Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN: 0976-9595
Research Article
DOI: 10.55218/JASR.202213118

NOVEL Co(II) Ni(II) AND Cu(II) COMPLEXES OF TRIDENTATE ONS DONOR SCHIFF BASE LIGANDS: SYNTHESIS, CHARACTERIZATION, DNA CLEAVAGE, MOLECULAR DOCKING, ANTIOXIDANT AND ANTIMICROBIAL STUDIES

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ABSTRACT

A new Schiff base ligand 2-((2-furylmethylene)amino)benzenethiol of the type ONS has been synthesized from 2-aminothiophenol and 2-furfuraldehyde and complexed with Co(II), Ni(II) and Cu(II) metal ions. The synthesized ligand and complexes [Co(II),Ni(II) &Cu(II)] were characterized using various spectral techniques viz., IR, NMR, EI-MS and thermogravimetric analysis. The geometry of the synthesized complexes was confirmed by electronic spectra, magnetic moment measurements and EPR analysis. DNA cleavage studies were performed using Gel electrophoresis method. The antibacterial activity of all the metal complexes studied against Gram positive bacterial strains *Bacillus substilis*, *Staphylococcus aureus*, and also Gram negative bacteria *Escherichia coli, Pseudomonas fluorescens and Klebsiella pneumoniae* with comparison to Gentamycin. Antifungal activities were carried out against two fungal strains i.e. *Aspergillus Niger* and *Candida albicans*. Schiff base and its metal complexes were also screened for *in vitro* antioxidant activity and was observed that the Co(II) complex shows enhanced activity when compared to ligand and other metal complexes and was further substantiated by molecular docking studies.

Keywords: Schiff base, Metal complexes, DNA cleavage, Antioxidant DPPH assay, Docking.

1. INTRODUCTION

In the current scenario, the microbial infections are considered as the main cause of sickness and mortality and these are mainly targeted on immunosuppressed individuals, especially in patients subject to cancer chemotherapy or organ transplantation [1], diabetic patients [2]. Schiff bases have important position in metal coordination chemistry owing to their stability, chelation ability and biological applications. These ligands are in various classes of organic compounds due to which they are capable of forming stable complexes with transition as well as rare-earth metals in varying oxidation states. They can have multiple hapticity towards the metals, depending on the type of oxidation state of the central metal atom and the ligand structure [3].

In last few years, great deal of interest has been developed on Schiff base transition metal complexes containing oxygen, nitrogen and sulphur as donor atoms in the ligand backbone. Because, these complexes have wide range of catalysts for oxidation [4], reduction [5], hydrolysis [6], organic and inorganic transformations [7], these metal complexes have been developed to possess antibiotic, antiepileptic, antimicrobial, anticonvulsant, antioxidant, antiproliferative, etc., properties [8]. Earlier works have revealed that most of the drugs contain metal complexes rather than organic compounds [9]. According to cell biologists, DNA is considered to be the primary and main target molecule for most of the anticancer as well as antiviral treatments. Investigations on DNA interactions with small molecules are observed to be crucial for designing new kind of pharmaceutical drugs. Some of the metal complexes interacting with DNA could facilitate the breaking of DNA strands by suitable methods [10, 11]. These results gave us motivation to carry out this research work.

In this study, we reported the synthesis, spectroscopic characterization of the Schiff base from 2-aminothiophenol and 2-furfuraldehyde. The metal Schiff

base complexes of Co(II), Ni(II) and Cu(II) were synthesized and characterized by several spectroscopic techniques. The prepared Schiff base and metal complexes were tested for various biological properties.

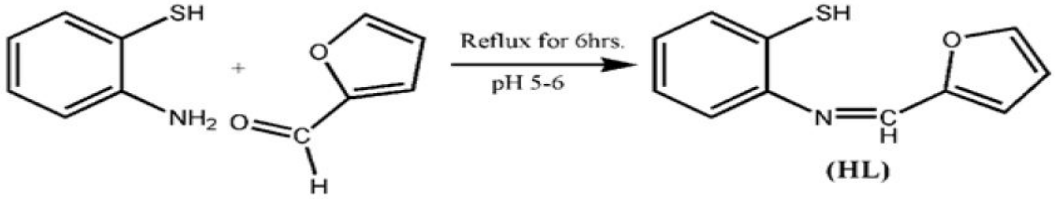
2. EXPERIMENTAL

2-aminothiophenol and 2-furfuraldehyde were purchased from Aldrich. The metal salts $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$ were purchased from Himedia. The solvents and buffer used were of analytical grade and hence no further purification was required. Inoculums for biological studies were purchased from 'The Microbial Type Culture Collection (MTCC), Chandigarh, India. Rose Bengal agar was used for the determination of susceptibility of fungal strains in antifungal agents which was purchased from Himedia.

The elemental analysis (C, H, N and S) data were analyzed using FLASH EA1112 model elemental analyzer. The metal contents present in the complexes were estimated as per the standard procedure [12]. The ^1H NMR spectrum was recorded on a Bruker Advance (III) HD Nanobay 400 MHz spectrometer using $CDCl_3$ solvent. The FT-IR spectra were recorded using Shimadzu model 8400S Spectrometer as KBr disks in the range of 400-4000 cm^{-1}. The electronic spectra were recorded from Shimadzu model UV-1700 spectrophotometer using DMSO as the solvent with the complex concentration of $1x10^{-3}$ M. Magnetic susceptibilities of all the complexes were obtained on a modified Hertz SG8-5HJ model gouy magnetic balance involving $CuSO_4.5H_2O$ as the calibrant. The molar conductivities of the complexes in DMF were measured using Elico model SX 80 conductivity-bridge. The EPR studies were carried out on ESR-JEOL model JES-FA200 spectrometer.

2.1. Synthesis of Schiff base ligand

A hot solution(60°C) of 2-aminothiophenol (2.50g, 10mmol) in 25mL methanol was mixed with a hot solution (60°C) of 2-furfuraldehyde (1.94g,10mmol) in the same solvent and then 3-4 drops of glacial acetic acid were added. The reaction mixture was refluxed for 6hours and then cooled to room temperature. The solid product formed was separated by filtration, purified by recrystallization from methanol, washed with diethyl ether and then dried in vacuum over anhydrous calcium chloride. The Schiff base 2-[(2-furylmethylene)amino] benzenethiol (scheme:1) was characterized by FT-IR , EI-MS and ^1H NMR spectroscopy.



(HL)

Yield:72%, m.p: 172°C, Anal. Found.(%): C, 65.00; H, 4.46; N, 6.89; S, 15.78; EI-MS: m/z, 203.1; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1610 cm^{-1}, $\nu_{(C-O-C)}$, 1257 cm^{-1}; ^1H NMR(δ, ppm in $CDCl_3$)8.799(C=N),1.56 (-SH)

Scheme 1: Schematic representation for the synthesis of ligand

2.2. Synthesis of metal complexes

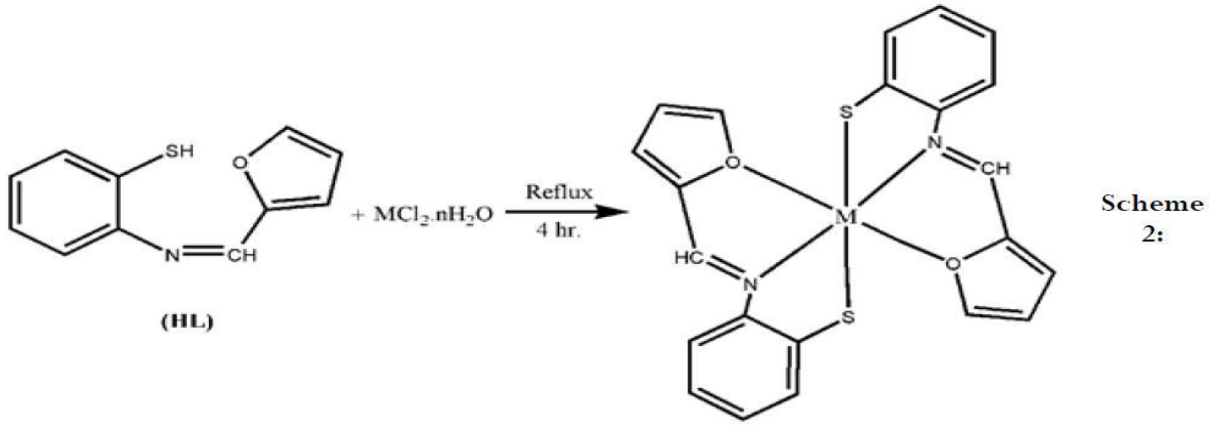
The Schiff base ligand (4 mmol; 0.80 g) was dissolved in 25 mL methanol. To this, 25 mL methanolic solution of 2mmol of the metal salt ($CoCl_2.6H_2O$-0.48g, $NiCl_2.6H_2O$-0.42g, $CuCl_2.2H_2O$- 0.34g) was added drop wise. Then the mixture was refluxed for 4 hours under stirring. The resulting solution was then slowly evaporated at room temperature whereupon the product obtained (scheme:2) was washed several times with methanol and dried in vacuum over anhydrous calcium chloride.

CoL_2: Yield: 52%; Brown color; Anal. Found (%): C, 41.65; H, 4.73; N, 4.44;S, 10.03; Co, 18.78; ESI-MS: m/z,465.2; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1586 cm^{-1}, $\nu_{(COC)}$, 1287 cm^{-1};$\nu_{(M-O)}$, 596; $\nu_{(M-N)}$, 540;$\nu_{(M-S)}$, 433; Λ_m ($Smol^{-1}cm^2$) 5.87; μ_{eff} (BM) 3.83; UV-Vis. in DMSO, nm (transition): 275 (LMCT).

NiL_2: Yield: 53%; Yellowish green color; Anal. Found (%): C, 57.15; H, 3.38; N, 6.25;S, 13.80; Ni, 12.71; ESI-MS: m/z, 464.20; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1554cm^{-1}, $\nu_{(COC)}$, 1296cm^{-1}; $\nu_{(M-O)}$, 568; $\nu_{(M-N)}$, 480;$\nu_{(M-S)}$, 434;

Λ_m($Smol^{-1}cm^2$) 3.94; μ_{eff} (BM) 2.93; UV-Vis. in DMSO, nm (transition): 260 (LMCT).

[CuL_2]: Yield: 42%; Black color; Anal. Found (%):C, 56.40; H, 3.50; N, 5.89;S, 13.50; Cu,13.51; ESI-MS: m/z,469.70; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1534 cm^{-1}, $\nu_{(COC)}$, 1298 cm^{-1} ; $\nu_{(M-O)}$, 578; $\nu_{(M-N)}$, 528; $\nu_{(M-S)}$, 434; Λ_m($Smol^{-1}cm^2$) 1.99; μ_{eff} (BM) 1.97; UV-Vis. in DMSO, nm (transition): 275 (LMCT).



Scheme 2:

Schematic representation for the synthesis of complexes

2.3. DNA cleavage studies

2.3.1. DNA isolation from bacteria

Escherichia coli cells were inoculated into LB broth and incubated at 37°C overnight. For DNA isolation, 2mL of the cultured bacterial strained was taken in an Eppendorf tube and the centrifuged at 4000 rpm for 10 minutes. After decanting the supernatant, the pellet was resuspended in 875 µL of TE buffer (pH 8.0) by gentle mixing. To this cell suspension, 100 µL of 10% SDS and 5µL of Proteinase K were added and mixed well. For the effective cell lysis, this mixture was kept at 37°C for an hour in an incubator. 1 mL of phenol-chloroform blend was added to the substance, blended well by upsetting and incubated at room temperature for 5 minutes. After incubation, the substance were centrifuged at 10,000 rpm for 10 minutes at 4°C and the supernatant was gathered utilizing slice tips and was moved to a new tube. Wide bore or slice tips are utilized to limit the DNA shearing while seclusion. To the supernatant, 100µL of 5M sodium acetate was added and was mixed gently. The genomic DNA was precipitated using 1mL of ice cold isopropanol and mixed gently until white strands of genomic DNA precipitates out. The tube was centrifuged at 14,000 rpm for 10 minutes, the supernatant was decanted, and DNA pellet was washed with 1mL of 70% ethanol. After washing, the DNA pellet was again centrifuged at 14,000 rpm for 10 minutes and supernatant was decanted. The DNA pellet was then air-dried for 5 minutes at room temperature and 200µL of TE buffer or Nuclease free water was added to redissolve the pellet. The quality and quantity of the isolated DNA was analyzed by agarose gel electrophoresis and UV spectroscopy [13-15]. The isolated genomic DNA was used for DNA cleavage studies.

2.3.2. DNA cleavage study

The DNA cleavage activity of test samples was studied using gel electrophoresis method [16]. The DNA stock solution was prepared in nuclease free water. This stock solution was diluted in 0.1M sodium phosphate buffer (pH-7.4). The DNA (1.5 µg) was treated with various concentrations (25, 50 and 100µg) of the test samples in a reaction volume of 15µl and incubated at 37°C for 2hours. After incubation, the treated DNA as well as control DNA were subjected to agarose gel electrophoresis in 1% gel in 0.5 % TAE buffer for 30 minutes.

2.4. Antioxidant activity

Radical scavenging activity of the Schiff base and its metal complexes against stable 2,2- diphenyl 2-picrylhydrazyl hydrate (DPPH) were determined according to Brand-William method [17] with slight modification. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in color (from deep violet to light yellow) was measured at the optical density 515 nm on a UV visible spectrophotometer.

For DPPH assay, the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1 mg/mL; w/v). A 60µM solution of

DPPH in methanol was freshly prepared and a 3.9mL of this solution was mixed with 100µl of test sample at various concentrations (6.25, 12.5, 25, 50, 100 µg/mL). The tubes were kept in the dark for 15 minutes at room temperature while the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank. Also, IC_{50} values for the synthesized derivatives were calculated and tabulated (Table 1).

Radical scavenging activity was calculated by the following formula.

Percentage inhibition = {(Absorbance of control at 0 min - Absorbance of test)/ Absorbance of control at 15 min} X 100

2.5. Antimicrobial studies

2.5.1. Antibacterial assay

Agar well diffusion method was utilized to assess the antimicrobial action of the metal complexes [18]. 15-20 mL of Mueller-Hinton agar was poured on glass petri plates of same size and permitted to harden. Normalized inoculum of the test organism was consistently spread on the outside of the plates using sterile cotton swab. Four wells each of diameter 9 mm (20 mm apart from one another) were punched with a sterile cork borer. The test sample (40 and 80 µL) was added into the wells T1 & T2 from 100mg/mL stock. In the positive well (+) Gentamycin was added (40µL from 4mg/mL stock) and in the negative well (-) the solvent used for the sample dilution was added. The plates were incubated for 24 hours at 36°C under aerobic conditions. After incubation, the plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm.

2.5.2. Antifungal assay

The antifungal activity of the metal complexes was also evaluated by Agar well diffusion method. The sterilized 15- 20mL of Rose Bengal agar was poured on glass petri plates of same size and allowed to solidify. After the solidification, the wells (4 wells/ plate) made with a sterile cork borer of diameter 8mm (20mm apart from one another) were punched aseptically in each plate. The standardized inoculums of test organisms were uniformly spread on the surface of these solidified media using sterile cotton swab. The test volumes (40µL &80µL) of the sample at desired concentrations were added to the first two wells; one well with 20mg of Clotrimazole as positive control and other one with DMSO as negative control. At that point, the agar plates were incubated under appropriate conditions relying on the test microorganism. After incubation, clear zone was noticed. Restraint of the contagious development was measured in mm.

2.6. Molecular docking study

The two-dimensional (2D) structures of the compounds were drawn using ChemDrawUltra and were converted to .pdb format using Chem3D Ultra. The X-ray crystal structure of protein (PDB ID: 3MNG) [19] was retrieved from the RCSB protein data bank (http://www.rcsb.org/pdb) for molecular docking study. Protein and grid preparation were made using the AutoDock Tool 4.2 [20]. Hydrogen atoms were added to the protein molecule. Gasteiger partial atomic charges were added to the optimized complexes and protein. Molecular docking study of complexes was performed with the AutoDockVina [21]. The protein was loaded into AutoDockTool 4.2, creating a PDBQT file that contains a structure with hydrogens in all polar residues. The best conformation was picked with the lowest docked energy after the docking search was finished. Ten runs were performed in all cases per each ligand structure, and for each run the best pose was saved. The interactions of complex with protein, including hydrogen bonds and hydrophobic interactions, were analyzed using Discovery studio 4.0 client [22] and PyMol [23].

3. RESULTS AND DISCUSSION

The Schiff base ligand **L** is synthesized by the condensation of 2-aminophenol and 2-furfuraldehyde and it is complexed with Co(II), Ni(II) and Cu(II) metal ions. The ligand is stable in air and soluble in common organic solvents like chloroform, ethanol, and methanol. The synthesized complexes are stable in air and soluble in DMF and DMSO etc., but insoluble in common organic solvents. Several attempts taken in obtaining a single crystal suitable for X-ray crystallography were failed. However, the analytical, spectroscopic and magnetic data assist us to predict the probable structure of the synthesized complexes. All the synthesized compounds give satisfactory elementary analytical results.

3.1. Vibrational spectroscopy

The FT-IR spectra of ligand (L) and the respective metal complexes (CoL, NiL and CuL) are depicted in Fig.1.

The IRspectrum of the ligand shows an intense peak at 1610 cm^{-1} which correspond to azomethine group. The medium to sharp peak at 1295cm^{-1} was due to ν(C-O-C) stretching vibration of furan in the ligand. This band was shifted to 1287-1298cm^{-1} in metal complexes indicating the possible coordination of the azomethine nitrogen (M-N). The participation of the SH group in chelation was ascertained from the shift of the ν_{asym}(CS) and ν_{sym}(CS) from 717 and 752 $cm^{-1}$to lower or higher wave numbers in the spectra of the complexes [24]. New bands were found in the complexes in the regions 578-596 (furan O), which are assigned to ν(M-O) stretching vibrations for the complexes. The bands at 480-540 have been assigned to ν(M-N) mode in metal complexes. The ν(M-S) bands appeared at 433-434 for metal complexes [25]. Therefore, from the IR spectra, it can be concluded that the ligand behaves to beuni-negatively tritentate coordinated to the metal ions *via*., azomethine N, furan O, thiophenol S.

3.2. ^{1}H-NMRspectrum

The ^{1}H-NMR spectrum of the ligand L (Fig.2) shows a signal at δ 8.795(m, 1H) which is assigned to azomethine proton. The signals in the range δ 7.202-7.541(m, 3H) are due to aromatic protons and the signals in the range δ 6.872-7.184 (m, 3H) is assigned to the furan hyrogens. The signal at δ 1.56(s, 1H) is assigned to thiophenol hydrogen.

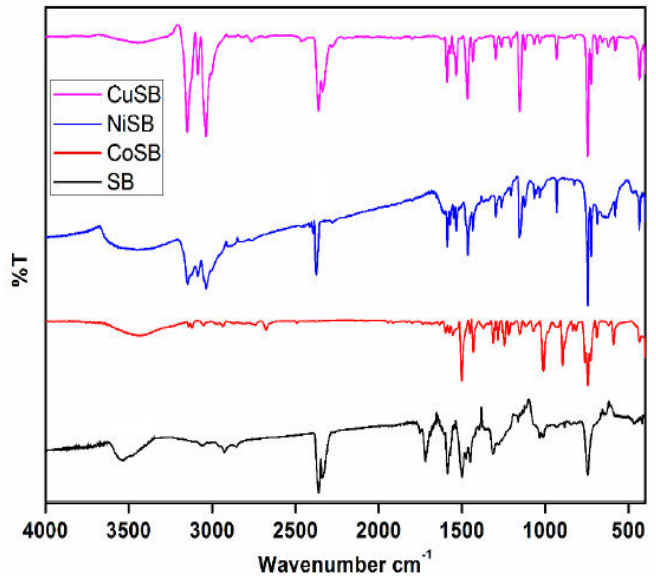


Fig. 1: IR spectra of HL and metal complexes

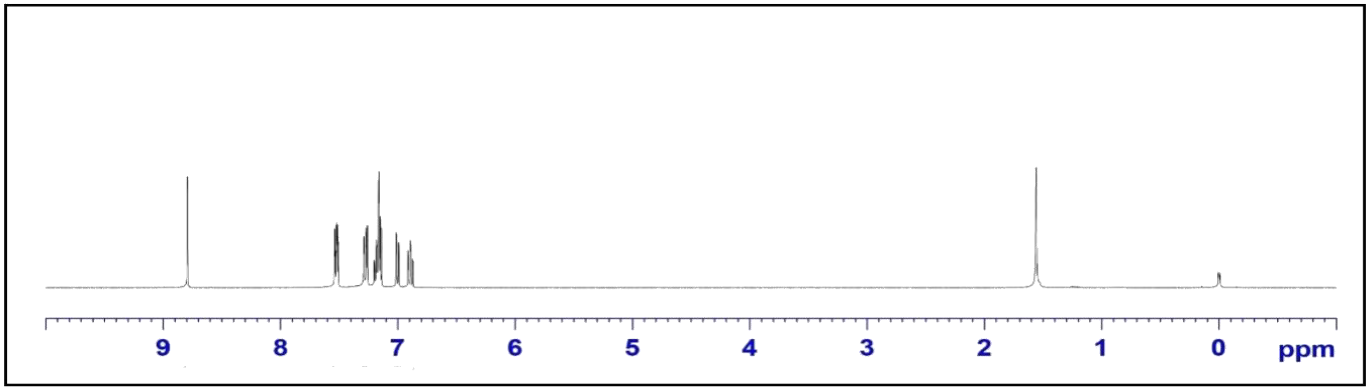


Fig. 2: ^{1}H NMR spectra of Schiff base Ligand

3.3. Mass spectra

The EI mass spectrum (Fig.3) of ligand shows the molecular ion (M^+) peak at m/z = 202.1 corresponding to the molecular weight of the ligand having the molecular formula $C_{11}H_9ONS$. The ion of m/z = 202.1 undergoes deprotanation to give peak at m/z = 201.10 . The ion m/z = 201.1 undergoes fragmentation to produce a peak at m/z = 173.10 corresponding to the formula $C_{10}H_{10}NS$ which is formed by losing the methanol moiety and it further undergoes deprotonation to give the ion m/z = 172.10 with the molecular formula $C_{10}H_9NS$. The fragmentation from m/z = 172.10 leads to a peak at m/z = 146.10 with formula C_8H_5NS which is formed by losing one molecule of ethylene unit. The peak at m/z = 128 is obtained from the fragmentation of m/z = 146.10 corresponds to the formula C_7H_4NS is due to the removal of one methylene group.

In addition, ESI-MS studies of the complexes were performed. The molecular ion peak in the spectra of the complexes CoL, NiL and CuL are in expected consistency with the molecular weight and also the

various other peaks are reliable with the different fragments of the complexes. The ESI-mass spectrum of the complex CoL displays the molecular ion peak at m/z = 463.85 which is exactly in accordance with the molecular weight of the complex.The complex NiL (Fig.4), a molecular ion peak at m/z = 465.30 is in correct coherence with its molecular weight. The complex CuLa molecular ion peak at m/z = 469.23 which is matched perfectly with its molecular weight. Thus, mass spectral analysis substantiates well with the proposed chemical structure of the complexes CoL, NiL and CuL.

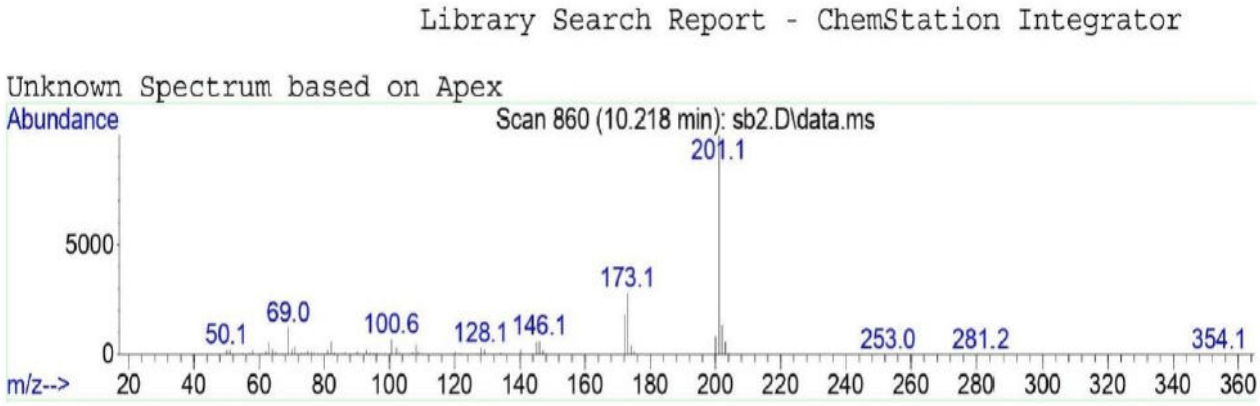


Fig. 3: Mass spectra of Ligand

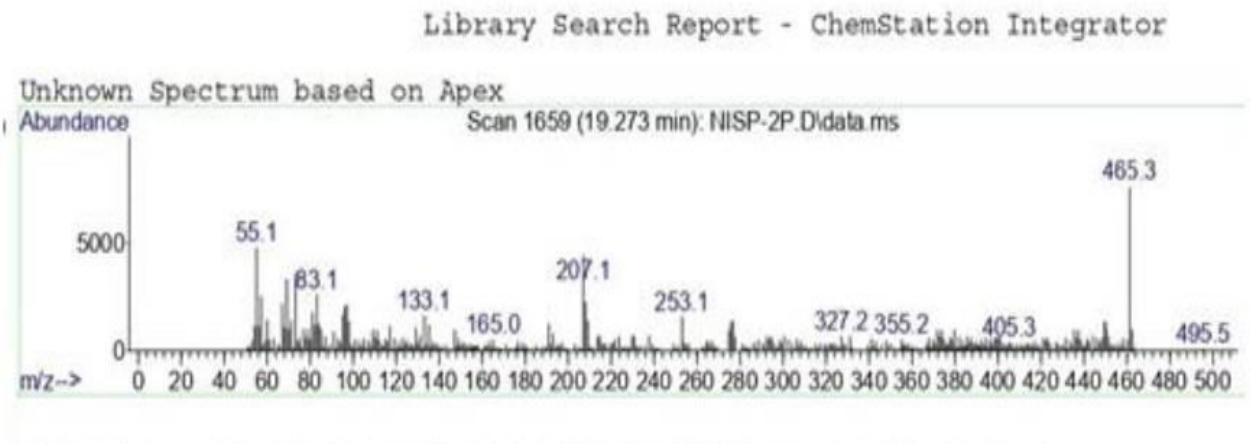


Fig. 4: Mass spectra of NiL

3.4. Electronic spectra, molar conductance and magnetic measurements

The electronic spectra of the complexes are given in Fig.5. The UV-Vis spectrum of CoL exhibits ligand field absorption at 340 nm which is consistent with the π- π* transition of the ligand. The ligand-metal charge transfer band is noticed at 277 nm. The electronic spectrum of NiL complex exhibits ligand field absorption at 320nm which is consistent with the π- π* transition of the ligand while the ligand-metal charge transfer band is observed at 230 nm. The electronic spectrum of CuL complex exhibits ligand field absorption at 310 nm which is consistent with the π- π* transition of the ligand whereas ligand to metal charge transfer band is

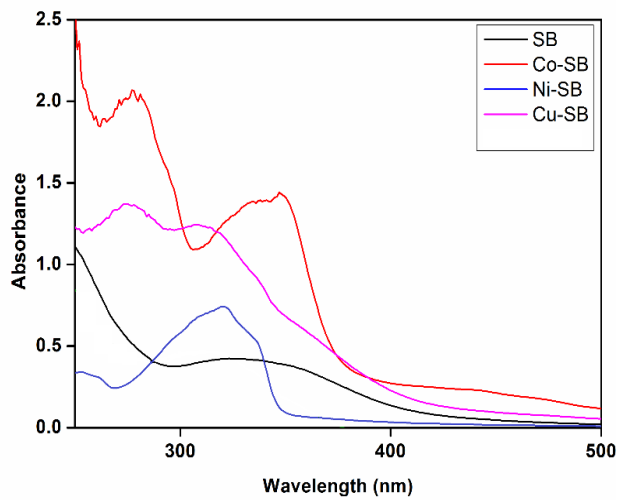


Fig. 5: UV spectra of ligand and complexes

observed at 275 nm. The magnetic moment value of 3.83 BM [26] for CoL complex indicate that the complex is in high spin environment.The molar conductance values of CoL, NiL and CuL are 5.87, 3.94 and 1.99which suggests the non-electrolytic nature of the complexes [27].

3.5. EPR Spectral Studies

The liquid nitrogen temperature (LNT) X-band EPR spectrum of the macrocyclic Cu(II) complex is appeared in Fig.6.

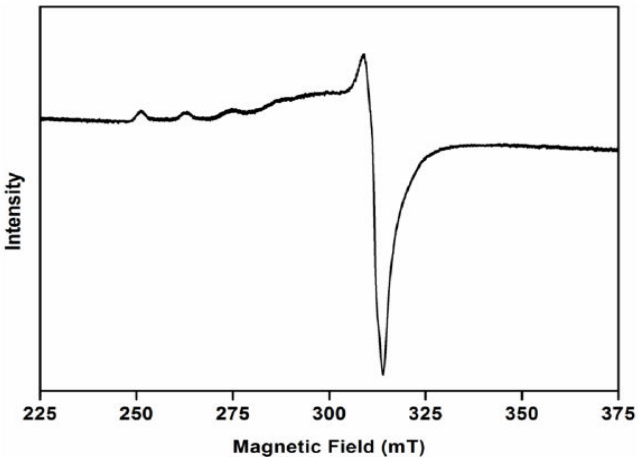


Fig. 6: EPR spectrum of Cu(II) complex

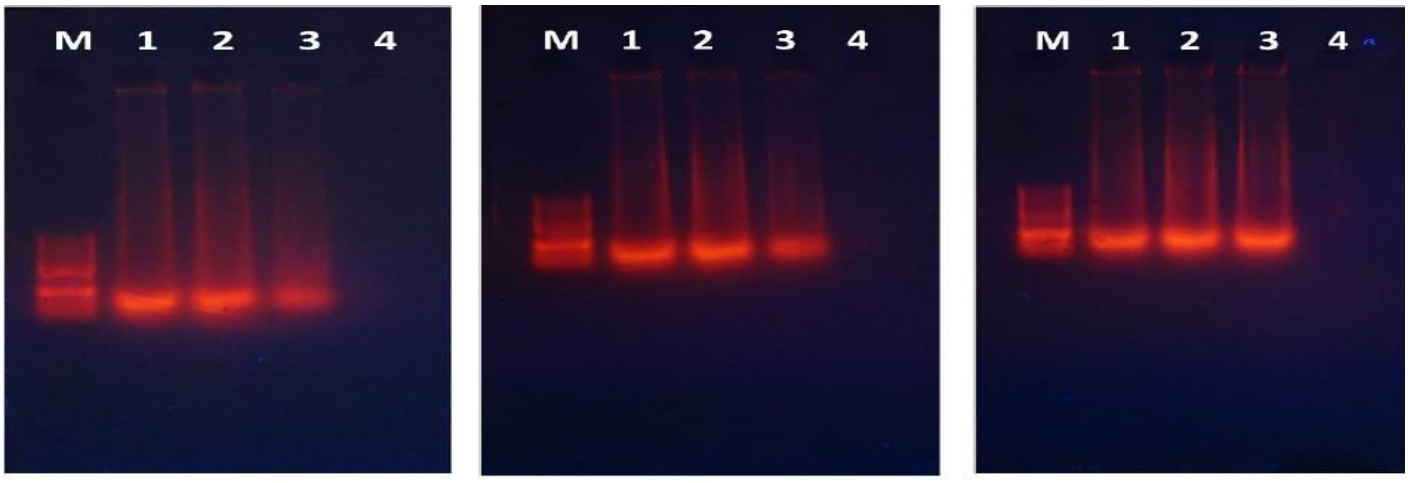
The hyperfine structure noticed relates to ONS coordination mode in octahedral complex. The $g_{ll}$and $g_{\perp}$are calculated to be 2.32 and 2.01 respectively. The g_{av}value can be calculated by the following equation.

$$g_{av}^2 = \frac{1}{3}[2g_{\perp}^2 + g_{ll}^2]$$

The g_{av}value is found to be 2.22 which is a characteristic of octahedral geometry with rhombic distortion.

3.6. DNA cleavage Activity

The metal complexes were studied for their DNA cleavage activity at three different concentrations (25µg, 50µg, 100µg) by gel electrophoresis method against DNA of *E.coli* are shown in Fig.7. The recognition of DNA by metal complexes has been supported by the cleavage of DNA which is related with the redox-active or photoactivated metal complexes [28]. The electrophoretic analysis clearly showed that the metal complexes have acted with DNA since a difference was noticed in molecular weight of the control and the treated DNA samples. This difference in band of lanes of complexes was observed compared to that of control DNA of *E.coli* which are due to the relaxation of circular DNA into its linear form. This shows that the control DNA alone doesn't show any cleavage whereas the metal complexes do show. At lower concentration (50µg), Co and Ni complexes show partial cleavage but Cu complex doesn't show any cleavage. Whereas at higher concentration (100µg), Co, Ni and Cu complexes show complete cleavage of DNA of *E. coli*. The compound at 100µg concentration could be observed to cleave DNA and hence can be concluded that the compounds inhibited the growth of pathogenic *E.coli* bacteria.



(a) M- DNA, ladder 1 - Control- Untreated DNA (E coli Genomic DNA), 2 - 25µg CoL + E coli Genomic DNA, 3 - 50µg CoL + E coli Genomic DNA, 4 - 100µg CoL + E coli Genomic DNA, (b) M- DNA, ladder 1 - Control- Untreated DNA (E coli Genomic DNA), 2 - 25µg NiL + E coli Genomic DNA, 3 - 50µg NiL + E coli Genomic DNA, 4 - 100µg NiL + E coli Genomic DNA and (c) (a) M- DNA, ladder 1 - Control- Untreated DNA (E coli Genomic DNA), 2 - 25µg CuL + E coli Genomic DNA, 3 - 50µg CuL + E coli Genomic DNA, 4 - 100µg CuL + E coli Genomic DNA.

Fig. 7: DNA cleavage activity of CoL, NiL&CuL

3.7. Antioxidant studies

Most of the investigations are considered as DPPH method to evaluate the antioxidant activity of their targets due to its procedure is simple and reliable. The results for DPPH radical scavenging activity of the synthesised compounds are provided in Table 1. The IC_{50} value for each compound was calculated by using scatter graph and linear regression. The IC_{50} value of the complex is inversely proportional to their antioxidant activity i.e. Lower IC_{50} value reflects better DPPH radical scavenging activity. From the table, it was clearly observed that, the complexes show better antioxidant activity than the ligand. A considerable increase in the percent of scavenging activity is found with increase in concentration of the compounds. It was important to note that Co complex show better radical scavenging activity than the other complexes.

3.8. Antimicrobial studies

3.8.1. Antibacterial activity

The metal complexes reported here was evaluated for antibacterial activity and tabulated in Table 2. The values of zone of inhibition were measured in millimetre. The inhibitory zone data reveals that metal complexes shows good antibacterial activity against Gram positive bacterial strains *Bacillus subtilis*, *Staphylococcus aureus* and against Gram negative bacterial strain *Klebsiella pneumoniae*. The metal complexes demonstrated wonderful antibacterial activity because of chelation of metal with Schiff base ligand synergistically expanding its impact [28-31]. Such expanded activity of the metal chelation complexes can be clarified based on Overtone's cell permeability concept and Tweedy's chelation theory [32]. The liposolubility plays major role on the antibacterial activity against Gram positive. On chelation, the polarity of the metal atom decreases by partial sharing of its positive charge with donor atoms (O, N and S) and possible π-electron delocalization over the ring. This increases the lipophilic nature of the metal chelate thereby favouring its permeation through the lipid layers of the membranes of the microorganism and thus blocks the metal binding sites on enzymes of microorganisms [33, 34]. It was clear that CuL complex shows better antibacterial activity among all of them.

Table 1: Antioxidant activity of Ligand and complexes

Concentration (µg/mL)	Percentage inhibition				
	Ascorbic acid (standard)	Ligand (L)	CoL	NiL	CuL
6.25	7.03	0	0	4.60	0
12.50	42.19	1.40	0	11.65	10.46
25	82.6	4.34	0.27	23.27	18.20
50	99.48	10.10	27.62	24.29	21.96
100	123.68	25.57	80.05	53.45	48.65
IC_{50}	**20.62**	**191.9**	**70.44**	**94.4**	**103.41**

Table 2: Antibacterial activity of CoL, NiL and CuL

Organism	Zone of inhibition(mm)						
	Standard	CoL		NiL		CuL	
	Gentamycin (80 µg)	400 (µg)	800 (µg)	400 (µg)	800 (µg)	400 (µg)	800 (µg)
Escherichia coli	25	-	-	-	-	-	11
Pseudomonas fluorescens	28	-	-	-	-	-	-
Bacillus substilis	26	11	12	15	17	14	17
Staphylococcus aureus	26	10	13	13	16	18	21
Klebsiella pneumoniae	26	-	12	13	16	12	15

3.8.2. Antifungal activity

The synthesized metal complexes were evaluated for *invitro* antifungal activity against fungal strains *Candida albicans* and *Aspergillus niger*. The complexes showed variable antifungal activities (Table 3) against the fungal strains. The experimental data indicates that all complexes show an appreciable activity against *Aspergillus niger*. Their activity increases generally with increase in concentration of the complexes. The Cu(II) complexes show better activity against *Aspergillus niger* than Co(II), Ni(II) complexes and standard clotrimazole. It show almost equal activity as clotrimazole against *Candida albicans* whereas the Co(II) and Ni(II) do not show any activity against *Candida albicans*.

3.9. Docking studies

Docking studies of the metal complexes were carried out on the protein structure. The docking poses of the metal complexes and the protein showed the hydrophobic and hydrogen bonding interactions. The docking site on the protein target was defined by establishing a grid box with the dimensions of X: 65 Y: 68 Z: 73 Å, with a grid spacing of 0.375 Å, entered on X: 52.84 Y:0.135 Z: 21.80 Å. Docking studies was carried out for the ligand, metal complexes and the standard ascorbic acid. The free ligand shows π-π interaction with the His88 with a distance of 5.54Å. It clears that there is hydrophobic interaction with the Pro53 and Val56. Also, it has one hydrogen bonding interaction with the Lys49.

The ligand shows the binding energy of -3.6kcal/mole. In the presence of Co complex it has higher binding energy of -7.7kcal/mole. It was tightly held with the protein through hydrogen and hydrophobic interaction with the short distance of 1.8Å. Next to Co, Ni complex shows moderate binding affinity of -6.4kcal/mole. It shows π-sulphur interaction with the Leu96. Hydrogen bonding interactions are found between Val67 and Pro100. Finally, Cu complex shows poor binding with the protein of binding energy (-3.4kcal/mole) which shows only one hydrophobic binding with the Pro53 amino acid residues. Docking of ascorbic acid is also carried out which shows higher binding affinity of -8.0kcal/mole. The docking results also showed that Co complex has higher binding with the protein. These results are comparable with the experimental one.

Table 3: Antifungal activity of CoL, NiL&CuL

Organism	Zone of inhibition(mm)						
	Standard Clotrimazole (200 µg)	CoL		NiL		CuL	
		400 (µg)	800 (µg)	400 (µg)	800 (µg)	400 (µg)	800 (µg)
Candida albicans	20	-	-	-	-	11	18
Aspergillus niger	19	-	11	-	12	15	25

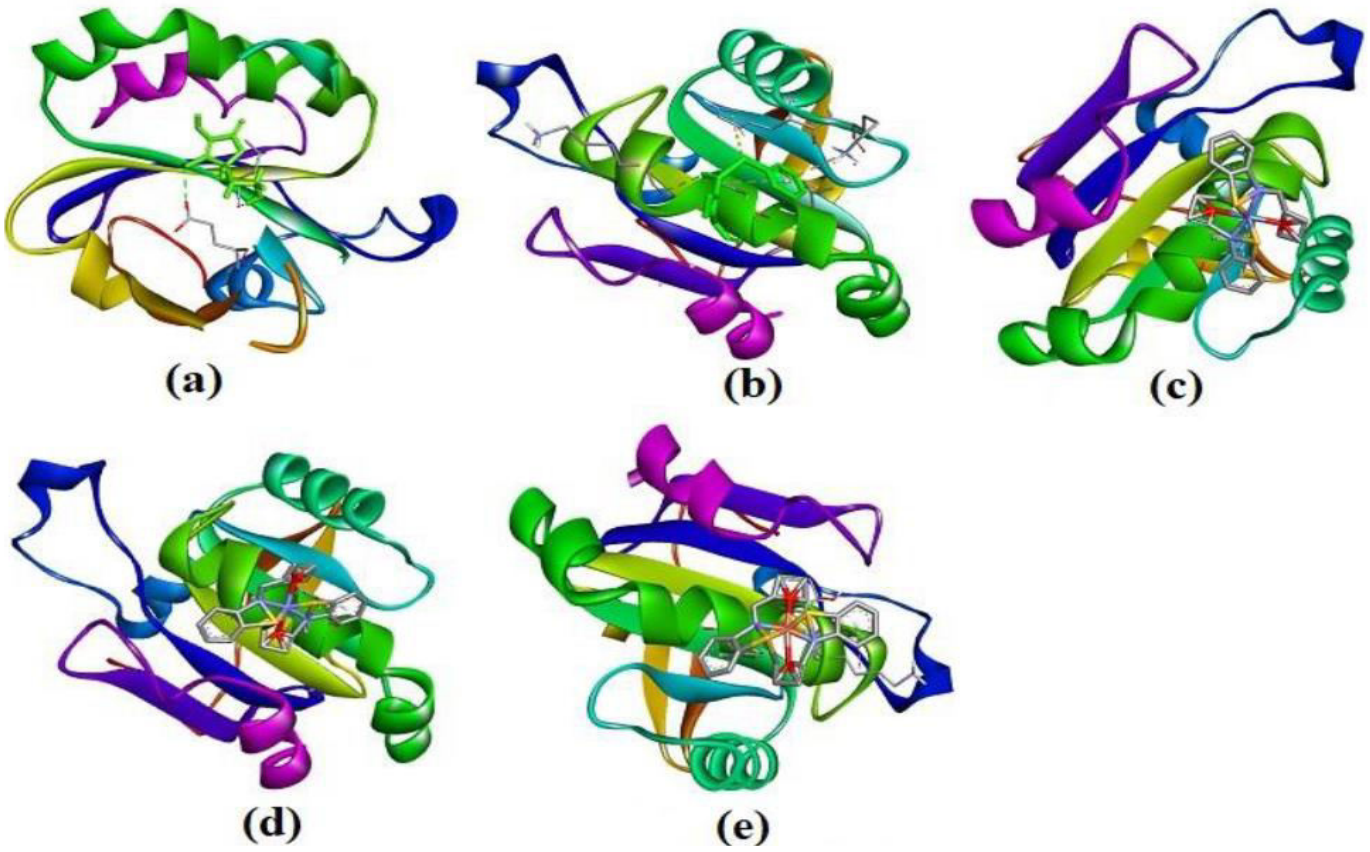


Fig. 8: Docked poses of (a) Standard ascorbic acid, (b) Ligand, (c) CoL, (d) NiL and (e) CuL

4. CONCLUSION

On the basis of the above results, the Schiff base ligand acts as tridentate (ONS) chelating agent coordinated with metal ions through oxygen atom of furfural moiety, azomethine nitrogen and Sulphur atom of the thiophenol *via* deprotonation. Analytical, spectral and magnetic studies revealed the mononuclear nature of the all the complexes. The Co(II), Ni(II) and Cu(II) complexes displayed octahedral geometry. The DNA cleavage studies revealed that all the three complexes have demonstrated complete cleavage of genomic DNA of *E. coli*. The results obtained from antioxidant activity clearly indicate that Co(II) complex exhibits good scavenging activity. The antimicrobial activity of Schiff base is upgraded upon complexation with metal ions particularly Cu(II) indicated promising activity contrasted with standard and end up being essential for the growth-inhibitor effect. Binding mode analysis has indicated a reasonable connection between experimental study and estimated energy of binding.

Conflict of interest

None declared

5. REFERENCES

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