SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF Cu (II) AND Co (II) SCHIFF BASE COMPLEXES

BY:

ALEMAYEHU TUFA

A Thesis Submitted to

Department of Chemistry,

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Chemistry

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

September, 2017

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Co-Advisor: Dr. Milkyas Endale

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APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defense by Alemayehu Tufa have read and evaluated his thesis entitled "**Synthesis ,Characterization and Antibacterial Activity of Cu(II) and Co(II) Schiff base complexes**" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master's in Chemistry

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ADVISOR'S APPROVAL SHEET

To: The Chemistry Department

Subject: Thesis Submission

This is to certify that the thesis entitled "**Synthesis, Characterization and Antibacterial Activity of Cu (II) and Co (II) Schiff base complexes**" submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry, the Graduate program of the department of Chemistry, and has been carried out by Alemayehu Tufa Id. No GSS/0205/05, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

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Milkyas Endale (Ph. D)	_____	_____
Name of Co-Advisor	Signature	Date

DECLARATION

I hereby declare that this MSc Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Alemayehu Tufa

Signature: _____

This MSc thesis has been submitted for examination with my approval as thesis advisor

Major Advisor

Name: _____

Signature: _____

Co-Advisor

Name: _____

Signature: _____

Date of submission: _____

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LIST OF ABBREVIATIONS AND SYMBOLS

L	Ligand
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
B.M	Bohr Magneton
μ_{eff}	Effective Magnetic Moment
AAS	Atomic Absorption Spectrophotometer
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transform Infrared Spectroscopy
MIC	Minimum Inhibition Concentration
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV-Vis	Ultra Violet Visible
ppm	Parts Per Million

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ABSTRACT

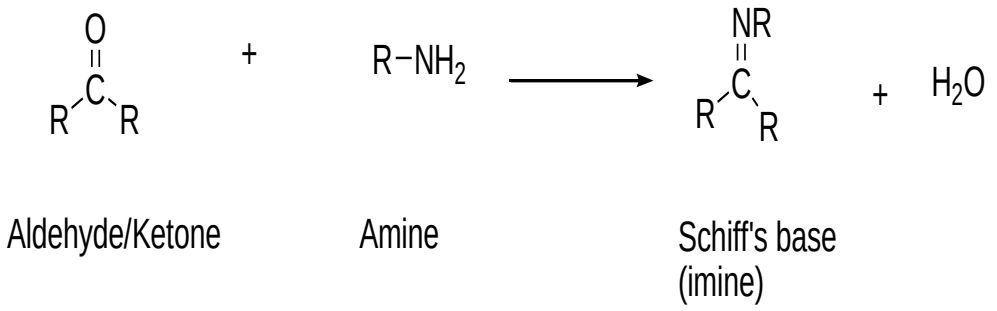
Interaction of metal ions with N, O and S atoms of Schiff bases have gained much attention in recent years due to their various biological activity such as ant malarial, anticancer, antibacterial, antifungal and ant tubercular. In this project, two new Cu (II) and Co (II) metal complexes of Aniline and Vanillin Schiff base were synthesized and screened for their antibacterial activity. The structures and geometry of Schiff base and two complexes were confirmed by elemental analysis (H, C and N), ^{1}H-NMR, ^{13}C-NMR, FT-IR, AAS and Magnetic susceptibility measurements. The Schiff base behaving as tridentate O,N,O donor employing one azomethine nitrogen, one hydroxyl oxygen and one methoxy oxygen atom. The electronic spectra of Co (II) complex exhibited band at 438nm, which were assigned to the d-d transition type $^{4}T_{1}g \rightarrow {}^{4}T_{1}g$ (F) for a high spin octahedral geometry whereas the electronic spectra of Cu (II) complex exhibited band at 404nm due to LMCT and spectra at 533nm due to d-d transition type $^{3}A_{2}g \rightarrow {}^{3}T_{2}g$ for a low spin suggest a distorted octahedral geometry. The synthesized Schiff base and Cu (II) and Co (II) complexes were screened for their antibacterial activity against Escherichia coli, S.aureus, K.pneumonia and Protein mirabilis. Overall, the antibacterial tests revealed that the Schiff base showed a very promising activity (17mm) against K.pneumonia compared to that of gentamicin (15mm) and Cu (II) complex also showed promising activity (13mm) against E. coli compared to that of gentamicin (14mm).

***Key words/Phrases**: Antibacterial Activity, Synthesis, Schiff base, Vanillin, metal Complexes*

1. INTRODUCTION

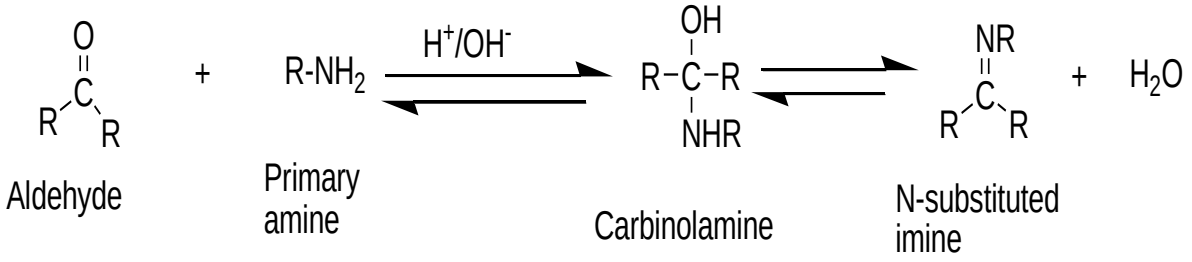
1.1 Background of the study

Recent advancement in inorganic chemistry has brought much attention about the synthesis of diverse metal complexes of various organic donor ligands for therapeutic uses. The earliest accounts on the usage of transition metal complexes as anti-cancer agents and leukemia started in the 16th century [1,2].The development of metal based drugs with pharmacological potential and unique therapeutic properties has been possible due to the fact that metals possess various oxidation states and the ability to interact with negatively charged molecules [3]. A Schiff's base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by C=N-R group. It is usually formed by condensation of an aromatic aldehyde or ketone with a primary amine according to the following scheme (Scheme 1):



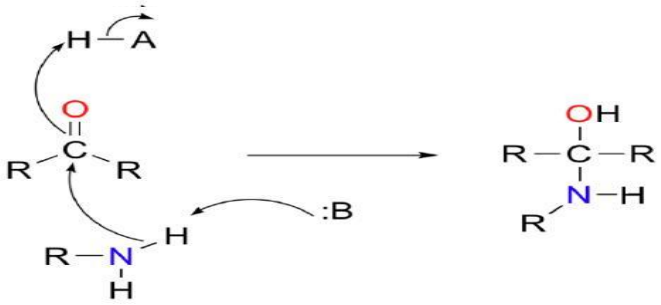
Scheme 1: Condensation reaction of aldehyde or ketone with primary amine

Where R, may be an alkyl or an aryl group. Schiff's bases that contain aryl substituents are substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable. Schiff's bases of aliphatic aldehydes are relatively unstable and readily polymerizable [4, 5] while those of aromatic aldehydes having effective conjugation are more stable [6, 7]. The formation of a Schiff's base from an aldehydes or ketones is a reversible reaction and generally takes place under acid or base catalysis, or upon heating (Scheme 2).

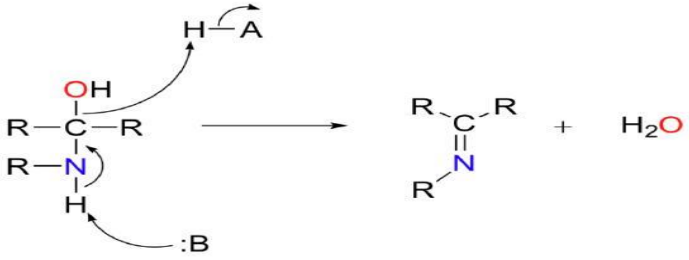


Scheme 2: Reversible reaction including carbinolamine as intermediate through formation of Schiff's base

Mechanistically, the formation of imines involves two steps. First, the amine nitrogen acts as a nucleophile, attacking the carbonyl carbon. This is closely analogous to hemiacetal and hemiketal formation



What happens next step is that the nitrogen is deprotonated, and the electrons from this N-H bond 'push' the oxygen Hoffa C=N double of bond the (an imines) carbon, and a displaced water molecule.



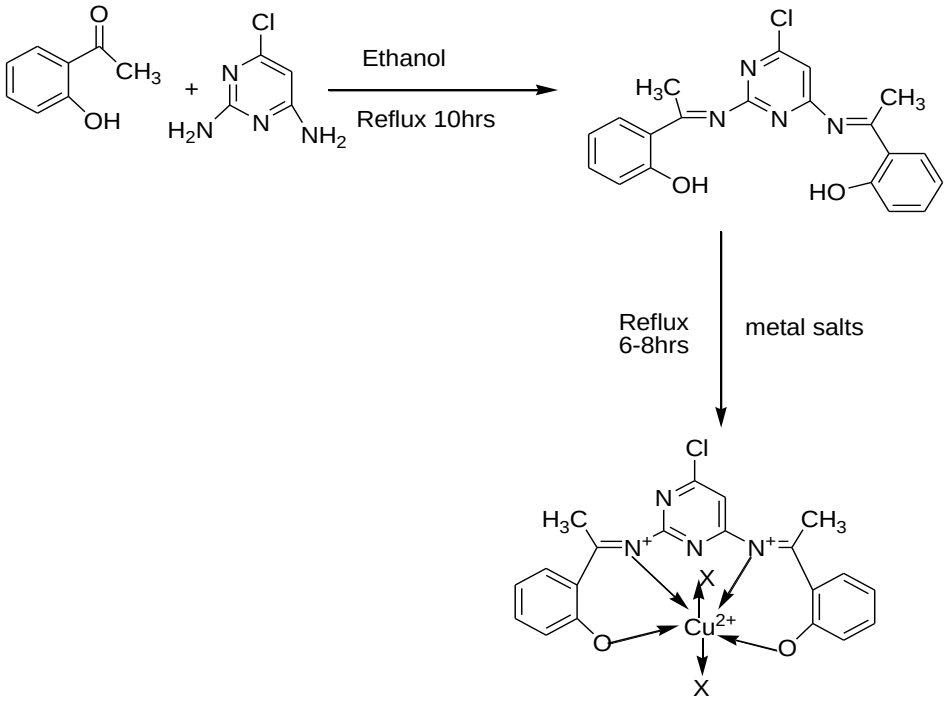
Scheme 3: Mechanistic explanation of the formation of Schiff base

The formation is generally driven to the completion by separation of the product or removal of water, or both. Many Schiff's bases can be hydrolyzed back to their aldehydes or ketones and amines in aqueous acidic or basic media. The mechanism of Schiff's base formation is another variation on the theme of neucleophilic addition to the carbonyl group. In this case, the neucleophile is the amine. In the first part of

the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine. The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration.

Preparation methods of Schiff base complexes :(By direct interaction of the Schiff base with the metal salts)

The Schiff's base can be synthesized without using the metal ion and then followed by addition of the metal ion as salt solution for complex formation. This can be easily described by the following example.

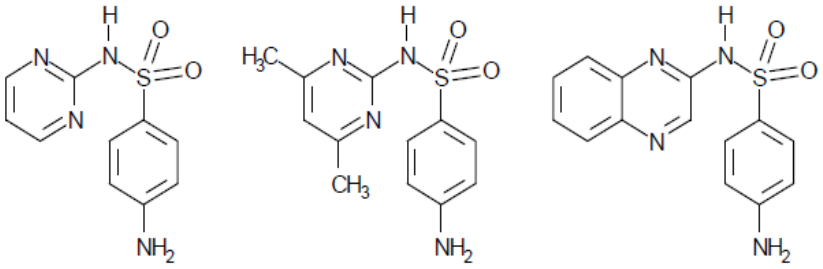


Scheme 4: Preparation of Schiff base complex by direct method.

A variety of Schiff's bases can be obtained by changing aldehydes or amines. Thus synthesis of large number of Schiff's bases with diverse structural features could be possible with ease. They can have additional donor groups like oxygen, sulphur, phosphorus *etc*. which makes them good candidates for metal ion complexation and for mimicking biological systems. They can be functionalized by the insertion of appropriate groups in the aliphatic or aromatic chains.

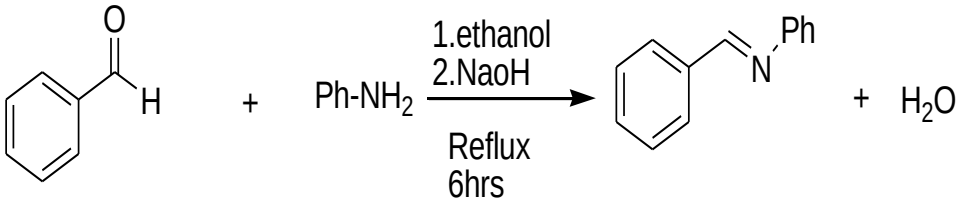
Sulfonamides are antibacterial drugs, which are used in human and veterinary medicine. Especially in

veterinary medicine sulfonamides are well-established for treating diseases of the respiratory, gastrointestinal, and urinary tract. Furthermore, they are very often the preferred choice for the medical treatment of rodents, since those in general have a broad intolerance against antibiotics (particularly penicillin. Structures of sulfonamides known by the name sulfadiazine (**1**), sulfadimidine (**2**), and sulfaquinoxaline (**3**) are shown below.



1 **2** **3**

Primary and secondary amines react with benzensulfonyl chloride as described in the discussion of the Hinsberg test**.** These reactions are examples of nucleophilic acyl substitution. Many times, reactions with acid chlorides are carried out in a basic solution to avoid the formation of HCl. The synthesis of new sulfonamides has got more attention of researchers for their previous success in the field of pharmaceutical sciences and medicinal chemistry. In recent studies, new sulfonamides have been synthesized by the reaction of p-toluene sulphonyl chloride with essential amino acids (histidine and tryptophan) and amino group containing drugs such as levetiracetam (anticonvulsant), famotidine (antiulcer), celecoxib (NSAID), ribavirin (antiviral), tranexamic acid (antifibrinolytic), furosemide (diuretic), aspartame (monosaccharide sweetener), and nicotinamide (vitamin B).



Scheme 4 Reaction of amine with benzaldehyde

The Schiff bases and their metal complexes have more importance recently [8] because of their variety of applications in many fields: biological, inorganic and analytical chemistry [9]. They have been found to possess the pharmacological activities such as anti -malarial [9, 10], anticancer [11a, b], antibacterial [12],

antifungal [13], and antimicrobial [14a]. Schiff's bases derived from aromatic amines and aromatic aldehyde have a wide variety of applications in many fields as sulfonamide Schiff's bases have been reported to posses-antimicrobial activity [14a]. In this project, two new Cu (II) and Co (II) metal complexes of Aniline and Vanillin Schiff base were synthesized and screened for their antibacterial activity

1.2. Statement of the problem

Purely synthetic antimicrobial drugs against the protozoal malady dozing disorder and the spirochaet ailment of syphilis were developed by Ehrlich in 1910 [15]. Microorganisms; diseases-causing types, have been able to develop resistance in order to fight back man's hostility which sought to deprive them off their territory using antimicrobial agent [16]. Apparently, several alternative synthetic antimicrobial agents including metal Schiff base complex derivatives have been synthesized and tested. In order to fill this gap, this project is intended to synthesize a new series of Co (II) and Cu (II) complexes and screen for antibacterial activity.

1.3. Significance of the study

Understanding the structure of some transition metal complexes was useful in pharmaceutical and industrial applications. This study focuses on the synthesis, characterization and antimicrobial study of some transition metal complexes. Therefore, this study provides important information about the structural information, geometry, mode of synthesis, and antimicrobial activity of metal complexes.

1.4 Justification of the study

Inorganic elements play crucial role in biological and biological medical process, and it is evident that many organic compounds used in medicine do not have purely organic mode of action. Some are activated or biotransfered by metal ions metabolism

Metal complexes have been known and used since time immoral to treat most of the diseases affecting human and animals, therefore researchers have found them to be a better choice for bioactive coordination compounds. The introduction of synthesis of bioactive drugs, however, changed the trend and attracted many to turn to use them on the expense of Schiff base metal complexes drugs. In this project, the synthesis and characterization of bioactive Schiff base and metal complexes were done including screened of their biological activity against selected strains of microorganism.

1.5 Objectives

1.5.1 General Objective

The overall objective of this project is to synthesize, characterize and screen for antibacterial activity of Co (II) and Cu (II) metal complexes of aniline-vanillin Schiff base.

1.5.2 Specific Objectives

- To synthesize aniline-vanillin Schiff base ligand derivative.
- To synthesize Co (II) and Cu (II) Schiff base complexes.
- To characterize the synthesized Schiff base and it's complexes of Co (II), and Cu (II) using spectroscopic, elemental analysis and magnetic susceptibility techniques.
- To screen antibacterial activity of Co (II) and Cu (II) metal complexes against selected strains of microorganisms

2. LITERATURE REVIEW

2.1 Schiff base-metal complexes

The chemistry of Schiff base-metal complexes has fascinated several chemists in different parts of the globe in search of promising chemotherapeutic agents for disease control. The ease with which the Schiff base ligands are designed and synthesized have made them to be referred to as 'fortunate ligands', possessing azomethine derivatives, the C=N linkage that is essential for biological activity, including: antibacterial, antifungal, antioxidant, anticancer and diuretic activity studied as model molecules for biological, analytical and industrial applications have made this part of inorganic chemistry highly interesting [8- 9].

Application of Schiff bases and their transition metal complexes have shown abroad biological activity in the area of DNA binding studies, bacterial and fungal inhibition, herbicides, insecticides, nematocides and rodenticides. Metal complexes of Schiff bases application can also be found in the areas like free radical chains termination, as antileishmanial agents, as radiotracers in nuclear medicine, anti–inflammatory, plant growth hormone regulator/ booster, cytotoxic activity [17-19].

2.2 Schiff bases and their complexes as biologically active agents

Schiff bases are widely studied ligands which coordinate to metal ions via azomethine nitrogen. In azomethine subordinates, the >C=N assembly is crucial for biological activity. The presence of this assemblage have made its complexes to be considered as imperative stereo-chemical models in main group and transition metal coordination chemistry because of straight forwardness in preparation and assortment in structure [10]. Interaction of metal ions with N, O and S atoms from Schiff bases organic compounds have gained much attention in recent years and have produced an enthusiastic arrangement of ligands, possessing characteristic features that can be adjusted by introducing unusual organic substituent’s, which bring about variety in the fundamental donor properties [8,20].

The use of Schiff bases in biological or therapeutic applications as promising drug agents or biological probes and analytical tools have been reported by several researchers. Also, the bioactivity of Schiff base compounds as antibacterial [12], anticancer [11], antifungal [13], antiviral agents [17-19] have been reported. There has been increasing focus on binding of small molecules such as Schiff base compounds to DNA [9, 20]. Their utilization in cancer prevention, sustenance bundles and as an O_2 sensor/ identifier has

been reported [11].

Furthermore, Schiff bases are found in diverse natural, semi-synthetic, and synthetic compounds and have been established to be significant for their biological activities. In the past few decades, Schiff base ligands and their metal complexes have been amongst the most widely examined coordination compounds. This can be attributed to their inexorably essential as biochemical, and scientific reagents. Schiff base complexes have been reported to offer ease and flexibility in their synthetic technique, diverse properties, and use as biologically active compounds.

2.3 Aniline

Aniline is an organic chemical compound, specifically a primary aromatic amine .It consists of benzene ring attached to an amino group .Aniline is oily and although colorless, it can be slowly oxidized and resin tied in air to form impurities which can give it a red-brown tint. Its boiling point is 184℃ and its melting point is -6℃. It is a liquid at room temperature. Aniline reacts with strong acids to form salts containing the anilinium (or phenyl ammonium) ion (C_6H_5-NH_3^+) and acts with acyl halides such as acetyl chloride (ethanoyl chloride, CH_3COCl) to form amides. Like phenols, aniline derivatives are highly reactive in electrophilic substitution reaction. For example, substitution of aniline produces sulfanic acid, which can be converted to sulfanilamide. Sulfanilamide is one of the sulfa drugs which were widely used as antibacterial in the 20^{th} century. Aniline was first isolated from the destructive distillation of indigo in 1826 by *OttoUnverdorben.* In 1834, Friedrich Runge isolated from coal tar a substance which produced a beautiful blue color on treatment with chloride of lime; this he named Kyanol or Cyanol. In 1841C.I,Fritzscheshowed that treating indigo with caustic potash it yielded an oil, which he named aniline, from the specific name of indigo-yielding plants, indigofera anil, anil being derived from the Sanskrit, dark-blue.

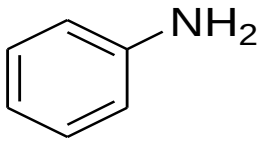


Figure 1: Chemical structure of Aniline

Both aliphatic amines and Aromatic amines are basic in nature. The aliphatic amines particularly are more basic than the aromatic amines. For instance, ethanamine and aniline, the former is more basic than the later because of the inductive electron donating effect by the alkyl group on the nitrogen atom. This increases the electron density on nitrogen and as a result gets protonated easily. Aniline is less basic because the non-bonding electron pair on the nitrogen atom is involved in resonance with the benzene ring. This tends to decrease the electron density on the nitrogen atom in aniline.

Secondary amine is more basic than a primary amine because it has two alkyl groups which donate electron inductively to the nitrogen atom. The tertiary amine is less basic than the other two. The reason is that a tertiary amine though has three alkyl groups which can donate electrons to the nitrogen atoms, but this atom is sterically hindered for protonation due to the presence of three sizeable alkyl groups.

2.4 Vanillin

Vanillin is a phenolic aldehyde organic compound with the molecular formula $C_8H_8O_3$. The functional groups include aldehyde, ether, and phenol. It is the primary component of the extract of the vanilla bean. Vanillin Schiff bases have been demonstrated to possess polyvalent metal ions [22]. Condensation product of vanillin with amines confers biological activity; as well as having good complexation ability with metal ions [23-25]. There are only few reports on the synthesis and physicochemical studies of vanillin Schiff-bases and their metal (II) complexes. In this project synthesis, characterization and antibacterial studies of vanillin derivative Schiff-base Co (II) and Cu (II) complexes was conducted.

Vanillin derivatives gained considerable interest in this work because of its potential biological activities, such as antiviral, antibacterial, antimalaria and antitumor activities.

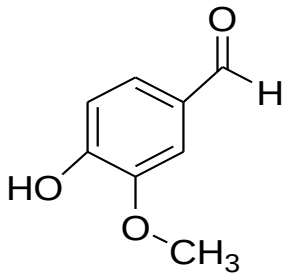
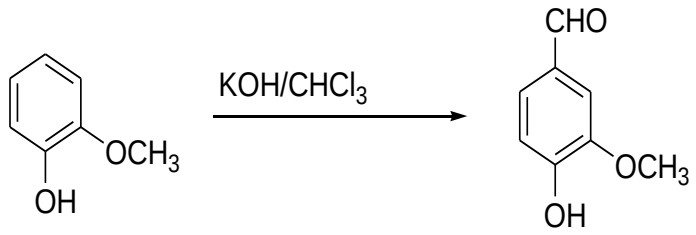


Figure 2 : Chemical structure of 4-hydroxy-3-methoxybenzaldehyde (Vanillin)

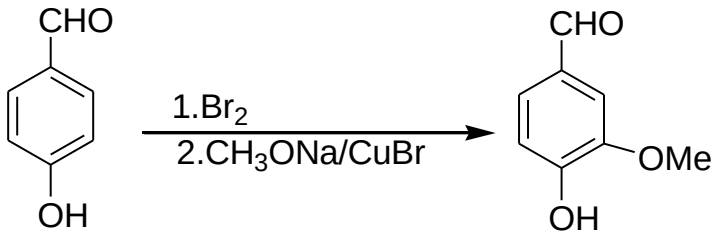
2.4.1 Different Synthetic procedure of vanillin

Karl Reimer et al synthesized in (1876) vanillin from guaiacol.More dramatic is the Riemer-Tiemer method (1876). It is obtained from the eugenol react with the potassium hydroxide and last product found out the vanillin when it refluxed with an alkaline solution of chloroform.[26]



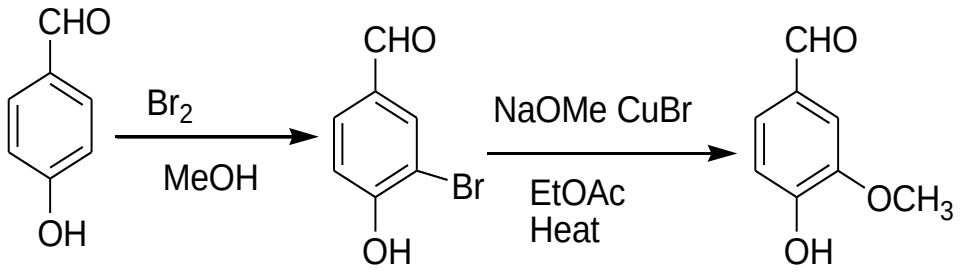
Scheme 5: Synthesis vanillin from eugenol

In October 2007 Mayo Yamamoto of the International Medical Center of Japan won an 1g Nobel Prize for developing a way to extract vanillin from cow dung [27] The developed a convenient two step synthesis of vanillin using electrophilic aromatic substitution, followed by an organometallic methoxylation procedure using copper bromide and sodium methoxide. [28]



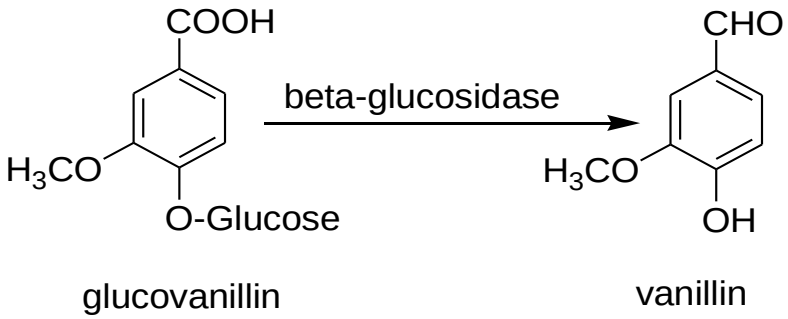
Scheme 6: Synthesis of vanillin from 4- hydroxybenzldehyde

Vanillin synthetic preparation from 4-Hydroxybenzaldehyde involves electrophilic bromination of 4-hydroxybenzaldehyde, and copper-catalyzed methoxylation, given the vanilla fragrance. This method involves two steps, the bromination of 4-hydroxybenzaldehyde to give 3-bromo-4-hydroxybenzaldehyde and Cu-mediated coupling of with methoxide to give the regioselectivity. The initial monobromo product disproportionates easily to starting material and 3, 5-dibromo-4 hydroxybenzaldehyde. Hence, the bromination is complete 30 seconds and the reaction mixture is then carrying directly on to the next step. The Br is replaced with OCH_3 in the presence of Cu- catalyst a pathway that probably involves oxidative addition and reductive elimination Reaction. [29, 30]



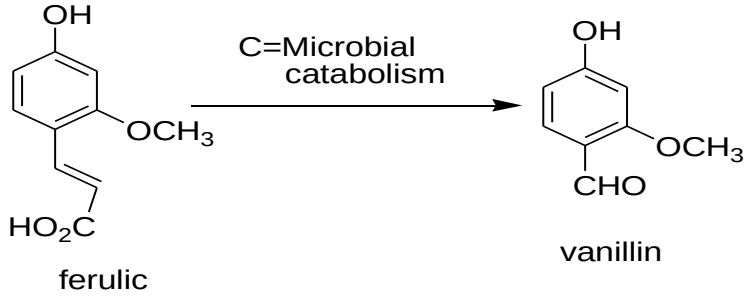
Scheme 7: Synthesis of vanillin from 4-Hydroxybenzaldehyde

Vanillin synthesis from the conversion of glucovanillin by beta –glycosidase enzyme and produced the vanillin [31]**.**



Scheme 8: Synthesis of vanillin from glucovanillin

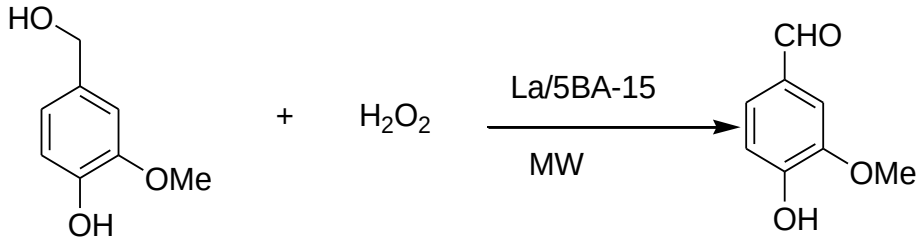
Vanillin synthesis from ferulic acid involves one step by microbial catabolism to give vanillin (Scheme 10) [32]



Scheme 9: Synthesis of vanillin from ferulic

The conversions of *O*-benzylvanillic and vanillin were examined by using whole cells and enzyme preparations of *Nocardia* sp. strain NRRL 5646. With growing cultures, decarboxylated (69% yield. In resting *Nocardia* cells in buffer, 4-*O*-benzylvanillic acid was converted to the corresponding alcohol product without decarboxylation. Purified *Nocardia* carboxylic acid reductase, an ATP and NADPH-

dependent enzyme, quantitatively reduced *O*-benzylvanillic to vanillin [33].



Scheme 10: Synthesis of vanillin from *O*-benzylvanillic

2.5 Schiff bases and their metal complexes as antifungal and antibacterial agents

Infectious diseases that are directly related to bacteria exhibiting multiple resistances to antibiotics have increased world's mortality rate. Therefore, the need to develop novel antibacterial drugs with excellent mechanism of action and structural-activity relationship has become an urgent biomedical necessity [8, 34]. Schiff base ligands and their biologically active complexes which provide potential sites for biochemically active compounds have been concentrated broadly in the course of the last few decade [35] for their antimicrobial [36] and anticancer [37] properties. Schiff base metal complexes are among the versatile models used as organic oxygen transporter [38-40].

Transition metals existing in various naturally occurring bimolecular are key to life process; hence, they can coordinate with O- or N-terminals from proteins in mixed models. They have an important role in the compliance and utility of living macromolecules [41-42], acting as an antimicrobial agent [43].

2.6 Copper

Copper is a bio-essential element with truly unique chemical characteristics in its two relevant oxidation states I and II [44]. According to its position as the highest homologue of group XV in the periodic table, copper is a very special element. The metal-ligand interaction in Cu (II) complexes is frequently ionic and favors the stabilization of Cu (II) state through the pronounced Jahn-Teller distortion. Different extent of axial elongation of the octahedral geometries was produced. Copper is being an essential trace element, is present in parts per million concentration ranges in biological systems. The functions as a key cofactor in a diverse array of biological oxidation reduction reactions [45].Copper containing proteins (hemocyanin, tyrosines, catecol, okidase etc.,) are involved in various processes in living systems [46, 47].

2.7 Cobalt

A wide variety of Co (II) complexes are known to bind dioxygen more or less reversibly and are therefore frequently studied as model compounds for natural oxygen carriers and for their use in oxygen storage, as well as in organic synthesis due to their catalytic properties under mild condition [48].In this respect, Co(II) complexes with N, O-donor ligands containing binding units suitable for the coordination of metal ion. In aprotic solvents, at atmospheric pressure and room temperature, cobalt chelated complexes with Schiff bases catalyses the oxygenation of indole, flavones, nitroalkanes, hydrazones, olefins, etc [49]. The cobalt (II) complexes with Schiff base ligands which coordinate through N, O donor atoms have been extensively studied as oxygen carriers and also as catalyst for water splitting systems [50].

2.8 Effect of complexation on biological activity

The transition metal ions are responsible for the proper functioning of different enzymes. If their concentration exceeds acertain level, then their toxic effects are evident.

It has been found that the activity of the biometals is attained through the formation of complexes and the thermodynamic and kinetic properties of the complexes govern the mode of biological action. Sometimes, the permeability, i.e., lipophilicity of drugs increased through the formation of chelates *in vivo* and the drug action is significantly increased due to much more effective penetration of the drug into the site of action.

Interaction of various metal ions with antibiotics may enhance or suppress their antimicrobial activity but usually in many cases the pharmacological activity of antibiotics after complexation with metals is enhanced as compared to that of the free ligands [51]. Generally it has been observed that transition metal complexes have greater activity and less toxic effects. The preparation and study of inorganic compounds containing biologically important ligands is made easier because certain metal ions are active in many biological processes. The fact that copper, together with magnesium, calcium, iron, zinc, cobalt, chromium, vanadium and manganese are essential metallic elements and exhibit great biological activity when associated with certain metal-protein complexes, participating in oxygen transport, electronic transfer reactions or the storage of ions [52], has created enormous interest in the study of these systems containing these metals [53].

2.8.1 Antimicrobial activity

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that cures a bacterial infection.

In the last years, the attention in this field was oriented to inorganic species among the organic ones. Although many complexes showed a good antimicrobial activity so far only a few are used as metallo antibiotics (antiseptics and antimicrobial) or disinfectants [54]. So far a good antimicrobial activity was observed for complexes bearing a biocation [55] and a multidentate ligand. Among biocations, copper is preferred having in view: (i) the low human toxicity associated with the both presence of albumin in the plasma and the metalotionein in the cytosal; (ii) the borderline character and the fact that forms the most stable complexes among the Irving William series of cations; (iii) the stereo chemical versatility; (iv) the easiness to change its oxidation state and; (v) the known biological activity including the ability to inhibit enzyme,

Different microbial species were used to screen the possible antimicrobial activity of the synthesized metal complexes. Of the species used, *Staphylococcus aureus* is one of the most common gram-positive bacteria causing food poisoning. Its source is not the food itself, but the humans who contaminate foods after they have been processed. Gram-negative bacteria are represented by *Escherichia coli*, which belong to the normal flora of humans. However, an enterohemmoragic strain of *E. coli* has caused serious cases of food poisoning and preservatives to eliminate its growth are needed. A clearly visible spoiling agent of bakery products is the mold that forms black-centered spots on the surface of products is *Aspergillus Niger*. A typical opportunist, *Candida albicans* is the microbe responsible for most clinical yeast infections, e.g., in mouth infections.

3. MATERIALS AND METHODS

3.1 Instruments

The UV spectra of compounds were recorded by UV-Vis Spectrophotometer (UV-950, Shimadzu). The FT-IR spectra were record by a Perkin-Elmer FT-IR 1650 spectrophotometer in wave number region of 4000-400 cm^{-1} as KBr pellet. The NMR spectra was recorded using Bruker avance 400MHz using DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. The magnetic susceptibility data were measured by (MSB-AUTO, (Sherwood scientific) magnetic balance). The purity of the Schiff base and its complexes were confirmed by thin layer chromatography (TLC) on silica gel plates and TLC visualization was realized by UV-light and iodine**.**

3.2 Chemicals and reagents

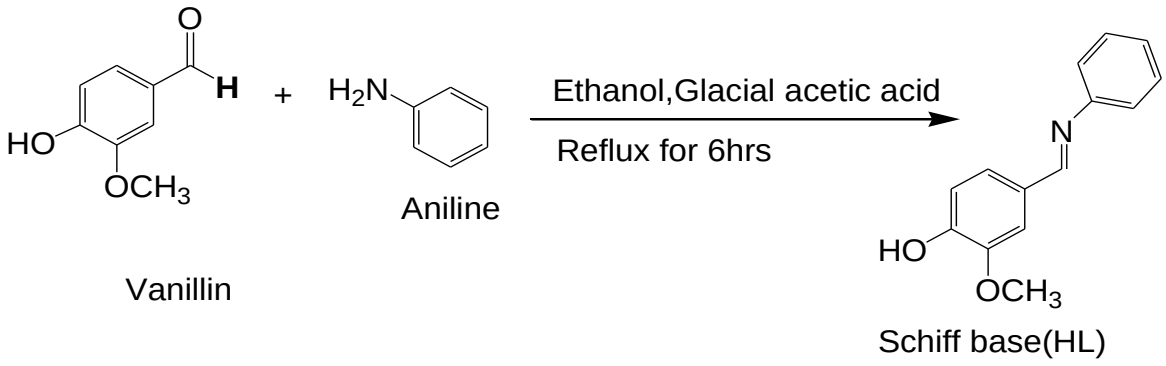
Analytical grade reagents and chemicals were used without further purification. The reagents and chemicals used in this project include: Aniline, Vanillin, 35%HCl, Glacial acetic acid, NaOH, Silca gel, metal salts such as $CoCl_2.6H_2O$ and $Cu(CH_3COO)_2.H_2O$ were used. The solvents include, DMSO-d_6, distilled water, ethanol, Methanol, n-hexane, Ethyl acetate, dichloro methane and diethyl ether (Aldrich) was used in this study.

3.3 Experimental sections

Synthesis of the required Schiff base ligand and metal complexes was carried out in Applied Chemistry program PG Research laboratory of Adama Science and Technology University. ^{1}H-NMR and ^{13}C-NMR, FT-IR, UV-Vis spectroscopy and Magnetic susceptibility was carried out in chemistry department of Addis Ababa University.

3.3.1 Synthesis of Aniline-Vanillin derivatives Schiff base ligand

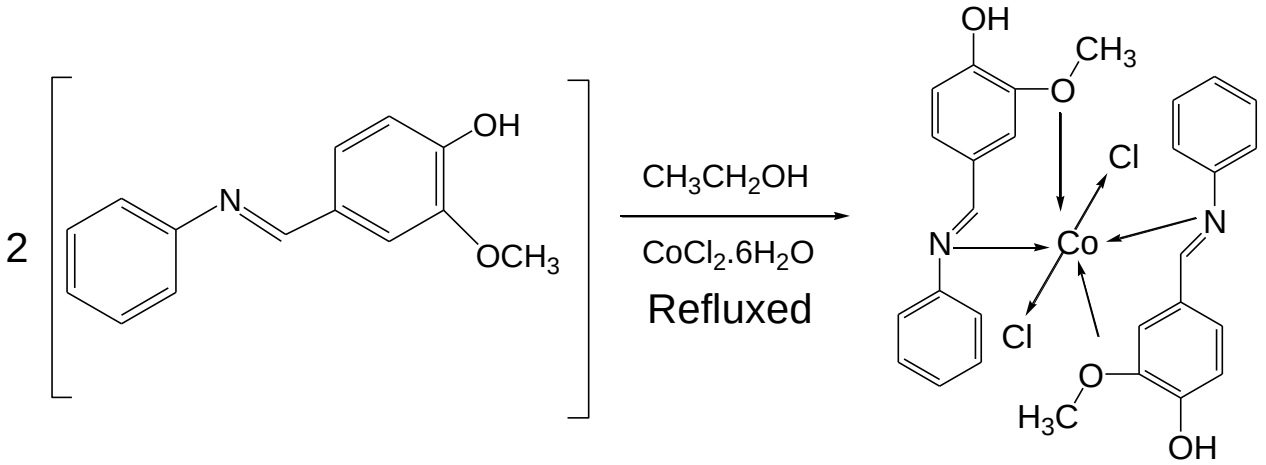
Equimolar ratio of vanillin (3.04g, 20mmol) was mixed with Aniline (1.86g, 20mmol) in 40mL ethanol and a few drops of glacial acetic acid were added as catalyst. The reaction mixture was heated under reflux for 6h at 60℃ (See Scheme 12).The product obtained was filtered using wakmann filter paper number one and washed with n-hexane and diethyl ether and dried in vacuum desiccators' overnight. A yellow precipitate of the ligand was formed and the purity was checked by TLC (81% yield).



Scheme 11: Synthesis of Schiff base from Vanillin and Aniline

3.3.2 Synthesis of Aniline-Vanillin derivative Schiff base ligand Co (II) complex

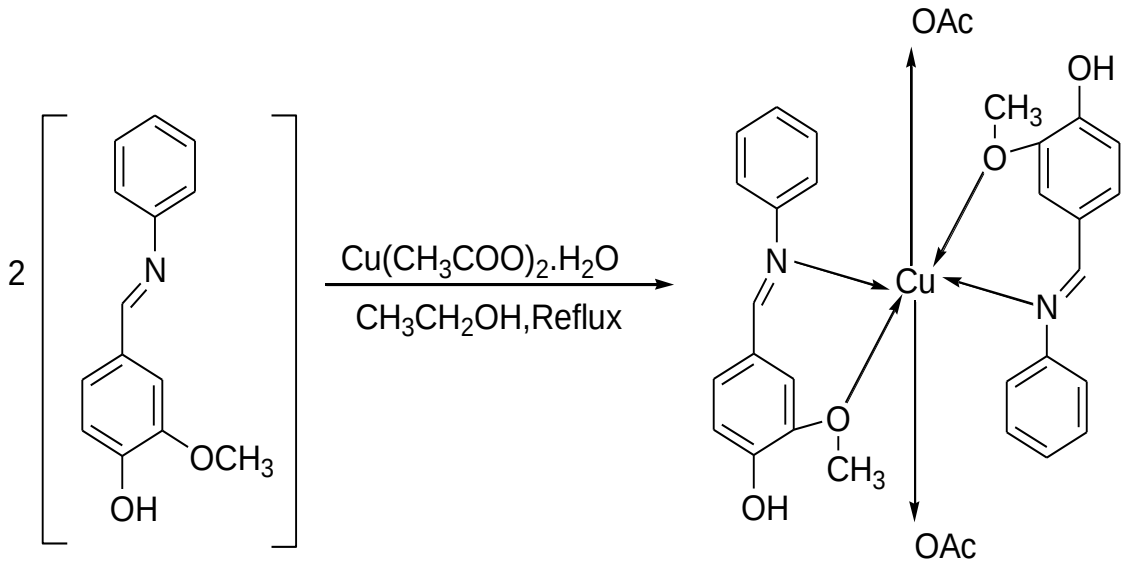
Vanillin-Aniline derivative of Schiff base Co(II) complex were prepared by mixing 25mL of (1.36g, 6mmol) ethanolic solution of Schiff base with 25mL ethanolic solution of (0.714g, 3mmol) of $CoCl_2.6H_2O$ metal (II) salt keeping ligand-metal ratio (2:1) in ethanol and a few drops of glacial acetic acid were added in drops to act as catalyst for the reaction. The reaction mixture was refluxed for 4h on water bath until precipitate was obtained. The colored (Co^{+2}-brown) precipitate so formed was filtered using wakmann filter paper number one and then washed several times using small portions of distilled water and n-hexane until to be free from impurities and re-crystallized from the same solvents and dried in vacuum over $CaCl_2$.(See Scheme 13)



Scheme13: Proposed structure of Co (II) Schiff base complex

3.3.3 Synthesis of Aniline-Vanillin derivative Schiff base ligand Cu (II) Complex.

The metal complex of Cu(II) were prepared by mixing the ethanolic solution(0.59g,3mmol) of ($Cu(CH_3COO)_2.H_2O$ and (1.36g,6mmol) of the ligand keeping metal: ligand ratio of 1:2 in 30mL ethanol and a few drops of glacial acetic acid were added in drops to act as catalyst for the reaction . The solution was heated under refluxed in water bath for 6h at 75℃.The precipitate so formed was filtered using wak mann filter paper number one and washed several times with distilled water and n-hexane until to be removed unreactive metal if any, to get the light green pure product. Finally, the product was dried in vacuum desiccators' overnight and recrystallized from ethanol (See Scheme 14).



Scheme 13: Proposed structure of Cu (II) Schiff base complex

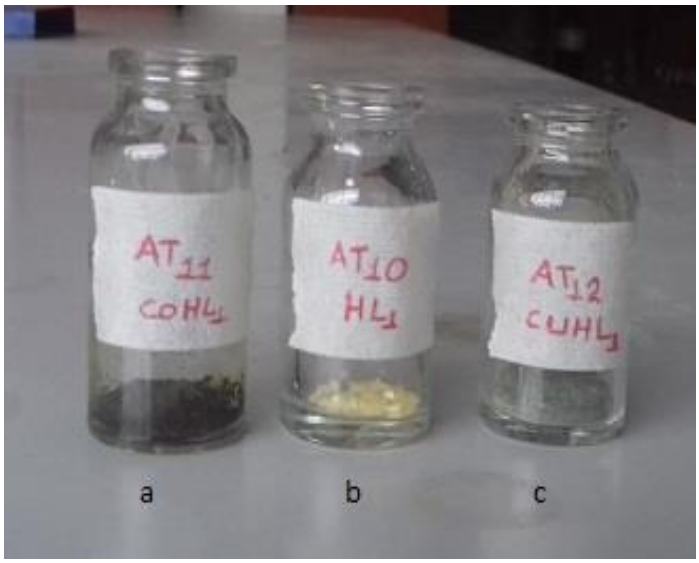


Figure 3: (a) Precipitate of Co (II) complex (b) yellow crystal of Schiff base (c) Precipitate of Cu (II) Complex .

3.4 Test of Purity of the Products

The purity of the Schiff base and the complexes was checked by using the thin layer chromatography (TLC) method, conducted on silica coated aluminum plates as stationary phase and dichloromethane, ethyl acetate and n-hexane as a mobile phase. A single spot in all samples indicates that Schiff base and complexes are may be pure.

Figure 4 TLC profile of CuHL1 Complex

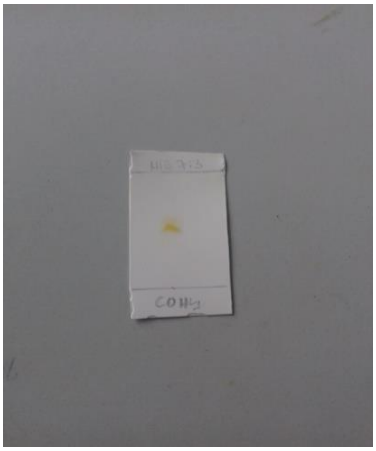


Figure 5 TLC profile of CoHL1 complex

3.5 Characterization Techniques

3.5.1 Spectroscopic techniques

The Schiff base and its Co(II) and Cu(II) complexes were characterized by means of ^{1}H-NMR,^{13}C-NMR,FT-IR,elemental analysis, magnetic susceptibility and UV-Vis spectroscopy. NMR spectral data was applied to establish the nature and structure of Schiff bases, as well as their complexes synthesized. The NMR spectra of Schiff bases were recorded in Dimethylsulfoxide (DMSO-d_6) solution, using tetramethylsilane (TMS) as internal standard.

The FT- IR Spectra was recorded using KBr pellet in the region of 4000-400cm^{-1}. Far IR spectrum was recorded in the region of 750-250cm^{-1} using IR Prestige 21-Shimadzu spectrophotometer. The UV-Visible spectra of the complexes was recorded on Shimadzu UV-1700 spectrometer in the range of 220-600nm in DMF solution.

3.5.2 Elemental Analysis of Schiff base and its metal complexes

Elemental analysis was applied to determine the percentage composition of C,H,N and metal ions found in the synthesized Schiff base as well as the complexes. The elemental analysis data was recorded in EA 1112 Flash CHNS/O- analyzer in conditions of carrier gas flow rate of 120 mL/min, reference flow rate 100 mL/min, oxygen flow rate 250 mL/min; furnace temperature of 900℃ and oven temperature of 75℃. Four calibration points for every component and samples were run in duplicate and the average values are to be taken.

3.5.3 Magnetic Susceptibility

Magnetic susceptibility measurement of the powdered Co (II) and Cu (II) complexes was recorded at 21℃ using (MSB-AUTO, (Sherwood scientific) magnetic balance) model; 7550, Hg [Co (SCN) $_4$] as the standard. The molar susceptibility, χ_m, is calculated by:

$$\chi_m = \chi \text{ (F.W.in g mol-1) and has c.g.s. units of erg•G}^{-2}\text{ mol}^{-1}.$$

The values for effective magnetic moment μ_{eff} of Co (II) and Cu (II) complexes under investigation were calculated from the equation given below:

$$\mu_{eff} = 2.828(\chi AT)^{1/2}$$

Where T is temperature in Kelvin scale.

The measured μ_{eff} can be compared to the calculated value, μs, from the spin-only formula. The diamagnetism factors for common ligands and ions as well as the Pascal constants used to calculate the diamagnetic corrections for complex ligands can be found:

$$\boldsymbol{\mu_{eff}} = (8\,\chi_{corr}T)^{1/2}$$

In addition, using the value obtained for the effective magnetic moment of each complex, the number of unpaired electrons present in the metal ions was also calculated by using the relation:

$$\boldsymbol{\mu_{eff}} = [n\,(n+1)]^{1/2}$$

Where n is number of unpaired electron.

However, the diamagnetic contribution is always negative; the corrected molar susceptibility should always be greater than the uncorrected value [56]

3.5.4. Molecular weight determination (Rast's method)

In this method 0.1 g of the ligand was mixed with 1 g of camphor. The mixture was ground and melted in a crucible to ensure homogeneity. The mixture was taken in a capillary tube sealed at one end and the freezing point of the molten mixture was determined using melting point apparatus. Then $\Delta T_{f's}$ was noted and molecular weight of compound (Ligand) was calculated from the Rast's formula as follows:

$$M = K_f \times w \times 1000\ \Delta T_{f's} \times W$$

Where: K_f = Molecular depression (Cryoscopic) constant for camphor (39.7)

w = Weight of unknown compound (ligand), W= Weight of camphor

ΔT = Depression of melting point

3.6 Antibacterial Activity

The *in vitro* biological screening effects of the investigated compounds were tested against selected bacterial strains *E.coli*, *S. aureus*, *K.pneumonia* and *Protein mirabilis* by the cup plate method at 0.05 mg/mL concentration. The bacterial cultures were inoculated in nutrient broth (inoculation medium) and incubated overnight at 37℃°. Inoculated medium containing 24 h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get a uniform distribution. This solution was poured (25 mL in each dish) into Petri dishes and then allowed to attain room temperature. Wells (6 mm in diameter) were punched carefully using a sterile cork borer and were filled with test solution 200 μL. The plate is allow to stand for an hour in order to facilitate the diffusion of the drug solutions, then the plates were incubated at 37℃ for 24h and the diameter of the zone of inhibition was measured [57]. The results were compared with those of standard drug Gentamicin for bacterial activity of the same concentration as that of the test compounds under identical conditions.

4. RESULTS AND DISCUSSION

4.1 Physical Characteristics of Schiff base and its metal complexes

Physical properties of the synthesized compounds along with analytical data are summarized in Table-1 below. The Schiff base ligand on interaction with Co (II) and Cu (II) formed complexes with moderate yields (53-57%). Elemental analyses data of the observed and calculated values for hydrogen, carbon and nitrogen compositions of the Schiff base and its metal complexes are in good agreement. The melting points of the free ligand and its metal complexes was found to be within the range of 134-180℃. The complexes vary in color depending on metal ions and are generally soluble in most organic solvents such as Dimethylsulfoxide (DMSO-d_6), dimethylformamide (DMF), hot methanol, hot ethanol, acetone, dichloromethane and distilled water, but insoluble in n-hexane and diethyl ether.

Table 1 Physical characteristics and analytical data for the Schiff base ligand and the metal (II) complexes

Compound	Molecular formula	Molar mass(g/mol)	Color	Melting point(℃)	Yield (%)
HL_1	$C_{14}H_{13}NO_2$	227	Yellow	180	84
$[Cu(L_2)]$	$Cu(C_{14}H_{13}NO_2)_2$	651	Light green	145	53
$[Co(L_2)]$	$Co(C_{14}H_{13}NO_2)_2$	619	Brown	134	57

Table 2 Elemental Analysis data of Schiff base and its metal complexes.

Compound	Molecular formula	Molecular mass g/mol	Elemental analysis,%				
				C	H	N	M
HL_1	$C_{14}H_{13}NO_2$	227	Calc	74.008	5.727	6.167	-
			Found	(73.18)	(5.76)	(6.01)	-
$[Co(L_2)]$	$Co(C_{14}H_{13}NO_2)_2$	619	Calc	54.28	6.14	4.52	9.21
			Found	(53.92)	(6.26)	(4.48)	9.18
$[Cu(L_2)]$	$Cu(C_{14}H_{13}NO_2)_2$	651	Calc	58.98	5.22	4.30	9.37
			Found	(57.68)	(4.93)	(4.02)	9.34

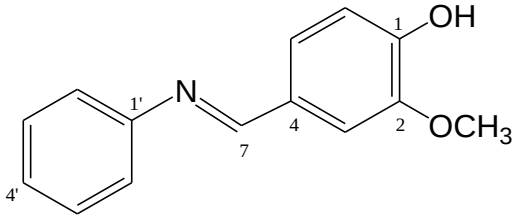
4.2 Characterization Techniques

4.2.1 NMR Spectral studies of Schiff base ligand (HL)

The NMR data of the Schiff base ligand are given in (Table-3). ^{1}H NMR spectrum of the free ligand (Appendix 1) (DMSO-d_6) revealed a singlet peaks at δ_H 3.8, 9.8 and δ 8.5 suggesting the presence of methoxy, hydroxyl, and proton of CH=N group respectively. There are eight aromatic protons from the two substituted benzene rings are clearly evident at δ 6.9-7.6[58, 59].

The ^{13}C-NMR spectrum (Appendix II) of the free ligand revealed peak at δ_C 160.5 attributed to carbon atom of azomethine (N=CH) group. A signal at δ_C 152.4 and 150.7 attributed to sp^{2}oxygenated quaternary aromatic carbons where a hydroxyl and methoxy group is attached. The signal appearing δ_C 148.5 belongs to quaternary aromatic carbon (C-N) group where the nitrogen is attached. The eight signals between 129-110.9ppm belong to aromatic methines. The presence of peak at δ_C 56ppm confirms the presence of methoxy group in the Schiff base ligand.

Table 3 ^{1}H NMR and ^{13}C NMR spectra data of Schiff base ligand ($C_{14}H_{13}NO_2$).



Position	**^{13}C-NMR** (δ,ppm)	**^{1}H-NMR** (δ,ppm)
1	152.4	9.8 (OH)
2	150.7	-
3	115.8	7.1 (d, J= 1.2)
4	129.5	-
5	110.9	7.2 (dd, J= 1.2, 8.0)
6	124.7	6.95 (d. J= 8.0)
7	160.6	8.4 (-N=CH)-
OCH_3	56.00	3.8
1’	148.52	-
2’,6’	125.7	7.65
3’,5’	121.3	7.3
4’	128.4	7.3

4.2.2 FT-IR Spectral interpretation

The IR frequencies of the complexes are given in (Table-4). The IR spectrum of free ligand is compared with that of complexes in order to determine the co-ordination sites that may have involved in chelation. The bands had been observed at 3050-3090cm^{-1} and 2953-2935 cm^{-1} in the spectra of both Schiff bases and Co (II) and Cu (II) complexes are attributed to hydroxyl group and aliphatic sp3 C-H vibrations, respectively. There is significant shift observed for the vibration of the hydroxyl group in the complexes as pet the presence of broad peaks at 3600-3200cm^{-1} and 3421-3176cm^{-1} for Co (II) and Cu (II) complexes, respectively. This suggests that the oxygen atom of the hydroxyl group does not participate in coordination.

A very weak intensity bands had been observed at 3090cm^{-1} in the spectra of Schiff base ligand and 3088 for Co (II) complex and 3050 cm^{-1} for Cu (II) complexes are due to hydroxyl group. Similarly, the presence of aliphatic sp3 C-H is evident from the vibrations at 2935 cm^{-1}, 2953 cm^{-1} and 2950 cm^{-1} for free ligand, Co(II) and Cu(II) complexes, respectively.

Another strong bands appeared at 1585 cm^{-1} in the spectra of Schiff base ligand is the characteristics of azomethine ν(C=N) stretching vibration. This vibration underwent a bath chromic shift to lower and higher frequency region at 1583cm^{-1} and 1646cm^{-1} upon complexation with Co (II) and Cu (II), respectively (Table 4). This is attributed to the bonding affinity of nitrogen atom of the azomethine group of the Schiff base to the respective metal ions directly linked to the donation of electrons from nitrogen to the empty d-orbital's of the metal atom [60].The presence of aromatic rings has been identified by the characteristic ring vibrations of sp2 CH of aromatic ring at 1500cm^{-1} regions.

The strong intensity bands had been observed at 1428 cm^{-1}in the spectra of the Schiff base ligand which indicates ν(C—O) stretching frequencies which has been shifted towards the lower frequency region around 1284and 1304cm^{-1} in the spectra of Co (II) and Cu (II) complex, respectively. This suggest that the coordination of the metal ion with oxygen atom of the methoxy (O-CH_3) group.

The band appeared at 1516 cm^{-1} was assigned to C=C stretching frequency of the aromatic group of the free ligand, which has been shifted to 1515cm^{-1} and 1585cm^{-1} for Co(II) and Cu(II) complex, respectively. The appearance of new band between 552-510 cm^{-1} and 492-483 cm^{-1}are indicate the formation of M-N and M-O bond in the complexes [61, 62].

Table 4 IR spectra data of metal complexes

Compound	ν(C=N) cm^{-1}	ν(O-H) cm^{-1}	ν(C=C) cm^{-1}	ν(C-O) cm^{-1}	ν(C-H) Aromatic stretching cm^{-1}	ν(C-H) Aliphatic stretching cm^{-1}	ν(M-O) cm^{-1}	ν(M-N) cm^{-1}
HL	1585	3413	1516	1428	3090	2935	-	-
$[Co(L_2)]$	1583	-	1515	1284	3088	2953	552	492
$[Cu(L_2)]$	1646	3421-3176	1585	1304	3050	2950	510	483

4.2.3 Magnetic susceptibility measurement

The magnetic properties of complexes in terms of unpaired electrons and their magnetic or spin properties are useful in determining structural features in transition metal compounds. Complexes that contain unpaired electrons are paramagnetic and are attracted into magnetic fields. Diamagnetic compounds are those with no unpaired electrons repelled by a magnetic field. The magnetic moments of the complexes which were measured at (21℃) are noted in (Table 5). The measured gram magnetic susceptibility of Co (II) and Cu (II) complexes at 21℃ was 1.606 $x10^{-5}$ and 1.855 x 10^{-6}cgs unit, respectively. The molecular weight of the Co (II) complex was 619 g/mol and that of Cu (II) complex was 651 g/mol. In the case of Co (II) complexes, the high spin and low spin μ_{so} value should be 3.88 BM and 1.73 BM, respectively. The Co (II) complex μ_{obs} value was 4.83 BM. This result confirms that the Co (II) complex should be high spin complex with 3 unpaired electrons and paramagnetic.

In the case of Cu (II) complex, μ_{so} value should be 1.73 BM with one unpaired electron. The Cu (II) complex observed μ_{obs} value was 1.69BM. This result confirms that the Cu (II) complex should be low spin complex with one unpaired electrons and paramagnetic. From the calculated magnetic moment values and literature data, the expected geometry was octahedral and distorted octahedral geometry for Co (II) complex and Cu (II) complex, respectively. The high observed spin values indicates spin-exchange interaction between metal ions and spin orbital coupling [63]

Table 5 Magnetic susceptibility and magnetic moment data

Compound	M.wt ($g.mol^{-1}$)	Gram Susceptibility (Cgs)	Molar conductivity(X_{mol}) (Cgs)	Diamagnetic correction (XD) Cgs	μ_{eff} (BM)
$[Co(L_2)]$	619	1.606×10^{-5}	9.94×10^{-3}	9.92×10^{-3}	4.83
$[Cu(L_2)]$	651	1.855×10^{-6}	1.22×10^{-3}	1.21×10^{-3}	1.69

4.2.4 The electronic spectral data

The electronic absorption spectra of Schiff base and its metal (II) complexes were recorded in the range of 600nm to 220nm at room temperature.

4.2.4.1 Electronic spectral studies of Schiff base

There are three absorption bands in the spectra of Schiff base ligand which can be assigned $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. These transitions can be shifted towards lower and higher frequencies due to the coordination of the Schiff base to the metal ions [64]. The spectral bands which was observed at 313nm (31,948 cm^{-1}) and 280nm (35,714cm^{-1}) can be attributed to $n \rightarrow \pi^*$ transitions of C=N and C-O chromophores. The spectra bands which was observed at 231nm (43,290cm^{-1}) can be attributed to $\pi \rightarrow \pi^*$ transition of C=C chromophore.

4.2.4.2 Electronic spectral studies of Co (II) complex

The electronic spectra of Co (II) complex exhibited band at 438nm, which were assigned to the transition $^4T_1g \rightarrow {}^4T_1g$ (F) for a high spin octahedral geometry [65]. The band at 231nm (43,290cm^{-1}), 285nm (34722cm^{-1}) and 328nm (30395cm^{-1}) was assigned to charge transfer transitions. The magnetic susceptibility measurements (4.83 B.M) for Co (II) complex are also three unpaired electrons per Co (II) ion consistent with their octahedral environment [66].

4.2.4.3 Electronic spectral studies of Cu (II) complex

The electronic spectra of Cu (II) complex exhibited band at 404nm (24,752cm^{-1}) due to LMCT and spectra at 533nm (18,762cm^{-1}) which were assigned to the d-d transition $^{3}A_{2}g \rightarrow {}^{3}T_{2}g$ for a low spin distorted octahedral geometry [67]. The band at 232nm (43,103cm^{-1}), 285nm (34,722cm^{-1}) and 318nm (31,446cm^{-1}) was assigned to internal charge transfer of the Schiff base. The magnetic susceptibility measurements (1.69 B.M) for Cu (II) complex are also one unpaired electrons per Cu (II) ion consistent with their distorted octahedral environment.

Table 6 : Electronic spectra data of SBs and its metal complexes

S No.	Compound	λ_{max} (nm)	Assignments	μ_{eff} (B.M)
1	$L=C_{14}H_{13}NO_2$	231	$\pi \rightarrow \pi^*$	-
		280	$\pi \rightarrow \pi^*$	
		313	$n \rightarrow \pi^*$	
2	$[Co(L)_2]$	231	INCT	4.83
		285	INCT	
		328	INCT	
		438	$^{4}T_{1}g \rightarrow {}^{4}T_{1}g$ (F)	
3	$[Cu(L)_2]$	232	INCT	1.69
		285	INCT	
		318	INCT	
		404	LMCT	
		533	$^{3}A_{2}g \rightarrow {}^{3}T_{2}g$	

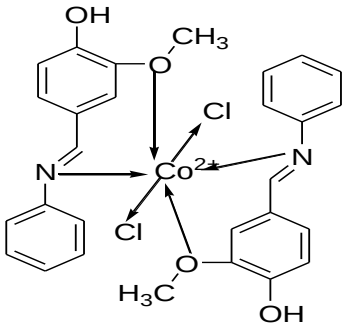


Figure 6: Proposed structure of Co (II) complexes

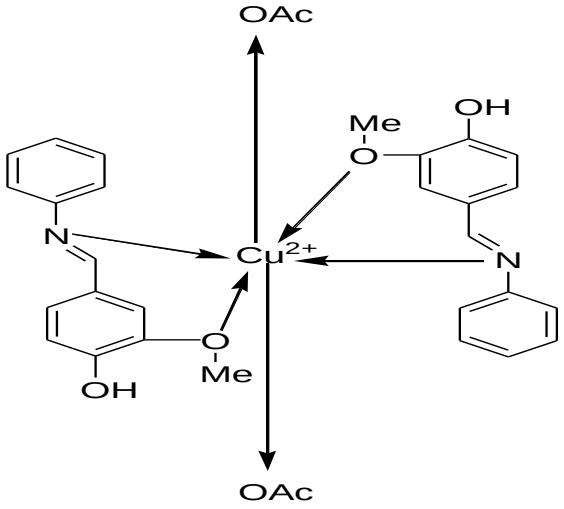


Figure 7: Proposed structure of Cu (II) complex

4.3 Antibacterial activity

The result of *in vitro* antibacterial activity of the Schiff base ligand and its Co (II) and Cu (II) complexes are presented in Table 6. Gentamicin was used as positive standard and methanol was used as negative control for these antibacterial activities. Zone of Inhibition of the Schiff base ligand and its metal complexes were determined at a concentration of 0.05 mg/ml. The Schiff base, Co (II) and Cu (II) complexes were found with promising antibacterial activity against four selected pathogenic bacterial strains (*S.aureus, E.coli, K.Pneumonia* and *Protein mirabilis).*However, the Schiff base ligand showed more activity towards *K.Pneumonia* when compared to that of standard drug gentamicin and Co (II) and Cu (II) complexes. The lower antibacterial activity of metal complexes is may be due to strong interaction between the imine moieties and the metal ions. This type of interaction reduces the activity of imine moieties to ward inhibition of bacterial activities [68, 69].

Cu (II) complex showed more antibacterial activity when compared to the Schiff base and Co (II) complex against *E. coli*, but less active compared to the standard drug. In addition, Co (II) complex is much better than the Schiff base and Cu (II) complexes in its antibacterial activity against *S. aureus* and *protein mirabilis* with zone of inhibition of 13mm. Both complexes and Schiff base are low activity against *E.coli* and *protein mirabilis* when compared to Gentamicin. The variation in the activity of different metal complexes against different microorganisms depends on the impermeability of the cell or differences in the ribosomes in the microbial cells. In the present study, the low activity of Schiff base and some metal complexes against different bacterial strain compared to the standard gentamicin may be due to their low lipophilicity, because of which the penetration of the compound through the lipid membrane was decreased, and they couldn't block or inhibit the growth of microorganisms [70].Generally, the Schiff base showed a very promising activity (17mm) against *K.pneumonia* compared to that standard (15mm) and the Cu (II) complex also showed promising activity (13mm) against E. coli compared to that of standard drug (14mm).

Table 7: Antibacterial activities of the synthesized Schiff base and its metal complexes.

S.No	Compound	Inhibition zone			
		Bacteria			
		Gram +ve	Gram –ve		
		S.aureus (mm)	E.coli (mm)	K.pneumonia (mm)	Protein mirabilis (mm)
1	HL_1	9	12	17	10
2	$[Co(L_2)]$	13	6	8	13
3	$[Cu(L_2)]$	8	13	8	10
7	Methanol(control)	-	-	-	-
8	Gentamicin	21	14	15	20

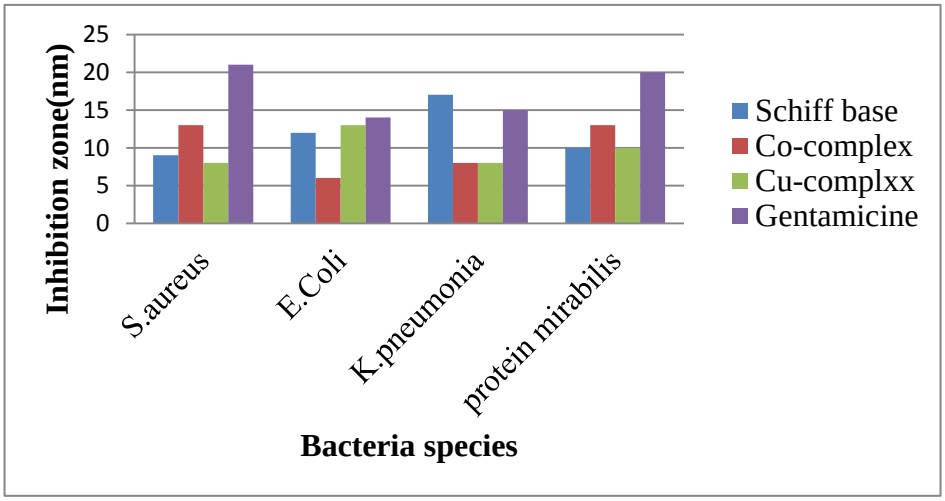


Figure7: The antibacterial activity of Schiff base and its metal complexes

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

A novel Schiff base ligand was synthesized by the reaction of aniline and vanillin in 1:1 molar ratio with a molecular formula of $C_{14}H_{13}NO_2$. The complexes of Co (II) and Cu (II) were synthesized by direct reaction of the synthesized ligand with $CoCl_2.6H_2O$ and Cu $(CH_3COO)_2.H_2O$ metal salts both in 2:1 ratio. The synthesized ligand and its metal complexes were fully characterized by FT-IR, UV-Vis, ^{1}H-NMR,^{13}C -NMR, Elemental analyzer, AAS and magnetic susceptibility measurements. From the electronic spectrum and magnetic moment data it can be showed that the Co (II) and Cu (II) complexes formed have octahedral and distorted octahedral geometry respectively. The IR spectra of Schiff base and its metal complexes imply that the Schiff base ligand behaves as basic bidentate ligand coordination through the Azomethine nitrogen atom and methoxy of oxygen atom. The fifth and sixth coordination sites being occupied by chloride and OA_C .The screening of biological activities of ligand and its complexes were conducted against four bacteria (*S.aureus, E.coli, and K.Pneumonia* and *Protein mirabilis).* Generally, the Schiff base showed a very promising activity (17mm) against *K.pneumonia* compared to that of standard (15mm) and the Cu (II) complex also showed promising activity (13mm) against E. coli compared to that of standard drug (14mm).

5.2 Recommendations

- The synthesized Schiff base and its metal complexes need further studies on other bacterial and fungal strains.
- Further work is recommended to synthesize a series of Cu (II) and Co (II) analogues of vanillin-aniline Schiff base ligand and conduct SAR study to search for a potential drug candidate.

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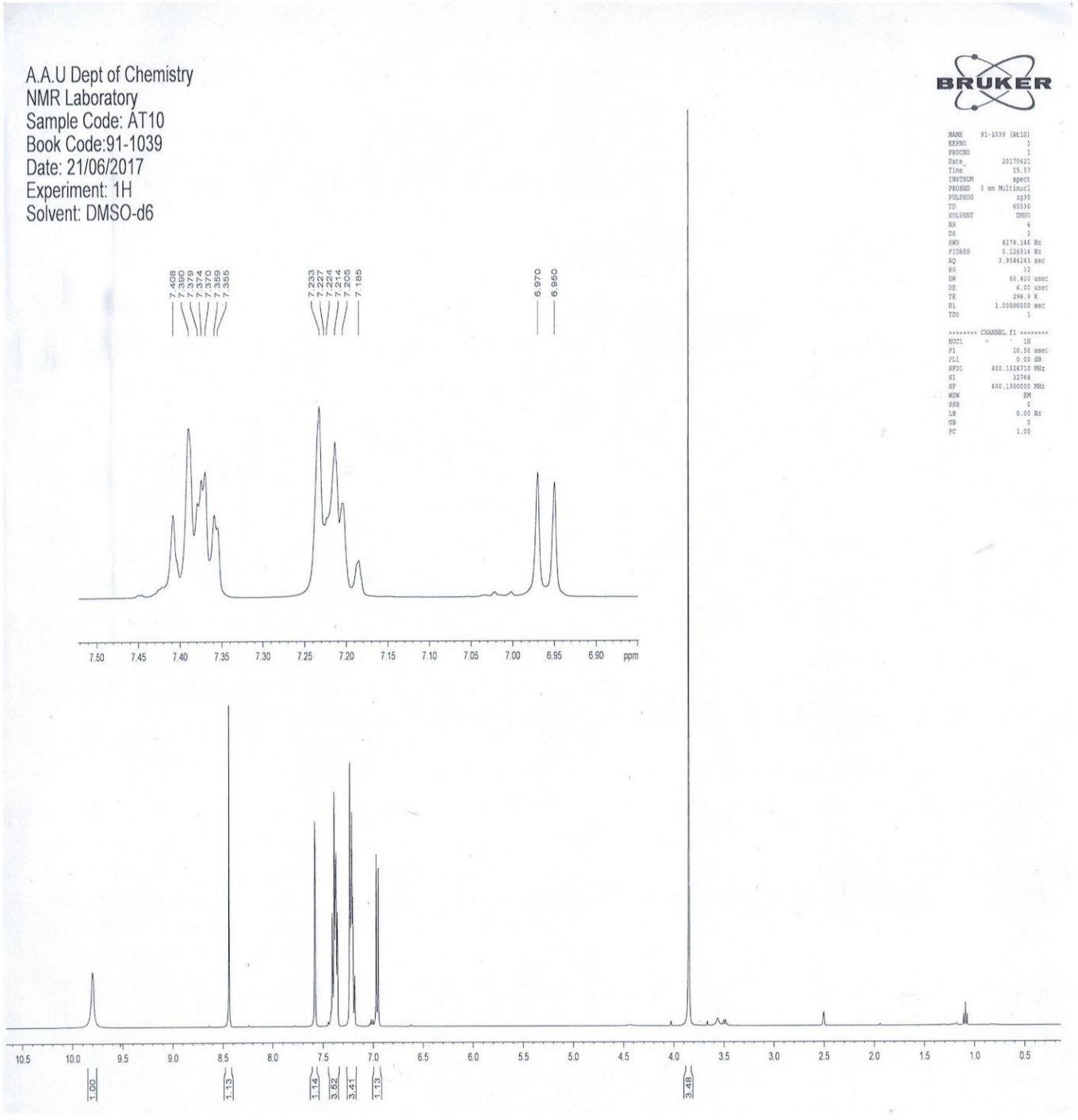
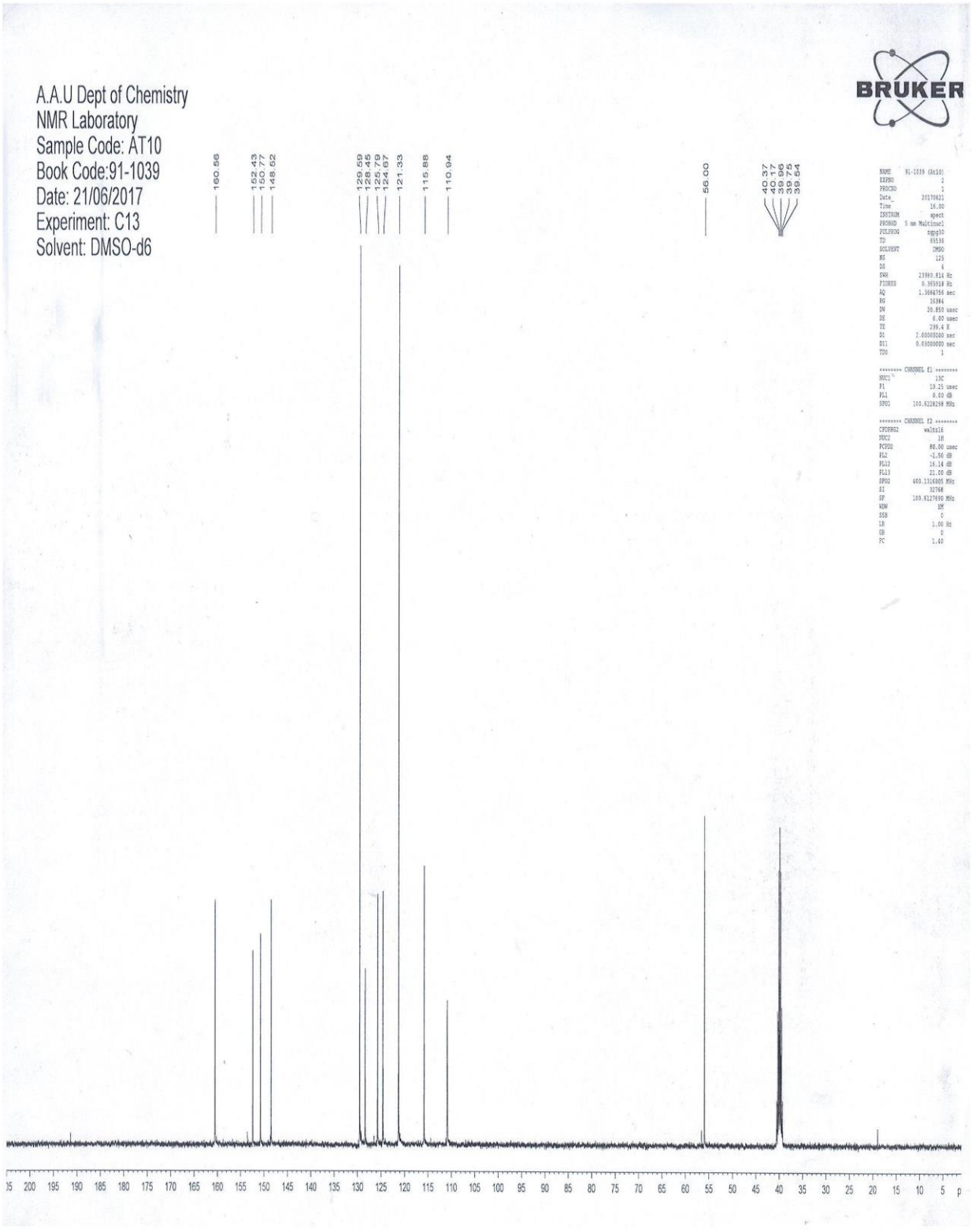
APPENDICES

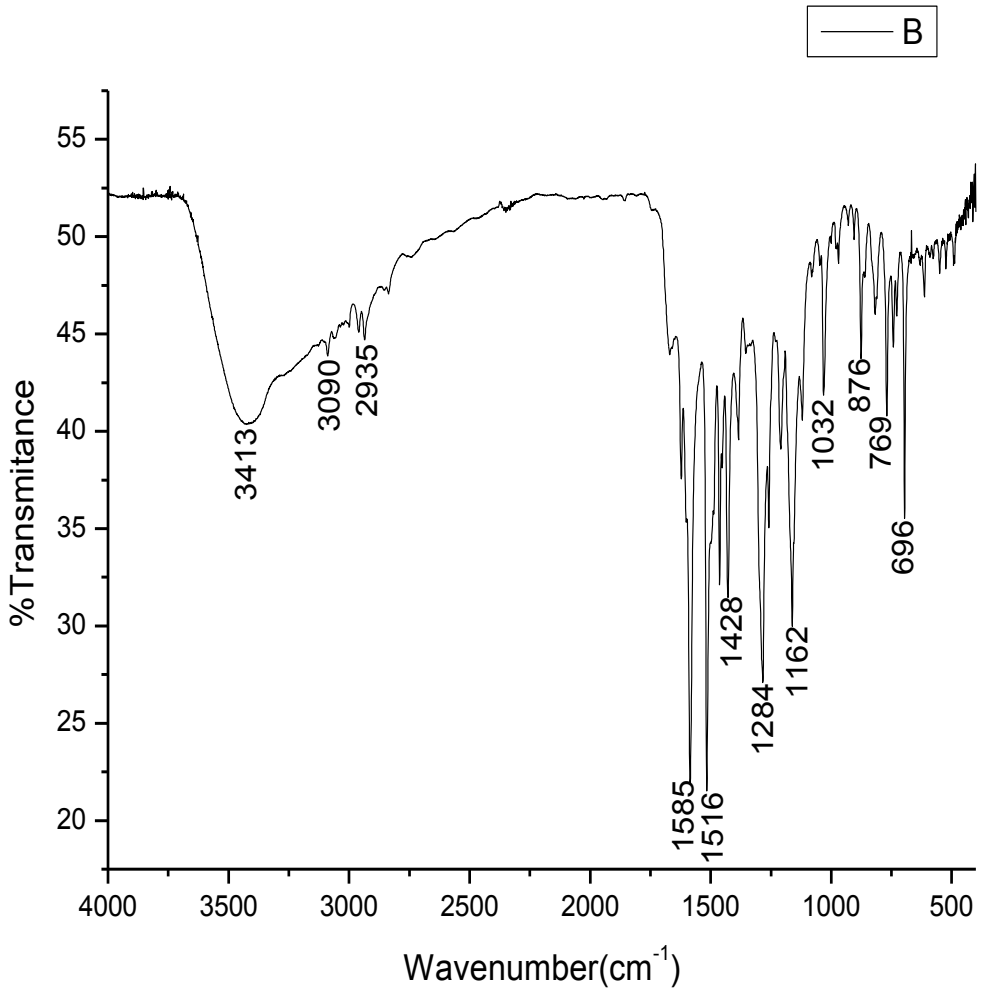
Appendix 1: ^{1}H- NMR spectrum of Schiff base ligand.



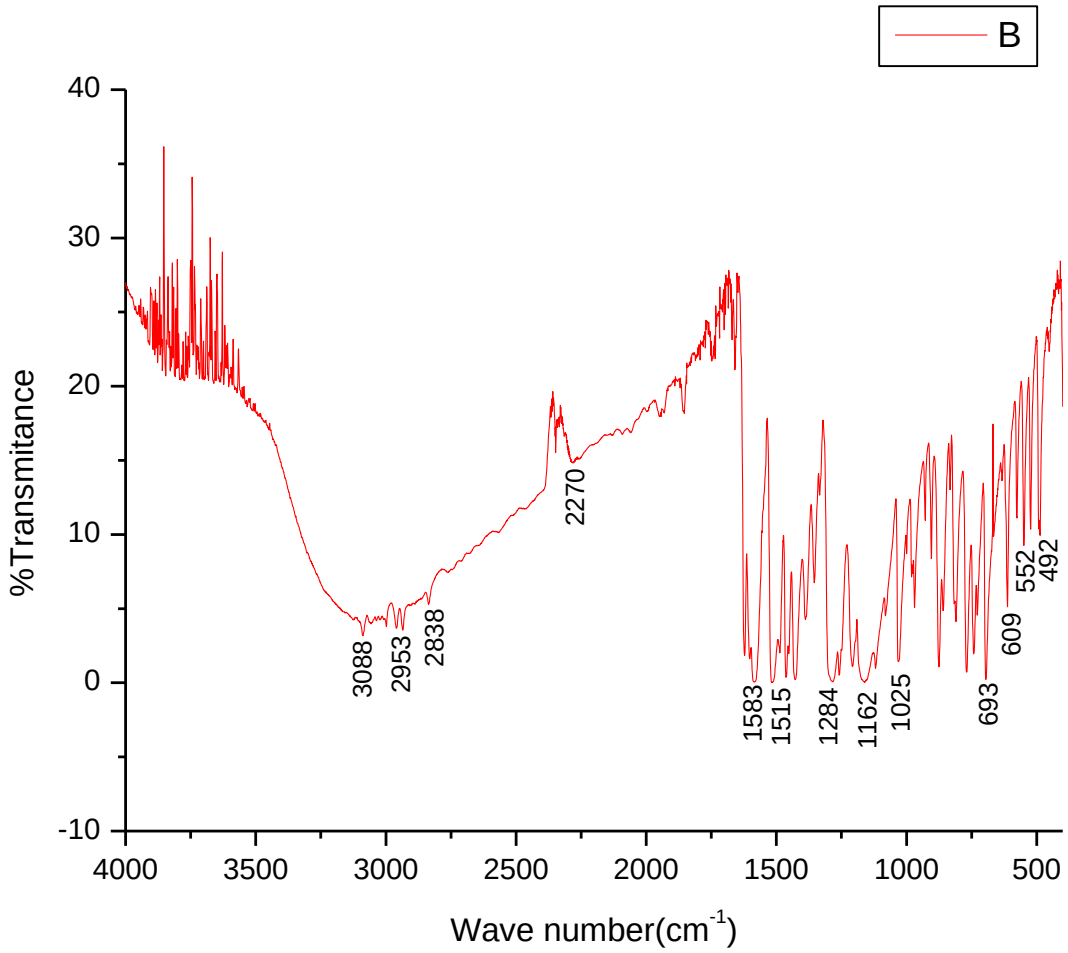
Appendix 2: ^{13} C -NMR spectrum of Schiff base ligand



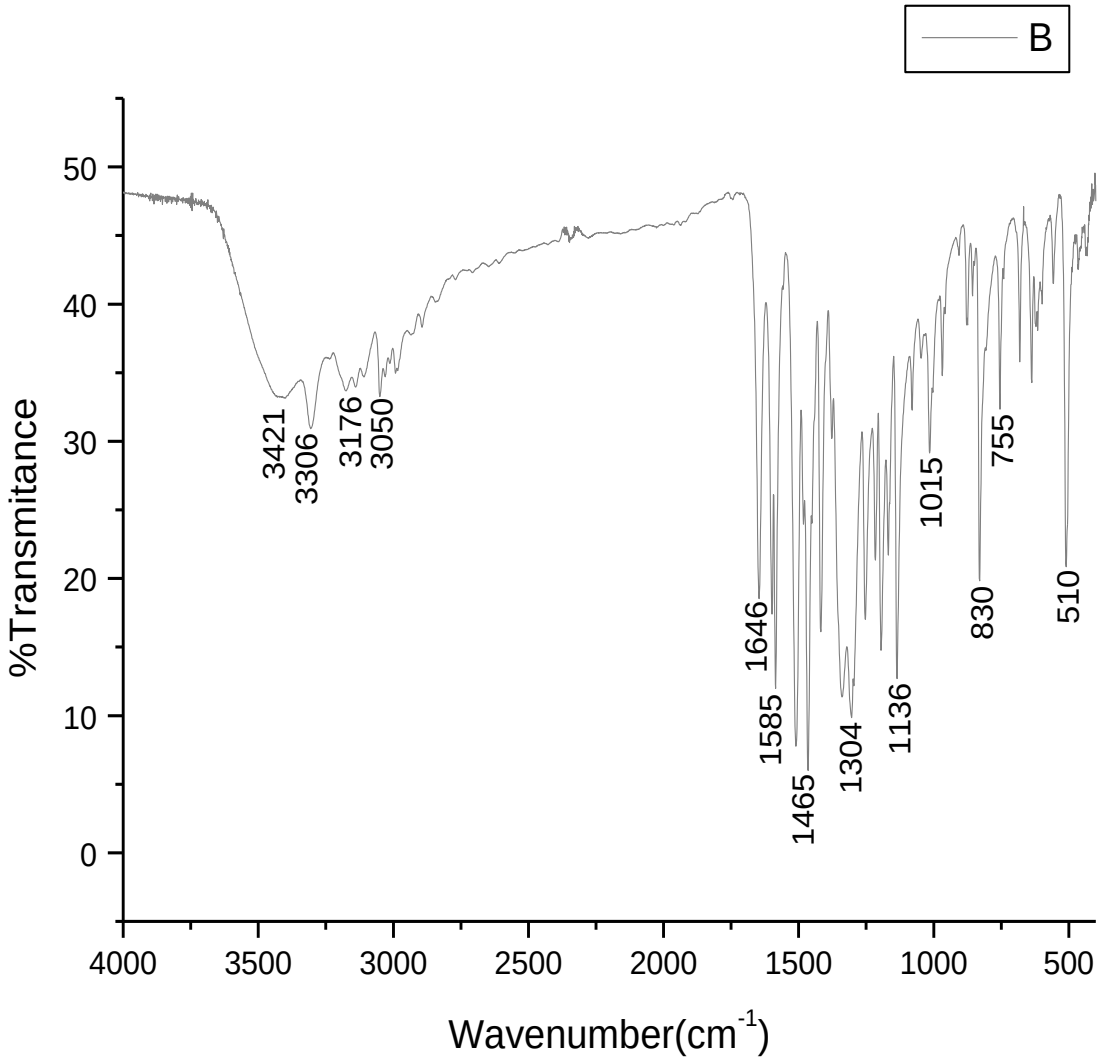
Appendix 3: FT-IR Spectrum of Schiff base.



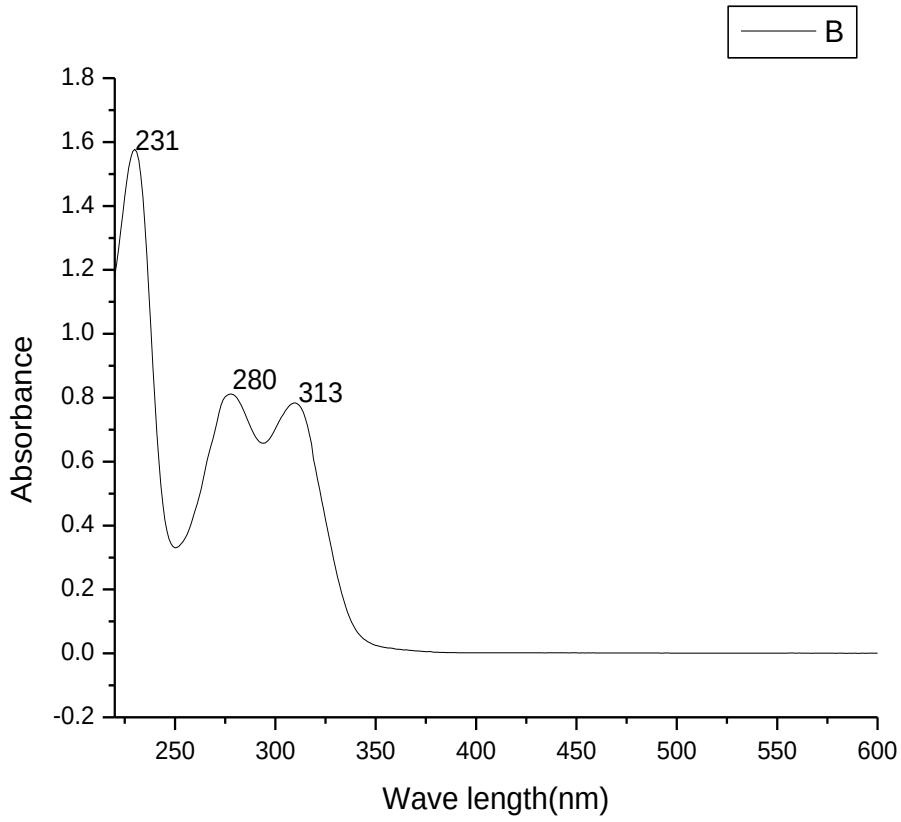
Appendix 4: FT-IR Spectrum of Co (II) Complex.



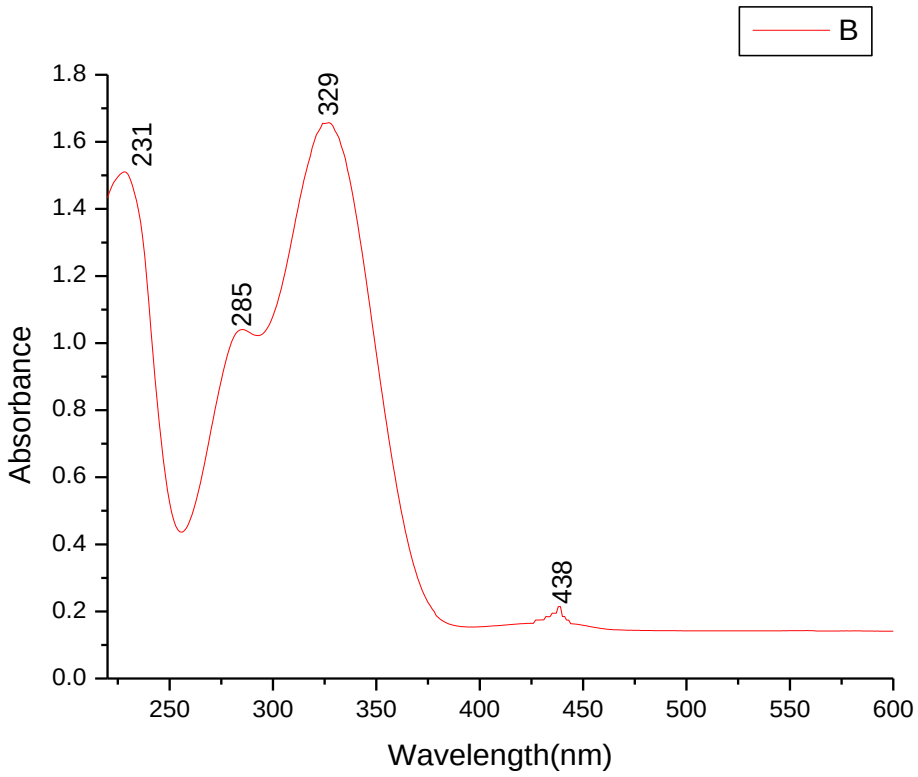
Appendix 5: FT-IR spectrum of Cu (II) complex



Appendix 6: UV-Vis Spectrum of Schiff base.



Appendix 7: UV-Vis spectrum of Co (II) complexes



Appendix 8: UV-Vis spectrum of Cu (II) complex