

Synthesis, Characterization and Biological Activity of Iron(II) Complex With 1, 10 –Phenanthroline And L-Threonine as Ligands

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Abstract- Due to diverse structural features of iron (II) complexes, resulting from various coordination modes of the ligands and varied coordination geometries of the metal centers [iron (II) chloride]. Hence this research therefore seeks on ways to defeat drug resistance by intercalating metal ion into the vicinity of the drugs. This metal complex has been found to resolve the issue of some radical detoxification and management of disorder caused by impaired iron homeostasis.

I. INTRODUCTION

Metal complex have recorded their existence in several biological system as well as in medical field from the evolution of life. Some of those as hemoglobin (transportation of oxygen in red blood cells), chlorophyll (photosynthesis in green plant) and a few natural metalloenzymes (vitamin B₁₂) have already established their significance prominently in bio chemistry. These type of complexes are composed by association of organic moiety and a metal ion. The first metal conjugate were synthesis by Sophus Jorgensen in Denmark in the middle of 1870. In 1893, Alfred Werner discovered a series of cobalt complexes as a potential therapeutic agents, and bagged lauress in the form of Nobel prize in 1913. Further, a lot of invention occur in the field of coordination chemistry and some of those metal complexes are used as therapeutic agent significantly for diseases, e.g. syphilis was cured by using Salvarsan (Keppler, *et al.*; 1991; Sekhon, 2011).

The bio-activity of metal complex obvious depend on the nature of functional groups of a ligand, metal atoms as well as on their oxidation state. Research and chemist received much attention and interest in bioinorganic chemistry of metal complexes after the discovery of Cisplatin on different types of malignancy. Though it is remarkable efficient for treating verity of malignancy but this platinum complexes exhibited considerable draw backs in cancer therapy as neurotoxicity, nephrotoxicity and

emetogenesis, and finally are not orally available (Jung *et al.*, 2007). These limitation endorsed researchers to improve alternative approaches based on various metals along with substantial biological activities in pointed target. Among the first row of transition metals as divalent ions, copper (II) is reflecting notable properties due to Ligand Field Stabilization Energy (LFSE) favoring various plausible geometry due to Jahn- Teller distortions of d⁹ electronic configuration (Haas *et al.*, 2009). These features provoke concerned chemist to develop copper (II) and Iron (II) complexes as alternatives to platinum based drugs.

The chemistry metal complexes is more popular now than before especially in the design of more biologically active drugs. Metal ions are known to effect the active of many drugs. The efficacy of the drugs in coordination with a metal are boast in many cases. Metal ion plays a basic role in a large number of widely differing medicinal process in reliance on their concentration and either contribute towards the health of organism or course toxicity. Several complexes act as (Anti-bacterial, anti-fungal, anti-viral and anti-cancer) bioactivity and have been found to be more antimicrobial than the ligands themselves these compounds are played in the field of medicine, bioinorganic chemistry and are used as a starting material for the synthesis of new catalysis and drugs. Research journal of pharmaceutical, biological and chemical sciences (Al-Noor *et al.*, 2017)

1.1.0. Meal Complex

According to Werner (1893), a metal complex as the compound formed from a Lewis acid (electron-pair acceptor) and a Lewis base (electron-pair donor). A complex is an ion or a compound consisting of a central metal ion or atom surrounded by and datively bonded to other ions or molecules called ligands (Oxtoby *et al.*, 2002) .A ligand is an ion or molecule

containing at least one atom having a lone pair electrons which can be donated to the central cation or atom to form a dative bond. Example of ligand anions like Cl^- , CN^- , OH^- and molecules like CO , H_2O , NH_3 . The most common complexes are aqueous metal ions. A metal ion does not exist on its own in an aqueous solution. Therefore, for any metal M, $\text{M}^{n+}(\text{aq})$ means $[\text{M}(\text{H}_2\text{O})_n]^{m+}$; where n is the coordination number of the central metal ion, that is the number of ligands bonded to the central metal ion. Thus, the aqueous Iron (II) ion exists as $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$, the aqueous copper(II) ion exists as $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$ and the aqueous zinc ion exist as $[\text{Zn}(\text{H}_2\text{O})_4]^{2+}$. The strong tendency for the d -block metals to form such a large number of stable complexes is due to two reasons

- (a) The small sizes and comparatively high charges of d -block metal ions favour the acceptance of lone pair electrons from ligand.
- (b) The availabilities of vacant low-lying d -orbitals enables metal ion to accept lone pair electrons from ligands (Wong *et al.*, 2002).

Compounds that contain a coordination complex are called coordination compounds. Coordination compounds and complexes are distinct chemical species - their properties and behavior are different from the metal atom/ion and ligands from which they are composed. The coordination sphere of a coordination compound or complex consists of the central metal atom/ion plus its attached ligands. The coordination sphere is usually enclosed in brackets when written in a formula. The coordination number is the number of donor atoms bonded to the central metal atom/ion.

1.1.1 The Biological Importance of Coordination Complexes of Metal Ions

metals are known since ancient times. Egyptians is said to have used copper to sterilize water in 3000 BC, the Chinese used gold in medicine in 2500 BC and Hippocrates was using metals such as Cu, Fe, Zn, Na, K, to cure diseases. The great majority of the element of the periodic table are metals (about 80%) and most of them are involve in life processes or have been used as drugs. The coordination chemistry of alkali, alkaline earth metals and toxic cations and their complexation aspect for which stability constants and selectivity have been determined with some macrocyclic cryptates are illustrated below

The applications of the above cryptates for the elimination of the radioactive cations have been shown for cryptate, $^{85}\text{Sr}^{2+}$, $^{224}\text{Ra}^{2+}$, $^{140}\text{Ba}^{2+}$. The complexation selectivities were: $\text{Sr}^{2+}/\text{Ca}^{2+} = 4.103$, $\text{Ra}^{2+}/\text{Ca}^{2+} = 1.6.102$, $\text{Ba}^{2+}/\text{Ca}^{2+} = 1.3.105$ and $\text{Sr}^{2+}/\text{K}^+ = 4.102$, $\text{Ra}^{2+}/\text{K}^+ = 16$, $\text{Ba}^{2+}/\text{K}^+ = 1.3.104$. All in favor of the radionuclide. The experiments were performed in vivo (on rats) and the excretion of radioactive cations in faeces and urine were greatly enhanced in the presence of the cryptates. As detoxification agents of heavy metals were also used several cryptates which formed strong binding with the toxic heavy metals (Cd^{2+} , Hg^{2+} , Pb^{2+}) and weak binding with biologically essential cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+}). For Ca^{2+} the cryptates 1-3,6-8 were used successfully (selectivities $\text{Hg}^{2+}/\text{Ca}^{2+}$ from 1013 to 1025, $\text{Hg}^{2+}/\text{Zn}^{2+}$ from 10^{11} to 10^{19} and for Pb^{2+} the cryptates 2,3,6-10 were found to be the best (selectivities $\text{Pb}^{2+}/\text{Ca}^{2+}$ from 10^6 to 10^{14} , $\text{Pb}^{2+}/\text{Zn}^{2+}$ from 10^5 to 10^{10}). Extraction and transport of cations were also possible with these cryptates and they have found a large variety of applications in analytical chemistry.

The biological effects of occupational and environmental exposure to chromium have been reported recently. In a report the occupational exposure to Chromium compounds in industry of production of the metal and applications of the metals, such as chrome plating chromium chemicals and pigments and in various other industries, such as in leather tanning, production of Cr catalysts, paints textiles, corrosion inhibitors and others has been studied. In all these cases Cr exists in the bivalent and hexavalent state. Chromium is a human carcinogen. Lung cancers have been reported in chromate-exposed workers in the production of alizarin dyes in 1911 and 1935 (Kessissoglou, 1995).

Lack of body iron is common in cancer patients and it is associated with complications in surgery and in animal experiments. The transport of iron and other metal ions by the blood plasma is achieved through the formation of protein complexes. Several of most important complexes are based on porphine, a compound of carbon, nitrogen and hydrogen (Oxtoby *et al.*, 2002). Copper is recognized as an essential metallo-element and is primarily associated with copper-dependent cellular enzymes. Metals are also used as inorganic drugs for many diseases. The cisplatin ($\text{cis-Pt}(\text{NH}_3)_2\text{Cl}_2$) is the first member of a new class of potent antitumor drugs belonging to

metal coordination complexes which are being introduced in Medicine. Another valuable property is the selective binding of certain metallic species.2009).

1.1.2 Transition metal

A transition metal is one which forms one or more stable ions which have incompletely filled d orbitals. The partial filled *d* electron shells of the transition metal elements causes ranges of physical properties and chemical reactions different from those of the main group elements. The presence of unpaired electrons in the transition metal elements and their compounds, the availability of low-lying unoccupied orbitals and the facility with which transition metal oxidation states change all give rise to a fascinating and rich chemistry. On the basis of this definition, scandium and zinc do not count as transition metals - even though they are members of the d block. The general properties of transition elements include; High melting points, Variable oxidation state, Formation of coloured ions, complex ion formation. Other properties include high boiling points, high electrical conductivity, and malleability. The transition metals also have d-orbitals which are loosely bound. The first row transition metals generally form high spin complexes. The second and third row transition metals generally form low spin complexes. Transition metals are also able to absorb light. Organometallic substances play an important role in the catalysis of reactions in organic chemistry and biochemistry (Oxtoby *et al.*, 2002).

Although they are used widely as simple substances and alloys, we typically encounter only iron, nickel, copper, silver, gold, platinum, or titanium in everyday life. However, molecular complexes, organometallic compounds, and solid- state compounds such as oxides, sulfides, and halides of transition metals are used in the most active research areas in modern inorganic chemistry. Transition elements are metallic elements that have incomplete d or f shells in the neutral or cationic states. They are called also transition metals and make up 56 of the 103 elements. These transition metals are classified into the d-orbital metals which consist of 3d elements from Sc to Cu, 4d elements from Y to Ag, and 5d elements from Hf to Au, and f-block metals, which consist of lanthanoid elements from La to Lu and actinoid elements from Ac to Lr. Although Sc and Y belong to the d-block, their properties are similar to those of lanthanoids (Saatchi *et al.*, 2005)

1.1.3 General properties of transition element

These include high melting point, variable oxidation state, formation of coloured ions, complex ion formation, high electrical conductivity and malleability. The transition metals also have partially filled d-orbitals which are loosely bound.

1.2 Biological Importance of Ferrous Chloride (FeCl₂.4H₂O)

Iron is a biologically important transition metal as it is also vital to life - it is one of the few trace elements needed for organisms to sustain life. It has three main biological roles:

- i. Transport oxygen from lungs to cells; it is used to bind to enzymes throughout the body, such as in Hemoglobin to transport oxygen throughout the human body in blood.
- ii. Energy Production Iron is used in the conversation of sugar, fats, and proteins into adenosine triphosphate, ATP.
- iii. Catalase Production Iron is involved with the production of catalase and this is important because catalase protects the body from free radical damage.

Rich sources of iron in food include; red meat, soybean, white flour products, seafood, and sunflower seeds despite its uses in biological systems, an excess amount of iron can be detrimental to the human body. First, iron can cause enzyme dysfunctions by replacing other vital minerals. All these essential minerals compete for binding sites in enzymes, and when iron replaces the competing mineral, it causes the enzyme to malfunction. Second, when iron replaces other elements in the body, it also causes inflammation. Iron attracts oxygen and when in excess, the free radical oxygen damages the surrounding body tissue. In addition, as a carrier for oxygen, iron promotes bacterial growth by feeding it oxygen, leading to chronic infections (Diabetes, Nervous System Diseases, Hypertension and Cardiac Conditions, Kidney Problems). Iron can mostly be found in the pancreas, joints, liver, and intestines

1.3 1, 10 -Phenanthroline Complexes

1, 10-phenanthroline (phen) is a classic nitrogen heterocyclic and chelating bidentate ligand for metal ions. It has played a vital role in the progress of coordination chemistry and still it continues to be of considerable interest as versatile starting material for

organic, inorganic and supramolecular chemistry. Phen has been chosen as the main ligand to make a few series of ternary metal (II) complexes in this project. Phen is a versatile starting material due to its rigidity, planarity, aromaticity, basicity and chelating capability (Bencini and Lippolis, 2010), hydrophobic, electron-poor heteroaromatic system whose nitrogen atoms are beautifully placed to act cooperatively in cation binding. After Brandt *et al.*, (1954) reviewed the metal complexes of 1,10 phenanthroline, extensive research was carried out on the coordination chemistry of phen. It played an important role in the development of coordination chemistry (Sammes and Yohioglu, 1994, Luman and Castellano, 2004). As phen could coordinate to many metal ions in the periodic table, phen-based complexes have been actively studied for their catalytic, redox, photochemical and photophysical (luminescence) properties (Scaltrito *et al.*, 2000, Armaoli, 2001; Luman and Castellano, 2004, Bossert and Daniel, 2008, Lavie-Cambot *et al.*, 2008). These structural features determine its coordination ability towards metal ions.

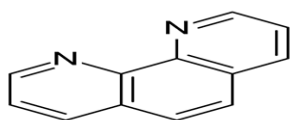


Fig: 1.1: 1,10-phenanthroline

1.3.0 Applications of 1, 10-phenanthroline-based materials

Due to its super-ability to coordinate many metal ions, o-phen and its derivatives are frequently used in many processes involving metal complexes, in which they can be featured in many roles; for example, as ligands for catalysis or as stabilizing agents for nanoparticle synthesis. Beside photodegradation (photosensitizer) properties, antibacterial and antifungal properties of phen-based were reported. A series of ternary phen-based complexes were synthesized by Shebi *et al.*, (2010) and their antibacterial and antifungal properties were investigated.

These metal complexes are $[\text{Fe}(\text{phen})(\text{H}_3\text{L})]\text{Cl}\cdot 4\text{H}_2\text{O}$, $[\text{Co}(\text{phen})(\text{H}_3\text{L})]\text{Cl}\cdot 4\text{H}_2\text{O}$. Phen has been used as important heterocyclic ligands for a large number of metal complexes that play an important role in a variety of important technological and medicinal applications; for example, promising applications in the field of

electroluminescent materials, organic light-emitting devices (OLED), organic semiconductors and therapeutic agents, due to their ability to bind or interact with the DNA biomacromolecule. Heffeter *et al.*, (2006) showed that the $[\text{tris}(1,10 \text{ phenanthroline}) \text{ lanthanum(III)}] \text{trithiocyanate}$ exerts anticancer activity via potent induction of cell cycle arrest and has promising in vivo anticancer activity against a human colon cancer xenograft.

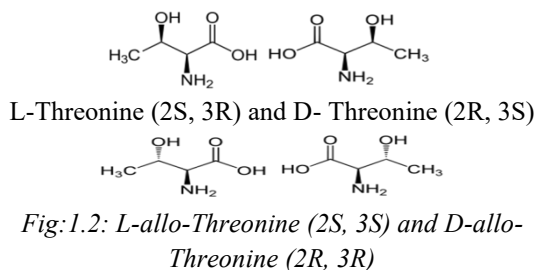
1.3.1 Chemistry of 1, 10-Phenanthrolines and derivatives

O-Phens have attracted special interest from researchers due to their various structural and chemical properties; for example, rigidity, planarity, aromaticity, basicity, and chelating capability, which makes them versatile starting materials in the area of materials science and in many fields of chemistry; for example, analytical, organic, inorganic, bioorganic, supramolecular coordination, and catalysis chemistry, as well as in the synthesis of photoactive molecules and as a versatile electron transfer reagent. O-Phens serve as versatile building blocks for the construction of linear or macrocyclic oligophenanthrolines (Chesneau *et al.*, 2007). Copper (I) complexes of oligophenanthroline ligands have been exploited for the preparation of catenanes. In the context of coordination chemistry, the generation of new molecular architectures is driven by the continued interest in researching their chemical and biological properties. The number of studies of the interactions of heterocyclic ligands with metal ions is growing. In this context, systems containing o-phen ligands as key components are emerging as luminescent sensors for the efficient detection of metal ions. Phen are useful in stabilizing low oxidation states (Ajibola, 2013).

1.4 Threonine Complexes

Threonine is one of the 20 amino acids that constitute proteins. It is a chiral alpha-amino acid bearing an alcohol group. This essential amino acid is a polar uncharged amino acid. Therefore; threonine has to be obtained from dietary sources. Threonine can exist in four possible stereoisomers with the following configuration: (2S, 3R), (2R, 3S), (2S, 3S) and (2R, 3R). The name L-threonine is mostly used for one single form chemically known as (2S, 3R)-2-amino-3-hydroxybutanoic acid and also D-threonine is used for (2R, 3S)-2-amino-3-hydroxybutanoic acid. Such pair of chiral molecules is known as Enantiomers and can be differentiated by their optical rotation. Optical

rotation is the turning of the plane of linearly polarized light about the direction of motion as light travels through certain materials (Carey, 2013). L-threonine is one of three indispensable amino acids since mammals do not possess the necessary enzymes for the transamination of threonine. Another form, called L-allo-threonine, is rarely present in nature. In plants and microorganisms, threonine is synthesized from aspartic acid via alpha-aspartyl-semialdehyde and homoserine



Enantiomers of chiral metal complexes were discovered to be able to function as structural probes of DNA (Qu *et al.*, 2001). Hence, enantiomers of chiral complexes, especially chiral amino acid complexes, have attracted considerable attention from researchers (Nakabayashi *et al.*, 2004; Zhang *et al.*, 2009; Chetana *et al.*, 2009; Rao *et al.*, 2017). Zhang *et al.* (2009) synthesized a ternary iron (II) complex, $[\text{Fe}(\text{phen})(\text{L-thr})(\text{H}_2\text{O})](\text{ClO}_4)$ and determined its crystal structure. L-threonine is an important essential amino acid. It has a range of roles in the human body. It helps to support the immune system and healthy digestion. This amino acid is also essential for tissue development and maintenance. Therefore, it plays a key role in heart and liver health. In addition, the central nervous system also requires adequate concentrations of this amino acid. This helps to support cognitive function (Azzam *et al.*, 2011).

Most people acquire healthy levels of threonine through their diet. In cases where a deficiency is identified supplements may be required. However, it is important to only take threonine supplementation under the guidance of a medical practitioner. Too much of this amino acid is dangerous. It can cause liver damage and ammonia toxicity (Hamard *et al.*, 2010). Beside anticancer property, some amino acids complexes were reported to exhibit nucleolytic property (Rao *et al.*, 2007). In 2007, two ternary copper (II) complexes were synthesized and investigated for their nucleolytic property. It acts as antitumor activity against MCF-7 cell line. Even

though quite a number of studies were done on amino acid complexes, there are very studies on DNA binding and biological properties of ternary metal (II) complexes with an enantiomeric pairs of amino acid.

1.5 Aim

The aim of this research is to synthesize and characterize the metal complexes of iron (II) with 1, 10- phenanthroline and threonine and investigate their biological activity.

1.6 Objectives

The aim would be achieved via the following objectives:

- To synthesize iron (II) chloride with 1, 10 - phenanthroline and threonine,
- To characterize metal (II) complexes in solid and solution and
- To conduct biological studies on selected Fe^{2+} complex.

1.7 Statement of the Problem

Due to the increase in resistance of microorganisms against the existing antibiotics and drugs, and that the therapy associated with most anticancer drugs is accompanied by severe side effects, such as nephrotoxicity, neurotoxicity, ototoxicity, and emetogenicity, which limit their widespread use (Bharti and Singh, 2009). These and several other limitations must be overcome in order to have an effective remediation. Thus, the quest for improving and or synthesizing new compounds of therapeutic tools for medical treatment, of high anti-microbial activity and of reduced or no toxic side-effects.

II. MATERIALS AND METHODS

All reagent and solvent used in the synthesis process were of analytical grade and were used without further purification. L-threonine (98%), iron (II) chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) and deionized water were purchased from Steve Moore chemical Co. chemical, equipment industrial, hospital equipment; No 2 Sokoto road opposite V.I.O office, Zaria. 1,10-phenanthroline, methanol and ammonia were obtained from the departmental chemical store. All apparatus used were washed and rinsed before the commencement of the lab work.

2.1 Apparatus

Measuring cylinder, filter paper, beaker, conical flask, spatula, analytical weighing balance, stirrer, thermometer and hot plate

2.2 Standardization of Ammonia Solution

Standard solution is a solution whose concentration is known. Ammonia solution was standardized to 1.0M from a stock solution. The molar concentration is first calculated from the specific gravity, molar mass and percentage purity using the following formulae.

$$\text{Molar concentration} = \frac{\text{specific gravity} \times \text{percentage purity} \times 10}{\text{Molar mass}}$$

This was followed by applying the dilution law

$$C_1V_1 = C_2V_2 \dots\dots\dots \text{eqn (1)}$$

2.3 Preparation of [Fe(Phen)(L Thr)(H₂O)Cl].2H₂

In the preparation of [Fe(phen)(L-thr)(H₂O)Cl].2H₂O, iron(II) chloride (0.34g, 1mmol) in deionized water (20mL) and L-threonine (0.24g, 1mmol) in 1.0 M NH₃ solution (40mL) were mixed and stirred continuously at room temperature for 10 minutes. This is followed by addition of a methanolic solution (10mL) of phenanthroline (0.34g, 1mmol) to the above mixture and it was observed that the colour change from brown to amber (wine). The resultant mixture was heated using heating mantle until the volume of the solution reduced approximately 20mL. The solution was then allowed to stand for period of three (3) days overnight in order for the solution to undergo sedimentation and the solid product formed on the following days collected by suction filtration and washed with cold deionized water

III. RESULTS AND DISCUSSION

Discussion of results was carried out based on the various isolates from the synthesis of metal complex.

Table1: Determination of Inhibitory Activity (Sensitivity Test) of Metal Complex on the Test Organisms.

	Diameter of zone of inhibition (mm) of complex (µg/ml) at varying concentrations.	
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Test organisms	100	50	25	12.5	Control (ciprofloxacin)
<i>Staphylococcus aureus</i>	28	25	23	18	35
<i>Bacillus subtilis</i>	19	15	10	-	32
<i>Escherichia coli</i>	17	15	13	-	37
<i>Salmonella typhi</i>	15	11	-	-	38

Key: - = No activity

The table (1) of metal complex shows activity of organisms at zone of inhibition of the complex of *Staphylococcus aureus* at 100, 50, 25 and 12.5 µg/ml except *Bacillus subtilis* and *Escherichia coli* which shows no activity of organism at 12.5 µg/ml and *Salmonella typhi* at 25 and 12.5 µg/ml.

Table2: Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the Metal Complex Against the Test Organisms.

Test organisms	Concentration of the metal complex (µg/ml)	
	M.I.C	M.B.C
<i>Staphylococcus aureus</i>	25	-
<i>Bacillus subtilis</i>	50	-
<i>Escherichia coli</i>	50	-
<i>Salmonella typhi</i>	50	-

KEY: µg/ml = microgram per milliliter

-No growth occurred i.e. zones of inhibition/susceptibility of organism to the metal complex

MIC= Growth of organisms i.e. resistance of organism to the metal complex.

In Table 2, it can be observed that the least concentration required to inhibit growth of *Staphylococcus aureus* is 25 µg/ml while for the others (*Salmonella typhi*, *Escherichia coli* and *Bacillus Subtilis*), 50 µg/ml is the threshold

concentration required to stop their growth. However no cidal effect was observed on the microbes by the complex. This infer that the complex compound is only bacteristatic but not bactericidal.

Table3: Determination of Inhibitory Activity (Sensitivity Test) Of 1, 10-Phenanthroline on the Test

	Diameter of zone of inhibition (mm) of 1,10-phenanthroline (µg/ml) at varying concentrations.				
Test organisms	10 0	50	25	12. 5	Control (ciprofloxacin)
<i>Staphylococcus aureus</i>	32	30	28	25	35
<i>Bacillus subtilis</i>	34	29	27	22	32
<i>Escherichia coli</i>	33	28	25	22	37
<i>Salmonella typhi</i>	34	30	27	25	38

The table (3) of 1,10-phenanthroline shows activity of organisms at zone of inhibition of the ligand of the tested organisms; *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* which decreases as its move from the highest value to the lowest value at varying concentrations.

Table4: Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of 1, 10-Phenanthroline against the Test Organisms.

	Concentration of 1,10-phenanthroline (µg/ml)	
Test organisms	M.I.C	M.B.C
<i>Staphylococcus aureus</i>	1.56	3.125
<i>Bacillus subtilis</i>	6.25	12.5
<i>Escherichia coli</i>	3.125	6.25
<i>Salmonella typhi</i>	1.56	3.125

From Table 4, the Phenanthroline compound is both bacteristatic and bactericidal at the respective concentrations. With the highest activity on *Bacillus subtilis* at MIC of 6.25 µg/ml and MBC of 12.5 µg/ml.

Table 5: Determination of Inhibitory Activity (Sensitivity Test) Of L-Threonine on the Test Organisms.

	Diameter of zone of inhibition (mm) of L-threonine (µg/ml) at varying concentrations.				
Test organisms	10 0	5 0	2 5	12. 5	Control (ciprofloxacin)
<i>Staphylococcus aureus</i>	-	-	-	-	35
<i>Bacillus subtilis</i>	-	-	-	-	32
<i>Escherichia coli</i>	-	-	-	-	37
<i>Salmonella typhi</i>	-	-	-	-	38

KEYS: - =No activity

MM= millimeter

µg/ml= microgram per milliliter

The table (5) of L-threonine shows no activity of organisms at zone of inhibition of the ligand of the tested organisms at varying concentration.

Table 6: Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (Mbc) Of L-Threonine Against the Test Organisms.

	Concentration of L-threonine (µg/ml)	
Test organisms	M.I.C	M.B.C
<i>Staphylococcus aureus</i>	-	-
<i>Bacillus subtilis</i>	-	-
<i>Escherichia coli</i>	-	-
<i>Salmonella typhi</i>	-	-

KEYS: - =No activity

MM= millimeter

µg/ml= microgram per milliliter

From Table 6, this shows that threonine is neither bacteriostatic nor bactericidal at the respective concentrations.

Table7: Determination of Inhibitory Activity (Sensitivity Test) Of FeCl₂ on the Test Organisms.

	Diameter of zone of inhibition (mm) of FeCl ₂ (µg/ml) at varying conc.	
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Test organisms	100	50	25	12.5	Control (ciprofloxacin)
<i>Staphylococcus aureus</i>	29	27	22	17	35
<i>Bacillus subtilis</i>	28	23	19	15	32
<i>Escherichia coli</i>	30	25	20	17	37
<i>Salmonella typhi</i>	31	28	25	19	38

The table (7) of iron (ii) chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) shows activity of organisms at zone of inhibition of the metal chloride of the tested organisms ;staphylococcus aureus, bacillus subtilis, escherichia coli and salmonella typhi which decreases as its move from the highest value to the lowest value at varying concentrations.

Table 8: Determination of Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ against the Test Organisms.

	Concentration of FeCl_2 ($\mu\text{g/ml}$) for ligand	
Test organisms	M.I.C	M.B.C
<i>Staphylococcus aureus</i>	3.125	6.25
<i>Bacillus subtilis</i>	3.125	6.25
<i>Escherichia coli</i>	3.125	6.25
<i>Salmonella typhi</i>	6.25	12.5

From Table 4, the Phenanthroline compound is both bacteristatic and bactericidal at the respective concentrations. With the highest activity on *Bacillus subtilis* at MIC of 6.25 $\mu\text{g/ml}$ and MBC of 12.5 $\mu\text{g/ml}$.

4.2 Ftir Spectroscopy Interpretation and Discussion

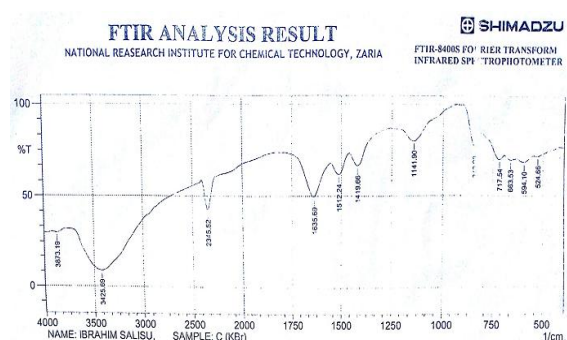


Fig 4.1 : 1,10-phenanthroline

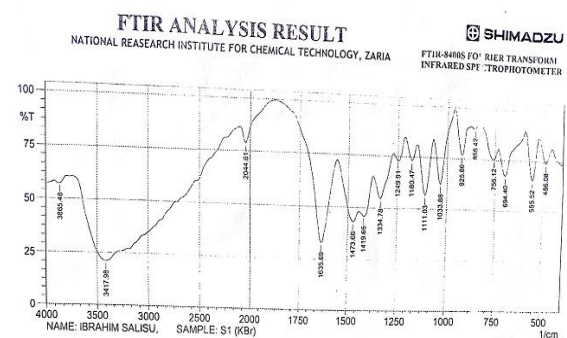


Fig 4.2: L-threonine

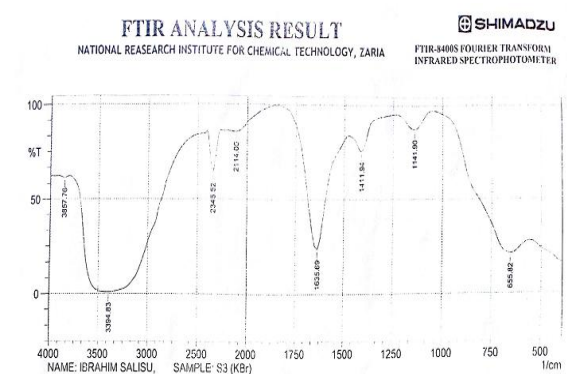


Fig 4.3: Iron (II) chloride

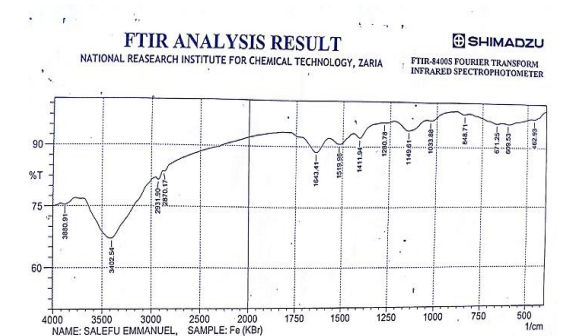


Fig 4.4: metal complex

Bands	Peaks (cm^{-1})	Remark
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N-H	3425	1,10-Phenanthroline
C=N	1636	
C=C	1512	
N-H	3418	L-Threonine
O-H	3418	
C=O	1636	
C-N	1334	
C-O	1144	
O-H	3403	Complex
N-H	3403	
C=O	1643	
C-N	1520	
C-O	1150	
O-H	3394	Iron(II) chloride
C=O	1635	
C-O	1411	
C-Cl	655	

From the table1 and fig. 4.1-4.4: The free amino acid (COO^-) is observed at frequency 1636 cm^{-1} (fig. 4.2) while the corresponding(COO^-) stretching vibration complex shifted to higher frequency 1643 cm^{-1} suggesting the terminal coordination mode of carboxylate group of threonine to the zinc ion (Yang *et al.*, 2003). The free amino acid (C-N) stretching occur at approximately 1334 cm^{-1} and this band appear to have shifted to a lower frequency 1120 cm^{-1} for its complex, suggesting coordination of the amino nitrogen atom to the Zn(II) center, FTIR analyses suggest the bidentate coordination of amino acid ligand to metal through $-\text{COO}^-$ and $-\text{NH}_2$ groups (Versiane *et al.*, 2006).

In addition, the (C=N) and (C=C) stretching frequencies of free phen ligand are observed at 1636 and 1512 cm^{-1} (fig. 4.3). In the infrared spectra of complex, the (C=N) stretching band shifted to a higher frequency of 1643 cm^{-1} . The (C=C) stretching peak is also observed at a higher frequency compared to that of the free phen ligand, at 1520 cm^{-1} in the complex. Both (C=N) and (C=C) stretching shifted to a higher frequency upon complexation (Jin and Ranford 2000; Zhang *et al.*, 2004). The literature value of (M-N), (M-O) and (M-Cl) stretching frequencies are found below 400 cm^{-1} . These peaks are beyond the detection of the FTIR spectrophotometer used (Reddy *et al.*, 2006).

IV. CONCLUSION

This study illustrates that the metal complex could be a potential antimicrobial activities worthy of further investigations. The pure isolates gotten from the various ligands and iron (II) chloride were bactericidal against the following organisms tested against; *Staphylococcus aureus*, *Bacillus Subtilis*, *Salmonella typhi* and *Escherichia coli* at different MIC and MBC. These may account for the activity recorded for its biological activities that is the determination of minimum inhibitory concentrations and minimum bactericidal concentrations of the metal complex against the test organisms, it shows that both the M.I.C and M.B.C of 1,10-phenanthroline and iron(ii)chloride has ability to inhibit growth of organisms (i.e. resistance of organism to the metal complex) and to kill the organisms (no growth occurred i.e. zones of inhibition/susceptibility of organism to the ligand) and the metal complex also has the ability to inhibit growth of organisms (i.e. resistance of organism to the metal complex) and no growth of organisms was observed; except L-threonine which possess none of the characteristics list above. These may also account for the broad spectrum activity observed for it against the test organisms.

4.1 RECOMMENDATION

Based on the findings from this research work, it is recommended that:

- Pharmaceutical industries in Nigeria should isolate and purify active component of metal (II) complex to produce antibacterial drug.
- Having evaluated the antibacterial activities of metal complex of all the isolated, tested. It is therefore recommended that the spectroscopy analysis should be conducted to explore the active component in the metal complex.
- It is also recommended that other bacterial and fungal isolates should be tested particularly on the iron (II) chloride and metal complex to determine its broad spectrum of activities.

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