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Synthesis, spectral characterizations, antioxidant, and antibacterial studies of novel Schiff base and its metal complexes

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A new Schiff base ligand has been produced by refluxing 8-hydroxyjulolidine-9-carboxaldehyde with H-imidazo [1,2-a]pyridin-6-amine in methanol using an acetic acid catalyst. The ligand has been studied by elemental analysis, IR, ^{1}H and ^{13}C NMR, and ESI-MS, resulting in an 86% yield. Metal complexes of Fe(II), Co(II), Ni(II), Cu(II), and Zn(II) have then been formed by reacting them with metal acetate salts. The coordination of azomethine nitrogen and phenolic oxygen has been established through characterization. The antioxidant capability has been tested using a DPPH radical scavenging experiment, which has revealed that metal complexes outperformed free ligands. The disc diffusion method has been used to assess antibacterial activity against Gram-positive and Gram-negative bacteria, and metal complexes demonstrate superior inhibition zones.

Keywords: Schiff base chemistry, Bioinorganic complexes, Free radical scavenging, Antibacterial agents

Schiff bases are organic entities marked by an imine group (>C=N–) and are extensively applied in producing pigments and dyes, as well as in catalytic systems and polymer stabilization processes[1-3]. The condensation of primary amines with carbonyl compounds such as aldehydes and ketones leads to the formation of Schiff bases[4,5]. Schiff bases are valued for their structural simplicity, synthetic accessibility, and wide-ranging functional applications[6,7]. The introduction of various substituents and heterocyclic moieties into Schiff base frameworks has enabled the design of compounds with tailored physicochemical and biological properties[8,9].

A stable and adaptable metal complex is formed when Schiff bases easily coordinate with a range of metal ions due to the lone pair of electrons on the azomethine nitrogen. The thermal, chemical, and biological characteristics of these metal complexes are frequently better than those of their free ligands[10-12]. For their many biological properties, such as their antibacterial[13], anticancer[14], antioxidant[15], anti-inflammatory[16], antiviral[17], and enzyme-inhibitory[18] properties, transition metal complexes of Schiff bases have been thoroughly investigated. The imine linkage is stabilized by the coordination of metal ions, which also enhances the ligands' lipophilicity and membrane permeability, which frequently results in higher potency and bioavailability[19-22]. Potential prospects for therapeutic development, these heterocyclic Schiff bases and their related metal complexes have shown noteworthy activity against a variety of microbial strains and cancer cell lines[23,24]. Additionally, these ligands' chelating efficacy is increased by the inclusion of extra donor sites, which enables the production of complexes with a variety of geometries and coordination environments[25,26].

Beyond medicinal applications, Schiff base metal complexes have found utility in catalysis, electrochemical sensors, optical materials, and photochemical systems. Their tuneable electronic structures, ligand field properties, and ability to stabilize metals in various oxidation states make them valuable in both homogeneous and heterogeneous catalytic processes[27-30].

In view of their broad applications and significant potential, the present study focuses on the synthesis, characterization, and biological evaluation of novel Schiff base ligands derived from heterocyclic precursors and their transition metal complexes. Particular emphasis is placed on investigating their antimicrobial, and antioxidant properties.

Materials and Methods

Materials

Analytical-grade chemicals and reagents were all utilized without additional purification. We purchased

H-imidazo[1,2-a]pyridin-6-amine and 8-hydroxy-julolidine-9-carboxaldehyde from reliable chemical vendors. As metal sources, Fe, Co, Ni, Cu, and Zn metal(II) acetate salts were employed. High grade solvents, including methanol, ethanol, and acetic acid, were acquired from commercial vendors. The standard antioxidant ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were acquired from Sigma-Aldrich for use in biological tests. Certified microbiological vendors provided the bacterial strains and nutrient agar used in the antibacterial testing, which were kept in accordance with guidelines.

Instrumental methods

All synthesized compounds throughout the study were characterized by elemental microanalysis (C, H, and N) performed with a Perkin Elmer-400 elemental analyser. The melting points of the compounds were determined using a Polmon-MP-96 apparatus. FT-IR spectra were recorded in the range of 4000 to 500 cm^{-1} using KBr pellets on a Perkin Elmer-337 FT-IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer, using TMS as the internal standard. Mass spectrometry data were collected on a VG-AUTOSPEC instrument employing ethanol as the eluent in the mobile phase. Powered XRD patterns were recorded using a Bruker D8 Advance diffractometer equipped with Cu Kα radiation (λ = 1.5406 Å).

Experimental Section

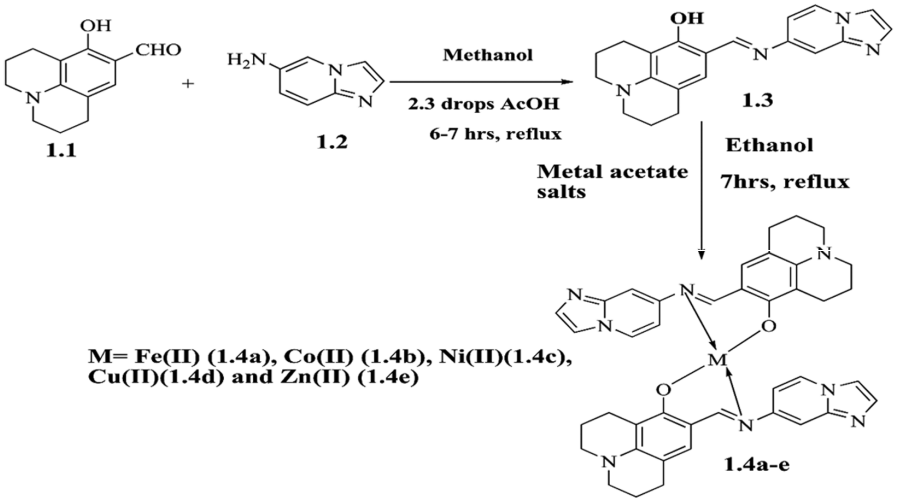
Synthesis of ligand

A mixture of 8-Hydroxyjulolidine-9-carboxaldehyde (1 mmol) and *H*-imidazo[1,2-a]pyridin-6-amine (1 mmol) was dissolved in hot Methanol (20 mL), 2-3 drops acetic acid and stirred under reflux for 6–7 hours. The progress of the reaction was monitored by TLC using ethyl acetate in hexane (2:9) solvent system. Upon completion, the reaction mixture was cooled to RT, and the yellow precipitate formed was filtered, washed with cold ethanol, and dried under vacuum. The product was recrystallized from ethanol to afford pure ligand[31,32] (Scheme 1).

Analytical data: M.F: $C_{20}H_{20}N_4O$**,** Colour: Dark Yellow, Yield: 86%, M.P: 181-183°C, ^{1}H NMR (400 MHz, DMSO, δ): 9.85 (OH, 1H, s), 8.96 (CH=N, 1H, s), 7.98-8-00 (Ar, 1H, d, *J*= 6.8 MHz), 7.72-7.74 (imidazole ring Hs, 2H, d, *J*= 8.8 MHz), 7.41 (Ar, 1H, s), 7.19 (Ar, 1H, s), 6.91-6.93 (Ar, 1H, d, *J*= 9.2 MHz), 3.32-3.34 (2×N-CH_2, 4H, m), 2.67-2.74 (2×Ar-CH_2, 4H, m), 2.67-2.74 (2×Ar-CH_2, 4H, m), 1.84 2.67-2.74 (2×CH_2, 4H, m) (Fig. 1). ^{13}C NMR (400 MHz, DMSO, δ): 162.02 (C=N), 158.12 (C–OH), 148.69, 143.96, 136.35, 134.02, 130.91, 127.22, 123.98, 121.79, 117.07, 116.13, 112.21, 49.28, 48.97, 26.80, 20.78, 20.69, 20.14. IR (KBr, cm^{-1}): 3488 (OH**)**, 1639 **(**C=N**)**, 1253 **(**C-O**)** (Fig. 2). ESI-MS, m/z): Calculated 332; found: 333 $[M+1]^{+}$. E. analysis: Caltd %: C, 72.27; H, 6.06; N, 16.86. Found %: C, 72.23; H, 6.03; N, 16.89; O, 4.77.

Synthesis of 3d series metal complexes

20 mL of pure ethanol was used to dissolve the Schiff base ligand (**1.3**) (1 mmol). Then, 1 mmol of the corresponding metal(II) acetate salts—Fe(II), Co(II), Ni(II), Cu(II), and Zn(II)—were added drop wise to this solution while being constantly stirred. After the reaction mixture was refluxed for seven



Scheme 1 — Synthesis of Schiff base ligand and its metal complexes

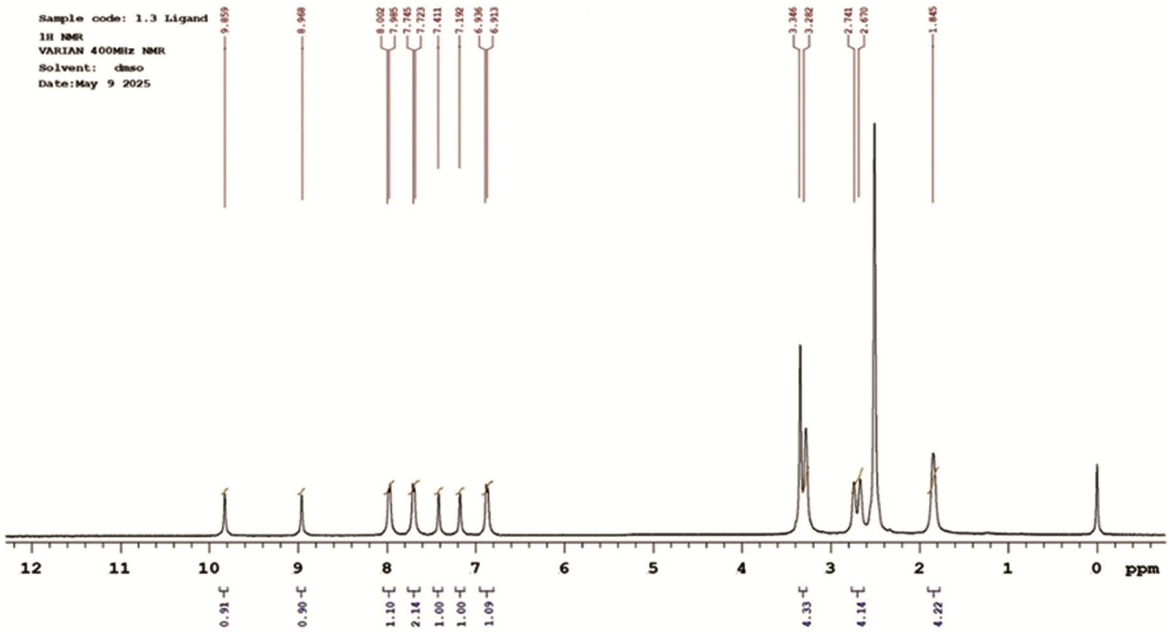


Fig. 1 — ^{1}H NMR spectra of ligand **1.3**

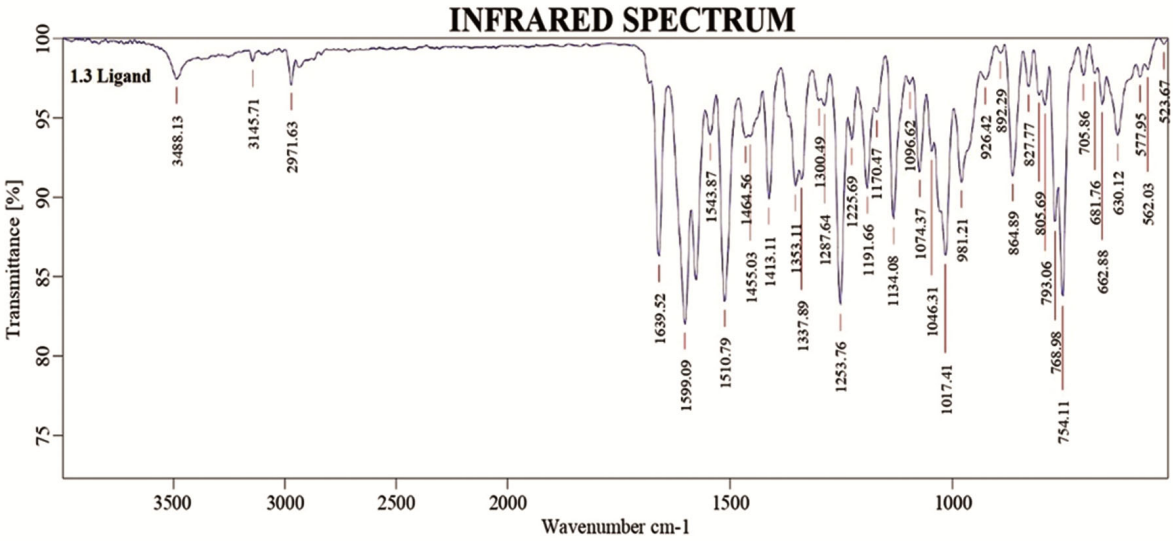


Fig. 2 — IR spectra of ligand **1.3**

hours, TLC was used to track the reaction's development using the proper solvent system. Solid metal complexes were formed when the reaction mixture was allowed to cool to 30°C. These complexes were then filtered, extensively cleaned with cold ethanol to eliminate any remaining ligand and impurities, and dried in a desiccator over anhydrous calcium chloride[33,34].

Analytical data

Fe complex: M.F: $C_{40}H_{38}FeN_8O_2$**,** Colour: Brown, Yield: 65%, M.P: 263-265°C, IR (KBr, cm^{-1}): 1618 **(**C=N**)**, 1230 **(**C-O**),** 622 (M–O), 589 (M–N)**.** ESI-MS, m/z): Calculated 718; found: 719 [M+1]$^{+}$. E. analysis: Caltd %: C, 66.85; H, 5.33; N, 15.59. Found %: C, 66.81; H, 5.30; N, 15.62.

Co complex: M.F: $C_{40}H_{38}CoN_8O_2$, Colour: Dark Green, Yield: 63%, M.P: 275-277°C, IR (KBr, cm^{-1}): 1616 **(**C=N**)**, 1231 **(**C-O**),** 623 (M–O), 591 (M–N)**.** ESI-MS, m/z): Calculated 721; found: 722 [M+1]$^{+}$. E. analysis: Caltd %: C, 66.57; H, 5.31; N, 15.53. Found %: C, 66.52; H, 5.34; N, 15.50.

Ni complex: M.F: $C_{40}H_{38}N_8NiO_2$**,** Colour: Green, Yield: 68%, M.P: 284-286°C, IR (KBr, cm^{-1}): 1615

(C=N**)**, 1248 **(**C-O**),** 633 (M–O), 588 (M–N)**.** ESI-MS, m/z): Calculated 721; found: 722 $[M+1]^+$. E. analysis: Caltd %: C, 66.59; H, 5.31; N, 15.53. Found %: C, 66.56; H, 5.34; N, 15.49.

Cu complex: M.F: $C_{40}H_{38}CuN_8O_2$**,** Colour: Dark Blue, Yield: 69%, M.P: 271-273°C, IR (KBr, cm^{-1}): 1614 **(**C=N**)**, 1235 **(**C-O**),** 632 (M–O), 579 (M–N)**.** ESI-MS, m/z): Calculated 726; found: 727 $[M+1]^+$. E. analysis: Caltd %: C, 66.14; H, 5.27; N, 15.43. Found %: C, 66.11; H, 5.24; N, 15.47.

Zn complex: M.F: $C_{40}H_{38}N_8O_2Zn$**,** Colour: Pale Orange, Yield: 62 %, M.P: 259-261°C, IR (KBr, cm^{-1}): 1615 **(**C=N**)**, 1234 **(**C-O**),** 627 (M–O), 559 (M–N)**.** ESI-MS, m/z): Calculated 728; found: 729 $[M+1]^+$. E. analysis: Caltd %: C, 65.98; H, 5.26; N, 15.39. Found %: C, 65.95; H, 5.29; N, 15.36.

Biological Experiments

DPPH Assay

The antioxidant potential of the synthesized Schiff base ligand (1.3) and its corresponding metal complexes Fe(II) (1.4a), Co(II) (1.4b), Ni(II) (1.4c), Cu(II) (1.4d), and Zn(II) (1.4e) was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. A 0.1 mM DPPH solution was freshly prepared in ethanol and stored in a dark container to prevent photo degradation. Various concentrations (100–700 µg/µL) of each test compound were prepared in ethanol. In each assay, 2 mL of DPPH solution was combined with 2 mL of the sample solution in separate test tubes. A control containing 2 mL of ethanol and 2 mL of DPPH was also prepared. The mixtures were vigorously shaken and incubated in the dark for 30 minutes at RT. Subsequently, absorbance was recorded at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as a reference standard. The percentage radical scavenging activity was determined using the following formula[35-37]:

$$DPPH\ scavenging\ (\%) = \frac{A_o - A}{A_o} x\,100$$

Where Ao is the absorbance of the control (DPPH solution without sample) and A is the absorbance of the test sample.

Antibacterial Screening

The antibacterial activity of the synthesized Schiff base ligand (**1.3**) and its metal complexes, namely Fe(II) (1.4a), Co(II) (1.4b), Ni(II) (1.4c), Cu(II) (1.4d), and Zn(II) (1.4e), was evaluated using the disc diffusion method. The compounds were tested against Gram-positive bacteria (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*K. pneumoniae* and *E. coli*). Each bacterial strain was cultured on nutrient agar broth, and wells were carefully created in the agar plates. Subsequently, 20 µL of each test sample (1 mg/mL in DMSO) was introduced into the wells. The plates were then refrigerated for 10–15 minutes to allow diffusion and subsequently incubated at 37°C for 24 hours. The antibacterial efficacy was determined by measuring the diameter of the inhibition zones (in millimetres) formed around the wells[38-40].

Results and Discussions

IR spectra

Infrared spectra is an excellent method for detecting functional groups and verifying metal coordination in Schiff base ligands and metal complexes. Compound **1.3** exhibits distinct absorption bands at 3488 cm^{-1} (O-H stretch), 1639 cm^{-1} (C=N stretch), and 1253 cm^{-1} (C-O stretch) (Table 1). The O-H band disappears when complexed with Fe, Co, Ni, Cu, and Zn (compounds **1.4a-1.4e**), indicating deprotonation and phenolic oxygen involvement in coordination (Table 1). The C=N stretching frequency decreases to lower wavenumbers (1614-1618 cm^{-1}), indicating coordination through azomethine nitrogen. New bands in the 559-633 cm^{-1} area confirm complex formation and metal binding sites. These bands are attributed to metal-oxygen (M-O) and metal-nitrogen (M-N) vibrations.

Mass spectra

The molecular weights of the Schiff base ligand (**1.3**) and its metal complexes (**1.4a–1.4e**) were verified by mass spectrometry (Table 2). The

Table 1 — IR data of Ligand and its metal complexes

Compd	ν(O–H)	ν(C=N)	ν (C-O)	ν(M–O)	ν(M–N)
1.3 (Ligand)	3488	1639	1253	-	-
1.4a (Fe)	-	1618	1230	622	589
1.4b (Co)	-	1616	1231	623	591
1.4c (Ni)	-	1615	1248	633	588
1.4d (Cu)	-	1614	1235	632	579
1.4e (Zn)	-	1615	1234	627	559

Table 2 — Molecular ion peaks of ligand and metal complexes

Compd	Molecular Ion Peak	Calculated (m/z)	Observed (m/z)
1.3 (Ligand)	$[M+1]^+$	332	333
1.4a (Fe)	$[M+1]^+$	718	719
1.4b (Co)	$[M+1]^+$	721	722
1.4c (Ni)	$[M+1]^+$	721	722
1.4d (Cu)	$[M+1]^+$	726	727
1.4e (Zn)	$[M+1]^+$	728	729

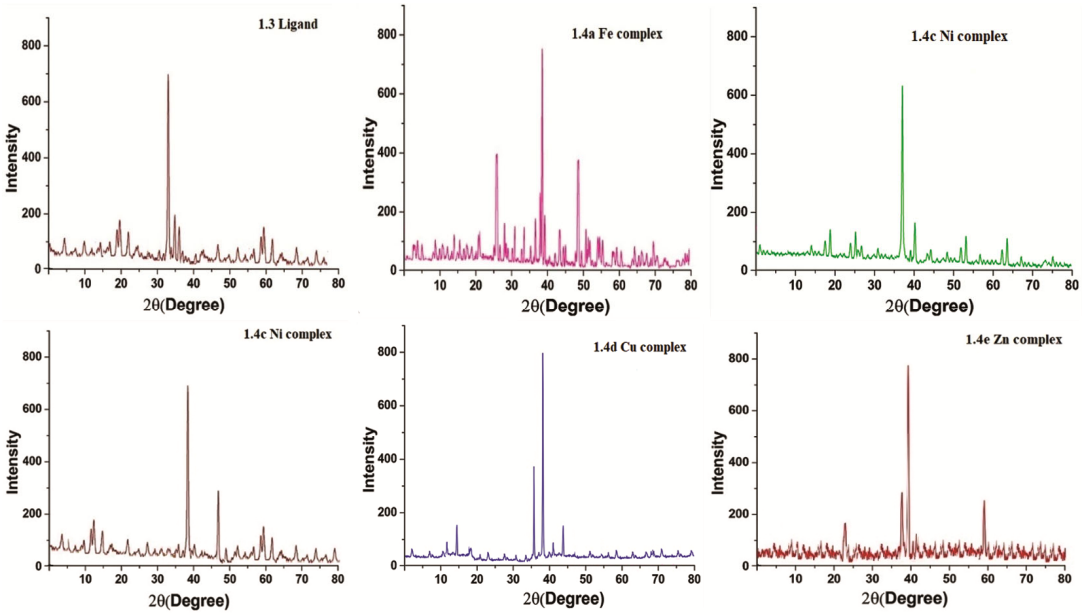


Fig. 3 — XRD images of Schiff ligand and its Fe, Co, Ni, Cu and Zn metal complexes

expected molecular formulas were confirmed by the tight match between the computed molecular ion peaks $[M+1]^+$ and the measured m/z values. The metal complexes had peaks that were in line with their compositions: Fe (719), Co (722), Ni (722), Cu (727), and Zn (729), whereas the ligand displayed a molecular ion peak at m/z 333 (calculated 332). These outcomes attest to the ligand and its metal complexes' effective production and purity.

Powder XRD analysis

A potent method for examining the crystalline nature, phase purity, and particle size estimation of synthetic substances is powder X-ray diffraction (PXRD). Within the 2θ range of 10° to 80°, the Schiff base ligand (1.3) and its metal complexes (1.4a–1.4e) were examined for PXRD patterns. The crystalline character of the ligand and its metal complexes is demonstrated by the distinct diffraction peaks visible in the diffractograms (Fig. 3).

The free ligand's crystalline structure was confirmed by the PXRD pattern, which showed distinct and sharp peaks with an average crystallite size of 10.1 nm. Peak locations and intensities changed noticeably upon coordination with metal ions, suggesting that new crystalline metal–ligand frameworks had been successfully formed. The Fe(II) complex (1.4a) showed distinct peaks and a calculated crystallite size of 11.3 nm, while the Co(II) complex (1.4b) exhibited a slightly higher crystallite size of 13.14 nm, reflecting enhanced crystal packing upon metal coordination. Similarly, the Ni(II) complex (1.4c) showed well-resolved diffraction peaks with an average size of 11.8 nm, and the Cu(II) complex (1.4d) revealed the highest crystallinity with a crystallite size of 14.0 nm, suggesting a more compact and ordered structure.

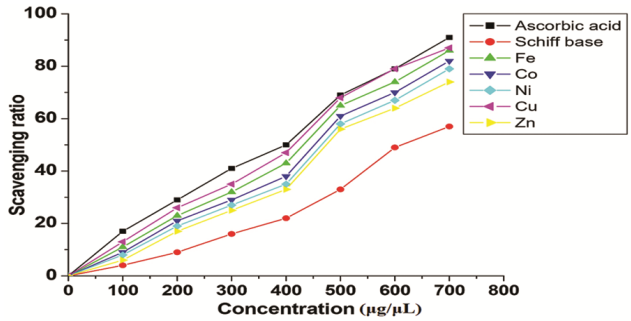


Fig. 4 — Antioxidant activity of Schiff base and its metal complexes *via* DPPH assay

Biological investigations

Antioxidant Activity

The antioxidant activity of the synthesized Schiff base ligand and its metal complexes with Fe(II), Co(II), Ni(II), Cu(II), and Zn(II) was evaluated using the DPPH free radical scavenging method, with ascorbic acid as the standard (Fig. 4, Table 3). The percentage inhibition increased with rising concentrations for all compounds. Among the tested complexes, the Cu(II) complex exhibited the highest scavenging activity, reaching 87% at 700 µg/µL,

Table 3 — Molecular ion peaks of ligand and metal complexes DPPH free radical scavenging activity (%) of Schiff base ligand and its metal complexes at different concentrations (µg/µL)

Concentration (µg/µL)	A.A	Schiff base	Fe	Co	Ni	Cu	Zn
100	17	4	11	9	8	13	6
200	29	9	23	21	19	26	17
300	41	16	32	29	27	35	25
400	50	22	43	38	35	47	33
500	69	33	65	61	58	68	56
600	79	49	74	70	67	79	64
700	91	57	86	82	79	87	74

A.A= Ascorbic acid

Table 4 — Bacterial Inhibition Zones (mm) of Schiff base and its metal complexes

Compd	Gram (+) Bacterial (Inhibition Zone in mm)		Gram (-) Bacterial (Inhibition Zone in mm)	
	B. subtilis	*S. aureus*	*K. pneumonia*	*E. coli*
Schiff base	12	11	9	10
Fe	12	15	11	13
Co	17	15	14	15
Ni	20	19	17	18
Cu	24	23	20	21
Zn	24	22	19	20
Ampicillin	26	23	18	21

followed by the Fe(II) (86%), Co(II) (82%), Ni(II) (79%), and Zn(II) (74%) complexes at the same concentration. The free Schiff base ligand displayed comparatively lower antioxidant activity, peaking at 57% at 700 µg/µL. The enhanced antioxidant potential of the metal complexes compared to the free ligand can be attributed to the coordination of metal ions, which reduces the LUMO energy levels of the ligand and facilitates efficient electron transfer to the DPPH radicals. The superior activity of the Cu(II) complex is likely due to its favourable redox properties and higher electron-donating ability. Based on the results, the antioxidant activity order was found to be: Cu(II) > Fe(II) > Co(II) > Ni(II) > Zn(II) > Schiff base.

Antibacterial assays

In this study, the antibacterial effects of a Schiff base ligand and its metal complexes were tested. The Cu and zinc Zn complexes were the most effective, with inhibition zones between 17 mm and 24 mm. Nickel (Ni) complexes also showed good activity, followed by cobalt (Co) with moderate effects (Fig. 5, Table 4). The iron (Fe) complex and the Schiff base alone had the weakest activity, producing inhibition

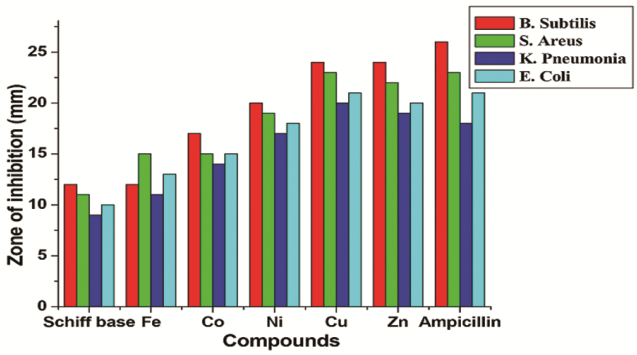


Fig. 5 — Zone of inhibition analysis of Schiff base and its metal complexes

zones of 9 mm to 15 mm. The overall order of activity was: Cu > Zn > Ni > Co > Fe > Schiff base. These results show that combining the Schiff base with certain metals, especially Cu and Zn, can significantly improve its antibacterial properties.

Conclusions

The synthesis and complexation of the Schiff base ligand with Fe(II), Co(II), Ni(II), Cu(II), and Zn(II) ions were verified by elemental and spectroscopic studies. Metal complexes had a substantially better DPPH radical scavenging ability than the free ligand, according to antioxidant tests; the Cu(II) complex demonstrated an 87% inhibition. According to antibacterial research, the most effective complexes against both Gram-positive and Gram-negative bacteria were Cu(II) and Zn(II). According to these findings, metal coordination improves the ligand's capacity for electron transfer and bioactivity, underscoring its potential as a strong antibacterial and antioxidant agent.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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