

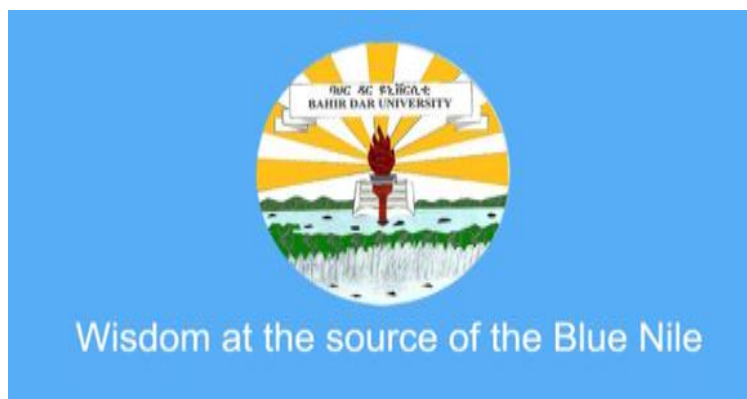
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Synthesis, Characterizations and Biological Activities Investigations of New Complex of Cobalt (II) Containing Resorcinide alone and Resorcinide py Mixed with 2, 2 - Bipyridine

Hiko, Hebo

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BAHIR DAR UNIVERSITY
COLLEGE OF SCIENCE
DEPARTMENT OF CHEMISTRY

**Synthesis, Characterizations and Biological Activities Investigations
of New Complex of Cobalt (II) Containing Resorcinide alone and
Resorcinide Mixed with 2, 2'-Bipyridine**

By

Hiko Hebo

Advisor: Atakilt Abebe (PhD)

September, 2019

Bahir Dar, Ethiopia

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New Complex of Cobalt (II) Containing Resorcinide alone and
Resorcinide Mixed with 2, 2'-Bipyridine

A Thesis Submitted To College of Science Department of Chemistry
Bahir Dar University

In Partial Fulfilment of the Requirement for the Degree of Masters of
Science in Chemistry (Inorganic)

By

Hiko Hebo

Advisor: Atakilt Abebe (PhD)

September, 2019

Bahir Dar, Ethiopia

APPROVAL SHEET

As thesis research advisor, I here certify that I have read and evaluate this thesis prepared under my guidance, by Hiko Hebo entitled “**Synthesis, Characterizations and Biological Activities Investigations of New Complex of Cobalt (II) Containing Resorcinide alone and Resorcinide Mixed with 2, 2'-Bipyridine**” I recommend that the thesis is to be submitted as fulfilling the requirement.

I hereby certify that I have read this thesis prepared under my supervision and recommended that the thesis to be accepted as fulfilling the requirement.

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(Advisor)		

Being the examiner of this thesis entitled “synthesis and biological activities investigation mixed ligand complex of cobalt (II) containing 2, 2-Bipyridine and resorcinol” I certify that the thesis is sufficient to full fill the requirement of degree of Master of Science in Inorganic Chemistry.

_____	_____	_____
External examiner	signature	Date

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Internalexaminer-1	signature	Date

_____	_____	_____
Internalexaminer-2	signature	Date

DECLARATION

I declare that the work described in this thesis is my original work done under the supervision of my advisor **Atakilt Abebe (PhD)** at the Department of Chemistry, Bahir Dar University for the degree of **M.Sc.** in Chemistry. I also declare that the work embodied in this thesis is the results of my own research and where work of any other researcher has been used, that has been duly acknowledged.

Name: Hiko Hebo _____ :

Signature: _____

Place: Bahir Dar University, Bahir Dar

Date of Submission: _____

ABSTRACT

*The increase in the mortality rate linked with infectious diseases is directly related to bacteria that demonstrate multiple resistances to antibiotics. The shortage of effective treatments is the major cause of this problem. The development of new antibacterial agents with novel and more efficient mechanisms of action is an alarming medical need. Based on this, in this work, two new cobalt (II) complexes with formula of typical protic acid, $H_2[Co(R)_2]$ and $H_2[Co(Bipy)(R)_2]$ (Where R= Resorcinide, Bipy=2,2'-bipyridine,) were synthesized. The synthesis was checked using halide test, and spectroscopic (ICP-OES, FT-IR, UV/Vis, 1H NMR, and ESI-MS) analysis. Square planar and octahedral geometries were proposed, respectively. The complexes were also tested for their in vitro anti bacterial activity against two Gram-positive *Staphylococcus aureus* and *Staphylococcus pyogenes* and two Gram negative *Escherichia coli* and *Klebsiella pneumonia* bacterial strains. The results were compared with the commercially available antibacterial agents (gentamicine). The antibacterial activities of the complexes were found to be active against all the tested bacterial strain and demonstrated wide spectrum and better inhibiting activity even on Gram negative bacteria (*E. coli* and *K. pneumonia*). Thus, after cytotoxicity investigations, these complexes could be considered as alternative drugs.*

Key words: Protic acid, cobalt (II) complexes, Resorcinide, 2,2-Bipyridine, antibacterial activities.

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SYMBOL AND ABBREVIATIONS

¹ HNMR.....	Proton Nuclear Magnetic Resonance spectroscopy
Bipy.....	2, 2'-Bipyridine
DNA.....	Deoxyribonucleic acid
FT-IR.....	Fourier-transform Infrared
g/mol.....	Gram per mole
ICP-OES.....	Inductively coupled plasma optical emission spectroscopy
IZ.....	Inhibition zone
LMCT.....	Ligand to metal charge transfer
M.w.....	Molecular weight
M'pt.....	Melting point
mg/L.....	Milligram per litre
MHA.....	MuellerHintonagar
MIC.....	Minimum inhibitory concentration
MLCT.....	Metal-to-ligandcharge transfer
mm.....	Millimetre
R.....	Resorcinol
UV-Vis.....	Ultraviolet-Visible spectroscopy

INTRODUCTION

1.1. Back Ground of the Study

Transition metal ions are playing important roles in biological processes in organisms. They are found coordinated either at the active sites or as structural components of a good number of enzymes. Thus, the study of the coordination chemistry of biologically important metal ions with mixed ligand has been one of the recent developments in the field of bioinorganic chemistry (*EL Hamdani et al., 2016*). Mixed ligand complexes with metal ion bound to two or more different, and biologically important ligand have aroused interest as model for metallo-enzymes. It is well established that mixed ligand complexes play a decisive role in enzyme activation and storage and transport of active substances (*Alka Choudhary et al., 2011*).

Moreover, the versatile coordination geometries arise from various oxidation states, results in metal ions and their complexes having potentials in medicinal application that could be complementary to the crude drugs which are sources of medicinal synthesis still obtained from wild growing material (*Gray et al., 2003, Abrams et al., 1993*). In general, transition metal complexes have numerous features that lead them to their application in many areas of modern medicine (*Orving et al, 1999*).

Consequently, pharmaceutical industries have considered them as alternatives to conventional drug which originates from wild sources. This is done by tuning the properties of the transition metals by coordinating with different ligand (*Wilkins et al., 1974, Lawrence et al., 2010, Searset al., 2010*).

These include stabilization of different oxidation state and modulation of the solvophilicity, electrophilicity, and nucleophilic properties of the metal ion (*Tolman et al., 1997, Boyer et al., 2010, Goo et al., 2015*). Recent studies reported on the structure and chemical characteristics of various metal complexes (*Joel et al., 2015, Terrón et al., 2007*).

Mixed ligand of cobalt(II) complexes are found to be particularly useful due to their potential to bind DNA (*Agwara et al., 2008*). A recent study on the incorporation of good intercalator containing aromatic ring such as 2,2'-bipyridine and 1,10-phenanthroline found high affinity between DNA base pair and their planar structure

through stacking interaction(Aroundnaguir et al., 1999).However little or no report is obtained that suggests the synthesis, characterization and biological activities of new complexes of cobalt (II) containing resorcinide (R) alone and resorcinide mixed with 2,2'-Bipyridine(Bipy). The purpose of this thesis is therefore; to fill this gap that the structure invented from such a composition, we believe, results non covalent interactions between the base pair of DNA and the components of the complex inhibits its replication and transcription.

1.2.Statement of the problem

The increase in the mortality rate linked with infectious diseases is directly related to bacteria that demonstrate multiple resistances to antibiotics. The shortage of effective treatments is the major cause of this problem. The development of new antibacterial agents with novel and more efficient mechanisms of action is an alarming medical need.

1.3.Significance of the study

To overcome the alarming problem of the microbial resistance to antibiotics, the discovery of novel active compound against new target is the matter of urgency. Based on this, pharmaceutical industries are searching for synthesizing alternative compound to medicinal preparation derived from the wild growing plants. However, plant based drug have shortened the life span of the origins. To alleviate this problem, synthesis and investigation of different metal complexes have been carried out and promising results are being found. The actions of these complexes against the microbes were reported to be through non covalent interaction with the DNA. The significance of this thesis is, therefore, complementing the effort of investigating new mixed ligand complexes, containing different ligands having various features. As a result, the ligands increase the activity of the complex against the microbes by covalent interactions in addition to the non covalent interactions with the DNA.

1.4.Objective of the study

1.4.1.General objective

To synthesize, characterize, and investigate the antibacterial activities of complex of Co (II) containing resorcinide alone and resorcinide mixed with 2, 2'-bipyridine.

1.4.2. Specific objectives

- ✿ To synthesize new complex of Co(II) containing resorcinide alone and resorcinide mixed with 2, 2'-bipyridine.
- ✿ To characterize the synthesized complexes by different physico-chemical and spectroscopic techniques and consequently propose the structure of all complexes.
- ✿ To study antibacterial activities of the synthesized metal complexes.

LITERATURE REVIEW

2.1. The Chemistry of mixed ligand complexes

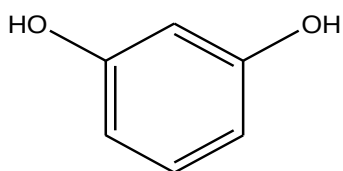
The concepts of mixed ligand complexes are always fascinating to the chemistry community that interest in synthesis of mixed ligand complexes is raising. This is because these complexes contained at least two different kinds of ligands with different properties associated with the same metal ion.

The combined effect of the ligands with different properties on the metal ion in a complex results in a set of new properties. This includes stabilization of different oxidation states and modulation of the solvophilicity, electrophilic and nucleophilic properties of the metal ion.

While coordinating, the properties of the ligands themselves are also modified. For instance, the biological activities of free 1, 10-Phenanthroline and 2, 2'-Bipyridil can significantly change after complex formation (Abebe et al., 2016).

2.2. The Chemistry of Resorcinol

The chemical name of resorcinol is 1, 3-benzene diol. The synonyms are: resourcing; 1,3- dihydroxy benzene; 3- hydroxyl cyclo hexa diene-1-ol ; m- di hydroxy benzene; m- benzene diol. Resorcinol has molecular weight of 110.11 g/mol with molecular formula $C_6H_6O_2$. Resorcinol occurs as white needle-like crystals, plate, flake, rhombic tablets, or powder with a faint, characteristics odour. It is soluble in water, alcohol, ether, glycerol, acetic acid, chloroform, dimethylsulfoxide and acetone. The boiling point of resorcinol is $280^{\circ}C$. The melting point of resorcinol ranges between $109^{\circ}C$ and $111^{\circ}C$. Its density is $1.2717g/cm^3$ (EFSA, 2010).



Scheme 1: Structure of Resorcinol

2.2.1. Uses of resorcinol

Resorcinol has been used for medicinal purposes since the 19th century. At first it was given orally to treat digestive disorders in children until, by the beginning of the

20th century, numerous reports of poisoning had appeared. However, because of its antiseptic activity, the substance continued to be used with considerable success for the local treatment of various skin disorders such as acne, seborrhoeic dermatitis, eczema, psoriasis, corns, and warts. (Graham et al., 1922).

Resorcinol is an important chemical intermediate in the manufacture of speciality chemicals, such as hexylresorcinol-amino salicylic acid, and light screening agents for the protection of plastics from exposure to UV-light.

Other uses include the manufacture of dyestuffs, pharmaceuticals, fungicidal, creams and lotions, explosive primers, a chain extender for urethane elastomers, and a treatment to improve the mechanical and chemical resistance of paper machine fabrics (R Schmiedal et al., 2000). A specialized medical use of resorcinol is in biological glues (gelatine-resorcinol-formaldehyde glue) for cardiovascular surgery, in particular aortic operations (Bachet et al., 1999).

The chemistry of molecular resorcinol as a ligand could not be found from literature. This is probably due to its inability to coordinate as the lone pair of electrons on the oxygen of OH group may be in the aromatic system. To help this, systematic selection of appropriate solvent may help to ionize and make an extra lone pair of electrons that can be involved in the coordination.

2.3. The chemistry of 2, 2'-bipyridine

2, 2'-Bipyridine is a heterocyclic organic compound with the formula $C_{10}H_8N_2$. Heterocyclics are a class of organic compounds in which one or more of the ring carbon atoms have been replaced by another element such as nitrogen, oxygen, and sulphur (Sharu et al., 2011).

These compounds have a broad range of applications which include as optical brightening agents, antioxidants, corrosion inhibitors, and additives with a variety of other functions. They are also widely distributed in nature. Many are of fundamental importance to living systems; for example, identification of the nucleic acid bases, which are derivatives of pyrimidine and purine ring systems, as being crucial to the mechanism of replication (Radalla, 2010).

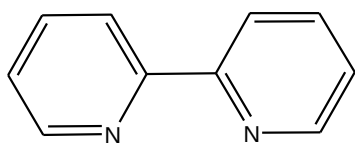
One of the reasons for widespread use of heterocyclic compounds is that their structures can be subtly manipulated to achieve the required functional modification. Such variations may include differences in acidity or basicity, susceptibility to attack

by electrophiles or nucleophiles and polarity which are the direct consequences of variation in electronic distribution across the molecular frame. The interactions are mediated by the bridging aromatic heterocycles, with communication between the metal centres gradually taking place through the π - system of the ligand.

There are different classes of ligands that incorporate heterocyclic rings. Examples of heterocyclic aromatics include; furan – five membered ring with one oxygen atom, pyridine - six membered ring systems with one nitrogen atom, Pyrrole -five membered ring with nitrogen in the ring, thiophene - five membered ring systems with sulphur in the ring etc (Gilchrist et al.,1972). 2, 2'- Bipyridine is a colourless solid and important isomer of the bipyridine family.

It is a bidentate chelating ligand, forming complexes with many transition metals. 2, 2'-Bipyridine consists of two pyridine molecules joined by a single bond thus known as bipyridyls (George et al., 2004).

Pyridine derivatives play significant role in many biological systems as the component of several vitamins, nucleic acids, enzymes and proteins (Altaf et al., 2015). 2,2'-Bipyridine is characterized by two inward-pointing nitrogen donor atoms being held together and therefore, pre-organized for strong metal binding it can bond to metal atoms (ions) using the lone pairs of electrons on the nitrogen atoms. In this ligand, the σ -donation is complemented by the π -acceptor ability giving the complex formed greater stability.



Scheme 2: The structure of 2, 2'-bipyridine

2.4.The chemistry of Co (II) complexes

Cobalt is naturally occurring element that appear in the first transition series of group nine (VIIB) of the periodic table along with iron and nickel. Cobalt commonly occurs in the 0, +2, and +3 valance states. Compound containing cobalt in the -1,+1,+4 and +5 states are few and uncommon(Liang et al.,2007).

There is over growing interest in investigating cobalt and other transition metal complexes for their interaction with DNA.This may be partly influenced by the results

of extensive investigation into two areas of research, viz. i. The binding specificity of small organic molecules for their possible modulation and inhibition of DNA replication, transcription and recombination, and (ii) anticancer, antiviral and antibacterial drugs (Valko et al., 2005).

The binding properties of cobalt with calf thymus DNA were studied by several methods, and experimental results showed that the size and shape of the intercalated ligand had an important effects on the binding affinity of the complexes with DNA (Chin et al., 2011). Moreover, cobalt is an element of biological interest because its biological role is mainly focused on its presence in the active centre of vitamin B₁₂, which regulates indirectly the synthesis of DNA.

Cobalt is also involved in the co-enzyme of vitamin B₁₂, and is used as a supplement of the vitamin (Arslantas et al., 2007). Since the first reported studies into the biological activity of cobalt complexes in 1952, many cobalt complexes of biological interest have been reported with the most structurally characterized showing antitumor, antimicrobial and antifungal activity Cobalt (II) complexes having octahedral geometries show a remarkable intercalative binding affinity as well as DNA cleavage properties (Sadler et al., 1991).

2.4.1. Toxicity of Cobalt (II) compounds

Although many studies have reported toxic and carcinogenic effects induced when humans and animals are exposed to certain metals, cobalt is generally not considered to be a very toxic element. Most toxicity studies have been concerned with Co (II) metal ions, surgical implants, or cobalt metal dust, with one notable example of cobalt-induced mortality from drinking large quantities of beer that contained cobalt chloride or cobalt sulphate as a foam stabilizer (Gomathi et al., 2013). Generally, the toxicity of cobalt is relatively low compared to many other metals in soil; however it is toxic and carcinogenic at higher concentrations. Effects on the lungs, including asthma, pneumonia, and wheezing, have been found in workers who breathed high levels of cobalt in the air. Studies revealed that workers exposed to cobalt in an electrochemical plant producing cobalt and workers exposed to cobalt-containing hard metal compounds exhibited significantly higher risk of developing lung cancer (Liang et al., 2007). Plants, animals and humans can all be affected by high cobalt concentrations in the environment.

2.4.2. Antibacterial applications of cobalt (II) complexes

A number of researches have been done in developing Co (II) complexes of Schiff bases for their antimicrobial and antifungal properties. However Co (II) complexes were found ineffective against Gram-negative bacteria because of their hydrophobicity and large molecular weight (Gujarathi et al., 2013).

2.4.3. Mode of action

Although the exact mechanism is not understood biochemically, mode of action of antimicrobial may involve different targets in microorganism.

- ✪ Denaturation of one or more proteins of the cell, as results of which the normal cellular process are impaired.
- ✪ Deactivate various cellular enzymes, which play important role in various metabolic pathways of this microorganism.
- ✪ Formation of hydrogen bond via the azomethine group with the active center of cell constituents, resulting interference with the normal process (Dhanaraj et al., 2008).

2.4.4. Antimicrobial resistance mechanism

Based on published literatures and the guidelines of clinical and laboratory standards institute (CLSI), antimicrobial resistance can be defined as an ability of microorganisms to resist the effects of one or more antimicrobial agents that they are originally sensitive to. The emergence of antimicrobial resistance has become a major threat to public health and even causes huge losses in agriculture and industry around the world. Better understanding of the resistance mechanisms of bacteria to antimicrobial agents can therefore guide the use of existing agents with improved activity and develop more efficient new ones.

Many resistance mechanisms for bacteria to combat antimicrobial agents have been found in the past decades. General resistance mechanisms include alterations of target sites, limited diffusions or impermeabilities, enzymatic modifications, efflux pumps and genetic adaptations.

Moreover, different resistance mechanisms will be employed by one given bacterium to protect themselves from one given antimicrobial agent or different ones (Zhou et al., 2015). General resistance mechanisms include alterations of target sites, limited diffusions or impermeabilities, enzymatic modifications and genetic adaptations

(Anthony et al., 2015).

Agwara and his co-workers synthesized mixed-ligand complexes containing 1, 10-henanthroline and 2,2'-bipyridine as $[\text{Co}(\text{Bipy})(\text{Phen})_2](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$. Antimicrobial studies showed that there is increased antibacterial activity of the metal ion up on coordination to the ligands. The Co (II) complexes were tested against different bacterial strains.

The in vitro antibacterial screening effects of the synthesized compounds were tested against five bacterial strains namely, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Klebsiella pneumonia*. It is found that the metal complexes have greater inhibitory effects than the free ligands. The antibacterial results evidently showed that the activity of the mixed ligand complex increase up on the coordination of the ligands with the metals. (Agwara et al., 2010).

Furthermore, various mixed ligands of cobalt (II) including $[\text{Co}(\text{Phen})(\text{Bipy})(\text{H}_2\text{O})]$ Zerubable Mogas, 2017, $[\text{Co}(\text{Phen})(\text{Act})(\text{H}_2\text{O})]\text{Cl}_2$ by Akililu Melese, 2018 were synthesized and characterized by different MSc students of Bahir Dar University, College of Science, department of chemistry. In these studies, the complexes showed good antimicrobial activity. These findings and reports showed the possibility and need of designating new complexes by slight modifications in the compositions.

To sum up, our literature survey showed the absence of any report on the chemistry of complexes of Co (II) containing resorcinide $\text{H}_2[\text{Co}(\text{R}_2)]$ as well as resorcinide mixed with 2,2'-bipyridine $\text{H}_2[\text{Co}(\text{Bipy})(\text{R})_2]$.

Thus, this study attempts to focus on synthesis and biological activity investigation of new complexes of cobalt (II) containing resorcinide alone and resorcinide mixed with 2, 2'-bipyridine. This composition of ligands in a complex as such, we believe, results both covalent and non covalent interactions between the base pairs of DNA and inhibits its transcription and replication.

EXPERIMENTAL SECTION

3.1. Chemicals

Chemicals used in this work are the following: CoCl_2 (Cobalt(II) chloride anhydrous) (BOH Poole.BH15 1TD England), 2,2'-Bipyridine, Resorcinol (Himedia laboratory, pvt, ltd, India), silver nitrate, sodium sulphate, acetone, acetonitrile, chloroform, sulphuric acid (Sigma Aldrich), methanol (Hi Media Laboratories Ltd., India), KBr, dichloromethane, tetrahydrofuran and Mueller Hinton agar, (BLULUX Laboratories Ltd., India), and nitric acid (T.V. Industrial Estate, India). All these chemicals were purchased from sigma-Aldrich and used as received and all solvents were purchased from Blulux Laboratories (p) and used without further purification.

3.2. Instruments and methods

The electronic spectra were recorded in the 200–800 nm regions on Sanyo SP65 UV/Vis spectrophotometer. IR spectra were recorded using KBr discs in the 4000–400 cm^{-1} region on AVATAR 330 FT-IR, Thermo Nicolet spectrophotometer. Cobalt content was determined by PerkinElmer, Optima 7300 V HF Version ICP-OES spectrometer, 7 mg and 7.5 mg for $\text{H}_2[\text{Co}(\text{R})_2]$ and $\text{H}_2[\text{Co}(\text{Bipy})(\text{R})_2]$ respectively in a mixture of concentrated HNO_3 , HCl and HClO_4 and diluting them using distilled water. Melting was determined using STONE, STAFFORDSHIRE, ST15 OSA, UK, and digital melting point apparatus. And also ^1H -NMR and EIS-MS were used

3.3. Synthesis

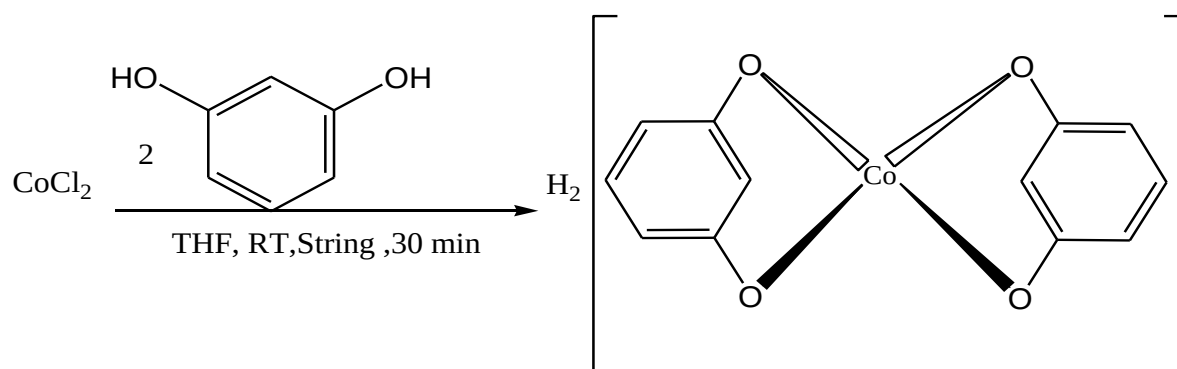
3.3.1. Synthesis of the complexes

The complexes were synthesized by reacting the metal salt with resorcinol followed by stoichiometric amount of, 2,2'-bipyridine using THF solvent as a reacting medium in 1:2:1 mole ratio, i.e. one mole of metal salt: two mole of resorcinol and one mole of 2, 2'-Bipyridine.

3.3.2.Synthesis of $H_2 [Co(R)_2]$

$CoCl_2$ (0.59gm, 4.50 mmol) was dissolved in 30 ml of tetrahydrofuran (THF) in a 100 ml round bottom flask. While the solution was stirring magnetically in water bath at room temperature a solution of resorcinol (1 gm, 9.00 mmol) in 30 ml of THF was added drop wise slowly from the burette in duration of 30 minutes with continuous monitoring of colour change and a homogeneous solution was obtained. Rotary evaporator was used to remove the solvent. Reddish brown powder was collected, dried at room temperature and kept in desiccators.

Yield: **.865 g, (81.068 %)**. During the synthesis process the reaction progress was monitored by the colour change with time.

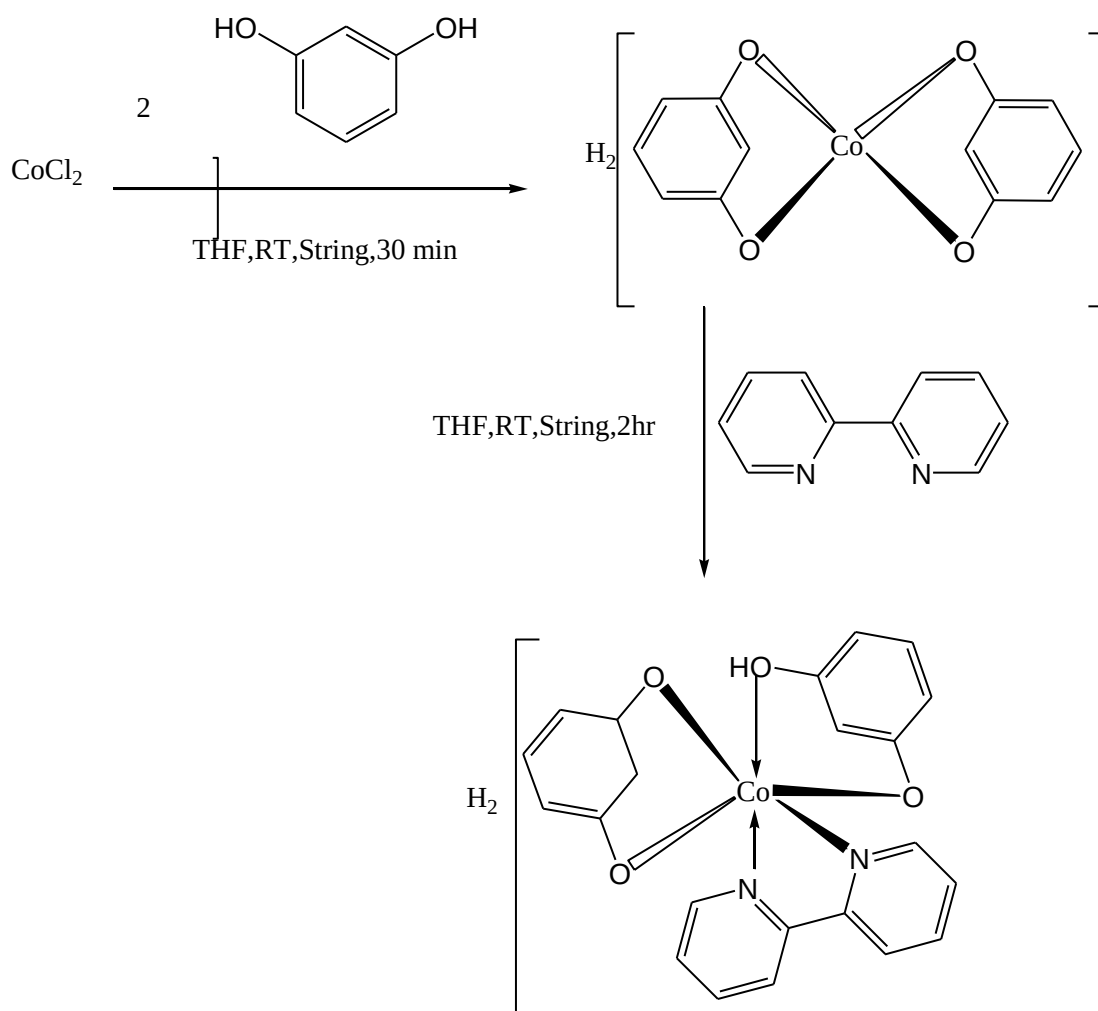


Scheme 3: The synthesis path of $H_2 [Co (R)_2]$

3.3.3. Synthesis of $H_2 [Co (Bipy)(R)_2]$

$H_2[Co((R)_2)]$ (0.43 gm, 1.50 mmol) was dissolved in a 30 ml of tetrahydrofuran (THF) in a 100 ml round bottom flask. While the solution was stirring magnetically in water bath at room temperature a solution 2,2'-bipyridine (0.242 gm, 1.50 mmol) in 30 ml of THF was added drop wise slowly from the burette for duration of 2 h with continuous monitoring of colour change and a blue green homogeneous solution was obtained. Rotary evaporator was used to remove the solvent and washed three times with acetonitrile and blue green powder was collected, dried at room temperature and kept in desiccators.

Yield: **0.672 gm, (84.375%)**. The synthesis strategy is indicated in scheme 4 below.



Scheme 4: The synthesis path of $H_2 [Co (Bipy)(R)_2]$

3.4. Physicochemical characterizations

3.4.1. Determination of acidity of the complexes

The acidity of $H_2[Co(R)_2]$ and $H_2[(Bipy)(R)_2]$ were examined using universal indicator. Universal indicator is pH indicator composed of a solution of several compounds that undergo several colour changes over pH value range from 4-14. The colour is used to indicate pH directly. Therefore, universal indicator is used to determine acidity or alkalinity of various substances (www.reference.com).

3.4.2. Solubility test

The solubility of $H_2[Co(R)_2]$ and $H_2[(Bipy)(R)_2]$ were examined using different solvents and the results are indicated in **table 1**.

3.4.3. Melting point /decomposition temperature determination

The melting points of the synthesized complexes were determined by taking small amount of complexes in capillary tube by using digital melting point apparatus and the results are presented in **table 2**.

3.5. Analysis of Elements

3.5.1. Quantitative Metal determination

The metal composition was deduced by decomposing 7mg and 7.5mg of $H_2[Co(R)]$ and $H_2[Co(Bipy)(R)_2]$ complex respectively via digestion in 5ml of HNO_3 , 5ml of perchloric acid and 15 ml of HCl until clear solution was observed. Then the vessels were removed from heat source and allowed to cool, and then the sample was added to 50 ml volumetric flask and diluted by adding distilled water until the volume of the solution became 50 ml to make solution of known concentration, and the metal content was recorded using ICP-OES. Based on the absorbance data, the concentration of Co (II) complexes were calculated and described in (**table-4**).

3.6. Spectroscopic Characterization

3.6.1. FT-IR spectra of the ligands and the Co (II) complexes

The IR spectra of the ligands and the complexes were recorded in KBr pellet in 4000-400 cm^{-1} range to determine the coordination of the ligands with the metal ion and to

identify the specific coordination site of the ligands with the metal center as the data displayed in (table-5).

3.6.2.Ultra violet-visible spectra

The electronic spectra of the free ligands, metal salt and new complexes have been determined in distilled water solution in the range of 200-800 nm at room temperature and the results shown in (table 6).

3.7. Antibacterial studies

The In Vitro antibacterial activity of ligands and their metal complexes have been carried out against two gram negative bacterial strain; *E. Coli*(ATCC-278431) and *K.pnuemonia*(ATTC-986605).and two gram positive, *S. aureus*(ATCC-1784) and *S.Pyogen*) bacterial strain. The ligands and metal complexes have been dissolved in the appropriate solvent to give a concentration of 150ppm (stock solution).

3.7.1. Antibacterial assay

A 24 hr fresh culture was obtained by inoculation of respective bacteria in double strength nutrient broth followed by incubation at 37°C. The stock solution of ligands and their metal complexes have been serially diluted in tube containing 1 ml of sterile doubles strength nutrient broth to get a concentration of 100 to 3.12µg/ml and then inoculated with 100 µl of suspension of respective organisms in sterile saline (*E.Coil*, *K.pnuemonia*, *S.aureus* and *S.Pyogen*). The inoculated tubes have been incubated at 37°C for 24 hr and minimum inhibitory concentrations (MIC) were determined.

3.7.2. Antibacterial activity tests

In vitro antibacterial screening of the ligands, 2,2-'Bipyridine, Resorcinol, the salt CoCl_2 and the synthesized complexes was generally performed by disc diffusion methods. These tests were carried out in the Bahir Dar university microbiology Laboratory Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia. For this antibacterial investigation two gram negative bacterial strain; *E.coli* and *K.pnuemonia* and two gram positive *S.aureus* and *S.Pyogen* bacterial strain were obtained from the Bahir Dar university microbiology Laboratory. Antibacterial activities were evaluated by measuring the diameter of zone of inhibition around the hole. The compounds were considered as active when IZ was greater than 6 mm (Agwara et al., 2010). The plates were observed for zones of inhibition after 24 h

incubation at 37 °C. The diameters of the zone of inhibition produced by the complexes were compared with a standard antibiotic agent (gentamicine). The minimum inhibitory concentration against each bacterium was determined by preparing aqueous solutions of different concentrations of the complexes.

3.7.3. Quality control

Reference strain of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 will be used as quality control. The evaluation of the antimicrobial activity was followed after the incubation period by the measurement of the diameter of the inhibition zones. The zones of 19 mm or greater (including the diameter of the disc) were considered of high antibacterial activity. Depending on the diameter of the inhibition zone, the tested complexes would be divided into groups of their various antimicrobial activities. For the tested compounds the activity index (%) will be calculated using the formula; % Activity Index = $\frac{A-B}{B} * 100$

Where: A – Zone of inhibition of test compound (diameter)

B – Zone of inhibition of reference (gentamicine)

The bacteria used in this study were two Gram negative such as, *Escherichia coli* and *Klebsiella pneumonia* and two Gram positive such as *staphylococcus aureus* and *streptococcus pyogenes*. The bacterial cultures were taken to Bahir Dar university microbiology department and maintained in appropriate slants at 4°C until use and the antibacterial tests were carried out at Bahir Dar University, Department of Biology, Microbiology Laboratory, Bahir Dar, Ethiopia.

RESULTS AND DISCUSSIONS

4.1. Physical Characterization

Solubility, melting point, appearance and colour of all reagents and complexes are presented in (table 1 and 2). The synthesized complexes are significantly different from the starting materials in the properties listed. This presents a clue of the conversion of the starting materials.

4.1.1. Solubility test

Solubility is often said to be one of a characteristic property of a substance. It indicates whether the hardness and/or softness of the component ions of the complex match (HSAB-principle). Those complexes with significant mismatch in the hardness and/or softness of the component ions demonstrate dissolution in a range of solvents of different polarities.

On the other hand, those complexes having comparatively matching hardness and/or softness of the component ions show dissolution in limited solvents. The solubility experiment results indicate that both $H_2[Co(R)_2]$ and $H_2[Co(Bipy)(R)_2]$ show similar solubility in polar solvents like water, ethanol and acetone.

However, $H_2[Co(Bipy)(R)_2]$ is partially soluble in non polar solvents like chloroform, and insoluble in diethylether as shown in table 1.

This is attributed to the increase in the hardness of the complex following the coordination of 2,2'-bipyridine. This is because, 2, 2'-bipyridine is a poor σ -base but a strong π -acid. The later phenomena diminish the negative charge whereby the polarity decreases. Thus, the solubility difference further confirms the formation of new product with new character.

4.2. Determination of the acidic property of the complexes

The colour of $H_2[Co(R)_2]$ was yellowish green and $H_2[Co(R)_2(Bipy)]$ was green this confirmed that both complexes were acidic in nature which strongly agrees with the proposed formula.

Resorcinide is a σ and π -base ligand which makes the anion of $H_2[Co(R)_2]$ very soft consequently, nearly complete dissociation is expected. This is evident from the pH value.

On the other hand, after the coordination of the σ -acid and π -base 2,2' bipyridine, due to the reduction of electron density, the softness decreases and the dissociation of $H_2[Co(R)_2(Bipy)]$ is smaller than the former which strongly agrees with the acidity investigation experiment.

Table 1: the solubility of the synthesized Co(II) complexes, and the ligands

Solvent	Complexes		Ligand	
	$H_2[Co(R)_2]$	$H_2[Co(Bipy)(R)_2]$	Resorcinol	2,2'-Bipyridine
Distilled water	Soluble	Partially soluble	Soluble	soluble
Ethanol	Soluble	Soluble	Soluble	Soluble
Tetra hydro furan	Insoluble	insoluble	Soluble	Soluble
Acetone	Soluble	Soluble	Soluble	Soluble
Chloroform	Partially soluble	Insoluble	Insoluble	Insoluble
Diethyl ether	In soluble	Insoluble	Insoluble	Insoluble

4.2.1. Melting point/decomposition temperature and Colour

Melting point is the temperature at which the solids substances melts or fused. $H_2[Co(R)_2]$ melted at 80 °C and $H_2[Co(Bipy)(R)_2]$ did not melt until 360 °C.

As presented in **table 2**. The complexes showed sharp melting points indicating that they are pure and very different from the starting materials.

Furthermore, the significant difference between the two complexes is the result of the change in the anion of the complex from soft $H_2[Co(R)_2]$ to hard $[Co(Bipy)(R)_2]$.

The later phenomena creates a stronger interaction between the hard H^+ and harder $[Co(Bipy)(R)_2]$ consequently requires higher heat energy.

Table 2: The physical characteristics of the ligands, salts and the complexes

compound	M.w(gm)	M'pt(°C)	Appearance	colour	Yield (%)
2,2-Bipyridine	156.19	73	Needle like fine crystal	white	
Resorcinol	110.11	112	Needle like fine crystal	white	
CoCl ₂	129.83		Crystalline	Blue	
H ₂ [Co(R)]	276.93	80	Powder	Reddish-Brown	81.068
H ₂ [Co(Bipy)(R)]	432.93	> 360	Powder	Blue-green	84.375

4.3. Analysis of the Elements

4.3.1. Determination of Metal content via ICP-OES

The data obtained from ICP-OES (**table-3**) shows the very good agreement between the calculated and experimental Co(II) ion contents in both in H₂[Co(R)₂] and H₂[Co(Bipy)(R)₂] which is confirmatory evidence for the achievement of the intended compounds.

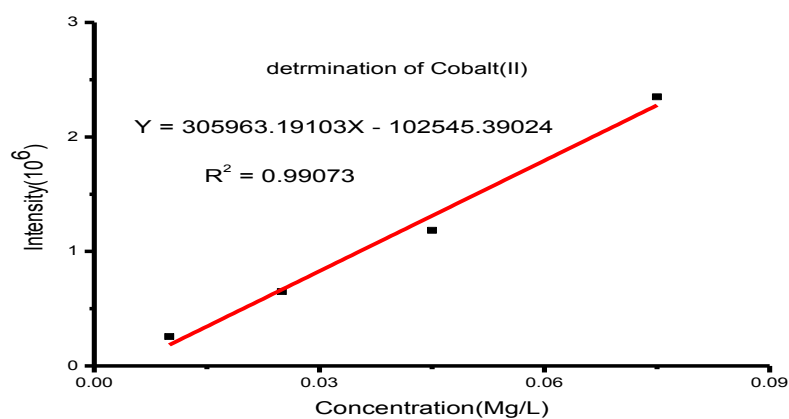


Figure 1: Determination of cobalt from complexes

Table 3: The composition of the cobalt in the complexes

Complexes			Metal to ligand ratio
	Calculated(mg/L)	Experimental(mg/L)	
H ₂ [Co(R) ₂]	1.468	1.437	1;2
H ₂ [Co(Bipy)(R) ₂]	6.809	6.463	1:1:2

4.4. Spectroscopic Characterization

4.4.1. FT-IR Spectroscopy

Infrared spectroscopy can be used as a good analytical tool to confirm the complexation of the metal ions with the organic ligands (Abdullah et al., 2014). Here the Infrared spectroscopy of the ligands and complexes were recorded to gain information about the binding modes of the ligand to the corresponding metals.

The IR spectra of the free ligand and complexes were recorded in the range 4000 cm⁻¹ to 400 cm⁻¹ (**table-4**). The broad band at 3292 cm⁻¹ due to the O-H groups in the metal complexes due to the hygroscopic nature of the complex and water of crystallization of KBr disc.

The characteristic bands in free resorcinol at 3400 cm⁻¹(s), 2884 cm⁻¹(s,br) and 1605 cm⁻¹(s) are due to (O-H), (C-H) and (C=C) respectively (**Figure -2**), shifted to 3382 cm⁻¹(s), 2642 cm⁻¹, 1603 cm⁻¹, in H₂[Co(R)₂], indicated coordination of resorcinide . The broadness of the band at 3382 cm⁻¹ (s and b) in H₂[Co(R)₂] is presumably due to increasing in the number of coordination and water of crystallization exist due to hygroscopic nature of the complex and water of KBr. (**Figure 2 and 4**).

.The characteristic bands in free 2,2'-bipyridine at 3050 cm⁻¹(s), 1575 cm⁻¹(s), and 1442 cm⁻¹(s) due to ν (C-H), ν (C=N) and ν (C=C) respectively (**Figure-2**), shifted to 3058 cm⁻¹, 1605 cm⁻¹ and 1438 in H₂[Co(Bipy)(R)₂] showed the coordination of 2,2'-bipyridine and resorcinide (**figure-3 and 5**). The changes in absorption frequencies and strengths confirm that 2, 2'-bipyridine and resorcinide was coordinated to the central metal ions. Identification of the peaks for ν (M-O) and ν (M-N) is difficult because these bands can be observed between 400- 800 cm⁻¹. The peaks of weak

intensity observed in the region of 520 cm^{-1} can be assigned to $\nu(\text{Co-O})$ in $\text{H}_2[\text{Co}(\text{R})_2]$ in (figure-4) and 636 cm^{-1} $\nu(\text{Co-N})$ in $\text{H}_2[\text{Co}(\text{Bipy})\text{R}_2]$ in (figure-5) respectively. Generally the IR spectral indicated that the ligands were coordinated to the central metal through oxygen atom of resorcinide and nitrogen atom of 2, 2'-bipyridine.

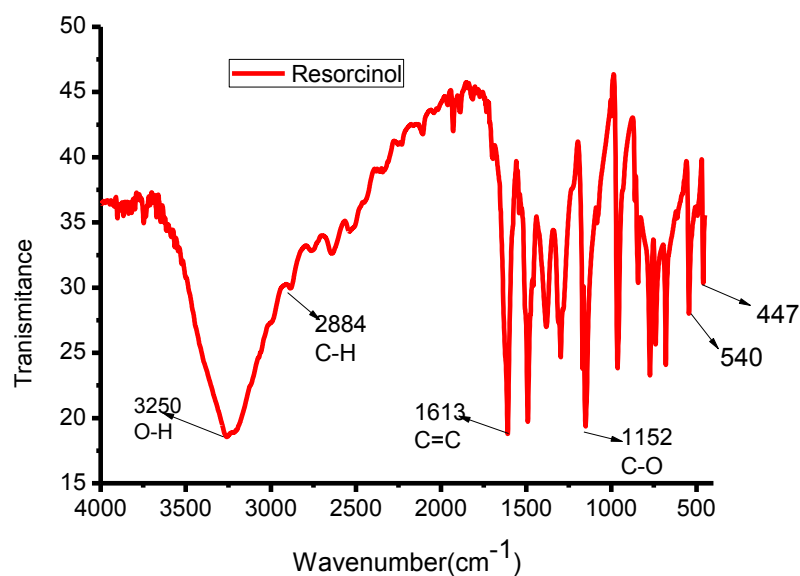


Figure 2: The IR spectra of resorcinol

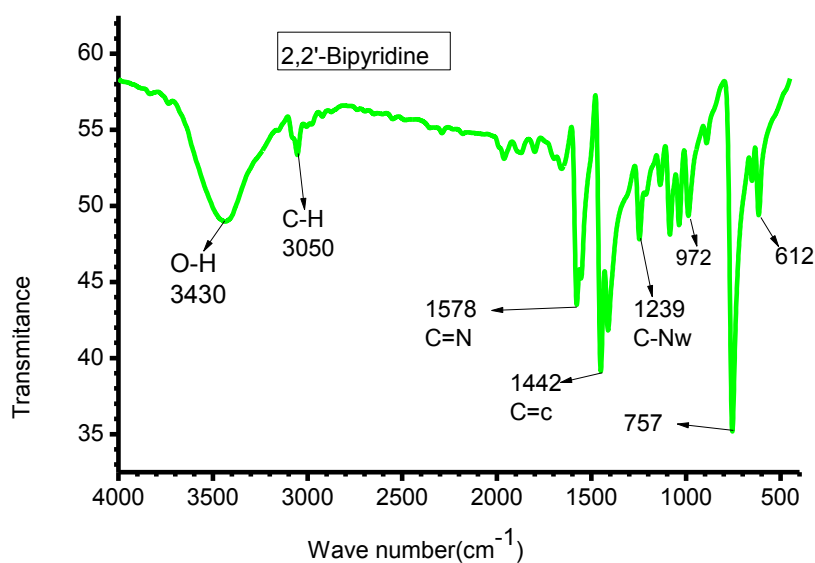


Figure 3: The IR spectra of 2, 2-bipyridine

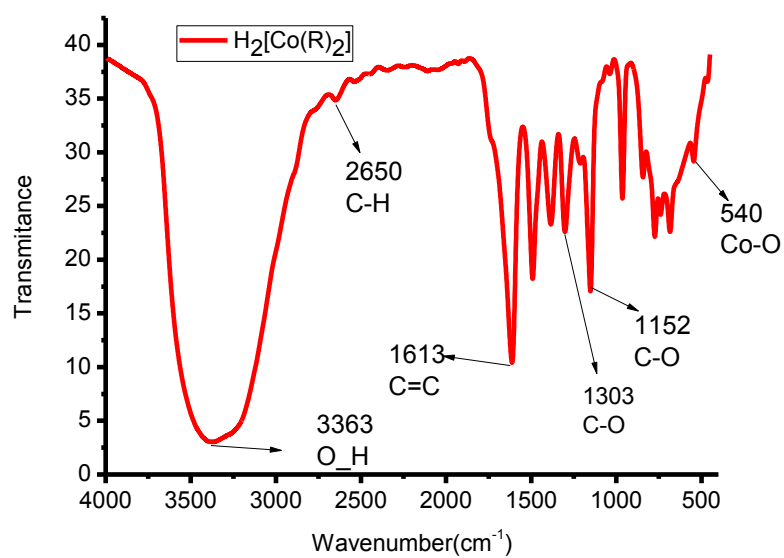


Figure 4: The IR Spectra of $H_2[Co(R)_2]$

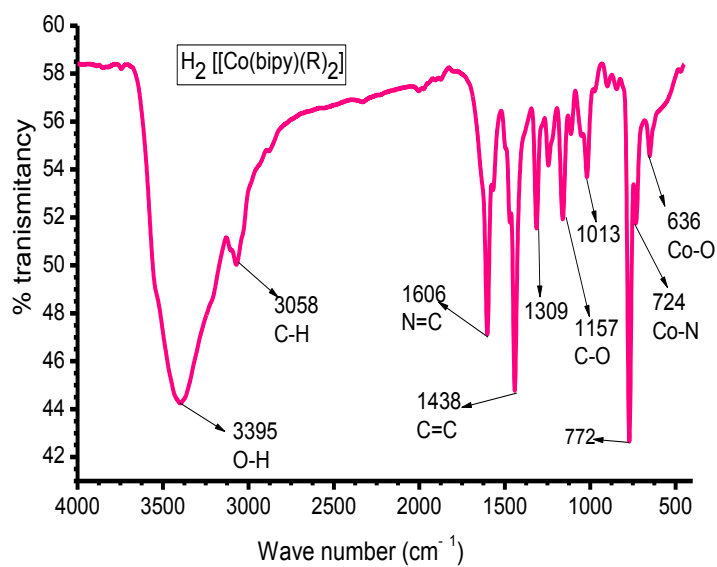


Figure 5: The IR Spectra of $[Co (Bipy)(R)_2]$

Table 4: Characteristic bands of Infrared spectra of the ligands and Co (II) complexes

Note: b= broad, m= medium, s = strong, w =weak

Compounds	Absorption frequencies(Cm^{-1})								
	$\nu(\text{O-H})$	$\nu(\text{N-H})$	$\nu(\text{C-H})$	$\nu(\text{C=C})$	$\nu(\text{C=N})$	$\nu(\text{C-O})$	$\nu(\text{C-N})$	$\nu(\text{Co-N})$	$\nu(\text{Co-O})$
2,2'-Bipyridine	-	-	3050(w)	1442(s)	1578(m)	-	1309(w)	-	-
Resorcinol	3250s	-	2884	1613	-	1152	-	-	-
$\text{H}_2[\text{Co}(\text{R})]$	3363s,b	-	2650w	1614	-	1303	-	-	540
$\text{H}_2[\text{Co}(\text{Bipy})(\text{R})_2]$	3395s,	-	3058	1438	1606	1157	1309	724	636

4.4.2. Uv-visible studies

Table 5: Uv-Visible absorption of complexes in (nm)

Compound	solvent	$\pi - \pi^*$	n- π^*
2,2-bipyridine	methanol	204,234	282
Resorcinol	methanol	274	
H[Co(R) ₂]	methanol	204,273	
H ₂ [Co(Bipy)(R) ₂]	methanol	223	306

The Uv-visible spectra of the ligands and complexes were recorded in methanol in wave length ranges of 200-800 nm. Typically, a shift in Uv-Visible maximum is expected in changing from a ligand to a metal complex, due to the modifications in electronic distribution.

As presented in the **table 6**, the bands of free ligands 2,2'- bipyridine were observed at 204 and 234 nm that of resorcinol was observed at 274 nm. The highest band could be because of $\pi - \pi^*$ transition of the aromatic system (C=C), and the other band might be due to then n- π^* (C=N).The Uv-Vis spectra of the H₂[Co(R)₂] showed two absorption bands in the ultraviolet region at 204 and 274 nm in methanol. While the peak shift in free 2,2-bipyridine ligands the new complex H₂[Co(Bipy)(R)₂] from 234 nm to 306 nm attributed to the Ligand to metal charge transfer(LMCT), which suggest the formation of complex(Mohamed et al.,2011).

The d-d transition in the Co(II) ions are not easily observable or forbidden transition in the Uv-Vis spectra(Liu et al.,2013) absorption of complexes may be explained by assuming different environments around the metal ion following the coordination.

The electronic spectra of the ligands are displayed in (**figure6-9**) and **table 6**.

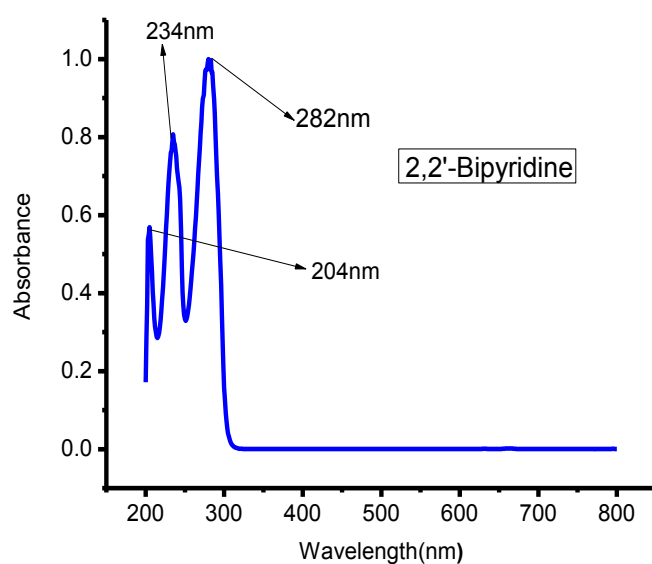


Figure 6: The Uv-vis spectra of 2, 2'-Bipyridine

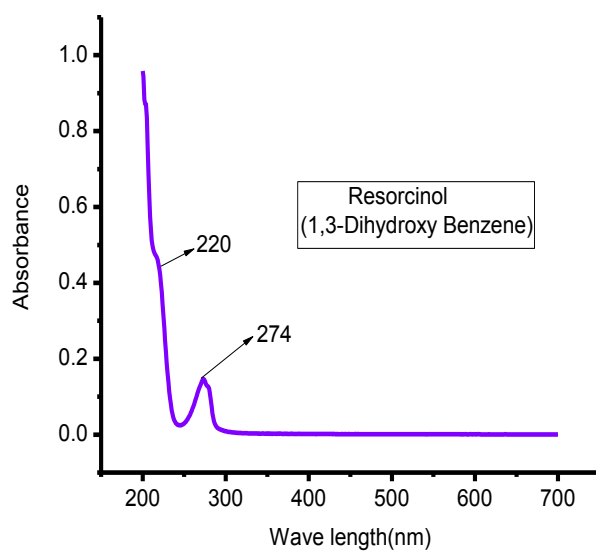


Figure 7: The Uv-Visible spectra of Resorcinol

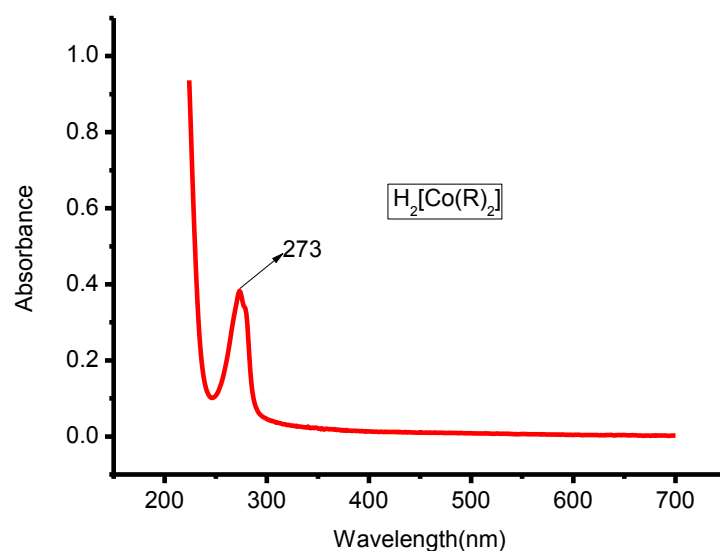


Figure 8: The UV-vis spectra of $H_2[Co(R)_2]$

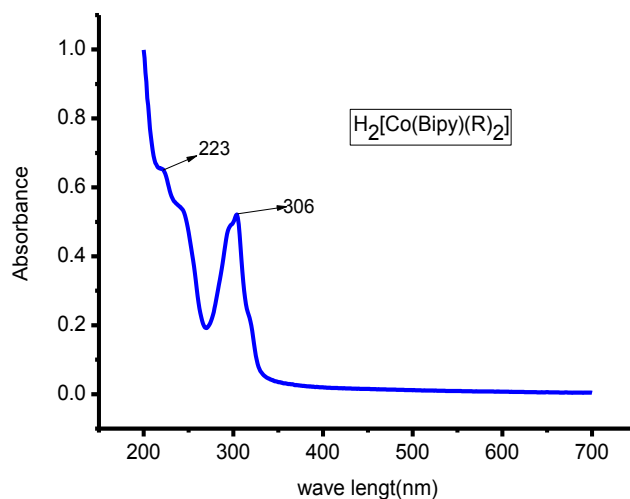


Figure 9: The UV-vis spectra of $[Co(Bipy)(R)_2]$

4.4.3. 1H -NMR Spectroscopy

The 1H -NMR spectra of precursor complex was recorded in H_2O-d_2 (figure 11 and 12). The comparative study between the 1H -NMR spectrum of the resorcinol alone in $DMSO-d_6$ (<http://www.hanhanggroup.com/nmr/nmr-en/RB03030010.html>) and the complex revealed the presence of resorcinol ligand in the complex in the form of Resorcinide. The 1H -NMR spectrum of $H_2[Co(R)_2]$ showed 8 proton signals in

aromatic region at $\delta = 6-7$ ppm, which is attributed to Resorcinide ring protons. In addition, the absence of the $-OH$ signal in the 1H -NMR spectrum of the complex confirmed the deprotonated (anionic) form of the resorcinol ligands coordinated with Co (II).

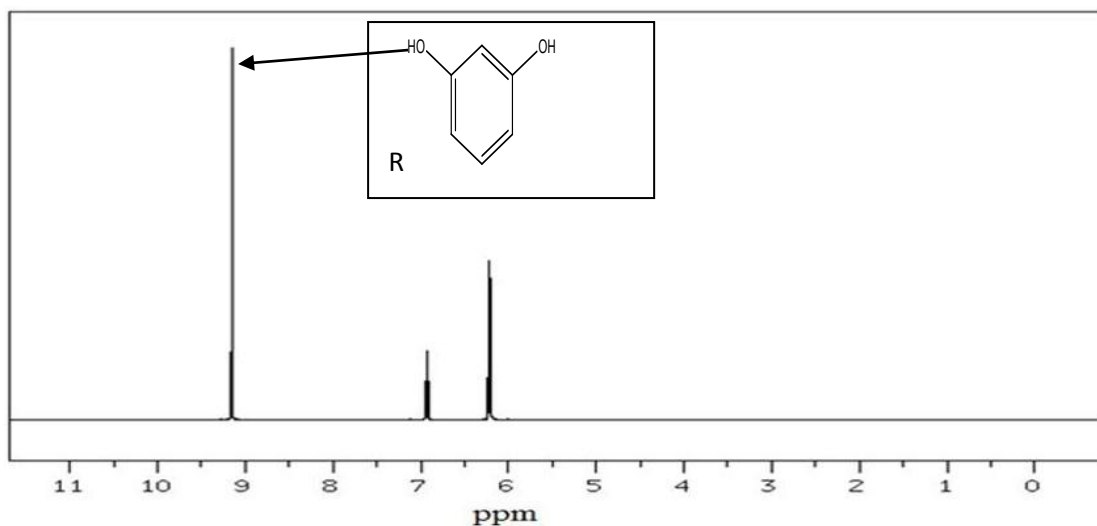


Figure 10: The 1H -NMR of Resorcinol

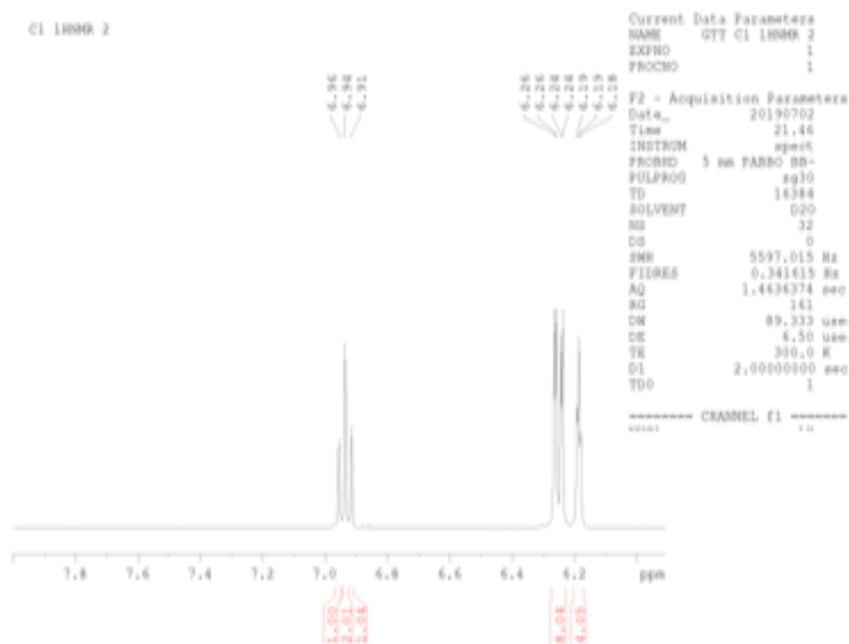


Figure 11: The 1H NMR of $H_2[Co(R)_2]$

4.4.4. MS spectroscopy

The ESI-MS spectrum of the complexes recorded dissolving in water showed characteristic molecular ion peaks at $M/z = 274$, $H_2[Co(R)_2]$, which is agreed with calculated molar mass of the complex. (Figure 12). This characterization compounded the evidences found in the former techniques in confirming the achievement of the intended complexes.

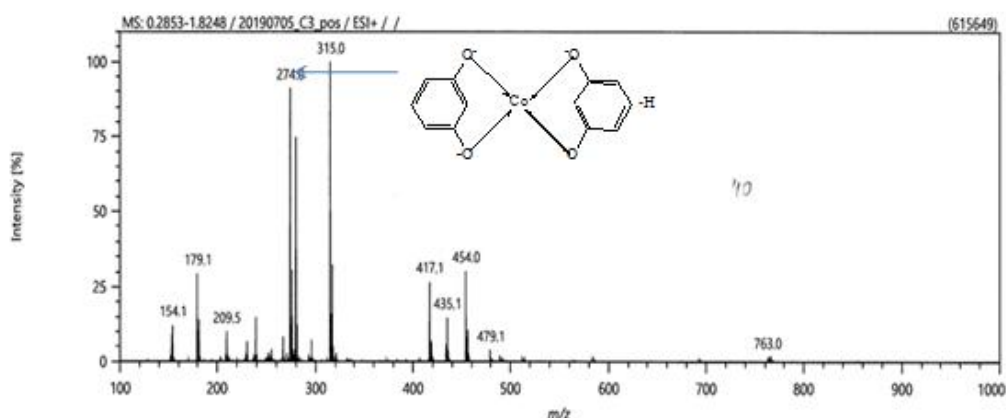


Figure 12: The mass spectra of $H_2[Co(R)_2]$

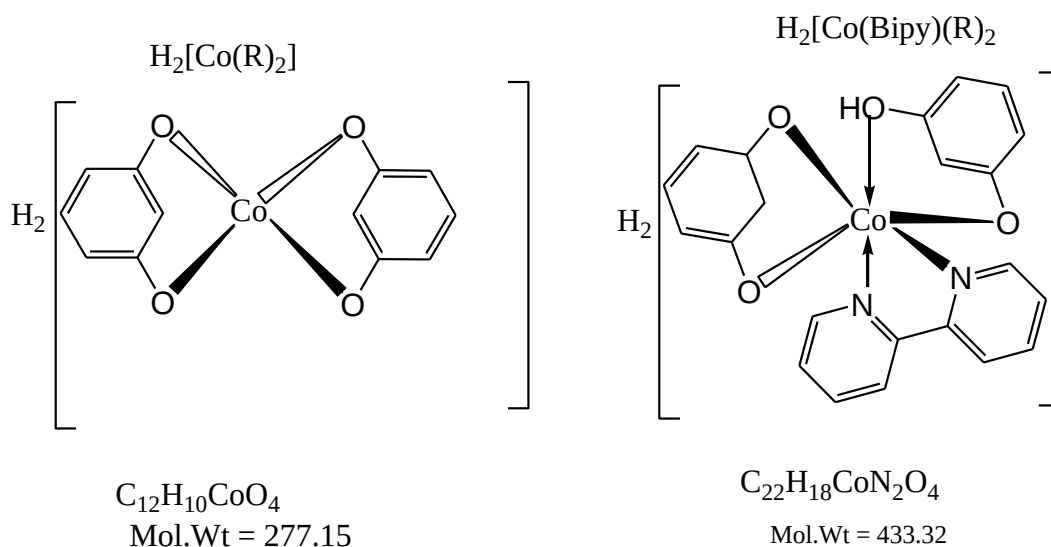
4.4.5. Structural investigation

The pH values of the complexes revealed that they were acidic (Arrhenius type) in nature implying that the hydrogen ion acts as a counter ion in both $H_2[Co(R)_2]$ and $H_2[Co(Bipy)(R)_2]$. The attempts to determine the presence of chloride ion in the outer sphere of the complexes verified no ionizable chloride.

The percentage of Co were obtained experimentally, by using ICP-OES after digesting the complexes in a mixture of concentrated HNO_3 , HCl and Per chlorate acid followed by dilution, agreed with the theoretical values of metal estimation. The observed shifts in band position and appearance of new non-ligand bands in the complexes infrared spectra confirmed the successful coordination of ligands to metal center in the corresponding complexes.

The UV-Visible spectra proved an electronic redistribution upon ligand coordination, particularly showed the bands attributed to the coordination of 2,2'-bipyridine from $H_2[Co(R)]$ to $H_2[Co(Bipy)(R)_2]$. The 1H NMR spectra confirmed the presence of

Resorcinide (dianionic bidentate chelating) ligand $H_2[Co(R)_2]$ complex. Furthermore, the ESI-MS results of complexes being in an excellent agreement with the molecular weight of proposed structures of C (II)-complexes further confirmed the composition and in turn the proposed structures of both Co (II)-complexes (Scheme-5).



Scheme 5: Proposed structure of $H_2[Co(R)_2]$ and $H_2[Co(Bipy)(R)_2]$

4.5. Antibacterial activity testing

In vitro biological screening effect of the ligands, salt, and their Co(II) complexes were studied against two gram negative *E. coli*, *K. pneumoniae* and two gram positive *S. aureus* and *S. pyogenes* shown in (figure-12) the Petri dish were incubated at 37 °C and inhibition zone were recorded after 24 hrs of incubation. The inhibition zone formed by these complexes against the tested bacterial strain determined the antibacterial activities of the synthetic complexes. The antibacterial activities of the free ligands, Metal salts, synthesized complexes and commercial available drugs (gentamicine) were described in (table-6).

The ligand 2,2-bipyridine exhibited great antimicrobial activity with high inhibition zone, While resorcinol has no significant effects on *E. coli* and *K. pneumoniae* as presented in the (table -6). $H_2[Co(R)_2]$ show high inhibition zone towards all tested bacterial strain relative to the reference gentamicine, except on the *S. aureus* show less IZ compared to gentamicine.

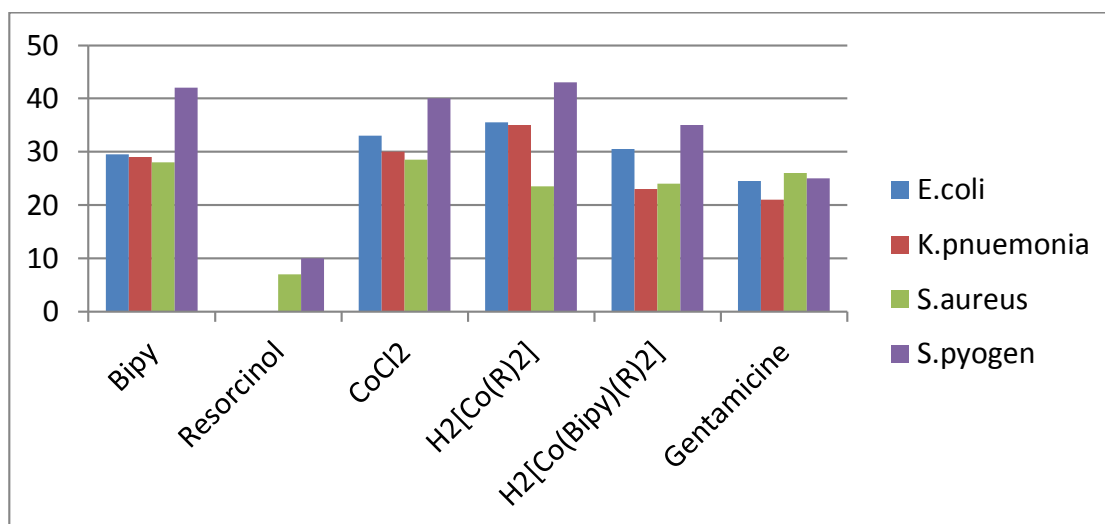
The new complex, $H_2[Co(Bipy)(R)_2]$ has also exhibit high inhibition zone toward all tested bacterial strain compared to the commercial drugs. Generally the free ligand resorcinol show relatively low activity againisting the pathogens with low inhibition zones. The metal salt also showed a relative effect on tested bacterial strain as well both the synthesized complexes are active and against all the tested bacterial strain as described below in (table6).

Table 6: Anti bacterial activity of salt, ligands, complexes and RA Mean value

Bacteria	Type (Gram strain)	Anti microbial activity(IZ,diameter in mm,) $\mu \pm s.d$					
		Bipy	R	CoCl ₂	H ₂ [Co(R) ₂]	H ₂ Co(Bipy)(R) ₂]	RA
<i>E.Coil</i>	Negative	29.5 $\pm$ 0.7	0.0	33 $\pm$ 4.2	35.5 $\pm$ 4.2	30.5 $\pm$ 4.9	24.5 $\pm$ 3.5
<i>K.pnuemonia</i>	Negative	29 $\pm$ 0.1	0.0	30 $\pm$ 0.2	35 $\pm$ 0.1	23 $\pm$ 0.0	21 $\pm$ 0.1
<i>S. aureus</i>	Positive	28 $\pm$ 0.2	7 $\pm$ 0.4	28.5	23.5 $\pm$ 0.0	24 $\pm$ 0.0	26 $\pm$ 0.0
<i>S.Pyogen</i>	Positive	42 $\pm$ 0 .5	10 $\pm$ 0.6	40	43 $\pm$ 0.0	35 $\pm$ 0.2	25 $\pm$ 0.1

RA = reference antibiotic, Bipy =2,2-bipyridine, R = resorcinol

Figure 13: The bar graph for the comparisons of the anti bacterial activity of salts, ligands, the synthesized complexes, and gentamicine



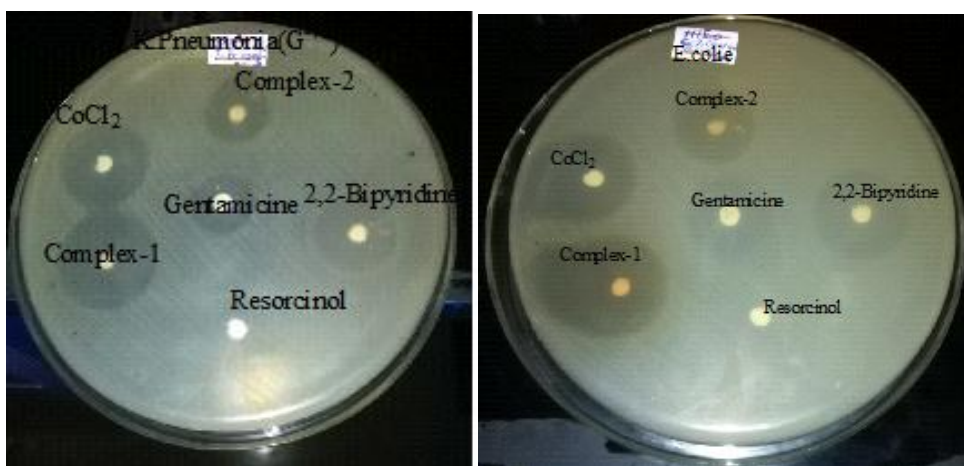
The comparative inhibition of the complexes with the reference gentamicine was indicated by the percent activity indexes. The result indicated that the complexes are very active against bacteria even more active than reference drug except on *S.aureus*.

For the tested compounds the activity indices (%) was calculated by the formula:-

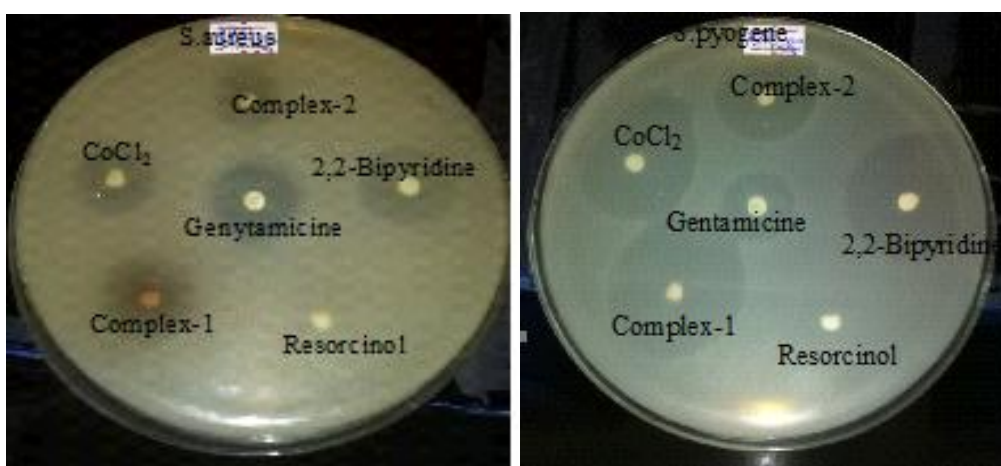
$$\% \text{Activity index} = \frac{\text{IZ of test compound(mm)} - \text{IZ of reference drug(mm)}}{\text{IZ of reference drug(mm)}}$$

Table 7: The % activity indices of the Co(II) complexes compared to gentamicine

Complexes	%activity index of Co(II) complexes			
	<i>E.coli</i>	<i>K.pnuemonia</i>	<i>S.aureus</i>	<i>S.pyogen</i>
H ₂ [Co(R) ₂]	+44.89	+66.67	-9.62	+72.00
H ₂ [Co(Bipy)(R) ₂]	+24.48	+9.52	-7.69	+40.00



a. *Klebsiella pneumoniae* and *E. coli* (Gram negative)



b. Gram-positive bacteria (*S. Pyogen* & *S. aureus*)

Figure 14: Biological activities of cobalt salt, ligand (rescinol & 2, 2-Bipy) and Co (II) complexes

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

New cobalt (II) complexes of resorcinide alone $H_2[Co(R)]$ and new complexes of resorcinide mixed with 2,2'-bipyridine $H_2[Co(Bipy)(R)_2]$ have been synthesized and characterized by analytical and spectroscopic (UV-Vis, IR, ICP-OES, 1H -NMR, ESI-MS) techniques. On the basis of analytical and spectroscopic data square planar geometry has been proposed for $H_2[Co(R)]$ complex, while octahedral geometry has been proposed for the new mixed ligand complex.

The biological activities investigation of complex, $H_2[Co(R)_2]$ showed higher inhibition on *E.coil*, *K.pneumonia*, and *S.Pyogen* relative to both second complex and commercial drug (Gentamicine). The new mixed ligand complex also showed higher inhibition on *E.Coil* and *S.Pyogen* compared to gentamicine, and showed less inhibition on *K.pneumonia* and *S.aureus* compared to commercially available drug.

Gentamicine showed less inhibition than the target complex toward *E.coli* and *S.Pyogen* indicate that the final synthesized complex has high degree of inhibition even compared with most commercially available antibiotic toward *E.Coil* and *S.Pyogen*.

5.2. Recommendation

The work done in this thesis could be further studied including different types of nucleobase and transition metal salts and testing their antibacterial activities on some other bacterial pathogen.

- ✿ Thermo gravimetric analysis is helpful to confirm the absence or presence of water in the complexes and thermal stability.
- ✿ The complexes $H_2[Co(R)]$ and $H_2[Co(Bipy)(R)_2]$ can be used as drug after studying of their cytotoxicity effects.
- ✿ X-ray crystallography analysis is also beneficial for further study of the length of chemical bonds and molecular structure of the product obtained in the solid state.

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