Synthesis, Characterization and Biological Activity Study of a New Schiff Base-Oxime Derived Ligand and Its Complexes with Some Metal Ions

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Abstract: A new ligand, (3Z)-3-((2-amino-4,5-dimethylphenyl)imino)butan-2-one oxime (L), was created in this research by combining 4,5-dimethyl-1,2-phenylenediamine with diacetyl monooxime in boiling CCl_4. The ligand's structure was confirmed using elemental analysis and various spectroscopic techniques including FT-IR, UV, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Co(II), Ni(II), Cu(II), and Zn(II) metal ions were complexed with a specific ligand by reacting their chloride salts with the ligand in methanol at a 1:1 molar ratio under reflux conditions. The complexes' structure was confirmed using elemental analysis, spectroscopic methods, magnetic moments, and molar conductivity investigations. The complexes exhibited tetrahedral, square planar, and octahedral geometries for Zn(II), Ni(II), Co(II), Cu(II), and, respectively. In addition to all that has been mentioned, We also obtained the biological activity of the prepared compounds against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli) at different concentrations of the ligand and the prepared complexes. It was found that some of them were effective at certain concentrations, while others were not effective. In addition, the effectiveness of the cobalt complex with bacteria was examined in the presence of the antibiotic (Levofloxacin), and it was found to be effective.

1 INTRODUCTION

Hugo Schiff, a German scientist, coined the term "Schiff base" in 1864 to characterize the compounds resulting from the combination of primary amines with carbonyl compounds. Despite originating over a decade before the establishment of coordination chemistry, Schiff bases continue to hold significance as ligands. They represent a vital category of compounds distinguished by the presence of a double bond (-C=N-) between carbon and nitrogen atoms [1]. These compounds exhibit various noteworthy attributes, such as high thermal stability, flame retardancy, and degradability. As a result, they have been widely employed in catalyst development, gas separation, and drug release industries [2]. Schiff bases stand out as among the most extensively utilized organic compounds, showcasing a diverse array of biological activities. These include but are not limited to antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties [3]. Oximes and their derivatives are important bioactive ligands widely used in coordination chemistry. Oximes are formed through the condensation of hydroxylamine with aldehydes or ketones. The oxime functional group exhibits amphoteric properties, with the azomethine group's nitrogen (C=N) being slightly basic and the hydroxyl group (OH) slightly acidic [4]. Advancements in both inorganic and bioinorganic chemistry have sparked greater interest in Schiff base complexes. This is driven by the recognition that numerous such complexes can potentially serve as models for emulating biomolecules [5]. Imine-oxime ligands have played a crucial role in coordination chemistry, exhibiting distinct chelation behaviours. They find various applications in analytical chemistry, including metal ion extraction and spectrophotometric determination. Moreover, certain imine-oxime complexes have been employed in catalyzing numerous organic reactions [6]. The biological activity of these compounds is frequently linked to their capacity to form complexes with the metals found in biosystems [7]. Additionally, certain hydrazones and oximes have found applications as herbicides, insecticides, nematocides, rodenticides, and plant growth regulators. They are also utilized as plasticizers and

stabilizers for polymers [8]. In addition, they play a significant role in applications in the fields of medicine, industry, and analysis [9]. The number of amine-imine-oxime complexes that have been reported is very low, even though a significant number of oximes and associated transition metal ion complexes have been investigated [10].

2 MATERIALS, METHODS, AND EXPERIMENTAL TECHNIQUES

2.1 Chemicals and Reagents

The synthetic work was carried out exclusively with analytical-grade substances. Among the main reagents employed were diacetyl monoxime (99%, Merck, Germany) and 4,5-dimethyl-1,2-phenylenediamine (97%, Sigma, USA). Additional salts included cesium iodide (98%, Merck, Germany), cobalt(II) chloride hexahydrate, copper(II) chloride dihydrate, nickel(II) chloride hexahydrate, and zinc(II) chloride (all ≥99%, Sigma, USA). Methanol of 99% purity (Merck, Germany) was used as the principal solvent.

2.2 Analytical and Spectroscopic Characterization

To ensure proper identification of the obtained products, a series of instrumental methods was applied. Elemental analyses (C, H, N) were performed with an Eager 300 (EA112). Infrared spectra were obtained on a SHIMADZU 8400S spectrophotometer, applying both KBr and CsI pellets. Proton and carbon NMR (^1H and ^13C) were measured on a Bruker 400 MHz instrument. UV–Vis spectra were registered using quartz cuvettes of 1 cm path length. The magnetic properties of the complexes were evaluated at room temperature with a Sherwood Scientific balance. Molar conductance was measured on a Starter 3000℃ conductivity meter [11].

2.3 Synthetic Strategy

2.3.1 Ligand Preparation

The ligand (3Z)-3-((2-amino-4,5-dimethylphenyl)imino)butan-2-one oxime (L) was obtained by condensation of 4,5-dimethyl-1,2-phenylenediamine (2.1 g, 15 mmol) with diacetylmonoxime (1.55 g, 15 mmol) in carbon tetrachloride (15 mL). The reaction was maintained under reflux with continuous stirring for 3 h. During the course of the process, the solution initially appeared dark brown and later produced a light creamy solid. The precipitate was isolated by filtration, washed sequentially with CCl4 (5 mL) and cold ethanol (3 mL), and finally dried. Yield: 1.6 g (50%); colour: creamy; m.p.: 130–134 °C.

2.3.2 Synthesis of Metal Complexes

Complexes were synthesized by reacting the ligand with the respective metal salts in methanolic solution:

- [L ZnCl]Cl (C1): Ligand L (0.3 g, 1 mmol) in 10 mL methanol was mixed with ZnCl2 (0.136 g, 1 mmol) dissolved in 5 mL methanol. The mixture was refluxed at 95 °C for 3 h. A light green solid was obtained after cooling and filtration. Yield: 0.2 g (57%); m.p.: 200–206 °C.
- [L CoCl(H2O)2]Cl (C2): Prepared analogously, substituting cobalt(II) chloride hexahydrate (0.237 g, 1 mmol) for zinc chloride. The obtained product was dark green. Yield: 0.26 g (76.4%); m.p.: 183–185 °C.
- [L CuCl(H2O)2]Cl (C3): Using copper(II) chloride dihydrate (0.17 g, 1 mmol), a dark green solid was isolated. Yield: 0.23 g (67.6%); m.p.: 155–160 °C.
- L·NiCl2 (C4): Reaction with nickel(II) chloride hexahydrate (0.23 g, 1 mmol) afforded a red compound. Yield: 0.22 g (64.7%); m.p.: 266–270 °C.

2.4 Antibacterial Screening

The newly obtained compounds were subjected to antimicrobial tests against Escherichia coli and Staphylococcus aureus. A disc diffusion method was applied. Three concentrations were prepared: 100 µg/mL (stock), 50 µg/mL (diluted in DMSO), and 25 µg/mL. The inhibitory effects were evaluated after incubation.

2.5 Culture Medium Preparation

Mueller–Hinton agar was prepared by dissolving 38 g of the medium in 1 L of distilled water, sterilized by autoclaving at 121 °C and 15 psi for 15 min. Once cooled, it was poured into sterile Petri dishes and stored under refrigeration. During testing, 100 µL of each compound solution was applied into wells, with sterile distilled water used as positive and negative controls. Incubation was carried out at 37 °C for 24 h [12], [13].

3 RESULT AND DISCUSSION

3.1 Synthesis and Characterization of the Ligand and Complexes

The Schiff base–oxime ligand (L) was obtained via condensation of diacetylmonoxime with 4,5-dimethyl-o-

phenylenediamine (Fig. 1). Its elemental analysis data matched well with the theoretically calculated values (Table 1), confirming the proposed molecular formula. Structural verification was additionally supported by a combination of spectroscopic techniques, including IR, UV-Vis, ^1H and ^13C NMR, and EI-MS. Complexes of L with Co(II), Cu(II), Ni(II), and Zn(II) were synthesized through reactions of the ligand with the corresponding hydrated metal chlorides in methanol at reflux, maintaining a 1:1 molar ratio (Fig. 2). The microanalytical and metal content results for all complexes (Table 1) further confirmed their compositions.

Table 1: Elemental analyses data and physical properties of ligands and their complexes.

No	Molecular Formula	M.wt g/mol	Yield%	Colour	m.p°C
L	$C_{12}H_{17}N_3O$	219.2	50%	Creamy	130-136
C_1	$C_{12}H_{17}Cl_2ZnN_3O$	355.5	57%	light green	200-206
C_2	$C_{12}H_{21}Cl_2CoN_3O_3$	385.1	76.4%	Dark green	183-185
C_3	$C_{12}H_{21}Cl_2CuN_3O_3$	389.7	67.6%	Dark green	155-160
C_4	$C_{12}H_{17}Cl_2Ni\ N_3O$	348.8	64.7%	Red	266-270

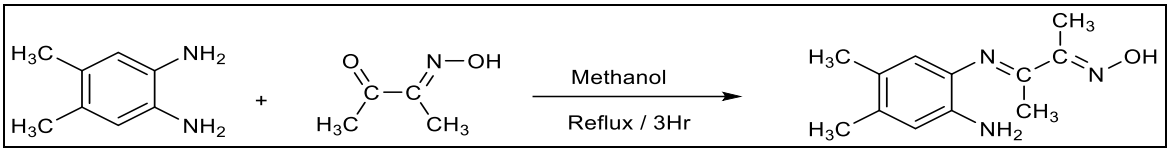

Figure 1: Preparation route of the ligands.

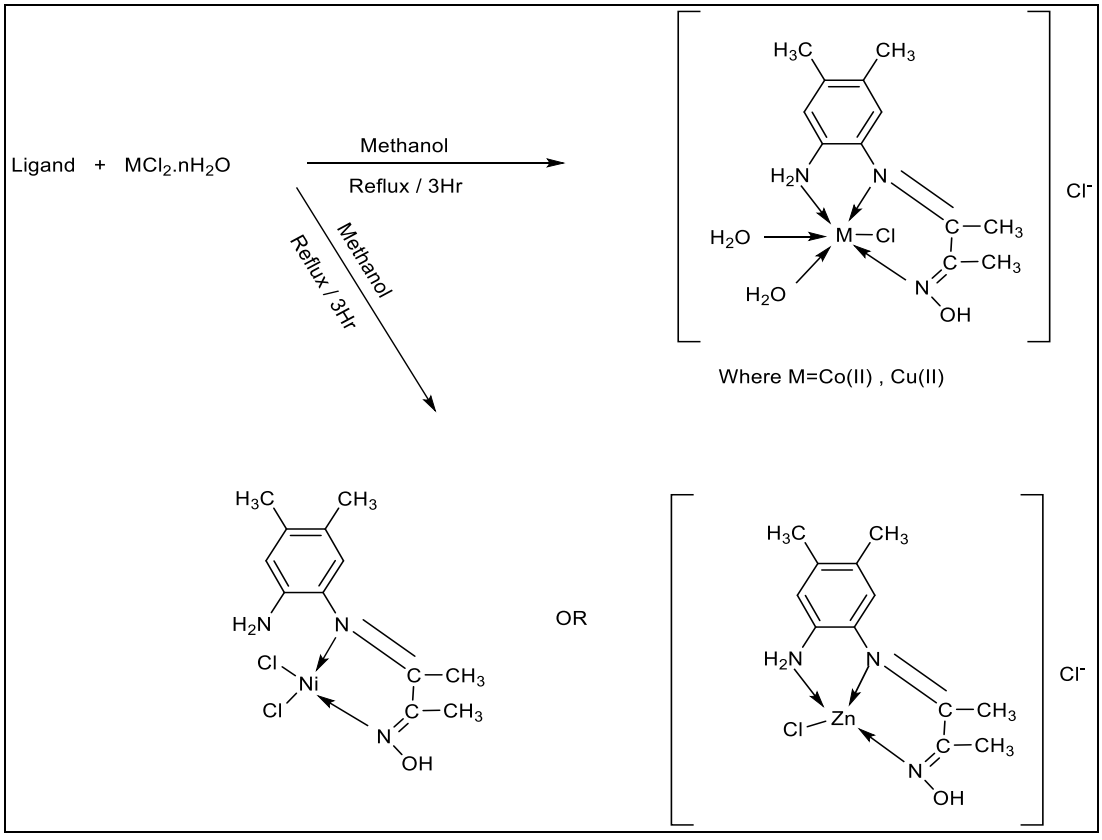

Figure 2: Preparation route of the Complexes.

3.2 Infrared Spectroscopy

The IR spectrum of ligand L displayed several diagnostic bands: ν(NH) stretching at 3379 and 3155 cm^{-1}, ν(OH) of the oxime group at 3286 cm^{-1}, and ν(C=N) of imine and oxime at 1627 and 1581 cm^{-1}, respectively. Upon complexation, the ν(C=N) signals shifted to lower frequencies (~1573 cm^{-1}), reflecting coordination of the azomethine and oxime nitrogens with the metal centers. The ν(N–O) band of the free ligand at 1134 cm^{-1} shifted to 1165–1265 cm^{-1} in the complexes, also supporting metal–ligand bonding. New absorptions at 475–425 cm^{-1} and 300–246 cm^{-1} were observed in all complexes, assignable to ν(M–N) and ν(M–Cl), respectively, and were absent in the free ligand spectrum [14], [15]. Additionally, the spectra of Co(II) and Cu(II) complexes showed features due to coordinated water molecules (e.g., 609, 1774, 3518 cm^{-1} for LCo; 864, 1797 cm^{-1} for LCu). (Fig. 3-4, Table 2).

Table 2: Infrared spectra for ligands and their complexes

No	V(N-H) Assm. Ssm.	*V(C*=N) *Imine*	*V(C*=N) Oxime	ν(N-O)	V(M-N)	ν(M-Cl)
L^1	3379m 3155m	1627s	1581m	1134m	-----	-----
C_1	3270m 3170w	1573m	1458m	1165m	475w	246m
C_2	3170w	1573w	1381w	1226m	439w	300w
C_3	3194 3156	1627	1404	1265w	432w	262w
C_4	3170w	1573s	1442w.b	1242m	425w	250w

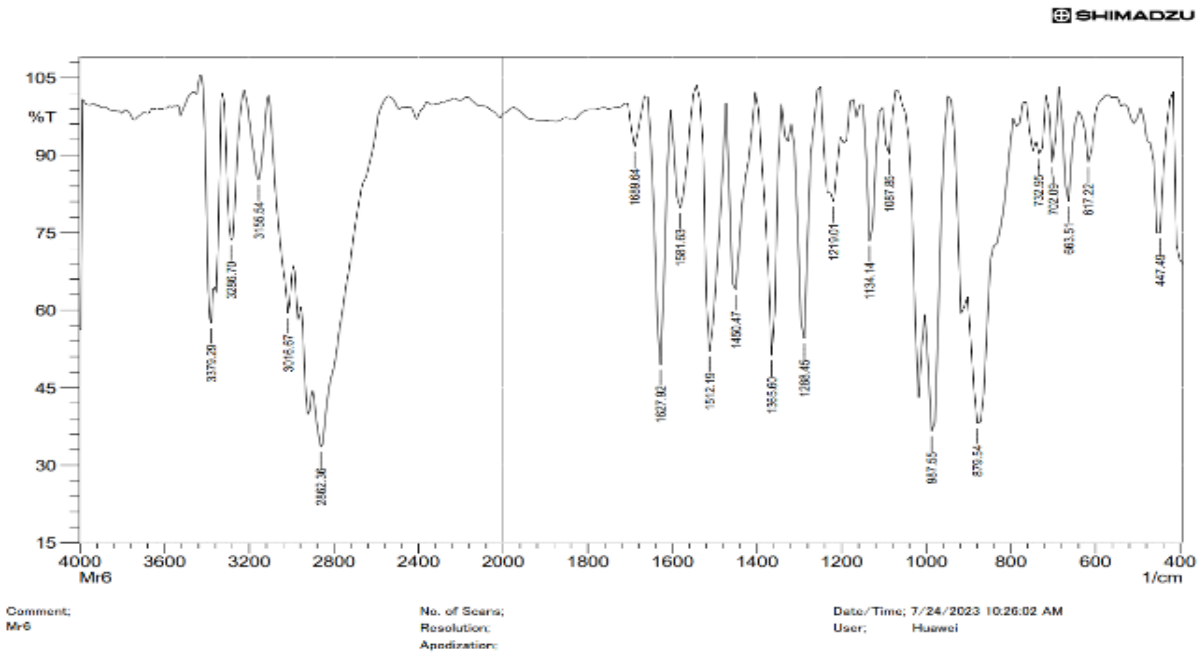

Figure 3: FT – IR spectrum of of the Ligand.

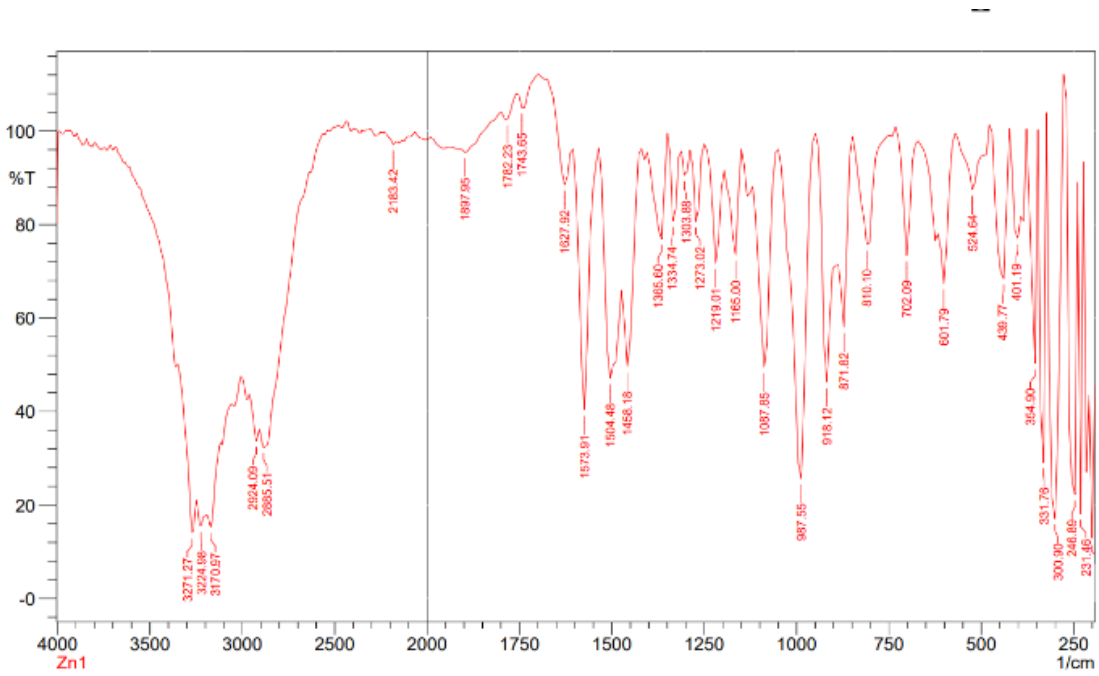

Figure 4: FT – IR spectrum of the [L ZnCl] Cl complex.

3.3 Electronic Absorption Spectra

The UV–Vis spectrum of the free ligand (Fig. 5) exhibited a strong band at 314 nm (31,847 cm^{-1}, ε = 1265 $L \cdot mol^{-1} \cdot cm^{-1}$), attributed to π→π* and n→π* transitions. In the LCu complex, a ligand-centered transition appeared at 258 nm (38,760 cm^{-1}), accompanied by a charge-transfer band at 364 nm. Weak d–d transitions at 662 and 930 nm were consistent with an octahedral geometry around Cu(II) [2], [16]. The LCo complex (Fig. 6) displayed intense bands at 256 and 272 nm (π→π* and n→π*) along with a charge transfer peak at 320 nm. A weak absorption at 500 nm was assigned to the ^4T1g → ^4A2g transition, also supporting an octahedral environment [17], [18].

For LNi, the spectrum showed π→π* and n→π* bands at 324 nm (30,864 cm^{-1}), while additional absorptions at 564 and 644 nm corresponded to ^1A1g → ^1B1g and ^1A1g → ^1A2g transitions, consistent with a square-planar geometry [18], [19]. In the LZn spectrum, intense absorptions at 256 and 318 nm were characteristic of ligand-centered and charge transfer transitions typical for tetrahedral Zn(II) complexes [17], [19]. Full spectral data are summarized in Table 3.

3.4 Conductivity and Magnetic Properties

Molar conductivity values (43–65 $S \cdot cm^2 \cdot mol^{-1}$) indicated that all complexes, except LNi, behaved as 1:1 electrolytes in solution, while Ni(II) complex was non-electrolytic [20] (Table 4). Magnetic moment measurements (Table 5) supported the proposed geometries: octahedral for Co(II) and Cu(II) (μ_eff = 2.19 BM), square planar for Ni(II) (μ_eff = 0.7 BM), and tetrahedral for Zn(II). These results are consistent with the spectral analyses [21].

3.5 NMR Spectroscopy

The ^1H NMR spectrum of ligand L in DMSO-d_6 (Fig. 7) displayed a diagnostic oxime proton at 11.40 ppm (singlet), which disappeared upon D_2O exchange. Aromatic and NH_2 protons resonated between 6.49–6.30 ppm, with NH_2 also vanishing after D_2O addition. Methyl groups were observed at 1.96, 2.29, and 2.63 ppm. In the LZn complex, these resonances shifted significantly: the NH_2 protons appeared at 4.24 ppm, and the oxime proton at 11.39 ppm, confirming coordination. The aromatic signals were shifted downfield, and methyl resonances appeared at 1.97, 2.40, and 2.61 ppm (Table 6).

The ^13C NMR spectrum of L (Fig. 8) revealed signals for C=N–OH at 164.78 ppm and C=N– at 153.49 ppm, along with aromatic carbons between 137–166 ppm. Methyl carbons appeared at 23.12, 20.21, 19.58, and 9.81 ppm [22]-[25]. In LZn, the oxime carbon signal shifted downfield to 153.16 ppm, confirming metal coordination. Other carbons showed comparable but slightly shifted signals (Table 7) [28].

Table 3: Electronic spectral data of L1 and its metal complexes.

No	λ nm	λ cm^{-1}λ	ΣMax (dm^3 mo^{-1} cm^{-1})	Assignment	Suggested geometry
L^1	314	31847	1265	Ligand Field	-----
C_1	256 318 372	39063 31447 26882	1796 1500 95	Ligand Field Charge transfer Charge transfer	Tetrahedral
C_2	256 272 320 500	39063 36765 31250 20000	1441 1114 673 130	Ligand Field Ligand Field Charge transfer $^4T_{1(g)}F \rightarrow {}^4A_{2(g)}F$	Octahedral
C_3	258 364 430 662 930	38760 27473 23256 15106 10753	2829 604 244 273 137	Ligand Field Charge transfer Charge transfer $^2B_{2(g)} \rightarrow {}^2B_{1(g)}$ $^2B_{2(g)} \rightarrow {}^2A_{1(g)}$	Octahedral
C_4	324 564 644	30864 17731 15530	1604 250 164	Charge transfer $^1A_{1(g)} \rightarrow {}^1B_{1(g)}$ $^1A_{1(g)} \rightarrow {}^1A_{2(g)}$	Square planar

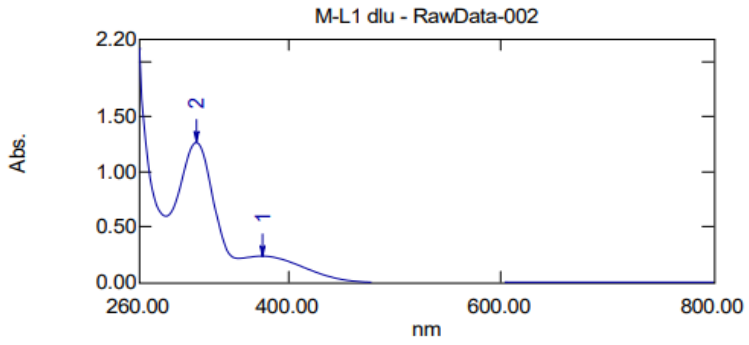

Figure 5: UV-VIS spectrum of the ligand.

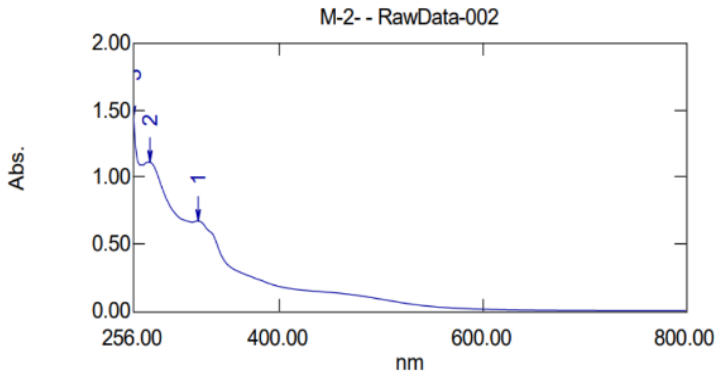

Figure 6: UV-VIS spectrum of the LCoCl (H2O)2]Cl complex.

Table 4: The molar conductance of the complexes.

COMP.	COMPLEX	Molar conductivity $S.cm^2.mole^{-1}$^
C_1	[LZnCl]Cl	43
C_2	[LCoCl $(H_2O)_2$]Cl	65
C_3	[LCuCl $(H_2O)_2$]Cl	136
C_4	$LNiCl_2$	43

Table 5: Magnetic Moment (μeff =B.M) of L1 complexes at 298K and suggested stereochemical structures.

Compound	μ_{eff}. B.M	Proposed Geometry
[LZnCl] Cl	diamagnetic	tetrahedral
[LCoCl $(H_2O)_2$] Cl	2.19	octahedral
[LCuCl $(H_2O)_2$] Cl	2.19	octahedral
$LNiCl_2$	0.7	Square planar

Table 6: 1HNMR data (δ, ppm) for the L1 ligand and L1Zn complex in DMSO-d6.

Funct. Group	δ(ppm) (Ligand)	δ(ppm) (C_1)
–C=N–OH	11.40 (s,1H)	11.39 (s, 1H)
C–H (ar)	(6.30-6.49) (d,2H)	(6.32-7.93) (d, 2H)
CH_3–C=N–OH	2.63 (s,3H)	2.61 (s, 3H)
CH_3–C=N–	1.97(s,3H).	2.40(s, 3H).
CH_3–C (ar)	2.29 (s,6H)	1.97 (d, 6H)
NH_2	4.24 (s,2H)	4.24 (s, 2H)

Table 7: ^{13}C nmr data for the ligand and L1Zn complex.

Funct. Group	δ(ppm) Ligand	δ(ppm) (C_1) Complex
–C_9=N–OH	163.92	152.28
C_7=N–	152.25	151.94
C_{13},C_5,C_4,C_{16},C_{15},C_{14}(ar)	(115.35-136)	(116.27-138.54)
C_{10}	21.88	21.90
C_1	18.97	18,99
C_2	18.34	17.88
C_8	8.56	8.59

Table 8: EI mass spectral data of L1 ligand.

Mass/charge m/z	Relative abundance
219	23
204	31
186	56
161	100
149	55
136	62
120	35
168	12
93	29
77.1	28
65	13
51	12

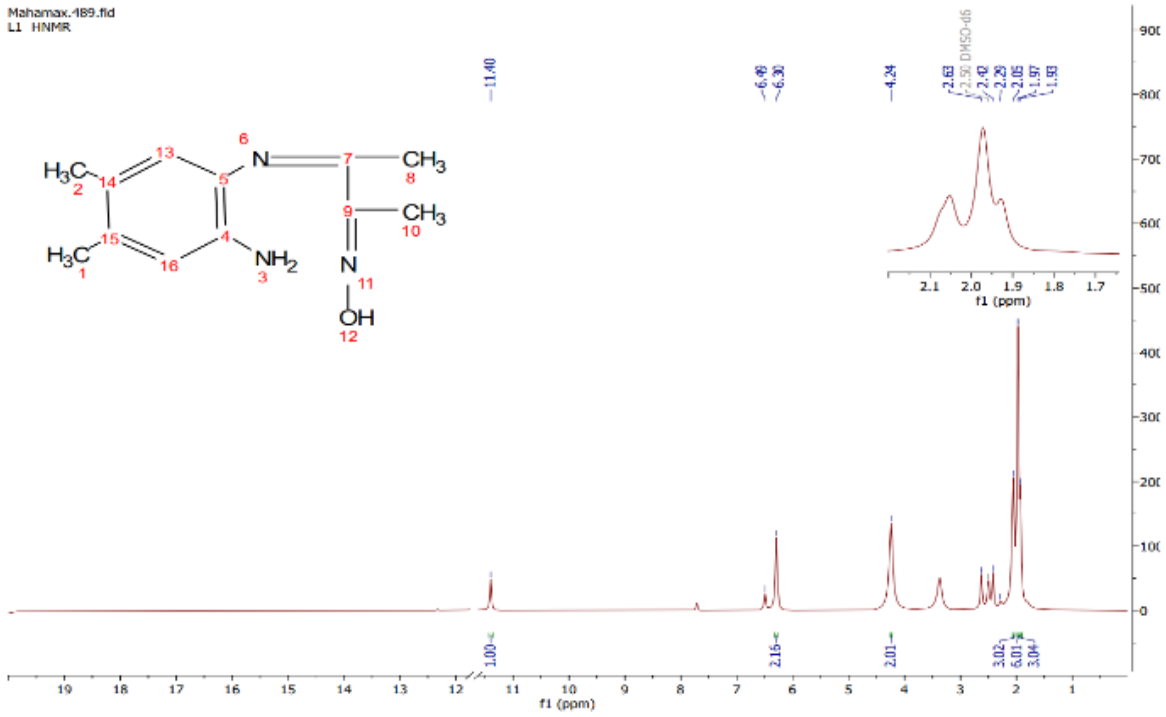

Figure 7: [1]Hnmr spectrum of the ligand.

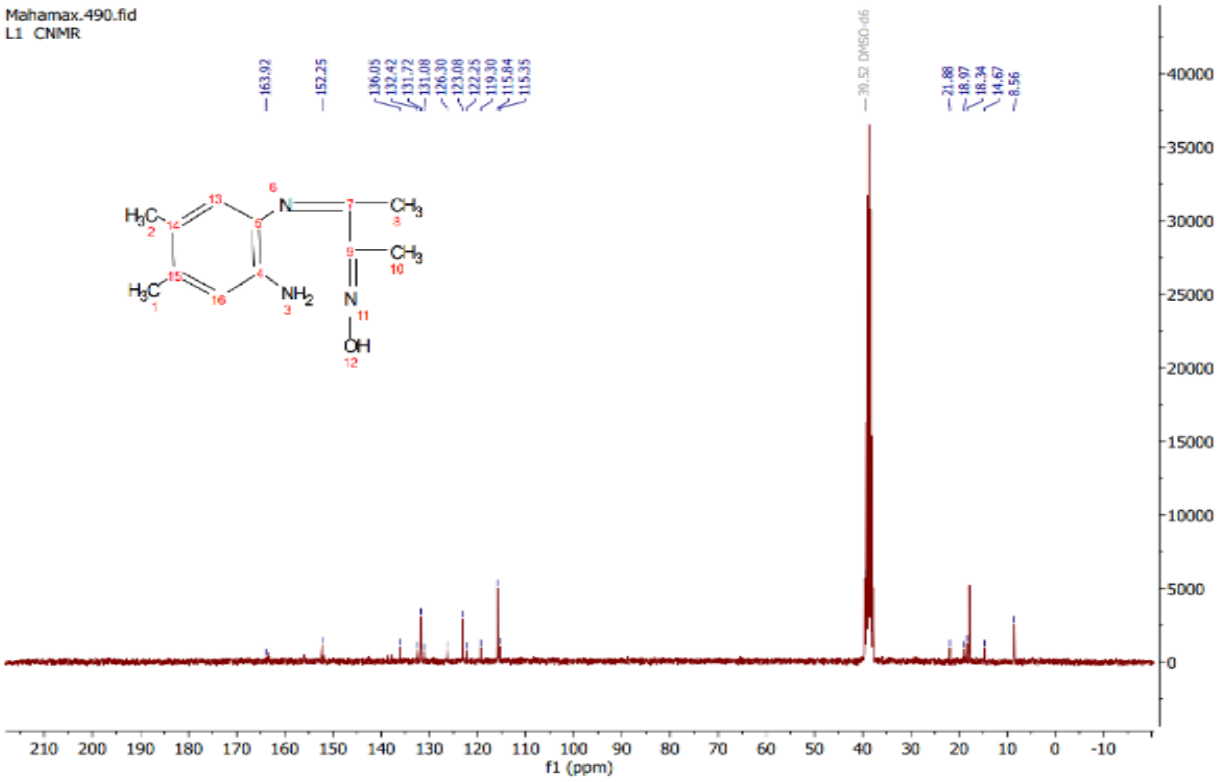

Figure 8: [13]Cnmr spectrum of the ligand.

Table 9: Biological activity and inhibition zone (mm) data for prepared ligand and their complexes.

Compound	Concentration, (µg/ml)	Staphylococcus Aureus, (G+)(mm)	Escherichia Coli, (G-)(mm)
L^1	100	25	20
	50	17	16
	25	R	R
C_1	100	18	17
	50	14	15
	25	R	R
C_2	100	34	30
	50	25	20
	25	19	15
	Levofloxacin Control	23	26
		R	R
C_3	100	21	18
	50	15	15
	25	13	14
C_4	100	R	17
	50	R	15
	25	R	14

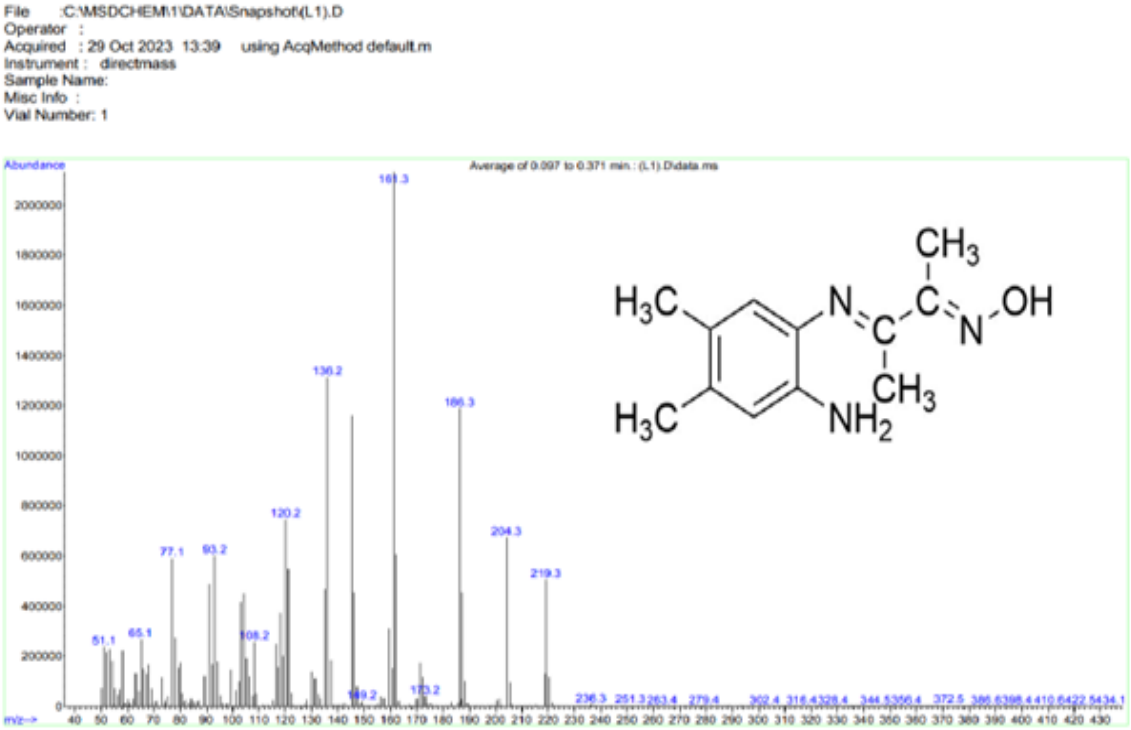

Figure 9: EI mass spectrum for the Ligand.

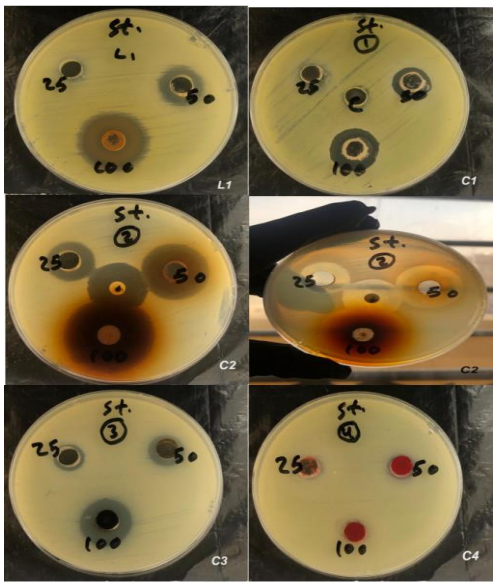

Figure 10. Evaluation of Antiviral Efficacy of the Ligand and Its Complexes [C1, C2, C3, C4] Against Staphylococcus aureus

3.6 Mass Spectrometry

The EI mass spectrum of the ligand displayed a molecular ion peak at m/z = 219 (M^+), matching the calculated molecular weight. Fragment ions are listed in Table 8 and illustrated in Figure 9. For LZn, the parent ion was observed at m/z = 355.57, again consistent with the expected formula.

3.7 Antibacterial Activity

The ligand and complexes were screened against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) at concentrations of 25, 50, and 100 µg/mL in DMSO. At the lowest concentration (25 µg/mL), LZn and the free ligand exhibited no inhibitory effect against S. aureus, while LCo and LCu showed measurable activity. Similarly, against E. coli, both L and LZn were inactive at 25 µg/mL, whereas all complexes except LNi were active at higher concentrations. Notably, the LNi complex showed no significant inhibition of S. aureus at any tested concentration (Table 9, Fig. 10) [29], [30].

4 CONCLUSIONS

Within the scope of this investigation, a novel Schiff base-oxime ligand was synthesized and characterized, in addition to its complexes with zinc (Zn^{2+}), cobalt (Co^{2+}), nickel (Ni^{2+}), and copper (Cu^{2+}), all of which were studied at reasonable relative ratios. Several methods, including magnetic susceptibility, molar conductivity, Fourier transform infrared spectroscopy, nuclear magnetic resonance (NMR) with ^{13}C and 1H, ultraviolet-visible spectroscopy, and mass spectroscopy, were utilized to investigate the complexes. Several of their structural characteristics were validated by the results that were obtained. The amine-oxime ligand and its metal complexes demonstrated activity that ranged from moderate to good against the two different species of bacteria that were utilized in this investigation. The geometric morphologies of the complexes were proposed, and the Zn^{2+} complex was found to be tetrahedral. On the other hand, the Co^{2+} and Cu^{2+} complexes were found to be octahedral, and the Ni^{2+} complex was found to be square planar. The article's principal objective is to catalyze ongoing study by providing readers with expansive insights into the astounding capabilities and multifarious complexity of the oxime moiety. This will be accomplished by providing readers with thorough information. Therefore, the continuous examination of these complexes may show to be a fruitful endeavour for future investigations. This prospect is supported by the fact that it is highly plausible.

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