

Potentially Bioactive New Cu(II), Ni(II) and Co(II) complexes: Synthesis, Spectroscopic, Physico – chemical investigations, DNA Cleavage, Antibacterial and Radical Scavenging Properties

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ABSTRACT

The mononuclear Schiff base Cu(II), Ni(II) and Co(II) complexes have been synthesized using N, N'-(indoline-2,3- diylidene)bis(4-nitroaniline) (L₁), and N, N'-(indoline-2,3- diylidene)bis(4-chloroaniline) (L₂). The metal complexes have been fully characterized with the help of analytical, conductivity, IR, electronic, and ¹H-NMR studies. The molar conductance value indicates that the complex is non-electrolyte in nature. The IR spectra of synthesized compounds confirmed the coordination of ligand to the metal ion. The UV-Vis and magnetic moment data of the complex suggest a distorted octahedral geometry. The Co(II) metal complex and the Schiff base ligand were cleaved supercoiled DNA by oxidative paths, and the nuclease activity of the cobalt complex was assessed by gel electrophoresis assay and the results that metal complex can cleave pUC19DNA in presence of H₂O₂ compared to the ligands. The antibacterial activities of the synthesized Cu(II) compound and the Schiff base ligand were tested in vitro against the sensitive organisms *Staphylococcus aureus*, *Bacillus subtilis* as Gram positive bacteria, *Escherichia coli* and *Klebsilla pneumonia* as Gram negative bacteria, and the results are discussed. We found that the antioxidant activity of the Ni(II) complex on DPPH is concentration dependent and the values are higher than that of the Schiff base ligand L₁ and L₂. The antioxidant activity of the complex was also investigated through the scavenging effect on DPPH.

KEYWORDS: Scavenging effect, Antibacterial assay, DNA Cleavage.

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I. INTRODUCTION

Schiff base ligands and their metal complexes have vast variety of applications in biological, clinical, analytical and industrial fields. Potentially bioactive Schiff base metal complex is a kind of attractive reagents due to specific activities of pharmacology and physiology [1]. Most of the metal ions play an important role in the biological

processes and the field of knowledge concerned with the application of inorganic chemistry to therapy or diagnosis of disease is medicinal inorganic chemistry [2]. People had a great interest in synthesis, DNA interaction and biological activity of Schiff base metal complexes in recent years. The reactive intermediates in oxidative DNA cleavage are generated via redox active metal

complex, the interaction of these intermediate with nucleobases can result in direct strand cleavage or in the formation of labile sites in the DNA and it forms transmission and expression of genetic information. The design of molecules exhibit affinity to DNA is a challenging area of research. Such molecules can act as a excellent chemotherapeutic agents that exert the biological activity through interaction with DNA [3]. Free radicals are produced under certain environmental conditions and during normal cellular functions in the body. Antioxidants thus play an important role to protect the human body against damage by the reactive oxygen species. The ability of Schiff bases and their metal complexes to scavenge free radicals is an important property [4]. Free radicals are capable of reacting indiscriminately with any molecules with which they come in contact. Research had shows a significant progress in utilization of the transition metal complexes as drugs to treat the several human diseases [5]. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. This activity of the transition metals has started the development of metal based drugs with promising the pharmacological application and may offer unique therapeutic opportunities. The advances in the organic chemistry provide better opportunities to use metal complexes as therapeutic agents [6].

Considerable effect has been devoted to the preparation and structure characterization of Schiff base Ni(II), Cu(II) and Co(II) metal complexes derived from (L₁) N, N'-(indoline-2,3-diyldene)bis(4-nitroaniline), and (L₂) N, N'-(indoline-2,3-diyldene)bis(4-chloroaniline). However little attention has been paid to systems in which the Schiff base (L₁) are derived from Isatin and p-nitroaniline, and the Schiff base (L₂) are derived from Isatin and p-chloroaniline have been synthesized and structurally characterized. Their spectral and biological studies like antibacterial, antioxidant and DNA cleavage activity have been discussed.

II. EXPERIMENTAL METHODS

Materials

Reagent grade chemicals were used without further purification in all the synthetic work. All solvents were purified by standard methods. Metal salts, Isatin, p-chloroaniline and p-nitroaniline were obtained from Aldrich and used as received. Ethanol, DMSO and DMF were used as solvents purchased from Merck and Loba chemicals.

Melting points of the synthesized compounds are determined in open capillaries and are uncorrected.

Analysis and Physical Measurements

Elemental analysis was carried out on a Carlo Erba Model 1106 elemental analyzer. IR spectra were recorded on a SHIMADZU model spectrophotometer on KBr disks in the range 4000– 400 cm⁻¹. Electronic spectral studies were carried out on a Perkin Elmer Lambda-25 spectrophotometer in the range 200– 800 nm. Molar conductivity was measured by using an ELICO CM 185 conductivity Bridge using freshly prepared solution of the complexes in DMF solution. The ¹H NMR spectra were recorded in DMSO-d₆ on a BRUKER ADVANCED III 400 MHz spectrophotometer using TMS as an internal reference.

Synthesis of Schiff base

Ligand 1: The Schiff base ligand was synthesized by adding 1-Indole-2,3-dione (1 mM) in 20 ml of ethanol and p-nitroaniline (2 mM) in 20 ml of ethanol. The mixture was refluxed for 2½ hrs. Then solution of the ligand was kept for slow evaporation and collected and dried in air [80].

Ligand 2: The Schiff base ligand was synthesized by adding 1-Indole-2,3-dione (1 mM) in 20 ml of ethanol and p-chloroaniline (2 mM) in 20 ml of ethanol. The mixture was refluxed for 2½ hrs. Then solution of the ligand was kept for slow evaporation and collected and dried in air.

Synthesis of mononuclear Schiff base metal complexes

The metal chlorides of [Ni(II), Cu(II) and Co(II)] (1 mM), the potential binucleating synthesized Schiff base ligand 1 (1 mM) and ligand 2 (1 mM) were dissolved in ethanol (20 ml) with constant stirring and the mixture was boiled under reflux for 4½ hrs. Then the volume of the reaction mixture was reduced by evaporation. The precipitated complex were filtered off, washed with ethanol and then in vacuo.

Biology

Antioxidant Assay

DPPH Radical Scavenging Activity

The scavenging ability determines the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. All of these compounds exhibit free radical scavenging ability at different concentrations [7]. The radical scavenging activity has been expressed as a percentage, and it is calculated using the following formula:

$$\% \text{ inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test sample}) / \text{Absorbance control}] \times 100}$$

IC₅₀ values were calculated for compounds, which exhibited the significant activity. IC₅₀ is defined as concentration sufficient to obtain 50% of maximum scavenging activity.

DNA Cleavage Experiment

Agarose Gel Electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples were prepared in DMSO. The gel electrophoresis experiments were performed by incubation of the samples containing 40 μM pUC18 DNA, 50 μM metal complexes and 50 μM H₂O₂ in tris-HCl buffer (pH 7.2) at 37°C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 μg cm⁻³ ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the experiments were performed at the room temperature [8].

Antimicrobial Assays

The biological activities of the synthesized Schiff's base ligands and its Cu(II) complex were screened for their antibacterial properties by the disc agar diffusion method in DMF solvent against *Staphylococcus aureus*, *Bacillus subtilis* are Gram-positive bacteria and *Escherichia coli* and *Klebsilla pneumonia* are Gram-negative bacteria [9].

III. RESULTS AND DISCUSSION

Chemistry

A new potentially bidentate Schiff base ligand (L₁) N, N'-(indoline-2,3-diylidene)bis(4-nitroaniline) and (L₂) N, N'-(indoline-2,3-diylidene)bis(4-chloroaniline) has been easily prepared from the reaction of Isatin and p-nitroaniline and Isatin and p-chloroaniline. Complexes of this Schiff base ligands were prepared with chlorides of [Ni(II), Cu(II) and Co(II)]. The molar conductivity values of these complexes (8.3-10.5) in DMF indicate that the complexes are non-electrolyte in nature. The complexes with general stoichiometry [ML₁L₂X₂], Where L₁L₂ = ligands, these are stable at room temperature. These metal complexes are soluble in DMF and DMSO. The spectral and analytical data are completely consistent with the proposed formulation as represented in Table 1.

IR Spectra and Coordination Mode

The IR spectra of the Schiff base ligands and their Cu(II), Ni(II) and Co(II) complexes can clarify the mode of bonding of Schiff base and the metal complexes. All the Schiff base ligands have the characteristic band of azomethine (C=N) group appeared in the range of 1600-1690 cm⁻¹. The free ligands L₁ and L₂ exhibits a strong ν(C=N) band at 1627.92 cm⁻¹ and 1612.49 cm⁻¹ [10] which provides a concrete evidence for the formation of the Schiff base. A relative decrease of ν(C=N) frequency to 1604.77, 1610.45 and 1603.26 cm⁻¹ supports the coordination of imine nitrogen atom with metal ion in complex. The band present in the region of 3109.25 cm⁻¹ may be assigned to C-H stretching vibrations in plane bending of phenyl group whereas C-H out of plane bending of phenyl group in the region of 840.96 cm⁻¹ and 686.66 cm⁻¹. The absorption band in the range of 1627.92 cm⁻¹ and 1612.49 cm⁻¹ may be assigned to ν(C=N) aromatic stretching vibrations.

Electronic spectra and magnetic moment

In order to propose a structure for the synthesized Ni(II), Cu(II), and Co(II) complexes, the electronic absorption spectra of freshly prepared DMF solution of the complexes was recorded at the room temperature and their spectral data are presented in Table 3. The electronic absorption spectra of the ligands N, N'-(indoline-2,3-diylidene)bis(4-nitroaniline) (L₁), N, N'-(indoline-2,3-diylidene)bis(4-chloroaniline) (L₂) and its metal complex have been studied with a view to obtain more information on the configuration of the complex [11]. The electronic spectra have been measured in DMF solution in the range of 200-800 nm at room temperature. In the electronic spectra of the Schiff base ligands L₁ and L₂ shows the intense absorption in the range of 310 nm and 305 nm due to π→π* transition and 390 nm and 400 nm due to n→π* transition involving the benzene rings and C=N groups [12]. The Ni(II), Cu(II), and Co(II) complexes of the show the low intensity bands at 550- 640 nm, which are assigned as the d-d transition of the metal ions. The Reddish brown colour complexes of Ni(II) shows the three d-d transition bands at 550, 580 and 610 nm which are assigned to ³A_{2g} (F) → ³T_{2g} (F) (ν₁), ³A_{2g} (F) → ³T_{1g} (F) (ν₂) and ³A_{2g} (F) → ³T_{1g} (P) (ν₃) transitions respectively, this indicates the distorted octahedral geometry around the nickel ion. The copper complex shows the band in the range of 600 nm. This spin allowed transitions are due to ²B_{1g} → ²A_{1g}, characteristic for distorted octahedral geometry. The d-d transition bands of

the Cobalt complexes in the range of 610, 630 and 650 nm. This also indicates the distorted octahedral geometry around the Co ion.

¹H NMR Spectra

The ¹H NMR Schiff base ligands N, N'-(indoline-2,3- diylidene)bis(4-nitroaniline) (L₁), N, N'-(indoline-2,3- diylidene)bis(4-chloroaniline) (L₂) was recorded in DMSO at room temperature and ¹H chemical shift are referenced to TMS. The proton of the aromatic rings shows the multiplets at the region of 6.6- 7.8 ppm in the ligand 1 and 6.4- 7.9 ppm in the ligand 2. The azomethine group in the Schiff base ligands L₁ and L₂ shows at 8.6 ppm and 8.5 ppm. The peaks at 10.4 and 10.5 in the ligand₁ and ligand₂ are assigned to be the N-H protons [13].

Antioxidant activity

DPPH Radical Scavenging Activity

The scavenging activity was increased by increasing the concentration of the compounds. DPPH antioxidant assay is based on the ability of decolourisation of DPPH (a stable free radical) in the presence of antioxidants [7]. This radical contains an odd electron responsible for absorbance at 517 nm. When DPPH accepts a electron donated by an antioxidant compound, the DPPH is decolorised which can be quantitatively measured by the changes in absorbance. In the present study, based on the IC₅₀ values, the scavenging activities of ligands and their complexes are calculated. From the results (Figure 2 & 3), it was found that the IC₅₀ values of [Ni(C₄₀H₂₆N₈O₄Cl₄)] is 6.8 mg/ml, it show good activity compared to remaining complexes and ligand. Ascorbic acid is used as standard.

DNA Cleavage by agarose gel electrophoresis

DNA cleavage is measured by relaxation of supercoiled pUC18 DNA to nicked circular conformation and linear conformation. During electrophoresis process supercoiled DNA migrates faster when compared with DNA in nicked and linear confirmations [14]. Co(II) complex has shown good comparable activity with other compounds and is evident by complete degradation of DNA resulting in the disappearance of bands on gel. The supercoiled plasmid DNA is completely degraded resulting in the disappearance of bands on agarose gel .Further the presence of a smear & decreased intensity of bands in the gel diagram indicates the presence of radical cleavage.

Antibacterial

In case of bacteriological studies, the results were compared with the Streptomycine as a

standard drug. It was observed that, [Cu(C₄₀H₂₆N₈O₄Cl₄)] complex was found potentially active against all the bacterial strains. The Cu(II) complex exhibit better antibacterial activity than their Schiff base ligands against all the four bacteria [15]. It was evident that, overall potency of the uncoordinated compounds has been enhanced on coordination with the metal ions.

IV. CONCLUSION

The synthesis of mononuclear Schiff base Cu(II), Ni(II) and Co(II) complexes derived from N, N'-(indoline-2,3- diylidene)bis(4-nitroaniline) (L₁) and N, N'-(indoline-2,3- diylidene)bis(4-chloroaniline) (L₂) are characterized. The complexes show the potent scavenging and antibacterial activity. The DNA Cleavage properties indicates that the Co(II) complex effectively cleave with DNA. The spectral and analytical data revealed that the complexes have the distorted octahedral geometry.

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AUTHORS CONTRIBUTIONS

All 4 authors participated in the practical work and writing of the manuscript. All authors have complete access to the study data that support the publication.

CONFLICT OF INTERESTS

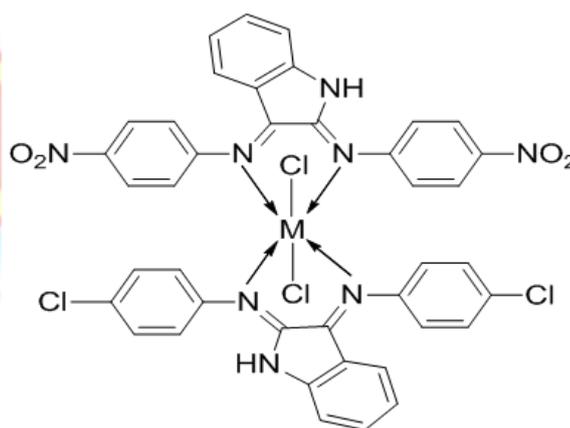
The authors declare that they have no conflict of interest

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FIGURES



M = Ni(II), Cu(II) and Co(II)

Fig 1. Proposed structure of the Schiff base metal complexes

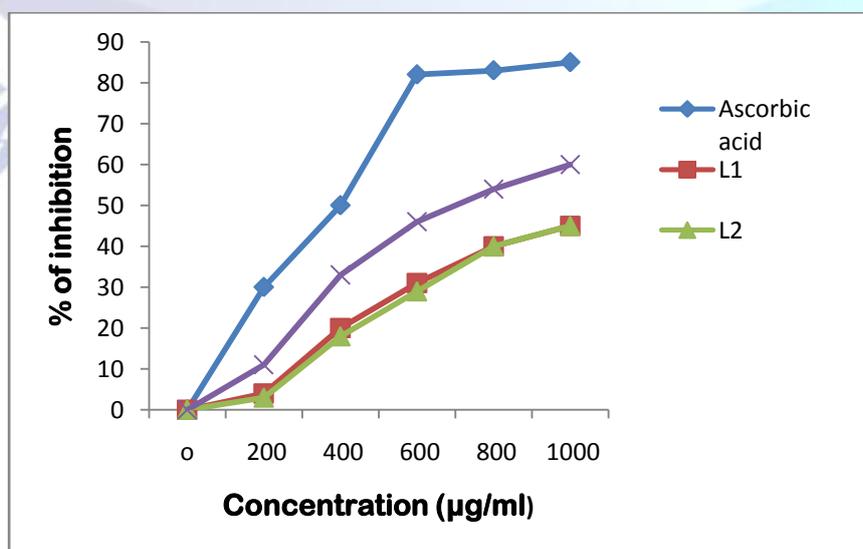


Fig 2. DPPH Scavenging activity of L₁, L₂ and its Ni(II) complex

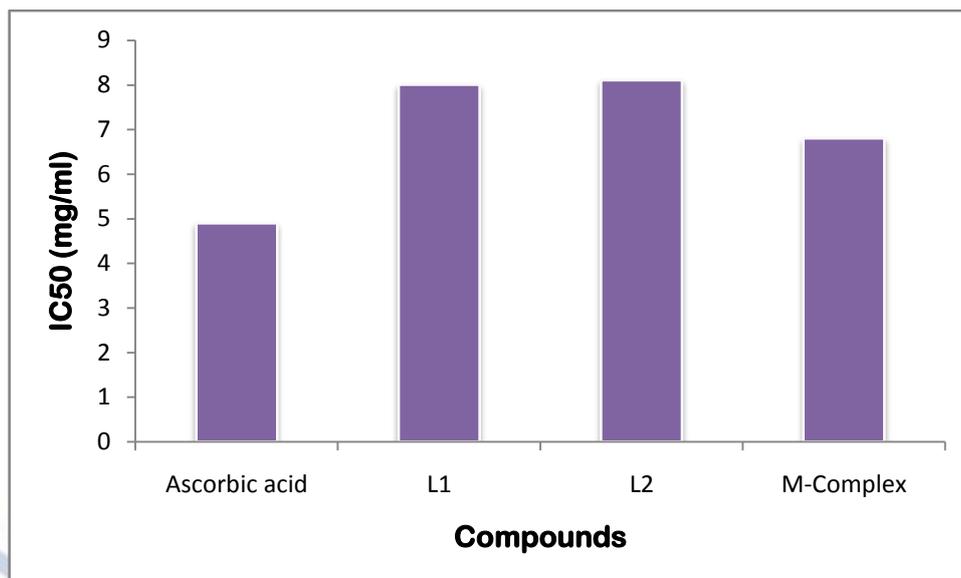
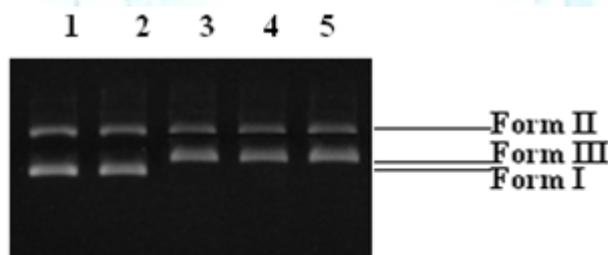


Fig 3. DPPH Scavenging capacities (IC₅₀ values) ligands and Ni(II) complex



Lane 1 – PUC 18 DNA - Control
 Lane 2 – DNA + H₂O₂ (1mM)
 Lane 3 – DNA + H₂O₂ (1mM) + L1 (50μM)
 Lane 4 – DNA + H₂O₂ (1mM) + L2 (50μM)
 Lane 5 – DNA + H₂O₂ (1mM) + Co (50μM)

Fig. 4. Agarose gel showing the results of electrophoresis of pUC18 DNA with the Schiff base metal complex : Lane 1, pUC18 DNA - Control; Lane 2, DNA + H₂O₂ (1mM); Lane 3, DNA + H₂O₂ (1mM) + L1 (50μM); Lane 4, DNA + H₂O₂ (1mM) + L2₄ (50μM) + H₂O₂; Lane 5, DNA + H₂O₂ (1mM) + Co -complex (50μM) + H₂O₂.

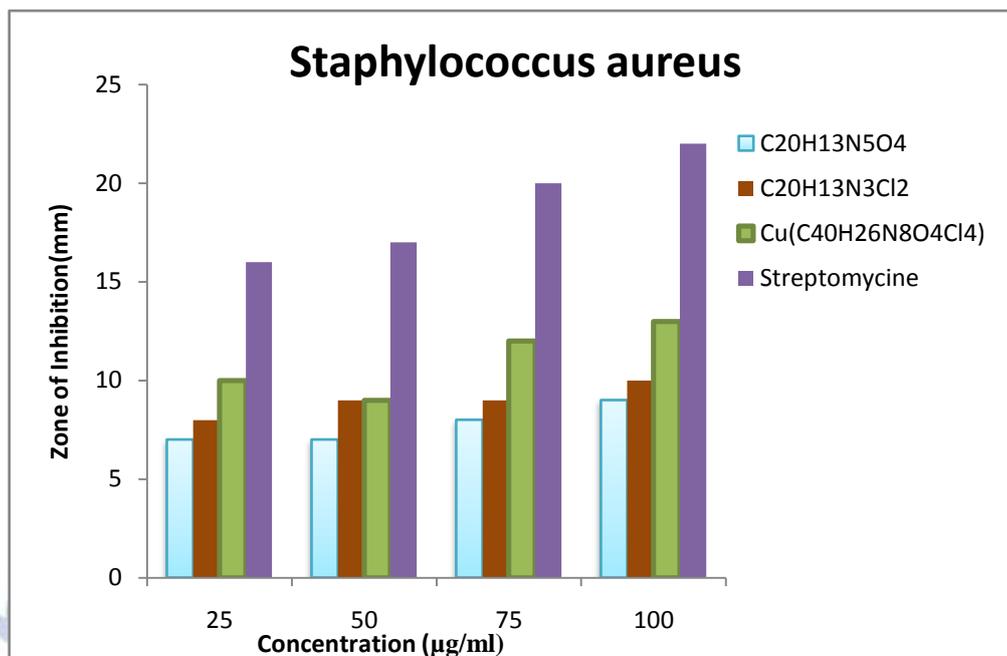


Fig 5. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against Staphylococcus aureus

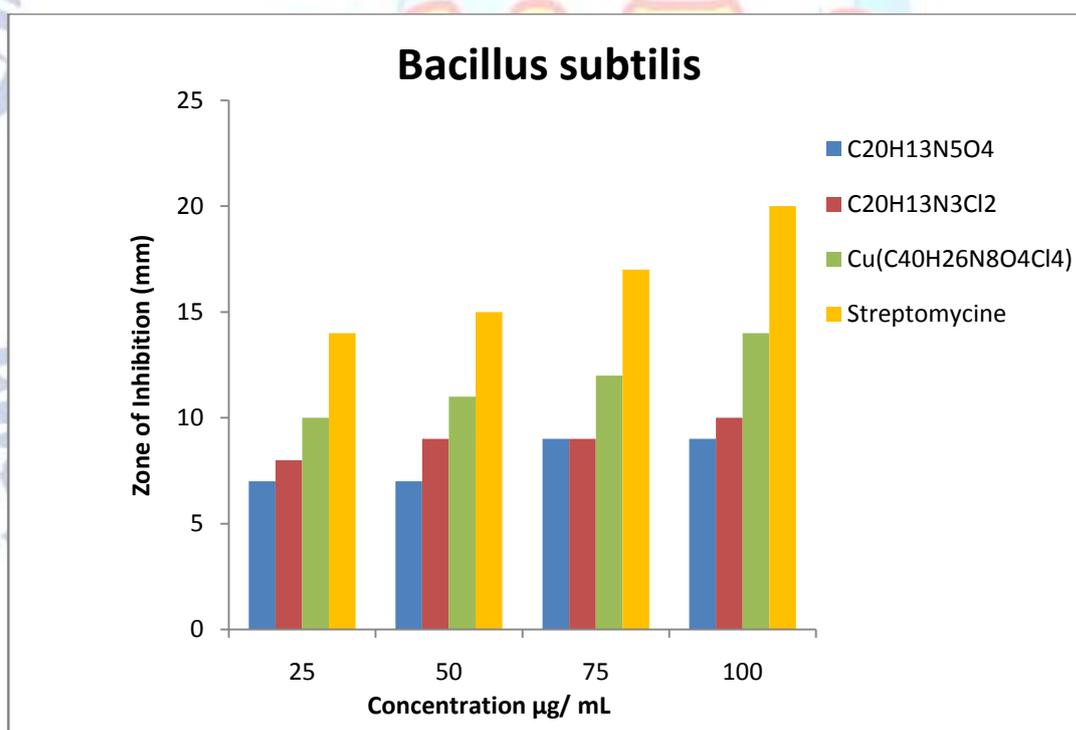


Fig 6. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against Bacillus subtilis

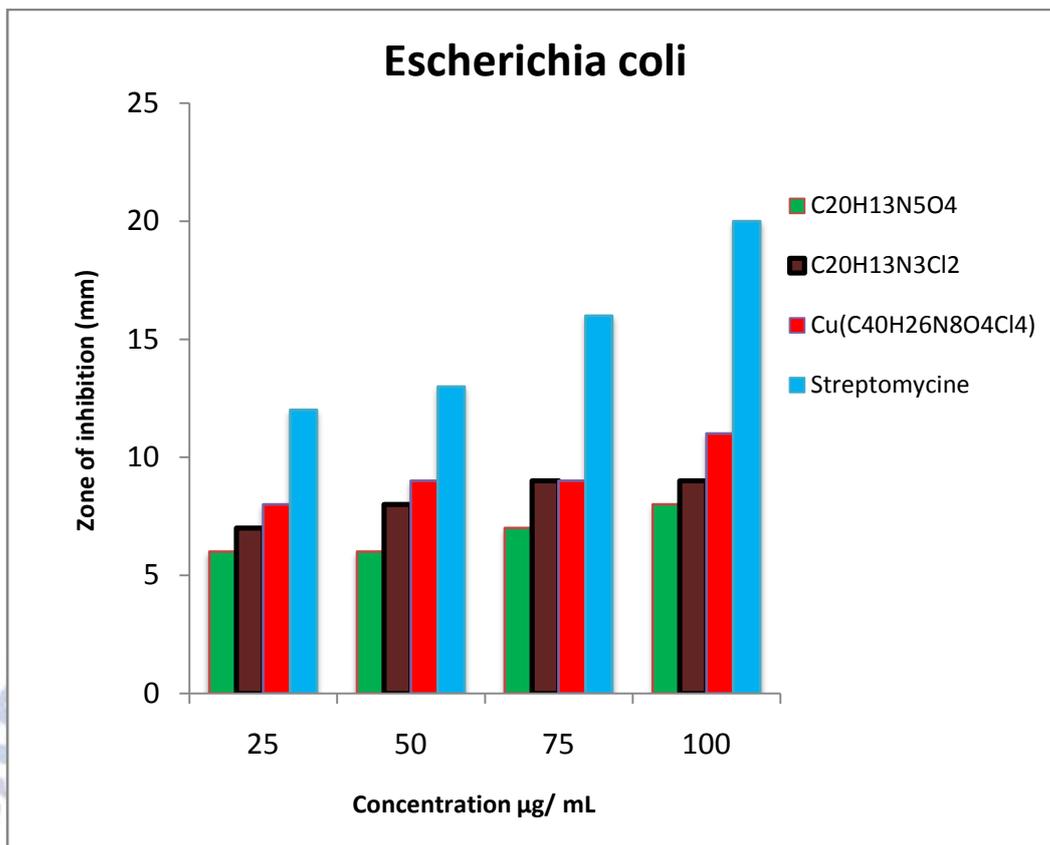


Fig 7. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against Escherichia coli

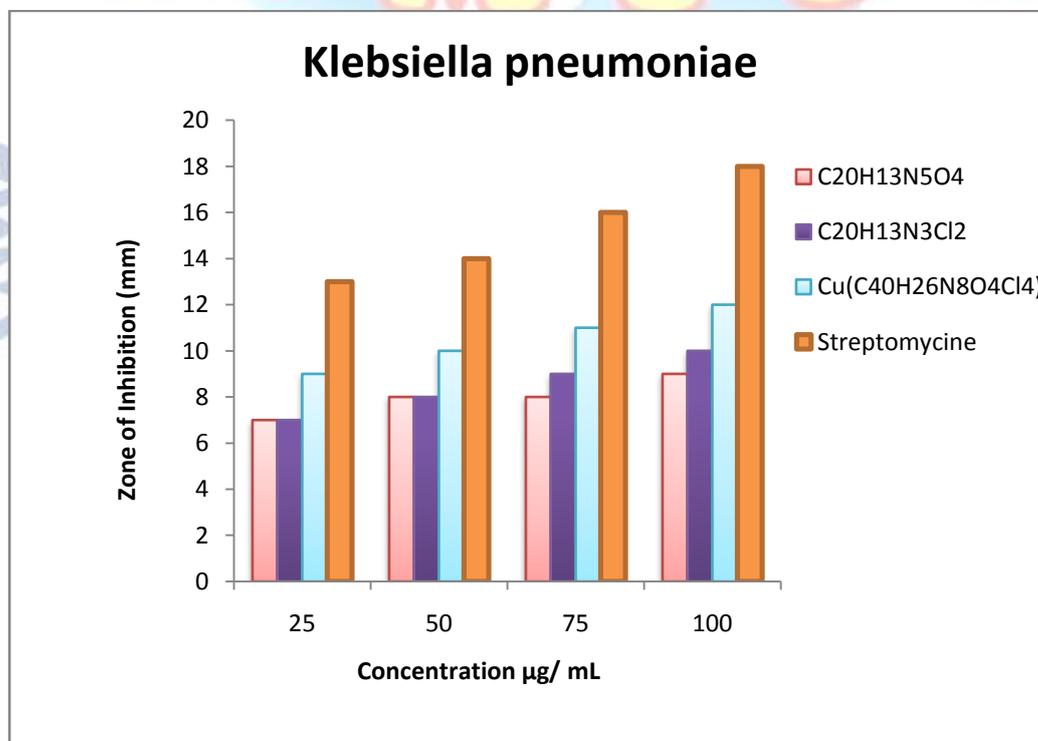


Fig 8. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against Klebsiella pneumonia

TABLES

Table 1. Analytical data for Schiff base ligands and its metal complexes

Compound s	Molecular formula	Color	Yield %	Melting point (°C)	Calculated (%)			
					C	H	N	Metal
L ₁	C ₂₀ H ₁₃ N ₅ O ₄	Yellowish orange	82	154	62.01 (62.03)	3.36 (3.38)	18.08 (18.10)	-
L ₂	C ₂₀ H ₁₃ N ₃ Cl ₄	Yellowish orange	80	152	65.75 (65.77)	3.56 (3.59)	11.51 (11.53)	-
NiL ₁ L ₂ X ₂	Ni(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	Reddish brown	75	>200	54.55 (54.58)	2.95 (2.97)	12.72 (12.75)	6.59 (6.61)
CuL ₁ L ₂ X ₂	Cu(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	Brownish Red	73	>200	54.24 (54.27)	2.94 (2.96)	12.66 (12.69)	7.12 (7.15)
CoL ₁ L ₂ X ₂	Co(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	Blackish brown	70	>200	54.55 (54.58)	2.95 (2.97)	12.72 (12.75)	6.59 (6.61)

Table 2. Important IR spectra bands of the ligands and its metal complexes

Compounds	ν (C=N) (cm ⁻¹)	ν (M-N) (cm ⁻¹)
C ₂₀ H ₁₃ N ₅ O ₄	1627.92	--
C ₂₀ H ₁₃ N ₃ Cl ₂	1612.49	--
Ni(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	1604.77	486.06
Cu(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	1610.45	470.06
Co(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	1603.26	465.06

Table 3. Electronic spectral data of the Schiff base ligand and its metal complexes

Compounds	Absorbance (nm)				Geometry of the complex
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L→M	d-d	
C ₂₀ H ₁₃ N ₅ O ₄	310	390	--	--	---
C ₂₀ H ₁₃ N ₃ Cl ₂	305	400	--	--	---
Ni(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	280	310	400	550,580,610	Distorted octahedral
Cu(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	300	320	400	640	Distorted Octahedral
Co(C ₄₀ H ₂₆ N ₈ O ₄ Cl ₄)	280	320	400	610, 630, 650	Distorted Octahedral