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Synthesis Characterization Spectroscopic Antimicrobial and Nuclease Activity of Schiff Base Complexes Derived From 4- Aminopyridine

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ABSTRACT: Cu(II), Co(II) and Ni(II) Schiff base complexes derived from di- α -formyl methoxybis(3-pentadecenylphenyl) methane and 4-aminopyridine have been synthesized and investigated by different physicochemical techniques. The resulting complexes were characterized by elemental analysis, molar conductance, IR, UV, $^1\text{H NMR}$, XRD and SEM. The synthesized compounds were tested for, anti-bacterial, antifungal, larvacidal, anti-inflammatory, anti-cancerous and Nuclease activity.

KEY WORDS: di- α -formyl methoxybis (3-pentadecenylphenyl) methane, 4-aminopyridine Schiff base, Nuclease activity, anti-bacterial, antifungal, larvacidal, anti-inflammatory and anti-cancerous activity.

I. INTRODUCTION

Schiff base is defined as the condensation product of a primary amine with a carbonyl compound is characterized by the presence of an azomethane group. Although this group is intermediate between $>C=C<$ and $>C=O$ groups. Carbonyl group is necessarily an end group; while atoms of both $>C=C<$ and $>C=N<$ can be located in internal position in chains or rings. Schiff bases ligands have occupied a central role in the development of coordination chemistry and biochemistry. Schiff base ligands forms stable complexes with different transition metal ions. The transition metal complexes have an interdisciplinary relation because of their extensive application in wide ranging areas from material science to biological science and leading to pharmaceutical science(1-5).

II. SIGNIFICANCE OF THE SYSTEM

One of the most promising nature sources of cardanol, 3-pentadecenylphenol from CSNL is a raw material very utilized in green chemistry works. The oil obtained from the shell are alkyl substituted phenolic compound with long side chain substituent at the meta position it under go polymerization and cross linked phenol group condensed with aldehyde and vice versa. It can be used as cationic exchange resin and incorporate a functional group in phenolic ring.

III. LITERATURE SURVEY

CSNL contain anacardic acid because of decarboxylation during roasting which convert anacardic acid to cardanol. CSNL constituents have been widely used as *synthon* for the preparation of many compounds with biological activities. Cardanol undergo reactions such as etherification, nitration, hydrogenation, epoxidation, sulfonation, and decarboxylation and esterification (Tyman, J H P 1996, Madhusudhan, V, Murthy, B G K & Sivasamban, M A 1984).

The meta substitution and long chain hydrocarbon molecules make the resin soluble in oils and impart properties such as high electric insulation and resistance against chemicals to the films. Manufacture of solid and semi solid resin or

novolac type of resins depends upon the nature of catalyst and the aldehyde compound. Cardanol resin varnishes have good electrical insulating properties and resistance against water and chemicals. They give superior quality varnishes, because of their high insulation and dielectric nature these varnishes are preferred to composition based on cresol or substituted phenol (Kim, SJ, kim, KS & Jang, H 2003).

By virtue, phenolic nature cardanol can be condensed with formaldehyde to give phenol formaldehyde type resin. The long chain CSNL imparts flexibility due to internal plasticizing, resulting in the formation of soft resin at elevated temperature unlike phenol formaldehyde resin which is hard (Bruno, E J 1970).

CSNL is used as a substitute for linseed oil in the manufacture of foundry core oil used as binders in foundry. Resin imparts good scratch hardness to sand core. It provides resistance to moisture and weathering, good green strength and surface finish to molded articles (Giller, A 1971).

Mineral oil additives are based on amino cardanol. Ether of hydrated cardanol and salt of its sulphonated ethers are found to improve the viscosity index of mineral oil and inhibit sludge formation and antioxidant properties (Nagar, R 1990).

IV. METHODOLOGY

Cardanol was obtained from M/S Sathya Cashew Chennai India, formaldehyde (37% solution), hydrochloric acid, epichlorohydrin, L-histidine, sodium hydroxide and other chemicals were used of GR/AR grade quality obtained from Merk chemicals. All the solvents used were purified by standard methods^[6]. Elemental analyses (C,H,N) were performed by using Elementar Vario EL III at STIC, CUSAT, Cochin. The IR spectra were recorded in KBr pellets using Shimadzu FTIR spectrometer (4000 – 400 cm⁻¹). The UV-Vis electronic spectra (200 – 800 nm) were recorded using Lab India 3000+ double beam spectrophotometer. The micro analytical data were collected using Perkin Elmer 2400 instrument. The metal ion intake were estimated by standard methods^[7].

A. SYNTHESIS OF SCHIFF BASE LIGAND WITH DFMPM AND 4-AMINOPYRIDINE

The Schiff base ligand was prepared by the reported methods^[8-9]. Equimolar ethanolic solution of DFMPM and L-histidine were mixed and refluxed for about an hour. Pour the reaction product in ice, (1+2) Schiff base ligand was obtained^[10]. The precipitated yellow compound was filtered washed with water and dried over anhydrous calcium chloride. The crude sample was recrystallised from 50% absolute alcohol yield=62%. Melting point =223⁰C.

B. Synthesis OF CU(II), CO(II) AND NI(II) schiff base metal complexes

All the metal complexes were prepared by mixing ethanolic solution of Schiff base ligand with the corresponding aqueous metal salt solution of Cu(II) nitrate, Co(II) nitrate and Ni(II) nitrate in 1:1 molar ratio. The resulting mixture was refluxed for about twelve hours at 70-80⁰C^[11]. A coloured solution appeared on standing. The complexes were filtered, washed with ethanol, diethyl ether, acetone and hot water and finally dried under vacuum at 90⁰C. The filtrates obtained in the above method were collected. The collections were used for the estimation of Cu(II), Co(II) and Ni(II) intake for complexation using standard methods^[12].

V. EXPERIMENTAL RESULTS

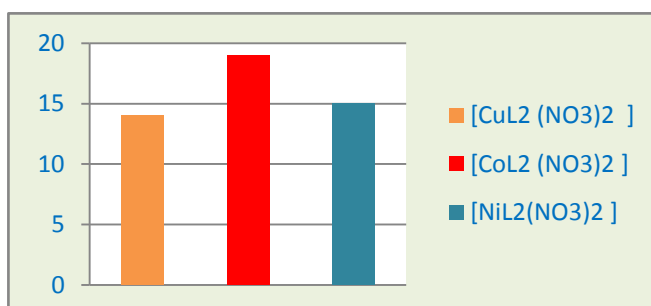
Physical and Analytical data of ligand and Metal complexes were depicted in Table 1. Complexes are in ML₂ composition, Cu(II), Co(II) and Ni(II) complexes are coloured solids, stable towards air and have high melting points above (250⁰C). The complexes are insoluble in water and common organic solvents but are soluble in DMF, CDCl₃, DMSO. Metal to ligand ratio in all the complexes to be 1:2 and their empirical formulae have been computed. Conductivity value was in the range 12-15 ohm⁻¹cm² mol⁻¹ table.2 & fig.1 shows complexes are non electrolytes in nature. For non-electrolytes conductivity was slightly higher it may be due to partial solvolysis of the complexes in DMSO medium⁽¹³⁻¹⁷⁾.

Table 7.1 Physical and Analytical data of ligand and Metal complexes

Complex / Ligand	Yield	Colour	Molecular formula	Mol. weight	Melting point	Elemental Analysis		
						C	H	N
Ligand L	61	grey	C ₆₉ H ₁₀₄ N ₄ O ₆	1084	232	74.41 (76.38)	7.74 (9.59)	3.66 (5.16)
[CuL ₂ (NO ₃) ₂]	57	ash	C ₁₃₈ H ₂₀₈ N ₁₀ O ₁₄	2291	>250	70.04 (72.20)	7.38 (9.07)	3.2 (6.10)
[CoL ₂ (NO ₃) ₂]	60	Brown	C ₁₃₈ H ₂₀₈ N ₁₀ O ₁₄	2286.93	>250	70.26 (72.40)	7.92 (9.09)	4.58 (6.12)
[NiL ₂ (NO ₃) ₂]	54	Brown	C ₁₃₈ H ₂₀₈ N ₁₀ O ₁₄	2286.69	>250	69.37 (72.41)	7.33 (9.09)	4.71 (6.12)

Table 2 & fig.1 Molar Conductance data of the complexes

Compounds	Molar conductance Ohm ⁻¹ cm ² mol ⁻¹
[CuL ₂ (NO ₃) ₂]	14
[CoL ₂ (NO ₃) ₂]	19
[NiL ₂ (NO ₃) ₂]	15



A. FT- IR spectrum analysis

The use of FTIR technique allows the detection of complex formations in solid phase and to point out the implication of the different functional groups of guest and host molecules in the inclusion process by analyzing the significant changes in the shape and position of the absorbance bands of ligand and complexes.

From table 3 & fig 2, ligand shows characteristic bands at 2767 cm⁻¹, 2990 cm⁻¹, 1651 cm⁻¹, 1499 cm⁻¹ and 1439 cm⁻¹ assignable to ν_{O-C}, ν_{C-H}, ν_{C=N}, ν_{C=O} and free -COOH; free (-O-H) stretching phenolic moiety, carbonyl (-C=O) and azomethine (-C=N), stretching modes, respectively. The spectra of metal of Cu(II), Co(II) and Ni(II) complexes fig. 2-5, showed bands at 3471- 3317cm⁻¹ which is assigned to ν_{O-H}, 2855 - 2845 cm⁻¹ for ν_{O-C}, 2929 - 2922 cm⁻¹ for ν_{C-H}, 1658 - 1644 identify presence of ν_{C=N} bond, 1499 cm⁻¹ point out bond at ν_{C=O} and bands at regions 626- 504cm⁻¹ and 413- 399cm⁻¹ can be assigned to ν_{M-N} and ν_{M-O} vibrations, respectively ⁽¹⁸⁻¹³⁾.

Table. 3 FT IR frequencies and UV-spectrum of the ligand and its complexes

Ligand/ Complexes	ν_{O-H} cm^{-1}	ν_{O-C} cm^{-1}	ν_{C-H} cm^{-1}	$\nu_{C=N}$ cm^{-1}	$\nu_{C=O}$ cm^{-1}	free CO OH cm^{-1}	ν_{M-N} cm^{-1}	ν_{M-O} cm^{-1}	λ max(nm)
Ligand L	-	2767	2990	1651	1499	1439	-	-	-
[CuL ₂ (NO ₃) ₂]	3356	2845	2929	1649	1499	1382	507	399	575,650
[CoL ₂ (NO ₃) ₂]	3471	2855	2929	1644	1499	1382	504	413	500,780
[NiL ₂ (NO ₃) ₂]	3317	2850	2922	1658	1499	1435	626	401	650,770

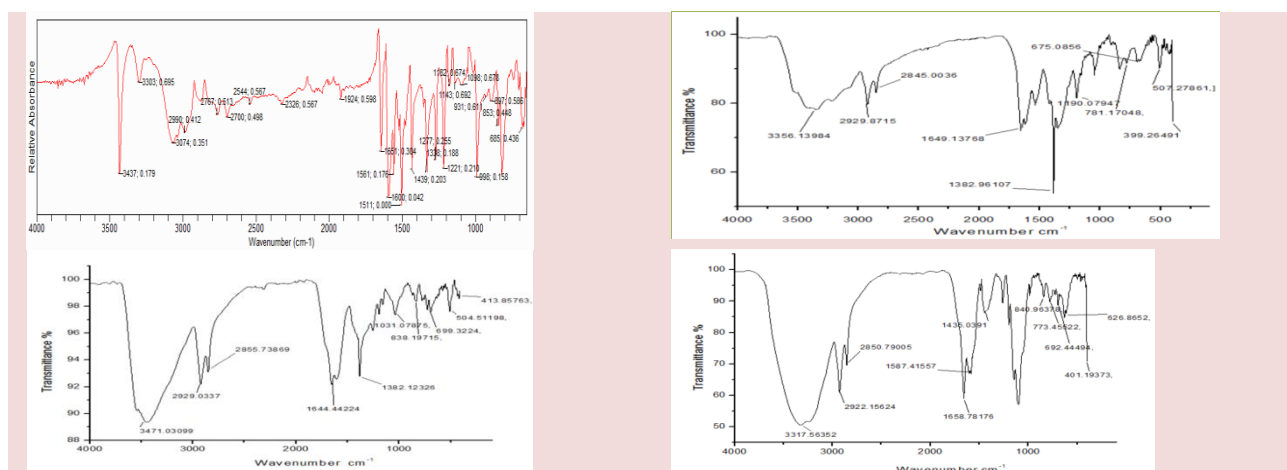


Fig.2,3,4,5 FTIR Spectrum of ligand, Cu(II), Co(II) and Ni(II) complex

B. UV-Visible spectrum analysis

Electronic absorption spectral data of the ligand and Schiff base metal complexes are given in Table 3. The electronic spectra of Cu(II) complex in DMSO show bands at 575 nm and 650 nm assignable to a ${}^2B_{1g} \rightarrow {}^2A_{1g}$ transition and charge transfer, respectively. The electronic spectral data coupled with magnetic moment and suggest square planar geometry for Cu(II) complex. Co(II) complex exhibits three bands at 500 nm and 780 nm assignable to ${}^4T_{1g} \rightarrow T_{2g}$ (F), ${}^4T_{1g} \rightarrow {}^4A_{2g}$ (F) transitions and charge transfer, respectively. These transitions indicate high spin octahedral geometry of the complex. In case of Ni(II) complex, the observed magnetic moment and the spectral bands at 650 nm and 770 nm assignable to ${}^6A_{1g} \rightarrow {}^4T_{1g}$, ${}^6A_{1g} \rightarrow {}^4T_{2g}$ and charge transfer respectively, indicate octahedral geometry (18-24).

C. ¹H NMR spectrum analysis

From Fig.6, the ligand shows a singlet at $\delta = 8.188$ ppm - $\delta = 8.174$ ppm due to the presence of H - C = N- group. A narrow band at $\delta = 7.186$ ppm - $\delta = 7.167$ ppm, due to - O-CH₂ -group. The doublet at $\delta = 6.804$ ppm - $\delta = 6.500$ ppm and $\delta = 5.413$ ppm - $\delta = 5.327$ ppm were due to the olefinic protons of the side chain and - O-CH₂ -group of the ligand. Multiplet at $\delta = 2.822$ ppm - $\delta = 2.002$ ppm is due to substituted H-C-C=O- group and band at $\delta = 1.579$ ppm - $\delta = 1.275$ ppm due to

substituted -CH₂-NH group. Fig.7 reveals the ¹H NMR spectrum of Cu(II) complex, a band at δ = 7.262ppm- δ = 7.189ppm exhibit the presence of H - C = N- group . The singlet at δ = 6.765 ppm - δ = 6.500 ppm and δ = 5.345ppm - δ = 5.327 ppm were due to the olefinic protons of the side chain and - O-CH₂ -group of the ligand. Singlet at δ = 7.028ppm - δ =7.011ppm, due to - O-CH₂ -group. Multiplet at δ =2.570 ppm -δ =2.015 ppm due to substituted H-C-C=O- group and band at δ = 1.564ppm - δ = 1.309ppm due to substituted -CH₂-NH group⁽²⁴⁾ .

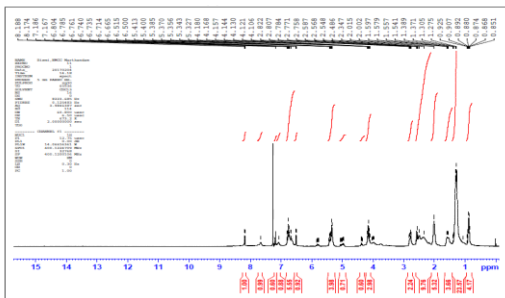


Fig.5 ¹H NMR spectrum of ligand

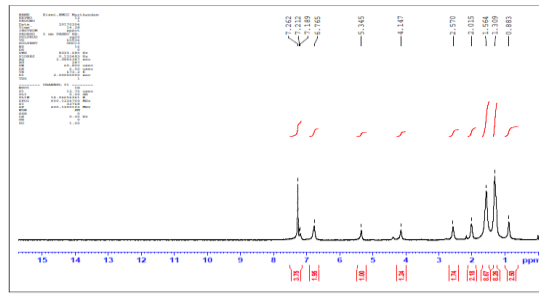


Fig.6 ¹H NMR spectrum of Cu(II) complex

Based on the observations in elemental analysis, FT-IR, electronic and ¹H NMR spectral studies, the proposed structure of Ligand (L) and metal Schiff base complexes were given in Fig.8-9.

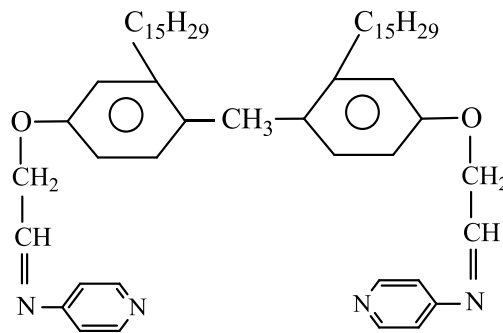


Fig .8 Structure of ligand

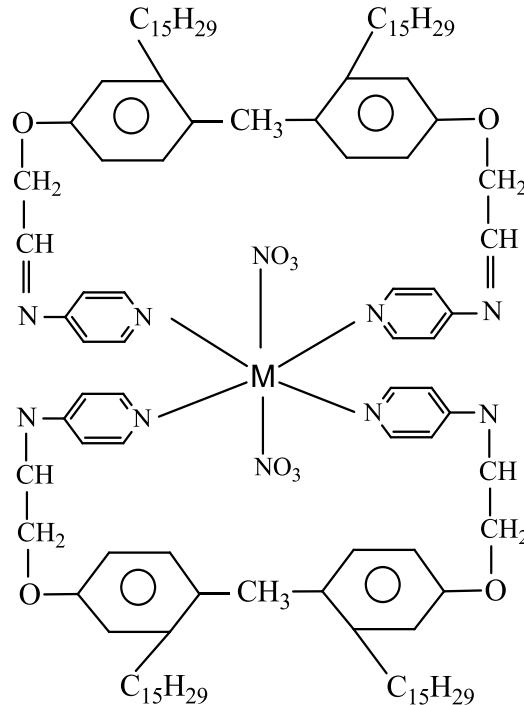


Fig.9 Structure of Schiff base complexes, M=Cu(II), Co(II) and Ni(II)

D. X-RAY diffraction analysis

X-ray diffractometry (XRD) is an instrumental technique that is used to identify minerals as well as other crystalline materials. In order to investigate the characteristic of the inclusion complexes, the XRD is the suitable equipment that can provide the information of the degree of crystallinity of the materials presents, possible deviation of the minerals from their ideal compositions and others. The diffractogram of Cu(II) complex is given in Fig.10 and datas of grain size in table.4. It is evident that the strong and broad peaks confirm that the complex formation and the appearance of large feeble peaks indicate micro crystalline. The grain size of the complexes was calculated using Scherrer's formula. The calculated grain size of the Cu(II) complex is 7.122nm These values suggested that the complexes are in nano crystalline size⁽²⁵⁾.

Complex	Grain size(nm)
[CuL ₂ (NO ₃) ₂]	7.122

Table 4 Grain size of the complexes

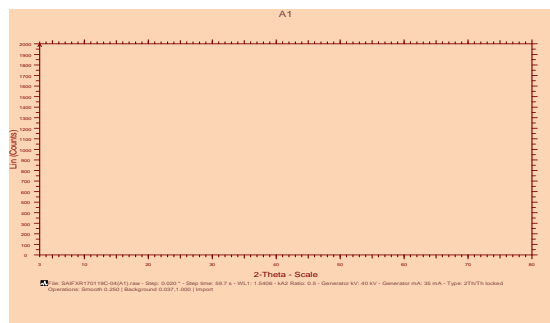


Fig.10 XRD spectrum of Cu(II) complex

E. SEM analysis

The surface morphology of the complexes has been examined using scanning electron microscope. SEM images of the samples in Fig 11 illustrate the interesting morphological evolution of Ni(II) complex. Ni(II) occur as shells and the images showed that the complex is micro crystalline in nature. Careful examination of the single crystal clearly indicated the nano scale size of the single crystal of the complex⁽²⁶⁾.

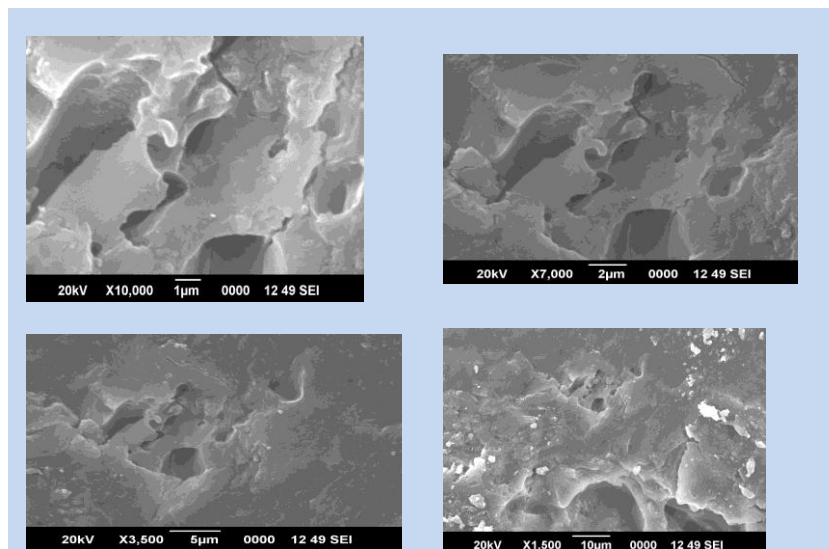


Fig.11 SEM images of Ni(II) complex at 1µm, 2µm, 5µm, 10µm

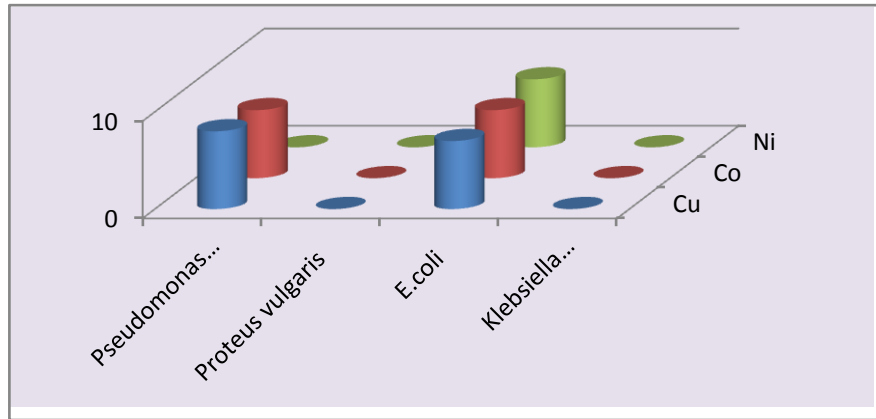
VI MICROBIOLOGI

A. ANTI-BACTERIAL ACTIVITY

Metal complexes of Schiff base were screened against bacterial species (*E. coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae*) Table.5 shows antibacterial datas of newly prepared Schiff base and its metal complexes, showed a higher effect on *E. coli* (Gram-negative bacteria). Schiff base complexes were screened by disc diffusion method. The activity order of the synthesized compounds is as follows: Cu(II) > Co(II) > Ni(II). The higher activity of the metal complexes may be owing to the effect of metal ions on the normal cell membrane . It clearly indicated that metal complexes showed a higher effect on *E. coli* (gram-negative bacteria)⁽²⁶⁾.

Table.5 Anti-bacterial activity data of complexes

Bacteria	Cu	Co	Ni
<i>Pseudomonas aeruginosa</i>	8mm	7mm	NZ
<i>Proteus vulgaris</i>	NZ	NZ	NZ
<i>E.coli</i>	7mm	7mm	7mm
<i>Klebsiella pneumoniae</i>	NZ	NZ	NZ

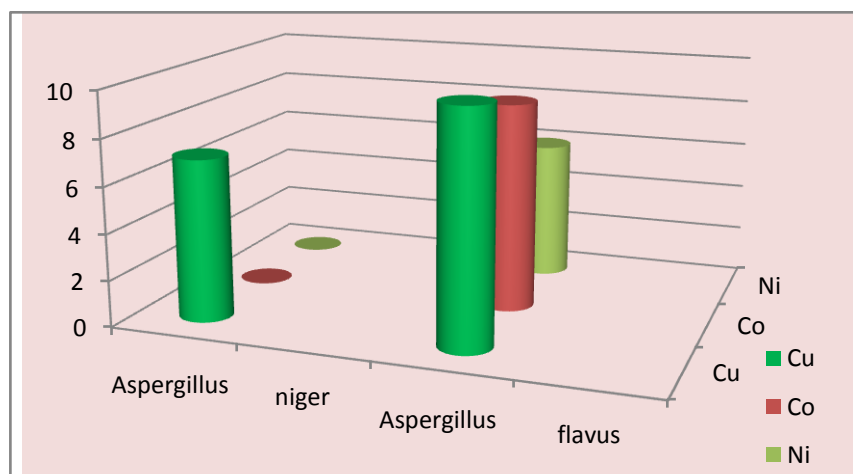


B. ANTI-FUNGAL ACTIVITY

Metal complexes Cu(II), Co(II) and Ni(II) are synthesized with Schiff bases derived from DFMPM and 4-aminopyridine, then tested against three fungi. It is clear that Cu(II) complex exhibit inhibition towards *Aspergillus niger* and *Aspergillus flavus*. The metal complexes gave better results against the growth of fungi. Cu(II) complexes show more activity than the other complexes. These results may be due to higher stability of Cu(II) complexes than the other complexes. It is found that the activity increases upon coordination. The increased activity of the metal chelates can be explained on the basis of chelation theory⁽²⁶⁾.

Table.5 Anti-fungal data of Schiff base complexes

FUNGA I	[CuL ₂ (NO ₃) ₂]	[CoL ₂ (NO ₃) ₂]	[NiL ₂ (NO ₃) ₂]
<i>Aspergillus niger</i>	7mm	NZ	NZ
<i>Aspergillus flavus</i>	10mm	9mm	6mm



C. Anti-inflammatory activity

Schiff base complexes were screened for their anti-inflammatory activities. . Bovine serum albumin and dichlorofenac sodium solution was used as the standard and distilled water as control. The percentage inhibition increases with increase in concentration. In the case of Invivo when concentration increases, there is a gradual increase in Percentage of inhibition for complexes. Co(II) complex exhibit statically significant activity in Invitro and Invivo percentage inhibition⁽²⁶⁾ . Table.6 Anti inflammatoractivity data of complexes

Table.6 Anti inflammatory activity data of complexes

Sample	Conc: ($\mu\text{g/ml}$)	Proteinase inhibitory % of inhibition	Proteinase denaturation %Inhibition
Cu(II)	25	61.95	17.46
	50	63.71	24.108
	100	68.25	30.642
Co(II)	25	57.06	9.42
	50	75.29	13.78
	100	78.27	29.96
Ni(II)	25	46.54	64.02
	50	53.23	54.07
	100	64.86	64.85

VII, NUCLEASE ACTIVITY

In the present study, metal complexes were incubated for 30 min at 37°C and the DNA was analyzed on 1.5% agarose gel in the presence of H_2O_2 as an oxidant. It was found that at very low concentration, the complexes exhibit nuclease activity in the presence of H_2O_2 . This is due to the formation of redox couple of the metal ions. In oxidative mechanism, metal ions in the complexes react with H_2O_2 to generate the OH which attacks at the C_3 positions of the sugar moiety and finally cleaves DNA⁽²⁶⁾ . Consequently these molecules find applications as pharmaceuticals mainly in the treatment of cancer.

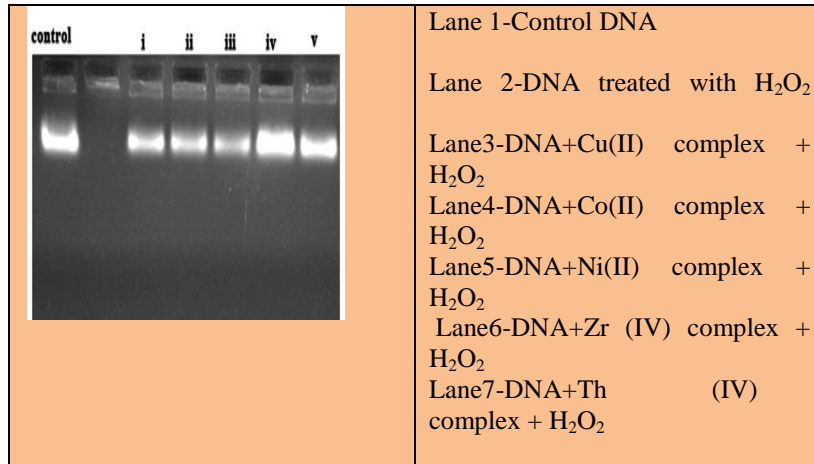


Fig.12 Gel electrophores diagram for complexes

A. Anti-cancer activity

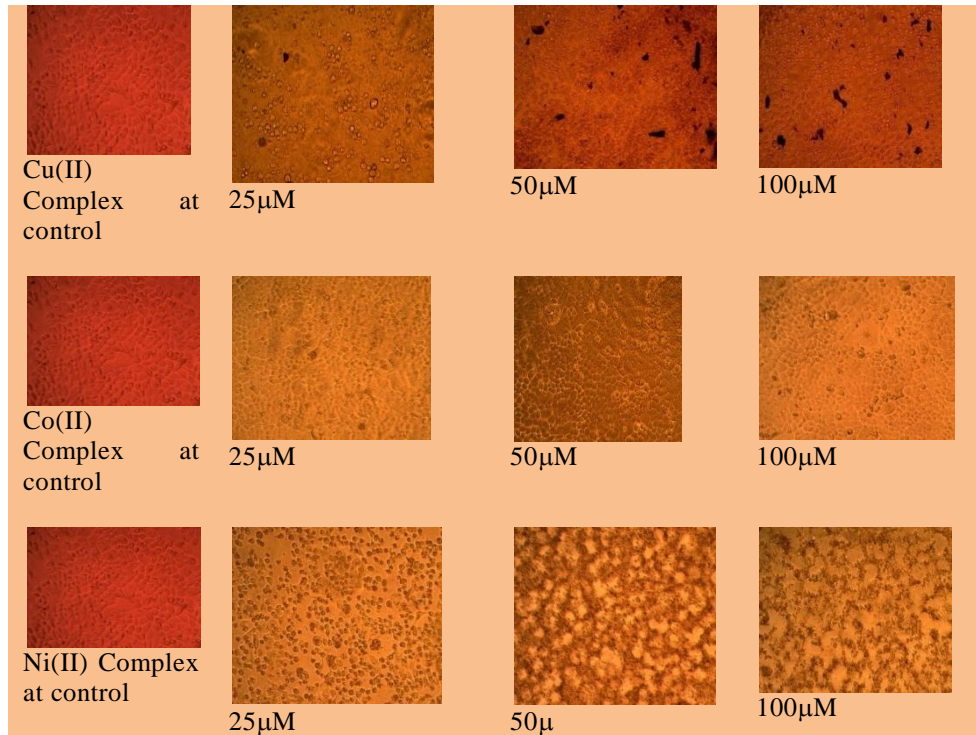
The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. HeLa (cervical cancer) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen). Observation from table and fig it is cleared that when concentration increases the viability decreases that means the cytotoxicity of the cells decreases there by the cells are not cytotoxic . When increase in concentration number of dead cells increases there by indicate the anti cancerous property of complexes. The inhibition ratio of the complexes are in the order Ni(II), Cu(II), and Co(II) ⁽²⁷⁾. The percentage of growth inhibition was calculated using the formula: % of viability =

$$\frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

- LD 50 VALUE (2A1) –45.4203 µg/ml LD 50 VALUE (2A2) –418.288µg/ml
- LD 50 VALUE (2A3) – 39.4812µg/ml LD 50 VALUE (2A4) – 177.416µg/ml
- LD 50 VALUE (2A5) – 271.44µg/ml

Table 7 Anti cancer activity data of complexes

Sample (µg/ml)	Concentration	Average OD at 540nm	Percentage Viability
Control		0.9852	100
Cu(II)			
	25	0.5866	59.54121
	50	0.34755	35.2771
	100	0.2953	29.97361
Co(II)		0	0
	25	0.8985	91.19976
	50	0.87715	89.03268
	100	0.8431	85.57653
Ni(II)		0	0
	25	0.5484	55.66382
	50	0.3673	37.28177
	100	0.1697	17.22493



B. ARVACIDAL ACTIVITY

The metal complex $[NiL_2(NO_3)_2]$ show enhanced larvacidal activity. The result obtained is presented in table.8. The action of larvacidal upset the normal behaviour and action of target organism. Chelation increases the liphophilic nature of central metal atom, which in turn favours the molecule in crossing the cell membrane of the microorganism and enhancing larvacidal activity of complexes. Brine shrimp cytotoxic assay of Ni(II) complex was performed to evaluate its cytotoxic activity. Sample exhibited 100% cytotoxic activity at 600 µg , 60% at 50 µg, 50% at 200 µg and 80% at 400 µg respectively ⁽²⁷⁾ .

Table.8 Brine Shrimp lethality assay of $[NiL_2(NO_3)_2]$

Concentration (µg/ml)	50 µg	200 µg	400 µg	600 µg	Control	-ve control
Number of brine shrimp per test sample	10	10	10	10	10	10
Average number of survivors	4	5	2	0	3	5
Average number of deaths	6	5	8	10	7	5

**VII. CONCLUSION**

In this work the synthesis and characterization of a Schiff base ligand derived from 4-aminopyridine and forms stable complexes with metals such as copper (II), nickel (II) cobalt (II) were carried out. The ligand and its complexes were characterized using spectral and analytical data. These analytical and spectral data suggests Cu(II), Co(II) and Ni(II) complex show tetrahedral geometry. The XRD and SEM studies indicated the nano crystalline nature of the complexes. The metal complexes have higher antimicrobial and antifungal activity. All the metal complexes showed a higher effect on E. coli for bacterial activity. Cu(II) complex show fungal activity in *Aspergillus niger* and *Aspergillus flavus*. The inhibition ratio of the complexes in MTT analysis are in the order Ni(II), Cu(II) and Co(II) when increase in concentration number of dead cells increases there by indicate the anti cancerous property of complexes.

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