International Journal of Multidisciplinary Research And Studies

Available online at
https://ijmras.com/index.php/ijmras/article/view/638
INTERNATIONAL JOURNAL OF
MULTIDISCIPLINARY RESEARCH AND STUDIES
ISSN: 2640 7272
Volume:06; Issue:10 (2023)
Doi: 10.33826/ijmras/v06i10.5

Green Synthesis and Antibacterial Study of Zinc Oxide (Zno) Nanoparticles

SIDRA GHAFOOR[1a*], MAHENDRA SINGH KUSHWAH[2b], NEELAM SHEHZADI[3c]

[1,3]Research Scholar department of Chemistry, University of Sialkot Punjab, Pakistan.

[2]Researcher Scholar Department of Pharmaceutical science, Rajiv Gandhi University M.P. India

***Contact Email, ORCID ID**: [a]sidraghafoorofficial@gmail.com, https://orcid.org/0000-3450-1824-4956-2 / [b]mahendrasingh.9276@gmail.com, https://orcid.org/0000-0002-6634-3757/ [c]chneelam981@gmail.com, https://orcid.org/0000-34602-7196-5472*

Abstract

With this work, the environmentally friendly manufacturing of ZnO NPs utilizing Catharanthus roseus leaves has been assessed. Here, a rapid and environmentally friendly method for producing zinc oxide nanoparticles is described: using Catharanthus roseus leaf extract as stabilizing and reducing agent. Different methods, including UV-Visible Spectroscopy, Fourier Transmission Spectroscopy (FTIR), and X-ray Diffraction (XRD) were used to analyze the green synthesized ZnO NPs. The size, morphology, crystallinity of the synthesized ZnO nano-particles were investigated via X-ray diffraction technique. It was found that ZnO possess crystallinity structure with 24.0 nm average size. The designed molecules exhibited good antibacterial activities against gram positive (*Bacillus subtilis*) and gram negative (*Escherichia coli*) and (*Pseudomonas aeruginosa) bacterial* strains. In the present work, (**NE-SMS-OA-01) have** been synthesized by utilizing two step reaction by exhibited significant antibacterial activity.

Keyword: Catharanthus Roseus Leaves, X-Ray Diffraction Technique, Fourier Transmission Spectroscopy, UV-Visible Spectroscopy, ZnO Nps.

1. INTRODUCTION

1.1. Nanotechnology

Simply said, nanotechnology is "technology at the nanometer scale." Without a doubt, during the past century, research has focused heavily on nanotechnology as a field numerous nanoscale material have been produced as a result of advances in nanotechnology. "Nanoparticles" (NPs) is defined as substances with a diameter of 100 nanometers or less. The foundation of nanotechnology is the capacity to build complex structures atom by atom utilizing a novel molecular organization. By controlling devices and structure at the atomic, molecular, and supramolecular levels and gaining knowledge of how to properly create and operate these devices, the goal is to take use of these properties. (Mansoori & Soelaiman, 2005)." A subfield of information science called nanotechnology is concerned with measuring events that occur on the nanoscale scale. It is a grouping of technologies that may be found in research domains including interfacial sciences, biochemistry, electronics, and other branches of knowledge. Concerning the creation and synthesis of nanomaterials, a substantial field of science is involved. Nanotechnology offers the fascinating possibility of resolving issues in a more effective way. (Abdullah et al., 2020).

1.2. Historical Background

Feynman suggested that the complete Encyclopedia Britannica would fit at the tip of a needle when the word "nanotechnology" was first used on December 29, 1959 at a Caltech American Physical Society conference. According to Subedi (1985), nanotechnology is the "control of separation, accumulation, and modification of material by single atoms or single molecules" (Professor Norio Taniguchi, Tokyo Science University). Despite the fact that scientists have been working with nanoparticles for thousands of years, their inability to comprehend the morphology of nanomaterials has greatly diminished their usefulness and effectiveness. However, the discipline of nanotechnology started to grow in the 1980s with the invention of the scanning electron microscope.

Since 1985, when researchers found the chemical known as buck minster fullerene, which resembled a football and had 60 carbon atoms, hundreds of millions of dollars and a growing number of individuals now have access to the chance to learn more about and use nanotechnology. A "fullerene" is often a carbon-based molecule with a hole, ellipse, or tubular form. (Chakraborty etal., 2018).

1.3. Nanoscale Materials

The application of nanoparticles may lead to the creation of new properties and an improvement in overall effectiveness. The integration of biomolecules and nanoparticles at the nanoscale is shown in Figure 1.1. The nanoscale size is significant for a variety of reasons, and there are several other reasons why this discipline has opened up new opportunities in science and technology. (Klabunde & Richards, 2019).

The following are instances of some of these factors:

1. One of the most important aspects of nanotechnology is the enhanced surface area to volume ratio that is found in most nanoscale materials. The discovery of novel phenomena involving subatomic particles is now possible as a result of this**.** (Ghorbanpour et al., 2016).
2. Since the ratio of surface to volume has grown and the reaction capacity of intercellular gaps and surface areas, chemical characteristics at this scale are advantageous for catalytic reactions.

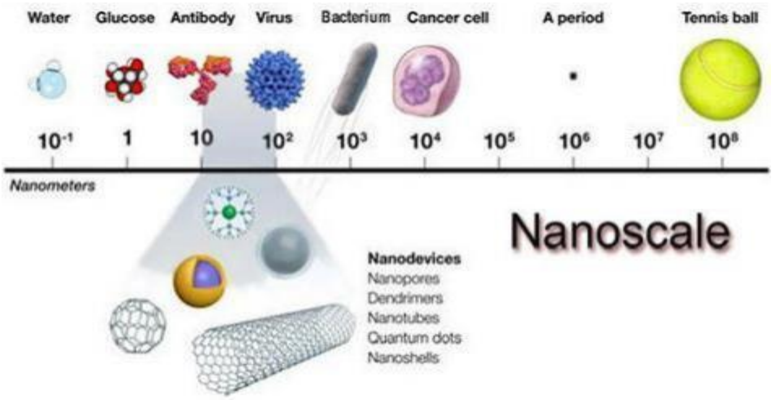


Figure 1.1. Nano scale integration of nanoparticles and biomolecules

1.3.1. Nanoparticles

Compounds known as nanoparticles are made up of nanostructured materials with at least one dimension falling within the range of 1- 100 nm. Nanoparticles differ from other kinds of bulk materials in terms of their chemical and physical characteristics because of their tiny size. Greater surface areas, more energy, and surface confinement are some of these characteristics. The amount of biologically active components present in the formation media has an impact on the morphology of nanoparticles in addition to the medium itself. (Monica & Cremonini, 2017). A comparison of the size of nanoparticles with that of a ball and the earth.

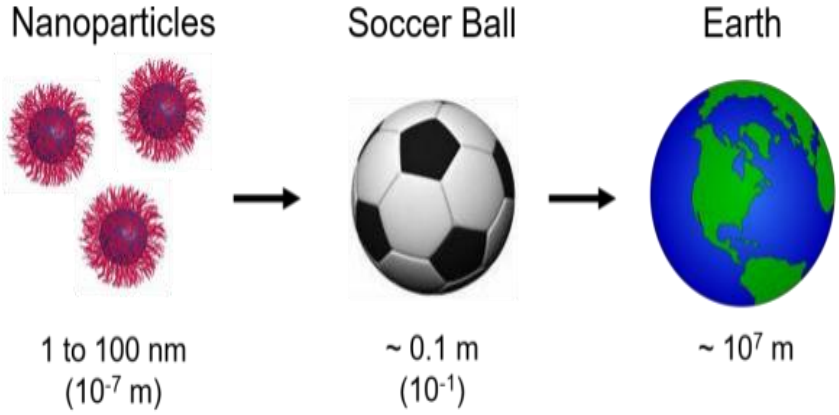


Figure 1.2: Size of nanoparticles

1.4. Classification of Nanoparticles

The nanoparticles are commonly categorized into following three types.

1) Organic Nanoparticles
2) Inorganic based Nanoparticles
3) Carbon based Nanoparticles

1.4.1. Organic Nanoparticles

Dendrimers, micelles, liposomes, ferritin, and other polymeric or organic nanoparticles are examples of common names. These nanoparticles may be thrown away and are safe. "Dendrite polymers have a number of significant characteristics that make them particularly desirable as functional materials for water purification. Since organic nanoparticles are efficient and can be introduced into specific areas is referred to as “targeted drug delivery "they are commonly used in biological applications like medicine delivery system. *(Tiwari et al., 2008)*

1.4.2. Inorganic Nanoparticles

Nanoparticles that lack carbon are referred to as inorganic. Metals a nanoparticle linked to metal oxides make up inorganic nanoparticles (Figure 1.2). Metals-based nanoparticles are those that are created from metals by destroying or constructing them into nanometer- sized particles. Nanoparticles may be made in almost all metals. Metal particles are very interested in copper nanoparticles since it is one of the most important metals in modern technology. (Salavati-niasari et al.,2008). The properties of metal oxide-based counterparts are alteres by the development of metal oxide -based nanoparticles. Along with being an essential ingredient in paint and superconductor products, they were also a crucial part of catalysis and the removal of hazardous waste. Aluminum oxide (Al2O3), cerium oxide (CeO2), iron oxide (Fe2O3), magnetite (Fe3O4), silicon dioxide (SiO2), titanium oxide (TiO2), and zinc oxide (ZnO) are the metal oxide nanoparticles that are most often formed. (Tai et al.,2007).

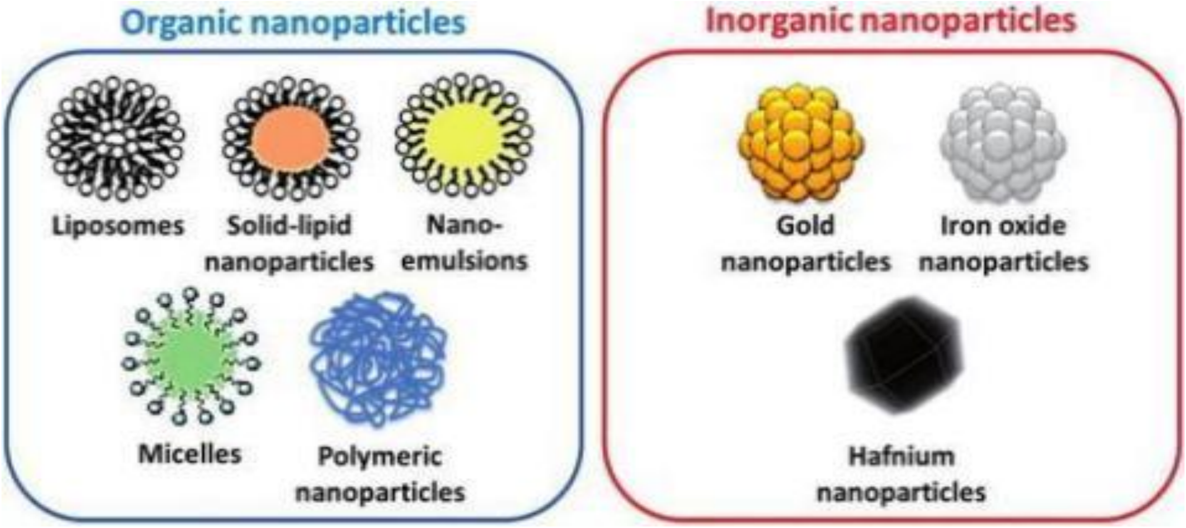


Figure 1.3: Classification of nanoparticles

1.4.3. Carbon Based Nanoparticles

Carbon-based nanoparticles are those that are entirely composed of carbon. Due to its flexibility, carbon nanostructures are one of the most fascinating nanoscale materials. The use of carbon nanostructures in biological applications including sensing and controlled drug administration might have unique impacts. (Siqueira & Oliveira, 2017). At the nanoscale level, it may be divided into carbon nanotubes (CNT), carbon black, activated carbon, carbon nanofibers, fullerenes, and graphene.

Nanoparticles can be categorized into a number of different groups based on their size, shape and chemical makeup. There is widespread usage of the kinds of carbon nanoparticles known as fullerenes and carbon nanotubes (CNTs). In contrast to allotropic carbon forms, fullerenes have spherical hollow cage nanomaterial. Their flexibility, electrical conductivity, and shape have inspired commercial use. Graphene sheets are rolled up to form carbon nanotubes. Single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) are the two types of carbon nanotubes. (*Aqel etal., 2012)*. The allotropic form of carbon is called graphene. A two- dimensional planar hexagonal honeycomb lattice structure made solely of carbon atoms is known as graphene. A graphene Nano sheet is wound into vacant spaces that have a honeycomb structure made of carbon atoms to form carbon nanotubes. The same graphene Nano-foils that are used to make carbon nanotubes are also utilized to make fullerene molecules, but they take on a conical or cupped from rather than coiling into tubes. Carbon black is a spherical, amorphous substance made of carbon with widths varying from 20 to 70 nanometer that is frequently used in the manufacture of carbon fiber. Strong particle interactions cause the particles to group together and form 500 nanometer-sized agglomerates. (Georgakilas et al., 2015)

1.5. Synthesis of nanoparticles

Nanoscale materials may be created using a variety of techniques. Top-down and bottom strategies, however, can be used to categories these methods. These techniques are further separated into various sub-classes based on the mechanism, reaction stage, and chosen protocols. (Khan at al, 2019)

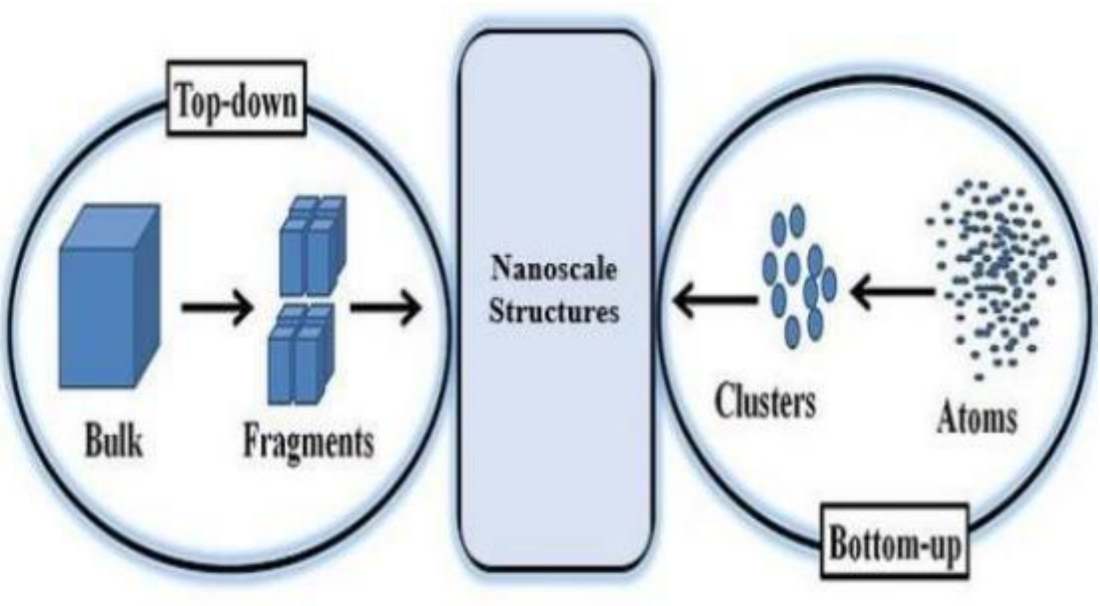


Figure 1.4. Nanoparticle synthesis approaches

1.5.1. Top-down approach

When using the top-down methods, the material is forced to be broken down into tiny bits by an outside force. There are two sorts of grinding when using a top-down strategy: dry and wet. When grains are refined, the surface energy of the particles rises, causing particle aggregation. (Horikoshi & Serpone, 2013).

1.5.2. Dry grinding method

In the dry grinding process, Solid materials are treated by means of standard method such a jet mill, hammer mill, roller mill, and tumbler mill in the dry grinding procedure as a result of shock, compression, or friction. It is challenging to produce particle sizes smaller than 3 m using grain refining processes because tiny particle condensation and pulverization occur simultaneously. (Horikoshi & Serpone, 2013).

1.5.3. Wet grinding method

A wet jet mill, planetary ball mill, flow tunnel beads mill, rolling ball mill, and rotating ball mill are a few examples of wet grinding techniques. Because the wet technique avoids the nanoparticles from condensing and makes it simple to obtain well disseminated nanoparticles, it is preferable to the dry method. Mechano-chemical and mechanical alloying methods are further in top-down strategies. (Horikoshi & Serpone, 2013).

1.5.4. Bottom-up approach

By breaking down gas or id molecules into tiny particles, this process creates nanoparticles. Bottom-up methods may be divided into two categories: liquid phase and gaseous phase. Chemical vapor deposition (CVD) includes a chemical process, whereas physical vapor deposition (PVD) depends on cooling the condensed material. While gaseous-phase procedures require expensive vacuum equipment, liquid-phase methods don’t, which has the ability drawback of being more expensive and inefficient. (Horikoshi & Serpone, 2013).

1.6. Methods of Nanoparticles Synthesis

Nanoparticles can be created through physical, chemical, or biological processes.

Table 1. 1Methods of NPs synthesis

Methods of NPs synthesis		
Physical methods	**Chemical methods**	**Biological methods**

1. Mechanical Approach 2. Spray pyrolysis 3. Ultrasonication 4. Vaporization 5. Pulsed Wire Discharge technique 6. Chemical Vapor Deposition (CVD) 7. Pulse Laser Ablation technique 8. Deposition of Ionized Cluster Beams	1. Sol-gel method 2. Synthesis using son chemical means Synthesis via Hydrothermal Process 4. The method of co- precipitation	1. Usage of Bacteria in NPs Synthesis 2. Algae-Based Synthesis 3. Usage of Plant Extracts

The requirements determine the choice of nanoparticle manufacturing methods. Every method has advantages and limitations, and the synthesis approach is selected based on the accessibility of the required materials. When production costs are an obstacle, chemical procedures are favored; physical approaches are better suited for small-scale applications. Different biological approaches are more or less significant. The top-down approach works well in lab testing but is not optimal for large-scale manufacturing. (Horikoshi & Serpone, 2013).

1.6.1. Physical Approach

The most often used methods in the physical approach to the creation of nanoparticles are optical cutting, high-energy ball grinding (HEBM), electro spraying, evaporation- condensation, and optical degradation. These methods can be applied to create abrasion, melting, evaporation, or condensation in the material. (Chetna Dhand et al., 2013).

1.6.2. Chemical Approach

The sol-gel method, micro emulsion technology, hydrothermal process, and chemical vapor manufacturing are some of the most often utilized chemical processes. Although chemical reduction can produce a lot of nanoparticles, the trash produced is harmful to the environment and the chemicals needed are toxic. (Chetna Dhand et al., 2013).

1.6.3. Biological Approach

One of the biological methods is the use of bacteria, fungi, or plants to produce nanoparticles. There is frequently no need to include wrapping and stabilizing agents from external sources in the process since the cells already contain reducing agents and other elements that serve as an opportunity capping and stabilizing agents. In biological systems, reducing agents can be found anywhere inside the cell. (Pantidos & Horsfall, 2014).

Antibacterial mechanisms of ZnO NPs synthesized from plant extracts

Although the primary antibacterial mechanism is still mostly unclear, No NPs generated through green synthesis show excellent antibacterial properties, Before ZnO may be extensively used as an antibacterial material, it is necessary to study their bactericidal processes. (Toxicol. Sci. 2011)

1.7. Conditions for the synthesis of NPs

To synthesize nanoparticles, a tool or technique must meet the following requirements: Control is maintained over particle size, size distribution, morphology, and crystal structure.

- Possibility of controlling the aggregation.
- improved effectiveness of nanomaterials (lower impurities).
- more accurate results.
- Fastest and more cost-effective production.

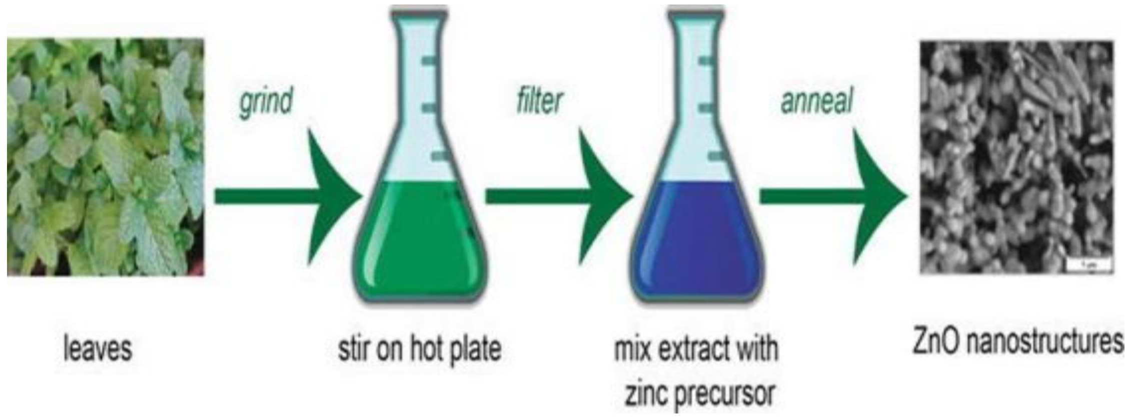
1.8. Green Synthesis of ZnO Nanoparticles

The total yearly production of ZnO-NP is about 550-33400 globally. ZnO is used in ointments in the pharmaceutical business. The degradation of the ecosystem and threats to the environment and public health are caused by commercial dye pollution and wastewater contamination from the textile, printing, and manufacturing sectors. Organic dyes are widely used and extremely hazardous to the environment. They are contaminants in wastewater. Pollutants have been removed from water and wastewater on a wide scale using photocatalysis. Because ZnO nanoparticles have a high surface-to-volume ratio, which facilitates the spread of bacteria and taint in the food and textile sectors, they possess strong antibacterial characteristics. Due to its optical properties, there are rumors that ZnO NPs can fight deadly, unrestrained illnesses like cancer. A variety of diseases caused by oxidative processes are shown to be reduced as a result of the plant's ability to reduce free radical levels. The alleged medicinal potentials of zinc-oxide nanoparticles indicate their antibacterial, antioxidant, and anticancer activities. Due to their abundance in metabolites, leaf extracts have been employed in the bulk of studies on green synthesis. In the fields of engineering medicine, and the physical sciences, green-mediated NPs have several uses. Both green synthetic and chemically generated ZnO NPs are equally effective against germs, however the biologically derived NPs have more potent antibacterial properties. As photocatalysts for removing heavy metals from wastewater, as anti-microbial and microbiological agents, as dietary additives, and in cosmetics, ZnO NPs are also widely and effectively utilized in a number of sensor applications. ZnO NPs affect the bacteria in the same way. However, in terms of antibacterial activity, the biologically obtained ZnO NPs excelled above the green and synthetically produced NPs Interestingly. (Trends Bioethanol. 2012).

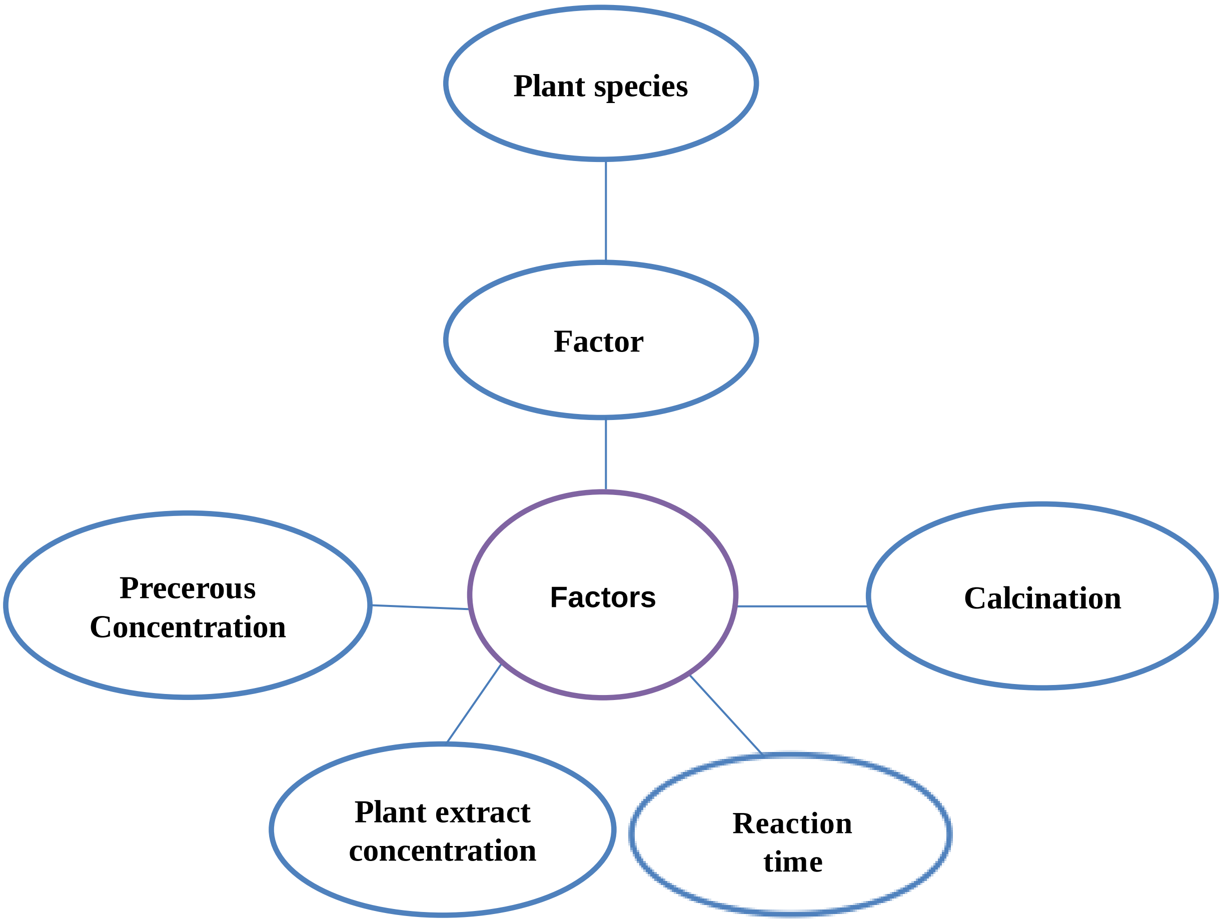
Green chemistry of ZnO nanoparticles

1.9. Factors influencing the antibacterial properties of ZnO NPs synthesized from

Bacterium types. Gram (negative) and Gram (positive) bacteria are distinguished by the characterization of their cell walls. Gram-positive bacteria have been discovered to be more responsive to zinc oxide NPs made from plants than Gram-negative bacteria. (Mater. Res. **2008**,).



Figure; 1.5 Plant extract of ZnO NPs



Figure; 1.6 Factor affecting the Plant extract

Factor affecting the extract

1.9.1 Plant Species: Several plant extracts, including those from the leaves of the cannabis sativa plant, the peltophorum pterocarpum plant, the Carissa edulis fruit, the Hagenia abyssinica passiflora fruit, and the Averrhoa carambola fruit, are used in the green synthesis of ZnO NPs to produce nanoparticles. (Appl Sci. 2020)

1.9.2 Plant Extract concentration:

A hot plate and magnetic stirrer operating at 700 rpm was used to mix 20 g of leaf powder into 100 ml of distilled water, which was then heated to 80°C for 90 minutes. Whatman No. 1 filter paper was used to filter the extract, which was then allowed to cool at ambient temperature before being further chilled. The supernatant was then taken off to be examined further (Microb Pathog. 2018)

1.9.3 Precerous concentration:

90 ml of Hagenia *abyssinica* plant leaf extract, 60 ml of 5.9265 g of zinc acetate dihydrate, and 60 ml of 1.08 g sodium hydroxide were mixed with a (3:2:2) ratio. (Int J Curr Eng Technol. 2014;)

1.9.4. Reaction time:

Hagenia abyssinica leaves were gathered, cleaned with distilled water, and let too dry in the shade to get rid of any moisture left in them. The following ingredients were combined in a 3:1:2: 1:2 ratio 90 ml of Hagenia abyssinica plant leaf extract, 60 ml of 5.9265g zinc acetate dihydrate, and 60ml of 1.08g sodium hydroxide. Even after 2 hours of spinning at 800 rpm, the mixture still produced yellow precipitate. Before drying for 8 hours at 100C, the precipitates were cleaned in ethanol and distilled water to remove impurities. The green synthetic ZnO NPs powder was calcined in a furnace for two hours at 400C. (Int J Curr Eng Technol. 2014;)

1.9.5. Calcination:

The process of calcination is used to make material more crystal like. If there are any impurities on the surface, they are also eliminated, and the temperature of the calcination process is used to change the state of the manufactured material. Typically, calcination temperature encourages the development of crystals. By decomposing ZnO at 400 °C, very crystalline pure phase nanoparticles were produced. The dense agglomeration of ZnO nanoparticles caused the average particle size to grow and the specific surface area to decrease when the calcination temperature climbed from 400 °C to 500 °C and 600 °C.

.

Figure 1.7 Calcination of extract

1.10. Zinc Oxide Nanoparticles:

Hagenia abyssinica leaf extract is used to create ZnO nanoparticles in a green manner. Large-scale pollution removal from water and wastewater has been accomplished by photocatalysis. Physically and chemically processes are used to create ZnO nanoparticles. Because of their high surface-to-volume ratio, which makes it simpler for germs to disseminate and penetrate, ZnO nanoparticles have strong antibacterial characterizes. Antioxidant potential were assessed using the DPPH and TRP tests, which yielded positive results, and antibacterial potential using the well diffusion method. A biological technique of synthesis could use bacteria or plants. Because natural compounds found in plants have the capacity to function as reducing and capping agents during synthetic procedures, the preparation of Nps from plant material is gaining popularity due to the usage of low chemical throughout the whole operation. (Trends Bioethanol. 2012).

- Nps are discovered to be less sssssshazardous than other metallic nanoparticles. There have been reports of these NPs having antibacterial properties. According to reports, ZnO NPs have the ability to combat dangerous, uncontrolled diseases like cancer. (J.A. Boutin, 2002)

- In the current study, we looked into synthesis of ZnO NPs using an environmentally benign method that involved in-situ reduction of Zn by F. vulgare extract.We also discussed the biological potential of environmentally friendly zinc oxide nanoparticles (ZnO-NPs) in terms of their antioxidant and antibacterial properties. (Sonochem 2009)

- As ZnO nanoparticles get smaller until they are nanoscale, their optical qualities start to matter. By using XRD, FT-IR, and SEM examination, the produced ZnO nanoparticles' structure, shape, and size were determined. Additionally, it was discovered that the ZnO NPs had a super paramagnetic behave and no coercivity. (Toxicol Sci 2015,)

- The benefits of green synthesis include inexpensive equipment, a straight experimental setup, ease of fabrication and control, uniform particle size, and the absence of the requirement for additional surface functionalizing agents. (Paneerselvam, 2009.)

1.11. Properties of Zinc oxide Nanoparticles

ZnO nanoparticles come in a different form, including rod-, star-, and isometric ones, and have a size range that spans 30- 150 nm. sometimes referred to as Calamine or Zinc White, is an inorganic substance. It is discovered naturally as the mineral zincite. The majority of its production is synthetic. It is a chemical substance that functions as a topical protector and has a moderate astringent and antiseptic effect.

1.11.1. Chemical Data

Table 1.2: Chemical properties of zinc oxide nanoparticles

Chemical Symbol	**ZnO**
Group	12
	Oxygen 16
Electronic Configuration	[Ar] $3d^{10} 4s^2$ Oxygen [He] $2s^2 2p^4$

1.11.2. Physical Properties

Table 1.3: Physical properties of zinc oxide nanoparticles

Element	Content (%)
Density	7. 13 g/cm^3
Molar Mass	81.379 g mol- 1

1.11.3. Thermal Properties

Table 1.4: Thermal properties of zinc oxide nanoparticles

Properties	Metrics
Melting Point	1,975°C
Boiling Point	2,360°C

1.12. Characterization Techniques

1.12.1. UV-Vis Spectrophotometer

UV-VIS spectroscopy is one of the most useful analytical methods, which serves as the foundation for a number of efficient techniques for detecting minute amounts of analyte in a sample. It deals with identifying the results of electromagnetic radiations interaction with absorbing substances like atoms or ions in the ultraviolet or visible area. (Hussain 2019).

The law behind it is Beer Lambert Law;

The amount of monochromatic radiation that may penetration solution depends on the solution thickness and the brightness of the incident light.

$$A = \log_{10}\left(\frac{I_o}{I}\right) = \epsilon c l$$

Figure;1.8 Ultraviolet visible spectroscopy

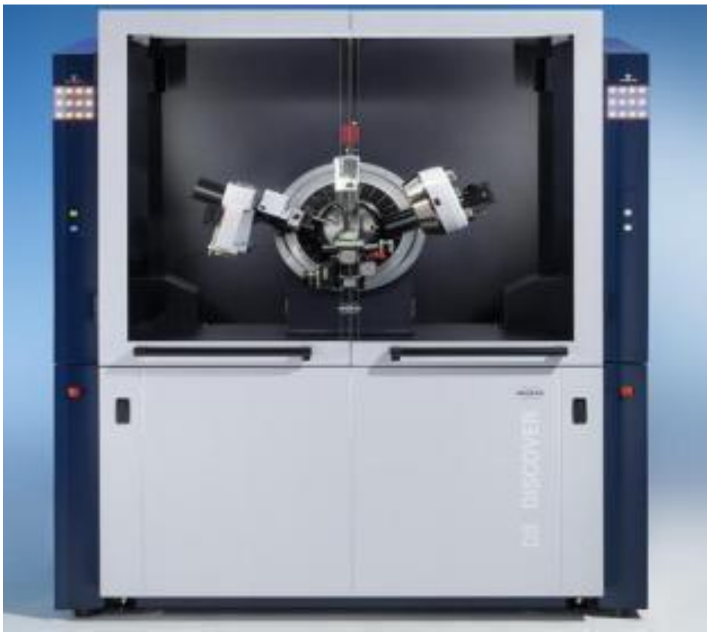
1.12.2. Scanning Electron Microscope

A type of microscope that may be used to examine the viewable portions of micro space and Nano space is the scanning electron microscope, or SEM for short. A light microscope is unable to display the complexity and minute features that may be observed with a (SEM). SEM maybe used to provide high magnification of three-dimensional to pographies. (figure 1.8) In addition to being pricey, the SEM is also rather big. This is a quick procedure that requires very little time for sample preparation. (Abdullah &Mohammed, 2019).

Figure 1.9: Scanning Electron Microscope

1.12.3. X-Ray Diffraction Spectroscope (XRD)

X-ray diffraction, often known as XRD, is a technique used to ascertain the crystallographic structure of a material in the field of materials science.



The investigation of a variety of substances, the method may be applied in several situations and in a variety of industries.

One of the most important uses of XRD analysis is to identify materials based on their diffraction patterns. A method called X-ray diffraction (XRD) may show how internal stresses and imperfections cause actual structures to deviate from ideal structures. (Bunaciu & Aboul-enein, 2015.

1.12.4. Fourier-transform infrared spectroscopy (FTIR):

It is possible to create an infrared spectrum that depicts the of matter three states using Fourier transform infrared spectroscopy (FTIR). Due to the simultaneous data gathering across a wide range, an FTIR analyzer may produce high-resolution spectrum information. Many more applications of infrared (IR) or Fourier transform infrared (FTIR) spectroscopy exist, from the analysis of cells and tissues to the investigation of extremely tiny molecules or chemical complexes. One of the most recent advancements in the area of the of infrared spectroscopy is infrared spectroscopy, which uses both synchrotron IR radiation and infrared microscopy. Tissue imaging is one of the most recent developments in this area. (Catherine Berthomieu & Hienerwadel Rainer, 2013).

Figure 1.11: Fourier Transform Infrared Spectroscopy

METHODOLOGY

In the current work, a green method for synthesizing zinc oxide nanoparticles (NPs) has been suggested utilizing Catharanthus roseus plant extract. A list of the tools, substances, and devices used during the experiment is provided in this chapter.

3.2. Required Apparatus

- Beakers
- Conical flasks
- Volumetric flasks
- Pipette
- Thermometer
- Sample vials
- Stirrer
- Foil
- Cotton
- Spatula
- Test tubes
- Hotplate

RESULTS AND DISCUSSION

4.1. Characterization of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles were characterized by FTIR and XRD.

4.1.1.FTIR Analysis of Zinc Oxide NPs:

The green synthesized zinc oxide nanoparticles were studied via Fourier transform infrared (FTIR) spectroscopy. Several well defined peaks, from 500–4000 cm- 1 peak, the successfully synthesis of zinc oxide nanoparticles. In the FTIR spectra of zinc oxide nanoparticles, there is an absorption at 3400 cm- 1 Which is attributed to O-H or N-H stretching. There is an absorption at 3010 cm-1 which indicate C-H stretching. There is an absorption at 2336 cm-1 which indicate O=C=O stretching. Other characteristics peaks at 1610 cm-1, 1278 cm-1, 1054 cm-1, and 670 cm-1 are also observed which are attributed to C=C stretching, C-N stretching, C-O stretching, and C-Br stretching, respectively. (Sci Semicond Process. 2015)

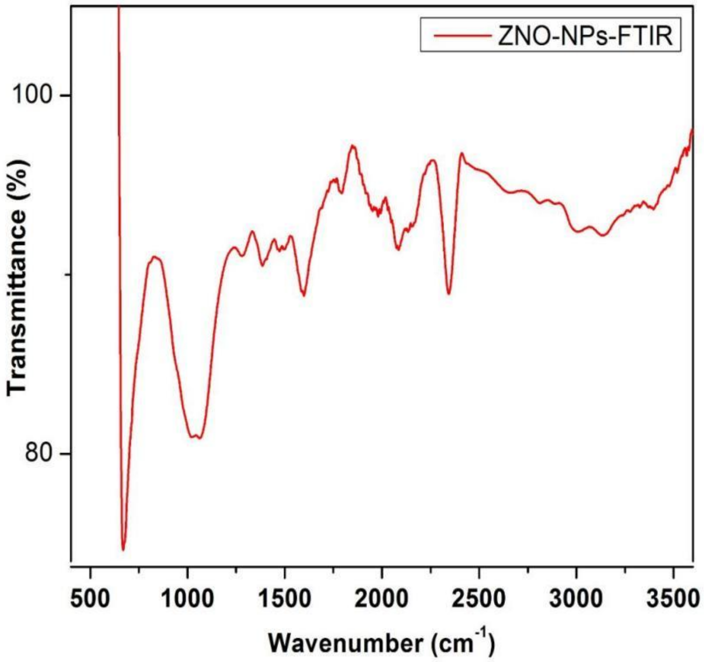


Figure 4.1 FTIR- spectroscopy

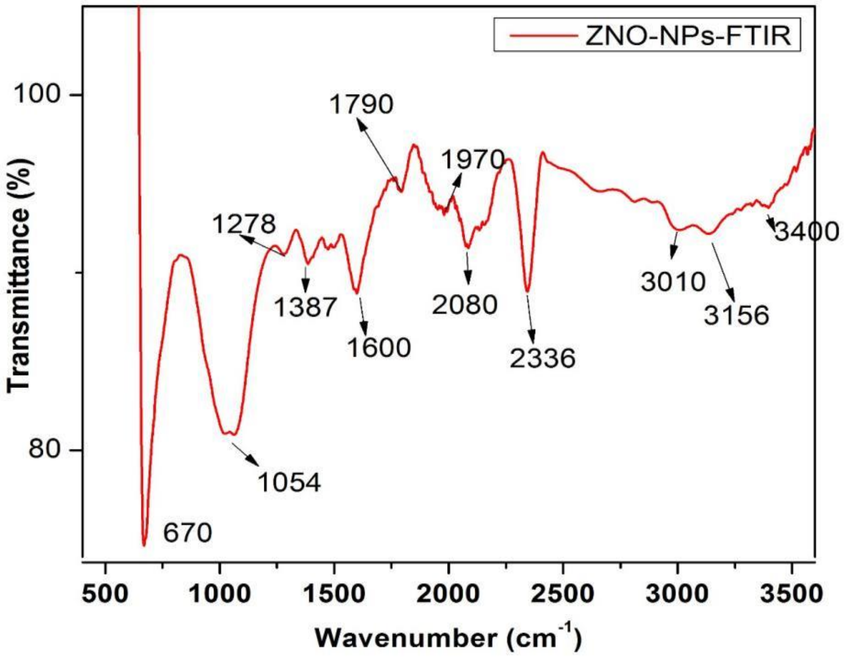


Figure 4.2 FTIR- spectroscopy

Frequency range (cm-1)	Functional Group
3400cm-1	O-H, N-H stretching
3010cm-1	C-H stretching
2336cm-1	O=C=O stretching
1600cm-1	C=C stretching
1278cm-1	C-N stretching

1054cm-1	C-O stretching
670cm-1	C-Br stretching

Table 4.1 Peak list of FTIR

4.1.2. XRD Analysis of ZnO NPs:

The XRD technique is a crucial for the characterization of crystal structures since it can be used to determine the lattice parameters of single crystals as well as the phase texture and stress of samples. Purity and particle size can also be evaluated using XRD methods, and also helpful X-rays are used to assess the morphology of nanoparticles. It can also be used to convey details on one-cell dimensions. XRD techniques are based on the elastic scattering of X-rays from materials exhibiting long-range order. Theta values range from 0 to 90, and intensity levels range from 0 to 300. To find the structure behaviors of the synthesized nanoparticles, we have used XRD analysis. As the results, shown in Fig. 4.3, four diffraction peaks were observed at 38.09°, 44.58°, 64.14° and 77.29° theta values, which corresponds to (111), (200), (220), and (311) crystal planes, respectively. The appeared signals are comparable with the reported literature which support the successful synthesis of ZnO nanoparticles via green approach. The results indicate that our designed nanoparticle is in of crystalline hexagonal Shape. (J Mol Struct. 2022)

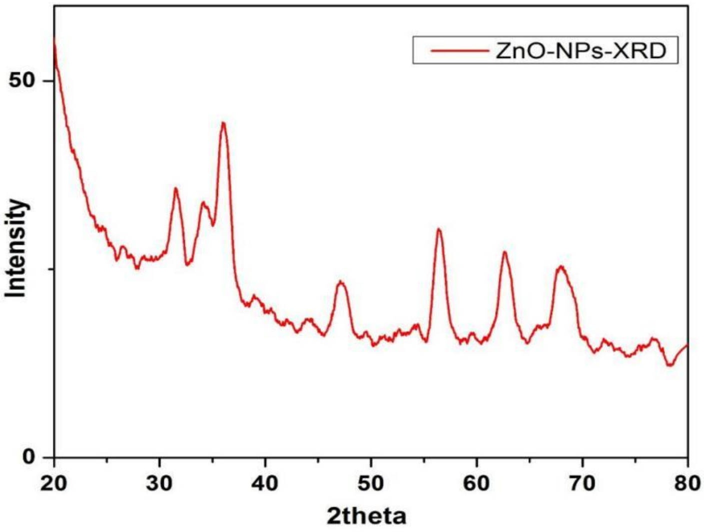


Figure: 4.2 XRD spectroscopy

Table 1.7 Peak list of XRD pattern of Zinc oxide Nanoparticles

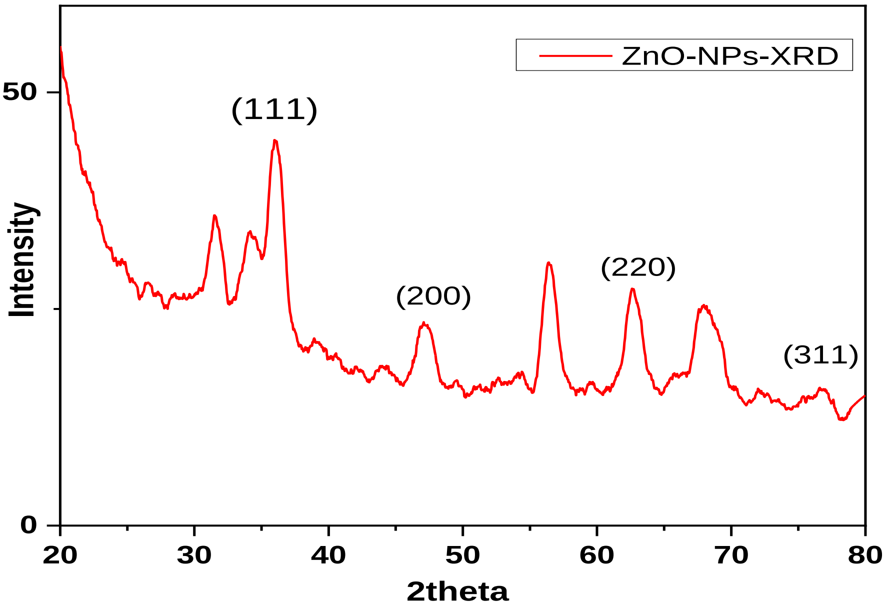


Figure: 4.3 XRD spectroscopy

The size of crystallites was calculated by the given Scherer equation.

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

Where,

D = crystallite size (nm)

K = 0.9 (Scherrer constant)

λ= 0.15406 (wavelength of x-ray)

$\boldsymbol{\beta}$ = Full width half maximum (FWHM) radians

θ = peak position (radian)

Sample	Peak position (2θ)	Peak orientation (Miller indices)	FWHM	Crystallite size (nm)
ZnO	56.62°	111	0.3231	27.9
	66.46°	200	0.2910	32.6
	68.26°	220	0.3678	26.0
	77.29°	311	1.0475	9.71

Mean value of Crystallite size = 24.0

Table 4.2 Peak list of XRD

4.2 Antibacterial Activity

The agar disk diffusion method was used to test the antibacterial activity of the synthesized drugs at the biotechnology lab at the University of Sialkot. The antibacterial activity of *Pseudomonas aeruginosa, Bacillus subtilis and*

Escherichia coli bacteria against each chemical was examined. The broth-dilution technique was used to determine MIC values.

4.2.1. Preparation of inoculums

Pure cultures of bacteria were added to L.B broth (PH=7) in test tubes and allowed to grow for 24 hours at 37°C. A sterile, isotonic solution was used to create bacterial suspensions. After that, bacterial dilution was made by combining 1 mL of turbid bacterial culture with 9 mL of distilled water in a test tube (9:1 dilution).

4.2.2. Preparation of Nutrient Agar Medium

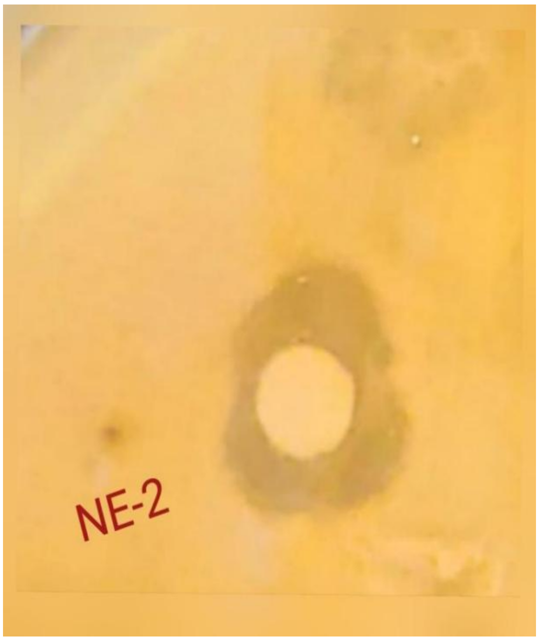
6.25g of nutrient agar powder were dissolved in 250mL of distilled water to make agar solution. Mix them thoroughly to dissolve them. It was sterilized in an autoclave for 15 minutes at 120°C while the pH was held constant at 7. All of the experimentation was done in a laminar flow after autoclaving, and waited for the liquid medium to settle before pouring it onto the clean petri dishes. To avoid contamination, the preparation of agar was be done in a sanitary setting.

4.2.3. Determination of Antibacterial Activity

Following the established approach, the agar disk diffusion method was used to assess the antibacterial activity of the ZnO nanoparticles. The sterilized nutrient agar medium was melted and then put in an amount of 20 ml onto petri plates, where it was left to cool and solidify for 30 minutes. A sterilized cotton swab is dipped into the inoculum and remove excess medium by pressing the swab onto the wall of the tube. Swab the surface area of the plate completely by rotating the plate. This technique is called lawn culture or carpet culture. Allowed the plates to dry for 5 minutes so that the medium absorbs the inoculum properly.

First sterilized the forceps with alcohol before picking up antibiotic discs. Discs should be placed at a distance of 24 mm. Lightly touch each disc with forceps to ensure that it is in good contact to avoid misplacement. For around 24 hours, plates were incubated in an incubator at 37°C with paraffin tape covering them. By measuring the millimeter diameter of the zone of inhibition and comparing it to standards, the antibacterial activity was determined.

Code	Gram- positive		
	Diameter of inhibition zone (mm) and MIC (µg/mL)		
	Bacillus subtilis	Positive control Diameter Ciprofloxacin	Negative control diameter Distilled water
NE-SMS-OA-01	11mm(100µg/mL)	45mm	0mm
NE-SMS-OA-01	13mm(200µg/mL)	45mm	0mm



Code	**Gram-negative**					
	Diameter of inhibition zone					
	E. coli	**Positive control Diameter Ciprofloxacin**	**Negative control Diameter Distilled water**	**P. aeruginosa**	**Positive control diameter Ciprofloxacin**	**Negative control diameter Distilled water**
NE-SMS-OA-01	**12mm (100µg/mL)**	**38mm**	**0mm**	**20mm (100µg/mL)**	**33mm**	**0mm**
NE-SMS-OA-01	13mm (200µg/mL)	38mm	0mm	29mm (200µg/mL)	33mm	0mm

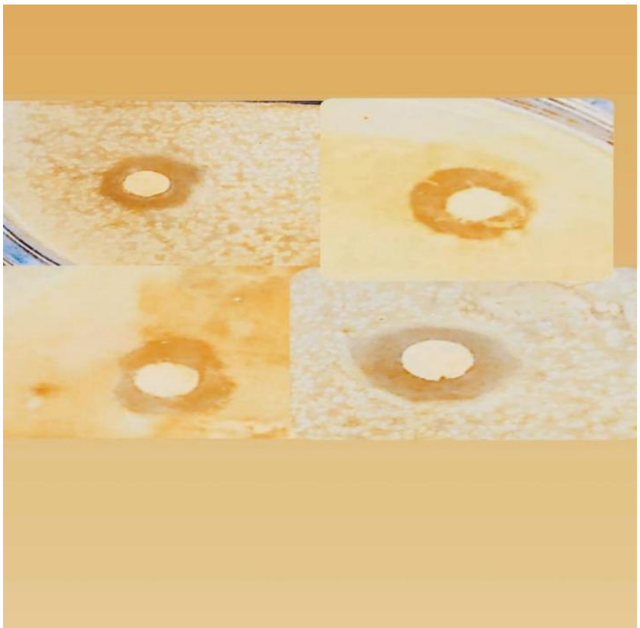


Figure 4.4 Antibacterial activity Experiment

MIC = Lowest conc. of sample inhibit growth + Highest conc. of sample inhibit growth /2

$$MIC = \frac{100+200}{2}$$

MIC = 300 µg/ml or 0.3 mg/ml

Concentration µg/ml of sample	400	300	200	100
Result	-	-	Maximum growth	Minimum growth

CONCLUSION

The leaves of the Catharanthus roseus plant were used to make zinc oxide nanoparticles, which were then characterized using several spectroscopic methods. FTIR, and XRD spectroscopy were used to analyze the synthesized nanoparticles. By using green production, zinc Oxide nanoparticles are inexpensive with the potential to be employed in a number of applications, such as antibacterial agents. According to the findings, the green zinc nanoparticles (NPs) and Demonstrated good antibacterial activity. The most promising inorganic materials with bactericidal activity are zinc oxide nanoparticles which are used in the formulation of medicinal medications, sanitizers, cosmetics, and food packaging procedures. Because of their tiny size (less than 100 nm) and high surface-to-volume ratio (NE-SMS-OA-01) showed the higher antibacterial activity against tested bacterial Strains.

REFERENCES

- Abdullah, A., & Mohammed, A. (2019). Scanning Electron Microscopy (SEM): A Review. In *Proceedings of the 2018 International Conference on Hydraulics and Pneumatics—HERVEX, Băile Govora, Romania* 1–9.
- Abdullah, J. A. A., Salah Eddine, L., Abderrhmane, B., Alonso González, M.,Guerrero, A., & Romero, A. (2020). Green synthesis and characterization ofzinc oxide nanoparticles by *Pheonix dactylifera* leaf extract and evaluation of their antioxidant activity. *Sustainable Chemistry and Pharmacy, 17,* 100280-100287.
- Alagiri, M., & Hamid, S. B. A. (2014). Green synthesis of α-Fe2O3 nanoparticles for photocatalytic application. *Journal of Materials Science: Materials in Electronics*, *25*(8), 3572-3577.
- Aqel, A., El-Nour, K. M. M. A., Ammar, R. A. A., & Al-Warthan, A. (2012). Carbonnanotubes, science and technology part (I) structure, synthesis and characterisation. *Arabian Journal of Chemistry*, *5*(1), 1–23.
- Bhuiyan, M. S. H., Miah, M. Y., Paul, S. C., Aka, T. Das, Saha, O., Rahaman, M. M.,Sharif, M. J. I., Habiba, O., & Ashaduzzaman, M. (2020). Green synthesis of zinc oxide nanoparticle using *Caricapapaya* leaf extract: application for photocatalytic degradation of remazol yellow RR dye and antibacterial activity. *Heliyon*, *6*(8), e04603.
- Bibi, I., Nazar, N., Ata, S., Sultan, M., Ali, A., Abbas, A... & Iqbal, M. (2019). Green synthesis of zinc oxide nanoparticles using pomegranate seeds extract and photocatalytic activity evaluation for the degradation of textile dye *ournal of Materials Research and Technology*, *8*(6), 6115-6124.
- Bunaciu, A. A., & Aboul-enein, H. Y. (2015). X-Ray Diffraction: Instrumentation and Applications Critical Reviews in Analytical Chemistry. *Analytical Chemistry*, 45, 289–299.

- Catherine Berthomieu, & Hienerwadel Rainer. (2013). Fourier transform infrared (FTIR) spectroscopy. *Photosynthesis Research*, 101, 157– 170.
- Chetna Dhand, A., Neeraj Dwivedi, B., Xian Jun Loh, C., Alice Ng Jie Ying, A.,Kumar, N., Verma, ad Roger W. Beuerman, ae R. L. and S., &
- Ramakrishna. (2013). Methods and Strategies for the Synthesis of Diverse Nanoparticles and their Applications: A Comprehensive Overview. *RSC Advances*, 1, 1– 100.
- characterization of zinc oxide nanoparticles using Ficus carica (common fig) dried fruit extract. *Journal of bioscience and bioengineering*, *127*(2), 241-245.
- Devi, H. S., Boda, M. A., Shah, M. A., Parveen, S., & Wani, A. H. (2019). Green synthesis of zin c oxide nanoparticles using Platanus orientalis leaf extract for antifungal activity. *Green Processing and Synthesis*, *8*(1), 38-45.
- zinc oxide nanoparticles using aqueous leaf extract of *Thymbraspicata* and evaluation of their antibacterial, antibiofilm, and antioxidant activity. *Inorganic and Nano-Metal Chemistry*, *51*(5), 683–692.
- Georgakilas,V., Perman, J. A., Tucek,J., & Zboril, R. (2015). Broad family of carbon nanoallotropes: classification, chemistry, and applications of fullerenes, carbon dots, nanotubes, graphene, nanodiamonds, and combined superstructures. *Chemical reviews*, *115*(11), 4744-4822.
- Ghorbanpour, M., Manika, K., & Varma, A. (Eds.). (2017). *Nanoscience andplant- soil systems.* Springer International Publishing.
- Horikoshi, S., & Serpone, N. (2013). Introduction to nanoparticles. *Microwaves in Nanoparticle Synthesis: Fundamentals and Applications*, 1–24.
- Hussain, A. F. (2019). UV-Visible spectrometry. In *Proceedings of the 2018 International Conference on Hydraulics and Pneumatics—HERVEX, Băile Govora, Romania* 1–6.
- Issa, B., Obaidat, I. M., Albiss, B. A., & Haik, Y. (2013). Magnetic nanoparticles:
- surface effects and properties related to biomedicine applications.
- *International Journal ofMolecular Sciences*, *14*(11), 21266-21305.

- Jadoun, S., Arif, R., Kumari, N., Rajesh, J., & Meena, K. (2020). Green synthesis of nanoparticles using plant extracts: a review. *Environmental Chemistry Letters*,19,355-374.
- Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, *12*(7), 908-931.
- Klabunde, K. J., & Richards, R. M. (Eds.). (2009). *Nanoscale materials in chemistry*.
- Pantidos, N., & Horsfall, L. E. (2014). Biological synthesis of metallic nanoparticles by bacteria, fungi and plants. *Journal of Nanomedicine & Nanotechnology*, *5*(5),
- Siqueira, J. R., & Oliveira, O. N. (2017). *Carbon-Based Nanomaterials.* 233–249
- Subedi, S. K. (1985). An introduction to nanotechnology and its implications. *Himalayan Physics*, 5, 78-81.
- Tiwari, D. K., Behari, J., & Sen, P. (2008). Application of Nanoparticles in Waste Water Treatment. *Cit.*