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Synthesis, Characterization, and Study of Anti-Microbial Properties of Fe (III) Schiff Base Complex Derived from Hydrazone and Benzaldehyde

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Abstract: In this study, a hydrazone Schiff base ligand and its Fe(III) complex were synthesized. The hydrazone ligand was derived from hydrazone and benzaldehyde, and its antimicrobial properties were systematically evaluated. The synthesis of the Schiff base ligand was done by the condensation of 2-(2-aminothiazol-4-yl)acetohydrazide with 2-nitrobenzaldehyde, followed by complexation with $FeCl_3$ in a 1:2 molar ratio. Characterization was achieved using spectroscopic and analytical techniques, including ^{1}H-NMR, FTIR, UV-Vis, and XRD, which confirmed the successful formation of the ligand and its coordination to Fe(III). The ^{1}H-NMR spectrum confirmed the formation of the imine linkage (δ 8.09 ppm), while FTIR and UV-Vis data supported the presence of C=N bonding and metal-to-ligand charge transfer, respectively. XRD showed an amorphous structure consistent with flexible hydrazone linkages. Antimicrobial activity was screened against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes,* and *Candida albicans* using the agar well diffusion method. The Fe(III) complex demonstrated superior inhibitory activity compared to the free ligand, with the most significant enhancements observed against *Staphylococcus aureus* (20 mm vs. 16 mm) and *Salmonella typhi* (20 mm vs. 12 mm). These results suggest that metal coordination substantially enhances the antimicrobial efficacy of Schiff base ligands. The synthesized Fe(III) complex has potential as a lead candidate for the development of novel antimicrobial agents, given the rising issue of multidrug resistance.

I. Introduction

Recently, antimicrobial resistance (AMR) has become a significant threat to public health, rendering common antibiotics less effective against bacterial and fungal infections. This has prompted the search for new therapeutic agents that are less harmful, less toxic, and highly effective.[1] Among these, Schiff bases have gained interest due to their ability to act as multidentate ligands, facilitating the formation of stable metal complexes with various geometries and electronic properties. These molecules were first described by German chemist Hugo Schiff in 1864 and are a class of organic compounds characterised by C=N bonds formed through a condensation reaction between an aldehyde or ketone and a primary imine.[2-4]

There are different derivatives of Schiff bases, one of which is the hydrazone derivative, classified as azomethine compounds and derived from hydrazides, with the general structure $R^1R^2C{=}NNR^3R^4$. The unique structural feature of hydrazones contributes to their physical, structural, chemical, biological, and pharmacological properties.[5] Schiff base hydrazones play a crucial role in discovering new biologically active compounds to combat multidrug-resistant microbial infections.[6] They have been reported to possess promising biological properties, such as antibacterial, antifungal, and anticancer, among others.[7]

However, the complexation of Schiff base compounds with metals has a considerable effect on their chemical composition and the characteristics of the metal complexes formed, as well as the types of metal ions generated, thereby affecting their biological functions and applications.[5] The Schiff base complexes have a diverse range of applications in catalysis, polymer science, and dye production, and exhibit a variety of biological activities, including antimicrobial, anticancer, antioxidant, antimalarial, analgesic, antiviral, antipyretic, and antidiabetic effects.[8] The coordination of these ligands with transition metals, such as iron(III), further enhances their bioactivity by modifying electronic properties, enhancing lipophilicity, and facilitating targeted interactions with biological targets, including DNA or proteins.[9]

The major focus of this study was to synthesise and characterise the Fe(III) Schiff base complex. The ligand and the complex were thoroughly examined using various physicochemical and spectroscopic techniques to elucidate their structures and understand their properties. The antimicrobial evaluations of the ligand and complex were evaluated against five clinical test organisms, including two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*), two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), and a fungal strain (*Candida albicans*).

II. Materials and Methods

In this study, we utilized ethyl-2-amino-4-thiazoleacetate ($C_7H_{10}N_2O_2S$), hydrazine hydrate ($N_2H_4.H_2O$), 2-nitrobenzaldehyde ($C_7H_5NO_3$), Iron chloride ($FeCl_3$), water, ethanol, methanol, and Dimethyl Sulfoxide (C_2H_6OS). All reagents and solvents were of analytical grade and obtained from Sigma–Aldrich Chemie GmbH, Germany, through the local suppliers

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III. Methods

The synthesis of the hydrazone Schiff base ligand and its metal complex was achieved using the condensation method, while the antimicrobial properties were evaluated using the well diffusion method in agar.

Synthesis of the Hydrazone Schiff Base Ligand

To synthesise the ligand, 2-(2-aminothiazol-4-yl)acetohydrazide was first synthesised from a 1:1 mole ratio of ethyl-2-amino-4-thiazoleacetate and hydrazine hydrate. 13.75 mL (0.22 moles) of $N_2H_4.H_2O$ was introduced to 40g (0.22 moles) of ethyl-2-amino-4-thiazoleacetate in 165 mL of ethanol. The mixture was then refluxed in a 500 mL round-bottom flask on a heating mantle for 6 hours. The solution was left to crystallize for 24 hours. The white crystals obtained were filtered and air-dried (Yield, 36.2g, 95%, M.P., 142˚C). After this, the 2-(2-aminothiazol-4-yl)acetohydrazide was reacted with 2-nitrobenzaldehyde via a 1:1 molar ratio.

2-(2-aminothiazol-4-yl)acetohydrazide (10g, 0.058mol) was mixed with 2-nitrobenzaldehyde (10g, 0.06mol) in ethanol (200 mL) and refluxed for 6 hours in a 500 mL round-bottom flask on a heating mantle. The solution was left to crystallise for 24 hours. The light-yellow amorphous powder obtained was filtered, recrystallised, and dried over silica gel in a desiccator.[10] (Yield, 9.8g, 55.2%, M.P., 150˚C)

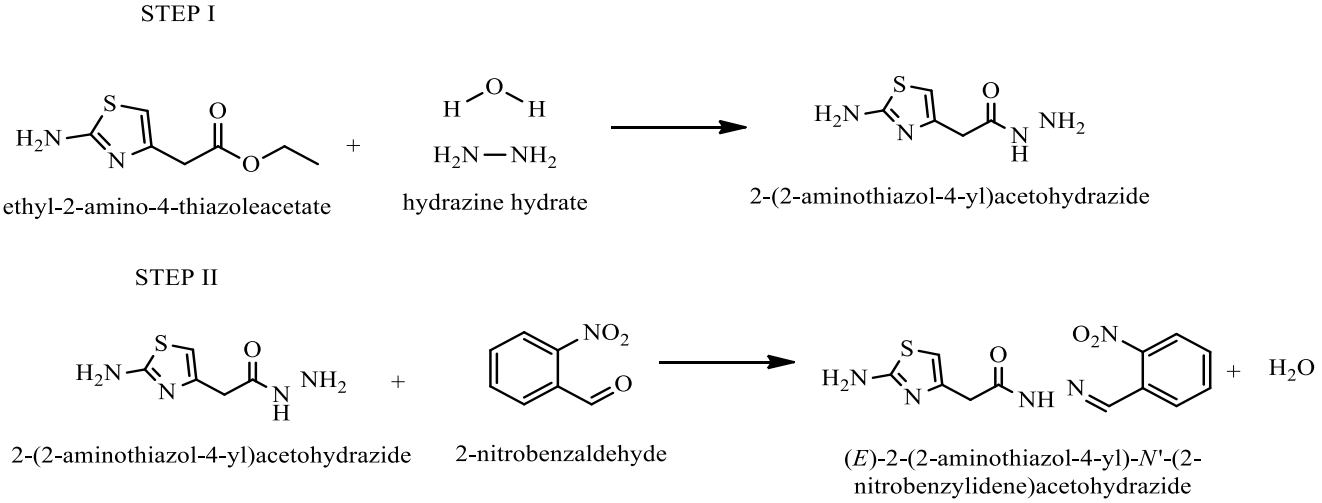

Figure 1: Synthetic route for the Ligand

Synthesis of the Metal Complex

The metal complex was prepared by reacting aqueous solutions of the metal salt ($FeCl_3$) with ethanoic solutions of the ligand in a 1:2 molar ratio.

The ligand (E)-3-((2-(2-(2-aminothiazol-4-yl)acetyl)hydrazono)methyl)-4-nitrobenzene-1-ylium (1.0g, 0.00328 mol) was dissolved in 10 mL of ethanol in a round-bottom flask, and warmed for 30 seconds on a heating mantle. The $FeCl_3$ (0.226g, 0.00164 mol) was dissolved in 10 mL of water in a separate round-bottom flask. The ligand was then added gently, while stirring continuously, to the Fe^{3+} solution, and the mixture was refluxed for 4 hours in a 250 mL round-bottom flask on a heating mantle. The solution was left to crystallise for 24 hours. The brown crystals obtained were filtered and dried over silica gel in a desiccator (Yield, 0.94g, 80%, M.P., 160˚C)

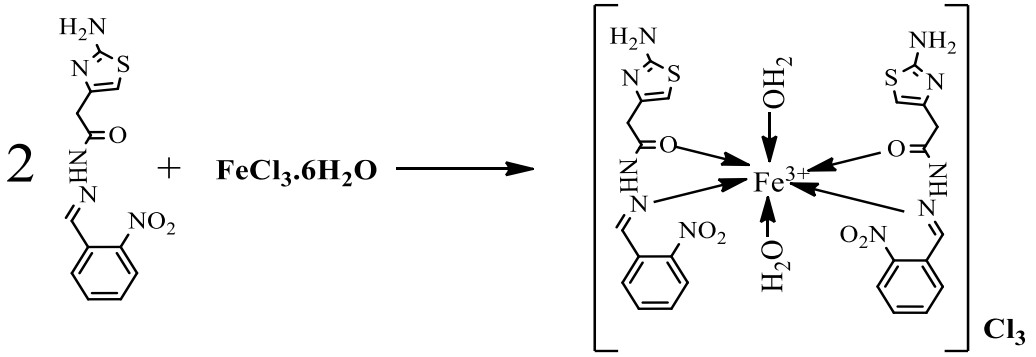

Figure 2: Synthetic Route for the Metal Complex

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Characterisation of the Synthesised Schiff Base Ligand and Metal Complex

The synthesised ligand was characterised by proton Nuclear Magnetic Resonance (^{1}H-NMR), infrared (IR) spectroscopy, XRD, and UV-Vis Spectroscopy, while the complex was characterised by UV-Vis Spectroscopy. All these aided in the structure elucidation

^{1}H-NMR

The ^{1}H spectra (dimethyl sulfoxide DMSO-d6) were recorded on a Bruker Avance 400. The chemical shifts in the ^{1}H are referenced to the residual solvent resonance external TMS (tetramethylsilane). The splitting of proton resonances in the reported ^{1}H NMR spectra is defined as a singlet, doublet, and multiplet[11].

FTIR

The FTIR spectrum of the compounds in Nujol was obtained using an FTIR—8400S Fourier Transform Infrared Spectrophotometer. The spectra were recorded within the frequency range of 4000 cm^{-1} to 400 cm^{-1} using an FT-IR spectrometer (Bruker, model Alpha II) with a detector at a resolution of 4 cm-1 and 32 scans for the sample [12].

Absorption and fluorescence spectroscopy

The UV-visible spectra were obtained using a UV-2500PC Series Spectrometer. 0.01g of the sample was dissolved in DMSO, and the resulting solution was then poured into a cuvette. At first, a blank solution (DMSO) was scanned in the ore to calibrate it. The cuvette containing the sample was then placed in the sample chamber of the UV-Vis spectrophotometer. The scan range used was 800-200nm, and the absorbance range was from -0.100 to -0.300A. The samples were scanned, and the result was recorded.

X-Ray Crystallography/Diffraction

Single-crystal X-ray diffraction data for the ligands and complexes were collected on the CrysAlis CCD diffractometer (CrysAlis CCD). The intensity data were collected at 150(2) K, and the structures were solved by direct methods using SHELXS-97, and each refinement was carried out by full-matrix least-squares on *F2* (SHELXL-97) with anisotropic displacement parameters for non-hydrogen atoms, and the remainder of the hydrogen atoms were placed in specific points and left to ride on the parent atom[13].

Anti-Microbial Screening

Anti-Bacterial Analysis

The synthesized compounds were screened for their antibacterial activity using the Disc Diffusion method[14] against two Gram-positive bacteria (Staphylococcus aureus and Streptococcus pyogenes) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*). The test compounds were prepared at a concentration of 200 μg/mL. Solvent control, that is, DMSO, was also maintained throughout the experiment. Nutrient agar plates were prepared under sterile conditions and incubated overnight to detect contamination. Approximately 0.2 mL of working stock culture was transferred into separate nutrient agar plates and spread thoroughly using a glass spreader. Whatman No. 1 discs (6mm in diameter) were impregnated with the test compounds and dissolved in DMSO (200 μg/mL) for approximately 30 minutes. A commercially available drug disc (Levofloxacin 10 µg/disc) was used as a positive reference standard. Negative controls were also prepared by impregnating a disc of the same size with DMSO solvent. The discs were placed on the inoculated agar plates and incubated at 37 ± 1°C for approximately 18–24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition (mm) against the test organism.

Antifungal Studies

The antifungal activity of the synthesised compounds was examined with a fungal strain, namely *Candida albicans*. The drug amphotericin B was used as a standard. Sabouraud's dextrose agar (SDA) medium was used for the growth of fungi, and testing was done in Sabouraud's dextrose broth (SDB) medium. The subculture and the viable count were carried out by the same procedure as done in antibacterial studies, except the temperature was maintained at 28 ± 1◦C for about 72h

IV. Results and Discussions

^{1}H-NMR

The recorded spectrum in DMSO-d_6 in Figure 3 revealed key structural features consistent with the proposed molecular structure. The imine proton (–CH=N–), a hallmark of Schiff base formation, appears as a distinct singlet at 8.09 ppm, confirming the successful condensation between the aldehyde and hydrazide moieties. Aromatic protons from both the 2-nitrobenzylidene and thiazole rings resonate in the region of 6.3–8.6 ppm. Broad signals at 6.82 ppm (NH_2) and 11.69 ppm (NH) indicate exchangeable protons, consistent with the presence of thiazole-4-amine and hydrazone functionalities. A multiplet at 3.80 ppm (2H) corresponds to the methylene protons of the –CO–CH_2– group adjacent to the thiazole ring.

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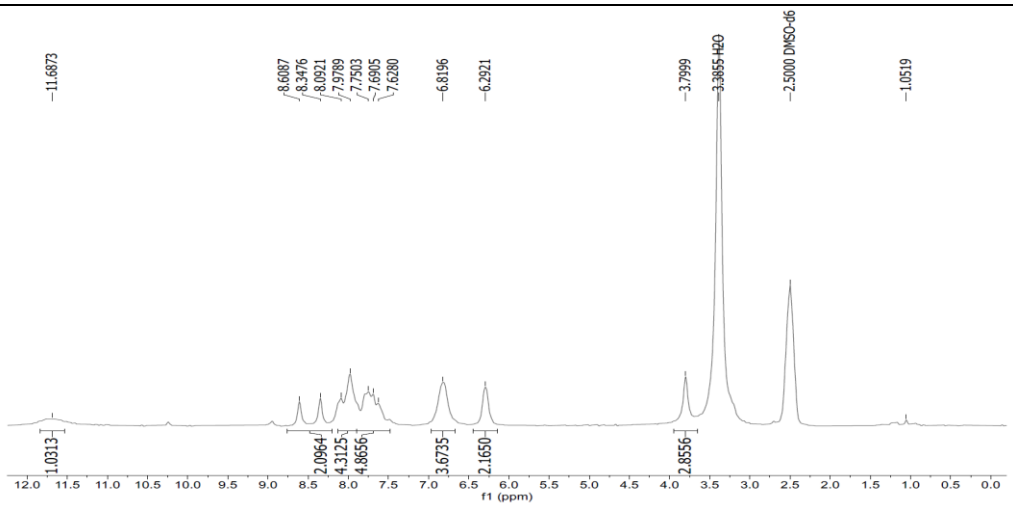

Figure 3: ¹H NMR spectrum of the Ligand (2-[2-[(2-nitrobenzylidene) hydrazinyl]-2-oxoethyl] thiazol-4-amine)

FTIR

The FTIR spectrum in Figure 4 confirms the formation of a Hydrazone Schiff base Ligand, characterized by a strong aromatic C–H stretching peak at 3092.85 cm^{-1} and N–H stretching bands at 2596.03 cm^{-1} and 2478.95 cm^{-1}, indicating the presence of amine functionalities. The C=N stretching at 1301.90 cm^{-1} and nitro group stretching at 1364.28 cm^{-1} verify the imine linkage and retention of the NO_2 group, respectively, while the C–S stretch at 600.05 cm^{-1} confirms the thiazole ring.

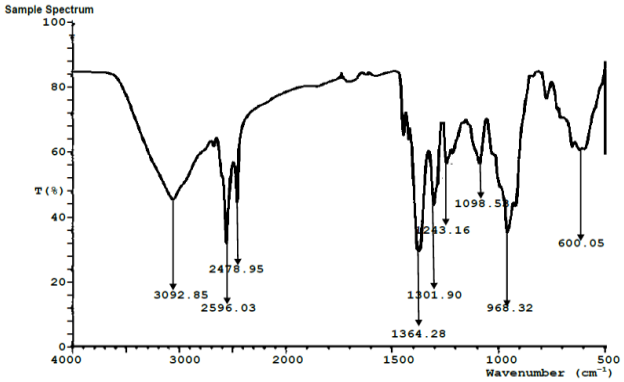

Figure 4: FTIR spectrum of 2-[2-[(2-nitrobenzylidene) hydrazinyl]-2-oxoethyl] thiazol-4-amine

X-Ray Crystallography/Diffraction

The XRD pattern of the Schiff base ligand in Figure 5 revealed a predominantly amorphous structure, characterized by broad, diffuse peaks at 2θ values of 39.41°, 44.00°, and 46.00°, corresponding to the (001), (010), and (110) planes, indicative of a disordered organic framework with flexible hydrazone linkages and steric hindrance.

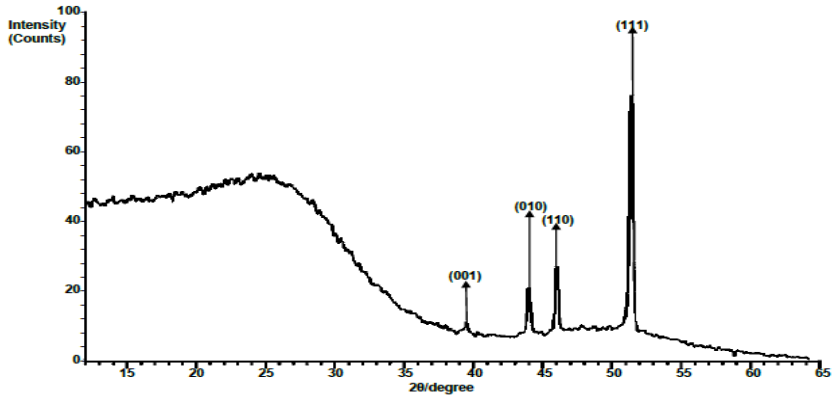

Figure 5: XRD spectrum of 2-[2-[(2-nitrobenzylidene) hydrazinyl]-2-oxoethyl] thiazol-4-amine

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UV-visible of the Ligand and Fe Complex

From the UV-Vis spectrum of the ligand and complex in Figure 6(A and B), the Free ligand exhibited a notable absorption peak at approximately 270–280 nm, with an absorbance of 0.03–0.035, indicative of π→π* transitions within the conjugated system, which reflects the extended conjugation of the aromatic and imine groups in the Schiff base ligand. A secondary peak appeared near 250 nm with an absorbance of about 0.025, suggesting possible n→π* transitions or electronic effects from the hydrazone structure. When complexed, the UV-Vis spectrum of the Fe(III) Schiff base complex exhibits a prominent absorption peak at approximately 230 nm with an absorbance of 0.02, indicating a strong π→π* transition associated with the aromatic system of the benzaldehyde-derived ligand. Additionally, a broader absorption band around 260–280 nm with decreasing intensity suggests possible n→π* transitions or metal-to-ligand charge transfer (MLCT) interactions, consistent with the coordination of Fe(III) to the hydrazone moiety.

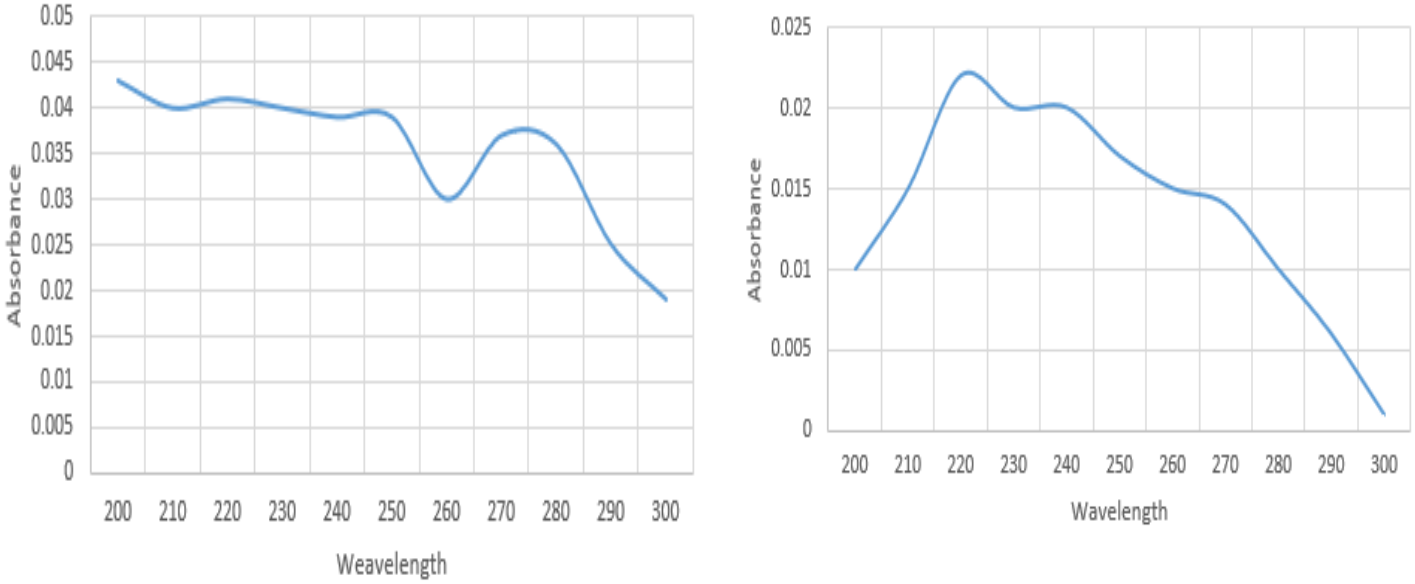

Figure 6: UV-Vis Absorption Bands of the Ligand (A) and Fe complexes (B)

Anti-Microbial Screening

The antimicrobial Susceptibility of the synthesised compounds was evaluated against five clinical test organisms, including two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*), two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), and a fungal strain (*Candida albicans*). The agar well diffusion method revealed varying degrees of inhibition, highlighting structure–activity relationships among the ligand and metal

Test Organisms/Zones of Inhibition (mm)					
Compounds	***Escherichia coli***	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Salmonella typhi***	***Candida albicans***
Ligand	20	16	-	12	12
L – Fe	22	20	12	20	18

Table 1: Antimicrobial Susceptibility Testing of the Synthesised Ligand and Complex

The antimicrobial activity data indicate that the L-Fe complex exhibits enhanced zones of inhibition compared to the free ligand across all tested organisms, with the most significant improvement observed against *Staphylococcus aureus* (20 mm vs. 16 mm) and *Salmonella typhi* (20 mm vs. 12 mm), suggesting that Fe(III) coordination amplifies the compound's efficacy. The broad-spectrum activity of the L-Fe, particularly against both bacterial strains (*Escherichia coli*, *Streptococcus pyogenes*) and the fungal strain (*Candida albicans*), highlights its potential as a versatile antimicrobial agent

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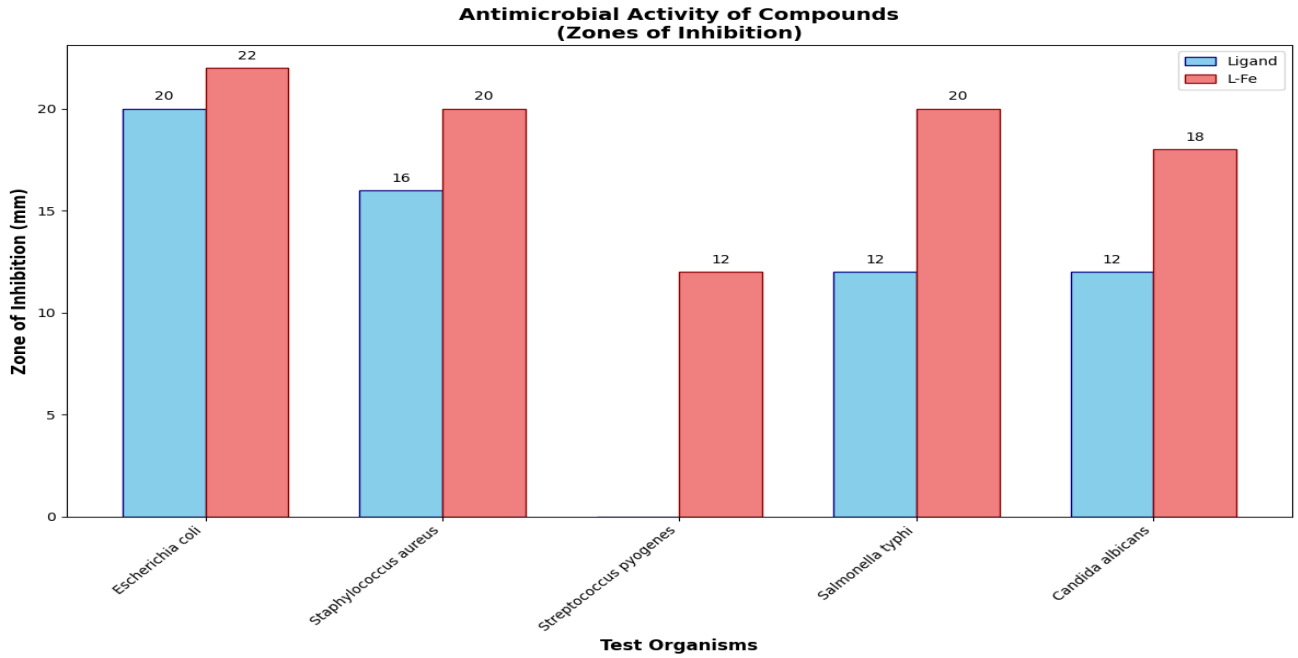

Figure 7: Comparative Antimicrobial Efficacy: Zones of Inhibition for Ligand and L-Fe Complex

V. Conclusion

This study successfully demonstrates the synthesis, characterization, and antimicrobial properties of a Schiff base ligand derived from 2-(2-aminothiazol-4-yl)acetohydrazide and 2-nitrobenzaldehyde. The Schiff base ligand was complexed with iron (III). To ensure the successful synthesis and characterization of these compounds, we relied on detailed spectroscopic analysis. The UV-Vis spectra of the Fe(III) complex consistently showed absorption bands indicating $\pi \rightarrow \pi^*$ transitions and metal-to-ligand charge transfer, which confirmed the formation of stable coordination complexes. Both the Schiff base and its Fe(III) complex were evaluated against different microorganisms. The antimicrobial results revealed enhanced inhibition zones by the metal complex, particularly against *Staphylococcus aureus* and *Salmonella typhi*. Through antimicrobial assessments, especially by measuring the inhibition zones, we found that the Fe(III) Schiff base complex exhibited much stronger germ-fighting activity than the free Schiff base ligand. This enhanced improvement shows the potential of Schiff base–metal complexes as effective antimicrobial agents.

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