

**GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLE
USING *ARTOCARPUS HIRSUTUS* SEED EXTRACT AND IN
VITRO CHARACTERIZATION OF THEIR POTENTIAL
ANTIBACTERIAL AND CYTOTOXIC ACTIVITY**

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Pharmacy

By

S. RUPPANA (39050042)

K. YESHWANTH (39050060)



SCHOOL OF PHARMACY

SATHYABAMA INSTITUTE OF SCIENCE & TECHNOLOGY

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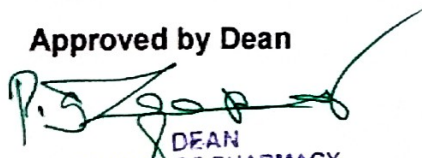
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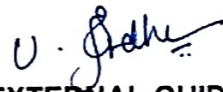
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Declaration

We do hereby declare that the work presented in the thesis entitled **“GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLE USING ARTOCARPUS HIRSUTUS SEED EXTRACT IN VITRO CHARACTERIZATION OF THEIR POTENTIAL ANTIBACTERIAL AND CYTOTOXIC ACTIVITY”**, submitted by us to the **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY**, Chennai-600119 for the award of the degree of **Bachelor of Pharmacy (2019-2023)** by Research is an original record of research work carried out by us under the supervision of our Internal Guide **Dr.N.Delhiraj, M.Pharm , Ph.D. ,Professor, Department of Pharmacy** and our External Guide **Dr.Sudha Uthaman, M.Sc., Ph.D., Scientist “C”, International Research Center**. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship, associateship etc. In keeping with the general practice in reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

Place

Date

S. Ruppana

S. Ruppana (39050042)

K. Yeshwanth

K. Yeshwanth (39050060)

1.INTRODUCTION

1.1. Medicinal plants

Medicinal plants refer to a plant comprising active ingredients or secondary metabolites that possess biological activity. A whole plant may be medicinally active or plant parts. *Herbal medicine* are medicinal preparations comprising active ingredients obtained from the herbal plant. The product can be made from the whole plant or any part. Preparations from by-product herbal plants such as oils, gums, and other secretions are also considered as herbal medicine.

Medicinal plants are a core component at primary health care level due to availability, acceptability, compatibility, and affordability. Medicinal plants have long been utilized in traditional medicine and worldwide ethnomedicine. People are encouraging indigenous production and processing of these medicinal plants to use in different cultures and religion for the treatment of various diseases. It is estimated that about 75-80% of people of developing countries and about 25% of people of developed countries depend either directly or indirectly on medicinal plants for the first line of treatment.¹

1.1.1. Phytoconstituents and its medicinal uses

Phytoconstituents are chemical compounds that naturally occur in plants. Some of them are responsible for color and other organoleptic properties. Various phytoconstituents present in plants include alkaloid, flavonoid, glycoside, terpenoid, tannin, saponin, Cardiac glycoside, anthraquinone, steroid and reducing sugar.^{2,3}

Table 1.1 Phytoconstituents and its medicinal uses.

Phytoconstituents	Example	Medicinal use
Alkaloid	1.Curarine 2.Atropine	Muscle relaxant Dilation of pupil
Glycoside	1.Digitalis 2.Ginseng	Congestive heart failure. Anticancer, Antidiabetic Antioxidant
Tannins	1.Eucalyptus 2.Red mangrove	Diabetes, Ulcer Wound Healing, Diarrhea
Terpenoids	1.Ocimum gratissimum leaf 2. Ruta chalepensis leaves.	Anti-inflammatory, Antibacterial, Antiviral, Antimalarial, Anticancer
Lectin and Polypeptide	Mannose-specific agglutinin, Fabatin	Antimicrobial
Phenols and Polyphenols	Cinnamic acid, Catechol	Antimicrobial Anthelmintic
Saponins	Glycyrrhiza	Antiulcer, Respiratory disorders.
Flavonoids	Quercetin	May constrain inflammation and tumour growth.

1.2. Extraction

Extraction as the term is used pharmaceutically, involves the separation of medicinally active portions of plant from the inactive or inert components by using selective solvents in standard extraction procedures.

1.2.1. Solvents involved in extraction

The solvent used for the extraction of medicinal plants is also known as the menstruum. The choice of solvent depends on the type of plant, part of plant to be extracted, nature of the bio-active compounds, and the availability of solvent.⁴

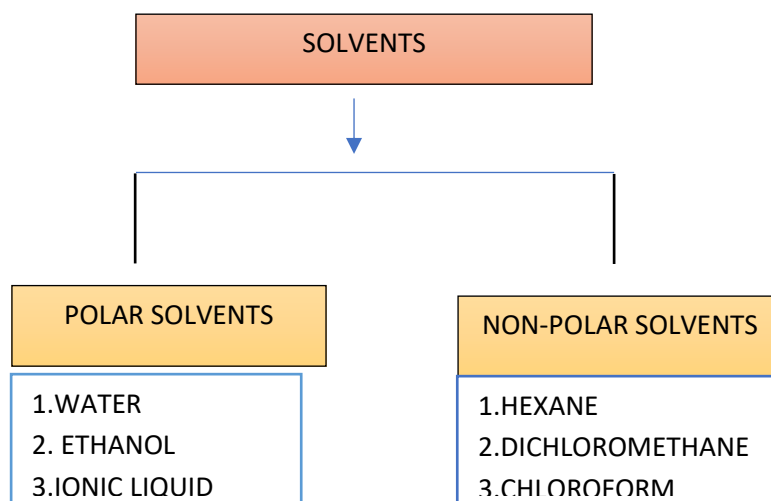


Figure:1.1 Different solvents used for extraction.

1.2.2. Factors to be considered in the selection of solvent

Various factors enumerated below should be taken into consideration when choosing a solvent of extraction.

- a) *Selectivity*: The ability of a chosen solvent to extract the active constituent and leave the inert material.
- b) *Safety*: Ideal solvent of extraction should be nontoxic and nonflammable.
- c) *Cost*: It should be as cheap as possible.
- d) *Reactivity*: Suitable solvent of extraction should not react with the extract.
- e) *Recovery*: The solvent of extraction should be quickly recovered and separated from the extract.
- f) *Viscosity*: Should be of low viscosity to allow ease of penetration.
- g) *Boiling temperature*: Solvent boiling temperature should be as low as possible to prevent degradation by heat.

1.2.3. Different Methods of extraction

- *Maceration:* In this extraction coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside a container; the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days. The content is stirred periodically to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermolabile plant material.⁴
- *Infusion:* The drug material is grinded into fine powder, and then placed inside a clean container. The extraction solvent hot or cold is then poured on top of the drug material, soaked, and kept for a short period of time. This method is suitable for extraction bioactive constituents that are readily soluble. In addition, it is an appropriate method for preparation of fresh extract before use. The solvent to sample ratio is usually 4:1 or 16:1 depending on the intended use.
- *Decoction:* This is a process that involves continuous hot extraction using specified volume of water as a solvent. A dried, grinded, and powdered plant material is placed into a clean container. Water is then poured and stirred. Heat is then applied throughout the process to hasten the extraction. The process is lasted for a short duration usually about 15min. The ratio of solvent to crude drug is usually 4:1 or 16:1. It is used for extraction of water soluble and heat stable plant material.^{5,6}
- *Percolation:* The apparatus used in this process is called percolator. It is a narrow-cone-shaped glass vessel with opening at both ends. A dried, grinded, and finely powdered plant material is moistened with the solvent of extraction in a clean container. More quantity of solvent is added, and the mixture is kept for a period of 4h. Subsequently, the content is then transferred into percolator with the lower end closed and allow to stand for a period of 24h. The solvent of extraction is then poured from the top until the drug material is completely saturated. The lower part of the percolator is then opened, and the liquid allowed to drip slowly. Some quantity of solvent was added continuously, and the extraction taken place by gravitational force, pushing the solvent through the

drug material downward. The addition of solvent stopped when the volume of solvent added reached 75% of the intended quantity of the entire preparations. The extract is separated by filtration followed by decantation. The marc is then expressed and final amount of solvent added to get required volume.^{8,9}

- *Soxhlet extraction:* This process is otherwise known as continuous hot extraction. The apparatus is called Soxhlet extractor made up of glass. It consists of a round bottom flask, extraction chamber, siphon tube, and condenser at the top. A dried, grinded, and finely powdered plant material is placed inside porous bag (thimble) made up of a clean cloth or strong filter paper and tightly closed. The extraction solvent is poured into the bottom flask, followed by the thimble into the extraction chamber. The solvent is then heated from the bottom flask, evaporates, and passes through the condenser where it condenses and flow down to the extraction chamber and extracts the drug by coming in contact. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask. The entire process continues repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind. This method is suitable for plant material that is partially soluble in the chosen solvent and for plant materials with insoluble impurities. However, it is not a suitable method for thermolabile plant materials.^{9,10}



Figure:1.2 Soxhlet extraction

1.3. Nanoparticle

Nanotechnology refers to an emerging field of science that includes synthesis and development of various nanomaterials. Nanoparticles can be defined as objects ranging in size from 1-100nm that due to their size may differ from the bulk material. Nanomaterials act as a bridge between bulk matters and atoms, molecules or cluster structures. The physicochemical properties of the nanomaterials change with the size or volume of their surface. Metal nanoparticles are now gaining importance industrially. Within the range of 1-100nm nanoparticles generally exhibit very interesting chemical, physical and electrical properties. These properties include thermal, optical and magnetic behavior, conductivity, redox potential etc.¹¹

1.3.1. Applications of nanoparticle in the field of medicine

Nanoparticles used in various medical filed including cancer therapy, Gene therapy, Acquired Immuno Deficiency Syndrome therapy and radiation. Some important application of nanoparticles is listed below.

- 1) Nanoparticles in the treatment of kidney disease:** Ferumoxytol has been included into nanoparticles for treatment of patients with chronic kidney disease or end-stage renal disease who do not have enough erythropoietin.¹²
- 2) Nanoparticles in treatment of Alzheimer's disease:** Nanoparticle - mediated medication or diagnosis and delivery is one of the newest approaches for increasing CNS Penetration for diagnosis and treatment of Alzheimer's disease. PNPs are promising candidates among the various nanocarriers used because, in addition to being able to open the tight junctions of the Blood Brain Barrier, they effectively conceal the membrane barrier confirming characterizations of the drug molecule, prolonging drug release and protecting drug against enzymatic hydrolysis.
- 3) Anticancer Agents:** Nano-oncology is a new discipline of medicine which makes use of nanoparticle in the treatment of cancer. Usage of nanoparticles improves cancer cell targeting overcomes cancer tissue multidrug resistance. PLGA is a widely polymer for making nanoparticles and has been utilized to make drug-loaded nanoparticles for cancer therapy due to its biocompatibility and long-term drug release¹³.

4) Nanoparticles for Treatment of tuberculosis by chemotherapy:

Anti-Tuberculosis medicines loaded nanoparticles was due to their changed release behavior following oral administration. Rifampin, Isoniazid and pyrazinamide are the three major medicines co-incorporated in PLG-nanoparticles. It improves the efficacy of loaded Anti-TB drugs.¹⁴

5) Drug targeting to infectious diseases by nanoparticles:

The physical and chemical properties of nanoparticles are being used as a tool to treat variety of infectious disorders. The use of therapeutic medicine loaded on a nano-vector boosted efficacy of drug against infectious disorders. Polyethylene glycol-modified carbon nanotubes are the most common type of non-viral deliver system due to their enhanced pharmacokinetic and toxic characteristics. These are efficient carriers of bioactive molecule in delivery of specific drugs for treatment of infectious illnesses.¹¹⁻¹⁴

6) Nanoparticles topical drug delivery for skin diseases:

Polymeric nanoparticle (PNP) is most extensively used nanoparticle for medication administration on the skin. PNPs are made from chitosan and alginate used to treat acne compared to benzoyl peroxide showed improved antibacterial efficacy against *Propionibacterium acnes*. Polymeric nanoparticles, electro-spun fibers mats have high surface area volume ratio, which help in dispersion of both hydrophilic and hydrophobic medications for ideal for topical drug administration. Liposomes, solid lipid nanoparticles and nanostructured lipid carriers (NLC) all cling to the surface of the skin. Liposomes, solid lipid nanoparticles (NLC) bind to skin surface, allowing lipid exchange between stratum corneum outermost layers and the carrier for better medication penetration.^{11,14}

- 7) Nano emulsions:** Nano emulsions and self-emulsified drug delivery system (SEDDS) is a way to increase bioavailability of medicines of low aqueous solubility. Nano emulsions are non-homogeneous systems made up of immiscible liquids where one is disseminated as droplets in the other. When integrated into aqueous phase under mild mixing, SEDDS are the isotropic mixes of oil, surfactant, co-surfactant and drug that produces oil-in water (o/w) nano emulsions. These substances improve the oral bioavailability of weakly water-soluble medicines. Small size of droplets reduces the surface tension between the oil droplet and aqueous medium of gastrointestinal tract for more uniform and widespread drug distribution in the gut.¹⁶
- 8) Solid Lipid nanoparticles (SLN):** SLN were produced as a substitute to emulsions, liposomes and PNP as a colloid drug delivery in a controlled manner. SLN are prepared from solid lipids and stabilized by surfactant. It offers several benefits for drug delivery and other particles including superior tolerability, biodegradability, high bioavailability via ocular route, and targeted impact on the brain.^{11,16}
- 9) Nano capsules:** Nano capsules and Nanospheres vary in the former are carriers where drug is contained in a core enclosed by polymeric membrane, while latter are structures where drug is disseminated through polymeric matrix. PNPs can be thought of as matrix in which drug is equally distributed. Across or inside of the polymeric matrix, medication is dissolved, entrapped or encapsulated. PNPs are excellent alternative for cancer therapy and other applications due to their capacity to customize medication delivery.¹⁶
- 10) Polymeric Nanoparticles:** Polymeric nanoparticles (PNPs) are mostly biodegradable and biocompatible. PNPs are divided into vesicular systems and matrix system. Natural PNPs prevailed over traditional delivery system, due to their higher efficiency and effectiveness' are potential intracellular and site -targeting systems.^{11,16}

1.3.2. Metal-based nanoparticles:

Metal-based nanoparticles are characterized by small size and high surface area. Their size, surface charge, and shape all influence their cellular uptake. The functionalities present on the surface of some metal nanoparticles enhance their cellular interactions.

1.3.2.1. Antibacterial activity:

Nanoparticles exhibit antibacterial activity resulting from their ability to produce reactive oxygen species that damage the bacteria and their ability to bind to DNA or RNA, thereby hindering microbial replication processes. Metal-based nanoparticles that have been reported with antibacterial activity include silver, iron, iron oxide, copper oxide, zinc oxide, aluminum oxide, titanium dioxide, gold and gallium nanoparticles. The mechanisms of antibacterial action with regard to specific metal nanoparticles explained as follows;

Silver nanoparticles interact with bacteria and release silver ion resulting in deactivation of the cellular enzymes, hindered membrane permeability and cell death. The antibacterial activity of **iron oxide nanoparticles** is via oxidative stress generated by ROS, resulting in the damage of the proteins and DNA in the bacteria.

Copper based nanoparticles were characterized by tapered spears, that they were in contact with the bacterial cell, they can penetrate easily into the bacterial cell, resulting in cell death. These nanoparticles killed the cells by destroying the cell membranes.¹⁷

Zinc oxide nanoparticles act as antibacterial agents by altering the permeability of the membrane and inducing oxidative stress resulting in bacterial cell death.

Titanium dioxide nanoparticles antibacterial activity is attributed to their interaction with the bacteria cell upon which photocatalytic action results in enhanced cell permeability, causing accelerated photo-oxidation of the intracellular components and cell death.

The antibacterial activity of gold nanoparticles is due to the penetration ability of the nanoparticles via the cell wall reduced adenosine triphosphate synthase activities which disrupt the metabolic process and a decline of the subunit of the ribosome for tRNA binding, thus resulting in a collapse of the biological mechanism. The nanoparticle interfered with the bacteria protein and cytoplasm, causing cell death.

Table 1.2: Antibacterial activity of metal-based nanoparticle.¹⁷

S.No	Metalbased nanoparticle	Effective against
1.	Silver	Escherichia coli, Staphylococcus aureus.
2.	Iron Oxide	Pseudomonas aeruginosa, Serratia marcescens.
3.	Zinc Oxide	Klebsiella pneumonia, Bacillus subtilis
4.	Gold	Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis

1.3.2.2. Cytotoxic activity:

Metal-based nanoparticles usually produce unique biological effects at the cellular level by affecting transmembrane and basic cellular processes, such as cell division, proliferation, apoptosis, and regulation of signal transduction pathways. The cytotoxicity mechanisms of metal-based Nanoparticle are mainly associated with oxidative stress, inflammatory response, autophagy, and apoptosis. Oxidative stress is typically evaluated according to ROS and superoxide dismutase (SOD) levels, the inflammatory response is evaluated by inflammatory factors, and autophagy and apoptosis are detected by DNA damage.

Table 1.3: Cytotoxic activity of metal-based nanoparticles.¹⁸

S.No	Nanoparticles	Typical effect
1.	Aluminium oxide	Impaired learning and memory
2.	Titanium oxide	Neuronal damage
3.	Zinc oxide	Immunotoxicity
4.	Copper	Liver fibrosis Immunosuppressive effect
5.	Silver	Dose dependent DNA damage
6.	Nickel	Significant Reproductive toxicity

1.3.3. Methods to synthesize nanoparticle

The synthesis of nanoparticles of controlled size, shape and also the dispersity of materials at the nanometer scale length is gaining importance for its antimicrobial and anticancer properties. Synthesis of nanomaterials using biological entities represents the interrelation between nanotechnology and biotechnology. Nanoparticles have been produced by physical and chemical methods for a long time, but recently the critical role of microorganisms and biological systems in production of metal nanoparticles have been well established and this area is rapidly developing due to tremendous success obtained and ease of formation of nanoparticle.

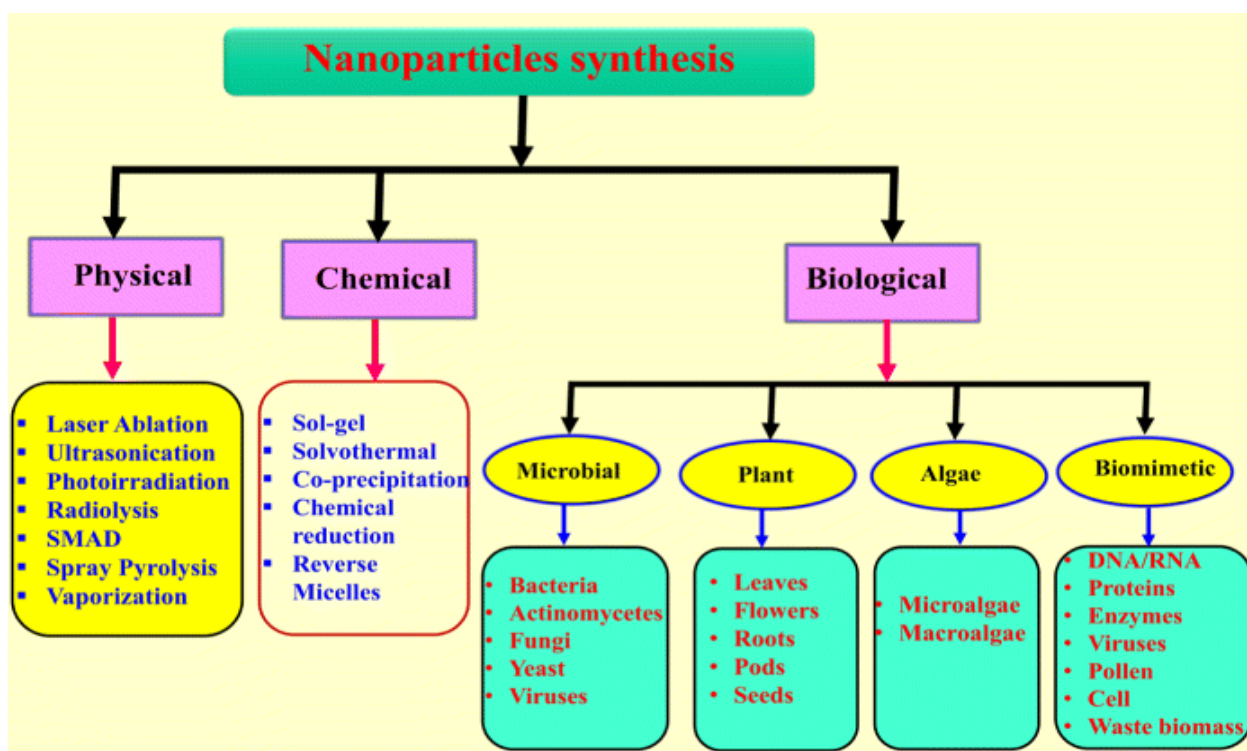


Figure: 1.3 Methods to synthesize nanoparticle

Physical methods

- **Ball milling:** Ingenious approaches for the creation of nanoparticles. Forms of mills used are planetary, vibratory, rod, tumbler. The container contains hard balls made up of steel or carbide. Nanocrystalline Co, Cr, W, Ag-Fe, are synthesized using this method. The ratio of balls to materials is 2:1. The container is filled with inert gas or air and is rotated at high speed around the central axis. The materials are pressed between the walls of the container and balls. The speed and duration of milling play a significant role in synthesizing nanoparticles of optimum size¹⁹.
- **Pulse laser ablation:** The target sample is placed inside a vacuum chamber. The high-pulsed laser beam is focused on the sample and plasma is generated, which is formerly transformed into a colloidal solution of nanoparticles. The second-harmonic group type laser is frequently used to formulate nanoparticles. Elements affecting the final creation are the type of laser, some pulses, type of solvent, pulsing time^{19,20}.

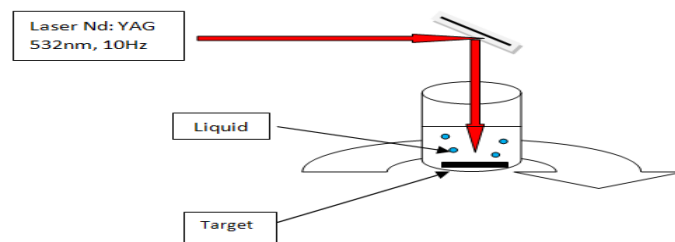


Figure:1.4 Pulse laser Ablation

- **Ionized cluster beam deposition:** This method was developed in 1985. The main aim of this method is to obtain high-quality single-crystalline thin films. The arrangement comprises a source of evaporation, a nozzle through which material can expand into the chamber, an electron beam to ionize the clusters, an arrangement to accelerate the clusters, and a substrate on which nanoparticle film can be deposited, all housed in a suitable vacuum chamber. After impact with an electron beam, collections get ionized. Due to applied hastening voltage, the clusters are focused near the substrate. It is likely to control the energy with which the clusters hit the substrate by monitoring the

accelerating voltage. Steady clusters of certain materials would need significant energy to break their bonds and would rather favour remaining as small as clusters of particles. Hence the films of nanocrystalline material using an ionized cluster beam can be produced.¹⁹

- **Chemical vapor deposition:** A thin film of gaseous reactant is deposited on the substrate at around 300-1200°C. A chemical reaction occurs between heated substrate and combining gas as an outcome thin film of product formed on the surface of the substrate. The applied pressure varies in the range of 100-10⁵Pa. There are many variants of CVD like Metallo Organic CVD, Atomic Layer Epitaxy, Vapor Phase Epitaxy, Plasma Enhanced CVD. The advantages of this technique are stiff, uniform, robust and highly pure nanoparticles are manufactured. The by-products formed on the substrate have to be conveyed back to the gaseous phase eliminating them from the substrate. Cold wall and hot wall are two techniques by which substrates are heated. In the hot wall arrangement, the deposition can take place even on reactor walls. This is evaded in cold wall strategy. Gas pressure and the substrate temperature ultimately affects the growth rate and quality of film.
- **Laser pyrolysis:** The process of synthesis of nanoparticles by using a laser is known as laser pyrolysis. An intense laser beam is focused to decompose the mixture of reactant gases in the existence of some inactive gas like helium or argon. The gas pressure shows a significant part in determining the particle sizes and their distribution.

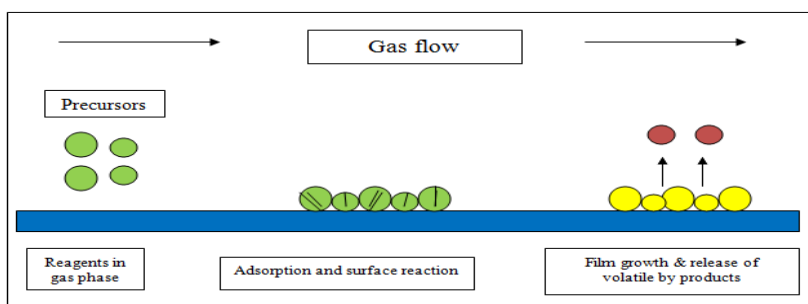


Figure: 1.5 Laser Pyrolysis

Chemical methods

- **Sol-gel method:** It comprises the condensation, hydrolysis, and thermal decomposition of metal alkoxides or metal precursors in solution. A stable solution is formed, known as the sol. Upon hydrolysis or condensation, the gel is formed with increased viscosity. The particle size can be monitored by changing precursor concentration, temperature, and pH values. A mature step is mandatory to empower the development of solid mass it may take a few days in which the removal of the solvent, Ostwald ripening, and phase alteration could happen. The unstable reagents are detached to produce nanoparticles.

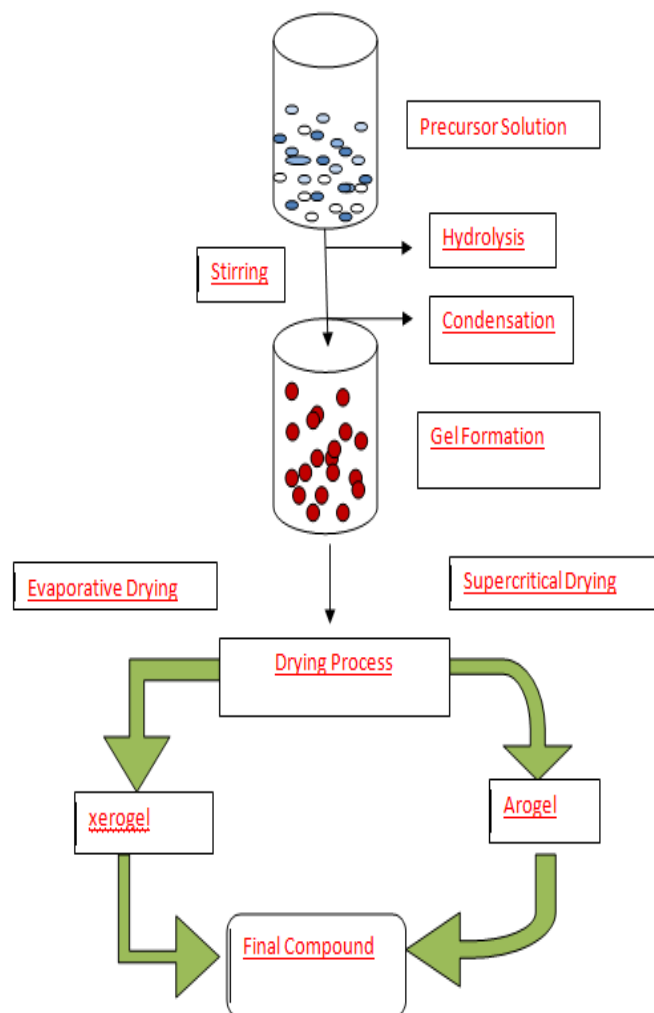


Figure:1.6 Sol-Gel Method

- **Co-precipitation method:** It is a wet chemical process, also called a solvent displacement method. Polymer phase can be synthetic or natural; polymer solvents are ethanol, acetone, hexane, and nonsolvent polymer. Nanoparticles are produced by rapid diffusion of polymer-solvent into a nonsolvent polymer phase by mixing the polymer solution at last. Nanoparticles are produced by interfacial tension at two phases

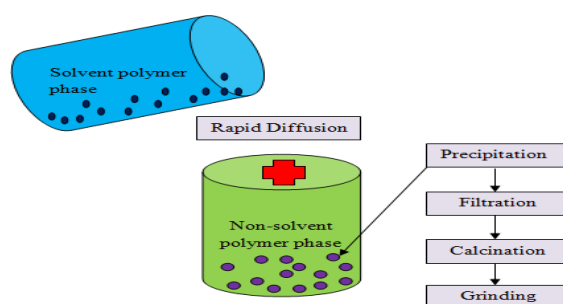


Figure: 1.7 Coprecipitation Method

- **Hydrothermal synthesis:** It is one of the most usually used methods for the preparation of nanoparticles. It is principally a chemical reaction-based approach. Hydrothermal synthesis involves a broad temperature range from room temperature to very high temperatures for the synthesis of nanoparticles. This method has a wide range of advantages over physical and biological methods. The nanomaterials generated through hydrothermal synthesis may be unstable at higher temperature ranges.

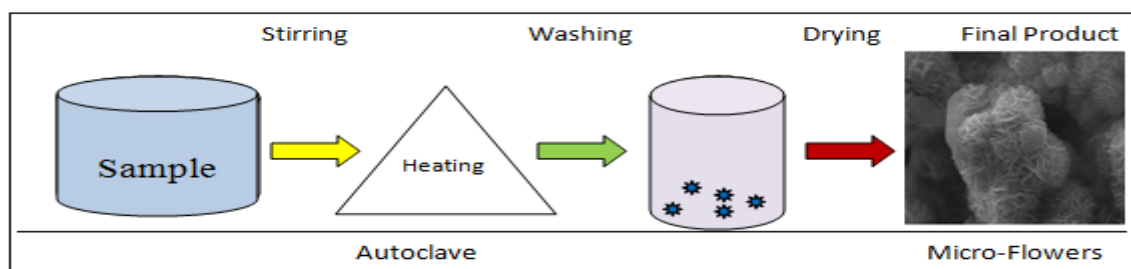


Figure: 1.8 Hydrothermal synthesis.¹⁹

- **Inert gas condensation method:** This process is broadly implemented for the creation of metal nanoparticles. The inactive gas compression technique, in which nanoparticles are produced via vanishing of metallic source in an inactive gas, had been widely used to yield fine nanoparticles. Metals are vaporized at a reasonable rate at an attainable temperature. Copper metal nanoparticles are synthesized by subjecting metal inside a chamber containing argon or helium or neon where the metal is vaporized. Once the atom is boil-off immediately loss its energy, by the cooling of the vaporized atom with inert gas. The gases cool by liquid nitrogen, to form nanoparticles in the series of 2-100nm.

Biological methods

- **Synthesis using microorganism:** In recent years synthesis of nanoparticles using microorganisms have gained more attention due to cost-effectiveness and eco-friendliness. There are two techniques by which nanoparticles can be synthesized from a microorganism, one is extracellular biosynthesis and another is intracellular biosynthesis. Certain microbes are capable of separating metal ions. *Pseudomonas stutzeri* Ag295 is frequently found in silver mines, accomplished by collecting silver inside or outside the cell walls. Different types of reductase enzymes exist in microorganisms thus can store and detoxify heavy metals. *Klebsiella pneumonia* can be used to produce Cadmium nanoparticles.²⁰
- **Synthesis using plant extracts:** Plant extracts show a vital character in the biosynthesis of nanoparticles. This process is also recognized as green synthesis or a green process of manufacture of nanoparticles. Leaves of the geranium herb (*Pelargonium graveolens*) have been used to manufacture gold nanoparticles. 1 ml of 1 mmol aqueous solution of silver nitrate is added to 5 ml of the plant extract to synthesize silver nanoparticles. The same procedure is followed for synthesis from alcoholic extract, The plant extract, along with silver nitrate, is kept in a shaker at 150 rpm in the dark.²⁰

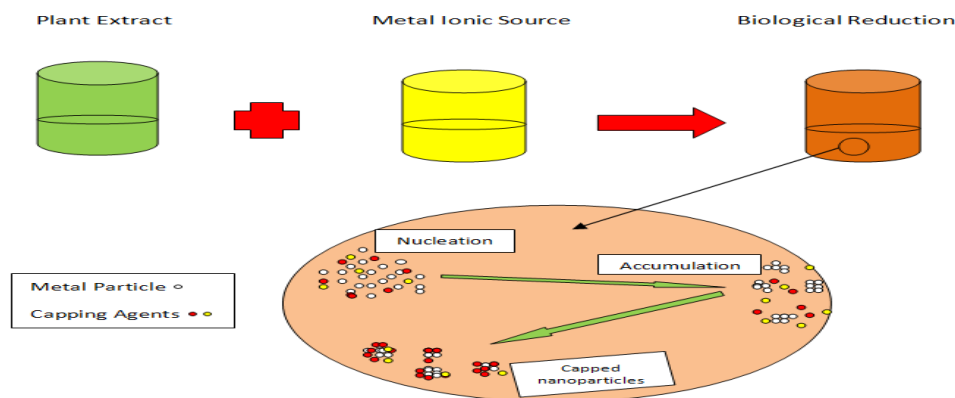


Figure: 1.9 Synthesize using Plant Extract

- **Synthesis using algae:** Preparation of algae extract in Aqueous Solvent or an organic solvent by heat or boiling it for a definite period. Preparation of molar solution of ionic metallic complex. Incubation of algae solution and molar solution of ionic metallic complexes followed whichever by nonstop stirring or without stirring for a definite period under controlled conditions. Nanoparticles synthesis is dose dependent process and depends on the type of algae used. The biomolecules peptides, pigments and polysaccharides are accountable for the reduction of metals. Nanoparticles synthesis using algae takes a shorter duration than other biosynthesizing methods.

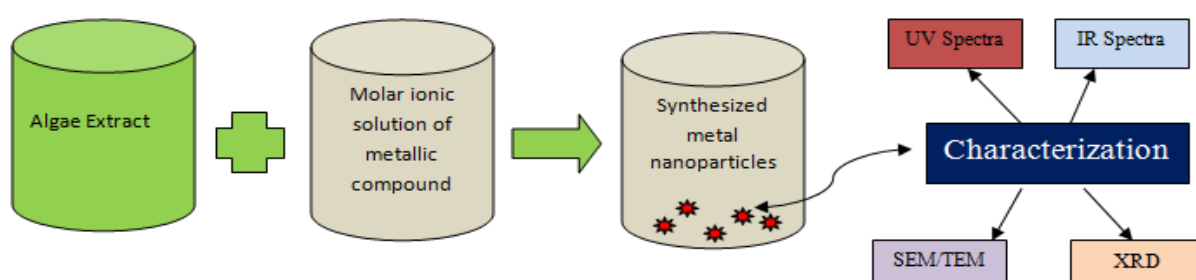


Figure:1.10 Synthesize using Algae

1.3.4. Characterization techniques

Nanoparticle characterization is performed to assess the surface area and porosity, pore size, solubility, particle size distribution, aggregation, hydrated surface analysis, zeta potential, wettability, adsorption potential and shape, size of the interactive surface, crystallinity, fractal dimensions, orientation, and the intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials. Several techniques can be used to determine nanoparticle parameters, including ultraviolet- (UV) visible spectroscopy, atomic force microscopy (AFM), transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), dual polarization interferometry, nuclear magnetic resonance (NMR), nanoparticle tracking analysis (NTA) for evaluation of Brownian motion, and particle size analysis.²⁰

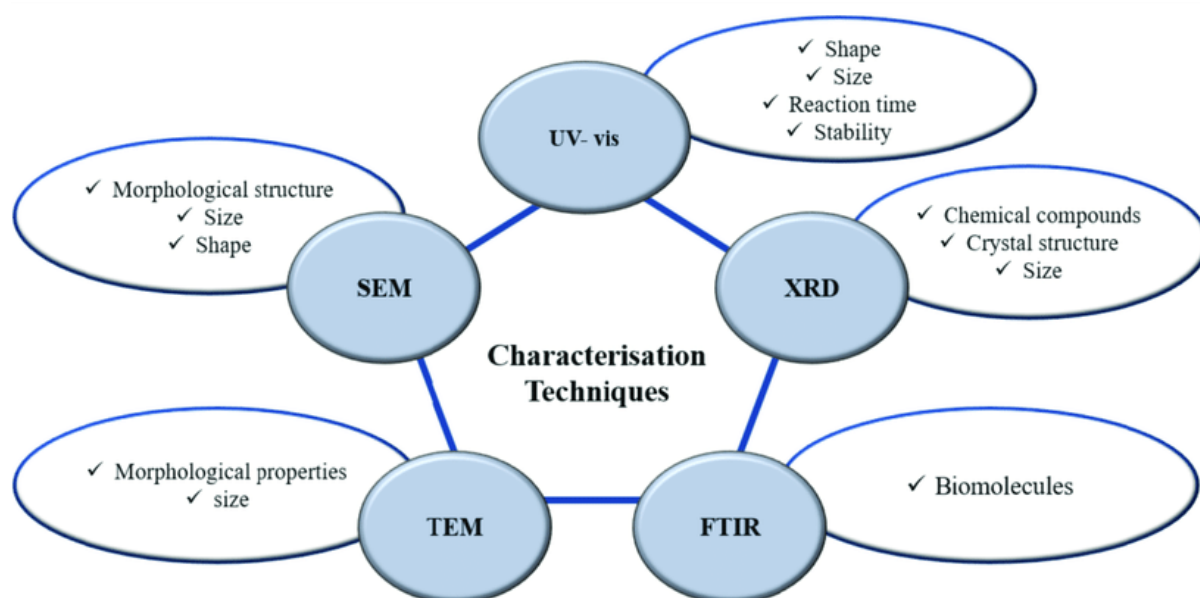


Figure:1.11 Characterization of Nanoparticles

➤ UV-VISIBLE SPECTROSCOPY

UV-visible spectroscopy is used to confirm the formation of various types of nanoparticles by measuring Plasmon resonance and evaluating the collective oscillations of conduction band electrons in response to electromagnetic waves. This provides information about the size, structure, stability, and aggregation of the nanoparticles. Metal nanoparticles are associated with specific absorbance bands in characteristic spectra when the incident light enters into resonance with the conduction band electrons on the surface of the nanoparticle.^{20,21}

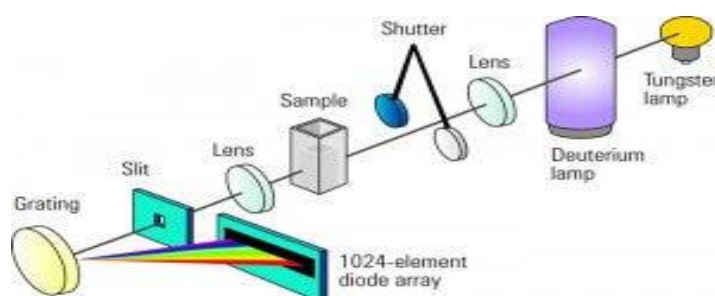


Figure:1.12 UV-Visible Spectroscopy

➤ SCANNING ELECTRON MICROSCOPY (SEM)

SEM is another technique used to characterize the morphology of nanoparticles through direct visualization. This method is based on electron microscopy and offers several advantages for morphological and size analysis; however, it is also associated with several disadvantages, such as the ability to provide only limited information about the size distribution and true population average. The instrument has features that include an electron gun, condenser lenses, and a vacuum system. SEM produces three types of principal images: external X-ray maps, backscattered electron images, and secondary electron images. For SEM analysis, the nanoparticle solution is dried into a powder, mounted on a sample holder, and coated with a conductive metal such as gold, gold/palladium alloy, platinum, osmium, iridium, tungsten, chromium, or graphite using a sputter coater. Next, a beam of high-energy electrons is directed to the sample to generate a variety of signals on the surface of the specimens. The signals received from the sample exposed to electron beams are recorded by a detector that reveals information about the samples, including their texture (external morphology), crystalline structure, and chemical composition and orientation of the

materials in the sample. For a successful analysis, the nanoparticles should be able to withstand vacuum pressure and the adverse effects of the electron beam, which can damage Nano polymers. In most cases, data for a selected area of the surface of the nanomaterial are collected and a two-dimensional image with spatial variations is displayed. Despite these advantages, this technique is time consuming and costly and often requires complementary information about the size distribution

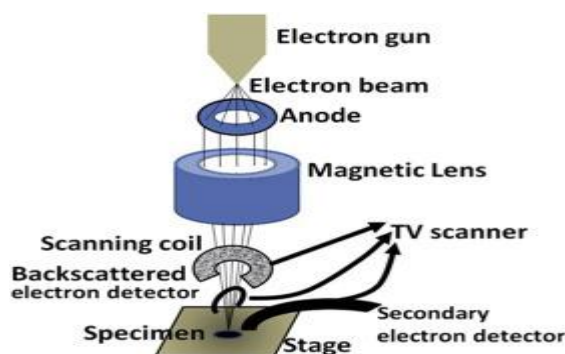


Figure:1.13 Scanning Electron Microscopy

➤ **Dynamic Light Scattering (DLS)**

Dynamic light scattering (DLS), otherwise known as photon-correlation spectroscopy, is one of the fastest and most popular methods for determining particle size distribution. DLS is widely used to measure the size of Brownian nanoparticles in colloidal suspensions. When a monochromatic beam of light (laser) is directed onto a solution of spherical particles in Brownian motion, a Doppler shift occurs when the light hits the moving particles, thereby changing the wavelength of the incoming beam of light by a value related to particle size. Accordingly, DLS enables computation of size distribution and nanoparticle motion in the medium can be computed by measuring the diffusion coefficient of the particle.²¹

➤ **X-Ray Photoelectron Spectroscopy (XPS)**

XPS is used to study the mechanism of reaction that occurs on the surface of magnetic nanoparticles, assess the bonding characteristics of the different elements involved, and confirm the structure and different elements present in the magnetic nanoparticles.

➤ Fourier Transform -Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy is conducted to identify the functional groups present on nanoparticles. Using FT-IR analysis, the infrared emission spectrum, absorption, photoconductivity, or Raman scattering of a solid, liquid, or gas can be evaluated. The spectrum represents a fingerprint of the nanoparticles consisting of absorption peaks that correspond to the frequencies of vibrations between the bonds of atoms in the nanoparticle. Since each type of nanoparticle contains a unique combination of atoms, we can identify functional groups present inside the nanoparticles based on the FT-IR spectra. This can help facilitate nanoparticle synthesis using green technology. The number of functional groups present in the nanomaterial can be determined by the size of the peaks of the spectrum. The transmission spectra for the nanoparticles are obtained by the formation of thin, transparent potassium bromide (KBr) pellets containing the compound of interest. The KBr mixtures are placed in a vacuum line overnight prior to pellet formation, and the pellets are again placed in the vacuum line before use. The transmission spectra are obtained after purging in dry air and background corrected relative to a reference blank sample (KBr). With the application of modern software tools, quantitative analysis of the nanoparticles can be completed within a few seconds.^{20,21}

➤ X-Ray Diffraction

XRD is used to assess the crystallinity of synthesized nanoparticles. This technique is employed to identify and quantitatively examine various crystalline forms or the elemental composition of natural and manufactured nanoparticles. To accomplish this, the structure and lattice parameters of the diffracted powder specimen are analyzed by measuring the angle of diffraction, when X-ray beam are made to incident on them. Particle size is also determined based on the width of the X-ray peaks using the Scherrer formula.²¹

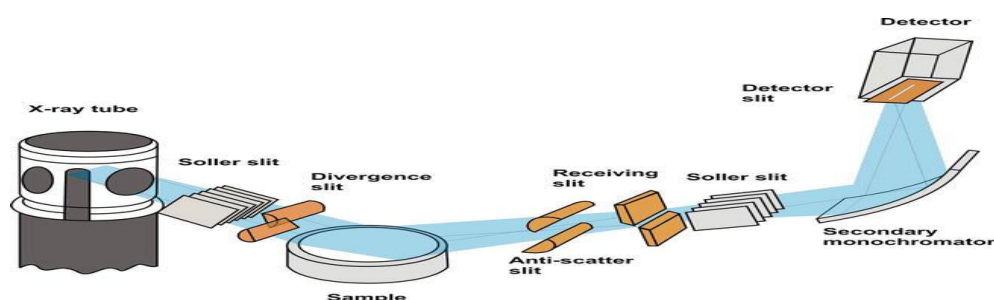


Figure:1.14 X-Ray Diffraction

➤ **Energy Dispersive X-Ray Spectra (EDX)**

Energy-dispersive X-ray analysis (EDAX) is a technique used for the measurement of nanoparticles by SEM. In this technique, the nanoparticles are analyzed by activation using an EDS X-ray spectrophotometer, which is generally present in modern SEM. The individual separated nanoparticles are deposited on a suitable substrate that does not interfere in the characterization of nanoparticles. The basic principle of EDAX is a generation of X-rays from a specimen through the electron beam. The X-rays are generated according to the characteristics and nature of the elements present in the sample. Hence, this technique also can be used to measure the energy of emitted X-rays. This method gives accurate results for not only element detection, but also to determine their concentration after maintaining the ideal instrumental conditions. There are three principal components present in basic EDAX systems. These components include the X-ray detector, pulse processor to measure the voltage with respect to X-ray energies, and a computer system. The X-ray detector is positioned to intercept X-rays emitted from the specimen. The emitted X-rays from the sample are detected by an X-ray detector. Upon entering the detector, an X-ray generates a small current, which is then converted into a voltage pulse. The voltage pulse is dependent on the X-ray energy. Histograms of this data may then be plotted following measurement of the voltage pulse for a 60-s period. This histogram represents the X-ray energy spectrum through which elemental analysis can be done. Some samples emit low energy X-rays and these low or weak signals can be increased by use of higher beam currents or increasing imaging times. An increased solid angle of the EDS detector may also resolve this problem. EDAX mapping of nanoparticles for longer acquisition time with higher magnification does not represent ideal.²¹

2. LITERATURE SURVEY

Suma VK et.al²² reported about antioxidant activity of *Artocarpus hirsutus*. Seed were taken from fruit and it is washed thoroughly and it is sun dried. It was extracted by employing nine different solvents like Hexane, Toluene, Chloroform, Ethyl acetate, Acetone, Ethanol, Petroleum ether and water. Phytoconstituents present in seed extract were confirmed by various phytochemical test. For alkaloids -Mayers test, Dragendroff test, Wagers test. For flavonoids -Ferric chloride and lead acetate. For Glycoside -killers killani. For terpenoids – Libermann buchard, Salkowski. For anticancer activity DPPH free radical scavenging assay were done. These studies revealed that ethanolic, methanolic and water extract have potent antioxidant activity compared to other extracts.

Akhil Hari et.al²³ reported about various phytoconstituents and pharmacological activities such as Antioxidant activity, Antidiabetic activity.

Suma VK et.al²⁴ was worked on evaluation of morphological, microscopic and powder characteristics of *A. Hirsutus* seed extract. Seeds of *Artocarpus hirsutus* were collected from Palakkad district kerala. Seeds were sun -dried and observed under naked eye and botanical characters were evaluated. Seeds are reported as pearl white in colour which is smooth and as well shining. Seeds are elliptical in shape. Hilum is prominent, circular at subterminal part of seed. Upper surface is convex and dorsal surface is concave.

Anu V et.al²⁵ worked on green synthesise of silver nanoparticle using leaf extract of *Artocarpus hirsutus*. Aerial and tender leaf of plant were collected and washed thoroughly in tap water. Aqueous and methanolic extraction was used to extract the plant. Silver nanoparticles were synthesized using silver nitrate and 1 ml of aqueous extract of plant material. Synthesized silver nanoparticles were characterized using UV-Visible spectroscopy. Potential antimicrobial activity of silver nanoparticle was studied using well diffusion method against *Staphylococcus Aerus* and *E. Coli*. Hence, synthesized nanoparticle was reported against antimicrobial activity.

Darren Yi Sern low et.al²⁶ worked on green synthesise of zinc oxide nanoparticles using seed extract of *Swietenia macrophylla*. Seeds were dried and ground finely and it is soaked in ethanol at room temperature for 72 hours. Extract was filtered from residue and evaporated at 40 °C using rotatory vaccum evaporator in order to produce yellow crude ethanolic extract. zinc oxide nanoparticles were synthesized using zinc nitrate reagent.

Makari HK et.al²⁷ have worked on Synthesis, characterization and studies on Anti-bacterial activity of colloidal Silver Nanoparticles from *Artocarpus hirsutus* fruit extract. For the study they using the fruit extracts of *Artocarpus* commonly known as Wild jack. The fruit pulp of *Artocarpus* was dried, powered and boiled with distilled water for the extract preparation. The extracts were treated with silver nitrate which was reduced to form Pure *Artocarpus hirsutus* Silver Nanoparticles. The extract was evaluated by UV visible spectroscopy. The size of particle was characterized by DLS instrument. Biomolecules present in the extract was identified by FT IR spectra. Crystalline nature was studied by XRD (X ray diffraction). Energy dispersive X ray spectroscopy analysis was performed to check the purity of the extract. The Antibacterial Activity was evaluated against the strains of *Streptococcus pneumonia*, *Salmonella typhi*, *Pseudomonas aeurginosa*, *Xanthomonascampestris* and *Bacillus subtilis* by Standard disc diffusion method. DPPH free radical scavenging assay was conducted for Antioxidant it shows high percentage of activity. Through these studies they have evaluated that spherical silver nanoparticle with a size of 30-100nm have been synthesized through Biogenic synthesis in the presence of aqueous extracts of *Artocarpus hirsutus* and also shows a potential activity of Antimicrobial, reducing and stabilizing activity.

Shailendra solanki et.al²⁸ have worked on Biochemical composition of pulp and seeds of wild jack (*Artocarpus hirsutus* lam) fruit. They carried out a biochemical profiling of ripe fruits and seeds of wild jack. The nutritional value of wild jack was ascertained by determining biochemical profiles of fruit and seed by comparing with profile of other *Artocarpus hirsutus* fruit like jack fruit and bread fruit. It is composed of soluble sugars, starch, carotenoids, ascorbic acid, GAE total phenol. They suggest that wild jack fruit deserves a minor fruit species of high nutritional importance and considered as a potential fruit crop species for further study.

Shanmugapriya K et.al.²⁹ have worked on Phytochemical screening of *Artocarpus hirsutus* and its antimicrobial potential. Phytoconstituents obtained from the medicinal plants have effective toxicity against microorganism by different mechanisms such as Extraction, screening, identification of bioactive substances. The plant matters are shade dried and powdered then extracted with acetone and ethanol solvents. The plant extracts are subjected to phytochemical screening for the presence of various active compounds. The extracts are then used to evaluate the anti-bacterial and anti-fungal activity against the strains of *E. coli*, *Staphylococcus aureus*, *Klebsiella* spp, *Enterobacter* sp, *Aspergillus tamarii*, *Aspergillus fumigates*, and *Aspergillus Niger*. The activities carried out by Standard disc diffusion method. Phytochemicals analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, carbohydrate which are known to exhibit medicinal activities. Anti-microbial Activity of fruit and leaf extract of *Artocarpus hirsutus* exhibit good inhibitory activity against the selected strains. Acetone extract showed the maximum zone of inhibition and the ethanolic extract showed low inhibitory activity against the strains. Acetone extracts showed good anti-bacterial activity and the ethanolic extract showed a good anti-fungal activity.

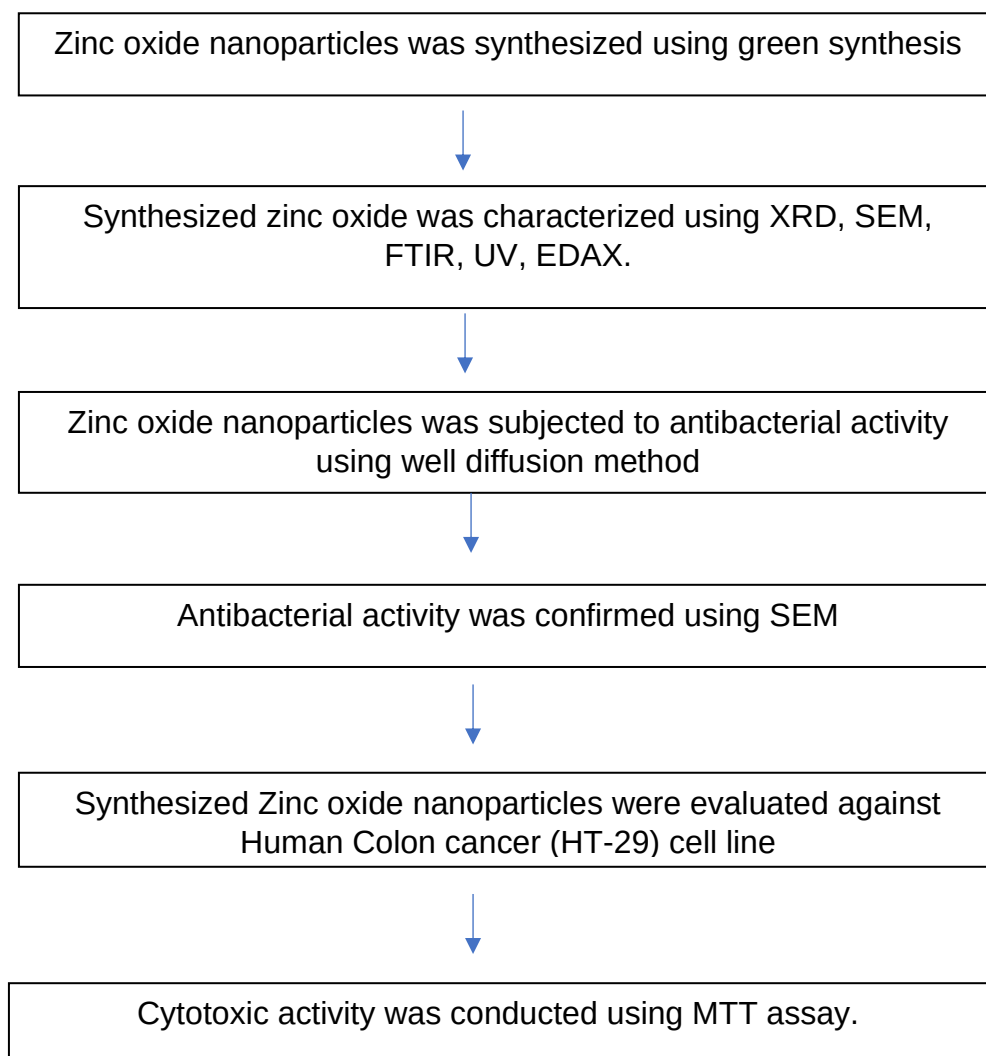
Shobha N et.al.³⁰ worked on green synthesis of Zinc oxide nanoparticle using the seed extract of *Ricinus Communio*. Synthesized nanoparticle was characterized using X-RD, FT-IR. Synthesized zinc oxide was subjected to antioxidant activity by DPPH free radical Scavenging as well as FRAP activity. This study reveals Zinc oxide has high potential to scavenge free radicals. Antifungal activity was also evaluated against *Aspergillus* spp, *Rhizopus* spp. yeast and penicillium was studied using diffusion method. This study indicates Maximum zone of inhibition was observed in Zinc oxide nanoparticles against *Aspergillus* and *Penicillium* at 30ug/ml concentration.

Suresh J et.al.³¹ worked on green synthesis of Zinc Oxide Nanoparticle using Insulin Plant. Synthesized Zinc oxide nanoparticles was characterized using XRD, FTIR.

3.Aim and Scope

Aim of the work: To synthesize zinc oxide nanoparticle from *Artocarpus hirsutus* and to evaluate antibacterial and cytotoxic activity.

Scope of the work:



4.EXPERIMENTAL WORK

4.1. Plant Profile of *Artocarpus hirsutus*

Wild Jackfruit having the Latin name *Artocarpus hirsutus* Lam. *Artocarpus heterophyllus* (Jack fruit), *Artocarpus altilis* (Bread fruit), *Artocarpus hirsutus* (Wild jack) and *Artocarpus Camansi* (Bread nut) are important species belonging to this genus.

It is common in Western Ghats from north Karnataka to Malabar Coast and Travancore. *Artocarpus hirsutus*, is a tropical evergreen tree species that is native to India, primarily in Kerala. Jack fruit trees are widely grown in Bangladesh, Burma, Indonesia china, srilanka, Thailand, Philippines, Parts of Africa, Australia, Brazil and Florida.

4.1.1. Description of plant

Artocarpus hirsutus grows at an altitude ranging from sea level at an elevation of 1000 metres in places with an annual rainfall of 1500mm or more. It is an evergreen tree with dense crown growing up to 50 metres tall. The heartwood is yellowish -brown; sapwood white. Wood is moderately hard, durable, it lasts well in water and is not attacked by white ants. Fruits are edible, bright yellow, ovoid covered with spines, seeds ovoid and white. *Artocarpus hirsutus* required warm humid climate heavy rainfall and thrives in any type of soil. Tree is propagated through seeds or by grafting, flowering season is from December to January and fruits get ripped in May and June.



Figure: 4.1 *Artocarpus hirsutus* Plant

4.1.2 Scientific classification of *Artocarpus hirsutus*

- ✓ Kingdom-Plantae
- ✓ Division-Angiosperms
- ✓ Phylum-Eudicots
- ✓ Class-Rosids
- ✓ Order-Rosales
- ✓ Family-Moraceae
- ✓ Tribe-Artocarpeae
- ✓ Genus-Artocarpus
- ✓ Species-hirsutus
- ✓ Species authority-Lam

4.1.3 Vernacular Names

- ✓ **English** – Wild Jack
- ✓ **Malayalam**- Ayani, Anjili, Ayaniplavu, Annali, Annili, Aini, Ayari
- ✓ **Marathi**- Pat-phanas, Ranphanas
- ✓ **Tamil**- Kattuppala. Akkini, Anjili
- ✓ **Telugu**- Pejuta.
- ✓ **Kannada**- Hebbalasu, hebbe-lasu

4.1.4. Phytoconstituents present in *Artocarpus hirsutus*

Phytoconstituents present in the plant are alkaloid, flavonoid, glycoside, terpenoid, tannin, saponin, glycoside, anthraquinone, steroid and reducing sugar, protein.

Table 4.1: Phytoconstituents Present in *Artocarpus hirsutus*

S.NO	PARTS OF PLANT	PHYTOCONSTITUENTS AND NUTRITIONS PRESENT
1.	Fruit	Carbohydrate, protein, fat, fibre, calcium, Phosphorus, iron, Thiamine.
2.	Seed	Starch, protein- Jacalin, Mineral and fibre content.
3.	Bark	Betullic acid, Cycloheterophyllin, Triterpenic compounds like heterophyllol and tannin
4.	Root	Ursolic acid, B sitosterol, betulinic acid, Cycloartenone, Heteroflavones A, Flavanones

Table 4.2: Medicinal Uses

S.NO	PARTS OF PLANT	MEDICINAL USES
1.	Bark	Cures ulcer, Diarrhea, Pimples, Piles
2.	Latex	Treatment of Asthma
3.	Seeds	Act as an Appetizer
4.	Burnt leaves ash	Treatment of Abdominal Problems
5.	Dry leaves	Treatment of Hydrocele, Bubo
6.	Ripe fruits	Appetizing, Constipating and Aphrodisiac
7.	Roasted seed powder mixed with Honey	Treatment of Asthma
8.	Fruit oil	Treatment of skin disease
9.	Stem bark Extract	Ulceration, Reduces gastric Secretory volume.
10.	Roasted seed extract	Aphrodisiac properties.
11.	Leaves	Treatment of Asthma, Diabetes, Gall bladder and ringworm infection
12.	Bark paste with coconut oil	Snake bite.
13.	Bark ash with coconut oil	Applied topically for ringworm infection.

4.2. Materials :

4.2.1. Chemicals used

Table 4.3: Chemicals used

1.	Zinc acetate	Lab chem.Pvt.Ltd.
2.	Dimethyl sulphoxide	Sigma Aldrich
3.	Glutaraldehyde	Sigma Aldrich
4.	Nutrient Agar	Himedia Laboratories.Pvt. Ltd
5.	Agar powder	Himedia Laboratories.Pvt. Ltd
6.	Nutrient broth	Himedia Laboratories.Pvt. Ltd
7.	HT-29 cancer cell line	National center for cell science, Pune
8.	Ciprofloxacin	Himedia Laboratories
9.	Ethanol	SRL Pvt.Ltd
10.	Methanol	SRL.PVT. Ltd

4.2.2. Instruments used

Table 4.4: Instruments used

NAME OF THE INSTRUMENT	MODEL NAME
UV-Visible Spectroscopy	(UV 3000+ from LABINDIA)
Scanning Electron Microscopy	(FEI Quanta 200)
X-Ray Diffraction	Malvern PAN analytical
Fourier Transform Infrared Spectroscopy	JASCO FTIR-6300

4.3. Methods:

4.3.1 Collection of plant material

Mature and ripe fruits of *A. hirsutus* were collected from Mannarkkad area of Palakkad district Kerala. Healthy plants which give rise to fruits were selected. Seeds were sun-dried for 7 days to remove moisture and to prevent decomposition of thermolabile compounds.

4.3.2. Preparation of extracts

Seeds were taken out from fruits, washed properly.

For preparation of aqueous extract 10g of seeds was chopped into pieces which is boiled in 100 ml of distilled water at 60°C for 20mins. Prepared solution is allowed to cool for some time. The solution was filtered using Whatman grade 1 filter paper.

For preparation of ethanolic extraction 10g of the chopped seed was soaked in 40ml of ethanol in a sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminum foil and shaken vigorously. The mixture was left to stand for 72 hours at room temperature of 25°C. The mixture was then filtered using Whatman No. 1 filter paper. Thereafter, the filtrate was evaporated to dryness by means of a rotary evaporator attached to a vacuum pump. The extracts were stored in refrigerator until needed for further analysis.

For preparation methanolic extraction about 10g of chopped seeds was mixed with 100 mL methanol in a conical flask and kept in rotary shaker at 150 rpm for 24h. After 24h, it was filtered and the solvent was evaporated to make the final volume to be one-fourth of the initial volume. It was stored at 4°C for further studies.



Figure 4.2 Seed Extract

4.3.3. Phytochemical Screening

1. Molisch test

2-3 drops of Molisch's reagent must be added to seed extract in a test tube and mixed well. Few drops of concentrated sulphuric acid must be added drop-wise along the walls of the test tube to facilitate the formation of a layer. The development of a purple ring at the layer formed by the concentrated acid is a positive indicator for carbohydrate.

2. Glycosides test

The extract of the substance was mixed with a little amount of anthrone on a watch glass. One drop of concentrated sulphuric acid was added made into a paste and warmed gently over water bath. Appearance of dark green colour confirms presence of glycoside.

3. Saponins test

1ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins.

4. Test for Tannins

Ferric chloride test: 2ml of the aqueous solution of the extract were added to a few drops of 10% Ferric chloride solution. The occurrence of blackish blue colour showed the presence of gallic tannins and a green-blackish colour indicated presence of catechol tannins.

5. General test for Alkaloids

The powdered seed material was boiled with 5 ml of dilute sulphuric acid and filtered. The filtrate was used for the following tests

- (a) To the filtrate, a few drops of Dragendorff's reagent was added and shaken well. Formation of orange red precipitate confirms the presence of alkaloid.
- (b) To the filtrate, few drops of Wagner's reagent was added and shaken well. Formation of reddish-brown precipitate. Confirms the presence of alkaloid.
- (c) To the filtrate, few drops of Hager's reagent was added and shaken well. Formation of yellow precipitate. Confirms the presence of alkaloid.
- (d) To the filtrate, few drops of Mayer's reagent was added and shaken well. Formation of white precipitate. Confirms the presence of alkaloid.

6.Flavonoid test: Shinado's test:

- a) The seed extract was shaken with 5 ml of alcohol and filtered. To the filtrate, a piece of magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Appearance Red color. Confirms the presence of flavonoid.
- b) The seed extract was shaken with 5 ml of alcohol and filtered. To the filtrate, 10% sodium hydroxide solution or ammonia was added. Appearance Dark yellow colour. Confirms the presence of flavonoids.
- c) The seed extract was shaken with 5 ml of alcohol and filtered. To the filtrate 1 ml of lead acetate solution was added. Yellow precipitate was formed. Confirms the presence of flavonoid.

7.Noller's test

The seed extract was warmed with tin and thionyl chloride. Appearance Pink color. Confirms the presence of terpenoids.

8.Biuret test

Seed extract of 0.5mg add equal volume of 40%NaOH solution and two drops of 1%copper sulphate solution was added. Appearance of violet colour indicates presence of protein.

9.Ninhydrin test

About 0.5 mg of seed extract was taken and 2 drops of freshly prepared 0.2%ninhydrin reagent were added and heated. Appearance of pink or purple colour indicates presence of Amino acids.

Table :4.5 Phytochemical Screening

S.NO	Phytoconstituents	Identification test
1.	Carbohydrate	Molisch test
2.	Flavonoids	Shinoda test
3.	Alkaloid	Mayers test, Wagers test, Dragendroff test
4.	Terpenoids	Nollers test
5.	Tannins	Ferric chloride
7.	Glycoside	Kiillers Kilani
8.	Protein	Biuret test
9.	Amino acids	Ninhydrin test

4.4 Preparation of zinc oxide nanoparticle

The freshly prepared *Artocarpus hirsutus* seed extract was used for the synthesis of zinc oxide nanoparticles. 0.1 M of aqueous zinc acetate solution and 25 ml of fresh seed extract were prepared for the synthesis of zinc oxide nanoparticles. The seed extract of 25 ml was titrated dropwise against 100ml of zinc acetate solution and kept for overnight reduction. The obtained zinc oxide nanoparticle was centrifuged at 10000 rpm for 10mins and the pellets were resuspended in acetone and dried.³²

4.4.1. Characterization of zinc oxide nanoparticles

Zinc oxide nanoparticles were characterized by an X-Ray diffractometer with operating at a voltage of 40kV and current of 30mA with Cu K (α) radiation. UV 3000+ from LABINDIA was used to measure the UV-Visible absorbance spectrum. Fourier Transform Infrared Spectroscopy (JASCO International, FTIR-6300) was used to confirm the presence of chemical bonds. (FEI Quanta 200) Scanning Electron Microscopy (SEM) was used to identify the structural events of the Zinc oxide nanoparticles.

4.5 Antibacterial Activity

To determine the antibacterial activity of zinc oxide nanoparticles by well diffusion method, two pathogenic strains *E. aerogenes* and *L. monocytogenes* were chosen. The weight of 10 mg of the nanoparticles is diluted in 1 ml of Dimethyl sulphoxide (DMSO) which is sonicated for 2 -3 minutes for the distribution of nanoparticles. A well with a diameter of 6 mm was made on the agar to load with a required concentration of the drug. After incubation of the test plates for 24 h at 37°C, the zone of inhibition was measured. This measurement was used to determine the activity of the nanoparticle against chosen pathogenic strains. Ciprofloxacin (20 µg/ml) was used as a positive control.³³

4.6. Determination of Minimum Inhibitory Concentration

Dimethyl sulphoxide of 10% was used to dissolve the test material where the initial test concentration was diluted serially in 96 well plates then inoculated with 5 ul suspension that contains 10⁸ CFU ml⁻¹ of bacteria. At 600 nm, the culture intensity of each well was measured to compare with the untreated control. The minimum inhibitory concentration of the zinc oxide nanoparticle is determined as the low concentration that inhibits bacterial growth.³⁴

4.7. Antibacterial Action of Zinc Oxide Nanoparticles on Bacterial Pathogens

Zinc oxide nanoparticle action is studied against bacterial pathogens and investigated using SEM analysis. To study its analysis, the bacterial broth was centrifuged at 10000 rpm for 10 min, with resuspension of pellets in Phosphate buffer saline. The procedure is repeated thrice and the obtained pellet is incubated with 5% glutaraldehyde at room temperature for 30 min. The incubated pellets were subjected to centrifugation, followed by pellet suspension in phosphate buffer and distilled water. After drying, the samples were observed in a scanning electron microscope to explicate the mechanism of zinc oxide nanoparticles against bacteria.

4.8. Cytotoxicity Assay

Nanoparticles against the human colorectal adenocarcinoma cell line (HT29). The colon cancer cell line was layered separate in 96 well plates with a concentration of 1×10^4 cells in Dulbecco's Modified Eagle medium (DMEM), then perhaps the cell lines were treated with different test concentrations (25–500 g/ml) of nanoparticle and incubated for 24 hours. At 570nm, the development of colour intensity was assessed. The MTT assay was used to determine cell viability, from which the cytotoxic concentration was The MTT assay was used to investigate the cytotoxicity of the synthesized zinc oxide estimated.³⁵

5.Results and Discussion

5.1. Qualitative Phytochemical study

Artocarpus hirsutus is rich in its phytochemical constituents and the phytochemical study on seed extract of *Artocarpus hirsutus* were carried out.

Qualitative analysis revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, phenols, terpenoids, and carbohydrates in the aqueous seed extract of *Artocarpus hirsutus* whereas diterpenes and glycosides are absent in the methanolic seed extract is clearly depicted in Table 5.1. This finding encouraged the utilization of *Artocarpus hirsutus* aqueous seed extract for antimicrobial activity.

Table 5.1: Phytochemical Test

Test	Aqueous extract	Ethanollic extract	Methanolic extract
Carbohydrates	Positive	Positive	Positive
Alkaloids	Positive	Negative	Negative
Flavonoids	Positive	Negative	Positive
Phenols	Positive	Negative	Positive
Saponins	Positive	Negative	Negative
Tannins	Positive	Positive	Negative
Glycosides	Positive	Negative	Negative
Terpenoids	Positive	Negative	Negative
Protein	Positive	Positive	Positive
Amino acid	Positive	Negative	Positive

5.2. X- Ray Diffraction analysis (XRD)

XRD spectrum of prepared sample of *Artocarpus hirsutus* was clearly depicted in Figure: 5.1. The pattern of zinc oxide nanoparticle where the peaks correspond to the JCPDS card No. (36-1451) and secondary phase or impurity peaks were observed that confirmed the complete reduction of the precursor.

The 2θ values of XRD at 31.67° , 34.43° , 36.33° , 47.69° , 56.41° , 62.81° , 66.45° , 67.90° , 69.06° , 72.56° and 76.92° corresponds to the planes (100) (002) (101) (102) (110) (103) (200) (112) (201) (004) (202) respectively that confirms the presence of Zinc oxide nanoparticles

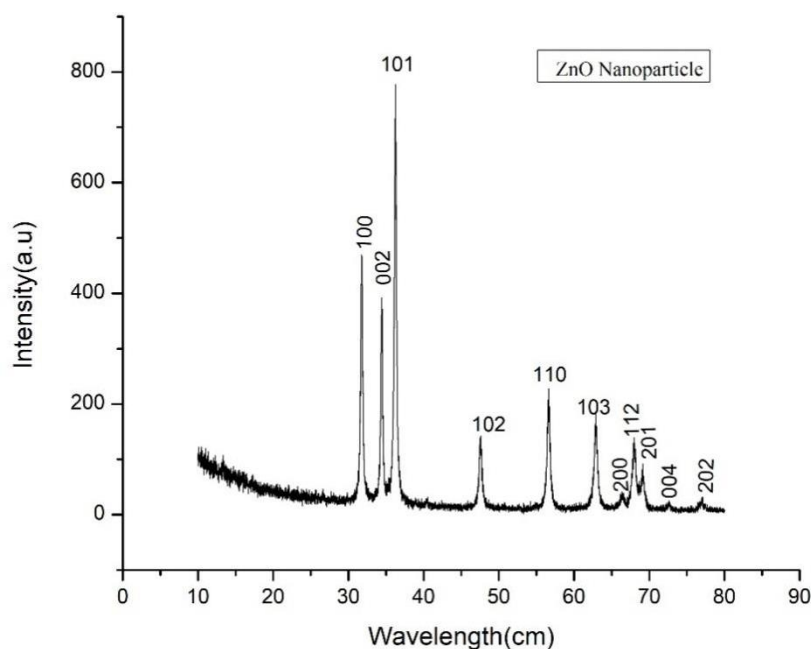


Figure: 5.1. X-Ray Diffraction analysis of *Artocarpus hirsutus*

Broad peaks correspond to the particle size of the nanoparticle while sharp peaks indicate the crystallinity of the nanoparticle. The average particle size of zinc oxide nanoparticles was calculated according to Scherrer equation $D = k\lambda / \beta \cos\theta$ and the particle size was about 22.52 nm.³²

5.3. Scanning Electron Microscopy (SEM)

SEM images of zinc oxide nanoparticles synthesized from *Artocarpus hirsutus* seed extract at a magnification of 120000x is shown in Figure:5.2. Rod and spherical-shaped nanoparticles were observed from these micrographs and the particles were 15 to 42 nm in size. Particles were seen to be agglomerated and this agglomeration of nanoparticles might have resulted because of the high surface energy of the nanoparticles.³⁶

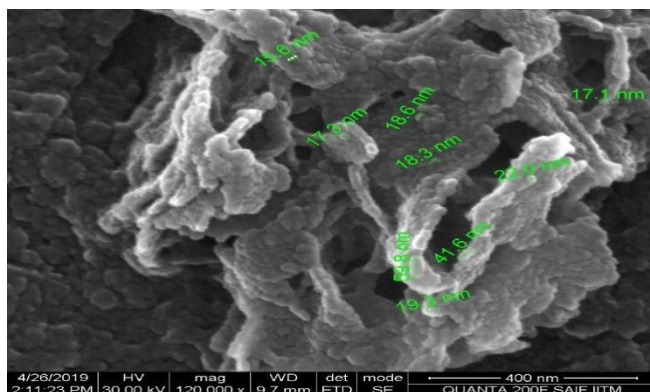


Figure: 5.2 Scanning electron microscopy of zinc oxide nanoparticle

5.4 Energy Dispersive X-ray Analysis (EDAX)

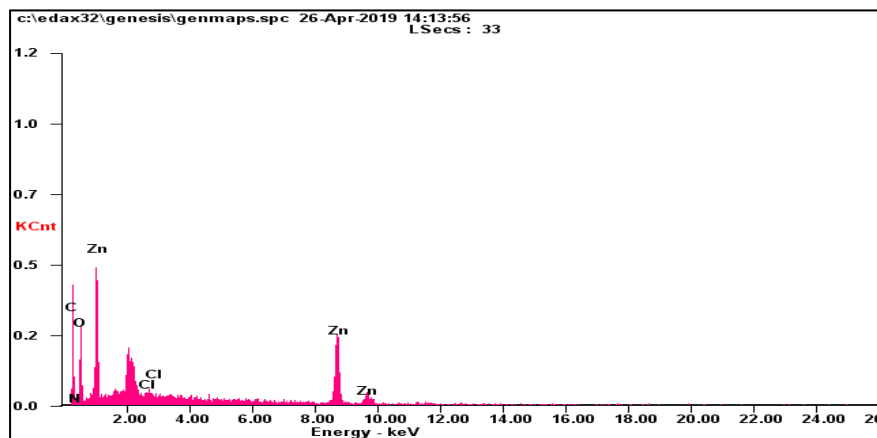


Figure: 5.3 Energy dispersive X-ray analysis of zinc oxide nanoparticle

The X ray spectrum of the prepared sample from *A. hirsutus* seed extract is represented in Figure 5.3 gives the elemental composition of the sample.

The presence of zinc and oxygen in the spectrum confirmed the presence of zinc oxide nanoparticles and the presence of carbon due to the use of zinc acetate dihydrate as a precursor for the synthesis. The presence of proteins/enzymes in *A. hirsutus* seed extract cause weaker signals from chlorine in the spectrum.

5.5 UV- Visible Spectroscopy

UV-Vis spectroscopic analysis of prepared sample from *Artocarpus hirsutus* seed extract is represented in Figure: 5.4 was performed to determine the optical band gaps of all the synthesized samples.³⁷

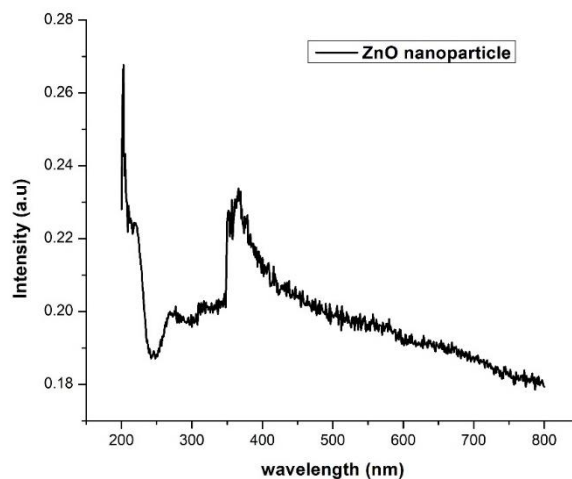


Figure: 5.4 UV-Visible spectroscopy of Zinc oxide nanoparticle

Zinc oxide UV-vis spectrum revealed absorption spectra at 266 and 365 nm that are related to blue emission (221 to 378 nm) which confirms the presence of Zinc oxide nanoparticles synthesized using *Artocarpus hirsutus* seed extract

5.6 Fourier Transform Infrared Spectroscopy

FTIR spectra of Zinc oxide nanoparticles are recorded in the band from 400 cm^{-1} to 4500 cm^{-1} as shown in Figure:5.5a. Bands at 1112 cm^{-1} correspond to C-N symmetric stretching vibration. The band at 1218, 1365 and 1748 cm^{-1} are due to the asymmetrical and symmetrical stretching of the zinc carboxylate and the band at 3032 cm^{-1} is assigned to the O-H stretching mode of the hydroxyl group. Hence, this IR spectroscopic study confirmed the presence of Zinc oxide nanoparticles.

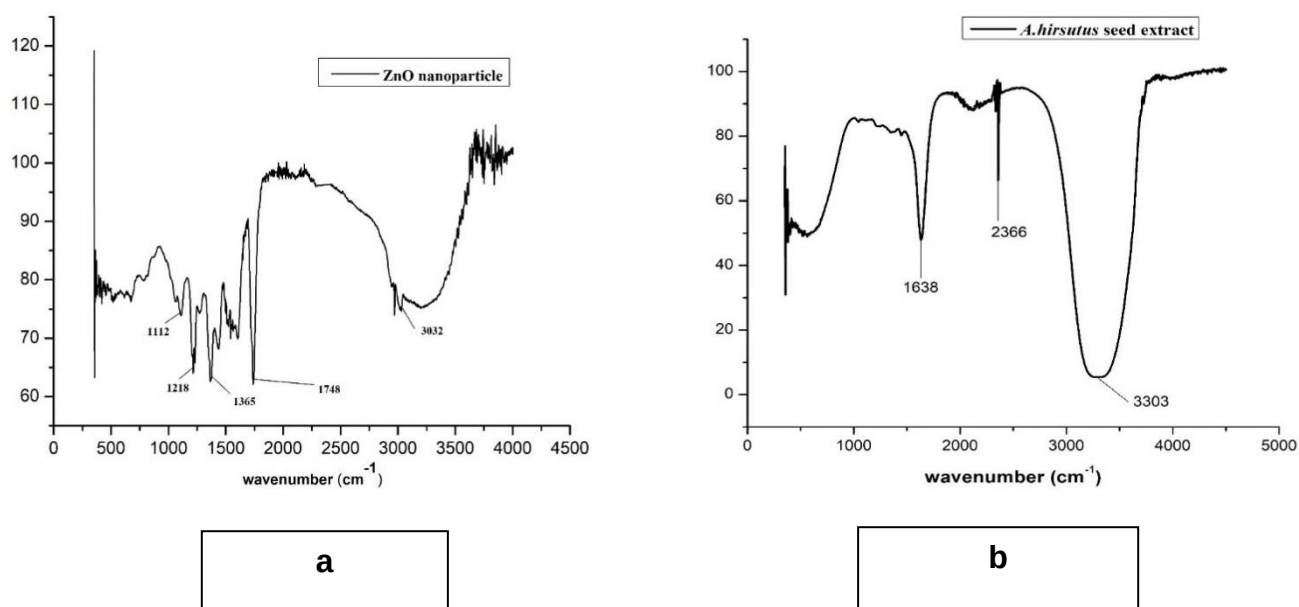


Figure:5.5 Fourier Transform Infrared spectrums:

a) Zinc oxide nanoparticle synthesized from *A. hirsutus* seed extract

b) *A. hirsutus* seed extract

FTIR spectra of seed extract are recorded from 400 cm⁻¹ to 5000 cm⁻¹ and sharp bands were observed at 1638 cm⁻¹ and 2356 cm⁻¹ whereas the band was measured at 3303 cm⁻¹ as depicted in Fig:5.5 b. The band at 1638 cm⁻¹ corresponds to the characteristic amino acids containing NH₂ groups and amide band. The band at 2356 cm⁻¹ is due to the N-H and C-O stretching of amides and amino acids. Furthermore, the band at 3303 cm⁻¹ corresponds to the N-H stretching of amides. Hence, this FTIR spectroscopic study confirmed the involvement of amides, amino acids and proteins present in *A. hirsutus* seed that act as the capping and stabilizing agent in the synthesis of Zinc oxide nanoparticles.³⁸

5.7. Antibacterial Activity of Zinc oxide nanoparticles

The antibacterial activity of Zinc oxide nanoparticles was evaluated against two pathogenic bacterial strains: Gram-negative (*E. aerogenes*) and Gram-positive (*L. monocytogenes*) using the agar well diffusion method and the zone of inhibition of sample was measured. Zone of inhibition of synthesized zinc oxide nanoparticle against *E. aerogenes* and *L. monocytogenes* was depicted in Figure :5.6 .

Zone of inhibition at 250 μg concentration for *E. aerogenes* and 500 μg concentration for *L. monocytogenes*.

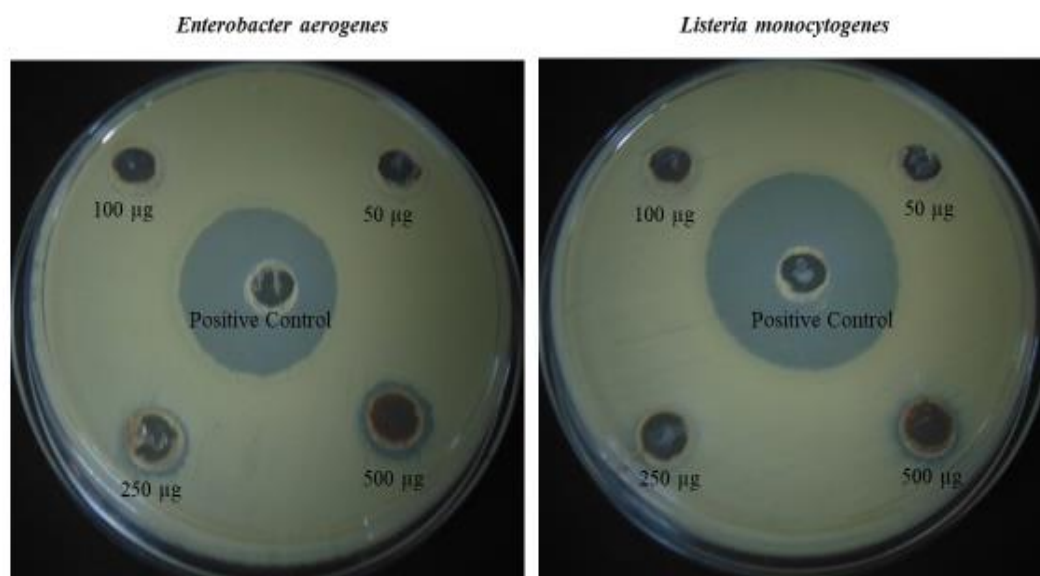


Figure: 5.6. Zone of inhibition of synthesized Zinc oxide nanoparticle against *E. aerogenes* and *L. monocytogenes*.

Hence, Zinc oxide nanoparticles synthesized using the seed extract of *A. hirsutus* exhibited higher antibacterial activity on gram-negative strain and also with increasing nanoparticle concentration zone of inhibition is enhanced.

5.8 Minimum Inhibitory Concentration (MIC) of Zinc oxide nanoparticles

In our study, the minimum inhibitory concentration value of Zinc oxide nanoparticles was observed at 125 $\mu\text{g}/\text{ml}$ for *E. aerogenes* and 250 $\mu\text{g}/\text{ml}$ for *L. monocytogenes* which is depicted in figure:5.7.

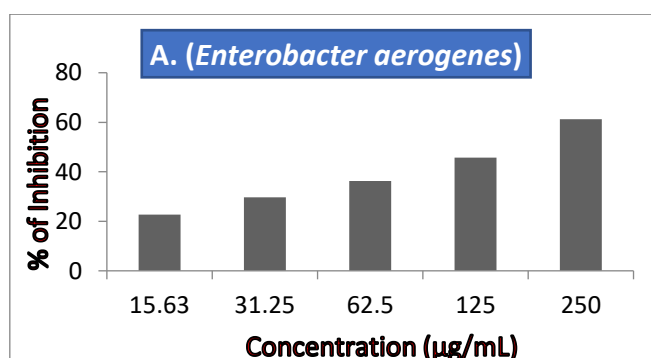
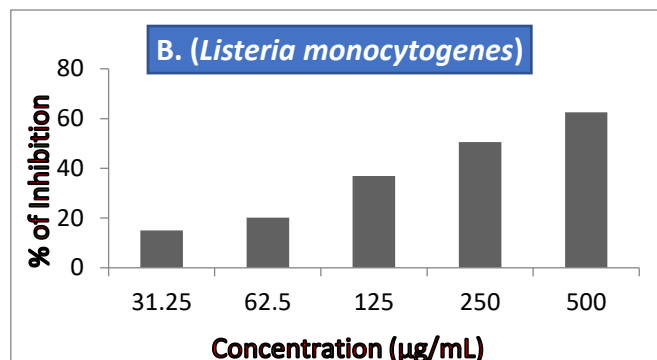


Figure :5.7 Minimum inhibitory concentration zinc oxide against *Enterobacter aerogenes*



***Minimum inhibitory concentration zinc oxide against
B. Listeria monocytogenes***

Zinc oxide nanoparticles exhibit higher antibacterial activity on gram-negative bacteria than gram-positive bacteria. This may be due to the absence of cell wall in gram-negative bacterial strains and also, they have a higher negative charge on the cell surface that enables strong interaction between the nanoparticles and the gram-negative bacteria.

5.9. Antibacterial Action of Zinc oxide nanoparticles

The antibacterial action of Zinc oxide nanoparticles against bacteria is studied through Scanning Electron Microscopy. Untreated bacterial cells were depicted in Figure 5.8 a and Figure 5.8 b these bacterial cells retained their original morphology. Morphological changes were observed in the treated groups in both bacterial strains were depicted in Figure:5.8 c and Figure:5.8 d.

The changes in the bacterial cell wall are caused by the interaction of Zinc oxide nanoparticles with bacteria. These results coincide with the report of that zinc oxide nanoparticles damage the membrane by the release of hydrogen peroxide or by the affinity of the nanoparticle with the bacterial surface. Results of antibacterial activity and MIC demonstrates that the *E. aerogenes* are more susceptible to Zinc oxide nanoparticles than gram-positive *L. monocytogenes*. This is because the MIC of Zinc oxide nanoparticles is high enough to produce hydrogen peroxide that causes easy membrane rupture in *E. aerogenes* in comparison to *L. monocytogenes*.

This breakage causes the release of cellular components leading to bacterial cell death. These results are in support of recent study reports that Zinc oxide nanoparticles exhibit pronounced antibacterial activity by the induction of oxidative stress and by the generation of ROS that causes cell membrane disruption. Thus,

[illegible]

(ii) *L. monocytogenes*: b) control d) treated with Zinc oxide nanoparticles

5.10. Cytotoxicity Study

According to our findings, the cytotoxicity of Zinc oxide nanoparticles on HT-29 cells demonstrated a decrease in cell viability as the nanoparticle concentration increased which is represented in figure:5.9. The IC_{50} value of Zinc oxide nanoparticles synthesized from the seed extract of *Artocarpus hirsutus* was 479 $\mu\text{g/ml}$, using which the cell viability was being investigated.

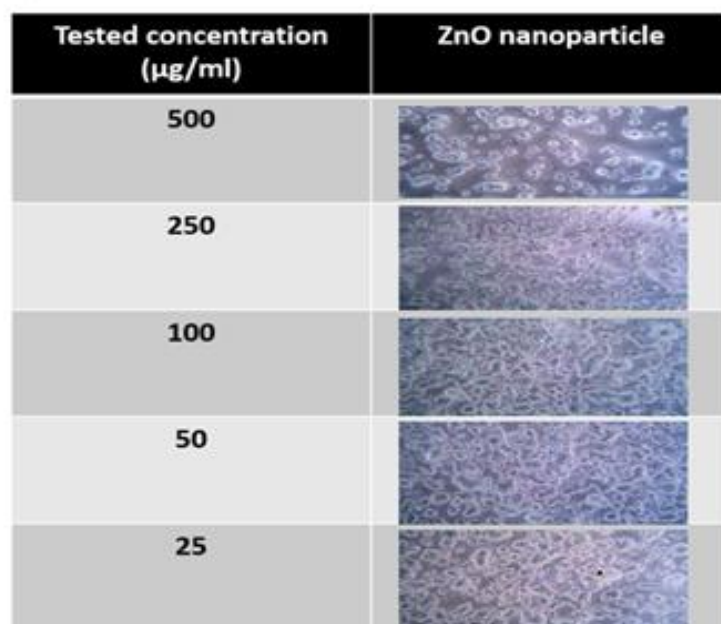


Figure:5.9 Morphological analysis of Zinc oxide nanoparticles on HT29 colon cancer cells

Our results demonstrate that tiny nanoparticles can easily enter the tumor matrix, resulting in cell death. This cytotoxicity of the produced nanoparticles was due to the presence of biomolecules on their surface. Thus, Zinc oxide nanoparticles synthesized biologically are cytotoxic to HT29 colon cancer cells.³⁹

Summary

In the current study, the nanoparticles have been successfully synthesized using *Artocarpus hirsutus* seed extract.

The formation of Zinc oxide nanoparticles was confirmed by X-Ray Diffraction confirms the crystallinity nature of Zinc oxide nanoparticles and the Scanning electron microscopy images confirmed the rod and spherical shaped nanoparticles with the particle size between 20-40 nm.

Fourier Transform Infrared Spectroscopy analysis revealed the presence of amides and proteins in the seed extract of *Artocarpus hirsutus* that acts as the reducing agent for the synthesis of Zinc oxide nanoparticles.

Antibacterial activity of the synthesized Zinc oxide nanoparticles was investigated against gastrointestinal bacteria and the results of our study exhibited higher susceptibility of Zinc oxide nanoparticles to *E. aerogenes* than *L. monocytogenes* with an increase in the concentration.

Scanning electron microscopy images on the action of Zinc oxide nanoparticles exposed the breakage of the cell membrane that leads to rupture of cellular components eventually leading to bacterial cell death.

Thus, our study reveals that Zinc oxide nanoparticles have potential bactericidal reactivity that can be used to treat bacterial infections in alternative to conventional antibiotics.

Cytotoxic activity of zinc oxide nanoparticles was evaluated through in-vitro studies on HT-29 colon cancer cells. The current study reveals the potential applicability of Zinc oxide nanoparticles from *A. hirsutus* for in vitro anticancer activity on colon cancer cell lines, as well as some more advanced research of anti-colon cancer therapeutic discovery.

These results suggest that an alternative source of nano nutrients could be green produced Zinc oxide Nanoparticles using *Artocarpus hirsutus* seed extract.

Conclusion

To determine the aggregating qualities and their effects on plant behavior, more research is needed. By developing protocols to prevent variances such as the quantity of bio constituents in plant and product morphology, normalization of the plant - mediated process in terms of natural sources and processing methods is a highly promising area.

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