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CHEMICAL NUCLEASE STUDIES OF CU(II) AND NI(II) COMPLEXES WITH SCHIFF BASE DERIVED FROM 2-HYDROXY-3-METHYL BENZALDEHYDE AND 2-CHLORO-5-AMINO PYRIDINE

Chetana PR*[1], Sahana S[1], Policegoudra RS[2], Aradhya S[3]

[1]Department of Chemistry, Central College Campus, Bangalore University, Bangalore, Karnataka, India
[2]Department of Biotechnology, Defense Research Laboratory, Tezpur, Assam, India.
[3]Department of Fruit and Vegetable Technology, CFTRI, Mysore, Karnataka, India.

ABSTRACT

Schiff base ligands are easily prepared by the condensation of aldehydes and amines. These compounds are also known to be imines or azomethines. Lone pair of nitrogen atom of the azomethine group is of considerable interest due to its chemical and biological significance. Thus, Schiff base ligands well coordinate with the various transition metals and stabilize them at different oxidation states. The complexes of Cu(II) and Ni(II) with Schiff base derived from benzaldehyde and amine have been synthesized and characterized by H^1-NMR, Mass, UV-Visible and elemental analysis. The Schiff base and its Cu(II)and, Ni(II) complex were soluble in organic solvents such as dimethylsulfoxide, dichloromethane and acetonitrile. The molar conductance of the complexes are measured to be low implies that the complexes are non-electrolytic nature. The spectrophotometric studies of the complex compounds revealed 1:2metal to ligand ratio. The DNA cleavage studies of the complexes have been investigated and both the complexes showed extensive cleavage of DNA. However, Cu(II) complexes showed better cleavage activity compared to Ni(II) complex. The complexes were screened for their *in-vitro* antimicrobial activity using various strains of gram positive and gram negative bacteria- *Bacillus mycoides, Bacillus subtilis, Escherichia coli, Micrococcusluteus, Proteus mirabilis, Pseudomonas aeruginosa* and *Yersinia enterocolitica*. The Schiff base ligand showed higher antibacterial activity than the respective complexes.

Keywords: Cu(II) and Ni(II) complex, Chemical nuclease activity, antibacterial activity.

INTRODUCTION

Interest in the preparation and characterization of transition metal complexes of Schiff bases with nitrogen and oxygen donors has gained interest among the researchers in the recent past [1-5]. The Schiff base ligands are considered to be good chelating agents and the ease of preparation by the condensation of aldehydes and amines with –OH functional group close to the azomethine group made Schiff bases as special class of ligands [6-9]. The Schiff bases derived from various amines and aldehydes have been widely investigated [10-11] and find applications in biomimetic catalytic reactions, materials chemistry and industry [12-13]. Schiff bases having chelation with nitrogen and oxygenetcdonors and their complexes have been used as drugs and reported to possess a wide variety of biological activities against bacteria, fungi and certain type of tumors and also they possess many biochemical, clinical and pharmacological properties [14-17].

Copper is a biologically important metal and more number of enzymes depend on copper for their activity [18].Copper Schiff complexes are important bioactive compounds *in vitro* and *in vivo* and used as potential drugs for therapeutic drugs in various diseases. Thus owing to its biological significance, coordination chemistry of copper attracts much attention.

Ni(II) complexes have found several important

Corresponding Author:-**Chetana PR**Email: pr.chetana@gmail.com

applications in medicine and very recently they have been screened for inhibition of cancer cell proliferation [19-20]. In the last 20 years transition metal complexes have become increasingly important as drugs and artificial nucleases. One target of designing Ni(II) complexes is developing more reactive chemical systems that can efficiently cleave DNA under physiological conditions [21-23]. A literature survey revealed that no work has been done on the condensation of 2-chloro-5-amino pyridine with 2-hydroxy-3-methylbenzaldehyde. Hence, in this communication, the synthesis, characterization, antimicrobial and DNA studies of transition metal complexes containing Schiff base derived from 2-chloro-5-amino pyridine with 2-hydroxy-3-methylbenzaldehyde is described.Further, in continuation of our work on transition metal complexes with Schiff base ligands [24], metal complexes have been synthesized, characterized and evaluated the biological activities. In this paper, we report the experimental results on two new complexes such as CuL_2/NiL_2whereL=2-{[(6-chloropyridin-3-yl)imino]methyl}-6-methylphenol. This paper is mainly focused on exploring the trend in DNA binding affinities, efficiency of DNA cleavage and its antimicrobial activities.

EXPERIMENTAL

Materials

All reagents were of AR grade and used as received. Solvents used for spectroscopic studies are purified by standard procedures [25].The super coiled (SC) pUC 19 DNA (CsClpurified) was purchased from Bangalore Genei, (India). Agarose (Molecular biology Grade), ethidium bromide (EB) were obtained from Sigma (USA). Tris(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer was prepared by doubly distilled water.

The elemental analysis were carried out using Vario-micro CHNS15106062 analyser. IR spectral studies were carried out by Shimadzu spectrophotometer form 4000-400 cm^{-1} using KBr pellets. UV-Visible spectra were recorded on Shimadzu UV-3101 PC spectrophotometer using dimethylformamide as solvent. ^{1}H NMR and ESI-MS data of the compounds were recorded using Bruker DSX 300 MHZ Solid-state NMR spectrometer and MicroTOF LC BrukerDaltonicsspectrometer.Cyclicvoltammetric experiments were performed using CHI600E electrochemical instruments and melting points were checked by melting point apparatus used in laboratories.

SYNTHESIS

Preparation of the Schiff base 2-MPP (2-{[(6-chloropyridin-3-yl)imino]methyl}-6-methylphenol).

The Schiff base ligand 2-methyl -2-(pyridine-3-yliminomethyl)phenol was synthesized with the following procedure.2-Hydroxy-3-methylbenzaldehyde (1mmol) in ethanol was added to ethanolic solution of 2-chloro-5-aminopyriidne (1mmol) and the mixture was refluxed at 45 ^{0}C for about 7-8 hrs. The reaction was monitored by TLC and the yellowish brown needle like crystals were collected on cooling. The solid was washed with ethanol and then with diethyletherand finally dried under vacuum. The ligand with good yield was obtained.

Preparation of bis[2-methyl-2-(pyridine-3-yliminomethyl)phenol]Cu(II) and Ni(II) complexes (ML_2) where L is 2-{[(6-chloropyridin-3-yl)imino]methyl}-6-methylphenol.

ML_2 complex was synthesized by themethanolic solution of metal chloride/acetate (1 mmol) was reacted with 2-MPP (2 mmol) dissolved 1:2 ratio acetonitrile and methanol under stirred condition at room temperature and monitored by TLC. The resulting precipitate was filtered off, washed with methanol and air dried. The obtained precipitate was dissolved dimethylformamide to obtain the pure compound.

ANTIMICROBIAL STUDIES

The antimicrobial activity of the synthesized complexes and ligands were evaluated against pathogenic bacterial strains like *Bacillus mycoides, Bacillus subtilis, Escherichia coli, Micrococcusluteus, Proteus mirabilis, Pseudomonas aeruginosa* and *Yersinia enterocolitica*. The test organisms were maintained onnutrient Agar slants. The reported procedure of Mukerji.et.al [26] agar well diffusion method was followed to determine the antibacterial activity.

The bacterial culture was centrifuged at 8000 rpm for 10 mins and suspended in saline to obtain a suspension of 10^5 CFU per ml and used for the assay. The bacterial suspension was transferred to sterile petri plate and mixed with molten nutrient agar medium and allowed to solidify. About 2mg/ml concentrated solution of the sample (75 µl) was added to well and incubated at 37 ^{0}C. The diameter of the inhibition zone determines the antibacterial activity.

DNA CLEAVAGE STUDIES

The cleavage of SC pUC19 DNA by the ligand and its complexes (ML_2) were studied by agarose gel electrophoresis. 3-mercaptopropionoic acid (MPA) (5 mmol) served as the reducing agent for the chemical nuclease activity. The reactions were carried out under dark conditions at 25 ^{0}C. agarose gel electrophoresis was done to determine the extent of cleavage of SCDNA (0.2µg) in 50 mmolTris-HCl buffer (pH 7.2) containing 50 mmolNaCl treated with the ligand and metal complexes. The concentrations of the ligands/complexes and the additives in the buffer were diluted to the final volume of 2µl using Tris-HCl buffer. The SC pUC19 DNA samples were incubated at 37 ^{0}C for duration of 1 hr followed by addition of loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol (2µl) and solution was finally loaded on to agarose gel (0.8%) containing ethidium bromide(1µg/ml). Electrophoresis was

carried out in a dark room for 2 hrs at 60V in Tris-acetate EDTA buffer (TAE). UV light was used for visualization of the bands that were photographed. The extent of DNA cleavage was measured from the intensities of the bands using the UVTEC gel Documentation system. Due corrections were made for the low level of the nicked circular (NC) form present in the original super coiled (SC) DNA. Sample and for the low affinity of EB binding to SC compared to NC and linear forms of DNA [27].

STUDIES ON DNA INTERACTION

The UV absorbance at 260 and 280 nm of the CT-DNA solutions in 5 mMTris-HCl buffer (pH 7.2) gave a ratio of 1.9, indicating that the DNA was free from protein [28]. The concentration of CT-DNA was measured from band intensity at 260 nm with a known ε value (6600 cm-1) [29]. Absorption titration measurements were done by varying the concentrations of the CT DNA, keeping the metal complex concentration constant in 5 mMTris-HCl/5mM NaCl buffer. Samples were kept for equilibrium before recording each spectrum. The intrinsic binding constant (K_b) for the interaction of the complexes with CT DNA were obtained from the absorption spectral titration data using the following equation:

$$(\varepsilon_a-\varepsilon_f)/ (\varepsilon_a- \varepsilon_f)=(b-(b^2-2K_b{}^2C_t[DNA]/s)^{1/2})/2K_bC_t \quad (1)$$

Where $b= 1+K_bC_{t+}K_b[DNA]/2S$; $\varepsilon_a$the extinction coefficient observed for the charge transfer absorption band ata given DNA concentration; ε_f, the extinction coefficient of the complex free in solution; ε_b, the extinction coefficient ofthe complex when fully bound to DNA; K_b, the equilibriumbinding constant; C_t, the total metal complex concentration;[DNA], the DNA concentration in nucleotides;and s, the binding site size in base pairs [30].

CYCLIC VOLTAMMETRIC STUDIES

Cyclic voltammetric experiments were performed at room temperature in water: DMF under oxygen free conditions created by purging pure nitrogen gas with CHI 600E electrochemical instruments. A three electrode system was used: a glassy carbon working electrode, an Ag^+/AgCl reference electrode and Pt wire counter electrode. The working electrode was polished with 1.0, 0.3, 0.05 µm alumina prior to each experiment. Through the experiment oxygen-free nitrogen was bubbled through the solution for ten minutes. Voltammetric experiments were performed at room temperature.

RESULTS AND DISCUSSION

Synthesis and General Aspects

A set of two different novel Cu(II) and Ni(II) complexes were prepared with high yield. The analytical data for the complexes indicate ML_n stoichiometry for all the complexes where L=4-MPP, M= Cu and Ni and n=2. The melting points of all complexes are above 300 ^{0}C, the complexes are stable in air. All the complexes are insoluble in common organic solvents and soluble in dimethyl lformamide (DMF) and dimethylsulfoxide (DMSO). The molar conductance of Cu complex in DMF (10^{-3}M) solution, fall in the range of 12 $ohm^{-1}cm^{-2}mol^{-1}$, indicating the non-electrolytic nature of complex, i.e. the anions are coordinated to the metal ions (Table 1) [31].

IR spectra

IR spectra usually provide a lot of information on coordination reactions. The IR spectra for our studied complexes give information about the coordination of the ligand to metal. The IR spectrum of the ligand indicate that the ν(C=N) band of ligand at 1608 cm^{-1} is due to the azomethine linkage which were shifted towards lower frequency indicating that the ligand coordinate to metal ions via the azomethine nitrogen. The absence of peak due to the phenolic OH group at 3412cm^{-1} suggests the coordination of the ligand to the metal via deprotonation which infers that azomethine-nitrogen and phenolic oxygen are the coordination sites of the bidentate ligand.

Electronic spectra

The electronic spectra of ligands and complexes are measured in acetonitrile at room temperature over 200-800nm range and presented in (Fig. 1 (a) (b)). The Ligands and the complexes exhibit similar UV spectra. In the UV spectrum of ligand the peaks appear at 243 and 265 nm attributed to π- π* of phenyl ring transitionand a peak attributed to the azomethine group transition is observed at 335 nm. In the spectra of the complexes, the bands of the azomethine chromophore bands are shifted and indicated that the imine nitrogen atom is involved in coordination to the metal ion.

H^1-NMR spectra

Further, evidence for the coordinating mode of the ligand is obtained by the ^{1}H-NMR studies. ^{1}H-NMR spectra data is recorded in $CDCl_3$. The ligand is characterized by 8 signals at 12.81(S), 8.61(S), 8.33 (S), 6.82-7.58 (M) and 2.31(S) which are assigned to –OH, -N=CH-, =N-CH-, aromatic protons and methyl protons, respectively. The presence of -N=CH- proton signal at 8.61 in the ligand (L) confirms the formation of condensation of 5-chlorosalicylaldehyde with 2-chloro-5-aminopyridine. In the ^{1}H NMR spectra of complexes (1-2), the absence of proton at 12.81 indicating that phenolic proton is absent in complexes. (Fig. 2 (a) (b) (c)).This information suggest the adjustment of electronic current upon coordination of>C=O group to the metal ions.

Mass Spectra

The ESI-MS of Schiff base ligand and their complexes showed molecular ion peaks which were in perfect agreement with their molecular formula. The mass spectrum of 4-MPP ($C_{13}H_{11}ClN_2O$) as shown in Figure shows a molecular ion peak at m/z 246, which is in

accordance with the proposed formula of the ligand. The mass spectra of the complexes $CuCl_2(C_{26}H_{24}Cl_2N_4O_2Cu)$ and $NiL_2(C_{26}H_{24}Cl_2N_4O_2Ni)$are at 560 and 555 m/z in ESI +ve mode, respectively (Figure 3 (a) (b) (c)).

Cyclic Voltametry

The copper complex (0.001M in DMF) was scanned in the potential range of -1.0 V to 1.0V in deareated condition with scan rate 0.1V/s. The numerical results with scan rate 0.1v/s are given in Table 1. A cathodic peak observed in the voltammograms in the range Epc = 0.15 to 0.07 V evidences the reduction of metallic species, Cu(II) to Cu(I) [32]. The reverse scan shows two anodic peaks with potentials in the range Epa_1 = -0.1 to -0.5V and Epa_2 = 0.35 to 0.68V corresponding to the oxidation reactions, Cu(I) to Cu(II) and Cu(II) t0 Cu(III). The Cu(II) complex display a quasireversible cyclic voltammetric response which can be assigned to the Cu(II)/Cu(I) couple near 0.45 V in DMF –Tris buffer.

Antibacterial activity

The in-vitro antibacterial activity of the Schiff base, and their Cu(II) and Ni(II) complexes were evaluated against four gram positive bacteria's-*Bacillus mycoides*, *Bacillus subtilis*, *Micrococcusluteus*, *Proteus mirabilis*and three gram negative bacteria's *Pseudomonas aeruginosa* and *Yersinia enterocolitica*, *Escherichia coli* and the findings were tabulated in Table 2. DMSO (blank) andAmpicillin was used as controls .In general, the activity against gram negative bacteria is higher than those of gram positive bacteria this may be due to the greater lipophilic nature of the Schiff bases than their metal complexes [33] and both the complexes showedantibacterial activity which are more or less similar towards both the strains of bacteria.The factors that govern antibacterial activities are strongly dependent on the central metal ion and the coordination numbers and also due to the presence of nitrogen and sulfur donor groups [34-36].

DNA cleavage studies

The DNA cleavage activity of the complexes in the presence of reducing agent 3-mercaptopropionic acid (MPA, 5mM) is investigated by agarose gel electrophoresis using super coiled (SC) plasmid pUC19 DNA (0.2µg, 3.33µM NP) in 50 mM Trish HCl/50 mMNaCl buffer (pH, 7.2) and the copper(II) complex. Selected DNA cleavage data are given in the Table 3. The complexes Cu(II) and Ni(II) shows efficient "chemical nuclease" activity. Control experiments with MPA or the complex alone do not show any apparent conversion of SC to its nicked-circular (NC) form. The maximum cleavage was exhibited by Cu(II) complex at 40 µm concentration (Fig. 4). The DNA cleavage of complexes in the presence of MPA probably proceeds through the hydroxyl radical pathway as proposed by Sigman [36].

DNA binding studies

It was reported that DNA binding mode and affinity are affected by a number of factors, such as planarity of the ligands [37]the coordination geometry, the ligand donor atom type [38] the metal ion type, and its flexible valence [39]. The binding of the Cu(II) and Ni(II) complexes with the calf thymus (CT) DNA has been studied by electronic absorption spectra technique. The absorption signals of the Cu(II) complex as a function of increasing concentration of CT DNA is shown in Fig. 5. It is clear from graph that the minor bathochromic shift of 1-3nm along with significant hypochromicity. K_b and *s* values for the Cu(II) complex are 2.1 (±0.8) x 10^6 and 0.38(±0.06), respectively.

Table 1. Analytical and physical data of the ligand L and its complexes

Compounds (Formula)	Mol. Mass	[a]Mol Cond	[b]ΔE_p (V)	MP ^{0}C	N%		C%		H%	
					Exp	Obt	Exp	Obt	Exp	Obt
4-MPP (L) ($C_{13}H_{11}ClN_2O$)	246.7	---	---	130	11.36	11.31	63.29	63.22	4.49	4.55
CuL_2 (1) ($C_{26}H_{24}Cl_2N_4O_2Cu$)	560	12.34	0.441	301	10.02	9.98	55.87	55.79	4.33	4.38
NiL_2 (2) ($C_{26}H_{24}Cl_2N_4O_2Ni$)	555	---	---	319	10.11	10.06	56.36	56.40	4.37	4.32

[a]Molar Conductance = Λ_M ($\Omega^{-1}cm^2M^{-1}$) in DMF at 25 0C, [b]Cyclicvoltametry, Cu(II)/Cu(I) couple in DMF-0.1M KCl, $\Delta Ep = Ep_a - Ep_c$are the anodic and cathodic peak potentials, respectively. Scan rate = 0.1 mV.

Table 2. The values of zone inhibition (mm) of microorganisms for the L and its metal complexes

	Bacteria	4-MPP	CuL_2	NiL_2
Gram Positive	*Bacillusmycoides* (MTCC 645)	14	--	--
	Bacillussubtilis (MTCC 441)	14	10	10
	Micrococcusluteus (MTCC 106)	11	--	--
	Proteus mirabilis (MTCC 743)	15	12	11
	Pseudomonasaeruginosa (MTCC 741)	21	23	21

Gram Negative	*Yersiniaenterocolitica* (MTCC 4848)	16	12	11
	Escherichia coli (MTCC 443)	11	10	10

Table 3. Selected cleavage data of SC pUC19 by ligand and its Cu(II) and Ni(II) complexes

Lane no	Complex	[a]NC%	Lane no	Complex	[a]NC%
1	DNA control	0	7	DNA+ H_2O_2+ Cu(II) complex (40µm)	9
2	DNA+ L (40µm)	8	8	DNA+Ni(II) complex(40µm)	5
3	DNA+MPA+L(40µm)	25	9	DNA+MPA+Ni(II) complex(40µm)	50
4	DNA+H_2O_2+L(40µm)	6	10	DNA+ H_2O_2+Ni(II) complex (60µm)	8
5	DNA+Cu(II) complex (40µm)	5	11	DNA+MPA	5
6	DNA+MPA+ Cu(II) complex (40µm)	65	12	DNA+ H_2O_2	4

Fig 1(a). The Electronic spectrum of ligand L and its Cu(II) and Ni(II) complexes

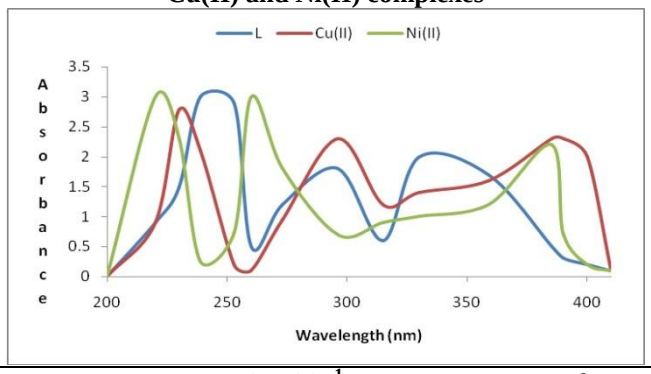


Fig 1(b). The d-d bands of complexes Cu(II) and Ni(II) complexes

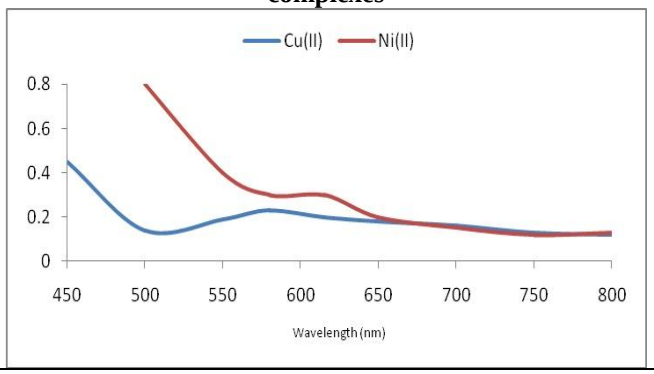


Fig 2(a). ^{1}H -NMR spectrum of 2-methyl -2-(pyridine-3-yliminomethyl)phenol

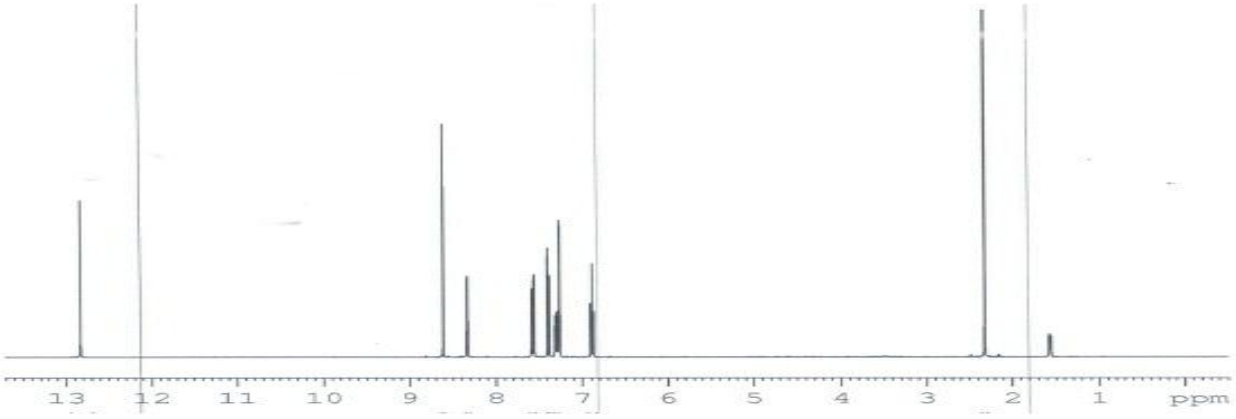


Fig 2(b). ^{1}H -NMR spectrum of CuL_2

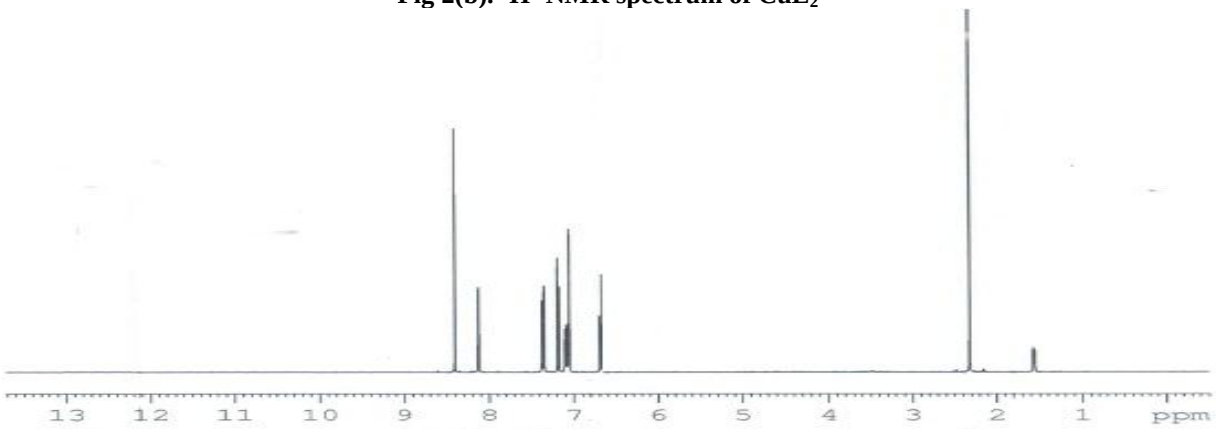


Fig 2(c). ^{1}H -NMR spectrum of NiL_2

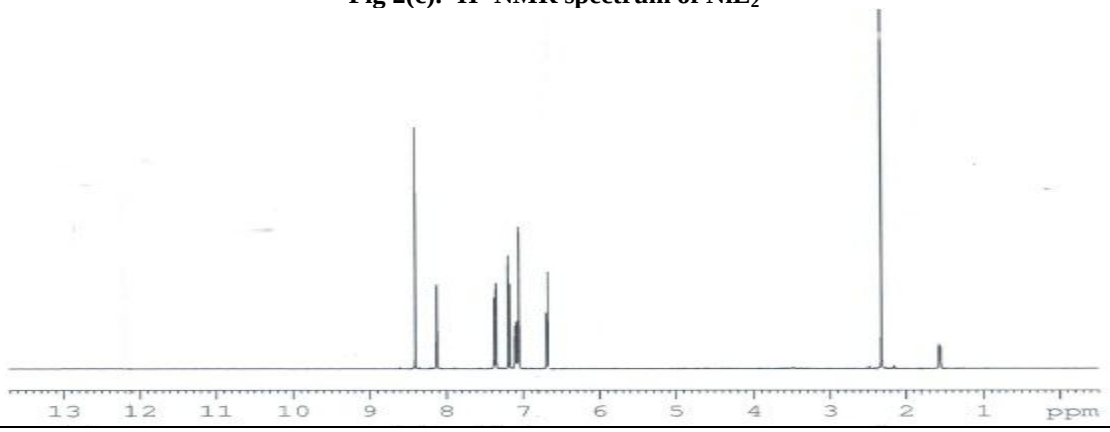


Fig 3(a). Mass spectrum of 4-MPP

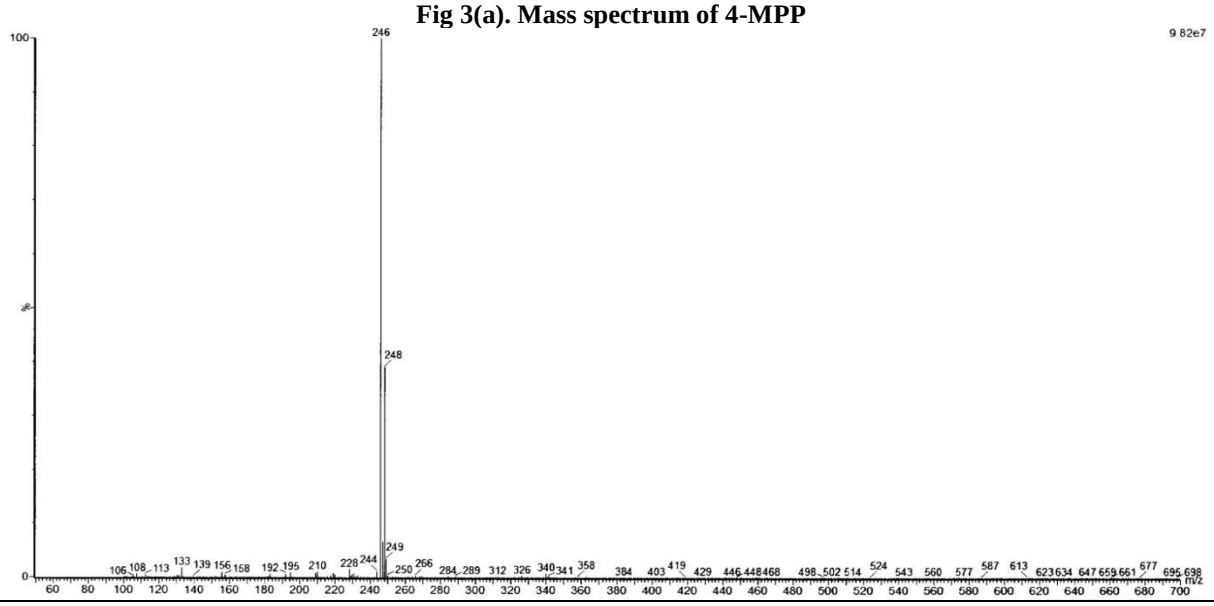


Fig 3(b). Mass spectrum of $Cu(L)_2$

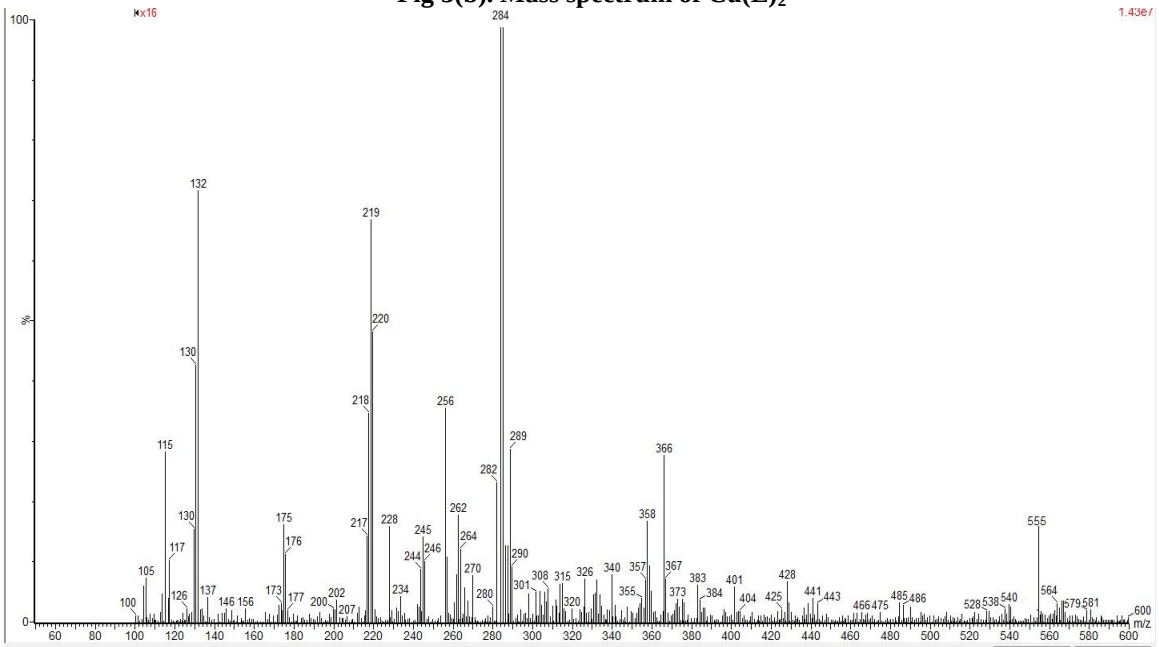


Fig 3(c). Mass spectrum of $Ni(L)_2$

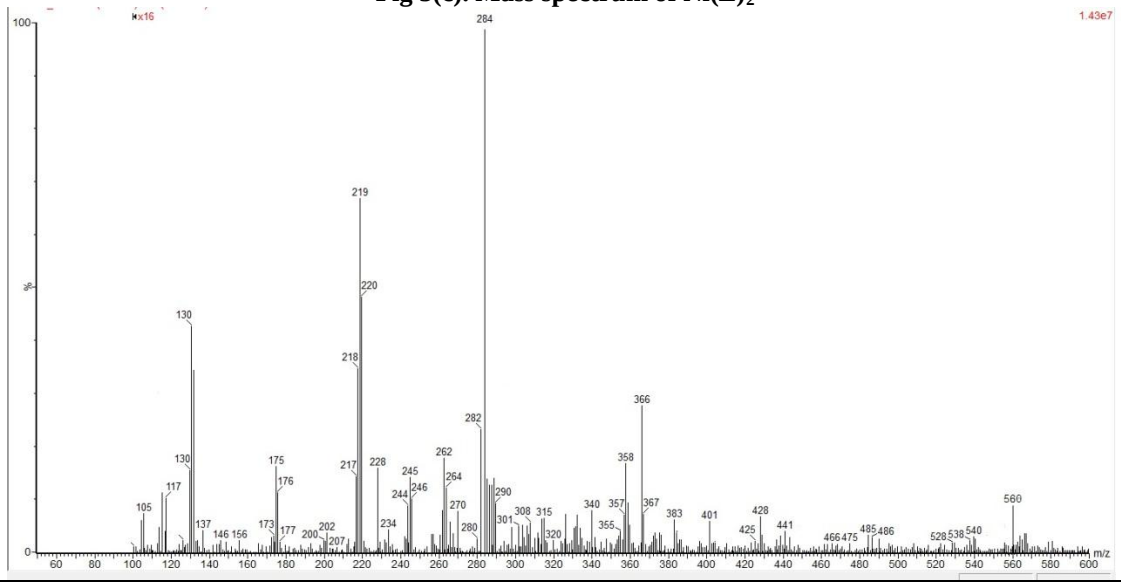
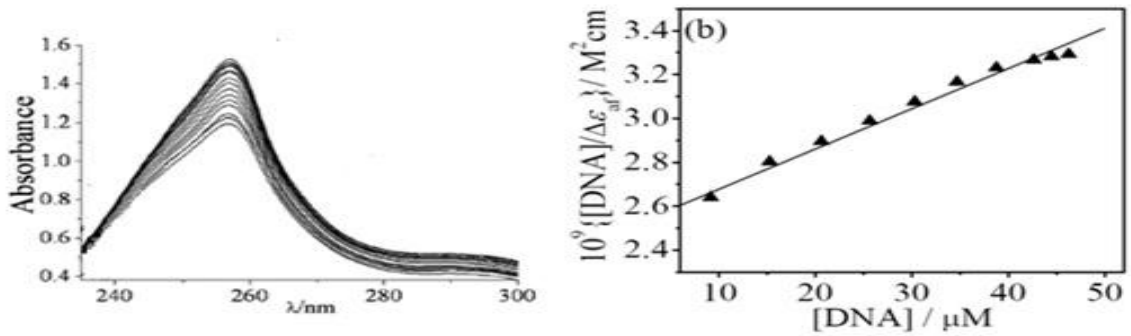
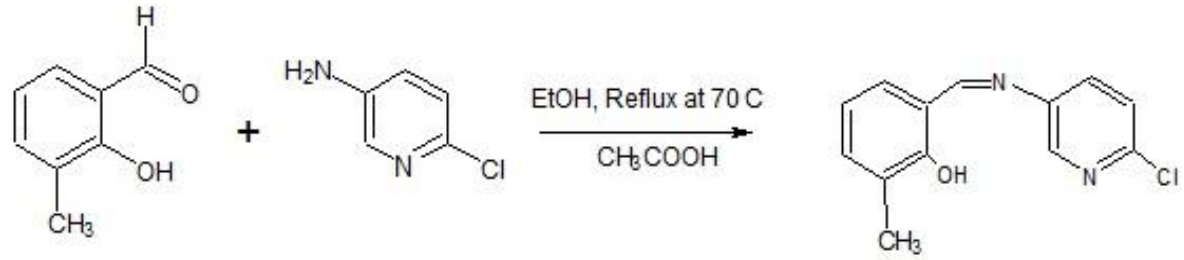


Fig 4. Gel electrophoresis diagram showing the cleavage of SC pUC DNA (0.2 µg, 3.33µM) by ligand and its complexes (30 µM) in 50 mMNaCl buffer (pH 7.2) in the presence of MPA (500 µM). Reaction details are given in Table 4 in which entry number correspondence to the lane number of the Figure

Fig 5. Absorption spectral traces on addition of CT-DNA to the solution of $Cu(L)_2$ and the best least squares fit of $\Delta\mathcal{E}_{af}/\Delta\mathcal{E}_{bf}$vs [DNA]

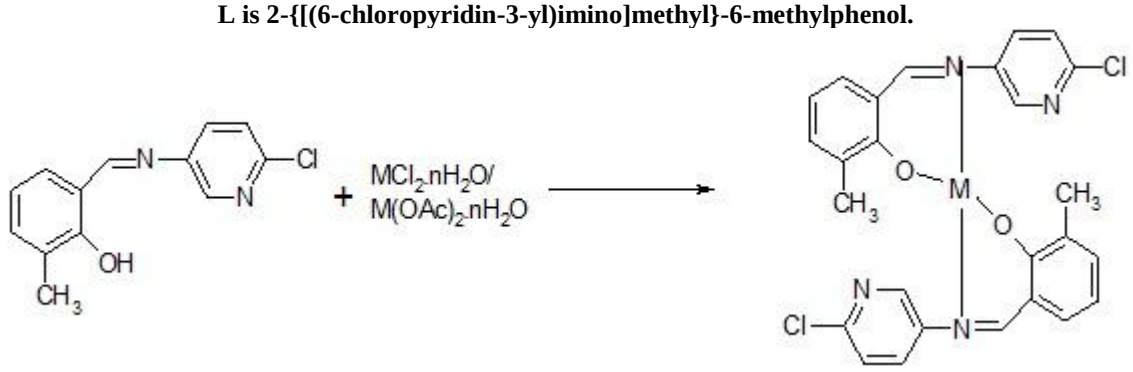


Scheme 1. Preparation of Schiff base ligand2-MPP.



2-hydroxy-3-methyl2-chloro-5-amino 2-MPP benzaldehydepyridine

Scheme 2. Preparation of bis[2-methyl-2-(pyridine-3-yliminomethyl)phenol]Cu(II) and Ni(II) complexes (ML_2) where L is 2-{[(6-chloropyridin-3-yl)imino]methyl}-6-methylphenol.



CONCLUSION

The complexes of Cu(II) and Ni(II) with Schiff base derived from benzaldehyde and amine have been synthesized and characterized by H^1-NMR, Mass, UV-Visible and elemental analysis.The ligand is found to have higher biological activities as compared to their corresponding metal complexes againstfour gram positive bacteria's-*Bacillus mycoides, Bacillus subtilis, Micrococcusluteus, Proteus mirabilis* and three gram negative bacteria's *Pseudomonas aeruginosa* and *Yersinia enterocolitica*, *Escherichiacoli*.The DNA interactions of the prepared complexes were evaluated by absorption method and results showed the binding affinity of the complexes to DNA.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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