



Synthesis and Characterization of a Schiff Base Derived from 6-Methylcoumarin and 2-aminophenol and its Complexes Using Cobalt (II) and Copper (II)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Schiff bases and their metal complexes has been a subject of research for a long period till date. Various researchers are focusing on synthesis of various Schiff bases with different metal salts to form complexes and their unique properties tried to be identified. The coumarin possess a lot of medicinal values and it was reported that incorporating of metal ion into its structure increases its activity compared to the coumarin based ligand as reported in literature. Such an attempt made here to synthesize a Schiff base from 6-methylcoumarin and 2-aminophenol and its two metal complexes using cobalt and copper (II) chloride salts. Synthesis was confirmed by FTIR and

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antimicrobial activity of the ligand (Schiff base) and the metal complex were determined by agar well diffusion method against some bacterial and fungal strains. The melting point of the Ligand was 158°C and that of Schiff bases were found to be 230°C and 264°C for Co (II) and Cu (II) respectively. Molar conductance values of Co (II) and Cu (II) complexes were 15.04 and 12.76 $\mu\text{Scm}^{-1}\text{mol}^{-1}$ respectively. The result from activity revealed an enhanced antimicrobial activity of the metal complexes over their corresponding Schiff bases in a concentrations dependent manner.

Keywords: Schiff bases; ligand; metal complexes; antimicrobial.

1. INTRODUCTION

Coumarins belong to a family of large and extensively studied compounds containing 2H-1-benzopyran-2-one core structure, which consist of fused benzene and alpha-pyrone rings. This heterocyclic system is also known as 1,2-benzopyrone, 2H-chromen-2-one, 2-oxo-1,2-benzopyran or o-hydroxycinnamic acid lactone. The coumarin nucleus is a recurring motif in both natural and synthetic compounds that exhibit a broad spectrum of biological properties including anticoagulant, anti-inflammatory, antioxidant, antiviral, antimicrobial and anticancer agents as well as enzyme inhibitors [1]. The history of coumarins can be traced back to 1820 when H.A. Vogel first isolated the simplest member of this family –coumarin from the *tonka beans*. Later on, this compound was first synthesized by W.M. Perkin in 1868 [2,3,4], and discovered both natural and synthetic coumarins were endowed with a great therapeutic potential due to the wide spectrum of biological properties including anticancer, antimicrobial, antiviral, anti-inflammatory, neuroprotective and antioxidant activities [5,6,7]. Hence, the coumarin skeleton can be foresighted as a privileged scaffold for the design and synthesis of pharmacologically active compounds [8,9,10,11 and 12]. Additionally, physicochemical properties and biological activities of coumarins might be enhanced by combining coumarin moiety with other chemical species such as metal ions. The literature survey reveals that several properties of the organometallic complexes offer great opportunities in the development of new compounds with specific and new modes of action. In fact, incorporation of metals such as cobalt, copper, zinc, silver, platinum, palladium, or iridium, into ligand molecules with biological activity has been implemented in the development of novel coumarin-based complexes with better pharmacological activity [13]. As a consequence, a large number of coumarin –based metal complexes have been synthesized in order to obtain more potent

molecules [14,15]. Metal complexes of Schiff bases are specifically of interest in bioinorganic chemistry because many of these complexes provide biological models for use in understanding the structure of biomolecules and biological processes [16,17,18].

2. EXPERIMENTAL

2.1 Materials

All chemicals reagents and solvents used were of analytical grade and were used without further purification. The metal (II) salts used are Cobalt (II) Chloride hexahydrate and Copper (II) Chloride dihydrate. All glasswares used were thoroughly washed with detergent and repeatedly rinsed with tap water and distilled water was finally used and then dried in an oven at 110°C. The melting points were determined using a Gallenkamp Melting Point Apparatus, the infrared (IR) spectra of the compounds were recorded using Cary 630 FTIR Spectrometer, conductivity and magnetic susceptibility values all determined using the Jen way 4010 conductivity meter and Sherwood Scientific MSB –MKI Magnetic susceptibility balance respectively. The Sensitive Balance used was a Labtech electronic scale BL 3002 Japan, which was used in all measurements involving weight or mass.

2.2 Method

Synthesis of the Schiff base (ligand): 3.2g (0.02mole) of 6-methylcoumarin in 20cm³ ethanol was added to 2.18g (0.02mole) of 2-aminophenol in 30cm³ ethanol, the mixture refluxed for 5 hours while stirring, the volume reduced to one-half of its original volume by heating on a water bath. The heated mixture was allowed to cool at room temperature and light-brown crystals obtained which were filtered, recrystallized using ethanol and then dried in a desiccator over anhydrous calcium chloride for 48 hours.

Synthesis of the metal (II) complexes: The Cobalt (II) and Copper (II) complexes were synthesized by mixing 0.75g (0.003mole) of each of the metal salts and 1.5g (0.006mole) of the Schiff base, (that is in a ratio of 1:2) in ethanol the mixture heated at reflux for 5hours in each case the complex formed was filtered, washed several times with hot ethanol and then dried over CaCl_2 in a desiccators [41].

3. CHARACTERIZATION TECHNIQUES

3.1 Solubility Test

The solubility test was carried out for both the Schiff base and the two complexes, the test was done using ethanol, methanol, petroleum ether, benzene, water, chloroform and dimethyl sulphoxide (DMSO). From each of the three samples 20mg was taken and dissolved into about 4cm^3 of the corresponding solvent in a test tube.

3.2 Melting Point

The melting point of the synthesized ligand as well as the decomposition temperature of the metal complexes were recorded using a Gallenkamp Melting Point Apparatus with a thermometer range of $0\text{-}360^\circ\text{C}$

3.3 Molar Conductance

The molar conductance of each of the two metal complexes was determined using a Jen way 4010 conductivity meter at a concentration of 10^{-3} M in DMSO. The measurement is used to determine whether a given compound is an electrolyte or non-electrolyte when in solution. Molar conductance is the conductivity of an electrolytic solution divided by the molar concentration of the electrolyte, and therefore measures the efficiency with which a given electrolyte conducts electric current in solution.

3.4 Magnetic Susceptibility Measurements

Magnetic susceptibility measurements are widely used to study the magnetic properties of transition metal complexes. The magnetic properties are due to the presence of paired or unpaired electrons in the filled or partially filled d-orbitals in the outer shell of these compounds.

3.5 Infrared (IR)

The IR spectra of the ligand and complexes were recorded on FT-IR-91184 (FTIR Spectrum II)

model in the range $450\text{-}4000\text{ cm}^{-1}$ using KBr pellets. This spectra is use to determine the coordinating atoms and the relative strength of the bonds.

3.6 Antibacterial Studies

The Antibacterial studies of the Schiff base and the metal complexes were carried out using *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* test organisms and Gentamicin as control and three fungal strains *Aspergillus fumigates*, *Candida albican* and *Mucor sps.* and Ketoconazole as control for the fungi. Using Agar-well diffusion method, the test organisms were prepared by dissolving them in peptone water and they were smeared over the surface of Muller Hilton Agar for the bacterial strain and potato dextrose agar for the fungal strains. The tests were carried out in triplicates i.e. the ligand and the complex were dissolved separately in DMSO. Three different concentrations used (15mg/ml, 30mg/ml, and 60mg/ml). A well was made on the agar medium inoculated with test organisms. The well was filled with the test solution using a micropipette and the plate was incubated 24 hours for the bacteria and 72 hours for fungi at 37°C . The results were obtained by measuring the diameter (in mm) of the zone of inhibition of the activities of the Schiff base and metal complexes against the test organisms [19].

4. RESULTS AND DISCUSSION

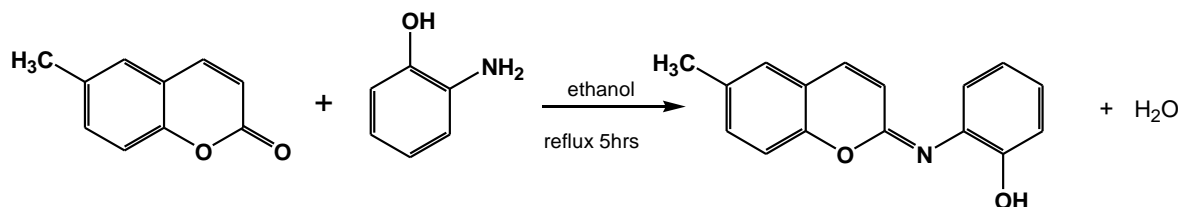
The results of the physical properties and the infrared of the ligand and that of the complexes are given in the following tables and discussed as follows.

4.1 Physical Properties of Ligand and its Metal Complexes

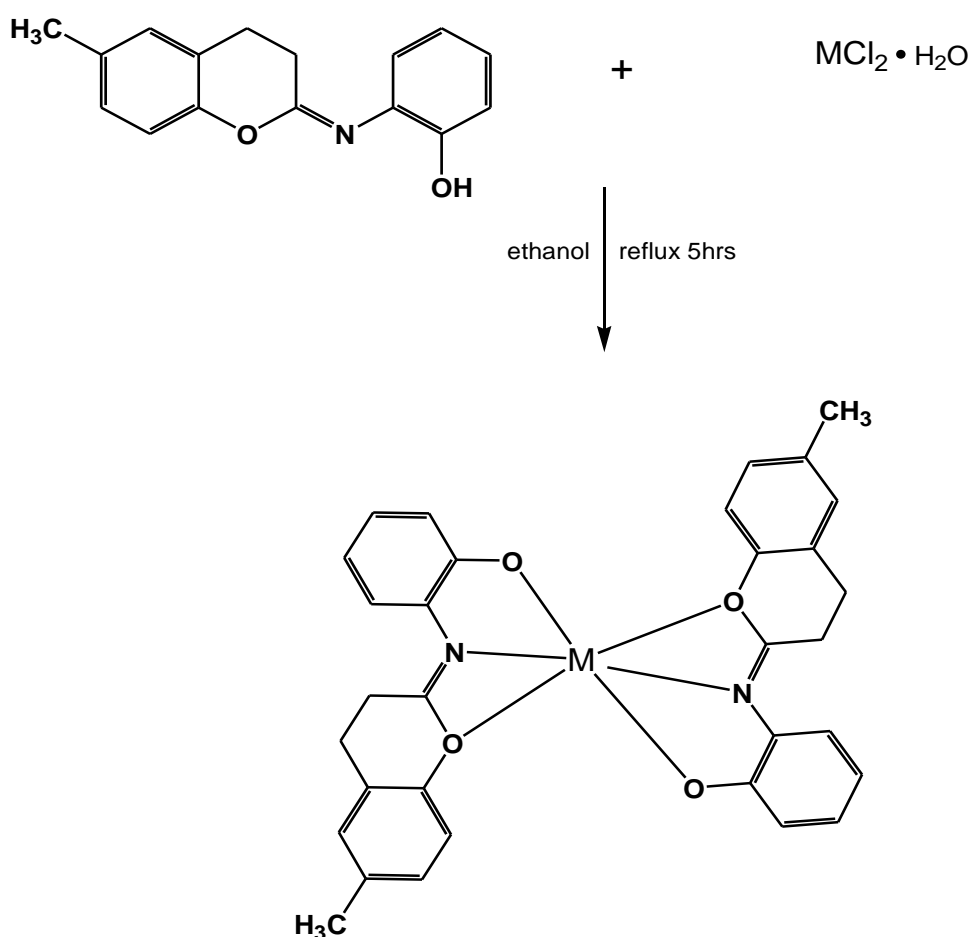
The reaction between 6-methyl coumarin with 2-aminophenol in a molar ratio of 1:1 produced the Schiff base ligand a pale brown coloured substance with percentage yield of 60.60 % (scheme 1). The interaction between the Schiff base ligand and the metal (II) ions in ratio of 2:1 ($\text{L}_2\text{:M}$) giving a reddish-brown cobalt complex with percentage yield of 71.80 % and a black coloured copper complex with a yield of 79.20% (scheme 2)The melting point of the ligand and the decomposition temperature of the cobalt and copper metal complexes found to be 158°C and 230°C and 264°C respectively (Table 1). The high melting point indicates that the compounds are stable and not easily decomposed [20]. The

higher melting points of the complexes also suggest the chelating effect of the ligand, as chelating ligands form more stable complexes. In another finding as quoted by Daniel and Kiremine [21,22] that the difference in melting point between ligand and their complexes proved

that the new compounds form are different from the parental ligands. Moreover, lower melting point of ligand than their respective complexes shows that complexation increases the intra-atomic bonding effect of particles of the compounds”.



Scheme 1. Synthesis of the Schiff base ligand



Scheme 2. Synthesis of the Schiff base metal complexes [M = Co, Cu]

Table 1. Some physical properties of the ligand and their complexes

Compound	Colour	M/Point (°C)	Yield (%)	Conductivity (μScm ⁻¹ mol ⁻¹)
Ligand	Pale-brown	158	60.60	-
Co complex	Reddish-brown	230	71.80	15.04
Cu complex	Black	264	79.20	12.76

4.2 Solubility Test of Ligand and its Metal Complexes

For the solubility the result in Table 2 revealed the ligand and the two complexes were tested in different solvents, the result shows none of the complexes nor the ligand was soluble in water this indicating all have low polarity. Ethanol and methanol dissolved the ligand and the two complexes, also the ligand and the complexes are all soluble in DMSO. Generally, transition metal complexes with organic ligands are relatively non-polar. As a result they are soluble in organic solvents but not in polar like the water. At molecular level, solubility is controlled by intermolecular forces. However, a simple empirical rule Likes dissolves like and is based on the polarity of the systems that is polar solvents and non-polar molecules in non-polar solvents [23].

4.3 Conductivity Measurement of Metal Complexes in 10^{-3} M DMSO

The molar conductivity values of the complexes in 10^{-3} M DMSO solution (Table 1) showed that of cobalt as $15.04\mu\text{Scm}^{-1}$ and $12.76\mu\text{Scm}^{-1}$ for the copper complex, the low values suggest the complexes are non-electrolytes [24,25] therefore occurs as a neutral solution. In 2006, Deligonul and Tumer [26] also ascertained that low value of molar conductance indicates non-electrolytic nature of a complex. The conductance of a solution is a measure of the ability of that solution to conduct a current. The use of conductivity enables the determination of ions in solution. The results obtained from magnetic susceptibility measurement (Table 3) revealed that the two complexes are all paramagnetic, which is due to the presence of unpaired electrons in the d-orbital of the metal ions, as such this make the complexes to also be less stable.

4.4 IR Spectra of the Schiff base and its metal complexes

"Fourier Transform Infrared Spectroscopy (FT-IR), an instrumental method of analysis based on the fundamental principles of molecular

spectroscopy, the FT-IR was utilized in order to identify the presence of certain functional groups in the synthesized compounds, and as an approach to confirm the identity of the compounds through the collection of absorption frequencies. The principle behind the usage is that the stretching modes of a ligand changes upon complexation due to subsequent changes in the positions of the bands appearing in the spectrum [27]. The IR spectra were assigned by comparing the spectra of the ligand with that of the metal complexes and also with literature reports on similar systems [28,29,30]. In the IR spectra of the ligand as shown in (Table 4) revealed a medium sharp band at 1599cm^{-1} , which was assigned to $\nu_{\text{C=N}}$ stretching of azomethine group [31]. In the complexes these bands has shifted to a lower region 1547cm^{-1} and 1592cm^{-1} for Cu (II) and Co (II) complexes respectively, these bands has suggested the coordination of azomethine nitrogen to the respective metal atoms due to complexation. [32,33]. The shift of the wave number is expected as a result of the coordination of the nitrogen atom of the azomethine group to the metal ions, which causes reduction in electron density in the azomethine link [34]. IR spectrum of the ligand shows a strong band at 3376cm^{-1} due to $\nu(\text{OH})$ ligand stretching vibrations. This band is absent in the spectra of the complexes indicating the dissociation of the phenolic proton on complexation and involvement of phenolic anionic oxygen in coordination [31]. A stretching vibration due to $\nu(\text{C-O})$ at 1268cm^{-1} was observed in the free ligand, [35,36,24] and shifted to higher frequencies of 1275cm^{-1} in copper and 1286cm^{-1} for the cobalt complexes; this shifts proved that there is coordination of the phenolic oxygen leading to the formation of C-O-M bond. The ligand showed a strong absorption band due to C-N which occurred at 1462cm^{-1} and shifted to a new position of 1487cm^{-1} in both complexes. This new value ascertained the nitrogen atom of the aminophenol has been involved in complexation [37,38]. From the IR results it may be understood that the Schiff base ligand coordinated to the metal ions through the azomethine nitrogen, hydroxyl group and the hetero oxygen of the ligand.

Table 2. Solubility of the ligand and their complexes

Compound	Ethanol	Methanol	Pet. Ether	Benzene	Water	Chloroform	DMSO
Schiff base	S	S	IS	SS	IS	SS	S
Co(L ₂)	S	S	IS	IS	IS	S	S
Cu(L ₂)	S	S	IS	S	IS	S	S

Key: S = Soluble SS = Slightly Soluble IS = Insoluble

Table 3. Magnetic susceptibility test result of the complexes

Complex	Co (L ₂)	Cu(L ₂)
Magnetic Susceptibility	13720 × 10 ⁻⁹	666.67 × 10 ⁻⁹

Table 4. Relevant infrared bands of the ligand and their complexes

Compound	$\nu(\text{O-H})$	$\nu(\text{C=N})$	$\nu(\text{C=O})$	$\nu(\text{C=C})$	$\nu(\text{C-N})$	$\nu(\text{M-N})$	$\nu(\text{M-O})\text{cm}^{-1}$
Ligand	3376	1599	1269	1514	1462	-	-
Co(L ₂)	-	1592	1286	1487	1487	739	437
Cu(L ₂)	-	1547	1275	1577	1487	757	448

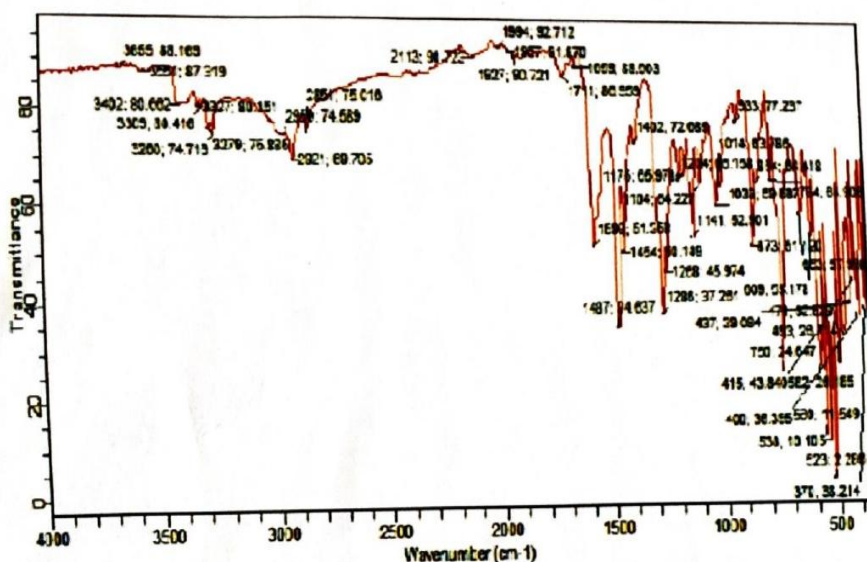


Fig. 1. FTIR of Cobalt (II) complex

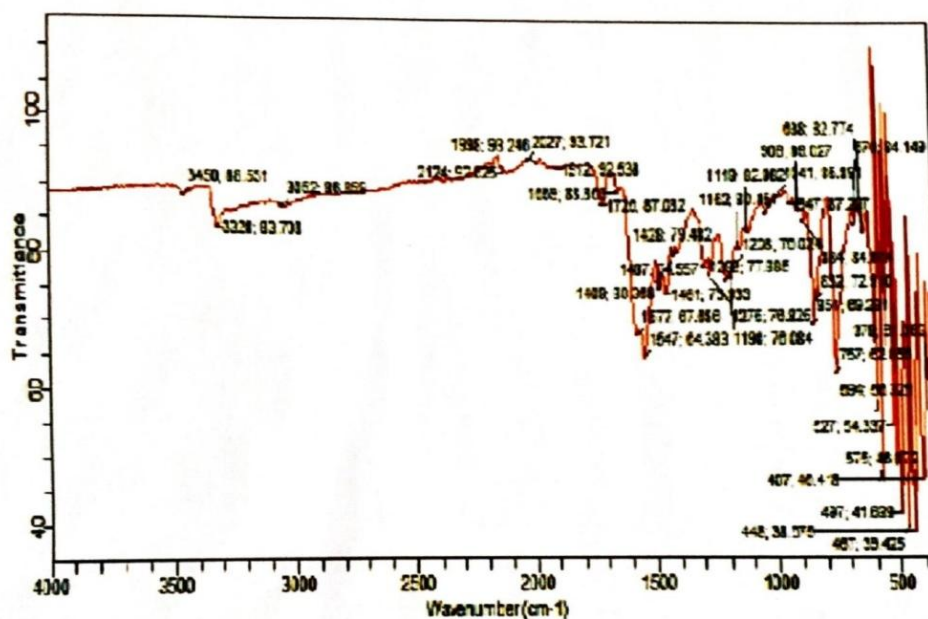


Fig. 2. FTIR of Copper (II) complex

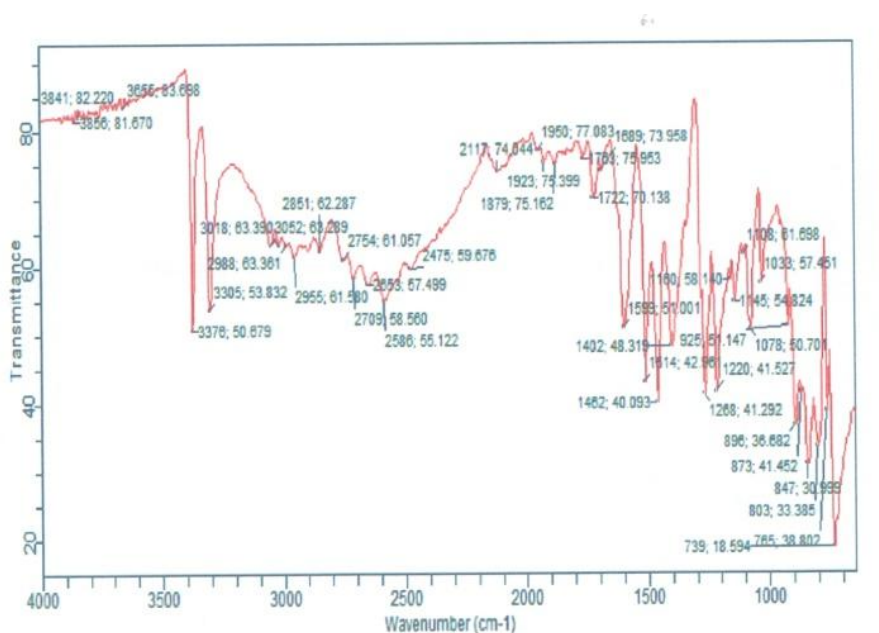


Fig. 3. FTIR of Schiff base

Table 5. Antimicrobial sensitivity test of Schiff base ligand and metal complexes showing zone of inhibition against bacterial and fungal isolates at various concentrations (mg/mL) using agar well diffusion method

Concentraions (mg/mL)	Co(L ₂)			Cu(L ₂)			Ligand			Gentamycin/ Ketoconazole (80/200)
	60	30	15	60	30	15	60	30	15	
<i>Staphylococcus aureus</i>	20mm	16mm	12mm	19mm	17mm	12mm	14mm	11mm	9mm	26mm
<i>Escherichia coli</i>	14mm	12mm	11mm	15mm	13mm	10mm	16mm	14mm	12mm	21mm
<i>Salmonella typhi</i>	20mm	16mm	14mm	18mm	16mm	14mm	11mm	10mm	-	17mm
<i>Candida albican</i>	-	-	-	18mm	15mm	13mm	18mm	16mm	12mm	19mm
<i>Aspergillus fumigates</i>	12mm	10mm	-	13mm	10mm	-	14mm	11mm	9mm	25mm
<i>Mucor spp.</i>	11mm	9mm	8mm	16mm	13mm	-	15mm	12mm	10	25mm

KEY: mg/mL - milligram per milliliter; mm - millimeter; Gentamycin – As antibacterial; Ketoconazole – As antifungal

4.5 Antimicrobial Activity

The Schiff base ligand and the two metal complexes were screened for antibacterial activity (*in vitro*) by agar well diffusion method against three bacterial strains and three fungal strains. Gram-positive bacteria such as *Staphylococcus aureus*, Gram-negative bacteria such as *Escherichia coli*, *Salmonella typhi* and three fungal strains *Aspergillus fumigates*, *Candida albican* and *Mucor spp.* were used for the test. The results of the biological activity indicated that the compounds are active against both gram (-) and (+) bacteria. The Schiff base ligand alone is slightly effective against all strains of the micro-organisms. The Cu(II) complex showed moderate activity against all bacterial and fungal strains compared to the control antibiotics used, and it was observed the effectiveness increases as with concentration

,except against *Aspergillus fumigates* and *Mucor spp.*in which the complex showed no action at a concentration of 15mg/mL. The cobalt complex showed high activity against *Aspergillus fumigates*, *Salmonella typhi* and moderate activity against *Escherichia coli*, and *Mucorsps.* but no activity against *Candida albican*, Conclusively the value for the minimum inhibition concentration (MIC) shows the Schiff base is more active against the unicellular fungus (*Candida albicans*) and Gram negative bacteria *Escherichia coli* this is because lower MIC value indicates higher drug activity, the compounds are therefore promising antimicrobials subject to *In vivo* study outcome.

5. CONCLUSION

The synthesized Schiff base ligand and the metal (II) complexes and also their biological potentials

investigated aiming at contributing towards search for novel metal complexes that exhibit various biological activities. The coordination through phenolic oxygen and imino nitrogen atom of the tridentate Schiff base ligand to the metal ions confirmed using the FTIR. The insolubility of the ligand and the two complexes in water may be due to their non-polar nature. The low molar conductance values of the metal (II) complexes showed their non-electrolytic nature. The infrared spectral data of the ligand when compared to those of the metal (II) complexes showed that coordination of the metal ion to the ligand is via the azomethine nitrogen, hydroxyl group of the ligand and the hetero oxygen in the coumarin. Their antimicrobial action has shown that the ligand is effective against the tested organisms but the complexes were observed to exhibit higher antibacterial action. The azomethine derivatives bearing C=N linkage is essential for biological activity, several azomethine have been reported to possess remarkable antibacterial, antifungal, anticancer and anti-malarial activities [39-41].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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