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Synthesis, Characterization, DNA binding studies and biological activities of a copper II complex containing of 1,10 phenanthroline, L. Alanine and urea

L. Selvarani and M.N. Arumugam*

Department of Chemistry, Thiruvalluvar University, Serkadu.

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ABSTRACT

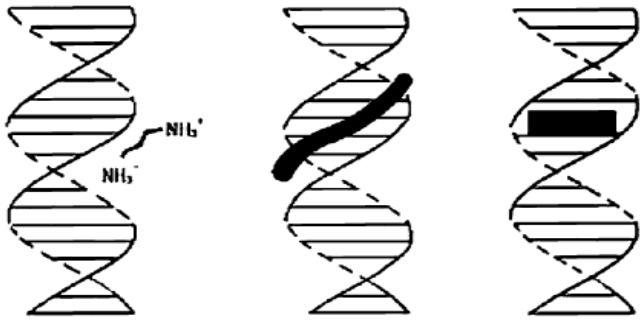
A copper (II) complex, [cu(phen)(L-Ala)U](ClO_4) (phen=1,10- phenanthroline, L-Ala =L-alanine,U =urea), has been synthesized and characterized by Infra-red, EPR spectral and elemental analysis methods. The interaction with calf-thymus DNA has been studied by means of electronic absorption spectroscopy, emission spectroscopy, viscosity and cyclic voltammetry has been conducted to assess their interaction between their complex and DNA. A sample of copper II complex was tested for its Antimicrobial Activities and it was found to have good antimicrobial activities.

Introduction

The interaction and reaction of metal complexes with DNA has long been the subject of intense investigation in relation to the development of new reagents for biotechnology and medicine. Studies of small molecules, which react at specific sites along a DNA strand as reactive models for protein–nucleic acid interactions, provide routes toward rational drug design as well as means to develop sensitive chemical probes for DNA. A number of metal chelates have been used as probes of DNA structure in solution, as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents (1-10). Over the past decade there has been substantial interest in the design and study of DNA binding properties of potential redox and spectroscopically active Cu(II/I) and Ru(II/III) complexes (11-13) as new chemical nucleases(14-21).

Thus, copper complexes containing phen (phen = 1,10-phenanthroline) ligands have been shown to be useful probes for DNA duplexes [22]. They show antiviral activity by their interaction with nucleic acid templates and inhibit proviral DNA synthesis (23). Sigman and his co-workers(24-27) have shown that the cationic complex $[Cu(phen)_2]^{2+}$ in the presence of molecular oxygen and a reducing agent, acts as an efficient nuclease by oxidative cleavage mechanism with a high preference for double-stranded DNA. Also, there are a few reports of copper complexes cleaving DNA hydrolytically (15-28) and several other copper-based synthetic nucleases have been also reported (29-34). Over the past several years there has been continuous interest in determining the mode and extent of binding of metal complexes to DNA, as such information's are important for understanding the cleavage properties of metal complexes in order to develop cleaving agents for probing nucleic acid structures and for other applications.

Metal complexes are known to bind to DNA via both covalent and non-covalent interactions. In covalent binding the labile ligand of the complexes is replaced by a nitrogen base of DNA such as guanine N_7. In fact, cisplatin, an important antitumor drug is thought to bind to DNA through an intrastrand cross link between neighboring guanine residues created by covalent binding to two soft purine nitrogen atoms. On the other hand, the non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of cationic metal complexes along outside of DNA helix, in the minor and major groove, hydrogen bonding and Vander Waals interactions of functionalities bound along the groove of the DNA helix, on the outside of the helix (external electrostatic), the coordination anion interacting with the nucleic acid cation through electrostatic interaction. Intercalation involves the partial insertion of aromatic heterocyclic rings of ligands between the DNA base pairs. Three non-covalent binding modes of metal complex bond to DNA



External electrostatic groove intercalation

In order to find anti carcinogens that can recognize and cleave DNA, people synthesized and Developed many kinds of complexes. Among these complexes, metals or ligands can be varied in an easily controlled way to facilitate the individual applications. Copper is a bioessential element with relevant oxidation states. More than a dozen of enzymes that depend on copper for their activity have been identified; the metabolic conversions catalyzed by all of these enzymes are oxidative. Due to their importance in biological processes, Copper (II) complexes synthesis and activity studies have been the focus from different perspectives.

In this work, we have synthesized and characterized a Copper II complex. The central atom copper II is coordinated with two Nitrogen atoms of the 1,10 Phennanthroline and one

Tele:

E-mail addresses: santhapandari@gmail.com

carboxylate oxygen and one amino nitrogen atoms of L.Alanine and one nitrogen atom of the urea ligand. The DNA binding of the copper (II) complex has been investigated by means of electronic absorption spectroscopy, emission spectroscopy, viscosity and cyclic voltammetry measurements. This result would be helpful for understanding the binding mode of the complex to DNA further, and it also lays a foundation for developing new useful DNA probes and effective inorganic complex nucleases, We have also reported the antimicrobial activities of a sample of copper(II) complex against Gram +ve and Gram –ve bacteria and fungus.

Experimental

Materials

All reagents and chemicals were procured from commercial sources. Solvents used for electrochemical and spectroscopic studies were purified by standard procedures. The stock solution of CT-DNA was prepared by dissolving the solid DNA in doubly distilled water and was stored at 4ºC. The concentration of the nucleotide was determined by UV absorption spectroscopy using the molar absorption coefficient at 260 nm [35] (6600 $M^{-1}cm^{-1}$). A solution of CT- DNA in the tris–HCl buffer (pH 7.4) gave an absorbance ratio, A_{260}/A_{280}, between 1.8 and 1.9, indicating that the DNA was sufficiently free of protein [36]. This DNA solution was not purified further. Common reagents such as ethanol, anhydrous ether, $CuSO_4$ $5H_2O$, NaOH, $NaClO_4$ and 1,10-phenanthroline are all analytical grade and used as received. L-Alanine, tris(hydroxymethyl) aminomethane (tris) and ethidium bromide (EB) were purchased from sigma.

Physical measurements

The carbon, hydrogen and nitrogen contents of samples were determined at SAIF, Lucknow, India. Absorption spectra was recorded on a UV–VIS–NIR Cary300 Spectrophotometer using cuvettes of cm^{-1} path length, and emission spectra was recorded on a JASCO FP 770 spectrofluorimeter FT-IR spectra were recorded on a FT-IR Perkin Elmer spectrophotometer with samples prepared as KBr pellets. EPR spectra was recorded on Varian E-112 EPR spectrometer at room temperature and at LNT (Liquid nitrogen temperature, 77 K), A three-electrode system comprising of a glassy carbon working electrode, a platinum wire auxillary electrode and a saturated calomel reference (SCE) electrode was used for voltammetric work. The buffer solution (50 mM NaCl-5 mM Tris–HCl) was used as supporting electrolyte. The antimicrobial screening studies was carried out at Arcot microlab,

Viscosity experiments was conducted on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained at 25 ± 0.1 ˚C. Titrations was performed for the complexes (1–10µM), and each compound was introduced in to the CT-DNA solution (10 lM) present in the viscometer. Data are presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of the compound to CT-DNA, where η is the viscosity of CT-DNA in the presence of the compound and η_0 is the viscosity of CT-DNA alone. Viscosity values were calculated from the observed flow time of CT-DNA containing solutions corrected from the flow time of buffer alone (t_0), $\eta = t - t_0$. Cyclic voltammetry experiments was performed at room temperature under an inert atmosphere (Nitrogen) with a conventional three-electrode electrochemical cell, and using a CHI-420 electrochemical. The three-electrode system used in this work consists of a glassy carbon electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode, and a Pt foil auxiliary electrode. Solutions were prepared by dissolving the complexes in DMF and 0.1 M $NaClO_4$ was used as supporting electrolyte.

Synthesis of complex [cu(phen)(L-Ala)u](ClO$_4$)

The ligand Urea (0.1802g) (Fig.1) was dissolved in ethanol, and to this a solution of Copper II complex[cu(phen)(L-Ala)(H_2O)]ClO_4 (1.347g) was added slowly with constant stirring for 2 hours with heating. After cooling the solution at room temperature the blue colour precipitate obtained was filtered and dried in vacuum. yield 85% *Anal.* Calcd. For the m.f . $CuC_{16}N_5H_{18}ClO_7$ C, 40.32.11; H, 4.18; N, 13.83 % Found: C, 40.30; H, 4.19; N, 13.85 %

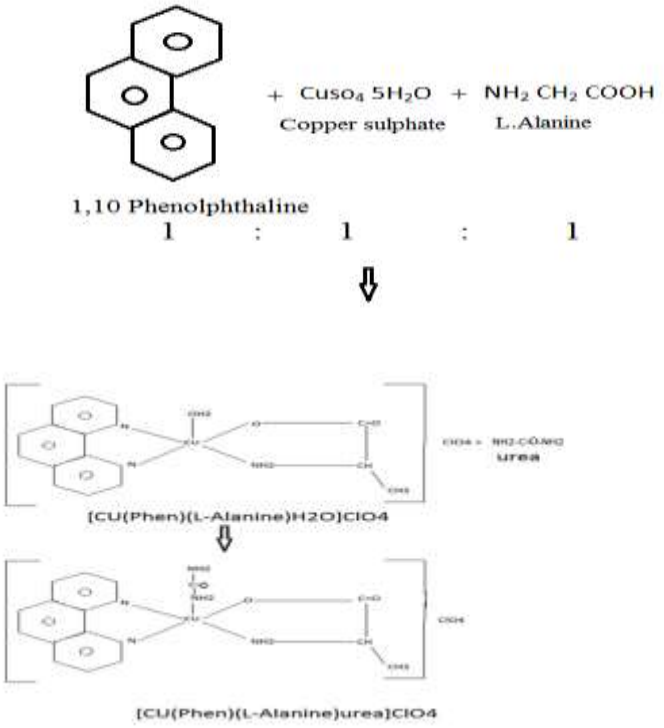


Fig 1. ***Synthesis of complex [cu(phen)(L-Ala)u](ClO$_4$)***

Results and discussion

The complex are stable in atmospheric condition and soluble in ethanol , Methanol, DMF, DMSO, and acetonitrile. The melting point value of the complex is 275^{0}C.

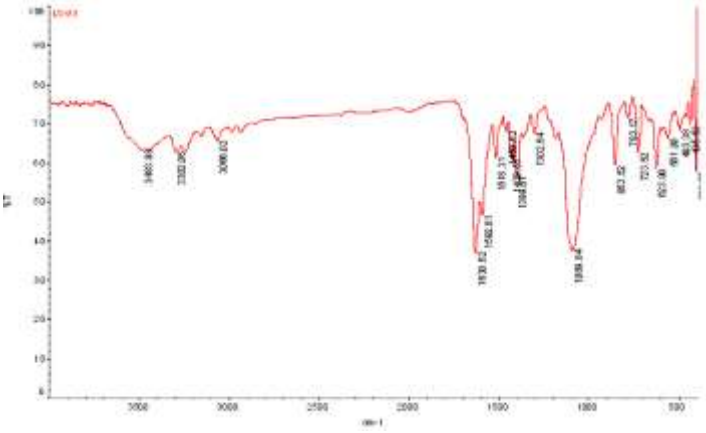


Fig 2

IR Spectra

IR of [Cu(Phen)(L.AL)(U)]ClO4 complex (Fig.2) can be attributed to the ring stretching frequencies [ν (C=C) and ν (C=N)] of 1,10-phenanthroline.[37] The IR values, δ (C– H) 723cm-1 and 623 cm-1 observed for phenanthroline are shifted to 853 cm-1 and 782 cm-1. These shifts can be explained by the fact that each of the two nitrogen atoms of phenanthroline ligands donates a pair of electrons to the central copper metal forming a coordinate covalent bond.[38] The bands around 3066

cm-1 can be attributed to the ring stretching frequencies (C=C) of 1,10-phenanthroline. The broad band observed around 3483 cm-1 and 3302 cm-1 is assigned to the N–H stretching of L-Alanine[39] and the band around 1089 cm-1 has been assigned to ν (Cl–O) of perchlorate anion.[37]

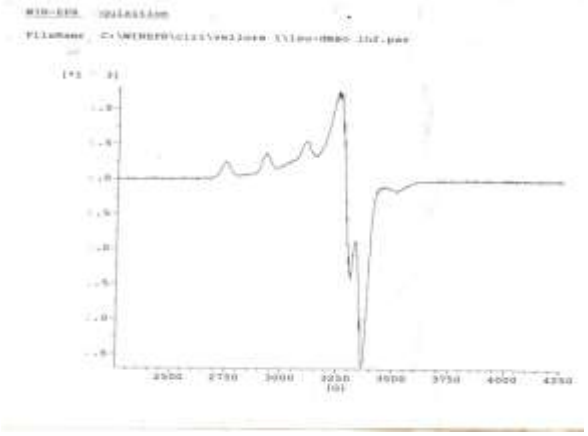


Fig 3

EPR Spectra

The solid state EPR spectra of the copper (II) complexwere recorded in X-band frequencies shows (Fig3). At liquid nitrogen temperature the complex exhibits well defined single isotropic feature near g = 2.13. Such isotropic lines are usually the results of inter molecular spin exchange, which broaden the lines. This intermolecular type of spin exchange is caused by the strong spin coupling which occurs during a coupling of two paramagnetic species.

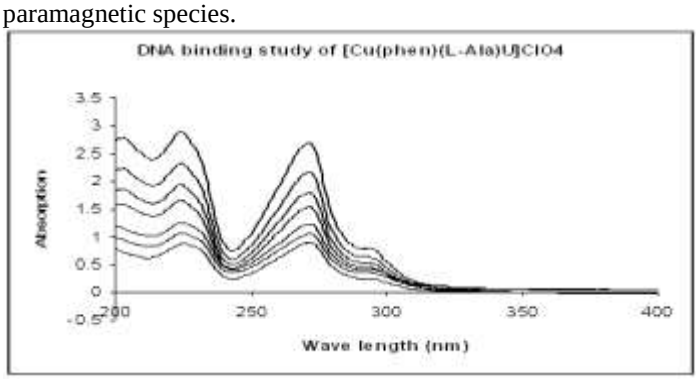


Fig. 4 Absorption spectra of [Cu(phen)(L-Ala)U](ClO$_4$) in the absence(a) and In the presence of increasing amounts of DNA(b,c,d,e,f,g), [Complex] = 15mM, [DNA] = 0.5- 2 mM. Arrow shows the absorbance change up on increasing DNA concentrations. Inset: plots of [DNA]/ (εa-εf) versus [DNA] for the titration of DNA with complexes.

Spectroscopic studies on DNA interaction

Electronic absorption spectra

Electronic absorption spectroscopy is usually utilized to determine the binding of complexes with the DNA helix. A complex bound to DNA through intercalation is characterized by the change in absorbance (hypochromism) and red shift in wavelength, due to the intercalative mode involving a strong stacking interaction between the aromatic chromophore and the DNA base pairs. The extent of hypochromism is commonly consistent with the strength of the intercalative interaction [40-42]. The absorption spectra of the complex in the absence and presence of CT DNA are shown in (Fig.4). In the UV. region, copper(II) complexe exhibit intense absorption bands: one at ca.260 nm which is attributed to the ligand-to-metal charge-transfer absorption (LMCT), With increasing CT DNA, the absorption bands of the complexes are affected, resulting in the obvious tendency of hypochromism and slight shifts to longer wavelengths, which indicates that the copper(II) complex can interact with CT DNA. So the binding of the copper(II) complex are expected to involve intercalation of an aromatic moiety ring between the DNA base pairs and the complexes.

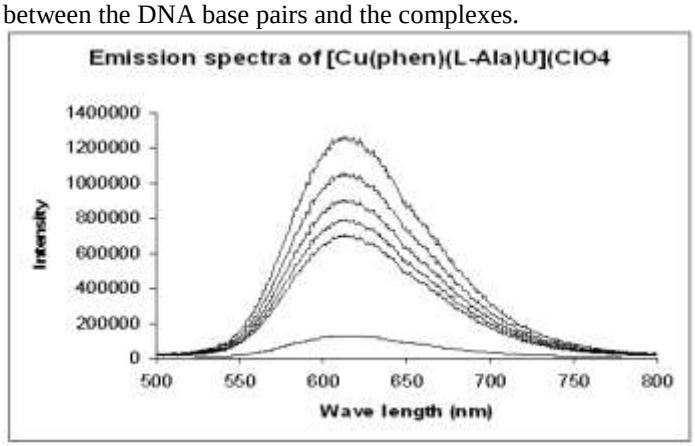


Fig 5. Emission spectra of Peak (a) EB bound to DNA in the absence(---) and in the presence of Complex [Cu(phen)(L-Ala)U](ClO$_4$), and Peak(b-f) [DNA] =4 mM,[Complex] = 0-20 mM. The arrow shows the intensity changes on increasing the complex concentration.

Fluorescence Emission spectra

As the copper (II) complexes are non-emissive, competitive binding studies with EB were carried out to gain support for the mode of binding of the complexes with DNA. The study involves addition of the complexes to DNA pretreated with EB ([DNA]/ [EthBr] =1) and then measurement of intensity of emission. The observed enhancement in emission intensity of EB bound to DNA is due to intercalation of the fluorophore in between the base pairs of DNA and stabilisation of its excited state (Fig. 5). [43]. Addition of all the complexes to CT-DNA incubated with EB decreases the DNA induced enhancement in emission to the same extent. This suggests that the complexes displace DNA-bound EB and bind to DNA at the intercalation sites with almost the same affinity, which is consistent with the above spectral results suggesting partial intercalation of the phen ring.

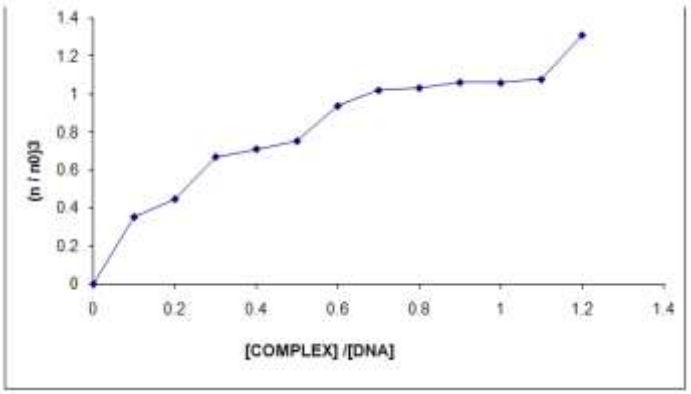


Fig.6 Viscocity of *[cu(phen)(L-Ala)u](ClO$_4$)* Effect of increasing amounts of *[cu(phen)(L-Ala)u](ClO$_4$)*on the relative viscosity of calf thymus DNA increased significantly

Viscosity studies

To explore further the interaction between the copper (II) complex and DNA, viscosity measurements were carried out on CT DNA by varying the concentration of the complex. Spectroscopic data are necessary, but insufficient to support an intercalative binding mode. Hydrodynamic measurements which are sensitive to length increases (i.e. viscosity, sedimentation et. al) are regarded as the least ambiguous and the most critical tests of binding on solution in the absence of crystallographic

structure data [44]. A classical intercalation mode causes a significant increase in the viscosity of DNA solution due to the increase in separation of the base pairs at intercalations sites and hence to an increase in overall DNA contours length. A partial and/or nonclassical intercalation of ligand would reduce the DNA viscosity [45]. The effects of the copper (II) complex on the viscosity of CT DNA solution are given in (fig.6). The plot shows that complex had a reverse effect on the relative viscosity of the CT DNA. With addition of the complex, the relative viscosity of DNA increased. Since the increase is far less than that observed for an intercalator such as EB. This observations leads us to support the above spectral studies which suggest that the complex interact with DNA via partial intercalation between DNA base pairs, which is similar to the interaction of $[Cu(phen)2]^{2+}$ with DNA [46,47].

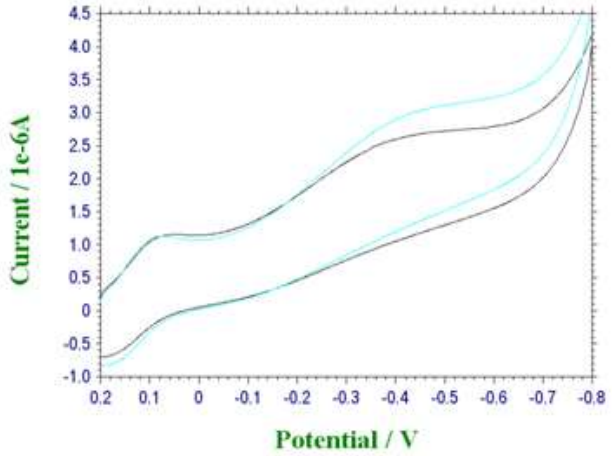


Fig 7. Cyclic voltammetric study of [Cu(phen)(L-Ala)U](ClO$_4$)

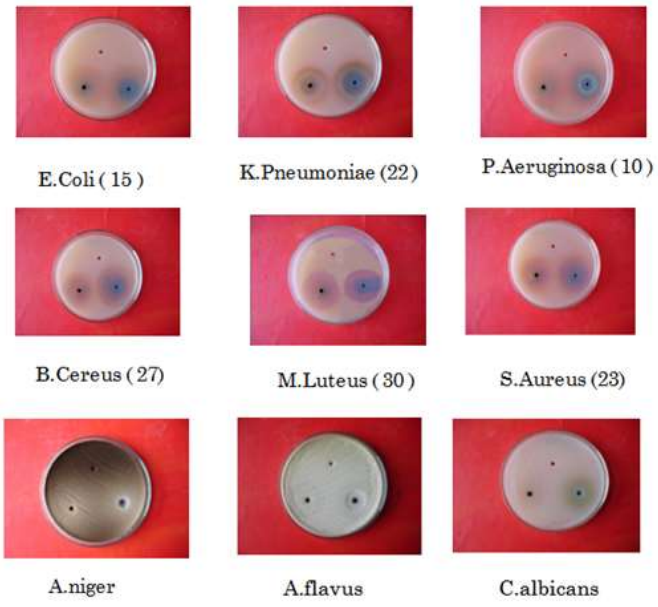


Fig 8. Antimicrobial Activity of *[cu(phen)(L-Ala)u](ClO$_4$)*

Cyclic voltammetric study

Cyclic voltammetric techniques was employed to study the interaction of the present redox active metal complex with DNA with a view to further explore the DNA binding modes assessed from the above spectral and viscometric studies. Typical cyclic voltammetry (CV) behaviors of [Cu(Phen)(L- A)(U)]ClO4 in the absence and presence of CT-DNA are shown in (Fig.7). The cyclic voltammogram of [Cu(Phen)(L-A)(U)]ClO4 in the absence of DNA featured reduction of Cu(II) to the Cu(I) form at a cathodic peak potential [48], Epc of -0.66 V and anodic peak pontial, Epa of -0.42 V. The separation of the anodic and cathodic peak potentials, Ep = -0.24 V. The formal potential E1/2, taken as average of Epc and Epa, is -0.54 V in the absence of DNA. The presence of DNA in the solution at the same concentration of [Cu(Phen)(L-A)(U)]ClO4 causes a considerable decrease in the voltammetric current coupled with a slight shift in the E1/2(E1/2 = -0.52 V) to less negative potential. The drop of the voltammetric currents in the presence of CT-DNA can be attributed to diffusion of the metal complex bound to the large, slowly diffusing DNA molecule. Obviously, E1/2 undergoes a positive shift (25 mV) after forming aggregation with DNA, suggesting that the copper complex bind to DNA mainly by intercalation binding mode [49], and this result also proves the results obtained from viscosity and absorption spectrum studies again.

Microbial assay

The copper (II) complex was screened in vitro for its microbial activity against certain pathogenic bacterial and fungal species(Fig.8) using disc diffusion method. The complex was found to exhibit considerable activity against Gram positive and Gram negative bacteria and the fungus C. albicans. The test solutions were prepared in dimethyl sulphoxide and the results of the antimicrobial activities are summarized in Table 1.

Table 1. Antimicrobial Activity of *[cu(phen)(L-Ala)u](ClO$_4$)* complex

S.No	Name of Test Organisms	zone of Inhibition in mm for
1.	*Staphylococcus aureus*	*23*
2.	*Micrococcus luteus*	*30*
3.	*Bacillus cereus*	*27*
4.	*Escherichia coli*	*15*
5.	*Klebsiella Pneumoniae*	*22*
6.	*Pseudomonas aeruginosa*	*10*
s.no	Name of Test Organisms	zone of Inhibition in mm
1	Aspergillus niger	7
2.	Aspergillus flavus	-
3.	Candida albicans	-

Zoroddu et al. [50] Have reported that copper complex show any significant activity against the Gram positive and Gram negative bacteria. Recently Patel et al. have indicated that the copper (II) complex with L-phenylalanine has exhibited considerable activity against some human pathogens [51]. In our biological experiments, using copper (II) complex, we have observed antibacterial activity against Gram positive bacteria Staphylococcus aureus and B. subtilis and Gram negative bacteria E. coli and Pseudomonas aeruginosa. The copper (II) complex has shown high activity against Gram positive than Gram negative bacteria. The copper (II) complex is also very active against the fungus C. albicans than the standard antifungal drug, clotrimazole. In our biological experiments, using copper(II) complex we have observed considerable antibacterial activity against Gram +ve bacteria and Gram ⁻ve bacteria. The copper(II) complex has shown a high activity against Gram +ve than Gram ⁻ve bacteria. The copper(II) complex is low active against the fungus . It may be concluded that our copper(II) complex inhibits the growth of bacteria and fungi .

Conclusion

A Copper (II) complex, [Cu(phen)(L-Ala)U](ClO$_4$) (phen=1,10- phenanthroline, L-Ala=L-Alanine, U=urea), has been synthesized and characterized. The interaction with calf-thymus DNA has been studied by means of electronic absorptions pectroscopy, emission spectroscopy, and viscosity methods conducted to assess their interaction between their

complex and DNA. The results suggest that the Copper (II) complex was shown to intercalate mode of DNA Binding. A sample of copper II complex was tested for its Antimicrobial Activities and it was found to have good antimicrobial activities.

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