Biosynthesis of zinc nanoparticles using *Moringa oleifera Lam.* seed extract

Kalaiyarasi,C
(16PZO005)

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

In partial fulfilment of the requirements for the Degree of

MASTER OF SCIENCE IN ZOOLOGY

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10.4.18
Signature of the Head of the Department

10/4/18
Signature of the Supervisor

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Contents

CONTENTS

4 **RESULTS AND DISCUSSION**

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Nanotechnology is an emerging field of science with its increasing applications in diverse areas for the development of new materials at nanoscale levels. It is the technological innovation in the 21st century which employs active researches in modern material science (Albrecht *et al.,* 2006).

Nanoscience and nanotechnology represent new and enabling platforms that promise to provide a broad range of novel uses and improved technologies for environmental, biological and other scientific applications. In science and engineering, sustainable nanotechnology is successful in giving solutions for the challenges in various sectors such as medicine, catalysis, industrial and agricultural activities. In the past decades, research and development in nanoscience and technology is growing rapidly throughout entire universe, hence the government and industrial sectors have steadily increased its investment in this area. Fundamentally, nanotechnology is about manipulating and making materials at atomic and molecular level (Mata *et al.,* 2012).

Nanoparticles are used immensely due to its small size and physical properties which are reportedly shown to change the performance of any other material which is in contact with these tiny particles.

In today's era of development in research, nanoparticles are the key component which is being used widely. As the name suggests these are particles having the dimension of nano size, which in other words referred as one billionth or 10^{-9}. These particles are measured in nanometre scale with the size range of 1 - 100nm. Nanoparticles are more effective because of its novel characterization and exceptional features and also the surface area to volume ratio of nanoparticles, which makes them more reactive than bigger molecules (Nour *et al.,* 2010).

Nanoparticles possess definite physicochemical properties such as optical (Pattanayak and Nayak, 2013), magnetic (Firdhouse and Lalitha, 2015), catalytic (Nasrollahzadeh, 2016) and antimicrobial properties at the nanostage level (Nam *et al.,* 2016) which characteristically consequences the superior chemical reactivity, biological activity, and catalytic behaviour compared to larger particles of the identical chemical composition (Hussein, 2014 and Kholoud *et al.,* 2010). Nanomaterials are “deliberately engineered” to guide the improvement of special properties at the nanoscale level. Nanomaterials may have superior bioavailability than larger units, ensuring in greater utilization of individual cells, tissues and organs. Nanomaterials that gain admission to our bodies just penetrate the biological membranes and access cells, tissues and organs. Materials with size 300 nm can be taken up by individual cells while nanometers that quantify below 70 nm can still used up by cells nuclei, where they can cause principal damage (Nasrollahzadeh, 2016).

The nanoparticles have drawn more attention from researchers worldwide because of their specific characteristics (size, shape, and distribution) which could be used in different fields of applications (Zarger *et al.,* 2011). The synthesis of nanoparticles is an emerging highlight of the intersection of nano-biotechnology which has received increasing attention due to a growing need to develop environmentally benign technologies in material synthesis. Various chemical and physical methods are being explored for nanoparticles production but most of the techniques involved are capital intensive. The chemical method includes photochemical (Sharma *et al.,* 2009), chemical reduction (Guzman *et al.,* 2009), ionization radiation method and classical chemical method in which reducing agents such as hydrazine, hydrogen etc., were used (Nour *et al.,* 2010). These processes produce a huge number of nanoparticles within a short period of time. But, it produces hazardous byproducts which may have the deleterious effect on the nanoparticle synthesis and may be toxic to human health (Sharma *et al.,* 2013). The reducing and capping agents are added externally for the synthesis of metal nanoparticles.

The physical method includes milling, pyrolysis, condensation, and evaporation (Gurav *et al.,* 2014). These processes are energy and time consuming since it takes a lot of time to achieve thermal stability. The laser ablation method depends on duration and wavelength of the laser. Moreover, nanoparticles size and shape gets modified when the laser is passed (Mahfouz *et al., 2008).*

Thus, the conventional methods used in nanoparticles synthesis make use of harmful chemicals and solvents in synthesis process with the addition of artificial preservatives or capping agents as stabilizers thus preventing the applications in biomedical and clinical fields. Hence, there is an emergent need for the improvement of environmental friendly, biocompatible, dependable and synthetic methods to evade some undesired ecological and health effects (Mrinmoy *et al.,* 2008).

Hence, sustainable initiatives have been taken to use green chemistry approach to improve and protect environmental issues. Development of cost effective and ecofriendly methods to synthesize nanomaterials seems to be a challenge for researchers (Mukherjee *et al.,* 2001 and Pasupuleti *et al.,* 2013). Some simpler techniques add the valuation to the synthesis productivity and operability of the nanoparticles. The biological systems have positioned themselves as one of the best candidature for the nanoparticle generation. Being safe and ecofriendly they have grabbed enormous attention in the field of nanotechnology (Paul and Yadav, 2014 and Narayanan and Natarajan, 2010).

In the biological method, plants and microbes are used for biosynthesis of nanoparticles. When microbes are employed for the synthesis of metal nanoparticles, the rate of synthesis is very slow when compared to the plant mediated synthesis, since phytochemicals present in the plant extracts act as reducing and capping agent (Ahmed *et al.,* 2015).

The green synthesis of metal nanoparticles using biological material as the reducing and stabilizing agents has attracted a lot of attention and consideration in pharmaceuticals and biomedical field. Metal nanoparticles have grabbed the attention of researchers due to their unique as well as dynamic properties which do not exist in its bulk form (Paul *et al.,* 2015 and Shankar *et al.,* 2004). In recent times, metal nanoparticles are broadly exploited in various fields such as agriculture, cosmetics, paints, catalysis, food coatings and textiles. In industries, these magnetic nanoparticles cover a broad spectrum of biomedical applications (Rai and Ingle, 2012). Each nanoparticle needs a specific property in a particular field to give its full potential.

Zinc is typically the second most abundant transition metal in organisms after iron and the only metal represented in all six enzyme classes. Zinc is one of the essential micronutrients required for optimum crop growth. Higher plants generally absorb Zn as a divalent cation (Zn^{2+}), which acts either as the metal component of enzymes or a regulatory co-factor of a large number of enzymes (Khan *et al.,* 2004 and Hafeez *et al.,* 2013).

Zinc plays an important role in chlorophyll production, pollen function, fertilization and germination (Cakmak, 2008) and in biomass production (Kaya *et al.,* 2000). It plays a fundamental role in protecting and maintaining structural stability of cell membranes. It is used for protein synthesis, membrane function, cell elongation and tolerance to environmental stresses (Cakmak, 2000).

Zinc is non-toxic, highly flexible in its coordination and makes stable associations with macromolecules (Bertholf, 1988 and Vallee and Aulid, 1990). It plays an important role in cell proliferation, since there are zinc finger motifs present in a wide variety of transcription and replication factors. Consequently, in zinc deficiency, cell proliferation does not take place, so that highly-proliferating cells (e.g. cells of the immune system or the skin) are considered to be sensitive indicators for a lack of zinc. Furthermore, zinc plays a distinct role in apoptosis (Zalewski and Forbes, 1993) and signal transduction (Beyersmann and

Haase, 2001). Zinc in normal soil ranges from 10-300 $mgKg^{-1}$. In general, concentration of zinc such as 500 mgKg-1 may be toxic to crops resulting reduced crop yield (Alloway, 2008).

Synthesis of zinc nanoparticles exhibit new and improved properties with larger particles of the bulk materials due to the variation in specific characteristics such as size, distribution and morphology of the particles (Senjen and Iuminato,2009).

Moringa oleifera Lam. is selected as the candidate plant for the synthesis of nanoparticles which is commonly known as “horseradish tree” or “drumstick tree”. It is a small tree (7–12 m high) with thick grey bark, fragrant white flowers, and long green pods (Makkar and Becker, 1997) which belongs to the family *Moringaceae.* It is found in the Middle East, African and Asian countries and highly adaptable and tolerate drought (Garima *et al.,* 2011). *Moringa oleifera* seed kernels contain a significant amount of oil (up to 40%) with a high-quality fatty acid composition (oleic acid > 70%) which after refining, shows a notable resistance to oxidative degradation (Anwar *et al.,* 2005). The leaves, are rich in proteins, minerals, β-carotene and antioxidants and are used in traditional medicine (Leone *et al.,* 2015). The tree is endowed with various life giving medicinal properties, such as to treat anemia, blindness, arthritis, hyperthyroidism, rheumatism, epilepsy, Chrohn's disease, antiherpes simplex virus, gout and sexually transmitted diseases (Mulh *et al.,* 2011; Monera and Maponga, 2012 and Dao and Kabore 2015).

In the present an investigation was made to synthesis zinc oxide nanoparticles using the seed extract of *Moringa oleifera Lam.* The bio-reduction of zinc ions into respective nanoparticles mediated by *Moringa oleifera* seed extract is chemically complex but environmentally benign. A broad variability of metabolites present in the seed extract possesses antioxidant or reducing properties that aids in the immediate reduction of the zinc ions into nanostructured zinc oxide.

Photocatalytic activity of zinc oxide nanoparticles offers a promising method for waste water treatment (Srinivasa *et al.,* 2012).

Crystal violet is one of the most common pollutants discharged from the industries directly or indirectly into water sources causing water pollution (Ameta *et al.,* 2013). Zinc oxide exhibits very good photochemical reactivity and efficiently degrades toxic water pollutants released from textile and dying industries by utilizing natural source of energy, sunlight. This is due to the presence of many active sites and fabrication of hydroxyl radicals on zinc oxide surface (Baruah *et al.,* 2009 and Kajbafvala *et al.,* 2012).

The present work has been targeted for the potential applications of nanoparticles on living organisms, including flora and fauna both because of their pollution free and eco-friendly approach. The exposure of plants to nanoparticles and the effects of such an exposure on plant systems could open a new path of research in the field of nano-agronomy. In order to recognize the possible advantages of nanoparticles in agriculture, it is essential to investigate the penetration, accumulation and transport of nanoparticles inside the plants. Taking these aspects into consideration, both affirmative and pessimistic effects of nanoparticles are experiential in living plants (Panda and Khan, 2004).

Zinc plays important role in modulating growth and germination in various plant including rice but there is little or no information on physiological impact of nanosized zinc(Zn NP) on seed germination which is tried to address in the present study (Kramer, 1995).

Thus with this background the present study has been formulated with following objectives :

1. To synthesis the zinc oxide nanoparticles using the seed extract of *Moringa oleifera Lam.*
2. To characterize the biosynthesized nanoparticles by UV-Vis spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy Dispersive X-ray spectroscopy (EDX).
3. To determine the particle size of the biosynthesized nanoparticles.
4. To analyse the phytochemicals present in the biosynthesized nanoparticles.
5. To assess the antimicrobial activity of the biosynthesized zinc nanoparticle.
6. To study the photocatalytic activity of the biosynthesized zinc oxide nanoparticles against the textile dye crystal violet.
7. To assess the phytotoxicity of the synthesized zinc oxide nanoparticles using green gram seeds.

Review of Literature

2. REVIEW OF LITERATURE

The review of literature for the present study *"Biosynthesis of zinc nanoparticles using Moringa oleifera Lam. seed extract"* is presented under the following headings.

2.1. Scenario on Nanotechnology
2.2. Nanoparticles – An overview
2.3. Classification of nanoparticles
    2.3.1. Metallic nanoparticles
    2.3.2. Nonmetallic nanoparticles
2.4. Properties of nanoparticles
    2.4.1. Physical and chemical properties of nanoparticles
    2.4.2. Biological properties of nanoparticles
2.5. Applications of nanoparticles
    2.5.1. Application of nanoparticles in medical field
    2.5.2. Applications of nanoparticles as Biosensors
    2.5.3. Applications of nanoparticles in food industry
    2.5.4. Applications of nanoparticles in textile industry
2.6. Zinc oxide nanoparticles
2.7. Synthesis and characterization of nanoparticles using plant extract
2.8. Photocatalytic activity of nanoparticles against textile dyes

2.1. SCENARIO ON NANOTECHNOLOGY

Nanotechnology is the manipulation of materials at atomic level by the combination of chemistry, physics, biology and engineering amongst others. Nanotechnology is the art and science of manipulating matter at the atomic or molecular scale and holds the promise of providing significant improvements in technologies for protecting the environment (Schmid, 2010).

According to National Nanotechnology Initiative (NNI) USA, nanotechnology is defined as research and technology development at the atomic, molecular or macromolecular levels using a length scale of approximately one to one hundred nanometer in any dimension. The creation and use of structures, devices and systems that have novel properties and functions because of their small size and the ability to control or manipulate matter on an atomic scale (USEPA, 2007).

Nanotechnology has been used to develop nanomaterials, nanodevices and various nano systems. The nanomaterials are most advanced in scientific knowledge as well as in commercial applications. Reduction in the size of nanoparticles brings significant changes in their physical properties in comparison to bulk materials nanoscale materials can be defined as those particles whose characteristic length scale lies within the nanometric range, i.e. in the range between one and several hundreds of nanometers preferably between 1-100 nm (Rana and Kalaichelvan, 2013).

The magnetic nanoparticles reveals the superior imaging characteristics and have therapeutics features in the nanomedicine field for the breast cancer applications (Yallapu *et al.,* 2012). Now a day's nanoparticles have strong emphasis in the technological developments. The nanomaterials have sublime physical and chemical properties which is more efficient than the bulk materials. These unique properties occur when there is a change in some features like variation of size and the band gap energy (Pathak *et al.,* 2012).

The technology has excellent prospects for exploitation across the medical, pharmaceutical, biotechnology, engineering, manufacturing, tele-communications and information technology markets. Nanotechnology plays a central role in the recent technological advances and in the areas of disease diagnosis, drug design and drug delivery. The nanotechnological application to disease treatment, diagnosis, monitoring and to the control of biological systems have been referred to as 'nanomedicine' (Moghimi *et al.,* 2005).

2.2. NANOPARTICLES - AN OVERVIEW

The history of nanoparticle usage dates back to the 9th century when the artisans of Mesopotamia used nanoparticles to generate glittering effects to pots. The properties of nanoparticles were proved in 1857 in Faraday's famous paper, "Experimental relations of gold and other metals to light". The end of this period was the appearance of conditions for managed nanotechnology development, which was facilitated by scientific and technical revolution (Tolochko, 2010). It is now widely accepted that nanotechnology is emerging as a major factor for commercial success in the 21st century and is regarded as "the next industrial revolution".

The nanoparticles synthesis and utilization by man can be traced back to over one thousand years ago. Ancient Chinese produced ink based on ultrafine powders by collecting smoke resulting from candle burning. However, systematic studies on nanoscale materials started only some decades ago. The first artificial nanopowder syntheses were reported in Japan in 1960s. Nanostructured metal particles were obtained by using a gas-condensation method. From then on, the preparation of nanopowders received more and more attention, motivated by their potentially useful properties in many technological respects (Krejcova *et al.,* 2013)

Physical, chemical and a biological method has been used to synthesize nanomaterials of particular size and shape. Although ultrasonic fields and photochemical reduction techniques have been used successfully to produce nanoparticles but they remain expensive and involve the use of hazardous chemicals. Therefore, there is a growing concern to develop simple, cost-effective, ecofriendly and sustainable method, to synthesis nanoparticles of different compositions, sizes and shapes.

Chaudhary *et al.* (2011) reported that "Ayurvedic bhasmas" are in nanometer dimensions and are considered as nanomedicine free from toxicity in therapeutic doses. Bhasmas produced by biological methods of nanoparticles is prescribed with several medicines in the ayurvedic field, which is one of the most ancient applications of nanomedicine.

The nanomaterial research was mainly focused in the area of materials science, mainly in development of microelectronic and optoelectronic devices. However, in last 10 years, the dramatic size dependent optical, electronic, magnetic, and mechanical properties of nanoparticles have attracted attention of researchers from almost every science including biology (Tan *et al.,* 2004).

Nanofluids are generally defined as suspended nanoparticles in solution either using surfactant or surface charge technology. Other nanostructures include nanorods, nano whiskers, nanoforms, nanopyramids and other nanocomposites. Development research is underway in nanoelectronics and photonics materials, such as bio nanomaterials, biomarkers, bio-diagnostics biosensors, and related nano materials for use in polymers, textiles, fuel cell layers, composites and solar energy materials (Annika *et al.,* 2017).

2.3. CLASSIFICATION OF NANOPARTICLES

2.3.1. Metallic nanoparticles

A variety of chemical and physical procedures are used for the synthesis of metallic nanoparticles, but there are major problems such as toxicity, release of hazardous by products and high energy consumption (Kang *et al.,* 2010).

Today these materials can be synthesized and modified with various chemical functional groups which allow them to be conjugated with antibodies, ligands and drugs of interest. Thus, it opens a wide range of potential applications in biotechnology, magnetic separation, and preconcentration of target analytes, targeted drug delivery, and vehicles for gene and drug delivery and more importantly diagnostic imaging (Kaushik *et al.,* 2010).

2.3.2. Nonmetallic nanoparticles

The method use salts that have hydrophobic ions of opposite charge to the nanoparticles, which site in the oil layer and thus reduce the coulombic repulsion between the particles in the organic phase, allowing the particles size close approximately to each other at the interface. The advantage of this method is that because it does not require the surface of the particles to be modified and it allows nonmetallic particles including TiO_2 and SiO_2 to be assembled into dense interfacial layers using the same procedure as is used for metallic particles.

Many natural things contain nanometer-scale features, for example human teeth, bones, sea algae and micro-organisms. Also the natural weathering of iron oxides and silicate minerals, can produce colloids and meteorites may even contain nano diamonds (Chen *et al.,* 2011).

2.4. PROPERTIES OF NANOPARTICLES

2.4.1. Physical and chemical properties of nanoparticles

Nanoparticles often have unique physical and chemical properties. For example, the electronic, optical, and chemical properties of nanoparticles may be very different from those of each component in the bulk. At the nanoscale, materials behave very differently compared to larger scales and it is still very difficult to predict the physical and chemical properties of particles of such a very small size (Manojsingh *et al.,* 2010).

The principal parameters of nanoparticles are their shape, size, surface characteristics and inner structure. Nanoparticles can be encountered as aerosols (solids or liquids in air), suspensions (solids in liquids) or as emulsions (liquids in liquids). In the presence of certain chemicals, properties of nanoparticles may be modified. Indirectly such agents can stabilize against coagulation or aggregation by conserving particle charge and by modifying the outmost layer of the particle.

The composition of a specific nanoparticle can be very complex, depending on what interactions it has had with other chemicals or particles and on its life time. The chemical processes taking place on the surfaces of nanoparticles are also very complicated and remain largely unknown. Nanoparticles have different ways of interacting with each other. They can remain free or group together depending on the attractive or repulsive interaction forces between them, which remain difficult to characterize. Nanoparticles suspended in gas tend to stick to each other more readily than in liquids (Liufu *et al.,* 2004).

In this size range, the physical, chemical and biological properties of the nanoparticle change in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk material. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes, etc. Nanoparticle having one or more dimensions of the order of 100nm or less, have attracted considerably attraction due to their unusual and fascinating properties, with various applications, over their bulk counterparts. Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles (Liu *et al.,* 2011, Tiwari *et al.,* 2008 and Mohanapriya *et al.,* 2008).

Once the particle size is reduced below 100nm, the solid particles begin to demonstrate unusual properties from the bulk material based on quantum mechanics (Bhusan, 2007). The surface related properties and the quantum properties play a fundamental role in making the difference in the properties of the bulk material with that of the nanoparticles (Roduner, 2006).

Depending on the material used to produce nanoparticles, properties like solubility, transparency, color, absorption or emission wavelength, conductivity, melting point and catalytic behavior are changed only by varying the particle size. Properties like dispersibility, conductivity, catalytic behavior and optical properties alter with different surface properties of the particle (Tiwari *et al.,* 2008).

If the surface properties are not controlled, nanoparticles quickly turn into larger particles due to agglomeration. Most of the size dependent effects are then lost. For the application of nanoparticles, it is therefore crucial to control their agglomeration behavior. Dispersed nanoparticles are needed in order to retain their specific properties for the technological applications as shown in (Borm *et al.,* 2006).

2.4.2. Biological properties of nanoparticles

Bio nanotechnology has been defined as nanotechnology that uses biological models for guidance and deriving inspiration from nature which promises great potential for the improvement of scaffolds with specific biological properties. This is an attractive and powerful approach for the production of scaffolds at nanoscale (Taylor, 2007 and Rajeshkumar *et al.,* 2012). Bio nanotechnology has come out as incorporation among biotechnology and nanotechnology for developing various scaffolds and environmental friendly technology for synthesis of nanoparticles.

The use of nanoparticles is gaining impetus in the present as they possess defined optical, chemical and mechanical properties. Among them, the metallic nanoparticles are most promising as they have outstanding microbial properties because of their large surface area to volume ratio (Gong *et al.,* 2007). It also represents a cost-effective substitute for chemical and physical methods of nanoparticles formation (Ahmad *et al.,* 2005).

Nanoparticle size and morphology between different metals when synthesized using the same microorganism (Mohanpriya *et al.,* 2008). This is also true when considering the use of plants for synthesizing nanoparticles. Plants are advantageous and ecofriendly when compared with bacteria and fungi since it avoids the use of specific, well-conditioned culture preparation and isolation techniques that tend to be expensive and elaborate. Biosynthesis of nanoparticles using plants or plant-based extracts tends to be safe, have relatively short production times, and have a lower cultivation cost compared to other biological systems (Mann, 1996).

Properties of the plants extract such as its concentration, metal salt concentration, reaction time, reaction solution pH and temperature significantly influence the quality, size, and morphology of the synthesized nanoparticles (Mittal *et al.,* 2013).

Bacteria of different sizes and shapes can be used as biotemplates for inorganic material synthesis. They can act as bio-scaffold for mineralization or take an active part in nanoparticle synthesis (Hussein, 2005 and Jayaseelan *et al.,* 2012). The major bacterial species used for the synthesis of metallic nanoparticles include *Actinobacter sp., Escherichia coli, Klebsiella pneumoniae, Lactobacillus sp., Bacillus cereus, Corynebacterium sp.,* and *Pseudomonas sp.* Bacteria are known to synthesis metallic nanoparticles either by intracellular or extracellular mechanisms (Mohanpriya 2008).

In microorganism, culturing methods are very important, since the optimization parameters such as nutrients, light, medium pH, temperature, mixing speed, and buffer strength can significantly increase enzyme activity (Iravani, 2011). Recently, the biological synthesis of nanoparticles using plants and plant extracts appears to bean attractive alternative to conventional chemical synthesis and the more complex culturing and isolation techniques needed for many microorganisms. Moreover, combinations of molecules found in plant extracts perform as both reducing and stabilizing (capping) agents during nanoparticle synthesis (Singh *et al.,* 2010).

Synthesize a variety of nanoparticles along with composition, particle size range, and morphology. In particular, selecting the appropriate culture media for a specific bacterium and the particular metallic salt is important since these two parameters form the basis of nanoparticle synthesis and can influence particle yield (Roh *et al.,* 2001).

Biosynthesis of nanoparticles using fungi is widespread among many research groups globally and the synthesis occurs at both extracellular and intracellular locations. For example, fungi such as *Aspergillus sp., Fusarium sp., and Penicillium sp*. Possess high intracellular metal uptake volumes and the synthesized particles tend to be smaller in size (Mukherjee *et al.,* 2002).

2.5. APPLICATIONS OF NANOPARTICLES

Nanoparticles are used in many fields to combat the problems encountred in the environment. Fig. 1 shows the applications of nanoparticles in different fields**.**

2.5.1. Application of nanoparticles in medical field

Nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine (Curtis and Wilkinson, 2001). Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging (Waren and Nie, 1998), sensing (Vaseashta and Malinovska, 2005), targeted drug delivery (Langer, 2001), gene delivery systems (Roy *et al.,* 1999) and artificial implants (Sachlos *et al.,* 2006). Hence, nanosized organic and inorganic particles are finding increasing attention in medical applications due to their amenability to biological functionalization (Xu *et al.,* 2006). Based on enhanced effectiveness, the new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer (Farokhzad *et al.,* 2006) and fight human pathogens like bacteria (Stoimenov *et al.,* 2002; Sondi and Sondi, 2004; Panacek *et al.,* 2006; Morones *et al.,* 2005; Baker *et al.,* 2005).

Nanoparticles has also been modified for early detection of Alzheimer's disease biomarkers in biological fluids as well as delivery of bioactive molecules directly to brain. Although nanotechnology is expected to have a huge impact on the development of “smart” drug delivery and devices against Alzheimer's disease, a crucial gap still to be filled concerns the elucidation of its etiology, for which a great deal of effort is required (Brambilla *et al.,* 2011). Nanoparticles are used in fluorescent biological labels (Bruchez *et al.,* 1998), drug and gene delivery (Mah *et al.,* 2000), bio detection of pathogens (Edelstein *et al.,* 2000), detection of proteins (Nam et al 2003), probing of DNA structure (Mahtabe *et al.,* 1995), tissue engineering (De la Ishla *et al.,* 2003), tumour destruction via heating (hyperthermia) (Yoshida *et al.,* 1999), separation and purification of biological molecules and cells (*Molday et al., 1982*), MRI contrast enhancement (*Weissleder et al., 1990*) and phagokinetic studies (*Parak et al., 2002*).

2.5.2. Applications of nanoparticles as Biosensors

Nanoparticles (NPs), with colorful light-scattering properties, have unique advantages and are comparable to optical probes with various fluorescent dyes. Metal nanoparticles with large diameter (>30 nm) exhibit strong light scattering in the visible region. This can be used directly for light scattering labels in biochemical assay. Nano sensors are capable of providing data through unique technology, could find wide application in monitoring our personal health, the food we eat, and our environmental health. The performances of nanobiochemical sensors are excellent in terms of sensitivity, selectivity, linearity, stability, response time, and reproducibility compared to the traditional biosensors. The nanoparticles labeling procedure is very simple, and the biochemical activity of the labeled compound is almost unaffected. This new approach is critically useful in preventing interference between chemically related analytes. Many nanosensors have been developed to detect diseases, and few are glucose (Wang *et al.,* 2009), and choline (Wang and Musamaeh, 2003), nicotinamide adenine dinucleotide (Gopalan *et al.,* 2009), lactate (Parra *et al.,* 2006), triglyceride (Vijayalakshmi *et al.,* 2008), and urea nanosensors (Seo *et al.,* 1993). Nanoparticles have also been developed in bioassays for detection of Human immunoglobulin G (Cui *et al.,* 2001), Steroids (Macara and Lannigan, 2005) etc.

2.5.3. Applications of nanoparticles in food industry

In food industry, to ensure the food safety several novel nanoparticles, in the form of micelles, liposomes, nanoemulsions and biopolymeric nanoparticles were developed (Yih and Al-Fandi, 2006; Nasongkla *et al.,*2006;

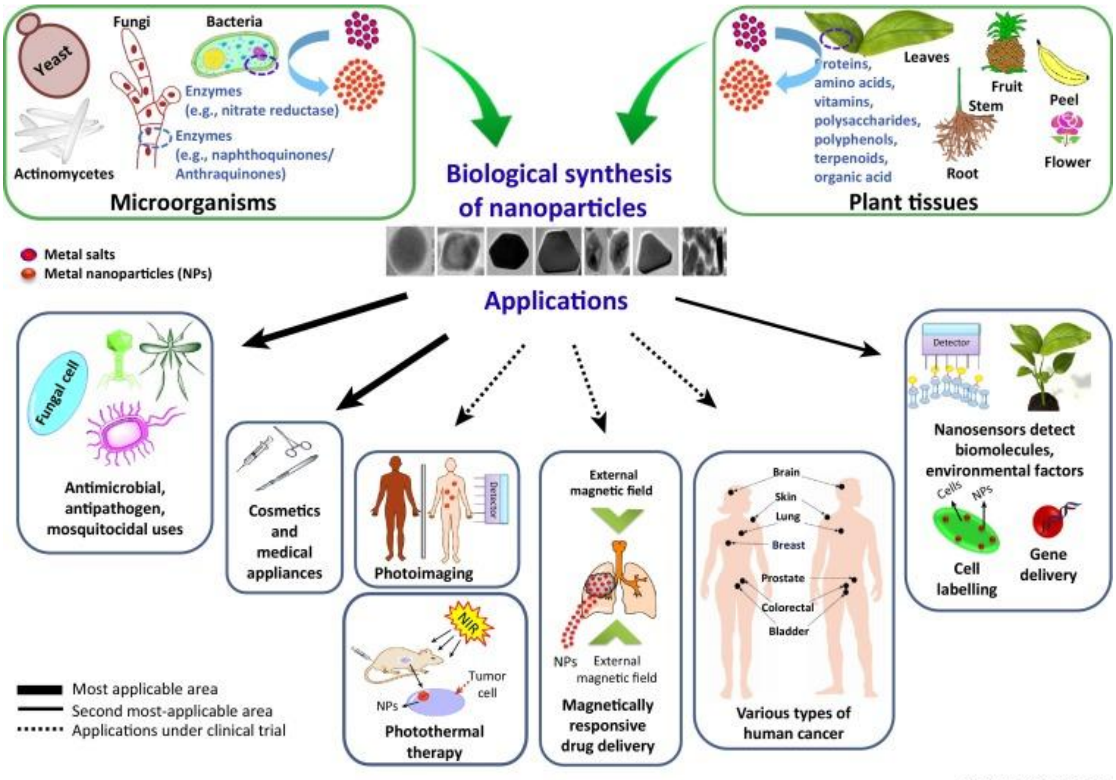
Esposito *et al.,* 2005; Ligler *et al.,* 2003). Some examples of the use of nanotechnology in food products are cooking oils that contain nutraceuticals within nanocapsules, nanoencapsulated flavor enhancers and nanoparticles that have the ability to selectively bind and remove chemicals from food.

2.5.4. Applications of nanoparticles in textile industry

The novel properties and low material consumption amount has attracted global interest across various disciplines and industries. The textile sector a fast-growing industry has health concerns along with customer satisfaction have made functionally finished fabric a fast paced one. It soon became more important for antimicrobial finished fabrics to protect the wearer from bacteria than it was to simply protect the garment from fiber degradation (Yadav *et al.,* 2006)The need for antimicrobial fabrics goes hand in hand with the rise in resistant strains of microorganisms. Functional textiles include everything from antimicrobial finished textiles, to durable, or permanent press finished garments, to textiles with self-cleaning properties, and also textiles with nanotechnology (Rajendran *et al.,* 2010; El-Rafie *et al.,* 2010). Coated antimicrobial sutures have also been developed to aid fast would healing without microbial infection (Dubas *et al.,* 2011).

In waste water treatment basically four classes of nanoscale materials are being evaluated as functional materials for water purification i.e., metal-containing nanoparticles, carbonaceous nanomaterial’s, zeolites and dendrimers. Carbon nanotubes and nanofibers also show some positive result. Nanomaterial’s reveal good result than other techniques and is used in water treatment because of its high surface area (surface/volume ratio) (Tiwari *et al.,* 2008).

Manufacturing and nanocrystalline materials provide very interesting substances for material science since their properties deviate from respective bulk material in a size dependent manner. Manufacture nanoparticles display physicochemical characteristics that induce unique electrical, mechanical, optical and imaging properties that are extremely looked-for in certain applications within the medical, commercial, and ecological sectors (Dong *et al.,* 2014 and Ma, 2003 and Todescato *et al.,* 2016).



Trends in Biotechnology

Fig. 1. Applications of nanoparticles in different fields

2.6. ZINC OXIDE NANOPARTICLES

Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) appears geologically as a white crumbly salt known as “goslarite” (Haidinger, 1845). Zinc sulphide has attracted much attention owing to its wide applications, such as UV light emitting diodes, efficient phosphors in flat-panel displays, and photocatalysis. Zinc sulphide have the capabilities for applications in areas such as non-linear optical devices fast optical switches and they have been studied extensively (Jayalakshmi and Rao, 2006). Fig. 2 depicts the applications of zinc oxide nanoparticles.

Nano structured zinc sulphide such as nanocrystals, nanowires and nanobelts exhibits excellent optical and electronic performances, which differ much from the bulk zinc sulphide material due to the three-dimensional electrons and holes confinement in a small volume. The surface of a nanoparticle is more important than the bulk because nanoparticle have larger surface to volume ratios, surface atoms are bound by weaker forces because of missing neighbours, which leads to high surface reactivity (Behboudnia *et al.,* 2005)

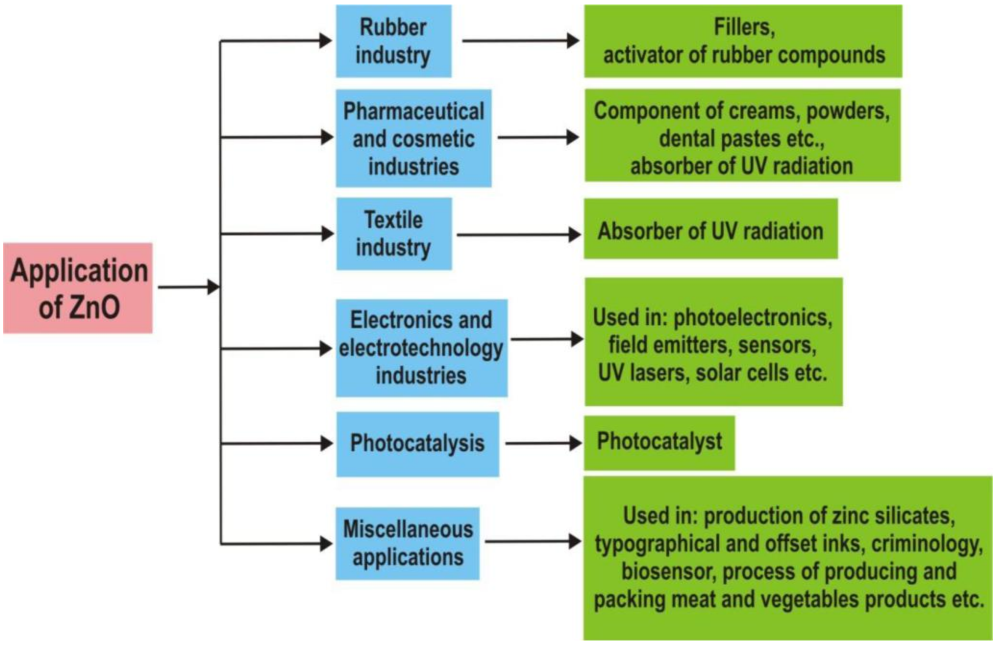


Fig. 2 – Application of zinc oxide nanoparticles

Zinc nanoparticles, nanodots or nanopowder are spherical or faceted high surface area metal particles. Nanoscale zinc particles are typically 20-40 nm with specific surface area in the 30 - 50 m/g range and also available in with an average particle size of 100 nm range with a specific surface area of approximately 7 m/g. Nano zinc Particles are also available in Ultra high purity and high purity and coated and dispersed forms (Herlemann *et al.,* 2017).

2.7. SYNTHESIS AND CHARACTERISATION OF NANOPARTICLE USING PLANT EXTRACTS

The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction. A number of plants are being currently investigated for their role in the synthesis of nanoparticles (Torresday *et al.,* 2002). Fig. 3a and b shows the synthesis and characterization of zinc nanoparticles. Certain plants are known to accumulated higher concentration of metals compared to others and such plants are termed as hyper accumulators. The plants investigated, *Brassica juncea* had better metal accumulating and later assimilating it as nanoparticles (Bali *et al.,* 2006).

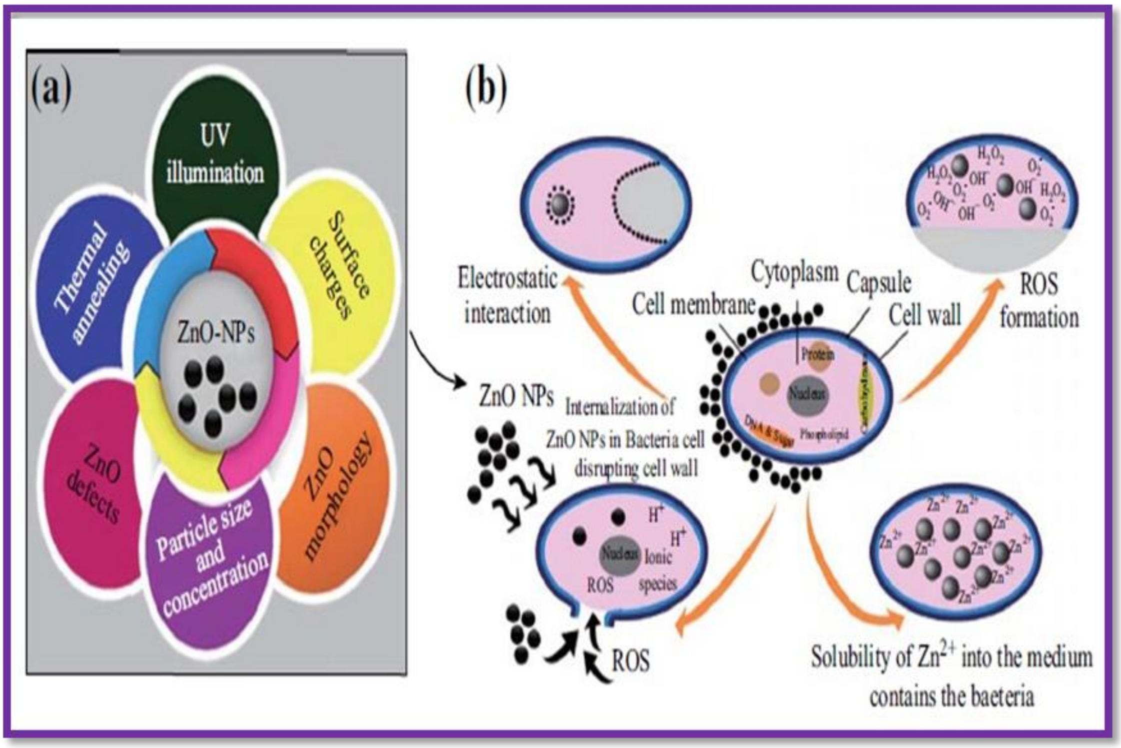


Fig. 3 – Synthesis and characterization of zinc nanoparticles

The synthesis of nanoparticles to have a better control over the particle size, distribution, purity, morphology, quantity and quality by employing as environment friendly economically processes has always been a challenge for the research (Hahn, 1997).

Rajiv *et al* (2013) studied the bio-fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L. and its size-dependent antifungal activity against plant fungal pathogens by inexpensive, ecofriendly and simple method. Highly stable, spherical and hexagonal zinc oxide nanoparticles were synthesized using different concentrations (25% and 50%) of parthenium leaf extracts. Both the concentrations of the leaf extract act as reducing and capping agent for conversion of nanoparticles. Formation of zinc oxide nanoparticles have been confirmed by UV–Vis absorption spectroscopy, X-Ray diffraction (XRD), Fourier Trans-form Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analysis with Energy Dispersive X-Ray Analysis (EDX). The parthenium mediated zinc oxide nanoparticles were explored for the antifungal activity against plant fungal pathogens. Highest zone of inhibition was observed against *Aspergillus flavus* and *Aspergillus niger*, which proved it to be a good antifungal agent.

Ramesh *et al* (2014) studied the green synthesis of zinc oxide nanoparticles using *Cassia auriculata* by mixing 1mM zinc acetate with aqueous extract of flower. The formation of nanoparticles was monitored by visualizing color changes and it was confirmed by Scanning Electron microscope, UV-Vis spectrophotometer and Fourier Transform Infra-Red spectroscopy.

Sivaraj *et al* (2014) investigated the biogenic zinc oxide nanoparticles synthesis using *Tabernaemontana divaricate* leaf extract and its anticancer activity against MCF-7 breast cancer cell lines. Highly stable spherical zinc oxide nanoparticles were synthesized using 50% concentration of the selected *trbernaemontana* leaf extract. Formation of zinc oxide nanoparticles have been characterized by X-ray diffraction (XRD), Raman spectroscopy and Transmission Electron Microscopy (TEM) analysis. *Trabernaemontana* mediated zinc oxide nanoparticles showed effective cytotoxic effect against MCF-7 breast cancer cell lines by MTT assay. The study supports the benefit of using biological method for synthesizing zinc oxide nanoparticles for anticancer activities.

Elumalai *et al* (2015) analysed the green synthesis of zinc oxide nanoparticles using *Moringa oleifera* leaf extract and evaluated its antimicrobial activity. The synthesized ZnO nanoparticles were characterized using various techniques such as UV–Vis absorption spectroscopy, X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), energy dispersive X-ray analysis (EDX), Fourier transform infrared spectroscopy (FT-IR) and photoluminescence spectroscopy (PL). XRD analysis revealed the wurtzite hexagonal structure of ZnO nanoprticles. FT-IR confirmed the presence of functional groups of both leaf extract and ZnO nanoparticles. The particles size, morphology and topography was determined from FE-SEM. The intense and narrow width of zinc and oxygen have high purity and crystalline nature which was identified using EDX. UV–Vis absorption showed the characteristic absorption peak of ZnO nanoparticles. The results of antimicrobial activities revealed that maximum zone of inhibition against Gram positive bacteria, followed by Gram negative bacteria.

Shah *et al* (2015) studied the synthesis of zinc oxide nanoparticles using the aqueous extract of green tea *Camellia sinensis* leaves and evaluated for antimicrobial efficacy against the selected microbes. The synthesis of zinc nanoparticles were characterized by UV-Vis spectroscopy, particle size analyser and Scanning Electron Microscopy. The synthesized zinc nanoparticles showed significant antimicrobial activity against Gram positive and Gram negative bacteria as well as against the fungal isolates. Hence the green tea leaf extracts can be effectively used for synthesizing zinc nanoparticles, and can be used as an alternative to existing antimicrobial agents.

Devasenan *et al* (2016) discussed the green synthesis and characterization of zinc nanoparticle using *Andrographis paniculata* leaf extract. The structural characterization of synthesized nanoparticles was carried using XRD, EDX and SEM. The optical characterization was carried out using UV – Vis and FT – IR analysis. The results showed that the leaf extract is optimum for the synthesis of zinc nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The synthesized zinc nanoparticles can be used for various applications due to its eco – friendly, non-toxic and compatibility for pharmaceutical and other applications.

Yedurkar *et al* (2016) reported the green synthesis of zinc oxide nanoparticles using *Ixora coccinea* leaf extract. Formation of zinc oxide nanoparticles has been confirmed by UV-Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), Dynamic light scattering analysis (DLS), zeta potential study and Scanning Electron Microscope with the Energy Dispersive X-ray studies (EDX). Dynamic light scattering analysis shows average particle size of 145.1 nm whereas high zeta potential value confirms the stability of formed zinc oxide nanoparticles. The Scanning Electron Microscope reveals the spherical morphology of nanoparticles and Energy Dispersive X-ray analysis confirms the formation of highly pure zinc oxide nanoparticles. The zinc oxide nanoparticles from *Ixora* *coccinea* leaves are expected to have applications in biomedical, cosmetic industries, biotechnology, sensors, medical, catalysis, optical device, coatings, drug delivery and water remediation and also may be applied for electronic and magneto-electric devices. This new eco-friendly approach of synthesis is a novel, cheap, and convenient technique suitable for large scale commercial production.

Chaudhuri and Malodia (2017) biosynthesized the zinc oxide nanoparticles using leaf extract of *Calotropis gigantea.* The combination of 200mM zinc acetate and 15 ml of leaf extract was ideal for the synthesis of less than 20 nm size of highly monodisperse crystalline nanoparticles. Synthesized nanoparticles were characterized through UV–Vis spectroscopy, dynamic light scattering, X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy, Energy Dispersive X-ray, and Atomic Force Microscopy. The UV–Vis absorption maxima showed peak near 350 nm, which is characteristic of ZnO nanoparticles. DLS data showed a single peak at 11 nm (100%). XRD analysis showed that these are highly crystalline ZnO nanoparticles having an average size of 10 nm. FT-IR spectra recorded to identify the biomolecules involved in the synthesis process. SEM images showed that the particles were spherical in nature. The presence of zinc and was confirmed by EDX 2D and 3D images of ZnO nanoparticles were obtained by AFM studies, which indicated the monodisperse nature. Significant enhancement of growth was observed in Neem *(Azadirachta indica)*, Karanj *(Pongamia pinnata)*, and Milkwood-pine *(Alstonia scholaris)* seedlings in foliar spraying ZnO nanoparticles to nursery stage of tree seedlings.

Suganya and Mahalingam (2017) attempted on the green synthesis and characterization of zinc sulphide nanoparticle from edilble *Mushroom*. The biosynthesis of zinc nanoparticles was made from the aqueous extract of edible mushroom, *Pleurotus florida* and authenticated using UV- Vis spectroscopic analysis. The pure form of zinc nanoparticles was sequentially subjected for FT-IR, XRD, SEM, EDX and TEM analysis for nanostructural characterization. In order to evaluate the biopotential properties, different concentrations of zinc sulphide was used to assess the seed germination of green gram and the study reveals that zinc nanoparticles positively influence the germination of green gram.

Kumar *et al* (2017) biosynthesized of zinc sulphide nanoparticles using plant extract of *Phyllanthus niruri* and assessed their antimicrobial activity. Biosynthesized zinc nanoparticles enhibited crystalline structure formation which was confirmed by using X-ray diffraction, scanning electron microscopy potrays the surface morphology of zinc nanoparticles, FT-IR reveals the different functional group of biosynthesized zinc nanoparticles and optical absorbance were evaluated by UV-Visible spectrophotometer. The antimicrobial activity of biosynthesizedzinc nanoparticles using *Phyllanthus niruri* methanol plant extract were determined by disc diffusion method against various pathogenic microorganisms.

Suresh *et al* (2018) synthesized and characterized the zinc oxide nanoparticles using insulin plant *Costus pictus* and investigated its antimicrobial as well as anticancer activities. FT-IR studies confirm the presence of biomolecules and XRD reveals the formation of pure hexagonal phase structures of ZnO nanoparticles. The surface morphologies of ZnO nanoparticles observed under the scanning electron microscope (SEM) suggested that most ZnO crystallites are hexagonal. EDX analysis confirms the presence of primarily zinc and oxygen. The biosynthesized zinc oxide nanoparticles exhibited strong antimicrobial behavior against the selected bacterial and fungal species and also anticancer activity against Daltons lymphoma ascites (DLA) cells.

2.8. PHOTOCATALYTIC ACTIVITY OF NANOPARTICLES AGAINST TEXTILE DYES

Textile attention of the readers may be drawn to the fact that with rapid growth of textile industries across the globe, use of organic non-biodegradable dyes is continuously increasing, and considered to be major causes of concern due to their impact on human health of metal nanoparticles are known to promote degradation of dyes. The catalytic efficiency of zinc nanoparticles was investigated under solar light irradiation for the degradation of organic dyes. The synthesized nanoparticles showed pronounced photocatalytic activity in degradation of organic dyes which indicates the adsorption of reactants on catalysts for efficient reduction.

Shahwan *et al* (2011) utilized the green synthesis of iron nanoparticles and their application as a fenton like catalyst for the degradation of aqueous cationic and anionic dyes. The iron nanoparticles for decolorization of aqueous solutions containing methylene blue (MB) and methyl orange(MO)dyes. The related experiments investigated the removal kinetics and the effect of concentration for both MB and MO. The concentrations of dyes in aqueous solution were monitored using ultraviolet visible (UV -vis) spectroscopy. The results indicated fast removal of the dyes with the kinetic data of MB following a second order removal rate, while those of MO were closer to a first order removal rate. The loading experiments indicated almost complete removal of both dyes from water over a wide range of concentration, 10-200 mg L^{-1}.

Kodihalli *et al* (2012) synthesized the electrochemical and photocatalytic property of zinc oxide nanoparticles. The band gaps for synthesized zinc oxide nanoparticles were 3.07, 3.12 and 3.13 eV, respectively, based on the results of diffuse reflectance spectra (DRS). The electrochemically synthesized zinc oxide powder was used as photocatalysts for UV-induced degradation of Methylene blue (MB). Photodegradation was also found to be function of exposure time and dye solution pH. It has been found that as-synthesized powder has excellent photocatalytic activity with 92% degradation of MB, indicating ZnO nanoparticles can play an important role as a semiconductor photocatalyst.

Ayodhya *et al* (2013) synthesized, characterization of ZnS nanoparticles by Coprecipitation method using various capping agents - Photocatalytic activity and Kinetic study. ZnS nanoparticles are prepared by coprecipitation method using various capping agents like PVP (polyvinyl pyrrolidine), PVA (polyvinyl alcohol) and PEG-4000 (polyethylene glycol). The photo catalytic degradation of xylene orange (XO) by the nanoparticles shows that these act as photo catalysts under sunlight irradiation.

Bandekar *et al* (2014) reported the Synthesis, characterization and photocatalytic activity of PVP stabilized ZnO and modified ZnO nanostructures. Sunlight driven photocatalytic degradation of methylene blue (MB) was studied for ZnO nanostructures synthesized by hydrothermal, nonchemical and precipitation methods using polyvinyl pyrrolidine (PVP) as the capping agent. The ZnO nanostructures were further decorated with Ag nanoparticles to enhance its dye degradation efficiency. The Ag decorated ZnO nanoparticles exhibited a higher degradation rate as compared to pure ZnO nanoparticles which was independent of pH.

Arshadi *et al* (2015) studied the preparation of iron nanoparticles loaded *Spondias purpurea* seed waste as an excellent adsorbent for removal of phosphate from synthetic and natural waters. The effects of various parameters, such as contact time, pH, concentration, reusability and temperature were studied. The adsorption of phosphate ions has been studied in terms of pseudo-first- and -second-order kinetics, and the Freundlich, and Langmuir isotherms models have also been used to the equilibrium adsorption data. The selected adsorbent removed phosphate from the natural water significantly.

Paul *et al* (2015) studied the green synthesis of silver nanoparticles using dried biomass of *Diplazium esculentum* (retz) sw. and their photocatalytic and anticoagulative activities. The biosynthesized silver nanoparticles were studied as catalyst in degradation of methylene blue (MB) and rhodamine B (RhB) dyes under solar light illumination. The ability of silver nanoparticles to inhibit coagulation of human blood plasma was also investigated.

Kumar *et al* (2017) reported the biosynthesis of tin oxide nanoparticles using *Psidium guajava* leaf extract photocatalytic dye degradation under sunlight. The photocatalytic activity of the nanoparticles was analysed for the photo degradation of reactive yellow 186 dye under sunlight. SnO_2 nanoparticles within size range 8-10 nm effectively degraded 90% of the dye within 180 min at a rate constant of $0.00476 min^{-1}$.

Quan *et al* (2018) reported the decolouration and degradation efficiency acid orange 7 under different pH value, temperature and dosage conditions with suitable conditions the removal rate of AO7 reached about 98.4% after 2h. The degradation intermediate products were investigated using HPLC-MS, and the potential degradation of AO7 was determined.

Materials and Methods

3. MATERIALS AND METHODS

The methodology adopted for the present study *"Biosynthesis of zinc nanoparticles using Moringa oleifera Lam. seed extract"* was carried out under following headings:

3.1. Collection of the sample
3.2. Preparation of the seed extract
3.3. Biosynthesis of zinc oxide nanoparticles
3.4. Characterization of zinc oxide nanoparticles
    3.4.1. UV-Vis Spectroscopy
    3.4.2. Fourier Transform Infrared Spectroscopy (FT-IR)
    3.4.3. Scanning Electron microscopy (SEM) and Energy Dispersive X- Ray Analysis Spectroscopy (EDX)
    3.4.4. X-Ray Diffractometry (XRD)
    3.4.5. Particle size determination
3.5. Phytochemical analysis of biosynthesized zinc oxide nanoparticles
3.6. Antimicrobial activity of biosynthesized zinc oxide nanoparticles
3.7. Photocatalytic activity
3.8. Phytotoxicity study

3.1. COLLECTION AND IDENDIFICATION OF SEED MATERIALS

Fresh pods of *Moringa oleifera* was collected during the month of December from Krishnagiri, Tamil Nadu, India (Plate 1- a,b). The seeds were separated from the pods and were shade dried for 10 days at room temperature and powdered using mortar and pestle (Plate 1- c,d). The air-dried sample was stored at 4°C for further study. The sample was authentically identified at Botanical Survey of India Tamil Nadu Agricultural University, Coimbatore (Appendix 1).

3.2. PREPARATION OF THE SEED EXTRACT

The powdered seed of *Moringa oleifera* (5g) was dissolved in 150ml of double distilled water and the mixture was boiled at 100°C for 10 minutes, and cooled at room temperature. The extract was filtered through Whatman Filter paper No. 1 and the filtrate was stored at 4°C for further experiment.

3.3. BIOSYNTHESIS OF ZINC OXIDE NANOPARTICLES

The aqueous solution of zinc sulfate was prepared in different concentrations (1mM – 5mM) and used for the synthesis of zinc oxide nanoparticles. The *Moringa oleifera* seed extract was added to each concentration of zinc sulfate at varying volume (1 – 5ml) and kept at sunlight for different time intervals (0 – 45 minutes), to observe the pale white colour indicating the synthesis of nanoparticles.

The biosynthesized zinc oxide nanoparticles were centrifuged at 1000rpm for 5 minutes to obtain the white precipitate which served as zinc oxide nanoparticles. The obtained zinc oxide nanoparticles (500mg/100ml) was dissolved in 100ml of double distilled water and stored at 4^{o}C for further analysis. The precipitate was collected to separate tube for using further throughout the experiment. The schematic representation of zinc oxide nanoparticles synthesis using *Moringa oleifera* seed extract is given Fig. 4.

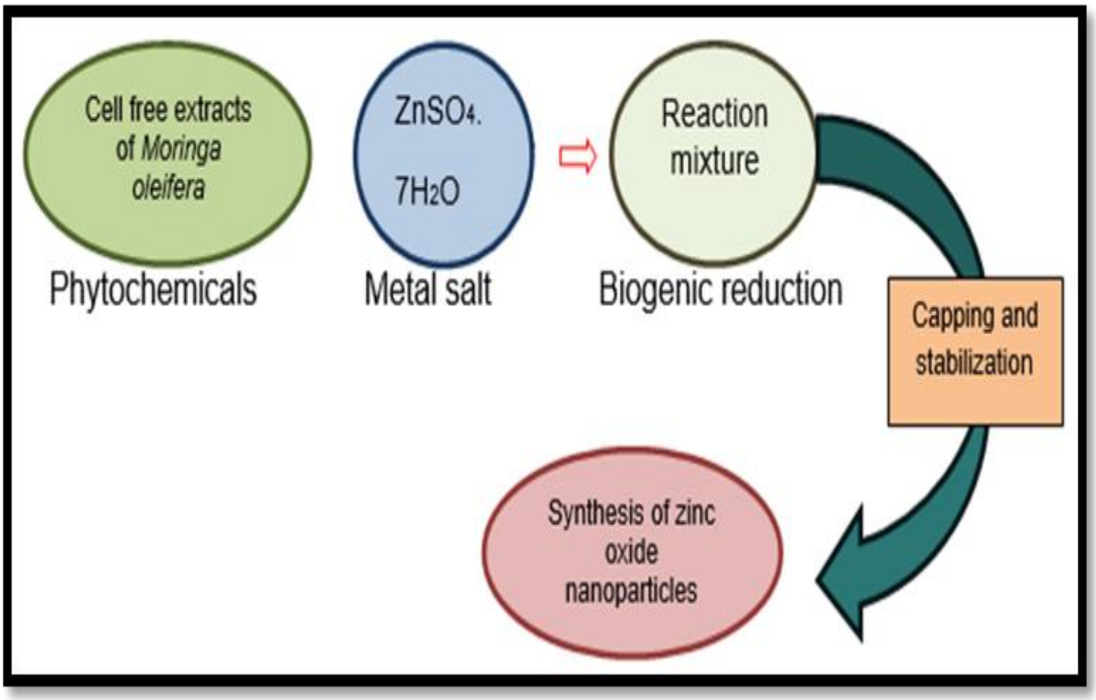
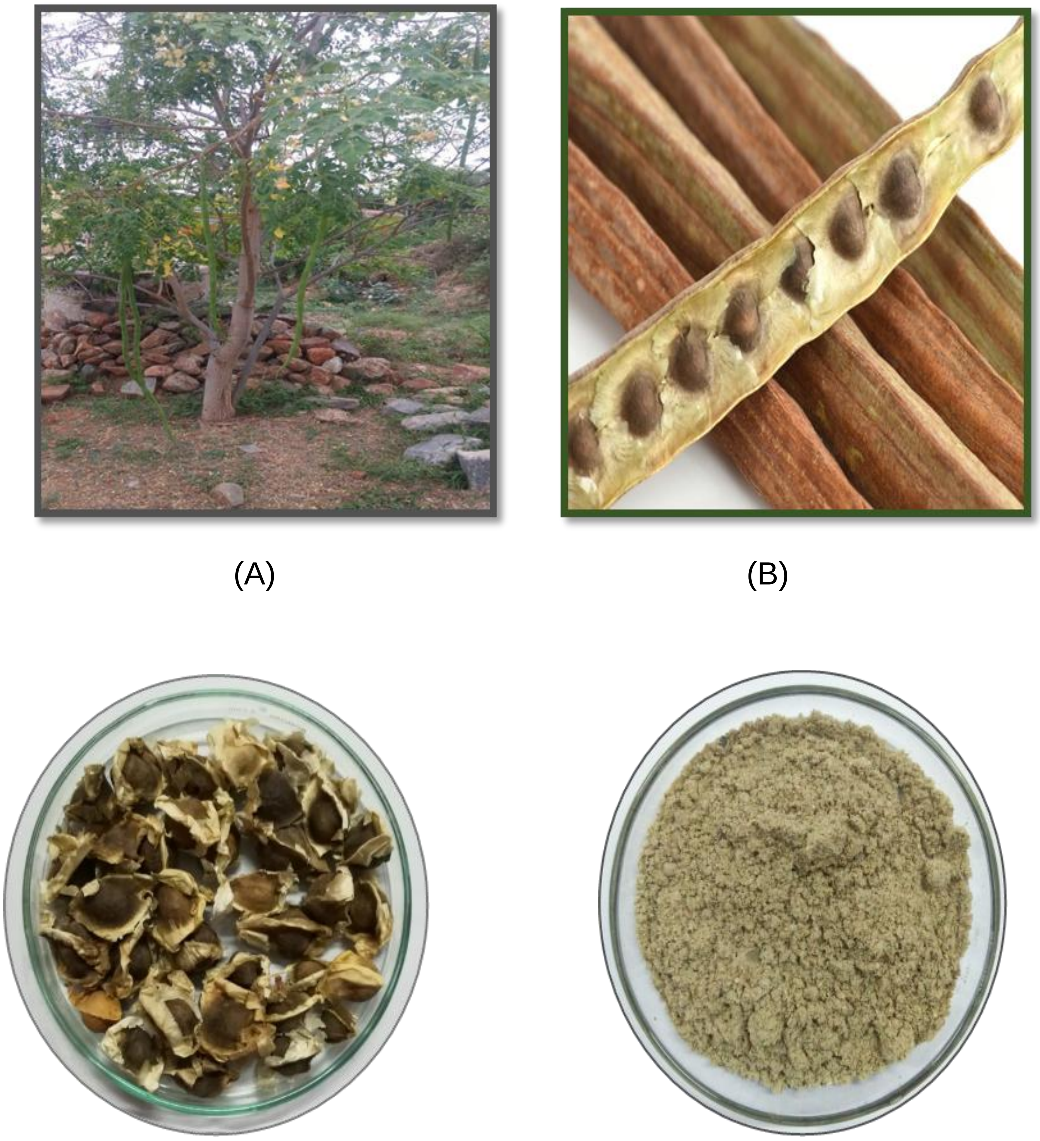


Fig. 4- Schematic representation of zinc oxide nanoparticles

PLATE. 1

MORINGA OLEIFERA – THE CANDIDATE TREE



(A)

(B)

(C)

(D)

a) *M. oleifera* tree, b) Pods of *M. oleifera*,

c) *M. oleifera* seeds and d) Powder

3.4. CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES

3.4.1. UV-Vis spectroscopy

The biosynthesized zinc oxide nanoparticles was measured for its maximum absorbance using UV–Vis using ultraviolet and visible absorption spectroscopy in the range of 200–800 nm.

3.4.2. Fourier transform infrared spectroscopy (FT-IR)

FT-IR measurement was carried out to identify the possible biomolecules responsible for the reduction and stabilization of nanoparticles using KBr pellet method in FT-IR Spectrophotometer. The FT-IR spectrum for the dried and powdered zinc oxide nanoparticles was recorded in the wavelength interval of 4000 to 800 cm^{-1} with the resolution of $4cm^{-1}$ by Perkin Elmer Spectrum.

3.4.3. Scanning electron microscopy (SEM) and EDX analysis

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

3.4.4. X-Ray diffraction (XRD) analysis

To identify the composition phase of the synthesized nanoparticles, X-Ray diffraction (XRD) analysis was performed using shimadzu 6000 with Cu-Kα radiation source at the wavelength of $1.5406A^{o}$, operated at 40KV / 30mA over 2θ range of $20 - 80^{o}$ and maintained at a scan speed of $50min^{-1}$.

3.4.5. Particle size analysis

The particle size range of the synthesized zinc oxide nanoparticles was determined using a particle size analyzer. Particle size was determined based on the Brownian motion of the scattering of laser light by the nanoparticles.

3.5. PHYTOCHEMIC ALANALYSIS OF BIOSYNTHESIZED ZINCOXIDE NANOPARTICLES

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

IDENTIFICATION OF FLAVONOIDS

Shinoda test

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

Ammonia test

Filter paper strips were dipped in the biosynthesized nanoparticles and ammoniated change in the filter paper colour to yellow indicated the presence of flavonoids. About 10ml of concentrated H_2SO_4 was added to the yellow coloured filter paper. Disappearance of yellow colour confirms the presence of flavonoids.

IDENTIFICATION OF ALKALOIDS

Dragondroff's test

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

Hager's test

To 1ml of the zinc oxide nanoparticles 1ml of Hager's reagents was added. Formation of orange precipitate indicate the presence of alkaloids.

IDENTIFICATION OF STEROIDS

Libermann - Buchard test

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

Salkowski test

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

IDENTIFICATION OF POLYPHENOLS

Ferric chloride test

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

IDENTIFICATION OF GLYCOSIDES

Legal test

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

IDENTIFICATION OF TANNINS

Braemer’s test

To 5ml of the synthesized zinc oxide nanoparticles10ml of distilled water was added, boiled and filtered. To the filtrate few drops of 10% ferric chloride was added. Appearance of green, blue, brown colour indicates the presence of tannins.

IDENTIFICATION OF SAPONINS

Sodium bicarbonate test

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

IDENTIFICATION OF CARBOHYDRATES

Benedict’s test

To 0.5ml of the synthesized zinc nanoparticles 2ml of benedict’s solution was added. Formation of reddish brown precipitate indicates the presence of carbohydrates.

IDENTIFICATION OF PROTEINS AND AMINOACIDS

Biuret test

The synthesized zinc nanoparticles was treated with equal volume of 40% sodium hydroxide and two drops of 1% copper sulphate solution. Pink or purple colour indicates the presence of proteins.

3.6. ANTIMICROBIAL ACTIVITY OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES

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

3.7. PHOTOCATALYTIC EXPERIMENTS

The photocatalytic activity of zinc nanoparticles was evaluated by assessing the decolorization of crystal violet dye solution. Various concentrations of crystal violet dye (1-5mg) was taken in a 100ml conical flask separately. To this 1ml (5mg) of biosynthesized zinc nanoparticles was added and kept for 10 minutes to enhance the physical absorption of dye molecules. The experimental setup was placed in sunlight under constant stirring. The samples (5mL) were collected at regular intervals from the reaction mixture and centrifuged to remove the suspended particles and the absorbance was measured spectrophotometrically at 590nm. The percentage dye removal was calculated using the formula,

$$\text{Dye removal (\%)} = \frac{\text{Initial absorbance of dye - Final absorbance dye}}{\text{Initial absorbance of dye}}$$

3.8. PHYTOTOXICITY STUDY

Zinc is used as a source of micronutrient to plants by farmers, since it can be easily absorbed by plants. The seedling growth and its vigour index was assessed under invitro conditions using green gram seeds. The green gram seeds were washed thoroughly using double distilled water to remove the impurities present in it. The green gram seeds were soaked in 20ml of distilled which served as water control (T_1), zinc oxide nanoparticles(T_2), *Moringa oleifera* seed extract (T_3) and zinc sulfate solution (T_4) separately for 5-6 hours. The treated green gram seeds were then transferred to petri plates lined with a thin layer of cotton. Each petri plates were then watered with 5ml of the test samples regularly and kept in dark place for 7 days. The experiment was conducted in triplicates. After 7 days of incubation, the biometric parameters such as seed germination, shoot length, root length and vigour index was recorded. The protrusion of radial through seed coat was taken as the criterion for germination.

The seed germination was calculated using the formula, Germination percentage = Number of seeds germinated / Number of seeds sown x 100

The maximum length of each shoot and root were recorded in centimetre and vigour index is calculated as follows,

Vigour index = Germination percentage x (seed length + root length) (Abdul Baki and Anderson, 1973).

Results and Discussion

4. RESULTS AND DISCUSSION

The results of the present study entitled "*Biosynthesis of zinc nanoparticles using Moringa oleifera Lam. seed extract"* is discussed under the following headings.

4.1. Visual observation of biosynthesized zinc nanoparticles

4.2. Characterization of biosynthesis of zinc oxide nanoparticles

- 4.2.1. UV-Visible spectroscopy
- 4.2.2. Fourier Transform Infra-Red Spectroscopy (FT-IR)analysis
- 4.2.3. Scanning electron microscopy (SEM) and EDX analysis
- 4.2.4. X-ray diffraction (XRD) analysis
- 4.2.5. Particle size

4.3. Phytochemical analysis of biosynthesized zinc oxide nanoparticles

4.4. Antimicrobial activity of biosynthesized zinc oxide nanoparticles

- 4.4.1. Antibacterial activity of biosynthesized ZnO nanoparticles
- 4.4.2. Antifungal activity of biosynthesized ZnO nanoparticles

4.5. Photocatalytic activity of biosynthesized ZnO nanoparticles

4.6. Phytotoxicity activity biosynthesized ZnO nanoparticles

4.1. Visual observation of synthesized zinc nanoparticles

Increasing concentration of zinc sulfate (1mM-5mM) and seed extract of *Moringa oleifera* (1ml-5ml) were used to optimize the synthesis of zinc nanoparticles at different incubation period (0-45 minutes). A pale white precipitate was obtained with 1ml of 2mM zinc sulfate incubated with 3ml of *Moringa oleifera* seed extract within 15 minutes which clearly indicates the formation of zinc nanoparticles (Plate 2 and 3). The pale white pellet was collected and dried in hot air oven to yield zinc oxide nanoparticles, and subjected to spectral analysis.

PLATE 2

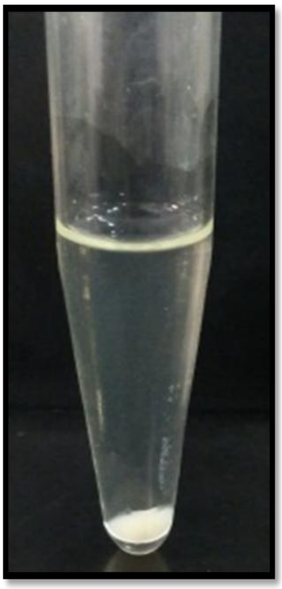
BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES AT OPTIMIZED CONDITIONS

(A) (B) (C)

(A) Zinc sulfate solution ($ZnSO_4$), (B) Moringa olifera seed extract
(C) Zinc nanoparticles

PLATE 3

PELLET FORMATION OF ZINC OXIDE NANOPARTICLES



4.2. characterization of biosynthesis of zinc oxide nanoparticles

4.2.1. UV-Visible spectroscopy

The formation of pale white precipitate was due to the surface plasma resonance and reduction of zinc ions by seed extract of *Moringa oleifera*. The frequency and the width of surface plasmon absorption depends on the shape and size of metal nanoparticles and also on the dielectric of the metal and the surrounding medium (Rai et al., 2006).

The pellet obtained was dispersed in water to record the absorption spectra using UV-Vis spectrophotometer at 200 to 800 nm. UV-vis spectroscopy helps in analysing the formation of metal nanoparticles in aqueous solution. UV-Vis absorption spectrum revealed that the synthesized zinc nanoparticles showed an absorption peak at 223.5 nm (Fig. 5)

Thus, the result strongly suggests that *Moringa oleifera* seed extract could be a better source for the biosynthesis of zinc nanoparticles.

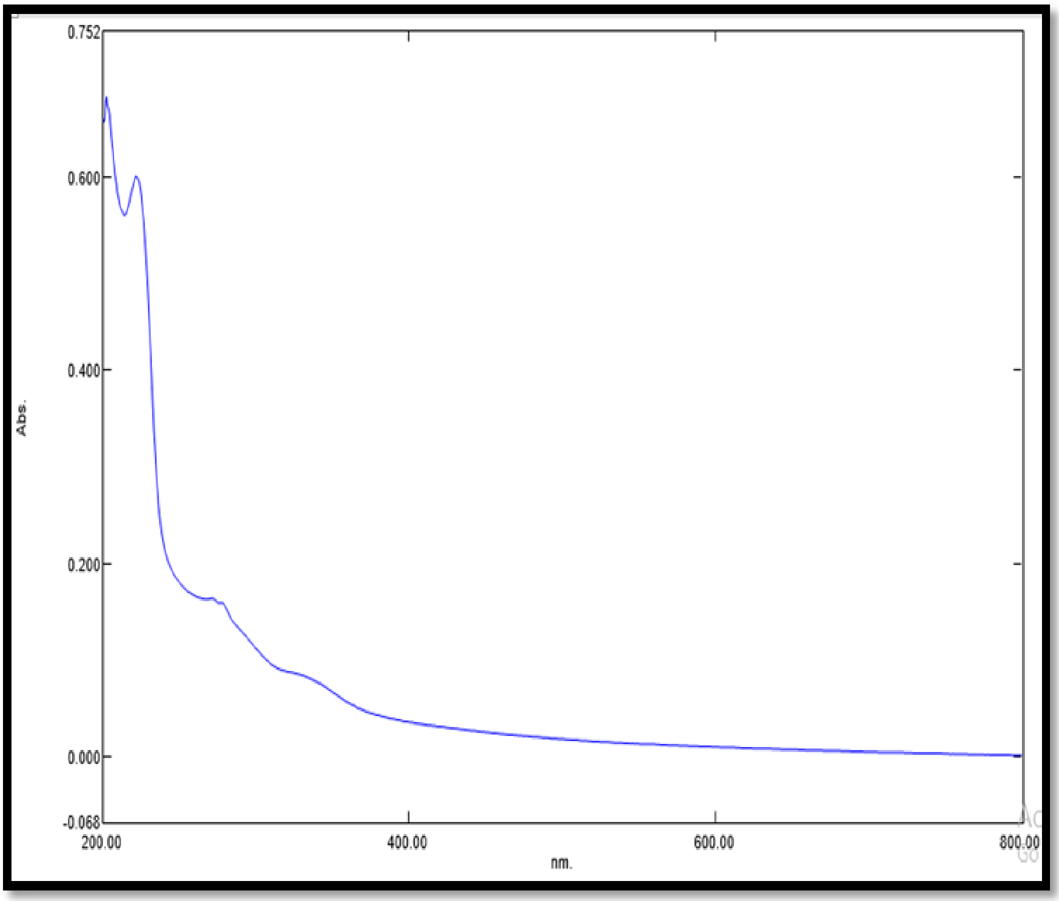


Fig. 5 - UV-Vis spectra of biosynthesized ZnO nanoparticles.

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

Fourier Transform Infra-Red Spectroscopy (FT-IR) analysis helps to identify the biomolecules in the seed extracts which plays the role in the reduction and stabilization of green synthesis of nanoparticles (Senthilkumar and Sivakumar, 2014).

The FT-IR Spectra of the zinc oxide nanoparticles were also recorded to identify the functional groups of the extract involved in the reduction of synthesis of zinc oxide nanoparticles. The FT-IR spectra of the synthesized zinc oxide nanoparticles was shown in Fig 6. The peaks at 3398.57, 2364.73, 2160.27, 1643.35, 1543.05, 1388.75, 1323.17, 1122.57, 1049.28 cm^{-1} are prominent in the FT-IR Spectrum. The peak at 3398 cm^{-1} was assigned to O-H and N-H stretching, while the peak at 1543 cm^{-1} and 1388.75 cm^{-1} may be ascribed to O-H and -C-O stretching modes. The band which appeared at 2364.73 and 2160.27 cm^{-1} might be due to C=C stretching. The peak at 1643.35cm^{-1} corresponds to C=O stretching in carbonyl groups, while the peak at 1049.28 cm^{-1} was assigned to the C=O stretch of the alcoholic group. The FT-IR studies have confirmed the fact that the amide group form proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e.,capping of zinc nanoparticles) to prevent agglomeration and thereby stabilize the medium.

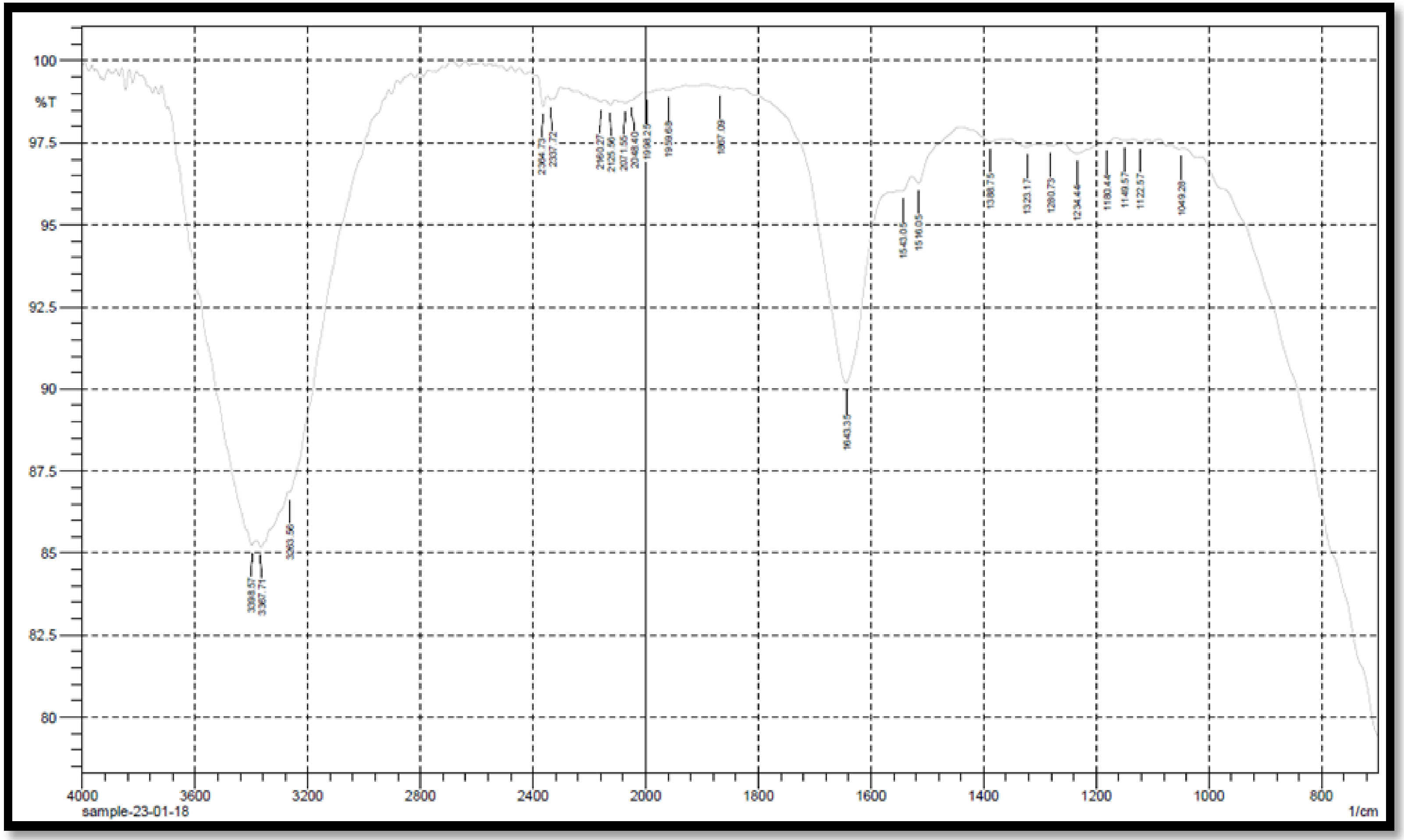


Fig. 6 - FT-IR absorption spectrum of synthesized zinc oxide nanoparticles

Singh and Gopal, (2012) reported that the biomolecules might act as stabilizing or growth terminator of zinc oxide nanoparticles as well as they might act as a linker molecule between two or more zinc oxide nanoparticles in making self-assembly.

Thus, the FTIR spectra indicate the seed extract mediated zinc oxide nanoparticles sample is rich in polyphenols, carboxylic acid, amino acids and proteins. The capping and stabilization of zinc oxide nanoparticles may be due coordination with OH, -NH, C=O, C=N. It may conclude that the presence of higher percentage of phenolic group molecules is responsible for the reduction process and the amino acids and amide linkages in protein are responsible for the stabilization of the zinc oxide nanoparticles (Awwad *et al.,* 2014). From the results of FT-IR studies, it has been clear that the functional groups of these diverse metabolites have reacted with metal ions and reduced their size into range. Moreover, they have also capped around the synthesized nanoparticles, thus providing stability as well as biocompatibility.

4.2.3. Scanning electron microscopy SEM and EDX analysis

The morphology of the nanostructures was studied using scanning electron microscopy (SEM). Fig. 7 represents the SEM image of the obtained zinc oxide nanoparticles. The synthesized zinc oxide nanoparticles showed individual zinc particles as well as a number of aggregates. The zinc oxide nanoparticles were also found to be spongy in nature. To gain further insight into the features of zinc oxide nanoparticles, the analysis of the sample was performed using EDX techniques. The energy dispersive spectra of the samples obtained from the SEM-EDX analysis shows that the sample prepared by the above route has pure zinc oxide phases (Kumar *et al.,* 2013). The EDX studies of Fig. 8 present a peak between 2KV and 3KV, which are directly related to zinc in the tested material. Thus, the results indicated that the reaction product was composed of high purity zinc oxide nanoparticles, which was also confirmed in X-Ray diffraction studies.

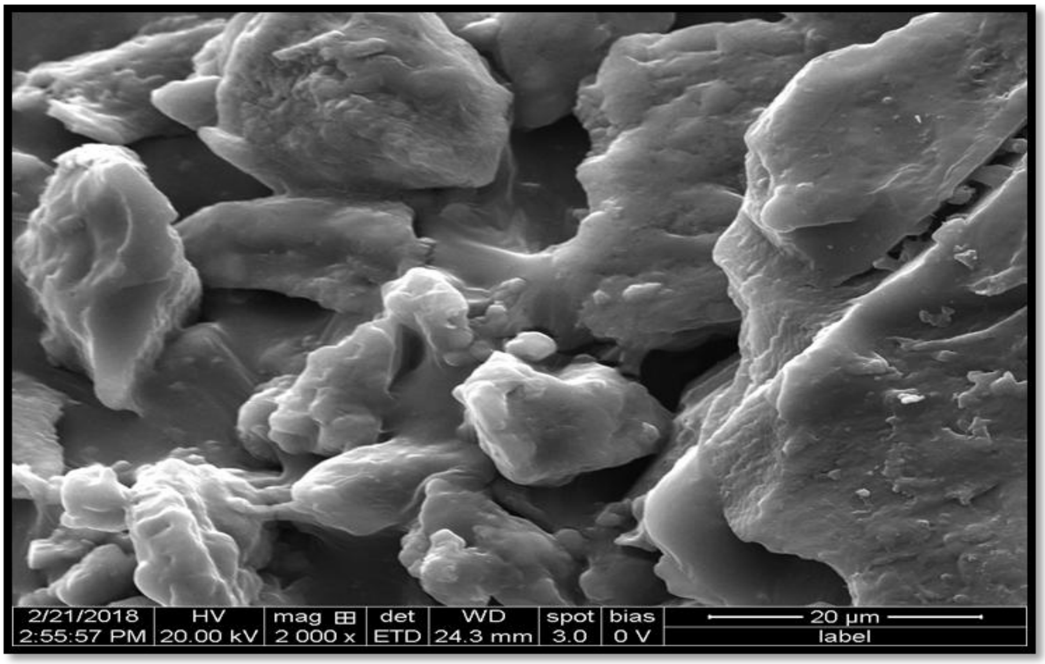
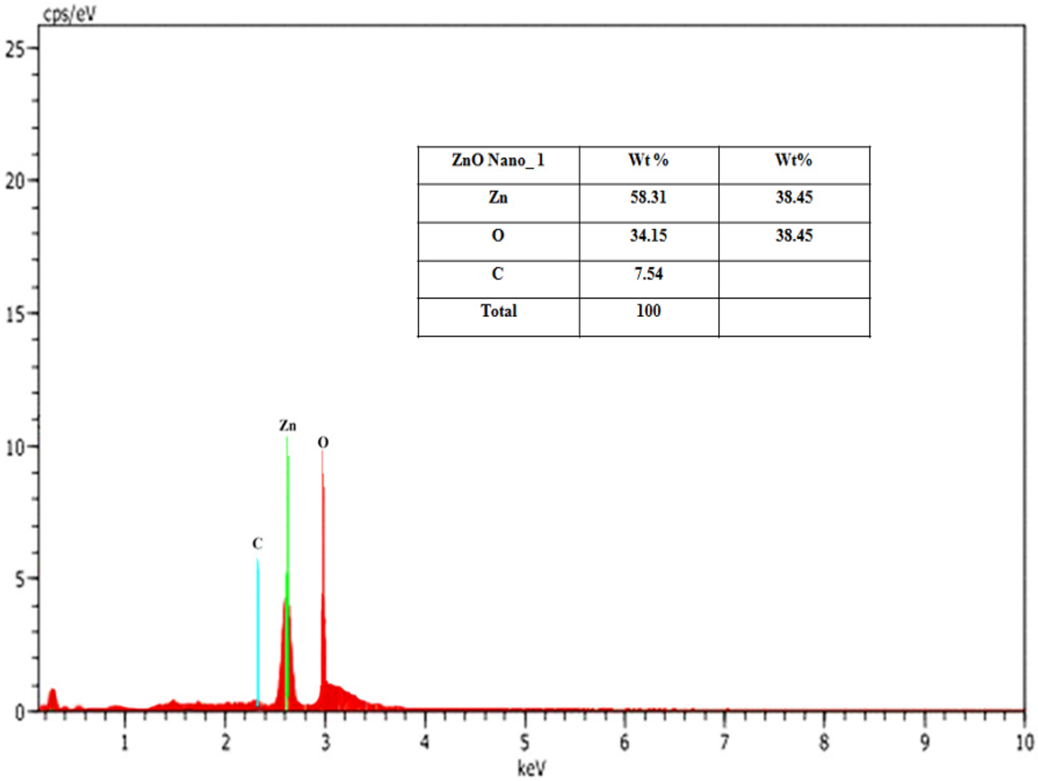


Fig. 7 – SEM image of biosynthesized zinc nanoparticles



ZnO Nano_1	Wt %	Wt%
Zn	58.31	38.45
O	34.15	38.45
C	7.54	
Total	100	

Fig. 8 - Energy dispersive X-ray (EDX) spectrum of synthesized zinc nanoparticles

4.2.4. X-ray diffraction (XRD) analysis

The XRD pattern of synthesized zinc nanoparticles was depicted in Fig. 9. Five main characterization diffraction peaks for zinc were observed at 2θ values of 31.5^o, 39.2^o, 47.4^o, 56.5^o and 67.8^o which are indexed to the 100,002,102,100,200 reflections of face centred cubic (fcc) structure of zinc. The narrow and strong diffraction peaks indicate the crystalline nature of zinc oxide nanoparticles.

Similar such result was observed by Jamdagni *et al.* (2016) who confirmed the crystalline nature of zinc oxide nanoparticles by using the flower extract of *Nyctanthes arbor-tristis*

4.2.5. Particle size

Particle size and distribution are the important characterisation of zinc nanoparticles which are determined as saturation solubility dissolution velocity, physical stability or even biological performance (Khodaovaskaya *et al.,* 2012). Fig.10 shows the Dynamic light scattering (DLS) pattern of the suspension of the zinc nanoparticles synthesized using *Moringa oleifera* seed extract. The size distribution histogram of DLS indicates that the particles obtained was 15.3nm.

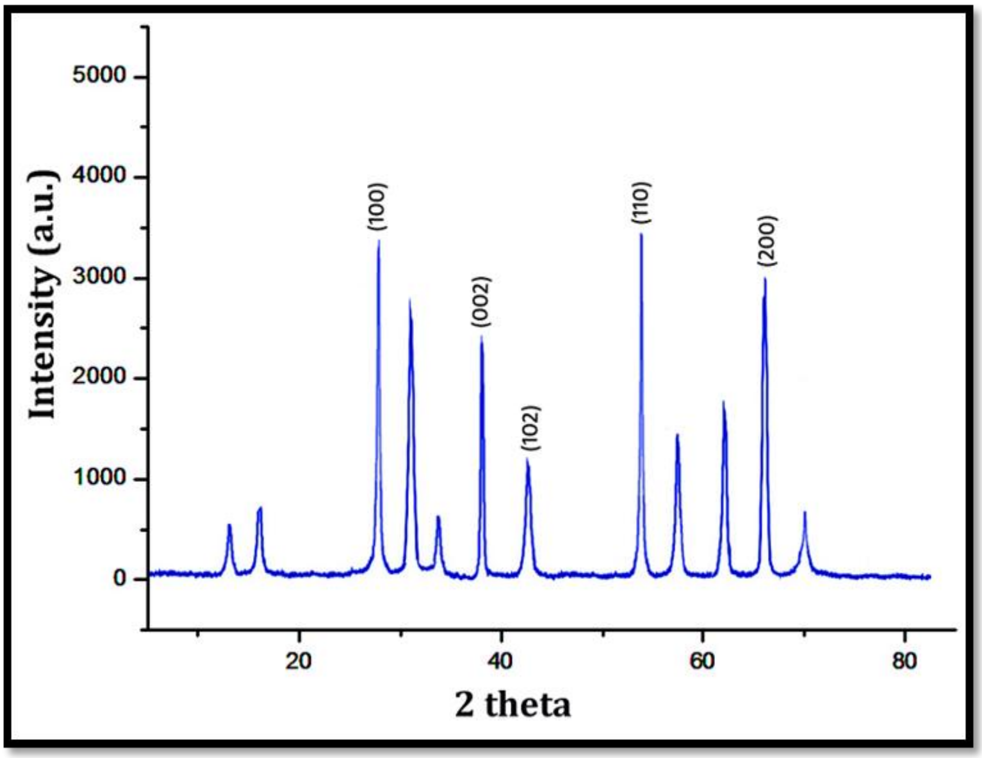


Fig. 9 – XRD pattern of biosynthesized zinc nanoparticles

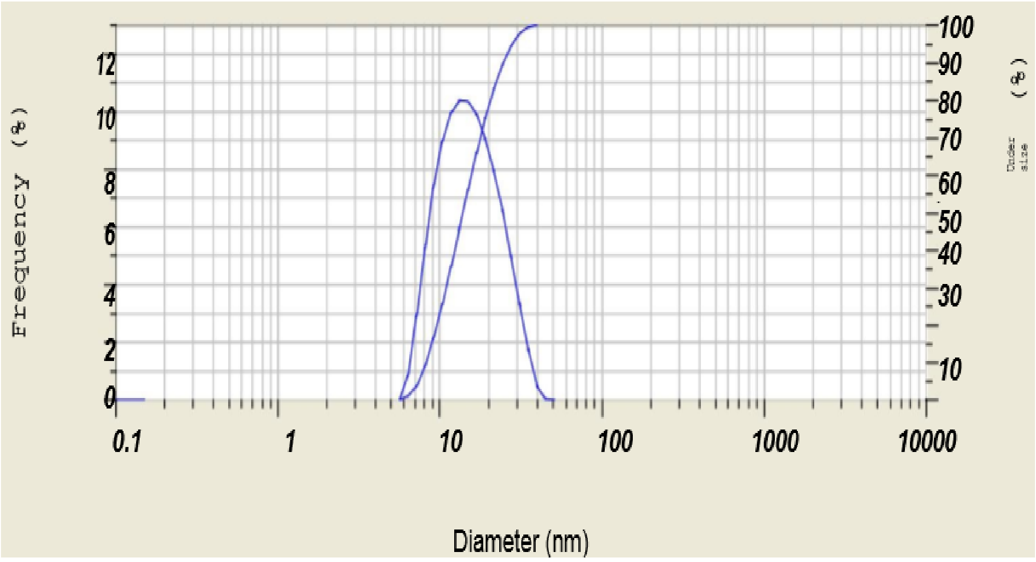


Fig. 10 – Particle size of biosynthesized zinc nanoparticles

4.3. PHYTOCHEMICAL ANALYSIS OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES

The phytochemical analysis is one of the tools to assess the preliminary phytochemicals chemo profiling and marker compound using modern analytical techniques. The preliminary phytochemical screening reveals the presence of akaloid, flavonoid, steroid, glycoside, polyphenols, protein and carbohydrate were present in the synthesized zinc oxide nanoparticles. Whereas tannins, terpenoids were absent Table 1.

Table. 1- Phytochemical screening of phytosynthesized zinc nanoparticles

S.No	Phytochemical	Zinc oxide nanoparticles
1	**FLAVONOIDS**	
	Shinodatest	+
	Ammonia test	+
	Lead acetate test	+
2	**ALKALOIDS**	
	Dragondroff’s test	+
	Hager’s test	+
	Wagner’s test	+
3	**STEROIDS**	
	Libermann-Buchard test	+
	Salkowski test	+
4	**POLYPHENOLS**	
	Ferric chloride	+
5	**GLYCOSIDES**	
	Legal	+
6	**TANNINS**	
	Braemers test	-
7	**SAPONINS**	

	Sodium bicarbonate	**+**
8	**CARBOHYDRAE**	
	Molisch test	**+**
	Fehling’s test	**+**
9	**TERPENOIDS**	**-**
10	**PROTEINS/AMINO ACIDS**	
	Biuret test	**+**

The results of the present study coincides with the findings of Esther and Taiwo. (2012) who reported that the reduction was ascribed to the phenolics, terpenoids, polysaccharides and flavones present in the *Cinnamomum camphora* extract and these nanoparticles also exhibit high bactericidal activity. Flavonoids and terpenoids present in the *Eucalyplus hybrid* leaf extract were claimed to be responsible for the stabilization of nanoparticles (Dubey *et al.,* 2009).

Keshanwani *et al.* (2009) also reported that the leaf extract of Datura metal contains alkaloids, proteins, enzymes, amino acids, alcoholic compounds and polysaccharides which are responsible for the reduction of the zinc ions to nanoparticles.

4.4. ANTIMICROBIAL ACTIVITY OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES

4.4.1. Antibacterial activity of biosynthesized ZnO nanoparticles

The antibacterial activity of zinc oxide nanoparticles was evaluated by measuring the zone of inhibition against selected bacterial isolates. The zone of inhibition of the selected bacterial isolates against zinc oxide nanoparticles and *Moringa oleifera* seed extract were compared with the standard antibiotic chloramphenicol and the results are tabulated in Table 2. The results indicated that zinc oxide nanoparticles synthesized from *Moringa oleifera* seed extract showed effective antibacterial activity against all Gram negative bacteria when compared with Gram positive bacteria. The results clearly indicated that the zone of inhibition exhibited by zinc oxide nanoparticles was maximum in S*almonella typhi* (30mm), followed by *Pseudomonas* aeruginosa (28mm), and

Kelbsiella pneumoniea (23mm) and *Shigella Flexneri* (22mm). The minimum zone of inhibition was recorded in P*roteus vulgaris* (19mm) and *Vibrio cholera* (16mm). The zone of inhibition recorded in Gram positive bacteria Streptococcus epidermis and Staphylococcus aureus was 12mm and 10mm respectively which was low when compared with Gram negative bacteria. The same trend of antibacterial activity was noticed in Streptococcus epidermis and Staphylococcus aureus which exhibited 10mm and 9mm zone of inhibition respectively in *Moringa oleifera* seed extracts (Plate 4).

Moringa oleifera seed extract exhibited a remarkable zone of inhibition against *Salmonella typhi* (23mm), P*seudomonas aeruginosa* (21mm), Kle*bsiella pneumoniae* (20mm), S*higella Flexneri* (18mm), and a weakest activity against *Proteus vulgaris* (15mm) and *Vibrio cholerae*(12mm).

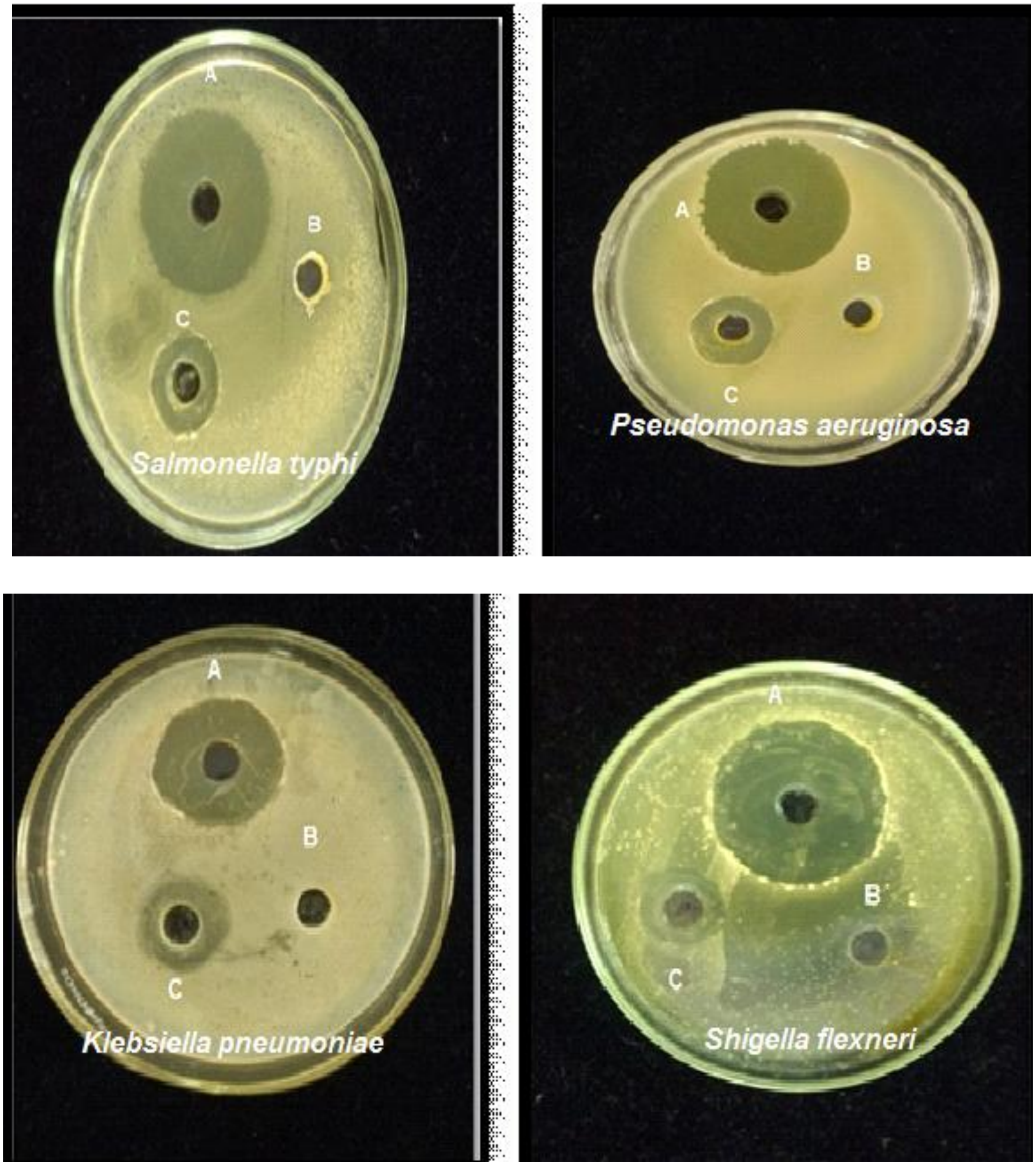
Thus, the zone of inhibition exhibited by zinc oxide nanoparticles were remarkable when compared with the standard antibiotic chloramphenicol which ranged from 15 to 40 mm.

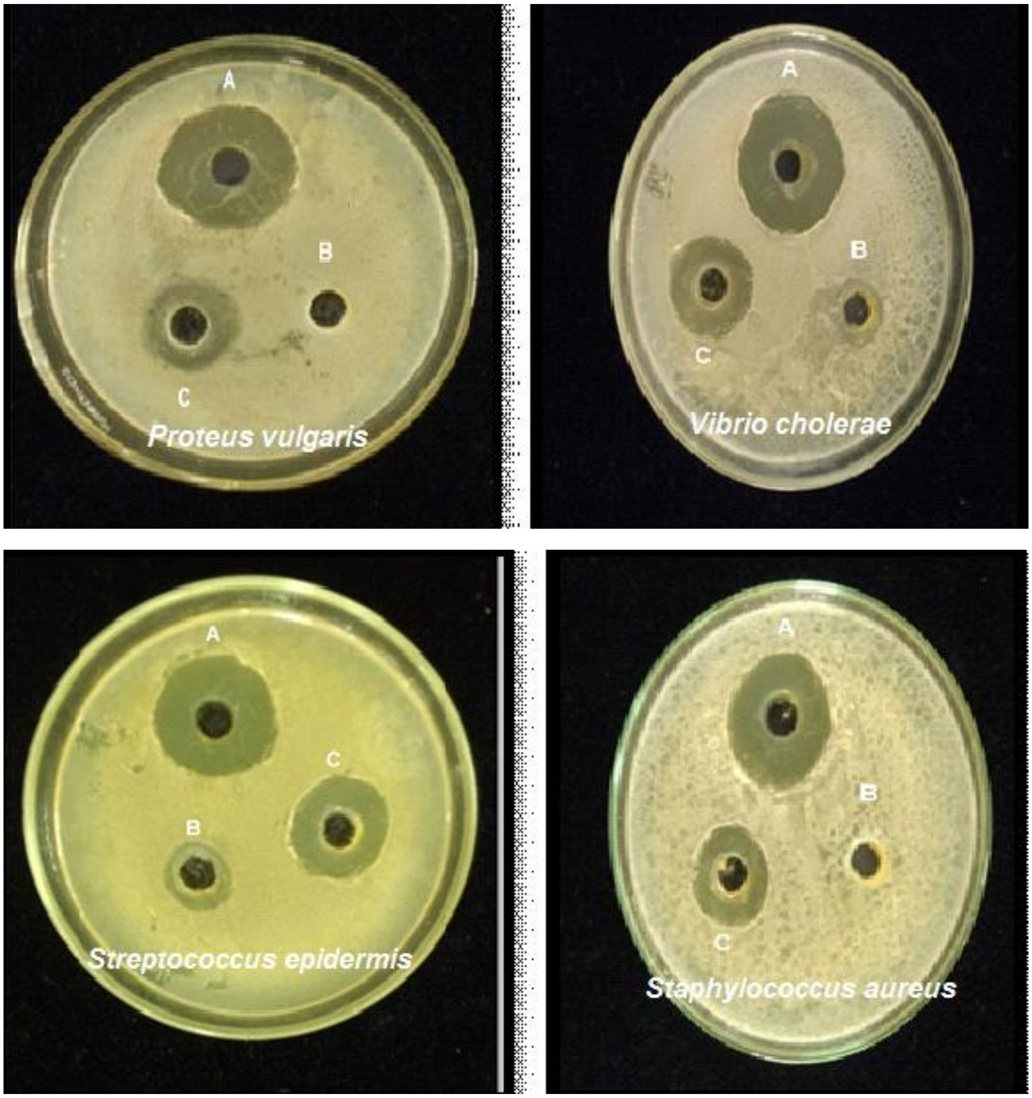
Table. 2 - Antibacterial activity of biosynthesized zinc oxide nanoparticles and seed extract

Bacteria isolates	**Zone of inhibition (mm)**		
	Moringa oleifera **seed extract**	**Zinc oxide nanoparticle ($ZnSO_4$)**	**Chloramphenicol**
Gram Negative Bacteria			
Salmonella typhi	23	30	33
Pseudomonas aeruginosa	21	28	30
Klebsiella pneumoniae	20	23	40
Shigella flexneri	18	22	39
Proteus vulgaris	15	19	25
Vibrio cholerae	12	16	23
Gram Positive Bacteria			
Streptococcus epidermis	10	12	18
Staphylococcus aureus	9	10	15

PLATE. 5

ANTIBACTERIAL ACTIVITY OF BIOSYNTHESIZED ZINC NANOPARTICLES





A. Chloramphenicol

B. *Moringa oleifera* seed extract

C. Zinc oxide nanoparticles

The remarkable zone of inhibition by zinc oxide nanoparticles might be due to its attachment to the surface of cell membrane which inhibits the permeability of bacterial cell and also the activity of respiratory enzymes of bacteria. It also enhances the outflow of cytoplasmic contents which damages its membranes and leads to the death of bacteria. The binding of zinc oxide nanoparticles to the cell surface depends on the surface area available for interaction. Smaller the particles, larger the surface area for interaction which may results in effective bactericidal activity than the larger particles (Panatak *et al.,* 2006).

Zinc oxide nanoparticles have the tremendous applications as antimicrobial agents and possess strong antibacterial activity against high temperature and pressure resistant spores (Nicole *et al.,* 2008 and Neal, 2008). It is believed that the antimicrobial activity of the zinc oxide nanoparticles might be either due to the generation of hydrogen peroxide or due to the electrostatic binding of the particles on the microbial surface (Zhang *et al.,* 2007). Antibacterial activity of zinc oxide nanoparticles is of tremendous practical applications in designing microbial resistant articles (Sharma *et al.,* 2010) for preserving food and wood products (Rajiv *et al.,* 2007),cosmetics, novel nanomedicines (Moritz and Moritz, 2013) wound dressing (Shalumon *et al.,* 2011) and disinfecting agents.

The results of the present study coincides with the findings of Khan *et al.* (2011) who reported that Gram negative bacteria were active when compared with Gram positive bacteria which might be due to the difference in the composition and thickness of the peptidoglycan layer in the cell wall. It has also been suggested that the interaction of biosynthesized nanoparticles with cell wall may increase the membrane permeability by forming pores or pits and thereby leads to the bacteria (Sondi and Salopek-Sondi 2004 and Morons *et al.*, 2005).

The reduction in the inhibition zone might be attributed to the easy permeability of nanoparticles into the bacterial cell which leads to disorganization of the cell membrane and changes in protein levels (Premanathan *et al.,* 2003; Ameta *et al.,* 2013 and Balusam *et al.,* 2012).

Morones *et al.* (2005) and Baker *et al.* (2005), suggested that the biosynthesized nanoparticles may penetrate into the bacterial cell and cause damage by interacting with phosphorous and sulphur present in DNA.

Thus, the biosynthesized zinc nanoparticles can be used as potential antimicrobial agents and helps to overcome the hurdles in managing microbial diseases posed by development of resistance to conventional fungicides and bactericides.

4.4.2. Antifungal activity of biosynthesized zinc nanoparticles

The antifungal activity of biosynthesized zinc oxide nanoparticles was tested against *Trichoderma viridae, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus and Rhizopus* and compared with Fluconazole which served as control and tabulated the results were in Table 3 and Plate 6 shows the antifungal activity of prepared zinc nanoparticles. The antifungal activity of the synthesized zinc nanoparticles exhibited highest zone of inhibition against *Trichoderma viridiae* (30mm), *Aspergillus niger* (28mm), *Aspergillus flavus* (27mm), *Aspergillus fumigatus* (24mm) and the weakest zone of inhibition against *Rhizopus stolonifer*(22mm). The *Moringa oleifera* exhibited the zone of inhibition against *Trichoderma* (27mm), followed by *Aspergillus niger* (23mm), *Aspergillus flavus* (20mm), *Aspergillus fumigatus* (18mm) and the weakest zone of inhibition against *Rhizopus* (16mm). Thus, the results indicated the zone of inhibition against the tested fungal isolated were remarkable when compared with the study antibiotic Fluconazole (25 to 32mm).

Table. 3 - Antifungal activity of biosynthesized zinc oxide nanoparticles and seed extract

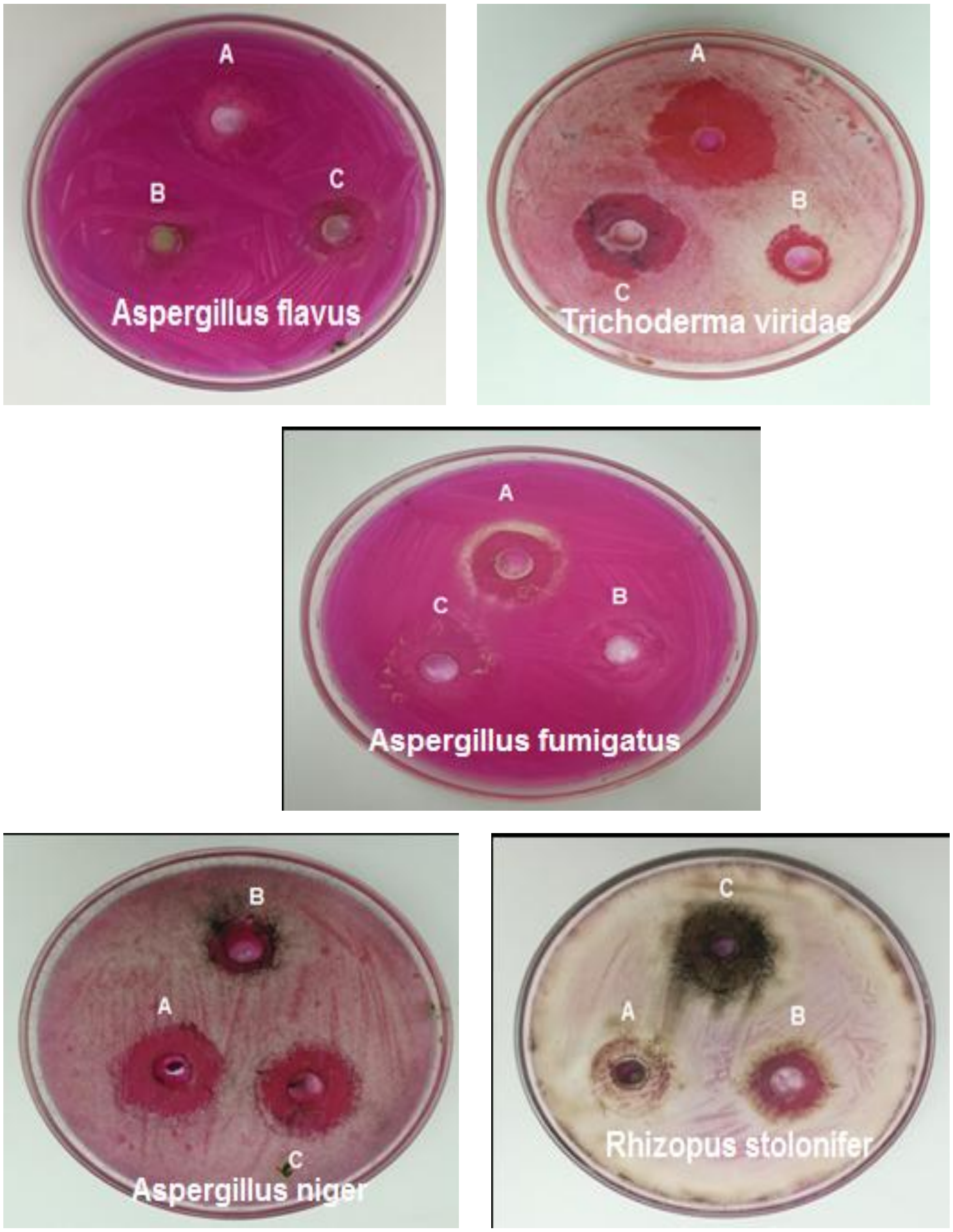
Fungal isolates	Zone of inhibition		
	Moringa oleifera seed extract	**Zinc oxide nanoparticles**	**Fluconazole**
Trichoderma viridae	27	30	32
Aspergillus nigar	23	28	30
Aspergillus flavus	20	27	29
Aspergillus fumigatus	18	24	27
Rhizopus stolonifer	16	22	25

The zinc nanoparticles may directly damage the cell envelope of fungi by penetrating the cell and then binds to the DNA forming a complex which prevents the DNA replication by rupturing the hydrogen bonds between adjacent purines and pyrimidines moieties.

Kim et al. (2007) reported that spherical zinc nanoparticles showed potent activity against *Candida albicans* compared with that of commercially available antifungal agents.

PLATE. 5

ANTIFUNGAL ACTIVITY OF BIOSYNTHESIZED ZINC NANOPARTICLES



A – Fluconazole

B – *Moringa oleifera* seed extract

C – Zinc oxide nanoparticles

4.5. PHOTOCATALYTIC ACTIVITY

The catalytic efficiency of zinc nanoparticles was investigated under solar light irradiation for the degradation of crystal violet in aqueous solution. The dye decolouration was observed at different concentrations of crystal violet (10-50mg/l) inoculated with 1ml (5mg) of zinc oxide nanoparticles. The catalytic reaction was monitored spectrophotometrically by following the decrease of absorbance at λ_{max} 540nm with time intervals of (0-45min). The results of the dye decolouration was depicted in Table 4 and Plate 6 shows the decolourisation of crystal violet.

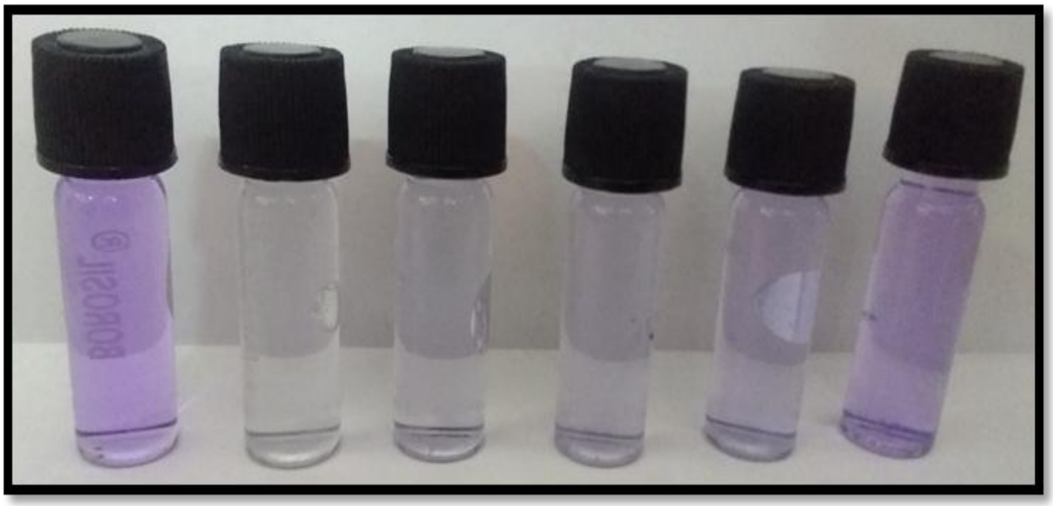
Table 4 - Photocatalytic activity of zinc oxide nanoparticles

Concentration of dye (mg/l)	Percent decolourisation
10	94
20	68
30	42
40	35
50	27

The intense violet colour of the solution faded and became colourless during the process. It was observed that discolouration of dye occurred within 3 minutes in 10mg/l dye (94%) indicating that zinc nanoparticles cause structural changes and the removal of chromophoric group from the dye. At 20 and 30mg/l dye concentration, the removal efficiency was recorded as 68% and 42% within 8 and 30 minutes respectively. Further decolouration was observed after 45 minutes of solar irradiation in 40 and 50mg/l dye concentration (35% and 27% respectively).

PLATE. 7

DECOLOURISATION OF ZINC OXIDE NANOPARTICLES UNDER SOLAR IRRADIATION



It was reported by Kumar *et al* (2013) and Kansal *et al* (2008) that the solar light was found to be more effective than other irradiation techniques for dye degradation. When the dye is exposed to sunlight, the photons hit the nanoparticles present in the colloidal mixture and the electrons at the particle surface are excited (Yu *et al.,* 2012). The dissolved oxygen molecules in the reacting medium accept the excited electrons from particle surface and are converted into oxygen anion radicals. These radicals break the organic dye into simpler organic molecules leading to the rapid degradation of the dye (Houas *et al.,* 2001 and Ameta *et al.,* 2013).

Therefore, the biosynthesized zinc oxide nanoparticles may act as a stable and efficiency photocatalyst for degradation of crystal violet under visible light irradiation.

4.6. PHYTOTOXICITY ACTIVITY

The results of germination percentage, root length, shoot length, fresh weight, dry weight and vigour index of green gram seedling grown with distilled water (T_1), Zinc nanoparticles aqueous solution (T_2), *Moringa oleifera* seed extract (T_3) and Zinc sulfate solution (T_4) on 7th day favoured the growth of the experimental plant and the results were depicted in Table 5 and Plate shows the growth of green gram on 7th day.

A maximum of 97 percent germination was recorded in green gram seeds grown with distilled water (T_1) followed by 90 percent in zinc nanoparticles (T_2). A minimum of 77 percent germination was recorded in seeds grown with *Moringa oleifera* seed extract (T_3) followed by 63 percent zinc sulfate solution (T_4).

The values recorded for the shoot length of green gram seedling were 9.36cm, 5.3cm, 1.0cm and 0.07cm in T_1, T_2, T_3 and T_4 respectively. The values recorded for the seedling grown in highly significant when distilled water (T_1), zinc nanoparticles (T_2), *Moringa oleifera* seed extract (T_3) and compared with zinc sulfate solution (T_4). The root length of green gram was maximum (2.69cm) in followed T_2 (1.52cm) and minimum (0.09cm) in T_3 and T_4 (0.01cm) showed an increase in root length when compared to T_1 and T_4 plants.

Table 5. phytotoxicity activity of zinc oxide nanoparticles

Treatments	Shoot length	Root length	Germination percentage	Vigour index
T_1	2.69 ± 0.5656	9.36 ± 1.50	96.6	1164
T_2	1.52 ± 0.541	5.3 ± 0.925	90	614
T_3	0.90 ± 0.077	1 ± 0.244	76.6	150
T_4	0.01 ± 0	0.07 ± 0.0054	63.3	11

The values are mean ± SD

T_1 – Distilled water

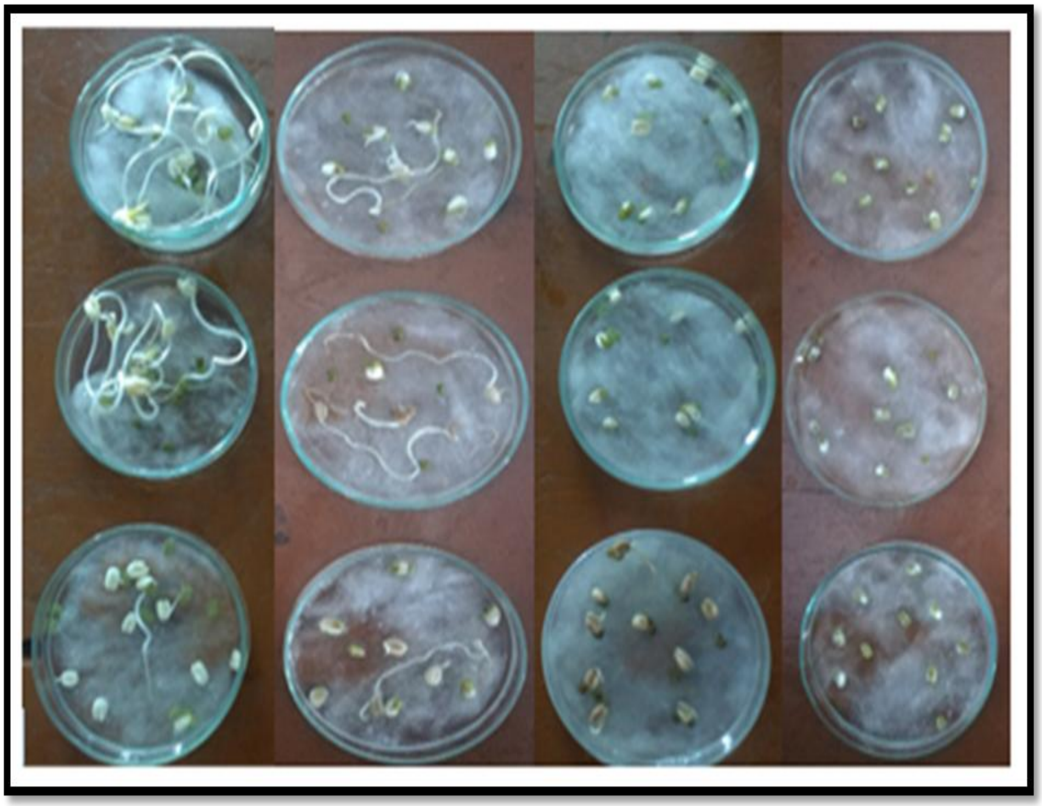
T_2 – Zinc oxide nanoparticles

T_3 – Seed extract of *Moringa oleifera*

T_4 – Zinc sulfate solution

PLATE. 8

PHOTOTOXICITY ACTIVITY USING GREEN GRAM SEEDS



T_1 T_2 T_3 T_4

T_1 - **Distilled water, T_2- Zinc oxide nanoparticles, T_3- *Moringa oleifera* and T_4- Zinc sulfate solution**

PLATE 8

SHOOT LENGTH AND ROOT LENGTH OF GREEN GRAM SEEDLINGS

(T_1) (T_2) (T_3) (T_4)

T_1- Distilled water, T_2- *Moringa oleifera* seed extract

T_3- Zinc sulfate solution and T_4- Zinc oxide nanoparticles

Phytotoxicity in higher plants should be investigated in order to develop a comprehensive toxicity profile for nanoparticles (USEPA, 2005). Seed germination and root elongation is a rapid and widely used acute phytotoxicity test with several advantages: such as sensitivity, simplicity, low cost and suitability for unstable chemicals or samples (Munzuroglu and Geckil, 2002; Wang *et al.,* 2001).

Germination involves the movement and mobilization of metal ions like zinc, so that it may be utilized efficiently and plants emerging from seeds with low Zn have poor seedling vigour (Takahasi *et al.,* 2009).

Zinc is an essential nutrient which plays important roles in numerous physiological processes in plants such as serving as a cofactor for many enzymes and as the key structural motifs in transcriptional regulatory proteins (Ishimeru *et al.,* 2011).

Ajouri *et al* (2004) reported that seed priming with Zn was very effective in improving seed germination and seedling development in barley. These results may indicate that high Zn concentration in seeds has very important physiological roles during seed germination and early seedling growth.

Several studies have shown that zinc affects plant metabolism and its nutrient uptake by roots and other physiological processes. The results with green gram seeds reveals that zinc nanoparticle caused increase of radicle and plumule length in green gram. Reviewed the roles of zinc in protecting plant cells from damage by reactive oxygen species and its effect on plant metabolism has also been well reviewed (Cakmak, 2008).

The decrease in the shoot length and root length in green gram treated with zinc sulfate might be due its high solubility and easy up take by plants and also the plant falls off quickly. Moreover, zinc sulfate has a large salt index which may damage the sensitive plants in high temperature. Studies suggested that zinc oxide nanoparticles are absorbed by plants to a larger extent contrasting to zinc sulfate salt (Prasad *et al.,* 2012).

Summary and Conclusion

5. SUMMARY AND CONCLUSION

The biological production of metal nanoparticles is becoming a very important field in chemistry, biology, and materials science. Metal nanoparticles have been produced chemically and physically for a long time however, their biological production has only been investigated very recently. The biological reduction of metals by plant extracts has been known and the reduction products were not studied. The rapid biological synthesis of zinc nanoparticles using seed extract of *Moringa oleifera* provides an environmental friendly, simple and efficient route for synthesis of nanoparticles. The use of plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents.

Findings of the study

The characterization of zinc ions exposed to *Moringa oleifera* extract was indicated by pale white colour with crystal within 15 minutes using 2mM zinc sulfate with 3ml *Moringa oleifera* extract.

- UV-Vis spectrum confirms the reduction of zinc ions to zinc nanoparticles.
- The biomolecules responsible for the formation of zinc nanoparticles are identified using FT-IR spectroscopy which act as stabilizing agent.
- The SEM analysis showed spongy aggregated nanostructured zinc oxide nanoparticles.
- EDX analysis confirmed the reduction of zinc oxide to zinc nanoparticles with high stability.
- XRD studies revealed that zinc nanoparticles obtained are crystalline in nature.
- Phytochemical screening of synthesized zinc nanoparticles and Moringa extract of *Moringa oleifera* reveals the presence of Alkaloid, Flavonoid, Steroid, Glycoside, polyphenols, Protein and Carbohydrate. These phytoconstituents proved to be effective stabilizing and capping agent in the reduction of Zn^{2+} ions inti zinc nanoparticles.
- The size of the biosynthesized zinc oxide nanoparticles was 15.3nm.

- The antimicrobial study of the synthesized zinc nanoparticles exhibited high potent activity against all tested bacterial and fungal isolates. The synthesized zinc nanoparticles have high bactericidal activity in Gram negative bacteria than the Gram positive bacteria.
- The photocatalytic activity studies revealed the potential of the zinc oxide nanoparticles to decompose the crystal violet dye.
- Phytotoxicity studies revealed high germination grand seedling growth in distilled water followed by zinc oxide nanoparticles. Moringa oleifera seed extract and zinc sulfate solution showed moderate and minimum growth.

Thus, developing a reliable eco-friendly process for the synthesis of zinc oxide nanoparticles is still in its infancy and more research needs to be focused on the mechanism of nanoparticle formation which may lead to fine tuning of the process and ultimately leading to the synthesis of nanoparticles with a strict control over the size and shape parameters.

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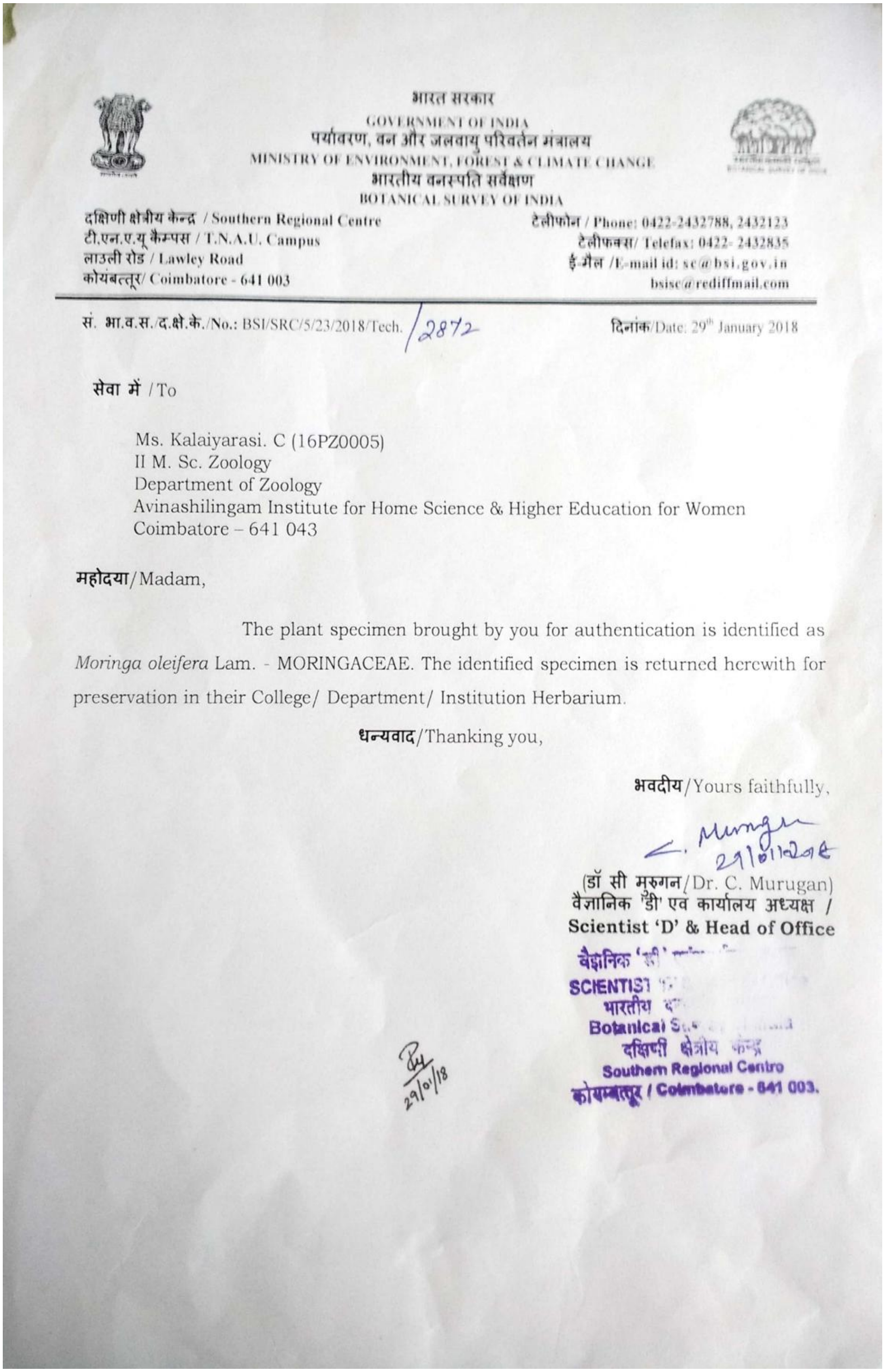
Appendix



भारत सरकार
GOVERNMENT OF INDIA
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MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2018/Tech. /2872

दिनांक/Date: 29th January 2018

सेवा में / To

Ms. Kalaiyarasi. C (16PZ0005)
II M. Sc. Zoology
Department of Zoology
Avinashilingam Institute for Home Science & Higher Education for Women
Coimbatore – 641 043

महोदया/Madam,

The plant specimen brought by you for authentication is identified as *Moringa oleifera* Lam. - MORINGACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

C. Murugan
29/01/2018

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

वैज्ञानिक 'डी' [illegible]
SCIENTIST [illegible]
भारतीय [illegible]
Botanical S[illegible]
दक्षिणी क्षेत्रीय केन्द्र
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29/01/18

APPENDIX. 2

ANTIBACTERIAL ACTIVITY OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES - AGAR WELL DIFFUSION METHOD

Principle

The antimicrobials present in the synthesized zinc nanoparticles and Moringa oleifera seed extract are allowed to diffuse out into the medium and interact in a plate seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as three will be a confluent lawn of growth. The diameter of zone of inhibition was measured in millimetres.

Reagents

1. Muller Hinton Agar Medium

The medium was prepared by dissolving 38g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 Ibs pressure at 1210C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient agar medium (1L)

One liter of nutrient broth was prepared by dissolving 13g of commercially available nutrient broth medium in 1000ml distilled water and boiled to dissolve the medium completely. The medium was suspensed as desired and sterilized autoclaving at 15 Ibs pressure at (121oC) for 15 minutes.

3. Chloramphenicol powder (Standard antibiotic)

Procedure

Petriplates containing sterile 20ml Muller Hinton Agar Medium was seeded with 24hr culture of bacterial strains separately. Three wells were cut and 20µl of the Moringa oleifera seed extract, zinc nanoparticles and chloramphenicol was added separately. The plates were then incubated at37oC for 24hr and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well.

APPENDIX. 3

ANTIFUNGAL ACTIVITY OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES - AGAR WELL METHOD

Principle

The fungicidal effect of the synthesized zinc oxide nanoparticles and M*oringa oleifera* seed extract can be assessed by the inhibition of mycelial growth of the fungus and was observed as a zone of inhibition near the wells.

Reagents

1. Rose bengal chloramphenicol agar medium (1L)

The commercially available rose bengal chloramphenicol agar medium (32.15g) was suspended in 1000 ml of distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 Ibs pressure (120^{0}C) for 15 minutes.

2. Fluconozole (standerd antifugal agent)

Procedure

Sterile rose bengal chloramphenicol agar medium was prepared and poured on to the petriplates. Fungal strains were swebbed on the plate's separately. Wells were cut and 20µl of the *Moringa oleifera* seed extract extract, zinc oxide nanoparticles, fluconozole was added separately. The antifungal effect was observed as zones of inhibition and expressed in millimeter.