



Synthesis, Spectroscopic Characterization and *In Vitro* Antimicrobial Studies of Copper (II) and Iron (III) Complexes Derived from (E)-N-Benzylidene Schiff Base Ligand

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Abstract

This study reported the synthesis of the novel Schiff base ligand (E)-N-benzylideneacetamide, which was synthesized by refluxing equimolar amount of benzaldehyde and acetamide. The synthesized Schiff base was subsequently reacted with Fe(III) and Cu(II) metal salts to form the corresponding metal complexes. The compounds were characterized using FT-IR, Molar conductance measurement, Melting point and decomposition temperature studies, solubility test and in vitro antimicrobial activity studies using Agar well diffusion method against Pseudomonas aeruginosa, E. coli and Aspergillus niger at 50, 25 and 12.5 mg/mL respectively. The spectroscopic data indicated that the Schiff base ligand acted as a bidentate and coordinated to the metal via imine nitrogen and deprotonated oxygen atoms. The molar conductances were found to be 14 and 19 ohm¹cm²mol⁻¹ for the Fe(III) and Cu(II) complexes respectively, indicating that they are non-electrolytic in nature. The Schiff base have melting point of about 80 - 81°C while the complexes decomposed around 120 -150 °C. The solubility test of the compounds indicated they are most dissoluble in water, acetone, DMF and DMSO but indissoluble completely in methanol, ethanol and methylated spirit. The results of the antimicrobial studies revealed that the metal complexes exhibited better antibacterial activity than the ligand but less compared to the standard drugs (amoxicillin). Whereas, both ligand and the complexes were non active against the tested Aspergillus niger.

Keywords: Antimicrobial, acetamide, benzaldehyde, spectroscopic characterization and Schiff base.

1. Introduction

Schiff bases are aldehyde or ketones –like compounds, formed by condensation of primary amines with carbonyl compounds and were first discovered by German chemist Hugo Schiff in 1864. It has been established that the azomethine linkage in Schiff base is responsible for the biological activities such as in the treatment of cancer (Yang *et al.*, 2008), antibacterial (Wang *et al.*, 2014) antiviral agent (Singh *et al.*, 2008). The common method of synthesizing Schiff base metal complex is condensation method which usually takes place under refluxed using a solvent media. Copper(II) complexes are particularly attractive since copper is known to play a significant role in naturally occurring biological systems while the Iron(III) complexes have been known to alter the external electromagnetism or ultrasound for magnetic resonance imaging (MRI) (Sheng *et al.*, 2008), antimicrobial activity (Tanaka *et al.*, 2010; Patel *et al.*, 2000) and for its DNA damaging property (Phaniband *et al.*, 2012). However, Antimicrobial resistance to most of our commercially available antibiotics, is being regarded as one of the greatest challenges faced by researchers today and thus necessitated the need to search for new compounds with potential effects against various pathogenic bacteria and fungi in order to arrest these problems. Since metal ions have unique effects on these pathogens as far as medicinal inorganic chemistry is concerned, it gives a direction for the synthesis of new drugs (Bruijninx and Sadler, 2008). Most of the organic ligands fall within this category of antibiotics and hence coordination of metal ion with the ligands, potentially increases the



biological activity of these ligands and hence prompted us to carry out this work. Numerous studies conducted indicated that metal complexes showed enhanced activity than the uncomplexed free ligands (Raman *et al.*, 2006). However, a large number of Schiff base metal complexes have been studied for their interesting properties such as antibacterial, antifungal, anticancer and antimalarial. These properties resulted in increasing the interest of researchers to study this kind of compounds. However, based on the scientific investigation discovered from the literature, there is paucity of information on synthesis, spectroscopic characterization and in *vitro* antimicrobial studies of Cu(II) and Fe(III) complexes derived from (E)-N-benzylideneacetamide Schiff base ligand.

2. Materials and Methods

The following materials were used; fume cupboard, capillary tube, thermometer, Kern ABS-N analytical balance, FT-IR (Cary 630, Agilent), Conductivity meter (Jenway 4020), melting point apparatus, beakers, conical flasks, stirrer, magnetic bead, filter paper, glass funnel, dropper etc. Whereas analar grade chemicals from BDH chemical company were purchased and used upon received, which include; acetamide, benzaldehyde, $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$. While solvents such as ethanol, acetone, methanol, methylated spirit and n-hexane were obtained from chemical store of Chemistry Department, ATBU-Bauchi.

2.1 Synthesis of Schiff Base Ligand

The Schiff base ligand was synthesized by microwave Irradiation method reported by (Kalshetty *et al.*, 2017). It is a temperature controlled method used to minimize the reaction time and improve yield. To a solution of acetamide (2.95 g, 0.05 moles) in 10 mL of ethanol, a solution of benzaldehyde (5.3 g, 0.05 moles) in 10 mL ethanol was added into a conical flask. Few drops of 10% NaOH were added to adjust the pH of the solution. The reaction mixture in the conical flask was placed into the microwave oven. Illuminated for 8 min, allowed to cool, resulting in the formation of a precipitate. The precipitate formed was filtered, purified using ethanol and evaporate the moisture at ambient temperature.

2.2 Synthesis of Metal Complexes

The Cu(II) complex was synthesized by the dissolution of Cu(II) salt (4.275 g, 0.025 moles) in 10 mL of ethanol, (7.35 g, 0.05 moles) of the synthesized Schiff base ligand was added to the solution in metal to ligand ratio of 1:2. Few drops of 10% NaOH were added to the reaction mixture to maintain pH of about 6.0-7.0 and the mixture was introduced into the microwave oven. Illuminated for 8 min, allowed cooling and the obtained precipitate was collected by filtration, purified using ethanol and evaporate the moisture at ambient temperature. The same procedure was used to synthesized the Fe(III) complex using the Schiff base ligand.

2.3 In vitro Antimicrobial Activity

The synthesized Schiff base ligand, its Cu(II) and Fe(III) complexes and the standard drugs were studied for their in *vitro* antimicrobial activities against two bacterial strains: *E. coli* and *P. auregenosa* and one fungal strain: *A. Niger*. The culture media used for the analysis was nutrient ager (NA). The mentioned media was used for the sensitivity test and the determination of minimum inhibitory concentration (MIC). The compounds were screened using the Agar well diffusion method (Chohan and Kausar, 2000).

3.0 Results and Discussion

3.1 Physical Properties of the Compounds

The physical properties of the Schiff base and its Cu(II) and Fe(III) complexes reported in (Table 4.1) showed that the complexes are colored and this is as a result of shift of electron(s) from lower energy orbitals to higher energy orbitals of the *d*-block metals and are stable at room temperature which may be attributed to the higher decomposition temperatures, suggesting a good thermal stability of the compounds (Aliyu and Ado, 2011). The percentage yield of the ligands and complexes were obtained in a moderately good yield (65-75%) as presented in Table 4.1 and these values are similar to (60-70%) reputed by (Bello *et al.*, 2018).

Table 1: Physical Properties of the Schiff base ligand and their Fe(III) and Cu(II) complexes

Compounds	M.W (gmol ⁻¹)	M.P (°C)	D.T (°C)	Colour	%yield
Schiff base	147	80-81	--	white	65
Fe(III)complex	203	--	120	Brown	67
Cu(II)complex	210.5	--	150	Pale Blue	75

Key: M.W = Molecular Weight, M.P= Melting Point, D.T= Decomposition Temperature

3.2 The Infrared spectral Data

The IR spectral data presented in Table 2, shows that the absorption frequency of the azomethine $\nu(\text{C}=\text{N})$ for the Schiff base ligand and the complexes were absorbed at 1664, 1653 and 1650 cm^{-1} for the Schiff base ligand, Fe(III) and Cu(II) complex respectively. In the complexes, the absorption bands for $\nu(\text{C}=\text{N})$ stretches have slightly shifted to a lower frequencies compared to that of the free Schiff base ligand and this might be due to the involvement of the azomethine nitrogen in coordination with the Cu(II) and Fe(III) ions respectively. However, this is in consensus with the findings described by Raman *et al.*, (2006) for indistinguishable metal complexes. The bands in the region 3300-3400 cm^{-1} in the spectra of the ligand and the complexes were accorded to $\nu(\text{OH})$ stretching vibrations which indicates that the phenolic $-\text{OH}$ group does not participate in bond formation. The considerable changes in frequencies observed by phenolic C-O stretches in the spectra of the ligand and complexes may be due to the participation of phenolic oxygen in the complexion with the metals via deprotonation (Bello *et al.*, 2018). The complexation of the metals with the ligands was further corroborated by the appearance of new peak intensity ligands bands in the region 655-540 cm^{-1} and 485-450 cm^{-1} in the spectra of the complexes which are assigned to $\nu(\text{M}-\text{O})$ and $\nu(\text{M}-\text{N})$ vibrations respectively.

Table 2: Infrared spectral data of the Schiff base ligand and their Fe(III) and Cu(II) complexes

Compound	$\nu(\text{C}=\text{N})$ (cm^{-1})	$\nu(\text{OH})$ (cm^{-1})	$\nu(\text{C}-\text{O})$ (cm^{-1})	$\nu(\text{M}-\text{O})$ (cm^{-1})	$\nu(\text{M}-\text{N})$ (cm^{-1})
Schiff base	1664	3295	--	--	--
Fe(III)complex	1653	3334	1233 654	485	--
Cu(II)complex	1650	3352	1262	540	450

3.3 Molar Conductance Studies

The molar conductance values of the Fe(III) and Cu(II) complexes presented in (Table 3) were found to be 14 and 19 $\text{ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$ respectively, which are relatively lower conductance values. This indicates that the complexes



do not dissociate into ions in aqueous solution to conduct electricity (non electrolytic) and thus concides with the findings of Geary, (1971).

Table 3: Molar conductance of Cu(II) and Fe(III) complexes in ($1 \times 10^{-3} \text{ mol dm}^{-3}$) DMSO solution

Compound	Specific Conductance ($\text{Mol dm}^{-3} \times 10^{-3}$)	Molar Conductance ($\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$)
Fe(III)complex	14×10^{-6}	14
Cu(II)complex	19×10^{-6}	19

3.4 Solubility Test

The presented result in (Table 4) shows that the synthesized Schiff base is dissoluble in water, acetone, DMF and DMSO but indissoluble in methanol, ethanol, N-hexane and methylated spirit. The solubility for its Cu(II) complex in the same solvents revealed that the complex was indissoluble in water, methanol, ethanol, acetone, methylated spirit but soluble DMF and DMSO₄. The solubility its Fe(III) complex was also tested with the same solvents and was found to be dissoluble in N-hexane, DMF and DMSO₄ but indissoluble in water, methanol, ethanol, acetone and methylated spirit (Manex, 2008).

4.0 Antimicrobial Studies

4.1 Minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations results presented in Table 5, revealed that the ligand showed inhibitory activity at 50 mg/mL against *p. aeruginosa*, whereas the complexes showed lower MIC values at 25 and 12.5 mg/mL for Cu(II) and Fe(III) complex respectively, compared to the MIC values of the ligand, which is higher, it indicates that the complexes exhibited enhanced activity than ligand. However, its ability to combine with or dissolve in lipids or fats and the presence of partially positive and negative charges might intensify the antimicrobial activities of the complexes compared to free ligand. Thus, the antibacterial property of metal complexes cannot be due to the presence of two or more separate coordinate bonds between polydentate ligand and the central metal atom only but it is a combination of the above contributions. The trend shows that Fe(III) complex has better activity than Cu(II) complex and ligand against the *p. aeruginosa*. However, both ligand and complexes exhibited higher MIC value than the standard drug (Amoxicilin) against the same organism. This testified that the standard drug showed better activity owing to its lower MIC value (6.25 mg/mL) compared to the other compounds (ligand and the complexes) and this can be justified by the fact that the standard drug (amoxicillin) being a broad spectrum antibiotic, made from active component and testified via series of clinical trials might likely be responsible for its higher activity against the tested microbe. For the isolate *E. coli*, the ligand and Cu(II) complex showed inhibitory activity at 25 mg/mL each, whereas Fe(III) complex and the standard drug indicated lower MIC values at 12.5 mg/mL each and this shows that the Fe(III) complex and the standard drugs, having lower MIC values, can be said to have better activity compared to the ligand and Cu(II) complex. For the fungal strain *A. niger*, the ligand and the complexes were said to be none active and this may be due to the non pathogenic nature of the isolate. But however, an activity was indicated at 12.5 mg/mL by the standard antibiotic (ketaconazole) which was used as the control.

Table 5: Minimum Inhibitory Concentration (mg/mL) of the Schiff base ligand and their Fe(III) and Cu(II) complexes

Compounds	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>A. niger</i>
Ligand	50	25	ND
Cu(II) complex	25	25	ND
Fe(III) complex	12.5	12.5	ND
Amoxicillin	6.25	12.5	--
Ketaconazole	--	--	12.5

KEY: ND= Not determined

5.0 Conclusion

The Schiff base ligand (E)-N-benzylideneacetamide and the Cu(II) and Fe(III) complexes have been synthesized and characterized by solubility testing, molar conductance studies, melting point and decomposition and infrared spectroscopy. The infrared analysis indicated that the metals atoms were coordinated with the ligand via azomethine nitrogen and oxygen of the phenolic group. The molar conductance values of the complexes obtained suggested their non-electrolytic nature. The melting point and decomposition temperatures suggested a good thermal stability. The *in vitro* antimicrobial activity indicated that the complexes demonstrated enhanced activity than the ligands but less compared to the standard drugs. Therefore, from those observations, it was concluded that the synthesized metal complexes can be potentially useful as biological agents.

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