Bayero Journal of Pure and Applied Sciences, 11(2): 136 - 141
Received: February, 2018
Accepted: November, 2018
ISSN 2006 – 6996

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND *IN VITRO* ANTIBACTERIAL ACTIVITY STUDIES OF Cu(II) COMPLEXES DERIVED FROM 6-HYDROXYFLAVONE SCHIFF BASES

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ABSTRACT

This study reported the synthesis of the ligands (E) – 4 – ((2-hydroxyphenyl)imino)-2-phenyl-4H-chromen-6-o1 (L^1) and (E) – 2 – ((6-hydroxy-2-phenyl-4H-chromen-4-ylidene)amino)benzaldehyde (L^2). These two Schiff base ligands were synthesized by refluxing equimolar amount of 6-hydroxyflavone with 2-aminophenol and 2-aminobenzoic acid as ligands L^1 and L^2 respectively. The synthesized Schiff bases were subsequently reacted with Cu(II) acetate to form the corresponding metal complexes C_1 and C_2. The compounds were characterized using FT-IR, molar conductance, melting point and decomposition temperature studies, solubility test, magnetic susceptibility studies and gravimetric analysis. The purity of the compounds were analysed using TLC. The spectroscopic data indicated that the Schiff base ligands acted as tridentate and coordinated to the metal via imine nitrogen and deprotonated phenolic oxygen atoms. The Schiff base ligands have melting point between 166 – 185 °C, whereas the Cu(II) complexes decomposes in the range 161 – 202 °C. Magnetic susceptibility studies showed that the complexes are paramagnetic in nature with the μ_{eff} values are in the range of 1.9 - 2.1 BM. Molar conductance values of the complexes were found to be in the range of 20.4 - 33.5 $\Omega^{-1}cm^2\ mol^{-1}$ indicating that they are non-electrolytic in nature. The solubility test of the compounds indicated that they are soluble in most organic solvents except water, acetone and tetrachloro methane in which are completely insoluble. The Cu(II) acetate, synthesized ligands and their metal complexes were studied for their in vitro antibacterial activity against two gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two gram negative bacteria (Escherichia coli and Salmonella typhi) using agar well diffusion method and the results indicated that the complexes were more active than the ligands and the Cu(II) acetate but less active compared to standard drug (ciprofloxacin).

Keywords: 6-hydroxyflavone, 2-aminophenol, 2-aminobenzoic acid, Schiff bases and antibacterial activity.

INTRODUCTION

Microbes have developed resistance to antibiotics which indicates necessity for the search of new compounds which have potential effects against various pathogenic bacteria. Metal ions have unique effects on these pathogens as far as medicinal inorganic chemistry is concerned and it gives a direction for the synthesis of new drugs (Bruijnincx and Sadler, 2008; Jyoti *et al.,* 2015). Most of the organic ligands fall within this category of antibiotics, since they have been used in the synthesis of drugs. However, coordination of the metal ion with ligands potentially increases the biological activity of these compounds. The synthesis and study of coordination compounds containing biologically important ligands have been an area of interest in recent years because of their wide applicability in biology, environment and other systems (Ahmed and Ibrahim, 2015). Transition metal complexes derived from Schiff base ligands are among the most widely studied coordination compounds (Refat *et al.,* 2013), perhaps due to biochemical and antimicrobial properties. Copper(II) complexes are particularly attractive since Cu(II) is known to play a significant role in naturally occurring biological systems as well as a pharmacological agent (Miura *et al.*, 1999). Flavones constitute one of the major classes of naturally occurring products, synthesis of flavones and their derivatives has attracted considerable attention owing to their significant biocidal (Kumar *et al.*, 2008), pharmaceutical (Patroniak *et al.,* 2005) and antioxidant activities (Yang *et al.*, 2008). They are naturally occurring heterocyclic compounds, widely distributed among higher plants in the form glycosides.

The 6-hydroxyflavone is a part of the important class of compounds of flavones, which occur naturally in plants and has potential beneficial effects for human health. Pharmacological and spectral studies of synthetic biomimetic copper complexes derived from 3-hydroxyflavone derivatives as anti inflammatory agents was carried out (Nagashri *et al* 2011).

Diana-carolina *et al.*, (2014), reported synthesis, characterization, crystal structure and antimicrobial activity of copper(II) complexes with thiosemicarbazone derived from 3-formyl-6-methylchromone. Therefore, this paper discusses the synthesis, spectroscopic characterization and *in vitro antimicrobial* activity studies of Cu(II) complexes derived 6-hydroxyflavone Schiff bases due to paucity of information.

MATERIALS AND METHODS

All glass wares were washed with detergent rinsed with distilled water and dried in an oven before use. Chemicals and solvents used were of analytical grade and purchased from BDH and Sigma-Aldrich, they were used without further purification. All weighing were carried out on a digital weighing balance (Sartorius ED 2245), melting point and decomposition temperature were determined using Gallenkamp melting point apparatus. Molar conductivity was determined using Jen way 4010 model, FT-IR Spectral analysis was recorded on a Fourier Transformed spectrophotometer cary 630 Agilent Technologies model within wavenumber range of 4000–650 cm^{-1}. The magnetic susceptibility measurement for the synthesized complexes was obtained at room temperature using magnetic susceptibility balance (MSB) (Sherwood scientific Cambridge). The gram magnetic susceptibility (X_g) of the complexes is thus evaluated using the relationship;

$$X_g = \frac{C \times L\,(R-Ro)}{10^9 \times M}$$

Where, C is the proportionality constant, 1.0, L is the length of sample in capillary column, R is the magnetic susceptibility of the filled capillary tube, Ro is the magnetic susceptibility of the empty capillary tube and M is the mass of sample in the capillary tube.

The molar magnetic susceptibility (Xm) was obtained using the formula;

$$Xm = Xg \times MW,$$

Where, *MW* is the molecular weight of the complex.

Hence, effective magnetic moments (μeff) for the complexes were evaluated using the expression;

$$\mu_{eff} = 2.83\sqrt{Xm \times T}$$

Where,T is the room temperature in Kelvin, and μ_{eff} is the effective magnetic moments in Bohr magneton (BM) (Jamil *et al.,* 2015).

Synthesis of Schiff base ligands

The Schiff base ligands (L^1 and L^2) were synthesized according to the procedure reported by Nagashri *et al.* (2011). This involves the condensation of 6-hydroxyflavone (0.1 g, 0.42 mmoles) with 2-aminophenol (0.05 g, 0.42 mmoles), 2-aminobenzoic acid (0.06 g, 0.42 mmoles) as L^1 and L^2 respectively and were dissolved in ethanol (40 mL). Acetic acid (1.0 mL) was also added to each of the solutions and refluxed for 3 hours on a water bath with stirring. The solution was cooled at room temperature and placed in ice to allow the formation of precipitate. The precipitate formed was filtered, washed with acetone, dried at room temperature and kept in a dessicator for further analysis.

Synthesis of the Cu(II) complexes

The complexes were synthesized as described by Nagashri *et al.,* (2011). This was done by dissolution of copper(II) acetate monohydrate (0.012 g, 0.06 mmoles) and ligand L^1 (0.04 g, 0.12 mmoles) in acetone (20 mL), that is metal to ligand ratio of 1:2. The solution was swirled and triethylamine was added drop-wise. The solution was stirred for 2 hours at room temperature and packed under ice which resulted in the formation of a precipitate. The precipitate was filtered, purified by washing several times with acetone and dried in a dessicator. The same procedure was applied to the other ligand (L^2).

In *Vitro* antibacterial activity

The synthesized Schiff base ligands, their Cu(II) metal complexes and the standard drug were studied for their *in vitro* antibacterial activity against four bacterial strains: *S. aureus, B. subtilis, E. Coli,* and *S. typhi*. The culture media used for the analysis was Nutrient agar (NA). The mentioned media was used for the minimum inhibitory concentration (MIC). The compounds were screened using the agar well diffusion method (Chohan and Kausar, 2000). The minimum inhibitory concentration (Table 5) was considered to be the lowest concentration that has completely inhibited the growth of the test organisms on agar plates.

The standard drug used as the control is ciprofloxacin (Broad spectrum antibacterial) purchased from rukayya pharmacy samaru, zaria.

RESULTS AND DISCUSSION

Physical properties of the compounds

The physical properties of the Schiff base ligands and Cu(II) complexes are presented in Table 1. All the compounds are coloured and were found to be stable at room temperature. The percentage yield of the compounds are obtained from 60.20 – 72.01 % for the Schiff base ligands and 58.30 – 60.10 % for the Cu(II) complexes, and these are in close agreement with the findings reported (60 – 76 %) by Nagashri *et al*, (2011) for similar Schiff base metal complexes. The moderately higher decomposition temperature suggested a good thermal stability of the complexes Aliyu and Ado, (2010).

Table 1: Physical properties of the compounds

Compounds	M.F	M.P (°C)	D.T (°C)	Colour	% Yield
L^1	$C_{21}H_{17}O_2N$	180	-	Brown	60.20
L^2	$C_{22}H_{17}NO_3$	183	-	Milky	72.01
C_1	$C_{42}H_{28}CuN_2O_8$	-	197-200	Light brown	58.30
C_2	$C_{44}H_{26}CuN_2O_8$	-	200-202	Pale blue	60.10

Key: M.F = Molecular formula, M.P = Melting point (°C), D.T = Decomposition temperature (°C),
L^1 = (E) – 4 – ((2-hydroxyphenyl)imino)-2-phenyl-4H-chromen-6-o1,
L^2 = (E) – 2 – ((6-hydroxy-2-phenyl-4H-chromen-4-ylidene)amino)benzaldehyde,
$C_1 = [Cu(L^1)_2]\cdot 3H_2O$ and
$C_2 = [Cu(L^2)_2]\cdot 3H_2O$.

Infrared spectral data

The IR spectral data (Table 2) of the schiff base ligands (L^1 and L^2) showed strong absorption bands at 1651 cm^{-1} each, which were due to azomethine vC=N. In the complexes, the absorption bands for vC=N have slightly shifted to lower frequencies of 1602 and 1561 cm^{-1} in the metal complexes C_1, and C_2 respectively and this might be due to the participation of azomethine nitrogen in the coordination with the copper ion and these are in agreement with the work of some researchers (Raman *et al.,* 2006; Nagashri *et al.*, 2011). Bands observed around 1575-1400 cm^{-1} in the spectra of the ligands and their Cu(II) complexes were due to v(C=C) stretching of the aromatic ring system. Similar results were obtained in the studies conducted by Heba, (2011). The compounds showed bands in the region 3400-3100 cm^{-1}, which were assigned to phenolic v(O-H) stretches (Aliyu and Abdullahi, 2009). These data are in close agreement with data reported by Prabhakara *et al.* (2016) for similar schiff base ligands and their metal complexes. Bands around 1300-1200 cm^{-1} in the spectra of the ligands were due to phenolic vC-O (Prabhakara *et al.,* 2016) and were observed to shift upward (1350-1400 cm^{-1}) in the spectra of the complexes and this confirms the participation of phenolic oxygens in complexation with the Cu metal and this observation suggest that –OH groups have been replaced by the metal ion forming M-O bonds via deprotonation (Shelke *et al.,* 2011).

Table 2: FT-IR data of the Schiff base ligands and their Cu(II) complexes

Compounds	v(C=N) (cm^{-1})	v(C=C) (cm^{-1})	v(C-O) (cm^{-1})	v(OH) (cm^{-1})
L^1	1651	1572	1285	3172
L_2	1651	1570	1285	3168
C_1	1602	1572	1356	3175
C_2	1561	1572	1356	3172

Key:
L^1 = (E) – 4 – ((2-hydroxyphenyl)imino)-2-phenyl-4H-chromen-6-o1,
L^2 = (E) – 2 – ((6-hydroxy-2-phenyl-4H-chromen-4-ylidene)amino)benzaldehyde,
$C_1 = [Cu(L^1)_2]\cdot 3H_2O$ and
$C_2 = [Cu(L^2)_2]\cdot 3H_2O$.

Molar Conductance Studies, magnetic susceptibility and solubility Test

The measured molar conductance of the complexes in 10^{-3} M dimethylsulphoxide determined as reported by Geary*,* (1971) are in the range of 20.4 - 33.5 ohm^{-1}cm^{2}mol^{-1}, which are relatively low, indicating their non electrolytic nature (Table 3). The magnetic moments of all the complexes were found to be in the range of 1.9 - 2.1 B.M at room temperature, suggesting an octahedral geometry around the copper(II) ions (Venkateswar *et al.,* 2007). The solubility test (Table 4) showed that the compounds are insoluble in acetone, water and tetrachloro methane but soluble in DMSO, methanol and ethanol. The water molecules associated with the complex formation are outside the coordination sphere because heating the hydrated complexes in the oven within temperature range of 80-110°C for 2-3 hours leads to dehydration, with corresponding weight losses of 6.5 and 7.5 %. Therefore, the percentage water of crystallization of the complexes was found to be 6.5 and 7.5 % for complexes C_1 and C_2 respectively.

Table 3: Molar conductivity measurements of the Complexes in 10^{-3}M DMSO

Complexes	Specific Conductance (ohm^{-1}cm^2)	Molar Conductance (ohm^{-1}cm^{2}mol^{-1})
C_1	20.4×10^{-6}	20.4
C_2	33.5×10^{-6}	33.5

Key: $C_1 = [Cu(L^1)_2]\cdot 3H_2O$, $C_2 = [Cu(L^2)_2]\cdot 3H_2O$, DMSO = dimethylsulphoxide

Table 4: The Solubility of the ligands and their Cu(II) complexes in some common solvents

Compound	Tetrachloro-methane	Acetone	DMSO	Ethanol	Methanol	Water
L^1	IS	IS	S	S	S	IS
L_2	IS	IS	S	S	S	IS
C_1	IS	IS	S	S	S	IS
C_2	IS	IS	S	S	S	IS

Key: IS = Insoluble, S = Soluble, SS= Slightly soluble, DMSO = dimethylsulphoxide

L^1 = (E) – 4 – ((2-hydroxyphenyl)imino)-2-phenyl-4H-chromen-6-o1,

L^2 = (E) – 2 – ((6-hydroxy-2-phenyl-4H-chromen-4-ylidene)amino)benzaldehyde

$C_1 = [Cu(L^1)_2]\cdot 3H_2O$ and $C_2 = [Cu(L^2)_2]\cdot 3H_2O$.

Antibacterial activity

The in *vitro* antibacterial activity of the synthesized compounds and standard drugs was carried out against four phatogens: *S. aureus, B. subtilis, E. coli* and *S. typhi* which are bacterial strains, two gram positive and two gram negative respectively. The MIC result presented in Table 5, shows that no activity was observed on the ligands against *B. subtilis* and *S. typhi,* but complexes C_1 and C_2 showed inhibitory activity of 50 and 25 mg/mL each against *B. subtilis* and *s .typhi* respectively. When compared to the standard drug having an activity of 32 mg/mL, the complexes indicated lower activity than the standard drug with respect to *B. subtilis* but better activity with regards to *S. typhi.* Similar results were obtained in the studies conducted by Jain *et al.* (2002). As far as *S. aureus*, complex C_2 has the best activity as all the other compounds and thus proving their potency as an antibacterial agent. On comparing with the standard drug, the complex (C_2) and its corresponding ligand L^2 and complex C_1 showed better activity than the standard drug. All the complexes showed the same inhibitory activity (25 mg/mL) against *E. coli.* Compared to the standard drugs and the ligands indicated that the complexes showed better inhibitory activity against the same organism. However, as expected, the complexes are more active than the ligand against the tested organisms. This may be due to the partial sharing of their positive charge with donor groups of the ligands and possible π-electron delocalisation on the aromatic rings which in turn increased the lipophilic character, favouring its permeation into the bacterial membrane, thus inhibiting the growth and possibly the death of the organisms (Raman *et al.*, 2006). Furthermore, better activity of the metal complexes than the ligands was explained based on Overtones concept (Anajaneyula *et al.,* 1986) and chelation theory (Dharamaraj *et al.,* 2001).

Table 5: Minimum inhibitory concentration of the compounds against the Test organisms

Compounds and	Bacteria			
Control	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*
L^1	35	ND	33	ND
L^2	20	ND	32	ND
C_1	25	50	25	25
C_2	12.5	50	25	25
Ciprofloxacin	30	32	30	32

Key: ND = Not detected, L^1 = (E) – 4 – ((2-hydroxyphenyl)imino)-2-phenyl-4H-chromen-6-o1,

L^2 = (E) – 2 – ((6-hydroxy-2-phenyl-4H-chromen-4-ylidene)amino)benzaldehyde

$C_1 = [Cu(L^1)_2]\cdot 3H_2O$ and $C_2 = [Cu(L^2)_2]\cdot 3H_2O$

From the analysis of the complexes, the following general molecular structures are proposed;

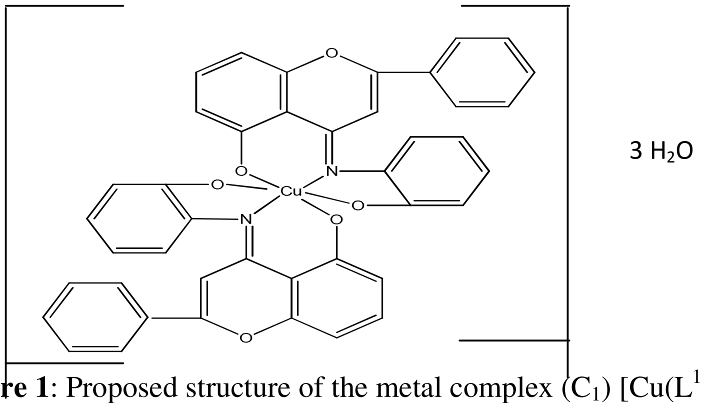

Figure 1: Proposed structure of the metal complex (C_1) $[Cu(L^1)_2]\cdot 3H_2O$

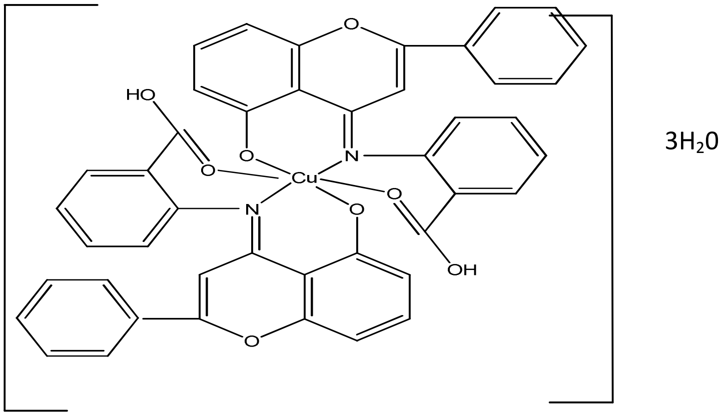

Figure 2: Proposed structure of the metal complex (C_2) [$Cu(L^2)_2$] ·$3H_2O$

CONCLUSION

In this paper the synthesis of Schiff base ligands derived from 6-hydroxyflavone with 2-aminophenol and 2-aminobenzoic acid and their Cu(II) complexes have been described. The Schiff base ligands coordinated through its azomethine nitrogen and oxygen atom after deprotonation and this was supported by infrared spectral data. The molar conductivity data of the complexes indicated that they are non electrolytes. The compounds are stable in air and soluble in solvents like methanol, ethanol DMSO but insoluble in acetone and tetrachloro methane. The *in vitro* antibacterial studies shows that the Cu(II) complexes have higher activities compared to the Schiff base ligand but less active compared to the standard drug (Ciprofloxacin).

Contribution of Authors

We will like to thank Prof. S.O. Idris and Dr. G.A Shallangwa of the Department of Chemistry ABU Zaria of their inspiring guidance and contribution towards the success of this research. Also, we will like to acknowledge the contribution of my colleagues Musa Muhammad and Aisha A. Abdullahi for their motivation.

Conflict of Interest

The authors of this paper declared no conflict of interest.

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