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Research Article

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The DNA cleavage and antimicrobial studies of Cu (II), Ni (II), Zn (II) and Co (II) complexes of 4- pyridine carboxaldehyde and Tryptophan

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ABSTRACT

The Schiff base complexes of Cu(II), Ni(II), Zn(II) and Co(II) have been synthesized from 4-pyridine carboxaldehyde with tryptophan. They have been characterized by UV, FT-IR techniques and their microbial and DNA cleavage activity have also been studied. The Schiff base and its metal complexes show a good activity against the bacteria; Klebsiella pneumonia, Pseudomonas aeroginosa, E.Coli, Staphylococus aureus,Proteus and fungi, Aspergillus niger. The antimicrobial results also indicate that the metal complexes of Cu(II), Ni(II), Zn(II) and Co(II) are better antimicrobial agents as compared to the Schiff bases. The DNA cleavage result reveals that all complexes showed enhanced nuclease activity.

Key Words: 4-Pyridine Carboxaldehyde, Tryptophan Antimicrobial activity, Spectral studies.

INTRODUCTION

The literature reveals that the schiff base ligands are excellent coordinating ligands. They form stable complex with different transition metal ions [1]. Schiff base ligand formed schiff base metal complexes with transition metal salts at their natural p^H. The formation of schiff base intermediate in reactions of biological importance is well documented [2]. Heterocyclic compounds do play important roles in regulating biological activities [3]. The pharmacological activity have been found to be highly depedent on the nature of the metal ion and the donor sequence of the ligands, different ligand shows different biological properties, though they may vary only slightly in their molecular structure [4].Transition metal complexes able to induce damages to nucleic acids may find useful applications as therapeutic agents [5-6] and especially provide efficient tools in molecular biology to study DNA and RNA structure and their function. The characterization of DNA recognition by small redox or photoactive transition metal complexes has been substantially aided by studying the DNA cleavage activity [7-10].Designing of metal complexes for cleaving DNA is currently an area of considerable interest from chemical as well as biological stand points and offers potential applications in the field of medicine in the post-genomic era. Transition metal complexes as an artificial nucleases are now areas of an extensive research due to their diverse structural features and reactivities [11].

EXPERIMENTAL SECTION

Chemicals

All the chemicals and solvents used were of A.R.grade. All the reagents used for the preparation of the Schiff bases were purchased from Sigma Aldrich and used as supplied. Metal salts were purchased from Loba chemie.

Measurements

C,H and N analyses were performed with Perkin Elmer 240 analyser. IR spectra were recorded in KBr pellets at Shimadzu FT-IR 8201 Spectrophotometer in 4000-200cm^{-1} technique. Electronic absorption Spectra were recorded on a Perkin- Elmer Lambda 35 spectrophotometer.

Synthesis of Schiff bases

Schiff bases have been synthesized by adding the methanolic solution of aldehyde with aminoacids in equimolar ratio. The reaction mixture was then refluxed on a water bath for about 4-5 hrs. The product obtained was filtered, thoroughly washed with ethanol, ether and dried.

Synthesis of Metal complexes

All the complexes were prepared by mixing hot ethanolic solution of Schiff base ligand with the corresponding aqueous metal salt solutions with constant stirring. The resulting mixture was refluxed on a water bath for about 4-6 hrs. A coloured product appeared on standing and cooling the solution. The complexes were filtered, thoroughly washed with ethanol, ether and dried under reduced pressure over anhydrous $Cacl_2$ in a desiccator.

Determination of antimicrobial activity

The invitro biological activity of the Schiff base and its metal complexes in DMSO were tested against the bacteria *Klebsiella pneumonia, Pseudomonas aeroginosa , E.Coli, Staphylococus aureus and Proteus* by disc diffusion method using nutrient agar as medium and *Amikacin* as control. The antifungal activities of the compounds were also tested by the in vitro well diffusion method against the fungi *Aspergillusniger*, on potato dextrose agar as the medium and *Flucanazole* as control. In a typical procedure, a well was made on agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated 24 hrs for bacteria at 37^0 C and 72 hrs for fungi at 30^0 C. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed at which the concentration was noted. The antimicrobial activities were estimated based on the size of inhibition zone in the discs [12-15]. From the results, the activity index was calculated using the following formula.

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Nuclease Activity studies

Gel Electrophoresis

Cleavage reactions were run between complexes and the calf Thymus DNA performed by gel electrophoresis [16] experiment and the solutions were diluted with loading dye using 1% agarose gel. 1X tris acetate EDTA buffer was prepared by mixing an appropriate concentration of 1ml 50 X TAE buffer with 49 ml distilled water, followed by addition of 0.5g of powdered agarose and mixed well. The solution was heated to boiling to dissolve agarose completely.The completely dissolved agarose gel solution was kept in the water bath at 65 ^{o}c. Then 3µlof ethidium bromide (0.5 µg /ml) was added to the above solution and mixed well. The warmed agarose was poured and clamped immediately with comb to form sample wells. After setting (30-40 ^{o}c at room temperature) comb was removed and taped. The gel was mounted into electrophoretic tank. Enough electroµphoretic buffers were added to cover the gel to a depth of about 1 mmol. The DNA sample (30 µm), 50µm metal complex and 500 µm H_2O_2 in 50 m mol tris-Hcl buffer (P^H=7.1) were mixed with loading dye and loaded into the well of the submerged gel using a micro pipette. The electric current was passed into running buffer. The sample was running from negative to positive pole. After 1-2 hours the gel was taken out from the buffer. After electrophoresis, the gel was photographed under UV transluminator [17].

Synthesis of Metal complexes

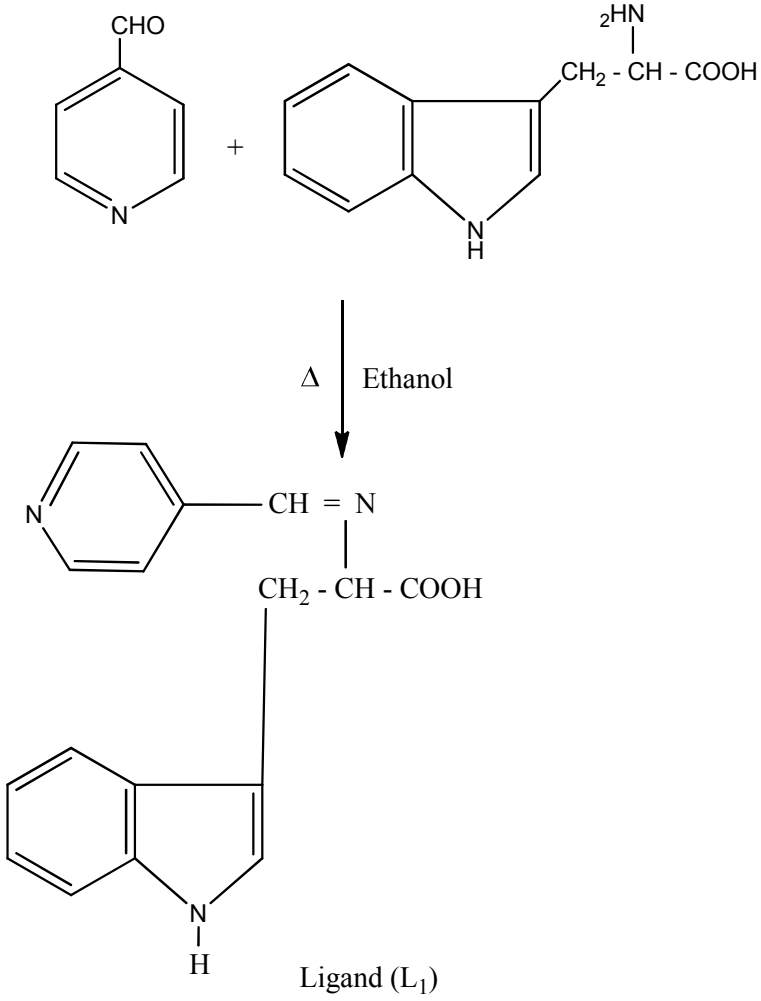
Hot methanolic solution of ligand L_1 and hot aqueous solution of Cu (II), Ni(II), Zn(II) and Co(II) metal salts were mixed with stirring. The mixture was refluxed for 2-3 hours at 70-80^0c on a water bath. On cooling coloured solid metal complex were precipitated .The precipitated product was filtered, washed with cold methanol and dried over anhydrous $Cacl_2$ in a desiccator.

Characterization

Elemental Analysis

The Schiff base and its metal complexes were subjected to elemental analysis. The results of elemental analysis (C, H and N) with molecular formula are presented in Table 1. The results obtained are in good agreement with those calculated for suggested formula.

Synthesis of Schiff base (L_1) : 4-Pyridine carboxaldehyde with tryptophan:



Scheme-1

Table.1 Analytical and Physical data of Schiff base and its complexes

Compound	Colour Yield (%)	M.P (^{0}c)	Calculated (found) %			μ (BM)
			C	H	N	
$C_{17}H_{15}N_3O_2(L_1)$	80% Colourless	185	69.62 (68.98)	5.12 (5.61)	14.33 (14.91).	
[Cu $L_1(No_3)_2$] $2H_2O$	65% Green	210	39.49(39.11)	3.68(2.89)	13.55(14.01)	1.9
[Ni $L_1(No_3)_2$] $2H_2O$	60% greenish yellow	202	39.87(41.01	3.71(3.99)	13.68(14.12)	3.1
[ZnL_1 $(No_3)_2$] $2H_2O$	50% Golden yellow	219	39.35(38.09)	3.66(3.72)	13.50(12.99)	3.0
[Co $L_1(No_3)_2$] $2H_2O$	45% Dark golden yellow	221	39.84(40.81	3.71(3.60)	13.67(14.20)	4.8

IR Spectra

The determination of coordinating atoms was made on the basis of the comparison of the IR spectra of the ligands and the complexes. Selected FT- IR frequencies are given in Table 2.

The IR spectrum of the free ligand is characterized by a band at 3406.63 cm^{-1} is assigned to the stretching vibration of N-H group . The band observed at 1667.59 cm^{-1} region in the ligand is assigned to the azomethine group (-C = N). The shift of this absorption band in metal complexes indicates coordination of the pyridine nitrogen atom with the metal ion. The Schiff base ligand show a peak at 846 cm^{-1}, 682cm^{-1}, 1357cm^{-1}, are due to the vibrations of 4-substituted pyrrole ring, CH_2 – group C-H string vibrations respectively. The IR spectrum of the free ligand is characterized by the strong bands at 2852cm^{-1}, and 2960 cm^{-1} which are attributed to the stretching frequencies of C – O and C - H respectively. In the complexes, the absorption bands in the range 2849-2815cm^{-1}, 2925-2900 cm^{-1} ,1618-1612 cm^{-1} are assigned to C – O, C – H and C = N respectively. Another absorption bands at 744-743 cm^{-1} is assigned to the coordinated nitrato group with the central metal atom and 699-648 cm^{-1} is assigned to M-O bond .In

addition, IR spectra of all metal complexes show a broad band around 3386-3246 and another band at 744-743 cm^{-1} at lower frequency region indicating the presence of water molecules in the coordination sphere [18].

The typical IR spectrum is given in figures 1,2,3,4 and 5.

Table 2. Selected FT-IR frequeneies (cm^{-1}) of the ligand and its complexes

Ligand/complex	υ_{O-H} (H_2O)	υ_{C-O}	υ_{C-H}	$\upsilon_{C=N}$	υ_{M-N}	υ_{M-O}
$C_{17}H_{15}N_3O_2$ (L_1)	-	2852.59	2960.41	1667.59	-	-
[Cu $L_1(No_3)_2$] $2H_2O$	3246.95	2815.11	2900.39	1618.01	699.53	744.42
[Ni $L_1(No_3)_2$] $2H_2O$	3331.48	2820.42	2905.31	1614.20	699.08	745.33
[Zn $L_1(No_3)_2$] $2H_2O$	3246.16	2849.58	2925.28	1618.28	698.67	743.97
[Co $L_1(No_3)_2$] $2H_2O$	3386.69	2818.21	2925.52	1612.08	648.48	744.55

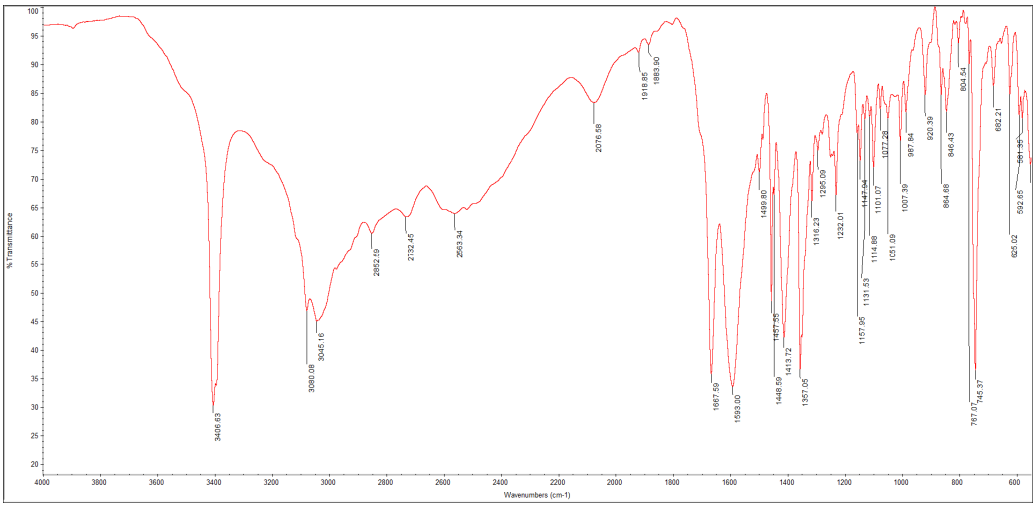


Fig 1.FTIR spectrum of a ligand

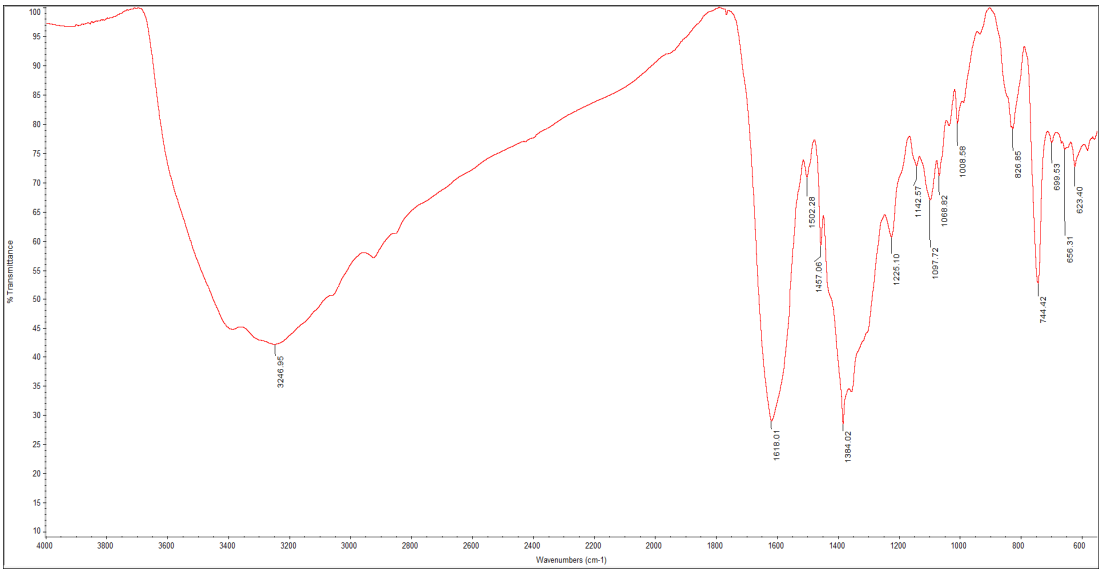


Fig 2.FTIR spectrum of Cu(II) complex

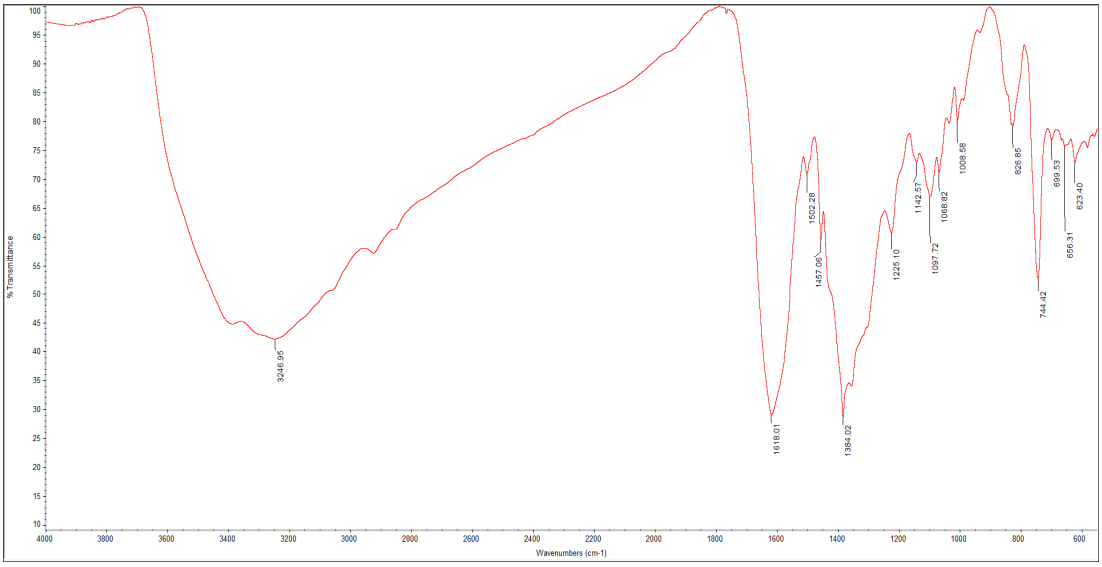


Fig 3.FTIR spectrum of Ni(II) complex

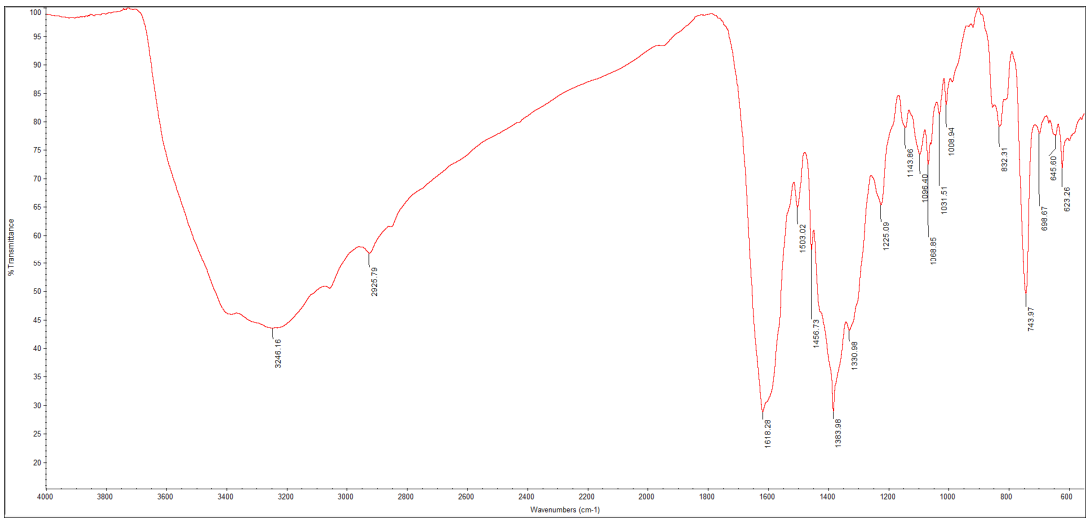


Fig4. FTIR spectrum of Zn(II) complex

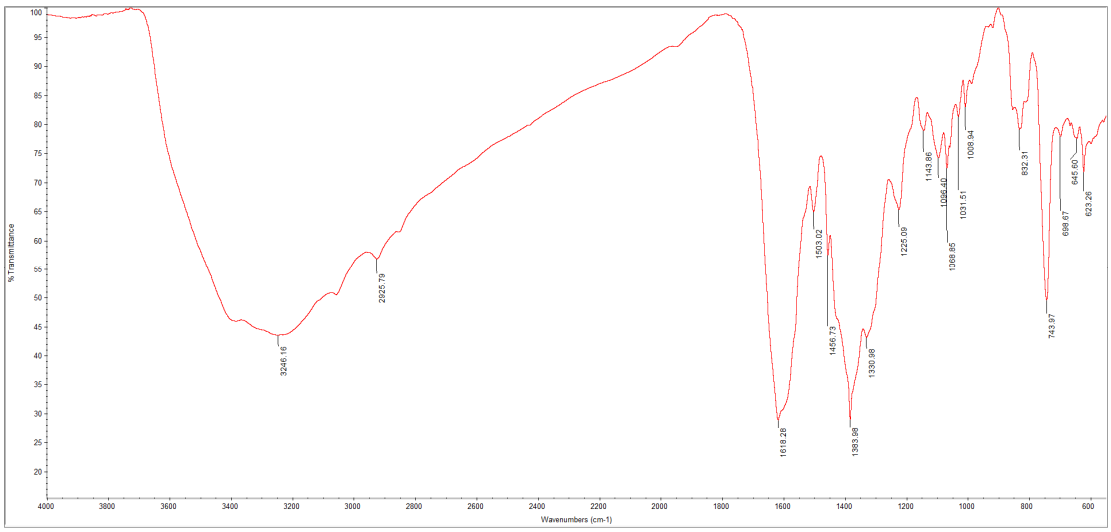


Fig 5. FTIR spectrum of Co(II) complex

Based on the above studies, the following structure is proposed. Fig. [6]

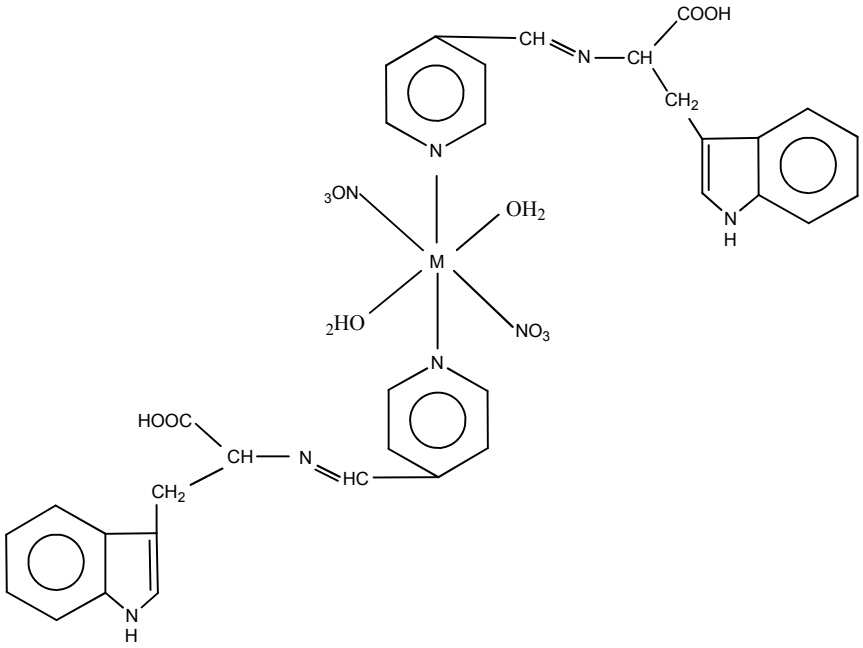


Fig 6 .Proposed structure of Schiff base complexes of Cu(II), Ni(II), Zn(II) and Co(II)
M= Cu (II), Ni(II), Zn(II) and Co(II)

DNA Cleavage activity:

The oxidative cleavage activity of the complexes was studied by gel electrophoresis using calf thymus DNA in tris-Hcl buffer. Selected CT - DNA cleavage activity of the gel diagram is shown in fig.7. The greater cleavage efficiency of the complexes compared to that of the control DNA is due to their efficient DNA - binding ability. All systems showed the same electrophoretic behavior and cleavage activity against CT - DNA.

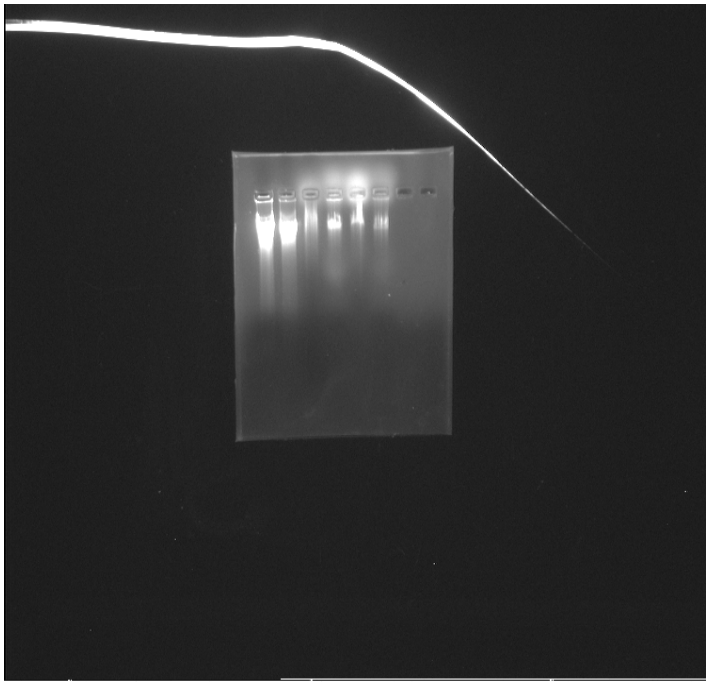


Fig .7 .Gel diagram for M (11) complexes

M= Cu(11), Ni(11), Zn(11) and Co(11)
Lane 1-Control DNA, Lane 2-DNA treated with DMSO, Lane 3-DNA+Cu (11) complex, Lane 4-DNA + Zn (11) complex, Lane 5 DNA + Ni (II) complex, Lane 6 DNA + Co (II) complex

Antimicrobial activity

The antimicrobial activity results of the Schiff base and its complexes are presented in Table 3.The Schiff base ligand and its complexes were characterized and screened for the biological activity. The complexes exhibited higher lethal effect on bacteria than their parent ligand [19].

The results of antibacterial activity substantiate the findings of earlier researchers that biologically active compounds become active and less biologically active compounds become more active upon coordination. Such enhancement in biological activity of metal complexes can be explained on the basis of overtone's concept and chelation theory [20]. According to overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage as only lipid soluble materials due to which liposolubility is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π -electron over the whole chelate ring and enhanced lipophilicity of the complex. Thus inhibiting the growth of bacteria and fungi are more potent than the parent Schiff base [21-22]. The antimicrobial activity significantly increased on coordination [23]. The present investigation suggest that all the metal complexes of the ligand bearing metal ion, pyridine ring, indole ring, -N=CH group have comparatively more biological activity.

Table.3Antimicrobial activities of ligand and its complexes

Ligand/ complex	Antibacterial activity (mm)					Antifungal activity (mm)
	Klebsieela pneuronia	*Pseudomonas aeroginosa*	***E. Coli***	***Staphylocous aureus***	*Proteus*	***Aspergillus niger***
$C_{17}H_{15}N_3O_2(L_1)$	6	7	7	8	9	8
$[Cu\ L_1(No_3)_2]\ 2H_2O$	8	8	9	10	11	10
$[Ni\ L_1(No_3)_2]\ 2H_2O$	8	10	8	10	9	10
$[Zn\ L_1\ (No_3)_2]\ 2H_2O$	9	8	11	9	10	7
$[Co\ L_1(No_3)_2]\ 2H_2O$	8	8	7	8	8	9
Amikacin	20	25	18	28	23	-
Flucanazole	-	-	-	-	-	28

Table .4 The activity index values of the ligand and its complexes

Ligand/complex	Antibacterial activity (mm)					Antifungal activity (mm)
	Klebsiella pneumonia	*Pseudomonas aeroginosa*	*E. Coli*	*Staphylococus aureus*	*Proteus*	*Aspergillus niger*
$C_{17}H_{15}N_3O_2(L_1)$	30	28	38	28	39	28
$[Cu\ L_1(No_3)_2]\ 2H_2O$	40	32	50	35	47	35
$[Ni\ L_1(No_3)_2]\ 2H_2O$	40	40	44	35	39	35
$[Zn\ L_1(No_3)_2]\ 2H_2O$	45	32	61	32	43	25
$[Co\ L_1(No_3)_2]\ 2H_2O$	40	32	38	28	34	32

CONCLUSION

The Schiff base ligand synthesized from 4-pyridine carboxaldehyde and tryptophan formed stable schiff base complexes with transition metal ions such as Copper (11), Nickel (11), Zinc (11), and cobalt (11) in ethanol. The ligand and its complexes are characterized using spectral and analytical data. From the spectral and stoichiometric analysis, hexacoordinated geometry was assigned for the metal complexes. The nucleolytic cleavage activity of all the complexes was examined on CT-DNA by using a gel electrophoresis experiment. All complexes showed significant nuclease activity. The antimicrobial studies reveal that the compounds possess significant activity against all the tested organisms.

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