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ORIGINAL ARTICLE

Bioactive Co(II), Ni(II), and Cu(II) Complexes of Thiophene-based Schiff base Ligands: Synthesis, Structural Characterization, DNA-Binding, Antioxidant, and Antimicrobial Studies

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ABSTRACT
A series of chelated metal complexes, [Co(LI)2] (1), [Ni(LI)2] (2), [Cu(LI)2] (3) [Co(LII)2] (4), [Ni(LII)2] (5) and [Cu(LII)2] (6) were designed and synthesized from newly synthesized Schiff bases, LI = 2-((E)-((thiophen-2-yl)methylimino)methyl)-4,6-di-tert-butylphenol and LII =2-((E)-((thiophen-2-yl) methylimino) methyl)-4,6-diiodophenol. The synthesized compounds were characterized by elemental analysis (CHN), nuclear magnetic resonance spectroscopy (NMR), electronic spectroscopy (UV-Vis), infrared spectroscopy (FT-IR), magnetic susceptibility (μeff) and electron spin resonance spectroscopy (ESR) Results of all the characterizations depicted that, geometry of square planar was exhibited by copper and nickel complexes, whereas octahedral geometry was observed in cobalt complexes around metal ion. The binding ability between these metal complexes and calf thymus DNA (CT-DNA) was investigated by UV-Vis and fluorescence spectroscopy which disclosed that, the complexes interacted to CT-DNA via an intercalation binding mode. Antioxidant activity of all complexes was determined by DPPH free radical scavenging experiment and showed prominent antioxidant activity. Further, the antibacterial and antifungal activities of all compounds were screened against bacterial and fungal strains via invitro disc diffusion method. These studies revealed that the complexes showed comparatively more antimicrobial activity than free ligands against tested microbial strains.
Keywords: *Binary metal complex; DNA interaction; Antioxidant; Antimicrobial.*

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INTRODUCTION

Biologically active metal complexes derived from Schiff base ligands are a significant set of bio-active entities. These metal complexes have received huge attention of inorganic biochemists due to their antimicrobial, antioxidant, antituberculosis, antiviral, anti-inflammatory, antiproliferative, anticancer and other bio-medicinal properties [1,2]. The metal complexes in combination with Schiff base plays an important role in coordination chemistry due to their variety of applications, such as ease of synthesis, changeable structural confirmations, variable binding ability with DNA, capability of DNA cleavage, catalytic and biological properties [3-6].

It is well known that the DNA is the important intracellular target for several drugs, which is evidenced by the interaction between the metal complexes and DNA leading to cell death by blocking the aggressive growth of cell division [7,8]. The Schiff base ligands coordinated with transition metal ions to form complexes, are special type of chelators and bonds with metal ions through nitrogen atom of azomethine group (–C=N–) and oxygen of phenolic –OH group, which intact to construct a variety of new frame works with interesting chemotherapeutic and bio-medical properties. The metal complexes are thermally stable

and actively participated in asymmetric catalysis, mainly involved in organic transformations as catalytic species and some of them are identified as oxygen carriers [9].

An extensive attention given towards the Schiff bases derivatives due to their well-defined chemotherapeutic activities explores new path way to design and development of anticancer agents [10, 11]. Further, it was found that the reactive oxygen species (ROS), such as $^{*}OH^{-}$ and $^{*}O_2^{-}$ radicals, induce the pathophysiological abnormalities and a variety of cancers in human body by breakdown the several proteins, lipids and DNA of biological tissues. Luckily, the human body was protected by antioxidants, due to their free radical scavenging property [12]. Hence, it was crucial point to development of potential drugs with both strong DNA interactions and antioxidant properties for quick remedy from miscellaneous type of cancers.

Biologically cobalt metal contributes an important role in many metabolic processes of living organisms. As a central metal ion in vitamin-B_{12}, regulates the normal functioning of brain and the nervous system. Earlier studies reveal that, most of the cobalt complexes significantly act as antioxidants, antimicrobial, antiproliferent and antiviral drugs [13, 14]. The literature survey noticed that, the nickel complexes are vital in redox enzyme systems as active catalysts and nickel supported complexes also exhibits catalytic activity in hydrogenation reactions [15,16]. Many of copper complexes reported as pharmacological agents and considered as potent anticancer drugs. These complexes induce their anticancer property via blocking the aggressive expansion of cell division by rupturing the cell DNA through an oxidative mechanism and are the best alternative to cisplatin drug [16].

In view of the above references, we have made a significant study on Co(II), Ni(II) and Cu(II) metal complexes derived from (thiophen-2-yl)methanamine Schiff base ligands and all complexes are employed to DNA binding, antioxidant activity, *invitro* antibacterial and antifungal activity investigations.

MATERIAL AND METHODS

Materials

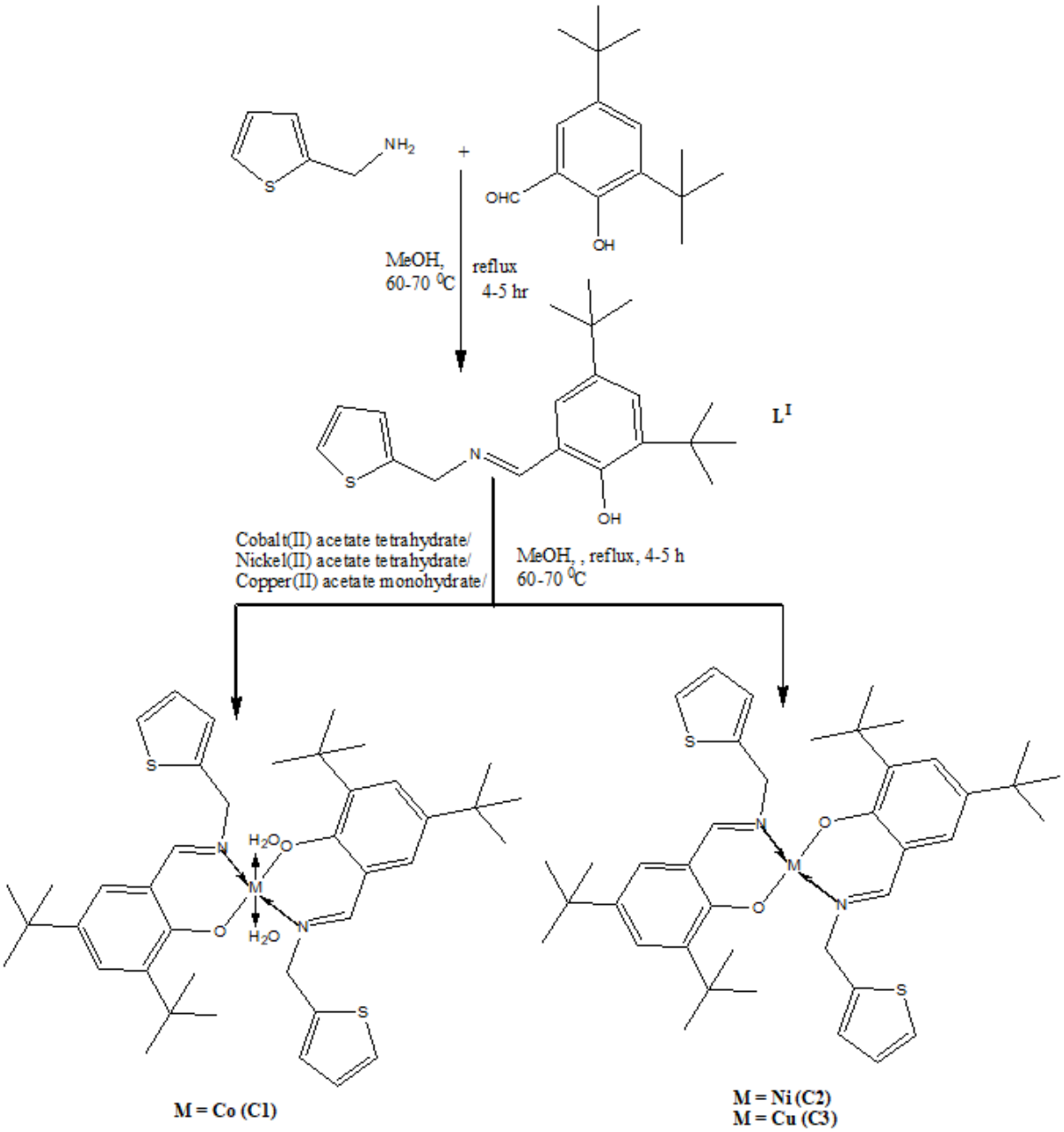
Chemicals, hydrated metal salts, $M(OAc)_2.xH_2O$ (M= Co, Ni and Cu) and solvents utilized in this experimental work are of analytical grade purchased from Hi-Media Ltd., Merck, Finar and Sigma–Aldrich Chemicals. The Calf-thymus DNA (CT–DNA) (stored at 4^{o}C) is procured from Genei, Bangalore, India. A Tris–HCl buffer (Tris-buffer, pH=7.2) solution of CT–DNA gives a ratio of 1.8–1.9 of UV absorbance at 260 and 280 nm, recommending, DNA used in this study was protein free [17].

Physical Instrumentation

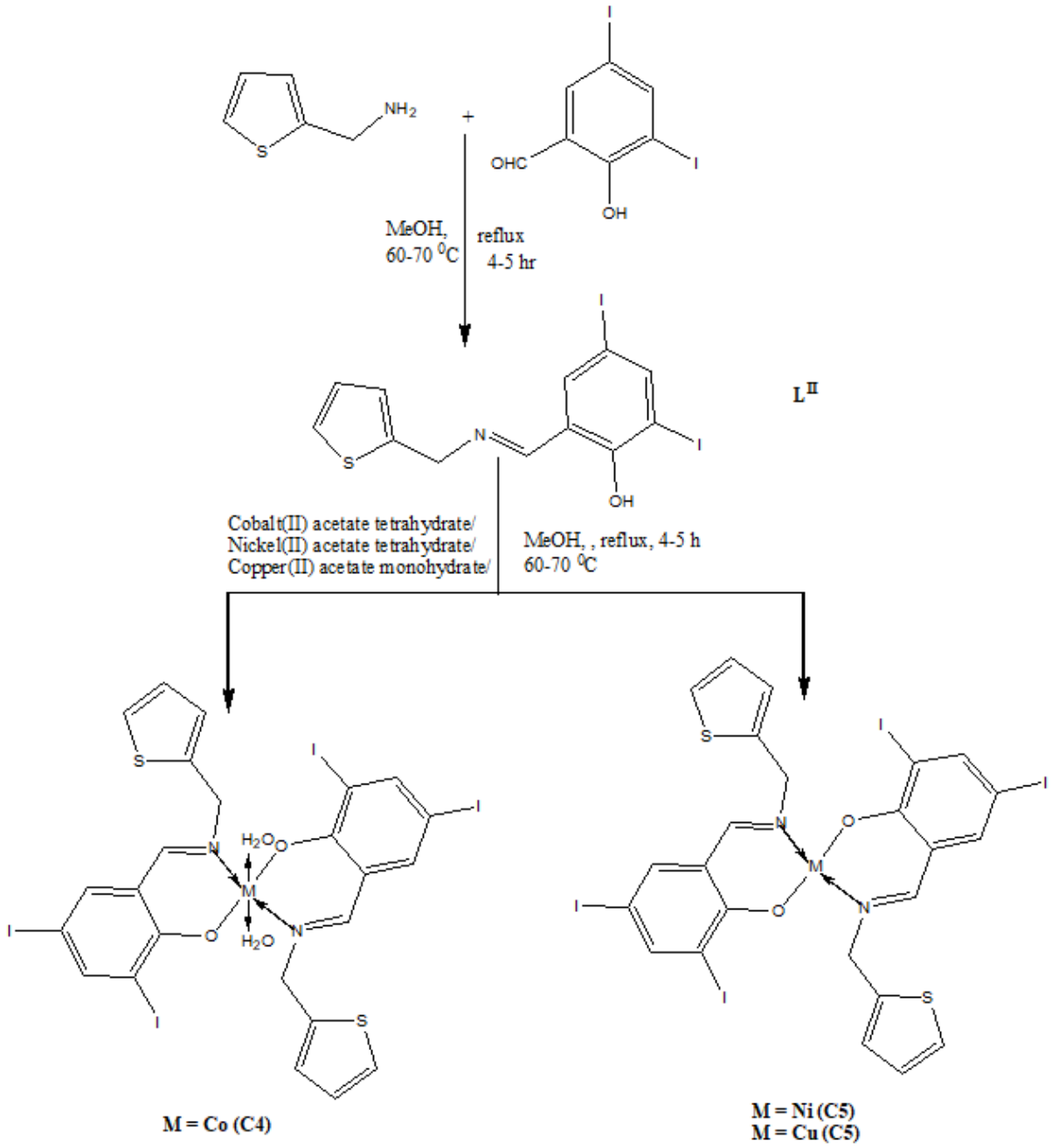
C, H, N analysis of compounds was carried out by Perkin-Elmer 240C (USA) elemental analyzer. The metal percentage of the complexes was calculated by atomic absorption spectroscopy after treatment of complexes with HNO_3 on GBC Avanta 1.0 AAS. The ^{1}H-NMR, ^{13}C-NMR spectra of Schiff base ligands and their metal complex are recorded on a Bruker 400 MHz NMR. FT-IR spectra of all compounds were recorded on Perkin–Elmer Infrared model 337 instruments in the range 4000–250cm^{-1} with KBr pellet method. Electronic spectra (200 to 800 nm range) of all compounds were recorded on Shimadzu UV–2600 spectrophotometer. Gouy balance model 7550 was used to investigate the magnetic moment values of metal complexes and the calibrant, $Hg[Co(NCS)_4]$ was used in this investigation. ESR spectroscopy of Cu (II) complexes was carried out in DMSO solvent using JEOL–Japan made JES–FA200 ESR Spectrometer at 77K. VG AUTOSPEC mass spectrometer was used for detrmmination of mass spectral data of all compounds. The polmon instrument, model No. MP–96 was used to find out the melting points of all compounds. The solution state UV absorption spectral studies and fluorescence quenching experiments were recorded on Shimadzu UV–2600 spectrophotometer and RF–5301PC (JASCO) spectrofluorometer respectively.

Synthesis of Schiff base ligands (L^{I}, L^{II})

Synthesis of Schiff bases ligands, **L^{I}** and **L^{II}** are carried out by mixing of hot methanolic solution (10 ml) of 3,5-di-tert-butyl-2-hydroxybenzaldehyde (0.136 g, 1 mmol)/ 2-hydroxy-3,5-diiodobenzaldehyde (0.156 g, 1 mmol) to hot stirred methanolic solution (20 ml) of (thiophen-2-yl)methanamine (0.178 g, 1 mmol) and the solution was refluxed for 2-3 hour at 60-70 ^{o}C on oil bath. Later the solution was allowed to cool for 1-2 hrs leads to settle down of coloured solid product. The product was filtered and gently rinse with Pet-ether and recrystallized from methanol. TLC technique was helped to check the purity of product and dried in vacuum desiccator loaded with anhydrous $CaCl_2$ (Scheme 1 & 2).



Scheme 1. Schematic representation for synthesis of **L**^{I} and its metal complexes (C**1**–C**3**).



Scheme 2. Schematic representation for synthesis of **L^{II}** and its metal complexes (C**4**–C**6**).

2-((E)-((thiophen-2-yl)methylimino)methyl)-4,6-di-tert-butylphenol *(L^{I})*
Mol. For.: $C_{20}H_{27}NSO$, M.P: 93 °C. Elemental Anal. (%) Calcd.: C, 72.90; H, 8.26; N, 4.25; S, 9.73. Found: C, 72.95; H, 8.36; N, 4.29; S, 9.78.^{1}H–NMR (400 MHz, solvent: $CDCl_3$): δ = 13.71 (s, 1 H), 8.39 (s, 1H), 7.37 (dd, J_1 = 1.75 Hz, J_2 = 7.78 Hz, 1H), 7.25 (m, 1H), 7.10 (s, 1H), 6.98 (m, 1H), 6.81 (s, 1H), 4.98 (s, 2H), 1.43 (s, 18H) shown. ^{13}C–NMR (100 MHz, solvent: $CDCl_3$): δ = 166.83, 158.00, 140.96, 140.17, 136.78, 127.24, 126.99, 126.15, 125.47, 125.02, 117.80, 57.31, 35.07, 34.16, 31.52, 29.43. FT-IR (KBr pellet) (cm^{-1}): $\upsilon_{(OH)}$ 3452, $\upsilon_{(CH=N)}$ 1631, $\upsilon_{(C-O)}$ 1167. UV-Visible (solvent: $CHCl_3$) λ_{max}/nm(cm^{-1}): 261 (38315), 320 (31250). ESI-Mass (*m/z):* 331 $[M+H]^+$.

2-((E)-((thiophen-2-yl)methylimino)methyl)-4,6-diiodophenol *(L^{II}):*
Mol. For.: $C_{12}H_9I_2NOS$, M.P: 98 °C. Elemental Anal. (%) Calcd.: C, 30.73; H, 1.93; N, 2.99; S, 6.84. Found: C, 30.79; H, 1.98; N, 3.05; S, 6.87. ^{1}H–NMR (400 MHz, solvent: $CDCl_3$): δ = 14.52 (s, 1H), 8.16 (s, 1H), 8.04 (d, J_1 = 2.08 Hz, 1H), 7.51 (d, J_1 = 2.08 Hz, 1H), 7.28 (d, J_1 = 4.76 Hz, 1H), 7.00 (d, J_1 = 3.51 Hz, 1H), 6.99 (s, 1H), 4.98 (s, 2H). ^{13}C–NMR (100 MHz, solvent: $CDCl_3$): δ = 163.36, 160.81, 148.77, 139.97, 138.86, 127.23,

126.41, 125.76, 119.86, 87.75, 79.19, 56.00. FT-IR (KBr pellet) (cm^{-1}): $\upsilon_{(OH)}$ 3446, $\upsilon_{(CH=N)}$ 1614, $\upsilon_{(C-O)}$ 1120. UV-Visible (solvent: $CHCl_3$) λ_{max}/nm (cm^{-1}): 259 (38610), 284 (35211), 336 (29761). ESI-Mass (*m/z):* 470 $[M+H]^+$.

Synthesis of Co(II), Ni(II) and Cu(II) complexes (C1–C6)

To a hot stirred methanolic solution of Schiff base ligand (**L^I/L^{II}**) (2 mmol), hot methanolic solution of metal acetates [$Co(CH_3COO)_2$. $4H_2O$/ $Ni(CH_3COO)_2$. $4H_2O$/ $Cu(CH_3COO)_2$. H_2O] (1 mmol) was added drop wise and reflux the 1:2 metal: ligand ratio solution for 2-3 hour at 70-80 ^oC leads to formation of coloured solid. The reaction mixture was left constant for 1-2 hrs to settle down the metal complex. The product was filtered and washed 2-3 times with methanol and petroleum ether. Filtered metal complexes were dried in vacuum desiccators loaded with anhydrous $CaCl_2$ (Scheme 1 & 2).

[Co(L^I)$_2$(H_2O)$_2$](C1)

Mol. For.: $C_{40}H_{56}CoS_2N_2O_4$, M.P: 109 ^oC, Elemental Anal. (%) Cal: C, 63.89; H, 7.51; N, 3.73, S, 8.53 Found: Cal: C, 63.95; H, 7.56; N, 3.77, S, 8.58. FT–IR (KBr pellet) (cm^{-1}): $\upsilon_{(OH)}$ 3441, $\upsilon_{(C=N)}$ 1623, $\upsilon_{(C-O)}$ 1171, $\upsilon_{(M-O)}$ 557, $\upsilon_{(M-N)}$ 531. UV-Visible (solvent: DMSO) λ_{max}/nm (cm^{-1}): 279 (35842), 396 (25252), 660 (15152). μ_{eff} (BM): 4.10. ESI-Mass (m/z): 753 $[M+H]^+$.

[Ni(L^I)$_2$](C2)

Mol. For.: $C_{40}H_{52}S_2N_2NiO_2$, M.P: 198 ^oC, Elemental Anal. (%) Cal: C, 67.13; H, 7.32; N, 3.91, S, 8.96. Found: Cal: C, 67.19; H, 7.22; N, 3.98, S, 8.97. FT–IR (KBr pellet) (cm^{-1}): $\upsilon_{(C=N)}$ 1597, $\upsilon_{(C-O)}$ 1173, $\upsilon_{(M-O)}$ 575, $\upsilon_{(M-N)}$ 527. UV-Visible (solvent: DMSO) λ_{max}/nm (cm^{-1}): 266 (37593), 412 (24272), 678 (14759). μ_{eff} (BM): Dia. Mag. ESI-Mass (m/z): **717** $[M+H]^+$.

[Cu(L^I)$_2$](C3)

Mol. For.: $C_{40}H_{52}CuN_2O_2S_2$, M.P: 201 ^oC, Elemental Anal. (%) Cal: C, 66.68; H, 7.27; N, 3.89, S, 8.90. Found: C, 66.78; H, 7.29; N, 3.92, S, 8.95. FT–IR (KBr pellet) (cm^{-1}): $\upsilon_{(C=N)}$ 1588, $\upsilon_{(C-O)}$ 1154, $\upsilon_{(M-O)}$ 553, $\upsilon_{(M-N)}$ 471. UV-Visible (solvent: DMSO) λ_{max}/nm (cm^{-1}): 264 (37878), 395 (25316), 552 (18116). μ_{eff} (BM): 1.76. ESI-Mass (m/z): 739 $[M+NH_4]^+$.

[Co(L^{II})$_2$(H_2O)$_2$](C4

Mol. For.: $C_{24}H_{20}I_4CoS_2N_2O_4$, M.P: 111 ^oC, Elemental Anal. (%) Cal: C, 27.96; H, 1.96; N, 2.72, S, 6.22. Found: C, 27.99; H, 2.05; N, 2.78, S, 6.27. FT–IR (KBr pellet) (cm^{-1}): $\upsilon_{(OH)}$ 3454, $\upsilon_{(C=N)}$ 1620, $\upsilon_{(C-O)}$ 1158, $\upsilon_{(M-O)}$ 536, $\upsilon_{(M-N)}$ 411. UV-Visible (solvent: DMSO) λ_{max}/nm (cm^{-1}): 277 (36101), 411 (24331), 653 (15314). μ_{eff} (BM): 3.98. ESI-Mass (m/z): 1049 $[M+NH_4]^+$.

[Ni(L^{II})$_2$](C5)

Mol. For.: $C_{24}H_{16}I_4N_2S_2NiO_2$, M.P: 199 ^oC, Elemental Anal. (%) Cal: C, 28.98; H, 1.62; N, 2.82, S, 6.45. Found: C, 28.99; H, 1.67; N, 2.86, S, 6.49. FT–IR (KBr pellet) (cm^{-1}): $\upsilon_{(C=N)}$ 1584, $\upsilon_{(C-O)}$ 1168, $\upsilon_{(M-O)}$ 575, $\upsilon_{(M-N)}$ 517. UV-Visible (solvent: DMSO) λ_{max}/nm (cm^{-1}): 255 (39216), 373 (26809), 503 (19881). μ_{eff} (BM): Dia. Mag. ESI-Mass (m/z): 1018 $[M+Na]^+$.

[Cu(L^{II})$_2$](C6)

Mol. For.: $C_{24}H_{16}I_4CuN_2O_2S_2$, M.P: 209 ^oC, Elemental Anal. (%) Cal: C, 28.83; H, 1.61; N, 2.80, S, 6.41. Found: C, 28.89; H, 1.67; N, 2.85, S, 6.47. FT–IR (KBr pellet) (cm^{-1}): $\upsilon_{(C=N)}$ 1607, $\upsilon_{(C-O)}$ 1166, $\upsilon_{(M-O)}$ 522, $\upsilon_{(M-N)}$ 440. UV-Visible (solvent: DMSO) λ_{max}/nm (cm^{-1}): 257 (38911), 318 (31447), 496 (20161). μ_{eff} (BM): 1.74. ESI-Mass (m/z): 1022 $[M+Na]^+$.

DNA binding techniques

UV–Vis absorption titrations

Tris-HCl buffer (pH=7.2) is used to determine the nature of interaction between metal complex and DNA at room temperature. The UV–Vis absorption titrations were carried out by maintaining the metal complex concentration (10 μM) as constant, whereas the Calf Thymus DNA (CT–DNA) concentration as variable (0–10 μM). At 260–280 nm wavelength range 1.8-1.9/1 ratio of UV-absorbance was shown by the buffer solution containing CT–DNA indicating that the CT–DNA was enoughly free of protein. The metal complexes (**1–6**) are moderately soluble in buffer solution due to this reason complexes are dissolved in dimethyl sulfoxide (DMSO) to prepare stock solution. The concentration of CT–DNA used in these experiments was determined by absorption spectroscopy with the known molar extinction coefficient of 6600 $M^{-1}Cm^{-1}$ at 260 nm [18], while determining the absorbance, to eliminate the absorbance of CT–DNA itself equivalent quantities of CT–DNA were added to complex solution as well as reference solution.

Fluorescence quenching assay

Fluorescence quenching assay was performed to further clear understanding of interaction between metal complex and DNA. Because of non-emissive nature of ethedium bromide (EB), the EB is mixed with CT–DNA to intensify the emissive nature and the solution was kept constant for 15 mints at room temperature for fluorescence quenching study. The fluorescence emission intensities of EB (12.5 μM)

bound CT–DNA were evaluated in the wavelength range of 500–750 nm with fixed excitation wavelength (λ=350 nm) by increasing complex concentration from 0–60 μM and CT-DNA concentration (125 μM) as constant. The Stern-Volmer constant, Ksv was calculated for each complex by using following equation [19].

$$I_0/I = 1 + K_{sv}[Q]$$

Where

I_0 = fluorescence emission intensity in the absence of complex

I = fluorescence intensity in the presence of complex

K_{sv} = Stern–Volmer constant which is a measure of the efficiency of quenching

[Q] = [metal complex] (concentration of quencher).

Antioxidant Activity

2, 2-diphenyl-2-picryl-hydrazyl (DPPH) solution was used to investigate the antioxidant property of all metal complexes (C**1**–C**6**) by Blois method with some adjustments [20]. On UV–Vis spectrophotometer, DPPH shows a strong absorption band at 517 nm due to odd electron. The metal complex samples were prepared in test tubes with variable concentrations (20-100 μM) along with standard (Ascarbic acid/ Vit-C). To each sample solution 1 ml of 0.3 μM concentrated DPPH solution was added and mixed thoroughly. The samples were incubated for 30-40 mints in dark. After incubation, the absorbances were recorded at 517 nm to investigate the antioxidant activity of metal complexes. The IC_{50} values (50% inhibition concentration) of complexes were calculated with regression lines.

Antimicrobial Investigations

Antibacterial activity

Antibacterial activity of synthesized ligands (**L^{I}**, **L^{II}**) and their metal complexes (C**1**–C**6**) were determined by *invitro* disc diffusion method [21]. All compounds were screened against two Gram positive bacterial strains, *Bacillus amyloliquefaciens (B. amyloliquefaciens), Staphylococcus aureus* (S. aureus), two Gram negative bacterial strains, *Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli)*. DMSO dissolved 1.0 mg/ml concentrated sample stock solutions (Schiff base ligands and metal complexes) were utilized for this biological investigation. In this activity, cultures of test organisms were maintained in beef extract and peptones containing nutrient agar media and sub cultured in petri dishes [22]. The medium (pH = 6.0-6.5) was sterilized in the autoclave at 121 ^{o}C (15 lbs) pressure for 15 min. The medium was cooled to 45-50 ^{o}C and poured in 20 mL volume in each Petri dish and allowed to solidify and inoculated with actively growing culture of *B. amyloliquefaciens*, *S. aureus*, *E. coli* and *P. aeruginosa* separately. The discs of Whatman filter paper No.41 having the diameter 6 mm were prepared and dipped in the test solution. After drying the disc, it was kept on antibiotic nutrient agar broth in petri plates and seeded with 0.1 mL of bacterial culture of above-mentioned strains, subsequently incubated for 24 h at 37 ^{o}C. After 24 h, the petri dishes were checked for growth inhibition zone. The activities of tested compounds were evaluated interms of inhibition zones (in mm). The drug, streptomycine was used as standered drug to compare the antibacterial activity

Antifungal activity

Two fungal strains, *Macrophomina phaseolina (M. phaseolina)* and *Sclerotium rolfsii (S. rolfsii)* were used to determine the antifungal activity of Schiff base ligands (**L^{I}**, **L^{II}**) and their metal complexes (C**1**–C**6**) by *invitro* disc diffusion method. In this study, cultures were maintained on potato starch and dextrose agar slants and sub cultured in petri dishes [23,24]. The medium (pH = 5.5–6.0) was sterilized in the autoclave at 121 ^{o}C with 15 lbs pressure for 20 min. The medium was cooled to 45-50 ^{o}C and poured in 20 ml volume in each petri dish and allowed to solidify. The 1 mg of testing sample was dissolved in 1 ml of DMSO and was utilized for this antifungal activity. After solidification of media, petri plates were inoculated with actively growing culture of *M. phaseolina* and *S. rolfsii* separately. The paper discs of 6 mm diameter were dipped in the test solution. After drying the disc, it was kept on potato dextrose agar in petri plates and seeded with respective fungal strains then incubated at 37 ^{o}C for 24 h. After 24 h, the petri dishes were checked for growth inhibition zone. The activities of tested compounds were evaluated interms of inhibition zones (in mm). The drug, mancozeb was used as standered drug to compare the antifungal activity.

RESULTS AND DISCUSSION

The Schiff base ligands (**L^{I}**, **L^{II}**) are yellow in colour and their metal complexes (C**1**–C**6**) are dark brown in colour. All complexes are thermally stable and non-hygroscopic at room temperature. The synthesized complexes are soluble in DMF and DMSO. The spectroscopic data confirmed that the ratio of metal to ligand is 1:2.

Structural characterization

Electronic spectra and magnetic susceptibility

Generally, the electronic spectroscopy is very helpful in the assessment of results provided by other structural evaluation techniques. In this electronic spectroscopy the spectral data was presented in **Table 1**. In these investigation free Schiff base ligands, **L^{I}** and **L^{II}** exhibited two high energy bands which are of direct structural significance. The one band appeared at 261 nm and 259 nm attributed to π-π* transitions of phenyl ring and other band appeared at 320 nm and 284, 336 nm correspond to n-π* transitions of azomethine group respectively. The transitions shown by the free ligands are shifted to higher or lower frequencies in their respective metal complexes. Along with these transitional bands further characteristic bands are appeared in the region of 496-660 nm corresponded to d-d transition bands [25].

The Co(II), Ni(II) and Cu(II) complexes showed d-d transitions are corresponding to $^{4}A_{1g} \rightarrow {}^{4}B_{1g}$, $^{1}A_{1g} \rightarrow {}^{1}A_{2g}$ and $^{2}B_{1g} \rightarrow {}^{2}E_{g}$ transition respectively and the magnetic moments for Co(II) complexes obtained a values of 4.10 BM (C**1**) and 3.98 BM (C**4**) which are consistent with octahedral geometry of Co(II) ion [26]. For the Ni(II) complexes are dia magnetic in nature. The magnetic moment values for Cu(II) complexes usually falls in the region of 1.76 BM to 1.74 BM which further supports a square planar environment around Cu(II) ions [27].

Table 1: Electronic spectral and magnetic moment values of L^{I}, L^{II} and their metal complexes (C1–C6).

Compound	π-π* (nm)	n-π* (nm)	d-d (nm)	μ_{eff} (BM)
L^{I}	261	320	-	-
L^{II}	259	284, 336	-	-
$[Co(L^{I})_2(H_2O)_2]$ (C**1**)	279	396	660	4.10
$[Ni(L^{I})_2]$ (C**2**)	266	412	552	-
$[Cu(L^{I})_2]$ (C**3**)	264	395	552	1.76
$[Co(L^{II})_2(H_2O)_2]$ (C**4**)	277	411	653	3.98
$[Ni(L^{II})_2]$ (C**5**)	255	373	503	-
$[Cu(L^{II})_2]$ (C**6**)	257	318	496	1.74

FT–IR spectroscopy

FT–IR spectral studies facilitate to know information regarding binding sites and coordination manner existing in between the metal ion and free bridged ligands. In the present study, the important IR bands are listed in **Table 2**. In IR spectra of free ligands (**L^{I}**, **L^{II}**) characteristic strong intense bands are observed in the region of 1631 cm^{-1} and 1614 cm^{-1} are due to the azomethine functional group (–C=N) stretching vibrations respectively. In metal complexes these bands are shifted to lower/higher stretching frequencies due to participation of azomethine nitrogen in bond formation with metal ion. In addition, free ligands (**L^{I}**, **L^{II}**) showed typical broad band's with medium intensity in the region of 3452 cm^{-1} and 3446 cm^{-1} attributed to stretching vibrations of phenolic –OH group respectively. Upon complexation these broad bands are disappeared in respective metal complexes which confirm the involvement of oxygen atom of –OH group in coordination with metal ion. Further, this is proved by shifting υ(-C-O) band at 1158-1173 cm^{-1} to low/high frequency region of metal complexes. A diffuse broad band at 3441 and 3454 cm^{-1} in complexes C1 and C4 explains the appearance in coordination sphere of water molecules which is later proved from the elemental and thermogram analysis of the complexes. Additionally, a new band was also noted at the regions from 575-522 cm^{-1} and 531-411 cm^{-1} due to υ(M-O), υ(M-N) bonds respectively [28].

Table 2: FT–IR data of L^{I}, L^{II} and their metal complexes (C1–C6)

Compound	$\nu_{(OH)}$ (cm^{-1})	$\nu_{(C=N)}$ (cm^{-1})	$\nu_{(C-O)}$(cm^{-1})	$\nu_{(M-O)}$ (cm^{-1})	$\nu_{(M-N)}$ (cm^{-1})
L^{I}	3452	1631	1167	–	–
L^{II}	3446	1614	1120	–	–
$[Co(L^{I})_2(H_2O)_2]$ (C**1**)	3441	1623	1171	557	531
$[Ni(L^{I})_2]$ (C**2**)	–	1597	1173	575	524
$[Cu(L^{I})_2]$ (C**3**)	–	1588	1154	553	471
$[Co(L^{II})_2(H_2O)_2]$ (C**4**)	3454	1620	1158	536	411
$[Ni(L^{II})_2]$ (C**5**)	–	1584	1168	575	517
$[Cu(L^{II})_2]$ (C**6**)	–	1607	1166	522	440

Mass spectral analysis

ESI-mass spectra of Schiff base ligands (**L^{I}**, **L^{II}**) and their respective metal complexes (C**1**–C**6**. The ESI-mass spectra of Schiff base ligands showed molecular ion peaks at m/z 331 $[M+H]^{+}$ and 470 $[M+H]^{+}$

respectively. The metal complexes displayed prominent peaks at m/z 753 (C**1**), 717 (C**2**), 739 (C**3**), 1049 (C**4**), 1018 (C**5**) and 1022 (C**6**) corresponding to $[M+H]^+$, $[M+H]^+$, $[M+NH_4]^+$, $[M+NH_4]^+$, $[M+Na]^+$ and $[M+Na]^+$ respectively. From the results, it is confirmed that all complexes (C**1**–C**6**) are in good agreement with 1:2 (Metal: Ligand) stoichiometric ratio by comparing their molecular formula weights with respective m/z values.

ESR spectra

An information regarding delocalization of unpaired electron around copper (II) ion was provided by electron spin resonance spectroscopy (ESR). Dimethyle sulphoxide (DMSO) solvent was utilised to record the X–band ESR spectra of synthesized Cu(II) complexes (C**3**, C**6**) at liquid nitrogen temperature (77 K). In the present investigation, the ESR parameter values $g_{||}$ and $g_{\perp}$ of complex C**3** found to be 2.08 and 2.04 where as complex C**6** have 2.06 and 2.03 respectively [29]. The ESR parameter values showed a pattern $g_{||} > g_{\perp} > g_e$ (2.0023) suggests the unpaired electron is located in $d_{x^2-y^2}$ orbital with ^2B_1g ground state, is a characteristic feature of square planar environment around metal centre. The $g_{||}$ values of both the complexes (C**3**, C**6**) are found to be less than 2.3 this is an indicative for existence of covalent nature between metal–ligand coordination bonds. The coupling interactions existing in between two Cu (II) centres within the complex are determined by computing the anisotropic parameter (G) from ESR spectrum. According to Kneubuhi's method, $G = (g_{||} -2) - (g_{\perp}-2)$ formula used to calculate the anisotropic parameter, G of the complexes. In present study, the G values for complex C**3** and C**6** are found to be 1.76 and 1.01 respectively, confirming the considerable exchange interactions in between Cu(II) ions within the complex [30,31].

DNA binding techniques

UV–Vis absorption titrations

Mode of interaction and binding strength of metal complexes with DNA were determined by globally acceptable electron absorption method, in which considerable changes in absorbance and wavelength were noticed. The bathochromic/ red shift is an indicative condition for strong stacking interactions i.e., intercalative interactions between the active portion of metal complex and nitrogenous base pairs of DNA. Mainly the non-covalent π-π* interactions are involved in formation of interactive binding between metal complex and CT–DNA leading to hypochromism. After this intercalation binding, π-π* transitions are also observed between π* orbitals of complex and π orbitals of DNA base pairs, due to these transitions from complex to DNA the lowering of π-π* transition energy is observed and leads to bathochromic shift. In this investigation, the increased concentration of DNA leads to decrease in π-π* transition intensity bands by 25–34 % (hypochromism) with 2–5 nm of bathochromic shift. Further, the intrinsic binding constants, Kb values of metal complexes with CT–DNA were explored from following equation by using UV–vis absorption spectral data [32].

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + 1/K_b(\varepsilon_b-\varepsilon_f)$$

Where [DNA] = concentration of DNA in the base pairs, ε_a = apparent coefficient of the complex in presence of DNA, ε_b = molar extinction coefficient of DNA bound form of complex, ε_f = molar extinction coefficient of free complex, K_b = intrinsic binding constant, calculated from the plot drawn between [DNA]/ $(\varepsilon_a-\varepsilon_f)$ Vs [DNA]. In the present study, the K_b values are found to be $3.12 \pm 0.02 \times 10^4$ M^{-1}(C**1**), $8.15 \pm 0.01 \times 10^4$ M^{-1}(C**2**), $1.49 \pm 0.01 \times 10^5$ M^{-1} (C**3**), $3.54 \pm 0.02 \times 10^4$ M^{-1} (C**4**), $9.52 \pm 0.01 \times 10^4$ M^{-1} (C**5**) and $1.98 \pm 0.01 \times 10^5$ M^{-1} (C**6**) respectively. From the result, it is observed that the Cu(II) complexes strongly interact with CT–DNA than Co(II) and Ni(II) complexes.

Fluorescence quenching assay

The binding nature of metal complexes (C1–C6) with DNA base pairs was additionally gets evidenced by fluorescence quenching investigations. Ethedium Bromide (EB) is a strong fluorescence active probe and emits strong fluorescence intensity in presence of CT–DNA, due to intercalative binding of EB with CT–DNA [33] where as it is non-emissive and showed weak fluorescence intensity in absence of DNA, due to quenching of free EB fluorescence nature by solvent molecules. Generally, the other DNA binding agents/ substances can quench the fluorescence intensity in their higher concentrations. The gradual quenching of fluorescence intensity of EB–DNA system was observed by successive increments of metal complex concentration [34]. This is due to displacement of EB from CT–DNA and clearly showed the potential competence of metal complex with EB in binding with CT–DNA. The Stern-Volmer quenching constant, K_{sv} values are determined from the slop of plot I_0/I Vs [Q]. In the present study, the K_{sv} values are found to be $1.09 \pm 0.01 \times 10^3$ M^{-1} (C1), $4.64 \pm 0.02 \times 10^3$ M^{-1} (C2), $1.97 \pm 0.02 \times 10^4$ M^{-1} (C3), $3.01 \pm 0.01 \times 10^3$ M^{-1} (C4), $7.91 \pm 0.02 \times 10^3$ M^{-1} (C5) and $2.23 \pm 0.01 \times 10^4$ M^{-1} (C6) respectively. Here the Cu(II) complexes showed higher fluorescence quenching activity than other complexes. The results are indicated an intercalative mode of binding exists in between the DNA and metal complexes along with this the Ksv values are well reliable with the UV–DNA binding constant (K_b) values.

Biological Investigations

Antioxidant activity

DPPH• (2,2-diphenyl-2-picryl-hydrazyl) free radical scavenging technique was employed to investigate the antioxidant proficiency of synthesized metal complexes (C**1–C6**). In the process of antioxidant activity experiment, the DPPH solution containing odd electron showed a strong absorption band at 517 nm in UV-vis spectroscopy. The stable DPPH• radical has purple colour in its solution form, in combination with metal complex (antioxidant) the DPPH• radical neutralized and converted in to 1,1-diphenyl-2-picryl-hydrazine, which was confirmed by turning of purple colour solution in to pale yellow colour and the antioxidant capacity can be measured by the decrease in its absorbance value at 517 nm. The following formula is used to measure the percentage scavenging activity of DPPH• free radical.

$$\text{Percentage scavenging activity (\%)} = \quad A_o - A_e / A_o \times 100$$

Where, A_o and A_e are absorbance of DPPH• in the absence and presence of antioxidant (metal complex). In the present study, it is observed that the free radical scavenging activity was increased by raising the metal complex concentration and the activity of complexes was expressed in terms of IC_{50} values (50 % inhibitory concentration) and were compared with IC_{50} values of Ascorbic acid (AA), standard for antioxidant activity [35]. Smaller the IC_{50} value indicates greater the antioxidant activity of metal complexes. The IC_{50} values of metal complexes found to be 7.72 µM (C**1**) 5.09 µM (C**2**) 3.74 µM (C**3**) 6.59 µM (C**4**), 4.36 µM (C**5**), 3.49 µM (C**6**) and 0.42 µM (**AA**) respectively. The antioxidant results explore that the Copper (II) complexes exhibited prominent antioxidant activity than Cobalt (II) and Nickel (II) complexes.

Antibacterial activity

Antibacterial activity study of ligands (L^I, L^{II}) and their metal complexes (C1–C6) were carried out separately by *invitro* disc diffusion method and compared with standard antibacterial drug Streptomycin at 1mg/ml concentration.

Table 3: Antimicrobial activity results of L^I, L^{II} and their metal complexes (C1–C6) at 1mg/mL concentration along with respective standard drugs at the same concentrations.

	Bacterium(mm)				Fungi(mm)	
	Gram-positive bacteria		Gram-negative bacteria			
Compound	*Bacillus amyloliquefaciens*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Sclerotium rolfsii*	*Macrophomina phaseolina*
L^I	8 ± 0.2	8 ± 0.1	7 ± 0.2	9 ± 0.1	8 ± 0.1	9 ± 0.2
L^{II}	10 ± 0.2	9 ± 0.2	9 ± 0.2	11 ± 0.2	9 ± 0.2	10 ± 0.2
$[Co(L^I)_2(H2O)_2]$ (C1)	15 ± 0.3	16 ± 0.3	16 ± 0.2	15 ± 0.2	17 ± 0.2	15 ± 0.2
$[Ni(L^I)_2]$ (C2)	16 ± 0.2	18 ± 0.2	19 ± 0.3	19 ± 0.2	18 ± 0.3	18 ± 0.3
$[Cu(L^I)_2]$ (C3)	23 ± 0.4	21 ± 0.2	22 ± 0.1	23 ± 0.4	21 ± 0.2	22 ± 0.1
$[Co(L^{II})_2(H2O)_2]$ (C4)	18 ± 0.1	17 ± 0.1	18 ± 0.2	16 ± 0.3	19 ± 0.1	17 ± 0.2
$[Ni(L^{II})_2]$ C(5)	20 ± 0.3	21 ± 0.2	20 ± 0.3	21 ± 0.1	20 ± 0.2	19 ± 0.2
$[Cu(L^{II})_2]$ (C6)	24 ± 0.2	23 ± 0.3	25 ± 0.2	24 ± 0.2	23 ± 0.3	25 ± 0.4
Streptomycin	34 ± 0.3	32 ± 0.2	31 ± 0.3	32 ± 0.2	-	-
Mancozeb	-	-	-	-	32 ± 0.3	33 ± 0.2

(±) Standard deviation; Zone of inhibition (mm)

All compounds were screened against two Gram positive bacterial strains *Staphylococcus aureus (S. aureus), Bacillus amyloliquefaciens (B. amyloliquefaciens)* and two Gram negative bacteria strains *Pseudomonas aeruginosa* (*P. aeruginosa)*, *Escherichia coli (E. coli)*. From the antibacterial screening it is observed that the metal complexes displayed higher antibacterial activity than free ligands and the antibacterial data was presented in Table 3. Among all complexes, (C1), (C4) complexes showed low antifungal activity and (C3), (C6) complexes showed high antifungal activity whereas (C2) and (C5) complexes exhibited intermediate activity in comparison with cobalt (C1, C4) and copper complexes (C3, C6). Overtone's concept [36] was given the explanation regarding the enhancement of potential antibacterial activity of metal complexes against bacterial strains. Permeability nature is the key point of Overtone's concept and according to this concept, the lipid membrane around the cell favours the passage of only lipid soluble materials. The atomic radius and electronegativities of metal ions may affect the lipid

soluble properties of metal complexes and intern enhances the liposolubility nature of metal complexes. This makes potential entry of metal complexes in to the cells and destroyed aggressively. The liposolubility nature of metal complexes is a key factor and controls the antibacterial activity [37]. In this investigation, the results are concluded that, the (C3), (C6) complexes have shown greater antibacterial activity than other complexes, due to small atomic radius and high electronegativity of Cu(II) ion than Co(II) and Ni(II) ions.

Antifungal activity

In-vitro antifungal activity of synthesized Schiff base ligands (L^{I}, L^{II}) and their metal complexes (C1–C6) along with antifungal standard drug, Mancozeb (1 mg/ml) are screened separately against two fungal strains *Macrophomina phaseolina (M. phaseolina), Sclerotium rolfsii (S. rolfsii)* by disc diffusion method. Table. 3 displayed the antifungal activity data of all compounds along with standard drug. The data explores that metal complexes (1–6) are more active than Schiff base ligands (L^{I}, L^{II}) as compared to the Mancozeb. From the results, it is observed that the activity of metal complexes depends upon the type of metal ion present in the complex. Among all complexes, (C1), (C4) complexes showed less antifungal activity and (C3), (C6) complexes showed more antifungal activity whereas (C2) and (C5) complexes exhibits intermediate activity in comparison with cobalt (C1,C4) and copper complexes (C3,C6). In this investigation, the comparative less activity of cobalt and nickel complexes than copper complexes may due to their low polarity nature. The increased antifungal activity of metal complexes may due to the effect of metal ions on the regular cell process, which can be explained by Tweedy's chelation theory [38]. On establishment of chelation, the overlapping of ligand orbitals with metal ion takes place and leads to partial sharing of the positive charge of the metal ion to the donor groups which intern reduces the polarity of the metal ion to a greater extent. Further, it increases the delocalization of π-electron cloud over the chelate ring leads to reduction in polar nature of metal ions, which enhances the penetration tendency of the metal complexes through the lipid membrane and blocks the metal binding sites in enzymes of microorganisms' intern inhibits the protein bio-synthesis pathway by disturbing the respiration process and thus restricts the growth of microorganisms [39].

The biological activity of compounds also depends upon the other factors such as dipole moment, solubility, nature of ligand, type of metal ion, geometry of complexes, stereo chemistry, coordination sites, concentration of compounds may the reasons for the higher antimicrobial activity of metal complexes [40-47].

CONCLUSION

New Co(II), Ni(II) and Cu(II) complexes (C1–C6) were synthesized from L^{I}, L^{II} schiffbase ligands and all compounds were thoroughly characterized by various spectroscopic techniques. The characterized spectro-analytical techniques have been revealed that the structure of the all Cu(II) and Ni(II) complexes are square planar, whereas Co(II) complexes owned a octahedral geometry around the metal ion. The metal complexes are employed to further analysis of their biological properties such as DNA binding interactions via electronic absorption studies, fluorescence quenching studies, antioxidant activity, antibacterial and antifungal studies. The DNA binding studies revealed that the intercalative binding mode is exist in between metal complexes and CT-DNA. From the results of antioxidant activity, the Cu(II) complexes shows moderately high antioxidant activity than Co(II) and Ni(II) complexes. Finally, the Schiffbase ligands and their metal complexes were screened *invitro* against bacterial and fungal strains. These studies reveale that the metal complexes are comparitively more effective than free schiffbase ligands in antibacterial and antifungal activities as compared to standard drugs, streptomycin and mancozeb respectively. Among these metal complexes Cu(II) complexes showed higher antimicrobial activity due to small atomic radius and high electronegativity of Cu(II) ion than Co(II) and Ni(II) ion. These *invitro* biological studies may explored the advanced posibilities to establish new bio-active metal complexes which may be pertain in the development of novel and potential antimicrobial drugs.

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