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Synthesis, Characterization and Antimicrobial Studies of Fe(II), Cu(II) and Cr(II) Complexes of (E)-2-hydroxy-5-nitro-N-ethylbenzylideneamine

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ABSTRACT

The escalating problem of antimicrobial resistance has renewed interest in metal coordinated curative agents, particularly Schiff base transition metal complexes, which combine structural versatility with promising biological activity. In this study, a Schiff base ligand, (E)-2-hydroxy-5-nitro-N-ethylbenzylideneamine, was prepared through condensation of 2-hydroxy-5-nitrobenzaldehyde with aminoethane in refluxing ethanol, and then reacted with Fe(II), Cu(II), and Cr(II) salts in a 2:1 molar ratio to yield their corresponding metal complexes. The synthesized ligand and complexes were characterized by Fourier-transform infrared (FT-IR) spectroscopy, magnetic susceptibility measurements, melting point determination, and solubility tests. FT-IR data confirmed Schiff base formation via the C=N stretching band at 1565 cm^{-1} and established metal coordination through characteristic M–N and M–O vibrations in the complexes, pointing to bidentate coordination through the imine nitrogen and phenolic oxygen donor atoms. Magnetic moment values of ~5.9 B.M. for Fe(II), ~4.9 B.M. for Cr(II), and ~1.9 B.M. for Cu(II) were recorded, consistent with high-spin octahedral geometry for Fe(II) and Cr(II), and square planar or distorted tetrahedral geometry for Cu(II). Antimicrobial screening against *Aspergillus niger* and *Salmonella* spp. using the agar well diffusion method revealed that all complexes outperformed the free ligand. The Fe(II) and Cu(II) complexes each recorded a maximum inhibition zone of 24 mm against Salmonella spp., while the Cr(II) complex achieved 22 mm against both test organisms at 1000 µg/mL. The improved bioactivity is ascribed to chelation effects that raise lipophilicity and facilitate penetration of microbial membranes. These findings indicate that the synthesized complexes hold real promise as candidates for novel antimicrobial agents.

Keywords:
Schiff base;
Metal complexes;
FT-IR;
Antimicrobial

INTRODUCTION

Over the past few decades, Schiff base metal complexes have drawn considerable attention in coordination and bioinorganic chemistry, largely because of the wide range of structural types they can adopt and their rich repertoire of biological activities. A Schiff base is typically obtained by condensing a primary amine with an aldehyde or ketone; the resulting azomethine linkage (–C=N–) acts as an efficient donor site for metal coordination (Jibril *et al.*, 2023). Because these ligands can stabilize metal ions across different oxidation states, they have been exploited for designing catalytic, magnetic, and therapeutically relevant complexes (Ingole, 2022).

Among the transition metals, iron, copper, and chromium are particularly worth studying because of their variable oxidation states and distinct coordination preferences. Iron and copper are indispensable in living systems, where they participate in oxygen transport, electron transfer chains, and enzyme-catalyzed reactions, while chromium complexes have been explored for therapeutic uses linked to their redox behavior (Al-Assafe and Al-Quaba, 2024). When these metal ions are bound to a biologically active ligand, the resulting complex generally shows stronger antimicrobial action than the free ligand alone, an observation widely explained by chelation-driven reductions in polarity and increases in lipophilicity (Raman *et al.*, 2007).

Antimicrobial resistance has become one of the most serious global health threats of our time, making the search for new classes of active compounds urgent. Metal complexes are attractive in this context because they operate through mechanisms that differ fundamentally

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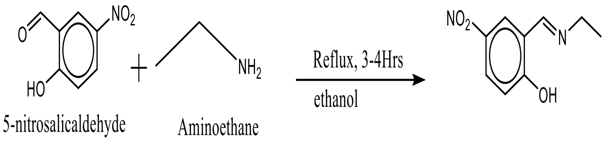
from those of conventional antibiotics, such as enzyme inhibition, disruption of the cell membrane, or the intracellular generation of reactive oxygen species (Biradar and Mruthyunjayaswamy, 2013). For this reason, Schiff base transition metal complexes have been tested against a broad spectrum of pathogenic bacteria and fungi, with many studies reporting activity levels that justify further development (Jibril *et al.,* 2023).

Against this backdrop, the present work describes the preparation, structural characterization, and antimicrobial evaluation of Fe(II), Cu(II), and Cr(II) complexes derived from a Schiff base ligand formed by condensing 2-hydroxy-5-nitrobenzaldehyde with aminoethane. The specific objectives were to determine how the ligand coordinates to each metal centre, to assign the coordination geometry of every complex from spectroscopic and magnetic data, and to evaluate the antimicrobial potency of each complex against *Aspergillus niger* and *Salmonella* spp.

MATERIALS AND METHODS

Synthesis of the Schiff Base Ligand

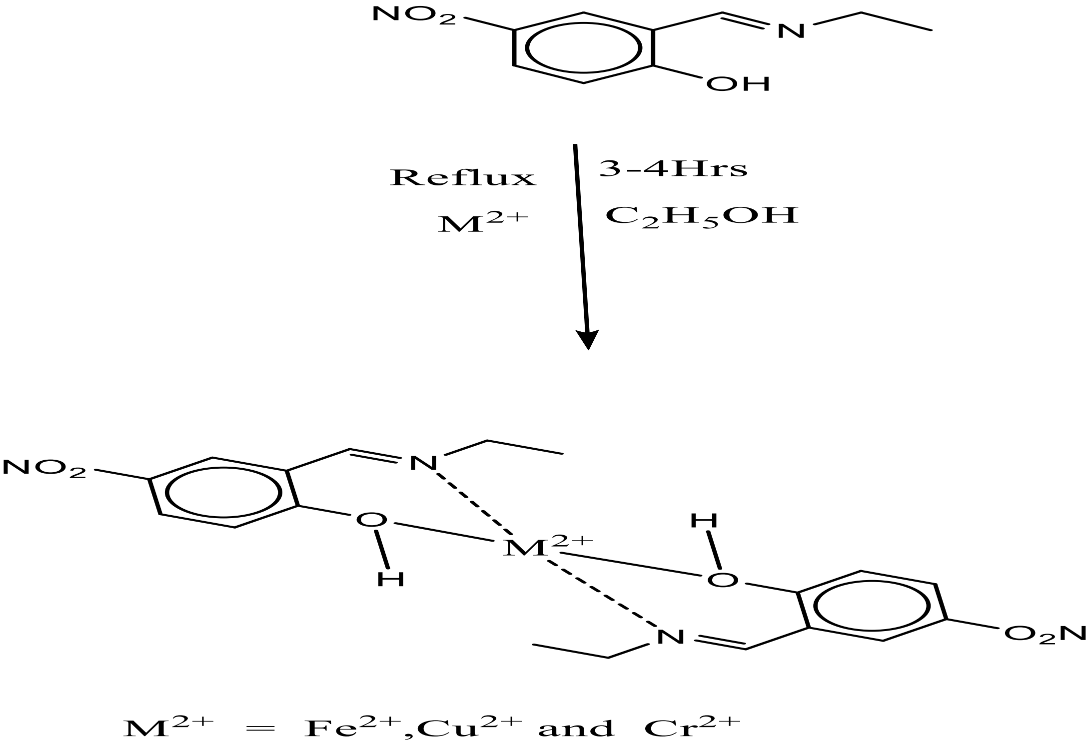
(7.2g, 43.1 mmol) of 2-hydroxy-5-nitrobenzaldehyde and aminoethane (1.94g) were combined and dissolved in 50 mL of ethanol. The reaction mixture was refluxed at 60–70 °C for 3 h with continuous stirring. Upon cooling to room temperature, a pale-yellow a pale-yellow solid precipitated out of solution; this product was collected by filtration, rinsed thoroughly with chilled ethanol, recrystallized from ethanol to improve purity, and subsequently dried over a desiccant



Scheme 1: Synthesis of the ligand

Synthesis of Metal (II) Complexes

The coordination complexes were synthesized by combining the prepared Schiff base ligand with the respective Fe(II), Cu(II), and Cr(II) metal salts at a molar ratio of 2:1 (ligand to metal). A solution of the ligand (0.1g, 0.515 mmol) in ethanol was admixed with 0.221 mmol of the selected metal salt, and the combined mixture was maintained under reflux for 3 to 4 hours. The precipitate obtained upon completion of the reaction was separated by filtration, washed successively with distilled water and ethanol, purified by recrystallization from ethanol, and air dried.



Scheme 2: Proposed structure of complex

Characterization and Antimicrobial Studies

The FTIR spectrum were measured over a range of 4000–400 cm⁻¹. Magnetic susceptibility measurements were performed at room temperature using the Gouy method. Antimicrobial activity was assessed using the agar well diffusion method against *Aspergillus niger* and *Salmonella* spp. at concentrations of 1000, 500, 250, and 125 µg/mL. Ciprofloxacin and fluconazole served as standard antibacterial and antifungal agents, respectively.

RESULTS AND DISCUSSION

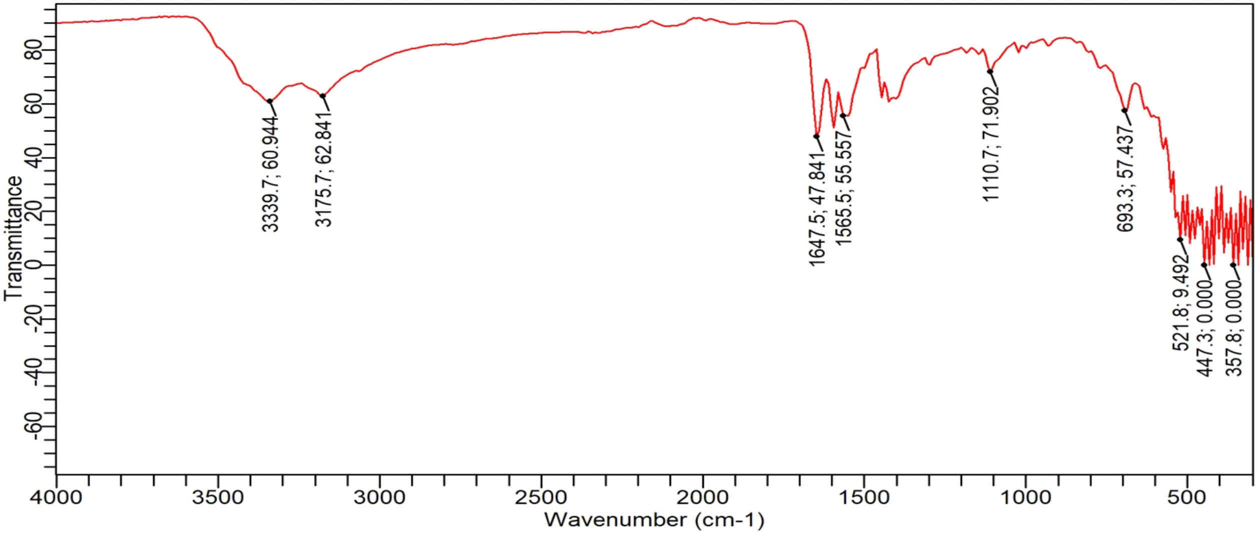


Fig:1: FTIR Spectrum of the ligand showing the Azomethine (C=N) at band 1565 cm^{-1}

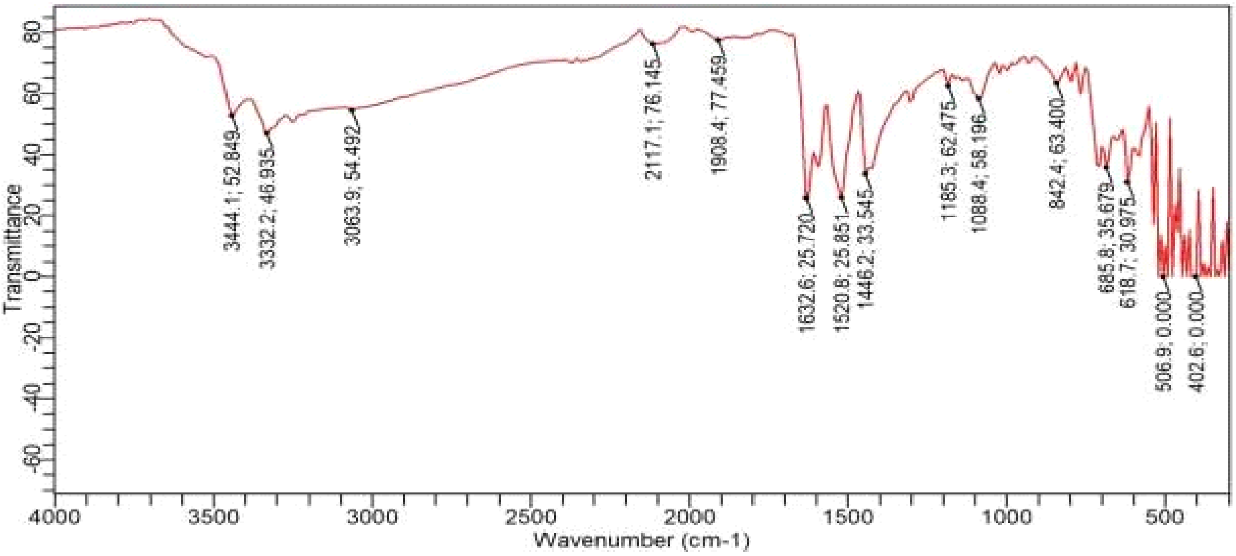


Fig2: FTIR Spectrum of Fe(II) complex showing the characteristics M-N and M-O band

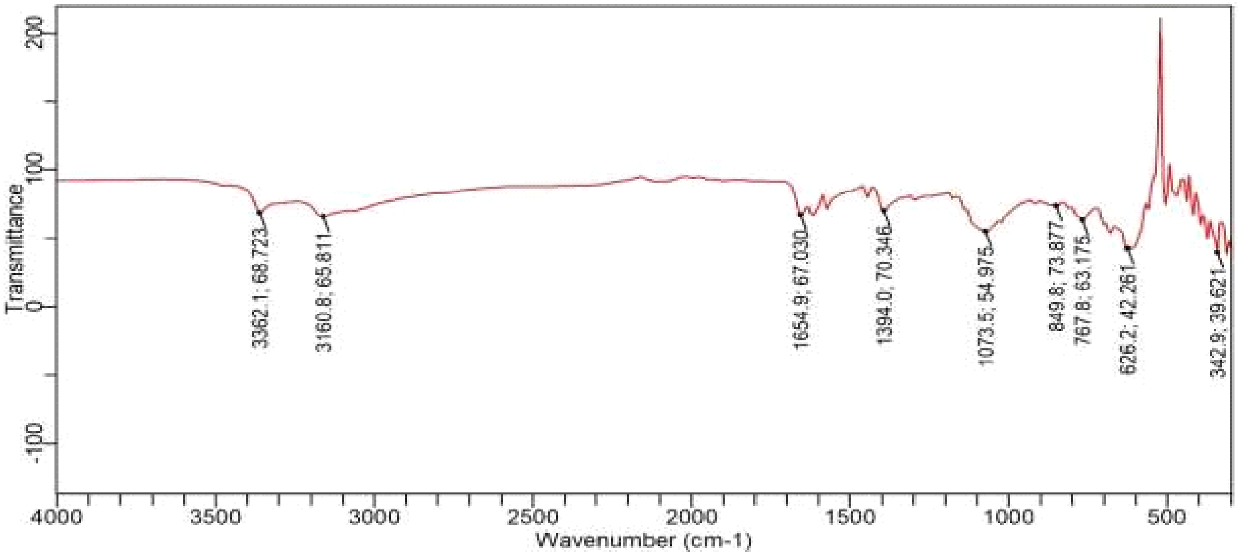


Fig3:FTIR Spectrum of Cu(II) complex indicating strong metal ligand interaction

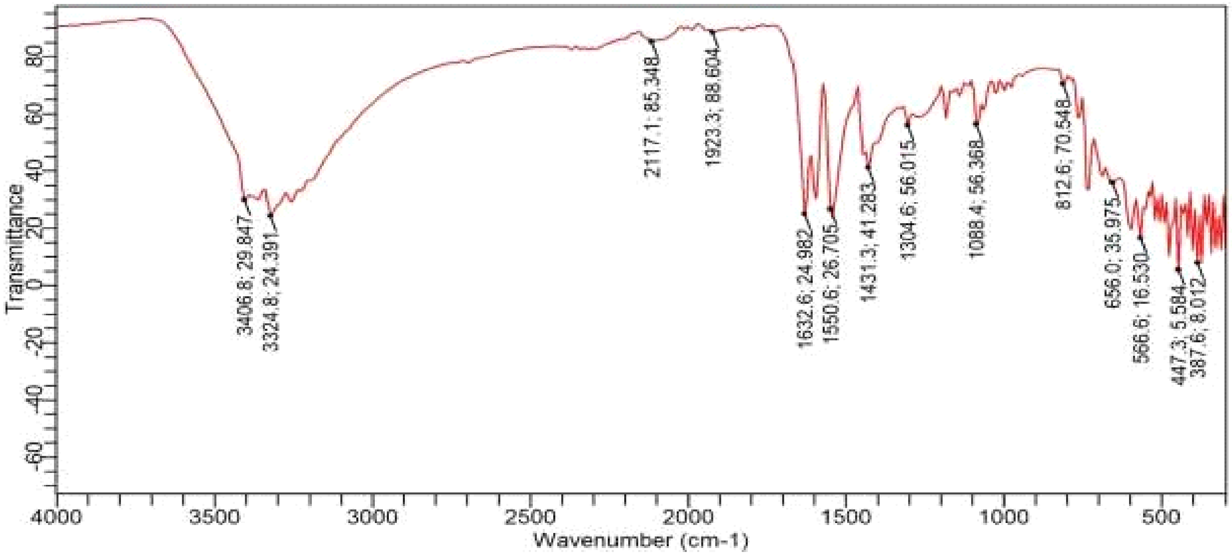


Fig4: FTIR Spectrum of Cr(II) complex to confirming bidentated coordination

Table 1: Physical properties of the Schiff base ligand and its metal(II) complexes

Compound	Molecular formula	M.W. (g mol⁻¹)	Colour	M.p. (°C)	Yield (%)	µeff (B.M.)
Ligand (L)	$C_9H_{10}N_2O_3$	194.19	Pale yellow	121	76.3	—
[Fe(L)₂]	$FeC_{18}H_{20}N_4O_6$	452.04	Brown	180	78.5	5.6–6.0
[Cu(L)₂]	$CuC_{18}H_{20}N_4O_6$	456.93	Dark green	215	74.7	1.8–2.1
[Cr(L)₂]	$CrC_{18}H_{20}N_4O_6$	448.28	Light brown	205	72.4	4.7–5.0

Table 1 presents an overview of the physical characteristics of the free ligand and its metal complexes. All complexes were recovered as stable crystalline solids displaying well defined and distinguishable colors. The Schiff base ligand was isolated in a satisfactory yield of 76.3%, reflecting the efficient progress of the condensation reaction between 5-nitrosalicylaldehyde and ethylamine. Such yields are typical for azomethine formation under mild reaction conditions and reflect the stability of the imine linkage (Sharma and Soni, 2016).

The metal complexes were obtained in yields spanning from 72.4% to 78.5%, an outcome that corroborates productive complex formation between the ligand and the respective metal centers. The Fe(II) complex yielded the highest amount, an observation consistent with a particularly favorable metal–ligand interaction, whereas the comparatively reduced yields of the Cu(II) and Cr(II) complexes are likely a consequence of inherent differences in their preferred coordination geometries and the kinetics governing the complexation reactions (Biradar and Mruthyunjayaswamy, 2013).

Table 2: FT-IR bands (cm^{-1}) of the ligand and metal(II) complexes

Compound	**ν(C=N)**	**ν(C–O)**	**ν(M–N)**	**ν(M–O)**
Ligand (L)	1565	1110	—	—
$[Fe(L)_2]$	1445	1088	680	505
$[Cu(L)_2]$	1650	1073	760	625
$[Cr(L)_2]$	1430	1088	655	565

FT-IR Spectral Analysis

The infrared spectrum of the uncoordinated ligand, a prominent absorption at approximately 1565 cm^{-1} marks the C=N stretching mode, which provides direct evidence that Schiff base formation was successful. On complexation, this band migrates to lower or higher frequencies in all three complexes, signaling that the azomethine nitrogen participates in metal binding. Additional absorptions appearing between 650 and 780 cm^{-1} and between 500 and 600 cm^{-1} are assigned to M–N and M–O stretching modes respectively, pointing to bidentate coordination through the imine nitrogen and the phenolic oxygen (Jibril *et al.*, 2023).

Table 3. Magnetic properties of the ligand and metal(II) complexes

Compound	**µeff (B.M.)**	**Unpaired electrons**	**Magnetic behavior**	**Proposed geometry**
Ligand (L)	0.00	0	Diamagnetic	—
$[Fe(L)_2]$	~5.9	4	Paramagnetic	High-spin octahedral
$[Cu(L)_2]$	~1.9	1	Paramagnetic	Square planar/distorted tetrahedral
$[Cr(L)_2]$	~4.9	4	Paramagnetic	High-spin octahedral

Magnetic Susceptibility Studies

The magnetic susceptibility data gathered at room temperature (**Table 3**) throw light on the electronic structure and probable geometry of each complex. The measured moment of approximately 5.9 B.M. for the Fe(II) complex and 4.9 B.M. for the Cr(II) complex both fall within the range expected for high-spin octahedral species with four unpaired electrons, while the Cu(II) value of about 1.9 B.M. is characteristic of a d^9 ion in a square planar or slightly distorted tetrahedral environment

Table 4. Antimicrobial activity (zone of inhibition in mm)

Complex	Microorganism	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL
$[Fe(L)_2]$	*Aspergillus niger*	21	17	15	11
	Salmonella spp.	24	15	10	8
$[Cu(L)_2]$	*Aspergillus niger*	18	12	10	8
	Salmonella spp.	24	19	10	6
$[Cr(L)_2]$	*Aspergillus niger*	22	18	12	10
	Salmonella spp.	22	16	12	10

Ciprofloxacin	*Salmonella spp.*	32	23	17	12
Fluconazole	*Aspergillus niger*	37	25	18	14

Antimicrobial Activity

Table 4 summarizes the antimicrobial activity of the synthesized Fe(II), Cu(II), and Cr(II) Schiff base complexes against *Aspergillusniger* and *Salmonella spp*. using the agar well diffusion method at four concentration levels: 1000, 500, 250, and 125 µg/mL. Every complex showed measurable growth inhibition against both test organisms, and the zones of inhibition widened as concentration increased, a dose-response pattern commonly seen for Schiff base complexes and consistent with a straightforward relationship between the amount of active compound and its biological effect (Raman *et al.,* 2007; Jibril *et al.,* 2023).

Looking at individual complexes, the Fe(II) complex stood out for its antibacterial potency against *Salmonella spp., s*howing a maximum inhibition zone of 24 mm at 1000 µg/mL, with progressively smaller zones at lower doses. This concentration-dependent decline suggests that the antibacterial effect is directly tied to the amount of active compound reaching the microbial target. The strong performance of this complex likely reflects the redox versatility of Fe(II), which enables it to interfere with bacterial enzyme function and damage key cellular components (Biradar and Mruthyunjayaswamy, 2013). This complex also showed meaningful antifungal activity against *A. niger*, which points to a broad-spectrum antimicrobial profile.

The Cu(II) complex also performed well against Salmonella spp., recording a maximum inhibition zone of 24 mm at 1000 µg/mL, essentially matching the Fe(II) complex in antibacterial potency. Its antifungal activity against *A. niger* was notably weaker, pointing to a degree of selectivity for bacteria over fungi. Cu(II) Schiff base complexes often show this pattern, and it is generally explained by the d^9 configuration of the Cu(II) ion, which supports redox cycling and the production of reactive oxygen species that damage bacterial metabolic pathways (Sengupta and Mukherjee, 2005; Jibril *et al.*, 2023).

The Cr(II) complex produced more balanced inhibition across both *A. nige*r and *Salmonella spp.*, producing inhibition zones of 22 mm against both test organisms at 1000 µg/mL. This even distribution of activity implies a wider antimicrobial spectrum and may stem from the particularly stable metal–ligand bond that Cr(II) forms with bidentate ligands, improving membrane penetration and intracellular accumulation of the complex (Raman *et al.*, 2007; Sharma and Soni, 2016).

Compared with the standard agents, ciprofloxacin and fluconazole produced larger inhibition zones at every concentration, as expected for established clinical drugs. Nevertheless, the clear dose-dependent inhibitory effects recorded for all three metal complexes confirm that tethering the Schiff base ligand to a transition metal ion substantially raises its antimicrobial potency. The most widely accepted explanation for this is chelation theory: by sharing its positive charge with the donor atoms of the ligand, the metal ion reduces overall polarity and raises lipophilicity, allowing the complex to cross the hydrophobic regions of microbial membranes more readily (Biradar and Mruthyunjayaswamy, 2013; Raman *et al.*, 2007).

The Fe(II) and Cr(II) complexes, in particular, show promising antibacterial and antifungal activities, supporting further investigation into their potential as metal based antimicrobial agent

Table 5. Solubility behavior of the ligand and metal(II) complexes

Solvent	**Ligand (L)**	**[Fe(L)$_2$]**	**[Cu(L)$_2$]**	**[Cr(L)$_2$]**
Distilled Water	IS	IS	IS	IS
Methanol	S	SS	S	S
Ethanol	S	SS	S	S
Chloroform	S	S	S	S

Benzene	SS	SS	SS	SS
DMSO	S	SS	SS	SS
Hexane	IS	SS	SS	SS
Acetone	S	S	SS	SS

Key: S = Soluble, SS = Sparingly Soluble IS = Insoluble

CONCLUSION

This study successfully describes the preparation and characterization of a Schiff base ligand derived from 2-hydroxy-5-nitrobenzaldehyde and aminoethane., along with its Fe(II), Cu(II), and Cr(II) metal complexes. FT-IR spectroscopy confirmed that the imine (C=N) bond formed correctly in the ligand and that the ligand attached to each metal through both the azomethine nitrogen and the phenolic oxygen in a bidentate fashion. The appearance of characteristic M–N and M–O vibrations in all three complexes leaves little doubt that coordination took place.

Magnetic susceptibility measurements gave moments of approximately 5.9 B.M. for Fe(II) and 4.9 B.M. for Cr(II), both pointing to high-spin octahedral arrangements, while the Cu(II) value of about 1.9 B.M. is in line with a square planar or distorted tetrahedral geometry. These assignments sit comfortably with the known electronic structures of each d-block metal and reinforce the structural picture drawn from the spectroscopic data.

Antimicrobial testing demonstrated that all three complexes were clearly more potent than the free ligand, confirming that metal coordination is the key factor driving the improvement. The Fe(II) complex produced the largest antibacterial zones against Salmonella spp. (24 mm at 1000 µg/mL), the Cu(II) complex matched it on antibacterial activity but was weaker against A. niger, and the Cr(II) complex produced the most even inhibition across *Aspergillus niger* and *Salmonella* both organisms (22 mm each at 1000 µg/mL). The pattern of results points clearly to chelation as the driver of enhanced activity, with the metal-bound complexes gaining the lipid affinity they need to penetrate and disrupt microbial membranes more effectively than the uncoordinated ligand.

Taken together, the results establish that these Schiff base metal complexes are credible leads for further antimicrobial research and offer concrete structure activity information that can guide the design of more potent derivatives. Future studies could explore additional metal ions, vary the substituents on the ligand backbone, and extend testing to a wider panel of clinically relevant pathogens.

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