



Synthesis, Characterisation, DNA-Binding Studies and antimicrobial activity of Copper (II) Complex with 1,10 Phenanthroline, L-Tyrosine and Thiosemicarbazide as Ligands

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ABSTRACT

A copper complex of the type $[\text{Cu}(\text{Phen})(\text{L-Tyr})(\text{TSC})](\text{ClO}_4)$, where Phen = 1,10 Phenanthroline, Tyr = Tyrosine and TSC = Thiosemicarbazide, was synthesised and characterised by elemental analysis, IR, conductivity measurement and EPR studies. DNA-binding properties have been studied by electronic absorption, emission, viscosity and cyclic voltametric methods. The results suggest that the copper(II) complex bind to DNA via different modes. Gel electrophoresis study reveals the fact that the copper complex cleaves super coiled pBR 322 DNA to nicked and linear forms in the presence and absence of ascorbic acid. The in vitro antimicrobial study indicates that the complex has good activity against gram positive, gram negative bacteria and fungus.

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Introduction

The interaction of transition metal complexes with DNA has long been a subject of intensive investigation with the perspective of development of newer materials for application in biotechnology and medicine. These investigations have resulted in the synthesis of many new metal complexes, which bind to DNA by non-covalent interactions such as electrostatic binding, groove binding and intercalative binding [1,2]. Hence, the current growing interest in small molecules that are capable of binding and cleaving DNA is related to their utility in the design and development of synthetic restriction enzymes, new drugs, DNA foot-printing agents, etc., and also to their ability to probe the structure of DNA itself [3].

Copper complexes of 1,10-phenanthroline and its derivatives are of great interest since they exhibit numerous biological activities such as antitumour[4], anti-Candida[5], antimycobacterial [6], antimicrobial [7], activities etc. Moreover, considerable attention has been focused on the use of phenanthroline complexes as intercalating agents of DNA [8] and as artificial nucleases [9-11].

Copper(II) is known to play a significant role in biological systems and also as a pharmacological agent. Synthetic copper(II) complexes have been reported to act as potential anticancer and cancer inhibiting agents [12-14], and a number of copper complexes [15,16] have been found to be active both in vitro and in vivo.. Very recently, Reedijk and co-workers have found that the complex $[\text{Cu}^{\text{II}}(\text{pyrimol})\text{Cl}]$ brings about efficient self-activated DNA cleavage and cytotoxic effects towards L1210 murine leukaemia and A2780 human ovarian carcinoma cell lines [17]. Sadler and co-workers have observed [4] that mixed ligand bis (salicylato) copper(II) complexes with diimines as co-ligands exhibit cytotoxic and antiviral activities. Very recently, Ng and co-workers have prepared ternary copper(II) complexes of ethylene diamine diacetic acid (H_2edda) and 1,10-phenanthroline, that strongly bind to DNA and also regulate apoptosis [18]. Palaniandavar, et. al., recently reported that

mixed ligand copper(II) complexes of diimines, that bind and cleave DNA and also exhibit anticancer activity which is more efficient than that of cisplatin[19]. The use of phenanthroline as the co-ligand in the above ternary complexes is of considerable interest because some of the phenanthroline containing copper complexes exhibit biological as well as pharmacological properties[20]. Palaniandavar and co-workers also reported that mixed ligand copper(II) [21] and ruthenium(II) [21-22] complexes exhibit prominent anticancer activities in which the diimine co-ligands play a pivotal role in the mechanisms underlying the induction of cell death. The mixed ligand palladium(II) and platinum(II) complexes containing amino acids have been shown to act as potential anticancer agents [23-24].

The study of ternary copper(II) complexes of diimines and amino acids as anticancer drugs is of interest because copper is a bio essential element but only in traces, and also, amino acids are present in all biological systems. Also, these mono positive complexes are expected to be more lipophilic than the corresponding dicationic complexes, thereby providing for greater uptake by cells and hence an enhanced cytotoxicity. Very recently, Guo and co-workers have found the ternary copper(II) complex of phenanthroline and L-threonine to cleave DNA oxidatively and exhibit cytotoxicity [25].

Further, some ternary copper(II) complexes are having macrocyclic bases such as 1,10 phenanthroline and its derivatives which are capable of DNA intercalation/binding in the presence of auxiliary ligands, were prepared and characterised. These complexes bind and cleave DNA effectively in the presence of a reductant or upon irradiation with UV or visible light [26-28].

Certain amino acid complexes are found to exhibit potent anti-tumour and artificial nuclease activity. The focus was shifted towards the development of ternary copper(II) complex of phenanthroline with amino acids and their DNA cleavage activity was investigated.

In this paper, the synthesis and characterisation of [Cu(Phen)(L-Tyr)(TSC)]ClO₄ complex by elemental analysis, conductivity measurement, UV-Visible spectra, IR and EPR spectra have been reported. The binding property of the complex to calf thymus DNA has been studied using different physico-chemical methods and the binding modes are discussed. The experimental results show that the copper(II) complex sample binds effectively with DNA-binding agents and cleaves its super-coiled form into nicked and linear forms. We have also reported the antimicrobial activities of copper(II) complex against gram positive, gram negative bacteria as well as fungus. It is generally believed that molecules which damage DNA and block DNA synthesis indirectly through inhibition of biosynthesis of precursor molecules for nucleic acids or disrupt hormonal regulation of cell growth would make them better candidates for development as anticancer agents [29]. Also, DNA has been identified as the possible primary molecular target [30] of metal-based anticancer agents such as cisplatin[31]. Therefore, we propose to investigate whether the complex exhibits DNA-binding and cleavage properties. The introduction of chirality via the amino acid may enhance the pharmacological behaviour of the copper complex by adopting a specific conformation and may also confer selective binding affinity for the chiral DNA. Furthermore, the carboxylate group of L-tyrosine has the potential to interact with sugar hydroxyl groups of DNA to enhance the DNA binding affinity and to provide recognition element, thus leading to the selective control of the metal-chelate nucleobase recognition process. Also, there has been considerable attention focused on the use of the small metal complexes containing diimines as recognition elements of DNA [32-35] and metal-based synthetic nucleases [35,10,9].

Experimental

Materials and Methods

The reagents such as ethanol, anhydrous ether, Cu(NO₃)₂·3H₂O, NaOH, NaClO₄·H₂O and 1,10 phenanthroline of analytical grade (sd fine chemical) are used in the same condition as received. L-Tyrosine was purchased from Aldrich. Disodium salts of calf-thymus DNA (CT DNA) was purchased from Aldrich. Ascorbic acid, tris HCl, NaCl and ethidium bromide were purchased from sd fine chemicals. The spectroscopic titration was carried out in the buffer (50 mM NaCl-5 mM Tris-HCl, pH 7.1) at room temperature. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance 1.8-1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [36]. Milli-Q water was used to prepare the solutions. Absorption spectral study was carried out by using UV-VIS-NIR Cary 300 spectrophotometer which is having cuvettes of 1 cm path length, and emission spectral study was carried out by using JASCO FP 770 spectrofluorimeter. The complex, [Cu(Phen)(L-Tyr)(H₂O)]ClO₄, was prepared as reported earlier in the literature [37].

Element analysis was performed by SAIF, Lucknow, India. The conductivity study was carried out by using an aqueous solution of complex with an Elico conductivity bridge type CM82 and a dip-type cell with cell constant 1.0. Absorption spectral study was carried out by using UV-VIS-NIR Cary 300 spectrophotometer having cuvettes of 1-cm path length, and emission spectral study was carried out by using JASCO FP 770 spectrofluorimeter. IR spectra were recorded on an FT-IR Perkin Elmer spectrophotometer.

Electron paramagnetic resonance spectra of the Copper(II) complex was obtained on a Bruker EMX EPR spectrometer. The spectra were recorded for solution of the Copper(II)

complex in acetonitrile : acetone (4 :1 v/v) solution at room temperature (RT) as well as at liquid nitrogen temperature (77 K). DPPH was used as the field marker.

The antimicrobial screening studies were carried out at micro labs, Arcot, India. The bacteria and fungus species were obtained from National Chemical Laboratory (NCL), Pune, India. Electrochemical measurements were recorded on an Electrochemical analyser CH Instrument version 5.01 and model-600C. A three-electrode system comprising a glassy carbon working electrode, a platinum wire auxiliary electrode and a saturated calomel reference (SCE) electrode was used for voltametric work. The buffer solution (50 mM NaCl-5 mM Tris-HCl) was used as the supporting electrolyte. Agarose gel electrophoresis method was carried out at micro labs, Arcot, India. Water purified using a Milli-Q system was used for all the present studies.

Synthesis of [Cu(Phen)(L-Tyr)(TSC)]ClO₄.

1.6241 g (0.003mM) of [Cu(Phen)(L-Tyr)(H₂O)]ClO₄ complex is dissolved in 15 ml water and mixed with equimolar concentration of thiosemicarbazide i.e., 0.2734 g (0.003mM) and stirred for one hour to get the blue coloured precipitate which was then filtered and washed with ethanol and then dried.

DNA Binding activity

The DNA binding experiments were performed at 30.0 ± 0.2° C. The DNA concentration per nucleotide was determined by electronic absorption spectroscopy using the known molar extinction coefficient value of 6600 M⁻¹ cm⁻¹ at 260 nm [38]. Absorption titration experiments of copper(II) complex samples in buffer solution (50 mM NaCl-5 mM Tris-HCl, pH 7.2) were performed by using a fixed complex concentration to which increments of the DNA stock solutions were added. Copper(II) complex-DNA solutions were allowed to incubate for 10 minutes before the absorption study was carried out.

For fluorescence-quenching experiments, DNA was pre-treated with ethidium bromide (EB) for 30 minutes. The copper(II) complex samples were then added to this mixture and their effect on the emission intensity was measured. Samples were excited at 450 nm and emission was observed between 500 nm and 800 nm.

Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature of 30.0±0.1 °C in a thermostatic water-bath. Calf-thymus DNA samples approximately 200 base pairs in average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility [39]. Flow time was measured with a digital stopwatch and each sample was measured three times and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio [40], where η is the viscosity of CT DNA in the presence of complex, and η_0 is the viscosity of CT DNA alone.

DNA cleavage

For the gel electrophoresis study, super coiled pBR322 DNA (0.1 µg) was treated with the copper(II) complex in 50 mM Tris-HCl-18 mM NaCl buffer, pH 7.2. The samples were electrophoresed for 3 hours at 50 V on a 0.8 % agarose gel in tris-acetic acid-EDTA buffer. The gel was stained with 0.5 µg of ethidium bromide and photographed under UV light.

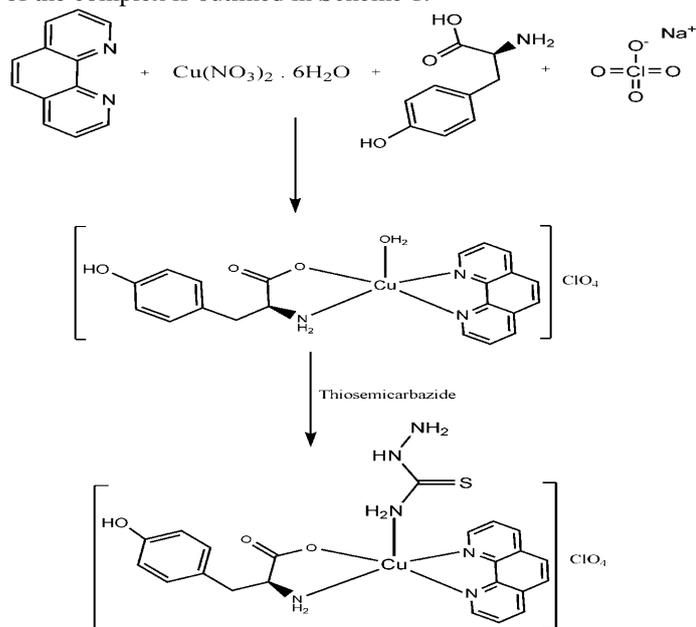
Microbial Assay

Antimicrobial analysis was followed using standard agar well diffusion method to study the antimicrobial activity of essential oils [41-43]. Each bacterial and fungal isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 10⁵ colony forming unit (CFU) per mL. They

were flood inoculated onto the surface of BHI agar and then dried. 5mm diameter wells were cut from agar using a sterile cork-borer and 30 μL (5 μg compound in 500 μL DMSO) of the sample solution were poured into the wells. The plates were incubated for 18 hours at 37 $^{\circ}\text{C}$ for bacteria and at room temperature for fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition in mm against the test microorganisms. DMSO was used as solvent control. Ciprofloxacin was used as reference antibacterial agent. Ketoconazole was used as a reference antifungal agent. The tests were carried out in triplicates.

Results and discussion

The elemental analysis data were found to be in good agreement with those of the calculated values. The Λ_M value of the complex in water is 82 $\text{Ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$, which indicated that the complex is a 1:1 electrolyte [44]. The synthetic strategy of the complex is outlined in Scheme 1.



Scheme-1. Synthetic strategy of Cu (II) complex

Characterisation

Elemental Analysis and Conductivity Measurements

The elemental analysis data are found to be in good agreement with those of the calculated values. The Λ_M values of the complexes indicate that the complexes are 1:1 electrolytes [44]. The values are given in table 1.

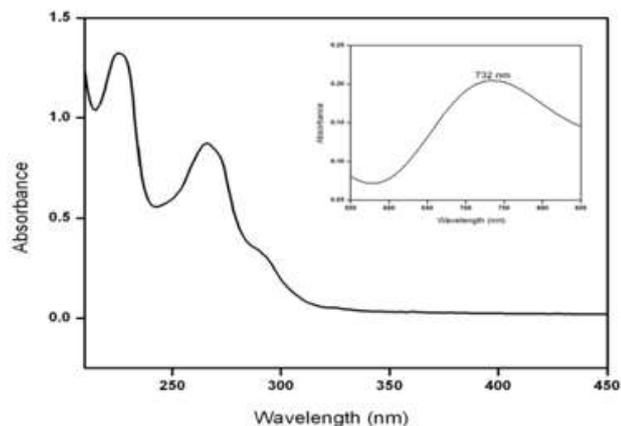


Fig 1. UV-Visible spectrum of $[\text{Cu}(\text{Phen})(\text{L-Tyr})(\text{TSC})]\text{ClO}_4$
UV Visible Spectra of the complexes

The complexes are one electron paramagnetic at room temperature, corresponding to d^9 electronic configuration for the

copper (II) center. The complexes display a copper (II) centered d-d bands 684nm and 732nm in addition to the ligands centered bands in the UV region of the electromagnetic spectra (Fig.1). The electronic spectra of the complexes are in good agreement with the previously reported square pyramidal geometry of the complexes[45,25,46].

In the UV-Vis region, the intense absorption bands which appeared from 230 nm to 275 nm is attributed to intraligand transitions. Another band which appeared around 265 nm is assigned to ligand field transitions[47]. The electronic absorption spectral data for Cu(II) complexes are given in the table 2.

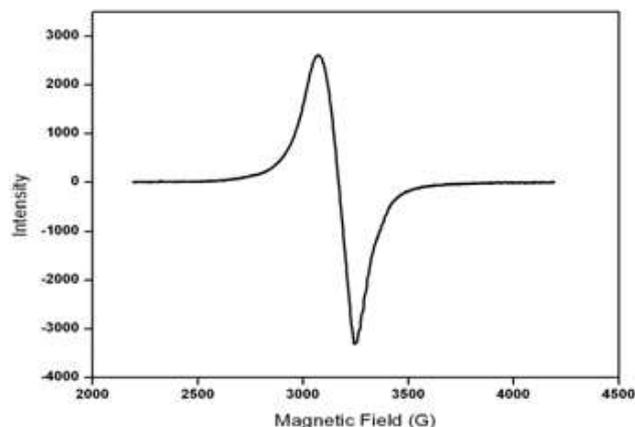


Fig 2. EPR spectrum of $[\text{Cu}(\text{Phen})(\text{L-Tyr})(\text{TSC})]\text{ClO}_4$ in DMSO at liquid nitrogen temperature

IR Spectra

Fig.2 shows the IR region the bands around 1589 cm^{-1} and 1329 cm^{-1} can be attributed to the ring stretching frequencies [ν (C=C) and ν (C=N)] of 1,10-phenanthroline. The IR values, δ (C-H) 722 cm^{-1} and 645 cm^{-1} observed for phenanthroline are shifted to 843 cm^{-1} and 804 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. The broad band observed around 3441 cm^{-1} is assigned to the N-H stretching of L- tyrosine and the band around 1098 cm^{-1} has been assigned to ν (Cl-O) of perchlorate anion.

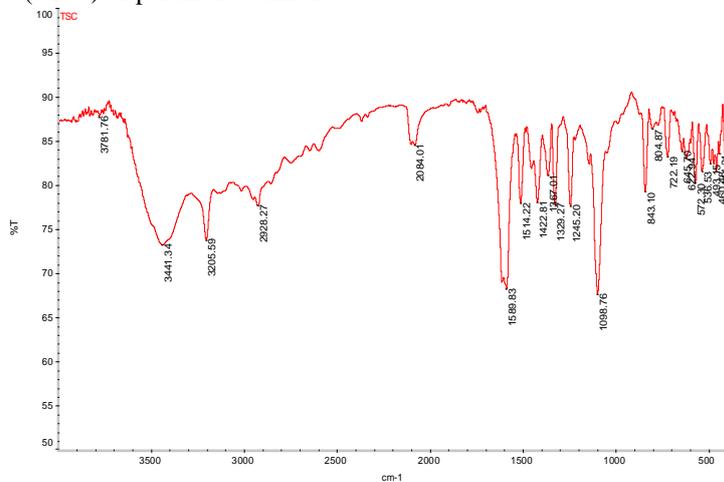


Fig 3. IR spectrum of $[\text{Cu}(\text{Phen})(\text{L-Tyr})(\text{TSC})]\text{ClO}_4$
EPR Spectra

The fig.3 shows EPR spectra of the copper(II) complex which exhibits well-defined single isotropic feature near g_{\perp} (perpendicular) value of 2.20 and g_{\parallel} (parallel) value of 2.08. Such isotropic lines are usually a result of intermolecular spin exchange which broadens the lines. This intermolecular type of

spin exchange is caused due to the strong spin coupling which occurs during a coupling of two paramagnetic species.

DNA Binding Studies

DNA Binding – Electronic absorption study

Electronic absorption spectroscopy was an effective method to examine the binding mode of DNA with metal complexes [45,46,48]. In general, hypochromism and red-shift are associated with the binding of the complex to the helix by an intercalative mode involving strong stacking interaction of the aromatic chromophore of the complex between the DNA base pairs.

Fig. 4 shows the UV absorption spectra of copper(II) complex in the absence and presence of DNA. In the ultraviolet region from 240 to 300 nm, the complex had strong absorption peak at 265 nm, besides a shoulder band around 294 nm. The absorption intensity of the copper(II) complex sample decreased (hypochromism) evidently after the addition of DNA, which indicated the interactions between DNA and the complex. We have observed minor red shift along with significant hypochromicity for the complex.

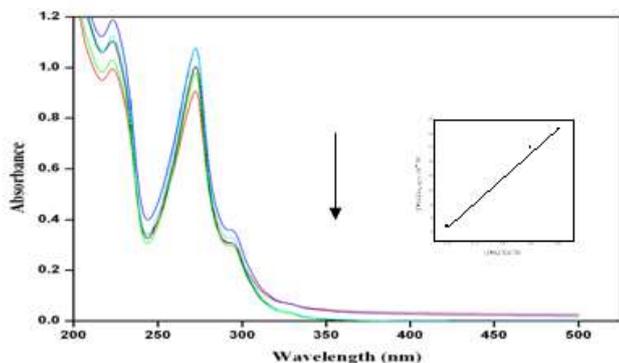


Fig 4. Electronic Absorption Spectra of [Cu(Phen)(L-Tyr)(TSC)]ClO₄ in the absence and in the presence of increasing amounts of DNA concentrations. [Complex] = 15 μM. [DNA] = (5,10,15,20,25) μM. Arrow shows the absorbance changes upon increasing DNA concentrations.

The copper(II) complex can bind to the double stranded DNA in different binding modes on the basis of their structure, charge and type of ligands. As DNA double helix possesses many hydrogen bonding sites which are accessible both in the minor and major grooves. The binding propensity of the phenanthroline complex is due to the presence of the extended planar aromatic ring in phenanthroline. The binding constant, K_b , was determined by using the following equation [49]:

$$[\text{DNA}] / (\epsilon_a - \epsilon_f) = [\text{DNA}] / (\epsilon_b - \epsilon_f) + 1 / K_b (\epsilon_b - \epsilon_f)$$

Where [DNA] is the concentration of DNA in base pairs, ϵ_a , ϵ_f and ϵ_b correspond to $A_{\text{obsd}}/[\text{Cu}]$, the extinction coefficient of the free copper complex and the extinction coefficient of the complex in the fully bound form, respectively, and K_b is the intrinsic binding constant. The ratio of the slope to intercept in the plot of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ versus [DNA] gives the value of K_b and for our copper(II) complex it is $3.6 \times 10^5 \text{ M}^{-1}$.

The K_b values obtained for our copper(II) complex is very similar than those for any other known simple mononuclear or binuclear copper(II) complexes including complexes such as $[\text{Cu}_2\text{phen}_2\text{Cl}_4]$ ($K_b = 4.75 \times 10^4 \text{ M}^{-1}$) [50], $[\text{Cu}(\text{Phen})_2\text{Cl}_2]$ ($K_b = 2.70 \times 10^3 \text{ M}^{-1}$) [51], $[\text{Cu}(\text{Phen})(\text{L-Thr})(\text{H}_2\text{O})]\text{ClO}_4$ ($K_b = 6.35 \times 10^3 \text{ M}^{-1}$) [25] and $[\text{Cu}(\text{L-Tyr})(\text{phen})]^+$ ($K_b = 4 \times 10^3 \text{ M}^{-1}$) [37].

DNA Binding – Emission spectral study

As the present copper(II) complex is non-emissive, ethidium bromide (EB) binding study was undertaken to gain support for the extent of binding of the complex with DNA.

Ethidium bromide (EB) was shown to emit intense fluorescence light in the presence of DNA, due to its strong intercalation between the adjacent DNA base pairs. It was previously reported that the fluorescent light could be quenched by the addition of a second molecule [52]. The quenching extent of the fluorescence of EB binding to DNA is used to determine the extent of binding between the second molecule and DNA. The addition of the complex to DNA pretreated with EB causes appreciable change in the emission intensity. This behaviour can be analysed through the Stern–Volmer equation [52], $I_0/I = 1 + K_{sv}r$, where I_0 and I are the fluorescence intensities in the absence and the presence of complex respectively. K_{sv} is a linear Stern–Volmer quenching constant, r is the ratio of the total concentration of complex to that of DNA. The quenching plot (Fig. 5) illustrates that the quenching of EB bound DNA by the copper(II) complex is in good agreement with the linear Stern–Volmer equation, which also indicates that the complex binds to DNA. In the plot of I_0/I versus $[\text{Complex}]/[\text{DNA}]$, K_{sv} is given by the ratio of the slope to intercept. The K_{sv} value for copper(II) complex thus obtained is 0.43. This suggests that our copper(II) complex binds strongly with DNA, which is also consistent with our absorption spectral result.

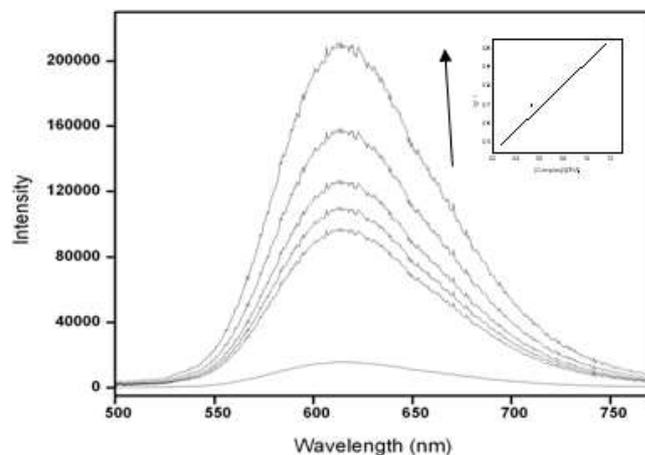


Fig 5. Emission Spectra of EB bound to DNA in the absence (a) and in the presence of [Cu(Phen)(L-Tyr)(TSC)]ClO₄. [complex] = 8,16,24,32,40 × 10⁻⁶ M. [DNA] = 3 × 10⁻⁵ M, [EB] = 3 × 10⁻⁵ M. Arrow shows the intensity changing upon increasing complex concentrations

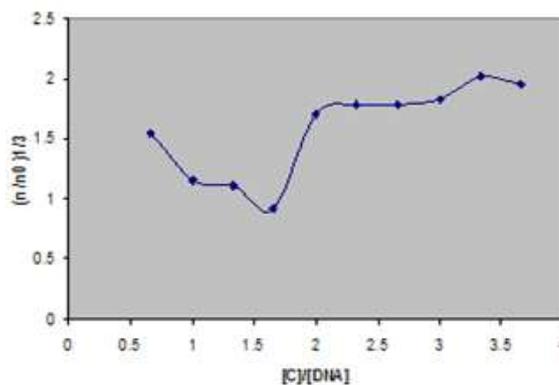


Fig.6. Effect of increasing amount of [Cu(Phen)(L-Tyr)(TSC)]ClO₄ (1,15,20,25,30,35,40,45,50 μM) on the relative viscosity of calf thymus DNA (15 μM) in 5mM Tris-HCl/50mM NaCl buffer

DNA Binding – Viscosity study

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.

Table 1. The Elemental Analysis and Molar Conductivity of the Copper(II) Complex

Name of the Complex	Carbon	Nitrogen	Hydrogen	Molar conductance Ohm ⁻¹ Cm ² Mol ⁻¹
[Cu(Phen)(L-Tyr)(TSC)]ClO ₄	43.00 (42.92)	13.68 (13.53)	3.77 (3.83)	82

Table 2. Electronic Absorption Spectral Data for Cu(II) complex

Name of the Complex	$\lambda_{\text{max}}(\text{nm})$	$\epsilon (\text{M}^{-1}\text{cm}^{-1})$
[Cu(Phen)(L-Tyr)(TSC)]ClO ₄	230	200000
	275	175000

Table 3. Antimicrobial activities of [Cu(Phen)(L-Tyr)(TSC)]ClO₄ complex

Name of the Complex	Diameter Zone of Inhibition (mm)								
	1	2	3	4	5	6	7	8	9
[Cu(Phen)(L-Tyr)(TSC)]ClO ₄	7	31	23	15	7	5	6	5	8

1.Staphylococcus aureus; 2.Micrococcus luteus; 3.Bacillus cereus 4.Escherichia coli;
5.Klebsiella pneumoniae; 6.Pseudomonas aeruginosa; 7.Aspergillus niger; 8.Aspergillus flavus; 9.Candida albicans; Solvent, DMSO (showed nil effect against the microorganisms under test).

Ciprofloxacin was used as reference antibacterial agent. Ketoconazole was used as a reference antifungal agent.

Spectroscopic data are necessary but insufficient to support an intercalative binding mode. Hydrodynamic measurements which are sensitive to length-increase (i.e. viscosity, sedimentation, etc.) are regarded as the least ambiguous and the most critical tests of binding on solution in the absence of crystallographic structure data [45]. 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 intercalation sites and hence to an increase in overall DNA contours length. A partial intercalation of ligand would reduce the DNA viscosity [53]. The effects of the copper(II) complex on the viscosity of CT DNA solution are given in fig. 6. The plot shows that the complex had a reverse effect on the relative viscosity of the CT DNA. With the addition of the complex, the relative viscosity of DNA changed. Since the change is far less than that observed for an intercalator such as EB, this observation 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[54-56].

Typical cyclic voltametry (CV) behaviours of our copper(II) in the absence and presence of CT-DNA are shown in fig. 7. The cyclic voltammogram of copper(II) in the absence of DNA featured reduction of copper(II) to the copper(I) form at cathodic potential, E_{pc} of -0.102 V and anodic peak potential, E_{pa} of -0.127V. The separation of the anodic and cathodic peak potentials, E_p = -0.025 V. The formal potential E_{1/2} was taken as the average of E_{pc} and E_{pa} is -0.114 V in the absence of DNA. The presence of DNA in the solution at the same concentration of copper(II) causes a considerable decrease in the voltametric current coupled with a slight shift in the potential (E_{1/2} = -0.109 V). The drop of the voltametric 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, E_{1/2} undergoes a positive shift after forming aggregation with DNA, suggesting that the copper complex binds to DNA mainly by intercalation binding mode [57] and this result also confirms the results obtained from viscosity and absorption spectrum studies again.

DNA Cleavage - Electrophoresis

The characterisation of DNA recognition by transition metal complex has been aided by the DNA cleavage chemistry that is associated with redox-active or photo activated metal complexes [58]. DNA cleavage is controlled by relaxation of super coiled circular form of pBR322 DNA into nicked circular form and linear form. When circular plasmid DNA is subjected to electrophoresis study, the fastest migration will be observed for the super coiled form (Form I). If one strand is cleaved, the super coils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated which migrates in between. DNA cleavage was analysed by monitoring the conversion of super coiled DNA (Form I) to nicked DNA (Form II) and linear DNA (Form III) in aerobic condition. Interestingly, we have found that this copper complex can cleave the super coiled DNA to nicked and linear DNA at the same time.

As shown in Fig. 8, with the increase of the complex concentration, the intensity of the circular supercoiled DNA (Form I) band was found to decrease, while that of nicked (Form II) and linear DNA (Form III) bands increase apparently. When the complex concentration was up to 20 μM (lane 4), the circular supercoiled DNA (Form I) band disappeared completely. When it is more than 40 μM (lane 6), the circular supercoiled DNA (Form I) band becomes extremely faint.

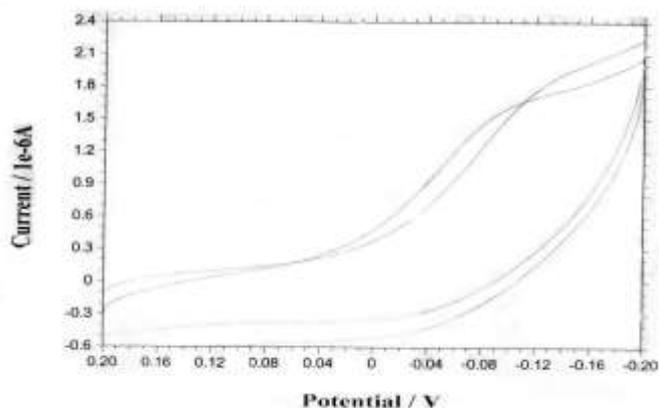


Fig 7. Cyclic voltammogram of [Cu(Phen)(L-Tyr)(TSC)]ClO₄ (1 mM) complex in the absence (—) and in the presence (---) of CT-DNA (1.5 x 10⁻⁵ M). 5 mM in buffer containing 50 mM NaCl–5 mM Tris–HCl, pH 7.2. Scan rate: 100 mV s⁻¹

DNA Binding – Cyclic voltametric study

Cyclic voltametric technique was employed to study the interaction of the present redox active metal complex with DNA with a view to further exploring the DNA binding modes assessed from the above spectral and viscometric studies.

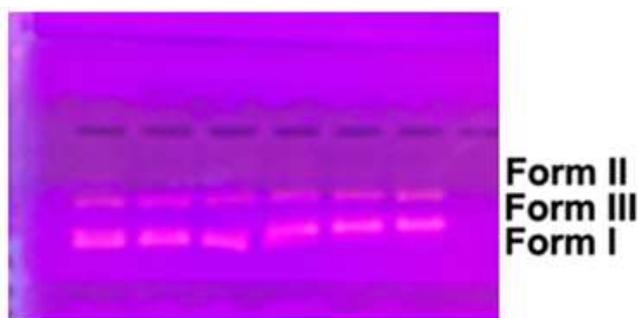


Fig 8. Electrophoretic behaviour of pBR322 DNA by [Cu(Phen)(L-Tyr)(TSC)]ClO₄. Lane 1 pBR322 DNA alone; Lane 2-6: DNA + copper (II) complex in the concentration of 10, 15, 20, 30, 40 μM

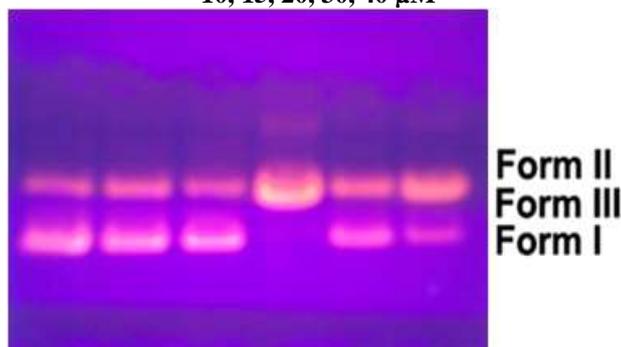


Fig 9. Electrophoretic behaviour of pBR322 DNA by [Cu(Phen)(L-Tyr)(TSC)]ClO₄. Lane 1: DNA alone, Lane 2: DNA + Complex (25 μM), Lane 3: DNA + Ascorbic Acid (100 μM), Lane 4: DNA + Ascorbic Acid (100 μM) + Complex (25 μM), Lane 5: DNA + Ascorbic Acid (500 μM), Lane 6: DNA + Ascorbic Acid (500 μM) + Complex (25 μM)

In order to establish the reactive species responsible for the cleavage of the plasmid DNA, we carried out the experiment in the presence of ascorbic acid as reducing agent (Fig.9). Compared with the control experiments using only the copper (II) complex or ascorbic acid (lane 2, lane 3 and lane 5), the experiment using both copper (II) complex and the same concentration of ascorbic acid (lane 4 and lane 6) showed that the supercoiled DNA (Form I) apparently convert to nicked (Form II) and linear DNA (Form III). Although the ascorbic acid concentration in lane 5 was fivefold of that in lane 3, there is little difference between these two bands. When we add the same concentration of the copper (II) complex to them, an obvious difference occurred. Compared with lane 4, the supercoiled DNA (Form I) completely disappeared and the linear DNA (form III) about to appear in lane 6. These results are similar to that observed for some Cu-salen complexes as chemical nucleases[59,10]. It is likely the generation of hydroxyl radical and/or activated oxygen mediated by the copper complex results in DNA cleavage. Further studies are being pursued to clarify the cleavage mechanism.

Antimicrobial Screening of [Cu(Phen)(L-Tyr)(TSC)]ClO₄ Complex

Table 3 shows that the copper(II) complex exhibits significant activity against the gram positive and gram negative bacteria. In our biological experiments using copper complex, we observed considerable antibacterial activity against gram positive bacteria *Micrococcus luteus* and *Bacillus cereus* and gram negative bacteria *E. coli* and *Klebsiella pneumoniae*. The copper complex has shown a good activity against gram positive than against gram negative bacteria. The complex also shows considerable activity against fungi. It may be concluded that our

copper(II) complex inhibits the growth of bacteria and fungi to a good extent.

Conclusion

In this paper, we have described new copper(II) complex. Further characterisation of the complex was achieved through physico-chemical and spectroscopic methods. The effectiveness of the binding of complex is being confirmed by means of hypochromism in the electronic spectral studies and change in intensity of emission in the case of emission spectral studies. Besides, the effectiveness of binding is also confirmed by the viscometric and cyclic voltametric studies. This shows that the complex interacts with DNA base pairs effectively. The supercoiled DNA is being cleaved in the electrophoresis by the complex which confirms that the complex is having the ability to act as a potent DNA cleaving agent. The copper(II) complex exhibits good antimicrobial activity.

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