SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL PROPERTIES OF Ni(II) AND Cu(II) COMPLEXES OF SCHIFF BASE DERIVED FROM FURFURAL SEMICARBAZONE

MSc. THESIS

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Synthesis, Characterization and Antimicrobial Properties of Ni (II) and Cu (II) Complexes of Schiff Base Derived From Furfural Semicarbazone

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As Thesis research advisors, we thereby certify that we have read and evaluated this thesis prepared under our guidance, by Gosa Buta Mekuria entitled**: “Synthesis, Characterization and Antimicrobial Properties of Ni(II) and Cu(II) Complexes of Schiff Base Derived from Furfural Semicarbazone.”**We recommend that it to be submitted as fulfilling the thesis requirement.

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As members of the *Board of Examiners of the M.Sc. Thesis Open Defense Examination,* We certify that we have read and evaluated the thesis prepared by Gosa Buta and examined the candidate. We recommend that the thesis be accepted as fulfilling the Thesis requirement for the *Degree of Master of Science in Chemistry.*

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DEDICATION

I dedicate this thesis manuscript to my Father Buta Mekuria, my Mother Jaware Katara and my wife Damo Roba for their affection, love and moral support during my study.

STATEMENT OF THE AUTHOR

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, data analysis and completion of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

This Thesis has been submitted in partial fulfillment of the requirement for MSc Degree in Chemistry at the Haramaya University and is deposited at the University Library. I solemnly declare that this Thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

The author was born in May, 1986 at Awandella, Munessa Woreda, East Arsi, Oromia region, Ethiopia. He attended Elementary and Secondary School in Dodola west Arsi. After completion of his Secondary School Education; he joined Haramaya University in 2006 and graduated with B.Ed degree in Chemistry in 2008. After that he worked as Preparatory Teacher in Nagele Borena and Shashemene Towns and then joined the School of Post Graduate program of Haramaya University in July, 2012 to persue M.Sc. study in chemistry.

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LIST OF ACRONYMS AND ABBREVATIONS

BM	Bohr Magneton
DMSO	Dimethyl Sulfoxide
FTIR	Fourier Transform Infrared
HPLC	High Performance Liquid Chromatography
LMCT	Ligand-Metal Charge Transfer
MLCT	Metal-Ligand Charge Transfer
Mp	Melting Point
υ	Stretching frequency(vibration)
M-L	Metal- Ligand
^{1}HNMR	Proton Nuclear Magnetic Resonance
χ_g	Gram Susceptibility
χ_m	Molar Susceptibility
μ_{eff}	Effective Magnetic Moment
NA	Nutrient Agar
µL	Micro Liter
Λ_m	Molar Conductance
MHA	Muller Hinton Agar
NMR	Nuclear Magnetic Resonance
PDA	Potato Dextrose Agar
TLC	Thin-Layer Chromatography
UV	Ultra- Violet
DEPT	Distortionless enhancement by polarization transfer
Uv-Vis	Ultra violet-Visible
CNH	Carbon, Nitrogen and Hydrogen

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ABSTRACT

Schiff base ligand and their Metal complex are important compounds owing to their wide range of biological activities. Metal complexes of Cu(II) and Ni(II)have been synthesized from the Schiff base ligand, furfural semicarbazone(L), derived from furfural and semicarbazide hydrogenchloride. Structural features of these complexes were obtained from their elemental analyses, magnetic susceptibility, molar conductance, chloride test, FT-IR, electronic spectra, ^1H , ^{13}C *NMR and DEPT-135 and Atomic Absorption Spectroscopic studies. The FTIR revealed that the ligand acts as a bidentate ligand coordinating through the oxygen of the carbonyl group and nitrogen of the azomethine group. The synthesized complexes showed the conductivity values of 154.14 and 6.12 S* $mol^{-1}cm^2$ *for* $[Cu(L)_2]Cl_2$ *and* $[Ni(L)_2 Cl_2]$*, in DMSO at 25* ^0C *which confirms the electrolytic and non electrolytic nature of the complexes, respectively. On the basis of spectral studies and magnetic susceptibility measurements, an octahedral geometry has been assigned for Ni(II)complex but square planar for Cu(II) complex. Antimicrobial activity test against bacteria (Escherichia coli, Staphylococcus aureus, Streptococcus Agalactia, and Salmonella) and fungi (Aspergillus niger, Fusarium oxisporium) strains revealed that the complexes were more bioactive than the corresponding free ligand and less than the standard drug. Cu (II) complex exhibited higher activity against Aspergillus niger str ain and Ni(II) complex exhibited higher activity against Escherichia coli strain. The biological activity of the ligand was enhanced when it is complexed with metal ions.*

Key words: *antimicrobial activities, Schiff's base ligand, spectral studies,* metal complex

1. INTRODUCTION

Metal complexes of Schiff bases have occupied a central place of importance in the development of coordination chemistry. Literature on these complexes range from the purely synthetic to modern physic-chemical as well as biochemically relevant antibacterial, antifungal, anti-inflammatory and anti-leukemic properties (Ali *et al*., 1974; Chohan *et al*., 2009 and Sumrra *et al*., 2016). Furthermore, these complexes find application as catalysts for a number of reactions such as oxygenation (Nishinaga *et al*., 1988), hydrolysis (Chakiraborty *et al*., 1998),electro -reduction (Zhao *et al*., 1998) and decomposition reactions (Sreekala *et al*., 1999).

Metal complexes derived from Schiff bases have been known since1840. Frist Schiff base type complexes were synthesized by the reaction of cupric acetate, salicylealdehyde and aqueous ammonia. He isolated a dark green crystalline product which was later known to be bis(salicylaldimino)copper(II).The aryl derivatives of these compound were isolated in1869 by Schiff (Schiff,1864). Since then a large number of Schiff base complexes have been prepared and even now they comprise the major portion of the current literature on the coordination chemistry. The chemistry of Schiff bases and their complexes have been repeatedly reviewed by several scholars (Elzahany *et al*., 2008; Valentina et *al*., 2009 and Shabani et al., 2010). Schiff bases can be represented by the general formula $R\text{-}CH{=}N\text{-}R^1$, where R and R^1 are various substituents. These compounds contain the azomethine group (-RC=N-). They are formed by the condensation of primary amines with compounds containing active carbonyl group.

$$RR^1\,CO \quad + \quad R^1NH_2 \longrightarrow RR^1C{=}NR \quad + \quad H_2O$$

The condensations for the formation of Schiff base depends up on the nature of amine and carbonyl compounds. Usually it is better to remove water formed during the reaction by distillation or by using an aziotrope-forming solvent (Crewe *et al*., 1953).This is necessary in the case of diary or aryl alkyle ketones. However, aldehydes and dialkylketones can usually be condensed with amines without the removal of water. Aromatic aldehydes react smoothly under mild condition and at relatively low temperatures in a suitable solvent. In the condensation of aromatic amines with aromatic aldehyde, electron withdrawing substituent's in the Para position of the amines decreases the rate of the reaction. When such group are in the Para position of the aldehyde the rate of the reaction is found to be increased (Pratt and Kamlet, 1961). The stability of the Schiff base complexes depends on the strength of the C=N bond, basicity of the imines group and steric factors. The presence of the second functional group with the replaceable hydrogen atom, preferably a hydroxyl group very near to the imines group allows the ligand to form a fairly stable four, five, or six membered rings on chelation to the metal atom. The role of the metal ion in these complexes seems to involve both stabilization and trapping of the Schiff base and in addition it also ensures the planarity of the system.

The general methods of the preparation of a Schiff base complexes are given below :-(a) complexation in basic medium; In this method the metal ions and the Schiff base are allowed to react in alcoholic or aqueous alcoholic solution containing a base like acetate or hydroxide. (b) Condensation method; the aldehyde metal complex formed by the interaction of metal ion and aldehyde is refluxed with a slight excess of amine solvents like ethanol, chloroform and dichloromethane.(c)Template reaction; the planar quadridentate complexes can be prepared by these method (Olszewski *et al*.,1964). The formation of macro cyclic rings is favored by the presence of the cation of appropriate size that can serve to hold the partially formed ligand in position as the remainder of the ring being synthesized. (d) Mechano-chemical method; in this method the complex is prepared by rubbing metal surfaces with ligand in the solution.

Systematic studies of Schiff base complexes have been done by many researchers (Pfeiffer *et al*., 1931; Villar *et al*., 2004; Venugopal and Jayushree, 2008; Sing and Srivastava, 2011, and Mary and Parameswaran, 2015). Because of the great synthetic flexibility, Schiff base of different structural types have been synthesized. The main reason for the enormous amount of work in this field is due to the wide possibilities of synthesizing a large number of Schiff base ligands with the single aldehyde, and each of these Schiff bases are able to form a considerable number of metal complexes. This is true with most of the aldehydes, especially with salicylaldehyde. In the exhaustive review on the metal complexes of the Schiff bases by Holm *et al*. (1966), more than half of the discussion is confined to the Schiff base complexes derived from salicylaldehyde. An interesting aspect is that the semicarbazones show a variety of coordi nation modes with transition metals. The coordination mode is influenced by the number and type of substituent. This is because the active donors the ligand varies depending up on the substituent. According to the reports, the coordination mode of the semicarbazone is very sensitive towards minor variations in the experimental conditions, the nature of the substituent on the carbonyl compound and the metal salt (Basuli *et al*., 2001).

Literature revealed that many Schiff bases derived from semicarbazidehydrochloride and their metal complexes have been synthesized, characterized and showed good antimicrobial activities (Chakraborty *et al*., 1994; Zhao et al., 1998; Sreekala *et al*.,1999; Singh *et al*., 2006; Pandey *et al*., 2012; and Wiegand *et al*., 2014). However, no work has been reported on the antimicrobial properties of furfural semicarbazone and its Ni(II) and Cu(II) complexes to the best of our knowledge. In this thesis work the Schiff base, furfural semicarbazone (L), and its Ni(II) and Cu(II) complexes were synthesized. The resulting Schiff base and its metal complexes were characterized by different spectral and analytical techniques. The antimicrobial properties of the synthesized compounds were evaluated against the selected bacterial and fungal species and the result were compared with the standards.

General Objective

Synthesis, characterization and antimicrobial Properties of Ni(II) and Cu(II) complexes of furfural semicarbazone.

Specific Objectives

i. Synthesis of a Schiff base, Furfural Semicarbazone (L) and its Ni(II) and Cu(II) complexes by condensation method.

ii. Characterization of the synthesized ligand and its metal complexes by using ^{1}H and ^{13}C NMR, electronic absorption spectra, AAS, melting point, conductance measurement, magnetic susceptibility and elemental analysis method towards elucidating the structure of the complexes.

iii. Studying antimicrobial (bactericidal and fungicidal) activities of the synthesized Schiff base and its Cu(II) and Ni(II) complexes against selected bacteria and fungi species by using paper disc method and comparing the results with standards.

2. LITRETURE REVIEW

2.1. Schiff's Bases

Schiff bases represent one of the most widely used families of organic compounds and their chemistry is essential material in many organic chemistry textbooks (March and Smith, 2007).These compounds which contain the azomethine (imine) group (–RC=N–) are usually prepared by the condensation of a primary amine with an active carbonyl compound (Kumari *et al.,* 2010). The first report of condensation reactions of primary amines with carbonyl compounds has been published by Hugo Schiff in the 1960s (Tidwell and Hugo Schiff, 2008). There after Schiff's bases have been extensively used as symmetric intermediates and as ligands for coordinating transition and inner transition metal ions, and recently also for co-ordinating anions (Xavier and Srividhya, 2014). The carbonyl group of the aldehyde gives aldimines while that of ketone gives ketoimines.

Schiff's base ligands may contain a variety of substituents with different electron-donating or electron-withdrawing groups, and therefore may have interesting chemical properties (Shapiro, 1998). They have been often used as chelating agents (ligands) in the field of coordination chemistry and Schiff's base metal complexes have been of great interest for many years. It is well known that O and N atoms play a key role at the active sites of numerous metallobiomolecules in the coordination with metals (Singh *et al.,* 2007).

2.2. Biological Importance of Schiff's Bases

Schiff's bases are important compounds owing to their wide range of biological activities (Lath *et al*., 2004) and industrial applications (Wang *et al.,* 2008). They have been found to possess the pharmacological activities such as ant malarial (Li *et al.,* 2003), anticancer (Villar *et al*., 2004), antibacterial (Venugopal *et al.,* 2008 and Bharti *et al*., 2010), antifungal (Pandey *et al.,* 2003 and Bharti *et al*., 2010), ant tubercular (Bhat *et al.,* 2005), anti inflammatory, antimicrobial (Wadher *et al.,* 2009) and antiviral (Karthikeyan *et al.,* 2006). They also serve as a back bone for the synthesis of various heterocyclic compounds. The presence of azomethine functional group is responsible for antimicrobial activity, which can be altered depending upon the type of substituent present on the aromatic rings (Singh *et al.,* 2007).

Later, a great number of sulfanilamide derivatives were synthesized, characterized and tested as antibacterial agents, and many derivatives are currently used for the treatment of bacterial infections. Such sulfonamide derivatives widely used in clinical medicine as pharmacological agents with a wide variety of biological actions, were designed from the simple sulfanilamide lead molecule (Alhassan *et al.*, 2004). These derivatives also known as anticancer and antiviral agents (Cheng *et al.*, 2010), and its metal complexes have been widely studied because they have industrial, anti-fungal, anti-bacterial, anticancer herbicidal applications (Cozzi, 2004), anti-tubercular activities (Ferreira *et al.*, 2009) and chelating abilities which give it remarkable attention (Ramana *et al.*, 2009). Sulfa drugs have attracted special attention from their therapeutic importance as they were used against a wide spectrum of bacterial ailments (Ahmed and Soliman, 2006).

2.3. Semicarbazones

Semicarbazonesareusuallyobtainedbythecondensationofsemicarbazidewithsuitablealdehydesandketones (Figure.1). The lone pair electron on the nitrogen of amide group is delocalized in to the carbonyl; thus forming partial double bond between primary amine nitrogen and carbonyl carbon

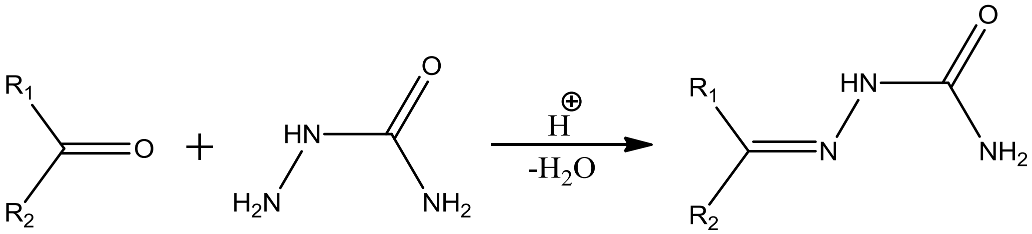

Figure 1: synthesis of the semicarbazone

An interesting fact is that the semicarbazones exist predominantly in the amido form in the solid state, whereas due to the interaction of the solvent molecules they can exhibit a amido-iminol tautomerism (Figure.2) in solution state. Amido form acts as a neutral ligand and the iminol form can deprotonate and serve as anionic ligand in metal complexes. Thus semicarbazones are versatile ligands in both neutral and anionic forms.

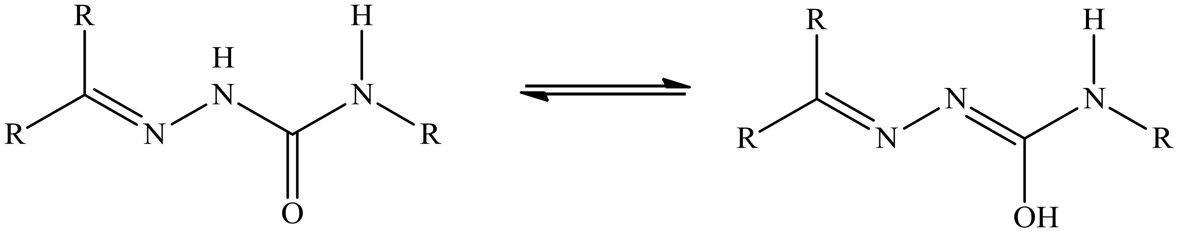

Figure 2: Amido-iminol Tautomerism of semicarbazone

Both tautomeric forms have an efficient electron delocalization along the semicarbazone moiety. Aromatic substituent's on the semicarbazone skeleton can further enhance the delocalization of electron charge density. These classes of compounds usually react with metallic cations giving complexes in which the semicarbazones behave as chelating ligands. Up on co-ordination to a metal center, the delocalization is further increased through the metal chelate rings. The coordination possibilities are further increased if the substituent has additional donor.

2.4. Binding Modes of Semicarbazones

A review of semicarbazones shows that the C=N–NH–CO-NH_2 back bone of unsubstituted semicarbazones in the solid state is usually planar, with O atom trans to the azomethine N Atom. However, few semicarbazones are exceptions to this rule (Cases *et al.*, 2000) although there are several electronic and steric factors that may contribute to the adoption of this rearrangement; the most important is probably that the trans arrangement places the amine and azomethine nitrogen atoms in relative position intermolecular hydrogen bonding (Brown and Agarwal, 1978).

An interesting aspect is that the semicarbazones show a variety of coordination modes with transition metals. The coordination mode is influenced by the number and type of substituents. This is because the active donor sites of the ligand vary depending up on the substituents. According to the reports, the coordination mode of the semicarbazone is very sensitive towards minor variations in the experimental conditions, the nature of the substituents on the carbonyl compound and the metal salt (Basuli et al., 2001).

The presence of di-2-pyridyl ketone at the carbonyl part attributes many interestining coordinating possibilities for the ligand systems under study. In most of the synthesized metal com plexes semicarbazones act as tridentate ligand and in some other cases, semicarbazones exhibit as potential quadridentate ones when the second pyridyl nitrogen of di-2-pyridyl ketone involves in coordination process. The ONO tridentate coordination mode of the semicarbazones is given below (Srukanth *et al*., 2004).

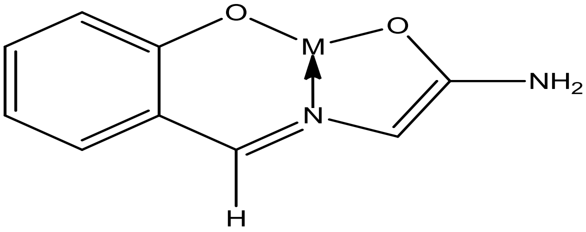

Figure 3: O,N,O-Tricoordination.

The different coordination modes of benzaldehyde semicarbazone are given below (Bassul *et al*., 1997).

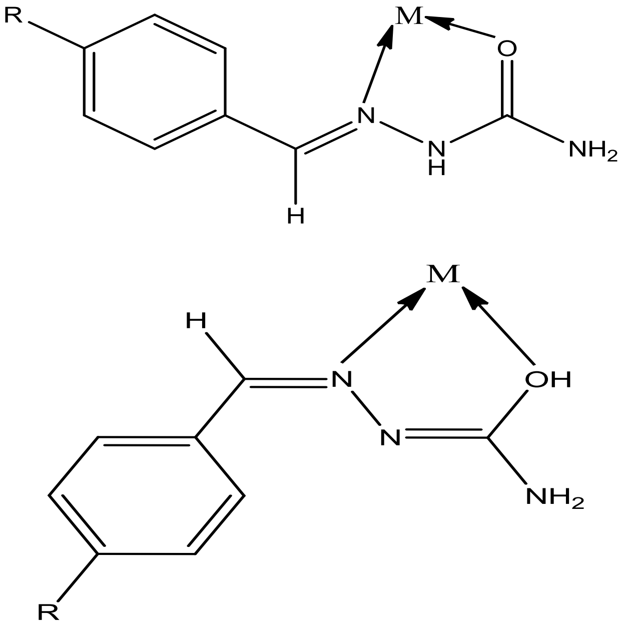

Figure 4: N, O-coordination forming a stable five membered chelate rings

2.5. Applications of Semicarbazones and their Complexes

The metal complexes of semicarbazones play an essential role in industrial chemistry (Wang *et al*., 2008), pharmaceutical (Wang *et al*., 2005) and used as catalysts (Sreekala *et al*., 1999), in various biological systems (Latha *et al*., 2004), polymers (Levitin *et al*.,2005) and dyes (Befta,1985),besides some uses as ant fertility and enzymatic agents. The biological properties of semicarbazones are often related to metal ion coordination. Firstly, lipophilicity, which controls the rate of entry in to the cell is modified by coordination (Farrel, 2002).Also, the metal complex can be more active than the free ligand. The mechanism of action can involve binding to a metal *in vivo* or the metal complex may be a vehicle for activation of the ligand as the cytotoxic agent.

Semicarbazones of aromatic and unsaturated carbonyl compounds have anticonvulsant properties (Zahra *et al*., 2005). Moreover, coordination may lead to significant reduction of drug- resistance (West *et al*., 1991). They are also used as spectrophotometric agents as well for the analysis of metal ions (Atalay and Akgemci, 1998) and were used in the quailtative organic analysis of carbonyl compounds (Kolbetal. 2003). Furthermore, semicarbazones ,which can also be regarded as urea derivatives, have gained considerable importance (Hutchins and Chapma, 1994) in the design of enzyme inhibitors (Lam *et al*.,1994), as replacement for the amide (CO NH) bond in peptidomimetics (Chorev and Goodman, 1996) and as sources of self complementary bidirectional hydrogen bonding motif in supra molecular chemistry (Zhao *et al*., 1990). Since peptides have poor metabolic stability and limited oral absorption, they are rarely useful drug candidates.

Epilepsies are common and frequently devastating and affect around1–2% of the world population. The convulsions of approximately 25% of epileptics are in adequately cotrolled by the standard drug therapy (Namara *et al*., 2001). The number of drugs useful for the treatment of epilepsy is remarkably small.Fewer than 20 drugs are currently marketed in the United States and of these, only five or six are widely used (Kadaba *et al*., 2002). Semicarbazones are a class of compounds which shows anticonvulsant activity (Ibrahim*etal*.,2014).

Arylsemicarbazone can be orally adminitered and are more active as anticonvulsants than mephenytoin or phenobarbital, besides the fact that they generally exhibit low or absent neurotoxicity (Dimmock and Baker,1994)

Review reported on the anticonvusant activity of thiosemicarbazones, semicarbazones and hydrazones derived from aromatic and unsaturated carbonyl compounds as well as from other precursors (Berado and Gambino, 2004) Cotrast to thiosemicarbazones literature records fewer examples of semicarbazone presenting signifcant anticancer and cytotoxic activity but some nitro, naphtopyran, and fluorine derivatives showed anti leukemia effect in mice (Pandeya and Dimmock, 1993). Several N^4-substituted semicarbazone derivatives of o- and p-chloro benzaldehyde and 2,6-dichlorobenzaldehyde exhibit potentantihypertensive effects (Warren and Woodward, 2014). The orally administered drugnaftazone (1,2 naphtoquinonesemicarbazone)Protects the vascular systemthrough an inhibitory effect on nitric oxide synthesisas reported by Sogni *et al*. (2004).

Schiff base complexes having oxygen coordination is important both structurally and biologically. These type of compounds include Schiff base complexes derived from semicarbazide, monoacids, aminophenol, etc. (Casellato *et al*., 1978). The oxygen atoms are coordinated in most cases to a transition metal ion to secure the stability of the complexes. In solution semicarbazone exists as an equilibrium mixture of keto (I) and enol (II) forms.

$R{=}N{-}N(H){-}C({=}O){-}NH_2$

(l)

$R{=}N{-}N{=}C(OH){-}NH_2$

(ll)

Figure 5: Tautomeric structure of Semicarbazone

Semicarbazone acts as a bidentate or tridentate ligand depending on the aldehyde residue attached to semicarbazide moiety. In most of the complexes semicarbazone remain in trans configuration. Salicylealdehyde semicarbazone a potentially tridentate ligand, is found to act as a bidentate mono anion because of the non-participation of the carbonyl oxygen of the semicarbazone moiety(Kravtsov *et al.*,1981).This seems to be a typical ligation behavior of aryl derivatives and has been termed the flip-tail bidenticity of semicarbazone ligand. The interaction of potentially tridentate dibasic tricarbazone ligand with cobalt(II)salt yields complex which was diamagnetic(Chandra et al.,1979).

2.6. Biological Importance of Schiff Bases and their Metal Complexes

Transition metal-Schiff's base complexes are of interest in inorganic chemistry and have been studied extensively (Tas *et al.,* 2002). The field of medicine has witnessed an increase in the number of complexes with therapeutic value, for example, cobalt (II) Schiff's base complexes are potential antiviral agents (Karthikeyan *et al.,* 2006), cis-dichlorodiamineplatinum (II) is an anti-cancer agent (Villar *et al*., 2004) and copper (II) Schiff's base complex is an anti-tubercular agent (Bleomink and Reedi, 1996).

Complexes of Ni (II), Cu (II), Zn (II), Mn (II), Cr (III), Fe (III) and Co (II) with a tridentate ligand, 4-hydroxy-4-methyl-2-pentanone-1*H*benzimidazole-2yl-hydrazone (H-HPBH) have been synthesized and tested for their antimicrobial activities(Neelamma *et al*.,2011). Antibacterial activities of these complexes were found in Chloramphenicol > Cu(II) > Ni(II) > Zn(II) > Co(II) >Cr(III) >Mn(II) > Fe(III) > ligand order against *Escherichia coli* and Chloramphenicol >Ni (II) > Cu (II) > Zn (II) >Mn (II)>Cr (III)> Fe (III)>Co (II) >ligand against *Staphylococusaureus.* Achut *et al.(*2009), also reported the antibacterial activities of Cu(II), Ni(II), Co(II), Mn(II) and Fe(III) complexes with an asymmetric tetra dentate Schiff base ligand derived from dehydroacetic acid, 4-methyl-*o*phenylenediamine and salicylic aldehyde against *Staphylococcus aureus* and *Escherichia coli* and fungicidal activity against *Asper gillusniger* and *Trichodermaviride.*Many other recent reports on metal complexes of Schiff bases have also drawn considerable attention due to their remarkable antifungal(Pandey et al.,2003 and Bharti etal.,2010),antibacterial(Venugopal *et al.,* 2008 and Bharti *et al*., 2010),antitumor,(Ferreira *et al.,* 2009) and anticanser activities(Villar *et al*., 2004).

Complexes of Co(II), Ni(II), Cu(II) and Zn(II) with condensation product of 1-formyl-2hydry-3- naphtholicarylamide with o-hydroxyl or o-methoxy aniline are useful as fumigants (Gupta *et al.*,2002). Ox vanadium complexes have been found strongly active against some type of Leukemia (Dong *et al.,* 2002). Transition metal complexes derived from a number of amino acids have been reported to have biological activity (Akhtar *et al.,* 2007). El-ajaily *et* *al.(*2007), reported the antibacterial activity of Ni (II) complex with salicyaldehyde and 2-amino-benzoic acid. Popova and Berova (1981) reported that copper is good for liver function, its level in blood and urine has influence in pregnancy disorders, nephritis hepatitis, leprosy, anemia and leukemia in children. Zinc plays an important role in biological systems being involved in cellular phenomena, e.g., enzyme catalysis (Berg and Lippard, 1994), apoptosis (Aoki *et al.,* 2003**)** and neurotransmission (Burdette and Lippard *et al.*, 2003). In addition, electroluminescent (EL) zinc complexes are very promising systems for their interesting properties such as electron transport ability, light emitting efficiency, high thermal stability, ease of sublimation, and great diversity of tunable electronic properties resulting from ligand substitution (He *et al.*, 2006).

2.7. Characterization

For structural studies of the synthesized ligand and its complexes, several methods including chemical analyses, infrared spectroscopy, UV-visible, ^{1}H and ^{13}C NMR, magnetic susceptibility measurements, conductometric measurements and atomic absorption spectroscopy are used. With the help of elemental analyses and molecular weight data, molecular formulae of compounds have been established. Infrared spectroscopy is used to identify and ascertain the presence of diverse groups, original and new bonds of ligand in coordination zone of the metal complex whereas UV-visible spectra is highly useful in the determination of complex geometry and energies of each of the d-d transitions and electronic charge transfer as well. Magnetic measurements impart valuable information regarding the magnetic nature of complexes and also the extent of pairing of electrons inside. Conductometric measurement data is used to know the electrolytic nature of complexes. Atomic absorption spectrophotometer on the other hand is also highly useful to determine the metal contents and metal to ligand (M:L) ratio of the complexes.

3. MATERIALS AND METHODS

3.1. Materials and Instruments

3.1.1. Reagents and chemicals

All the chemicals used in this study were of analytical grade. Solutions of PDA (potato dextrose agar), MHA (mueller hinton agar) were prepared in the laboratory. Solvents in synthetic work were used as supplied. The following chemicals were used in this study:- $NiCl_2.6H_2O$; $CuCl_2$.2H2O; Urea; Hydrazine hydrate; Semicarbazide hydrochloride; Sodium acetate and Furfural.

3.1.2. Instruments and Apparatus

The ^1H and ^{13}C NMR spectra of the samples were recorded in $CDCl_3$ on a Bruker Ultrashield TM 400 spectrometer using TMS as internal standard. The infrared spectra were recorded on a Fourier Transform Infrared (FT-IR) spectrophotometer (prestige- 21) in the range 4000-400 cm^{-1}. CHN elemental analyses were done using EA 1112 Flash CHNS/O- analyzer. Magnetic susceptibility measurements were done using a MSB-AUTO, (Sherwood Scientific) magnetic balance. All of them were recorded at Addis Ababa University, Department of Chemistry.

The melting points of the synthesized compounds were determined in open glass capillaries uing a Bibby Sterilin LTD, ST150SA, UK melting point apparatus. Electronic spectral measur ements were done using UV/Vis-SP65 SYANO spectrophotometer in 200-800 nm range. The molar conductivity measurements were carried out using Jenway digital conductivity meter (UK) at Haramaya University. Except ^1H and ^{13}C NMR, FT-IR, magnetic susceptibility measurements and elemental analysis, all the instrumental and synthetic works were carried out in Chemistry Department research laboratories of Haramaya University but antimicrobial evaluation has been done at Plant Science Department, Haramaya University. Different apparatus such as; Round bottom flask, Beaker, Crucible, Analytical Balance (OHAUS, made in Switzerland), Oven (Genlab, UK) and the like were used in this study.

3.2. Experimental Procedures

3.2.1. Synthesis of semicarbazidehydrochloride

An aqueous hydrazine solution (5.76g, 0.18mol) and urea (10.02g, 0.167mol) were reacted at temperature of 110^0C.The completion of the reaction was monitored with TLC. Semicarbazide was obtained as a white crystal after removal of water through suction filtration. The product was dried at room temperature (25^0C), and then its melting point was recorded to be 95^0C. In a round bottom flask 12.5g (1.67mol) of the white crystal was added with a sufficient amount of anhydrous hydrogen chloride and the mixture was stirred at room temperature for 15 min to form semicarbazide hydrochloride. The product was recovered by filtration and then dried at room temperature (25^0C). Finally the melting point of the product was recorded to be 165^0C. (Mistry and Guha, 1930)

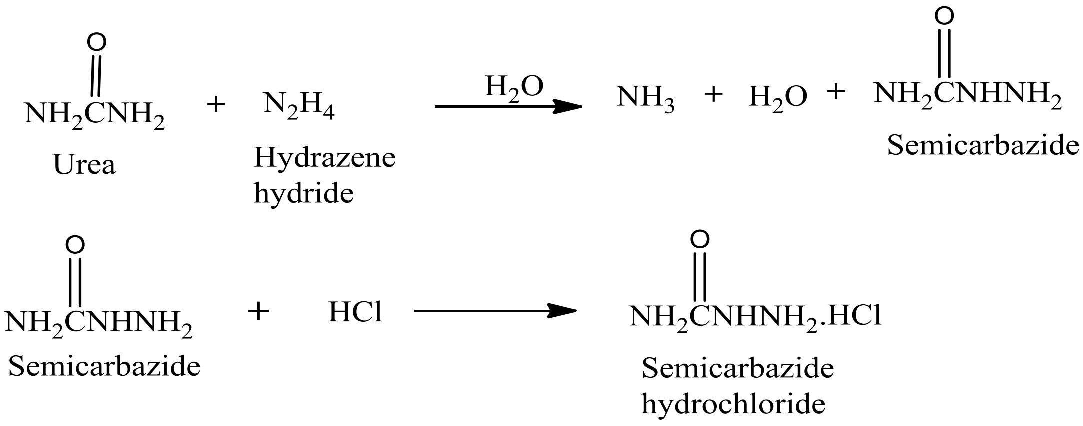

Figure 6: The synthetic route of semicarbazide hydrochloride from Urea, Hydrazenehydrate and Hydrochloric acid

3.2.2. Synthesis of the Schiff Base

The Schiff base was prepared by the procedure reported earlier (Mery and Paramaswaran, 2015). An aqueous solution of semicarbazide hydrochloride (2.23 g, 0.02 mol) and Furfural (2.44 mL, 0.02 mol) were mixed slowly in the presence of anhydrous sodium acetate (1.64 g, 0.02 mol). The mixture was stirred vigorously with the help of mechanical stirrer for an hour at room temperature (25^0C). On cooling a colored precipitate was observed. The product was filtered, washed with cold ethanol and dried in a desiccator under vacuum over P_4O_{10}. Finally a rosy brown colored solid product was collected and its melting point was measured to be 180^0C.

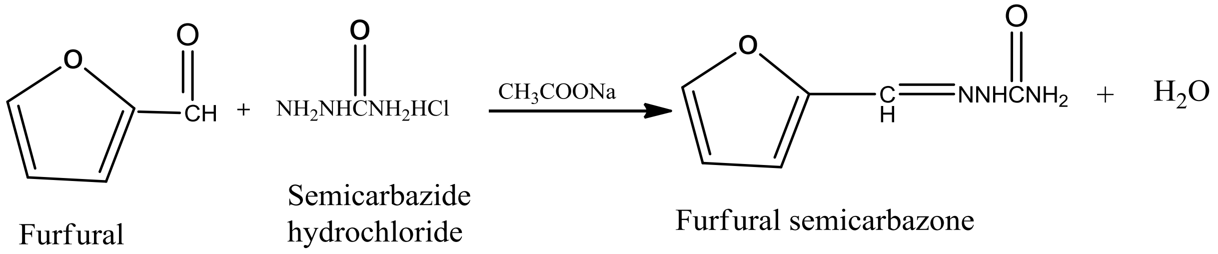

Figure 7: Synthesis of Furfuralsemicarbazone from Furfural and Semicarbazide Hydrochloride

3.2.3. Synthesis of Nickel (II) Complex of Furfuralsemicarbazone

Nickel (II) complex was prepared by mixing hot ethanolic solution (15 mL) of the precursor nickel (II) salt (2.376 g, 0.01 mol) and hot ethanolic solution (15 mL) of the corresponding ligand (3.063 g, 0.02 mol) in 1:2 molar ratio with constant stirring. The mixture was refluxed for 6 h at 95 ^{0}C. The progress of the reactions was monitored by TLC; Chloroform and Toluene in 9:1 ratio was mixed in TLC plate as the solvent system for TLC. After 6 h of refluxing, TLC evaluation showed that all starting material have been consumed, and the reaction was completed. On cooling a colored precipitate was observed. It was filtered, washed with cold Ethanol and distilled water, and dried in desiccator under vacuum over P_4O_{10}. Light grey color of the resulting complex was obtained and its melting point was found to be 240^0C. Finally purity of the complex was checked by using thin layer chromatography. (Sulekh C, *et al.*, 2013)

3.2.4. Synthesis of Copper (II) Complex of Furfuralsemicarbazone

To synthesize the title complex, hot ethanolic solution of $CuCl_2.2H_2O$ (1.70g, 0.01mol) was mixed with hot ethanolic solution of the ligand (3.063g, 0.02mol) in 1:2 molar ratio with constant stirring. The mixture was refluxed for 5 h at 80 ^{0}C.The progress of the reactions was monitored by TLC, Chloroform and Toluene in 9:1 ratio was mixed in a TLC plate as the solvent system for TLC. After 5 h of refluxing the TLC evaluation showed that all starting materials have been consumed, and the reaction was completed. On cooling, black color of the resulting complex was obtained. It was filtered, washed with absolute ethanol and dried in desiccator under vacuum over P_4O_{10}. Finally, the purity of the complex was checked by using thin layer chromatography (TLC) and melting point measurement. The melting point was found to be 239^{0}C (Sanjay, *et al*., 2016).

3.2.5. Test for Purity of the synthesized Compounds

3.2.5.1. TLC test

The purity of the ligand and its complexes were tested by using thin-layer chromatography (TLC) on silica gel thin layers using two component solvent systems, Chloroform and Toluene (9:1 ratio). Sample solutions in ethanol were spotted on warm plates as small drops using glass capillaries in series on a line 2 cm from the lower edge of the plate. The oven dried loaded plates were developed in rectangular glass chambers with ground-in-lids by ascending technique to obtain reproducible results. The development chambers were saturated with solvents before use. When the development had proceeded for 8 cm the plates were removed from the chamber.

3.2.5.2. Melting point

The ligand and its complexes were also separately exposed to capillary tube. The capillary tube was inserted in to melting point measuring apparatus and activated by heating at the point where the solid start to melt. Melting point was recorded. Melting point of the ligand was compared with that of its complexes.

3.2.6. Chloride ion test

From each complex, 30 mg was weighed out and digested in nitric acid to remove organic content and diluted to give a clear solution to which $AgNO_3$ solution was added in a drop wise manner. A white precipitate (silver chloride) was found in the Ni(II) complexe only, which was soluble in ammonia solution. This indicated the presence of the chloride ion in the complex solution. Quantitative determination was carried out by titration of the samples whose concentration was unknown, with standard solution of silver nitrate in the presence of 1ml of potassium chromate as an indicator. The end point was determined by the formation of red precipitate. The number of moles of chloride was calculated based on titration value.

3.3. Methods of Characterization

3.3.1. Physico-chemical methods of characterization

3.3.1.1. Elemental analysis

The elemental analysis for carbon, hydrogen, and nitrogen were performed by micro analytical methods. Ligand and its complexes were analyzed micro analytically for carbon, hydrogen and nitrogen contents to find out their composition.

3.3.1.2. Metal analysis using AAS

Metal content of each of the prepared complexes was determined using Atomic Absorption Spectroscopy (AAS). 20 mg of each of Cu(II) and Ni(II) complex was digested in 15 ml of concentrated HNO_3.The clear solution was diluted to 50 ml and 1ml of this solution is again diluted to 100 ml in a volumetric flask and the metal estimation was carried out on atomic absorption spectrometer. For sample analysis, five series of working standard metal solutions (in the optimum concentration range) were prepared by appropriate dilution of the metal stock solutions with distilled water. Based on the absorbance data, the concentrations of Cu(II) and Ni(II) in the complexes were calculated using the following formula:

$$\mathrm{M\%}= \frac{Concentration\ (ppm)\ xVolumedilutedto}{\text{Mass of sample taken}}\, x\, \frac{100}{1000}$$

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

3.3.1.3. Molar conductance measurements

The solution conductivities of 0.001M solution of Cu(II) and Ni(II) metal complexes of Furfural semicarbazone in DMSO were measured at 298K. The conductivity cell used in the present investigation was having cell constant 0.099. The instrument was calibrated using 0.005M KCl solution at room temperature.Finally from specific conductivity and molarity (M) of solution, the molar conductivity (Λ_m) was calculated by the equation(Philip, 2002; Barbucci- *et al., 2014*).

$$\Lambda_m = \frac{Kv1000}{M}$$

Where, M is molarity of a solution. The molar conductivity has the unit S cm^2 mol^{-1} or ohm^{-1} cm^2 mol^{-1}.

3.3.1.4. FTIR measurements of ligand and complexes

Fourier Transform Infrared (FT-IR) spectra of the ligand and its complexes were recorded over 4000-400 cm^{-1} in KBr medium. This gave information regarding the coordinating sites of the ligand in the metal complexes. Pellets were prepared by mixing about 1.0 mg of solid sample with 100 mg of dry, powdered KBr and grinding in a smooth agate mortar and pressing in a disk. Then the KBr disk with the sample was immediately put in the sample holder and FTIR spectra were recorded.

3.3.1.5. NMR (^{1}H, ^{13}C and DEPT ^{13}C) spectra of the ligand

^{1}HNMR spectrum of the ligand was studied to know position of hydrogen containing groups of the ligand. ^{13}CNMR spectrum of ligand was studied to know position of carbon containing groups of the ligand used to resolve organic structure from spectra. DEPT-135 spectrum of ligand was studied to distinguish carbon nuclei based on the number of proton attached to it.

3.3.1.6. The electronic absorption spectra and magnetic susceptibility studies

UV-Vis absorption spectra of the ligand and its complexes in 200-800 nm regions were recorded to identify and determine the energies of d-d electronic transitions and charge transfers. Magnetic susceptibility measurements of the complexes were done to know their magnetic properties and also the stereochemistry by calculating their magnetic moment.

3.4. Procedures for Antimicrobial Evaluation

Antimicrobial (antibacterial and antifungal) activities of the ligand and its complexes were tested *in vitro* against two bacteria and two fungi using disc diffusion method. The two bacteria and fungi were cultivated on Mueller hinton agar (MHA) and potato dextrose agar mediam respectively. The degree of bactericidal and fungicidal activities was determined by measuring diameter of inhibition zone and compared with that of the standard drug *chloramphenicol* and *bavistin* respectively (Mapari and Mangaonkar, 2011).

3.4.1. Inoculums preparation

The test bacterial strains, *Escherichia coli* and *Salmonella* (Gram-negative) and *Staphylococcus aureus and Streptococcus Agalactia* (Gram- positive), were transferred from the stock cultures and streaked on mueller hinton agar (MHA) plates and incubated for about 24 h. Bacteria were transferred using bacteriological loop to autoclaved MHA that was cooled to about 45 ^{0}C in water bath and mixed by gently swirling the flasks. The medium was then poured to sterile Petri dishes, allowed to solidify and used for the biotest. For test fungi, mycelia plugs from the stock cultures were transferred to PDA plates and incubated for 6 days. Then spores of the test fungi species were harvested by washing the surface of the colony using 10 ml sterile distilled water and transferred to 50 ml autoclaved PDA cooled to about 45 ^{0}C in a water bath. The medium containing spore suspension was poured to sterile plates, allowed to solidify and was used for the paper disc diffusion bioassay.

3.4.2. Preparation of test solution

The synthesized ligand and its complexes were dissolved in DMSO at a concentration of 20 mg/ml.

3.4.3. Testing for antifungal activity

Paper discs about 3 mm in diameter were cut from Watman-1filter paper with an office paper punch and placed in a beaker covered with aluminum foil and sterilized in an oven at 180 ^{0}C for 1 h. Aliquots of 20 µl of the sample solutions of ligand and its complexes were pipetted to the discs in three replications each. The paper discs impregnated with the sample solutions were then transferred using sterile forceps to PDA seeded with spore suspension of test fungi as described under inoculums preparation above. The petri dishes were incubated at 26 ^{0}C for 6 days. All the tests were performed in triplicate. The effectiveness of the samples was evaluated by measuring inhibition zone against the tested organisms.

3.4.4. Testing for antibacterial activity

Similar procedures were followed for testing antibacterial activities. Paper discs were transferred to Mueller hinton agar (MHA) plate seeded with bacteria and incubated at 37 ^{0}C for 24 hrs. All the tests were performed in triplicate. Antibacterial activity was evaluated by measuring the zone of inhibition against the tested organisms.

4. RESULTS AND DISCUSSION

4.1. Test for Purity of the Ligand and its Complexes

4.1.1. TLC tests

The purity of the ligand and its complexes were tested by using thin-layer chromatography (TLC) on silica gel thin layers using two component solvent systems, Chloroform and Toluene (9:1 ratio). All the synthesized compounds migrated as single spot revealing the purity of the compounds.

4.1.2. Melting point

For synthesized ligand and its metal complexes temperatures of sharp melting points were recorded to confirm their purity. The melting points of the synthesized complexes were higher than the free ligand, revealing the higher stability of the complexes compared to the free ligand.

4.1.3. Chloride test determination of chloride ions in the synthesized complexes

In qualitative test of Cu(II) Complex, a negative test for the inner coordination sphere of chloride ion(s) was observed, indicating the absence of directly coordinated chloride ion(s) to the central metal ions. There was however a positive test of presence of chloride ion in the outer coordinate sphere, confirming the presence of counter chloride in the complex. Quantitatively, the presence of two moles of chloride ions outside the coordination sphere was confirmed after titration of this sample by dilution law.

Qualitative test for nickel (II) complex revealed the presence of inner chloride ion(s) in coordination sphere, indicating the presence of directly coordinated chloride to the metal ion and negative tests for the outer coordinate sphere, confirming the absence of counter chloride ion in the complex.

4.2. Physico Chemical Characterization

4.2.1. Elemental analysis

Physical properties and elemental analyses data of the ligand and its metal complexes are presented in Table 1. The proposed molecular formulae of the synthesized compounds were consistent with the analyses data of C, H, N and metal content. For both Cu(II) and Ni(II) complexes the metal to ligand ratios of 1:2 were established. The reported Ni(II) and Cu(II) complexes confirmed the general composition of $[Ni(L)_2Cl_2]$ and $[Cu(L)_2]Cl_2$ with ligand (L) , respectively.

Table 1: Physical characteristics of the ligand and its complexes.

No	Compounds	Molecular Weight g/mol	Color	Mpt (^{0}C)	Yield (%)	Elemental analysis Calculated (Found)(%)			Metal (%) Calc. (found)
						C	H	N	
1	$C_6H_7N_3O_2$	153.141	Rosy brown	180	87	47.02 (46.98)	4.57 (4.56)	27.29 (27.24)	-
2	$[Cu(L)_2]Cl_2$	440.828	Black	239	92	32.66 (32.63)	3.19 (3.20)	19.055 (19.02)	14.42 (14.38)
3	$[Ni(L)_2Cl_2]$	435.9754	Light gray	240	93	33.029 (32.98)	3.21 (3.20)	19.267 (19.23)	13.46 (13.35)

4.2.2. Metal Analysis using AAS

The metal content in the complex was determined using atomic absorption spectroscopy (AAS).The experimental percentage of the metal in the complex was obtained from the AAS data using the formula:

$$\text{M\%} = \frac{Concentration\ (ppm)xVolume\ dilutedto}{\text{Mass of sample taken}}\, x\, \frac{100}{1000}$$

Based on the absorbance data 0.488 and 0.340, the concentration of metal ions in the complex were calculated to be 14.38 and 13.35 for Ni (II) and Cu(II)complexes, respectively. This showed the existence of Cu(II) and Ni(II) in synthesized complex, respectively and 1:2 metal to ligand ratio in the complex was confirmed. The calibration curves for synthesized metal complex appeared in (Appendix Figure 1 and 2).

4.2.3. Molar conductance

Complexes prepared in this work showed molar conductance values of 154.14 and 6.12S mol^{-1}cm^{2}in DMSO at room temperature for $[Cu(L)_2]Cl_2$ and $[Ni(L)_2 Cl_2]$,respectively. The Molar conductance of Ni(II) complex indicated its non electrolytic nature while Cu(II) complex was electrolytic with cation to anion ratio of 1:2

Table 2: Molar conductivity measurement data of the metal complexes

Complexes	Conductance of complex (µS)	Specific conductance x10^6 (Conductance due to complex. K (Ohm cm^2 mol^{-1})	Molar Conductance (Ω-1cm^2 mol^{-1})	Electrolytic nature	Cation to anion Ratio
$[Cu(L)_2]Cl_2$	287	284.13	154.14	Electrolyte	1:2
$[Ni(L)_2Cl_2]$	1224	1211.76	6.12	Non-electrolyte	-

Cell constant=0.99, Molarity =1 x10^{-3}M

4.2.4. FT-IR Spectral Studies

The IR spectrum of the Schiff base (Figure 8) was compared with its metal complexes (Figure 9 and 10) to study the binding mode of the ligand towards the metal ions. The band at 1656cm^{-1} in free ligand was due to the υ(C=N) and this band shifted to lower frequency side 1603-1602cm^{-1} in both complexes confirmed the involvement of nitrogen of the azomithine (C=N) group in coordination with the metal ions (Deepa and Aravindakshan, 2000; Sliverstein and Webser, 2005; Joseph *etal*.,2006; Nakamoto,2009).

The band at 1690 cm^{-1} in the free ligand was due to the υ(C=O) and this frequency shifted to lower side 1680-1669cm^{-1} in both complexes confirmed the involvement of oxygen of carbonyl group(C=O) in coordination with the metal ions (Youssef and Hagab, 2005; Nakamoto, 2009).

Further, the bonding of the ligand with the metal ions in both complexes were supported by the appearance of new bands at the low frequency region, which were not appeared in the free ligand, at 467- 434cm^{-1}and 495-485cm^{-1}regions. The bands were assigned to ʋ(M-O) and ʋ(M-N) vibrations for Cu(II) and Ni(II) complexes, respectively (Raman *et al*., 2008 and Nakamoto ,2009). The band at 403cm^{-1} was due to ʋ(M-Cl)stretching vibrations in the Ni(II)complex (Reddy, 2000; Gulcan *et al*.,2012). These confirmed the participation of the carbonyl oxygen and azomethine nitrogen atoms in coordination (Jose and JoyAnto, 2008).

The band due to N-H stretching of primary and secondary amine in the free ligand remains the same in the complexes. This indicate absence of participation of the primary and secondary amine nitrogen in complexation (Wang *et al*., 2004; Manrao *et al*., 2009; Sanna, 2012).

It was concluded from the IR spectra of the ligand and its metal complexes, the free ligand (L) acts as a bidentate in the investigated complexes, using carbonyl oxygen and azomethine nitrogen as donor atoms. As a result, the four and six coordination for Cu(II) and Ni(II) complexes, respectively were confirmed(Jose and JoyAnto, 2008; Arunachalam and Bhakyaraj, 2009).

Table 3: IR frequencies (cm^{-1}) characteristics groups of the ligand(L) and its complexes

Com pounds	ʋ(*C=N*)	ʋ(*C=O)*	ʋ(*M-N*)	ʋ(*M-O*)	ʋ(M-Cl)	ʋ(*N-H*) 2^0amide	ʋ(*N-H*) 1^0amide
$C_6H_7N_3O_2$	1656	1690	-	-	-	3453	3281
$[Cu(L)_2]Cl_2$	1602	1680	495	467	-	3452	3282
$[Ni(L)_2Cl_2]$	1603	1669	485	434	403	3453	3283

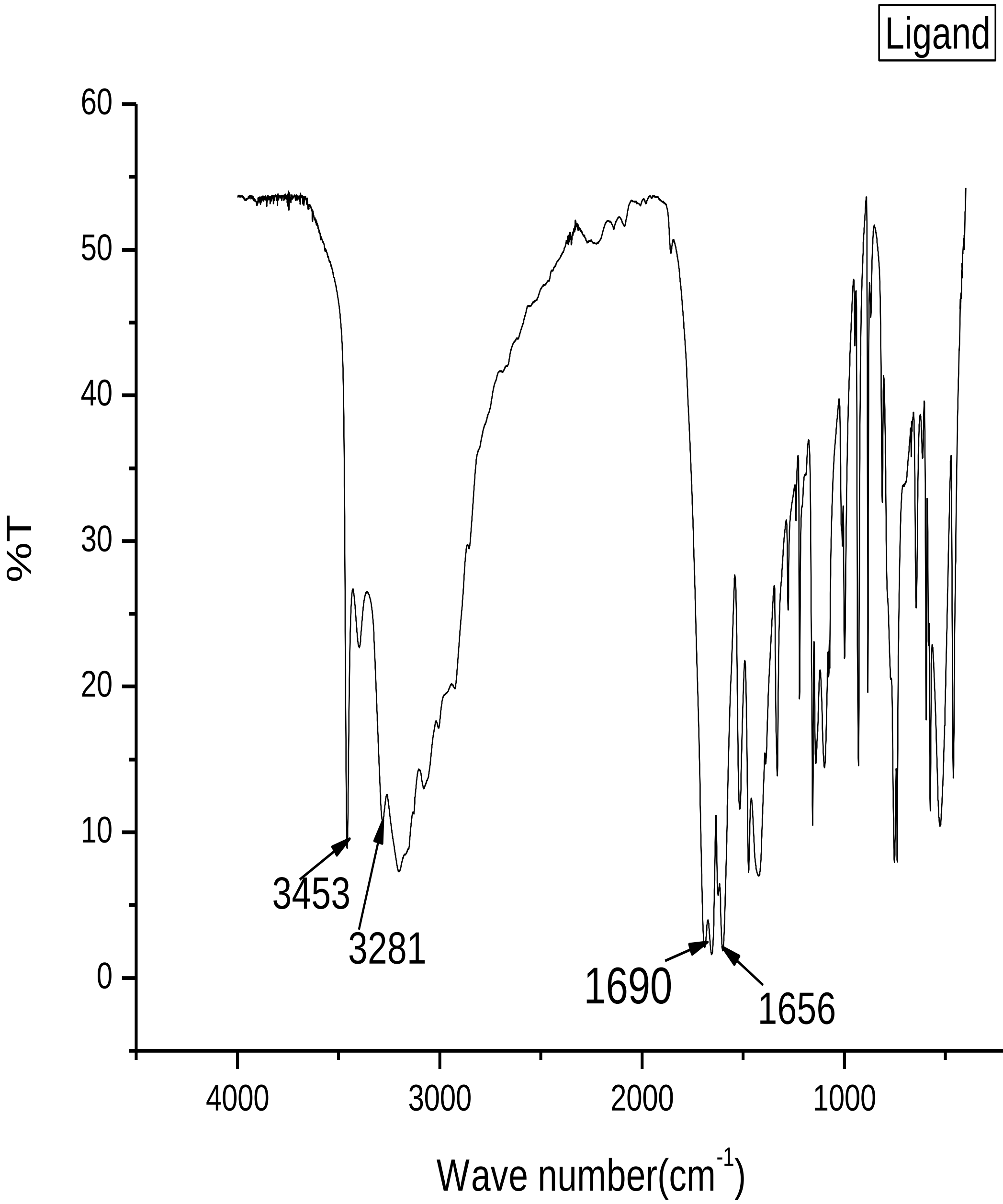

Figure 8: FTIR spectrum of Furfural semicarbazone.

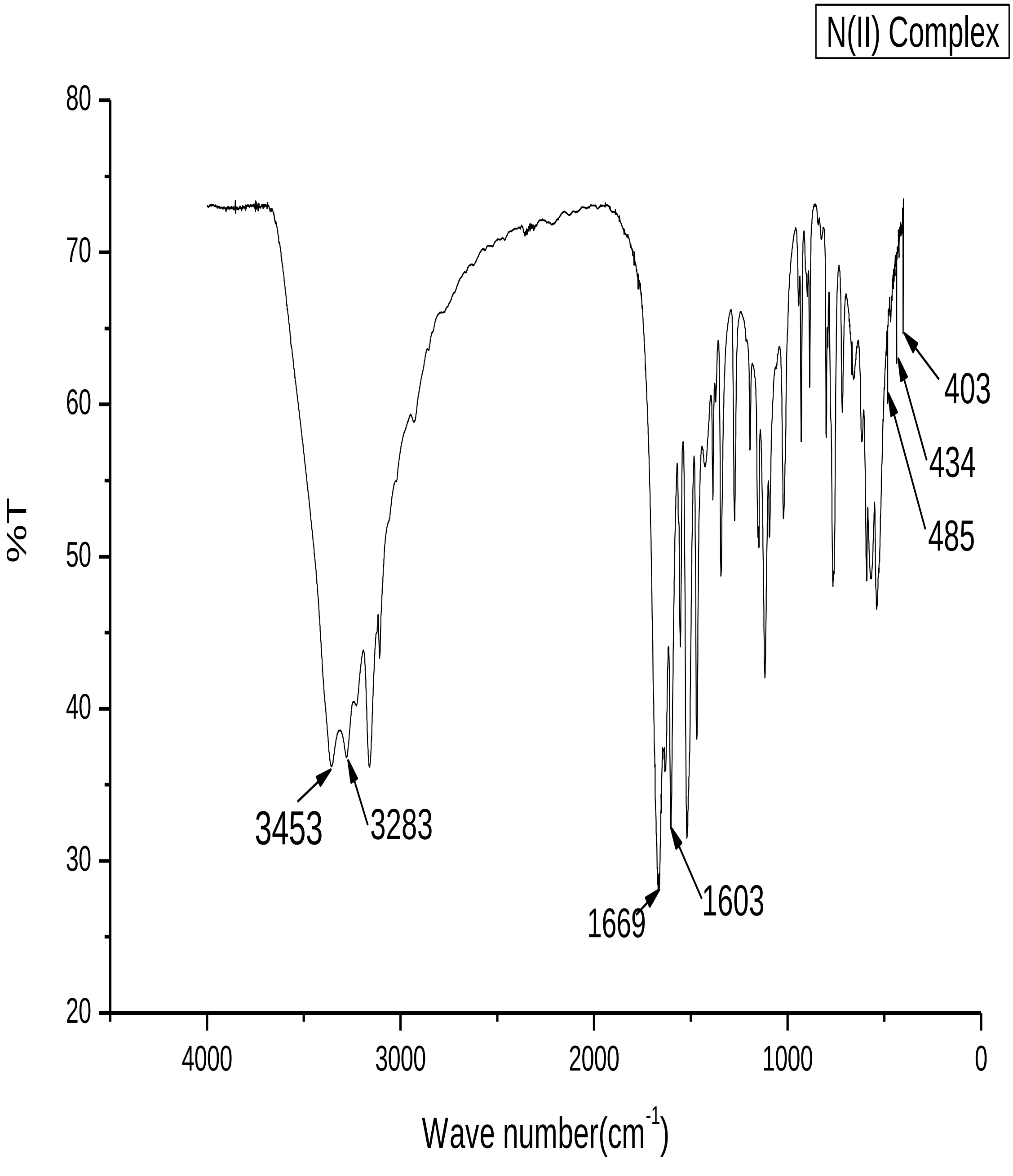

Figure 9: FTIR spectrum of Ni(II) Complex of Furfural semicarbazone

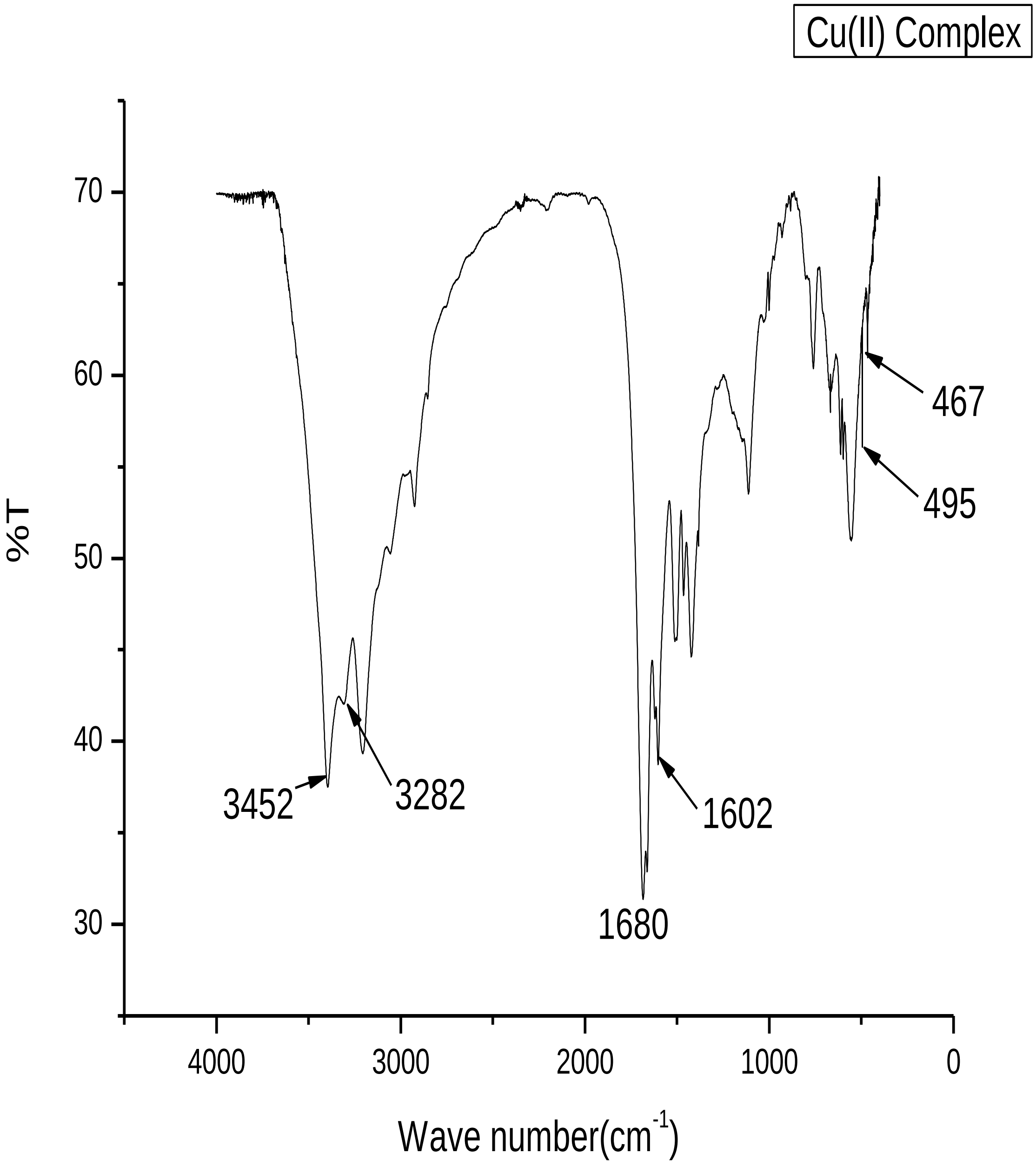

Figure 10: FTIR spectrum of Cu(II) Complex of Furfural semicarbazone

4.2.5. NMR (^{1}H, ^{13}C and DEPT^{13}C) Spectra of the ligand

With a view to receive verification of infrared structural inferences ^{1}H NMR spectrum of the ligand (L) was analyzed (Table 4). In this spectrum, the following chemical shift were attributed in the region; 6.388, 10.303, (6.566, 6.562, 6.789) ppm and 7.779 ppm, for their characteristic protonic groups H_2N-C=O, -HN-C=O, 2-furan(-CH,-CH, -CH),and HC=N, respectively (Prashanthi and Shiva, 2010).

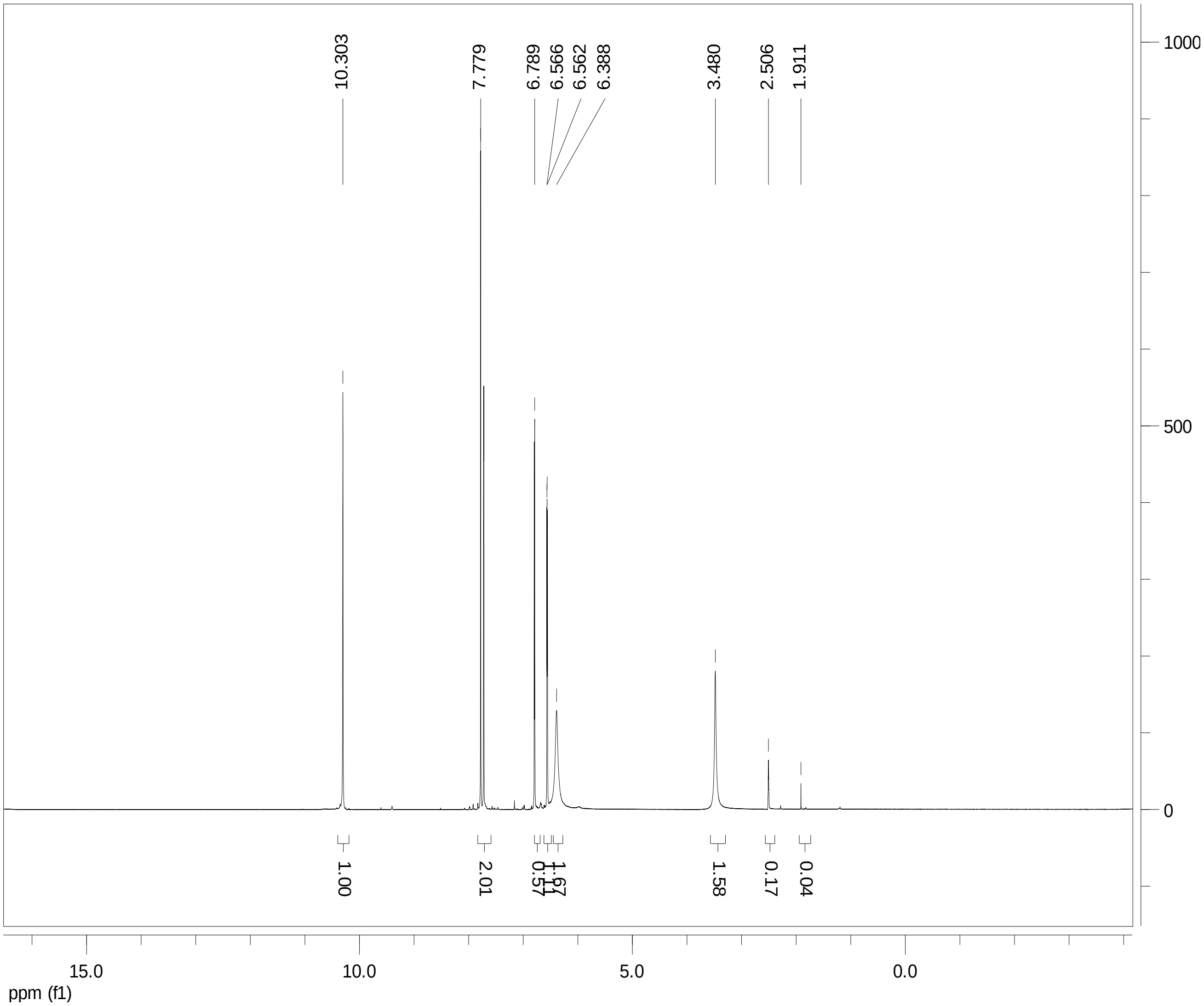

Figure 11: ^{1}H NMR Spectrum of Furfuralsemicarbazone

The ^{13}C NMR spectrum of the ligand (L) was analyzed (Table 5). In this spectrum, the following chemical shift were attributed in the region; (111.518, 112.431, 144.496 and 150.28) ppm, 130.59ppm and 157.134ppm for their characteristic carbonic groups of furan (-CH) aromatic ring, HC=N azomethine, carbonyl (-C=O) carbon, respectively in the ligand, (Prashanthi and Shiva, 2010).

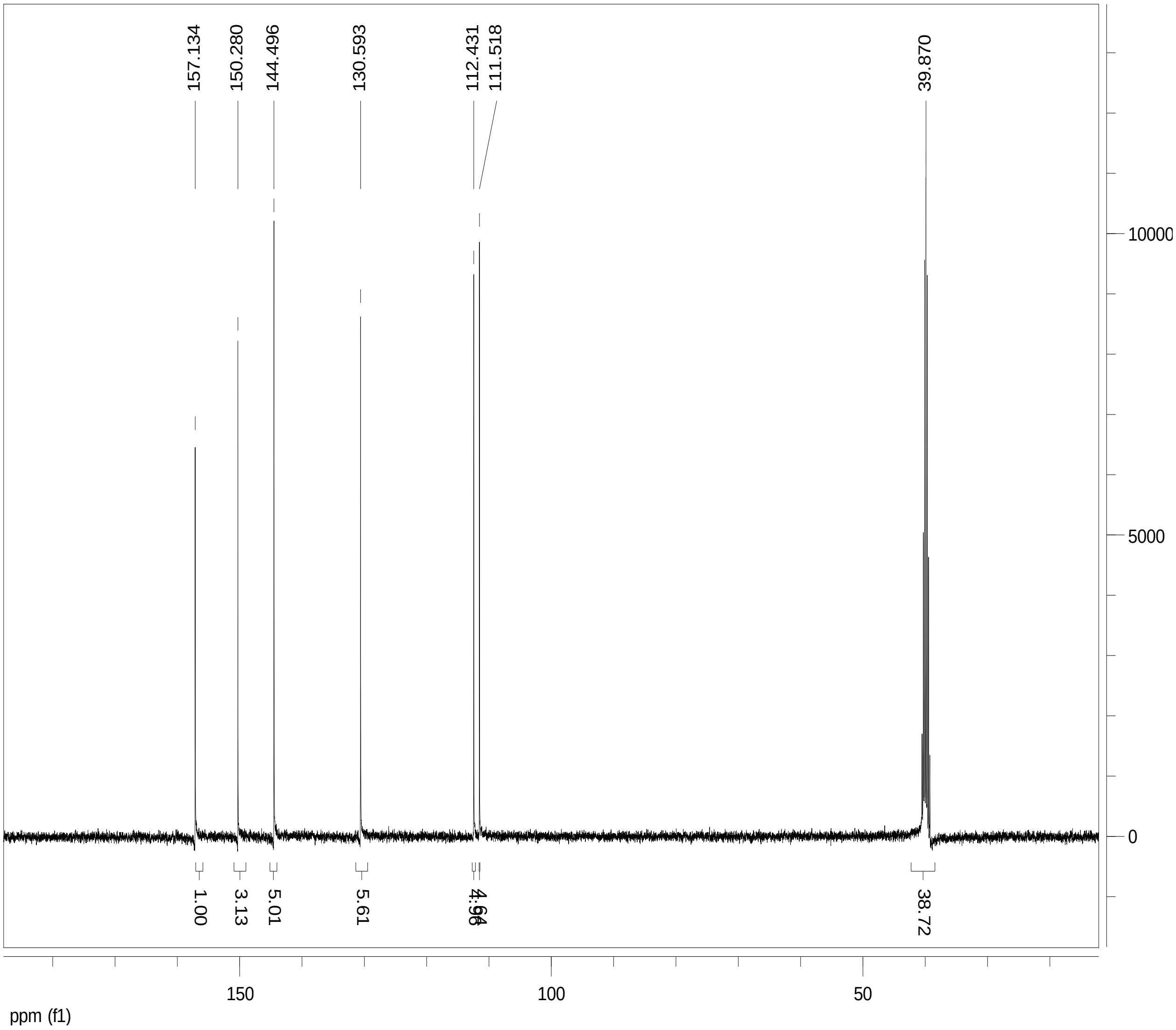

Figure 12: ^{13}C NMR Spectrum of Furfuralsemicarbazone

DEPT-135^{13}C Spectrum of Furfuralsemicarbazone was also analyzed (Table 6).From this spectrum it was confirmed the presence of five peeks corresponding to five protonated carbons with four CH from the proposed ligand (furfural semicarbazone) and one CH_3 from the solvent (Dimethyl Sulfoxide).The presence of all the characteristic protonated carbonic groups of ligand, two of2-furan (-CH,-CH,), O-CH and -CH=N were exhibited in the ligand spectrum.

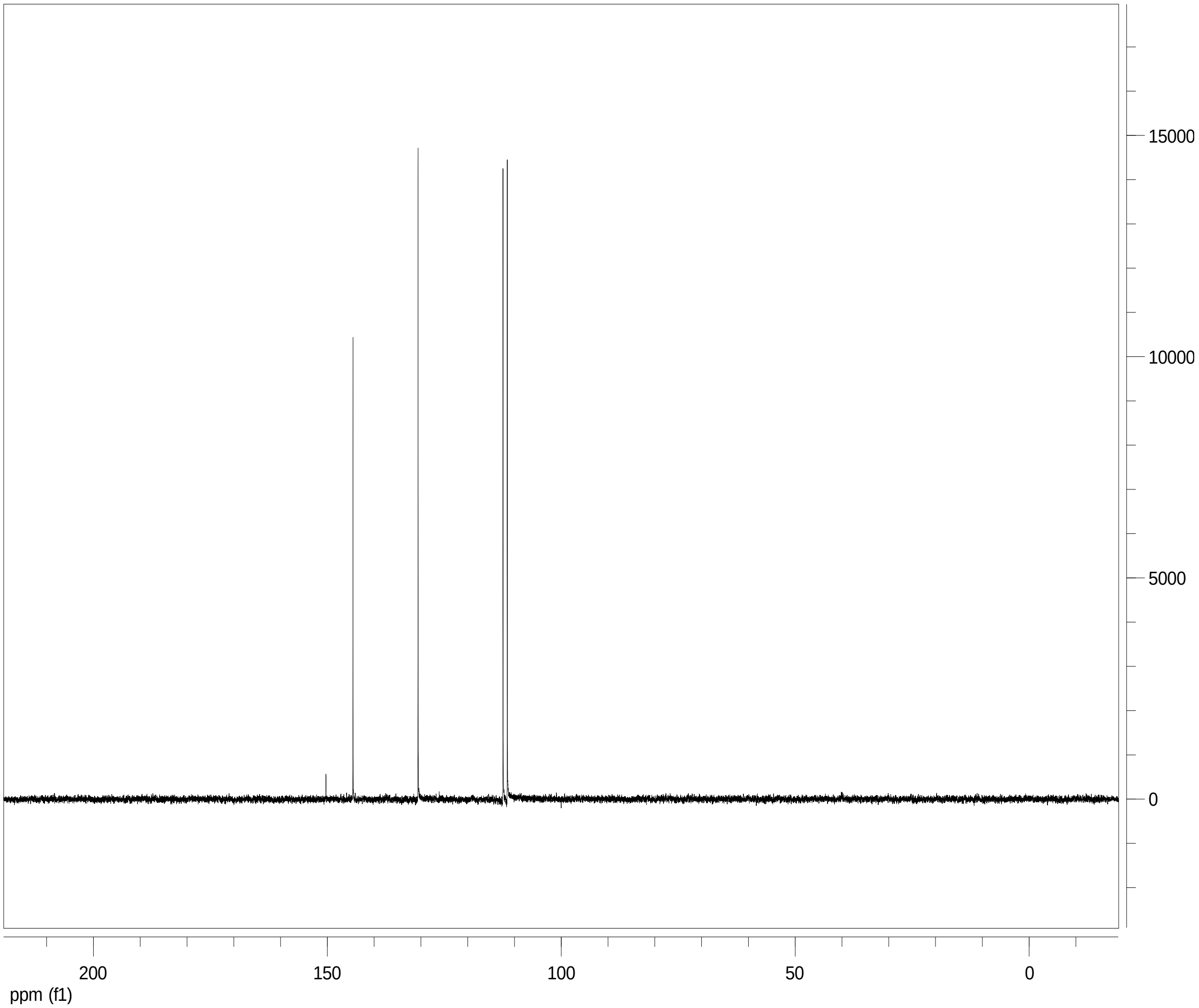

Figure 13: DEPT ^{13}C Spectrum of Furfuralsemicarbazone

Table 4: ^{1}H NMR spectral data of Furfural semicarbazone

Compounds	Chemical shift (δ(ppm))	Type of proton
$C_6H_7N_3O_2$	δ (6.388)	-NH_2 group
	δ (6.562)	2-Furan(-CH)
	δ(6.566,)	2-Furan(-CH)
	δ (6.789)	2-Furan(-CH)
	δ (7.779)	(HC=N)
	δ (10.303)	-NH

Table 5: ^{13}C NMR spectral data of the ligand

Compounds	Chemical shift(δ(ppm))	Types of carbon
$C_6H_7N_3O_2$	δ(111.518)	2-furan(-CH)
	δ(112.431)	2-furan(-CH)
	δ(130.593)	-HC=N(azomethine)group
	δ(144.496)	2-furan(-CH)
	δ(150.28)	O-C
	δ(157.134)	-C=O (carbonyl)group

Table 6: DEPT ^{13}C Spectra data of the ligand

Compounds	Chemical shift(δ(ppm))	Types of protonated carbon
$C_6H_7N_3O_2$	δ(116.1898)	2-furan(-CH)
	δ(131.7541)	2-furan(-CH)
	δ(145.1366)	-HC=N
	δ(150.8678)	O-CH

4.2.6. The Electronic Absorption Spectra and Magnetic Susceptibility Studies

The electronic absorption spectra of the Schiff base ligand (L) and its metal complexes were recorded in DMSO solution in the range of 200 to 800 nm and the data is presented in the table (Table 7).

The electronic absorption spectrum of free ligand (L) (figure 14) displayed one absorption peak at (336) nm (39762cm^{-1}) was assigned to n–π* electronic transition of the azomethine group (Taemel, 2011). The bands due to n–π* transition in the free ligand was shifted towards lower frequency at (321)nm (31152.65 cm^{-1}) and 320nm (31250cm^{-1}) for Ni(II) and Cu(II) complexes, confirming the coordination of the ligand through the azomethine nitrogen with the metallic ions (Bailar *et al*., 2009). The new bands for the synthesized complexes in the visible region ranging from 400 nm to 780 nm were the characteristic for inter metal d→d transition (Yusnita *et. al*., 2009).

The electronic absorption spectrum of Ni(II) complex (figure 15) displayed three absorption bands at (621)nm (16000 cm^{-1}),(540)nm(18518.52 cm^{-1}) and (405)nm(24691.36 cm^{-1}). These bands were due to d-d electron(spin allowed) transitions corresponding to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$, $^3A_{2g}(F)\rightarrow{}^3T_1g(P)$, respectively suggesting an octahedral geometry around Ni(II) ion. The magnetic susceptibility of the complexes give as information regarding the ma gnetic and geometry of the complexes. Room temperature (297K) magnetic moment value of Ni(II) complex to be 2.83 B.M.which is within the range of 2.8-3.5 B.M as reported for octahedral geometry (Chandrra *et al*., 2009).This value also indicates the paramagnetic nature of the synthesized complex. Therefore, the result of electronic spectral studies and magnetic measurement of this complex confirming octahedral geometry (Hunoor, 2010)

The electronic absorption spectrum of Cu(II) complex (figure 16) displayed bands at 620 nm (16,129.03 cm^{-1}) and 535nm(18691.59cm^{-}). These bands corresponding to the transitions $^2B_1g\rightarrow{}^2B_2g$ and $^2B_1g\rightarrow{}^2Eg$ respectively suggesting a square-planar geometry around Cu(II) ion**.** Room temperature (297K) magnetic moment value of the synthesized Cu(II) complex was 1.81 B.M. which is within the range of 1.73-1.87 B.M. for square planar complexes whereas for tetrahedral in the range of 2.0-2.2 B.M. (Hamil *et al*., 2009; Chandra *et al*., 2013). This value also indicates the paramagnetic nature of the synthesized complex with one unpaired electron. The absence of bands below 10,000cm-1 excluded the possibility of tetrahedral geometry for Cu(II) complexe (Mohammed and Abd El-Wahab, 2003; Akmal *et al*., 2013).The band at 365nm (27397cm^{-1}) was due to charge transfer transition.Therefore, the result of electronic spectral studies and magnetic measurement of this complex confirming a square planar geometry (Chandra and Gupta, 2005; Kothari and Sharma, 2011; Lever and Solomon, 2014).

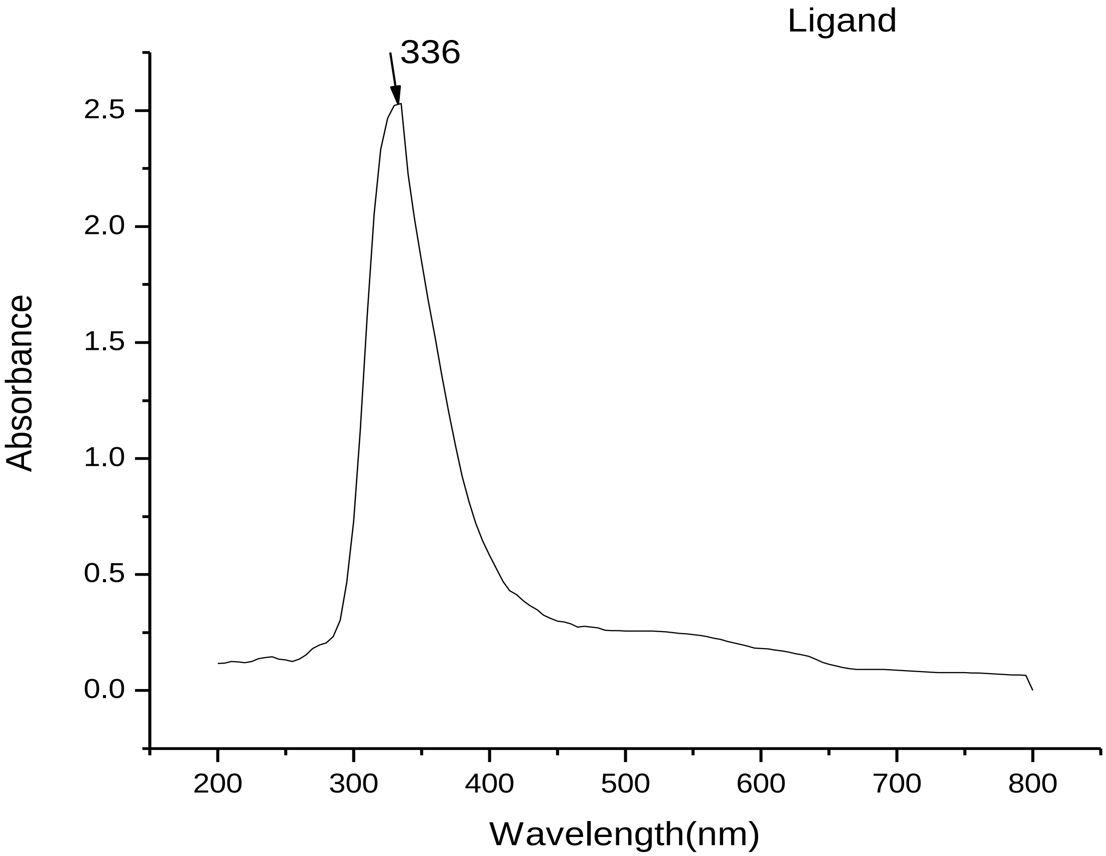

Figure 14: Electronic Absorption spectrum of Furfural semicarbazone.

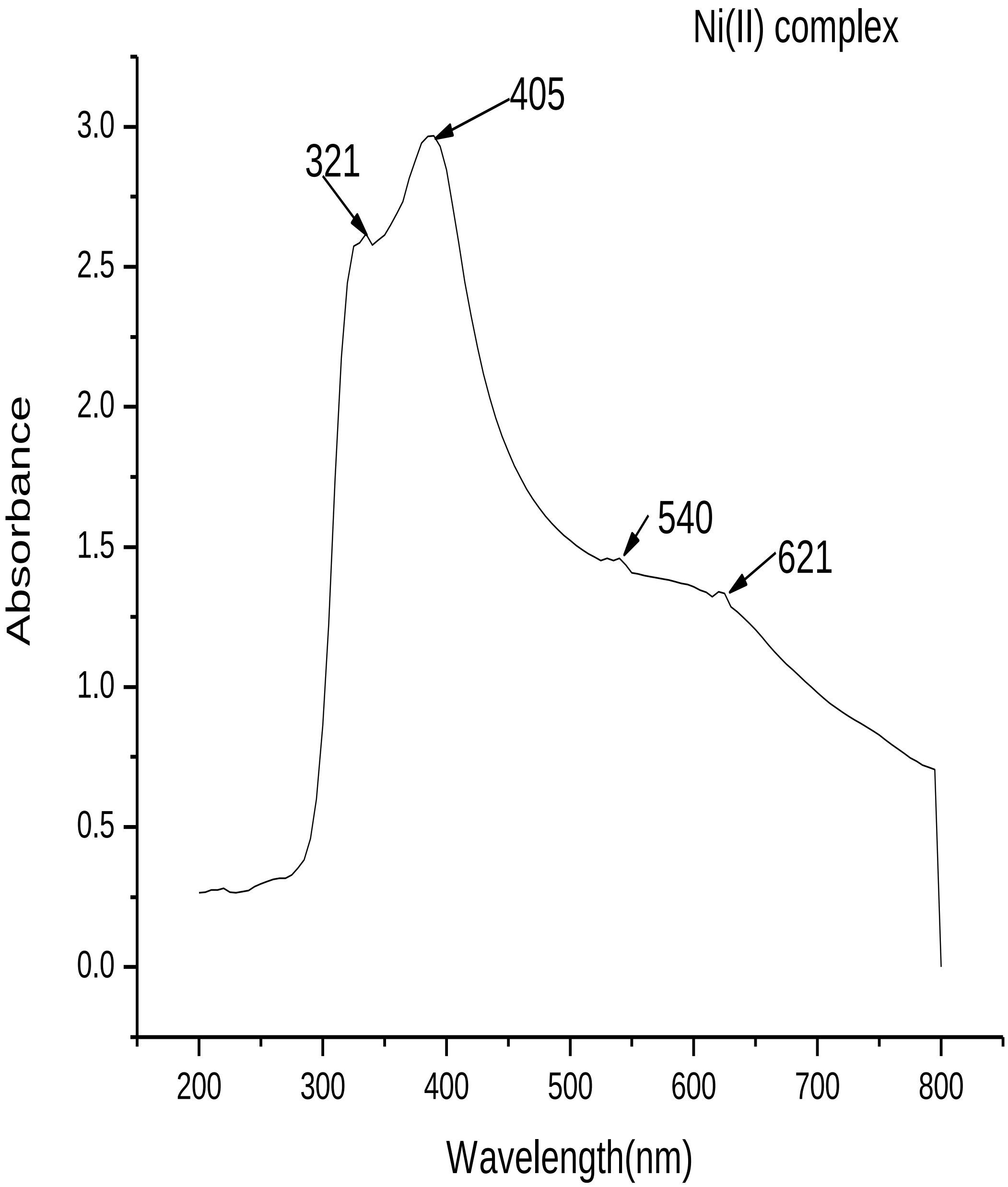

Figure 15: Electronic Absorption Spectrum of Ni(II) complex of Furfural Semicarbazone

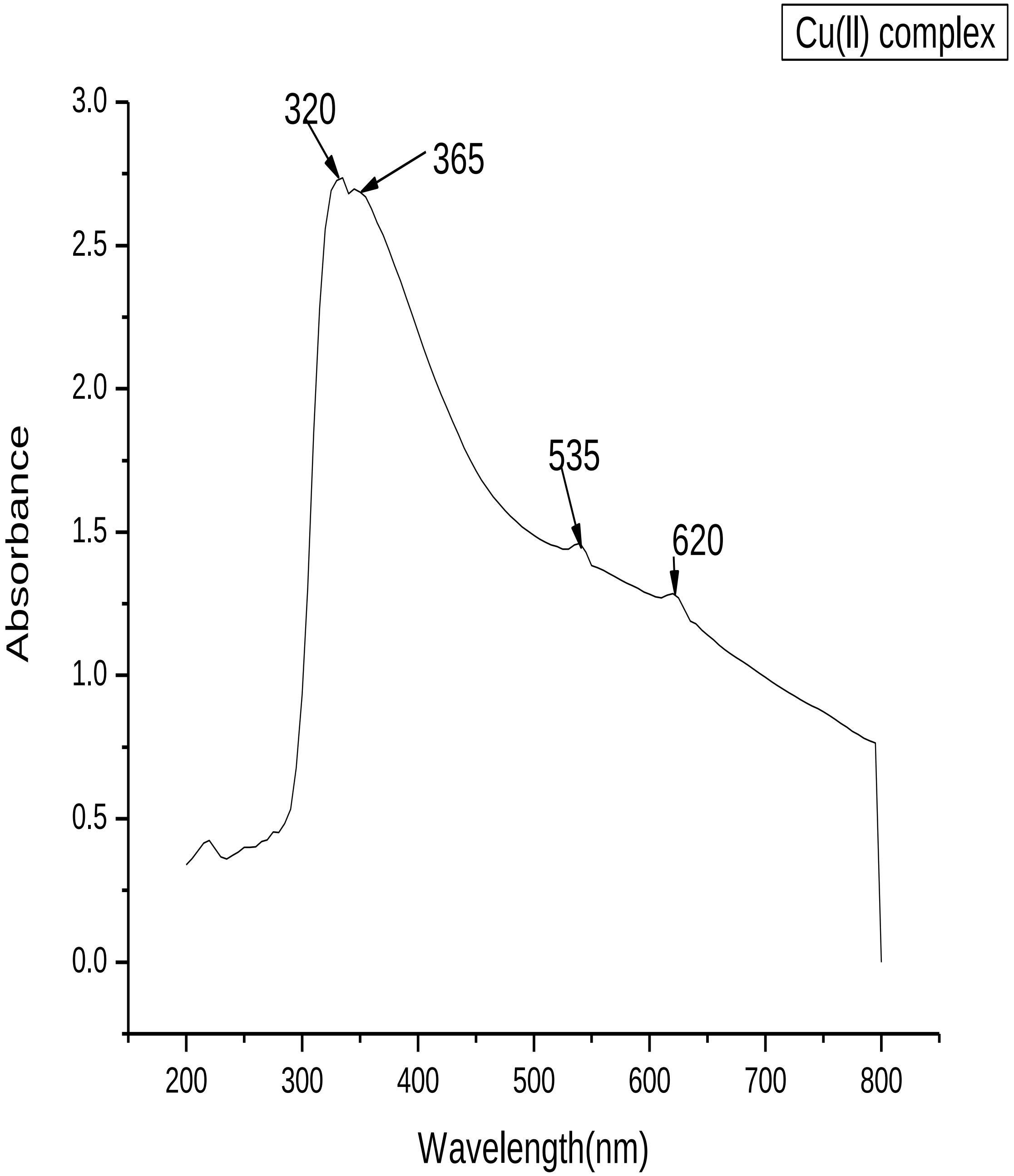

Figure 16: Electronic Absorption Spectrum of Cu(II) complex of Furfural semicarbazone

Table 7: Electronic absorption spectral data of the Schiff base and its complexes

Compound	Absorption Region λmax(cm^{-1})	Transitions Band assignments	Meff (BM)	Magnetic properties	Geometry
$[Ni(L)_2Cl_2]$	16000 18518.52 24691.36 31152.65	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ $^3A_{2g}(F) \rightarrow {}^3T1g(F)$ $^3A_{2g}(F) \rightarrow {}^3T1g(P)$ n–π*	2.83	Paramagnetic	Octahedral
$[Cu(L)_2]Cl_2$	16129.03 18691.59 27397 31250	$2B_1g \rightarrow 2B_2g$ $^2B_{1g} \rightarrow {}^2E_g$ CT band n–π*	1.81	paramagnetic	Square planar
$C_6H_7N_3O_2$	39762	n–π*	-	-	-

Table 8: Molar susceptibility and magnetic moment measurement data

Compounds	Molecular weight	Gram susceptibility (χ_g x10^6cgs)	Molar susceptibility X_M x10^6 cgs	Diamagnetic correction X_Dx10^6cgs	Effective Susceptibility Mcorr x10^6 cgs	Effective magnetic moment µeff (BM)
$C_6H_7N_3O_2$	153.141	-	-	-	-	-
$[Cu(L)_2]Cl_2$	440.734	3.127	1377.3	1444	1852	1.81
$[Ni(L)_2Cl_2]$	435.8814	6.952	3030.95	3097.65	2712.5	2.83

4.3. Proposed Structures of the synthesized Complexes

Based on the inferences made from elemental analysis, chloride test,conductometric measurement, IR, AAS and electronic absorbance spectral studies, the following general structures could be proposed for $[Ni(L)_2Cl_2]$ and $[Cu(L)_2]Cl_2$ complexes,
where L= Furfural semicarbazone.

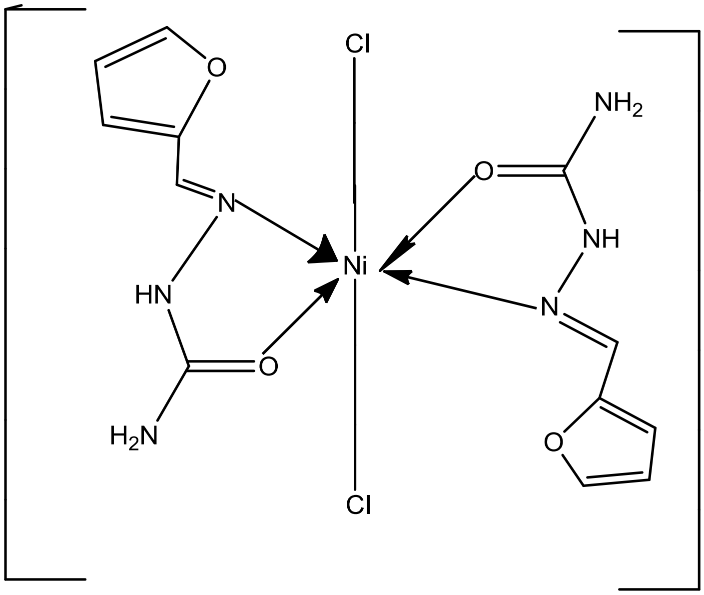

Figure 17: The proposed structure of Ni(II) complex of Furfuralsemicarbazone

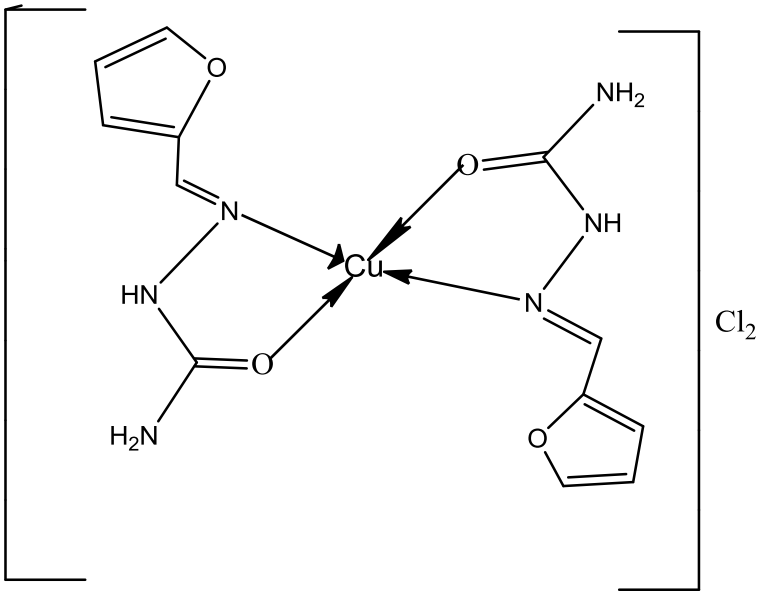

Figure 18: The proposed structure of Cu(II) complex of Furfural semicarbazone

4.4. Antimicrobial Studies

The ligand (L) and its metal complexes were screened against selected bacteria and fungi strains to examine their anti-bacterial and anti-fungal activities. The susceptibility of the bacteria and fungi towards metal complexes were tested by measuring zone of inhibition (in mm) and compared with that of the ligand (L) at a concentration of 20µg/mL. The results are presented in Table 9.

For antifungal activity, the free ligand and its Cu(II) and Ni(II) complexes were screened against *Fusarium oxisporium and Aspergillus niger*.They showed activities with inhibition zone s range of 10.5-18.2mm.Their activities against the selected fungal strains are summarized as; Bavistine (standard drag) > Cu(II) complex > Ni(II) complex > Free ligand (L).Cu(II) complex exhibited higher antifungal against fungal strain used. Antifungal activities of the complexes against fungal strains were better than the free ligand but lower than the standards.

For antibacterial activity, the ligand and its metal complexes were screened against *Escher ich iacoli*,and *Salmonella* gram negative(-Ve) and *Staphylococcus aureus,Streptococcus Agalactia* gram positive(+Ve)strains.They showed activities against *Staphylococcus aureus and Striptoc occus Agalactia* with inhibition zones range of 14.13-15.75mm and 11.56-15mm,respectively. Similarly the free ligand and its metal complexes had an activity against *Escherichia coli* and *Salmonella* with inhibition zones range of 10-17.31mm and 8.9-10.8,respectively. Their activities against the selected bacterial strains are summarized as; *Chloramphinicol* (standard drag) >Ni(II)complex > Cu(II) complex > Free ligand(L).Ni(II) complexes exhibited higher antibacterial activity than Cu(II) complex against all bacterial strains used. Here also the antibacterial activities of the ccomplexes against bacterial strains are better than that of the free ligand but lower than the standard.

Moreover the better antimicrobial activity of the complexes against the test microbes than the free ligand was explained by chelation theory (Wayne, 1999). Most of the pathogens were inhibited by the complexes. On coordination, the polarities of the metal ions are reduced due to overlap of the ligand orbital and partial sharing of the positive charge of the metal ions with the ligand donor atoms. This may increase the lipophilic character of the metal complexes, enabling it to permeate the lipid membrane and thus active in some considered bacteria while small inhibition in the ligand. While chelation is not the only criterion for antimicrobial activity, it may be an intricate blend of several contributions such as the nature of the metal ion and the ligand, the geometry of the metal complex, the lipophilicity, the steric, and the pharmacokinetic factors (Singh and Srivastava, 2011).

Table 9: Antimicrobial activity of the synthesized compounds at concentration of 20 μg/mL

Inhibition zone(mm)						
Compounds	Bacteria				Fungi	
	Staphylococcus Aureus (gram +)	*Striptococcus* Agalactia (gram +)	*Escherichia coli* (gram -)	*Salmonella* (gram -)	*Aspergillus niger*	*Fusarium oxisporium*
$[Ni(L)_2Cl_2]$	*14.44*	*15*	*17.31*	*10.8*	*13.3*	*12.7*
$[Cu(L)_2]Cl_2$	15.75	12.1	14	10.4	18.2	15
$C_6H_7N_3O_2$	14.13	11.56	10	8.9	10.5	0
DMSO	0	0	0	0	0	0
Chloramphenicol(Standard)	40	40	40	34	-	-
Bavistin (standard	-	-	-	-	35.5	31.5

Figure 19: Effect of as-synthesized compounds on *Asperiglles niger fungus*

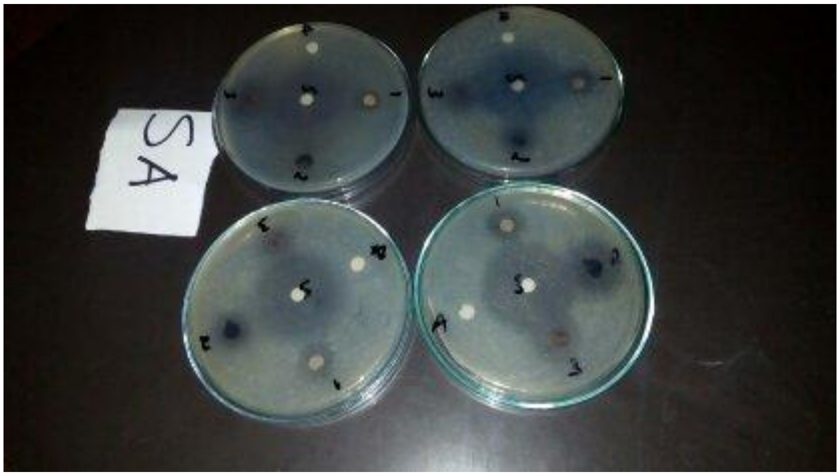

Figure 20: Effect of as-synthesized compounds on *Striptococcus* Agalactia *bacteria*

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary and Conclusions

The present work describes the synthesis, characterization and antimicrobial properties of Furfural semicarbazone ligand (L) derived from Furfural and semicarbazidehydrochloride and its Copper (II) and Nickel (II) complexes. The structure of the ligand and its complexes were elucidated on the basis of elemental analyses, molar conductivities, magnetic susceptibility measurements, IR spectra, UV–Vis., atomic absorption spectroscopy,^{1}H NMR,'and ^{13}C NMR spectra. Moreover, the synthesized Schiff's base ligand and its complexes were tested for their antimicrobial activities.

Chloride ion test suggested the presence of chloride ions inside the coordination sphere for Ni(II) complex and outside the coordination sphere for Cu(II) complex. Molar conductance indicates the non electrolytic nature of Nickel (II) complex and the electrolytic nature of Copper (II) complex.

The stoichiometry of the ligand and its complexes were confirmed by their elemental analysis, and AAS. Elemental analysis of ligand and its Cu(II) and Ni(II) complexes showed good agreement with the proposed structures of the ligand and its complexes .The AAS result confirmed the metal to ligand ratio of 1:2 in both complexes.

FT-IR studies revealed the coordination of the ligand through azomethine nitrogen and carbonyl oxygen atoms to the metal ion. Electronic spectral and magnetic data confirmed octahedral geometry for Ni(II) complex and square planer for Cu(II) complex

The antimicrobial evaluation of Ni(II) and Cu(II)complexes against the selected pathogenic bacterial and fungal strains exhibited higher antimicrobial activity than free ligand and less than the standard drug.Cu(II)complex exhibited higher activity against *Aspergillusniger* strain and Ni(II) complex exhibited higher activity against *Escherichia coli* strain. The biological activity of the ligand was enhanced when it is complexed with metal ion.

5.2. Recommendation

Based upon the results of antimicrobial activities of M(II) complexes, this study opens the way for further study on antimicrobial activities against fungi and bacteria species with higher concentration and with other microbes in addition to the microbes investigated in this study. The synthesized compound with different substituent may also be exploited for better antimicrobial activities, and diverse pharmaceutical properties. This gives hint for the future field of study in medicinal chemistry.

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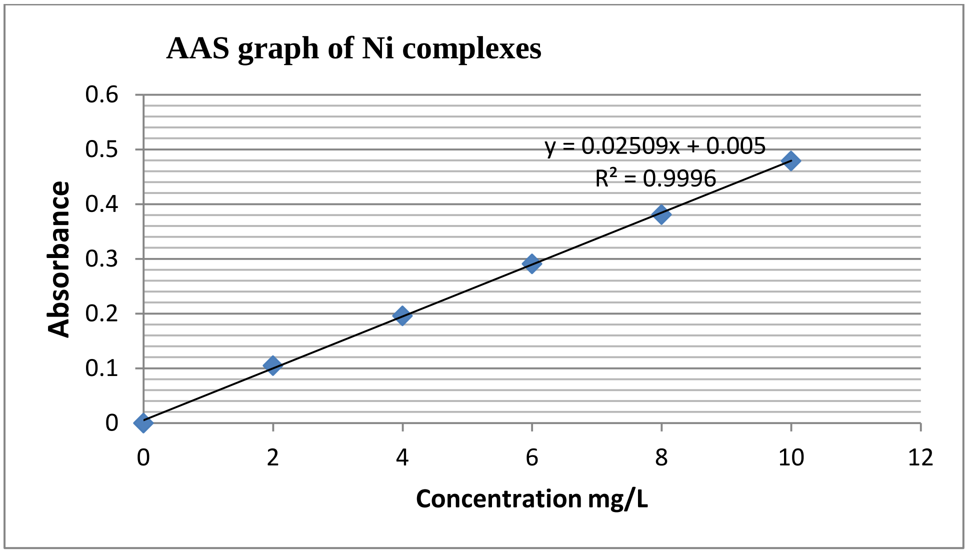
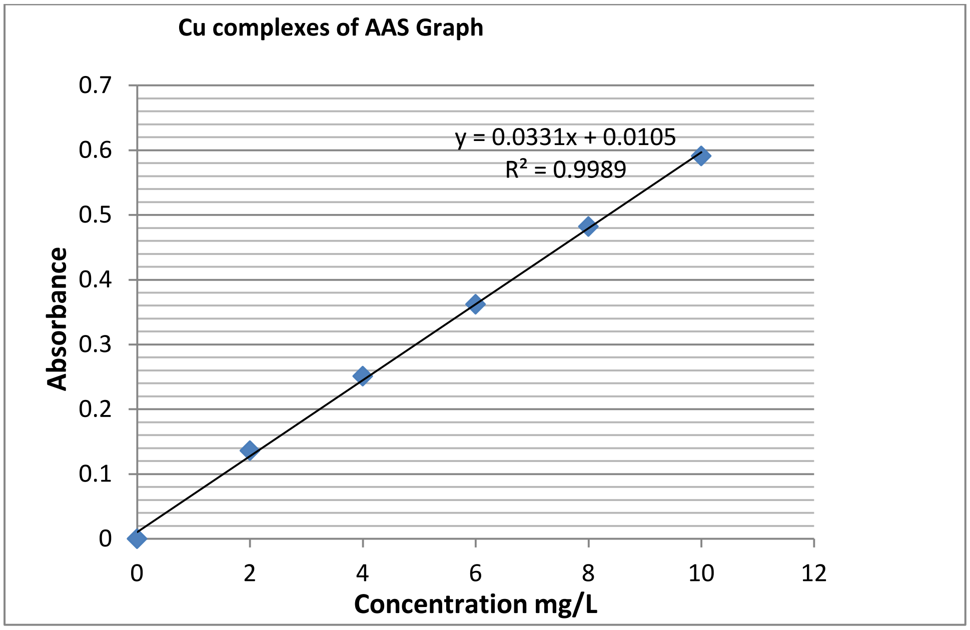
7. APPENDIX



Appendix figure .1: Calibration Curve of Ni(II) Complex of Furfural semicarbazone



Appendix figure 2: Calibration Curve of Cu(II) Complex of Furfuralsemicarbazone.