SYNTHESIS OF ZnO NANOPARTICLES USING LEAF EXTRACT OF *KALANCHOAE PETITIANA* AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES

BY

SOLOMON SORECHA

A THESIS SUBMITTED TO THE DEPARTMENT OF APPLIED CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

OFFICE OF GRADUATE STUDIES

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SEPTEMBER,2018

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APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defense by Solomon Sorecha have read and evaluated his thesis entitled " SYNTHESIS OF ZnO NANOPARTICLES USING LEAF EXTRACT OF *Kalanchoe petitiana* AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in Chemistry.

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DECLARATION

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

Name: Solomon Sorecha

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

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ADVISOR'S APPROVAL SHEET

To: Chemistry Department

Subject: Thesis Submission

This is to certify that the thesis entitled "SYNTHESIS OF ZnO NANOPARTICLES USING LEAF EXTRACT OF *Kalanchoae petitiana* and evaluation of its antibacterial activities" submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry, the Graduate program of the department of Chemistry, and has been carried out by Solomon Sorecha Id no.GSU/0506/06, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

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Name of Co-Advisor	Signature	Date

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ABBREVIATIONS

NPs	Nanoparticles
FTIR	Fourier Transform Infrared
XRD	X-ray Diffraction
USNSF	the United States National Science Foundation
VLS	vapor liquid solid
PVD	physical vapor deposition
ROS	Reactive oxygen species
TEM	Transmission electron microscope
FWHM	Full Width at Half Maximum
TGA	Thermal gravimetric analysis
nm	Nanometer
eV	electrovolt
kV	kilovolt
mA	miliamper
DTA	differential thermal analysis
SEM	scanning electron microscopy
EDX	Energy dispersive x-ray spectroscopy
Uv - vis	Ultra violet visible spectroscopy

ABSTRACT

Recently in nanotechnology research, synthesis of nanoparticle from green chemistry pathways has been preferred due to its natural biological reduction property which reduces the utilization and exposure of toxic chemical to the environment when compared to physical and chemical methods. The present study focuses on the green synthesis and characterization of zinc oxide nanoparticles using different ratios of *Kalanchoae petitiana* Leaf extract for antibacterial application on the drug resistant bacteria. Zinc acetate dihydrate were used as precursor to synthesize ZnO nanoparticles using the *Kalanchoae petitiana* leaf extract. The synthesized nanoparticles were characterized by advanced techniques like Thermo Gravimetric Analysis (TGA), X-Ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR). TGA was performed for ZnO nanoparticles to study the thermal stability of materials. The XRD analysis reveals all the synthesized particles have pure hexagonal phase with particle size in the range of 12 – 22 nm. The FT-IR analysis shows the interaction between the capping agents and the synthesized ZnO nanoparticle. The SEM analysis indicates the nanorods and hollow shape morphology and grain size increases with the decrease of the concentration of the capping agents. EDX spectrum showed the elemental composition of ZnO NPs. The synthesised ZnO NPs were applied for antibacterial activity against Klebsella Pneumonia, Escherichia coli, Staphylococcus aureus, and Entrococcius Spps bacteria using disc diffusion method. ZnO NPs at 3:1 ratio has greatest zone of inhibition (11mm,12mm,11mm) indicating that it has greatest antibacterial activity against gram-negative bacteria(E.coli and klebsella pneumonia) and gram-positive bacteria (S.aaureus) but resistant to gram- positive bacteria (Entrococcius spp).

Keywords: *Kalanchoae Petitiana,* Zinc oxide nanoparticle, characterization, Escherichia coli, Gentamicin

.

1. Introduction

1.1 Background

Nanotechnology can be defined as manipulation of matter through certain chemical and/or physical processes to create materials with specific properties which can be used in particular applications (Amrita, 2015). ZnO NPs have drawn attention of many researchers for their unique optical and chemical behaviors which can be easily tuned by changing the morphology (Surya, 2017). Within the large family of metal oxide NPs ZnO NPs have been used in various cutting edge applications like electronics, communication, sensors, cosmetics, environmental protection, biology and the medicinal industry. Moreover, ZnO NP has a tremendous potential in biological applications like biological sensing, biological labeling, gene delivery, drug delivery and nanomedicine along with its antibacterial, antifungal, acaricidal, pediculocidal, larvicidal and anti-diabetic activities (Nirannjan, 2015).

Metal oxide nanoparticles were synthesized by various physical and chemical methods. Some commonly used synthetic methods are non-sputtering, Solvothermal, reduction, sol-gel technique and electrochemical technique. But these methods are costly, employ toxic chemicals, high pressure, high energy requirement and potentially hazardous. Hence developing of reliable biosynthetic and environment friendly approach has added much importance because of its eco-friendly products, biocompatibility and economic viability in the long run and also to avoid adverse effects during their application especially in medical field (Vivek, 2017).

Biosynthesis of nanoparticles is a bottom up approach where the main reaction occurring is reduction/oxidation. The plant phytochemicals with antioxidant or reducing properties are usually responsible for the preparation of metal and metal oxide nanoparticles. The three ecofriendly and green chemistry perspective for the nanoparticle synthesis are the choice of the solvent medium, reducing agent and non-toxic material for the stabilization of nanoparticles (Vivek, 2017) .

Recently nanoparticle synthesis were achieved using plant extract such as azadirachta indica, camellia sinensis, Nyctanthes arbor-tristis, coriandrum, nelumbo nucifera, ocimum sanctum and several others which is compatible with the green chemistry principles. Among the various biosynthetic approaches, the use of plant extract has advantages such as easily available, safe to

handle and possess a broad viability of metabolites. The main phytochemicals responsible for the synthesis of nanoparticles are terpenoids, flavones, ketones, aldehydes, and amides (happy agarwal, 2017).

In this work green synthesis of ZnO NP using Kalanchoae petitiana leaves will be carried out. K. petitiana is distributed around the forest margins and open ever green bush land, often in areas 2000-3000 m above sea level. It is a perennial plant forming dense stands, whose stems are decumbent at base, and are up to 3 cm thick and 70 cm high (Raf Aerts, 2017).

In Ethiopian traditional medicine, the leaves of Kalanchoae petitiana (known as "Indahula" in Amharic and "Bosoke" in Afan Oromo) is used to treat blunt hand trauma sustained after a fall off a horse. This succulent plant is native to the highlands of Ethiopia, and its fresh leaves are reportedly used as dermal medicine. Heated leaves of *Kalancoae petitiana* are used as human medicine to relax sore muscles and as veterinary medicine to treat swelling and broken bones in cattle. The anti-inflammatory, antimicrobial, and wound healing properties of *Kalanchoae petitiana* have been demonstrated in vitro and in animal models (Raf Aerts, 2017).

1.2 Statement of the problem

During the past decade, a lot of serious issue was caused by the increase resistance of pathogen agents towards antibiotics, which has become a major issue in health care. A possible solution is novel application of metal oxide as antimicrobial activity by combining advance technologies of material science. A zinc oxide nanoparticle has proved to have the advantage of durability as well as chemical and physical stability over common organics and antibiotics compounds. So, this study focused to synthesize zinc oxide nanoparticles in green synthesis method using *Kalanchoae petitiana* leaves extract and evaluates its effect against antimicrobial agent Bacillus subtilis, Klebsella Pneumonia, Entrococcius Spps, and Escherichia coli

1.3. Objective of the Study

1.3.1 General objective

- Synthesis of ZnO nanoparticle from *Kalanchoae petitiana* leaves extract and evaluation of its antimicrobial activity against drug resistant bacteria

1.3.2. Specific objective

- To synthesize ZnO NPs through a green synthetic pathway using *Kalanchoae petitiana* leave extract
- To characterize synthesized ZnO NPs using Fourier Transform Infrared (FTIR) Spectroscopy, X-ray Diffraction (XRD),Scanning electron microscope(SEM),Electron diffraction spectroscopy(EDS), and Ultraviolet-Visible (UV-Vis) spectroscopy
- To investigate the effect of ZnO nanoparticle against antibacterial agent, *Klebsella Pneumonia, Escherichia coli, Staphylococcus aureus,* and *Entrococcius Spps* bacteria

1.4 Significance of the Study

This study provides as important information about the synthesis strategy of ZnO nanoparticles using *Kalanchoae petitiana* and its antimicrobial activity.This work also opens an opportunity for the researchers interested to work in this area.

1.5. Scope of the Study

The scope of this study was limited to synthesis of ZnO nanoparticles from *Kalanchoae petitiana* leaf extract, characterization of the ZnO nanoparticles using, FTIR, XRD,EDS ,SEM and UV-Vis spectroscopy technique, and antibacterial test of ZnO nanoparticles against *Klebsella pneumonia, Escherichia coli, Staphylococcus aureus,* and *Entrococcius Spps* bacteria.

2. Review of Literature

2.1. Nanotechnology

Nanotechnology is the science of production, manipulation and use of materials at subatomic level to produce novel products and processes (Dalia, 2015). It can be understood as a technology of design, fabrication and applications of nanostructures and nanomaterials, as well as fundamental understanding of physical properties and phenomena of nanomaterials and nanostructures (Dalia, 2015).

Nanotechnology is considered as the synthesis, characterization, and exploration of materials in the nanometer region (1–100 nm). In this technology, the pertinent materials are those whose structures exhibit new and considerably enhanced physicochemical and biological properties as well as distinct phenomena and functionalities as a result of the nanoscale size (Vidya, 2013). This nanoscale size generally confers larger surface areas to nanoparticles (NPs) compared with macro-sized particles. NPs are known as controlled or manipulated particles at the atomic level (1–100 nm). They show size-related properties significantly different from bulk materials. Given their small size, NPs have larger structures in comparison with their counterparts. This distinct property allows their possible applications in many fields such as biosensors, nanomedicine, and bionanotechnology (Amna, 2015).

Nanostructured materials have a larger percentage of atoms at their surface which lead to high surface reactivity. Thus, nanomaterials have witnessed recently significant importance in the basic and applied sciences as well as in bionanotechnology (Amna, 2015).

2.2. Nanoparticles

Nanoparticles in general refers to atomic or molecular aggregates with dimensions between 1 and 100 nm that can drastically modify their physico-chemical properties compared with the bulk material (K.K Yadav, 2017) . This reduction in size brings about significant changes in their physical properties with respect to those observed in bulk materials. The reduction in size leads to increase in the surface area to volume ratio, which leads to the appearance of surface effects related to the high number of surface atoms, as well as to a high specific area (Sanmath, 2016). 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 (Tamasa, 2013).

2.3. Metallic Oxide Nanoparticles

Metal oxides are an interesting class of inorganic materials that have been extensively explored and studied due to their wide range of structures and properties. The character of metal oxides is more complex than pure metals, with metal oxide bonding varying from nearly ionic to highly covalent and even metallic in nature (S. Devasenan, 2016). Metal oxides come in a variety of different forms, each possessing unique compositions, morphologies, structures, and physiochemical properties. In particular, metal oxide nanoparticles are of particular interest due to their unique and phenomenal optical, electronic, and magnetic properties. These unique properties give metal oxide nanoparticles significant industrial importance in a variety of applications that include catalytic processes, electronics, sensors, magnetic storage media, and solar energy conversion (Derek, 2017).

Metal oxide NPs can exhibit unique chemical properties due to their limited size and a high density of corner or edge surface sites. Particle size is expected to influence important groups of basic properties in any material. The properties such as structural characteristics, namely the lattice symmetry, cell parameters, and effect of size, are related to the electronic properties of the oxide and structural and electronic properties obviously drive the chemical properties of the solid and also by size in a simple classification (Sanmath, 2016).

2.4 ZnO Nanoparticles

ZnO nanoparticles are II–VI semiconductor with wide band gap energy, that is, 3.3 eV, and high excitation energy, that is, 60 eV. Thus, it can tolerate large electric fields, high temperature , and operations (Rajeshwari, 2014). These properties make ZnO nanoparticles to have exceptional electrical and optical properties, which makes them suitable for a wide range of applications such as biomedical, photocatalysts and solar cells (Anbuvannan, 2017).

Nowadays, ZnO has been widely investigated for plant protection products, fertilizers, soil improvement, and water high power purification and many others. Antimicrobially active

packaging is a new generation of nano-food packaging based on metal nanocomposites which are made by incorporating ZnO into polymer films (Ayesha, 2017) .

2.5. Synthesis of ZnO Nanoparticles

ZnO NP can be synthesized by various techniques such as physical, chemical, and biological methods. Radiolysis, ultrasonication, spray pyrolysis, photo irradiation, electro spinning, laser ablation etc. are some of the physical methods that have been employed for the synthesis of ZnO nanoparticles. However chemical methods have received much attention as compare to physical methods due to better control over size and shape of nanoparticles (Sangeetha, 2013). Chemical methods mainly include sol-gel method, solvothermal, co-precipitation and template based methods (Pramila, 2016). But biological synthesis of nanoparticles using bacteria, algae, fungi, actinomycetes and plants has gained attention as they are eco-friendly, simple and efficient. Among which plant extract based synthesis has been greatly acknowledged and explored with many plant species (M.Mankori, 2017).

2.5.1. Physical Method

The physical method often called top-down approach which includes high energy ball milling, melt mixing, physical vapor deposition, laser ablation, sputter deposition, electric arc deposition, and ion implantation (Mullana, 2013). In most physical/mechanical processes, the production rates of ZnO nanoparticles are very high and are mostly used for the industrial processes. In high energy ball milling method powdered material placed inside a ball mill is subjected to high energy collision from balls. Compared to the other physical methods it is a very efficient, cost effective, and simple technique for the preparation of ZnO nanoparticles (Sadraei R, 2016). Compared to the other physical method laser ablation technique utilizes a laser beam to remove particle from a solid or a liquid surface. At lower reflux, materials are heated by the energy absorbed through laser and evaporate, while, at higher refluxes, materials may convert into plasma. Other frequently studied methods used are vapor liquid solid (VLS), and physical vapor deposition (PVD). Physical vapor deposition (PVD) methods are used to coat the surfaces by depositing the metals. Two types of techniques, namely, evaporation and sputtering, are used in PVD. Sputtering refers to the mechanism of particle escape from the surface by striking high energy particles. The ions for sputtering process are supplied from plasma (Ayesha, 2017).

These methods hold certain advantages over chemical methods such as, no solvent contamination in prepared film and uniformity in nanoparticle formation (Tooba, 2017).

The use of costly equipment, high temperature and pressure, and large space area for setting up of machines, are some of the draw backs of using physical methods of synthesis (Happy Agarwal, 2017).

2.5.2. Chemical Method

Chemical methods have emerged to be indispensable for synthesizing nanocrystals of various types of materials. These methods are generally carried out under mild conditions and are relatively straightforward. One of the important factors that determine the quality of a synthetic procedure is the mono dispersity of the nanocrystals obtained. These methods allow for adequate control of the size and concentration of the dispersed particles (Aneesh, 2007). Chemical synthesis can be divided into liquid phase synthesis and gas phase synthesis. Liquid phase synthesis includes precipitation, co-precipitation method, colloidal methods, sol-gel processing, water–oil micro emulsions method, hydrothermal synthesis, solvothermal, and sonochemical, and polyol method. And vapor phase fabrication includes pyrolysis and inert gas condensation methods (Ayesha, 2017) .

In processes of synthesis of nanopowders based on precipitation, it is increasingly common for surfactants to be used to control the growth of particles. The presence of these compounds affects not only nucleation and particle growth, but also coagulation and flocculation of the particles (Agnieska, 2014).

Sol-gel techniques rely on colloidal chemistry. Sols are referred to the colloidal solution consisting of solid particles suspended in liquid phase having a diameter of a few hundred nanometers. An advantage of sol-gel methods is the insurance of synthesis of highly pure and uniform structured ZnO (Ayesha, 2017).

The hydrothermal method does not require the use of organic solvents or additional processing of the product (grinding and calcinations), which makes it a simple and environmentally friendly technique. This process has many advantages, including the possibility of carrying out the synthesis at low temperatures, the diverse shapes and dimensions of the resulting crystals depending on the composition of the starting mixture and the process temperature and pressure,

the high degree of crystallinity of the product, and the high purity of the material obtained (Agnieska, 2014).

Mostly the chemical methods of synthesis of nanoparticles are the bottom up approaches. These approaches include the miniaturization of material components (up to atomic level) with further self-assembly processes leading to the formation of nanostructures. During self-assembly the physical forces operating at nanoscale are used to combine basic units into larger stable structures. Typical examples are quantum dot formation during epitaxial growth and formation of nanoparticles from colloidal dispersion (Upsana, 2013).

Advantages of chemical methods over physical methods are that the techniques are not complicated, economic, diverse sizes and shapes can be synthesized, large quantities can be obtained in short span of time, doping of foreign atoms during synthesis is possible (Tooba, 2017).

The use of toxic chemicals which can prove to be hazardous for the environment and the person handling it is the main drawback of chemical method of synthesis (Happy Agarwal, 2017) .The chemical synthesis methods of ZnO NPs like chemical precipitation, hydrothermal method, pyrolysis, chemical vapor deposition, and so forth result in the presence of some toxic chemicals adsorbed on the surface that may have adverse effects in medical applications. There are some reactions in these chemical procedures which require high temperature and high pressure for their initiation while some reactions require inert atmosphere protection or inert conditions. Some chemical techniques also involve utilization of certain toxic matters such as H_2S, toxic template, and metallic precursors. The chemicals used for synthesis of nanoparticles and for their stabilization are toxic and lead to none eco-friendly by products (Sidra, 2014).

2.5.3. Biological /Green Synthesis Method

The production of nanoparticles through conventional physical and chemical methods results in toxic byproducts that are environmental hazards. Additionally, these particles cannot be used in medicine due to health-related issues, especially in clinical fields. Moreover, these techniques are complicated, costly, inefficient, and outdated (Arinjoy Datta, 2017). In recent years, there has been growing interest in the synthesis of environmentally friendly nanoparticles that do not produce toxic waste products during the manufacturing process. This can only be achieved

through benign synthesis procedures of a biological nature using biotechnological tools that are considered safe and ecologically sound for nanomaterial fabrication as an alternative to conventional physical and chemical methods. This has given rise to the concept of green technology or green nanobiotechnology (Jayanta, 2014).

In general, green nanobiotechnology means synthesizing nanoparticles or nanomaterials using biological routes such as those involving microorganisms, plants, and viruses or their byproducts, such as proteins and lipids, with the help of various biotechnological tools. (Anbuvannan, 2017) Nanoparticles produced by green technology are far superior to those manufactured with physical and chemical methods based on several aspects. For example, green techniques eliminate the use of expensive chemicals, consume less energy, and generate environmentally benign products and by products (Barnali, 2011).

Biological methods follow “bottom up” approaches in which the NPs are built from small entities by reduction and oxidation processes. The biological entities possess capping and stabilizing agents in the synthesis process. Enzymes, proteins, sugars, and phytochemicals like flavonoids, phenolics, terpenoids, cofactors, etc. mainly act as reducing and stabilizing agents during synthesis (Pijuskanti, 2016).

Plant parts like leaf, stem, root, fruit, and seed have been used for ZnO NPs synthesis because of the exclusive phytochemicals that they produce. Using natural extracts of plant parts is a very eco-friendly, cheap process and it does not involve usage of any intermediate base groups. It takes very less time, does not involve usage of costly equipment and precursor and gives a highly pure and quantity enriched product free of impurities (Robina ashraf, 2013). Plants are most preferred source of NPs synthesis because they lead to large-scale production and production of stable, varied in shape and size NP. Bio-reduction involves reducing metal ions or metal oxides NPs with the help of phytochemicals like polysaccharides, polyphenolic compounds, vitamins, amino acids, alkaloids, and terpenoids secreted from the plants (HappyAgarwal, 2017).

The chemical reaction process for the formation of zinc oxide nanoparticle from zinc acetate is shown in the following chemical equation

$$Zn(CH_3COO)_2.2H_2O + 2NaOH \longrightarrow Zn(OH)_2 + 2CH_3COONa + 2H_2O$$

$$Zn(OH)_2 + 2H_2O \longrightarrow Zn(OH)^{2+}_4 + 2H^+$$

$Zn(OH)^{2+}_4 \longleftrightarrow ZnO + 2OH^- + 2H_2O$

The limited evidence for the use of *Kalanchoae petitiana* in human and veterinary medicine is anecdotal, and the compounds responsible for the anti-inflammatory, antimicrobial, and wound-healing properties that have been demonstrated in vitro and in animal models have not yet been identified (Maria J.sierra, 2018). *Kalanchoae pinnata* and *Kalanchoe brasiliensis* are related species native to South America and are also known for their traditional medicinal uses, including veterinary medicine. A number of studies suggest that quercetin-derived flavonoid glycosides are the bioactive molecules that produce antiophidic, antinociceptive, antiedematogenic, and anti-inflammatory activities of Kalanchoae leaf and flower extracts. For *Kalanchoae petitiana*, quantitative data on its active components are lacking (Raf Aerts, 2017).

Bacteria are considered as a potential 'biofactory' for the synthesis of nanoparticles. The use of bacteria as a source of enzymes that can catalyze specific reactions leading to inorganic nanoparticles is a new rational biosynthesis strategy and use of enzymes, microbial enzymes, vitamins, polysaccharides, biodegradable polymers, microorganisms, and biological systems for synthesis of nanoparticles (Yadav, 2017). NP synthesis using bacteria has several disadvantages like screening of microbes is a time consuming process, careful monitoring of culture broth and the entire process is required to avoid the contamination, lack of control on NP size, shape and cost associated with the media used to grow bacteria is also very high (Happy Agarwal, 2017).

Three main steps followed for the synthesis of nanoparticles using a biological system are, the choice of solvent medium use, the choice of an eco friendly and environmentally benign reducing agent, and the choice of a nontoxic material as a capping agent is to stabilize the synthesized nanoparticles (Jayanta, 2014).

2.6. Application of ZnO Nanoparticles

Zinc oxide (ZnO) is not stranger to scientific study. In the past 100 years, it has produced as a subject of thousands of research papers, dating back as early as 1935. For its potential of ultra violet absorbance, wide chemistry, piezoelectricity and luminescence at high temperatures, ZnO has entered into industry, and now is one of the critical building blocks in today's modern society. It is found in paints, cosmetics, plastic and rubber manufacturing, electronics and

pharmaceuticals. More recently however, it has gained large interest for its semiconducting properties (Barnali, 2011).

Among these oxide nanoparticles, ZnO nanostructure material has gained much interest owing to its wide applications for various devices such as solar cells, transducers, transparent conducting electrodes, sensors, and catalysts. However, these properties of the pure bulk ZnO are not stable and cannot meet the increasing needs for the present applications. Gas sensors based on ZnO had already been developed for detection and control of gases such as CO, H_2, H_2S, NH_3, etc (Barnali, 2011).

ZnO nanoparticles embedded in polymer matrices like soluble starch are a good example of functional nanostructures with potential for applications such as UV-protection ability in textiles and sunscreens, and antibacterial finishes in medical textiles and inner wears. ZnO nanoparticles have successfully been dispersed inside a soluble starch matrix using a simple water-based technique. The stabilization of these nanoparticles was achieved by the presence of soluble starch in the reaction medium (Barnali, 2011).

2.6.1. Antibacterial Activities of ZnO Nanoparticles

According to The American Heritage Medical Dictionary 2007, antibacterial activity is defined as the action through which bacterial growth is disrupted. The antibacterial agents are basically some drugs which are capable to inhibit or destroy the bacterial cell and must not be harmful to the host cell. ZnO nanoparticles, synthesized by environmental friendly green approach, exhibit excellent biocompatibility and anti-bacterial activity (Pijuskanti, 2016).

The antibacterial activity was assumed to be due to per oxidation of membrane lipid by the NP-ROS interaction in the culture medium. Different ions, small molecules (such as H_2O_2), free radicals (like, OH^{-}, O^{2-}) or superoxide ions (such as O^{2-}) are examples of highly reactive ROS species which can be produced on the surface of metal oxide nanoparticles and can induce bacterial cell death. ROS-induced damages and bacterial death comprise oxidative stress, oxidative lesions and membrane lipid per oxidation. In addition, ROS can harm bacterial components such as proteins and nucleic acids (Slavika, 2016).

The physicochemical characteristics of metal oxide nanoparticles, such as size, crystal structure defects, composition and surface charge, are directly associated with enhanced antibacterial

effects. The synthesis and treatment procedures employed can tune these characteristics, and hence produce the desired antibacterial efficacy. For instance, nanoparticles of smaller sizes (less than 20 nm) can easily penetrate into bacterial cells and may release toxic metal ion upon dissolution. Thus, smaller particles are usually the most efficient antibacterial agents (Slavika, 2016).

3. Materials and Methods

3.1. Materials

3.1.1. Chemicals and solvents

The following chemicals and solvents were used in this research work. Zinc acetate dihydrate (Zn (CH_3COO) 2.2H2O , Sodium hydroxide (NaOH) (Ranchem Industry & Trading)), Ethanol absolute (99.9%) (Ranchem Industry & Trading) and deionized water (Ranchem Industry & Trading) were purchased. All chemicals were analytical grade and used without further purification

3.1.2 Apparatus

The laboratory apparatus used for this work were the following: beakers, conical flasks, funnel, volumetric flasks, borosilicate glass, scissor, mortar and pestle, Whatman filter paper No 1, crucible, cuvette, spatula, analytical balance with accuracy of 0.0001g ,magnetic stirrer with hot plate, drying oven and Muffle furnace.

3.2 Methods

3.2.1 Collection of Plant Material

For the preparation of leaves extract fresh leaves of kalanchoae petitiana were collected from Arsi Zone Kula district.

Figure 1: Morphological view of *Kalanchoae petitiana*

The leaves were thoroughly washed and rinsed with deionized water to remove the dust particles and then dried at room to remove the residual moisture. The dried leaves were cut and grinded into powder (S.Kavita, 2017).

3.2.2 Preparation of the Leaf Extract

10 g of plant powder was taken and added in 200 ml deionized water and heated for 20 min at 60˚C until the color of the solution were changed to light yellow color. After that, the plant extract was filtered with Whattman paper in order to remove the unwanted residues. Furthermore, the extract was stored in a refrigerator at 4˚C to use for synthesis of ZnO nanoparticle (S.Vennila, 2017).

3.2.3 Preparation of precursors and synthesis of zinc oxide nanoparticles

Zinc acetate dihydrate [$Zn(CH_3COO)_2.2H_2O$] was used as precursor to synthesize ZnO nanoparticles using aqueous extract of kalanchoae petitiana. 0.5M Zinc acetate solution was prepared by dissolving 27.48 g of Zinc acetate dihydrate in 250 mL deionized water using 250 mL volumetric flask and stored for further use. 0.5M NaOH was prepared using deionized water to be used as precipitating agent (S.vennila). Then six different samples were prepared by taking different ratios by volume of precursor salt solution and *Kalanchoae petitiana* leaf extract

Sample 1: *Kalanchoae* leaf extract: Zn $(CH_3COO)_2.2H_2O$ (1:1) ratio (25mL: 25mL) labeled as S_1

Sample 2*: kalanchoae* leaf extract: Zn $(CH_3COO)_2$. 2 H_2O (1:2) ratio (25mL: 50 mL) labeled as S_2

Sample 3: *kalanchoae* leaf extract: Zn $(CH_3COO)_2$. 2 H_2O (1:3) ratio (25mL: 75 mL) labeled as S_3

Sample 4: *kalanchoae l*eaf extract: Zn $(CH_3COO)_2$. 2 H_2O (1:5) ratio (25mL: 125 mL) labeled as S_4

Sample 5: *kalanchoae l*eaf extract: Zn $(CH_3COO)_2$. 2 H_2O (2:1) ratio (50mL: 25 mL) labeled as S_5

Sample 6: *kalanchoae* leaf extract: Zn $(CH_3COO)_2$. 2 H_2O (3:1) ratio (75mL: 25 mL) labeled as S_6

For sample 1: (1:1 ratio)

25 ml of *Kalanchoae petitiana* leaf extract and 25 mL of 0.5M NaOH were added to 25 mL of 0.5M $Zn(CH_3COO)_2.2H_2O$. Then the mixture was stirred continuously for 2 hrs with magnetic stirrer and resulted in solution with yellowish precipitate. The precipitate was filtered using Whatman filter paper No 1 and washed repeatedly with deionized water followed by ethanol in order to remove the impurities. Finally, the precipitate was dried in an oven at 60 ˚C for overnight and grinded to fine powder using agate mortar. The yellow powder obtained from the above method was calcined at 450 ˚C for 1 hrs

For sample 2: (1:2 ratio)

25 ml of *Kalanchoae petitiana* leaf extract and 50 mL of 0.5M NaOH were added to 50 mL of 0.5M $Zn(CH_3COO)_2.2H_2O$. Then the mixture was stirred continuously for 2 hrs with magnetic stirrer and resulted in solution with yellowish precipitate. The precipitate was filtered using Whatman filter paper No 1 and washed repeatedly with deionized water followed by ethanol in order to remove the impurities. Finally, the precipitate was dried in an oven at 60˚C for overnight and grinded to fine powder using agate mortar. The yellow powder obtained from the above method was calcined at 450 ˚C for 1hrs. The same procedures were also used for the preparations of samples 2, 3, 4, 5, and 6 using different precursor to plant extract ratios.

3.3 Characterization Methods of Zinc Oxide Nanoparticles

3.3.1. Thermo Gravimetric Analysis (TGA)

Thermo gravimetric analysis (TGA) was carried out using a simultaneous DTA-TG apparatus (DTA-60H, Shimadzu Co., Japan) to determine the calcinations temperature of the synthesized material. About 14.907 mg of uncalcinated ZnO nanoparticle samples were placed in a platinum crucible on the pan of the microbalance and heated from room temperature to 800˚C , using Al_2O_3 as inert material

3.3.2 X-Ray Diffraction (XRD)

The crystalline structure of the green synthesized zinc oxide nanoparticles were investigated by the X-ray diffraction using X-ray diffractometer (Bruker D8 Advance Diffractometer). XRD spectrum was recorded from 10˚ to 80˚ with 2θ angles using CuKα (λ =1.54 Å) radiation operated at 40kV and 30 mA. Debye Scherrer's equation indicated in equation 1, was used to estimate the crystalline size

$$D = \frac{0.94\lambda}{\beta cos\Theta} \quad \text{.....(1)}$$

Where, D is the Crystallite size, λ is the X – ray source wavelength, β is the Full Width at Half Maximum (FWHM) of a peak, and Θ is the Bragg diffraction angle.

3.3.3 Fourier transform infrared(FTIR)

FTIR measurements were carried out to identify the possible bio molecules (functional group) responsible for the reduction of zinc oxide nanoparticles. FTIR was used to characterize the nanoparticles using the powdered nanoparticles samples by KBr pellet method. The absorbance maxima were scanned by FTIR at the wavelength of 400- 4000 cm^{-1} (Saranya, 2017).

3.3.4 Scanning Electron Microscopy(SEM)

In this research work JSM-6480 LV SEM machine was employed to study the morphology of synthesized nanoparticles. The experiment was performed at an accelerating voltage of 30 kV. The slide was coated with platinum and after the platinum coating, the SEM image was taken. SEM reveals information about the surface morphology of materials making up the sample. SEM provides detailed high resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back scattered electron signal. In SEM characterization, nanoparticles powder is mounted on a sample holder followed by coating with a conductive metal. The sample is then scanned with focused fine beam of electrons. The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface.

3.3.5. Energy Dispersive x-ray spectroscopy (EDX)

The chemical composition of the green synthesized zinc oxide nanoparticles was estimated by energy-dispersive X-ray spectroscopy (EDX). Energy dispersive X-ray spectroscopy (EDX) is an analytical technique used to determine the elemental analysis or chemical characterization of a sample through interaction in between sample and source of X-ray excitation. At rest, every atom has definite arrangement of electron in discrete energy levels around the nucleus. When high energy beam of radiation hit the sample (matter), it may excite an electron from inner shell and create a hole over there. To compensate this vacancy an electrons from outer shell migrate to inner shell, while the difference in energy between outer and inner shell may be generated in the form of X-ray. As, every element has a definite electronic arrangement, when the sample is being hit with high energy electromagnetic radiations it will generate characteristic X-ray this is like fingerprint for every element. The number and energy of X-rays emitted from sample can be measured by a detector.

3.3.6 UV-Visible Spectroscopy

The optical characterization of ZnO NPs was carried out using UV-Vis Spectroscopy (Perkin–Elmer lambda 950 UV/VIS/NIR/ spectrometer) in the wave length region of 260 nm - 1100 nm. First a dimethyl sulfoxide solvent was inserted in a 1 cm cuvette as a reference and the dimethyl sulfoxide solution and ZnO nanoparticles was placed in the second cuvette compartment for UV – Vis absorbance measurement.

3.4 Method for Antibacterial Activity

Materials used for antibacterial activity of pure zinc oxide nanoparticles were Nutrient-broth , Nutrient agar, petriplates, antibiotic discs, cotton swabs, zinc oxide nanoparticle sample, *Klebsella Pneumonia , Escherichia coli, Staphylococcus Aureus,* and *Entococcius Spps* bacteria. Disc diffusion method was used for antimicrobial activity of zinc oxide nanoparticles (Haritha, 2011).

3.4.1 Preparation of Inoculum

Antimicrobial studies of synthesized nanoparticles were studied on four bacterial strains viz. *Klebsella pneumonia, Escherichia coli, Staphylococcus aureus,* and *Entrococcius Spps*. by using disc diffusion method. First fresh broth was prepared into test tubes and autoclaved at 121˚C for 15 minutes then a single colony was taken from 24 hrs old culture with a sterile wire loop and inoculated in a prepared broth and kept in an incubator at 37˚C to get the maximum growth (Sana, 2017).

3.4.2 Inoculation of test plate

Nutrient agar was prepared and sterilized. The agar suspension within 15 min was used to inoculate plates by dipping a sterile cotton-wool swab into the suspension and remove the excess by turning the swab against the side of the container. Then the inoculums were spread evenly over the entire surface of the plate by swabbing in three directions. Then the plate was allowed to dry before applying antibiotic to discs (Haritha, 2011).

3.4.3 Preparation of Antibiotic Discs

Discs which were used for antimicrobial activity were nitrofuration, tetracycline, nalidixic acid, Vancomycin, amoxyclav, gentamycin, ciprofloxarin, erythromycin, ceftazdine and Methicillin. Discs were firmly applied to the surface of an agar plate that has previously been dried. The contact with the agar was even (Haritha, 2011).

3.4.4 Disc Diffusion Method for Antibacterial Activity

Antibacterial tests were carried out separately using plant extract, as prepared ZnO, and calcinated ZnO by the disc diffusion method using the suspension of bacteria spread on nutrient agar. The swab was dipped into the broth culture of the organism and gently squeezed the swab against the inside of the tube to remove excess fluid. The swab was used to streak agar plate or a nutrient agar plate for a lawn of growth. This was best accomplished by streaking the plate in one direction, then streaking at right angles to the first streaking, and finally streaking diagonal. The inoculated plates were incubated at appropriate temperature for 24 hrs. Antibiotic discs were placed on the surface of the agar using a dispenser that dispenses multiple discs at the correct distance apart, or by obtaining individual discs and placing them on the surface of the agar using

flame sterilized forceps. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Finally we measured diameters (mm) of zones of inhibition of the control strain and test with a ruler, calipers (Haritha, 2011) .

4. Results and Discussion

4.1Thermo Gravimetric Analysis (TGA)

The uncalcinated synthesized ZnO nanoparticles using *Kalanchoae petitiana* were characterized by using TGA/DTA (Differential Thermal Analysis). The weight losses of the investigated materials were analyzed. Figure 2 shows the thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) of the uncalcined ZnO nanoparticles using *Kalanchoae petitiana*. TGA curve shows mass loss of the sample whereas DTA curve indicates the energy gain or loss during the process. TGA/DTA transition shows a loss of 21.104 % up to 450 ˚C. No considerable loss observed after this up to 800 ˚C. This indicates that after 450 ˚C there is no weight loss for the synthesized ZnO nanoparticles and the nanoparticle is thermally stable. This temperature was used for calcination of the synthesized ZnO nanoparticles.

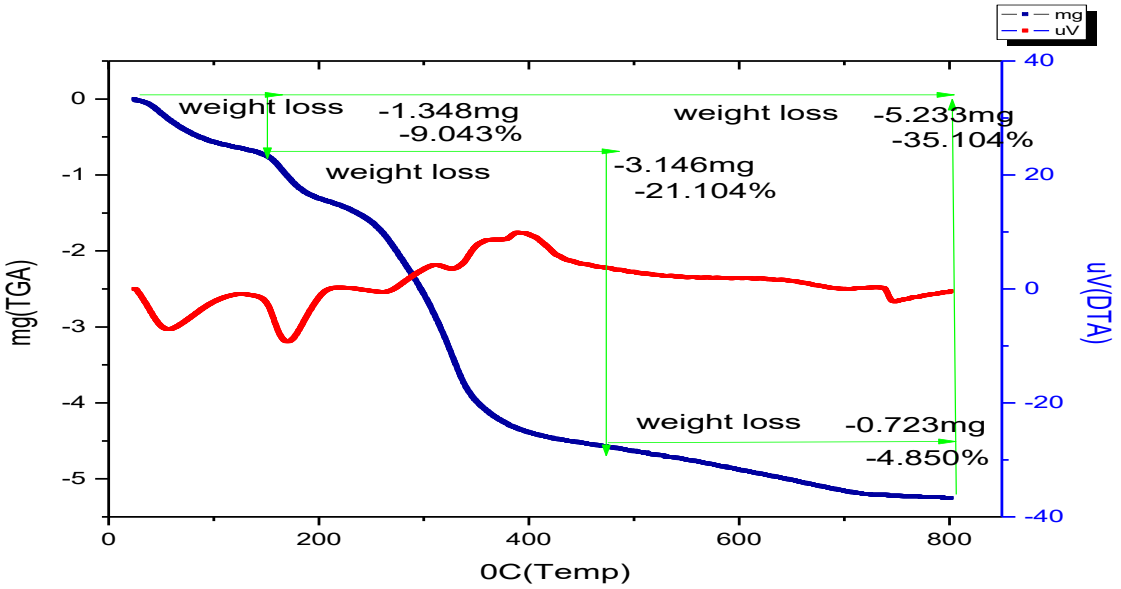


Figure 2: Thermal analysis result for uncalcined Zno Nanoparticle synthesized using Kalanchoae petitiana leaf extract

4.2. X-Ray Diffraction (XRD)

Figure 3 shows the XRD patterns of calcinated ZnO NPs synthesized using different ratios of aqueous solution of *Kalanchoae petitiana* plant extract and zinc acetate.

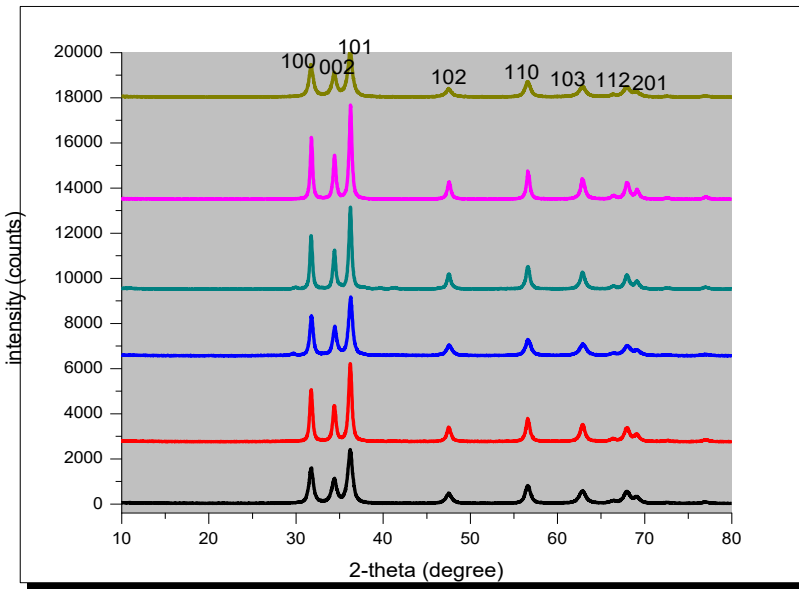


Figure3: XRD pattern of calcined ZnO nanoparticle synthesized using Kalanchoae petitiana and zinc acetate precursor

Table 1: FWHM values, crystalline sizes, d–spacing (d h k l) and Miller indices for calcined ZnO NPs synthesized from plant extract and zinc acetate precursor in different ratios

Samples	2Θ(degree)	FWHM values	Crystalline size	d-spacing	Miller indices
	31.7653	0.6183	13.9231	2.81472	100
	34.4129	0.69100	12.5913	2.60399	002
	36.2362	0.67400	12.2712	2.47703	101
	47.5276	0.80790	11.2248	1.91157	102
1:1	56.5861	0.72650	13.0450	1.62517	110
	62.8314	0.87200	11.1382	1.47781	103
	67.9638	0.88800	11.3125	1.37817	112
	69.0415	0.82000	12.2712	1.35926	201
	Average size	**12.22**			
	31.7652	0.41250	20.9855	2.81473	100
	34.4246	0.44610	18.5641	2.60313	002
	36.2431	0.45360	19.3067	2.47658	101
	47.5398	0.51640	17.6585	1.91111	102
	56.5945	0.52130	18.1000	1.62495	110
1:2	62.8593	0.61850	15.7391	1.47722	103
	67.9599	0.67630	14.7755	1.37824	112

	69.1015	0.54500	18.5604	1.35823	201
	Average size		**17.96**		
	31.8070	0.49240	17.4458	2.81113	100
	34.4680	0.54720	15.9121	2.59995	002
	36.2817	0.55550	15.5699	2.47403	101
1:3	47.5837	0.71310	12.7018	1.90945	102
	56.6332	0.68740	13.6604	1.62393	110
	62.9069	0.86490	11.2248	1.47622	103
	68.0383	0.95890	10.4173	1.37684	112
	69.0215	0.92660	10.8872	1.35961	201
	Average size		**13.47**		
	31.7671	0.35450	24.5424	2.81457	100
	34.4259	0.38490	21.6119	2.60303	002
	36.2523	0.38360	22.6250	2.47597	101
1:5	47.5470	0.46680	19.3067	1.91084	102
	56.6005	0.46180	20.3944	1.62479	110
	62.8636	0.53600	18.1000	1.47713	103
	67.9522	0.52670	19.0526	1.37837	112
	69.0447	0.59640	16.8372	1.35921	201
	Average size		**20.30**		
	31.7972	0.32270	26.8148	2.81197	100
	34.4464	0.37380	23.3548	2.60153	002
	36.2751	0.36380	24.1333	2.47447	101
2:1	47.5638	0.45120	20.1111	1.91020	102
	56.6299	0.40710	22.9841	1.62402	110
	62.8908	0.49340	19.8356	1.47656	103
	67.9847	0.50180	19.8356	1.37779	112
	69.1096	0.46730	21.6119	1.35809	201
	Average size		**22.33**		
	31.7629	0.59440	14.4800	2.81493	100
	34.4070	0.64110	13.5327	2.60442	002
	36.2252	0.62660	13.9231	2.47776	101

3:1	47.5127	0.75330	12.0667	1.91213	102
	56.5792	0.72200	13.0450	1.62535	110
	62.8460	0.82130	11.8689	1.47750	103
	67.9684	0.85330	11.7724	1.37808	112
	69.0215	0.86500	11.6774	1.35961	201
	Average size		**12.79**		

As indicated in Table 1 the ZnO NPs diffraction peaks observed at 2Ɵ of (1:1) 31.7653, 34.4129, 36.2362, 47.5276, 56.5861, 62.8314, 67.9638, 69.0415, for (1:2) 31.7652, 34.4246 ,36.2431, 47.5398,56.5945,62.8593, 67.9599,69.1015 , for (1:3) 31.8070,34.4680, 36.2817, 47.5837, 56.6332, 62.9069, 68.0383, 69.0215 , for (1:5) 31.7671,34.4259, 36.2523,47.5470, 56.6005, 62.8636, 67.9522, 69.0447 , for (2:1) 31.7972, 34.4464, 36.2751, 47.5638, 56.6299, 62.8908, 67.9847, 69. 1096 , and for (3:1) 31.7629, 34.4070, 36.2252, 47.5127, 56.5792, 62.8460, 67.9684, 69. 0215 are indexed as (100), (002), (101), (102), (110), (103), (112), (201) planes, which are in a good agreement with the literature report obtained (G.Bhumi, 2014) from the international center of diffraction Data card (JCPDS-36-1451), and confirmed the presence of zinc oxide particles. As observed from the XRD peaks no extra diffraction peaks were detected, indicating the phase purity of ZnO nanopowder (Akl M. Awwad, 2014). The sharp and narrow diffraction peaks suggest that the synthesized product has good crystallinity with relatively larger crystallites, and signifies the effects of experimental conditions on the nucleation and growth of the crystal (Joghee Suresh, 2018). The XRD pattern is identical to the hexagonal phase with Wurtzite structure with unit cell parameters a = 3.249 Å and c = 5.2 Å. The average crystalline size of nanoparticles was estimated using the Debye-Scherer formula. Accordingly the calculated value of crystalline size was found to be 12.22, 17.96, 12.27, 20.38, 22.33, and 12.79 for 1:1, 1:2, 1:3, 1:5, 2:1, and 3:1 ratios respectively.

As compared to ZnO nanoparticles synthesized in different ratios, XRD result showed that the average crystalline size of ZnO nanoparticles in 1:1 and 3:1 ratio has a smaller particle size. This difference may be due to the greater amount of leaf extract used during synthesis process resulting in more capping agents that in turn effectively stabilize the synthesized nanoparticles.

As we have seen from the data's given above in Table 1, the inconsistencies or lack of regularity in size of naoparticles were observed.This might depends on physical and chemical parameters

which mainly include temperature, pH, presence of specific enzyme, type of biomass, exposure time to substrate and the substrate concentration (Pramila, 2016) . Other authors explained the inconsistencies in the results of the nanoparticles size due to the sample preparation (Jose R.Peralta-velta, 2016)

Figure 4, shows the XRD patterns of uncalcinated ZnO NPs synthesized using 1:1 ratios of zinc acetate and plant extract

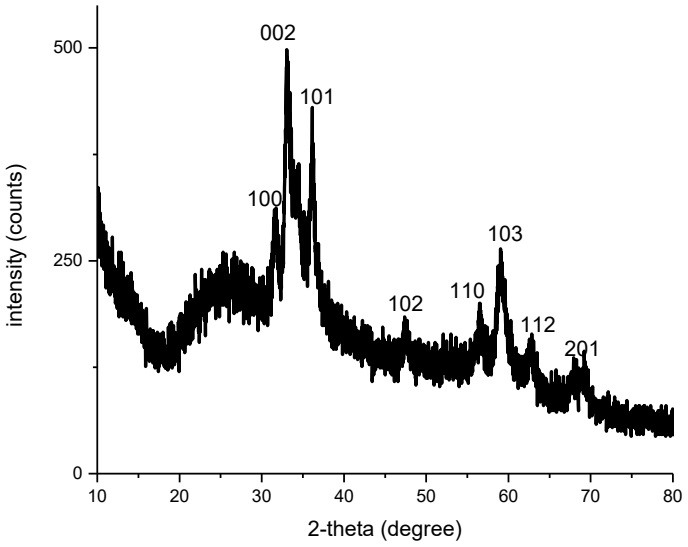


Figure 4: XRD pattern of uncalcined ZnO nanoparticle

The peak position with 2Θ values of uncalcined (1:1) ratio is 31.6825, 34.200, 36.0908, 47.4350, 56.4500,62.6800,67.8900,69.0300,which are indexed as,(100), (002), (101), (102), (110), (103),(112),(201) **,** which are in a good agreement with the literature report (S.Sai Ganesh, 2017) obtained from the international center of diffraction Data card (JCPDS-36-1451) . The location of the peaks was compared to the literature and the presence of zinc oxide particle was confirmed. No extra diffraction peaks were detected, indicating the phase purity of ZnO nanopowder (Akl M. Awwad, 2014) . The average crystalline size of the synthesized zinc oxide nanoparticle was calculated to be 10.8911 nm using debye-Scherrer equations. When we compared the XRD peak of uncalcined ZnO nanoparticles with that of calcined ZnO nanoparticles , calcined ZnO nanoparticles are more crystalline than that of uncalcined ZnO nanoparticles. This is due to the reason that as the temperature increases crystallity of the

nanoparticles also increases (Niranjan, 2014). And the size of uncalcined ZnO nanoparticles is smaller than calcined ZnO nanoparticles. This is because of the more phytochemicals available which stabilizes the formation of ZnO nanoparticles but calcined ZnO nanoparticles has lost those phytochemicals through calcinations at high temperature (Sana, 2017)

Table 2: FWHM values, crystalline sizes, d–spacing (d h k l) and Miller indices for uncalcined ZnO NPs with 1:1 ratios synthesized from plant extract and zinc acetate precursor

Sample	2Θ(degree)	FWHM values	Crystalline size	d-spacing	Miller indices
1:1 uncalcined	33.1400	0.8686	9.9862	2.70104	100
	34.3200	1.3800	6.2957	2.61080	002
	36.0908	0.8883	9.8503	2.48668	101
	47.4350	0.6900	13.1636	1.91509	102
	56.4500	0.9000	10.4928	1.62876	110
	62.6800	1.0400	9.3419	1.48102	103
	67.8900	0.9400	10.6471	1.37948	112
	69.0300	0.9800	10.2695	1.35946	201
	Average size		**10.00**		

4.3 Fourier transformed infrared (FTIR) spectroscopy analysis

Figure 5a shows the FTIR spectra of green synthesized calcinated ZnO nanoparticles. The spectra were recorded in the series of absorption band 4000 cm^{-1} to 500 cm^{-1}. The band at 3445 cm^{-1} is possibly attributed to the O-H stretching of hydroxyl group due to the absorbed water on the surface of the sample and the band at 2350 represents CO_2 molecule in the environment. C=O vibrations are observed at around 1642 cm^{-1} , N=O vibrations occur around 1396cm^{-1} and the peak at 1109 cm^{-1} occured due to the stretching vibration between CN bonds of amine . Moreover, absorption band at 501 cm^{-1} identifies the occurrence of ZnO nanoparticles. Thus, it can be observed from the FTIR spectrum that *Kalanchoae petitiana* leaf extract is significantly rich in different chemical groups such as –OH, -C=O,-N=O,-CN and amine. Further, the existence of hydroxyl group can also be probably due to the presence of phenols. The presence of phenols probably indicates the existence of polyphenolic tannins which also prevent aggregation, play an important role in the reduction and stabilization of ZnO nanoparticles. Thus,

physicochemical properties of *Kalanchoae petitiana* act as a bio template that prevents agglomeration.

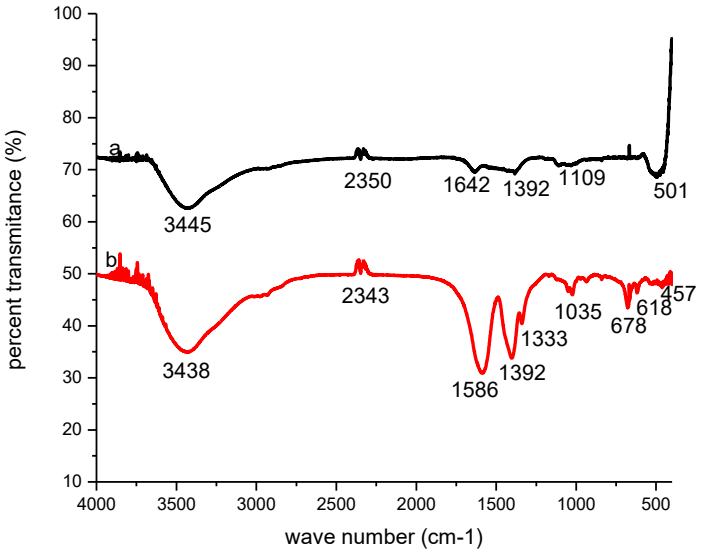
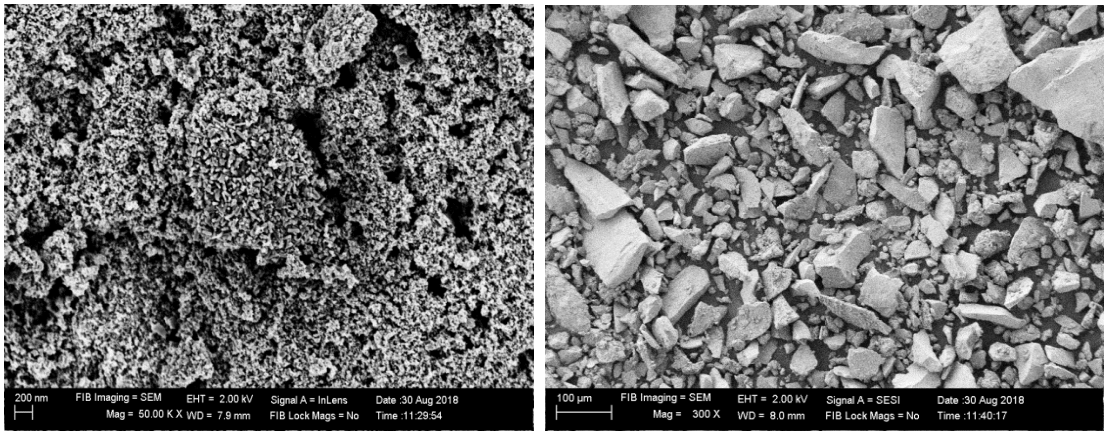


Figure 5: FT-IR spectrum for a) calcinated Zno NPs b) uncalcinated ZnO NPs

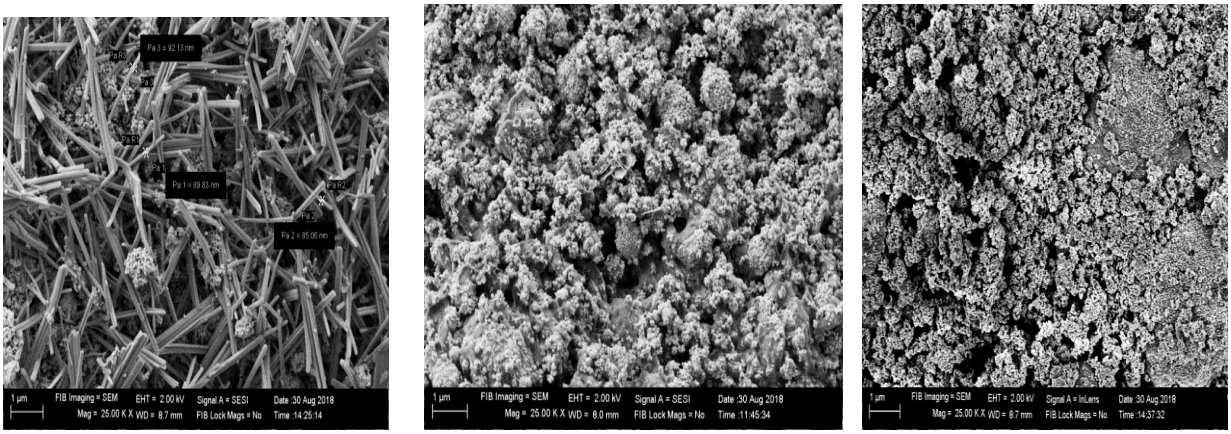
Figure 5b shows the FT-IR spectra of uncalcinated ZnO nanoparticle . In the spectrum, the wide absorption band around 3438 cm^{-1} is due to O-H stretching vibrations and the peak around 2343cm^{-1} reperesnts CO_2 in the enviroment.The peak around 1586 cm^{-1} is due to C=O stretching or asymmetric stretching vibration of –COOH,and that around 1392 cm^{-1} corresponds to bending vibration of –OH in phenols or symmetric stretching vibration of –COOH functional group.On the other hand, the peak around 1333 cm^{-1} indicates combination of C-O stretch and O-H bending.the peak around 1035 cm^{-1} represents C-O stretching vibrations from an alcohol and the peak around 678 cm^{-1} attributed to bending of CO_2 and that of peak 618 cm^{-1} represents C-H bending. As it can be seen the peak around 457 cm^{-1} indicates stretching vibrations of Zn-O bond. As compared to calcined ZnO NPs the FT-IR spectra of uncalcined ZnO NPs displayed a number of absorption peaks like,-OH,C=O,C-O,-COOH, and C-H. This might be due to chemically bonded functional groups of *Kalanchoae petitiana* leaf extract used as a capping agent during the synthesis of the materials which was not removed by washing with deionized water and ethanol.

4.4 Scanning electron Miroscope (SEM) analysis

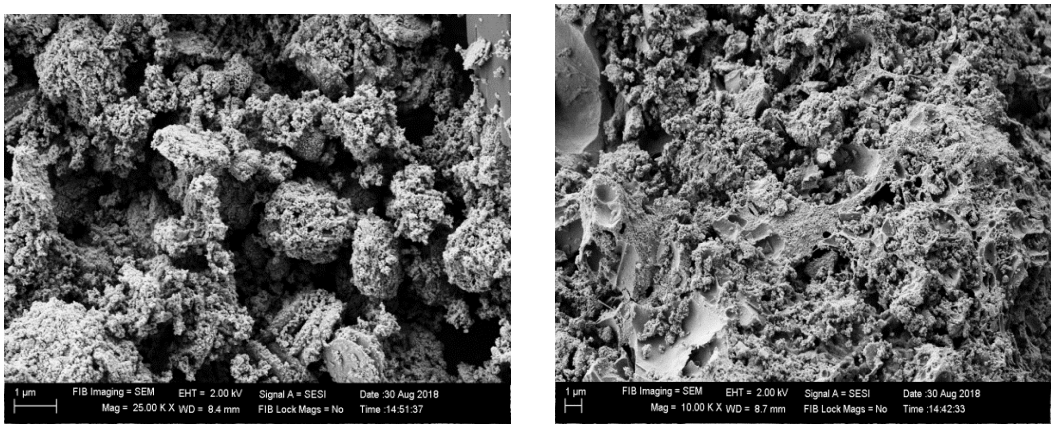
The morphology of ZnO nanoparticles were studied using SEM images. The surface morphology of the synthesized samples was viewed through high and low resolution scanning electron microscope. SEM images of ZnO NPs have different morphology as shown in Figure 7 (a-g). The morphology of uncalcined ZnO as indicated in Figure 7 (b) is not clearly identified. The SEM images of calcined ZnO indicate the agglomeration of particles that increases with the concentration of zinc acetate. The morphology of ZnO NPs synthesized from zinc acetate in different mixing ratio of reactants such as 1:1, 1:31:5, 2:1 and 3:1 have agglomerated nanorods, hollow structure and porous surface . The scanning electron microscope results confirmed that the grain sizes of the synthesized NPs were strongly dependent on mixing ratio of Zinc acetate & leave extracts under the same reaction conditions. The SEM image results also agreed with XRD results obtained, i.e. the crystal sizes in XRD with big size have also big shapes in SEM images. This indicates that ZnO NPs have successfully prepared using Zinc acetatedihydrate precursor and *kalanchoae petitiana* leaf extract.



(a) **(b)**



(c) **(d)** **(e)**



(f) **(g)**

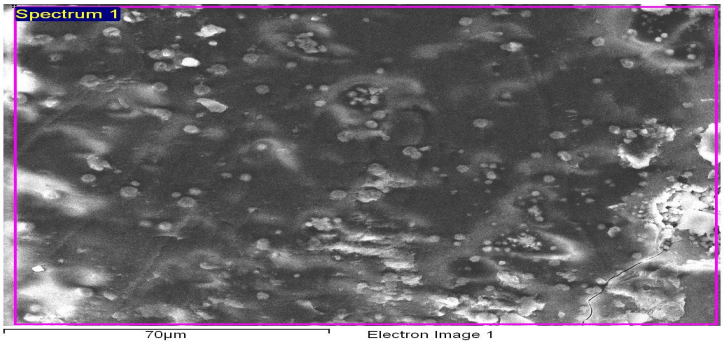
Figure 6: SEM images of ZnO Nanoparticles for: a) 1:1 calcinated B) 1:1 uncalcinated C) 1:2 d) 1:3 e) 1:5 f) 2:1 g) 3:1

4.5 Energy Dispersive X-ray spectroscopy (EDX) analysis

The composition of the samples was determined by energy dispersive X-Ray spectroscopy (EDX) using scanning microscope. The energy dispersive X-ray analysis of all ZnO nano particles using zinc acetate at different mixing ratios are shown in Figures 8 to 21. It is evident from the X-ray patterns of all the samples in which Zn and O are found in the respective spectrum.

From the X-ray patterns of Figures (7 -13), the sample contains largely the elements Zn and O in the spectrum with weight percentage of 97.88%, 75.98%, 85.99%%, 94.67%, 93.89%, 96.90%, 95.64%,and atomic percentage of 95.47%, 61.50%, 71.75%, 88.30%, 82.25%, 92.68% and 90.28 for 1:1,1:1un,1:2,1:3,1:5,2:1,and 3:1ratios respectively . This shows that there is very small amount of foreign material present in the spectrum and hence this confirms the purity of the sample of ZnO NPs synthesized from zinc acetate dihydrate

Element	Weight %	Atomic%
O k	27.78	59.03
Zn k	70.10	36.46



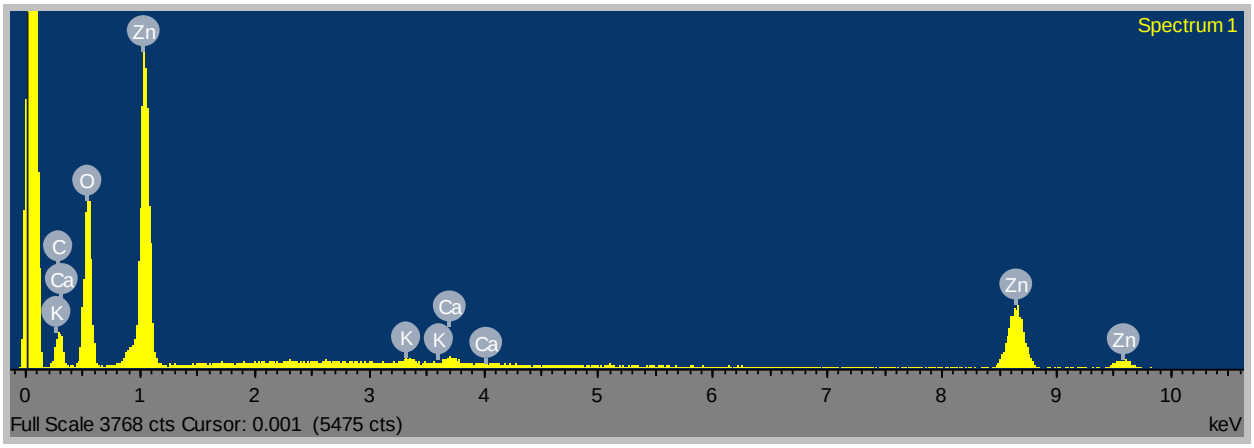
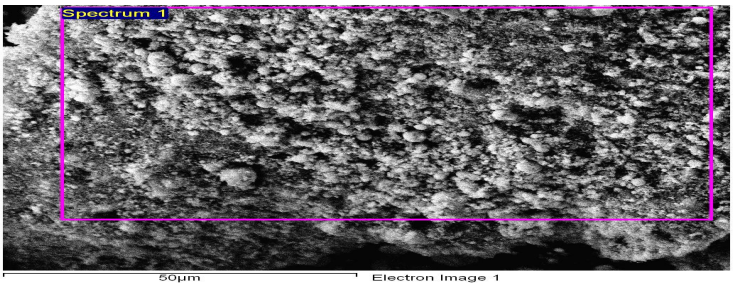


Figure 7: EDX spectrum of Zno nanoparticles synthesized from kalanchoae petitiana leaf extract and zinc acetate precursor in (1:1) ratio uncalcinated

Element	Weight %	Atomic %
O k	40.90	50.81
Zn k	35.18	10.69



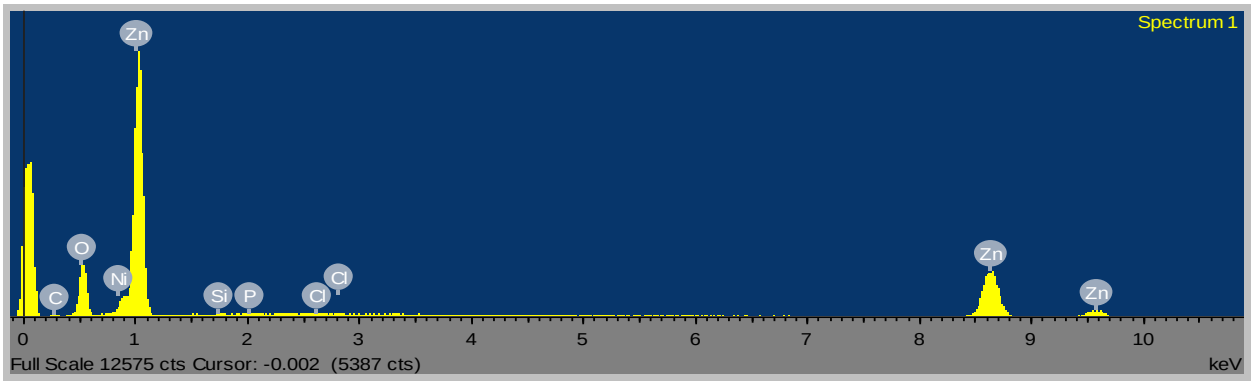
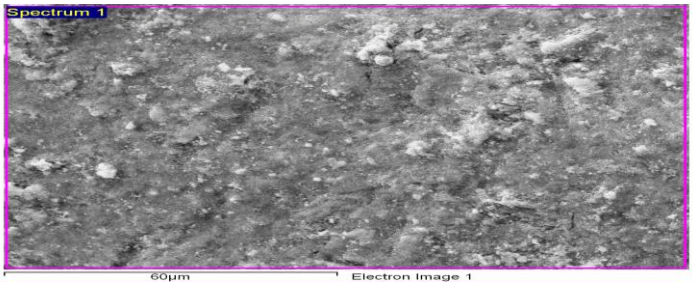


Figure 8: EDX spectrum of Zno nanoparticles synthesized from kalanchoae petitiana leaf extract and zinc acetate precursor in (1:1) ratio calcinated

Element	Weight %	Atomic %
O k	32.49	51.14
Zn k	53.50	20.61



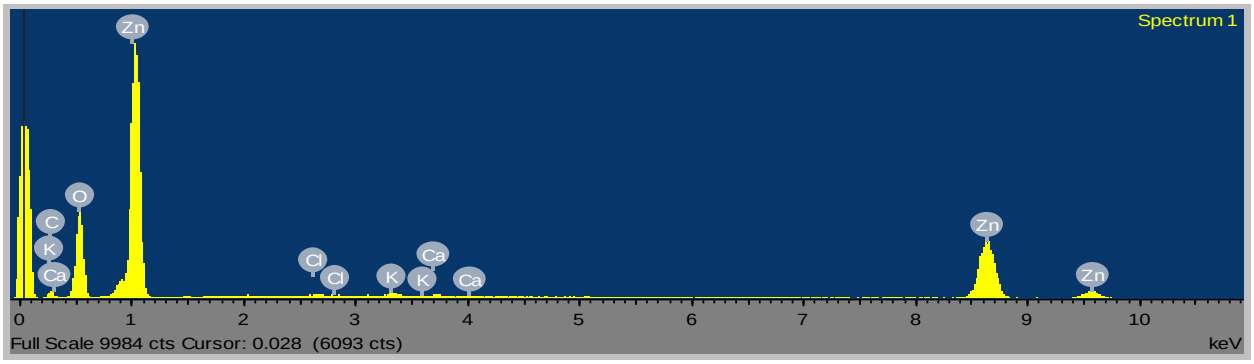
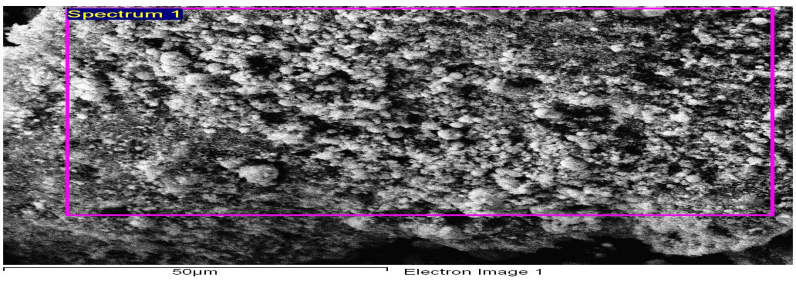


Figure 9: EDX spectrum of Zno nanoparticles synthesized from kalanchoae petitiana leaf extract and zinc acetate precursor in (1:2) ratio

Element	Weight %	Atomic %
O k	23.59	50.82
Zn k	71.08	37.48



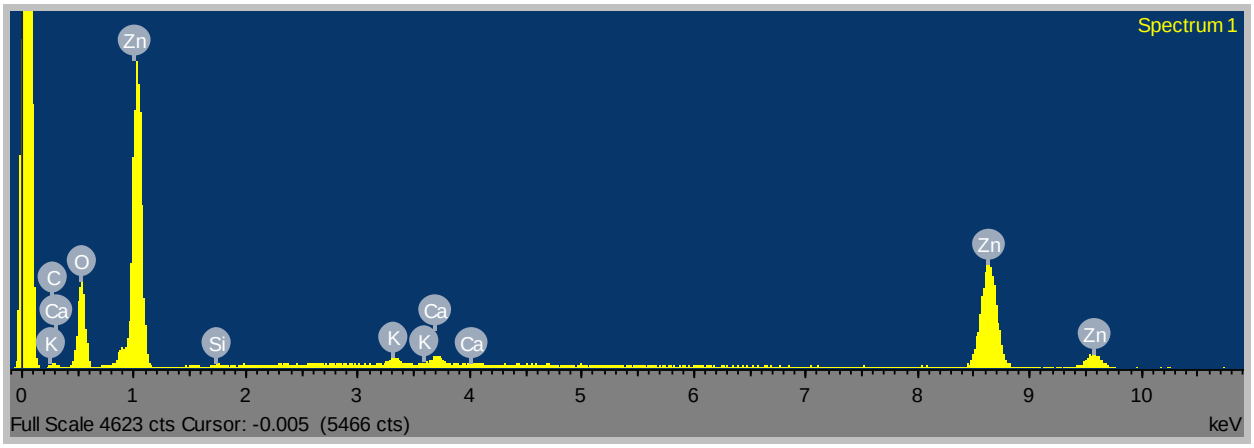
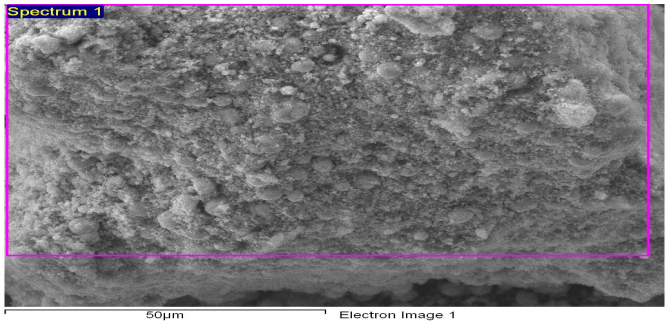


Figure 10: EDX spectrum of Zno nanoparticles synthesized from kalanchoae petitiana leaf extract and zinc acetate precursor in (1:3) ratio

Element	Weight %	Atomic %
O k	**18.38**	**41.01**
Zn k	**75.51**	**41.24**



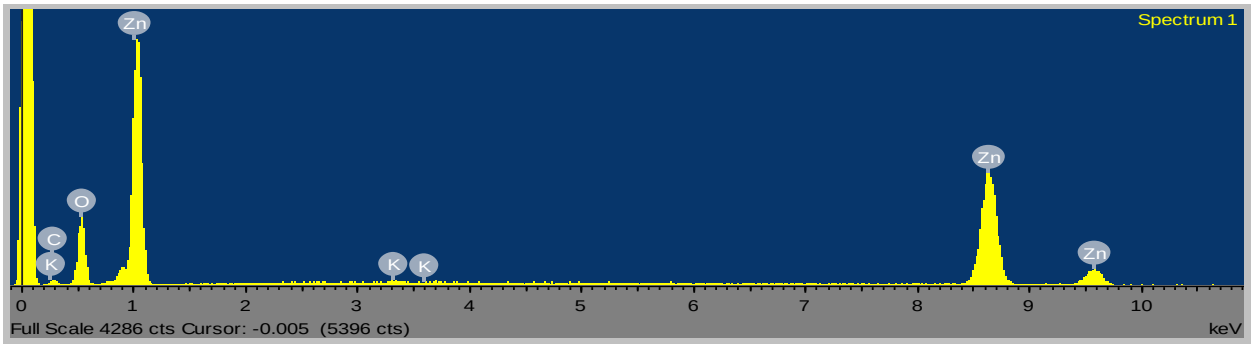
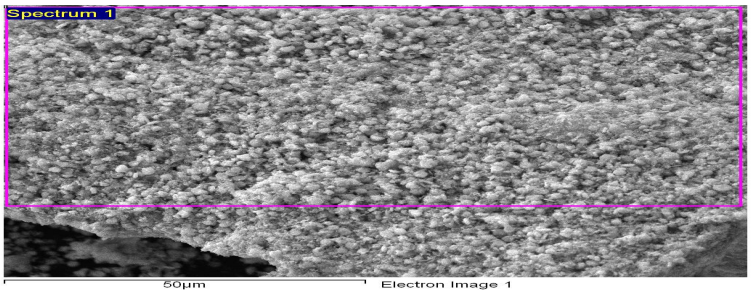


Figure 11: EDX spectrum of Zno nanoparticles synthesized from kalanchoae petitiana leaf extract and zinc acetate precursor in (1:5) ratio

Element	Weight %	Atomic %
O k	33.74	63.56
Zn k	63.16	29.12



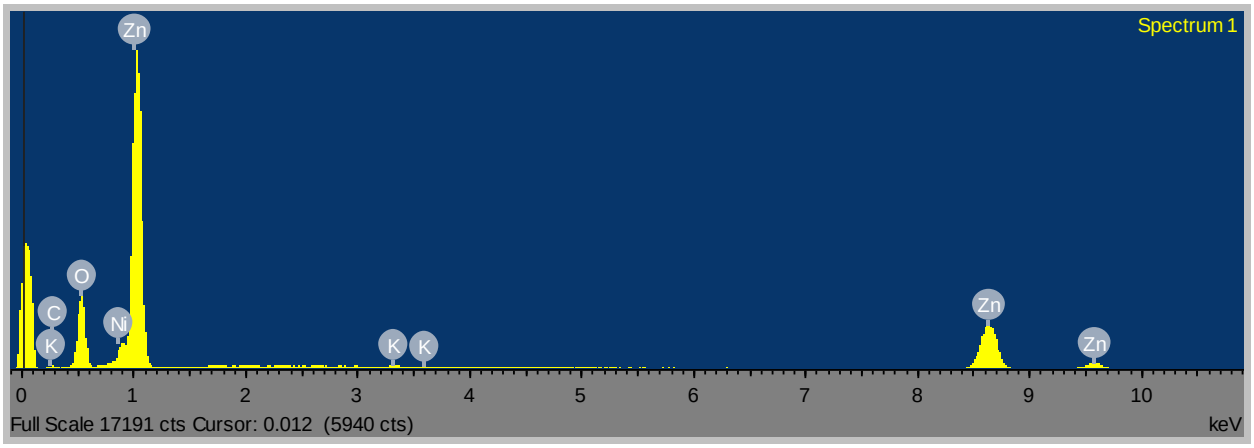
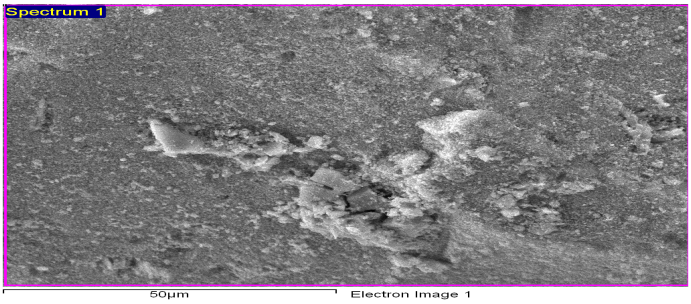


Figure 12: EDX spectrum of Zno nanoparticles synthesized from kalanchoae petitiana leaf extract and zinc acetate precursor in (2:1) ratio

Element	Weight %	Atomic %
O k	14.51	38.11
Zn k	81.13	52 .17



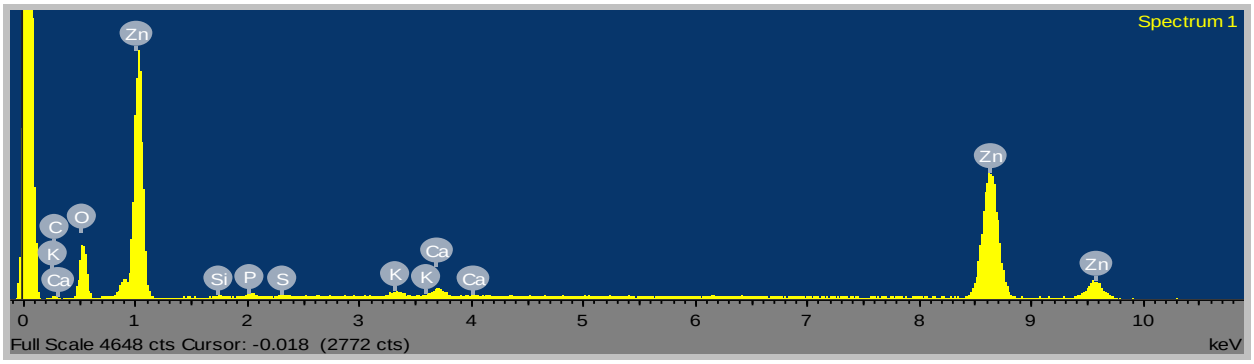


Figure 13: EDX spectrum of Zno nanoparticles synthesized from kalanchoae petitiana leaf extract and zinc acetate precursor in (3:1) ratio

4.6 Uv-Visible spectroscopy analysis

The UV-Vis spectra of the synthesized ZnO NPs by using different ratios of leaf extract and precursor salt were shown in Figure 20.

Figure 14 shows the UV visible spectra of calcined and uncalcined ZnO NPs sample using zinc acetate. It depicts the absorption spectrum of the green synthesized ZnO NPs with the absorption peak from 355-373 nm. The excitonic absorption peak observed due to zinc oxide nanoparticles lies much below the band gap wavelength of bulk zinc oxide 388 nm (Singh, 2009). The wavelength of (355-373nm) absorption peak confirms the occurrence of blue shifted absorption spectrum with respect to the bulk value (388 nm) of the ZnO NPs, due to the quantum confinement effect, which is good agreement of the previous report (Elumalai, 2015). The spectrum shows the characteristic absorbance peak of ZnO nanoparticles, which confirmed the presence of ZnO nanoparticles.

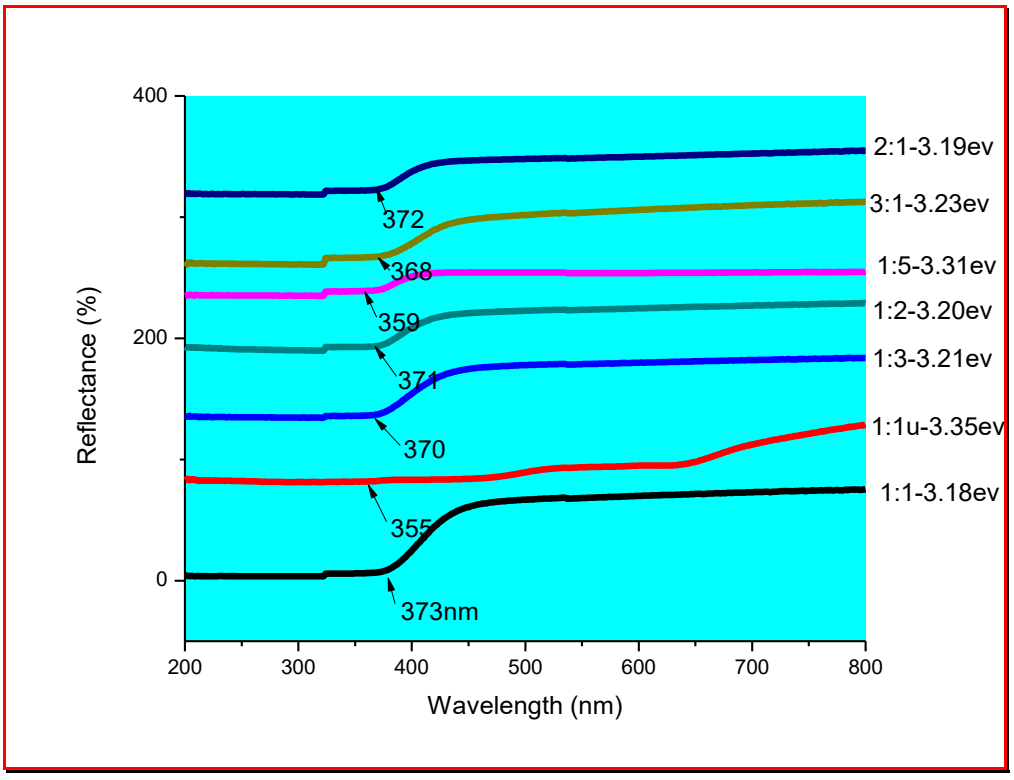


Figure14: Reflectance spectra of the synthesized ZnO NPs using 1:1, 1:1uncalcinated, 1:3, 1:2,1:5,2:1 and 3:1 leaves extract to precursor salt ratios by volume

The band gap energy (Eg) of ZnO was obtained from the wavelength value corresponding to the intersection point of the vertical and horizontal part of the spectrum, using the equations:

$$E_g = \frac{hc}{\lambda} ; E_g = \frac{1240}{\lambda}\ eV$$

The band gap energy corresponds to the absorption limit and can be roughly evaluated by the above relation. Where, E_g is the band gap energy (eV), h is the Planck's constant (6.626 x 10-34 J s), C is the light velocity (3 x108 m/s) and λ is the wavelength (nm). From Figure 22, the absorption edges are positioned at 373,355,370,371,359,368,and 372 respectively for1:1,1:1un,1:3,1:2,1:5,3:1, and 2:1ratios with the corresponding band gap value of 3.18,3.25,3.21,3.20,3.31,3.23,and3.19.From this result we can observe that the band gap energy decreases with an increase in leaf extract concentration and increases with an increase in concentration of precursor.

4.7 Antibacterial activities of ZnO Nanoparticles

The aim of the present study is to determine the antibacterial activity of ZnO nanoparticles against Gram-positive and Gram-negative bacteria. *Staphylococcus aureus (S.aureus), Escherichia coli (E. coli), klebsella*, and *Entrococcius spp* were used as test microorganisms. The antibacterial activity performance of ZnO nanoparticles was done by using disc diffusion method. The effects of particle size and concentration of plant extract and precursor ratio on the antibacterial activity of ZnO nanoparticles were studied using this method. These tests were performed in nutrient broth and nutrient agar following standard methods. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values.

Table 10 shows zone of inhibition (mm) of the tested samples of ZnO NPs using zinc acetate and *Kalanchoe petitiana* leaf extract at different ratios , plant extract alone ,and uncalcined ZnO using (1:1) ratio against four different bacterial strains were also given.

Table3: Zone of inhibition (mm) of ZnO NPs against gram positive and gram negative bacteria

Sample	Nanoparticles size	*E.coli*	*Klebsella pneumonia*	*S.aureus*	*Entrococcius spp*
1:1	12.22	8mm	7mm	8mm	6mm
1:2	17.96	6mm	6mm	6mm	6mm
1:3	13.47	6mm	6mm	7mm	6mm
1:5	20.30	6mm	6mm	6mm	6mm
2:1	22.33	7mm	8mm	8mm	6mm
3:1	12.79	11mm	12mm	11mm	6mm
1:1(uncalcinated)	10.00	13mm	11mm	9mm	6mm
Plant extract		6mm	8mm	6mm	6mm
Gentamycin		12mm	8mm	14mm	8mm

In Table 3 ZnO NPs synthesized from *kalanchoae petitiana* were sensitive to gram- negative bacteria (E.coli and klebsella pneumonia) and gram- positive bacteria (*S.aureus*) but they were not active against *Entrococcius spp.*

According to Table 3, ZnO NPs for 1:1 ratio has zone of inhibition 8mm for gram- negative bacteria (*E.coli*) and gram positive bacteria (*S.aureus*), but gram- positive bacteria (*Entrococcius spp*) showed resistance to this NPs. Both gram- negative and gram- positive bacteria were resistant to ZnO NP synthesized at 1:2 and 1:5 ratios.

ZnO NPs at 1:3 ratio showed zone of inhibition of 7mm for gram- positive bacteria (*S.aureus*) but it is resistant to the other three bacterias.ZnO NPs at 2:1 and 3:1 ratios were sensitive to gram-negative bacteria(*E.coli and klebsella pneumonia*) and gram- positive bacteria (*S.aureus*). but resistant to gram-positive bacteria *Entrococcius spp*.

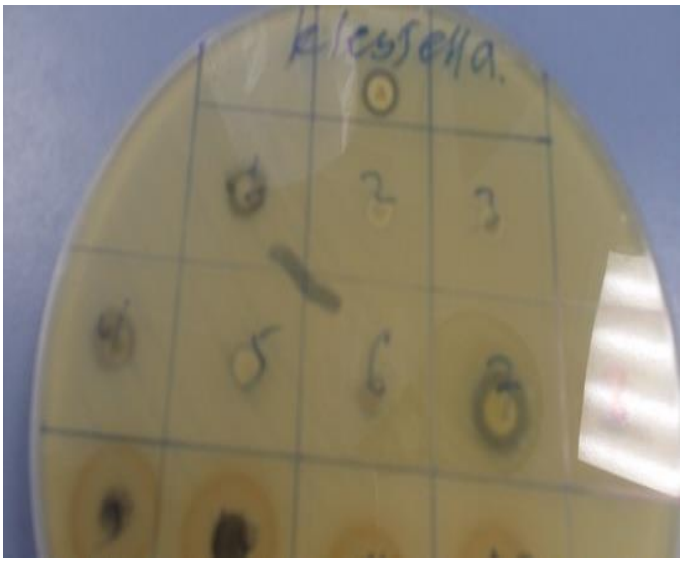
ZnO NPs at 3:1 ratio has greatest zone of inhibition (11mm,12mm,11mm) indicating that it has promissing antibacterial activity against gram-negative bacteria(*E.coli and klebsella pneumonia*) and gram- positive bacteria (*S.aureus*) but resistant to gram- positive bacteria (*Entrococcius*

spp). As the amount of leaf extract ratio increases the antibacterial activities of ZnO nanoparticles slightly increases.This could be due to decrease in the size of ZnO nanoparticles with increase in amount of leaf extract resulting in high surface area to volume ratio.

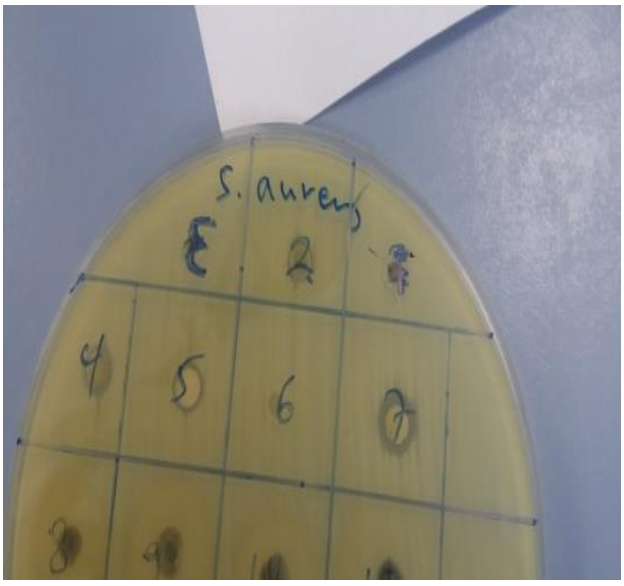
On Table 3, uncalcined ZnO NPs using (1:1) ratio has better zone of inhibition than calcined ZnO NPs. This indicates that uncalcined ZnO NPs have greater antibacterial effect than calcined one. This is because in uncalcined ZnO NPs there are some bioactive molecules which come from the leaf extract and offers a better effects to enhance the antibacterial properties of the synthesized *E.coli* ZnO nanoparticles.

From The table 3, zone of inhibition for plant extract is 8mm for gram negative bacteria (*klebsella Pneumonia*), and resistant against gram-negative bacteria , and gram-positive bacterias (*S.aureus and Entrococcius spp*). Uncalcined ZnO NP in 1:1ratio is sensitive towards both gram-negative (*E.coli and klebsella*) and gram-positive bacteria (*S.aureus and Entrococcius spp*). Calcined ZnO NP in 1:1 ratio has zone of inhibition 8mm for gram- negative bacteria (*E.coli*) and gram-positive bacteria (*S,aureus*) , and 7mm for gram-negative bacteria (*klebsella Pneumonia*), but it is resistant towards gram-positive bacteria (*Entrococcius spp*) . This indicates that the antibacterial effects of uncalcined ZnO NP is better than both calcined ZnO NP and plant extract. Possibly , there are two different reasons behind these results. Smaller particle size of uncalcined ZnO nanoparticle caused better penetration and better activity due to higher surface to volume ratio. On the other hand , larger particle size of calcined ZnO nanparticle showed less penetration and lower activity due to reduced surface to volume ratio than that of uncalcined ZnO nanoparticles. Nature of synthesized ZnO nanoparticles was another reason. Uncalcined ZnO nanoparticles get stabilized by several polyphenols and flavonoids which inhibit the growth of bacteria. But calcined ZnO nanoparticles calcinated at 450 ˚c for 2 hrs lost the associated bioactive stabilizer compounds (Niranjan, 2014).

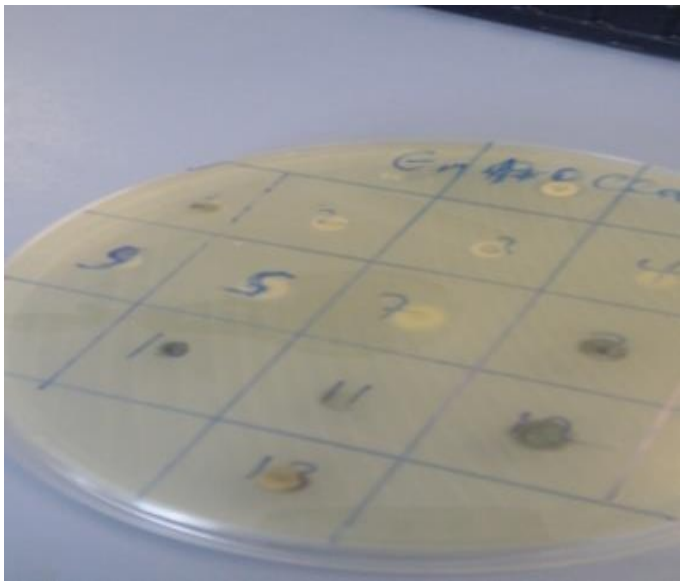
(a)



(b)



(c)



(d)

Figure15: Inhibition zone of ZnO NPs against a)E.coli b) Klebsella pneumonia c) S.aureus d) Entrococcius Spp

5. Conclusion and Recommendation

5.1 Conclusion

Zinc oxide nanoparticles were successfully synthesized by using biological green synthesis method using *Kalanchoae petitiana* leaf extract and characterized using XRD, FTIR,SEM,and UV-visible method.

The XRD confirms the formation of hexagonal wurtzite structure and the crystalline size (12-22nm) of the synthesized ZnO . FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by the leaf extract and the vibrational stretching around 501 cm^{-1} absorption peak supports the formation of ZnO nanoparticles. The SEM image analyses revealed as the ratio of the leave extract and zinc acetate affects the grain size of synthesized zinc oxide nanoparticles. The EDX studies also confirmed the composition of the synthesized nanoparticles were Zinc and oxygen.

The synthesized ZnO nanopartiles with 3:1 ratio showed significant antibacterial activities against gram positive bacteria and against gram negative bacteria including, E.*coli, klebsella pneumona, S.aureus, and Entrococcius spp*. All the synthesisized nanoparticles have not sensitive to *Entrococcius spp* since this bacterium has greatest resistance towards drugs. ZnO nanoparticles have significantly higher antibacterial effect on *Klebsella pneumonia* indicating that the synthesized ZnO NPs are more sensitive to gram negative bacteria. Compared with Gram negative bacteria, Gram positive bacteria are most resistant against drug . From the result obtained it suggested that the green synthesis is a better choice due to ecofriendliness.

5.2 Recommendation

Regarding future aspect of ZnO nanoparticles, one can conclude that although there are various reports regarding ZnO nanoparticles synthesis with various shapes and sizes, there is still a lack of quality synthesis in terms of crystallinity, size, and shape of the particles and the non-use of organic solvents, which may hinder its possible application in biomedical sciences (or in evaluation its antibacterial activity). Application of ZnO nanoparticles in the biological realm requires high quality ZnO nanoparticles in aqueous solution at neutral pH and physiological temperature, because biomolecules are very sensitive to changes in temperature and pH. During

the synthesis of ZnO nanoparticles from zinc acetate dihydrate and leaf extract, then washing the sample several times after calcination should be needed in order to remove the impurities from the products.In addition,even though it is costy in preparing different ratios of nanoparticles it is better to perform repeated tests instead of deciding in one test to get good results.

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