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BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

A Thesis on

“Synthesis, Structural Investigation and Assessment of Microbial Activity of Mononuclear and Homobinuclear complex of Iron (III) and 1, 10-phenanthroline using ethylenediamine as link’’

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Advisor: Getinet Tamiru (Ph.D.)

July 2020

Bahir Dar, Ethiopia

Master Thesis (Inorganic Chemistry Stream)

THESIS APPROVAL SHEET

I have read and evaluated this thesis entitled "**Synthesis, Structural Investigation and Assessment of Antibacterial Activities of New Mononuclear and Homobinuclear Iron (III) complex of 1, 10-phenanthroline using ethylenediamine as link**" Prepared by Aychal Arega under my supervision, I certify that the thesis is sufficient to full fill the requirement of the degree of master of science in inorganic chemistry.

Getinet Tamiru (PhD)	___________________	____________________
Name of Advisor	Signature	Date

As a member of the examination committee of the thesis entitled "**Synthesis, Structural Investigation and Assessment of Antibacterial Activities of New Mononuclear and Homobinuclear Iron (III) complexes of 1, 10-phenanthroline using ethylenediamine as link"** Prepared by Aychal Arega , I certify that the thesis is sufficient to full fill the requirement of the degree of Master of Science in Inorganic Chemistry.

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Name of Internal Examiner-2	Signature	Date

Bahir Dar University

July 2020

DECLARATION

I hereby declare that the work described in this thesis was carried out by me under the supervision of Dr. Getinet Tamiru at the Department of Chemistry, Bahir Dar University.
I also hereby confirm that the work embodied in this thesis report is an original investigation and has not been submitted in part or full for any other diploma or degree of this or any other university.

Aychal Arega	_______________	________________
Name of Student	Signature	Date of submission

Bahir Dar University

July 2020

Bahir Dar

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LIST OF ABBREVIATIONS AND SYMBOLS

ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
UV-Vis	Ultra Violet Visible spectroscopy
FT-IR	Furrier Transfer Infrared radiation spectroscopy
ESI-MS	Electron Spray Ionization Spectra
^{1}H-NMR	Proton Nuclear Magnetic Resonance
TG-DSC	Thermal Gravimetric Diffraction Scanning colorimetery
MS	Mass Spectra
ESR	Electron-spin resonance
Phen	Phenanthroline
Bpy	Bipyridine
Ac	Acetamide
HQ	Quinolinol
E.Coli	*Escherichia Coli*
K. Pneumonia	*Klebsiella Pneumonia*
S. Aurous	*Staphylococcus Aurous*
S. Payogen	*Staphylococcus Payogen*
Py	Pyridine
MHA	Mueller-Hinton Agar
MIC	Minimum Inhibition Concentration
LMCT	Ligand to Metal Charge Transfer
MLCT	Metal to Ligand Charge Transfer
IZ	Inhibition Zone
A.index	Activity index

Abstract

The improper use of antibiotics has led to an increase in drug-resistant bacteria against known antibiotics. To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active metal complexes derived from biologically active heterocyclic aromatic ligands for drug development against new targets is a matter of urgency. Based on this, in the present study two new Iron (III) complexes $[Fe(phen)_2(en)(H_2O)]Cl_3$ mononuclear and $[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$ binuclear were synthesized from $FeCl_3$, 1,10-phenanthroline monohydrate, and ethylenediamine by drop wise addition of ligand solution to the salt. The structures of the synthesized complexes were characterized using various Physico-chemical (Chloride test, molar conductance, solubility test, and melting point) and spectroscopic techniques (FTIR, UV-Vis, ICP-OES analysis). The change in color, solubility, melting point, peak position in the FT-IR and UV-Vis from the ligand indicate the formation of metal complexes. Based on the analytical and spectroscopic data Octahedral geometry were presumed to be achieved. The synthesized metal complexes have been evaluated for their *in-vitro* antibacterial activity against some Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) and Gram-negative (*Escherichia coli and Klebsiella Pneumonia*) bacteria by Disc diffusion method. The comparative antibacterial study was also conducted using commercial drug Gentamycin as reference material. The synthesized complexes showed even higher antibacterial activity on (*S. aurous and K. Pneumonia*) and comparable antibacterial activity on (*S.pyogenes and E.Coli*) compared to the standard antibiotic drug Gentamycin. Thus, the synthesized complexes may be considered as potential antibacterial agents after passing cytotoxicity testing.

Keywords: *Iron (III) complexes, Antibacterial Activity, 1, 10, phenanthroline, ethylenediamine.*

1- INTRODUCTION

1.1. Background of the study

Coordination chemistry is about tuning properties of metal ions using different ligands to form metal complexes with new properties upon the different modes of coordination. The pharmacological activities, properties, and their interaction ability within DNA/RNA base pairs can significantly change after complex formation [1-3]. The synthesized Metal-complexes have antimicrobial activity when administered and no more intention given to synthesize them earlier. However, due to the success of cisplatin as (anticancer), gold (anti-arthritic), bismuth(antiulcer) and iron(antimalarial) drug and the ability of transition metal complexes to bind DNA and RNA, there has been increasing interest in Medicinal inorganic chemistry using metal complexes as antimicrobial agents. Based on this, the synthesis of metal complexes with desired properties by changing the ligand has become an interesting research area. Designing new metal complexes with a new property, drug action, and therapeutic abilities has been part of this activity [4-8].

Transition-metal complexes in particular with N-and O – donor atoms have antimicrobial properties. These is due to stabilizing ability transition-metals for short-lived ligands, to synthesize metal complexes and it’s interaction ability with some negatively charged bio-molecules leads recent metal-based drug development [9-10]. The Heterocyclic ligands such as 1, 10 – phenanthroline, amino acids, and their metal complexes have a wide range of biological activities such as antibacterial, antifungal, antitumor, and antiviral activities. These properties are due to their coordination abilities with metal ions and pharmacological values of ligands take the attention of the scientific community as a focus of the investigation. These properties could be attributed to the presence of nitrogen (N) atom in the ligand moieties to interact with biological molecules. [3, 6, 11 -16]

Investigation of polynuclear metal complexes has been great interest nowadays because of their interesting magnetic properties. Particularly, the synthesis of iron (III) binuclear complexes, which differ in the type and kind of bridging moiety and ligands directly coordinated to the metal ion are many and part of this activity. Binuclear iron (III) complexes with metal centers linked by pyridine and imidazole derivates; cyano metallic complexes; pyridine N-oxide derivates; and oxo, oxalate and hydroxo-bridging complexes were reported in the previous work [17]. Literature shows interesting results obtained on the synthesis, characterization and antimicrobial activity of binuclear complexes of Iron (II) with Schiff Base shows better activity than salts upon coordination were reported [6, 18 -28]. A series of binuclear iron (III) complexes in which phen acts as a terminal ligand has been reported [21, 29]. Some mononuclear iron (III) complexes with Resorcinide, Phen, Py, Bipy and Ac differ in the type of ligand coordinated to metal ions has been reported by Bitew Fentahun 2019 (iron(III) with Resorcinide and Phen), Bekalu Molla 2018 (Iron (III) with Py and Ac), Melese Abiye 2018 (Iron (III) with Phen, Bipy and Ac) in Bahir Dar University but there is no reports have appeared before on the Binuclear mixed ligand complex of iron (III) with 1, 10-phenanthroline as a terminal ligand and ethylenediamine as a link. However, no report was found on the synthesis, characterization, and assessment of antibacterial activity of binuclear mixed ligand complexes of iron (III) Containing1, 10-phenanthroline, and ethylenediamine as a link.

Although, a large number of antibiotics are available for medical use rapid emergence of old and new antibiotic resistance is persisted and fast becoming a global concern which revealed the need for new classes of antimicrobial agents. The conventional solution obtained taking wild growing plants and animals as a source has become problematic. This problem has magnetized the attention of the scientific community, in general, to consider and investigate transition metal complexes as alternative solutions for this problem [30-32].To overcome this problem of bacterial resistance to the known antibiotics, there is a real perceived need for the discovery of new, novel active antibacterial compounds with a good spectrum of activity and a new mechanism of action against new targets is a matter of urgency [33-37]. From

This perspective, the synthesis of new metal complexes and assessment of their antibacterial activity has been considered by the global community as part of the solution [5, 7, 17, 31, 46, 47] and some binuclear [18-21, 23, 28] metal mixed-ligand complexes have been reported. To the best of our knowledge, there is no report to the antibacterial activity of binuclear Fe (III) mixed ligand complexes.

Therefore, to complement the efforts made by the world's scientific community in alleviating the aforementioned problem motivated us to look for a solution by synthesis and assessment of antibacterial activities of mononuclear and binuclear mixed-ligand Fe (III) complexes containing 1,10-phenanthroline and ethylenediamine in combined and there is no report has appeared earlier with this composition.

1.2 Statement of the problem

Antimicrobial drug resistance is one of our most serious health threats and serious public health problem. [18, 34, 38-42] Antibiotic resistance is rising to dangerously high levels and spreading globally, in all parts of the world. These were the most pressing public health problems, threatening our ability to treat common infectious diseases and poses a substantial threat to morbidity and mortality worldwide as a consequence million of the population are infected and died every year worldwide. This growing list of infections becoming harder and sometimes impossible, to treat as antibiotics become less effective [30, 34, 43-46]. To overcome the alarming problem of this microbial resistance to known antibiotics, the discovery of novel active compounds of metal complexes with new modes of action, properties, and mechanisms against new targets to the lipid layer of the antibiotic-resistant micro-organisms is a matter of urgency. Therefore, the Synthesis and application of transition metal complexes of nitrogen-containing heterocyclic Ligands exhibit versatile biological roles and microbial activities to complement the efforts made by the world scientific community in alleviating the problem. This study is, therefore, complementing the efforts made by the world scientific community in alleviating the problem by synthesis, investigate the structure and it's the antibacterial activity of new mixed ligand mononuclear and binuclear iron (III) complexes bridged by ligands with high lipophilicity, drug action upon coordination.

1.3. Significance of the Study

Antibiotic discovery needs to be re-energized; to rival the threat for the increasing number of resistant bacteria to known antibiotics. Strategies need to be designed to synthesize the metal complexes with a good spectrum of activity and new mechanisms of action to resistant bacteria. The synthesized metal complexes are effective for their antibacterial activity to have been considered as potent classes of drugs by the global community [10]. This study the synthesized metal complexes were more effective to bacteria where the antibacterial activity was Investigated address the problem arising due bacteria resistant to antibiotics considered to be widely manufactured in pharmacological industry and therapeutic applications in the health care system.

1.4. Objectives of the study

1.4.1. General Objective

The purpose of this work was to synthesize, characterize, and Investigate the antibacterial activity of Mononuclear and binuclear Fe (III) mixed ligand complex of 1, 10-phenanthroline, and Ethylenediamine.

1.4.2. Specific Objective

- To synthesize the Mononuclear complex (1) $[Fe(phen)_2(en)H_2O].Cl_3$ using 1:2:1 ratio of the Ferric chloride, Phenanthroline Monohydrated, ethylenediamine
- To synthesize the Binuclear complex (2) of $[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$ using 2:1 ratio of the precursor complex $[Fe\ (Phen)_2(H_2O)_2]Cl_3$ and Ethylenediamine
- To characterize the synthesized metal complexes using some analytical, and spectroscopic techniques.
- To investigate the antibacterial activity of the new complexes (1) and (2) against some Gram-positive and Gram-negative bacteria.
- To compare the antibacterial activities of the complexes with a commercially available drug.

2-REVIEW OF LITERATURE

2.1 Overview of the chemistry of ligands (1, 10-Phenanthroline, Ethylenediamine) and the metal iron

2.1.1 1, 10-phenanthroline

1, 10-phenanthroline is a classic, rigid planar, hydrophobic, electron-poor, π-acidic, chelating bidentate heteroaromatic and cyclic ligand makes its high ability to chelate many metal ions via its two nitrogen's to exert excellent *in vitro* antimicrobial activity against a broad-spectrum of bacterial, fungal pathogens. The melting point of 1, 10-phenanthroline is 117 ^{0}C. The antimicrobial activity of phen can be significantly modulated by modifying its structure. The development of metal-phen complexes offers the medicinal chemist an opportunity to expand such structural diversity by controlling the geometry and varying the oxidation states of the metal center, with the inclusion of appropriate auxiliary ligands in the structure, offering the opportunity to target different biochemical pathways in bacteria. [29]

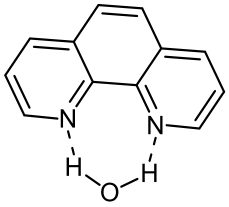


Figure 1: Structure of 1, 10 phenanthroline monohydrate.

2.1.2 Ethylenediamine

EDA has moderate acute toxicity in animals. It is a primary irritant; being corrosive when undiluted, and is also a skin sensitizer. EDA has not been tested for mutagenicity to current regulatory standards, and there are no assays for a clastogenic activity or for the potential to express activity in somatic cells *in vivo*. Thus, there is insufficient information to draw firm conclusions regarding the mutagenic potential of EDA. EDA was not carcinogenic in animals. EDA is a colorless to yellowish hygroscopic liquid with an ammonia-like odor. Its molecular weight is 60.12. It is a strongly alkaline, very volatile, pungent material, which fumes profusely in air. It has a melting point of about

8.5 °C, a boiling point of 116 °C and it is miscible with water and alcohol. The metal complexes of Ethylenediamine have high antimicrobial activity. [37]

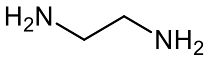


Figure 2: Structure of Ethylenediamine.

2.1.3 Metal Iron (Fe)

Most proteins in cells of living organisms require transition metal ion for their proper function. Iron is an essential element for the growth and development of all the scale of living organisms, and acquiring iron is crucial for the development of any pathogen. The ability of microorganisms to compete successfully with the host for iron is an important determinant of their virulence because it has antibacterial activity under suitable concentration. As a result, the longstanding battle between pathogens and hosts for iron acquisition is effective for the survival of the host organism. Iron (III) complexes were applicable for diagnostic imaging techniques in inorganic medicinal chemistry and known antimicrobial activity. Iron participates in a large number of cellular processes, the most important of which are oxygen transport, ATP generation, cell growth and proliferation, and detoxification. It is a co-enzyme or enzyme activator of ribonucleotide reductase, a key enzyme for DNA synthesis, which catalyzes the conversion of ribonucleotides to deoxyribonucleotides.[29,47, 48]

2.1.4 Iron (III) chloride

Anhydrous iron (III) chloride is an inorganic, a black, essential solid compound which has a molecular mass of 162.2 g/mol, melting point 37 ^{0}C with the molecular formula, $FeCl_3$ and soluble in water. It forms numerous additional compounds, especially with organic molecules that contain donor atom, for example, ethers, alcohols, aldehydes, ketones, and amines. Iron (III) chloride is hygroscopic and can absorb water from the atmosphere to produce a yellow hydrated ferric chloride, $FeCl_3.6H_2O$ having an octahedral Geometry of the complex. Anhydrous iron (III) chloride is fairly strong Lewis acid. It is used mostly in the pharmaceutical industry where it is used as a precursor for iron-bearing medicine anemia treatment [48] forms hydrogen chloride, which dissolves in the water forming hydrochloric acid.

2.2 Review on the synthesis, structural investigation and antimicrobial activity of some Fe (III) complexes with different ligands

Some reports have appeared before on synthesis, characterization of iron (III) complexes.

Li, F. et al. [19]**;** a novel oxalate-bridged binuclear iron (III) complex of aminophenol derivative ligands was synthesized. The structure of the synthesized binuclear iron (III) complexes were characterized by elemental analysis (C, H, N), X-ray crystallography, FT-IR, UV–Vis, magnetic susceptibility studies and cyclic voltammetry techniques in which its X-ray structure has been revealed that the synthesized complex having an octahedral arrangement and each iron (III) is coordinated by two oxygen atoms of the oxalate group, two amine nitrogens and two oxygen atoms of phenolate ligand. The Magnetic studies revealed the presence of weak anti-ferromagnetic interaction in this compound, which confirms the presence of a ligand-bridged structure.

Feng X.et.al.[21]; A novel binuclear iron(III) Schiff base complex has been synthesized from the mixture of $Fe(NO_3)_3$ and KSCN in suitable anhydrous methanol solvent was refluxed for 3 hr in 1:1 ratio. To the resulted mixture solution DMF solution of 5-nitrosalicylaldehyde and 1,3propanediamine was added in 2:1 ratio with constant stirring for 2.50 hr in which the two Fe Schiff base moieties were doubly bridged by two hydroxyl moieties. The structure of the synthesized binuclear iron (III) complexes were characterized by elemental analysis, X-ray diffraction analysis, magnetic susceptibility and spectroscopic techniques such as FT-IR to confirm its distorted octahedral geometry in which each iron(III) is six-coordinated by two N atoms from 1,3-propane diamine, two O atoms from 5-nitrosalicylaldehyde and two O atoms from lattice water.

T. Karimpour et al.[23]; has been synthesized a novel oxalate-bridged binuclear iron (III) complex of deprotonated tetradentate aminophenol ligand containing ethylenediamine moiety in the ligand and is a derivative form of an aminophenol was synthesized by addition of $FeCl_3$ to a tetradentate aminophenol and Triethylamine mixture in ethanol solution in a ratio of (1:1:2 mmol) under continuous stirring and then after An ethanol solution of $H_2C_2O_4$ and Triethylamine in (0.5: 1.00 mmol) ratio was added to the mixture. After 4 hr stirring, the initial blue color changed to bluish-purple is an indicator for the formation of the complex. The iron complex has been characterized by elemental analysis, X-ray crystallography, infrared spectroscopy, UV–Vis, magnetic susceptibility studies, and cyclic voltammetry techniques. X-ray structure analysis has revealed that each iron (III) is coordinated by two oxygen atoms from the oxalate group, two amine nitrogens, and two oxygen atoms from phenolate ligand in an octahedral arrangement. The temperature variable magnetic susceptibility exhibits quite strong anti-ferromagnetic coupling between the two iron (III) ion centers.

Jian, F.F, et al. [20]; a new oxo-bridged binuclear iron (III) complex of phenanthroline with other ligands have been Synthesized by reacting phen with $FeCl_3$. $6H_2O$ in 2:1 ratio using ethanol solvent solution and constant stirring of the reaction at which the PH is adjusted at PH=1 in dilute Sulphuric acid solution in which the sulfate anions in the lattice used the charge of the titled complex. The structure of the synthesized binuclear iron (III) complexes were characterized by elemental analysis, TG-DSC, X-ray diffraction analysis and spectroscopic techniques FT-IR to confirm the crystal structure the newly synthesized complex having two octahedrally coordinated iron(III) ions bridged with oxygen atoms formed a non-linear complex and each iron(III) ions are surrounded by two nitrogen from phenanthroline and four oxygen atoms from water and bridged Oxygen atom to form a distorted octahedral geometry.

Jinyu Li et al.[28]; has been synthesized one oxo-bridged binuclear iron(Ill) complex of Phenantroline with the mixture of $(NEt_4)_2[Fe_2OCl_6]$ and PhCOONa in suitable acetonitrile solvent with stirring for 2 hr, and then an acetonitrile solution of phenanthroline was added into a mixture with constant stirring for 5 hr. the structure of the synthesized complexes characterizing by using various techniques such as X-ray diffraction, IR, Magnetic susceptibility and elemental analysis which confirms each part in binuclear iron(Ill) complex possesses distorted octahedral Geometry. In which each iron atoms are surrounded by four nitrogen atoms from two phenanthrolines that are perpendicular to each other and two oxygen atoms one from the bridged and the other is from Benzoic acid to form a non-linear complex.

Bitew Fentahun [65] reported on the Synthesis, Structural Investigation and Assessment of Antibacterial Activities of New Iron (III) Complexes with Resorcinide Alone (H[FeR_2] and Mixed with 1,10 Phenantroline (H[FeR_2(Phen)]). *Bahirdar University*, **2019**. The structure of the synthesized complexes was characterized by using physicochemical, and spectroscopic techniques (UV-Vis, FT-IR, ^{1}H-NMR, ESI-MS and ICP-OES) confirms square planar geometry to the former and the later octahedral geometry was suggested respectively. Furthermore, the antibacterial activity of the synthesized metal complexes was evaluated against some Gram-positive (*staphylococcus aureus, Staphylococcus pyogenes*) and Gram-negative (*Escherichia coli, Klebsiella Pneumonia*) bacteria by Disc Diffusion method and both are effective in all bacterial strains when compared with the standard drug, gentamicin.

Melese Abeyie [66] reported on Synthesis, Structural Investigation, and Antibacterial Activity of Mixed Ligand Complexes of mononuclear Iron (III), Phen, Bpy and Ac *Bahirdar university*, **2018**. The physicochemical and spectroscopic data obtained were confirmed Coordination ligands to metal leads the successful formation of the new target [Fe(Phen)(H_2O)$_4$]Cl_3,[Fe(Phen)(Bpy)(H_2O)$_2$]Cl_3 and [Fe(Phen)(Bpy)(Ac)(H_2O)]Cl_3 complexes and Octahedral geometry was suggested. The *in-vitro* antibacterial activities of complexes were tested for antimicrobial activity against two Gram-positive bacteria (*Staphylococcus aureus and Streptococcus pyogenes*) and two Gram-negative bacteria (*Escherichia coli and Klebsiella Pneumonia*) by Disc diffusion method and all showed antibacterial activity. The complexes have comparable to Gentamycin in antibacterial activity against all tested bacterial strains except *Klebsiella Pneumonia*.

Bekalu Mola [67] reported on the Synthesis, Structural Investigation, and Assessment of Antibacterial Activities of Iron (III) Mixed Ligand Complexes of Bpy and Ac *Bahirdar University*, **2018**. The structures of the complexes were characterized by physic-chemical and spectroscopic techniques (UV-Vis, FT-IR, and ICP-OES). The antibacterial activities of the synthesized metal complexes were evaluated against Gram-positive (*staphylococcus aurous, Staphylococcus pyogenes*) and Gram-negative (*Escherichia coli, and Klebsiella Pneumonia*) bacteria by Disc Diffusion method and its antimicrobial activity were is smaller in all bacterial species when compared with Gentamycin. Furthermore; the minimum inhibitory concentrations of the complexes were determined.

D.A. House, P.J. Steel et al. [68] Has been reported the first monodentate and bidentate bridging ethylenediamine ligand cobalt complexes (cis-$[CoCl(en)_2(enH)]Cl\ (ZnCl_4)$ and cis μ-en$[CoCl(en)_2]_2Cl\ (ZnCl_4)$) in which their structure is characterized by X-ray crystallography revealed Octahedral geometry. But the antibacterial activities of the synthesized mononuclear and binuclear metal complexes were not investigated.

Getinet Tamiru et al. [17] reported two new ethylenediamine containing cobalt (II) mononuclear and ethylenediamine bridged binuclear mixed ligand complexes $[Co(phen)_2(Act)(en)]Cl_2$, and $[Co_2(phen)_4(Act)_2(en)]Cl_4$ and one $[Co(phen)_2(Act)(H_2O)]Cl_2.H_2O$ without ethylenediamine were synthesized from $CoCl_2.6H_2O$, 1,10-phenanthroline monohydrate, acetamide, ethylenediamine and characterized by using physicochemical and spectroscopic (ESI-MS, ICP-OES, FT-IR, and UV-Vis) technique's confirms octahedral geometry of the intended complexes was achieved. *In-vitro* antibacterial activity the synthesized metal complexes was tested and showed comparable antibacterial activity with the commercial drug Gentamycin.

These and all other binuclear iron (Ill) complexes containing Phenantroline and amine groups have been reported earlier but, there no reports have appeared before on the Binuclear mixed ligand complex of iron (III) with 1, 10-phenanthroline as a terminal ligand and ethylenediamine as a link and amazingly none of any binuclear iron (Ill) complexes have been tested their antimicrobial activity. Thus, in this study, we propose the synthesis, characterization, and assessment of antibacterial activity of Binuclear mixed ligand complexes of iron (III) Containing 1, 10-phenanthroline, and ethylenediamine as a link.

3 - MATERIALS AND METHODS

3.1. The methodology of the study

In this study, synthesis of iron (III) Complexes from metal salt, 1, 10 phenanthrolines and ethylenediamine followed by stoichiometric amount were conducted in 1:2:1:1 and 2:4:1:2 (Fe: phen: en: H_2O) molar ratio and this complex has been characterized by some physicochemical properties conductivity measurement, Melting point, Qualitative Chloride test, Quantitative chloride test and Spectroscopic techniques UV-Vis, FT-IR, ICP-OES, and their antimicrobial activity were tested in some gram-positive and gram-negative bacteria and carried out in Microbiology laboratory department of Biology, Bahir Dar University, Bahir Dar, Ethiopia.

3.2. Chemicals and Regents

The chemicals and reagents used for this investigation were ferric chloride salt ($FeCl_3$), 1, 10 Phenanthroline monohydrated(Lobachemie), Ethylenediamine, Mueller Hinton agar, Distilled water, Methanol(Loba Chemie), ethanol, Acetone, KBr, KCl, $AgNO_3$ (BDH Laboratory Supplies Poole), HCl (Loba Chemie), H_2SO_4 (Loba Chemie), HNO_3, $HClO_4$ (Neolab Chemie), NaOH, DMF, n-hexane, Chloroform(Fisher Scientific UK Limited chemical supplier), Diethyl ether(Loba Chemie) and DMSO.

3.3 Instruments and Method

During synthesis, characterization, and antimicrobial tests the following instruments and apparatus were used. FT-IR spectra were obtained in the region 4000-400 cm^{-1} in potassium bromide pellets. The metal analysis was performed by an inductively coupled plasma optical emission spectrometer, Optima 8000 (ICP-OES). The electronic absorption spectra in (200-800 nm) data were collected in distilled water from Cary 60 UV-visible spectrophotometer. The molar conductivity of the 10^{-3}M solution of the complexes was measured by JENWAY 4200 conductivity meter using 0.1M potassium chloride solution. The melting point was measured by Stuart SMP30 melting point apparatuses. Chlorination test was done thermogravimetrically using silver chloride precipitate obtained from a mixture solution of each complex in excess silver nitrate solution. The anti-microbial test was performed by using two gram-positive bacteria (*Staphylococcus aureus and Staphylococcus pyogenes*) and gram-negative bacteria

(*Escherichia coli and Klebsiella pneumonia*) using Muller Hinton agar media and Gentamycin as a standard drug. Generally, all the experimental activities (Synthesis of the complexes, the analytical analysis, instrumental analysis Except IR spectral data, and anti-microbial test) were carried out at Bahir Dar University postgraduate chemistry laboratory.

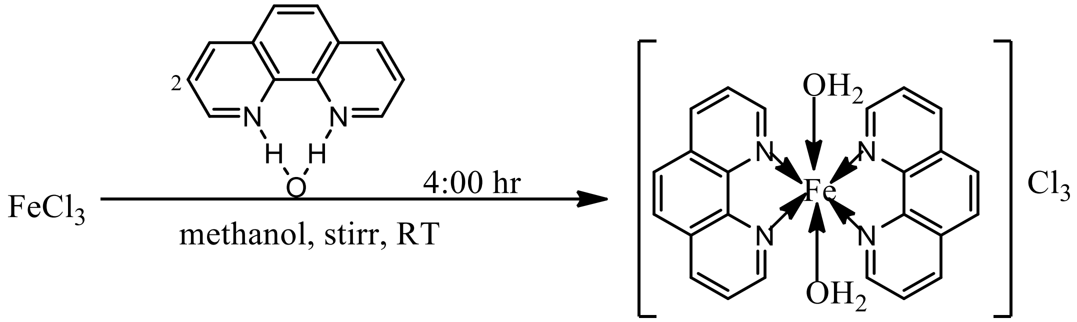
3.4. Synthesis

The preparation of coordination compounds and the transformation of one coordination compound into another form is the basis for a vast amount of synthetic methods in inorganic chemistry. Among the methods used in synthetic inorganic chemistry, a direct combination of Metal Salts with biologically active Ligands and replacement of one ligand by another in the complex was the most common type of reaction of coordination compounds usually carried out in solution is mostly applicable**.** [49] The phen-iron complexes were synthesized by combining the phenanthroline ligand with $FeCl_3$ in the presence of methanol solvent ***(Scheme 1, 2 and 3)***. Treatment of anhydrous $FeCl_3$ with monohydrate 1, 10-phenanthroline in 1:2 ($FeCl_3$: phen) mol ratio yielded precursor complex of $[Fe(Phen)_2(H_2O)_2]\ Cl_3$. The treatment of the precursor complex with ethylenediamine in 1:1 and 2:1 molar ratio formed a new complex $[Fe(Phen)_2(en)(H_2O)]\ Cl_3$ and $[Fe_2(Phen)_4(en)(H_2O)_2]$ Cl respectively. The resulting precipitates of metal complexes were then filtered, dried, weighed and analyzed

3.4.1. Synthesis of Complexes

3.4.1.1. Synthesis of $[Fe(phen)_2(H_2O)_2]\ Cl_3$

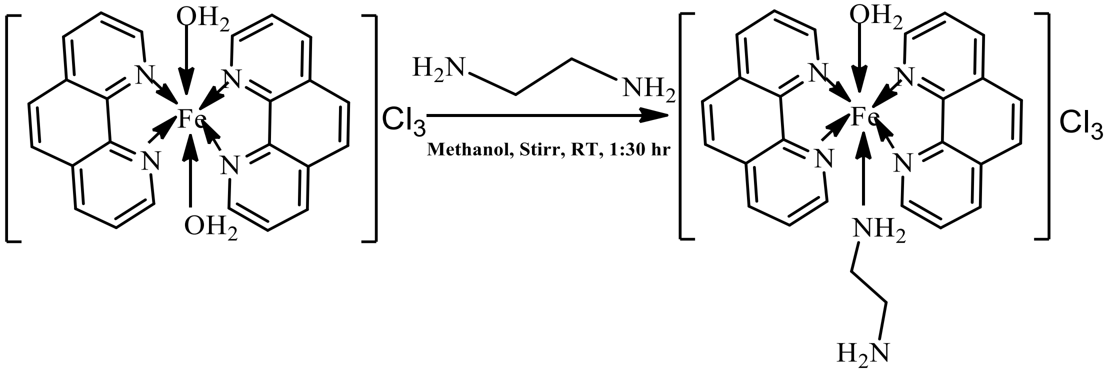
A 1.0 g (5.04 mmol) of 1, 10-phenanthroline monohydrated solution in 25 ml methanol was added dropwise slowly from a burette to a methanolic solution of 0.41 g (2.52 mmol) iron (III) chloride with constant stirring magnetically to form the precursor complex $[Fe(phen)_2(H_2O)_2]Cl_3$ by using 2:1 (phen: salt) ratio for 4:0 hr at room temperature. The reaction progress was monitored by TLC and color change. The solution was subjected to a rotary evaporator to remove the solvent. The solid was collected and washed three times with acetone. Yield: ***1.32 gm*** (94.286 %) The synthesis path is indicated in Scheme- 1 below.



Scheme 1: Synthesis of $[Fe(Phen)_2(H_2O)_2]Cl_3$

3.4.1.2. Synthesis of $[Fe(phen)_2(en)(H_2O)]Cl_3$

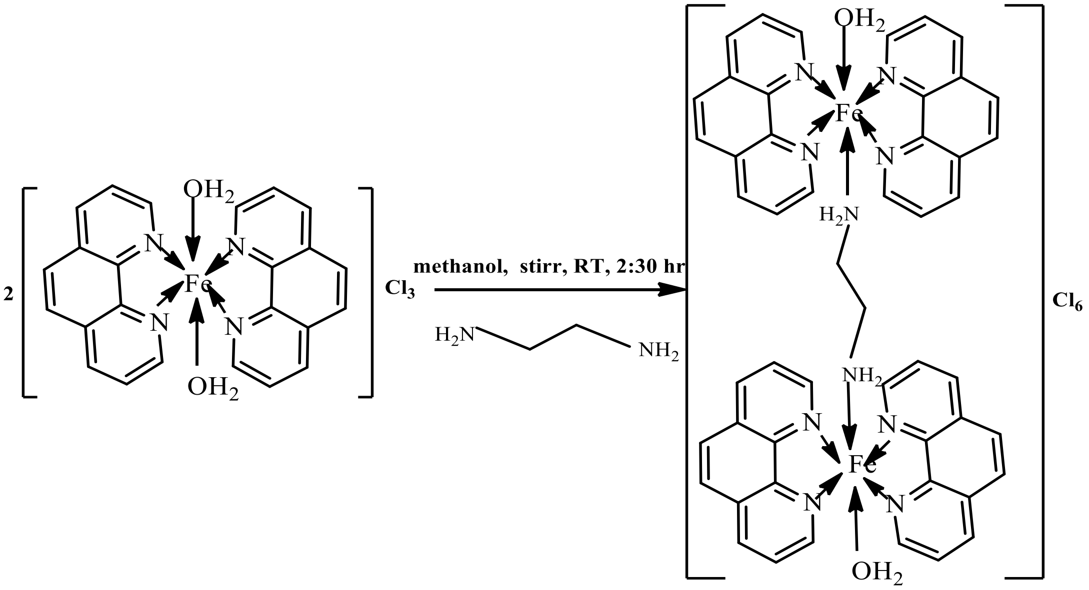
A 35µl (0.51 mmol) solution of ethylenediamine in 25 ml methanol was added dropwise slowly from a burette to a methanolic solution of the $[Fe(Phen)_2(H_2O)_2]Cl_3$ 0.2896 g (0.51 mmol) in 25 ml by using 1:1 ratio of the $[Fe(Phen)_2(H_2O)_2]Cl_3$ and Ethylenediamine for 1:30 hr at room temperature to obtained complex (1) $[Fe(phen)_2(en)(H_2O)]Cl_3$. The reaction progress was monitored by TLC and color change. The solution was subjected to a rotary evaporator to remove the solvent. The solid was collected and washed three times with acetone. Yield: ***0.28 gm*** (90.03 %) The synthesis path is indicated in Scheme- 2 below.



Scheme 2: Synthesis of $[Fe(Phen)_2(en)(H_2O)]Cl_3$

3.4.1.3. Synthesis of $[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$

35 µl (0.52 mmol) in 20 ml methanol solution of Ethylenediamine from the burette was added dropwise to a methanol solution (25 ml) of 0.558 g (1.03 mmol) of $[Fe(phen)_2(H_2O)_2]Cl_3$ by using 1:2 ratio of (en:$[Fe(phen)_2(H_2O)_2]Cl_3$) for 2:30 hr at room temperature and the reaction progress was monitored by TLC and color change. The solution was subjected to a rotary evaporator to remove the solvent. The solid was collected and washed three times with acetone. Yield: ***1.12 gm*** (94.91 %) The synthesis path is indicated in Scheme- 3 below.



Scheme 3: Synthesis of $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$

3.5. Characterization of Metal complexes

The synthesized metal complexes were characterized by the Physico-chemical characterization technique. Colors of the metal complexes were determined by visual observation and its solubility was tested using various polar solvents like water, methanol, ethanol, and with some non-polar solvents, acetone diethyl ether and Chloroform. The metal content of each complex was determined by using Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP-OES) instrument. Chloride ion content was determined by taking solutions of the complex and mixing to an aqueous solution of excess $AgNO_3$; then after, the precipitate is formed and the actual yield of silver chlorides was weighted and chlorides were calculated. The Electrical conductivity of the complex was recorded in solutions of the samples in di-ionized water using Digital conductivity meter after the conductivity meter was first calibrated by KCl solution in di-ionized water and Melting point of the complex was recorded by using Stuart SMP30 melting point apparatus. The IR spectra of the ligands and the synthesized complex were recorded in the KBr pellet in 400-4000cm^{-1} range with an FT-IR spectrophotometer to confirm the coordination of the ligands to the metal ion and to identify the particular coordination site of the ligands with the metal center. Electronic spectra of the ligand and synthesized complex solutions in distilled water were measured in the range of 200–800 nm of using a UV-Vis Spectrophotometer.

3.5.1. Physicochemical Measurements

3.5.1.1. Solubility Test of Fe (III) complexes

A very small amount Fe (III) complexes$[Fe(Phen)_2(H_2O)_2]Cl_3$, $[Fe(Phen)(en)(H_2O)]Cl_3$ and $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_3$ was taken in 10 mL beaker dissolved in 3 ml of the corresponding solvent and their solubility was tested in polar solvents like water, methanol Acetonitrile, Ethanol and some non-polar solvents like Chloroform, Acetone, Tetrahydrofuran, and their solubility was checked.

3.5.1.2. Qualitative Chloride Test

A small amount of each sample complex was dissolved in 3 mL of di-ionized water and 10 mL beaker. Then to each complex solution, an aqueous solution of excess $AgNO_3$ from another 10 mL beaker was mixed and the white precipitate was observed.

3.5.1.3 Quantitative Chloride Test

0.052 gm, 0.01gm and 0.06 gm of $[Fe(phen)_2(H_2O)_2]Cl_3$, $[Fe(phen)_2(en)(H_2O)]Cl_3$ and $[Fe_2(phen)_4(en)(H_2O)_2]Cl_3$ Fe (III) complexes was dissolved in 5 mL di-ionized water with 10 mL in separate beaker respectively. To each solution of the complex, an aqueous solution of excess $AgNO_3$ was added and a white precipitate was observed in all complex solutions. The white precipitate was formed filtered, washed three times with di-ionized water, dried, and weighed the yield of white precipitate (AgCl) of each complex.

3.5.1.4 Melting Point Determination

The melting point of precursor and new synthesized Fe (III) complexes was determined in the capillary tube using the Stuart SMP30 melting point apparatus. One side opened a capillary tube with a powdered metal complex was introduced. The capillary tube with sample complex was heated and the temperature was noted when the metal complex was first to get melted and decomposed.

3.5.1.5. Molar Conductivity Measurement

The conductivity of Fe (III) complexes was measured with JENWAY 4200 Digital conductivity meter by taking $1x10^{-3}M$ freshly prepared sample solution of each complex ambient temperature. The conductivity meter was standardized by a highly conductive solution of KCl. The conductivity electrode was dipped in each sample solution and the reading was recorded. The molar conductance of the complexes was determined using the relation:

$$\Lambda M = \frac{1000K}{C}$$

Where:

- ✓ ΛM- molar conductance (Scm^2mol^{-1})
- ✓ K- specific conductance (Scm^{-1})
- ✓ C-concentration of the sample ($mole/m^3$)

3.5.2 Spectroscopic Techniques

3.5.2.1. Metal Determination

0.003, 0.003, and 0.014 gm of complexes $[Fe(Phen)_2(H_2O)_2]Cl_3$, $[Fe(Phen)(en)(H_2O)]Cl_3$ and $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ was measured and added to a separate conical flask respectively. Each sample in vessels was dissolved with 10 ml of water and 5 ml of concentrated HNO_3, H_2SO_4, and 5 ml $HClO_4$ was added one after the other and heated for 3 hours. Heating was continued until the last 3 or 4 drops of the solution were left and the organic portion of the complexes presumed to be decomposed. The vessels were removed from the heat source and allowed to cool for 30 minutes to transfer into a 50 ml volumetric flask followed by the addition of de-ionized water to the meniscus of the flask. The solutions were subjected to Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP-OES) instrument and metal content of each complex were determined. The experimental percentage composition of complexes was determined using the relation:

$$\mathbf{Fe\ (\%) = \frac{C\ x\ V\ x\ 100}{m\ x\ 1000}}$$

Where:

- C- Average concentration read from the instrument mg/L
- V- Volume of metal complex solution
- m- Mass of the complex used in gram
- Fe(%) - Percentage of metal in complex

3.5.2.2. UV-Vis Spectroscopy Study

The UV spectral data of all complexes were obtained in Shimaduzu UV60 spectrophotometer by taking a small amount of each complex distilled water solution of each complex in the wavelength range of 200 to 800nm.

3.5.2.3. FT- IR Spectroscopy Study

The IR spectral data of iron complexes were obtained by taking around 1mg of the samples mixed with a small amount of dry KBr pellet in the region of 400 to 4000 cm^{-1} at room temperature.

3.6 Antibacterial Studies

The antibacterial activity of the ligand and their corresponding metal complexes were tested against two Gram-positive bacteria (*staphylococcus aureus* and *staphylococcus pyogenes*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) by disc diffusion method using Muller Hinton Agar (MHA) as nutrient medium and Gentamycin as control. The complexes would be tested at different concentration and their activities towards the bacteria were compared with the standard drug Gentamycin. The size of the zone of inhibition is usually related to the response given by the metal complexes to the bacterial growth. The active response of metal complexes to the bacterial growth is the highest size and zone of inhibition is an indicator for the more potent antimicrobial drugs. The cleared zone was measured by a ruler in mm and compared with the standard drug Gentamycin. [50, 51]

3.6.1 Media Used

The nutrient Mueller-Hinton Agar (MHA) was used for culturing the bacterial isolates.

3.6.2 Source of microorganism

The bacteria which are used for testing the ligands and metal complex in this work; was obtained from the Microbiology laboratory from the Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia.

3.6.3 *In- vitro* antibacterial assay

The antibacterial activities of the ligands and metal complexes were screened out against some Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes) and* Gram-Negative *(Escherichia coli and Klebsiella Pneumonia)* pathogens using the agar well disk diffusion method.

3.6.4 Antibacterial study of Iron (III) metal complexes and determination of their activity

The antibacterial activity of the ligands and complexes was performed against some Gram-positive and Gram-Negative bacteria by the agar disc diffusion method. The cultured bacteria were freshly prepared into a test tube and treated in an incubator at 37 ^{o}C for 24 hours under nutrient agar broth using the streak plate method. The plates and nutrient gar were sterilized in autoclaved steam sterilizer for 15 minutes at 121 ^{o}C and 1.5 bar pressure using steam sterilization method. For the experimental work, certain ml of incubated agar medium was transferred into dishes and the solid agar plates were swabbed with certain µl of the bacterial strain. After the adsorption of bacteria, wells of certain mm diameter were made by the sterile metallic borer and the fixed concentrations of Metal salt, ligands and their complexes at 700 mg/L in distilled water and methanol were carefully injected to the respective disc by using a dropper. Then all plates were incubated at 37 °C for 24 hr and the efficiency for killing bacteria of each ligand and complex was determined by measuring the zone of inhibition and compared with antibacterial drugs that were in use currently to compare their antibacterial activities of the synthesized complex regarding Gentamycin. A drop of each complex and ligand was added onto a disc.

Finally, the antibacterial activity of the compounds was compared with commercially available Gentamycin standard drugs and % activity index for the complex were calculated by using the formula as given below:

% of Activity Index = $\frac{A-B}{B}$**x100**

Where

- ✓ A- inhibition Zone of the tested complex
- ✓ B- inhibition Zone of reference

3.6.5. Minimum Inhibitory Concentration

Minimum inhibition concentration (MIC) of the target complex $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ was determined by using Muller Hinton agar disc diffusion method at concentrations of 100, 150,200, 250, 300, 500 and 700 mg/L. The plate and the medium were allowed to sterilize at 121 ^{0}C in a steam sterilizer. After sterilization, the medium was allowed to cool. Each of the cultures of the organism was inoculated uniformly on the surface of prepared agar medium in a petri-dish by using a spreader. wells of 6 mm diameter were punched carefully using sterile cork borer and these were filled with the test solution using a micropipette and the plates were incubated for 24 hours at 37 ^{0}C. The diameter of the inhibition zone (mm) was measured after 24 hours of incubation.

4 -RESULTS AND DISCUSSION

4.1. Physical Characterization Fe (III) Complexes

The physical and analytical data of complexes are presented in **Table: 1** and also the solubility of Metal complexes is presented in **Table: 2.** The Fe (III) complexes have a melting point in the binuclear complex and the decomposing temperature of the Mononuclear complex was greater than 95 ^{o}C differs from the melting point of ligands *viz.*1, 10-phenanthroline with melting point 117 ^{o}C. The high melting point and decomposing temperature of complexes confirmed the complexation of the ligands with the metal ion and their stability. Generally, the observed change in color, solubility, melting point and stability of complexes from starting reagents indicate the formation of new compounds with strong metal-ligand bond due to coordination.

The analytical data are consistent with the calculated results from the proposed empirical formula of each complex. During the Qualitative chloride test, the precipitation of the white cloudy precipitate of AgCl confirms the presence of chloride ion outside the coordination sphere of the complexes. During quantitative chloride estimation, the experimental values of chloride are very close to calculated values **(Table: 1)** which confirms the expected chloride ions are in the outer sphere of coordination in each iron complex, and hence the intended structure is most probably achieved. The observed small change between the calculated and experimental chloride content of complexes may be due to experimental error. Furthermore, the metal content in the complexes was determined spectroscopically using ICP-OES and the experimental reading of metal content from the instrument is within an excellent agreement to the calculated value**(Table:1)** which is confirmatory evidence for achievement of the structure of intended complexes. The small variation between calculated and experimental values may be attributed to the incomplete digestion of complexes. The conductivity of metal complexes in aqueous solution was determined by the concentration, charge, and mobility of the dissolved ions. Conductance measurement plays an important role to determine the ionic nature, mode of coordination, and structure of metal-complexes. The molar conductivity values of complexes in water with 10^{-3} M concentration were found to be in the range of (13.76- 79.2 S cm^2 mol^{-1}) (**Table: 1**) which confirmed that the synthesized complex non-electrolyte in nature [52]. The lower conductance of $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ compared to $[Fe(Phen)_2(en)(H_2O)]Cl_3$ and $[Fe(Phen)_2(H_2O)_2]Cl_3$ is a consequence of the increase in molar mass and the surface area which decreases the speed of the mobility of the ions, as a result the kinetic energy of the ions decreases and causes lower conductivity of the complexes. This further confirms the formation of the intended complex successfully. [5, 7, 17, 46]

Table 1: shows the physical and analytical data of precursor and new mixed ligand Fe (III) complexes

Complexes	M.W (g/mol)	Dec/ Melt Temp(° C)	Yield (%)	Conductivity S cm^2 mol^{-1}	% Cl Calcu (found)	% Fe Calcal (found)	Color
$[Fe(phen)_2(H_2O)_2]Cl_3$	558.67	>95	94.28	79.20	19 (18.6)	10.01(9.84)	saddle brown
$[Fe(phen)_2(en)(H_2O)]Cl_3$	600.69	110-118	90.03	53.23	18 (16)	9.32 (9.09)	dark brown
$[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$	1141.36	> 115	94.91	13. 61	18.6 (17.4)	4.91 (4.60)	brown

Table 2: The solubility of precursor and new mixed ligand Fe (III) complexes

Solvents	Water	Methanol	Ethanol	Aceto nitrile	Acetone	Chloro form	Diethyl ether	DCM
$[Fe(Phen)_2(H_2O)_2]Cl_3$	S	S	S	S	Ins	Ins	Ins	Ins
$[Fe(Phen)_2(en)(H_2O)]Cl_3$	S	S	S	S	Ins	Ins	Ins	Ins
$[Fe_2(Phen)_4(H_2O)_2]Cl_6$	S	S	S	S	Ins	Ins	Ins	Ins

Key: S= Soluble, Ins= Insoluble and DCM= Dichloromethane

4.2. IR Spectral Study of Ligands and their Fe (III) Complexes

To get further information about the coordination behavior of the ligand 1, 10-phenanthroline and Ethylenediamine with a metal ion, comparison of the infrared spectra of the free ligands and their complexes were necessary. The structural possibilities of the complexes depend upon the mode of coordination of the ligands. The complexation of the ligands with the central metal ion is confirmed by IR spectroscopy and it was primarily used to identify the bond types, structure, and functional groups of the ligands and the corresponding complex.

The principal IR vibrational bands of ligands, as well as their iron complexes, are presented in (**Table:3** and **Figure: 3).** In the infrared spectra, a peak around 3382 cm^{-1} is characteristic stretching $\nu_{O\text{-}H}$ in the free 1,10-phenanthroline monohydrate which is supportive evidence for the presence of water molecule in the ligand and hydrogen bond. Upon coordination the peak shifted to 3405 cm^{-1} in $[Fe(Phen)_2(H_2O)_2]Cl_3$, 3404 cm^{-1} $[Fe(Phen)_2(en)(H_2O)]Cl_3$ and 3393 cm^{-1} in $[Fe_2(Phen)_4(H_2O)_2]Cl_6$ is characteristic stretching $\nu_{O\text{-}H}$. This is confirmatory evidence for the presence of coordinated water in both complexes. Bands at 1645 cm^{-1} and 1585 cm^{-1} [5, 17, 46, 47] are characteristic for $\nu_{(C=C)}$ and $\nu_{(C=N)}$ stretching in 1, 10-phenanthroline monohydrate, shifted to 1617cm^{-1}(1) , 1617 cm^{-1}(2), 1627cm^{-1} (3) [17,46] and 1504 cm^{-1}(1), 1514 cm^{-1}(2), 1514 cm^{-1} (3) [17, 46, 53] is characteristic for $\nu_{(C=C)}$ and $\nu_{(C=N)}$ stretching in $[Fe(Phen)_2(H_2O)_2]Cl_3$(1), $[Fe(Phen)_2(en)(H_2O)]Cl_3$(2) and $[Fe_2(Phen)_4(H_2O)_2]Cl_6$(3) respectively. (**Figure: 3**) The observed shifts in the peaks position of complexes to lower frequency [54] compared to the ligand, is due to the electron redistribution in a ring decrease in bond order within 1,10-Phenantroline ligand. These clearly indicated the coordination of 1, 10-Phenantroline to Fe^{3+} through ring nitrogen.

Upon coordination, a broadband peak around 3465 cm^{-1} of ethylenediamine was disappeared in the mononuclear complex is a characteristic the N-H vibrational stretching of coordinated primary amine may be obscured to $\nu_{O\text{-}H}$ stretching was observed. A peak around 2942 cm^{-1} characteristic vibrational frequency for C-H (sp^3) stretching of ethylenediamine ligand shifts to 2921 cm^{-1} and 2911 cm^{-1} in $[Fe(Phen)_2(en)(H_2O)]Cl_3$ and $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ respectively are characteristic vibrational frequency for C-

H (sp^3) stretching. This is an indicator for the coordination of ethylenediamine to the metal. Whereas, the N-H vibrational stretching in $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ has not appeared. This is due to Ethylenediamine prefers the trans-form when it functions as a bridging group between two metal atoms and the coordination of the two Nitrogen atoms to the metal. As a result, the complex have null dipole movement and hence do not absorb the IR radiation may be the likely reason for the disappearance primary amine N-H stretching [17, 55-56]

Table 3: IR spectral data for ligands and their corresponding precursor and new mixed ligand Fe (III) complexes

Compounds	$\nu_{(O\text{-}H)}$ (H_2O)	$\nu_{(N\text{-}H)}$ (NH_2)	$\nu_{(C\text{-}H)}$ $(C{=}CH)$	$\nu_{(C\text{-}H)}$ (CH_3)	$\nu_{(C=C)}$	$\nu_{(C=N)}$	$\nu_{(C\text{-}N)}$	$\nu_{(Fe\text{-}O)}$
Ethylenediamine	-	3465	-	2942 2866	-	-	-	-
1, 10-Phenanthroline	3382	-	3056	-	1645	1585	1343	-
$[Fe(Phen)_2(H_2O)_2]Cl_3$	3405	-	3045	-	1617	1504	1335	638
$[Fe(Phen)_2(en)(H_2O)]Cl_3$	3404	-	3034	2921	1617	1514	1339	623
$[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$	3393	-	3034	2911	1627	1514	1329	638

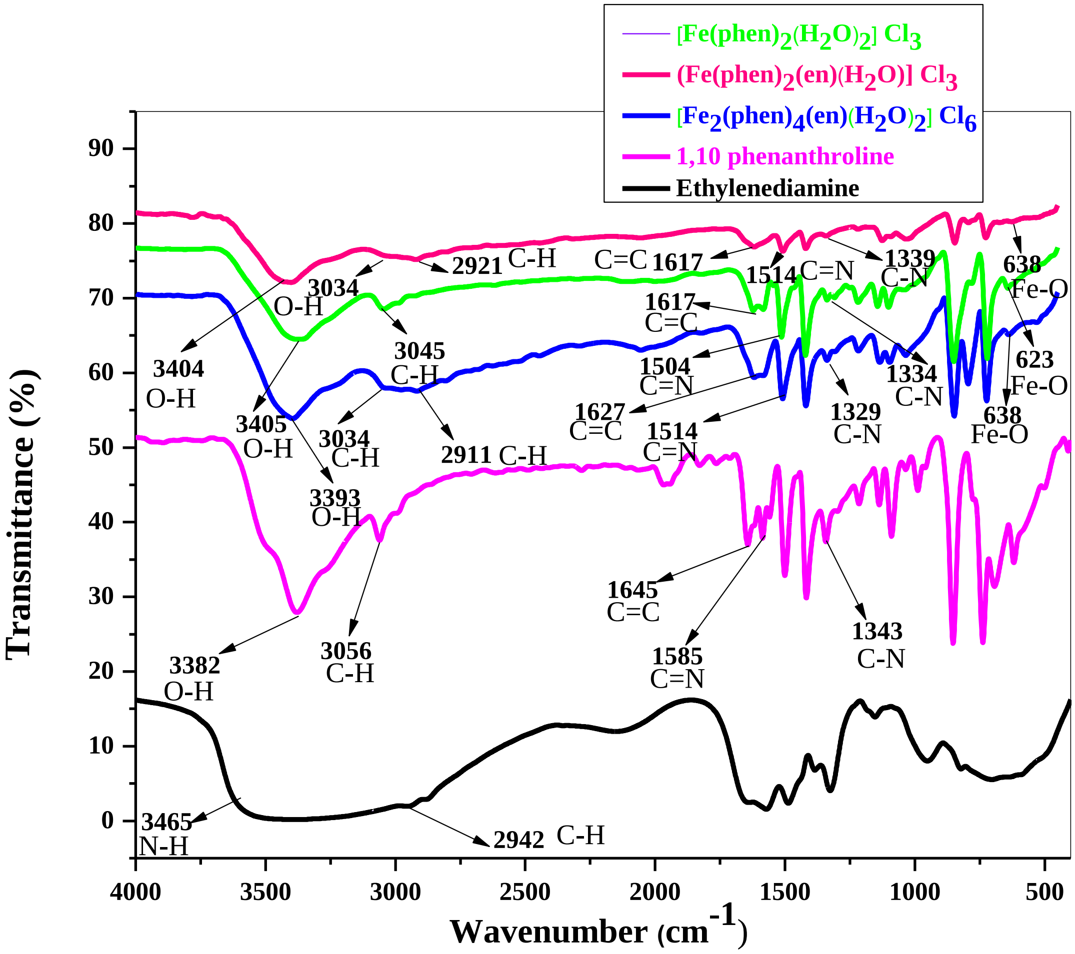


Figure 3: IR spectra of Ethylenediamine, 1,10-phenanthroline, [Fe(Phen)$_2$(H$_2$O)$_2$]Cl$_3$, [Fe(Phen)$_2$(en)(H$_2$O)]Cl$_3$ and [Fe$_2$(Phen)$_4$(H$_2$O)$_2$]Cl$_6$.

4.3. UV-Visible Spectral Study of Ligands, Metal Salt and Fe (III) Complexes

Ultraviolet-visible (UV/Vis) spectroscopy is the study of the electron transitions involved in the rearrangements of valence electrons. In the field inorganic chemistry, the color of transition metal complexes is commonly associated with the d-d transition which is Complementary to the color of light it absorbed during the transition. [50, 57, 58, 59, 60] In transition metal complexes a change in electron distribution between the metal and a ligand gives rise to charge transfer (CT) bands is an index for the coordination of ligands to metals and used to characterize the geometry of the complexes. The absorption in the ultraviolet region is attributed to transition within ligand orbital and those in the visible region are probably due to allowed metal to ligand charge transfer and d-d transition. [57] In this section, the principal electronic spectral data for metal salt, ligands, and their corresponding Fe (III) complexes were recorded in the wavelength range of 200- 800 nm, and their assignments are compiled and presented in **Table: 4** and **Figure: 4.**

Upon coordination of 1, 10- Phenanthroline to Fe(III), the intra-ligand charge transfer bands were shifted from 227 nm and 261 nm in 1,10-phenanthroline to 224 nm and 264 nm , 227 nm and 264 nm , 227 nm and 264 nm in $[Fe(Phen)_2(H_2O)_2]Cl_3$, $[Fe(phen)_2(en)(H_2O)]Cl_3$ and $[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$ respectively (**Figure: 4**), are characteristic for $\pi \rightarrow \pi^*$ (C=C) and $n \rightarrow \pi^*$ (C=N) transition. This is due to rearrangement of electrons during charge transfer. The up shifted band in the complexes for $n \rightarrow \pi^*$ (C=N) transition implies that decrease in band gap due to the likely interaction of phenanthroline and metal orbitals for bonding leads charge transfer absorption at lower energy. However, the blue shifted band in the precursor complexes is due to increase in band gap due to electronic repulsion of between phen (π) and metal d_π orbitals. upon coordination with 1,10- Phenanthroline bands at 545 nm, 553 nm and 563 nm in $FeCl_3$ showed a significant shift to 443 nm 458 nm and 468 nm in $[Fe(Phen)_2(H_2O)_2]Cl_3$, 502 nm, 507 nm, 512 nm in $[Fe(Phen)_2(en)(H_2O)]Cl_3$ and 562 nm 566 nm, 576 nm in $[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$ **(*Appendix 8-11*)** was assigned to $^2T_{2g}(I)\rightarrow{}^2A_{2g}(I)$, $^2T_{2g}\rightarrow{}^2T_{1g}(I)$ and $^2T_{2g}\rightarrow{}^2E_g(I)$ transitions respectively. [61, 62]

Upon coordination of non-band ligand Ethylenediamine with the precursor complex $[Fe(Phen)_2(H_2O)_2]Cl_3$, the band at 224 shifts to 227 in $[Fe(Phen)_2(en)(H_2O)]Cl_3$ for intra-ligand $\pi \rightarrow \pi^*$ (C=C) transition to the metal**.** Furthermore, the bands observed around the region of 443 nm,458 nm and 468 nm in $[Fe(phen)_2(H_2O)_2]Cl_3$ shifts to 502 nm, 507 nm and 512 nm indicates the coordination of Ethylenediamine to $[Fe(Phen)_2(H_2O)_2]Cl_3$ and assigned to $^2T_{2g}(I) \rightarrow{}^2A_{2g}(I)$, $^2T_{2g}(I) \rightarrow{}^2T_{1g}(I)$, $^2T_{2g}(I) \rightarrow{}^2E_g(I)$ transitions respectively. Thus, the shifts in the bands due to charge transfer, $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and d-d transition in $[Fe(phen)_2(H_2O)_2]Cl_3$, $[Fe(Phen)_2(en)(H_2O)]Cl_3$ and $[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$ upon ligand coordination were confirmatory evidence for the coordination of ligands to metals and probably the intended complexes with low spin octahedral geometry were achieved.

Table 4: UV-Vis spectral data for ligands, metal salt, and their corresponding precursor and new mixed ligand Fe (III) complexes.

Compounds	*Solvents*	*λ_{max} (nm)*	*Assignments*
1,10-Phenanthroline	Methanol	227	$\pi \rightarrow \pi^*$ (C=C)
		261	$n \rightarrow \pi^*$ (C=N)
Ethylenediamine	Water	-	-
$FeCl_3$	Water	267	LMCT
$[Fe(phen)_2(H_2O)_2]Cl_3$	Water	224	$\pi \rightarrow \pi^*$ (C=C)
		264	$n \rightarrow \pi^*$ (C=N)
		371	LMCT
		443,458,468	$^2T_{2g}\rightarrow{}^2A_{2g}$, $^2T_{2g}\rightarrow{}^2T_{1g}{}^2T_{2g}\rightarrow{}^2E_g$
$[Fe(Phen)_2(en)(H_2O)]Cl_3$	Water	227	$\pi \rightarrow \pi^*$ (C=C)
		264	$n \rightarrow \pi^*$ (C=N)
		354	LMCT
		502, 507,512	$^2T_{2g}\rightarrow{}^2A_{2g}$, $^2T_{2g}\rightarrow{}^2T_{1g}{}^2T_{2g}\rightarrow{}^2E_g$
$[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$	Water	227	$\pi \rightarrow \pi^*$ (C=C)
		264	$n \rightarrow \pi^*$ (C=N)
		348	LMCT
		562, 566, 576	$^2T_{2g}\rightarrow{}^2A_{2g}$, $^2T_{2g}\rightarrow{}^2T_{1g}{}^2T_{2g}\rightarrow{}^2E_g$

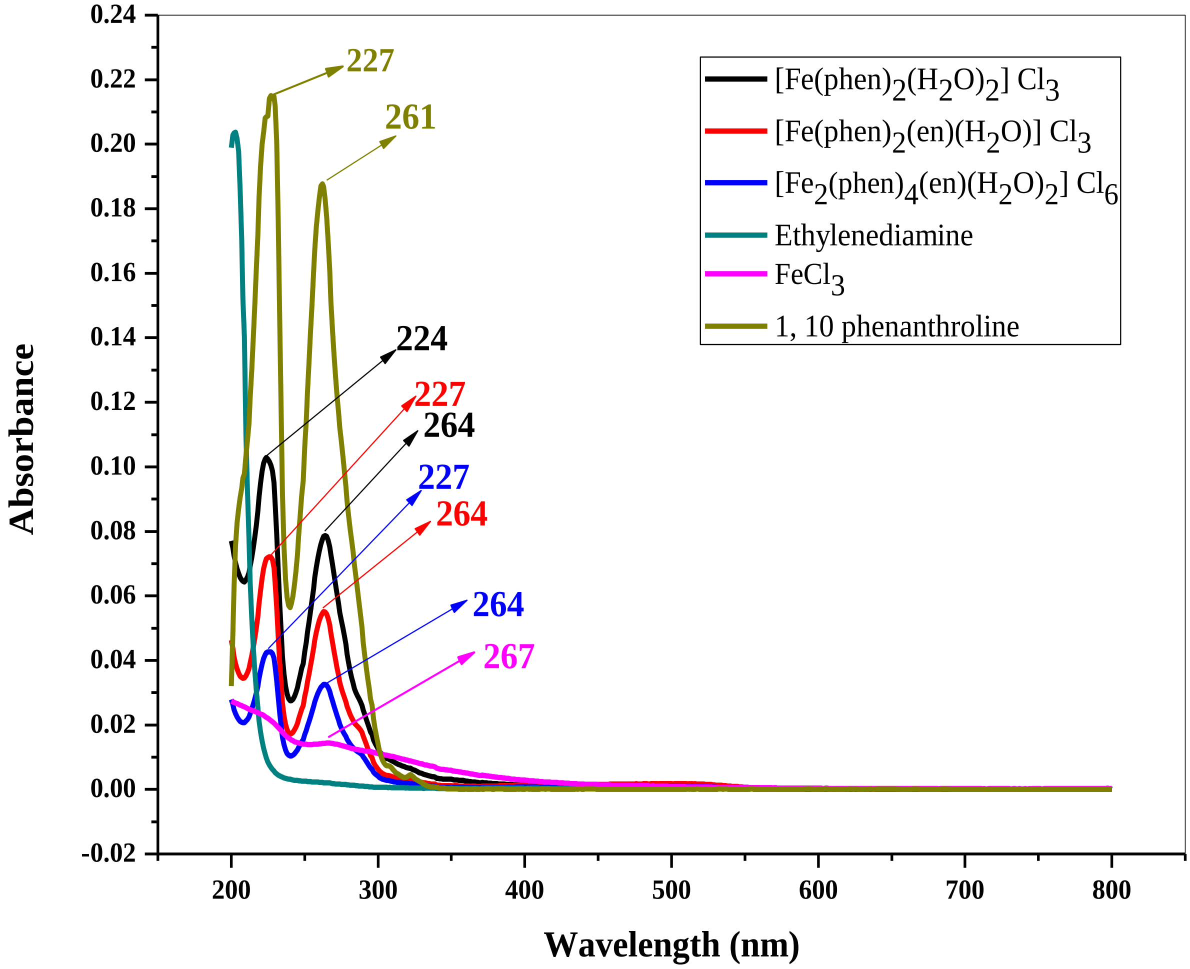


Figure 4: UV-Vis spectra of Ethylenediamine, $FeCl_3$, 1,10-phenanthroline and their Fe (III) Complexes

4.4. Structural Investigation

The metal content measurement in the ICP-OES data showed the formation of mononuclear and Binuclear Fe (III) complexes. The qualitative and quantitative chloride analysis is an affirmation for the presence of outer-sphere chloride. Based on IR spectral data; The observed shifts in band position in the complexes are confirmatory evidence for the successful coordination of ligands to metal center to form their corresponding metal complexes. Furthermore, water molecules are incorporated within the complex indicates coordinated water to metal through O atoms. The UV-Visible spectra showed that the electronic redistribution up on ligand coordination particularly showed the bands attributed to the coordination of 1, 10-phenanthroline from metal salt, and the change in the appearance of d-d transition bands which further support formation of the complex.

Hence based on the aforementioned analytical and spectral data the intended complex with the proposed binding site of the ligand 1, 10- phenanthroline, and Ethylenediamine through N donor and water O donor atoms in metal binding has been successfully achieved.

5.5. Antimicrobial Testing

4.6. Antibacterial Activity

Table 5: Antibacterial activity of the ligand, the metal salt, their complexes and Gentamicin

Compounds	**Antimicrobial activity mean zone of inhibition diameter (mm)**			
	Gram-positive bacteria		**Gram-negative bacteria**	
	S. aureus	**S. pyogenes**	**E. coli**	**K. pneumonia**
Methanol	0 ±0.00	0±0.00	0±0.00	0±.0.00
Ethylenediamine	27.16±1.18	21.83±6.83	23.66±1.41	18.83±2.12
1, 10 phenanthroline	37.49±4.69	41.3±0.00	38.33±0.00	35.99±4.71
$FeCl_3$	22.83±1.17	22.33±2.36	11.99±0.74	11.48±1.18
$[Fe(phen)_2(H_2O)_2]Cl_3$	31.0±0.00	30.99±1.88	27.66±3.44	26.66±10.83
$[Fe(phen)_2(en)(H_2O)]Cl_3$,	33.83±0.70	36.66±2.35	29.66±0.94	34.16±1.17
$[Fe_2(phen)_4(en)(H2O)_2]Cl_6$	35.16±2.71	33.5±5.00	28.83±2.58	35.49±0.23
Gentamycin	29.50±1.57	38.66±2.82	29.49±0.23	29.66±0.00

The metal complexes have higher antimicrobial activity than ligands and metal salts in all bacterial strains except Phenanthroline but the solvent does not have antibacterial activity. The metal salt has greater and better antibacterial activity in *S.aurous,* and *S.pyogenes* than *E.coli,* and *k.pneumonia*. This may happen due to the absence of liposolubility behavior of the metal salt which cannot penetrate the lipid membrane of Gram-negative bacteria. However, upon coordination, the antibacterial activity of metal complexes become enhanced and could be explained based on Overtone’s concept and Tweed’s chelation theory [5, 63, 64]. According to Overtone's concept and Tweed's chelation theory, the outer membrane which composed from lipopolysaccharides allows only lipid-soluble antibacterial drugs to pass through it, which is an important condition for antimicrobial activity. Upon coordination, the liposolubility of the metal ion was increased to a greater extent due to the overlap of the ligand orbitals with metals and partial sharing of the positive charge of the metal ion to the donor groups and also the liposolubility behavior of the ligand is slightly modified. Furthermore, it increases the delocalization of π electrons over the whole chelate ring and hence enhances the liposolubility of the complexes. This increased liposolubility of metal salts also enhances the penetration into the lipid membrane and interferes with the normal activities of the bacteria. [65, 66] The synthesized metal complexes have higher antibacterial activity against all bacterial strains [61-62, 67] and comparable antibacterial activity with the standard Gentamicin, particularly in *E.coli.* When Compared the two newly synthesized mixed ligand complexes, the new binuclear mixed ligand complex showed the greatest activity in *S. aureus,* and *K. pneumonia.*

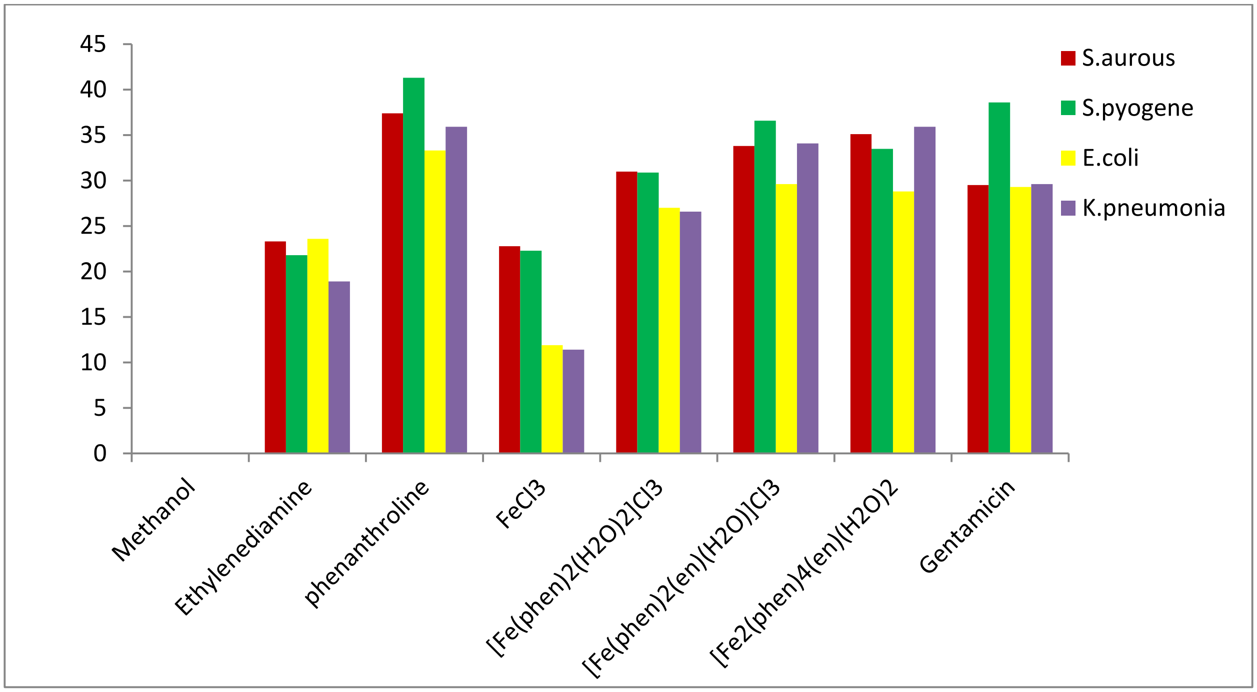
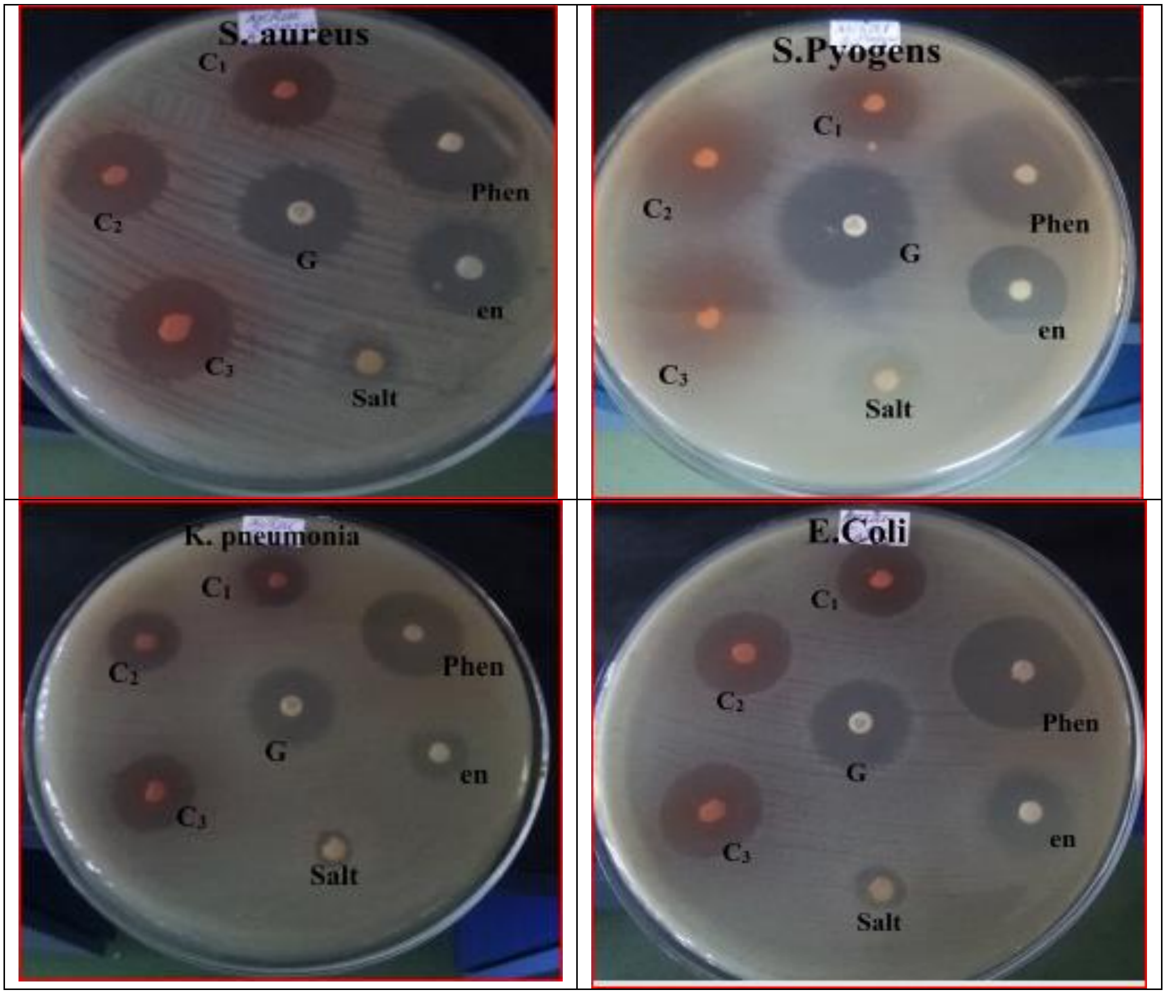


Figure 5: The graph showed the comparisons for the antibacterial activity of the metal salt, ligands, the synthesized complexes and the solvent regarding antibiotic drug(gentamyin)



Key:C_1=[Fe(phen)$_2$(H_2O)$_2$]Cl_3,C_2=[Fe(phen)$_2$(en)(H_2O)]Cl_3,C_3=[Fe_2(Phen)$_4$(en)(H_2O)$_2$] Cl_6 , Salt=$FeCl_3$, en=Ethylenediamine, Phen= 1, 10-Penanthroline and G = Gentamycin

Figure 6: The photographic images for the antibacterial activity of ligands, Salts, and complexes

4.7. Comparison of Activity of Fe (III) complexes and Gentamyin

The antibacterial activities (%) index data for the synthesized metal complexes against the tested bacteria compared to the standard drug Gentamicin was shown in **Table** -: below.

Table 6: The % activity indices of the Fe (III) complexes compared to gentamycin

Complexes	%activity index of Fe(III) complexes			
	S.aurous	*S.pyogen*	*E.coli*	*K.pneumonia*
$[Fe(Phen)_2(H_2O)_2]Cl_3$	5.08	-19.95	-7.85	-10.13
$[Fe(Phen)_2(en)(H_2O)]Cl_3$	14.58	-5.18	1.02	15.20
$[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$	18.98	-13.21	-1.7	21.28

The percent activity indexes of the new metal complexes $[Fe(Phen)_2(en)(H_2O)]Cl_3$ and $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ showed higher antibacterial activity against *Staphylococcus aureus*, and *K.pneumonia* and $[Fe(Phen)_2(en)(H_2O)]Cl_3$is more potent antibacterial agent against all bacterial strains except *Streptococcus pyogenes.* Furthermore, Interestingly the binuclear complex $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ revealed significantly high antibacterial activity than corresponding mononuclear complex and commercial drug Gentamicin against Gram negative bacteria, *K.pneumonia*.

4.8. Minimum Inhibitory Concentration(MIC) Assay of $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$

MIC is the lowest concentration of antimicrobial agent required to inhibit the growth of the Microorganisms in 24 hours. The Minimum Inhibitory Concentration informs to us the degree of resistance and important information about the resistance mechanism performed by series of dilution method for the newly synthesized metal complex, $[Fe_2(phen)_4(en)(H_2O)_2]Cl_6$ at 100 ppm, 150 ppm 250 ppm,300 ppm, 500 ppm and 700 ppm. The antibacterial activity of the metal complex is active against the bacterial growth in all the concentration range and bacterial strain. The minimum concentration sufficient to inhibit the growth of bacteria is100 ppm for all bacterial strains and the inhibition directly related to the concentration of the complex [33].

5-CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In this work, the intended complexes $[Fe(Phen)_2(en)(H_2O)]Cl_3$ and $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ were synthesized and characterized by various physico-chemical and spectroscopic techniques. The observed changes in color, melting/decomposition temperature, conductivity value, solubility data, and chloride test of the complexes compared to the corresponding ligands and metal salt confirms the coordination of ligands to metal. The UV-Vis together with other spectroscopic data's suggested octahedral geometry for both synthesized complexes. The antibacterial activity of the synthesized complex $[Fe(Phen)_2(en)(H_2O)]Cl_3$ and $[Fe_2(Phen)_4(en)(H_2O)_2]Cl_6$ have been investigated as compared to the standard antibiotic drug Gentamycin. The result revealed that metal complexes are effective against all the tested bacterial species and higher in activity than the iron (III) complexes synthesized before, *in Bahir Dar University*. This may be due to a slight change in composition leads new behavior and activity of metal complexes. Therefore, the synthesized complexes become potential alternative antibacterial drugs after the *in-vivo* cytotoxicity investigation.

5.2. Recommendation

Based on the physical and spectroscopic data obtained the octahedral geometry of newly mixed ligand complexes has been proposed. The structure of the synthesized metal complexes and the coordination mode of ligands to metals need further investigation by using advanced spectroscopic techniques such as X-ray crystallography, mass spectroscopy, and TGA. In this study, to elucidate the structure of the synthesized complex melting point, solubility test, conductivity measurement, and spectroscopic techniques such as UV-Visible, ICP-OES and IR have been used.

The antibacterial test of iron (III) complexes showed powerful activity against all the tested bacterial strains. The antibacterial activity of newly synthesized iron complexes to other strains of bacteria and the *in-vivo* activity of the complex needs further Investigation for application of it as potential antimicrobial agents.

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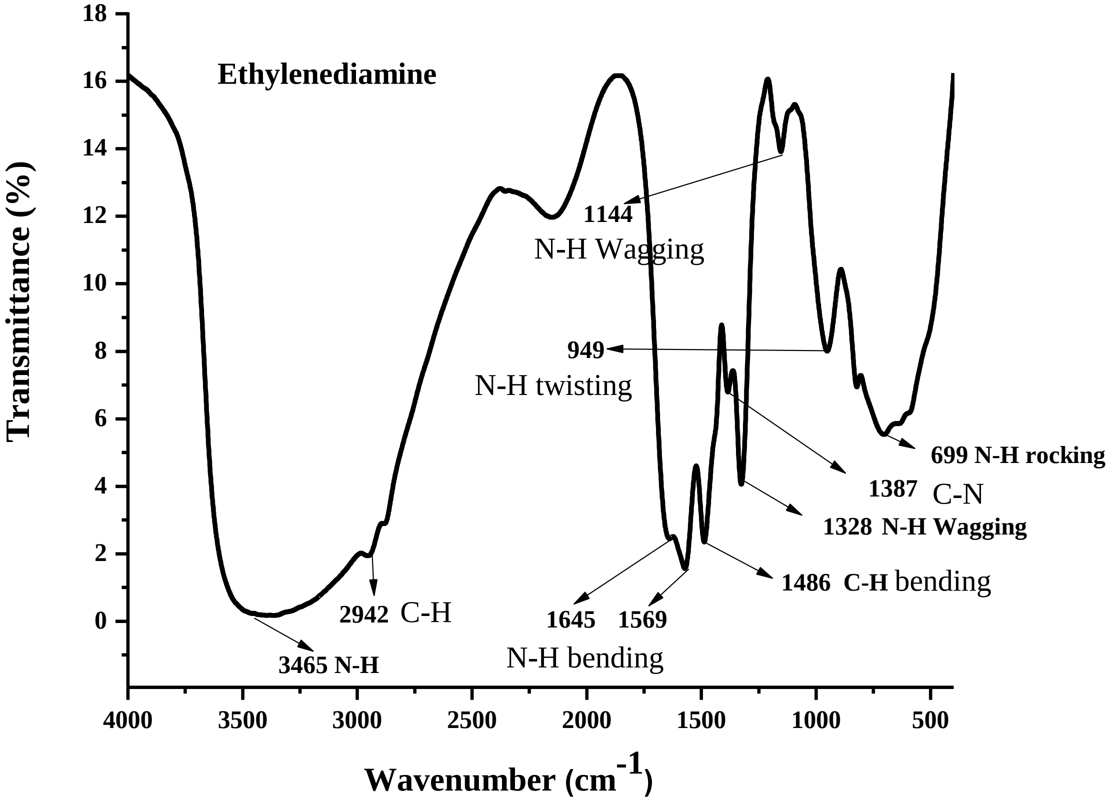
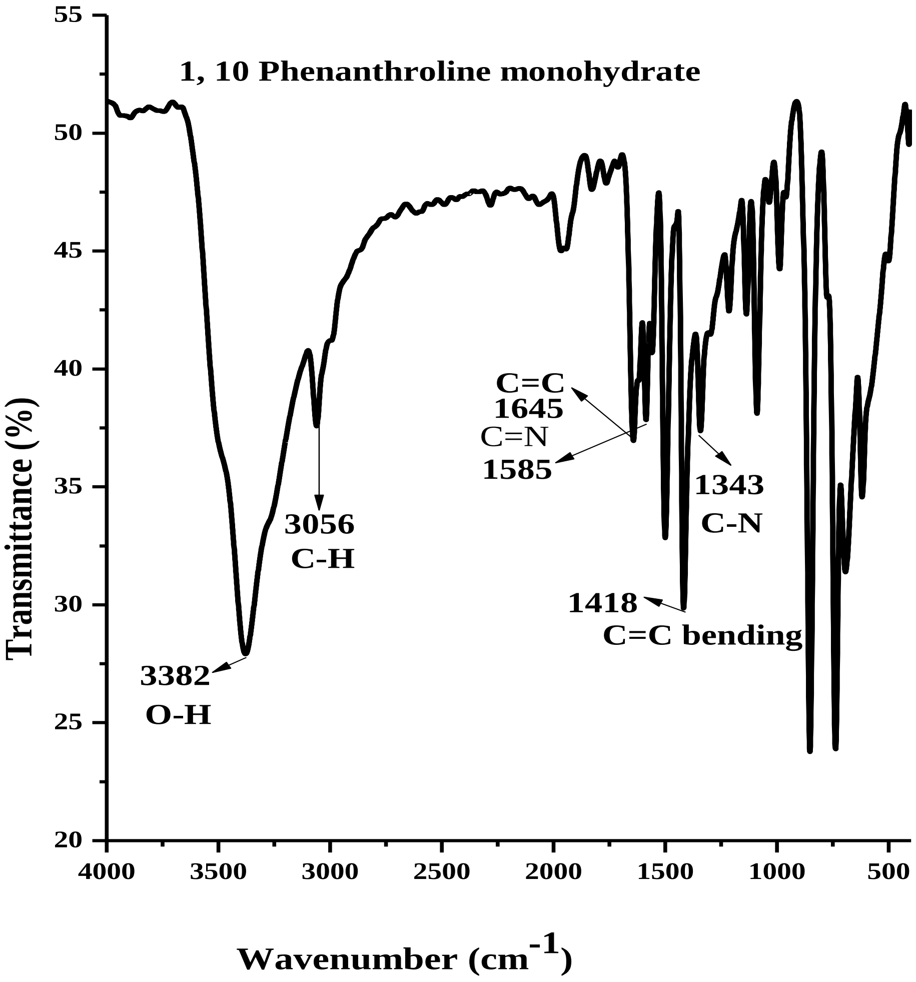
APPENDIXES

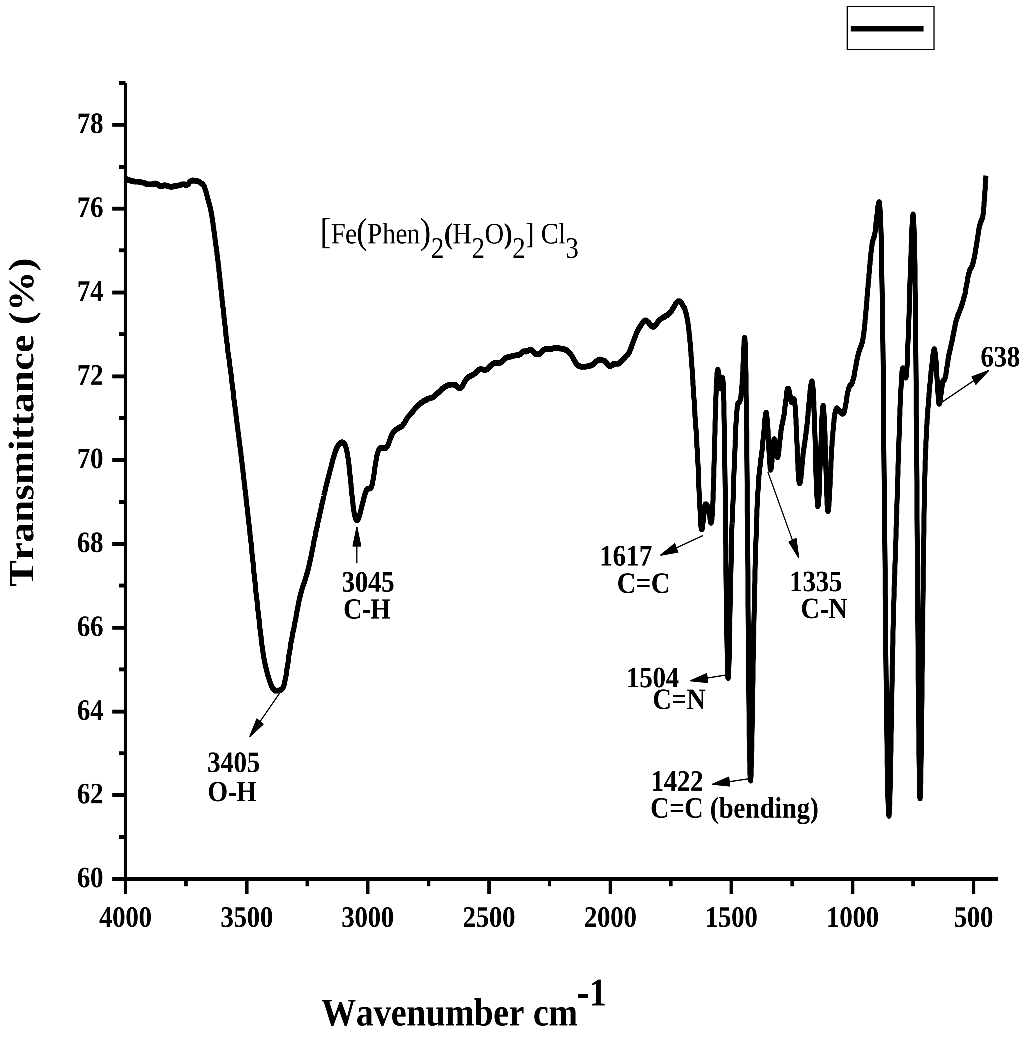
Appendix 1: IR Spectra of Ethylenediamine ligand



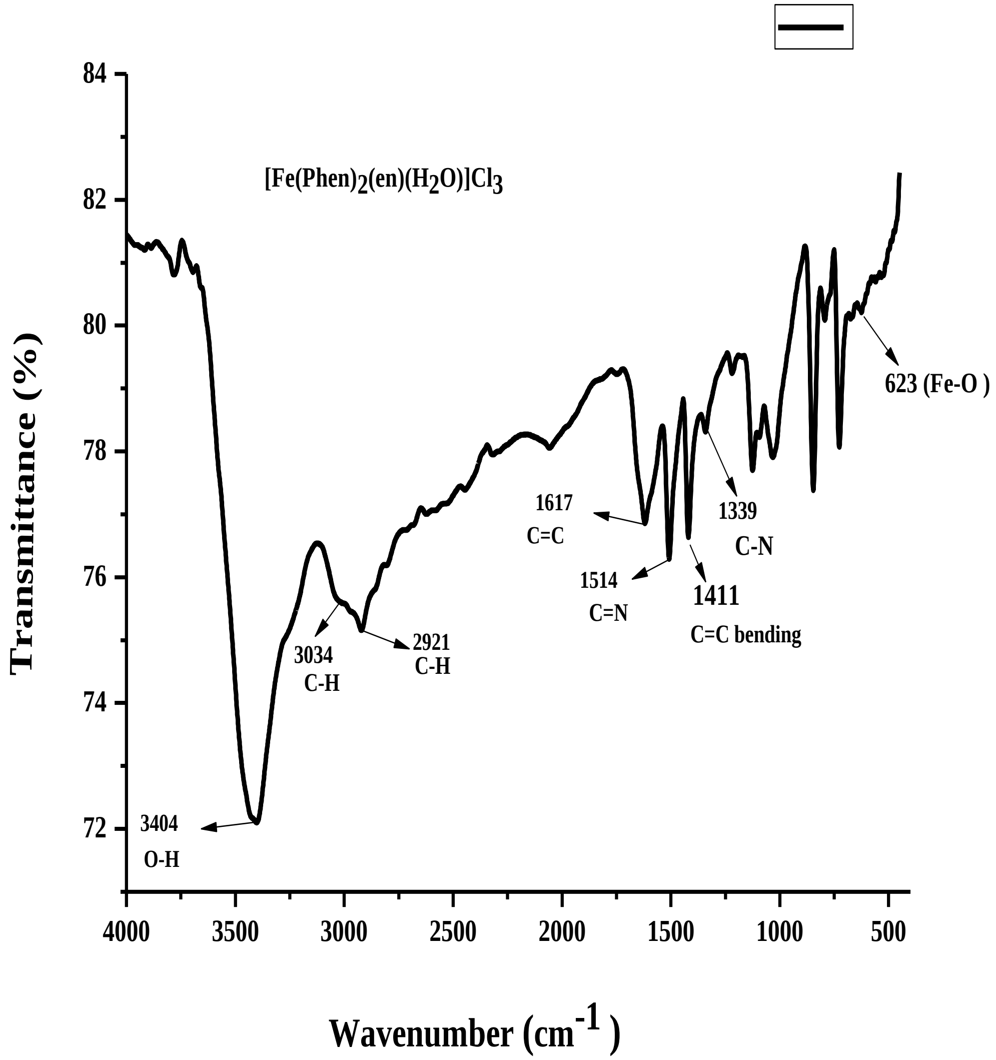
Appendix 2: IR spectra of 1, 10- Phenanthroline ligand



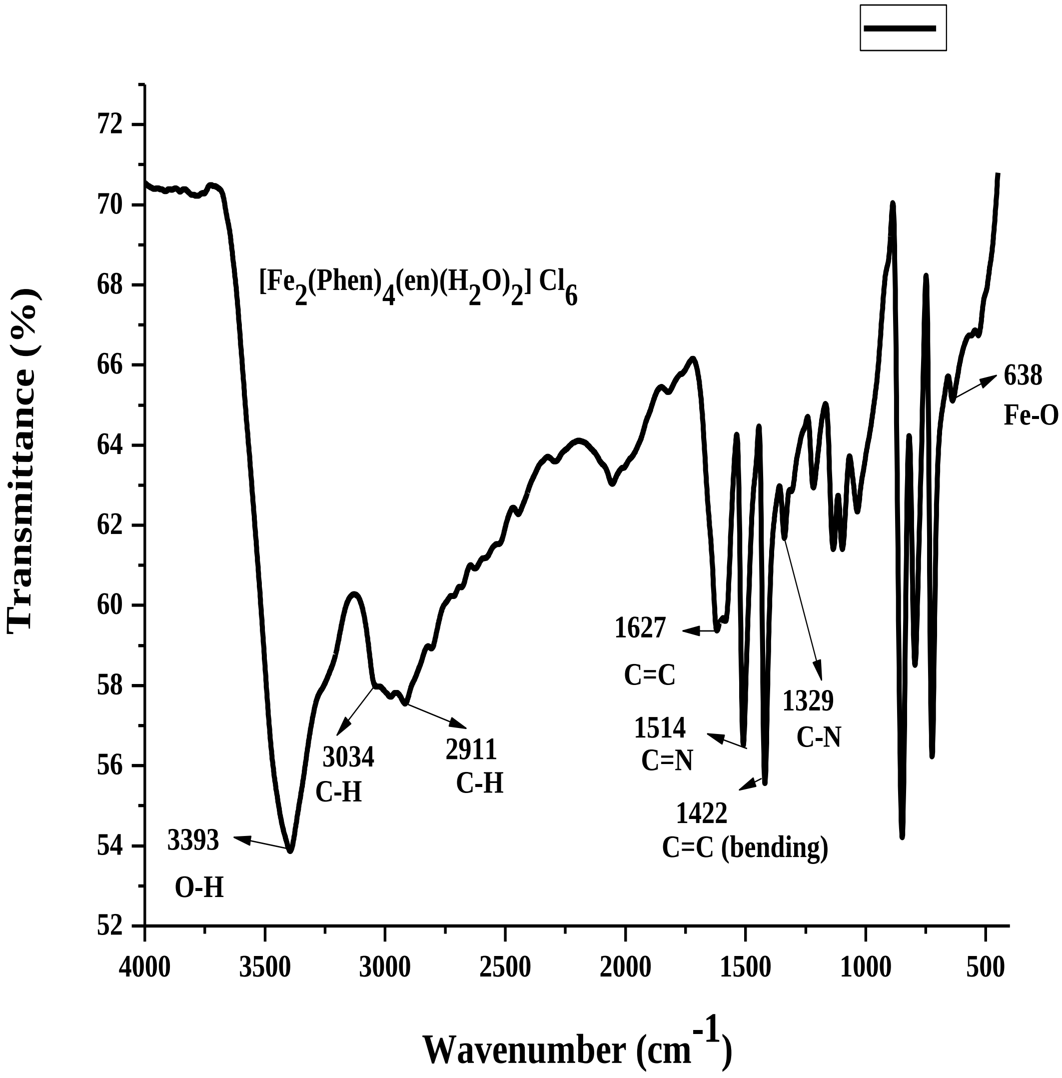
Appendix 3: IR Spectra of [Fe (phen)$_2$(H_2O)$_2$] Cl_3 Complex



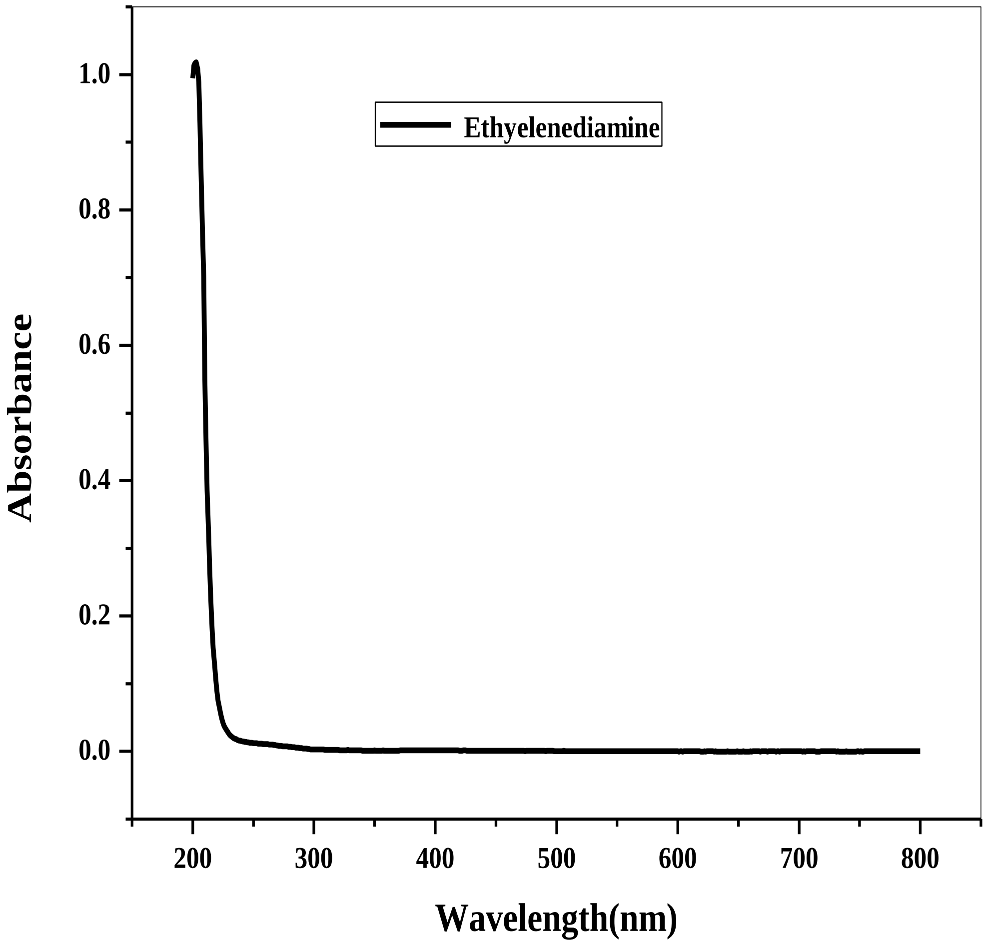
Appendix 4: IR Spectra of [Fe (phen)$_2$(en)(H$_2$O)$_2$] Cl$_3$ Complex.



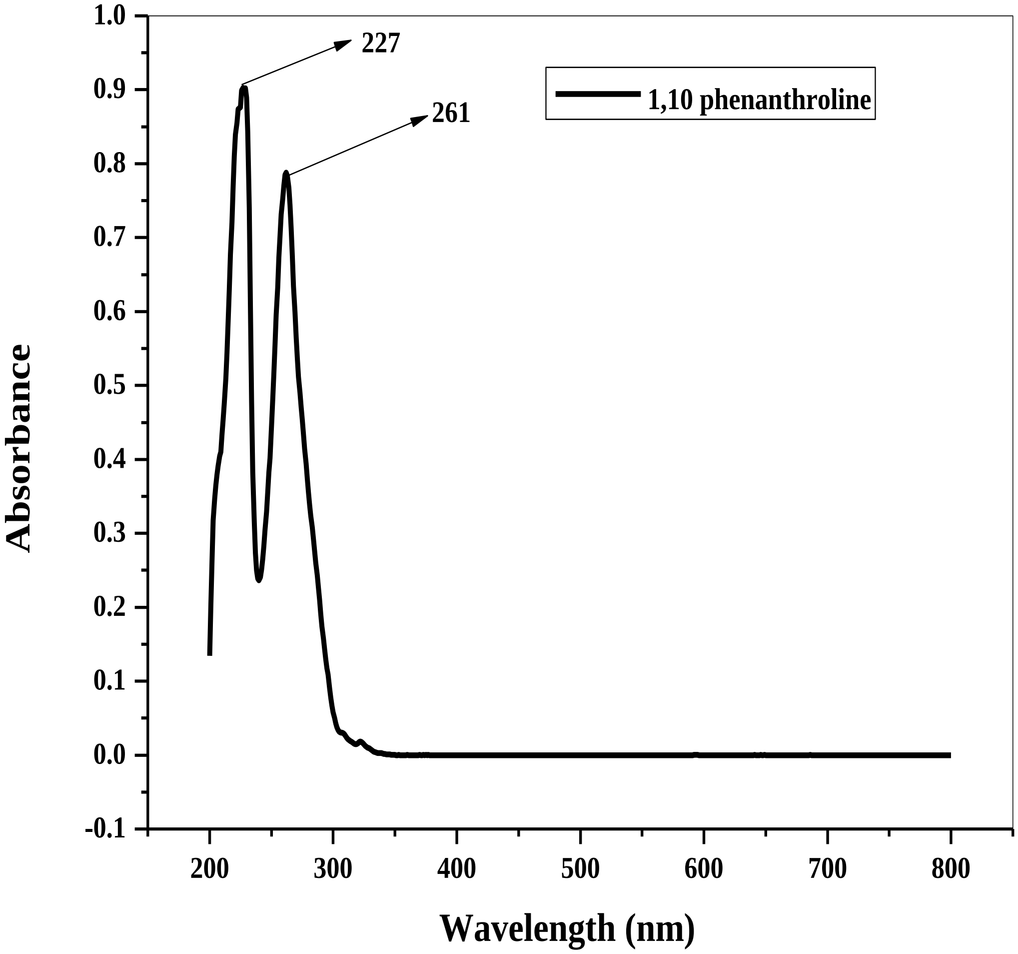
Appendix 5: IR Spectra of [Fe_2(phen)$_4$(en)(H_2O)$_2$] Cl_3 Complex.



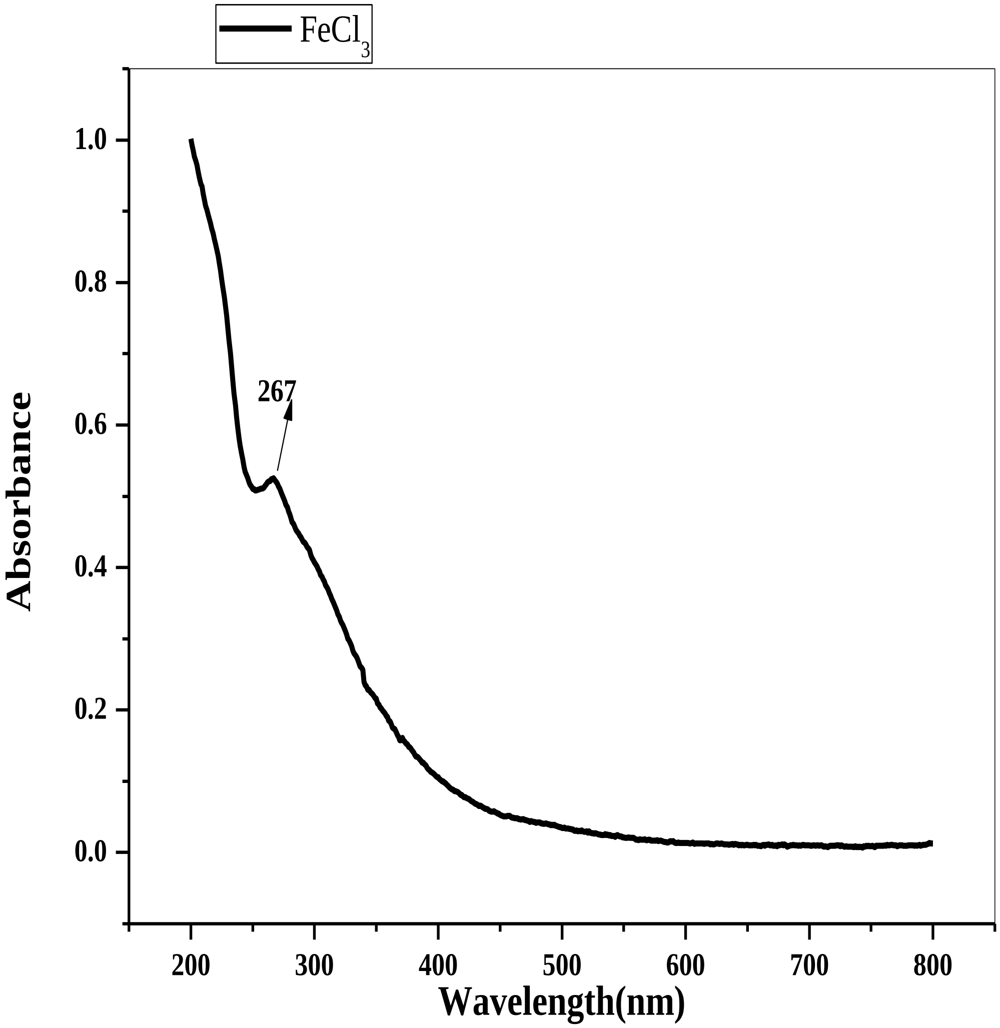
Appendix 6: Uv-Vis Spectra of Ethylenediamine ligand



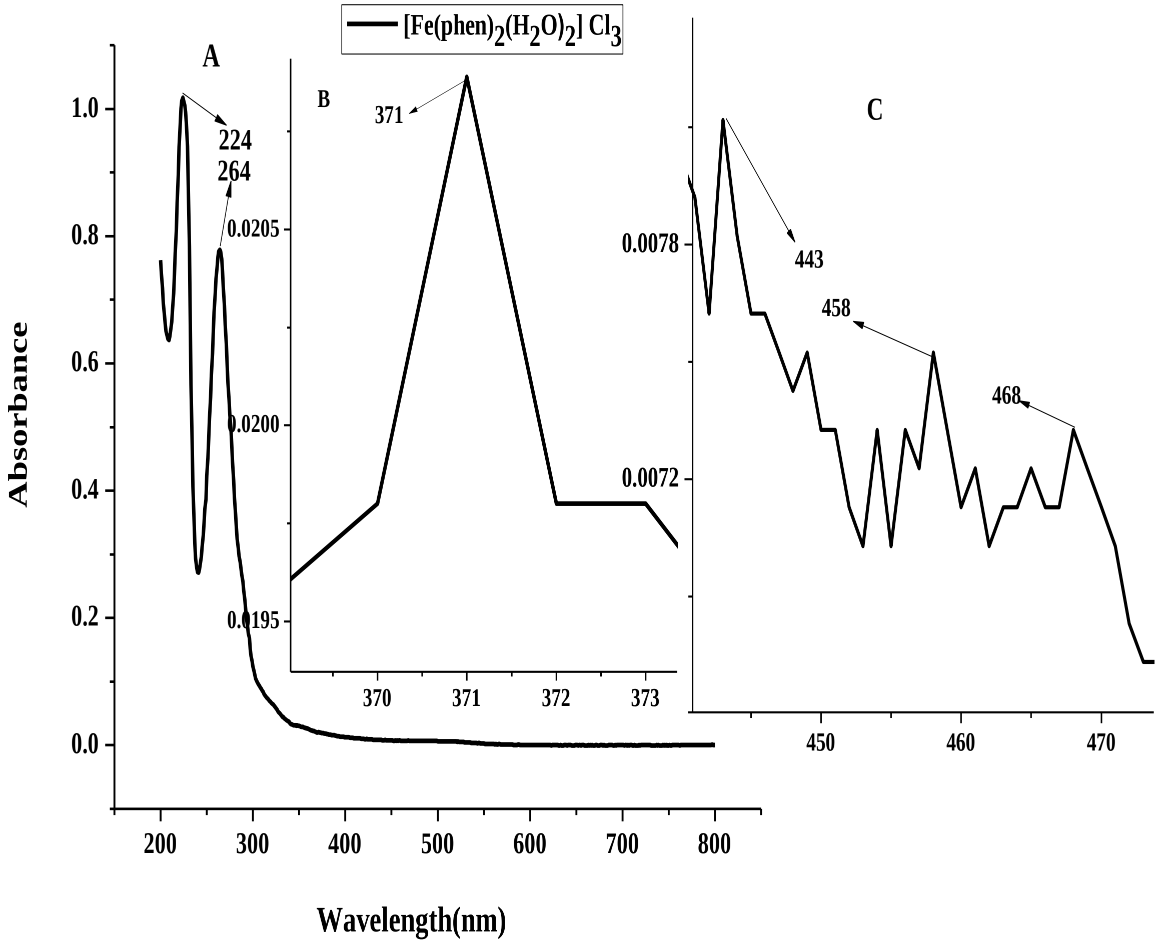
Appendix 7: UV-Vis Spectra of phenanthroline ligand



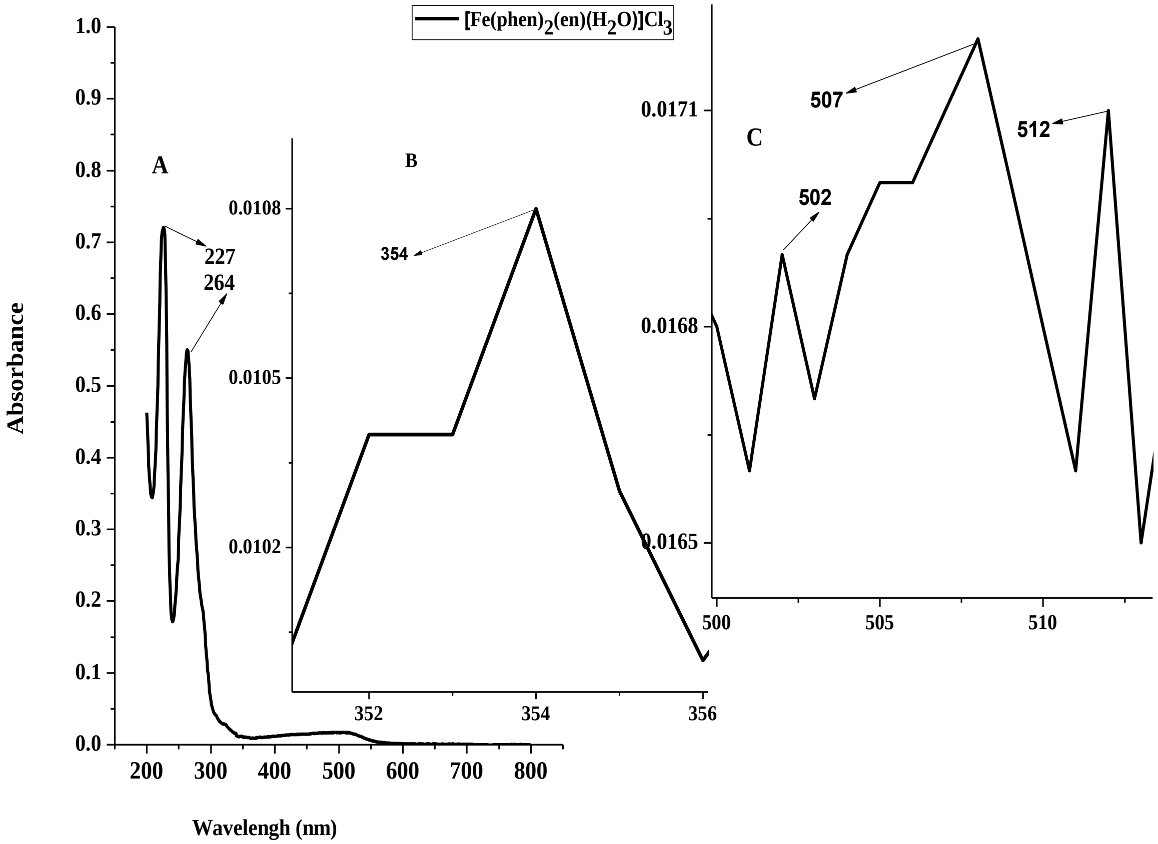
Appendix 8: Uv-Vis Spectra of Ferric Salt



Appendix 9: Uv-Vis Spectra of $[Fe(phen)_2(H_2O)_2]\ Cl_3$ Complex A) Normal B) Expanded for charge transfer band and C) expanded for the d-d transition band.



Appendix 10: Uv-Vis Spectra of $[Fe(phen)_2(en)(H_2O)_2]$ Cl_3 Complex. A) Normal B) Expanded for charge transfer band and C) expanded for the d-d transition band



Appendix 11 : Uv-Vis Spectra of $[Fe_2 (phen)_4(en)(H_2O)_2] Cl_6$ Complex A) Normal B) Expanded for charge transfer band and C) expanded for the d-d transition band