PROJECT REPORT

ON

GREEN CHEMICAL APPROACH FOR THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES: CHARACTERIZATION AND ANTIBACTERIAL STUDY

Submitted by

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Under the guidance of

Ms. PRIYA K
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In partial fulfilment for the award of the

Bachelor's Degree in Chemistry

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH
ST. TERESA'S COLLEGE (AUTONOMOUS)
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B.Sc. CHEMISTRY PROJECT REPORT

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This is to certify that the project work entitled "**GREEN CHEMICAL APPROACH FOR THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES: CHARACTERIZATION AND ANTIBACTERIAL STUDY**" is the work done by **ALEENA LIL PAUL, DILNA P K, SANDRA RAJU & ANU M**

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CERTIFICATE

This is to certify that the project work entitled "**GREEN CHEMICAL APPROACH FOR THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES: CHARACTERIZATION AND ANTIBACTERIAL STUDY"** is the work done by **ALEENA LIL PAUL, DILNA P K, SANDRA RAJU & ANU M** under my guidance in the partial fulfilment of the award of the Degree of Bachelor of Science in Chemistry at St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

Ms. PRIYA K
Project Guide

DECLARATION

We hereby declare that the project work entitled "**GREEN CHEMICAL APPROACH FOR THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES: CHARACTERIZATION AND ANTIBACTERIAL STUDY"** submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is a record of an original work done by us under the guidance of **Ms PRIYA K, GUEST FACULTY,** Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfilment of the requirements for the award of the Degree of Bachelor of Science in Chemistry.

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Chapter 1

Introduction

Nanotechnology is a newly emerging science as well as technology which deal with the study of materials in their nano size range (1-100 nm). In the nano size range, molecules are able to exhibit several unique properties that can be employed in a variety of scientific fields. Nanoparticles have already advanced a number of scientific fields, including textiles industry, food industry, biomedicine, information and communication technology and agriculture.

Metal oxide nanoparticles offer a bright and wide-ranging future for the biomedical field with the development of nanomaterials, particularly for antibacterial, anticancer drug/gene delivery, cell imaging, biosensing, and other applications. Due to their unique physical and chemical characteristics zinc oxide nanoparticles (ZnO NPs) emerged as one of the most significant metal oxide nanoparticles and are widely employed in various fields. It is generally known that zinc is an essential trace element which extensively exists in all body tissues. Compared with other metal oxide NPs, ZnO NPs with the comparatively inexpensive and relatively less toxic property exhibit excellent biomedical applications, such as anticancer, drug delivery, antibacterial, and diabetes treatment; anti-inflammation; wound healing; and bioimaging.

The Green nanotechnology is arising as a new branch of nanotechnology where plant mediated synthesis of nanoparticles had already got a wide attention due to its simplicity, eco-friendly nature, rapid rate of synthesis and less cost. The chemical synthesis followed by stabilization synthesized

ZnO NPs cause release of toxic by-products which are harmful to the ecosystem. Thus, plant mediated synthesis or green synthesis has emerged as the best alternative to chemical synthesis.[1]

1.1 NANOPARTICLES

A nanoparticle is a microscopic particle with a size between 1 and 100 nanometres. The physical and chemical characteristics of nanoparticles, which are invisible to the human sight, might differ significantly from those of their bigger material equivalents.

As a material gets closer to the atomic scale, its properties alter. When compared to bulk materials nanoparticles have a relatively significant surface area to volume ratio because of their extremely small size and the performance of the material is dominated by its surface atoms. As they are small enough to contain their electrons and induce quantum effects, nanoparticles can have unexpected optical, physical, and chemical capabilities.[2]

Nanoparticles can be classified according to their composition, dimension, application, physical and chemical properties etc. Some of them are:

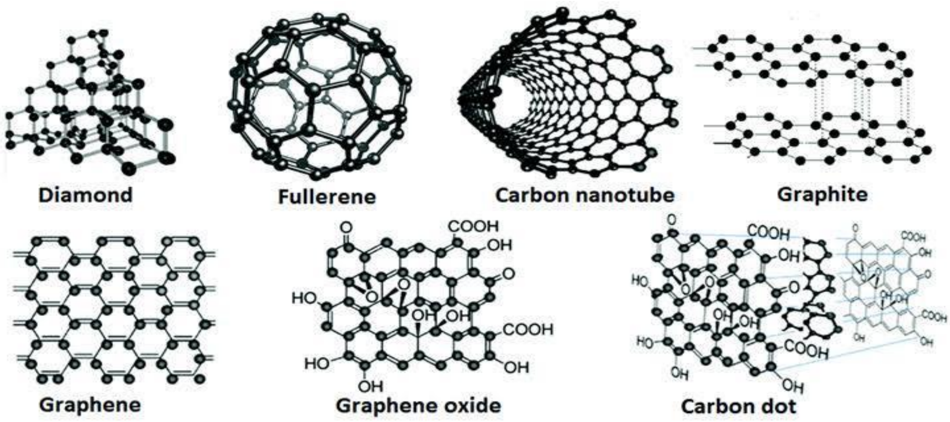
1.2 CLASSIFICATION OF NANOPARTICLES

1.2.1 Based on composition

Based on composition, nanoparticles are mainly classified into four.

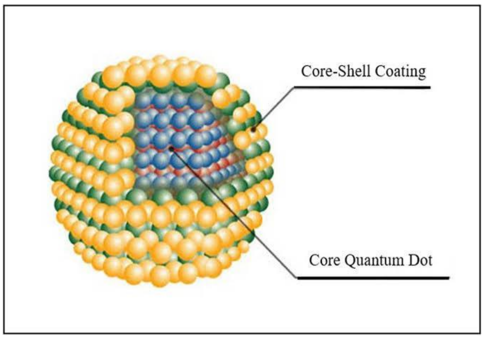
1.2.1.1 Carbon based nanoparticles

These nanomaterials are primarily made of carbon and are most frequently found as hollow spheres, ellipses, or tubes. Carbon nanomaterials that are spherical, elliptical, or cylindrical are referred to as fullerenes. Many potential applications for these particles exist, such as improved films and coatings, stronger and lighter materials, and electronic applications.[3]



1.2.1.2 Metal based nanoparticles

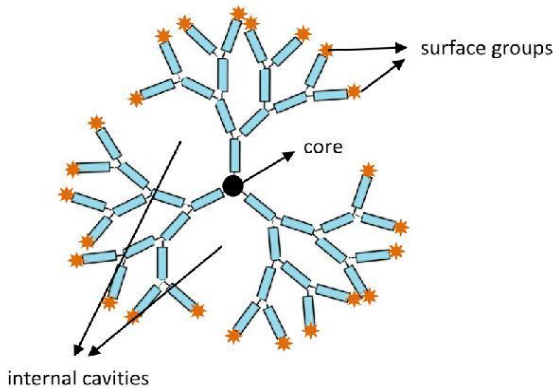
These nanomaterials include quantum dots, nanogold, nano silver and metal oxides such as zinc oxide, titanium dioxide etc. A quantum dot is a densely packed semiconductor crystal with a size of a few nanometres to a few hundred nanometres that contains hundreds or thousands of atoms. Quantum dots' optical characteristics alter depending on their size.[3]



Quantum Dot

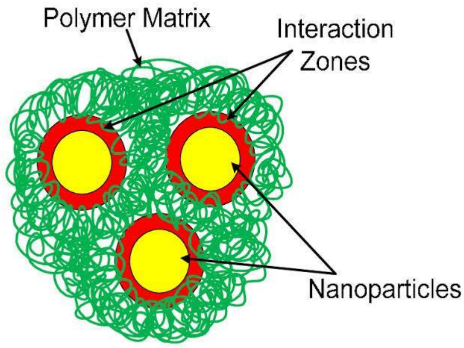
1.2.1.3 Dendrimers

These nanomaterials are nanosized polymers built from branched units. The surface of a dendrimer has numerous chain ends, which can be tailored to perform specific chemical functions. This property could also be useful for catalysis. Also, because three-dimensional dendrimers contain interior cavities into which other molecules could be placed, they may be useful for drug delivery.[3]



1.2.1.4 Composites

Composites combine nanoparticles with other nanoparticles or with larger, bulk-type materials. Nanoparticles, such as nanosized clays, are already being added to products ranging from auto parts to packaging materials, to enhance mechanical, thermal, barrier, and flame-retardant properties. [3]



A polymeric nanocomposite

1.2.2 Based on dimensions

The arrangement of atoms determines the characteristics of the nanoparticles. Based on dimension nanoparticles are divided into four as:

1.3.1 Zero dimensional nanoparticles

Zero dimensional nanoparticles have dimensions at the nanoscale that are 100 nm or larger. They have the following crucial characteristics: they are amorphous or crystalline, single crystalline or polycrystalline, and made of one or more chemical components. They have different shapes and forms, are contained in the matrix or exist separately, and have structures made of ceramic, metallic, or polymeric materials. Important examples are carbon dots, fullerenes, carbon nanotubes, graphene and graphene oxide.[4]

1.3.2 One dimensional nanoparticles

One-dimensional nanoparticles fall outside the nanoscale. It leads them to look like needle like-shaped nanomaterials. They include nanotubes, nanorods, nanowires.

1-D nanoparticles bear the following characteristics: They are amorphous or crystalline, Single crystalline or polycrystalline and they are chemically pure or impure. Also, they have standalone elements or embedded within another medium. They include metallic, ceramic, or polymeric nanoparticles.[4]

1.3.3 Two dimensional nanoparticles

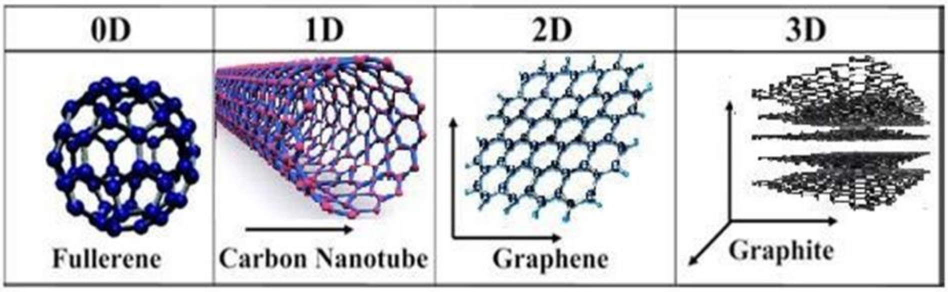
In 2 dimensional nanoparticles, two of the dimensions does not confine to the nanoscale. They exhibit 2-D shapes like a plate. Important examples for two-dimensional nanomaterials include nanofilms, nanolayers, graphene, nano coatings, and nanofilms.

They are amorphous or crystalline in arrangement and used as a single layer or as multilayer structures. They are made up of various chemical compositions and are deposited on a substrate or integrated into a surrounding. They include metallic, ceramic, or polymeric nanoparticles.[4]

1.3.4 Three dimensional nanoparticles

Three-dimensional nanomaterials do not confine to any shape, but they are like bulk nanomaterials and arranged in three dimensions. These materials have the arbitrarily sizes above 100 nm. Major examples are nanowires, nanotubes, and multi-nanolayers.

Common characteristics of 3D nanoparticles include: -Nanocrystalline arrangement formation of nanoparticles can exist as bulk nanomaterials. These materials exist in multiple arrangements, and crystals structures, for this reason, different orientations of existence are possible in 3-D structures. Based on the nanoscale arrangements, most of the 3-D nanoparticles can show different dispersion characteristics. These structures also exist with a bundle of nanotubes and multi-nanolayers.[4]



1.3. GENERAL PROPERTIES OF NANOPARTICLES

The principal parameters of nanoparticles are their shape, size, and the morphological sub-structure of the substance. Nanoparticles are presented as an aerosol (mostly solid or liquid phase in air), a suspension (mostly solid in liquids) or an emulsion (two liquid phases).
The important physical and chemical properties of nanomaterials are:

1.3.1 Physical Properties

- Size, shape, specific surface area, aspect ratio
- Agglomeration/aggregation state
- Size distribution
- Surface morphology/topography
- Structure, including crystallinity and defect structure
- Solubility. [5]

1.3.2 Chemical Properties

- Structural formula/molecular structure.
- Composition of nanomaterial (including degree of purity, known impurities or additives).
- Phase identity.
- Surface chemistry (composition, charge, tension, reactive sites, physical structure, photocatalytic properties, zeta potential).
- Hydrophilicity/lipophilicity.[6]

1.4 APPLICATIONS OF NANOPARTICLES

1.4.1 Next-Generation Computer Chips

The ability to fabricate nano-transistors allows integration of more transistors per space, thus enhancing chip performance, particularly with using highly conductive nanomaterials like carbon nanotubes.[7]

1.4.2 Automobiles with Greater Fuel Efficiency

Existing automobile engines waste substantial amounts of gasoline, thus adding to environmental pollution by burning the fuel incompletely. A traditional spark plug is not made to burn the gasoline totally and efficiently. This problem is amplified by faulty, or worn-out, spark plug electrodes. Since nanomaterials are harder, stronger and considerably more erosion-resistant and wear-resistant, they are currently being proposed for use as spark plugs.[7]

1.4.3 High-Sensitivity Sensors

Sensors use their sensitivity to detect the variations in different parameters they are programmed to measure. The parameters include chemical activity, thermal conductivity, electrical resistivity, magnetic permeability, and capacitance. All of these parameters depend a lot on the microstructure (grain size) of the materials used in the sensors. A variation in the sensor’s environment is revealed by the sensor material’s physical, chemical, or mechanical characteristics, which is leveraged for detection. [7]

1.4.4 High-Power Magnets

A magnet’s strength is measured in terms of saturation magnetization and coercivity values. These values will increase when there is a decrease in the grain size and an increase in the specific surface area (surface area per unit volume of the grains) of the grains. It has been demonstrated that magnets made of nanocrystalline yttrium-samarium-cobalt grains have highly uncommon magnetic properties because of their extremely large surface area.[7]

1.4.5 Elimination of Pollutants

Nanocrystalline materials have very large grain boundaries corresponding to their grain size. Therefore, they are very active with regards to their physical, chemical, and mechanical properties. Owing to their improved chemical activity, nanomaterials can be employed as catalysts to react with toxic and noxious gases such as nitrogen oxide and carbon monoxide, in power generation equipment and automobile catalytic converters, to avoid environmental pollution caused when gasoline and coal are burnt.[8]

1.5 ZINC OXIDE (ZnO)

Zinc is a transition metal with atomic number 30 and represented in the Periodic Table with the symbol ‘Zn’. Zinc is one of those advantageous metals that we make use of in daily life. It has a lustrous bluish-white color. The abundance of the zinc element is estimated at almost 65 grams for every ton of the Earth’s crust. The word ‘zinc’ has a German origin whereas its exact derivation goes beyond the Persian word ‘sing,’ which means stone.

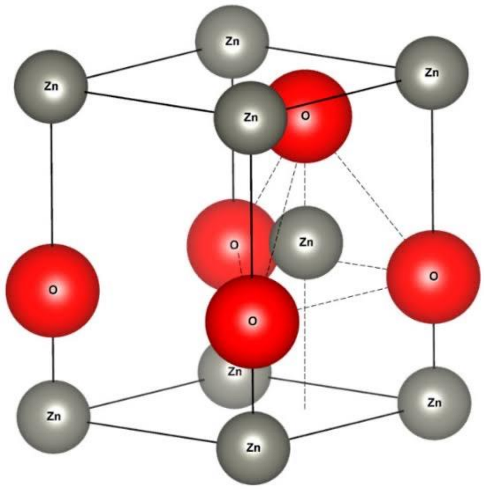
Zinc oxide is an inorganic compound with the formula ZnO. It is a white powder that is insoluble in water. ZnO is present in the Earth's crust as the mineral zincite. That being said, most ZnO used commercially is synthetic. It is commonly known as zinc white or calamine. The chemical reaction of zinc oxide is given by: 2Zn + O2 → 2ZnO

ZnO can show polymorphism and has three different structure that is rock-salt structure, wurtzite structure, and zinc blend structure. The wurtzite structure of ZnO is the most stable structure thermally. ZnO is the metal and non – metal elements combination forming an ionic compound.[9]

1.5.1 Crystal structure of ZnO

Crystalline ZnO has a wurtzite crystal structure at ambient conditions. Wurtzite structure of ZnO is composed of Zn^{2+} and O^{2-} ions. It has a hexagonal closed packing (HCP) arrangement of Zn^{2+} and O^{2-} ions. It has 6 atoms present per unit cell. In HCP structure it has 6 octahedral voids and 12 tetrahedral voids. The O^{2-} anions are present at the corners and faces of the hexagon. Both the Zn and O atoms have coordination number 4.

Each Zn^{2+} cation is surrounded by 4 O^{2-} anions and each O^{2-} anion surrounds by 4 S^{2+} cations. It has alternate layers of Zn^{2+} and O^{2-} ions i.e., O^{2-}, Zn^{2+}, O^{2-}, Zn^{2+}, and so on. Thus, it forms an ABAB-type structure. It has two asymmetric units in its unit cell of HCP.[9]



Wurtzite structure of ZnO

1.6 ZINC OXIDE NANOPARTICLES

ZnO nano particles are available as powders and dispersions. They exhibit antibacterial, anti-corrosive, antifungal and UV filtering properties. ZnO nanoparticles are nano-sized particles with size less than 100 nm. They can be prepared by several different methods. There are a variety of chemical methods, for example mechanochemical process, precipitation process, precipitation in the presence of surfactant, sol-gel method, solvo-thermal, hydrothermal, emulsion and micro-emulsion methods. The chemical method is the most cost-effective, reliable and environmentally friendly and also provides flexibility for controlling the size and shape of synthesized nanoparticles. Nanoparticles with high surface area to volume ratio are preferred and, to make these types, stabilization of the nanoparticles is important. Nanotechnology deals with controlling, modifying and fabricating materials, structures and devices with nanometre precision. It helps to understand the fundamental physics, chemistry, biology and technology of nanometre-scale object.

1.6.1 Applications of Zinc Oxide Nanoparticles

Due to its vast areas of application, various synthetic methods have been employed to grow a variety of ZnO nanostructures, including nanoparticles, nanowires, nanorods, nanotubes, nanobelts, and other complex morphologies. ZnO nanoparticles exhibit a range of beneficial optoelectronic properties including good transparency, high electron mobility, and a wide band gap.

The inorganic compound zinc oxide (ZnO) is synthesized at industrial scales worldwide, with an array of manufacturing applications. Powders and dispersions comprising ZnO are broadly used in the production of synthetic rubber, dermatological ointments, food additives, and more. Relatively recent research and development into the properties and applications of ZnO nanoparticles have explored the innate semiconducting nature of the compound. It is now widely used in the construction of printed electronics such as organic photovoltaics (OPVs) and organic light-emitting diodes (OLEDs).

Applications of ZNOs include the manufacture of rubber and cigarette filters; calamine lotion and creams and ointments used to treat skin diseases; an additive in the manufacture of concrete and ceramics; food products such as breakfast cereals; and as a coating agent in various paints.

Zinc oxide nanoparticles have been used to eliminate sulphur, arsenic from water because bulk ZnO cannot remove arsenic because nanoparticle have great surface area than bulk material. Zinc oxide have amazing application in diagnostics, biomolecular detection, micro-electronics. Zinc is the most widespread deficient micronutrient in the soil world over. In India, 40-42 per cent cultivated lands show Zn deficiency which is causing considerable reduction in yield. There is a need to supplement crop plants with zinc nutrient. If the crop plants are supplied with Zn in their nano-formulation, the nutrient use efficiency will be more. To become economic, the production of nanoparticles should be cheap and eco-friendly.

1.6.1.1 In anticancer therapies

ZnO nanoparticles show relatively high biocompatibility. They have an inherent nature of showing selective cytotoxicity against cancerous cells in in vitro condition compared with other nanoparticles. They can be further surface engineered to show increased selective cytotoxicity. ZnO nanoparticles have the unique ability to induce oxidative stress in cancer cells, which has been found to be one of the mechanisms of cytotoxicity of ZnO nanoparticles towards cancer cells. This property is due to the semiconductor nature of ZnO.[10]

1.6.1.2 In agriculture

Nanotechnology has a dominant position in transforming agriculture and food production. Zinc oxide nanoparticles (ZnO NPs) also have remarkable optical, physical, and antimicrobial properties and therefore have great potential to enhance agriculture. Zinc oxide NPs have potential to boost the yield and growth of food crops. The colloidal solution of zinc oxide nanoparticles is used as fertilizer. Nano-fertilizer is a plant nutrient which is more than a fertilizer because it not only supplies nutrients for the plant but also revives the soil to an organic state without the harmful factors of chemical fertilizer. [11,12]

1.6.1.3 In Devices

Intrinsic optical properties of ZnO nanostructures are being intensively studied for implementing photonic devices. Used in transparent electronics, ultraviolet (UV) light emitters, piezoelectric devices, chemical sensors, and spin electronics.[20]

1.6.1.4 Medicinal Applications

ZnO NPs play some potential role in CNS and perhaps during development processes of diseases through mediating neuronal excitability or even release of neurotransmitters. [13,18]

1.6.1.5 As Catalysts

ZnO is nontoxic; it can be used as photocatalytic degradation materials of environmental pollutants. Bulk and thin films of ZnO have demonstrated high sensitivity for many toxic gases. ZnO nanostructures exhibit high catalytic efficiency.[14]

1.6.1.6 As Anti-bacterial Agent

ZnO-NPs exhibit attractive antibacterial properties due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity. ZnO is a bio-safe material that possesses photo-oxidizing and photocatalysis impacts on chemical and biological species. One functional application of the ZnO antibacterial bioactivity was discussed in food packaging industry where ZnO-NPs are used as an antibacterial agent toward foodborne diseases. Proper incorporation of ZnO-NPs into packaging materials can cause interaction with foodborne pathogens, thereby releasing NPs onto food surface where they come in contact with pathogenic bacteria and cause the bacterial death and/or inhibition.[15]

1.6.1.7 In waste water treatment

ZnO nanoparticles have emerged as a valuable photocatalytic candidate in water and wastewater treatment due to their unique properties, including high oxidation capacity and good photocatalytic property. In addition to being environment-friendly, ZnO NPs are also compatible with organisms, which make them ideal for sewage treatment.[10]

1.6.1.8 As fillers

ZnO nanoparticles keep their mean size and morphology within the PC matrix but tend to form micron-sized agglomerates. Increasing the proportion of ZnO nano particles added to polycarbonate from 0 to 5 wt.% decreases the thermal stability of the polymer. The presence of the nano-filler increases the stiffness and reduces the ductility of polycarbonate.[18]

1.6.1.9 In cosmetics

ZnO are used as ingredients in sunscreens due to their UVA and UVB absorption capabilities. Nanoparticles of ZnO are widely used in sunscreens as UV filters starting at the size of 20 nm. They show better dispersion and leave a better cosmetic result.[18]

1.6.1.10 As sensors

ZnO nanoparticles have their own importance due to their vast area of applications, for example, hydrogen gas sensor, ethanol gas senor, vacuum pressure sensor, biosensor and etc. [18]

1.6.1.11 As food additives

ZnO is currently listed as a “generally recognized as safe (GRAS)” material by the Food and Drug Administration and also as food additive. [17,18]

1.6.1.12 In solar cells

Zinc oxide nanoparticles (ZnO NPs) are among the well-known electron transporting layers (ETLs) in flexible electronics, especially in organic solar cells (OSCs). This is due to their high mobility and transparency, annealing-free and solution-processable features, large-area fabrication technique compatibility, etc. [16,20]

1.7 GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES

There are a variety of chemical and physical preparation methods available for the fabrication of nanoparticles including radiation, chemical precipitation, photochemical methods, electrochemical, and Langmuir–Blodgett techniques, but these methods are often extremely expensive and non-environmentally friendly due to the use of toxic, combustible, and hazardous chemicals, which may pose potential environmental and biological risk and high energy requirement. The drawbacks of low production rate, structural particle deformation, and inhibition of particle growth are also encountered in these nanoparticles synthesis. Currently, there is a growing need to develop sustainable preparation of nanoparticles that get rid of using harmful organic chemical substances, since noble nanoparticles are widely applied to areas of human contact. To achieve the principle of green chemistry process, it leads to the search of green synthesis of nanoparticles

Nanotechnology is a developing field in biotechnology. The synthesis of nanoparticles is an important step in the field of nanotechnology. Therefore, materials scientists and nano chemists look forward to using eco- friendly substances to obtain metal nanoparticles. Nano biotechnology is dedicated to the synthesis of nanostructures from living organisms. In the biological use of nanoparticle synthesis, plants have found applications especially in the synthesis of nanoparticles. Using plants to synthesize nanoparticles may be superior to other biological processes that are not harmful to the environment because it eliminates the complex process of maintaining cell culture. If plants or their extracts are used to produce nanoparticles in an extracellular manner, in a controlled manner according to their size, dispersion, and shape, the biosynthesis process of nanoparticles will be more helpful. Plant use can also be appropriately scaled up to synthesize nanoparticles on a large scale. Overcoming the limitations of traditional methods, a green scheme for synthesizing nanoparticles has emerged. Plants and microorganisms are mainly used for the green synthesis of metal nanoparticles. Some of the nanoparticles showed strong antimicrobial effects against different plant pathogens. Compared with microorganisms, the use of plants to synthesize nanoparticles is on the rise, and has advantages compared with microorganisms, because plants have a wide range of bio-molecular variability, which can act as blocking/stabilizing agents and reducing agents, thereby increasing reduction rate and stability of synthetic nanoparticles. Compared with microorganisms, the synthesis of plant-derived nanoparticles is faster and more stable. The biosynthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods.

The green synthesis of ZnO nanoparticles has eco-friendly aspects and various biomedical applications. Neem and Tulsi plants are known for their medicinal properties like anti-bacterial, anti-fungal, anti-inflammatory activities and are easily available. The phytochemicals present in neem and tulsi namely terpenoids, alkaloids and flavanones, which act as reducing as well as capping agent in stabilizing the zinc oxide nanoparticles.[21]

1.8 OBJECTIVES OF THE WORK

- To synthesize ZnO NPs from leaves extract of neem and tulsi plants.
- To implement simple, cost-effective and environment friendly synthesis of ZnO NPs via green synthesis.
- To characterize the green synthesized ZnO NPs .
- To study the antibacterial property of ZnO nanoparticles.

Chapter 2

Materials and Methods

This chapter gives a brief description of the materials and experimental procedures adopted for the present investigation.

2.1 MATERIALS AND APPARATUS REQUIRED

2.1.1 For synthesis of ZnO NPs using neem leaves

- Neem Leaves
- Zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$)
- Filter Paper
- Beakers
- Conical flask
- Silica Crucible

2.1.2 For synthesis of ZnO NPs using tulsi leaves

- Tulsi leaves
- Zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$)
- Magnetic stirrer
- Filter paper
- Beakers
- Conical flask
- Silica Crucible

2.2 EXPERIMENTAL METHODS

2.2.1 Preparation of leaves extract

2.2.1.1 Using Neem Leaves

The leaves were washed in running water in order to separate it from dirt and dust. It was then dried under the shade for 2 days and again oven dried at 30°C for few hours. After complete drying, the leaves were powdered using a mixer grinder and stored in a separate container. About ten grams of extracted leaf sample was extracted with 100ml of water and boiled for 30-35 min with constant stirring and then filtered it to get 10% extract. It is then stored in a refrigerator.[22]

Powdered neem Leaves

Preparation of neem leaf extract

Filtration of extract

2.2.1.2 Using Tulsi Leaves

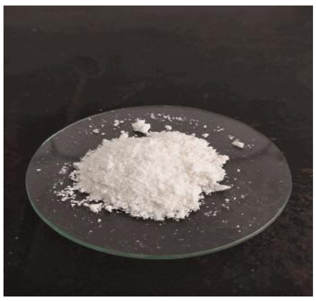
The fresh and healthy leaves were taken. The leaves were cleaned by washing with water and dried in sunlight for 2 days. Then to 10gm of dried leaves and 100 ml distilled water was taken in a 250 ml beaker and it was boiled for 10 minutes until solution turns to reddish colour. The solution was cooled at room temperature. The leaves extract was filtered using filter paper and stored in refrigerator. [23]

Dried Tulsi leaves Tulsi leaves extract

2.2.2 Synthesis of Zinc Oxide Nanoparticles

2.2.2.1 Using Neem Leaves Extract

The stored 10 percent extract was boiled on a hot water bath. When the leaf extract started to boil, 1 gram of Zinc nitrate was added to it and continuously stirred. The mixture was then boiled till it became a paste. It was then transferred to a silica crucible and heated at a temperature of 400°C in a muffle furnace for two hours and cooled. Zinc oxide nanoparticles were obtained as white powder and preserved it on a plastic container for further characterization.[22]



Silica crucible Containing Paste kept in muffle furnace

White powdered ZnO NPs

2.2.2.2 Using Tulsi Leaves extract

About 50 ml of the extract was boiled at 60-80°C using magnetic stirrer. 5g of zinc nitrate was added into the solution at 70°C and boiled until it turns into a deep reddish paste. The deep reddish paste was kept in a muffle furnace at 130°C and cooled. ZnO nanoparticles were obtained as yellow powder and preserved it on a plastic container for further characterization purposes.[23]

Silica crucible Containing Paste kept in muffle furnace

Yellow powdered ZnO NPs

2.3 CHARACTERIZATION TECHNIQUES

2.3.1 Ultraviolet spectroscopy

UV spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, it also potentially provides information on what is in the sample and at what concentration the sample is. UV-spectrophotometer analysis was done for preliminary confirmation of green synthesized ZnO nanoparticles. Small amount of green synthesized ZnO nanoparticles were dispersed in water by ultrasonication for 30 minutes. Dispersed samples were fed into UV-visible spectrophotometer and absorption peak of the samples were recorded with help of connected PC and the software SP-UV5.[24]

UV Spectrometer

2.3.2 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared Spectroscopy is commonly referred to as FTIR Analysis or FTIR Spectroscopy. This infrared spectroscopy method is used to identify organic, polymeric, and in some cases, inorganic materials. The FTIR test relies on infrared light to scan samples and observe bond properties. A beam source of various IR wavelength light is sent through a beam splitter, where half reaches a fixed mirror and half a mirror that moves with a constant velocity. These two split beams are then reflected and recombined to construct an interference pattern reflecting the constructive and destructive interference of the recombination. After, this interference pattern (or interferogram) is sent to the sample, and the transmitted portion of the interferogram is sent to a detector. After comparison with a reference sample beam spectrum in the detector, a Fourier transform is performed to obtain the full spectrum as a function of wavenumber. FTIR analysis services can identify compounds and the general type of material being analyzed when there are unknowns. Functional groups analysis of the synthesized ZnO nanoparticles were analyzed by FTIR spectrometer (Thermo Nicolet Avatar 370).[25]

FT-IR spectrometer

2.3.3 X-Ray Diffraction Spectroscopy

X-Ray diffraction analysis (XRD) is a non-destructive technique that provides detailed information about the crystallographic structure, chemical composition, and physical properties of a material. It is based on the constructive interference of monochromatic X-rays and a crystalline sample. X-rays are shorter wavelength electromagnetic radiation that are generated when electrically charged particles with sufficient energy are decelerated. In XRD, the generated X rays are collimated and directed to a nanomaterial sample, where the interaction of the incident rays with the sample produces a diffracted ray, which is then detected, processed, and counted. The intensity of the diffracted rays scattered at different angles of material are plotted to display a diffraction pattern. Each phase of the material produces a unique diffraction pattern due to the material's specific chemistry and atomic arrangement. The diffraction pattern is a simple sum of diffraction patterns of each phase. From the diffraction pattern size of nanoparticles can be determined using Debye-Scherrer equation and crystal structure can be identified. XRD analysis of the synthesized ZnO nanoparticles were carried out in an X-ray Powder diffractometer (Bruker AXS D8 Advance).[26]

X-ray diffractometer

2.3.4 Energy Dispersive X-ray Spectroscopy

Energy dispersive X-ray spectroscopy (EDAX) is a powerful technique that enables the user to analyze the elemental composition of a desired sample. When a sample is bombarded with a beam of high-energy electrons, the electrons in the target atoms are expelled from their inner shell, producing an electron vacancy. Subsequently, the electrons on the higher energy levels are transferred to the inner shell in a transition that releases energy in the form of X-rays. The generated X-rays are characteristic of every element. By measuring the characteristic X-ray energy and intensity released by each element, it is possible to have information on the elemental composition of a sample. It is used together with a scanning electron microscope (SEM), an EDX detector can generate more information about a sample than an SEM can alone. The elemental composition of the synthesized ZnO nanoparticles were analysed by SEM/EDS Spectrometer (Jeol JSM – 6390L/JED – 2300).[27]

EDAX Spectrometer

2.3.5 Scanning Electron Microscopy

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the sample with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the intensity of the detected signal to produce an image. Thin films of the sample were prepared by just placing a very small amount of sample on a copper grid. Then the film of the copper grid is allowed to dry and the morphology of sample is analyzed using a scanning electron microscope. SEM images of the synthesized ZnO nanoparticles were obtained by SEM/EDS Spectrometer (Jeol JSM – 6390L/JED – 2300).[28]

Scanning Electron Microscope

2.4 ANTIBACTERIAL STUDY

The antimicrobial activity of the nanoparticles synthesized were evaluated by disc diffusion method. The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The samples for antibacterial study were prepared by dissolving the ZnO nanoparticles in Dimethyl sulphoxide (DMSO).

Using aseptic techniques, a single pure colony was transferred into a 10 ml of nutrient broth and was placed in incubator at 37°C for overnight incubation. Sterile MHA plates were prepared and the bacterial inoculum was uniformly swabbed in the plate. Medium was seeded with bacterial culture of *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus.* Test samples of volume 20µl added to the disc and were placed over the agar plates. The plates were incubated at 37ºC for 18 hours a period sufficient for the growth. After incubation, the diameter of inhibitory zones formed around each well were measured in mm and recorded. The antibacterial activity was assayed by analyzing the diameter of the inhibition zone formed around the well.[29]

Chapter 3

Results and Discussion

3.1 CHARACTERIZATION

3.1.1 UV Spectroscopy

Absorbance peak of green synthesized ZnO NPs obtained in UV-wavelength range (280-375 nm), which confirmed their size in nano range. In UV-spectroscopy, zinc oxide nanoparticles synthesized using different plant extracts showed difference in their absorbance peaks (Fig. 1 & 2). ZnO NPs synthesized using neem extract exhibited absorbance peaks at 364 nm and that of tulsi was at 370 nm.

ZnO NPs synthesized using neem extract exhibited their absorption peak at the lower wavelength was supposed to have a smaller size than that synthesized using tulsi. When the size of bulk molecules gets reduced to nano range, their absorption peak gets shifted towards UV range from visible range. So, nanoparticles exhibiting absorption peak at lower wavelength have smaller size than the particles exhibiting absorption peak at higher wavelength. [22]

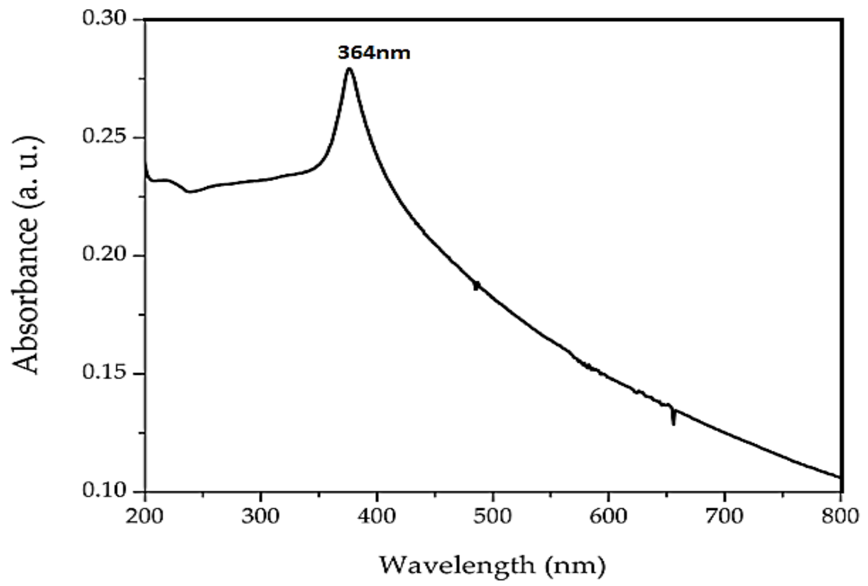


Fig 1: Absorption peak of ZnO NPs synthesized using Neem leaf extract in UV spectroscopy

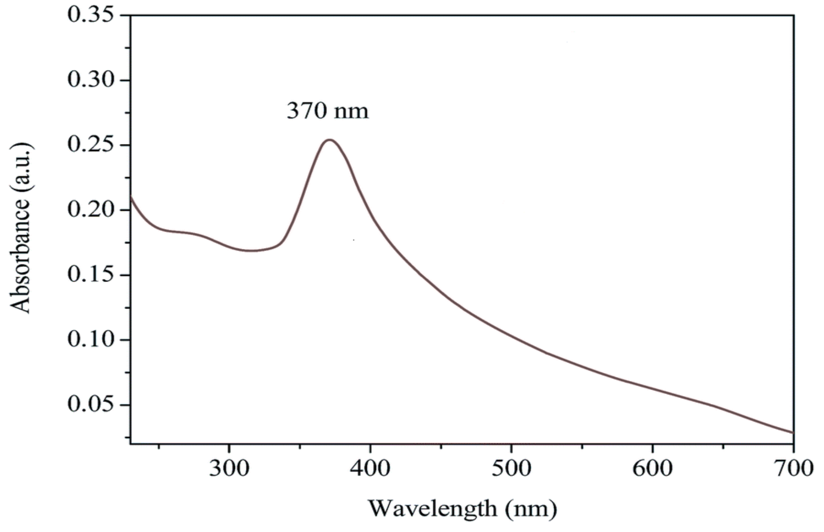


Fig 2: Absorption peak of ZnO NPs synthesized using Tulsi leaf extract in UV spectroscopy

3.1.2 FTIR Spectroscopy

The FTIR spectrum of ZnO particles using Neem is given in Fig 3. A series of absorption peaks from 1000-4000 cm^{-1} can be found, corresponding to the carboxylate and hydroxyl groups in the material. To be more specific, a broad band at 3439.65 cm^{-1} is assigned to the O-H stretching mode of hydroxyl group. Peaks at 1384.24cm^{-1} and 1428.51cm^{-1} is due to asymmetrical and symmetrical stretching of zinc carboxylate (COO^-). The carboxylate group probably comes from carboxylic acids present in the neem leaf extract and the hydroxyl group results from the hygroscopic nature of ZnO. The absorption peak in Fig 3. (Fingerprint region) at 432.90 cm^{-1} corresponds to hexagonal ZnO.

The FTIR spectrum of leaves extract of tulsi is given in Fig 4. A series of absorption peaks from 1000-4000 cm^{-1} can be found, corresponding to the carboxylate and hydroxyl groups in the materials. To be more specific, a broad band at 3396.79 cm^{-1} is assigned to the O-H stretching mode of hydroxyl group. Peak at 1384.38 is due to symmetrical stretching of zinc carboxylate (COO^-). The carboxylate group probably comes from carboxylic acids present in the tulsi leaf extract and the hydroxyl group results from the hygroscopic nature of ZnO. The absorption peak in Fig 4. (Finger print region) at 441.02 cm^{-1} corresponds to hexagonal ZnO.[30]

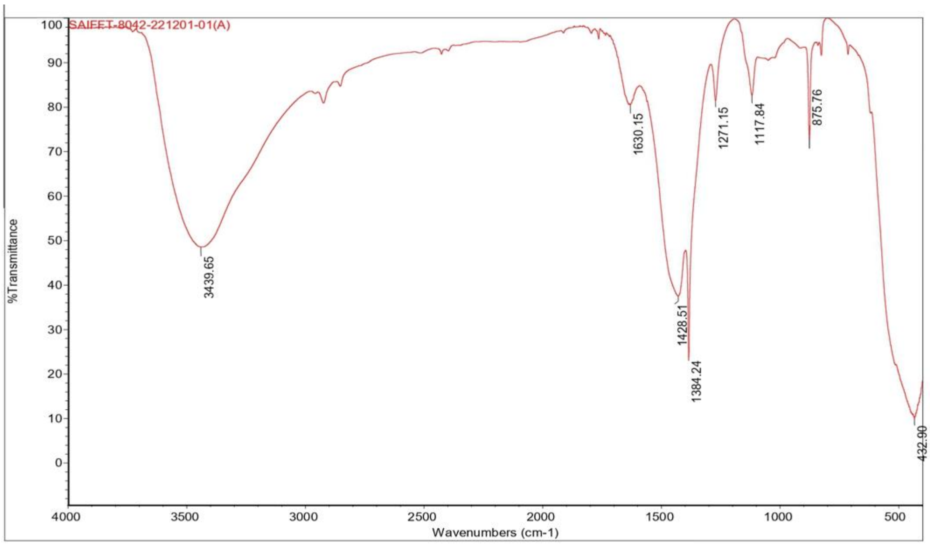


Fig 3: FTIR Spectra of ZnO nanoparticles synthesized using neem leaves extract

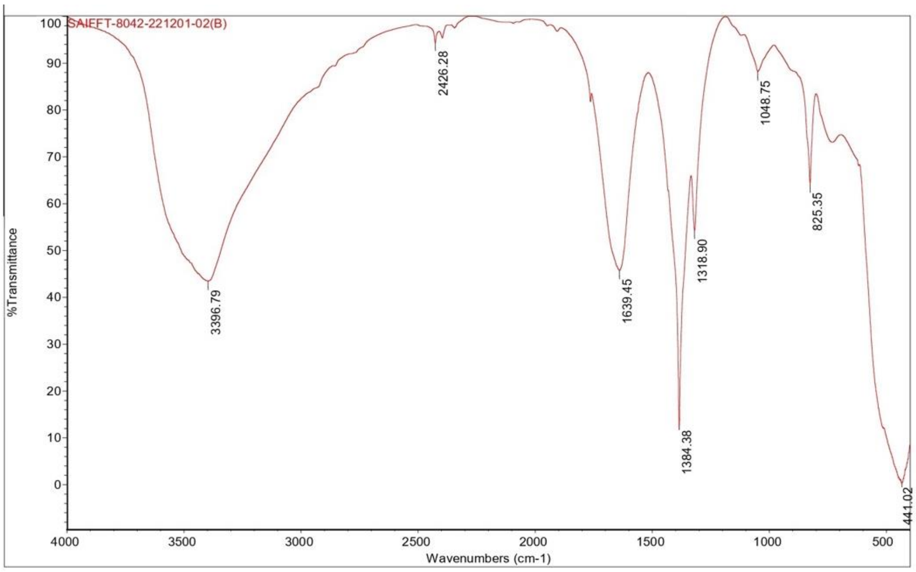


Fig 4: FTIR spectra of ZnO nanoparticles synthesized using tulsi leaves extract

3.1.3 EDAX Spectroscopy

The elemental composition of green synthesized nanoparticles was determined by EDAX spectroscopy (fig.5 & 6). ZnO NPs synthesized with neem leaf extract was found to have 19.52 weight percent of oxygen and 80.48 weight percent of zinc, whereas ZnO NPs synthesized with tulsi leaf extract was found to have 10.89 weight percent of oxygen and 89.11 weight percent of zinc (Table 1&2). Therefore, it is found that ZnO NPs synthesized from both neem and tulsi are pure and is in good agreement with the reported work.

Chemical composition of ZnO NPs synthesized using Neem leaves extract			
Element	Line type	Wt.%	Atomic%
O	K series	19.52	49.78
Zn	K series	80.48	50.22
Total		100	100

Table.1

Chemical composition of ZnO NPs synthesized using Tulsi leaves extract			
Element	Line type	Wt.%	Atomic%
O	K series	20.01	50.29
Zn	K series	79.99	49.79
Total		100	100

Table.2

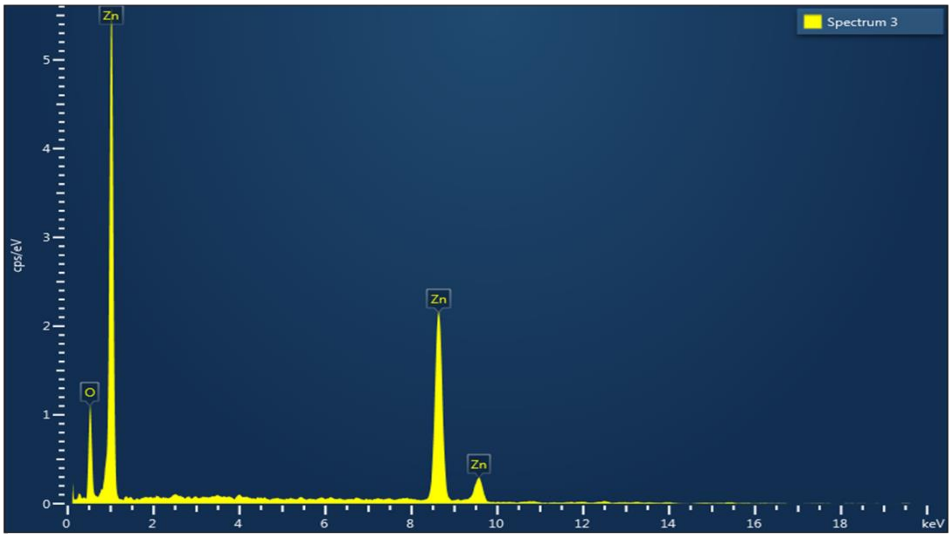


Fig.5 EDAX spectrum of ZnO NPs synthesized using neem leaves

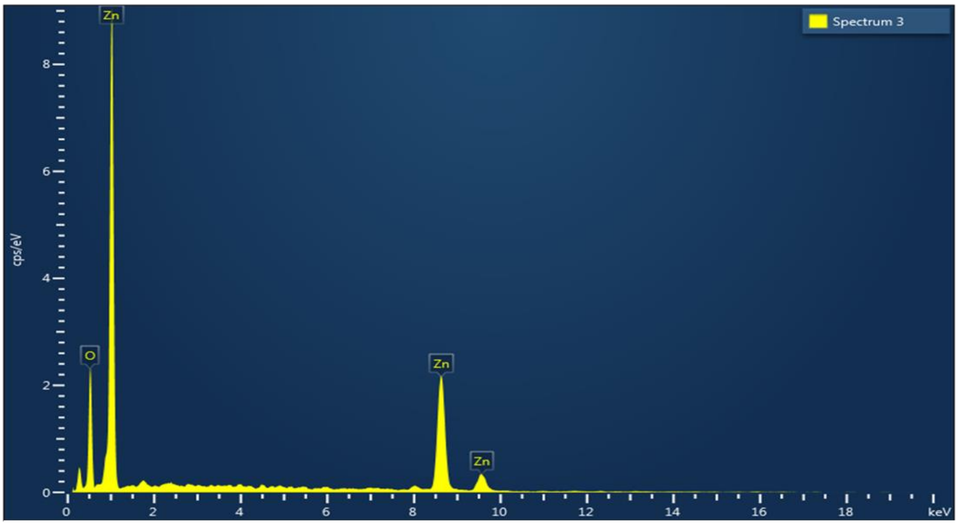


Fig.6 EDAX spectrum of ZnO NPs synthesized using tulsi leaves

3.1.4 XRD Spectroscopy

The particle size and nature of ZnO synthesized were determined using XRD. The X-ray powder diffraction was carried out with 30 kv, 30 mA with Cu kα radians at 2 θ angle. XRD pattern of the prepared Zinc oxide nano powder is shown in the figures. The average size of the nanoparticles is calculated using Debye Scherrer equation:

$$D=k\lambda/\beta\cos\theta$$

Where, D- crystalline size

λ- wavelength

β- Full width at the half maximum (FWHM)

K -Scherer's constant

Calculations of average nano-size of the particle:

A) ZnO NPs synthesized using neem leaf extract

PEAK 1:

$2\theta=31.919^{o}=0.557091644$ rad

$\theta=0.278545822$ rad

$\beta=0.823=0.01436406$ rad.

$\lambda=1.5406$ x 10-10 m

k=0.94

$D=k\lambda/\beta\cos\theta=1.115534835 \times 10^{-8}$ m $=11.15534835 \times 10^{-9}$ m

=11.15534835 nm

PEAK 2:

$2\theta=56.745^{o}=0.990387084$ rad

$\theta=0.495193542$ rad

$\beta=0.823=0.01436406$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

D=kλ/βcosθ=1.218963643 x 10^{-8} m =12.18963643 x 10^{-9} m

=12.18963643 nm

PEAK 3:

2θ=47.649º=0.831631935 rad

θ=0.415815967 rad

β=0.768=0.013404129 rad.

λ=1.5406 x 10^{-10} m

k=0.94

D=kλ/βcosθ= 1.25640975 x 10^{-8} m =12.5640975 x 10^{-9} m

=12.5640975 nm

The average particle size is **11.9696 nm**

B) ZnO NPs synthesized using tulsi leaf extract

PEAK 1:

2θ=18.879º=0.329500709 rad

θ=0.164750354 rad

β=0.476=0.0083077672 rad.

λ=1.5406 x 10^{-10} m

k=0.94

D=kλ/βcosθ= 1.673078804 x 10^{-8} m =16.73078804 x 10^{-9} m

=16.73078804 nm

PEAK 2:

$2\theta=22.749^{o}=0.397044952$ rad

$\Theta=0.198522476$ rad

$\beta=0.225=0.0039269908$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

$D=k\lambda/\beta\cos\theta= 3.561515637 \times 10^{-8}$ m $=35.61515637 \times 10^{-9}$ m

=35.61515637 nm

PEAK 3:

$2\theta=30.287^{o}=0.528607871$ rad

$\theta=0.264303935$ rad

$\beta=0.322 =0.056199602$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

$D=k\lambda/\beta\cos\theta= 2.527526944 \times 10^{-8}$ m $= 25.27526944 \times 10^{-9}$ m

=25.27526944nm

PEAK 4:

$2\theta=31.850^{o}=0.55588737$ rad

$\theta=0.277943685$ rad

$\beta=0.336 =0.0058643063$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

$D=k\lambda/\beta\cos\theta=2.43141531 \times 10^{-8}$ m $=24.3141531 \times 10^{-9}$ m

=24.3141531 nm

PEAK 5:

$2\theta=34.541^o=0.602854177$ rad

$\theta=0.301427088$ rad

$\beta=0.295=0.0051487213$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

$D=k\lambda/\beta\cos\theta=2.788793477 \times 10^{-8}m =27.88793477 \times 10^{-9}$ m

=27.88793477 nm

PEAK 6:

$2\theta=36.343^o=0.634479543$ rad

$\theta=0.317239771$ rad

$\beta=0.349=0.0060911991$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

$D=k\lambda/\beta\cos\theta= 2.369232883 \times 10^{-8}m =23.69232883 \times 10^{-9}$ m

=23.69232883 nm

PEAK 7:

$2\theta=47.601^o=0.830794177$ rad

$\theta=0.415397088$ rad

$\beta=0.469=0.0081855942$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

$D=k\lambda/\beta\cos\theta= 1.830751549 \times 10^{-8}m =18.30751549 \times 10^{-9}$ m

=18.30751549 nm

PEAK 8:

$2\theta=56.646^{o}=0.988659208$ rad

$\theta=0.494329604$ rad

$\beta=0.430=0.007504916$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

$D=k\lambda/\beta\cos\theta= 2.075438125\times 10^{-7}m =20.75438125\times 10^{-9}$ m

=20.75438125nm

PEAK 9:

$2\theta=62.952^{o}=1.09871697$ rad

$\theta=0.549358485$ rad

$\beta=0.505=0.0088139127$ rad.

$\lambda=1.5406 \times 10^{-10}$ m

k=0.94

$D=k\lambda/\beta\cos\theta=1.824036747 \times 10^{-8}m =18.24036747 \times 10^{-9}$ m

=18.24036747 nm

The average particle size is **23.4242 nm**

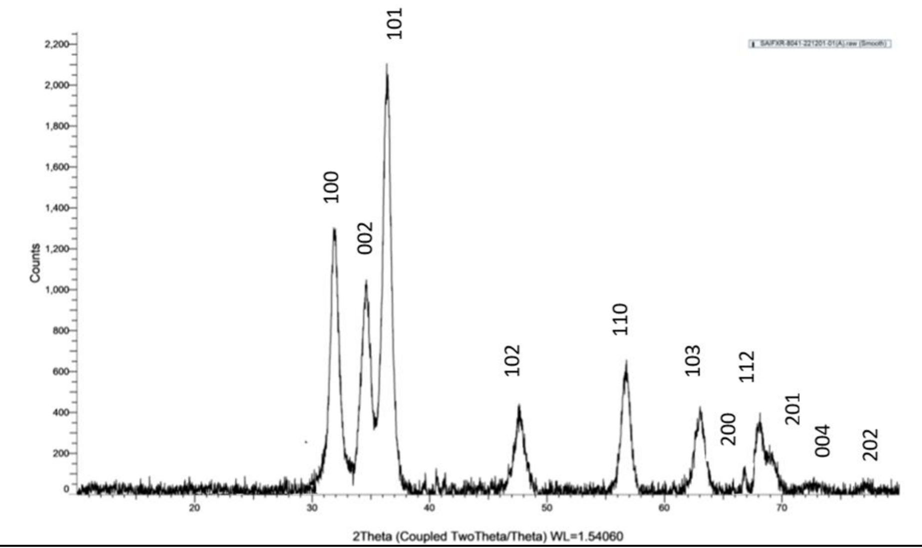


Fig 7: XRD pattern of ZnO NPs synthesized using neem leaves

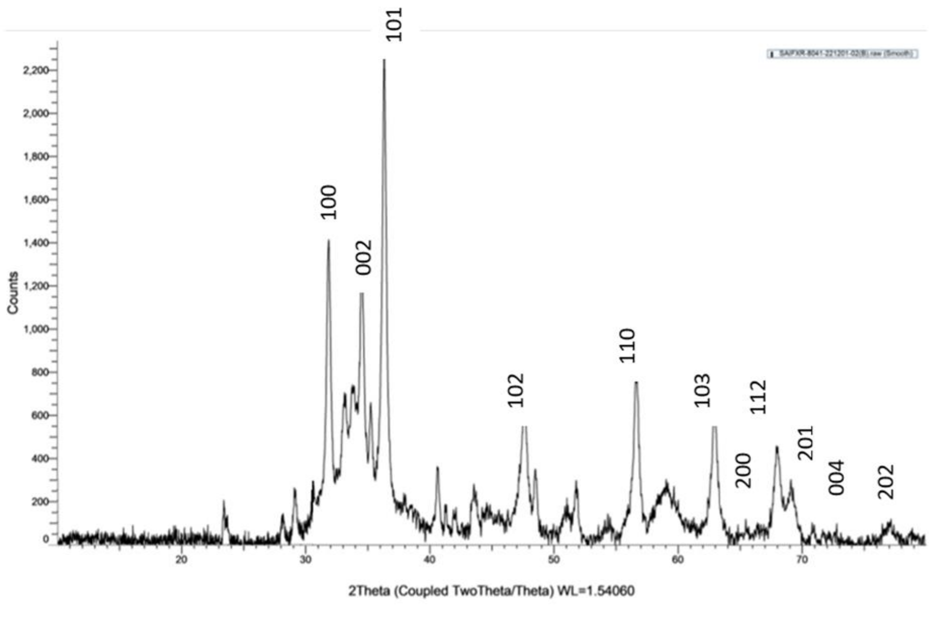


Fig 8: XRD pattern of ZnO NPs synthesized using tulsi leaves

The obtained XRD patterns of neem and tulsi (fig 7 & 8) were compared with the standard card of ZnO powder sample (JCPDS file no:36-1451) and the lattice points were plotted.

The observed diffraction peak of ZnO at $2\theta=36.19^{o}$ correspond to lattice plane (101).

The diffraction peaks located at 31.84◦, 34.52◦, 36.33◦, 47.63◦, 56.71◦, 62.96◦, 68.13◦, and 69.18◦ of figure1. have been keenly indexed as hexagonal wurtzite phase of ZnO which confirms the synthesized nanoparticles was free of impurities as it does not contain any characteristics XRD peaks other than ZnO peaks. The average particle size of particle obtained from neem leaf extract is 11.9696 nm. The average particle size obtained from tulsi leaf extract is 23.4242 nm.

3.1.5. Scanning Electron Microscopy

The SEM image of ZnO NPs synthesized from neem (Fig 9 & 10) and tulsi (Fig 11 & 12) leaves extract is given below.

SEM image of ZnO NPs using Neem leaves extract

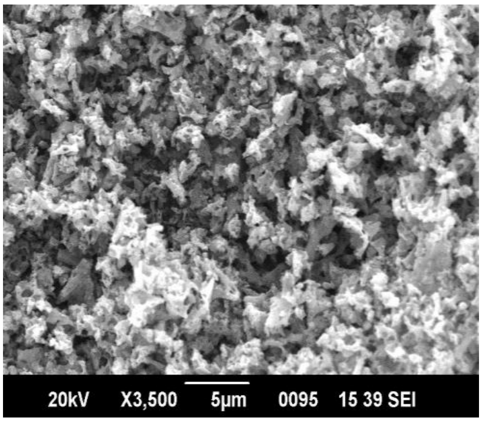


Fig.9

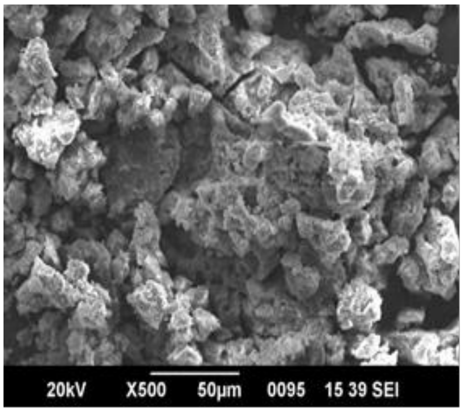


Fig.10

SEM image of ZnO Nps using tulsi leaves extract

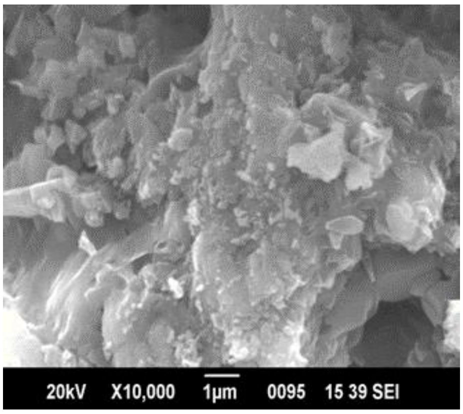


Fig.11

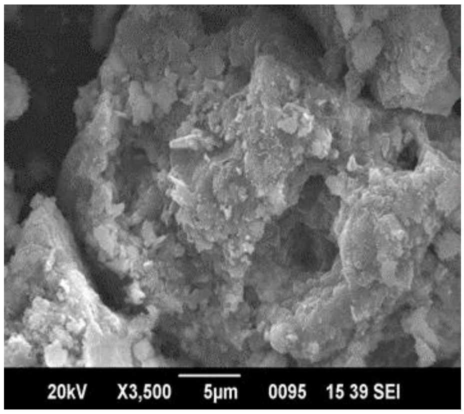


Fig.12

3.2 ANTIBACTERIAL STUDY

Antibacterial assay was conducted for four bacterial strain *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus*. From the evaluation of antibacterial study, it is seen that the ZnO NPs synthesized using neem leaf extract (F) showed a zone of inhibition of 12 mm against *Escherichia coli*, *Pseudomonas aeruginosa and Klebsiella pneumoniae*, while it was 14 mm zone of inhibition for ZnO NPs synthesized using Tulsi leaves (G). But both ZnO NPs showed no activity against *Staphylococcus aureus*.

Therefore, it is evident that the ZnO NPs synthesized from both neem (F) and tulsi(G) leaf extract shows antibacterial activity. Also, the ZnO NPs synthesized using tulsi leaf extract show comparable antibacterial activity (14 mm) as that of ZnO NPs synthesized using neem leaf extract (12 mm). The results of analysis are given in the table below. (Table 3)

Bacteria	**Zone of inhibition of ZnO NPs synthesized from neem leaf extract-F (mm)**	**Zone of inhibition of ZnO NPs synthesized from Tulsi leaf extract-G (mm)**
E. coli	12	14
P. aeruginosa	12	14
K. pneumoniae	12	14
S. aureus	nil	nil

Table.3

E. coli

K. pneumoniae

S. aureus

P. aeruginosa

Chapter 4

Conclusions

Conclusions of the study

- ZnO nanoparticles were successfully synthesized from leaves of Neem and Tulsi plants.
- Presence of ZnO was primarily determined by UV and FTIR spectroscopy.
- XRD spectroscopy confirmed ZnO nanoparticles by analysing the crystalline structure. Also, we found out the size of ZnO nanoparticles synthesized from neem and tulsi leaves extract as 11.96 nm and 23.42 nm respectively.
- The purity of samples was confirmed by EDAX spectroscopy.
- Morphological studies of the samples were also done by SEM.
- The antibacterial activity of synthesized ZnO nanoparticles has been studied. ZnO NPs synthesized using neem and tulsi leaves extract showed a zone of inhibition of 12 mm and 14 mm against *Escherichia coli*, *Pseudomonas aeruginosa and Klebsiella pneumoniae* respectively.
- The synthesized ZnO nanoparticles can be further studied for their anti-fungal and anti-cancer properties and also, they can be incorporated to biopolymers to produce nanocomposites.

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