Synthesis of Schiff base ligand, its novel Mn (II), Co (II), Ni (II), Cu (II), Zn (II), Pd (II) complexes: spectral investigation, biological applications, and Nanoparticles

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Keywords: FTIR, UV-VIS, MASS, TGA/DTA, TEM, EDX, XRD, and Microbial Activity.

Highlights :

Eco-friendly Synthesis: Manganese nanoparticles were successfully synthesized using a green approach, yielding particles in the size range of 40-95 nm.

UV-Visible Spectroscopy: Spectral analysis confirmed the formation of Co(II) and Ni(II) complexes. Nephelauxetic effect, covalence factor (b½), and percent covalence (δ%) were calculated to elucidate the metal-ligand bonding nature, indicating significant covalent character.

FTIR Analysis: Spectra revealed coordination through imino (–C=N) and hydroxy (–OH) functional groups, confirming successful Schiff base formation and metal complexation.

Mass Spectrometry: Molecular ion peaks corresponding to $[M+H]^+$ validated the formation of Schiff bases and their respective Cu(II) complex.

TGA/DTA: Confirm that two water molecules are coordinated in the complex.

ABSTRACT

Novel Schiff base ligand and its Mn (II), Co (II), Ni (II), Cu (II), Zn (II), and Pd (II) complexes have been prepared. By using a variety of analytical and spectroscopic techniques, the type of bonding between the ligand and the metal ions in the recently formed complexes was clarified. The Schiff base ligand acts as a tetradentate and coordinates with the metal ions through the azomethine-N and the phenolic-O centers, in a mono-deprotonated form. Except for the Zn (II) complex, which displayed an octahedral geometry, all complexes displayed an octahedral geometry. The TGA findings supported that the stability and decomposition properties of the metal complexes were entirely distinct from one another. The thermogram showed the decomposition of all investigated metal complexes above 200 °C in three, four, or five steps, and indicated the high thermal stability of these complexes. According to XRD patterns, the particles of these complexes were located at the nanoscale. Moreover, for all the samples analyzed, the TEM images showed uniform and homogeneous surface morphology. The biological activity reveals the high efficiency of the screened complexes as antibacterial agents. The antimicrobial activity of the ligand and its complexes was examined. The data obtained revealed that the metal ions in the complexes enhanced the antimicrobial activity compared to the free ligand.

INTRODUCTION:

Schiff bases have an important function as ligands, among the most widely used chemical compounds, despite being discovered in coordination chemistry over a century ago [1-2]. They serve as catalysts, intermediates in organic synthesis, dyes and pigments, and stabilisers for polymers [3-4]. An imine with the azomethine group [R_2-R_1-C=N-R^3] has been substituted for the nitrogen analogue of an aldehyde [R1-CH=O] or ketone [R_1-C=O-R_2] in which the carbonyl group [C=O] is condensed with a primary amine [R_3-NH_2]. R_1, R_2, and R_3 can be aryl, alkyl, heteroaryl, or cycloalkyl, among other types. Schiff bases' imine group [-C=N-] plays a unique role in producing these compounds, which have a wide range of biological activity [5].

The synthetic flexibility, selectivity, and sensitivity of Schiff bases to the central metal atom, along with their structural resemblance to natural biological compounds and the presence of the azomethine group (−N=CH−), have been extensively researched [6]. The azomethine group plays a crucial role in elucidating the mechanisms of transformation and racemization reactions. Schiff bases constitute a significant class of organic molecules with diverse applications in inorganic chemistry, biology, and analytical science [7-9]. Some of these compounds serve as dyes, polymer catalysts, and corrosion inhibitors. Additionally, due to their broad spectrum of biological activities [10], Schiff bases have become increasingly important in pharmacology and medicine.

Under varying reaction conditions, Schiff bases readily interact with metal ions, forming complexes that continue to captivate researchers due to their nitrogen and oxygen donor atoms. Schiff base transition metal complexes have been employed as biological models to enhance the understanding of biomolecular structure and function. When complexed with specific proteins, elements such as manganese, cobalt, nickel, copper, and zinc exhibit biological activity, contributing to ion storage, electron transfer processes, or oxygen transport within living systems [11-12].

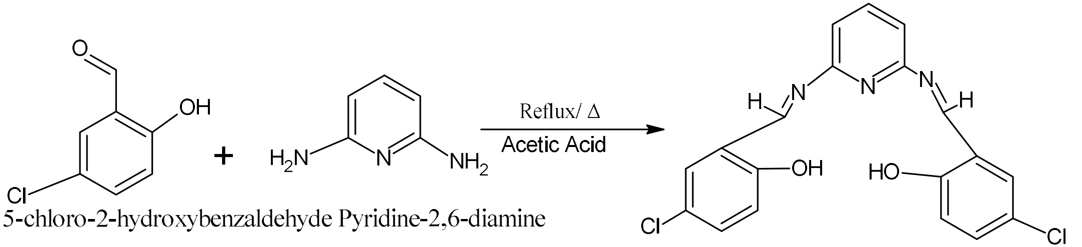
This study aimed to synthesize Mn (II), Co (II), Ni (II), Cu (II), Zn (II), and Pd (II) complexes, with a focus on spectral investigations, biological applications, and nanoparticle formation. These complexes were derived from Schiff bases obtained from pyridine-2,6-diamine and 5-chloro-2-hydroxybenzaldehyde. A variety of analytical techniques, including elemental analysis, electronic spectroscopy, mass spectra, infrared spectroscopy, SEM, TEM, EDX, TGA/DTA, and microbial studies, were employed to characterize and assess these compounds.

Material and Methods:

The chemical compounds pyridine-2,6-diamine and 5-chloro-2-hydroxybenzaldehyde. were purchased from Sigma-Aldrich and used directly without any purification process. SD Fine Chemicals supplied metal chlorides and nitrates.

Measurements: The prepared ligand complexes were subjected to (C, H, and N) elemental analyses using a 2400 Series II CHNS/O elemental analyser. Molar conductance measurements were carried out in DMSO using a CMD650 digital conductivity meter. Electronic absorption spectra were measured in DMSO using a Shimadzu NIR3101PC UV-VIS spectrometer. Infrared spectra were obtained as KBr pellets on a Perkin-Elmer. In the range of 4000 - 400 cm^{-1}. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) measurements were conducted using the Mettler Toledo Star System in the presence of atmospheric nitrogen, within a temperature range of 50°C to 1000°C. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) data were collected for nanoparticles using the Quanta 200 ESEM instrument from Icon Laboratory, Mumbai, as it detects dispersed electrons on the particle surface.

Syntheses of Schiff Base (L): One mole of pyridine-2,6-diamine (1.09 g) and two moles of 5-chloro-2-hydroxybenzaldehyde (3.12 g) were separately dissolved in ethanol. The solutions were then combined in a 2.1 molar ratio and refluxed in a water bath for two hours. As the mixture cooled to room temperature, a solid of distinct color formed. The solid was carefully separated using a sterile watch glass and stored overnight in a dark location before being utilized for further analysis.



5-chloro-2-hydroxybenzaldehyde Pyridine-2,6-diamine

4-chloro-2-{(*Z*)-[(6-{[(2-hydroxyphenyl)methylidene] amino}pyridin-2-yl)imino]methyl}4-chlorophenol

Preparation of Complexes: The Schiff base ligand, 4-chloro-2-{(Z)-[(6-{[(2-hydroxyphenyl) methylidene] amino} pyridin-2-yl)imino]methyl}-4-chlorophenol (3.86 g), was combined in equal molar ratios (0.02 mol) with Mn(II), Co(II), Ni(II), Cu(II), Zn(II), and Pd(II) salts to synthesize the ligand complexes. The reaction mixture was refluxed for three hours. To adjust the pH, a few drops of an alcoholic ammonia solution (1:1) were added. The resulting complexes were washed multiple times with hot ethanol until the filtrate became clear. After initial air drying, the filtrate was vacuum-dried using anhydrous $CaCl_2$. The yield ranged from 79% to 86%. The dried complexes were analyzed using spectroscopic and elemental techniques. The synthesized complexes were soluble in DMSO but insoluble in C_2H_5OH. The purity of the ligand complexes was confirmed using thin-layer chromatography (TLC)

Synthesis of metal complex nanoparticles: Nanoparticle synthesis was performed by mixing a metal ion solution with Schiff base at an equimolar concentration of 0.01 M. The mixture was refluxed for three hours, then transferred to a test tube and centrifuged at room temperature while being continuously stirred using a magnetic stirrer. During refluxing, two to three drops of alcoholic ammonia (1:1) were added dropwise, resulting in varied-coloured precipitates for Mn(II) ion. The precipitate was allowed to cool, filtered, and washed thoroughly with 50% pure alcohol and distilled water. The obtained complexes were dried for two hours at 60°C in a hot air oven. The final complex achieved a yield of 75-85% after centrifugation at 6000 RPM for four hours. High-quality coloured crystals were obtained. Following separation and drying, these crystals were stored at room temperature in a covered container. The synthesized nanoparticles were intended for characterization.

Result and Discussions:

The table below presents the analytical and physical parameters of the Schiff base complexes. All complexes are coloured and thermally stable at room temperature, except Zn(II). While the complexes are soluble in DMSO and DMF, the Schiff base dissolves in ethanol, methanol, and butanol. The complexes decomposed at high temperatures ranging from 255°C to 295°C, whereas the Schiff base melted within the range of 135°C to 145°C. The melting point (MP) and decomposition point (DP) were determined using an uncorrected open capillary method. Based on molar conductance measurements in DMSO at a concentration of 10^{-3} M, the complexes were classified as non-electrolytic[13-14]. Pd (II) concentrations were analyzed using atomic absorption spectroscopy (AAS), while metal content was determined through titration with 0.01M EDTA using various indicators [15-16].

Table-1: Physical and Analytical Parameters (*Experimental)

Complexes/Ligand	MP/DP°C	C%	N%	M%	Conductivity	**μ_{eff} BM**
($C_{19}H_{13}Cl_2N_3O_2$)	138-141	59.03	10.88	-	-	-
		58.88*	10.74*			
[$MnC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	270-273	29.36	5.41	7.70	6.58	5.08

		29.21*	5.34*	7.57*		
[Ni $C_{19}H_{11}Cl_2N_3O_2.2H_2O$]	278-281	29.22	5.38	7.52	7.14	3.11
		29.12*	5.29*	7.41*		
[$CoC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	255-258	29.21	5.37	7.54	8.18	4.37
		29.09*	5.27*	7.37*		
[$CuC_{19}H_{13}Cl_2N_3O_2.2H_2O$]	292-295	29.04	5.35	8.09	8.02	2.04
		28.92*	5.22*	7.97*		
[$ZnC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	262-265	28.97	5.34	8.30	7.44	0.00
		29.85*	5.20*	8.18*		
[$PdC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	289-292	27.54	5.07	12.84	7.18	-
		27.43*	4.95*	12.70*		

IR Spectroscopic Study: The FTIR spectrum of the Schiff base ligand exhibits a moderately intense band at 1670 cm⁻¹ and 1630 cm⁻¹, attributed to the ν(C=N) vibration of the azomethine group[17]. A band at 1434 cm⁻¹ confirms the presence of an aromatic ring. In Schiff base complexes, this characteristic is further validated by bands appearing at 1643 cm⁻¹ and 1615 cm⁻¹, corresponding to the ν(C=N) vibration.

The IR spectral data of the Ni(II) complex of the ligand reveal additional bands that are absent in the ligand's spectrum. These include bands in the 530-520 cm⁻¹, which correspond to νM–N vibrations, and bands in the 435-422 cm⁻¹ range, assigned to νM–O vibrations [18-19]. These spectral features indicate the involvement of hydroxy groups and the azomethine nitrogen in complexation with metal ions [20].

Table 2: FTIR of Schiff base ligand and metal complexes

L/Complexes	**νOH**	**νOH**	**νHC=N**	**νH₂O**	**νH₂O**	**νHC=N**	**νM-O**	**νM-N**
($C_{19}H_{13}Cl_2N_3O_2$)	3330	3380	1632	-	-	1652	-	-
[$MnC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	-	-	1618	3440	3493	1628	528	432
[$CoC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	-	-	1615	3435	3488	1630	524	428
[$NiC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	-	-	1612	3438	3485	1625	520	424
[$CuC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	-	-	1614	3442	3502	1621	523	425
[$ZnC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	-	-	1617	3444	3504	1624	527	430

Mass Spectra: The mass spectra of the ligand and the Cu (II) complex have been recorded. Due to the presence of molecular ions (M+), all the spectra exhibit parent peaks. By comparing the molecular formula weights of these complexes with their m/z values, the suggested molecular formula was validated. The following are the molecular ion (M+1or2) peaks found for different complexes: m/z 483.792 Cu (II) complex, m/z 386.232 (ligand). The spectra display peaks corresponding to different fragments resulting from the thermal cleavage of the complexes, in addition to peaks attributed to the molecular ions. The shards' stability was indicated by the peak intensity. By comparing experimental and theoretical values for all compounds under study, the elemental composition was verified. The typical ions seen in the first-order positive-ion mass spectra were [M+H] and[M+L][21] .

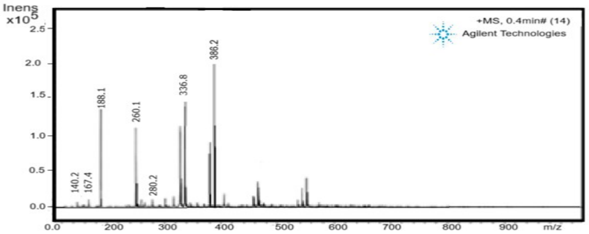


Figure 1 : Mass Spectra of ($C_{19}H_{13}Cl_2N_3O_2$)

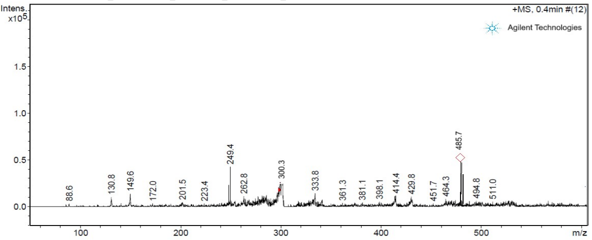


Figure 2 : Mass Spectra of [$CuC_{19}H_{11}Cl_2N_3O_2.2H_2O$]

Electronic spectra: The electronic spectral data of the Schiff base organometallic compounds and their metal complexes were recorded in DMSO and ethanol at a concentration of 0.002 mol/L. The nature of the spectra observed for all the complexes suggests an octahedral geometry around the central metal ions. Specifically, the Ni(II) and Co(II) complexes exhibit features consistent with six-coordinate, likely octahedral configurations. Furthermore, bonding parameters such as Racah interelectronic repulsion ($b^{1/2}$), Nephelauxetic parameter (β), Angular covalency (η), and Sinha's covalency parameter (δ%) have been calculated to support these structural assignments [22-24].

Table 3: Electronic spectra of Co(II) and Ni(II) Complexes

Complexes	Bands(cm⁻¹)	Assignments	Parameters
[$CoC_{19}H_{11}Cl_2N_3O_2.2H_2O$]	17375 21710	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	β = 0.9729 $b^{1/2}$= 0.02655 δ % =1.3464 η = 0.01327
	8310	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$,	β = 0.9689

[Ni $C_{19}H_{11}Cl_2N_3O_2.2H_2O$]	17790 25525	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ $^4A_{1g}(F) \rightarrow {}^4T_{1g}$ (P).	$b^{1/2}$= 0.0155 δ % =1.2098 η = 0.03159

Thermal analysis: Thermal analysis data for the [Pd $C_{19}H_{11}Cl_2N_3O_2.2H_2O$] complex is presented in the accompanying table. The experiments were conducted under a controlled nitrogen atmosphere with a heating rate of 20 °C min⁻¹, and weight loss was monitored from ambient temperature up to 800 °C. The thermogram of the palladium analogue revealed a total weight loss of approximately 79% within this temperature range, occurring in four distinct steps:

- Stage I (30–130 °C): Corresponds to the loss of water molecules present within the coordination sphere
- Stage II (130–280 °C): Indicates the removal of both coordinated water molecules along with partial degradation of the ligand
- Stage III (300–450 °C): Represents substantial decomposition of the ligand framework
- Stage IV (>450 °C): Associated with complete breakdown of the ligand moiety surrounding the metal ion[25]. Subsequently, the complex is transformed into its corresponding metal oxide, specifically rhodium oxide [26]. The existence of water molecules is further corroborated by distinct endothermic signals observed in the Differential Thermal Analysis (DTA) curves, which align with the weight loss stages in the Thermogravimetric Analysis (TGA) profile.

Table 4: Thermo gravimetric analysis data of the complex (* exothermic)

Complex	Temp.Range (°C)	Calculated value (%)	Experimental value (%)	Possible leaving groups
[Pd$C_{19}H_{11}Cl_2N_3O_2.2H_2O$]	20-180	6.75	6.20	H_2O,C
	180-345	20.20	19.97	H_2O,C_4H_2N
	345-450	23.50	23.44*	C_5H_5
	450-900	40.18	40.05	Pd oxide

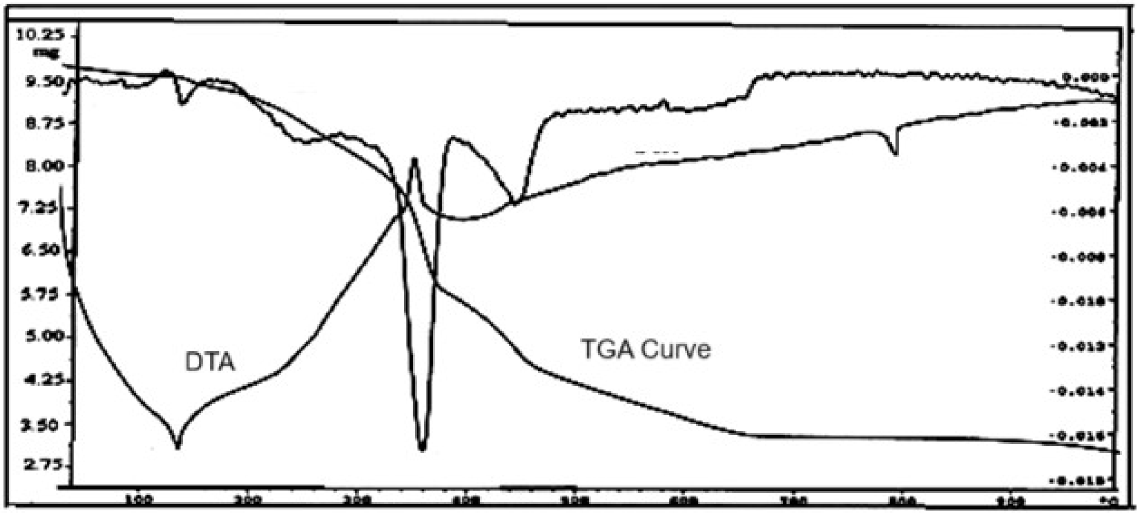


Figure 3: TGA/DTA of [Pd$C_{19}H_{11}Cl_2N_3O_2.2H_2O$]

SEM, TEM, and EDX:

Morphological analysis of Manganese nanoparticles (Mn-NPs) was conducted using Scanning Electron Microscopy (SEM)[27-28]. As illustrated in the Figures below, the SEM images at both low and high magnifications reveal that the Mn-NPs are predominantly spherical and evenly dispersed. Transmission Electron Microscopy (TEM) revealed that the nanoparticles exhibit sizes ranging between 40 and 95 nm. The smooth crystallite surfaces suggest a densely packed agglomeration without visible porosity. Notably, particle agglomeration is evident, attributed to the elevated surface energy of the nanoparticles, which promotes cluster formation and increased assembly size. This enlarged surface area contributes to improved energy storage potential. Additionally, Energy Dispersive X-ray Spectroscopy (EDX), presented in the below Figure, verified that Manganese is the principal elemental component.

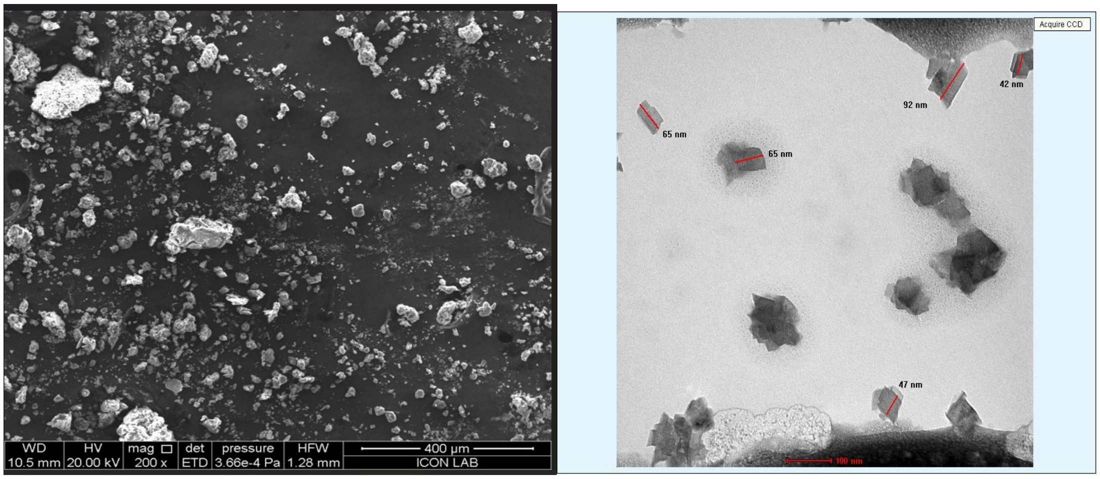
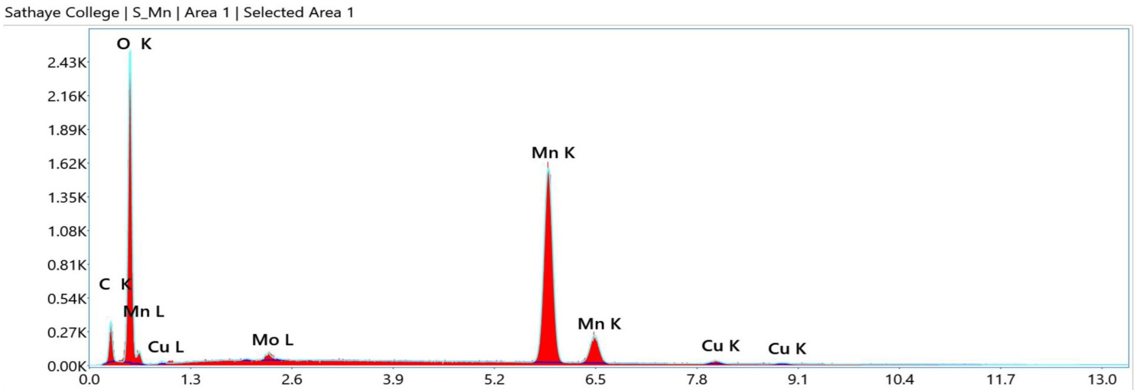


Figure 5: 3D-View of Schiff base and NI(II) Complex



Element	Wt.%	MDL	Atomic %	Net Int.	R	A	F
C K	20.3	1.00	35.4	57.4	0.8741	0.0943	1.0000
O K	36.8	0.22	48.4	501.1	0.8868	0.1766	1.0000
Mn K	40.5	0.37	15.5	718.9	0.9509	0.9782	1.0305
Cu K	1.4	0.60	0.5	13.1	0.9631	0.9552	1.0620
Mo L	1.0	0.31	0.2	17.5	0.9230	0.7290	1.0104

Figure 4: SEM, TEM, and EDX of Mn-NPs

Microbial activity: The antibacterial efficacy of metal complexes derived from Mn (II), Ni (II), Co (II), Cu (II), Pd (II), and Zn (II) ions with Schiff base ligands was systematically evaluated. The minimum inhibitory concentrations (MICs) were determined using the liquid dilution technique against four bacterial strains: Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and S. typhi [29-30]. Stock solutions were prepared at varying concentrations (2.5–50 μg/cm³) using an appropriate solvent system. To each test tube, 1 cm³ of the Schiff base or metal complex solution was added, followed by 0.2 cm³ of bacterial inoculum and 4.0 cm³ of sterile distilled water. After incubation for 24 hours, the samples were examined for turbidity, and the MIC was identified as the lowest concentration at which bacterial growth was inhibited.

Compared to free Schiff bases, their corresponding metal complexes exhibited significantly enhanced antibacterial activity. The increased potency of the metal complexes may be attributed to their ability to disrupt microbial cell processes, rendering them more cytotoxic than their parent ligands under identical experimental conditions. This phenomenon aligns with Tweedy's chelation theory[31], which postulates that chelation diminishes the polarity of the metal ion through partial charge sharing with donor groups and possible π-electron delocalization across the chelate ring [31]. Such interactions may amplify the permeability of the complex through microbial membranes, thereby improving antibacterial performance. Overall, the data indicate a progressive increase in microbial inhibition with increasing concentration of the metal complexes.

Table 5: Microbial Activity

Ligand/ Complexes	**Gram-Positive Bacteria**		**Gram-Negative Bacteria**	
	B. subtilis	**S. aureus**	**S. typhi**	**E. coli**
Ligand	15	12	14	15

Mn (II)	18	17	18	16
Ni(II)	23	19	22	19
Co(II)	22	16	18	21
Cu(II)	19	20	21	20
Zn(II)	23	18	22	17
Pd(II)	27	23	26	25

CONCLUSION

The Schiff base ligand was synthesized via a condensation reaction between 5-chloro-2-hydroxybenzaldehyde and pyridine-2,6-diamine in the presence of acetic acid, yielding 4-chloro-2-{(Z)-[(6-{[(2-hydroxyphenyl) methylidene] amino} pyridin-2-yl) imino] methyl}-4-chloro phenol. Metal complexes of this ligand were subsequently formed with Mn (II), Co (II), Ni (II), Cu (II), Zn (II), and Pd (II) ions. Infrared spectral analysis confirmed coordination through the nitrogen atom of the imine group and the oxygen atom of the hydroxyl group, indicative of covalent bonding within the complexes. All metal complexes displayed a six-coordinate geometry, where the Schiff base functioned as a monodentate ligand. Thermogravimetric and differential thermal analysis (TGA/DTA) further supported the presence of two coordinated water molecules within the coordination sphere. The electronic absorption spectra of Co (II) and Ni (II) complexes demonstrated characteristic bands consistent with covalent interactions. Parameters such as the nephelauxetic effect, covalence factor ($b^{½}$), and percent covalence (δ%) were calculated to elucidate the bonding nature. Cu (II) and Ni (II) complexes were found to adopt an octahedral geometry. Mass spectrometric analysis affirmed the molecular composition by matching observed molecular ions with theoretical mass values. Additionally, synthesized Mn-NPs exhibited particle sizes ranging from 40 to 95 nm. Biological assays demonstrated notable antimicrobial activity of the complexes against both Gram-positive and Gram-negative bacterial strains.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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