SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL STUDY OF SCHIFF BASE AND ITS COMPLEXES OF Ni(II) AND Zn(II)

Project work by

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In partial fulfillment of the requirements for the award of degree of

MASTER OF SCIENCE IN

PHARMACEUTICAL CHEMISTRY

Under the Guidance of

Dr. Sindhu Joseph

DEPARTMENT OF CHEMISTRY

BHARATA MATA COLLEGE, THRIKKAKARA

CERTIFICATE

This is to certify that this project work entitled **"Synthesis, characterization and antibacterial study of Schiff base and its complexes of Ni(ll)and Zn(ll)"** is an authentic record of the project work carried out by **STYGA SIVAN** of final MSc Chemistry under the supervision and guidance of **Dr. P. V**. **MOHANAN**, Professor of Department of Applied Chemistry, Cochin University of Science and Technology in the partial fulfillment of the requirement for the award of the degree of Master of Science in Pharmaceutical Chemistry of Bharata Mata college, Mahatma Gandhi University, Kottayam during the academic year 2018-2020.

Cochin-22

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CERTIFICATE

This is to certify that the project report entitled "**Synthesis, characterization and antibacterial study of Schiff base and its complexes of Ni(ll) and Zn(ll)**" is an authentic record of the project work carried out by **Ms.STYGA SIVAN** (Reg.no:180011017736) in partial fulfillment of the award of the degree of Master of Science in Pharmaceutical chemistry at Bharata Mata College, Thrikkakara affiliated to Mahatma Gandhi University, Kottayam under my guidance and supervision during 2018-2020.

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DECLARATION

I, Styga Sivan hereby declare that the work presented in this project entitled "**Synthesis, characterization and antibacterial study of Schiff Bases and its complexes of Ni(ll) and Zn(ll)"** is entirely original work which was carried independently under the supervision of **Dr. P. V Mohanan**, Professor of Department of Applied Chemistry, Cochin University of Science and Technology and has not been included in any other project submitted previously for the award of any other degree.

Thrikkakara STYGA SIVAN

June 2020

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STYGA SIVAN

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CHAPTER 1

INTRODUCTION

1.1 Schiff Base

Schiff bases are organic compounds having azomethine group (-C=N). Schiff bases are generally synthesized from the condensation reaction of primary amines and active carbonyl groups. Structurally a Schiff base is considered as a nitrogen analogue of an aldehyde or ketone where the carbonyl group has been replaced by an imine or azomethine group. The German Chemist, Nobel Prize winner, Hugo Schiff innovated Schiff base in 1864 [1]. Schiff bases are weak bases and hydrolysed by dilute mineral acid. But not hydrolysed by aqueous alkali. Schiff bases are very useful in medicinal and pharmaceutical field. Schiff bases exhibit broad range of biological activities such as antifungal, antibacterial, antimalarial, anti-inflammatory, antiviral and antipyretic properties [2].

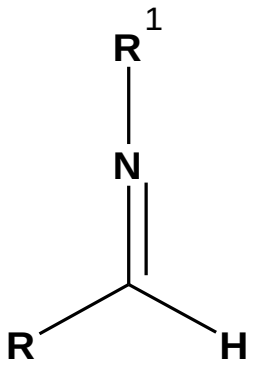


Figure: 1 Structure of Schiff base ligand

Schiff base contains a Carbon- Nitrogen double bond (-C=N-) with the nitrogen atom which should connected to an aryl or alkyl group, not a hydrogen atom i.e., R^1 is an alkyl or aryl group (not hydrogen atom). R may be hydrogen or alkyl or aryl group. Several studies showed that the presence of a lone pair of electrons in an SP^2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance. Because of the relative easiness of preparation, synthetic, flexibility and the special property of C=N group,

Schiff bases are generally excellent chelating agent [3]. Schiff bases are considered as subclass of imines, either secondary ketamines or secondary aldamines depending upon their structures. Potentially Schiff bases are forming stable complexes with metal ions. Therefore Schiff base ligands are essential compound in the coordination chemistry.

The synthesis of Schiff base from an aldehyde or ketone and amine is reversible reaction. The reaction occurs under the presence of acid or base catalyst upon heating [4]. Aryl substituted Schiff bases are substantially more stable and more readily synthesized. But alkyl substituted Schiff bases are relatively unstable. Due to the presence of effective conjugation, Schiff bases with aromatic substituted aldehydes are more stable, while those with aliphatic aldehydes are relatively unstable and easily polymerizable [5].

Mechanism of Schiff base formation includes nucleophilic addition reaction to the carbonyl group either aldehyde or ketone. In this reaction, amine is considered as nucleophile. In the first step of mechanism, amine reacts with electrophilic carbonyl carbon of aldehyde or ketone to form an unstable compound called carbinolamine. In the second step, carbinolamines loses water by either in the presence of acid or base. Dehydration of carbinolamine is the rate determining step. Amine should not be protonated to form non-nucleophilic nature. If amine is protonated, the equilibrium shifted to the left and carbinolamine cannot be formed. As a whole, the formation of Schiff base is two step reaction that is addition of H^+ followed by elimination of water.

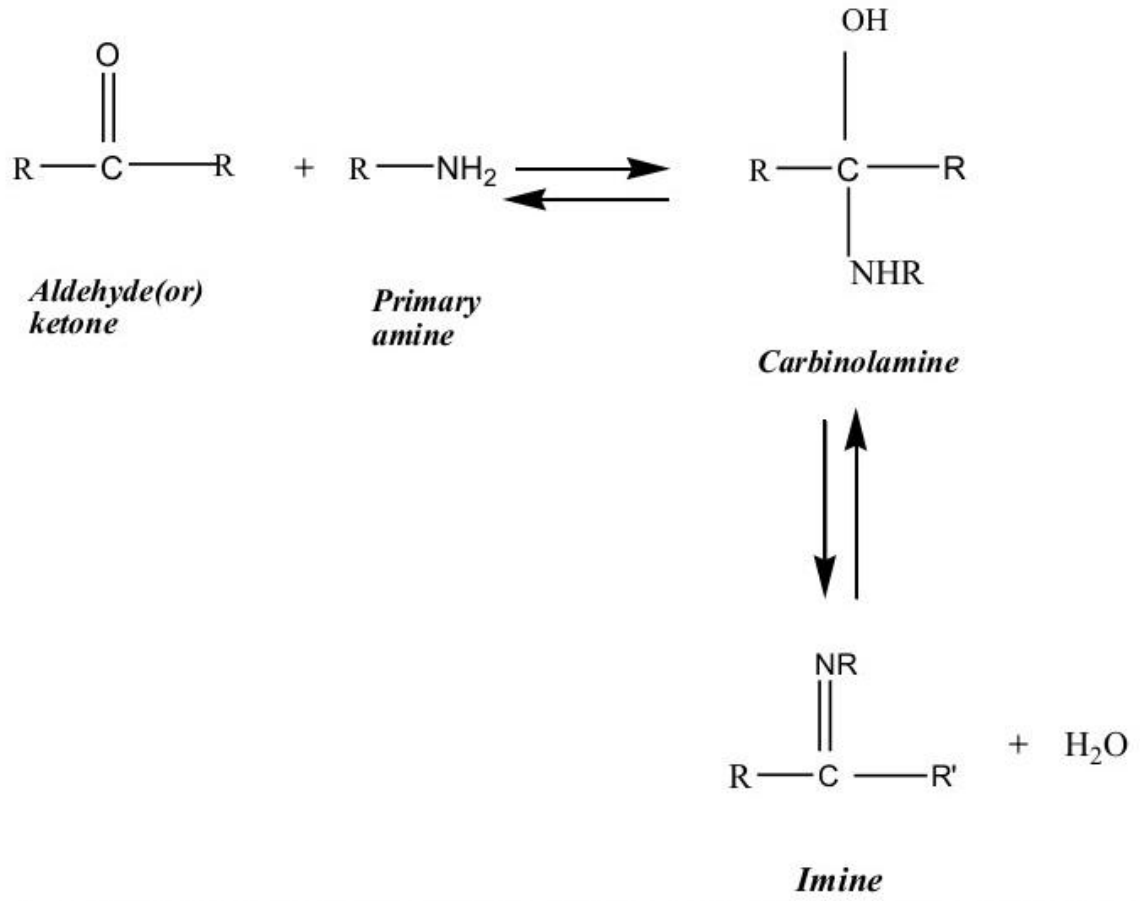


Figure: 2 General scheme for the formation of Schiff base

1.2 Application of Schiff bases

Schiff base ligands reacts with metal salts to give metal complexes of Schiff bases under the suitable experimental condition. Potentially Schiff base ligands are capable of forming stable complexes with metal ions. Schiff bases and their metal complexes are versatile compounds synthesized from the condensation of an amino compound with carbonyl and widely used for industrial purposes and also exhibit a broad range of biological activities including antifungal, antibacterial, antimalarial, anti-inflammatory, antiviral, and antipyretic properties.

Many Schiff base complexes show excellent catalytic activity in various reaction at high temperature (>100) and in the presence of moisture. Over the past few years, there have been many reports on their application in homogeneous and heterogeneous catalysis. The high thermal and moisture stabilities of many Schiff base complexes were useful attributes for their application as catalyst in reaction involving at high temperatures. The activity is usually

increased by complexation therefore to understand the properties of both ligands and metal can lead to synthesis of highly active compounds. The influence of certain metals on biological activity of these compounds and their intrinsic chemical interest as multidentate ligands has prompted a considerable increase in the study of their coordination behaviour [6].

Schiff base has wide variety of synthetic uses in organic chemistry, analytic chemistry, biological chemistry and inorganic chemistry. They also exhibit a broad range of biological activities including antifungal, antibacterial, anti-inflammatory, antimalarial, and antiviral and so on. The Schiff bases and their metal complexes showed a better catalytic activity in various reaction such as polymerization reaction, reduction of ketones, Henry reaction, epoxidation of alkenes, Diels alder reaction etc. Schiff bases act as an important intermediate in a number of enzymatic reaction which involving the interaction of an enzyme with an amino or carbonyl group of the substrate. Chemotherapeutic nature of Schiff base is become important in medicinal chemistry. Schiff bases are used as synthons in formation of the number of industrial and biologically active compounds such as formazans, 4-thiazolidins, and benzoxazine and so on, via, ring closure, cycloaddition and replacement reaction. Schiff bases are used as pigment, polymer stabilizer [7]. Schiff compound used as dyes. Schiff base is a good corrosion inhibitors [8].

1.3 Biological activities of Schiff base

1.3.1 Antibacterial activity

Schiff bases are compounds that destroy bacteria or suppresses their growth or their ability to reproduce. Many antibacterial products are used for cleaning hand washing purposes. The increased in the mortality rate associated with infectious diseases is directly related to bacteria that exhibit multiple resistance to antibiotics. The lack of effective treatments is the main cause of this problem. The development of the new antibacterial agents with novel and more efficient mechanisms of action is definitely an urgent medicinal need. Most of Schiff base ligands and its metal complexes are play important role to inhibit the growth of bacteria. For example, the Schiff base of N-salicylidene-2-hydroxylaniline (figure2) is used as antibacterial agents against the bacteria Mycobacterium tuberculosis [9].

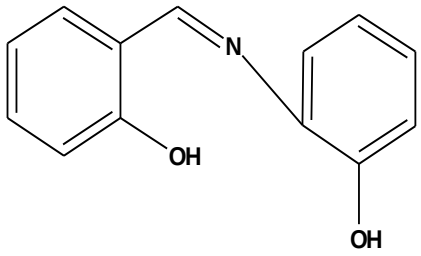
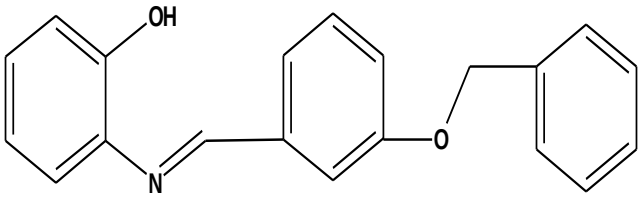
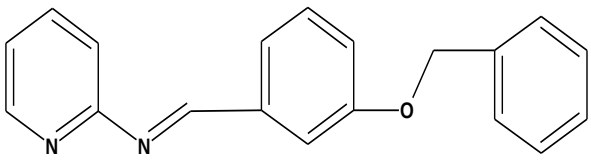


Figure: 3 N-salicylidene-2-hydroxyaniline

Demir muzazimoglu aysen et al synthesized 2 different Schiff bases and studied their antibacterial property. The Schiff base ligands were derived by reacting carbonyl of 4-benzyloxylbenzaldehyde with 2- aminophenol or 2-aminopyridine. The Schiff base of 2-(4-benzyloxyl-benzylidene) aminophenol (fig:3 compund a) synthesized from reaction between 4-benzyloxybenzaldehyde and 2-aminophenol. (4-benzyloxyl-benzylidene)pyridine-2-yl-amine (fig:3 compound b) synthesized from reaction between 4-benzyloxybenzaldehyde and 2-aminopyridene. Disc diffusion and micro dilution technique used for prepared compound against the 5 bacteria. I.e., Escherichia coli, Bacillus cereus, Bacillus subtilis, Streptococcus mutans and Staphylococcus aureus. The compound b exhibit moderate activity against Coli, B.subtilis, S.aureus, and B.cereus. The compound a showed moderate activity against E.coli, B.subtilis, S.mutans, S.aureus and B.cereus due to the presence of phenyl ring [10]



Compound a



Compound b

Figure: 4 Benzyloxybenzaldehyde derivative of Schiff bases

Certain metal complexes of Vo(ll), Co(ll), Rh(ll), Pd(ll) and Au(lll) with Schiff base of 2N-Salicylidene-5-(p-nitrophenyl)-1,3,4-thiadizole (figure 4) was reported by Emad yousif et al. The ligand and their metal complexes were tested for their antibacterial activity against Staphylococcus aureus, Salmonella typhi and Escherichia coli bacterial strains by agar diffusion method. All metal complexes showed moderate activity against the bacteria [11]

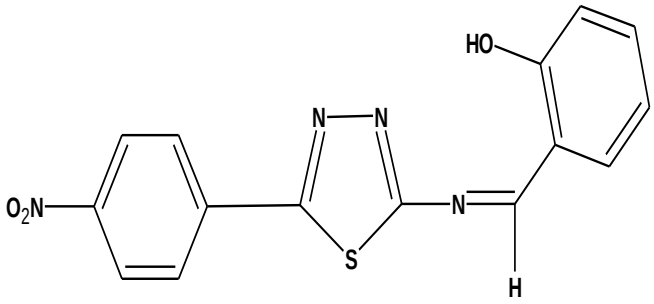


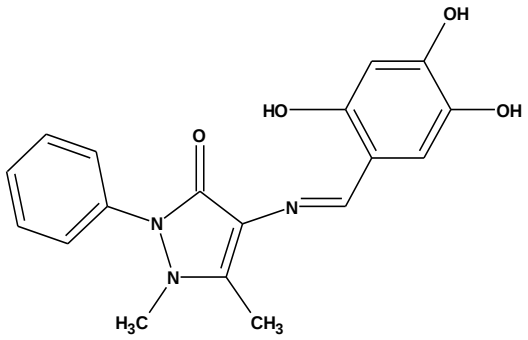
Figure:5 Schiff bases of 2N-salicylidene-5-(p-nitrophenyl)-1,3,4-thiadizole

1.3.2 Antioxidant activity

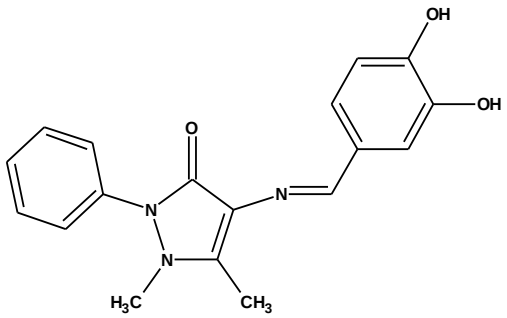
Antioxidant are compounds that prevent the oxidation. Antioxidants are plays important function to protect the human body against the damage by reactive oxygen species. Free radicals are produced in the oxidation reaction and leading to chain reaction that damage the cells of organisms. Schiff bases and metal complexes possesses potent antioxidant activity. P.Valentina et al reported antioxidant activity of some substituted 1,2,4-triazo-5-thione Schiff base. Series of 3-substituted 1,2,4-triazo-5-thione Schiff base were prepared from the ester of methyl paraben. Antioxidant activity of these compounds were evaluated by hydrogen peroxide scavenging method. The results showed that all these compounds have good antioxidant activity. The activity was shown as IC_{50} lies between 20 to 60 μg/ml [12].

Series of Schiff bases of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one were synthesized by Mohammal sayed alam et al from different substituted benzaldehyde. All these compounds were evaluated for antioxidant activities using DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The results showed that 4-(2,4,6-Trihydroxylbenzylideneamino)-1,5-dimethy-2-phenyl-1H-pyrazole-3-(2H)one (fig:5 compound a) and 4-(3,4-Dihydroxylbenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3-(2H)one(fig:5compound b) from all the synthesized compounds showed highest antioxidant activity due to the presence

of the hydroxyl substituted phenyl ring. IC_{50} values for the compounds a and b found to be 0.44 and 0.93 μM respectively comparable to that of ascorbic acid (IC_{50} 0.41 μM) which used as standard antioxidant agent [13].



Compound a



Compound

Figure:6 Schiff base derivatives of 4-amino-1,5-dimethyl-2-phenylpyrazole-3-one

1.3.3 Anticonvulsant activity

Anticonvulsant are used for treatment of epileptic seizures. It is also known as antiepileptic drugs or antiseizure drugs. The spread of the seizure within the drug also prevented by the action of anticonvulsant. Presently, Schiff base ligands and their metal complexes have been reported as anticonvulsants.

Schiff bases of N-methyl and N-acetyl isatin derivatives with different aryl amines were synthesized by Manjusha varma et al. All the prepared compounds were evaluated for

anticonvulsant activities against maximal electroshock (MES) and subcutaneous metrazole (ScMet). N-methyl-5-bromo-3-(p-chlorophenylimino) isatin (figure 6) from all synthesized compounds showed excellent anticonvulsant activity in MES and ScMet with LD_{50} >600 mg/kg. It showing excellent anticonvulsant activity than standard drug such as phenytoin, carbamazepine and valproic acid.

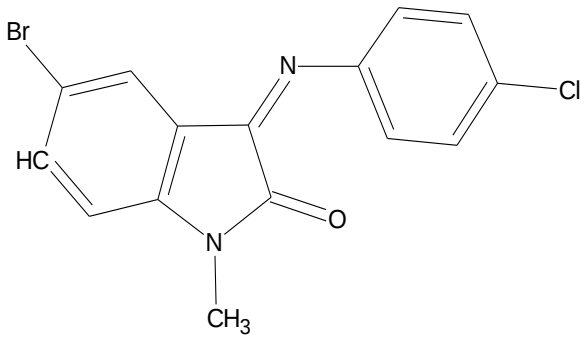


Figure:7 Isatin derivatives of Schiff base

The compound with chlorosubstituent in phenyl ring and bromosubstituent in isatin ring showed a broad spectrum of activity in both MES and ScMet without neurotoxicity at normal dose [14].

P. Paneersalvam et al synthesized series of Schiff bases of 3-amino-6,8-dibromo-2-phenylquinazolin-4-(3H)ones with different substituted aldehydes. All these compounds were investigated for anticonvulsant activity on albino mice against maximal electroshock method. Phenytoin was used as standard drug. In all these compounds, Schiff bases of 3-amino-6,8-dibromo-2-phylquinazolin-4-(3H)ones with styryl substituted aldehyde (figure:7) showed a very high anticonvulsant activity (82.74%) at dose level of 100 mg/kg b.w due to the presence of extended conjugation [15].

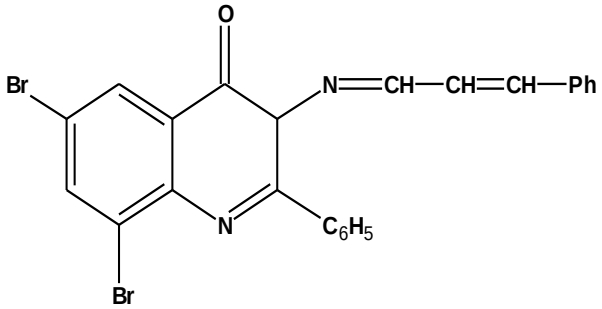


Figure: 8

1.3.4 Anticancer activity

Cancer is an uncontrolled growth of the cells and multistage of progressive disease. Cancerous disease can affects almost all body system. Anticancer activity is the effect for treatment of cancerous disease. Most of the Schiff bases and their metal complexes have been reported as anticancer agent.

Copper complex of Schiff base of 5-dimethyl-2-phenyl-4-[(pyridine-2-ylmethylene)-amino]-1,2-dihydropyrazol-3-one (figure:8) show a potent anticancerous activity when studied against human tumors cells such as humancervical cancer line (HeLa) and human breast cancer cell line (McF-7) [16].

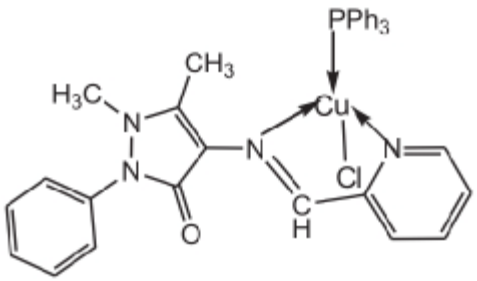


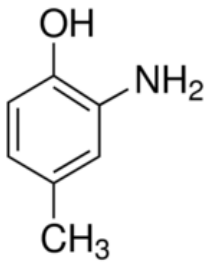
Figure:9 Cu complex of Schiff base.

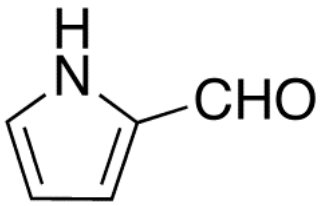
1.4 Importance of 2-amino-4-methylphenol, pyrrole-2-carboxaldehyde.

2-amino-4-methylphenol was used in the preparation of functionalized spiropyran derivatives of 2H-1,3-benzoxazinone. Its molecular formula is C_7H_9NO. It combines with acetylacetone in ethanol to form 4-(2-hydroxy-5-methylphenyl)imino-2-pentanone. It converted to form dihyrophenoxazinone by purified human haemoglobin. It occurs in brown powder. It used as sensitizer in allergy to disperse yellow3.

Pyrrole-2-carboxaldehyde containing a formyl group at the second position. It is considered as member of pyrroles and 1,3-thiazole-2-carboxaldehyde. Coffee and coffee products are containing pyrrole-2-carboxaldehyde. It occurs in pale yellow crystalline solid. Its molecular

formula is C_5H_5NO. The aldehyde group in C_5H_5NO undergo nucleophilic attack by amine to form Schiff bases. It is considered as pharmaceutical intermediate. Condensation of pyrrole-2-carboxaldehyde leads to form pyrrole-2-carboxaldehydesalicylhydrazone. It is used as starting material for the preparation of pyrrole-2-carboxylic acid by oxidation reaction.





2-amino-4-methylphenol **pyrrole-2-carboxaldehyde**

Figure 10

1.5 SCOPE OF PRESENT INVESTIGATION

Schiff bases are considered as very important organic compounds because of their ability to form complexes with transition metal ions and its pharmacological properties. Transition metal complexes containing Schiff bases have been of much interest over the last years, because of its various applications in biological process and potential application of designing new therapeutic agents. Many Schiff bases were found to satisfy the requirements for acting as catalyst for oxidation of organic substances. Selective oxidations of organic substances are important in chemical and photochemical industries due to the wide variety of products synthesized in this route. Many Schiff base complexes have antioxidant, anticancerous, antibacterial activities and so on. The wide applicability of these complexes inspired us to synthesize new Schiff base complexes.

CHAPTER II

MATERIALS AND METHODS

2.1 REAGENTS

1) Pyrrole-2-carboxaldehyde

2) 2-amino-4-methylphenol

3) Nickel acetate

4) Zinc acetate

2.2 SOLVENTS

1) Methanol

2) Petroleum ether

3) Chloroform

4) DMSO

2.3 INSTRUMENTAL TECHNIQUES

2.3.1 ELEMENTAL ANALYSIS

Elemental analysis is a process in which sample of some materials is analysed for its elemental composition. Elemental analysis can be quantitative and qualitative. The most common form of elemental analysis is CHN analysis which was done on a Vario EL III CHNS elemental analyzer at the SAIF, Cochin University of science and Technology, Kochi, India.

2.3.2 INFRARED SPECTROSCOPY

Spectroscopy which involves change in the vibrational energy levels due to the absorption of infra-red radiation is called infra-red spectroscopy. The vibrational state of a molecule can be probed in a variety of ways. The most direct way is infrared spectroscopy because vibrational transition typically acquire an amount of energy that corresponds to the infrared region of the

spectrum between 4000-400cm^{-1}. Radiation in the region can be utilizes in structure determination since the functional groups give rise to characteristics bands both in terms of intensity and position. Fourier transform infrared (FTIR) spectrometer is used as a common laboratory instrument in this technique.

Infrared spectra were recorded on a JASCO FT-IR spectrometer with KBr pellets at Department of Applied Chemistry, Cochin University of Science and Technology, Kochi, India.

2.3.3 MASS SPECTROSCOPY

Mass spectroscopy is an analytical technique that determining the molecular mass of the compound and its elemental composition. In a typical mass spectrometry, molecules are bombarded with highly energetic electrons. This may cause some of the sample's molecules to break into charged fragments or simply become charged without fragmenting. Some which are positive ions. Each ions are related to particular mass to charge ratio (m/z). It is used to prove the identity of a compound and establish structure for a new compound. It helps to establish exact molecular mass and molecular formula. In mass spectrometer the sample is introduced into high vacuum chamber where neutral molecules pass through a beam of electrons where ionization occurs.

The instrument used for Mass spectrometry is Waters 3100 Mass Detector using ESI technique designed for routine LC-MS analysis was recorded at Department of Applied Chemistry, CUSAT.

2.3.4 NMR SPECTROSCOPY

Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy or magnetic resonance spectroscopy, is a spectroscopic technique to observe local magnetic fields around atomic nuclei. It is used to investigate the properties of organic molecules to confirm the identity of substances. Under appropriate conditions in a magnetic field, a sample can absorb electromagnetic radiation in the radio frequency region at frequencies governed by the characteristics of the sample. Absorption is a function of certain nuclei in the molecule. As the fields are unique or highly characteristic to individual compounds, in modern organic chemistry practice, NMR spectroscopy provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. The difference in the absorption

position of a reference proton is called the chemical shift of the particular proton. In NMR spectrum, chemical shift is expressed in terms of parts per million (ppm). Tetramethylsilane (TMS) is taken as the reference. The most common types of NMR are proton and carbon-13 NMR spectroscopy, but it is applicable to any kind of sample that contains nuclei possessing spin.

^{1}H NMR and ^{13}C NMR spectra were recorded in chloroform on a Bruker advance 111400 MHz FT- NMR spectrometer using TMS as internal standard at SAIF, Cochin University of Science and Technology, Kochi, India.

2.3.5 ELECTRONIC SPECTROSCOPY

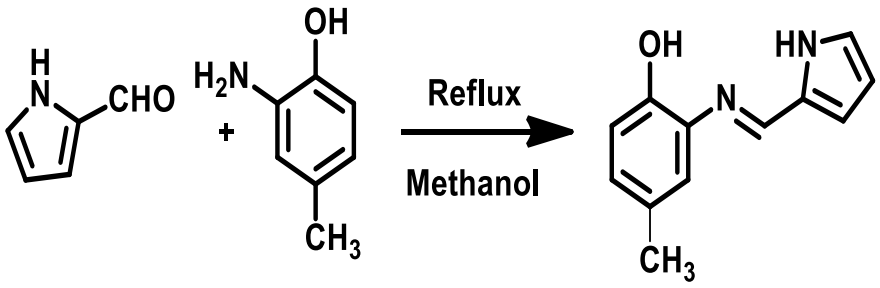
Electronic spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis) relies on the quantised nature of energy states. Given enough energy, an electron are excited from ground state to excited state. Without incentive, an electron will not transition to a higher level. Electron can be excited only by absorbing energy. Electrons in the excited state will relax back to its ground state by releasing energy as photons. Bonding and non-bonding electrons can absorbs energy in the form of ultraviolet or visible light and these electrons are excited to higher anti-bonding molecular orbitals. n$\rightarrow \pi^*$, $\pi \rightarrow \pi^*$, $\sigma \rightarrow \sigma^*$ and n $\rightarrow \sigma^*$ transition are the 4 possible type of transition. The electronic transition involved in metal complexes can be broadly classified into types. Ligand field transition or metal centered transition which include d-d transition and f-f transition, intra-ligand transition involving $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$ and n $\rightarrow \pi^*$ transition of ligand and charge transfer transition. Charge transfer transition are classified into three types, metal to ligand MLTC, ligand to metal LMTC and charge transfer to solvent.

Electronic spectra were recorded in DMSO on a Spectro UV-Vis Double Beam UVD-3500 spectrometer in the range 200 to 900nm at the Department of Applied Chemistry, Cochin University of Science and Technology, Kochi, India.

2.4 PREPARATION OF LIGAND AND ITS METAL COMPLEXES.

2.4.1 Synthesis of Schiff base from Pyrrole-2-carboxaldehyde and 2-amino-4-methylphenol (L)

Methanolic solution of 2-amino-4-methylphenol (8.2 mmol) were added to methanolic solution of pyrrole-2-carboxaldehyde (8.2 mmol). The mixture was refluxed on a boiling water bath for 5-6 h. The solvent evaporated slowly. Crystalline products obtained were filtered, washed with water, methanol and ether and recrystallized twice from ethanol methanol mixture. The yields of the product were determined.



Scheme: 1 Preparation of ligand L

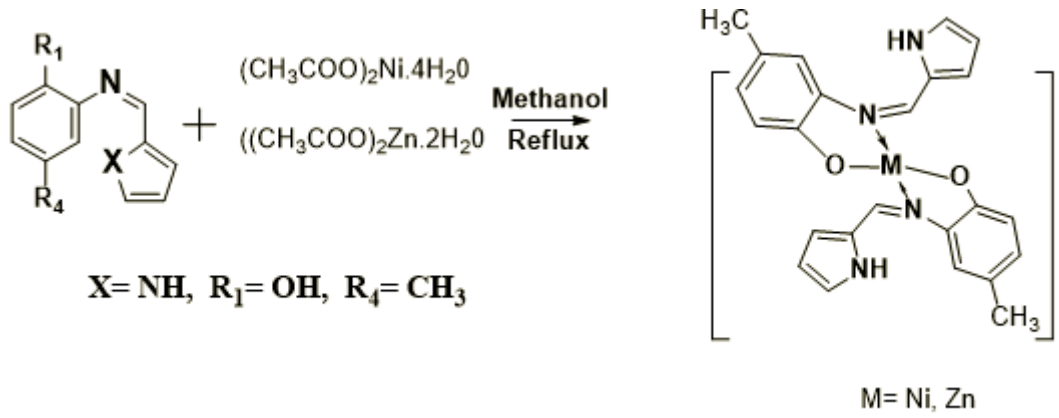
2.4.2 Synthesis of Ni(ll) Complex of L ($Ni(L)_2$)

A Solution of nickel acetate (2.48g, 0.01mol) in 25 ml methanol was added to the solution of the Schiff base, L (0.01mol) in 40 ml methanol. The solution was refluxed for three hours at 60^0C. Ni(ll) complexes were precipitated in hot. The product was washed with cold methanol and petroleum ether before drying over phosphorous pentoxide in a desiccator.

2.4.3 Synthesis of Zn(ll)Complex of L ($Zn(L)_2$)

A Solution of zinc acetate (2.19g, 0.01mol) in 25 ml methanol was added to the solution of the Schiff base, L(0.01mol) in 40 ml methanol. The solution was refluxed for three hours at 60^0C.

Zn(ll) complexes were precipitated in hot. The product was washed with cold methanol and petroleum ether before drying over phosphorous pentoxide in a desiccator.



X= NH, R_1= OH, R_4= CH_3

M= Ni, Zn

Scheme: 2 Preparation of metal complexes of L

CHAPTER III

RESULTS AND DISCUSSIONS

3.1 ELEMENTAL ANALYSIS

Elemental analysis obtained is in good agreement with the assigned chemical formula of the proposed structure of ligand and its metal complex. The analytical data for the ligand L, $Ni(L)_2$ and $Zn(L)_2$ are given the Table: 1

Table: 1 Elemental analysis

Complex	molecular weight	Colour (Yield %)	Elemental analysis Found (calc %)				μ_{eff} (B.M)	Molar conductance ($ohm^{-1}cm^2mole^{-1}$)
			C	H	N	Metal		
L	200.24	Light yellow (68)	71.98 (71.67)	6.04 (6.01)	13.99 (13.51)	-	-	-
$Ni(L)_2$	457.15	Dark green (70)	62.85 (63.05)	4.55 (4.85)	12.17 (12.26)	12.42 (12.84)	3.57	20
$Zn(L)_2$	463.86	Green (72)	61.90 (62.11)	4.78 (4.53)	12.03 (12.08)	13.90 (14.10)	-	3.5

3.2 INFRARED SPECTROSCOPY

The IR bands of Schiff bases L, $Ni(L)_2$ and $Zn(L)_2$ give important information about the various functional groups present in it. The band shows in the range 1630-1600cm^{-1} which shows strong band of azomethine υ (HC=N) group. The IR spectra of compounds shows stretching bands in the range 1290-1250cm^{-1}which indicate the phenolic C-O group. FT-IR spectral bands of Schiff bases and their spectra are given in the Table: 2.

Table: 2 Infrared spectral data of Schiff bases

Compound	υ(C=N)	υ(CO)
L	1620	1280
$Ni(L)_2$	1602	1254
$Zn(L)_2$	1602	1271

A strong absorption band is observed at 1620 cm^{-1} due to the presence of υ(C=N) azomethine group in free ligand L. This was shifted to 1602 cm^{-1} in both metal complexes of ligand L, which indicates the coordination of metal takes place through azomethine group. Band 3350 cm^{-1} is a characteristic band of phenolic υ(OH) group present in the ligand L. Band at 1280 cm^{-1} is due to the presence of phenolic υ(C-O) group in free ligand L. Phenolic moiety in complexation process is deprotonated, which confirmed by shift of υ(C-O) stretching band to a lower frequency. Band at 1254 cm^{-1} and 1271 cm^{-1} was assigned to the stretching band of group υ(C-O) for $Ni(L)_2$ and $Zn(L)_2$ respectively. The shift of υ(C-O) band to a lower frequency indicate the formation of stronger M-O bond and weakening of C-O bond.

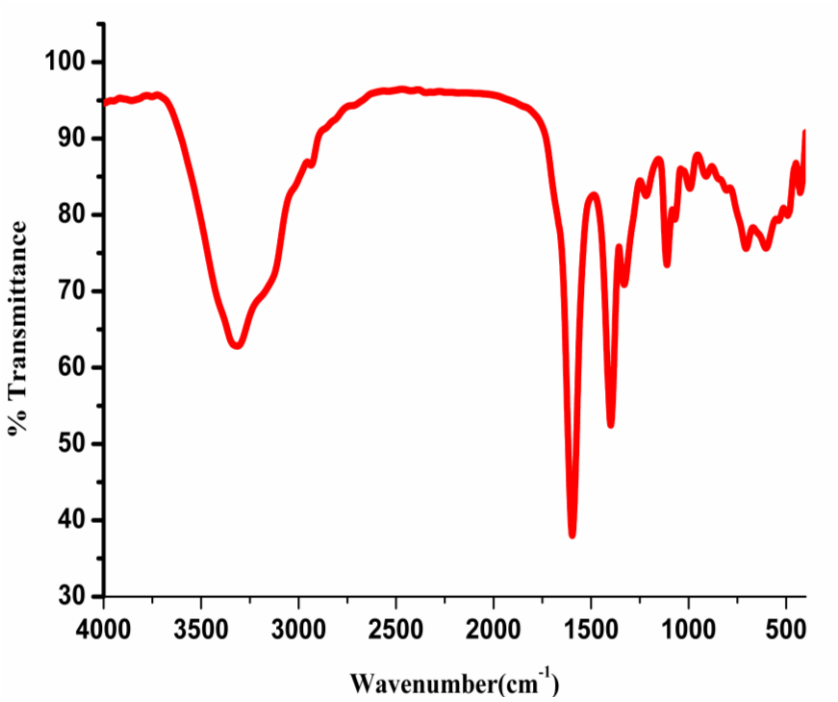


Figure: 11 IR spectrum of L

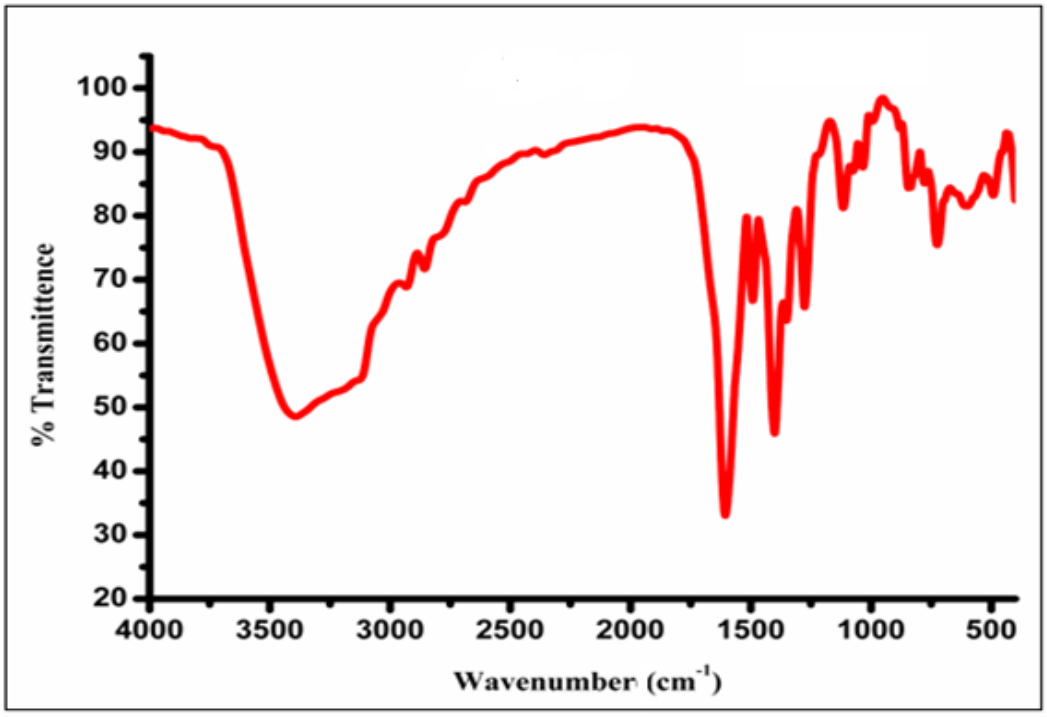


Figure: 12 IR spectrum of $Ni(L)_2$

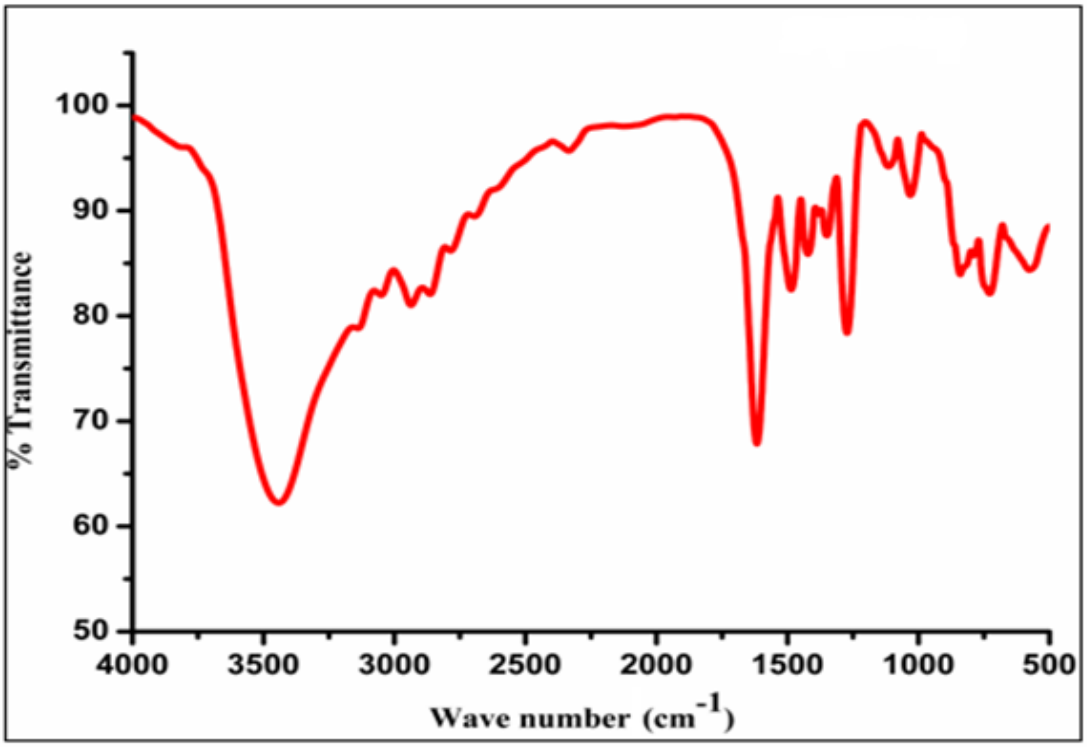


Figure: 13 IR spectrum of $Zn(L)_2$

3.3 NMR SPECTROSCOPY

For unknown compounds, NMR can either be used to match against spectral libraries or to infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as studying physical properties at the molecular level such as conformational exchange, phase changes, solubility and diffusion. The ^{1}H-NMR spectra of ligand L, The singlet at δ 8.4ppm is corresponds to the azomethine proton (-CH=N-). The singlet at 2.30ppm is corresponds to the methyl proton. The peaks at δ6.2ppm – δ7.1ppm corresponds to the aromatic protons. The broadness of the spectrum was due to the strong hydrogen bonding. ^{13}C-NMR spectra of L, the formation of the schiff base is supported by the presence of a peak at δ158 ppm corresponds to the azomethine carbon (C=N). The pyrrole carbons show signals at 121ppm, 111ppm, 115ppm, and 133ppm. Benzene carbons show signals at 119-146ppm. The methyl carbon show signal at 21ppm.

The ^{1}H NMR and ^{13}C NMR spectrum of the ligand L is recorded in $CDCl_3$ and DMSO respectively using TMS as internal standard

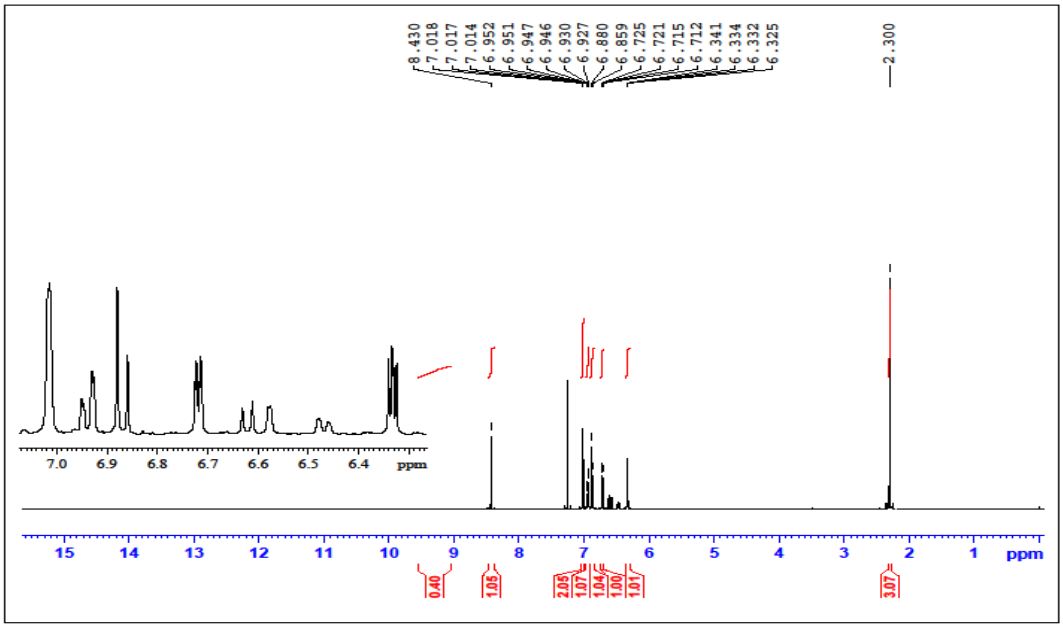


Figure: 14 ^{1}H NMR spectrum of L

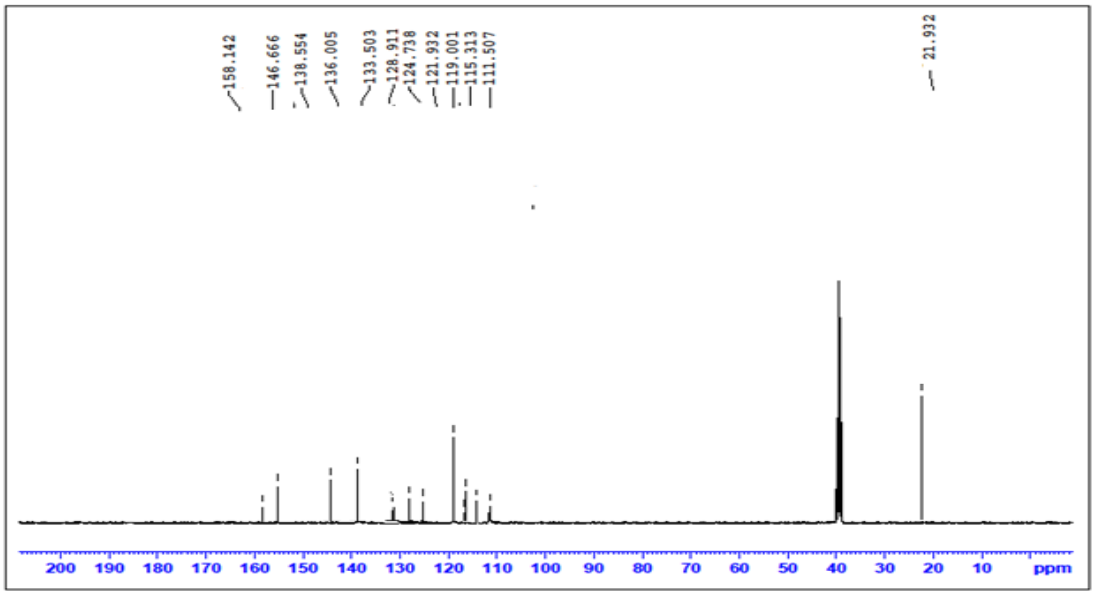


Figure: 15 ^{13}C NMR spectrum of L

3.4 MASS SPECTRUM OF L

Mass spectra is an accurate method used for determining the molecular mass of compound and its elemental analysis. Mass spectra (fig: 16) shows molecular weight of the ligand L as 200.24 and stable peak are obtained is 201.09.

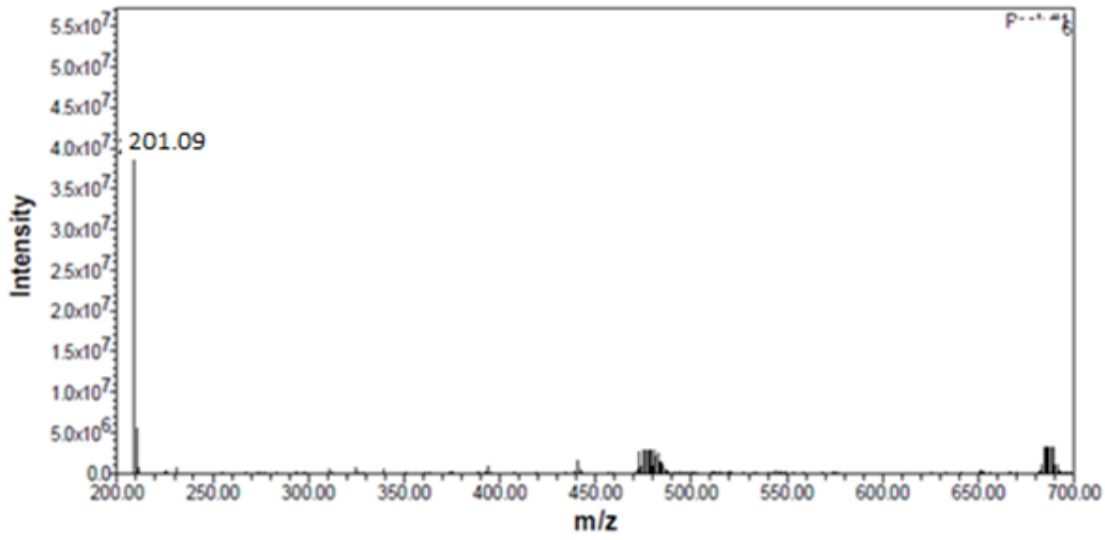


Figure: 16 Mass spectrum of L

3.5 UV-VISIBLE SPETROSCOPY

Electronic spectra are used to study the electronic structures and its dynamics in atom and molecules. The electronic spectra of ligand L, $Ni(L)_2$ and $Zn(L)_2$ were taken in DMSO. Transition within the aromatic ring assigned to π- π* transition. Transition within the C=N group assigned to be n-π* transition. The UV-Visible spectrum of ligand L, $Ni(L)_2$ and $Zn(L)_2$ showed in fig (17, 18 and 19).

The ligand L showed 2 bands at 275 nm and 355nm. The first band at 275 nm assigned to π- π* transition within aromatic ring. The second band at 355nm assigned to n-π* transition within the azomethine group.

The $Ni(L)_2$ showed 3 bands at 310 nm, 425 nm and 530 nm. The band at 310 nm attributed to intraligand transition, while the band at 425 nm was due to the charge transfer. The band at 530 nm was as result of d-d transition for $^3A_{2g} \rightarrow {}^3T_{1g}$ which indicates the tetrahedral structure of $Ni(L)_2$.

The $Zn(L)_2$ showed 3 bands at 290 nm, 360 nm and 465 nm. The band at 290 nm and 360 nm was due to the intraligand transition. The band at 465 nm signifies metal to charge transfer (MLCT) transition. There were no d-d transition for Zn(ll) complex in visible spectrum due to its complete d^{10} electronic configuration.

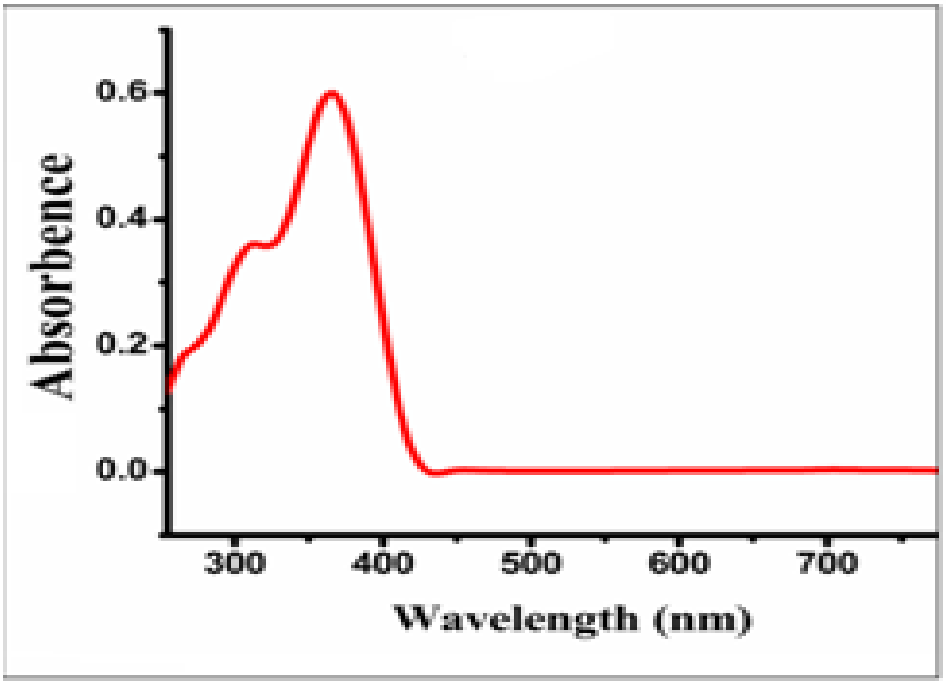


Figure: 17 electronic spectrum of L

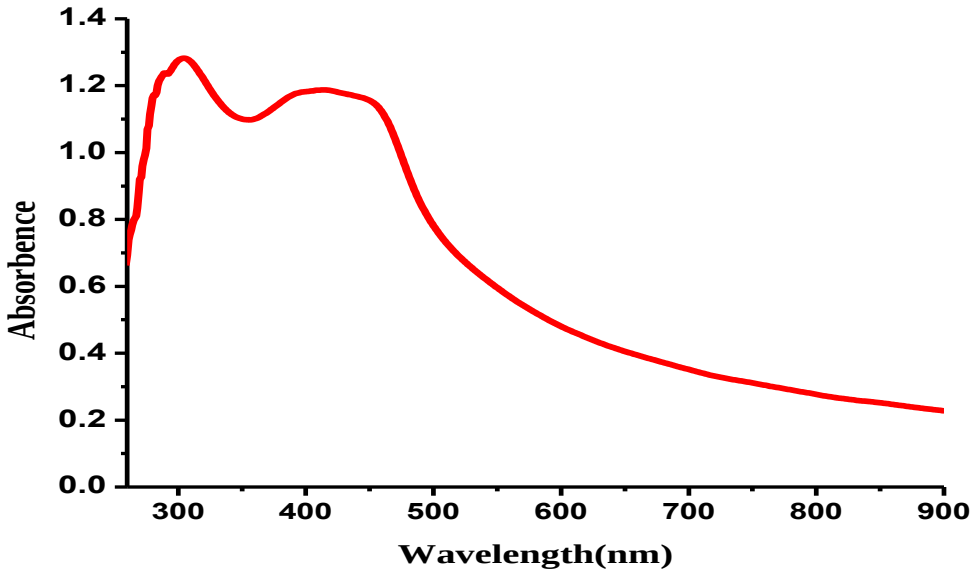


Figure: 18 electronic spectrum of $Ni(L)_2$

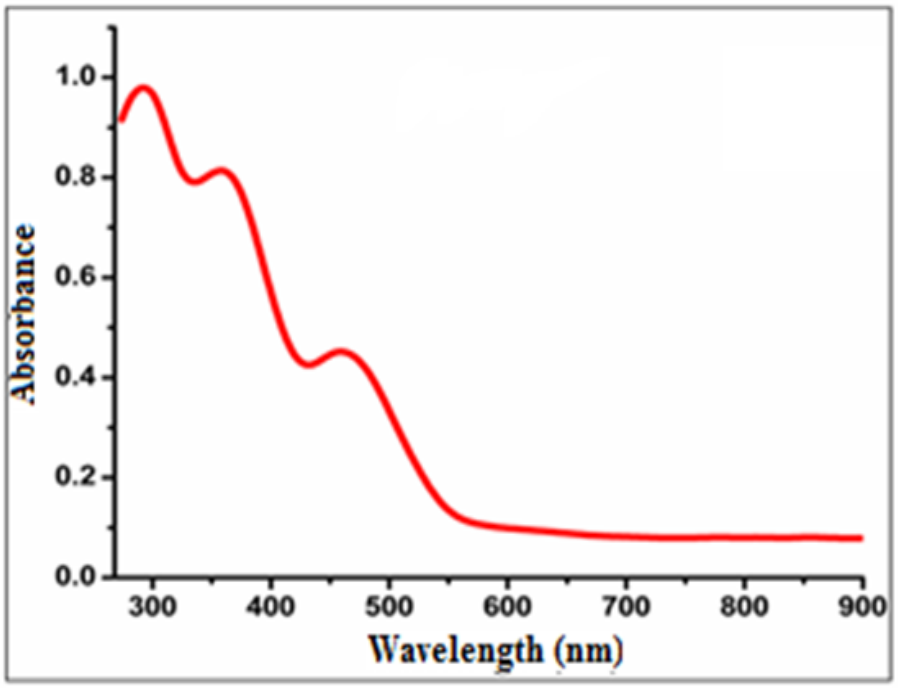


Figure: 19 electronic spectrum of $Zn(L)_2$

CHAPTER IV

ANTIBACTERIAL ACTIVITY STUDIES OF SCHIFF BASES

4.1 INDRODUCTION

Bacteria are unicellular microorganism. They are extremely tiny thus they cannot be seen individually unless viewed through microscopic. They are considered as prokaryotes which does not contain a nucleus. They are surrounded by protective cell wall containing peptidoglycan. Their basic shape is used to classify bacteria in 5 groups (fig: 20):-spherical (cocci) (a), rod (bacilli) (b), spiral (spirilla) (c), corkscrew (spirochaetes) (d) or comma (vibrios) (e).

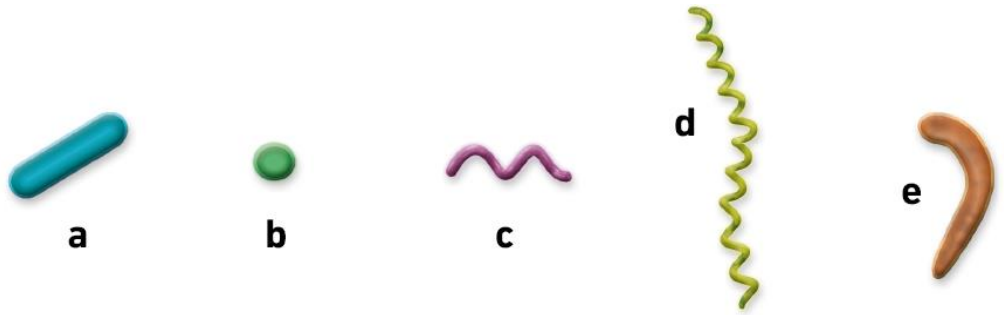


Figure: 20

According to the composition of prokaryotic cell wall, bacteria are classified into 2 major classes (fig:21) :-gram positive bacteria and gram negative bacteria [17].

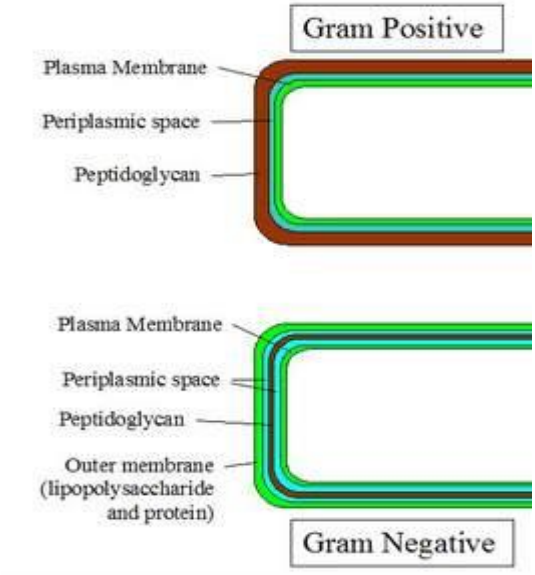


Figure: 21

Gram positive bacteria

The cell wall of gram positive bacteria is thick layer of peptidoglycan. They are the bacteria that give positive result to the gram stain test and take up the crystal violet stain. So they are known as gram positive bacteria. They appears as dark violet or purple colored when observed under microscope after gram staining and retain the crystal violet color after washing with alcohol. The cell wall is single layered, less elastic and more rigid. The rigidity of the cell wall is due to the high amount of peptidoglycan. The cell wall is contains teichoic acid and also muramic acid, which is around 16-20% of the total weight of cell. The cell wall is give the resistance to alkali. The outer membrane and lipopolysaccharides are also absent. The flagellar structure has 2 rings in basal body and it produce exotoxins. They have a surface layer known as S- layer, which is attached to the peptidoglycan layer.

Examples: Streptococcus, Clostridium,Lactobacillus [18,19]

Gram negative bacteria

The cell wall of gram negative bacteria is thin layer of peptidogycan. They are the bacteria that give the negative result to the gram stain test and not able to retain the crystal violet color. So they are known as gram negative bacteria. They appears as in red or pink colored when observed under the microscope after the gram staining and counterstain to safranin. The

cell wall is double layered, less rigid and more elastic. The elasticity of the cell wall is due to the less amount of peptidoglycan. The content of muramic acid is only 2-5% of the total weight. The cell wall is sensitive to alkali. The outer membrane and lipopolysaccharides are also present. The flagellar structure has 4 rings in basal body and it produce endotoxins. They have a surface layer known as S-layer, which is attached to the outer membrane.

Example: Vibrio,Rhizodium, Escherchia coli, Acetobacter [18,19]

Salmonella typhi

Salmonella typhi is gram negative bacteria of the family Enteriobacteriaceae. It is a rod shaped bacteria. It is a non-spore forming bacteria. It has 3 layer cell membrane. The first layer is the outer membrane, in the centre is a thin peptidoglycan layer and third layer is inner membrane. It is facultative anaerobic pathogen. It causes the typhoid fever. It inhibit the Lymphatic tissue of the liver, small intestine, spleen and blood stream of infected human [20]

Escherichia coli

Escherichia coli is gram negative bacteria of the family Enterobacteriaceae. The rod shape E.coli is a facultative anaerobe. Especially, the area of lower intestine in the warm-blooded organisms is contained E.coli. Many common bacterial infections are caused by the action of E.coli such as cholecystitis,bacteremia,cholangitis,urinary tract infection,pneumoniadiarrhea and meningitis.

Bacillus coagulans

Bacillus coagulans is gram positive bacteria of the family bacillaceae. The rod shaped Bacillus coagulans is a endospore forming bacteria. It is facultative anaerobe, grows optimally at 37^0C and pH in the range 5.5 to 6.2. It is a beneficial bacteria called probiotic. Its action may promote and protect health against infections. It also used to treat diarrhea. They stimulate the immune system, prevent the cancer, respiratory infection

Bacillus circulans

Bacillus circulans is rod shaped gram positive bacteria of the family bacillaceae. It is commonly involved in soil, sewage, food and infant bile. It is a endospore forming bacteria. It is facultative anaerobe, grows optimally at 30^0C-37^0C and pH in the range 6 to 9. It is a pathogen that causes fatal sepsis in an immunocompromised patient, which later leads to that patient death. Meningitis in human is caused by the action of bacillus circulans. It is also causes the abscesses [22].

Bacillus pumilus

Bacilllus pumilus is a gram positive bacteria, aerobic, spore-forming Bacillus, usuaaly found in the soil as a commensal and more commonly isolated in cultures as contaminants, but rarely implicated as a pathogen. B.pumilus has been reported to have caused sepsis in the newborns and the immunocompromised, central venous catheter infections and cutaneous infections. [23].

4.2 Antibacterial activity

Antibacterial compound is inhibit the growth of the bacteria without any side effects on the patients. Bacteria are found in different material that are in close contact with humans, foods, etc. So it is very essential to prevent the growth of the microorganism. Schiff base and its metal complexes are paly important role in prevention of growth of bacteria. Antibacterial activity of synthesized compounds were assessed using agar diffusion and minimum inhibition concentration method.

4.3 Experiment

4.3.1 Materials

1.Salmonella typhi

2.Bacillus coagulans

3.Bacillus pumills

4.Escherichia coli

5.Bacillus circulans

6.Muller Hinton agar

4.3.2 Methods

4.3.2.1 Agar Diffusion method

Antibacterial activity of synthesized compounds were evaluated by agar diffusion method. Test organism was inoculated onto a nutrient agar plate and incubated at 37 ◦C for 24 h to obtain the primary culture. Several discrete colonies were picked from the culture to make a bacterial suspension (10 mL) in a test tube using saline water. The turbidity of the suspension was compared with 0.5 Mc Farland standards to obtain 10^6-10^8 CFUs. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with (10µL) the compounds and were placed on the Mueller-Hinton agar surface. The plate was then incubated at 37°C for 18 to 24 hours depending on the species of bacteria used in the test. Ampicillin and dimethylsulphoxide (DMSO) were used as standard antibacterial drug and control solvent respectively. All the tests were performed in triplicates for those compounds that showed activity of more than 6.5 mm and their activity was recorded as average zone of inhibition

4.3.2.2 Minimum Inhibitory Concentration (MIC)

Resazurin based Microtiter Dilution Assay (RMDA)

The quantitative antimicrobial activity of the test compounds was evaluated using Resazurin based Microtiter Dilution Assay (RMDA). Under aseptic conditions, 96 well microtitre plates (HiMedia) were used for Resazurin based Microtitre Dilution Assay. The first row of microtiter plate was filled with 100 µL of test materials dissolved in sterile water. All the wells of microtitre plates were filled with 50µL of Luria broth. Two fold serial dilution (throughout the column) was achieved by starting transferring 50µL test material from first row to the subsequent wells in the next row of the same column and so that each well has 50µl of test material in serially descending concentrations. 2µL of resazurin solution as indicator was added in each well. Finally, a volume of 10 µL was taken from bacterial suspension and then added to each well to achieve a final concentration of 5×10^6 CFU/mL. To avoid the dehydration of bacterial culture, each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each microtitre plate had a set of 3 controls: (a) a column with ampicillin as positive control, (b) a column with all solutions with the exception of the test material and

(c) a column with all solutions except bacterial solution replaced by 10 μL of Luria broth. The plates were incubated at 37° C for 24 h. The color change in the well was then observed visually. Any color change observed from purple to pink or colorless was taken as positive. The pink color is due to the formation of resorufin. The lowest concentration of the sample at which no color change occurred was recorded as the MIC value. All the experiments were performed in triplicates. The average values were calculated for the MIC of test mater [24]

4.4 Results and Discussions

The antibacterial activity of synthesized compound and its metal (ll) complexes were evaluated by agar diffusion method against gram positive and gram negative microorganisms. Salmonella typhi, Bacillus coagulans, Bacillus pumills, Escherichia coli and Bacillus circulans were used as microoraganisms in this test. The higher antibacterial activity of compounds were evaluated by Minimum inhibitory concentration (MIC). Ampicillin was used as standard antibiotic drugs. The diameter of zone of inhibition and the MIC results for Schiff base and its metal (ll) complexes are shown in the table 3. A higher inhibition zone value is indicate of higher antibacterial activity. The compound L showed activity against Salmonella typhi, Escherichia coli and Bacillus circulans. The compound $Ni(L)_2$ showed activity against Salmonella typhi and Escherichia coli. The compound $Zn(L)_2$ showed good activity against Salmonella typhi, Bacillus coagulans, Bacillus pumills, Escherichia coli and Bacillus circulans.

Table: 3 Mean zone diameter and MIC of ligand L and its complexes of Ni(ll) and Zn(ll)

Bacteria	Inhibition zone (mm)			MIC (mg/ML)		
	L	$Ni(L)_2$	$Zn(L)_2$	L	$Ni(L)_2$	$Zn(L)_2$
Salmonella typhi	09	10	11	0.01	0.02	0.05

Bacillus coagulans	-	-	10	-	-	0.16
Bacillus pumills	-	-	11	-	-	0.08
Escherichia coli	10	11	10	0.16	0.08	0.08
Bacillus circulans	11	-	11	0.16	-	0.08

The L, $Ni(L)_2$, and $Zn(L)_2$ shows activity against salmonella typhi with inhibition zone diameter 09mm, 10mm, and 11mm respectively. Among these, $Zn(L)_2$ showed higher activity to inhibit the growth of Salmonella typhi. $Zn(L)_2$ had the highest activity against Bacillus coagulans and Bacillus pumills with inhibition zone diameter 10mm and 11mm respectively. But L and $Zn(L)_2$ shows no activity against Bacillus coagulans and Bacillus pumills. The L, $Ni(L)_2$ and $Zn(L)_2$ shows activity against Escherichia coli with inhibition zone diameter 10mm, 11mm and 10mm respectively. The Ni(II) complex (L) had a slightly higher activity than L and $Zn(L)_2$. The L and $Zn(L)_2$ shows activity with same inhibition zone diameter 11mm but $Ni(L)_2$ shows no activity against Bacillus circulans. Against all organisms, Zn(II)complex was found to be highly active in the bacterial species of Salmonella typhi, Bacillus coagulans, Bacillus pumills, Escherichia coli and Bacillus circulans.

The ligand L, $Ni(L)_2$ and $Zn(L)_2$ were active against Salmonella typhi at MIC of $0.01mgml^{-1}$, $0.02mgml^{-1}$, and $0.05mgml^{-1}$ respectively. In these, the MIC of ligand L was high as $0.01mgml^{-1}$. The $Zn(L)_2$ was active against Bacillus coagulans and Bacillus pumills at MIC of $0.16mgml^{-1}$ and $0.08mgml^{-1}$ respectively. MIC of $Ni(L)_2$ and $Zn(L)_2$ were observed as same as $0.08mgml^{-1}$ against Escherichia coli. But ligand L was active against E.coli only at 0.16mg/ml. The L and $Zn(L)_2$ were active against Bacillus circulans at MIC of 0.16mg/ml and 0.08mg/ml respectively. In these case, MIC of $Zn(L)_2$ was high as 0.08mg/ml.

CONCLUSION

The present work describes the synthesis and characterization of new heterocyclic Schiff Base and its complexes of Ni(ll) and Zn(ll). Schiff base has been synthesized by the condensation of 2-amino-4-methylphenol with pyrrole-2-carboxaldehyde and its Ni(ll) and Zn(ll) included in the first three chapters. All the synthesized Schiff bases were characterized by IR, UV-VIS, Mass, ^{1}H and ^{13}C NMR spectroscopic methods. All these studies give good evidence for the prepared structure for the Schiff bases. Besides these, this work also evaluates and studied the antibacterial activity of synthesized Schiff base and its Ni(ll) and Zn(ll) complexes which is briefly discussed in the last chapter. From the whole it is clear that these synthesized Schiff bases have significant role in diverse biological and pharmacological field.

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