SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF Co (II) AND Cu (II) COMPLEXES OF SCHIFF BASE DERIVED FROM *p*-DIMETHYLAMINO BENZALDEHYDE AND ETHYLENE DIAMINE

MSc THESIS

ADEFRIS ASEFA MAMO

AUGUST 2017

HARAMAYA UNIVERSITY, HARAMAYA

Synthesis, Characterization and Antimicrobial Studies of Co (II) and Cu (II) Complexes of Schiff Base Derived from *p*-Dimethyl Amino Benzaldehyde and Ethylene Diamine

A Thesis Submitted to the Department of Chemistry, Postgraduate Program Directorate

HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN CHEMISTRY

Adefris Asefa Mamo

August 2017

Haramaya University, Haramaya

HARAMAYA UNIVERSITY
POSTGRADUATE PROGRAM DIRECTORATE

I hereby certify that I have read and evaluated this Thesis entitled **"Synthesis Characterizati on and Antimicrobial Studies of Co (II) and Cu (II) Complexes of Schiff Base Derived from *p*-Dimethyl Amino Benzaldehyde and Ethylenediamine"** prepared under my guidance by Adefris Asefa. I recommended that it be submitted as fulfilling the thesis requirement

Major Advisor	Signature	Date
Co-Advisor	Signature	Date

As a member of the Board of Examiners of MSc Thesis Open Defence Examination, I certify that I have read and evaluated the Thesis prepared by Adefris Asefa and examined the candidate. I recommended that the Thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in chemistry.

Chair Person	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date

Final approval and acceptance of Thesis is contingent upon the submission of its final copy to the council of Graduate Studies (CGS) through the candidate's department or school graduate committee (SGC).

DEDICATION

I dedicate this thesis manuscript to my beloved family and my wife Lidiya Belayneh for their continuous support and encouragement during my study.

STATEMENT OF THE AUTHER

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, performing experiment, antimicrobial test, data analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

This Thesis is submitted in partial fulfillment of the requirements for an MSc degree at the Haramaya University. The Thesis is deposited in the Haramaya University Library and is made available to borrowers under the rules of the Library. I solemnly declare that this Thesis has not been submitted to any other institution anywhere for the award of degree, diploma or certificate.

Brief quotations from this Thesis may be made without special permission provided that accurate and complete acknowledgement of the source is made. Requests for permission for extended quotations from or reproduction of this Thesis in whole or in part may be granted by the Head of the School or Department when in his or her judgment the proposed use of the material is in the interest of scholar ship. In all other instances, however, permission must be obtained from the author of the thesis.

Name: Adefris Asefa Signature_____

Date: 19/08/2017

Department: Chemistry

BIOGRAPHICAL SKETCH

The author was born in February, 1969 G.C in Gololcha Woreda, Arsi zone of Oromiya Region, Ethiopia. He attended his elementary and secondary education at Mine Elementary School and Abomsa Secondary School, respectively. Following the successful completion of high school education, he joined Kotobe Colledge of Teacher Education and earned diploma in chemistry in 1989 G.C. After graduation, he thought for three years in Gamugofa Zone of Konso Secondary School and for six years Arsi Zone of Gara Gora Junior Secondary School. Then, he joined Haramaya University summer In-service program in 1998 G.C and graduated with B.Ed degree in chemistry in June 2003 G.C. Again he joined Haramaya University summer In-service program to persue his MSc study in chemistry in 2012 G.C. Presently he is teaching chemistry at Abomsa preparatory school, Arsi Zone, Oromiya region, Ethiopia.

ACKNOWLEDGEMENT

I would like to express my heartfelt thanks to Ministry of Education, for providing me the opportunity to join the program and access the necessary financial support to complete my study.

I am also very much indebted to my thesis research major advisor Dr Yiheyis Bogale because of his encouragement and effort that I came to the end.

My appreciation goes to my co-advisor Dr. Neelaiah Babu G. who was giving valuable advice and comments, and facilitating the resources required for conducting this research.

I am grateful for the Haramaya University Chemistry department staff for giving me genuine information requested for the research.

I wish to extend my gratitude to chemistry department laboratory technicians for their material and technical support in order to do the experiment in the university chemistry research laboratory.

My thanks go to Miss Haimanot Bizuneh, Haramaya University plant science department for her great support in order to do the antimicrobial test.

I would like to thank Dr.Yonas Chebude and Mr. Yohannis, Addis Ababa University, Department of Chemistry for running the samples.

I appreciate all my friends, colleagues and those who lend me a hand by contributing in one way or the other for the completion of this research work.

At last I would like to thank my wife Lidiya Belayneh for her great moral support and giving advice by controlling and guiding my family in order to complete my education and the research work.

ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
A. flavus	*Aspergillas flavus*
A. niger	*Aspergillas niger*
B.M.	Bohr magnetron
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
E.coli	*Escherichia coli*
FT-IR	Fourier Transform Infrared Spectrophotometer
IUPAC	International Union of Pure and Applied Chemistry
MLCT	Metal to Ligand Charge Transfer
MIC	Minimum Inhibitory Concentration of the Antimicrobial Agent
NMR	Nuclear Magnetic Resonance Spectroscopy
ppm	Parts per million
S.typhi	*Salmonella typhi*
S.aureus	*Staphylococcus aureus*
S.galatia	*Streptococcus galatia*
TMS	Tetra methyl silane
TLC	Thin Layer Chromatography
UV-Vis	Ultraviolet and visible

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

LIST OF FIGURES IN THE APPENDIX

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIALSTUDIES OF Co (II) AND Cu (II) COMPLEXES OF SCHIFF BASE DERIVED FROM *p*-DIMETHYL AMINO BENZALDEHYDE AND ETHYLENE DIAMINE

ABSTRACT

Schiff base ligands and their metal complexes are very important in medicinal and pharmaceutical fields because of their wide spectrum of biological activities. This research work describes the synthesis, characterization and antimicrobial investigation of Schiff base ligand derived from p-dimethyl amino benzaldehyde and ethylene diamine and its Co (II) and Cu (II) complexes. The ligand has been synthesized by condensation of p-dimethyl amino benzaldeyde and ethylene diamine. The complexes of Co (II) and Cu (II) with the synthesized ligand were prepared. The synthesized ligand was characterized by NMR spectra. The synthesized ligand and its complexes were characterized on the basis of elemental analysis, IR and electronic spectral data. The complexes were also characterized by molar conductance, AAS, and magnetic susceptibility measurements. Based on the spectral and analytical data octahedral geometry was proposed for both complexes. The conductivity data suggests electrolytic nature of both complexes. The antimicrobial activity of the synthesized ligand L and its Co (II) and Cu (II) complexes were evaluated against four bacteria (S.aureus, S.gatia, E.coli and S.typhi), and two fungi (A.flavus and A.niger) strains. The results obtained as such were compared with the values for standards. Both complexes exhibited higher antibacterial activity than the parent ligand. However the ligand and its metal complexes have shown no inhibitory effect on the tested fungi.

Key Words: *Schiff Base, Metal Complex, Octahedral Geometry, Antimicrobial Activity*

1. INTRODUCTION

1.1. Background of the Study

Coordination chemistry, widely developed in the last few decades, is highly considered in inorganic, organic, and biological fields. Coordination chemistry has always been a challenge to the chemists as it has more branches nowadays. In coordination chemistry Schiff base ligands, which can be synthesized from the condensation of primary amines with carbonyl compounds (Figure 1) play an important role (Sulekh and Seema, 2014).

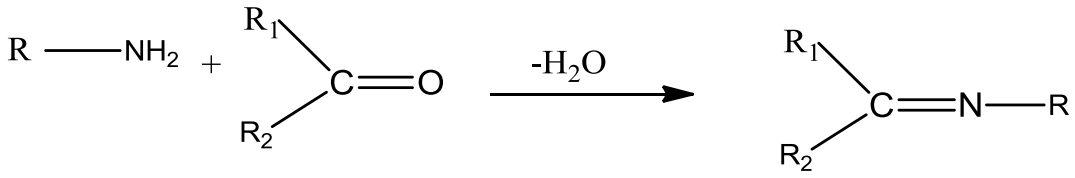


Figure1: Formation of Schiff base by condensation reaction (R group may be various substitute)

Schiff base complexes of transition metals have played prominent role in the development of coordination chemistry. They are among the polyhedron ligands which have been studied because of their industrial and biological applications (Gupta *et al*., 2012). The Schiff bases are more frequently applied for the welfare of mankind with the passage of time. The complexation of Schiff base ligands with transition metals increases the biological activities, such as antibacterial (Karthikeyan *et al*., 2006), antifungal (Singh *et al*., 2006), antidiabetic (Subbaro *et al*., 2014), antitumor (Prakash and Adhikari, 2011) and anticancer (Tyagi *et al*., 2014) activities. Keeping the above facts in mind, Schiff base ligand derived from *p*-dimethyl amino benzaldehyde with ethylene diamine and its Co (II), and Cu (II) complexes were synthesized and characterized with various analytical and spectroscopic techniques. The antibacterial activities of the Schiff base ligand and its metal complexes were studied by taking two gram positive bacteria (*Stappylococcus aureus* (*S.aureus*) and *Streptococcus galatia* (*S. galatia*)) and two gram negative bacteria (*Escherichia coli* and *Salmonella typhi*). The antifungal activities of the same were tested by taking *Aspergillas niger* and *aspergillas flavus* fungi. Finally the antibacterial activities were compared with chloramphinicol as

positive control for bacteria and the antifungal activities were compared with tilt as positive control for fungi. Dimethyl sulfoxide (DMSO) was taken as negative control for both bacteria and fungi.

1.2. Statement of the Problem

The increase in the mortality rate associated with infectious diseases is directly related to bacteria that exhibit multiple resistances to antibiotics. The lack of effective treatment is the main cause of this problem. The development of new antibacterial agent with novel and more efficient mechanisms of action is definitely an urgent medical need. Schiff bases and their metal complexes have been pointed to as promising antibacterial agents (Sulekha and Seema, 2014).

Fungal infections are not usually limited to the superficial tissues; indeed, a significant increase in life threatening systemic fungal infections has been reported. The fundamental reason for this is the increasing number of patients at risk, including those with advanced age, major surgery immune suppressive therapy, acquired immune deficiency syndrome (AIDS), cancer treatment, and solid-organ system. The search and development of more effective antifungal agents are mandatory and some Schiff bases are known to be promising antifungal agents (Sharma and Chandra, 2013).

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multidrug resistant microbial pathogens. In spite of the availability of a large number of antibiotics for medical use, at the same time the emergence of old and new antibiotic resistance microbes created in the last decade's revealed substantial medical need for new classes of antimicrobial agents. Due to the outbreak of infectious diseases caused by pathogenic bacteria and the development of antibiotic resistance, researchers are searching for new antibacterial and antifungal agents. There is a real perceived need for the discovery of new compounds. Therefore new antimicrobial agents would be synthesized for the treatment of resistant bacterial and fungal diseases and to fill the research gap.

1.3. Objective of the Study

1.3.1. General Objective

The general objective of this research work was to synthesize and characterize Schiff base complexes of transition metals that possess bioactivity against selected bacteria and fungi strains.

1.3.2. Specific Objectives

The specific objectives of the present research are

1. To synthesize a Schiff base ligand derived from *p*-dimethyl amino benzaldehyde and ethylene diamine and its Co (II) and Cu (II) complexes by condensation reaction.
2. To characterize the synthesized ligand and its Co (II) and Cu (II) complexes with various analytical and spectroscopic techniques
3. To evaluate the antibacterial and antifungal activities of the Schiff base ligand and its metal complexes and compared the result with the standard.

1.4. Significance of the Study

Schiff base complexes have remained an important and popular area of research due to their simple synthesis, versatility and diverse range of applications. Schiff bases have a central role in coordination chemistry as they form stable complexes with most of transition metals (Mukul *et al*., 2013). Some chelating agents have been developed for metal intoxication (Abdallah *et al*., 2012). Some metals have been used as drugs and diagnostic agents to treat a variety of diseases (Yadav *et al*., 2011). The pharmacological activities of these metal compounds depend on the metal ion, its ligands and the structure of the compounds. Certain metal ions penetrate in to bacteria and in activate their enzymes, or some metal ions can generate hydrogen peroxide, thus killing bacteria (Dai *et al.*, 2011*).*

Biologically relevant metal complexes have several requirements in terms of their synthetic design. First, a biologically active metal complex should have a sufficiently high

thermodynamic stability to deliver the metal to the active site. The metal-ligand binding Should be hydrolytically stable. The kinetics with which the metal ion undergoes ligation or deligation reactions is of great importance. The molecular weight of the metal complex is also critical. The compounds of low molecular weight with neutral charge and some water solubility are soluble in almost any medium and may slip through biological membranes by passive diffusion. Many biologically active compounds used as drugs posses modified pharmacological and toxicological potentials when administered in the form of metal based compounds. Various metal ions potentially and commonly used are cobalt, copper and zinc because of forming low molecular weight complexes and therefore proven to be more beneficial against several diseases (Sari *et al*., 2013).

Generally drug combinations have proven to be an essential feature of antimicrobial treatment due to a number of important considerations (Mohammed *et al*., 2015). To mention some, they: (i) increase activity through the use of compounds with synergistic or additive activity;(ii) thwart drug resistance;(iii) decrease required doses reducing both cost and the chance of toxic side effects and (iv) increase the spectrum of activity.

Based on the above facts in mind, this study focuses on the synthesis of Schiff base derived from *p*-dimethyl amino benzaldehyde and ethylene diamine followed by complexation with Co (II) and Cu (II) ions. Then the antimicrobial activity of the Schiff base and its metal complexes would be tested with two gram positive bacteria's (*S.aureus* and *S.galatia*),two gram negative bacteria (*E.coli* and *S.typhi*) and two fungi (*A.niger* and *A. flavus*) . The chemicals needed to synthesize the Schiff base and its metal complexes were available in our country and relatively cheap. Moreover, the synthesis of the Schiff base and its complexes are very easy and straightforward. The significance of this study was to synthesize compounds which are biologically active against bacteria.

1.5. Scope of the Study

The synthesis, characterization and antimicrobial activity of the Schiff base ligand derived from *p*- dimethyl amino benzaldehyde and ethylene diamine and its Co (II) and Cu (II) complexes would be analyzed with two Gram- positive bacteria's (*S.aureus* and *S.galatia*),

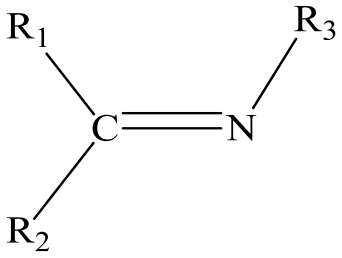
two Gram-negative bacteria *(S.typhi* and *E.coli*) and two fungi (*A.flavus* and *A.niger*). Finally the result would be compared with chloroamphinicol for bacteria and tilt for fungi as the standard reference.

2. LITERATURE REVIEW

2.1. Schiff Bases

Schiff bases are an important class of ligands in coordination chemistry due to not only their useful physical and chemical properties and large number of reactions they undergo but also because they have very wide use in industry and due to their interesting pharmacological as well as physiological activities (Misbahu *et al*., 2013). They are also important intermediate in the enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. It is possible to introduce different substituent in to the existing skeleton of the molecule, hence enabling the designing of the compounds with the suitable structural, electronic and biological properties (Panda and Chakravoty. 2005).

Schiff bases are a special class of ligands with a variety of donor atoms exhibiting interesting coordination modes towards various metals (Muhammad *et al*, 2015). They are generally bi-or tri-dentate ligands capable of forming very stable complexes with transition metals (Mukul *et al*., 2013). The field of Schiff base is fast developing, because of the wide variety and potential applications of their industrial, biological, analytical, medicinal, pharmaceutical and catalyst application (Belal *et al*., 2015). Therefore Schiff bases are essential ligands in modern coordination and medicinal chemistry. In organic synthesis, Schiff base reactions are useful in making carbon-nitrogen bonds (Sulek. *et al*., 2014).



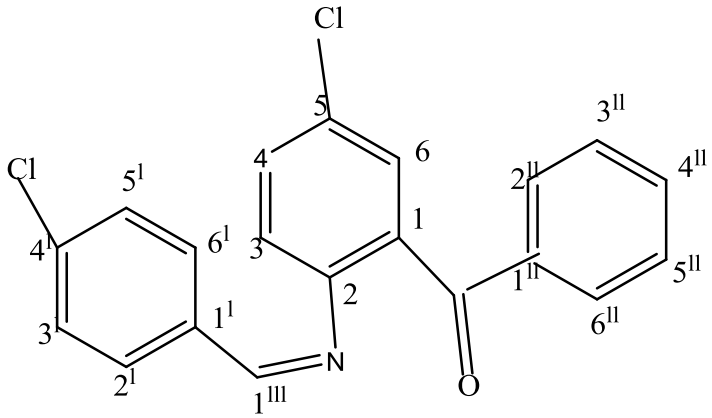
Where R_1, R_2 and R_3=alkyl or aryl

Figure2. General structure of a Schiff base

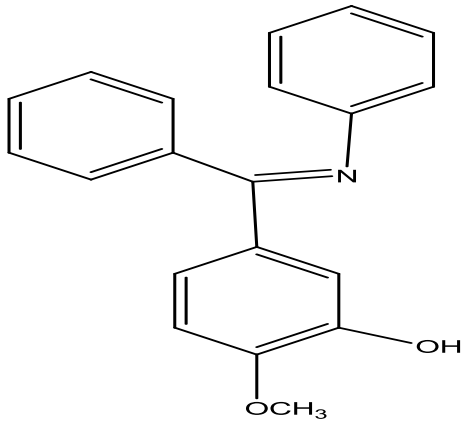
2.1.1. Schiff Base Ligands with Two or More Donor Atoms

Schiff base ligands regarded as ”privileged ligands” received a great attention because they are able to coordinate metals through imine nitrogen and another group usually linked to aldehyde or ketone (Kumar *et al*., 2013). Not only they have played a role in the development of coordination chemistry, but they can also found as key points in the development of inorganic biochemistry (Malik *et al*., 2011).These ligands containing donor atoms like N, O and S show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions (Sharma and Chandra, 2013).

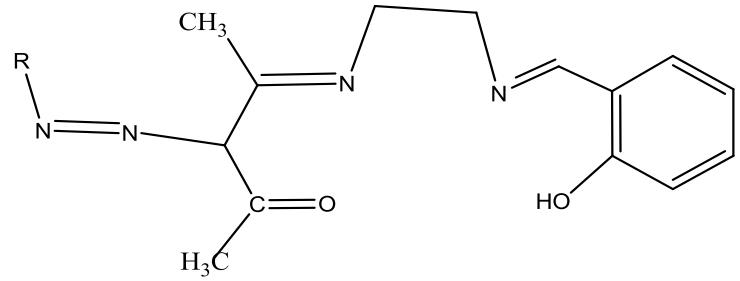
Schiff bases with both hard nitrogen and soft sulfur donor atoms in the ligand have high capability for coordinating with a wide range of metal ions to give stable and intensely colored metal complexes (Mukul *et al*., 2013). Metal Complexes with oxygen, nitrogen, and sulfur donor set have engendered much research because of their potential applications in fundamental and applied sciences due to their diverse roles in metallo–enzymes (Abdel-Nasser, 2014). The spectral studies of nitrogen-oxygen donor ligands suggest the bidentate nature of the ligand, which coordinate with all metal ions through imine nitrogen and phenolic oxygen. Four examples of polydentate ligands are shown in figure 3,



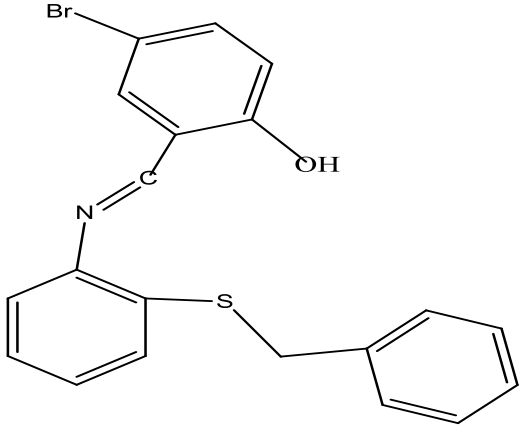
{5-Chloro-2-[(-4^{1}-chloro benzyledene)-imino] phenyl} phenyl) methanone Schiff base ligand



5-methoxy-2-(phenyl (phenyl imino) methyl) phenol Schiff base ligand



4-(1-Acetyl-2-{2-[(2-hydroxy-benzylidene)-amino]-ethyl amine}-propylazo)-N-pyrimidin-2-yl- Benzene sulfonamide [AHAPPS]



Bromo- N-[2-(benzyl thio)-phenyl] salicylaldimine,

Figure3: Structure of two or more donor Schiff base ligands

2.2. Schiff Base Metal Complexes

Schiff base metal complexes are synthesized from Schiff base ligand and metal salts (Subbaro et *al*., 2014). Schiff base complexes have remained an important and popular area of research due to their simple synthesis, versatility and diverse range of applications. Many potent antibacterial and antifungal compounds synthesized from Schiff base complexes (Joseph, 2013). It is known that the existence of metal ions bonded to biologically active com pounds may enhance their activities (Chandra and Agrawali, 2014). Schiff bases and their metal complexes have wide applications in food industry, analytical chemistry, dye industry, catalysis, agrochemical, and biological field (Fugu *et al*., 2013). Complexes of Schiff bases with some transition metals show significant biological notification including antibacterial, antifungal and anticancer activities (Belal *et al.,* 2015*).* Stable open and macrocyclic Schiff base ligands and their metal complexes have become the most well-known of all metal based drugs (Patil *et al*., 2013). In literature survey a number of research articles have been published on transition metal complexes of Cu (II), Co (II), Ni (II), Fe (III) and Zn (II) with Schiff bases derived from the condensation of salicyl aldehyde and o-amino phenol or 2-amino benzoic acid (Yiase *et al*., 2014). Azo-compounds have been studied widely because of their excellent thermal, antibacterial and optical recording medium.

2.3. Application of Metal Complexes

2.3.1. Antimicrobial Activity of Schiff Base Metal Complexes

Schiff base metal complexes are biologically more active than free ligands (Misbahu *et al*., 2013). The development of the field of bioinorganic chemistry has increased the interest in Schiff base since it has been recognized that many of these complexes may serve as models for biologically important species. Coordination compounds have been reported to act as enzyme inhibitor and are useful due to their pharmacological application.

More than thousands of articles have been published on the synthesis and biological activities of Schiff base metal complexes such as chromium, cobalt, iron, manganese, nickel and Copper (Ahmed *et al*., 2013). The azomethine (HC=N) linkage present in Schiff base ligand (s) and its metal (II) complexes show a wide range of biocidal activities such as antiviral (Fugu *et al.,* 2013), antitumor (Prakash and Adhikari, 2010), antioxidant (Kharadi, 2013),

DNAbinding and DNA cleavage (Dede*et al*., 2009), antibacterial (Karthikeyan *et al*., 2006),a ntifungal (Singh *et al*., 2006), anticancer (Tyagi*et al.*,2014*)* and anti diabetic (Subbaro *et al.*,2 014) activities

For decades, coordination chemistry of Schiff base ligands has been the subject of great interest. This interest comes from the fact that their metal complexes have found various applications in antimicrobial, antifungal and antitumor agents, catalysis and several other applications (Chohan *et al*., 1998).

Isatin is a versatile lead molecule for designing potential bioactive agents and its derivatives were reported to possess a broad spectrum of antiviral activities. Schiff bases of Isatin are known to possess a wide range of pharmacological properties that include antibacterial, antifungal and anti- HIV activities (Gupta *et al*., 2012).

The increased activity of metal chelates can be explained on the bases of chelation theory. The chelation tends to make ligand act as a more powerful and potential antibacterial agent, thus killing more of the bacteria than the ligand (Patel and Chaudhary, 2012). The antimicrobial screening concentration of the compounds was estimated from the minimum inhibitory concentration (MIC). MIC is the lowest inhibitory concentration of antimicrobial complex that will inhibit the growth of microorganisms (Misbahu *et al*., 2013). The chemical compounds broth medium at various concentration ranges from 20µg/ml to 200µg/ml.

Modern chemists still prepare Schiff bases and nowadays active and well-designed Schiff base ligands are considered "privileged ligands". In fact Schiff bases are able to stabilize many different metals in various oxidation states, controlling the performance of metals in a large variety of useful catalytic information (Cozz, 2004). Schiff bases and their metal complexes have wide applications in foodindustry, dye industry, analytical chemistry, Catalysis, agrochemical and biological field (Patil *et al*., 2013).

Recently in the treatment of cancer with a chemotherapeutic approach DNA is the target molecule. Cisplatin and its derivatives are widely used as anticancer drugs but they create several side effects such as anemia, diarrhea and neurotoxicity. As a result inorganic chemists are focusing their attention in the metal complexes from bioactive ligands (Subbaro *et al*., 2014). The introduction of chiral and achiral Schiff base metal complexes in proteins or

other biologically active molecules can be used to control different types of substrate activation. Molecular recognition and supramolecular interaction will almost be used to design the next generation of chiral Schiff base.

Metal Complexes play an essential role in agriculture, pharmaceutical and industrial chemistry. Medicinal application of metals can be traced way back almost 5000 years (Orving and Abrams, 2000). Metal centers being positively charged are favored to bind to negatively charged bio molecules: the constituents of proteins and nucleic acids offer excellent ligands for binding to metal ions. The pharmaceutical use of metal complexes has been investigated. Metal ions affect the well- being of humans in various ways (Sadler and LiH, 2001: Sakurai: *et al*., 2002). Several of these elements are indispensible for life and nature governs their uptake metabolism, and excretion. Consequently, their concentrations in a human body are well defined. When a metal ion combines with a ligand (drug), the resulting substance is said to be a complex. If the ligand (drug) which combines with the metal forms one or more rings with the metal the resulting structure is said to be chelates and the process is known as chelation (Wayne, 2009). Chelation therapy is a form of detoxication that means the chelator or chelating agent. E.g. EDTA which actively grabs the unwanted metal ions on two sides in a claw like fashion and either inactivates it or helps its removal from the body (Kramer *et al*., 2006).

Benzophenone derivatives of Schiff bases are very important compounds due to their various biological and physiochemical properties such as electrochemical, spectroscopic, metal complexation and adsorptive properties (Spinu, 2008) among others. The most important biological property of benzophenone derivatives is their ability to absorb abroad range of UV-radiation (200 to 350 nm). Due to this property the benzophenone derivatives were used as raw materials in the manufacture of sunscreen creams. These creams are helpful to avoid photo sensitization, photo toxicity or allergic reactions of patients with various medicinal treatments

A bidentate NO (nitrogen-oxygen) donor type Schiff base (HL) derived from 2-hydroxy-4-methoxy phenyl) phenyl methanone with aniline and its metal (II): M=Mn, Ni, Cu and Zn) has been synthesized. Antimicrobial activity of the ligand (HL) and its metal (II) complexes were screened against few bacteria (E.coli and S.aureus) and fungal species (*A. niger* and

Candida albican) by a well diffusion method using agar as a nutrient. All the complexes show considerable significant activity at higher concentration (50µg) against microorganisms with the help of overtones concept and the Tweedy chelation theory. Cu (II) complex showed a remarkable antimicrobial activity than the other complexes and free ligand. The structure of the complex was shown in figure 4 (Subbaro, 2014).

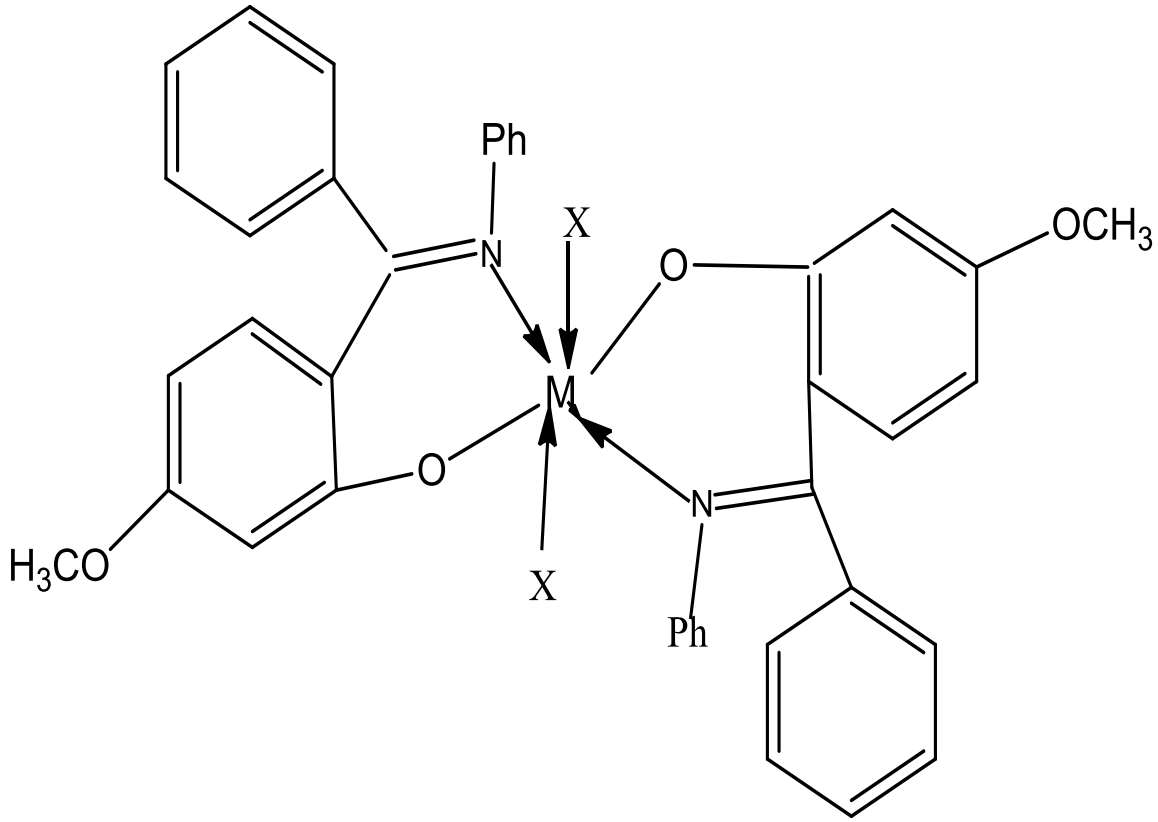


Figure4: General outlines of Schiff base metal (II) complexes

Where x=H_2O and M=Mn (II), Co (II) and Ni (II) complexes

2.3.2. Catalystic Activity of Schiff Base Metal Complexes

In the last decades Schiff base ligands have received more attention mainly because of their wide application in the field of catalysis and due to their antimicrobial, antituberculosis and antitumor activity. They easily form stable complexes with most transition metals. Many Schiff base complexes of metal ions show high catalystic activity. The development of the

field of bio inorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species. Coordination compounds have been reported to act as enzyme inhibitor and are useful due to their pharmacological application (Patel *et al*., 2000).

Metal-Salen complexes are the one used in catalysis. The more general term Salen-type is used in the literature to describe the class of [O, N, N, O] tetra dentate bis-Schiff bases. They are useful catalysts for opening epoxides for various oxidation reactions, to bind oxygen, to act as polymerization catalysts and to stabilize reactive cationic nickel complexes (Cozz, 2004).

The epoxidation of alkenes is one of the most widely studied reactions in organic chemistry since epoxides are key starting materials for a wide variety of products. The catalytic epoxidation of olefins by transition metal complexes is an area of intense research activity. Recently a large number of publications described the synthesis, structural study and catalytic activity of oxovanadium Schiff base complexes in oxidation of various organic substrates (Gholamhossein *et al*., 2012). Essentially in all these complexes the metal center is surrounded by N- O coordination environment. Sulfur-ligated transition metal complexes may mimic the ligation of certain bimolecules in proteins. Chelating nitrogen-sulfur ligands are potential models for synthesizing copper complexes as most copper metallo-enzymes contain the N, S donor set (Mukul *et al*., 2013).

Aromatic Schiff bases or their metal complexes catalyze reactions on oxygenation, hydrolysis, and electro reduction, and decomposition. Four coordinated Co (II) Schiff base chelate complexes show catalystic activity in oxygenation of alkenes. Metallo-porphynes oxidize phenols (naphthol). Some copper complexes, derived with amino acids, enhance (10-15 times) hydrolysis rate than simple complex exhibits Catalystic activity towards electro reduction of oxygen. Some metal complexes of a polymer bound Schiff base show catalystic activity on decomposition of hydrogen peroxide and oxidation of ascorbic acid. Cyanohydrins cobalt complexes exhibit Catalystic activity (Deshpande and Shah, 2003). Chiral Schiff base complexes are more selective in various reactions such as oxidation, hydroxylation, condensation and epoxidation. The oxidation of organic compounds is an important and widely used reaction in laboratory scale organic synthesis as well as in large

scale industries. There are hundreds of different reagents and methods available for the oxidation of organic compounds. The selections of solvent, oxidant, reaction conditions such as temperature, pressure and number of reaction steps are crucial in affecting the speed of reaction and the type and quantity of side-product produced.

The Schiff base transition metal complexes are a family of attractive oxidation catalysts for a variety of organic substrates because of their cheap and easy synthesis and their chemical and thermal stability. Important oxidation reactions include the transformation of alcohols to either the corresponding carbonyl compounds or carboxylic acids, the oxidation of sulfides to sulfoxide, alkenes to epoxides and diols, and he activation of hydrocarbons.

3. MATERIALS AND METHODS

3.1. Experimental Site

The synthesis of the Schiff base ligand derived from *p*-dimethyl amino benzaldehyde with ethylene diamine and the metal complexes was carried out at Haramaya University Chemistry Department Laboratory. Part of the characterization (UV, AAS, molar conductivity) and the antimicrobial activity of the Schiff base and its metal complexes were carried out at Haramaya University. The determination of elemental analysis, NMR, IR and magnetic susceptibility were done at Addis Ababa University, Department of Chemistry.

3.2. Materials and Apparatus

3.2.1. Chemicals and Reagents

Cobalt (II) chloride hydrate, copper (II) chloride hydrate, *p*-dimethyl amino benzaldehyde, ethylene diamine, ethanol, Sulfuric acid, Gram positive bacteria (*S.aureus* and *S. galatia*), Gram negative bacteria (*E. coli* and *S.typhi*), fungi (*A. flavus* and *A.niger*), chloroamphinicol, tilt, distilled water, deionized water, TMS, anhydrous calcium chloride , DMSO, n-hexane, ethyl acetate, $CDCl_3$, KBr, $AgNO_3$ and conc. HNO_3.

3.2.2. Instruments and Apparatus

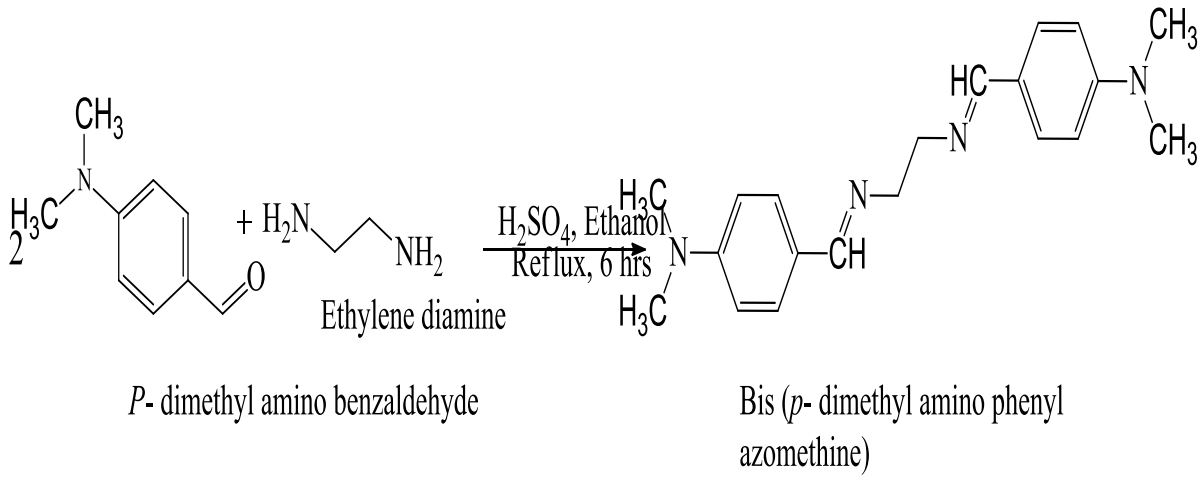
Méttler Toledo PB 602 (mass measuring apparatus) for measuring the mass of the reagents needed to synthesize the Schiff base and its metal complexes, electro thermal mantle for refluxing the samples, suction filtration apparatus for filtration of Schiff base and its metal complexes, thinlayer chromatography (TLC) plate for testing the purity of the ligand and its complexes, UV lamp (Chromato UVE) for visualizing thin layer chromatography, oven for drying the Schiff base, desicator for drying the metal complexes, electro thermal melting point apparatus for determining the melting point of the Schiff base and its metal complexes, Bruker AMX spectrometer (NMR) for characterizing the Schiff base. Fourier transform infrared spectrophotometer (FTIR), elemental (EA1112 Flash CHNS analyzer) and UV -Visible spectrophotometer for characterizing the Schiff base and its metal (II) complexes. Conductivity meter (Jenway 4310), atomic absorption spectrometer (AAS) and magnetic susceptibility apparatus for characterizing Co (II) and Cu (II) complexes.

3.3. Experimental Methods and Procedures

The ligand and its Co (II) and Cu (II) complexes were synthesized based on the established procedure with slight modification (Muhammad *et al*., 2015).

3.3.1. Preparation of the Ligand (L)

Ethanolic solution of ethylene diamine (0.01mol in 50ml ethanol) was mixed with ethanolic solution of *p*-dimethyl amino benzaldehyde (0.02mol in 50ml ethanol) (figure 5). To this mixture three drops of sulfuric acid was added. Then the mixture was stirred and refluxed in an electro thermal mantle for about 6h.The resulting solution was cooled and a colored crystal was obtained by keeping the reaction mixture overnight at ambient temperature (25^0C). The resulting colored product was separated by suction filtration and washed with cold ethanol. Recrystallization was carried out with ethanol for further purification. Finally the resulting colored product was dried in an oven.



P- dimethyl amino benzaldehyde

Bis (*p*- dimethyl amino phenyl azomethine)

Figure 5: General scheme for the synthesis of the ligand

3.3.2. Preparation of Cobalt (II) and Copper (II) Complexes

Ethanolic solution of the respective salts [hydrated cobalt (II) chloride and hydrated Copper (II) chloride] were mixed to hot stirred ethanolic solution of the ligand L in 2:1 (L: M) mol ratio. The reaction mixture was stirred at reflux on an electro thermal mantle for 2h. Excess solvent was evaporated to concentrate it to one third of its initial volume. The resulting solution was cooled. A colored precipitate was obtained by keeping reaction mixture over night at ambient temperature (25^0C).The resulting precipitate was filtered by suction filtration, and dried over anhydrous calcium chloride in a desiccator.

3.3.3. Test for the Purity of the Synthesized Schiff Base and its Complexes.

3.3.3.1. Melting point determination

The purity of the Schiff base ligand and its Co (II) and Cu (II) complexes were tested by measuring the melting point of the samples using a capillary in a melting point measuring apparatus.

3.3.3.2. Thin layer chromatography (TLC) test

The purity of the Schiff base ligand and its Co (II) and Cu (II) complexes were also tested by using thin layer chromatography (TLC) on silica gel thin layers using two component solvent systems: ethyl acetate and n-hexane. Small amounts of the samples (Schiff base and its complexes) were dissolved in ethanol. Ethyl acetate and *n*-hexane were mixed in 4.5 to 0.5 ratios to use as mobile phase. The samples dissolved in ethanol were added on TLC plate using a capillary by making a point for the samples (Schiff base and its complexes) on TLC by using a pencil. Then the TLC was immersed in a solution containing ethyl acetate and n-hexane in a flask. After a few minutes the TLC was taken out from the flask by the forceps, allowed to be dried in air and placed in a UV lamp to visualize the spots on TLC.

3.3.4. Chloride Ion Test in the Complexes

The qualitative and quantitative determination of ionizable and coordinated chloride (s) of the synthesized complexes was carried out by the following general methods. The qualitative determination of ionizable chloride was carried out by the addition of $AgNO_3$ solution in a

solution of each sample (Co (II) and Cu (II) complexes). Quantitative determination was carried out by drying the precipitated sample in an oven and measuring its mass.

For the quantitative determination of coordinated chloride the samples were dissolved in concentrated nitric acid and digested until a clear solution was obtained. To the digested solution 0.1N of $AgNO_3$ was added, the contents were further digested for 1h and allowed to stand for overnight. Then the resulting precipitate was filtered through sintered crucible which was initially cleaned, dried and weighed. The crucible was then dried at 110^0C in an oven to a constant weight. The amount of chloride in percentage was determined from the weight difference of the crucible.

3.4. Physicochemical Characterization of the Ligand and its Complexes

The balance was used for weighing mass of the reagents and mass of the products to determine the yield. The melting point of the Schiff base and its metal complexes were recorded with electro thermal melting point apparatus to determine their decomposition temperature in ^{0}C. The color of the Schiff base ligand and its Co (II) and Cu (II) complexes were determined by comparing their colors with the standard references.

3.4.1. Elemental Analysis of the Ligand and its Metal Complexes

The Schiff base ligand and its Co (II) and Cu (II) complexes were analyzed micro analytically for carbon, hydrogen, and nitrogen contents to check their composition. Elemental analysis was carried out on elemental (CHN) analyzer to compare the calculated mass with the experimental mass of the elements in the Schiff base and its metal complexes. The experiment was carried out in four replications (4 calibration points) for every component and average of the 4 replications per component (sample) was taken for the analysis (interpretation) of the result.

3.4.2. Metal Determination by Atomic Absorption Spectroscopy

Metal contents of the Co (II) and Cu (II) complexes were determined using flame atomic absorption spectroscopy (FAAS). 30 mg of each of the Co (II) and Cu (II) complexes was placed separately in a clean and dry beaker and 10ml of 69% conc. HNO_3 was added to each and the content heated gently in a hood until a few drops remained in each beaker. Then 5 ml

of 69% conc. HNO_3 was added to each beaker and heated slowly until a few drops remained by repeating three times and finally diluted with deionized water to 100 ml using volumetric flask. The above solutions were subjected to FAAS Studies after appropriate dilutions. Based on the absorbance data, the concentration of Co (II) and Cu (II) were calculated using the following formula shown below.

M (II) % = Concentration (PPm) ×volume diluted to/mass of sample taken x1000/100

The calibration curves of each metal ion was prepared by plotting absorbance of a function of their standard concentrations and used to calculate the amount of each metal ions present in each complex.

3.4.3. Infrared Spectra of the Ligand and its Metal Complexes

In order to determine the binding mode of the ligand to the metal in the complexes, the IR Spectrum of the free ligand was compared with the spectra of the metal complexes. The IR spectra of the ligand and its complexes were recorded over 4000-400cm^{-1} by FTIR spectrophotometer in KBr pellet that gave information regarding the coordinating sites of the ligands in the metal complexes.

3.4.4. Conductometric Measurements of the Complexes

In this research work Co (II) and Cu (II) Complexes were dissolved in DMSO and molar conductance measurements ($\wedge_m$) of $1x10^{-3}$M of their solutions at room temperature were measured by conductivity meter to determine the electrolytic nature of the metal complexes. 0.01M KCl was used to calibrate the instrument.

3.4.5. UV-Visible Absorption Spectra of ligand and its Co (II) and Cu (II) Complexes

UV-Visible absorption Spectra of the Schiff base ligand and its metal (II) complexes in 200-800nm regions were recorded to identify and determine the energies of n-π,* π-π,* d-d electronic transitions and electronic charge transfers. The UV-Visible spectra of the ligand and the complexes were recorded in DMSO at room temperature.

3.4.6. Nuclear Magnetic Resonance Spectra of the Ligand

3.4.6.1. ^{1}H and ^{13}C nuclear magnetic resonance spectra of the ligand

Both ^{1}H and ^{13}C NMR give information about the number of chemically non equivalent nuclei (non equivalent hydrogen's or non equivalent carbons). To understand the structure of the ligand, NMR spectra have been employed. ^{1}H and ^{13}C NMR spectra of the ligand were recorded by Bruker AMX-400 spectrometer in $CDCl_3$ to elucidate the chemical structures of the synthesized ligand. The chemical shifts were analyzed relative to tetra methyl silane (TMS) as an internal standard which has the chemical formula Si $(CH_3)_4$.The TMS peak is a single peak located at (δ= 0.0 ppm). CDCl3 was used as the solvent for the synthesized ligand to determine its structure.

3.4.6.2. Distortionless enhancement by polarization transfer

DEPT is an acronym for Distortionless enhancement by polarization transfer. This experiment allows for determining multiplicity of carbon atom substitution with hydrogen's .Signs of this experiment will reveal the substitution of carbon atoms:

In DEPT-135 signals from CH_2 will be negative, while CH and CH_3-positive.

In this research work DEPT-135 was carried out in $CDCl_3$ solvent by using Bruker AMX-400 spectrometer to distinguish CH_3 and CH positive from CH_2 negative.

3.4.7. Magnetic Susceptibility of the Complexes

Magnetic susceptibility is the quantitative measure of the extent to which a material may be magnetized in relation to a given applied magnetic field. It is denoted by the Greek letter Chi (χ). Particularly for the first row transition elements, give information about the number of unpaired electrons. The number of unpaired electrons provides information about the oxidation state and electron configuration.

$\chi_m = \chi_g$ (F.W. in g/mol)

$\mu_{eff} = 2.84\ (\chi_A T)^{1/2}$ B.M.

Where μ_{eff}= the effective magnetic moment

χ_A= the susceptibility per mol of the paramagnetic ion. The unit is in B.M (Bohr magnetron), which is a unit of magnetic moment.

X_m=molar susceptibility

χ_g=the susceptibility per gram of the paramagnetic ion

Diamagnetic corrections to the molar susceptibility are made to account for the inner core electrons, ligands, atoms and ions in the compound or material, which make the apparent susceptibility it really is from the contribution from the unpaired electrons.

$\chi_A=\chi_m$+ sum of all diamagnetic corrections.

The magnetic moments of Co (II) and Cu (II) complexes were recorded by magnetic balance to determine the magnetic nature and the geometry of the complexes.

3.5. Antimicrobial Studies

The main aim of the synthesis of any antimicrobial compound is to inhibit the causal microbe without any side effects on the patients. The biological activities of the parent Schiff base ligand and its metal (II) complexes in DMSO medium were screened against two gram positive bacterial strains (*S.aureus* and *S.galatia*), two gram negative bacterial strains *(E.coli* and *S.typhi*) and two fungal strains (*A.flavus* and *A.niger*) using Muller's test as the medium by a well diffusion method. All the experiments were made in three replicates for each compound. The obtained zone of inhibition (in mm) of the Schiff base and its metal (II) complexes were compared with chloroamphinicol for bacteria and tilt for fungi as the standard. The effectiveness of the compounds was determined by measuring the diameter of the inhibition zones (Yadav *et al*., 2011).

3.5.1. Preparation of Medium

Each bacterial and fungal stain was transferred from the culture and then streaked on Muller Hinton Agar (MHA) Plate and incubated for 24 h for bacterial strain and 36 h for fungal strain at 37℃ in an oven. Then the bacteria and fungi were transferred to autoclaved MHA that would be maintained at 45℃ in a water bath and would be mixed by vigorous swirling of

the flasks. Then the medium would be poured to sterilized petridishes, solidified and used for bio test.

3.5.2. Preparation of Sample Solutions

Solutions of ligand and complexes were prepared by dissolving their 10 mg samples in 2 ml dimethyl sulfoxide (DMSO which has no inhibitory activity) to get concentration of 5mg/ml and 20 mg/ml aliquots were used for bio test.

3.5. 3.Procedure for Antibacterial and Antifungal Activity

Whatmann No 1 filter paper was punctured with office puncture to get 6mm diameter paper disc. Then 20 mg/ml of solution of compounds were released over paper discs in three replications. The paper discs impregnated with the samples were transferred with sterile forceps to nutrient agar plate seed with microbes and incubated at 37^0C for 24 h for bacterial strains and at 37^0C for 36 h for fungal strains. Then the antimicrobial activity of the Schiff base ligand and its complexes were determined by measuring the diameter of the inhibition zones between the paper disc and the nutrient agar that contained microbial strains. Finally the effectiveness for the antibacterial and antifungal activities of the Schiff base ligand and its metal complexes were compared with chloroamphinicol for bacterial strains and tilt for fungal strains as positive control and DMSO was taken as negative control standard references. The inhibition zone of the Schiff base ligand, its Co (II) and Cu (II) complexes and the standards were measured and recorded in three replications. Finally the average of the triplicate was taken for each sample and the standards to evaluate the effectiveness of the samples.

4. RESULTS AND DISCUSSION

4.1. Characterization of the Ligand and its Metal (II) Complexes

The synthesized ligand was obtained as a pale yellow crystalline solid where as its Co (II) and Cu (II) complexes were obtained as orange and grey crystalline solids, respectively. The melting point of the ligand and its Co (II) and Cu (II) complexes were found to be (160 ± 1) ^{0}C, (215 ± 1) ^{0}C and (22 5± 1) ^{0}C, respectively (Table 1). The decomposition temperature of the samples indicated that the complexes were more stable than the Schiff base ligand at room temperature. The formation of single spot on TLC test for the synthesized ligand and its Co (II) and Cu (II) complexes indicated their purity. Both the synthesized ligand and its complexes were soluble in DMSO and slightly soluble in ethanol and water. The qualitative test for chloride in both complexes indicated the presence of chloride outside the coordination sphere and absence of coordinated chlorides. From the quantitative analysis the presence of two moles of chloride outside the coordination sphere was confirmed.

4.1.1. Elemental Analysis of the Ligand and its Metal (II) Complexes

Physical properties and elemental analysis data of the Schiff base ligand and its metal complexes are listed in Table1. The proposed molecular formulae of the synthesized compounds are consistent with their elemental analyses data of C, H, N and metal contents. For both Co (II) and Cu (II) complexes the metal ligand ratios of 1:2 were established. The data obtained was in a good agreement with the calculated values and showed the formation of 1:2 [metal: ligand] ratio.

Table1: Physical properties and elemental analyses data of the ligand and its metal complexes

Compounds	Molecular weight	Color	Elemental analysis Calculated (found)% C	H	N	Percent yield (%)	M (II) %	mp (^{0}C)
$C_{20}H_{26}N_4$	322	Pale yellow	73.53 (73.80)	7.72 (7.63)	18.39 (18.32)	85%	_	160 ± 1
$[CoL_2(H_2O)_2]Cl_2$	809.93	Orange	59.26 (59.33)	6.42 (6.38)	18.55 (18.23)	75%	7.27 (7.16)	215 ± 1
$[CuL_2(H_2O)_2]Cl_2$	814.5	Grey	58.93 (58.77)	6.3 (6.17)	13.75 (13.53)	71%	7.79 (7.66)	225 ± 1

Where L=Bis (*p*-dimethyl amino phenyl azomethine)

4.1.2. Molar Conductance of Co (II) and Cu (II) Complexes

Co (II) and Cu (II) complexes were dissolved in DMSO and molar conductance measurements of 1×10^{-3} M at room temperature were measured. Literature review showed that the limiting molar conductance values of complexes 40 Scm^2mol^{-1} and above, suggesting the electrolytic nature of the complexes (Prakash and Adhikari, 2010). The prepared Co (II) and Cu (II) complexes with molar conductance values of 171.8 Scm^2mol^{-1} and 151.7 Scm^2mol^{-1}, respectively indicated the electrolytic nature of the complexes. The cation to anion ratio was found to be 1:2.

4.1.3. Infrared Spectral Analysis of the Ligand and its Metal (II) Complexes

4.1.3.1. Infrared specteral analysis of the ligand

There were no bands for the carbonyl group υ (C=O) of *p*-dimethyl amino benzaldehyde and the amino group υ (NH_2) of the free ligand ethylene diamine in the synthesized Schiff base; bis (*p*-dimethyl amino phenylazomethine) L. This suggests that complete condensation takes place between the carbonyl group of benzaldehyde and the amino group of ethylene diamine in the Schiff base formation (Shakir *et al*., 2016).

In the IR spectrum of the free ligand L, a sharp intensity band at 1609cm^{-1} was assigned to the azomethine nitrogen (C=N) stretching frequency (Mohammed *et al*., 2015). The bands appearing at 807 cm^{-1}, 1363 cm^{-1} and 2852 cm^{-1}might be due to υ (C-H) out of plane bending of the aromatic ring, υ (C-H) bending and υ (C-H) stretching of the methyl groups, respectively. The bands appeared at 1038 cm^{-1} and 1163 cm^{-1} might be assigned to υ (C=C) aromatic stretching vibrations of the ring. IR spectrum of the ligand is shown in figure 6.

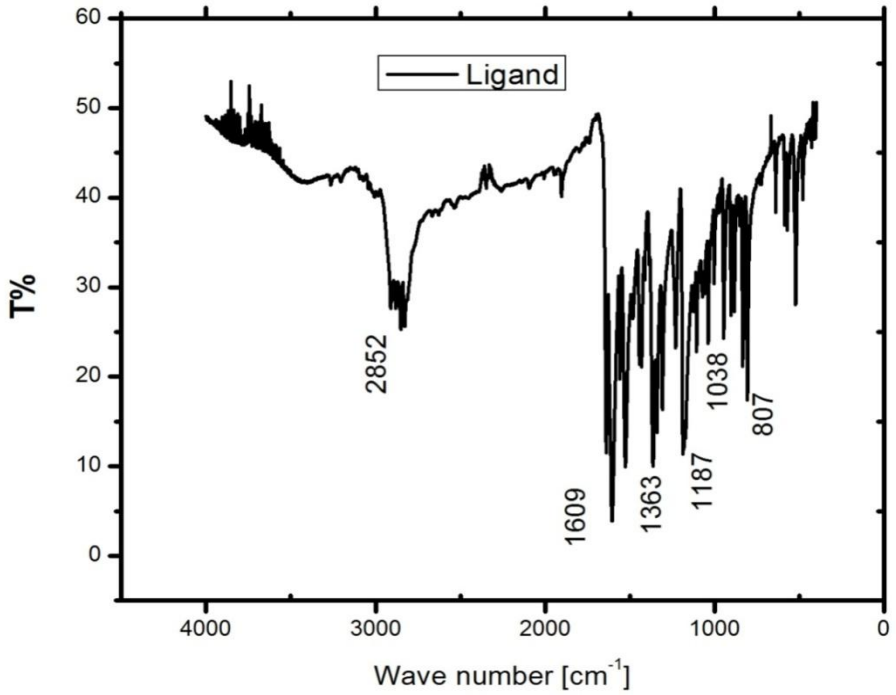


Figure 6: IR spectrum of the ligand

4.1.3.2. Infrared spectral analysis of Co (II) and Cu (II) complexes of the ligand L

The IR spectrum of the free ligand was compared with the spectra of the metal complexes in order to determine the coordination sites that could be involved in complex formation (Table 2). The band formed at1609 cm^{-1} in the free ligand L was shifted to lower frequency 1578 cm^{-1} for Co (II) and1582 cm^{-1} for Cu (II) complexes, suggesting the coordination of nitrogen of the azomethine group of the ligand with metal ions (Pal and Pal, 2015). Assignments in the far IR region are purely tentative because of various skeletal vibrations associated with metal-ligand vibrations. The appearance of new bands at595 cm^{-1} and 592 cm^{-1} might be assigned to υ (M-N) of Cu (II) and Co (II) complexes, respectively. The presence of coordinated water molecule in the complexes was confirmed by the broad band appeared at 3380 cm^{-1} for Co (II) and at 3304 cm^{-1} for Cu (II) complexes, respectively due to υ (O-H) water molecule. The appearance of new bands at516 cm^{-1} and 524 cm^{-1} might be assigned to υ (M-O) of Cu (II) and Co (II) complexes, respectively. From the IR spectral data, it is concluded that the ligand behaves as a neural bidentate ligand coordinated to M (II) ions, through two nitrogen of the azomethine group of the ligand to give five membered chelating complexes, which is the most stable ring size. The IR spectra of Co (II) and Cu (II) complexes are shown in figure 7 and 8, respectively.

Table 2.Characteristic IR spectral data of ligand Land its Co (II) and Cu (II) Complexes in cm^{-1}

Compound	υ (C=N)	υ (M-N)	Aromatic υ (C=C)	υ (O-H)	M-O
L	1609	-	1187, 1038	-	
$[CoL_2(H_2O)_2]Cl_2$	1578	592	1168,92 1058	3380	**524**
$[CuL_2(H_2O)_2]Cl_2$	1582	595	1169, 1045	3304	**516**

Where L= Bis (p- dimethyl amino phenyl azomethine)

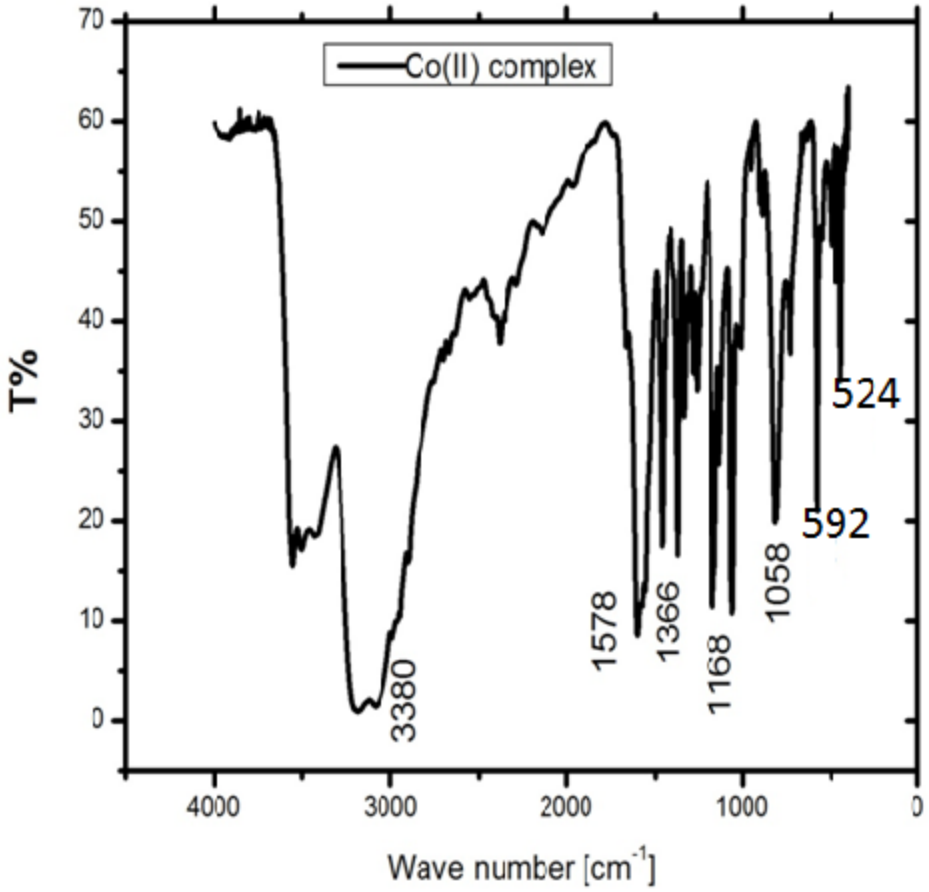


Figure7: IR spectrum of Co (II) complex

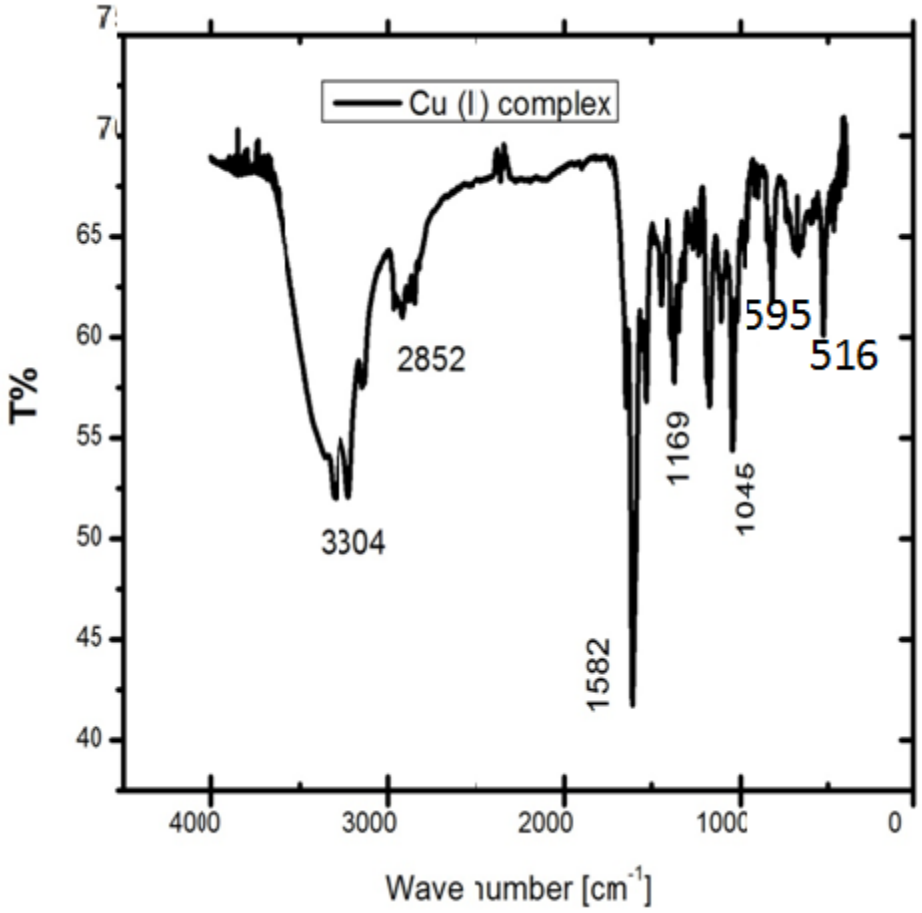


Figure 8: IR spectrum of Cu (II) complex

4.1.4. Nuclear Magnetic Resonance Spectral Analysis of the Ligand

4.1.4.1. ^{1}H and ^{13}C NMR spectral analysis of the ligand

The result of the ^{1}H and ^{13}C NMR for the ligand supported the interferences made based on the IR regarding the structure of the ligand. The NMR assignments are listed in Table 3. The signal at δ 8.2 ppm singlet in $^{1}HNMR$ spectrum indicated the presence of azomethine proton in the synthesized ligand. The signals assigned in the range δ 6.65-7.60 ppm were due to the aromatic protons of the benzene ring of the ligand. The signals at δ 3.9 ppm and δ 3.0 ppm were due to CH_2 and CH_3 protons of the ligand, respectively.

The ^{13}C NMR spectrum displayed signals at δ 40 ppm, δ 62 ppm, δ77ppm, δ (112-152) ppm and δ 162 ppm due to carbon of CH_3, aliphatic CH_2, $CDCl_3$, the C=C (aromatic ring),.and

azomethine groups (HC=N), respectively. The spectra of the ^{1}HNMR and ^{13}CNMRof the ligand is shown in figure 9 and 10, respectively.

4.1.4.2. Distortionless enhancement by polarization transfer (DEPT) NMR analysis spectrum

The DEPT-135 NMR experiment result of the chemical shift relative to TMS as an internal standard in $CDCl_3$ solvent showed four positive signals for CH_3 and CH and one negative signal for CH_2 as expected. The signals appeared at δ 40.17 ppm, δ 61.94 ppm, and δ 161.94 ppm were due to the (C-H) of CH_3, CH_2 and azomethine (HC=N) groups (Table 3) respectively. The signals assigned to δ 111.59 ppm and δ 129.57 ppm was due to Ar-H. DEPT-135 spectrum is given in Figure 11.

Table 3: NMR spectra analysis data for the ligand

Compound	^{1}H NMR			^{13}C NMR		DEPT-135	
	(δ, ppm)	Assignments	Multiplicity & no of protons	(δ, ppm)	Assignments	δ, ppm	Assignments
L	3.9	Aliphatic CH_2	t (4H)	62	Aliphatic CH_2	62	CH_2
	(6.65-7.60)	Aromatic H(C=C)	m (8H)	(112,125, 130,152)	Benzene ring (C=C)	112 &130	CH
	8.25	Azomethine H(C=N)	s(2H)	162	Azomethine (C=N)	162	CH
	3.0	Aliphatic CH_3	s(12H)	40	Aliphatic CH_3	40	CH_3

Where L=Bis (*p*-dimethyl amino phenyl azomethine)

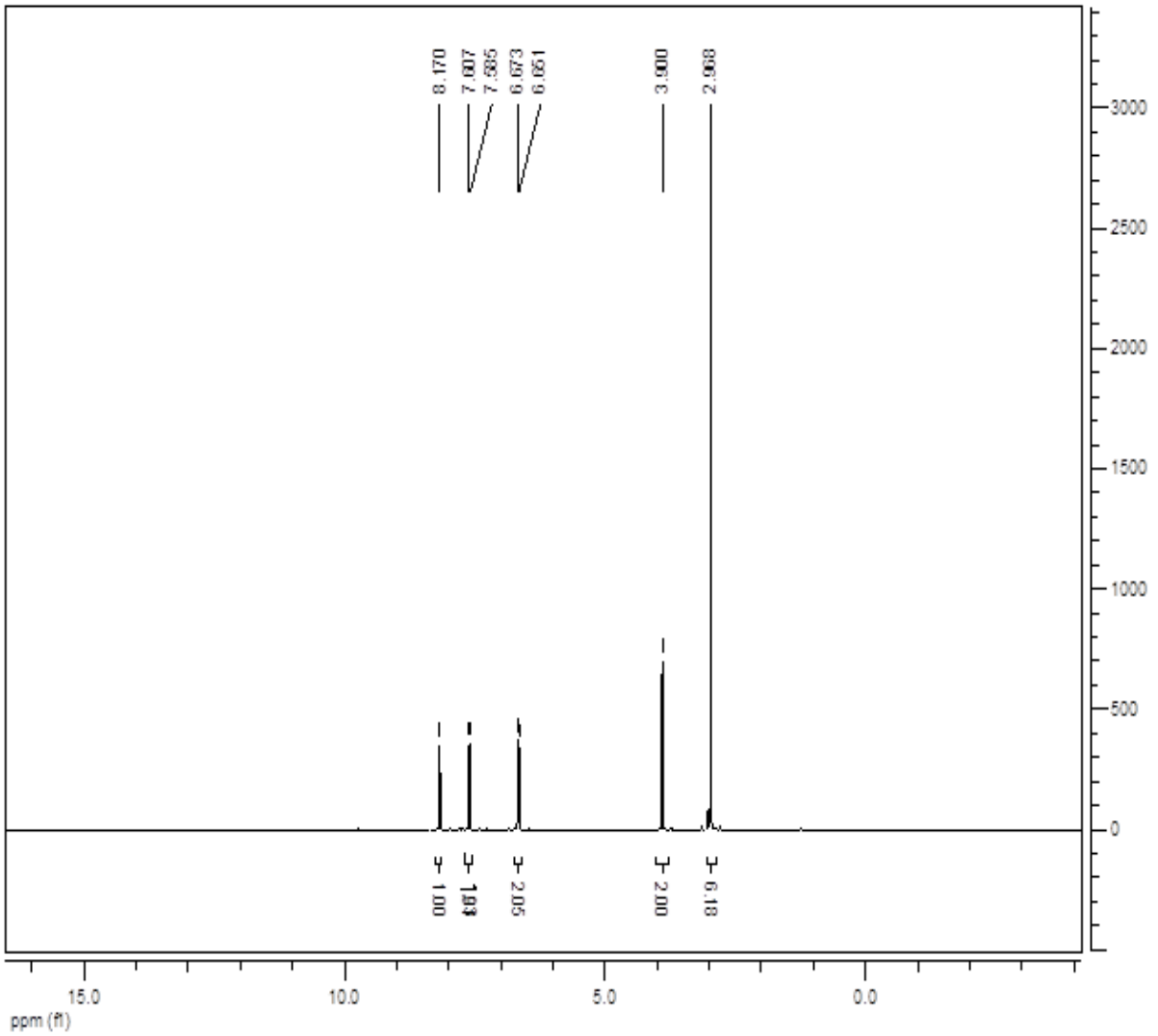


Figure 9: ^{1}HNMR spectrum of the ligand

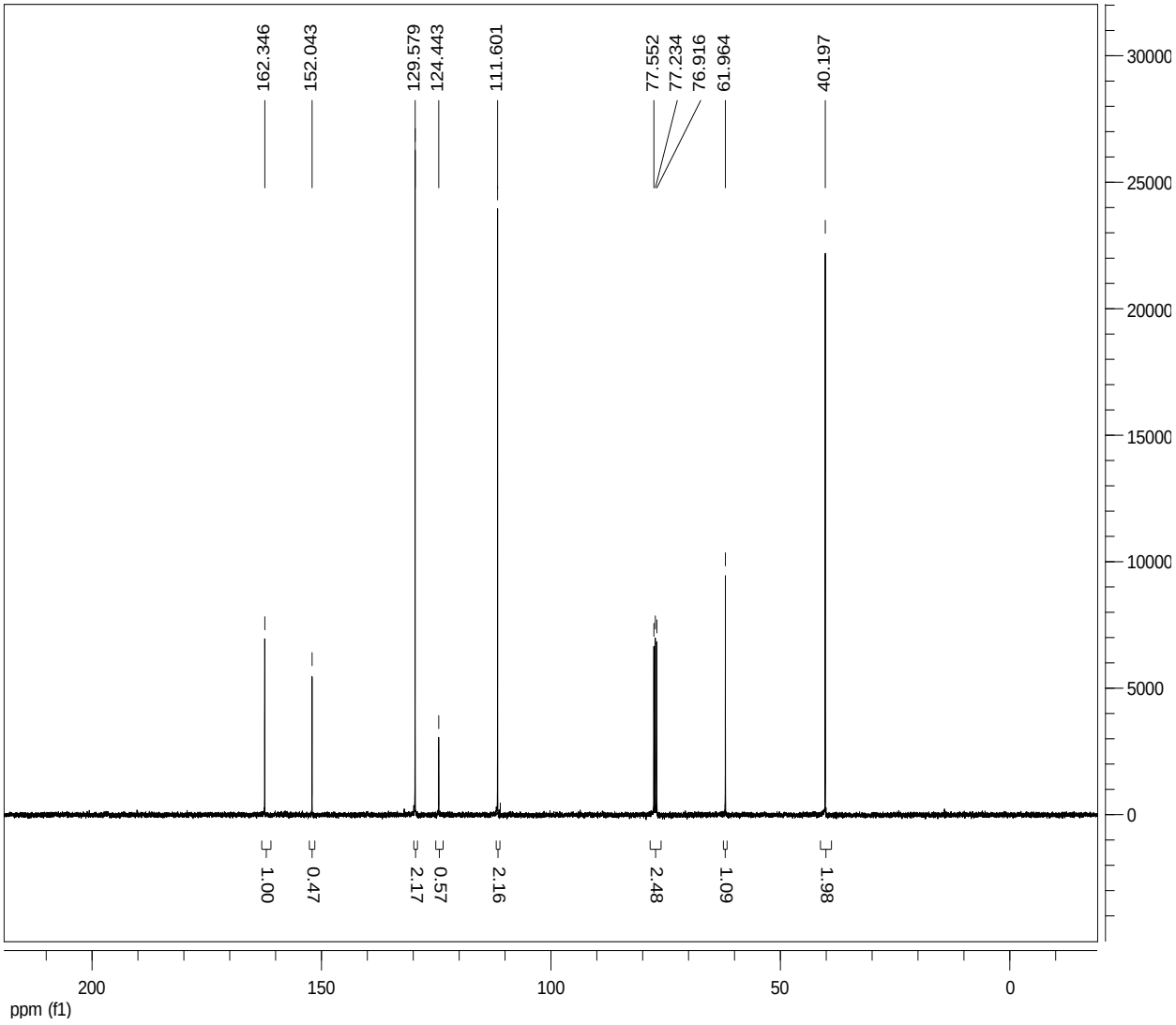


Figure 10: ^{13}C spectrum of the ligand

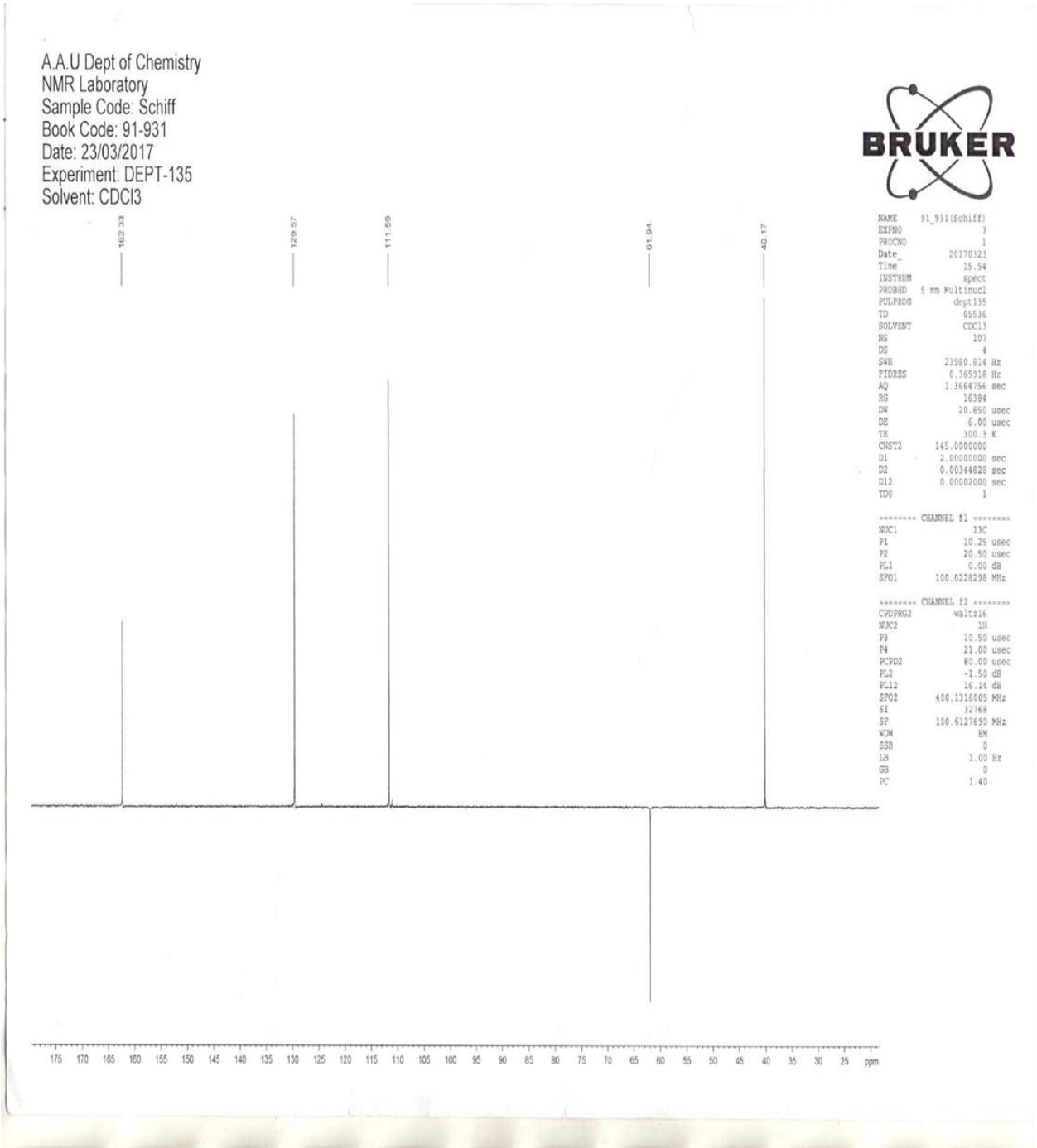


Figure 11: DEPT-135 spectrum of the ligand

4.1.5. Atomic Absorption Spectra of Co (II) and Cu (II) Complexes

The flame atomic absorption spectroscopy (FAAS) measurements of the synthesized complexes were conducted to determine the metal contents and metal to ligand ratio in Co (II) and Cu (II) complexes. The results obtained are listed in Table 4.The metal contents determined from AAS for Co (II) and Cu (II) complexes were consistent with the calculated value. The metal to ligand (M: L) ratio was found to be 1:2. The calibration curves of Co (II) and Cu (II) are shown on Appendix figure1 and 2 respectively.

Table 4.Atomic absorption analysis data for the metal-ligand complexes

Complexes	**Mol.Wt**	**M (II) ppm**	**A**	**M (II)% calculated**	**M (II) (%) found**	**M:L ratio**
$[CoL_2(H_2O)_2]Cl_2$	809.93	21.5	0.456	7.27	7.16	1:2
$[CuL_2(H_2O)_2]Cl_2$	814.5	23	2.26	7.79	7.66	1:2

Where L= Bis *(p*-dimethyl amino phenyl azomethine)

4.1.6. Electronic Absorption Spectra of the Ligand, L and its Co (II) and Cu (II) Complexes

The UV-Visible absorption spectrum of the synthesized ligand was characterized by one absorption band at 355 nm (28169 cm^{-1}) which might be assigned to n-π*and π-π*transitions associated with the azomethine chromophores (-HC=N) (Abdallah *et al*., 2012). The electronic spectrum of the ligand is shown in figure 12. In Co (II) and Cu (II) complexes, these bands shifted to the lower frequency region 388 nm (25;773 cm^{-1}) and 387 nm (25,839 cm^{-1}), respectively. This may be due to the coordination of the lone pair of electrons in azomethine nitrogen with the metal ions. The electronic absorption spectral data of the ligand, Co (II) and Cu complexes are shown in table 5.

Co (II) complex showed three peaks at 326 nm (30674 cm^{-1}), 388 nm (25773 cm^{-1}) and 472 nm (21186 cm^{-1}). The band at 326 nm (30675 cm^{-1}) might be due to the decrease in the Pi-

electrons of the ligand when coordinated with the metal ion. The band at 388 nm (25773 cm^{-1}) might be assigned to charge transfer transition from the metal ion to the ligand (MLCT) or the coordination of the lone pair of electrons in azomethine nitrogen with the metal ions. The band at 472 nm (21876 cm^{-1}) might be assigned to d-d transition.

Co (II) is d^7 ion that can exhibit both in octahedral and tetrahedral geometry. In octahedral Co (II) complexes three spin allowed transitions are expected corresponding to the transitions ^4T_1g (F) → ^4T_2g (F) (υ_1), ^4A_2g (F) ⟵ ^4T_1g (F) (υ_2) and ^4T_1g (F) → ^4T_1g (P) (υ_3). Patel *et al*., 2000, have reported three transitions corresponding to υ_1, υ_2, and υ_3 frequencies for octahedral Co (II) complex in the range 12000 cm^{-1}-20000 cm^{-1}. Even though three bands were expected for the Co (II) complex of the ligand L under present study, only one broad band was observed in the visible region. Similar result was reported earlier (Shashidhara and Goudar, 2006). This might be due to the merging of all the three d-d transition bands.

The electronic spectrum of the synthesized Cu (II) complex derived from the ligand L, showed three bands at328 nm (30487 cm^{-1}), 387 nm (25839 cm^{-1}) and 553 nm (18083 cm^{-1}). The band at 328 nm (30487 cm^{-1}) might be assigned to due to the decrease in the Pi- electrons of the ligand when coordinated with the metal ion. The band appearing at 387 nm (25839 cm^{-1}) might be due to charge transfer transition from the metal ion to the ligand (MLCT) or a shift of n-π^* transition of the ligand towards lower frequency (red shift), confirming the coordination of azomethine nitrogen of the ligand to the metal ion .The broad band appeared at 553 nm (18083cm^{-1}) could also be due to d-d transition. Literature reveled that distorted Octahedral Cu (II) complexes displayed a band in the region 13000 cm^{-1}-19000 cm^{-1} due to d-d transition, which was assigned to $^2T_2g \rightarrow {}^2Eg$ transition and is a characteristic of octahedral geometry (Rao, 2000). The observed broad band in the present Cu (II) complex of ligand L at 553 nm (18053 cm^1) assigned to $^2T_2g \rightarrow {}^2Eg$ transition, suggesting distorted octahedral geometry of the Cu (II) complex due to the coordination of two different ligands (the Schiff base ligand and water)) to the Cu (II) ion as expected by Jahn-Teller distortion. The UV-Visible absorption spectra of the synthesized Co (II) and Cu (II) complexes are shown in figure 13 and 14, respectively.

Table 5 Electronic absorption spectral analysis data of Co (II) and Cu (II) complexes

Compound	**Electronic spectral bands**	**Assignment**	**Geometry**
L	355nm (28169cm^{-1})	π-π*, n-π*	__
[CoL$_{2(}$H$_2$0)$_2$]Cl$_2$	326nm (30674 cm^{-1})	π-π*	Octahedral
	388nm (25773cm^{-1})	MLCT, n-π*	
	472 nm (21186cm $^{-1}$)	d-d	
[CuL$_2$(H$_2$O)$_2$]Cl$_2$	328 nm (30487cm^{-1})	π-π*	Octahedral
	387 nm (25839cm^{-1})	MLCT, n-π*	
	553nm (18083cm^{-1})	d-d (^{2}T$_2$g-^{2}Eg-)	

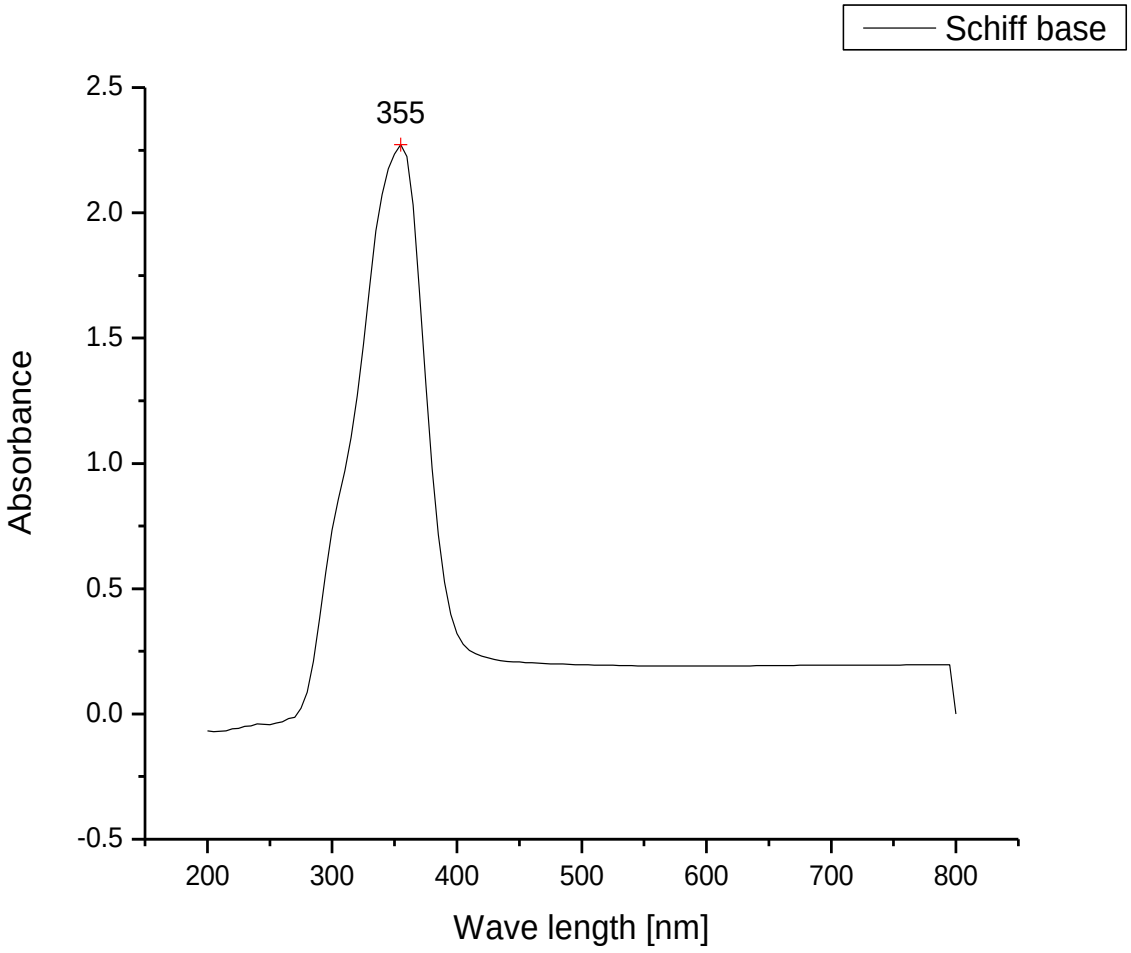


Figure 12: UV-Visible spectrum of Schiff base

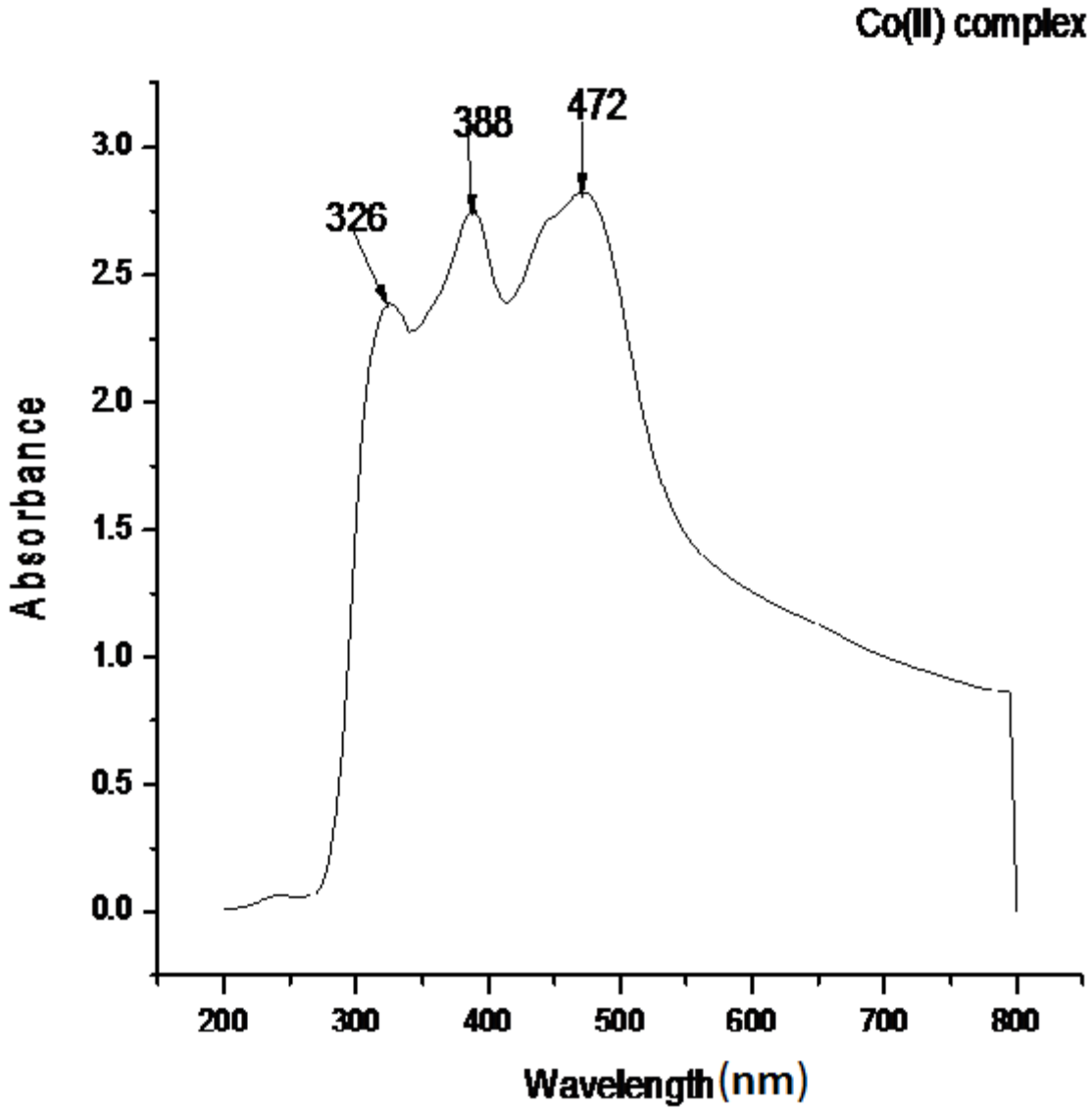


Figure 13: UV-Visible spectrum of Co (II) complex

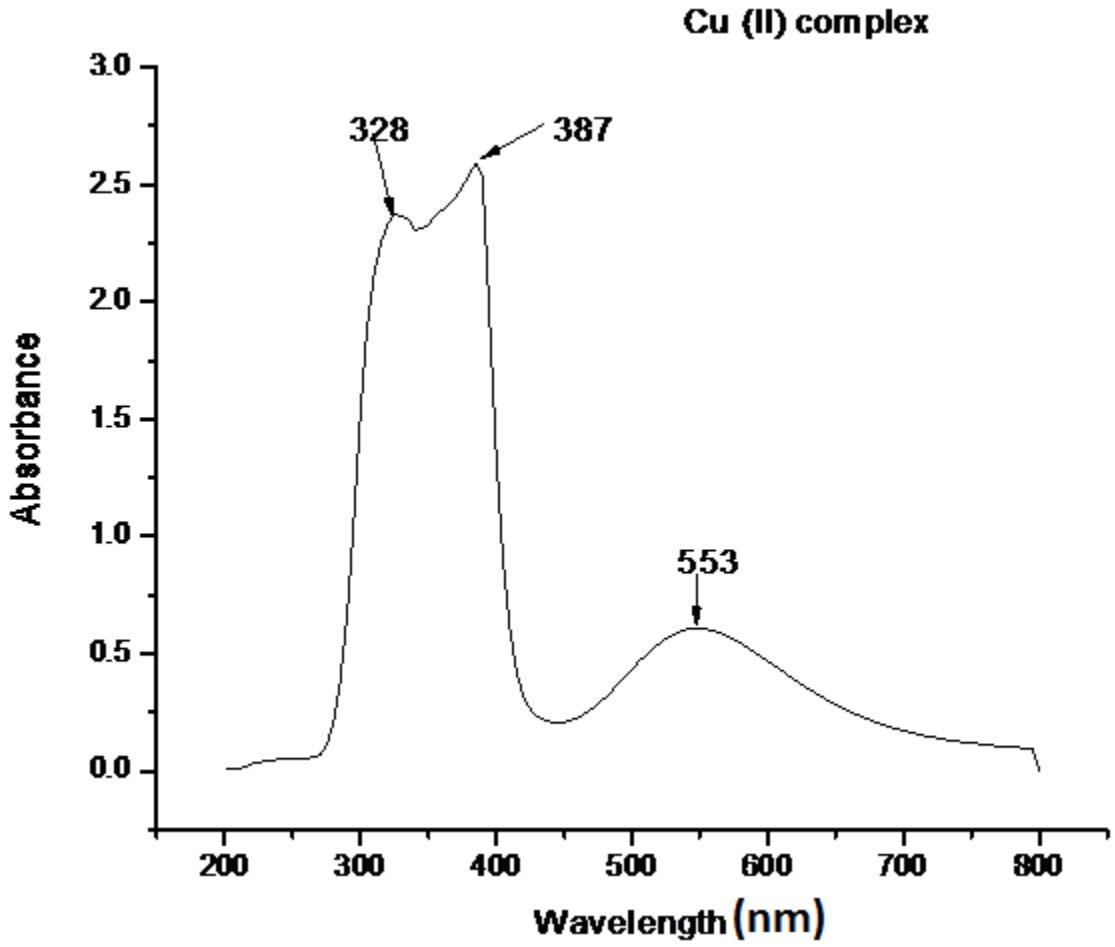


Figure 14: UV-Visible spectrum of Cu (II) complex

4.1.7. Magnetic Susceptibility of Co (II) and Cu (II) Complexes

The magnetic susceptibility measurements for Co (II) and Cu (II) complexes were recorded at room temperature (T=21^{0}C). The magnetic moment for Cu (II) complex of the ligand L was 1.85 B.M. The reported value for the mononuclear Cu (II) complex having no major spin interaction in octahedral geometry is 1.75-2.20 B.M (Mishra *et al*., 2009). Thus the present Cu (II) complex is octahedral geometry. This is in the support of the electronic absorption spectrum of the complex. In octahedral Co (II) complex, the ground state is "T_1g" and a large orbital contribution to the singlet state lowers the magnetic moment values for the various Co (II) complexes which are in the range 4.8-5.20 B.M for high spin interaction and 2.0-2.7 for low spin interaction in the octahedral geometry (Mishra *et al*., 2009). In the present investigation the observed magnetic moment value for Co (II) complex was 2.14 B.M. Although the electronic absorption spectrum of Co (II) complex couldn't help to decide the geometry of the complex, the magnetic moment value indicated octahedral geometry for the complex. The effective magnetic moment value is shown in Table 6.

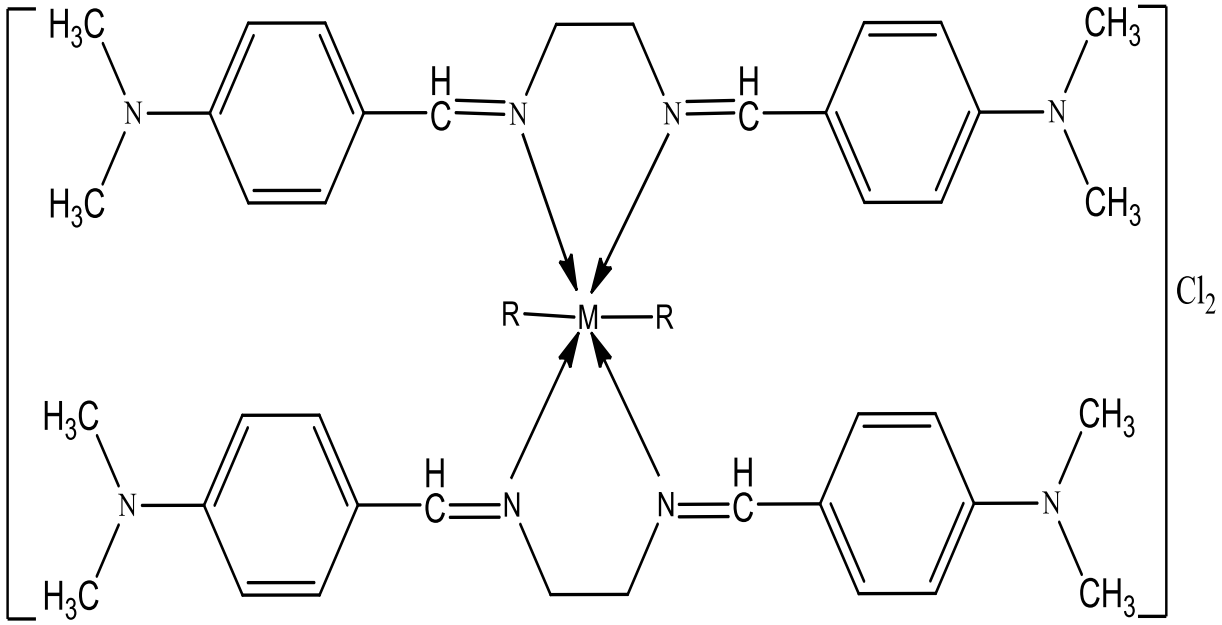
Table 6: Effective magnetic moment (μ_{eff}) of Co (II) and Cu (II) complexes

Sample	Molecular weight	Gram susceptibility (χ_g)	Molar susceptibility (χ_m)	Diamagnetic correction D	Effective susceptibility (X_A)	Effective magnetic moment μeff (B.M.)
Co (II) complex	809.93	1.107×10^{-6}	8.96×10^{-4}	1.038×10^{-3}	1.93×10^{-3}	2.14
Cu (II) complex	814.5	0.567×10^{-6}	4.61×10^{-4}	1.038×10^{-3}	1.5×10^{-3}	1.85

4.1.8. Suggested Structural Formula of Co (II) and Cu (II) Complexes

Based on the references made from elemental analyses, chloride test, Conductometric, IR spectroscopy, AAS, magnetic susceptibility and electronic absorption spectral studies the

following general structures could be proposed for $[CoL_2(H_2O)_2]Cl_2$ and $[CuL_2(H_2O)_2]Cl_2$ complexes (Figure 15).



Where M = Co (II) or Cu (II)

$R = H_2O$

Figure 15: Proposed structure of Co (II) and Cu (II) complexes

4.2. Antimicrobial Studies

The biological screening effects of the investigated compounds were tested against the bacterial species, *S.aureus* and *S. galatia* (gram positive bacteria), *E.coli* and *S.typhi* (gram negative bacteria), and fungal species (*A.niger* and *A. flavus*). The Schiff base and the complexes exhibited varying degrees of inhibitory effects on the growth of the tested bacterial and fungal species. The inhibition values (mm) of the compounds are summarized in Table 7 and 8.

Table 7: Inhibition values (mm) of the ligand L, and its Co (II) and Cu (II) complexes in the anti bacterial assay

Name of bacteria	Chloroamphinicol (20mg/ml)	DMSO (20mg/ml)	L (20mg/ml)	Co (II) complex (20mg/ml)	Cu (II) complex (20mg/ml))
	Inhibition (mm)	Inhibition (mm)	Inhibition (mm)	Inhibition (mm)	Inhibition (mm)
S.galatia	34.6	-	20.6	22.1	21.5
S.aureus	35	-	20.5	23.3	22.5
E.coli	25	-	12.5	12.6	13.5
S.typhi	33	-	20.3	22.1	20.8

Table 8: Inhibition values (mm) of the ligand L, and its Co (II) and Cu (II) complexes in the antifungal assay

Name of fungi	Tilt (20mg/ml).	DMSO (20mg/ml))	L (20mg/ml)	Co (II) complex (20mg/ml)	Cu (II) complex (20mg/ml)
	Inhibition (mm)	Inhibition (mm)	Inhibition (mm)	Inhibition (mm)	Inhibition (mm)
A. niger	27	-	-	-	-
A. *flavus*	28	-	-	-	-

On the bases of the data (Table 7) Cu (II) complex exhibited better antibacterial activity than Co (II) complex against *E.coli*. On the other hand Co (II) complex showed better antibacterial activity than Cu (II) complex against *S. aureus*, *S. galatia* and *S. typhi*. Both Co (II) and Cu (II) Complexes have higher antibacterial activity than the synthesized Schiff base ligand. Both the Schiff base ligand and the metal complexes have no inhibitory activities against the tested

fungi (*A. niger and A. flavus*). This might be due to the reason that the ligand contained only one functional group (azomethine group). The photograph of antibacterial and antifungal effects of the samples is shown in figure 16, 17 and 18, respectively.

The overall results showed that the metal chelates are more active than the ligand. This is explained on the basis of overtones concept and chelating theory (Wayne, 2009). According to the overtones concept, the lipid membrane surrounding the cell allows passage of only lipid-soluble materials, due to which lipo-solubility is a vital factor for controlling the antimicrobial activity. On the basis of chelation, the reduced polarity of the metal ion is due to the overlap of the ligand and the partial sharing of the positive charge of the metal ion with donor groups. For this reason, the complexes become very stable due to delocalization of π electrons. It enhances the penetration of the complexes in to lipid membranes and blocking of the metal binding sites in the enzymes of pathogens. These complexes also interfere in the respiration process of the cell and therefore block the synthesis of proteins, which restricts further growth of the pathogens.

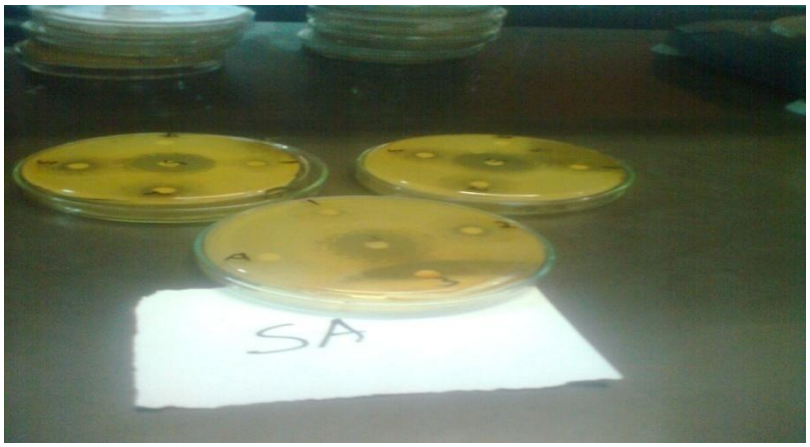


Figure 16: Antibacterial effect of the samples on S.aureus

Figure 17 Antibacterial effect of the samples on S.Typhi

Figure 18: Antifungal effect of the synthesized samples on A. Flavus

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary and Conclusion

In the present work, divalent metal complexes of NN donor type ligad derived from *p*-dimeth yl amino bezaldehyde and ethylenediamine were synthesized and characterized by various spectroscopic and analytical techniques. From the elemental analysis and AAS data the stoichiometry of metal to ligand ratio (M: L) was found to be 1:2.The electronic absorption spectra of the synthesized ligand and its complexes in DMSO have been investigated and the absorption bands due to n-π*, π-π*, charge transfer and d-d electronic transitions were observed in the compounds.

The Conductometric study indicates the electrolytic nature of both complexes. Both Co (II) and Cu (II) complexes were formulated as mononuclear $[CoL_2(H_2O)_2]Cl_2$ and $[CuL_2(H_2O)_2]Cl_2$ compounds. Based on the infrared results, it is concluded that the ligand behaves as a neutral bidentate ligand coordinated to the metal ion through the nitrogen atoms of azomethine to form the metal complexes. ^{1}H and ^{13}C NMR spectra justified the structure of the ligand. Both the magnetic susceptibility and electronic spectra studies indicated the octahedral structure of Co (II) and Cu (II) complexes.

The biological evaluation of the ligand and its complexes against the selected pathogenic bacterial and fungal strains showed that metal complexes exhibited higher antibacterial activity than the free ligand and less than the standard drug (chloramphinicol). However, both the synthesized Schiff base ligand and its Cu (II) and Co (II) complexes have no inhibitory activities on the tested fungi (*A.niger and A. Flavus*).

5.2. Recommendations

Based upon the results of antibacterial activities of the synthesized Schiff base ligand and its Co (II) and Cu (II) complexes, this study opens the way for further study on antibacterial activities against more bacterial species in addition to these four microbes investigated. The antifungal activity of the prepared Schiff base ligand against different microorganisms may be enhanced with chelation of other biological active metals. Moreover, the antimicrobial

activity of the synthesized ligand may be enhanced when complexed with the second and third row transition metal ions.

6. REFERENCES

Abdallah, S. M., Zayed, M. A. and Mohamed, G.G. 2012. Synthesis and spectroscopic characterization of new tetra dentate Schiff base and its coordination compounds NOON donor atoms and their antibacterial and antifungal activity. *Arabian Journal of Chemistry*, 3: 103-113

Abdel-Nasser, Alaghaz. 2014. Synthesis, Spectroscopic characterization, molecular modeling and potentiometric studies of Co (II), Ni (II) and Zn (II) Complexes with 1, 1-diamino hexane. *Journal of Molecular Structure*, 1072: 103-113.

Ahmed, R.M., yousif, E.I., Hasan, H.A. and Aljeboori M.J. 2013. Metal complexes of macrocyclic Schiff base ligand: *Preparation, characterization and biological activity*, 10:1155.

Belal, A., EI-Deena, M. and Farida, N. 2015. Synthesis, Spectroscopic coordination and biological activities of some transition metal complexes containing ONO tridentate Schiff base. *Journal of Molecular and Bimolecular Spectroscopy*, 149:771-787.

Chandra, S. and Agrawali, S. 2014. Spectroscopic characterization of Lanthanaid derived from hexadentate macrocyclic ligand. J*ournal of antifungal capacity of complexes*, 3: 21-25.

Chohan, Z.H., Praveen, M. and Ghaffar, A. 1998. "Synthesis, characterization and biological role of anions (Nitrate, Sulphate, Oxalate and Acetate) in Co (II), Cu (II) and Ni (II) metal chelates of some Schiff base derived from amino acids," Synthesis and reactivity in inorganic and metal- organic chemistry, 28: 1673-1687.

Cozz pier. 2004. Metal-Salem Schiff base complexes in catalysis. *Journal of Practical Aspects*, 17: 1087.

Dai, Hui-Xiong., Antonia, F., Stepan, Marks. Plumber, yang-HuiZhang and Jin-Quanyu. 2011. Stage diversification as a tool for drug discovery. *Journal of American Chemical Society*, 5: 16-20**.**

Dede, B., Karip, F. and Cenjiz, M.2009. Synthesis, crystal structure, and bioactivities of Ni (II) and Cu (II) Complexes. *Polyhedron*, 163: 148.

Deshpande, U.G. and Shah, J.R. 2003. Physico chemical studies on chelate polymers of Cr (III), Mn (II), Fe (II), Co (II) and Cu (II) with Schiff base. *Journal of macromolecular science*, 20: 355-363.

Fugu, M.B., Ndahi, N.P., Paul, B.B and Mustapha, A.N. 2013.Synthesis,characterization, and antimicrobial studies of some vanillin Schiff base metal (II) complexes. *Journal of Chemical Pharmacology*, 5(4): 22-28.

Gholamhossein, Grivani., Giuseppe, Bruno, Hadi, Amiri., Rudbari, Aliakbar., Dehno, Khalaj and Pegah, Pourie, Mouri. 2012. Synthesis, characterization and crystal structure determination of a new oxovanadium (IV) Schiff base complex: The catalytic activity in the epoxidation of cyclo octene. *Journal home page: www.esler.com*, 18: 15-20

Gupta, Y.K., Agrawali, S.C., Manawa, S.P. and Ram, Narian. 2012. Synthesis, characterization and antimicrobial studies of some transition metal complexes of Schiff bases. *Research journal of chemical sciences*, 2 (4): 68-71.

Joseph. 2013. Metal complexes as antimicrobial agents. *Journal of Saudi Chemical Society*, 17: 285-294.

Karthikeyan, M.S., Prasad, D.J., Poojary, B., Subramanyabhat, K., Holl, B.S. and Kumari, N. S. 2006. Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety. *Journal of Bio-organomolecular Chemistry*, 14:7482-7489.

Kharadi, G.J. 2013. Antioxidant, tautomeric and antibacterial studies of Fe (III) 1, 2, 4-triazole based complexes. *Journal of Molecular and Bimolecular Spectroscopy*, 110:311-316.

Kramer U., Cotter-Howells, J.D., Charlock, J.M., Baker, A.J. and Smith, J.A. 2006. Free histidine as metal chelator in plants that accumulates nickel. *Nature*, 379:635-638.

Kumar, D., Chard, S., Sharma, J. and Suriyan,P. 2013.Synthesis, specteral characterization and antimicrobial studies on the coordination compounds of metal ions with Schiff base containing both aliphatic and aromatic moieties. *Journal of Bioinorganic chemistry*, 10:36-40.

Malik, S., Ghosh, S. and Mitu, L. 2011. Complexes of some 3d-metals with a Schiff base derived from 5-acetamido-1, 3, 4-thiazole-2-sulphamide and their biological activity. *Journal of Serbian chemical Society*, 76(10): 1387-1394

Misbahu, Rahman. Muhammad, Imran.and Muhammad, Arif. 2013. Synthesis, characterizati on and invitro antimicrobial studies of Schiff bases derived from acetyl acetone and amino acids and their oxo vanadium (IV) Oxide. *American Journal of Applied Chemistry,* 1(4): 59-66.

Mishra, L.K., Jha, Y., Sinha, B.K., Kant, R. and Singh, R. 2009. Studies of chelates of, cobalt, nickel, copper, zinc and cadmium (II) with 4-amino-3, 5-dioxo-6-methanol-2, 3, 4, 5-tetrahydro-1, 2,4-tri-azine. *Journal of Indian Chemical Society*, 76: 65-66.

Muhammad, Aslam, Itrat, Anis, Night, Aza. and Rashad, Muhammad. 2015. Synthesis, chara cterization and antibacterial investigation of divalent complexes of NO donor ligand. *Journal* of *Saudi Chemical Society*, 19: 322-326.

Mukul, kalita, Prasanta, Gogoi., Pranjit, Barman, Bipul, Sarma., Alok, k. and Buragohain, Ranjan. 2013. A new series of Ni (II), Cu (II), Co (II) and Pd (II) complexes with an ONS donor Schiff base: Synthesis, crystal structure, catalytic properties and bioactivities. *Polyhedron*, 60: 17-19.

Orving, C. and Abraham, M.J. 2000. Medicinal inorganic chemistry introduction. *Chemical review*, 99: 2201-2204.

Pal and Pal. 2015. Ruthenium (II) complexes RuN_4O_2 spheres assembled via pyridine-imine –amide coordination. Synthesis, structures properties and prorogation behavior of coordinated amid, .*Journal of chemical transaction*: 2102-2106.

Panda, B.K. and Chakravoty, A. 2005. Spectroscopic properties of inorganic and organometal lic compounds. *Journal of Organometallic Chemistry*, 690: 3169-3175.

Patel, M.K., Patel, N.H., Patel, K.N., and Patel, M.N. 2000. Synthesis, structural characterization and biocidal studies of Cu (II), Ni (II), Zn (II), Co (II), Fe (III), Mn (II) and Cd (II) complexes derived Schiff bases (ONO) and bidentate (NN) hetrocyclic. *Journal of Indian council of chemists*, 17: 19-24.

Patel and Chaudhary. 2012. Synthesis, Characterization and antimicrobial studies on bivalent copper, nickel and cobalt complexes of thiosemicarbazone. *Internationa*l *Journal of Chemical Technological Research USA*, 4: 918-924.

Patil, A.R., Donde, K.J., Raut, S.S., Patil, V.R. and Loklande, R.S. 2013. Synthesis, characterization and biological activity of mixed ligand Co (II) complexes of Schiff

base 2-amino-4-nitro phenol-n-salicylidenewith some amino acids. *Journal of Chemical Pharmacology*, 4(2): 1413-1425.

Prakash, A. and Adhikar, D. 2010. Synthesis and characterization of bioactive zinc (II) and cadmium (II) complexes with new Schiff bases derived from 4-nitrobenzaldehYde and aceto phenone with ethylene di amine. *Journal of Molecular and Bimolecular Spectroscopy*, 76: 356-362.

Prakash, A. and Adhikari, D. 2011. Application of Schiff bases and their metal complexes. *International journal* of *chemical technology*, 3(4):1891-1896.

Rao, C.N.R. 2000. Chemical applications of infrared spectroscopy .*Newyork, Academic press USA*, 16:21-25.

Sari, N., Arslan, S., Logoglu, E. and Shakiyan. 2013. Spectroscopic and biological activity studies on tridentate Schiff base ligands and their transition metal complexes. *Indian Journal of Science*, 16: 283-288.

Sadler, P.J. and Lih, Sun. 2001. Coordination chemistry of metals in medicine. *Coordination chemical review*, 186:689-709.

Sakurai, H., Kojima,Y.,Yoshikawa, y., Kawabe, K., and Yasmi, H. 2002. Antibiotic vanadium (iv) and zinc (II) Complexes. *Coordination chemistry review*, 226. 186-198.

.Shakir, M., Khanam, S.S., Firdaus, F., Aatif, A.M., Al-Resayes and S.I. 2016. Synthesis, spectroscopic characterization, DNA interaction and antibacterial study of metal comp lexes of tetra azamacro cyclic Schiff base. *Journal of molecular and Biomolecar Spectroscopy*, 75: 312-315.

Sharma, A. K.and Chandra, S. 2013. Synthesis, structural and fungicidal studies of hydrazones based on coordination compounds. *Journal of Spectroscopy*, 103(5):96-100.

Shashidhara, G.M.and Goudar, T.R. 2006. Synthesis of Uranium (IV) complexes of Schiff bases. *Journal of the Indian chemical society*, 78:360-361.

.Singh, K., Barwa, M.S. and Tyagi, P. 2006. Synthesis, characterization and biological studies of Co (II),Ni (II),Cu (II) and Zn (II) complexes with bi dentate Schiff bases derived by hetrocyclic ketone. *European Journal of Medical chemistry*, 41:147-153.

Spinu, C. 2008. Biologically active transition metal chelates with a 2-thio phenocarboxy aldehyde derived Schiff base, Synthesis, characterization and antibacterial properties. *Journal of Chemistry*, 32:487-493.

Subbara, P., Ramu, A., Raman, N. and Dharmara, J. 2014. Synthesis, characterization, DNA interaction and pharmacological studies of substituted benzophenone derived Schiff base metal (II) complexes. *Journal of Saudi Chemical Society*, 19: 207-216.

Sulek, Chandra and Seema Gautam. 2014. Antibacterial and antifungal activity of Schiff base ligands and their metal complexes. *International Journal of Chemical and Pharmaceutical Sciences*, 5(2): 1.

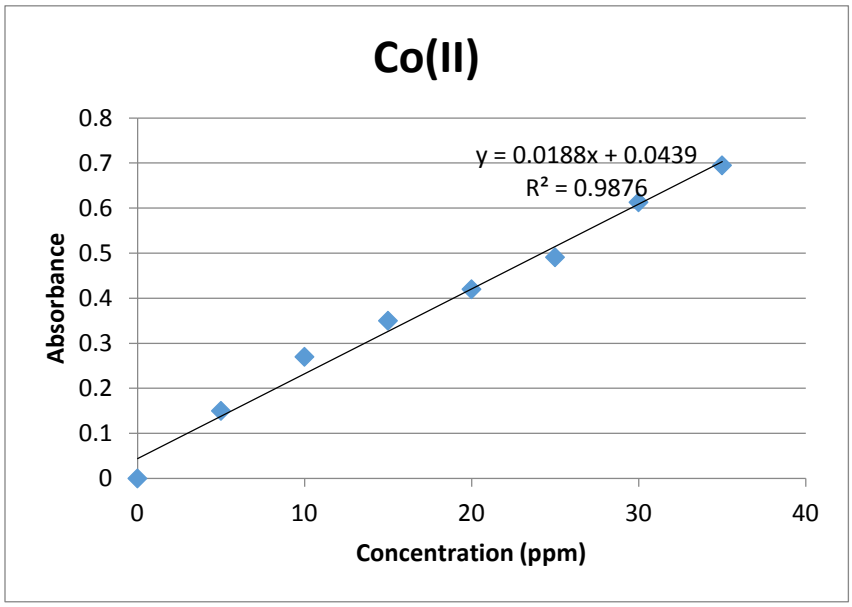
Tyagi, M., Chandra, S., Akhtar, J. and Chandra, D. 2014. Modern spectroscopic technique in the characterization of insensitive macro cyclic Schiff base ligand and its complexes: *Inhibitory activity against plant pathogenic fungi*, 118(24):1056-1061.

Wayne, R.B. 2009. Iron transport and storage in microorganisms, plants and animals. *Journal of the American college of nutrition*, 18: 368-369.

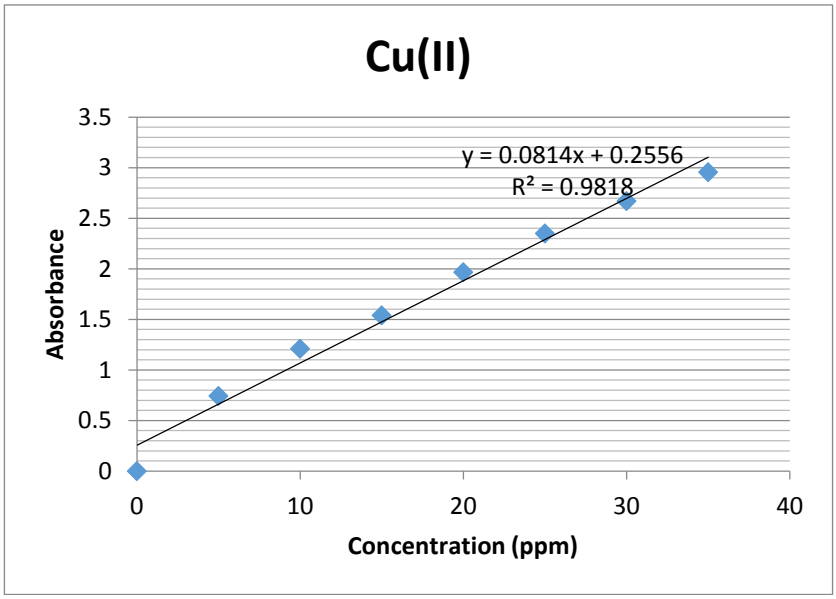
Yadav, P.S., Prakash, D. and Senthilkumar, G.P., 2011. Different Methods of Synthesis and Diverse Biological Activities. *International Journal of Pharmaceutical Science Drug Research*, 3: 01-07.

Yiase, S.G., Adejo, S.O., Gbertyo, J.A. and Eden, J. 2014. Synthesis, characterization and antimicrobial studies of salicylic acid complexes of some transition metals. 2014. *Journal of applied chemistry*, 7: 04-10

7. APPENDIX



Appendix figure1: Calibration curve of cobalt (II)



Appendix figure 2: Calibration curve of copper (II)