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SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL STUDIES OF NI(II), CU(II), AND ZN(II) COMPLEXES WITH A SCHIFF BASE DERIVED FROM 3-FORMYLCHROMONE AND BENZOHYDRAZIDE

*Hussaina Aminu Ibrahim, Umar Faruk Muhammed, Abdullahi Aliyu, Muhammad Shamsu Bunza

Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Federal University, Birnin Kebbi (FUBK), P.M.B 1157, Kebbi State, Nigeria

*Corresponding Author Email Address: hussainaai@gmail.com

ABSTRACT

A Schiff base ligand derived from condensation of 3-formylchromone and benzohydrazide was synthesized. Its Ni(II), Cu(II) and Zn(II) complexes were subsequently prepared and characterized. The characterization techniques included: molar conductivity measurement, magnetic susceptibility, IR spectroscopy, AAS spectroscopy, elemental analysis and thermal measurements. The Schiff base gave a melting point of 165^{0}C, while the complexes decomposed in range of (195-230)^{0}C indicating good thermal stability. The molar conductance values of the complexes ranging from (3.08-5.71) $\Omega^{-1}cm^2mol^{-1}$ suggested that all complexes are non-electrolytic in nature. The IR spectrum of the free ligand displayed a band at 1633cm^{-1} attributed to the azomethine (-C=N-) stretching vibration. This band shifted to lower wavenumbers (1603 -1655) cm^{-1} in the spectra of the complexes, confirming coordination through the azomethine Nitrogen. Magnetic susceptibility measurements indicated that all the complexes except that of Zn are paramagnetic and exhibited tetrahedral geometry. Elemental analysis data were consistent to 1:1 metal to ligand stoichiometry. Furthermore, the Schiff base and the complexes were evaluated for antibacterial and antifungal activity against some pathogenic microorganisms. Some of the compounds showed notable activity, while others exhibited no significant effect.

Keywords: Schiff base, condensation, metal complexes, antimicrobial, characterization, reflux

INTRODUCTION

Schiff bases were first by great German chemist, named Hugo Schiff in 1864 (Ashraff, Mahmood & Wajid, 2011). They are versatile ligands and are synthesized from the condensation of primary amine with carbonyl compounds (Prakash & Adhikari, 2011). In Schiff base, the oxygen atom of a carbonyl group (>C=O) is substituted with a nitrogen atom, forming an imine group (>C=NR), where R≠ H. while the term Schiff base is often used interchangeably with 'Azomethine'some definitions limit it specifically to secondary aldimines in which the carbon atom is directly bonded to a hydrogen atom(Vivek & Pandurangan, 2014). Schiff base are generally represented by the formula ($R_1R_2C=NR_3$) where R_1, R_2 and R_3 may be alkyl, aryl, cycloalkyl or heterocyclic groups though R_3 cannot be hydrogen. These compounds can function as mono-, di-, tri-, or tetra dentate ligands depending on the number and type of donor atom within the molecule. Upon coordination with metal ions, Schiff bases frequently form stable five or six membered chelating rings. Furthermore, when additional donor groups such as –OH, –NH_2 or –SH are present, the Schiff bases act as mixed-donor ligands capable of participating in bi-, tri-, tetra- or even higher coordination geometries. Schiff bases derived from aliphatic aldehydes tend to be relatively unstable and prone to polymerization, whereas those formed from aromatic aldehydes exhibit greater stability due to enhanced conjugation across the aromatic system (Arulmurugan, Kavitha & Venkatraman, 2010). They are widely used for various purposes which includes; industrial and exhibit a wide range of biological activities. Aromatic aldehydes with aliphatic or aromatic ketones give stable azomethine moiety. Modifications of the Schiff bases have proven very efficient with improved potency and less toxicity (Rajavel et al., 2013). On the other hand, Transition metal complexes of Schiff base are well known for almost two centuries; for example, in 1840, Ettling (as cited in (Abdul-Alim et al., 2015)) first isolated a dark green crystalline compound resulting from the reaction of cupric acetate, salicyladehyde and aqueous ammonia. However, a more detailed and systematic investigation into such complexes was not undertaken until in 1931 when Pfeiffer and colleagues as cited in (Hossain et al., 2017) reported a series of metal complexes derived from Schiff bases formed with salicylaldehyde and it substituted derivatives. Transition metals form Schiff base complexes; due to the fact that the Schiff base possesses a lone pair of electron that can donate to the empty orbital of the metal. These metal ions play crucial roles in various biological processes that are essential for sustaining life. They can also coordinate with O or N-terminals from proteins in a variety of models and play a crucial role in the conformation and function of biological macromolecules (Aliyu & Sani, 2012). Transition metal centers are especially appealing to the reversible recognition of nucleic acids due to their well- defined coordination geometries. In addition, their unique electrochemical and photophysical properties, enhance the functional potential of the resulting metal-ligand complexes as effective binding agents (Prasad, Agrawal & Sharma, 2002). These metal complexes also serve as structural models for biologically relevant species and are widely employed in biomimetic catalytic system (Ammar & Alaghaz, 2013). Researchers are presently concentrating on Schiff base transition metallic complexes originating from heterocyclic carbonyl derivatives owing to their distinctive focused of enticement in many different kinds of applications, including biological, clinical, therapeutic and pharmaceutical industries (Senthamil Selvan et al., 2024). In the present study, a schiff base derived from 3-formylchromone and benzohydrazide was synthesized and subsequently used to prepare its corresponding metal(II) complexes. Both the schiff base and its metal complexes were characterized using a range of analytical and spectroscopic techniques. Their antibacterial and antifungal effect was also determined against *Staphylococcus aureus* and *Eschirichia coli* for antibacterial and *Aspergillus fluvus,* and *Candida albican* for antifungal.

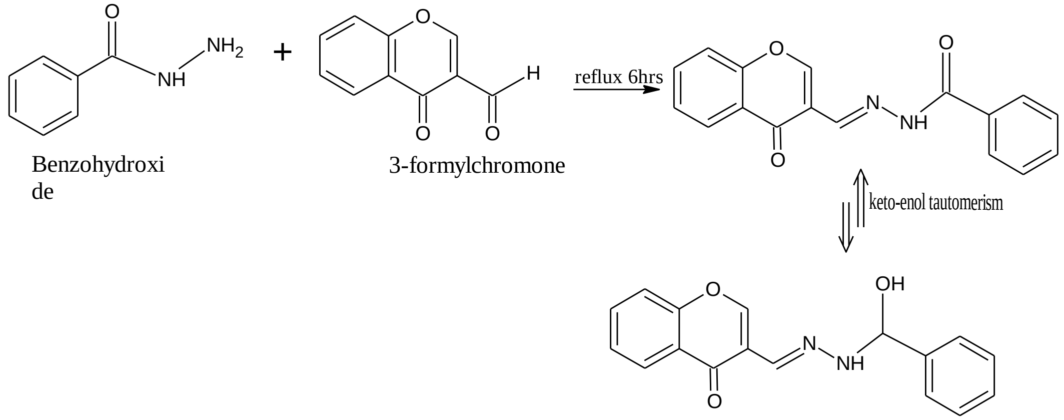
This work reports for the first time, a chromone-hydrazide hybrid Schiff base, coordinated with Ni(II), Cu(II) and Zn(II) triad and evaluates their comparative antimicrobial profiles within an identical ligand framework. By combining the chromone nucleus with benzohydrazide donor set, the study creates a previously under explored coordination environment, enabling direct structure-activity correlations across three biologically relevant d-block metals. Schiff base metal complexes are widely studied for their structural diversity and biological activities. Particular attention has been given to ligands derived from heteroaromatic aldehydes such as chromones and from hydrazide derivatives. However, limited research has focused on Schiff base complexes formed from *3-formylchromone* and *benzohydrazide*. In particular, the coordination chemistry of Ni(II), Cu(II), and Zn(II) with this ligand system remains largely unexplored. Previous studies often examined either chromone Schiff bases with other amines or benzohydrazide ligands without the chromone nucleus. Additionally, there is a lack of comparative studies involving these three transition metals within the same ligand framework, leaving a significant gap in understanding their structural and biological behaviors. This study seeks to address the identified gap by synthesizing, characterizing, and investigating the antimicrobial activities of Ni(II), Cu(II), and Zn(II) complexes with a Schiff base derived from *3-formylchromone* and *benzohydrazide*. The hybrid ligand system is expected to enhance coordination properties and potentially improve antimicrobial potency due to the synergistic contribution of the chromone nucleus and hydrazide group. The outcomes of this research will expand the chemistry of chromone-based Schiff bases and provide valuable insights for the development of novel antimicrobial agents.

MATERIALS AND METHODS

All chemicals employed in this study were purchased from commercial suppliers (Sigma/Aldrich), of analytical grade and were utilized as received, without further purification. Weighings were performed using Mettler electronic balance model H3OAR. All glassware were thoroughly cleaned with detergent, rinsed with distilled water and oven dried at 110^{o}C prior to use.

Synthesis of the Schiff base Ligand

The Schiff base ligand was synthesized following the procedure described by Jessica *et al* (15) with slight modification where necessary. A mixture of Benzohydrazide (25.0 mmol, 3.404g) and 3-formylchromone (25.0 mmol, 4.354g) was prepared by dissolving the compounds in 50ml of ethanol. Few drops of glacial acetic acid were added to the solution to catalyze the condensation reaction. The reaction mixture was stirred continuously and refluxed for 6 hrs. to ensure complete reaction. After completion, the resulting mixture was cooled in ice-cold water. The resulting solid product was filtered, concentrated and then dried in a desiccator over CaCl$_2$ for a week to obtain pure and stable Schiff base ligand (Ajibade & Ejidike, 2015).



Scheme 1: Synthetic pathway of the ligand adopted method of (Jessica et al., 2018)

Synthesis of the metal (II) Schiff Base Complexes

Metal(II) Schiff base complexes were synthesized by reacting the Schiff base ligand with the respective metal(II) salt **in a** 1:1 molar ratio. Specifically, A solution of the ligand 20 mmol(5.842g) was prepared in dimethylformamide (DMF), while separate solution of the metal salt (20 mmol) was made using 50cm^3 of methanol. The metal solution was added gradually to the ligand solution with continuous stirring. The reaction mixture was then refluxed for 8 hrs. to ensure complete complexation. After cooling at room temperature, the resulting precipitate were filtered, successfully washed with methanol, hexane and diethylether to remove impurities, and finally air dried. The purified complexes were stored in a dessicator over anhydrous calcium chloride for further use (Ajibade & Ejidike, 2015).

Physicochemical characterization.

The infrared (IR) spectra were obtained for both the Schiff base and its metal complexes using FTIR Carry 630 spectrophotometer (Agilent Technologies) in the range of 4000 - 650 cm^{-1} , employing KBr pellets. Elemental analysis for C, H and N were carried out using Perkin-Elmer 240 elemental analyzer. Molar conductivity measurement were performed using Jenway 4010 conductivity meter with DMSO as solvent. Melting point and decomposition temperatures were determined using a STUART SMP10 melting point apparatus. Magnetic susceptibility measurements of the complexes were conducted at room temperature on Gouy's balance.

Antibacterial and Antifungal Activity

Evaluation of the synthesized Schiff base ligand and its metal(II) complexes was carried out to determine their antimicrobial activity against selected microbial strains including *Staphylococcus aureus, Escherichia coli, Candida albican and Aspergillus fluvus*. Antimicrobial testing was conducted using a disc diffusion method. Stock solutions were prepared and serially diluted to obtain concentrations of 4000µg/ml, 2000µg/ml, 1000µg/ml and 500µg/ml using a double dilution technique. Sterile forceps were used to carefully place the prepared discs containing the ligand and metal complexes onto nutrient agar plates previously inoculated with the respective test organism. The plates were incubated at 37^{o}C for 24 hrs. for bacterial cultures and at 48 hrs. for fungi at room temperature. Following incubation, the zone of inhibition around each disc were measured in millimeters and compared with those produced by standard antimicrobial agents – Amoxicillin for bacteria and Nystatin for fungi.

RESULTS AND DISCUSSION

Physicochemical Properties of the Schiff base and its Metal (II) Complexes

The synthesized Schiff base and its metal (II) complexes were obtained in good yields, with their physical and chemical properties summarized in table 1. The Schiff base was obtained in yield of 92% while the Ni(II), Cu (II) and Zn(II) complexes were isolated in yield of 91%, 81% and 86% respectively the schiff base appears as pale yellow crystals, whereas the Ni(II), Cu (II) and Zn(II) complexes exhibited brown, green and yellow colors respectively. The Schiff base exhibited a melting point of 165^{O}C, while the decomposition temperatures of the metal(II) complexes were 239^{o}C, 195^{o}C and 220^{o}C respectively, indicating their stability under thermal conditions. Molar conductance values of the synthesized complexes were measured to be (5.71, 5.17 and 3.08) $\Omega^{-1}cm^{2}mol^{-1}$ respectively, suggesting their non-electrolytic nature, consistent with previously reported findings (Yushau & Salisu, 2011). The effective magnetic moment of the complexes were calculated as 3.9µB and 1.2µB for Ni(II) and Cu (II). The values are indicative of paramagnetic behavior, as expected for such complexes, while Zn(II) appeared to be diamagnetic (Cozzi, 2004).

Table 1: Physicochemical Properties of the Schiff base and its Metal (II) Complexes

Compounds	**Colour**	**% yield**	**M.P (OC)**	**D. Temp (OC)**	B.M. (µB)	**M.L(Ω-1cm2 mol-1)**
Ligand	Pale Yellow	92	165	-	-	**-**
[NiL(H2O)(oAc)]	Brown	91	-	230	3.9	5.71
[CuL(H2O)(oAc)]	Green	81	-	195	1.2	5.17
[ZnL(H2O)(oAc)].H2O	Yellow	86		220	-	3.08

Solubility Test

The solubility of the Schiff base and its metal(II) complexes was evaluated in water and several common organic solvents as summarized in table 2, the compounds exhibited high solubility in dimethylsulfoxide (DMSO) and Methanol, moderately soluble in ethanol and (DMF) and were found to be insoluble in petroleum ether and water. The observed solubility pattern suggest that both Schiff base and the complexes exhibit polar characteristics as evidence by their good solubility in polar aprotic (DMF and DMSO) and polar protic solvents (methanol and ethanol). Their insolubility in water and petroleum ether, a non-polar solvent indicate limited or no interaction with highly polar or non-polar media, respectively. This behavior is typical of coordination compounds with moderate polarity and support the presence of strong intra-molecular interactions and possibly extensive conjugation within the ligand framework (Ibrahim et al., 2021)

Table 2: Solubility profile of the Schiff base and its metal (II) complexes

Compound	Water	DMSO	methanol	Ethanol	DMF	CCl_4	Chloroform	P.Ether
Ligand	IS	S	S	S	SS	IS	SS	IS
[NiL(H2O)(oAc)]	IS	S	S	SS	S	IS	SS	IS
[CuL(H2O)(oAc)]	IS	S	S	SS	S	IS	S	IS
[ZnL(H2O)(oAc)].H2O	IS	S	S	SS	SS	IS	SS	IS

Key; S = soluble, SS = slightly soluble, IS = insoluble

Elemental Analysis

The elemental composition of the synthesized shiff base and its metal(II) complexes were determined and summarized in table 3.the experimentally obtained value shows good agreement with the calculated values, with slight deviation, which may be attributed to the retention of coordinated or lattice solvent molecules that are often difficult to remove completely during drying. In addition, the hygroscopic nature of a compounds may have contributed to variations in the hydrogen and carbon percentages(Cinarli, 2024). Nonetheless, the values obtained are within acceptable analytical ranges, thereby supporting the proposed molecular compositions of the ligand and its metal complexes. The elemental analysis of the free ligand indicated a molecular formula $C_{17}H_{12}N_{2}O_{3}$. For the metal complexes, the data supported the formulations of [NiL(H_2O)(oAc)], [CuL(H_2O)(oAc)] and [ZnL(H_2O)(oAc)].H_2O. the result suggest the formation of 1:1 metal to ligand complexes, with acetate ions acting as co-ligands.

Table 3. Elemental composition result of the Schiff base and its Metal (II) Complexes

Compound	C Observed (calculated)	H Observed (calculated)	N Observed (calculated)
Ligand	70.36 (69.86)	4.06 (4.14)	9.66 (9.58)
$[NiL(H_2O)(oAc)]$	52.27 (53.44)	4.35 (3.77)	9.06 (7.56)
$[CuL(H_2O)(oAc)]$	53.83 (52.83)	3.48 (3.73)	8.46 (7.48)
$[ZnL(H_2O)(oAc)].H_2O$	48.71 (50.51)	3.01 (4.01)	9.58 (8.20)

FTIR spectral Analysis

The FTIR spectrum of the schiffbase ligand exhibited a characteristic band at 1633cm^{-1}, corresponding to the v(C=N) stretching vibration of the azomethine group. In the metal(II) complexes, this band shifted to a lower frequencies appearing in the range of (1581-1611)cm^{-1}, indicating coordination of the azomethine Nitrogen to the metal centres (Mahmoud et al., 2016). Additionally, bands appearing in the regions (638-760)cm^{-1} and (452 - 496)cm-^{1} were attributed to v(M-N) and v(M-O) stretching vibrations respectively, further supporting the coordination of the ligand to the metal ions through nitrogen and oxygen donor atoms(Al-Shemary & Zaidan, 2016). Addional band observed in the range of (864-969)cm^{-}1 was assigned to coordinated water molecules, reinforcing the proposed structural formulations of the complexes(Aliyu & Toudou, 2012). These observations are consistent with literature reports for structurally related Schiff base metal complexes, reinforcing the proposed coordination mode and structural geometry of the synthesized compounds. The spectra are provided in the supplementary information figures (figure 1-7), while the key absorption bands are summarized in table 4.

Table 4. FT-IR spectral data of the Schiff base and its metal (II) complexes

Compound	υ(C=N) cm^{-1}	υ(M-N)cm^{-1}	υ(M-O)cm^{-1}	υ(OH/H$_2$O) cm^{-1}	υ(OH) cm^{-1}
Ligand	1633	-	-	-	
[NiL(H2O)(oAc)]	1581	764	496	-	864
[CuL(H2O)(oAc)]	1611	760	452	-	969
[ZnL(H2O)(oAc)].H2O	1599	638	489	3316	909

Antibacterial and antifungal Activity.

The biological activity of the Schiff base and its metal complexes was evaluated by the disc diffusion method using inhibition zone (IZ) diameter (mm) as a measure activity. All compounds were tested in vitro against *Staphylococcus aureus* and *Escheirichia coli (bacterial strains) and Aspergillus fluvus,* and *Candida albican* (fungal strains). The results are summarized in table 5 and 6. To rule out any influence of the solvent, DMSO was used as a negative control in parallel assays. It exhibited no inhibitory effect on any of the tested microbial strains, confirming that the observed activity was attributed solely to the rest compounds. The metal complexes showed greater antimicrobial activity compared to the free Schiff base ligand. This enhance activity may be attributed to increased lipophilicity of the metal complexes, which facilitates their penetration through the microbial cell membrane. The interaction of the complexes with cellular components such as lipoproteins may disrupt normal cell function, ultimately leading to cell death (Tadele, 2017). Furthermore, the thermal stability of the complexes suggests their potential applicability as antimicrobial agents under diverse environmental conditions. A similar trend was observed on the antifungal assays, where the complexes showed higher inhibitory activity than the free ligand against the isolates. These findings suggest that metal coordination enhances biological activity of Schiff base ligand (Badade et al., 2025).

Table 5. Antibacterial evaluation of the schiff base and its metal (II) complexes.

Isolates	Compound	Zone of Inhibition (mm)/Concentration (μg/cm^3) 4000 2000 1000 500		Control(mm)Amoxicillin 500μg/disc
Staph	Ligand	15	12	
ylococ	[NiL(H2O)(	9	-	31
cus	oAc)]	16	12	
aureus	[CuL(H2O)	8	-	
	(oAc)]	21	18	
	[ZnL(H2O)(	16	13	
	oAc)].H2O	15	13	
		12	8	
Esche	Ligand	10	8	
richia	[NiL(H2O)(	-	-	26
coli	oAc)]	16	14	
	[CuL(H2O)	10	8	
	(oAc)]	14	12	
	[ZnL(H2O)	8	-	
	(oAc)].H2	12	8	
	O	8	-	

Table 6. Antifungal evaluation of the schiff base and its metal (II) complexes.

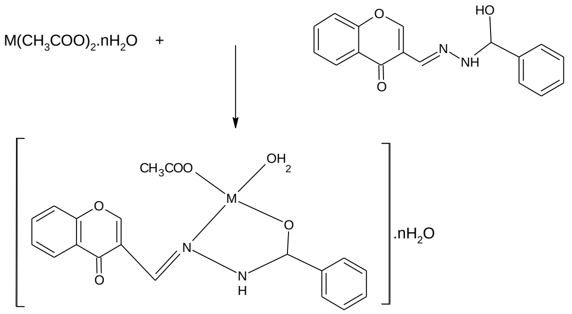
Isolates	Compound	Zone of Inhibition (mm)/Concentration (μg/cm^3) 4000 2000 1000 500	Control(mm) Nystatin 200mg
Candida albicans	Ligand [NiL(H2O)(oAc)] [CuL(H2O)(oAc)] [ZnL(H2O)(oAc)].H2O	14 12 10 8 17 15 11 9 16 14 12 9 16 13 8 -	30

Aspergill	Ligand		16	12	8	
us fluvus	[NiL(H2O)(oAc)]	-				40
	[CuL(H2O)(oAc)]		19		17	
	[ZnL(H2O)(oAc)].H	14	12			
	2O		18		17	
		13	10			
			18		16	
		15	12			

Conclusion

In this study a novel Schiff base of 3-formylchromone and benzohydrazide was synthesized and successfully complexed with Ni(II), Cu(II) and Zn(II) ion. The work introduce a chromone-hydrazide Schiff base and its Ni(II), Cu(II) and Zn(II) complexes, delivering the first comparative anti-microbial evaluation within this framework and revealing metal-dependent activity enhancement that guide future ligand/metal selection. Elemental analysis, FTIR spec, and magnetic susceptibility measurement confirm the formation of mononuclear complexes with a tetrahedral geometry. Elemental composition data support the formation of 1:1 metal-to-ligand stoichiometry. The FTIR revealed shift in characteristic bands, indicating coordination through azomethine N and phenolic O atoms of the ligand. The non-electrolytic nature of the complexes was revealed by conductivity measurement data. The solubility behavior of the ligand and its metal (II) complexes showed good solubility in methanol and dimethylsulphoxide, limited solubility in dimethylformamide and ethanol and complete insolubility in water and petroleum ether. This behavior is typical of coordination compounds with moderate polarity and support the presence of strong intra-molecular interactions and possibly extensive conjugation within the ligand framework. The synthesized compounds were evaluated for their antimicrobial and antifungal activities. The metal complexes exhibited significantly enhanced biological activity compared to the free Schiff base ligand. Likely due to increased lipophilicity and improved interaction with microbial cell membranes upon coordination. The work demonstrate utility of the Schiff base metal complexes as promising candidates for further development in coordination chemistry and bioinorganic applications; especially antibacterial and antifungal activities.

Below is the proposed structure of the complexes.



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Supplementary information

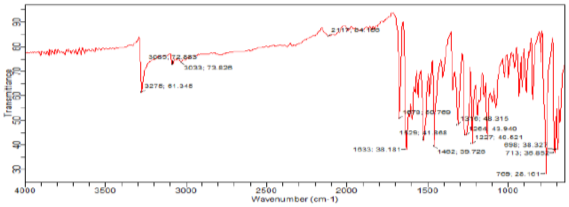

Figure 1: FTIR of the synthesized 3-formylchromonehydrazone

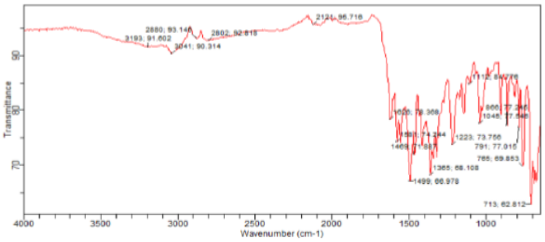

Figure 2: FTIR of Ni (II) complex

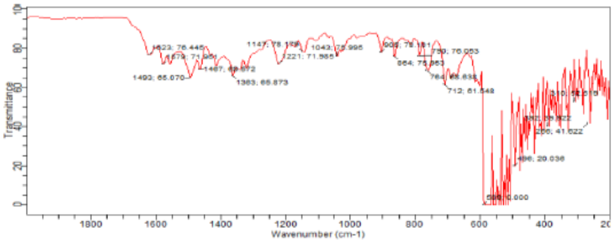

Figure 3: Lower region FTIR of Ni(II) complex

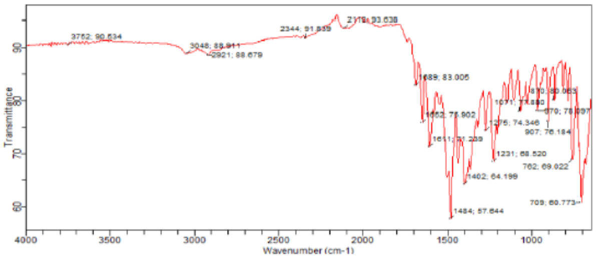

Figure 4: FTIR of Cu (II) complex

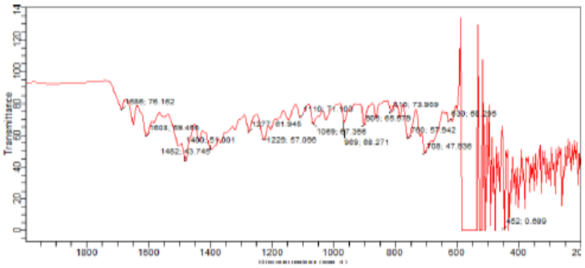

Figure 5: Lower region FTIR of Cu(II) complex

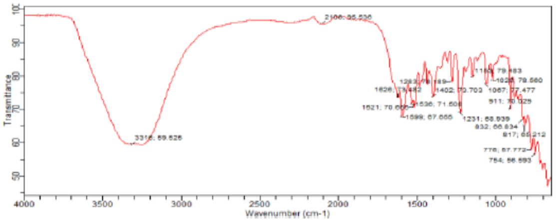

Figure 6: FTIR of Zn (II) complex

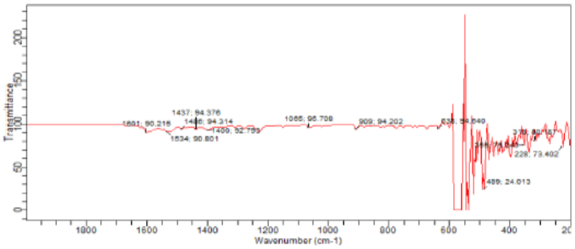

Figure 7: Lower region FTIR of Zn (II) complex