

Synthesis and Characterization of 4-Amino Antipyrine Based Schiff Base Complexes: Antimicrobial, Cytotoxicity and DNA Cleavage Studies

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Abstract— A series of novel Cu(II), Co(II), Ni(II) and Mn(II) complexes were synthesized from the Schiff base obtained by the condensation of 4-aminoantipyrine with furfuraldehyde and dl-cysteine. Their structural features and other properties were explored from the analytical and spectral methods. The measured low molar conductance values in DMF indicate that the complexes are non-electrolytes. The stoichiometry of the obtained complexes and the bonding modes of the ligand were found to depend on both nature of metal and ligand. The grain size of the complexes was calculated by Scherer's formula using powder XRD. The bio-efficacy of the ligand and their complexes has been examined against the growth of bacteria and fungi in vitro to evaluate their anti-microbial potential. The Schiff base and their metal complexes were evaluated using neuroblastoma SH-SY5Y cell lines. DNA-cleavage studies with CT-DNA have shown that more obvious activity against both Schiff base and its metal complexes in the presence of H₂O₂.

Keywords—Antimicrobial, 4-aminoantipyrine, cytotoxicity, dl-cysteine, DNA Cleavage, Schiff base.

INTRODUCTION

Coordination of an organic compound with metal, i.e., chelation causes a harsh change in the biological assets of the ligand and also their metal moiety (Sorenson, 1987; Macias et al., 2003; Chandra and Gupta, 2005). Biological actions of transition metal complexes derived from Schiff base ligand were one of the most exhaustively studied topics in coordination chemistry, due to their superior activities compared to non-Schiff base complexes [4-7]. The coordination chemistry of Schiff base complexes involving oxygen and nitrogen donor ligands has attracted considerable attention from the biochemists owing to their applications in catalysis and their relevance to bioinorganic systems (Shanker et al., 2009). There has been an enormous effort directed towards the development of novel chemical compounds able to arrest or reverse the development of cancer [9,10]. It has been reported that chelation was the cause and cure of many diseases including cancer. In recent

years, a great deal of interest in the synthesis and characterization of transition metal complexes of 4-aminoantipyrine derivatives has been extensively examined due to their wide applications in various fields corresponding biological, analytical and therapeutical. In addition, they have been probed due to their diverse biological possessions as antifungal, antibacterial, analgesic, sedative, antipyretic, anti-inflammatory agents (Chandra et al., 2009), anti-tumor and greater DNA binding ability (Raman et al., 2010). Schiff base metal complexes of amino acids have gained significance not only from the inorganic point of view, yet also because of their physiological and pharmacological activities [13,14]. On account of the importance of 4-aminoantipyrine Schiff base complexes, in this work we explore the synthesis of novel Schiff base metal complexes derived from 4-aminoantipyrine, furfuraldehyde, dl-cysteine and its metal complexes. They were characterized using analytical and spectral techniques. Furthermore, their biological studies like antimicrobial, cytotoxicity and DNA cleavage responses have been performed.

Experimental protocols

1. Reagents and Instruments

For the preparation of the Schiff base metal complexes, we were used analytical grade chemicals and were used without further purification. All reagents, 4-aminoantipyrine, furfuraldehyde, dl-cysteine and metal (II) chlorides were of Aldrich and Merck products. Commercial solvents were distilled and then used for the preparation of ligands and their complexes. Microanalyses (C, H and N) were performed using the Perkin Elmer elemental analyzer. Molar conductance measurements of the complexes (10⁻³ M) in DMF were carried out by using ELICO CM 185 conductivity Bridge. Infrared spectra were measured using KBr discs on a Jasco FT-IR-410 spectrometer in the range 4000-400cm⁻¹. The absorption spectra were recorded by using T80+double beam spectrophotometer at room temperature.

The ^1H NMR spectrum was recorded using a DMF- d_6 on a BRUKER ADVANCED III 400 MHz instrument. Cyclic voltammetric measurements were performed using electrochemical analyzer CH instruments electrochemical work station (Model 680) using a tetrabutylammonium perchlorate as a supporting electrolyte. A mass spectrum was recorded on Agilent6330 Ion Trap mass spectrometer. X-ray powder patterns were obtained with a Rigaku Miniflex II diffractometer.

2. Synthesis of Schiff base (L)

A methanolic solution of 4-aminoantipyrine (0.20 g, 1mmol) was added to a methanolic solution of furfuraldehyde (0.82 g, 1mmol) and amino acid [dl-cysteine) (0.121 g, 1mmol)] which was dissolved in alcoholic potash. The resultant mixture was refluxed for 4 h. The orange color solid product formed was filtered and recrystallized from ethanol.

L. Yield: 80%. Anal. Calc. for $\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_3\text{S}$: C, 59.30; H, 5.24; N, 14.57; O, 12.49; S, 8.33(%); Found: C, 59.30; H, 5.20; N, 14.57; O, 12.48; S, 8.34(%). IR (KBr pellet, cm^{-1}): 1483 ν (C=O); 1645 ν (HC=N); 2925 ν (S-H); 3431 ν (OH); ^1NMR (δ): (furfurylidine Moiety) 6.6–7.8 (m); (-CH₃) 2.4–2.5(s); (N-CH₃) 3.03–3.07 (s); (CH=N) 9.42 (s); (-SH) 1.2(s).

3. Synthesis of metal complexes

The Schiff base metal(II) complexes were prepared by mixing the appropriate molar quantity of ligand and metal salt using the following procedure. A solution of metal(II) chloride in methanol (1 mmol) was mixed with a methanolic solution of the Schiff base (2 mmol), and the resultant mixture was refluxed for 3 h with constant stirring. The solid complexes was filtered off and washed systematically with ethanol and then dried in slow evaporation. Schematic diagram for the synthesis of ligand and metal complexes was given in Figure 1.

(CuL) Yield: 80%. Anal. Calc. for $\text{C}_{38}\text{H}_{38}\text{CuN}_8\text{O}_6\text{S}_2$: C, 54.96; H, 4.61; N, 13.49; O, 7.65; S, 7.71; Cu, 7.66 (%); Found: C, 54.91; H, 4.57; N, 13.48; O, 7.62; S, 7.70; Cu, 7.65(%). IR (KBr pellet, cm^{-1}): 1493 ν (C=O); 1632 ν (HC=N); 2963 ν (S-H); 528 ν (M-O); 466 ν (M-N); μ_{eff} (BM): 1.93.

(CoL) Yield: 78%. Anal. Calc. for $\text{C}_{38}\text{H}_{38}\text{CoN}_8\text{O}_6\text{S}_2$: C, 55.27; H, 4.64; N, 13.57; O, 11.63; S, 7.75; Co, 7.14; Found: C, 55.21; H, 4.60; N, 13.05; O, 11.62; S, 7.74; Co, 7.15; IR (KBr pellet, cm^{-1}): 1485 ν (C=O); 1644 ν (HC=N); 2962 ν (S-H); 556 ν (M-O); 504 ν (M-N); μ_{eff} (BM): 4.74.

(NiL) Yield: 80%. Anal. Calc. for $\text{C}_{38}\text{H}_{38}\text{NiN}_8\text{O}_6\text{S}_2$: C, 55.28; H, 4.64; N, 13.58; O, 11.63; S, 7.77; Ni, 7.11; Found: C, 55.23; H, 4.60; N, 13.56; O, 11.62; S, 7.77; Ni, 7.10; IR (KBr pellet, cm^{-1}): 1488 ν (C=O); 1627 ν (HC=N); 2964 ν (S-H); 543 ν (M-O); 471 ν (M-N); μ_{eff} (BM): 3.17.

(MnL) Yield: 80%. Anal. Calc. for $\text{C}_{38}\text{H}_{38}\text{MnN}_8\text{O}_6\text{S}_2$: C, 55.54; H, 4.66; N, 13.63; O, 11.68; S, 7.80; Mn, 6.66; Found: C, 55.48; H, 4.62; N, 13.62; O, 11.68; S, 7.80; Mn, 6.69; IR (KBr pellet, cm^{-1}): 1486 ν (C=O); 1630 ν (HC=N); 2958 ν (S-H); 538 ν (M-O); 495 ν (M-N); μ_{eff} (BM): 5.78.

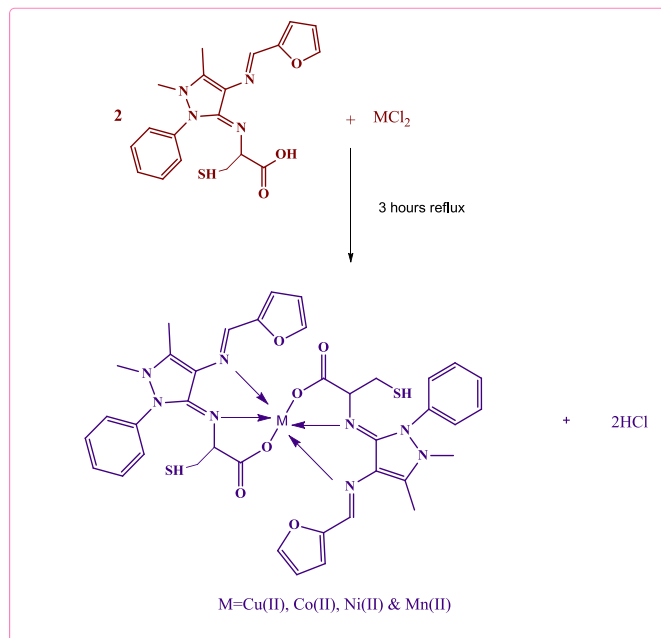


Figure 1. Synthetic route of Schiff base and its metal complexes

4. Antibacterial activity

In a typical procedure [15], the antibacterial activities of the compounds were evaluated by the disc diffusion method against the bacterial microorganisms. Newly synthesized Schiff base and its metal complexes were tested against *in vitro* antibacterial strains such as *S.aureus*, *Bacillus subtilis*, *K.pneumoniae* and *E.coli* were separately inoculated into nutrient broth and incubated at 37°C for 4–6 hours. Then broth culture of the test bacteria was seeded on Mueller-Hinton agar plates and spread evenly. Five wells of 6mm in diameter were cut on the seeded agar plates using a sterile cork borer. The different chemicals were poured into each well and the standard antibiotic disc (Ciprofloxacin) were placed on the center of the plate. The plates were incubated at 37°C for 24hrs after which the zones of inhibition round the wells resolute the antibacterial bustle of the extracts. Zone diameter was recorded as the differences between.

5 Anti-fungal activity

Fungicidal activity of testing compound was assessed against *Aspergillus Niger* and *Pencillium sp.* by cup-plate method[16]. The 5 mm diameter and 1 mm thickness of the disc was filled with the test solution using a micropipette and the plates were incubated at 37 °C for 72 h. During this period, the test solution was diffused and affected the growth of the vaccinated fungi. Later 36 h of incubation at 37 °C, the diameter of the inhibition was measured. Compounds screening auspicious antifungal activity were selected for minimum inhibitory concentration studies. The minimum inhibitory concentration was determined by assaying at concentrations of compounds along with standards at the same concentration. Minimum inhibitory concentration (MIC) was the lowermost concentration of an antimicrobial compound, inhibiting the visible growth of microorganisms

subsequent overnight incubation and are important in diagnostic laboratories endorsing resistance of microorganisms to antimicrobial agents and also to monitor the activity of new antimicrobial agents.

6. Cytotoxicity effect

The SH-SY5Y cells (Human neuroblastoma) were grown in DMEM-F12 medium supplemented with 10% FCS (fetal calf serum). Incubation of cells were routinely trypsinized and plated at $2 \times 10^5/5 \text{ cm}^2$ flasks. Schiff base and its Schiff base metal complexes were dissolved in DMSO at a concentration of 10, 25 and 50 μM and the solutions were kept at humidified atmosphere at -20°C until used. In generally 1% of the solvent (DMSO) was used in the cell viability assays. After 24 h of incubation, the cell viability was evaluated by using the Trypan Blue exclusion method.

a. DNA Cleavage studies

The DNA cleavage activity of the Schiff base and its metal complexes was examined by agarose gel electrophoresis method. pUC19 plasmid was educated, insulated and used as DNA for the experiment. Test compounds were prepared in DMF (1 mg/ml). Further 25 μg of the test samples was added to the insulated plasmid and incubated for 2 h at 37°C . Afterward incubation, 30 μl of plasmid DNA sample assorted with bromophenol blue dye (1:1) was overloaded into the electrophoresis chamber along with the control DNA, 5M FeSO_4 (treated with DNA) and standard DNA marker having TAE buffer (4.84 g Tris base, pH 8.0, 0.5 MEDTA/1 L). To end with, it was loaded onto an agarose gel and electrophoresed at 50 V constant voltage up to 30 min. Subsequently the gel was detached and stained with 10 $\mu\text{g/ml}$ ethidiumbromide and the image was taken in Versadoc (Biorad) imaging system. The fallouts were compared with standard DNA. Likewise the same system was followed in the presences of H_2O_2 .

2. RESULTS AND DISCUSSION

The Schiff base ligand and their Cu(II), Co(II), Ni(II) and Mn(II) complexes have been synthesized and characterized by spectral and elemental analytical data. They were found to be stable at room temperature. The ligand was soluble in common organic solvents and all the metal complexes were spontaneously soluble in DMF and DMSO.

1. Elemental analysis and molar conductivity measurements

The outcomes of elemental analysis for the metal complexes were in good agreement with the calculated values (Table1) showing that the complexes have 1:2 metal–ligand stoichiometry of the type ML_2 where in L acts as a tridentate ligand. The Schiff base metal(II) complexes were dissolved in DMF and the molar conductivities (10^{-3} M) of their solution at room temperature were measured. The lower conductance values (39–46 $\text{ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$) of the complexes support their non-electrolytic nature.

Co mpo und s	Molecular Formula	Calculated (found)%						Δm ($\Omega^1\text{c}$ m^2m ol^{-1})
		C	H	N	O	S	M	
Liga nd	$\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_3\text{S}$	59.3 (59.3)	5.24 (5.20)	14.57 (14.57)	12.49 (12.48)	8.33 (8.34)	-	-
Cu	$\text{C}_{38}\text{H}_{38}\text{CuN}_8\text{O}_6\text{S}_2$	54.96 (54.91)	4.61 (4.57)	13.49 (13.48)	7.65 (7.62)	7.71 (7.70)	7.66 (7.65)	42
Co	$\text{C}_{38}\text{H}_{38}\text{CoN}_8\text{O}_6\text{S}_2$	55.27 (55.21)	4.64 (4.60)	13.57 (13.05)	11.63 (11.62)	7.75 (7.74)	7.14 (7.15)	39
Ni	$\text{C}_{38}\text{H}_{38}\text{NiN}_8\text{O}_6\text{S}_2$	55.28 (55.23)	4.64 (4.60)	13.58 (13.56)	11.63 (11.62)	7.77 (7.77)	7.11 (7.10)	45
Mn	$\text{C}_{38}\text{H}_{38}\text{MnN}_8\text{O}_6\text{S}_2$	55.54 (55.48)	4.66 (4.62)	13.63 (13.62)	11.68 (11.68)	7.80 (7.80)	6.68 (6.69)	46

Table1. Analytical and Physical data of Schiff base ligand and their metal complexes.

2. Infrared Spectra

The coordination mode and sites of the ligand to the metal ions were investigated by comparing the infrared spectra of the free ligand with their metal complexes. The IR Spectra results were summarized in Table 2. The Schiff base ligand (L) show a band at 1645 cm^{-1} which was assigned to azomethine $\nu(\text{CH}=\text{N})$ linkage and the band were shifted towards lower frequencies in the spectra of their metal complexes ($1627\text{--}1644 \text{ cm}^{-1}$). The comparison of the IR spectra of the complexes with Schiff base indicates the involvement of the azomethine nitrogen in chelation with the metal ion [17]. The coordination of nitrogen to the metal ion could be expected to reduce the electron density of the azomethine link and thus causes a shift in the $\nu(\text{CH}=\text{N})$ group. The intensity peak of absorption in the Schiff base ligand around 3431 cm^{-1} due to the phenolic hydroxyl group of the Schiff base ligand and this peak of absorption disappears while chelation with metal ions. This confirms the coordination through phenolic $-\text{OH}$ and the central metal ion [18]. In addition, the ligand also exhibits a band at 2925 cm^{-1} due to two thionyl group ($-\text{SH}$) [19] originating from the amino acid which was shifted towards higher region at around $2958\text{--}2964 \text{ cm}^{-1}$ on complexation. The bands are observed at 1483 cm^{-1} for a free amino acid ligand ($-\text{C}=\text{O}$), for comparison, the display bands at $1486\text{--}1493 \text{ cm}^{-1}$, respectively for all the metal complexes. These results reveal that the carbonyl group of the amino acid ligand was involved in the complex formation. The coordination through the nitrogen of azomethine and oxygen of $\nu(\text{C}-\text{O})$ of ligand were further evidenced by the appearance of non-ligand bands around $466\text{--}504 \text{ cm}^{-1}$ and $528\text{--}556 \text{ cm}^{-1}$ were due to M-N and M-O bonds respectively [20,21].

Compo unds	-OH (v, cm ⁻¹)	-SH (v, cm ⁻¹)	C=N (v, cm ⁻¹)	C=O (v, cm ⁻¹)	M-O (v, cm ⁻¹)	M-N (v, cm ⁻¹)
Ligand	3431	2925	1645	1483	-	-
Cu-comple x	-	2963	1632	1493	528	466
Co-comple x	-	2962	1644	1485	556	504
Ni-comple x	-	2964	1627	1488	543	471
Mn-comple x	-	2958	1630	1486	538	495

Table 2. IR spectra of ligand and metal complexes

3. Electronic spectra

The electronic spectra of the Schiff base ligand and its metal complexes were recorded in DMF solution at room temperature and values are shown in Table 3. The band appears at 327 nm were attributed to n-π* transitions which are associated with the azomethine chromophore, which was shifted to longer wavelength (329-334 nm) for metal complexes [22,23]. All the complexes show the energy absorption bands in the region from 278-289 nm compare with ligand (263nm, which was confirmed by complexation (Figure 2). The electronic spectra of copper complex display the d-d transition bands in the region 794 nm, which were due to 2E_g → 2T_{2g} (F) transition. This d-d transition band strongly favors a distorted octahedral geometry around the metal ion [24]. Electronic spectra of cobalt complex display the d-d transition bands in the region 635 and 703 nm, which were assigned to the 4T_{1g} (F) → 4A_{2g} (F) and 4T_{1g} (F) → 4T_{2g}(F) transitions, respectively. These transitions correspond to the octahedral geometry and further supported by their magnetic susceptibility values (4.74 BM). The electronic spectra of Mn(II) complex display the absorption bands in the range 683, 552 and 528 nm. These absorption bands may be assigned to the 6A_{1g} → 4A_{1g}, 6A_{1g} → 4A_{2g} and 6A_{1g} → 4E_g, 4A_{1g} transitions, respectively. These bands suggest that the complexes possess an octahedral geometry. The absorption spectra of nickel complex display two d-d bands in the region of 712 and 406 nm respectively. This corresponds to 3A_{2g} (F) → 3T_{1g} (F) and 3A_{2g} (F) → 3T_{1g} (P) transitions, respectively, and existence the characteristic of an octahedral geometry. These were further supported by their magnetic susceptibility values 3.17 BM.

Compounds	π -π* (nm)	n-π* (nm)	L → M (nm)	d-d (nm)	Magnetic moment value(μ _{eff}) BM
Ligand	263	327	-	-	-
Cu-complex	287	332	409	794	1.93
Co-complex	289	329	437	635, 703	4.74
Ni-complex	285	334	443	712, 406	3.17
Mn-complex	278	331	439	683, 552 and 528	5.78

Table.3. Electronic spectroscopic and magnetic moment for ligand and their complexes

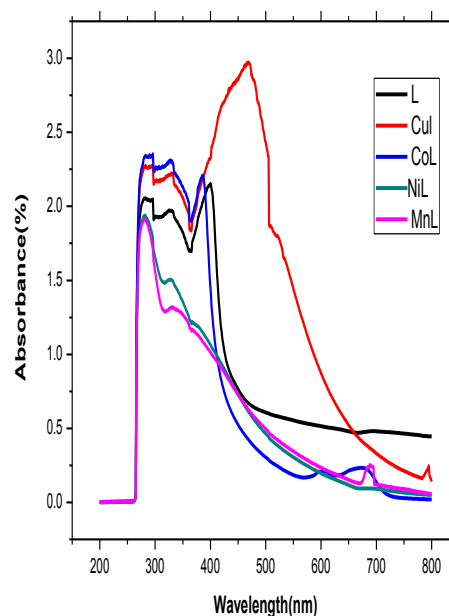


Figure 2. Electronic spectrum of Schiff base metal complexes

4. Cyclic Voltammetry

The cyclic voltammogram of Cu(II) complex showed a redox active peak in the potential range from 200-400 mV assigned to the Cu(II)/Cu(I) couple (Figure 3 & Table 4.1-4.2). A cyclic voltammogram displays a reduction peak at E_{pc} = 0.84 V with an associated oxidation peak at E_{pa} = 1.06 V and the peak separation value (ΔE_p) was 220 mV. The different current intensity of cathodic and anodic peaks found to be the quasi reversible behavior of simple one electron transfer reaction. The redox potential of Cu(II)/Cu(I) couple shown that, it was dependent on coordination number, nature and bulkiness of Schiff base ligand [25-26]. The cobalt complex exhibit one electron quasi reversible transfer process with a peak at E_{pa} = 0.86 V, E_{pc} = 0.97 V and ΔE_p = 110 mV. This gives evidence for quasi reversible Co(II)/Co(I) couple. The cyclic voltammogram of Ni(II) complex shown the anodic peak at E_{pa} = 0.37 and associated cathodic peak at E_{pc} = 0.51 V. This corresponds to quasi-reversibility of Ni(II)/Ni(I) couple with ΔE_p = 140 mV [27]. The Manganese(II) complex exhibit one electron quasi reversible transfer process with a peak at ΔE_p values falls in the range of 190 mV and the E_{1/2} values falls in the range of 0.47 V

corresponds to the quasi reversible of Mn(II)/ Mn(I)

Complexes	E _{pc} (V)	E _{pa} (V)	E _{1/2} (V)	ΔE _p (mV)
Cu-complex	0.84	1.06	0.95	220
Co-complex	0.86	0.97	0.91	110
Ni-complex	0.37	0.51	0.44	140
Mn-complex	0.38	0.57	0.47	190

couple[28].

Table.4.1. Electrochemical data of heterocyclic mononuclear Schiff base metal(II) complexes in DMF medium (Negative potential)

Complexes	E _{pc} (V)	E _{pa} (V)	E _{1/2} (V)	ΔE _p (mV)
Cu-complex	-1.40	-1.18	-1.99	220
Co-complex	-0.86	-0.75	-0.80	110
Ni-complex	-1.13	-0.96	-1.04	170
Mn-complex	-1.85	-1.71	-1.78	140

Table 4.2. Electrochemical data of heterocyclic mononuclear Schiff base metal(II) complexes in DMF medium (Positive potential)

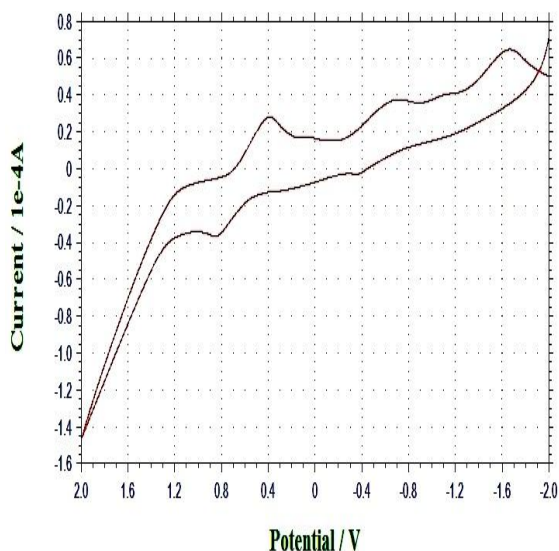


Figure 3. Cyclic voltammogram of Cu(II) Complex

5. ¹H NMR spectrum

The ¹H NMR spectrum of ligand (L) was recorded in DMF and Spectrum was shown in **Figure 4**. ¹H NMR spectrum of the Schiff base ligand shows peak singlet at 10.6 ppm due to -OH group present in free ligand. The peaks at 9.42 ppm corresponds to azomethine (-C=N) group[29]. The signal formed in the region from 6.6-7.8 ppm showed the multiplet due to furfurylidine moiety. The ligand also shows the following signals: (-CH₃ 2.4–2.5 δ(s)), (-N-CH₃ 3.03–3.07 δ (s)); and (-SH 1.2) δ (s) respectively. All the protons were found to be in their expected region. The conclusions drawn from these studies offer additional support to the mode of bonding discussed in their IR spectra. The number of protons

calculated from the integration curves and those attained from the values of the expected CHN analyses agree with each other.

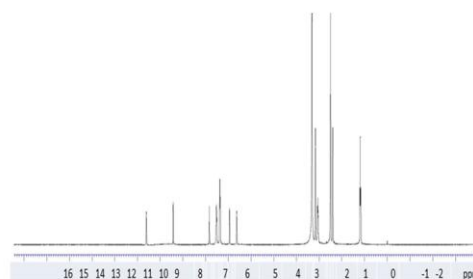


Figure 4. ¹H NMR Spectrum of Schiff base ligand

6. Mass spectra

Mass spectra afford an energetic clue for elucidating the structure of compounds. The mass spectra of the ligand (C₁₉H₂₀N₄O₃S) were recorded and their stoichiometric compositions were compared (**Figure 5**). The molecular ion peak for the ligand (L) was observed at 384.45 m/z which confirm the stoichiometry of the ligand. Elemental analysis values were in good agreement with the values calculated from molecular formula which was further supported by the mass studies.

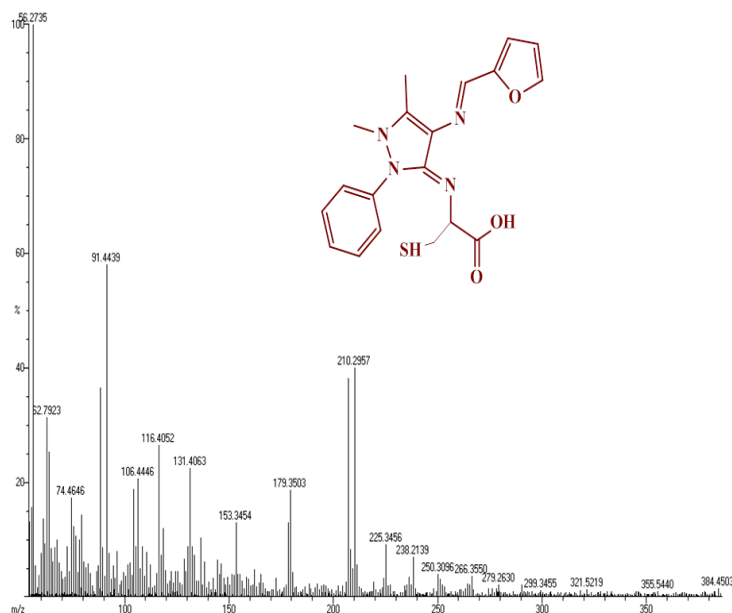


Figure 5. Mass Spectrum of Schiff base ligand

7. Powder XRD

In the absence of single crystal, X-ray powder data were especially useful to deduce accurate cell parameters. The diffraction pattern reveals the crystalline nature of the complex. Powder XRD patterns of Ni(II) and Mn(II) complexes recorded in the range 10–80° (θ) at wavelength 1.54 Å were shown in **Figure 6a & 6b**. The XRD pattern of the metal complexes shows well demarcated crystalline peaks

representing that the samples were crystalline in phase (Souaya et al., 2000). The metal complexes illustrated sharp crystalline XRD patterns, which differ considerably from that of the ligand. The appearance of crystallinity of the metal–Schiff base complexes were due to the inherent crystalline nature of the metallic compounds.

The calculated lattice parameter for Ni(II) and Mn(II) complexes was $a=b=c= 8.2263$ and 8.1925 . The grain size of the metal Schiff base complexes, d_{xrd} was calculated using Scherer's formula (Cullity, 1978).

$$d_{xrd} = 0.9\lambda / \beta \cos\theta$$

Where ' λ ' is the wavelength, ' β ' is the full width at half maximum of prominent intensity peak and θ is the diffraction angle.

The complexes have the average grain size values of 2.4-2.8 nm, suggesting that the complexes were in nanocrystalline regime.

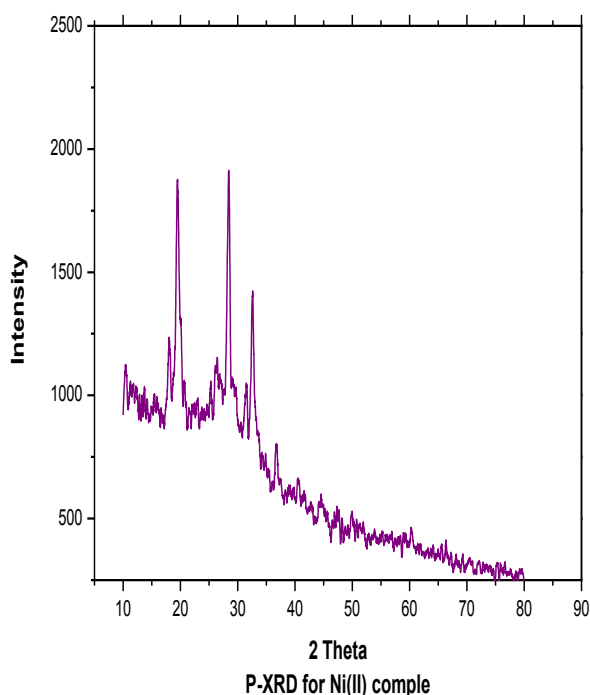


Figure 6(a). P-XRD for Ni(II) Complex

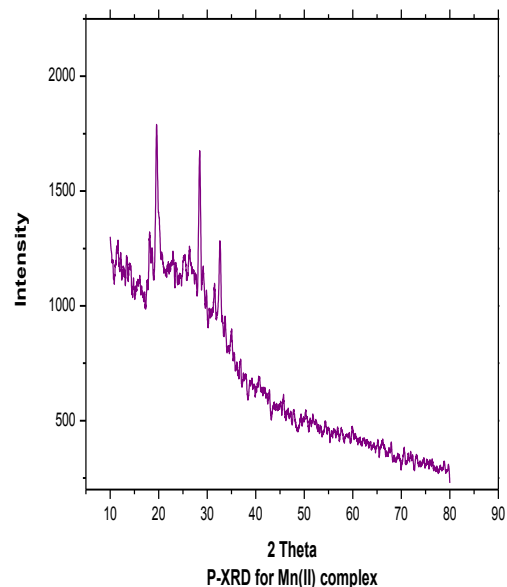


Figure 6(b). P-XRD for Mn (II) Complex

8. Anti-microbial Studies

The bacteriological activity of the Schiff base and its metal complexes were resolute against two gram positive (*Staphylococcus aureus*, *bacillus subtilis*) and two gram negative bacteria (*E.Coli*, *K.pneumoniae*) under different concentration. The antibacterial activity of ligand and their complexes were shown in **Figure 7**. The agar well diffusion method was employed for the bacteria with respect to Ciprofloxacin as standard drug. The copper complex showed better activity than other metal complexes for microorganisms. The increased activity of the copper complexes can be explained on the basis of Tweedy's theory. In addition, chelation may enhance or suppress the biochemical potential of bioactive organic species. It was suggested that chelation considerably reduces the charge of the metal ion mainly because of partial allocation of its positive charge with the donor groups and possible p-electron delocalization over the whole chelate ring which leads to increase in lipophilic character. Further, lipophilicity, which controls the rate of entry of molecules into the cell, was modified by coordination, so the metal complexes can become more active than the free ligand (Farrell, 2007). The higher activity of the metal complexes may be owing to the effect of metal ions on the normal cell membrane. Similarly, all the compounds screened for their antifungal activity, showed more than 60% inhibition of mycelial growth against *Aspergillus Niger* and *Penicillium sp* (Figure 8). The antimicrobial studies suggested that all the Schiff bases were found to be biologically active and their metal complexes showed significantly enhanced antibacterial and antifungal activity against microbial strains in comparison to the free ligands.

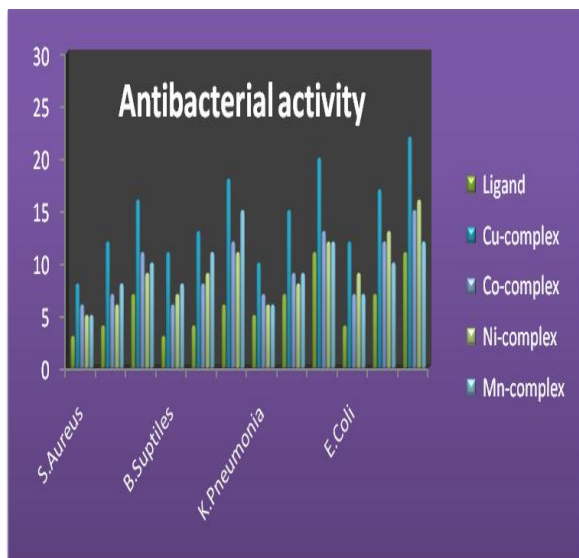


Figure 7. Antibacterial activity for schiff base ligand and its metal complexes

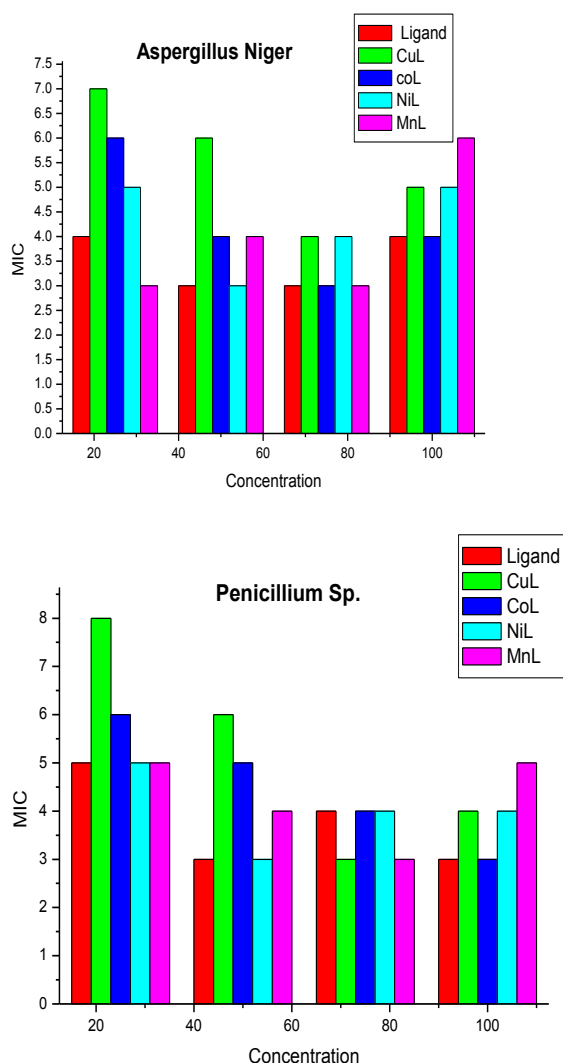


Figure 8. Anti-fungal activity of Schiff base ligand and their metal complexes

9. Cytotoxic Activity

The cytotoxic activity of Schiff base and its metal complexes was treated on neuroblastoma SH-SY5Y cell lines in the concentration of 10, 25 and 50 μ M. After 24 h of treatment, Cell viability performed by using the Trypan blue exclusion method shown in Figure 9. In our study revealed that the Schiff base and its metal complexes stimulate loss of cell proliferation on the increasing concentration for cell lines. From this observation, we noticed that the Schiff base and its complexes were non-toxic to the neuroblastoma SH-SY5Y cell lines [33]. Among the Schiff base and its metal complexes, we found that the Cu(II) complex act as a good antitumor agent on SH-SY5Y cell lines.

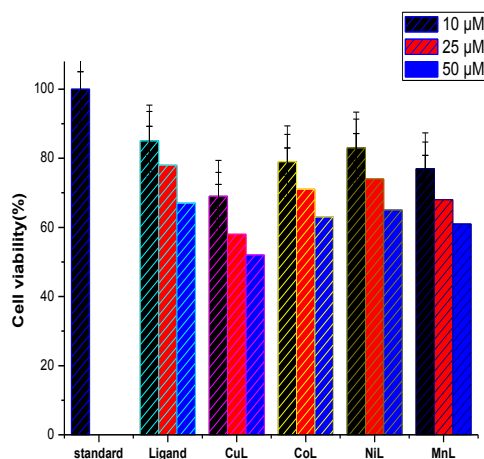


Figure 9. Percentage of cell viability versus different concentrations for SH-SY5Y cells exposed to the metal complexes after 24 h incubation.

10. DNA Cleavage Study

The DNA cleavage study is one of the gel electrophoresis methods. It was subjected to the most of the anticancer and antimicrobial therapies for cleaving the cells. This method was conducted by using agarose gel and the system containing pUC19 DNA alone, hydrogen peroxide and the synthesized compounds [34-35]. Among the Schiff base ligand and the metal complexes, Cu(II) and Co(II) complexes have, the more potential cleavage compares with others were shown in Figure 10.

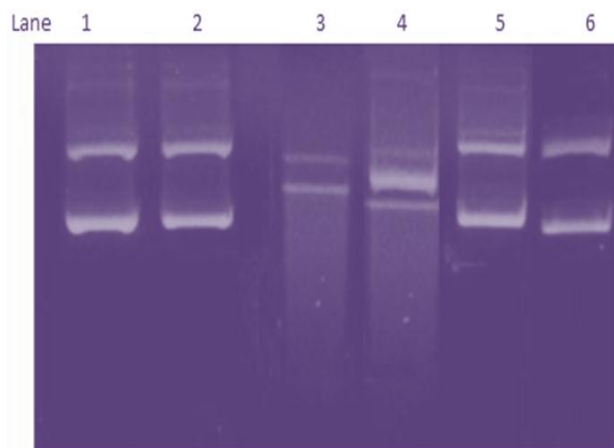


Figure 10 : Gel electrophoresis pattern of Schiff base and its metal complexes Lane 1: DNA alone; Lane 2: DNA+ H₂O₂+HL; Lane 3: DNA+ H₂O₂+CuHL; Lane 4: DNA+ H₂O₂+CoHL; Lane 5: DNA+ H₂O₂+NiHL; Lane 6: DNA+ H₂O₂+MnHL;

CONCLUSION

This work depicted the synthesis and characterization of 4-aminoantipyrine derived Schiff base and their metal(II) complexes. The Schiff base ligand were behaving as nitrogen and oxygen donor tridentate ligand. The bonding of ligand to metal ion was strengthened by elemental analyses, molar conductivities, cyclic voltammetry, IR spectra, UV-Vis., mass spectra, ¹H NMR spectra. All these studies predicted good evidence for the proposed structure. The Powder XRD data analyzed the nanocrystalline structure of Ni(II) and Mn(II) complexes. Antimicrobial study of the presently studied metal complexes showed that the general trend observed in inhibition potency against the bacterial and fungal strains. The result suggested that the antibacterial and antifungal activities of the ligand and their complexes exhibit high persuasive biocidal and fungicidal activity than the free ligand. Entire lines of cytotoxicity of Schiff base metal complexes possess a very distinct behavior in impacting the cell death. The Schiff base ligand and their metal complexes can excellently cleave the plasmid DNA in the presence of an oxidizing agent H₂O₂.

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