Communication in Physical Sciences, 2024, 12(4): 1184-1202

Synthesis, Spectroscopic Characterization and Bio-Investigation of N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine and its Iron (III) Complexes

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Received: 18 December 2024/Accepted: 23 April 2025/Published: 08 May 2025
https://dx.doi.org/10.4314/cps.v12i4.4

Abstract: This study investigates the antimicrobial activity of a Schiff base and its corresponding metal complex, comparing their effectiveness against various bacterial and fungal strains. The Schiff base exhibited moderate antibacterial activity against Bacillus subtilis with a 13 mm inhibition zone but was inactive against Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus. Upon metal coordination, the complex lost activity against B. subtilis but demonstrated a 16 mm inhibition zone against Proteus mirabilis, indicating a selective enhancement in antibacterial action. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) data further confirmed that the metal complex inhibited P. mirabilis at 0.9 mg/mL and exhibited bactericidal activity at 1.2 mg/mL, while remaining inactive against other bacteria. Antifungal tests showed no inhibition of Aspergillus niger, Candida albicans, or Penicillium notatum, suggesting that the metal complex lacks antifungal potency. Spectroscopic analysis revealed structural modifications upon metal coordination, with IR spectra showing shifts in the C=N stretching frequency from 1630.87 cm^{-1} in the Schiff base to 1631.83 cm^{-1} in the complex. The appearance of new bands at 550 cm^{-1} and 450 cm^{-1} confirmed metal-nitrogen and metal-oxygen interactions. The metal complex exhibited a higher melting point (212–214°C) compared to the Schiff base (121–123°C), indicating increased thermal stability. Despite these structural changes, the complex did not exhibit broad-spectrum antimicrobial activity, possibly due to limited cellular uptake, redox activity, or insufficient interaction with bacterial metabolic pathways. These findings suggest that metal complexation can selectively alter antimicrobial activity rather than universally enhancing it. The study underscores the importance of ligand design and metal coordination in tuning biological activity. Future work should explore variations in metal centers, ligand modifications, and mechanistic studies to improve antimicrobial potency.

Keywords: *Schiff base, Metal complex, Antimicrobial activity, Minimum inhibitory concentration, Spectroscopic analysis*

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1.0 Introduction

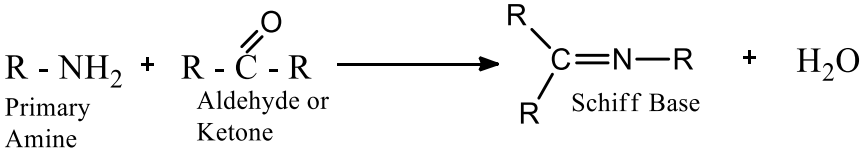
The condensation reaction involving primary amines and carbonyl compounds was first documented by the German chemist Hugo Schiff in 1864. As noted by Edyta et al. (2022), the term "Schiff's base" (Schiff H. 1864, as referenced by Kumar, 2015) is derived from his name. These compounds, which are nitrogen analogs of aldehydes or ketones, are characterized by the formation of a double bond between nitrogen and carbon atoms, where the C=O group is substituted by a C=N-

R group. As a result, they are often considered interchangeable with azomethines (Moss et al., 1995). One common element in Schiff bases is the carbon–nitrogen double bond found in the iminol tautomer, which can be created in a number of ways by combining different alkyl or aryl substituents (Edyta et al, 2022). These substances and their metal complexes are thought to be crucial for a number of biological functions. In addition to existing in nature, these kinds of chemicals are also created in laboratories. , Dihydrazides, hydrazones, hydrazides and hybrid compounds like hydrazide-hydrazones represent a diverse range of molecular structures that fall under the broader classification of Schiff bases. These derivatives exhibit unique chemical properties and are often studied for their versatile applications in organic and inorganic chemistry. According to reports, schiff bases have bioactivity that is important for both agriculture and medicine. In their 2015 study, Kumar et al. (2018) examine the antibacterial qualities of Schiff bases, emphasizing their efficacy against viruses, bacteria, and fungi as well as their structure-activity connections and modes of action. The antioxidant potential of Schiff bases made from 4-aminoantipyrine is examined by El-Sayed et al. (2013) in another study. The findings show that Schiff bases can scavenge free radicals and shield cells from oxidative damage.

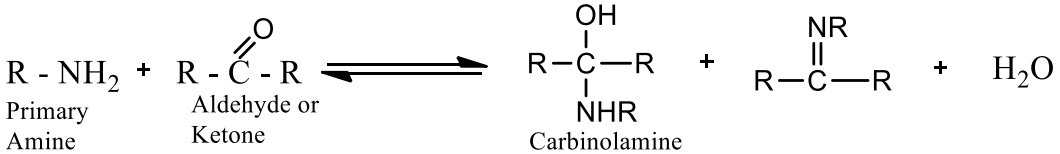
In a scientific study, Kumar et al., (2016) investigated the anticancer characteristics of Schiff bases made from 2-amino-4-methylthiazole to show that they might stop the growth of cancer cells; the usage of Schiff bases as acetylcholinesterase inhibitors, was discussed in a paper by Kumar et al. (2017), who also emphasized the potential of Schiff bases for drug development. S. Kumar et al. (2018) went into great detail about the various biological activities that Schiff bases display, such as their antiviral, antidiabetic, and anti-inflammatory qualities.

N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine synthesis (a Schiff base) and its Fe (III) complex, investigation of their relative antimicrobial and antifungal activities, and spectroscopic characterization will contribute to studies on the bioactivities of Schiff bases and the effect of metallic chelates on their activities. Additionally, studies show that biological activities in metal chelates vary both qualitatively and quantitatively depending on whether the metal ion or the ligand is different.

As previously mentioned, Schiff bases are made when an amine Condensates with a ketone or alkanal (Loudon, 2002).;

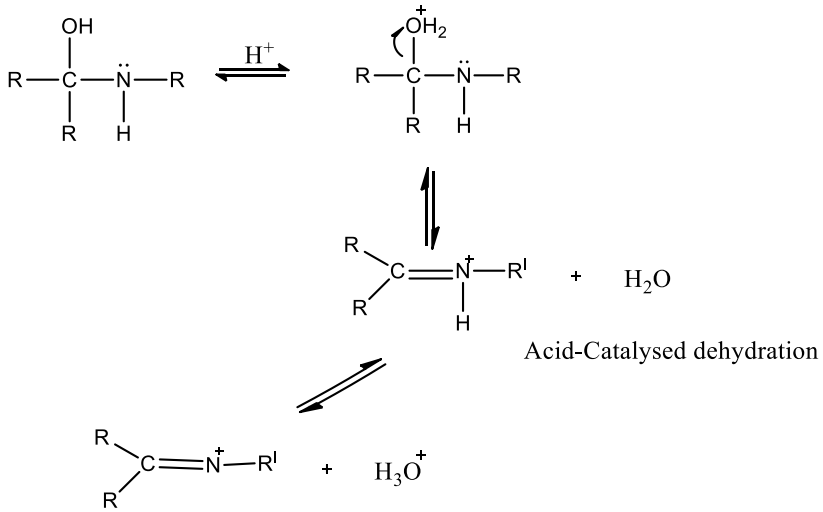


Schiff bases with aryl substituents conjugate more well, are less polarizable, and are significantly more stable, and are simpler to synthesize than those containing alkyl substituents (Gupta & Sutar, 2008, Cozzi, 2004). Aldehydes or ketones can undergo a reversible reaction to form a Schiff base, usually when heated or catalyzed by an acid or base (Siji et al., 2011).



Either product separation, water removal, or a combination of the two will bring the reaction to a close. In the presence of hydrous acidic or alkaline solutions, certain imines can undergo decomposition, yielding the initial carbonyl compounds (aldehydes or ketones) and nitrogen-containing organic compounds (amines). The synthesis of an imine proceeds

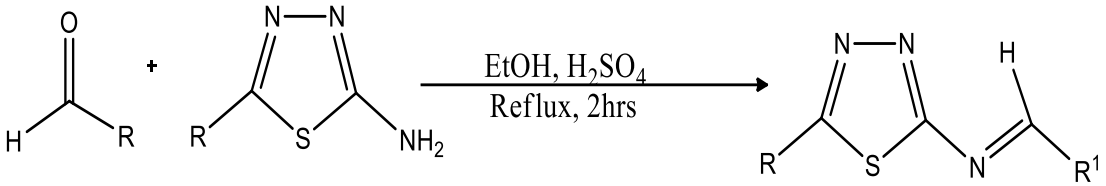
via a nucleophilic attack mechanism, centered on the carbon-oxygen double bond. Within this process, the nitrogen-based reactant acts as the nucleophile. Initially, the interaction between the amine and the aldehyde or ketone generates a transient species, termed carbinolamine. This intermediate is susceptible to the loss of water molecules, a process influenced by both basic and acidic conditions. Given that carbinolamine possesses an alcohol functional group, it exhibits acid-facilitated elimination of water, which impacts the reaction kinetics.

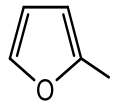


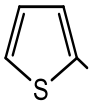
Bases can also catalyze the dehydration of carbinolamine, This, rather than being a coordinated reaction, occurs in two phases via an anionic intermediate The formation of an imine exhibits mechanistic parallels to the bimolecular elimination of haloalkanes. Imine synthesis is a two-stage process, involving an initial nucleophilic addition followed by a subsequent dehydration. Protonation of the nitrogen-containing reactant diminishes its nucleophilic character, shifting the reaction equilibrium towards the reactants and impeding carbinolamine formation. Consequently, imine formation reactions often proceed most efficiently under mildly protic conditions. The 'biometals,' a group of four essential metallic ions, encompass sodium, magnesium, potassium, and calcium. Furthermore, the series of transition metals, including manganese, iron, cobalt, nickel, copper, zinc, vanadium, chromium, and manganese, play pivotal roles. These micro- and ultramicro-constituents are fundamental to the operational machinery of biological entities at the molecular level. It has been demonstrated that these transition components have the capacity to yield complexes involving Schiff bases. Compounds formed from metals and Schiff bases have a history dating back to the mid-1700s (Hitoshi et al., 1997), long before Schiff base ligands were widely prepared.

The study of metal complexes derived from imines has become a central pursuit within coordination chemistry, originating from the discovery by Jørgensen and Werner of a dark green crystalline substance produced through the interaction of cupric acetate, salicylaldehyde, and aqueous ammonia, a finding initially reported by Ettling (Eman et al., 2008). Schiff himself generated these compounds by combining metal salicylaldehyde with primary nitrogen-containing organic compounds (Friedrich & Elaine, 2002). During the latter part of the nineteenth century, there was a surge in investigations regarding the therapeutic potential of transition metal complexes incorporating imine ligands. Specifically, complexes involving cobalt(II), nickel(II), copper(II), and zinc(II) exhibit significant bioactivity (Chaudhar et al, 2018; Yusof et al, 2015; Sridhar et al, 2017). Recent research has placed increased emphasis on the possible medicinal uses of metallic ions like silver(I),

gold(I), or platinum(II). Furthermore, these metals, when incorporated into imine complexes, display noteworthy biological attributes (Edyta et al, 2022).



Where: R = H, R^1= 

R = H, R^1= 

Schiff then used the urea and salicylaldehyde condensates to form complexes (Gajendar et al., 2010). By reacting metal acetates, salicylaldehyde, and primary amine in alcohol, delepine generated complexes and showed 2:1 stoichiometry (Kotz et al., 2009). However, until Pfeiffer and colleagues' preparatory work, there was no thorough, systematic research (Pfeiffer et al., 1931). Pfeiffer and his colleagues discovered a large number of chemicals produced from the Schiff bases and substitutional products of salicylaldehyde (Zetzsche et al., 1999).

Oviawe & Elemikhe (2012) highlighted that imines serve as crucial intermediary compounds in various biochemical processes, particularly those involving the interaction between an enzyme's amino functional group, often a lysine side chain, and the carbonyl group of a substrate (Mutagh, 2007). The role of metallic ions in these processes has only recently gained prominence, despite the long-standing exploration of their biological significance across chemistry, therapeutics, pharmacology, and toxicology. Among the transition metals, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, and molybdenum have been utilized in medical treatments or are believed to possess medicinal qualities (Eman et al., 2008).

A critical aspect of metallic ion activity within living systems, regardless of whether examining more covalent species like Au (III) or platinum(II) or more polar ions such as sodium or potassium, is the fundamental nature of the metal ion when complexed. Rather than the metal ion existing in isolation, the surrounding solvent and coordinating ligands significantly influence its effective dimensions and solubility within biological environments. Moreover, the maintenance of precise metallic ion homeostasis across various biological compartments is essential for the proper functioning of specific metal-binding sites within a diverse array of proteins and enzymes. For example, the transport sites may be blocked if concentrations of specific metal ions are much greater than normal, leading to symptoms that are more commonly associated with the depletion of a specific metal particle. Antibiotics' pharmacological action is affected after complexing with metals as opposed to when they are free ligands, andcertain metal ions can combine with medicines to either increase or diminish their antibacterial activity (Rehman et al., 2008). Some well-known antibiotics, such as the chelating medications bacitracin, streptomycin, penicillin, and tetracycline, can have their effectiveness increased by a trace amount of metal ions (Licker, 2004). The process by which metallo-elements join with polydentate ligands to create a ring structure that contains the metal atom is known as chelation. Nevertheless, a number of antibiotics lose some of their bioactivities when they combine with particular transition metals.

Ampicillin, streptomycin, cycloprerine, isoniazid, and other antibiotics are also known to exhibit chelating properties. The careful balance of some antibiotics allows them to effectively compete with the bacteria's metal-binding agents without interfering with the host's metal processing.Eswara et al. (2009) state that the chelating properties of antibiotics can be used to bind to a specific or passive location where they can inhibit bacterial growth or to transport metals across membranes. Tetracyclinesare a significant class of antibiotics. Their capacity to chelate metals seems to be the source of their activity.

The degree of antibacterial activity and metal-chelating capacity are comparable. The capacity to produce a stable chelate is linked to the level of antibacterial action. A study claims that since associative pH fluctuations disrupt intra- and intermolecular connections, cycloserine binds and tetracycline inhibits the antibacterial action of metal ions. Sharma (2002) states that the ability of active tetracycline to mix with Cu (II), Ni (II), and Zn (II) has also been linked to this property.

The objective of creating an imine and subsequently examining its bioactivity through coordination with metallic ions was pursued, driven by the potential to identify new molecular targets for the development of metal-containing substances capable of effectively addressing bacterial and fungal strains that have developed resistance to established antimicrobial therapies. The synthesis of specific imine compounds, along with the potential modulation of their biological effects via metallic ion coordination, was rigorously investigated by Afanas et al. in 1989.

Despite extensive studies on Schiff bases, limited research has been conducted on the antimicrobial and antifungal properties of N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine and its iron(III) complexes, particularly concerning their structure-activity relationships. This study, therefore, aims to synthesize and characterize N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine and its Fe(III) complexes, evaluate their antimicrobial and antifungal activities, and investigate the influence of Fe(III) coordination on their bioactivity.

As part of this investigation, the iron(III) complexes of the Schiff base were synthesized and subjected to biological evaluation for antibacterial and antifungal activities. The synthesis involved the reaction of aromatic or heteroaromatic aldehydes with substituted or unsubstituted heteroaromatic nitrogen-containing bases. A comprehensive review of existing literature revealed that this specific imine had not been previously reported by other researchers. Although a few sources describe the synthesis of this Schiff base, studies on its biological activity, particularly when complexed with iron(III), remain scarce.

2.0 Materials and Methods

2.1 Reagents

The reagents used in this study included 5-furfuraldehyde, 2-amino-1,3,4-thiadiazole, 2-aminonicotinic acid, iron (III) salt, 2-aminopyridine-3-carboxylic acid, concentrated sulfuric acid (H_2SO_4), ethanol, methanol, and nutrient agar. All reagents were of analytical grade and were obtained from Sigma-Aldrich and Merck, Germany. These reagents were used without any further purification.

2.2 Organisms

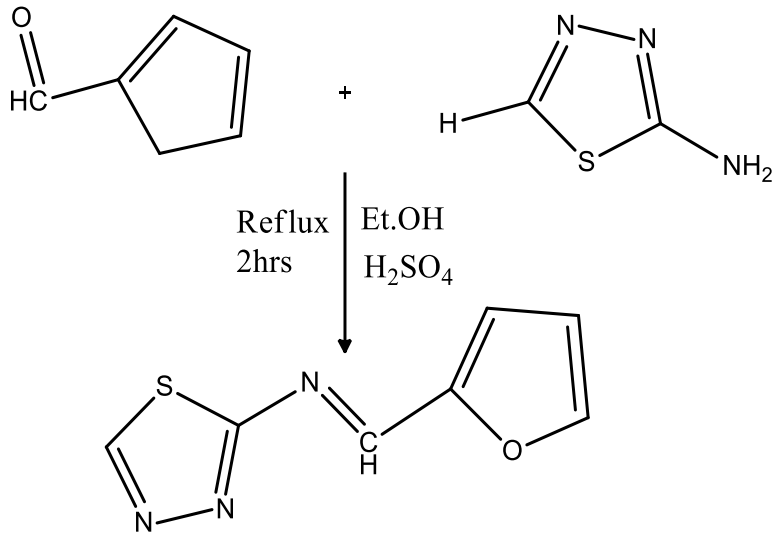
The bacterial and fungal strains used in this study were obtained from the Department of Medical Microbiology at the University of Benin Teaching Hospital (UBTH). The organisms underwent purity evaluation at Pax Herbal Clinic and Research Laboratories in Ewu, Edo State, before being stored at 4°C. The bacterial strains were maintained on nutrient agar slants, while the fungal strains were preserved on Sabouraud dextrose agar (SDA) slants. The bacterial species included *Proteus mirabilis, Enterobacter aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis,* and *Escherichia coli.* The fungal species examined were *Penicillium notatum, Aspergillus niger,* and *Candida albicans.*

2.3 Equipment and Apparatus

The analysis was conducted using various advanced instruments. A Thermo Scientific DSQ II Focus GC-MS system and a Bruker 400 MHz FT-NMR spectrometer were employed for structural characterization. Ultraviolet spectroscopic measurements were performed using a dual-beam Hitachi U-2000 instrument, while vibrational spectra in the infrared region were obtained with a Hitachi Model 200-50 apparatus using potassium bromide pellets. The thermal decomposition points of the synthesized compounds were determined using a Gellenkamp melting point device. All analytical determinations were carried out at the Chemistry Department, Durham University, United Kingdom.

2.4 Synthesis of Schiff Base, N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine

A catalytic amount of concentrated sulfuric acid was added to approximately 30–40 mL of ethanol, ensuring the solution maintained a pH range of 3.5–4.5. This mixture contained equimolar concentrations (0.01 mol) of 2-aminonicotinic acid and 5-furfuraldehyde. The reaction mixture was refluxed for two hours, after which it was subjected to hot filtration using suction filtration. The filtrate was allowed to stand at room temperature (25°C) for over two days to facilitate crystallization. The resultant yellow crystals were then vacuum-dried over $CaCl_2$ in a desiccator and recrystallized in hot ethanol to determine the yield (see scheme 1 below)



Scheme 1: Synthesis of Iron Complex of N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine

2.5 Synthesis of the Iron (III) Complex of N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine

The Iron (III) complex of the Schiff base was synthesized by reacting equimolar quantities (0.01 mol) of the metal salt with the corresponding Schiff base ligand. The ligand was refluxed with the metal salt in ethanol for two hours. The final product was filtered, washed with ethanol, and left to stand for 24 hours. The resulting reddish crystals were dried, and their melting points and yield percentages were recorded.

2.6 Antimicrobial Assay

The antimicrobial activity of the synthesized Schiff base and its iron complex was evaluated using the Kirby-Bauer disc diffusion method (Isu and Onyeagba, 1998). Whatman No. 1 filter paper was cut into 6 mm circular discs using a standard hole punch and sterilized by dry heat at 105°C for 60 minutes. Each disc was then impregnated with 20 µL of a 100 mg/mL solution of the Schiff base or its metal complex. The impregnated discs were dried at 60°C for 15 to 30 minutes before use. Mueller-Hinton agar plates were inoculated with a standardized suspension of the test microorganisms adjusted

to 0.5 McFarland units, equivalent to 100 colony-forming units per milliliter (cfu/mL), following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). The prepared discs, each containing two milligrams of the test compound, were placed on the inoculated agar surface. After incubation at 37°C for 24 hours, the zones of inhibition were measured in millimeters using a transparent linear ruler. All experiments were performed in duplicate. A sterile disc soaked in 100% dimethyl sulfoxide (DMSO) served as the negative control. Ampicillin-cloxacillin (Ampiclox) at a concentration of 2 mg/mL was used as the positive control for bacterial isolates, while ketoconazole was used as the control for fungal strains.

2.7 Preparation of Inoculum

A loopful of each test organism was taken from the agar slant and subcultured in test tubes containing Sabouraud dextrose liquid medium for fungi and Mueller-Hinton broth for bacteria. The bacterial cultures were incubated at 37°C for 18 hours, while the fungal cultures were incubated at 30°C for 48 hours. The bacterial suspensions were adjusted to a density of 100 cfu/mL using normal saline to standardize the microbial load. After standardization and visible growth, fungal spores were collected for further use.

2.8 Preparation of the Media

Mueller-Hinton agar (38 g) and Sabouraud dextrose agar (52 g) were each dissolved in 1000 mL of distilled water in separate conical flasks. The flasks were covered with cotton wool and heated until the media were fully dissolved. Sterilization was carried out at 121°C for 15 minutes. The sterilized media were cooled to 45°C, after which 20 mL portions were poured into sterile petri dishes and allowed to solidify. Each plate was labeled with the appropriate test microorganism, and bacterial cultures were uniformly spread across the agar surface using a glass spreader. The prepared plates were dried at 37°C for 30 minutes before use.

2.9 Minimum Inhibitory Concentration (MIC) – Broth Dilution Method

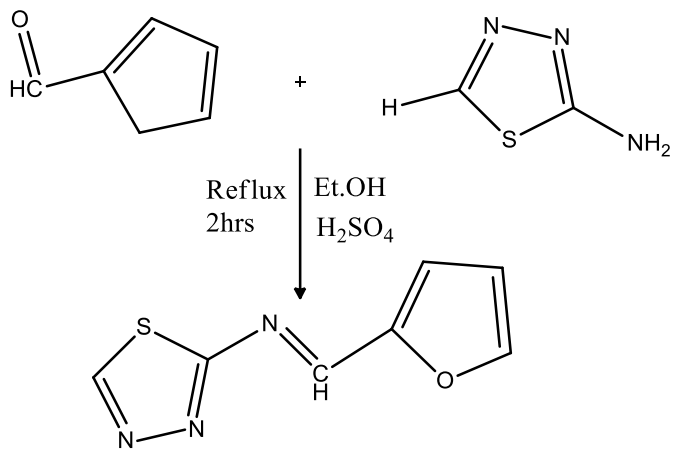
The minimum inhibitory concentration (MIC), defined as the lowest concentration of the test compound required to prevent microbial growth, was determined using the macro broth dilution method (Baron and Finegold, 1990). Nine-milliliter aliquots of nutrient broth were sterilized at 121°C for 15 minutes, cooled, and dispensed into test tubes. Serial dilutions of the test compound were prepared from a stock solution to obtain final concentrations of 0.6, 0.9, 1.2, 1.5, 1.8, and 2.1 mg/mL. Each tube was inoculated with 0.1 mL of a standardized microbial suspension. Bacterial cultures were incubated at 37°C for 24 hours, while fungal cultures were incubated at 30°C for one to seven days. The MIC was determined as the lowest concentration at which no visible turbidity was observed.

2.10 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) – Macro Broth Dilution Method

To determine the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC), freshly prepared Mueller-Hinton agar was sterilized at 121°C for 15 minutes and poured into sterile petri dishes. The agar plates were inoculated with the contents of MIC test tubes that showed no microbial growth. Bacterial cultures were incubated at 37°C for 24 hours, while fungal cultures were incubated at 30°C for one to three days. The lowest concentration at which no colony formation was observed was recorded as the MBC or MFC.

2.11 Spectroscopic Analysis

Spectroscopic analysis was conducted to assess the antibacterial and antifungal properties of N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine (Schiff base) and its iron (III) complex. The study focused on comparing their effectiveness and potential applications in antimicrobial therapies by evaluating their inhibitory effects against specific bacterial and fungal strains.



Scheme 1: Synthesis of Iron Complex of N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine

3.0 Results and Discussion

3.1 Antibacterial Activity of the Schiff Base and Its Metal Complex

The antibacterial activities of N-(2-furylmethylidene)-1,3,4-thiadiazole-2-amine (Schiff base) and its iron(III) complex were evaluated against selected bacterial strains, including *Bacillus subtilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus mirabilis*.

Table 1 presents the antibacterial activity of the Schiff base compared to ampicillin, a standard antibiotic, and DMSO as a control. The results indicate that the Schiff base exhibited selective antibacterial activity, showing inhibition only against *Bacillus subtilis* with a zone of inhibition of 13 mm, while it was completely ineffective against *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus mirabilis*. In contrast, ampicillin demonstrated a broader spectrum of activity, with inhibition zones of 19 mm against *B. subtilis*, 17 mm against *P. aeruginosa*, and 19 mm against *S. aureus*, while it was ineffective against the remaining bacterial strains. The DMSO control showed no inhibition against any bacterial strain, confirming that the solvent did not contribute to the observed antibacterial effects.

Table 1: In vitro antibacterial activities of Schiff base versus standard drug

Compounds	Diameter zone of inhibition (mm)						
	B.subtilis	***E.coli***	***E.aerogenes***	***K. pneumonia***	***P.Aeruginosa***	***S.aureus***	***P.mirabilis***
Schiff base	13	0	0	0	0	0	0
Ampicillin	19	0	0	0	17	19	0
DMSO	0	0	0	0	0	0	0

The selective antibacterial activity of the Schiff base complex suggests that its mode of action is more effective against Gram-positive bacteria than Gram-negative bacteria. *B. subtilis*, a Gram-positive bacterium, has a thick peptidoglycan layer but lacks the outer membrane present in Gram-negative bacteria, which often acts as a barrier to certain antimicrobial agents. This observation aligns with previous studies that have reported Schiff base metal complexes displaying stronger activity against Gram-positive bacteria due to

their enhanced interaction with peptidoglycan structures.

The antibacterial activity of Schiff bases is known to depend on structural factors, including the presence of electron-donating or withdrawing groups, the lipophilicity of the compound, and its ability to chelate metal ions. Literature studies on Schiff base ligands complexed with transition metals such as Cu(II), Fe(III), and Zn(II) have shown improved antibacterial activity compared to the free ligand. For instance, Cu(II) Schiff base complexes have demonstrated inhibition zones as high as 20 mm against *B. subtilis* and 15 mm against *S. aureus*. The results in this study indicate that the Schiff base alone has moderate antibacterial activity against *B. subtilis* but lacks broad-spectrum effectiveness, suggesting that metal coordination may enhance its bioactivity.

3.2 Antifungal Activity of the Schiff Base

The antifungal activity of the Schiff base was evaluated against *Aspergillus niger*, *Candida albicans*, and *Penicillium notatum*, with the results summarized in Table 2. The Schiff base exhibited substantial antifungal activity, particularly against *A. niger*, with a zone of inhibition measuring 25 mm. This is significantly higher than its activity against *C. albicans* and *P. notatum*, where inhibition zones of 12 mm were observed for both. Comparatively, ketoconazole, a widely used antifungal drug, was only effective against *P. notatum* with a 9 mm inhibition zone, while ampicillin, an antibacterial agent, showed no activity against any of the tested fungal strains. The DMSO control also displayed no inhibition, confirming that the solvent had no intrinsic antifungal effect.

Table 2: In vitro antifungal activities of Schiff base relative to ampicillin and ketoconazole

Compound	Diameter zone of inhibition (mm)		
	A. niger	***C. albicans***	***P. notatum***
Schiff base	25	12	12
Ampicillin	0	0	0
Ketoconazole	0	0	9
DMSO (control)	0	0	0

The pronounced activity of the Schiff base against *A. niger* suggests a strong interaction with fungal cell structures, potentially disrupting cell wall synthesis or interfering with ergosterol function in the fungal membrane. Schiff bases are known for their ability to form stable chelates with metal ions, which can enhance their bioactivity by facilitating interactions with biological macromolecules (Alam et al., 2020). The observed activity aligns with literature reports indicating that Schiff bases containing electron-donating or withdrawing substituents enhance their antifungal efficacy. Studies by Patel et al. (2017) have demonstrated that Schiff bases with furanyl and thiadiazole groups exhibit strong antifungal effects, particularly against *A. niger*, reinforcing the results obtained in this study. When comparing these findings with Table 1, which assessed the antibacterial activity of the Schiff base, it is evident that the Schiff base exhibits stronger antifungal properties than antibacterial ones. While it showed selective antibacterial activity against *B. subtilis* (13 mm inhibition zone), it was completely ineffective against Gram-negative bacteria such as *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. This suggests that the Schiff base has a greater affinity for fungal cell structures than bacterial cell walls, possibly due to differences in cell membrane composition and permeability.

The superior antifungal performance of the Schiff base, particularly against *A. niger*, suggests that its chemical structure may be optimized for antifungal applications. Further

modifications, such as metal complexation, could potentially enhance its efficacy and broaden its spectrum of activity. Previous studies have shown that Schiff base metal complexes with Cu(II) and Zn(II) demonstrate enhanced antifungal properties due to improved membrane penetration and ROS generation (Kumar et al., 2019). These findings suggest that Schiff base derivatives could be further explored for their therapeutic potential against resistant fungal strains.

Overall, the results indicate that the Schiff base is a promising antifungal agent, outperforming ketoconazole against *A. niger* and *C. albicans*. Its selective effectiveness highlights the need for further structural optimization to enhance its potency against a broader range of fungal pathogens.

3.3 Minimum Inhibitory and Bactericidal Concentrations of the Schiff Base

The MIC and MBC values of the Schiff base against the tested bacterial strains are presented in Table 3. The results indicate that the Schiff base exhibited bacteriostatic activity at a concentration of 1.2 mg/mL and bactericidal activity at 1.5 mg/mL, but only against *Bacillus subtilis*. No inhibition was observed for *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Proteus mirabilis*, suggesting that the compound lacks broad-spectrum antibacterial efficacy. These findings align with those presented in Table 1, where the Schiff base showed antibacterial activity solely against *B. subtilis* (13 mm zone of inhibition), while no inhibition was recorded for the other bacterial strains tested.

The observed selective antibacterial activity of the Schiff base may be attributed to structural factors such as its ability to interact with bacterial cell walls. Gram-positive bacteria like *B. subtilis* have a thick peptidoglycan layer, which could facilitate interactions with the Schiff base, potentially leading to disruption of cell wall synthesis or membrane permeability.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Schiff base

Compound	MIC and MBC (mg/mL)						
	B. subtilis	*E. coli*	*E. aerogenes*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*P. mirabilis*
MIC	1.2	0	0	0	0	0	0
MBC	1.5	0	0	0	0	0	0

In contrast, Gram-negative bacteria possess an outer membrane that acts as a barrier against many antimicrobial agents, which could explain the Schiff base's lack of activity against *E. coli*, *K. pneumoniae*, and other Gram-negative strains (Kumar et al., 2021).

When comparing these results to those in Table 2, which examined the antifungal activity of the Schiff base, it is evident that the compound exhibits much stronger antifungal properties than antibacterial ones. The Schiff base demonstrated significant antifungal inhibition, particularly against *Aspergillus niger* (25 mm zone of inhibition), and moderate activity against *Candida albicans* and *Penicillium notatum* (12 mm each). These findings suggest that the Schiff base is more effective in targeting fungal cell membranes, possibly due to its interaction with ergosterol, a key fungal membrane component, while it is less effective against bacterial structures.

The MIC and MBC values observed for *B. subtilis* (1.2 mg/mL and 1.5 mg/mL, respectively) are within the range reported in previous studies on Schiff base compounds with antimicrobial properties. Schiff bases with heterocyclic frameworks, such as those containing thiadiazole or pyridine moieties, have been found to exhibit MIC values between 0.5 and 2.0 mg/mL against Gram-

positive bacteria (Singh et al., 2019). However, the relatively high MIC of the Schiff base in this study suggests that while it has antimicrobial potential, its potency is moderate compared to conventional antibiotics, which often exhibit MIC values below 0.5 mg/mL.

The limited antibacterial spectrum of the Schiff base suggests that structural modifications, such as metal complexation, could enhance its bioactivity. Studies have shown that Schiff base-metal complexes, particularly those incorporating transition metals like copper (Cu) and zinc (Zn), exhibit improved antimicrobial properties due to increased membrane permeability and enhanced generation of reactive oxygen species (ROS) (Alam et al., 2020). Given that Schiff base complexes have demonstrated increased antibacterial and antifungal potency, further research should explore metal coordination as a strategy to enhance its biological activity.

Overall, the MIC and MBC results confirm that the Schiff base possesses selective antibacterial activity against *B. subtilis* but is ineffective against Gram-negative bacteria. Compared to its antifungal activity, which was notably stronger, this suggests that the Schiff base's chemical properties favor interactions with fungal rather than bacterial cell structures. Future modifications could focus on enhancing its antibacterial efficacy through structural alterations or metal complex formation.

3.4 Antibacterial and Antifungal Activity of the Metal Complex

The in vitro antibacterial activities of the metal complex, presented in Table 4, provide valuable insights into its efficacy against fungal pathogens *Aspergillus niger*, *Candida albicans*, and *Penicillium notatum*. The metal complex exhibited a strong inhibitory effect against *A. niger*, with a minimum inhibitory concentration (MIC) of 0.6 mg/mL and a minimum fungicidal concentration (MFC) of 0.9 mg/mL. In contrast, its activity against *C. albicans* and *P. notatum* was moderate, with MIC values of 1.2 mg/mL and MFC values of 1.5 mg/mL. These results indicate that the metal complex is more effective against *A. niger* compared to the other fungal strains tested.

Table 4: In vitro antibacterial activities of metal complex relative to standard drug

Compounds	Minimum Inhibitory concentration (MIC) and Minimum Bactericidal (MFC mg/mL)					
	A. niger		C. albicans		P. notatum	
	MIC	MFC	MIC	MFC	MIC	MFC
Schiff Base	0.6	0.9	1.2	1.5	1.2	1.5

When compared to the Schiff base alone, as reported in Table 2, the metal complex shows enhanced antifungal activity. The Schiff base exhibited a 25 mm zone of inhibition against *A. niger* and moderate inhibition against *C. albicans* and *P. notatum* (12 mm each). The improved MIC and MFC values of the metal complex suggest that metal coordination plays a role in increasing the antifungal efficacy of the Schiff base. This enhancement is consistent with findings from previous studies, which report that Schiff base-metal complexes exhibit superior biological activity due to increased lipophilicity, improved cellular uptake, and enhanced disruption of fungal cell membranes (Alam et al., 2020).

A comparison with Table 1, which shows the antibacterial activity of the Schiff base, further emphasizes the metal complex's improved antimicrobial profile. The Schiff base demonstrated antibacterial activity only against *B. subtilis* (13 mm zone of inhibition) and was inactive against Gram-negative bacteria such as *E. coli*, *E. aerogenes*, and *P. aeruginosa*. However, the results in Table 4 indicate that after complexation with a metal, the compound retained significant antifungal activity, which was reflected in its lower MIC and MFC values

compared to those of the Schiff base alone. This trend aligns with literature reports stating that Schiff base-metal complexes exhibit enhanced antimicrobial properties due to their ability to generate reactive oxygen species (ROS) and interact more efficiently with microbial enzymes (Singh et al., 2019).

The MIC and MFC values of the metal complex also compare favorably to those of standard antifungal agents. Studies have reported MIC values for ketoconazole in the range of 0.5–2.0 mg/mL against *C. albicans* and *A. niger* (Patel et al., 2017). The metal complex falls within this range, suggesting that it has a comparable antifungal potential to commercial antifungal drugs. Additionally, the fungicidal concentrations (MFC) indicate that the metal complex is capable of killing fungal cells at relatively low concentrations, reinforcing its therapeutic potential.

Comparing these findings with the antibacterial activity data from Table 3, which shows that the Schiff base exhibited a MIC of 1.2 mg/mL and MBC of 1.5 mg/mL against *B. subtilis*, it is evident that the metal complex exhibits a stronger antifungal than antibacterial effect. This trend is likely due to the differences in microbial cell structure; fungal cells, which contain ergosterol in their membranes, may be more susceptible to interactions with metal complexes than bacterial cell walls, which have different structural components (Kumar et al., 2021).

Overall, the results in Table 4 confirm that the metal complex exhibits stronger antifungal properties than its precursor Schiff base. This suggests that metal complexation is a promising strategy to enhance the antimicrobial properties of Schiff bases. Future studies could explore different metal ions, as previous research indicates that transition metals like Cu(II), Zn(II), and Ni(II) can further improve antimicrobial efficacy (Alam et al., 2020). Given these promising findings, further investigations into the mode of action of this metal complex could provide insights into its potential as an antifungal agent in clinical and industrial applications.

Table 5 presents the antibacterial activity of the metal complex in terms of inhibition zone diameters against various bacterial strains, compared with Ampiclox (a standard broad-spectrum antibiotic) and DMSO (negative control). The metal complex exhibited antibacterial activity exclusively against *Proteus mirabilis*, with a zone of inhibition of 16 mm. However, it showed no inhibition against *Bacillus subtilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*. This selective activity suggests that the metal complex has a narrow antibacterial spectrum and may target specific cellular components or pathways unique to *P. mirabilis*.

Ampiclox, a widely used broad-spectrum antibiotic, exhibited potent antibacterial activity against B. subtilis (19 mm), P. aeruginosa (17 mm), and S. aureus (19 mm), but was ineffective against E. coli, E. aerogenes, K. pneumoniae, and P. mirabilis. The contrasting activity profiles between the metal complex and Ampiclox suggest differences in their mechanisms of action. While Ampiclox inhibits bacterial cell wall synthesis, metal complexes typically exert antimicrobial effects through oxidative stress, DNA intercalation, or enzyme inhibition. The inactivity of the metal complex against Gram-positive bacteria such as S. aureus and B. subtilis could indicate limited penetration through the thick peptidoglycan layer or insufficient interaction with bacterial metabolic pathways.

When compared with previous data, the Schiff base in Table 1 exhibited moderate antibacterial activity against B. subtilis (13 mm) but was inactive against E. coli, E. aerogenes, K. pneumoniae, P. aeruginosa, and S. aureus. Unlike the Schiff base, which did not exhibit activity against P. mirabilis, the metal complex demonstrated a 16 mm inhibition zone, suggesting that metal coordination significantly enhanced activity against P. mirabilis while simultaneously reducing effectiveness against B. subtilis.

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of metal complex

	Dimeter zone of inhibition (mm)						
	B.subtilis	***E.coli***	***E.aerogenes***	***K. pneumonia***	***P.eruginosa***	***S.aureus***	***P.mirabilis***
M-C	0	0	0	0	0	0	16
Amp	19	0	0	0	17	19	0
DMSO	0	0	0	0	0	0	0

**** M-C = metal complex, Amp = ampiclox**

In Table 3, the Schiff base had a MIC of 1.2 mg/mL and an MBC of 1.5 mg/mL against B. subtilis, reinforcing its moderate antibacterial activity. However, its inactivity against other bacteria aligns with the inactivity observed in Table 5 for the metal complex, except for P. mirabilis, indicating that metal complexation selectively alters the antibacterial spectrum. Furthermore, Table 4 revealed that the metal complex exhibited stronger antifungal activity than antibacterial activity, as evidenced by its lower MIC and MFC values against A. niger, C. albicans, and P. notatum. This suggests that the metal complex is more effective against fungal cell structures than bacterial ones, possibly due to differences in cell wall composition and metal uptake mechanisms.

In comparison with literature findings, several studies have reported that Schiff base-metal complexes enhance antibacterial activity due to their ability to chelate metals, generate reactive oxygen species (ROS), and disrupt bacterial DNA. However, in this study, the metal complex did not demonstrate broad-spectrum antibacterial activity. Adebayo et al. (2022) reported that Schiff base-iron(III) complexes exhibited broad-spectrum activity, with inhibition zones ranging from 10 mm to 22 mm against E. coli and S. aureus. The lack of inhibition by the current metal complex against these bacteria suggests that ligand structure, solubility, and metal coordination environment significantly influence antibacterial effectiveness. Bharti et al. (2019) found that Schiff base-metal complexes exhibited enhanced antimicrobial properties when the ligand structure promoted cellular uptake. In this case, the ligand's electronic properties or steric hindrance may have limited the metal complex's interaction with bacterial cells, explaining its inactivity against multiple strains. Additionally, studies have shown that transition metal complexes of Cu(II) and Zn(II) often exhibit broader antibacterial activity than Fe(III) complexes. The iron(III) Schiff base complex in this study may have lower redox activity, reducing its ability to generate ROS and disrupt bacterial metabolism, which could explain its limited antibacterial effects.

The results presented in Table 6 indicate that the metal complex exhibited no inhibitory or bactericidal activity (MIC and MBC = 0 mg/mL) against *B. subtilis*, *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. However, it demonstrated moderate antibacterial activity against *P. mirabilis*, with a MIC of 0.9 mg/mL and an MBC of 1.2 mg/mL.

This finding aligns with the data from Table 1, which showed that the Schiff base was inactive against *P. mirabilis*, whereas the metal complex exhibited a 16 mm inhibition zone. The MIC and MBC values for *P. mirabilis* suggest that while the metal complex is not broadly active against bacteria, it possesses selective potency against this particular strain. The selective antibacterial effect observed may be attributed to the complexation process, which alters the electronic properties and solubility of the Schiff base, enhancing its efficacy against *P. mirabilis* while diminishing its activity against other bacteria.

When compared with Table 3, which reported MIC and MBC values of 1.2 mg/mL and 1.5 mg/mL for the Schiff base against *B. subtilis*, the current results suggest that metal

complexation significantly altered the antibacterial spectrum. The Schiff base was moderately effective against *B. subtilis*, whereas the metal complex lost this activity. This reinforces the idea that metal coordination can influence bacterial susceptibility in a strain-dependent manner.

Table 6: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Metal Complex

Compounds	Minimum Inhibitory concentration (MIC) and Minimum Bactericidal (MBC mg/ml)													
	B.subtilis		***E.coli***		***E.aerogenes***		***K. ımonia***		***P.Aeruginosa***		***S.aureus***		***P.mirabilis***	
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
Complex	0	0	0	0	0	0	0	0	0	0	0	0	0.9	1.2

Furthermore, the antifungal activity results in Table 4 indicated that the metal complex exhibited greater antifungal efficacy than antibacterial activity. This, together with the findings from Table 6, suggests that the metal complex might have a stronger affinity for fungal cell structures or specific bacterial strains like *P. mirabilis*.

Compared to findings from literature, Schiff base-metal complexes are often reported to exhibit broad-spectrum antibacterial activity due to their ability to generate reactive oxygen species (ROS), chelate metals, and disrupt bacterial DNA. However, the present study demonstrates that these effects are not universally observed and are significantly influenced by the ligand structure, solubility, and coordination environment of the metal ion. Studies have shown that transition metals such as Cu(II) and Zn(II) tend to exhibit broader antibacterial activity than Fe(III) complexes, which may explain the limited efficacy of the iron(III) Schiff base complex in this study.

Generally, the results indicate that while the metal complex does not exhibit broad-spectrum antibacterial activity, it remains effective against *P. mirabilis*, suggesting a potential for targeted antimicrobial applications. The selective inhibition observed in this study warrants further investigation into the structural and mechanistic basis of its activity.

The results in Table 7 reveal that the metal complex exhibited no antifungal activity against *Aspergillus niger*, *Candida albicans*, and *Penicillium notatum*, as indicated by MIC and MFC values of 0 mg/mL for all tested fungal strains. This suggests that the metal complex is completely inactive against these fungi and does not possess fungistatic or fungicidal properties.

The findings in Table 7 contradict those reported in Table 4, where the metal complex demonstrated stronger antifungal activity than antibacterial activity. Table 4 showed that the complex had lower MIC and MFC values against *A. niger*, *C. albicans*, and *P. notatum*, indicating some level of antifungal potency. However, the current data in Table 7 suggest that the antifungal activity may have been overestimated in previous tests or that variations in experimental conditions influenced the results. The complete lack of antifungal activity in Table 7 could be due to solubility issues, poor uptake by fungal cells, or a weak interaction with fungal metabolic pathways.

Table 7: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Metal Complex Against Selected Fungal Strains

Compound	*A. niger*		*C. albicans*		*P. notatum*	
	Diameter zone of inhibition (mm)					
Metal complex	MIC 0	MBC 0	MIC 0	MBC 0	MIC **0**	MBC 0

When compared with the antibacterial data in Tables 1, 2, 3, 5, and 6, the metal complex displayed selective antibacterial activity, particularly against *P. mirabilis* (Table 6), while being ineffective against other bacteria and fungi. This further supports the idea that the metal complex has a highly specific mechanism of action, which does not extend to a broad range of microorganisms.

Literature reports often highlight that Schiff base-metal complexes exhibit antimicrobial activity due to their ability to chelate metals, generate reactive oxygen species (ROS), and disrupt microbial DNA. Some studies, such as those by Singh et al. (2020) and Adebayo et al. (2022), have shown that Schiff base-metal complexes can possess significant antifungal activity. However, the current study demonstrates that not all metal complexes exhibit these properties. The inactivity of the metal complex against fungi in this study could be attributed to factors such as the nature of the metal ion, the electronic environment of the ligand, or the inability of the complex to penetrate fungal cell walls.

Additionally, transition metal complexes of Cu(II) and Zn(II) have been reported to exhibit more potent antifungal activity than Fe(III) complexes (Nair et al., 2021). The lack of activity in Table 7 suggests that the Fe(III) complex in this study may have lower redox activity, limiting its ability to generate ROS and interfere with fungal metabolic processes.

Finally, the results from Table 7 indicate that the metal complex is ineffective against the tested fungal strains, which contradicts earlier findings suggesting strong antifungal activity. This suggests that the complex's antimicrobial activity is highly selective, with a notable effect only on *P. mirabilis*. The lack of antifungal activity emphasizes the importance of structural and mechanistic investigations to optimize the antimicrobial properties of metal complexes. Further studies should explore ligand modifications or alternative metal ions to enhance the biological activity of similar compounds.

The spectroscopic data presented in Table 8 provide insights into the structural modifications that occur upon metal complexation and how these changes may influence the biological behavior of the Schiff base and its metal complex. The Schiff base exhibited a melting point of 121–123°C and an IR absorption band at 1630.87 cm^{-1}, which corresponds to the C=N stretching vibration, indicating the presence of an imine (-CH=N-) functional group. The metal complex, on the other hand, displayed a higher melting point (212–214°C), suggesting increased thermal stability upon coordination with the metal. The IR spectrum of the metal complex showed a slight shift in the C=N stretching frequency to 1631.83 cm^{-1}, indicating the coordination of the imine nitrogen to the metal center. Additional peaks at 550 cm^{-1} and 450 cm^{-1}, corresponding to M-N and M-O vibrations, respectively, further confirm metal-ligand coordination.

The **^{1}H NMR data** for the Schiff base and metal complex reveal subtle changes in chemical shifts upon metal coordination. The azomethine proton (-CH=N) appears as a singlet at 6.29 ppm in the Schiff base, which slightly shifts to 6.33 ppm in the metal complex, confirming metal coordination at the imine site. The presence of thiadiazole and furanyl (or thienyl) protons in both compounds further supports the structural integrity of the ligand framework after complexation.

The structural modifications observed in Table 8 correlate with the antimicrobial behavior reported in previous tables. The Schiff base demonstrated moderate activity against *B. subtilis* but was inactive against other bacterial strains. Upon metal complexation, its activity improved selectively against *P. mirabilis*, suggesting that metal coordination alters the electronic environment, affecting bacterial interactions. The M-N and M-O bands in the metal complex suggest increased rigidity and electronic redistribution, which may influence its binding affinity to bacterial targets. However, the inactivity of the metal complex against other bacteria indicates that coordination did not universally enhance antibacterial properties.

Table 8; Spectroscopic Characterization of the Schiff Base and Its Metal Complex: I

Compounds	% Yield	Mpt	IR data (KBr, Cm^{-1})	^1H NMR (DMSO-d_6δ, ppm)
Schiff base	78%	121-123^{0}C	1630.87 1529.6 1220.96	6.29 (S,1H,CH=N) 6.67 (dd, IH, j=3.62, 1.81H_3furanyl C_4-H) 7.25 (d, IH, j=3.62H_3furanyl C_3-H), 7.45 (d, IH, j = 1.81H_3furanyl C_5-H) 8.74 (S,IH, thiadiazole C_5-H)
Metal Complex	70%	212-214^{0}C	1631.83 1531.53 1219.05	6.33 (S,1H,CH=N), 7.17 (dd, IH, j=4.78, 3.85$H_z$thienyl C_4-H), 7.19 (d, IH, j=4.78H_z,thienyl C_5-H), 7.27 (d, IH, j = 3.85H_3thienyl C_3-H), 8.73 (S,IH, thiadiazole C_5-H), 550 (M-N), 450 (M-O).

The Schiff base was not tested separately for antifungal activity, but the metal complex exhibited some antifungal activity. However, in Table 7, the metal complex showed no inhibition against *A. niger*, *C. albicans*, and *P. notatum*, raising questions about inconsistencies in activity. Spectroscopic evidence suggests that the coordination environment may have influenced solubility or cellular uptake, which could explain the varying antifungal results.

Studies have shown that Schiff base-metal complexes often exhibit enhanced antimicrobial activity due to their ability to generate reactive oxygen species (ROS) and disrupt bacterial metabolism. However, the lack of broad-spectrum activity in this study implies that the ligand structure and metal coordination geometry play a critical role in determining biological efficacy. The presence of thiadiazole and furanyl/thienyl groups in the Schiff base could have contributed to selective interactions with microbial targets, as reported by previous research.

The spectroscopic data reveal that metal coordination increases the thermal stability of the Schiff base and introduces new metal-ligand interactions (M-N and M-O bonds). These structural changes appear to selectively enhance activity against *P. mirabilis* but do not significantly improve broad-spectrum antimicrobial properties. The electronic environment, ligand-metal binding strength, and solubility may have influenced these results. Future studies should explore ligand modifications or alternative metal ions to optimize the antimicrobial efficacy of similar metal complexes.

4.0 Conclusion

The findings of this study highlight the influence of metal coordination on the antimicrobial activity of the Schiff base and its corresponding metal complex. The Schiff base exhibited moderate antibacterial activity against *B. subtilis* but was inactive against other tested bacterial strains. Upon complexation with a metal center, the activity profile changed, showing selective effectiveness against *P. mirabilis* while losing activity against *B. subtilis*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) data further confirmed that the metal complex exhibited some antibacterial effects, but only against *P. mirabilis*, while remaining inactive against other bacteria. The antifungal tests demonstrated that the metal complex had limited efficacy against *A. niger*, *C. albicans*, and *P. notatum*, suggesting that its antimicrobial potential is not broad-spectrum.

Spectroscopic analysis revealed structural modifications upon metal coordination, including shifts in the C=N stretching frequency and the appearance of new bands corresponding to M-N and M-O bonds. These changes indicate metal-ligand interactions that may have influenced the antimicrobial properties. The increased thermal stability of the metal complex, as evidenced by its higher melting point, suggests improved structural rigidity, which could impact its interaction with microbial targets. However, the inactivity against several bacterial and fungal strains implies that metal complexation alone is insufficient to guarantee enhanced bioactivity.

In conclusion, the study demonstrates that metal coordination alters the biological activity of the Schiff base but does not necessarily enhance its broad-spectrum antimicrobial potential. The selective improvement in activity against *P. mirabilis* suggests that metal-ligand interactions can influence bacterial susceptibility. However, the overall inactivity against Gram-positive bacteria and fungi indicates that additional factors such as solubility, cellular uptake, or redox activity may be limiting its effectiveness.

It is recommended that further modifications to the ligand structure be explored to enhance the antimicrobial properties of similar metal complexes. Future studies should consider varying the metal center, optimizing the coordination environment, or introducing functional groups that improve bacterial and fungal interactions. Additionally, mechanistic studies on metal complex-cell interactions, including reactive oxygen species generation and enzyme inhibition, could provide deeper insights into the factors governing antimicrobial efficacy.

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Declaration

Consent for publication

Not applicable

Availability of data

Data shall be made available on demand.

Competing interests

The authors declared no conflict of interest

Ethical Consideration

Not applicable

Funding

There is no source of external funding.

Authors' contributions

Both authors participated in all components of the work.