

Synthesis, Characterization and Antimicrobial Activity of Cu (II), Co (II) and Ni (II) Histidine Complexes

U. Lawal¹ R. A. Wuana² S. I. Eneji²

1. Department of Chemical sciences, Federal University, Wukari. Taraba State

2. Department of Chemistry, University of Agriculture, Makurdi. Benue State

Abstract

Ternary complexes of histidine amino acid (Schiff base) were synthesized, characterized and tested against multi-drug resistant pathogens. The metal ion centers included Cu (II), Ni (II) and Co (II). These complexes were characterized using physico-chemical and spectroscopic analytical methods. All the complexes are found to be considerably soluble in both polar and non-polar solvents including methanol, ethanol, butanol, acetone, ethyl acetate, and benzene and di ethyl-ether. Electronic Absorption Studies using FT-IR spectrophotometer revealed ν (O-H), ν (C=O), ν (C=N), ν (M-N) and ν (M-O) occurred between 3410 - 3417, 1751 - 1753, 1519 - 1521, 671 - 678, 439 - 470 cm^{-1} respectively in complexes and ligand. UV-Visible was further used to elucidate the complexes resulting in transitions characteristics of the ligand and complexes. The Schiff base showed no antimicrobial activity at various therapeutic concentrations. However, the metal complexes exhibited broad spectrum antibiotic activities against the multi-drug resistant pathogens at minimum inhibitory concentration ($\text{MIC} \leq 200 \mu\text{g/ml}$). The metal complexes showed strong activity against the isolates at medium and high concentrations, the bacteria strains included *E.coli*, *P. aeruginosa*, *S. typhi* and *S. aureus* and the fungi strains of *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. The bioactivity recorded against these multi drug resistant pathogens indicates the potentials of these complexes for further therapeutic studies.

Keywords: Histidine complex, synthesis, characterisation, antimicrobial activity, pathogens.

INTRODUCTION

Antimicrobial resistance to therapeutic drugs is fast becoming a global concern with rapid increase of resistant bacteria. Established treatable conditions are now becoming untreatable, examples are methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococcus* (VRE).^[1] *M. tuberculosis* (Strain no: H37Rv ATCC 27294) is also showing drug resistance to isoniazid, rifampicin, ethambutol.

The multi-drug resistant list also extends to gram negative organisms like *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Salmonella typhi* and also Gram-positive *Bacillus subtilis* bacterial strains. *Candida albicans*, *Aspergillus flavus*, *Fusarium solani*, and *Candida glaberata* are some fungal pathogens are also showing the resistance feature against established drugs.^[2]

This new trend of infectious diseases still remains an important and challenging one because of a combination of factors including new discoveries of infectious diseases and the increasing number of multi-drug resistant microbes. There is a pressing necessity for the finding of novel compounds and complexes with better antimicrobial activities, preferably acting through a diverse exploit mechanism. The action mechanism should be different from the current classes of antimicrobial agents and drugs to which many clinically active microbial agents are now resistant. Given the disturbing upsurge in infectious maladies instigated by diverse pathogens and the expansion of multi-drug resistance, investigators are examining new different antibacterial representatives. Consequently, fresh anti-pathogenic representatives with distinct action mechanisms and nano-technological properties have to be synthesized for the treatment of multi-drug resistant bacterial diseases.

This study aims to synthesize, characterize and carry out antimicrobial activities of Cu (II), Co (II) and Ni (II) metals complexes with Amino acid (histidine) ligand. This project is limited to the elementary processes of organometallic based drug design and evaluation. This procedure includes the synthesis of the proposed complexes, their physico-chemical and instrumental characterization and testing the antimicrobial effectiveness of these complexes.

Materials and methods

Analytical grade reagents were purchased from sigma-Aldrich chemicals. All reagents were used directly without further modifications. All glass wares were properly washed with dilute nitric and distilled water and oven dried. The solubility of the metal complexes was tested using various polar solvents including water, methanol, ethanol, butanol, acetone, ethyl acetate and non-polar solvents like benzene and diethyl ether. Melting points of both the Schiff base ligand and it corresponding complexes was determined by an electrical melting point determination apparatus. Electronic Absorption Studies indicated the UV-Visible spectra in the region 200-1100 nm were measured by systronics double beam spectrophotometer 2202 model using calibrated quartz cells. The metal complexes were subjected to IR spectral analysis to determine molecular interaction and functional group, structural elucidation and bond vibration of each of the complexes. The in vitro anti-microbial

screening was performed. Nutrient agar and Sabouraud Glucose agar were used as culture media. The multi-drug resistant pathogens used include Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and Gram-positive *Staphylococcus aureus* and the fungi samples include *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger* for testing antibacterial activity, and was collected from the Baurau Dikko Hospital clinical laboratory, Kaduna.

Preparation of the Schiff base (Ligand)

All the required chemicals were used as bought without further modification. 20 mmol of amino acid histidine was added into 30 ml of water and solution stirred, 20 mmol acetylacetone was also added to 10 ml of ethanol, and mixed well. Both solutions were then mixed together. The mixture was refluxed for 6 hours at 50°C. During refluxing the color of the solution spun to yellow at first and later spun to orange.^[2]

Recovery of the Ligand

Upon the completion of the reaction, the reaction mixture was transferred to a beaker and cooled on an ice bath to afford a solid product. After cooling the mixture, solid product was settled at the bottom of the beaker. The solid product was recovered by the following steps, evaporation of excess solution from the mixture by heating the solution on a hot plate and after heating the solution mixture was kept in an ice bath for a few minutes to make it cool and finally solid residue was filtered with a filter paper.^[3]

Preparation of metal complexes

Exactly 500 mg of histidine ligand was added into 20 mL hot methanol and mixed well. At the same time, 500 mg of the metal chlorides were taken and mixed with 20 mL of ethanol with a magnetic stirrer. Then both solutions were mixed and refluxed for 4 hours in a refluxing unit at a temperature of 45°C. After the completion of the reaction, the solution mixture was transferred into a beaker and then cooled on an ice bath. Once the solution was cooled the solid product (metal complex) was settled down at the bottom of the beaker and filtered.^[4]

Recovery of metal complex

The solid product was obtained by filtering with filter paper. The obtained solid product was washed with ethanol twice, washed with ether and finally washed again with ethanol. The filtrate product was air dried to afford the desired product and the product was handled very carefully as the product may be hygroscopic in nature, long exposure to air can damage.^[5]

Antibacterial and Antifungal bioassay

Antibacterial assay

Muller Hilton Agar was prepared and sterilized according to manufacturer standard. 20 mL of media was poured into the Petri plates and allowed for solidify. The bacterial lawn culture was made using sterile cotton swab and labelled. A metallic borer was used to make wells in the media with a center of 24 mm. the suggested concentration 50 µL of the test sample 100 mg/mL in water was poured in the respective wells. These plates were then incubated immediately at 37°C for 24 hours to facilitate healthy condition of bacterial growth. The antibacterial activity was determined by measuring the diameter of zones showing total inhibition in millimeters (mm). Growth inhibition was compared with the DMSO.^[6]

Antifungal assay

The antifungal activities of these metal complexes were studied against three fungal strains, *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. Sabouraud dextrose agar was prepared according to manufacturer's instructions and sterilized in an autoclave. The culture plates were pour-plated just like Muller Hilton Agar case. Upon the solidification of the media, respective fungal spore were swab on it. A sterile metallic borer was used to make wells in the media with centers of 24 mm. The commended concentration of the test sample, 100 mg/mL in water was introduced in these wells. The plates were incubated at 300°C for 72 hours. The outcomes were chronicled as zones of inhibition in millimeter (mm).^[7]

Minimum inhibitory concentration

The minimum inhibitory concentration of the complexes was determined using the tube dilution method. The following concentrations 300, 200 and 100 in µg/mL of the metal complexes solution were prepared. Cleaned test tubes were used; the different concentration of metal complexes was made, the volume of medium made up to 20 mL with nutrient broth after which the control was prepared with 2 mL of nutrient broth without any metal complex and sterilized the medium at 121°C temperature at 15 lbs pressure for 15 minutes in autoclave. At the end of the sterilization, the medium was allowed to cool and 0.2 mL of overnight cultures of each organism was dispensed into sterile medium and incubated for 24 hours and the activity measured.^[7]

RESULTS

Table 1, shows the physical properties of the ligand and complexes

| Compounds | Colour | Melting Point | Yield (%) |
|---|--------|---------------|-----------|
| [NiHis ₂ (H ₂ O) ₂] | Green | 166°C | 62.75 |
| [CuHis ₂ (H ₂ O) ₂] | Green | 160°C | 70.45 |
| [CoHis ₂ (H ₂ O) ₂] | Pink | 163°C | 68.35 |
| Schiff base | Yellow | 244°C | 66.20 |

Table 2, shows the Infrared Frequencies (in cm⁻¹) of the ligand and Metal Complexes.

| S/N | Compounds | v(O-H) | v(C=O) | v(C=N) | v(M-N) | v(M-O) |
|-----|---|--------|--------|--------|--------|--------|
| 1. | [NiHis ₂ (H ₂ O) ₂] | 3410 | 1751 | 1519 | 678 | 447 |
| 2. | [CuHis ₂ (H ₂ O) ₂] | 3410 | 1751 | 1519 | 671 | 439 |
| 3. | [CoHis ₂ (H ₂ O) ₂] | 3417 | 1753 | 1521 | 671 | 470 |
| 4. | Schiff base | - | 1751 | 1519 | - | - |

Table 3, The antibacterial activity of the Schiff base ligand and its metal complexes in millimeters (mm) are shown below.

| S/N | Name of Bacteria Pathogens | Zones of inhibition of ligand and metal complexes in millimeter (mm) | | | | |
|-----|----------------------------|--|----|----|----|------|
| | | Histidine | Cu | Ni | Co | DMSO |
| 1. | Escherichiacoli | 00 | 25 | 22 | 23 | 00 |
| 2. | Pseudomonas aeruginosa | 00 | 23 | 18 | 23 | 00 |
| 3. | Salmonella typhi | 00 | 20 | 24 | 24 | 00 |
| 4. | Staphylococcus aureus | 00 | 27 | 25 | 29 | 00 |

Table 4; Minimum Inhibitory Concentration of the metal complexes against bacterial pathogens

| S/N | Bacteria | Compounds | Zones of inhibition of ligand and metal complexes. | | |
|-----|----------------------|---|--|-------|-------|
| | | | 300µg | 200µg | 100µg |
| 1. | <i>E.coli</i> | [NiL ₂ (H ₂ O) ₂] | + | + | - |
| | | [CuL ₂ (H ₂ O) ₂] | + | + | - |
| | | [CoL ₂ (H ₂ O) ₂] | + | + | - |
| 2. | <i>P. aeruginosa</i> | [NiL ₂ (H ₂ O) ₂] | + | + | - |
| | | [CuL ₂ (H ₂ O) ₂] | + | + | - |
| | | [CoL ₂ (H ₂ O) ₂] | + | + | - |
| 3. | <i>S. typhi</i> | [NiL ₂ (H ₂ O) ₂] | + | + | - |
| | | [CuL ₂ (H ₂ O) ₂] | + | + | - |
| | | [CoL ₂ (H ₂ O) ₂] | + | + | - |
| 4. | <i>S. aureus</i> | [NiL ₂ (H ₂ O) ₂] | + | + | - |
| | | [CuL ₂ (H ₂ O) ₂] | + | + | - |
| | | [CoL ₂ (H ₂ O) ₂] | + | + | - |

Table-5; Antifungal activity of the Schiff base ligand and its metal complexes in millimeters (mm)

| S/N | Name of Fungi Pathogens | Zones of inhibition of ligand and metal complexes in millimeter (mm) | | | | |
|-----|-------------------------|--|----|----|----|------|
| | | Histidine | Cu | Ni | Co | DMSO |
| 1. | Candida albicans | 00 | 24 | 26 | 28 | 00 |
| 2. | Aspergillus flavus | 00 | 18 | 27 | 26 | 00 |
| 3. | Aspergillus niger | 00 | 22 | 26 | 27 | 00 |

Table-6; Minimum Inhibitory Concentration of metal complexes against fungi pathogens

| S/N | Fungi | Compound | Zones of inhibition of ligand and metal complexes. | | |
|-----|---------------------------|---|--|-------|-------|
| | | | 300µg | 200µg | 100µg |
| 1. | <i>Candida albicans</i> | [NiHis ₂ (H ₂ O) ₂] | + | + | - |
| | | [CuHis ₂ (H ₂ O) ₂] | + | + | - |
| | | [CoHis ₂ (H ₂ O) ₂] | + | + | - |
| 2. | <i>Aspergillus flavus</i> | [NiHis ₂ (H ₂ O) ₂] | + | + | - |
| | | [CuHis ₂ (H ₂ O) ₂] | + | + | - |
| | | [CoHis ₂ (H ₂ O) ₂] | + | + | - |
| 3. | <i>Aspergillus niger</i> | [NiHis ₂ (H ₂ O) ₂] | + | + | - |
| | | [CuHis ₂ (H ₂ O) ₂] | + | + | - |
| | | [CoHis ₂ (H ₂ O) ₂] | + | + | - |

DISCUSSION

The Schiff base ligand was prepared by refluxing calculated amounts of amino acid histidine with acetylacetone in ethanol and water. The metal complexes were prepared by using hydrated metal chloride salts with the amino acid histidine (Schiff base) in appropriate molar ratios of metal-ligand as illustrated. [3] The Schiff base which is yellow in colour had a melting point of 244°C and percentage yield of 66.20%. The complexes of nickel (II), copper (II) and Cobalt (II) synthesized are blue, green and pink in colour and have decomposition temperatures of 166°C, 160°C and 163°C, and percentage yield of 62.75, 70.45 and 68.35% respectively as shown in Table 1. The metal complexes were found to be soluble in all the polar solvents dissolved in including water, methanol, ethanol, butanol, acetone, ethyl acetate and in the non-polar solvents of benzene and di ethyl-ether. The diverse coordination geometry of transition metals opens an exciting window for the discovery of novel and potent metallodrugs with novel mechanisms of action. [8]

Moreover, Electronic Absorption Studies indicated the UV-Visible spectra in the region 200-1100 nm were measured by systronics double beam spectrophotometer 2202 model using calibrated quartz cells. The measurements were carried out at room temperature. The electronic absorption spectrum of the Schiff base ligand indicated two absorption bands around 340 nm and 420 nm correspondingly. The first band around 340 nm corresponded to $\pi \rightarrow \pi^*$ of the azomethine group and the second band at 420 nm corresponded to $\pi \rightarrow \pi^*$ transitions. The distinctive electronic absorption spectrum of the Co (II) complexes. The electronic spectra of Co(II) complexes display absorption bands in the region of 8000-10000 cm^{-1} and 18000-20000 cm^{-1} consequent to the transitions ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_1) and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_3) respectively (Underhill and Billing1966). In the current work Co (II) complexes shows the absorption bands in the region 240 nm and 384 nm corresponding to ν_1 and ν_3 transitions respectively. The ν_2 band is not observed due to strong proximity to ν_3 transition. These bands are distinguishing of high spin octahedral Co (II) complexes. [2] Upon complexation, the further reduced polarity of Co (II) ion was due to ligand orbital overlap and partial positive charge sharing of the Co (II) ion with donating lone pair groups. Furthermore, the delocalization of π -electrons upsurges over the whole ring and expands the lipophilicity of the whole complexes. The amplified lipophilicity enhances the chances of the infiltration of the compounds into lipid membranes and obstructing spots of the metal binding in the enzymes of pathogens. These organometallic compounds also alter the respiration route of the pathogenic cells and thus stopping the protein synthesis consequently limiting additional development of these organisms. [9] The Ni(II) complex indicated three bands at 246, 261 and 266 nm ascribed to the ${}^3A_{2g} \rightarrow {}^3T_{2g}$ (ν_1), ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$ (ν_2) and ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$ (ν_3) transitions in that order, which is an indication of the octahedral geometry around Ni(II) ion center. This also holds true for the Cu(II) complex result similar to other reported complexes. [12] The electronic spectra of Cu(II) complexes displayed two important bands, first a low-intensity band at about 16,243 cm^{-1} which is due to ${}^2E_g \rightarrow {}^2T_{2g}$ transition and another high-intensity band at 26,366 cm^{-1} is assigned to ligand-metal charge transfer. A distorted octahedral geometry is suggested around the Cu(II) ion on the basis of electronic spectra. [10] Upon complexation, the further reduced polarity of Cu (II) ion was due to ligand orbital overlap and partial positive charge sharing of the Cu (II) ion with donating lone pair groups. Furthermore, the delocalization of π -electrons upsurges over the whole ring and expands the lipophilicity of the whole complexes.

The infrared spectra of the amino acid histidine Schiff base showed a band at 1632 cm^{-1} indicative of $\nu(C=N)$ stretching frequencies. Similar bands are observable in the spectra of the corresponding metal complexes, but in lower frequencies, indicating that the Schiff base has coordinated to the respective metal ions. The coordination positions of the histidine amino acid in the M(II) complexes are confirmed by observing the well-built bands in the far IR spectra region. The bands in the spectra of the metal (II) complexes in the ranges of 460 – 480 and 630 - 625 cm^{-1} progressively are assigned to the vibrational modes of $\nu(M-N)$ and $\nu(M-O)$ confirming the complexation of the Schiff base to the individual metal ions as in the Table 2. The tough bands at 2922 and

2900 cm^{-1} are indicative of the $\nu(\text{O-H})$ stretching frequencies for coordinated water in M (II) Schiff base complexes. ^[11]

The dissociation constant of the amino histidine Schiff base as reported ^[1] is 8.81, demonstrating that it is a weak acid. The empirical formula of the metal (II) ions, water content and the Schiff base from their known percentage compositions in the complexes, suggested the general formula $[\text{MHis}_2(\text{H}_2\text{O})_2]$.

The antibacterial activity evaluation of the Schiff base and the metal (II) complexes have been determined in comparison with the standard Dimethyl sulfoxide (DMSO). Organometallic drugs consist of basically two parts: the drug itself, usually a hydroxamic acid, and an ancillary ligand. The mechanism of action involves dissociation of the complex inside the bacteria target promoting the release of the drug to its target. ^[12] The diameter of inhibition zone in (mm) was measured for each treatment. The organisms were found to be resistant to the Schiff base at all concentrations. However, the metal (II) complexes showed strong activity against the isolates at medium and high concentrations as shown in Tables 3 and 4.

CONCLUSION

In view of these results of the bioactivities of these complexes synthesized, it has shown that histidine complexes have the potentials against multi drug resistant pathogens. Nevertheless, further studies on the mechanism of growth inhibition, toxicity and invitro analysis are needed, in order to evaluate their potential of therapeutic applications. The novel approach of organometallic complexes in their biological activity against these multi drug resistant pathogens gives more rooms for further experimentations and analysis especially when the complexes mimics naturally occurring complexes in the living system.

REFERENCES

1. David K. Henderson, (2006). American Journal of Infection Control, 34(5)46-54
2. Chohan, Z. H., Hazoor A. S., Moulay H. Y., Taibi B. H. (2010). Some new biologically active metal-based sulfonamide, *European Journal of Medicinal Chemistry* 4(5)2893-2901
3. Subhasish S., Dharumadurai D., Sarvanan C., and Annamalai P. (2009). Synthesis, Characterization And Antimicrobial Activity Of Cobalt Metal Complex Against Multi Drug Resistant Bacterial And Fungal Pathogens. *Facta Universitatis Series: Physics, Chemistry and Technology*. 7(1)73 – 80.
4. Aliyu H. N. and Mohammed A. S. (2012) Synthesis, Spectrophotometric and Biological Activity of Nickel (II) and Copper (II) Complexes with Schiff Base Derived from Acetyl acetone and Histidine. *Global Advanced Research Journal of Microbiology* 1(5) pp. 067-071
5. Mark E., E., Galina N. N., Nadezhda Y., Krainova, V. P., and Lapikova, A. A., (2000). *Journal of Inorganic Biochemistry*, 8(1)285-293
6. Bandow, J. E., and Metzler, N. N. (2009). New ways of killing the beast: Prospects for inorganic-organic hybrid nanomaterials as antibacterial agents. *Journal of BioChem*, 10(18), 2847-2850.
7. Malghe Y.S., Prabhu R.C. and Raut, R.W. (2009). Synthesis, characterization and biological activities of mixed ligand Zr (IV) complexes. *Acta Poloniae Pharmaceutica- Druge Research*, 66(1), 45-50.
8. Janiaj C. (2000). A Critical account on -stacking in metal complexes with aromatic nitrogen containing ligands, *Journal Chemical Society. Dalton Transactions*. 78(3)79-85.
9. Zafar A. S., Shahid, M., Khalid, M. and Kumar S. (2009) Antimicrobial and SOD activities of novel transition metal ternary complexes of iminodiacetic acid containing a-diimine as auxiliary ligand. *European Journal of Medicinal Chemistry* (44)2517-2522,
10. Rahman, M.A., Imran T. and Islam S. (2013). Antioxidative, antimicrobial and cytotoxic effects of the phenolics of *Leea indica* leaf extract. *Saudi Journal of Biological Sciences*. 20(3): 213-225.
11. Bellú S., Hure, E. M. Trapé, M., Trossero, C., Molina, G., Drogo, C., Williams, P. A. M., Atria, A. M., Muñoz Acevedo, J. C., Zacchino, S., Sortino, M., Campagnoli D. and Rizzotto, M. (2005) Synthesis, Structure and Antifungal Properties of Co (II)-sulfathiazolate complexes *Polyhedron* 2(4)501-509
12. O'Connell, K. M., Hodgkinson, J. T., Sore, H. F., Welch, M., Salmond, G. P., and Spring, D. R. (2013). Combating multi-drug resistant bacteria: Current strategies for the discovery of novel antibacterials. *Angewandte Chemie International Edition*, 52(41), 10706-10733.
13. Ahmad, J. and Jemal, M. A. (2013). Breast Cancer Metastasis and Drug Resistance, *Springer New York*, pp. 1.
14. Pagès, J. M., Kascàková, S., Maigre, L., Allam, A., Alimi, M., Chevalier, J., and Artaud, I. (2013). New peptide-based antimicrobials for tackling drug resistance in bacteria: Single-cell fluorescence imaging. *ACS Medicinal Chemistry Letters*, 4(6), 556-559.
15. Pagès, J. M., Kascàková, S., Maigre, L., Allam, A., Alimi, M., Chevalier, J., and Artaud, I. (2013). New peptide-based antimicrobials for tackling drug resistance in bacteria: Single-cell fluorescence imaging. *ACS Medicinal Chemistry Letters*, 4(6), 556-559.