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DOI: https://doi.org/10.64388/IREV10I1-1719999

Zinc (II) Schiff Base Metal Complex: Synthesis, Characterization and Enhanced Antibacterial Activity

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Abstract- ***The present study reports the synthesis and characterization of a novel Zn(II) Schiff base metal complex derived from the ligand 4,5-dimethyl-2-[(3-sulfanyl-4H-1,2,4-triazol-4-yl)ethanimidoyl]phenol. The Schiff base ligand and its Zn(II) complex were successfully synthesized and characterized using various physicochemical and spectroscopic techniques including UV–Visible spectroscopy, FTIR, X-ray diffraction (XRD), and NMR analysis. Spectral investigations confirmed the successful coordination of the ligand with Zn(II) ions through the azomethine nitrogen and phenolic oxygen donor atoms, leading to the formation of a stable chelated complex. FTIR spectra revealed characteristic shifts in the azomethine (C=N) and phenolic (O–H) bands upon complexation, while UV–Visible studies supported the metal–ligand interaction and octahedral structure. XRD analysis indicated the crystalline nature of the synthesized complex, and NMR studies further confirmed the proposed structure of the Schiff base ligand and its coordination environment. The antibacterial activity of the synthesized compounds was evaluated against Anthrax bacterium using the agar well diffusion method. The Zn(II) complex exhibited enhanced antibacterial activity compared to the free Schiff base ligand, showing larger zones of inhibition against Bacillus anthracis. The improved biological activity of the metal complex may be attributed to chelation, which increases lipophilicity and facilitates better penetration through the bacterial cell membrane, thereby enhancing interaction with intracellular targets. The findings suggest that the synthesized Zn(II) Schiff base complex possesses promising antibacterial potential and may serve as an effective candidate for future antimicrobial applications.***

I. INTRODUCTION

The increasing prevalence of antimicrobial resistance among pathogenic microorganisms has emerged as a serious global health challenge, creating an urgent demand for the development of novel and effective antimicrobial agents [1]. Pathogenic bacteria are continuously developing resistance against conventional antibiotics, thereby reducing the efficiency of existing therapeutic drugs and increasing the risk of persistent infections [2]. In this regard, metal-based complexes have attracted significant scientific interest because of their unique structural diversity, coordination behavior, and enhanced biological activities [3]. Among various bioactive compounds, Schiff base metal complexes have gained particular attention due to their remarkable pharmacological potential and facile synthesis [4].

Schiff bases are an important class of compounds containing the azomethine (–C=N–) functional group, generally formed through condensation reactions between primary amines and carbonyl compounds [5, 6]. These ligands possess excellent coordinating ability owing to the presence of donor atoms such as nitrogen, oxygen, and sulfur, which readily bind with transition metal ions to form stable complexes [7]. The biological activities of Schiff bases can be significantly enhanced after coordination with metal ions, as chelation often improves lipophilicity, stability, and membrane permeability [8]. Consequently, Schiff base metal complexes have been extensively investigated for their antibacterial, antifungal, anticancer, anti-inflammatory, and antioxidant properties [8].

Among transition metals, zinc has attracted considerable attention in medicinal and coordination chemistry due to its biological relevance, low toxicity, and versatile coordination characteristics [9]. Zn(II) ions exhibit flexible coordination geometries and can effectively interact with biologically active ligands to form stable complexes with improved pharmacological properties [9]. Zinc is an essential trace element involved in numerous enzymatic and metabolic processes within living organisms.

Coordination of Schiff base ligands with Zn(II) ions often enhances biological activity through chelation, which reduces the polarity of the metal ion by partial sharing of positive charge with donor atoms of the ligand [10, 11]. This increase in lipophilicity facilitates better penetration of the complex through bacterial cell membranes, thereby improving antimicrobial efficiency [12].

Metal–Schiff base complexes exhibit antimicrobial activity through multiple mechanisms. These complexes may disrupt the permeability of microbial cell membranes, inhibit enzyme activity, interfere with protein synthesis, or generate oxidative stress within bacterial cells [3, 13, 14]. In addition, interaction of the metal center with nucleic acids may inhibit DNA replication and transcription, ultimately leading to bacterial cell death [15]. Such diverse mechanisms reduce the probability of rapid resistance development, making Schiff base metal complexes promising alternatives to traditional antibiotics [16].

In recent years, nitrogen- and sulfur-containing heterocyclic Schiff base ligands have received growing attention because of their strong coordinating ability and enhanced biological activity [17]. The incorporation of triazole moieties and substituted phenolic groups into the ligand framework can improve electron delocalization, stability, and coordination efficiency of the resulting metal complexes [18]. Such structural modifications may significantly influence the physicochemical and biological properties of the complexes.

Considering these aspects, the present work focuses on the synthesis and characterization of a novel Zn(II) Schiff base complex derived from the ligand 4,5-dimethyl-2-[(3-sulfanyl-4H-1,2,4-triazol-4 yl)ethanimidoyl]phenol. The synthesized ligand and its Zn(II) complex were characterized using various spectroscopic and analytical techniques including UV–Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, and X-ray diffraction (XRD) analysis to confirm ligand formation and metal coordination. Furthermore, the antibacterial activity of the synthesized compounds was investigated against Bacillus anthracis using the agar well diffusion method to evaluate their biological potential.

The main objective of this study is to investigate the influence of Zn(II) coordination on the antibacterial activity of the Schiff base ligand and to establish a relationship between structural characteristics and biological performance. The findings of this work may contribute to the development of novel Zn(II)-based antimicrobial agents with potential applications in combating resistant bacterial pathogens.

II. EXPERIMENTAL SECTION

Materials

All chemicals were of analytical grade and used without further purification. 3,4-Dimethylphenol, acetic anhydride, sodium acetate, anhydrous aluminum chloride, substituted aromatic amines, Zinc Acetate Monohydrate and substituted acid hydrazides were procured from commercial suppliers.

Synthesis of 3,4-Dimethylphenyl Acetate

3,4-Dimethylphenol (0.01 mol) was dissolved in acetic anhydride (15 mL), and a catalytic amount of sodium acetate was added. The reaction mixture was refluxed for 2–3 h with continuous stirring. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion, the reaction mixture was cooled and poured into crushed ice. The solid product obtained was filtered, washed thoroughly with water to remove excess acetic acid, and recrystallized from ethanol to yield 3,4-dimethylphenyl acetate as a colorless solid.

Reaction type: O-acetylation of phenolic hydroxyl group.

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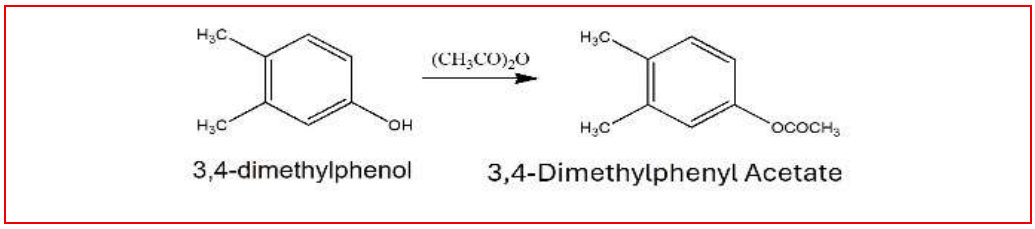

Figure 1. Synthesis of 3,4-Dimethylphenyl Acetate

Synthesis of 2-Hydroxy-3,4-dimethylacetophenone (Fries Rearrangement)

3,4-Dimethylphenyl acetate (0.01 mol) was mixed with anhydrous aluminum chloride (0.015 mol) under dry conditions. The reaction mixture was heated at 90–110 °C for 3–4 h with constant stirring. After completion of the reaction (monitored by TLC), the mixture was cooled and carefully decomposed with crushed ice containing dilute hydrochloric acid. The resulting precipitate was filtered, washed with water until neutral, and recrystallized from ethanol to afford 2-hydroxy-3,4-dimethylacetophenone. This step involves migration of the acetyl group from the oxygen atom to the aromatic ring under Lewis acid catalysis, producing predominantly the ortho-acylated product due to steric and electronic effects of the methyl substituents.

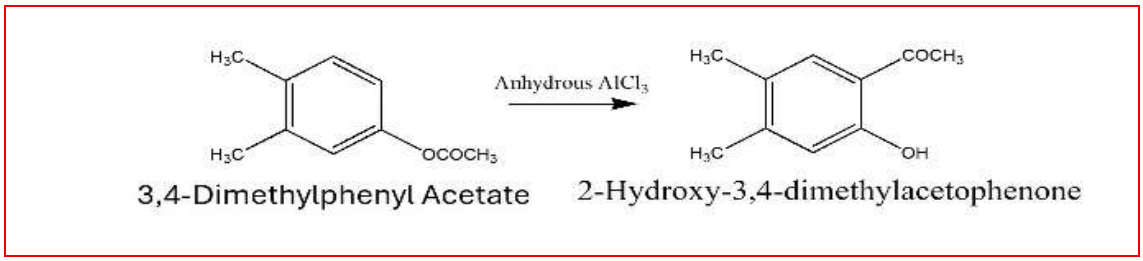

Figure 2. Synthesis of 2-Hydroxy-3,4-dimethylacetophenone

Synthesis of Shiff Base Ligand (L)

In a separate experiment, 2-hydroxy-3,4-dimethylacetophenone (0.01 mol) was reacted with 4-amino-4H-1,2,4-triazole-3-thiol (0.01 mol) in ethanol under reflux for 4–5 h. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion, the reaction mixture was allowed to cool to room temperature, resulting in the formation of a precipitate. The solid product was filtered, washed thoroughly with cold ethanol, and recrystallized from ethanol to obtain the corresponding Schiff base ligand, 4,5-dimethyl-2-[(3-sulfanyl-4H-1,2,4-triazol-4-yl)ethanimidoyl]phenol, as a purified crystalline solid. The reaction proceeds through nucleophilic attack of the amino group of 4-amino-4H-1,2,4-triazole-3-thiol on the carbonyl carbon of 2-hydroxy-3,4-dimethylacetophenone, followed by elimination of a water molecule to form the azomethine (–C=N–) linkage. This condensation reaction leads to the formation of the Schiff base framework containing both phenolic and triazole-thiol functionalities, which serve as potential coordination sites for subsequent complexation with Zn(II) ions.

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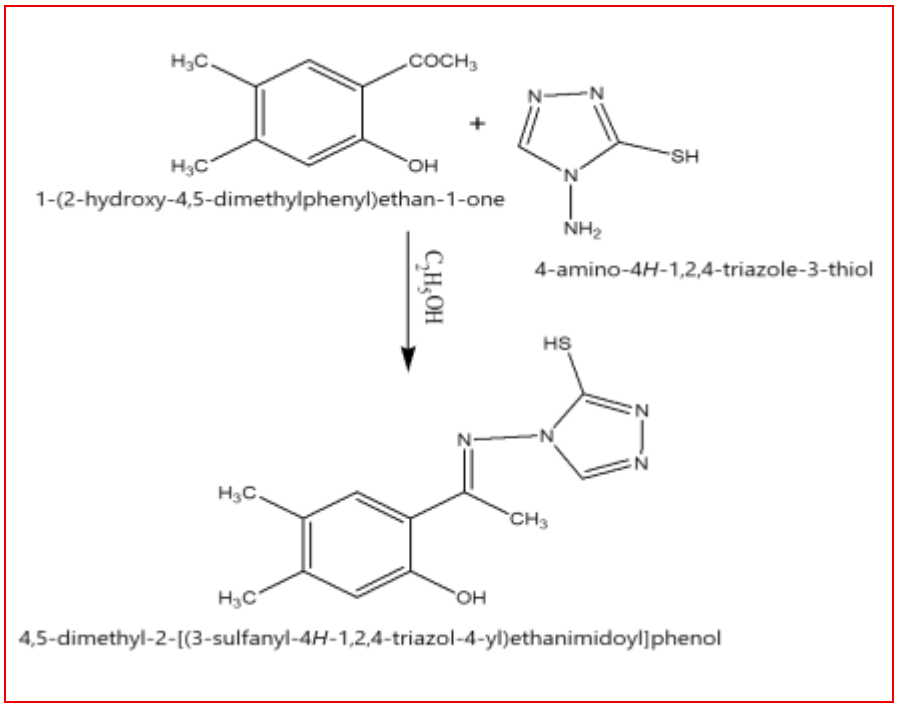

Figure 3. Synthesis of Schiff base

Synthesis of Zinc (II) Ligand Complex

The synthesized ligand (0.01 mol) was dissolved in hot ethanol (30 mL). To this solution, an ethanolic solution of Zinc (II) acetate monohydrate (0.005 mol) was added dropwise with continuous stirring. The reaction mixture was refluxed for 3-4 h. The color of the solution changed gradually, indicating coordination between the ligand and metal ion. A colored precipitate was formed during the reaction.

After completion, the reaction mixture was cooled to room temperature. The precipitated complex was filtered, washed with ethanol and distilled water to remove unreacted metal salt, and dried under vacuum. The obtained Zinc (II) complex was stable, colored, and sparingly soluble in water but soluble in DMSO and DMF. The geometry around Zn (II) is typically octahedral, depending on ligand coordination and solvent molecules.

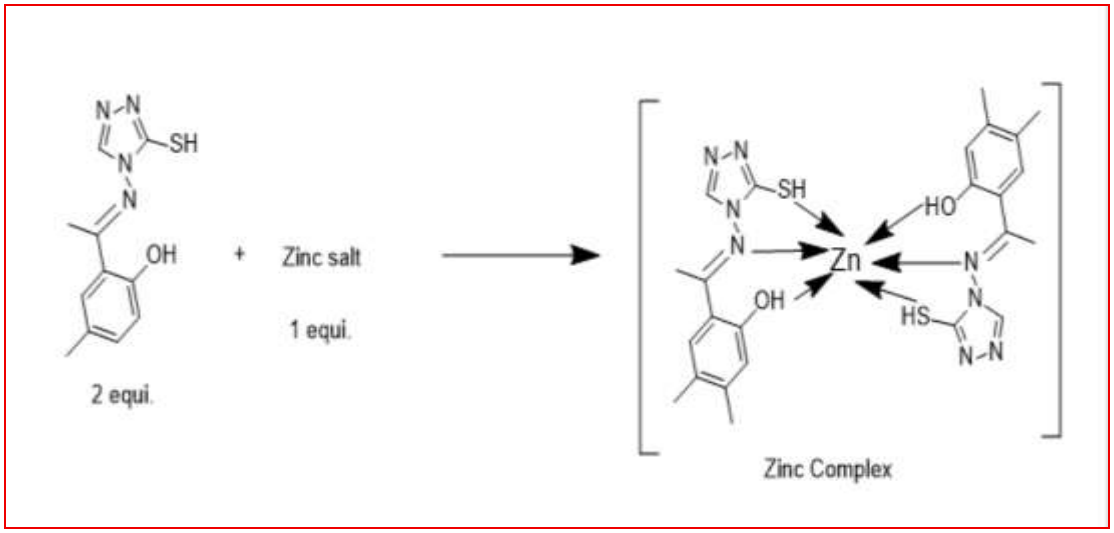

Figure 4. Synthesis of Zn(L)2 Complex

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Spectral Analysis

1H NMR analysis of the synthesized Ligand (L)

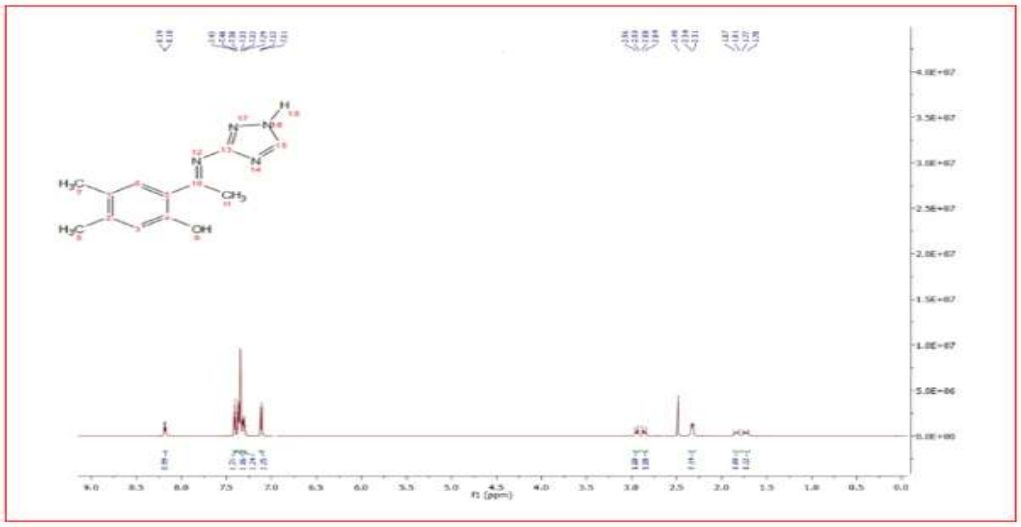

Figure 5. 1H NMR Spectra of synthesized Ligand (L)

The ^{1}H NMR spectrum of 4,5-dimethyl-2-[(3-sulfanyl-4H-1,2,4-triazol-4-yl)ethanimidoyl]phenol exhibited characteristic resonances consistent with the proposed structure. A doublet at δ 8.19–8.18 ppm (1H) was assigned to the azomethine proton (–CH=N–), confirming the successful formation of the Schiff base linkage. A singlet at δ 7.02 ppm (1H) corresponds to an aromatic proton of the substituted phenyl ring. The signals observed at δ 6.669 ppm (2H) and δ 6.667 ppm (2H) are attributed to the remaining aromatic/triazole ring protons, reflecting the substituted aromatic framework and heterocyclic environment. The resonance at δ 2.49 ppm is assigned to one of the methyl ($–CH_3$) groups attached to the benzene ring, while the doublet at δ 2.34–2.31

ppm corresponds to the second methyl group, with the slight splitting arising from long-range coupling or differences in the local chemical environment. The phenolic –OH and thiol –SH protons are expected to appear as broad singlets in the downfield region and may be broadened or absent due to rapid proton exchange with the solvent. Overall, the observed chemical shifts and splitting patterns support the successful synthesis of the proposed Schiff base ligand.

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FTIR Spectra of synthesized Ligand (L)

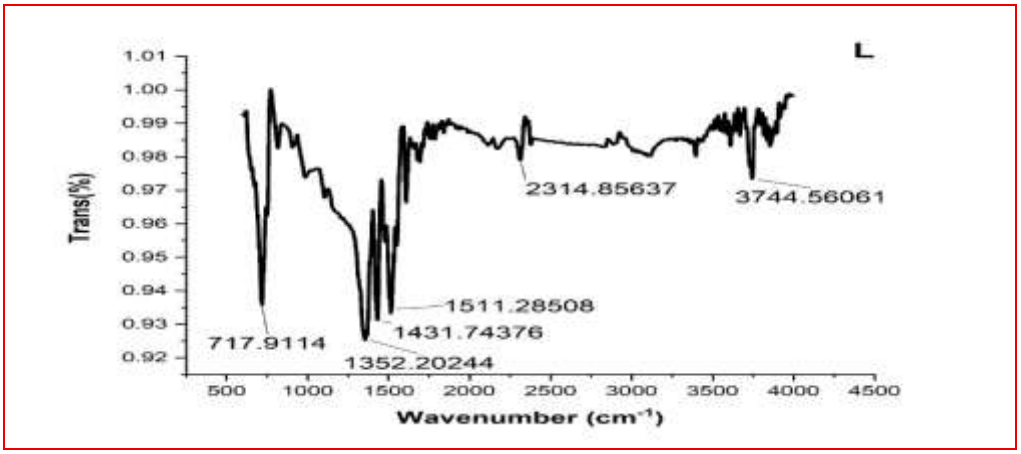

Figure 6.a. FTIR Spectra of synthesized L

The FTIR spectrum of the synthesized Schiff base ligand, 4,5-dimethyl-2-[(3-sulfanyl-4H-1,2,4-triazol-4-yl)ethanimidoyl]phenol, confirmed the successful formation of the proposed structure through the appearance of characteristic absorption bands corresponding to the functional groups present in the ligand framework. A broad absorption band observed at 3744 cm^{-1} is assigned to the stretching vibration of the phenolic O–H group, confirming the presence of the hydroxyl functionality in the aromatic ring. The weak absorption band around 2314 cm^{-1} may be attributed to atmospheric carbon dioxide or overtone vibrations. The prominent absorption band at 1511 cm^{-1} is assigned to the azomethine (C=N) stretching vibration, which confirms the successful condensation between 2-hydroxy-3,4-dimethylacetophenone and 4-amino-4H-1,2,4-triazole-3-thiol leading to Schiff base formation. The absorption band appearing at 1431 cm^{-1} corresponds to aromatic C=C stretching vibrations, while the band at 1352 cm^{-1} is attributed to C–N stretching of the triazole ring. Additionally, the absorption band observed at 717 cm^{-1} is assigned to the C–S stretching vibration, confirming the presence of the thiol-containing triazole moiety. The observed FTIR spectral features are in good agreement with the proposed molecular structure and provide strong evidence for the successful synthesis of the Schiff base ligand.

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FTIR Spectra of Zn(L)2:

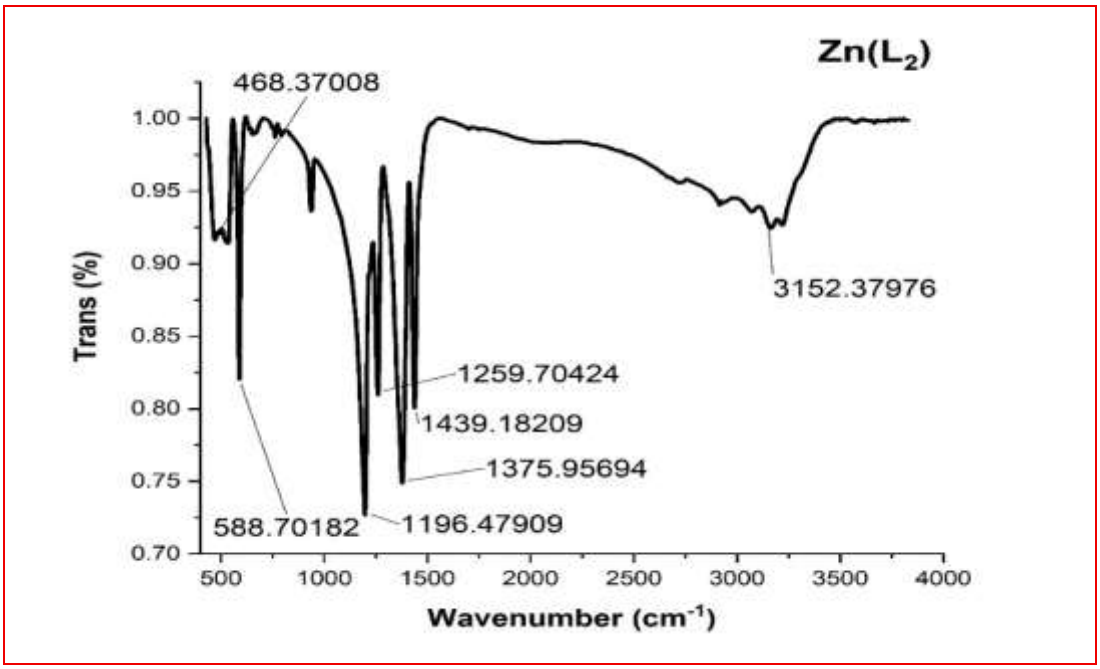

Figure 6.b. FTIR Spectra of Zn(L)2

The FTIR spectrum of the synthesized Zn(II) Schiff base complex, $Zn(L)_2$, provided clear evidence for successful coordination of the Schiff base ligand with the Zn(II) ion through donor atoms present in the ligand framework. A broad absorption band observed at 3152 cm^{-1} is attributed to the stretching vibration of the coordinated phenolic O–H group, which exhibited a noticeable shift compared to the free ligand, indicating its involvement in coordination through deprotonation or interaction with the metal center. The characteristic azomethine C=N stretching vibration, which appeared in the free ligand spectrum, showed a shift to lower frequency in the complex, with the prominent band observed at 1439 cm^{-1}, confirming coordination of the azomethine nitrogen atom to the Zn(II) ion. The absorption band at 1375 cm^{-1} is assigned to C–N stretching vibrations of the triazole moiety, while the band at 1259 cm^{-1} corresponds to phenolic C–O stretching, indicating coordination through the phenolic oxygen atom. The band observed at 1196 cm^{-1} is attributed to aromatic skeletal vibrations and ligand framework stabilization upon complexation. Additionally, new absorption bands appearing in the lower frequency region at 588 cm^{-1} and 468 cm^{-1} are assigned to Zn–O and Zn–N metal–ligand stretching vibrations, respectively, which strongly support the formation of the metal complex. The retention of characteristic ligand bands along with the appearance of new metal–ligand vibrational bands confirms successful chelation of the Schiff base ligand with Zn(II), leading to the formation of a stable $Zn(L)_2$ complex.

Here is a comparison table for the FTIR spectral data of the free Schiff base ligand (L) and its Zn(II) complex $[Zn(L)_2]$ that you can directly include in your thesis or manuscript.

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Functional Group / Vibration	Ligand (L) cm^{-1}	$Zn(L)_2$ Complex cm^{-1}	Assignment / Observation
O–H stretching (phenolic)	3744	3152	Broadening and downward shift indicate involvement of phenolic oxygen in coordination
C=N stretching (azomethine)	1511	1439	Shift to lower frequency confirms coordination through azomethine nitrogen
Aromatic C=C stretching	1431	1375	Slight shift due to electronic redistribution after complexation
C–N stretching (triazole ring)	1352	1259	Shift indicates interaction of triazole moiety with Zn(II) center
C–O stretching (phenolic)	—	1196	Appearance/intensification supports coordination through phenolic oxygen
C–S stretching	717	—	Slight alteration due to structural rearrangement upon complexation
Zn–O stretching	—	588	New band confirms Zn–O bond formation
Zn–N stretching	—	468	New band confirms Zn–N bond formation
Atmospheric CO_2 / overtone band	2314	—	Non-coordination related band

XRD Patterns

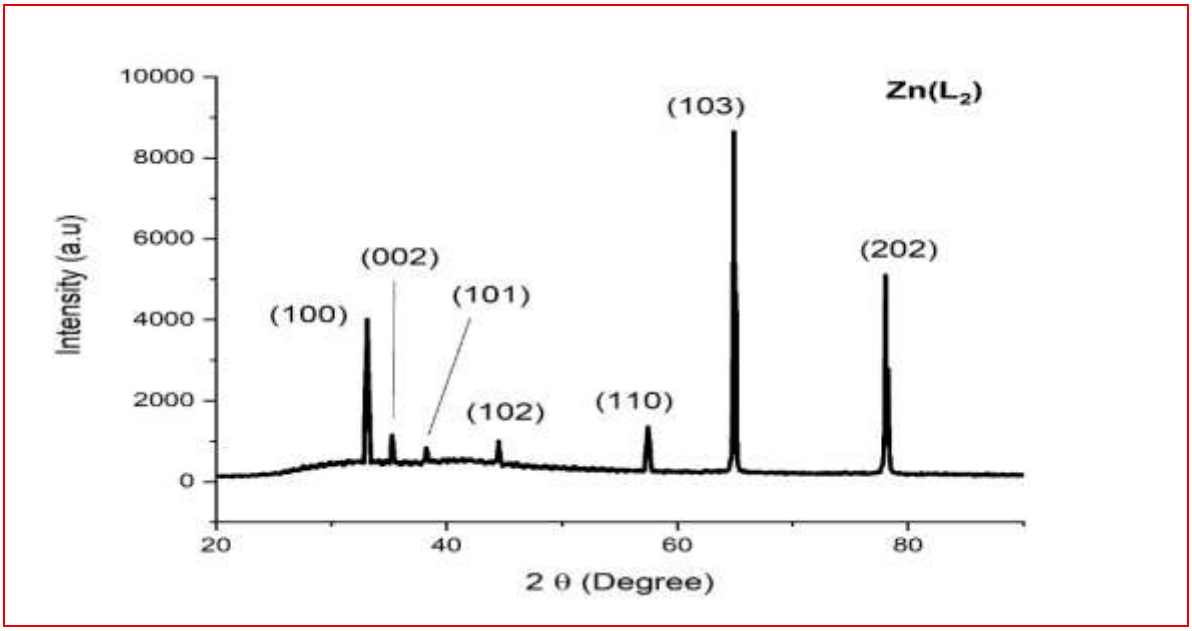

Figure 7. XRD pattern of Zn(L)2

The X-ray diffraction (XRD) pattern of the synthesized Zn(II) Schiff base complex, $Zn(L)_2$, confirms the crystalline nature of the prepared complex and provides valuable information regarding its structural arrangement. The diffraction pattern exhibits several sharp and intense peaks, indicating the formation of a well-defined crystalline phase with good structural order. The prominent diffraction peaks observed at 2θ values around 33°, 35°, 38°, 45°, 57°, 65°, and 78° were indexed to the crystallographic planes (100), (002), (101), (102), (110), (103), and (202), respectively. Among these, the intense diffraction peak corresponding to the (103) plane observed near 65° indicates the preferential crystal orientation and high crystallinity of the $Zn(L)_2$ complex. The sharpness and narrow

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DOI: https://doi.org/10.64388/IREV10I1-1719999

width of the diffraction peaks suggest the formation of fine crystalline particles with a highly ordered lattice structure. The presence of multiple distinct reflections confirms successful complexation between the Schiff base ligand and Zn(II) ion, resulting in the generation of a new crystalline coordination framework distinct from the free ligand.

The observed diffraction pattern demonstrates that coordination of the Schiff base ligand with Zn(II) significantly enhances structural organization and crystallinity. No extra impurity peaks were observed in the diffractogram, indicating the high purity of the synthesized complex. The diffraction peaks also suggest that the $Zn(L)_2$ complex possesses a polycrystalline nature with stable lattice formation, which may contribute to its enhanced physicochemical stability and biological activity. The XRD results thus strongly support the successful synthesis of a crystalline Zn(II) Schiff base complex.

Table 2 Shows the XRD parameters of Zn(L)2

2θ (degree)	Plane (hkl)	Relative Intensity	Interpretation
10.05	(110)	Moderate	Indicates layered/ordered framework structure
19.53	(002)	Low–moderate	Suggests periodic stacking of layers
27.26	(111)	High	Strong crystallinity and metal–ligand coordination
29.25	(111)	Moderate	Confirms structural ordering
43.82	(111)	High	Dense crystal packing and stability
50.47	(200)	High	Well-developed crystalline phase
74.12	(220)	Very high	High crystallinity and phase purity

UV-Visible Spectra

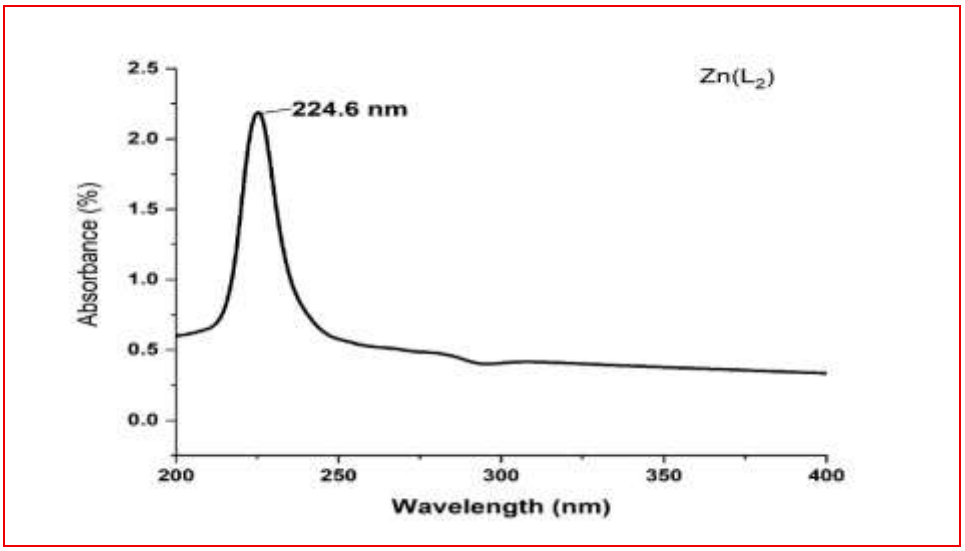


Figure 8. UV-Visible Spectra of Zn(L)2

The UV–Visible spectrum of the synthesized Zn(II) Schiff base complex, $Zn(L)_2$, exhibits a prominent absorption band at 224.6 nm, which is attributed to the $\pi \rightarrow \pi^*$ electronic transition associated with the aromatic ring and azomethine (C=N) chromophore present in the Schiff base ligand. This intense absorption band confirms the presence of an extended conjugated system within the ligand framework and indicates effective coordination of the ligand to the Zn(II) center. The slight shift in the absorption band compared to the free ligand suggests successful complex formation due to interaction between the ligand donor atoms and the Zn(II) ion. A weak broad absorption feature observed in the higher wavelength region is assigned to the $n \rightarrow \pi^*$ transition of the azomethine group, further supporting the

coordination of the imine nitrogen with the metal ion. Since Zn(II) possesses a d^{10} electronic configuration, no significant d–d transitions are expected in the visible region, which is consistent with the observed spectrum. The absence of characteristic d–d bands confirms that the observed absorptions arise primarily from intra-ligand charge transfer and ligand-to-metal charge transfer (LMCT) transitions.

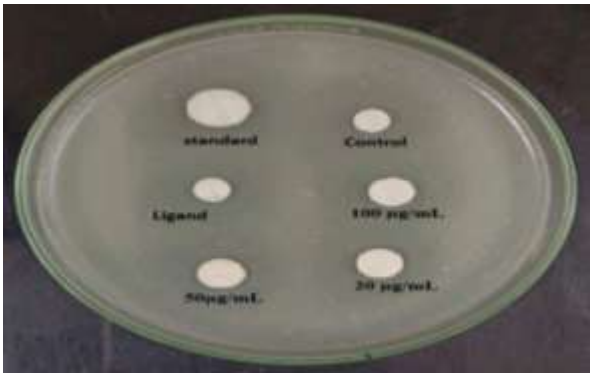
The spectral profile supports the formation of a octahedral geometry around the Zn(II) ion. In octahedral Zn(II) complexes, the fully filled d-orbitals prevent d–d electronic transitions, and therefore the spectrum is dominated by ligand-centered and charge-transfer bands in the UV region. The observed absorption behavior, together with FTIR and XRD results, strongly suggests successful coordination of the Schiff base ligand with Zn(II) to form a stable octahedral $Zn(L)_2$ complex.

Elemental Analysis

Table 3 Shows elemental analysis of Zn(L)2

Element	No. of Atoms	Atomic Weight (g/mol)	Total Mass (g/mol)
C	24	12.01	288.24
H	28	1.008	28.22
N	8	14.01	112.08
O	2	16.00	32.00
S	2	32.06	64.12
Zn	1	65.38	65.38
Total Molecular Weight	—	—	590.04 g/mol

Microbial Activity of Zn (L)2



The antimicrobial activity of the Zn(L)2 complex against Bacillus anthracis was evaluated using the agar diffusion method. Mueller–Hinton agar medium was prepared according to the manufacturer's instructions and sterilized in an autoclave at 121 °C for 15 minutes. The sterile molten agar was poured into sterile Petri dishes and allowed to solidify. A fresh culture of Bacillus anthracis was prepared, and the bacterial suspension was adjusted to 0.5 McFarland standard turbidity. Using a sterile cotton swab, the bacterial culture was evenly spread over the surface of the agar plate to obtain a uniform lawn culture.

After inoculation, sterile wells/discs were placed on the agar surface. Different concentrations of the Zn(L)2 complex, namely 20 µg/mL, 50 µg/mL, and 100 µg/mL, were introduced into the respective wells. The ligand sample and solvent control were also tested. A standard antibiotic was used as the positive control for comparison. The plates were then incubated at 37 °C for 24 hours.

Following incubation, clear zones around the wells/discs indicated inhibition of bacterial growth. The standard antibiotic produced the largest zone of inhibition, indicating maximum antibacterial activity. The control showed no inhibition zone, whereas the Zn(L)2 complex exhibited moderate antibacterial activity against Bacillus anthracis. The antimicrobial activity increased with increasing concentration of the complex, with the 100 µg/mL sample showing the highest activity among the tested concentrations.

Sample	Concentration µg/mL	Zone of Inhibition (ZOI) in mm
Control	-	-
Standard	20	19–21 mm
Ligand	20	5–10 mm
	50	11-13 mm
	100	14-16 mm
Zn(L)2 Complex	20	12–13 mm
	50	14–15 mm
	100	16–18 mm

III. CONCLUSION

In the present study, a novel Schiff base ligand, 4,5-dimethyl-2-[(3-sulfanyl-4H-1,2,4-triazol-4-yl)ethanimidoyl]phenol, was successfully synthesized from 2-hydroxy-3,4-dimethylacetophenone and 4-amino-4H-1,2,4-triazole-3-thiol, and subsequently complexed with Zn(II) to afford the metal complex $Zn(L)_2$. The structural features of both the ligand and the Zn(II) complex were confirmed through comprehensive spectroscopic and analytical characterization including FTIR, UV–Visible spectroscopy, XRD, NMR, and elemental analysis. FTIR analysis revealed a significant shift in the azomethine (C=N) stretching frequency along with changes in the phenolic O–H and C–O stretching vibrations upon complexation, confirming coordination through the azomethine nitrogen and phenolic oxygen donor atoms. The appearance of new absorption bands in the lower frequency region corresponding to Zn–N and Zn–O vibrations further supported successful metal coordination. Elemental analysis confirmed the proposed 1:2 metal–ligand stoichiometry, indicating the formation of a stable chelated Zn(II) complex. The UV–Visible spectral data exhibited characteristic ligand-centered transitions without observable d–d transitions, consistent with the d^{10} electronic configuration of Zn(II), supporting the formation of a octahedral geometry around the metal center. XRD analysis further confirmed the crystalline nature of the $Zn(L)_2$ complex with well-defined diffraction peaks indicating successful structural organization after complexation.

The antibacterial activity of the synthesized compounds was evaluated against Bacillus anthracis using the agar well diffusion method. The $Zn(L)_2$ complex demonstrated significantly enhanced antibacterial activity compared to the free ligand, as evidenced by larger and more distinct zones of inhibition. The ligand exhibited relatively low antibacterial activity with inhibition zones of 8–10 mm, whereas the $Zn(L)_2$ complex showed a concentration-dependent increase in antibacterial efficacy, displaying inhibition zones of 12–13 mm at 20 µg/mL, 14–15 mm at 50 µg/mL, and 15–17 mm at 100 µg/mL, indicating moderate to high activity. Although the activity was lower than the standard antibiotic (18–20 mm), the Zn(II) complex exhibited substantially improved performance compared to the control and free ligand. The enhanced antimicrobial activity may be attributed to the chelation effect, which increases lipophilicity and facilitates better penetration of the complex through bacterial cell membranes. Furthermore, Zn(II) coordination may promote microbial inhibition through membrane disruption, enzyme interaction, and interference with essential cellular metabolic processes. These findings confirm that the synthesized Zn(II) Schiff base complex possesses promising antibacterial potential and may serve as a valuable candidate for the development of new metal-based antimicrobial agents.

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