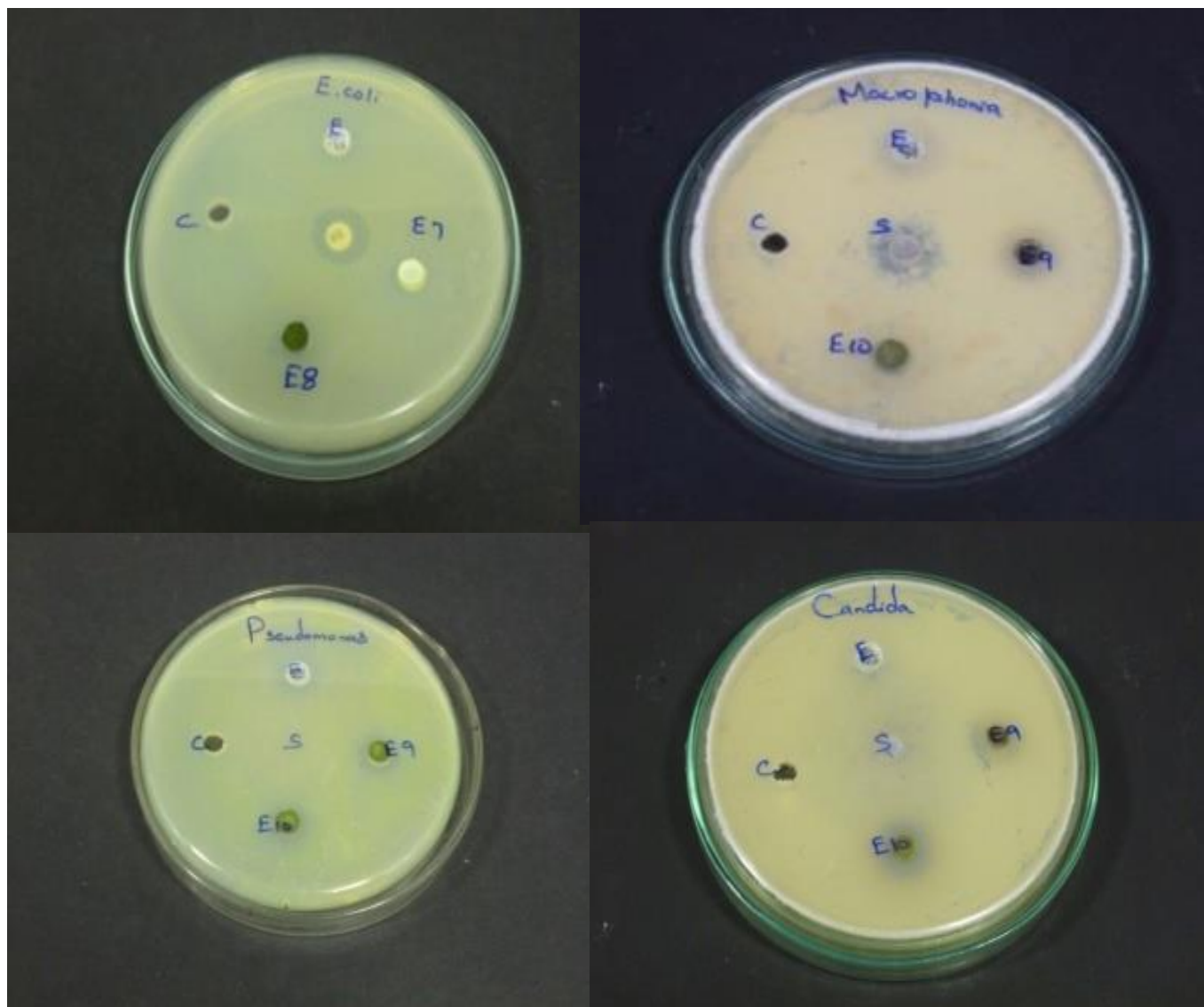


Fig.4. Images of Zone Inhibition of ligand L1 and metal complexes **against** bacteria and fungi.

5.3. Cytotoxic Activity

The in vitro cytotoxic activities of the synthesized Schiff base ligand L1 and their metal complexes are studied on breast cancer cell lines (MCF-7) by applying the MTT colorimetric assay. The calculated IC_{50} values, that is, the concentration ($\mu\text{g/ml}$) of a compound able to cause 50% of cell

death with respect to the control culture, are presented in Figure 5. Cyclophosphamide is used as a reference compound. The Cu(II) complexes are cytotoxic to the breast cancer cell lines with IC_{50} value of 55.52 and 58.96 $\mu\text{g}\cdot\text{cm}^{-3}$. The different activities are currently being investigated in terms of the mechanism of action of these compounds at the cellular level [22].

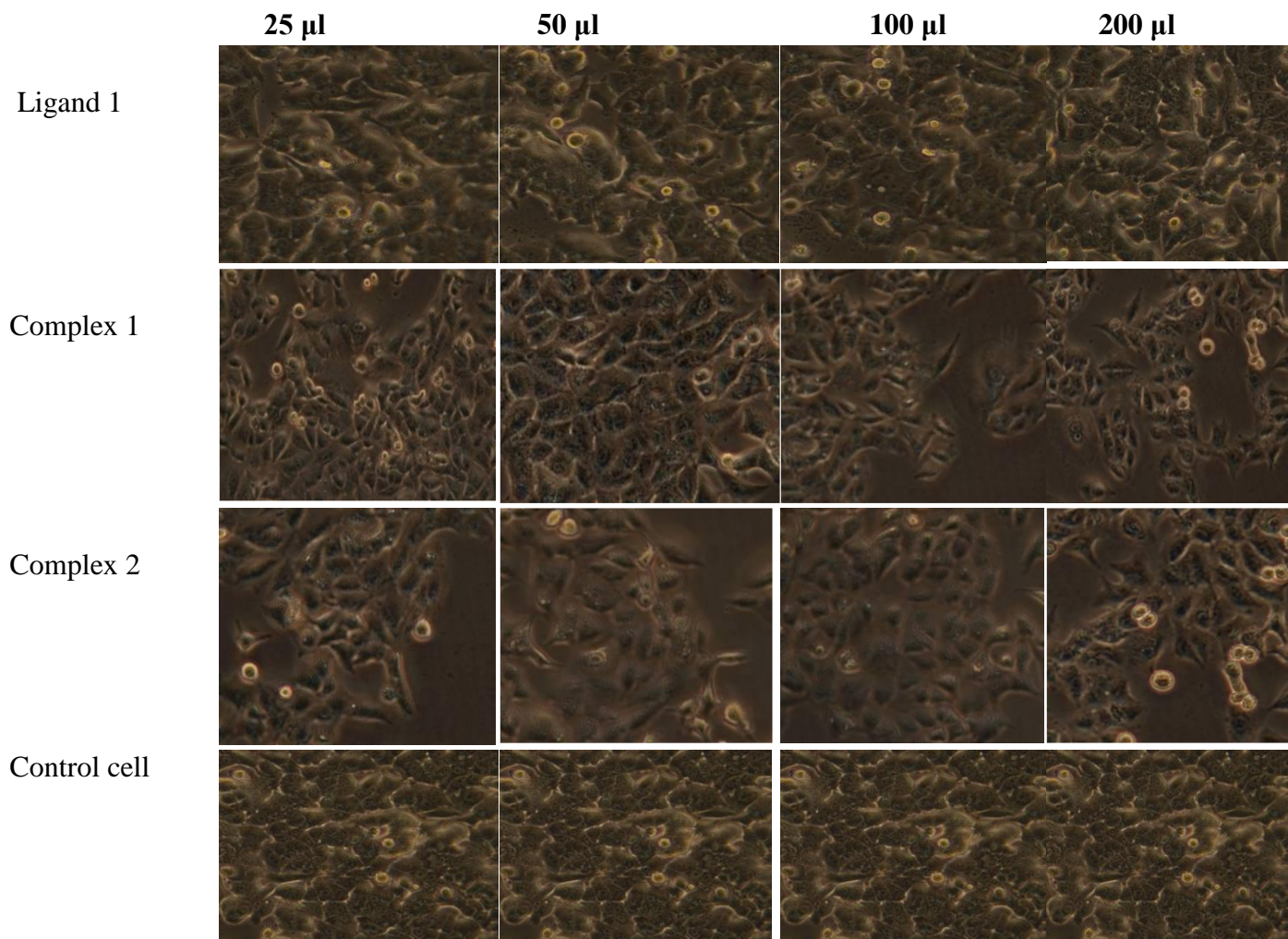


Fig.5. Morphological images of the ligand L1 and Cu(II) metal complexes. The cells are observed under phase contrast microscope $\times 100$.

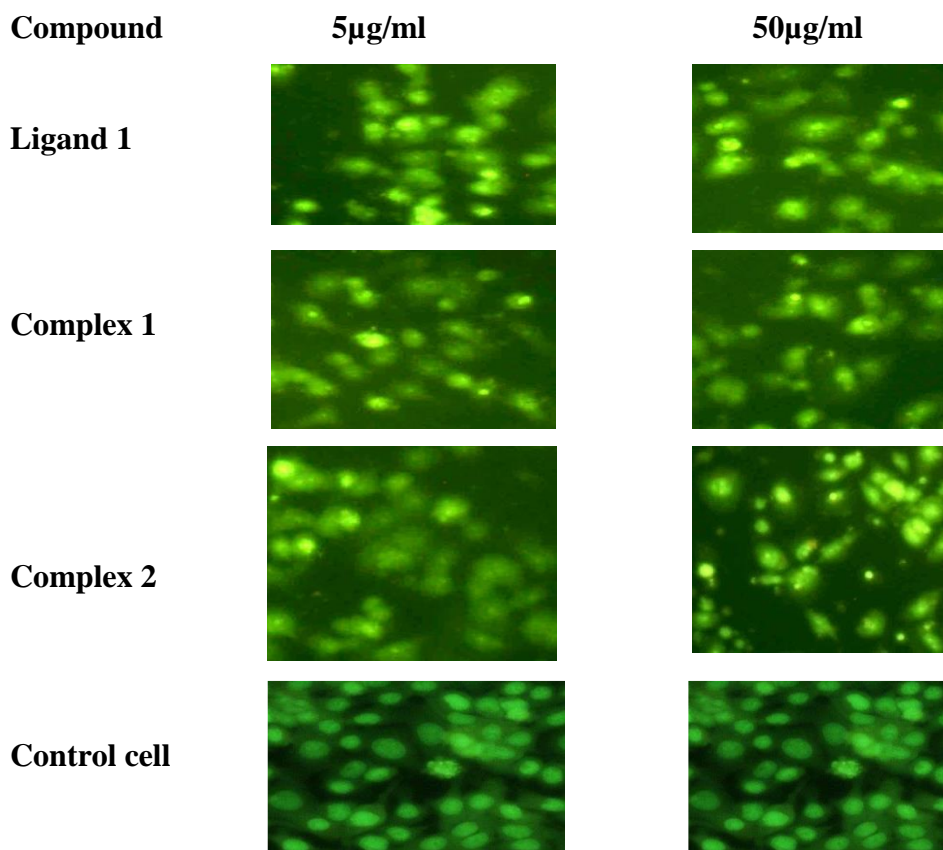


Fig.6.Fluorescent Staining Studies of the synthesized ligand (L1), Cu(II) Complexes and control cell.

6. CONCLUSION

The new ligand viz 1-(3-ethoxy-2-hydroxy benzilidene) -4-phenyl semicarbazide L1 have been synthesized. Cu(II) complexes have been prepared by using L1 and characterized by various spectral techniques. From IR spectral data, it has been concluded that the ligand, L1 behave as a mono basic bidentate ligand coordinating through deprotonated phenolic oxygen and the azomethine nitrogen atom. Analysis of EPR spectra, shows that the site symmetry of Cu(II) ion in Cu(II) complex is ascertained to be a rhombically distorted octahedron with $2A_1(dx^2-y^2)$ as the ground state.

Further, the promising results have been observed for the antimicrobial screening especially for the metal complexes against bacteria and fungi it is attributed to the fact that the metal complexes are potentially active against bacterial cells. The free ligand and its metal complexes show anticancer activity against MCF-7 cells. As far as the biological activities are concerned, the complexes have shown an appreciable activity. The catalytic activity of CuLbpy is tested with benzaldehyde as a model substrate. Results show that Copper catalyzed system is still a better method in terms of reaction time and catalyst loading because only 1 mol % catalytic loading is required for the effective reaction.

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