GREEN SYNTHESIS OF IRON OXIDE NANOPARTICLES USING *OCIMUM LAMIIFOLIOUM* FOR ANTI- BACTERIAL ACTIVITIES

By: Motuma Ejigu

A Thesis Submitted to the Applied Chemistry Program

In Partial Fulfillment of the Requirements for the degree of Master of Science in Applied Chemistry

School of Applied Natural Science

Adama Science and Technology University

September, 2018

Adama, Ethiopia

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APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defense by Motuma Ejigu have read and evaluated his thesis entitled "**GREEN SYNTHESIS OF IRON OXIDE NANOPARTICLES USING *OCIUM LAIIFOLIUM*: FOR ANTI BACTERIAL ACTIVITIES** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in Chemistry.

Advisor	Signature	Date
Co - Advisor	Signature	Date
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DECLARATION

I hereby declare that this M.Sc Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Motuma Ejigu

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This M.Sc Thesis has been submitted for examination with our approval as thesis advisors.

Advisor Name: Dr. Fedlu Kedir (PhD) Co-advisor Name: Dr. Enyew Amare (PhD)

Signature:_____ Signature:_____

Date of submission:_____

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TABLE OF CONTENTS

Pages

List of Table

LIST OF FIGURES

LIST OF ABBREVIATIONS

IONPs	Iron oxide nanoparticles
IR	Infrared
XRD	X – Ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
E.Coli	Escherichia Coli
DNA	Deoxyribonucleic acid
ROS	Reactive Oxygen Species
DMF	N, N – dimethyl form amide
PVP	Poly n-Vinyl pyrrolidine
TFATEB	tetra – n – tetra – fluroborate
eV	Electron volt
meV	milielectron volt
LED	Light Emitting Diodes
TGA	Thermal Gravimetric Analysis
Nm	nanometer
NPs	nanoparticles
DTA	Deferential thermal analysis
MM	mille meter
mA	mille ampere
KV	kilo volte
ROS	reactive oxygen species
COD	chemical oxygen demand
TDD	Targeted drug delivery

MRI	magnetic Resonance interference
EMU	Electro Mass Unit

ABSTRACT

The progress of green chemistry in the synthesis of nanoparticles using plants has attracted a great attention nowadays due to its low cost, simple, non-toxic and environmental-friendly nature. The present study focuses on the green synthesis and characterization of Iron oxide nanoparticles using different ratios of ocimum Lamiifolium leaf extract for antibacterial application on the drug resistant bacteria. Iron (II) and Iron (III) hydrated chloride were used as precursor to synthesize Fe_3O_4 nanoparticles using the Ocimum Lamiifolium leaf extract. $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ used to synthesis Fe_3O_4 nanoparticles. The synthesized nanoparticles were characterized by advanced techniques like Thermo Gravimetric Analysis (TGA), X-Ray Diffraction (XRD) , Fourier Transform Infrared Spectroscopy (FT-IR), UV-Visible spectroscopy, scanning electron microscopy (SEM) with EDS along with the evaluation of their antibacterial activity against gram positive and gram negative bacteria with a view to explore their pharmaceutical applications.

Iron oxide nanoparticles were synthesized by co precipitation method using ferric chloride and NaOH as precursor to study the thermal stability of materials. The XRD analysis reveals all the synthesized particles have pure hexagonal phase with particle size in the range of 13 – 37 nm. The FT-IR analysis shows the interaction between the capping agents and the synthesized Fe_3O_4 nanoparticle. These Fe_3O_4-NPs fabricated through biosynthesis method, are promising candidate in various applications like biomedical and utilizing as recyclable magnetic nano-catalyst for organic reactions. The study on antibacterial activity of synthesized Fe_3O_4 NPs, against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa* have better performance with the reference to the selected standards penicillin. The iron oxide nanoparticles showed their antibacterial properties on both gram positive and gram negative bacterial strains. As the diameter of the zone of inhibition is high, we can conclude that iron oxide is a very effective antibacterial agent. .

Keywords: *Ocimum Lamiifolium,* Iron oxide nanoparticle, characterization, Escherichia coli, Pseudomonasoeruginose, Staphylococcus aureus and Bacillus subtilus, penicillin**.**

1. INTRODUCTION

1.1 Background

Green synthesis of nanoparticles using plant extract is the novel method to develop environmentally being nanoparticles which can be used in numerous biomedical applications. Many plant species and herbs suggested the presence of anti-oxidative and antimicrobial constituents (Hirasa *et al*., 2003). Among the important constituents' in plants, phenolic compounds are mainly involved in the cell defense system against free radicals (Szeto *et al.,* 2002). A number of phenolic compounds with strong antioxidant and antimicrobial activities have been identified in plants, especially in those belonging to the Lamiaceae family. Traditional medicinal plants have long been used in Ethiopia to treat human and livestock ailments. Despite a well-documented rich tradition of medicinal plant use in the country, their direct antimicrobial effects are still poorly known. In this study, reducing and stabilizing properties of Ocimum lamiifolium in iron Oxide nanoparticles (Fe_3O_4) synthesis. The objective of the present study is to synthesize Fe_3O_4 NPs using plant extract of leaves of Ocium Lamifolium as reducing and stabilizing agent and test their antibacterial activity. The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in plants. These compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant (Raj *et al*., 2010). Generally, leaves are the favorable storage site for desired compounds. A variety of laboratory methods can be used to evaluate or screen the in vitro antibacterial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods.

Ociumum Lamiifolium is a perennial evergreen shrub having oblong, ovate green colored leaves (0.5-5 meter height), oppositely arranged having pubescent leaf surface, narrow at the base and deeply serrated (Kashyap et al., 2011). The genus Ocimum is cultivated for its extraordinary essential oil which display many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent (Kashyap *et al.,* 2011). It has wide range of therapeutic effects like antimicrobial, antispasmodic, bactericide, carminative, anthelmintic, hepatoprotective, antiviral, larvicidal, remedy of coughs, colds, measles, abdominal pains, diarrhea, insect repellent, particularly against mosquitoes and

storage pest control. The essential oils obtained from this plant as repellent against nuisance biting insects and malaria vector (kashyap *et al*., 2011).

Iron oxides are chemical compounds composed of iron and oxygen. There are many known iron oxides. In this paper we are focusing on iron (II, III) oxide, magnetite (Fe_3O_4). It is mixture of Iron (II) and Iron (III) oxides of a red brown iron compounds. IONPs are widely used for numerous applications in solid state. It is an oxide which used intensively for both fundamental science interests and technological applications. Fe_3O_4 has the face centered cubic spinel structure, based on 32 O_2−ions and close-packed along the direction (Martinez *et al* 2012). Magnetite is biocompatible and therefore it is one of the most extensively used biomaterials for different applications like cell separation, drug delivery in cancer therapy, magnetic induced hyperthermia, magnetic resonance imaging (MRI) and others antibacterial activities (Kashyap *et al.,* 2011) . Iron oxide nanoparticle(IONP)is a particle defined as a small object that behaves as a whole unit in terms of its transport and properties, usually ranging from 1 to 100 nanometers (nm). The nanomaterial's may provide solutions for technological and environmental challenges in the areas of antibacterial activities, solar energy conversion, catalysis, biology biomedical science, and water treatment. Nanoparticles possess very high surface to volume ratios. This property can be utilized in the scientific fields, where high surface area is needed. As an example, in the catalytic industry, some nanoparticles have actually proven to be good catalysts (Jiang *et al*.,2004). Moreover, the nanoparticles show bactericidal effects. There has been an increasing interest in the synthesis of nanosized crystalline IONPs because of their large surface areas, unusual adsorptive properties, surface defects, and fast diffusivities (Weng *et al.*, 2007) . Iron oxide nanoparticles, due to their small size, super paramagnetic properties and controllable parameters, can interact directly with various microorganisms without posing a risk for human body. These nanoparticles can interact directly with the microbial cells causing damage to the envelope and thus rendering the bacterial cell defenseless or, in some cases, disrupting the metabolic functions and causing the destruction of the cell (Furno *et al*., 2004). Chemical and physical methods may successfully produce pure, well-defined nanoparticles, but these techniques are more expensive, energy consuming and potentially toxic to the environment. We are considering an environmentally friendly technique to produce, IONPs using extracellular biosynthesis. Biosynthetic methods can employ either microorganism cells or plant extract for

nanoparticles synthesis. An exciting branch of biosynthesis of nanoparticles is the application of plant extracts to the biosynthesis reaction. Recently, the green processes synthesis of nanoparticles are evolving into an important branch of nanotechnology (Sharam *et al*.,2009) .The choice of plants in the present paper has been made based on their medical application or antibacterial activities (Satyajit *et al*.,2014) and (Albert *et al*.,1992) .In this paper we present a rapid method for iron oxide nanoparticles synthesis using plant Ocimum lamiifolium leaves extract and their characterization and inhibitory effects against Gram-negative and Gram-positive bacteria (Thamima *et al*,2014). Nanoparticles are also widely used in medicinal fields, electronics, sensors, optics, catalysis photoelectron chemical devices, drug delivery, biosensors, bio-imaging, antimicrobial activities, food preservation and photonics.

1.2. Statement of the problem

During the last decade, a rapid increment in the development of new antibacterial materials has been observed as consequence to the spread of antibiotic resistant infections, which has become a major issue in health care. A possible alternative is the synthesis of new inorganic compounds with antimicrobial properties. IONPs have the advantage of durability as well as chemical and physical stability over common organics and antibiotics compounds. 'Green' synthesis methods of nanomaterial reduce or eliminate toxic substances from the environment. Green synthesis of nanoparticles by inactivated plant tissue and plant extracts makes use of environmental friendly, non-toxic and safe reagents (Mahdavi *et al* 2013).

The genus Ocimum Lamiiflium is cultivated for its extraordinary essential oil which display many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent (Kashyp *et al*., 2011). It has wide range of therapeutic effects like antimicrobial, antispasmodic, bactericide, carminative, anthelmintic, hepatoprotective, antiviral, larvicidal, remedy of coughs, colds, measles, abdominal pains, diarrhea, insect repellent, particularly against mosquitoes and storage pest control. The essential oils obtained from this plant used as repellent against nuisance biting insects and malaria vector (kashyp *et al*., 2011). The plant has also been in use successfully in the management of diabetes mellitus. However, its increased use has not been accompanied by an increase in the quantity, quality and accessibility of clinical evidence to support traditional medicine practitioner's claims.

In the past few years, a wide range of work has been done in producing new drugs due to the resistance of plant extracts nanoparticles to the current drugs. This work is a novel way of synthesizing Fe_3O_4 nanoparticles with the plant extracts and it is also an attempt to study the antibacterial properties of Fe_3O_4 nanoparticles. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient from *Ocimum lamiifolium* plant with $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ precursor. So, in We have this work we have planned to synthesis pure and modified IO nanoparticles which are cost effective, from most common cheap precursors and in green synthesis method and investigate the effect of pure & modified IO nanoparticle antimicrobial agent against gram-negative (*Escherichia coli* and *Pseudomonas oeruginose*) and gram-positive (*Staphylococcus aureus* and *Bacillus subtilus*). Iron oxide will be of particular interest due to its distinctive physical and chemical properties. *Ocimumlamiifolium* leaf extract will be selected as it is of high medicinal value and it does not require any sample preparation and hence is cost-effective (Rajan *et al*.,2011).

The antimicrobial activity of Iron oxide nanoparticles is well known and hence we made use of this property to inhibit growth of two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis)* and two gram negative bacteria *(Escherichia coli* and *pseudomonas aerudinosa*) using disc well diffusion method. These four bacterial strains were selected as they are highly contagious. Hence, the potential antimicrobial activity of Iron oxide nanoparticles against these pathogenic bacteria was evaluated in this work. These four different types of pathogenic bacteria have been chosen for the study because of the following reasons:

Staphylococcus aureus is a gram-positive, round-shaped bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin (Firouzabadi, *et al.*, 2015).

Bacillus subtilis: Gram-positive coccoid bacterium causes pneumonia, meningitis, sepsis (Haritha *et al*., 2011).

***E. coli*:** Gram- negative bacteria, virulent strain causes food poisoning, urinary tract infection, neonatal meningitis (Haritha *et al*., 2011).

Pseudomonas aeruginosa is Gram-negative bacteria; a primary habitat of P.aeruginosa is human and animal gastro-intestinal tract, water, sewage, soil and vegetation. It is physiologically versatile and flourishes as a saprophyte in warm moist situations in the human environment, including sinks, drains, respirators, humidifiers, etc.

1.3 Objective

1.3.1 General Objectives

The general objective of this research work is to synthesis iron oxide nanoparticles through green method using a plant extract of *Ocimumlamiifolium* and study its antibacterial activity.

1.3.2 Specific Objectives

The specific objective of this research is:

- to synthesize magnetite iron oxide nanoparticles using leaf extract of OcimumlLamiifolium and Iron Precursor salts ($FeCl_3.6H_2O$ and $FeCl_2.4H_2O$).
- to characterize the synthesized magnetite iron oxide nanoparticles using X-ray diffraction (XRD) technique, thermogravimetric analysis (TGA), UV-Visible spectroscopy, Scanning electron microscopy (SEM), and Fourier transform- infrared (FT-IR) spectroscopy.
- to test the antibacterial activity of synthesized iron oxide nanoparticles against gram positive and gram negative bacteria (*S. aureus* , *Bacillus subtilis* and *E. coli*, *pseudomonas aerudinosa*) respectively.

1.4. Significance of the Study

The effect of magnetite IONPs and medicinal plant extracts from Ocimumlamiifolium leaf synthesis in laboratory combined with bacteria grown on Agar pelt *S. Aureus, Bacillus subtilis* ,and *E. coli, pseudomonas aerudinosa* .Test the antibacterial activities of nanoparticles, due to the resistance to presently available antibiotics has necessitated the search for new antibacterial agents or a combination of drugs to be able to combat new resistant pathogenic bacteria. The plant extracts of IONPs how it affects against some resistant bacteria, therefore we will check this possibility in our study by using Ocimum Lamiifolum traditional medicinal plants. This study was providing important information about the synthesis strategy of IONPs and modified Iron Oxide nanoparticles and its antimicrobial activity.

1.5 The scope of the study

The study covers the synthesis and applications on antibacterial activities of IONPs by using Ocimum Lamiifolium plant leaf collected from Oromia Regional state west Shewa Zone Chire Kebele. The study limited on how the nanoparticles used in as anti-bacterial activities by converting and stabilizing its magnetic properties. To synthesis of Fe_3O_4 NPs in using leaf extract of Ocium lamiimfolium and Iron precursor salts and evaluate their antibacterial activities. The characterization of the nanoparticles is might be also limited to XRD, FT-IR, and ATG technique, the application of IONPs is on *S. Aureus, Bacillus subtilus* and *E. coli, pseudomonas aerudinosa* bacteria. The Agar Well Diffusion Assay was employed with modifications as described by (Irshad *et al.,* 2012).

2. REVIEWS OF LITERATURE.

2.1. Nanoparticles

Nanoparticle is defined as a small object that behaves as a whole unit in terms. Particles are further classified according to size; in terms of diameter. Fine particles cover a range between 100 and 2500 nm. On the other hand, ultrafine particles are sized between 1 and 100 nm. It is the dimension in the range of 1–100 nm acts as a bridge between bulk materials and atomic or molecular structures. They possess remarkable and interesting properties owing to their small sizes, large surface area with free dangling bonds and higher reactivity over their bulk cousins.

2.2. Iron Oxide Nano particles

Magnetite (Fe_3O_4) is differs from most other iron oxides in that it contains both divalent and trivalent iron. Fe_3O_4 has a cubic inverse spinel structure that consists of a cubic close packed array of oxide ions, where all of the Fe^{2+} ions occupy half of the octahedral sites and the Fe^{3+} are split evenly across the remaining octahedral sites and the tetrahedral sites. Iron oxide nano particles were synthesized by co precipitation method using ferric chloride, ferrous chloride and NaOH as precursors. The crystal structure of the sample is analyzed by means of XRD-6100 diffract meter (Sharam *et al*.2009), and the patterns will be recorded with Copper Kα radiation (λ=1.54060 A°). Molecular analysis of the samples is performed by Fourier transform infrared (FT-IR) spectroscopy using IR affinity spectrometer, recorded in the wave number range of 400–4,000 cm^{-1}. During the green synthesis of iron oxide nanoparticles; both the aqueous leaf extract and the precursor salt solution were mixed in different ratios proportions. After the addition of leaf extract to the salt solution, the color changed from yellowish to black. The reaction mixture will centrifuged at 10,000 rpm for 15 min. The supernatant will be discarded and the pellets will repeatedly wash with distilled water and dried for the evaporation of aqueous phase in hot air oven (Mahmoud *et al*, 2011).

2.3. Properties of iron oxide nanoparticle (IONPs)

Semiconductors with the dimensions in the nanometer are important because of their electrical, optical and chemical properties which can be tuned by the changing the size of particles. Fe_3O_4 NPs exhibit ferrimagnetism's at room temperature, with the saturation magnetization reaching to 92 emu g^{-1}. It is noteworthy that many properties of IONPs depend on their size and shapes

.Nano particles may or may not exhibit size-related properties that differ, significantly from those observed in fine particles or bulk materials (Buzea *et al*., 2007). Iron oxides are common compounds, which are widespread in nature and can be readily synthesized in the laboratory. Magnetic iron oxides have served humans for centuries. It is considered as one of the most desirable materials for various applications due to its inherent biocompatible nature and stability of oxidation as well as its good magnetic properties (Bosak *et al*., 2011). It also exhibits modest photo catalytic activity and reparability. High magnetization of Fe_3O_4 has potential applications for cleaning polluted water with the help of magnetic separation. As a result, magnetic properties, as an important symbolic characteristic of IONPs, are noticeable for study. Iron oxide nanoparticles (IONPs) are widely used for numerous applications from solid state in antibacterial activities and to biomedical treatments. For all these applications, one needs to control simultaneously numerous mutually related properties of IONPs, such as composition, structure, morphology, crystallinity, magnetic, saturation magnetization etc. In general, IONPs become super paramagnetic at room temperature when the size of IONPs is below about 15 nm, meaning that the thermal energy can overcome the anisotropy energy barrier of a single nanoparticle.

2.3.1 Electrical Properties

The fundamental study of the electrical properties of IO nanostructures is crucial for developing their future applications in nanoelectronics. Fe_3O_4has the face centered cubic spinal structure, based on $32O^{2-}$ions and close-packed along the direction (Martinez *et al*, 2012). In stoichio metric magnetite Fe (II)/Fe (III) = 1/2, and the divalent irons may be partly or fully replaced by other divalent ions (Co, Mn, Zn). Thus Fe_3O_4 can be both an n- and p-type semiconductor. However, Fe_3O_4 has the lowest resistivity among iron oxides due to its small band gap (0.1 eV) (Boxall *et al.*, 1996).

2.3.2 Magnetic Properties

Understanding the correlation between the magnetic properties and the size and shape of IONPs is a prerequisite for widespread applications of magnetism in data storage and bio-separation areas (Zhang *et al.,* 2011). IONPs exhibit ferromagnetism at room temperature, while the saturated magnetization reaching to 92 emu g^{-1} (Yamaura *et al.,* 2004). It is noteworthy that many properties IONPs depend on their size and shape. The magnetic properties are strongly influenced by the morphology of the Fe_3O_4 NPs. In general, IONPs become super paramagnetic

at room temperature when the size of IONPs is below about 15 nm, meaning that the thermal energy can overcome the anisotropy energy barrier of a single nanoparticle. However, aggregation among super paramagnetic IONPs is a common phenomenon. The unique combination of high magnetization and paramagnetic behavior opens these materials to a very wide range of applications. Particularly, the possibilities of nanoparticle modification by biologically active compounds to use them in controlled drug delivery systems, as agents in magnetic resonance imaging and for magnetic-induced tumor treatment via hyperthermia are very interesting (Chikov *et al*.,1993).

UV-visible spectroscopy is most widely used technique to investigate the optical properties of the particles. The color change from colorless to black indicated the formation of iron oxide nano particles. UV-visible spectroscopy analysis will be done in the range of 200-800 nm and the maximum absorbance will observed at 285 and 324 nm regions for the formation of iron oxide nanoparticles due to the excitation of surface plasma on vibrations.

2.3.3 Chemical Properties

Magnetite is the inorganic compound with the formula Fe_3O_4. It is one of the three main oxides of iron, and iron (II, III) oxide (Fe_3O_4), which occurs naturally as the mineral magnetite. It is the main source of the iron for the steel industry. Magnetite which contains Iron (III) oxide is paramagnetic, reddish brown, and readily attacked by acids. Rust is often called iron (III) oxide, and to some extent, this label is useful, because rust shares several properties and has a similar composition. To a chemist, rust is considered an ill-defined material, described as hydrated ferric oxide (Senthil *et al*.,2012).

2.4. Synthesis of Iron Oxide nanoparticles (IONPS)

The synthesis of magnetite (Fe_3O_4) nanoparticles was done by chemical combustion. The required amount of ferric Chlorides and ferous chlorides mixture (0.1 M) will be dissolved in 100 mL of distilled water under the magnetic stirrer for 10 min. The fuel sodium hydroxide (0.1 M) was dissolved in20 mL of distilled water. Fuel solution was mixed with oxidizer solution which was under stirring followed by mixing with sample. The whole solution was kept under stirring for 15 min for stirring. The solution will be placed on a hot plate to initiate the reaction. When the temperature was started to increase, the solution boiled and fumes gushed forth from the solution; as the temperature increased above 80 ^{0}C, the solution started to evaporated leading

to an increase in the viscosity of the liquid and smouldering started eventually self-ignition took place forming the final product (Fe_3O_4). The powder was collected from the beaker and calcinated for 1 h at 400 ^{0}C. The collected powder were characterized and used for antibacterial activities (Liang *et al*.,2011).

2.4.1. Physical method

Physical methods developed for synthesis of IONPs often require special equipment's or operational control. The physical method often called top-down approach which includes methods like diffusion, irradiation, thermal decomposition and arc discharge etc. The thermal decomposition method is one of the significant top-down approaches. It is used for the creation of mono disperse nanoparticles. These approaches use larger initial structures or macroscopic units which can be externally controlled in the processing of nanostructures. Etching through the mask; ball milling and application of severe plastic deformation are common examples of physical top-down method of synthesis of nanoparticles. The top-down method of synthesis is a model which generates on a larger scale for further reduction of Nano scale. Mostly the top-down methods are not cheap and quick to manufacture. This method is slow and not suitable for large scale production (Singh *et al*.,2010).

2.4.2. Chemical method

Moderate reaction conditions and convenient synthetic manipulation have rendered chemical methods an attractive option for accessing IO nanoparticles. Traditional chemical routes that are frequently used in recent years include sol-gel approach, chemical precipitation and colloidal synthesis. Surfactants or polymer based preparative method for IO nanomaterial's are also quite popular to avoid agglomeration and achieve better control of IONPs. Removal of surfactants or polymers, however, poses serious limitations to such methods. Hence, delicate and precise control of experimental factors for the synthesis of nanoparticles with desired morphologies is a cherished goal for future device applications. Homogeneous chemical precipitation method is often considered economically viable for preparation of mono disperse metal oxide particles of different shapes and sizes. The method also provides better control of chemical and morphological characteristics (Bathacharjee *et al.*, 2011). Mostly the chemical methods of synthesis of nanoparticles are the bottom up approaches. These approaches include the miniaturization of material components (up to atomic level) with further self-assembly processes

leading to the formation of nanostructures. During self-assembly the physical forces operating at Nano scale are used to combine basic units into larger stable structures. Typical examples are quantum dot formation during epitaxial growth and formation of nanoparticles from colloidal dispersion. In these methods different metal particles are reduced to form nanoparticles. Various types of metal particles used like Sodium boro hydrate and Sodium citrate (Jana,*et al*,2000). Other chemical reagents used are N, N-dimethylformamide (DMF), Poly (N-vinyl pyrrolidine (PVP), ethyl alcohol, tetra-n-tetra-fluroborate (TFATEB), etc. (Raj *et al.*, 2008). This method of synthesis of nanoparticles is cheap and quick to manufacture but it is slow and not suitable for the large scale production.

2.4.3. Green Synthesis of IONPs

Green synthesis method is proved as beneficial over other methods which are implemented for the synthesis of nanoparticles. Green synthesis methods are eco-friendly approach and compatible for pharmaceutical and other biomedical applications, as the toxic chemicals are not used in these methods (Willner *et al.*, 2007). Besides, this method does not require high pressure and temperature. Synthesis of various nanoparticles by microorganisms is used more than the other techniques due to its eco- friendly nature. The use of environmentally beneficial materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of Iron oxide nanoparticles proposes abundant benefits of eco-friendliness and compatibility for pharmaceutical and many other biomedical applications. The rapid biosynthesis of iron oxide NPs (<100 nm) was done by adding Eucalyptus globulus leaf extracting the aqueous solution of $FeCl_3$ (Balamurugan *et al*, 2014). In an another rapid single step, green synthesis of Iron oxide NPs occurred using aqueous sorghum extracts as both reducing and capping agent, the remediation and treatment of hazardous waste of water treated by IONPs. $FeCl_3$ the extracts of different parts of plants were used to produce IONPs at 50–60^{o}C (Balamurugan *et al*, 2014). The reported that stem and leaf extracts of Calotropisprocera did not produce NPs while the stem extract of Euphorbia produced NPs of minimum ranging 13–21 nm with spherical morphology

2.5. Applications of IONPs

IONPs are used in various applications like magnetic storage, catalysis and biological applications in bone tissue engineering and in antibacterial activity. It is a nanoparticles

exhibited strong antibacterial activity against bacterial species. In some disciple area the following application are out lined.

2.5.1. Water Treatment

Spherical IONPs were synthesized via a facile one step method using Eucalypt leaf extract for green treatment of eutrophic waste water (Balamurugan *et al.,*2014) produced NPs effectively catalyzed H_2O_2 degradation which made them useful for environment are mediate and treatment of hazardous waste .They reported that 72 % of total nitrogen30 % of total phosphorus, 85 % of chemical oxygen demand (COD) were removed respectively by the produced NPs. Thus NPs may play great role in remediation of waste water, in another experiment, (Balamurugan *et al.*, 2014). Synthesized IONPs (20–80) nm, spherical using extract of green tea and eucalyptus. Iron NPs were reactive towards nitrate in comparison to the traditional chemically prepared Fe_3O_4 NPs. The biologically produced NPs showed significant in sit remediation of waste water especially in nitrate contaminated site. Green nanomaterial's have a wide scope in the treatment of surface water, groundwater and wastewater contaminated by toxic metal ions, organic and inorganic solutes and microorganisms (Gonzale z. *et al*., 2013). Self-cleaning Nano scale surface coatings may clean chemicals used in regular maintenance. Iron oxide NPs are considerable interest because of their rapidly developing applications for disinfection of water and remediation of heavy metals from soils (Mahadvi *et al.*,2013).

2.5.2. Biomedical applications

The biocompatibility and toxicity of IONPs are important criteria to take into account for their biomedical applications. Parameters determining biocompatibility and toxicity are the nature of the magnetically responsive component, and the final size of the composite particles including their core and the coatings (shell). Ideally, composite IONPs must also have a high magnetization so that their movement in the blood can be controlled with an external magnetic field until it is immobilized close to the targeted pathologic tissue (Salabas *et al*, 2007). Magnetic IONPs with a long blood retention time, biodegradability and low toxicity have emerged as one of the biomedical applications in vitro and in vivo. Some biomedical applications require surface functionalized.

2.5.3. In vivo Applications

IONPs have a large surface area and can be engineered to provide a large number of functional groups for cross-linking to tumor-targeting legends such as monoclonal (Sundarm *et al*., 2012). Especially, the magnetic properties of IONPs can be used in numerous in vivo applications, be divided into three main groups: (I) magnetic vectors that can be directed by means of a magnetic field gradient towards a certain location, such as in the case of targeted drug delivery; (ii) magnetic contrast agents in MRI and (iii) hyperthermia or thermo ablation agents, where the magnetic particles are heated selectively by application of high-frequency magnetic field(Pascal *et al.*,1998).

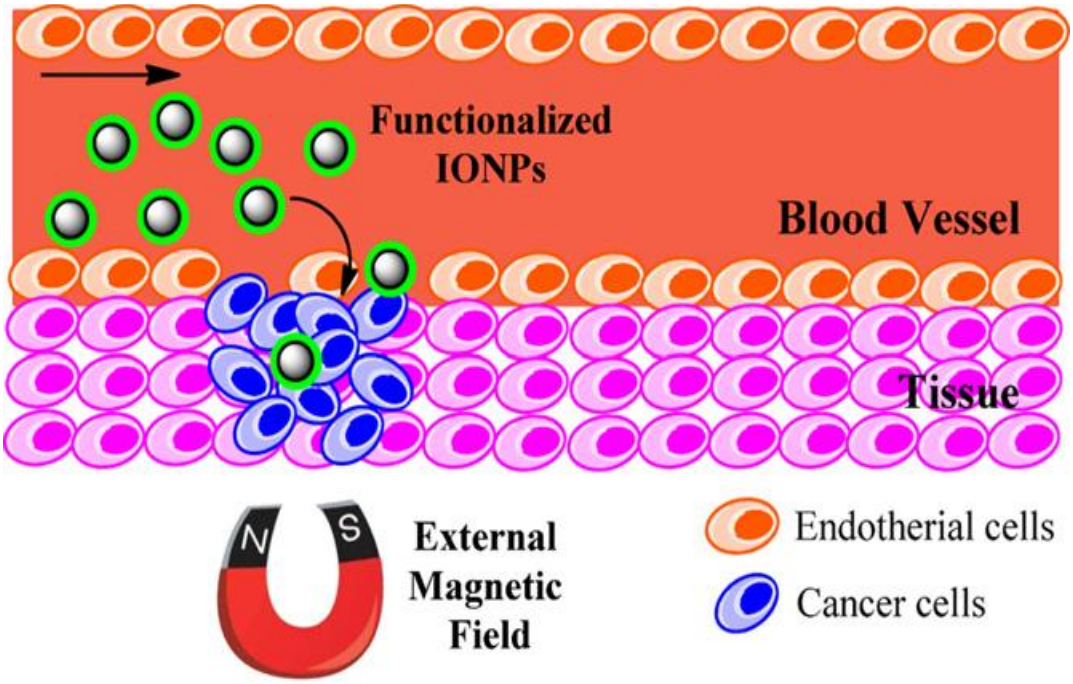

Figure1: Representation of magnetic nanoparticle-based drug delivery system: these magnetic carriers concentrate at the targeted site using an external high-gradient magnetic field. After accumulation of the magnetic carrier at the target tumor site in vivo, drugs are released.

2.5.4. Targeted Drug Delivery

In traditional drug delivery systems, such as oral ingestion or intravascular injection, the medication is distributed throughout the body in blood circulation. For most therapeutic agents,

however, only a small portion of the medication affected organ and there is reduced drug diffusion through biological barriers causing a high reaches the incidence of adverse effects drug Targeted drug delivery (TDD) seeks to concentrate the medication in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues and crossing the biological barriers by active accumulation or an active targeting strategy(Duran *et al.*,2008). Magnetic IONPs-based drug targeting is a promising cancer treatment method for avoiding the side effects of conventional chemotherapy by reducing the systemic distribution of drugs and lowering the doses of cytotoxic compounds (Cao *et al.*, 2008). As shown in Figure 1, functionalized IONPs as carrier can deliver a wide range of drugs to all areas in the body. Hence the efficient intracellular delivery of NPs is one of the main factors in enhancing the efficacy of the encapsulation therapeutic agent. Generally, magnetic IONPs are used as the core and biocompatible components act as functionalized shell to form the core–shell structure for TDD carriers, and the drugs are bound or encapsulated into the polymer matrix. In a drug carrier system, the sizes, surface properties, and stability are the crucial features. Partially, the IONPs should be small enough to penetrate through the capillary bed. However, if the diameter of the IONPs is smaller than 10 nm, they will be rapidly removed through extravasations and renal clearance. Therefore, IONPs with a ranging from 10 to 100 nm are optimal for intravenous injection and have the most prolonged blood circulation times (Laurent*et al.*, 2008).Additionally, IONPs with the positive charges is better internalized by human breast cancer cells than those IONPs with negative charge. However, intake of these IONPs also depends upon cell type. IONPs with hydrophobic surface are easily adsorbed at the protein surface and show a low circulation time (Arruebo *et al.*, 2014).

2.6. Antibacterial Activity

IONPs showed antibacterial effect against gram-positive as well as gram-negative bacteria which clearly indicates that these nanoparticles are effective antibacterial agents. Many antibacterial studies were made using different nanoparticles. The reason for the bactericidal activity is due to the presence of reactive oxygen species (ROS) generated by different nanoparticles (Yamamoto *et al.*, 2001). Chemical interaction between hydrogen peroxide and membrane proteins or between the chemical produced in the presence of Fe_3O_4 nanoparticles and the outer bi layer of bacteria could be the reason for the antibacterial activity of Fe_3O_4. The hydrogen peroxide produced enters the cell membrane of bacteria and kills them. It is also noted that nanoparticles

continue to be in interaction with dead bacteria once the hydrogen peroxide is generated; thus foiling further bacterial action and continue to produce and release hydrogen peroxide to the medium (Padmavathy *et al.*,2008). The possible mechanism of action is that the metalnano particles are carrying the positive charges and the microbes are having the negative charges which create the electromagnetic attraction between the nanoparticles and the microbes. When the attraction is made, the microbes get oxidized and die instantly (Rezaei-Zarchi *et al.*, 2010). Generally, the nanomaterial's release ions, which react with the thiol groups(–SH) of the proteins present on the bacterial cell surface which leads to cell lyses (Zhang *et al*,2009).The central mechanism that caused the antibacterial activity by the particles might be through oxidative stress caused by ROS (Mahdy *et al*,2012). ROS includes radicals like superoxide radicals (O_2^-), hydroxyl radicals (-OH) and hydrogen peroxide (H_2O_2); and singlet oxygen ($^1O_2$) could be the reason damaging the proteins and DNA in the bacteria .ROS could have been produced by the present metal oxide (iron oxide) leading to the inhibition of most pathogenic bacteria like *S. aurous and E. coli* . A related studies were explained by (Kim *et al*, 2001) in which hydrogen peroxide (H_2O_2) was generated when Fe^{2+} responded with oxygen. The ferrous irons reacted with the produced H_2O_2 subsequently through Fenton reaction and thus leading to creating hydroxyl radicals which damage the biological macro-molecules (Touati *et. al.*, 2000). The nanoparticles can also produce bactericidal effects as verified by a few authors have verified. A few authors like (Lee *et al*, 2008) stated that the iron nanoparticles caused the inactivation of membranes *S. Aureus, Bacillus subtilus and E. coli, pseudomonas aerudinosa* bacteria's.

3. Materials and Methods

Study Site

IONPs synthesis and most of characterization were done at the Department of Chemistry in Adama Science and Technology University. Antibacterial activity of synthesized iron oxide nanoparticles was evaluated in laboratory of department of applied biology at Adama Science and Technology University. The plant sample collection site, Chire kebele, is located at 40 km far from Addis Ababa to the west.

3.1. Collection and Preparation of the Plant Materials

The plant used in this study was collected from its native habitat on the basis of ethno-botanical information. It was collected from village of Chire Kebele of Oromia Regional state. For this study, the part of the plant collected was the leaves. Botanical identity of the plant was authenticated by an acknowledged authority in taxonomy and a voucher specimen deposited at the Addis Ababa University. Green leaves was collected and dried at room temperature away from direct sunlight for one week. The dried leaves was separately grounded using a mechanical grinder into fine powder. The powdered plant materials was kept at room temperature away from direct sunlight in closed, dry plastic air tight bags ready for extraction.

Figure 2: Ocimum lamiifolium(photograph taken by Motuma Ejigu in September, 2017)

3.2 Preparation of the Aqueous Extracts of OciumLamiifolium

One hundred grams of the powdered plant material were extracted in 1 liter distilled water at 80°C for 30min. The mixture was left to cool to room temperature and then decanted into dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was filtered using filter papers under vacuum pump.

Figure 3: Powderd plant sample for Ocimum lamiifolium **(Damakase)**

3.3 Biogenic Synthesis of Fe_3O_4 nanoparticles using Ocimum Lamiifolium as Reducing Agent.

3.3.1 Reagents and Chemicals

For the synthesis of iron oxide nanoparticles, Ocimum Lamiifolium leaf extract was used as the reducing agent, Ferric chloride hexahydrate ($FeCl_3.6H_2O$, 99%) and ferrous chloride tetra hydrate ($FeCl_2.4H_2O$, 99.5%) were purchased from local market, manufactured in Guangodong guangzhou Science and Technology (China). All reagents in this research were analytical grade and were used as received without further purification. All aqueous solutions were freshly prepared using distilled water.

3.3.2. Green Synthesis of Iron Oxide Nano particles

$FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ were used as precursor to synthesize Fe_3O_4 nanoparticles using aqueous extract of Ocimum Lamiifolum leaf, 1g $FeCl_2.4H_2O$ and 3g $FeCl_3.6H_2O$ was dissolved in 100 mL distilled water using volumetric flask and stored for further use. 20 ml of 1M NaOH (0.8gms) was measured and dissolved in distilled water in a beaker. After 5 min, the above prepared NaOH aqueous solution was added to the mixture drop wise to be used as precipitating

agent (Amlan *et al*., 2014). Then five different samples were prepared by taking different ratios by volume of plant leaf extract to precursor salt solution.

Mechanism of the Fe_3O_4 -NPs formation from Ocimum lamiifolium leaf Extract: The present work focused on the development of biosynthetic method for the production of Fe_3O_4-NPs using Osmium Lamiifolium leaf extract. The reduction potential of Osmium Lamiifolium leaf Extract polyphenols in plant is sufficient to reduce metals. Decreasing in pH during the formation of Fe_3O_4-NPs signifies the involvement of the OH group in the reduction process. The formation of Fe_3O_4-NPs with Osmium Lamiifolium/polyphenols occurs via the following steps: (1) complexation with Fe salts,(2) simultaneous reduction of Fe (III) capping with oxidized polyphenols/plant. The color of the iron solution/Osmium Lamiifolium extract solutions at room temperature rapidly changed from yellow to dark brown, indicating the formation of Fe_3O_4-MNPs in the plant extract. The proposed green synthesis method for Fe_3O_4-NPs was found to be constructive and extremely reproducible.

Sample 1: Osmium Lamiifolum Leaf Extract to precursor solution (1:1) Ratio (50:50 mL) labeled as **S1**

Sample 2: Osmium Lamiifolum Leaf Extract to precursor solution (1:2) Ratio (50:100 mL) labeled as **S2**

Sample 3: Osmium Lamiifolum Leaf Extract to precursor solution (1:5) Ratio (20:100 mL) labeled as **S3**

Sample 4: Osmium Lamiifolum Leaf Extract to precursor solution (2:1) Ratio (100:50mL) labeled as **S4**

Sample 5: Osmium Lamiifolum Leaf Extract to precursor solution (5:1) Ratio (100:20mL) labeled as **S5**

Magnetite nanoparticles (Fe_3O_4) synthesized as follows: 1 g of $FeCl_2.4H_2O$ and 3 g of $FeCl_3.6H_2O$ (1:2 molar ratio) were dissolved in 100 mL of sterile distilled water in a 250 mL beaker and heated at 80℃ under mild stirring using magnetic stirrer and under atmospheric pressure. After 10 minutes, different ratios (50:50),(50:100),(20:100),(100:50) and(100:20) mL of the aqueous solution of Osmium Lamiifolium leaf extract with of precursor salt solution were heated, immediately the yellowish color of the mixture changed to black color. After 5 minutes, 20 mL of sodium hydroxide aqueous solution was added to the mixture with rate 3 mL/min for allowing the magnetite precipitations uniformly. From the first addition of sodium hydroxide the reddish mixture changed to black suspended particles. The mixture was allowed to cool down to room temperature and the magnetite nanoparticles were obtained by decantation, dilution with distilled water and centrifugation to remove heavy biomaterials of Osmium Lamiifolium leaf extract. The magnetite nanoparticles were purified by dispersing in distilled water and centrifugation three times; the magnetite nanoparticles after purification were dried at 160℃ in oven for 1hr. The black powder obtained from the above method was calcined at 400^0C for 1 hr and grinded to fine powder using agate mortar and ready for XRD, TGA and FTIR analysis. The type of Furnace used for calcination is Sx-2.5-10 Box Type Resistance Furnace.

3.4. Characterization of Green Synthesized Fe_3O_4 Nano Materials

Once the IONPs are synthesized, their conformational details about shape, size, disparity, homogeneity as well as surface morphology are determined by using various techniques (Mahmoudi M. *et al*.2011). The common techniques used for characterizing Fe_3O_4 NPs are as follows: Thermal gravity analysis (TGA), X-Ray diffraction (XRD), Fourier Trans infrared (FTIR) spectroscopy, UV-Visible spectroscopy, scanning electron microscopy (SEM) with EDS along with the evaluation of their antibacterial activity against gram positive and gram negative bacteria with a view to explore their pharmaceutical applications.

3.4.1.UV-Vis absorption spectroscopy

Uv–Vis spectra were employed to examine the size and shape of NPs in aqueous suspension (Nath *et.al*, 2013). Wavelengths from 300 to 800 nm are normally used for characterization of NPs ranging in size from about 2–100 nm (Feldheim *et al*, 2002). The films of Fe_3O_4 nano films were prepared and energy gap(E_g) of the nano materials was determined using the formula in Equation 1.

$$\boldsymbol{E_g = \frac{1240}{\lambda}} \qquad (1)$$

Where λ(nm) is the wavelength of the absorption edge in the spectrum. The energy gaps of samples show mostly blue shift and exhibit distinct synergistic effects.

3.4.2. FT-IR Spectroscopy

FTIR spectroscopy was used to determine the nature of functional groups or metabolites present on the surface of NPs which might be responsible for reduction and stabilization of NPs and also to determine the vibration modes of Fe-O bond in Fe_3O_4 nanoparticle (Elango *et al.*, 2015). The sample prepared for FT-IR detection was, *Osmium lamiifolum* leaf extract to Iron chloride salt precursor solution (1:2) ratio (50:100 mL). Measurements of Fe_3O_4 NPs were performed by putting the powder of the synthesized NPs on KBr plate (Elango *et al*, 2015).

3.4.3. X-Ray Diffraction technique

To characterize crystal structure and phase of the powder Fe_3O_4 nanoparticle, small amount of the powders was take and ground to fine particle and analyzed by XRD equipped with a Cu target for generating a Cu Kα radiation with λ = 0.154 nm. The 2θ record ranging from 40– 640 while the accelerating voltage and applied current is held at 40 kV and 50mA, respectively. The particle size (D) of the powders is calculating by Scherer's Formula using Equation 2 below.

$$\boldsymbol{D = \frac{K\lambda}{\beta Cos\theta}} \qquad (2)$$

Where k=0. 9 Scherrer constant, λ is the wavelength of the Cu-Kα radiations, β is the full width at half maximum and θ is the angle obtained from 2θ values corresponding to maximum intensity peak in XRD pattern.

3.4.4 Scanning Electron Micro scope (SEM) and EDX

The morphology and size of NPs were characterized by SEM (Zeng *et al.*, 2012). Morphologies and thickness of the samples were investigated by scanning electron Microscope (SEM) instrument operating at constant beam energy. To prepare the sample small amount of the synthesize powder was taken and placed in the pellet maker. The powder was then compressed with high pressure. The SEM images are capture at different magnification levels and the morphological features of the particles were studied. The elemental analysis or chemical composition of the synthesize powder was doing by using energy dispersive x-ray at the Electron Microscope Unit (EMU). The operation was done at excitation energy of 20 K eV with scan or acquisition time of 600s (Volinsky A A 2011)

3. 5. Methods of Antibacterial Test

Materials used for antimicrobial activities are nutrient agar, petri plates; Para film tape, incubator, and Fe_3O_4 nanoparticle sample, gram- negative and gram-positive bacteria. Agar well diffusion method was used to determine the inhibition zone.

3.5.1 Agar well diffusion method

Antibacterial testing was performed against gram-negative (*Escherichia coli* and *P.seudomonas*) and gram-positive (*Staphylococcus Aureus* and *Bacillus subtilus*) bacteria by agar well diffusion

method. Bacterial strains were obtained at Adama Science and Technology University, Biology Department Laboratory. For bacterial growth nutrient agar medium was used which contains beef extract, peptone, sodium chloride, yeast and distilled water at pH 7. The agar well-diffusion technique was used for this work that reported by (Chung *et al.*, 1998). Agar well diffusion method is widely used to evaluate the anti- microbial activity of plants or microbial extracts (Valgas *et al.*, 2007). Similarly to the procedure used in disk-diffusion method, the agar plate surface is in occulted by spreading a volume of the microbial in-oculus mover the entire agar surface. Then, a hole with a diameter of 6 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100mL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. It is carried out in Petri plates. 500 µL of microbe's cultures of age 18–24 h was added to Petri plates and nutrient agar was poured. The medium were solidified, holes also made and each hole packed with different concentrations of nanoparticles ranging from 50, 75, 100 µg/mL one after the other. The plates has been wrapped in zones are then recorded in millimeter. Para film tape and transferred to incubator and maintained at 37 °C for 24 hr. The inhibition zones are then recorded in millimeter (Valgas,*et al*,2007).

3.5.2. Antibacterial Activity

Fe_3O_4 showed antibacterial effect against gram-positive as well as gram-negative bacteria which were tested by using different concentration of nanoparticles are antibacterial agents. In the control, low and high concentrations of Fe_3O_4 were shown, respectively. Many antibacterial studies were made using different nanoparticles. The reason for the bactericidal activity from nanoparticles was due to the presence of reactive oxygen species (ROS) generated by different nanoparticles (Yamamoto *et al.*, 2011). Chemical interaction between hydrogen peroxide and membrane proteins or between the chemical produced in the presence of Fe_3O_4 nanoparticles and the outer bi layer of bacteria could be the reason for the antibacterial activity of Fe_3O_4. The hydrogen peroxide produced enters the cell membrane of bacteria and kill them (Touat *et al*., 2000).

4. Result and Discussion

In this paper, we mainly presented the characterization and application of Iron oxide nanoparticles synthesized by biological method from Iron (II) and Iron (III) hydrated chloride salt.

4.1. UV-visible spectroscopy

UV-visible spectroscopy is most widely used technique to investigate the optical properties of the particles. UV-visible spectroscopy analysis was done in the range of 200-800 nm. It is spectrum of magnetite iron oxide nanoparticles in the Ocimium lamiifolium extract is shown in Figure 4. The absorption peaks at wavelengths of 340 nm indicate the formation of magnetite iron nanoparticles. The UV-Vis spectra of uncalcined Fe_3O_4 NP prepared by using Iron salt precursor and Ocimium lamiifolium leaf by five different ratio were shown in Figure 4. The absorption peak of the as-prepared nano Fe_3O_4 was found at around 330 nm and its sharp absorption feature indicates that the Fe_3O_4 NPs are monodispersed in nature.

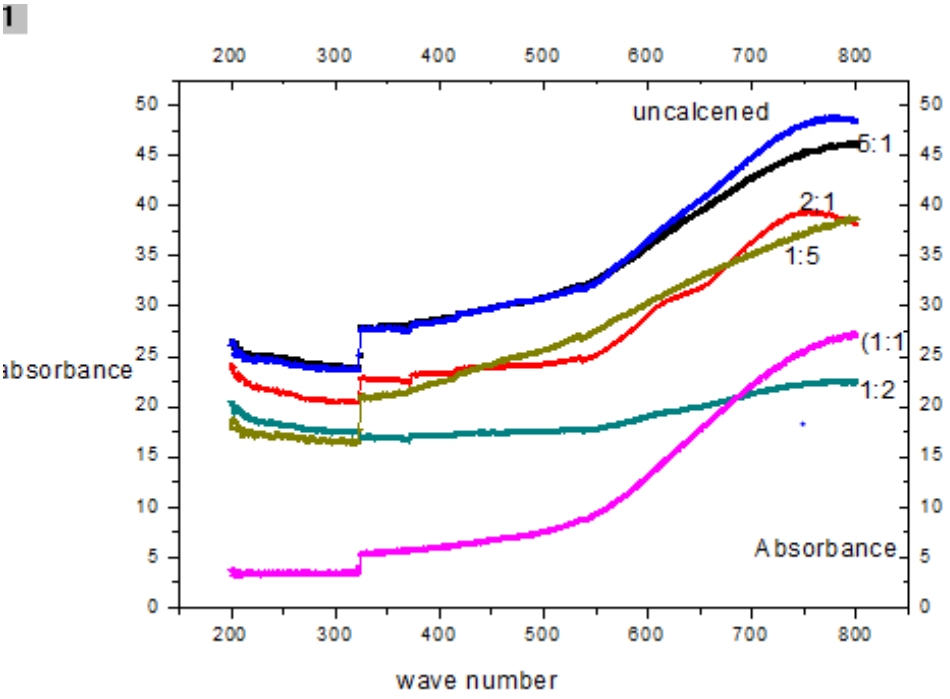

Figure 4. UV-Vis spectra of IONP synthesis by using Ocimum lamiifolum extract.

4.2. Thermo Gravimetric Analysis (TGA)

The uncalcinated green synthesized Fe_3O_4 nanoparticles using Iron (II) and Iron (III) chloride precursor were characterized by using TGA/DTA (Differential Thermal Analysis). The weight losses of the investigated materials were analyzed. Figure **4** shows the thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) of the uncalcinated Fe_3O_4 nanoparticles synthesized using Iron chloride precursor and plant extracted solution. TGA curve shows mass loss of the sample whereas DTA curve indicates the energy gain or loss during the process. TGA/DTA transition shows a loss of 43.795 % up to 400 ^{0}C. No considerable loss is observed after this up to 800C^0. TGA-DTA data for Fe $(OH)_2$ precursor registered strong endothermic peak at 500 C^0. This indicates that after 500 ^{0}C there is no weight loss for the synthesized Fe_3O_4 nanoparticles and the nanoparticle is thermally stable. This temperature was used for calcination of the synthesized Fe_3O_4 nanoparticles using Iron (II) and Iron (III) precursor.

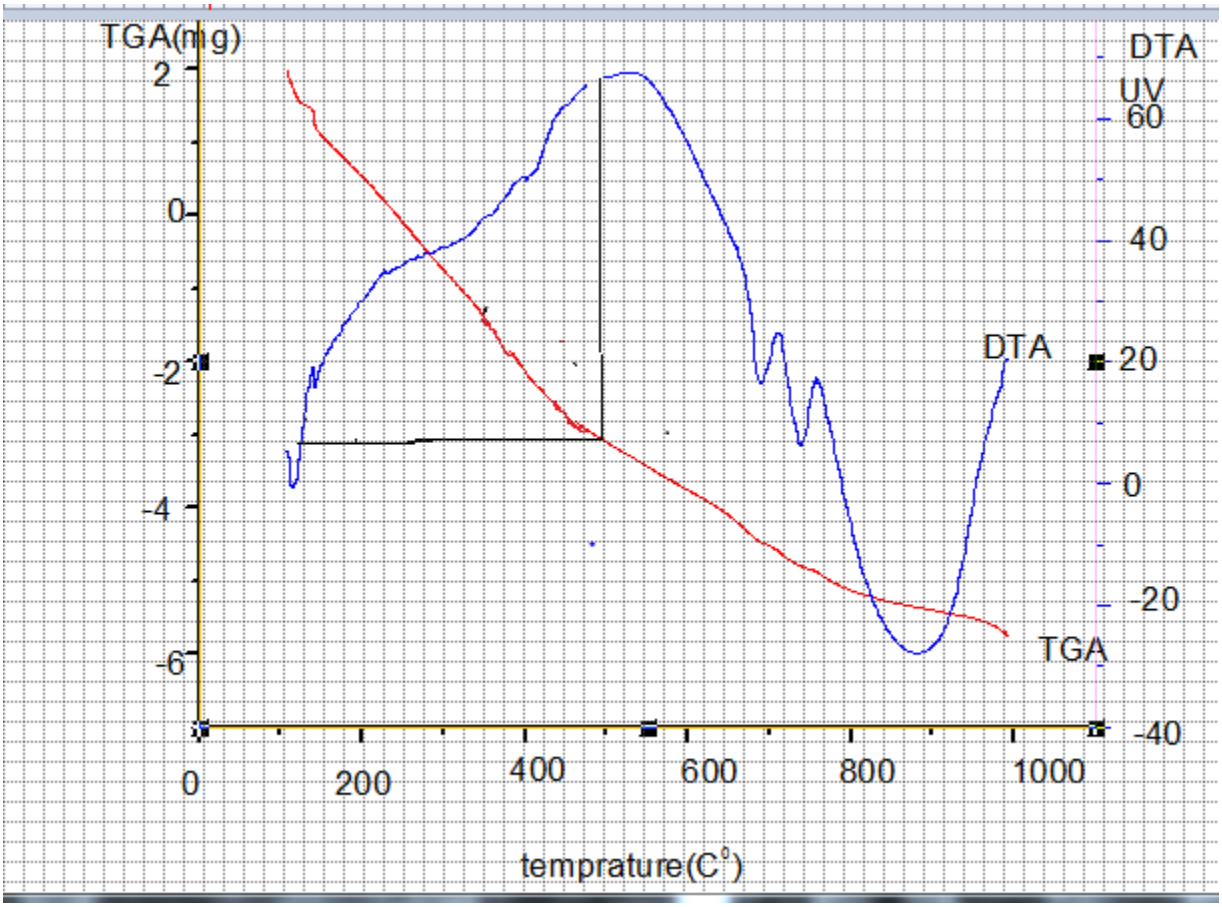

Figure 5: Thermal AnalysisTGA \DTA result for uncalcinated Fe_3O_4

4.2. X-Ray diffraction

The XRD pattern of the magnetite Iron Oxide nanoparticles (MIONPs) shown in Figure **6**, shows that all the obtained experimental peaks were compared with theoretically generated peaks and they were processed using powder X (Dong, 1999) and indexed with origin software. The XRD analysis contains five different ratios of plant extract to salt precursor solutions. The first categories were that plant extract is constant and salt precursor varied ,the ratio of plant extract to salt precursor solution were 1:1,1:2,1:5, the diffract- gram demonstrates the intense peaks at thevaluesof2θwithcorrespondtothelatticeplaneof(1:1)ratio:31.7(200),35.6(311),45.4(220), 33.1(111),35.9(440),63.1(222),45.4(220),40.5(400);theratio(1:2)35.6(311),31.5(200),33.2 (220),31.7(311),45.1(440),45.4(220),49.5(222),54(111)andtheratio(1:5)35.6(110),3.1(104),31.6(200),45.4(311),49.4(220),54.1(214),62.1(300),64(012.They matched the diffraction peaks for pure magnetite (Fe_3O_4) from the reference database (JCPDS 1 No.19-629). The average crystallite size was evaluated by using the lattice constant (ao) was calculated the'd' value and with their respective (h k l) parameters. The average crystalline structure was determined by using Debye Scherer's formula which is in between 13-37 nm. Figure **5,** shows that the results of the diffraction pattern of the formation of cubic spinel structure for all the samples. The strongest reflection comes from (311) plane, which denotes the spinel phase, also other strongest peaks of maximum intensity was obtained from the (200) plane in all the samples due to the presence of NaCl which obtained asabyproduct(Marangoni*et a*l., 2012).The second categories were ratio,(2:1)and(5:1)the2 θ,value with corresponding to lattice plane26.2(200),19.6(311),22.1(220),32.3(440),34.8(220),33(111),26.6(400),39.9(511)and the ratio(5:1)2θ,(35.6),(36.10),(63.1),(62.6),(31.6),(33.1)(34.70(30.1)which correspond to the lattice plane(119),(401),(206),(109),(111)(226) (211)(201) respectively. In the ratio 2:1 and5:1 the broad diffraction peak were formed. This broaden peak formed may be due to the difference in plant extract used in synthesizing processes larger than the other ratios. A broad diffraction peak was represented in Figure 5d and 5e, the calculated particle size were smaller than the others

ratios i.e, 28.5nm and 13.4nm respectively, which is attributed by leaf extracted to synthesis nanoparticles. The decreasing in intensity or shifting the peak suggested that the leaf extract/iron oxide nanoparticles have interaction with biomolecules present in the leaf extract. The crystallite size of synthesized leaf extract /iron oxide nanoparticles can be calculated using the Debye-Scherrer equation (Raman and Doble, et al., 2014), which reveals a relationship between X-ray diffraction peak broadening and crystallite size. Using the equation, the estimated average mean size of synthesized leaf extract/iron oxide nanoparticles was 29.40 nm, which was calculated from the full-width of the leaf extract/iron oxide nanoparticles diffraction peak at all 2θ. Based on the particle size, the particle synthesized leaf extract/iron oxide nanoparticles was in the range of nanoparticle size. This figured out to be high purity crystalline, as no impurity peak was observed. The difference in crystal size may be due to difference in leaf extract and precursors used for making Fe_3O_4 NPs. A definite line broadening of the diffraction peaks also resembles that the synthesized particles are in nanometer range and no characteristic peak were observedotherthanFe_3O_4peaks.

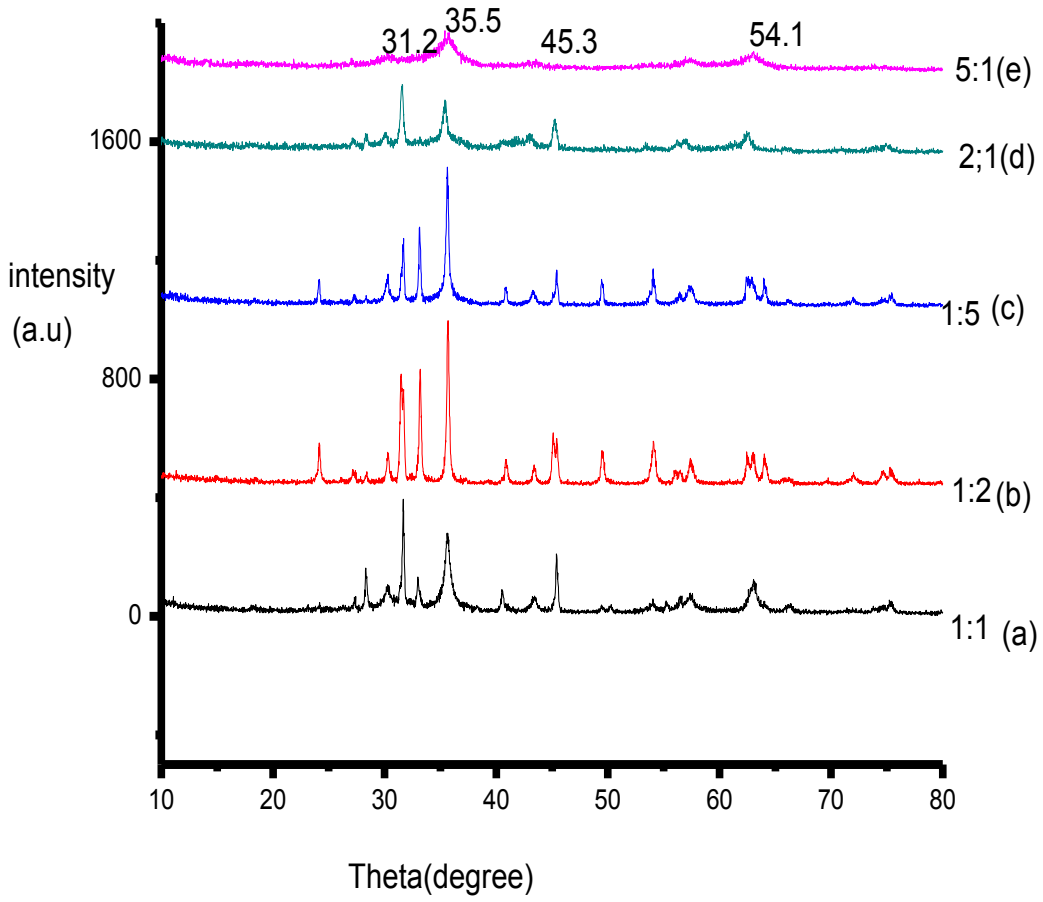

Figure6. XRD pattern of Fe_3O_4 nanoparticles a) ratio (1:1) nanoparticles b) ratio (1:2) Synthesized Fe_3O_4 nanoparticles c) ratio (1:5) nanoparticles d) ratio (2:1) nanoparticlesand e) ratio (5:1) synthesized Fe_3O_4 nanoparticles.

The XRD pattern of Fe_3O_4 nanoparticles observed in Figure 7, using plant extract to salt solution precursor in 1:1 ratio was studied and the sharp intense peak obtained at 2θ , 31.69, 35.61, 45.42, 33.06, 35.95, 63.04, 45.42 and 40.55 corresponds to the lattice plane (200), (311), (220), (111), (440), (220), (220) and (400), respectively. All the diffraction peaks can be well indexed to the hexagonal phase. Crystallographic planes proving the structure of plant extract/iron oxide nanoparticles to be cubic structure (Ref. No. 01-075-0449).

Table 1: FWHM values, Crystallite sizes, d–spacing (d h k l) and Miller indices for S1 (1:1) ratio.

(degree)	FWHM (degree)	Crystallite size(nm)	d-spacing	Miller Indices h k l
31.69	0.1768	46.9	2.8208	2 0 0
33.02	0.1608	51.7	2.7111	1 1 1
35.62	0.4880	17.1	2.5186	3 1 1
35.95	0.3200	27.3	2.4956	4 4 0
40.55	0.2300	36.8	2.2224	4 0 0
45.42	0.4390	55.8	1.9950	2 2 0
63.04	0.4560	20.4	1.4733	2 2 2
Average c.size		37.0		

As compared to IO nanoparticles synthesized using iron chloride salt and Osmium Lamiifolum leaf extract in 1:1 ratio, XRD result showed that the average crystallite size of IO nanoparticles in 1:1 ratio has a good particle size sharp XRD peaks. This difference may be due to the equal amount of leaf extract and salt precursor solution used during synthesis process resulting in more capping agents that in turn effectively stabilize the synthesized nanoparticles.

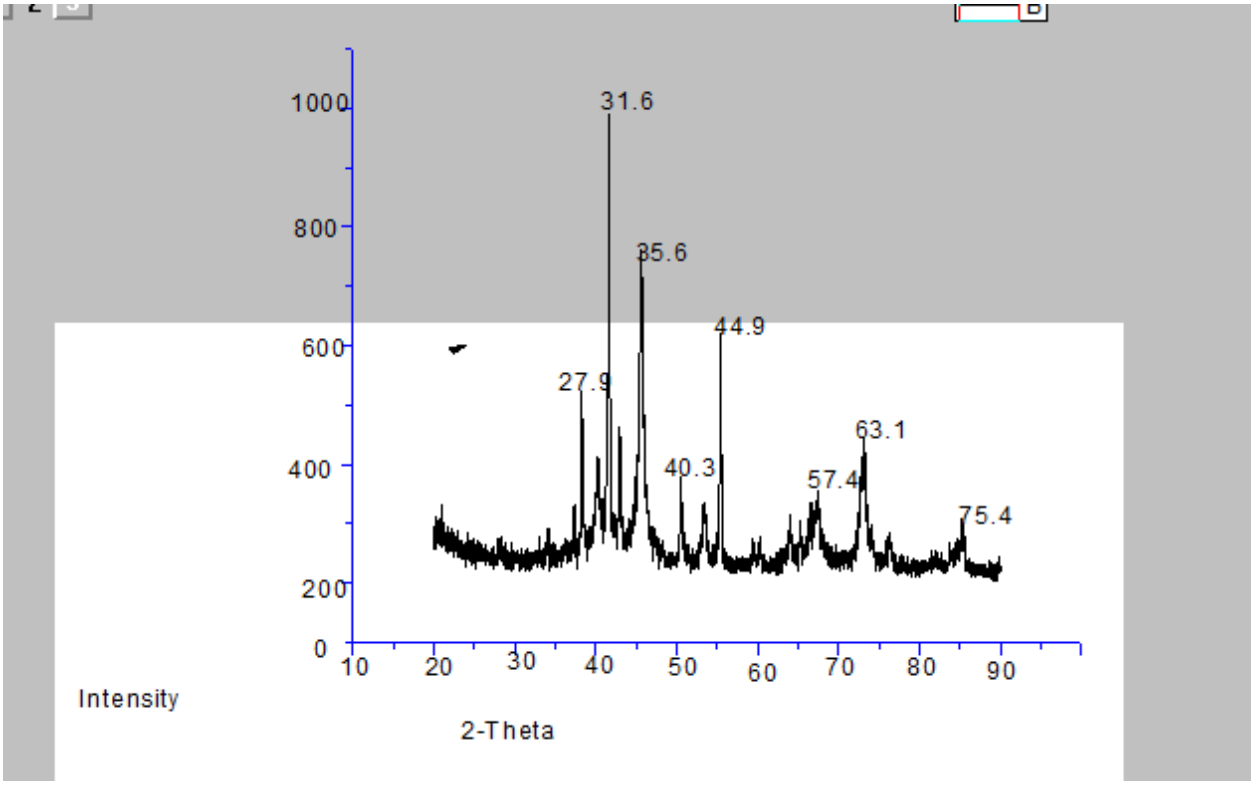

Figure 7: XRD pattern for Fe_3O_4 nanoparticles synthesized Osmium lamiifolum extract and Iron chloride salt by the ratio (1:1).

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Table 2: Different angles and their corresponding FWHM values and sizes of Fe_3O_4 NPs using Iron (II) and Iron (III) chloride salt precursors ratio (1:2 and 2:1)

2θ(degree)	FWHM (degree)	Size of Fe_3O_4 NPs(nm) ratio (1:2) S2	2θ (degree)	FWHM (degree)	Size of Fe_3O_4 NP(nm)ratio (2:1) S4
35.68	0.2381	35.08	31.56	0.3140	26.29
31.50	0.2456	33.64	35.40	0.4244	19.65
33.20	0.2116	39.26	45.24	0.3900	22.07
31.70	0.2278	36.28	62.56	0.2880	32.30
45.11	0.2615	32.92	62.24	0.2666	34.82
45.43	0.2300	37.45	56.20	0.2733	33.00
49.05	0.2689	32.53	56.82	0.3400	26.60
54.08	0.3333	26.80	57.04	0.1267	39.94
Average crystallite size		**30.83**	Average crystallite size		**28.58**

Table 3: Different angles and their corresponding FWHM values and sizes of Fe_3O_4 NPs using Iron(II) and Iron (III) chloride salt precursors ratio (1:5 and 5:1).

2θ(degree)	FWHM (degree)	Size of Fe_3O_4 NPs(nm) ratio (1:5) S3	2θ (degree)	FWHM (degree)	Size of Fe_3O_4 NP(nm)ratio (5:1) S5
35.63	0.231	33.47	35.64	0.8100	10.34
33.16	0.203	40.88	36.17	0.6000	12.71
31.67	0.233	35.44	63.16	0.6400	14.71
45.41	0.212	33.80	31.69	0.5000	15.60
49.47	0.220	39.82	33.03	0.5200	16.53
54.07	0.190	47.62	62.64	0.6000	15.96
62.46	0.255	36.37	34.73	0.6400	13.20
64.01	0.214	43.72	30.16	0.9200	9.42
Average crystallite size		**37.73**	Average crystallite size		**13.93**

4.3. Fourier Transform-Infrared (FT-IR) Spectroscopy Analysis

FT-IR gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterization of a substance. The infrared spectrum of an organic compound provides a unique fingerprint, which is readily distinguished from the absorption patterns of all other compounds; only optical isomers absorb in exactly the same way. Hence FT-IR is an important technique for identification and characterization of a substance.

The FT-IR spectra *of Osmium lamiifolum*/iron oxide nanoparticles were shown in Figure 8. Based on the FT-IR spectrum of *Osmium Lamiifolium* Figure 8(a), shows the FT-IR of IO

nanoparticles which was calcinated sample. These peaks represent the following bonding in the sample confirms the reducing agent role in the formation of Fe_3O_4-NPs. The peak at 3405 cm^{-1} corresponds to the –OH bond stretching denotes the aqueous phase as well as the reduction of the Ferric chloride, 1625 cm^{-1} corresponds to the C=O bond stretching denotes the phytochemicals present in the plant extract and amino acids which stabilize as well as act as a capping agents, 1471 cm^{-1} $–CH_2$ (Chen *et al*., 2011). Remaining unclear peaks represents small amount of organic acids which is responsible for the low pH of the sample which helps to the synthesis of the Fe_3O_4-NPs. The strong peak at 565 cm^{-1} corresponds to the inorganic stretching indicates the Fe_3O_4-NPs (Karaoglu *et al.*, 2011). The presences of magnetite nanoparticles can be seen by strong absorption band at around 580 and 449 cm-^{1} which, corresponding to the Fe-O stretching band of bulk magnetite (Fe_3O_4). These results revealed that the C=O groups were bonded on the magnetite particle surface. Overall the observation confirms the presence of protein in Osmium Lamiifolium leaf extract, which acts as a reducing agent and stabilizer for magnetite nanoparticles (Mahnaz *et al*., 2013).

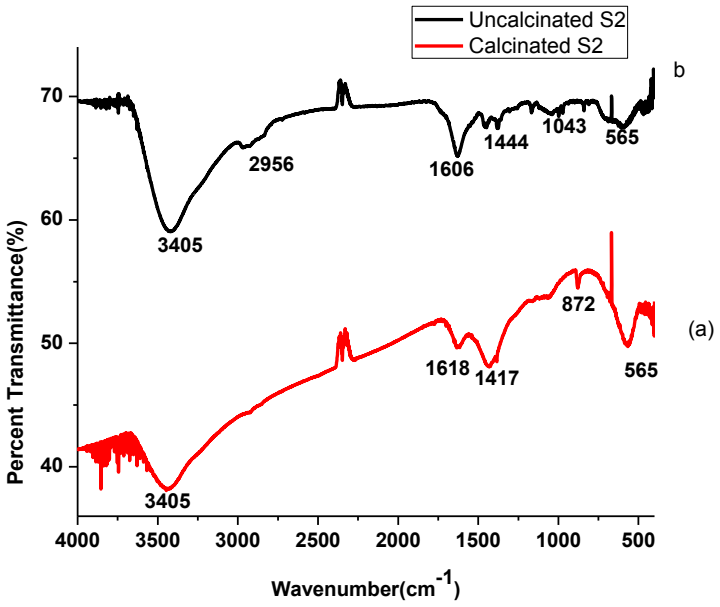


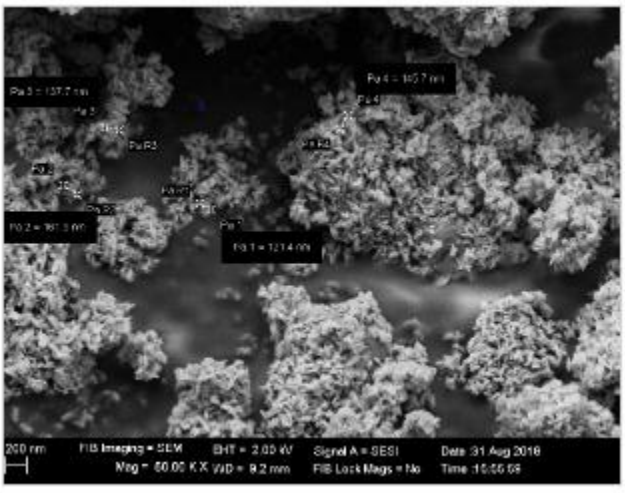
Figure 8: FT-IR spectrum for calcinated Fe_3O_4 NPs and uncalcinated precursors**.**

Figure 8(b) also Show the FT-IR spectra of the green synthesized Fe_3O_4 NPs using Leaf extract of *Ocium Lamiifolium* of iron (II) and Iron (III) salt before calcinations. In the spectrum, the wide absorption peak around 3405 cm $^{-1}$ is due to O-H stretching vibrations and 2956cm.$^{-1}$ Absorption peak shows that C-H stretching for carbohydrate. Aromatic C=C ring stretching absorption occurs around 1606 C$^{-1.}$ Strong intensity at 1444 cm $^{-1}$ is due to C-CH_3 bending and C-O stretching and vibrations of aldehydes and ketones. The strong peak at 565 cm-^{1} corresponds to the inorganic stretching indicates the Fe_3O_4-NPs. The presences of Magnetite nanoparticles can be seen by strong absorption band in between 580 and 449 cm-^{1} which, corresponding to the Fe-O stretching band of bulk magnetite (Fe_3O_4) (Karaoglu *et al.*, 2011).These results revealed that the C=O groups were bonded on the magnetite particle surface. Overall the observation confirms the presence of protein in Osmium Lamiifolium leaf extract, which acts as a reducing agent and stabilizer for Magnetite nanoparticles Ocimium lamiifolum (Mahnaz *et al*., 2013).

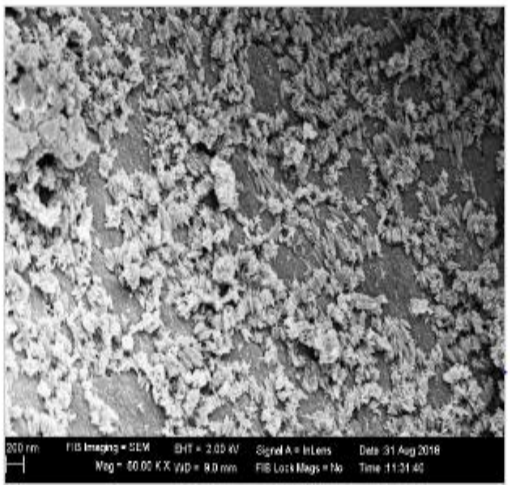
4.4.Scanning Electron Microscopy (SEM): To further confirm the role of extracts in synthesizing iron oxide NPs we used SEM microscope to show the size and morphology of Fe_3O_4-NPs using Ocimium lamiifolum extracts can be seen in figure 9. This figure clearly

shows the cubic morphology of the IONPs, with an average particle size of 37 nm, in which few NPs were agglomerated. In comparison to chemical synthesis of Fe_3O_4-NPs different concentrations slight aggregation of Fe_3O_4-NPs was observed. This outcome can be explained by the fact that the polyphenols concentrations in ocimium lamiifolium extract plays a key role in the formation of the final structures and size of these green synthesized Fe_3O_4-NPs.

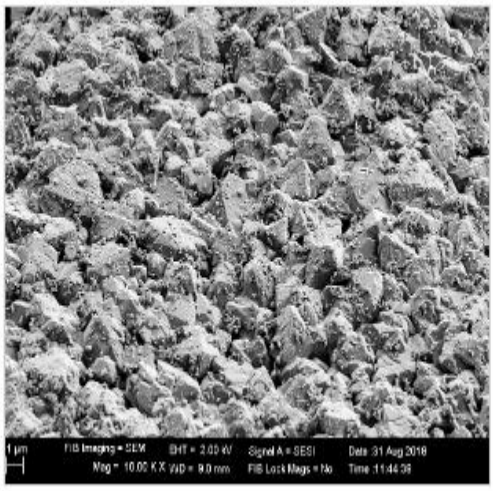
The morphology of Fe_3O_4 nanoparticles were studied using SEM images. The surface morphology of the synthesized samples was viewed through the high resolution scanning electron microscope. SEM images of as-grown low dimensional iron oxide NPs are presented in Figure 9.Figure 9a -f shows the low to high magnified images of NPs grown by hydrothermal Process .The morphology of uncalcined Fe_3O_4 as indicated in Figure 9 (a) is not clearly identified. The SEM images of calcined Fe_3O_4 on (Figure 9(b - f) indicate the agglomeration of particles that increases with the concentration of Fe_3O_4. The morphology of Fe_3O_4 NPs synthesized in different mixing ratio of precorsore to plant extract such as 1:1, 1:2,1:5, 2:1 and 5:1 have agglomerated spherical shapes. On the SEM image of cluster, flake-like and agglomerated spherical shapes were observed. The scanning electron microscope results confirmed that the grain sizes of the synthesized NPs were strongly dependent on mixing ratio of Iron Chloride salt& leave extracts under the same reaction conditions



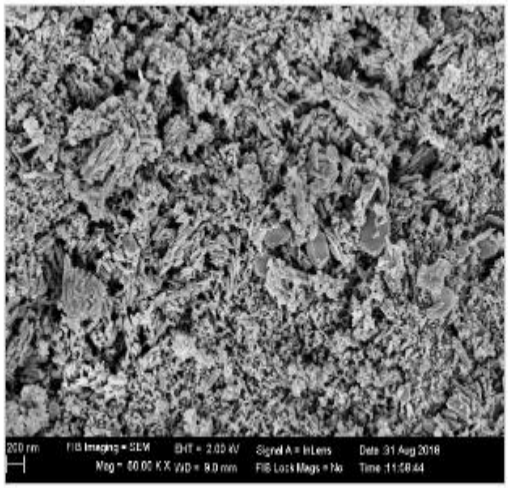
(a)



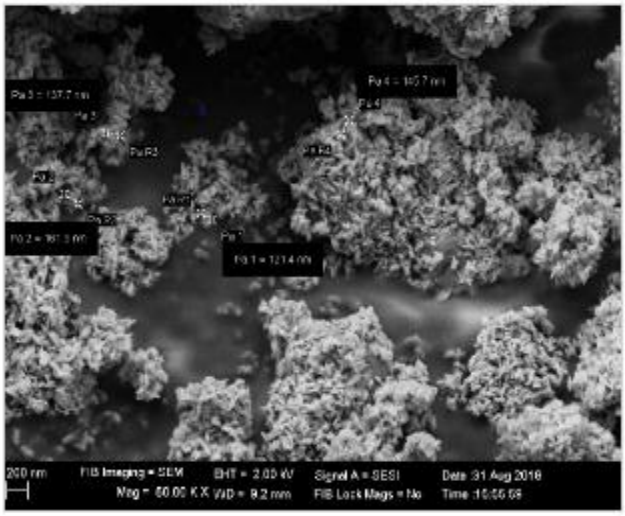
(b)



(c)

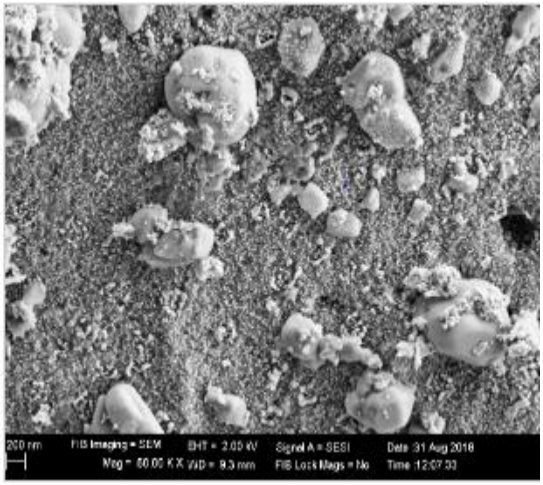


(d)



c

(e)



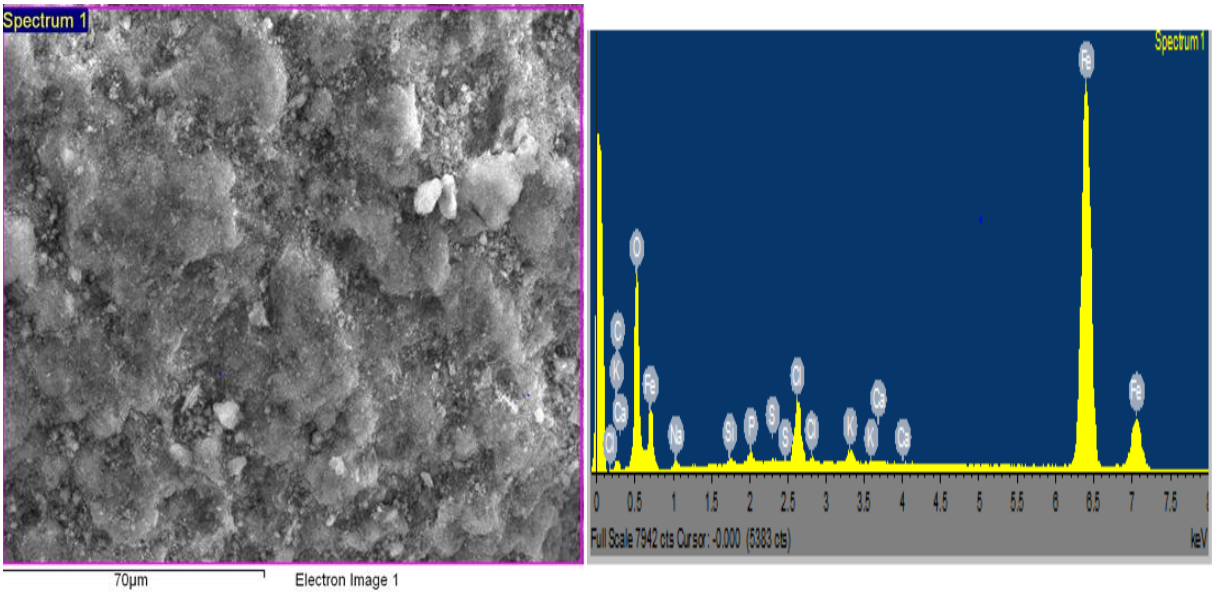
(f)

Figure9(a-f). The SEM image of Fe_3O_4-IONPs green synthesized using Ocimium lamiifolium extract

The SEM image results also agreed with XRD results obtained, i.e. the crystal sizes in XRD with big size have also big shapes in SEM images. This indicates that Fe_3O_4 NPs have successfully prepared using Iron Chloride salt precursor within a nano range. The SEM images clearly show micro structural homogeneities and remarkably different morphology for Fe_3O_4 powder.

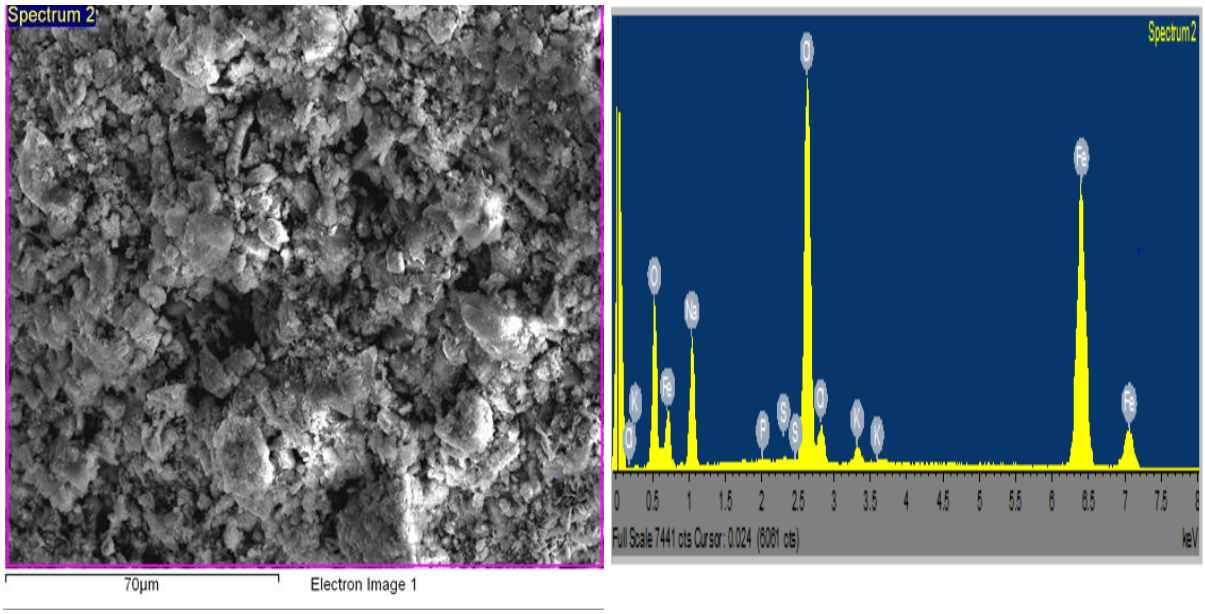
4.5. Energy Dispersive X-Ray Spectroscopy (EDX) Analysis

The composition of the samples was determined by energy dispersive X-Ray spectroscopy (EDX) using scanning microscope. The energy dispersive X-ray analysis of all Fe_3O_4 nano particles using iron Chloride salt at different mixing ratios with plant extracts are shown in Figures 10. It is evident from the X-ray patterns of all the samples in which Iron and Oxygen are found in the respective spectrum. Fig10. depicts the distribution size and identification of the iron elements using by SEM image and EDX. SEM image supports the TEM results that the iron oxide nanoparticles have small size (Fig. 10a). Each element has a unique atomic structure; EDX provides information about the chemical composition of the compound. EDX is an interaction between X-rays and the compound being investigated. Therefore, when this analysis is carried out, the X-rays that are reflected off the compound give peaks. The amplitude of the peaks obtained help to identify the elements present in the compound being studied. The peak amplitude of iron starts from 0.7 to 7 keV. Fig. 10b confirms the presence of the iron elements in the compounds using EDX. The results also demonstrate the high percentage of iron present in the particles. The EDX spectra revealed the presence of iron peaks in three different areas (0.7, 6.5 and 7.0).



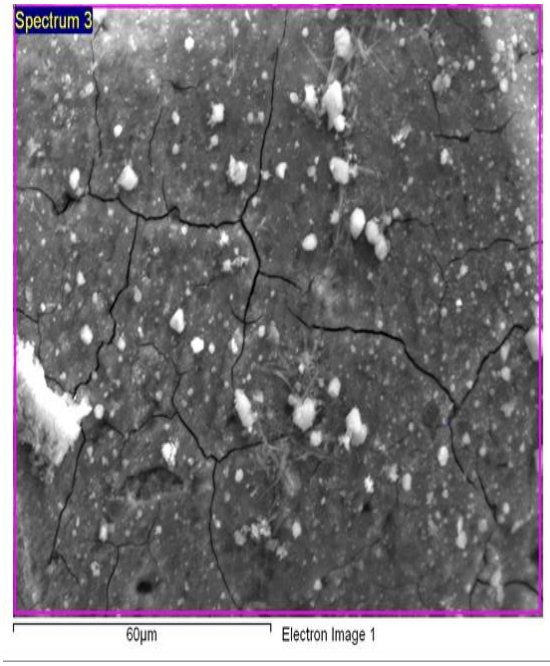
(a) (b) EDX

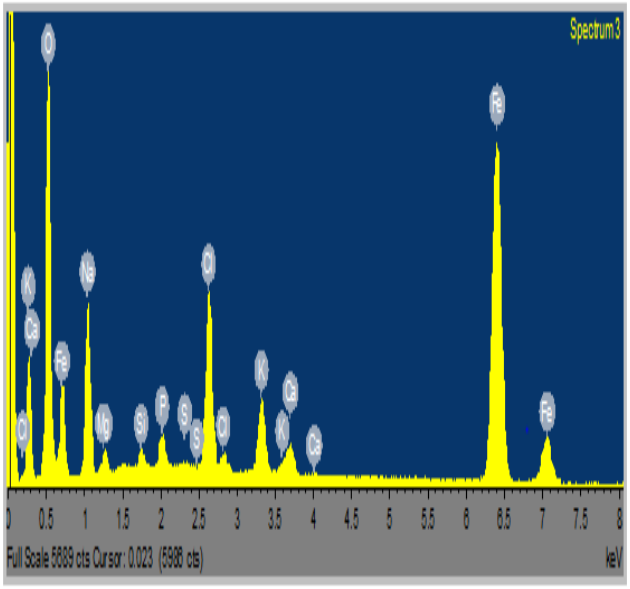
Fig. 10. SEM micrographs (a) and EDX spectra of the Ocimium lamiifolium iron oxide nanoparticles (b) of sample (1:1)



(c) **(d) EDX**

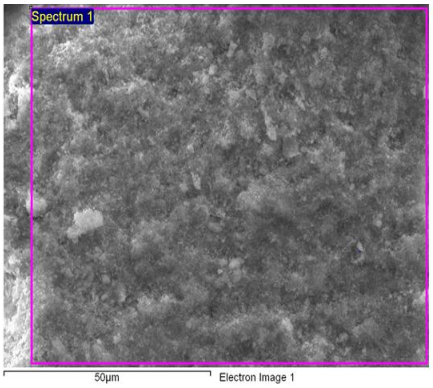
Fig. 10. SEM micrographs (c) and EDX spectra of the Ocimium lamiifolium iron oxide nanoparticles (d) of sample (1:2)

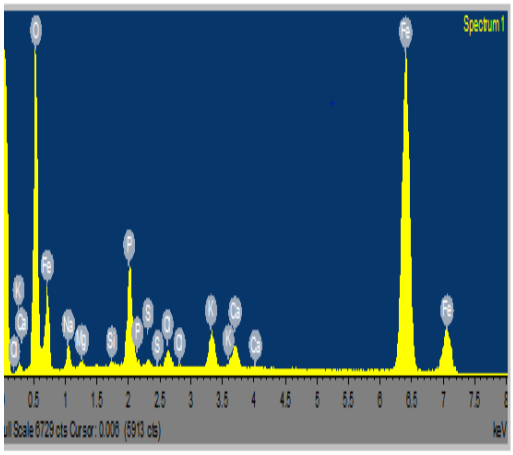




(e) **(f)** **EDX**

Fig10. SEM micrographs (e) and EDX spectra of the Ocimium lamiifolium iron oxide nanoparticles (f) of sample (2:1)





(g) **(h) EDX**

Fig10. SEM micrographs (g) and EDX spectra of the Ocimium lamiifolium iron oxide nanoparticles (h) of sample (5:1)

4.6 Antibacterial activity

Iron oxide nanoparticles showed antibacterial effect against gram-positive as well as gram-negative bacteria which clearly indicates that these nanoparticles were effective antibacterial agents. Figure 8(a) and (b) shows zone of inhibition of Fe_3O_4 nanoparticles against bacteria's. Many antibacterial studies were made using different concentration nanoparticles. The reason for the bactericidal activity is due to the presence of reactive oxygen species (ROS) generated by different nanoparticle. Chemicals interaction between hydrogen peroxide and membrane proteins or between the chemical produced in the presence of Fe_3O_4 nanoparticles and the outer bilayer of bacteria could be the reason for the antibacterial activity of Fe_3O_4. The hydrogen peroxide produced enters the cell membrane of bacteria and kills them. It is also noted that nanoparticles continue to be in interaction with dead bacteria once the hydrogen peroxide is generated; thus foiling further bacterial action and continue to produce and release hydrogen peroxide to the medium. The results of antibacterial studies clearly suggest that the Fe_3O_4 nanoparticles synthesized using *Osmium lamiifolum* extract has a far better antibacterial activity even at a large concentration dose when compared to small concentration IO nanoparticles. The mechanism of action of Fe_3O_4 nanoparticles has not been established yet. Nano materials were reported to exhibit broad spectrum biocidal activity towards various microorganisms like bacteria, cancer, and viruses (Ikigai *et al.,* 1990). Previous studies have reported that the membrane proteins were

inactivated by nanoparticles, which decreases the membrane permeability causing the cellular death (Mohanpuria *et al*., 2008). Nano materials also produce retardation in the bacterial adhesion and development of bio-film; hence it is easy to destroy the microbes (Gou *et al*., 2010). Polyphenols on the other hand were reported to possess various beneficial activities like antibacterial, antiviral, antifungal and anticancer activities. Polyphenols were also reported to inhibit exotoxins found in bacteria (Tanapon *et al*., 2010),With these background data, we can suggest that the bio stabilized Fe_3O_4 nanoparticles capped by large ratio of extracted plant polyphenols has better activity nanoparticles to induce membrane damage and cell death of gram-negative(*Pseudomonas aeruginosa, E. coli)* and gram-positive (*Staphylococcusaureus, Bacillus subtili*) produced in the presence of Fe_3O_4 nanoparticles and the outer bilayer Fe_3O_4 nanoparticles at various concentrations (50, 75, and 100 μg/ml) were tested for antibacterial. The antibacterial activity performance of Fe_3O_4 nanoparticles was done by using disc diffusion method. The effects of particle size and concentration on the antibacterial activity of Fe_3O_4 nanoparticles were studied using this method. These tests were performed in nutrient broth and nutrient agar following standard methods. This method was well documented and standard zones of inhibition have been determined for susceptible and resistant values. The zone of inhibition increases with the increase in Iron oxide nanoparticle concentration and in increase particle size.

Table 4. **Zone of inhibition produced by biosynthesized Fe_3O_4 nanoparticles and positive control (Penicillin)**

Sample composition	S1(1:1) 37nm			S2(1:2) 30.83nm			S3(1:5) 37.73nm			S4(2:1) 28.58nm			S5(5:1) 13.93nm		
Inhibition by bacteria (mm).	C1	C2	C3	C1	c2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
E.Coli(-ve)	12	14	11	6	6	6	6	6	6	6	6	8	15	18	20
P.aeruginosa	12	6	10	6	6	6	6	6	10	6	6	6	12	14	15

(-ve)														
Bocillus subtilus (+ve**)**	12	12	13	6	12	13	6	6	6	6	6	6	6	6	6
S.aureus(+ve)	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Penicillin	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

According to Table **4** Fe_3O_4 NPs using sample ratio (1:1) all the concentrations were sensitive to gram positive bacteria (*Bacillus subtilis*) and gram negative bacteria (*E. coli* and *p.aeruginose)* but they are resistant to *S. aureus*. The results showed that Fe_3O_4 nanoparticles have antibacterial activities towards gram positive bacteria and gram negative bacteria (*E.coli*) and P.aeruginese but resistant to *S.aureus.*

Gram-positive bacteria seemed to be more resistant to Fe_3O_4 nanoparticles than Gram-negative bacteria. It was found that the antibacterial activity of IONPs nanoparticles increased with decreasing particle size and increasing powder concentration. Their anti-bacterial activity was found higher than reference antibiotic penicillin to pical dosage forms. All the bacteria are exposed to positive control penicillin all of them were not inhibited that means they are resistant. In Table 4 zone of inhibition (mm) of the tested samples of IONPs using Iron salt precursor and *Ocimum Lamiifolum* leaf extract at different ratios .The activities of Fe_3O_4 against four different bacterial strains were also given. According to Table **4,** IONPs using plant to salt precursor solution 1:1 ratio was resistant to gram positive S.aureus bacterial strains. IONPs using the ratio1:1and 1:5 have greatest zone of inhibition(20 mm) indicating that it has greatest antibacterial activity against *E.coil* and P.aeruginose (gram negative bacteria) but it is resistant to *Bacillus subtilis S.aureus*(gram positive bacteria). IONPs at 1:1 and 1:2 ratios have zone of inhibition 13 mm. Table **4** how's that *S.aureus* (gram positive) bacteria resistant IONPs in all ratio and concentration level. This indicates that they have strong resistant activity against IONPs

(a) (b)

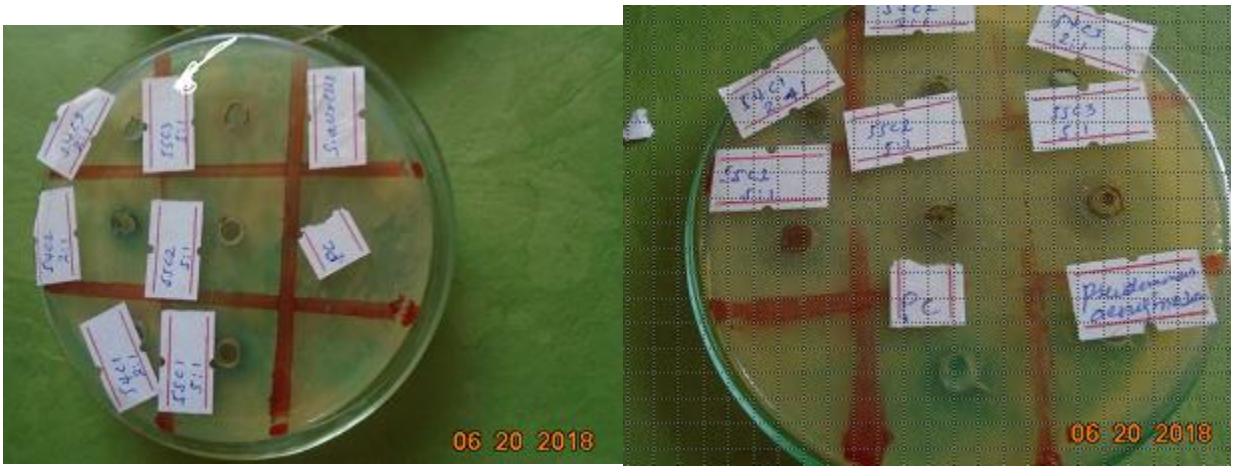


Figure 11: Zone of inhibition of Fe_3O_4 nanoparticles against (a) *Staphylococcus Aureus*, and *P.seudomonas Aereus*

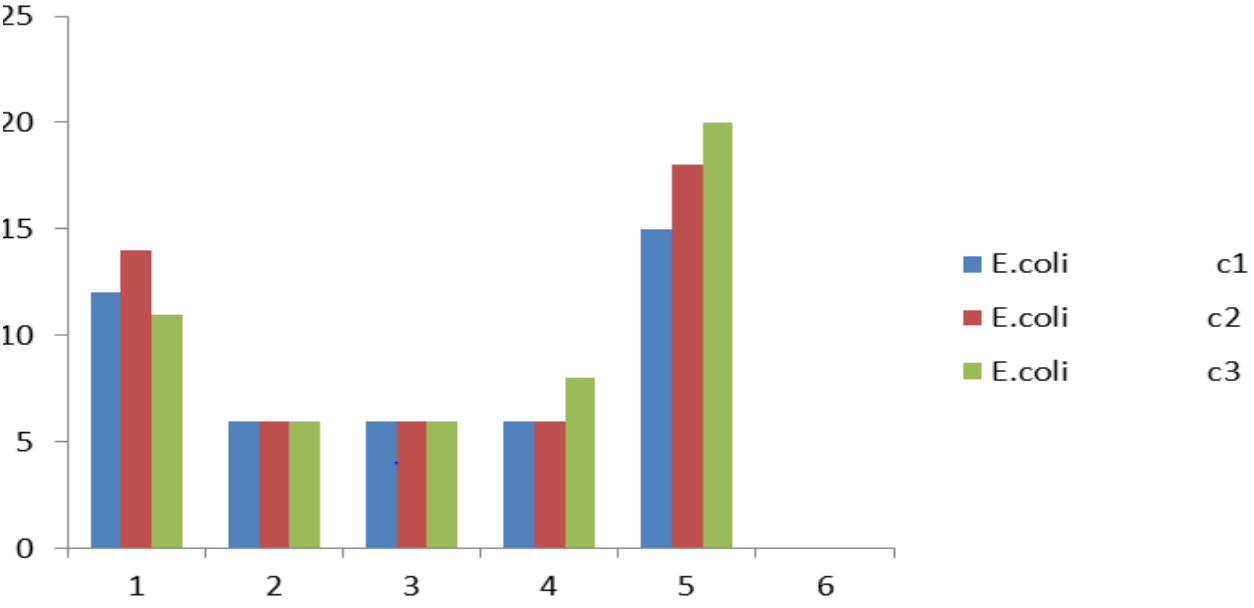


Fig. 11 (a) bargraph shows the inhibition zone versus concentration of sample (1-5) on E.coli bacteria.

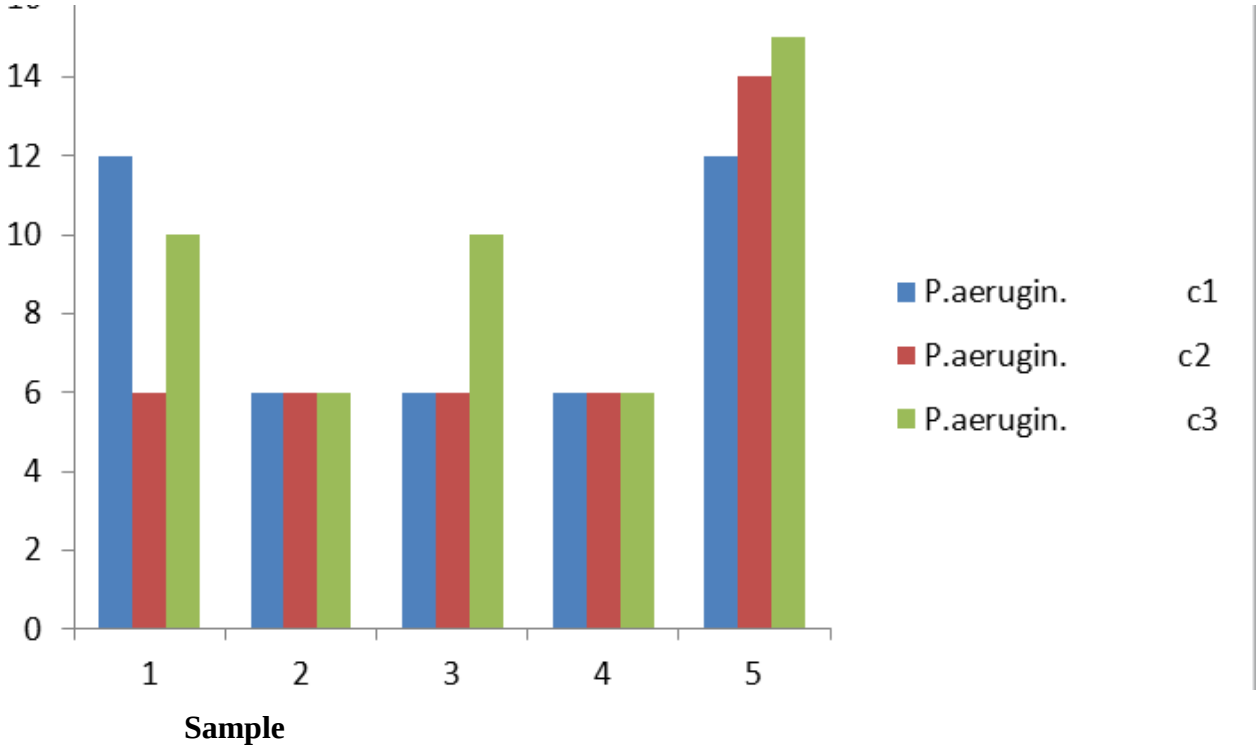


Fig. 11 (b) barograph shows the inhibition zone versus concentration of sample (1-5) on P.aerugin bacteria.

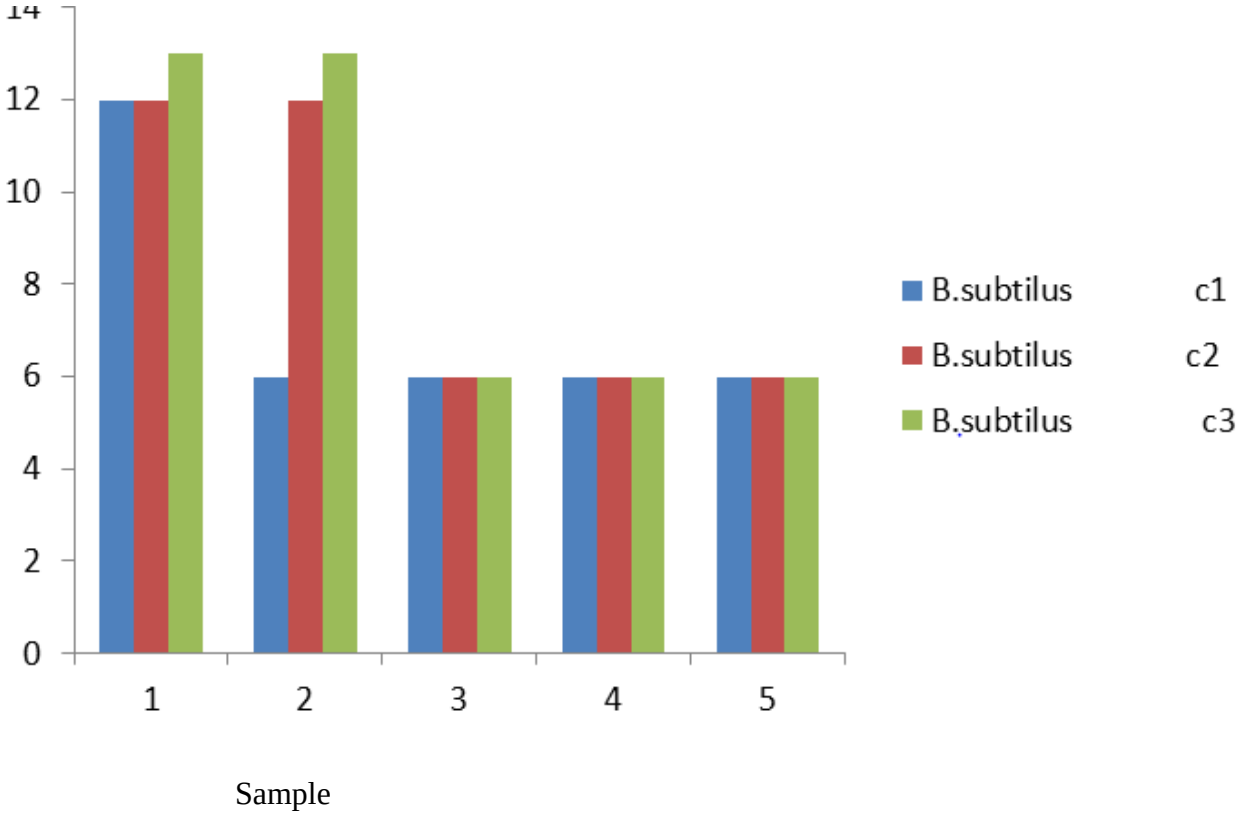


Fig. 11(c) barograph shows the inhibition zone versus concentration of sample (1-5) on B.subtilus bacteria.

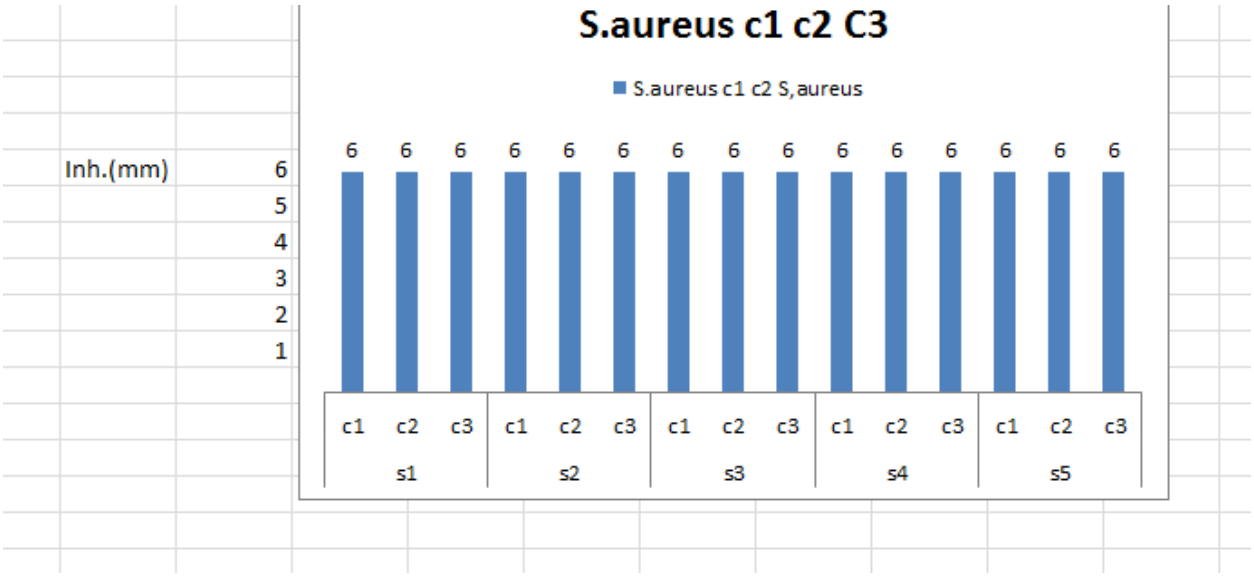


Fig. 11 (d) barograph shows the inhibition zone versus concentration of sample (1-5) on S.aereus bacteria.

5. CONCLUSION

The main objective of the study was to synthesize pure Fe_3O_4 nanoparticles using leaf extract of *Osmium Lamiifolium* and to study their antibacterial activity against drug resistant bacteria. Fe_3O_4 NPs were successfully synthesized by the biological synthesis method using aqueous leaf extracts of Osmium Lamiifolium and Iron Chloride salt solution and characterized by advanced techniques.

The XRD study confirms the formation of hexagonal magnetite structure and the crystallite size (13 – 37nm) of the synthesized Fe_3O_4 nanoparticles while the vibrational stretching around 565 cm^{-1} & the characteristic absorption peak observed at 311 nm were also supports the formation of Fe_3O_4 nanoparticles. Functional group bands observed at 3405, 2956 and 2932 cm^{-1} have been assigned to stretching vibrations of the amines, O–H stretching of alcohols and C–H stretching of alkanes respectively for NPs using. Fourier transformer infrared (FT-IR) spectroscopy of the green synthesized Fe_3O_4 nanoparticles were characterized and recorded using a FT-IR

spectrophotometer in order to analyze and detect surface functional groups of the biosynthesized Fe_3O_4 nanoparticles at the scanning range of 4000 - 400 cm^{-1}. The crystallite of the synthesized iron oxide nanoparticles was found from XRD analysis, which is in an agreement with the result obtained from the TEM. The FT-IR results of the nanoparticles were shown that Ocimium lamiifolum was successfully coted in iron oxide nanoparticles which is confirm by TGA. This method is inexpensive and environmentally friendly leading to the preparation of very small iron oxide nanoparticles.

The synthesized Fe_3O_4 with (1:1) ratio Fe_3O_4 nanoparticles showed significant antibacterial activity against gram positive and gram negative bacteria, including *Bacillus subtilis,* and *Escherichia coli and P.aeruginosa but resistance on S.aureus*. All the synthesized nanoparticles have not sensitive to *S.aureus* it may be this bacterium has greatest resistance towards drugs. Fe_3O_4 nanoparticles have significantly higher antibacterial effect on *E. coli* indicating that the synthesized Fe_3O_4 NPs are more sensitive to gram negative bacteria. Compared with gram-negativeitive bacteria, Gram-positive bacteria are most resistant against antibodies because of their impenetrable cell wall. The iron oxide nanoparticles showed their antibacterial properties on both gram positive and gram negative bacterial strains, but do not *S. aerus*. As the diameter of the zone of inhibition is high, we can conclude that iron oxide is a very effective antibacterial agent.

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Lett. 65 1882