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RESEARCH ARTICLE

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION, THERMAL BEHAVIOR, AND ANTIMICROBIAL AND ITS EVALUATION OF SCHIFF BASE TRANSITION METAL COMPLEXES DERIVED FROM ISONICOTINIC ACID HYDRAZIDE

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ABSTRACT

A novel Schiff base ligand, isonicotinic acid (1-(3,4-dimethoxyphenyl) ethylidene) hydrazide (INADPEH), was synthesized from condensation of isonicotinic acid hydrazide with 1-(3,4-dimethoxyphenyl)ethan-1-one. The Schiff base and its metal (cobalt, copper, nickle, and iron) complexes were characterized by elemental analysis, molar conductivity, magnetic moment values, UV-Vis, FTIR, and thermogravimetric analysis (TGA), and were obtained in good yields. The structure of the ligand was also confirmed by ^{1}H NMR spectroscopy. Analytical and spectral data confirmed that INADPEH acts as a bidentate ligand, coordinating through the azomethine nitrogen and carbonyl oxygen. FTIR and UV-Vis spectra and magnetic susceptibility values collectively suggested predominantly octahedral geometries for the complexes. Thermal studies indicated stepwise decomposition of the complexes, with metal oxides as final residues. The antimicrobial activities of the compounds were tested against *Escherichia coli, Staphylococcus aureus, Candida albicans,* and *Aspergillus niger.* The antibacterial activity results revealed that the free ligand showed lower activity compared to the cobalt and copper complexes, with the copper complex exhibiting highest potency, surpassing the standard in some cases. This study highlights the enhanced biological efficacy arising from the significant role of metal complexation and recommends the potential applications of these hydrazone metal complexes as antimicrobial agents.

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INTRODUCTION

Schiff bases ligand and their transition metal complexes have gained significant interest because of their diverse structures, flexible coordination abilities, and broad practical uses in fields such as catalysis, materials science, and medicinal chemistry (1-2). The azomethine group (-C=N-) not only helps stabilize the ligand but also plays a crucial role in influencing biological activities. Various transition metal complexes of Schiff bases have been found to show remarkable antibacterial, antifungal, anticancer, and antioxidant effects compared to the original ligands from which they are derived (3). Isonicotinic acid hydrazide derivatives are of particular pharmacological relevance due to their antimicrobial activity and potential as anti-tubercular agents. Coordination of such ligands with transition metals is known to significantly modify their physicochemical and biological properties (4). In this study, we report the synthesis, spectral and thermal characterization, and antimicrobial evaluation of Schiff base complexes derived from isonicotinic acid hydrazide 1-(3,4-dimethoxyphenyl)ethan-1-one.

MATERIALS AND METHODS

All the chemicals employed were of analytical grade, and the solvents were purified before use. Metal salts $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$ and $FeCl_3 \cdot 6H_2O$ were obtained commercially.

Scheme of Ligand Synthesis: The ligand INADPEH was obtained by refluxing equimolar amounts of isonicotinic acid hydrazide with 1-(3,4-dimethoxyphenyl)ethan-1-one in ethanol for 8 h. The product was filtered and then washed with ethanol and air dried. Yield: 81.4%, M.P. 185-189 °C.

Synthesis of Metal Complexes: The metal complexes formation was achieved by refluxing the Schiff base ligand and appropriate metal salts in ethanol at a 2:1 molar ration for 2 hours. The products were subsequently filtered, washed with ethanol and dried in vacuo.

Analytical and Physical Measurements: Elemental analysis(C, H, N) was performed by using Elementar Vario EL

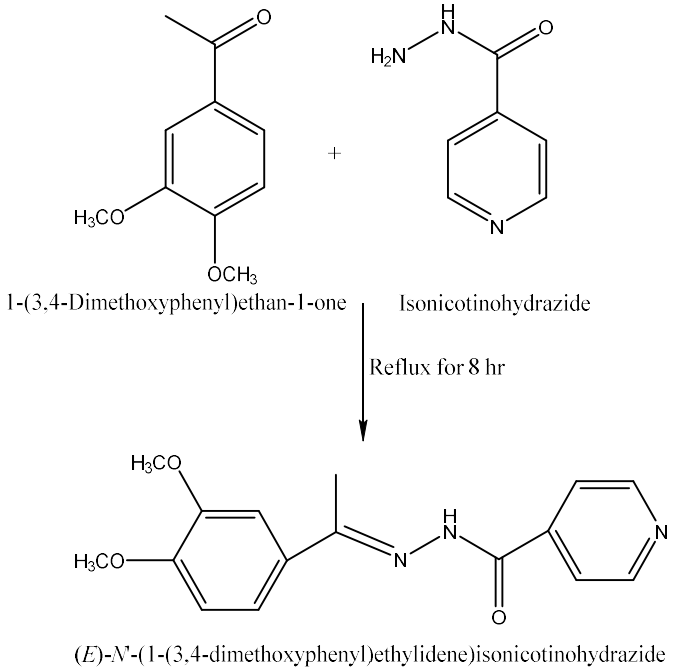

Figure 1. Scheme for the synthesis of the ligand

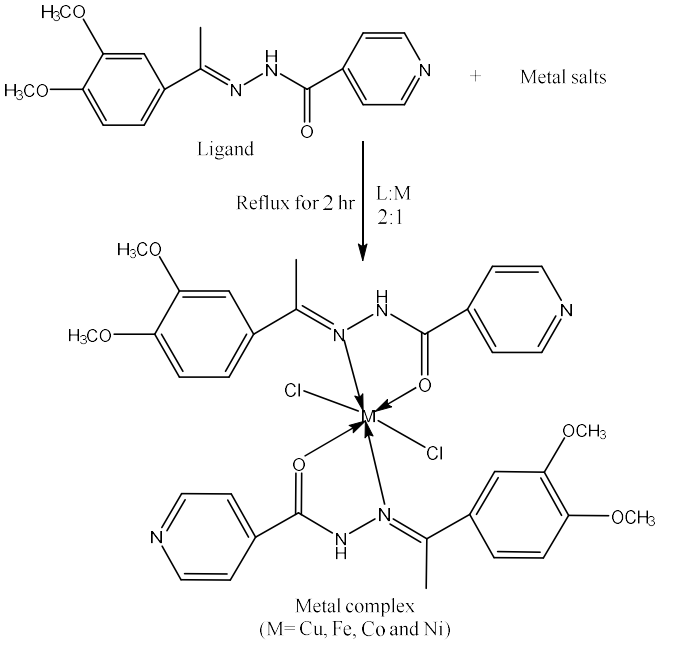

Figure 2. Synthesis of Schiff base metal complexes

cube V4.0.11 Elementar Analysensysteme GmbH, molar conductance (10^{-4} M, DMSO), FTIR (KBr pellets, (at 4000-400 $cm^{-1)}$,^{1}H NMR (DMSO-d_6), UV-Vis (methanol, 10^{-3} M), magnetic susceptibility, TGA (N_2 gas, 30-800°C and 20 °C/min), and PXRD (Cu-Kα radiation) were employed for the characterization of the Schiff base ligand and its metal complexes. Chlorine content in the metal complexes was determined using Mohr's method, and the results were consistent with the proposed molecular formulae.

Antimicrobial Studies: Antibacterial (tested against *S. aureus* and *E. coli*) and antifungal (against *C. albicans* and *A. niger)* activities were assessed by the agar well diffusion method at concentrations of 20-80 µl (10 mg/ml solutions). Ciprofloxacin and Ketoconazole served as standard drugs.

RESULTS AND DISCUSSION

Analytical and Physical Data: When analyzing the composition of the ligand and its corresponding metal complexes, the results matched up closely with what theory predicted, confirming that these compounds formed just as intended (5). The yields for the complexes were above 34 percent and they did not break down until hitting pretty sharp melting point, elevated temperatures, which means they're slightly tough against heat. It's interesting how the appearance changed: the ligand itself was a soft yellow, but once it mixed with metals, each complex took on its own bold color cobalt looked blush purple, copper turned olive green, nickel gave off a lavender shade, and iron settled into brown. These colors reflect what's happening inside their structures, with d-d transitions and charge-transfer effects that are distinct for transition metals. Looking at molar conductivity (between 46 and 60.7 $\Omega^{-1}cm^2mol^{-1}$), most of these complexes didn't behave like typical electrolytes, except for the iron (III) compound, which was a bit higher just as expect for its structure, $(FeL_2Cl_2)Cl$. What this means is that chloride ions are bound to the metal in cobalt, copper, and nickel complexes, while iron keeps one chloride out as a counter-ion, which lines up with what's been reported for similar octahedral iron complexes in previous researches (6-7).

FTIR Spectral Analysis: The FTIR spectra of the Schiff base ligand showed a distinct azomethine (ν C=N) band at 1541 cm^{-1}. Upon complexation, this absorption band which shifted to lower frequency range (1509-1549 cm^{-1}). These observed shift suggests the involvement of the azomethine nitrogen in binding to the metal ion (8). Similarly, the carbonyl band ν C=O of the Schiff base ligand appeared at 1665 cm^{-1} and showed a shift in the complexes, suggesting bonding through the carbonyl oxygen (9-10). The amide N-H stretching band remained unaffected, excluding its involvement in coordination. In addition, new absorption bands corresponding to ν M-N (759-767 cm^{-1}), ν M-O (533-657 cm^{-1}), and ν M-Cl (428-509 cm^{-1}) appeared in the complexes, these results provide additional confirmation of bidentate binding through the azomethine nitrogen and carbonyl oxygen (11-12). The observation is consistent with earlier studies where Schiff bases derived from hydrazides typically acts as bidentate ligands, stabilizing octahedral geometries around transition metals.

^{1}H NMR Spectroscopy: The ^{1}H NMR spectral of the ligand (L) recorded in DMSO- d_6 displayed expected chemical shifts for all protons. Singlets corresponding to the methoxy (δ 3.48 and 3.82 ppm) and methyl (δ 2.37 ppm) groups confirmed substitution on the aromatic ring. The aromatic and pyridine protons appeared in the δ 7.0-8.7 ppm range. Importantly, a broad singlet observed at δ 11.09 ppm was attributed to the amidic proton, confirming condensation between the hydrazide and ketone moieties (13). The absence of any aldehydic proton resonance further substantiated Schiff base formation.

Magnetic Moments and Electronic Spectral Studies: The electronic spectra of the Schiff base ligand showed bands at λ_{max} 258 and 385 nm, assignable to π-π^* and n-π^* transitions of the azomethine group. The coordination of ligand with metal showed band shifts to longer wavelengths, indicating ligand-to-metal charge transfer (LMCT). The Co(II) complex displayed multiple bands at λ_{max} 231, 335, 516, and 593nm and

Table 1. Physico-chemical parameter and analytical data of ligand and its metal complexes

Compounds Name	Color	Yield	Melting Point	Observed (Theoretical %)				Molar conductivity ^m (mol^{-1} cm^2 ohm^{-1})
				C	H	N	Cl	
Ligand, L ($C_{16}H_{17}N_3O_3$)	Pale yellow	81.4%	185-189	64.69 (64.20)	5.64 (5.72)	14.95 (14.04)	-	60.7
CoL_2Cl_2	Blush Purple	44.5%	>250	52.69 (52.76)	4.64 (4.7)	10.95 (11.54)	9.63 (9.73)	55.3
CuL_2Cl_2	Olive Green	61.25%	>250	51.81 (52.43)	4.15 (4.67)	11.65 (11.46)	9.54 (9.67)	46
NiL_2Cl_2	Light Lavendra	74.3%	>250	51.81 (52.78)	4.15 (4.71)	11.45 (11.54)	9.66 (9.76)	56.7
$(FeL_2Cl_2)Cl$	Brown	34.2%	>250	51.81 (52.98)	4.15 (4.72)	11.29 (11.59)	9.74 (9.77)	52.4

Table 2. Selected FTIR spectral data ν(cm^{-1}) of Schiff base ligand and its complexes

Name	ν C=N	ν C=O	ν M-N	ν M-O	ν M-Cl
Isonicotinic Acid (1-(3,4-dimethoxy-phenyl) ethylidene)Hydrazide	1541	1665	-	-	-
Complex-Co(II)	1549	1651	760	539	428
Complex-Cu(II)	1510	1670	765	545	466
Complex-Ni(II)	1509	1651	767	533	454
Complex-Fe(III)	1543	1651	759	657	509

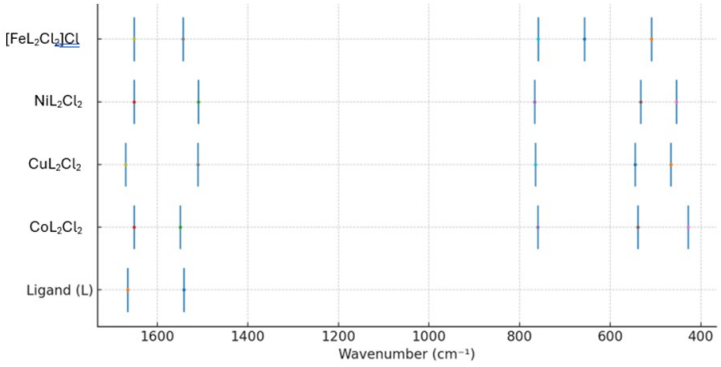

Figure 3. IR spectrum of ligand (isonicotinic acid (1-(3,4-dimethoxyphenyl) ethylidene) hydrazide) and their transition metal complexes

Table 4. Electronic spectral and magnetic moment data of Schiff base ligand and its complexes with corresponding assignments

Compounds name	M.M	Absorption band positions (nm)	Possible transitions	Geometry
Ligand ($C_{16}H_{17}N_3O_3$)	-	258, 385	π-π* and n-π*	-
CoL_2Cl_2	4.87	231, 335, 516, 593	LMCT, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, and $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	octahedral
CuL_2Cl_2	1.87	278, 312	(LMCT)	distorted octahedral
NiL_2Cl_2	2.96	262, 350, 564	LMCT, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$	octahedral
$(FeL_2Cl_2)Cl$	1.58	393, 265	$^6A_1 \rightarrow {}^4T_2(G)$ and LMCT	octahedral

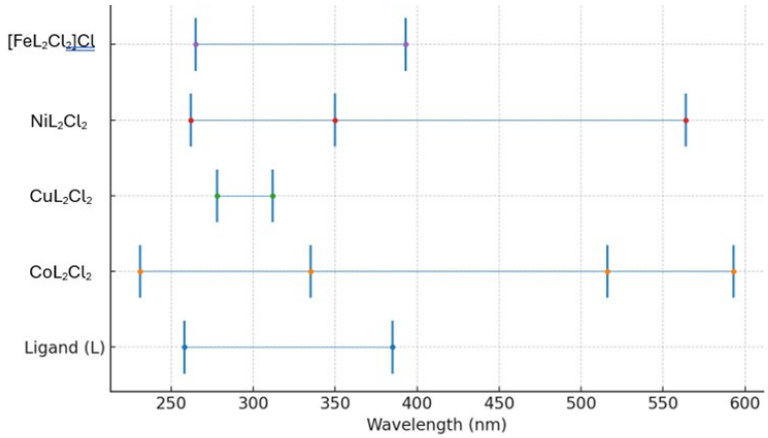

Figure 4. UV-Vis spectrum of ligand (isonicotinic acid (1-(3,4-dimethoxyphenyl) ethylidene) hydrazide) and its metal complexes

Table 5. Ligand and its complexes of crystallite size

Complex	2θ	Crystallite size
Ligand, L $C_{16}H_{17}N_3O_3$	12.8141 degrees	444.37 Å (44.44 nm)
Cobalt	29.692 degrees	327.74 Å (32.8 nm)
Copper	15.475 degrees	273.97 Å (27.4 nm)
Nickle	12.6746 degrees	410 Å (41.0 nm)
Iron	29.695 degrees	507.32 Å (50.7 nm)

assignable were respectively to LMCT, d-d transitions $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, and $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, confirming an octahedral geometry. The magnetic moment (4.87 BM) was consistent with a high-spin Co(II) configuration (8). Cu(II) complex exhibited abroad absorption at λ_{max} 278,312 nm, attributed to LMCT. The absence of well-resolved d-d bands is typical of distorted octahedral Cu(II) complexes due to Jahn-Teller distortion. The magnetic moment (1.87 BM) confirmed one unpaired electron (14). Ni(II) complex exhibited absorption bands at λ_{max} 262, 350 and 564 nm, which were assigned to LMCT, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ transitions respectively, which supported an octahedral geometry. The moment (2.96 BM) was consistent with two unpaired electrons (15). For Fe(III) complex displayed weak d-d transitions at λ_{max} 265 and 393 nm along with LMCT and $^6A_1 \rightarrow {}^4T_2(G)$ bands, consistent with ahigh-spin octahedral d^5 system. The magnetic moment (1.58 BM) was lower than expected, possibly due to ant ferromagnetic interaction (16). These observations establish that all metal complexes adopt octahedral geometries, whereas Cu(II) showing a distorted environment.

Thermal Studies (TGA): Thermo gravimetric analysis (TGA) confirmed multiple decomposition steps of all complexes. Co(II) complex was stable up to 200 ℃ and a major decomposition event between 400-600 ℃ results in 51.7% weight loss (calcd. 51.1%), corresponding to the degradation of the compound's organic framework and stepwise decomposition gave the residue of metal oxide CoO. Cu(II) complex exhibited a major weight loss (70.9%) between 150-400 ℃ corresponded to ligand and decomposition and the final residue observed as metal oxide (CuO). Ni(II) complex was undergoing nearly complete degradation (95% weight loss) and left the metal oxide as a residue (NiO). For Fe(III) complex which followed by a major decomposition of the organic framework between 300–600 °C. The final residue matched Fe_3O_4 (29.1%), consistent with magnetite formation at high temperature. The close match between observed and calculated weight losses confirmed the proposed compositions (17). Complexation with metal ions enhanced the thermal stability of the ligand, particularly delaying major decomposition beyond 300 ℃, suggesting strong coordination and potential for high-temperature applications.

PXRD (Powder X-ray Diffraction): The crystalline nature of the complexes was confirmed by PXRD analysis. Crystalline sizes estimated using by the Scherrer equation ranged from 27.4 to 50.7 nm, placing them in the nanocrystalline regime (18). Such nanoscale crystallinity is of interest since it can significantly influence both the catalytic and biological properties of metal complexes, enhancing their reactivity and antimicrobial potential.

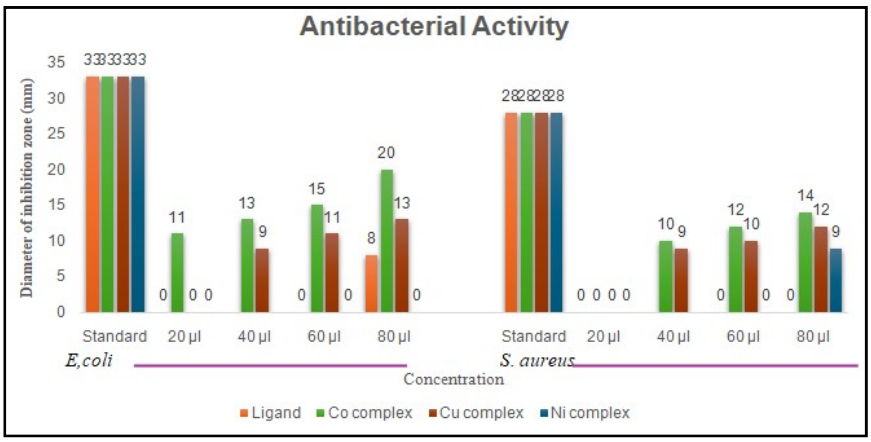

Figure 3. Graph of diameter of inhibition zone (mm) against *E. coli and S. aureus*

Table 6. Diameter of inhibition zone (mm) of Schiff base ligand and its complexes against *E. coli and S. aureus* bacteria

S. No.	Samples	Activity against *E. coli* bacteria					Activity against *S. aureus* bacteria				
		Diameter of inhibition zone (mm)					Diameter of inhibition zone (mm)				
		Standard	20 µl	40 µl	60 µl	80 µl	Standard	20 µl	40 µl	60 µl	80 µl
1	Ligand ($C_{16}H_{17}N_3O_3$)	33	-	-	-	8	28	-	-	-	-
2	CoL_2Cl_2	33	11	13	15	20	28	-	10	12	14
3	CuL_2Cl_2	33	-	9	11	13	28	-	9	10	12
4	NiL_2Cl_2	33	-	-	-	-	28	-	-	-	9

Table 7. Diameter of inhibition zone (mm) of Schiff base ligand and its complexes against *C. albicans and A. niger* fungal

S. No.	Samples	Activity against *C. albicans* fungal					Activity against *A. niger* fungal				
		Diameter of inhibition zone (mm)					Diameter of inhibition zone (mm)				
		Standard	20 µl	40 µl	60 µl	80 µl	Standard	20 µl	40 µl	60 µl	80 µl
1	Ligand ($C_{16}H_{17}N_3O_3$)	23	-	-	-	-	31	-	11	12	15
2	CoL_2Cl_2	23	-	-	9	11	31	-	9	13	17
3	CuL_2Cl_2	23	11	16	18	20	31	13	16	21	24
4	NiL_2Cl_2	23	-	-	-	10	31	-	-	-	9

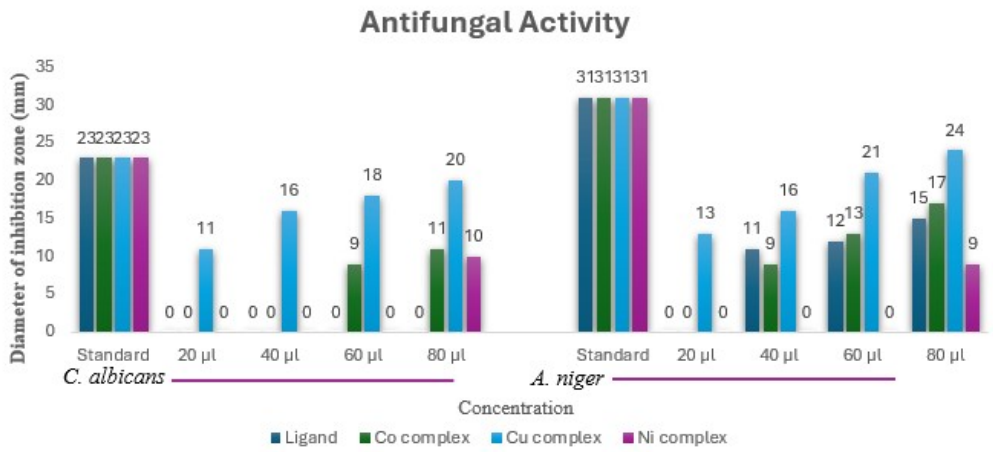

Figure 4. Graph of diameter of inhibition zone (mm) against *C. albicans and A. niger* fungal

The crystallite sizes of the ligand and its metal complexes were estimated using the Scherrer equation, and the results are summarized in Table 5. The free Schiff base ligand exhibited a crystallite size of 44.4nm which confirming its nanocrystalline nature. Upon coordination with transition metal ions, significant variations in crystallite size were observed. The Co(II) (32.8) and Cu(II) (27.4) complexes displayed reduced crystallite sizes in comparison to the ligand, indicating that metal coordination causes lattice strain and limits crystallite development. In comparison, the Ni(II) complex (41nm) displayed a size similar to that of the ligand, suggesting that the incorporation of Ni(II) does not significantly change the dimensions of the crystalline domain. Notably, the Fe(III) complex (50.7 nm) demonstrated the greatest crystallite size, indicating improved crystallinity and superior lattice arrangement in the iron-coordinated system. In general, the sizes of the Schiff base complex crystallites range from 27 to 51 nm, and the noted differences are due to variations in ionic radii, coordination geometry, and the extent of distortion caused by the specific metal ions (18). These results emphasize the impact of metal coordination on the crystalline characteristics of Schiff base complexes, which could subsequently influence their stability and functional attributes

Antimicrobial Activity: The results of the antimicrobial activity showed a distinct improvement with metal complexation when compared to the free ligand tested against *Escherichia coli, Staphylococcus aureus, Candida albicans,* and *Aspergillus niger.* The Co(II) complex exhibited highest activity (20mm at 80 µl), while the copper showed 13mm. The free ligand showed weak inhibition (8mm), while Ni(II) was inactive. Dose-dependent antibacterial activity was observed for the cobalt and copper complexes, whereas the ligand was inactive against *S. aureus.* The Cu(II) complex showed remarkable activity (20mm) against *C. albicans*, nearly comparable to the standard drug ketoconazole, while Co(II) was moderately active. Against *A. niger,* Cu(II) complex surpassed even the standard drug at higher concentrations (24mm vs 23mm), highlighting its strong antifungal potential. The enhanced antimicrobial effect of cobalt and copper complexes can be attributed to increased lipophilicity upon chelation, which facilitates penetration of microbial cell membranes (19). Additionally, transition metal ions may promote redox reactions inside microbial cells, disrupting DNA or protein functions. The relatively weak activity of the Ni(II) complex suggests that the geometry and electronic properties of the metal center strongly influence biological activity (20, 3).

CONCLUSION

A new Shiff base ligand and its transition metal complexes were successfully synthesized and comprehensively characterized. Spectral and thermal studies confirmed octahedral coordinated, while PXRD indicated nanoscale crystallinity. Antimicrobial evaluation demonstrated that Co(II) and Cu(II) complexes exhibit promising antibacterial and antifungal activity, with Cu(II) showing superior efficacy. These results highlight the potential of Schiff base transition metal complexes as leads for developing novel antimicrobial agent.

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