Biobased green approach to synthesise iron nanoparticles using *Phyllanthus reticulatus* (Poir.) aqueous extract

Siva Dharshini, R

(16PZO011)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

In partial fulfilment of the requirements for the Degree of

MASTER OF SCIENCE IN ZOOLOGY

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Signature of the Supervisor

Acknowledgement

ACKNOWLEDGEMENT

The author owes her sincere thanks and gratitude to **Thiru. Dr. P. R. Krishna Kumar,** Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for all the amenities provided for the conduct of the project.

The author place on her gratitude and heartfelt thanks to **Dr. (Mrs.) Premavathy Vijayan, M.Sc., M.Ed., Dip. Spl. Edn., M.Phil., Ph.D.,** Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for extending all possible help towards the completion of the study.

The author feel greatly thankful to **Dr. (Mrs.) S. Kowsalya, M.Sc., M.Phil., Ph.D.,** Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her continued interest and valuable guidance in the conduct of this project work.

The author is greatly indebted and thankful to **Dr. (Mrs.) A. Parvathi**, **M.Sc., Dip. Ed., M.Phil., Ph.D.,** Dean, Faculty of Science, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her continued interest and constant support in the conduct of project work.

The author expresses her deep sense of gratitude and special thanks to **Mrs. E. Santhi, M.Sc., Dip. Ed., M.Phil.,** Associate Professor and Head, Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for the constant support given during the period of investigation.

The author is highly grateful and indebted to her guide **Dr. (Mrs.) M. Poonkothai, M.Sc., M. Phil., Ph.D.,** Assistant Professor (SS), Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for the guidance rendered at every stage of the dissertation. Without her dynamic guidance, valuable suggestions, untiring help, meticulous efforts and enduring support, this study would never have seen the light of the day.

The author takes this opportunity to thank the **Staff Members** and **Lab Assistants** in the Department of Zoology for their help to carry out the study.

The author wishes to express the fact that the empathy and inspiration from her father **Mr. R. Rajathirajan,** mother **Mrs. R. Vijayalakshmi,** brother **R. Senthil Amarnath,** sister **R.Siva sri saranya** and **friends** who helped her to complete this endeavor.

Above all the author thanks **God Almighty** for his immense mercy for the successful completion of her studies.

Contents

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Nanotechnology was currently ruling the world in various fields and has significant operations in the production of future tools in the nanoscale. Nano was derived from the Greek word for dwarf which is pooled with technology to indicate operations occurring on the scale of 10^{-9} meters. The scales of nanotechnology are obvious first and vital delineator of various activities, artifacts, tools, knowledges and structures comprise the technological domain. Nanotechnology was the further miniaturization of existing techniques and processes.

Fascinatedly, nanotechnology is not on the nanoscale but rather is focused on the micron level. The term nanotechnology was used to symbolize smaller rather than scale, as evinced by the cooperation between the National and Aeronautics and Space Administration (NASA) and the U. S. Department of Defense to develop and launch three 10 kg spacecraft called as nanosatellites to examine the satellite coordination (Martin *et al.,* 1999).

There are many factors in nanotechnology than a simple delimitation of nanometers. Nanoparticles were significant to the high extent and it has very interesting applications and properties related to the various fields such as medicine, nutrition and energy resources (Chandran *et al*., 2006).

Numerous processes were used for synthesis of nanoparticles such as physical, chemical, enzymatic and biological methods. Physical methods includes plasma arcing, ball mining, thermal evaporate, spray pyrolysis, ultra-thin films, pulsed laser desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epistaxis and diffusion flame synthesis of nanoparticles (Joerger *et al*., 2000).

Correspondingly, the chemical processes also involved in the nanoparticle synthesis like electro deposition, sol-gel process, chemical solution deposition, chemical vapour deposition (Panigrahi *et al.,* 2004 and Oliveira *et al.,* 2005), soft chemical method, Langmuir Boldgett method, catalytic route, hydrolysis (Pileni, 1997), co-precipitation method and wet chemical method (Gan *et al.,* 2012).

The above listed physical and chemical methods were subjected with elevated radiation and extremely concentrated reductants and stabilizing agents that are injurious to environment as well as the human health.

Green synthesis of metallic nanoparticles has accumulated an ultimate interest over the last decade due to their distinctive properties that make them applicable in various fields of science and technology. Metal nanoparticles that are synthesized by using plants have emerged as nontoxic and ecofriendly approach (Naseem and Farrukh, 2015).

The interest in synthesizing nanoparticles in an easy and environmental friendly way has been increasing in the recent years. Physical and chemical methods are conventionally used for synthesis of nanoparticles, however due to limitations of these methods, the focus of research has been recently shifted towards the development of clean and eco-friendly synthesis protocols (Prabhu and Poulose, 2012).

Comparatively, biological method for the synthesis of nanoparticles is the bioreduction method and less energy consuming (Sathishkumar *et al.,* 2009). Although the biological methods are using plant extracts, bacteria, fungi, micro algae such as cyanobacteria, diatom, seaweed (macroalgae) and enzymes (Iravani *et al.,* 2011).

The biobased synthesis of monodispersed nanoparticles with specific size and shapes has the major complexity in biological material science. Also, it was vital in its advantages in the pharmacology to cure various bacterial and viral diseases (Song *et al.,* 2008).

Biological methods are entertained over the complete compensation of classical synthesis processes due to the enormous availability of more biological resources and eco- friendly pollution free procedures. These wide biodiversity of the natural resources was highly reconnoitered for the nanomaterials synthesis (Monda *et al.,* 2011).

Recent studies on the biosynthesis of nanostructures like wires, particles, flowers and tubes were executed successfully. They are highly potent in the applications in various fields such as treatment, diagnosis, development of surgical nanodevices and commercial product manufacturing (Bar *et al.,* 2009).

Nanomedicine imparts a major healthcare sector in treatment of chronic diseases. Therefore, biobased synthesis of nanoparticles was reflected as building blocks for future generation to prevent the prevalence of various diseases (Cruz *et al.,* 2016).

Currently, plant mediated nanoparticles were placed the hotspot due to its immense application in various fields on accordance with their physio- chemical properties. Various metallic nanoparticles such as iron, gold, silver, platinum, zinc, copper, titanium oxide, magnetite and nickel were synthesized using plants.

The different parts of plant were stem, root, fruit, seed, callus, peel, leaves and flower used to synthesis metallic nanoparticles. Biosynthesis will get transformed the shapes and size of the nanoparticles (Chandran *et al.,* 2006 and Dubey *et al.,* 2010). Nano sized inorganic particles of either simple or complex nature, display unique, physical and chemical properties and represent an increasingly important material in the development of novel nanodevices which can be used in numerous physical, biological, biomedical and pharmaceutical applications (Loureiro *et al.,* 2016; Martis *et al.,* 2012; Nikalje, 2015).

Iron was the major constituent in every living being and it was the vital component of blood. Iron was the essential component for plants and animals in traces. It is the most ubiquitous of the transition metals and the fourth most plentiful element in the Earth's crust and is the structural backbone for modern infrastructure. It is therefore ironic that as a nanoparticle, iron has been somewhat neglected in favor of its own oxides, as well as other metals such as cobalt, nickel, gold, and platinum. This is unfortunate, but understandable. Irons reactivity is important in macroscopic applications (particularly rusting), but is a dominant concern at the nanoscale.

Finely divided iron has long been known to be pyrophoric, which is a major reason that iron nanoparticles have not been more fully studied to date. This extreme reactivity has traditionally made iron nanoparticles difficult to study and inconvenient for practical applications. Iron however has a great deal to offer at the nanoscale, including very potent magnetic and catalytic properties. Recent work has begun to take advantage of irons potential, and work in this field appears to be blossoming (Huber, 2005).

Iron oxide particles such as magnetite (Fe_3O_4) or its oxidized form maghemite (Fe_2O_3) are the most commonly employed for biomedical applications (Ali *et al.,* 2016). There are number of potential biomedical applications for magnetic iron nanoparticles, which include the labeling and magnetic separation of biological materials, directed drug delivery, MRI contrast enhancement, and hyperthermia treatment (Pankhurst, 2003).

Iron nanoparticles were highly efficient in their antimicrobial activity because these metal oxides have high affinity towards antimicrobial agent. Mechanism between iron oxide nanoparticles and bacteria interface is discovered by Arakha *et al.* (2015) for evaluation of antimicrobial activity. The biosynthesized iron nanoparticles will hold positive surface potential to have better surface for bacterial attachment thereby enhance production of reactive oxygen species (ROS) in the culture media. Parallel studies were investigated by Lee *et al.* (2008) and Sneha *et al.* (2011) on the antibacterial activity of zero valent iron nanoparticles (nano Fe^0).

Even today, we have limited studies for contaminant bioremediation in the waste water treatment applying iron nanoparticles. Currently, Devatha *et al.* (2016) established the green synthesis of iron nanoparticles using plants for the waste water treatment. Literature survey reveals the significance of green synthesis of iron nanoparticles in various fields due to the benefit of environmental friendliness and cost effective approach.

The application of iron nanoparticles as we compared with the micrometric particles is due to high efficiency in reduction reactions, high reactivity, due to the

high surface area, mobility and filtration efficiency when used in technologies for remedying a certain environment (Zhou *et al.,* 2015). The nanosize remain in suspension for a long period of time, it facilitates the different known applications such as water treatment and wastewater (Elliot and Zhang, 2001).

Iron is one of the vital components for plant growth and it also contributes to the photosynthetic reactions. Iron is also involved in the enzymatic reactions and contributes in RNA synthesis and enhances the enactment of photosystems in plants (Sheykhbaglou et al. 2010). Some studies regarding impact of iron oxide upon the plant growth evinced an optimistic influence in cereals, illuminated on the basis of significance of iron in the vegetal organism. Iron oxide may be a source of iron for the plant development. The biosynthesis of siderophores by plant was assumed to be enthused with the iron from iron oxides (Racuciu *et al.,* 2007).

Silver, copper and iron have been extensively applied on various crops effecting positively and negatively depending on crop and concentration of nanoparticles applied. Due to mixed sort of effects of these nanoparticles on plants, present study was planned to have a deep view about green gram response towards these nanoparticles since it forms a major component in the diet. Hence, to quantify the possible effect of iron nanoparticles phytotoxicity assay was carried out.

The plant selected for the biosynthesis of iron nanoparticles mediated by leaf extract was *Phyllanthus reticulatus* (Poir.) which is cosmopolitan in distribution and found mostly in hedges or waste places. It was widespread in India and occurs throughout tropical Africa, except in the most humid and equatorial areas. It was commonly called as *Black-Honey* shrub and in Tamil it was called as “Kattukizhanelli”, which belongs to the family Phyllanthaceae.

The leaves of the plant contains tannic acid and gum. It is reported to be used as diuretic, attenuant, astringent, antidiabetic, antidiarrheal and a cooling agent (Nadkarni and Nadkarni, 2009). The plant has the highest degree of traditional medicinal applications all over the world. The Asante people in Ghana

give a soup made of *Phyllanthus reticulatus* leaves boiled with palm oil to women after childbirth. Sap from the stem is used as eye drops to cure conjunctivitis and soreness. In Southern Africa powdered leaves are topically applied to sores, including venereal sores, burns, suppurations and chafes. In Tanzania crushed leaves are rubbed on the body of malaria patients. In Sudan and Southern Africa the leaves and bark are reputed to be diuretic and cooling. In Zanzibar the plant is considered as a remedy for anemia and intestinal hemorrhage.

In India the powdered leaves are pounded with cubebs (*Piper cubeba* L.) and camphor into tablets for sucking against bleeding gums and the leaves are also used in the treatment of diabetes. In Philippines leaves are applied locally against pinworms. A root decoction is taken against gonorrhoea and other venereal diseases and also against diarrhoea accompanied by mild anal bleeding. In Malaysia the stem and leaves are rubbed on the chest against asthma and leaf decoction is drunk to treat a sore throat, against snakebites, mental problems and diarrhoea.

Although iron nanoparticles possess the broad spectrum applications in the magnetic field because of its magnetic properties, catalytic application, biomedical applications, environmental applications and diagnostic applications, the commercial applications of iron nanoparticles are limited, but the scientific implications have been broad. Much progress has been made, but there is much more work to do.

Hence with this background the present study was formulated with the following objectives:

- Biobased green approach to synthesise iron nanoparticles using *Phyllanthus reticulatus* leaf extract
- Characterisation of the biosynthesized iron nanoparticles by UV- Vis spectroscopy, FT- IR, XRD, DLS, SEM and EDX.
- Phytochemical screening of biosynthesized iron nanoparticles.

- Assessment of antimicrobial activity of biosynthesized iron nanoparticles
- Evaluation of phytotoxicity of biosynthesized iron nanoparticles
- Assessment of photocatalytic activity of biosynthesized iron nanoparticles

Review of Literature

1. REVIEW OF LIERATURE

The review of literature pertaining to the present study “Biobased green approach to synthesize iron nanoparticles using *Phyllanthus reticularis* (Poir.) leaf extract” is presented under the following headings:

2.1 CAPSULISATION OF NANOTECHNOLOGY

2.2 ORIGIN OF NANOTECHNOLOGY

2.3 CLASSIFICATION OF NANOPARTICLES

- 2.3.1 Carbon based nanoparticles
- 2.3.2 Metal based nanoparticles
- 2.3.3 Ceramic nanoparticles
- 2.3.4 Semiconductor nanoparticles
- 2.3.5 Polymeric nanoparticles
- 2.3.6 Lipid based nanoparticles

2.4 PHYSICO CHEMICAL PROPERTIES OF NANOPARTICLES

- 2.4.1 Electronic and optical properties
- 2.4.2 Magnetic properties
- 2.4.3 Mechanical properties
- 2.4.4 Thermal properties

2.5 APPLICATIONS OF NANOPARTICLES IN VARIOUS FIELDS

- 2.5.1 Drugs and medications
- 2.5.2 Manufacturing and materials
- 2.5.3 Environment

2.6 METHODS OF NANOPARTICLES SYNTHESIS

- 2.6.1 Top down method
- 2.6.2 Bottom up method
- 2.6.3 Other methods of preparation

2.7 IRON NANOPARTICLES

- 2.7.1 Synthesis of iron nanoparticles

2.8 SYNTHESIS OF NANOPARTICLES USING PLANT EXTRACT

2.9 PHOTOCATALYTIC ACTIVITY OF NANOPARTICLES

2.9.1 Mechanism of photocatalytic activity using nanoparticles

2.9.2 Photocatalytic activity of nanoparticles on pollutants

2.1 CAPSULISATION OF NANOTECHNOLOGY

Nanotechnology is the leading scientific and engineering field at present, since it is a combination of different interdisciplinary fields like physics, chemistry, informatics, biology, medicine and engineering. It was the evolving technology with huge advantageous applications. Novel nano biomaterial and nanodevices are well contrived and well ordered by nanotechnology techniques, which consider and alter the properties and functions of living and non-living environment, at measures below 100nm. Based on the nature of size below 100nm,nanoparticles are economically beneficial and important in future and its applications are widespread in electronic and mechanical devices, in optical and magnetic components, quantum computing, tissue engineering and other biotechnologies. The revolving nano products around the world in the form of nano engineered materials are going to place a positive impact in the real life (Logothetidis, 2012).

The term nanotechnology comes from the Greek numerical. Nano means a billionth and technology means application of scientific knowledge for practical purposes. Nanoscale science studies the phenomena, properties and response of materials at atomic, molecular and macromolecular scales (Sattler, 2010 and Bhushan, 2004).

In the energy generation tragedy, the demand on the consumption of fuel and carbondioxide emissions and alternative energy resources based on the innovative technology was highly promoted in the industries and markets. Renewing solar energy using nanomaterials and systems like organic photovoltaics give a great technological effect due to its large scale and low cost properties.

The trending nanoscience and its products require the parallel progress in their applications. Growing innovations in nanoscience will

required in the near future and existing ones will have to be improved in the area of better resolution and sensitivity for elements and molecular species.

Significant applications of nanoscience and nanotechnology place in the areas of pharmaceutics, cosmetics, processed food, chemical engineering, high- performance materials, electronics, precision mechanics, optics, energy production and environmental sciences (Logothetidis, 2012).

As an emerging field, over 50,000 articles are published annually worldwide in recent years and more than 2,500 patents are filed at major patent office's such as the European Patent Office (Huang *et al.,* 2011). Nanotechnology renovate new avenues of research and lead a new, beneficial and sometimes unusual applications.

Nanotechnology is the innovative field in research and nanomaterials are used in many industries as additive agents. For example, the Chinese used gold nanoparticles as an organic dye to introduce red colour into their ceramic materials for the thousand years of practice. Carbon black particles make rubber tires wear resistant, nanofibers are used for insulation and reinforcement of composites, iron oxide creates the magnetic material used in disk drives and audio-video tapes, and nano-zinc oxides and titanium are used as sun blocks for UV rays (Pokropivny *et al.,* 2007).

Nanoparticles are never as cheap to manufacture as the corresponding bulk material, but in a catalytic application one can achieve a kinetic improvement to more than counterbalance the increased cost (Huber, 2005). The improvement may come from two sources: the obviously large increase in relative surface area of a nanoparticle as compared to larger particulates, and a change in reactivity in the nanoparticle. Nanoparticles do not necessarily behave in the same manner as larger particles in chemical reactions, but tend to be much more reactive. The particles possess an enormous amount of energy in their high surface-to-volume ratios, which changes their reactivity. For example, nanoparticles have a general tendency to adsorb species very readily, which has obvious kinetic advantages. But nanoparticles cannot be simply assumed to always

react in the same manner but more quickly. A recent example of this principle is that platinum nanoparticles have been shown to have a lower reactivity per unit of surface area than corresponding bulk materials in fuel-cell applications (Antoine,2001).

Nanoparticles and nano thin layers play a major role in the industries for making some other products like magnetic recording tapes, computer hard drivers, bumpers on cars, solid-state compasses, safety and reflective coatings of eyeglasses and windows, automobile catalyst converters, metal cutting tools, dental bonding agents, tennis ball, burn and wound dressing, ink etc.

Novel nanomaterials make the effective impact in medication for fatal diseases such as brain tumours and Alzheimer's disease. Computers are made with nanoparticles and enhance the performance based on the changing dimensions (Simonis *et al*., 2014). Nanomaterials have the specialized properties such as nano carbon tubes, fullerenes, quantum dots, quantum wires, nanofibers and nanocomposites etc. While new methodologies and instrumentation have to be emerged in order to encourage our knowledge and depth on their properties.

Effective applications of nanotechnology in clinical sciences are the creation of nanoscale devices for improved therapy and diagnostics for human beings. Nano robots are used as carriers for the delivery of therapeutic agents, detectors against prenatal disease and the metabolic defects (Sahoo, S. K and Labhasetwar, 2003).

2.2 ORIGIN OF NANOTECHNOLOGY

Nanotechnology was firstly presented by Nobel laureate Richard P. Feyman on the occasion of his special lecture during 1959 on "There's Plenty of Room at the Bottom", that made many innovative developments in the field of nanotechnology. Nanoparticles are ranged from the dimension less than at least 100nm (Laurent *et al*., 2010). Depending upon the shape of the materials, nanoparticles will be of zero/one/two/three dimension (Tiwari *et al*., 2012). These nanoparticles showed the

characteristic variation based on the ratio of nanoshell thickness and concentration that will get applied for the bioimaging applications (Dreaden *et al*., 2012). Nanoparticles consists of triple layer such as surface layer, which may become functionalized with a variety of small molecules, metal ions, surfactants and polymers, shell layer chemically different material from the in all aspects and the core, essential central part of the nanoparticle (Shin *et al.,* 2016).

The only particles between 1 to 10nm in size can be regarded as a nanoparticle, in the order of SI units system (Sergeev *et al*., 2001) or that anything between 1 to 1000nm is a nanoparticle (Klabunde, 2001). Though all would agree that a particle whose size were all greater 1000nm is no longer a nanoparticle. The term nanoparticle is a relatively new, only common in late 1970s, and while being used by many researchers, it was restricted by others (Pokropivny, 2007).

2.3 CLASSIFICATION OF NANOPARTICLES

Nanoparticles are classified into distinct categories depending on its morphometries and chemical properties. On the basis of physical and chemical characters, nanoparticles are classified as follows:

2.3.1 CARBON BASED NANOPARTICLES

` Carbon based nanoparticles such fullerenes and carbon nanotubes are commercially advanced due to their electrical conductivity, high strength, structure, electron affinity and versatility (Astefanei *et al*., 2015). Carbon nanotubes are elongated, tubular structure with 1–2 nm in diameter (Ibrahim, 2013). These can be predicted as metallic or semiconducting reliant on their diameter telicity (Aqel *et al*., 2012). They are synthesized by carbon precursor deposits especially the atomic carbon and also by chemical vapour deposition (Elliott *et al.,* 2013). They are also used as the fillers (Saeed and Khan, 2016), effective gas adsorbents for the environment protection and as support medium for different catalysts (Mabena *et al.,* 2011) (Ngoy *et al*., 2014).

2.3.2 METAL NANOPARTICLES

Metal nanoparticles are only made up of metal precursors. By the nature of its localized surface plasmon resonance character, they possess specific optional electrical properties. Nano particles of alkali and noble metals such as copper, silver and gold have a broad absorption band in the visible zone of the electromagnetic solar spectrum. The facet, size and shape are well-ordered for the synthesis of metal nanoparticles which is specific in the cutting edge materials (Dreaden *et al.,* 2012).

2.3.3 CERAMIC NANOPARTICLES

Ceramic nanoparticles possess amorphous, polycrystalline, dense, porous and hollow properties. Based on those functions, its synthesis will differ by the methods of inorganic solids, synthesized via heat and successive cooling (Sigmund *et al.,* 2006). Its applications are catalysis, photo catalysis, photo degradation of dyes and imaging (Thomas *et al.*, 2015).

2.3.4 SEMICONDUCTOR NANOPARTICLES

Semiconductor nano particles possess both the properties of metals and nonmetals (Ali *et al.,* 2017 and Khan *et al*., 2017). As an example, variants of semiconductor nanoparticles are found to be efficient in water splitting applications, based on their appropriate band gap and band edge (Hisatomi *et al*., 2014).

2.3.5 POLYMERIC NANOPARTICLES

They are mainly used as the nano spheres or nano capsular shaped possess thousands of applicatiions in many research fields (Abouelmagd *et al.,* 2016) (Mansha *et al.,* 2017).

2.3.6 LIPID BASED NANOPARTICLES:

Lipid based nanoparticles are made up of lipid moieties which is greatly used in biomedical applications. Like metal nanoparticles, lipid nanoparticles possess a solid core and matrix of soluble lipophilic molecules. Surfactants and emulsifiers are used as the stabilizing agent of the external core of these nanoparticles (Rawat *et al.,* 2011). Lipid

nanotechnology was renowned for the clinical applications such as drug carriers (Puri *et al.,* 2009) and RNA release in cancer treatment (Gujrati *et al.,* 2014).

2.4 PHYSICOCHEMICAL PROPERTIES OF NANOPARTICLES

The nanoparticles have various physical and chemical properties like surface area, electronic, magnetic, mechanical, optical and thermal properties. They also play a major role in the applications as same as proteins and their functions are also based on the properties of the nanoparticles. Some of the properties are as follows:

2.4.1 ELECTRONIC AND OPTICAL PROPERTIES

The electronic and optical properties are interdependent with each other. Like noble metals nanoparticles have size dependent optical properties and they exhibit a strong UV visible band. This band will lead when the incident photon frequency is constant with the collective excitation of the conduction electrons and it is referred to as localized surface plasma resonance(LSPR). It is well established that the peak wavelength of the LSPR spectrum is based on the size, shape and interparticle spacing of the nano particles as well as its own dielectric properties and those of its environment such as substrate, solvents and adsorbates (Eustis and El-Sayed, 2006). There is no scattering expected from the bulk, upon the light interaction, instead they set into a standing resonance conditions, which is responsible for LSPR in these nano particles (Khlebtsov and Dykman, 2010).

2.4.2 MAGNETIC PROPERTIES

Magnetic properties of the nanoparticles are effectively investigated by the researchers than the electric properties. They are used as heterogenous and homogenous catalysis, biomedicine, magnetic fluids, data storage magnetic resonance imaging (MRI) and in environmental remediation like water decontamination. The literature and researches denotes that nanoparticles perform best when the size is less than critical value i.e., 10-20 nm (Reiss and Hütten, 2005). At such low nanoscale the

magnetic properties of nanoparticles are prominent effective and these priceless nanoparticles can be used in various applications (Faivre and Bennet, 2016; Priyadarshana *et al.,* 2015; Zhu *et al.,* 1997). These magnetic properties are also depends upon the synthetic preparation and method like solvo thermal (Qi *et al.,* 2016), co- precipitation, micro emulsion, thermal decomposition and flame spray synthesis etc. (Wu *et al.,* 2008).

2.4.3 MECHANICAL PROPERTIES

The specific mechanical properties of the nano properties permit the researchers to improve the novel applications in many important fields like tribology, surface engineering, nanofabrication and nano manufacturing. Various mechanical parameters like elastic modulus, hardness, stress and strain, adhesion and friction are present to know the mechanical nature of nano particles. Associate with these parameters, surface coating, coagulation, lubrication etc. also aid to mechanical properties of nanoparticles (Guo *et al*., 2014). Profitable results in these areas generally need a deep insight of the basics of the mechanical properties of nano particles, such as elastic modulus and hardness, movement law, fiction and interfacial adhesion and its size dependent features (Guo *et al*., 2014).

2.4.4 THERMAL PROPERTIES

The metals nanoparticles have thermal conductivities greater than those of fluids in solid form. The fluid containing suspended solid particles are estimated to display significantly enhanced thermal conductivities related to those of alternative heat transfer fluids. Because of the transfer of heat in the surface, it is anticipatable to use the particles with large total surface area which also enhances the stability (Lee *et al.,* 1999). Recently it was proved that nanofluids such as cupric and aluminium oxide in water and ethylene exhibit advance thermal conductivity (Cao, 2002).

2.5 APPLICATIONS OF NANOPARTICLES

Nanoparticles are broadly used as the economically beneficial materials in many fields. Some of the important applications are,

2.5.1 DRUGS AND MEDICATIONS

Nanoparticles are simple and possess the unique physico chemical properties and it represent core part in nanodevices which can be used in numerous physical, biological, biomedical and pharmaceutical applications (Loureiro *et al.,* 2016; Martis *et al.,* 2012; Nikalje, 2015). Nanoparticles act as a efficient carriers in the drug delivery of the optimum dosage and results in increased treatment efficiency with low side effects and improved patient compliance (Alexis *et al.,* 2008). Iron oxide particles such as magnetite and magnesite are prominently used in biomedical applications (Ali *et al.,* 2014). The optical properties of the nanoparticles are main feature for the selection of nanomaterials in biological and cell imaging as well as for photo thermal therapeutic applications. The development of hydrophilic nanoparticles as drug carrier has represented over the last few years as an important challenge. Among the different approaches, polyethylene oxide (PEO), poly lactic acids (PLA) nanoparticles have been revealed as very promising system for the intravenous administration of drugs (Calvo *et al.,* 1997).

Magnetic iron oxide nanoparticles (MNPs) with appropriate surface chemistry exhibit many interesting properties that can be exploited in a variety of biomedical applications such as magnetic resonance imaging contrast enhancement, tissue repair, hyperthermia, drug delivery and in cell separation(Kanagasubbulakshmi and Kadirvelu., 2017).

Iron oxide nanoparticles with specific surface chemistry are used for various *in vivo* applications such as MRI, contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, hyperthermia, drugs delivery and cell separation. Over the few decades, the researchers are focused in the developing biodegradable nanoparticles as the drug delivery carriers (Zhang and Saltzman, 2013). Liposomes have been used as a potential drug carrier instead of conventional dosage forms because of their unique advantages which include ability to protect drugs from degradation, target to the site of action and reduce the noxiousness and other side effects. However developmental work on liposome drugs has been

restricted due to inherent health issues such as squat encapsulation efficiency, rapid water leakage in the commodity of blood components poor storage and stability etc. On the other hand, polymeric nanoparticles promise some critical advantages over these materials (liposomes). For instance, nanoparticles help to increase the ratability of drugs or problems and possess convenient controlled drug release properties.

Most of the semiconductor and metallic nanoparticles are potent for cancer diagnosis and treatment on the basis of Surface Plasmon resonance (SPR) (Huang *et al.,* 2014). Nanoparticles show the antineoplastic activity with good efficiency and low toxicity (Chen *et al.,* 2005). The antimicrobial characteristics of inorganic nanoparticles improve the potential as compared to organic compounds, which are relatively toxic to the biological systems (Hajipour *et al.,* 2012). These nanoparticles are functionalized with various groups to overcome the microbial species selectively. TiO_2, ZnO, $BiVO_4$, Cu- and Ni based nanoparticles have been utilized for this purpose due to their suitable antibacterial efficacies (Akhavan *et al*., 2011; Pant *et al.,*2013; Qu *et al.,* 2016; Yin *et al.,* 2016).

2.5.2 Manufacturing and materials

The benefits of nanotechnology have been acknowledged by many manufacturers at high and low level, marketable products such as microelectronics, aerospace and pharmaceutical industries (Weiss *et al*., 2006). The unique plasmon absorbance features of these noble metals nanoparticles have been exploited for a wide variety of applications including chemical sensors and biosensors (Unser *et al*., 2015).

2.5.3 ENVIRONMENT

The nanoparticles in industries and household applications results in the release of nanomaterials into the environment. Assessing the effect of nanoparticles in the environment will necessitate on their mobility, reactivity, ecotoxicity and persistency (Ripp and Henry, 2011 and Zhuang and Gentry, 2011).

Most of environmental applications of nanotechnology fall into three categories namely environmentally benign sustainable products (e.g. green chemistry or pollution prevention), remediation of materials contaminated with hazardous substances and sensors for environmental stages (Tratnyek and Johnson, 2006).

Photodegradation by nanoparticles is also very common practice and many nanomaterials are utilized for this purpose. Rogozea *et al.* (2016) also used NiO/ZnO nanoparticles modified with silica which has the high surface area and facilitated the efficient photodegradation reaction. Rogozea *et al*. (2017) also reported the synthesis of variety of nanoparticles and their optical, florescence and degradation applications.

The removal of heavy metals such as mercury, lead, thallium, cadmium and arsenic from natural water has attracted considerable attention because of their adverse effects on environmental and human health. Iron oxide nanoparticles are an effective sorbent material for this toxic soft material. So, for no measurements of engineered nanoparticles in the environment have been available due to the absence of analytical methods which will able to quantify trace concentration of nanoparticles (Mueller and Nowack, 2008).

2.6 Methods of Nanoparticle synthesis

Different preparation techniques are engineered for nanoparticles syntheisis. Two approaches are well known for the preparation of ultrafine particles from the ancient times. The first one is the breakdown method which deals with the external force that acts on the solid and leads it to break into smaller particles. The second is the buildup method that produces nanoparticles starting from atoms of gas or liquids based on the atomic transformations or condensations (Shukla et al., 2017). Fig 1 depicts different approaches for the synthesis of nanoparticles.

2.6.1 Top down method

In the breakdown method the solid substances are broken and it was further classified into dry and wet grinding. A characteristic of particles in

grain refining processes is the surface energy increases, and causes the aggregation of particles to increase, In the dry grinding method the solid substance is ground as a result of a shock, compression or by friction using such popular methods such as a jet mill, hammer mill, shearing mill, roller mill, shock shearing mill, ball mill, and tumbling mill. Since condensation of small particles also takes place simultaneously with pulverization, it is diffi cult to obtain particle sizes of less than 3μm by grain refining. On the other hand, wet grinding of a solid substrate is carried out using tumbling ball mill, vibratory ball mill, planetary ball mill, centrifugal fluid mill, agitating beads mill, flow conduit beads mill, annular gap beads mill or wet jet mill. Compared with the dry method, the wet process is suitable for preventing the condensation of the nanoparticles so formed, and thus it is possible to obtain highly dispersed nanoparticles (Horikoshi and Serpone, 2013).

Other than the above, the mechanochemical method and the mechanical alloying method are also known as top-down methods.

2.6.2 Bottom up method

The bottom-up approach is roughly divided into gaseous phase methods and liquid phase methods. For the former, the chemical vapour deposition method (CVD) involves a chemical reaction, whereas the physical vapor deposition method (PVD) uses cooling of the evaporated material. Although the gaseous phase methods minimize the occurrence of organic impurities in the particles compared to the liquid phase methods, they necessitate the use of complicated vacuum equipment whose disadvantages are the high costs involved and low productivity. The CVD procedure can produce ultrafine particles of less than 1 μm by the chemical reaction occurring in the gaseous phase. The manufacture of nanoparticles of 10 to 100 nm is possible by careful control of the reaction. Performing the high temperature chemical reaction in the CVD method which requires heat sources such as a chemical flame, a plasma process, a laser, or an electric furnace. In the PVD method, the solid material or liquid material is evaporated and the resulting vapor is cooled rapidly, yielding the desired

nanoparticles. To achieve evaporation of the materials one can use an arc discharge method. The simple thermal decomposition method has been particularly fruitful in the production of metal oxide or other types of particles and has been used extensively as a preferred synthetic method in the industrial world (Iravani., 2011).

2.6.3 Other methods of preparation

For many years, liquid phase methods have been used to prepare nanoparticles. They can be sub-divided into liquid/liquid methods, and sedimentation methods. Chemical reduction of metal ions is a typical example of a liquid/ liquid method, whose principal advantage is the facile fabrication of particles of various shapes, such as nanorods, nanowires, nanoprisms, nanoplates, and hollow nanoparticles. With the chemical reduction method it is possible to fine-tune the form (shape) and size of the nanoparticles by changing the reducing agent, the dispersing agent, the reaction time and the temperature. The chemical reduction method carries out chemical reduction of the metal ions to their 0 oxidation states (i.e., $Mn^{+} \rightarrow M^{0}$). The process uses non-complicated equipment or instruments, and can yield large quantities of nanoparticles at a low cost in a short time. Of particular interest in this regard is the use of microwave radiation as the heat source that can produce high quality nanoparticles in a short time period. Besides the chemical reduction method which adds a reducing agent (direct reduction method), other reduction methods are known, such as photo reduction using gamma rays, ultrasonic waves, and liquid plasma which can be used to prepare nanoparticles. These methods do not use a chemical reducing substance which has the attractive feature and no extraneous impurities are added to the nanoparticles (Horikosh and Serpone, 2013).

Other than these methods, spray drying, spray pyrolysis, solvothermal synthesis, and the supercritical method are also known. The general technique in the sedimentation method is a sol–gel process, which has been used extensively for the fabrication of metal oxide nanoparticles.

This procedure transforms a solution of a metal alkoxide into a sol by hydrolysis, followed by polycondensation to a gel. (Brinker *et al.,* 1990).

The wet process (liquid phase method) guarantees a high dispersivity of nanoparticles compared to the dry method. However, if the resulting nanoparticles are dried, aggregation of the particles soon follows. In this case, re-dispersion can be carried out according to the process used in the solid phase method.

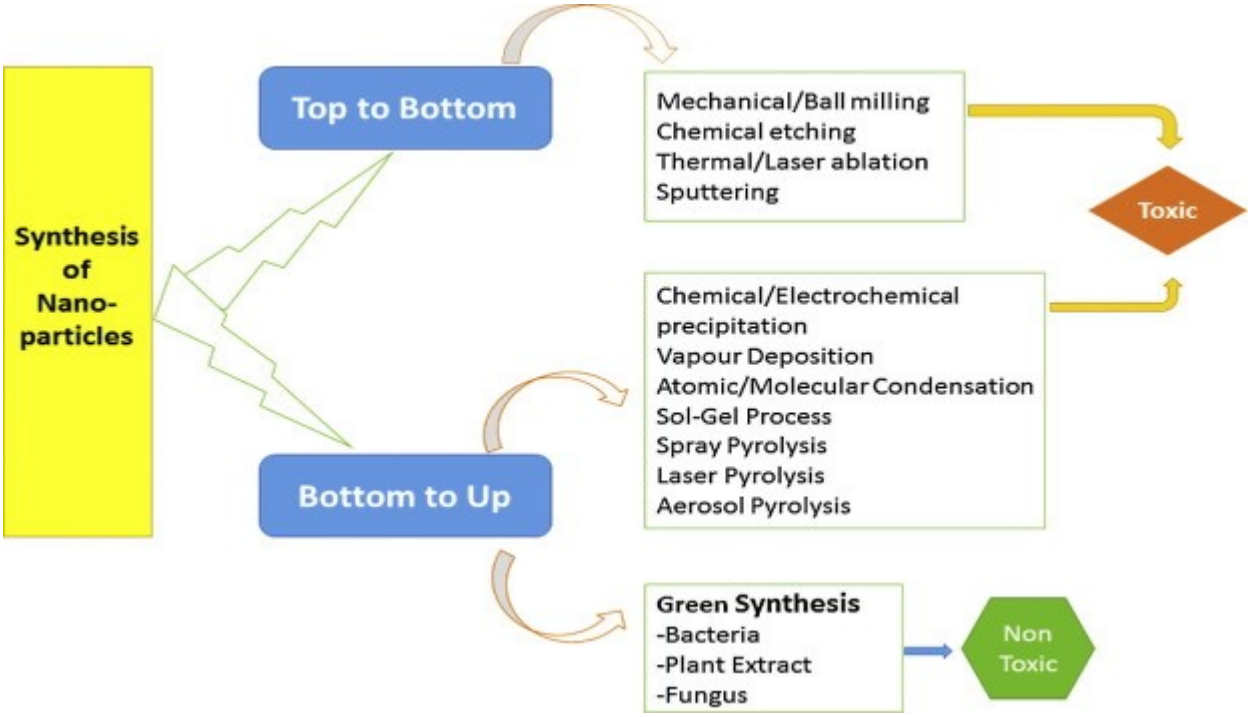


Fig: 1 Different approaches of synthesis of nanoparticles

2.7 IRON NANOPARTICLES

Iron is the most cosmopolitan of the transition metals the fourth most element in the Earth's crust and the structural boon of modern infrastructure. Iron has the major role in the macroscopic applications and also concerned higher in the nanoscale level. This extreme reactivity blocks the study on nanoparticles and made it difficult and inconvenient for practical applications. However iron nanoparticles are very potent due to its magnetic and catalytic properties. Recent research has begun to make advantage of iron's potential and work in this field appears to be blossoming.

Some of the particles studied were nanoparticles, but investigating their synthesis they were polydisperse and give the distinct shape. Theories are designed to describe the expected magnetic properties of iron nanoparticle and it will be inconvenient while the lack of independent measure of size and shape. There was transformed interest in grounded iron, as the properties could be correlated with the size and shape. By the early 1960s, the theory defining the magnetism of iron nanoparticles was fully formed and highly accepted by the practical experiments (Kittel, 1946, Hebd Seances,1965, Bean *et al*., 1959 and Jacobs *et al.,*1963). Research on iron nanoparticles are continued likely due to the new synthetic methods as well as interest in new applications of iron nanoparticles (Fig. 2).

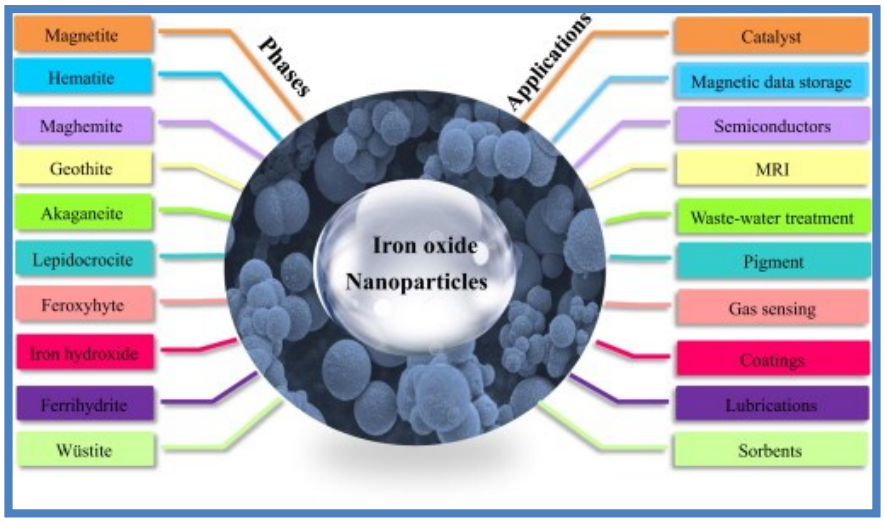


Fig .2. Applications of Iron oxide Nanoparticles

Iron oxide is present everywhere in different forms. The well-known forms are Magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and hematite (α-Fe2O3) (Cornel and Schwertmann, 1996 and Chan and Ellis, 2004). By the special features of iron nanomaterials like size, high surface area to volume ratios and superparamagnetism the many new innovations are came in the synthesis and their utilization (McHenry and Laughlin, 2000; Afkhami *et al*., 2010; Pan *et al.,* 2010). Its ability to rheostat or modify matter in a nanoscale could offer supreme resourcefulness (Boyer *et al.,* 2010 and

Dias *et al.,* 2011). In addition to this, iron nanoparticles have low toxic effect, chemical contribute and companionship with the biological systems illustrate a remarkable prospect in permutation with biotechnology (Huang *et al.,* 2003; Roco, 2003; Gupta and Gupta, 2005).

These specific features along with the application of iron oxide nanomaterials as we compare with the raw iron oxide materials were shown in the Fig. 3. Progresses in nanomaterials synthesis aid the exact resist of surface by producing monodisperse and outline ordered iron oxide nanoparticles (Bautista *et al.,* 2005 and Li and Somorjai, 2010). Many biologically mediated methods and techniques require the massive improvement.

Along with them, the novelty in iron oxide nanomaterials in the environment may gave a industrious research and further more researches were needed for the dig the potentials of the novel nanomaterials. Largely, nanomaterials would be sound to prevent combination and provide a little deposition, to promise their reactivity and flexibility (Schrick *et al.,* 2004 and Kanel *et al.,* 2007).

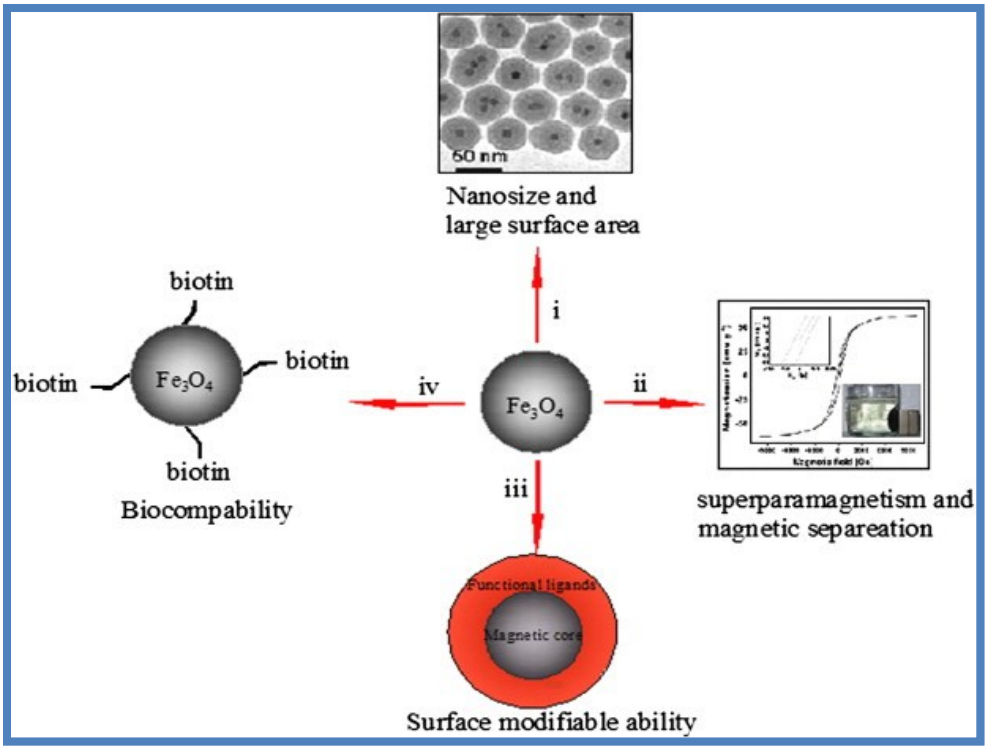


Fig. 3- Overview of iron nanomaterials

2.7.1 Synthesis of iron nanoparticles

Iron nanoparticles can be produced through high-energy milling (Kerekes *et al.,* 2002), though this is not the most common method of forming iron nanoparticles it does have advantages. The primary advantage is the ease with which this method can be scaled up. For the economical production of large quantities of nanoparticles, milling is difficult to compete with. The drawback, however is that the product tends to be very polydisperse in size and irregular in shape. In addition to standard milling procedures, composites can be produced directly by co-milling iron and a matrix material, such as alumina. Additionally, reactive milling can produce iron in situ by co-milling magnetite and aluminum. Materials produced through these two processes are surprisingly similar, producing iron nanoparticles of around 12 nm, and having a reduction in saturation magnetization of about 25% relative to bulk iron.

The microemulsion method is employed to synthesize iron nanoparticles with an average size of ∽3 nm using trioctyl phosphine oxide (TOPO) as a stabilizing agent. The morphology, structure, and composition of the nanoparticles are studied by transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and UV–VIS spectroscopy. The iron nanoparticles show a remarkable surface-enhanced Raman scattering (SERS) activity, and the scheme of iron nanoparticle-on-electrode is successfully used in the *in situ* SERS study of adsorbed molecules. Electrocatalysis over the iron nanoparticles is demonstrated in the highly efficient and selective reduction of H_2O_2 in the presence of oxygen (Guo *et al.,* 2001).

A water-in-oil micro emulsion method has been applied for the preparation of silica-coated iron oxide nanoparticles. Three different nonionic surfactants (Triton X-100, Igepal CO-520, and Brij-97) have been used for the preparation of micro emulsions, and their effects on the particle size, crystallinity, and the magnetic properties have been studied. The iron oxide nanoparticles are formed by the coprecipitation reaction of ferrous and ferric salts with inorganic bases. A strong base, NaOH, and a comparatively mild base, NH_4OH, have been used in each surfactant to observe whether the basicity has some influence on the crystallization process during particle formation. Transmission electron microscopy, X-ray electron diffraction, and superconducting quantum interference device magnetometry have been employed to study both uncoated and silica-coated iron oxide nanoparticles. All these particles show magnetic behavior close to that of superparamagnetic materials. By use of this method, magnetic nanoparticles as small as 1−2 nm and of very uniform size (percentage standard deviation is less than 10%) have been synthesized. A uniform silica coating as thin as 1nm encapsulating the bare nanoparticles is formed by the base-catalyzed hydrolysis and the polymerization reaction of tetraethyl orthosilicate in micro emulsion. All experimental results are also compared with those for particles synthesized in pure water (Santra *et al.,* 2001).

2.8 SYNTHESIS OF NANOPARTICLES USING PLANT EXTRACT

Pattanayak and Nayak, (2013) used aqueous extract of ten plants species at room temperature for the iron nanoparticles synthesis. Synthesized nanoparticles were characterized using UV-visible spectrophotometer and SEM methods. The change in color and pH was observed significantly. Novelty of this present study is that the plant extract is very cost effective and ecofriendly and thus can be economic and effective alternative for the large scale synthesis of iron nanoparticles.

Wang *et al.* (2014) firstly synthesized the iron nanoparticles through a one-step room-temperature biosynthetic route using eucalyptus leaf extracts (EL-Fe NPs). Scanning electron microscopy (SEM) and X-ray energy-dispersive spectrometer (EDS) confirmed the successful synthesis of the spheroidal iron nanoparticles. Furthermore, X-ray diffraction (XRD) and Fourier Transform Infrared spectrometer (FTIR) indicated that some polyphenols are bound to the surfaces of EL-Fe NPs as a capping/stabilizing agent. Reactivity of EL-Fe NPs was evaluated for the treatment of swine wastewater and results indicated that 71.7% of total N and 84.5% of COD were removed, respectively. This demonstrated the tremendous potential of EL-Fe NPs for *in situ* remediation of eutrophic wastewater.

An eco-friendly green synthesis of iron oxide nanoparticles using leaf extract of *Ocimum sanctum* was investigated by Balamurughan *et al*., (2014). UV visible spectra showed the maximum absorbance of 285 and 324 nm due to the excitation of surface plasmon vibrations in the iron oxide nanoparticles formation. FTIR spectrum exhibited the characteristic band at 618 cm^{-1} which indicated the Fe-O stretching of Fe_2O_3 nanoparticles. The XRD spectrum showed three different diffraction peaks corresponding to the crystal planes of crystalline Fe_2O_3. The sharp peaks indicated the crystallinity and purity of iron oxide nanoparticles. The average particle size of the synthesized iron oxide nanoparticles was estimated to be 47nm using

the Scherrer equation. The formation of Fe_2O_3 nanoparticles as well as their morphological dimensions in the SEM study revealed that the particles were aggregated. Transmission electron microscope image of iron oxide nanoparticles showed that the nanoparticles size was below 20 nm.

Nasrollahzadeh et al. (2015) reported that the green synthesis of Pd/Fe3O4 nanoparticles using Euphorbia condylocarpa M. bieb root extract as reducing agents and stabilizers and their catalytic applications in ligand- and copper-free Sonogashira and Suzuki coupling reactions. This method has such advantages as high yields, simple methodology and easy work up. In addition, the catalyst can be recovered by using a magnet and reused several times without significant loss of its catalytic activity. The catalyst was characterized using UV–vis, powder XRD, SEM and EDS techniques.

Shojaee and Shahri, (2016) proposed the green synthesis of iron oxide magnetic nanoparticles (Fe_3O_4-MNPs) using the aqueous extract of White tea (*Camelia sinensis*) as a reducing and capping agent. The formation of Fe_3O_4-MNPs was observed by the change of colour from colourless to dark brown by the addition the leaf extract. The properties and morphology of the Fe_3O_4 -MNPs is evaluated using FT-IR, XRD and SEM. The average particle diameter as determined by SEM was found to be 35 nm. X-ray diffraction demonstrated that the nanoparticles are crystalline in nature, with spinel shape. These Fe_3O_4 -MNPs fabricated through biosynthesis method, are promising candidate in various applications like biomedical and utilizing as recyclable magnetic nano-catalyst for organic reactions.

Jeyasundari, (2017) handled a very cheap and simple conventional heating method to obtain the iron nanoparticles (FeNPs) using the extract obtained from the leaves of *Psidium guajava* plant. The obtained iron nanoparticles were characterized by UV-Vis spectroscopy, FTIR spectroscopy, XRD, Cyclic Voltammetry, SEM, EDS and the antibacterial activity was studied against *Bacillus cereus, Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus* by the standard disc diffusion method. The synthesized

Fe nanoparticles were found to be 27nm which was confirmed by XRD and they exhibited a significant antibacterial activity.

Gottimukkala, (2017) reported that the green synthesis of iron nanoparticles has been achieved using *Camellia sinensis* leaf extract which reduced iron ions into iron nanoparticles at room temperature. The synthesized iron nanoparticles were characterized using Scanning Electron Microscope (SEM) and Fourier Transform Infrared (FTIR) analysis. This study shows that the iron nanoparticles can be synthesized using *Camellia sinensis* leaf extract as a reducing agent.

Kanagasubbulakshmi and Kadirvelu.,(2017) reported the green synthesis of iron oxide nanoparticles (Fe_3O_4-NPs) using *Lagenaria siceraria* leaves extract and their characteristics were studied by using UV-visible spectrophotometer, SEM, EDX, XRD, Zeta sizer, and FT-IR. The synthesized Fe_3O_4-nanoparticles were naturally stabilized, cubic shaped and the size ranged from 30 - 100 nm. The phytochemicals present in the leaf had a main role as reducing agent and assists to the ecofriendly synthesis of Fe_3O_4-NPs with enhanced antioxidant property. FT- IR shows that the functional groups present on the nanoparticles are mainly –OH and –COOH which makes it hydrophilic, The antimicrobial property of synthesised Fe_3O_4-nanoparticles was evaluated against Gram negative (*Escherchia coli),* Gram positive (*Staphylococcus aureus)* bacteria in which the zone of inhibition was found to be more in *Escherchia coli*, then *Staphylococcus aureus*. Thus naturally stabilised Fe_3O_4-NPs with herbal property can be used in various biological applications.

An eco-friendly green synthesis of iron oxide nanoparticles using leaf extract of *Leucas aspera* was investigated by Veeramanikandan *et al.* (2017). The characteristics of the obtained Fe_3O_4 nanoparticles were studied using UV-Visible spectrophotometer, FTIR, SEM with EDX, XRD and HPLC. The synthesized Iron oxide nanoparticles were effectively utilized for the antibacterial activity and antioxidant studies. The maximum zone of inhibition was found to be high in Gram negative bacteria when compared to Gram

positive bacteria. This green method of synthesizing Fe_3O_4 nanoparticles could also be extended to fabricate other industrially important metal oxides. This simple, low cost and greener method for development of nanoparticles may be valuable in environmental, biotechnological and biomedical applications.

2.9 PHOTOCATALYTIC ACTIVITY OF NANOPARTICLES

2.9.1 Mechanism of photocatalytic activity using nanoparticles

In the first step in the photocatalytic activity of nanoparticles, oxide nanostructure come in contact with light, creating a photo- generated electron and a hole. The photogenerated electron reacts with oxygen molecule to form superoxide free radical in the second step. In the third step, the hole reacts with water and hydroxyl ions to produce highly hydroxyl radicals. These superoxide and hydroxyl free radicals react violently with dye and decompose or degradation the dye totally which depends on morphological and crystal structure of the phytochemical. Fig. 4 represents the photocatalytic activity of iron nanoparticles.

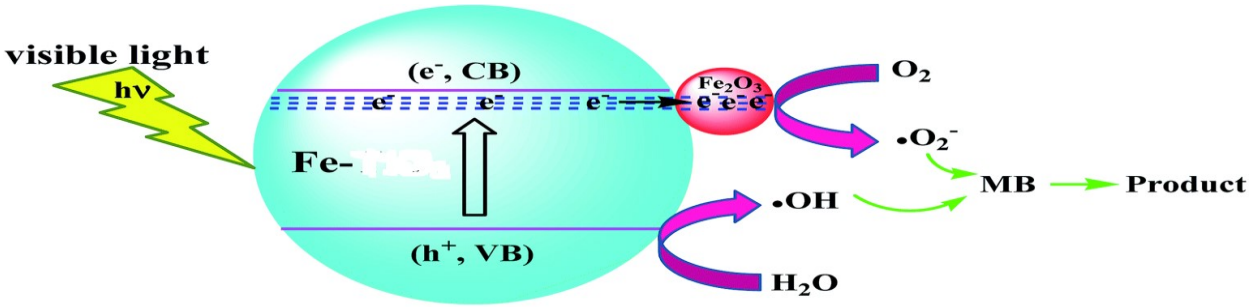


Fig- 4 Photocatalytic activity of iron nanoparticles

2.9.2 Photocatalytic effect of nanoparticles on pollutants

Shahwan *et al*. (2011) reported the production of iron nanoparticles were produced using the extracts of green tea leaves. The obtained nanoparticles were then utilized as a Fenton-like catalyst for decolorization of aqueous solutions containing methylene blue (MB) and methyl orange (MO) dyes and monitored using ultraviolet–visible (UV–vis) spectroscopy. The results indicated fast removal of the dyes with the kinetic data of MB following a second order removal rate, while those of MO were closer to a first order removal rate. The loading experiments indicated

almost complete removal of both dyes from water over a wide range of concentration, (10–200 mgl^{-1}). Compared with iron nanoparticles produced by borohydride reduction, green tea iron nanoparticles demonstrated more effective capability as a Fenton-like catalyst, both in terms of kinetics and percentage removal.

Weng *et al*. (2013) used the green tea extract for the synthesis of iron nanoparticles and degrade malachite green in aqueous solution. The results show that the damage to morphology and increase in in size of green tea Fe NPs after reaction with malachite green were observed by SEM and EDS. XRD shows that there are few changes in the characteristic peaks of green tea Fe NPs before and after reaction. UV- vis spectroscopy shows that the absorption peak of MG decreased. The factors impacting on the removal efficiency of malachite green, including the initial solution pH, the initial concentration of MG, the dosage of GT- Fe NPs, and the reaction temperature, was also investigated. It emerged that 96% of MG was removed with a 50mg/L at 298K

Poguberović *et al.,* (2016) reported that the production of nano zero-valent iron nanoparticles, using the extract from natural products, increased in recent years as it represents green and environmentally friendly method. Synthesis of green zerovalent iron nanoparticles (nZVI) using oak, mulberry and cherry leaf extracts proved to be a promising approach for As(III) and Cr(VI) removal from aqueous solutions. Characterization of produced green nZVI materials confirmed the formation of nanosize zero-valentiron particles within the size of 10–30 nm. Nanoparticles were spherical in shape and represented stable material with minimum agglomeration observed by TEM and SEM morphology measurements. Batch experiments revealed that the adsorption kinetics followed pseudo-second order rate equation. The obtained adsorption isotherm data could be well described by the Freundlich model. In addition, the effect of pH showed that varying the initial pH value had a great effect on As(III) and Cr(VI) removal. This study indicated that nZVI could be produced by low cost and non-toxic method

with oak, mulberry and cherry leaf extracts and potentially be used as a new green material for remediation of water matrices contaminated with As(III) and Cr(VI).

Seru et al. (2017) emphasised the importance of fabricating iron nanoparticles using leaf and seed extracts of Moringa olifera as an alternative to traditional method The green application of synthesized nanoparticles for the removal of nitrate ion (NO_3-) from surface and ground water was also investigated. The batch adsorption resulting showed an enhanced removal of NO_3 (85 %) by *Moringa Olifera* seed -FeNPs and MOL-FeNPs respectively as compared to *M. oleifera* extracts. FeNPs were successfully fabricated using M. oleifera extracts, giving an alternative method to nitrate removal from surface and ground water.

Quan *et al.* (2018) used iron nanoparticle (INP) coated on palygorskite (P), cationic surfactant cetyltrimethyl ammonium bromide (CTMAB) to modify its surface properties through ion exchange. The structure of the composited P-INP/CTMAB was characterized by various analytical methods. The results showed that the long chain of CTMAB was coated on the surface of P-INP without intercalation effect. The morphology of P-INP was thus not obviously changed after the modification, but the special specific surface area increased from 30.63 m2 g−1 to 127.04 m2 g−1. The decoloration and degradation efficiency of P-INP/CTMAB towards acid orange 7 (AO7) was examined under different pH value, temperature and dosage conditions. The results showed that the reaction was preferred to the acid ic environment andits first-order reaction rate constant (kobs) linearly depended on the dosage. With suitable conditions, the removal rate of AO7 and total organic carbon (TOC) by P-INP/CTMABreachedabout98.4%and59.21%after2h,respectively.Thesevalue swereboth higher than that of the unmodified composition P-INP, suggesting the synergistic effects in surface adsorption and the following removal reaction. The degradation intermediate products were investigated

using HPLC-MS, and the potential degradation pathway of AO7 by P-INP/CTMAB was determined.

Bisnoi *et al.* (2018) described a novel and eco-friendly, room temperature synthesis of magnetic iron oxide nanoparticles (MIONPs) utilising the fruit waste extract of *Cynometra ramiflora*,. The photocatalytic ability of the nanoparticles was demonstrated by the degradation of methylene blue dye under sunlight irradiation. The reusability and the stability of the magnetic iron oxide nanoparticles were tested for five cycles which ascertained the potential photocatalytic activity. Thus, these nanoparticles could play a vital role in the environmental catalytic remediation of polluted water.

Materials and Methods

3. MATERIALS AND METHODS

The methodology adopted for the present study "Biobased green approach to synthesise iron nanoparticles using *Phyllanthus reticularis* (Poir.) leaf extract" was carried out under following headings:

3.1 Collection and identification of the plant material

3.2 Preparation of plant extract

3.3 Biobased green synthesis of iron oxide nanoparticles

3.4 Characterization of biosynthesized iron oxide nanoparticles

- 3.4.1 UV- visible spectroscopy analysis
- 3.4.2 Fourier Transform Infra-Red Spectroscopy (FT- IR) analysis
- 3.4.3 Scanning Electron Microscopy (SEM) and Energy Dispersion X-Ray (EDX) analysis
- 3.4.4 X Ray Diffraction (XRD) analysis
- 3.4.5 Dynamic Light Scattering (DLS) analysis

3.5 Photocatalytic degradation activity

3.6 Phytotoxicity assay

3.7 Phytochemical analysis of biosynthesized iron oxide nanoparticles

3.8 Antimicrobial activity of biosynthesized iron oxide nanoparticles

3.1 COLLECTION AND IDENTIFICATION OF THE PLANT MATERIAL

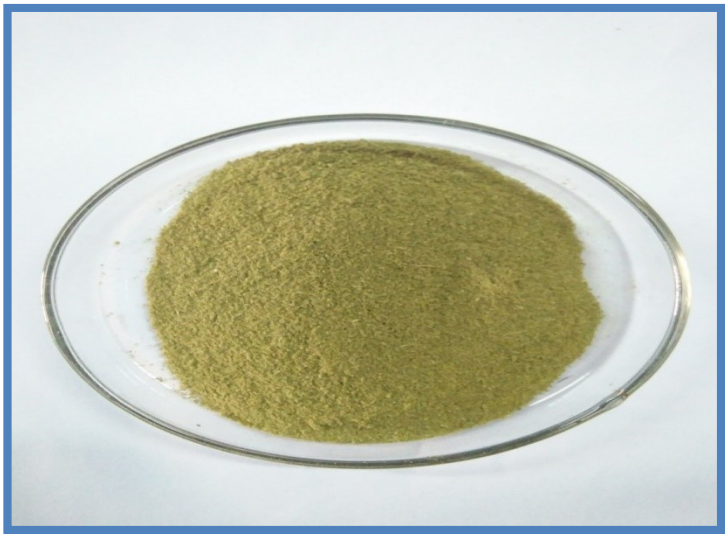
The leaves of *Phyllanthus reticulatus* were collected in Mohanur, Nammakkal district, Tamilnadu during the month of October (Plate 1). The collected leaf sample was authentically identified by Botanical Survey of India, Tamilnadu Agricultural University, Coimbatore, Tamilnadu. The voucher specimen number was BSI/SRC/5/23/2018/Tech./2873 (Appendix 1). The leaves were primarily cleaned with running tap water, followed by deionized distilled water for surface sterilization. The surface cleaned leaves were shade dried under the room temperature and the dried leaves were crushed into fine powder and stored in airtight container at room temperature for further studies (Plate 2).

PLATE 1

Phyllanthus reticulatus

PLATE 2

Leaf powder of *Phyllanthus reticulatus*



3.2 PREPARATION OF PLANT EXTRACT

About 10g of finely powdered leaf *Phyllanthus reticulatus* leaf was weighed and dissolved in 100ml of distilled water and boiled at 100^0C for 10mins. The contents were filtered through Whatman No.1 filter paper to get the clear filtered extract and extract was stored under refrigeration at 4^0C for further use.

3.3 BIOBASED GREEN SYNTHESIS OF IRON OXIDE NANOPARTICLES

Ferrous sulphate was used in the preparation of iron oxide nanoparticles. The ferrous sulphate was prepared in different concentrations ranging from 1 - 5 mM and to each concentration 1 – 5 ml of the leaf extract was added separately and the reaction time was varied from 0 – 10 minutes. The setup was incubated at room temperature. At the optimized parameters the reduction of Fe^+ to Fe^0 was confirmed by the colour change of solution from yellow to black.

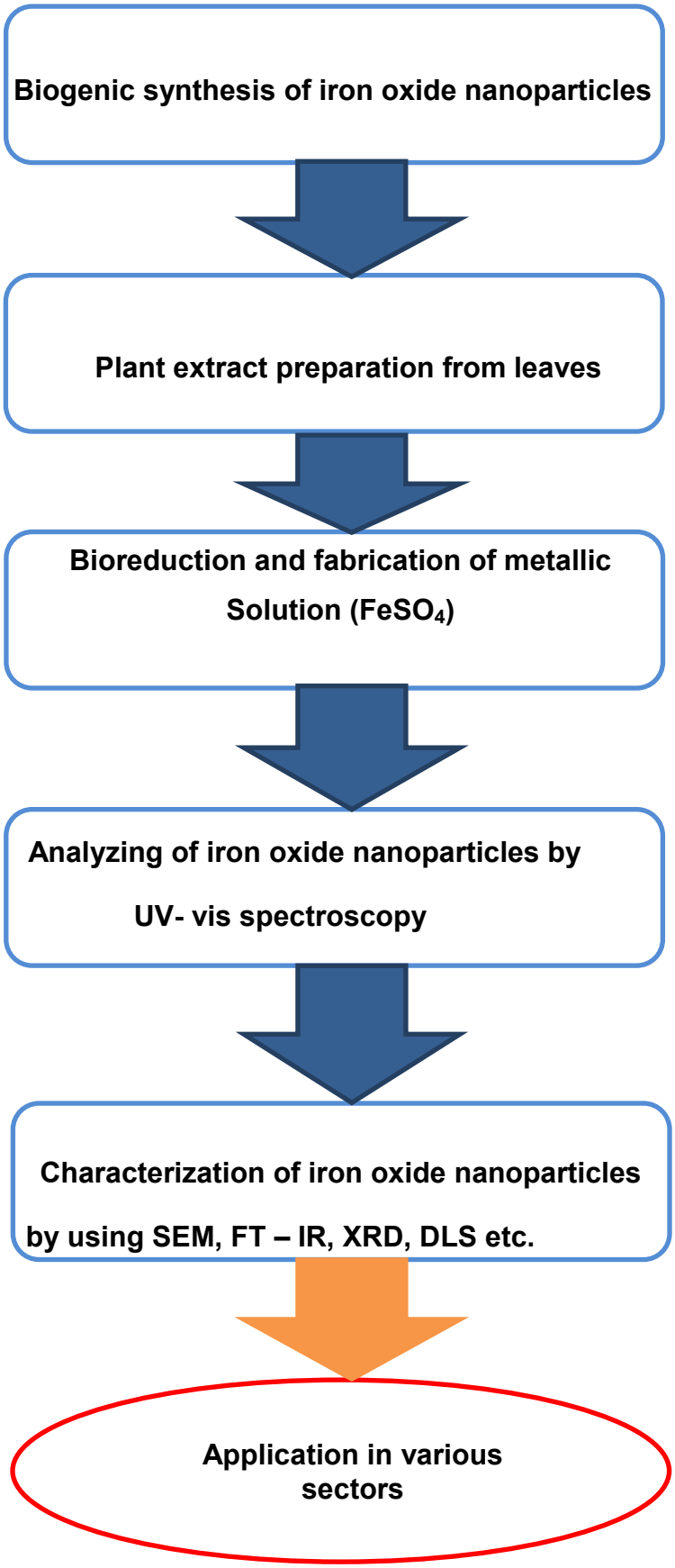
The biosynthesized iron oxide nanoparticles were centrifuged at 1000 rpm for 5 minutes to obtain the black precipitate which served as iron oxide nanoparticles. The obtained iron oxide nanoparticles was dissolved in 100 ml of double distilled water and stored at 4^0C for further analysis.

The schematic representation of iron oxide nanoparticles synthesis using *Phyllanthus reticulatus* leaf extract is given (Fig. 5).

3.4 CHARACTERIZATION OF BIOSYNTHESISED IRON OXIDE NANOPARTICLES

To evaluate the maximum production of iron oxide nanoparticles at the standard point, it is a prerequisite to characterize the samples. The sample was characterized by UV- visible spectroscopy, FT-IR spectroscopy, Scanning Electron Microscopy, EDX analysis, X- Ray diffraction and Dynamic Light Scattering.

Fig. 5– BIOGENIC SYNTHESIS OF IRON OXIDE NANOPARTICLES



3.4.1 UV- visible spectroscopy analysis

The initial characterization of biosynthesized iron oxide nanoparticles was carried out using UV- vis spectroscopy (HITACHI model U- 2800 Spectrophotometer). The reduction of iron ions was monitored from 200-800 nm by UV- vis double beam spectrophotometer after 10- fold dilution of the sample with deionized water.

3.4.2 Fourier Transform Infra-Red Spectroscopy (FT- IR) analysis

The FT- IR analysis was carried out on dried iron oxide nanoparticles through KBr pellet method using FT- IR spectrum (Shimadzu) and the spectrum was recorded in the mid- IR region between 4000- 800 cm^{-1}.

3.4.3 Scanning Electron Microscopy (SEM) – Energy Dispersion X- Ray (EDX) Analysis

Thin film of biosynthesized iron oxide nanoparticles from *Phyllanthus reticulatus*
was prepared on a carbon coated copper grid and dried. The sample was sonicated and analysed at different magnifications with gold sputtering at a potential of 20kV prior to recording SEM. For EDX analysis the iron oxide nanoparticles were dried and drop coated on to carbon film, and then performed using the NOVA-450 instrument.

3.4.4 X Ray Diffraction analysis

In XRD spectra, the pelletized iron oxide nanoparticles was prepared by drop coating on a glass slide. The XRD spectra was recorded using X- ray diffractometer (Shimadzu XRD- 6000/6100 model) operated at a tube voltage of 30kV and a tube current of 30 mA with CuKα radiation by using 2θ from 20-80^0.

3.4.5 Dynamic Light Scattering (DLS) analysis

Dynamic light scattering principle shows the particle size distribution in the sample. The particle size range of the synthesized iron oxide nanoparticles was determined using a particle size analyzer. Particle size was determined

based on the Brownian motion of the scattering of laser light by the nanoparticles.

3.5 PHOTOCATALYTIC DEGRADATION ACTIVITY

Photocatalytic degradation activity of iron oxide nanoparticles was evaluated by the disintegration of methylene blue dye under sunlight irradiation. 500mg of iron oxide nanoparticles were dispersed in 100ml of distilled water. A series of test tubes containing different concentrations of methylene blue (10 - 50mg/L) was taken and 1ml of prepared iron oxide nanoparticles solution was added separately. The experimental setup was kept over the sunlight irradiation between 11am to 1pm to observe the colour change in the dye. The resultant solution was monitored in the wavelength range of 680nm in a colorimeter and the percentage dye removal was calculated by,

$$\text{Dye removal (\%)} = (C_0 - C) / C_0 \times 100\%$$

Where,

C_0 is the initial concentration of dye

C is the dye concentration of dye at time.

3.6 PHYTOTOXICITY ASSAY

The green gram seeds (120 numbers) subjected for germination assay was immersed in 10% sodium hypochlorite solution for 10 min to ensure surface sterility (USEPA, 1996). After surface sterilization the seeds were soaked in distilled water (T_1), plant extract (T_2), ferrous sulphate (T_3) and iron oxide nanoparticles (T_4) suspensions for about 24h and rinsed thrice with distilled water. The experiment was conducted in triplicates. A layer of cotton was placed on each petri dish and 5ml of the selected test medium was added separately. Seeds were then transferred onto the cotton layer, with 10 seeds per dish and 1 cm distance between each seed. The plates were watered regularly with the selected treatment. Petri dishes were covered and placed in the dark chamber for 3 days. After 3rd day of incubation, germination percentage, shoot length, root length and vigour index of the green seedlings were recorded.

The seed germination was calculated using the formula,

$$\textbf{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

The maximum length of each shoot and root were recorded in centimeter.

Vigour index is calculated as the product of germination percentage and plant height.

Vigour index = Germination percentage x (seed length + root length)

(Abdul Baki and Anderson, 1973)

3.7 PHYTOCHEMICAL ANALYSIS OF SYNTHESIZED IRON OXIDE NANOPARTICLES

The biosynthesized iron oxide nanoparticles was screened for the presence of phytochemicals namely flavonoids, alkaloids, steroids, terpenoids, polyphenols, glycosides, tannins, saponins, proteins, amino acids and carbohydrates according to the method proposed by Sofowora (1993).

Identification of flavonoids

Shinoda test

To 5 g of the biosynthesized iron oxide nanoparticles, 50ml of ethanol was added and heated in a boiling water bath. To the ethanolic solution, 1 drop of concentrated hydrochloric acid and few pieces of magnesium fillings were added and kept at room temperature for 10 – 15 minutes. Formation of red colour indicates the presence of flavonoids.

Ammonia test

Filter paper strips were dipped in the biosynthesized iron oxide nanoparticles and ammoniated change in the filter paper colour to yellow indicates the presence of flavonoids. About 10ml of concentrated sulfuric acid was added to the yellow coloured filter paper. Disappearence of yellow colour confirms the presence of flavonoids.

Identification of alkaloids

Dragondroff's test

To 1ml of the biosynthesized iron oxide nanoparticles, 1ml of Dragondroff's reagent was added. Formation of orange precipitate indicates the presence of alkaloids.

Hager's test

To 1ml of the biosynthesized iron oxide nanoparticles, 1ml of Hager's reagent was added. Formation of orange precipitate indicates the presence of alkaloids.

Identification of steroids/terpenoids

Libermann - Buchard test

To the synthesized iron oxide nanoparticles, 2ml of chloroform was added followed by 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid. Development of rosy red colour which quickly changes to bluish green indicates the presence of sterols.

Salkowski test

To the synthesized iron oxide nanoparticles, chloroform was added and shaken well with equal amount of concentrated sulphuric acid. Appearance of red colour in the chloroform layer and green fluorescence in the acid layer indicates the presence of sterols.

Identification of polyphenols

Ferric chloride test

To 2ml of synthesized iron oxide nanoparticles, 2ml of ferric chloride solution was added. Formation of deep bluish green solution indicates the presence of phenols.

Identification of glycosides

Legal test

The synthesized iron oxide nanoparticle (2 ml) was dissolved in few drops of pyridine. To this a drop of 2% (w/v) sodium nitroprusside solution and a drop of 20% sodium hydroxide solution were added. Appearance of pink or deep red colour indicates the presence of glycosides.

Identification of tannins

Braemer's test

To 5ml of synthesized iron oxide nanoparticles, 10ml of water was added, boiled and filtered. To the filtrate few drops of 10% of ferric chloride was added. A dark green, blue or brown colour indicates the presence of tannins.

Identification of saponins

Sodium bicarbonate test

To 2ml of synthesized iron oxide nanoparticles, few drops of sodium bicarbonate was added and shaken well. Formation of honeycomb indicates the presence of saponins.

Identification of proteins and aminoacids

Biuret test

The biosynthesized iron oxide nanoparticle was treated with equal volume of 40% sodium hydroxide and two drops of 1% copper sulphate solution. Pink or purple colour indicates the presence of proteins and free aminoacids.

Identification of carbohydrates

Benedict's test

To 0.5ml of the synthesized iron oxide nanoparticles, 2ml of benedict's solution was added. Formation of reddish brown precipitate indicates the presence of carbohydrates.

3.8. ANTIMICROBIAL ACTIVITY OF SYNTHESIZED IRON OXIDE NANOPARTICLES

The antibacterial and antifungal activity of the synthesized iron oxide nanoparticles was assessed by strandard agar well diffusion method (Bauer *et al.,* 1966) (Appendix II and III). The bacterial cultures (*Klebsiella pneumoniae, Streptococcus epidermis, Shigella flexneri, Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Vibrio cholerae* and *Pseudomonas aeroginosa*) and fungal cultures (*Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Trichoderma viridae* and *Rhizopus sp.)* were used in the present study. Sterile Muller Hinton Agar and Rose Bengal Chloramphenicol Agar media was poured onto petri plates aspetically and allowed to solidify. Three wells were bored on the agar plates with the help of cork borer (0.6cm diameter) separately. The selected bacterial and fungal cultures were swabbed on the respective medium separately. To each well 20µl of the biosynthesized nanoparticles, aqueous extract of *P. reticulatus* leaves and the standard antibiotics chloramphenicol (bacteria) and fluconazole (fungi) which served as positive control were added separately. The plates were then incubated at 37^{o}C for 24hrs (bacteria) and at room temperature for 5 days (fungi). To determine the antimicrobial activity, the diameter of inhibition zone was measured and expressed in millimeter.

Results andDiscussion

4. RESULTS AND DISCUSSION

The results of the present study entitled “Biobased green approach to synthesise iron nanoparticles using *Phyllanthus reticulatus* Poir. leaf extract” is discussed under the following headings:

4.1 Effect of concentration of ferrous sulphate, leaf extract and reaction time on the formation of iron oxide nanoparticles

4.2 Characterization of green synthesized iron oxide nanoparticles

4.2.1 UV - Visible Spectroscopy analysis

4.2.2 Fourier Transform- Infra Red Spectroscopy (FT- IR) analysis

4.2.3 Scanning Electron Microscopy (SEM) and Energy Dispersion X-Ray (EDX) analysis

4.2.4 X- Ray diffraction (XRD) analysis

4.2.5 Dynamic Light Scattering (DLS) analysis

4.3 Photocatalytic activity of biosynthesized iron oxide nanoparticles for the removal of methylene blue dye

4.4 Phytotoxicity assay

4.5 Phytochemical screening of biosynthesized iron oxide nanoparticles

4.6 Antimicrobial activity of the biosynthesized iron oxide nanoparticles

4.6.1 Antibacterial activity of the biosynthesized iron oxide nanoparticles

4.6.2 Antifungal activity of the biosynthesized iron oxide nanoparticles

4.1 Effect of the concentration of ferrous sulphate, leaf extract and reaction time on the formation of iron nanoparticles

Different parameters such as concentration of ferrous sulphate solution (1- 5mM), leaf extract (1-5ml) and reaction time (0-10 minutes) were optimized for the significant synthesis of iron nanoparticles. All these parameters have been identified as the factors affecting the production of iron nanoparticles. Among the different concentrations of ferrous sulphate solution and plant extract used with the varying time intervals, the colour change from yellow to black was well observed within a second using 2mM of ferrous sulphate with 1ml of leaf extract indicating the presence of iron nanoparticles. Plate 3 represents the development of iron nanoparticles under optimized parameters.

Visual perception of black colour depicting the formation of iron oxide nanoparticles was observed using the extracts of *Lantana camara*, *Mimosa pudica* extract, *Eichhornia crassipes* (Prabhakar, 2017), green tea, *Eucalyptus globulus* (Huang *et al.,* 2014; Markova *et al.,* 2014; Madhavi *et al.,* 2013).

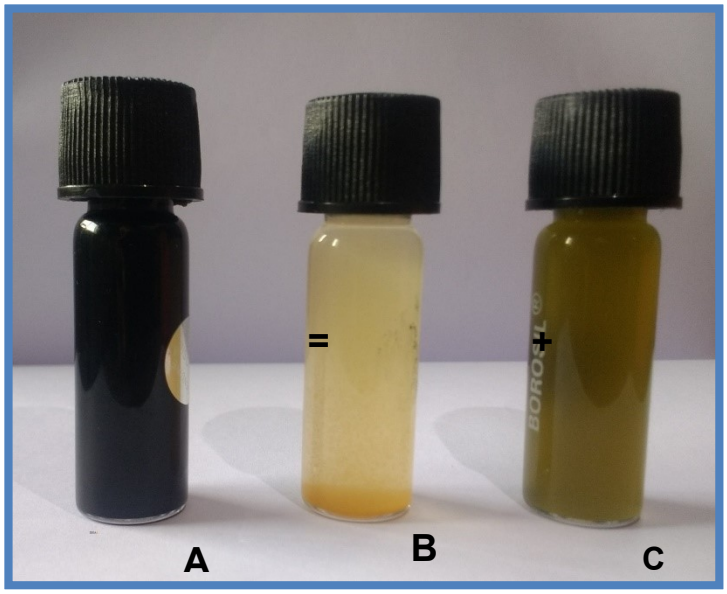
The change in the colour of the solution represented that the ferrous sulphate was reduced to ferrous ions due to the reduction. The reaction mechanism might be due to the formation of a complex when the plant extract was added to the metal which breaks the –OH bond and forms a partial bond with the metal ion. Further there might be breakage of the partial bond and the transfer of electrons to reduce the metal ions to nanoparticles (Gottimukkala, 2017). Hence it was assured that biomolecules present in the leaf extract may function as reducing agent and capping agent.

The results of the present study correlates with the findings of Jeyasundari (2017) who observed black colour solution when 1mM ferric chloride solution was added to the fresh leaves of *Psidium guajava*.

The formation of iron oxide nanoparticles was observed with *Cynometra ramiflora* (Bishnoi *et al.,* 2018), *Cypressus sempevirens* (Ebrahiminezhad *et al.,* 2017) and *Eichhornia crassipes* (Jagathesan and Rajiv, 2018) which supports the findings of the present study.

PLATE 3

SYNTHESIS OF IRON OXIDE NANOPARTICLES USING LEAF EXTRACTS OF *Phyllanthus reticulatus*



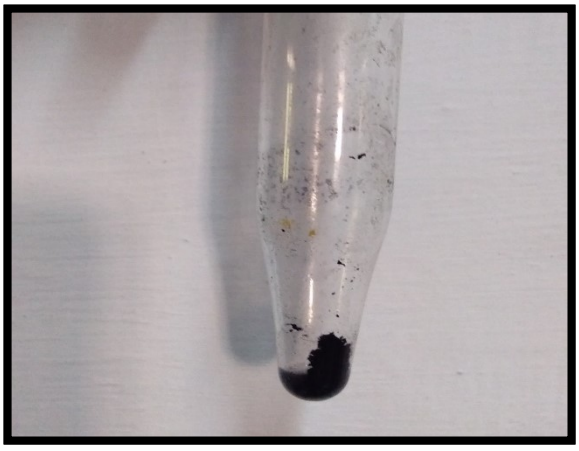
A- Biosynthesized iron oxide nanoparticles

B- Ferrous Sulphate solution

C- Leaf extract of *Phyllanthus reticulatus*

PLATE 4

PELLET OF IRON OXIDE NANOPARTICLES



4.2 Characterisation of green synthesized iron oxide nanoparticles

4.2.1 UV- Vis Spectroscopy analysis

The green synthesized iron oxide nanoparticles mediated by *Phyllanthus reticulatus* was monitored first visually and then observed under the UV- Visible spectroscopy. During visual monitoring a gradual shift in the colour of the mixture was observed from yellow to black within a second of reaction time at room temperature. UV- Vis spectral analysis was done at the range of 200- 800 nm and a broad band absorption was recorded around 229 nm (Fig. 6).

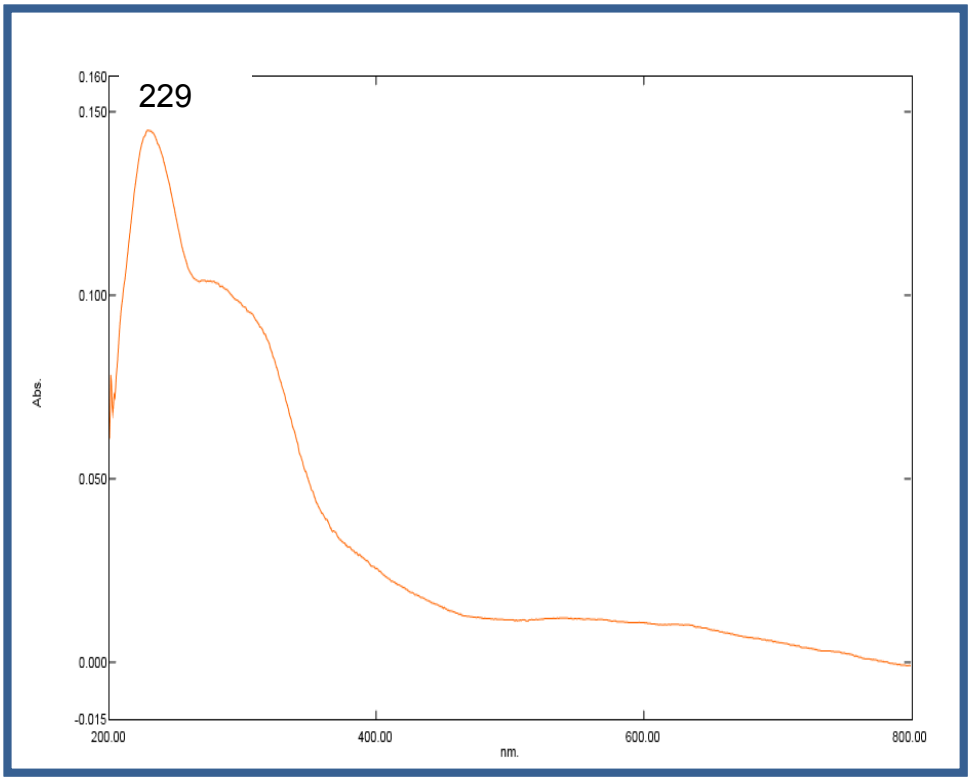


Fig. 6 UV- vis spectrum of synthesized iron oxide nanoparticles

Incubation of *Phyllanthus reticulatus* leaf extract in different concentrations leads to a change in the plasmon resonance band that eventually resulted in the appearance of yellow to black colour (Bindhani and Panigrahi, 2014). Mulvaney (1996) reported that the intensity of the colour arises from the surface plasmons, which are dipole oscillation arising when an

electromagnetic field in the visible range is coupled to the collective oscillations of conduction electrons.

Parallel studies were observed in the findings using various plants and spices (Pattanayak *et al.,* 2013), *Lagenaria siceraria* (Kanagasubbulakshmi and Kadirvelu, 2017) and in P*sidium guajava* (Jeyasundari *et al.,* 2017).

4.2.2 Fourier Transform Infra-Red Spectroscopy (FT- IR) analysis

Fourier Transform Infra-Red Spectroscopy (FT- IR) analysis was observed for the leaf extract and iron oxide nanoparticles are shown in the Fig.7. It was carried out to determine the functional groups present in the leaf extract and their liable connection in the synthesis of iron oxide nanoparticles. The strong absorption band at 3367.71 cm^{-1} and 3255.84 cm^{-1} indicates the OH stretching vibrations and the presence of alcohols and phenols (Litvin *et al.,* 2012). Nasrollahzadeh *et al.* (2015) reported the peaks at 3375 – 3000 cm^{-1} in FT- IR spectrum indicating the presence of flavonoids and other phenolics in the synthesized nanoparticles. The peak at 2985.8cm^{-1} indicated the C-H stretching vibrations of alkanes. The absorption band at 2353.16cm^{-1} corresponds to the –OH stretching of carboxylic acids. The sharp peak at 1639.49 cm^{-1} can be assigned to the amide band of the proteins released by *Phyllanthus reticulatus* leaves. The absorption peak at 1473.62cm^{-1} depicts the C=C stretching of aromatic compounds. The FT- IR spectrum of iron oxide nanoparticles stretching in the range of 1380-1240 cm^{-1} indicates C-N stretch of aliphatic amines. Based on the above results different functional groups were observed in the biosynthesized iron oxide nanoparticles which confirm the presence of phenols, amines, carboxyl and carbonyl groups.

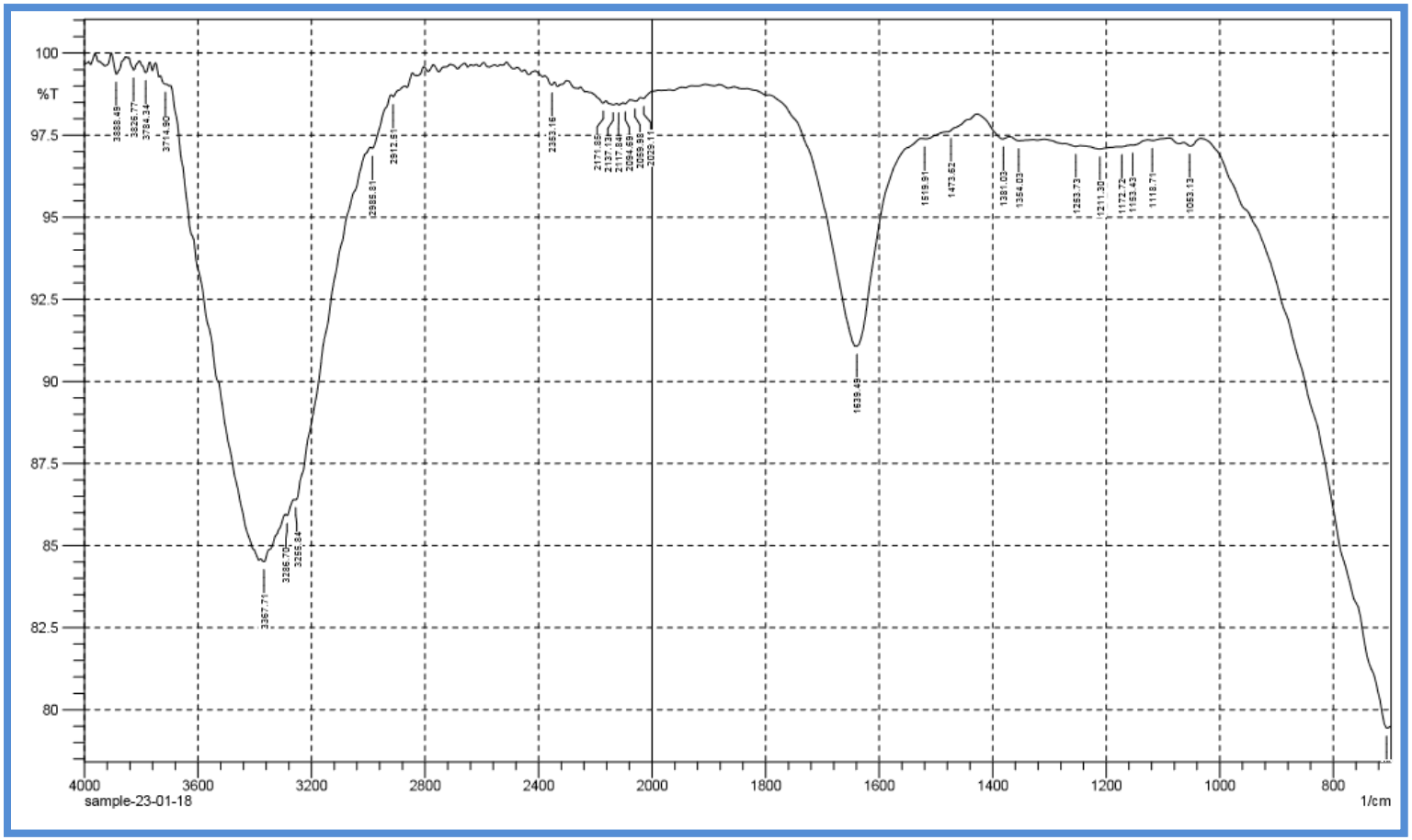


Fig.7- FT – IR absorption spectra of biosynthesized iron oxide nanoparticles

It has been reported by Lin *et al.* (2005) that the carbonyl groups from the amino acid residues and peptides of proteins have a strong affinity to bind metals. So that protein can act as encapsulating agent and thus protect the nanoparticles from agglomeration. The amide linkages in proteins and polypeptides give well known signature in IR region.

Presence of organic groups in the FT- IR spectrum of iron oxide nanoparticles indicated that bioactive compounds from *P. reticulatus* act as capping agent and decorate the surface of prepared particles. This organic coating increases the chemical and colloidal stability of iron oxide nanoparticles and also can increase the biocompatibility of nanoparticles (Ebrahiminezhad *et al.,* 2015 and Gholami *et al.,* 2015).

From the analysis of FT- IR study, it was revealed that the carbonyl group from the amino acid residue, carbohydrates and phytochemical constituents has the stronger ability to bind metal nanoparticles (capping of iron oxide nanoparticles) to prevent the agglomeration and thereby stabilize the medium. This suggests that biological molecules could possibly perform the dual function of formation and stabilization of iron oxide nanoparticles in the aqueous medium (Jeyasundari *et al.,* 2017).

Das *et al*. (2010) reported that the polyphenols may bound to the surface of biosynthesized iron oxide nanoparticles and thereby stabilizes them through interacting with free amino or carboxylic groups. Smulec *et al*. (2011) also suggested that polyphenols play a key role in the synthesis of nanoparticles and can directly reduce iron ions to iron nanoparticles. Similarly, Kumar *et al.* (2013) also highlighted that the polyphenols present in the biological resources act both as reducing and capping or stabilizing agent and hence seems to be an alternative to chemical methods in which uses of hazardous reducing, capping or stabilizing agents and toxic expensive organic solvents are inevitable.

Shah *et al.* (2015) also stated that presence of hydroxyl, carboxyl and amino functional groups may act as a precursor in metal ion reduction and also as capping agent to form the robust coating on the metal nanoparticles and leads to the colour change from yellowish brown to black.

Nasrollahzadeh *et al.* (2015) reported that the flavonoids and other phenolics in the plant extract could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. Thombre *et al*. (2014) also identified the presence of flavonoids, terpenoids in the biosynthesized silver nanoparticles using *Eichhornia crassipes* which act as an reducing or capping agent.

The results are consistent with the previous studies on iron nanoparticles synthesis using the extracts of *Terminalia chebula* (Kumar *et al.,* 2013), *Ocimum sanctum* (Balamurughan *et al.,* 2014), tree leaf extracts (Machado *et al.,* 2015), *Mangifera indica, Azadirachta indica, Murraya koineggei* and *Magnolia champaca* (Devatha *et al.,* 2016).

4.2.3 Scanning Electron Microscopy (SEM) and Energy Dispersion X- ray (EDX) analysis

Scanning electron microscopic images were employed to analyze the morphology and size of nanoparticles that are formed in presented study. SEM is able to provide images of three dimensional objects because in its normal mode of operation it records not the electrons passing thorough the specimen but the secondary electrons that are released from the sample by the electron beam impinging on it.

SEM images revealed that the synthesized iron oxide nanoparticles were aggregated as irregular sphere shapes with rough surfaces and shown in the Fig. 8. The morphology of the nanoparticles mostly appeared to be a porous and spongy which might be due to bioreduction of iron salts by *P. reticulatus* extract. Also, it can be explained by the fact that the phytochemicals present in *P. reticulatus* extract plays a role in formation of final structure and particle size of these green synthesised iron nanoparticles (Mahdavi *et al.,* 2013).

Similarly, Jeyasundari *et al.,* 2017 reported that the irregular morphological appearance that iron oxide particles show the spherical shape as the characteristic of metallic nanoparticles and also observed the irregular morphological appearance which occurred due to agglomeration of iron oxide nanoparticles due to its adhesive nature.

The EDX spectrum was recorded after documenting the electron micrographs in the spot-profile mode by focusing on the densely occupied iron oxide nanoparticles region. The EDX spectrum showed intense peak of Cl - 10.12% and

O - 38.32% and also showed a strong signal of pure Fe with a weight percentage of 51.56% (Fig. 9). In addition, the EDX spectrum also showed signal for Cl and O illustrating the existence of macromolecule as stabilizing agent on the surface of iron nanoparticles.

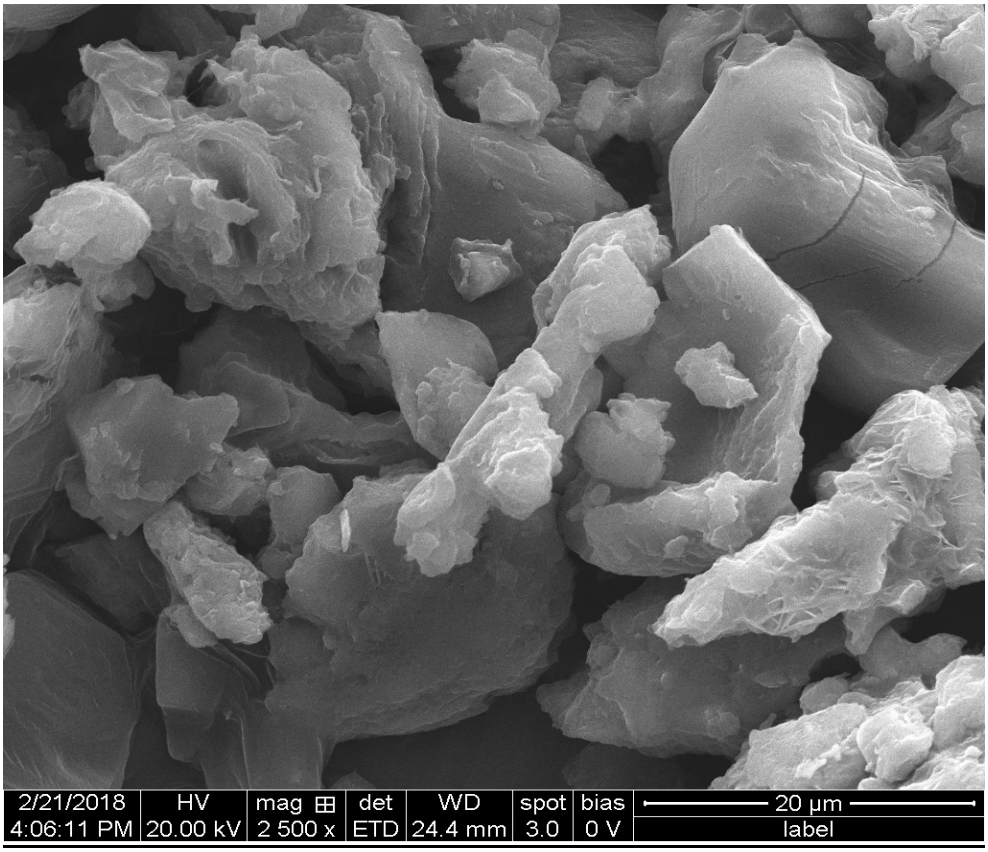


Fig. 8 SEM image of biosynthesised iron oxide nanoparticles

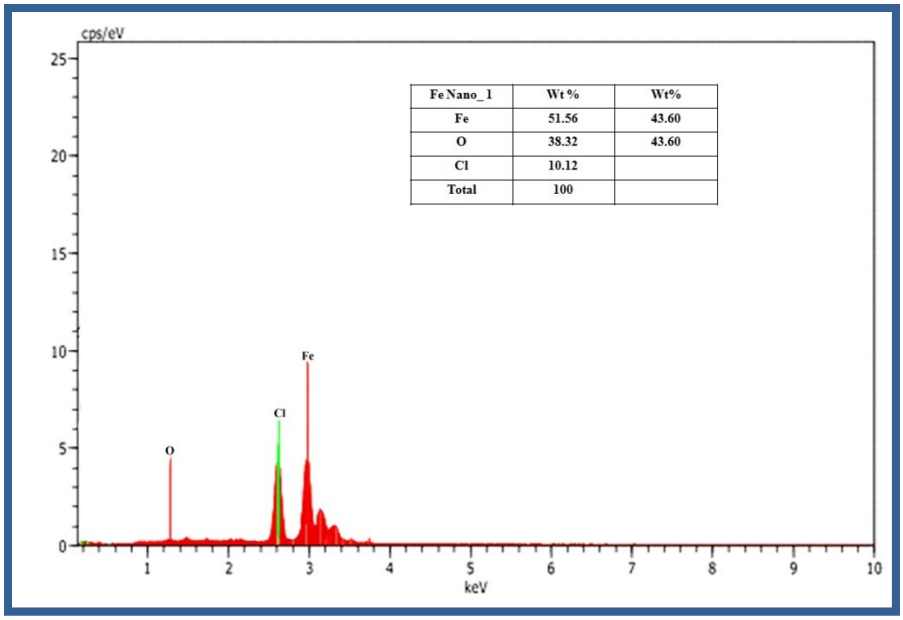


Fig. 9- EDX spectrum of synthesized iron oxide nanoparticles

The various other signals namely O and Cl detected may be from the biological phytoconstituents capped to the surface of iron oxide nanoparticles. The O signals may indicate the formation of iron oxide nanoparticles (Kumar *et al.,* 2013).

The weight composition of Fe synthesized by *Phyllanthus reticulatus* was relatively greater than that of the extracts of green tea and eucalyptus leaf whose percentage was reported to be 27.8% and 16.17% due to the highest phenolic content of the candidate plant (Machado *et al.,* 2013 and Wang *et al.,* 2014).

4.2.3 X- Ray Diffraction (XRD) analysis

The nature and phase composition of iron oxide nanoparticles were identified by X- ray diffractometer with Bragg's angle ranging from 20^0 and 80^0. The XRD pattern of biosynthesized iron oxide nanoparticles is shown in the Fig. 10. The presence of several broad Bragg peaks in the biosynthesized iron nanoparticles corresponds to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) orientations respectively. Thus the iron oxide nanoparticles are found to be crystalline in nature.

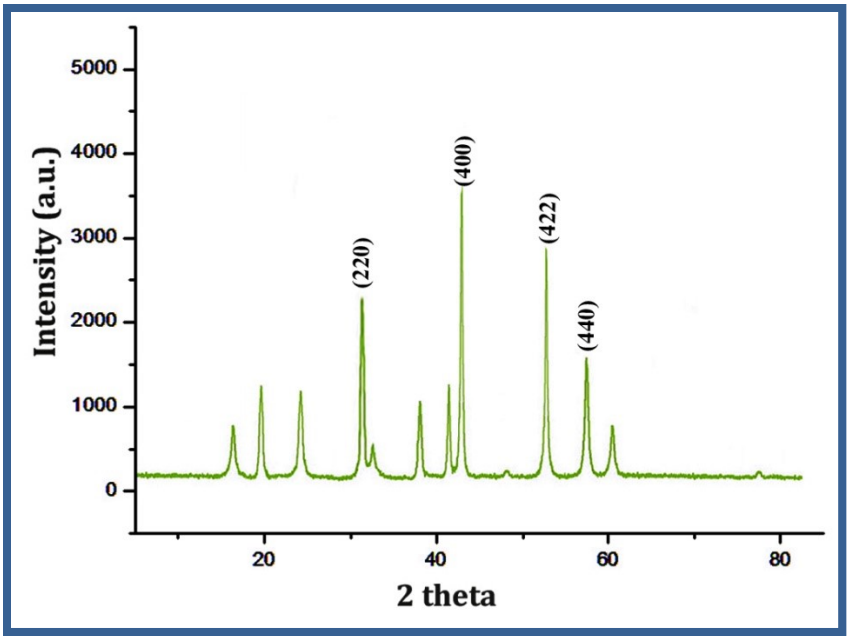


Fig. 10- XRD pattern of biosynthesized iron oxide nanoparticles

The XRD spectrum with sharp peaks for the biosynthesized nanoparticles also well establishes their crystalline nature and supported the EDAX results (Ljaz *et al.,* 2017). Thus it can be concluded that the green fuel played a profound role in controlling the particle size.

4.2.5 Dynamic Light Scattering (DLS)

Dynamic light scattering is a widely used technique for the determination of particle size in colloidal solution. The average size of the particles, size distribution, and polydispersity index (PDI) of the synthesized iron oxide nanoparticles were determined by this technique.

The resulting particle size of iron oxide nanoparticles exhibits the size distribution of 185.6 nm (Fig.11). These results are consistent with the UV- Vis spectra where broadness of absorption peak is proportional with the size of the particle. Similarly, the *Mimosa pudica* (Prabhakar *et al.,* 2017) based iron nanoparticles showed comparatively larger size distribution of 65 - 230 nm. These results are consistent with the UV-vis spectra, where broadness of absorption peak was observed. Particle size and distribution are the major characteristics to be determined for a nanoparticles based on

its saturation solubility, dissolution velocity, physical stability or even biological performance (Visahl and Agarwal., 2011).

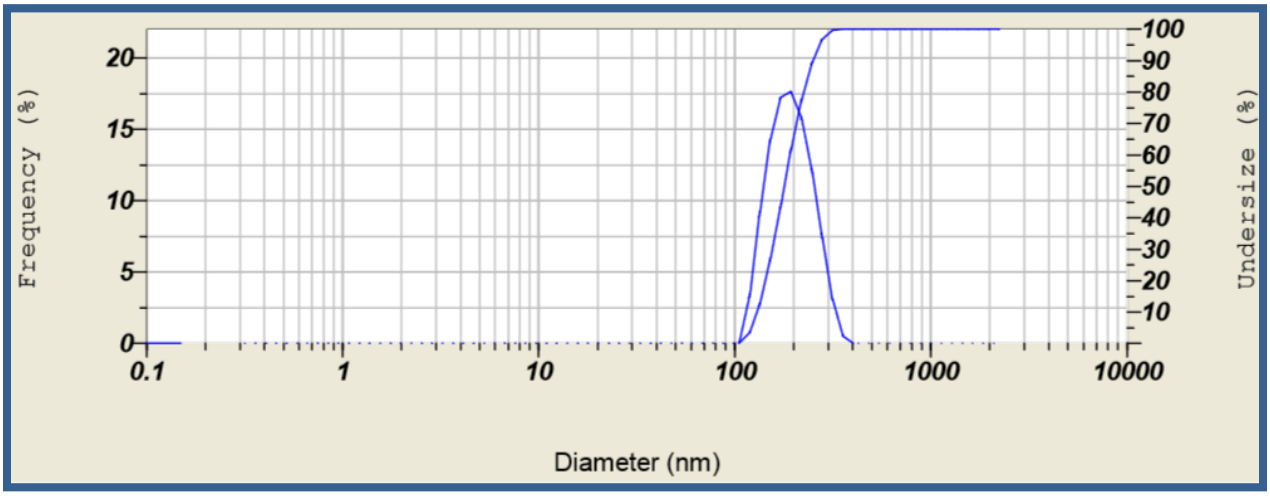


Fig. 11- Particle size of biosynthesized iron oxide nanoparticles

4.3 Photocatalytic activity of biosynthesized nanoparticles for the removal of methylene blue dye

Photocatalytic activity of biosynthesized iron oxide nanoparticles was evaluated by the degradation of methylene blue as a classical pollutant under solar irradiation. Dye degradation was visually detected by gradual change in the colour of the dye solution from deep blue to colourless as shown in the Plate 5. It was noted that as the concentration of dye increases the absorption value decreases at λ 680 nm, i.e the concentration is indirectly proportional to the absorbance value.

The control exhibited no change in colouration during exposure to sunlight and the iron oxide nanoparticles completely disintegrated the methylene blue dye within 10 seconds in 10mg/L dye (98%), whereas the colour change was decreased in 20mg/L (87%) and 30mg/ L (66%) was noted at 25 and 30 minutes of incubation respectively. Minimum decolourisation was observed in 40mg/L (27%) and 50mg/L (15%) inoculated with 1ml of iron oxide nanoparticles (Table 1).

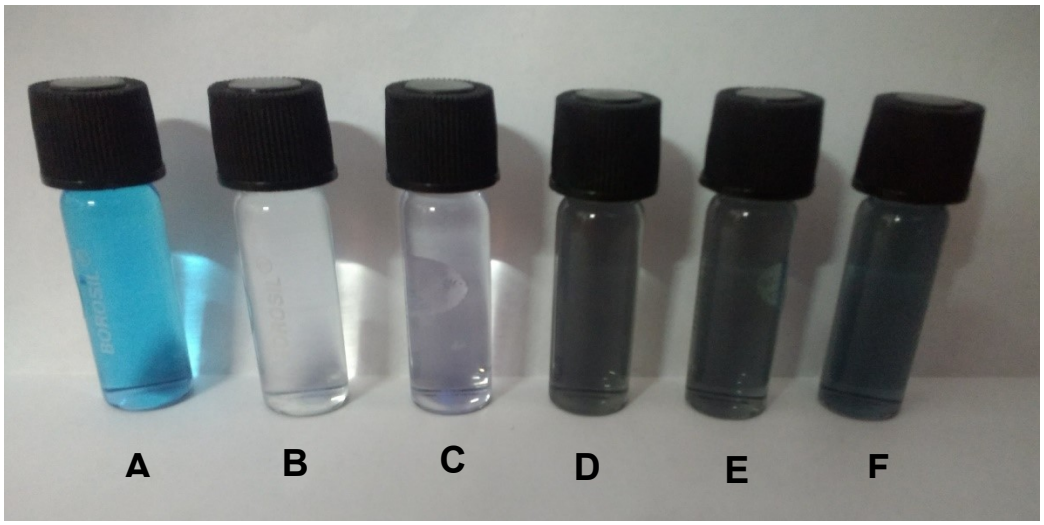
Table 1 –Decolourisation of methylene blue using iron oxide nanoparticles

DYE CONCENTRATION (mg/L)	DECOLOURISATION (%)
10	98
20	87
30	66
40	27
50	15

The mechanism of degradation of dye took place due to the generation of surface plasmons under resonant excitation with molecular environment (Bryukhanov *et al.,* 2015). It was also described by Maji *et al.* (2012) that the mechanism of degradation of dye might be due to photo-absorption, electrons and holes generation, charge carriers transfer and the recombination of carrier with the dye molecules. The high surface area of iron oxide nanoparticles in the present study assist the methylene blue degradation and increase the formation of hydroxyl radicals.

PLATE 5

Photocatalytic activity of biosynthesized iron oxide nanoparticles



A- Control (Methylene blue Dye)

B- Photocatalytic activity of iron oxide nanoparticles at 10mg/L

C- Photocatalytic activity of iron oxide nanoparticles at 20mg/L

D- Photocatalytic activity of iron oxide nanoparticles at 30mg/L

E- Photocatalytic activity of iron oxide nanoparticles at 40mg/L

F- Photocatalytic activity of iron oxide nanoparticles at 50mg/L

The results of the present study were in agreement with Muthukumar and Maheswaran (2015) who investigated the degradation of methyl orange and methylene blue using *Amaranthus spinosus* leaf extract and chemically synthesized (i.e. using $NaBH_4$) iron nanoparticles. Shahwan *et al*. (2011) also reported that iron nanoparticles which were synthesized by using leaf extract of green tea were able to remove 80% of methyl orange dye within an hour of irradiation. Nevertheless, ultra- small nanoparticles can afford larger surface area as compared to iron nanoparticles which could be more effective for a wide range of applications as catalyst and absorbent. Muthukumar and Matheswaran (2015) stated that heightened photocatalytic activity was exhibited only by the larger surface area and lesser band gap of iron oxide nanoparticles.

The results of the present study coincides with the findings of Shahwan *et al.* (2011) who reported 80% removal of methylene blue dye within 5 minutes of sunlight irradiation and the remaining concentration of dye was decolourised after 200 minutes under optimized conditions. Similar studies were performed using iron nanoparticles to remove malachite green by green tea leaf extract (Weng *et al.,* 2013), indigo carmine by *Azadirachta indica* and methyl orange by benzoate supported nanoscale zero valent iron (Chen *et al.,* 2011).

Thus the remediation with *Phyllanthus reticulatus* mediated iron oxide nanoparticles synthesis seems to be a potent tool to encounter the environmental consequences.

4.4 Phytotoxicity assay

The results of germination percentage, shoot length, root length and vigour index of green gram seeds grown with distilled water (T_1), *Phyllanthus reticulatus* leaf extract (T_2), ferrous sulphate solution (T_3) and biosynthesized iron nanoparticles (T_4) were depicted in table 2. Plate 6 and 7 portrays the germination of green gram seeds in different treatments .

Biometric parameters

The germination percentage was maximum T_1 (97%) followed T_4 (93%), T_2 (90%) and T_3 (83%). Similar trend was noticed in the vigour index of green gram seeds (Table 2). The values observed for the shoot lengths of green gram were 3.83, 3.54, 1.54 and 0.74 cm in T_1, T_4, T_2 and T_3 respectively. The root length was heightened in T_1 (2.92 cm) followed by T_4 (1.35 cm) and T_2 (0.69 cm). The least root length was observed in T_3 (0.16 cm). T_1 (Control) showed an increase in shoot and root length when compared with T_4 (iron oxide nanoparticles). T_2 and T_3 showed the moderate and minimum shoot and root length when compared with T_1. Plate 6 portrays the shoot and root length green gram seedlings grown with different treatments.

The pores present in the seed coat facilitate the selective permeability and interaction between media and plant will be partial till the radicles emerge and interact with the growth medium (Wierzbicka and Obidzinska, 1998). The aqueous medium will fill the intercellular spaces of parenchyma smoothing the transport of soluble nutrients (Dongen *et al.,* 2003).

Phytotoxicity profile of nanomaterials is highly empirical and preliminary and the effects of nanoparticles elemental composition, size and stability has to be known. Seed germination and seedling growth are being widely used to test the phytotoxicity of chemical species such as engineered nanomaterials which may be released into the environment. Seed germination and root elongation is a rapid and widely used acute phytotoxicity test with several advantages like sensitivity, simplicity, low cost and suitability for unstable chemicals or samples (Munzuroglu and Geckil, 2002 and Wang *et al.,* 2001). Germination is normally known as a physiological process beginning with water imbibition by seeds and culminating in the emergence of the rootlet (Kordan, 1992). In this study, seeds showing emergence of radicle or cotyledon coming out of the seed coat were recorded as being germinated.

Seed coat plays a very important role in protecting the embryo from harmful external factors. Seed coats can have selective permeability (Wierzbicka and Obidzinska, 1998). Pollutants, though having obviously inhibitory effect on root growth, may not affect germination if they cannot pass through seed coats. This may explain that seed germination in this study was not greatly altered by nanoparticles.

Table 2 - Phytotoxicity assay of green gram seeds grown with different treatments

Treatments	Germination Percentage	Shoot Length (cm)	Root Length (cm)	Vigour Index
T_1	97	3.83 ± 0.24	2.92 ± 0.03	655
T_2	90	1.25 ± 0.81	0.69 ± 0.60	175
T_3	83	0.74 ± 0.02	0.16 ± 0.24	75
T_4	93	3.54 ± 0	1.35 ± 0.18	455

Values of Mean ± Standard Deviation

Where, **T_1 -** Distilled water (Control), **T_2 -** Leaf extract of *P. reticulatus*

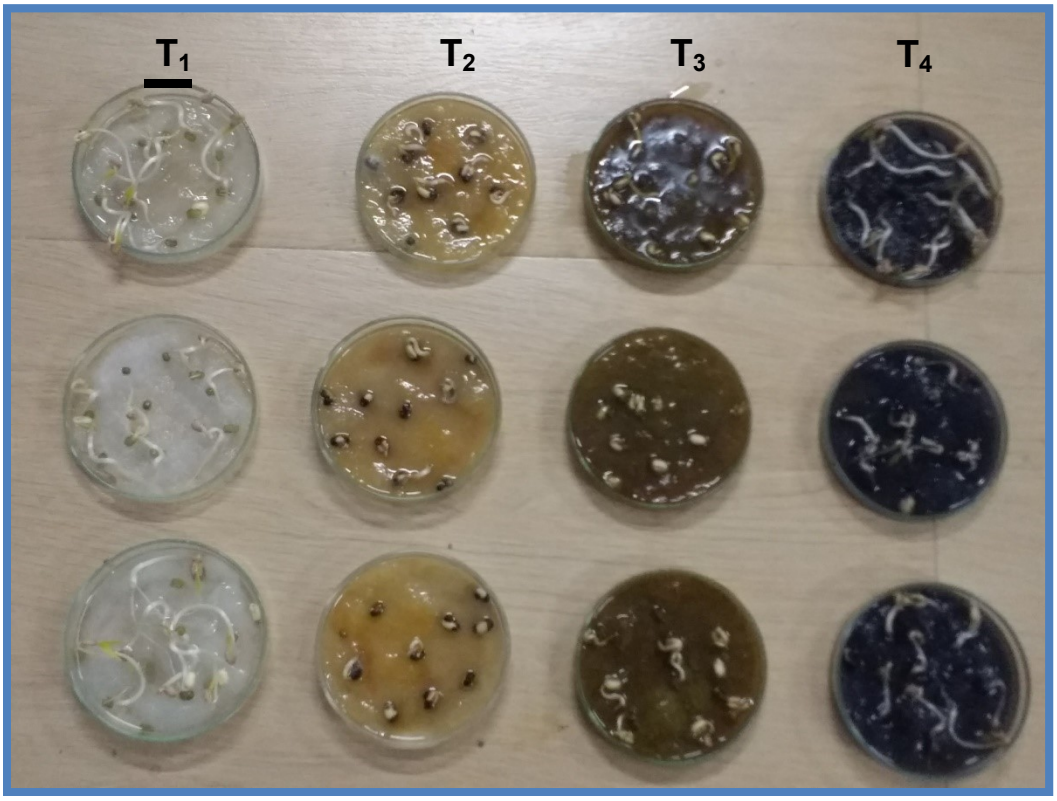
T_3 - Ferrous sulphate solution, **T_4 -** Biosynthesised iron oxide nanoparticles

Increase in the germination and seedling growth by biosynthesized iron oxide nanoparticles shows that iron with plant extract might have acted as stabilizer and proves to be non- toxic in nature. It has also been anticipated for the bioremediation of pollutants (Barrena, 2009 and Yavuz, 2006). Literature also says that iron oxide nanoparticles act as non- toxic material in many fields (Ju- Nam and Lead, 2008) and also perform as biostimulant and micronutrient (Li *et al.,* 2015).

Ma *et al*. (2013) indicated that the nano zero valent iron is phytotoxic to plants at concentrations often used in the field. Several mechanism could be attributed to the phytotoxicity of nano zero valent iron. The formation of black coating on root surface could effectively block the root membrane pores and interfere with the water and nutrient uptake process.

PLATE 6

Germination of green gram seeds using different treatments



T_1- Distilled water (Control)

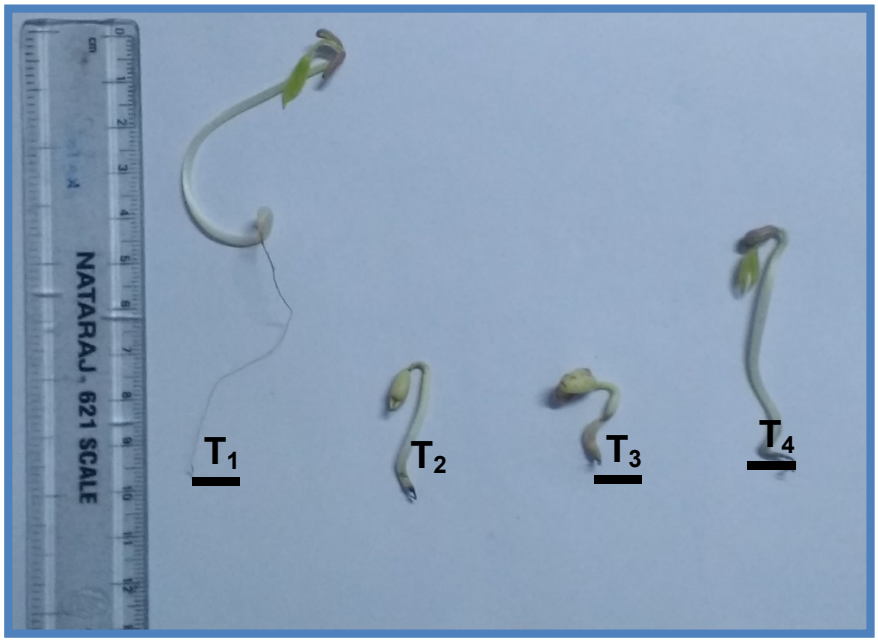
T_2- Ferrous sulphate solution

T_3- Leaf extract of *P. reticulatus*

T_4- Biosynthesised iron oxide nanoparticle

PLATE 7

Shoot and Root length of green gram seedlings grown using different treatments



T_1 - Distilled water (Control)

T_2 - Leaf extract of *P. reticulatus*

T_3 - Ferrous sulphate solution

T_4 - Biosynthesised iron oxide nanoparticles

Earlier literature has documented that under reduced condition, ferrous ion, a byproduct of iron oxidation could be further oxidized to its less soluble from ferric iron by the oxidative agents that are released from plant roots, thus creating a cover of an insoluble Fe^{3+} compound on the root surface. The black coating on plant root could also result from the direct deposition of nano zero valent iron on the surface. It is likely that black materials were a combination of both ferric iron oxides and zero valent irons.

Regardless of the nature of the black plaque, it could block the membrane pores and substantially reduce the effectiveness of root uptake of water and nutrients.

Decrease in the seedling growth might be due to the block in the pores by the iron sulphate which might disturb the absorption of nutrients and water and also decreases the transpiration rate by formation of iron plaque on root surface (Ma *et al*., 2013).

Radicles, after penetrating the seed coats, could contact the nanoparticles directly. Therefore, root elongation of sensitive plant species would have a dose-dependent response. Since root are the first target tissue to confront with excess concentrations of pollutants, toxic symptoms seem to appear more in roots rather than shoots (Sresty and Rao, 1999).

Decrease in the root length of green gram seedlings grown with ferrous sulphate solution (T_3) showed dark colouration while the roots of control plant showed heathy root with no colour indicating the nil deposition of the chemicals on the roots. Hence the green gram seeds treated with ferrous sulphate solution might have inhibited the growth of roots due to high accumulation of the chemicals.

4.5 Phytochemical screening of *Phyllanthus reticulatus* leaf extract and biosynthesized iron oxide nanoparticles

The plants contain various phytochemicals with enormous medicinal values and highly attracted by the people on the basis of natural and safe mode in comparison with synthetic drugs (Ahmad, 2010). Green synthesized iron oxide nanoparticles were screened for the presence of phytoconstituents by carrying out the qualitative chemical tests, which depicts the presence of flavonoids, alkaloids, proteins, amino acids, steroids, polyphenols, glycosides, tannins and saponins and the results were tabulated in the table 3.

Table 3- Phytochemical screening of green synthesized iron oxide nanoparticles

S.No	Phytochemicals	Iron oxide nanoparticles
1	**FLAVONOIDS**	
	Shinoda test	+

	Ammonia test	+
	Lead acetate test	+
2	**ALKALOIDS**	
	Dragondroff's test	+
	Hager's test	+
	Wagner's test	+
3	**STEROIDS**	
	Libermann-Buchard test	+
	Salkowski test	+
4	**POLYPHENOLS**	
	Ferric chloride test	+
	GLYCOSIDES	
5	Legal's test	+
6	**TANNINS**	+
7	**SAPONINS**	
	Sodium bicarbonate test	+
8	**CARBOHYDRATE**	
	Molisch test	-
	Fehling's test	-
9	**TERPENOIDS**	+
10	**PROTEIN/AMINO ACIDS**	
	Biuret test	+

Water soluble heterocyclic compunds such as flavonoids and alkaloids were mainly responsible for the reduction and stabilization of nanoparticles. The results of the present study implied that flavonoids, alkaloids, proteins, amino acids, steroids, polyphenols, glycosides, tannins and saponins present in the leaf extract of *Phyllanthus reticulatus* play a major role in the reduction of Fe^{3+} (Kiruba and Alagumuthu, 2014).

Reduction kinetics was the major component which drives the nucleation with control and growth of nanoparticles. The metal ions were reduced to nano zero valent metallic particles using flavonoid contents of the plant as green (without any hazardous impact on the environment) chemicals (reducing agents) which can be used as an alternative to the commonly used reducing agent (Nasrollahzadeh *et al*., 2015).

The presence of phenolics and other phytochemicals in *Phyllanthus reticulatus* not only result in effective reduction of iron oxide nanoparticles but their chemical framework is also effective at wrapping around the iron oxide nanoparticles to provide excellent robustness against agglomeration. Phenolic compounds are the complex of hydroxyl and carboxyl groups, which play a major role in the binding of Fe ions and responsible for the reduction of Fe ions to iron oxide nanoparticles (Sharma, 2009 and Kanchana *et al.,* 2010). It may also inactivate the iron ions by chelating and additionally suppressing the superoxide driven Fenton reaction, which is believed to be the most important source of reactive oxygen species (ROS). Therefore, plants with high content of phenolic compounds are one of the best candidates for nanoparticle synthesis (Iravani, 2011).

Saponins also upkeep the stability of the biosynthesized iron oxide nanoparticles. Polyphenols in biosynthesized iron oxide nanoparticles almost play a major role in the controlling the aggregation of the nanoparticles and do improve their dispersion by acting as a capping agent. It also act as the binder and form a ligand to Fe^{+3}, which ultimately reduces Fe^{+2}. Further with the formation of some intermediate compound through hydrolysis, Fe^{2+} gets reduced to Fe^{0} (Prabhakar *et al.,* 2017). Terpenoids present in the leaf extract mediated the reduction of iron ions by oxidation of aldehyde groups in the molecules to carboxylic acid.

The amino acids present in the biosynthesized iron oxide nanoparticles may stabilize and act as a capping agent. The presence of tannins, flavonoids and glycosides also contribute to the capping of iron to prevent agglomeration. which helps to bind the metals. Saponins also upkeep the stability of the biosynthesized iron oxide nanoparticles. Similarly, Elavazhagan et al, (2011) reported that saponins present in the aqueous leaf extract of *Memecylon edule* also contributed to the reduction of iron ions to nanosized iron oxide nanoparticles The flavonoids and phenolic compounds which

play a major role in the binding of Fe ions and responsible for the reduction of Fe ions to iron oxide nanoparticles (Sharma., 2009).

4.6 Antimicrobial activity of the biosynthesized iron nanoparticles

4.6.1 Antibacterial activity of biosynthesized iron nanoparticles

The antibacterial activity of iron oxide nanoparticles was investigated by measuring the zone of inhibition against the selected bacterial isolates. The zone of inhibition of the selected bacterial isolates against iron oxide nanoparticles and leaf extract was compared with the standard antibiotic chloramphenicol and the results were tabulated in table 4. Plate 8 shows the zone of inhibition of selected bacterial isolates against iron oxide nanoparticles and leaf extract. The bacterial isolates showed highest zone of inhibition against Gram negative bacteria namely*, Proteus vulgaris* (25 mm), *Vibrio cholera* (25 mm), *Shigella flexneri* (23 mm), *Salmonella typhi* (20 mm), *Klebsiella pneumoniae* (15 mm) and *Pseudomonas aeruginosa (32 mm)* whereas Gram positive bacteria namely, *Streptococcus epidermis* (12 mm) and *Staphylococcus aureus* (15 mm) exhibited minimum zone of inhibition for iron oxide nanoparticles.

Thus, among the selected bacterial isolates Gram negative bacteria exhibited highest antibacterial activity when compared with Gram positive bacteria. This greater bactericidal activity against Gram negative bacteria is ascribed to the variation in cell membrane of the bacteria. The Gram negative bacteria consist of a thin layer of cell wall membrane, its thickness ranged from 7- 8 nm and made up of peptiglycans and lipopolysaccharides (Snega *et al.,* 2015). The thickness of the cell wall of the Gram positive bacteria ranged from 20- 80 nm and made up of large number of mucopeptides, lipoteichoic acids and murein (Ravichandran *et al.,* 2014). In addition, *Staphylococcus aureus* has an antioxidant enzyme and shows a strong oxidant resistance (Zhao *et al.,* 2006). Thus, super oxide anions, hydroxyl radicals and released iron ions can easily penetrate into the bacteria and destroyed them. Hence the biosynthesized iron nanoparticles revealed remarkable antibacterial activity when compared with the leaf extract and the zone of inhibition exhibited by the synthesized nanoparticles was significant when compared with the standard antibiotic chloaramphenicol.

The antibacterial activity was caused based on the fact that iron ions released form iron oxide nanoparticles infused the bacterial cell membrane by attaching to the

negatively charged cell wall (Ren *et al.,* 2009 and Sankar *et al.,* 2014). Iron ions may indulged in cross linkage of nucleic acid strands by binding them with DNA of bacteria. This may leads to the disordered helical structure of DNA molecule which may be due to the denaturation of proteins and irregular of some biochemical processes in the cell, it may results the complete destruction of the bacterial cell (Yallappa *et al.,* 2013).

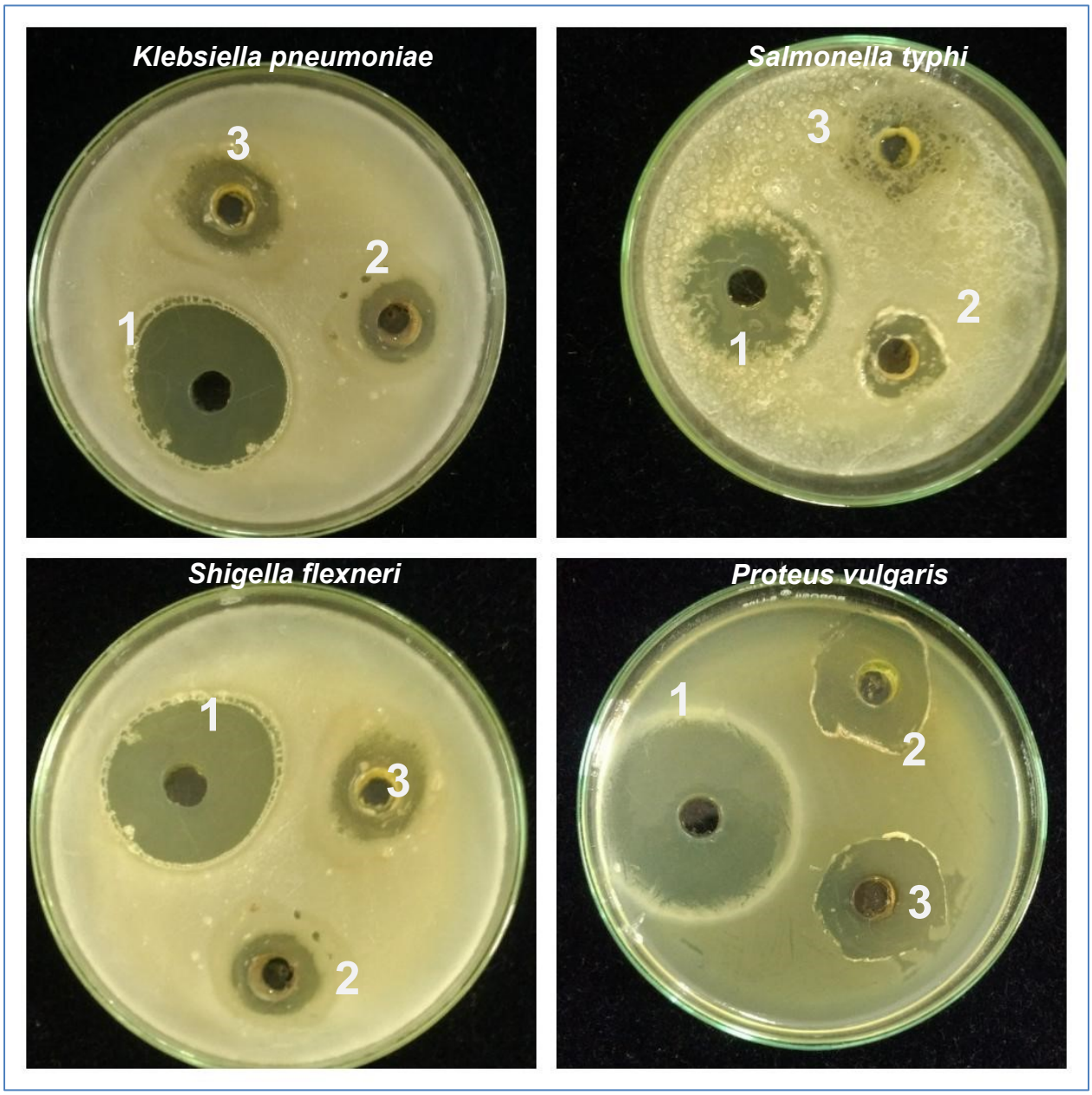
Das et al. (2013) evaluated the factors that are endanger the sensitivity of bacteria to cupric oxide nanoparticles, such as size of particles, temperature of synthesis of the nanoparticles, structure of bacterial cell wall, the degree of contact of the nanoparticles with bacteria. Bacterial cell membrane has abundance of sulfur containing proteins with iron oxide nanoparticles might have reacted and affects the viability of bacterial cell leading to increased permeability and iron oxide nanoparticles may also react with phosphorus present inside the DNA replication, inactivates and may inhibit enzyme activity which in turn cause the death of bacteria (Ravishankar *et al.,* 2011).

Table 4 - Antibacterial activity of the biosynthesized iron oxide nanoparticles and leaf extract

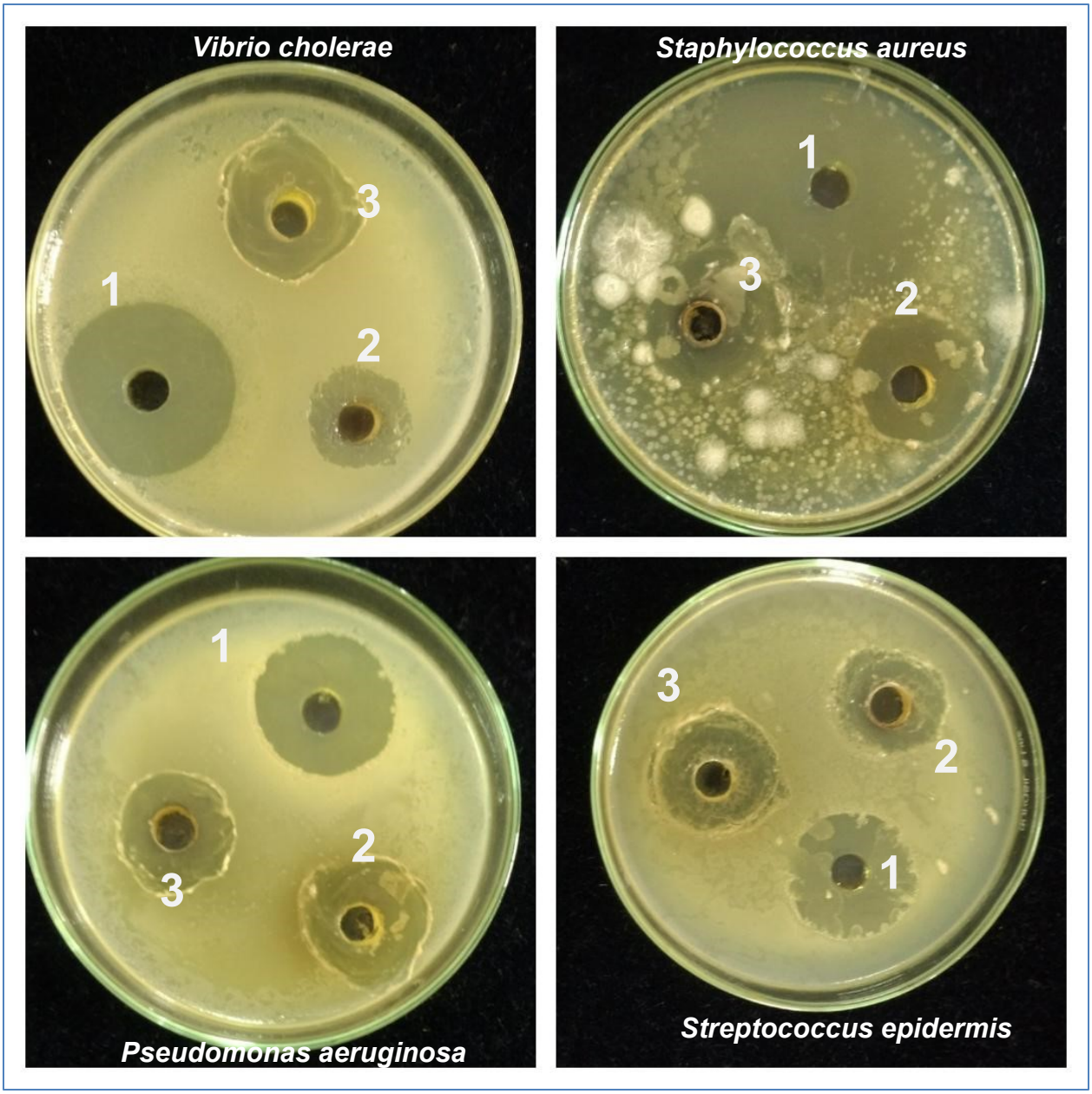
Bacterial isolates	Zone of inhibition (mm)		
	*P. reticulatus leaf e*xtract	Iron oxide (Fe_2O_3) nanoparticles	Chloramphenicol
Gram negative bacteria			
Proteus vulgaris	10	25	30
Vibrio chlorae	18	25	32
Shigella flexneri	12	23	36
Salmonella typhi	12	20	32
Klebsiella pneumoniae	13	15	35
Pseudomonas aeruginosa	50	32	42
Gram positive bacteria			
Staphylococcus aureus	10	15	35
Streptococcus epidermis	15	12	31

PLATE 8

Antibacterial activity of biosynthesized iron oxide nanoparticles



1



1- Chloramphenicol

2- Leaf extract of *P. reticulatus*

3- Biosynthesised iron oxide nanoparticles

Moreover, the rate of diffusion of nanoparticles was also a important characteristic against the bacterial strains (Goswami *et al.,* 2015). The evidences showed that the biosynthesized nanoparticles have the greater efficiency and least genotoxicity and cytotoxicity over the healthy cells, when compared with the chemically synthesized nanoparticles (Lima *et al.,* 2012). The presence of biologically active compounds in the leaf extract was beneficially involved in the inhibition of bacterial pathogens (Furneri *et al.,* 2002). The antibacterial activity was also liable to the higher surface area and smaller crystalline size (Hameed *et al.,* 2013). The bactericidal effect of iron nanoparticles was mainly depends on the electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cell wall and it is fundamental for the antibacterial activity. This interaction leads to the formation reactive oxygen species which replaced as the antibacterial agent (Xia, 2008) and (Burello and Worth, 2011).

Tong et al. (2012) reported that released ions of nanoparticles interact with the thiol(-SH) group of the protein present on the bacterial cell wall that leads to the denaturation of surface protein along with the loss of cell membrane permeability, subsequently cause cell death.

Wang *et al.* (2007) reported that the rough surface for metallic nanoparticles may intensify the bactericidal capabilities of bacteria. UV analysis showed strong absorption peak at 230 nm for iron oxide nanoparticles. Correspondingly, iron as compared to its bulk, forms low band gap energy which results in its photoexcitation at room temperature (Abbas *et al.,* 2015). This may assist in mass production of reactive oxygen species inside bacterial cellular environment, and intoleration of reactive oxygen species caused by iron nanoparticles leads to the faulty in the biosynthetic machinery of bacteria and cause the cell lethality (Maqbool *et al.,* 2016).

As Gomathi et al. (2017) reported that antibacterial activity of nanoparticles were associated with several mechanisms such as generation of Reactive Oxygen Species like super oxide anions (O_2^-) and hydroxyl radicals (OH), the presence of iron ions on iron oxide nanoparticles are making bind with sulphhydryl groups which direct to denaturation of proteins in the bacteria (Patil *et al.,* 2012) and release of iron ions from the iron oxide nanoparticles which simply penerate into the cell wall and cause severe

damage to the bacteria and kill them. Morever, nanosized iron oxide nanoparticles were attached to the bacteria and disturb the usual function of bacteria and hence damage severely to outer surface of the bacteria such as DNA, lipids and proteins.

4.6.2 Antifungal activity of the biosynthesized iron nanoparticles

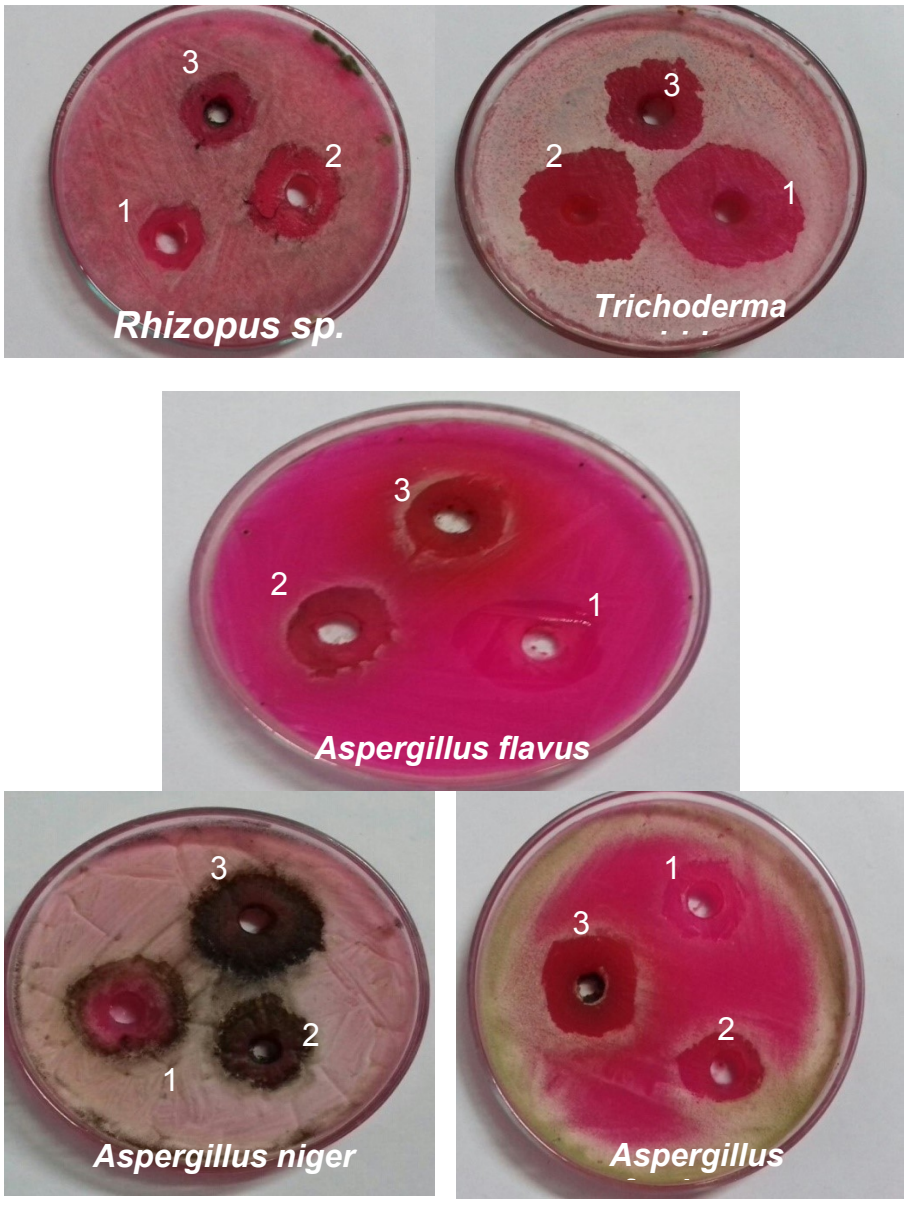
The antifungal activity of the green synthesized iron oxide nanoparticles was compared with the standard antibiotic flucanozole and depicted in the table- 5 and Plate 9. The fungicidal activity of iron oxide nanoparticles was found to be maximum in *Rhizopus sp.* and *Trichoderma viridae* with zone of inhibition of 31mm and 30mm respectively and showing greater competency with antifungal agent (Flucanozole). *Aspergillus fumigatus* and *Aspergillus niger* showed a zone of inhibition of 25 mm and followed by *Aspergillus flavus* (18mm) respectively. Among the tested fungal isolates *Rhizopus* exhibited 25mm zone of inhibition followed by *Aspergillus niger* (22mm) and *Aspergillus fumigatus* (20 mm) in the leaf extract of *Phyllanthus reticulatus*. Minimum zone of inhibition was recorded in *Trichoderma viridae (*15 mm) and *Aspergillus flavus* (13 mm).

Fungal isolates	**Zone of inhibition (mm)**		
	Phyllanthus reticulatus	**Iron oxide nanoparticles (Fe_2O_3)**	**Fluconazole**
Rhizopus sp.	25	31	35
Trichoderma viridae	15	30	40
Aspergillus niger	22	25	30
Aspergillus fumigatus	20	25	30
Aspergillus flavus	13	18	20

Table 5- Antifungal activity of the biosynthesized nanoparticles and leaf extract

PLATE 9

Antifungal activity of biosynthesized iron oxide nanoparticles



1- **Flucanozole**

2- Leaf extract

3- Iron oxide nanoparticles

The antimicrobial efficiency was present because of the electromagnetic interactions and free radical generation when present in the abrupt vicinity of the lipid membrane, leads to the production of lethal hydroxyl radical (OH) when present in immediate vicinity of the lipid membrane (Xia *et al.,* 2008). Reactive oxygen species may cause oxidative deterioration of cell membrane lipids, (Howlett and Avery, 1997) denaturing the cell membrane permeability with leakage of potassium ions, ultimately causing cell death (Tong *et al.,* 2012). Naseem and Farrukh (2015) reported that biosynthesized iron nanoparticles mediated by *Lawsonia inermis* and *Gardenia jasminoides* have the biomedical properties and had several reimbursements such as aptness for medical and pharmaceutical suggestions.

Thus the biosynthesized iron oxide nanoparticles seems to exhibit effective zone of inhibition against the selected fungal isolates and were moderate when compared with standard antifungal agent Flucanozole.

Summary and Conclusion

5. SUMMARY AND CONCLUSION

There is a demand in nanotechnology to improve credible and eco- friendly procedures for synthesis of various metal oxide nanoparticles using plant extracts which utilizes no additional surfactants/ polymers as capping and reducing agents. Hence, green synthesis of iron oxide nanoparticles using plant extract is a simple, environmentally friendly, pollutant - free and low - cost approach that could also be extended to fabricate other industrially important metal nanoparticles.

The present study was focused on the development of green method for the synthesis of iron oxide nanoparticles using the leaf extract of *Phyllanthus reticulatus,* under room temperature. The synthesized iron oxide nanoparticles prepared were characterized by visual perception, UV – vis spectroscopy, FT - IR, XRD, particle size, SEM and EDX analysis. The synthesized iron oxide nanoparticles were analyzed for its photocatalytic activity, phytotoxicity and antimicrobial activity and also screened for phytochemical anaysis.

Findings of the study

- The characterization of iron ions exposed to leaf extract was indicated by colour change from yellow to black within a second using 2 mM ferrous sulphate with 1 ml of plant extract.
- UV- vis spectrum confirms the reduction of iron ions to iron oxide nanoparticles.
- The biomolecules responsible for the formation of iron oxide nanoparticles are identified using FT - IR spectroscopy
- SEM analysis revealed that the synthesized iron oxide nanoparticles were aggregated as irregular sphere shapes with rough surfaces
- The EDX spectrum showed a strong signal of pure Fe with a weight percentage of 51.56%
- XRD studies revealed that iron oxide nanoparticles obtained are crystalline in nature.
- The size of the biosynthesized iron oxide was 185.6 nm.

- Photocatalytic activity revealed the efficiency of iron oxide nanoparticles as the photocatalyst to degrade methylene blue.
- Phytotoxicity assay confirms the potential of biosynthesized iron oxide nanoparticles as a fertilizer for the growth of green gram seeds.
- Phytochemical screening of biosynthesized iron oxide nanoparticles reveals the presence of proteins, aminoacids, saponins, terpenoids, alkaloids, flavonoids, tannins, glycosides, steroids and polyphenols. These biomolecules proved to be effective stabilizing and capping agent in the reduction of Fe^{+} ions into iron oxide nanoparticles.
- The antimicrobial study of the biosynthesized iron oxide nanoparticles exhibited high potent activity against all tested bacterial and fungal isolates. The biosynthesized iron oxide nanoparticles have high bactericidial activity in Gram negative bacteria than the Gram positive bacteria.

Hence, to concluded that the biosynthesis of iron oxide nanoparticles using *Phyllanthus reticulatus* leaf extract is a cost effective, simple and ecofriendly method that excludes the hazards arising out of the use of harmful reducing/ capping agents. This ecofriendly method could be a competitive alternative to the conventional physical/ chemical methods used for synthesis of iron oxide nanoparticle and thus has a potential to use in biomedical applications and will play an important role in opto – electronics and medical devices in near future. In this regard, use of plant extract for the synthesis of nanoparticles can form an immense impact in coming decades, to combact threatened diseases.

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APPENDIX

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी.एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003
टेलीफोन / Phone: 0422-2432788, 2432123
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2018/Tech./2873 दिनांक/Date: 29th January 2018

सेवा में / To

Ms. Siva Dharshini. R (16PZ0011)
II M. Sc. Zoology
Department of Zoology
Avinashilingam Institute for Home Science & Higher Education for Women
Coimbatore – 641 043

महोदया/Madam,

The plant specimen brought by you for authentication is identified as *Phyllanthus reticulatus* Poir. - PHYLLANTHACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

29/01/2018

(डॉ सी मुरुगन/Dr. C. Murugan)
वैज्ञानिक 'डी' एवं कार्यालय अध्यक्ष /
Scientist 'D' & Head of Office

वैज्ञानिक 'डी' एवंकार्यालयअध्यक्ष
SCIENTIST 'D' & Head of Office
भारतीय दनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयम्बत्तूर / Coimbatore - 641 003.

29/01/2018

APPENDIX II

Antibacterial activity

The antibacterial activity was assessed by agar well diffusion method against the selected microbes.

Agar - well diffusion method

Principle

The antimicrobials present in the synthesized iron oxide nanoparticles are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition was measured in millimeters.

Reagents

1. Muller Hinton Agar Medium (1L)

   The medium was prepared by dissolving 38 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121^0C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25 – 30 ml/plate) while still molten.

2. Nutrient broth (1L)

   One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient broth medium (HiMedia) in 1000 ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes.

3. Chloramphenicol (Standard antibacterial agent / Reference antibiotic)

Procedure

Petriplates containing 20 ml Muller Hinton agar medium was seeded with 24 hr culture of bacterial strains separately. Well were cut and 20 µl of the leaf extract, synthesized iron oxide nanoparticles and chloramphenicol were added separately. The plates were incubated at 37^0C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). The plant extract and chloramphenicol was used as a control.

APPENDIX III

Antifungal activity

Principle

The fungicidal effect of leaf extract can be assessed by the inhibition of mycelial growth of the fungus and was observed as a zone of inhibition near the well.

Reagents

1. Rose Bengal Chloramphenicol Agar medium (1L)

   The 32.15 g of commercially available Rose bengal chloramphenicol agar medium (Himedia) was suspended in 1000 ml of distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 lbs pressure (121^0C) for 15 minutes.
2. Flucanozole (standard antifungal agent

Procedure

Sterile Rose bengal chloramphenicol agar medium was prepared and poured onto the petriplates and swabbed with fungal strains separately. Wells were cut and 20 µl of the leaf extract, synthesized iron oxide nanoparticles and flucanozole were added to each well separately. The plates were incubated at room temperature for 5 days and examined for the presence of zone of inhibition. The diameters of the inhibition zone were measured and recorded (Schlumbaum *et al*., 1986)