



Electrochemical Studies and Antimicrobial Properties of Synthesized Green Mediated Metal Oxide Nanoparticles

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DECLARATION

I, **Ugochi Lydia Ifeanyichukwu**, hereby declare that the dissertation titled **“Electrochemical Characterization and Antimicrobial Properties of Synthesized Green Mediated Metal Oxide Nanoparticles”** submitted for the degree of Masters of Science in Biology (Molecular Microbiology) and the work contained herein is my own work in design and execution and has not previously, in its entirety or part, been submitted to another university for a degree. I further declare that all the materials contained herein have been duly acknowledged.

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DEDICATION

To my Lord and personal saviour Jesus Christ, am grateful for everything sweet baby Jesus.

To my Dad, His Royal Highness, Sir Gilbert “Barzoo” Ifeanyichukwu Ogoegbunam, you live forever in my heart.

Dearest Mummy, Lady Patricia Egoyibo “AsaBarzoo” Ogoegbunam, you are my rock.

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DISSERTATION OUTLINE

This study consists of two major chapters that have been submitted for publication in peer-reviewed international accredited Journals. The chapters contained therein are not projected to be individual articles but describe the research work that has been performed to achieve the aim and objectives of this study.

Chapter one presents the general introduction of the study, aim, objectives and outline of the research.

Chapter two presents the general literature review of the study.

Chapter three outlines an overview of the antimicrobial properties of zinc oxide nanoparticles.

Chapter four presents the phytochemicals that were in pomegranate leaf and flower extracts and also the antimicrobial properties of the plant extracts.

Chapter five outlines the synthesis process and characterization techniques of the synthesized metal oxide nanoparticles.

Chapter six presents data on the antimicrobial efficiency of the synthesized metal oxide nanoparticles.

GENERAL ABSTRACT

Nanoparticles synthesized through alternate biological methods are known to be more biocompatible with no toxic effects. The biological method of synthesis relies on the ability of organic compounds to reduce metal ions and stabilize them into nanoparticles. In this study, a green approach was reported for the fabrication of zinc and iron oxide nanoparticles using extracts from leaf and flower of pomegranate (*Punica granatum*) plant. The obtained extracts were evaluated for phytochemicals that are expected to function as capping, reducing and stabilizing agents during synthesis. Primary phytochemical analysis of the extracts indicated the presence of bioactive components including phenol, tannins, flavonoids and alkaloids. The biologically stable nanoparticles obtained were characterized spectroscopically and electrochemically. X-ray diffraction analysis (XRD) of the particles revealed the elemental components and nature of the synthesized particles. The XRD pattern spectrum obtained indicated a crystalline structure for the zinc oxide nanoparticles and an amorphous nature for the iron oxide nanoparticles. Morphology of the nanoparticles as shown by Scanning electron microscopy (SEM) was unevenly shaped for the ZnO-NPs and evenly spherical for the Fe₃O₄-NPs. The functional groups involved in stabilization, reduction and capping were confirmed using FTIR. Energy Dispersive X-Ray (EDX) analysis of the synthesized nanoparticles revealed compositions the oxides with peaks of zinc and oxygen components for the ZnO-NPs and iron and oxygen for the Fe₃O₄-NPs. Confirmation of the nanoparticles by UV-Vis analysis showed absorption bands of 284 nm and 357 nm for pomegranate leaf and flower extract mediated ZnO-NPs respectively while Fe₃O₄-NPs absorbance was at 308 nm and 310 nm respectively for leaf and flower mediated nanoparticles. The nanoparticles were further characterized electrochemically using a cyclic voltammetry tool to

evaluate their electrochemical properties. The Voltammogram obtained from the electrochemical characterization of the synthesized zinc oxide nanoparticles and iron oxide nanoparticles showed good electrochemical behaviour of the nanoparticles, which indicates that they can be used as biocatalysts. Evaluation of the antimicrobial efficacy of the fabricated nanoparticles showed that ZnO-NPs were effective against all selected pathogenic strains; *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Salmonella diarizonae*, *Salmonella typhi*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Moraxella catarrhalis*, *Aeromonas hydrophilia*, and *Listeria monocytogenes* used in the analysis. Iron oxide nanoparticles also showed activity against the pathogenic strains with the exception of *Klebsiella pneumoniae* in the case of iron oxide synthesized via pomegranate flower (Fe₃O₄-NPs -PF). The effectiveness of the nanoparticles could be linked to their sizes and shapes as obtained using transmission electron microscopy. Our reports also showed that an increase in concentration of the nanoparticles from 50 µg/ml to 5000 µg/ml led to an increase in the antibacterial effect exerted by the nanoparticles (4.33 mm to 23mm), and this suggests that both ZnO-NPs and Fe₃O₄-NPs can effectively be employed as an alternative to conventional antibiotics. However, further research is required to understand the mechanism of action and the possibility of any dangerous effects of their use.

Keywords: Antimicrobial activity, Electrochemical Characterisation, Spectroscopic Characterization, Iron oxide nanoparticle, Zinc oxide nanoparticle.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL NAME
AE	Auxiliary Electrode
AMR	Antimicrobial resistance
ATCC	American type culture collection
CFU	Colony forming unit
CV	Cyclic voltammetry
DMF	N-N-dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDX	Energy dispersive x-ray
EIS	Electrochemical impedance spectroscopy
Fe₃O₄	Iron oxide
Fe₃O₄-NPs	Iron oxide nanoparticle
FTIR	Fourier-transform infrared spectroscopy
MeO-NPs	Metal oxide nanoparticle
MHA	Muller Hinton agar
MIC	Minimum inhibitory concentration
NDM⁻¹	New Delhi metallo-β-lactamase 1
NPs	Nanoparticle
RE	Reference elcetrode
ROS	Reactive Oxygen Specie
SEM	Scanning electron microscope

TEM	Transmission electron microscope
TSB	Tryptic soy broth
WE	Working electrode
XRD	X-ray diffraction
ZnO	Zinc oxide
ZnO-NPs	Zinc oxide nanoparticle
Fe₃O₄-NPs-PL	Iron oxide nanoparticle from pomegranate leaf
Fe₃O₄-NPs-PF	Iron oxide nanoparticle from pomegranate flower
ZnO-NPs-PL	Zinc oxide nanoparticle from pomegranate leaf
ZnO-NPs-PF	Zinc oxide nanoparticle from pomegranate flower

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CHAPTER ONE

INTRODUCTION AND PROBLEM STATEMENT

1.1 General introduction

Electrochemical studies of properties of synthesized nanoparticles is of great interest to researchers today. Due to the versatility of nanomaterials, study of their electrochemical properties would allow incorporation of the unique properties to different applications such as electrochemical sensors, bioanalytical devices, nanosensors and biosensors (Malik et al., 2013, O' Riordan & Barry, 2016). The electrochemical properties of nanoparticles are evaluated using varying dynamic electrochemical techniques. Recently, electrochemical studies have been carried out in different fields of application such as analysis of pharmaceutical, environmental, industrial, and biological samples (Bakirhan et al., 2017). Data obtained from electrochemical studies of a sample most often show a relationship to its molecular structure and biological activity (Grieshaber et al., 2008). The physiochemical properties of these materials also allows its effective application in degradation of bacterial species.

Infectious diseases are mainly caused by microorganisms, especially nosocomial bacteria (Hemeg 2017, Wang *et al.*, 2017). These bacterial infections spur health challenges and mortality globally (Raghunath & Perumal 2017, Baptista *et al.*, 2018, Singh *et al.*, 2018). Concurrently, the emergence and re-emergence of old infectious diseases coupled with the emergence of multidrug and antibiotic resistant bacterial strains has dampened the primary health care system globally (Dizaj *et al.*, 2014, Vega-Jiménez *et al.*, 2019). The standard means of treatment of bacterial infectious diseases is by the use of antibiotics (Hemeng, 2017, Raghunath & Perumal, 2017,

Wang *et al.*, 2017, Singh *et al.*, 2018). However, the indiscriminate and unmonitored use of these antibiotics has permitted the generation of bacterial strains that are resistant to this form of treatment (Karaman *et al.*, 2017, Vega-Jiménez *et al.*, 2019). In addition, biofilm formation by the infectious bacteria impedes antibiotic treatment (Bjarnsholt 2013, Wu *et al.*, 2014, Karaman *et al.*, 2017).

Despite the wide range of effective antibiotics available worldwide, bacterial infections are on the rise (Beyth *et al.*, 2015, Wang *et al.*, 2017). The efficacy of major antibiotics lies in their ability to target the bacterial cell wall synthesis, translational machinery and DNA replication machinery (Wang *et al.*, 2017, Gold *et al.*, 2018). The production of antibiotic inactivating enzymes (such as beta lactamase or aminoglycosides), alteration of bacterial structure or cell components (such as the cell wall in vancomycin resistance and ribosomes in tetracycline resistance) and the action of efflux pumps that expel antibiotics are major ways through which bacteria can alter or lessen antibiotic potency (Knetsch *et al.*, 2011, Munita & Arias, 2016, Wang *et al.*, 2017, Gold *et al.*, 2018, Peterson & Kaur, 2018). The situation is worsened due to the resistance against a broad range of beta lactam antibiotics including the carbapenems conferred on most bacteria by the newly discovered New Delhi metallo- β -lactamase 1 (NDM¹) gene (Karaman *et al.*, 2017, Wang *et al.*, 2017, Shaikh *et al.*, 2019, Kumarasamy *et al.*, 2010).

The use of antimicrobial peptides, bacteriocins, flavonoids, competitive exclusions, and phage therapy have been proposed as alternate means of combating bacterial infections (Joerger 2003, Cotter *et al.*, 2012, Sharma *et al.*, 2018). However, owing to the threat of increased multi drug resistance and biofilm forming pathogens, there is an urgent need to seek for long term active, antimicrobial compounds with a new systematic outlook (Hemeng, 2017, Escárcega-González *et al.*, 2018, Gold *et al.*,

2018). An emerging strategy offering a new approach to the menace caused by antibiotic-resistant microbes involves the use of nanomaterials, which is a rapidly developing branch in science and technology (Salem *et al.*, 2015, Hannefa *et al.*, 2017).

Nanoparticles are particles that have well defined sizes and shapes, with varying unique properties, which make them suitable therapeutic agents (Wang *et al.*, 2013, Ealias & Saravanakumar, 2017, Hannefa *et al.*, 2017, Bejarano *et al.*, 2018, Jeevanandam *et al.*, 2018) as they are inert and non-toxic (Ibrahim *et al.*, 2017, Baptista *et al.*, 2018). Studies have shown that these particles have demonstrated broad-spectrum antibacterial properties against gram positive and gram negative microorganisms (Wang *et al.*, 2017, Baptista *et al.*, 2018, Escárcega-González *et al.*, 2018). Their ability to interact with microbes and exert antimicrobial effectiveness is linked to their well-defined size (Dizaj *et al.*, 2014, Khezerlou *et al.*, 2018), shape, roughness, zeta potential and large surface area to volume ratio (Hemeng, 2017, Gold *et al.*, 2018, Escárcega-González *et al.*, 2018). A nanoparticle is believed to act directly with the bacterial cell wall without having to penetrate the cell (Wang *et al.*, 2017, Fernando *et al.*, 2018). This suggests that nanoparticles can reduce the incidence of antimicrobial resistance (Wang *et al.*, 2017, Fernando *et al.*, 2018).

Over the centuries, the antimicrobial properties of metals have been exploited (Dizaj *et al.*, 2014, Karaman *et al.*, 2017, Singh *et al.*, 2018, Fernando *et al.*, 2018). They exert their antimicrobial effects by the generation of reactive oxygen species, (Agarwal *et al.*, 2018, Akbar *et al.*, 2018) disruption of membrane (Karaman *et al.*, 2017) and damage of cellular components (Sirelkhatim *et al.*, 2015, Singh *et al.*, 2018, Baptista *et al.*, 2018). Metal oxides are also applicable environmentally where they act as catalysts that quicken the reduction or removal of chemicals that are toxic and can

cause environmental hazards (Santhoshkumar *et al.*, 2017, Védrine, 2017). The conversion of bulk metal to nano-sized metal enhances it, for example, solubility of zinc oxide increases with decrease in size (Raghupathi *et al.*, 2011, Singh *et al.*, 2018, Khezerlou *et al.*, 2018). Due to its stability and high antimicrobial activity, metal and inorganic metal oxide-based nanoparticles are receiving more attention (Salem *et al.*, 2015, Vega-Jiménez *et al.*, 2019). The activities of different metal oxides nanoparticles have been investigated (Basnet *et al.*, 2018).

Zinc oxide serves as a key component in catalysis, drug delivery, microelectronics, optoelectronic devices, antioxidant and antimicrobial activities (Khan *et al.*, 2016, Jiang *et al.*, 2018). Zinc oxide nanoparticles have effectively been used in paint production, manufacturing of cosmetics, plastics, rubber, skin products including baby powder, antidandruff shampoos and antiseptic ointments (Sharma *et al.*, 2010, Kołodziejczak-Radzimska & Jesionowski 2014). As an antibacterial agent, Roselli *et al.* (2003) reported that zinc oxide can inhibit the adhesion and internalization of enterotoxigenic *Escherichia coli* (ETEC) into the enterocytes. Almoudi *et al.* (2018) recounted that even at a lower concentration, zinc exhibited an effective antibacterial activity against the growth of *Streptococcus mutans*. Brayner *et al.* (2006) reported that zinc oxide nanoparticles reduce the ability of microbes to be viable or attach on medical surfaces. Zinc oxide nanoparticles target prokaryotic cells (Gunalan *et al.*, 2012, Hsueh *et al.*, 2015) and their anti-cancer activity has also been reported (Selvakumari *et al.*, 2015, Jiang *et al.*, 2018, Sivakumar *et al.*, 2018). Addition of plant extract during metal oxide nanoparticle synthesis allows production of nanomaterials with surface free of hazardous material, which enable their application in medicine (Pirtarighat *et al.*, 2019).

The use of plant extracts in the biosynthesis of green nanoparticles is simple, rapid, and has more economic and eco-friendly benefits over the chemical and physical methods whereby different plant parts have been used (Suzan *et al.*, 2014, Das *et al.*, 2017, Escárcega-González *et al.*, 2018, Singh *et al.*, 2018). Plant extracts are rich in phytochemicals, which act as reducing, capping and stabilizing agents (Suresh *et al.*, 2018, Rajeshkuma *et al.*, 2018). Synthesis of metal oxide nanoparticles using plants has been reported (Rajakumar *et al.*, 2017, Vijayakumar *et al.*, 2018).

The pomegranate plant (*Punica granatum L.*) originates from central Asia, but is widely distributed geographically (Shaygannia *et al.*, 2015). It has nutritional, medicinal and ornamental properties (Teixeira da Silva *et al.*, 2013, Shaygannia *et al.*, 2015). The fruits contain a number of medically active phytochemicals including tannins, flavonoids, alkaloids, organic acids, triterpenes and steroids (Ismail *et al.*, 2012, Redha *et al.*, 2018) that confer hypolipidemic, antioxidant, antiviral, anti-neoplastic, anticancer, antibacterial, anti-diabetic, anti-diarrheal, helminthic, vascular and digestive protection, as well as immunomodulatory effects (Wang *et al.*, 2010, Ismail *et al.*, 2012, Teixeira da Silva *et al.*, 2013). It is used as a herbal cure for cancer, diarrhoea, diabetes, blood pressure, leprosy, dysentery, haemorrhages, bronchitis, dyspepsia and inflammation. (Wang *et al.*, 2013, Teixeira da Silva *et al.*, 2013). The pomegranate industry is a fast growing industry in South Africa, with cultivation starting in the early 2000s. Pomegranate farming is done mostly in the Western Cape and Northern Cape Province of the country.

The present work is designed to investigate and evaluate the electrochemical effect and antimicrobial properties of zinc oxide and iron oxide nanoparticles synthesized from fruit and leaf extract of pomegranate (*Punica granatum L.*)

1.2 Problem statement

A challenging factor in the health system globally is the constant outbreak and re-emergence of infectious disease. These infections are caused by varying pathogens, most of which are resistant to traditional and conventional methods of treatment using antibiotics. Therefore, scientists are researching for a more effective and reliable antimicrobial agent to curb the menace caused by antibiotic resistant organisms. Recently, antimicrobial nanoparticles and nanodrug carriers for antibiotic delivery have been explored in infectious disease treatment. Metal oxide and metal nanostructures have been reported to possess antimicrobial properties that can be utilized in the control of infectious diseases. The application of nanoparticles, however, reaches far more than biomedical applications. Different methods can be used for the synthesis of nanoparticles including chemical reduction, photochemical reactions, electrochemical techniques and the green chemistry route. The conventional physical and chemical route of synthesis is gradually being substituted by the biological technique of synthesis. This is because, in the green synthesis, the technique is less expensive, with minimal risk of secondary pollution as no toxic chemical is required as a capping or reducing agent (Moritz & Geszke-Moritz, 2013) Natural extracts from plants are readily available and the process of their use for nanoparticle synthesis is cheap, biocompatible, and environmentally friendly, and does not require the use of heavy machinery or expending energy. The green route also proves to be less time consuming (Jiang et al., 2018).

1.3 Aim and objective

The aim of the study was to synthesize nanoparticles of zinc oxide and iron oxide using the alternative green technique. The synthesized nanoparticles were characterized spectroscopically and electrochemically. Also, the antibacterial potential of the synthesized *Punica granatum* leaf and flower mediated zinc oxide and iron oxide nanoparticles were evaluated.

1.4 Objectives

The aim of this study was achieved under the following objectives:

- synthesize metal oxide nanoparticles of zinc and iron using pomegranate leaf and flower extracts.
- screen for the phytochemical constituents of the crude plant extract.
- characterize the synthesized nanoparticles to check for the electron transfer properties.
- evaluate the antimicrobial properties of synthesized nanoparticles using varying techniques.
- evaluate the effect of electrochemical studies on the antimicrobial properties

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CHAPTER TWO

GENERAL LITERATURE REVIEW

2.1 Nanotechnology and its applications

An important and currently expanding area of research in science and technology is the field of nanotechnology (Wong *et al.*, 2006, Ali *et al.*, 2016). Nanotechnology is a science that manipulates and exploits very small particles (nanomaterial) at the level of atoms and molecules to create new large-scale materials that possess a new or improved property (Adams & Barbante, 2013, Nikalje, 2015, Schmidt & Storsberg, 2015, Ahmed *et al.*, 2016, Vijayaraghavan & Ashokkumar, 2017). The study of this occurrence is termed nanoscience (Sahoo *et al.*, 2007, Sorbiun *et al.*, 2018). Nanotechnology is a currently developing field with considerable benefits (Sahoo *et al.*, 2007, Mohammad *et al.*, 2017). It allows the creation of high valued products with magnified characteristics and applicable function (Logothetidis, 2012, Abiodun-Solanke *et al.*, 2014, Alkandari, *et al.*, 2017).

Nanotechnology can be employed in providing solutions to global health, climatic and energy challenges (Diallo *et al.*, 2013, Mohammad *et al.*, 2017, Gao *et al.*, 2018). It offers a high prospect in science and technology, allowing the conversion of fundamental research into successful innovations (Dizaj *et al.*, 2014, Singh *et al.*, 2017, Umar *et al.*, 2018). The field of nanotechnology enables the formation of nanomaterials such as nanoparticles, carbon nanotubes, fullerenes, quantum dots, quantum wires, nanofibers, and nanocomposites (Mohamed, 2017, Baptista *et al.*, 2018). This nanomaterial formed usually has unique properties that allow for its diverse applications (Sahoo *et al.*, 2007, Logothetidis, 2012, Vijayaraghavan & Ashokkumar 2017, Akbar *et al.*, 2018). Products that contain this engineered

nanomaterial are already in the public domain. Such products include metals, ceramics, polymers, smart textiles, cosmetics, sunscreens, electronics, paints and varnishes (Vijaya Shanti *et al.*, 2011, Logothetidis, 2012).

Historically, the first discussion on nanotechnology is attributed to the speech delivered at Caltech in the year 1959 (published 1960) by an American physicist Richard Feynman (Sahoo *et al.*, 2007, Bhatia, 2016). In the speech titled “There's Plenty of Room at the Bottom”, he talked about manipulation and controlling things on a small scale. He also described scaling down of letters to the size that would allow the whole *Encyclopaedia Britannica* to fit on the head of a pin (Tolochko, 2009). The Japanese scientist Norio Taniguchi however first used the term nanotechnology in the year 1974, in a publication titled “production technology that creates objects and features on the order of a nanometre” (Abiodun-Solanke *et al.*, 2014, Alkandari, *et al.*, 2017, Das *et al.*, 2017). Professor Kevie E. Drexler, an American engineer, also proposed the use of the term “nanotechnology” which could be used interchangeably with “molecular technology” in his 1986 book titled “Engines of Creation: the coming era of Nanotechnology” (Tolochko, 2009, Das *et al.*, 2017).

2.2 Applications of nanotechnology

Nanotechnology has applications in several scientific and research fields, and aims to improve existing ideas and also to develop new ones (Logothetidis, 2012, Qu *et al.*, 2012, Schmidt & Storsberg, 2015). The platform strives to provide solutions to most challenges faced by humans ranging from health care, energy, and agriculture to the least basic human need (Vijaya Shanti *et al.*, 2011). Over the years, various impactful advancements have been made in the nanotechnology field, and its application extends to electronics, materials, environment, robotics, healthcare, information

technology, pharmaceuticals, agriculture, transport etc. (Vijaya Shanti *et al.*, 2011).

Figure 1 represents some applications of nanotechnology.

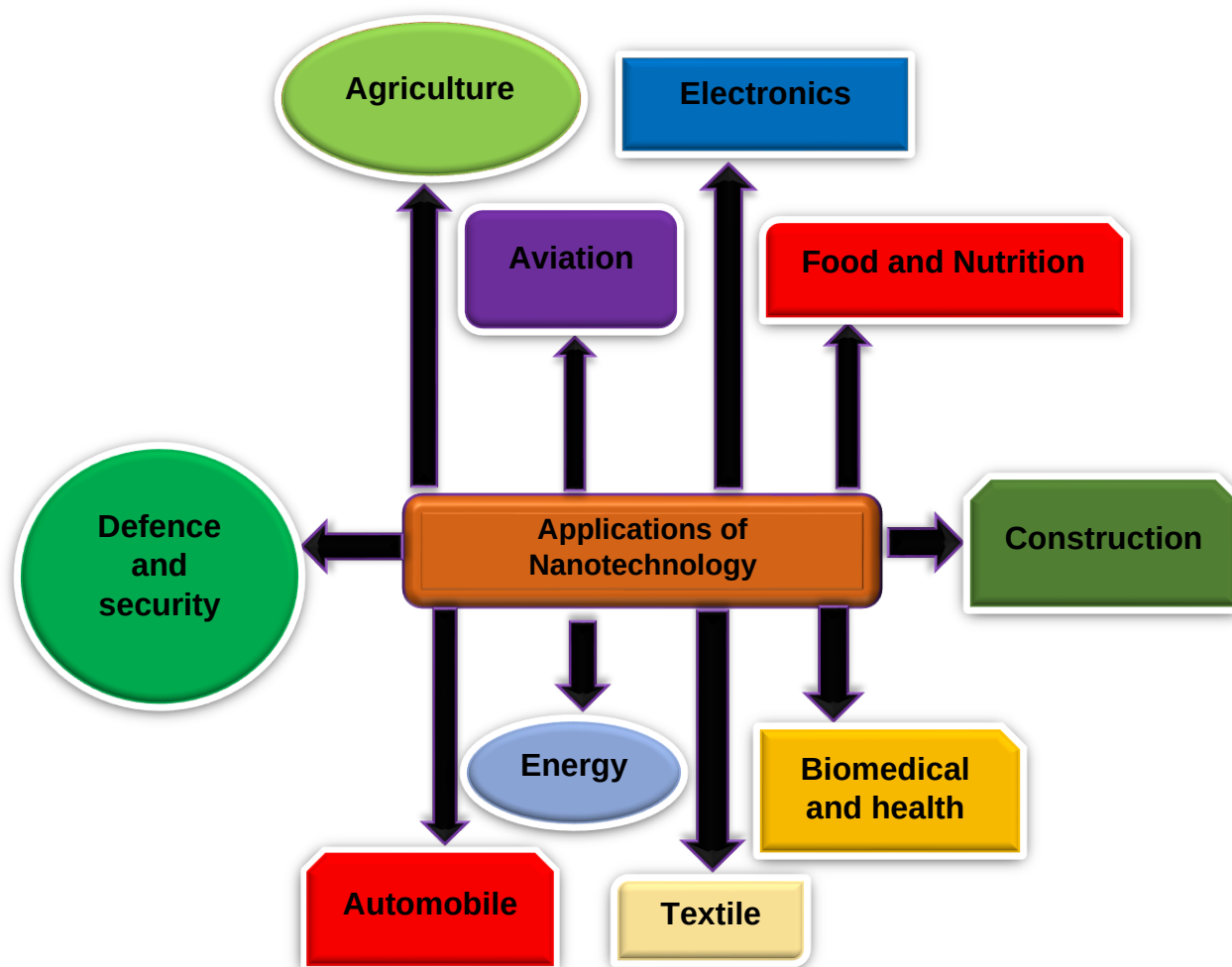


Figure 2.1: Some applications of nanotechnology

2.2.1 Application of nanotechnology in medicine

Diseases such as diabetes, Parkinson's disease, cancer, and other severe inflammatory or infectious diseases like tuberculosis and HIV are a major challenge in the world health care system (Nikalje, 2015). Nanotechnological advancement in the

medical (nanomedicine) and pharmaceutical world is progressing rapidly (Sahoo *et al.*, 2007). Workable applications of Nanomedicine involve the use of nanomaterials and nanoelectronic biosensors to aid diagnosis, monitoring, early detection, and prevention of diseases (Nikalje, 2015, Das *et al.*, 2017, Mohammad *et al.*, 2017). Nano devices such as gold nanoparticles, when applied in the area of gene sequencing, where the gold nanoparticle is tagged with short segments of DNA, effectively detects the genetic sequence in a sample (Nikalje, 2015). The benefits of nanotechnology in the field of medicine and healthcare cannot be overemphasized.

In pharmaceutical companies, nanotechnology is used in the manufacture of new and innovative drugs and drug products, and as a means of providing measures to the pharmacokinetic and pharmacodynamics of drugs produced (Bhatia, 2016, Soares *et al.*, 2018). In dentistry, nanomaterials and biotechnologies like tissue engineering and nanorobots are deployed in the diagnosis and management of dental associated malady (Abiodun-Solanke *et al.*, 2014, Mirsasaani *et al.*, 2019). Nanotechnology has been applied in other areas of health care such as in drug delivery, where nanoparticles are used as carriers to deliver therapeutic drugs to desired sites in time without causing harm (Vijaya Shanti *et al.*, 2011, Patel *et al.*, 2015, Bhatia, 2016), magnetic hyperthermia for cancer treatment (Giustini *et al.*, 2010, Chang *et al.*, 2018), Magnetic Resonance Imaging (MRI) contrast agent, magnetic separation, controlled drug release, cellular therapy, tissue engineering, and antibiotic resistance (Gupta & Gupta, 2005, Nikalje, 2015, Naseri *et al.*, 2017).

2.2.2 Application of nanotechnology in agriculture

Nano-based compounds like nanofertilizers, nanofungicides, and nanopesticides, which improve crop yield, reduce the amount of sprayed chemicals, and minimize nutrient losses in fertilization, are important applications of nanotechnology in the agricultural sector (He *et al.*, 2018, Marchiol, 2018). Nanotechnology can also be applied in biosecurity and health assurance of agricultural products (Singh *et al.*, 2015), formulation of new methods to identify pathogenic agents causing crop and livestock diseases, and production of drug delivery systems for the treatment of such diseases (Mukherjee *et al.*, 2015, Prasad *et al.*, 2017). Nano-capsules and nano-emulsions are used as smart delivery systems for disease and pest control (Mukherjee *et al.*, 2015). NPs are also used as a seed treatment for various plants prior to seed sowing (Aslaniet *et al.*, 2014; Srivastava, 2014). Other applications of nanotechnology in agriculture include monitoring of soil quality using nanosensors (Prasad *et al.*, 2017), water management, post-harvest technology, as well as analysis of gene expression and regulation (Abobatta, 2018).

2.2.3 Application of nanotechnology in the food industry

The application of nanotechnology in the field of food science and food Microbiology is aimed at providing safe and quality food for consumers (Singh *et al.*, 2017, Nasrollahzadeh, *et al.*, 2019). Nanotechnology is being applied in food processing, food packaging, functional food development, food safety, detection of foodborne pathogens, and shelf-life extension of food and/or food products (Prasad *et al.*, 2017, Singh *et al.*, 2017). In food processing, nutrients, supplements, nanosized organic additives and animal feed are delivered to specific action sites using nanocarrier systems (Umami & Siddiquee, 2018, Nasrollahzadeh *et al.*, 2019). Polymer

nanocomposites as well as inorganic nanoparticles are employed in food packaging due to their antimicrobial activities (Singh *et al.*, 2017, Hernández-Muñoz *et al.*, 2019). Nanosensors are employed in the detection of pathogenic contaminants in food. Nanotechnology is also applied in food security, manufacturing, processing, and shipping of food products (Umami & Siddiquee, 2018, Nasrollahzadeh *et al.*, 2019).

2.2.4 Application of nanotechnology in the environment

Nanoparticles can also be applied in environmental remediation (nanobioremediation), pollution prevention (detection, monitoring, and remediation), and wastewater treatment (permeable reactive barriers, membrane filtration, adsorption) (Ibrahim *et al.*, 2016, Cecchin *et al.*, 2017, Nasrollahzadeh *et al.*, 2019). Due to their absorption properties, nanoscale metal oxides such as zinc oxide and titanium dioxide, especially for their photocatalytic property, are deployed as nanoabsorbents for the removal of organic compounds during water treatment (Qu *et al.*, 2012, Ibrahim *et al.*, 2016, Mohamed, 2017). Carbon nanotubes are deployed for the removal of organic pollutants in the environment and heavy metals from water due to their accessible adsorption sites (Ibrahim *et al.*, 2016, Ealias & Saravanakumar, 2017). Nanosensors can aid in the detection of contamination in water, measure air and water quality, and detect toxic gas leaks in the environment (Dahman, 2017, Mohamed, 2017).

2.2.5 Application of nanotechnology in energy

The worldwide energy demand is continuously growing as it is required in various spheres of life and applied in different fields (Christian, 2013). Nanotechnology aims to solve problems of low and ineffective energy supply (Deshmukh & Katariya, 2013). In the energy field, nanoparticles are therefore used to improve the efficiency of fuel production and consumption (Serrano *et al.*, 2009, Iavicoli *et al.*, 2014). They are

employed in nanobioengineering of enzymes, thermoelectric materials, prototype solar panels, batteries, and aerogels. In addition, they are important in the conversion of waste heat in computers, automobiles, homes, power plants, etc. to usable electrical power (Ali *et al.*, 2016). The generation of electricity from solar, wind and geothermal sources can be facilitated by nanotechnology (Deshmukh & Katariya, 2013). For a more effective optical path, and ability to control energy band gap, nanoscale materials are included in the photovoltaic cell devices used in producing electricity from the sun (Serrano *et al.*, 2009). Nanotechnology plays a role in the storage and distribution of energy. Owing to their good electrical conductivity and high surface area, carbon nanotube based electrodes in batteries are used to generate electricity (Deshmukh & Katariya, 2013).

2.2.6 Application of nanotechnology in aviation and defence

Nanotechnology also cuts across the defence and aviation fields where they are applied in nano-composites, nano-coating, sensors and electronics, fuel additives, energy devices, and smart materials (Matsui, 2005). Researchers are carrying out different studies to inculcate nano based components into advanced missile, aviation, and autonomous air and ground systems (Ruffin *et al.*, 2011). Nano based devices, components and materials that have been integrated in defence artillery, promise a cutting-edge advancement in the military arena (Kurahatti *et al.*, 2010). Conventional constituents are being replaced by nanocomposites, due to their ability to withstand hostile conditions, which makes them useful in aviation for the production of complex gears (Gopi *et al.*, 2017, Joshi *et al.*, 2016).

2.2.7 Application of nanotechnology in construction

In construction, iron oxide pigments are used in colouring concrete, bricks, tiles, concrete interlocking blocks (CIB) and other construction materials for high colour strength (Lee *et al.*, 2003). Mechanical properties, structural efficiency, durability, strength and stability of the concrete are enhanced by the addition of nanosilica (Hanus *et al.*, 2013, Papadaki *et al.*, 2017). Stronger concretes and steels are made with the addition of nanoparticles, and carbon nanotubes can replace steel in construction (Ealias & Saravanakuma, 2017, Papadaki *et al.*, 2017). Nanoparticles are applied to coat glazing due to their sterile and anti-fouling properties, and the ability to break down volatile organic compounds and pollutants (Rana *et al.*, 2009). The addition of nanoparticles to paint makes it corrosion resistant and antimicrobial, thereby resisting the formation of moulds on walls (Ealias & Saravanakuma, 2017). Nanoparticles with antimicrobial and antifungal properties, like the silver nanoparticle, zinc oxide and magnesium oxide nanoparticles, are added to paint to protect its beauty by preventing growth of microbes (Hanus *et al.*, 2013).

2.2.8 Application of nanotechnology in automotive and electronics

In the automotive industry, nanoparticles are used as additives in catalysts and lubricants, nanocoatings, fuel cells, composite fillers, and smart materials (Ali *et al.*, 2016). On the other hand, in the electronic industry, they function in printed electronics, carbon nanotubes, nanoscale memory, nanowires, and quantum dots (Matsui, 2005).

2.2.9 Application of nanotechnology in textile

The application of nanotechnology in the textile industry has resulted in increased sturdiness, coziness, and sanitary properties, as well as reduced costs of textile

production (Patra & Gouda, 2013). Application of nanotechnology in textiles can be seen in sensors, nanofibers, coatings, and smart materials (Ali *et al.*, 2016). Due to their large surface area to volume ratio, nanoparticles confer high affinity to textiles, leading to the production of fabric with high functionality, which is easy to feel and durable (Wong *et al.*, 2006). Nanoparticles with antimicrobial properties are integrated in textile manufacturing to avoid the growth of bacteria and fungi that cause odour and itching (Patra & Gouda, 2013). Zinc oxide nanoparticles are used in textile production for their antibacterial and UV blocking properties (Singh *et al.*, 2016). Coating in textile production involves the use of nanoparticles, a surfactant and a carrier medium that modifies the surface of the textile (Crammer *et al.*, 2003). The benefit of the use of nanotechnology in textiles includes water repellence, soil resistance, wrinkle resistance, anti-bacterial and flame retardation properties, anti-static and UV-protection as well as improvement of dyeability (Wong *et al.*, 2006). Figure 2 represents the application of nanotechnology in the textile industry.

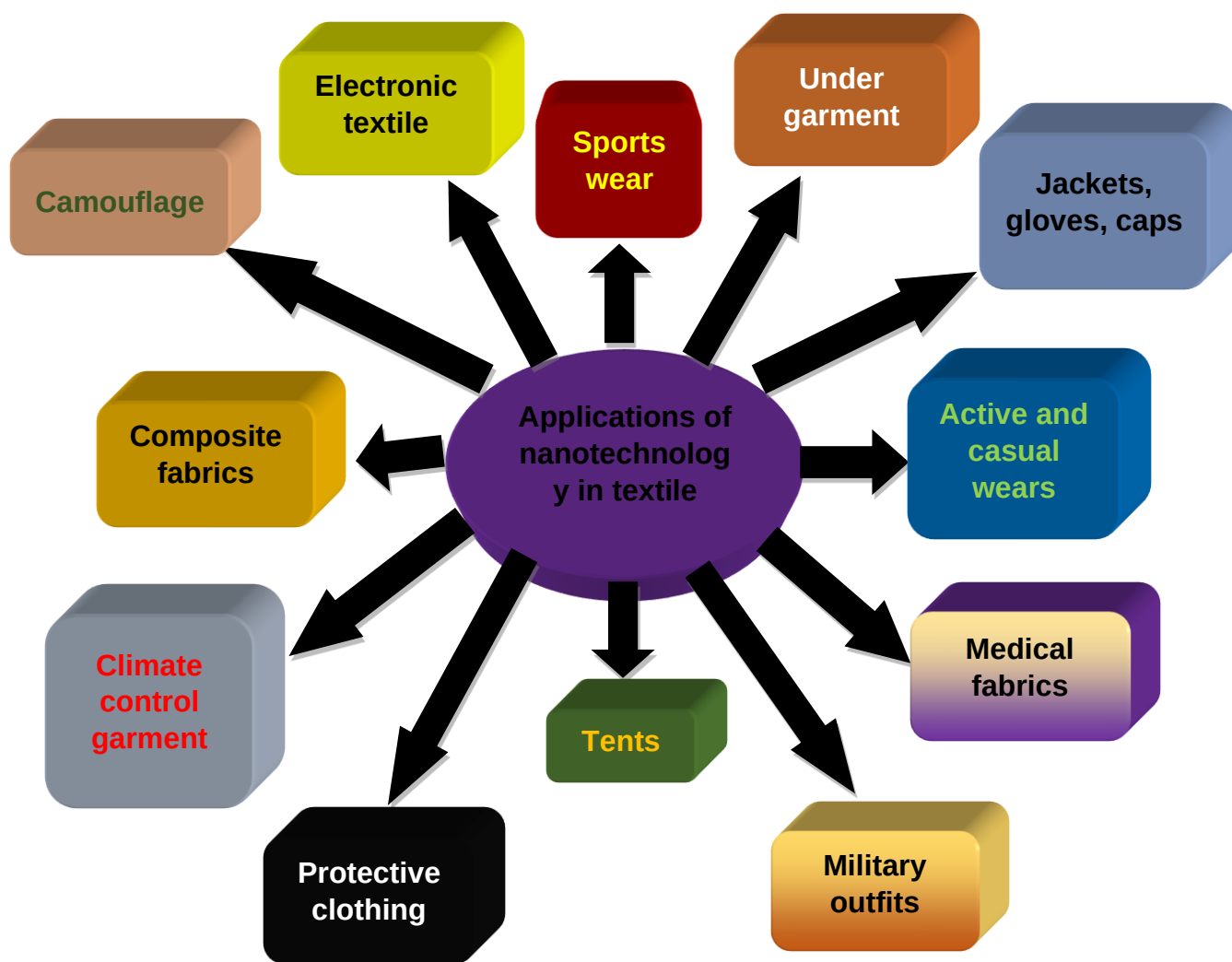


Figure 2.2: Representation of nanotechnological applications in textile

2.3 Nanoparticles

Nanoparticles are basically particles whose diameter are in the range of 1 to 100 nanometres (Nikalje, 2015, Khan *et al.*, 2017, Ibrahim *et al.*, 2017, Fernando *et al.*, 2018), that exhibit size dependent characteristics that are usually different from the same larger compound (Mohamed, 2017, Jeevanandam *et al.*, 2018). The Nobel Prize winner P. Ehrlich proposed nanoparticles in 1906 as “magic bullets” with unique properties having various application potentials ((Himmelweit, 1960, Khandel & Shahi, 2016, Basnet *et al.*, 2018). They are composed of either carbon, metal, metal oxide or

organic matter (Hassan, 2015, Ealias & Saravankumar, 2017). They are of various sizes, shapes and dimensions (Ealias and Saravanakumar, 2017, Wang & Wang, 2014) and can have either uniform or irregular surfaces (Ealias and Saravanakumar, 2017).

A number of nanoparticles assume a crystalline or unstructured form with distinct or multi crystal solids that are loose or agglomerated (Ealias & Saravanakumar, 2017, Sharma *et al.*, 2017). The particle comprises of a surface layer that functions with different small molecules, metal ions, surfactants and polymers, a shell layer that is totally different from the core chemically, and a core (most vital) which is the nanoparticle itself (Christian *et al.*, 2008, Shin *et al.*, 2016, Swamy *et al.*, 2018). They are chemically more reactive than their bulk counterparts, due to their large surface area to volume ratio (Dobrucka & Długaszewska, 2016, Mohamed, 2017). Their unique properties influence their behaviour (Khan *et al.*, 2017). Due to their size distribution, small size and shape, nanoparticles exhibit characteristic physicochemical, optical and biological properties which enable an exciting and new approach in studying their applications (Wang & Wang, 2014, Fawcett *et al.*, 2017, Anwar *et al.*, 2018, Sorbium *et al.*, 2018).

The classification of nanoparticles into groups such as fullerenes, metal nanoparticle, ceramic nanoparticle, and polymeric nanoparticle is due to their varying properties (Khan *et al.*, 2017, Sharma *et al.*, 2017). They are currently receiving great attention due to their impact in improving many sectors of the economy, including consumer products, pharmaceuticals, cosmetics, transportation, energy and agriculture. They are therefore increasingly produced for a wide range of new applications within different industries (Novack & Bucheli, 2007, Ahmed *et al.*, 2016). A very interesting application

of nanoparticles in the scope of life sciences is their use as “smart” delivery systems (González-Melendi *et al.*, 2007, Patra *et al.*, 2018).

In the field of biology, the application of nanoparticle serves in imaging of biological processes at the cellular level (Mendes, 2013, Wang and Wang, 2014). Nanoparticles have also shown strong resistant activities against most microorganisms (Agarwal *et al.*, 2017, Singh *et al.*, 2018). Another important application of nanoparticles is in biosensors (Hills & Mirkins, 2006, Singh *et al.*, 2016) to detect and separate pathogens and capture or select specific cells that are found at low frequency (Wang and Wang, 2014). They have been employed in the detection and capturing of tumour cells (Miller *et al.*, 2010, Huang *et al.*, 2018). In nanochemistry, nanoparticles aid immobilization and enhancement of catalyst (Cipolatti *et al.*, 2016). In recent times, industries like the food, health, cosmetic, textile and manufacturing industries all incorporate nanoparticles into their products (Fouda *et al.*, 2018).

2.4 Properties of nanoparticles

Nanoparticles possess physical properties that are dissimilar from their bulk counterparts (Ealias & Saravanakumar, 2017, Javeendam *et al.*, 2017, Sorbium *et al.*, 2018). Nanoparticles are chemically reactive, have large surface area, are optically active and exhibit strong mechanical properties (Khan *et al.*, 2017). These special physiochemical properties, which are mainly due to their nanoscale features such as high surface area to volume ratio, allow for the application of nanoparticles in various fields (Ealias & Saravanakumar, 2017, Mohamed, 2017, Pirtarighat *et al.*, 2018).

2.4.1 Optical properties

Optical properties of nanomaterials such as reflection, transmission, absorption and emission of light are unlike that of bulk material (Patra *et al.*, 2012, Ross *et al.*, 2016, Jeevanandam *et al.*, 2018) and they depend on the size, shape, chemical composition and internal electronic structures of the nanomaterial (Chouhan, 2018, Zhang *et al.*, 2018). At nanoscale, electrons do not move freely, and as such, they react differently to light (Chouhan, 2018). The excitation of surface plasmons in metallic nanoparticles brings about the unique optical properties of metal nanoparticles (Garcia, 2011, Guo & Barnard, 2013, Anwar *et al.*, 2018). This excitation band results when the incident photon frequency is constant with the collective excitation of the conduction electrons, and is known as the localized surface plasma resonance (LSPR) (Olson *et al.*, 2015, Khan *et al.*, 2017). The optical properties of nanoparticles can be exploited, making them useful in applications including solar cells, imaging, sensing and constructing nanostructures (Patra *et al.*, 2012, Ross *et al.*, 2016).

2.4.2 Electrical properties

The high surface area, ability to absorb different bioactive molecules and excellent electrical conductivity make nanoparticles good materials for creating electrochemical biosensors (Llobet, 2013, Lee *et al.*, 2019). The electrical property of some nanoparticles such as graphene makes it highly sensitive to foreign molecules (Lee *et al.*, 2019). Carbon nanotubes are sensitive to their chemical environment due to their electrical properties (Llobet, 2013). Electrical properties are size dependent (Makhlouf *et al.*, 2009), and support applications in nanoelectronics (Khan *et al.*, 2017).

2.4.3 Mechanical properties

Nanoparticles have mechanical properties that allow for exciting applications in different fields (Guo & Barnard, 2013). Mechanical properties include hardness, elastic modulus, friction, interfacial adhesion, tensile and compressive behaviour (Guo & Barnard, 2013, Hasan, 2016). The structural perfection of a nanomaterial enhances its mechanical properties (Hasan, 2016). In orthopaedic surgery, the long term stability of prostheses is influenced by the mechanical properties of acrylic (Shi *et al.*, 2006).

2.4.4 Thermal properties

Thermal properties of nanomaterials are useful for their various applications (Khan *et al.*, 2017). Studies have shown that there is a difference between the thermal property of nanostructure and that of its bulk counterpart (Zhang & Li, 2010). Thermal properties can be impacted by doping (Shaikh *et al.*, 2007).

2.4.5 Magnetic properties

The unique magnetic properties of nanomaterials differ from that of the bulk materials and allow for the exciting technological applications such as magnetic recording, biological sensors, and contrast agents (Jeon *et al.*, 2006, Mørup *et al.*, 2017). The magnetic properties depend on the material's electron configuration, which is strongly related to the geometrical structure, and on temperature (Tamuliene *et al.*, 2012).

2.5 Classification of nanoparticles

Nanoparticles can be classified based on their origin (Hochella *et al.*, 2015, Jeevanandam *et al.*, 2018), dimension (Tiwari *et al.*, 2012), and composition, or other

characteristics such as physical and chemical characteristics (Strambeanu *et al.*, 2014).

2.5.1 Classification based on origin

Based on Origin or source, nanoparticles can either be of natural origin or synthesized (Hochella *et al.*, 2015, Jeevanandam *et al.*, 2018). Naturally occurring nanoparticles are produced either by biological species or through human activities (Jeevanandam *et al.*, 2018). These natural occurring nanomaterials can be found on the earth's sphere (Hochella *et al.*, 2016). Volcanic eruptions, forest fires, dust, storms, meteoritic deterioration, air currents, and human activities like quarry materials to exhaust gas, thermal power or cement plant stacks, engine jets or fireworks, lead to the formation of natural nanoparticles (Strambeanu *et al.*, 2014, Jeevanandam *et al.*, 2018). The synthesized or engineered nanoparticles are produced by mechanical grinding, engine exhaust and smoke, or are synthesized by physical, chemical, biological or hybrid methods (Jeevanandam *et al.*, 2018).

2.5.2 Classification of nanoparticle based on dimension

Dimensionally, nanoparticles are classified as zero, one, two or three based on the movement of electrons along the dimension in the nanoparticle (Hochella *et al.*, 2016, Ealias & Saravanakumar, 2017, Khan *et al.*, 2017, Jeevanandam *et al.*, 2018). Quantum dots, nanodots and nanospheres are all synthesized examples of zero dimensional (0D) nanoparticles (Tiwari *et al.*, 2012). They have their electrons captured in a space that has no dimension (Jeevanandam *et al.*, 2018) and 0D nanoparticles have their length, height and breadth fixed at a point (Ealias & Saravanakumar, 2017). One dimensional nanoparticles have varying applications. Their electrons can move along the x-axis and they include nanorods, nanowires,

nanotubes and nanoribbons (Lal Pal *et al.*, 2011, Tiwari *et al.*, 2012, Jeevanandam *et al.*, 2018). The two dimensional nanoparticles exhibit low dimensional characteristics in comparison with their larger counterparts and they possess length and breadth parameters with a unique shape dependent characteristic (Tiwari *et al.*, 2012; Ealias & Saravanakumar, 2017). Electron movement is along the x and y axes (Jeevanandam *et al.*, 2018). For three dimensional nanoparticles, the electron movement is on the x, y, and z- axes (Jeevanandam *et al.*, 2018). They include nanoballs (dendritic structures), nanocoils, nanocones, nanopillers and nanoflowers (Tiwari *et al.*, 2012).

2.5.3 Classification based on physical and chemical characteristics.

This classification scheme encompasses classification based on material makeup (Jeevanandam *et al.*, 2018), structural core (Purbia & Paria, 2015), functionality, (Wang & Wang, 2014), physical and chemical characteristics (Khan *et al.*, 2017). Nanoparticles can therefore be classified as:

2.5.3.1 Carbon based nanoparticles

Nanoparticles in this class are made up of carbon and are usually in an ellipsoid, sphere or hollow tube form (Jeevanandam *et al.*, 2018). Representatives in this group include fullerenes, graphene, carbon nanotubes (CNT), carbon nanofibers, carbon onions and carbon black (Ealias & Saravanakumar, 2017, Khan *et al.*, 2017, Jeevanandam *et al.*, 2018). They have applications as lubricants, data storage and several other medical, environmental and commercial applications (Bhatia, 2016, Tyagi, 2016, Khan *et al.*, 2017).

2.5.3.2 Inorganic-based nanoparticles

Metal and metal oxide nanoparticles fall under the inorganic based nanomaterial (Jeevanandam *et al.*, 2018). Metal and metal oxide based nanoparticles possess unique chemical, physical and strong antimicrobial properties (Beyth *et al.*, 2015, Khan *et al.*, 2017). These nanoparticles are very small in size, and have localized surface plasmon which enhances their optical property (Harish *et al.*, 2018). Metal oxide and metal nanoparticles are synthesized by the addition of oxidizing or reducing agents and have applications in several fields (Khan *et al.*, 2017, Rastogi *et al.*, 2017).

2.5.3.3 Organic based nanoparticles

Organic based nanoparticles are mainly lipid or polymeric solid compounds (Allouche, 2012). Structurally, they exist as nanospheres (Mora-Huertas *et al.*, 2010, Allouche, 2012, Khan *et al.* 2017). Nanoparticles in this class are mostly non-toxic, sensitive to thermal and electromagnetic radiation and biodegradable (Wang & Wang, 2014, Ealias & Saravanakumar, 2017). Representatives include dendrimers, liposomes, ferritin and micelles (Ealias & Saravanakumar, 2017, Khan *et al.*, 2017, Jeevanandam *et al.*, 2018). Owing to their unique properties and form, organic based nanoparticles are usually applied in the biomedical field, especially in drug delivery, as they can be injected directly to target sites (Bhatia, 2016, Tyagi, 2016).

2.5.3.4 Composite-based nanoparticles

A composite based nanoparticle is a combination of two or more components of a nanoscale material (Wang & Huang, 2014). The composites may be any combination of carbon-based, metal-based, or organic-based nanomaterials, with any form of metal, ceramic, or polymer bulk material (Jeevanandam *et al.*, 2018). Figure 3 represents nanoparticle classification based on origin, dimension and physicochemical characteristics.

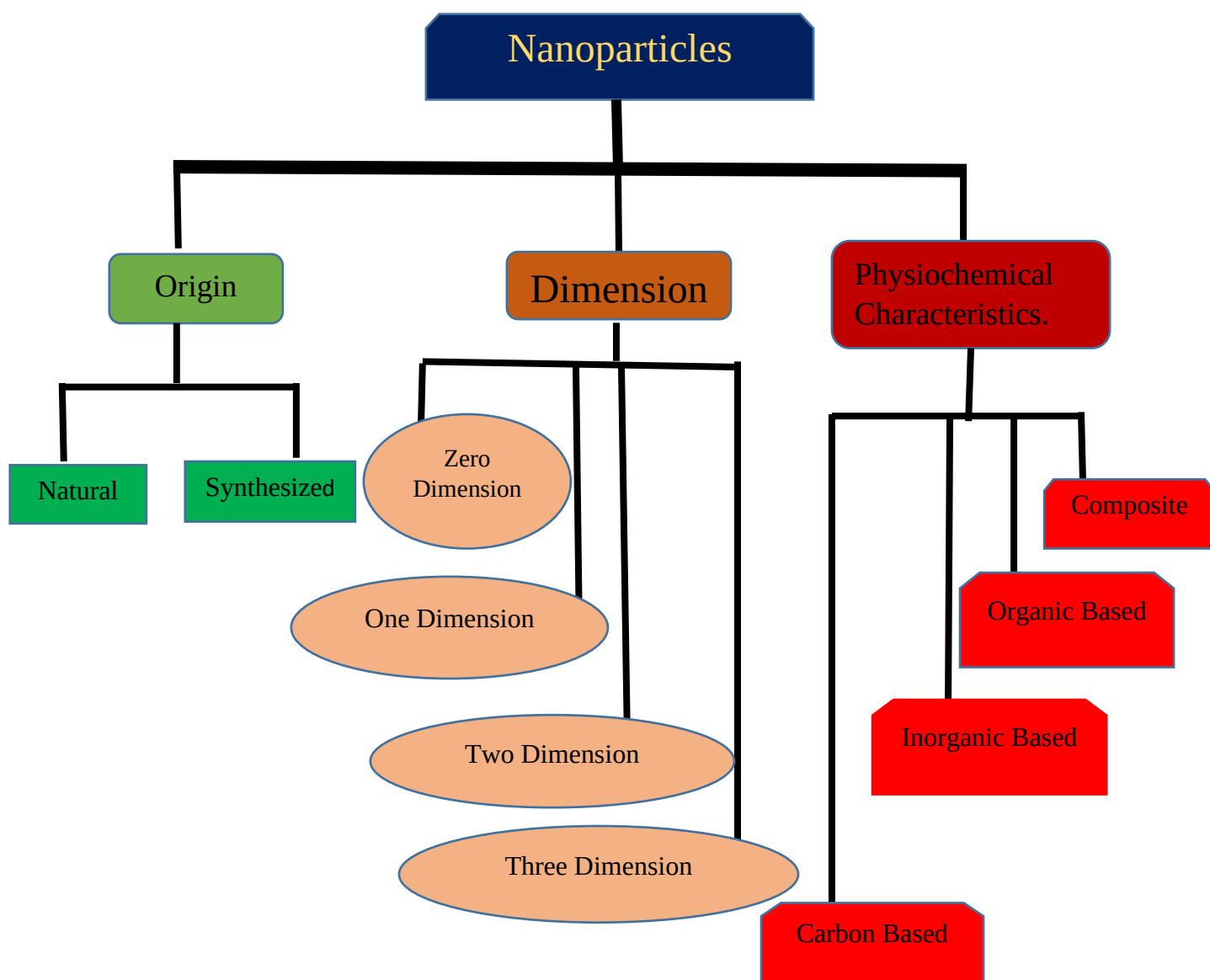


Figure 2.3: Representation of the classification of nanoparticles

2.6 Synthesis of nanoparticles

The synthesis of nanoparticles follows two approaches based on the method of nanoparticle formation: top down and bottom up approach (Nikalje, 2015, Agarwal *et al.*, 2017, Sorbiun *et al.*, 2018). The top down approach involves breaking down of appropriate starting material into smaller sizes by physical or chemical means (Razavi *et al.*, 2015, Agarwal *et al.*, 2017, Fawcett *et al.*, 2017). Bottom up approach involves manipulation of small sized molecules to clusters and then to nanoparticles (Fawcett *et al.*, 2017, Devatha & Thalla, 2018). Nanoparticles with specific size, shape, and characteristics can be synthesized using chemical, physical or biological methods (Khan *et al.*, 2017, Rane *et al.*, 2018, Jeevandam *et al.*, 2016, Anwar, 2018). The physical and chemical methods of synthesis are not frequently carried out (Kumar *et al.*, 2015, Chaudhary *et al.*, 2018). This is due to drawbacks such as the use of costly equipment, high energy, low material conversion, and production of hazardous waste generated from the chemical reducing agents used during the process (Ahmed *et al.*, 2016, Agarwal *et al.*, 2017, Sorbiun *et al.*, 2018).

2.6.1 Physical method of nanoparticle synthesis

Physical method of synthesis is achieved with the use of high temperature, high pressure and costly instruments that require large areas of space (Dhand *et al.*, 2015, Agarwal *et al.*, 2017). Nanoparticles are synthesized through physical techniques such as mechanical milling, sputtering, physical vapour deposition, laser ablation, electrospraying, inert gas condensation, laser pyrolysis, and melt mixing (Figure 4) (Dhand *et al.*, 2015, Jeevandam *et al.*, 2016).

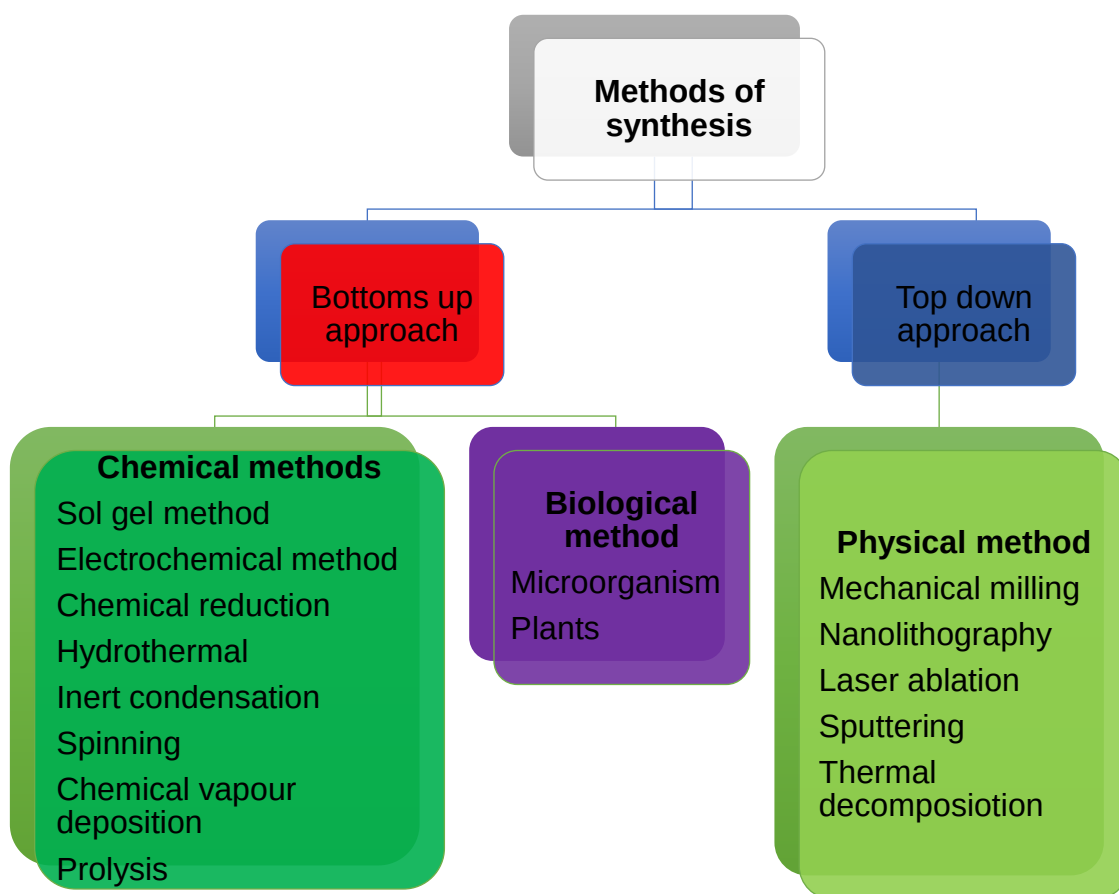


Figure 2.4: Representation of the various techniques used in nanoparticle synthesis

2.6.1.1 Mechanical milling

Mechanical milling is a simple and economical top down process that uses an appropriate milling medium combined with a high energy mill containing a powder charge to reduce or blend particle size (Thakur *et al.*, 2012, Virji & Stefaniak, 2014, Jeevanandam *et al.*, 2016). Nanoparticles are mechanically alloyed (MA) using the solid state high-energy ball mill (Ghorbani, 2014, Wang *et al.*, 2018, Ostovan *et al.*, 2019). The mechanical mill allows for high yield synthesis of various nanocomposites, aluminium, copper-based nanoalloys, wear resistant spray coatings, and oxide and carbide strengthened aluminium alloys (Yugandhar *et al.*, 2017, Ostovan *et al.*, 2019). Synthesis is achieved by milling and post annealing or by mechanical activation (Thakur *et al.*, 2012, Yugandhar *et al.*, 2017). Mechanical milling can take place at

room temperature with the resultant nanoparticles being fine and of uniform dispersion (Ghasemi *et al.*, 2018, Nunes *et al.*, 2019). However, they are of broad geometrical distributions, contain impurities and defects in shape and size (Virji & Stefaniak, 2014). Characteristics of engineered nanomaterials obtained from the mechanical milling process are determined by the power exerted, the medium used (e.g. tungsten carbide ball), the control solution, speed and time (Virji & Stefaniak, 2014).

2.6.1.2 Laser ablation

The laser ablation technique is a fast, simple technique of metal nanoparticle synthesis that does not require the use of chemical precursors (Sportelli *et al.*, 2018, Reza Sadrolhosseini *et al.*, 2019). The technique involves the use of light amplification by stimulated emission of radiation (LASER) as a source of energy to remove the solid target (Kim *et al.*, 2017, Mansoureh & Parisa, 2018, Rane *et al.*, 2018). The process involves the use of high energy to evaporate light absorbing material from a solid surface, thermal evaporation and breaking of chemical bond (Kim *et al.*, 2017). Different kinds of nanoparticles can be generated from this technique and it does not require the use of toxic chemical precursors (Reza Sadrolhosseini *et al.*, 2019). The purity of the final product is usually high, though size distribution is difficult to control (Kim *et al.*, 2017, Reza Sadrolhosseini *et al.*, 2019). The laser parameters (such as wavelength, pulse repetition rate, pulse width, pulse energy), as well as environmental parameters (vacuum, a controlled gas atmosphere or a liquid) in which generation occurs determine the properties of the nanoparticles produced using the laser ablation technique (Ortaç, *et al.*, 2017, Sportelli *et al.*, 2018).

2.6.1.3 Sputtering

Sputtering is a top down technique used to fabricate thin films, nanostructured coatings, and nanoparticles of various materials, including metallic oxide, nitride, and

carbide films (Asanithi *et al.*, 2012, Nguyen & Yonezawa, 2018, Verma *et al.*, 2018, Nunes *et al.*, 2019). In this technique, energetic gas ions are concentrated on a solid metal target, and atoms or molecules are knocked off and deposited on a substrate to produce thin films (Nguyen & Yonezawa, 2018). The production of nanoparticles using this technique requires highly controlled conditions like low pressure (Nguyen & Yonezawa, 2018). Nanoparticles fabricated from this technique are highly pure and have narrow size distribution and high surface area (Asanithi *et al.*, 2012, Alexeeva *et al.*, 2016, Verma *et al.*, 2018). The size of a particle produced from sputtering is dependent on the target-substrate distance, deposition time and sputtering current (Alexeeva *et al.*, 2016).

2.6.1.4 Thermal decomposition

The synthesis of monodispersed metal nanoparticles can be achieved using the thermal decomposition technique (Sharifi *et al.*, 2016, Odulareu *et al.*, 2019). This technique requires a high temperature thermal furnace used for vaporizing the source materials and facilitating the deposition of the nanostructures with thickness ranging from Angstrom to microns (Prasanna *et al.*, 2019). Nanoparticles obtained from thermal decomposition synthesis are usually highly crystalline, size controlled with narrow particle size distribution (Betancourt-Galindo *et al.*, 2014, Effenberger *et al.*, 2017, Odulareu *et al.*, 2019). The size of the obtained nanoparticles is determined by the duration of synthesis, temperature, concentration of reactant, stabilizing and capping agent (Majidi *et al.*, 2014, Odulareu *et al.*, 2019).

2.6.2 Chemical method

The chemical method involves the use of chemicals that are generally toxic to humans and the environment (Gudikandula & Charya-Maringanti, 2016, Sorbiun *et al.*, 2018).

The deposition of toxic chemical surface contaminants on chemically synthesized nanoparticles renders the nanoparticle unfit for medical application (Razavi *et al.*, 2015, Fawcett *et al.*, 2017, Swamy *et al.*, 2018). Other shortcomings to the use of chemical and physical methods include challenging and uneconomical purifications, the need for additional stabilizing agents because of undesirable clusters of nanoparticles, and high cost of production (Parveen *et al.*, 2016, Mukherjee & Patra, 2017, Swamy *et al.*, 2018).

2.6.2.1 Sol-gel

The sol-gel technique is one of the chemical methods used for nanoparticle synthesis. Hydrolysis, poly-condensation, drying and heating are the four steps followed in this technique (Majidi *et al.*, 2014, Virji *et al.*, 2014, Kalpana *et al.*, 2017). The metalorganic compound precursor is hydrolysed to yield a stable metal precursor, followed by condensation to form a metal hydroxide network (Virji & Stefaniak, 2014, Stankic *et al.*, 2016). Drying and heating is done to yield particles that are ultrafine and in a desired crystalline phase (Majidi *et al.*, 2014, Virji *et al.*, 2014). The kinetic, growth, hydrolysis and condensation reactions can be affected by the type of solvent, temperature, catalyst, pH, additives and mechanical agitation (Majidi *et al.*, 2014).

2.6.2.2 Chemical vapour deposition (CVD)

The CVD technique is usually carried out in a reaction chamber at ambient pressure (Ealias & Saravanakumar, 2017). In this technique, substrates are heated at high temperatures and exposed to the gaseous precursor, leading to the deposition of a thin film onto the substrate, which will either generate decomposition or a reaction to form nanomaterials (Stankic *et al.*, 2016, Ealias & Saravanakumar, 2017).

2.6.2.3 Hydrothermal

The hydrothermal method of synthesis is also known as the solvothermal method, especially when solvents other than water are used (Majidi *et al.*, 2014, Virji *et al.*, 2014, Kalpana *et al.*, 2017). This is a method of synthesis employed in the preparation of various nanomaterials including ultrafine nanoparticles (Stankic *et al.*, 2016, Kalpana *et al.*, 2017). The correct solvent, high temperature and high pressure conditions are prerequisites in the hydrothermal/solvothermal synthesis method (Majidi *et al.*, 2014, Prasanna *et al.*, 2019). Generally, the method involves dissolving the solid starting material in a suitable solvent and subjecting it to high temperature and pressure leading to particle formation (Virji *et al.*, 2014, Stankic *et al.*, 2016, Prasanna *et al.*, 2019). Particle formation is affected by both chemical (type, composition and concentration of the reactants, ratio-solvent/reducing agent) and thermodynamic factors (temperature, pressure and reaction time) (Majidi *et al.*, 2014, Stankic *et al.*, 2016).

2.6.2.4 Electrochemical method

Particles obtained from the electrochemical method of synthesis are usually pure, stable, and of superior property (Gurrappa & Binder, 2008, Singaravelan & Alwar, 2015). This method gives room for controlling the size of the particle formed by adjusting current density (Khaydavo *et al.*, 2008, Abdel-Karim, 2016). In this method, two inexpensive electrodes are set up in which the anode and cathode are made from the bulk metal (Anand *et al.*, 2015).

2.6.3 Biological method

The biological route of nanoparticle synthesis offers more advantages in that the process is more cost effective, simple to conduct, safe, eco-friendly, biocompatible

and offers wider applications than the conventional chemical and physical methods (Razavi *et al.*, 2015, Adelere & Agbaje, 2016, Parveen *et al.*, 2016, Mukherjee & Patra, 2017, Swamy *et al.*, 2018, Sorbiun *et al.*, 2018). It is therefore a more economical, sustainable, and effective method of synthesis (Agarwal *et al.*, 2017, Vijayakumar *et al.*, 2018). Biosynthesis of nanoparticles is an approach that involves the use of environmentally benign materials or bioresources such as whole cells, metabolites or extracts from plants, microorganisms, and even proteins, polysaccharides and synthetic polymers for nanoparticle synthesis (Ahmed *et al.*, 2016, Fawcett *et al.*, 2017, Anwar *et al.*, 2018, Agarwal *et al.*, 2018, Devatha & Thalla, 2018, Swamy *et al.*, 2018). Adelere and Agbaje (2016) documented the preparation of metallic nanoparticles from agro-wastes, enzymes, and pigments of biological origin. These bioresources employed in the synthesis of nanoparticles have been reported to have high bioreduction ability. Various sizes and shapes of nanoparticles can be synthesized using this technique (Vijayaraghavan & Ashokkumar, 2017).

2.6.3.1 Plant-mediated synthesis

In the biological method of synthesis, plant mediated synthesis is the most preferred approach due to its several benefits (Da *et al.*, 2017, Sorbiun *et al.*, 2018). This is due to the fact that plants are readily available, not harmful in most cases, non-pathogenic, bio-compatible, ecological, and contain metabolites, amongst others. Figure 5 represents the benefits of plant mediated nanoparticle synthesis. Nanoparticles derived from plant mediated synthesis are mostly stable and exhibit greater antimicrobial activity (Rajakumar *et al.*, 2017, Singh *et al.*, 2017, Anwar *et al.*, 2018, Devatha & Thalla, 2018). Plants contain active phytochemicals such as terpenoids, flavones, ketones, aldehydes, proteins, amino acids, vitamins, alkaloids, tannins, phenols, saponins, and polysaccharides (Adelere & Agbaje, 2016, Vijayaraghavan &

Ashokkumar, 2017). These biomolecules act as guides or support as they serve in the formation, reducing and stabilizing roles in nanoparticle synthesis (Agarwal *et al.*, 2017, Ovais *et al.*, 2018, Sorbiun *et al.*, 2018).

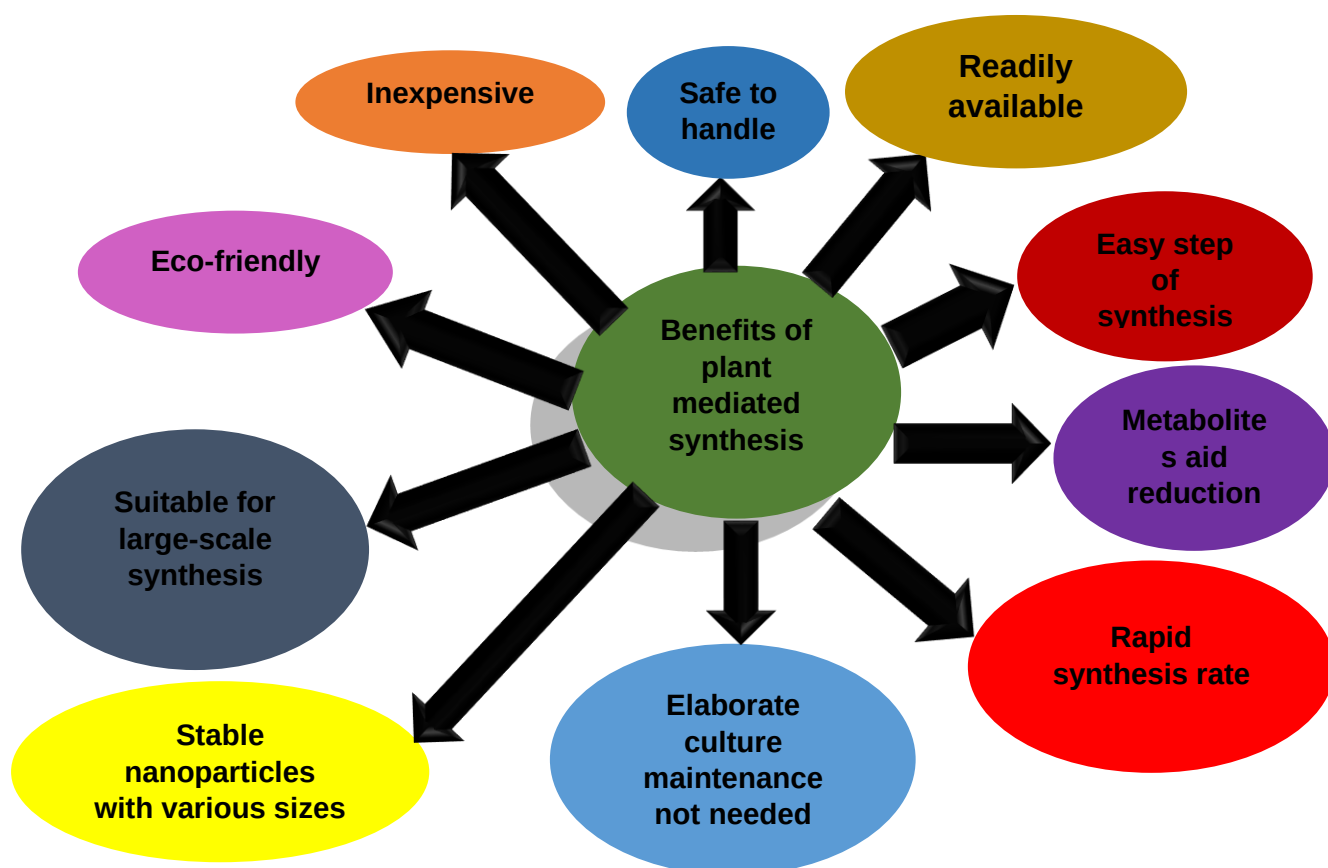


Figure 2.5: Representation of the benefits of plant mediated synthesis method.

Plant parts used in synthesis include stem, leaf, flower, fruit, root, latex, seed and seed coat (Figure 6) (Adelere & Agbaje, 2016, Singh *et al.*, 2016, Sorbiun *et al.*, 2018). During synthesis, these plant parts are used in powdered form or as aqueous extracts, which are mixed with the metal salt at room temperature, and the nanoparticles are synthesized within a short period of time (Shan *et al.*, 2015). The quality, size and shape of the synthesized nanoparticles are affected by properties of the plant extract

used, concentration of the metal salt, pH of the solution used in the reaction, reaction time and temperature (Shan *et al.*, 2015, Singh *et al.*, 2016). Several plants and plant parts have been employed in the synthesis of nanoparticles. Bhuyan *et al.* (2015) synthesized pure, spherically shaped zinc oxide nanoparticles of size 9.5 to 25.5nm from leaves of *Azadirachta indica* (Neem) which acted as a reducing and stabilizing agent. The obtained zinc oxide nanoparticles showed great antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*. In another study, Azizi *et al.* (2016) described the effect of annealing temperature on antimicrobial properties of zinc oxide nanoparticle which were formed from zinc acetate dehydrate salt using *Anchusa italic* plant. Anwar *et al.* (2018) used the bark of *Monothecha buxifolia* (Flac) plant to synthesize silver nanoparticles from silver nitrate salt. The plant extract solution was mixed with the salt solution and stirred for four hours. A visible colour change was observed which suggests nanoparticle formation (Azizi *et al.*, 2016, Basnet *et al.*, 2018).

In a study by Chaudhary *et al.* (2018), zinc oxide nanoparticle formation was confirmed when the colourless zinc sulphate salt solution mixed with greenish aqueous extract of *Aloe vera* peel yielded a yellowish-white suspension. TEM characterization showed hexagonally shaped zinc oxide nanoparticles with size ranging from 500-220nm. Wang *et al.* (2014) synthesized spheroidal iron oxide nanoparticles (Fe_3O_4 -Nps) from eucalyptus leaf extract which contains phenols, carboxyl and carbonyl functional group. The polyphenol group in the eucalyptus extract contains epicatechin and quercetin-glucoronides that play a major role in the synthesis process and reduce iron ions to zero valent particles. Different plants like *Magnifera indica*, *Murraya koenigii*, *Azadiracta indica*, and *Magnolia champaca* were used to synthesize iron oxide between the size of 96-110nm and 99-129nm (Devatha *et al.*, 2016). Khalil *et al.*

(2017) synthesized pure crystalline iron oxide nanoparticles from the aqueous leaf extract of *Sagettia thea* plant and investigated their pharmacological properties. Figure 2.6 represents different plant parts used in nanoparticle synthesis while Table 2.1 shows a list of some nanoparticles synthesized from various plants.

TABLE 2.1: Nanoparticles synthesized from different plants

S/N	Nanoparticle	Plant Used	References
1.	Silver	<i>Salvia spinose</i>	Pirtarighat <i>et al.</i> , 2018
		<i>Punica granatum</i> (Pomegranate) fruit peel	Shanmugavadivu <i>et al.</i> , 2014
		Latex of <i>Carica papaya</i> (Paw-paw)	Chandrasekaran <i>et al.</i> , 2016
		<i>Costus afer</i> leaf	Elemike <i>et al.</i> , 2017
		<i>Caltropis procera</i>	Salem <i>et al.</i> , 2015
		<i>Syzygium alternifolium</i> (Wt.) Walp	Yugandhar <i>et al.</i> , 2015
		Bark of <i>Plumbago zeylanica</i>	Priya Velemmal <i>et al.</i> , 2016
2.	Zinc oxide	Root Extract of <i>Zingiber officinale</i>	Raj & Jayalakshmy 2015
		Aqueous Leaf Extract of <i>Talinum triangulare</i>	Elemike <i>et al.</i> , 2016
		Leaf extract of <i>Andrographis paniculata</i>	Rajakumar <i>et al.</i> , 2017
		<i>Mangifera indica</i> leaves	Raheshkumar <i>et al.</i> , 2018
		<i>Passiflora caerulea</i> L.	Santhoshkumar <i>et al.</i> , 2017
		Stem bark of <i>Albizia lebbeck</i>	Umar <i>et al.</i> , 2018
		<i>Atalantia monophylla</i> leaf	Vijaykumar <i>et al.</i> , 2018
		<i>Caltropis procera</i>	Salem <i>et al.</i> , 2015
		Flower extract of <i>Trifolium pratense</i>	Dobrucka & Długaszewska 2016
		<i>Euphorbia jatropa</i> latex	Geetha <i>et al.</i> , 2016
3.	Iron oxide	<i>Mangifera indica</i> , <i>Murraya koenigii</i> , <i>Azadirachta indica</i> and <i>Magnolia champaca</i> .	Devatha <i>et al.</i> , 2016
		Aqueous extract of Seaweed (<i>Sargassum muticum</i>)	Namvar <i>et al.</i> , 2014
		<i>Lawsonia inermis</i> (Henna)	Chauhan & Upadhyay 2019
		<i>Sageretia thea</i> (osbeck)	Khalil <i>et al.</i> , 2017
		<i>Eichhornia crassipes</i>	Jagathesan & Rajiv 2018
		<i>Excoecaria cochinchinensis</i> Lour	Lin <i>et al.</i> , 2018
		<i>Platanus orientalis</i>	Devi <i>et al.</i> , 2018
		Alfalfa	Herrera-Becerra <i>et al.</i> , 2007
		<i>Azadirachta indica</i>	Samrot <i>et al.</i> , 2018

4.	Copper oxide	<i>Punica granatum (Pomegranate) peel</i>	Kaur <i>et al.</i> , 2014
		Flower of <i>Cordia sebestena</i>	Prakash <i>et al.</i> , 2018
		Leaf of <i>Juglans regia</i>	Asemani & Anarjan 2019
		Aloe Vera leaf	Kumar <i>et al.</i> , 2015
5.	Gold	Bark of <i>Plumbago zeylanica</i>	Priya Velemmal <i>et al.</i> , 2016
		<i>Ziziphus zizyphus</i> (Jujube)	Aljabali <i>et al.</i> , 2018
		<i>Terminalia catappa</i>	Ankamwar 2010
		<i>Polyscias scutellaria</i>	Yulizar <i>et al.</i> , 2017
6.	Nickel oxide	<i>Aegle maemelos</i> leaf	Ezhilarasi <i>et al.</i> , 2018
		<i>Rhamnus virgata</i>	Iqbal <i>et al.</i> , 2019
		<i>Moringa oleifera</i>	Ezhilarasi <i>et al.</i> , 2016, Mamuru & Jaji 2015
		<i>Gymnema sylvestre</i> leaf	Vijaya Kumar <i>et al.</i> , 2019
		<i>Ageratum conyzoides</i> leaf	Wardani <i>et al.</i> , 2019
7.	Manganese oxide	Flower bud of <i>Syzygium aromaticum</i> L. (Dry cloves)	Kumar <i>et al.</i> , 2017
		<i>Gardenia resinifera</i> leaves	Manjula <i>et al.</i> , 2019
		<i>Citrus limon</i> (Lemon extract)	Haneefa <i>et al.</i> , 2017
	Manganese dioxide nanoparticle	<i>Matricaria chamomilla</i> L	Ogunyemi <i>et al.</i> , 2019
		<i>Yucca gloriosa</i> leaf	Souri <i>et al.</i> , 2018
8.	Magnesium oxide	<i>Matricaria chamomilla</i> L	Ogunyemi <i>et al.</i> , 2019
		<i>Rosmarinus officinalis</i> L. (Rosemary)	Abdallah <i>et al.</i> , 2019
		<i>Artemisia abrotanum</i> Herba Extract	Dobrucka 2016
		<i>Azadirachta indica</i> (Neem leaves)	Moorthy <i>et al.</i> , 2015
		Peels of <i>Nephelium lappaceum</i> L.	Suresh <i>et al.</i> , 2014
9.	Titanium dioxide	<i>Cassia fistula</i>	Swathi <i>et al.</i> , 2019
		<i>Nyctanthes Arbor-Tristis</i> leaves	Sundrarajam & Gowri 2011
		<i>Psidium guajava</i>	Santhoskumar <i>et al.</i> , 2014
10.	Aluminium oxide	<i>Cymbopogon citratus</i> (Lemon grass)	Ansari <i>et al.</i> , 2014

2.6.3.1.1 Mechanism of nanoparticle formation with plants

Plants contain biomolecules such as proteins, amino acids, enzymes, flavonoids, and terpenoids that serve as stabilising and reducing agents for the synthesis of nanoparticles (Adelere & Agbaje, 2016, Zheng *et al.*, 2016, Umamaheswari *et al.*, 2018). The use of plants in the synthesis of nanoparticles has taken centre stage because it is a less expensive, ecological and faster technique (Dobrucka and Długaszewska, 2016, Rajeshkumar & Bharath, 2017, Jamdagni *et al.*, 2018). The mechanism and components responsible for nanoparticle synthesis are still not clearly explained, although biomolecules and secondary metabolites of plants are believed to be major role players in biosynthesis, as they serve as capping and stabilizing agents (Imran Din & Rani, 2016, Basnet *et al.*, 2018, Pirtarighat *et al.*, 2018). During synthesis, a change in colour of the reaction mixture usually indicates the formation of nanoparticles (Azizi *et al.*, 2016, Rajakumar *et al.*, 2017, Santhoshkumar *et al.*, 2017, Pirtarighat *et al.*, 2018). However, confirmation of nanoparticle and identification of biomolecules involved in its formation is determined using the FTIR (Shanmugavadivu *et al.*, 2014, Vijayakumar *et al.*, 2018).

The mechanism of nanoparticle formation varies with the plant species (Singh *et al.*, 2016). Ahmad *et al.* (2012) suggest that the abundantly naturally occurring phenolic compound known as ellagic acid is responsible for nanoparticle formation through its electron loss capacity. Vijaykumar *et al.* (2018) reported that the phytochemicals found in the leaf extract of *A. monophylla* act as a bio-reductant reducing zinc ions to nanostructured zinc oxide. Three stages are encountered in the mechanism of plant mediated synthesis of nanoparticles. This involves the activation stage whereby metal ions are reduced to the metal to undergo nucleation, the growth phase where small nanoparticles are merged into a larger size also known as Ostwald ripening and finally

the termination stage which determines the shape of the nanoparticle (Makarov *et al.*, 2014, Shan *et al.*, 2015, Imran Din & Rani, 2016). The concentration of the reactant, pH, reaction time, and temperature all affect nucleation and formation of stabilized nanoparticles (Makarov *et al.*, 2014, Shan *et al.*, 2015).

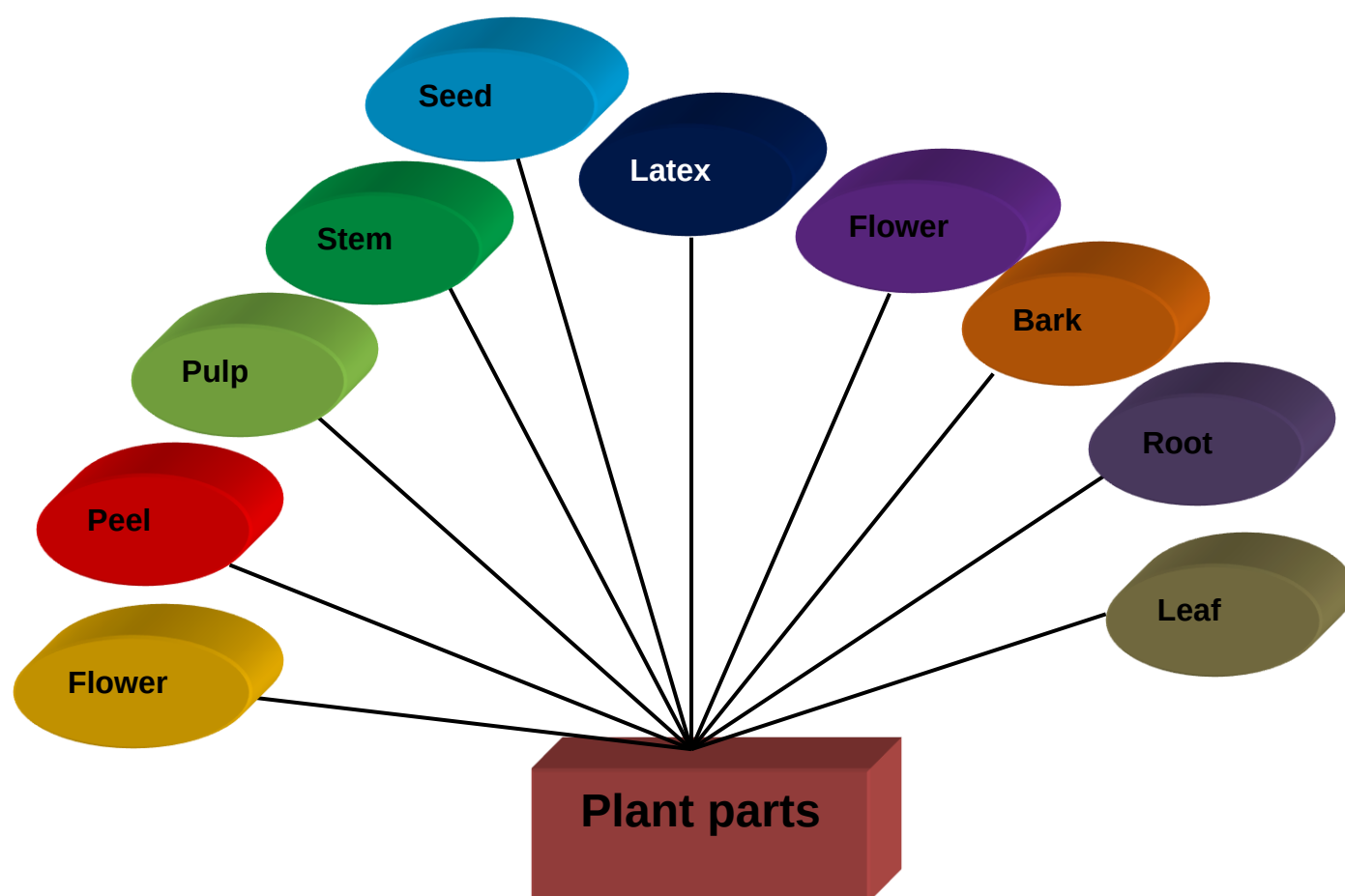


Figure 2.6: Representation of the different plant parts used in biosynthesis of nanoparticles

2.6.3.2 Microbial mediated synthesis

Microbial mediated synthesis involves the use of microbes and microbial metabolites for nanoparticle synthesis (Reverberi *et al.*, 2017, Sathiyarayanan *et al.*, 2017). Bacteria are commonly used in biosynthesis because bacterial cells can easily be manipulated and cultured under controlled conditions (Swamy *et al.*, 2018). Synthesis of nanoparticles by microorganisms can be extracellular or intracellular, based on the location of nanoparticle formation (Hulkoti & Taranath, 2014, Shah *et al.*, 2015, Reverberi *et al.*, 2017, Gothandam *et al.*, 2018). The use of microorganisms has some drawbacks in that the process is generally time consuming, tiresome, requires high aseptic conditions, has a slow synthesis rate, and high cell culture maintenance (Narayanan & Sakthivel, 2010, Raj & Jayalakshmi, 2015, Ahmed *et al.*, 2016, Sorbiun *et al.*, 2018, Agarwal *et al.*, 2017, Swamy *et al.*, 2018, Basnet *et al.*, 2018). However, the process has some advantages which include the fact that microorganisms, especially bacteria, are everywhere and can easily adapt to extreme conditions, are fast growing and easy to manipulate (Pantidos & Horsefall, 2014, Swamy *et al.*, 2018).

In the use of fungi for the synthesis of nanoparticles, the biomass is easy to handle, which allows for scale up of large biomass capacity and large scale nanoparticle production (Pantidos & Horsefall, 2014, Agarwal *et al.*, 2017, Aftab, 2018, Devatha, & Thalla, 2018). Microbes are useful in several biological applications such as biomineralization, bioremediation, and bioleaching and they are effectively used in reducing environmental pollution (Das *et al.*, 2017, Swamy *et al.*, 2018). This is because microbes have the ability to adsorb and accumulate metals from their environment (Swamy *et al.*, 2018).

In microbial mediated synthesis of nanoparticles, the microorganism confines ions from its surrounding and converts the ions into matching metal with the help of

enzymes produced by microbial cellular metabolism (Li *et al.*, 2011, Das *et al.*, 2017, Gothandam *et al.*, 2018). The process flow involves isolation and culturing of a specific microbial strain in a specific nutrient, and addition of a corresponding metal salt into the culture media where nanoparticles are required. The culture is maintained at a specific pH and temperature and time is allowed for the chelating redox reactions to occur.

Nanoparticle formation is indicated by a colour change of media. Harvested nanoparticles can be further characterized using scanning electron microscopy, Zeta sizer, energy dispersive x-ray analysis, x-ray diffraction, and transmission electron microscopy. (Li *et al.*, 2011, Chokriwal *et al.*, 2014, Aftab, 2018). Microbial mechanism of action in nanoparticle synthesis differs with species (Singh *et al.*, 2016). Some mechanisms of action may include alteration of solubility and toxicity through reduction or oxidation, lack of specific metal transport system, biosorption, extracellular complexation or precipitation of metals, bioaccumulation and efflux system (Hulkoti & Taranath, 2014., Razavi *et al.*, 2015, Aftab, 2018). Microbe-mediated nanoparticles have several applications in medicine, for example in cancer treatment and treatment of bacterial infections. They also have applications in biosensing and in bioimaging (Ibrahem *et al.*, 2017). Figure 2.7 is an illustration of the process for microbial mediated biosynthesis of nanoparticles.

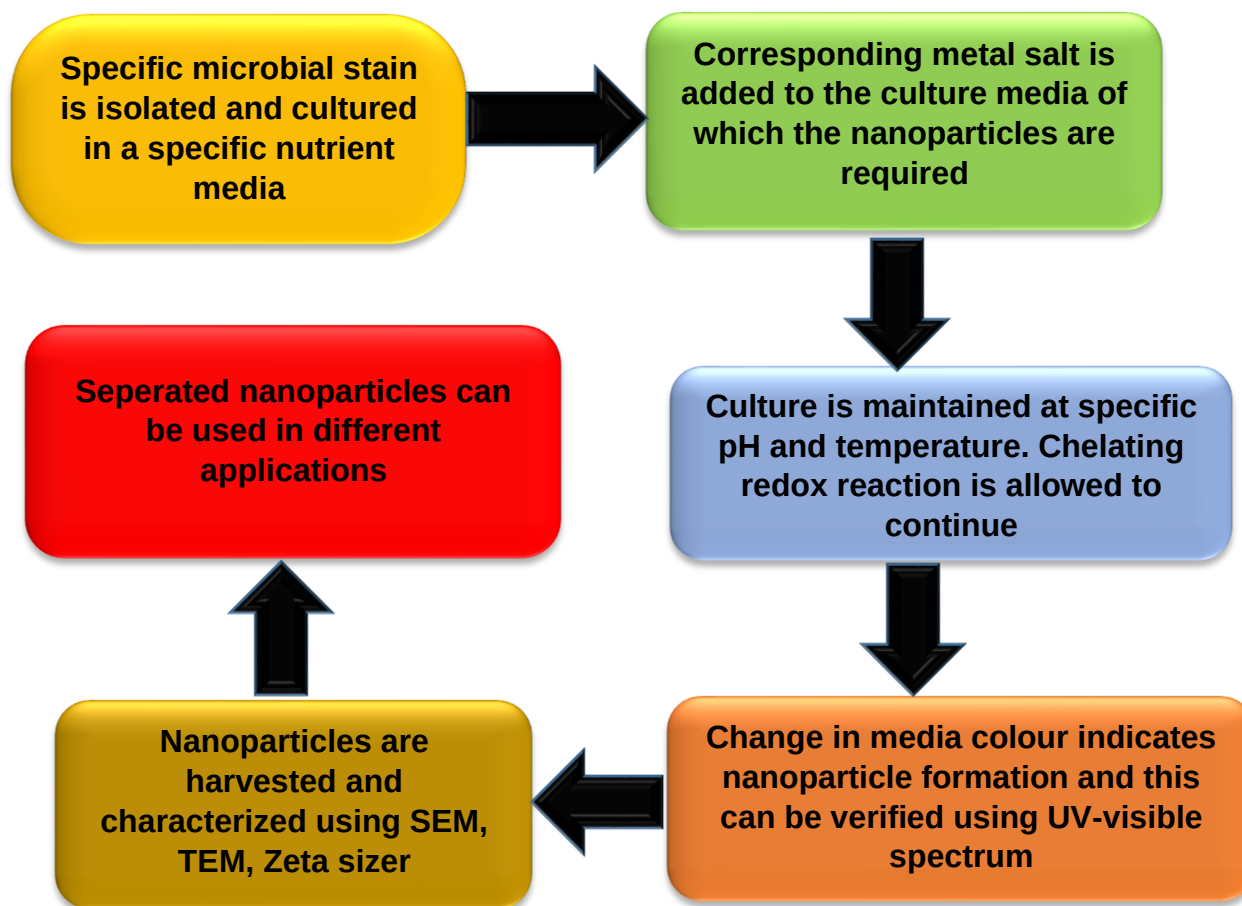


Figure 2.7: Mechanism for biosynthesis of nanoparticles using microorganism.

2.7 Characterisation of nanoparticles

Nanoparticles have several applications and therefore there is a need for their characterization, which is majorly done to ascertain their several physical, chemical, and biological properties (Kumar & Dixit, 2017, Mourdikoudis *et al.*, 2018). Nanoparticles can be characterised using microscopic or non-microscopic techniques. Characterization following biosynthesis is crucial, to enable identification of shape, size, chemical composition, surface and dispersity (Bhatia *et al.*, 2016, Khandel & Shahi 2016, Chouhan 2018). There are varyious tools or techniques that can be employed in nanoparticle characterization (Ealias & Saravanakumar, 2017, Kumar & Dixit, 2017, Chouhan *et al.*, 2018) and they include X-ray diffraction (XRD), Fourier transform infrared spectroscopy, infrared (IR), Brunauer–Emmett–Teller (BET) UV-Visible (UV-Vis) spectroscopy, Transmission electron microscopy (TEM), Scanning

electron microscopy (SEM), Energy dispersive X-ray analysis (EDX), X-ray photo-electron spectroscopy (XPS) (Khan *et al.*, 2017, Basnet *et al.*, 2018). Other techniques used in characterization may include ion-particle probe, thermodynamic, tilted laser microscopy, zeta potential measurements, isopycnic centrifugation, hydrophobic interaction, electrophoresis and fractionation (Ali *et al.*, 2016). Figure 2.8 illustrates various techniques used for nanoparticle characterisation.

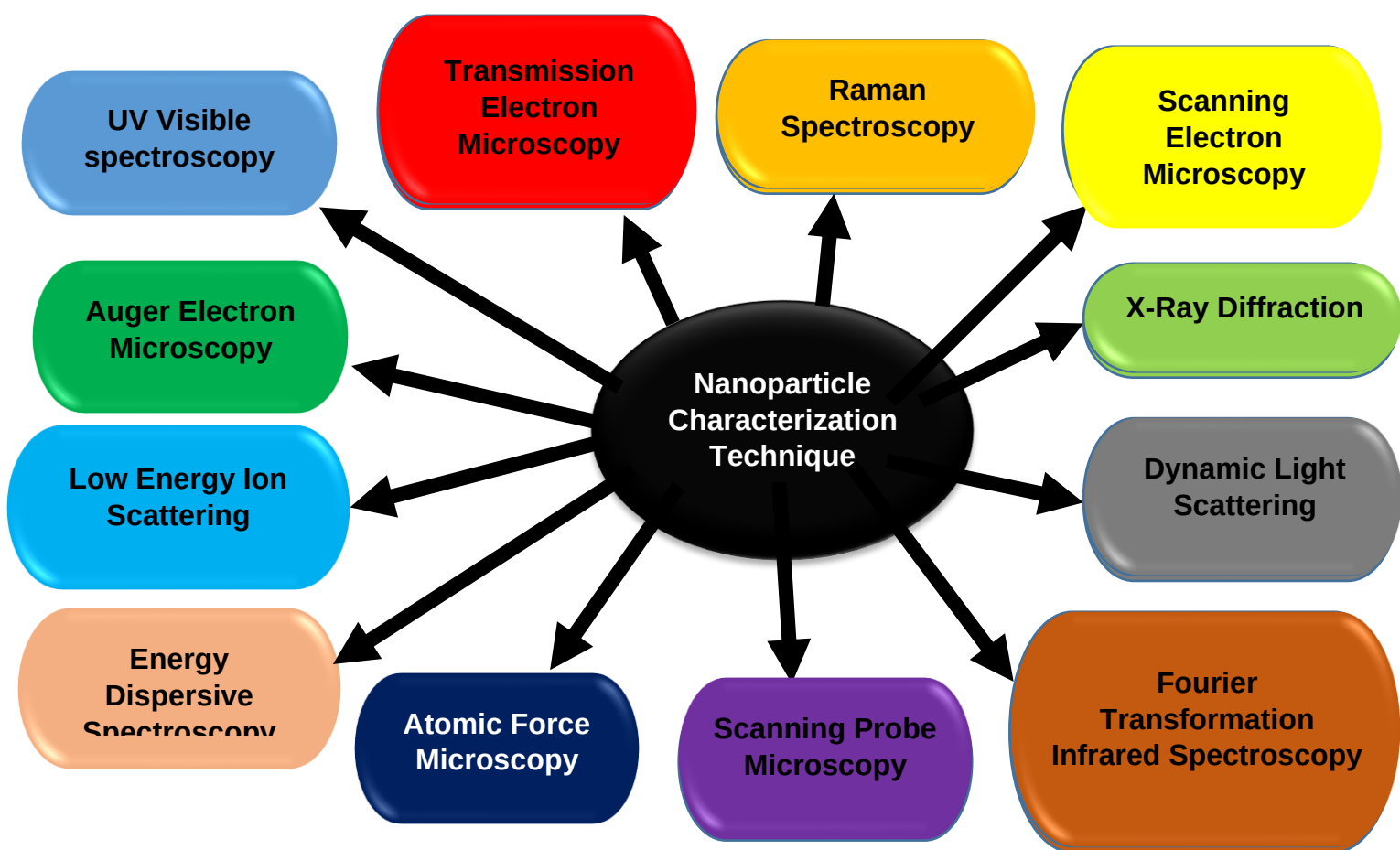


Figure 2.8: Characterization techniques of nanoparticle.

2.7.1 UV-visible (UV-Vis) spectroscopy

The UV-Visible (UV-Vis) spectroscopy is a low cost, simple, fast and sensitive characterization technique (Rajeshkumar & Bharath, 2017, Mourdikoudis *et al.*, 2018) employed to evaluate the stability, formation, absorbance and optical properties of bio-nanoparticles in solution (Talam *et al.*, 2012, Gangadoo *et al.*, 2014, Heera & Shanmugam, 2015, Dobrucka & Długaszewska, 2016). It allows for the study of the absorbance and optical band gap energy of the nanoparticles (Basnet *et al.*, 2018) as well as the size and molar concentration of zerovalent nanoparticles (Mourdikoudis *et al.*, 2018).

2.7.2 Fourier transform infrared spectroscopy (FTIR)

This technique identifies the type of functional groups and structural features of biological extracts with nanoparticles (Gangadoo *et al.*, 2014, Heera & Shanmugam, 2015, Shah *et al.*, 2015). FTIR measures infrared intensity versus wavelength of light and allows for the study of the vibrational characteristic of the nanoparticles (Heera & Shanmugam, 2015, Khan *et al.*, 2017, Mourdikoudis *et al.*, 2018). FTIR elucidates the biomolecules involved in reduction during nanoparticle synthesis (Dobrucka & Długaszewska, 2016, Rajeshkumar & Bharath, 2017).

2.7.3 Scanning electron microscopy (SEM)

SEM characterization is an important electronic microscopic technique to determine the size and shape of nanomaterials (Gangadoo *et al.*, 2014, Shanmugavadivu *et al.*, 2014, Bhatia, 2016, Sorbium, 2018). The technique is based on the principle of electronic scanning and aims at providing information about the nanoparticle at a nanoscale (Khan *et al.*, 2017) and structural information of the bulk materials

(Gangadoo *et al.*, 2014). The technique allows for the study of the morphological features of nanomaterials (Dobrucka & Długaszewska, 2016). Santhoshkumar *et al.* (2017) reported SEM images of magnification range between 2 μm –200 nm, indicating the presence of spherical-shaped nanoparticles with mean average diameter of 70 nm.

2.7.4 Transmission electron microscopy (TEM)

TEM is a characterization technique for nano-biomaterial mostly used in combination with atomic force microscopy (AFM) (Gangadoo *et al.*, 2014). TEM is an important high spatial resolution structural and chemical characterization tool that facilitates the understanding of the crystalline characteristics and inter-particle interaction of nanomaterials (Geetha *et al.*, 2016, Harish *et al.*, 2018). Its principle is based on electronic transmittance and it provides information on the sub-nanometre resolution of a sample (Gangadoo *et al.*, 2014, Khan *et al.*, 2017).

2.7.5 X-ray diffraction (XRD)

X-ray diffraction is an analytical technique that allows for the study of the crystalline structure, the nature of the phase, lattice parameters and crystalline grain size of nanoparticles (Ealias & Saravanakumar, 2017, Rajeshkumar & Bharath, 2017, Basnet *et al.*, 2018). The Derby Scherer's equation is usually applied in rough estimation of the crystalline grain size (Khan *et al.*, 2017, Chouhan, 2018, Harish *et al.*, 2018, Mourdikoudis *et al.*, 2018).

2.7.6 Electrochemical characterisation of nanoparticles

Electrochemistry is a branch of science that focuses on chemical changes that occur due to the passage of electric current and production of electrical energy by a chemical reaction (Ciobanu *et al.*, 2007, Zoski, 2007, Nnamchi & Obayi, 2018). Reactions involving electron transfer are investigated and measured in electrochemistry (Elgrishi *et al.*, 2017). Electrochemical characterization of substances is done to understand the basic electrochemistry of the substance (Choudhary *et al.*, 2017, Elgrishi *et al.*, 2017). It involves the use of different important characterization techniques to measure electrochemical signals such as potential, charge or current, and thereby determining the chemical reactivity of the analyte (Choudhary *et al.*, 2017, Nnamchi & Obayi, 2018). Synthesized nanoparticles are electrochemically characterized to understand their electrochemical behaviour and evaluate their electron properties (Alexeyeva *et al.*, 2010, Mohammad & Arfin, 2014, Choudhary *et al.*, 2017).

Electrochemical characterization techniques have the advantage of sensitivity, selectivity, accuracy and reliability (Pisoschi *et al.*, 2015, Nnamchi & Obayi, 2018). They can therefore be deployed in clinical and environmental analysis (Manea, 2014, Martín-Yerga, 2019). Most electrochemical characterisation tools are easily made-up, less expensive, simple and easy to move around (Laborda *et al.*, 2017, Nnamchi & Obayi, 2018). Electrochemical characterization can be performed with the aid of an electrode system electrochemical cell. An electrochemical cell is a device that generates voltage and current from chemical reactions and can be of two, three or four electrode systems (Choudhary *et al.*, 2017). An electrode is an electrical conductor whose electron energy can be modulated by the application of an external power source voltage.

The three electrode system electrochemical cell is commonly employed in electrochemistry (Nnamchi & Obayi, 2018). The electrode system is inclusive of the working electrode (WE), reference electrode (RE), and auxiliary electrode (AE) (Koyun *et al.*, 2012). The working electrode is typically a cathode and it is the electrode where the reaction occurs (Bard & Faulkner 2001, Ciobanu *et al.*, 2007, Nnamchi & Obayi 2018). The RE considered as the anode is made up of phases with constant composition and allows the potential of the WE to be determined accurately (Bard & Faulkner, 2001, Ciobanu *et al.*, 2007, Choudhary *et al.*, 2017, Nnamchi & Obayi, 2018). The CE/AE simply completes the circuit, acting to jolt off excess current away from the reference electrode (Bard & Faulkner 2001, Ciobanu *et al.*, 2007).

2.7.6.1 Electrochemical techniques for nanomaterials characterization

Cyclic voltammetry, Square wave voltammetry chronoamperometry (CA), chronopotentiometry (CP), galvanostatic charge discharge (GCD) and Electrochemical impedance spectroscopy (EIS) are some tools employed in advanced electrochemical study of nanoparticles (Manea, 2014., Choudhary *et al.*, 2017). The knowledge of the particle to be analysed, its application and limitations to an extent determines the suitable technique for analysis (Nnamchi & Obayi, 2018). A typical electrochemical workstation is composed of a potentiostat (to control voltage between electrodes), an efficient generator and a system that records and displays time (t), current (i) and electric potential (E), that is connected to an electrochemical cell. Recently, the modern electrochemical workstation is composed of a potentiostat and relevant control software on one end, and the electrochemical cell setup (Bard & Faulkner, 2001, Nnamchi & Obayi, 2018).

2.7.6.2 Cyclic voltammetry (CV)

Cyclic voltammetry is a low cost, resourceful, simple and sensitive technique that aids understanding of the structural relationship, potential and characteristics of different substances by monitoring the electrochemical behaviour of the substance (Boltalina, 2017, Nnamchi & Obayi, 2018, Chooto *et al.*, 2019). The technique allows for the study of the electrochemical properties of different electroactive substances in solution (Koyun *et al.*, 2012, Arfin & Mohammad, 2013, Manea, 2014) or in solid state (Kim *et al.*, 2010, Boltalina, 2017, Nnamchi & Obayi, 2018). Vidal & Muñoz (2013) reported that series of cyclic voltammetry measurements can be carried out to study the interaction between proteins and solid surfaces under potentiodynamic conditions. Mamuru & Jaji (2015) investigated the voltammetric behavior of nickel towards a one electron transfer redox probe. They observed that the electron transfer rate constant and apparent heterogeneous electron transfer constant were relatively high, indicating that nickel nanoparticles can transfer electrons quickly, which is beneficial to biosensor application. Kim *et al.*, (2010) studied the electrochemical behavior of electrodeposition of CdSe and CdTe thin film using the cyclic voltammetry technique. The technique allows for swift information on the thermodynamic redox processes, kinetic heterogeneous electron transfer reaction, coupled chemical reaction and adsorption process of substances (Manea, 2014, Nnamchi & Obayi, 2018). Analysis and characterization of large compounds that involve electron transfer can be carried out using the cyclic voltammetry technique (Li *et al.*, 2018, Chooto *et al.*, 2019). Generally, the CV is usually made up of a conventional three electrode system which consists of a working electrode (the anode or cathode), a counter electrode placed in the cathode chamber and a reference electrode placed in the anode chamber close to the anode (Kim *et al.*, 2010, Li *et al.*, 2018).

In the CV technique, potential relative to the reference electrode is scanned at the working electrode, ensuing current flows through the counter electrode and is observed in a supporting unstirred analyte (Manea, 2014, Nnamchi & Obayi, 2018). The technique is based on the linear back and forth sweeping of potentials at a constant sweep rate in volts/seconds using a triangular potential waveform, (Valero-Vidal & Igual-Muñoz, 2013, Pisoschi *et al.*, 2015, Choudhry *et al.*, 2017, Sequeira 2017). Usually the sweep is reversed at a specific switching potential, hence the name cyclic voltammetry (Bard & Faulkner, 2001). Subsequent current which oxidizes or reduces the analyte is recorded and plotted against the potential applied to obtain the cyclic voltammogram curve (Kim *et al.*, 2010, Sequeira, 2017, Nnamchi & Obayi, 2018). Accurate analysis of the curve obtained can explain the redox potential of the analyte, and the diffusion coefficients of species taking part in the electrochemical reaction (Valero-Vidal & Igual-Muñoz 2013, Sequeira, 2017, Nnamchi & Obayi, 2018). The CV experiment can be carried out with one cycle or multiple potential cycles (Choudhry *et al.*, 2017).

2.8 Metal oxide nanoparticles

Metal oxide-based nanomaterials have shown exceptional surface to bulk ratio dependent physicochemical properties (Stankic *et al.*, 2016, Rastogi *et al.*, 2017). These surface specific physicochemical properties of metal oxide permit its application in several fields including gas sensors, fuel cells, advanced ceramics, chemical sensors, biosensors, batteries, solar cells, pyroelectric, super capacitors, catalysts, and anticorrosion coatings (Corr, 2012, Prasanna *et al.*, 2019). A combination of metallic element and oxygen leads to the formation of metal oxide. The various electronic structures of metal oxides enable it give off a metallic, semi conductive and

insulative appeal (Fernandez-Garcia & Rodriguez, 2009, Jeevanandam, *et al.*, 2016, Gangwar *et al.*, 2016). When in nanoscale form, the properties of metal oxides are heightened, unlike their larger counterparts (Jeevandam *et al.*, 2016, Ealias & Saravanakumar, 2017, Chouhan, 2018). Semiconducting metal oxides are of three types: intrinsic i-type which has insulating and conductive properties; the n-type that exhibits excellent electronic properties as they have free electrons as charge carriers and the p-types which are electron-deficit semiconductors with a cation-deficient oxide that provides the additional electrons for reactivity (Raghunath and Perumal, 2017, Yim *et al.*, 2018, Zhang, 2019).

The interaction of metal oxide nanoparticles and the biological system is made possible owing to these features that confer unique chemical and physical properties to the metal oxide nanoparticles (Sirelkhatim *et al.*, 2015, Raghunath and Perumal, 2017). Nanomaterials of different metal oxides have been investigated, including zinc oxide, iron oxide, tin oxide, gallium oxide, copper oxide, nikel oxide amongst others (Akin & Sonmezoglu, 2018, Prasanna *et al.*, 2019). Metal oxide nanoparticles are synthesized following the chemical, physical or biology technique of synthesis (Stankic *et al.*, 2016, Ibrahim *et al.*, 2018, Singh *et al.*, 2018, Vijayakumar *et al.*, 2018). Biosynthesis of metal oxide nanoparticles is more cost effective than the conventional physical and chemical method of synthesis (Basnet *et al.*, 2018).

Synthesized metal oxide nanoparticles show unique properties and have varying applications (Fawcett, 2017, Basnet *et al.*, 2018, Vijayakumar *et al.*, 2018). For example, zinc oxide nanoparticles are applied as nanoabsorbents due to their thermal and chemical stability as well as their antimicrobial properties (Kecili & Hussain, 2018, Yuvaraja *et al.*, 2018, Zarfar *et al.*, 2018). Titanium oxide, silica oxide, aluminium oxide, zirconia oxide, zinc oxide, and cerium oxide nanoparticles are used for the

modification of surface coatings in nanofillers (Ameen *et al.*, 2013, Pruna, 2019). Nerve agents such as sulfur, mustard and phosphorus can be neutralized using magnesium oxide and zinc oxide nanoparticles (Turaga *et al.*, 2012, Sadeghi *et al.*, 2016). Photodegradation of pollutants in wastewater is achieved using photocatalytic semiconducting metal oxides like TiO_2 and ZnO (Ameta *et al.*, 2012, Bedia *et al.*, 2019). UV absorption, specific colour absorption in the visible region, dichroism, and photoluminescence are specific optical properties shown by metal oxide nanoparticles which allow their application in the fields of solar cells, light-emitting diodes, transistors, optoelectronic packaging, and coatings (Yu *et al.*, 2016, Soytaş *et al.*, 2019). Metal oxide nanoparticles are applied in the production of cosmetics, toothpaste, skin care products, (Lohani *et al.*, 2014, Zhang *et al.*, 2015, Kaul *et al.*, 2018), antibacterial and air cleaning products, paints, and for decomposing organic matter in wastewater (Ali *et al.*, 2016, Rastogi *et al.*, 2017, Pavithra *et al.*, 2019).

Conductivity and chemical activities of metal oxide nanoparticles can be heightened by modifying their surface properties (Neouze & Schubert, 2008, Stankic *et al.*, 2016, Soytaş *et al.*, 2019). In polymer nanocomposite formation, metal oxide nanoparticles are modified and employed to generally improve thermal stability, toughness, glass transition temperature, optical, tensile strength, etc. (Seabra & Durán, 2015, Prasanna *et al.*, 2019).

Metal oxide nanoparticles have different properties that increase their ability to resist microorganisms (Wang *et al.*, 2017, Siddiqi *et al.*, 2018, Vega-Jiménez *et al.*, 2019). This can be seen in calcium oxide and magnesium oxide nanoparticles that serve as good antimicrobial agents due to their alkaline property (Tang *et al.*, 2014, Cai *et al.*, 2018, Nguyen *et al.*, 2018). The microcidal property of cerium oxide nanoparticle is linked to its electrostatic nature (Dahle & Arai, 2015, Charbgoon *et al.*, 2017). Metal

oxide nanoparticles are also receiving heightened focus due to their unique electronic and optical properties (Sangeetha *et al.*, 2011, Agarwal *et al.*, 2018, Siddiqi *et al.*, 2018). Nanoparticles usually have potentially distinguishing properties from bulk species, especially due to their size and shape (Fernández-Garcia, & Rodriguez, 2007, Basnet *et al.*, 2018). Biological activities of some metals oxides like Fe₃O₄, TiO₂, CuO and ZnO have been investigated.

2.9 Zinc oxide nanoparticles: properties and applications

Oxides of zinc are n-type semiconducting inorganic compounds that have applications in transparent electronics, ultraviolet (UV) light emitters, piezoelectric devices, chemical sensors, spin electronics, personal care products, coatings and paints (Sonmez *et al.*, 2012, Khelladi & Sari, 2013, Murali *et al.*, 2017). The unique physical and chemical properties possessed by zinc oxide account for its varied functionality (Kołodziejczak-Radzimska & Jesionowski, 2014, Siddiqi *et al.*, 2018). Its piezoelectric and pyro-electric properties, coupled with a high exciton binding energy of 60 meV at room temperature, allows for its application in optoelectronic devices (Wang, 2004, Coleman & Jagadish, 2006, Talam *et al.*, 2012, Fu & Fu, 2015). Zinc oxide is stable under harsh conditions, has a high refractive index and can be used in pigments and coatings (Liu *et al.*, 2009).

Structurally there are three crystals of zinc oxide; hexagonal wurtzite, which is most stable thermodynamically, the cubic zinc-blende structure (metastable) and a rarely-observed cubic rock-salt which is only stable under extreme pressure (Coleman & Jagadish, 2006, Moezzi *et al.*, 2012). The mechanical properties of zinc are reported to be influenced by its crystal coordination (Kołodziejczak-Radzimska & Jesionowski, 2014). Different shapes and sizes of zinc oxide nanoparticles can be produced through

precipitation, sol-gel process, chemical microemulsion, hydrothermal, vapour phase transparent, solvothermal, physical fragmentation and biosynthetic methods (Khatami *et al.*, 2018, Umamaheswari *et al.*, 2018).

In the olden days, the antibacterial activity of zinc oxide was exploited as it was incorporated into medical ointment used in treating skin infections (William *et al.*, 1979, Moezzi *et al.*, 2012, Siddiqi *et al.*, 2018). Zinc oxide nanoparticles produced through green synthesis have been shown to be more stable, and possess better antibacterial activity against a wide range of organisms. They are safe for use in humans as they exhibit selective toxicity towards a variety of abnormal and cancer cell lines, a potential for use in cancer therapy (Jones *et al.*, 2008, Namvar *et al.*, 2015, Murali *et al.*, 2017). The antibacterial activity of ZnO may be dependent on the particle size, shape, concentration and exposure time (Agarwal *et al.*, 2018). They exert their antibacterial activity on a microbe by first damaging the bacterial cell wall, then penetrating, and finally accumulating in the cell membrane (Ibrahem *et al.*, 2018, Siddiqi *et al.*, 2018). The antimicrobial properties of ZnO-NPs have led to its application in agriculture, medicine, biomedicine, pharmaceuticals, biotechnology and food packaging (Raj & Jayalakshmy, 2015, Khatami *et al.*, 2018, Siddiqi *et al.*, 2018). ZnO-NPs greatly absorb UV-light and as such have considerable applications in textile, deodorants, cosmetics and sunscreen production (Basnet *et al.*, 2018, Jiang *et al.*, 2018). Zinc oxide nanoparticle's high binding energy property allows its use in photoelectronics, acoustic devices commonly used for high frequency filtering, field emitter and photovoltaic cells (Basnet *et al.*, 2018). Dyes and toxic compounds that are responsible for water contamination can be decomposed by ZnO-NPs, due to its high photocatalytic activity (Santhoshkumar *et al.*, 2017, Bhuyan *et al.*, 2018). In the

ceramic industry, zinc oxide nanoparticle is a significant material used due to its hardness, rigidity and piezoelectric constant (Matinise *et al.*, 2017).

2.10 Iron oxide nanoparticles

Iron oxides are naturally occurring complex chemical compounds whose functions are dependent on size, shape and thermodynamic properties (Guo & Barnard, 2013, Wu *et al.*, 2015, Iacob *et al.*, 2016). Iron oxide is used in various industrial applications (Jolivet *et al.*, 2004), and acts as a reagent in different ecosystem biogeochemical processes (Guo & Barnard, 2013, Sangaiya & Jayaprakash, 2018). Chemically, iron oxide comprises of oxygen and iron. It occurs as a divalent or trivalent compound or as a combination of both (Egirani, *et al.*, 2018). Amongst the widely known general oxides of iron, the haematite (α -Fe₂O₃), magnetite (Fe₃O₄) and maghemite (γ -Fe₂O₃) are mostly studied (Wu *et al.*, 2015, Ali *et al.*, 2016). This is due to the fact that they have detailed distinctive biochemical, magnetic, and catalytic properties that allow their applicability in various fields (Wu *et al.*, 2015).

Haematite (α -Fe₂O₃) is weakly ferromagnetic at room temperature (Martinez *et al.*, 2015), an n-type semiconductor and the most stable iron oxide (Guo & Barnard, 2013, Sangaiya & Jayaprakash, 2018). It has a band gap of 2.3 eV, with a conduction band that is made up of empty d-orbitals of Fe³⁺ and valence band comprised of occupied 3d crystal field orbitals of Fe³⁺ with some mixture from the O 2p non-bonding orbital (Zainullina *et al.*, 2006, Katikaneani *et al.*, 2016). Magnetite (Fe₃O₄) is ferromagnetic, meaning that even in the presence or absence of an external magnetic field, they exhibit spontaneous magnetization (Assa *et al.*, 2016). It can exist as both divalent and trivalent ions and can be both n-type and p-type semiconductors (Wu *et al.*, 2015, Liu & Di Valentin 2017, Sequeira 2018). Maghemite (γ -Fe₂O₃) is an n-type

semiconductor with a band gap of 2.0eV and shows ferromagnetic properties (Assa *et al.*, 2016).

Iron oxides have various industrial, biomedical and biotechnological applications (Assa *et al.*, 2016). Figure 2.9 shows a schematic representation of haematite, magnetite, and maghemite.

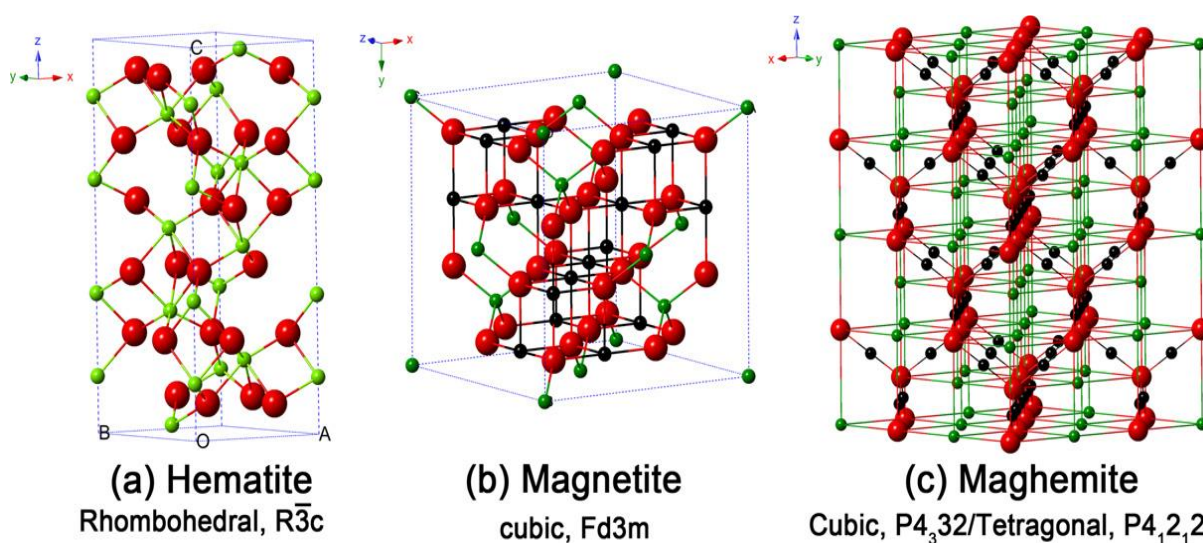


Figure 2.9: Schematic representation of (a) Haematite (α -Fe₂O₃) (b) Magnetite (Fe₃O₄) (c) Maghemite (γ -Fe₂O₃). (Wu *et al.*, 2015)

Magnetic metallic nanoparticles, composed of magnetic elements and their oxides, (Assa *et al.*, 2016) have received great attention from researchers recently because of their characteristic structural changes (Herrera-Becerra *et al.*, 2007, Samrot *et al.*, 2018). Nanoparticles of iron oxides are remarkable transition metal oxide nanoparticles that exhibit antibacterial, biocompatibility and magnetic properties that allow their usage in the treatment of diseases and infections (Chifiriuc & Grumezescu, 2016, Vasantharaj *et al.*, 2018). The synthesis of iron oxide nanoparticles (Fe₃O₄

(magnetite) or –same (maghemite) can lead to the production of very small sized (10-20 nm) particles with superparamagnetic properties (Assa *et al.*, 2016, Arais *et al.*, 2018). Size, shape and stability affect functionality of the synthesized iron oxide nanoparticle (Arias *et al.*, 2016). Concurrently, the shape, size and make-up of the iron oxide nanoparticle is dependent on parameters like the type of iron salt used in synthesis, the pH value, the precursor used and the ionic strength of the medium involved in the experiment (Wu *et al.*, 2015, Assa *et al.*, 2016).

The superparamagnetic and biocompatibility properties of iron oxide nanoparticles make their application in biotechnology and medicine feasible (Assa *et al.*, 2016, Arais *et al.*, 2018, Sequeira 2018, Gul *et al.*, 2019, Ansari *et al.*, 2019). Other properties of iron oxide nanoparticles which include high surface area, band gap, easy separation under external magnetic fields and its physical and chemical stability, boost the application of iron oxide nanoparticles in various fields (Karlsson *et al.*, 2015, Ali *et al.*, 2016, Khalil *et al.*, 2017). The production of iron oxide nanoparticles can be done through the chemical, physical or biological route of synthesis (Arais *et al.*, 2016, Iacob *et al.*, 2016). Common chemical methods used include thermal decomposition, hydrothermal synthesis, reverse micelles, co-precipitation, micro-emulsion technology, sol–gel synthesis, sonochemical reactions, thermolysis and hydrolysis of precursors, electrospray synthesis, flow injection synthesis and colloidal chemistry-based methods (Assa *et al.*, 2016, Ansari *et al.*, 2019).

Nanoparticles produced using some of these chemical methods show compromised magnetic properties, are easily agglomerated, poorly distributed, and come in uneven sizes (Arais *et al.*, 2016). The methods allow for the production of toxic harmful wastes (Sorbiun *et al.*, 2018). The physical method of synthesis is usually costly and requires a lot of energy (Khalil *et al.*, 2017, Chauhan & Upadhyay, 2019). The biological route

creates room for the use of plants or other biological agents (Deshmukh *et al.*, 2019). Due to their rich phytochemical bioactive components, researchers tend to use more medicinal plants in biological synthesis of nanoparticles (Khalil *et al.*, 2017). Prolonged storage of iron oxide nanoparticles leads to agglomeration and reduced energy linked to its high surface to volume ratio (Wu *et al.*, 2015, Gul *et al.*, 2019). Iron oxide nanoparticles if not uncapped are very reactive and prone to oxidation which can damage its magnetic property (Makarov *et al.*, 2014, Assa *et al.*, 2016, Gul *et al.*, 2019).

2.10.1 Green synthesis of iron oxide nanoparticles

Iron oxide nanoparticles produced using the green route are more stable, less toxic to humans and depict excellent antimicrobial properties (Khalil *et al.*, 2017, Devi *et al.*, 2018). It is, therefore, an efficient, biocompatible, safe and cheap method of nanoparticle production (Namvar *et al.*, 2014, Arias *et al.*, 2018). This involves the use of biological material such as plants, enzymes, and microorganisms in synthesis (Devatha *et al.*, 2016, Chauhan & Upadhyay, 2019). The biological substances contain active biomolecules that act as reducing, capping and stabilizing agents (Khalil *et al.*, 2017). The availability of plants and plant parts has permitted extensive research work to be carried out on the synthesis of nanoparticles (Khalil *et al.*, 2017).

Numerous plants and their parts have been used in Fe₃O₄-NPs synthesis (Chauhan & Upadhyay 2019). Khalil *et al.*, (2017) reported the synthesis of highly crystalline pure iron oxide maghemite nanoparticles via aqueous extract of *Sagretia thea* (Osbeck). Other plants/plant parts that have been used in Fe₃O₄-NPs synthesis include *Eichhornia crassipes* (Jagathesan & Rajiv, 2018), *Lawsonia inermis* (Henna) (Chauhan & Upadhyay, 2019), *Hordeum vulgare* and *Rumex acetosa* (Makarov *et al.*,

2014). Fenugreek seed extract (Deshmukh *et al.*, 2019), *Excoecaria cochinchinensis* leaves (Lin *et al.*, 2018), *Platanus orientalis* (Devi *et al.*, 2018), Alfalfa (Herrera-Becerra *et al.*, 2007), *Ruellia tuberosa* leaf extract (Vasantharaj *et al.*, 2018), and *Azadirachta indica* leaf extract (Samrot *et al.*, 2018) have also been used.

2.10.2 Properties of iron oxide nanoparticles

Iron oxide nanoparticles have presented interesting physical, chemical, thermal, electrical magnetic and mechanical properties, in addition to appropriate surface characteristic features (Sundaram *et al.*, 2012, Mahdavi *et al.*, 2013). Most properties of iron oxide nanoparticles are size and shape dependent (Wu *et al.*, 2015). Magnetic Fe₃O₄-NPs are inexpensive to produce, physically and chemically stable, biocompatible, and environmentally safe (Wu *et al.*, 2015, Arias *et al.*, 2018). In addition to its magnetic property, Fe₃O₄-NPs also show semiconducting properties, and low toxicity which also enables its diverse application in the biomedical field (Sangaiya & Jayaprakash, 2018, Vallabani & Singh, 2018). Based on the magnetic properties, Fe₃O₄-NPs can either exhibit diamagnetism, paramagnetism, ferromagnetism, antiferromagnetism, or ferrimagnetism (Ansari *et al.*, 2019).

2.10.3. Applications of iron oxide nanoparticles

Iron oxide nanoparticles, especially those made up of magnetite (Fe₃O₄) and maghemite (γ-Fe₂O₃), exhibit biocompatible and super paramagnetic properties, which allow their application in biomedicine (Assa *et al.*, 2016, Ansari *et al.*, 2019). The application of iron oxide nanoparticles in this field includes their use in safe labelling of endothelial progenitor cells, magnetic resonance imaging, cell separation and detection, tissue repair, magnetic hyperthermia and drug delivery (Arias *et al.*, 2018, Devi *et al.*, 2018, Deshmukh *et al.*, 2019). Iron oxide nanoparticles are applied

in wastewater treatment (Samrot *et al.*, 2018, Chauhan & Upadhyay, 2019). Wang *et al.* (2014) reported the application of iron oxide synthesized from eucalyptus leaf extracts in a swine wastewater and observed the removal of 71.7% of total nitrogen, 30.4% of total phosphorus, and 84.5% of carbon oxygen demand. In addition, iron oxide nanoparticles are relevant in the textile, paint, and cosmetic industries (Khalil 2017). These nanoparticles have shown potential in environmental remediation to control environmental pollution (Saif *et al.*, 2016, Vasanthara *et al.*, 2018), and in diagnostic and material engineering (Khalil *et al.*, Gul *et al.*, 2019). The application of iron oxide nanoparticle extends to its use as a nanocatalyst, heavy metal nanoabsorbents, dyes, and as antibiotic degraders and nanobiocides (Vasantharaj *et al.*, 2018, Samrot *et al.*, 2018, Lin *et al.*, 2018). Furthermore, iron oxide nanoparticles have been used as high-performance anodes in lithium ion batteries, photoelectrochemical cells, high performance CO gas sensors, catalysts for the decomposition of phenols and in the oxidation of benzyl alcohols with periodic acid (Devi *et al.*, 2018). They are also used as carriers for anticancer, anti-inflammatory, and anticonvulsant agents. They are used in the management of pathogenic microbes as antibacterial and antifungal agents (Arias *et al.*, 2018, Vasanthara *et al.*, 2018).

2.11 Antimicrobial activity/mechanism of metal oxide nanoparticles.

Metals and metal ions are fundamental for life functions and biological processes of organisms (Porcheron *et al.*, 2013). Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Colbalt (Co), Nitrogen (N), Copper (Cu), Zinc (Zn), Selenium (Se) and Molybdenum (Mo) are essential metals required for normal functioning of organisms, but when in excess they can cause harmful effects to the cell (Lemire *et al.*, 2013). Oxidative stress, disruption

of protein synthesis or membrane, electrostatic interactions that alter the prokaryotic cell wall and enzyme or DNA pathways through ROS production are reported mechanisms of antimicrobial action of metal and metal oxides (Lemire *et al.*, 2013, Gold *et al.*, 2018). Formation of biofilm on membrane surfaces follows the planktonic and initial interaction phase, followed by development and maturation phase and finally the bacteria detachment phase (Zunita *et al.*, 2018). Prevention of biofilm formation and attachment can be minimized by washing, scrubbing or by the use of a biocidal chemical substance (Galié *et al.*, 2018). Oxides of metals have the capability of serving as a biocide to control pathogenic microorganism and prevent primary interaction and development stages of biofilm formation (Wang *et al.*, 2017, Dzianach *et al.*, 2019).

Metal oxide nanoparticles (MeO-NPs) however, possess interesting antibacterial/biocidal properties which are currently being explored as a solution to the challenge of antimicrobial resistance and biofilm formation exhibited by organisms (Azam *et al.*, 2012, Wang *et al.*, 2017, Zunita *et al.*, 2018). MeO-NPs display specific interaction with bacterial growth population and basic cell components (Gold *et al.*, 2018). The antibiotic resistance mechanism displayed by bacteria is not pertinent to nanoparticles, because nanoparticles, on direct contact with bacteria, penetrate the cell wall, and give off several mechanisms of action such as damage to the membrane and bacterial cell wall, damage to proteins and internal components of bacteria, release of ions, DNA damage and oxidative stress. To combat this, the bacteria would have to also give off simultaneous gene mutation, which is highly unlikely (Wang *et al.*, 2017, Kadiyala *et al.*, 2018, Niño-Martínez *et al.*, 2019). Besides their excellent antimicrobial properties, MeO-NPs also portray selective toxicity to normal and cancer cell (Rasmussen *et al.*, 2010).

The extent of MeO-NPs bactericidal action on bacteria would however depend on their size, shape, stability and concentration used on a bacterial population (Hrenovic *et al.*, 2012, Dizaj *et al.*, 2014). The mechanism by which MeO-NPs exert their antibacterial activity is still an ongoing research study and in addition, several nanoparticles are being developed and there is a need to identify the most promising candidate and also develop a standard protocol for evaluation of the antimicrobial efficiency of MeO-NPs (Dizaj *et al.*, 2014, Kadiyala *et al.*, 2018). Owing to their small size, diverse morphology and immense surface area per unit volume, MeO-NPs can interact with bacteria and biofilm, inducing toxicity through different mechanisms (Kadiyala *et al.*, 2018, Gold *et al.*, 2018). Reported mechanisms of action of MeO-NPs include contact with the nanoparticle, which incites different effects both within and outside the bacterial cell, reactive oxygen specie formation (ROS), electrostatic interactions, accumulation and release of ions (Jeevanandam *et al.*, 2019, Vega-Jiménez *et al.*, 2019). Gold *et al.* (2018) report that the first step of antimicrobial action taken by zinc oxide nanoparticle is by bacterial membrane attachment, which causes ROS formation, resulting in “pitting” and finally cell damage.

In a study carried out by Liu *et al.* (2009), the antimicrobial mechanism of action observed on *E. coli* O157:H7 treated with zinc oxide nanoparticles from Raman spectroscopy, TEM and SEM images is destruction of cell membrane’s lipid and protein, which leads to leakage of intracellular contents and cell membrane disorganization. Chen *et al.* (2019) investigated the bactericidal activity of *Carica papaya* biosynthesized copper oxide nanoparticles (CuO-NPs) against *Ralstonia solanacearum*. TEM images showed damage to cell morphological structure. They also reported a reduced quantity of cellular components on CuO-NPs treated cells.

Antimicrobial properties of magnesium oxide nanoparticles are linked to their exclusive oxide vacancies and crystalline structures (Krishnamoorthy *et al.*, 2012, Tang *et al.*, 2014, Chen *et al.*, 2019). Nguyen *et al.* (2018) reported the disruption of *S. epidermis* biofilm, 24 hours after addition of 1.6mg/ml of magnesium oxide nanoparticles. TEM images revealing bactericidal mechanism of copper oxide nanoparticles (CuO-NPs) as damage to cell morphological structure was reported by Chen *et al.*, (2019). They also cited reduced expression of cellular components of *Ralstonia solanacearium* cells treated with CuO-NPs. Absorption of nanoparticles by bacteria can lead to depolarization of cell walls, making the cell wall more permeable, allowing unregulated flow of cellular content and the cell wall also becomes hazy signifying disintegration of the cell wall (Slavin *et al.*, 2017).

From a study, Yan *et al.* (2019) presumed that the main mechanism of action of Ag-NPs is the induction of oxidative stress (ROS) and membrane. Figure 2.10 is a diagrammatical representation of proposed mechanisms of action of metal oxide nanoparticles while Figure 2.11 is an illustration of some antibacterial mechanism of action exhibited by nanoparticles.

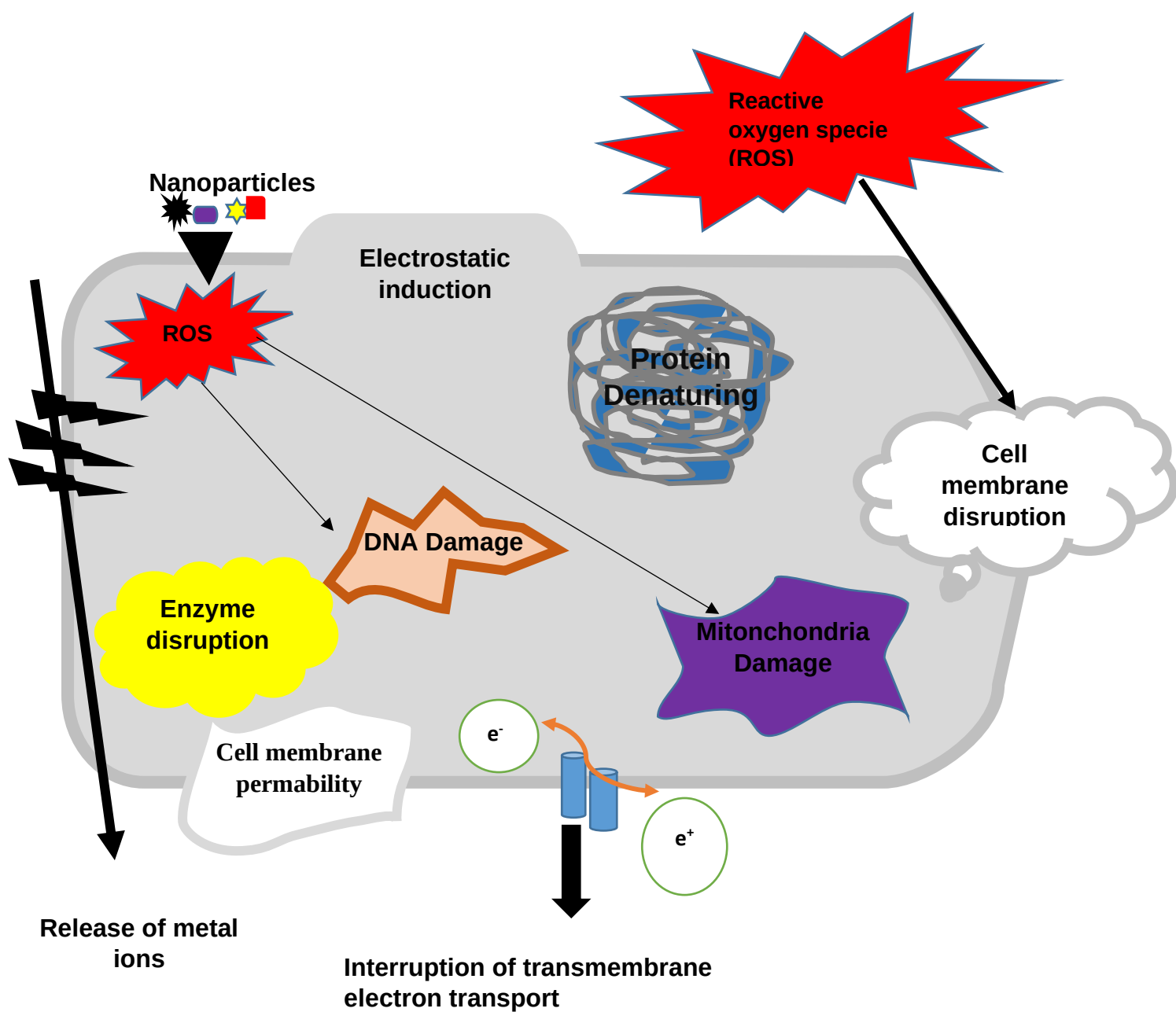


Figure 2.10: Diagrammatical representation of the proposed metal oxide nanoparticle mechanism of action. Adapted from (Kwon & Huh 2011, Vega-Jiménez et al., 2019)

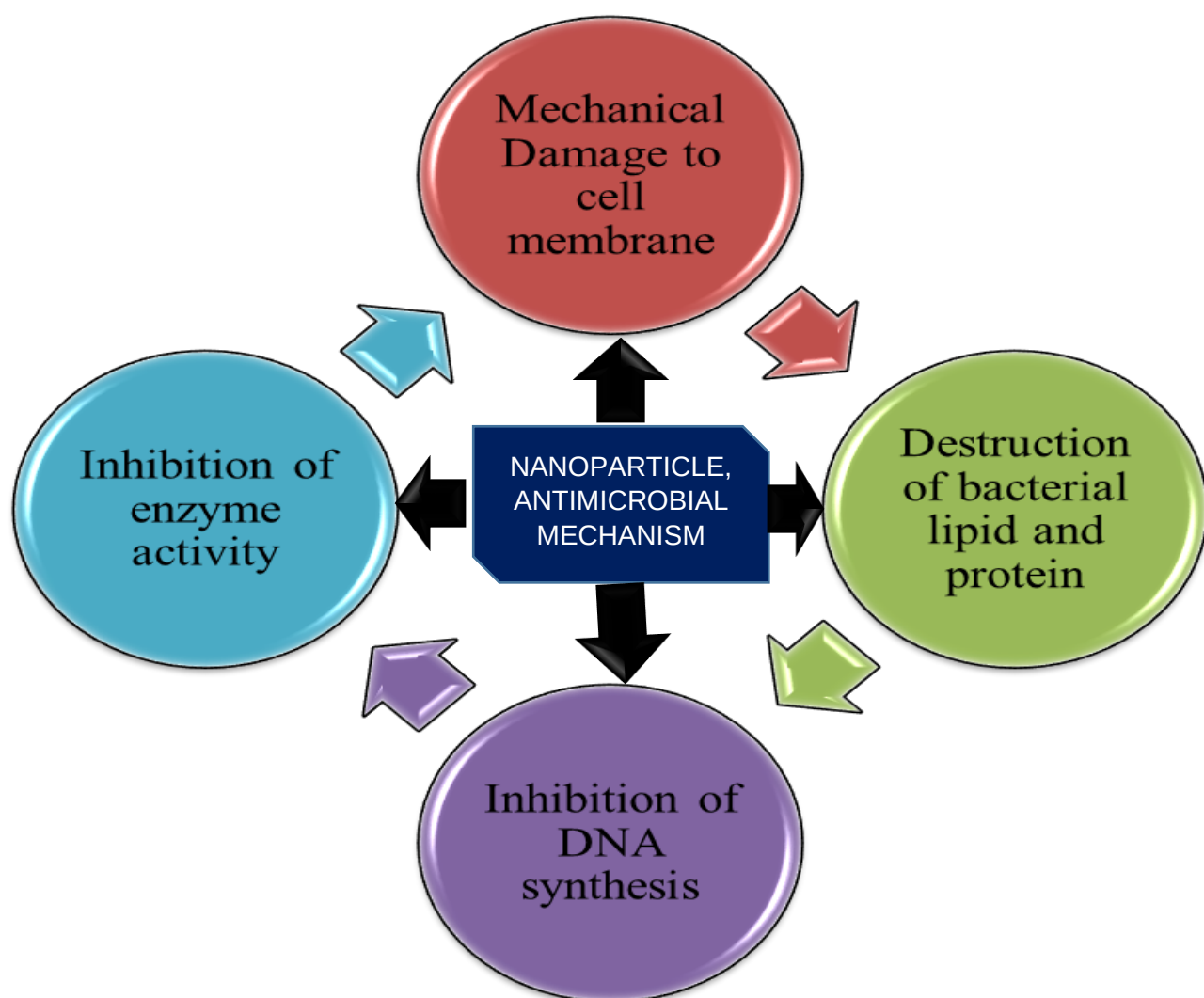


Figure 2.11: Some antibacterial mechanism of action exhibited by nanoparticles.

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CHAPTER THREE

Zinc oxide nanoparticles synthesized from plants: potential agents for antimicrobial applications

3.1 Abstract

The treatment of infections caused by bacteria pathogens is typically achieved through the administration of antibiotics. In recent years, bacterial pathogens have developed resistance against almost all conventional antibiotics that are used routinely for therapeutic processes. An alternative to antibiotic treatment is the use of antimicrobial nanoparticles known as nanoantibiotics. Nanoparticles can be synthesized using different chemical and physical methods, but the use of the biological synthetic technique is currently gaining significant attention. This is due to its simplicity, cost effectiveness, biocompatibility and its potential to be applied in a wide variety of applications. Moreover, physical and chemical synthetic protocols require more expensive equipment and usually produce chemicals that are generally toxic to humans and the environment. Biological synthetic processes make use of biomolecules from plant extracts, microorganisms, and more recently agro wastes, enzymes or plant and microbial pigments in order to produce nanoparticles. The production of nanoparticles from extracts of medicinal plants is rewarding, as such plants have been used for decades even in rural communities worldwide for the treatment and management of a variety of infections in both humans and animals. Most recently, zinc oxide nanoparticles (ZnO-NPs) have been reported to possess antifungal, antibacterial, wound healing, and antioxidant properties. This review focuses on the synthesis and characterisation of ZnO-NPs from plants and the

evaluation of their antimicrobial properties to provide possible evidence of their usage in bio-control applications.

3.2 Introduction

Nanoparticles (NPs) are small sized particles ranging from 1- 100 nanometres in diameter (Ahmed *et al.*, 2016, Khan *et al.*, 2017, Santhoshkumar *et al.*, 2017), that possess excellent and unique properties due to their large surface area to volume ratio (Iravani, 2011, Ealias & Saravanakumar, 2017, Fawcett *et al.*, 2017). The unique physicochemical characteristics of nanoparticles such as electrical and thermal conductivity, catalytic activity, light absorption and scattering resulting in enhanced performance over their bulk counterparts have drawn great attention, especially with recent technological advancements (Ibrahim *et al.*, 2016, Jeevanandam *et al.*, 2018). Based on their material makeup, nanomaterials can be classified as carbon-based, inorganic based, organic based or composite based nanoparticles (Ealias & Saravanakumar, 2017, Khan *et al.*, 2017, Jeevanandam *et al.*, 2018). Carbon based nanomaterials contain carbon, and are mostly fabricated from laser ablation, arc discharge and chemical vapour deposition methods (Elliot *et al.*, 2013, Jeevanandam *et al.*, 2018). They, therefore, occur as hollow tubes, ellipsoids or spheres and representatives include fullerenes (C_{60}), carbon nanotubes (CNTs), carbon nanofibers, carbon black, graphene (Gr) and carbon ions (Kumar & Kumbhat, 2016, Ealias & Saravanakumar, 2017, Khan *et al.*, 2017,).

Combinations of organic and inorganic materials make up the inorganic based hybrid nanomaterials. Inorganic nanoparticles comprise of the metal and metal oxide nanoparticles (Ealias & Saravanakumar, 2017, Jeevanandam *et al.*, 2018, Umamaheswari *et al.*, 2018). Examples include silver (Ag), iron oxide (Fe_3O_4), titanium

oxide (TiO₂), copper oxide (CuO), and zinc oxide (ZnO), known for their active microbial properties (Beyth *et al.*, 2015, Kahraman *et al.*, 2018) while organic based nanoparticles are made up of organic matter such as lipids or polymeric compounds (Kumar and Lal, 2014, Jeevanandam *et al.*, 2018). However, composite based nanomaterials are either comprised of combinations of carbon based and metal based materials, or organic based materials, with forms of metal, ceramic or polymer bulk material (Calandra *et al.*, 2013, Jeevanandam *et al.*, 2018). Based on their overall forms or dimensions, these materials can either be zero dimensional (0D), one dimensional (1D), two dimensional (2D) or three dimensional (3D) (Tiwari *et al.*, 2012, Bhatia *et al.*, 2016, Khan *et al.*, 2017). The length, height, and breadth in zero dimensional nanomaterials are fixed at a point, e.g. nanotube. For one dimensional such as graphene, it is made up of one parameter, while two dimensional has length and breadth, e.g. the carbon nanotube. For three dimensional nanoparticles like the gold nanoparticle, all three parameters are in place, i.e. length, height and breadth (Ealias & Saravanakumar, 2017). Based on their origin, nanoparticles are classified as natural or synthetic (Hochella *et al.*, 2015, Sharma *et al.*, 2015, Jeevanandam *et al.*, 2018). Naturally occurring nanoparticles, as the name implies, are formed through natural processes and are found everywhere in the universe (Hochella *et al.*, 2015), while synthetic (engineered) nanomaterials are produced by mechanical grinding, engine exhaust and smoke, or are synthesized by physical, chemical, biological or hybrid methods (Hochella *et al.*, 2015, Sharma *et al.*, 2015, Jeevanandam *et al.*, 2018).

Three different techniques, namely physical, chemical or biological processes, can be used in the synthesis of nanoparticles (Kumar *et al.*, 2015, Saba, 2015, Khan *et al.*, 2017). However, the syntheses of nanoparticles using physical and chemical

processes are faced with many challenges that range from the use of costly equipments, high energy requirements, low material conversion as well as the production of hazardous waste from chemical reducing agents used during the processes (Shanmugavadivu *et al.*, 2014, Kumar *et al.*, 2015). In addition, chemical techniques expose humans to toxic chemicals that have severely harmful effects, or result in environmental contamination (Kumar *et al.*, 2015, Adelere & Agbaje 2016). It has been documented that chemically synthesised nanoparticles have the ability to retain toxic chemical surface impurities, rendering them unfit for most medical applications (Vidya *et al.*, 2013, Saba, 2015, Fawcett *et al.*, 2017). Moreover, the need to engage in challenging and wasteful purifications, the addition of stabilizing agents due to the undesirable cluster of chemically produced nanoparticles, as well as the high cost of production, amongst other problems, reduce the utilization of chemical synthetic processes (Shanmugavadivu *et al.*, 2014, Kahraman *et al.*, 2018). Based on these challenges, the biological technique, which offers more advantages due to its cost effectiveness, simple to perform protocols, safety, eco-friendliness and biocompatibility, is most often preferred over the conventional chemical and physical methods, especially as the final products are widely used in a variety of applications (Saba, 2015, Adelere and Agbaje, 2016).

The procedures that are designed to synthesise nanoparticles using biological techniques make use of plants and plant extracts, microorganisms as well as organic products (Aziz *et al.*, 2016, Ibrahim *et al.*, 2016, Agarwal *et al.*, 2018). Zinc oxide is an essential inorganic white powder, with a wide and direct band-gap semiconducting characteristic (Geetha, *et al.*, 2016 Matinise *et al.*, 2017) and it is insoluble in water. It holds some distinctive physical, biological and chemical properties (Vijayakumar *et al.*, 2018) such as high chemical stability, high electrochemical coupling coefficient,

antibacterial properties, broad range of radiation absorption, paramagnetic characteristic and high photostability (Ludi & Niederberger, 2012, Bhuyan *et al.*, 2015; Basnet *et al.*, 2018).

Different protocols can be employed in the synthesis of ZnO-NPs (Kolekar *et al.*, 2013, Dobrucka & Długaszewska 2016, Singh *et al.*, 2017). Synthesized ZnO-NPs have been shown to have varying unique properties and can be applied in various fields (Rouhi *et al.*, 2013, Santhoshkumar *et al.*, 2017). They have shown not just antimicrobial properties but also anti-inflammatory and anti-cancerous properties (Rouhi *et al.*, 2013, Jamdagni *et al.*, 2018, Umar *et al.*, 2018).

3.3 Zinc oxide nanoparticles

Broad attention is being directed to zinc oxide nanoparticles by many researchers due to their extensive applicability and excellent electrical, chemical, optical and magnetic properties (Prashanth *et al.*, 2015, Raj & Jayalakshmy, 2015, Vandana *et al.*, 2018). Zinc oxide (ZnO) is an n-type semiconducting inorganic metal oxide that has O₂ holes and interstitial zinc matrix which allows it to absorb UV rays with a wavelength shorter than 380nm (Gluba *et al.*, 2013, Agarwal *et al.*, 2017, Karaköse *et al.*, 2017, Nonomura 2017, Akbarian *et al.*, 2018, Basnet *et al.*, 2018, Fu & Fu, 2018). The wide band gap energy of 3.37 eV, possessed by zinc oxide material enables its usage in optoelectronics turf (Hussain *et al.*, 2013, Vandana *et al.*, 2018, Vijaykumar *et al.*, 2018). At room temperature, ZnO has a large exciton binding energy of 60 meV (Geetha *et al.*, 2016, Matinise *et al.*, 2017, Akbarian *et al.*, 2018, Khatami *et al.*, 2018). This ensures its efficient luminescent and photovoltaic characteristics (Hussain *et al.*, 2013, Basnet *et al.*, 2018). Generally, zinc oxide is a group II-VI multifunctional material of low toxicity (Geetha *et al.*, 2016, Santhoshkumar *et al.*, 2017,

Mohammadian *et al.*, 2018, Vijaykumar *et al.*, 2018). It has a high refractive index of about 1.9, is inexpensive and has antibacterial properties (Salem *et al.*, 2015, Mohammadian *et al.*, 2018).

Zinc oxide possesses other unique chemical and physical properties such as electrochemical and thermal stability, broad radiation absorption range, high photostability, paramagnetic nature, large piezoelectric constant, and strong pyroelectric coefficient (Hussain *et al.*, 2013, Kołodziejczak-Radzimska and Jesionowski 2014., Karaköse *et al.*, 2017, Vandana *et al.*, 2018). In human body tissue, zinc is an essential element found in trace amounts and it is a major constituent of various enzymes (Jiang *et al.*, 2018, Siddiqi *et al.*, 2018). Most physiological functions in eukaryotes such as cellular signal recognition, second messenger metabolism, and protein kinase amongst others are moderated by zinc (Kambe *et al.*, 2015, Siddiq *et al.*, 2018). Structurally, zinc oxide appears as a hexagonal wurtzite crystal (Ludi & Niederberger, 2013, Geetha, *et al.*, 2016) and has been declared as a safe material by the US food and drug administration (Bhuyan *et al.*, 2015, Jiang *et al.*, 2018, Siddiqi *et al.*, 2018, Vijaykumar *et al.*, 2018). Reports indicate that zinc oxide nanoparticles have been produced following chemical, physical or biological techniques. (Ochieng *et al.*, 2015, Prashanth *et al.*, 2015, Karaköse *et al.*, 2017, Santhoshkumar *et al.*, 2017, Akbarian *et al.*, 2018, Sadatzadeh *et al.* 2018). Green synthesized zinc oxide nanoparticles show versatility and have unique antimicrobial properties (Santhoshkumar *et al.*, 2017, Suresh *et al.*, 2017, Kahraman *et al.*, 2018). Biosynthesized zinc oxide nanoparticles can be applied in ultraviolet diodes, transistors and photovoltaic cells, due to their unusual optical and electronic properties (Karaköse *et al.*, 2017, Santhoshkumar *et al.* 2017).

Owing to the biocompatibility of zinc oxide nanoparticles and their anti-bacterial and disinfecting properties, they are applied in biomedicine and production of skin care ointments (Santhoshkumar *et al.*, 2017, Basnet *et al.*, 2018). Zinc oxide is also used in the cosmetic industry for the production of sunscreen lotion because it is transparent, less adhesive and can absorb UV radiation (Basnet *et al.*, 2018, Siddiqi *et al.*, 2018). Its enhanced pyroelectric property allows its use as a sensor and photocatalyst in the production of hydrogen (Kołodziejczak-Radzimska & Jesionowski, 2014, Raj *et al.*, 2015). Zinc oxide nanoparticles can also be used in many other fields including concrete production, electronics, food preservation, electro technology, and textile production (Jiang *et al.*, 2018, Siddiqi *et al.*, 2018). Depending on the technique followed during nanoparticle synthesis, zinc oxide nanoparticles may appear structurally as nanorods, nanoplates, nanospheres, nanoboxes, hexagonal, tripods, nanowires, nanotubes, nanorings, nanocages, and nanoflowers (Wahab *et al.*, 2012, Sirelkhatim *et al.*, 2015, Baskoutas, 2018). Figure 3 shows a diagrammatical representation of some structures of zinc oxide.

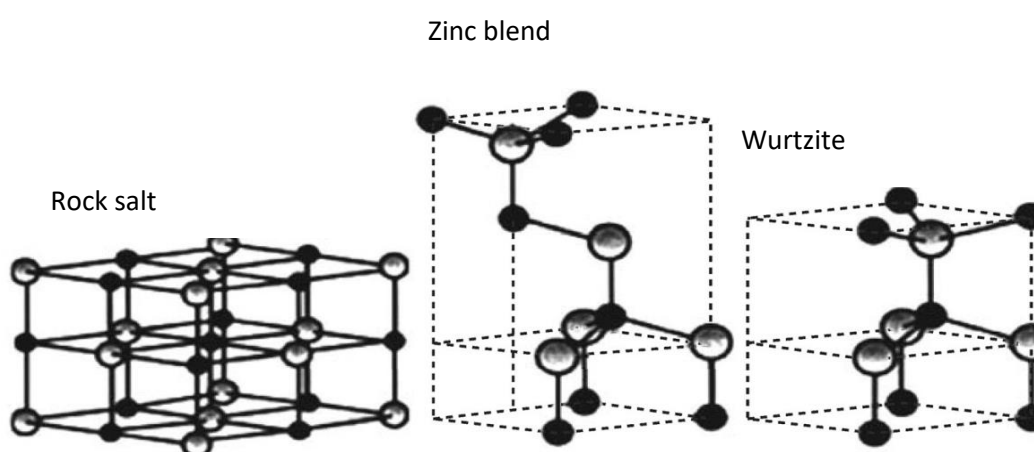


Figure 3.1: Zinc oxide crystal structures. Adapted from (Sirelkhatim *et al.*, 2015)

3.4 Green synthesis of zinc oxide nanoparticles from plant extracts

The green method of nanoparticle synthesis termed biosynthesis has taken over from chemical and physical methods of synthesis (Bhuyan *et al.*, 2015, Dobrucka and Długaszewska 2016, Agarwal *et al.*, 2018). In biosynthesis of nanoparticles, organic sources like plant parts, microorganisms and agro waste, are deployed (Adelere & Agbaje 2016, Agarwal *et al.*, 2018). The use of these organic sources stems from the fact that they are generally eco-friendly, cheaply sourced, biocompatible, can produce large scale end products (Fu & Fu, 2015, Saba 2015) and do not lead to the production or use of harmful toxic elements (Gunalan *et al.*, 2012, Adelere & Agbaje 2016, Jamdagni *et al.*, 2018). Different plant parts such as the seeds, flowers, bark, leaves, and stem are often the preferred choice in biosynthesis (Akhtar *et al.*, 2013, Azizi *et al.*, 2016, Agarwal *et al.*, 2017, Kahraman *et al.*, 2018). This is because of the simplicity and effectiveness of the process of synthesis, and the high yield of product obtainable that is free of impurities and does not cause harm to the living system (Akhtar *et al.*, 2013, Agarwal *et al.*, 2017, Kahraman *et al.*, 2018). In addition, biosynthesis does not require the use of toxic chemicals and synthesis occurs at neutral pH and ambient temperature (Agarwal *et al.*, 2019).

Nanoparticles generated through plant assisted biosynthesis have been shown to be more stable with well-defined shape and size (Vanathi *et al.*, 2014, Bhuyan *et al.*, 2015, Zheng *et al.*, 2016, Umamaheswari *et al.*, 2018). Plant part extracts contain biomolecules or phytochemicals such as amino acids, vitamins, terpenoids, alkaloids, flavones, polyphenol and polysaccharides that have varying functions and properties such as acting as capping and reducing agents (Adelere & Agbaje 2016, Zheng *et al.*, 2016, Umamaheswari *et al.*, 2018). For example, Bhuyan *et al.* (2015) reported the

bio-reduction activity of Neem leaf extract, which contains phytochemicals such as flavones, organic acid and quinone that reduce zinc ion to zinc oxide nanoparticles. Medicinal plant extract biomolecules tend to attach to the surface of generated nanoparticles during the synthesis process and exhibit this surface effect when applied medically (Azizi *et al.*, 2016).

In the synthesis of zinc oxide nanoparticles from plant parts like flower or leaves, the protocol involves aqueous extraction of the plant part. Fresh, healthy leaves or flower parts are collected from available sites and then thoroughly washed with running tap water to remove dust, and rewashed with distilled water. The washed part is shade-dried at room temperature until all moisture is lost. Then the dried plant part is blended to yield fine powder, and a portion is weighed and boiled in distilled water for a few minutes. The aqueous extract is then allowed to cool and filtered using Whatman No. 1 filter paper and stored at 4°C for further use. A measured volume of the extract is mixed with an aqueous solution of precursor (zinc nitrate, zinc acetate or zinc sulphate) while stirring. Sodium hydroxide (NaOH) solution is added, which leads to the formation of a whitish precipitate and the pH is maintained at 12 (Bhuyan *et al.*, 2015, Umamaheswari *et al.*, 2018, Umar 2018). The mixture is centrifuged, and pellets washed and air-dried in a hot air oven to obtain nanoparticle crystals.

Characterization of synthesized nanoparticles is carried out using X-ray diffraction (XRD), Energy Dispersion Analysis of X-ray (EDAX), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Field Emission Scanning Electron Microscopy (FE-SEM), Atomic Force Microscopy (AFM), Thermal-gravimetric Differential Thermal Analysis (TG-DTA), Photoluminescence Analysis (PL), X-ray Photoelectron Microscopy (XPS), Raman Spectroscopy, UV-Visible Diffuse Reflectance Spectroscopy (UV-DRS), and

Dynamic Light Scattering (DLS) (Umamaheswari *et al.*, 2018). The synthesized zinc oxide nanoparticles have shown antimicrobial activities against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhi*, *Bacillus megaterium*, *Staphylococcus epidermidis* (Bhuyan *et al.*, 2015, Azizi *et al.*, 2016, Ibrahim *et al.*, 2017, Murali *et al.*, 2017, Fouda *et al.*, 2018, Vijay Kumar *et al.*, 2018, Umar *et al.*, 2018). Table 3.1 shows various plant extracts used in synthesis of zinc oxide nanoparticles and their applications.

TABLE 3.1: Various plant extracts used in the synthesis of zinc oxide nanoparticles and their applications

S/N	Plant used	Plant part	Precursor used	Size (nm)	Shape(s)	Functional group	Application	Reference
1	<i>Urticadioica</i> L. (Common Nettle)	Leaves	Zinc nitrate hexahydrate	19-28	Hexagonal /Spherical	O-H stretching, C=C stretching, C-N, C=O free carbonyl group and aldehyde C-H.	Antibacterial	Akbarian <i>et al.</i> , 2018
2	<i>Ceropegia candelabrum</i> L.	Leaves	Zinc nitrate hexahydrate	12-35	Hexagonal/Spherical	Carbonyl group (C=O), alkanes (C-H), ester (C-O), aliphatic (C-N),	Antibacterial and antioxidant	Murali <i>et al.</i> , 2017
3	<i>Stevia rebaudiana</i> (Stevia)	Leaves	Zinc acetate	10-90	Rectangular, square, and oval bars shapes	—	Antimicrobial and antileishmanial	Khatami <i>et al.</i> , 2018
4	<i>Zingiber officinale</i> (Ginger).	Root	Zinc acetate dihydrate	30-50	Spherical	O-H, N-H , C=C and C-H bond stretching	-	Raj & Jayalakshmy 2015
5	<i>Andrographis paniculata</i> (Green chireta)	Leaves	Zinc nitrate hexahydrate	57-60	Spherical and hexagonal	O-H stretching of alcohols, C-H bending, C-N stretching vibrations of aliphatic and aromatic amines, C-O stretching of polysaccharides, alcohols and phenolic groups	Anti-oxidant, anti-diabetic, and anti-inflammatory	Rajakumar <i>et al.</i> , 2017
6	<i>Corymbiacitriodora</i> (Lemon scented gum)	Leaves	Zinc nitrate hexahydrate	47	Hexagonal wurtzite	—	Electrochemical biosensor	Zheng <i>et al.</i> , 2016
7	<i>Mangifera indica</i> (mango)	Leaves	Zinc nitrate	45-60	Spherical and hexagonal quartzite	—	Antioxidant and cytotoxicity	Rakeshkumar <i>et al.</i> , 2018
8	<i>Eichhornia crassipes</i> (Water hyacinth)	Leaves	Zinc nitrate	32±4	Spherical shape	—	-	Vanathi <i>et al.</i> , 2014
9	<i>Azadirachta indica</i> (Neem)	Leaves	Zinc acetate dihydrate	9.6-25.5	Spherical	Amide II stretching band, C-N stretching band of aliphatic, aromatic amide, an aliphatic amine, alcohol, phenol, secondary amine, C-H of alkane and aromatics, C = C-H of alkynes, C = O, C-C of an alkane.	Antibacterial and photocatalytic	Bhuyan <i>et al.</i> , 2015
10	<i>Aloe vera</i>	Leaves	Zinc acetate		—	—	Antimicrobial	Gunalan <i>et al.</i> , 2012
11	<i>Sargassum muticum</i> (brown)	Seaweed	Zinc acetate dihydrate	30-57	Hexagonal	C-O vibration associated with a C -O-SO ₃ and hydroxyl groups, (NH) C≡O stretch of protein.	Biomedical	Azizi <i>et al.</i> , 2014

12	<i>Moringa oleifera</i>	Leaves	Zinc nitrate hexahydrate	16-31.9	Spherical	O-H, NH ₂ , H ₃ CO, HO-C≡O and C-H aromatic stretch band, N=C=S stretch of thiamine, C=O, C=N, NH, C=C aromatic stretch, C-H, C=C, N-H	Electrochemistry	Matinise <i>et al.</i> , 2017
13	<i>Trifolium pratense</i>	Flower	Zinc nitrate	60–70	Spherical	Hydroxyl group (O–H). –C–O and –C–O–C, C=C stretching modes	Antimicrobial	Dobrucka & Długaszewska 2016
14	<i>Nyctanthes arbor-tristis.</i>	Flower	Zinc acetate dihydrate	12–32	—	H, OH, N-H stretch, C≡C stretch of alkynes, C≡O, amine –NH Stretch, aromatic amines, C-N stretch of aliphatic amines, alkanes and C-H bend in alkynes,	Antifungal	Jamdagni <i>et al.</i> , 2018
15	<i>Caltropis procera</i>	Fruits and leave		88–100	Hexagonal wurtzite structure	—	antimicrobial	Salem <i>et al.</i> , 2015
16	<i>Euphorbia jatropa</i>	Latex	Zinc Nitrate	6-18	Hexagonal	O-H group stretching of O-H, H-bonded single bridge	-	Geetha <i>et al.</i> , 2016
17	<i>Poncirus trifoliata</i>	Fruit	Zinc nitrate	36.2	Spherical	O–H stretch, N–H bend, –C–H stretch (alkanes), C–H (aromatics) and –C=C–H (alkynes). C=C bend, C–C stretch of aromatic ring, C–N stretch mode in aliphatic amines.	Catalytic	Nagajyothi, <i>et al.</i> , 2013
18	<i>Hibiscus subdariffa</i>	Leaf	Zinc acetate	12-46	Dumbbell shaped	C=C Stretch of aromatic amine, aromatic C-H, C=C-C, -C-C=C and C=C, alcoholic -C-OH, -NH ₂ of secondary amine and -C=O aromatic ring, glycosidic linkage of C-O-C and secondary alcoholic group	antibacterial	Bala <i>et al.</i> , 2015
19	<i>Punica granatum</i> (Pomegranate)	Peel	Zinc nitrate	50-100	Spherical and square	-	Antimicrobial	Mishra & Sharma 2015
20	<i>Passiflora caerulea</i>	Leaves	Zinc acetate	30–50	Hexagonal	OH stretch vibrations, C = C stretch in aromatic ring, C = O stretch in polyphenols, C –N stretch of Amide-I in protein, C –O stretch in amino acid, C –N stretch, C –H bend, C- Alkyl chloride, Hexagonal phase ZnO, N –H stretch amide group, C = C stretch alkane group, C –H bend of alkane group, C –N stretch amine group, C –Br stretch alkyl Halide	-	Santhoshkumar <i>et al.</i> , 2017
21	<i>Psidium guajava</i> (guava)	Leaves	Zinc acetate	21.9	Spherical, hexagonal	O-H stretch, aliphatic C-H stretch, C-O, C-C, C-N, 1° and 2° Alcohol, O-H, C-O, C=C, C-H.	Antibacterial	

22	<i>Azadirachta indica</i>	Leaves	Zinc nitrate hexahydrate	18	Spherical	O-H, C-H stretching of polyols, C-C stretch of aromatic rings, O-H, C-OH vibrations of polyols, C-N stretch, N-H of amines, C-O stretch of carboxylic acid.	Antibacterial	Elumalai <i>et al.</i> , 2015
23	<i>Costus pictus D. Don</i>	Leaves	Zinc nitrate	20–80	Hexagonal and spherical	O–H stretch, C=O, C–F stretch of mono- and poly-fluorinated, aromatic Ring, alcohol C–O stretch, C–H stretch of aromatic aldehyde, carbonyl,	Antimicrobial and anticancer	Suresh <i>et al.</i> , 2018
24	<i>Camellia sinensis</i> (green tea)	Leaves	Zinc acetate dihydrate	16	Hexagonal	O–H stretch in water, water, alcohol and phenols, N–H stretch in amines. C–H stretch in alkanes. O–H stretch in carboxylic acid, C=C stretch in aromatic ring, C=O stretch in polyphenols, C–N stretch of amide-I protein, C–O–C stretching in polysaccharides, C–O stretch in amino acid, C–H out of plane bending	Antibacterial and antifungal.	Senthilkumar & Thirumal 2014
25	<i>Cassia fistula</i>	Leaves	Zinc nitrate hexahydrate	5–15	Hexagonal wurtzite, Sponge-like irregular Shape	-	Photodegradative, antioxidant and antibacterial	Suresh <i>et al.</i> , 2015
26	<i>Aloe barbadensis miller</i>	Leaves	Zinc nitrate	25-40	Spherical	–CO–C–, –C–O– and –C=C–.		Sangeetha <i>et al.</i> , 2011
27	<i>Tectona grandis</i> leaf	Leaves	Zinc nitrate		Spherical	O–H stretch, –C=C stretching vibration of alkynes, C=O hydroxyl (or) carboxyl, nitrate ions,	Biomedical	Senthilkumar <i>et al.</i> , 2017
28	<i>Gossypium hirsutum</i> (Cotton seed plant)	Seed	Zinc acetate dihydrate	18.9-30.5	Hexagonal	stretch hydroxyl group, C = C stretch of the aromatic ring, C-O	-	Sadatzaadeh <i>et al.</i> , 2018
29	<i>Plectranthus amboinicus</i>	Leaves	Zinc nitrate	50-180	Rod shaped	stretch band of zinc and oxygen, C–O, C–OSO ₃	Photocatalytic	Fu & Fu 2015
30	<i>Anchusa Italica</i>	Flower	Zinc acetate dihydrate	8-14	Hexagonal	O–H, C–H, C=O stretch of diisobutyl phthalate, O–H and C–OH vibration	Antimicrobial	Azizi <i>et al.</i> , 2016
31	<i>Atalantia monophylla</i>	Leaves	Zinc acetate dihydrates	30	Hexagonal wurtzite structure	O-H stretch, Hydroxy group and H-bond, C-H, –CN, C=O, C=C, C=H, OH bend, C-H bend in alkane, C-S linkage, O-H stretch, N≡O stretch, C-N of aromatic amines, alkyl halides.	Antimicrobial	Vijayakumar <i>et al.</i> , 2018
32	<i>Allium cepa</i> (onion bulb)	Leafy bulb	Zinc nitrate	20-100	Nano rod	N-H stretch of proteins, O-H stretch, C=O stretching of esters, aromatics, ring C-C stretch of phenyl, alkanes, C-O stretch in ring stretch of	Antimicrobial	Tensingh & Lega 2018

						phenyl, aliphatic amines, alcohols, carboxylic acids, ester, ether, carbohydrate and alkyl halides, N-H bend primary amines, C-O stretch alcohols, carboxylic acids, ester and ethers.		
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3.5 Antimicrobial properties of zinc oxide nanoparticles

Globally, antimicrobial resistance (AMR) continues to pose a major challenge (Sakeena *et al.*, 2018, Vijayakumar *et al.*, 2018). The increase of these resistant microbes can be credited to the inapt antimicrobial drug usage and the unhampered accessibility of antimicrobial agents (Ibrahim *et al.*, 2017, Wang *et al.*, 2017, Sakeena *et al.*, 2018). Multi drug resistant pathogens hinder management of bacterial infections and continue to be a bottleneck in the use of antimicrobial therapy for the treatment of infections (Dabbous *et al.*, 2014, Beyth *et al.*, 2015). Several cases involving methicillin resistant *Staphylococcus aureus*, vancomycin resistant enterococci, penicillin-resistant *Streptococcus pneumoniae* and an increase in infection caused by fluoroquinolone-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant *Klebsiella* species, as well as *Acinetobacter* species have been reported (Jones *et al.*, 2008, Dabbous *et al.*, 2014, Khan *et al.*, 2016, Karaman *et al.*, 2017). Foodborne pathogenic microorganisms when present in food tend to ruin the freshness and longevity (Mostafa, 2015, Rawat, 2015). These microbial contaminants are controlled by the addition of antimicrobial agents during food processing or packaging (Huh & Kwon, 2011, Xie *et al.*, 2011, Khan *et al.*, 2016). Long term solutions to curb the challenge of infectious disease and emergence of antibacterial resistance strains, and also the use of antimicrobials as food additives are therefore required (Moritz & Geszke-Moritz, 2013, Agarwal *et al.*, 2018, Vijayakumar *et al.*, 2018).

Antimicrobial nanomaterials (nanoantibiotics) are being explored currently as an alternative to the use of conventional antibiotics (Jones *et al.*, 2008, Huh & Kwon 2011, Khan *et al.*, 2016, Agarwal *et al.*, 2017, Sorbiun *et al.*, 2018). These small sized materials have been reported to be less toxic, more effective on resistant bacteria,

very competent in drug delivery and have shown diverse enhanced biological functions (Azizi *et al.*, 2016, Ibrahim *et al.*, 2017, Raghunath & Perumal, 2017). Inorganic metal oxide nanoparticles such as silver (Ag), silver oxide (Ag₂O), titanium dioxide (TiO₂), silicon (Si), copper oxide (CuO), zinc oxide (ZnO), gold (Au), calcium oxide (CaO) and magnesium oxide (MgO) are being used because they have shown great antimicrobial activities (Agarwal *et al.*, 2018, Sorbiun *et al.*, 2018), enhanced by their large surface area to volume ratio (Raghupathi *et al.*, 2011, Dizaj *et al.*, 2014, Sirelkhatim *et al.*, 2015, Siddiq *et al.*, 2018).

In ancient Egypt, during the reign of King Pharaoh, records show that zinc oxide has always been used in ointments for the treatment of injuries and boils (Siddiqi *et al.*, 2018, Tensingh & Lega, 2018). It has appealed to many researchers due to its wide-ranging antibacterial activity on different organisms (Salem *et al.*, 2015, Sirelkhatim *et al.*, 2015). However, the mechanism of antibacterial action is not yet fully understood (Dizaj *et al.*, 2014, Sirelkhatim *et al.*, 2015, Raghunath and Perumal, 2017). Some studies have suggested that its antibacterial activity may be linked to the generation of reactive oxygen species (ROS) which include H₂O₂ (hydrogen peroxide), OH⁻ (hydroxyl radicals) and O₂⁻² (peroxide) which could lead to cell wall damage, thus making the bacterial membrane very permeable (Dizaj *et al.*, 2014, Beyth *et al.*, 2015, Sirelkhatim *et al.*, 2015, Raghunath and Perumal, 2017). In addition, proton motive force might be lost, causing internalization of nanoparticles and uptake of toxic dissolved zinc (Dizaj *et al.*, 2014, Sirelkhatim *et al.*, 2015). This eventually leads to weakness of the mitochondria (Raghunath and Perumal, 2017, Agarwal *et al.*, 2018, Siddiqi *et al.*, 2018), outflow of intracellular materials and release in gene expression of oxidative stress which inhibits cell growth and consequently leads to cell death (Bhuyan *et al.*, 2015, Sirelkhatim *et al.*, 2015). Tiwari *et al.* (2018), in a study reported

on the mechanism of ROS production, lipid peroxidation, loss of membrane integrity, membrane disruptions, membrane leakage of reducing sugar, protein and DNA of carbapenem resistant strain of *Acinetobacter baumannii* when treated with synthesized zinc oxide nanoparticles. Another suggested mechanism is electrostatic induction (Raghupathi *et al.*, 2011, Xie *et al.*, 2011, Raghunath and Perumal, 2017). The bacterial cell wall, which is negatively charged, is characterized by its ability to bind or absorb aqueous metal over a large pH range due to its low isoelectric point. Metal oxide NPs which are positively charged. on interaction with bacterial cells cause electrostatic attraction leading to the accumulation of the metal oxide nanoparticle on the cell surface (Dizaj *et al.*, 2014, Raghunath and Perumal, 2017, Jiang *et al.*, 2018, Khezerlou *et al.*, 2018, Sorbiun *et al.*, 2018).

Electrostatic induction is higher in a gram negative bacterial cell due to its lipopolysaccharide high negatively charged region (Sirelkhatim *et al.*, 2015, Wang *et al.*, 2015). As the metal oxide nanoparticle attaches to the bacterial cell wall, its structure is altered, and the membrane becomes highly permeable, allowing more inflow of metal oxide nanoparticles which in turn leads to destruction of lipids in the cell membrane, and ultimately leakage of the cellular content (Agarwal *et al.*, 2018, Jiang *et al.*, 2018, Sorbiun *et al.*, 2018). From SEM and TEM images, zinc oxide acts by first damaging bacterial cell wall before penetrating and accumulating in the cell membrane (Sirelkhatim *et al.*, 2015). Liu *et al.* (2009) reported membrane deformation and intracellular structure disorganization of *E. coli* 0157:H7 that was treated with a TSB media containing 12 mmol^{-1} zinc oxide nanoparticles. The study also revealed many null cells found in the bacterial sample, which suggests the leaking out of the intracellular content due to damage and distortion of the cell membrane. Microbial survival also depends on metal ion homeostasis (Chandrangsu *et al.*, 2017). When

there is an excess of metal and metal ion, toxicity and metabolic function disorder occurs (Jaishankar *et al.*, 2014, Raghunath and Perumal, 2017). Disturbance in metal and metal ion homeostasis is another mechanism by which metal oxide nanoparticles exert their antimicrobial powers (Niño-Martínez *et al.*, 2019). In this case, the metal oxide nanoparticle, on interaction with the bacterial cell, releases toxic and abrasive metal ion by hydrolysis, dissolution or adsorption (Wang *et al.*, 2016). The released scratchy metal ion lyses the bacterial cell wall causing physical damage to the bacterial cell, leading to bacterial cell death (Bhuyan *et al.*, 2015, Sirelkhatim *et al.*, 2015). Zinc oxide nanoparticles also exert antimicrobial efficacy by protein, DNA, or lipid denaturing and enzyme dysfunction, aided by the generation of ROS (Huh & Kwon, 2011, Hsueh *et al.*, 2015, Salem *et al.*, 2015, Agarwal *et al.*, 2018). Pati *et al.* (2014) reported bacterial cell membrane disruption, reduced cell surface hydrophobicity, and downregulation of the transcription of oxidative stress-resistance genes in bacteria by zinc oxide nanoparticles.

Physiochemical properties such as size, shape, charge, and concentration of zinc oxide nanoparticle are known to regulate its antibacterial activity (Raghupathi *et al.*, 2011, Moritz & Geszke-Moritz 2013, Salem *et al.*, 2015, Raghunath and Perumal, 2017, Tensingh & Lega 2018). The smaller the size, the better the antibacterial activity due to its ability to penetrate the membrane easily (Hsueh *et al.*, 2015, Agarwal *et al.*, 2018, Khatami, *et al.*, 2018). High concentration of zinc oxide nanoparticles is essential for antibacterial efficacy as reported by Dobrucka and Długaszewska, (2016). Ibrahim *et al.* (2017) also reported that the inhibitory effect of zinc oxide nanoparticles on *Eschericia coli* and *Staphylococcus aureus* was significantly increased with the increase in concentration. Bhuyan *et al.*, (2015) reported an inhibition in bacterial cell growth resulting from an increase in the concentration of zinc

oxide nanoparticles. The gram negative bacterial cell wall is composed of a peptidoglycan layer, an outer membrane that confers resistance to hydrophobic compounds, and a lipopolysaccharide layer that increases its negative charge (Hajipour *et al.*, 2012, Slavin *et al.*, 2017, Wang *et al.*, 2017). In contrast, the gram positive bacterial cell consists of a thick peptidoglycan layer (Agarwal *et al.*, 2018) attached to teichoic acid and pores that allow the penetration of foreign materials (Hajipour *et al.*, 2012, Wang *et al.*, 2017). The bacterial cell wall aims to protect the cell against mechanical damage as well as offering strength and shape and rigidity (Wang *et al.*, 2017, Agarwal *et al.*, 2018). Due to the lipopolysaccharide, lipoprotein and phospholipid content of a gram negative cell wall, studies have shown that the antimicrobial effect of nanoparticles is less on Gram negative bacteria like *Escherichia coli* and higher on Gram positive bacteria such as *Staphylococcus aureus* (Emami-Karvani & Chehrazai, 2012, Hsueh *et al.*, 2015, Wang *et al.*, 2017). However, other factors such as growth rate, ability to form biofilm, and properties of nanoparticles, can influence susceptibility or resistance of bacteria to nanoparticles apart from its cell wall feature (Hajipour *et al.*, 2012, Wang *et al.*, 2017). Owing to the greater negative charge on Gram negative bacteria than on Gram positive bacteria, electrostatic attraction is higher on gram negative strains (Raghunath and Perumal, 2017).

The toxicity of zinc oxide nanoparticles is focused on damage to the microbial cell where the nanoparticle enters and accumulates in the cytoplasm (Siddiqi *et al.*, 2018). The photocatalytic property of zinc improves its antimicrobial activity (Dizaj *et al.*, 2014). Doping of zinc oxide nanoparticles using other metals like titanium has also been reported to improve its antimicrobial effectiveness (Dizaj *et al.*, 2014, Sirelkhatim *et al.*, 2015, Jamdagni *et al.*, 2018). Zinc oxide nanoparticles have been reported to have antimicrobial activity against gram negative, gram positive bacteria and on high

temperature and pressure resistant spores (Xie *et al.*, 2011, Dizaj *et al.*, 2014, Bala *et al.*, 2015). Table 3.2 shows assumed antimicrobial mechanisms adopted by biosynthesized zinc oxide nanoparticles and proposed biomolecules of plants involved in the synthesis. Figure 3.2 illustrates some applications of zinc oxide nanoparticles.

Table 3.2: Assumed antimicrobial mechanism of biosynthesized zinc oxide nanoparticles

S/ N	Plant used and part	Precursor used	Biomolecules involved in synthesis	Bacteria species	Assumed antimicrobial mechanism	Reference
1	Leaves of <i>Urticadioica L.</i> (Common Nettle)	Zinc nitrate hexahydrate	-	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>	Membrane disruption and high rate of generation of surface oxygen species	Akbarian <i>et al.</i> , 2018
2	Leaves of <i>Ceropegia candelabrum</i>	Zinc nitrate hexahydrate	NH (amine) group	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , and <i>Escherichia coli</i> and <i>Salmonella typhi</i> .	Membrane disruption and high rate of generation of surface oxygen species	Murali <i>et al.</i> , 2017
3	Leaves of <i>Stevia rebaudiana</i> (Stevia)	zinc acetate	Carbohydrates (glycosylated Steviol), amino acids, fat	<i>Leishmaniosis major</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i>	Membrane and cell wall destruction	Khatami <i>et al.</i> , 2018
4	Stem bark of <i>Albizia lebbeck</i>	Zinc nitrate	Carboxylic acid, alcohol, amines.	<i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Klebsiellapneumoniae</i> , and <i>Salmonella typhi</i>	—	Umar <i>et al.</i> , 2019
5	Leaves of <i>Azadirachta indica</i> (Neem)	Zinc acetate dihydrate	Flavones, organic acids, quinines, terpenoids, reducing sugars and phenolic groups	<i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , <i>Escherichia coli</i>	Bacterial membrane penetration and inactivation of respiratory enzymes, outflow of cytoplasmic contents, which eventually damages its membrane and ultimately kills the bacteria	Bhuyan <i>et al.</i> , 2015
6	Leaves of <i>Anchusa italic italica</i>	zinc acetate	Carboxylic acid, carbonyl and alcohols	<i>Bacillus megaterium</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Salmonella typhimurium</i>	Disruption of the cell walls, leakage of cell contents and finally leads to the death of pathogens	Azizi <i>et al.</i> , 2016
7	Leaves of <i>Atalantia monophyll</i>		Tannin, organic acids, terpenoids and Aromatic dicarboxylic acid, amides	<i>Bacillus subtilis</i> MTCC 121, <i>Bacillus cereus</i> MTCC 430, <i>Staphylococcus aureus</i>	Cell membrane Penetration	Vijayakumar <i>et al.</i> , 2018

				MTCC 737, <i>E. coli</i> MTCC 1303, <i>Pseudomonas aeruginosa</i> MTCC 429, <i>Klebsiella pneumoniae</i> MTCC 109, <i>Candida albicans</i> MTCC 227 and <i>Aspergillus niger</i> MTCC 281		
8	<i>Aloe vera</i> leaves	Zinc nitrate	-	<i>S. aureus</i> , <i>Serratia marcescens</i> , <i>Proteus mirabilis</i> , <i>Citrobacter freundii</i> , and fungal strains; <i>Aspergillus flavus</i> , <i>Aspergillus nidulans</i> , <i>Trichoderma harzianum</i> , and <i>Rhizopus stolonifer</i>	Release of oxygen species on the surface of Zinc Oxide, damage to microorganisms. Reaction with hydrogen ions to produce molecules of H ₂ O ₂ . Penetration of cell membrane by generated H ₂ O ₂ and eventual cell death.	Gunalan <i>et al.</i> , 2012
9	<i>Sargassum muticum</i> weed	Zinc acetate dehydrate	Amino, sulfate, carboxyl and hydroxyl groups	-	-	Azizi <i>et al.</i> , 2014
10	<i>Trifolium pretense</i> (red clover) leaves	-	Anthocyanins, phenolic acids and small amounts of tannins, carotene, essential oils and vitamins C and E. T.	<i>S. aureus</i> ATCC 4163, <i>E. coli</i> ATCC 25922, <i>P. aeruginosa</i> ATCC 6749,	Damage of the bacterial cell membrane and the extrusion of the cytoplasmic contents thereby resulting in the death of the bacterium	Dobrucka& Długaszewska (2016)
11	<i>Caltropis procera</i>	zinc nitrate	Flavones, organic acids, and quinones	<i>Vibrio cholerae</i> and enterotoxigenic <i>Escherichia coli</i> (ETEC)	Target of bacterial metabolic pathways	Salem <i>et al.</i> , 2015
12	<i>Hibiscus subdariffa</i> flower	zinc acetate solution	Phenolic compounds,	<i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	ROS generation, plasma membranes permeability, ion leaching, Hydroxylation of cell wall triggered by the hydroxyl group of polyphenols, cell damaged due to ruptured cell wall and pore formation,	Bala <i>et al.</i> , 2015

			flavonoids, saponins, tannins and alkaloids, amines		leakage of ions and cellular materials, and ultimately cell death	
13	<i>Punica granatum peel</i>	Zinc nitrate	-	<i>Proteus vulgaris</i> and <i>Aspergillus niger</i>	Membrane disruption by zinc ion, leakage of proteins and nucleic acid	Mishra & Sharma 2015
14	<i>Passiflora caerulea</i>	Zinc acetate	Amines and alkanes, terpenoids, flavonoids, alkaloids	<i>E.coli</i> , <i>Streptococcus sp.</i> , <i>Enterococcus sp.</i> , <i>Klebsiella spp.</i>	—	Santhoshkumar <i>et al.</i> , 2017
16	<i>Azadirachta indica</i>	zinc nitrate hexahydrate	Amine, alcohol, ketone, carboxylic acid	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus subtilis</i> , <i>Proteus mirabilis</i> , <i>E. coli</i> and fungi strains such as <i>Candida albicans</i> and <i>Candida tropicalis</i>	Generation of reactive oxygen species (ROS), increase of H ₂ O ₂ concentration from the surface of ZnO and cell membrane penetration	Elumalai <i>et al.</i> , 2015
17	<i>Costus pictus D. Don leaves</i>	Zinc nitrate	Glycone steroids, flavoniodes, diosgenin,	<i>Staphylococcus aureus</i> , <i>Bacillus. subtilis</i> , <i>E. coli</i> , <i>S. paratyphi</i> , <i>C. albicans</i> (MTCC 3100) and <i>A. niger</i> (MTCC 1344)	Disruption of bacterial membrane with high rates of surface oxygen species generation and finally cause the death of pathogens -	Suresh <i>et al.</i> , 2018
18	<i>Camellia sinensis</i> (green tea) leaves	Zinc acetate dihydrate	Polyphenols, carboxylic acid, polysaccharide, amino acid and proteins	<i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> and Gram-positive bacteria: <i>Staphylococcus aureus</i>	-	Senthilkumar & Sivakumar 2014
19	<i>Cassia fistula</i>	zinc nitrate hexahydrate	Polyphenols , flavonoids	<i>Klebsiella aerogenes</i> , <i>Escherichia coli</i> , <i>Plasmodium desmolyticum</i> and <i>Staphylococcus aureus</i> .		Suresh <i>et al.</i> , 2015
20	<i>Catharanthus roseus</i>	Zinc nitrate	Phenolic compounds, terpenoids or			

			proteins			
21	<i>Tectona grandis</i> leaf		Phenols and flavonoids	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> and Gram negative (<i>Salmonella paratyphi</i> , <i>Escherichia coli</i>		Senthilkumar <i>et al.</i> , 2017
22	Leafy bulb of <i>Allium cepa</i> (onion bulb)	zinc nitrate	Phenolic acids, polyphenol, and flavonoid	<i>E. coli</i> and <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> and two fungal pathogens; <i>Aspergillus niger</i> and <i>Aspergillus flavus</i> .	Direct contact of ZnO NPs with cell walls, destruction of bacterial cell integrity, liberation of antimicrobial ions mainly Zn ⁺² ions.	Tensingh&Lega 2018

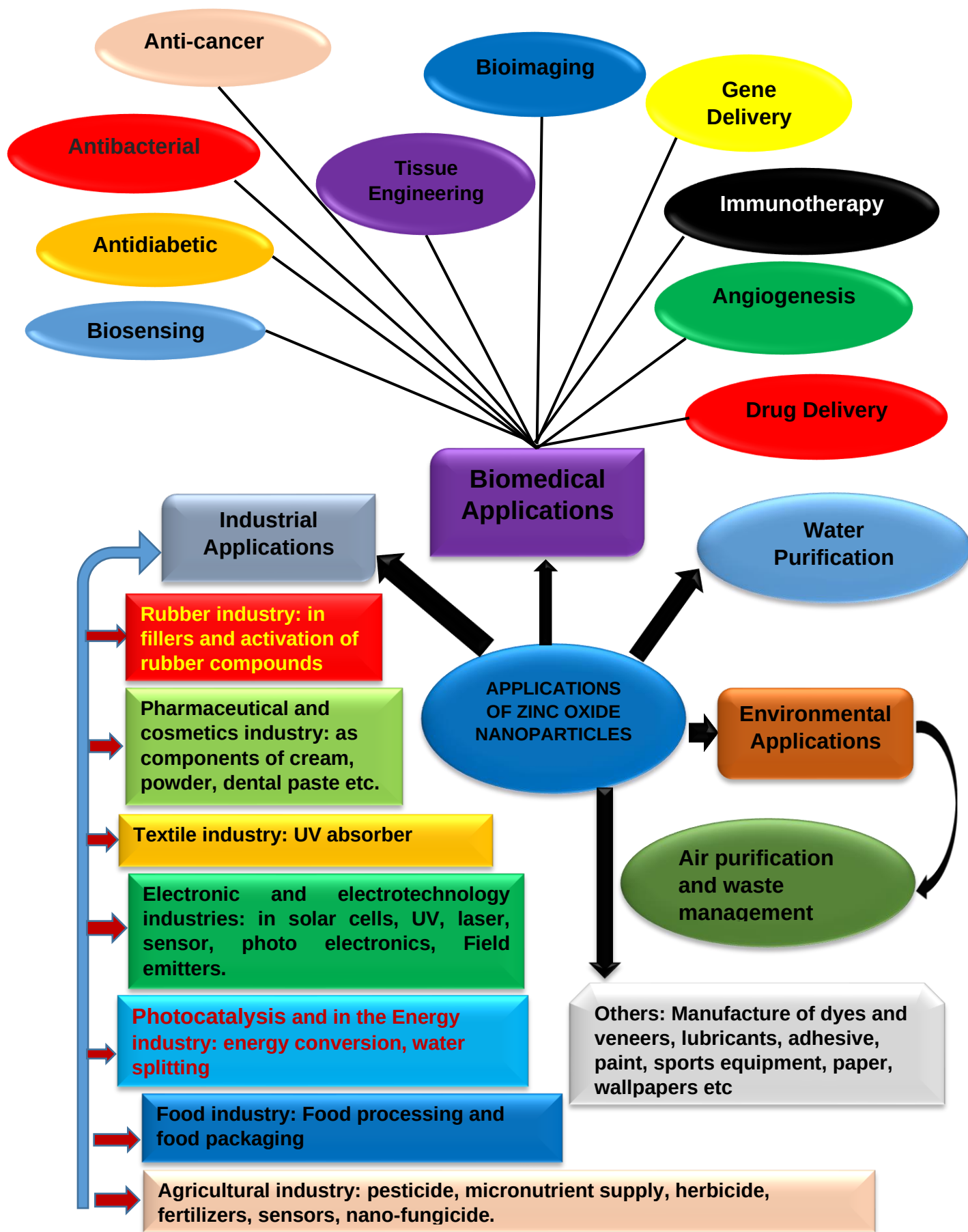


Figure 3.2: Some applications of Zinc Oxide

3.6 Conclusion

A number of studies have been carried out on the varying biomedical applications of zinc oxide nanoparticles. Nano scaled zinc oxide have shown anticancer, antidiabetic, antimicrobial, anti-inflammatory, and wound healing activities. Zinc oxide nanoparticles also play a role in agriculture, food, cosmetics agriculture, energy and other industrial applications. Zinc oxide nanoparticles can be prepared conventionally or unconventionally. However, preparation of nanoparticles via the green route employing plants has been considered as safe, less toxic and allows production of more stable and biocompatible zinc oxide nanoparticles. Different plants and their parts have been used in synthesis, producing enhanced chemical, physical and biological property embedded zinc oxide nanoparticles.

Owing to the ability of zinc oxide nanoparticles to prevent growth or kill off a broad spectrum of microorganisms, they can be used as proficient therapeutics in place of the orthodox antibiotics which microorganisms are garnering resistance against. Ability of zinc oxide to exert antimicrobial effect effectively is reliant on dose concentration and size of synthesized particle. The antibacterial mechanism of action of nanoparticles is not clearly known but studies have shown that nanoparticles show antibacterial ability by causing damage and disorganization of the bacterial cell membrane, generation of ROS which damage lipids, proteins, carbohydrates and cell DNA, release of toxic ions, and protein peroxidation. Zinc oxide nanoparticles particularly prevent efflux pumping of zinc ion, thereby increasing the concentration of local zinc ion.

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CHAPTER 4

Pomegranate leaves and flowers: preliminary phytochemical and antibacterial evaluation

4.1 Abstract

The pomegranate (*Punica granatum*) plant is an ancient, mystical, fruit bearing deciduous plant belonging to the Punicaceae family. The leaves and other plant parts serve as therapeutics in treatment of different ailments. In this study, the flower and leaves of the pomegranate plant were subjected to methanolic and aqueous extraction process and screened for their phytochemical constituents and antimicrobial activity. The active biocompounds found in the extract are tannins, phenols, and alkaloids, which are linked to its antimicrobial and medicinal efficacy. The obtained aqueous and methanolic extracts were tested for antimicrobial efficacy against Gram-positive *Staphylococcus aureus*, *Bacillus cerus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria momcytogenes* and Gram-negative *Aeromonas hydrophilia*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Escherichia coli*, *Salmonella diarizonae*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Results showed that bacterial growth of *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Bacillus cerus*, *Aeromonas hydrophilia*, *Moraxella catarrhalis*, *Escherichia coli*, *Salmonella diarizonae* and *Salmonella typhi* was inhibited by the methanolic and aqueous extracts of leaves and flowers, with the exception of the aqueous flower extract that showed no inhibition.

4.2 Introduction

The use of plants and plant parts in synthesis of nanoparticles is gradually gaining importance in current research and development in material science and technology (Singh *et al.*, 2018). Several plants, especially medicinal plants, have been employed to synthesize various nanoparticles (Saha & Dutta Gupta, 2016, Aritonang *et al.*, 2019). Some medicinal plants and plant parts reported in the synthesis of nanoparticles include, *Passiflora caerulea* L. (Passifloraceae) a medicinal plant native to north America (Santhoshkumar *et al.*, 2017), Chamomile flower (*Matricaria chamomilla* L.), Olive leaves (*Olea europaea*) and red tomato fruit (*Lycopersicon esculentum* M.) (Ogunyemi *et al.*, 2019), Bark of *Albizia lebbbeck* (Umar *et al.*, 2018), *Euphorbia hirta* L. (Monakari & Shekhawat 2017), *Costus pictus* D. Don (Suresh *et al.*, 2018).

The pomegranate plant (*Punica granatum*) is also a medicinal plant, used in traditional treatment of ailments over the years (Bhowmik *et al.*, 2013, Redha *et al.*, 2018). Therapeutic properties of the pomegranate have been utilized in treatment and prevention of cancers, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, mastitis, acne, folliculitis, piles, allergic dermatitis, scalds, diarrhoea and dysentery (Duman *et al.*, 2009, Sreedevi *et al.*, 2017). The various plant parts including the seed, juice, flower, leaves, root, fruit, and bark are said to have remarkable antibacterial, anti inflammatory and pharmacologic activity (Lansky & Newman, 2007, Shayganma *et al.*, 2015). Historically, the plant is said to have originated from the Middle East, then migrated throughout the Mediterranean, eastward to China and India, and on to the American Southwest, California and Mexico in the New World (Duman *et al.*, 2009, Sreedevi *et al.*, 2017, Yan *et al.*, 2017). Several religious and spiritual affirmations have been linked to the pomegranate fruit as being mystical,

associated with fertility and described as god's good creation (Lansky *et al.*, 2000, Rahimi *et al.*, 2012, Wu & Tian 2017, Redha *et al.*, 2018).

Pomegranates, belonging to the family of Punicaceae, are small thorny tree shrubs that grows about 5 to 10 meters high, with red or white flowers that occur as either single blossoms or clusters of several blossoms (Sreedevi *et al.*, 2017, Redha *et al.*, 2018). The plant possesses various biologically active compounds and nutrients (Yan *et al.*, 2017, Abiola *et al.*, 2018). These biologically active biomolecules (phytochemicals) of plants like carbohydrates, proteins, alkaloids, tannins, flavonoids, phenol, aldehydes, ketones, saponins and cardiac glycosides have the potential to reduce metal salt into nanoparticles (Singh *et al.*, 2018, Suresh *et al.*, 2018, Ogunyemi *et al.*, 2019). Studies have shown that the pomegranate plant has a high number of phenolic compounds including punicalagin, gallic acid, ellagic acid, chlorogenic acid, 25 caffeic acid, punicalin, apigenin, quercetin, pelargonidin, cyanidin, granatin A and 26 granatin B (Wu & Tian 2017, Mohamad Sukri *et al.*, 2019). The plant is also loaded with antioxidants such as tannin, polyphenol, flavonoid, tocopherols, anthocyanins and vitamins C (Passafiume *et al.*, 2019). The bioactivities of these phytochemicals are mainly due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and also may have a metallic chelating potential (Elfalleh *et al.*, 2012). This study aims to evaluate and establish the antimicrobial properties and phytochemicals present in leaf and flower extracts of the pomegranate plant used in the biosynthesis of zinc oxide nanoparticles and iron oxide nanoparticles discussed in the preceding chapters. Figure 4.1, shows pictures of sun-dried and powdered pomegranate leave and flower



A. Pomegranate sun dried leaf, (b) Pomegranate leaf powder (c) Pomegranate flower (d) Pomegranate sun dried flower powder

Figure 4.1: Photograph of (a) pomegranate leaves (b) ground pomegranate leave (c) Pomegranate flower (d) ground pomegranate flower

4.3 Materials and method

4.3.1 Preparation of leaf and flower extract

Leaves and flowers of pomegranate plant (*Punica granatum* L.) were obtained from Unit 1 and Unit 6 areas in Mafikeng, North West Province, South Africa. The leaves and flowers of the plant were carefully separated and thoroughly washed with tap water and then distilled water to remove dust particles. They were dried in shade and finely powdered using an electric blender. Aqueous and methanolic extracts of pomegranate leaves and flowers were prepared by soaking ten grams of powdered materials in distilled water and 50% methanol separately. The mixtures were placed in a shaker for 24 hours to obtain extracts. The extracts were filtered and then evaporated to dryness under controlled temperature (35-40°C) using a rotary vacuum evaporator. The extracts were stored in airtight containers under refrigeration. These

dried extracts were re-constituted in respective solvents and used for further phytochemical analysis.

4.4 Primary phytochemical screening of pomegranate leaves and flower

The following phytochemical screening was done following the methods as described by Sreedevi et al. 2017 with slight modifications

4.4.1 Test for phenol

1ml of extract was placed in a test tube, to which drops of 5% ferric chloride was added. Formation of deep blue or black colour indicates the presence of phenol.

4.4.2 Test for tannins

2 ml of each extract was put into clean test tubes, and then drops of 10% alcoholic ferric chloride solution were added. A change to blue or greenish colour solution indicated the presence of tannins.

4.4.3 Test for alkaloids

Wagner's reagent was first prepared by dissolving 0.63 g of iodine and 1g of potassium iodide in 50 ml distilled water. 2 ml of each extract was placed in tubes and 3-5 drops of the prepared Wagner's reagent was added. Formation of reddish brown precipitate (or colouration) indicates the presence of alkaloids.

4.4.4 Test for anthraquinone

Few drops of 1% hydrochloric acid were added to 1 ml of the plant extracts. Appearance of red colour precipitate indicates the presence of anthraquinone.

4.4.5 Test for reducing sugar

A few drops of Benedict's reagent were added to 2ml of plant extracts in tubes. The tubes were kept in a boiling water bath for a few minutes. Formation of reddish brown precipitate is an indicator of positive result showing the presence of reducing sugar.

4.4.6 Test for flavanoids

2 ml of extracts was treated with a few drops of 20% sodium hydroxide solution. Then dilute hydrochloric acid was added. Formation of intense yellow colour on addition of sodium hydroxide, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

4.4.7 Test for coumarins

2 ml of the extract was treated with a few drops of 10% sodium hydroxide. Appearance of yellow colour indicates the presence of coumarins.

4.4.8 Test for saponins

2 ml of extract was added to 6 ml of distilled water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of Saponins.

4.4.9 Test for terpenoids

1 ml of chloroform was added to 2 ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

4.5 Antimicrobial evaluation of pomegranate leaves and flower extract

The antimicrobial activity of pomegranate aqueous and methanolic extract of leaves and flowers was determined using the agar well diffusion method. Bacteria chosen for the antibacterial assay were obtained from American Type Culture Collection (ATCC) as lyophilisates in ampoules which are: *Staphylococcus aureus* (ATCC 25923), *Eschericia coli* (ATCC 25922), *Streptococcus pneumoniae* (ATCC 27336), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cerus* (ATCC 10876), *Moraxella catarrhalis* (ATCC 25240), *Aeromonas hydrophilia* (ATCC 7966), *Salmonella diarizonae* (ATCC 12325), *Enterococcus fecalis* (ATCC 29212), *Enterococcus faceium* (ATCC 6569), *Salmonella typhi*, *Klebsiella pneumoniae* (ATCC 13883), and *Listeria monocytogen*. Bacterial culture was grown on 10 mL Luria broth and incubated overnight at 37°C. The next day, bacterial cultures were standardized with Luria broth to an optical density of range 0.08 - 0.1 at 630 nm using the spectrophotometer. 100µl of the standard bacterial concentrations were inoculated onto Muller Hinton agar plates and spread using a sterile swab stick. Inoculated plates were left to dry, and wells were bored into the agar using a sterile cork borer. The wells were carefully filled with the prepared solution of the extract at a concentration of 5mg/ml. The plates were allowed to stand in the laboratory biosafety cabinet for about 2 hours before being incubated at 37°C for 24 h. After this time the plates were observed for the zones of inhibition which were measured across the diameter (in mm). The susceptibilities of the isolates to the leaf and flower extract were compared with those of tetracycline (0.1 mg/ml) and ampicillin (1 mg/ml) used as standard antimicrobial drug. The experiment was carried out in triplicate.

4.6 Results and discussion

4.6.1 Phytochemical analysis

Table 4.1: Primary phytochemical analysis of methanolic and aqueous extracts

S/N	Phytochemical constituent	Aqueous leaf extract	Aqueous flower extract	Methanolic leaf extract	Methanolic flower extract
1.	Phenol	+	+	+	+
2.	Tannins	+	+	+	+
3.	Alkaloid	+	+	+	+
4.	Anthraquinone	-	-	-	-
5.	Reducing sugar	+	+	+	+
6.	Flavonoids	+	+	+	+
7.	Coumarins	+	+	+	+
8.	Saponins	-	-	+	+
9.	Terpenoids	-	-	-	+
Key: (+) = present, (-) = Absent					

Aqueous and methanolic extract of ground fresh and healthy flower and leaf of the pomegranate plant was analysed to detect the chemical constituents. Phytochemical analysis of extracts assists in detecting sources of bioactive molecules implicated in plants. The primary photochemical analysis of pomegranate leaf and flower was carried out in this study to determine their basic biomolecules. From Table 4.1 above, it can be seen that the aqueous extracts of both flower and leaf contained phenols, tannins, alkaloid, reducing sugar, flavonoids and coumarines, but was negative for saponines, trepenoid and anthraquinone. In contrast, the methanolic extract of

pomegranate leaf was positive for phenols, tannins, alkaloid, reducing sugar, flavonoids and coumarines, with exception of trepenoid and anthraquinone. Methanolic extract of flower only lacked anthraquinone. These phytochemicals present in the leaf and flower extracts have been implicated as reducing and stabilizing agents during biosynthesis of nanoparticles. For example, gallic and punicalagum (Phenol compound) have been reported to contribute to the reduction and stabilizing of Zn^{2+} ions to stable Zn atoms in aqueous solution during the biosynthesis process (Mohamad Sukri *et al.*, 2019). Phenolic compounds are made up of phenol, more polyphenol aromatic rings and a hydroxyl group attached structurally. Due to the presence of the hydroxyl and phenolic ring, phenolic compound containing extracts are said to have antioxidant properties and other health benefits.

4.6.2 Antimicrobial evaluation of pomegranate leaves and flower extract

The antimicrobial activity of the methanolic and aqueous extract of the pomegranate leaf and flower were analysed using the agar well diffusion method. The aqueous leaf extracts obtained were found to be effective against the bacteria *S. diarizonae*, *K. pneumoniae*, *M. catarrhalis*, *S. typhimurium*, *B. cerus*, and *E. coli*. The aqueous flower extract showed no activity to any bacteria except for *S. typhimurium*. Methanolic extract of leaf exhibited its antimicrobial efficacy over *K. pneumonia*, *S. diarizonae*, *M. catarrhalis*, *S. typhi*, *B. cerus*, *S. pneumonia* and *E. coli* while methanolic extract of flower had activity over *S. diarizonae*, *M. catarrhalis*, and *S. typhi*. The antimicrobial activity was depicted by a clear inhibition zone that ranges from 10 mm – 24 mm (Table 4.2). *S. diarizonae* exhibited the highest zone of inhibiton while *E. coli* showed the lowest inhibition zone for the aqueous extract of leaf, *S. typhi* showed the highest inhibition zone for methanolic extract of leaf and flower. The overall highest inhibition

shown was obtained for *S. typhi* and the lowest for *E. coli*. Methanol extract showed higher antibacterial activities than the other extracts, similarly leaves were found to have the maximum zone of inhibition when compared to flower. Studies have reported the antimicrobial capability of pomegranate plant parts. Malviya *et al.* (2013) reported on the antimicrobial potential of the aqueous, methanolic, and ethanolic extract of the pomegranate peel against *S. aureus*, *E. aerogenes*, *S. typhi* and *K. pneumonia*. Rajesh (2014) reports that apart from the antimicrobial activity of *Punica granatum* against cariogenic oral bacterial, *Punica granatum* also has antifungal efficacy against *Candida*. Antimicrobial effectiveness of the various *Punica granatum* plant part (ethanolic extract) againsts *E. coli* was evaluated and reported by Shaza *et al.* (2004). Major bioactive molecules found in the plant such as tannins and polyphenols have been attributed to its ability to effectively show antimicrobial activity. Polyphenols are able to interact with proteins and as such disturbing microbial co-aggregation, while tannins precipitate proteins, and act on the cell surface. Tannins are toxic, and are able to reduce enzyme production, precipitate proteins, bind vitamins and sugar required by the microbes and act on the cell surface, causing cell death (Betanzos-Cabrera *et al.*, 2015, Ali *et al.*, 2018).

Table 4.2: Antimicrobial activity of Pomegranate leaf and flower extract (methanolic and aqueous)

Bacterial Strain	Zones of inhibitions			
	Aqueous Leaf Extract	Aqueous Flower Extract	Methanolic Leaf Extract	Methanolic Flower Extract
<i>Streptococcus pneumoniae</i>	12	-	-	14
<i>Salmonella diarizonae</i>	24	-	20	22
<i>Moraxella catarrhalis</i>	18	-	15	22
<i>Enterococcus faecium</i>	-	-	-	-
<i>Salmonella typhi</i>	22	20	24	24
<i>Bacillus cerus</i>	16	-	16	-
<i>Staphylococcus aureus</i>	-	-	20	-
<i>Eschericia coli</i>	10	-	15	-
<i>Aeromonas hydrophilia</i>	14	-	13	-
<i>Psedomonas aeruginosa</i>	-	-	-	-
<i>Listeria monocytogenes</i>	-	-	-	-
<i>Klebsiella pneumoniae</i>	14	-	14	-
<i>Enterococcus faecalis</i>	-	-	-	-
Key: - = No zone of inhibition observed.				

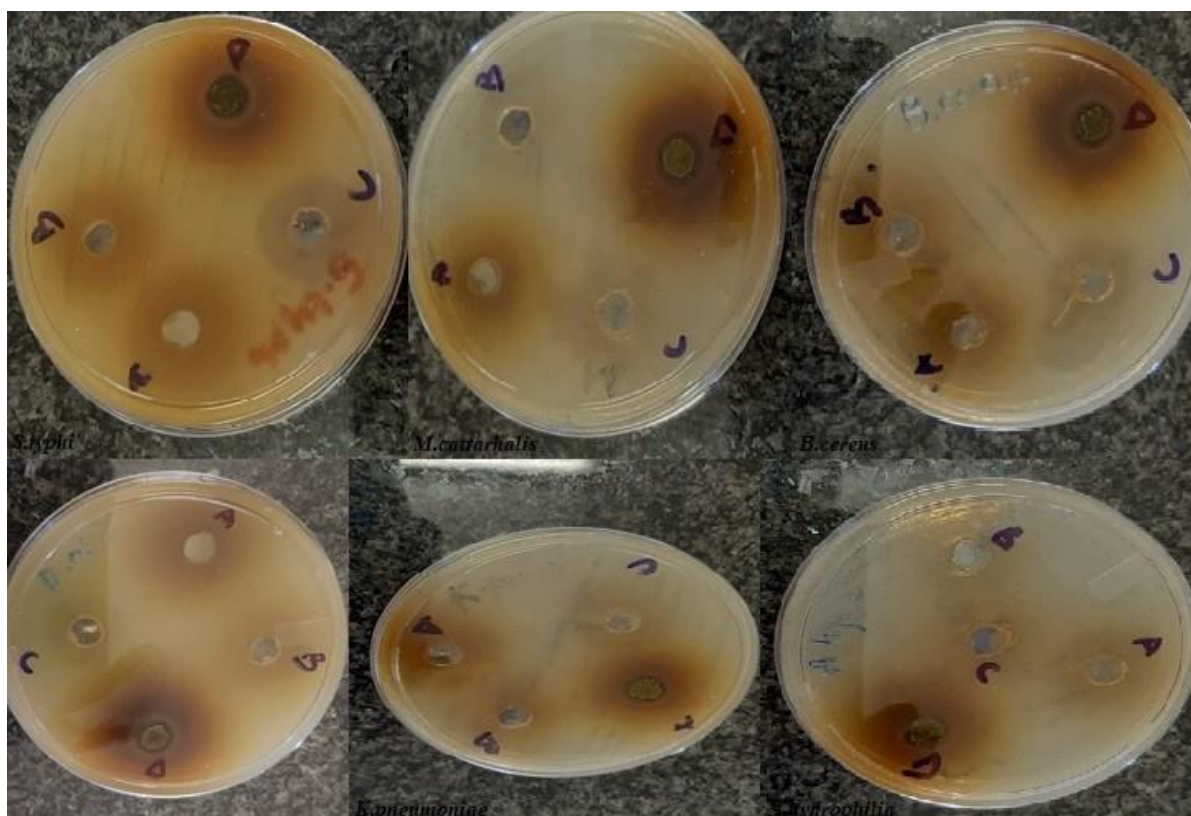


Figure 4.2: Zones of inhibitions observed when aqueous and methanolic extracts were tested against pathogenic strains.

Keys: Aqueous leaf extract is denoted by letter A.
 Aqueous flower extract is represented as B.
 Methanolic leaf extract is represented as C
 Methanolic flower extract is represented as D

4.7 Conclusion

The use of medicinal plants in therapeutics is a life long practice in developing and some developed nations. Pomegranate plant (*Punica granatum*) is a well known medicinal plant employed in treatment of ailments. In this study, phytochemical screening and antimicrobial efficiency of pomegranate aqueous and methanolic leaf and flower extract was evaluated. Phytochemical screening of the methanolic and aqueous extracts of the pomegranate leaf and flower shows that they contain phenols, tannins, alkaloid, flavonoids, coumarins, saponines and terpenoid. These bioactive

molecules possess properties that promote the antimicrobial efficiency of the plant. The extracts were tested against bacterial strains and they exhibited antibacterial activity against different pathogenic strains including *S. aureus*, *E. coli*, *S. typhi*, *B. cerus*, *M. catarrhalis*, *S. diarizonae*, *K. pneumonia*, *S. pneumonia* and *A. hydrophilia*. Pomegranate plant also contains other bioactive compounds such as gallic acid, quercetin and myricetin which have been implicated as having antimicrobial properties. The antimicrobial effectiveness of the extract is dependent on the constituent bioactive metabolites. The ability of the plant extract to show antimicrobial activity may be an indication that the plant contains metabolic content or compounds with broad antibiotic spectrum. These constituents are said to possess antimicrobial capabilities which make them a suitable therapeutic.

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CHAPTER 5

Spectroscopic and electrochemical characterisation of synthesized metal oxide nanoparticles from pomegranate (*Punica gratum*) extract

5.1 Abstract

Metal oxides of zinc and iron nanoparticles were prepared using a simple, safe, non-toxic, fast and cheap green method of synthesis. Nanoparticles produced using this method are known to be biocompatible and nontoxic. In the study, zinc oxide and iron oxide nanoparticles were successfully synthesized using pomegranate leaves and flower extract. The extract contains phytochemicals that functioned as capping, reducing and stabilizing agents. The nanoparticles obtained from this method were characterized by using energy dispersive X-ray analysis (EDX), and X-ray diffractive analysis (XRD), which revealed the elemental components and nature of the synthesized particles. The zinc oxide nanoparticles obtained showed a crystalline structure while the iron oxide was of an amorphous nature. Morphology of the particle was obtained from Scanning electron microscopy (SEM) analysis which showed an uneven shape for the zinc oxide and spherical shape for the iron oxide. Also the average particle size of ZnO-NPs-PL and ZnO-NPs-PF were 57.75nm and 52.50 nm respectively. TEM analysis of the particles revealed spherical iron oxide nanoparticles with average particle size of 49.59 nm for Fe₃O₄-NPs-PL and 34.30 nm for Fe₃O₄-NPs-PF. The functional groups involved in stabilization, reduction and capping were confirmed using FTIR. The confirmation of nanoparticles by UV-Vis analysis showed an absorption band of 284 nm and 357 nm for the zinc oxide nanoparticle mediated via pomegranate leaf and flower extract respectively, while iron oxide nanoparticle absorbance was at 308 nm and 310 nm for leaf and flower mediated particles. The

obtained particles were also characterized electrochemically using the cyclic voltammetry to evaluate their electrochemical properties.

5.2 Introduction

The area of science and technology has been greatly impacted by nanotechnology (Basnet *et al.*, 2018, Martín-Yerga, 2019). Nanotechnology involves the manipulation of bulk material, to produce small sized materials with properties more enhanced than its bulk counterpart (Heera & Shanmugam, 2015, Testolin *et al.*, 2019). The unique properties of synthesized nanomaterial are exploited and applied to create important products and services (Firdhouse & Lalitha, 2015, Moeinzadeh & Jabbari, 2019). Size, geometry, surface charge and chemical modification are major contributing factors to properties displayed by the engineered nanomaterial (Mamuru & Jaji, 2015, Rajeshkumar & Bharath, 2017). Nanoparticles of metal oxide have attracted much attention from researchers due to their remarkable properties and diverse technological applicability in fields such as biomedicine, engineering, electronics, catalysis, electrocatalysis, and sensors (Nguyen *et al.*, 2018, Testolin *et al.*, 2019). Several applications of nanoparticles have been reported, especially in the medical and environmental fields (Salata, 2004, Khandel & Shahi, 2018). Biological properties of nanomaterial can be influenced by their physical and chemical properties (Rajeshkumar & Bharath, 2017).

New nanomaterials are constantly being synthesized, and one of the worrying concerns about the use of nanoparticles is their possible toxicity to humans and the environment (Ray *et al.*, 2009, Rajasekhar & Kanchi, 2018). This has resulted in a need to create an analytical method platform for detection and characterization of these engineered nanoparticles (Mourdikoudis *et al.*, 2018). Characterization of

nanoparticles provides information on their features (Kumar & Dixit, 2017). Detection and characterization of nanoparticles can be carried out using one of the several available spectroscopic and surface characterization techniques like ultraviolet and visible spectroscopy, vibrational spectroscopy, electron- and ion spectrometry, X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX; EDX systems are attachments to TEM or SEM), atomic force microscopy (AFM), and dynamic light scattering (DLS) (Ali *et al.*, 2016, Rajeshkumar & Bharath, 2017, Sequeira *et al.*, 2018).

Apart from the listed characterization techniques, complementary electrochemical characterisation of the nanoparticles is also essential (Testolin *et al.*, 2019). Electrochemical characterization techniques include cyclic voltammetry (CV), voltammetric, differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS), and polarographic techniques (Nnamchi & Obayi, 2018). Electrochemical characterization is mainly carried out to study the catalytic behaviour and electrode surface reaction of the materials (Nnamchi & Obayi, 2018, Jeremiah Aoki & Chen, 2019). Detection and characterization of nanomaterial can aid applications in biosensing (MacKenzie *et al.*, 2009, Yerga *et al.*, 2019). Cyclic voltammetry is an electrochemical technique that offers electrochemical information of the reduction and oxidation process of a molecular species (Manea, 2014., Elgrishi *et al.*, 2017), and the stability constant of the interface between metal salt and organic molecule (Abd El-Hady *et al.*, 2018). In this technique, potential applied to an electrode, resultant current and intensity obtained including voltage applied to a working electrode can all be monitored (Kim *et al.*, 2010). The cyclic voltammetry gives information about properties of analyte in solution, thermodynamic data of a reaction,

diffusion coefficient, heterogeneous rate constant, surface coverage, quantity of electricity, temperature, scan rate and effect of different concentration of reactants (Manea, 2014., Abd El-Hady *et al.*, 2018, Durairaj *et al.*, 2018).

In this study, zinc oxide nanoparticles and iron oxide nanoparticles were synthesized via pomegranate leaves and flowers. Synthesized nanoparticles were characterized structurally and electrochemically.

5.3 Materials and method

Zinc nitrate hexahydrate, ferric chloride (Merck SA), ultrapure water of 18.2 MX resistivity obtained from a Milli-Q Water System (Millipore Corp., Bedford, MA, USA) were used throughout for the preparation of electrochemical solutions. All solutions were prepared using distilled water.

5.3.1 Collection and preparation of plant extract



Figure 5.1 (a): Pictorial representation of the preparation process for Pomegranate (*Punica granatum*) leaf extract.

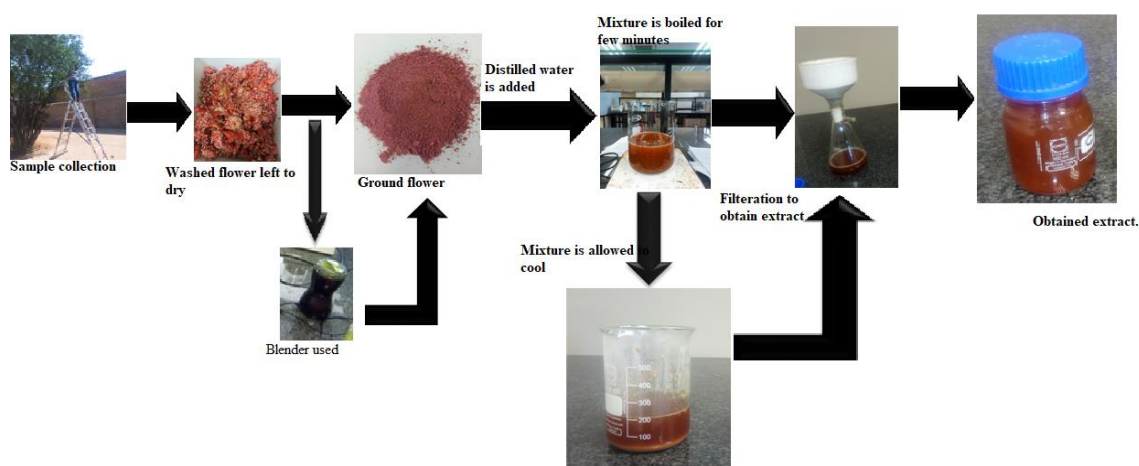


Figure 5.1 (b): Pictorial representation of the preparation process for Pomegranate (*Punica granatum*) flower extract.

Healthy leaves and flowers of pomegranates were collected from Unit 6 in Mafikeng, North West province South Africa. The leaves and flowers were washed separately with tap water to remove dust particles, then washed again with distilled water and dried for 4-6 days. The dried leaves and flowers were each separately shredded and ground to a fine powder, then stored in a properly labelled bottle for further use. About 10g of the leaf and flower powder were weighed out and put into a well labelled beaker, and 100 ml sterile double distilled water was added to each. The flower and leaf mixtures were heated for 20 minutes at 60°C. The obtained extract was allowed to cool down and filtered using Whatman filter paper. The filtrate was collected in a well labelled Erlenmeyer flask and stored at 4°C for further use. Figures 5.1(a) and 5.1(b) is a representation of the preparatory procedure for the pomegranate leaf and flower extract.

5.3.2 Synthesis of zinc oxide nanoparticles

Zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] (Merck) was used as a precursor to synthesize zinc oxide nanoparticles in this study. 1mM Zinc nitrate hexahydrate solution was prepared using sterile double distilled water and stored in a brown bottle at 4°C for further use to synthesize zinc oxide nanoparticles via leaf and flower of pomegranate. 0.1 M Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) solution was prepared by dissolving 6.58 g in 300 ml double-distilled water. 10 ml of the aqueous leaf and flower pomegranate extracts were each slowly added drop wise into the solution under magnetic stirring at 60°C for roughly 2 h to obtain a complex formation. The complex formed after stirring was collected and centrifuged at 10 000 rpm for 10 minutes and the pellets collected. The separated pellets were dried in an oven at 80°C for 8 h and preserved in airtight bottles for further studies.

5.3.3 Synthesis of iron oxide nanoparticles

The iron oxide nanoparticles were prepared using ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) as iron precursor and pomegranate flower and leaf extract as reducing agent and stabilizer. Iron oxide nanoparticles (Fe_3O_4 -NPs) of pomegranate leaves and flowers were prepared by adding 0.1 M FeCl_3 solution to the pomegranate extract solution in a 1:1 volume ratio. The solution immediately turned black in colour indicating formation of Fe_3O_4 complexes with the reduction process. The mixture was stirred for 60 minute and then allowed to stand at room temperature for another 30 min. The obtained colloidal suspension was centrifuged and washed several times with ethanol and then dried at 40 °C under vacuum to obtain the Fe_3O_4 nanoparticles.

5.3.4 Characterization of the synthesized metal oxide nanoparticles

The resulting synthesized metal oxide nanoparticles were characterized by UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Fourier transform infra-red spectrophotometry (FTIR). The elemental composition and morphology were examined using SEM-EDX.

5.3.4.1 UV-visible spectroscopy

UV-Vis spectroscopy is a simple, fast, sensitive and selective characterization technique that enables determination of the formation and stability of nanoparticles (Mourdikoudis *et al.*, 2018). In this technique, light is sent through a sample and the amount of UV light or radiation absorbed by the sample is measured or calculated. The metal oxide nanoparticles (zinc oxide and iron oxide) synthesized from pomegranate leaves and flower were weighed out (2µg) and distilled water added then sonicated. The resulting solutions were poured into cuvettes and spectra in the range of 200-800 nm were determined using the UV- visible spectrometer.

5.3.4.2 X-ray diffraction analysis (XRD)

X-ray diffraction analysis is a technique that gives information about the morphology and crystalline nature of a particle (Heera & Shanmugam 2015). The XRD measures symmetry, size, and shape. In this technique, the X-ray diffraction spectra provide the structural fingerprint of the crystal particle. Synthesized nanoparticles were analysed in powdered form using an X-ray diffractometer of angle 2θ in the range 20° - 80° .

5.3.4.3 Fourier transform infra-red spectrophotometer (FTIR)

FTIR Spectra of the powdered samples of the synthesized metal oxide nanoparticles were measured using the Cary 600 series FTIR spectrometer, USA. FTIR analysis

was carried out to identify the surface functional groups and also to detect the stretching and bending vibrations in the pomegranate extract and the nanomaterial samples. For FTIR measurements, a small amount of powdered NPs was placed on round-shaped selenide plates for FTIR spectral analysis.

5.3.4.4 Scanning electron microscopy (SEM)- Energy Dispersive X-ray analysis (EDX)

Morphology, microstructure and the elemental composition of the synthesized metal oxide nanoparticle was examined using SEM with EDX. Scanning electron microscopy (SEM) images were recorded using Hitachi 3600 SEM instrument and energy dispersive X-ray analysis (EDX) by using the Thermo Fisher Scientific Ultradry (Madison, WI, USA) instrument.

5.3.4.5 Transmission electron microscopy (TEM)

TEM analysis aims to provide information on the shape and size distribution of synthesized nanoparticles while confirming the existence of metal oxide nanoparticles in the synthesized samples. TEM analysis was done using the transmission electron microscope, JEOLJEM 2100 electron microscope (Japan) operated at 200kv accelerating voltage and connected to an Energy dispersive spectrophotometer (EDX). In performing the analysis, 2 mg of each sample was weighed out into a sample bottle and about 4 ml ethanol solution was added. The mixture was sonicated for 10 mins to disperse the particles, using a digital ultrasonic cleaner. A drop of the sonicated mixture was placed on a carbon coated copper grid and allowed to dry for about 5 mins.

5.4 Electrochemical properties of synthesized metal oxides nanoparticles

5.4.1 Preparation of modified electrode

A stock solution of zinc oxide nanoparticle and iron oxide nanoparticle paste was prepared by dispersing 2 mg each of zinc oxide nanoparticles and iron oxide nanoparticles into different properly labelled sample glass bottle containing 3-4 drops of Dimethylformamide (DMF). The mixture was sonicated for over 1 h and about 5 μ l aliquot of each sonicated paste was strategically casted onto the screen printed platinum electrode using the drop dry method. Modified screen printed platinum electrode was air dried for further cyclic voltammetry analysis.

Electrochemical study of the synthesized nanoparticles was done using cyclic voltammetry. This is to ascertain that electrodes were successfully modified and also to obtain information on the electron transport and electro-catalytic property of the bare, modified zinc oxide and iron oxide nanoparticles. The Cyclic voltammetry (CV) study was performed on μ Stat200 bipotentiostats (Metrohm, South Africa) driven by a drop view 200 software using a screen printed platinum electrode which already includes the conventional three electrode configuration. The three-electrode configuration is composed of a screen-printed platinum working electrode, a silver pseudo-reference electrode and a platinum counter electrode.

5.5 Results and discussion

5.5.1 Synthesis of nanoparticles

From the above study, during biosynthesis of zinc oxide nanoparticles using pomegranate leaves and flower, zinc nitrate hexahydrate served as a precursor. On addition of the plant extracts (leaf and flower) with greenish and pinkish-brown colour respectively to the colourless zinc nitrate hexahydrate solution, a yellowish-white precipitate occurred, as shown in figure 5.2(a) below, indicating the presence of zinc oxide nanoparticles. A similar colour change was observed by Rajakumar *et al.* (2017) on addition of greenish *A. paniculata* leaf extract to the zinc nitrate precursor. Bhuyan *et al.* (2015) also reported formation of a whitish precipitate. In contrast, Umar *et al.* (2018) observed a change in colour from brown to a dark brown colour on addition of *Albizia lebbbeck* leaf extract to the precursor zinc acetate.

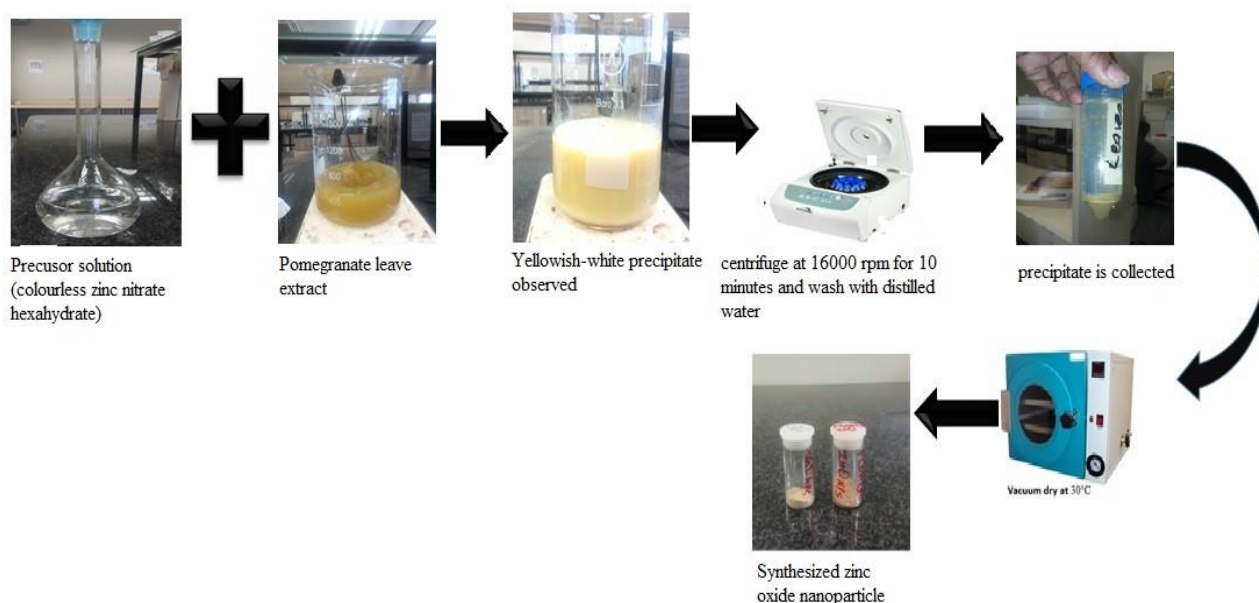


Figure 5.2 (a): Synthesis of zinc oxide nanoparticles via pomegranate leaf extracts (pictorial representation)

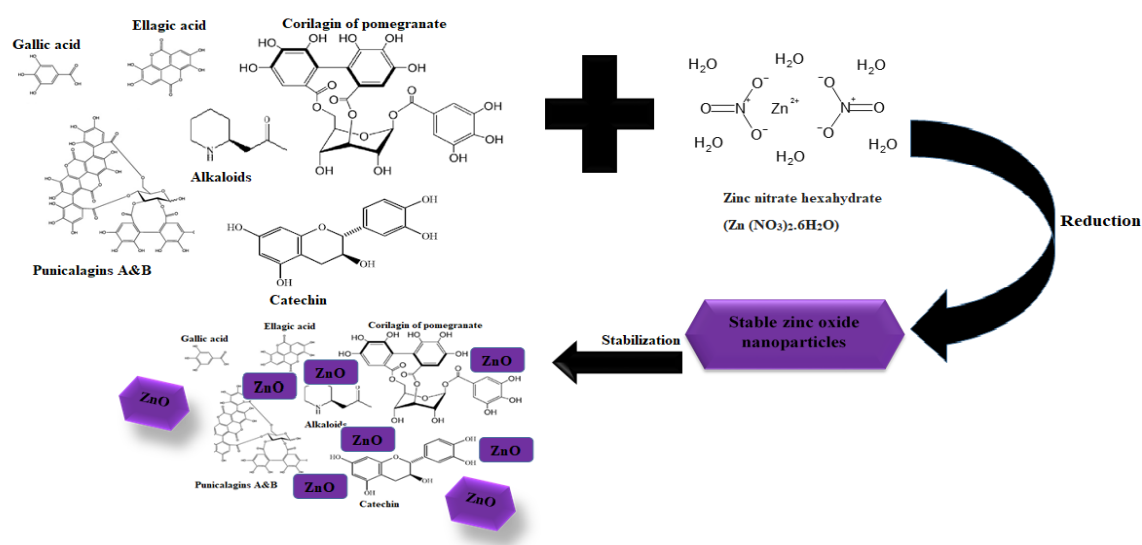


Figure 5.2 (b): Mechanism of action between Pomegranate extract with zinc oxide to form zinc oxide nanoparticles

For iron oxide nanoparticle, the presence of iron oxide nanoparticles formed from reduction of iron salt was observable by the appearance of an intense black colour change on addition of the plant extract to the light yellow coloured precursor (0.1 M ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) salt solution. Similar results were reported by Irshad *et al.* (2017), where a change to black colour was recounted on addition of *Punica granatum* peel extract to the yellow coloured ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) precursor salt solution. Vasantharaj *et al.* (2018) also reported a colour change to brownish black on addition of *R. tubrosa* leaf extract to the FeSO_4 salt solution that served as a precursor. In a study by Da'na *et al.* (2018), formation of iron oxide nanoparticles was confirmed with the formation of a black precipitate on addition of the *Acacia nilotica* pod extract to the ferric chloride precursor. However, Devi *et al.* (2018) reported a change to dark brown on introducing the *Platanus orientalis* leaf extract and slowly to red brown colour in synthesis of iron oxide nanoparticles. Change in colour signifies excitation of plasmon vibration from metal nanoparticles. Other

researchers such as Samrot *et al.* (2018) reported formation of a black precipitate on addition of *Azadirachta indica* leaf extract to $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ salt solutions within a few minutes.

5.5.2 Ultraviolet-visible (UV-vis) spectroscopy

The confirmation of formation of the metal oxide nanoparticles (zinc oxide nanoparticle and iron oxide nanoparticle) was performed by analysis with UV-Vis spectroscopic technique with wavelength range of 200 - 800 nm. UV-Vis absorption spectra for zinc oxide nanoparticles synthesized from pomegranate leaf (ZnO-NPs-PL) showed an absorbance peak at 284 nm and another strong peak at 357 nm (Figure 5.3), while in the case of zinc oxide nanoparticles synthesized from flower (ZnO-NPs-PF), an excitation absorption peak was observed at 345 nm (Figure 5.4). The shift in peak to a lower wavelength is caused by the presence of a blueshift which is observed as particle size reduces. The absorption peaks obtained show formation of zinc oxide nanoparticles following the green synthesis method, and also indicate combined vibration of electrons of the nanoparticles with the light waves. The peak values obtained are close to the characteristic line of the light absorption range of zinc oxide nanoparticle at 360–380 nm. A similar result was reported by Ezealisiji *et al* (2019), where a strong peak at 359 nm was obtained. In a study conducted by Tensingh & Lega (2018), where nanorod shaped zinc oxide nanoparticles with particle size range of 20-100 nm were synthesized, a corresponding absorption peak at 354 nm was obtained. A *Magnifera indica* mediated synthesis of zinc oxide nanoparticles study carried out by Rajeshkumar *et al.* (2018) yielded an absorption peak of 355nm. In the same vein, Khatami *et al.* (2018) synthesized rectangular shaped nanoparticles with size 50 ± 10 nm that showed a surface plasmon resonance absorption peak at 360 nm.

Saputra & Yulizar (2017) report a maximum absorption peak of 359 nm, Rajakumar *et al.* (2017) obtained a strong absorbance peak in the 358 nm range, which is also in the range of the peak value in this study. In contrast, Sadatzadeh *et al.* (2018) reported a peak value of 255 nm for zinc oxide nanoparticles mediated by aqueous extract of cotton seed plant. Figures 5.5 and 5.6 show the band gap energy which was calculated using Tauc relation. ZnO-NPs-PL showed a band gap of 3.70 eV, which is slightly higher than that reported in literature of 3.3 eV. The increase in band gap is attributed to a decrease in particle size caused by confined quantum size effect (Akbarian *et al.*, 2018). For ZnO-NPs-PF, the direct energy band gap obtained is at 2.69 eV. Other researchers like Bhuyan *et al.* (2015) reported a direct band gap energy of 3.87 eV from *Azadirachta indica* leaf mediated zinc oxide nanoparticles, while Azizi *et al.* (2016) reported band gaps of 3.30 and 3.27 eV from *Anchusa italic* flower extract mediated zinc oxide nanoparticles, which is close to the direct band gap energy of 2.60 eV reported in this ZnO-NPs-PF study. For semiconductors, direct band gap is influenced by particle size, and a decrease in size automatically denotes an increase in optical band gap. The direct band gap graph is obtained by plotting $h\nu$ versus $(\alpha h\nu)^2$.

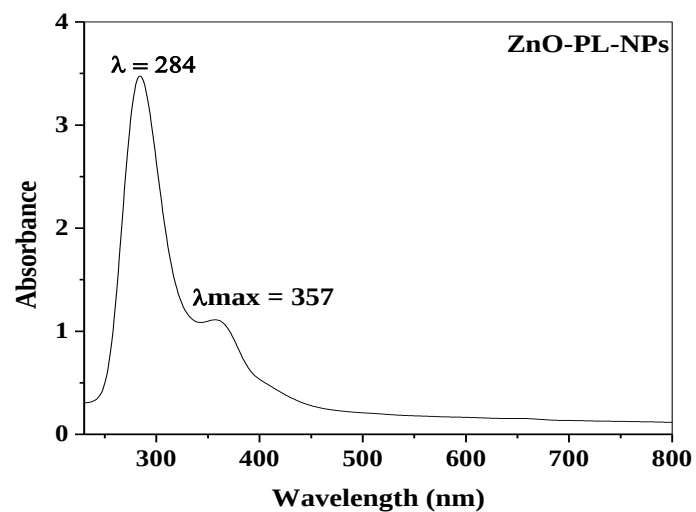


Figure 5.3: UV-visible spectra of zinc oxide nanoparticles synthesized from pomegranate leaf extract.

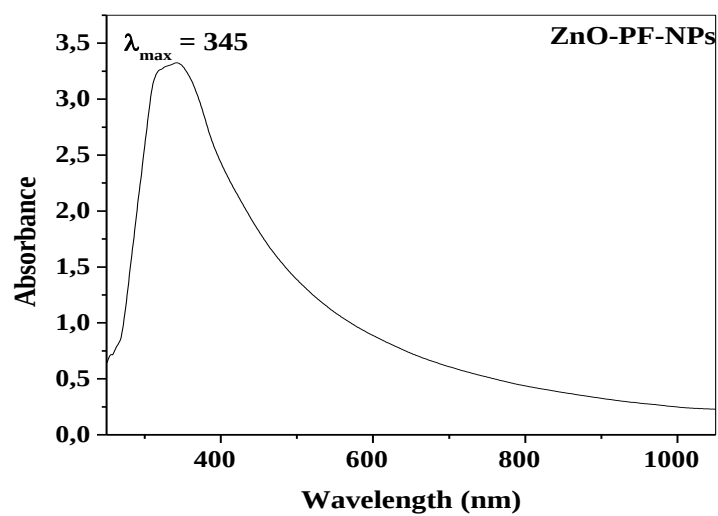


Figure 5.4: UV-visible spectra of zinc oxide nanoparticles synthesized from pomegranate flower extract.

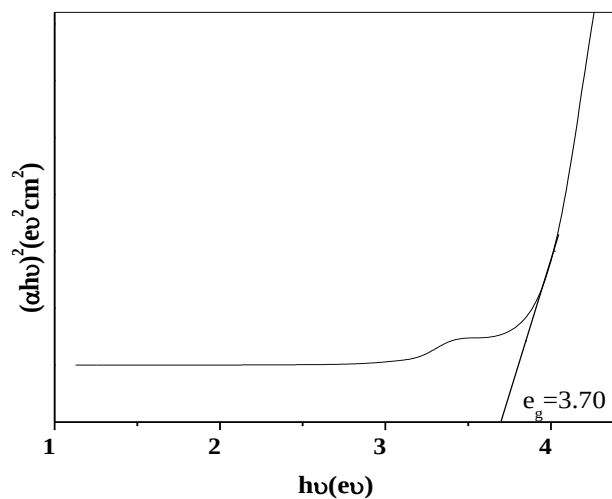


Figure 5.5: Direct optical band gap of zinc oxide nanoparticles synthesized from pomegranate leaf extract.

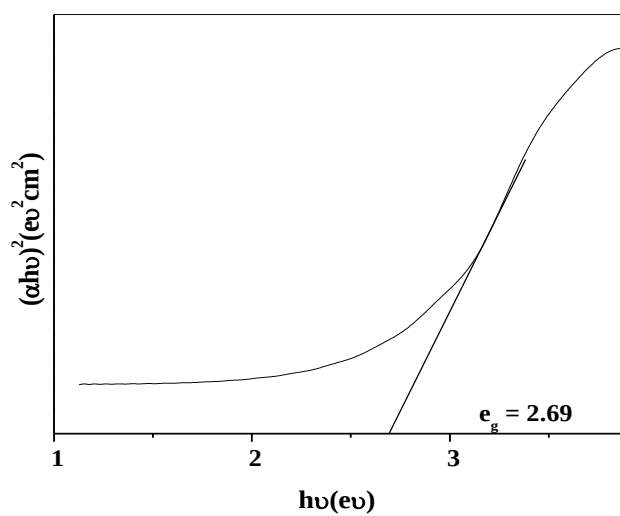


Figure 5.6: Direct optical band gap of zinc oxide nanoparticles synthesized from pomegranate flower extract.

For pomegranate leaf and flower extract mediated synthesis of iron oxide nanoparticles, Uv-vis spectra were recorded in the 200-800 nm range and strong absorption peaks were produced in the visible regions of 308 nm and 310 nm respectively, as shown in Figures 5.7 and 5.8, indicating stability of particles in solution and formation of iron oxide nanoparticles. Similar spectra were also observed in studies conducted by Neupane *et al.* (2019), where an absorption peak of 315 nm was reported for Himalayan honey mediated iron oxide nanoparticles. An absorption peak of 310 nm was reported by Saranya *et al.* (2019), from *Musa Ornata* flower sheath mediated iron oxide nanoparticles. The absorption peak maxima obtained were close to the range of observed UV spectra of 307 nm for Fe₃O₄ reported by Rahman *et al.* (2017). Devi *et al.* reported a UV spectrum for 350 nm for iron oxide nanoparticles synthesized from *Plantanus orientalis* leaves, and Jagathesan (2018) observed strong maximum absorption peaks at 379 nm with a band gap energy of 3.9 eV. Synthesis of *Ocimum sanctum* leaf mediated iron oxide nanoparticles, reported by Karpagavinayagam & Vedhi (2018), yielded peaks in the range of 298-301 nm. Similar peaks were observed at regions of 285 nm and 345 nm as reported by Balamurugan *et al.* (2014). Band gap energy was obtained following the Taucs relationship plot. Fe₃O₄-NPs -PL band gap energy was 2.30 eV, while that of Fe₃O₄-NPs -PF was 2.50 eV following the plot of $h\nu$ versus $(\alpha h\nu)^2$. (Figures 5.9 and 5.10)

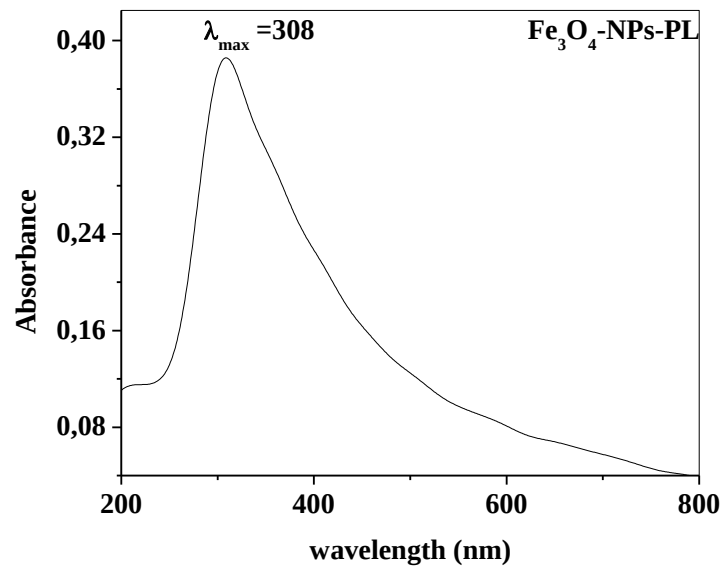


Figure 5.7: UV-visible spectra of iron oxide nanoparticle synthesized from pomegranate leaf extract.

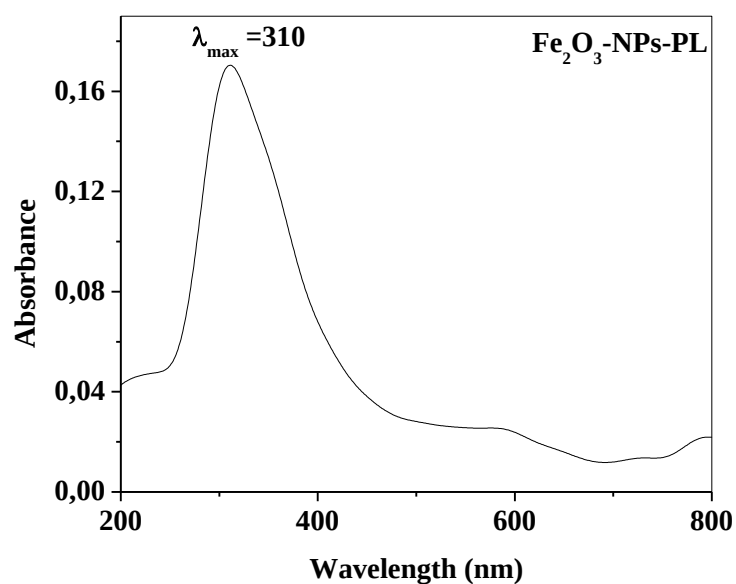


Figure 5.8: UV-visible spectra of iron oxide nanoparticle synthesized from pomegranate flower extract.

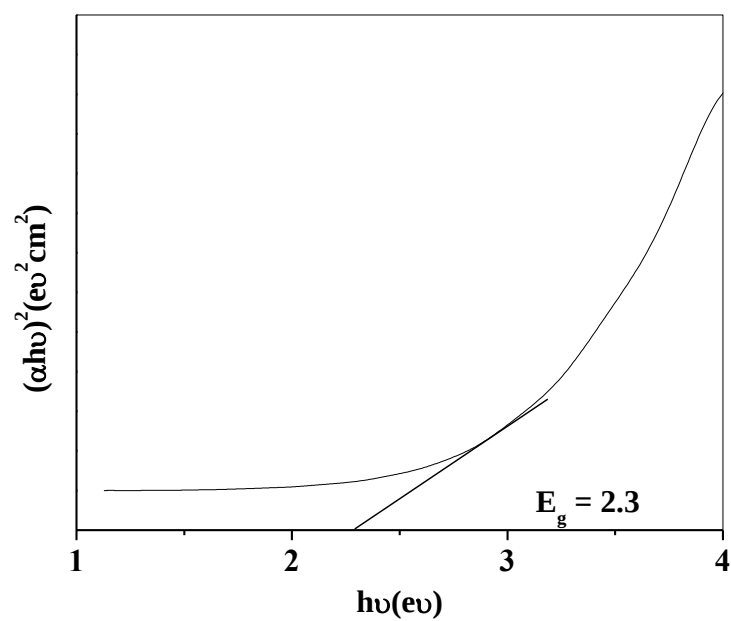


Figure 5.9: Direct optical band gap of iron oxide nanoparticles synthesized from pomegranate leaf extract.

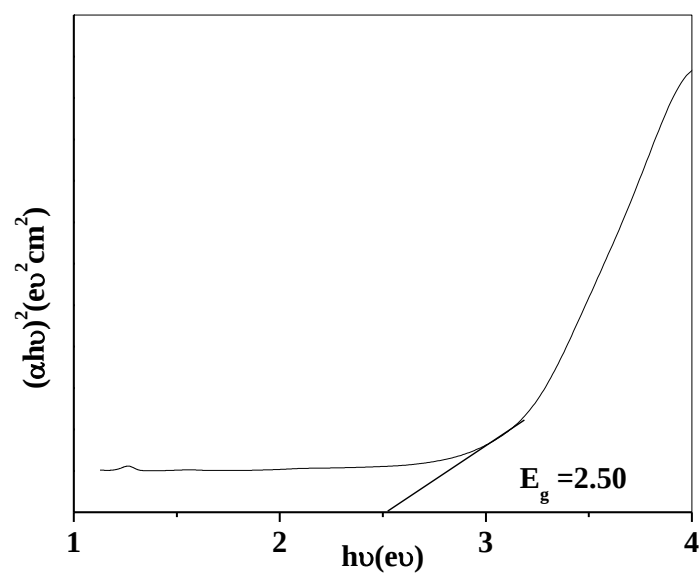


Figure 5.10: Direct optical band gap of iron oxide nanoparticles synthesized from pomegranate flower extract.

5.5.3 Fourier transform infrared spectroscopy (FTIR)

The purpose for FTIR analysis for the synthesized metal oxides is to determine the position and relative sizes of all absorptions or peaks in the infrared region. FTIR analysis helps to identify the functional groups present in the compound on the surface on the nanoparticles, thereby providing molecular information of the molecules and biomolecules present in the plant extracts that participated in synthesis of nanoparticles by acting as capping and reducing agents. FTIR analysis for this study was done in the spectral range of 4000 to 400 cm^{-1} . From the study conducted, the IR spectrum of the synthesized aqueous pomegranate leaf and flower mediated zinc oxide nanoparticles is depicted in Figures 5.13 and 5.14, while the spectra for the plant extracts are shown in Figures 5.11 and 5.12. Strong peaks that show chemical structures for pomegranate leaf extract were observed at 3560 and 3700 cm^{-1} , which fall within the range of 3000-3700 cm^{-1} . This absorption range confirms the presence of O–H stretch, free hydroxyl group, which signifies that the pomegranate leaf contains flavonoids, polyphenol and alcohol functional group that have different hydrogen bonds. Every bond type observed in FTIR analysis has a different frequency of vibration. Absorbance peaks were also obtained at 2936 cm^{-1} indicating the O–H stretch bond of carboxylic acid functional group, and –C–H stretch of alkanes. Less pronounced peak at 2100 cm^{-1} represents –C $\equiv$ C– stretch of alkynes and C $\equiv$ N stretch of nitriles. Not so prominent peaks in the 1715-1727 cm^{-1} range confirm the presence of C=O carbonyl group in the molecule, C=O stretch of ketones, aldehydes, saturated aliphates and stretch of α , β unsaturated esters. Less prominent absorption peak of 1588 cm^{-1} is indicative of C–C stretching (in ring) of aromatics and N–H bend of primary amines. Other absorption peaks obtained at 1352 cm^{-1} are representative of C–H rock of alkanes and N=O symmetric stretching of nitro compounds. Peaks at 1215 cm^{-1}

represent the C-O stretch of alcohols, carboxylic acids, esters and ethers. 1053 cm^{-1} peak represents the C-N stretch of aliphatic amines. In addition, a less pronounced peak representing the N-H wag of primary and secondary amines, and C-H out of plane bend of aromatics indicated by 765 cm^{-1} . A peak of 565 cm^{-1} shows the presence of C-Br stretch of alkyl halides.

Bands for pomegranate flower were obtained at peaks 541 cm^{-1} , 752 cm^{-1} , 1053 cm^{-1} , 1215 cm^{-1} , 1339 cm^{-1} , 1613 cm^{-1} , 1727 cm^{-1} , 2026 cm^{-1} , 2175 cm^{-1} , 3060 cm^{-1} and 3585 cm^{-1} . The peak seen at 541 cm^{-1} represents medium of C-Br stretch of alkyl halides, 752 cm^{-1} denotes the C-Cl stretch of alkyl halides, C-O out of plane bending of aromatics, N-H wag of primary and secondary amine, and a medium =C-H bend of alkenes. 1053 cm^{-1} represents the -C-O stretch of alcohols, carboxylic acid esters and ethers functional group. 1215 cm^{-1} peak is representative of C-N stretch of aliphatic amine and a medium C-O stretch of alcohol. Absorbance peak 1352 cm^{-1} indicates N-O stretch of nitro compounds and a weak C-H rock of the alkane functional group. Peak 1613 cm^{-1} represents N-H bend of primary amine and peak 1727 cm^{-1} represents C=O stretch of ketones, aldehydes, saturated aliphatic, $\alpha\beta$ -unsaturated ester, and stretch of carbonyls. The band at 2175 cm^{-1} is due to a weak $\text{C}\equiv\text{C}$ stretch of alkyne group. The band seen at 3060 cm^{-1} indicates O-H stretch of carboxylic acids. The band at 3585 cm^{-1} is due to O-H stretch, H-bonded of alcohols, free hydroxyl O-H stretch vibration of phenol functional group. The obtained peaks are similar to those reported by Ghaidaa *et al.* (2016) and Haniff Nisha *et al.* (2015).

TABLE 5.1: IR absorption rate for study

S/N	Wave Number						Surface Functional group
	Pomegranate leaf extract	Pomegranate flower extract	ZnO-NPs-PL	ZnO-NPs-PF	Fe ₃ O ₄ -NPs-PL	Fe ₃ O ₄ -NPs-PL	
1	565	541	536	516	516	516	C-Br Alkyl Halide
2	765	752	690		752	725	C-Cl Alkyl Halide
3	-	-	891	891	-	-	=C-H Alkenes
4	1053	1053	1053	1065	1078	1065	C-O Alcohol
5	1215-1352	1215-1339	1389	1389	1364	1352	C-F Alkyl halides
6	1588	1613	1600	1588	1588	1588	N-H Amide
7	1727	1727	-	-	-	-	Cyclic Ketone
8	2100	2026-2175	-	-	2125	-	-C≡C- Alkynes
9	2936		2324	2312	2337	2337-2998	C-H Alkane
10	-	3060	-	-	3023	-	=C-H Alkene
11	-	3585	3394	3398	3210	3210	O-H Alcohol

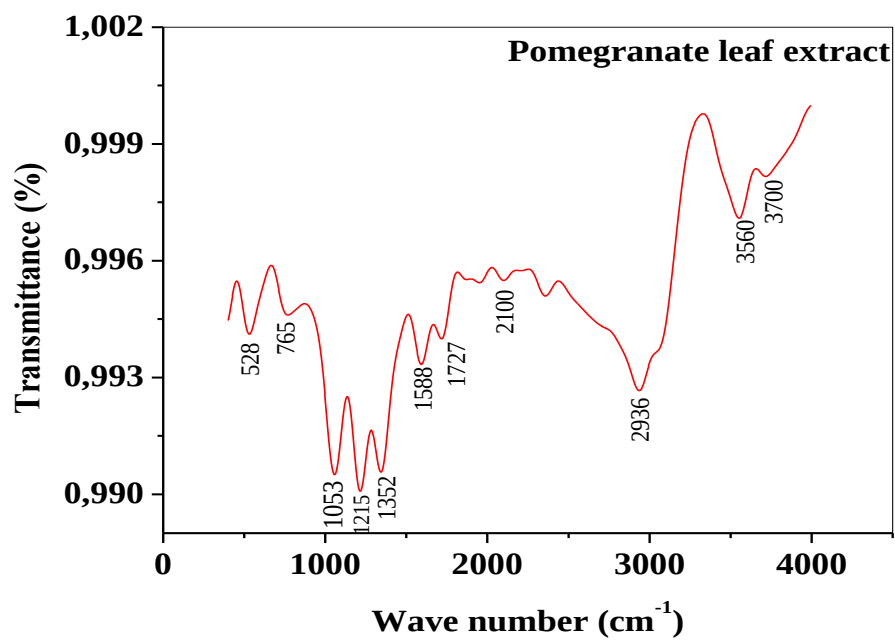


Figure 5.11: FTIR spectrum for Pomegranate leaf extract.

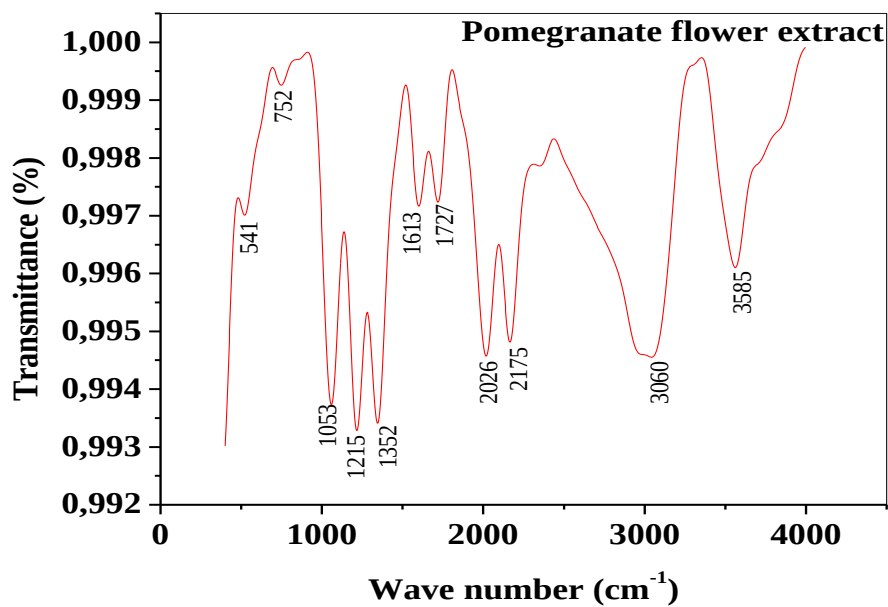


Figure 5.12: FTIR spectrum of Pomegranate flower extract.

FTIR spectra for zinc oxide nanoparticles prepared from pomegranate leaf and flower extracts are shown in Figures 5.13 and 5.14. ZnO-NPs-PL absorbed at peak values 536, 690, 891, 1053, 1389, 1600, 2063, 2324, and 3394 cm^{-1} , while for ZnO-NPs-PF, absorption peaks were seen at 516, 891, 1065, 1389, 1588, 2075, 2312, 3398 cm^{-1} . The bands observed at 536 cm^{-1} (ZnO-NPs-L) and 516 cm^{-1} (ZnO-NPs-F) correspond to the stretch band of zinc and oxygen (Zn-O), confirming synthesis of zinc oxide nanoparticles. Broad peaks absorbed at 3411 cm^{-1} and 3398 cm^{-1} for ZnO-NPs-L and ZnO-NPs-F respectively can be attributed to asymmetric and symmetric stretching of H-O-H vibration mode of phenol (Akbarian *et al.*, 2018). Similarly, Sadatzadeh *et al.* (2018) report a peak of 3445.03 cm^{-1} (stretch hydroxyl group (O-H)) for aqueous extract of cotton seed mediated zinc oxide nanoparticles. Broad bands at 1600 cm^{-1} (ZnO-NPs-L) and 1588 cm^{-1} (ZnO-NPs-F) may correspond to a medium C=C ring stretch absorption of aromatics and N-H bend of primary amine where a shift in band to lower frequency is observed. A not so pronounced peak of 1053 cm^{-1} (ZnO-NPs-L) and a pronounced peak of 1065 cm^{-1} (ZnO-NPs-F) may indicate C-O stretch of alcohols, carboxylic acids, esters, ethers and C-N stretching of aliphatic amines. The bands at 891 cm^{-1} represent C-H out of plane bending of aromatics and N-H wag of primary and secondary amines, while the peak of 690 cm^{-1} observed in ZnO-NPs-L only indicates the presence of C-Br stretch of alkyl halides. Peaks of almost similar range were reported by Azizi *et al.* (2016) for essential oil of *A. italica* mediated zinc oxide nanoparticles. FTIR analysis of zinc oxide nanoparticles from *Andrographis paniculata* leaf extract also showed similar peaks to those reported by Rajakumar *et al.* (2017). A shift in vibration band of the carboxylic groups (from 2936 cm^{-1} of PL extract and 3060 cm^{-1} of PF extract to a lower wavelength of 2655 cm^{-1} (ZnO-NPs-L) and 2658

cm^{-1} (ZnO-NPs-F)), and phenol group (from $3560\text{--}3700\text{ cm}^{-1}$ range for PL extract and 3585 cm^{-1} PF extract to a lower wavelength of 3411 cm^{-1} (ZnO-NPs-L) and 3398 cm^{-1} (ZnO-NPs-F)), coupled with the absence of ketone in the ZnO-NPs-L and ZnO-NPs-F spectral, signifies that there was an interaction between the plant leaf and flower extracts and the metal salts. The absence of ketones in the spectrum for synthesized ZnO-NPs-L and ZnO-NPs-F may probably mean that the ketone was used up during synthesis of zinc oxide nanoparticles where it acted as a reducing and capping agent. Carboxylic and alcohol compounds can bind metals and may form metal nanoparticles by stabilizing the medium and preventing agglomeration (Umar *et al.*, 2018). The presence of functional groups within the leaf and flower extract of pomegranate plant such as amines, alcohols, ketones, phenol, carboxylic acid and alkenes may have contributed to the reduction of Zn^{2+} . In addition, bio-capping during synthesis can be attributed to the action of carboxylic and phenolic acid, while stability of synthesized zinc oxide nanoparticles is linked to the presence of free amino and carboxylic group interaction with the zinc oxide nanoparticle surface.

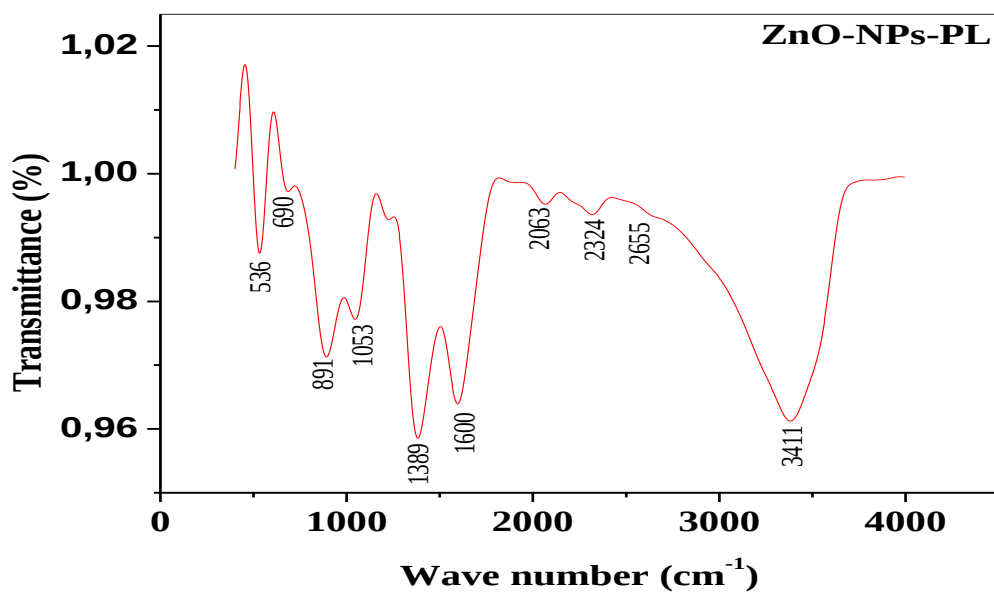


Figure 5.13: FTIR spectrum of Pomegranate leaf extract mediated zinc oxide nanoparticles

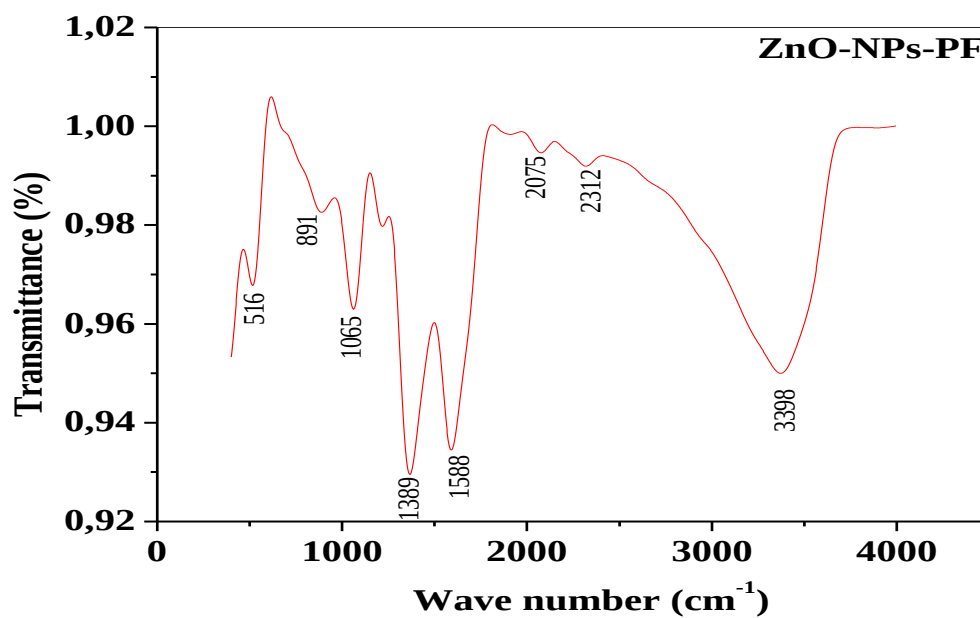


Figure 5.14: FTIR spectrum of Pomegranate flower extract mediated zinc oxide nanoparticles

As seen in Figures 5.15 and 5.16, the FTIR spectra of the synthesized iron oxide nanoparticle mediated via pomegranate leaf and flower aqueous extract showed peaks at values, 516 cm^{-1} , 725 cm^{-1} , 1078 cm^{-1} , 1364 cm^{-1} , 1588 cm^{-1} , 2125 cm^{-1} , 2337 cm^{-1} , 3023 cm^{-1} and 3210 for $\text{Fe}_3\text{O}_4\text{-PL}$, peak values were seen at 516 cm^{-1} , 727 cm^{-1} , 1065 cm^{-1} , 1352 cm^{-1} , 1588 cm^{-1} , 2088 cm^{-1} , 2337 cm^{-1} , 2998 cm^{-1} and 3172 cm^{-1} for $\text{Fe}_3\text{O}_4\text{-PF}$. The presence of iron oxide nanoparticle is confirmed by the peak 516 cm^{-1} (Fe-O vibrations) obtained in both the leaf and flower mediated iron oxide nanoparticle spectra. Deshmukh *et al.* (2018) report a closely related strong peak in the range of 586 cm^{-1} indicating the presence of $\text{Fe}_3\text{O}_4\text{-NPs}$ for fenugreek seed extract mediated Fe_3O_4 . An absorption peak at 500 cm^{-1} was reported by Khalil *et al.* (2017), which shows the Fe-O stretch vibrations of iron oxide nanoparticles. Absorption peaks at 725 cm^{-1} ($\text{Fe}_3\text{O}_4\text{-PL}$) and 727 cm^{-1} ($\text{Fe}_3\text{O}_4\text{-PF}$) indicate C-Cl stretch of alkyl halides, C-H “oops” aromatics and N-H wag of primary and secondary amines. 1078 cm^{-1} and 1065 cm^{-1} lies in the C-O stretching vibrations of alcohols, carboxylic acid, esters and ether domain. Medium -C-H rock band of alkanes can be observed in the 1364 cm^{-1} peak for $\text{Fe}_3\text{O}_4\text{-L}$ while the peak at 1352 cm^{-1} derived from $\text{Fe}_3\text{O}_4\text{-F}$ conforms to the N-O symmetric stretching of nitro compounds and -C-H rock of alkanes. The N-H bend of primary amine is represented by the peak at 1558 cm^{-1} ($\text{Fe}_3\text{O}_4\text{-L}$ and $\text{Fe}_3\text{O}_4\text{-F}$), while peaks of 2125 cm^{-1} ($\text{Fe}_3\text{O}_4\text{-L}$) denote the -C $\equiv$ C-stretching of alkynes which shifted to a lower wavelength in case of $\text{Fe}_3\text{O}_4\text{-F}$ where a close peak of 2088 cm^{-1} was obtained. For $\text{Fe}_3\text{O}_4\text{-L}$, peak 3023 cm^{-1} refers to the =C-H stretching of alkenes, and C-H stretching of aromatics, while peak 2998 cm^{-1} of $\text{Fe}_3\text{O}_4\text{-F}$ denotes the presence of C-

H stretching of alkanes. The strong absorption peak at 3210 cm^{-1} ($\text{Fe}_3\text{O}_4\text{-PL}$) and 3222 cm^{-1} ($\text{Fe}_3\text{O}_4\text{-PF}$) is assigned to H–O–H stretching of alcohol and phenolic compounds. Similar observation of peaks was reported by Khalil *et al.* (2017) and Devi *et al.* (2018). The C=O stretch indicates the presence of flavonoids and may be responsible for reduction and stabilization process of nanoparticle synthesis. In plant mediated synthesis of nanoparticles FTIR analysis shows that the phytochemicals, amines and oxygen bearing functional groups such as phenols, carboxyl, and carbonyl are responsible for the synthesis and capping of iron oxide nanoparticles. The nucleophilic aromatic ring possessed by phenolic acids such as gallic and ellagic compounds found in the pomegranate plant, has the capability of chelating metal, and as such acts as reducing agent (Ali *et al.*, 2017, Ovais *et al.*, 2018). Despite being subjected to high drying temperatures, these bioactive biomolecules adhere to the surface of the green mediated synthesized $\text{Fe}_3\text{O}_4\text{-NPs}$.

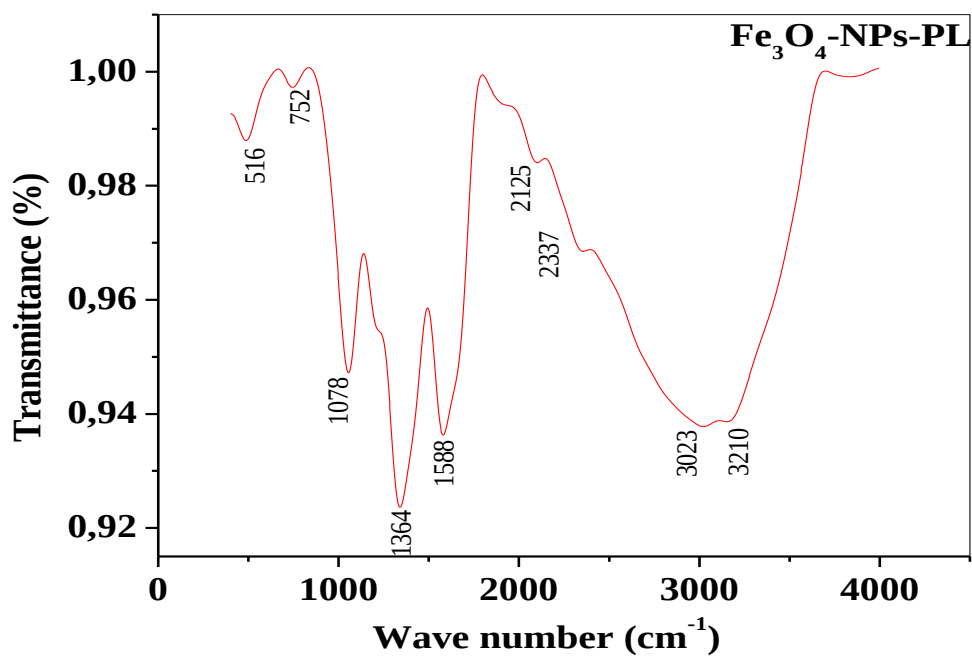


Figure 5.15: FTIR Spectrum Pomegranate leaf extract mediated iron oxide

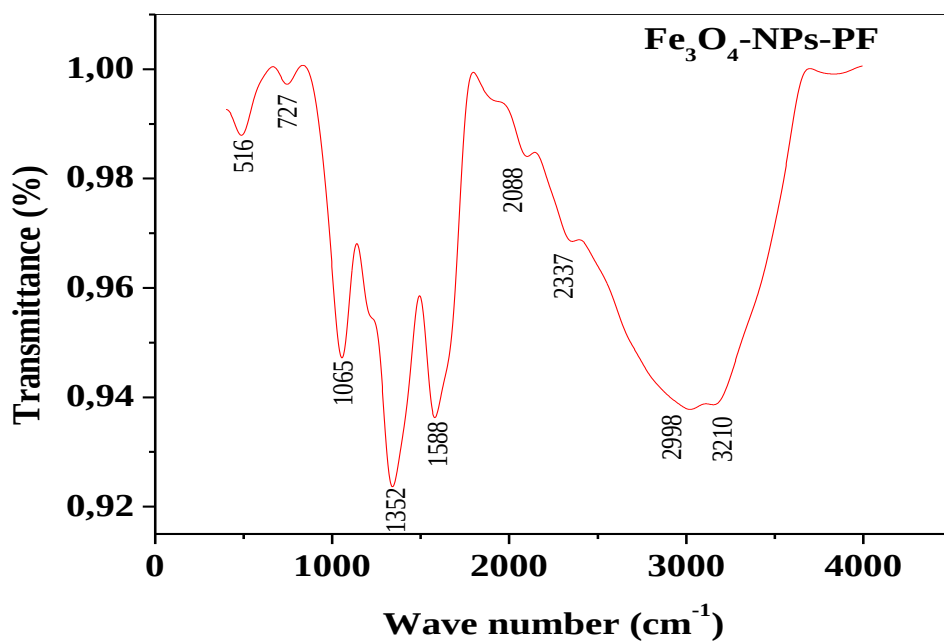


Figure 5.16: FTIR Spectrum Pomegranate flower extract mediated iron oxide

5.5.4 X-ray diffraction (XRD) analysis

Characterization of biosynthesized zinc oxide nanoparticles via pomegranate leaf and flower were analysed by X-ray diffraction. This was done to ascertain the purity and crystalline structure of the metal oxide nanoparticles (Khatami *et al.*, 2018, Umamaheswari *et al.*, 2018). Obtained diffraction peaks at 2θ values as shown in Figure 5.17 were 31.71° , 34.34° , 36.25° , 47.54° , 56.62° , 62.76° , 68.00° , 69.10° , 72.43° , and 72.43° for zinc oxide nanoparticles synthesized via pomegranate leaf extract, and (Figure 5.18) 31.74° , 34.42° , 36.13° , 47.54° , 56.53° , 62.84° , 67.93° , 69.05° , and 72.69° for the pomegranate flower extract mediated zinc oxide nanoparticles. These peak values relate to the crystal or lattice plane of (100), (002), (101), (102), (110), (103), (200), (112), (201) and (202), according to the Joint Committee on Powder Diffraction Studies Standards (JCPDS Card number 008, 82-1042 and 5-0664). The correspondent plane also known as Bragg's reflection line, suggests that the synthesized metal oxide nanoparticles might be of spherical crystalline structure of zinc oxide nanoparticles. Similar results were obtained by Fu & Fu. (2015), who biosynthesized zinc oxide nanoparticles via *Plectranthus amboinicus* leaf extract and reported diffraction peaks at 31.899° , 34.420° , 36.145° , 47.987° , 56.502° , 63.101° , 67.958° and 69.014° that correspond to (100), (002), (101), (102), (110), (103), (112) and (201). Vanathi *et al.* (2014), Bhuyan *et al.* (2015), Azizi *et al.* (2016), Fouda *et al.* (2017) and Vijayakumar *et al.* (2018) all reported similar diffraction peaks. The XRD spectrum of pomegranate leaf and flower mediated zinc oxide nanoparticles obtained from the study showed no other external peaks, indicating purity of the synthesized nanoparticles and suggesting the green method of synthesis used can be deployed to obtain nanoparticles of high purity (Akabrain *et al.*, 2018). From the figures, the XRD pattern shows both strong broad and narrow diffraction peaks which signifies that the

biosynthesized zinc oxide nanoparticles are crystalline in nature (Bhuyan *et al.*, 2015). However, the broad peak is an indication that the nanoparticles are small and fine particles (nanoscale crystalline sized particles) while the narrow or low intensity peak is an indication of low crystallinity of the nanoparticles (Azizi *et al.*, 2016). From the XRD spectral pattern, the mean particle diameter of the biosynthesized zinc oxide nanoparticle can be calculated following the line width of maximum intensity reflection peak using the Derby Scherer equation (Murali *et al.*, 2017, Rajakumar *et al.*, 2017):

Equation 1:

$$d = \frac{k\lambda}{\beta \cos \theta}$$

Where d denotes the mean particle size,

k = 0.89 (Shape factor)

λ =x-ray wavelength (1.5406 Å)

β denotes full width at half maximum (FWHM) in radians

θ denotes the Bragg diffraction angle.

From the above equation, the crystalline particle size derived for pomegranate leaf mediated zinc oxide nanoparticle and pomegranate flower mediated zinc oxide nanoparticle was 57.75 nm and 52.50 nm respectively. These are close to the particle size values reported by Yuvakkumar *et al.* (2014) and Dhanemozhi *et al.* (2017), who obtained “D” values of 50.95 nm and 54.84 nm respectively. In contrast, Khatami *et al.* (2018), Rajeshkumar *et al.* (2018) and Vanathi *et al.* (2014) obtained lesser “D” values of 47.70 nm, 47.70 nm and 32 nm respectively.

For pomegranate leaf and flower mediated iron oxide nanoparticles, XRD analysis to obtain the phase and crystalline nature, yielded an insufficient peak without distinguishing peak. The XRD spectrum (Figure 5.19 and 5.20) was devoid of peak

but shows humps which indicates that the synthesized iron oxide nanoparticle is amorphous in nature and not crystalline. However, a broad peak was observed from 20 to 30 of 2θ value. This can be said to be due to the presence of organic compounds found in the leaf and flower extract that function in capping and stabilizing nanoparticles (Kheshtzar *et al.*, 2019). Similar observations were reported by Irshad *et al.* (2017), Kheshtzar *et al.* (2019) and Kouhbanani *et al.* (2019) for Punica granatum peel extract mediated iron oxide nanoparticles, pine needle leaves mediated iron oxide nanoparticles, and aqueous leaf extract of *Teucrium polium* mediated iron oxide nanoparticles respectively.

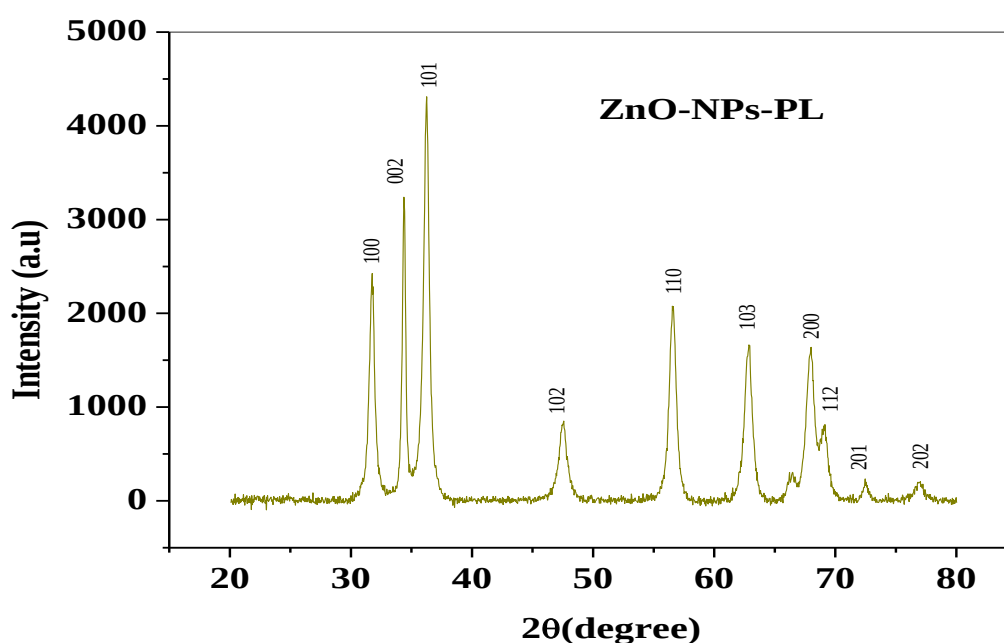


Figure 5.17: XRD Spectra of Pomegranate leaf extract mediated zinc oxide nanoparticle.

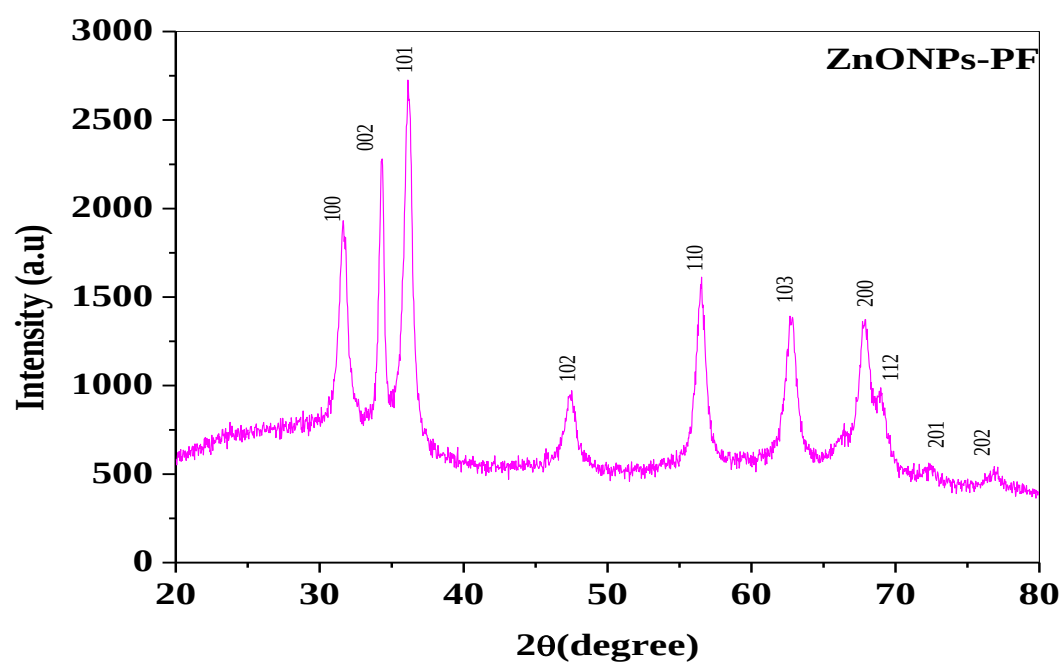


Figure 5.18: XRD Spectra of Pomegranate flower extract mediated zinc oxide nanoparticle

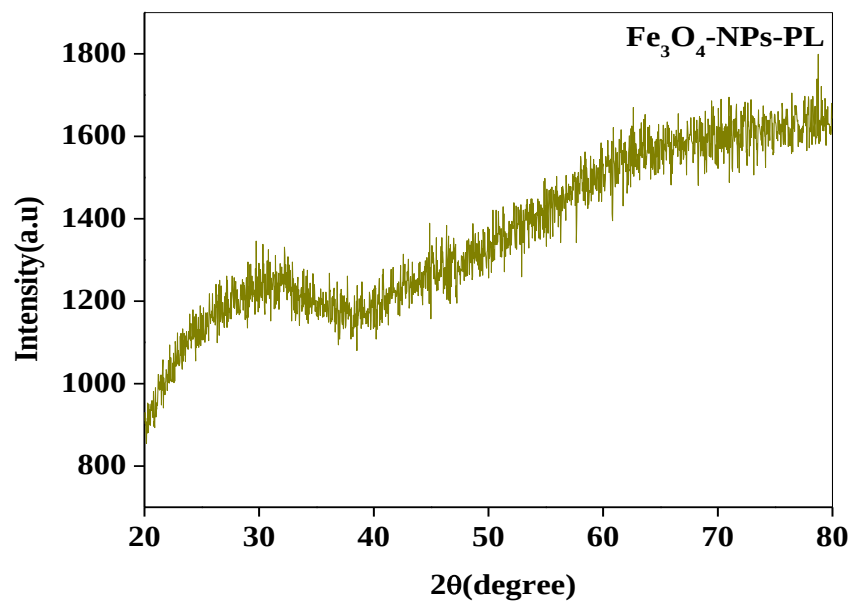


Figure 5.19: XRD Spectra of Pomegranate leaf extract mediated iron oxide nanoparticle

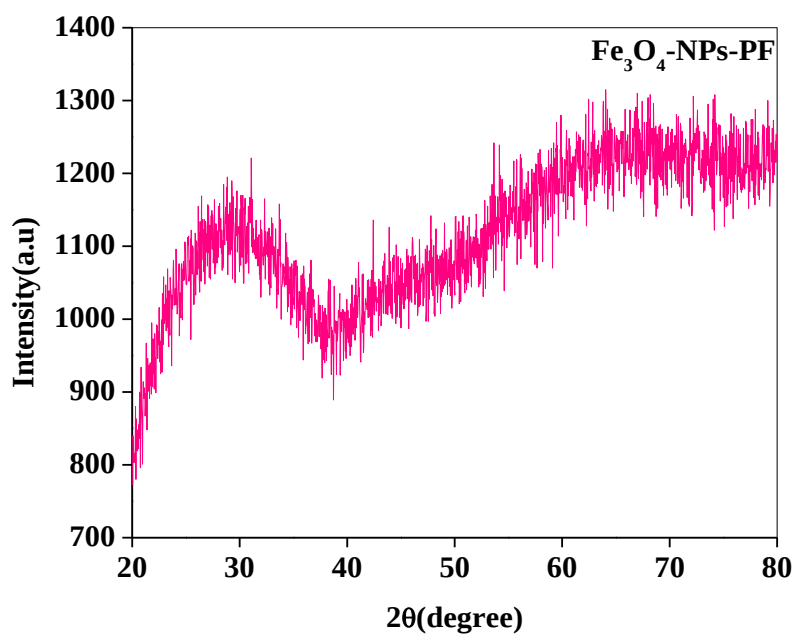


Figure 5.20: XRD Spectra of Pomegranate flower extract mediated iron oxide nanoparticle

5.5.5 Scanning electron microscopy with energy dispersive x-ray (EDX)

SEM imaging with EDX analysis was done to further confirm presence of nanoparticles synthesized. It aims to provide the morphology or microstructure of biosynthesized nanoparticles. From SEM images of Figure 5.21a and 5.22a, the zinc oxide nanoparticle synthesized from pomegranate leaf (ZnO-NPs-PL) shows an unevenly roundlike structure, which is similar to that reported by Prashanth *et al.* (2015), Umar *et al.* (2018) and Wali *et al.* (2019), also the presence of agglomerated nanoparticles can be seen on the SEM image. The SEM image of ZnO-NPs-PF (Figure 5.22a) also reveals an irregularly shaped microstructure with protrusion which seems flower-like. EDX-analysis to confirm elemental compositions of the synthesized nanoparticles reveals the presence of zinc and oxygen components. The elemental analysis of the nanoparticles also shows 77.24% of zinc and 22.74% of oxygen elements for ZnO-NPS-PL (Figure 5.21b), while ZnO-NPs-PF (Figure 5.22b) shows 72.75% zinc and 27.25% oxygen elemental composition. This indicates that the synthesized nanoparticle is in its purest form.

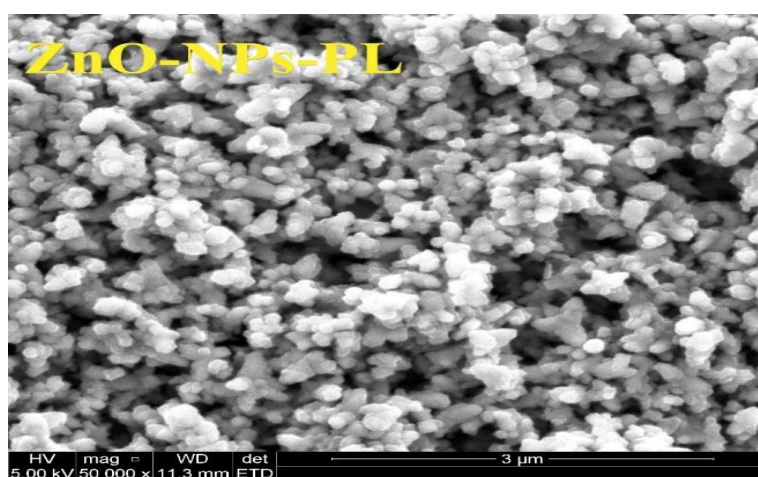


Figure 5.21a: SEM image of pomegranate leaf mediated zinc oxide nanoparticle.

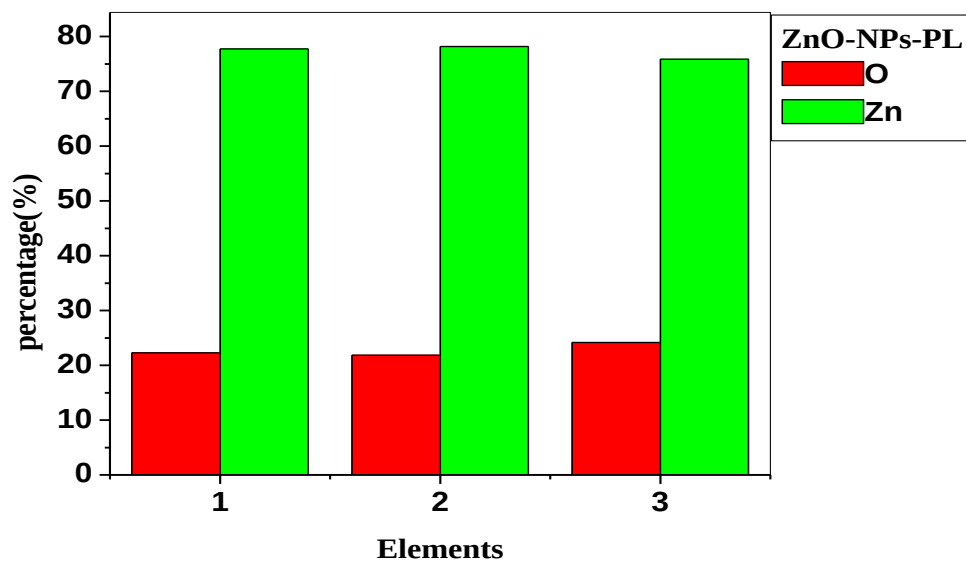


Figure 5.21b: EDX spectrum of pomegranate leaf mediated zinc oxide nanoparticle.

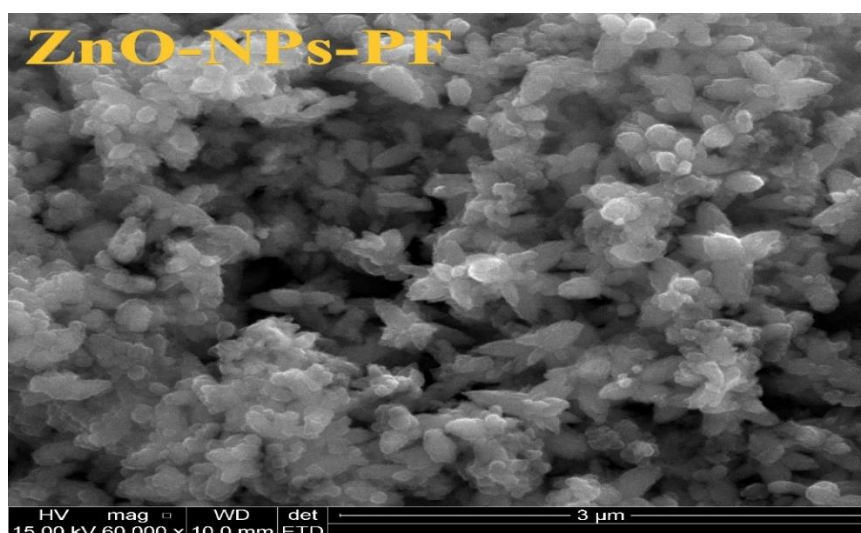


Figure 5.22a: SEM image of pomegranate flower mediated zinc oxide

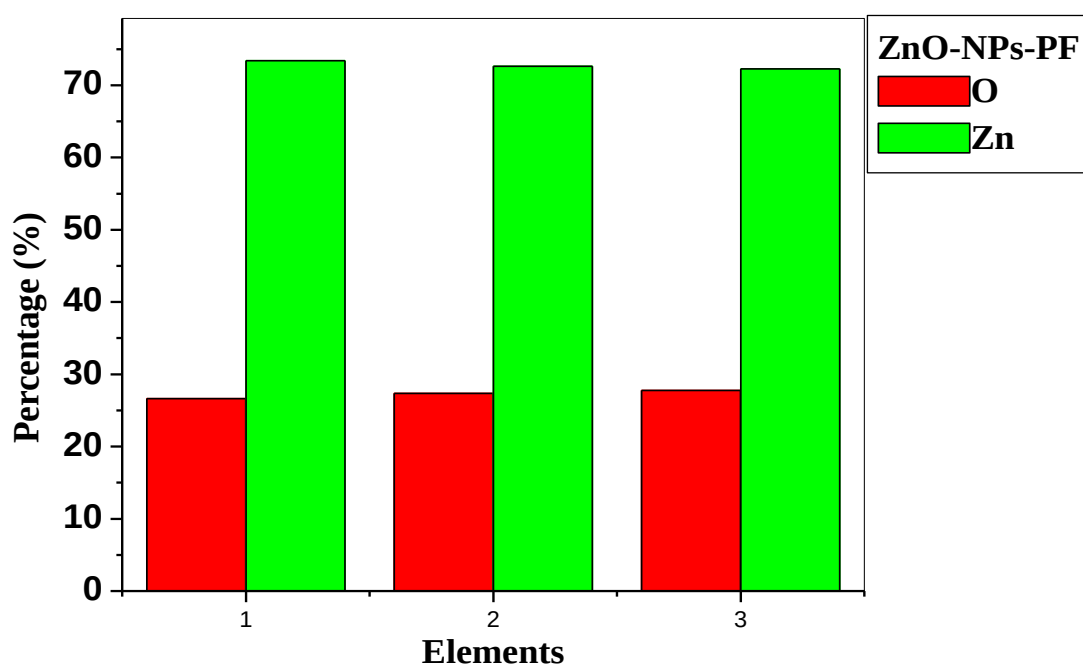


Figure 5.22b: EDX spectrum of pomegranate flower mediated zinc oxide nanoparticle.

Figure 5.23a and 5.24a reveal the SEM imaging for iron oxide nanoparticle synthesized from pomegranate flower and leaf, and show a discrete spherical shaped structure. Agglomerated nanoparticles were also revealed. The EDX confirmed the presence of both iron and oxygen in the nanoparticle. Other elemental signals observed such as Chlorine (Cl) could have originated from the precursor $\text{Fe}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ used in the synthesis process, while the phosphorous (P) and potassium (K) originated from the plant pomegranate, as they are part of the elemental constituents of the plant (Dumlu *et al.*, 2007). The elemental analysis of the Fe_3O_4 -NPs -PL shows 22.67% iron, 69.10% oxygen, 4.86% phosphorus, 0.90% chlorine and 2.47% potassium. Fe_3O_4 -NPs -PF shows 26.21% iron, 68.44%

oxygen, 2.71% phosphorus, 0.63% chlorine and 2.01% potassium. (See Figures 5.23b and 5.24b)

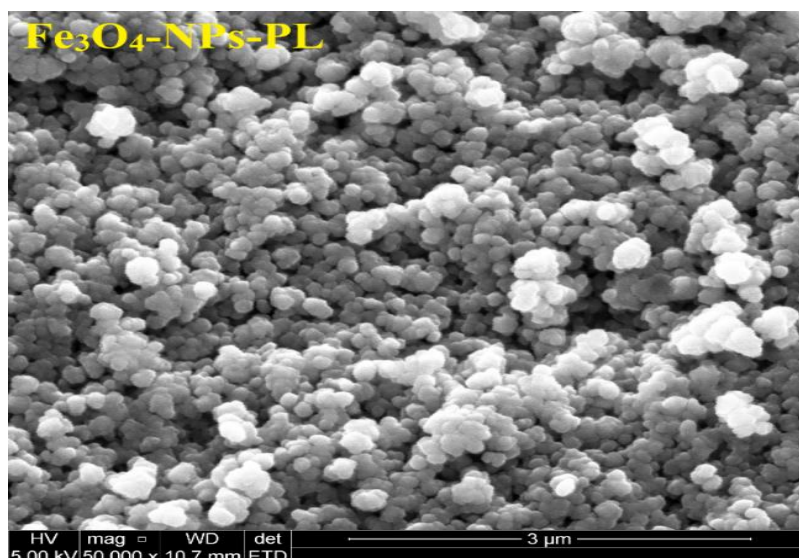


Figure 5.23a: SEM image of pomegranate leaf mediated iron oxide nanoparticle.

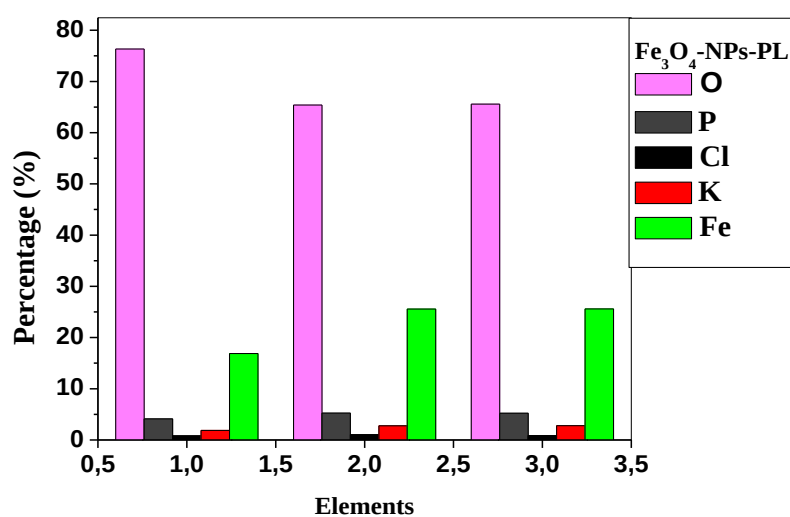


Figure 5.23b: EDX spectrum of pomegranate leaf mediated iron oxide nanoparticle.

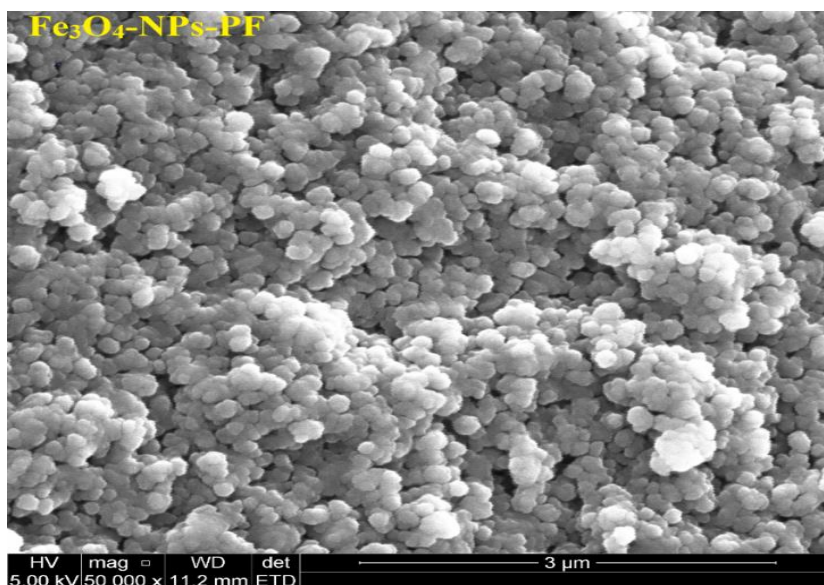


Figure 5.24a: SEM image of pomegranate flower mediated iron oxide nanoparticle.

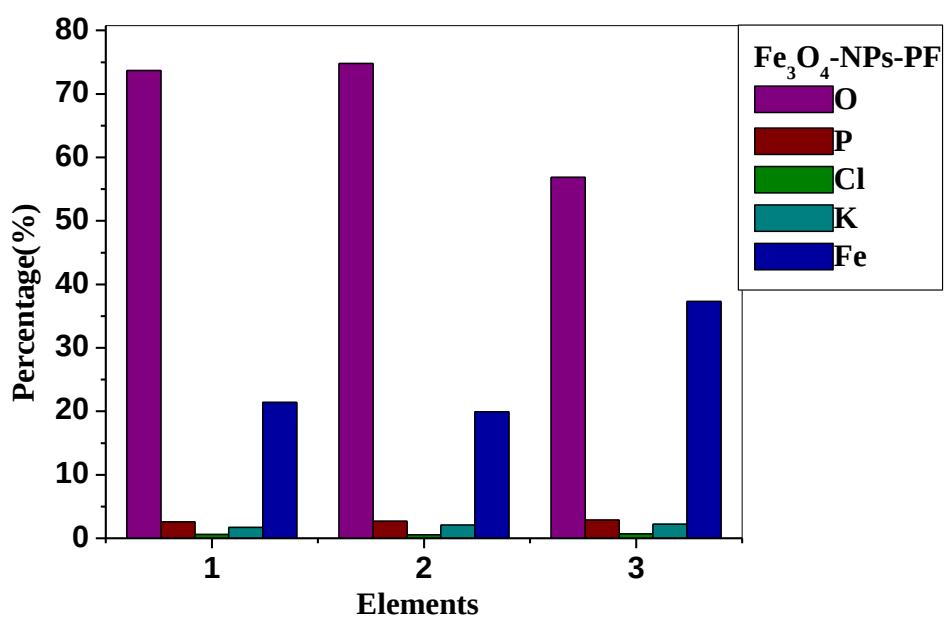


Figure 5.24b: EDX spectrum of pomegranate leaf mediated iron oxide nanoparticle.

5.5.6 Transmission electron microscopy

TEM micrographs of nanoparticles obtained showed that ZnO-NPs-PL and ZnO-NPs-PF were flower-like structures as seen in Figures 5.25a and 5.25b. This conforms to the SEM images obtained for ZnO-NPs-PF. In accordance to TEM images obtained in Figures 5.25c and 5.25e, Fe₃O₄-NPs-PL and Fe₃O₄-NPs-PF are indicated to be almost spherical in shape. Average particle size distribution for the obtained particles were determined using Image J application where particles were measured at random and represented using a histogram. From the obtained histogram, average particle size distribution for Fe₃O₄-NPs-PL is 49.59 nm while that of Fe₃O₄-NPs-PF is 34.30nm as shown in Figures 5.25d and 5.25f. Obtained TEM images also indicated agglomeration of the particles to each other. This occurrence can be linked to the presence of biological components of the plant extract wound around the nanoparticles resulting in particles sticking together.

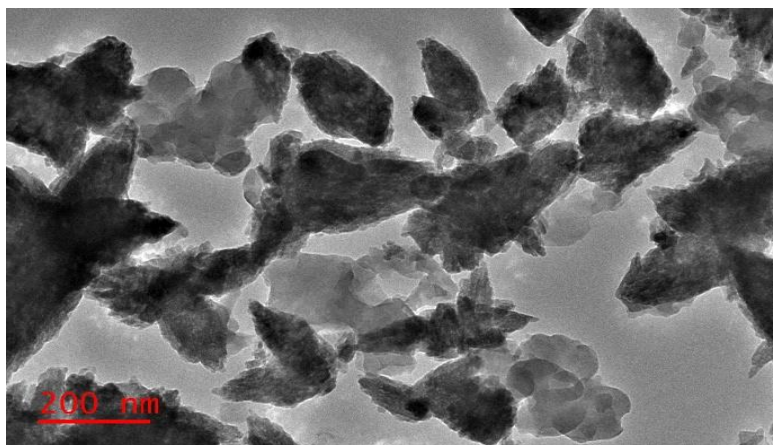


Figure 5.25a: Typical TEM image of synthesized ZnO-NPs-PL

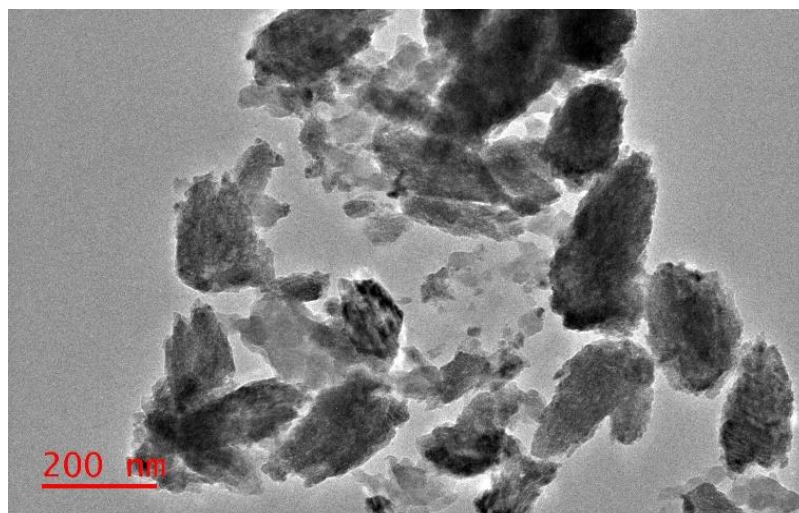
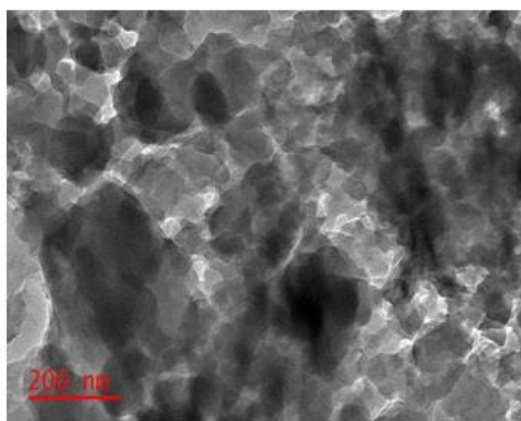
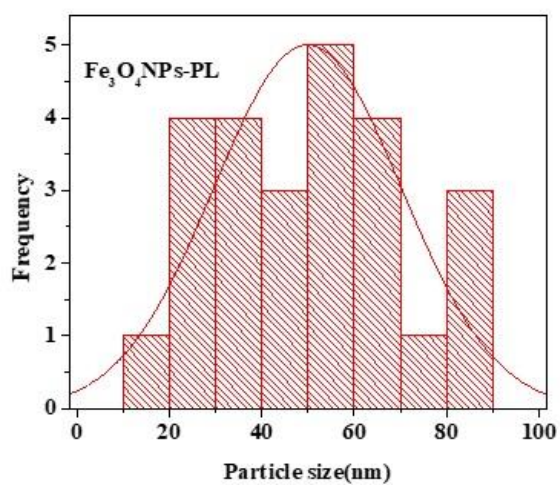


Figure 5.25b: Typical TEM image of synthesized ZnO-NPs-PF



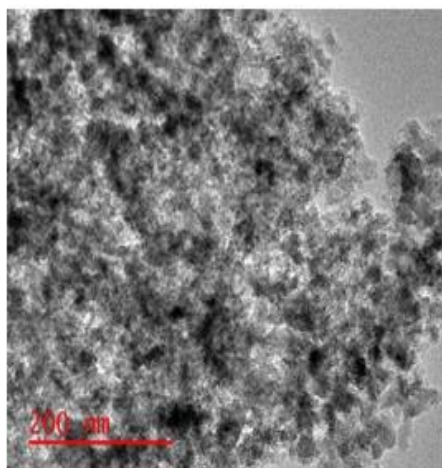
(c)



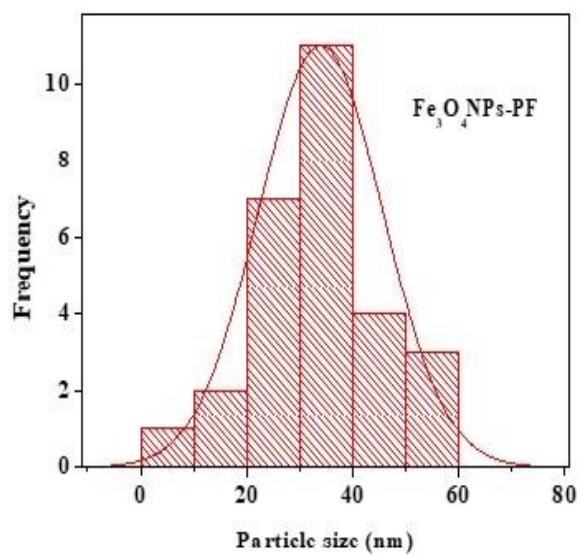
(d)

Figure 5.25c: Typical TEM image of synthesized Fe₃O₄-NPs-PL and,

Figure 5.25d: Histogram showing corresponding particle size distributions for Fe₃O₄-NPs-PL



(e)



(f)

Figure 5.25e: Typical TEM image of synthesized Fe_3O_4 -NPs-PF and,
Figure 5.25f: Histogram showing corresponding particle size distributions for Fe_3O_4 -NPs-

5.6 Electrochemical characterization

5.6.1 Cyclic voltammetry

Cyclic voltammetry experiments aim to determine the electrochemical properties of analytes that can be oxidized or reduced electrochemically. In the study, the charge properties of the bare screen printed electrode (SPCE), modified screen printed electrodes of synthesized iron oxide nanoparticles (SPCE-Fe₃O₄-NPs-PL, SPCE-Fe₃O₄-NPs-PF) and zinc oxide nanoparticles (SPCE-ZnO-NPs-PL, SPCE-ZnO-NPs-PF) mediated via pomegranate leaf and flower, were evaluated using the cyclic voltammetry experiment. The analysis was conducted using 5 mM Fe(CN)₆⁴⁻/[Fe(CN)₆]³⁻ in 0.1 M phosphate buffer solution (PBS), at scan rates of 25, 50, 75, 100, 125 and 150, 175, 300 mV/s. Obtained voltammogram results of the modified electrodes are shown in Figures 5.26 (a), and 5.27 (a).

Following the voltammogram of Figure 5.26a, a pair of redox peaks was observed at the modified electrodes. For SPCE-Fe₃O₄-NPs-PL, the first redox peak was observed in the 147 mV/s region and the second in the 429 mV/s region. The first redox peaks were assigned to the Fe(CN)₆⁴⁻/[Fe(CN)₆]³⁻ while the second peaks were ascribed to the modified SPCE-Fe₃O₄. The anodic current density of each electrode in Fe(CN)₆⁴⁻/[Fe(CN)₆]³⁻ redox probe follows the order: SPCE (13.312 μA) < SPCE-Fe₃O₄-NPs-PL (13.762 μA), < SPCE-Fe₃O₄-NPs-PF (16.691 μA). Following the obtained anodic peaks order, it is evident that higher current responses were recorded in the modified electrodes than in the bare (SPCE). This can be linked to the electronic conductivity of Fe₃O₄. The SPCE-Fe₃O₄-NPs-PF, however, records a higher electrochemical response than the SPCE-Fe₃O₄-NPs-PL, signifying that the Fe₃O₄-NPs-PF is more conductive than the Fe₃O₄-NPs-PL. In respect to the ratio of the anodic to cathodic

current peaks (I_{pa}/I_{pc}), obtained values for SPCE-Fe₃O₄-NPs-PL and SPCE-Fe₃O₄-NPs-PF were 0.6550 and 0.7861 respectively, which is almost equivalent to 1. This indicates that the CV electrochemical process was reversible (Namchi & Obayi 2018).

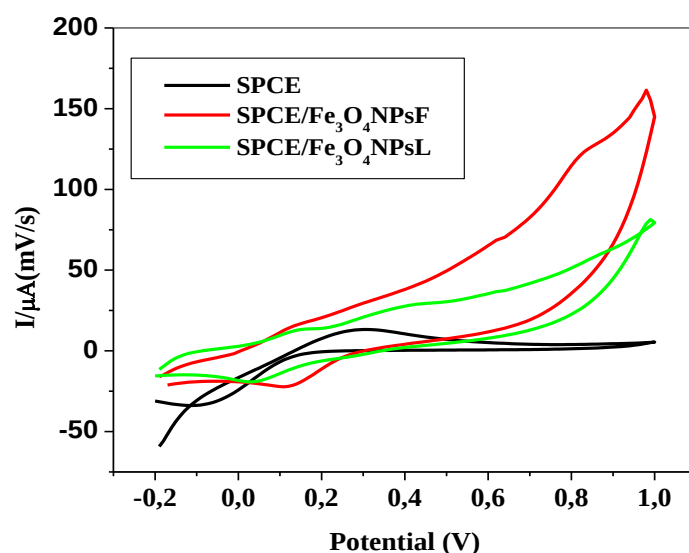


Figure 5.26a: Cyclic voltammograms of bare SPCE, modified SPCE-Fe₃O₄-NPs-PL, and modified SPCE-Fe₃O₄-NPs-PF.

5.6.1.1 Scan rate effect (Iron oxide nanoparticle)

Scan rate study was done from 25-300 mV/s. From the obtained scan rate voltammogram in Figures 5.26(b) and 5.26(c), representing Fe₃O₄-NPs-PL and Fe₃O₄-NPs-PF respectively, there was an increase in both the anodic peak current (I_{pa}) and cathodic peak current (I_{pc}) as scan rate increased, and a shift in potential to the right was also observed.

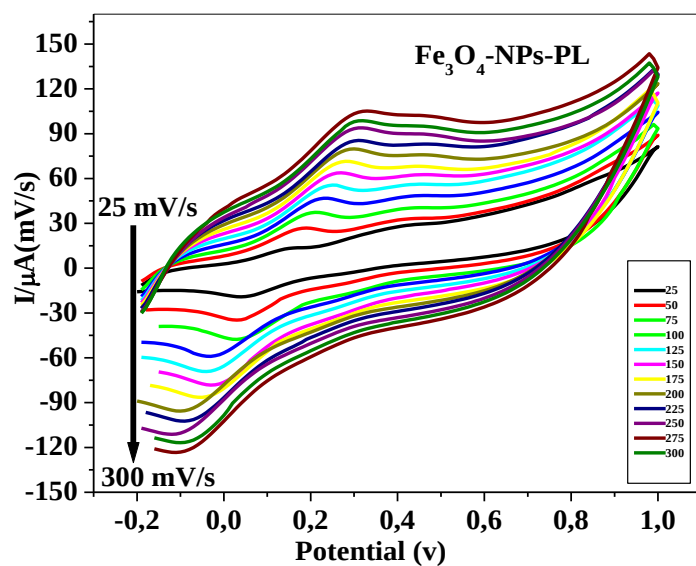


Figure 5.26b: Cyclic voltammogram obtained for SPCE- $\text{Fe}_3\text{O}_4\text{-NPs-PL}$ in FECN solution prepared in 0.1 M PBS (scan rate: 25–300 Mv/s; inner to outer)

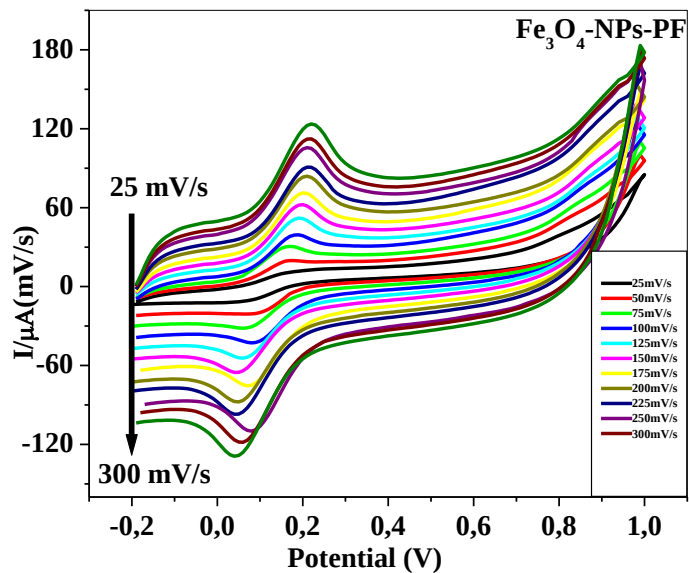


Figure 5.26c: Cyclic voltammogram obtained for SPCE- $\text{Fe}_3\text{O}_4\text{-NPs-PF}$ in FECN solution prepared in 0.1 M PBS (scan rate: 25–300 Mv/s; inner to outer)

In addition, a linear analysis plot of anodic current as a function of square root of scan rate is shown in Figures 5.26(d) and 5.26(e). The plots reveal that the anodic and cathodic peak current (I_{pa}) varies linearly with an increase in scan rate. The regression value of $R^2 = 0.99$ was obtained for the modified electrodes (SPCE- Fe_3O_4 -NPs-PL and SPCE- Fe_3O_4 -NPs-PF) which validates that the electron transfer is under diffusion controlled process as reported in literature.

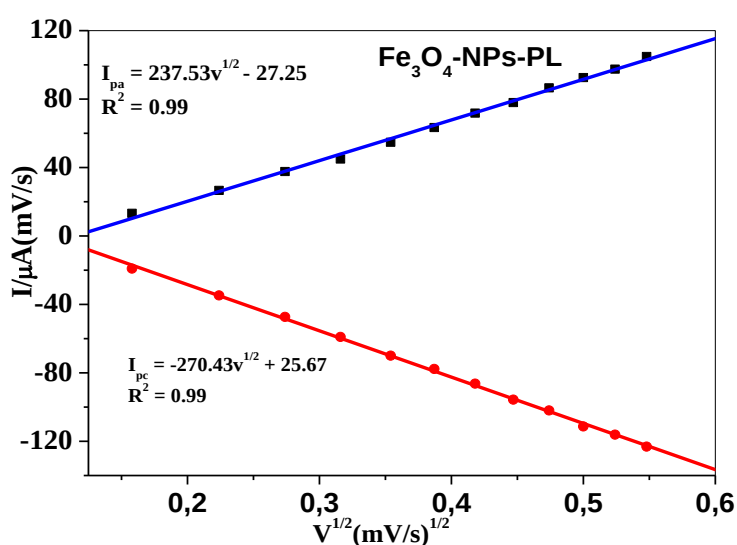


Figure 5.26d: Linear plot of I_{pa} versus $V^{1/2}$ and I_{pc} versus $V^{1/2}$ for SPCE- Fe_3O_4 -NPs-PL in 5Mm $\text{Fe}(\text{CN})_6^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ solution prepared in 0.1M PBS.

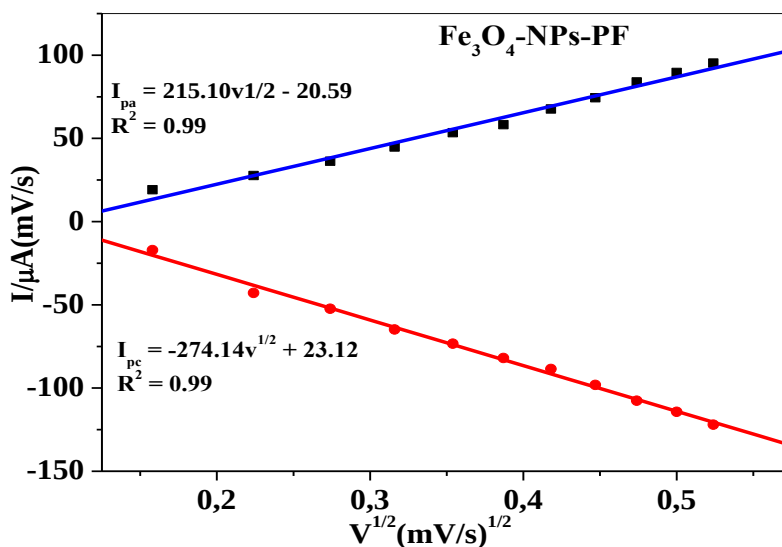


Figure 5.26e: Linear plot of I_{pa} versus $V^{1/2}$ and I_{pc} versus $V^{1/2}$ for SPCE-Fe₃O₄-NPs-PF in 5 Mm Fe(CN)₆⁴⁻/[Fe(CN)₆]³⁻ solution prepared in 0.1M PBS.

The electrochemical behaviour of modified electrodes for zinc oxide nanoparticles synthesized via pomegranate leaf and flower (SPCE-ZnO-NPs-PL and SPCE-ZnO-NPs-PF) and the bare (SPCE) were also analysed using the CV technique. From the voltammogram obtained, the first redox peak was obtained in the 141 mV/s region and the second peak at 832 mV/s, and this can be associated with the redox process of zinc oxide nanoparticle. There was absence of an oxidation peak, relatively a reduction peak was observed for the modified electrodes. This signifies that the zinc oxide nanoparticles obtained, shows characteristics that resembles but not really capacitive. It also explains that the electrocatalysis of the obtained zinc oxide nanoparticle is a quasi-irreversible reaction. Similar results were reported by Matinise *et al.* (2017) and Kahraman *et al.* (2018). The reduction peak values obtained were 35 mV/s and

172mV/s for modified electrode for the SPCE-ZnO-NPs-PL and SPCE-ZnO-NPs-PF respectively. This signifies reduction of ZnO to metallic Zn.

Following the order: SPCE (-33.4 μA) < SPCE-ZnO-NPs-PL (-11.35 μA), < SPCE-ZnO-NPs-PF (-7.03 μA), in comparison to the bare SPCE, the modified electrodes exhibited higher reduction current, due to their catalytic activity.

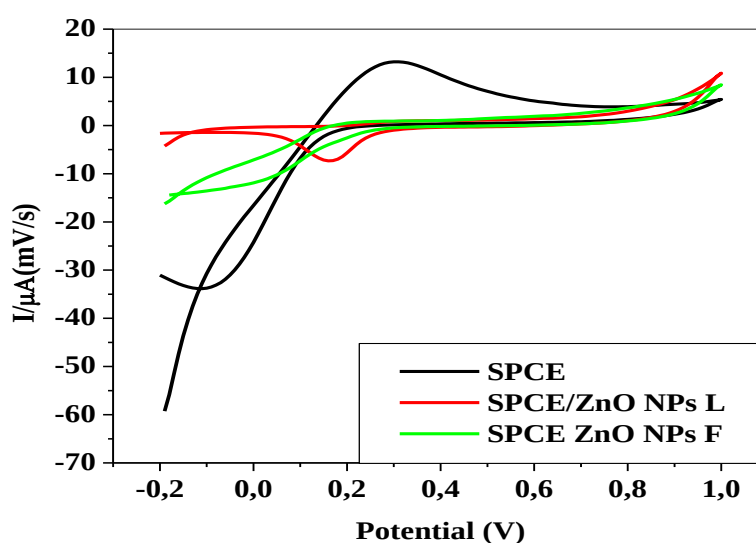


Figure 5.27a: Cyclic voltammograms of bare SPCE, modified SPCE-ZnO-NPs-PL, and modified SPCE- ZnO-NPs-PF.

5.6.1.2 Scan rate effect (Zinc oxide nanoparticle)

Electrochemical activity of a material can be estimated by monitoring peak current in the cathodic and anodic scan. The scan rate of the synthesized zinc oxide nanoparticle was studied using cyclic voltammetry. Based on the voltammogram obtained, observation shows that as the scan rate increased, there was a linear increase in the reduction peak, and the reduction peak shifted slightly to a more negative value. This can be caused by the fast faradaic redox reaction (Matinise *et al.*, 2017). Thereby it

can be deduced that the synthesized ZnO-NPs have portrayed good electrochemical behaviour.

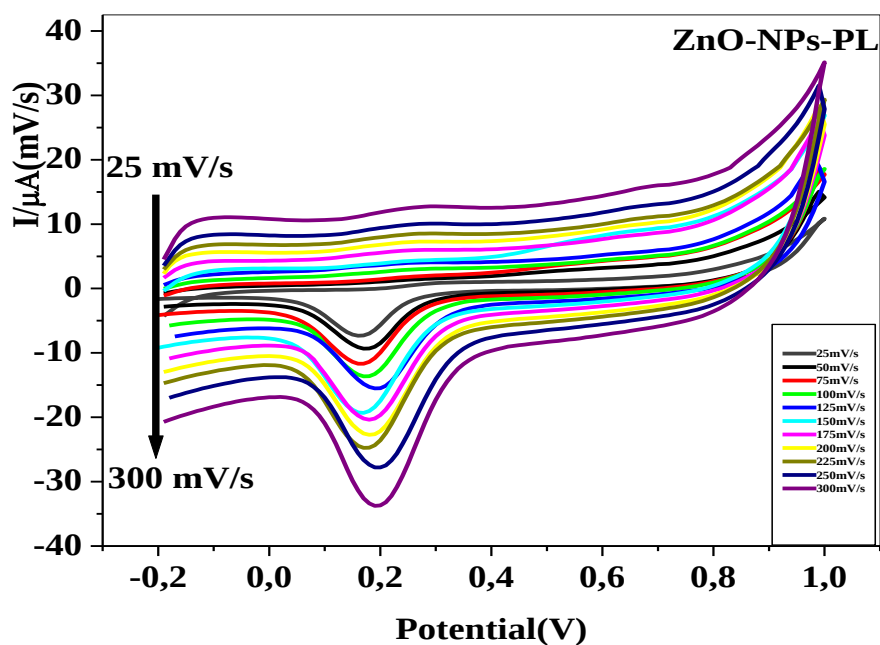


Figure 5.27b: Cyclic voltammogram obtained for SPCE-ZnO-NPs-PF in FECN solution prepared in 0.1 M PBS (scan rate: 25–300 Mv/s; inner to outer)

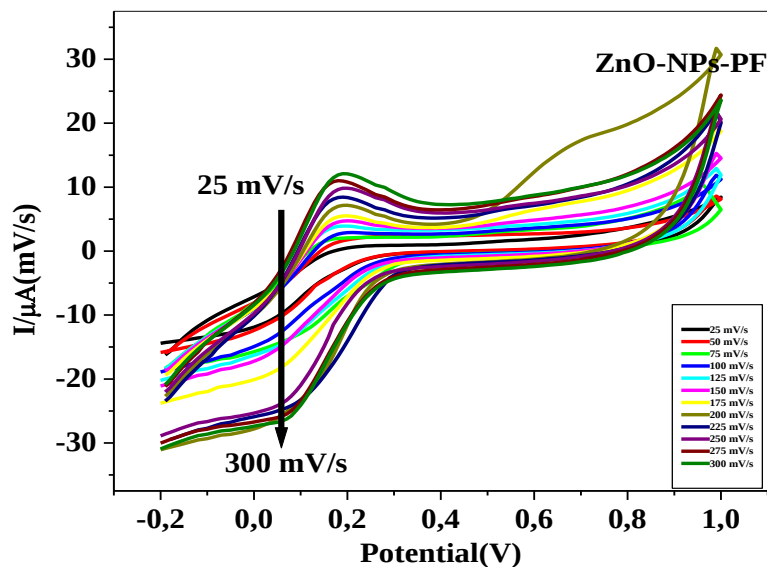


Figure 5.27c: Cyclic voltammogram obtained for SPCE-ZnO-NPs-PL in FECN solution prepared in 0.1 M PBS (scan rate: 25–300 Mv/s; inner to outer)

In the plot of peak current height against the square root of scan rate as shown in Figures 5.27(d) and 5.27(e), regression values for the modified electrodes were obtained at $R^2 = 0.97$ for the SPCE-ZnO-NPs-PL, while the regression value for modified SPCE-ZnO-NPs-PF was at $R^2 = 0.97$ and 0.96 for the anodic and cathodic plot respectively, thereby indicating a diffusion controlled process.

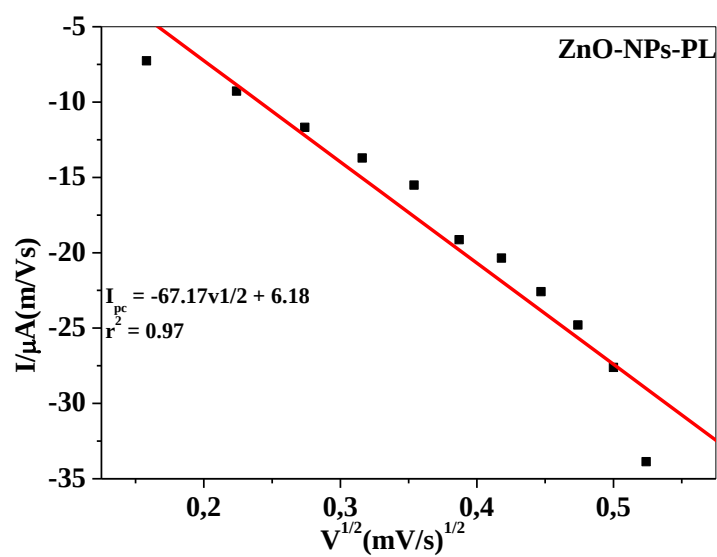


Figure 5.27d: Linear plot of I_{pa} versus $V^{1/2}$ and I_{pc} versus $V^{1/2}$ for SPCE-ZnO-NPs-PL in 5Mm $Fe(CN)_6^{4-}/[Fe(CN)_6]^{3-}$ solution prepared in 0.1M PBS.

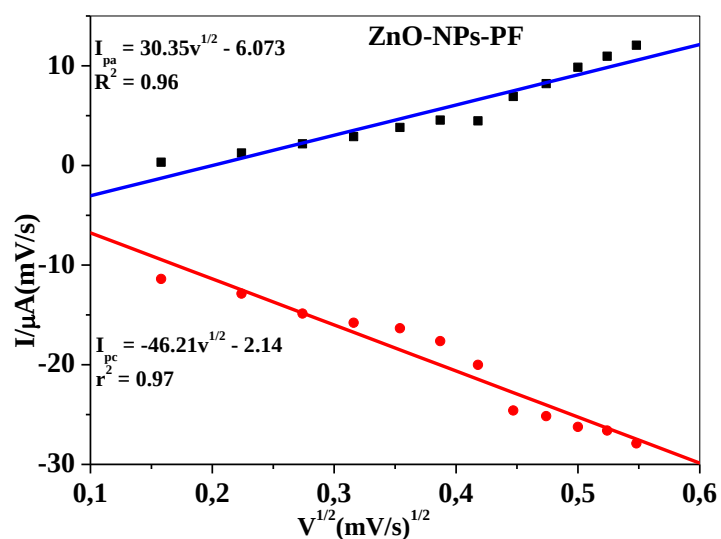


Figure 5.27e: Linear plot of I_{pa} versus $V^{1/2}$ and I_{pc} versus $V^{1/2}$ for SPCE-ZnO-NPs-PL in 5Mm $Fe(CN)_6^{4-}/[Fe(CN)_6]^{3-}$ solution prepared in 0.1M PBS.

5.7 Conclusion

Metal oxide nanoparticles of zinc and iron were biosynthesized via leaves and flower extract of the pomegranate (*Punica granatum*) plant. The extract contains phytochemicals and metabolites that functioned as capping, reducing and stabilizing agents during the synthesis process. A visual change in colour from greenish yellow (leaf) and pinkish brown (flower) to a yellow-whitish precipitate for zinc oxide and black for iron oxide during the synthesis process indicates formation of zinc oxide and iron oxide nanoparticles. Confirmation of the formation of nanoparticles from the process was determined using different spectroscopic and electrochemical characterization tools. The UV-absorbance maximum of the synthesized zinc oxide nanoparticle via leaf and flower extract were 284 nm and 345 nm respectively. Obtained UV-absorbance maximum for iron oxide nanoparticles for leaf and flower extract were 308nm for the former and 310 nm for the latter. A slight shift (blue shift) was obtained in the absorption maximum of 411nm for flower extract and 326 nm of the ferric chloride hexahydrate precursor to a shorter wavelength of 310 nm for the Fe₃O₄-NPs-PF. A blue shift was also obtained as absorption maximum shifted from 412 nm for leaf extract and 326 nm for the precursor to 308nm. FTIR analysis of the biosynthesized nanoparticles revealed peaks with IR absorption values of 536 cm⁻¹ for ZnO-NPs-PL, 516 cm⁻¹ for ZnO-NPs-PL, 516 cm⁻¹ for Fe₃O₄-NPs-PL and 516 cm⁻¹ for Fe₃O₄-NPs-PF. These values obtained are in the reported IR range for Zn-O bond linkage and Fe-O vibration mode. No other peak was observed in the range, thereby confirming formation of zinc oxide nanoparticles and iron oxide nanoparticles. FTIR of the aqueous pomegranate (*Punica granatum*) flower and leaf extract reveals presence of bioactive phytochemicals like phenols, alcohol, aldehyde and amine, which were

the surface active molecules that were implicated in stabilizing, reducing and capping functions during synthesis. XRD analysis revealed the purity and crystalline structure of the zinc oxide nanoparticles and an amorphous structure for the iron oxide nanoparticles. In addition, the Derby Scherer equation gave a mean diameter size for the ZnO-NPs-PL as 57.75 nm and 52.50 nm for the ZnO-NPs-PL. Surface morphology of the synthesized metal oxide was characterized by scanning electron microscopy, which showed irregular spherically shaped zinc and iron oxide nanoparticles with agglomerations. The elemental composition analysis using EDX revealed zinc and oxygen constituent for the biosynthesized zinc oxide nanoparticles (ZnO-NPs-PL, ZnO-NPs-PF) and iron and oxygen composition where seen in iron oxide nanoparticles (Fe_3O_4 -NPs-PL, Fe_3O_4 -NPs-PF). In addition, CV analysis for electrochemical characterisation of the biosynthesized nanoparticles describes the electron transport and electrocatalytic properties of the bio-synthesized metal oxide nanoparticles. From the voltammograms showing the important anodic and cathodic current peaks, it can be said that the synthesized metal oxide nanoparticles exhibit good electrochemical activities, and as such, they are potential excellent candidates for electrocatalysts in electrochemical applications.

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CHAPTER 6

Evaluation of the antimicrobial properties of synthesized metal oxide nanoparticles from pomegranate (*Punica gratum*) extract.

6.1 Abstract

An alternative approach to the conventional use of antibiotics is to develop and use nanoparticles, which have been found to effectively target and inhibit the growth of bacteria cells. Studies have shown that nanoparticles can elicit various mechanisms of action against bacteria cells at the same time, thereby making it difficult for them to produce any form of resistance. This study aims at evaluating the antimicrobial efficacy of synthesized iron oxide and zinc oxide nanoparticles produced using aqueous extracts of pomegranate leaves and flowers designated ZnO-NPs-PL, ZnO-NPs-PF, Fe₃O₄-NPs –PL and Fe₃O₄-NPs -PF. The synthesized particles were tested against thirteen pathogenic bacteria strains: *S. aureus*, *B. cerus*, *P. aeruginosa*, *K. pneumoniae*, *S. pneumonia*, *S. diarizonae*, *S. typhi*, *E. faecalis*, *E. faecium*, *E. coli*, *M. catarrhalis*, *A. hydrophilia*, and *L. monocytogenes*. Generally, zinc oxide nanoparticles (ZnO-NPs-PL and ZnO-NPs-PF) produced antibacterial properties [ZnO-NPs-PL – 50 µg/ml (6.33±0.58 – 13.33±1.15) 100µg/ml (9.67±1.53 – 15.17±0.29) 1000 µg/ml (11.17±0.29 – 17.33±1.15) 5000 µg/ml (13.00 – 19.33±1.15)] [ZnO-NPs-PF – 50 µg/ml (5.67±0.58 – 12.67±0.58) 100µg/ml (8.67±1.15 – 15.83±0.76) 1000 µg/ml (10.67±1.15 – 17.33±1.15) 5000 µg/ml (14.00 – 21.50±0.50)] against all the thirteen pathogenic strains. Similarly, iron oxide nanoparticles (Fe₃O₄-NPs –PL and Fe₃O₄-NPs –PF) were also active against the pathogenic strains [Fe₃O₄-NPs-PL – 50 µg/ml (5.33±0.58 – 12.77±0.40) 100µg/ml (8 – 15.83±0.15) 1000 µg/ml (9.93±0.12 – 17.90±0.17) 5000 µg/ml (11.77±0.25 – 23.00)] [Fe₃O₄-NPs –PF – 50 µg/ml (4.33±0.58 – 9.77±0.40)]

100µg/ml (6.33 ± 0.58 – 13.33 ± 0.58) 1000 µg/ml (10 – 17.17 ± 0.29) 5000 µg/ml (12 – 20.27 ± 0.25)] except for *K. pneumoniae* that was resistant to the iron oxide nanoparticle (Fe₃O₄-NPs -PF) synthesized from pomegranate flower extract. Based on data from SEM and TEM characterisation, we suggest that the effectiveness of the nanoparticles could be linked to differences in their sizes and shapes. In addition, our data reveal that increasing the concentration of the extract in nanoparticle leads to an increase in its antibacterial effect. Our data also reveal that zinc oxide nanoparticles and iron oxide nanoparticles can effectively be employed as alternative antibacterial agents. Further studies are required to understand the mechanism of actions of these nanoparticles as well as their cytotoxicity of epithelial cells.

6.2 Introduction

Bacterial pathogens cause infections that are typically treated using antibiotics (Wang *et al.*, 2017, Gold *et al.*, 2018). Antibiotics are antibacterial agents that inhibit the growth of bacterial pathogens through different modes of action ranging from inhibition of enzyme action, interfering with DNA, RNA and protein synthesis (Wang *et al.*, 2017, Singh *et al.*, 2018). These processes ultimately result in the disruption of the bacteria cell membrane structure leading to cell death (Wang *et al.*, 2017, Singh *et al.*, 2018). Recent studies have demonstrated that bacterial pathogens are exhibiting resistance against a variety of antibiotics (Jones *et al.*, 2008, Singh *et al.*, 2018), thereby limiting effectiveness of these agents (Singh *et al.*, 2018, Vega-Jiménez *et al.*, 2019). Contrary to the modes of actions of antibiotics, bacteria strains are able to express antimicrobial resistance by: (1) altering the target of antibiotics by expressing genes that code for an alternate version of the antibiotic target (Rai *et al.*, 2016, Whitehouse *et al.*, 2018); (2) developing enzymes that can degrade or modify the drug (Gold *et al.*, 2018,

Ruddaraju *et al.*, 2019); (3) ensuring reduced uptake of antimicrobial drugs or acting as efflux pumps that push out the drugs (Rai *et al.*, 2016, Singh *et al.*, 2018); and (4) formation of biofilm layers around the bacteria cell thus limiting or reducing its exposure to antibiotics (Ruddaraju *et al.*, 2019, Shaikh *et al.*, 2019). The ever-increasing occurrence of bacterial resistance among pathogenic bacteria that is most often caused by the the inappropriate or misuse of antibiotics (Raghunath and Perumal, 2017), coupled with limited surveillance data as well as the recent increase in biofilm-associated infections in humans, has led to the search for more effective agents and strategies to combat antimicrobial resistance (Huh & Kwon 2011, Swain *et al.*, 2014).

Owing to their antibacterial activity, different inorganic metals and their oxides, such as zinc oxide, copper oxide, titanium oxide and magnesium oxide are being exploited as antimicrobial agents since they are stable, robust and have a long shelf-life (Raghupathi *et al.*, 2011, Salem *et al.*, 2015, Raghunath & Perumal, 2017). Metals and metal oxides have been used in the past for treatment of infections and illnesses (Brandelli *et al.*, 2017, Gold *et al.*, 2018, Singh *et al.*, 2018). Metals exert their antimicrobial strength on Gram-positive and Gram-negative bacteria by selectively upsetting the process required for cell growth (Lemire *et al.*, 2013, Gold *et al.*, 2018). The antimicrobial mechanism of action of these metal oxides involves the release of metal ions, which are absorbed by microbial cell membranes, eliciting interactions with functional groups of protein and nucleic acid, thereby inhibiting enzyme activity (Wang *et al.*, 2017, Shaikh *et al.*, 2019). This results in a change in cell structure and finally inhibits the microorganism (Wang *et al.*, 2017, Shaikh *et al.*, 2019). In addition to these, another mechanism of action exhibited by metal oxides is the production of reactive oxygen species (ROS), which triggers electrostatic interaction, thus altering the

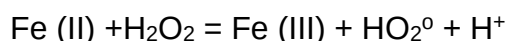
prokaryotic cell wall and enzyme or DNA pathways (Gold *et al.*, 2018, Ruddaraju *et al.*, 2019).

Nanoparticles are small sized objects with diameter of about 1-100 nm and they have a wide range of applications (Salem *et al.*, 2015, Wang *et al.*, 2017). Metals and their oxides can be synthesized into nanoparticles (Niño-Martínez *et al.*, 2019) which have applications in medicine, biotechnology and many other industrial applications (Sorbiun *et al.*, 2018). In recent times, metal and metal oxide formulated nanoparticles, especially those synthesized from plant extracts, are constantly being explored as alternative bio-control agents (Raghunath and Perumal, 2016, Kadiyala *et al.*, 2018, Singh *et al.*, 2018, Vega-Jiménez *et al.*, 2019). In addition, these biosynthesized metal and metal oxide nanoparticles have produced very valuable results when utilized as treatment agents against some cases of infections caused by multidrug resistant Gram-negative and Gram-positive bacteria (Dizaj *et al.*, 2014, Brandelli *et al.*, 2017, Vega-Jiménez *et al.*, 2019). In the biosynthesis of nanoparticles, the plant extracts function as reducing, stabilizing and capping agents (Saif *et al.*, 2016, Ruddaraju *et al.*, 2019, Zhu *et al.*, 2019).

The pomegranate plant is reported to possess important biomolecules and metabolites including organic acids, polyphenols, flavonoids, anthocyanins, alkaloids, fatty acids, and vitamins (Shayganma *et al.*, 2015). The high phenolic content of pomegranate has been reported to account for its antimicrobial, antihelminthic, anti-inflammatory and antioxidant properties (Ismail *et al.*, 2012). However, the effectiveness of synthesized metal and metal oxide nanoparticles is greatly related to their stability, size, size distribution, surface functionality, morphology, shape and the type of material used in synthesis (Moritz & Geszke-Moritz, 2013, Dizaj *et al.*, 2014, Swain *et al.*, 2014, Tang *et al.*, 2014, Khezerlou *et al.*, 2018).

As stated earlier, studies have shown that the smaller the size of the nanoparticle, the larger its surface to volume ratio, and thus the higher its antimicrobial efficacy (Dizaj *et al.*, 2014, Brandelli *et al.*, 2017, Sorbiun *et al.*, 2018, Niño-Martínez *et al.*, 2019, Vega-Jiménez *et al.*, 2019). This is because small sized nanoparticles can interact directly with the cell membrane of bacterial pathogens (Sorbiun *et al.*, 2018). In terms of shape, spherically-shaped nanoparticles have been shown to have more antibacterial capabilities (Aderibigbe 2017, Shahzad *et al.*, 2019).

The antimicrobial activities of the magnetic iron oxide nanoparticles (Fe₃O₄-NPs) arise from different interactions, such as membrane depolarization which destroys cell integrity (Vega-Jiménez *et al.*, 2019), photocatalytic generation of reactive oxygen species (ROS) which affect the respiratory system, damaging bacterial cellular and viral components (Huh & Kwon 2011, Gunalan *et al.*, 2012), damage of cellular DNA (Ansari *et al.*, 2017, Saqib *et al.*, 2018, Vasantharaj *et al.*, 2018), release of metal ions that affect homeostatis and protein coordination (Arias *et al.*, 2018). When iron oxide nanoparticles come in contact with bacterial medium, both Fe³⁺ and Fe²⁺ undergo oxidation-reduction reactions through the Fenton reaction or Haber-Weiss cycle, leading to ROS production (Lemire *et al.*, 2013, Arakha *et al.*, 2015, Wang *et al.*, 2017).



In the Fenton reaction, enough quantity of free radicals (HO₂[•] and OH[•]) are formed to cause stress on the bacterial cell (Arakha *et al.*, 2015, Wang *et al.*, 2017). Gabrielyan *et al.* (2019) studied the antimicrobial activity of Fe₃O₄-NPs against *Escherichia coli* BW 25113 and Gram-positive *Enterococcus hirae* ATCC 9790. They suggested that the reactive oxygen species, together with superoxide radicals and singlet generated

by Fe₃O₄ NPs, could be the leading cause of the inhibitory activity noticed. The antimicrobial effect of iron oxide nanoparticle is also largely dependent on concentration. Gabrielyan *et al.* (2019) and Ansari *et al.* (2017) observed that at 40 µg/ml concentration, zones of inhibition shown by Fe₃O₄ nanoparticles against *Bacillus cerus* and *Klebsiella pneumoniae* were at 15 mm and 13 mm respectively, but at a higher concentration of 80 µg/ml, inhibition zones increased to 26 mm and 22 mm.

Despite all these, it is very important to investigate the possible cytotoxic effects of synthesised nanoparticles in humans (Khezerlou *et al.*, 2018, Vega-Jiménez *et al.*, 2019), regardless of their antibacterial potential.

Nanoparticles can interact with biological molecules; this can give them biomedical applications, especially in the area of cancer diagnosis and treatment (Namvar *et al.*, 2015, Assa *et al.*, 2016). The zinc oxide nanoparticle is one of the most studied inorganic metal oxide nanoparticles (Tang *et al.*, 2014), and this is attributed to its stability under harsh conditions, predominant antimicrobial properties, and low toxicity to humans (Dizaj *et al.*, 2014 Tang *et al.*, 2014, Akbar *et al.*, 2018). Several studies have shown that zinc oxide nanoparticles have great antimicrobial activity against several microorganisms (Jones *et al.*, 2008) and may prevent biofilm formation (Salem *et al.*, 2015). In a previous study, Akbar *et al.* (2018) synthesized 20 nm sized zinc oxide nanoparticles that were tested against *Salmonella typhimurium* and *Staphylococcus aureus* and reported that the nanoparticles showed potent antimicrobial effects against the tested bacteria. Salem *et al.* (2015), also evaluated the antimicrobial effects of zinc oxide nanoparticles against *Vibrio cholera* and enterotoxic *Escherichia coli*, while Chaudhary *et al.* (2018) assessed the antimicrobial effect of zinc oxide nanoparticles against the following pathogenic organisms: *Staphylococcus epidermidis* (MTCC-3382), *Staphylococcus epidermidis* (MTCC-

3382), *Klebsiella pneumoniae* (MTCC-3384), *Escherichia coli* (MTCC-41) and fungi, *Aspergillus niger* (MTCC-404) and *Aspergillus oryzae* (MTCC-3107).

6.3 Materials and Method

6.3.1 Bacteria strains

The bacterial strains used in this study were American type culture collection strains purchased from BioMérieux South Africa while the *Salmonella typhi* was obtained from the Microbiology culture collection unit at the Department of Microbiology, North West University, South Africa. The bacteria species, strain numbers and the morphological characteristics are outlined in Table 6.1.

Table 6.1: List of Bacterial strains

S/N	Bacteria	Strain Number	Gram stain
1	<i>Staphylococcus aureus</i>	ATCC 25923	Gram-positive
2	<i>Eschericia coli</i>	ATCC 25922	Gram-negative
3	<i>Streptococcus pneumoniae</i>	ATCC 27336	Gram-positive
4	<i>Pseudomonas aeruginosa</i>	ATCC 27853	Gram-negative
5	<i>Bacillus cerus</i>	ATCC 10876	Gram-positive
6	<i>Moraxella catarrhalis</i>	ATCC 25240	Gram-negative
7	<i>Aeromonas hydrophilia</i>	ATCC 7966	Gram-negative
8	<i>Salmonella diarizonae</i>	ATCC 12325	Gram-negative
9	<i>Enterococcus fecalis</i>	ATCC 29212	Gram-positive
10	<i>Listeria monocytogenes</i>	ATCC 19115	Gram-positive
11	<i>Enterococcus faceium</i>	ATCC 6569	Gram-positive
12	<i>Klebsiella pneumoniae</i>	ATCC 13883	Gram-negative
13	<i>Salmonella typhi</i> ,	Lab isolate	Gram-negative

6.3.2 Preparation of nanoparticle suspensions

Varying concentrations of synthesized metal oxide nanoparticle (50 µg/ml, 100 µg/ml, 1000 µg/ml and 5000 µg/ml) as outlined in chapter 5 were prepared by adding a known quantity (µg or mg) of each particle (ZnO-NPs-Pomegranate Leaf, ZnO-NPs-Pomegranate Flower, FeO-NPs-Pomegranate Leaf, FeO-NPs-Pomegranate Flower) to a measured volume of dimethyl sulfoxide (DMSO) organic solvent. The mixture was allowed to sonicate for 24 h.

6.3.2 Antimicrobial activity of the synthesized metal oxides nanoparticles

The antimicrobial activities of the synthesized metal oxide (zinc oxide and iron oxide) nanoparticles were evaluated against thirteen pathogenic bacterial strains that are frequently associated with both food poisoning diseases and nosocomial infections in humans.

6.3.3 Preparation of bacteria inoculums

Each pure bacterial strain was sub-cultured in Tryptic soy broth (TSB) and incubated aerobically at 37 °C for 24 h. The absorbance of the bacteria cultures was adjusted to attain viable cell count of 10^7 CFU/mL (optical density of 0.4 - 0.5) at 630 nm using spectrophotometer (model, MB-580, Shenzhen Huisong Technology Development Co., Ltd, Shenzhen, China).

6.3.5 Antibiogram test of bacterial strain

As an internal control, the bacterial strains assessed in this study were screened against standard antibiotics using the Kirby-Bauer method on Muller-Hinton agar plates. Antibiotic discs; Augmentine (30 µg), Gentamicin (30µg), Amikacin (30 µg), Norfloxacin (10 µg), Ciprofloxacin (5 µg), Cefotaxime (30 µg), Tetracycline (30 µg) and Ampicillin (10 µg). Pre-adjusted overnight cultures (10^6 cfu/mL) spread-plated on Mueller-Hinton agar plates. The different antibiotic discs were placed at equitable distances on the inoculated agar using sterile forceps. The plates were incubated aerobically at 37°C for 24 h. Antibiotic inhibition zone diameter (AIZD) data were measured in millimetres and recorded. The AIZD data were used to classify isolates as either resistant, intermediate resistant or susceptible to a particular antibiotic using standard reference values (Ashley *et al.*, 2011, Maxson *et al.*, 2018).

6.3.6 Antibacterial activity of biosynthesized zinc oxide nanoparticle and ironoxide nanoparticle.

The agar well diffusion method as previously described by Azam *et al.* (2012) was used to evaluate antimicrobial activities of the synthesized metal oxides nanoparticles, but with slight modifications. Aliquots of 100 µL of the 10^6 cfu/mL bacterial culture was spread-plated on Mueller-Hinton agar and plates to prepare a lawn. The plates were left to stand for 10 mins and 8 mm wells were punched on the agar. An aliquot of 100 µL of a 50 µg/ml nanoparticle was poured into each of well on all plate. The plates were left to stand for 1 hour in the biosafety cabinet to ensure even diffusion of the samples into the agar, and later incubated at 37°C for 24 hours. Bacteria growth inhibition zone diameter data were measured in millimetres and recorded. The experiment was performed in triplicate and pooled data was statistically analysed

using the statistical package for the social sciences (SPSS) software version 20.0 IBM cooperation. Positive control, Gentamicin antibiotic (30 µg) and negative control Dimethyl sulfoxide were included in this experiment.

6.3.7 Determination of minimum inhibitory concentrations (MIC) of the synthesized metal oxides nanoparticles

The lowest concentration of an antimicrobial agent that can inhibit microbial growth after 24 hours of incubation is known as the Minimum inhibitory concentration (MIC) (Salem *et al.*, 2015). In order to evaluate the efficiency of the synthesized nanoparticles in controlling the pathogenic bacterial growth, the most effective synthesized metal oxide nanoparticles that exhibited strong antibacterial activity at 5000 µg/mL were used to determine the MIC based on the microbroth dilution method in 96 well microtiter plates. Two-fold dilution of the initial nanoparticle concentration (5000 µg/mL) was aseptically prepared by transferring 100 µL of the sterile nanoparticles into 100 µL of sterile tryptic soy broth in a microtitre well plate to obtain a 2500 µg/mL concentration. The process was repeated several times to obtain other concentrations (1250µg/ml, 625µg/ml, 312.5µg/ml, 156.25 µg/ml, 78.13µg/ml, 39.06µg/ml and 19.5µg/ml) of the nanoparticles. Aliquots of 100 µL of the 10⁶ cfu/mL standardized bacterial suspension (OD_{630 nm} = 0.1) was added to the wells and the plates were incubated at 37°C for 24 h. Following the overnight incubation, the optical density (OD₆₃₀) of the plates were measured for absorbance using the HEALES full automatic microplate reader MB-580 (Shenzhen Huisong Technology), to determine the minimum inhibitory concentration that prevented growth of bacterial cells.

6.3.8 Killing time kinetics

In order to determine the killing time, fabricated zinc oxide nanoparticles were reconstituted in dimethyl sulfoxide to obtain different concentrations (5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL). The different concentrations of the nanoparticles were transferred into the wells of a microtiter containing 100 μ L of Tryptic soy broth. An aliquot of 100 μ L of the bacteria culture was added to the wells and incubated at 37°C. Optical density was determined after every hour and recorded. The killing time kinetics that indicates the reaction of the cells when exposed to the nanoparticles was evaluated by a plot of log of the optical density versus time.

6.3.9 Statistical analysis

Data obtained from anti-microbial activity experiments were analysed by one-way analysis of variance (ANOVA) using the statistical package for the social sciences (SPSS) software version 20.0 IBM cooperation, North Castle, NY, USA. Significant mean difference was compared using New Duncan's Multiple Range Test (DMRT) and a significant difference defined as $P \leq 0.05$.

6.4 Results

6.4.1 Antibigram test

Antibacterial resistance remains a major bottleneck to the treatment of infectious disease both in healthcare systems, animal husbandry and in the food production sector. The antibiogram test against the pathogenic strains (Table 6.1) used in the study was evaluated by the disc diffusion method, following the guidelines of the clinical laboratory standard institute (CLSI). The results were represented by measuring the diameter of zones of inhibitions in mm. Table 6.2 gives an overview of

the report of the antibiogram test, and Figure 6.1 is a pictorial representation of the conducted antibiogram test. From the results represented in Table 6.2, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. pneumonia* ATCC 27336, *B. cerus* ATCC 10876, *M. catarrhalis* ATCC 25240, *A. hydrophilia* ATCC 7966, *S. diarizonae* ATCC 12325, *E. fecalis* ATCC 29212, *E. faecium* ATCC 6569, and *K. pneumonia* ATCC 13883 exhibited susceptibility to Tetracycline, while *L. monocytogenes* ATCC 19115 showed resistivity to Tetracycline antibiotics.

S. aureus ATCC 25923, *E. coli* ATCC 25922, *A. hydrophilia* ATCC 7966, *E. fecalis* ATCC 29212, *K. pneumonia* ATCC 13883, and *M. catarrhalis* ATCC 25240 were resistant to Ampicilin, but *B. cerus* ATCC 10876, *E. faecium* ATCC 6569, *S. diarizonae* ATCC 12325, *S. pneumoniae* ATCC 27336, and *L. monocytogenes* ATCC 19115 were susceptible to Ampicillin. All thirteen pathogenic strains used in the study showed susceptibility to Amikacin, Augumentin and Gentamicin. With the exception of *E. faecium*, all other pathogenic strains in the study were susceptible to Ciprofloxacin and Norfloxacin. *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *A. hydrophilia* ATCC 7966, *S. diarizonae* ATCC 12325, *B. cerus* ATCC 10876, *E. faecium* ATCC 6569, *K. pneumonia* ATCC 13883, *M. catarrhalis* ATCC 25240, *S. pneumoniae* ATCC 27336, and *L. monocytogenes* ATCC 19115, were resistant to Ceftoxamine however *S. typhi*, *E. fecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 exhibited susceptibility to the *Ceftoxamine* antibiotic.

Generally, Cefalexin (CFX) and Ampicillin were the the most resistant antibiotics while Amikacin, Augumentin and Gentamicin were the most effective antibiotics. (See appendix for zones of inhibition measurement in mm).

Table 6.2: Antibigram Resistant Profiles of the isolates

S/N	Bacterial Isolates	TET	AMP	AK	GEN	AUG	CIP	NOR	CFX
1	<i>Staphylococcus aureus</i>	S	R	S	S	S	S	S	R
2	<i>Salmonella typhi</i>	*	*	S	S	S	S	S	S
3	<i>Escherichia coli</i>	S	R	S	S	S	S	S	R
4	<i>Aeromonas hydrophilia</i>	S	R	S	S	S	S	S	R
5	<i>Enterococcus faecalis</i>	S	R	S	S	S	S	S	S
6	<i>Pseudomonas aeruginosa</i>	*	*	S	S	S	S	S	S
7	<i>Bacillus cereus</i>	S	S	S	S	S	S	S	R
8	<i>Enterococcus faecium</i>	S	S	S	S	S	R	R	R
9	<i>Salmonella diarizonae</i>	S	S	S	S	S	S	S	R
10	<i>Klebsiella pneumoniae</i>	S	R	S	S	S	S	S	R
11	<i>Moraxella catarrhalis</i>	S	R	S	S	S	S	S	R
12	<i>Streptococcus pneumoniae</i>	S	S	S	S	S	S	S	R
13	<i>Listeria monocytogenes</i>	R	S	S	S	S	S	S	R
Key: S indicates susceptibility, R indicates resistance. * indicates not evaluated TET (Tetracycline), AMP (Ampicilline), AK (Amikacin), GEN (Gentamicin), AUG (Augmentine), CIP (ciprofloxacin), NOR (Norfloxacin). CFX (Ceftoxamine)									

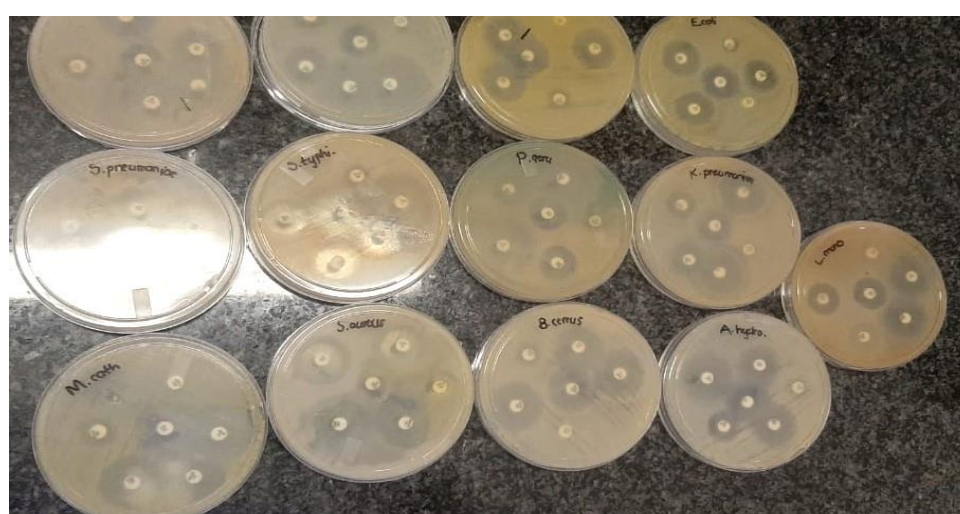


Figure 6.1: Antibigram tests showing zones of inhibitions against pathogenic strains.

6.4.2. Antibacterial activity of synthesized metal oxides nanoparticles

Table 6.3a: Mean zones of inhibition value (mm) produced by zinc oxide nanoparticle mediated via pomegranate (*Punica granatum*) leaf extract.

S/N	Bacterial Isolates	50µg/ml (mm)	100µg/ml (mm)	1000µg/ml (mm)	5000µg/ml (mm)
1	<i>Staphylococcus aureus</i>	7.00±1.00 ^a	10.50±0.87 ^a	11.33±0.58 ^a	13.00 ^a
2	<i>Salmonella typhi</i>	6.33±0.58 ^a	10.00 ^a	11.17±0.29 ^a	14.33±0.58 ^a
3	<i>Escherichia coli</i>	13.33±1.15 ^d	13.83±0.76 ^c	15.00±1.00 ^b	15.67±1.53 ^a
4	<i>Aeromonas hydrophilia</i>	11.00±2.65 ^b	12.33±0.58 ^a	13.33±0.58 ^a	14.67±1.15 ^a
5	<i>Bacillus cereus</i>	10.33±2.52 ^b	13.67±0.58 ^c	15.33±0.58 ^b	14.33±1.5 ^a
6	<i>Listeria monocytogenes</i>	10.00±1.00 ^b	12.00±1.00 ^a	12.33±1.15 ^a	13.67±1.53 ^a
7	<i>Enterococcus faecalis</i>	6.33±0.29 ^a	14.33±0.58 ^d	17.33±1.15 ^d	18.00 ^b
8	<i>Pseudomonas aeruginosa</i>	9.67±1.53 ^b	11.33±0.58 ^a	12.67±1.53 ^a	15.33±1.53 ^a
9	<i>Enterococcus faecium</i>	12.67±0.58 ^c	15.17±0.29 ^e	16.67±1.53 ^c	19.33±1.15 ^c
10	<i>Salmonella diarizonae</i>	10.83±1.26 ^b	12.00±1.73 ^a	15.33±1.15 ^b	19.00±1.00 ^c
11	<i>Klebsiella pneumoniae</i>	9.67±1.53 ^b	11.67±1.53 ^a	12.00±1.00 ^a	14.00±2.65 ^a
12	<i>Moraxella catarrhalis</i>	8.67±1.15 ^b	9.67±1.53 ^a	13.33±2.08 ^a	14.00±2.65 ^a
13	<i>Streptococcus pneumoniae</i>	10.67±2.31 ^b	12.67±0.58 ^b	14.67±1.53 ^a	16.67±0.58 ^a
In each concentration column, all values with the same alphabetic subscript are not significantly different.					

Table 6.3b: Mean zones of inhibition value (mm) produced by zinc oxide nanoparticle mediated via pomegranate (*Punica granatum*) flower extract.

S/N	Bacterial Isolates	50µg/ml (mm)	100µg/ml (mm)	1000µg/ml (mm)	5000µg/ml (mm)
1	<i>Staphylococcus aureus</i>	7.00±1.00 ^a	12.33±0.58 ^c	12.50±0.50 ^b	15.00±1.00 ^a
2	<i>Salmonella typhi</i>	5.67±0.58 ^a	8.67±1.15 ^a	12.00±1.00 ^a	14.53±0.23 ^a
3	<i>Escherichia coli</i>	8.67±0.58 ^c	10.00±1.00 ^a	14.00±1.00 ^c	14.50±0.50 ^a
4	<i>Aeromonas hydrophilia</i>	10.33±2.05 ^e	13.83±0.29 ^d	17.33±1.15 ^g	17.50±0.50 ^e
5	<i>Bacillus cereus</i>	10.67±2.08 ^e	12.33±0.58 ^c	14.50±1.32 ^d	16.33±0.58 ^c
6	<i>Listeria monocytogenes</i>	6.33±0.58 ^a	14.33±0.58 ^d	14.67±0.58 ^e	16.50±0.50 ^d
7	<i>Enterococcus faecalis</i>	7.33±1.15 ^a	9.67±0.58 ^a	12.00±1.00 ^a	16.17±0.15 ^c
8	<i>Pseudomonas aeruginosa</i>	12.67±0.58 ^g	10.00±1.00 ^a	13.00 ^b	14.00 ^a
9	<i>Enterococcus faecium</i>	9.67±0.58 ^d	15.83±0.76 ^e	18.00 ^h	21.50±0.50 ^g
10	<i>Salmonella diarizonae</i>	10.67±0.58 ^e	10.00 ^a	16.00±1.00 ^f	18.50±0.50 ^e
11	<i>Klebsiella pneumoniae</i>	8.00±1.00 ^b	11.00±1.00 ^b	10.67±1.15 ^a	14.00 ^a
12	<i>Moraxella catarrhalis</i>	12.00±0.50 ^f	12.00±1.00 ^c	11.67±1.15 ^a	15.33±1.44 ^b
13	<i>Streptococcus pneumoniae</i>	10.67±2.30 ^e	14.00±1.00 ^d	16.00 ^f	19.00±0.50 ^f

Table 6.4a: Mean zones of inhibition value (mm) produced by iron oxide nanoparticle mediated via pomegranate (*Punica granatum*) leaf extract.

S/N	Bacterial Isolates	50µg/ml (mm)	100µg/ml (mm)	1000µg/ml (mm)	5000µg/ml (mm)
1	<i>Staphylococcus aureus</i>	6.33±0.58 ^b	13.00±0.10 ^f	14.20±0.20 ^c	15.50±0.50 ^b
2	<i>Salmonella typhi</i>	5.93±0.11 ^a	10.87±0.23 ^d	14.90±0.17 ^d	15.93±0.58 ^c
3	<i>Escherichia coli</i>	6.67±0.58 ^b	8.17±0.29 ^b	14.33±0.58 ^c	19.83±0.29 ^e
4	<i>Aeromonas hydrophilia</i>	6.33±0.58 ^b	10.60±0.53 ^c	15.10±0.36 ^d	23.00 ^g
5	<i>Bacillus cereus</i>	10.50±0.50 ^d	13.33±0.42 ^f	15.23±0.25 ^d	19.70±0.30 ^e
6	<i>Listeria monocytogenes</i>	6.47±0.42 ^b	10.33±0.58 ^c	13.70±0.61 ^c	15.33±0.61 ^b
7	<i>Enterococcus faecalis</i>	11.50±0.50 ^e	15.27±0.25 ^g	17.87±0.23 ^f	19.67±0.58 ^e
8	<i>Pseudomonas aeruginosa</i>	8.67±0.58 ^c	12.83±0.29 ^f	15.57±0.40 ^e	20.00 ^e
9	<i>Enterococcus faecium</i>	8.17±0.29 ^c	12.07±0.12 ^e	15.27±1.10 ^d	15.17±1.04 ^b
10	<i>Salmonella diarizonae</i>	12.77±0.40 ^f	15.83±0.15 ^h	17.90±0.17 ^f	21.17±0.15 ^f
11	<i>Klebsiella pneumoniae</i>	11.00 ^d	13.13±0.12 ^f	14.50±1.50 ^c	18.13±0.15 ^d
12	<i>Moraxella catarrhalis</i>	6.17±0.29 ^b	8.00 ^b	12.50±0.50 ^b	14.93±0.12 ^b
13	<i>Streptococcus pneumoniae</i>	5.33±0.58 ^a	5.83±0.15 ^h	9.93±0.12 ^a	11.77±0.25 ^a

Table 6.4b: Mean zones of inhibition value (mm) produced by iron oxide nanoparticle mediated via pomegranate (*Punica granatum*) flower extract.

S/N	Bacterial Isolates	50µg/ml (mm)	100µg/ml (mm)	1000µg/ml (mm)	5000µg/ml (mm)
1	<i>Staphylococcus aureus</i>	6.33±0.58 ^c	9.07±0.12 ^d	10.33±0.29 ^b	15.00±0.20 ^c
2	<i>Salmonella typhi</i>	5.67±0.58 ^c	11.07±0.12 ^e	12.93±0.12 ^d	15.00 ^c
3	<i>Escherichia coli</i>	5.67±0.58 ^c	10.43±0.51 ^e	12.00 ^c	12.57±0.59 ^b
4	<i>Aeromonas hydrophilia</i>	8.10±0.17 ^f	10.93±0.12 ^e	16.50±0.50 ^g	17.93±0.45 ^d
5	<i>Bacillus cereus</i>	9.77±0.40 ^g	12.67±0.58 ^g	14.00±1.00 ^e	16.00±1.04 ^c
6	<i>Listeria monocytogenes</i>	6.00 ^c	7.67±0.58 ^c	13.00 ^d	15.47±0.55 ^c
7	<i>Enterococcus faecalis</i>	7.00±1.00 ^e	13.00 ^g	14.67±0.58 ^f	15.37±1.55 ^c
8	<i>Pseudomonas aeruginosa</i>	.0000 ^a	.0000 ^a	.0000 ^a	.0000 ^a
9	<i>Enterococcus faecium</i>	8.60±0.36 ^f	11.67±0.58 ^f	13.33±0.58 ^d	15.10±0.53 ^c
10	<i>Salmonella diarizonae</i>	8.33±0.58 ^f	13.33±0.58 ^g	17.17±0.29 ^g	20.27±0.25 ^e
11	<i>Klebsiella pneumoniae</i>	5.33±0.58 ^c	7.33±0.58 ^c	10.00 ^b	12.00 ^b
12	<i>Moraxella catarrhalis</i>	4.33±0.58 ^b	6.33±0.58 ^b	10.50±0.50 ^b	12.43±0.51 ^b
13	<i>Streptococcus pneumoniae</i>	.0000 ^a	.0000 ^a	.0000 ^a	.0000 ^a

The antibacterial efficacy of the synthesized zinc oxide nanoparticles and iron oxide nanoparticles was evaluated against 13 pathogenic strains using the agar well diffusion method. Different concentrations (50µg/ml, 500µg/ml, 1000µg/ml and 5000µg/ml) of the nanoparticles was prepared and used. Synthesized zinc oxide nanoparticle mediated via pomegranate leaf extract in this study is represented as ZnO-NPs-PL while zinc oxide nanoparticle mediated via pomegranate flower extract is represented as ZnO-NPs-PF. Iron oxide nanoparticles from pomegranate leaf extract is captioned Fe₃O₄-NPs -PL and that from flower extract is Fe₃O₄-NPs-PF. Antimicrobial activity exhibited by all synthesized nanoparticles, which prevents the

growth of bacteria, can be seen in the form of the clear zone of inhibition, as seen in Figure 6.2. From Table 6.3 (a), using the 5000µg/ml concentration as a standard, the synthesized zinc oxide nanoparticle exhibited susceptibility against all bacterial strains used in the study. *E. faecium* ATCC 6569 and *S. diarizonae* ATCC 12325 showed more susceptibility with high zones of inhibition (mean value) of 19.33±1.15mm and 19.00± mm respectively for ZnO-NPs-PL. In respect to 5000µg/ml ZnO-NPs-PF, *E. faecium*, *S. pneumonia* and *S. diarizonae*, exhibited high susceptibility with zones of inhibition at 21.50±0.50mm, 19.00±0.50mm and 17.50±0.50mm respectively. The ability of zinc oxide nanoparticles to exert antimicrobial activity has been documented. In a study by Umar *et al.* (2018), the antimicrobial activity of zinc oxide nanoparticles synthesized via *A. lebbeck* stem extract was evaluated against *B. cereus*, *S. aureus*, *E. coli*, *K. pneumoniae* and *S. typhi*. Khatami *et al.* (2018) documented the antiparasitic and antimicrobial effect of stevia mediated, rectangular shaped zinc oxide nanoparticles against *L. major*, *S. aureus* and *E. coli* and showed that an increase in concentration of zinc oxide nanoparticles elicited maximum cytotoxic effect. Ibrahim *et al.* (2017) also recounted the concentration dependent manner of zinc oxide nanoparticles. Nazoori & Kariminik (2018) investigated the antimicrobial efficiency of zinc oxide nanoparticles against ten (10) human pathogenic organisms, and reported that the minimum concentration of zinc oxide that can inhibit or prevent the growth of most of *S. aureus*, *S. marcescens* and *E. coli* was 2.5mg/ml of synthesized ZnO-NPs, while the minimum concentration required to kill off the bacterial strain was 10mg/ml. In another study by Akbar *et al.* (2018), ZnO nanoparticles were synthesized and their antimicrobial potential against food borne pathogens *S. typhimurium* and *S. aureus* were evaluated. The mode of antimicrobial action by nanoparticles is still not clear, but studies have suggested that zinc oxide nanoparticles exert antibacterial effect on

microorganisms by ROS production (Tiwari *et al.*, 2018), membrane leakage of intracellular content, DNA and protein damage (Jiang *et al.*, 2018), and toxic ion release (Niño-Martínez *et al.*, 2019). Zinc oxide is generally regarded as safe by the Food and Drug Administration (FDA). Zinc oxide nanoparticles have different applications such as in food science where they serve as preservatives, in pharmaceuticals as drug delivery agents and in industrial applications like textiles.

Table 6.3 (b), shows the mean zone of inhibition (in mm) produced by iron oxide nanoparticles. Susceptibility against all 13 pathogenic strains was produced by Fe₃O₄-NPs-PL. In the case of Fe₃O₄-NPs-PF, all strains showed susceptibility, with the exception of *P. aeruginosa* ATCC 27853 and *S. pneumonia* ATCC 27336, which showed resistivity against the Fe₃O₄-NPs -PF. *A. hydrophilia* ATCC 7966 showed high susceptibility for Fe₃O₄-NPs-PL with a mean zone of inhibition value of 23 mm for 5000 µg/ml Fe₃O₄-NPs-PL while *S. pneumonia* ATCC 27336 showed least susceptibility with an 11.17 ± 0.25 mm zone of inhibition. High zones of inhibition for Fe₃O₄-NPs -PF at 20.27 ± 0.25 mm were shown by *S. diarizonae* ATCC 12325 and 17.93 ± 0.45 mm for *A. hydrophilia* ATCC 7966. In a report by Behera *et al.* (2012), chemically synthesized Fe₃O₄-NPs did not have any activity against *P. aeruginosa*. In another study by Caamano & Carrillo Morales (2016), no activity was seen when chemically prepared iron oxide nanoparticle was tested against *S. pneumonia*. However, in the present study, significant activity was shown by biogenic Fe₃O₄-NPs-PL against *P. aeruginosa* and *S. pneumonia*, but no activity was recorded on testing the strains with biogenic iron oxide nanoparticles synthesized via pomegranate flower extract (Fe₃O₄-NPs -PF). Increase in zones of inhibition with increase in nanoparticle concentration has also been reported for iron oxide nanoparticles. Based on zones of

inhibition diameter obtained, Da'na *et al.* (2018) demonstrated that Fe₃O₄-NPs synthesized via *Acacia nilotica* seedless pod extract had effective antibacterial activity against *Candida*, *S. aureus*, *Salmonella*, *Marsa*, and *E. coli*. They also explained that a low concentration of Fe₃O₄-NPs causes a weak antibacterial activity while a higher concentration causes a greater antibacterial activity. Naseem & Farrukh (2015) investigated the antibacterial effects of Fe₃O₄-NPs synthesized via the extracts of *Lawsonia inermis* and *Gardenia jasminoides* against *E. coli*, *S. enterica*, and *P. mirabilis* and reported on the efficiency of the nanoparticles.

The ability of the prepared zinc oxide nanoparticle and iron oxide nanoparticle to show activity against the tested pathogenic stains could be attributed to oxidative stress caused by ROS generation which leads to protein and bacterial DNA damage (Tiwari *et al.*, 2018). In the study, the prepared metal oxide nanoparticles could have produced ROS that elicited inhibition against the bacterial strains. In a study by Padmavathy & Vijayaraghavan, (2008), high generation of ROS was cited as the main cause of bacterial cell death on treatment with synthesized zinc oxide nanoparticle. Also, in another study by Raghupathi *et al.* (2011), generation of ROS like hydrogen peroxide, hydroxyl radicals and superoxide anions in the presence of UV light which caused cell toxicity was reported as the mechanism of antibacterial inhibitions. The antibacterial effect of zinc oxide nanoparticle on multi drug resistant ESKAPE pathogen *Acinetobacter baumannii* was reported by Tiwari *et al.* (2018), who recount that ROS generation, which caused oxidative degradation of lipid, and leakage of membrane reducing sugar, proteins and DNA and eventual loss of cell viability, was the mode of action for the zinc oxide nanoparticle. From the tables 6.3(a), 6.3(b), 6.4(a) and 6.4(b) above, it is evident that enhanced antibacterial activity of metal oxide nanoparticles increases with concentration. With an increase in nanoparticle concentration, bacterial

growth generally reduces. A similar report was given by Liu *et al.* (2009), who demonstrated that an increase in concentration of the prepared nanoparticles increased antimicrobial activity of zinc oxide nanoparticle against *E. coli*. Pati *et al.* (2014) also reported on dose dependent activity of nanoparticles against bacterial strains. On assessing the antibacterial effect of Fe₃O₄-NPs on *E. coli* and *B. subtilis*, Arakha *et al.* (2015) hinted that formation of high amounts of ROS by Fe₃O₄-NPs is the main mechanism by which Fe₃O₄-NPs exerts its bactericidal action on microorganisms.

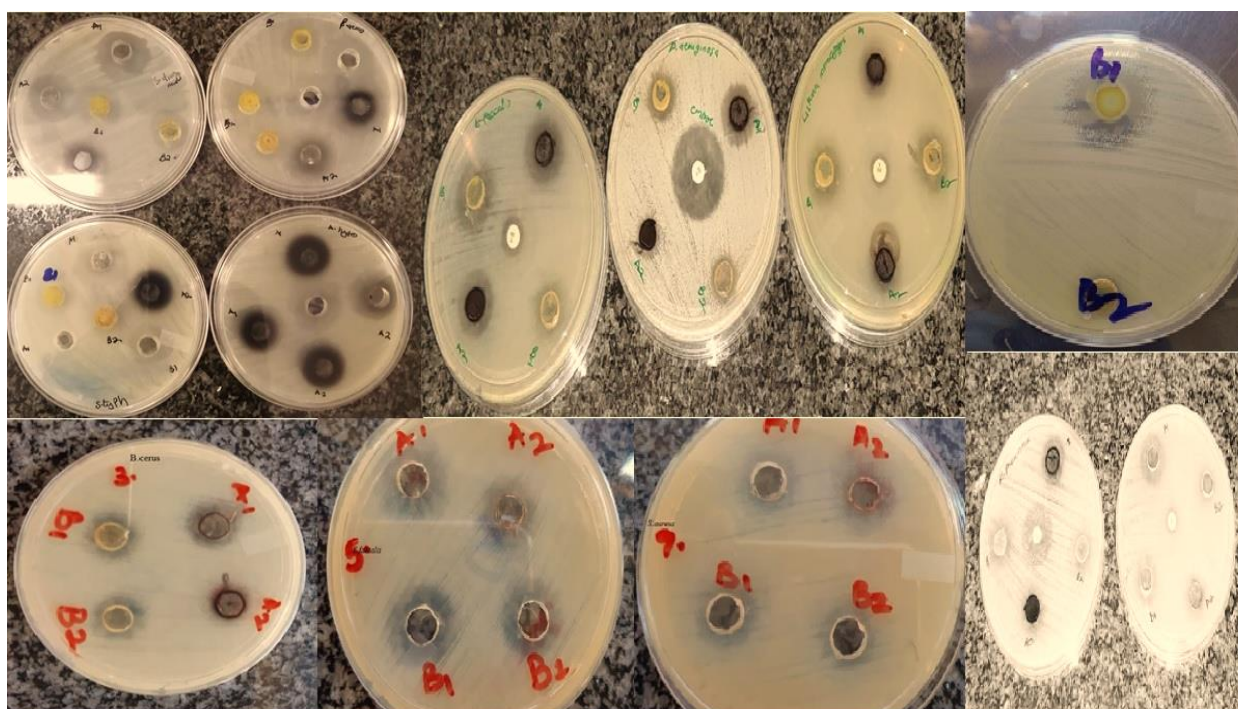


Figure 6.2: Plates showing the zones of inhibitions obtained on testing biosynthesized nanoparticles against bacterial strains.

A1= Fe₃O₄-NPs-PL, A2= Fe₃O₄-NPs-PF,
B1-ZnO-NPs-PL, B2-ZnO-NPs-PF,

6.4.3 Effect of different concentration of nanoparticles on its antibacterial effectiveness.

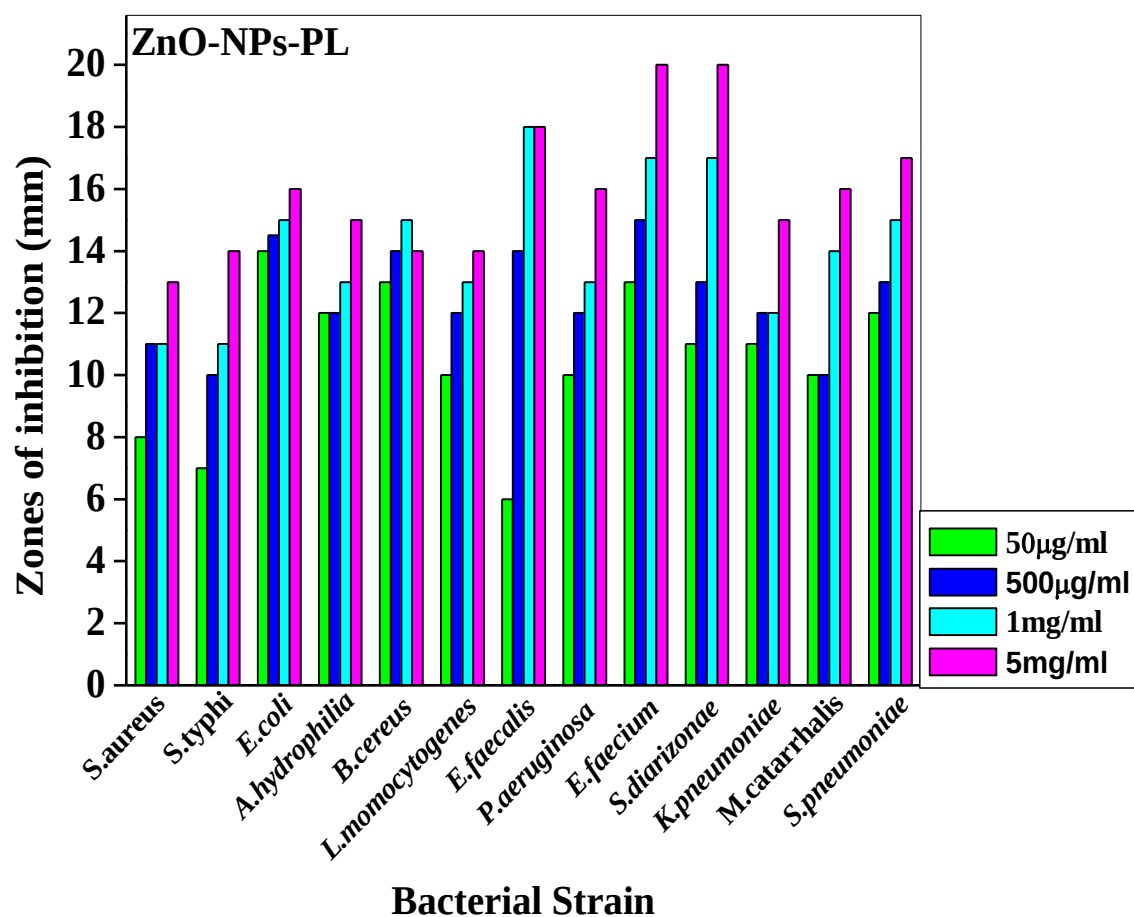


Figure 6.3 (a) Bar graphs showing the effect of different concentration (50 µg/ml, 500 µg/ml, 1 mg/ml, and 5 mg/ml) on antimicrobial activity of ZnO-NPs-PL against various microorganism.

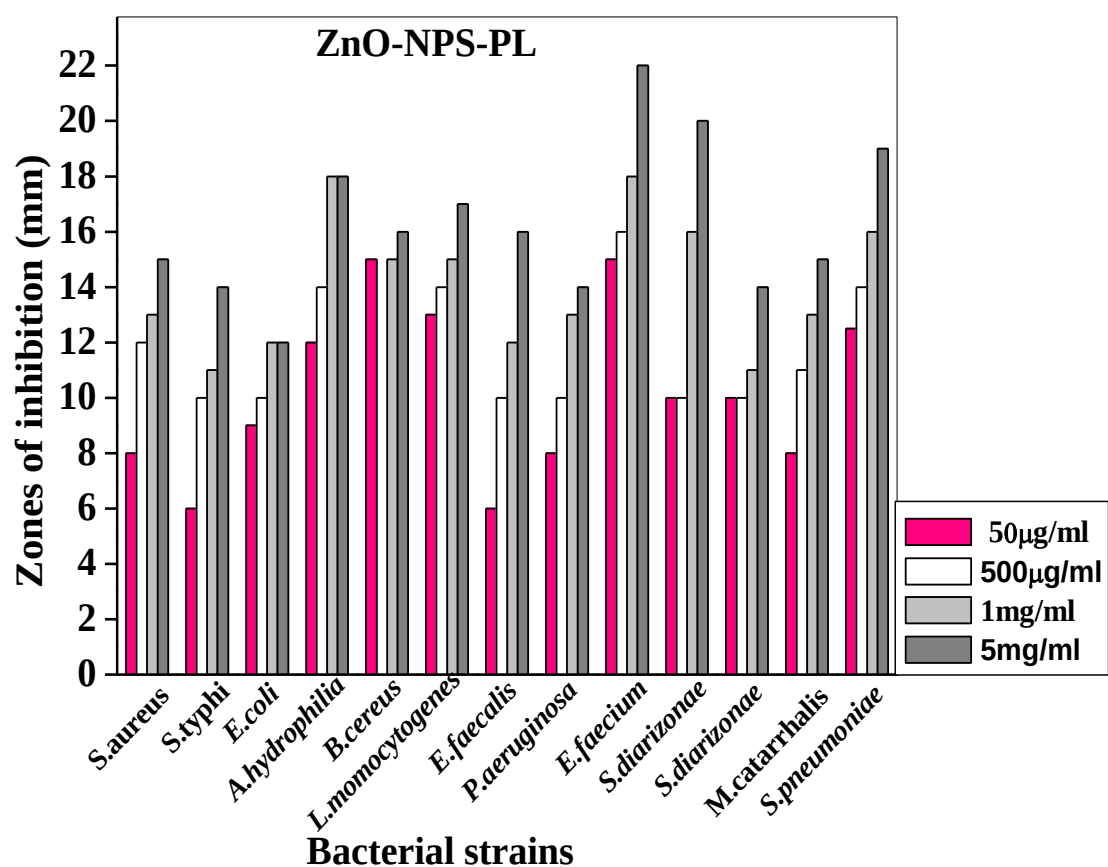


Figure 6.3 (b) Bar graphs showing the effect of different concentration (50 µg/ml, 500 µg/ml, 1 mg/ml, and 5 mg/ml) on antimicrobial activity of ZnO-NPs-PF against various microorganism

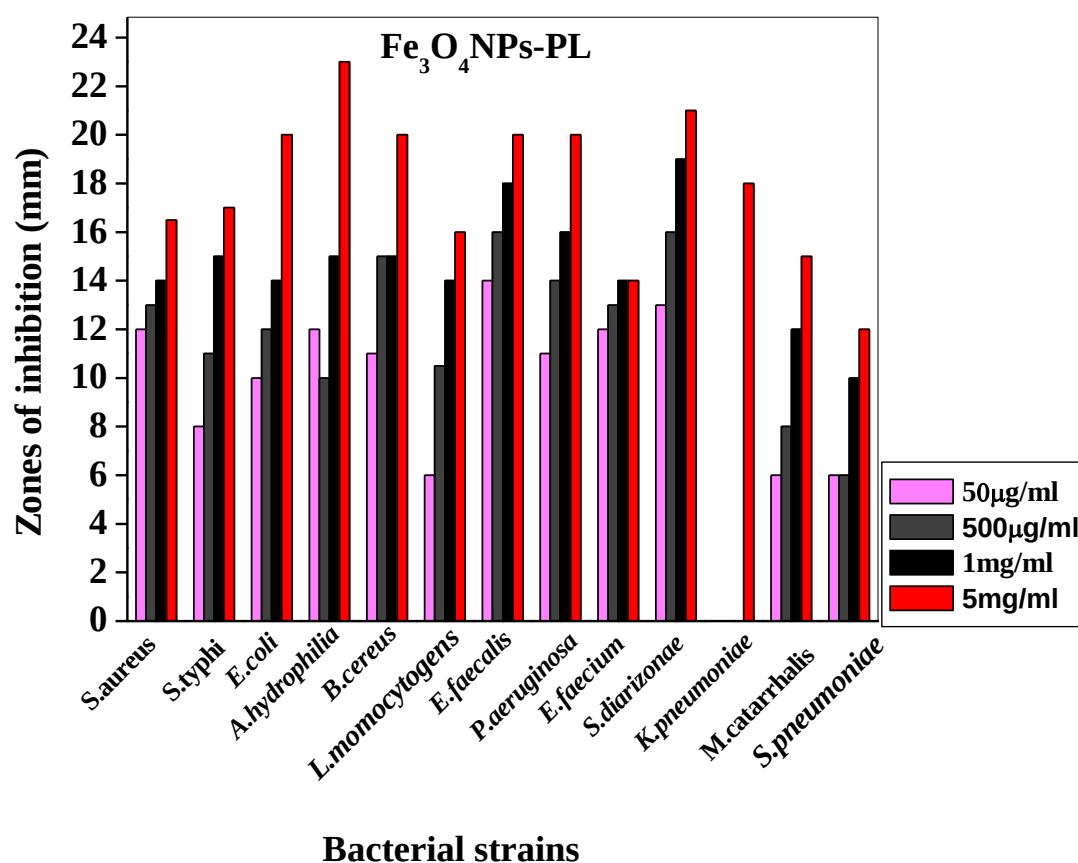


Figure 6.3 (c) Bar graphs showing the effect of different concentration (50 µg/ml, 500 µg/ml, 1 mg/ml, and 5 mg/ml) on antimicrobial activity of Fe₃O₄-NPs -PL against various microorganism

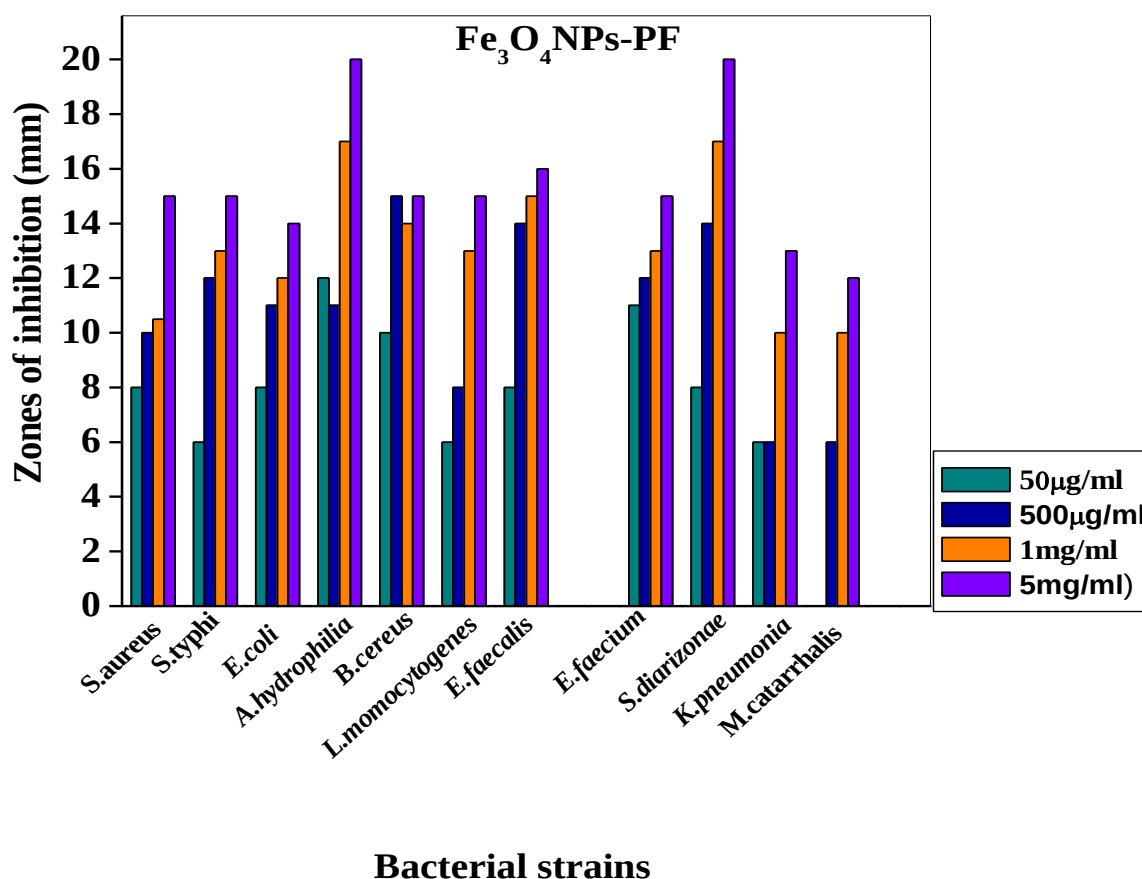


Figure 6.3 (d) Bar graphs showing the effect of different concentration (50 µg/ml, 500 µg/ml, 1 mg/ml, and 5 mg/ml) on antimicrobial activity of Fe₃O₄-NPs -PF against various microorganism

6.5.4 Determination of minimum inhibitory concentrations (MIC) of the synthesized metal oxides nanoparticles.

The antibacterial effect of the prepared iron oxide and zinc oxide nanoparticles mediated via pomegranate leaf and flower extracts were also evaluated using the minimum inhibitory concentration technique. This was done to ascertain the lowest concentration of the prepared metal oxide nanoparticle that can inhibit or stop the bacterial growth. From the tests carried out on the 96 well titre plate, the lowest concentration of ZnO-NPs-PF that could completely inhibit growth of *S. aureus*, *E.*

faecalis, *E. coli*, *E. faecium*, *L. monocytogens* and *K. pneumonia*, *S. pneumonia* and *S. typhi* were at 1250 µg/ml while for *B. cereus* and *M. catarrhalis*, minimum inhibitory concentration was achieved at 0.61 ± 0.54 µg/ml. MIC values for *P. aeruginosa*, *A. hydrophilia* were 0.83 ± 0.36 µg/ml, and 0.82 ± 0.36 µg/ml respectively. For ZnO-NPS–PL, minimum inhibitory concentrations against *B. cerus*, *P. aeruginosa*, *E. feacalis*, *A. hydrophilia*, *S. pneumonia*, *E. faecium*, *S. aureus*, *E. coli*, *L. monocytogenes*, *M. catarrhalis*, *S. typhi* and *K. pneumonia* were 0.93 ± 0.54 µg/ml, 0.88 ± 0.6 µg/ml, 1.25 mg/ml, 0.85 ± 0.6 µg/ml, 0.93 ± 0.54 µg/ml, 0.816 ± 0.375 µg/ml, 1250 µg/ml, 1250 µg/ml, 0.71 ± 0.48 µg/ml, 1250 µg/ml, 0.6 µg/ml and 1250 µg/ml respectively. For Fe₃O₄-NPs-PL and Fe₃O₄-NPs-PF, MIC was obtained at 1250 µg/ml concentrations for all bacterial strains evaluated with the exception of *S. pneumonia* and *S. aureus*, for which inhibitory concentrations were obtained at 2500 µg/ml.

6.4.5 Killing time

The survivability of the different pathogenic strains was evaluated in different concentrations of synthesized metal oxide nanoparticles. Growth analysis was measured and recorded by monitoring the optical density at 630 nm over time. From the graph obtained in Figure 6.4(a) at 5000 µg/ml of zinc oxide nanoparticle, it can be seen that growth of the pathogenic strains *Staphylococcus aureus* ATCC 25923, *Eschericia coli* ATCC 25922, *Streptococcus pneumoniae* ATCC 27336, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 10876, *Moraxella catarrhalis* ATCC 25240, *Aeromonas hydophilia* ATCC 7966, *Salmonella diarizonae* ATCC 12325, *Enterococcus fecalis* ATCC 29212, *Enterococcus faceium* ATCC 6569, *Salmonella typhi*, and *Listeria monocytogenes* ATCC 19115 were totally inhibited, although *B.cereus* and the *P.aeruginosa* strains exhibited growth, or were in the lag phase,

where the bacterial were adjusting to the media they were placed in, till the second hour, where a very sharp decline in growth profile was observed. At 2.5mg (Figure 6.4b), effective inhibition was also observed. In the case of the *E. coli* strain, cellular activity occurred from 0 hour to the first (1) hour, after which the exponential phase occurred from hour two until hour four, with zinc oxide nanoparticles disrupting the metabolic activity, and by the fifth hour there was a stationary phase, which subsequently led to the death phase at the sixth hour. Figures 6.4(c), 6.4(d) and 6.4(e) show the effect of time on survivability in different concentrations of zinc oxide nanoparticles of *M. catarrhalis*, representing gram-negative organisms, and *S. aureus* for gram-positive organisms.

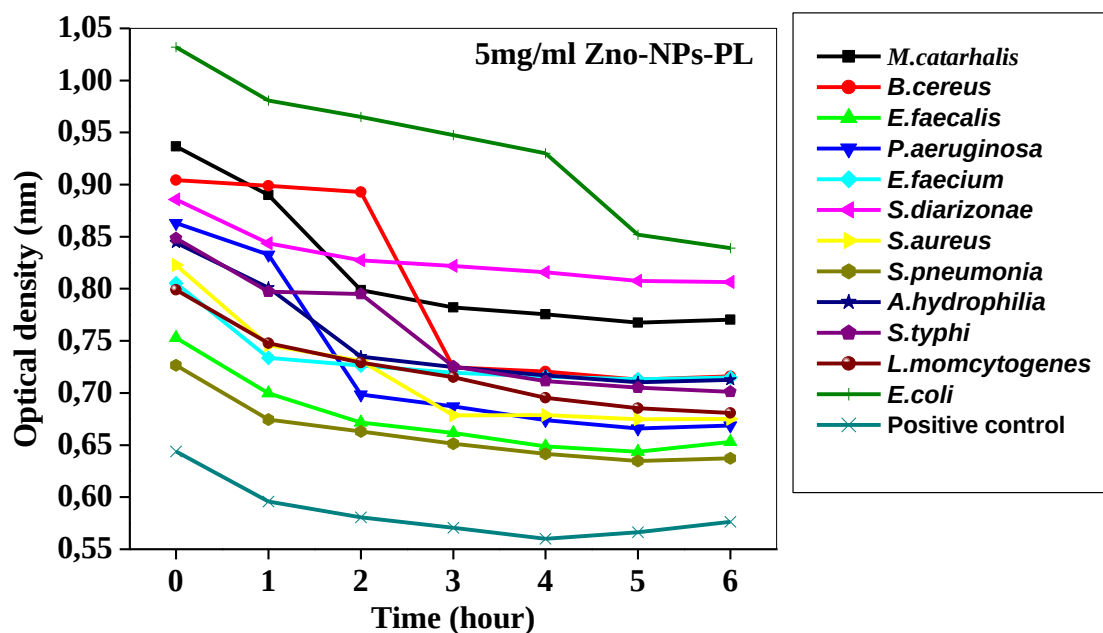


Figure 6.4a: Effect of time on persistence (survivability) of individual pathogenic strain in 5 mg/ml zinc oxide nanoparticle.

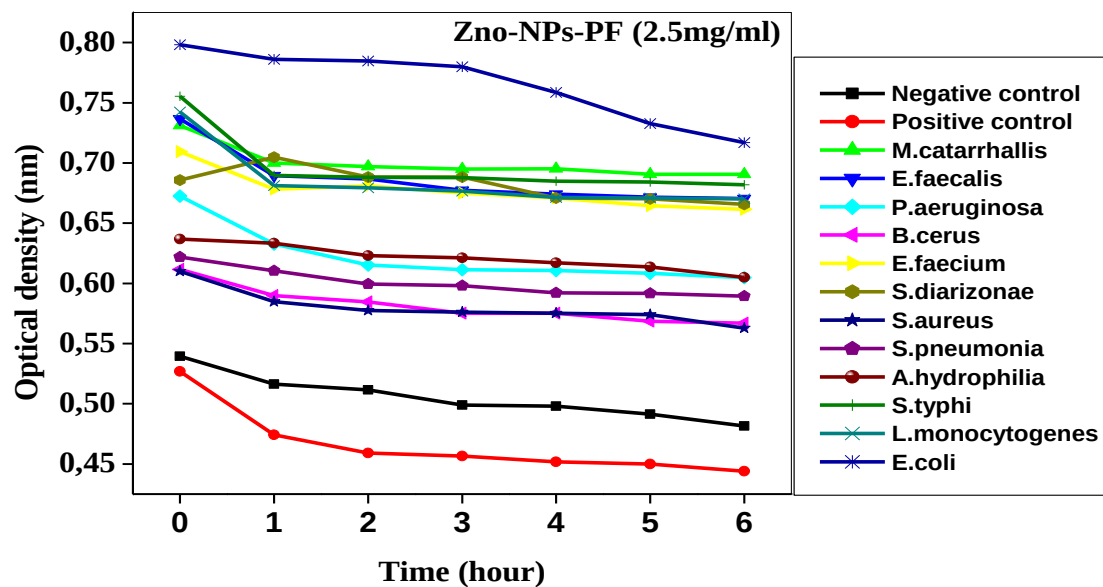


Figure 6.4b: Effect of time on persistence (survivability) of individual pathogenic strain in 2.5 mg/ml zinc oxide nanoparticle.

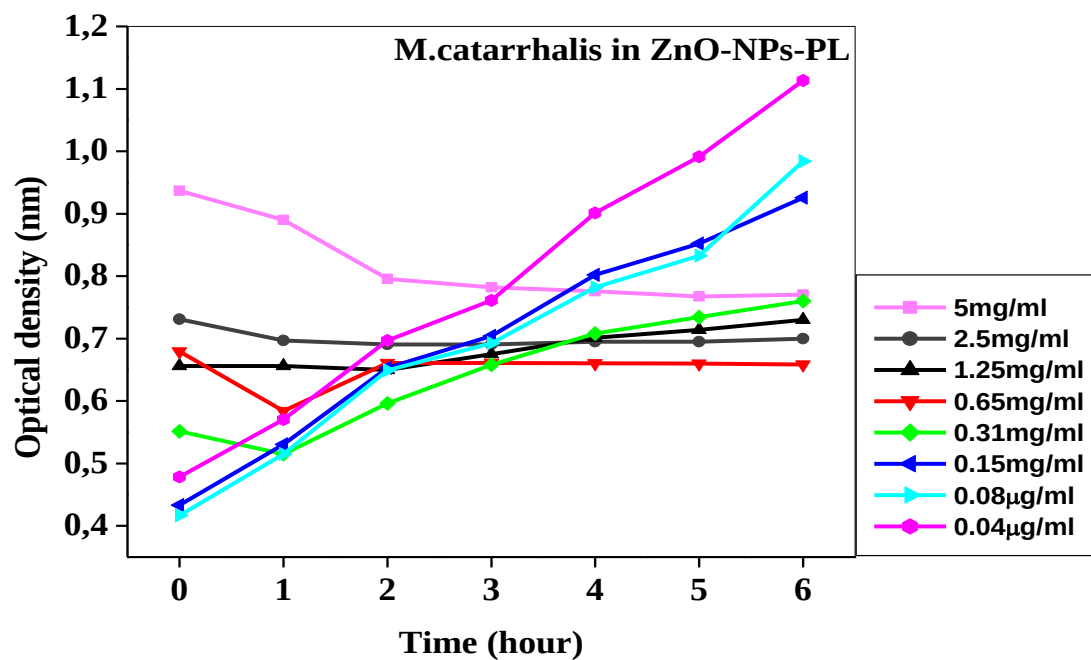


Figure 6.4c: Effect of time on persistence (survivability) of *M. catarrhalis* in zinc oxide nanoparticle.

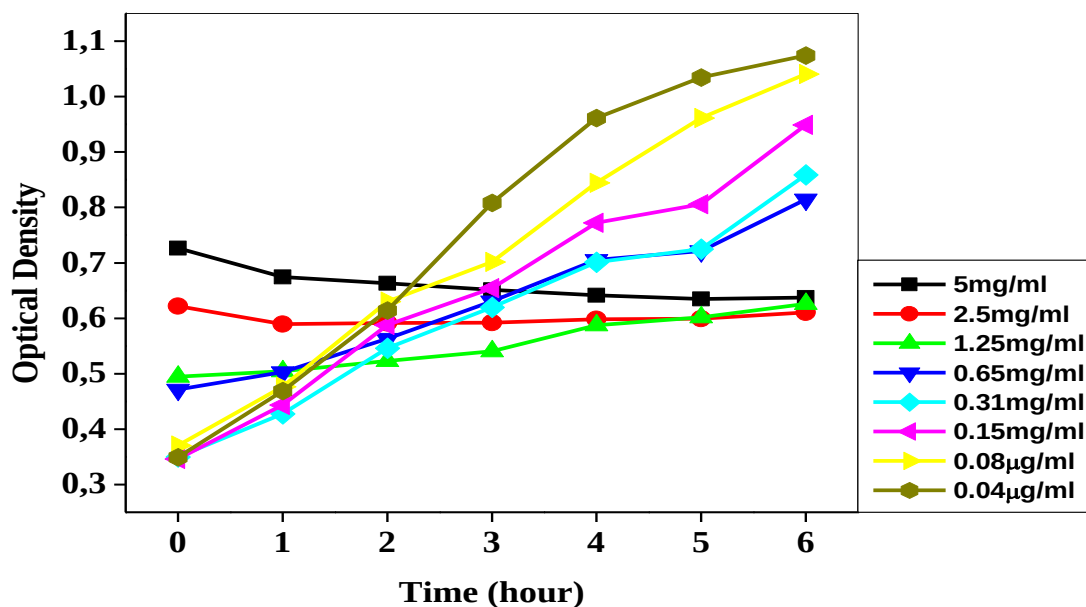


Figure 6.4d: Effect of time on persistence (survivability) of *S. aureus* in zinc oxide nanoparticle.

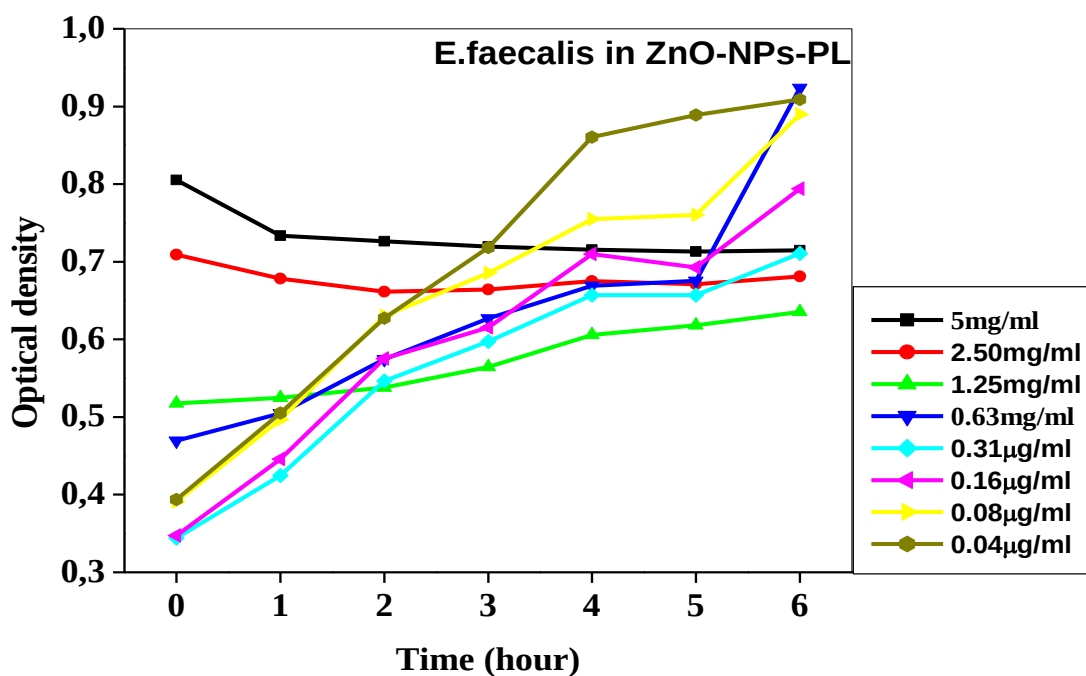


Figure 6.4e: Effect of time on persistence (survivability) of *E. faecalis* in zinc oxide nanoparticle.

6.5 Conclusion

The effects of different concentrations of zinc oxide and iron oxide nanoparticles against pathogenic bacterial strains were evaluated. High concentrations of nanoparticles at 5 mg/ml effectively inhibited growth of bacteria. From zones of inhibitions obtained, iron oxide nanoparticles synthesized from leaf (Fe_3O_4 -NPs -PL) showed a more promising antibacterial activity, but zinc oxide mediated from leaf and flower showed consistency, inhibiting all bacterial strains used in the analysis. The mechanism of action of inhibiting bacteria growth is suggested to be by production of ROS on attachment to the bacterial cell membrane, thereby causing damage of cell membrane and protein dysfunction. Liu *et al* (2009) report that on treatment of *E. coli* with zinc oxide nanoparticles, the particles fixed onto the cell membrane of *E. coli*, causing deformity of the membrane and disorganization of the intracellular structures. Kadiyala *et al.* (2018) investigated the antibacterial effect of zinc oxide nanoparticles on multi-drug resistant *S. aureus* and indicated that generation of ROS alone cannot be the main antibacterial mode of action. Arakha *et al.* (2015) studied the antimicrobial effect of iron oxide nanoparticles against *E. coli* and reported ROS generation, which consequently causes oxidative stress that damages bacterial cells, as an antibacterial mechanism of action for iron oxide nanoparticles. An increase in concentration of nanoparticle yielded a higher zone of inhibition, signifying a greater antibacterial effect. From the analysis carried out, the minimum concentration of zinc oxide and iron oxide nanoparticles that could effectively inhibit growth of microorganisms used in the study ranged from 0.6 $\mu\text{g/ml}$ to 2500 $\mu\text{g/ml}$, depending on the bacterial strain.

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CHAPTER 7

7.1 General conclusion and future research prospects

A major challenge of the public health sector is the issue of infectious diseases caused by bacterial pathogens, coupled with emergence of new unknown infections and re-emergence of old diseases. Several health system policies are in place to assist tackle the threat fostered by infectious disease. Traditionally, the use of antibiotics is one recognized means of treating bacterial infectious disease. One major problem associated with the conventional use of antibiotics as a means of treatment is the issue of antibacterial resistance. Bacteria causing infections are developing mechanisms to avert the effect of antibiotics. There is therefore a need to find other forms or mechanisms to fight the issue of antibacterial resistance, thereby addressing the global threat associated with resistance mechanisms produced by bacteria.

An emerging field offering a way out is the nanotechnological field. Several opportunities abound in the field of nanotechnology, allowing its applications in medicine, pharmaceuticals, industry, environment, food security and preservation, energy production and consumption, agriculture, electronics, optics and so forth. The booming field of nanotechnology has allowed production of small sized particles possessing enhanced characteristics and function. Advances being made in nanotechnology offer a solution to the menace of antibacterial resistance. Metal and metal oxides have been shown to possess antimicrobial ability. Nanoparticles of metal and metal oxide are said to have enhanced antibacterial properties. In curbing the problem of antibiotic resistance, the use of nanoparticles in place of conventional antibiotics offers a solution. Production of nanoparticles can be done by orthodox or alternative means, however the alternative green route of nanoparticle production is

being increasingly employed due to safety, biocompatibility and eco-friendliness of the methods involved. In green synthesis, no toxic waste is generated, and it involves the use of biological resources such as plants, enzymes, and microorganisms. The use of plant parts for synthesis is the preferred approach due to several benefits. Nanoparticles produced using plant extract are generally more stable. Metal oxide nanoparticles have been reported to show enhanced antimicrobial effectiveness and this is linked to their large surface area, which is proportional to particle size. On contact with a bacterial cell, an interaction occurs which is mostly electrostatic, and multiple mechanisms of action are elicited, thereby inactivating the bacterial cell. Other mechanisms of action exhibited by metal oxide nanoparticle include generation of ROS, release of toxic ions, and destruction and disorganization of intracellular membrane contents. In this work, zinc oxide nanoparticles and iron oxide nanoparticles were prepared using pomegranate leaf and flower extract. The extracts performed as reducing, capping and stabilizing agents. Formation of nanoparticles during the synthesis process was indicated by a colour change. The phytochemical content of the leaf and flower of *Punica granatum* extract was assessed, and alkaloid, anthocyanin, phenol, tanins etc were observed as major constituents of the extract of the plant part. Spectroscopic characterisation of the nanoparticles was done to confirm formation. Electrochemical characterization was also conducted using cyclic voltammetry to ascertain the electrochemical behaviour of the nanoparticles. The obtained voltammogram signified that the synthesized zinc oxide nanoparticles and iron oxide nanoparticles mediated via the pomegranate leaf and flower extract possess good electrochemical properties, which would allow their use as probable catalyst candidates in electrochemistry and electrochemical applications. The antimicrobial characteristics of the synthesized metal oxide nanoparticles (ZnO-NPs-PL, ZnO-NPs-

PF, Fe₃O₄-NPs-PL, Fe₃O₄-NPs-PF) were also evaluated. The effectiveness of the obtained ZnO-NPs-PL, ZnO-NPs-PF, Fe₃O₄-NPs -PL, Fe₃O₄-NPs -PF was checked against 13 pathogenic bacterial strains. ZnO-NPs-PL, and ZnO-NPs-PF, showed more antibacterial activity than the Fe₃O₄-NPs-PL and Fe₃O₄-NPs-PF. The effect of concentration on the ability to exert antimicrobial activity was also investigated, and the results showed that an increase in nanoparticle concentration is directly proportional to an increase in antimicrobial effect exerted by the nanoparticle. MIC values obtained from the experiment indicate that, even at low concentration, the effect of nanoparticles is still evident as they can inhibit microbial growth to an extent.

7.2 FUTURE RESEARCH PROSPECTS

Different types of nanoparticles are being produced constantly, using the biosynthesis method. This calls for a need to develop a unifying standard protocol for investigation of the antibacterial potency of the biosynthesized metal oxide nanoparticles, to enable comparative study on the antibacterial capability against bacterial strains. The exact mechanisms by which nanoparticles exert antibacterial potency also need to be investigated, as the mechanism of action is still unclear. Also studies are required to find which properties of the nanoparticles govern the antibacterial activity and cytotoxicity to mammalian cells. Intricate research studies are also needed to find the specific mechanism of reaction and identification of the protein and enzyme involved in biosynthesis.

APPENDIX

Appendix 1: Media Used

Appendix 1A: Muller Hinton Agar

Composition

Ingredients	In Gram/litre
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0

Final pH 7.3 ± 0.1 at 25°C

Appendix 1B: Tryptic soy broth

Composition

Ingredients	In Gram/litre
Pancreatic digest of casein	17.0
Enzymatic digest of soya bean*	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.5
Glucose	2.5

Final pH 7.3±0.2 @ 25°C

Appendix 2

Appendix Table 2A: Inhibition zone diameters obtained using the pomegranate plant extract

Bacterial Strain	Zones of Inhibition (mm)											
	Aqueous Leaf Extract			Aqueous Flower Extract			Methanolic Leaf Extract			Methanolic Flower Extract		
<i>Streptococcus pneumoniae</i>	14	12	11		-			-		14	14	13.5
<i>Salmonella diarizonae</i>	22	24	26		-		19	20	20	22	22	22
<i>Moraxella catarrhalis</i>	16	18	20		-		*	15	15	22	22	22
<i>Enterococcus faecium</i>			-		-			-			-	
<i>Salmonella typhi</i>	22	22	22		-		23.5	24	24	24	24	24
<i>Bacillus cereus</i>	14	17	17		-		16	16	16		-	
<i>Staphylococcus aureus</i>			-		-		20	15	22		-	
<i>Escherichia coli</i>	10	10	10		-		14	15	17		-	
<i>Aeromonas hydrophilia</i>	13	15	14		-		14	13	16		-	
<i>Pseudomonas aeruginosa</i>			-		-			-			-	
<i>Listeria monocytogenes</i>			-		-			-			-	
<i>Klebsiella pneumoniae</i>	13.5	15	14		-		17	13	13		-	
<i>Enterococcus faecalis</i>			-		-			-			-	

Key: - = No zone of inhibition observed.

Appendix Table 2B: Inhibition zone diameters obtained using the 50 µg/ml of zinc oxide leaf nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-50 µg/ml zinc oxide nanoparticle Leaf (ZnO-NPs-PL)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	8	7	6	7	1
<i>Salmonella typhi</i>	7	6	6	6,333333	0,57735
<i>Escherichia coli</i>	14	12	14	13,33333	1,154701
<i>Aeromonas hydrophilia</i>	12	13	8	11	2,645751
<i>Bacillus cereus</i>	13	10	8	10,33333	2,516611
<i>Listeria monocytogenes</i>	10	11	9	10	1
<i>Enterococcus faecalis</i>	6	6,5	6,5	6,333333	0,288675
<i>Pseudomonas aeruginosa</i>	10	11	8	9,666667	1,527525
<i>Enterococcus faecium</i>	13	13	12	12,66667	0,57735
<i>Salmonella diarizonae</i>	11	9,5	12	10,83333	1,258306
<i>Klebsiella pneumoniae</i>	11	10	8	9,666667	1,527525
<i>Moraxella catarrhalis</i>	10	8	8	8,666667	1,154701
<i>Streptococcus pneumoniae</i>	12	8	12	10,66667	2,309401

Appendix Table 2C: Inhibition zone diameters obtained using the 500 µg/ml of zinc oxide leaf nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-500 µg/ml zinc oxide nanoparticle Leaf (ZnO-NPs-PL)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	11	11	9,5	10,5	0,866025
<i>Salmonella typhi</i>	10	10	10	10	0
<i>Escherichia coli</i>	14.5	13	14	13,5	0,707107
<i>Aeromonas hydrophilia</i>	12	13	12	12,33333	0,57735
<i>Bacillus cereus</i>	14	14	13	13,66667	0,57735
<i>Listeria monocytogenes</i>	12	11	13	12	1
<i>Enterococcus faecalis</i>	14	15	14	14,33333	0,57735
<i>Pseudomonas aeruginosa</i>	12	11	11	11,33333	0,57735
<i>Enterococcus faecium</i>	15	15	15,5	15,16667	0,288675
<i>Salmonella diarizonae</i>	13	10	13	12	1,732051
<i>Klebsiella pneumoniae</i>	12	10	13	11,66667	1,527525
<i>Moraxella catarrhalis</i>	10	11	8	9,666667	1,527525
<i>Streptococcus pneumoniae</i>	13	13	12	12,66667	0,57735

Appendix Table 2D: Inhibition zone diameters obtained using the 1 mg/ml of zinc oxide leaf nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-1 mg/ml zinc oxide nanoparticle Leaf (ZnO-NPs-PL)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	11	11	12	11,33333	0,57735
<i>Salmonella typhi</i>	11	11	11,5	11,16667	0,288675
<i>Escherichia coli</i>	15	16	14	15	1
<i>Aeromonas hydrophilia</i>	13	13	14	13,33333	0,57735
<i>Bacillus cereus</i>	15	16	15	15,33333	0,57735
<i>Listeria monocytogenes</i>	13	11	13	12,33333	1,154701
<i>Enterococcus faecalis</i>	16	18	18	17,33333	1,154701
<i>Pseudomonas aeruginosa</i>	13	11	14	12,66667	1,527525
<i>Enterococcus faecium</i>	17	15	18	16,66667	1,527525
<i>Salmonella diarizonae</i>	16	14	16	15,33333	1,154701
<i>Klebsiella pneumoniae</i>	12	11	13	12	1
<i>Moraxella catarrhalis</i>	14	11	15	13,33333	2,081666
<i>Streptococcus pneumoniae</i>	15	13	16	14,66667	1,527525

Appendix Table 2E: Inhibition zone diameters obtained using the 5 mg/ml of zinc oxide leaf nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-5 mg/ml zinc oxide nanoparticle Leaf (ZnO-NPs-PL)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	13	13	13	13	0
<i>Salmonella typhi</i>	14	15	14	14,33333	0,57735
<i>Escherichia coli</i>	16	17	14	15,66667	1,527525
<i>Aeromonas hydrophilia</i>	16	14	14	14,66667	1,154701
<i>Bacillus cereus</i>	13	15	15	14,33333	1,154701
<i>Listeria monocytogenes</i>	15	12	14	13,66667	1,527525
<i>Enterococcus faecalis</i>	18	18	18	18	0
<i>Pseudomonas aeruginosa</i>	14	15	17	15,33333	1,527525
<i>Enterococcus faecium</i>	20	20	18	19,33333	1,154701
<i>Salmonella diarizonae</i>	20	18	19	19	1
<i>Klebsiella pneumoniae</i>	15	11	16	14	2,645751
<i>Moraxella catarrhalis</i>	16	11	15	14	2,645751
<i>Streptococcus pneumoniae</i>	17	17	16	16,66667	0,57735

Appendix Table 2F: Inhibition zone diameters obtained using the 50 µg/ml of zinc oxide flower nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-50 µg/ml zinc oxide nanoparticle Flower(ZnO-NPs-PF)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	8	7	6	7	1
<i>Salmonella typhi</i>	6	6	5	5,66667	0,5773503
<i>Escherichia coli</i>	9	8	9	8,66667	0,5773503
<i>Aeromonas hydrophilia</i>	12	11	8	10,3333	2,081666
<i>Bacillus cereus</i>	12	10	11	11	1
<i>Listeria monocytogenes</i>	13	9	10	10,6667	2,081666
<i>Enterococcus faecalis</i>	7	6	6	6,33333	0,5773503
<i>Pseudomonas aeruginosa</i>	8	8	6	7,33333	1,1547005
<i>Enterococcus faecium</i>	13	12	13	12,6667	0,5773503
<i>Salmonella diarizonae</i>	10	9	10	9,66667	0,5773503
<i>Klebsiella pneumoniae</i>	10	11	11	10,6667	0,5773503
<i>Moraxella catarrhalis</i>	8	7	9	8	1
<i>Streptococcus pneumoniae</i>	12.5	11,5	12	11,75	0,3535534

Appendix Table 2G: Inhibition zone diameters obtained using the 500 µg/ml of zinc oxide flower nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-500 µg/ml zinc oxide nanoparticle Flower(ZnO-NPs-PF)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	12	13	12	12,3333	0,57735
<i>Salmonella typhi</i>	10	8	8	8,66667	1,1547
<i>Escherichia coli</i>	10	11	9	10	1
<i>Aeromonas hydrophilia</i>	14	13,5	14	13,8333	0,28868
<i>Bacillus cereus</i>	*	12	13	12,5	0,70711
<i>Listeria monocytogenes</i>	14	15	14	14,3333	0,57735
<i>Enterococcus faecalis</i>	10	10	9	9,66667	0,57735
<i>Pseudomonas aeruginosa</i>	10	11	9	10	1
<i>Enterococcus faecium</i>	16	15	16,5	15,8333	0,76376
<i>Salmonella diarizonae</i>	10	9	10	9,66667	0,57735
<i>Klebsiella pneumoniae</i>	10	11	12	11	1
<i>Moraxella catarrhalis</i>	11	12	13	12	1
<i>Streptococcus pneumoniae</i>	14	15	13	14	1

Appendix Table 2H: Inhibition zone diameters obtained using the 1 mg/ml of zinc oxide flower nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-1 mg/ml zinc oxide nanoparticle Flower(ZnO-NPs-PL)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	13	12	12,5	12,5	0,5
<i>Salmonella typhi</i>	11	12	13	12	1
<i>Escherichia coli</i>	13	14	15	14	1
<i>Aeromonas hydrophilia</i>	18	16	18	17,3333	1,1547
<i>Bacillus cereus</i>	15	13	15,5	14,5	1,32288
<i>Listeria monocytogenes</i>	15	14	*	14,5	0,70711
<i>Enterococcus faecalis</i>	12	13	11	12	1
<i>Pseudomonas aeruginosa</i>	13	13	13	13	0
<i>Enterococcus faecium</i>	18	18	18	18	0
<i>Salmonella diarizonae</i>	16	15	17	16	1
<i>Klebsiella pneumoniae</i>	12	10	10	10,6667	1,1547
<i>Moraxella catarrhalis</i>	13	11	11	11,6667	1,1547
<i>Streptococcus pneumoniae</i>	16	16	16	16	0

Appendix Table 2I: Inhibition zone diameters obtained using the 5 mg/ml of zinc oxide flower nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-5 mg/ml zinc oxide nanoparticle Flower (IONPs-PF)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	16	14	15	15	
<i>Salmonella typhi</i>	14,4	14,8	14,4	14,53333	0,23094
<i>Escherichia coli</i>	14	14,5	15	14,5	0,5
<i>Aeromonas hydrophilia</i>	17	17,5	18	18	0,5
<i>Bacillus cereus</i>	16	16	17	16,33333	0,57735
<i>Listeria monocytogenes</i>	16,5	17	16	16,5	0,5
<i>Enterococcus faecalis</i>	16,2	16	16,3	16,16667	0,152753
<i>Pseudomonas aeruginosa</i>	14	14	14	14	0
<i>Enterococcus faecium</i>	21	21,5	22	21,5	0,5
<i>Salmonella diarizonae</i>	18,5	18	19	18,5	0,5
<i>Klebsiella pneumoniae</i>	14	14	14	14	0
<i>Moraxella catarrhalis</i>	14,5	14,5	17,1	15,36667	1,501111
<i>Streptococcus pneumoniae</i>	18,5	19,5	19	19	0,5

Appendix Table 2J: Inhibition zone diameters obtained using the 50 µg/ml iron oxide leaf nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-50 µg/ml iron oxide nanoparticle Leaf (IONPs-PL)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	6	7	6	6,33333	0,5773503
<i>Salmonella typhi</i>	8	6	5	6,33333	1,5275252
<i>Escherichia coli</i>	6	7	7	6,66667	0,5773503
<i>Aeromonas hydrophilia</i>	6	6	8	6,66667	1,1547005
<i>Bacillus cereus</i>	11	10	11	10,6667	0,5773503
<i>Listeria monocytogenes</i>	6	7	5,5	6,16667	0,7637626
<i>Enterococcus faecalis</i>	12	11	12	11,6667	0,5773503
<i>Pseudomonas aeruginosa</i>	11	8	6	8,33333	2,5166115
<i>Enterococcus faecium</i>	8	8	9	8,33333	0,5773503
<i>Salmonella diarizonae</i>	13	13	12	12,6667	0,5773503
<i>Klebsiella pneumoniae</i>	11	11	11	11	0
<i>Moraxella catarrhalis</i>	6	7	6	6,33333	0,5773503
<i>Streptococcus pneumoniae</i>	6	5	5	5,33333	0,5773503

Appendix Table 2K: Inhibition zone diameters obtained using the 500 µg/ml iron oxide leaf nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-500 µg/ml iron oxide nanoparticle Leaf (IONPs-PL)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	13	13,1	12,9	13	0,1
<i>Salmonella typhi</i>	11	11	10,6	10,86667	0,23094
<i>Escherichia coli</i>	11	8	8	9	1,732051
<i>Aeromonas hydrophilia</i>	10	11	10,8	10,6	0,52915
<i>Bacillus cereus</i>	13	14	13,2	13,4	0,52915
<i>Listeria monocytogenes</i>	11	10	10	10,33333	0,57735
<i>Enterococcus faecalis</i>	15	15,3	15,5	15,26667	0,251661
<i>Pseudomonas aeruginosa</i>	13	13,2	12,8	13	0,2
<i>Enterococcus faecium</i>	12	12	12,2	12,06667	0,11547
<i>Salmonella diarizonae</i>	15,7	16	15,8	15,83333	0,152753
<i>Klebsiella pneumoniae</i>	13	13,2	13,2	13,13333	0,11547
<i>Moraxella catarrhalis</i>	8	8	8	8	0
<i>Streptococcus pneumoniae</i>	6	5,7	5,8	5,833333	0,152753

Appendix Table 2L: Inhibition zone diameters obtained using the 1 mg/ml iron oxide leaf nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-1 mg/ml iron oxide nanoparticle Leaf (IONPs-PL)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	14	15	13,8	14,26667	0,64291
<i>Salmonella typhi</i>	15	15,5	14,7	15,06667	0,404145
<i>Escherichia coli</i>	14	14	15	14,33333	0,57735
<i>Aeromonas hydrophilia</i>	15	15,5	14,8	15,1	0,360555
<i>Bacillus cereus</i>	15	15,2	15,5	15,23333	0,251661
<i>Listeria monocytogenes</i>	14	13	14,1	13,7	0,608276
<i>Enterococcus faecalis</i>	18	17,6	18	17,86667	0,23094
<i>Pseudomonas aeruginosa</i>	16	15,2	15,5	15,56667	0,404145
<i>Enterococcus faecium</i>	17	14	15,8	15,6	1,509967
<i>Salmonella diarizonae</i>	18	18	17,8	18,33333	0,57735
<i>Klebsiella pneumoniae</i>	16	14,5	13	14,5	1,5
<i>Moraxella catarrhalis</i>	12	13	12,5	12,5	0,5
<i>Streptococcus pneumoniae</i>	10	10	9,8	9,933333	0,11547

Appendix Table 2M: Inhibition zone diameters obtained using the 5 mg/ml iron oxide leaf nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-5 mg/ml iron oxide nanoparticle Leaf (IONPs-PF)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	15	16	15,5	15,5	0,5
<i>Salmonella typhi</i>	16	15,9	15,9	15,93333	0,057735
<i>Escherichia coli</i>	20	20	19,5	19,83333	0,288675
<i>Aeromonas hydrophilia</i>	23	23	23	23	0
<i>Bacillus cereus</i>	20	19,7	19,4	19,7	0,3
<i>Listeria monocytogenes</i>	16	15,2	14,8	15,33333	0,61101
<i>Enterococcus faecalis</i>	20	20	19	19,66667	0,57735
<i>Pseudomonas aeruginosa</i>	20	20	20	20	0
<i>Enterococcus faecium</i>	14	15,5	16,5	15,33333	1,258306
<i>Salmonella diarizonae</i>	21	21,5	21,3	21,26667	0,251661
<i>Klebsiella pneumoniae</i>	18	18,3	18	18,1	0,173205
<i>Moraxella catarrhalis</i>	15	15	14,6	14,86667	0,23094
<i>Streptococcus pneumoniae</i>	12	11,8	11,5	11,76667	0,251661

Appendix Table 2N: Inhibition zone diameters obtained using the 50 µg/ml iron oxide flower nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-50 µg/ml iron oxide nanoparticle Flower (IONPs-PF)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	6	7	6	6,33333	0,5773503
<i>Salmonella typhi</i>	6	6	5	5,66667	0,5773503
<i>Escherichia coli</i>	5	6	6	5,66667	0,5773503
<i>Aeromonas hydrophilia</i>	8	11	8	9	1,7320508
<i>Bacillus cereus</i>	10	10	9	9,66667	0,5773503
<i>Listeria monocytogenes</i>	6	6	6	6	0
<i>Enterococcus faecalis</i>	8	6	6	6,66667	1,1547005
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0
<i>Enterococcus faecium</i>	9	8	8,5	8,5	0,5
<i>Salmonella diarizonae</i>	8	9	8	8,33333	0,5773503
<i>Klebsiella pneumoniae</i>	6	5	5	5,33333	0,5773503
<i>Moraxella catarrhalis</i>	4	4	5	4,33333	0,5773503
<i>Streptococcus pneumoniae</i>	0	0	0	0	0

Appendix Table 2O: Inhibition zone diameters obtained using the 500 µg/ml iron oxide flower nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-500 µg/ml iron oxide nanoparticle Flower (IONPs-PF)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	10	9	9	9,333333	0,57735
<i>Salmonella typhi</i>	11,5	11	11	11,16667	0,288675
<i>Escherichia coli</i>	11	10	10,5	10,5	0,5
<i>Aeromonas hydrophilia</i>	11	11	9,5	10,5	0,866025
<i>Bacillus cereus</i>	13	12	13	12,66667	0,57735
<i>Listeria monocytogenes</i>	8	7	8	7,666667	0,57735
<i>Enterococcus faecalis</i>	13	13	13	13	0
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0
<i>Enterococcus faecium</i>	12	12	11	11,66667	0,57735
<i>Salmonella diarizonae</i>	14	13	13	13,33333	0,57735
<i>Klebsiella pneumoniae</i>	7	8	7	7,333333	0,57735
<i>Moraxella catarrhalis</i>	6	6	7	6,333333	0,57735
<i>Streptococcus pneumoniae</i>	0	0	0	0	0

Appendix Table 2P: Inhibition zone diameters obtained using the 1 mg/ml iron oxide flower nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-1 mg/ml iron oxide nanoparticle Flower (IONPs-PF)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	10.5	10	10,5	10,25	0,353553
<i>uSalmonella typhi</i>	13	12	13	12,66667	0,57735
<i>Escherichia coli</i>	12	12	12	12	0
<i>Aeromonas hydrophilia</i>	16,8	16	16,8	16,53333	0,46188
<i>Bacillus cereus</i>	14	13	15,5	14,16667	1,258306
<i>Listeria monocytogenes</i>	13	13	13	13	0
<i>Enterococcus faecalis</i>	15	15	14	14,66667	0,57735
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0
<i>Enterococcus faecium</i>	13	14	13	13,33333	0,57735
<i>Salmonella diarizonae</i>	16	16,8	16	16,26667	0,46188
<i>Klebsiella pneumoniae</i>	10	10	10	10	0
<i>Moraxella catarrhalis</i>	10	9	11	10	1
<i>Streptococcus pneumoniae</i>	0	0	0	0	0

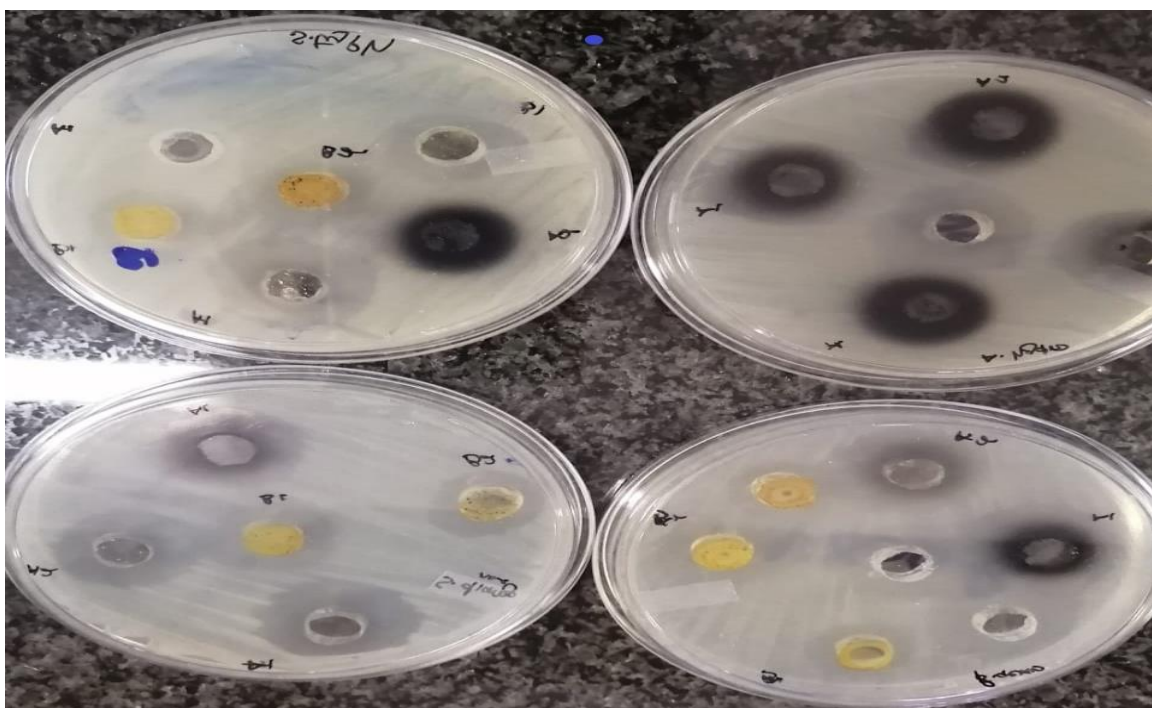
Appendix Table 2Q: Inhibition zone diameters obtained using the 5 mg/ml iron oxide flower nanoparticle

Bacterial strains	Zones of Inhibitions(mm)-5 mg/ml iron oxide nanoparticle Flower (IONPs-PF)				
	Plate 1	Plate 2	Plate 3	Average	Standard Deviation
<i>Staphylococcus aureus</i>	15	15	14	14,66667	0,57735
<i>Salmonella typhi</i>	15	15	15	15	0
<i>Escherichia coli</i>	13,1	12,8	11,9	12,6	0,6245
<i>Aeromonas hydrophilia</i>	18,4	17,9	17,2	17,83333	0,602771
<i>Bacillus cereus</i>	16,5	14,8	16,7	16	1,044031
<i>Listeria monocytogenes</i>	15,5	16	14,9	15,46667	0,550757
<i>Enterococcus faecalis</i>	16,5	16,3	16	16,26667	0,251661
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0
<i>Enterococcus faecium</i>	15,3	14,5	15,5	15,1	0,52915
<i>Salmonella diarizonae</i>	20	20,5	20,3	20,26667	0,251661
<i>Klebsiella pneumoniae</i>	12	12	12	12	0
<i>Moraxella catarrhalis</i>	12	12,3	13	12,43333	0,51316
<i>Streptococcus pneumoniae</i>	0	0	0	0	0

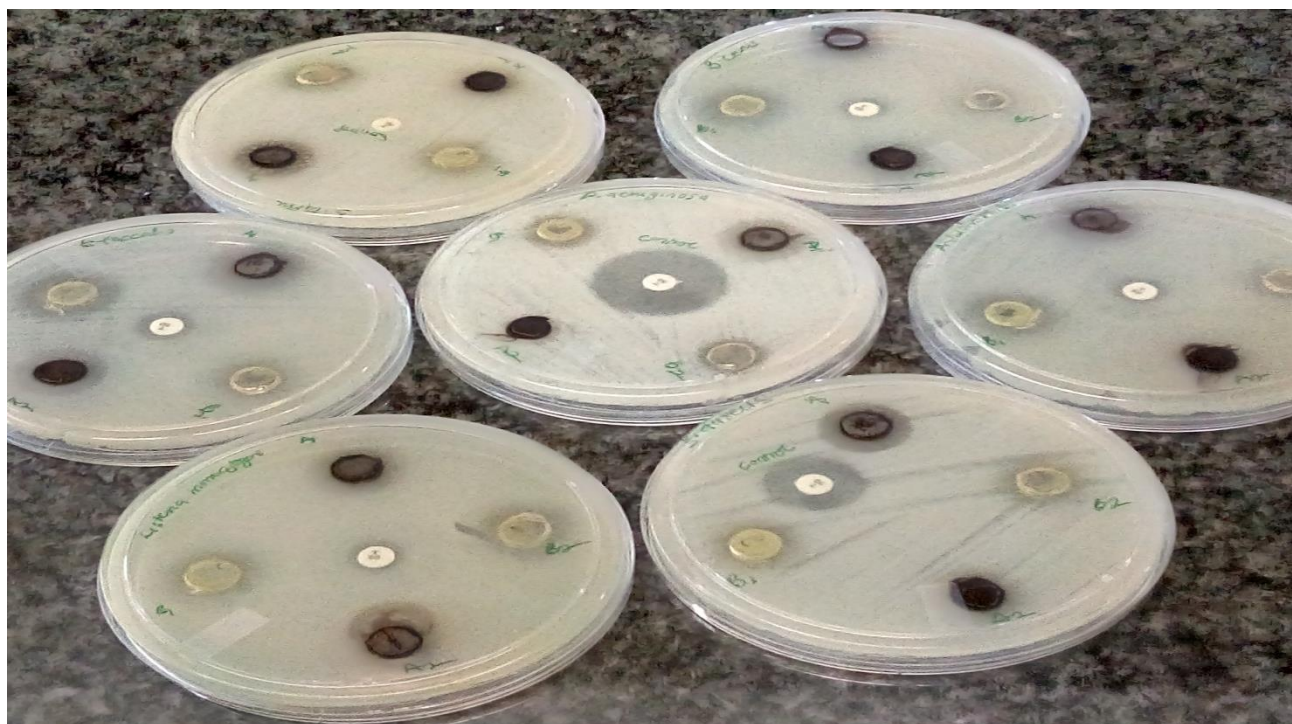
Appendix 3:



Appendix 3A: Image showing Petri dishes of the antimicrobial activity of methanolic and aqueous leaf and flower extract of *Punica granatum*.



Appendix 3B: Image showing Petri dishes of the antimicrobial activity zinc oxide and iron oxide nanoparticles



Appendix 3D: Image showing obtained Iron oxide nanoparticle.



Appendix 3E: Image showing obtained zinc oxide nanoparticle.

