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Synthesis, Characterization and Antimicrobial Studies of Schiff Base Derived from 2'-hydroxyacetophenone and 4,5-diaminopyrimidine and its Mn(II) and Ni(II) Complexes

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ABSTRACT

A novel Schiff base ligand was synthesized by condensing 2'-hydroxyacetophenone with 4,5-diaminopyrimidine. This Schiff base was utilized to prepare transition metal complexes of Mn(II) and Ni(II). Characterization of the ligand and its metal complexes was carried out based on solubility test, thermal stability (melting/decomposition temperatures), Fourier Transformed Infrared (FTIR) spectroscopy, conductivity measurements, magnetic susceptibility measurements, UV-Vis spectroscopy, and Job's method of continuous variation for determination of the average number of coordinated ligand to the metal ion. The compounds were soluble in DMF and DMSO, sparingly soluble in polar solvents, but insoluble in non-polar solvents. The azomethine peak in the FTIR spectra of the Schiff base was observed at 1603 cm^{-1}, which as a result of complexation shifted to the lower frequencies of 1581 and 1594 cm-1 in the Mn and Ni complex respectively. UV spectra revealed absorption at 246nm for the ligand while those of Mn and Ni complex shifted to 247 and 253nm respectively for π-π* transition in the aromatic ring, also n-π* transitions of the azomethine group in the ligand was observed at 328nm while that of the complexes shifted to 352 and 305nm for Mn and Ni complexes respectively. The metal to ligand ratio was found to be 1:2 by Job's method of continuous variation. The antimicrobial activities of the Schiff base and its metal complexes were evaluated against *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli*, and *Klebsiella pneumoniae*, as well as two fungal isolates, *Aspergillus flavus* and *Aspergillus nigar*. The results showed that the metal complexes exhibited higher antimicrobial activity than the Schiff base, which might be due to complexation or metal ion toxicity. However, their activity was lower than that of the standard drugs ciprofloxacin (antibacterial) and ketoconazole (antifungal).

Keywords: 2'-hydroxyacetophenone; 4,5-diaminopyrimidine; Antimicrobial activity; Chelation; Schiff Base

INTRODUCTION

In 1864, German chemist Hugo Schiff introduced a class of compounds known as Schiff Bases, produced through the condensation reaction between primary amines and carbonyl compounds (aldehydes or ketones) (Raczuk *et al.,* 2022). A Schiff base is characterized by a carbon-nitrogen double bond (C=N), with the nitrogen attached to either an aryl or an alkyl group. The general structure is $R_1R_2C=NR_3$, where R_3 is an alkyl or phenyl group (Ünlü *et al.,* 2023).

Schiff bases derived from aniline are referred to as anils. Bis-Schiff bases, particularly those formed from salicylaldehyde and ethylenediamine, are termed salen-type compounds (Tariq *et al*., 2024). Aromatic Schiff bases are typically more stable and easier to synthesize than their aliphatic counterparts due to extended conjugation (Reddy *et al.,* 2024).

The imine (-C=N-) functional group plays a vital role in the biological activities of Schiff bases, which include antimicrobial, anticancer, anti-inflammatory, antiviral, and analgesic effects (Karatas *et al.,* 2024). Additionally, Schiff base metal complexes are prominent in catalysis, material science, supramolecular chemistry, and biomedical applications (Kargar *et al.,* 2024).

Their photophysical properties make them useful in optoelectronic devices such as Light Emitting Diodes and solar cells (Middya *et al.,* 2023). Schiff bases and their metal complexes are especially promising as antimicrobial agents (Kamaleddin *et al.,* 2016; Sani *et al.,* 2022; Sumit *et al.,* 2024). Moreover, computational studies have shown Schiff base derivatives to be active against viral targets like the SARS-CoV-2 main protease (Ünlü *et al.,* 2023).

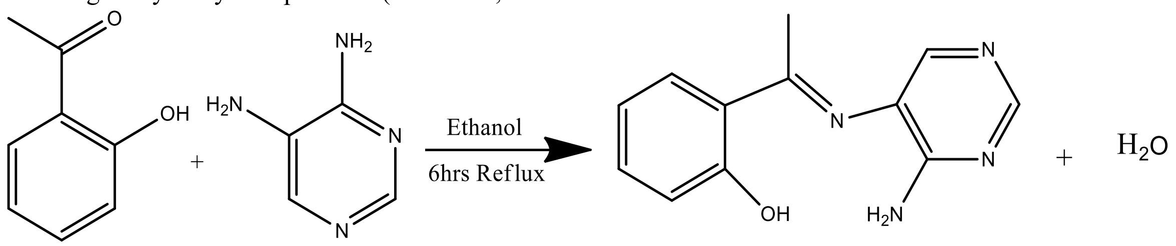
MATERIALS AND METHODS

Chemicals and Reagents

All reagents used were of analytical grade and used without further purification. 2’-Hydroxyacetophenone and 4,5-diaminopyrimidine were purchased from Sigma Aldrich.

Synthesis of Schiff base Ligand

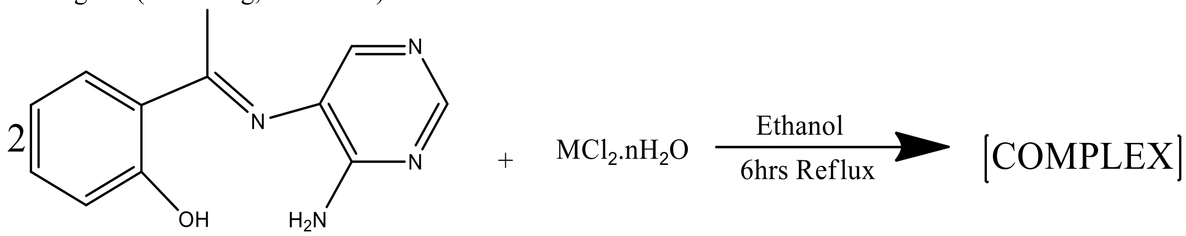
The Schiff base ligand was synthesized by refluxing 2’-hydroxyacetophenone (6.019 mL, 0.05 mmol) in 40 mL absolute ethanol (Scheme 1). This solution was added dropwise to an ethanolic solution of 4,5-diaminopyrimidine (5.506 g, 0.05 mmol). The mixture was refluxed for 6 hours. Upon cooling and concentration, a brownish-yellow solid was obtained which was filtered, washed with ethanol, recrystallized, and dried in a desiccator (Diganta *et al.,* 2017).



Scheme 1: Reaction for the Preparation of Schiff Base

Synthesis of Metal Complexes

The metal complexes were synthesized by mixing $MnCl_2{\cdot}4H_2O$ (0.19791 g, 1.0 mmol) or $NiCl_2{\cdot}6H_2O$ (0.23769 g, 1.0 mmol) with the Schiff base ligand (0.45654 g, 2.0 mmol) in ethanol in a 1:2 molar ratio (Scheme 2). The solutions were refluxed for 6 hours at 78 °C. The resulting precipitates were filtered, washed, and dried in a desiccator (Jayaseelan *et al.,* 2011).



Where M = Mn^{2+}, Ni^{2+}.

Scheme 2: Complex Formation

Determination of Melting point of Schiff base and Decomposition Temperature of the metal complexes

Average melting point of the Schiff base and the decomposition temperature of the Metal complexes was determined using Stuart SMP10 Melting point Apparatus as shown in Table 1.

Determination of Water of Crystallization

Exactly 0.2g of each complex prepared was placed in an oven at 110ºC for 24hours and continuously until a constant weight was achieved. The percentage water of crystallization was calculated using equation 1.

$$\%\ \text{of water of Crystallization} = \frac{W2-W3}{W2-W1} \times 100 \qquad (1)$$

$$[ML].H_2O = \frac{\text{Molar Mass of XH2O}}{\text{Molar Mass of the [ML]}} \times 100 \qquad (2)$$

Where;
W_1 = weight of the watch glass W_2 = weight of the watch glass and complex before heating
W_3 = weight of the watch glass and sample after heating M = Metal Complex L = Ligand.

Magnetic Susceptibility Measurement

The magnetic susceptibility measurements of complexes was determined using magnetic susceptibility balance MKI Sherwood science ltd via equation 3.

$$Xg = \frac{C\times L(R-R^{o})}{109M} \qquad (3)$$

Where;
Xg= Grams magnetic moment C = 1 (Constant) L = Sample Length (cm)
R = Reading of the sample in the tube Rº = Reading of the empty tube
M = W1-W2 = Mass of the Sample in the tube (mg) W1 = Weight of empty tube
W2 = Weight of sample and tube

The Molar Magnetic Susceptibility was calculated using equation 4;
Xm = Xg(Molar Mass) (4)
The Magnetic Moment was calculated using equation 5;
μeff = 2.828√(XmT) (5)
Where;
2.828 = Constant T = Absolute Temperature

Job's method of continuous variation

3mmol solution of the ligand and the metal salt were prepared in DMF. The following ligand to metal salt ratio (cm^3); 15:01, 13:03, 11:05, 09:07, 07:09, 05:11, 03:13, and 01:15, were taken from the ligand solution and each of the metal salts solutions respectively. A total volume of $16cm^3$ was maintained (in that order) throughout the process and the mole fraction of the ligand was calculated in each mixture. The solutions of the metal salts were scanned (as blank) to find the wavelength of maximum absorption (λ_{max}) for that particular metal ion. The machine was fixed at λ_{max} (in each case) before taking the absorbance values. The absorbance values were extrapolated against mole fraction of the Ligand and the number of coordinated ligand (coordination number) was determined using equation 6.

$$n = \frac{Xi}{1-Xi} \quad (6)$$

Where n= number of coordinated ligand, Xi = ligand mole fraction at maximum absorbance.

UV-Visible Spectroscopy Measurement

UV-Visible spectroscopic measurement of the Schiff base and complexes formed were measured using PerkinEmer UV Winlab Data Processor and Viewer Version 1.00.00 in the range of 200-700nm. $\pi-\pi*$, $n-\pi*$ absorption measurements were compared (Seema *et al.* 2024).

Antimicrobial Test

Preparation of Concentrations of Ligand and complexes for Antimicrobial Sensitivity Test

0.002g of Schiff base and the complexes was weighed and diluted into 1ml of dimethylsulphoxide (DMSO). The mixture was allowed to dissolved completely which gives a stock concentrations of 2000µg/ml, and other working concentrations from each stock concentrations of doubling serial dilution method of 1000µg/ml, 500µg/ml respectively (Zahid *et al,* 2005).

Preparation of Media

Sensitivity testing medium was prepared according to manufacturer's specifications e.g. 48.5g of Muller Hinton Agar, and 39g potato dextrose agar was weighed and dissolved each into 1000ml of distilled water, the mixture was allowed to dissolved completely and it's then autoclaved at 121ºC for 15minutes. After autoclaving time was reached, the medium was poured into sterile petri dishes, and allowed to solidify at 45 ºC.

Standardization of Test Organisms

The test organism selected was standardized with 0.5ml of normal saline and 0.5ml of Macfland Standard by comparing the turbidity of each organism with which can be matched with that of 0.5ml of Macfland standard (Zahid *et al,* 2002).

RESULTS

Physical Properties

The physical properties of the Schiff base and its metal (II) complexes are summarized in Table 1. The Schiff base appeared orange with a melting point of 192 °C, while the metal complexes formed colored solids with decomposition temperatures of 216 °C and 245 °C for the Mn (II) and Ni(II) complexes, respectively. The ligand yielded 79% while Mn and Ni complexes yielded 82 and 72% respectively.

Table 1: Physical properties of Schiff base and its metal complexes

Compound	Color	Melting point (ºC)	Decomposition Temperature (ºC)	Percentage Yield%
L	Orange	192	-	79
$[MnL_2].3H_2O$	Pale Green	-	216	82
$[NiL_2].3H_2O$	Light	-	245	72

L = $C_{12}H_{12}N_4O$

Solubility

The ligand and its complexes were found to be soluble in polar aprotic solvents like DMF and DMSO, slightly soluble in ethanol and methanol, and insoluble in non-polar solvents like carbon tetrachloride, benzene, and diethyl ether.

Table 2: Solubility profile of compounds

Compound	Dist. H_2O	Ethanol	Methanol	DEE	CCl_4	Benzene	Acetone	DMF	DMSO
L	SS	SS	SS	IS	IS	IS	SS	S	S
$[MnL_2].3H_2O$	SS	SS	SS	IS	IS	IS	SS	S	S
$[NiL_2]. 3H_2O$	SS	SS	SS	IS	IS	IS	SS	S	S

L= $C_{12}H_{12}N_4O$; (S = Soluble; SS = Slightly Soluble; IS = Insoluble); DMF =Dimethyl Formamide; DMSO = Dimethyl Sulfoxide; DEE = Diethylether; CCl_4 = Carbon tetrachloride

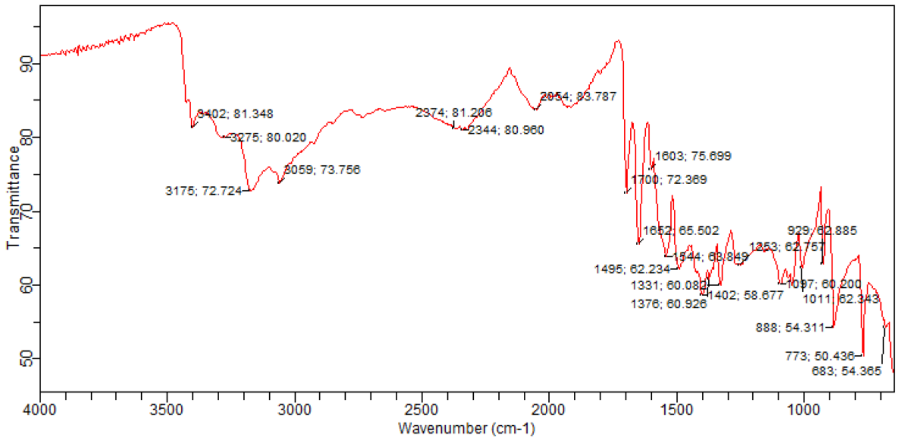
FTIR Spectroscopy

FTIR spectra confirmed the formation of Schiff base and its complexes. A strong band at 1603 cm⁻¹ (C=N stretch) was observed in the ligand. This band shifted to lower wavenumbers in the metal complexes, 1581 and 1594 cm⁻¹ for Mn and Ni complexes respectively, indicating coordination via the azomethine nitrogen. M–N and M–O bands appeared in the ranges of 435–483 cm⁻¹ and 521–587 cm⁻¹, respectively.

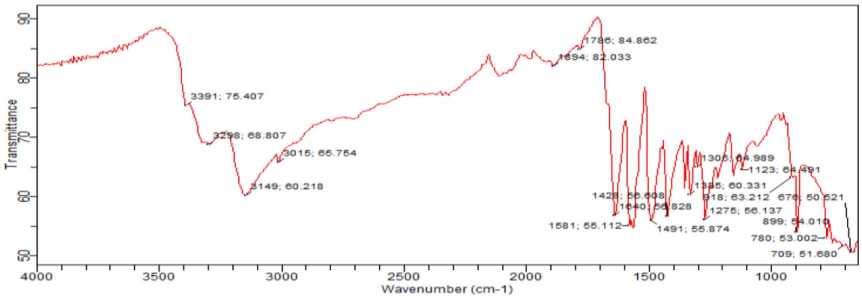
Table 3: FTIR data (cm⁻¹)

Compound	v(C=N)	v(OH)	v(O-H$_2$)	v(M-N)	v(M-O)
L	1603	3402	-	-	-
[MnL_2].3H_2O	1581	-	3391	483	587
[NiL_2]. 3H_2O	1594	-	3436	435	521

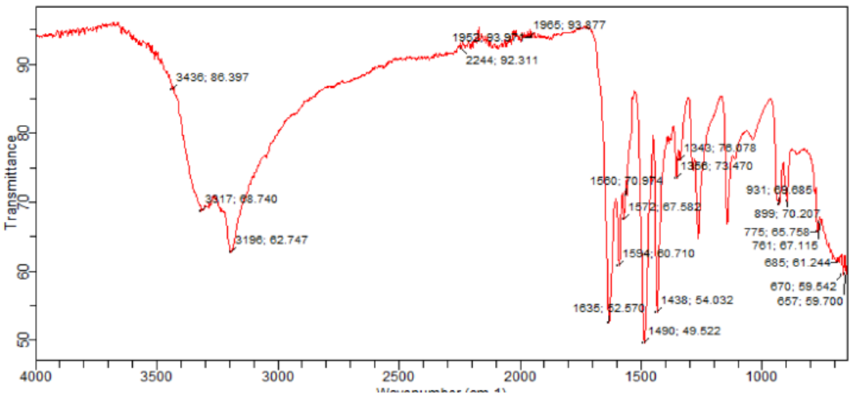
L = $C_{12}H_{12}N_4O$



(a)



(b)



(c)

Figure 1: Infrared spectra of (a) Schiff base; (b) Mn(II) Complex; (c) Ni(II) Camplex

Conductivity Measurements

The low molar conductivity values (23-24 Ω⁻¹cm²mol⁻¹) in DMSO suggest non-electrolytic behavior for the complexes (Siraj and Sanusi, 2021).

Table 4: Conductivity Measurement Data of Complexes in DMSO (Dimethylsulphoxide)

Complex	Concentration($moldm^{-3}$)	Specific Conductance ($\Omega^{-1}cm^{-1}mol^{-1}$)	Molar Conductance ($\Omega^{-1}cm^{2}mol^{-1}$)
$[MnL_2].3H_2O$	1.0×10^{-3}	23×10^{-6}	23
$[NiL_2].3H_2O$	1.0×10^{-3}	24×10^{-6}	24

Magnetic Susceptibility data

Magnetic measurements confirmed the paramagnetic nature of the complexes. The observed magnetic moments indicate high-spin octahedral geometry. (Table 5).

Table 5: Magnetic susceptibility data

Complex	Xg (g/mol)	Xm (g/mol)	μ_{eff}(B.M)	Magnetic Property	Number of Unpaired Electrons
$[MnL_2].3H_2O$	2.40952×10^{-5}	1.363×10^{-2}	5.70	Paramagnetic	5
$[NiL_2].3H_2O$	5.25362×10^{-6}	2.991×10^{-3}	2.67	Paramagnetic	2

UV-Visible Spectra

Electronic spectra (Table 6) showed characteristic π-π* and n-π* transitions for aromatic and azomethine groups, with shifts upon complexation

Table 6: UV-Vis spectral data (nm)

Compound	**π – π*** (Aromatic)	**π – π*** (C=N)	n- **π** (C-O)	n- **π** (C-N)	n- **π** (C=N)
L	246	236	308	302	328
$[MnL_2].3H_2O$	247	238	321	310	352
$[NiL_2].3H_2O$	253	240	261	296	305

L = $C_{12}H_{12}N_4O$

Job's Method of Continuous Variation

Job's plots indicated a 1:2 metal-to-ligand stoichiometry for both complexes.

Table 7: Job's method data

Complex	**Mole Fraction (Xi)**	**Average Number of Coordinated Ligands (n)**	**M:L Ratio**
$[MnL_2].3H_2O$	0.68	2.125	1:2
$[NiL_2].3H_2O$	0.68	2.125	1:2

Mole fraction (Xi) Average number of coordinated ligand (n) and Ligand (L)

Antimicrobial Analysis

Antimicrobial activity (inhibition zone diameter, mm) of the Schiff base ligand and its metal (II) complexes against selected microbial strains. Ciprofloxacin and ketoconazole were used as standard antibacterial and antifungal agents, respectively.

Table 8: Antimicrobial activity results for Schiff base and the metal complexes

Compound	**Zone of Inhibition (mm)**							
		Anti-bacterial				**Anti-fungal**		
	Concentration(μg/ml)	***Staph. Aureus***	***Strept. Pneumonia***	***Escherichia Coli***	***Kleb. Pneumonia***	***Aspergillus Flavus***	***Aspergillus Niger***	**Aspergillus Niger**
Schiff Base(L)	2000	15	16	13	16	14	13	13
	1000	13	14	11	13	11	10	10
	500	11	12	9	11	9	8	8
$[MnL_2].3H_2O$	2000	19	15	19	18	16	18	18
	1000	16	12	17	16	14	16	16
	500	14	00	15	14	00	14	14
$[NiL_2].3H_2O$	2000	17	16	15	19	16	15	15
	1000	15	14	13	17	14	13	13
	500	12	13	00	13	11	00	00
Ciprofloxacin	2000	30	28	25	32	`-	-	22
Ketoconazole	2000	-	-	-	-	27	22	

DISCUSSION

The successful condensation of 2'-hydroxyacetophenone with 4,5-diaminopyrimidine yielded an orange Schiff base with a melting point of 192 °C and percentage yield of approximately 79%. The subsequent complexation with Mn(II) and Ni(II) salts resulted in colored complexes with decomposition temperatures of 216 °C and 245 °C, respectively, indicating thermal stability of the synthesized complexes.

The appearance of a strong C=N stretching band at 1603 cm^{-1} in the Schiff base, and its shift to lower frequencies (1581 cm^{-1} for Mn(II) and 1594 cm^{-1} for Ni(II)) in the complexes, confirms coordination through the azomethine nitrogen. Additionally, new bands in the regions of 483-435 cm^{-1} and 587-521 cm^{-1} in the complexes suggest metal-nitrogen and metal-oxygen bonding, respectively, supporting a bidentate coordination mode. These observations are consistent with the reports of (Salihu *et al.,* 2022; Sani *et al.,* 2022; Reddy *et al.,* 2023).

The solubility profiles indicate that both the ligand and its complexes are soluble in polar aprotic solvents such as DMSO and DMF, while they remain insoluble in non-polar solvents. This is typical of metal-organic compounds with polar functional groups.

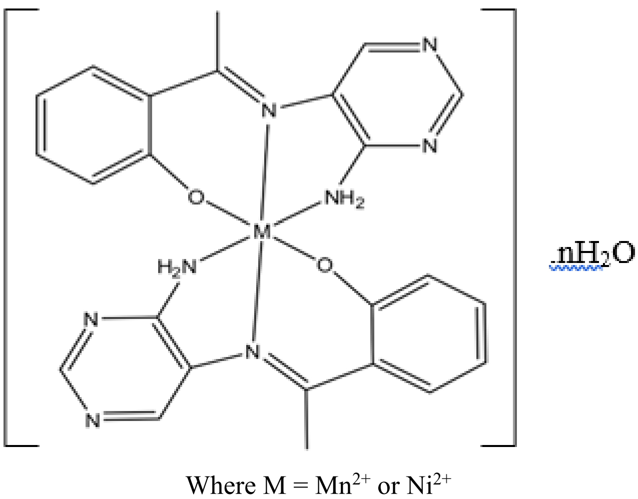
Low molar conductivity values (23-24 $\Omega^{-1}cm^2mol^{-1}$) in DMSO suggest that the metal complexes to be non-electrolytes, indicating that no counter-ions are present outside the coordination sphere (Geary, 1971; Siraj and Sanusi, 2021).

UV-Visible spectroscopic analysis revealed π-π* and n-π* transitions, with bathochromic shifts observed in the complexes. This shift suggests changes in the ligand's electronic environment due to metal coordination. Bands between 236-253 nm correspond to π-π* transitions, while n-π* transitions are observed between 261-352 nm. These shifts support complex formation (Seema *et al.,* 2024).

Magnetic moment values of 5.70 BM for Mn(II) and 2.67 BM for Ni(II) complexes are in agreement with high-spin octahedral geometries. The paramagnetic nature and number of unpaired electrons (5 for Mn(II), 2 for Ni(II)) also support this conclusion (Kumar *et al.,* 2024; Seema *et al.,* 2024).

Job's method of continuous variation confirmed the stoichiometry of metal-to-ligand as 1:2 for both Mn(II) and Ni(II) complexes, indicating bidentate ligand behavior with two ligand molecules coordinating to each metal ion.

Antimicrobial studies revealed moderate to strong activity of the Schiff base and its metal complexes against both bacterial and fungal strains. The metal complexes displayed enhanced activity relative to the free ligand, attributed to the chelation effect, which increases the lipophilic character of the complex, facilitating permeation through microbial cell membranes. However, the antimicrobial activities of the metal complexes were still lower than those of standard drugs (ciprofloxacin and ketoconazole). These findings align with earlier reports on Schiff base metal complexes (Singh *et al.,* 2012; Salihu *et al.,* 2022). The proposed structure of the complex is;



Where M = Mn^{2+} or Ni^{2+}

CONCLUSION

A new Schiff base was synthesized from the condensation of 2'-hydroxyacetophenone with 4,5-diaminopyrimidine, and its Mn(II) and Ni(II) complexes were successfully prepared. Characterization of the ligand and complexes were carried out using melting/decomposition temperature analysis, solubility testing, FTIR, UV-Vis spectroscopy, magnetic susceptibility, conductivity measurements, and Job's method of continuous variation.

Spectral and magnetic data confirmed the coordination of the ligand through the azomethine nitrogen and hydroxyl oxygen atoms, suggesting bidentate binding. Shifts in FTIR and UV-Vis spectra, along with Job's plots, indicated the formation of stable 1:2 (metal : ligand) complexes with octahedral geometry.

Antimicrobial screening showed that the metal complexes have higher activity than the free Schiff base, especially at higher concentrations, though their activity was lower than that of standard drugs. This enhancement in activity is attributed to the chelation effect, which improves the penetration of metal complexes into microbial cells.

The study confirms that Schiff base metal complexes are promising candidates for further research into biologically active coordination compounds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding publication of this manuscript.

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