SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND ANTI-INFLAMMATORY INVESTIGATIONS OF ACETAMINOPHEN COMPLEXES OF Cu(II), Mg(II) and Zn(II) IONS

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY

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JULY, 2018

DECLARATION

I declare that the work in this dissertation entitled**"Synthesis, Characterisation, Antimicrobial and Anti-inflammatory Investigations of Acetaminophen Complexes of Zn(II), Mg(II) and Cu(II) Ions"** has been carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled, **"Synthesis, Characterization, Antimicrobial and Anti-inflammatory Investigations of Acetaminophen Complexes of Cu(II), Mg(II) and Zn(II) Ions"** by **Akwu John FARUNA** meets the regulations governing the award of the Degree of Masters of Science in Analytical Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to God Almighty my creator, my source of inspiration. He has been the source of my strength throughout this programme.

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ABSTRACT

Copper, magnesium and zinc complexes of acetaminophen were synthesized and characterized by infrared spectroscopy, UV- Visible spectroscopy, melting point, X-Ray diffraction analysis and conductivity measurements. On the basis of this study, it is proven that acetaminophen acts as a bidentate ligand coordinated to the metal ions through phenol and carbonyl oxygen atoms. Antibacterial and antifungal activities of the complexes were determined against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Candida albicans*. The results indicate that acetaminophen showed activity against *Pseudomonas aeruginosa* (18 mm and 15 mm) and *Staphylococcus aureus* (20 mm, 17 mm, 14 mm and 10 mm) respectively. *Z*inc complex of acetaminophen also showed activity against *Pseudomonas aeruginosa* (20 mm, 15 mm and 12 mm). Magnesium complex of acetaminophen showed activity against *Staphylococcus aureus* (19 mm, 15 mm and 13 mm), *Salmonella typhi* (15 mm and 12 mm) and *Pseudomonas aeruginosa* (27, 18 and 13 mm). Copper complex of acetaminophen showed activity against *Staphylococcus aureus* (23 mm, 20 mm, 17 mm and 15 mm), *Bacillus subtilis* (21 mm, 18 mm, 16 mm and 14 mm), *Escherichia coli* (22 mm, 19 mm, 17 mm and 14 mm), *Salmonella typhi* (18 mm, 16 mm, 14 mm and10 mm) and *Pseudomonas aeruginosa* (24 mm, 21 mm, 19 mm and 16 mm). Acetaminophen, magnesium and zinc complexes of acetaminophen showed the same inhibitory activity (50 mg/mL) against *Pseudomonas aeruginosa.* Zinc and magnesium complexes showed the same inhibitory strength (50 mg/mL) against *Staphylococcus aureus*. Copper complex of acetaminophen was found to be the best as it exhibits the lowest minimum inhibitory concentration (12.5 mg/mL). The lethal dose determination (LD_{50}) of zinc, copper and magnesium complexes of acetaminophen were 289 mg/kg, 3808 mg/kg and 1265 mg/kg respectively. The complexes of copper and magnesium were considered slightly toxic while zinc

complex of acetaminophen was considered moderately toxic. Anti-inflammatory activity of the synthesized complexes has been carried out by paw edema method in rats. The maximum percentage anti-Inflammatory effects of zinc, magnesium and copper complexes of acetaminophen were 26.9%, 18.9% and 32.4% respectively.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Acetaminophen and its Properties

Acetaminophen, also known as Paracetamol, is a medication used to treat pain and fever. It is typically used for the relief of mild to moderate pain reliever (*Meremikwu, and Oyo-Ita, 2002).* It is often sold in combination with other ingredients such as cold medications and combination with opioid pain medication. Acetaminophen is also used for more severe pain such as cancer pain and pain after surgery (*Hochhauser, 2014*). The chemical name of acetaminophen is para-acetyl aminophenol, and this is where acet-amino-phen comes from. Scientists or medical professionals refer to acetaminophen by using APAP (Larson *et al*., 2005). Acetaminophen is a p-aminophenol derivative with analgesic and antipyretic activities.

Acetaminophen is the most commonly used drug ingredient in America (FDA, 2012). More than fifty years of consumers use and scientific investigation has established acetaminophen as safe and effective when used as directed at recommended doses. However, like any medicine, there is a limit to how much should be taken in one day. The maximum daily dose of acetaminophen is 4,000 milligrams (US FDA, 2012).

Acetaminophen is typically used orally, or rectally, and it is used intravenously. It can also be used in the hospital health service. For example, in the postoperative pain therapy, it has been shown that administration of intravenously paracetamol especially during the first hour of treatment is more efficient in reducing pain intensity than given orally. This is done to quickly decrease high fever or in the case when another route of administration is not possible (Pasero and Stannard, 2012).

1.2 Disease-causing Micro-organisms

Microorganisms are the causative agents (pathogens) in many infectious diseases. The organisms involved include pathogenic bacteria, causing diseases such as plague, tuberculosis and anthrax; protozoa, causing diseases such as malaria, sleeping sickness, dysentery and toxoplasmosis; and fungi, causing diseases such as ringworm, candidiasis and histoplasmosis (Eckburg *et al.,* 2006). While some microbes play an important part in our daily lives by keeping us healthy, others are nothing but bad news. These "bad-news" microbes are called disease-causing microbes and can make humans, animals and plants sick by causing infection and disease. Most microbes belong to four major groups: bacteria, viruses, protozoa or fungi. Disease-causing microbes can also be called pathogens, germs or bugs and are responsible for causing infectious diseases (Ishige *et al*., 2005).

1.3 Importance of Metal-Based Complexes

Metal-based complexes offer a rich environment to build upon a variety of distinct molecular structures that confer a wide spectrum of coordination numbers and geometries, as well as kinetic properties, that cannot be realized with conventional carbon-based compounds (Cohen, 2007). The partially filled d-orbitals in transition metals impart interesting electronic properties that can act as suitable probes in the design of anticancer agents (Hambley, 2007). The oxidation state of a metal is also an important consideration in the design of coordination compounds, given that it allows the participation in biological redox chemistry and plays an influential role in optimal dose and bioavailability of the agent administered (Thompson and Orvig, 2003). Furthermore, the ability to undergo ligand-exchanged reactions offers a myriad of opportunities for metals to interact and coordinate to biological molecules. Furthermore, when designing metal-based therapeutics, one is not restricted solely by metals selected by nature and can take advantage of the unique properties of

nonessential metals, including other 1st and 2nd row transition metals and metals that can impart additional utility not found naturally (Haas and Franz, 2009). Most notable is the design of radio-pharmaceuticals that exploit the radioactive properties of metals that are commonly used in the diagnosis of cancer and other medicinal applications (Haas and Franz, 2009).

1.4 Statement of Research Problem

Literature data contain numerous references on the synthesis, characterization, and antibacterial activities of paracetamol complexes with some metal complexes (Sadler and Zijien., 1998; Lawal and Obaleye., 2007; Adediji *et al*., 2011; Bamigboye *et al.,* 2012;Refat*et al.*, 2013; Harminder *et al*., 2013; Osowole *et al.,* 2013a; Osowole *et al.,* 2013b). Most researchers obtained paracetamol tablets from pharmaceutical stores. Paracetamol tablets contain excipients, which may affect the characterization of the metal complexes. Synthesis, characterization, antimicrobial and anti-inflammatory activity of Complexes derived from Acetaminophen were less studied. There are some analgesics (Acetaminophen) that are commonly associated with anti-inflammatory drugs but that have no anti-inflammatory effects (Ottani *et al*., 2006; Dani *et al*., 2007)

1.5 Justification

Coordination complexes in which the Centre is a metal atom are gaining increasing importance in the design of drugs when the drugs (ligand) form complexes with metals. In spite of a large number of antimicrobial and chemotherapeutic drugs available for medical use, the emergence of new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. There is need for the discovery of new compounds with antimicrobial activity, which is distinct from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant. Therefore, it was necessary to study Acetaminophen-metal complexes, because of their analgesic, antipyretic and chemotherapeutic properties. We hereby report

the synthesis and characterization of acetaminophen with Mg(II), Zn(II), and Cu(II)complexes and the determination of their antimicrobial and anti-inflammatory properties.

1.6 Aim

The aim of this research was to synthesize, characterize and to investigate the antimicrobial and anti-inflammatory properties of Mg(II), Zn(II), and Cu(II)as the central atoms using acetaminophen as the complexing agent or ligand.

1.7 Objectives

The objectives of the study were to:

I prepare of the solid complexes of Mg(II), Zn(II), and Cu(II)ions of acetaminophen;

II characterize the metal-acetaminophen complexes using UV-Vis spectrometer, Infrared Spectroscopy, magnetic susceptibility and molar conductance;

III estimate the crystallite size of the complexes using X-Ray diffractometer;

IV determine the antimicrobial activity of the solid complexes of Mg(II), Zn(II), and Cu(II) ions of acetaminophen on bacteria and fungi;

V investigate the acute toxicity of the solid complexes of Mg(II), Zn(II), and Cu(II) ions of acetaminophen;

VI investigate the anti-Inflammatory property of the solid complexes of Mg(II), Zn(II), and Cu(II) ions of acetaminophen.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Medications that Contain Acetaminophen as their Active Ingredient

Acetaminophen is the most common drug ingredient in America. There are more than 600 medicines that contain acetaminophen as an active ingredient, including prescription (Rx) and over-the-counter (OTC) medicines such as Tylenol. Acetaminophen is an external analgesic that works as a pain reliever (for mild to moderate pain) and fever reducer (U.S. FDA, 2014).

Acetaminophen is a generic name para-acetyl aminophenol and there are more than six thousand medicines that contain acetaminophen. Medicines that contain acetaminophen are over-the-counter (OTC) brands such as Excedrin, Paracetamol, Midol, Mucinex, Sinus Max, Nyquil, Robitussin, Sudafed, Triaminic, Tylenol and prescription medicines like Endocet, Percocet and Vicodin. Sometimes, acetaminophen is the only active drug ingredient in a medicine. This is a single-ingredient medicine like regular strength Tylenol and extra strength Tylenol. Acetaminophen can also be found in many products with more than one active ingredient (combination medicines), such as Vicks Nyquil Severe Cold or Percocet (*Hamilton, 2013; Raymond et al., 2017*).

2.2 Chemical Properties of Acetaminophen

Acetaminophen (Figure 1.1) consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the Para (1,4) pattern (Bertolini *et al.,* 2006). The amide group is acetamide (ethanamide). It is an extensively conjugated system, as the lone pair of electrons on the hydroxyl oxygen, the benzene pi cloud, the nitrogen lone pair of electrons, the p-orbital on the carbonyl carbon, and the lone pair of electrons on the carbonyl oxygen is all conjugated. The presences of two activating groups also make the benzene ring highly reactive toward electrophilic aromatic

substitution. The conjugation greatly reduce the basicity of the oxygen and the nitrogen, while making the hydroxyl group acidic through delocalization of charge developed on the phenoxide anion.

Figure 2.1: Acetaminophen

Paracetamol (Figure 2.1) is part of the class of drugs known as "aniline analgesics". It is the only such drug still in use today (Bertolini *et al.,* 2006). It is not considered a Nonsteroidal Anti-Inflammatory Drug (NSAID) because it does not exhibit significant anti-inflammatory activity (Viswanathan *et al.,* 2008). This is despite the evidence that paracetamol and NSAIDs have some similar pharmacological activity (Byrant *et al.,* 2007).

2.3 Maximum Daily Limit of Acetaminophen

The amount of acetaminophen in an individual product is included on the medicine label and is measured in milligrams (mg). For some populations, such as people with underlying liver disease or those who drink three or more alcoholic beverages every day, the daily limit, may be less (US FDA, 2014). Most OTC medicines for adults that contain acetaminophen have one of the following three strengths:

- 325 mg per tablet/capsule or liquid dose: “regular strength”
- 500 mg per tablet/capsule or liquid dose: “extra strength”
- 650 mg per tablet/capsule: “extended relief”

For acetaminophen-containing prescription combination medicines such as Percocet and Vicodin, the amount of acetaminophen is now limited to 325 mg per tablet/capsule. Following a request made by the U.S.

Food and Drug Administration (FDA) to manufacturers to lower the dose of acetaminophen in these medicines. Prior to this change, many prescription opioid medicines contained unit doses of acetaminophen of 500 mg or higher. Limiting the amount of acetaminophen per dosage unit of these prescription combination medicines is expected to help reduce the risk of severe liver injury from inadvertent acetaminophen overdose (US FDA, 2014). With different strengths of acetaminophen across many different types of medicine, it is critical to read and follow medicine labels to understand dosage strength and unique dosing directions for each product and to avoid taking multiple acetaminophen-containing medicines at the same time.

2.4 Health Effects of Acetaminophen

Taking more acetaminophen than directed and exceeding the maximum daily dose of 4,000 mg is considered an overdose and can lead to severe liver damage (U.S. FDA, 2012). Like many drugs, acetaminophen is metabolized in the liver, which is an essential organ that filters the blood coming from the digestive tract before passing it to the rest of the body. Most acetaminophen is broken down in the liver and converted into harmless substances that are excreted in the urine.

The percent of overall acetaminophen users who exceed the maximum labeled dose when using the medicine is very low, and in the vast majority of instances it does not result in side effects or adverse events such as liver damage. However, acetaminophen overdose is the leading cause of acute liver failure (ALF), and is responsible for nearly half of all ALF cases (Larson *et al.*, 2005). Acute liver failure is rare in the United States, with an estimated 2,000 cases per year. According to the U.S. Liver Failure Study Group (a consortium of investigators and clinical centers that consolidate data), acetaminophen-related ALF results in roughly 450 deaths each year (Fontana and Adams, 2006). It is important to note that the number of deaths is primarily due to intentional overdose of acetaminophen (suicide or attempted self-harm), while accidental or unintentional overdose is linked to an estimated 100-150 deaths each year (Fontana and Adams, 2006).

2.5 Coordination Complexes

A coordination complex consists of a central atom or ion, which is usually metallic and is called the coordination center, and a surrounding array of bound molecules or ions, that are in turn known as ligands or complexing agents (Lawrence, 2010). Many metal-containing compounds, especially those of transition metals, are coordination complexes. Coordination complexes whose center is a metal atom is called a metal complex (Lawrence, 2010).The atom within a ligand that is bonded to the central metal atom or ion is called the donor atom. In a typical complex, a metal ion is bonded to several donor atoms, which can be the same or different. A polydentate (multiple bonded) ligand is a molecule or ion that bonds to the central atom through several of the ligand's atoms; ligands with 2, 3, 4 or even 6 bonds to the central atom are common. These complexes are called chelate complexes; the formation of such complexes is called chelation, complexation, and coordination.

The number of donor atoms attached to the central atom or ion is called the coordination number. The most common coordination numbers are 2, 4, and especially 6. A hydrated ion is one kind of a complex ion (or simply a complex), a species formed between a central metal ion and one or more surrounding ligands, molecules or ions that contain at least one lone pair of electrons. If all the ligands are monodentate, then the number of donor atoms equals the number of ligands. For example, the cobalt (II) hexahydrate ion or the hexaaquacobalt(II) ion $[Co(H_2O)_6]^{2+}$ is a hydrated-complex ion that consists of six water molecules attached to a metal ion Co. The oxidation state and the coordination number reflect the number of bonds formed between the metal ion and the ligands in the complex ion. However, the coordination number of $Pt(en)_2^{2+}$ is 4 (rather than 2) since it has two bidentate ligands, which contain four donor atoms in total (Cotton *et al*., 2009).

2.6 Microorganisms

A microorganism or microbe is a microscopic organism, which may be multicellular (Madigan and Martinko., 2006) or single-celled. The study of microorganisms is called microbiology. Microorganisms are very diverse and include all bacteria, archaea and most protozoa. This group also contains some fungi, algae, and some micro-animals such as rotifers. Many macroscopic animals and plants have microscopic juvenile stages. Some microbiologists classify viruses and viroids as microorganisms, but others consider these as nonliving.

2.6.1 Bacteria

Bacteria constitute a large domain of prokaryotic microorganisms. Bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste and the deep portions of Earth's crust. The largest number exists in the gut flora, and a large number on the skin (*Rappe and Giovannoni, 2003)*. The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, though many are beneficial particularly in the gut flora. However several species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy, and bubonic plague. The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people per year, mostly in sub-Saharan Africa (*Ishige et al., 2005).*

Bacteria also live in symbiotic and parasitic relationships with plants and animals. Most bacteria have not been characterized, and only about half of the bacterial phyla have species that can be grown in the laboratory (*Fredricksonet al.*, 2004). The study of bacteria is known as

bacteriology, a branch of microbiology. Some common examples of bacteria are discussed below.

Staphylococcus aureus

Staphylococcus aureus (*S. aureus*) is a Gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative aerobe that can grow without the need for oxygen (Masalha, 2016). Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning (Tong *et al.*, 2015). Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine (Masalha, 2016). While *S. aureus* usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population, it can cause various skin and soft tissue infections (Tong *et al.*, 2015) particularly when skin or mucosal barriers have been breached.

S. aureus infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person, and contact with objects used by an infected person such as towels, sheets, clothing, or athletic equipment. Prosthetic joints put a person at particular risk of septic arthritis, staphylococcal endocarditis (infection of the heart valves), and pneumonia (Tong *et al*, 2015).

Bacillus subtilis

Bacillus subtilis (*B. subtilis*) is a Gram-positive bacterium, rod-shaped and catalase-positive. *B. subtilis* cells are about 4-10 micrometers (μm) long and 0.25–1.0 μm in diameter (Yu et al., 2013). As with other members of the genus Bacillus, it can form an endospore, to survive extreme environmental conditions of temperature and desiccation (Madigan and Martinko, 2006). *B. subtili*s is heavily flagellated, which gives it the ability to move quickly in liquids. *B. subtilis* have proven highly amenable to genetic manipulation, and has become widely adopted as a model organism for laboratory studies, especially of sporulation, which is a simplified example of cellular differentiation. In terms of popularity as a laboratory model organism, *B. subtilis* is often considered as the Gram-positive equivalent of *Escherichia coli*, an extensively studied Gram-negative bacterium (Yu *et al.,* 2013).

Bacillus subtilis is commonly found in the upper layer of the soil, and evidence exists that *B. subtilis* is a normal gut commensal in humans. A 2009 study compared the density of spores found in soil (about 106 spores per gram) to that found in human feces (about 104 spores per gram). The number of spores found in the human gut was too high to be attributed solely to consumption through food contamination (Hong *et al.,* 2009).

Escherichia coli

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2 (Bentley and Meganathan, 2001) and preventing colonization of the intestine with pathogenic bacteria. *E. coli* is expelled into the environment within fecal

matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards (Russell and Jarvin, 2001).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a prototypical "multidrug resistant (MDR) pathogen" recognised for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – especially nosocomial infections such as ventilator-associated pneumonia and various sepsis syndromes (Klockgether *et al.*, 2011). *Pseudomonas aeruginosa* is a facultative anaerobe, as it is well adapted to proliferate in conditions of partial or total oxygen depletion. This organism can achieve anaerobic growth with nitrate or nitrite as a terminal electron acceptor. When oxygen, nitrate, and nitrite are absent, it is able to ferment arginine and pyruvate by substrate-level phosphorylation *(Schobert and Jahn, 2010).* Adaptation to microaerobic or anaerobic environments is essential for certain lifestyles of *P. aeruginosa*, for example, during lung infection in cystic fibrosis and primary ciliary dyskinesia, where thick layers of lung mucus and alginate surrounding mucoid bacterial cells can limit the diffusion of oxygen. *P. aeruginosa* growth within the human body can be asymptomatic until the bacteria form a biofilm, which overwhelms the immune system. These biofilms are found in the lungs of cystic fibrosis and primary ciliary dyskinesia, and can prove fatal *(Gerard, 2016;* Leach and Kevin, 2016).

Salmonella typhi

Worldwide, typhoid fever affects roughly 17 million people annually, causing nearly 600,000 deaths *(Hendriksen et al., 2011*). The causative agent, *Salmonella enterica typhi* (referred

to as *Salmonella typhi*), is an obligate parasite that has no known natural reservoir outside of humans. The encounter of humans to *S. typhi* is made via fecal-oral route from infected individuals to healthy ones. Poor hygiene of patients shedding the organism can lead to secondary infection, as well as consumption of shellfish from polluted bodies of water. The most common source of infection, however, is drinking water tainted by urine and feces of infected individuals *(Hendriksen et al., 2011*).

2.6.2 Fungi

A fungus is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, Fungi, which is separate from the other eukaryotic life kingdoms of plants and animals (Bowman and Free, 2006).

A characteristic that places fungi in a different kingdom from plants, bacteria, and some protists is chitin in their cell walls. Similar to animals, fungi are heterotrophs; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi do not photosynthesize. Growth is their means of mobility, except for spores (a few of which are flagellated), which may travel through the air or water. Fungi are the principal decomposers in ecological systems. These and other differences place fungi in a single group of related organisms, named the *Eumycota (*Muhailand*Bruhn*,2005), an interpretation that is also strongly supported by molecular phylogenetics. This fungal group is distinct from the structurally similar myxomycetes (slime molds) and oomycetes (water molds). The discipline of biology devoted to the study of fungi is known as mycology. In the past, mycology was regarded

as a branch of botany, although it is now known fungi are genetically more closely related to animals than to plants (Muhail and *Bruhn*, 2005).

Candida albicans

Candida albicans is a dimorphic fungus that grows both as yeast and as filamentous cells. It is one of the few species of the Candida genus that cause the infection candidiasis in humans (Erdogan and Rao, 2015). *C. albicans* is responsible for 50–90% of all cases of candidiasis in humans (Martin *et al.*, 2013). Systemic fungal infections (fungemias) including those by *C. albicans* have emerged as important causes of morbidity and mortality in immune compromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation) (Martin *et al.*, 2013). *C. albicans* biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by *C. albicans* have become a cause of major health concerns. About 85-95% of vaginal infection, cases are responsible for physician office visits every year (Martin *et al.*, 2013).

Aspergillus niger

Aspergillus niger is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mould on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called "black mould") (Samson *et al*., 2001).Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins. Other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggests some true *A. niger* strains do produce Ochratoxin A (*Schusteret al.*, 2002).

2.7 Antimicrobials

Antibacterial is a type of antimicrobial drugs that kills or inhibits the growth of microorganisms (*Kellermayer, 2013)*. A limited number of antibiotics also possess anti-protozoal activity (*Kellermayer, 2013)*. Antibiotics are not effective against viruses such as the common cold or influenza, and their inappropriate use allows the emergence of resistant organisms (Brooks, 2015). Drugs, which inhibit viruses, are termed antiviral drugs or antiviral rather than antibiotics. Some sources distinguish between antibacterial and antibiotic; antibacterial are used in soaps and disinfectants, while antibiotics are used as medicine (Rollins *et al.*, 2016).

Antibiotics are used to treat or prevent bacterial infections, and sometimes, protozoan infections. Metronidazole for example is effective against a number of parasitic diseases, when an infection is suspected of being responsible for an illness but the responsible pathogen has not been identified, an empiric therapy is adopted. This involves the administration of a broad-spectrum antibiotic based on the signs and symptoms presented and are initiated pending laboratory results that can take several days (Rollins *et al.*, 2016).

When the responsible pathogenic microorganism is known or has been identified, definitive therapy can start. This will usually involve the use of a narrow-spectrum antibiotic. The choice of antibiotic given will also be based on its cost. Identification is critically important as it can reduce the cost and toxicity of the antibiotic therapy and reduce the possibility of the emergence of antimicrobial resistance (Rollins *et al.*, 2016).

2.8 Antifungals

An antifungal medication, also known as an antimycotic medication, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Such drugs are usually obtained by a doctor's prescription, but a few are available OTC (over-the-counter). Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism with fewer adverse effects to the host. Unlike bacteria, fungi and humans share a common ancestor. Thus, fungal and human cells are similar at the biological level. This makes it more difficult to discover drugs that target fungi without affecting human cells. As a consequence, many antifungal drugs cause side-effects. Some of these side-effects can be life-threatening if the drugs are not used properly (Brilhante *et al*., 2015). Apart from side effects like altered estrogen levels and liver damage, many antifungal medicines can cause allergic reactions in people (Kyriakidis *et al*., 2017).

Fungal infections commonly treated with antifungals include: Ringworm, athlete's foot, fungal nail infection and viginal thrush (Sutton *et al*., 2017). There are also more serious fungal infections that develop deep inside the body tissues, which may need to be treated in hospital, examples are: aspergillosis, which affects the lungs, and fungal meningitis, which affects the brain. Antifungal medicines are available as: **topical antifungals –** a cream, gel, ointment or spray you can apply directly to your skin, hair or nails, **oral antifungals –** a capsule, tablet or liquid medicine that you swallow, **intravenous antifungals –** an injection into a vein in your arm, usually given in hospital, and **intravaginal antifungal pessaries –** small, soft tablets you can insert into the vagina (Hope *et al*., 2017).

2.9 Inflammation and Non-steroidal Anti-inflammatory Drugs

Inflammation is a complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (*Ferrero-Miliani et al., 2007).* It is a protective response involving blood vessels, immune cells, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair.

The classical signs of inflammation are heat, pain, redness, swelling, and loss of function. Inflammation is a generic response, and therefore, it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen (Abbas and *Lichtman, 2009).* Too little inflammation could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the organism. In contrast, chronic inflammation may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). Inflammation is therefore normally closely regulated by the body (Abbas and *Lichtman, 2009*).

2.9.1 Types of Inflammation

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues (Kumar *et al*., 2004). A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is

characterized by simultaneous destruction and healing of the tissue from the inflammatory process (Hall, 2011).

2.9.2 Non-steroidal anti-inflammatory drugs

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system to block pain signaling to the brain (Ottani *et al*., 2006).Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by counteracting the cyclo-oxygenase (COX) enzyme. On its own, COX enzyme synthesizes prostaglandins, creating inflammation. In whole, the NSAIDs prevent the prostaglandins from ever being synthesized, reducing or eliminating the pain (Hinz and *Cheremina*, 2008).Some common examples of NSAIDs are aspirin, ibuprofen, and naproxen. The newer specific COX-inhibitors are not classified together with the traditional NSAIDs even though they presumably share the same mode of action.

On the other hand, there are analgesics that are commonly associated with anti-inflammatory drugs but that have no anti-inflammatory effects. An example is acetaminophen. As opposed to NSAIDs, which reduce pain and inflammation by inhibiting COX enzymes, Acetaminophen has been shown to block the reuptake of endocannabinoids (Ottani *et al*., 2006), which only reduces pain, likely explaining why it has minimal effect on inflammation. Acetaminophen is sometimes combined with an NSAID (in place of an opioid) in clinic practice to enhance the pain relief of the NSAID while still receiving the injury/disease modulating effect of NSAID-induced inflammation reduction (Dani *et al.,* 2007)

2.10 Role of Metals in the Body

Metals are integral parts of many structural and functional components in the body, and the critical role of metals in physiological and pathological processes has always been of interest to researchers. The use of metals to restore the normal healthy physiology of the body either by direct administration of essential metals, or by chelating out excess or toxic metals, or using them as carriers for targeted drug delivery, or for tagging biomolecules for diagnostics, are all techniques that may be classified under the general heading of metallo-pharmacology(Andreini *et al*., 2008).

In nature, many biological systems make extensive use of metal ions, such as zinc and copper, which play critical roles in the normal functioning of organisms (Orvig and Abrams, 1999). Transition metals such as copper, iron, and manganese, among others, are involved in multiple biological processes, from electron transfer to catalysis to structural roles, and are frequently associated with active sites of proteins and enzymes (Orvig and Abrams, 1999). However, deregulation of some of these essential metals during normal biochemical processing has been implicated in the development of various pathological disorders, such as cancer (Yaman *et al*, 2007). These cellular functions only require the “trace metals” in miniscule but tightly regulated amounts (Mjos and Orvic, 2014; Wang *et al*., 2015). By comparison, other metals such as arsenic, cadmium, chromium, and nickel are less beneficial since they produce a wide range of toxic side effects, including carcinogenesis (Thompson and Orvig, 2003).

Many metal-containing compounds have been utilized throughout history to treat a wide variety of disorders (Chen *et al*., 2009). In Medicinal Chemistry, metal complexes have gained favor as diagnostic tools, antibacterial and anticancer agents (Yan *et al*., 2005). However, the clinical use of many metal-containing compounds is restricted due to dose-dependent toxicity and resistance coupled with a narrow spectrum of activity (Criado *et al*., 2003). In addition to numerous platinum analog, other metal complexes containing metal ions such as zinc(II), copper(II), gold, and copper chelating agents have

received considerable attention as potential anticancer agents (Daniel *et al*., 2005). Additionally, the investigation of ruthenium-containing compounds in clinical trials attests to the rich potential of utilizing non-platinum metal-based compounds in the treatment of cancer (Hartinger *et al*., 2008). Furthermore, metals that take advantage of their unique physiochemical properties have been utilized as powerful tools in cancer diagnosis (Bowen and Orvig, 2008).

2.11 Properties of Metal Ions and Metal-Based Complexes

An important property of metals is that they form positively charged ions in aqueous solution that can bind to negatively charged biological molecules. Thus, the charge can be fine-tuned depending on the coordination environment involved, leading to the generation of a species that can be cationic, anionic, or neutral. Additionally, metal ions with high electron affinity can significantly polarize groups that are coordinated to them, fostering the generation of hydrolysis reactions (Haas and Franz, 2009).

In recent years, the field of medicinal inorganic chemistry has received considerable attention in the design of anticancer agents (Jakupec*et al*., 2008).Metal-containing compounds offer many advantages over conventional carbon-based compounds in the development of new medicinal compounds. These advantages are due to their ability to coordinate ligands in a three dimensional configuration, thus allowing functionalization of groups that can be tailored towards defined molecular targets (Meggers, 2009).

2.11.1 Metal Complexes as Antimicrobial Agents

Medicinal inorganic chemistry is rich in metal or metalloid-based drugs, including Paul for the treatment of syphilis, antiarthritic gold preparations and diagnostic agents for magnetic resonance imaging (Gd, Mn, Fe) among others (Higazy *et al*., 2010). The field of bioinorganic chemistry, which deals with the study of role of metal complexes in biological system, has opened a new horizon for scientific research in coordination compounds. A large number of compounds are important from the biological

point of view. Some metals are essential for biological functions and are found in enzymes and cofactors required for various processes. For example, hemoglobin in red blood cells contains an iron porphyrin complex, which is used for oxygen transport and storage in the body. Chlorophyll in green plants, which is responsible for photosynthetic process, contains a magnesium porphyrin complex. Metals such as copper, zinc, iron and manganese are incorporated into catalytic proteins (the metalloenzymes), which facilitate a multitude of chemical reactions needed for life (Katsarou *et al*., 2008). Today medicinal inorganic chemistry remains a field of great promise with many challenges. Biologically relevant metal complexes have several requirements in terms of their synthetic design. First, a biologically active metal complex should have a sufficiently high thermodynamic stability to deliver the metal to the active site. The metal-ligand binding should be hydrolytically stable. The kinetics with which the metal ion undergoes ligation or deligation reactions is of great importance. The molecular weight of the metal complex is also critical. The compounds of low molecular weight with neutral charge and some water solubility are soluble in almost any medium and may slip through biological membranes by passive diffusion (Kim *et al*., 1998).

2.11.2 Synthesis and Characterization of Transition Metal Complexes of 4-Amino-5- Pyridyl-4h-1,2,4-Triazole-3-Thiol

Many coordination complexes of Ni(II), Cu(II), Zn(II), Cd(II) and Sn(II) with 4-amino-5-(pyridyl)-4H-1,2,4-triazole-3-thiol, as a ligand successfully synthesized in alcoholic medium was reported by Raghad *et al*. (2013). The prepared complexes were characterized quantitatively and qualitatively by using: micro elemental analysis, FTIR spectroscopy, UV-visible spectroscopy, ^{1}H and ^{13}C NMR, magnetic susceptibility and conductivity measurements. This triazole ligand acts as bidentate ligand that coordinates to the metal ions through sulphur and amine group. According to the spectral data of the complexes, a tetrahedral geometry was suggested for these complexes except Cu (II) complexes, which exhibit a square planar geometry (Raghad *et al*., 2013). The ligand 4-amino-5-(pyridyl)-4H-1,2,4-triazole-

3-thiol was successfully synthesized. The ligand was treated with different metal ion salts to afford the corresponding complexes. It was observed that the ligand coordinated through amino and thiol groups to the metal atom leading to the formation of five-member ring chelate. Square planar geometry was proposed for the copper complex. The other complexes were proposed to be tetrahedral (Raghad *et al*., 2013).

2.11.3 Synthesis and Characterization of some Transition Metal Complexes Derived from Bidentate Schiff Base Ligand

Schiff bases have often been used as chelating ligands in coordination chemistry, they are more effective as a chelating agent when they bear supporting and stabilizing group like -OH close to -HC=N- group. Bidentate group containing imine groups have also been used as the modulators of structure and electronic properties of transition metal centers. The ligand Schiff base finds its applications as the analytical reagent for determination of metals (Praveen *et al.,* 2007). Transition metal complexes containing the Schiff base ligands have been of interest for many years.

Preparation of Schiff Base

Sharma and Manish (2013) prepared the Schiff base by condensation of 3-(3-nitrophenyl)-1-phenyl-1H-pyrazole-4- carbaldehyde (0.01 mol) with *o*-amino phenol (0.01 M) in ethanol (30 ml), and the mixture was refluxed for 8 hrs. The progress of reaction was monitored by TLC (solvent system, benzene: acetone- 8:2). After completion of the reaction, the reaction mixture was poured over crushed ice. The separated solid product was filtered and washed with cold saturated sodium bisulphate solution and recrystallized with ethanol. 2-(3-(3-nitrophenyl)-1-phenyl-1H-pyrazole-4-yl) methylene amino) phenol and its transition metal complexes have been synthesized in 2:1 molar ratio of ligand to metal ion. They have been characterized by the analytical, spectral, thermal and magnetic studies. The results of these investigations support the suggested structure of the metal complexes. Molar conductance values and analytical data suggest that the complexes are neutral in Dimethyl sulfoxide (Sharma and

Manish, 2013). The UV/Visible spectrum and magnetic susceptibility value suggested the octahedral geometry of complexes. Thermal analysis of metal complexes indicates the presence of coordinated water molecules in the complexes. Thermo-gravimetric analysis supported the octahedral environment around the metal ion. Complete analysis suggests a plausible octahedral structure of metal complex (Sharma and Manish, 2013).

2.12 Applications of Transition Metal Complexes in Drugs and Cosmetics

Transition metal complexes are cationic, neutral or anionic species in which a transition metal is coordinated by ligands (Cox, 2005). Transition metal complexes often have spectacular colors caused by electronic transitions by the absorption of light. For this reason, they are often applied as pigments. Most transitions that are related to colored metal complexes are either d–d transitions or charge transfer bands. In a d–d transition, an electron in a d-orbital on the metal is excited by a photon to another d-orbital of higher energy. A charge transfer band entails promotion of an electron from a metal-based orbital into an empty ligand-based orbital (Metal-to-Ligand Charge Transfer or MLCT). The converse also occurs: excitation of an electron in a ligand-based orbital into an empty metal-based orbital (Ligand to Metal Charge Transfer or LMCT). These phenomena can be observed with the aid of electronic spectroscopy; also known as UV-Vis (Harris and Bertolucci., 1989).

Research has shown significant progress in the utilization of transition metal complexes as drugs to treat several human diseases. Transition metals show different oxidation states and can interact with a number of negatively charged molecules. Advances in inorganic chemistry provide better opportunities to use metal complexes as therapeutic agents. The mode of action of metal complexes on living organism is differing from non-metals. These complexes show a great diversity in action (Hariprasath *et al.*, 2010). Medicinal inorganic chemistry can exploit the properties of metal ions for the

design of new drugs. This leads to the clinical application of chemotherapeutic agents for cancer treatment, such as cisplatin (Pieter *et al.*, 2008).

The use of transition metal complexes as therapeutic compounds has become more and more pronounced. These complexes offer a great diversity in their action; they do not only have anti-cancer properties but have also been used as anti-inflammatory, anti-infective and anti-diabetic compounds. Development of transition metal complexes as drugs is not an easy task; considerable effort is required to get a compound of interest. Beside all these limitations and side effects, transition metal complexes are still the most widely used chemotherapeutic agents and make a large contribution to medicinal therapeutics in a way that is unimaginable a few years back. (Rafique *et al.,* 2010). Transition metal complexes are also important in catalysis, materials synthesis, photochemistry, and biological systems.

2.13 Antibacterial Properties of Some Heteroleptic Metal (II) Complexes of Paracetamol and Vanillin

Osowale *et al.* (2014) studied the suitability of Paracetamol and Vanillin in forming low spin, novel heteroleptic, octahedral metal(II) complexes and the potentials of the metal(II) complexes as broad-spectrum antibacterial agents' in-vitro. Heteroleptic metal(II) complexes of Paracetamol (HL) and Vanillin(HL1) were synthesized and characterized by percentage metal analysis, infrared and electronic spectroscopies, room temperature magnetic moments, melting points, conductance measurements. They also studied the antibacterial activity of the synthesized complexes as well as their metal-free ligands using the agar diffusion technique (Osowale *et al.,* 2014).

Infrared spectra data confirmed that the strong and medium bands at 3328 cm^{-1} and 3184 cm^{-1} in Paracetamol and Vanillin were assigned as υOH band (Osowale *et al.,* 2014). This band in Paracetamol was absent in the metal complexes, due to the coordination of the phenol oxygen atom. On the contrary, the *v*OH band in Vanillin still remained but shifted to 3247-3116 cm^{-1}, due to the coordination

of the phenol oxygen atom without deprotonation (Osowale *et al.,* 2014). The broad band at 3500 cm^{-1} in the metal complexes were assigned to *v*(OH) of coordinated water. The sharp bands in the range 1666-1503 cm^{-1} in Paracetamol and Vanillin were assigned to υC=O stretching vibrations. These bands shifted to 1759-1553 cm^{-1} in the metal complexes, confirming coordination through their carbonyl oxygen atoms (Harminder, 2013). Furthermore, the new bands in the range 586 - 503 cm^{-1}, 440-425 cm^{-1} and 388-356 cm^{-1} in the spectra of the metal complexes were assigned to υ(M-N), *v*(M-O) and *v*(M-Cl) respectively. These bands were absent in the spectra of Paracetamol and Vanillin, a further evidence of coordination (Harminder, 2013). Furthermore, the nikel (II) complex showed high spin low spin octahedral equilibrium, and the copper (II) complex was dimeric and anti-ferromagnetic. The molar conductance of the metal (II) complexes in dimethyl sulfoxide showed that the complexes were covalent. Interestingly, the in-vitro antibacterial studies on these metal (II) complexes, Paracetamol and Vanillin against *Bacillus cereus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella oxytoca and Staphylococcus aureus*showed that the Cobalt (II) complex had the best antibacterial activity, being active against all the bacteria used with inhibitory zones in the range, 19.0-26.0 mm proving its potentials as a broad spectrum antibacterial agent.

2.14 Antimicrobial Properties of Ibuprofen Analgesic Drug Complexes with Certain Metal Ions

Solid-state compounds of general formula Ln(L)3, in which L is ibuprofen and Ln stands for trivalent La, Ce, Pr, Nd, Sm and Eu, have been synthesized. Simultaneous Thermogravimetry and Differential Thermal Analysis (TG-DTA), X-ray powder diffractometry (DRX), complexometry, fourier-transformed infrared spectroscopy (FTIR) and thermogravimetry coupled to fourier-transformed infrared spectroscopy (TG-FTIR) were used to characterize these compounds (Galico *et al*., 2014).

A novel Gold (I) complex with ibuprofen was synthesized and characterized by chemical and spectroscopic measurements (Fiori *et al*., 2011) that elemental analysis led to the composition of $AuC_{14}H_{18}O_2N$. Infrared, ^{1}H and ^{13}C NMR data suggest that coordination of the ibuprofen ligand to Au(I) is carried out through the oxygen atom of the carboxylic group in a monodentate form. An antibiotic sensitive profile indicated the antibacterial activity *in-vitro* of the complex against Gram-negative (*E. coli and P. aeruginosa*) and Gram-positive (*S. aureus*) microorganisms.

Metal complexes have been applied as medicinal agents in the treatment of different human infections and diseases. Currently, there are Platinum (II) complexes that are being used clinically as antitumor drugs, silver and gold compounds are used as antibacterial and antiarthritic agents (Sadler and Sue, 1994). More recently, gold compounds are found to be considerable important in the treatment of rheumatoid arthritis, with emphasis on uses of auranofin, solganol and miochrysine (Fricker, 2007; Youn *et al*., 2006). Several other reports on this subject have also been published and many of them are cited in most articles and reviews (Clarke, 2003; Zhang and Lippard, 2003; Ang and Dyson, 2006; Dyson and Sava, 2006; Habtemariam *et al*., 2006; Hartinger *et al*., 2006; Kostova, 2006).

Ang and Dyson (2006) reported that some ruthenium complexes exhibit antitumor activity, which may be involved in the protein and DNA binding capacity of the metal. Due to the growth of antibiotic-resistant bacteria, new metallo-pharmaceuticals with antibacterial activities have been synthesized and described in the literature (Ang and Dyson, 2006). Ibuprofen is a well-known anti-inflammatory, analgesic and antipyretic drug that has been found recently to slow down the proliferation of colon cancer cells effectively (Ang and Dyson, 2006). The aim of the study was focused on studying the spectroscopic, thermal and evaluating antimicrobial investigations of ibuprofen analgesic drug complexes formulas with Cu (II), Ni (II), Ag (I), Hg (II), UO_2 (II), Cd (II), Ba (II) and Al (III) metal ions. Complexes of Cu (II), Ni (II), Ag (I), Hg (II), UO_2 (II), Cd (II), Ba (II) and Al (III) with ibuprofen

ligand as to evaluate the biological activity were synthesized and characterized by elemental analysis, conductometry, magnetic susceptibility, UV-VIS, IR, ^{1}H-NMR spectroscopy and thermal analysis. The IR spectral data suggested that the ibuprofen ligand behaves as a monobasic bidentate ligand towards the central metal ion with deprotonated carboxylic group (Ahmed *et al*., 2015). From the microanalytical data, the stoichiometry of the complexes 1:1, 1:2 and 1:3 (metal: ligand) was found. The metal complexes were screened for antibacterial activity against Gram-negative organisms as *Escherichia coli* and *Pseudomonas aeruginosa,* Gram-positive bacteria as *Bacillus subtilis and Bacillus cereus* and antifungal organisms such as *Aspergillus niger, Aspergillus flavus* and *Aspergillus oryzae*. The resulted Ibuprophen compounds have proved the thermal stability feature (Ahmed *et al*., 2015). The antimicrobial activities of the metal complexes of Ibuprophen recorded a significant effect against some bacteria and fungi (Ahmed *et al*., 2015).

2.15 Antimicrobial Activities of Some Mixed Drug Metal (II) Complexes of Sulfamethoxazole and Paracetamol

Infectious diseases remain a crucial and challenging problem because of a combination of factors including rising infectious diseases and the increasing number of multi-drug resistant pathogens. Thus, there is still need to discover new compounds with enhanced antimicrobial activities to combat drug resistance menace as corroborated by Jegede (2005). Paracetamol is a mild analgesic with weak anti-inflammatory activity, commonly used for the relief of aches and pains (Roberts *et al.,* 2001). However, overdose of Paracetamol may cause liver damage as validated by Larson *et al.* (2005). Sulfamethoxazole is in the class of Sulfonamides, which are

used as antibacterial agents. This is because they interfere with p-amino benzoic acid (PABA) in the biosynthesis of tetrahydrofolic acid, which is essential for the metabolic process of Bacteria.

Sulfamethoxazole is a bacteriostatic antibiotic, used in combination therapy with Trimethoprim for the treatment of urinary tract infection. Many metal drug complexes have been found to have better antimicrobial and anticancer activities than classical drugs like cis-platin and Cloxacillin (Sadler and Zijien 1998; Lawal and Obaleye 2007; Bamigboye *et al.,* 2012; Harminder *et al* 2013;Osowole *et al.,* 2013a; Osowole *et al.,* 2013b).

Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes of mixed drug Paracetamol (HL) and Sulfamethoxazole (HL1) were synthesized and characterized by room temperature magnetic moments, melting points, percentage metal, conductance measurements, infrared and electronic spectroscopies (Oluwatoosin *et al*., 2014). The percentage metal analysis showed that the complexes were mostly analyzed as $[M(HL)(HL_1)\ X_2]$. nH2O, X = Cl or NO_3, and [M(HL)(HL1) (SO4)]. nH_2O. Infrared spectra data confirmed that coordination was through phenol and carbonyl oxygen atoms of Paracetamol, while the coordination in Sulfamethoxazole was through the nitrogen and oxygen atoms of the amine and sulphone groups. The room temperature magnetic moment and electronic spectra data indicated that all the metal (II) complexes were monomeric and octahedral, with the exception of the Cu (II) complex, which was dimeric and anti-ferromagnetic. Furthermore, the Fe(II) complex exhibited high spin low spin octahedral equilibrium. The molar conductance measurements of the metal (II) complexes in DMSO confirm that the complexes were all covalent, with the exception of the nickel (II) complex, which was electrolytic. Interestingly, the in-vitro antimicrobial studies on these mixed drug metal(II) complexes, Paracetamol and Sulfamethoxazole against *Escherichia spp, Streptococcus spp, Proteus sp, Candida albicans, Salmonella sp, Bacillus spp, Staphylococcus spp,* and *Pseudomonas spp* showed that $[Co(HL)(HL_1)Cl_2].2H_2O$, $[Cu(HL)(HL_1)(NO_3)]2.H_2O$ and $[Cu(HL)(HL1)SO_4].H_2O$ have higher inhibitory zones than Streptomycin (2.0-29.0 mm) against these microbes with the exceptions of *Escherichia Spp*, and inhibitory zones range of 19.0-28.0, 17.0-27.0 and 13.0-29.0 mm respectively, proving their potentials as broad-spectrum antimicrobial agents (Oluwatoosin *et al*., 2014).

2.16 *In Vitro* Antibacterial Activity of Ibuprofen and Acetaminophen

Acetaminophen is a synthetic non-opiate derivative of ρ-aminophenol, while ibuprofen is one of phenyl propionic acid derivatives (Mcevoy *et al*., 1993). These chemical agents have anti-inflammatory, antipyretic, and analgesic activity in both animals and humans (Minous and pearson, 2003; Polidori *et al*., 2006; Walsh, 2006). Available information about the relationship between bacteria and ibuprofen or acetaminophen is focused on the anti-inflammatory action of these agents on immune system that is stimulated by bacterial infections and not on the antibacterial activities. Ibuprofens found limit the effect of *E. coli* endotoxin on several physiological activities of rabbits (Celik*et al*., 2002) and human (Benard *et al*., 1997). It also reduces the inflammation in mouse lung resulting from *Pseudomonas aerogenosa* infection with no effect on the bacteria itself. Otherwise, ibuprofen showed no antibacterial effects on *Campylobacter pyloriin* in human body and on *Mycobacterium tuberculosis* in mice (Byrne *et al*., 2007) Complexes of acetaminophen with Co, Ni or Fe element (not acetaminophen alone) revealed variable inhibitory effects on *E. coli*, while complexes of acetaminophen showed no antibacterial effects on *Serratia* and *Bacillus subtilis* (Lawal and Obaleye, 2007).

Al- Janabi (2010) tested Ibuprofen and acetaminophen for antibacterial activity against seven isolates of bacteria including gram-positive bacteria (*Staphylococci aureus* and *Bacillus subtilis*) and gram-negative bacteria (*E. coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Salmonella typhi* and *Paracoccus yeei*). Spectrophotometer assay was applied to determine the antibacterial activities of ibuprofen and acetaminophen. Three controls were included in his study: Ampicilline sodium (20 µg/ml); cefotaxime sodium (20 µg/ml) and chemical free medium. Among seven strains of isolated bacteria, *Enterobacter aerogenes* and *Enterobacter cloacae* exhibited more resistance to ibuprofen and acetaminophen. These strains are the species most commonly encountered in clinical specimens. They are part of commensal enteric flora

(Winnie *et al*., 2006). *Enterobacter cloacae* has been reported to be resistant to many antibiotics (Al- Janabi, 2010). It needs high MIC of pexiganan to inhibit with the ability to survive for long periods after post antibiotic effect of meropenem. In addition, the resistance to cephalosporins had been observed due to the production of a stable cephalosporinase (Al- Janabi, 2010).

2.17 Antibacterial Activity of a Zn (II) Coordination Polymer Based on a Paddle-Wheel Cluster.

The antibacterial activity of the complex was assayed against *Escherichia coli*, and *Staphylococcus aureus*. The complex had some antibacterial activity to both of the two model bacterial strains, which means that the antibacterial activity has relations with the complex. The two-fold serial dilution method was employed to further investigate the antibacterial activity of the complex. The results indicated that the Minimum Bactericidal Concentrations (MBC) of the complex against *Escherichia coli,* and *Staphylococcus aureus* are 0.0625 and 0.125 mg/mL, respectively, which are similar to those reported for Zn(II) complexes (Keypour *et al.,* 2017). Their antibacterial activity was evaluated by using MBC benchmark. The binuclear Zn (II) complex shows excellent and long-term antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

2.18 Antimicrobial, antioxidant and DNA cleavage studies of Cu(II) complexes of formyl chromone Schiff bases

Synthesis of chromone derivatives have been of special interest due to their biological activities including antimycobacterial, anticonvulsant, antimicrobial, anticancer, antioxidant and mushroom tyrosinase inhibition activities. These derivatives also serve as intermediates to many products of pharmaceuticals, agrochemicals and dyestuffs. In addition, 3 formylchromone and its derivatives inhibit a human protein phosphatase PTP1B.

Schiff bases are important classes of ligands in catalysis and organic synthesis (Datta *et al*., 2002). Furthermore, these Schiff bases and their metal complexes have a variety of biological, clinical,

analytical and industrial applications. Schiff bases of 3-formyl chromone and its complexes also have a variety of applications in biological, clinical and pharmacological areas (Khan *et al*., 2010). A huge number of literature reports are available on DNA binding studies of formyl chromone Schiff bases and their metal complexes (Khan *et al*., 2010). Knowledge of DNA binding parameters of these molecules explores their potential applications, since their binding mode could be associated with their ability to cause DNA damage (Kumar *et al.,* 2011).

Kavitha *et al. (*2013) In their study synthesized 3-formyl chromone Schiff bases and their Cu (II) complexes. All synthesized complexes were characterized by using various spectroscopic techniques. The results revealed that all the ligands were coordinated to the Cu(II) ions in tridentate manner and geometrical structures of these complexes were found to be tetragonally distorted octahedral. From the thermograms the numbers of lattice water molecules present in the complexes were calculated. The well-defined crystalline and homogeneous nature of the Copper-Metal (II) complexes was observed from Powder X-Ray Diffraction (PXRD) and Scanning Electron Microscope (SEM) studies. The Schiff bases and their Cu(II) complexes are fluorescent in nature. All the Cu (II) complexes exhibited prominent antimicrobial activity than ligands (Kavitha *et al*., 2013).

2.19 Anti-Inflammatory and Analgesic Activities of Complexes of Zn (II) With 1,10- Phenanthroline and some Amino Acids

Hossain *et al.* (2013) prepared New mixed ligand complexes of Zn(II) with 1,10- Phenanthroline and some amino acids in the solid form and characterized by elemental analysis, conductivity, magnetic moment measurement, FT-IR, 1H-NMR, 13C-NMR and FAB+ mass studies. To examine anti-inflammatory and analgesic activities of its complexes, some Swiss albino mice of 05-07 weeks' old were taken. One dose of the test compounds of 10 mg/kg was selected throughout the research work. The anti-inflammatory activity (Kota *et al*., 2011) of the test compounds was determined using the carrageenan-

induced mice paw edema inhibition method employing 1.0% carrageenan solution as the phlogistic agent. The test compounds were administered orally as suspensions in 3 % DMSO, 30 min before the injection of the phlogistic agents, at dose level of 10 mg/kg body weight. Diclofenac sodium was used as a standard at a dose level of 10 mg/kg body weight. 3 % DMSO served as a control. Groups of four Swiss albino mice of either sex were used in each experiment. The volume of paw edema was measured with the help of plethysmograph by mercury displacement method at 0 h (immediately after injection of carrageenan). Then, the volume of paw edema was observed at 1, 2, 3 and 4 h.

The analgesic activity of the test samples was studied (Ahmed *et al., 2004*) using acetic acid-induced writhing model in mice. Swiss albino mice of either sex were divided into control, standard and different test groups contain three mice in each. The control group received 3% DMSO and standard group was treated with diclofenac sodium at a dose level of 10 mg/kg. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.6% acetic acid but diclofenac sodium was administered 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 30 min. These four compounds showed positive effects as anti-inflammatory and analgesic agents. Anti-inflammatory and analgesic activities of the test compounds at 50 mg/kg were quite comparable to those of standard drugs at 10 mg/kg (Ahmed *et al., 2004*).

2.20 Metal Complexes Used as Anti-Inflammatory Agents

Inflammation, angiogenesis and remodeling are self-limiting processes under normal healing conditions. Angiogenesis is a fundamental process for normal and abnormal tissue growth and repair, which consists of recruiting endothelial cells towards an angiogenic stimulus (Crans *et al*., 2013). Vanadium has therapeutic importance and is known as a cofactor for metalloenzymes. In addition to being a cation, vanadium has analogy with phosphorus and as such is a potent inhibitor for

phosphorylases. Because speciation can change the metal's existence in cationic or anionic form, speciation has profound effects on biological systems (Crans *et al*., 2013).

Shraddha and Mishra (2014) studied the effects of the Vanadium Schiff base complexes and Carrageenan induced paw edema in an experimental model of inflammatory angiogenesis induced by a sponge implant in order to characterize probable synergistic effects between the metal and the standard drug on experimental models. The effects of the vanadium complexes on the selected experimental model of the fibro-vascular tissue were investigated. They concluded that the anti-inflammatory activity of the complexes was moderate.

2.21 Antifungal Activity of Organometallic Complexes of Cholic Acid.

Cholic acid, also known as 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid, is a primary bile acidthat is insoluble in water (soluble in alcohol and acetic acid), it is a white crystalline substance (*Colleen et al., 2007).* Salts of cholic acid are called cholates. Cholic acid, along with chenodeoxycholic acid, is one of the two major bile acids produced by the liver, where it is synthesized from cholesterol. These two major bile acids are roughly equal in concentration in humans. Cholic acid, formulated as Cholbam capsules, is approved by the United States Food and Drug Administration as a treatment for children and adults with bile acid synthesis disorders due to single enzyme defects, and for peroxisomal disorders (*Colleen et al., 2007)*.The antifungal activity of organometallic compounds of cholic acid was based on Poisoned Food Technique (Perrucci *et al.*, 1994) and done against *Aspergillus flavus* and *Aspergillus niger* collected. The antifungal activity of these compounds was tested by agar plate diffusion method using Ketoconazole as standard and 10, 20, 50,100 mg/ml of compounds.

Metal chelates are both polar and non polar which make them suitable for permeation to the cells and tissues. Changing hydrophilicity and lipophilicity probably leads to a decrease in solubility and permeability barriers of cell, which in turn enhances both bioavailability of chemotherapeutics and potentiality. Cholic acid and its metal complexes are one of the novel groups among the metallic complexes having the following characteristics.

1) Good antibacterial as well as antifungal activity.

2) Metal chelates have a higher activity than the free ligand.

Increase in lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of metal binding sites on the enzymes of the microorganism (Tripathi, 2012). Metal complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms. The inhibition of microbial growth by cholic acid and its complexes with divalent metallic salts was found to be in the following order:

$Mn^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} < Zn^{2+} < Cd^{2+}$

The result showed that the activity enhanced from Mn^{2+} to Cd^{2+} as charge density increases due to electrons draining from metal to ligand by back donation facilitating the intra-molecular hydrogen bonding between coordinated ligands and slowly approaching the chelating character, which subsequently favor lipophilic nature of central metal ion in permeation through the lipid layer of membrane. Cd^{2+}, Zn^{2+}, Cu^{2+} and Ag^{1+} complexes possess high degree of inhibition, which may be attributed to the greater number of d-electrons, which increases the electrostatic field around metal ion (Tripathi, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

All chemicals were of analytical grade. Acetaminophen (product of Sigma Aldrich) was obtained from Bristol Scientific Company, Lagos, Nigeria. Zinc Chloride Hexahydrate ($ZnCl_2.6H_2O$), Magnesium Chloride Hexahydrate ($MgCl_2.6H_2O$) and Copper (II) Chloride dihydrate ($CuCl_2.2H_2O$) were obtained from the Department of Chemistry, Ahmadu Bello University, Zaria. The albino rats were housed in standard animal cages in the animal house section of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

3.2 Preparation of Solid Complexes

The metal complexes (Cu(II), Mg(II) and Zn(II)) were prepared according to the method used by Oluwatoosin *et al*. (2014). Copper (II) complex was prepared by the addition of(0.51 g, 3 mmol) of

$CuCl_2.2H_2O$ in 20mL of distilled water to (0.91 g, 6 mmol) of acetaminophen in 60 mL of distilled water, the mixture was stirred using magnetic stirrer at room temperature until the dissolution of acetaminophen occurred, the mixture was heated in water bath for 6 hours. The solution was allowed to cool down overnight during which brown crystals were obtained. The crystals were filtered off, washed with distilled water and dried in a desiccator for about 48 hours.

Magnesium (II) complex was prepared by the addition of(0.61 g, 3 mmol) of $MgCl_2.6H_2O$ in 20mL of distilled water to (0.91 g, 6 mmol) of acetaminophen in 60 mL of distilled water, the mixture was stirred using magnetic stirrer at room temperature until the dissolution of acetaminophen occurred, the mixture was heated in water bath for 6 hours. The solution was allowed to cool down overnight during which brown crystals were obtained. The crystals were filtered off, washed with distilled water and dried in a desiccator for about 48 hours (Oluwatoosin *et al*., 2014).

Zinc (II) complex was prepared by the addition of (0.73 g, 3 mmol) of $ZnCl_2.6H_2O$ in 20mL of distilled water to (0.91 g, 6 mmol) of acetaminophen in 60 mL of distilled water, the mixture was stirred using magnetic stirrer at room temperature until the dissolution of acetaminophen occurred, the mixture was heated in water bath for 6 hours. The solution was allowed to cool down overnight during which brown crystals were obtained. The crystals were filtered off, washed with distilled water and dried in a desiccator for about 48 hours (Oluwatoosin *et al*., 2014).

3.3 Physical Measurement

The melting point of acetaminophen complexes was determined using automatic melting point SMP40. Conductivity of the metal complexes of acetaminophen was measured in methanol solution using conductivity meter (Refat*et al.,* 2013). The UV/Visible spectral measurements of the metal complexes were made by using Cary 300 UV/Visible spectrophotometer in the range of 200-400 nm in the Multi user laboratory, Ahmadu Bello University, Zaria (Refat *et al.,* 2013). The Infrared spectra of

acetaminophen and the metal complexes were recorded using Cary 630 FTIR (Refat *et al.,* 2013) on 4000-650 cm^{-1} spectral range in the Multi user laboratory, Ahmadu Bello University, Zaria.

3.3.1. Magnetic susceptibility

The magnetic susceptibility measurement for the synthesized complexes was obtained at room temperature using an AUTO (Sherwood scientific) magnetic susceptibility balance (MSB). This was done by first taking the magnetic measurement of an empty capillary tube (which is always negative since glass is diamagnetic) recorded as R_o then that of capillary tube with pre-weighed amount of sample as R. The length of the sample in the column was taken as L, the proportionality constant (C) is always 1.0, mass (M) of the sample alone weighed from the digital weighing balance was also recorded. The gram susceptibility (X_g) of the complexes was thus evaluated using the relationship in equation 3.1 (Lotz, 2008; Sharwood-Scientific, 2017).

$$X_g = \frac{C \times L\,(R - Ro)}{10^9 \times M} \quad \text{Eq. 3.1}$$

A negative value of X_g indicates that the complex is diamagnetic while a positive value is indicative of a paramagnetic behavior. The molar susceptibility (Xm) was then obtained using the standard formulae in equation 3.2

$$Xm = Xg \times MM \quad \text{Eq. 3.2}$$

Where MM is the molecular mass of the complex. Hence, effective magnetic moments (μeff) for the complexes was evaluated using the expression in equation 3.3

$$\mu eff = 2.83\sqrt{Xm \times T} \quad \text{Eq. 3.3}$$

Where T is the room temperature in Kelvin, and μeffis the effective magnetic moments in Bohr magnetons (BM).

3.3.2 Crystallite size of the metal complexes

X-ray diffraction pattern were recorded using X-Ray diffractometer (Pradeep *et al*., 2014) at Umar Musa Yaradua University, Katsina. The diffraction pattern of complexes was recorded between 2θ ranging from 5° to 65°. The crystalite size of the samples was estimated using the Scherrer's formula. According to Scherrer's equation, the crystallite size is given by

$$t = \frac{0.9\,\lambda}{B\cos\theta} \quad \text{Eq. 3.4}$$

where t is the crystal thickness (in nm), B is half width (in radians), θ is the Bragg angle and λ is the wavelength. The crystallite sizes corresponding to each diffraction maxima were determined from the measurement of the half width of the diffraction peak (Kavitha *et al.,* 2013).

3.4 Antimicrobial activity

Antimicrobial tests were carried out in the Department of Microbiology Laboratory, Ahmadu Bello University, Zaria. Adult albino rats were used for acute toxicity and anti-inflammatory tests.

3.4.1. Test Organisms

The test organisms used for this analysis were clinical isolates of bacteria and fungi obtained from the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria. The isolates were *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeroginosa*, *Candida albicans* and *Aspergillus niger.*

3.4.2 Culture Media

The culture media used for the analysis were Mueller-Hinton Agar (MHA), Muller Hinton Broth (MHB), Potato Dextrose Agar (PDA), and Nutrient Agar (NA). The mentioned media were used for sensitivity test, determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal

Concentration (MBC). All media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes (Offiong and Martelli., 1994).

3.4.3 Turbidity standard for inoculum preparation-McFarland standard

The standard was prepared by adding 0.5 mL of 0.048 M barium chloride (1.17 %w/v $Bacl_2.2H_2O$) to 99.5 mL of 0.18 M sulfuric acid (1% v/v) with constant stirring and was transferred to a tube similar to that used in growing the growth culture. The accurate density of the turbidity standard was determined by taking the absorbance reading at 625 nm on the spectrophotometer. Absorbance value of 0.008 to 1.10 was considered equivalent to McFarland. The standard was vigorously agitated on a vortex mixer each time before use (Araujo and Pina-Vaz, 2004).

3.4.4 Determination of Inhibitory Activity (susceptibility test) of acetaminophen and the metal complexes using Agar Well Diffusion Method

The standard inocula of both the bacterial and fungal isolates were streaked on sterilized Mueller Hinton and Potato Dextrose Agar plates with the aid of sterile swab sticks. Four wells were punched on each inoculated agar plate with a sterile cork borer. The wells were properly labeled according to different concentrations of the acetaminophen and the synthesized complexes which were 100, 50, 25, 12.5, 6.25 mg/ml. Each well was filled up with approximately 0.2 mL of the complexes.

The inoculated plates with the complexes were allowed to stand on the bench for about one hour; to enable the samples to diffuse on the agar. The Mueller Hinton agar plates were then inoculated at 37°C for 24 hours for plates of Mueller Hinton agar while the plates of potato dextrose agar were incubated at room temperature for about 5 days.

At the end of the incubation period, the plates were observed for evidence of inhibition, which appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of the zones was measured using a transparent ruler calibrated in millimeter and the result

was recorded (Kavitha *et al.,* 2013). The zone of inhibition was measured in mm and compared with standard antibiotics. All the experiments were performed in triplicates and the average zones of inhibition was recorded. The compounds, which showed significant activities, were selected to determine the MIC using disc diffusion technique (Kavitha *et al.,* 2013).

3.4.5 Determination of Minimum Inhibitory Concentration of Acetaminophen and the Metal Complexes against the selected Bacterial and Fungal Isolates

The Minimum Inhibitory Concentration was determined using tube dilution method with the Mueller Hinton broth used as a diluent. The lowest concentration of acetaminophen and the metal complexes showing inhibition for each organism when the complexes were tested during sensitivity test was serially diluted in the test tubes containing Mueller Hinton broth. The organisms were inoculated into each tube containing the broth and the complexes. The inoculated tubes were then incubated at 37°C for 24 hours (Al-Janabi, 2010).

At the end of the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion, the lowest concentration in the series without visible sign of growth was considered the Minimum Inhibitory Concentration (MIC). The result was also recorded (Tripathi, 2013; Aamer *et al*., 2015).

3.4.6 Determination of Minimum Bactericidal Concentration of Acetaminophen and the Metal Complexes against the selected Bacterial and Fungal Isolates

This is to determine whether the antimicrobial effect of the samples is Bacteriostatic or Bactericidal. The result from the Minimum Inhibitory Concentration was used to determine the Minimum Bactericidal Concentration of the metal complexes. A sterilized wire loop was dipped into the test tubes that did not show turbidity (clear) in the MIC test and a loopful was taken and streaked on a sterile nutrient agar plates. The plates were incubated at 37°C overnight. At the end of incubation

period, the plates were examined for the presence or absence of growth. The least concentration that does not show any growth of the tested isolates was considered the Minimum Bactericidal concentration of magnesium (II), zinc (II) and Copper (II) complexes (Aamer *et al*., 2015).

3.5 Acute Toxicity Study

The LD_{50} (Median Lethal Dose) determination was conducted orally in rats using the method described by Lorke (1983) with modification. This method was carried out in two phases. In the first phase, the animals were divided into three groups containing three rats each. All the rats in the groups were administered with copper-acetaminophen complex (Cu(II)-AC) at three different concentrations (10, 100 and 1000 mg/kg body weight) orally. These animals were observed for signs of toxicity and death for 24 hours.

In the second phase, three rats were treated with Cu(II)-AC at doses of 1600, 2900 and 5000 mg/kg and observed for 24 hours for signs of toxicity and death. This same method was used carryout the LD_{50} of magnesium-acetaminophen complex (Mg(II)-AC) and zinc-acetaminophen complex (Zn(II)-AC) in the first phase. In the second phase, three rats were treated with Zn(II)-AC at doses of 140, 225 and 600 mg/kg (Lorke, 1983), and observed for 24 hours for signs of toxicity and death. In the second phase, four rats were also treated with Mg(II)-AC at doses of 600, 1000, 1600 and 2900 mg/kg (Lorke, 1983) and observed for 24 hours for signs of toxicity and death.

3.6. Anti-inflammatory study

Anti-inflammatory activity was determined by the paw edema method in rats used by Girish and Khan (2016). The rats were divided into five groups (n= 5), each containing 5 rats. Group 1 served as the control (saline). Group II was given piroxicam orally (20 mg/kg) as a standard drug. Groups III, IV and V received 200, 400 and 600 mg/kg of Cu(II)-AC complex by oral administration respectively. One hour later, 0.1ml of freshly prepared carrageenan suspension was injected into the sub planter region of the left hind paw of each rat. The paw diameter was measured with the aid of a digital caliper at 0, 1, 2, 3, 4 and 5 hours, after the injection of carrageenan.

This method was used to determine the anti- inflammatory activity of Zn(II)-AC and Mg(II)-AC where the rats were divided into five groups each. Groups 1 served as the control, while groups II were given piroxicam orally (20 mg/kg) as standard drug. Groups III, IV and V received 50, 25, 12.5 and 200, 150 100 mg/kg respectively.Mean increase in the paw diameter was measured. The percentage inhibition of edema compared with that of the control was taken as anti-inflammatory activity. The percentage (%) inhibition of the inflammation was calculated from the formula in equation 3.5 (Ghule *et al.,* 2011).

Percentage (%) inhibition $= \frac{1-\mathrm{Dt}}{\mathrm{Do}} \times 100$.....Eq 3.5

Where Do is the average inflammation (hind paw edema) of the control group of rats at a given time Dt is the average inflammation of the sample administered at the same time.

3.6.1 Statistical Analysis

All values were expressed as mean ±standard error of mean (S. E. M) and the analyzed by one-way analysis of variance (ANOVA) followed by tukey's post-hoc test. Graph pad prism version 5 was used for the analysis. Values of $p<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Measurement

The reactions of Acetaminophen with magnesium chloride and zinc chloride gave white solid complexes while the reaction of Acetaminophen (AC) with copper (II) chloride gave a brown solid complex. The purity of the three complexes was checked by taking the melting points. The solubility of the complexes was carried out by dissolving the complexes in ethanol, water, methanol and Dimethyl sulfoxide (Table 4.1).

The decomposition temperature, molecular formula, percentage yield and molar conductance of acetaminophen (ligand), Zn(II), Mg(II) and Cu(II) complexes prepared are presented in Table 4.2. The complexes were obtained in good yield, the molar conductance value of zinc, magnesium and copper complexes were 0.02, 0.03 and 0.05 (mS/cm) respectively.

The electronic absorption spectral data and magnetic susceptibility of acetaminophen, magnesium, zinc and copper complexes are presented in Table 4.3. The shift in lambda max is and evidence of complexation. The observed magnetic moment in the table falls within the range observed for octahedral geometry (Koushik *et al* 2007).

The Fourier-transform infrared spectroscopy (FTIR) data 0f acetaminophen, magnesium, zinc, magnesium and copper complexes are presented in Table 4.4. The Infrared spectrum of acetaminophen showed bands at 3321, 3160, 3108 and 1551 cm^{-1}. Magnesium complex showed bands at 3324 and 1547 cm^{-1}, zinc complex showed bands at 3205, 3321 and 1551 cm^{-1}, while copper complex showed bands at 3317 and 1561.

The crystallite size and XRD patterns are presented in Figure 4.1 and Table 4.5. The peak width is influenced by the size of the crystal and peak intensity is influenced by the position of atom.

Table 4.1: Solubility of complexes in some solvents

Compounds	Ethanol	DMSO	Methanol	Water
Cu(II)-AC	S	S	S	SS
Mg(II)-AC	S	S	S	SS
Zn(II)-AC	S	S	S	SS

S= Soluble, SS= Sparingly Soluble, DMSO= Dimethyl Sulfoxide, AC= Acetaminophen

Table 4.2 Physical data of Acetaminophen and the solid complexes

Complexes	Color	MP/DT (^{o}C)	Molecular Formula	Percentage yield (%)	Molar Conductance (mS/cm)
AC	White	169.6-170.4	$C_8H_9NO_2$		
Zn(II)-AC	White	165.1-168.1	$ZnC_{16}H_{22}N_2O_6Cl_2$	64	0.02
Mg(II)-AC	White	165.6-167.8	$MgC_{16}H_{22}N_2O_6Cl_2$	60	0.03
Cu(II)-AC	Brown	149.3-150.9	$CuC_{16}H_{22}N_2O_6Cl_2$	58	0.05

MP = Melting Point; DT = Decomposition Temperature; AC = Acetaminophen; Zn(II)-AC= Zinc complex; Mg(II)-AC = Magnesium complex; Cu(II)-AC = Copper complex

Table 4.3 **Electronic absorption spectral data and magnetic susceptibility of Acetaminophen, Mg(II)-AC, Zn(II)-AC, Cu(II)-AC**

ligand/complexes	Absorption (nm)	μeff (B.M)
Acetaminophen	256	

Mg(II)-AC	293, 209, 206	0.44
Zn(II)-AC	295, 215, 206	0.75
Cu(II)-AC	293, 210	1.35

μeff = effective magnetic moment, AC = Acetaminophen; Zn(II)-AC= Zinc complex; Mg(II)-AC= Magnesium complex; Cu(II)-AC = Copper complex

Table 4.4 FTIR Spectral data of Acetaminophen and the solid complexes

FTIR cm^{-1}			
ligand/complexes	$V_{(O-H)}$	$V_{(N-H)}$	$V_{(C=O)}$
Acetaminophen	3108, 3160	3321	1651
Mg(II)-AC	-	3324	1547
Zn(II)-AC	3205	3321	1551
Cu(II)-AC	-	3317	1561

Zn(II)-AC= Zinc complex; Mg(II)-AC= Magnesium complex; Cu(II)-AC = Copper complex

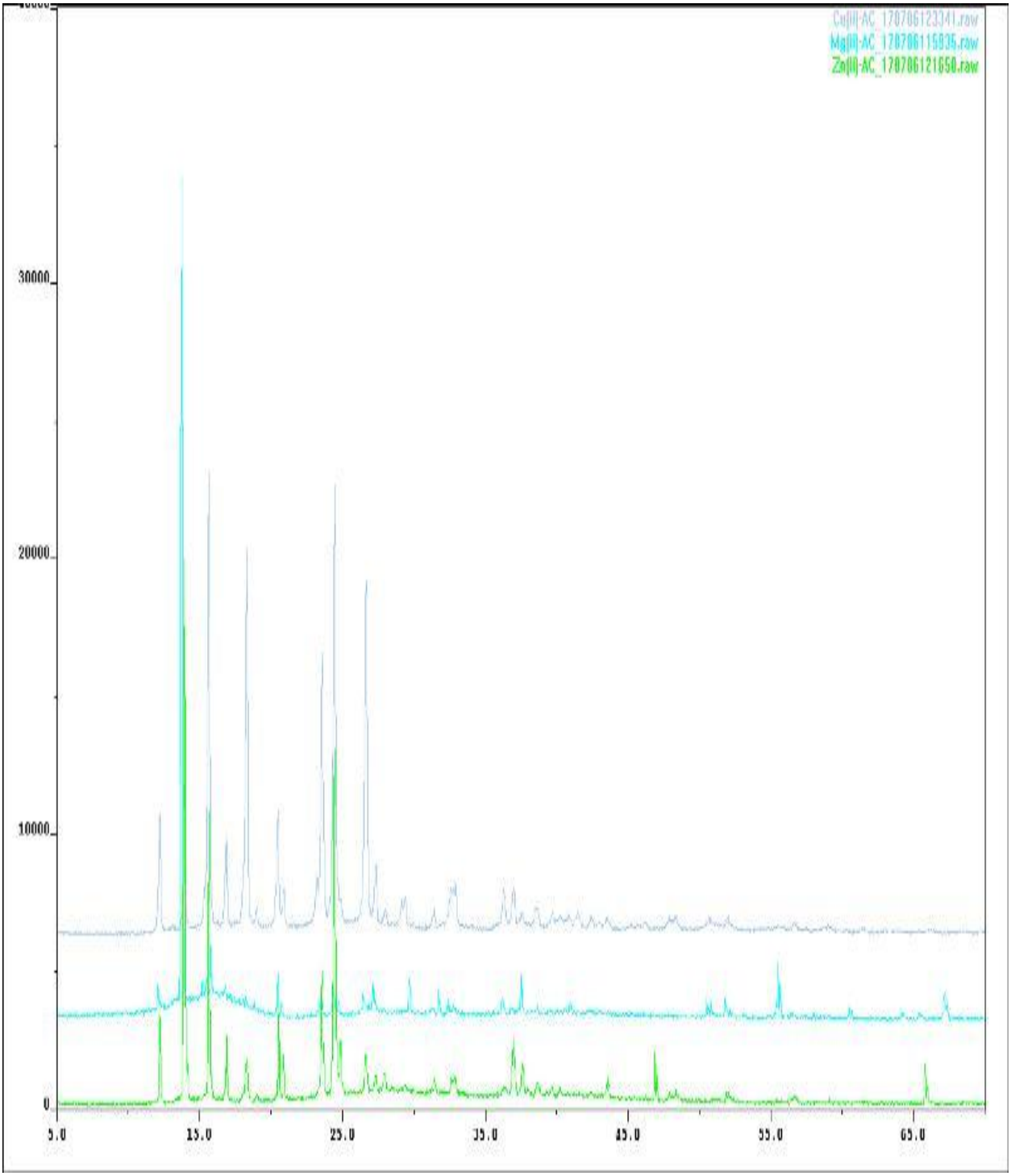
Cu[II]-AC_170706123341.raw
Mg[II]-AC_170706115935.raw
Zn[II]-AC_170706121650.raw
30000
20000
10000
0
5.0
15.0
25.0
35.0
45.0
55.0
65.0

Figure 4.1: XRD Patterns of Cu(II)-AC, Mg(II)-AC and Zn(II)-AC complexes

Table 4.5 Crystalite size of the Complexes

Complexes	d_{xrd} (nm)
Mg(II)-AC	73.36
Zn(II)-AC	84.03
Cu(II)-AC	58.01

Zn(II)-AC= Zinc complex; Mg(II)-AC= Magnesium complex; Cu(II)-AC = Copper complex, d_{xrd}=Crystallite Size

4.2 Anti-Microbial Activity of Metal-Acetaminophen Complexes

Acetaminophen, copper, zinc and magnesium complexes were subjected to antimicrobial activity on the following microorganisms; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. From the results below, it is known that Zn(II)-AC, Mg(II)-AC and Cu(II)-AC showed more antibacterial activity than acetaminophen (Tables 4.6 to 4.11).

4.3 Minimum Inhibitory Concentration of Acetaminophen and Its Complexes

The minimum concentrations of Mg(II)-AC and Zn(II)-AC required to inhibit the growth of *Staphylococcus aureus* and *Salmonella typhi* was 50 mg/ml, the minimum concentrations of Acetaminophen, Mg(II)-AC and Zn(II)-AC required to inhibit the growth of *Pseudomonas aeruginosa* is 25 mg/ml, while the minimum inhibitory concentrations of Cu(II)-AC required to inhibit the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* were 25, 25, 25, 50 and 50 mg/ml respectively (Table 4.12). The minimum bactericidal concentration (Table 4.13) indicates that Cu(II)-AC showed bactericidal activity against all the bacteria at 50, 50, 50, 25 and 25 mg/ml for *Bacillus subtilis* (Gram-positive),*Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), *Salmonella typhi* (Gram-negative), *Pseudomonas aeruginosa* (Gram-negative) respectively.

4.4 Acute lethal and Anti-inflammatory effects of Zinc, Copper and Magnesium Complexes

The LD_{50} of zinc, copper and magnesium complexes were 289, 3808 and 1265 mg/kg respectively (Tables 4.14 to 4.16). The average inflammation, % inhibition and S. E. M of the complexes were presented in Tables 4.17 to 4.19.

Table 4.6: The Susceptibility test of Acetaminophen (AC) at varying concentrations

Test organism	Diameter of Zone of Inhibition (mm) at Varying Concentrations (mg/mL)			
	50	25	12.5	6.25
Staphylococcus aureus				
Bacillus subtilis				
Escherichia coli				
Salmonella typhi				
Pseudomonas aeruginosa	18	15		
Candida albicans				
Aspergillus niger				

Table 4.7: The Susceptibility test of Mg(II)-AC at varying concentrations

Test organism	Diameter of Zone of Inhibition (mm) at Varying Concentrations (mg/mL)

	50	25	12.5	6.25
Staphylococcus aureus	19	15	13	
Bacillus subtilis				
Escherichia coli				
Salmonella typhi	15	12		
Pseudomonas aeruginosa	27	18	13	
Candida albicans				
Aspergillus niger				

Table 4.8: The Susceptibility test of Zn(II)-AC at varying concentrations

Test organism	Diameter of Zone of Inhibition (mm) at Varying Concentrations (mg/mL)			
	50	25	12.5	6.25
Staphylococcus aureus	20	17	14	10
Bacillus subtilis				
Escherichia coli				
Salmonella typhi	20	15	12	

Pseudomonas aeruginosa

Candida albicans

Aspergillus niger

Table 4.9: The Susceptibility test of Cu(II)-AC at varying concentrations

	Diameter of Zone of Inhibition (mm) at Varying Concentrations

Test organism	(mg/mL)			
	50	25	12.5	6.25
Staphylococcus aureus	23	20	17	15
Bacillus subtilis	21	18	16	14
Escherichia coli	22	19	17	14
Salmonella typhi	18	16	14	10
Pseudomonas aeruginosa	24	21	19	16
Candida albicans				
Aspergillus niger				

Table 4.10: The Susceptibility test of Ciprofloxacin/Fluconazole (Controls) at varying concentrations

Test organism	Diameter of Zone of Inhibition (mm) at Varying Concentrations (mg/mL)			
	50	25	12.5	6.25
Ciprofloxacin				
Staphylococcus aureus	38	30	25	20
Bacillus subtilis	39	32	28	25
Escherichia coli	30	25	22	19
Salmonella typhi	37	32	28	25

Pseudomonas aeruginosa	25	21	18	16
Fluconazole				
Candida albicans	36	32	28	25
Aspergillus niger	25	20	15	13

Table 4.11: The Susceptibility Testing of selected isolates to metal chlorides

	Diameter of Zone of Inhibition (mm) at Varying Concentrations (mg/mL)											
	$ZnCl_2$				$CuCl_2$				$MgCl_2$			
	50	20	12.5	6.25	50	25	12.5	6.25	50	25	12.5	6.25
Test organisms												
Staphylococcus aureus									16	14	13	
Bacillus subtilis					17	18	15	10				
Escherichia coli												
Salmonella typhi					19	18	13	12				
Pseudomonas aeruginosa					20	16	15	11				
Candida albicans												
Aspergillus niger												

Table 4.12 Minimum inhibitory concentration (MIC mg/mL) of acetaminophen and the Complexes against the test organisms

Test Organisms	AC	Mg(II)-AC	Zn(II)-AC	Cu(II)-AC	Controls	$CuCl_2$
Staphylococcus aureus		50		25	6.25	
Bacillus subtilis				25	12.5	50
Escherichia coli				25	12.5	
Salmonella typhi		50	50	12.5	12.5	25
Pseudomonas aeruginosa	25	25	25	12.5	6.25	25
Candida albican					6.25	
Aspergillus niger					25	

Table 4.13 Minimum bactericidal concentration (MBC mg/ml) of acetaminophen and the complexes against the test organisms

Test Organisms	AC	Mg(II)-AC	Zn(II)-AC	Cu(II)-AC	Controls	CuCl2
Staphylococcus aureus				50	12.5	
Bacillus subtilis				50	25	

Escherichia coli				50	25	
Salmonella typhi				25	25	25
Pseudomonas aeruginosa	50	50	50	25	12.5	25
Candida albican					50	
Aspergillus niger					12.5	

TABLE 4.14: Acute lethal effect of Cu(II)-AC Administered orally to white Albino Rats.

Experiment	Dose (mg/kg)	No of Dead Rats after 24hrs
Phase 1	10	0/3
	100	0/3
	1,000	0/3
Phase 2	1,600	0/1
	2,900	0/1
	5,000	1/1

Lethal Dose (LD_{50}) $= \sqrt{2900 \times 5000}$

$= \sqrt{14500000}$

= 3808 mg/kg

Table 4.15 Acute lethal effect of Zn(II)-AC Administered orally to white Albino Rats.

Experiment	Dose (mg/kg)	No of Dead Rats after 24hrs
Phase 1	10	0/3

		100	0/3
		1,000	3/3
	Phase 2	140	0/1
		225	0/1
		370	1/1
		600	1/1

Lethal Dose (LD_{50}) = $\sqrt{225 \times 370}$

$= \sqrt{83250}$

= 289 mg/kg

Table 4.16 Acute lethal effect of Mg(II)-AC Administered orally to white Albino Rats.

Experiment	Dose (mg/kg)	No of Dead Rats after 24hrs
Phase 1	10	0/3
	100	0/3
	1,000	1/3
Phase 2	600	0/1
	1,000	0/1

	1,600	1/1
	2,900	1/1

Lethal Dose (LD_{50}) $= \sqrt{1000 \times 1600}$

$= \sqrt{1600000}$

= 1265 mg/kg

Table 4.17: Effect of Cu(II)-AC on carrageenan- induced rat paw edema

		edema diameter(mm)/Time interval (hour) [mean of inhibition]					
			1 hr	2 hrs	3 hrs	4 hrs	5 hrs
Group (n=5)	Dose(mg/kg)	0 hr	*	*	*	*	*
Saline(control)	10mL/kg	1.886±0.0745	4.306±0.24[a]	4.548±0.174[a]	4.764±0.1901[a]	4.736±0.0832[a]	4.65±0.20[a]
Piroxicam	20mL/kg	1.974±0.0999	3.278±0.1949[b] [23.8]	3.202±0.3816[b] [29.6]	3.042±0.3474[b] [36.1]	3.106±0.4067[b] [34.4]	3.04±0.3236[b] [34.6]
Cu(II)-AC	200mg/Ml	1.95±0.0841	3.326±0.1632[b] [22.8]	3.912±0.1721 [14.0]	3.49±0.2453[b] [22.5]	3.818±0.219[b] [19.4]	3.542±0.2859[b] [23.8]
	400mg/Ml	2.072±0.0835	2.874±0.0673[b] [33.3]	3.778±0.3187 [16.9]	3.602±0.3006[b] [24.4]	3.756±0.0931[b] [20.7]	3.606±0.1333[b] [22.4]

Cu(II)-AC							
			2.978±0.1338[b]	3.498±0.1413	3.22±0.1106[b]	3.22±0.1106[b]	2.894±0.1969[b]
Cu(II)-AC	600mg/Ml	2.004±0.1753	[30.8]	[23.1]	[32.4]	[29.5]	[37.7]

*ANOVA:indicates that comparison at the 5% level is statistically significant. Means having different superscripts ([a,b]) are significantly different (P<0.05), [] = % Inhibition

Table 4.18: Effect of Zn(II)-AC on carrageenan- induced rat paw edema

edema diameter(mm)/Time interval (hour)

		[mean of inhibition]					
		0hr	1hr	2hrs	3hrs	4hrs	5hrs
Saline(control)	10mL/kg	2.178±0.0845	4.25±0.1328[a]	4.962±0.1207[a]	5.726±0.2761[a]	4.674±0.1735[a]	5.138±0.1927[a]
piroxicam	20mL/kg	2.172±0.0136	2.764±0.1504[b,c] [35]	2.904±0.096[b,c] [41.5]	3.168±0.2205[b] [42.7]	3.096±0.2105[b,c] [33.8]	2.83±0.0829[b,c] [44.9]
Zn(II)-AC	50mg/Ml	2.178±0.0845	3.754±0.0769[d] [11.7]	4.072±0.1569[d] [17.9]	4.126±0.2791[b] [25.3]	3.83±0.2123[b] [18.1]	3.634±0.1944[b] [29.3]
Zn(II)-AC	25mg/Ml	2.028±0.0564	3.708±0.1024[d] [12.8]	4.54±0.3146[d] [8.5]	4.042±0.2278[b] [26.9]	3.76±0.2532[b] [19.6]	3.414±0.1887[b] [33.6]

			4.192±0.1218[d]	4.726±0.2979[d]	4.09±0.2071[b]	3.978±0.0656[d]	3.742±0.1619[b,d]
Zn(II)-AC	12.5mg/Ml	2.166±0.0647	[0.09]	[0.7]	[26.0]	[14.9]	[27.1]

ANOVA: indicates that comparison at the 5% level is statistically significant. Means having different superscripts ([a,b]) are significantly different (P<0.05), [ ] = % Inhibition

Table 4.19: Effect of Mg(II)-AC on carrageenan- induced rat paw edema

		edema diameter(mm)/Time interval (hour) [mean of inhibition]					
Group(n=5)	Dose(mg/kg)	0h	1h	2h	3h	4h	5h
Saline(control)	10mL/kg	2.148±0.0627	4.51±0.1339[a]	4.606±0.0501	4.738±0.0883[a]	4.654±0.0509	4.626±0.081[a]

			2.47±0.0278[b,c]	2.476±0.0427[b,c]	2.53±0.0673[b,c]	2.414±0.0682	2.322±0.0445[b,c]
piroxicam	20mL/kg	2.09±0.0453	[45.1]	[47.1]	[46.5]	[49.5]	[49.6]
			3.63±0.0249[b,d]	3.816±0.0238[b,d]	.844±0.0277[b,d]	3.836±0.0273	3.8±0.0141[b,d]
Mg(II)-AC	200mg/Ml	2.12±0.0432	[18.8]	[18.1]	[17.9]	[17.9]	[19.7]
			3.66±0.1964[b,d]	3.774±0.1839[bd]	3.888±0.0622[b,d]	3.82±0.1074	3.714±0.1023[b,d]
Mg(II)-AC	150mg/Ml	2.14±0.077	[19.5]	[17.2]	[18.9]	[17.6]	[17.9]
			3.992±0.1254[b,d]	4.276±0.2085[d]	4.206±0.2015[b,d]	4.424±0.2424	3.936±0.2647[b,d]
Mg(II)-AC	100mg/Ml	2.004±0.1753	[11.5]	[7.8]	[7]	[4.9]	[10.6]

ANOVA: indicates that comparison at the 5% level is statistically significant. Means having different superscripts ([a,b,c,d]) are significantly different ($P<0.05$), [] = % Inhibition

CHAPTER FIVE

5.0 DISCUSSION

The reactions of Acetaminophen with the metal (II) chlorides (Zn, and Mg) gave white solid complexes while that of acetaminophen with copper (II) chloride gave a brown solid complex (Table 4.2), according to equations 5.1- 5.1

$ZnCl_2.6H_2O + 2(C_8H_9NO_2) \rightarrow [Zn(C_8H_9NO_2)_2(H_2O)\ Cl]\ Cl.\ H_2O + 4H_2O$…….eq 5.1

$MgCl_2.6H_2O + 2(C_8H_9NO_2) \rightarrow [Mg(C_8H_9NO_2)_2\ (H_2O)Cl]\ Cl.\ H_2O + 4H_2O$…….eq 5.2

$CuCl_2.2H_2O + 2(C_8H_9NO_2) \rightarrow [Cu(C_8H_9NO_2)_2(H_2O)Cl]\ Cl.\ H_2O$……..eq 5.3

All the complexes were obtained in good yield (Table 4.2). The purity of the complexes was checked by taking the melting points. The sharp melting point of the complexes is an indication of the purity of the complexes (Refat *et al.,* 2013). The solubility of acetaminophen and the metal complexes was determined by dissolving the complexes in ethanol, water, methanol and dimethyl sulfoxide. All the complexes were soluble in ethanol, methanol and dimethyl sulfoxide but were found to be sparingly soluble in water (Table 4.1).The molar conductivity of the complexes was determined in methanol. The molar conductance values (Table 4.2) were in the range of 0.02mS/cm to 0.05 mS/cm. The low molar conductivity of the complexes is an indication of the non-electrolytic or covalent nature of the metal complexes (Refat *et al.,* 2013). Analytical data of the compounds, together with their physical properties are consistent with proposed molecular formula.

The electronic absorption spectral data and magnetic moment values of acetaminophen and its metal complexes are given in Table 4.3. The electronic absorption bands showed the absorption spectra of acetaminophen, in the range of 200-400 nm, (Table 4.3). The electronic spectra of acetaminophen show bands at (256nm). There was a shift in the bands of the metal complexes due to coordination

(Oluwatoosin *et al.,* 2014). As presented in Table 4.3, Cu(II)-AC, Zn(II)-AC, Mg(II)-AC showed electronic transition at 210, 293 nm, 206, 209, 293 nm 206, 215, and 295 nm respectively. The observed magnetic moment of Cu (II) complex was 1.35 B.M, which falls within the range observed for distorted octahedral geometry (Koushik*et al* 2007). The observed magnetic moment of Zn(II)-AC is 0.75 B.M and assumed a 6-coordinated octahedral geometry (Saha *et al.,*2000). Mg(II)-AC had an effective magnetic moment of 0.44 B.M, suggesting octahedral geometry (Conpolat and Kaya, 2004).

5.1 Infrared Spectra of Acetaminophen and its Complexes

The IR spectra provide valuable information regarding the nature of functional groups and their bonding where if present. In order to study the binding mode of acetaminophen to the metal ions, the Infrared spectrum of acetaminophen was compared with the Infrared spectra of the metal complexes. On comparison, the band positions of various vibrations were ascertained (Table 4.4).

The Fourier Transform Infrared Spectrum of acetaminophen (Table 4.4) showed some characteristic stretching bands at 3321, 3160, 3108 and 1651 cm^{-1}. In the analysis of acetaminophen, -NH group shows absorption band at 3321 cm^{-1}, the absorption band at 3160 and 3108 cm^{-1} can be assigned to –OH stretching vibration (Coates, 2000). The strong absorption band at 1651 cm^{-1} in acetaminophen spectrum is indicative of a carbonyl group (Figure 5.1).

5.1.1 Infrared Spectra of Mg(II)-AC Zn(II)-AC and Cu(II)-AC

In the analysis of Mg(II)-AC, several bands were modified. The disappearance of the characteristic –OH bands at 3108 and 3160$cm^{-1}$is an evidence of coordination through this group. The shifting of the carbonyl group from 1651 to 1547 cm^{-1} is also an indication of coordination through the carbonyl group. Comparing the absorption bands of AC and Mg(II)-AC, since there is no significant shift in the –NH group, then the –NH group is not involved in complex formation.

Infrared spectrum of Zn(II)-AC was also studied. The shift in stretching frequency of –OH bands from 3160- 3108 to 3205 $cm^{-1}$is observed after the formation of Zn(II)-ACcomplex due to the coordination. The insignificant shift in the –NH group is an indication that –NH is not involved in the formation of Zn(II)-AC and the shifting of the carbonyl group from 1651 to 1551 cm^{-1} is also an indication of coordination through the carbonyl group (Gulam and Manohar, 2014). By the analysis of Cu(II)-AC, several bands have been modified. The shift in the characteristic –OH bands at 3160- 3108 cm^{-1} is an evidence of coordination through this group. The shifting of the carbonyl group from 1651 to 1561 cm^{-1} is also an indication of coordination through the carbonyl group. Comparing the absorption bands of AC and Cu(II)-AC, there was no significant shift in the –NH group, therefore, the –NH group was not involved in complex formation (Gulam and Manohar, 2014).

5.2 Crystallite Size of the Metal Complexes

The Powder XRD patterns of Cu(II)-AC, Mg(II)-AC and Zn(II)-AC complexes are shown in Figure 5.1. The crystallite sizes are given in Table 4.5. The Powder XRD patterns of acetaminophen and its complexes were recorded. The XRD patterns of all complexes are similar and suggest that all the complexes possess similar structure (Kavitha *et al.,* 2013).

5.3 Proposed Structure of the Synthesized Complexes

The proposed structure of acetaminophen complexes of Mg(II), Zn(II) and Cu(II) ions was derived from the decomposition temperature, infrared, molar conductance, UV–Visible, magnetic susceptibility and X-Ray diffraction analysis data. Thus, from the infrared spectra, acetaminophen is seen as a bidentate ligand, coordinated to the metal ions-oxygen atoms of the hydroxyl and carbonyl groups. From the molar conductance data, the complexes are found to be non-electrolytes. On the basis of the above observations, octahedral geometry structure is suggested for the investigated complexes (Figure 5.1).

Figure 5.1: The proposed structure of $[M(C_8H_9NO_2)_2(H_2O)\ Cl]\ Cl.\ H_2O$

M = Zn, Mg and Cu

5.4 Antimicrobial activity of Acetaminophen, Zn(II)-AC, Mg(II)-AC and Cu(II)-AC against the Test Microorganisms

The susceptibility of the isolates to the metal complexes and acetaminophen at various concentrations (100, 50, 12.5 and 6.25 mg/ml) was carried out. Table 4.6 shows the activity of acetaminophen against *Pseudomonas aeruginosa* (18 and 15 mm) at 50 and 25 mg/ml. Acetaminophen showed no significant effect against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli, Salmonella typhi* and *Candida albicans*. The inactivity of acetaminophen was attributed to their probable lipophilic character (Obaleye *et al*., 2011).

Mg(II)-AC (Table 4.7) showed activity against three of the six microorganisms, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* with the inhibitory zones of 19.0, 15.0 and 13.0 mm respectively at the concentrations of 50, 25 and 12.5 mg/ml respectively for *Staphylococcus aureus*, 15 and 12 mm at 50 and 25 mg/ml for *Salmonella typhi* and 27, 18 and 13 mm for *Pseudomonas aeruginosa*. Mg(II)-AC was inactive against *Bacillus subtilis*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. The test of Mg(II)-AC on some bacteria isolates revealed that Mg(II)-AC possess antimicrobial activities compared to acetaminophen which showed activity against only *Pseudomonas aeruginosa.*

Zn(II)-AC showed inhibitory activity against *Staphylococcus aureus* (Table 4.8) with the inhibitory zones of 20, 17, 14 and 10 mm at 50, 25, 12.5 and 6.25 mg/ml respectively. It also showed inhibitory activity against *Pseudomonas aeruginosa* with inhibitory zones of 20, 15 and 12 mm at 50, 25 and 12.5 mg/ml respectively.

Cu(II)-AC (Table 4.9) showed inhibitory activity against all the bacteria, *Bacillus subtilis* (21, 18, 16 and 14 mm), *Staphylococcus aureus* (23, 20, 17 and 15 mm), *Escherichia coli* (22, 19, 17 and 14 mm), *Salmonella typhi* (18, 16, 14 and 10 mm) and *Pseudomonas aeruginosa* (24, 21, 19 and 16 mm) at 50, 25, 12.5 and 6.25 mg/ml. Cu(II)-AC showed no activity against *Candida albicans* and *Aspergillus niger*. This is due to chelation, which reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with donor groups of the ligand, which increases lipophilic character, favouring its permeation through lipid layers of the bacterial membrane (Osowale *et al.,* 2014). Zn(II)-AC had the least antibacterial activity while Cu(II)-AC had the best antibacterial activity, being active against all the bacteria, proving its potential as broad spectrum of antibacterial agent.

5.5 Susceptibility of Ciprofloxacin, Fluconazole, Copper Chloride, Magnesium Chloride and zinc chloride against the Test Microorganisms

Ciprofloxacin (Table 4.10) showed inhibitory activity against all the bacteria *Bacillus subtilis* (39, 32, 28 and 25 mm), *Staphylococcus aureus* (38, 30, 25 and 20 mm),*Escherichia coli*(30, 25, 22 and 19 mm), *Salmonella typhi* (37, 32, 28 and 25 mm) and *Pseudomonas aeruginosa* (25, 21, 18 and 16 mm) at 50, 25, 12.5 and 6.25 mg/ml. while Fluconazole showed activity against *Candida albicans* (36, 32,28 and 25 mm) and *Aspergillus niger* (25, 20, 15 and 13 mm).

Copper Chloride (Table 4.1) showed inhibitory activity against all the bacteria; *Bacillus subtilis* (18, 17, 15 and 10 mm), *Salmonella typhi* (19, 18, 13 and 12 mm) and *Pseudomonas aeruginosa* (20, 16, 15 and 11 mm) at 50, 25, 12.5 and 6.25 mg/ml. Copper Chloride showed no activity against *Staphylococcus aureus*, *Escherichia coli.* Magnesium Chloride showed activity against *Staphylococcus aureus* while Zinc Chloride showed no activity against any test organisms.

The analysis of the results showed a good antimicrobial activity of the metal complexes relatively superior to their ligands. This is in line with the work of (Osowale *et al.,* 2014) who showed

that the high antimicrobial activity of the complexes is due to chelation, which reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with donor groups of the ligand, which increases lipophilic character, favouring its permeation through lipid layers of the bacterial membrane.

Ciprofloxacin, Fluconazole, Cu(II)-AC, Zn(II)-AC and Mg(II)-AC produced significantly sized zones of inhibition against the tested bacteria and fungi. $ZnCl_2$ produced no observable inhibitory effect against the test organisms (Table 4.6 to Table 4.11). However, the overall results indicate that the three metal complexes showed better inhibitory activity than acetaminophen against the organisms used under the same experimental conditions. Cu(II)-AC being active against all the test bacteria showed the best Inhibitory effects.

5.6 Minimum Inhibitory Concentration and Minimum Inhibitory Concentration of Acetaminophen, Ciprofloxacin, Fluconazole, Cu(II)-AC, Zn(II)-AC and Mg(II)-AC

At the end of the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion, the lowest concentration in the series without visible sign of growth was considered to be the minimum inhibitory concentration (MIC) as shown in Table 4.12. The minimum concentration of Mg(II)-AC and Zn(II)-AC required to inhibit the growth of *Staphylococcus aureus* and *Salmonella typhi* was 50 mg/ml, the minimum concentration of AC, Mg(II)-AC and Zn(II)-AC required to inhibit the growth of *Pseudomonas aeruginosa* was 25 mg/ml, while the minimum inhibitory concentrations of Cu(II)-AC required to inhibit the growth of bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* were 25, 25, 25, 12.5 and 12.5 mg/ml respectively.

The MBC determines the lowest concentration of an antibacterial agent required to kill a particular bacterium (*Cushnieet al*., 2007). The MIC result was used to determine the MBC.

Acetaminophen, Zn(II)-AC and Mg(II)-AC showed bactericidal activity against *Pseudomonas aeruginosa*at 50 mg/ml but are bacteriostatic against *Bacillus subtilis* (Gram-positive),*Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), *Salmonella typhi* (Gram-negative).

Mg(II)-AC and Zn(II)-AC showed the same inhibitory strength against *Staphylococcus aureus* and *Salmonella typhi.* Acetaminophen, Mg(II)-AC and Zn(II)-AC showed the same inhibitory strength against *Pseudomonas aeruginosa* while Cu(II)-AC showed inhibitory strength higher than Mg(II)-AC and Zn(II)-AC. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were more susceptible to Cu(II)-AC showing the lowest MIC and MBC values of 12.5 and 25 mg/mL. This is a great importance as it has been reported that some organisms have developed resistance to many antibiotics, which sometimes makes its clinical management difficult (Adewunmi *et al.,* 2001). This result agreed with the work of Lawal and Obaleye (2007) who showed that metal complexes were found to have more antimicrobial activity than the ligand.

The differences in the susceptibilities of the isolates to the complexes can be related to the cell wall composition of organisms. Gram-positive bacteria have cell wall composed of peptidoglycan with techoid acid in between, so gram-positive bacteria are more susceptible than gram-negative bacteria that have their cell wall surrounded by bi-lipid layers of gram-negative lipoglycans and lipoproteins, which prevent the penetration of antibiotics through their cell wall (Lee *et al.,* 2009).

5.7 Acute Toxicity

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance (Walum, 1998).

The results for Acute toxicity study of Cu(II)-AC on rats showed that no mortality was recorded in any of the test groups within 24 hours in the first phase. In the second phase, mortality was recorded at 5000 mg/kg.The result for Acute toxicity study of Zn(II)-AC on rats showed that all the rats in the third group died within 24 hours in the first phase. In the second phase, mortality was recorded at 370 and 600 mg/kg.

The lethal dose determination (LD_{50}), after the administration of Mg(II)-AC showed that one rat died within 24 hours in the first phase. In the second phase, mortality was recorded at 1600 and 2900 mg/kg. Based on the results, further specific doses were administered to calculate the LD_{50}.

Then the LD_{50} is calculated by the formula: $\sqrt{D_o \times D_t}$..... Eq 5.1

D_o= Highest dose that gave no mortality

D_t= Lowest dose that gave mortality

According to Hodge and Sterner toxicity scale (2005), substances with lethal dose (LD_{50}) ≥5000mg/kg are considered to be practically non-toxic, 500 - 5000 mg/kg are considered to be slightly toxic, 50 - 500 mg/kg are considered to be moderately toxic, 1 - 50 mg/kg are considered to be highly toxic, Less than 1 mg/kg are considered to be extremely toxic.

From Table 4.14 to 4.16, the LD_{50} of Zn(II)-AC, Cu(II)-AC and Mg(II)-AC were 289 mg/kg, 3808 mg/kg and 1265 mg/kg respectively. So the three complexes were considered to be moderately toxic and slightly toxic. Below 30 % of the LD_{50} was used to carry out anti-inflammatory activity of the complexes.

5.8 Anti-inflammatory Activity

The rats were divided into five groups. The injection of 0.1ml of freshly prepared carrageenan suspension into the sub planter region of the left hind paw of Group 1 (normal saline) produced an edema reaching its maximum (4.764±0.4) at the third hour (Girish and Khan, 2016).

The test complexes in general showed maximum inhibition percentage at about 3 hrs. After 4 hrs it goes on reducing and reaches a minimum at about 5 hrs. The percentage anti-inflammatory effect of Cu(II)-AC (Table 4.17) at the peak of carrageenan-induced edema at the third hour were 22.5 %, 24.4 %, 32.4 % at respective doses of 200, 400 and 600 mg/kg compared to 36.1% for piroxicam at 20 mg/kg. At the fourth and fifth hour, there was an increase in the percentage Anti-inflammatory effects and a decrease in the volume of edema. Cu(II)-AC (200, 400 and 600 mg/kg) produced a significant ($p<0.05$) decrease in the size of the paw oedema as shown in Table 4.17.

From Table 4.18, the percentage anti-inflammatory effect of Zn(II)-AC at doses of 50, 25 and 12.5 mg/ml at the 3rd hour was 25.3 %, 26.9 % and 26 %. The percentage anti-inflammatory effect of Piroxicam at the third hour was 42.7% at 20mg/kg. There was an increase in percentage Anti-inflammatory effect at the 4th and 5th hours. Zn(II)-AC produced a significant ($p<0.05$) decrease in the size of the paw oedema as shown in Table 4.18. Zn(II)-AC (50, 25 and 12.5mg/kg) was more effective than normal saline, but not as effective as piroxicam (10 mg/kg)

There was also a general decrease in the diameter of the edema and increase in the percentage anti-inflammatory effect of at the fourth and fifth hour for Mg(II)-AC (Table 4.19) and the percentage anti-inflammatory effect at its peak was at the third hour (for the three doses :200, 150 and 100 mg/kg) were 17.9 %, 18.9 % and 7 %. Mg(II)-AC produced a significant ($p<0.05$) decrease in the size of the paw oedema as shown in Table 4.19. Mg(II)-AC (200, 150 and 100 mg/kg) was more effective than normal saline, but not as effective as piroxicam (10 mg/kg)

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Cu(II)-AC, Mg(II)-AC and Zn(II)-AC have been synthesized and characterized by FTIR spectroscopy, electronic spectroscopy, conductivity measurement and XRD. It was proven that the formation of complexes occurred via both C=O and –OH groups from Acetaminophen.

The antibacterial studies showed that all the metal complexes had better antibacterial activity than acetaminophen and their metal chlorides. Based on the observations made, the three complexes were considered to be moderately toxic and slightly toxic according to Hodge and sterner (2005).

There was also a general decrease in the diameter of the edema. Increase in the percentage Anti-inflammatory effect was observed at the fourth and fifth hours for the three complexes, and was at its peak was in the third hour. The percentage anti-inflammatory effect of piroxicam at the 3rd hour was 46.5%. Since the percentage anti-inflammatory effects of the complexes are higher than that of the normal saline, we can conclude that the complexes have anti-inflammatory activity.

6.2 Recommendations

Based on the findings of this study, the following recommendations are hereby made:

i. Analgesic activity of Cu(II)-AC, Mg(II)-AC and Zn(II)-AC complexes should be determined to compare the analgesic activity of Acetaminophen and the synthesized complexes.

ii. Median effective dose (ED_{50}) of Cu(II)-AC, Mg(II)-AC and Zn(II)-AC should be carried out to determine the dose that will producea therapeutic response.

iii. There is need for further research on In-vivo antimicrobial activity of the synthesized complexes.

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APPENDICES

Appendix I: List of Apparatus and Equipment

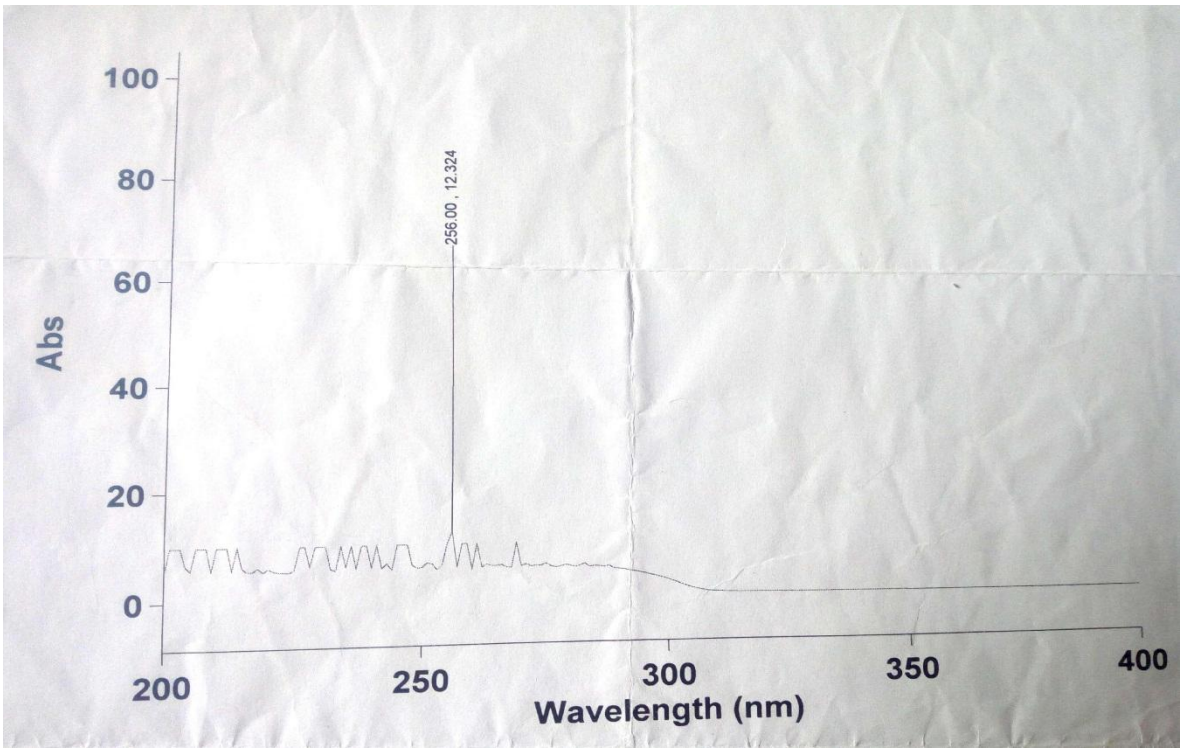
1. Conical Flasks
2. Beakers
3. Volumetric Flasks
4. Measuring cylinder
5. Wash bottle
6. Funnel
7. Filter paper
8. Desiccator
9. Analytical balance
10. Water bath

11. Magnetic stirrer
12. Pipette
13. Spatula
14. Conductivity meter
15. Digital caliper
16. Glass rod

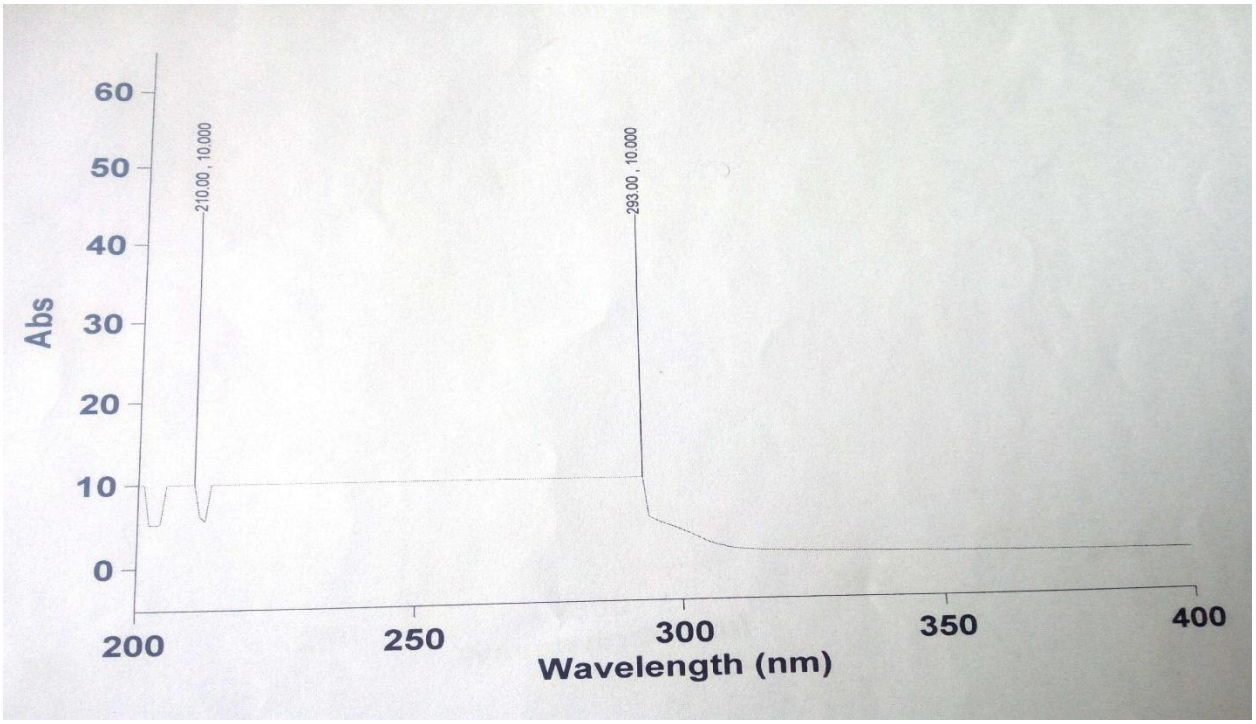
List of Reagents and Chemicals

1. $ZnCl_2$. $6H_2O$
2. $MgCl_2$. $6H_2O$
3. $CuCl_2$. $2H_2O$
4. Acetaminophen
5. Methanol
6. Ethanol
7. Chloroform
8. Dimethyl sulfoxide
9. Distilled water
10. Inoculation loop
11. Incubator
12. Petriplate
13. Ruler

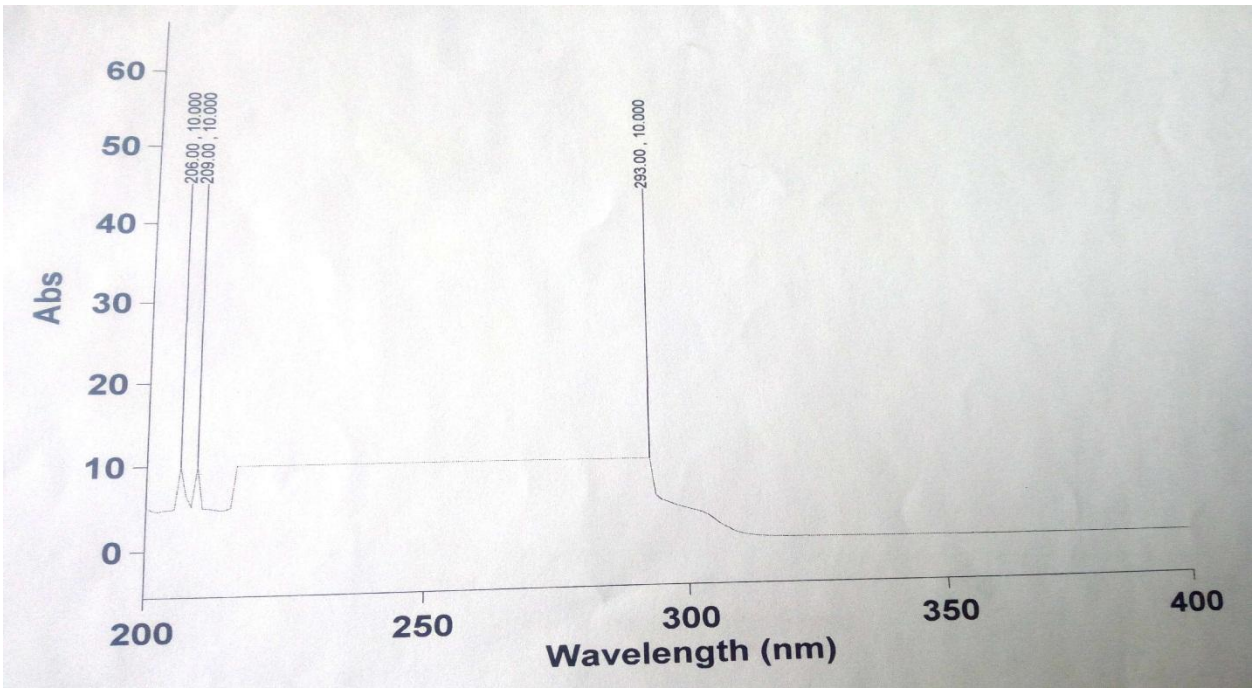
Appendix II: UV Spectra of Acetaminophen


100
80
60
40
20
0
Abs
256.00 , 12.324
200
250
300
350
400
Wavelength (nm)

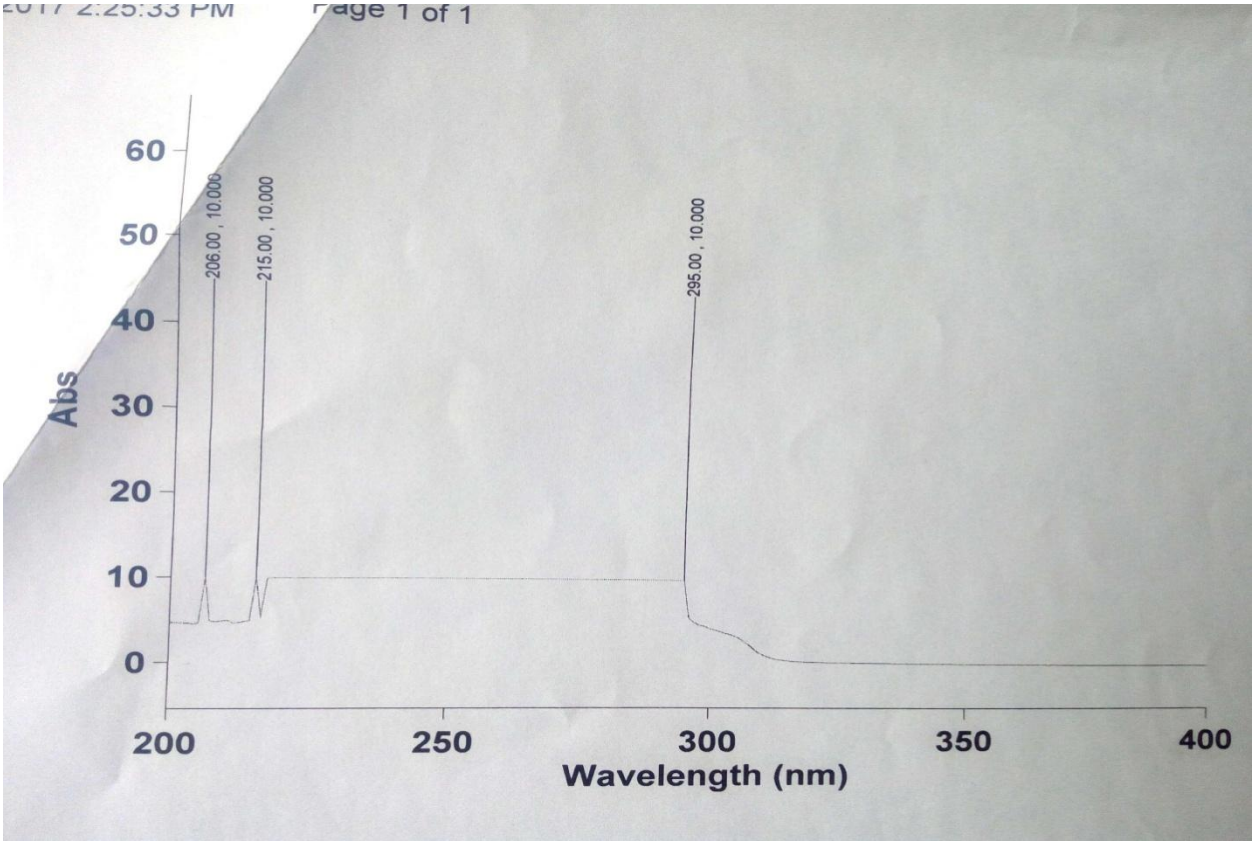
Appendix III:UV Spectra of Acetaminophen- Copper complex



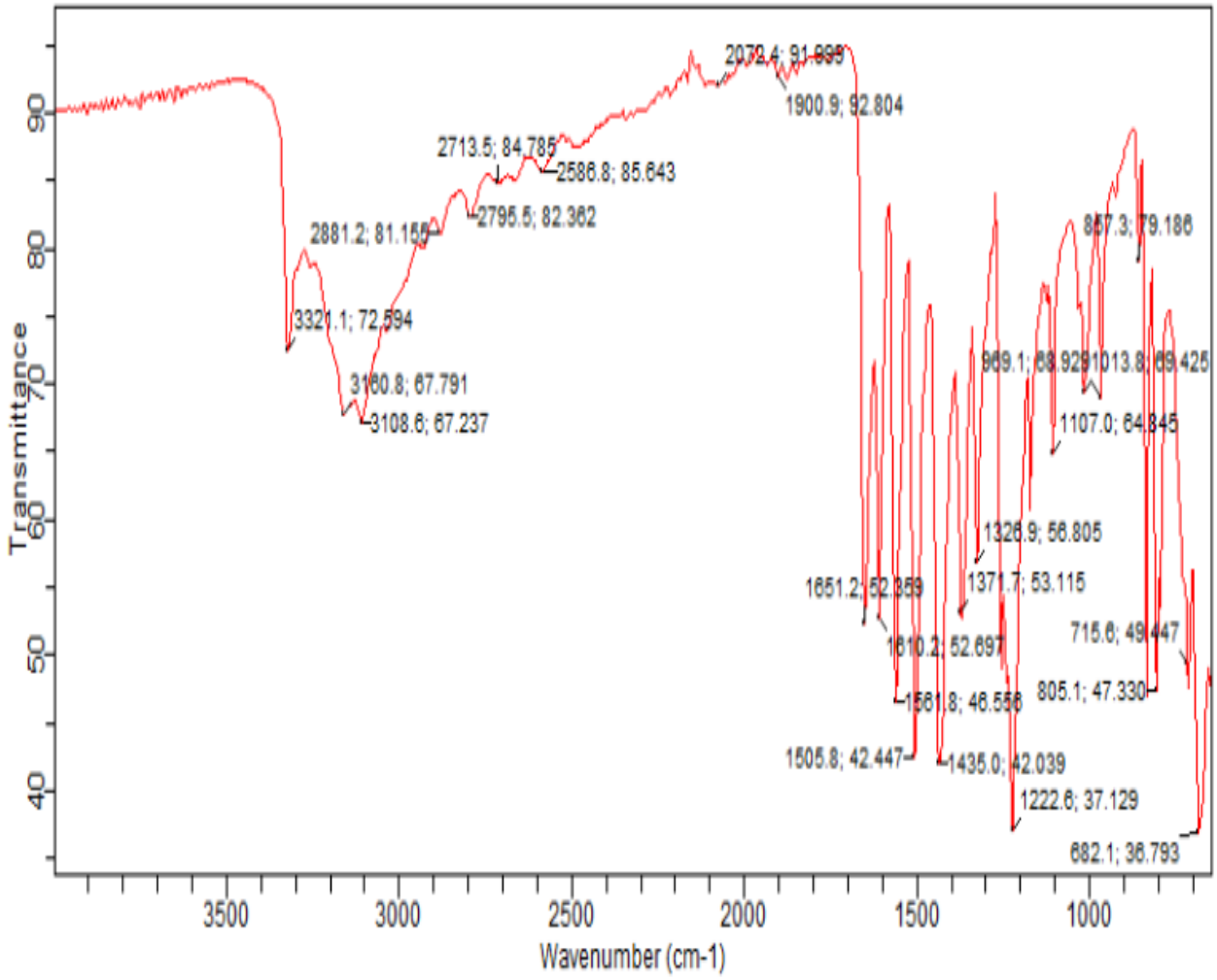
Appendix IV: UV Spectra of Acetaminophen- Zinc complex



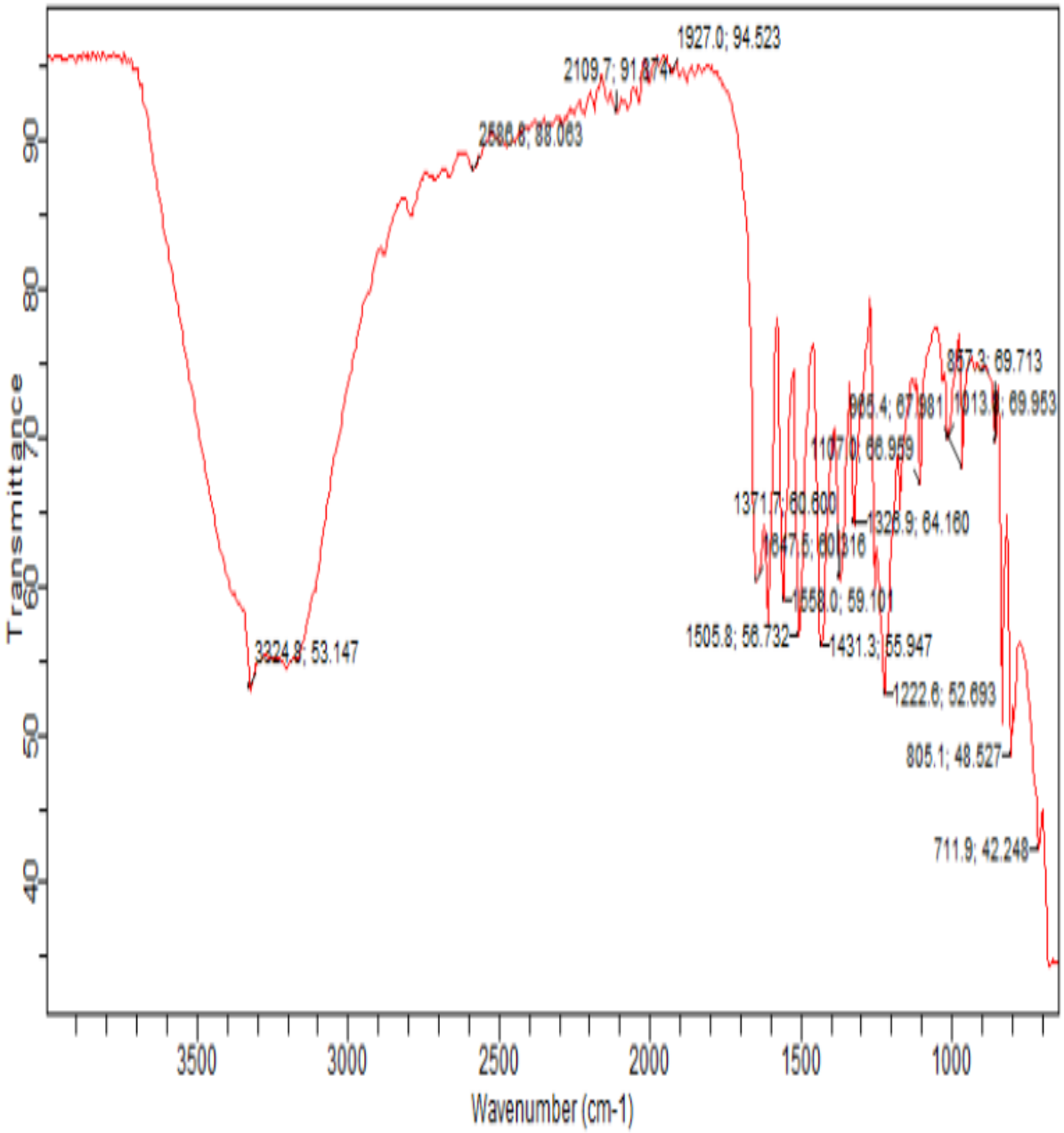
Appendix V: UV Spectra of Acetaminophen- Magnesium complex



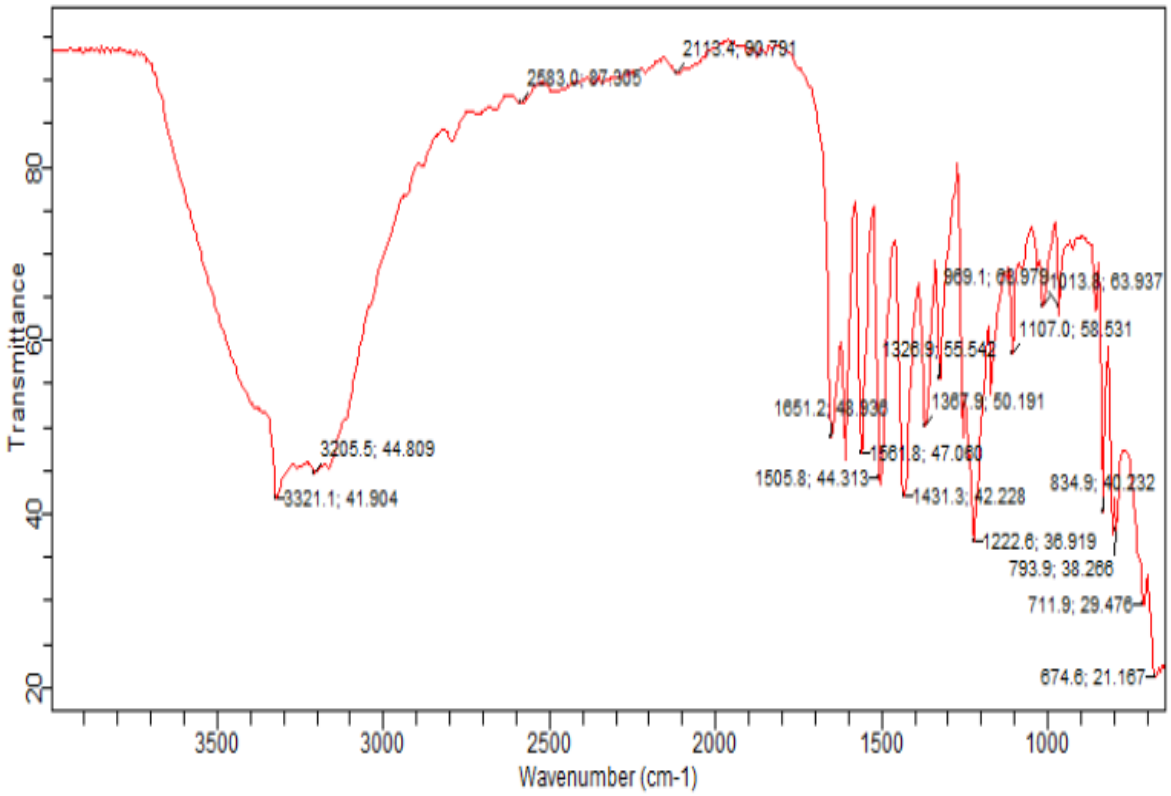
Appendix VI: FTIR spectra of Acetaminophen


Transmittance
Wavenumber (cm-1)
3500
3000
2500
2000
1500
1000
90
80
70
60
50
40
2881.2; 81.155
3321.1; 72.694
3160.8; 67.791
3108.6; 67.237
2713.5; 84.785
2586.8; 85.643
2795.5; 82.362
1900.9; 92.804
857.3; 79.186
1107.0; 64.845
1326.9; 56.805
1371.7; 53.115
1651.2; 52.359
1610.2; 52.697
1561.8; 46.556
1505.8; 42.447
1435.0; 42.039
1222.6; 37.129
715.6; 49.447
805.1; 47.330
682.1; 36.793

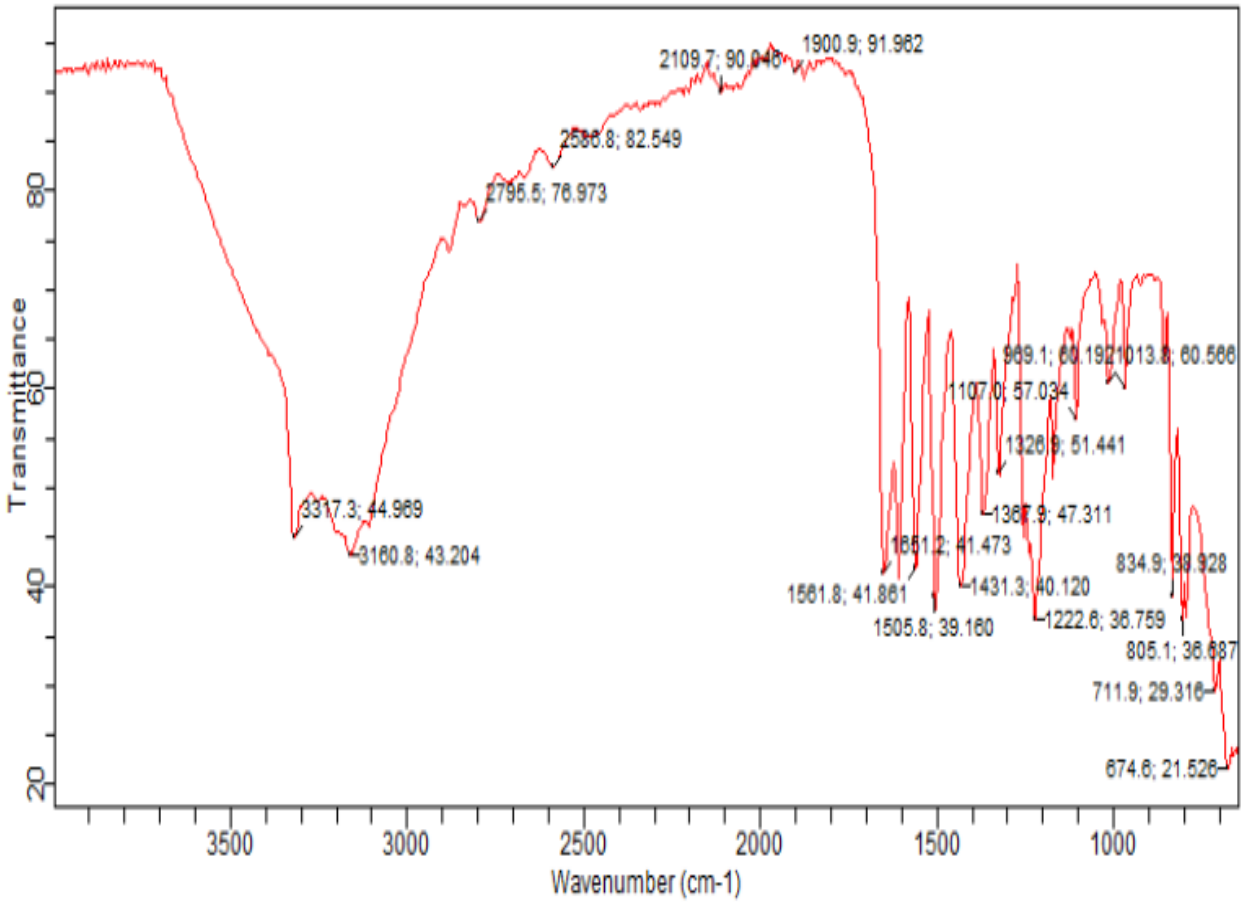
Appendix VII: FTIR spectra of Mg(II)-AC Complex


1927.0; 94.523
2109.7; 91.874
2586.8; 88.063
3324.8; 53.147
1371.7; 60.600
1647.5; 60.316
1326.9; 64.160
1558.0; 59.101
1505.8; 56.732
1431.3; 55.947
1222.6; 52.693
805.1; 48.527
711.9; 42.248
857.3; 69.713
1013.8; 69.953
1107.0; 66.959
Transmittance
Wavenumber (cm-1)

Appendix VIII: **FTIR spectra of Zn(II)-AC Complex**



Appendix IX: FTIR spectra of Cu(II)-AC Complex



Appendix X: Anti-inflammatory Activity of Cu(II)-AC

Group(mg/mL)		weight(g)	0h(mm)	1h(mm)	2h(mm)	3h(mm)	4h(mm)	5h(mm)
		181	1.75	4.88	4.47	4.76	4.62	4.7
	I	184	1.97	4.26	4.7	5.4	4.9	4.8
(saline)		245	2.14	4.8	5.14	4.9	4.7	4.29
		132	1.8	3.63	4.26	4.33	4.95	4.72
		101	1.77	3.96	4.17	4.43	4.51	4.72
	II	118	1.77	2.85	2.37	2.64	2.59	2.77
(Piroxicam)		159	2.26	3.3	3.16	2.74	3.02	2.88
		126	1.74	3.17	2.81	2.7	2.58	2.89
		186	2.12	4	4.63	4.43	4.7	4.28
		106	1.98	3.09	3.04	2.7	2.64	2.38
		113	1.83	3.19	3.6	2.79	3.47	2.96
	III	147	1.81	2.94	3.77	3.62	3.28	2.81

Cu(II)-AC		155	1.96	3.64	3.98	3.17	4.05	3.78
(200)		139	1.88	3.08	3.66	3.62	3.77	3.83
		243	2.27	3.78	4.55	5.25	4.52	4.33
		168	1.85	2.7	2.83	2.84	3.76	3.38
	IV	162	1.99	2.78	2.91	3.98	3.97	3.88
Cu(II)-AC		135	2.07	3.01	4.77	4.49	3.95	3.9
(400)		218	2.36	2.83	3.45	3.06	3.5	3.23
		163	2.09	3.05	3.93	3.64	3.6	3.64
		140	2.57	3.23	3.13	3.07	3.07	2.78
	V	212	1.69	3.08	3.71	3.03	3.03	3.58
Cu(II)-AC		126	2.04	3.25	3.91	3.5	3.5	2.93
(600)		126	2.14	2.75	3.28	3.48	3.48	2.82
		121	1.6	2.58	3.46	3.02	3.02	2.36

Appendix XI: Anti-inflammatory Activity of Zn(II)-AC

Group (mg/mL)	weight(g)	0h(mm)	1h(mm)	2h(mm)	3h(mm)	4h(mm)	5h(mm)
	163	2.04	4.3	4.98	4.84	4.62	5.19
I	132	2.5	4.38	4.94	5.41	4.16	5.3
(saline)	164	2.15	4.65	4.93	4.99	4.71	4.53
	124	2.16	3.91	4.6	5.94	5.25	4.97
	142	2.04	4.01	5.36	6.45	4.63	5.7
	124	2.16	2.45	2.81	3.18	3.01	2.85
II	122	2.13	2.78	3.11	3	2.78	2.77
piroxicam	151	2.19	2.56	2.79	2.82	2.96	2.61
	128	2.21	2.71	3.15	4.01	3.92	3.12
	117	2.17	3.32	2.66	2.83	2.81	2.8
	162	2.04	3.78	3.53	3.91	3.45	3.35
III	153	2.5	3.81	3.92	3.4	3.4	3.04
Zn(II)-AC	186	2.15	3.58	4.21	3.79	3.67	3.74
(50)	168	2.16	4	4.38	4.6	4.12	3.94
	135	2.04	3.6	4.32	4.93	4.51	4.1
	195	1.81	3.98	3.37	3.88	3.44	3.47
IV	156	2.09	3.75	4.94	3.67	3.23	3.08
Zn(II)-AC	125	2.03	3.6	4.87	3.97	3.51	3.39
(25)	138	2.12	3.38	4.41	3.76	3.97	3.04
	155	2.09	3.83	5.11	4.93	4.65	4.09
	155	2.1	4.41	4.15	3.69	3.91	3.18
V	196	2.13	3.96	4.52	3.91	3.95	3.64
Zn(II)-AC	155	2.16	3.98	4.55	3.74	3.85	3.8

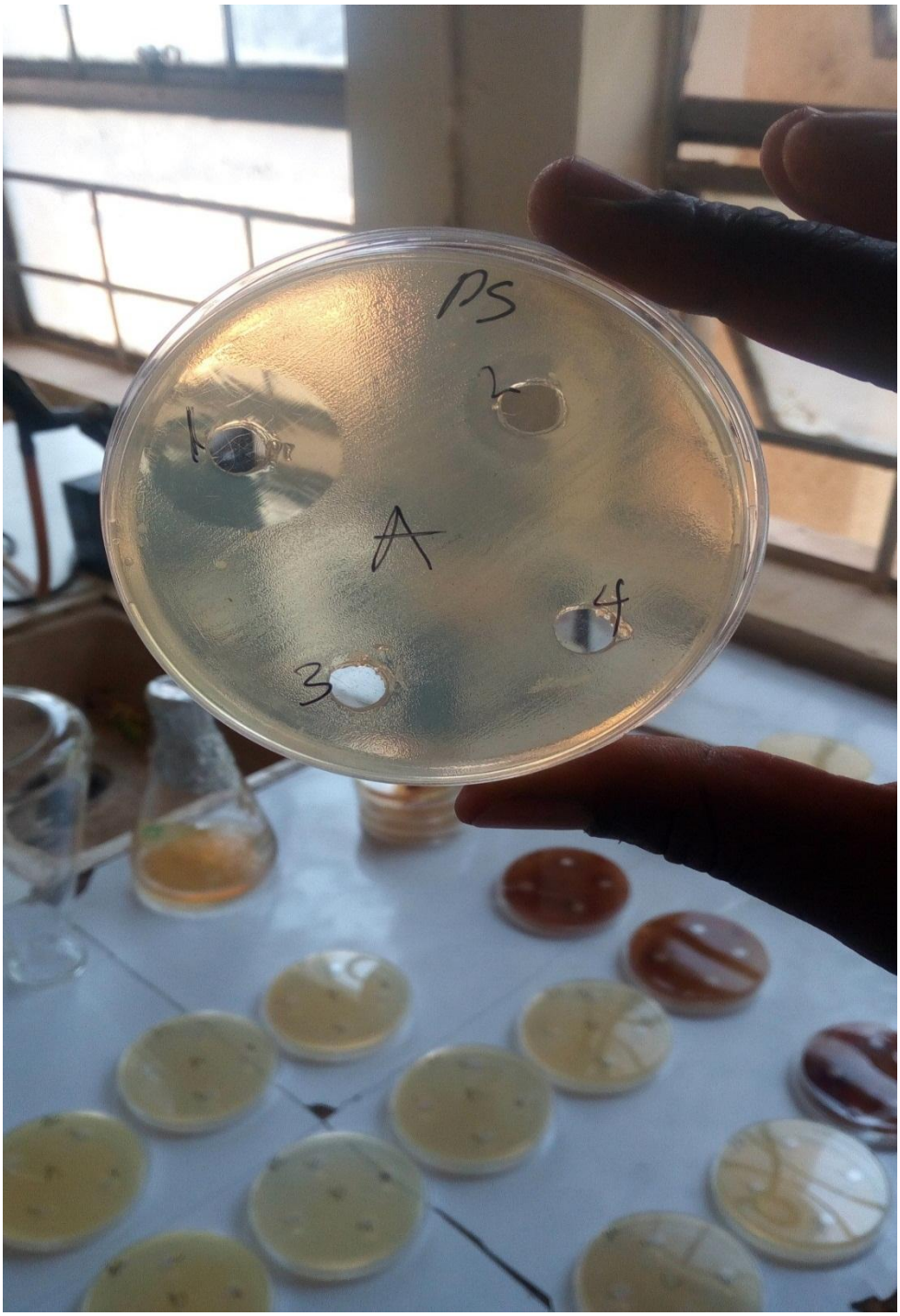
(12.5)	176	2.41	4.63	5.53	4.32	4.23	4.12
	219	2.03	4.25	5.88	4.79	3.95	3.97

Appendix XII: Anti-inflammatory Activity of Mg(II)-AC

Group (mg/mL)	weight(g)	0h(mm)	1h(mm)	2h(mm)	3h(mm)	4h(mm)	5h(mm)
	163	2.19	4.31	4.51	4.58	4.77	4.63
I	184	1.98	4.41	4.57	4.73	4.69	4.65
(saline)	118	2.32	5.04	4.5	4.86	4.74	4.7
	204	2.22	4.37	4.72	5	4.51	4.82
	146	2.03	4.42	4.73	4.52	4.56	4.33
	161	2.21	2.45	2.64	2.52	2.66	2.28
II	125	1.98	2.42	2.43	2.61	2.34	2.19
(piroxicam)	133	2.15	2.55	2.41	2.74	2.42	2.44
	158	2.12	2.41	2.48	2.41	2.25	2.3
	126	1.99	2.52	2.42	2.37	2.4	2.4
	155	2.18	3.59	3.86	3.82	3.79	3.81

III	146	2.22	3.64	3.76	3.79	3.78	3.78
Mg(II)-AC (200)	135	1.97	3.72	3.81	3.82	3.81	3.77
	128	2.09	3.62	3.77	3.84	3.88	3.85
	200	2.14	3.58	3.88	3.95	3.92	3.79
	165	1.92	3	3.5	3.87	4.11	3.83
IV	140	2.26	3.64	3.74	4.01	3.97	3.91
Mg(II)-AC (150)	153	2.18	3.98	3.38	3.74	3.52	3.33
	156	2	4.13	4.44	4.05	3.86	3.81
	145	2.06	3.55	3.81	3.77	3.64	3.69
	163	2.17	4.22	4.31	4.68	4.62	4.53
V	176	2.22	3.97	4.67	4.31	4.22	3.83
Mg(II)-AC (100)	154	2.02	4.08	4.33	4.57	4.75	4.43
	122	1.99	4.17	4.58	4.73	4.97	4.57
	170	2.08	3.52	3.49	3.74	3.58	3.32

Appendix XIII: **Susceptibility test of Acetaminophen and the metal complexes using Agar Well Diffusion Method.**


PS
A
3
4

Appendix XIV: **Minimum Inhibitory Concentration (MIC) of Acetaminophen and the metal complexes**

Appendix XV: Inflamed carrageenan induced edema in rats

Appendix XVI: Inflamed carrageenan induced oedema in rats

Appendix XVII: Digital Caliper

OFF
ON
ZERO
.mm/inch
STAINLESS
HARDENED