
Synthesis, Characterization and Antimicrobial Activity of Cu(II) And Zn(II)
Metal Complexes from 2-[(2-Hydroxyphenyl)Imino]Methyl Phenol

By

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ABSTRACT

The Schiff base was synthesized by refluxing equimolar amount of 2-aminophenol and Salicylaldehyde. It was subsequently reacted with metal(II) salts to form the corresponding metal(II) complexes. The compounds were characterized by solubility test, melting point and decomposition temperature, magnetic susceptibility, Atomic absorption spectrophotometry (A.A.S), FTIR analysis, molar conductance. The compounds were observed to be soluble in Dimethylsulfoxide (DMSO), Dimethylformamide (DMF), insoluble in water, methanol, ethyl ether, n-hexane, ethyl acetate, petroleum, ether, but slightly soluble in acetone, ethanol and chloroform. The melting point and decomposition temperature of the Schiff base and its metal(II) complexes were found to be within the range of 69.5-263 °C. The magnetic moment value of Cu complex is 1.82 BM but Zn complex is diamagnetic. The elemental analyses of the complexes were observed using A.A.S and the metal to ligand ratio was established to be 1:1. Molar conductivity analyses indicated that the ligand acts as neutral and tridentate coordinated through azomethine nitrogen atom to the central metal ion and the oxygen atoms of the phenolic group. Molar conductance values of the metal(II) complexes were found to be in the range of 11.69-13.07 $\Omega^{-1}\text{cm}^2 \text{mol}^{-1}$. The ligand and its metal(II) complexes were also screened for antibacterial and antifungal activities against three bacteria isolates (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*) and three fungi isolates (*Aspergillus flavus*, *Mucor iudicus*, and *Aspergillus niger*) by disc diffusion method respectively, and the result indicated that the complexes were more active than the free ligand.

Keywords: Schiff base, Salicylaldehyde, 2-aminophenol, 2-hydroxybenzylaldehyde, Synthesis, Characterization, Metal complex.

INTRODUCTION

Katarzyna, *et al.*, (2014) reported the synthesis of Schiff bases which are condensation products of primary amines and carbonyl compounds, and the Schiff bases were first discovered by a German chemist Hugo Schiff who won the Nobel prize in 1864. The Schiff base (also known as imine or azomethine) is an analogue of a ketone or aldehyde in which the carbonyl group (C=O) has been replaced by an imine or azomethine group. Schiff base ligands are essential in the field of coordination chemistry, especially in the development of complexes of Schiff bases because these compounds are potentially capable of forming stable complexes with metal ions, Sauza *et al.*, (1985).

Prasad *et al.*, (2014) reported that, Schiff bases are important intermediates for the synthesis of various bioactive compounds. Furthermore, they are reported to show a variety of biological activities including antibacterial, antifungal, anticancer and herbicidal activities and have many applications as ligands in coordination chemistry of transition metals. There are various applications of Schiff bases and their metal chelates in biological systems catalysis, dying processes, and analytical applications.

Schiff base is a compound that contains azomethine group ($>C=N-$) connected to an aryl or alkyl group. It can be synthesized from an amine and a carbonyl compound by nucleophilic addition forming a hemi-aminal group followed by dehydration to generate an imine compound, Uddin *et al.*, (2014).

Mounika *et al.*, (2010) also reported that, Schiff bases are considered as a very important class of organic compounds, which have wide applications in many biological aspects.

Schiff bases are aldehyde or ketone-like compounds in which the carbonyl group is replaced by an imine or azomethine group, they are widely used for industrial purposes and also exhibit a broad range of biological activities, such as antimalarial, antibacterial, antifungal, and antiviral. An overview of synthetic methodologies used for the preparation of Schiff bases have been described. Schiff bases are some of the most widely used organic compounds. They are used as pigments, dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers. Schiff bases have also been shown to exhibit a broad range of biological activities including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties and also used in the design and development of anticancer chemotherapeutic agents. Imine or azomethine groups are present in various natural, natural-derived, and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities. Schiff base ligands are able to coordinate too many different metals and stabilized in various oxidation states, Cleiton, *et al.*, (2010).

Xavier, and Srividhya, (2014) synthesized Schiff base which have significant importance in Chemistry, especially in the development of Schiff base complexes, because Schiff base compounds are potentially capable of forming stable complexes with metal ions, many Schiff base complexes show excellent catalytic activity in various reaction at high temperature and in the presence of moisture, the Schiff bases have very flexible and different structures, a wide range of Schiff base compounds and their behavior have been studied because these compounds have very flexible and diverse structure. Schiff bases are generally bi, tri, or tetra-dentate chelate ligands and form very stable complexes with metal ions. Their chemical and physical properties in various field such as preparative uses, identification, or protection and determination of aldehyde or ketones, purification of carbonyl and amino compounds or production of these compounds in complex or sensitive reactions have been studied by various workers.

This research aimed at the synthesis of Schiff base from 2-[(2-hydroxyphenylimino)methyl]phenol and to prepare its metal(II) complexes of Copper and Zinc. Characterize and study their antibacterial and antifungal activities

MATERIALS AND METHODS

Materials

All glass wares used in this research work were properly washed with detergent, rinsed with distilled water and dried in an oven at 110 °C. All reagents were of analytical grade and were used without further purification. Conductivity measurement was carried out in DMSO solvent using Jenway 4010 conductivity meter. The FTIR spectral analyses were recorded using SHIMADZU FTIR 8400S model. Melting point and decomposition temperature measurements were obtained using a SMP10 STUART melting point apparatus. The *in vitro* antimicrobial screening was performed using disc diffusion method at Department of Biological Science, Bayero University, Kano.

Preparation of Schiff base (Ligand)

2-aminophenol (1.09 g, 0.01 mol) in 40 cm³ ethanol was added to 2-hydroxybenzaldehyde (1.06 cm³, 0.01 mol). The resulting solution was refluxed for 2 hours. It was then concentrated and allowed to cool. The mixture was filtered, washed thoroughly with ethanol. The precipitate was dried over calcium chloride (CaCl₂) in a desiccator, Abdullahi and Gareth, (2013).

Synthesis of metal(II) complexes

The hydrated metal(II) chloride (0.01 mol) in 40 cm³ ethanol was added to a solution of Schiff base (2.13 g, 0.01 mol) in 40 cm³ ethanol. The resulting mixture was refluxed for 2 hrs. It was then concentrated and allowed to cool. The precipitate was filtered, washed thoroughly with ethanol. The precipitate was dried over calcium chloride (CaCl₂) in a desiccator, Abdullahi and Gareth, (2013).

Determination of metal ion in the metal (II) complex

Three stock solutions of each metal complex for determination of metal ion contents in the metal(II) complexes were prepared by digesting 0.04 g of each metal(II) complex in 1:3 aqua regia (i.e 4 cm³ HNO₃ acid): (12 cm³ HCl) acids for 15-20 mins, then the resulting solutions were filtered and added to mark in 100 cm³ Volumetric flask with distilled water. The calibration solutions were obtained by serial dilution of these three stock solutions with distilled water and the concentrations of metal ions were measured against the blank solution using Atomic absorption spectrophotometry Hassan *et al.*, (2013).

Determination of Water of Crystallization

0.2 g of each complex was placed in a cleaned crucible of known weight. The crucible and its content were placed in an oven at 110 °C until constant weight was obtained. The percentage of water of crystallization in the complex was calculated using the following equation below, Vogel, (1972).

$$\text{Percentage of water of Crystallization} = \frac{\text{weight loss}}{\text{weight of the complex taken}} \times 100 \%$$

Antibacterial Activity

The *in vitro* antibacterial activity of the Schiff base and its metal(II) complexes was assayed by agar disc diffusion method using cultures of *staphylococcus aureus*, *Escherichia coli*, and *salmonella typhi* using Amoxicillin as the reference drug, and DMSO-wetted disc was used as negative control, Khan *et al.*, (2014).

Antifungal Activity

The antifungal activity of the Schiff base and its corresponding metal(II) complexes was tested against three fungal isolates (*Aspergillus flavus*, *Aspergillus niger* and *Mucor iudicus*) using various concentrations by disc diffusion method. Ketoconazole was used as reference drug and DMSO as a negative control, Khan *et al.*, (2014).

RESULTS AND DISCUSSION

The results of the various analysis of the synthesized 2-[(2-hydroxyphenyl) imino]methyl} phenol and its metal complexes of Cu(II); and Zn(II) are presented in the following tables

RESULTS

Table 4.1: Physical Properties of the ligand and its metal(II) complexes

Compound	F/ weight(gmol ⁻¹)	Colour	% yield	M.P(°C)	Dec. Temp.(°C)
Ligand (L)	213.00	Red	69.50	190	-
[CuL(H ₂ O) ₃].H ₂ O	364.54	Green	81.50	-	263
[ZnL(H ₂ O) ₃].H ₂ O	420.37	Yellow	76.60	-	215

Ligand (L) = C₁₃H₉NO₂

Dec. Temp =Decomposition temperature

Table 4.2: Solubility test of the Schiff base and its metal(II) complexes

Compound/Solvent	Ligand(L)	[CuL(H ₂ O) ₃].H ₂ O	[ZnL(H ₂ O) ₃].H ₂ O
Water(H ₂ O)	IS	IS	IS
Methanol	IS	IS	IS
Ethanol	SS	SS	SS
Ethyl ether	IS	IS	IS
N-hexane	IS	IS	IS
Ethyl acetate	S	IS	IS
Chloroform	IS	SS	SS
P/ ether	IS	IS	IS
Acetone	S	SS	SS
DMF	S	S	S
DMSO	S	S	S

KEY: IS= insoluble, S= soluble, and SS= slightly soluble. Ligand (L) =C₁₃H₉NO₂

DMF= Dimethylformamide, and DMSO= Dimethylsulphoxide.

Table 4.3: Molar conductance of the metal(II) complexes in (1x10⁻³ moldm⁻³) DMSO solution

Compound	Concentration (moldm ⁻³) x10 ⁻³	Specific Conductance (Ohm ⁻¹ cm ⁻¹) x10 ⁻⁶	Molar Conductance (Ohm ⁻¹ cm ² mol ⁻¹)
[CuL(H ₂ O) ₃].H ₂ O	1.00	13.07	13.07
[ZnL(H ₂ O) ₃].H ₂ O	1.00	11.69	11.69

Ligand (L) =C₁₃H₉NO₂

Table 4.4: Magnetic Susceptibility values of the metal(II) complexes

Compound	Gram Susceptibility (X _g) (g ⁻¹)	Molar Susceptibility (X _m) (mol ⁻¹)	Paramagnetic Contribution (X _p)	μ _{eff} (B.M)
[CuL(H ₂ O) ₃].H ₂ O	4.569x10 ⁻⁶	1.254x10 ⁻³	1.392x10 ⁻³	1.82
[ZnL(H ₂ O) ₃].H ₂ O	-1.409x10 ⁻⁷	Dia

Ligand (L) = C₁₃H₉NO₂**Table 4.5:** Determination of metal ion in the metal(II) complexes

Compound	Metal ion % Observed	Metal ion % Calculated
[CuL(H ₂ O) ₃].H ₂ O	15.49	17.41
[ZnL(H ₂ O) ₃].H ₂ O	14.46	16.24

Ligand (L) = C₁₃H₉NO₂**Table 4.6:** Infrared Spectral data of the ligand and its metal(II) complexes

Compound	ν(C=N) (cm ⁻¹)	ν(OH) (cm ⁻¹)	ν(H ₂ O) (cm ⁻¹)	ν(C-O) (cm ⁻¹)	ν(M-N) (cm ⁻¹)	ν(M-O) (cm ⁻¹)
Ligand(L)	1627	3404	—	1229	—	—
[CuL(H ₂ O) ₃].H ₂ O	1614	—	3404	1289	519	432
[ZnL(H ₂ O) ₃].H ₂ O	1626	—	3404	1285	601	490

Ligand (L) = C₁₃H₉NO₂**Table 4.7:** Antibacterial activity inhibition zones of the ligand and its metal(II) complexes.

Compound	Concentration (μg/cm ³)	Bacterial inhibition zones (mm)		
		<i>Staphylococcus aureus</i> (mm)	<i>Escherichia Coli</i> (mm)	<i>Salmonella typhi</i> (mm)
Ligand (L)	1000	07	09	08
	2000	06	08	10
	3000	09	07	09
	2000	12	15	14
	3000	14	20	17
[CuL(H ₂ O) ₃].H ₂ O	1000	11	11	10
	2000	12	15	15
	3000	16	18	14
[ZnL(H ₂ O) ₃].H ₂ O	1000	09	10	11
	2000	11	13	13
	3000	13	18	16
Amoxicillin	1000	14	16	17
	2000	17	21	20
30μg/cm ³	3000	24	29	25

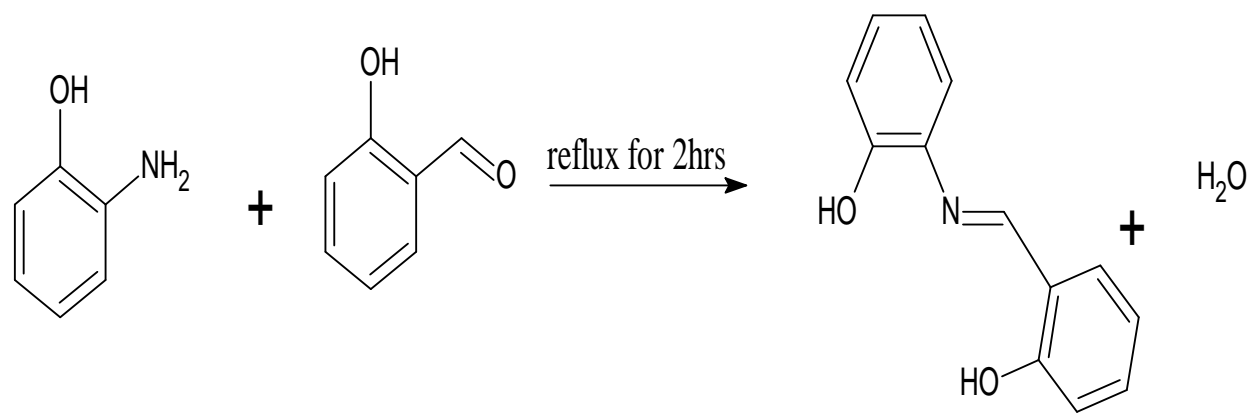
Ligand (L) = C₁₃H₉NO₂

Table 4.8: Antifungal activity inhibition zones of the ligand and its metal(II) Complexes.

Compound	Concentration ($\mu\text{g}/\text{cm}^3$)	Fungal inhibition zones (mm)		
		<i>Aspergillus flarus</i> (mm)	<i>Mucor iudicus</i> (mm)	<i>Aspergillus Niger</i> (mm)
Ligand (L)	1000	06	03	04
	2000	08	05	06
	3000	09	07	07
	2000	14	15	10
	3000	17	18	18
[CuL(H ₂ O) ₃].H ₂ O	1000	11	09	11
	2000	15	12	14
	3000	18	17	17
[ZnL(H ₂ O) ₃].H ₂ O	1000	10	11	12
	2000	12	16	15
	3000	20	20	19
Ketoconazole	1000	16	15	13
	2000	18	20	19
200mg	3000	27	26	23

Ligand (L) = C₁₃H₉NO₂**DISCUSSION**

The Schiff base obtained was prepared as reported by Abdullahi and Gareth, (2013).



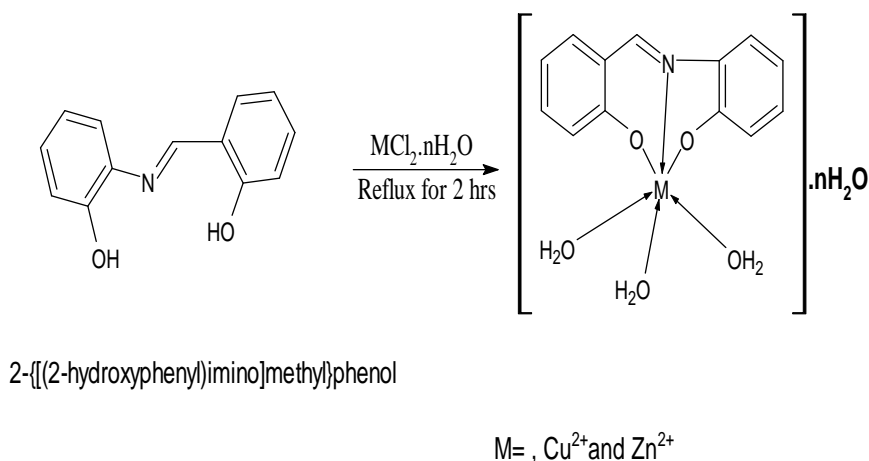
2-aminophenol

2-hydroxybenzaldehyde

2-[(2-hydroxyphenyl)imino]methylphenol

Scheme 1.2: Preparation of Schiff base

The percentage yield of the Schiff base is 69.5%, a melting point of 190 °C and is red in colour. The interaction of the Schiff base with metal(II) chloride yielded the metal(II) complexes of Cu(II) and Zn(II) as can be seen below, Abdullahi and Gareth, (2013) **Scheme 1.3.**



Scheme 1.3: Synthesis of Metal complexes

The complexes are Green and yellow, which yields at 81.50 %, and 76.60 % respectively, these complexes showed appreciable yields as compared with similar report by Mounika *et al.*, (2013). All the metal(II) complexes were found to contain molecules of water of hydration as evident by the presence of peaks in the range of $3403\text{-}3405\text{ cm}^{-1}$, with decomposition temperatures of $263\text{ }^{\circ}\text{C}$, and $215\text{ }^{\circ}\text{C}$ which indicated thermal stability with similar report presented by Kriza *et al.*, (2010). The result is shown in (Table 4.1). The ligand was tested for solubility in some common solvents, it was found to be insoluble in water, methanol, ethyl ether, n-hexane, chloroform, and petroleum. It is Soluble in ethyl acetate, acetone, DMF, and DMSO, this may be attributed to its fairly high polarity while slightly soluble in ethanol only. The solubility tests of metal(II) complexes in the same solvents indicated that all the complexes were soluble in DMSO, and DMF. But they were insoluble in water, methanol, ethyl ether, n-hexane, petroleum ether, ethyl acetate, chloroform, this may be due to poor polarity. Cu(II), and Zn(II) complexes were slightly soluble in ethanol, acetone and chloroform. The molar conductance values of the metal(II) complexes were found to be in the range of $11.69\text{-}13.07\ \Omega^{-1}\text{ cm}^2\text{ mol}^{-1}$ which is low, revealing the non-electrolytic nature of the metal(II) complexes. This is in agreement with the table reported by Elena *et al.*, (2013).

The results obtained is presented in (Table 4.3). The magnetic moments of the complexes were measured at room temperature. Cu(II) complex is found to be paramagnetic while Zn(II) complex which is diamagnetic in nature as reported in (Table 4.4) above. The calculated magnetic moments value of Cu(II) complex is 1.82 B.M and it is in good agreement with a report by Shariffat *et al.*, (2014). Gurbuz *et al.*, (2013) reported that high range value of magnetic moment was supported for octahedral, The percentage compositions of Cu^{2+} , and Zn^{2+} were determined as 15.49, and 14.46, respectively, as shown in (Table 4.5) above. The percentages of metal ions in the complexes and water of hydration were used to determine the percentage composition of the ligand by subtracting the sum of the percentages of metal ions and water of hydration from hundred, and then the empirical formula of each complex was obtained by dividing the percentages of metal, ligand and water of hydration by atomic weight and formula weight respectively. Vogel (1972). The results obtained suggested the proposed general molecular formula as $[ML(H_2O)_3].nH_2O$. The infrared spectrum of the free Schiff base showed a strong band at 1627 cm^{-1} which was due to $\nu(C=N)$

vibrational mode, confirming the formation of a Schiff base. This band shifted to the range of 1614-1626 cm^{-1} in the spectra of the metal(II) complexes supporting coordination through the nitrogen of azomethine $\nu(\text{C}=\text{N})$ to the metal ion. In addition, a band at 3404 cm^{-1} was assigned to $\nu(\text{O}-\text{H})$ stretching vibration in the spectrum of the free Schiff base, Mounika *et al.*, (2010). A (C-O) vibrational peak at 1229 cm^{-1} in the spectrum of the ligand was shifted to higher field in the range of 1285-1289 cm^{-1} in the spectra of the complexes was attributed to coordination via phenolic oxygen atom $\nu(\text{C}-\text{O})$. The emergence of bands at 3404 cm^{-1} in the spectra of the metal(II) complexes demonstrating the presence of water molecules. The new bands in the range of 519-601 cm^{-1} and 432-490 cm^{-1} in the spectra of metal(II) complexes were attributed to $\nu(\text{M}-\text{N})$ and $\nu(\text{M}-\text{O})$ respectively, similar peaks for the same functional groups were reported by Mounika *et al.*, (2010). The ligand and its metal(II) complexes were also screened for antibacterial and antifungal activities against three bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*) and three fungi (*Aspergillus flavus*, *mucor iudicus*, and *Aspergillus niger*) by disc diffusion method respectively. Comparing the activities of the compounds, it was observed that the complexes have higher activity than the ligand but lesser than reference drug as showed in tables 4.7 and 4.8 respectively. The Compounds showed relatively the same activity with similar compounds prepared by Aurora *et al.*, (2009).

From the results of analyses of the metal(II) complexes and the available literature report, the proposed general molecular structure of the complexes is presented below:

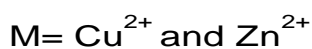
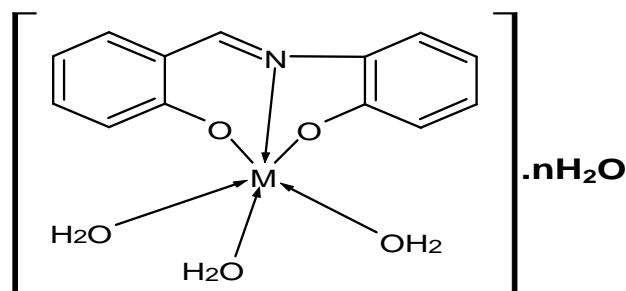


Figure 1.2: the proposed general molecular structure of the complexes.

CONCLUSION

The 2-[[2-(2-hydroxyphenyl)imino]methyl] phenol and its metal complexes of Cu(II) and Zn(II) have been synthesized and characterized by solubility, melting point and decomposition temperature, atomic absorption spectrophotometry (A.A.S), Infrared spectroscopy, molar conductance, magnetic susceptibility. The elemental analyses of the complexes and determination of water of hydration established that metal: ligand ratio is 1:1. Molar conductivity analyses indicated that the ligand acts as tridentate coordinated through azomethine nitrogen atom to the central metal ion and the oxygen atoms of the phenolic group. The molar conductance values of the complexes obtained are low, these low values confirming the non-electrolytic nature of the

complexes. The melting point and decomposition temperature of the ligand and its metal(II) complexes showed good thermal stability. The magnetic moment values of the complexes and infrared spectral data demonstrated an octahedral geometry for Cu(II) complex while Zn(II) complex which is diamagnetic. The ligand and its metal(II) complexes of Cu(II) and Zn(II) have also been screened for antimicrobial activity against the three bacteria isolates; (*staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*) and three fungi isolates; (*Aspergillus flavus*, *Mucor indicus*, and *Aspergillus niger*) by disc diffusion method respectively. The complexes were found to be more active than the ligand but less than the reference drugs (Amoxicillin and Ketoconazole) used respectively.

RECOMMENDATION

Instrumental analyses such as X-ray crystallographic, proton (¹H) and carbon-13 (¹³C) NMR and UV spectroscopic analysis needed to be employed to the compounds. Toxicity test of the Schiff base and its metal(II) complexes needed to be carried out to ascertain the level of toxicity of the complexes.

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