SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SCHIFF BASE, 4-CHLORO-2-{(E)-[(4-FLUOROPHENYL) IMINO]METHYL}PHENOL AND ITS TRANSITION METAL (II) COMPLEXES

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Synthesis, Characterization and Antibacterial Activity of Schiff Base, 4-Chloro-2-{(E)-[(4-Fluorophenyl)Imino]Methyl}Phenol and Its Transition Metal (II) Complexes

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry of the Jomo Kenyatta University of Agriculture and Technology

2021

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature……..Date ……..

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

This research work is dedicated to my parents, Philemon and Rose, my lovely wife Felistas, my children, Beverly, Ramah and Amaliah, my brothers, Boaz and Paul and my sister, Caroline, with love. Thank you for everything.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired ImmunoDeficiency Syndrome
ALA	L-alanine
ARG	L-arginine
ASPA	L-aspartic acid
ATS	2-aminomethylthiophenyl-4-bromosalicylaldehyde
BS	5-bromosalicylaldehyde
CFPIMP	4-chloro-2-{(E)-[(4-fluorophenyl)imino]methyl}phenol
CT-DNA	Calf thymus-Deoxyribonucleic acid
DFT	Density functional theory
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
HIS	L-histidine
HL	Aminobenzoic Acid
ILT	Intra-Ligand Transitions
IR	Infrared Spectroscopy
LCT	Ligand Centred Transitions
MIC	Minimum Inhibitory Concentration
MLCT	Metal to Ligand Charge Transfer
M^{n+}	Metal ion
m.p	Melting point
NMR	Nuclear Magnetic Resonance

PHALA	L-phenylalanine
r.t	room temperature
UV-Vis	Ultraviolet-Visible Spectroscopy

ABSTRACT

The mortality rate in the world continues to increase due to severe challenge of multidrug resistance in treating a host of bacterial infections. Therefore, an effort to develop new antibacterial agents with novel mechanisms of action, higher activity and improved selectivity to address and counter this antibiotic resistance is important. This will avert significant threats posed to human and animal survival. Schiff base transition metal (II) complexes have exhibited great promise with regard to structural modification of existing drugs and reversing bacterial resistance. In this study, new Mn (II), Co (II), Ni (II), Cu (II) and Zn (II) complexes of the Schiff base ligand, L, 4-chloro-2-{(E)-[(4-fluorophenyl)imino]methyl}phenol derived from 5-chlorosalicylaldehyde and 4-fluoroaniline were synthesized. The ligand and the complexes obtained were characterized by Fourier-Transform Infrared, Ultraviolet-visible, and Nuclear Magnetic Resonance Spectroscopy. The elemental analysis data showed that the metal complexes formed had the general formulae $[ML_2(H_2O)_2]$, where M = Mn (II), Co (II), Ni (II), Cu (II) and Zn (II). The spectroscopic data showed that "O" and "N" donor atoms of the Schiff base ligand participated in coordination to the transition metal (II) ions. An octahedral geometry was thus proposed for these complexes. Molar conductance studies on the complexes indicated they were non-electrolytic in nature. The Schiff base ligand and its transition metal (II) complexes were tested *in vitro* to evaluate their antibacterial activity against Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus typhi*) using the disc diffusion method. The evaluation results revealed that the transition metal (II) complexes exhibited higher antibacterial activity than the free Schiff base ligand against the same bacterial strain. The increased activity of the complexes might be due to partial sharing of the positive charge of metal ion with the donor groups of the Schiff base ligand.

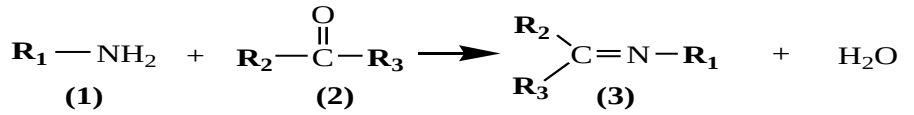
CHAPTER ONE

INTRODUCTION

1.1 Background information

In recent years, the number of people suffering from multidrug-resistant bacterial infections has sharply increased, leaving humanity without any choice but to search for new treatment options and strategies (Dickey *et al*., 2017). The wide occurrence of multidrug resistance in bacterial infections has necessitated the development of new and more potent molecules with desired properties that could circumvent this resistance. A successful strategy has been the use of metallo-drugs with the potential to treat multidrug-resistant infections more efficiently. As a class of molecules, Schiff bases have been of considerable interest, owing to their versatile metal chelating properties, inherent biological activities and flexibility to modify the structure to fine-tune it for a particular biological application. Schiff base-based metallo-drugs are thus being researched on to develop new anti-bacterial therapies (Malik *et al*., 2018; Nema *et al*., 2016).

Schiff bases, named after Hugo Schiff (Schiff, 1864), are formed when a primary amine **(1)** reacts with an aldehyde or a ketone **(2)**. Structurally, a Schiff base **(3)**, also known as imine or azomethine, is a nitrogen cognate of an aldehyde or ketone in which the carbonyl group (C=O) has been supplanted by an azomethine group (C=N-R) as shown in the Scheme 1.1.



Scheme 1.1: General preparation method for Schiff base

According to (Ghara *et al*., 2017), imines of aliphatic aldehydes are relatively unstable and readily polymerizable while those of aromatic aldehydes having effective conjugation are more stable. These bases are considered important compounds because of their wide range of biological activities, and also their use as ligands when coordinated to transition metal ions. Schiff base ligands usually coordinate to a metal ion through the imine nitrogen atom, but coordination *via* other functional groups, e.g. through oxygen or carbon, has also been reported by (Guo *et al*., 2007).

Schiff bases continue to be of great interest, owing to their inherent biological activities, versatile metal binding properties, and flexibility to modify the structure to fine-tune it for a particular biological application (Malik *et al*., 2018). Imines derived from fluoroaniline and salicyladehyde, specifically, have been considered as potential pharmaceutically interesting compounds. This is because, several of the members of this family have shown antiviral, antitumor or antimicrobial activities (Ma *et al*., 2002; Siddiqui *et al*., 2006). These biological activities are attributed to the anil group, the C=N linkage. Thus, Schiff bases are being researched upon deeply owing to their unique and important role in coordination chemistry and medicine.

1.2 Statement of the problem

In recent years, ailments caused by bacterial infections have increased significantly and are becoming a serious global health concern. For instance, Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus typhi*) that infect humans and animals cause various illnesses such as fever, diarrhea and related complications. Eradication of these diseases and associated complications continues to be hampered by drug resistance developed by these disease-causing organisms. Gram positive bacteria have a thick peptidoglycan layer and no outer lipid membrane while Gram negative bacteria have a thin peptidoglycan layer and have an outer lipid membrane.

Therefore, this highlights a great need for a continued search for new compounds with novel and more efficient mechanisms of action against these disease-causing pathogens. Schiff base-metal complexes are promising antibacterial agents, which can be used against these drug resistant microbes.

1.3 Justification of the study

The mortality rate in the world has increased sharply due to various infectious diseases that are directly related to bacterial multiple resistance to antibiotics. This has left humanity without any choice but to search for new and strategic treatment options that could circumvent the multidrug resistance problem. Therefore, there is need to develop novel drug analogues or active agents for the treatment of diseases with better mechanisms of action and structural-activity compared to the classical antibacterial organic molecules. Hence, efforts are being intensified towards the synthesis of metal based active agents with higher efficacy, increased selectivity for disease-causing organisms, lowered toxicity and wider spectrum of activity. Schiff base ligands and their metal ion complexes have been established as potential biochemically active agents.

Schiff base metal complexes which usually contain nitrogen, sulphur or oxygen as donor atoms have become increasingly important. The bases can bind with different metal centres involving various coordination sites and allow successful synthesis of metal complexes. The high affinity of the Schiff base for the transition metal ions is utilized in preparation of their solid complexes. The interaction of these ligands and metal ions give complexes of different geometries with promising antibacterial activities (Divya *et al.*, 2017).

1.4 Hypothesis

Transition metal (II) ions do not significantly enhance the antibacterial activity of Schiff bases against drug resistant bacterial strains.

1.5 Objectives

1.5.1 General Objective

The main objective of this project was to synthesize and characterize the Schiff base, 4-chloro-2-{(E)-[(4-fluorophenyl)imino]methyl}phenol and its metal (II) complexes of Mn (II), Co (II), Ni (II), Cu (II), and Zn (II) and, determine their antibacterial activity.

1.5.2 Specific Objectives

The specific objectives of this project were to:

1. Synthesize and characterize the Schiff base, 4-chloro-2-{(E)-[(4-fluorophenyl)imino]methyl}phenol from 5-chlorosalicylaldehyde and 4-fluoroaniline.
2. Synthesize and characterize complexes of Mn (II), Co (II), Ni (II), Cu (II), and Zn (II) with Schiff base, 4-chloro-2-{(E)-[(4-fluorophenyl)imino]methyl}phenol.
3. Conduct bioassay of the Schiff base ligand and its metal (II) complexes against Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus typhi*).

CHAPTER TWO

LITERATURE REVIEW

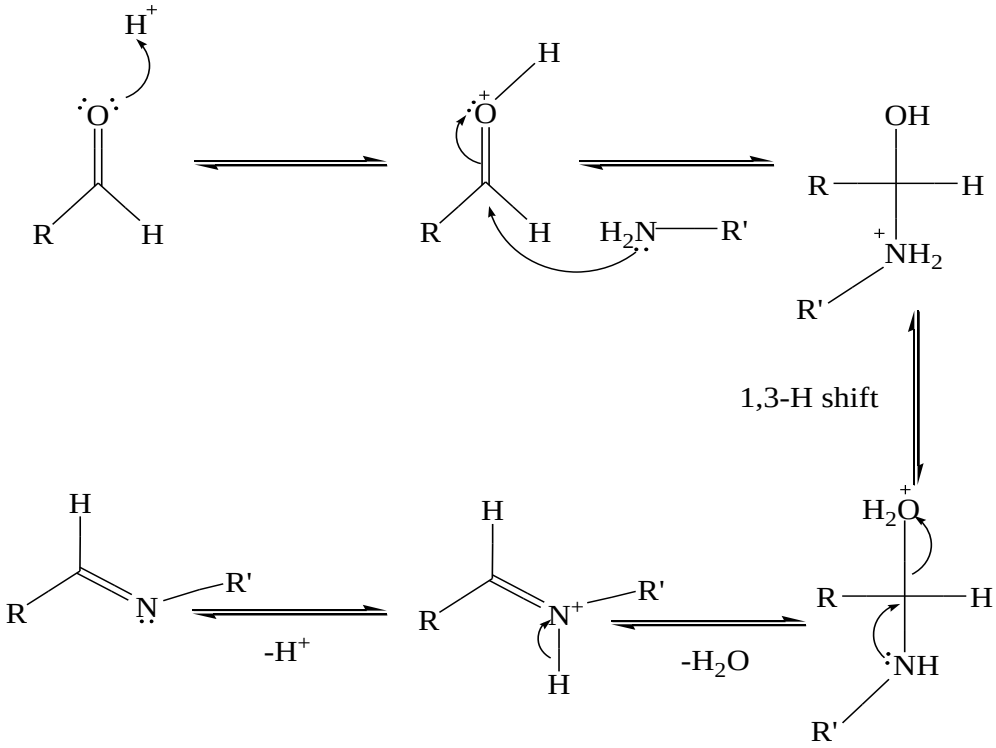
2.1 Schiff bases

Schiff bases are condensation products of primary amines with carbonyl compounds and were first reported by Hugo Schiff in 1864 (Schiff, 1864). According to Kejal and co-workers, these compounds form an important class of widely used organic compounds with a wide variety of applications in many fields including analytical, biological, and inorganic chemistry (Kajal *et al*., 2013).

These Schiff bases have gained importance in medicinal and pharmaceutical fields due to their broad spectrum of biological activities which include among others, analgesic (Mishra *et al*., 2011; Sondhi *et al*., 2006), anti-inflammatory (Sathe *et al*., 2011), antimicrobial (Mounika *et al*., 2010), anticonvulsant (Ajit Kumar and Pandeya, 2012), antitubercular (Aboul-Fadl *et al*., 2003), anticancer (Ali *et al*., 2012; Miri *et al*., 2013), antioxidant (Danyi *et al*., 2006) and antihelminthic (Avaji *et al*., 2009).

2.1.1 Synthesis of Schiff base

Schiff base ligands are easily synthesized and form complexes with most metal ions. The mechanism of Schiff base synthetic reaction involves a nucleophilic attack of the primary amine through its lone pair of electrons on the electrophilic carbonyl carbon. A 1,3-H shift follows, which facilitates the removal of water to give the protonated imine, with subsequent disassociation of this species to give the Schiff base product as shown in the Scheme 2.1(Aljamali *et al*., 2019).



Scheme 2.1: Mechanism of imine formation (Aljamali *et al*., 2019)

According to Kailas and co-workers, four common methods, namely microwave, room temperature, grindstone and reflux, for synthesizing Schiff bases have been advanced and vary in reaction time, product and percentage yield (Kailas *et al*., 2016). The microwave method of synthesis is temperature controlled and takes a shorter time to proceed to completion. The pH of an ethanolic solution of the aldehyde and the primary amine in a reaction vessel is adjusted by addition of a few drops of sodium hydroxide. The reaction vessel is placed in a microwave and the mixture irradiated for a few minutes. After irradiation, the mixture is instantly cooled by addition of cold water. The precipitate obtained is collected by filtration, dried, recrystallized from ethanol and dried at room temperature (Kailas *et al*., 2016).

Room temperature method entails the addition of a solution of an aldehyde to a primary amine in ethanolic solution in a reaction vessel. The pH of the resulting mixture is adjusted by addition of two to three drops of sodium hydroxide. The reaction mixture is

then magnetically stirred for one to two hours at room temperature and cold water added to precipitate out the product. The precipitate obtained is collected by filtration, dried, recrystallized from ethanol and dried at room temperature. This method gives the best yield and easily forms the product (Kailas *et al*., 2016).

Grindstone method is a newly developed and green way of synthesizing Schiff bases. The pH of an ethanolic solution of the aldehyde and the primary amine in a mortar is adjusted by addition of a few drops of citric acid. The solution is then ground using a pestle for five to ten minutes. After the reaction is complete, cold water is added to precipitate out the product. The precipitate obtained is collected by filtration, dried, recrystallized from ethanol and dried at room temperature (Kailas *et al*., 2016).

Reflux method is a commonly used method for the synthesis of Schiff base ligands. The pH of an ethanolic solution of the aldehyde and the primary amine in a round bottomed flask is adjusted by addition of a few drops of sodium hydroxide. The reaction mixture is then refluxed with controlled heating for two to three hours and then cold water added to precipitate out the product. The precipitate is collected by filtration, dried, recrystallized from ethanol and dried at room temperature (Kailas *et al*., 2016).

In this study, the room temperature method was used to prepare the Schiff base ligand because the method is cheap and affords higher product yield. The complexes on the other hand were prepared using the aqueous based reflux method. This is because the method is simple, low cost and gives the desired product with precise control over reaction parameters.

2.1.2 Application of Schiff bases

Schiff bases derived from aromatic and aliphatic amines and aldehydes show a lot of biological importance as well as wide range of applications (Kumar *et al*., 2009). These include application in coordination chemistry, agriculture, in synthesis and chemical

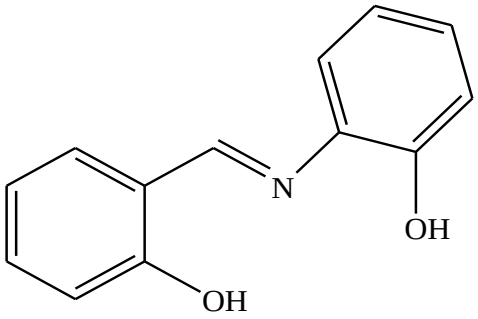
analysis, and in medicine and pharmacy. Application in coordination chemistry, medicine and pharmacy is highlighted.

2.1.2.1 Application of Schiff bases in coordination chemistry

In coordination chemistry, Schiff bases are popular due to their excellent chelating ability to transition metal ions through azomethine moiety(Maher and Mohammed, 2018). The ability of several Schiff base molecules to participate in chelation process is due to the ease with which they provide the lone pair of electrons on nitrogen atom caused by the high electronegative nature of the nitrogen atom. Metal chelates having five or six membered ring system acquires high stability. The stability of metal chelates depends on the strength of the azomethine linkage, basicity of the imino group and steric factors due to the other groups present in the ligand (Garnovskii *et al*., 1993). The relative ease of preparation, synthetic flexibility and the peculiar binding nature of the azomethine linkage makes the Schiff bases excellent chelating molecules (Memon *et al*., 2014).

2.1.2.2 Application of Schiff bases in medicine and pharmacy

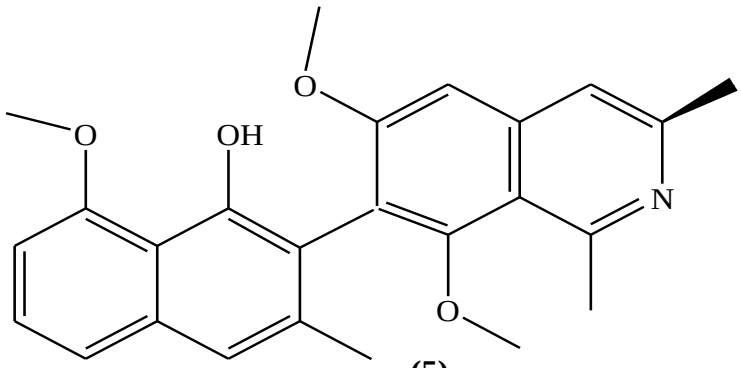
Schiff bases have a broad range of biological properties which include: antitumor, antiviral, antifungal, antibacterial activities due to an imine group, –N=CH–, which helps in understanding the mechanism of transamination and racemization reaction in biological system (Radecka-Paryzek *et al*., 2007). They are also used in the treatment of diabetes and AIDS (Brodowska andLodyga-Chruscinska, 2014). As biological models, they help in understanding the structure of biomolecules and biological processes occurring in living organisms. The bases have also been identified as promising antibacterial agents and often tested as antimalarial (Da Silva *et al*., 2011). For example, N-(Salicyldene)-2-hydroxyaniline **(4)** (Figure 2.1) is active against *M. tuberculosis* (Boghaei *et al*., 2008; Prashanthi *et al*., 2008).



(4)

Figure 2.1: Structure of N-(Salicyldene)-2-hydroxyaniline (Boghaei *et al.*, 2008)

The bases also form part of antimalarial drugs. An example of a compound with such antimalarial effect is Ancistrocladidine **(5)** (Figure 2.2), a secondary metabolite produced by plants of the families *Ancistrocladaceae* and *Dioncophyllaceae*, and possessing an imine group in its molecular structure (Da Silva *et al*., 2011).



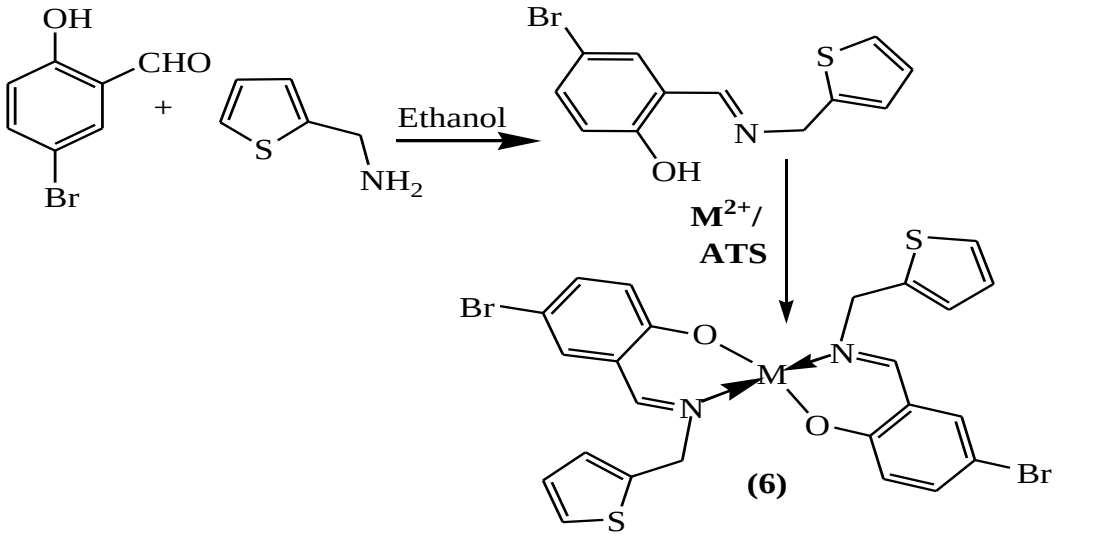
(5)

Figure 2.2: Structure of ancistrocladidine (Da Silva *et al*., 2011)

(Jesmin *et al*., 2010) reported imine derivatives of N-hydroxy-N'-aminoguanidine with a high antitumor activity. The imine blocks ribonucleotide reductase in tumor cells, and is used in the treatment of leukemia

2.1.3 Synthesis and antibacterial activity of Schiff base metal complexes

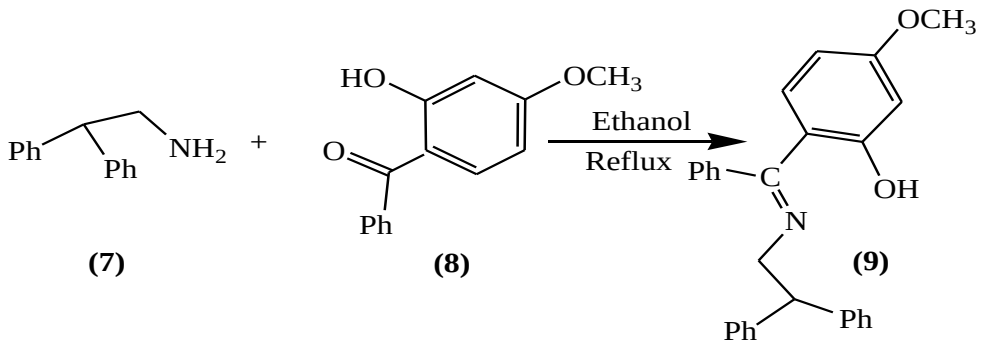
According to (Wang *et al*., 2007), Schiff bases are known to form stable transition metal complexes because they are generally bi- or tri- dentate ligands. It is also known that the existence of metal ions bonded to biologically active compounds enhance their activities. (El-Sherif and Eldebss, 2011) screened 2-aminomethylthiophenyl-4-bromosalicylaldehyde (ATS) Schiff base **(6)** (Scheme 2.2) and its metal complexes for their antimicrobial activities using the disc diffusion method against bacteria. The results of antimicrobial activity showed that the metal complexes exhibited antimicrobial properties with enhanced inhibitory activity compared to the parent ligand under the same experimental conditions.



Scheme 2.2: Synthesis of Schiff base ligand and its metal (II) complexes (El-Sherif and Eldebss, 2011)

(Sujarani and Ramu, 2013), prepared biologically important complexes of Cu (II), Co (II), Mn (II) and Ni (II) with a neutral bidentate Schiff base **(9)** derived from 2,2-diphenylethanamine **(7)** and 2-hydroxy-4-methoxy benzophenone **(8)** (Scheme 2.3). The interaction investigations of these complexes with CT-DNA using spectroscopic and electrophoresis techniques have indicated an evidence for groove binding of the DNA

with the metal complexes (Palanimurugan *et al*., 2019), and in addition, the complexes showed their efficient antimicrobial activities against *E. coli* and *S. aureus* bacterial species.



Scheme 2.3: Synthesis of 2-((2,2-diphenylethylimino)(phenyl)methyl-5-methoxyphenol) (Sujarani and Ramu, 2013)

According to Prasad *et al*., 2011 and Palanimurugan *et al*., 2019,copper (II) complex **(10)** of quinolin-4(3*H*)-one Schiff base ligand (Figure 2.3) derived from 3-amino-2-methyl-4(3*H*)-quinazolinone with diverse substituted aromatic aldehydes showed efficient antimicrobial activity against the bacteria *E. coli* and *S. aureus*. The DNA interaction investigations suggest that the copper (II) complex acts as an avid binding and cleaving agent than the free Schiff base ligand.

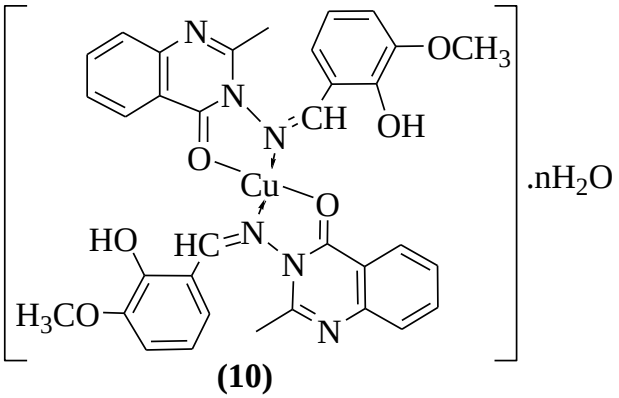
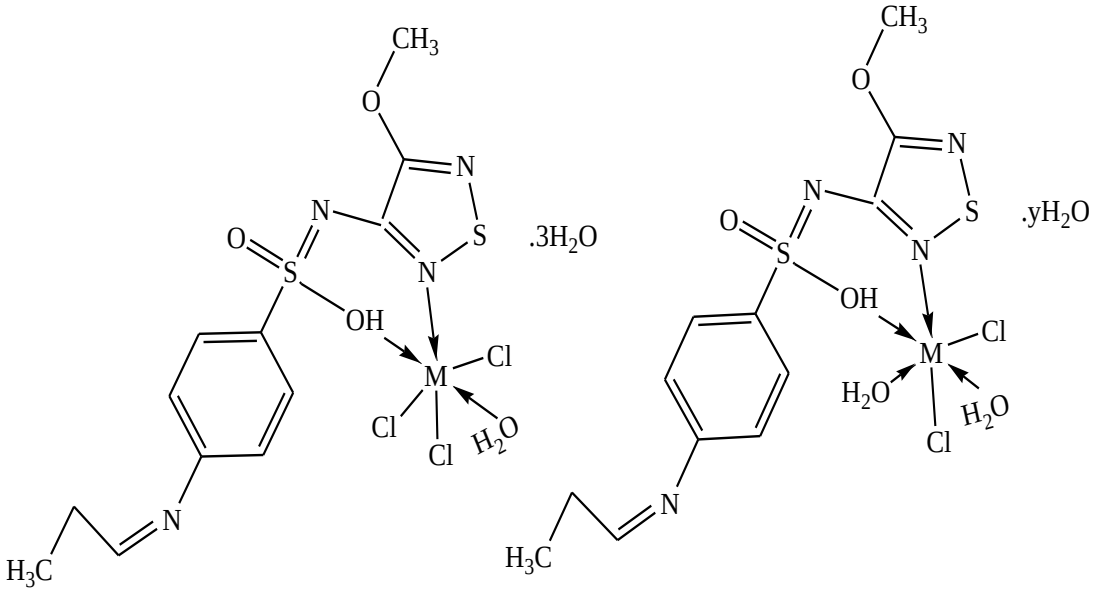


Figure 2.3: Structure of Copper (II) complex of quinoline-4(3H)-one (Prasad *et al*., 2011)

(Mohamed *et al*., 2010) reported Cr (III), Fe (III), Mn (II), Co (II), Ni (II), Cu (II), and Zn (II) complexes (11) (Figure 2.4) of a Schiff base derived from condensation of sulphametrole and varelaldehyde. The Schiff base and its complexes were screened against bacterial species *E. coli* and *S. aureus*. The Schiff base and its metal complexes showed a higher effect on *E. coli*, Gram-negative bacteria, and *S. aureus*, Gram-positive bacteria. The membrane of Gram-negative bacteria is surrounded by an outer membrane containing lipopolysaccharides. The synthesized Schiff base and its metal complexes were able to combine with the lipophilic layer in order to enhance the membrane permeability of the Gram-negative bacteria. The lipid membrane surrounding the cell favours the passage of only lipid soluble materials. The Schiff base and its metal complexes were more toxic on *S. aureus* than on *E. coli*, probably due to the sulphonic, hydroxy, methoxy, sulphur and propyl groups, which might have interacted with the double membrane.



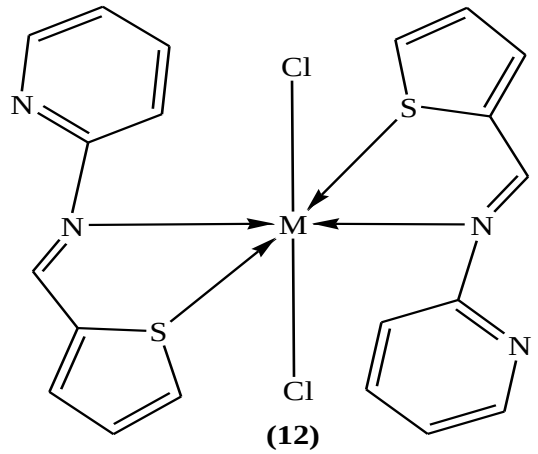
Where M = Cr (III) and Fe (III)

Where M = Mn (II), Fe (II), Co (II), Ni (II), Cu (II) and Zn (II)

(11)

Figure 2.4: Structural formulae of metal complexes of a Schiff base derived from sulphametrole and varelaldehyde (Mohamed *et al*., 2010)

(Spînu *et al.*, 2008) reported the preparation, isolation, and characterization of a new bidentate Schiff base and its metal complexes **(12)** of Fe (II), Co (II), Ni (II) and Cu (II) (Figure 2.5). The Schiff base was derived from the condensation of 2-thiophenecarboxaldehyde with 2-aminopyridine. The complexes were examined for their antibacterial activities on *E. coli*, *S. aureus* and *P. aeruginosa* using the disk diffusion method. The metal chelates showed very good antibacterial activity than the free Schiff base ligand. The growth of *E. coli* was inhibited to a greater extent by the Co (II) and Fe (II) complexes. Thus, these complexes could reasonably be used for the treatment of some common diseases caused by *E. coli*.



(12)

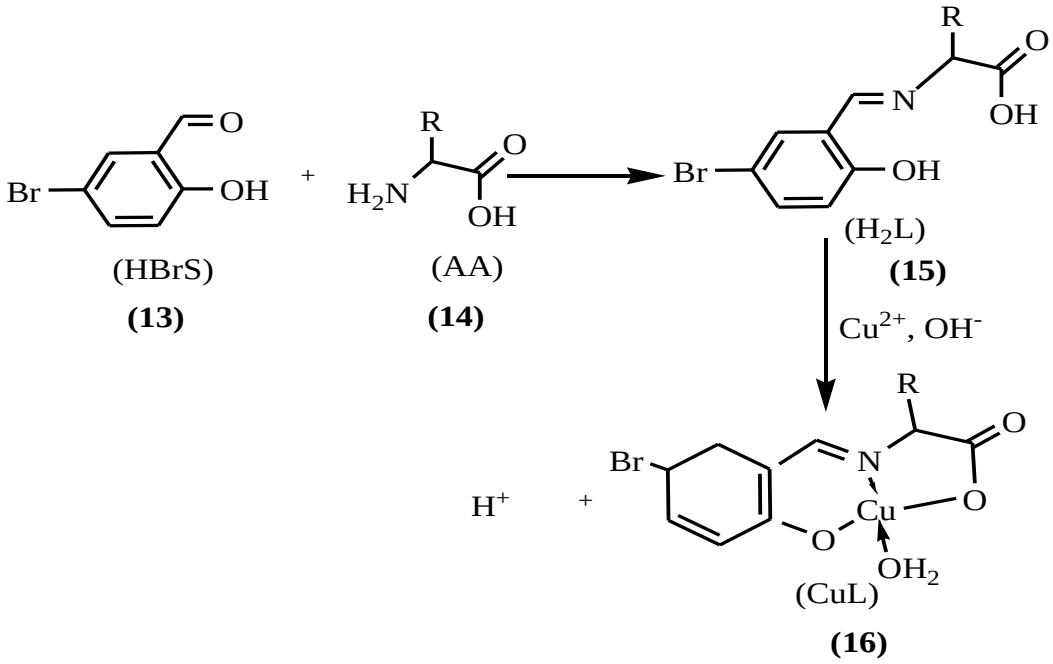
Where M = Fe (II), Co (II), Ni (II) and Cu (II)

Figure 2.5: Structure of the complexes (Spînu *et al.*, 2008)

Abdel-Rahman and co-workers synthesized iron (II) complexes based on amino acid Schiff base ligands from condensation of 5-bromosalicylaldehyde (BS) and α-amino acids (L-alanine (ALA), L-phenylalanine (PHALA), L-aspartic acid (ASPA), L-histidine (HIS) and L-arginine (ARG)). The structure of the complexes was validated using quantum mechanics calculations based on accurate DFT methods. Geometry optimization of the Fe-Schiff base amino acid complexes showed that all complexes had octahedral geometry. The compounds were screened for their *in vitro* antibacterial activity against three types of bacteria, *E. coli*, *P. aeruginosa* and *B. cereus* using disk diffusion method. The results of these studies indicated that the metal complexes

exhibited a stronger antibacterial and antifungal efficacy than their corresponding Schiff base amino acid ligands. The DNA interaction studies of these complexes at pH = 7.2, by electronic absorption spectra and viscosity measurements indicated that they could bind to DNA via intercalative mode (Abdel-Rahman *et al*., 2013).

(Abu-Dief and Nassr, 2015), synthesized and characterized Cu (II) complexes **(16)** of Schiff bases **(15)** derived from the condensation between 5-bromosalicylaldehyde (BS) **(13)** and α-amino acids **(14)** (L-alanine, L-phenylalanine, L-aspartic acid, L-histidine and L-arginine) (Scheme 2.4).



HBrS = 5-bromosalicylaldehyde, AA = amino acid (ala, phala, aspa, his or arg), H_2L = Schiff base ligand, CuL = Cu (II) Schiff base amino acid complexes.

Scheme 2.4: Synthesis of Schiff base amino acid Cu (II) complexes (Abu-Dief and Nassr 2015)

They indicated that the Schiff bases of the amino acids: L-alanine, L-phenylalanine, L-histidine and L-arginine were tridentate ligands which coordinate to Cu (II) via azomethine nitrogen, deprotonated carboxylate oxygen and phenolic oxygen while L-aspartic was tetradentate due to the coordination of the second carboxylate group.

Magnetic moments and electronic spectra data suggested a square planar geometry for all Cu (II) complexes except bromosalicylaldehydine aspartate complex which has a distorted tetrahedral structure. The *in vitro* bioassays for the representative Schiff bases and their Cu (II) complexes against two Gram-positive bacteria, *M. luteus* and *B. cereus* and one Gram-negative bacteria, *P. aeruginosa*, showed that all the complexes were active against the organisms more than the free Schiff base ligands. The antibacterial activity increased with the increase in concentration of test solution containing the complexes. The metal complexes are promising antibacterial agents compared to the parent Schiff base ligand (Abu-Dief and Nassr, 2015).

The coordination complexes **(17, 18, 19** and **20)** of Co (II), Ni (II) and Cu (II) (Figures 2.6 and 2.7) with the Schiff bases derived from isatin with 3-chloro-4-fluoroaniline and 2-pyridinecarboxaldehyde with 4-aminoantipyrine have been synthesized by conventional as well as microwave methods by Mishra and co-worker (Mishra and Kumar, 2013). The Schiff base and metal complexes showed good activity against the bacterial species *S. aureus, E. coli* and *S. fecalis.* The antibacterial results also indicated that the metal complexes were better antibacterial agents as compared to the free Schiff bases.

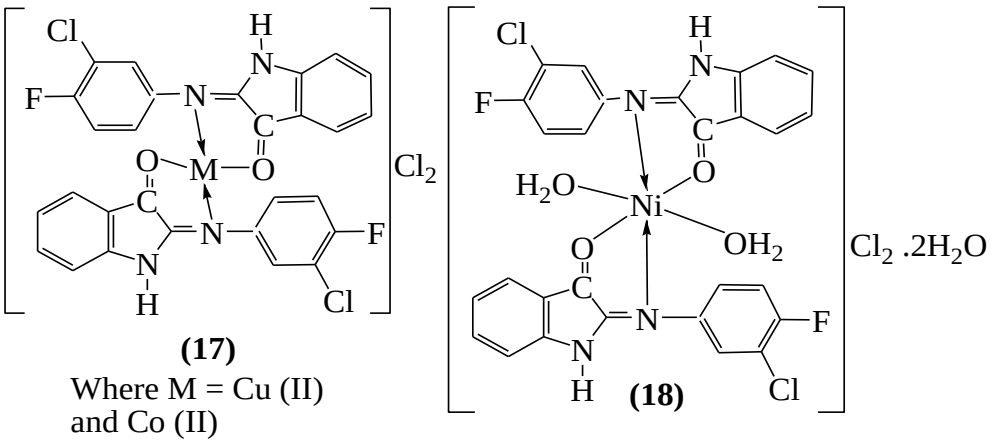


Figure 2.6: Structures of metal complexes of Isatin-3- Chloro-4-Floroaniline ligand (Mishra and Kumar, 2013)

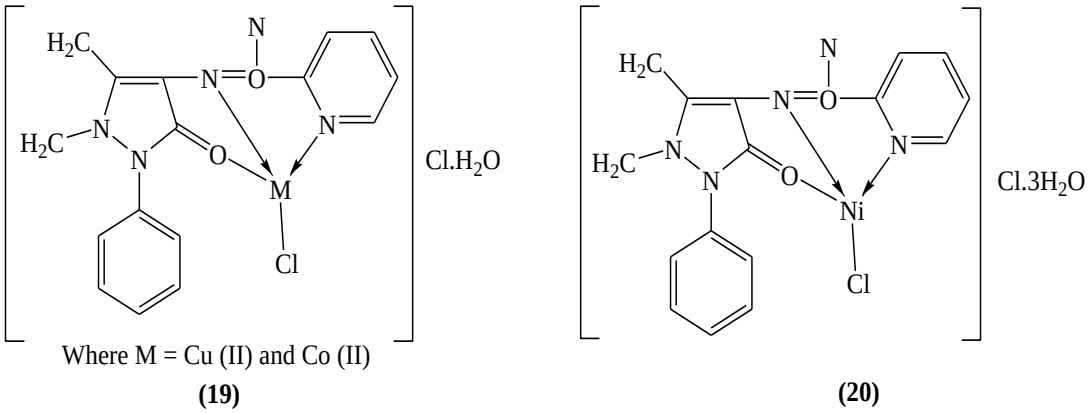


Figure 2.7: Structures of metal complexes of 2-Pyridinecarboxylidene-4-Aminoantipyrine (Mishra and Kumar, 2013)

Metal complexes (21, 22 and 23) of Schiff base derived from 2-thiophenecarboxaldehyde and 2-aminobenzoic acid (HL) and Fe (III) or Co (II) or Ni (II) or UO_2 (II) (Figure 2.8) showed a good antibacterial activity against *E. coli*, *P. aeruginosa* and *S. pyogenes*. Fe (III), Cu (II), Zn (II) and UO_2 (II) complexes but not Co (II) complex caused inhibition for *E. coli*. Fe (III), Co (II), Cu (II), Zn (II) and UO_2 (II) complexes were all specialized in inhibiting Gram-positive bacterial strains (*S. pyogenes* and *P. aeruginosa*) in the sense that they could be structurally modified to achieve the desired molecule, targeting a particular bacterial strain. The importance of this unique property of the investigated Schiff base complexes lies in the fact that, it could be applied safely in the treatment of infections caused by any of these particular strains (Mohamed *et al*., 2005).

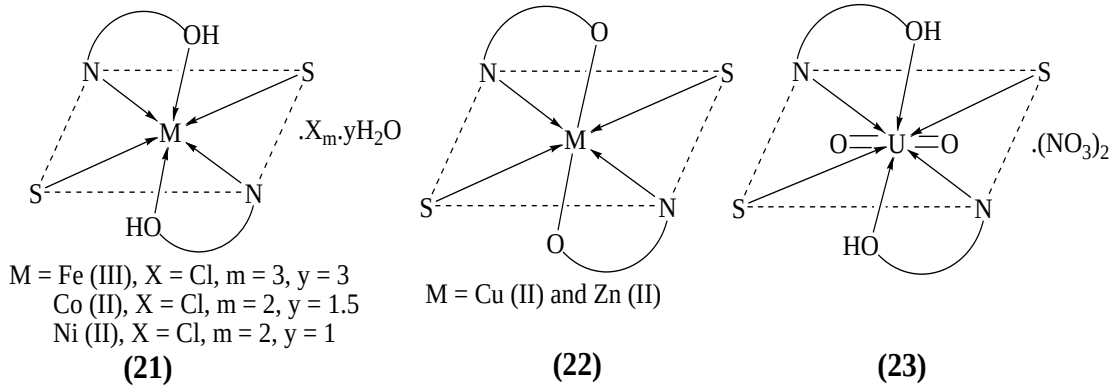


Figure 2.8: Structural formulas of HL - metal complexes (Mohamed *et al*., 2005)

Schiff base complexes **(24 and 25)** of Co (II), Ni (II), Cu (II) and Zn (II) incorporating indole-3-carboxaldehyde and *m*-aminobenzoic acid (Figure 2.9) were screened by disk diffusion method and their activity order was as follows: Cu (II) > Co (II) > Ni (II) > Zn (II) > Ligand. The higher activity of the metal complexes may be owing to the effect of metal ions on the normal cell membrane. Metal chelates bear both polar and nonpolar properties and this makes them suitable for permeation to the cells and tissues. In addition, chelation may enhance or suppress the biochemical potential of bioactive organic species (Nair *et al*., 2012).

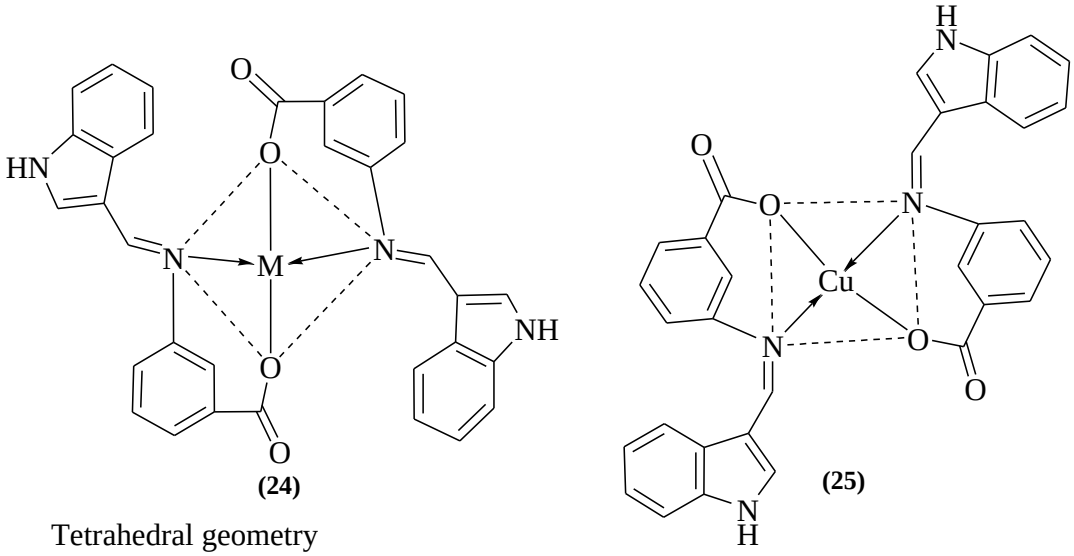
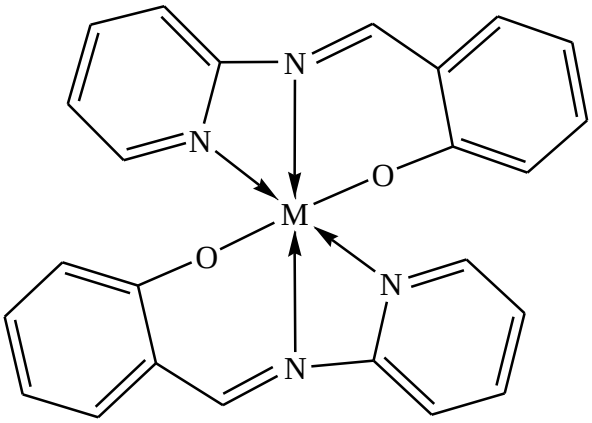


Figure 2.9: Structure of Schiff base metal complexes (Nair *et al*., 2012)

Transition metal [Co (II), Cu (II), Ni (II) and Zn (II)] complexes **(26)** of substituted pyridine Schiff-bases (derived from substituted aminopyridine and salicylaldehyde) (Figure 2.10) were synthesized by Chohan and co-workers (Chohan *et al.*, 1999) in order to evaluate the effect of metal ions upon chelation on antibacterial activity against the bacterial strains *E. coli*, *S. aureus*, and *P. aeruginosa*. The complexed Schiff bases were more active against one or more bacterial species as compared to uncomplexed Schiff-bases.

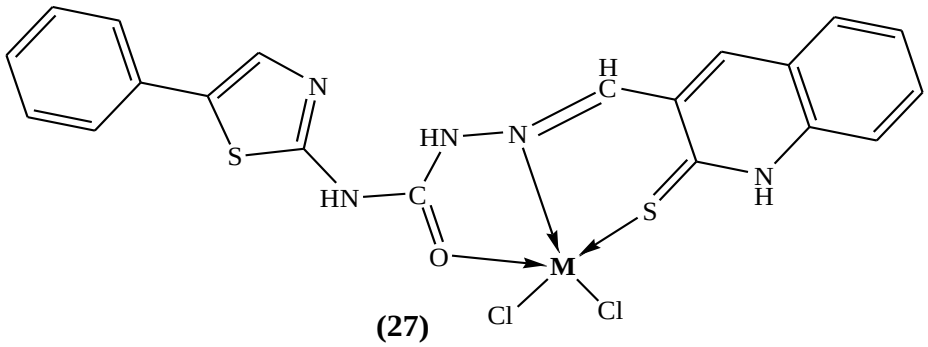


(26)

Where M = Co (II), Ni (II), Cu (II) or Zn (II)

Figure 2.10: Structure of the metal (II) complexes (Chohan *et al*., 1999)

In another study, Yernale and Bennikallu synthesized some novel Co (II), Ni (II), and Zn (II) metal complexes **(27)** of a Schiff base obtained from N-(4-phenylthiazol-2-yl)hydrazinecarboxamide and 2-thioxo-1,2-dihydroquinoline-3-carbaldehyde. The complexes exhibited good antibacterial activities against *E. aerogenes* and *P. aeruginosa* bacteria (Yernale and Bennikallu 2014). The biological results showed that the metal complexes were more active compared with the ligand, which was explained on the basis of chelation theory (Mishra *et al*., 2012).



(27)

M = Co, Ni, Zn

Figure 2.11: Structure of Co, Ni and Zn metal complexes (Yernale and Bennikallu, 2014)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

The chemicals and reagents used in this research were of analytical grade sourced from MilliporeSigma Chemicals Company. They included: 5-chlorosalicylaldehyde (98%), 4-fluoroaniline (99%), Manganese (II) chloride tetrahydrate (98%), Cobalt (II) chloride hexahydrate (99%), anhydrous Nickel (II) chloride for analysis (98%), anhydrous Copper (II) chloride for analysis (99%) and anhydrous Zinc Chloride (>98%) were used without further purification. The solvents, diethyl ether (99.5%), methanol (99.8%), absolute ethanol (99.5%), glacial acetic acid (99.8%), and dimethylsulphoxide (99.5%) were used without further purification.

3.2 General methods

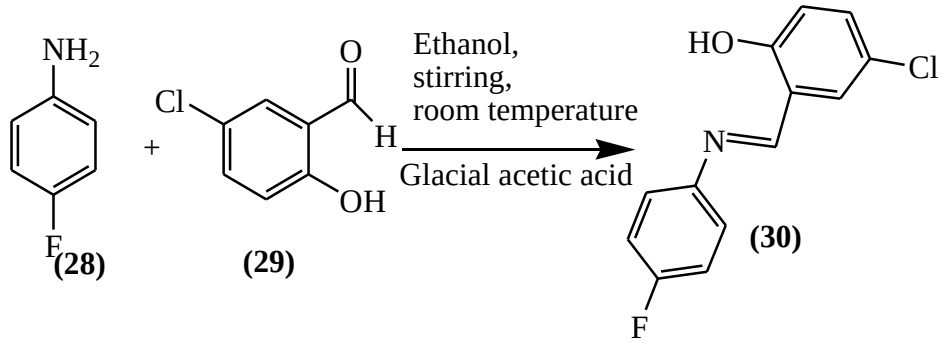
The uncorrected melting point of the ligand and its metal (II) complexes were determined by taking a small amount of compound in a capillary tube closed at one end and placed in a SANYO Gallenkamp melting point apparatus (model MPD350BM3.5) and the temperatures at which the compounds melted were noted. The ^{1}H-NMR and ^{13}C-NMR spectra of the Schiff base ligand and its metal complexes were recorded in deuterated DMSO on a Bruker Avance III HD Nanobay 400 MHz NMR spectrometer equipped with a 5 mm BBO probe at room temperature. Tetramethylsilane was used as internal standard and chemical shifts given in δ. Elemental analyses (C, H, and N %) were recorded on a LECO CNHS-932 micro-analyzer. FT-IR spectra of the ligand and its metal complexes were recorded on a SHIMADZU FTIR-8400 spectrophotometer in the 400-4000cm^{-1} range using KBr matrix. Electronic spectra of the ligand and complexes in solution were scanned in the range 200 - 800 nm on a AOE Instruments UV - 1800 PC spectrophotometer. The samples were dissolved in 5 mL HPLC grade acetonitrile and then placed in quartz cuvette of 1 cm optical path. The molar

conductivity measurement of the Schiff base ligand and its metal (II) complexes in DMSO 10^{-3} M solutions were carried out on a SIBATA Conductivity meter Model SC – 17A, at room temperature.

3.3 Synthesis of Schiff base, 4-Chloro-2-{(E)-[(4-Fluoro-phenyl)Imino]Methyl}Phenol

The Schiff base ligand, (**L)** (Scheme 3.1) was prepared according to literature methods with a few modifications (Kailas *et al.*, 2016). 4-Fluoroaniline **(28)** (0.3549 g; 3.19 mmol) was added to a 30 ml magnetically stirred ethanolic solution of 5-Chlorosalicylaldehyde **(29)** (0.5000 g; 3.19 mmol) in a 50 mL round-bottomed flask at room temperature (Scheme 6). Two drops (0.2 ml) of glacial acetic acid were added to the mixture, to adjust its pH to ≃6, upon which a thick yellow orange precipitate formed instantly. The solid product was separated by filtration and purified by re-crystallization from ethanol. Shiny, needle-shaped yellow-orange crystals were formed (Plate 3.1). The crystals were washed with cold ethanol and diethyl ether to remove unreacted amine and aldehyde. The crystals were air-dried. Yield (92.0%, 0.7336 g), m.p = 89 – 91 °C, molar conductance (Λ) = 5 $\Omega^{-1}cm^2mol^{-1}$. Elemental analysis data for $C_{13}H_9ClFNO$ (FW = 249.67), found: C, 62.63 %; H, 3.59 %; N, 5.50 % calculated: C, 62.48 %; H, 3.60 %; N, 5.61 %. FT-IR (KBr, disc cm^{-1}) 3386.4 υ(O-H), 1601.0 υ(HC=N), 1277.0 υ(C-O). UV-Vis (acetonitrile) λ_{max} (nm) 225.3, 269.1 and 345.1.^{1}H NMR (ppm d_6-DMSO, 400MHz): δ 8.92 (s, 1H, CH=N), 12.85 (s, 1H, OH), 7.43 – 7.49 (m, 7ArH). ^{13}C NMR (ppm d_6-DMSO): 117,119,121, 123,131,133,144,159, 160 and 163; MS: *m/z* 249 [M^+ + 1].

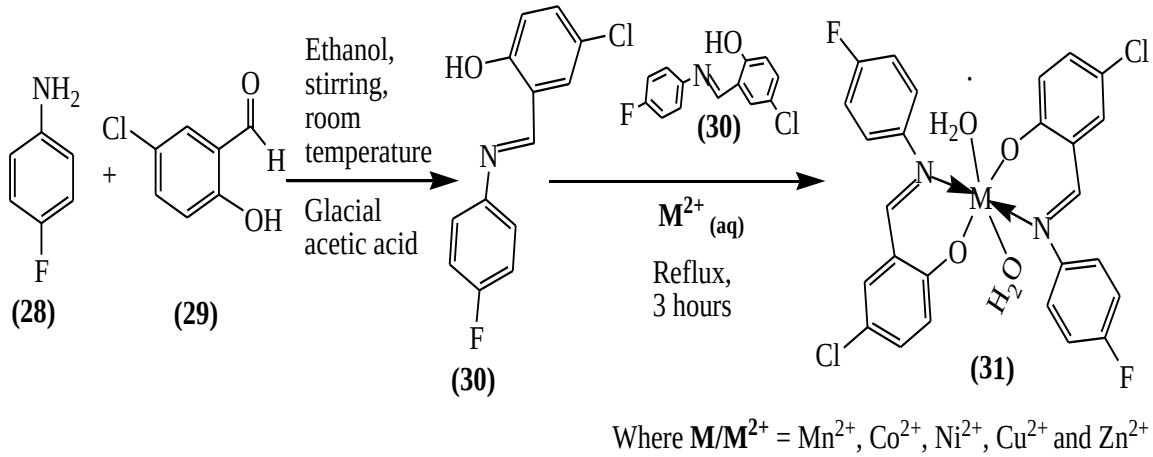
Plate 3.1: A photograph showing crystals of the synthesized ligand, L ($C_{13}H_9ClFNO$)



Scheme 3.1: Synthesis of the Schiff base ligand, L ($C_{13}H_9ClFNO$)

3.4 Synthesis of Schiff base metal (II) complexes

The Schiff base metal (II) complexes, (**31)** (Scheme 3.2), were prepared by reacting the Schiff base with the metal (II) ions as per the literature method (Kailas *et al*., 2016). A solution of the metal salt (0.1 mmol) in 10 ml hot methanol at 50 °C was added drop wise to a solution of the Schiff base ligand (0.0500 g; 0.2 mmol) in 20 ml of hot methanol at 50 °C in a 50 mL round-bottomed flask. The resulting mixture was magnetically stirred and refluxed for 3 hours on an oil bath at 65 °C whereupon a complex precipitated. The precipitate was obtained by filtration, washed with methanol, hot ethanol and diethyl ether to remove unreacted Schiff base and metal (II) salt.



Scheme 3.2: Synthesis of Schiff base ligand and its transition metal (II) complexes

3.4.1 Schiff Base Manganese (II) Complex

A solution of Manganese (II) chloride tetrahydrate (0.0198 g; 0.1 mmol) was used. A grey solid complex (Plate 3.2) was obtained and air-dried. Yield (65.03%, 0.0383 g), m.p> 350 °C, molar conductance (Λ) = 21 $\Omega^{-1}cm^2mol^{-1}$. Elemental analysis data for $C_{26}H_{20}Cl_2F_2MnN_2O_4$ (FW = 588.28), found: C, 53.08 %; H, 3.43 %; N, 4.72 % calculated: C, 53.04 %; H, 3.40 %; N, 4.76 %. FT-IR (KBr, disc cm^{-1}) 3423.0 υ(O-H), 1596.2 υ(HC=N), 1212.7 υ(C-O), 697.0 υ(H_2O), 526.6 υ(Mn-N), 489.1 υ(Mn-O). UV-Vis (acetonitrile) λ_{max} (nm) 221.4, 251.8 and 342.1

Plate 3.2: A photograph of solid $[MnL_2(H_2O)_2]$ complex

3.4.2 Schiff base Cobalt (II) complex

A solution of Cobalt (II) chloride hexahydrate (0.0239 g; 0.1 mmol) was used. A dark brown solid complex (Plate 3.3) was obtained and air-dried. Yield (70.99%, 0.421 g), m.p> 350 °C, molar conductance (Λ) = 19 $\Omega^{-1}cm^2mol^{-1}$. Elemental analysis data for $C_{26}H_{20}Cl_2F_2CoN_2O_4$ (FW = 592.27), found: C, 52.69 %; H, 3.35 %; N, 4.88 % calculated: C, 52.68 %; H, 3.38 %; N, 4.73 %. FT-IR (KBr, disc cm^{-1}) 3427.1 υ(O-H), 1597.4 υ(HC=N), 1213.3 υ(C-O), 692.6 υ(H_2O), 530.6 υ(Co-N), 488.3 υ(Co-O). UV-Vis (acetonitrile) λ_{max} (nm) 249.1 and 401.9

Plate 3.3: A photograph of solid $[CoL_2(H_2O)_2]$ complex

3.4.3 Schiff base Nickel (II) complex

A solution of anhydrous Nickel (II) chloride (0.0130 g; 0.1 mmol) was used. A red brown solid complex (Plate 3.4) was obtained and air-dried. Yield (61.05%, 0.0362 g), m.p> 350 °C, molar conductance (Λ) = 20 $\Omega^{-1}cm^2mol^{-1}$. Elemental analysis data for $C_{26}H_{20}Cl_2F_2NiN_2O_4$ (FW = 592.03), found: C, 52.68 %; H, 3.36 %; N, 4.70 % calculated: C, 52.70 %; H, 3.38 %; N, 4.73 %. FT-IR (KBr, disc cm^{-1}) 3415.7 υ(O-H), 1595.7 υ(HC=N), 1210.8 υ(C-O), 697.9 υ(H_2O), 526.4 υ(Ni-N), 489.2 υ(Ni-O). UV-Vis (acetonitrile) λ_{max} (nm) 245.2, 325.1 and 421.9.^{1}H NMR (ppm d_6-DMSO, 400MHz): δ 8.87 (s, 1H, CH=N), 7.12 – 7.15 (m, 7ArH); ^{13}C NMR (ppm d_6-DMSO): 118, 122, 132, 133, 162, and 165.

Plate 3.4: A photograph of solid $[NiL_2(H_2O)_2]$ complex

3.4.4 Schiff base Copper (II) complex

A solution of anhydrous Copper (II) chloride (0.0136 g; 0.1 mmol) was used. A brown solid complex (Plate 3.5) was obtained and air-dried. Yield (73.91%, 0.0442 g), m.p> 350 °C, molar conductance (Λ) = 16 $\Omega^{-1}cm^2mol^{-1}$. Elemental analysis data for $C_{26}H_{20}Cl_2F_2CuN_2O_4$ (FW = 596.89), found: C, 52.21 %; H, 3.34 %; N, 4.65 % calculated: C, 52.27 %; H, 3.35 %; N, 4.69 %. FT-IR (KBr, disc cm^{-1}) 3446.6 υ(O-H), 1597.1 υ(HC=N), 1214.7 υ(C-O), 694.8 υ(H_2O), 530.1 υ(Cu-N), 488.7 υ(Cu-O). UV-Vis (acetonitrile) λ_{max} (nm) 229.1, 266.1 and 349.7

Plate 3.5: A photograph of solid $[CuL_2(H_2O)_2]$ complex

3.4.5 Schiff base Zinc (II) complex

A solution of anhydrous Zinc (II) chloride (0.0137 g; 0.1 mmol) was used. A light yellow solid complex (Plate 3.6) was obtained and air-dried. Yield (63.11%, 0.0378 g), m.p = 265-268 °C, molar conductance (Λ) = 19 $\Omega^{-1}cm^2mol^{-1}$. Elemental analysis data for $C_{26}H_{20}Cl_2F_2ZnN_2O_4$ (FW = 598.73), found: C, 52.09 %; H, 3.30 %; N, 4.64 % calculated: C, 52.11 %; H, 3.34 %; N, 4.68 %. FT-IR (KBr, disc cm^{-1}) 3417.2 υ(O-H), 1596.6 υ(HC=N), 1214.3 υ(C-O), 699.7 υ(H_2O), 527.8 υ(Zn-N), 489.7 υ(Zn-O). UV-Vis (acetonitrile) λ_{max} (nm) 223.4, 303.2 and 343.9.^{1}H NMR (ppm d_6-DMSO, 400MHz): δ 8.55 (s, 1H, CH=N), 7.18 – 7.39 (m, 7ArH); ^{13}C NMR (ppm d_6-DMSO): 116, 117, 124, 125, 162, and 165.

Plate 3.6: A photograph of solid $[ZnL_2(H_2O)_2]$ complex

3.5 Biological assays

The synthesized ligand and its transition metal (II) complexes were screened *in vitro* for their antibacterial activities against bacterial strains: *E. coli, P. aeruginosa, B. subtilis* and *S. typhi,* using disc diffusion method (Murray *et al*., 1995; Zaidan *et al*., 2006). The disc diffusion method was used because of its convenience, efficiency, simplicity, low cost, ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided. The only disadvantage of this method is lack of automation of the test.

The medium was prepared by dissolving 28 g of nutrient agar in 1 litre of freshly distilled water. It was allowed to soak for 15 minutes and then boiled on a water bath until the agar was completely dissolved. The mixture was sterilized by autoclaving at 120 °C for 15 minutes and then introduced into previously washed and sterilized Petri

dishes to a uniform depth of 4 mm and stored at 50 °C for inoculation. The inoculation was done with the help of a platinum wire loop which was sterilized by heating to red hot in a flame, cooled and then used for the application of bacterial strains.

The stock solution (1 mg ml^{-1}) of the test chemical compound was prepared by dissolving 20 mg of the test compound in 20 ml of dimethyl sulfoxide (DMSO) solvent. From this stock solution, 10, 20, 30 and 40 µgml^{-1}solutions were prepared by dilution with DMSO. Standard antibacterial drugs (Amoxyclav, Nalidixic acid and Gentamicin) were used as positive controls for comparison with the synthesized metal complexes while the solvent DMSO was used as control for each dilution. Whatman filter paper (No. 1) discs of 7 mm diameter were sterilized in an autoclave and then soaked in the desired concentration of the synthesized compounds and allowed to dry before application on the bacteria grown agar plates.

The paper discs were then placed aseptically in the Petri dishes containing nutrient agar media seeded with *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. typhi* separately using sterilized forceps and then incubated for 24 hours at 37 °C. The diameters of the zones of inhibition were measured after 24 hours of incubation. The antibacterial activities were calculated as a mean ± SD of three replicates (Table 4.8). The zones of inhibition (Plates 3.7–3.12) were measured using a Vernier caliper in millimeters (mm) and the following criteria applied; low activity (1-6 mm), moderate activity (7-10 mm), high activity (11-15 mm), very high activity (≥16) and no activity(-) (Zaidan *et al*., 2006).

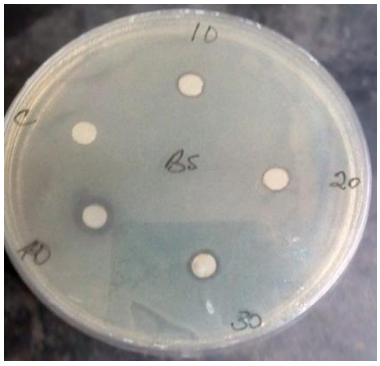


Plate 3.7: Zone of inhibition of $[ZnL_2(H_2O)_2]$ complex against *B. subtilis*

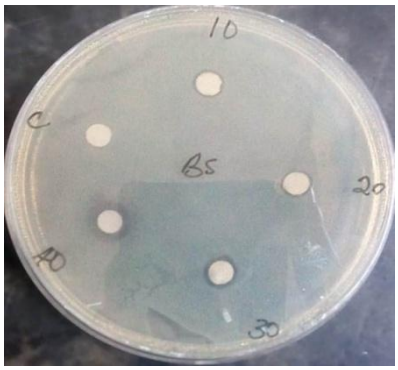


Plate3.8: Zone of inhibition of $[CoL_2(H_2O)_2]$ complex against *B. subtilis*

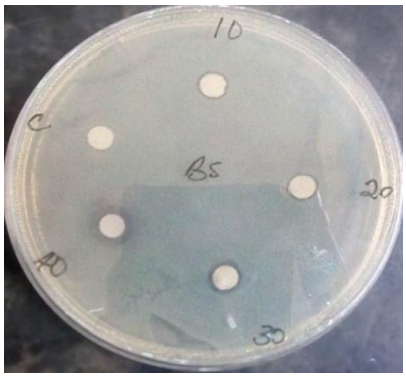


Plate 3.9: Zone of inhibition of $[CuL_2(H_2O)_2]$ complex against *B. subtilis*

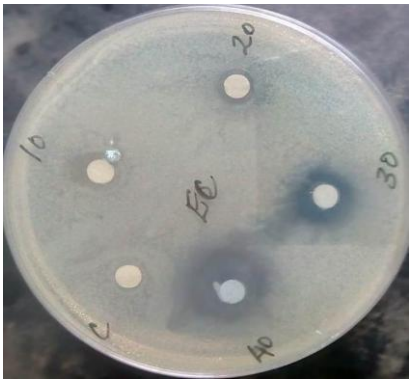


Plate 3.10: Zone of inhibition of $[NiL_2 (H_2O)_2]$ complex against *E. coli*

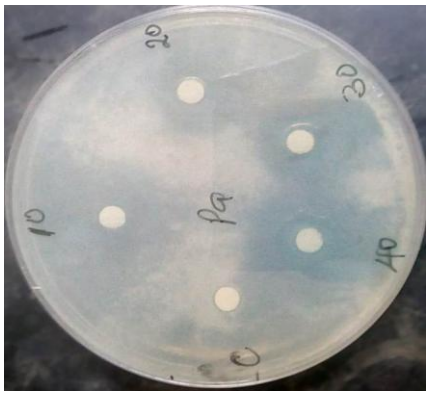


Plate 3.11: Zone of inhibition of $[ZnL_2(H_2O)_2]$ complex against *P. aeruginosa*

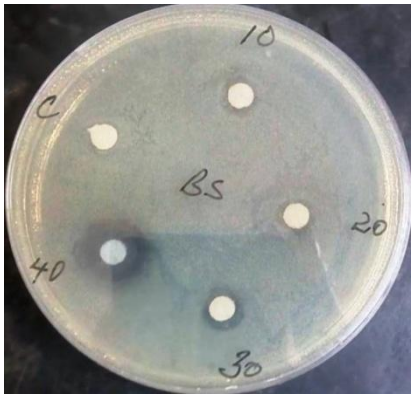


Plate 3.12: Zone of inhibition of $[MnL_2(H_2O)_2]$ complex against *B. subtilis*

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 General

The Schiff base ligand in this study was first synthesized by Feng (Feng, 2014) using reflux method. In this research, however, the ligand was prepared using the room temperature method which afforded a higher yield. The Schiff base ligand, 4-chloro-2-{(E)-[(4-fluorophenyl)imino]methyl}phenol ($C_{13}H_9ClFNO$), L, was prepared by reacting5-chlorosalicylaldehyde with 4-fluoroaniline in ethanolic medium. The metal (II) complexes of the ligand were prepared using the reflux method because the method is simple, low cost and gives the desired product with precise control over reaction parameters. The bidentate ligand, 4-chloro-2-{(E)-[(4-fluorophenyl)imino]methyl}phenol ($C_{13}H_9ClFNO$), L, reacted readily with the metal (II) ions in the mole ratio of 2:1 to yield the metal (II) complexes. The complexes are new and thus reported for the first time (Ommenya *et al*., 2020).

The suggested molecular formulae of the ligand and metal (II) complexes have been achieved from micro-analytical data in combination with Fourier-Transform Infrared, Ultraviolet-visible, and Nuclear Magnetic Resonance spectroscopic techniques. From the elemental analysis data, the metal complexes formed have the general formulae $[ML_2(H_2O)_2]$, where M = Mn (II), Co (II), Ni (II), Cu (II) and Zn (II) and L = $C_{13}H_8ClFNO$ (deprotonated Schiff base ligand). The imine and its metal (II) complexes are coloured and air-moisture stable. The colour changes observed during the syntheses of the metal complexes (Table 4.2)supported the metal ligand interaction which was further reinforced by conductivity values. The ligand is soluble in hot ethanol, hot methanol, acetonitrile, dimethylformamide and DMSO. The complexes are insoluble in water, common organic solvents such as methanol, dichloromethane, ethanol, and acetone but soluble in DMSO, acetonitrile and DMF at room temperature.

The melting points of the complexes were higher than that of the ligand (Table 4.2) indicating that the complexes are more stable than the ligand thermally. The chemical equations showing the preparation of the Schiff base ligand and its metal (II) complexes are represented in Scheme 3.2.

Table 4.1: Physical data of Schiff Base ligand, L and its metal (II) complexes

Compound	Colour	Empirical formula	Molecular weight	% yield
L	Yellow orange	$C_{13}H_9ClFNO$	249.67	92.00 (0.7336 g)
$[MnL_2(H_2O)_2]$	Grey	$C_{26}H_{20}Cl_2F_2MnN_2O_4$	588.28	65.03 (0.0383 g)
$[CoL_2(H_2O)_2]$	Dark brown	$C_{26}H_{20}Cl_2F_2CoN_2O_4$	592.27	70.99 (0.4210 g)
$[NiL_2(H_2O)_2]$	Red brown	$C_{26}H_{20}Cl_2F_2NiN_2O_4$	592.03	61.05 (0.0362 g)
$[CuL_2(H_2O)_2]$	Brown	$C_{26}H_{20}Cl_2F_2CuN_2O_4$	596.89	73.91 (0.0442 g)
$[ZnL_2(H_2O)_2]$	Light yellow	$C_{26}H_{20}Cl_2F_2ZnN_2O_4$	598.73	63.11 (0.0378 g)

Where L = Schiff base ligand L, 4-Chloro-2-{(E)-[(4-Fluorophenyl)Imino]Methyl}Phenol

4.1.1 Conductivity measurements

The molar conductance values of the synthesized compounds in DMSO 10^{-3} M were measured at room temperature (Table 4.2). The conductance values of the synthesized compounds were below 50 $\Omega^{-1}cm^2mol^{-1}$, indicating their non-electrolytic nature (Chen *et al*., 1993; Geary, 1971). This suggested that there were no ions present outside the coordination sphere of the complexesi.e, the complexes were neutral.

Table 4.2: Melting points and conductivity measurements of the ligand (L) and its complexes

Compound	Melting point (^oC)	Conductance ($\Omega^{-1}cm^2mol^{-1}$)
L	133-135	5
$[MnL_2(H_2O)_2]$	> 350	21
$[CoL_2(H_2O)_2]$	> 350	19
$[NiL_2(H_2O)_2]$	> 350	20
$[CuL_2(H_2O)_2]$	>350	16
$[ZnL_2(H_2O)_2]$	265-268	19

4.1.2 Electronic spectral analysis

The electronic spectral data of the Schiff base ligand and its metal (II) complexes are given in Table 4.3 below and also at the experimental section. The Schiff base ligand showed three major bands at 225.3 nm, 269.1 nm and 345.1 nm (Figure 4.1). The band at 225.3 nm is due to the $\pi - \pi^*$ transition of the ligand centered transitions (LCT) of benzene. The band appearing at 269.1 nm is as a result of non-bonding electrons present on the nitrogen of the azomethine group (-HC=N) due to $n - \pi^*$ transition. The band at 345.1 nm is due to $n - \pi^*$ transition of the phenolic group (Boghaei *et al*., 2008; Shaker *et al*., 2013).

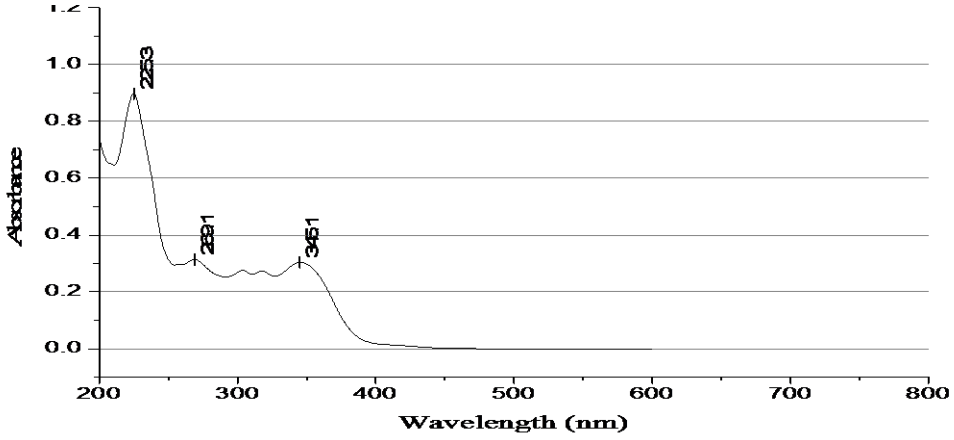


Figure 4.1: UV-vis spectrum of the Schiff base ligand

Table 4.3: Assignment of the important λ_{max} (nm) of the Schiff base ligand and its complexes

Compound	λ_{max} (nm)	Transition	Assignment	Geometry
L	225.3	$\pi - \pi^*$	LCT	----------
	269.1	$n - \pi^*$		
	345.1	$n - \pi^*$		
$[MnL_2(H_2O)_2]$	221.4	$\pi - \pi^*$	ILT	Octahedral
	251.8	$n - \pi^*$		
	342.1	$d - \pi^*$	$^6A_{1g} \rightarrow {}^4E_g$	
$[CoL_2(H_2O)_2]$	249.1	$n - \pi^*$	ILT	Octahedral
	401.9.	$d - \pi^*$	$^3T_{1g}$ (F) $\rightarrow {}^3T_{2g}$	
$[NiL_2(H_2O)_2]$	245.2	$\pi - \pi^*$	ILT	Octahedral
	325.1	$n - \pi^*$	MLCT	
	421.9	$d - \pi^*$	$^3A_{2g} \rightarrow {}^3T_{2g}$	
$[CuL_2(H_2O)_2]$	229.1	$\pi - \pi^*$	ILT	Octahedral
	266.1	$n - \pi^*$		
	349.7	$d - \pi^*$	$^3T_{1g} \rightarrow {}^3T_{2g}$	
$[ZnL_2(H_2O)_2]$	223.4	$\pi - \pi^*$	ILT	Octahedral
	303.2	$n - \pi^*$		
	343.9		MLCT	

Where L= Schiff base ligand, 4-Chloro-2-{(E)-[(4-Fluorophenyl)Imino]Methyl}Phenol; LCT = Ligand Centred Transitions; ILT = Intra-Ligand Transitions; MLCT = Metal to Ligand Charge Transfer

The UV-vis spectra of the Schiff base metal (II) complexes (Figure 4.2) displayed similar absorption spectra as the ligand which is either shifted to the blue or red region.

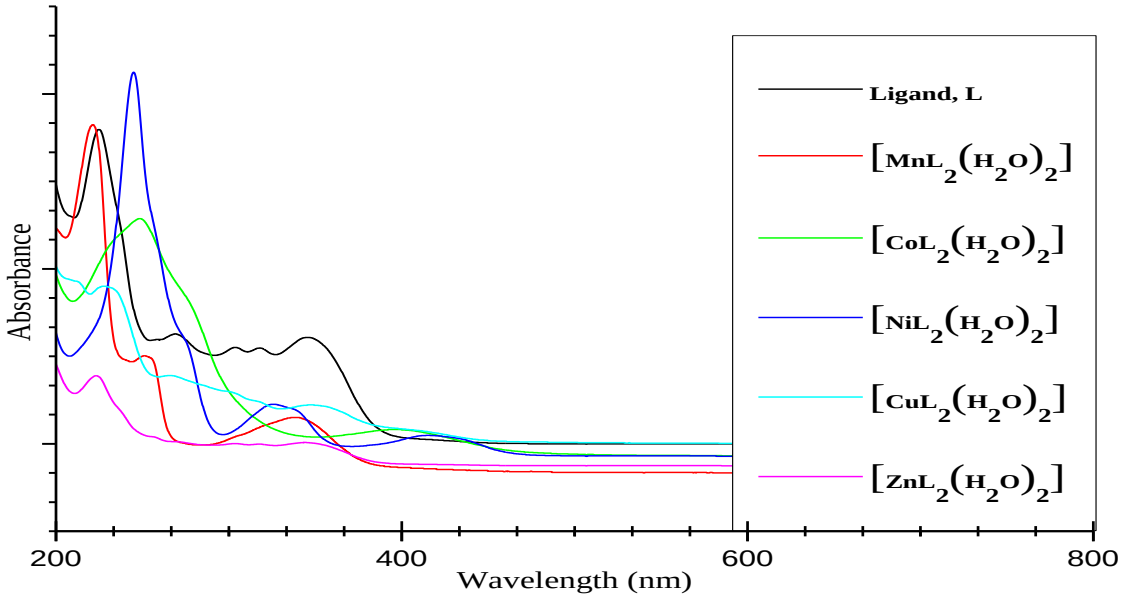


Figure 4.2: UV-vis spectra of Schiff base and its metal (II) complexes

The electronic spectrum of the Mn (II) complex shifted towards shorter wavelength and gave three bands at 221.4 nm, 251.8 nm and 342.1 nm (Appendix I). The bands at 221.4 nm and 251.8 nm were due to intra-ligand transition and the band at 342.1 nm was as a result of d-d low spin transition for $^{6}A_{1g} \rightarrow {}^{4}E_{g}$ which is suggestive of octahedral geometry around Mn (II) ion (Abou-Hussein and Linert, 2014; Bertini, 2009).

In the Co (II) complex, only two bands were observed at 249.1 nm and 401.9 nm (Appendix II). This was a shift towards longer wavelength in relation to the spectrum of the Schiff base ligand. The band at 249.1 nm was due to intra-ligand transition while the band at 401.9 nm was as a result of d-d transition for $^{3}T_{1g}$ (F) $\rightarrow$ $^{3}T_{2g}$ which is within the range for octahedral configuration as reported in many octahedral cobalt (II) complexes (Abou-Hussein and Linert, 2014; El-Sherif *et al*., 2012; Mohamed *et al*., 2010).

The electronic spectrum of Ni (II) complex shows that the bands shifted to the red with three bands at 245.2 nm, 325.1 nm and 421.9 nm (Appendix III). The band at 245.2 nm is due to intra-ligand transition while the band at 325.1 nm was due to charge transfer. The observed band at 421.9 nm was as a result of d-d transition for $^{3}A_{2g} \rightarrow {}^{3}T_{2g}$ which

favours an octahedral geometry for the Ni (II) complex (Bertini, 2009; Chohan *et al*., 1998).

Copper (II) complex spectrum showed three bands at 229.1 nm, 266.1 nm and 349.7 nm (Appendix IV), which was a red shift. The bands at 229.1 nm and 266.1 nm were due to intra-ligand transition and the one at 349.7 nm was as a result of d-d spin allowed transition for $^3T_{1g} \rightarrow {}^3T_{2g}$. This transition is suggestive of octahedral geometry around Cu (II) ion (Nair *et al*., 2012).

Zn (II) complex displayed absorption bands at 223.4 nm, 303.2 nm and 343.9 nm (Appendix V). The bands at 223.4 nm and 303.2 nm were due to intra-ligand transition and the band at 343.9 nm was assignable to the metal to ligand charge transfer (MLCT) transition. An octahedral geometry is proposed for this complex based on its analytical, conductance and spectral data. This is further supported by its diamagnetic nature and absence of d-d band, due to its complete d^{10} electronic configuration (Aupers *et al*., 1998; Chohan *et al*., 1998; Selwood, 1956).

The absence of any band below 118nm eliminates the possibility of a tetrahedral environment in these complexes (Chohan *et al*., 2011). It can also be concluded that a shift in the spectra of the complexes, with respect to the spectrum of the Schiff base ligand, bathochromically or hypsochromically, indicated coordination. Assignment of the important λ_{max} (nm) data of the Schiff base ligand and its metal (II) complexes are given at the experimental section.

4.1.3 Mass spectra

The mass spectrum of the Schiff base ligand (Appendix VI) showed a molecular ion peak at *m/z* 249 (M^++1) which is consistent with the molecular weight of the Schiff base ligand 249. This data shown by mass spectra strongly confirmed the formation of the ligand.

4.1.4 FT-IR spectral analysis

The binding mode of the Schiff base ligand to the metal ions in the complexes was studied by comparing the FT-IR spectrum of the free ligand with the spectra of the complexes. The FT-IR data of the ligand and its metal (II) complexes together with assignments for most of the major peaks (Appendix VII) are given at the experimental section and in Table 4.4 below.

Table 4.4: Selected FT-IR absorption bands (cm^{-1}) of Schiff base ligand and its complexes

Compound	υ(O-H)	υ(C=N)	υ(C-O)	υ(H_2O)	υ(M-N)	υ(M-O)
L	3386.4	1601.0	1277.0	-	-	-
$[MnL_2(H_2O)_2]$	3423.0	1596.2	1212.7	697.0	526.6	489.1
$[CoL_2(H_2O)_2]$	3427.1	1597.4	1213.3	692.6	530.6	488.3
$[NiL_2(H_2O)_2]$	3415.7	1595.7	1210.8	697.9	526.4	489.2
$[CuL_2(H_2O)_2]$	3446.6	1597.1	1214.7	694.8	530.1	488.7
$[ZnL_2(H_2O)_2]$	3417.2	1596.6	1214.3	699.7	527.8	489.7

Where L= Schiff base ligand, 4-Chloro-2-{(E)-[(4-Fluorophenyl)Imino]Methyl}Phenol

The azomethine vibration, υ(C=N), of the ligand at 1601.0 cm^{-1} shifted to lower wave numbers after complexation, i.e 1596.2, 1597.4, 1595.7, 1597.1 and 1596.6 cm^{-1} for Manganese (II), Cobalt (II), Nickel (II), Copper (II) and Zinc (II) complexes, respectively (Appendices VIII - XIII). This indicated coordination of Schiff base through the azomethine nitrogen (Alias *et al.*, 2014).

Moreover, the appearance of additional weak bands in the region 526.4 – 530.6 cm^{-1} and 488.3 – 489.7 cm^{-1}(Table 4.4)attributed to υ (M-N) and υ (M-O), respectively (Ferraro, 1971), further confirmed complexation (Nakamoto, 1992). This showed that the Schiff base ligand coordinated to the metal via “N” and “O” atoms.

The FT-IR spectra of the complexes also showed strong bands in the 3140.3 – 3446.6 cm^{-1} region (Table 4.4), suggesting the presence of coordinated/lattice water in the

complexes. This was further confirmed by the appearance of non-ligand band in 692.6 – 699.7 cm^{-1} region, assignable to the rocking mode of water (Nakamoto, 2009).

In the free Schiff base ligand, the band at 1277 cm^{-1} due to υ(C-O, phenolic) shifted to lower wave number by 62.3 – 66.2 cm^{-1} in the complexes indicating the coordination of the phenolic oxygen atom to the metal ion (Tandon *et al*., 1986). Therefore it can be concluded that coordination took place via phenolic oxygen and azomethine nitrogen of the Schiff base ligand molecule.

4.1.5 Elemental analysis

In order to resolve the molecular formulae of the complexes, elemental analysis of these compounds was carried out. It also enabled the comparison of the theoretical and calculated values. The micro-analysis data (Table 4.5) suggested that all the complexes were mononuclear where two moles of the ligand and two moles of water molecules were coordinated to the central metal atom. The data therefore suggested that the metal to ligand ratio in the complex was 1:2 and the general formula for the complexes as $[M(L)_2(H_2O)_2]$ (M = Mn (II), Co (II), Ni (II), Cu(II), Zn (II); L = deprotonated imine) (Hammam *et al*., 2015; Sobana, Gnana, and Princela, 2015). The theoretical (calculated) values were found to be in good agreement with the experimental values.

Table 4.5: Elemental analysis data of ligand and its metal (II) complexes

Compound	%C: Found (Calc.)	%H: Found (Calc.)	%N: Found (Calc.)
L	62.63(62.48)	3.59(3.60)	5.50(5.61)
$[MnL_2(H_2O)_2]$	53.08(53.04)	3.43(3.40)	4.72(4.76)
$[CoL_2(H_2O)_2]$	52.69(52.68)	3.35(3.38)	4.88(4.73)
$[NiL_2(H_2O)_2]$	52.68(52.70)	3.36(3.38)	4.70(4.73)
$[CuL_2(H_2O)_2]$	52.21(52.27)	3.34(3.35)	4.65(4.69)
$[ZnL_2(H_2O)_2]$	52.09(52.11)	3.30(3.34)	4.64(4.68)

Where L= Schiff base ligand, 4-Chloro-2-{(E)-[(4-Fluorophenyl)Imino]Methyl}Phenol

Calc. = Calculated

4.1.6 NMR spectral analysis

The ^{1}H and ^{13}C NMR spectra of the Schiff base ligand and its diamagnetic Ni (II) and Zn (II) complexes were recorded in DMSO-d_6. The ^{1}H NMR spectrum of the Schiff base showed a singlet peak at δ = 8.92 ppm (Table 4.6 and Figure 4.3) corresponding to the azomethine proton (– N=CH–) (El-Sherif, 2011 and Munde, 2010), an indication that the Schiff base was formed during the condensation reaction. Observation of a peak at δ = 162.82 ppm (Appendix XIV and Table 4.7) in the ^{13}C-NMR spectrum was further proof that the ligand was successfully synthesized (Agarwala, Hingorani and Puri, 1994).

The azomethine proton peak of Schiff base shifted up field in the ^{1}H NMR spectra of the Ni (II) and Zn (II) complexes (δ = 8.87- 8.90 ppm and 8.55 ppm respectively). The up field shifting of azomethine proton in Ni (II) and Zn (II) complexes was attributed to the discharging of electronic cloud towards the Ni (II) and Zn (II) ion indicating coordination through the azomethine nitrogen to the metallic ion (Gunther, 1995). The expected ^{1}H NMR peak, at δ=12.85 ppm, assignable to the phenolic proton in the free Schiff base ligand (Aruland Manjula, 2013) was absent in the spectra of the two complexes. This confirmed the deprotonation of the phenolic group and coordination of the negatively charged oxygen species to the metal cation. This observation further proves the neutral nature of the metal (II) complexes and explains the non-electrolytic behavior of the complexes.

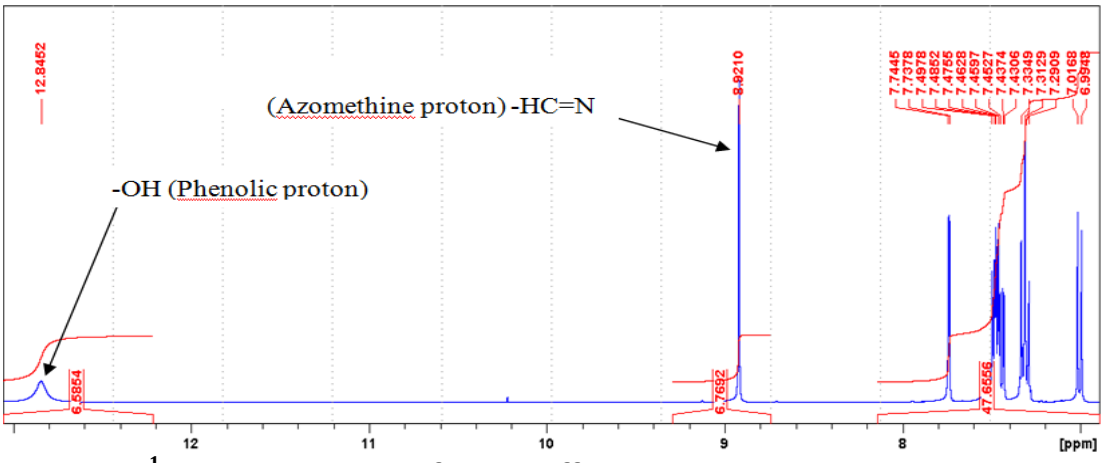


Figure 4.3: ^{1}H-NMR spectrum of the Schiff base ligand

The ^{13}C NMR peaks for the azomethine carbon atom and phenolic group carbons of the coordinated Schiff base ligand were respectively observed at δ = 165 ppm and 162 ppm, in the spectra of the Ni (II) complex, a 2 ppm upfield shift from that observed for the free ligand, due to coordination (Agarwala, Hingorani and Puri, 1994; Canpolat and Yazici, 2007) (Appendix XVI). Thus, the ^{1}H NMR and ^{13}C NMR spectra confirmed the monobasic bidentate nature of the ligand, already suggested by the FT-IR spectral studies. Furthermore, the number of protons calculated from the integration curves and obtained values of the expected C H N analysis agreed well (Gunther, 1995; Nyquist, 2001).

Table 4.6: ^{1}H-NMR data for the free ligand and its diamagnetic Ni (II) and Zn (II) complexes

Compound	Chemical shift (δ, ppm) and Assignment
L	8.92 (singlet, 1H, CH=N) 12.85 (singlet, 1H, OH) 7.43 – 7.49 (multiplet, 7ArH)
$[NiL_2(H_2O)_2]$	8.87 (singlet, 1H, CH=N) 7.12 – 7.15 (multiplet, 7ArH)
$[ZnL_2(H_2O)_2]$	8.55 (singlet, 1H, CH=N) 7.18 – 7.39 (multiplet, 7ArH)

Where L= Schiff base ligand, 4-Chloro-2-{(E)-[(4-Fluorophenyl)Imino]Methyl}Phenol

Table 4.7: ^{13}C-NMR data for the free ligand and its diamagnetic Ni (II) and Zn (II) complexes

Compound	Chemical shift (δ, ppm) and Assignment
L	123 –117 (Ar, C=C) 163 (HC=N) 133 (C-O)
$[NiL_2(H_2O)_2]$	122 - 118 (Ar, C=C) 162 (HC=N) 132 (C-O)
$[ZnL_2(H_2O)_2]$	124 - 116 (Ar, C=C) 162 (HC=N) 125 (C-O)

4.1.7 Antibacterial activity

The tests were carried out on two Gram-positive and two Gram-negative bacterial strains according to standard procedure (Choudhary, 2001; Murray *et al*., 1995). The results were recorded in Tables 4.8, 4.9, 4.10 and 4.11and represented using bar graphs (Figures 4.4, 4.5, 4.6 and 4.7). The ability of the Schiff base ligand and its metal (II) complexes to inhibit the growth of the bacteria was compared to those of the known standard antibacterial drugs, Amoxyclav (AMC), Nalidixic acid (NA) and Gentamicin (GEN). The Schiff base ligand and its metal (II) complexes demonstrated abilities to inhibit bacterial growth.

Table 4.8: Antibacterial screening data for the ligand and its metal (II) complexes (10 µg/ml)

Sample		**Zone of inhibition in mm* against bacterial strains:**			
		Gram negative		**Gram positive**	
		E. coli	***P. aeruginosa***	***B. subtilis***	***S. typhi***
L	(A)	7.3±0.5	8.4±0.5	7.7±0.9	8.4±0.5
$[MnL_2(H_2O)_2]$	(B)	8.1±0.5	10.7±0.8	9.7±0.5	9.3±0.5
$[CoL_2(H_2O)_2]$	(C)	9.0±0.9	9.7±1.2	8.3±0.5	9.4±1.2
$[NiL_2(H_2O)_2]$	(D)	9.5±0.8	11.3±0.5	9.7±0.5	8.6±0.5
$[CuL_2(H_2O)_2]$	(E)	8.1±0.8	10.3±1.2	8.6±0.5	8.7±0.5
$[ZnL_2(H_2O)_2]$	(F)	7.6±0.9	10.0±0.5	8.4±0.9	10.1±0.9
Amoxyclav (AMC)	(G)	8.0±0.0	-	-	-
Nalidixic acid (NA)	(H)	12.0±1.0	20.0±2.0	13.0±1.0	30.5±0.5
Gentamicin (GEN)	(I)	9.0±0.0	14.0±0.0	9.5±0.5	10.5±1.5

* = average of three replicates ± SD

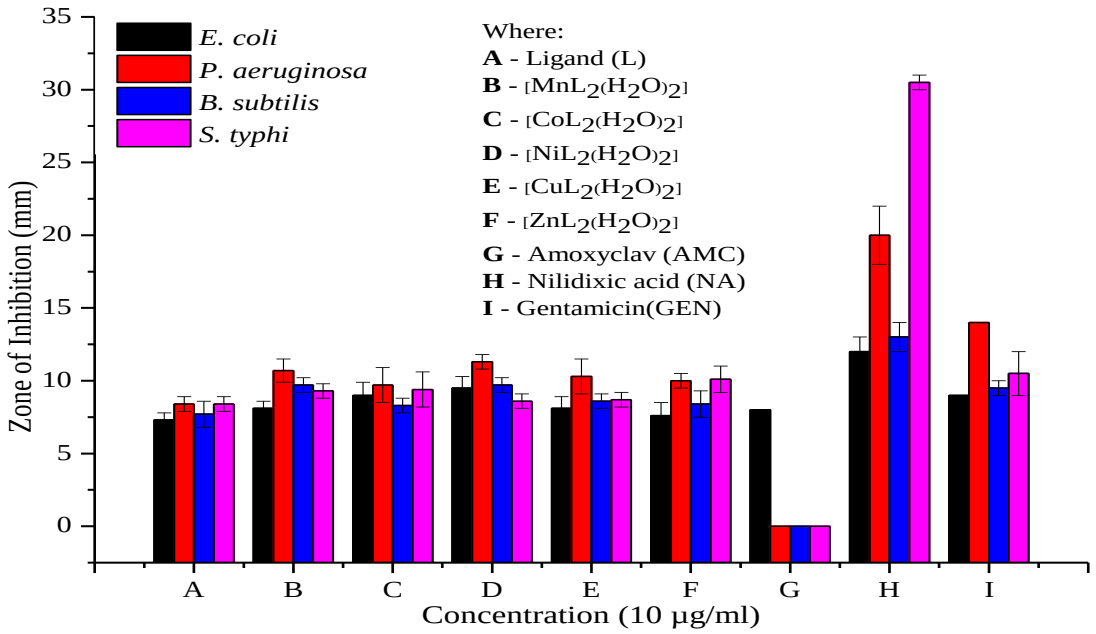


Figure 4.4: Bar graphs showing the effect of Schiff base ligand (A), its metal (II) complexes (B – F) at 10 µgml^{-1}and the standard drugs (G – I) towards *E. coli, P. aeruginosa, B. subtilis* and *S. typhi*

Table 4.9: Antibacterial screening data for the ligand and its metal (II) complexes (20 µg/ml)

Sample		**Zone of inhibition in mm* against bacterial strains:**			
		Gram negative		**Gram positive**	
		E. coli	***P. aeruginosa***	***B. subtilis***	***S. typhi***
L	(A)	7.5±0.5	8.7±0.5	7.9±0.9	8.6±0.5
$[MnL_2(H_2O)_2]$	(B)	8.6±0.5	11.0±0.8	10.3±0.8	9.6±0.5
$[CoL_2(H_2O)_2]$	(C)	9.2±0.8	10.7±1.4	8.9±0.9	9.7±0.5
$[NiL_2(H_2O)_2]$	(D)	10.0±0.8	11.8±0.5	9.9±0.5	8.8±0.5
$[CuL_2(H_2O)_2]$	(E)	8.3±0.5	10.6±1.2	9.3±0.5	8.9±0.5
$[ZnL_2(H_2O)_2]$	(F)	7.8±0.5	10.4±1.4	8.9±0.9	10.3±0.9
Amoxyclav (AMC)	(G)	8.0±0.0	-	-	-
Nalidixic acid (NA)	(H)	12.0±1.0	20.0±2.0	13.0±1.0	30.5±0.5
Gentamicin (GEN)	(I)	9.0±0.0	14.0±0.0	9.5±0.5	10.5±1.5

* = average of three replicates ± SD.

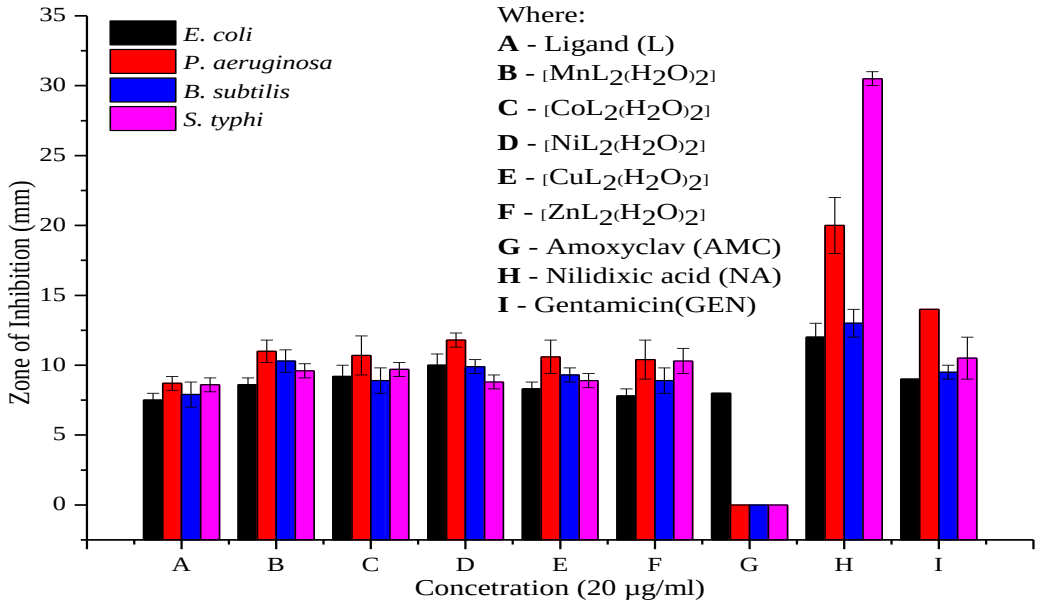


Figure 4.5: Bar graphs showing the effect of Schiff base ligand (A), its metal (II) complexes (B – F) at 20 µgml−1and the standard drugs (G – I) towards *E. coli, P. aeruginosa, B. subtilis* and *S. typhi*.

Table 4.10: Antibacterial screening data for the ligand and its metal (II) complexes (30 µg/ml)

Sample		**Zone of inhibition in mm* against bacterial strains:**			
		Gram negative		**Gram positive**	
		E. coli	***P. aeruginosa***	***B. subtilis***	***S. typhi***
L	(A)	7.7±0.5	8.9±0.8	8.5±0.8	8.8±0.5
$[MnL_2(H_2O)_2]$	(B)	8.9±0.5	12.±0.5	10.9±0.5	9.8±0.5
$[CoL_2(H_2O)_2]$	(C)	9.8±0.5	11.3±1.2	9.3±0.5	11.0±0.8
$[NiL_2(H_2O)_2]$	(D)	10.4±0.8	12.4±0.5	10.8±0.5	9.0±0.8
$[CuL_2(H_2O)_2]$	(E)	8.6±0.5	10.9±1.2	10.0±0.8	9.3±0.5
$[ZnL_2(H_2O)_2]$	(F)	8.1±0.5	10.9±1.2	9.7±0.5	11.0±1.2
Amoxyclav (AMC)	(G)	8.0±0.0	-	-	-
Nalidixic acid (NA)	(H)	12.0±1.0	20.0±2.0	13.0±1.0	30.5±0.5
Gentamicin (GEN)	(I)	9.0±0.0	14.0±0.0	9.5±0.5	10.5±1.5

* = average of three replicates ± SD.

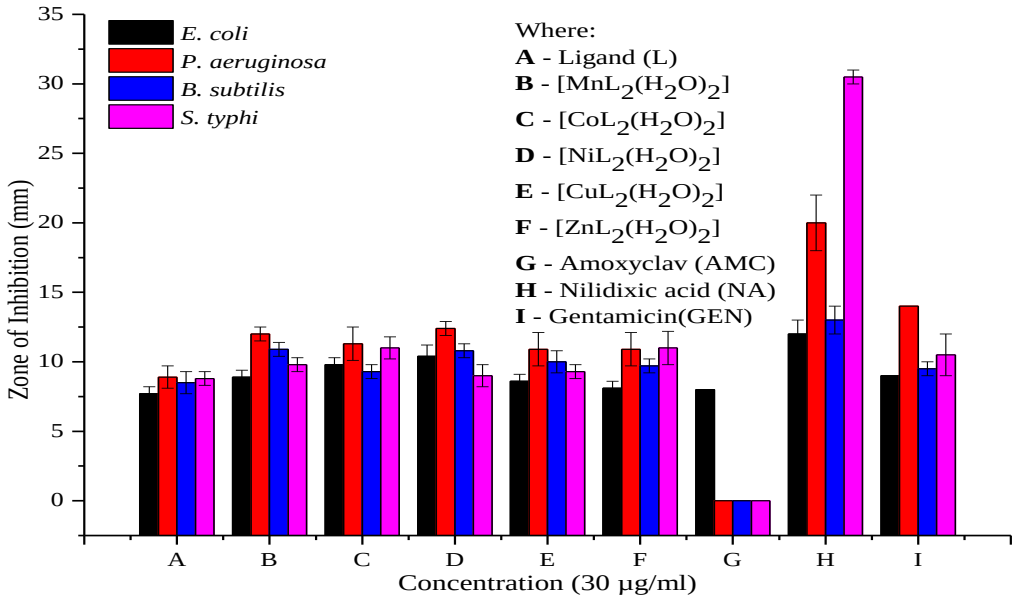


Figure 4.6: Bar graphs showing the effect of Schiff base ligand (A), its metal (II) complexes (B – F) at 30 µgml−1and the standard drugs (G – I) towards *E. coli, P. aeruginosa, B. subtilis* and *S. typhi*.

Table 4.11: Antibacterial screening data for the ligand and its metal (II) complexes (40 µg/ml)

Sample		**Zone of inhibition in mm* against bacterial strains:**			
		Gram negative		**Gram positive**	
		E. coli	***P. aeruginosa***	***B. subtilis***	***S. typhi***
L	(A)	8.0±0.5	10.0±0.8	9.0±0.8	8.9±0.5
$[MnL_2(H_2O)_2]$	(B)	9.7±0.5	14.7±0.5	11.7±0.5	10.2±0.5
$[CoL_2(H_2O)_2]$	(C)	10.0±0.8	12.0±0.8	10.2±0.5	11.7±0.5
$[NiL_2(H_2O)_2]$	(D)	11.3±0.9	12.7±1.2	11.0±0.8	9.3±0.8
$[CuL_2(H_2O)_2]$	(E)	8.9±0.9	11.7±1.2	10.7±0.5	9.6±0.5
$[ZnL_2(H_2O)_2]$	(F)	8.3±0.5	11.4±0.8	9.9±0.9	11.6±1.4
Amoxyclav (AMC)	(G)	8.0±0.0	-	-	-
Nalidixic acid (NA)	(H)	12.0±1.0	20.0±2.0	13.0±1.0	30.5±0.5
Gentamicin (GEN)	(I)	9.0±0.0	14.0±0.0	9.5±0.5	10.5±1.5

* = average of three replicates ± SD.

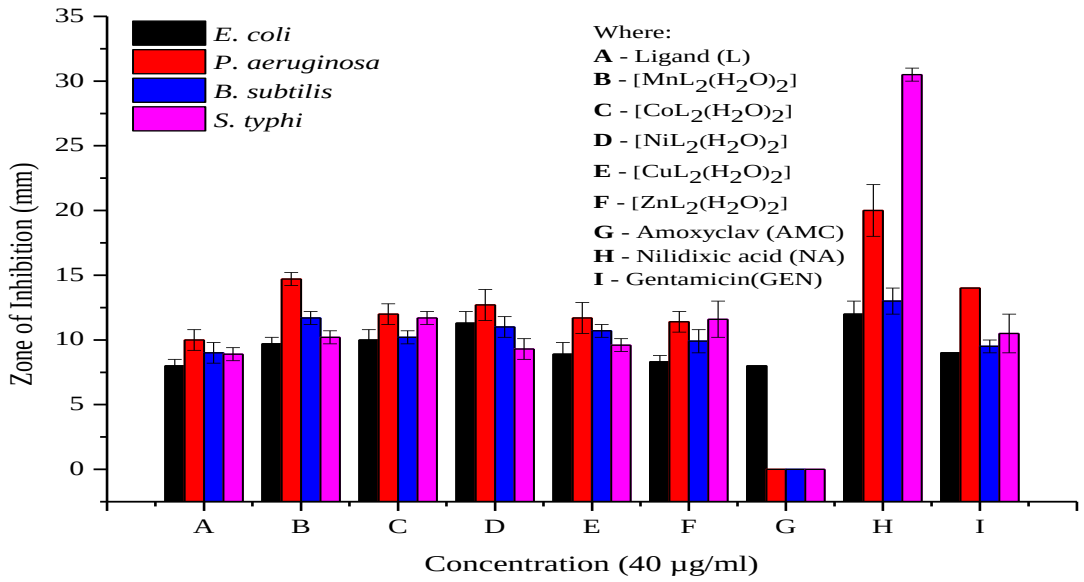


Figure 4.7: Bar graphs showing the effect of Schiff base ligand (A), its metal (II) complexes (B – F) at 40 µgml−1and the standard drugs (G – I) towards *E. coli, P. aeruginosa, B. subtilis* and *S. typhi.*

Research has shown that coordination of a ligand, with the potential to inhibit bacterial growth, evokes antibacterial growth inhibition ability (Nyawade *et al*., 2015). Results based on the synthesized compounds indicate that nickel (II) complex had the highest activity against *E. coli* while manganese (II) complex showed maximum inhibition zone against *P. aeruginosa* and*B. subtilis*at 40 µg/ml. The zinc (II) complex was slightly more active than the ligand against *E. coli.* The cobalt (II) complex had a slightly higher activity than the ligand against *P. aeruginosa* and *B. subtilis.* The nickel (II) complex performed better than the ligand against *S. typhi* at 40 µg/ml. It is worth noting that the Schiff base and its metal (II) complexes inhibited the growth of Amoxyclav-resistant *P. aeruginosa*, *B. subtilis* and *S. typhi.* The ligand was mildly active towards all the four bacterial strains probably due the presence of hydroxyl and azomethine groups in its structure (Zaidan *et al*., 2006).

Overall, the inhibition zones of Schiff base ligand and its complexes at different concentrations showed that the complexes had enhanced bactericidal activity than the ligand (Revanasiddappa *et al*., 2008) and the standard drug Amoxyclav (AMC) against

all the bacterial strains studied. It should be further observed that the complexes delivered better antibacterial activity at their higher concentration. The increase in the antibacterial activity of the metal (II) complexes may be due to the effect of the metal ion on the normal state of the bacterial cell process (Naeimi and Moradian, 2017). Research has shown that the structural components possessing additional (C=N) bond with nitrogen and oxygen donor systems inhibit enzyme activity due to their deactivation by metal coordination (Al-Amier *et al*., 2012 and Jesmin *et al*., 2008).

In the light of Tweedy's chelation theory, polarity of the metal ion is greatly reduced as a result of overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups (Mahajan *et al*., 2007). This enhances the delocalization of the π e^- s over the entire complex ring thereby promoting the lipophilicity of the chelate. Therefore, chelation enhances the lipophilic character of the central metal ion hence increasing the hydrophobic character and liposolubility of the complex (Mishra *et al*., 2012). This favors the complex permeation through the lipid layers of the cell membrane of the bacteria (Figure 4.8).

Once it penetrates the cell, the complexes block the metal binding sites on enzymes of microorganisms. This in turn disturbs the respiration process of the cell by curtailing the synthesis of proteins, which restricts further growth of the organism (Bagihalli *et al* 2008; El-Sherif and Eldebss, 2011). Thus, there is hope that thesecomplexes could reasonably be used in designing more potent antibacterial agents for the treatment of some common diseases caused by *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. typhi*.

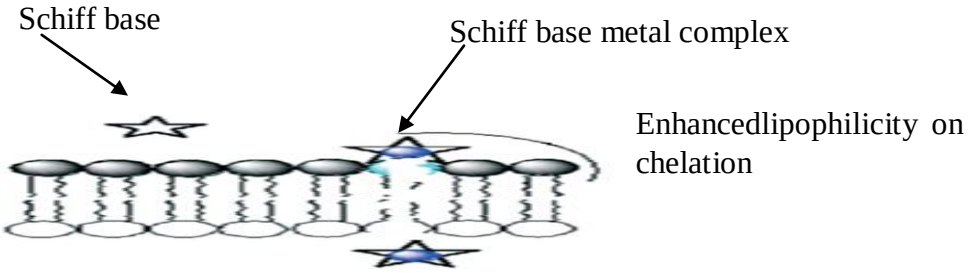


Figure 4.8: Schematic diagram showing enhanced lipophilicity of metal complexes on chelation favoring easy penetration of the complexes into the bacterial lipid membrane (Mishra *et al*., 2012)

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

i. The ligand and its complexes were successfully synthesized and characterized.
ii. The UV-vis, FT-IR and ^{1}H NMR spectral studies showed that the ligand coordinated to the metal (II) ion via the azomethine nitrogen and phenolic oxygen.
iii. Octahedral structure has been proposed for the metal (II) complexes.
iv. Based on conductance data, the synthesized compounds are non-electrolytes.
v. The complexes exhibited enhanced antibacterial properties relative to the parent ligand under the same experimental conditions.
vi. Ni (II) complex was the most effective against all the microorganisms while *E. coli* was the most resistant bacterial strain.
vii. The inhibitory ability of the Schiff base and its metal (II) complexes towards these bacterial strains increased with increasing concentration.

5.2 Recommendations

i. Attempts to grow a single crystal of each of these synthesized compounds did not yield fruits, only amorphous solid complexes were generated. Further studies on how to generate crystals of these complexes should be pursued.
ii. Only bacterial activity of the synthesized compounds was studied. Other antimicrobial properties such as antiviral, antifungal, etc, of these compounds should be investigated.
iii. It is recommended that toxicity tests and MIC of these compounds be explored.
iv. It is also recommended that these compounds be used in designing more potent antibacterial agents for the treatment of some common diseases caused by *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. typhi*

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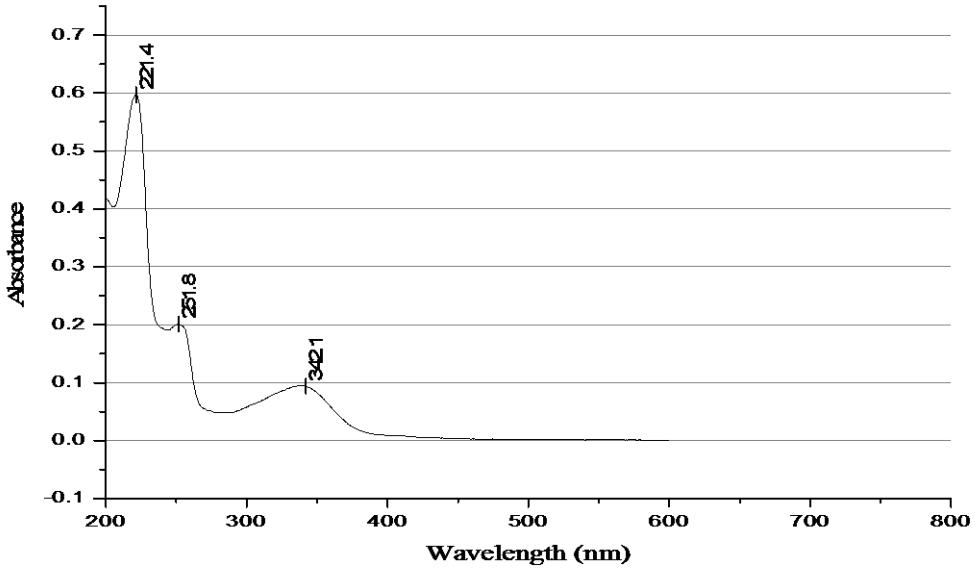
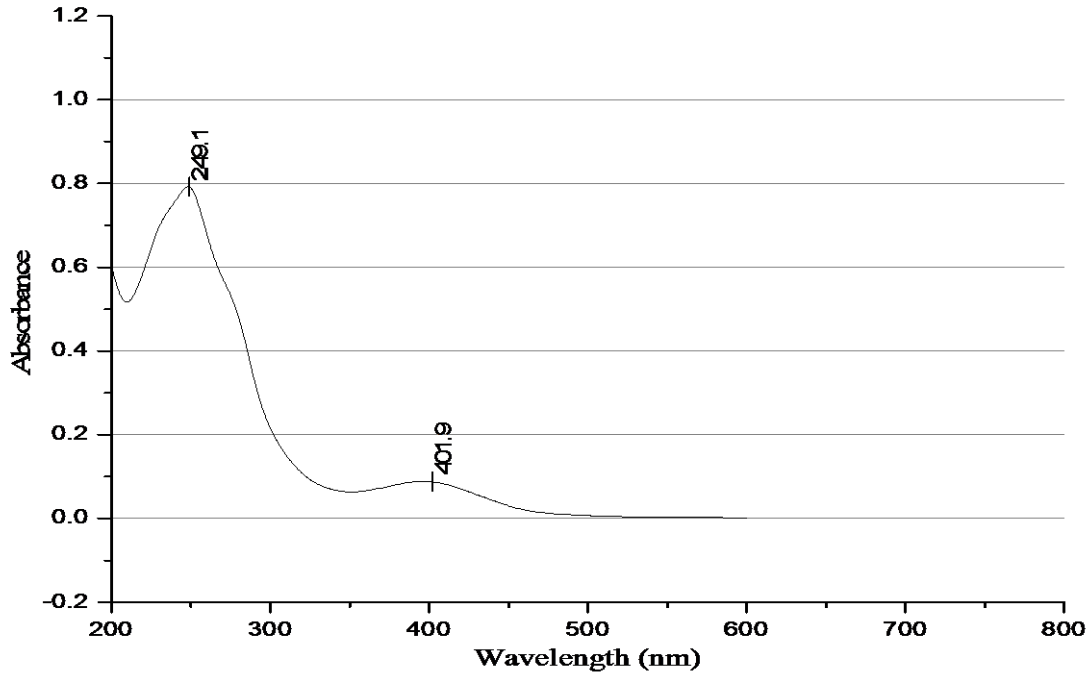
APPENDICES

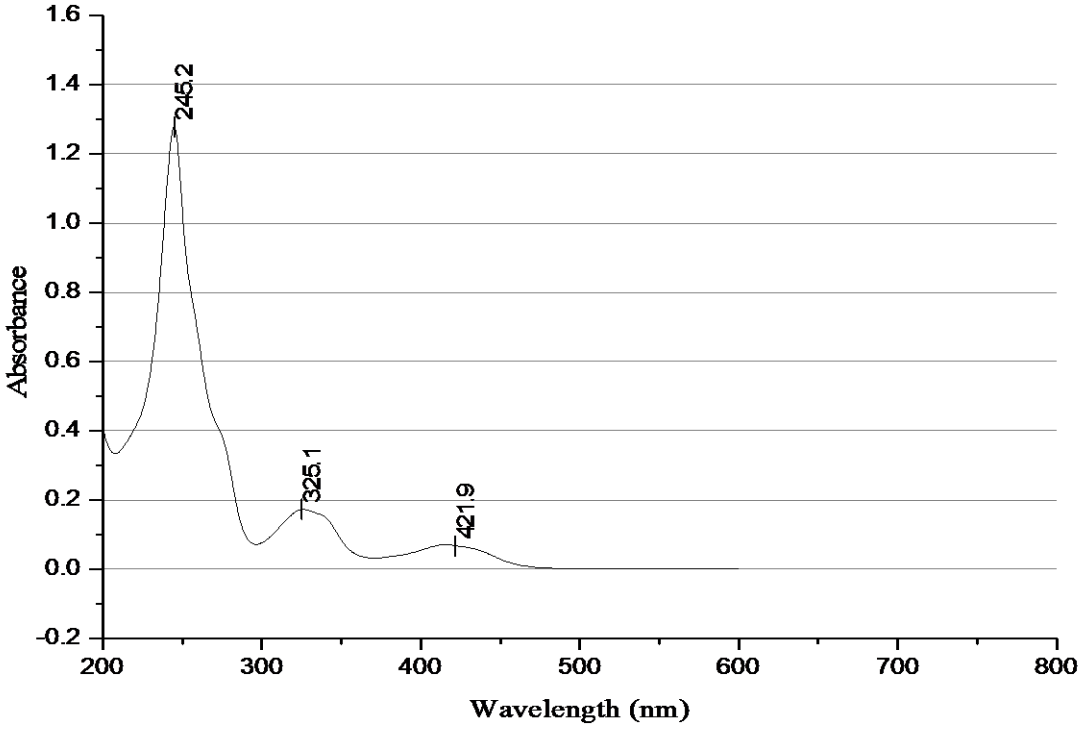
Appendix I: UV-vis spectrum for $[MnL_2(H_2O)_2]$ complex



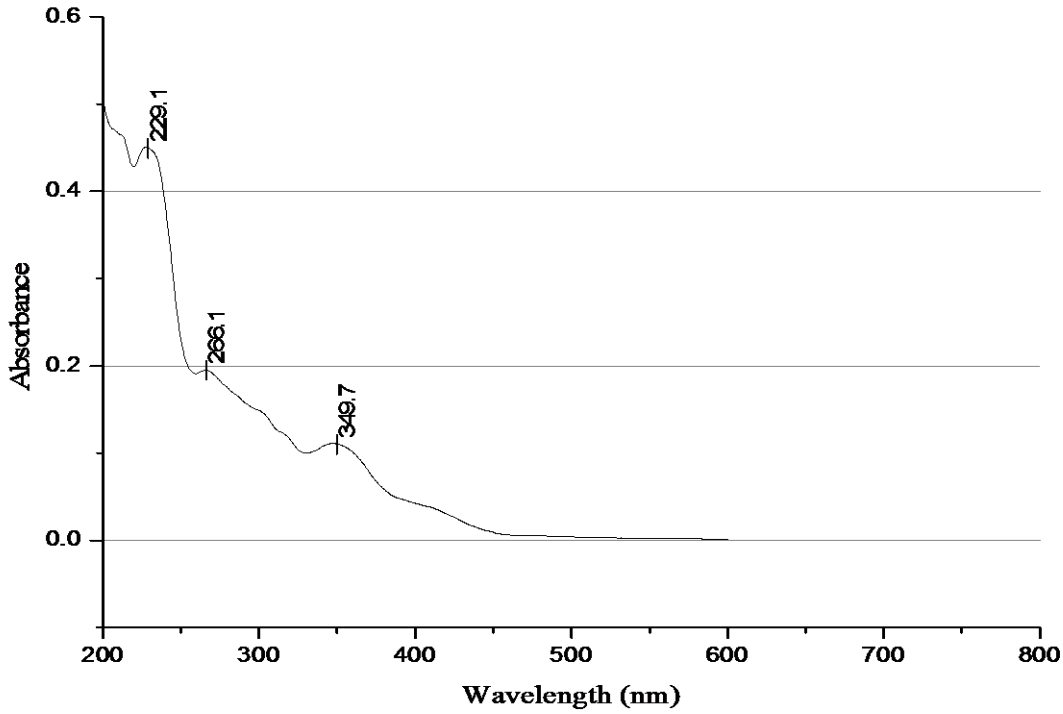
Appendix II: UV-vis spectrum for $[CoL_2(H_2O)_2]$ complex



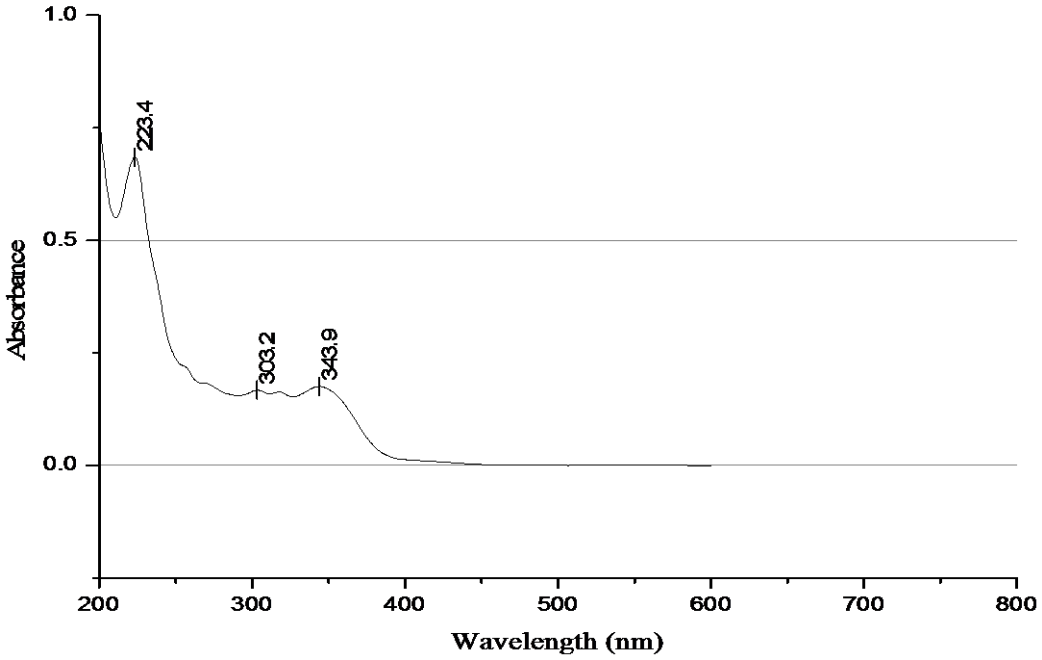
Appendix III: UV-vis spectrum for $[NiL_2(H_2O)_2]$ complex



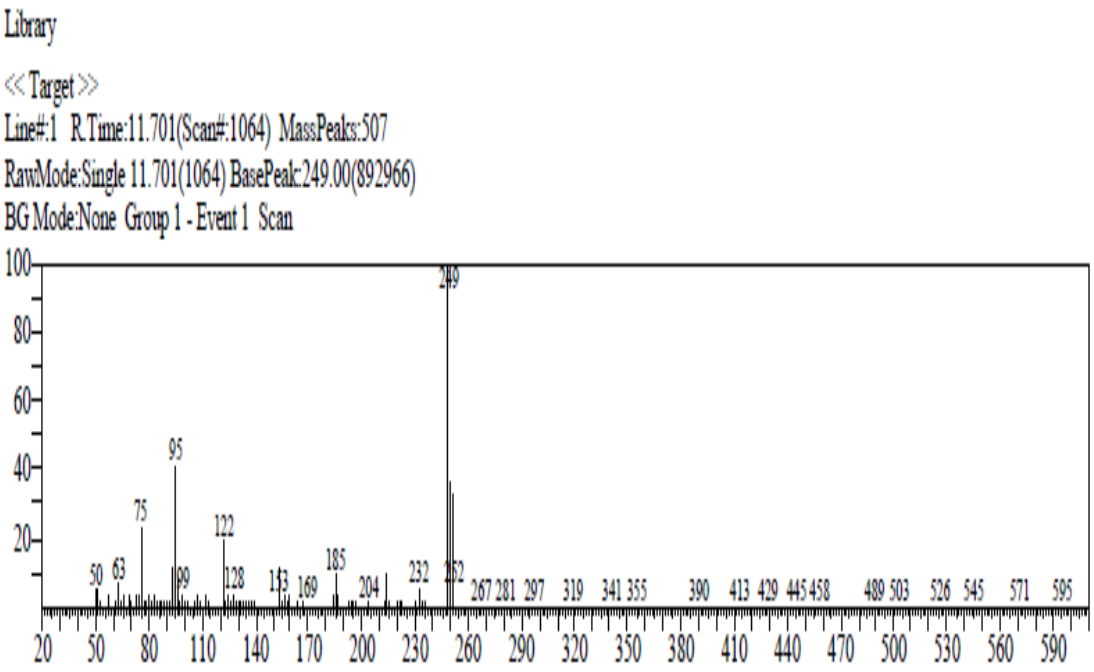
Appendix IV: UV-vis spectrum for $[CuL_2(H_2O)_2]$ complex



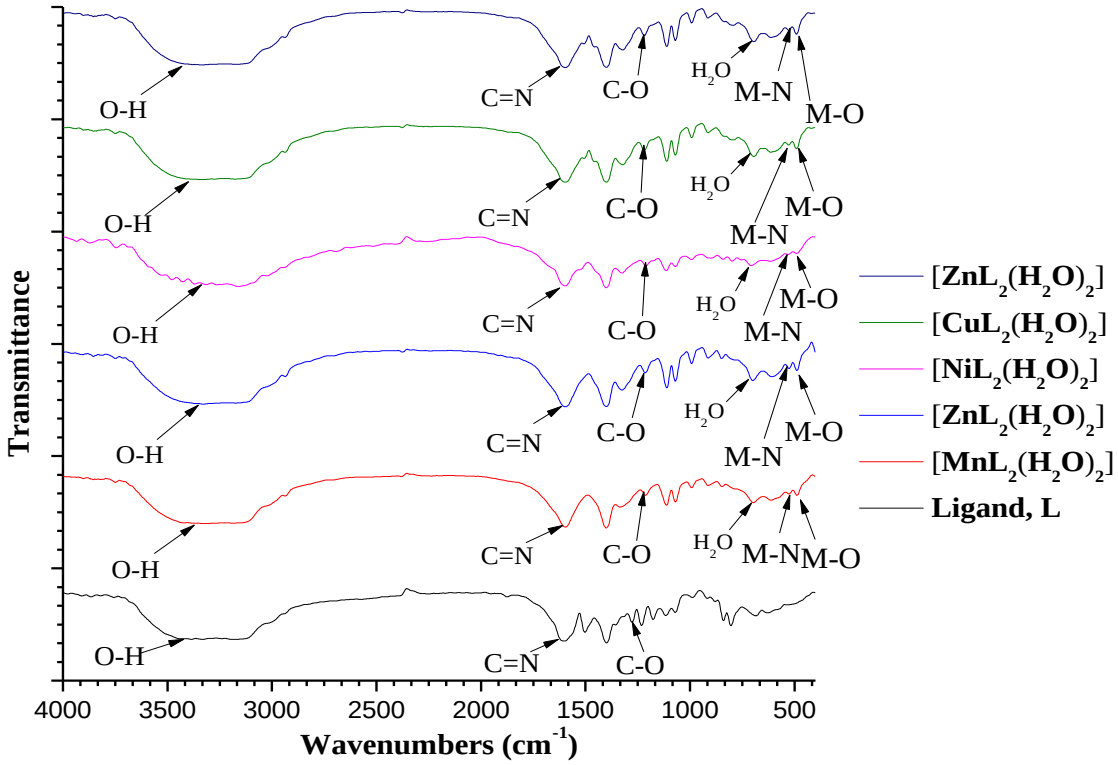
Appendix V: UV-vis spectrum for $[ZnL_2(H_2O)_2]$ complex



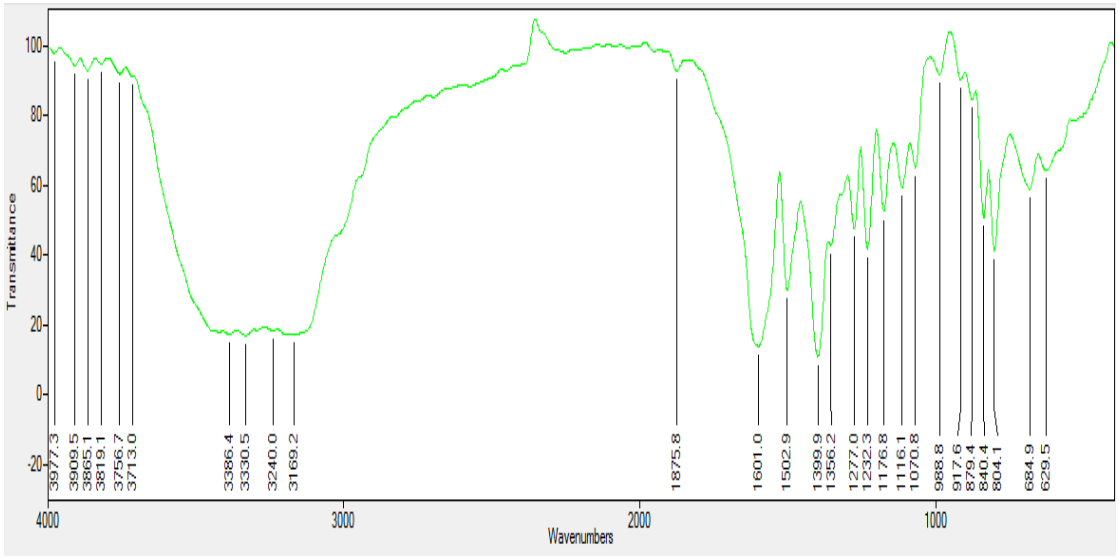
Appendix VI: Mass spectrum of the Schiff base ligand, L



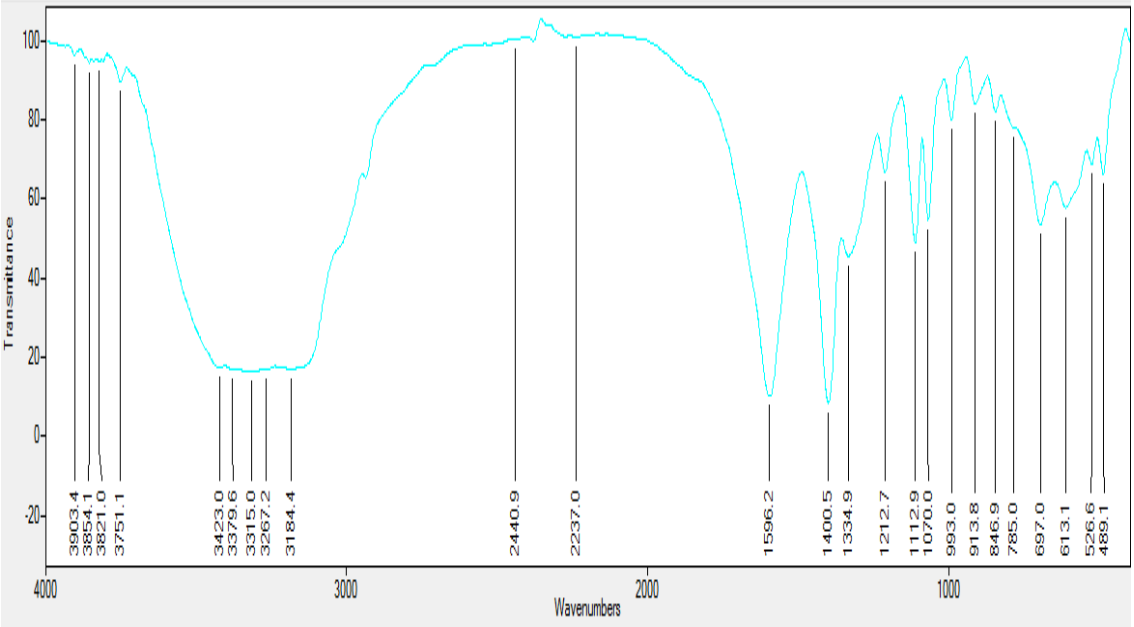
Appendix VII: FT-IR spectra of Schiff base ligand and its metal (II) complexes



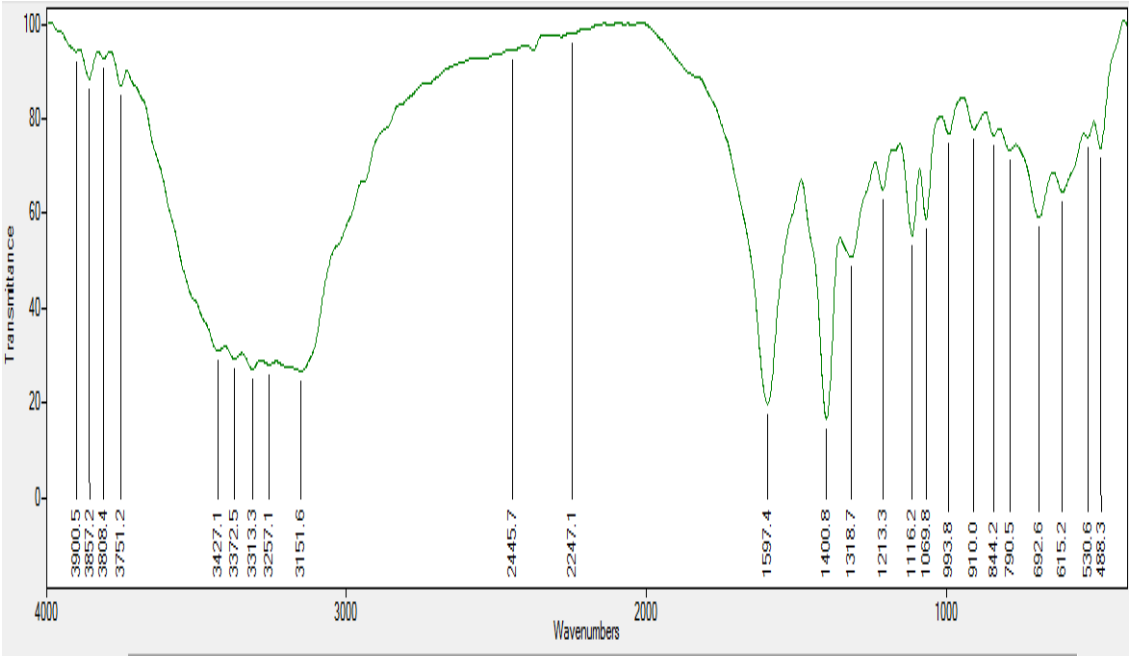
Appendix VIII: FT- IR Spectrum of Schiff base ligang, L



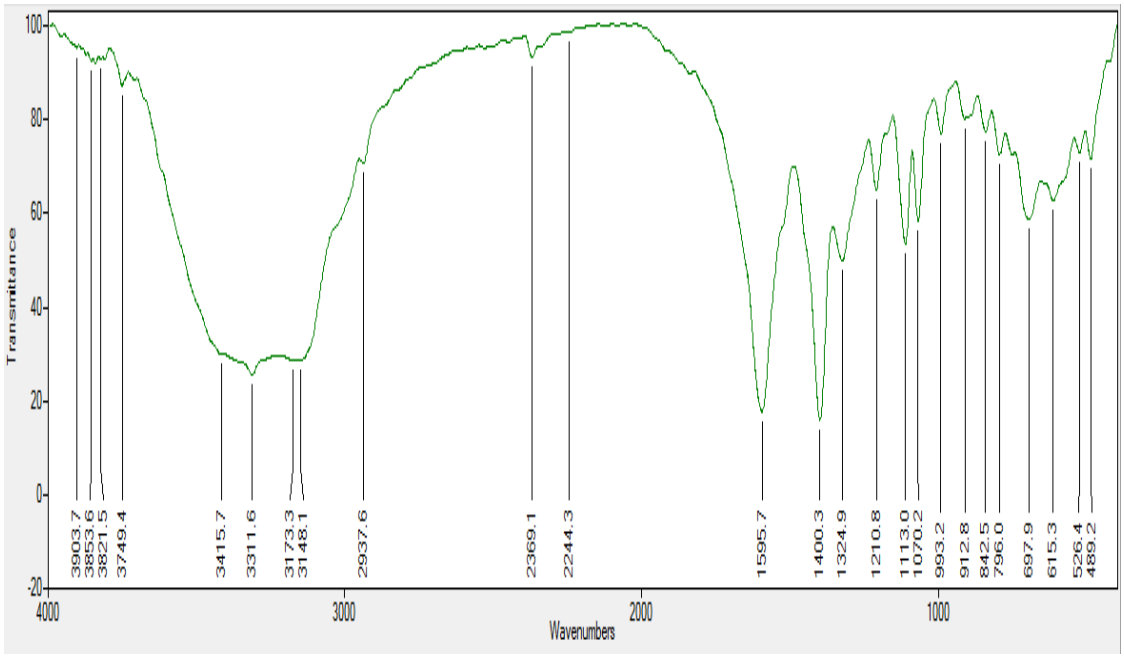
Appendix IX: FT-IR Spectrum of $[MnL_2(H_2O)_2]$ complex



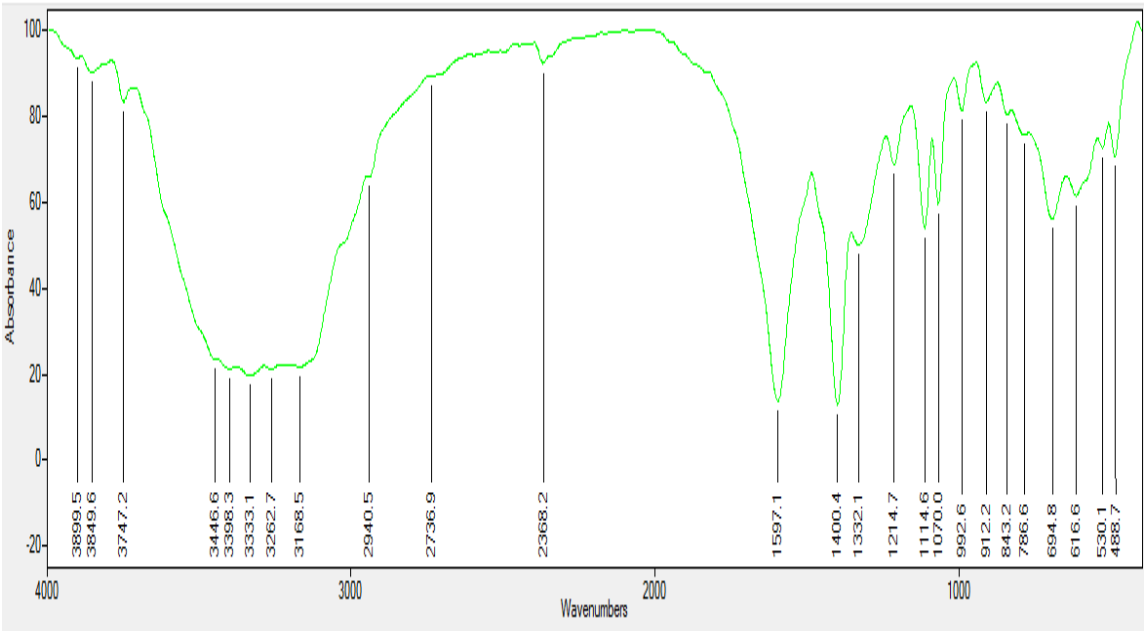
Appendix X: FT-IR Spectrum of $[CoL_2(H_2O)_2]$ complex



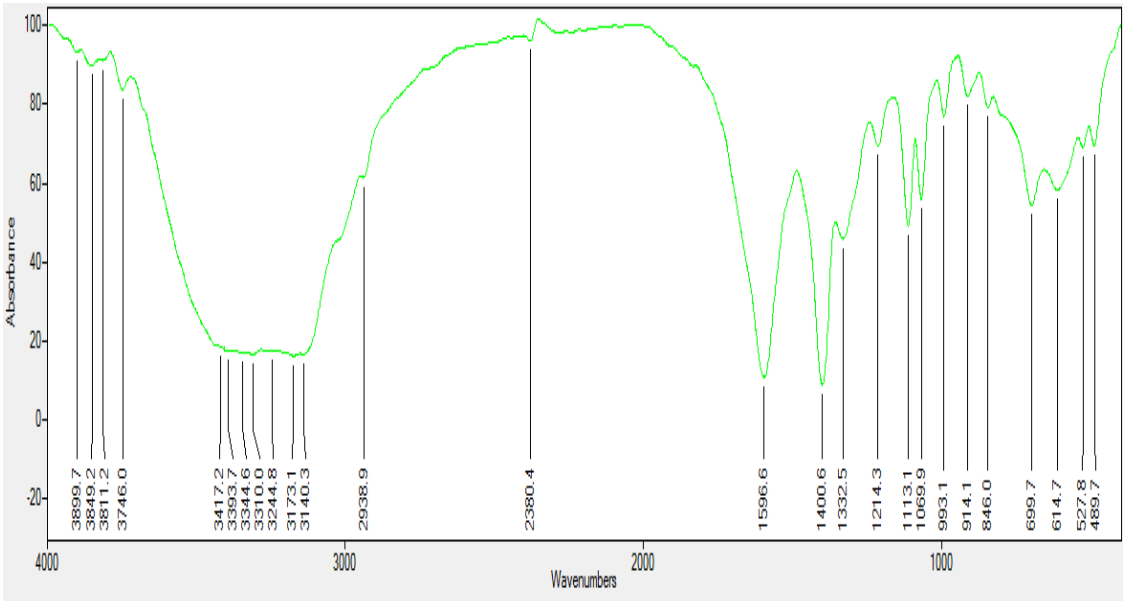
Appendix XI: FT-IR Spectrum of $[NiL_2(H_2O)_2]$ complex



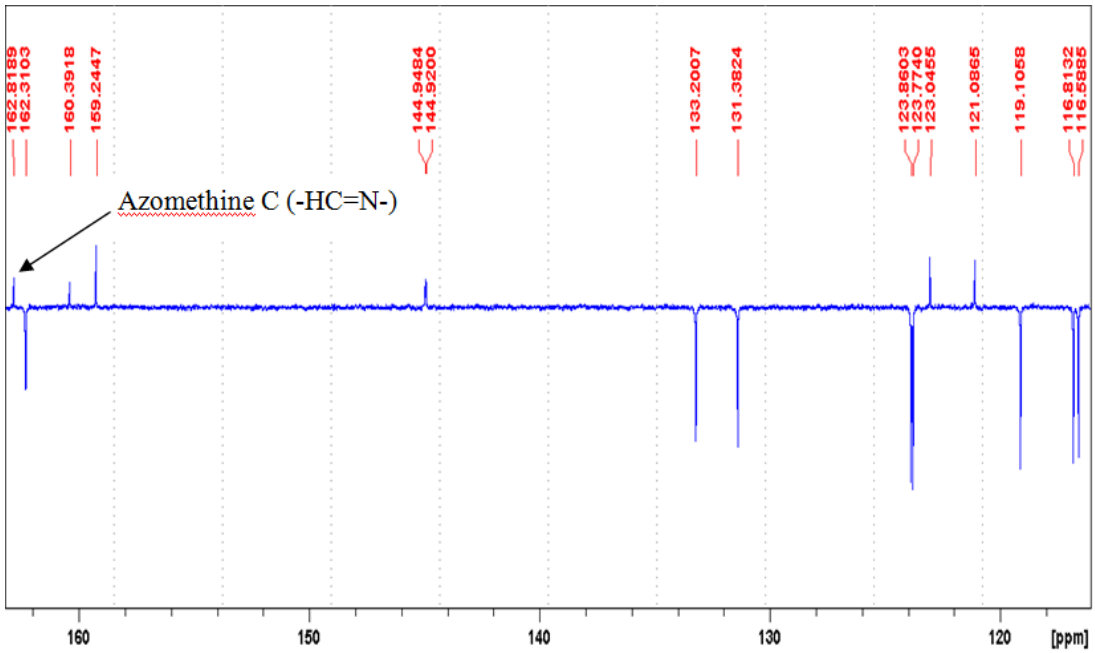
Appendix XII: FT-IR Spectrum of $[CuL_2(H_2O)_2]$ complex



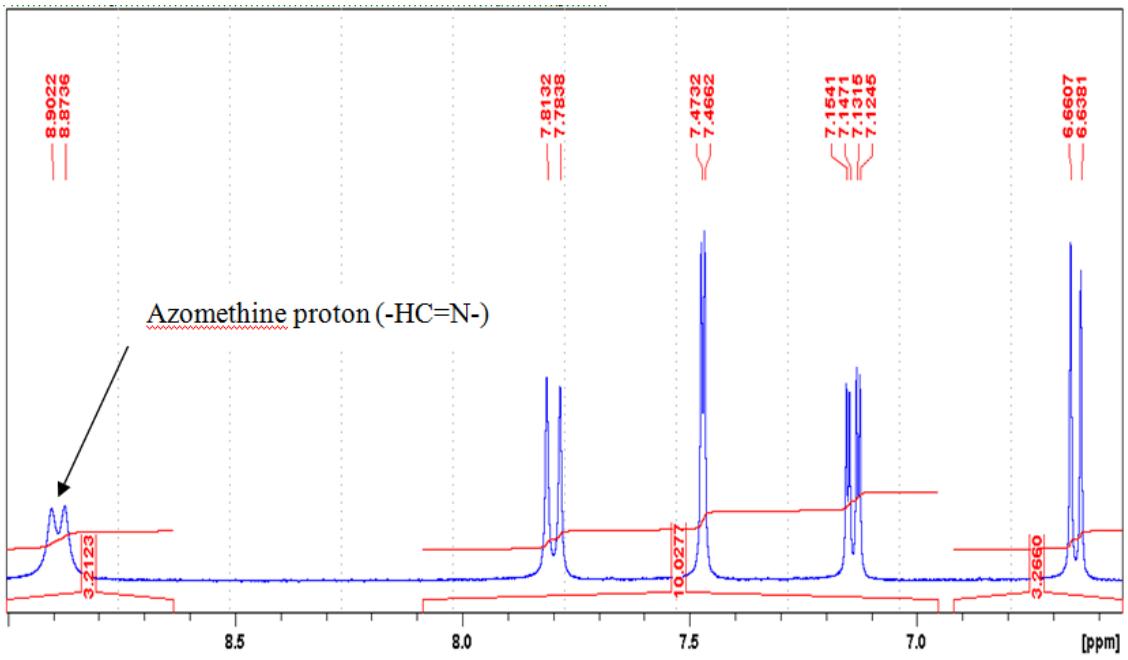
Appendix XIII: FT-IR Spectrum of $[ZnL_2(H_2O)_2]$ complex



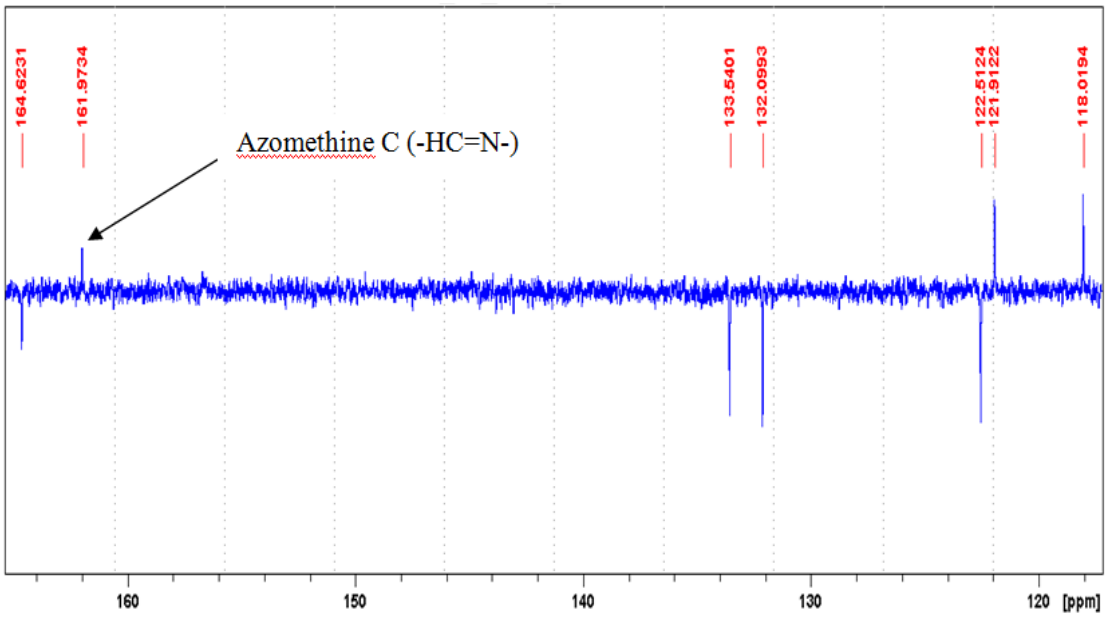
Appendix XIV: ^{13}C-NMR spectrum of the Schiff base ligand, L



Appendix XV: ^{1}H-NMR spectrum of $[NiL_2(H_2O)_2]$ complex



Appendix XVI: ^{13}C-NMR spectrum of $[NiL_2(H_2O)_2]$ complex



Appendix XVII: ^{1}H-NMR spectrum of $[ZnL_2(H_2O)_2]$ complex