

DNA CLEAVAGE, ANTIOXIDANT AND ANTIBACTERIAL STUDIES OF COPPER(II) COMPLEX DERIVED FROM N, N'-(1, 2 PHENYLENEBIS(METHANYLYLIDENE)) BIS(4-NITROANILINE)

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ABSTRACT

Objective: The aim of the present work was to synthesize the Schiff base ligand and its Copper(II) metal complex from the condensation of o-phthalaldehyde and p-nitroaniline. The synthesized Schiff base and its metal complex were characterized by elemental analysis, FTIR, ¹HNMR and UV spectral studies. **Results:** The study reveals that the Schiff base ligand implicated the N₄ donor atom. On the basis of spectral data, distorted octahedral geometry is proposed for the Cu(II) complex. The Schiff base and its metal complex were screened for their antibacterial, antioxidant and DNA cleavage activities. **Conclusion:** The Cu(II) complex exhibited good antimicrobial activity when compared to the free ligand.

KEYWORDS: Schiff base, antibacterial, antioxidant activities and DNA cleavage

INTRODUCTION:

Schiff base acts as active ligands due to the presence of low electronegativity of nitrogen, of the azomethine group (>C=N), lone pair of electrons on the nitrogen atom, electron donating character of the double bond [1-2]. An interesting application of Schiff bases is their use as an effective corrosion inhibitor, which is based on their ability to spontaneously form a monolayer on the surface to be protected. Schiff base and its metal complex had a variety of applications including biological, clinical, analytical and industrial in addition to their important roles in catalysis and organic synthesis. The present developments of Schiff bases and metal complexes are more fascinating. Metal complexes of Schiff bases have been extensively studied due to synthetic flexibility, selectivity and sensitivity towards a variety of metal atoms. The strong attraction for the bonding of Schiff bases to inner and non-inner transition metal ions is essential in the synthesis of metal complex. The aim of this work deals with the synthesis, spectral

characterization like elemental analysis, FTIR, UV and $^1\text{H-NMR}$, antibacterial, antioxidant, DNA cleavage activities of mononuclear Schiff base Cu(II) metal complex [3-4].

Analysis and physical measurement

The elemental analysis (Carbon, Hydrogen and Nitrogen) was performed on a Carlo Erba 1106 instrument. Molar conductivity of the complex in DMF solution was measured using ELICO CM 185 conductivity Bridge. The Infrared spectra were recorded on the Perkin Elmer FT-IR-8300 model spectrometer using KBr disc in the region $4000 - 200 \text{ cm}^{-1}$. Electronic absorption spectra in the UV-Vis range were recorded on Double beam spectrometer Cyber lab between $200 - 800 \text{ nm}$ by using DMF as the solvent. $^1\text{H-NMR}$ spectra of the ligand were recorded on Bruker Spectrometer at 400 MHz in DMSO. The chemical shifts are given in PPM, using Tetramethylsilane (TMS) as the internal reference.

Synthesis of N, N'-(1, 2-phenylenebis(methanylylidene))bis(4-nitroaniline)

The Schiff base ligand was synthesized by adding o-phthalaldehyde (1 mM) in 20 ml of ethanol, and p-nitroaniline (2 mM) in 20 ml of ethanol. This condensation reaction was carried out by using acid catalyst (few drops of HCl). The mixture was refluxed for $2 \frac{1}{2}$ hrs. After completion of the reaction, the mixture was reduced to half of its original volume using water bath and kept aside at room temperature and coloured precipitate was collected and dried in air [5].

Synthesis of mononuclear Schiff base metal complex

The ethanolic solution of synthesized ligand (2 mM) was added drop wise to an ethanolic solution of the metal salt (1 mM) with constant stirring, and the mixture was boiled under reflux for $4 \frac{1}{2}$ hrs. Then, the volume of the reaction mixture was reduced by evaporation. The precipitated complex were filtered off, washed with ethanol and then dried in vacuo [6].

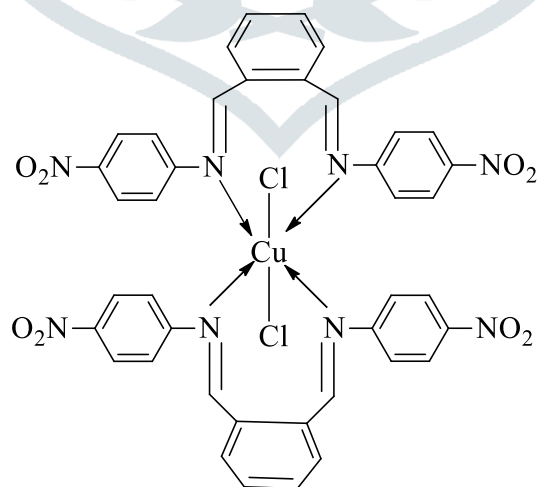


Fig 1. Synthesis of Cu(II) Schiff base metal complex

DNA cleavage activity

Gel electrophoresis

The DNA cleavage of supercoiled pUC18DNA promoted by metal complex was proceeded by addition of reaction mixture (40µl). The reaction mixture (40µl) containing pUC18DNA, 50mM Tris-HCl, pH 7.4, 50 mm NaCl, 10 mM H₂O₂ added in a different volume, followed by adding Millipore water for final volume. Then the mixed solutions were incubated at 37° C for 1 hr. They checked by agarose electrophoresis methods [7].

Antibacterial activity

The antibacterial screening effects of the Schiff base ligand and their corresponding Cu(II) complex were tested against four bacterial strains namely *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsilla pneumonia* and *Escherichia coli* by disc diffusion method, using nutrient agar medium. The compounds were dissolved in DMF. The plates were incubated aerobically at 37° C for 24 hours and the antimicrobial activity was assessed by measuring the inhibition of microbial growth around the well. This method involves the exposure of the zone inhibition toward the diffusion of micro-organism on agar plate [8].

Total antioxidant capacity assay

- Different concentrations (10 µl, 20 µl, 30 µl, 40 µl and 50 µl) of synthesized compounds were taken and 1ml of reagent solution was added. [Reagent solution: 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM Ammonium molybdate.
- The tubes were capped and incubated in thermal block at 95°C for 90 minutes.
- After the time interval the tubes were cool down at room temperature.
- The absorbance was recorded at 695 nm using shimadzu UV 1800 spectrophotometer [9].

RESULTS AND DISCUSSION:

The synthesized compounds i.e. Schiff base and its Cu(II) complex were coloured, stable in air and non-hygroscopic. They were sparingly soluble in common organic solvents; however, these synthesized compounds were completely soluble in DMF and DMSO. Schiff base and its metal complex were obtained in good yield. The molar conductance value at 10⁻³ M concentration were too low to account for any dissociation of the complex in DMF. Hence, the Cu(II) complex may be regarded as nonelectrolyte. The spectral and analytical data showed the formula of the complex is ML₂X₂ type, where L = Schiff base ligand X = chloride ions and M = Cu(II). The elemental analysis data shows the formation of Cu(II) complex is in 2:1 [L:M] stoichiometry ratio.

Table 1. Analytical and physical data of synthesized N, N'-(1, 2-phenylenebis(methanylylidene))bis(4-nitroaniline) and its corresponding metal complex

Compound	Molecular formula	Colour	Yield %	Melting point (° C)	% of Nitrogen		% of Metal	
					Cal	Exp	Cal	Exp
L	C ₂₀ H ₁₄ N ₄ O ₄	Yellowish orange	75	145	15.0	14.8	-	-
CuL ₂ X ₂	Cu(C ₄₀ H ₂₈ N ₈ O ₈ Cl ₂)	Black	70	>200	12.6	12.4	7.2	6.8

IR spectral studies

The important IR frequencies of the Schiff base and its Cu (II) complex are presented in Table 2. In order to study the binding mode of the Schiff base to metal ions, the IR spectrum of the free Schiff base was compared with the IR spectrum of the metal complex. The comparative analysis of the IR spectra of the complex and of the free ligand revealed that the absorption band characteristic of the stretching vibrations of $\nu(\text{C}=\text{N})$ (azomethine group) is shifted towards lower wave number from 1655.34 cm^{-1} in the spectrum of the ligand to 1649.42 cm^{-1} in the complex. This indicates the coordination of the nitrogen of this group with the metal ion. In addition, the appearance of new band at 335.10 cm^{-1} showing the involvement of M–Cl bond in complex formation, supporting the IR spectral study. Further coordination of metal to azomethine nitrogen is confirmed by a new band at 530.88 cm^{-1} with the involvement of nitrogen in the coordination with metal ion [10] due to (M–N).

Table 2. IR spectral data (4000–200 cm^{-1}) of Schiff base and its metal complex

Compounds	$\nu(\text{C}=\text{N})$ (cm^{-1})	$\nu(\text{M}-\text{Cl})$ (cm^{-1})	$\nu(\text{M}-\text{N})$ (cm^{-1})
C ₂₀ H ₁₄ N ₄ O ₄	1655.34	--	--
Cu(C ₄₀ H ₂₈ N ₈ O ₈ Cl ₂)	1649.42	335.10	530.88

Electronic spectra and magnetic moment studies

The electronic spectral data of the synthesized compounds in DMF solution are displayed in Table 3. Electronic spectra of the ligand and Cu(C₄₀H₂₈N₈O₈Cl₂) complex were obtained in DMF medium. The nature of the ligand field around the metal ion was deduced from the electronic spectra. The ${}^2\text{E}_g$ and ${}^2\text{T}_{2g}$ states of the octahedral Cu(II) (d^9) split under the influence of the distortion and the distortion can be such as to cause the ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$ transition to remain unresolved in the spectra. Further the d-d transition showed

a strong band at 655 nm for Cu(II) complex. This is due to ${}^2B_{1g} \rightarrow {}^2E_g$ transition suggesting that Cu(II) is in distorted octahedral geometry [11]. In the spectrum of the Schiff base ligand, the absorption band observed at 365 and 312 nm were assigned to intra-ligand $\pi \rightarrow \pi^*$ transition and the band at 382 and 378 nm were assigned due to $n \rightarrow \pi^*$ transition associated with the azomethine chromophore ($-C=N$). The magnetic moment of 1.97 B.M. falls within the range normally observed for octahedral Cu(II) complex.

Table 3. Electronic Spectral data of Schiff base ligand and its Cu(II) complex

Compound	Electronic spectra (nm)				Geometry of the complex
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L→M	d-d	
$C_{20}H_{14}N_4O_4$	365	382	-	-	-
$Cu(C_{40}H_{28}N_8O_8Cl_2)$	312	378	410	655	Distorted Octahedral

1H – NMR Spectra

The 1H NMR spectrum of the Schiff base was recorded in DMSO- d_6 at room temperature. The 1H NMR spectrum of the Schiff base ligand exhibits well resolved signals and significant differences in their chemical shifts.

Two different type of protons were identified

- The multiplets at around 6.6-7.9 ppm are assigned to aromatic protons,
- The azomethine group in the Schiff base appears 8.48 ppm was observed [12].

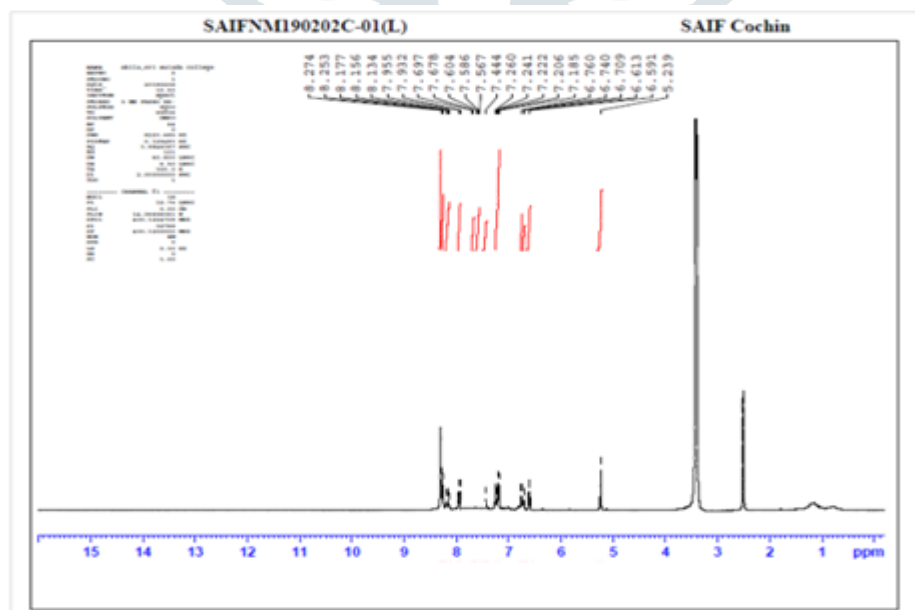


Fig 2. 1H NMR spectrum of $C_{20}H_{14}N_4O_4$

Antibacterial screening

The antibacterial activity of the ligand and its $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex was studied by using Steptomycin as positive standard. The Cu(II) complex was found to show bactericidal activity against selected types of bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsilla pneumonia* respectively. In general, complexes have been shown to be more effective than the free ligand and the same was observed in this study, i.e., the Cu(II) complex is more active than the parent ligand. The variation in the activity of complex against different organisms depends either on the impermeability of cells of the microbes or differences in the ribosomes of microbial cells [13]. The higher antibacterial activity of M-complex than the free Schiff base ligand can be explained by chelation theory. From the results it is observed that $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex is more active than the free ligand .

Anti-oxidant assay

The newly synthesized ligand and its $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex were investigated for their antioxidant properties by total antioxidant capacity assay [14]. Fig 4 & 5 and Table 5 & 6 demonstrate the free radical scavenging activity of $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex and its ligand. The decreasing absorbance as well as the lower IC_{50} value indicated the higher antioxidant activity of test compounds. From the result it is observed that the free radical scavenging activity of complex is better than $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_4$ free ligand but lower than that of standard ascorbic acid (vitamin C).

Table 4. Standard Antioxidant Activity

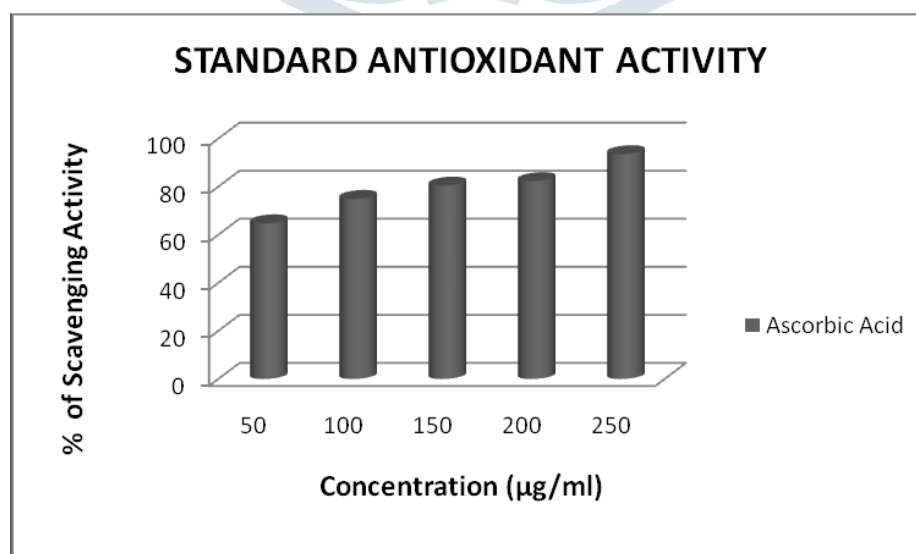
S.No.	Standard	Concentration ($\mu\text{g/ml}$)	Antioxidant Activity% of inhibition
			TAC (695nm)
1.	Ascorbic Acid	50	64.71
2.	used as a Standard (OD Value)	100	74.88
3.		150	80.43
4.		200	82.28
5.		250	93.52

Table 5. Free radical scavenging activities of (L) in Total Antioxidant Capacity assay (%).

S.No.	Compound Concentration ($\mu\text{g/ml}$)	TAC Assaay		
		Absorbance	% of SCV	IC ₅₀
1.	10	0.507	34.79	63.34
2.	20	0.480	35.02	
3.	30	0.478	37.97	
4.	40	0.420	40.79	
5.	50	0.373	41.10	

Table 6. Free radical scavenging activities of (Cu) in Total Antioxidant Capacity assay (%).

S.No.	Compound Concentration ($\mu\text{g/ml}$)	TAC Assaay		
		Absorbance	% of SCV	IC ₅₀
1.	10	0.521	46.83	23.26
2.	20	0.502	48.77	
3.	30	0.474	51.63	
4.	40	0.469	52.14	
5.	50	0.369	62.34	

**Fig 3. Total Antioxidant Capacity of Ascorbic acid**

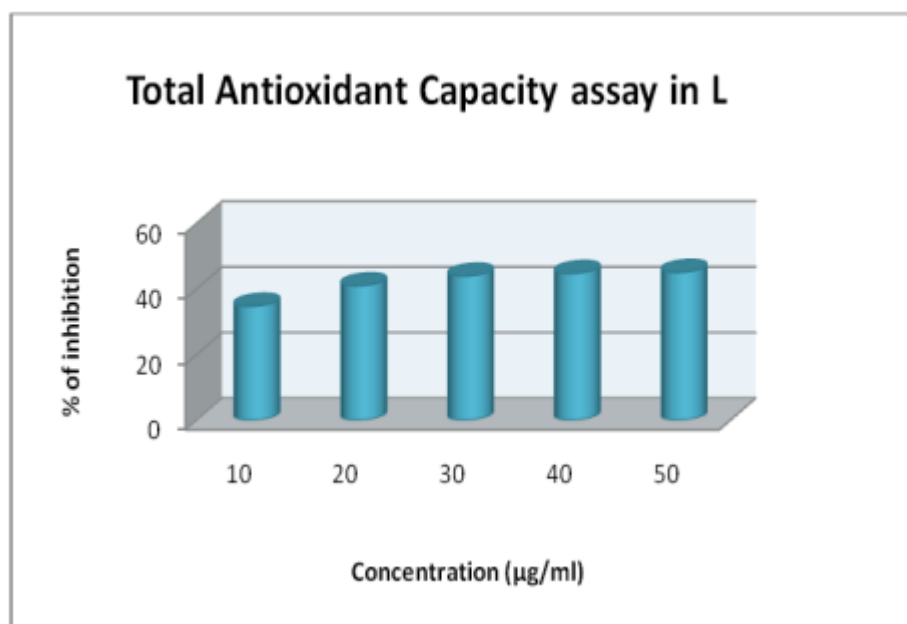


Fig 4. Total Antioxidant Capacity of $C_{20}H_{14}N_4O_4$

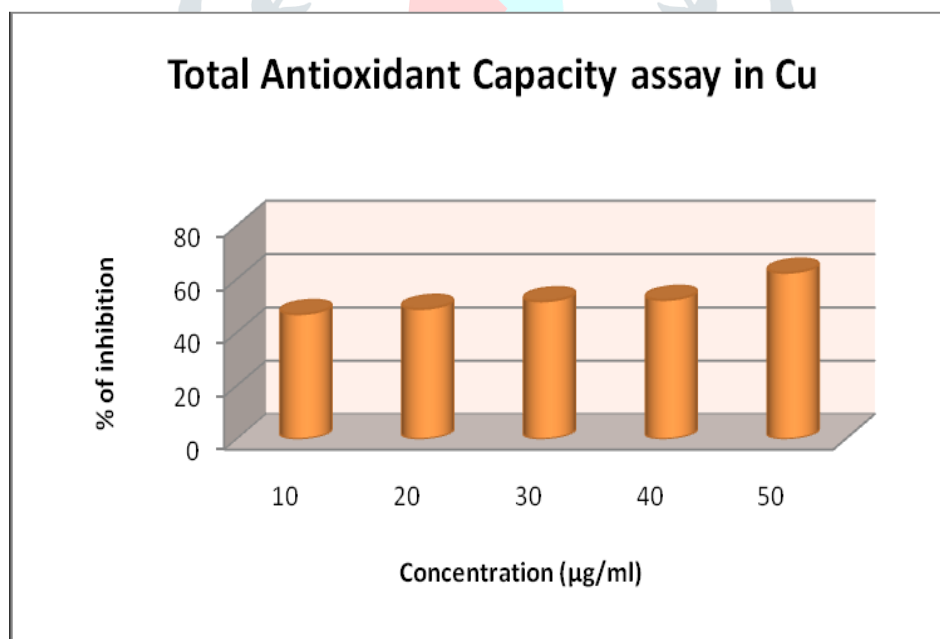


Fig 5. Total Antioxidant Capacity of $Cu(C_{40}H_{28}N_8O_8Cl_2)$

DNA cleavage study

Gel electrophoresis experiments were performed using pUC18DNA with ligand and $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex in presence of H_2O_2 . The results of DNA cleavage studies have been shown in Fig 6. $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex exhibit cleavage ability at low concentration ($40 \mu\text{M}$). The ligand exhibits significant activity in the presence of oxidant. Also the activity was much higher for the $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex in presence of H_2O_2 . When pUC18DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coil form (Form I). If scission occurs on one strand (nicking), the super coil will relax to generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated. From Fig 6, the $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex show more activity in the presence of oxidant which may be due to the reaction of hydroxyl radical with DNA. $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex were able to convert DNA (Form I) into linear form (Form III). The mononuclear $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex was found to be highly active in cleaving DNA than the free ligand [15].

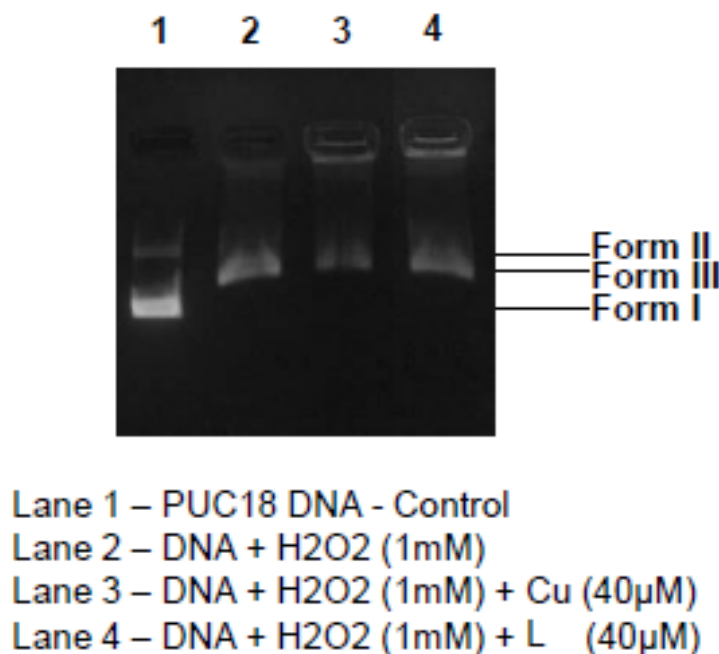


Fig 6. Agarose gel electrophoresis pattern showing effect of pUC18DNA induced by H_2O_2 and metal complex. Lane 1-DNA alone; Lane 2- DNA alone + H_2O_2 ; Lane 3-DNA + $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ + H_2O_2 ; Lane 4-DNA + $\text{C}_{20}\text{H}_{14}\text{N}_4\text{O}_4$ + H_2O_2 .

CONCLUSION

On the basis of various physico-chemical and spectral data presented and discussed in this paper, the $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex may tentatively be suggested to have distorted octahedral geometry. The coordination and the type of geometry of mononuclear copper(II) complex were determined by IR, electronic, magnetic and molar conductivity studies. Lower molar conductivity value obtained for the complex indicates that it is a non-electrolyte. The electronic and magnetic susceptibility studies reveal that the geometry of the newly synthesized $\text{Cu}(\text{C}_{40}\text{H}_{28}\text{N}_8\text{O}_8\text{Cl}_2)$ complex is distorted octahedral. The

therapeutic promise of the investigated Cu(II) complex were found to exhibit higher antibacterial activity than the ligand. The interaction of Schiff base and Cu(II) complex with pUC18DNA were investigated by gel electrophoresis and concluded that Cu(II) complex cleave DNA effectively in the presence of H₂O₂. Moreover, the ligand and its complex were screened for antioxidant activity and the Cu(C₄₀H₂₈N₈O₈Cl₂) complex showed better activity when compared to ligand.

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