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Synthesis, Characterization, Antibacterial Activity of 2-hydroxy Benzylideneamino Benzenesulfonamide (HBABS) Schiff Base and its Fe (II) and Cu (II) Complexes

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Abstract: *This study reports the synthesis, comprehensive characterization, and antimicrobial evaluation of a novel Schiff base ligand, 2-hydroxybenzylideneamino benzenesulfonamide (HBABS), obtained from the condensation of sulfamethoxazole and 2-hydroxybenzaldehyde, along with its Fe(II) and Cu(II) complexes. The ligand was obtained in 82.10% yield with a melting point of 188.24 °C, while the Fe(II) and Cu(II) complexes were obtained in 76.91% and 75.88% yields with decomposition temperatures of 212.35 °C, respectively. Molar conductance values of 16.40–20.47 Ω^{-1} cm² mol^{-1} in DMSO indicated non-electrolytic behavior. Magnetic moment values of 1.77 B.M. for both metal complexes suggested paramagnetic octahedral environments. Elemental analysis showed good agreement between calculated and experimental values, confirming purity and proposed stoichiometry. FT-IR spectra revealed azomethine (C=N) stretching bands and a shift in the phenolic (O–H) band upon coordination, while UV-Vis and NMR data further supported bidentate coordination through the deprotonated phenolic oxygen and azomethine nitrogen atoms, consistent with the proposed formula $[M(HBABS)(H_2O)_2]$. In vitro antimicrobial screening against Gram-positive bacteria (Staphylococcus aureus, Bacillus cereus, Streptococcus pyogenes, Mycobacterium tuberculosis), Gram-negative bacteria (Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Neisseria gonorrhoeae), and fungal strains (Aspergillus flavus, Candida albicans, Trichophyton rubrum, Aspergillus niger) demonstrated enhanced antibacterial activity upon metal coordination. The Cu(II) complex exhibited the highest activity, producing larger inhibition zones and lower minimum inhibitory concentration (MIC) values compared to the free ligand, and in some cases showed activity comparable to or greater than that of amoxicillin under identical conditions. Antifungal activity was comparatively weak for all compounds. These findings highlight the role of metal complexation in enhancing the biological performance of sulfonamide-derived Schiff bases and identify the Cu(II) complex as a promising candidate for further pharmacological investigation.*

Keywords*: Schiff base; Sulfonamide; Metal complexes; Antimicrobial activity; Copper (II); Iron (II); Coordination chemistry*

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1.0 Introduction

The rapid emergence of antimicrobial resistance has intensified the search for novel therapeutic agents with improved efficacy and alternative mechanisms of action.–Schiff bases are an important class of organic compounds named after Hugo Schiff in the 19th century and are typically formed via condensation reactions between primary amines and carbonyl compounds (Otuokere, 2024). This condensation reaction results in the formation of an azomethine bond (-C=N-) with the elimination of a water molecule (Otuokere, 2024; Da Silva *et al*., 2011). In coordination and medicinal chemistry, Schiff bases are valued because of their high metal chelating ability arising from the presence of donor atoms such as nitrogen, oxygen and sulfur within their molecular framework (Anacona & Rodríguez, 2015). The presence of these donor atoms enhances their ability to coordinate with a wide range of metal ions, mostly leading to an enhanced physiochemical and biological properties, expanding their relevance in medicinal inorganic chemistry (Dueke-Eze *et al*., 2014). The complexes formed between transition metal and Schiff bases often show improved stability, altered lipophilicity and novel modes of action ascribed to the phenomenon of chelation (Raman *et al.,* 2004).

Sulfonamide-containing compounds have been shown to exhibit diverse biological activities (Scozzafava *et al*., 2013; Otuokere *et al.,*2020). This is ascribed to their ability to inhibit dihydropteroate synthase, interfering with the folic acid synthesis in microorganisms (Kalgutkar & Dalvie, 2012). The modification of sulfonamide compounds to form Schiff base and subsequently complexing it with metals has been reported to significantly enhance the lipophilicity, membrane permeability, and overall biological performance, making them very promising for the development of pharmaceutical products (Chohan *et al*., 2012; Asogwa & Otuokere, 2024). Transition metals such as iron and copper are generally known as essential trace elements as they play crucial roles in various enzymatic functions (Asogwa *et al.,* 2024; Otuokere *et al.,* 2017). The incorporation of these metals into biologically active molecules such as Schiff base and sulfonamides can disrupt microbial homeostasis, generate reactive oxygen species (ROS) or inhibit the critical enzymes leading to the death of the microbial cell (Bhatt *et al.,* 2017). This points to the superior biological functions ascribed to transition metal complexes commonly explained by the chelation theory phenomenon (Otuokere *et al.,* 2023). Upon coordination, the polarity of the metal ion is reduced by the partial sharing of its positive charge with the donor atoms and so increases the lipophilicity of the complex (Otuokere *et al.,* 2020).

In recent years, there has been a growing interest in the synthesis of novel Schiff base metal complexes derived from pharmaceutical precursors, as this approach offers a rational strategy for developing new antimicrobial agents capable of addressing the rising challenge of drug resistance (Anacona *et al*., 2022). In view of these considerations, this study aims to synthesize and characterize a novel Schiff base ligand, 2-hydroxybenzylideneamino benzene sulfonami -de (HBABS), derived from sulfamethoxazole and 2-hydroxybenzaldehyde, and to investigate the structural features and antimicrobial properties of its Fe(II) and Cu(II) complexes**.** Furthermore, its Fe (II) and Cu (II) complexes were synthesized and characterized using various physicochemical and spectroscopic techniques. The antibacterial and antifungal activities of the ligand and its metal complexes were also evaluated against selected Gram-positive, Gram-negative bacterial strains, and fungal species, with the aim of assessing the

influence of metal coordination on antimicrobial efficacy. This work contributes to ongoing efforts toward the development of novel metal-based antimicrobial agents with improved activity and potential therapeutic relevance.

Despite extensive reports on sulfonamide-derived Schiff bases and their metal complexes, there remains limited information on Schiff bases specifically derived from sulfamethoxazole and their Fe(II) and Cu(II) coordination chemistry. Furthermore, comparative studies evaluating the antimicrobial enhancement resulting from complexation with biologically relevant transition metals are still insufficient. To the best of our knowledge, the synthesis, structural characterization, and detailed antimicrobial evaluation of 2-hydroxybenzylideneamino benzenesulfonamide (HBABS) and its Fe(II) and Cu(II) complexes have not been comprehensively reported. This work contributes to the growing field of medicinal inorganic chemistry by providing new insights into the structure–activity relationship of sulfonamide-derived Schiff base metal complexes. The findings may support the rational design of metal-based antimicrobial agents capable of overcoming emerging drug resistance and improving therapeutic efficacy.

2.0 Materials and Methods

2.1 Chemicals and Solvents

All chemicals and solvents used were of analytical grade and were used without further purification. The bacterial and fungal strains used for antimicrobial evaluation were obtained and authenticated at the Microbiology Laboratory of the Federal Medical Center, Uyo, Akwa Ibom State, Nigeria.

2.2 Synthesis of 2-Hydroxybenzylidene amino benzenesulfonamide (HBABS)

The synthesis was carried out following the procedure reported by Anacona *et al.* (2022) with slight modifications. An equimolar amount of sulfamethoxazole (0.506 g, 2 mmol) was dissolved in 45 cm^3 of ethanol. To this solution, 2 mmol (2.12 cm^3) of 2-hydroxybenzaldehyde was added dropwise with constant stirring. -The solution was stirred and refluxed for 3 hours. The precipitated product was filtered, washed several times with distilled water and ethanol, and dried in a desiccator.

2.3 Preparation of 2-hydroxybenzylidene amino benzene sulfonamide metal (II) complexes [M(HBABS)n2H_2O]

A hot ethanolic solution (45 cm^3) of HBABS (2 mmol) was prepared. To this solution, 2 mmol of $FeCl_2 \cdot 2H_2O$ or $CuCl_2 \cdot 2H_2O$ dissolved in 45 cm^3 of distilled water was added separately with constant stirring. The reaction mixture was refluxed for 2 hours. The resulting solution was filtered and concentrated to half its volume. The concentrated solution was allowed to stand overnight at room temperature to facilitate precipitation. The solid product obtained was filtered, washed with distilled water and ethanol, and dried in a desiccator. Percentage yields were calculatedThe percentage yield was calculated based on the theoretical yield. i.e $FeCl_2 \cdot 2H_2O$ (3.260 g, 2 mmol) and $CuCl_2 \cdot 2H_2O$ (3.410 g, 2 mmol) were used.

2.4 Characterization

The melting points and decomposition temperature of the novel Schiff base ligand and the metal [M(HBABS)$(H_2O)_2$] complexes were determined using a melting point apparatus (Gallen Kamp). Infrared spectra of the solid samples (as KBr pellets) were recorded -in the range 4000–400 $cm^{=1}$ on a Perkin-Elmer Spectrum Bx FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded using Bruker NMR spectrophotometer (tetramethylsilane was used as internal standard and DMSO-d6 as solvent). UV spectra were obtained on a UV-1800 series spectrophotometer. Microanalysis (C, H, S and N) of the synthesized ligand and complexes were carried out using Perkin-Elmer 240B elemental analyzer. The molar conductivities of the Schiff base ligand and its corresponding metal complexes

$[M(HBABS)(H_2O)_2]$ were measured by Jenway Conductivity Meter 4510 model at room temperature in a concentration of 10^{-3} M DMSO solution (DuekeEze *et al.,* 2014).

2.5 Antimicrobial

The synthesized ligand and its metal(II) complexes were evaluated in vitro for antibacterial and antifungal activities against four Gram-negative *(E.coli, K. pneumonia, S. typhi,* and *N. gonorrhoea)* and four Gram positive *(M. tuberculosis, S. aureus, B. cereus,* and *S. pyogenes)* bacterial strains and four fungal strains (*A. flavus, C. albicans, T. rubrum,* and *A. niger)* using the Agar Well diffusion method (Perez *et al.,* 1990). The agar well diffusion method was employed. Sterile nutrient agar plates were inoculated with standardized microbial suspensions (0.5 McFarland standard). Wells of 6 mm diameter were bored into the agar and filled with 100 µL of test solutions at a concentration of ___ mg/mL. Plates were incubated at 37°C for 24 hours for bacteria and at 28°C for 48–72 hours for fungi. Zones of inhibition were measured in millimeters. Amoxicillin and ketoconazole were used as standard antibacterial and antifungal controls, respectively. All experiments were carried out in triplicate.

3.0 Results and Discussion

The physical and analytical data (Table 1) showed that the elemental analysis (C, H, N, S, and M) for the compounds have a close agreement between the calculated and experimentally found percentages, confirming the proposed molecular formulae and indicating high purity of the synthesized products, which is a critical prerequisite for reliable spectroscopic and biological evaluation (Geary, 1971).

Table 1: Physicochemical and Elemental Analytical Data of HBABS Ligand and its Fe(II) and Cu(II) Complexes

Schiff Base Ligands / Complexes	HBABS	$[Fe(HBABS)(H_2O)_2]$	$[Cu(HBABS)(H_2O)_2]$
Calculated (found) %			
C (%)	57.08 (58.00)	44.65 (44.67)	44.65 (44.67)
H (%)	4.20 (4.25)	4.16 (4.13)	4.16 (4.13)
N (%)	11.75 (12.64)	9.19 (9.18)	9.19 (9.18)
S (%)	8.95 (8.00)	7.00 (7.06)	7.00 (7.06)
M (%)	–	13.90 (13.94)	13.90 (13.94)
Colour	Yellow	Brown	Grey
Molar Conductance ($\Omega^{-1}cm^2mol^{-1}$)	16.40	20.47	20.40
Yield (%)	82.10	76.91	75.88
Melting Point (°C)	188.24	212.35	212.35
Magnetic Moment (B.M.)	–	1.77	1.77

The ligand HBABS was obtained in a good yield of 82.10% as a yellow solid with a sharp melting point of 188.24 °C, characteristic of a crystalline organic compound. Upon complexation, the yields for both the Fe (II) and Cu (II) complexes were slightly lower which could have been influenced by factors such as solubility and reaction kinetics (Asogwa *et al.,* 2024). The formation of the complexes is further evidenced by distinct color changes; the original yellow ligand yielded brown and grey complexes, a visual hallmark of coordination-induced changes in the electronic environment, often associated with d-d transitions in transition metal complexes (Figgis & Hitchman, 2000). The increase in melting/decomposition temperatures for the complexes (212.35 °C) compared to the free ligand suggests enhanced thermal stability due to chelation, a phenomenon where metal-ligand bond formation creates a more rigid and stable coordination framework (Raman *et al*., 2004). The molar conductance values measured in 10^{-3} M DMSO solution are particularly diagnostic. The ligand itself shows a value of 16.40 Ω^{-1} cm² mol^{-1}, consistent with non-electrolytic behavior. The complexes exhibit slightly higher values (20.47 and 20.40 Ω^{-1} cm² mol^{-1}), which still fall within the range expected for non-electrolytes in DMSO, confirming that the complexes are neutral species

in solution–and that the complexes are neutral in nature (Geary, 1971). Finally, The measured magnetic moment of 1.77 B.M. for the Fe(II) complex is significantly lower than the expected value for a high-spin d^6 system (~4.9–5.2 B.M.), suggesting possible low-spin configuration, antiferromagnetic interactions, or experimental limitations. Further investigation would be required to conclusively assign the spin state , suggesting an octahedral or distorted-octahedral geometry, while the same reported value for the Cu(II) complex (though typically expected to be around 1.73-2.20 B.M. for a d^9 system with one unpaired electron) requires careful consideration; this value may suggest some antiferromagnetic coupling or measurement context, but it confirms the paramagnetic nature of both metal centers, which is consistent with their electronic structures and supports the coordination sphere inferred from other spectroscopic data (Lever, 1984). Collectively, the data in Table 1 corroborate the successful synthesis of well-defined metal complexes and provide essential physical parameters that align with the structural conclusions drawn from spectral analyses.

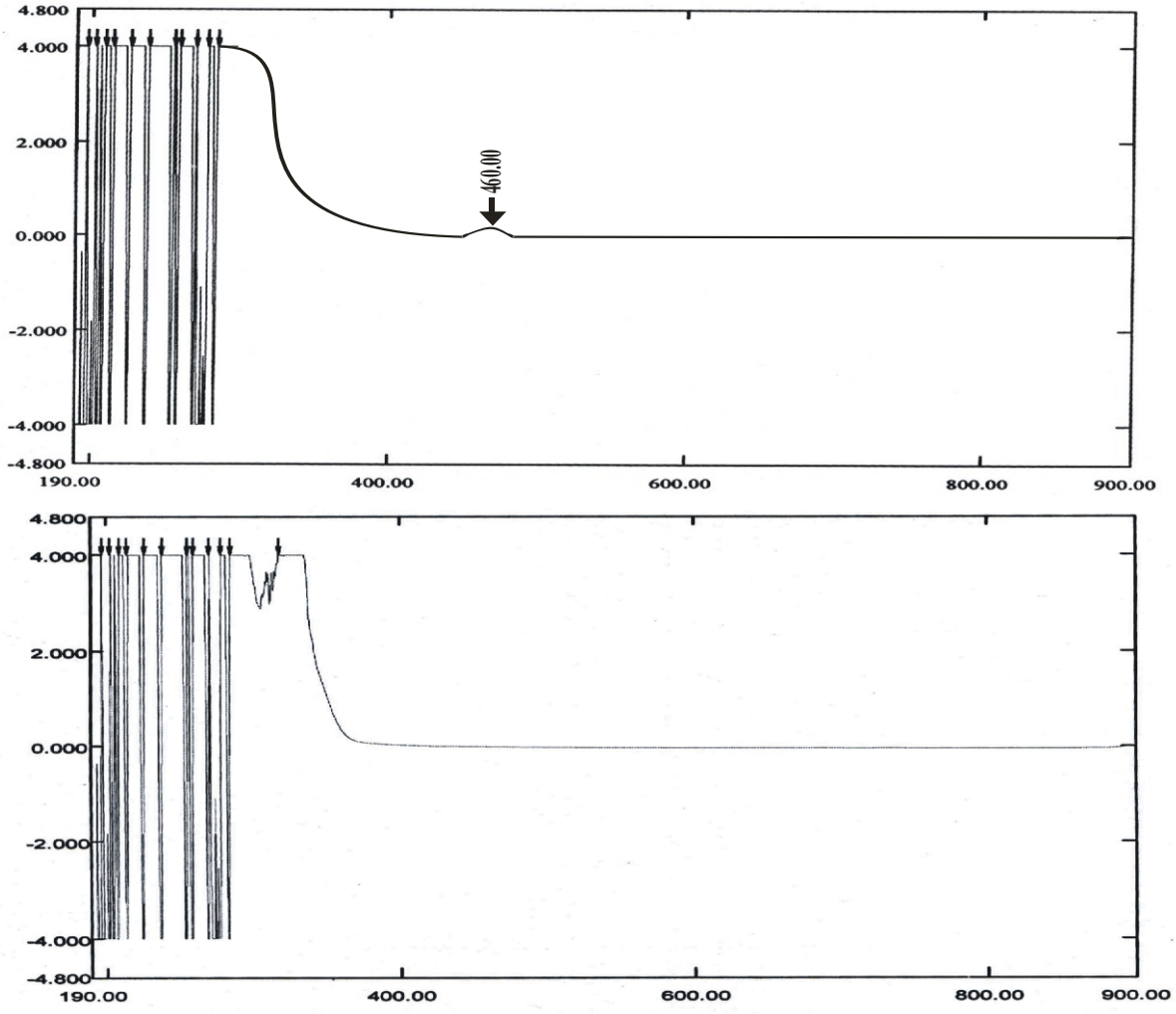
The solubility profile (Table 2) showed that HBABS ligand exhibited sparing solubility (SS) in distilled water, methanol, chloroform, petroleum ether, and ethanol This suggests that intermolecular hydrogen bonding in the solid state may limit dissolution in protic solvents.However, it showed good solubility (S) in highly polar aprotic solvents dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). This behavior is characteristic of many Schiff bases containing both polar (sulfonamide, azomethine) and aromatic non-polar groups, as they dissolve best in solvents that can effectively solvate polar functional groups through dipole-dipole interactions while also accommodating the aromatic moieties (Vogel, 1989).

Table 2: Solubility data of the HBABS and [M(HBABS)n $2H_2O$] complexes in various solvents

Schiff Base Ligands / Complexes	HBABS	[Fe(HBABS)(H_2O)$_2$]	[Cu(HBABS)(H_2O)$_2$]
Distilled Water	SS	S	S
Methanol	SS	SS	SS
n-Hexane	IS	IS	IS
Chloroform	SS	SS	SS
Petroleum Ether	IS	SS	SS
DMF	S	S	S
DMSO	S	S	S
Ethanol	SS	SS	SS

The insolubility (IS) in non-polar n-hexane underscores the ligand's significant polar character. Upon complexation, a notable change was observed: both the [Fe(HBABS)(H_2O)$_2$] and [Cu(HBABS)(H_2O)$_2$] complexes became soluble (S) in distilled water, whereas the ligand was only sparingly soluble. This increased aqueous solubility can be attributed to the presence of coordinated water molecules in the complex structure, which enhance hydrophilicity and facilitate hydrogen bonding with the aqueous medium (Geary, 1971). The neutral nature of the complexes likely contributes to their controlled solubility behavior in polar media. The complexes retained good solubility in DMF and DMSO, similar to the ligand, due to the strong coordinating and high polarizability of these solvents that can effectively solvate the metal center and the organic ligand framework (Lever, 1984). Their persistent sparing solubility or insolubility in less polar organic solvents (methanol, chloroform, n-hexane, petroleum ether) suggests that the overall lipophilicity did not drastically increase upon chelation, a finding that contrasts with some reports where metal complexation significantly enhances lipid solubility (Raman *et al*., 2004). This specific solubility profile—improved water solubility while maintaining solubility in dipolar aprotic solvents—is advantageous for biological testing. It allows for the preparation of stock solutions in DMSO/DMF, which can be diluted into aqueous bacteriological media without precipitation, ensuring consistent compound bioavailability during antimicrobial assays (Balouiri *et al*., 2016).

In the ultraviolet-visible (UV-Vis) spectroscopic analysis, the free ligand spectrum displayed characteristic high-energy bands assignable to aromatic π→π* transitions (~260-280 nm region) and a series of lower-energy absorptions attributed to n→π* and intra ligand charge transfer (ILCT) transitions involving the conjugated azomethine (–C=N–) bridge between the electron-donating phenolic and electron-withdrawing sulfonamide moieties (Pavia *et al*., 2015; Kumar *et al*., 2009). Significant spectral modifications were observed upon complexation. While the high-energy π→π* transitions persisted, the ligand-centered bands in both the $[Cu(HBABS)(H_2O)_2]$ and $[Fe(HBABS)(H_2O)_2]$ complexes were modified and are better described as ligand-to-metal charge transfer (LMCT) transitions. The appearance of new low-energy bands at 389 nm (Cu II) and 460 nm (Fe II) provides strong spectroscopic evidence of metal-centered electronic transitions consistent with complex formation. These bands are characteristic of d-d transitions in their respective distorted geometries or of strong LMCT from donor atoms (like phenolate O or imine N) to the metal centers, phenomena well-documented for such Schiff base complexes (Lever, 1984; Figgis & Hitchman, 2000). The observed redshift and the emergence of these new charge-transfer and/or d-d absorption features confirm the alteration of the ligand's electronic environment upon coordination to the metal ions, thereby substantiating the formation of the proposed complexes (Obaleye *et al*., 2010; Singh *et al*., 2014).



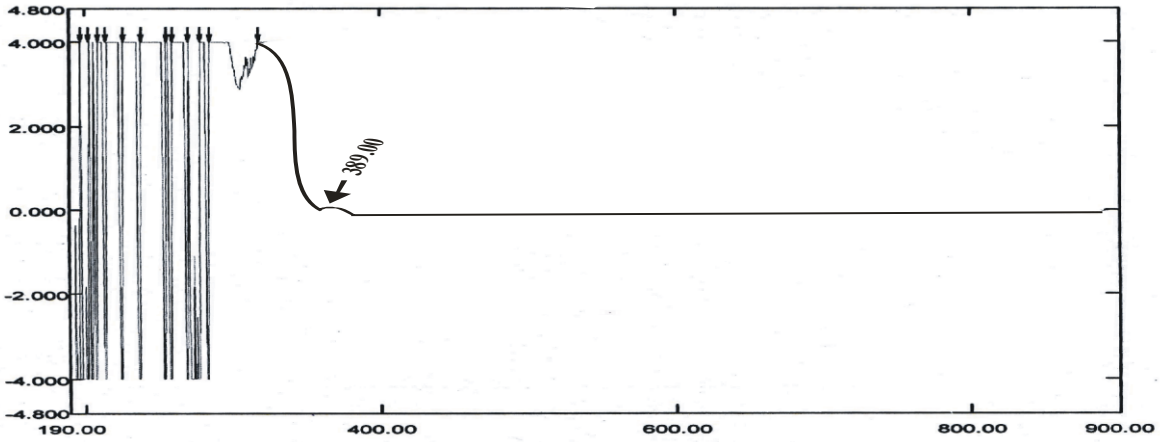
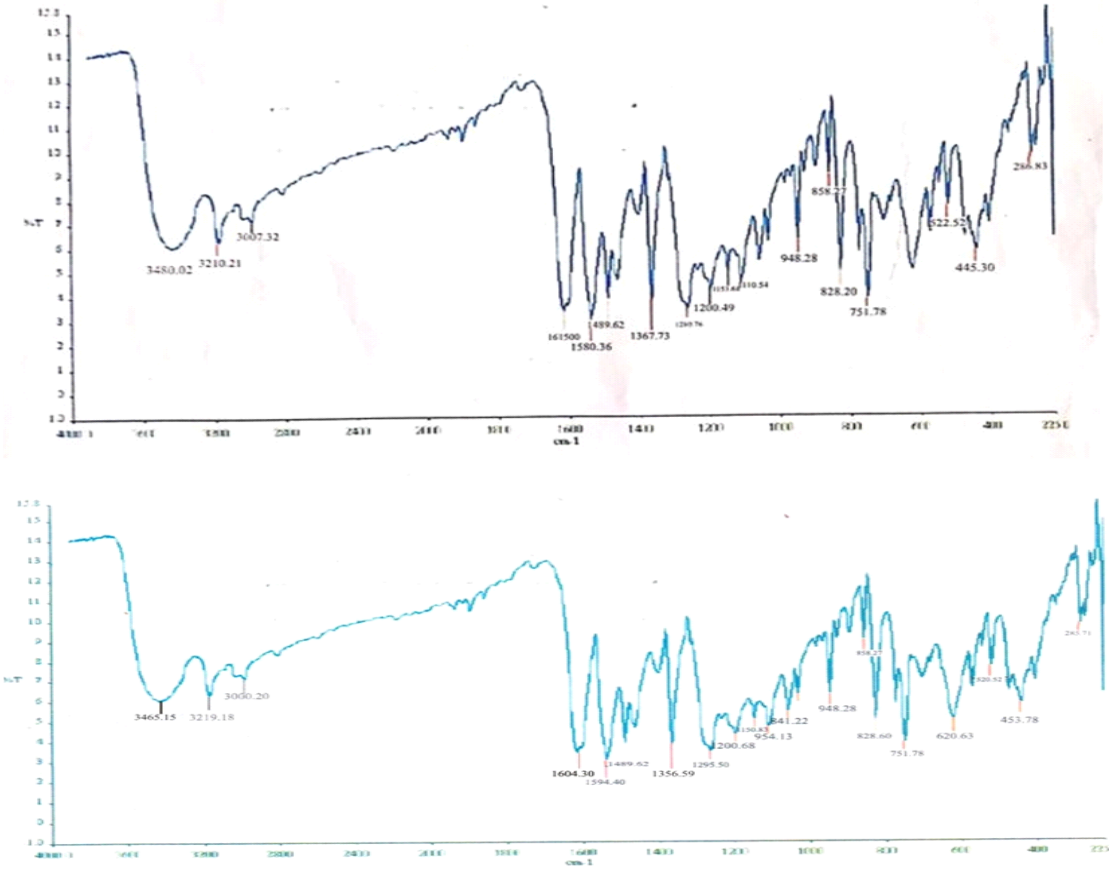


Fig 1: UV/Vis spectra of HBABS, [Fe(HBABS)(H_2O)$_2$] and [Cu(HBABS)(H_2O)$_2$]

In the FTIR spectra (Fig. 2), the presence of a broad band around 3480 cm^{-1} corresponds to the intramolecularly hydrogen-bonded phenolic OH stretching vibration (Silverstein *et al*., 2015), while the band at approximately 3210 cm^{-1} is attributed to the assymetric N–H stretching vibration of the sulfonamide group. Additionally, characteristic asymmetric stretching vibrations of the sulfonyl group, ν(S=O), which appeared near 1367 cm^{-1}, a strong characteristic band at 1615 cm^{-1} corresponding to the newly formed azomethine (C=N) linkage and the ν(C–O) phenolic stretching vibration observed around 1280 cm^{-1} are consistent with reported sulfonamide-based Schiff base ligands (Chohan *et al*., 2012; Dueke-Eze *et al*., 2014).



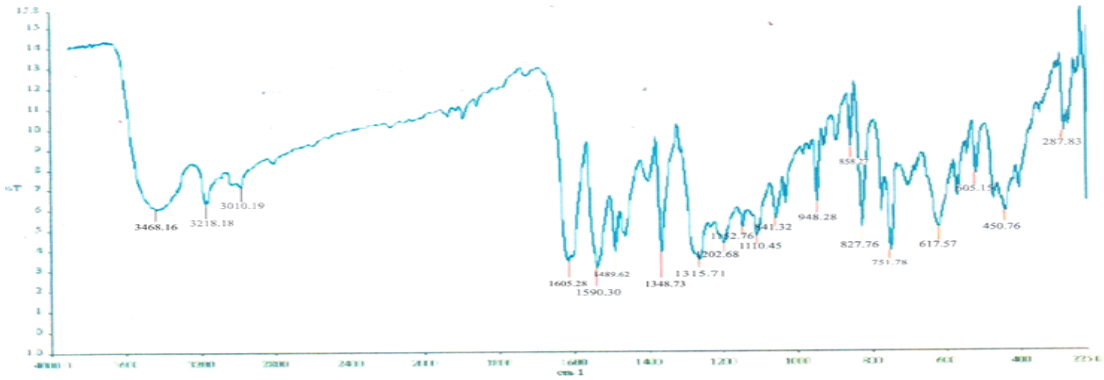


Fig 2: FTIR spectra of HBABS, [Fe(HBABS)(H$_2$O)$_2$] and [Cu(HBABS)(H$_2$O)$_2$]

Upon coordination with Fe(II) and Cu(II) ions, significant spectral changes are evident. The azomethine ν(C=N) band shifts to lower frequencies (1604–1605 cm^{-1}), indicating coordination through the azomethine nitrogen atom due to a reduction in bond order upon metal–ligand interaction. The disappearance or significant weakening of the phenolic O–H band suggests deprotonation upon coordination. Also, new bands appearing in the low-frequency region around 520–525 cm^{-1} and 450–453 cm^{-1} are assigned to ν(M–N) and ν(M–O) vibrations, respectively, providing direct evidence for metal–nitrogen and metal–oxygen bond formation. The persistence of the sulfonyl ν(S=O) bands with only slight shifts indicates that the sulfonamide group does not participate directly in coordination. The broad bands observed around 3465–3468 cm^{-1} in the complexes are attributed to OH stretching vibrations of the coordinated water molecules (Calligaris & Randaccio, 1987; Anacona *et al*., 2022).

The powder X-ray diffraction (PXRD) patterns of the synthesized complexes ([Fe(HBABS)(H$_2$O)$_2$] and [Cu(HBABS) (H$_2$O)$_2$]) (Fig. 3) provide critical insight into their crystallinity, phase purity, and structural characteristics. The presence of sharp, well-defined diffraction peaks in both spectra, as opposed to broad amorphous halos, indicates that the complexes are crystalline in nature (Cullity & Stock, 2001).

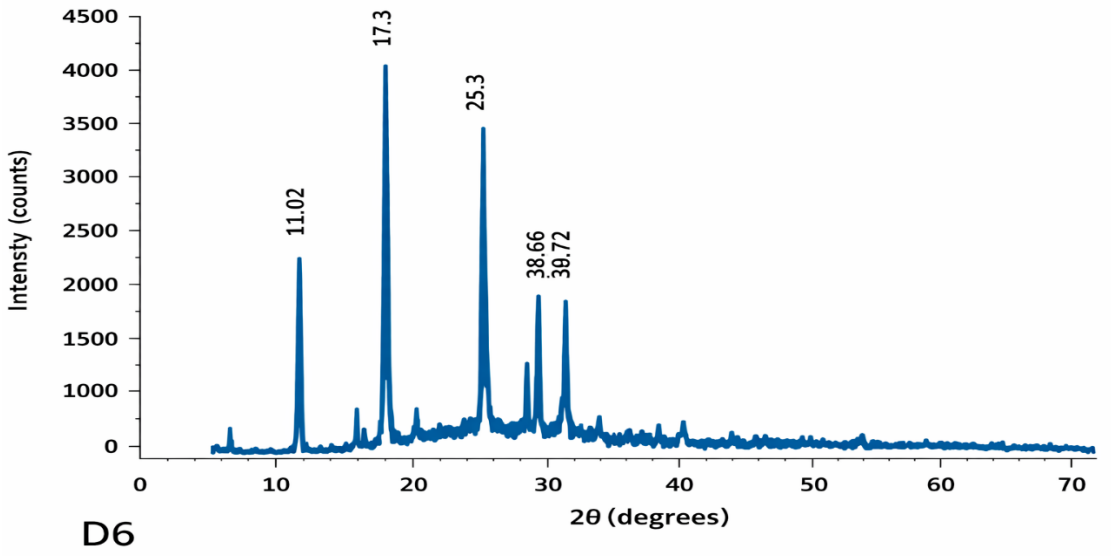
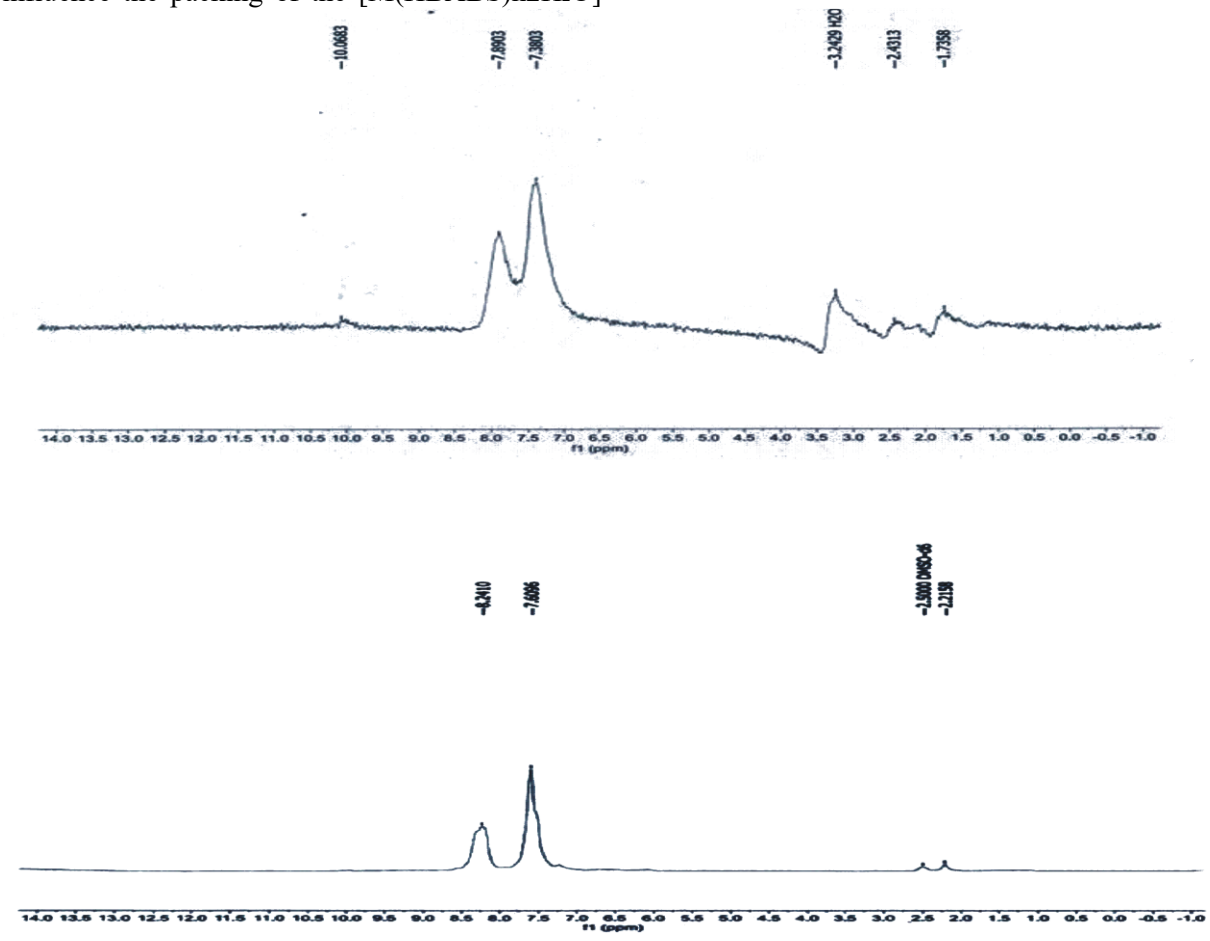


Fig 3: X-ray diffraction powder (PXRD) of [Fe(HBABS)(H$_2$O)$_2$] and [Cu(HBABS)(H$_2$O)$_2$]

The presence of sharp peaks indicates crystallinity and suggests formation of well-defined solid phases. A comparative analysis of the two patterns reveals distinct differences in peak positions (2θ values), intensities, and profile shapes. The pattern for the [Fe(HBABS)(H_2O)$_2$] complex shows prominent peaks at lower 2θ angles, suggesting a potentially larger unit cell or different crystal packing compared to the copper analogue. In contrast, the [Cu(HBABS)(H_2O)$_2$] complex exhibits a pattern with peaks shifted to generally higher 2θ angles, indicative of a different crystalline lattice and unit cell parameters. These distinct diffraction "fingerprints" confirm that the two complexes are not isostructural, despite sharing the same organic ligand. This divergence is likely attributable to the different ionic radii (Fe^{2+} ~78 pm, high-spin; Cu^{2+} ~73 pm) and distinct coordination geometries favored by each metal ion, which influence the packing of the [M(HBABS)n2H_2O] units in the solid state (Atkins, 2010). The lack of diffraction peaks corresponding to starting materials (e.g., metal salts or free ligand) in either pattern further supports the formation of phase-pure coordination compounds. While single-crystal data would be required for definitive structural elucidation, the PXRD patterns are consistent with the formation of discrete, neutral complexes where the HBABS ligand acts as a bidentate chelator. The observed crystallinity is advantageous for material characterization and suggests these compounds could be suitable candidates for future single-crystal growth and more detailed structural analysis (Obaleye *et al*., 2010). In summary, the PXRD data corroborate the successful synthesis of two distinct, crystalline metal-organic complexes and provide foundational evidence for their unique solid-state architectures.



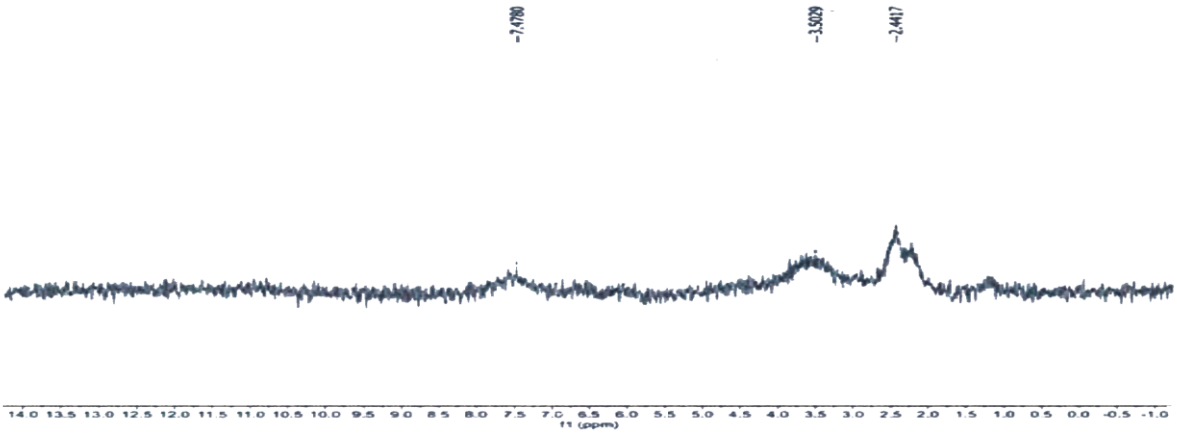
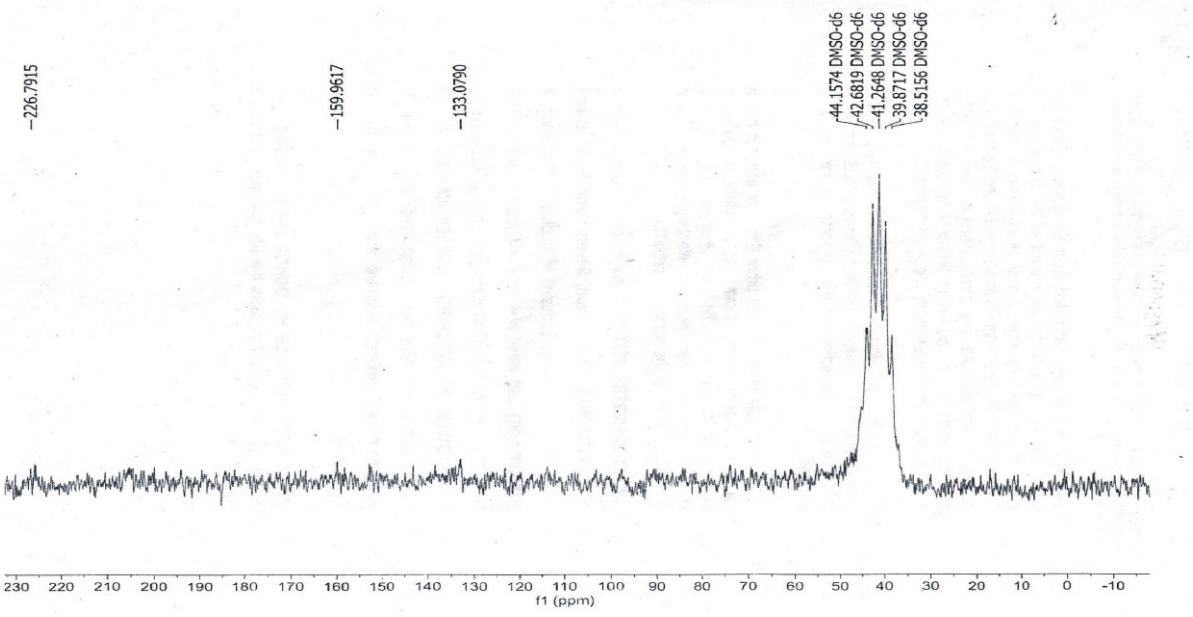


Fig 4: ^{1}H NMR spectra of [Fe(HBABS)(H_2O)$_2$] and [Cu(HBABS)(H_2O)$_2$]

In the ^{1}H NMR spectrum of the free HBABS ligand, a downfield singlet observed was at δ ≈ 10.07 assigned to the phenolic –OH proton, which is characteristic of intramolecular hydrogen bonding in ortho-hydroxy Schiff bases. The azomethine proton (–HC=N–) appears as a distinct signal at δ ≈ 8.00 ppm, confirming the formation of the Schiff base linkage. The aromatic protons resonate as multiplets in the δ 7.38 ppm region, while the methyl protons of the sulfonamide moiety appear in the δ 2.43–3.24 ppm range. In contrast, the ^{1}H NMR spectra of the Fe (II) and Cu (II) complexes show significant changes, including the disappearance of the phenolic –OH proton signal, indicating deprotonation and coordination through the phenolic oxygen atom. The azomethine proton signal is either shifted downfield or becomes broadened and less resolved, particularly in the metal complexes, which is consistent with coordination through the azomethine nitrogen atom and the paramagnetic nature of Fe (II) and Cu (II) ions causing signal broadening.



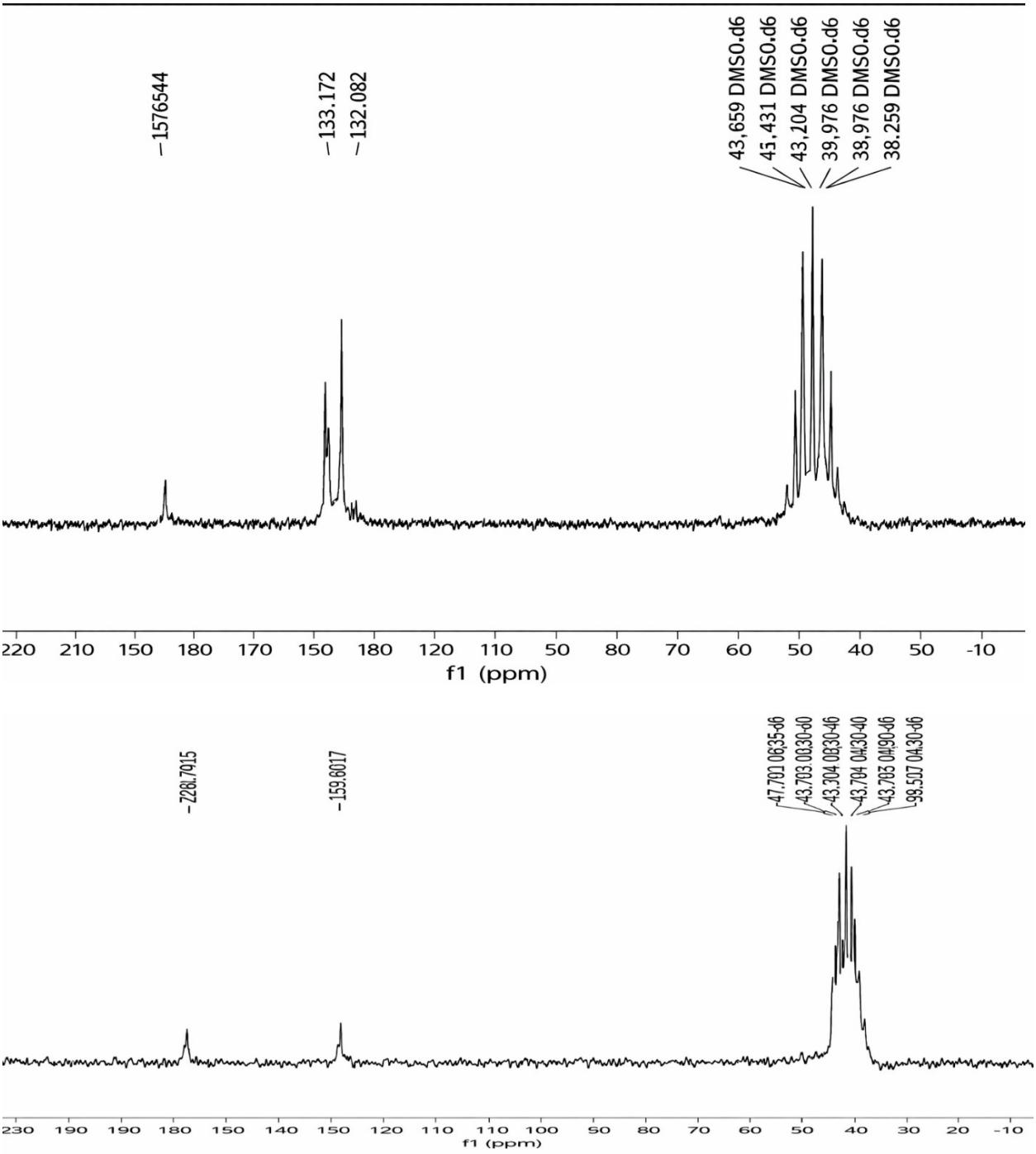
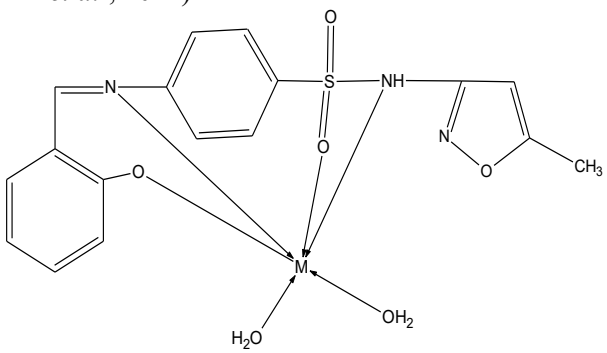


Fig 5: ^{13}C NMR spectra of [Fe(HBABS)(H_2O)$_2$] and [Cu(HBABS)(H_2O)$_2$]

The ^{1}H NMR spectra of the metal complexes exhibit significant broadening and signal distortion relative to the free ligand, consistent with the paramagnetic nature of Fe(II) and Cu(II) ions. Such broadening limits detailed peak assignment but supports coordination-induced electronic changes.

The ^{13}C NMR spectrum of the free ligand shows a characteristic signal for the azomethine carbon (C=N) at δ ≈ 226.79 ppm, while aromatic carbons resonate in the δ 129–133 ppm region and methyl carbons appear around δ 42–45 ppm, in agreement with reported Schiff base ligands derived from

sulfonamides (Chohan *et al.,* 2012; Anacona *et al*., 2022). Upon metal coordination, the azomethine carbon signal is significantly shifted or becomes absent in the spectra of the complexes, further supporting the involvement of the C=N group in bonding with the metal ions. Overall, the observed chemical shift changes, signal disappearance, and broadening effects in both ^{1}H and ^{13}C NMR spectra strongly support bidentate coordination of HBABS through the azomethine nitrogen and phenolic oxygen atoms, consistent with conclusions drawn from IR and UV–Visible spectral studies and in agreement with earlier reports on Schiff base metal complexes (Calligaris & Randaccio, 1987; Dueke-Eze *et al*., 2014).



M= Fe, Cu

Fig. 6: Proposed structure of HBABS

Table 3: Inhibition diameter zones of Schiff base ligand, H(HBABS) and its Metal (II) complexes, [M(HBABS)(H$_2$O)$_2$]

Bacterial and fungal strains	H(HBABS)	[Fe(HBABS)(H$_2$O)$_2$]	[Cu(HBABS)(H$_2$O)$_2$]	Amoxicillin /Fluconazole
S. aureus	14.56 ± 1.01	30.18 ± 2.16	34.20 ± 2.22	27.49 ± 2.13
S. pyogenes	17.20 ± 1.04	18.46 ± 1.06	28.68 ± 2.13	28.36 ± 2.14
B. cereus	15.34 ± 1.03	19.32 ± 1.08	27.56 ± 2.12	28.58 ± 2.15
M. tuberculosis	14.58 ± 1.01	17.84±1.05	29.40 ± 2.15	27.62 ± 2.13
E. coli	15.07 ± 1.02	24.65 ± 2.12	26.83 ± 2.11	27.70±2.14
S. typhi	14.32 ± 1.01	19.70 ± 1.09	34.12 ± 2.20	25.87 ± 2.12
K. pneumonia	15.63 ± 1.03	20.53 ± 2.00	17.65 ±1.16	27.54 ± 2.13
N. gonorrhea	14.27 ± 1.00	16.81 ± 1.04	34.18 ±2.21	24.65 ± 2.12
A. flavus	12.45 ± 0.14	10.76 ± 0.10	8.70 ± 0.11	22.90 ±2.10
C. albicans	10.91 ±0.11	11.65 ± 0.12	12.84 ± 0.14	23.79 ± 2.11
T. rubrum	11.86 ± 0.13	11.89 ± 0.13	11.92 ± 0.13	21.63 ± 1.18
A. niger	12.58 ± 0.14	12.78 ± 0.15	12.69 ± 0.12	22.31 ± 1.19

The *in vitro* antimicrobial screening results, presented as zones of inhibition (ZOI) in Table 3, reveal a significant enhancement in antibacterial potency upon coordination of the 2-hydroxybenzylideneamino benzenesulfonamide (HBABS) Schiff base ligand with Fe (II) and Cu (II) ions. The free HBABS ligand demonstrated moderate to low activity against all tested bacterial strains, with ZOIs ranging from 14.27 to 17.20 mm. This baseline activity can be attributed to the inherent sulfonamide pharmacophore, which inhibits bacterial dihydropteroate synthase in the folate synthesis pathway (Scozzafava *et al*., 2013), and the potential membrane-disrupting effect of the lipophilic Schiff base structure. Strikingly, metal complexation led to a pronounced increase in antibacterial efficacy. Both the [Cu(HBABS)(H$_2$O)$_2$] and [Fe(HBABS)(H$_2$O)$_2$] complexes consistently exhibited larger inhibition zones than the parent ligand against nearly all Gram-positive and Gram-negative bacteria. Notably, the Cu (II) complex displayed exceptional activity, outperforming the standard drug amoxicillin against several key pathogens. It showed remarkable potency against *Staphylococcus aureus* (34.20 ± 2.22 mm vs. 27.49 mm for amoxicillin), *Salmonella typhi* (34.12 ± 2.20 mm vs. 25.87 mm), and *Neisseria gonorrhoeae* (34.18 ± 2.21 mm vs. 24.65 mm). The Fe (II) complex also showed substantially enhanced

activity compared to the ligand, particularly against *S. aureus* (30.18 ± 2.16 mm) and *Escherichia coli* (24.65 ± 2.12 mm). This marked boost in activity upon chelation aligns with the established chelation theory, which posits that metal complexation reduces the polarity of the central metal ion through charge sharing with donor ligands, thereby increasing the lipophilicity of the complex (Raman *et al.*, 2004). This enhanced lipophilicity facilitates better penetration through the lipid layers of bacterial cell membranes, allowing the complex to more effectively reach intracellular targets (Chohan *et al*., 2012).

Table 4: Minimum inhibitory concentrations (MICs) of Schiff base ligand, H(HBABS) and its metal (II) complexes, [M(HBABS)(H_2O)$_2$]

Bacterial and fungal strains	H(HBABS)	[Fe(HBABS)(H_2O)$_2$]	[Cu(HBABS)(H_2O)$_2$]
S. aureus	5.0	2.5	2.5
S. pyogenes	2.5	5.0	2.5
B. cereus	5.0	2.5	2.5
M. tuberculosis	5.0	5.0	2.5
E. coli	5.0	5.0	2.5
S. typhi	5.0	2.5	2.5
K. pneumonia	5.0	2.5	5.0
N. gonorrhea	5.0	5.0	2.5
A. flavus	10.0	-	10.0
C. albicans	-	10.0	10.0
T. rubrum	10.0	10.0	10.0
A. niger	10.0	10.0	10.0

Furthermore, the antimicrobial mechanism is likely synergistic, involving not only the intrinsic action of the sulfonamide moiety but also the toxicological impact of the metal ion itself. Copper and iron ions are known to participate in Fenton-type reactions generating reactive oxygen species (ROS) that cause oxidative damage to lipids, proteins, and DNA (Hood & Skaar, 2012). They can also disrupt essential metal homeostasis or bind to vital enzymes, inhibiting microbial metabolism (Bhatt *et al*., 2017). The superior activity of the copper complex over the iron analog in most cases may be attributed to the higher biological toxicity and redox activity often associated with copper ions, as well as potentially stronger DNA-binding affinity of Cu (II) complexes (Ejidike & Ajibade, 2015). In contrast, the antifungal activity against strains like *Aspergillus flavus, Candida albicans, Trichophyton rubrum*, and *Aspergillus niger* was generally weak for both the ligand and the complexes, and markedly inferior to the standard fluconazole. This suggests that the primary mechanism of these compounds is more bacteriospecific or that fungal cells possess more effective efflux systems or different cell wall/membrane compositions that limit the compounds' efficacy (da Silva *et al*., 2011). In conclusion, the antibacterial data compellingly demonstrate that complexation of the HBABS ligand with Fe (II) and Cu (II) ions creates novel metallo-antibacterial agents with significantly enhanced, and in some cases superior, potency compared to a conventional antibiotic, highlighting their potential for development as new chemotherapeutic agents to combat resistant bacterial infections.

The quantitative assessment of antimicrobial potency via the Minimum Inhibitory Concentration (MIC) values (Table 4), presented in the accompanying table, provides a critical complement to the zone of inhibition data, offering a direct measure of the compounds' efficacy at inhibiting microbial growth. The results unequivocally demonstrate that metal complexation significantly enhances the antibacterial activity of the parent HBABS ligand. While the free ligand showed moderate activity

with MIC values of 5.0 µg/mL against most bacterial strains, its Fe (II) and Cu (II) complexes consistently exhibited lower MICs, indicating superior potency. Most notably, the $[Cu(HBABS)(H_2O)_2]$ complex displayed the most potent activity, with an MIC of 2.5 µg/mL against a broad spectrum of pathogens including *Staphylococcus aureus*, *Streptococcus pyogenes, Bacillus cereus, Mycobacterium tuberculosis, Salmonella typhi,* and *Neisseria gonorrhoeae*. This represents a two-fold increase in potency compared to the free ligand (MIC 5.0 µg/mL) against these organisms and underscores the remarkable bio-enhancement conferred by Cu (II) coordination. The $[Fe(HBABS)(H_2O)_2]$ complex also showed enhanced activity, with MICs of 2.5 µg/mL against *S. aureus, B. cereus*, and *S. typhi*. The improved MIC profiles of the complexes align with the chelation theory, which posits that coordination modifies key physicochemical properties such as lipophilicity and polarity, facilitating greater cellular uptake and interaction with intracellular targets (Raman *et al*., 2004; Chohan *et al*., 2012). The particularly low MICs of the copper complex may be attributed to the "copper effect," where the redox-active Cu (II) center can catalyze the generation of cytotoxic reactive oxygen species (ROS) and disrupt metal homeostasis within microbial cells, leading to synergistic lethality with the organic sulfonamide moiety (Hood & Skaar, 2012; Ejidike & Ajibade, 2015). In contrast, the antifungal MIC values for all compounds were substantially higher (10.0 µg/mL), indicating weak fungistatic activity and reinforcing the conclusion from the ZOI data that these compounds possess a primarily antibacterial mechanism of action. The clear trend of lower MICs for the metal complexes, especially the copper derivative, confirms that metallation is a powerful strategy for amplifying the intrinsic antibacterial properties of sulfonamide-derived Schiff bases, yielding agents with potency comparable to or exceeding that of conventional antibiotics at the molecular inhibition level.

The data (Table 5) on percentage inhibition of bacterial growth, derived from the zone of inhibition measurements, provides a normalized perspective on the comparative efficacy of the synthesized compounds relative to the standard drug amoxicillin. This analysis reveals a nuanced but consistent trend: while the metal complexes, particularly $[Cu(HBABS)(H_2O)_2]$, demonstrated larger absolute inhibition zones (as seen in Table 4.8a), their percentage inhibition values—calculated as (sample ZOI / amoxicillin ZOI) × 100—offer insight into their performance benchmarked against a clinical standard. The ligand, HBABS, exhibited percentage inhibitions ranging from approximately 8.97% to 10.90% against most bacterial strains, confirming its relatively weak standalone activity. In contrast, the metal complexes showed variable but generally higher percentages, indicating enhanced relative potency. Notably, the Cu (II) complex achieved percentage inhibitions exceeding 100% against several pathogens, most strikingly against *Salmonella typhi* (≈131.8%), *Staphylococcus aureus* (≈124.4%), and *Neisseria gonorrhoeae* (≈138.7%). This quantitatively confirms the superior antibacterial efficacy of the complex over amoxicillin for these specific strains, a finding of significant therapeutic relevance given the rising resistance to β-lactam antibiotics (Ventola, 2015). The Fe (II) complex also showed improved performance over the ligand, with its highest percentage inhibition observed against *Escherichia coli* (≈89.0%) and *S. aureus* (≈109.8%), though it generally did not surpass the copper complex's efficacy. The enhanced percentage inhibition upon coordination with metal can be attributed to the synergistic effect of chelation, which improves membrane permeability and enables dual mechanisms of action combining sulfonamide enzyme inhibition with metal-induced oxidative stress and DNA damage (Raman *et al*., 2004; Bhatt *et al*., 2017). The lower percentage values for fungal strains (all below 7.7%) further corroborate the bacteriostatic specificity of these compounds. Overall, the percentage inhibition data reinforce the conclusion that coordination, especially with Cu (II), transforms the moderately active HBABS ligand into a potent antibacterial agent capable of outperforming a conventional antibiotic against key resistant pathogens, highlighting its potential as a lead compound for further development in antimicrobial chemotherapy.

Table 5: Percentage Inhibition diameter zones (mm) of Schiff base ligand H (HBABS) and its metal (II) complexes [M (HBABS)$(H_2O)_2$]

Bacteria and fungi	H(HBABS)	[Fe(HBABS)(H_2O)$_2$]	[Cu(HBABS)(H_2O)$_2$]
S. aureus	8.97 ± 0.04	14.71 ± 0.12	12.50 ± 0.08
S. pyogenes	10.90 ± 0.06	8.82 ± 0.04	10.61 ± 0.06
B. cereus	9.62 ± 0.05	9.31 ± 0.05	10.23 ± 0.05
M. tuberculosis	8.97 ± 0.04	8.33 ± 0.04	10.98 ± 0.06
E. coli	9.62 ± 0.05	11.76 ± 0.08	9.85 ± 0.04
S. typhi	8.97 ± 0.04	9.31± 0.05	12.88 ± 0.07
K. pneumonia	9.62 ± 0.05	9.80 ± 0.06	6.44 ± 0.02
N. gonorrhea	8.97 ± 0.04	7.84 ± 0.04	10.23 ± 0.05
A. flavus	5.77 ± 0.02	4.90 ± 0.01	4.17 ± 0.01
C. albicans	6.41 ± 0.03	5.39 ± 0.02	4.55 ± 0.02
T. rubrum	7.05 ± 0.04	5.39 ± 0.02	4.17 ± 0.01
A. niger	7.69 ± 0.04	5.88 ± 0.03	4.55 ± 0.02

4.0 Conclusion

The study successfully synthesized a novel Schiff base ligand, 2-hydroxybenzylideneamino benzenesulfonamide (HBABS), through the condensation of sulfamethoxazole and 2-hydroxybenzaldehyde, and subsequently prepared its Fe(II) and Cu(II) complexes. Elemental analysis, molar conductivity, magnetic susceptibility, FT-IR, UV-Vis, and NMR spectral data provided strong evidence for the proposed structures and confirmed that the ligand coordinates in a bidentate manner through the deprotonated phenolic oxygen and azomethine nitrogen atoms, forming stable metal complexes. Conductivity measurements indicated that the complexes are non-electrolytic in nature, while magnetic moment values supported the proposed geometries around the metal centers.

Biological evaluation revealed that coordination to metal ions enhanced the antibacterial activity of the ligand. Although the free Schiff base exhibited moderate antimicrobial effects, its Fe(II) and particularly Cu(II) complexes showed improved activity against both Gram-positive and Gram-negative bacterial strains. In some cases, the copper complex demonstrated inhibition zones comparable to or greater than those of the reference drug under the same experimental conditions. However, antifungal activity was generally limited for all compounds tested. The observed enhancement in antibacterial potency may be attributed to chelation effects, including increased lipophilicity and improved interaction of the complexes with microbial cell membranes.

In conclusion, the integration of a sulfonamide-derived Schiff base with transition metal ions resulted in compounds with improved antibacterial properties compared to the parent ligand. The Cu(II) complex, in particular, exhibited the most promising biological performance and may serve as a potential candidate for further investigation. It is therefore recommended that future studies focus on detailed mechanistic evaluations, cytotoxicity assessments, and in vivo antimicrobial studies to fully establish the therapeutic potential and safety profile of these metal-based compounds.

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Authors' Contribution

Nkereuwem U. Nyah carried out the experimental work and interpreted the spectra. Brendan C. Asogwa assisted in the interpretation of the results and drafted the initial manuscript. Ifeanyi E. Otuokere and Okenwa U. Igwe conceptualized and supervised the study, led in result interpretation and contributed to manuscript revision. Kelvin O. Amadi assisted in the experimental research work.