



**ANTI - ALZHEIMER, ANTICANCER AND ANTIMICROBIAL ASSESSMENT OF  
NOVEL TRIDENTATE AZO-SCHIFF BASE LIGAND AND IT'S METAL  
COMPLEXES**

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**ABSTRACT:** A series of metal complexes i.e. M=Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and VO(II) have been prepared by using novel azo-Schiff base ligand 4-((E)-(5-bromo-2-hydroxy-3-((E)-(3-hydroxypyridin-2-yl)diazenyl)benzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one and characterized by using spectroscopic techniques like: FT-IR spectra, <sup>1</sup>H-NMR spectra, Mass spectra, UV-Visible spectra, Elemental analysis, Thermal analysis, X-Ray powder diffraction, Molar conductivity etc. The anti-Alzheimer activity of the Schiff base ligand (L) and its metal complexes was conducted. This assessment involved testing against the SHSY-5Y Neuroblastoma cell line by using 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Anticancer activity was done by using MCF-7 human breast cancer cell line using Sulfo-Rhodamine-B-stain assay. Additionally antimicrobial assessments vs gram +ve bacteria and gram -ve bacteria and fungus.

**KEYWORDS:** Azo halogenated salicylaldehyde; Azo-imine; Anticancer; 4-aminoantipyrine; Antibacterial.

**INTRODUCTION:**

The azo-Schiff base molecules having pair of azo (R-N=N-R') and azomethine (R-C=N-R'). The azo-Schiff base i.e., Azo-imine molecules are significant ligand in inorganic and Bio-inorganic chemistry. They have created large applications in diverse field of science both biologically and chemically. These types of molecules have previously been scrutinized and are still being scrutinized for their diverse properties<sup>I,II</sup>. The azo-imine ligand and its transition metal complexes exhibit exciting biological activities like antibacterial, antifungal, intertubercular, hypertensive and anticancer activities<sup>III-V</sup>. As the azo molecule associated with novel category of chemical compounds, there is a growing interest in scientific research due to their amazing utility in many fields<sup>VI</sup>. The azo-imine was utilized in the applications of diverse field like dyeing pigment, polymer paper, paint and coating agency<sup>VII-IX</sup>. Derivatives of antipyrine Schiff base ligands exhibit antiparasitic agents and its metal complexes of Pt(II) and Co(II) ions exhibit antitumor agent<sup>X</sup>. The azo (-N=N-) groups and imine groups (>C=N-)

on azo-imine ligand are positioned in such manner that donation or participation of these two groups with a metal (II) atom is unacceptable. Hence preferred coordination of imine group at the same time the azo (-N=N-) group is left free and lack of coordination has been observed<sup>XI-XIV</sup>. Isatin based, Isovanillin based, 4-aminoantipyrine based Schiff base ligand exhibits anti-Alzheimer, anticancer, antioxidant activity<sup>XV-XVII</sup>. The azo-imine molecules contain heterogeneous ring which have tremendous importance to scientists and researchers because of their excellent biological, industrial, pharmaceutical and analytical applications i.e., they were utilized as guides and in the production of thin films. Also, they have tremendous importance in industries and utilized in the production of plastics, food colouring, optical switches, optical data storage, cosmetics, nonlinear optics, liquid crystal displays and photovoltaic devices<sup>XVIII-XIX</sup>. Azo molecules are utilized in food processing due to nontoxic effect, non-hyperactivity effect and little allergic reactions<sup>XX</sup>. The coordination complexes of transition metal ion with azo-imine ligand are contemporary attraction because the amazing chemical, physical, catalytic, photophysical, photochemical and diverse material applications<sup>XXI</sup>. They are interesting due to their electronic properties, sensitivity, synthetic adaptability, selectivity close to transition metal ion<sup>XXII</sup>. The azo-imine molecules and their transition metal complexes can engage in medical and pharmacological industry like antitumor, antioxidant, drug delivery molecular and drug polymer<sup>XXIII-XXIV</sup>. In view of above literature survey, we have focused on design and development of a novel Azo halogenated salicylaldehyde and their Schiff base ligand with 4-aminoantipyrine and synthesize the metal complexes.

## EXPERIMENTAL

### Chemicals and Materials

All the reagents were used of AR grade and purchased from Spectrochem Pvt. Ltd. Solvents such as ethyl alcohol; methyl alcohol was distilled and purified before use. The purity of the product and progress of reaction tracked by TLC (thin layer chromatography) using Aluminium sheet precoated with silica gel in petroleum ether-ethyl acetate mixed solvents. The structure of synthesized Azo halogenated salicylaldehyde, azo-imine ligands and its metal complexes were primarily confirmed by IR-spectra, recorded on a FTIR-spectrophotometer SHIMADZU as pressed KBr disks in region of 4000-400 $\text{cm}^{-1}$ . The <sup>1</sup>H-NMR spectra were performed on a BRUKER 500MHz<sup>1</sup>H-NMR instrument by  $\text{CDCl}_3$  as a solvent and tetramethylsilane ( $(\text{CH}_3)_4\text{Si}$ ) as an internal standard and also chemical shift values were reported in  $\delta$  parts per million (ppm). The study of thermal analysis of the complexes were recorded on TA instruments Trios V4.4.0.41128 TG/DSC thermal system under nitrogen atmosphere. EI-MS spectra were recorded on a BRUKER compass data analysis 4.2. The UV-visible spectrums were recorded on SHIMADZU Model 1600UV-spectrophotometer in the range of 800-200nm

### Synthesis of Azo halogenated salicylaldehyde (AHS)

The Azo halogenated salicylaldehyde named (E)-5-bromo-2-hydroxy-3-((3-hydroxypyridin-2-yl) diazenyl) benzaldehyde has been synthesized by the diazotization coupling reaction<sup>XXV</sup>.

**Solution 1:** In 100ml beaker 4.092g (0.0372mol) of 2-Amino-3-hydroxypyridine, added 25ml concentrated hydrochloric acid and 20ml cold distilled water. The solution was cooled at 0°C by using an ice-salt bath.

**Solution 2:** In a 100ml beaker 3.105g (0.045mol) of sodium nitrite dissolved in 15ml distilled water and the solution cooled at 0°C by using an ice-salt bath. When solution 1 and solution 2 reached 0°C temperature, solution 2 was added drop wise to solution 1 with constant stirring. While addition the reaction temp. Maintained below 10°C. A pinch of urea was added for decompose the excess nitrous acid and filtered the solution.

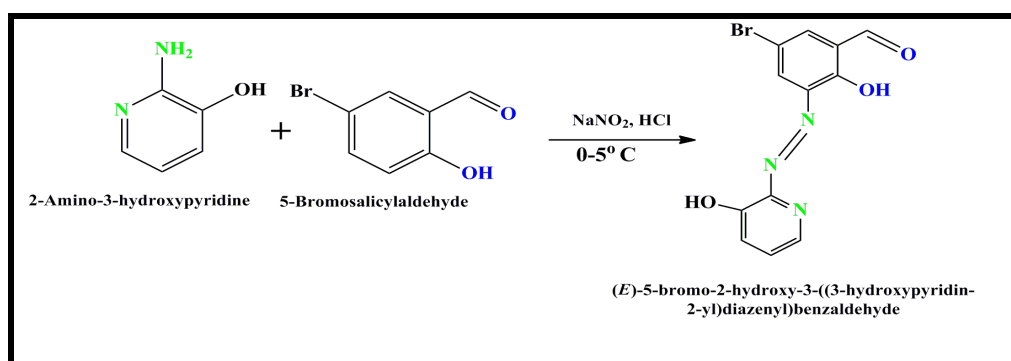
**Solution 3:** 7.477g (0.0372mol) of 5-Bromosalicylaldehyde dissolved in 10% of NaOH solution and stirred to make clear solution, cooled the solution to 0°C in ice-salt bath. Then added the above filtrate of solution 1 and Solution 2 to this aldehyde solution with constant stirring. After completion of the addition, the reaction mixture allowed to stand for 10 min in ice-salt bath. Rosy brown coloured product observed, Filtered, washes with cold distilled water and recrystallized and stored in air tight container. (**Scheme 1**)

### Synthesis of New azo-Schiff base ligand (L)

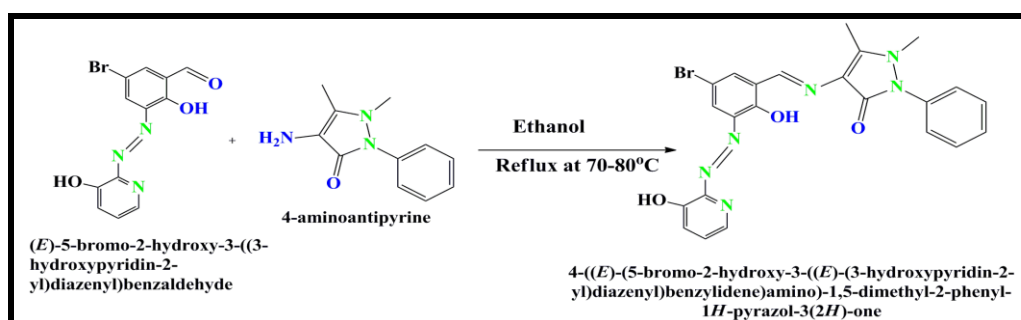
3.22g (0.01mol) of azo halogenated salicylaldehyde was dissolved in 30ml hot ethyl alcohol and 2.03g (0.01mol) of 4-aminoantipyrene was dissolved in 30ml ethyl alcohol separately. These both ethanolic solutions were mixed together and refluxed for 2-3 hours at 70-80°C. The reaction monitored by thin layer chromatography (TLC). The yellow-coloured solid obtained in reaction mixture at ambient temperature. The yellow solid product was filtered, washed with hot ethanol. (**Scheme 2**)

### Synthesis of metal complexes

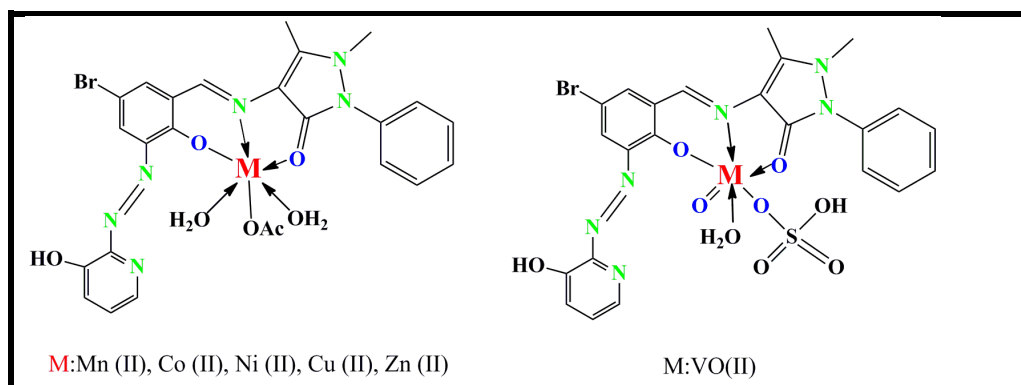
The metal complexes were synthesized by addition of equimolar solutions of acetate of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and sulphate of VO(II) to the azo-Schiff base ligand (L) (0.001mol) in 20ml hot ethyl alcohol at 70-90°C. The mixture was refluxed for 4-6 hour and resulting solid metal complexes obtained in reaction mixture. Metal complex filtered off, washed with ethyl alcohol and dried under vacuum. (**Scheme 3**)



**Scheme 1.** Synthesis of azo halogenated salicylaldehyde (AHS)



**Scheme 2.** Synthesis of novel azo-Schiff base ligand (L)



**Scheme 3.** Proposed structures of metal complexes

### Anti-Alzheimer assessment

The assessment of the Anti-Alzheimer of L and its metal complexes was carried out using 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay on the SHSY-5Y Neuroblastoma Cell line. The SHSY-5Y Neuroblastoma Cell line incubated at concentration of  $1 \times 10^4$  cells/ml in culture medium for 24 hrs at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  seeded at concentration (70 $\mu\text{l}$ )  $10^4$  cells/well in 100 $\mu\text{l}$  culture medium. 100 $\mu\text{l}$  Sample into micro plates respectively. Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated in 5%  $\text{CO}_2$  in  $\text{CO}_2$  incubator for 24 hrs at  $37^\circ\text{C}$ . After incubation, the medium was completely removed and added 20  $\mu\text{l}$  of MTT reagent. After addition of MTT, cells incubated for 4 hrs at  $37^\circ\text{C}$  in  $\text{CO}_2$  incubator. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark coloured formazan by viable cells only. After removing the medium completely, added 200 $\mu\text{l}$  of DMSO and incubate at  $37^\circ\text{C}$ . Triplicate samples were analysed by measuring the absorbance of each sample by an Elisa microplate reader at a wavelength of 570 nm.

### Anticancer assessment

Anticancer assessment of L and metal complexes was tested by using SRB (Sulfo-Rhodamine-B-Stain) assay on MCF-7 human breast cancer cell line. The cell line grows in a medium that contain 2 mM L-glutamine + 10 % fetal bovine serum. The densities of plates were 100  $\mu\text{L}$ , the cells were placed on 96 well microtiter plates for 24 hours, and an inoculated plate was incubated at a temperature of  $37^\circ\text{C}$  with 5 %  $\text{CO}_2$ , 95 % air and 100 % relative humidity. The 1 mg/ml solution of L and metal complexes were prepared in dimethyl sulfoxide (DMSO) and diluted up to 100  $\mu\text{g}$  /ml by water. 100  $\mu\text{L}$  of diluted solution added into microtiter plate was incubated for 48 hours. To complete the process, cold trichloroacetic acid (TCA) was added to wash the stain. The cell line was fixed, rinsed and stain with Sulfo-Rhodamine-B-Stain. The excess stain was rinsed with glacial acetic acid ( $\text{CH}_3\text{COOH}$ ) and the bound dye stain was eluted with 10 mM trizma base at 540 nm and reference wavelength of 690 nm the absorbance was measured and compared with standard 5-fluorouracil.

### Antimicrobial assessment

The antimicrobial assessment was done with the help of agar disk diffusion method as described by NCCLS 2002. Tetracycline and fluconazole used as +ve control for bacteria and fungi respectively. Firstly, petri dish was made by Mueller-Hinton agar (MHA) for bacteria and Potato Dextrose agar (PDA) for yeast. The preparation of McFarland 0.5 turbidity standards by using 0.5 ml barium chloride dihydrate ( $\text{BaCl}_2 \cdot \text{H}_2\text{O}$ ) solution was added to 99.5 ml of sulfuric acid with constant stirring to preserve suspension and stored in dark room at  $22^\circ\text{C}$  to  $25^\circ\text{C}$ . After that application of ligand and metal complexes to inoculated agar plates and each plate was observed. The zone of inhibition was uniformly circular and had confluent

lawn of growth. The diameters of the zones of complete inhibition which were assessed by the unaided eye and measured along with diameter in millimeter, through the use of sliding calipers.

### RESULTS AND DISCUSSION:

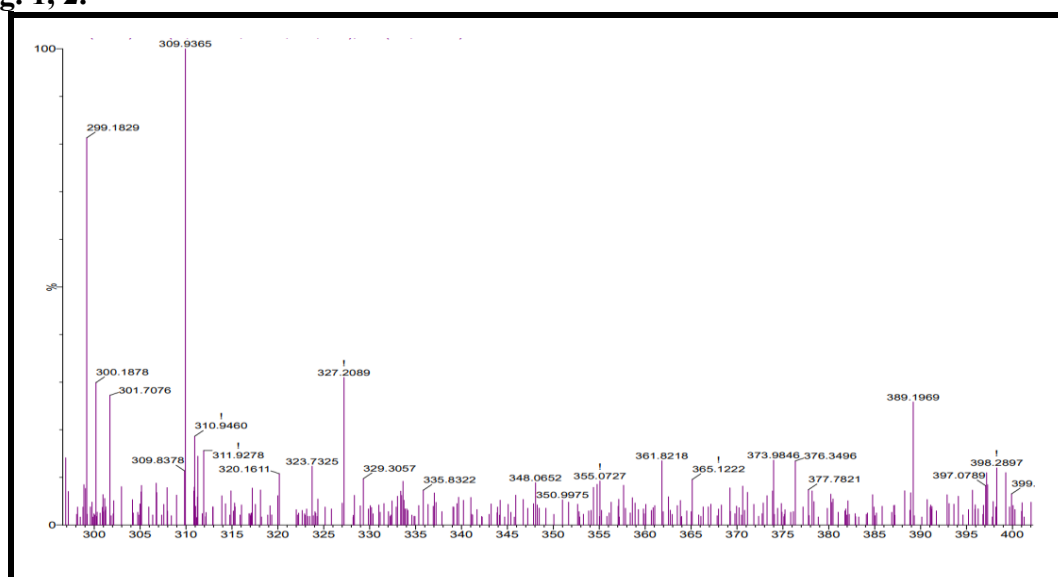
Synthesized azo halogenated salicylaldehyde, azo-Schiff base ligand and its metal complexes having diverse colours, freely soluble in ethanol, methanol, DMSO. They are stable in air. Some physical and chemical properties of AHS, L and metal complexes are recorded in **Table 1**.

**Table 1.** The physical and chemical data of AHS, L and metal complexes

Sr. No.	Sample	Colour	M.P. (°C)	Yield (%)	Elemental Analysis Found % (Calculated %)		
					C	H	N
1.	AHS	Rosy Brown	110°C	62%	44.21 (44.74)	2.20 (2.50)	12.65 (13.05)
2.	Ligand (L)	Pale Yellow	230°C	72%	53.80 (54.45)	3.52 (3.74)	15.90 (16.55)
3.	Mn (II) complex	Dark Yellow	>300	65%	46.80 (46.50)	4.15 (3.76)	12.70 (12.05)
4.	Co (II) complex	Brown	>300	81%	45.80 (46.24)	3.45 (3.74)	11.10 (11.98)
5.	Ni (II) complex	Pastel Green	>300	54%	45.65 (46.25)	3.41 (3.74)	11.21 (11.99)
6.	Cu (II) complex	Green	>300	71%	45.20 (45.93)	3.19 (3.71)	11.23 (11.90)
7.	Zn (II) complex	Yellow	>300	80%	45.65 (45.82)	3.55 (3.70)	11.53 (11.87)
8.	VO (II) complex	Emerald	>300	60%	45.98 (46.72)	2.95 (3.41)	13.87 (14.21)

### Mass spectra of AHS and L

The mass spectra show a peak at  $m/z$  323.73(M+1) and  $m/z$  at 507.94 indicates that formation of azo halogenated salicylaldehyde (AHS) and azo-Schiff base ligand (L) respectively shown in **Fig. 1, 2**.



**Fig. 1:** Mass spectrum of AHS

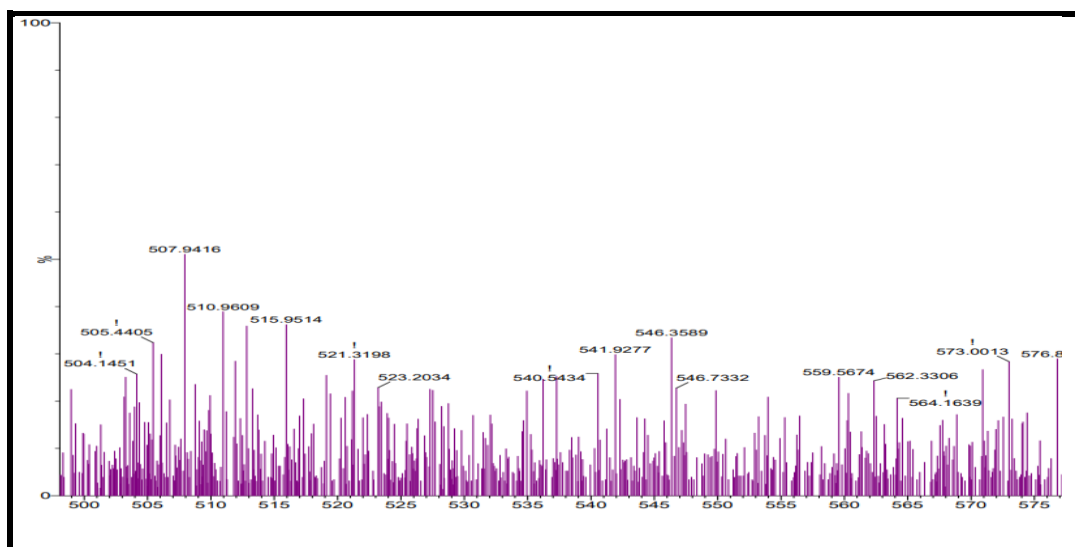


Fig. 2. Mass spectrum of L

### <sup>1</sup>H-NMR Spectra of AHS and L

In the spectrum of azo halogenated salicylaldehyde exposed signals at  $\delta=10.93$  ppm belongs to protons of hydroxyl group (-OH), the values  $\delta=6.90$ ppm- $7.68$ ppm multiplets exhibit phenyl ring,  $\delta=9.84$  ppm belongs to aldehyde proton (-CHO) it clearly indicates that formation of azo halogenated salicylaldehyde. The spectrum of azo-Schiff base ligand exposed signals a  $\delta=13.40$ ppm belongs to intramolecular hydrogen bonding of hydroxyl group (-OH) with 4-aminoantipyrine carbonyl group.  $\delta=9.79$ ppm belongs to the azomethine (HC=N) proton.  $\delta=7.31$ ppm- $7.57$ ppm belongs to proton of phenyl rings  $\delta=2.47$ ppm,  $3.25$ ppm belongs to  $>C-CH_3$  and  $>N-CH_3$ . Respectively<sup>XXVI</sup> presented in Fig. 3.

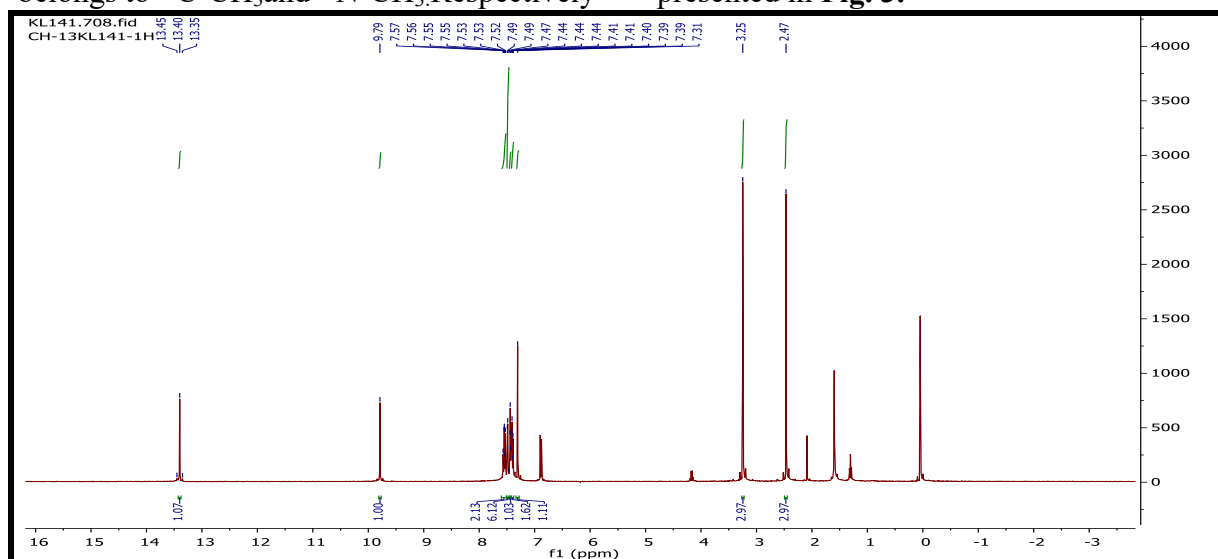


Fig. 3. <sup>1</sup>H-NMR Spectrum of azo-Schiff base ligand

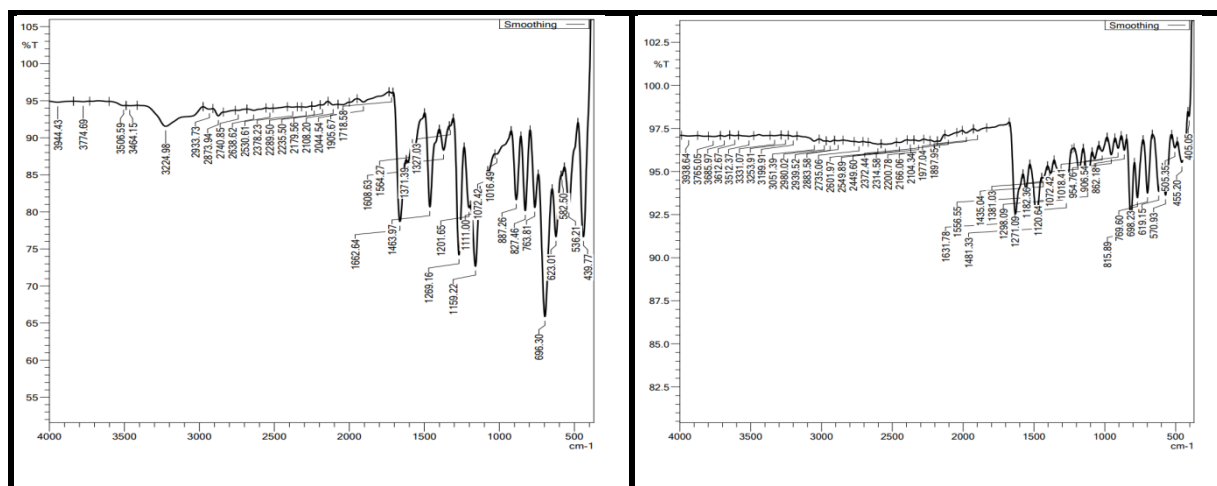
### IR spectra of AHS, L and metal complexes

The IR spectral data of azo halogenated salicylaldehyde, azo-Schiff base ligand along with its metal complexes enlisted in Table 2 and illustrated in Fig. 4, Fig. 5. The FT-IR spectrum of complexes was analysed with free azo-Schiff base ligand to determine the frequencies changes during the complexation i.e. The loss and shifting of IR peak were occur during the complexation process. The peak of azomethine group in azo Schiff base ligand (L) was found at  $1556\text{cm}^{-1}$  and shifted to lower frequency ranging from  $1460-1552\text{cm}^{-1}$  in the spectrum of all metal complexes. This clearly indicates that coordination of nitrogen atoms to the central

metal ions<sup>XXVII</sup>. The peak of carbonyl group is observed in azo-Schiff base ligand at  $1631\text{cm}^{-1}$  and shifted to lower or higher frequency ranging from  $1583\text{-}1635\text{cm}^{-1}$  it indicates that the donation of carbonyl oxygen in coordination<sup>XXVIII</sup>. The peak of phenolic hydroxyl group in azo Schiff base ligand is observed at  $3254$  and  $3512\text{cm}^{-1}$ . The peak of  $3512\text{cm}^{-1}$  is completely disappear in metal complexes, it indicates that the one phenolic oxygen atom involved in coordination by deprotonation. This was further proved via the peak of phenolic C-O in azo-Schiff base ligand is observed at  $1271\text{cm}^{-1}$  shifted to higher frequency from  $1282\text{-}1311\text{cm}^{-1}$ <sup>XXIX, XXX</sup>. The peak of azo group is observed at  $1381\text{cm}^{-1}$  in azo-Schiff base ligand. The peak of  $>\text{C-Br}$  observed at  $660\text{-}696\text{cm}^{-1}$  in azo-Schiff base ligand and metal complexes<sup>XXVI</sup>. The new bands observed in metal complexes at  $441\text{-}464\text{cm}^{-1}$  and  $580\text{-}586\text{cm}^{-1}$  assigned to M-N and M-O respectively.

**Table 2.** IR data of AHS, L and metal complexes

Frequencies in $\text{cm}^{-1}$									
Sr. No	Sample	-OH	$>\text{C}=\text{N}-$	$\text{C}=\text{O}$ (4-AAP)	$-\text{N}=\text{N}-$	$>\text{C-Br}$	$>\text{C-O}$	M-N	M-O
1.	AHS	3224,3506	--	--	1371	696	1269	--	--
2.	Ligand (L)	3254, 3512	1556	1631	1381	690	1271	--	--
3.	Mn (II)	3197	1460	1614	1373	675	1282	464	586
4.	Co (II)	3238	1552	1635	1379	671	1290	447	588
5.	Ni (II)	3215	1506	1577	1386	680	1292	447	584
6.	Cu (II)	3273	1504	1598	1384	686	1311	449	584
7.	Zn (II)	3224	1527	1624	1379	680	1303	443	586
8.	VO (II)	3203	1469	1583	1360	688	1303	441	580



**Fig. 4.** FTIR Spectrum of AHS and L



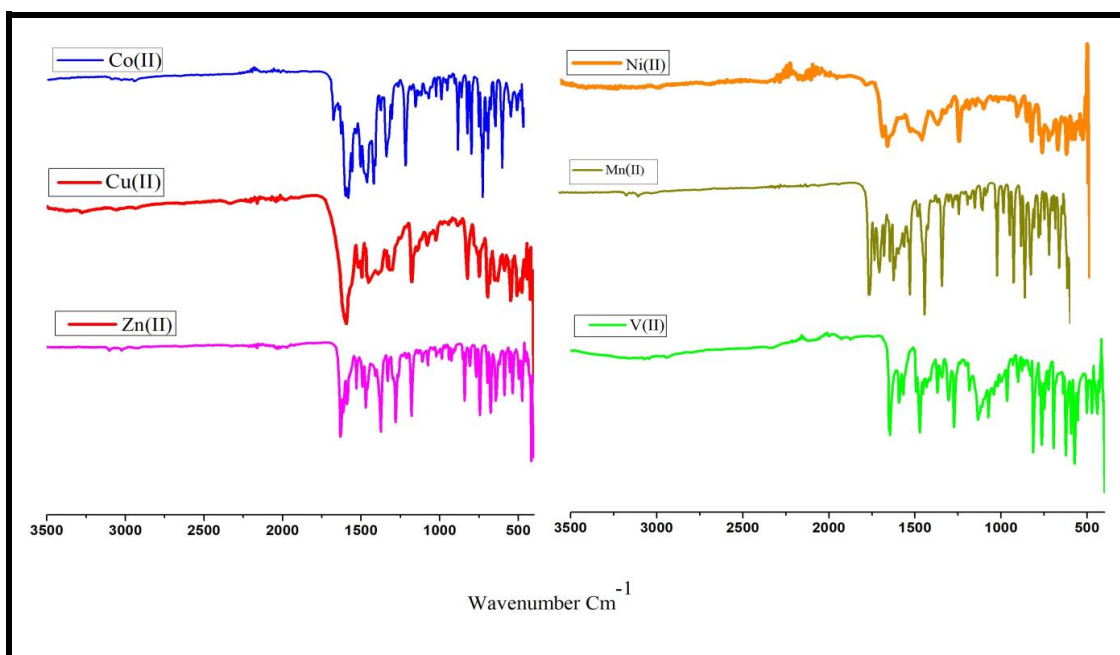


Fig. 5. FTIR Spectrum of metal complexes

#### UV-Visible spectra of L and metal complexes

The UV-Vis spectrum of azo-Schiff base ligand (L) and its metal complexes are listed in **Table 3** and presented in **Fig. 6**. The UV-Vis spectra were analysed in dimethylsulfoxide (DMSO) solvent in the region 200-600nm and molar range of 50000 to 16666cm<sup>-1</sup> <sup>XXVI, XXXI</sup>. The UV-Vis spectra were used to characteristics of the ligand field surrounding the central metal ion.

#### Measurement of Molar conductivity

Molar conductance measurements ( $\Lambda_m$ ) of azo-Schiff base ligand and metal complexes performed by using dimethyl sulfoxide (DMSO) solvent at concentration of 10<sup>-3</sup>M at RT. They show conductivity value ranged between 3.6-8.0 S.mol<sup>-1</sup> cm<sup>2</sup> that non-electrolytic in nature<sup>XXXII</sup> listed in **Table 3**.

**Table 3.** UV-Visible data of L and its metal complexes

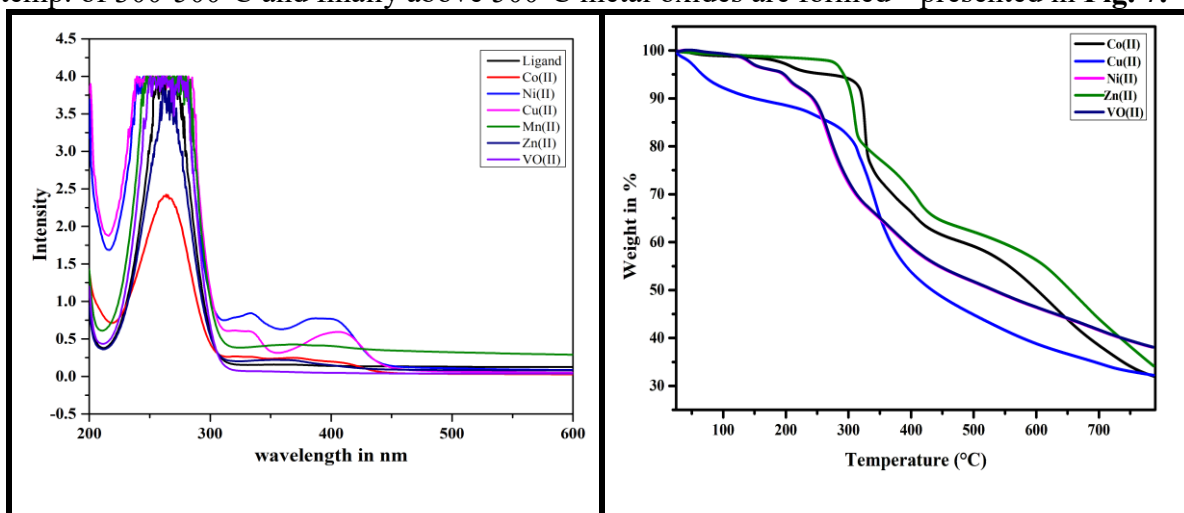
Sr. No.	Sample	$\lambda_{max}$ nm	Absorption Bands in cm <sup>-1</sup>	Tentative Transitions	Molar conductance in S.mol <sup>-1</sup> cm <sup>2</sup>
1.	Ligand (L)	290	38461	$n \rightarrow \pi^*$	1.6
		260	34482	$\pi \rightarrow \pi^*$	
2.	Mn(II)	360	27777	M→L, CT	6.2
3.	Co(II)	410	24390	${}^4T_{1g} \rightarrow {}^4T_{2g}$	6.2
		365	27397	${}^4T_{1g} \rightarrow {}^4T_{1g(P)}$	
		330	30303	${}^4T_{1g} \rightarrow {}^4A_{2g}$	
4.	Ni(II)	405	24691	${}^3A_{2g} \rightarrow {}^3T_{2g}$	8.0
		380	26315	${}^3A_{2g} \rightarrow {}^3T_{1g(f)}$	
		335	29850	${}^3A_{2g} \rightarrow {}^3T_{1g(P)}$	
5.	Cu(II)	415	24096	${}^2E_g \rightarrow {}^2T_{2g}$	6.9
		355	28169	${}^2E_g \rightarrow {}^2T_{2g}$	
		340	29411	L→M, CT	
6.	Zn(II)	355	28169	M→L, CT	5.1



7.	VO(II)	345	28985	${}^2B_2 \rightarrow {}^2E$	7.6
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### Thermal analysis

In the present investigation thermal analysis was performed to get significant details about the thermal stability of the metal complexes and whether the coordinated molecules are bounded inside the inner coordination sphere to the metal or outside<sup>XXXIII-XXXV</sup>. The thermal stability of metal complexes was examined from room temperature to 800°C in atmosphere of Nitrogen. Thermo-gravimetric analysis shows that the complexes decompose in three (3) steps, in initial step ionic water (up to 200°C) and coordinated water (200-300°C) molecule decomposes<sup>XXXVI</sup> then in the last step the loss of acetate group and ligand takes place, further stable metal oxides formed. The loss of acetate molecule and ligand take place in the range of temp. of 300-500°C and finally above 500°C metal oxides are formed<sup>37</sup> presented in **Fig. 7**.



**Fig. 6.** UV Spectra of L and metal complexes

**Fig. 7.** TGA of metal complexes

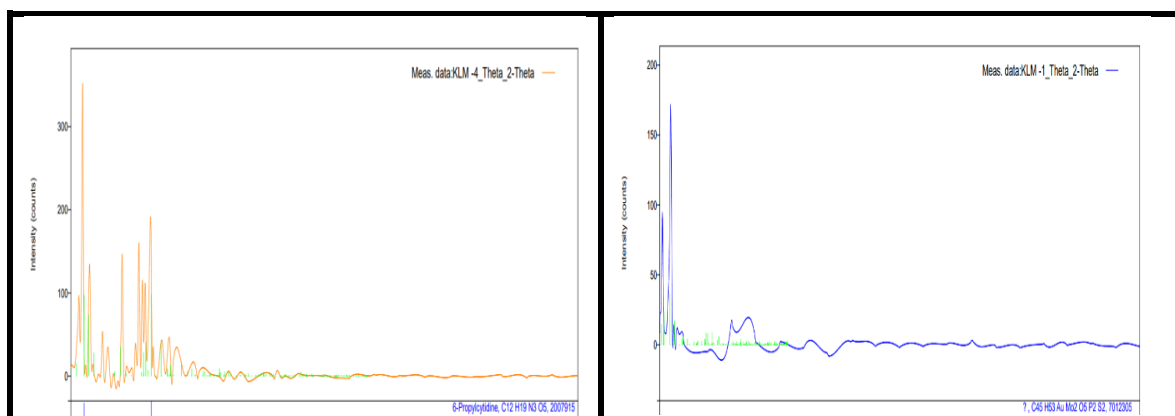
### Powder X-Ray Diffraction

The P-XRD of metal complexes derived from ligand were scanned in the range from  $2\theta = 20 - 80^\circ$  at wave length of 1.540Å. All information is given in **Table 4** and **Fig. 8**. The diffraction pattern exhibits the crystalline nature of metal complexes. Among the metal complexes Mn(II), Co(II), Cu(II), Zn(II) and VO(II) shows the monoclinic crystal system, the Ni(II) shows triclinic crystal system<sup>XXIX</sup>.

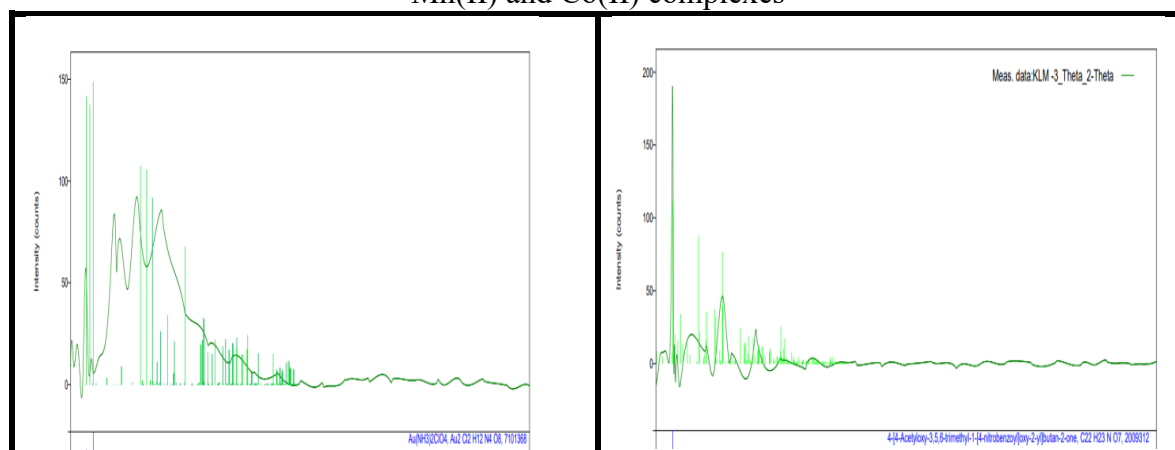
**Table 4.** P-XRD spectral data of metal complexes

Complexes	Mn(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)	VO(II)
No. of reflections	27	10	14	25	29	32
Maxima( $2\theta$ )	$57.11^0$	$30.84^0$	$49.08^0$	$40.68^0$	$35.97^0$	$40.40^0$
Intensity	9.5a.u.	100 a.u.	100 a.u.	100a.u.	100 a.u.	100 a. u
d value(Å)	10.508Å	14.428 Å	10.861Å	11.793Å	13.56 Å	10.352Å
Lattice constant (Å)	a=16.7340, b=7.8300, c= 12.0280	a=12.2628, b=12.867, c=15.5143	a=7.6791, b=7.9129, c=11.5690	a=12.1860, b= 8.7763, c= 20.4128	a=11.482,b =9.8752, c=27.2800	a=10.915 b=17.606, c=11.629
Unit cell volume	1376.793	2276.111	605.268	2112.681	3076.990	2119.380

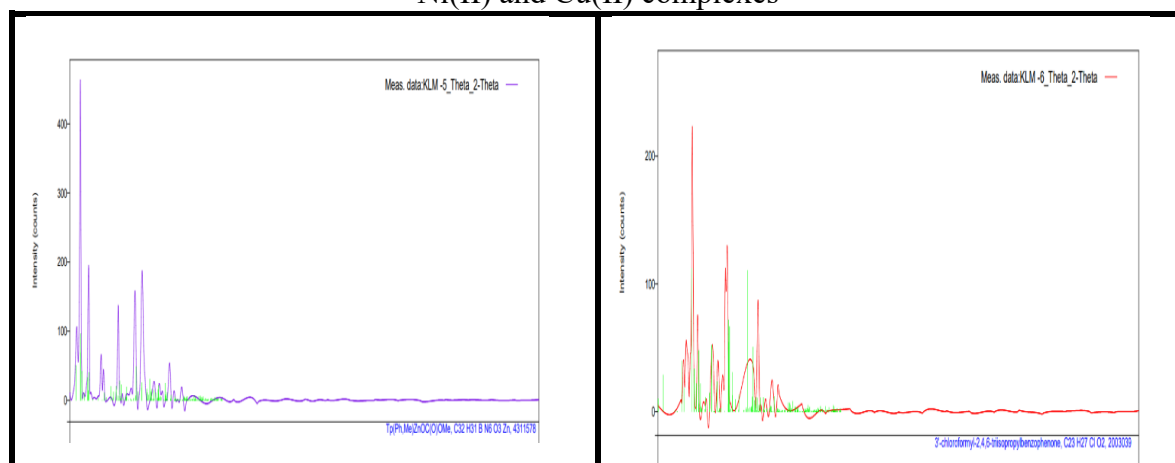
Axis and axis angle	$a \neq b \neq c$ and $\alpha = \gamma = 90^\circ \neq \beta$	$a \neq b \neq c$ and $\alpha = \gamma = 90^\circ \neq \beta$	$a \neq b \neq c$ and $\alpha \neq \beta \neq \gamma \neq 90^\circ$	$a \neq b \neq c$ and $\alpha = \gamma = 90^\circ \neq \beta$	$a \neq b \neq c$ and $\alpha = \gamma = 90^\circ \neq \beta$	$a \neq b \neq c$ and $\alpha = \gamma = 90^\circ \neq \beta$
Z Value	4	2	2	4	4	4
Crystal system	Monoclinic	Monoclinic	Triclinic	Monoclinic	Monoclinic	Monoclinic



Mn(II) and Co(II) complexes



Ni(II) and Cu(II) complexes



Zn(II) and VO(II) complexes

**Fig. 8.** P-XRD of metal complexes

**Anti-Alzheimer assessment**

The Anti-Alzheimer activity of synthesized azo-Schiff base ligand and their Co(II), Cu(II) and Zn(II) complexes were determined against SHSY-5Y Neuroblastoma Cell line with different concentrations at 25, 50,100 µg/ml. The present study revealed that the Cu(II) and Zn(II) complex demonstrated the better activity with IC50 value at 52.95, 50.31 µg/ml. The Co(II) complex shows lower activity with IC50 value at 68.55 µg/ml compared with other metal complexes.. From the finding we can conclude that Cu(II) and Zn(II) complexes were non-toxic to SHSY-5Y Neuroblastoma Cell line and exhibit significant neuroprotection than ligand in the comparison with standard reference drug tacrine<sup>XXXVIII, XXXIX</sup>. The Cu(II) and Zn(II) complexes of Schiff base ligand are often dysregulated in neurodegenerative diseases. In the Anti-Alzheimer activity increased cell viability is better because protecting the SHSY-5Y Neuroblastoma Cell line from damage which is desirable for neuroprotection and shown in **Table 5**.

**Table 5.** Anti-Alzheimer assessment of L and metal complexes

Sr. No	Compounds	Concentration of Sample(µg/ml)	Cell viability (%)	IC50
1.	TAC(Standard)	25	70.20	48.63
		50	77.41	
		100	78.68	
2.	L	25	39.80	78.56
		50	47.41	
		100	49.87	
3.	Co(II) complex	25	42.54	68.55
		50	48.78	
		100	60.69	
4.	Cu (II) complex	25	67.76	52.95
		50	68.73	
		100	72.62	
5.	Zn (II) complex	25	68.68	50.31
		50	70.40	
		100	78.06	

#### Anticancer Assessment

Anticancer assessment of the azo-Schiff base ligand (L) and its metal complexes was examined on MCF-7 human breast cancer cell line by using standard 5-fluorouracil. The synthesized metal complexes of Co(II) exhibit greater activity than azo-Schiff ligand (L) in the comparison with standard 5-fluorouracil<sup>XL,XLI</sup> listed in **Table 6**.

**Table 6.** Anticancer assessment of L and metal complexes

Sr. No	Sample	Concentration of Sample(µg/ml)	Inhibition (%)
1.	5-fluorouracil(standard)	20µg/ml	69.66
2.	Ligand (L)	100µg/ml	43.18
3.	Co (II) complex	100µg/ml	65.75
4.	Cu (II) complex	100µg/ml	50.29
5.	Zn (II) complex	100µg/ml	44.47

#### Antibacterial Assessment

The Antibacterial assessment of the azo-Schiff base ligand (L) and its metal complexes was screened in comparison with gram positive bacteria such as *Bacillus subtilis*, *Staphylococcus Aureus* and gram-negative bacteria like *Pseudomonas aeruginosa*, *Klebsiella pneumonia* by disc diffusion method. Tetracycline used as standard antibiotic. Metal complexes of Ni(II)

and Cu(II) exhibit higher activity than azo-Schiff base ligand <sup>XLII</sup> but less than standard antibiotic Tetracycline listed in **Table 7**.

### Antifungal Assessment

The Antifungal assessment of the synthesized azo-Schiff base ligand and its metal complexes was screened in comparison with *Penicillium Chrysogenum*, *Tricoderma Viride* and *Aspergillus Niger* by disc diffusion method. Fluconazole used as standard. Metal complexes of Co(II), Ni(II), Cu(II), Zn(II) and VO(II) exhibit better activity than azo-Schiff base ligand <sup>XLII</sup> but less than standard fluconazole listed in **Table 7**.

**Table 7.** Antibacterial and antifungal assessments L and metal complexes

Antibacterial Assessment								
Test Organism	Zone of inhibition (In mm)							
	Standard	L	Mn(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)	VO(II)
<i>S.aureus</i>	19	8	--	--	8.5	16	--	--
<i>B.subtilis</i>	25	--	--	11	11	10	--	9
<i>K.pneumoniae</i>	20	8	--	--	11	11.5	9	9
<i>P.aeruginosa</i>	19	--	--	--	19	13.5	--	--
Antifungal Assessment								
<i>P.Chrysogenum</i>	25	--	18	18	25	28	19	14
<i>T.Viride</i>	35	8	--	15	32.2	32.2	20	17.5
<i>A. Niger</i>	26	--	--	10	25	25	10	--

### CONCLUSION:

In this research work, we have synthesized novel azo-Schiff base ligand and its metal complexes. Spectral, elemental and thermal analysis confirms that the ligand adopted tridentate behaviour, bonded with metal through azomethine nitrogen, carbonyl oxygen and phenolic oxygen atom. The P-XRD analysis of the complexes indicating diverse crystal systems such as monoclinic and triclinic. In the Anti-Alzheimer assessment, the Cu(II) and Zn(II) complex shows better activity than the ligand and compared with standard tacrine. Metal complexes of Co(II) exhibit significant anticancer activity than ligand compared with standard 5-fluorouracil using MCF-7 human breast cancer cell line. The metal complexes show greater biological assessment than ligand but less than standard drug and the antifungal assessment exhibit greater activity compared with antibacterial assessment.

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### CONFLICT OF INTERESTS:

We all authors declare that there is no conflict of interest.

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