



Synthesis, Spectral Sharacterization and Antimicrobial Studies of Ni(II), Co(II) and Mn(II) Complexes with Schiff Base Ligand derived from 3,5-Dibromosalicylaldehyde

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Abstract

A novel Schiff base ligand was created by combining 2-amino-4-chloro-6-methylpyrimidine with 3,5-Dibromosalicylaldehyde through a condensation reaction. Metal complexes were formed by reacting the Schiff base with Iron nitrate in an ethanol solution. These complexes were then collected, cleaned, and dried. The Schiff base appears pale yellow, while the complexes with Ni(II), Co(II), and Mn(II) exhibit a light yellow color. Characterization of the synthesized compounds was performed using FT-IR, ¹H-NMR, and UV-Vis methods for the ligands, along with FT-IR and UV-Vis for the complexes, as well as assessing all reactions via TLC, molar conductivity, and magnetic susceptibility measurements. The complexes exhibit paramagnetic properties. Molar conductivity assessments showed that all the complexes act as non-electrolytes when dissolved in DMSO. An octahedral structure was determined for all complexes. The ligands function as bidentate (L) through their phenolic (OH) group and azomethine nitrogen. Both the ligand and its complexes were tested for antifungal and antibacterial effects against different strains, including *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme*, *Aspergillus flavus*, and *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *B. subtilis*. The findings reveal that the complexes demonstrated significant antifungal and antibacterial activity.

Keywords: Heterocyclic Schiff bases, 2-amino-4-chloro-6-methylpyrimidine, 3,5-Dibromosalicylaldehyde, Antimicrobial Activity.

Introduction

An area of Bio-inorganic Chemistry is still a strong-growing expanse for research which despite its designation it covers several disciplines of Chemistry and Biology. Recent Inorganic chemistry as it is nowadays in its empire has grown into a standard discipline due to the standard growth of Schiff base metal complexes. of Schiff base metal compounds. Presently, numerous researchers and industrial teams are focused on the synthesis of Schiff base ligands and their complexes with transition metal ions¹. The ligands are produced through the condensation of a primary amine with an active carbonyl group, incorporating the azomethine group. They yield stable Schiff base ligands, particularly when the amine and the carbonyl components have another functional group close enough to the condensation site to create a five or six-membered chelate ring during chelation^{2,3}. There exists a wealth of literature discussing metal chelates formed by Schiff bases. Ligands that feature oxygen and nitrogen donor atoms are coordinated to metal ions and have garnered notable interest due to their diverse applications in catalytic, industrial, and biological areas, including uses as antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and other pharmacological applications^{4,5}.

The significance of transition metals as crucial metallic elements, along with their notable biological activity linked to specific metal-protein complexes involved in oxygen transport and ion storage electronic transfer reactions, has sparked considerable interest in studying systems with these metals⁶⁻⁸. Schiff bases and their compounds in water have been investigated for their intriguing and valuable properties. This document details the synthesis of the complexes formed with Schiff base ligands⁹.

Metals play a crucial role in cellular structures and have been naturally selected to participate in vital biochemical functions required by living beings¹⁰. The formation of positively charged ions that can dissolve easily in biological fluids is a key characteristic of metal ions that greatly lose electrons. These metal ions carry a charge that enables them to interact and bind with biological molecules^{11,12}. In the body, metals are involved in transferring electrons and transporting oxygen. They are favored due to their broad range of antimicrobial properties and their lack of disruption to the host's defense mechanisms¹³. Transition metals provide specific benefits, such as acting as agents that bind to DNA. Consequently, this research examined transition metal coordination compounds that possess pharmacological properties, including Zn(II), Cu(II), Mn(II), Fe(II), and Co(II) from the periodic table¹⁴.

Moreover, within the cellular structure of living organisms, both micro and macro elements that include these metals are present. Various therapeutic agents such as antibiotics, antibacterial agents¹⁵, antiviral drugs¹⁶, antiparasitics¹⁷, anti-HIV medications¹⁸, anti-diabetes treatments¹⁹, radio-sensitizers²⁰, and anti-cancer agents²¹ are being developed through the innovative use of metallo complexes and clinical applications of these metal compounds. Therefore, in this study, we aim to create metal complex ligands derived from Schiff bases, which are formed from Salicylaldehyde alongside alanine and serine groups, utilizing oxygen-deficient linkages with metal chlorides. This approach may help to combat antibiotic resistance²².

A review of existing literature shows that there has been no research conducted on Schiff base transition metal complexes created from 2-amino-4-chloro-6-methylpyrimidine and 3,5-Dibromosalicylaldehyde. In this report, we describe the creation of a bidentate Schiff base resulting from the condensation of 2-amino-4-chloro-6-methylpyrimidine and 3,5-Dibromosalicylaldehyde (Figure-5). We synthesized solid complexes of these ligands with Ni(II), Co(II), and Mn(II) and conducted characterization through various physicochemical techniques.

Materials and Methods

Reagents and solvents: The synthesis of the ligand and metal complex involved using 2-amino-4-chloro-6-methylpyrimidine (Aldrich Sigma), 3,5-Dibromosalicylaldehyde, and metal nitrate of analytical grade.

Synthesis of ligand: The ligand was created by altering existing procedures²³⁻²⁵. The Schiff base ligand was synthesized by heating a mixture of 0.01mol (1.2015g) of 3,5-Dibromosalicylaldehyde and 0.01mol (1.2710g) of 2-amino-4-chloro-6-methylpyrimidine in 50ml of super dry ethanol for approximately 4 hours. Once the Schiff base was formed, it was allowed to cool to room temperature, then collected through filtration, recrystallized in ethanol, and finally dried in a vacuum with anhydrous calcium chloride, yielding 78%.

Synthesis of metal complexes: To a heated solution of the ligand (2 mol) in ethanol (25 ml) and a solution of metal nitrate (1 mol) in ethanol (25 ml), constant stirring was applied. The pH of the mixture was adjusted to 7-8 by introducing a 10% solution of alcoholic ammonia and the mixture was refluxed for around 3 hours. The solid metal complex that formed was filtered while hot, washed with hot ethanol, and dried in vacuum desiccators over calcium chloride, resulting in a yield of 70%.

Physical Measurement: IR spectra were recorded on FTIR(ATR)-BRUKER -TENSOR37 spectrometer using KBr pellets in the range of 4000-400cm⁻¹. ¹H-NMR Varian mercury 300MHZ spectra of ligand were measured in CDCl₃ using TMS as internal standard. X-RD were recorded on BRUKER D8 Advance. TGA- DTA were recorded on Shimadzu.

The carbon, hydrogen and nitrogen contents were determined on Elementar model vario EL-III. The UV-visible spectra of the complexes were recorded on model Jasco V-530 UV-Vis spectrometer. Molar conductance of complexes was measured on Elico CM 180 conductivity meter using 10⁻⁴ M solution in DMSO. Magnetic susceptibility measurements of the metal chelates were done on a Guoy balance at room temperature using Hg[Co(SCN)₄] as a calibrant.

Results and Discussion

The Schiff bases made from 2-amino-4-chloro-6-methylpyrimidine and its metal complexes are used in biology, medicine, and analytical procedures, among other areas. By reacting with various carbonyl compounds, 2-amino-4-chloro-6-methylpyrimidine has improved its coordination ability. An attempt was made to use 3,5-Dibromosalicylaldehyde to produce Schiff bases from 2-amino-4-chloro-6-methylpyrimidine. Tables-1 and 2 show the ligand's and its metal complexes' physical characteristics, microanalytical data, and molar conductance. The complexes' analytical data show a 2:1 molar ratio (ligand to metal), which is consistent with the general formula [ML(H₂O)₂], where M stands for Ni(II), Co(II), and Mn(II). Ni(II), Co(II), and Mn(II) complexes' magnetic susceptibilities at room temperature point to a high spin octahedral structure with two water molecules bonded to the metal ion. TGA-DTA analysis confirmed the presence of the two connected water molecules. The metal chelates' poor conductance in DMSO solutions validates their non-electrolyte properties (Table-1).

Molar Conductivity Measurements: The metal (II) complexes were dissolved in DMSO, and the molar conductivity of a 10⁻⁴M solution was recorded at room temperature. The reduced conductivity readings of the complexes indicate that these compounds behave as non-electrolytes.

¹H-NMR spectra of ligand: The ¹H-NMR spectra of the unbound ligand at ambient temperature display the following resonances: 2.35 δ (s, 3H, Methyl hydrogen attached to the pyrimidine structure), 2.35δ (s, 3H, Methyl hydrogen linked to the phenyl group), 5.47δ (s, 1H, Phenolic (OH) hydrogen within the pyrimidine structure), 6.77δ (s, 1H, Hydrogen associated with the pyrimidine structure), 7.84δ (s, 1H, hydrogen connected to the azomethine carbon), 7.2-7.42δ (D, 4H, Aromatic Ha, Hb, protons belonging to the phenyl group).

IR Spectra: The infrared spectra of the complexes are analyzed alongside the ligand to identify potential alterations occurring during the formation of the complexes. The peaks observed at 3363, 1678, 1516, 1309, and 1186cm⁻¹ correspond to the stretching modes for OH (intramolecular hydrogen bonding), C=C (aromatic), C=N (azomethine), C-N (aryl azomethine), and C-O (phenolic), respectively²⁶⁻²⁸.

The lack of a faint broad band within the 3200-3400 cm^{-1} range in the spectra of the metal complexes indicates the loss of the intramolecular hydrogen bonded OH group upon complexation, leading to the coordination of the phenolic oxygen with the metal ion. This conclusion is further reinforced by the observed downward shift in the C-O (phenolic) frequency when compared to the free ligand²⁹. Upon complexation, the C=N³⁰ band experiences a shift to a lower wave number compared to the free ligand, indicating the coordination of the nitrogen atom from the azomethine group to the metal ion. The C-N band also shows a shift to a lower wave number in relation to the free ligand. The IR spectra of the metal chelates reveal additional bands in the 500-600 and 400-500 cm^{-1} regions, which can be attributed to M-O and M-N vibrations, respectively³¹. The spectra for Cu (II) display a prominent band in the 3050-3600 cm^{-1} region, indicating the presence of coordinated water within these metal complexes. The existence of coordinated water is confirmed by a non-ligand band appearing in the 830-840 cm^{-1} region, associated with the rocking motion of water. The presence of coordinated water is further validated through TGA/DTA analysis of these complexes. Consequently, it is inferred that coordination occurs via the phenolic oxygen and the azomethine nitrogen of the ligand molecule.

Thermogravimetric analysis: The changing TGA alongside the percentage mass reductions at various stages has been documented. The concurrent TGA/DTA examination of Co(II) was conducted from room temperature to 1000 $^{\circ}\text{C}$ under a nitrogen environment, utilizing $\alpha\text{-Al}_2\text{O}_3$ as a benchmark. An evaluation of the thermogram related to the complexes revealed that Co(II) complexes ligandL (refer to Figure 1) exhibit a two-

step decomposition process. The initial weight reduction of 5.61% within the temperature range of 50-195 $^{\circ}\text{C}$ can be associated with the loss of two molecules of lattice water (calculated at 6.50%). The anhydrous form is unstable at elevated temperatures, leading to swift decomposition between 195 and 570 $^{\circ}\text{C}$, resulting in a 79.45% mass loss linked to the decomposition of the complex (calculated at 79.14%) in the second stage. The decomposition process concludes with the formation of a stable metal oxide residue, CoO, observed at 11.23% (calculated at 14.35%). The kinetic and thermodynamic parameters, such as activation energy (E_a), frequency factor (Z), change in entropy ($-\Delta S$), and change in free energy (ΔG) for the non-isothermal decomposition of the complexes were established using the Horowitz-Metzger method³², with values provided in Table-3. The computed activation energy values for the complexes are relatively modest, suggesting the autocatalytic influence of the metal ion on the thermal breakdown of the complex. The negative activation entropy value indicates that the activated complexes were more structured compared to the slower reaction. This increased order might result from the polarization of bonds in the activated state, possibly occurring due to charge transfer interactions³³.

Magnetic measurements and electronic absorption spectra:

The electronic spectral analysis depicted in Figure-3 for nickel (II) metal complexes with Schiff bases was conducted in a DMSO solution. The absorption spectrum corresponding to the Ni(II) complex displays peaks at 13812 cm^{-1} and 30030 cm^{-1} , which are attributed to the transitions of ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$ and charge transfer in an octahedral environment, respectively³⁴. The nickel (II) complexes exhibited diamagnetic properties.

Table-1: Physical characterization, analytical and molar conductance data of compounds.

Compound Molecular formula	Mol. Wt.	M.P. Decomp temp. $^{\circ}\text{C}$	Colour	Molar Conduc. Mho. $\text{cm}^2\text{mol}^{-1}$
L	244	98	Yellow	---
Co-L	587	>300	Dark Yellow	13.27
Ni-L	595	>300	Yellow	14.22
Mn-L	601	>300	Yellow	12.20

Table-2: Elemental Analysis of Co(II), Ni(II) and Mn(II) Complex.

Compound	% Found (Calculated)			
	C	H	N	M
L	52.54 (53.23)	3.53 (3.82)	16.60 (16.85)	----
Co-L	44.45 (44.32)	3.38 (3.30)	14.17 (14.16)	9.91 (9.87)
Ni-L	45.46 (45.42)	3.34 (3.32)	15.17 (15.16)	10.61 (10.63)
Mn-L	46.41 (45.42)	3.28 (3.37)	16.10 (16.12)	10.71 (10.73)

Table-3: The kinetic and thermodynamic parameters for decomposition of metal complexes.

Complex	Step	Decomp. Temp. ($^{\circ}\text{C}$)	n	Ea (kJmole^{-1})	Z (S^{-1})	ΔS ($\text{JK}^{-1}\text{mole}^{-1}$)	ΔG (kJmole^{-1})	Correlation coefficient
Co-L	I	370	1.3	11.88	2.26×10^4	-167.97	24.86	0.933
Ni-L	I	375	1.4	11.78	2.23×10^4	-168.95	24.76	0.923

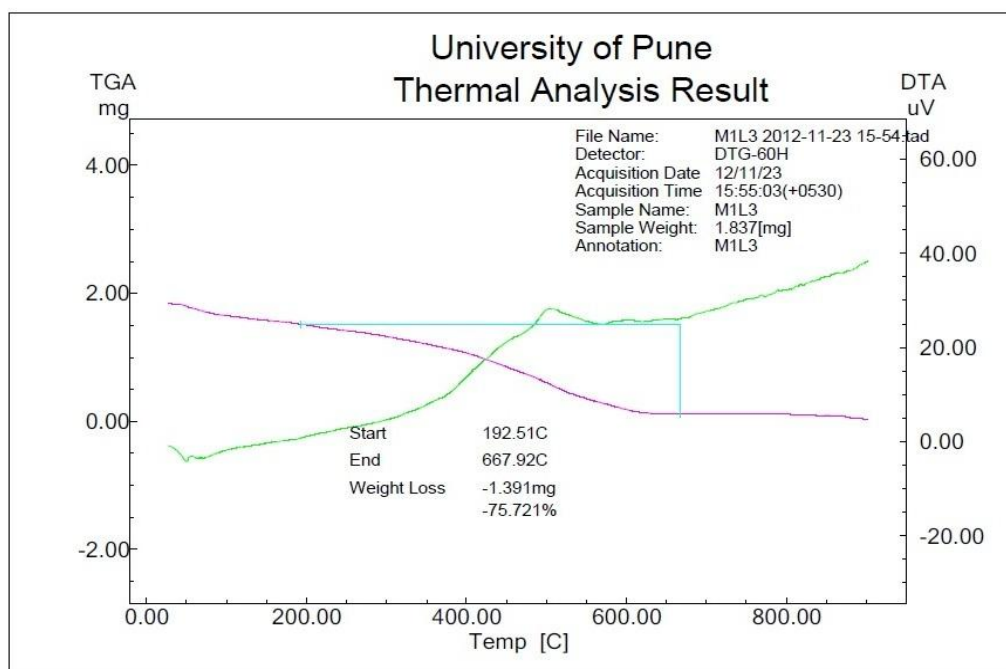


Figure-1: TGA-DTA Curve of Co(II) Complex of Ligand L.

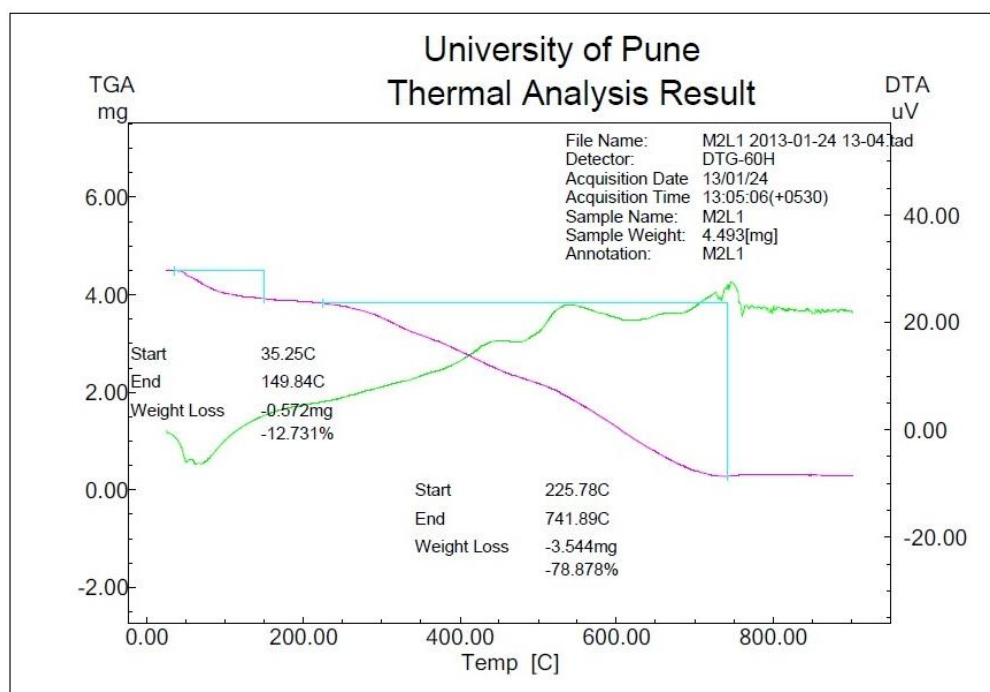
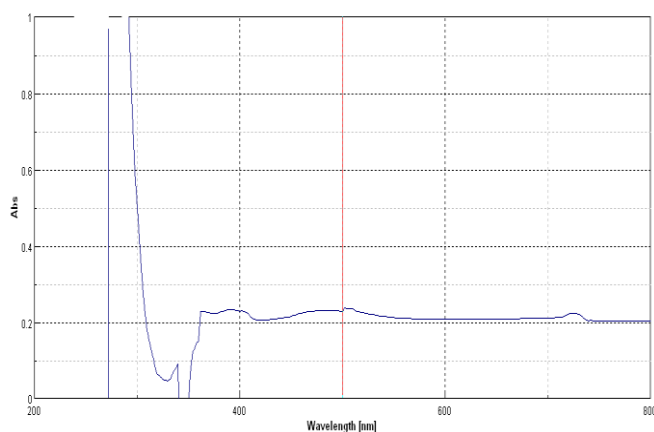


Figure-2: TGA-DTA Curve of Ni(II) Complex of Ligand L.

Powder x-ray diffraction: The X-ray diffractogram for Co (II) complexes of L8 was recorded over the range of 20 to 80° using a wavelength of 1.543 Å (Figure-4). The resulting diffractogram along with its data illustrates the 2θ values for all peaks, their relative intensities, and the inter-planar distances (d-values). In the diffractogram of Co(II) complex of L, there were fifteen reflections, with the highest peak appearing at 2θ = 12.89° which corresponds to a d value of 6.86 Å. The x-ray diffraction pattern for these complexes concerning significant peaks with relative intensities exceeding 10% was analyzed using computer software³⁵. This indexing approach also provides Miller indices (hkl), parameters of the unit cell, and the volume of the unit cell. The unit cell for the Co(II) complex of L produced lattice constants of a= 9.76 Å, b=10.24 Å, c= 27.24 Å, and a unit cell volume of V=2722. 43096 Å³. Along with these parameters, it was verified that the conditions a = b = c and α = β = γ = 90°,

necessary for the sample to exhibit a Monoclinic structure, were met. Therefore, it can be inferred that the Co(II) complex possesses an Orthorhombic crystal system. Also, it can be deduced that the Co (II) complex of L has a monoclinic crystal system. The density values obtained for the complexes were assessed using the specific gravity method³⁶ and measured at 0.8968gcm⁻³ for the Co (II) complexes. With the determined density values, molecular weights of the complexes, Avogadro's number, and the unit cell volume were calculated. The number of molecules per unit cell was obtained using the equation $\rho = nM/NV$; the results pertained to the Co (II) complexes. Using these data, the theoretical density was calculated and found to be 0.8858gcm⁻³ for each respective complex. A comparison between the experimental and theoretical densities reveals a strong correlation within the bounds of experimental error³⁷.



Wavelength	Absorption
778	0.205
742	0.206
724	0.226
502	0.239
484	0.233
402	0.233
392	0.235
362	0.230
340	0.092

Figure-3: Electronic Absorption Spectra of Ni(II) Complex of Ligand L.

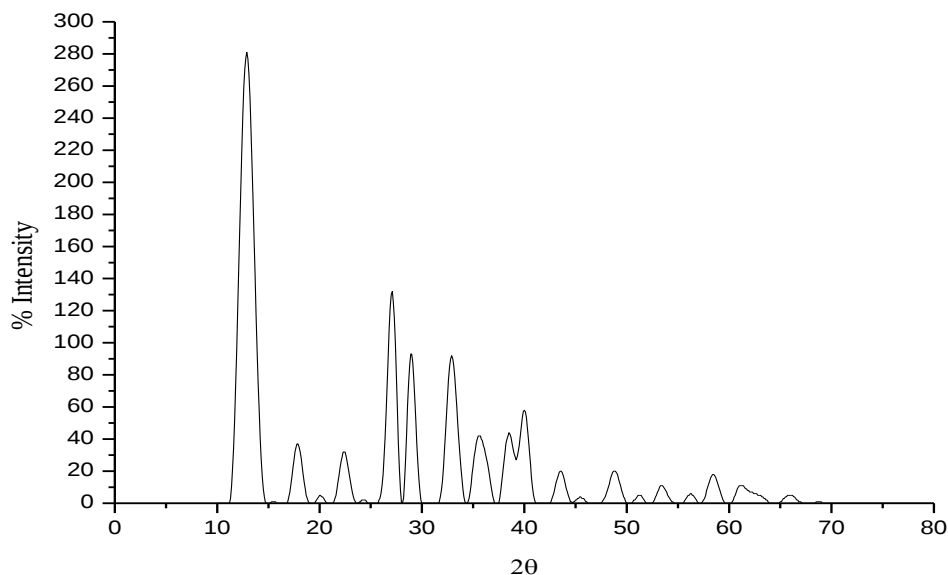


Figure-4: X-ray Diffractogram of Co (II) complex of L.

Antibacterial activity: The antifungal and antibacterial properties of both the ligand and metal complexes were evaluated in a laboratory setting against various fungi including *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moniliforme*, and *Aspergillus flavus*, along with bacteria such as *E. coli*, *B. subtilis*, *S. aureus*, and *Bacillus subtilis* using the paper disc diffusion method^{38,39}. The compounds were assessed

at concentrations of 1% and 2% in DMSO and were compared with established antibiotics like *griseofulvin* and *penicillin*. The findings indicate that the effectiveness of the metal chelates in inhibiting growth is superior to that of the ligand itself, and these results are consistent with earlier research regarding the comparative efficacy of the free ligand versus its complexes^{40,41}.

Table-4: Antifungal activity of ligands.

Test Compound	Antifungal Growth							
	<i>Aspergillus niger</i>		<i>Penicillium chrysogenum</i>		<i>Fusarium moniliforme</i>		<i>Aspergillus flavus</i>	
	1%	2%	1%	2%	1%	2%	1%	2%
L	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Co-L	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Ni-L	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Mn-L	-ve	-ve	RG	-ve	-ve	-ve	-ve	-ve
+ve control	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-ve control (Griseofulvin)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Ligand & Metal: +ve – Growth (Antifungal Activity absent) -ve - Growth (Antifungal Activity present) RG - Reduced Growth (More than 50% reduction in growth observed).

Table-5: Antibacterial activity of ligands and their metal complexes.

Test Compound	Diameter of inhibition zone (mm)							
	<i>E. coli</i>		<i>Salmonella typhi</i>		<i>Staphylococcus aureus</i>		<i>Bacillus subtilis</i>	
	1%	2%	1%	2%	1%	2%	1%	2%
L	12mm	17mm	13mm	16mm	15mm	18mm	16mm	19mm
Co-L	12mm	14mm	13mm	15mm	18mm	21mm	11mm	13mm
Ni-L	14mm	16mm	15mm	17mm	19mm	23mm	15mm	17mm
Mn-L	17mm	18mm	14mm	16mm	20mm	24mm	18mm	20mm
DMSO	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Penicillin	14mm	14mm	18mm	18mm	31mm	31mm	19mm	19mm

Ligand & Metal: -ve - No Antibacterial Activity Zone of inhibition - ---mm

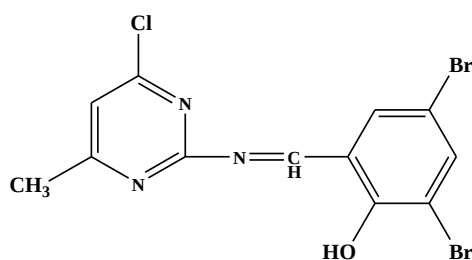


Figure-5: Structure of Schiff Base Ligand L.

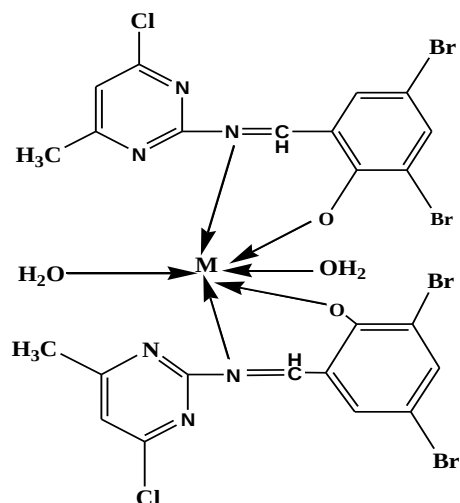


Figure-6: The proposed Structure of the Metal complexes. [When M= Ni(II), Co(II) And Mn(II)].

Conclusion

Considering the preceding discussion, we suggest an octahedral structure for the Ni(II), Co(II), and Mn(II) complexes. Based on the physical, chemical, and spectral information provided earlier, it can be inferred that the ligand functions as dibasic, not bidentate, coordinating through imino nitrogen and phenolic oxygen as shown in Figure-6. The complexes exhibit biological activity and demonstrate improved antimicrobial properties when compared to the unbound ligand. Thermal analysis indicates the complexes have stability against heat. The X-ray analysis points to an orthorhombic crystal system for the complexes involving Ni(II), Co(II), and Mn(II).

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