Spectroscopic and Biological Investigations of 4-[(1E)-N-(2-aminophenyl) ethanimidoyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol and its Copper (II) Complex

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ABSTRACT

This study reports the synthesis, characterization, and antimicrobial evaluation of a novel Schiff base ligand (4-[(1E)-N-(2-aminophenyl) ethanimidoyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol) derived from 4-acyl pyrazolone and its copper (II) complex. The work aims to correlate the structural modifications induced by metal coordination with variations in biological activity. The experimental procedure involved the condensation of 4-acyl pyrazolone with 1,2-diaminobenzene to form the Schiff base ligand, followed by complexation with copper (II) chloride dihydrate ($CuCl_2·2H_2O$) to yield the corresponding Cu (II) complex. Structural elucidation was achieved using elemental analysis, molar conductivity, infrared (IR), ultraviolet–visible (UV–Vis), proton nuclear magnetic resonance (^1H NMR), and gas chromatography–mass spectrometry (GC–MS) techniques. Spectroscopic analyses confirmed the formation of the Schiff base through the characteristic azomethine (C=N) absorption at 1636 cm^{-1} and coordination to Cu(II) via N,N,O donor sites, as evidenced by metal–ligand (M–L) bands at 667.2 cm^{-1}. The Cu(II) complex displayed a higher melting point and molar conductivity than the free ligand, indicating greater thermal stability and a non-electrolytic nature. Antimicrobial activities were assessed against Salmonella typhi, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, *and* Candida albicans using the agar well diffusion method following CLSI standards. The Cu(II) complex exhibited enhanced antimicrobial efficacy compared to the free ligand, except against S.aureus. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) values further confirmed improved potency upon complexation. The increased activity of the Cu (II) complex is attributed to enhanced lipophilicity and cell membrane permeability in accordance with Tweedy's chelation theory. Overall, the synthesized Cu (II) complex demonstrates promising potential for development as a broad-spectrum antibacterial and antifungal agent.

Keywords: Schiff base ligand, Copper(II) complex, Antimicrobial activity, Spectroscopic characterization.

INTRODUCTION

Schiff bases are an important family of organic compounds formed by the condensation of primary amines with aldehydes or ketones to form an imine/azomethine group (C=N). These compounds have attracted interest because of their structural flexibility, ease of preparation, and diverse coordination behaviour. When coordinated to transition-metal ions, Schiff bases often exhibit improved biological and catalytic activities due to changes in their electronic and steric environments (Ceramella *et al*., 2022; Manohar *et al*., 2022). Over the years, Schiff-base metal complexes have found applications as antibacterial, antifungal, anticancer, and antioxidant agents, demonstrating that coordination can significantly modulate the pharmacological potential of the parent ligands (Silas *et al.*, 2025)

Within this broad class, derivatives of 4-acylpyrazolone occupy a special position because of the chemical versatility of the pyrazolone moiety (Okolo *et al*., 2025). The 4-acyl-5-pyrazolone system behaves as a β-dicarbonyl framework that undergoes keto–enol tautomerism and coordinates metals through oxygen and nitrogen donor atoms. These properties make 4-acylpyrazolones excellent building blocks for new Schiff-base ligands and their metal complexes (Idemudia *et al*,, 2016). Studies have shown that transition metals such as copper, nickel, and zinc readily form stable chelates with acylpyrazolone derivatives, producing geometrically diverse complexes with interesting optical and biological properties (Shaikh *et al*., 2022).

Spectroscopic characterization provides essential insight into the formation and bonding patterns of such compounds. In the infrared region, the appearance of a strong azomethine (C=N) stretching vibration around 1600–1650 cm^{-1} confirms the condensation reaction, whereas coordination to a metal center is usually accompanied by shifts in characteristic bands and the emergence of new metal–ligand vibrations below 700 cm^{-1} (Ahmed *et al*., 2022). Complementary data from ultraviolet–visible, nuclear magnetic resonance, elemental analysis, and molar-conductivity measurements aid in deducing the coordination environment, stoichiometry, and geometry of the complexes (Xi *et al*., 2020). Together, these techniques allow reliable correlation between structural features and physicochemical behavior.

The enhancement of antimicrobial activity observed after metal complexation of Schiff bases has often been explained through chelation and lipophilicity concepts. According to Tweedy's chelation theory, the partial sharing of metal ion positive charge with the donor atoms of the ligand reduces metal polarity, delocalizes electron density over the chelate ring, and increases overall lipophilicity. This facilitates diffusion of the complex across microbial cell membranes and improves interaction with intracellular targets (Alezzy *et al.*, 2022). Overtone's principle similarly proposes that lipid-soluble compounds pass more easily through cell envelopes, accounting for the higher activity of coordinated species (Nandini & Amutha Selvi, 2025).

Copper(II) complexes garners significant attention because Cu(II) possesses variable coordination geometries, moderate redox potential, and intrinsic antimicrobial capability. Schiff bases derived from 4-acylpyrazolone and coordinated to Cu(II) have been reported to exhibit square-planar or distorted octahedral structures and it tends to display stronger biological effects than their free ligands (Barad *et al*., 2023; Wang *et al.*, 2019)

This investigation focuses on the synthesis of a Schiff base formed by condensing a 4-acylpyrazolone derivative with an aromatic diamine and its subsequent complexation with Cu(II) ions. Comprehensive spectroscopic analyses: FT-IR, UV–Vis, ^{1}H NMR, elemental analysis, molar-conductivity, and GC–MS were employed to elucidate the structural features of both the ligand and the complex. Their antimicrobial efficacy was then examined against selected bacterial and fungal strains to assess how coordination influences biological activity. This study therefore bridges structural chemistry and bioactivity, aiming to clarify and analyse how metal–ligand interactions in acyl pyrazolone based Schiff bases contribute to enhanced antimicrobial performance.

MATERIALS AND METHODS

All reagents and solvents utilized in this study were of analytical reagent grade and were used without further purification. Ethyl acetoacetate ($C_6H_{10}O_3$), phenylhydrazine ($C_6H_8N_2$), diethyl ether, ethanol, 1,4-dioxane, acetyl chloride, and hydrochloric acid were procured from Sigma-Aldrich. Additional reagents and metal salts were obtained from Loba Chemie. Distilled water of high purity was also used as supplied. Culture media such as nutrient agar, Sabouraud's dextrose agar, and culture broth were obtained from the Department of Microbiology, Federal Polytechnic Nekede, Imo State, Nigeria.

Instrumentation and Measurements analyses (C, H, N, and O) were performed on a Perkin Elmer 2400 Series II elemental analyzer. Molar conductivity measurements were recorded using a Philips PW 9506 conductivity meter. The solubility of the synthesized Schiff base ligand and its copper (II) complex was determined in various solvents including water, ethanol, methanol, DMSO, petroleum ether, and n-hexane. Infrared (IR) spectra were obtained in KBr discs using an Agilent Cary 630 FTIR spectrophotometer, with absorption frequencies reported in the range of 4000–650 cm^{-1}. The proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Nanalysis X685 spectrometer operating at 60 MHz, using deuterated DMSO (d_6) as the solvent. Gas chromatography–mass spectrometry (GC–MS) analyses were conducted on a Varian 3800/4000 GC–MS system with a run time of 45 minutes and a mass range up to 500/1000 m/z. Ultraviolet–visible (UV–Vis) spectra were recorded using an Agilent Cary 630 UV spectrophotometer within the wavelength range of 200–800 nm.

METHODS

Synthesis of Tridentate Schiff Base Ligand (4-[(1E)-N-(2-aminophenyl) ethanimidoyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol)

Step 1: Synthesis of 3-Methyl-1-Phenyl -Pyrazol-5-one {Precursor-P}

The precursor ligand, designated as **P**, was synthesized following the method previously described by Furniss *et al.* (1989), with slight modifications (Figure 1). Ethyl acetoacetate (49 ml, 0.384 mol) was mixed with phenyl hydrazine (36.5ml, 0.370 mol) in a large evaporating dish. The reaction mixture was heated over a boiling water bath inside a fume cupboard and continuously stirred with a stirrer for approximately two hours. As the reaction proceeded, a reddish syrup-like mass was formed. After heating for two hours, the mixture was removed from the water bath and allowed to cool for about five minutes. Diethyl ether (100 ml) was then added, and the mixture was stirred vigorously, leading to gradual solidification of the syrup. The resulting solid, which was insoluble in ether, was collected and washed thoroughly with diethyl ether (100 mL) to remove impurities. The washing was repeated, the crude product was then recrystallized from ethanol (100 mL) to yield pale yellow crystals. Yield**:** 78%; M.p**.:** 127°C; Molecular formula**:** $C_{10}H_{10}N_2O$; IR (KBr, cm^{-1}): 3125.4 (N–H stretch), 2924.1 (C–H stretch), 1697.2 (C=O), 1496.5 (C=C stretch), 1343.7 (C–N stretch), 1198 (C–N cyclic); GC–MS (m/z): Calc., 174; Found, 174.0.

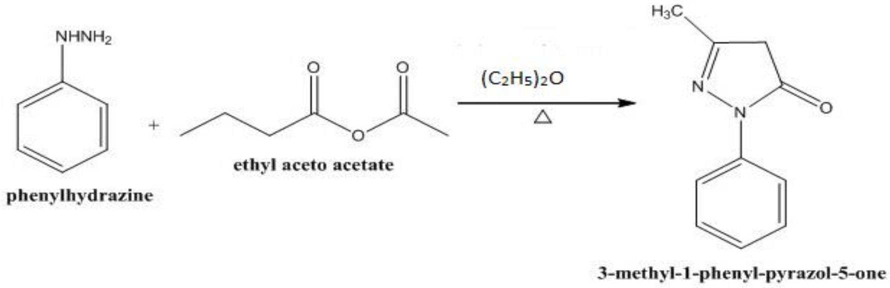
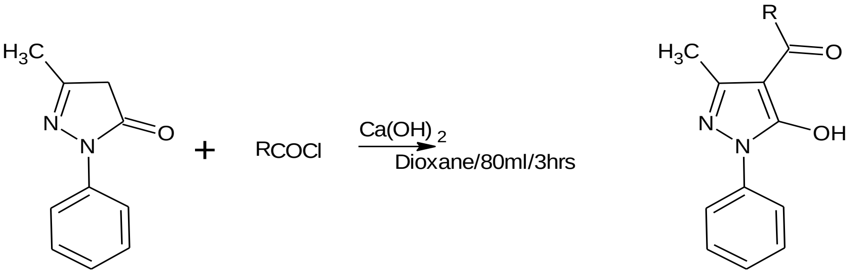


Figure 1: synthesis of 3 methyl-1-phenyl-pyrazol-5-one (P)

STEP 2: Synthesis of Acyl Pyrazolone (4-acetyl-3-methyl-1-phenyl-5-pyrazolone)

The compound 4-acetyl-3-methyl-1-phenyl-5-pyrazolone was synthesized following a slightly modified procedure of Jensen (1959), (Figure 2). 3-methyl-1-phenyl-5-pyrazolone (17.4 g, 0.1 mol) was dissolved in 80 mL of 1,4-dioxane contained in a three-necked round-bottom flask equipped with a reflux condenser and a magnetic stirrer. The solution was gently warmed and stirred until complete dissolution was achieved. Anhydrous calcium hydroxide (12 g) was then added to the reaction mixture, and heating was continued. Acetyl chloride (7.1 mL, 0.1 mol) was introduced dropwise over a period of two minutes, resulting in the formation of a thick paste. The reaction mixture was subsequently refluxed for three hours. After completion of reflux, the mixture was allowed to cool for about 20 minutes and then poured into 200 mL of cold dilute hydrochloric acid (2 M) with continuous stirring. Acidification decomposed the calcium complex, leading to the precipitation of cream-colored crystals of 4-acetyl-3-methyl-1-phenyl-5-pyrazolone. The product was filtered, washed with cold water, and dried. Yield: 50%; M.p.**:** 242 °C.

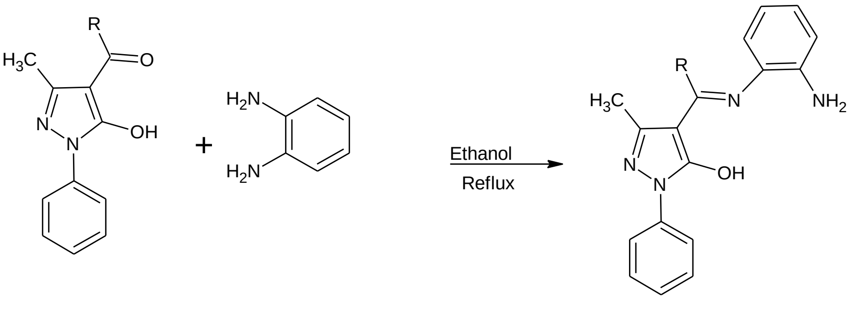


` $R = CH_3$

Figure 2: synthesis of 4-acetyl-3-methyl-1 -phenyl-5-pyrazolone

STEP 3: Procedure for the Synthesis of Tridentate Schiff Base Ligand (4-[(1E)-N-(2-aminophenyl)ethanimidoyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol).

The Schiff base ligand, 4-[(1E)-N-(2-aminophenyl)ethanimidoyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (L), was synthesized according to the procedure described by Nasridas *et al.* (2015), with slight modifications (Scheme 3). An ethanolic hot solution (50 mL) of 1,2-diaminobenzene (1.08 g, 0.01 mol) was mixed with an equimolar ethanolic solution (50 mL) of 4-acetyl-3-methyl-1-phenyl-5-pyrazolone (2.16 g, 0.01 mol). The resulting mixture was refluxed for three hours in the presence of a few drops of glacial acetic acid as a catalyst. After refluxing, the reaction mixture was allowed to cool to room temperature and left to stand overnight. Cream-colored crystalline precipitates were obtained, filtered, washed with cold ethanol, and dried to yield the Schiff base ligand of excellent purity. Yield: 73% , M.P: 180 °C $C_{18}H_{18}N_4O$ (L), Anal. Found(%) : C, 66.99; H, 6.20; N, 18.29;O, 5.53; Calc(%): C, 70.11; H, 6.54; N, 18.17; O,5.18. IR (KBr, cm-1-): 3496.2 (N-H Stretch), 3236.3 (O-H), 2961 (C-H Stretch), 1636.3 (azomethine C=N), 1561.8 (C=C Stretch), 1323,2 (C-N Stretch), 1073 (C-O):. UV-Vis.(DMSO) λmax(nm);298, ε (8.16 x 104), H NMR (DMSO-d6, 400 MHz) δ (ppm): 6.85–6.50 (m, 1H, Ar–H), 5.10 (s, 2H, OH or NH2), 1.23 (s, 1.5H, CH3).. GC-MS (m/z): cal., 308; found, 308.2.



$R = CH_3$

Figure 3: Synthesis of Tridentate Schiff Base Ligand (4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol).

Synthesis of the Copper Complex 4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol (CuL_2)

The copper (II) complex of the Schiff base ligand was synthesized by reflux condensation. The Schiff base ligand, 4-[(1E)-N-(2-aminophenyl) ethanimidoyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (1.54 g, 5 mmol), was dissolved in 100 ml of ethanol and stirred for approximately five minutes to obtain a homogeneous solution. Separately, copper (II) chloride dihydrate (0.85 g, 5 mmol) was dissolved in 20 ml of ethanol. Both solutions were gently warmed for a few seconds to ensure complete dissolution while preventing solvent evaporation, after which they were mixed together. The resulting mixture was refluxed for six hours under continuous stirring. During reflux, the solution was gradually concentrated to about half of its original volume. Upon cooling, the solid product formed was filtered, washed repeatedly with hot water and then with hot ethanol to remove any unreacted materials or impurities. The Black-coloured complex obtained was dried under vacuum and recrystallized from dimethylformamide (DMF) to yield a pure copper (II) complex suitable for further characterization.). Yield: 75%, M.p.: >250 °C, $C_{36}H_{34}CuN_8O_2$ (CoL_2), Anal. Found (%) : C, 63.61; H, 5.63; N, 16.57;O, 4.75; Cu, 9.44. Calc (%): C, 63.56; H, 5.93; N, 16.47; O,4.70; Cu, 9.34. IR (KBr, cm-1-): 3339.4 (N-H Stretch), 3179.4 (O-H), 1617.1 (azomethine C=N), 1524.5 (C=C Stretch), 1233.7(C-N Stretch), 1103.3 (C-O), 667.2 (M-L): UV-Vis.(DMSO) λmax (nm);286, ε (3.58 x 104). GC-MS (m/z): cal., 679.5; found, 6. Below (Figure 4) is a schematic equation of the synthesis of the Metal complex (Copper Complex of 4-[(1E)-N-(2aminophenyl)ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol).

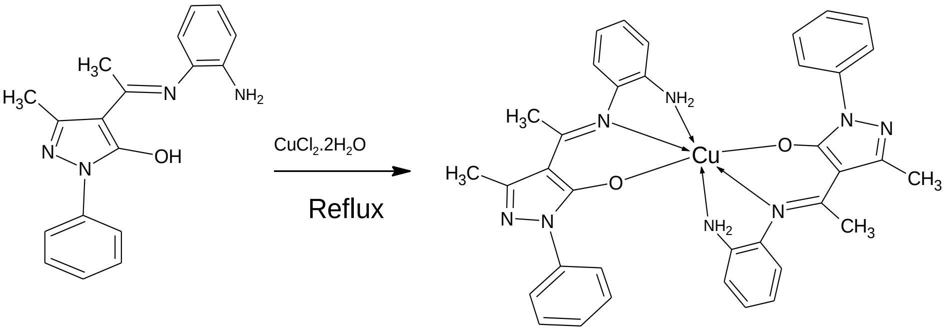


Figure 4: Equation of the synthesis of the Metal complex (Copper Complex 4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol).

Antimicrobial/ Antifungal Analyses of the Schiff base Ligand (L) and its Cu (II) Complex (CuL_2)

The antibacterial and antifungal activities of the Schiff base ligand (L) and its Copper(II) complex (CuL_2) were evaluated using the disc diffusion method (Jayarajan *et al.*, 2010). Test organisms included *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans*. The compounds (200 µg/mL in DMSO) were tested on nutrient or Sabouraud agar plates, with Levofloxacin and Nystatin serving as positive controls, and DMSO as the negative control. Plates were incubated at 37 ± 1°C for bacteria and 28 ± 1°C for fungi, and activity was determined by measuring inhibition zones (mm).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal /Fungicidal (MFC/MBC)

Concentrations (MBC/MFC) were determined by the broth dilution method. Serial dilutions of the test compounds were prepared in suitable broth media and inoculated with standardized microbial suspensions. After incubation, the MIC was recorded as the lowest concentration showing no visible growth, while the MBC/MFC was the lowest concentration showing no microbial recovery on agar plates (Weigand *et al.*, 2008).

RESULTS AND DISCUSSION

The precursor compound, 3-methyl-1-phenyl-pyrazol-5-one, underwent acylation with acetyl chloride to produce an acyl pyrazolone derivative. This intermediate was subsequently condensed with 1,2 diamino benzene to yield the Schiff base, 4-[(1E)-N-(2-aminophenyl)ethanimidoyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol. The resulting ligand was then reacted with copper (II) chloride dihydrate to form the corresponding copper (II) complex. The precursor, Schiff base ligand, and its copper complex were characterized using various analytical and spectroscopic techniques.

Physicochemical Properties of the Pyrazolone (3-Methyl-1-Phenyl -Pyrazol-5-one) Schiff base Ligands (L) and its Cu (II) Complex (CuL_2)

The synthesized compounds exhibited distinct physical properties that support their structural transformation. Table 1 shows the formation of the Schiff base ligand (L) resulted in a cream-colored compound with a melting point (180 °C) and moderate conductivity (10.8 µS/cm), upon coordination with Cu(II**)** ions, the resulting complex (CuL_2) appeared as a black solid with a higher melting point (>250 °C) and slightly higher conductivity (15.9 µS/cm). These changes are consistent with metal–ligand chelation, which typically enhances thermal stability and slightly alters the electronic environment, producing intense coloration due to d–d transitions. The progressive increase in melting point and conductivity values confirms successful Schiff base formation and complexation with copper (II). The data align with previous findings that Schiff base metal complexes are thermally stable, non-electrolytic, and exhibit strong metal–ligand interactions through azomethine nitrogen, enolic oxygen and primary amine nitrogen atoms (Ogbuagu *et al*., 2025).

Table 1 Physicochemical Properties of the Schiff base Ligands (4-[(1E)-N-(2-aminophenyl) ethanimidoyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol) and its Cu (II) Complex (CuL_2)

Compounds	**Molecular Formula**	**% Yield**	**Colour**	**Melting Point (∘C)**	**Conductivity (µs/cm)**
L	$C_{18}H_{18}N_4O$	73	Cream	180	10.8
CuL_2	$C_{36}H_{34}CuN_8O_2$	55	Black	>250	15.9

L- 4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol

CuL_2- Cu (II) Complex 4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol

Solubility Profile of L and CuL_2 in various solvents

Table 2 shows solubility profile of Schiff base, and copper complex. Schiff base ligand (**L**) showed **slight solubility** in DMSO, ethanol, and methanol but was **insoluble** in petroleum ether, *n*-hexane, and water, indicating a **moderately polar nature**. In contrast, the **Cu (II) complex (CuL_2)** was in**soluble in polar solvents** (DMSO, ethanol, and methanol) and **nonpolar solvents,** reflecting decreased polarity due to **metal–ligand coordination. C**oordination induces reduction in polarity and increased lattice stability. These observations further support the formation of a stable Cu(II) complex.

Table 2 Solubility Profile of Schiff base Ligand (L) and its Cu (II) Complexes (CuL_2) in various solvents

Compounds			**Solvents**			
	DMSO	Petroleum Ether	N-Hexane	Ethanol	Methanol	Water
L	s	is	is	s	s	s
CuL_2	ss	ss	ss	ss	ss	ss

Infrared Spectral Analysis

The IR spectral data of the Schiff base ligand (**L**)**,** and its copper(II) complex (**CuL_2**) are presented in Table 3. The emergence of an **azomethine (C=N) stretch at 1636.3 cm⁻¹,** indicating successful **Schiff base formation** through condensation of the carbonyl group with an amine (Fig 5). In the spectrum of the **Cu(II) complex**, the **C=N stretching frequency shifted to a lower value (1617.1 cm⁻¹**), suggesting **coordination of the azomethine nitrogen** to the metal ion. The **O–H stretching vibration** observed at 3236.3 cm⁻¹ in the ligand also shifted to **3179.4 cm⁻¹** in the complex, implying **involvement of the enolic oxygen** in chelation and a downward shift of the C-N stretching frequency(Fig 6). Additionally, new absorption bands appearing around **667 cm⁻¹** correspond to **M–O/M–N vibrations**, confirming the formation of metal–ligand bonds. The observed shifts in the C=N, O–H, C-N and C–O frequencies, along with the presence of new metal-sensitive bands, collectively support a **tridentate coordination mode** of the ligand through **azomethine nitrogen, enol oxygen** and diamino nitrogen atoms. These findings align with literature reports on pyrazolone-based Schiff base metal complexes (Achonye *et al*,. 2024).

Table 3: Selected Infra-red absorption bands of Schiff base Ligand (L) and its Cu (II) Complexes (CuL_2)

S/N	Compounds	(N-H)	(O-H)	(C-H)	(C=N)	(C=C)	(C-N)	(C-O)	M-L
1	**$C_{18}H_{18}N_4O$ [L]**	3496.2	3236.3	2961	1636.3	1561.8	1323.2	1073.5	
2	**$C_{36}H_{34}CuN_8O_2$ (CuL_2**	3339.4	3179.4	-	1617.1	1524.5	1233.7	1103.3	667

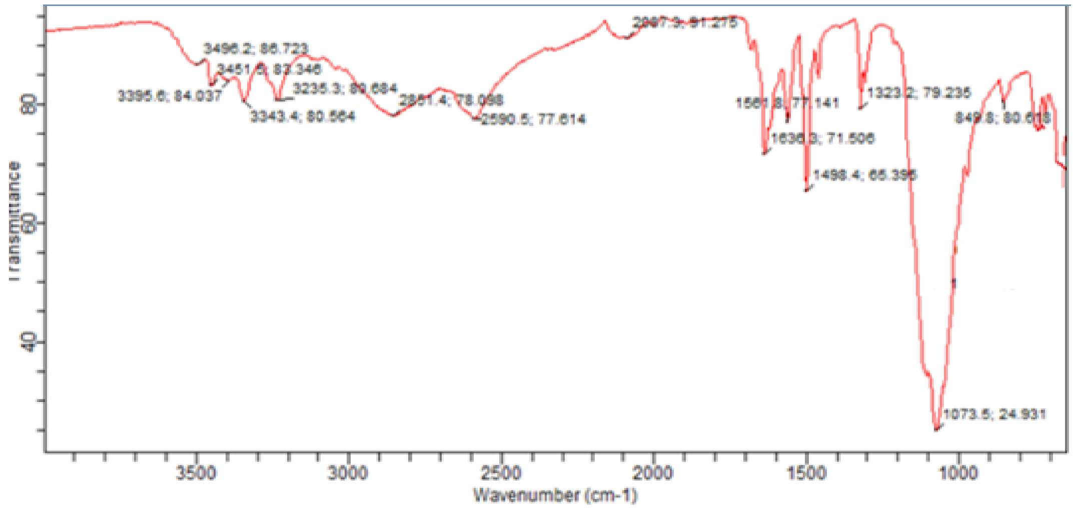


Fig 5: IR Spectrum for Schiff base Ligand -L (4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol)

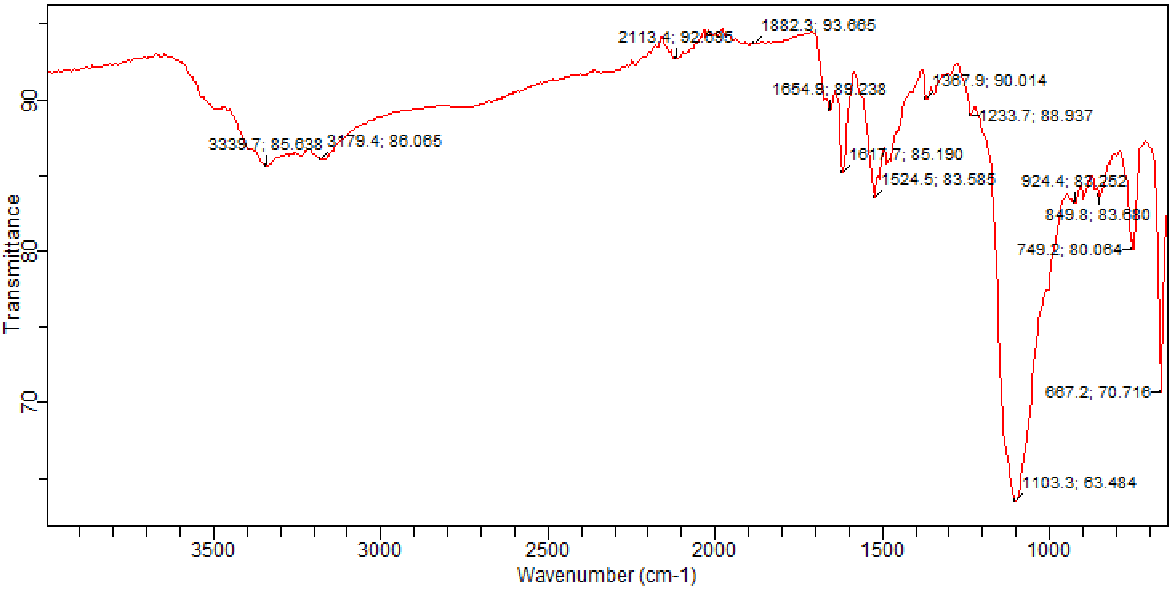


Fig 6: IR Spectrum for Cu (II) Complex

Percentage Elemental Analysis

Table 4 shows elemental analysis of the synthesized Schiff base ligand (L) and its copper (II) complex (CuL_2) showed good agreement between the experimental and calculated values, confirming the purity and proposed molecular formula of the compounds. For the ligand, the experimental values correspond well with the calculated values. In the copper (II) complex (CuL_2), the experimental values closely match the theoretical data. The slight reduction in the percentages of carbon, hydrogen, and nitrogen upon complexation indicates successful coordination of the ligand to the copper (II) ion

Table 4: Percentage Elemental Analysis of L and CuL_2

Compounds	**Molar Mass (g/mol)**	**%C**	**%H**	**%N**	**%O**	**%M**
L($C_{18}H_{18}N_4O$)	306	69.99 (70.11)	6.20 (6.54)	18.29 (18.17)	5.53 (5.18)	-
CuL_2 ($C_{36}H_{34}CuN_8O_2$)	679.5	63.61 (63.56)	5.63 (5.93)	16.57 (16.47)	4.75 (4.70)	9.44 (9.34)

Gas Chromatography-Mass Spectroscopy Analysis of Schiff base Ligand (L)and its Cu(II) complex (CuL_2)

The mass spectral analysis (Table 5) supports the formation of the Schiff base ligand and its copper (II) complex. The Schiff base ligand (L) displayed a molecular ion peak at m/z 308.2 (Fig 7), which agrees with its calculated mass (308 g/mol). Fragment ions at m/z 293, 275, 232, 136, 125, and 58 correspond to the loss of methyl, amine, and aromatic groups, indicating cleavage around the azomethine and pyrazolone moieties. The copper(II) complex (CuL_2) recorded a molecular ion peak at m/z 680.8 (Fig 8), which is in close agreement with the calculated molecular weight (679.5 g/mol). The major fragment peaks at m/z 662.2, 603.1, and 510.0 represent stepwise loss of ligand fragments and small neutral species, suggesting a stable metal-ligand coordination structure.

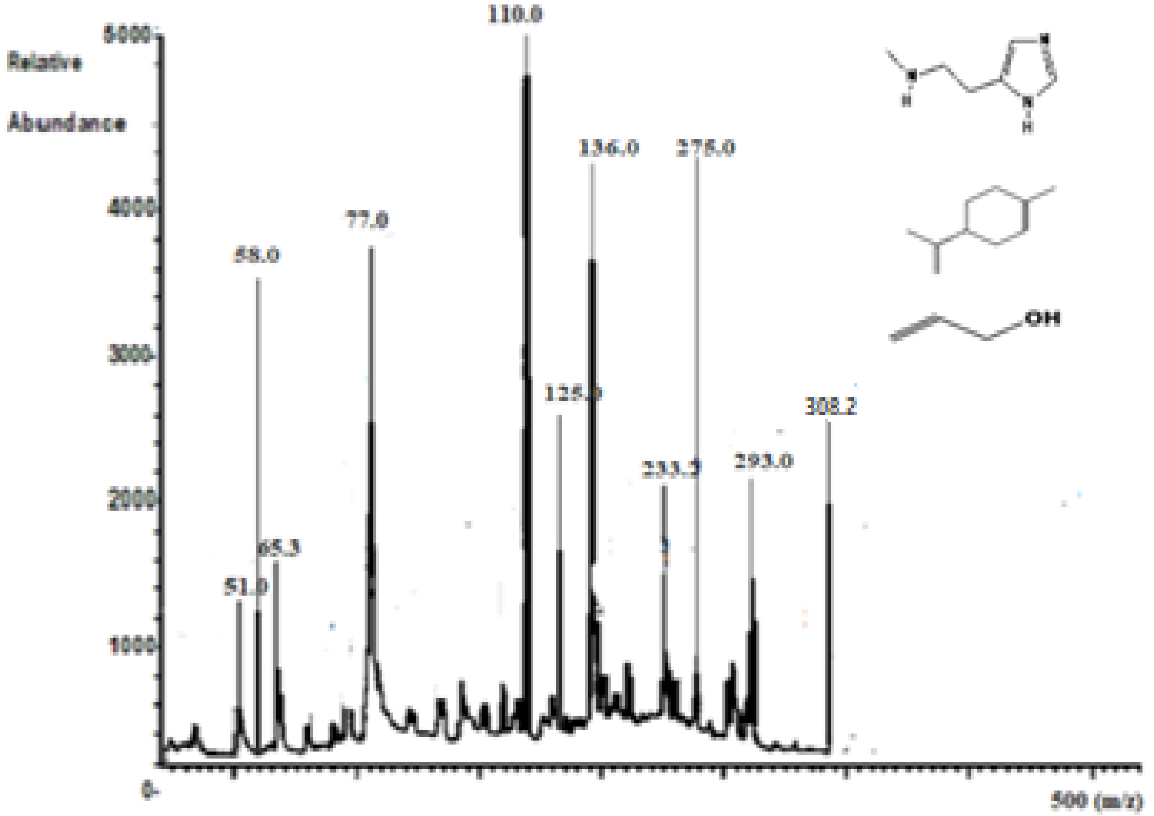


Fig 7 GC-MS spectrum of Schiff base Ligand (L) with a few fragment structure

The presence of a characteristic isotopic pattern for copper around m/z 680.8 further confirms metal the coordination number of six (6) was deduced from the molecular ion value of the copper(II) complex (CuL_2), which indicates two (2) tridentate ligands coordinate to the metal ion which is indicative of an octahedral geometry around the Cu(II)centre. Overall, the close correspondence between the experimental and theoretical data from both elemental and mass spectral analyses confirms the successful synthesis, purity, and proposed structural formulations of the Schiff base ligand and its Cu(II) complex.

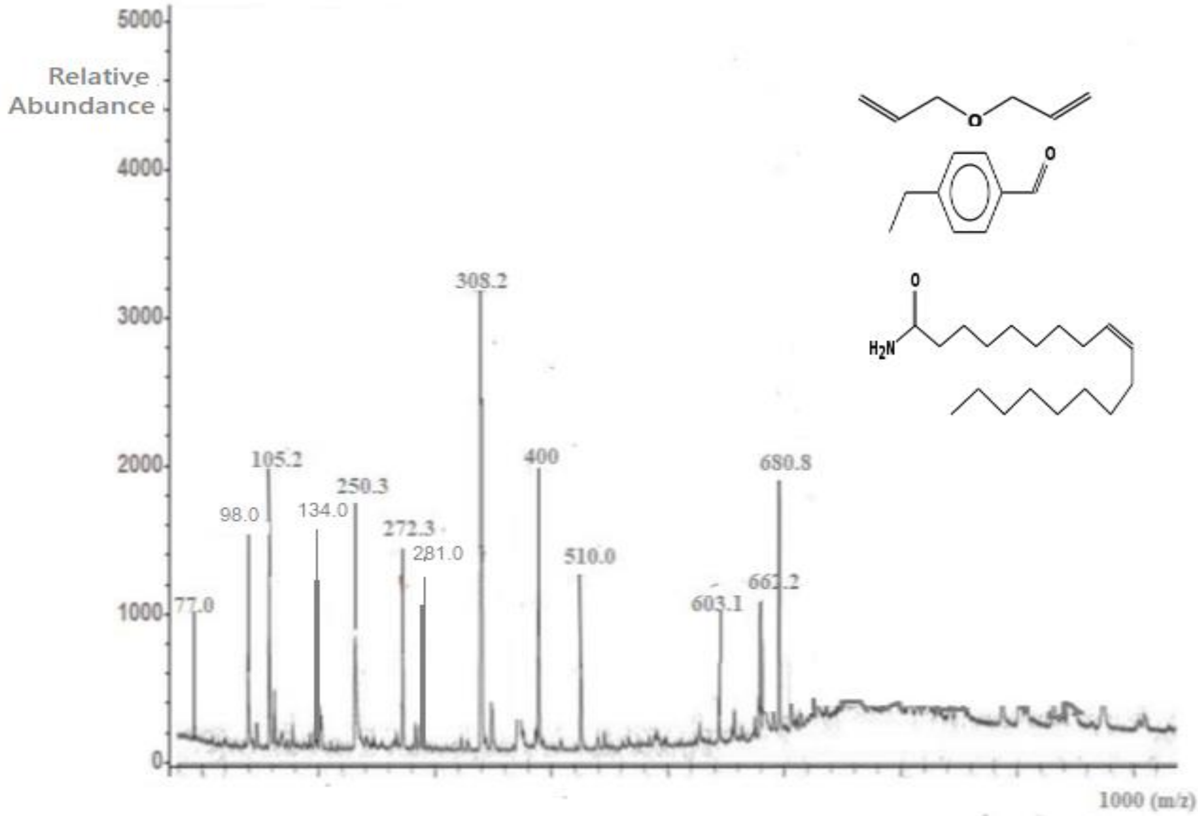


Fig 8: GC-MS spectrum of Cu(II) metal complex (CuL_2) with a few fragment structure

Table 5: Gas Chromatography-Mass Spectroscopy Analysis of Schiff base Ligand (L)and its Cu(II) complex (CuL).

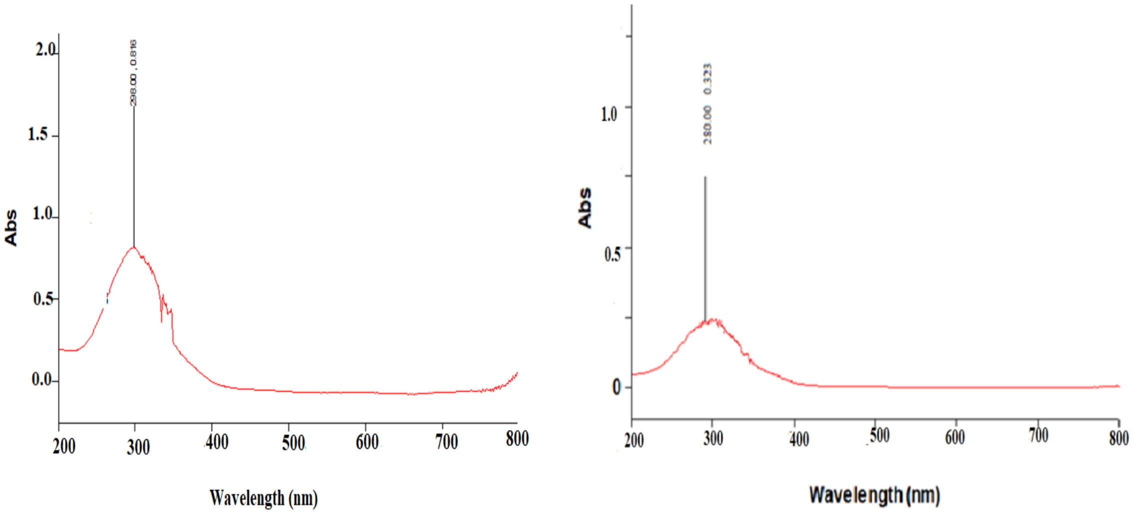
Compounds	Calculated Molecular Mass (g/mol)	Observed Molecular ion (M^+)	Observed Fragment ions	Coordination Number
L ($C_{18}H_{18}N_4O$)	308	308.2	58.0, 125.0, 136.0, 232.2, 275.0, 293.0	-
CuL_2($C_{36}H_{34}CuN_8O_2$)	679.5	680.8	662.2, 603.1, 510.0	6

L- 4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol

CuL_2- Cu (II) Complex 4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol

Ultra Violet -Visible Spectra Analysis of L and CuL_2

Table 6 shows the UV–visible spectra of the Schiff base ligand (L) and its copper(II) complex (CuL_2), the UV-Vis Spectra of L exhibit strong absorption peaks at 298 nm ($\varepsilon = 8.16 \times 10^4$ $L \cdot mol^{-1} \cdot cm^{-1}$) and that of CuL_2 a peak of 286 nm ($\varepsilon = 3.58 \times 10^4$ $L \cdot mol^{-1} \cdot cm^{-1}$). These absorptions are associated with electronic transitions within the ligand's conjugated system (Fig 9), primarily of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ types. A slight shift of the absorption band toward a shorter wavelength in the complex, along with a reduction in intensity, indicates that metal coordination affects the electronic environment of the ligand, leading to minor adjustments in orbital energy levels and transition probabilities. The absence of any observable d–d band in the spectrum of the copper (II) complex is attributed to the transitions are both spin- and Laporte-forbidden, making them intrinsically weak (ε values are low). These low-intensity bands were overshadowed by the much stronger ligand-based transitions occurring in the UV region. Consequently, the spectral features observed are dominated by ligand-centred excitations.



(a) **(b)**

FIG 9: UV-VIS Spectrum (a) Schiff Base Ligand L (b) Cu(II) Complex (CuL_2)

TABLE 6: Ultra Violet -Visible Spectral Data of L and CuL_2

Compounds	**Maximum wavelength Λ_{max} (nm)**	**Molar Absorptivity ε (L mol^{-1} cm^{-1})**	**Electronic transitions**
$C_{18}H_{18}N_4O$ (L)	298	8.16×10^4	$\pi - \pi^*$ $n - \pi^*$
$C_{36}H_{40}CuN_8O_2$ (CuL_2)	286	3.58×10^4	$\pi - \pi^*$ $n - \pi^*$

^{1}HNMR Spectral Analysis of 4-[(1E)-N-(2aminophenyl) ethanimidoyl] -3-methyl-1-phenyl-1H-pyrazol-5-ol (L)

Table 7 shows the ^{1}HNMR analysis in DMSO of the Schiff base Ligand. The data support the formation of a Schiff-base-type compound containing aromatic and aliphatic regions, along with a hydrogen-bonded hydroxyl and amine functionality. The presence of aromatic resonances between 6.5–6.9 ppm and a downfield exchangeable proton at 5.1 ppm is consistent with a **conjugated enol-imine system** typical of 4-acetylpyrazolone derivatives. The small methyl singlet at 1.23 ppm further confirms substitution on the ligand backbone.

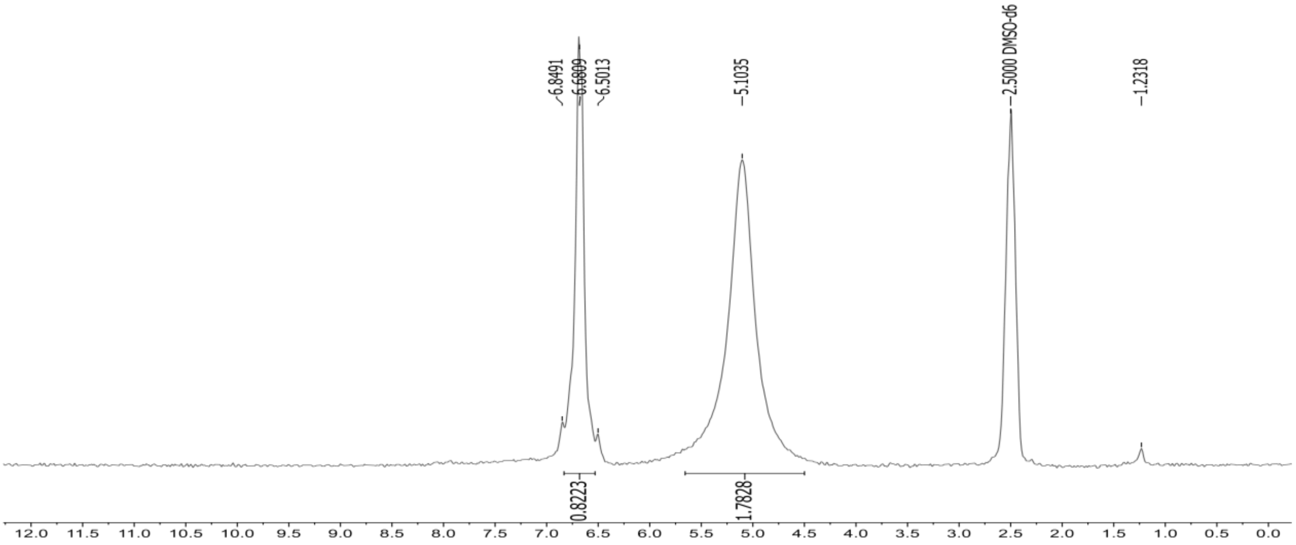


Fig 10: H’NMR Spectrum of Schiff base Ligand (L)

Table 7- ^{1}H NMR Spectral Data (in DMSO-d_6) for a Schiff base Ligand(L)

Chemical Shift (ppm)	Multiplicity	Integration	Tentative Assignment	Proton Type
6.8491, 6.6809, 6.5013	Multiplet (appears as three distinct peaks)	0.8223	Aromatic protons	Ar-H
5.1035	Singlet	1.7828	-OH or N-H	Hydroxyl or Amine proton
2.5000	Singlet	Not integrated (solvent peak)	DMSO-d6	Solvent
1.2318	Singlet	Appears as a small peak	CH3	Methyl proton

Antimicrobial Susceptibility Analysis of Schiff base Ligand (L) and its Cu(II) complex (CuL_2)

The antimicrobial activity of the Schiff base ligand (L) and its copper (II) complex (CuL_2) was evaluated against selected Gram-positive, Gram-negative, and fungal strains, namely *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans*. The results were compared with standard antibiotics: Levofloxacin (LEV) for bacterial strains and Nystatin (NY) for fungal strain (Table 8). The Schiff base ligand (L) exhibited moderate antibacterial and antifungal activity, with the highest inhibition zone recorded against *S. aureus*, indicating significant potency against Gram-positive bacteria. However, its activity against *E. coli* and *S. pyogenes* were comparatively lower, reflecting limited efficacy against some Gram-negative and Gram-positive organisms. Upon complexation with copper (II) ion, there was a notable enhancement in antimicrobial activity across almost all tested microorganisms(Fig 11). The Cu(II) complex (CuL_2) showed the highest zone of inhibition against *C. albicans* and *S. typhi* , followed by *S. pyogenes* and *E. coli* . This increase in activity upon chelation supports the chelation theory. This enhances lipophilicity and facilitates penetration through microbial cell membranes, thereby improving biological efficacy (Zoubi, 2013). Comparatively, the CuL_2 complex demonstrated comparable or superior activity to the reference drugs in certain cases. For instance, against *S. typhi* and *C. albicans*, CuL_2 (26 mm and 32 mm, respectively) surpassed LEV (20 mm) and NY (28 mm). However, LEV remained more effective against *E. coli* and *S. aureus* (32 mm and 36 mm, respectively). Overall, the data indicate that metal complexation significantly improves the antimicrobial potential of the parent Schiff base, making CuL_2 a promising candidate for further biological evaluation (Ogbuagu et al 2025).

Table 8: Antimicrobial Susceptibility data of Schiff base ligand (L) and its Cu(II) complex (CuL_2)

Test Organisms **Formulation/Zone of Inhibition (mm)**

Compounds	*S. typhi*	*E. Coli*	*S. Aureus*	*S.Pyogenes*	*C. albicans*
L	22	14	32	16	20
CuL_2	26	20	30	26	32
LEV	20	32	36	28	-
NY	-	-	-	-	28

Key:

mm = Millimeter

Clinical Laboratory Standard Institute guideline for antimicrobial agents (CLSI)

Resistant (0 – 12 mm) **Susceptible** (16 mm and above) **LEV** = Levofloxacin

NY = Nystatin - = Not determined **µg/ml** = Microgram per millilitre ND = Not detected

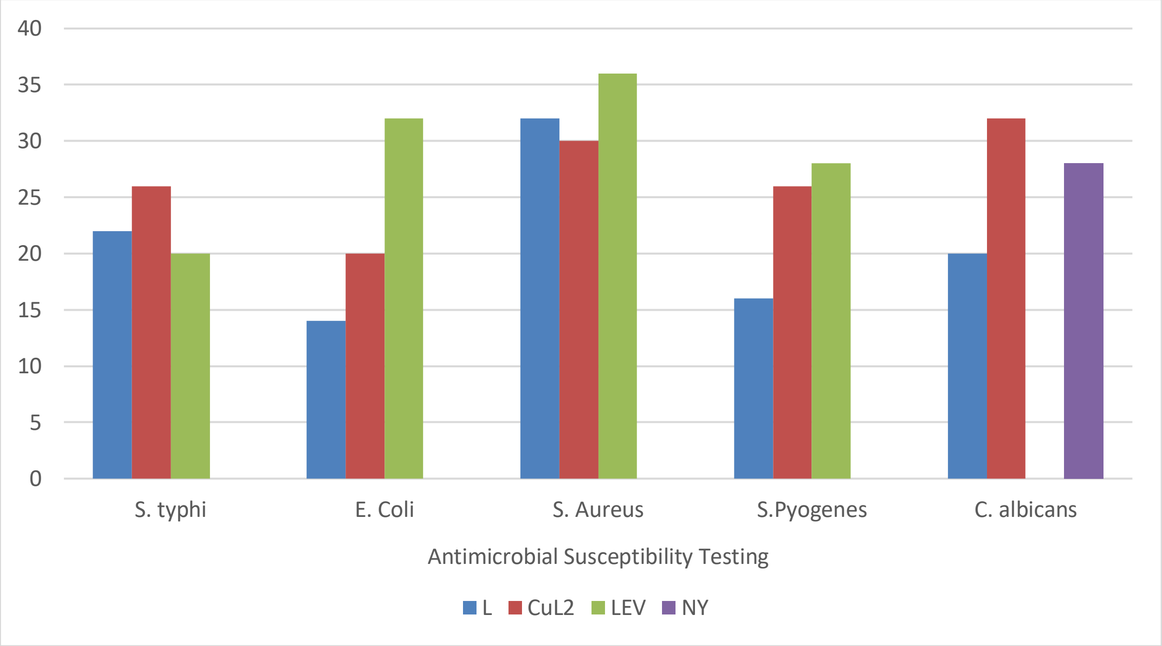


Fig 11: Antimicrobial Susceptibility Bar chart of Schiff base ligand (L) and its Cu(II) complex (CuL_2)

The MIC (Minimum inhibitory concentration) data (Table 9) reveals the Schiff base ligand (L) and its copper (II) complex (CuL_2) exhibit **broad-spectrum antimicrobial activity** against the tested bacterial and fungal strains. However, a **distinct improvement in activity is observed upon complexation with Cu (II)**, as evidenced by the generally lower MIC values of CuL_2 compared with the free ligand. The Schiff base ligand (L) displayed the **highest sensitivity** against *Staphylococcus aureus* (MIC = 62.5 µg/mL), indicating strong inhibition toward Gram-positive bacteria. In contrast, higher MIC values (500 µg/mL) were recorded for *E. coli* and *S. pyogenes*, suggesting lower susceptibility of these organisms to the free ligand (Fig 12a). The moderate inhibition against *S. typhi* and *C. albicans* (250 µg/mL each) indicates partial effectiveness against Gram-negative and fungal species. Upon complexation with copper(II), there was a **twofold reduction in MIC values** for most organisms, signifying enhanced inhibitory potency. The CuL_2 complex exhibited MIC values of 125 µg/mL against *S. typhi* and *C. albicans*, and 250 µg/mL against *E. coli* and *S. pyogenes*, while maintaining the same MIC (62.5 µg/mL) against *S. aureus*. This demonstrates that chelation substantially increases the biological efficacy of the ligand, likely by improving its lipophilicity and cell membrane permeability (Jayarajan et al, 2010).

Table 9: Minimum Inhibitory Concentration analysis data for L and CuL_2

Test organisms **Formulations/Concentrations (µg/ml)**

Compounds	***S. typhi***	***E. Coli***	***S. Aureus***	***S.Pyogenes***	***C. albicans***
L	250	500	62.5	500	250
CuL_2	125	250	62.5	250	125

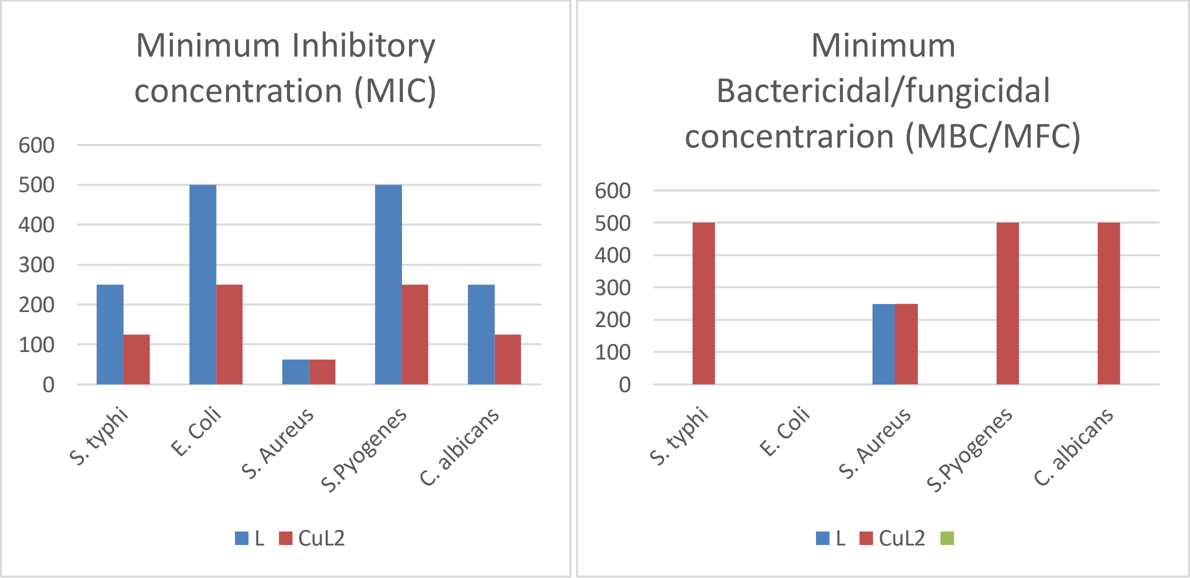
This observation aligns with the **chelation theory** (Lever, 1984; Mohamed *et al*,, 2006), which explains that complex formation reduces the polarity of the metal ion and enhances its ability to penetrate microbial cell membranes. Consequently, the Cu(II) complex becomes more effective at interacting with intra-cellular bio-molecules, leading to greater antimicrobial activity. Overall, the results confirm that **metal complexation significantly enhances the antimicrobial potential** of the Schiff base ligand, with CuL_2 showing promising activity comparable to standard antimicrobial agents in similar studies.

The MBC/MFC activities of the Schiff base ligand (L) and its copper(II) complex (CuL_2). The ligand (L) exhibited limited bactericidal activity(Fig 12b), showing an MBC value of 250 µg/mL only against *S. aureus*, while no lethal effect was observed against *S. typhi*, *E. coli*, *S. pyogenes*, and *C. albicans* up to the highest tested concentration (>500 µg/mL). Complexation with copper(II) significantly broadened the antimicrobial spectrum of the ligand. The Cu(II) complex (CuL_2) demonstrated bactericidal/fungicidal activity against *S. aureus* (MBC = 250 µg/mL), *S. typhi* (MBC = 500 µg/mL), *S. pyogenes* (MBC = 500 µg/mL), and *C. albicans* (MFC = 500 µg/mL), whereas *E. coli* remained resistant (MBC > 500 µg/mL). The enhanced activity of the complex compared to the free ligand is attributed to the chelation effect, which increases lipophilicity and facilitates penetration through the microbial cell membrane, leading to greater disruption of essential biological processes (Table 10). Among all the tested organisms, *S. aureus* was the most susceptible, with both the ligand and its Cu(II) complex exhibiting bactericidal activity at 250 µg/mL. The broader activity of CuL_2 against both Gram-positive and Gram-negative bacteria, as well as *C. albicans*, indicates that metal coordination enhances the antimicrobial potential of the ligand.

Table 10: Minimum Bactericidal/Fungicidal Concentration data for L and CuL_2

Test organisms **Formulations/Concentrations (µg/ml)**

Compounds	***S. typhi***	***E. Coli***	***S. Aureus***	***S.Pyogenes***	***C. albicans***
L	ND	ND	250	ND	ND
CuL_2	500	ND	250	500	500



(a) (b)

Fig 12; (a)Minimum Inhibitory Concentration MIC (b) Minimum bactericidal /Fungicidal concentration (MBC/MFC)

CONCLUSION

A novel Schiff base ligand derived from 4-acyl pyrazolone and its Cu(II) complex were successfully synthesized and thoroughly characterized using spectroscopic and analytical methods. The results from IR, UV–Vis, ^1H NMR, and GC–MS analyses confirmed the formation of the azomethine linkage and coordination through the nitrogen and oxygen donor atoms, while elemental and conductivity measurements verified the proposed stoichiometry and non-electrolytic nature of the Cu(II) complex. Observed spectral shifts, enhanced thermal stability, and the emergence of M–O and M–N bands collectively evidenced strong metal–ligand bonding. Biological evaluation revealed that coordination with copper(II) markedly enhanced the antimicrobial properties of the ligand. As supported by chelation theory, complexation reduces the metal ion's polarity, increases lipophilicity, and facilitates membrane permeability—thereby strengthening interactions with microbial biomolecules. The free ligand displayed limited activity, while the Cu(II) complex exhibited broader antimicrobial and antifungal efficacy, particularly against *Staphylococcus aureus*.

Overall, these findings demonstrate that metal complexation not only stabilizes the Schiff base framework but also significantly enhances its bioactivity. The synthesized Cu(II) complex shows promising broad-spectrum antimicrobial potential, indicating that pyrazolone-derived Schiff base metal complexes could serve as valuable scaffolds for the design of new antimicrobial agents

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