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## Synthesis, Characterization and Biological Activities of Heterocyclic Schiff Base and Its Metal Complexes

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### ABSTRACT

*Metal complexes of some transition metal ions viz Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) have been synthesized from a Schiff base (L) formed by the condensation of 3-amino-5-bromobenzofuran-2-carboxamide and 2-hydroxy-1-naphthaldehyde. Synthesized compounds have been studied by various spectroscopic techniques and evaluated for their DNA cleavage and antimicrobial activities. Antibacterial activities of ligand and its metal complexes have shown that the metal complexes are more active than their corresponding ligand.*

**Keywords:** Schiff base, Metal Complexes, Spectral studies, Biological activity studies.

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### INTRODUCTION

Schiff bases derived from Benzofuran are studied extensively due to their easy synthesis, stability towards normal environmental conditions and having excellent biological properties such as antibacterial and antifungal activities [1]. Bromine is one of among 92 naturally occurring chemical elements in the nature and is the 28<sup>th</sup> most essential element needed for tissue development in all animals including us. A recent study shows that certain fruit fly *Drosophila melanogaster* dies when bromine removed from their diet, but found survived when bromine kept restored. This result has important applications for human disease [2]. It is found that the introduction of the bromine into the ligand enhances its biological activities[3]. In view of the importance of the benzofuran Schiff base and its metal complexes in the animal kingdom and in continuation of our work on the study of Schiff base and their metal complexes, herein we report new Schiff base formed by the fusion of biologically active heterocyclic aldehydes 2-hydroxy-1-naphthaldehyde and 3-amino-5-bromobenzofuran-2-carboxamide could result in valuable chemical and biological properties. Furthermore, insertion of metal ions like Co (II), Ni (II), Cu (II), Zn (II), Cd (II) and Hg (II) could result in synthesizing most biologically active compounds and also for the design of new therapeutic agents with a special significance in medicinal chemistry [4]. All the synthesized compounds have been studied by various spectroscopic techniques and evaluated for their different biological activities.

## MATERIALS AND METHODS

All the chemicals used are of AR grade. Benzofuran-2-carboxamide was synthesized as per the literature procedure [5]. The metal content were determined according to the *Vogel's* procedure [6].

**Synthesis of Schiff base: 5-Bromo-3-(((2-hydroxynaphthalen-1-yl) methylene) amine) benzofuran-2-carboxamide [L]:** A mixture of 3-amino-5-bromobenzofuran-2-carboxamide (0.1 mol) and 2-hydroxy-1-naphthaldehyde (0.1 mol) in 30 mL ethanol are refluxed on water bath for about 8h in the presence of a few drops of glacial acetic acid with occasional shaking. The product that was separated out as crystalline solids on cooling was collected and recrystallized from ethanol (Fig. 1)

[C<sub>19</sub>H<sub>21</sub>BrN<sub>2</sub>O<sub>2</sub>] [L]: Molecular Weight = 408.81, M.P. = 210°C, Yield= 65%.

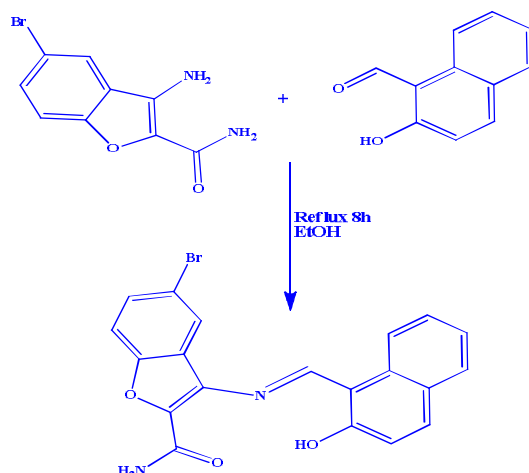


Fig. 1. Synthesis of Schiff base [L]

**General procedure for preparation of metal complexes of Co (II), Ni (II), Cu (II), Zn (II), Cd (II) and Hg (II) with the Schiff base (L):** A Metal chloride (0.01 mol) was mixed with a Schiff base (0.01 mol) in 30 mL ethanolic medium and reaction mixture was refluxed for about 3h on a water bath, then an aqueous solution of sodium acetate was added to the mixture to adjust the pH between 5 to 6 and further refluxed for about an hour. The complexes separated were filtered off, washed with distilled water, then with alcohol, and finally dried in vacuum over fused calcium chloride.

**Physical Measurements:** The FTIR spectra of a Schiff base and its metal(II) complexes were recorded in KBr pellets in the range of 4000 – 400 cm<sup>-1</sup> at 'SAIF IIT Bombay'. The proton magnetic resonance spectra of Schiff base ligand was recorded on 'AMX-400 NMR Spectrometer at 'SAIF IIT Bombay' using TMS as an internal standard and DMSO-d<sub>6</sub> as a solvent. Thermogravimetric studies of the complexes were carried out on a Parkin Elmar Thermal Analyser at a heating rate of 10 °C per min from 28-1000 °C in nitrogen atmosphere. The electronic spectral analysis of the Co(II), Ni(II) and Cu(II) complexes were carried out on an Elico-SL-164 double beam UV-Visible spectrophotometer in the range 250 – 900 nm in DMF (10<sup>-3</sup> M) solution. Molar conductivity measurement was recorded on an Elico CM-180 conductivity bridge in DMF (10<sup>-3</sup> M) solution using dip-type conductivity cell fitted with a platinum electrode. The powder X-ray diffraction pattern of ligand [L] and its Zn(II) complex have been recorded with Cu-Kα X-ray source in the range 3-80°. In the light of interesting antimicrobial activities, the synthesized ligands and their metal complexes were screened for their antibacterial activity by agar diffusion method [7,8] at 'BioGenics' Hubballi Activity was determined by measuring the diameter of the zone showing complete inhibition.

**DNA cleavage experiment:** All the metal complexes are screened for their DNA cleavage activity [8]. DNA cleavage experiments were done according to the procedure followed in our previous research article [9].

## RESULTS AND DISCUSSION

The physical appearance and analytical results show that all the metal complexes are of the type  $ML_2$  (Where  $M = Co/Ni/Cu/Zn/Cd/Hg$ ) (Table -1) and possess 1:2 metal –Ligand ratio. Lower molar conductance values indicate the non-electrolytic nature of the complexes. All the synthesized complexes are, stable and non-hygroscopic at normal temperature and pressure with high ( $>300\text{ }^{\circ}C$ ) melting points.

**Infrared spectral studies:** IR spectral study of ligand and complexes has an immense importance in the evaluation most characteristic frequency appeared at a comparative interpretation of IR spectra of nature, of the metal ligand bond, molecular symmetry, electronic distribution and stability of the complexes [10-12]. The important IR spectral bands for the synthesized ligand (L) and its metal complexes are presented in the Table-2. In the IR spectra of the ligand the data suggest that ligand (L) acts as a bidentate ligand in the synthesized complexes, by means of carbonyl oxygen and azomethine nitrogen as donor atoms. The IR spectrum of the ligand (L) shows the A strong band at  $3284\text{ cm}^{-1}$  attributed to  $\nu$  (N-H) stretching vibrations of the  $NH_2$  group [12], which is unchanged in the complexes indicating non-involvement of the nitrogen atom of  $NH_2$  group. The band at  $1680\text{ cm}^{-1}$  is due to the  $\nu$  (C=O) stretch and this frequency shifts to a lower value in the complex confirming the involvement of the (C=O) in the coordination with the metal ions[13].The stretching vibrational band of the (C=N) group of a ligand lies at a frequency of  $1609\text{ cm}^{-1}$ [14] and this band get shifted to the lower frequency in all the complexes and become weaker in some cases. This suggests the coordination of the C=N group through metal ions, additionally the bonding is supported by the appearance of new bands in the complexes those are absent in the ligand spectra in the region  $503\text{-}560$  and  $420\text{-}436\text{ cm}^{-1}$ . are assigned to  $\nu(M-N)$  and  $\nu(M-O)$  stretching vibrations respectively[12]. This indicates that carbonyl oxygen and azomethine nitrogen atoms are involved in coordination. Another medium intensity band, at  $\nu(3356)\text{ cm}^{-1}$  in the free ligands due to  $\nu(-OH)$ , was absent in the complexes, indicating deprotonation of the Schiff bases prior to coordination.

**UV-Vis Spectral Studies:** On the basis of electronic spectral data of the Co(II), Ni(II) and Cu(II) complexes and ligand field parameters such as  $Dq$ ,  $B'$ , L.F.S.E and  $\nu_2/\nu_1$  ratio[15] were calculated and recorded in the Table-3. The Co (II) ion possesses the electronic structure  $3d^7$  and occurs in both octahedral and tetrahedral geometry. The present Co(II) complexes exhibit two absorption bands at  $16150$  and  $20441\text{ cm}^{-1}$  in DMF ( $10^{-3}\text{ M}$ ) solution corresponding to  ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F) (\nu_2)$  and  ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) (\nu_3)$  transitions respectively, in an octahedral environment [14]. The  $\nu_1$  values were calculated using band fitting procedure [15]. The electronic spectra of Ni(II) complex exhibit two bands at  $15821$  and  $25581\text{ cm}^{-1}$ . These transitions are assigned to  ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F) \nu_2$  and  ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P) (\nu_3)$  respectively, in an octahedral environment. The Cu(II) ion with  $d^9$  configuration in the complex can be either a distorted octahedral or a tetrahedral. The cubic octahedral coordinated Cu(II) ion has the ground state  ${}^2E_g(t_{2g})^6, (e_g)^3$ . The only excited state should then be  ${}^2T_{2g}(t_{2g})^5, (e_g)^4$ , the energy difference being  $10Dq$ . The  ${}^2E_g$  ground state is highly susceptible to a Jahn (Teller configuration instability and then no regular octahedral coordinated Cu (II) complexes should exist and this is in accordance with the experimental findings of Orgal and Dunita [16]. The Cu(II) complex exhibit a single broad asymmetric band in the region  $13600\text{-}16350\text{ cm}^{-1}$ , the asymmetry being on the lower energy side. These observations suggest that the Cu (II) complex has a tetragonally distorted octahedral structure. The broadness of the band may be due to dynamic Jahn–Teller distortion.

**Table-1:** Physical characterization, analytical, molar conductance and magnetic susceptibility data of the ligand and the complexes

Molecular formula	F.W g mol <sup>-1</sup>	M.P. °C	Yield %	Elemental analysis (%) found/(calculated)				$\mu_{\text{eff}}$ B.M.	$\Lambda_M^\dagger$
				C	H	N	M		
Ligand (L) [C <sub>20</sub> H <sub>13</sub> BrN <sub>2</sub> O <sub>3</sub> ]	409.23	220	63	53.50 (53.62)	05.09 (5.44)	07.80 (7.20)	--		
[Co(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	875.38	348	72	54.88 (53.96)	2.76 (2.08)	6.40 (6.01)	6.73 (5.88)	4.86	13.60
[Ni(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	875.14	312	66	54.90 (53.33)	2.76 (2.12)	6.40 (6.17)	6.71 (6.15)	2.96	9.81
[Cu(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	880.00	310	65	54.59 (53.99)	2.75 (2.21)	6.37 (6.01)	7.22 (6.96)	1.67	08.10
[Zn(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	881.83	320	75	54.48 (53.42)	2.74 (2.00)	6.35 (5.89)	7.41 (6.96)	----	10.30
[Cd(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	928.86	330	68	51.72 (50.85)	2.60 (2.55)	6.03 (5.55)	12.10 (11.63)	----	13.32
[Hg(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	1017.04	340	75	47.24 (46.54)	2.38 (2.20)	5.51 (4.88)	19.72 (18.01)	----	12.10

 $\dagger \text{ohm}^{-1} \text{cm}^2 \text{mole}^{-1}$ **Table-2:** The important IR frequencies (cm<sup>-1</sup>) of ligand (L) and its metal complexes

Compound	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
Ligand (L) [C <sub>20</sub> H <sub>13</sub> BrN <sub>2</sub> O <sub>3</sub> ]	1680	1609	—	—
[Co(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ] <sub>n</sub>	1640	1600	503	425
[Ni(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	1655	1590	505	420
[Cu(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	1656	1585	560	435
[Zn(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	1666	1586	535	430
[Cd(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	1651	1588	541	432
[Hg(C <sub>20</sub> H <sub>12</sub> BrN <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> ]	1648	1587	542	436

**MASS Spectral Studies:** The mass spectrum is a pattern representing the distribution of ions by mass-to-charge ratio and gives valuable information about The molecular mass of the compounds. In the Fig.2 spectrum shows a well-defined peak at  $m/z = 409.2$  (M+1) which is exactly one mass unit more than that of the molecular mass of the ligand calculated from empirical formula (Table-1), this confirms the formation of the ligand (L).

**<sup>1</sup>HNMR Spectral Studies:** The <sup>1</sup>HNMR spectrum of the ligand (L) was recorded in DMSO-d<sub>6</sub> solvent Fig.3. The ligand (L), shows amide proton [-CONH<sub>2</sub>] at  $\delta$  10.028 (s, 1H) as a singlet. The azomethine proton [-N=CH-] has appeared at  $\delta$  8.436 (s, 1H) as a singlet, nine aromatic protons have resonated in the region  $\delta$  7.14–8.40 (m, 9H) as multiplet. The signal at  $\delta$  14.738 (s, H, -OH) is due to the proton of -OH group of phenyl ring [17].

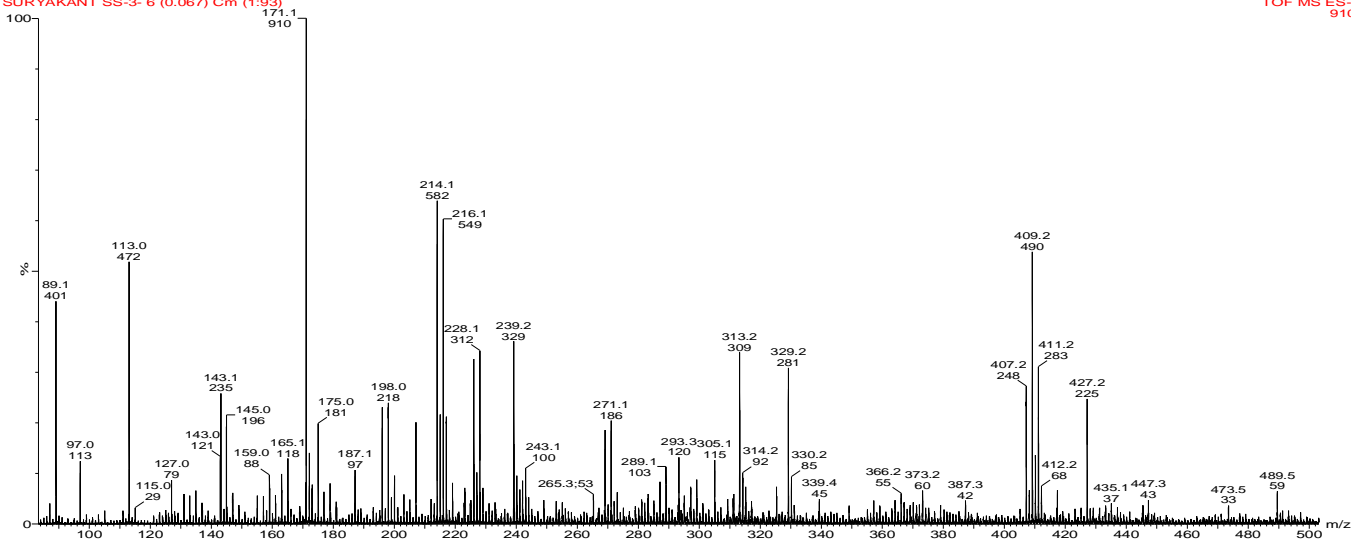
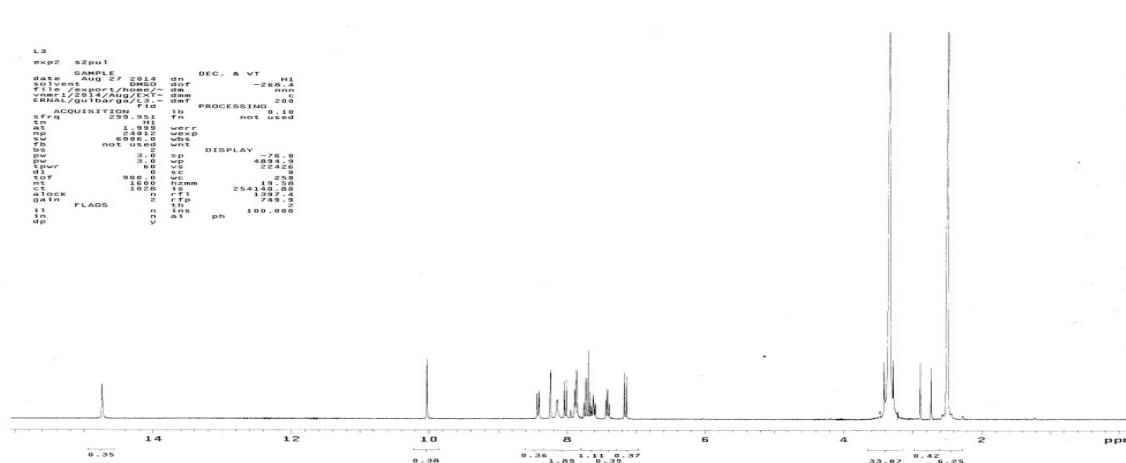
**Table. 3.** Electronic spectral bands and ligand field parameters of the Co(II), Ni(II) and Cu(II) complexes in DMF ( $10^{-3}$  M) solution

Complex	Transitions ( $\text{cm}^{-1}$ )			$Dq^\dagger$	$B'^\dagger$	$\beta$	$v_2/v_1$	LFSC (K.cal)
	$v_1^*$	$v_2$	$v_3$					
[Co(L) <sub>2</sub> ]	7300	1615 0	2044 1	864	937	0.97	2.2	14.81
[Ni(L) <sub>2</sub> ]	9920	1582 1	2558 1	992	775	0.75	1.6	34.02
[Cu(L) <sub>2</sub> ]	13600	- 16350		149 8	----	----		25.67

$L=(C_{20}H_{12}BrN_2O_3)$ ,  $^\dagger \text{cm}^{-1}$ , \*Calculated values

WATERS, Q-TOF MICROMASS (LC-MS)  
SURYAKANT SS-3-6 (0.067) Cm (1:93)

SAIF/CIL,PANJAB UNIVERSITY,CHANDIGARH  
TOF MS ES-910

**Fig.2-** LC-Mass spectrum of the Ligand [L]**Fig.3.**  $^1\text{H}$ NMR spectrum of the Ligand [L]

**ESR Spectral Analysis:** The ESR spectrum of Cu(II) complex at room temperature displayed a poorly resolved broad asymmetric signal with Hamiltonian parameters range  $g_{\parallel} = 2.4689$  and  $g_{\perp} = 2.0960$ ,  $g_{iso} = 2.2203$ ,  $g_{avg} = 2.2273$ ,  $A_{\parallel} = -0.0206$  and  $A_{\perp} = -0.0057$ . These values are in agreement with values for distorted octahedral structure with no hyperfine splitting in the parallel features. It is difficult to interpret quantitatively this broadband. If the exchange interaction parameters  $G$  (4.998) in the copper (II) complex is more than 4, indicating the negligible of copper-copper exchange coupling interaction between copper (II) centers in the solid state. [18]

**Powder X-ray Diffraction Studies:** The powder X-ray diffraction pattern of the ligand [L] is depicted in Fig. 4, shows seventeen reflections in the range 14-57°. The  $2\theta$  value with maximum intensity peak for the ligand was found to be 24.866° corresponding to interplanar distance 3.57778 Å. All the main peaks have been indexed by trial and error methods [19-21]. The interplanar distance 'd' calculated from the positions of the intense peaks using Bragg's relationship as given in equation  $n\lambda = 2d\sin\theta$ . The interplanar distance values obtained has been compared with calculated values. The comparison of these values revealed a good agreement between calculated and observed 'd' values. The unit cell calculations have been made for cubic symmetry of the ligand and its metal complexes. The characteristic of the cubic system is that  $\sin^2\theta$  values have a common factor. The cell parameters have been calculated by using the equation.

$$\sin^2\theta = \frac{\lambda^2}{4a^2}(h^2 + k^2 + l^2)$$

In the spectra there are no forbidden peaks indicating the cubic system in the complex. The powder X-ray diffraction pattern of Zn(II) complex is depicted in Fig. 5, shows fifteen reflections in the range 8-57°. The  $2\theta$  value with maximum intensity peak for the complex was found to be 44.434° corresponding to interplanar distance 2.0372 Å. In the spectra there are forbidden peaks such as 15, 23, 27 and 39 indicating the absence of a cubic system in the complex.

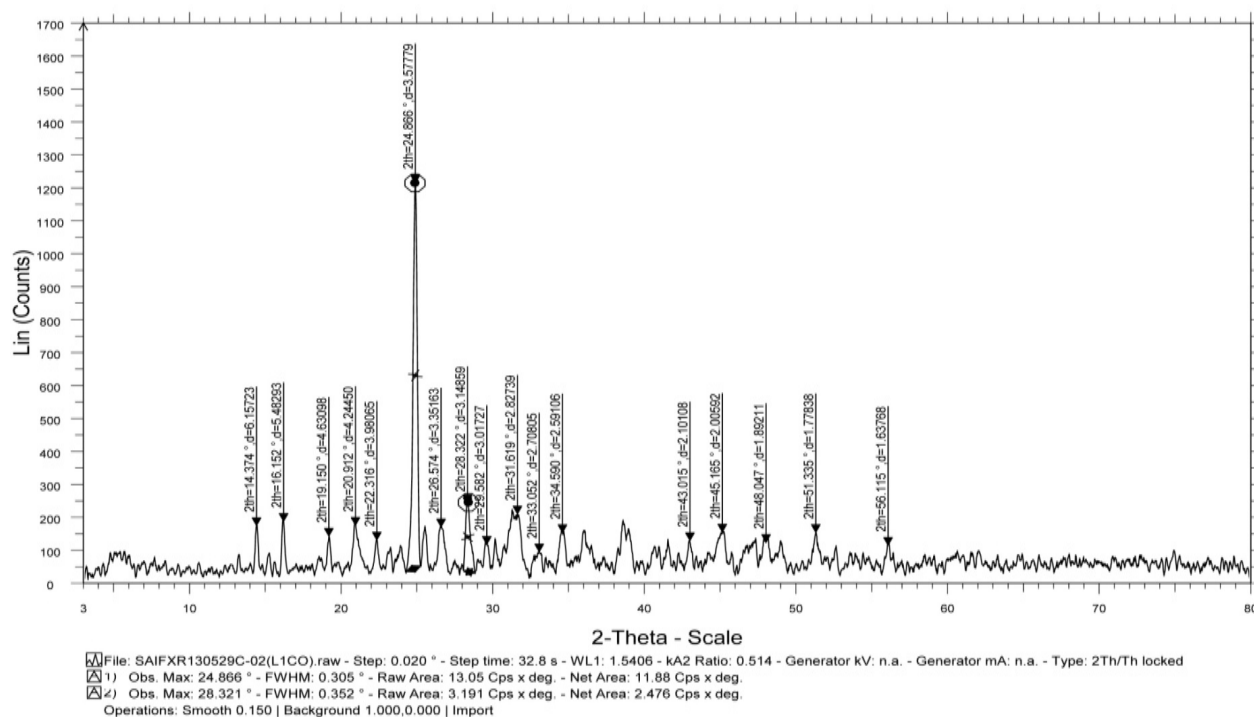


Fig. 4. Powder X-ray Diffraction Spectrum of the ligand [L]



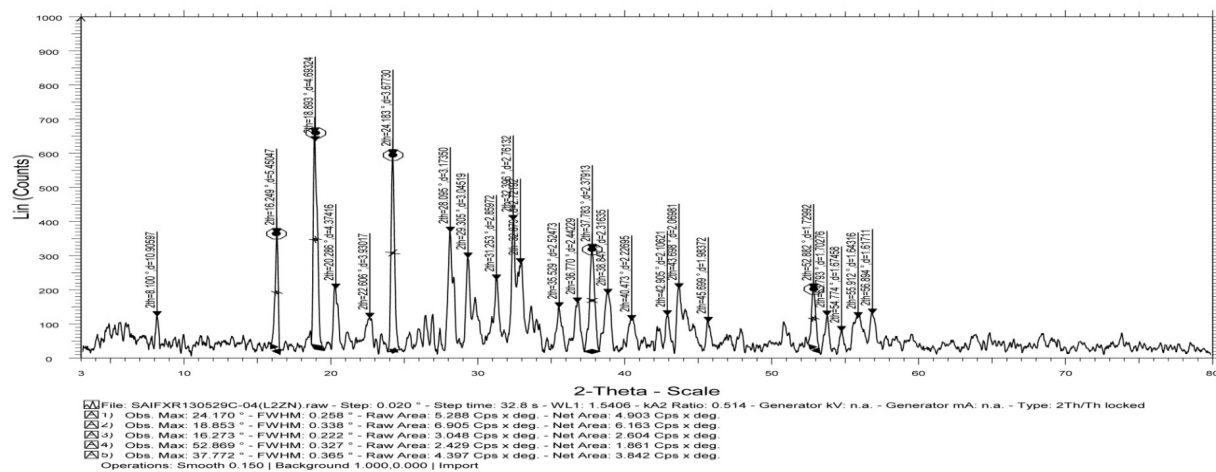


Fig. 5. Powder X-ray Diffraction Spectrum of the Zinc complex of the ligand [L]

**Antibacterial Activity:** The *in vitro* antibacterial activity of Schiff base (L) and its metal complexes have been studied by agar diffusion method in DMF as a solvent against the bacteria *Escheria coli* and *S. aureus* in two concentrations of 25 and 12.5  $\mu\text{g mL}^{-1}$  ( Fig. 6). Gentamycin is used as a standard antibacterial drug. The results showed that the metal chelates are more toxic than its parent ligand against the same bacteria and under similar experimental conditions. The effect of metal toxicity on bacterial cell can be explained on the basis of Tweedy's Chelation theory [22]. Chelation reduces the polarity of the metal ion by sharing its positive charge with donor atoms and possible  $\pi$ -electron delocalization over the chelate ring. This kind of chelation increases the lipophilic character of the metal atom which consequently helps its penetration through the bacterial cell wall disturbing the cell processes. Metal ion on complexation also interrupts the respiration of the bacteria and blocks its protein synthesis which restricts further growth[23,24].

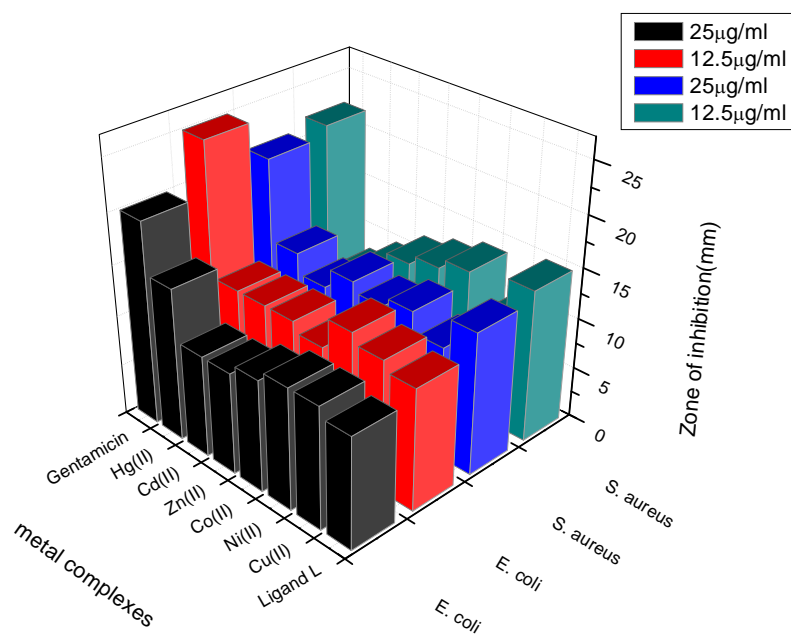


Fig. 6. Antibacterial activity of Ligand [L] and its metal complexes

**Electrophoretic analysis:** The Schiff base metal complexes subjected to DNA cleavage analysis by agarose gel Electrophoretic method and represented in the fig 7. The gel after the electrophoresis clearly revealed that the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The complete cleavage of DNA was observed by Co(II) and Ni(II) complexes (lane Na1 and Na2 respectively) and partial cleavage of DNA was observed by complexes Cu(II) (lane Na3) and Zn(II) (lane Na4) complexes. The differences observed in the bands of the complexes (lane Na-Na4) compared to that of the control DNA. This shows that the control DNA alone does not show any apparent cleavage whereas complexes shown. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes has not been clear. The results indicated the important role of metal ions in these isolated DNA cleavage reactions. As the compound was observed to cleave the DNA, it can be concluded that the compounds inhibit the growth of the pathogenic organism by cleaving the genome [25].

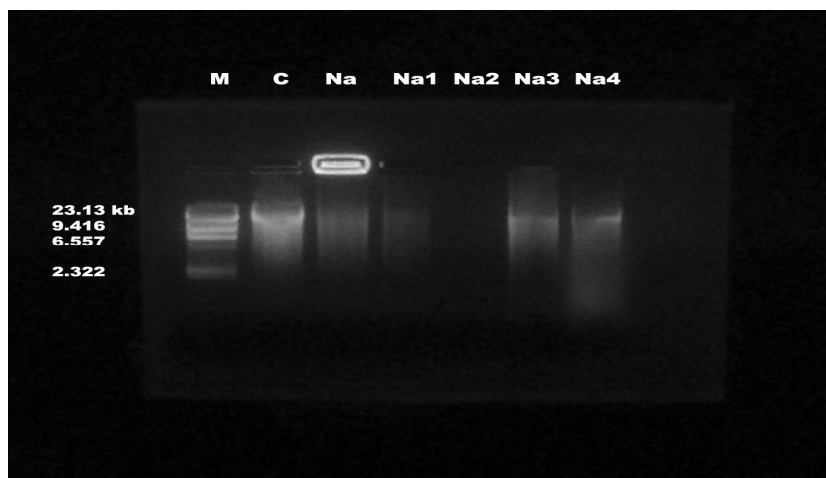


Fig. 7. DNA Cleavage study of the Ligand [L] and its Co(II), Ni(II), Cu(II) and Zn(II) complexes

## APPLICATIONS

Above synthesized ligand and its metal complexes are good chelating agents possess good antibacterial activity. Synthesized all complexes have cleaved the DNA completely or partially hence can acts as good DNA cleaving agents.

## CONCLUSIONS

On the basis of above results we conclude that the newly synthesized Schiff base acts as a monobasic tridentate ligand and it coordinates through the oxygen of the amide group, nitrogen of the azomethine group and oxygen of the hydroxyl group.

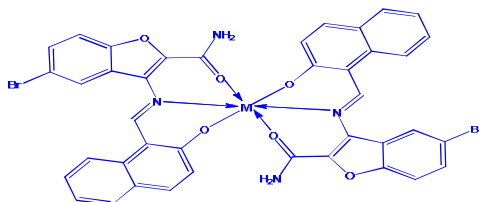


Fig.8. Proposed structure of Metal (II) complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) or Hg(II) of the Schiff base (L)



Analytical and spectral investigation reveals probable structures for all the complexes as dimeric octahedral. Antibacterial investigation shows that the metal complexes are moderately more active than its ligand [L] DNA cleavage study shows that two of the four complex cleaved the DNA fragment completely.

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