SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF Ni(II) AND Cu(II) COMPLEXES WITH SCHIFF BASE DERIVED FROM 2-ACETYL-5-METHYLFURAN GLYOXIME HYDRAZONE

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ABSTRACT
A hydrazine Schiff base has been synthesized by the interaction of ethanolic solution of antiglyoxime hydrazine with 2-acetyl-5-methylfuran, to obtain the corresponding Schiff base L. The complexes of Ni(II) and Cu(II) of the Schiff base were synthesized and studied. The Schiff base and its metal(II) complexes characterized based on solubility, percentage yield, elemental analysis, melting/decomposition temperatures, infra-red spectral (FT-IR), conductivity measurement, magnetic susceptibility and water of crystallisation. The synthesized Schiff base and the complexes are coloured and stable at room temperature. Based on the results the prepared Schiff base and synthesized complexes are relatively soluble in most organic solvents but insoluble in distilled water. The melting point temperature of the ligand was 118°C while the decomposition temperature for metal complexes were 308 and 300°C respectively. The molar magnetic susceptibility of the complexes were 3.55 and 2.10 x 10⁻³ erg.G⁻¹mol⁻¹. The molar conductance values of 2.17 and 2.43ohm⁻¹cm²mol⁻¹ indicating non electrolytic nature of the complexes. The spectral data of the Schiff base showed band of 1584cm⁻¹, revealing the formation of the azomethine. A bands of 791cm⁻¹ indicate the metal nitrogen bond. The elemental analysis determination of the complexes and the Schiff base showed the metal ligand ratio of 1:2 (M:2L). The antibacterial and antifungal activities of the Schiff base and the metal(II) complexes were evaluated using disc diffusion method. The antibacterial assay was carried out on three pathogenic bacteria, Staphylococcus aureus, Staphylococcus epidermidis and Streptococcus pneumonia and two fungi viz: Candida utilis and Saccharomyces cerevisiae. All the Schiff base and the metal(II) compounds showed some antibacterial and antifungal activity (8mm – 12mm inhibition zone) but having lower activity when compared to control drugs.

Keywords: Ni(II) and Cu(II) complexes with hydrazine Schiff Base

INTRODUCTION
Schiff bases are condensation products of primary amines with aldehydes or ketones they were first reported by Hugo Schiff in 1864. The most common structural feature of these compounds is the azomethine group (C=N) with the general formula RHC=N-R' where R and R' are alkyl, aryl, cyclo or heterocyclic groups which may be variously substituted, these compounds are also known as amines, imines, or azomethines (Ashraf et al., 2011; Aliyu and Sani, 2012). The most common method used in preparation of Schiff base is through the condensation of carbonyl compounds (aldehydes and ketones) and primary amine. The reaction follows simple nucleophilic addition, but gives an intermediate compound called carbinolamine. The compound is unstable and easily loses water molecule of hydration, the hydration process step is very slow hence it is the rate determining step of the reaction and it is acid/or base catalyzed reaction. The removal of product or separation of water from the reaction mixture assists the formation the product (Olatekan and Bakare, 2015). Schiff base metal complexes have been widely studied because they have industrial, antibacterial, antifungal, antiviral, anticancer, anticonvulsant and herbicidal applications (Adeola, 2010). Some complexes containing nitrogen and oxygen donor atoms in their structures are effective as stereospecific catalysts for oxidation, reduction, hydrolysis, biological activity and other transformation of organic and inorganic chemistry (Eman, 2015). Schiff base metal complexes exhibit a large number of biological activities which include antibacterial, antifungal, antimalarial, antiproliferative, antiinflammatory, antiviral and antipyretic activities, they also used in agricultural and industrial applications (Thilagavathi et al., 2015). Schiff base and their metal complexes were found in various biological systems, polymers, medicinal and pharmaceutical field. They were also use in birth control, food packaging, allergic inhibition reducing activity (Parvez et al., 2014). Hydrazones are also a class of Schiff base containing C≡NHR₂ as their functional group, they are formed by the condensation of substituted hydrazine with aldehyde or ketone (John, 2008). Therefore they are related to aldehydes or ketones by the replacement of the oxygen by NH₂ functional group. Hydrazones also constitute an important class of biologically active drugs molecules which has attracted attention of medical chemists due to their antimicrobial, antihypertensive, analgesic, anti-inflammatoy, anti-tuberculosis, antiproliferative and antimalarial activities. Hydrazones are present in many of the bioactive heterocyclic compounds that are of very important use because of their various biological and clinical applications. Hydrazone-based coupling methods are used in medical biotechnology as anticonvulsant, antidepressant, analgesic, anti-inflammatory, antiplatelet, antimalarial, antimicrobial, anticancer, vasodilator, antiviral, anti-HIV, antidiabetic and trypanocidal activities. (Mohammed et al., 2018).
Coordination chemistry has been considerably enriched due to the synthesis and characterization of a large number of transition metal complexes in which the metals are coordinated through sulphur, nitrogen or oxygen. Metal complexes of ligands containing ONS donors usually show antibacterial, antifungal and antitumor activities (Saeed et al., 2015). They are also useful in industries for preparation of pigments, dyes, catalysts, organic and inorganic synthesis, polymer stabilizers and optical sensors (Naeimi et al., 2007 and Ibrahim et al., 2007).

Hydrazones Schiff base having nitrogen, oxygen or sulphur donors have been reported by several researchers due to their wide range of applications in both pharmacological and non-pharmacological fields. (Sridhar S. K. 2002). Rauf Abdul et al., 2008 and Thiagarajan G. 2012 reported that the presence of heteroatom in hydrazone Schiff base enhance bioactivity. In this regard an attempt is made to carry out the research. This research work is concern with the synthesis, characterization and biological evaluation of hydrazone Schiff base metal (II) complexes.

**Materials and Methods**

All glass wares used in this work were properly washed with detergent, rinsed several times with tap water. They were then soaked in a concentrated solution of nitric acid for about 2 – 3 hours after which they were rinsed 3 – 4 times with distilled water and dried in an oven maintained at 110°C. All reagents and solvents used were of Analar grade and were used as supplied without further purification. All weighing were done on Stuart SMP 10 melting point apparatus. Determination of water of hydration was done on drying oven model DHO – 307, Jenway. The magnetic susceptibility measurements were also carried out using conductivity meter DDS – 307, Jenway. The magnetic susceptibility measurements were carried out using magnetic measurement balance Sherwood scientific MK 01 model at room temperature. Three bacterial isolates viz: *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pneumonia* and two fungal isolates viz: *Candida utilis* and *Saccharomyces cerevisiae* were obtained from Yusuf Dantsoho Memorial Hospital and the isolates were identified in the micro biology lab, department of applied biology Kaduna Polytechnic for in vitro antimicrobial screening, using ampicillin capsule and nyastatin purchased from Magrib pharmaceutical store Tudun Wada Kaduna as positive control.

**Preparation of Glyoximehydrazine**

A solution of NaOH (0.4g, 10mmol) in 1cm$^3$ of distilled water was mixed with 10cm$^3$ of ethanol and 0.6cm$^3$ of hydrazinium hydroxide and cooled to 5°C. A solution of chloroglyoxime (1.225g, 10mmol) in 5cm$^3$ of ethanol was added drop wise with stirring into the prepared mixture maintained at 5°C. Stirring was continued for 15min at the same temperature to complete the reaction. The precipitate that formed was filtered, washed with cold ethanol and dried in a desiccator over phosphorus pentaoxide.

**Preparation of 2-acetyl-5-methylfuran glyoxime hydrazone (L$'$)**

A cooled solution 5°C of 2-acetyl-5-methylfuran (1mmol) in ethanol was added drop wise into a cooled solution 5°C containing 1mmol of glyoximehydrazine and 3-5 drops of acetic acid with constant stirring. After the addition of the substituted furan was completed, the solution was stirred for 4-6 hours at room temperature. The resulting precipitate (L$'$), was separated, washed with water, ethanol and dried over phosphorus pentaoxide in a desiccator.

The equation for the reaction is presented below:

\[
\text{ketone} + \text{glyoximehydrazine} \rightarrow \text{Schiff base L'}
\]

**Scheme 1.0: Preparation of the Schiff base L$'$**

**Synthesis of Metal(II) Complexes**

A solution of Ni(II) and Cu(II) chlorides (1mmol) in 20cm$^3$ of distilled water were added to 2mmol of Schiff base ligand (L$'$) in 15cm$^3$ of ethanol with constant stirring. An initial sharp decrease of pH of the solution from 3.5 to 3.5 was observed. After raising the pH to 5.5 using aqueous NaOH solution, the reaction mixture was kept in a hot water bath (60°C) for 2 hours to complete the precipitation. Then the precipitated complexes were filtered, washed with water and dried in a desiccator over phosphorus pentaoxide. The general equation for the reaction is:
Where $M = \text{Ni}^{2+}$ or $\text{Cu}^{2+}$

Scheme 2.0: Preparation of Ni(II) and Cu(II) complexes.

### Solubility Test
The solubility of the ligands and the metal(II) complexes were determined by taking approximately 0.01g of the sample and put in a dried clean test tube followed by few drops of the solvent used and shaken. The solvents used were: distilled water, methanol, ethanol, acetone, chloroform, tetrachlomethane, hexane, DMSO, DMF and benzene

### Determination of Water of Hydration
Water of hydration of the prepared complex compounds was determined by weighing 0.2g each of the compounds in a watch glass and put in an oven maintained at temperature of 110°C until a constant weight was obtained. The average loss of weight was recorded after cooling as weight of water of hydration (Vogel, 1978).

### Molar Conductance Measurements
A $10^{-3}$M solution of each complex compounds was prepared using DMSO as solvent, and the electrode of the conductivity meter were inserted and the values recorded, this is the observed conductance and the corresponding specific conductance was also calculated (Moamens, 2013). All the measurements were carried out at room temperature. Knowing the cell constant, the specific conductance was obtained.

Specific conductance = cell constant x observed conductance. Furthermore, the molar conductance was calculated using the equation:

$$\text{Molar Conductance} = \frac{1000K}{C}$$

Where: $C$ is the molar concentration and $K$ is the specific conductance.

### Determination of Metal ions in the Metal(II) Complexes
A 0.05g of each metal (II) complex compound was accurately weighed and heated with a mixture of 3cm$^3$ concentrated nitric acid $\text{HNO}_3$ (1M) and 2cm$^3$ of hydrogen peroxide $\text{H}_2\text{O}_2$ to dryness, then 3cm$^3$ of dilute hydrochloric acid (0.1M) was added to dissolve the residue and a clear solution was obtained. The solution was allowed to cool and diluted to 100cm$^3$ with distilled water. The calibration solutions were made by further diluting 10cm$^3$ of the stock solutions with distilled water to 100cm$^3$mark of volumetric flask. The concentration of the metal ions was then measured against the blank solution using (AAS) Atomic Absorption Spectrophotometer (Anu et al., 2013).

### Magnetic Susceptibility Measurement
All recordings were done at room temperature for the samples and the magnetic susceptibility ($X_g$) were calculated using the relation.

$$X_g = \frac{C(L)(R_1 - R_0)}{1 \times 10^9 (\text{m})} \text{erg. G}^{-2} \text{g}^{-1}$$

Where:
- $X_g$ = Gram magnetic Susceptibility of the complex
- $C$ = Balance calibration constant, (which is consider as equal to 1)
- $L$ = Length of the sample inside the tube (in cm)
- $R_0$ = Initial reading of the empty glass tube
- $R_1$ = Final reading of the glass tube with sample
- $W_0$ = Initial mass of the empty glass tube
- $W_1$ = Final mass of the glass tube with the sample

And $W_1 - W_0 = m$ (Lanchanshire, 2000).
Evaluation of Antibacterial and Antifungal effects of synthesized Schiff bases and their Metal(II) complexes

Antibacterial and antifungal activity of the synthesized Schiff base and their Cu(II) and Ni(II) complexes were tested using agar well diffusion method on Mueller Hinton agar (MHA) and potato dextrose agar (PDA) sterile plates. The MHA and the PDA were prepared according to the manufacturer’s instruction at 121°C for 15 minutes. The three bacterial isolates *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pneumonia* which are gram positive (G+) and two fungi viz: *Candida utilis* and *Saccharomyces cerevisiae*.

**RESULTS AND DISCUSSION**

**Table 1:** Physical Properties of Schiff base (L′) and its Metal(II) complexes.

| Compound | Molecular formula | Colour   | Molecular weight (g/mol) | Percentage yield (%) | Melting Temp. (°C) | Decomp Temp. (°C) |
|----------|-------------------|----------|--------------------------|----------------------|-------------------|-------------------|
| L′       | C₉H₁₂N₄O₃        | Orange   | 224.2188                 | 65                   | 118               | -                 |
| [NiL₂′]_4H₂O | [Ni(C₉H₁₂N₄O₃)₂]_4H₂O | Green    | 577.1726                 | 65                   | -                 | 308               |
| [CuL₂′]_2H₂O | [Cu(C₉H₁₂N₄O₃)₂]_2H₂O | Green    | 545.9982                 | 60                   | -                 | 300               |

L′ = 2-acetyl-5-methylfuran glyoxime hydrazone.

**Table 2:** The Solubility Data of the Schiff base (L′) and its Metal(II) Complexes.

| SOLVENT | L′ | DistH₂O | MeOH | EtOH | Acetone | Hexane | CHCl₃ | CCl₄ | DMF | C₆H₆ | DMSO |
|---------|----|---------|------|------|---------|--------|-------|------|-----|------|------|
| L′      | IS | S       | S    | S    | SS      | SS     | SS    | SS   | SS  | SS   | SS   |
| [NiL₂′]_4H₂O | SS | S       | SS   | S    | SS      | SS     | SS    | SS   | SS  | SS   | S    |
| [CuL₂′]_2H₂O | IS | S       | S    | S    | SS      | SS     | SS    | SS   | SS  | SS   | S    |

Key: S = soluble, SS = slightly soluble and IS = Insoluble.

**Table 3:** Results for the Determination of the Metal Concentration in the Metal (II) Complexes

| COMPOUND | Absorbance (y) | Concentration (x)(mg/l) | Metal percentage(%) |
|----------|----------------|-------------------------|---------------------|
| [NiL₂′]_4H₂O | 0.070          | 51.47                   | 10.29               |
| [CuL₂′]_2H₂O | 0.990          | 51.32                   | 10.26               |

L′ = 2-acetyl-5-methylfuran glyoxime hydrazone

**Table 4:** IR Spectral Data (cm⁻¹) for the Schiff base (L′) and its Metal(II) Complexes.

| Compound | ν(N-H) | ν(O-H) | ν(C=N)oxime | ν(C=N)hydrazone | ν(C-H) | ν(M-N) |
|----------|--------|--------|-------------|-----------------|--------|--------|
| L′       | 3156   | 3085   | 1584        | 1640            | 2947-2918 | -     |
| [NiL₂′]_4H₂O | 3156   | 3115   | 1640        | 1640            | 2947-2918 | 791   |
| [CuL₂′]_2H₂O | 3353   | 3095   | 1640        | 1640            | 2947-2880 | 791   |

L′ = 2-acetyl-5-methylfuran glyoxime hydrazone

**Table 5:** Microanalysis Data for the Schiff base (L′) and its Metal(II) Complexes.

| Compound | Molecular Weight (g/mol) | Elemental %Found (Calculated) |
|----------|-------------------------|-------------------------------|
|          |                         | C    | H    | N    | M    |
| L′       | 224.2188                | 48.67(48.21) | 6.72(5.39) | 22.43(24.99) | -     |
| [NiL₂′]_4H₂O | 577.1726     | 37.84(37.46) | 3.93(3.84) | 19.32(19.41) | 10.29(10.17) |
| [CuL₂′]_2H₂O | 545.9982     | 37.10(39.60) | 4.85(4.06) | 20.40(20.52) | 10.26(11.64) |

L′ = 2-acetyl-5-methylfuran glyoxime hydrazone
Table 6: Molar Conductance Measurement for metal(II) complexes.

| Compound                  | Molecular Mass (g/mol) | Specific Conductance (ohm cm⁻¹) | Molar Conductance (ohm cm² mol⁻¹) |
|---------------------------|------------------------|--------------------------------|----------------------------------|
| [NiL₂]₂.4H₂O             | 577.1726               | 2.17x10⁶                       | 2.17                             |
| [CuL₂]₂.2H₂O             | 545.9982               | 2.43x10⁶                       | 2.43                             |

L’ = 2-acetyl-5-methylfuran glyoximehydrazone

Table 7: Magnetic Susceptibility Data for Metal(II) Complexes.

| Compound                  | Mass susceptibility Xg(erg.G²g⁻¹) | Molar Susceptibility Xm(erg.G²mol⁻¹) | Number Of unpaired Electron(s) | Geometry |
|---------------------------|-----------------------------------|-------------------------------------|---------------------------------|----------|
| [NiL₂]₂.4H₂O             | 6.16 x 10⁻⁶                      | 3.55 x 10⁻⁵                        | 2                              | Tetrahedral |
| [CuL₂]₂.2H₂O             | 3.85 x 10⁻⁶                      | 2.10 x 10⁻⁵                        | 1                              | Tetrahedral |

L’ = 2-acetyl-5-methylfuran glyoximehydrazone

Table 8: Antibacterial Sensitivity Test for the Schiff base (L’) and its Metal(II) Complexes

| Compound                  | Concentration (µg/ml) | Bacterial Zone of Inhibition (mm) |
|---------------------------|-----------------------|-----------------------------------|
|                           |                       | Staphylococcus aureus | Staphylococcus epidermidis | Streptococcus pneumoniae |
| L’                        | 600                   | 06                      | 08                           | 10                        | 06 |
|                           | 300                   | 06                      | 06                           | 06                        | 06 |
|                           | 150                   | 06                      | 06                           | 06                        | 06 |
| [NiL₂]₂.4H₂O             | 600                   | 06                      | 06                           | 06                        | 06 |
|                           | 300                   | 06                      | 06                           | 06                        | 06 |
|                           | 150                   | 06                      | 06                           | 06                        | 06 |
| [CuL₂]₂.2H₂O             | 600                   | 16                      | 14                           | 14                        | 10 |
|                           | 300                   | 10                      | 10                           | 10                        | 10 |
|                           | 150                   | 10                      | 10                           | 10                        | 10 |
| 300                       | 20                    | 18                      | 17                           | 17                        | 17 |
| 600                       | 18                    | 18                      | 18                           | 18                        | 18 |
| 300                       | 14                    | 14                      | 14                           | 14                        | 14 |
| 150                       | 10                    | 10                      | 10                           | 10                        | 10 |
| Amphicillin               |                       | 06                      | 06                           | 06                        | 06 |
| (control)                |                       | 06                      | 06                           | 06                        | 06 |

L’ = 2-acetyl-5-methylfuran glyoximehydrazone

Table 9: Antifungal Sensitivity Test on the Schiff base (L’) and its Metal(II) Complexes

| Compound                  | Concentration(µg/ml) | Fungal zone of Inhibition (mm) |
|---------------------------|----------------------|-------------------------------|
|                           |                      | candida utilis | saccharomyces cerevisiae    |
| L’                        | 600                  | 06              | 06                          |
|                           | 300                  | 06              | 06                          |
|                           | 150                  | 06              | 06                          |
| [NiL₂]₂.4H₂O             | 600                  | 08              | 08                          |
|                           | 300                  | 06              | 06                          |
|                           | 150                  | 06              | 06                          |
| [CuL₂]₂.2H₂O             | 600                  | 06              | 06                          |
|                           | 300                  | 06              | 06                          |
|                           | 150                  | 06              | 06                          |
| Nystatin(control)         | 600                  | 22              | 20                          |
|                           | 300                  | 18              | 18                          |
|                           | 150                  | 15              | 16                          |

L’ = 2-acetyl-5-methylfuran glyoximehydrazone
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DISCUSSION

The reaction of the prepared antichloroglyoxime with hydrazinium hydroxide in the presence of aqueous NaOH, produced antichloroglyoximehydrazine(GH). The antichloroglyoximehydrazine was separately reacted with 2-acetyl-5-methylfuran, to produce Schiff base hydrazone ligand labelled L’. The reaction between the Schiff base hydrazone in the molar ratio of 2:1 and aqueous solution of Ni(II) and Cu(II) chlorides in ethanol produced the respective metal(II) complexes having various colours. The colour of transition metal complexes are as a result of d-d transitions of electrons between energy levels. The percentage yield of the Schiff base hydrazone and the corresponding metal(II) complexes were also calculated. The melting points of the hydrazones Schiff base and decomposition temperatures of the metal(II) complex compounds were also recorded (Table 1). Solubility of all the Schiff base and their metal(II) complexes were also determined in some common organic solvents in which the Schiff bases are soluble in methanol and ethanol but insoluble in DMSO. All the metal(II) complexes are insoluble in distilled water but soluble in DMSO, the results were shown in Table 2. The metal concentration in the complex compounds were determined using atomic absorption spectrophotometer (AAS) which were used to calculate the percentage of the metal(II) ion in the complex and the result obtained agreed with the theoretical values, the values were recorded in Table 3. The important IR frequencies of the complexes and Schiff base are produced in Tables 4. The IR spectra of Schiff base show a sharp and strong intensity bands around 3156cm⁻¹ and 1584cm⁻¹ assigned to ν(N-H) and ν(C=N) respectively. The strong band due to azomethine function and amide carbonyl ν(C=O) appeared around 1640 and 1660cm⁻¹ respectively. The IR spectra of the Schiff base show a broad medium intensity band in the region of 3430cm⁻¹ and the band due to ν(C-O) oxime is located in the region of 1520cm⁻¹ due to oxime-OH. The absence of ν(O-H) band in all the complexes suggest involvement of oxygen of the oxime group in hydrogen bonds formation. In addition, another weak band were also observed at in the range of 791cm⁻¹ which may be attributed to ν(M-N).

Table 5 showed the results of micro elemental analysis for the Schiff base hydrazone and their Ni(II) and Cu(II) complexes, the results obtained from elemental analysis were almost the same as the results calculated theoretically. Molar conductance measurements were carried out in 10-3mol dm⁻³ DMSO at 25OC (at room temperature), molar conductivity values of the complexes are given in Table 6 molar conductance of the synthesized Ni(II) and Cu(II) were 2.17 and 2.43 ohm⁻¹cm²mol⁻¹ respectively these low values agrees with those obtained in the literature. The molar conductivity result revealed the non-electrolytic nature of the complexes (Jones and Fleming, 2010). Tables 7 showed the magnetic susceptibility results of the complexes taken under room temperature. The values for the magnetic moments for all the metal(II) complexes conformed to the tetrahedral geometry, the Schiff base ligands all have magnetic moments values suggesting high spin complexes. The antibacterial assay carried out on three gram negative Staphylococcus aureus, Staphylococcus epidermidis and Streptococcus pneumonia bacteria using ampicillin capsule purchased from Magrib pharmaceutical store Tidun wada Kaduna as control during the experiment, zones of inhibition based upon size around each of the discs were measured in millimetre and recorded (Table 8). The antifungal results of (L´) Schiff base hydrazone shown against the two fungal isolates indicate no activity at any concentrations with the zone of inhibition were only within the disc i.e 6mm. Moderate zones inhibition were observed for the two fungal isolates at the remaining concentrations (Table 9).

CONCLUSION

The hydrazone Schiff base and their Ni(II) and Cu(II) complexes were successively prepared and analysed. The results obtained of percentage yield for the ligand is 65% and the metal(II) complexes are 65 and 60% respectively. The metal ligand ratio for all the metal(II) complexes from elemental analysis was found to be 1:2. The magnetic behavior of all the complex compounds have been determined and found to be paramagnetic and tetrahedral shapes. The relatively low molar conductance values indicated their non-electrolytic nature. The Infra-red spectral data of the ligand when compare to those of their respective metal(II) complexes showed that coordination of the metal ion to the ligand is via azomethine nitrogen and phenolic (OH) of the hydrazone Schiff base.

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