



Spectral characterization, biological activities and DNA cleaving studies of 1-[(6-Methyl-pyridin-2-ylimino)-methyl]-naphthalen-2-ol and its metal(II) complexes

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ABSTRACT Metal complexes derived from 2-hydroxy-naphthalene-1-carbaldehyde, 6-methyl-pyridin-2-ylamine and M(II) salts in ethanolic solution are reported and characterized based on elemental analyses, IR, UV, molar conductance and magnetic moment. The complexes are found to have the formulae ML_2 , where M= Co(II), Ni(II), and Cu(II) and L = 1-[(6-Methyl-pyridin-2-ylimino)-methyl]-naphthalen-2-ol. The molar conductance data reveal that the chelates are non-electrolytes. IR spectra show that the Schiff bases are coordinated to the metal ions in a tridentate manner with ONN donor sites. From the magnetic moment values, it is found that the geometrical structures of these complexes are octahedral. The synthesized ligand, in comparison to their metal complexes also were screened for their antibacterial activity against bacterial species, *Bacillus subtilis*, *Staphylococcus aureus* (as Gram-positive bacteria) and *Klebsilla pneumonia* and *Escherichia coli* (as Gram-negative bacteria). The activity data show that the metal complexes to be more potent/antibacterial than the parent organic ligands against one or more bacterial species.

KEYWORDS : Transition metal complexes, IR, Conductance, Biological activity.

Introduction

Schiff bases have gained significance because of physiological and pharmacological actions associated with them. A large number of Schiff base compounds are frequently used as ligands in coordination chemistry by considering their metal binding ability. Schiff base metal complexes have ability to reversibly bind oxygen in epoxidation reactions [1], biological activity [2], catalytic activity in hydrogenation of olefins [3] and photochromic properties [4]. Tridentate Schiff bases are well known to coordinate with various metal ions forming stable compounds and some of these complexes are recognized as oxygen carriers [5]. In view of recent interest in the energetic of metal ligand binding in metal chelates involving N, O donor ligands [6] we started to study Schiff base complexes derived from tridentate ligands involving an N_2O_2 donor atoms. However literature survey reveals that little work has been done on tridentate Schiff base complexes of Co(II), Ni(II), and Cu(II) metal ions derived from 1-[(6-Methyl-pyridin-2-ylimino)-methyl]-naphthalen-2-ol. The spectral parameters were calculated as well as the antibiologicial screening of these complexes and the free ligand against different bacterial species have been reported.

Experimental

Materials

All the chemicals were purchased from Merck and used were of Analar grade. Solvents were purified and dried before use according to the standard method [7].

Analytical and physical measurements

Carbon, hydrogen and nitrogen were obtained using a Perkin- Elmer elemental analyzer. The metal contents were determined by complexometric titrations with EDTA [8]. The IR spectra were recorded in KBr pellets on a JASCO FT/IR-410 spectrometer in the range $400-4000\text{ cm}^{-1}$. Electronic spectra were recorded on a Perkin-Elmer Lambda-25 UV-vis spectrometer using MeOH as solvent. Conductivity measurements were made on freshly prepared 10^{-3} M solutions in DMF at room temperature with a coronation digital conductivity meter. NMR signals were obtained from Bruker Avance III, 400MHz model spectrometer. EPR spectra of compounds were recorded on an E-112 ESR Spectrometer with X band microwave frequency (9.5 GHz).

Synthesis of 1-[(6-Methyl-pyridin-2-ylimino)-methyl]-naphthalen-2-ol

To an ethanolic solution of 2-hydroxy-naphthalene-1-carbaldehyde (1mmol), ethanolic solution of 6-methyl-pyridin-2-ylamine (1 mmol) was added and the mixture was stirred until complete dissolution. After

the addition of the equimolar amount of 2-hydroxy-naphthalene-1-carbaldehyde and 6-methyl-pyridin-2-ylamine the mixture was stirred for 3 hrs at room temperature and a dark solution was formed [8]. After evaporation of this solution, a dark yellow product was formed.

Synthesize of Mononuclear Schiff base metal complexes

A mixture of synthesized 1-[(6-Methyl-pyridin-2-ylimino)-methyl]-naphthalen-2-ol (2 mmol) was added to an ethanolic solution of solid metal acetate salt (1 mmol). The reaction mixture was stirred for 3 hrs at room temperature [9]. The precipitate was filtered, washed with diethyl ether and the collected solid was air dried overnight and then dried in a desiccator, whereupon dark color precipitate was formed. The structure of complexes was confirmed by spectroscopic techniques.

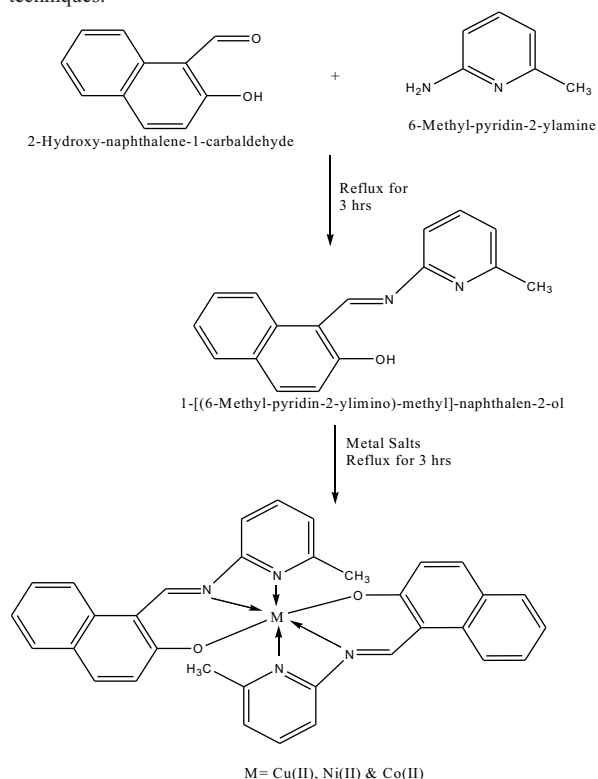


Figure 1: The structures of Schiff base and their complexes**Antimicrobial activity**

The ligand and its complexes were tested against the bacterial species *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsilla pneumonia* and *Escherichia coli*. These studies were carried out using *Streptomycine* as standard antibacterial agent by disc diffusion method [10]. The test solutions were prepared in DMSO. Diffusion method [11] was used to evaluate the antimicrobial activities of the tested compounds as follows: 0.5 ml spore suspension (106 to 107 spore ml^{-1}) of each of the investigated organisms was added to a sterile agar medium just before solidification, then poured into sterile Petri dishes (9 cm in diameter) and left to solidify. Using sterile cork borer (6mm in diameter), wells were made in each dish, then 0.1 ml of the tested compounds dissolved in DMSO were poured into three wells and the dishes were incubated at 37°C for 48 h (for bacteria) where clear or inhibition zones were detected around each well.

Results and discussion

The analytical and physical data of the Schiff base ligand and its complexes are given in Table 1. The complexes are soluble in DMF and DMSO and are insoluble in some common organic solvents. The analytical data show that the metal to ligand ratio is 1:2 in all the complexes. The composition of the complexes is ML_2 where (M = Co(II), Ni(II), and Cu(II)). The low molar conductance values of 10^{-3}M solutions in DMF showed them to be non-electrolytes [12].

Table 1: Analytical data of the Schiff base ligand and its mononuclear metal complexes

Compounds	M.F	M.Wt	Colour	M.Pt	Molar conductivity $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$	Elemental analysis			
						C	H	N	M
L	$\text{C}_{16}\text{H}_{13}\text{NO}_2$	251.28	Yellow	135	--	76.44 (76.40)	5.20 (5.17)	5.58 (5.57)	-
CuL	$\text{C}_{34}\text{H}_{26}\text{N}_4\text{O}_2\text{Cu}$	586.14	Dark Green	250	7.6	68.07 (68.05)	4.25 (4.22)	4.96 (4.94)	11.13
CoL	$\text{C}_{34}\text{H}_{26}\text{N}_4\text{O}_2\text{Co}$	581.28	Dark Brown	210	5.3	68.63 (68.60)	4.28 (4.25)	5.01 (4.99)	10.51
NiL	$\text{C}_{34}\text{H}_{26}\text{N}_4\text{O}_2\text{Ni}$	581.52	Brown	262	8.2	68.66 (68.65)	4.28 (4.26)	5.00 (4.98)	10.30

Infrared spectra

The IR spectral data of Cu(II), Ni(II) and Co(II) complexes are given in Table 2. In the Schiff base ligand, the strong band observed at 1644.21 cm^{-1} can be assigned to the $\nu(\text{C-N})$ azomethine stretching vibration. On complexation, this band was shifted to lower frequency in the $1610\text{--}1601\text{ cm}^{-1}$ range indicating the coordination of the azomethine nitrogen atom to the central metal ion [13]. The spectra of the complexes show broad bands in the 3401.15 cm^{-1} range, which can be attributed to the stretching vibration of the O-H group and its absence in the complexes is due to the involvement of the phenol O in bonding to the metal ions. In the complexes, weak bands in the $557.12\text{--}581.20$ and $442.35\text{--}470.59\text{ cm}^{-1}$ range, can be attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively [14]. From the IR results, it may be concluded that the Schiff base ligand is tridentate and coordinates with the metal ion through the phenolic oxygen and azomethine nitrogen groups.

Table 2: Infrared spectroscopic data of the Schiff base ligand and its mononuclear metal complexes

Compounds	$\nu(\text{O-H})$ (cm^{-1})	$\nu(\text{C=N})$ (cm^{-1})	$\nu(\text{C-C})$ (cm^{-1})	$\nu(\text{C=C})$ (cm^{-1})	$\nu(\text{M-N})$ (cm^{-1})	$\nu(\text{M-O})$ (cm^{-1})
$\text{C}_{16}\text{H}_{13}\text{NO}_2$	3401.15	1644.21	1262.50	1581.27	--	--
$\text{C}_{34}\text{H}_{26}\text{N}_4\text{O}_2\text{Cu}$	--	1610.11	1310.30	1511.42	557.12	470.59

$\text{C}_{34}\text{H}_{26}\text{N}_4\text{O}_2\text{Ni}$	--	1603.12	1300.23	1558.21	581.20	468.21
$\text{C}_{34}\text{H}_{26}\text{N}_4\text{O}_2\text{Co}$	--	1601.22	1280.11	1583.63	563.23	442.35

Electronic spectra

The electronic spectrum of the Schiff base ligand shows a broad band at 317 nm , which is assigned to $\pi\text{--}\pi^*$ transition of the C=N chromophore. On complexation, this band was shifted to lower wavelength region, suggesting the coordination of azomethine nitrogen with the metal ion.

The electronic spectra of all the free ligands showed two types of transitions, the first one appeared at range 288 nm which can be assigned to $\pi\text{--}\pi^*$ transition was due to transitions involving molecular orbitals located on the phenolic chromophore. This peak have been shifted in the spectra of the complexes; this is may be due to the donation of a lone pair of electrons by the oxygen of the phenoxy group to the central metal atom [15]. The second type of transitions appeared at range 397 nm which can be assigned to $n\text{--}\pi^*$ transition was due to transitions involving molecular orbitals of the C=N chromophore and the benzene ring. These bands have also been shifted upon complexation indicated that, the imine group nitrogen atom appears to be coordinated to the metal ion [16].

Copper(II) complex gave only one band due to ${}^3\text{E}_g\text{--}{}^2\text{T}_{2g}$ transition at 655 cm^{-1} and charge transfer transition was observed in the range of 455 nm . This electronic spectral data conclude the suggested octahedral geometry for the synthesized Copper(II) complex. The magnetic moment for Cu(II) complex is 1.67 B.M. this clearly shows that there is no major spin interactions [17]. The octahedral geometry for the dark coloured complex of Cobalt(II) complex as confirmed by magnetic moment 4.81 B.M. the observation of two bands in the visible region. The Ni(II) complex shows bands at $550, 670\text{ nm}$ respectively corresponds to ${}^6\text{A}_1g\text{--}{}^4\text{E}_g(4\text{D})$, ${}^6\text{A}_1g\text{--}{}^4\text{T}_2g(4\text{G})$ transitions which are compatible to an octahedral geometry around Ni(II) ion [18].

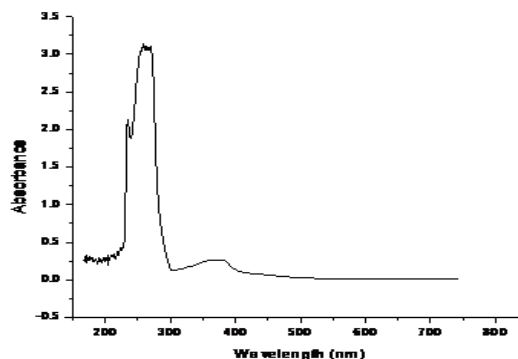
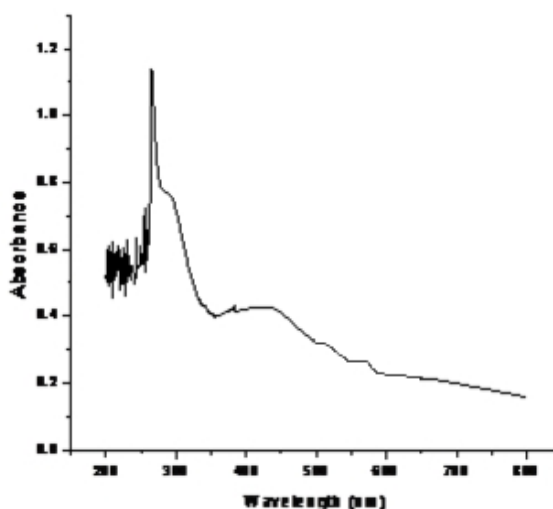
**Figure 2. Electronic spectrum of $\text{C}_{16}\text{H}_{13}\text{NO}_2$ ligand**

Figure 3. Electronic spectrum of $C_{34}H_{26}N_4O_2Cu$ complex

¹H NMR spectral studies of synthesized ligand indicated signals at 6.3-7.7 ppm corresponding to aromatic protons (m, 28 Ar-H) and at 8.7 ppm due to azomethine moiety (C=N). A strong signals at 10.4 ppm assignable (S, 1H) due to phenolic OH group. These signals provide structural arrangement for the synthesized ligand [19].

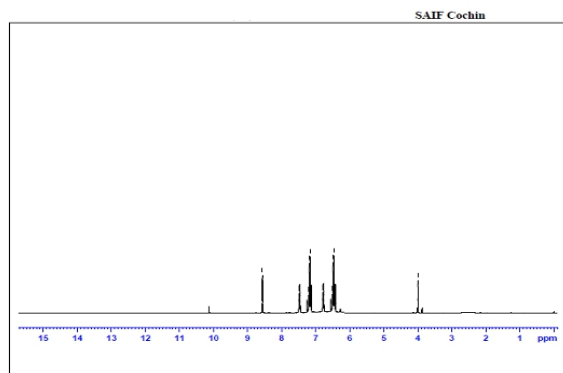


Figure 4. NMR Spectra of Schiff base ligand

The ESR spectrum of the Cu(II) complex shows hyperfine splitting and the g_{\parallel} , g_{\perp} values are 2.29 and 2.03. The observed $g_{\parallel} > g_{\perp}$ is typical for Cu(II) having one unpaired electron in a dx^2-y^2 orbital. The g_{av} value, calculated according to the relation $= 1/3 (g_{\parallel} + 2g_{\perp})$, $g_{av}(2.256) > g_s(2.052) > g_i(2.002)$, which suggest that this complex has a distorted octahedral geometry [20].

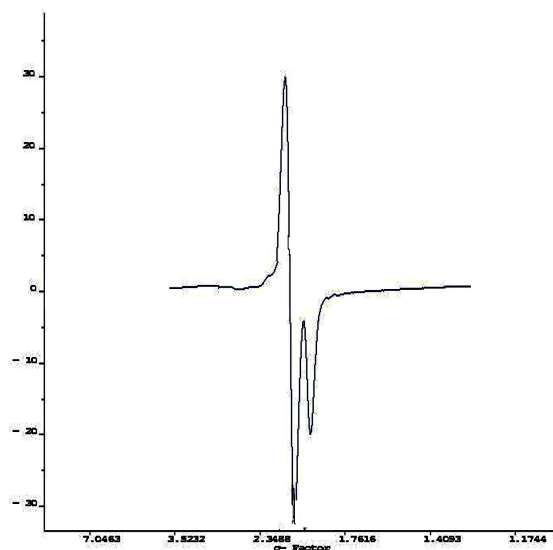


Figure 5. EPR Spectra of $C_{34}H_{26}N_4O_2Cu$ complex

Redox Behaviour
The electrochemical behaviour was studied by cyclic voltammetry in DMF containing 10^{-1} M tetra(n-butyl) ammonium perchlorate over the range of 1.2 to -2.0 V. The cyclic voltammograms for the mononuclear complexes are shown in Fig 6 – 8. A three-electrode configuration was used, comprising a glassy carbon electrode as the working electrode, a Pt-wire as the auxiliary electrode, and an Ag/AgCl electrode as the reference electrode. The electrochemical data such as cathodic peak potential (Epc) and anodic peak potential (Epa) were measured.

Cyclic voltammogram of $C_{34}H_{26}N_4O_2Cu$ complex shows that the ΔE_p values falls in the range of 120 mV which shows quasireversible reduction waves. The $E_{1/2}$ values falls in the range of -1.24 V indicate that each couple corresponds to one electron transfer process. Controlled potential electrolysis was carried out at 100 mVs^{-1} and the experiment reports that each couple corresponds to one electron transfer process. So, the processes are assigned as follows.

$Cu^{II}Cu^I$

The Cu(II) complexes show a quasireversible oxidation waves, which

is assigned as a Cu(I)/Cu(II) couple. The ΔE_p values falls in the range of 180 mV are suggest the each couple was quasireversible. The $E_{1/2}$ values falls in the range of 0.99 V indicate that each couple corresponds to one electron transfer process.

The cyclic voltammogram of $C_{34}H_{26}N_4O_2Ni$ complex shows that the ΔE_p values of Ni(II) falls in the range of 160 mV which shows quasireversible reduction waves. The $E_{1/2}$ values falls in the range of -1.07 V indicate that each couple corresponds to one electron transfer process. Controlled potential electrolysis was carried out at 100 mVs^{-1} and the experiment reports that each couple correspond to one electron transfer process. So, the processes are assigned as follows.

$Ni^{II}Ni^I$

The Nickel(II) complexes show a quasireversible oxidation waves, which is assigned as a Ni(II)/Ni(III) couple. The ΔE_p values falls in the range of 190 mV are suggest that each couple was quasireversible. The $E_{1/2}$ values falls in the range of 1.02 V indicate that each couple corresponds to one electron transfer process [21].

The $C_{34}H_{26}N_4O_2Co$ complex exhibit one electron quasi reversible transfer process with a peak at ΔE_p values falls in the range of 200 mV. The $E_{1/2}$ values falls in the range of -1.08 V corresponding to reduction waves. This gives evidence for quasi reversible Co(II)/Co(I) couple. Also, ΔE_p values falls in the range of 120 mV. The $E_{1/2}$ values falls in the range of 1.07 V corresponding to oxidation waves.

$Co^{II}Co^I$

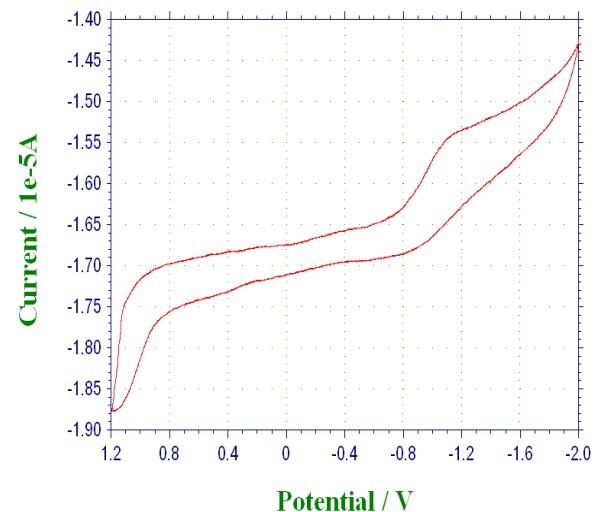


Figure 6. Cyclic Voltammogram of $C_{34}H_{26}N_4O_2Cu$ complex

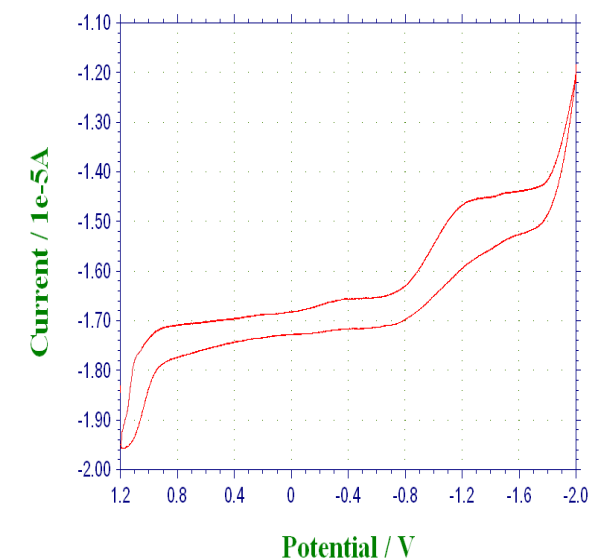


Figure 7. Cyclic Voltammogram of $C_{34}H_{26}N_4O_2Ni$ complex

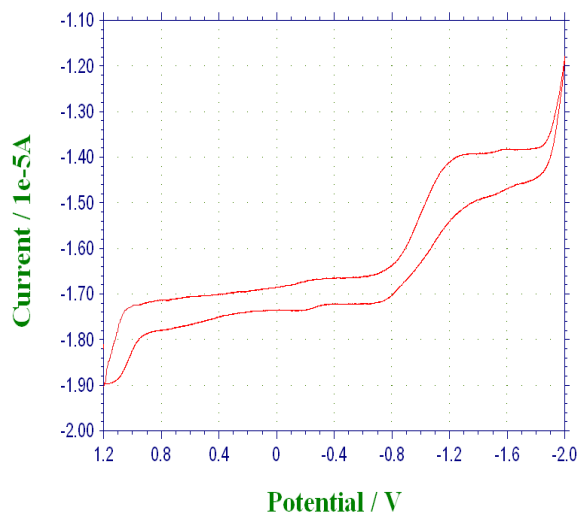


Figure 8. Cyclic Voltammogram of $C_{34}H_{26}N_4O_2Co$ complex

Antibacterial activity
The antibacterial activities of all prepared ligand and its complexes have been screened against two gram positive pathogenic strains such as *Staphylococcus aureus*, *Bacillus subtilis* and two gram negative pathogenic strains such as *Escherichia coli* and *Klebsiella* species. The ligand and their complexes are less effective towards other bacteria. Also, it was noted that the complexes showed greater inhibition than the ligand. It was evident that overall potency of the ligand was enhanced on coordination with the metal ions. This enhancement in the activity may be efficient on the basis that ligand mainly possess C=N bond [22].

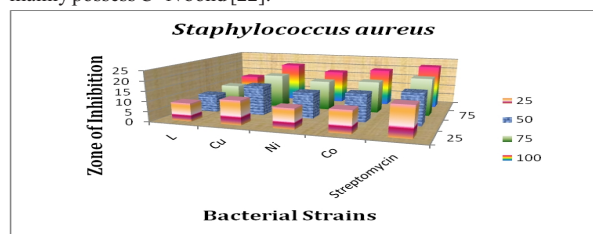


Figure 9. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complexes against *Staphylococcus aureus*

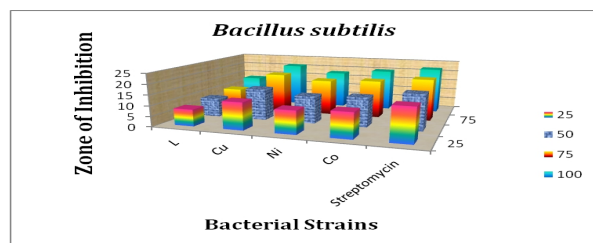


Figure 10. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complexes against *Bacillus subtilis*

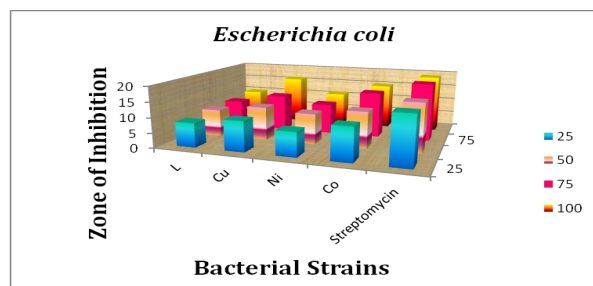


Figure 11. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complexes against *Escherichia coli*

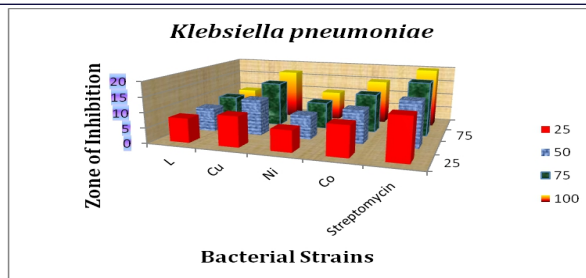


Figure 12. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complexes against *Klebsiella pneumoniae*

DNA cleavage studies

There are a number of agents, which exert their effect by inhibiting enzymes that act upon DNA. These inhibitions results from binding of agents to the enzyme's site of interaction on the DNA and not direct enzyme inactivation. Transition metals have been seen to inhibit DNA repair enzymes. When DNA is run on horizontal gel using electrophoresis, the fastest migration will be observed for the open circular form (Form I). If one strand is cleaved a slower-moving linear form (Form II) is observed. The gel electrophoretic separation of pUC18DNA induced by the Schiff base metal complexes was conducted at 35 °C in the presence of H_2O_2 as an oxidant and is given in

Fig. 13. The DNA cleavage efficiency of the complex was due to the different binding affinity of the complex to DNA. Control experiment using DNA alone (lane 1) does not show any significant cleavage even on longer exposure time. From the observed results we conclude that the copper complex (lane 3) cleave DNA as compared to control DNA. Probably this may be due to the formation of redox couple of the metal ions and its behavior. It is also thought that most cleavage cases are caused by copper ions reacting with H_2O_2 to produce the diffusible hydroxyl radical ($-OH$) or molecular oxygen, which may damage DNA through Fenton type chemistry [23].

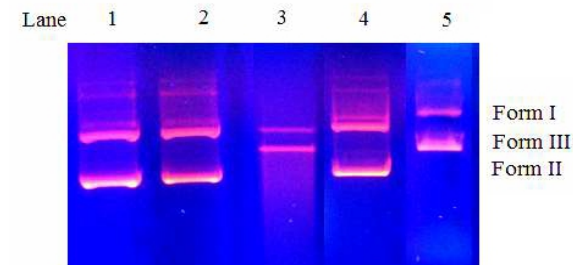


Figure 13. Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H_2O_2 and metal complexes. Lane 1-DNA alone; Lane 2- DNA alone + H_2O_2 ; Lane 3-DNA + Cu complex + H_2O_2 ; Lane 4-DNA + Co complex + H_2O_2 ; Lane 5-DNA + Ni complex.

CONCLUSION

The newly synthesized ligand was used to prepare Cu(II), Ni(II) and Co(II) complexes. The data obtained from these studies were in good agreement with the proposed structure and composition of the ligand and the metal complexes. Distorted octahedral geometry was confirmed for the metal complexes by electronic absorption spectroscopy, ESR spectroscopy and the magnetic moment values. *In vitro* antibacterial studies showed that metal complexes were more biologically active than the free ligand. Cu(II) complex effectively cleave with DNA than the other complexes.

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