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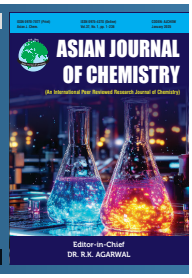

Ultrasonic-Assisted Synthesis, Characterization and Bioactivity of Azomethine based Some Transition Metal(II) Complexes

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The present investigation focuses on the synthesis and characterization of coordination compounds of transition metal ions incorporating azomethine ligand, which show a wide range of biological activities. The structure of the ligand and its metal complexes were confirmed by elemental analysis, spectroscopic studies (UV-vis, FTIR and LC-MS) and magnetic susceptibility measurements. The azomethine ligand was obtained by sonicating 5-bromo-2-hydroxy-3-methoxybenzaldehyde and ethane-1,2-diamine in a 2:1 M ratio. The utilization of sonochemical synthesis techniques for ligand preparation adds an eco-friendly aspect to the process. In particular, the metal-coordinated complexes, which feature azomethine moieties with nitrogen and oxygen atoms for coordination and halogen groups, have attracted significant interest due to their notable bioactive profile. The prepared ligand and its coordinated complexes were evaluated for antimicrobial activities against bacterial microbes (*Streptomyces aureus* and *Pseudomonas aeruginosa*) and fungi (*Trichoderma viride* and *Aspergillus niger*). The antioxidant activity, as measured by the DPPH method and H_2O_2 methods, indicates that the coordinated complexes, particularly those of cobalt, nickel and copper, exhibit greater activity than their parent ligand.

Keywords: Azomethine, Mononuclear metal complexes, Antimicrobial activity, Antioxidant activity, Sonochemical.

INTRODUCTION

Azomethine is a class of organic compounds with a strong coordination affinity and synthesized by treating amines with aldehydes and ketones [1,2]. Azomethine ligands with strong denticity have numerous biological as well as the industrial applications [3,4]. The straightforward reaction, adaptability and extensive range of uses for their metal chelates highlighted the area of interest [5,6]. The integration of two active components within a single compound results in improved physicochemical and biological properties of this versatile category of chelators and their associated chelated complexes. Owing to their numerous uses in materials and medical science, transition metallic complexes have garnered interest [7-9]. The integration of the structural array with imine functionality facilitates the development of the metal complexes with defined characteristics and functionalities [10]. The framework, reactance and activity depend on the interactions of metal complexes with multidentate ligands [11,12].

Azomethine ligands from *o*-hydroxybenzaldehyde and its analogs are versatile [13]. The ligands possess N and O donor sites, which enable them to bind a variety of transition metal ions. Investigations into ligand derivatives by researchers could potentially result in the development of new medicinal drugs [14-16]. Schiff base compounds can incorporate multiple substituents and electron acceptor/donor groups to generate additional energy sublevels [17]. The optics and photovoltaic cells [18] subsequently utilize intramolecular electron delocalization, which these molecules exhibit through larger electronic transitions and charge transfers from metal to ligand or ligand to metal [19].

To permit the structural alterations, it is important to implement eco-friendly approaches and also avoid to obtain undesired side products, sonochemistry is a relatively more efficient method as compare to the conventional method. Thus, in this work, the ultrasonic assisted synthesis, characterization and biological evaluation of 6,6'-(ethane-1,2-diyl*bis*(azaneylylidene))*bis*(methaneylylidene))*bis*(methaneylylidene))*bis*(4-bromo-2-methoxyphenol) derived from *o*-vanillin derivative

with ethylenediamine and its metal complexes were conducted. The compounds were characterized with various analytical techniques like elemental analysis, electronic spectra, FTIR, ^1H & ^{13}C NMR spectroscopic studies.

EXPERIMENTAL

All the commercial grade chemicals and solvents were utilized throughout the experiment. The open capillary approach determined the melting points of the compounds and are uncorrected. TLC evaluated the progress of synthesis of the azomethine ligand and metal(II) complexes and UV radiation exposed the spots. UV-Vis 1800 Shimadzu Spectrophotometer was used to record the absorbance spectra. The FTIR analysis was carried out in a FTIR spectra Perkin-Elmer spectrum two under dry air using horizontal attenuated total reflectance phenomenon in the scanning range of 4000–400 cm^{-1} at room temperature. LC-MS with electrospray ionization and the Waters Micromass Q-TOF micro high-resolution were used to record the mass spectra. The C, H and N elements were analyzed using the Elementar Vario EL III elemental analyzer. The Spectra lab model 40D Ultrasonic cleaner instrument with a powerful transducer at 40 KHz and 250W power was used for ultrasonic-assisted reactions.

Synthesis of azomethine ligand (L): An eco-friendly reaction using ultrasonication technique to condense *o*-vanillin derivatives with ethylenediamine. In brief, a solution of 5-bromo-2-hydroxy-3-methoxybenzaldehyde (2 mmol) in 5 mL of ethanol containing few drops of glacial acetic acid and sonicated for 2 min. To this solution, ethylenediamine (1 mmol) in 5 mL ethanol was added dropwise and again sonicated for 30 min. The precipitate was separated with filtration, washed thrice with 60% alcoholic solution and then recrystallized (**Scheme-I**). TLC, using ethyl acetate and pet. ether as mobile phase, was determined to check the purity of product. The obtained product was soluble in polar solvents. Colour: flake yellow, yield: 86.70%, m.p.: 180 ºC. Elemental anal. of $C_{18}H_{18}N_2O_4Br_2$, calcd. (found) (%): C, 44.42 (43.74); H, 3.70 (3.43); N, 5.75 (5.65); FTIR (KBr, ν_{max}, cm^{-1}): 3431 (-OH), 1613 (-C=N), 1396 (-CN), 1274 (-CO); ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 13.54 (s, H, -OH phenolic), 8.23 (s, H, -CH=N), 6.96-7.26 (2H, m, Ar), 3.87 (3H, s, $-OCH_3$), 3.96 (2H, q, $-CH_2$); ^{13}C NMR (500 MHz, $CDCl_3$) δ ppm: 56.30, 59.22, 109.46, 117.20, 119.01, 125.02, 149.33, 151.02, 165.64; LC-MS (TOF MS ES+) for $C_{18}H_{18}N_2O_4Br_2$ (*m/z*): 487 $[M+1]^+$ 485 $[M-1]^+$; λ_{max} (nm): 336 π–π*, 428 n–π.

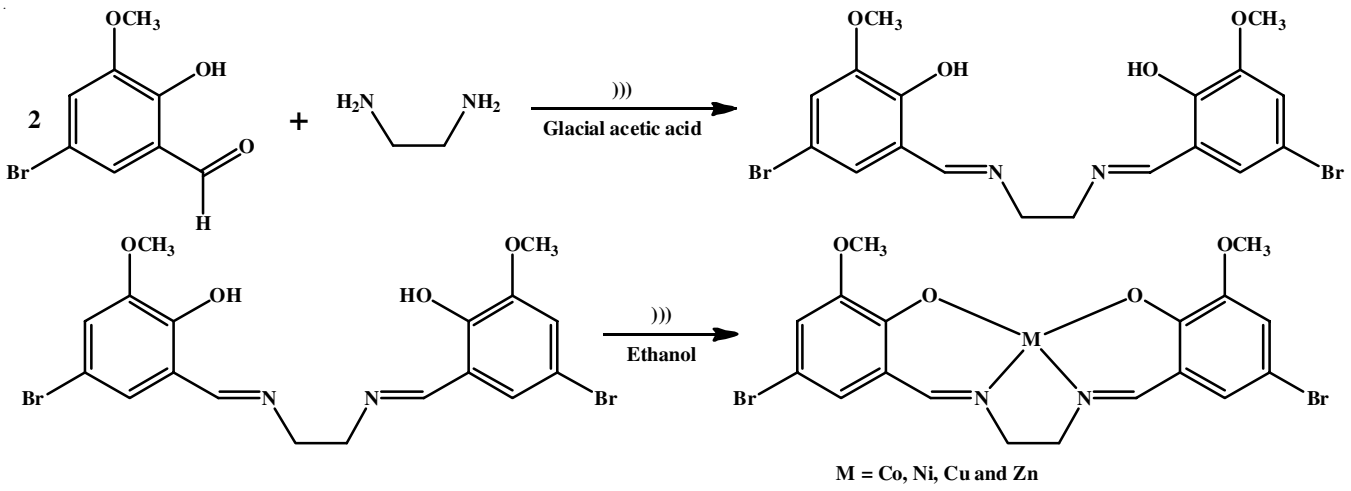
General method for the metal complexation of ligand: The metal complexes were obtained by adding the respective alcoholic solution of metal salts (1 mmol) in dropwise manner to azomethine ligand (1 mmol) while mixing constantly. TLC evaluated the reaction mixture after 2 h of sonication. After centrifugation, the obtain colour precipitate was washed thoroughly with ethanol followed by water and finally dried in a vacuum (**Scheme-I**).

[CoL] complex: Yield: 78%, colour: brown; m.p. = >300 ºC; Elemental anal. of $C_{18}H_{16}N_2O_4Br_2Co$, calcd. (found) % for (%): C, 39.85 (35.54); H, 2.95 (3.43); N, 5.16 (4.43); LC-MS (*m/z*): calculated 542.18; observed 542; ICP-MS: 10.40% of Co element; λ_{max} (nm):300 π–π*, 382 n–π*; FTIR (KBr, ν_{max}, cm^{-1}): 1641 (-C=N), 1460 (-C=C), 1226 (-CO), 679 (M-O), 561 (M-N). μ_{eff}: 1.165 B.M. Conductance (Λ_m Ω^{-1} cm^2 mol^{-1}): 43.08 in DMF.

[NiL·H_2O] complex: Yield: 79%, colour: greenish-orange; m.p.: >300 ºC; Elemental anal. of $C_{18}H_{16}N_2O_4Br_2Ni$, calcd. (found) (%): C, 39.78 (36.91); H, 2.94 (3.14); N, 5.15 (4.76); LC-MS (*m/z*): calculated 542.9; observed 564.88; ICP-MS: 5.40% of Ni element; λ_{max} (nm):321 π–π*, 352 n–π*; FTIR (KBr, ν_{max}, cm^{-1}): 1620 (-C=N), 1460 (-C=C), 1239 (-CO), 695 (M-O), 569 (M-N). μ_{eff}: 0.0146 B.M. Conductance (Λ_m Ω^{-1} cm^2 mol^{-1}): 11.71 in DMF.

[CuL] complex: Yield: 71%, colour: black; m.p.: >300 ºC; Elemental anal. of $C_{18}H_{16}N_2O_4Br_2Cu$, calcd. (found) (%): C, 39.43 (36.04); H, 2.92 (3.22); N, 5.11 (4.71); LC-MS (*m/z*): calculated 547.726; observed 547.887; ICP-MS: 4.30% of Cu element; λ_{max} (nm):303 π–π*, 381 n–π*; FTIR (KBr, ν_{max}, cm^{-1}): 1641 (-C=N), 1457 (-C=C), 1242 (-CO), 692 (M-O), 568 (M-N). μ_{eff}: 1.059 B M. Conductance (Λ_m Ω^{-1} cm^2 mol^{-1}): 22.29 in DMF.

[ZnL] complex: Yield: 89%, colour: yellow; m.p.: >300 ºC; Elemental anal. of $C_{18}H_{16}N_2O_4Br_2Zn$, calcd. (found) (%): C, 39.30 (37.93); H, 2.91 (3.05); N, 5.09 (4.81); LC-MS (*m/z*): calculated 549.56; observed 550.86; ICP-MS: 10.70% of Zn element; λ_{max} (nm):363 π–π*, 391 n–π*; FTIR (KBr, ν_{max}, cm^{-1}):



Scheme-I: Synthetic route for azomethine ligand and it coordinated complexes

1635 (-C=N), 1457 (-C=C), 1239 (-CO), 686 (M-O), 561 (M-N). μ_{eff}: 0.054 B M. Conductance (Λ_m Ω^{-1} cm^2 mol^{-1}): 14.69 in DMF.

Biological activities

Antibacterial assay: The antibacterial assay method of disc diffusion on *Streptomyces aureus* and *Pseudomonas aeruginosa* at 100 µg/mL. The agar nutrient media was poured into a sterile glass dish and permitted to set at ambient temperature for 24 h to ensure proper sterilization. After solidifying, 100 µL of specified microbial suspension was evenly distributed across the glass plate media [20]. The Whatman paper No. 1 (5 mm) was soaked and then kept it evenly in the inoculated petri plates. Streptomycin served as the standard drugs, whereas filter paper discs impregnated with DMF functioned as the reference. The culture cell dishes were incubated at 37 ºC for 1 day. All trials were conducted in triplicate and the mean measurements for each chemical were recorded.

Antifungal assay: The disc diffusion technique [21] was employed to test azomethine ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes against fungi (*Trichoderma viride* and *Aspergillus niger*) at 100 µg/mL concentration in DMF solvent. Potato dextrose agar (PDA) was poured onto a cell culture dish and allowed to solidify at room temperature for 24 h to ensure proper sterilization. After 24 h, 0.1 mL of specified fungal suspension was evenly distributed over the media on the glass plate. The Whatman Paper No. 1 discs of 5 mm diameter were immersed in designated amounts of solution and placed them in three equal positions within the inoculated petri plates. In this study, nystatin was used as standard, while DMF as control. Petri dishes were heated up at 37 ºC for 72 h. All experiments were performed in triplicate and the mean values for each compound were recorded.

DPPH radical quenching method: The evaluation of the oxidative assay of azomethine ligand and its coordinated complexes was done by the DPPH method. DPPH radicals react as well as combine with present reducing agents, resulting in the pairing of electrons with appropriate hydrazine [22]. The colour loss of the examined azomethine ligand and its metal complexes is stoichiometrically dependent on the number of electrons accepted and the substances that can donate electrons or hydrogen atoms. The solution of azomethine ligand and its metal complexes were prepared in DMSO and 1 mL of 100 µg/mL [23] solution was transferred to a test tube, followed by the addition of 4 mL of 0.1 mM methanolic solution of DPPH, which was constantly agitated until a clear solution was obtained. After 30 min, the test tubes were kept in a dark environment. Using a spectrophotometer, the optical density at 517 nm was determined. In this study, ascorbic acid was used as standard for comparison. Three duplicates of the radical scavenging activity assay were performed and the percentage (%) was computed using the following formula:

$$\text{DPPH scavenging activity (\%)} = \frac{A_o - A_1}{A_o} \times 100$$

where A_o represents the optical density of blank (without compound); A_1 is the optical density of the compound.

Hydrogen peroxide radical scavenging assay: H_2O_2 scavenging radical action of the prepared azomethine ligand and its metal(II) complexes was evaluated by following the reported method [24]. The Fenton reaction was responsible for the formation of hydroxyl ($OH^{\bullet}$) radical ions in the aqueous solution. The reaction mixtures in the assay contained 2.5 mL of pH 7.4 phosphate buffer (0.15 M), 0.5 mL of 114 µM safranin, 1 mL of 945 µM EDTA-Fe(II), 1 mL of 3% H_2O_2 and 100 µg/mL of azomethine ligand solution in DMSO. The transparent, homogeneous solution was obtained by vigorously and continuously shaking this mixture. The control was the reaction mixture that did not contain the test compound. The tested reaction mixtures were incubated in an aqueous medium at 37 ºC for 1 h. The absorbance of the evaluated compounds was measured at 520 nm. The following formula was used to determine the scavenging ratio (%):

$$\text{Scavenging ratio (\%)} = \frac{A_i - A_o}{A_c - A_o} \times 100$$

where A_i is the optical density of tested compound in the reaction mixture; A_o is the optical density of the solution without compoun; A_c represents the optical density of blank, containing EDTA-Fe (II) and H_2O_2 solutions.

RESULTS AND DISCUSSION

The N_2O_2 donor symmetrical azomethine ligand and its metal complexes were successfully synthesized by ultrasonic method in a 1:1 M ratio. The compounds are readily soluble in polar solvents. The elemental amounts of cobalt (10.40%), nickel (5.40%), copper (4.30%) and zinc (10.70%) estimated with inductively coupled plasma mass spectrometry (ICP-MS) are in good accordance with the calculated values for the complexes, which can confirm the structure.

Molar conductivity: The molar conductivities of the azomethine ligand based metal(II) complexes with a concentration of 10^{-3} M solutions were determined at ambient temperature. The complexes exhibited molar conductivity values ranging from 11 to 45 Λ_m Ω^{-1} cm^2 mol^{-1}. The range of conductivity values suggests that all the metal(II) complexes are non-conductive in nature.

Magnetic susceptibility: The magnetic moment of the metal(II) complexes was determined using a vibrating sample magnetometer (VSM). The [CoL] compound shows the paramagnetic moment 1.165 B M., which matches for Co(II) d^7 configuration with dsp^2 hybridization, corresponding to square planar geometry, typically resulting in a low-spin configuration due to the strong ligand field of tetradentate salen ligand. The [NiL·H_2O] complex was characterized by its distorted octahedral geometry and diamagnetic nature. The [CuL] complex has square planar and paramagnetic in nature, with a magnetic moment of 1.059 B.M. The [ZnL] complex shows tetrahedral geometry with sp^3 hybridization and diamagnetic nature. Fig. 1 displays the VSM spectrum of metal complexes containing azomethine ligand.

UV-Visible studies: The electromagnetic spectrum in the methanolic exhibits absorption of the high energy range of 290-310 nm and 380-410 nm, which are assigned to the π–π* and n–π* shifts, respectively. The *d-d* transition for the Co(II) complex occurred below 550 nm, indicative of a square planar

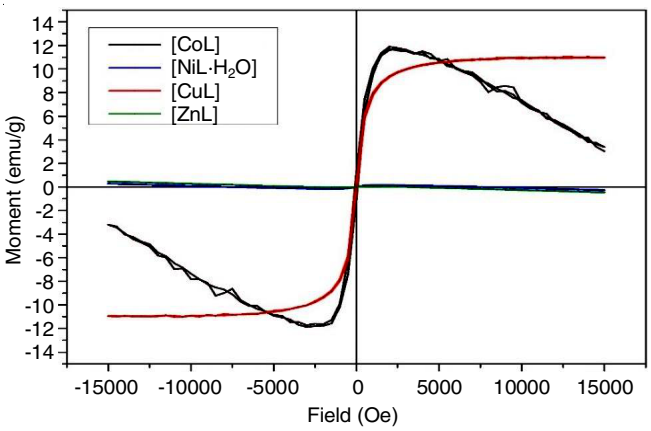


Fig. 1. VSM spectrum of metal complexes of azomethine ligand

structure. For Ni(II) complex, 321 π–π* and 352 n–π* shifts, respectively, whereas copper(II) complex shows 303 π–π* and 381 n–π* transitions. The zinc(II) complex show tetrahedral geometry with 363 π–π* and 391 n–π* transitions. Fig. 2 shows the UV-Vis spectrum of organic moiety and their coordinated compounds.

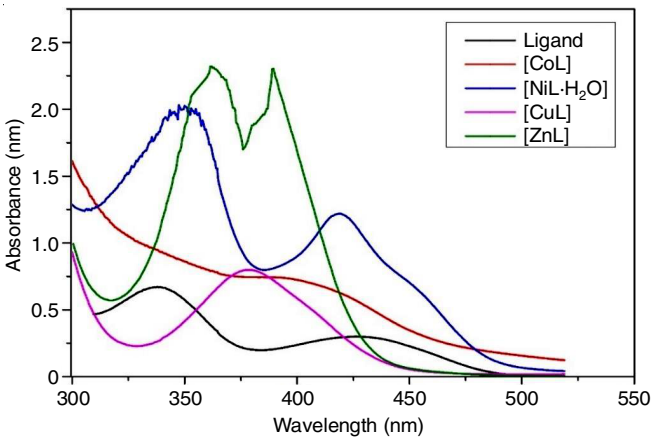


Fig. 2. UV-vis band of parent ligand and coordinated complexes

FTIR studies: A characteristic band at 3431 cm^{-1} is due to phenolic -OH group present in the azomethine ligand, however, this peak disappeared in the metal complexes. Moreover, in the metal complexes, the ligand's C-N absorption band at 1641 cm^{-1} experiences slight shifts to lower frequencies, while the coordination of imine nitrogen occured at 1457 cm^{-1} (Fig. 3). The appearance of bands in the ranges of 695-670 cm^{-1} and 590-561 cm^{-1}, respectively, provides supplementary indication for coordination of nitrogen (M-N) and oxygen (M-O), which is consistent with the literature [25].

Mass spectrum: The mass spectrum of azomethine ligand $[C_{18}H_{18}N_2O_4Br_2]$ in the current study reveals a $M^{+\bullet}$ ion peak at *m/z* 487 corresponds to [M+1] peak and *m/z* 485 resembles to [M-1] peak. In addition, the spectrum displays the peak at *m/z* 273 corresponds to $[C_{10}H_{11}N_2O_2Br]$ ion. The spectrum of [CoL] complex show $M^{+\bullet}$ ion and base peaks at *m/z* 542 with [M+2] and [M+4] peak at 544 and 546 respectively. The spectra of $[NiL·H_2O]$ complex show $M^{+\bullet}$ ion and base peaks at *m/z* 564 with [M+2] and [M+4] peak at 566 and 568 respectively. The

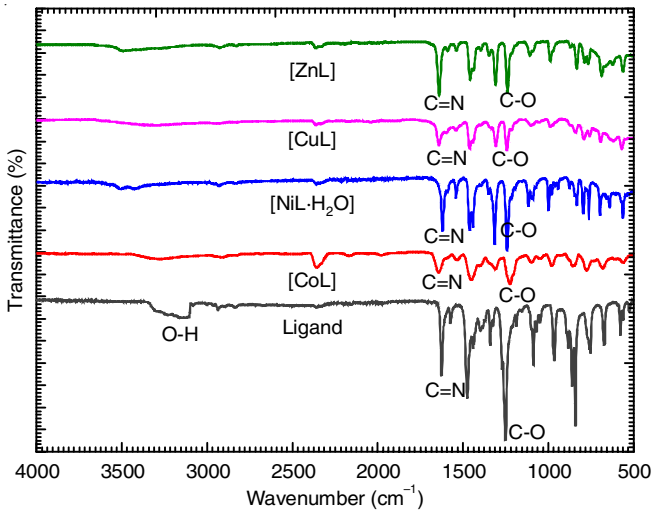


Fig. 3. FTIR spectrum of ligand and their coordinated complexes

spectra of [CuL] complex show $M^{+\bullet}$ ion and base peaks at *m/z* 547 with [M+2] and [M+4] peak at 549 and 551, respectively. The spectra of [ZnL] complex show $M^{+\bullet}$ ion and base peaks at *m/z* 550 with [M+2] and [M+4] peak at 552 and 554, respectively. The stoichiometry of the complexes is confirmed by the *m/z* of all azomethine ligand fragments and their coordinated complexes.

Antimicrobial activity: The antibacterial efficacy of azomethine ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes was evaluated against *Streptomyces aureus* and *Pseudomonas aeruginosa*. The metal(II) complexes show significant activity against *S. aureus* and *P. aeruginosa*. The antibacterial activity data of synthesized azomethine ligand and their metal(II) complexes are shown in Table-1. The resulting results show that the synthesized complexes were found to be more effective and improved antibacterial activity as compared to free ligand but less than the standard. The presence of the -NH, -C=N and -OH groups in the azomethine moiety has significance for the antibacterial action which demonstrates substantial activity [26]. Generally, the metal(II) complexes demonstrate enhanced antibacterial properties relative to their azomethine ligand after binding with metal ions. Coordination primarily decreases the metal ion's polarity by distributing its partial positive charge across the donating groups within the binding ring system [27, 28]. This method enhances the hydrophobic nature of the central metal atom, enabling more efficient permeation through the

TABLE-1
ANTIMICROBIAL ACTIVITY DATA OF
LIGANDS AND THEIR COMPLEXES (-ve) MEANS
(NO ANTIBACTERIAL ACTIVITY OBSERVED)

Compounds	Zone of inhibition (mm)	
	Streptomyces aureus (Gram-positive)	*Pseudomonas aeruginosa* (Gram-negative)
Ligand	7	-ve
[CoL]	10	12
$[NiL·H_2O]$	9	13
[CuL]	10	9
[ZnL]	-ve	-ve
DMF	-ve	-ve
Streptomycin	19	18

lipid layer of microorganisms [29]. Some factors, such as coordinating sites, hydrophilicity, lipophilicity, the constitution of the acceptor and donor atoms, the topology of assembly and the inclusion of co-ligand have significant impacts on the activity against bacteria [30].

Moreover, the Co(II), Ni(II), Cu(II) and Zn(II) complexes are more effective against monoderm (*S. aureus*) than diderm (*P. aeruginosa*) bacteria [31]. It can be inferred that the antimicrobial properties of the coordinated compounds are contingent upon the outer layer of the microbe's cell wall. Gram-negative microbes have fragile cells made of a peptidoglycan and enclosed by a second fat sheath holding endotoxin and conjugated proteins, while Gram-positive bacteria have dense cell layers composed of numerous sheets of teichoic acids and peptidoglycan. Variations in cell wall architecture can affect the antibacterial susceptibility, with specific drugs being effective solely against Gram-positive bacteria, excluding Gram-negative bacteria [32].

Antifungal activity: The antifungal activity of azomethine ligand and their Co(II), Ni(II), Cu(II) and Zn(II) complexes were tested in contrast to *Trichoderma viride* and *Aspergillus niger*. The inhibitory results were compared to the conventional drug nystatin. Based on Table-2, it was observed that ligand has negligible as well as no antifungal activity. Zn(II) complex show good antifungal activity as compared to Co(II) and Ni(II) and their corresponding ligand. Moreover, the tested compounds are more actively showing antifungal activity in *A. niger* as compared to *T. viride.* These variations in the antifungal efficacy of the coordinated complexes against various strains mainly depend on differences in the ribosome of bacterial cells or on the rigidity of the microorganism cells [33].

TABLE-2
ANTIFUNGAL ACTIVITY DATA OF VM-251 LIGANDS AND THEIR METAL COORDINATE COMPLEXES, (-ve) MEANS NO ANTIFUNGAL ACTIVITY OBSERVED

Compounds	Zone of inhibition (mm)	
	Trichoderma viride	*Aspergillus niger*
Ligand	-ve	-ve
[CoL]	-ve	-ve
[NiL·H_2O]	7	-ve
[CuL]	8	10
[ZnL]	11	12
DMF	-ve	-ve
Nystatin	17	19

Antioxidant activity: The investigation of antioxidant efficacy revealed that azomethine ligand and their complexes showed potent to moderate scavenging activity but less as compared to the standard.

DPPH radical scavenging activity: The azomethine ligand showed moderate to low DPPH activity as compared to standard ascorbic acid, though after coordination with metal ions, the antioxidant properties are enhanced as compared to ligand. Among the Cu(II) complex showed significantly higher DPPH activity than other complexes (Table-3).

Hydrogen peroxide scavenging activity: The hydrogen peroxide scavenging activity data showed that azomethine ligand and their metal complexes exhibited effective antioxidant activity. The antioxidant activity of the imine molecule enhanced on complexation with Co(II), Ni(II) and Cu(II) ions, while the Zn(II) complex shows near similar to free ligand. The Cu(II) complex exhibited higher hydrogen peroxide activity as compared with other complexes but less than the standard BHT (Table-4). The azomethine ligand containing -OCH_3 groups in their structure exhibited an effective and more H_2O_2 scavenging activity.

TABLE-3
SCAVENGING ACTIVITY DATA FOR DPPH RADICALS OF SALEN LIGANDS AND THEIR COORDINATED COMPOUNDS

Compounds	Inhibition (%)
Ligand	31.9
[CoL]	46.0
[NiL·H_2O]	40.1
[CuL]	67.6
[ZnL]	32.1
Ascorbic acid	86.4

TABLE-4
HYDROGEN PEROXIDE (H_2O_2) SCAVENGING ACTIVITY OF ORGANIC LIGANDS AND THEIR COORDINATED COMPOUNDS

Compounds	Inhibition (%)
Ligand	31.9
[CoL]	43.9
[NiL·H_2O]	68.1
[CuL]	72.6
[ZnL]	42.1
BHT	88.4

Conclusion

A new azomethine ligand and its four transition metal(II) complexes were synthesized and characterized using chemical composition, spectroscopic, vibrating sample magnetometer and molar conductivity data. The bactericidal activity of the compounds was performed against *Streptomyces aureus* and *Pseudomonas aeruginosa* bacteria strains in comparison to conventional pharmaceutical drugs streptomycin. The data showed that the bactericidal activity was enhanced when the ligand was complexed. Similarly, the antifungal activity against *Trichoderma viride* and *Aspergillus niger* were also found to be effective. Copper complexes exceed cobalt, nickel and zinc complexes in terms of antioxidant activities.

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CONFLICT OF INTEREST

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

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