



## Synthesis, Characterisation and Antimicrobial Studies on 2 - [(2 - Hydroxyphenyl) Methylidene] Hydrazine-1-Carbothioamide and its Ni(II) and Cu(II) Complexes

<sup>1</sup>Sani, U. and <sup>2</sup>Yahaya, M.

<sup>1</sup>Department of Pure and Industrial Chemistry, Bayero University, Kano, Nigeria

<sup>2</sup>Department of Chemistry, Sule Lamido University Kafin Hausa, Jigawa State, Nigeria

Email: [sumar.chm@buk.edu.ng](mailto:sumar.chm@buk.edu.ng); [musbahu.yahaya@yahoo.com](mailto:musbahu.yahaya@yahoo.com)

### ABSTRACT

2-[(2-hydroxyphenyl)methylidene]hydrazine-1-carbothioamide was obtained by the condensation of salicylaldehyde and thiosemicarbazone. Ni(II) and Cu(II) complexes were prepared and characterized by elemental (CHNOSO) analysis, FTIR, UV – visible, magnetic susceptibility, and molar conductance measurements. The ligand sharp peak at  $1616\text{cm}^{-1}$  in the FTIR spectra indicating the formation of azomethine ( $\text{C}=\text{N}$ ) bond. A shift shows band to the band regions  $1597\text{cm}^{-1}$  and  $1510\text{cm}^{-1}$  confirmed the coordination of the azomethine nitrogen with Nickel(II) and Cu(II) ions respectively. The ligand formed a chelate with 1:2 metal : ligand stoichiometry. All the compounds have been screened for antibacterial and antifungal activity by disc diffusion method against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* as well as *Aspergillus flavus* bacterial and fungal isolates respectively. The compounds are found to be active against the tested isolates.

**Keywords:** Schiff base, Characterisation, Ligand, Antibacterial, Antifungal

### INTRODUCTION

Schiff base is a nitrogen analog of an aldehyde or ketone in which the  $\text{C}=\text{O}$  group is replaced by an imine or azomethine,  $\text{C}=\text{N}-\text{R}$  group (Cleinton, *et al.*, 2011). It is usually formed by condensation of an aldehyde or ketone with a primary amine. Schiff bases that contain aryl substituents are substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable. Schiff bases of aliphatic aldehydes are relatively unstable and readily polymerizable while those of aromatic aldehydes having effective conjugation are more stable (Xavier, *et al.*, 2014). A wide range of Schiff base compounds have been studied because they have very flexible and diverse structure. Schiff bases are essential in the field of coordination chemistry, especially in the development of complexes because these compounds are potentially capable of forming complexes with metal ions (Brodowska, *et al.*, 2014). A Schiff base behaves as a Flexi-dentate ligand and commonly co-ordinates through the O atom of the de -protonated phenolic group and the N atom of the azomethine group (Xavier, *et al.*, 2014). In Schiff base azomethane nitrogen and other donor atoms like oxygen play a vital role in co-ordination chemistry. Hence an attempt is made to study the

interaction of reduced Schiff base with transition of metals of biological interest and to investigate the co- ordination chemistry of such interactions. This paper reports synthesis, characterisation and antimicrobial studies of schiff base derived from salicylaldehyde and thiosemicarbazide and its Ni(II) and Cu(II) complexes.

### MATERIALS AND METHOD

The solvents and chemicals used in this work were of Analar grade. All the glass wares used were washed thoroughly with detergent, rinsed with distilled water and dried in an oven at  $110^{\circ}\text{C}$ . Melting point and decomposition temperatures were determined on Guoming RY-2 melting point Tester. Infrared spectral analyses were recorded using Shimadzu FTIR-8400S Spectrophotometer. Electrical conductivity measurements were carried out using conductivity meter model Jenway 4010, while UV- visible spectral measurements were done on a PerkinElmer Lambda 35 UV-visible spectrophotometer. Magnetic susceptibilities of complexes were determined on MBS MK1 Magnetic susceptibility Balance at  $25^{\circ}\text{C}$ . Elemental analysis was done using Perkin Elmer Series 11 (CHNS/O) Analyser 2400 at micro analytical lab of King Fahd University of Petroleum and Minerals. Bacterial

and fungal isolates were obtained from Aminu Kano Teaching Hospital and identified at Microbiology department, Bayero University Kano. Nutrient agar and PDA were as bacterial and fungal media respectively.

### Synthesis of the Schiff base

Hot ethanolic solution of thiosemicarbazide (1.822g, 20mmol) and salicylaldehyde (2.1ml, 20mmol) were taken in a round bottom flask. 1ml of acetic acid was added and the mixture was refluxed at 80°C for two hours. On cooling a white precipitate was obtained. It was filtered, washed with ethanol, recrystallised and dried over CaCl<sub>2</sub>. (Sulekh, *et al.*, 2012)

### Synthesis of the complexes

Hot methanolic solution of the metal (II) acetate (5mmol) and hot methanolic solution of the ligand (10mmol) were mixed together with constant stirring. The mixture was refluxed for four hours at 80°C. On cooling, precipitate was obtained. It was filtered, washed with methanol and dried over CaCl<sub>2</sub> (Sulekh, *et al.*, 2012).

### Determination of Metal to Schiff base Ratio

The number of ligand coordinated to the metal ion was determined using Job's method of continuous variation (Angelici, 1971). Methanolic solution of the ligand (3mmol) and the metal acetates were prepared. The following Ligand to Metal ion ratio (ml); 1:15, 3:13, 5:11, 7:9, 9:7, 5:11, 3:13, 1:15 were taken from the ligand solution and each of the metal acetate solutions respectively. A total volume of 16ml was maintained (in that order) throughout the process and the mole fraction of the ligand was calculated in each mixture. The solutions of the metal acetate were scanned (as blank) to find the wavelength of maximum absorption ( $\lambda_{max}$ ) for that particular metal ion (Angelici, 1971). The machine was fixed at  $\lambda_{max}$  398nm and 685nm (in each case) for nickel and copper metal salt solutions respectively before taking the absorbance values of the metal-ligand mixture. The absorbance values were plotted against the mole fraction. Average number of coordinated ligand to the metal ion was obtained from that plot.

### Antibacterial Activity Test

The *in vitro* antibacterial property of the Schiff base ligand and the metal complexes were assayed using three bacterial isolates: *Staphylococcus aureus*, *Escherichia coli* and

*Salmonella typhi*, using disc diffusion method (Sharma, *et al.*, 2009). The suspension of each microorganism was rubbed onto the surface of solidified nutrient agar (N.A) already poured into Petri dishes. Different concentrations (250, 500 and 1000µg/disc) of the Schiff base ligand and the metal complexes in DMSO were prepared through simple serial dilution and placed on the culture media before incubation at 37°C for 24 hours. Activities were determined by measuring (in mm) the diameter of the zone showing complete inhibition and comparing the values with centriasone and gentamicin as standards.

### Antifungal Activity Test

The *in vitro* antifungal activity of the Schiff base ligand and its metal complexes were studied on two fungi namely; *Aspergillus flavus* and *Mucor sp* using disc diffusion method (Sharma, *et al.*, 2009). The fungal suspensions were rubbed onto the surface of solidified Potato Dextrose Agar (PDA) already poured into Petri dishes. Different concentrations (250, 500 and 1000µg/disc) of the Schiff base ligand and the metal complexes in DMSO were prepared through serial dilution and placed on the culture media. This was left to stand at room temperature for about 48 hours. Activities were determined by measuring (in mm) the diameter of the zone showing complete inhibition and comparing the result with katoconazole as standard.

## RESULTS AND DISCUSSION

The Schiff base have been prepared by condensation of salicylaldehyde and thiosemicarbazide as a white crystalline solid. The nickel (II) and copper (II) Schiff base complexes have been prepared using the adopted procedure reported by Sulekh, *et al.*, 2012, the compounds are green and brown respectively (Table 1). The melting point of the schiff base is 217°C. The decomposition temperatures of nickel (II) and copper (II) Schiff base complexes are 280°C and 263°C respectively, which show that the complexes have higher thermal stability. The complexes are insoluble in water and common organic solvents, but are readily soluble in DMSO. This shows that they are non ionic (Table 2). The molar conductance values of nickel (II) and copper (II) schiff base complexes in  $1 \times 10^{-3}M$  DMSO solution were 1.38 and 1.21  $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$  respectively, suggesting their non electrolytic nature as shown in Table 3 (Geary, 1971).

**Table 1: Physical Properties of the Ligand and its Corresponding Metal (II) Complexes**

Compound	Colour	Percentage yield (%)	Melting/Decomp. Temp (°C)	Elemental analysis Theoretical (Found)			
				%C	%H	%N	%S
Ligand (L)	White	90	217	49.21 (49.74)	4.65 (4.71)	21.52 (21.45)	16.42 (14.80)
[NiL <sub>2</sub> ]	Green	89	280	42.98 (41.47)	3.61 (2.92)	18.79 (18.71)	14.34 (14.79)
[CuL <sub>2</sub> ]	Brown	74	263	42.51 (39.79)	3.57 (3.46)	18.59 (16.37)	14.19 (13.42)

L= 2-[(2-hydroxyphenyl)methylidene]hydrazine-1-carbothioamide

**Table 2: Solubility of the Ligand and its Metal (II) Complexes in water and Some Common Organic Solvents**

Compound	Solvent									
	Dis. H <sub>2</sub> O	Methanol	Ethanol	Chloroform	CCl <sub>4</sub>	Ether	Acetone	DMF	DMSO	
Ligand	IS	S	S	IS	IS	S	IS	S	S	
[NiL <sub>2</sub> ]	IS	IS	IS	IS	IS	IS	IS	IS	S	
[CuL <sub>2</sub> ]	IS	IS	IS	IS	IS	IS	IS	IS	S	

S= Soluble, SS= Slightly Soluble and IS= Insoluble

In the IR spectrum of ligand, the characteristic  $\nu(\text{OH})$  band was observed at  $3429\text{ cm}^{-1}$ . The absence of this band in the IR spectra of the complexes indicates the coordination through the phenolic oxygen (Julekha, 2014). The  $\nu(\text{C}=\text{S})$  was seen at  $833\text{ cm}^{-1}$  in the ligand while its metal complexes showed some absorptions at lower frequency of  $763$  and  $817\text{ cm}^{-1}$  respectively. The down shift of this value in the spectra ligand is due to the co-ordination of the thiocarbonyl sulphur to the transition metal ion in the complexes (Monika, *et al.*, 2012). A band at  $1616\text{ cm}^{-1}$  in free schiff base is due to  $\nu(\text{C}=\text{N})$  vibration. The shifting of this band to lower frequency ( $1593$  and  $1510\text{ cm}^{-1}$ ) in the nickel (II) and copper (II) complexes when compared to free ligand respectively, suggests the coordination of metal ion through nitrogen atom of azomethine group (Mounika, *et al.*, 2010). New bands, which are not present in the spectrum of ligand appeared in the spectra of complexes in the range of  $615\text{--}709\text{ cm}^{-1}$  and  $468\text{--}472\text{ cm}^{-1}$ , corresponding to  $\nu(\text{M-O})$  and  $\nu(\text{M-N})$  vibrations

respectively (Table 4). The appearance of  $\nu(\text{M-O})$  and  $\nu(\text{M-N})$  vibrations support the involvement N and O atoms in complexation with nickel (II) and copper (II) ions (Mounika, *et al.*, 2010).

Determination of the Metal to ligand ratio was achieved by using Job's method of continuous variation which suggest 1:2 Metal-ligand ratios in both complexes (Fig.1 and Fig.2) (Angelici, 1971). This metal/ligand ratio was confirmed by estimating the carbon, hydrogen nitrogen and sulphur contents of the complexes. The Elemental analysis (Table 1) of the ligand and its metal complexes show good agreement with the proposed structures of the ligand and complexes (Fig.3 and Fig.4 respectively).

The magnetic moments of Ni(II) complexes was 2.7 B.M within the range of 2.5-3.5 B.M for octahedral Ni(II) complexes (Aderoju, *et al.*, 2015). The effective magnetic moment value for Cu (II) complex was 1.64 B.M which was characteristic of mononuclear, Cu (II) ( $d^9$ ) one unpaired electron per Cu (II) ion (Mounika, *et al.*, 2010).

**Table 3: Conductivity Measurement for the Metal (II) Complexes**

Compound	Electrical Conductance ( $\text{ohm}^{-1}\text{cm}^{-1}$ )	Molar Conductance ( $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ )
[Ni L <sub>2</sub> ]	$1.38 \times 10^{-6}$	1.38
[Cu L <sub>2</sub> ]	$1.21 \times 10^{-6}$	1.21

L= 2-[(2-hydroxyphenyl)methylidene]hydrazine-1-carbothioamide

**Table 4: Selected Vibration Frequencies of the Schiff base and the Metal Complexes**

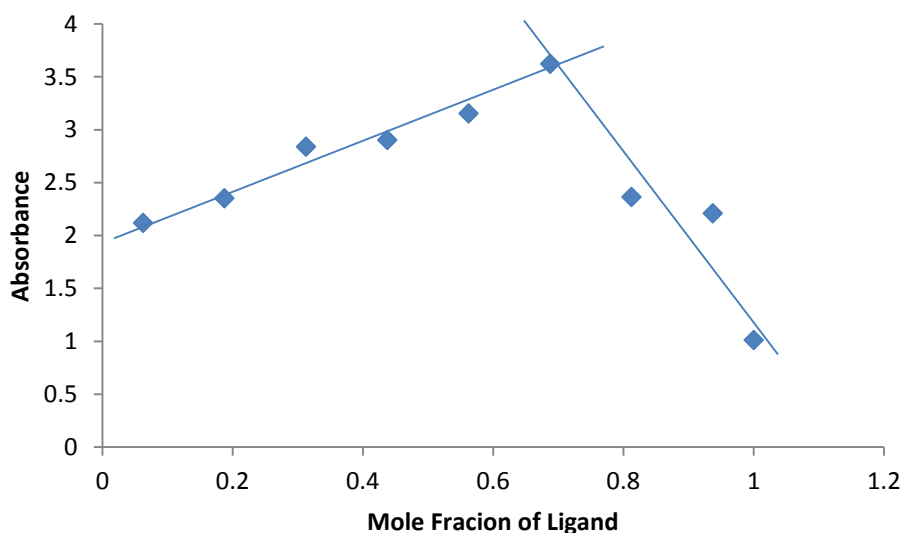
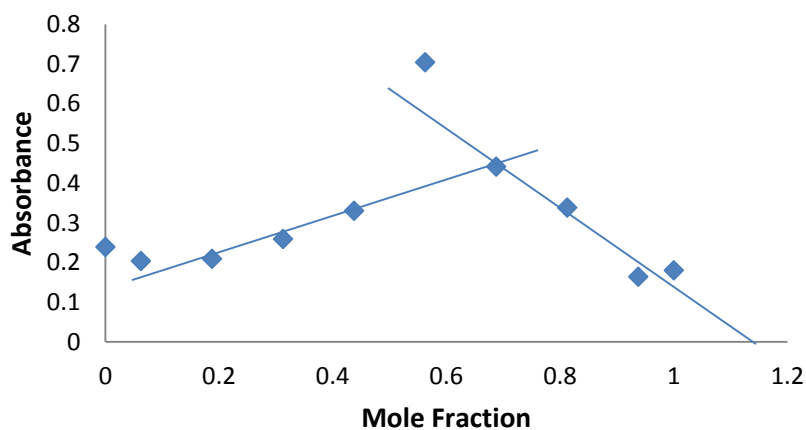
Compound	$\nu(\text{O-H})\text{cm}^{-1}$	$\nu(\text{C=N})\text{cm}^{-1}$	$\nu(\text{C=S})\text{cm}^{-1}$	$\nu(\text{M-O})\text{cm}^{-1}$	$\nu(\text{M-N})\text{cm}^{-1}$
Ligand (LH)	3429	1616	833	---	---
[Ni L <sub>2</sub> ]	---	1597	763	615	472
[Cu L <sub>2</sub> ]	---	1510	817	709	468

L= 2-[(2-hydroxyphenyl)methylidene]hydrazine-1-carbothioamide

**Table 5: Molar Magnetic Susceptibility ( $X_M$ ) and Effective Magnetic Moments ( $\mu_{\text{eff}}$ ) of Complexes**

Compound	$X_g$ (ergG <sup>-2</sup> g <sup>-1</sup> )	$X_M$ (ergG <sup>-2</sup> mol <sup>-1</sup> )	$\mu_{\text{eff}}$ (B. M)
[Ni L <sub>2</sub> ]	$7.0028 \times 10^{-6}$	$3.1300 \times 10^{-3}$	2.7
[Cu L <sub>2</sub> ]	$2.5000 \times 10^{-6}$	$1.1300 \times 10^{-3}$	1.64

L= 2-[(2-hydroxyphenyl)methylidene]hydrazine-1-carbothioamide

**Fig.1: Plots of Absorbance against Ligand Mole Fraction for Ni<sup>2+</sup>****Fig.2: Plots of Absorbance against Ligand Mole Fraction for Cu<sup>2+</sup>**

**Table 6: Antibacterial Screening of the Ligand and its Metal (II) Complexes**

Compound	Zone of Inhibition (mm)/Concentration( $\mu\text{g/ml}$ )								
	<i>Stap. aureus</i>			<i>E. coli</i>			<i>Salmonella typhi</i>		
	250	500	1000	250	500	1000	250	500	1000
<b>Ligand</b>	10	12	14	8	10	12	11	14	20
<b>[Ni L<sub>2</sub>]</b>	18	24	30	8	14	12	13	16	25
<b>[Cu L<sub>2</sub>]</b>	26	28	30	22	26	30	26	28	30

L= 2-[(2-hydroxyphenyl)methylidene]hydrazine-1-carbothioamide

**Table 7: Antifungal Screening of the Ligand and its Metal (II) Complexes**

Compound	Zone of Inhibition (mm)/Concentration( $\mu\text{g/ml}$ )					
	<i>Aspergillus Flarus</i>			<i>Mucor sp.</i>		
	250	500	1000	250	500	1000
<b>Ligand</b>	8	9	12	NZI	NZI	NZI
<b>[Ni L<sub>2</sub>]</b>	11	14	16	NZI	NZI	NZI
<b>[Cu L<sub>2</sub>]</b>	10	12	16	NZI	NZI	NZI

L= 2-[(2-hydroxyphenyl)methylidene]hydrazine-1-carbothioamide

NZI = No Zone of Inhibition,

The *in vitro* antibacterial activity of the ligand and its complexes have been carried out against bacterial isolates (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*) using disc diffusion method in DMSO as a solvent. A comparative study of the zone of inhibition of Schiff base and its complexes indicate that metal complexes exhibit higher antibacterial activity than the free ligand and the same is indicated from the results given in the table 6. This is probably due to the greater lipophilic nature of the complexes. Such increased in activity of the metal chelates can be explained on the basis of Overton's concept and Tweedy's chelation theory (Thangadurai, *et al.*, 2001). The results indicate that copper complex shows higher activity against all the three isolates over the ligand and nickel complex (Table 6). At 100 $\mu\text{g/ml}$ , the complexes show similar/greater activity against *Staphylococcus aureus* and

*Salmonella typhi* as compared to the Centriasonone and Gentamicin as standard respectively.

The antifungal activity of the ligand and its metal complexes were tested against *Aspergillus flavus* and *Mucor sp* using disc diffusion method. The antifungal activity data indicate that the ligand and its complexes were inactive against *Mucor sp* but active against *Aspergillus flavus*. Ligand has shown a lesser activity against *Aspergillus flavus* as compared to the metal complexes and this can be explained by Overton's concept and Tweedy's chelation theory. The results revealed that the ligand and its metal complexes were less active against *Aspergillus flavus* as compared with Ketoconazole as standard.

Based on UV-visible, molar conductance, magnetic moment data, the proposed structure of the Schiff base and the complexes are presented in Fig. 3 and Fig. 4 respectively.

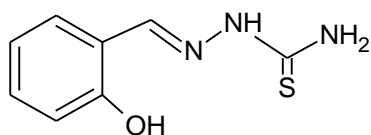
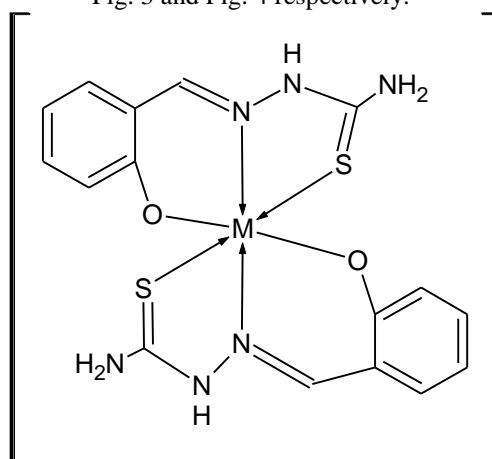


Fig 3: Proposed structure of Schiff base



M = Ni<sup>2+</sup> and Cu<sup>2+</sup>

Fig 4: Proposed structure of metal (II) complexes

## CONCLUSION

The ligand and its metal complexes of Ni(II) and Cu(II) have been prepared and characterized. The Job's method of continuous variation shows that the metal-ligand stoichiometry in the complexes is 1:2 which was confirmed by elemental analysis. All the complexes are non-electrolytes in DMSO. The FTIR spectral data show that azomethine bond was formed as a result of condensation between salicylaldehyde and thiosemicarbazide. It was involved in coordinating through nitrogen atom of the azomethine, thiocarbonyl sulphur and oxygen atom of hydroxyl group.

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