GREEN SYNTHESIS OF TiO_2 NANOPARTICLES

USING AQUEOUS EXTRACT OF *CHROMOLAENA ODORATA:* EXPLORING ITS ANTIBACTERIAL ACTIVITIES

Dissertation submitted to

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In partial fulfillment of the

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Master of Science in
PHARMACEUTICAL CHEMISTRY

BY

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CERTIFICATE

This is to certify that the project report entitled "GREEN SYNTHESIS OF TiO_2 NANOPARTICLE USING AQUEOUS EXTRACT OF *CHROMOLAENA ODORATA*: EXPLORING ITS ANTIBACTERIAL ACTIVITIES" is an authentic record of the project work carried out by Ms. NADIYA A A (Reg.no:210011012085) in partial fulfillment of the award of the degree of Master of Science in Pharmaceutical Chemistry at Bharata Mata College, Thrikkakara affiliated to Mahatma Gandhi University, Kottayam under my guidance and supervision.

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DECLARATION

I, NADIYA A A do hereby declare that the project report entitled GREEN SYNTHESIS OF TiO_2 NANOPARTICLE USING AQUEOUS EXTRACT OF *CHROMOLAENA ODORATA:* EXPLORING ITS ANTIBACTERIAL ACTIVITIES is a bona-fide record of the work submitted to mahatma Gandhi university in partial fulfillment of the requirement for the award of the degree of M.Sc in Pharmaceutical Chemistry carried out by me under the guidance of Dr. ANU K JOHN, Assistant Professor, Department of Chemistry, Bharata Mata College, Thrikkakara.

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Date:

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CONTENTS

ABSTRACT

Metal Nanoparticle have attracted much attention in recent decades due to their high surface area, more active site promote the reaction time ,increase the yield of product, selectivity, tunable morphologies and plays an important role in catalytic application. Nanoparticle catalyst easily separated and can be recycled. In this Study, we discuss the synthesis of TiO2 Nanoparticles by green Manner. It was conducted using Leaf extract of the plant Chromolaena odorata by Hydrothermal Method. In this Method, create a high-temperature, high-pressure reaction environment by heating the reaction system and pressurizing it. The Synthesized Nanoparticle were charecterized by, X-ray diffraction and FTIR Spectroscopy. By using different Bacteria, we tested the Antibacterial Activity of TiO2 Nanoparticles. This Method offers a simple, ecofriendly and costeffective way of producing TiO2 Nanomaterials which can be used for a variety of applications.

CHAPTER 1

INTRODUCTION

NANOTECHNOLOGY

Nanotechnology is a field that focuses on manipulating matter at the atomic and molecular level, typically between 1 and 100 nm. It might be used in a variety of fields, including medicine, water purification, information technology, and the production of lightweight materials. Nanotechnologies are centered on the manufacture and manipulation of materials at the nanoscale. Scaling up from single atomic groupings or reducing or improving bulk materials can also accomplish this. The basis of nanotechnology is nanoparticles which show distinctive physical and chemical properties.

The standard definition of NPs is a collection of particles between 1 and 100 nm in size. They exhibit a range of size-dependent properties and can include different kinds of atoms (or molecules).Due to one kind physical and chemical characteristics, Titanium nanoparticles (TiNPs) are being hired an increasing number of in a ramification of industries which include medicinal drug, meals, health care, purchaser items, and industrial packages. Nanotechnology has revolutionized many industries by enabling the development of new materials, products, and technologies with enhanced properties and functionalities. It has applications in fields such as electronics, medicine, energy, environmental remediation, and agriculture. In electronics, nanotechnology has led to the miniaturization of devices, allowing for faster and more efficient electronic components. Nanomaterials, such as carbon nanotubes and graphene, have shown promise in creating smaller, faster, and more flexible electronic devices. In medicine, nanotechnology has opened up new possibilities for targeted drug delivery, imaging, and diagnostics. Nanoparticles can be designed to specifically target cancer cells, deliver drugs directly to the site of disease, and enhance the effectiveness of treatments. The preparation of TiNPs using Green chemistry has fantastic capability. The goal of green chemistry is to carry out chemical processes in a way that makes its byproducts and end products less harmful or hazardous for the environment, living things, and other living things. Ti NPs are made using a lot of green synthesis techniques because they use low-cost, environmentally friendly processes that increase yield.

Green chemistry principle are listed below.

1. Protection : It is better to prevent waste generation rather than cleaning it up afterwards.

2. Atom Economy : Synthetic methodologies ought to be maximize the absorption of all substance used during the process into final result.

3. Chemical Synthesis with lower Risk : Synthetic methods must be developed wherever possible to use and make chemicals that are safe for both people and the surrounding.

4. improving chemical designs : The least harmful chemical products that can nevertheless fulfill the required function should be produced.

5. improved accessories and solvents: When using supplementary compound, such as solvents or separating agents, care must be taken to ensure that they are safe.

6. energy-saving design : Energy need in chemical processes should be minimized to reduce environmental impact.

7. Use of Renewable raw material : a raw material ought be renewable rather than depleting.

8. Decrease Derivatization : Unnecessary derivatization should be avoided to minimize waste generation

9. In catalysis, more selective catalytic reagents outperform stoichiometric reagents.

10. Creating a Degradable Design : Chemical goods should be created to decompose into harmless compounds after usage, lessening their negative effects on the environment.

11. Pollution Prevention Through Real-Time Analysis: The development of analytical methodology is necessary to identify and stop the emergence of dangerous compounds during chemical reactions.

12. Essentially risk-free chemistry for preventing accidents: Substances and processes that are less prone to accidents and releases should be chosen to minimize the risk to human health and the environment..

Chemists and researchers can use these ideas as a guide to create chemical processes and goods that are ecologically benign and sustainable.

NANOMATERIALS

The phrase associated with nanotechnology is "nanomaterial." Because it complies with all nanotechnology principles. Materials classified as a group of substances with at least one dimension less than or equal to about 100 nm are those made from blocks of nanoparticles. In the universe, a single nanomaterial is so tiny that it cannot be seen with the human eye. To examine them, we always need a powerful electron microscope. The following are the various organizations' differing definitions of the term "nanomaterial":-

International Organization for Standardization: A material can be described as having nanoscale properties if any of its outward dimensions or internal structure is between 1 and 100 nanometers. European Commission: It is defined as a natural, unintentional, or artificial material that contains particles in an unbound state, as an aggregate, or as an agglomeration, and When at least 50% of the particles have one or more external dimensions that fall between 1 nm and 100 nm in the number size distribution. The number size distribution barrier of 50% may, in some circumstances and where justified by issues with the environment, health, safety, or competitiveness, be substituted by a threshold between 1 and 50%. The major classification of nanoparticles is.

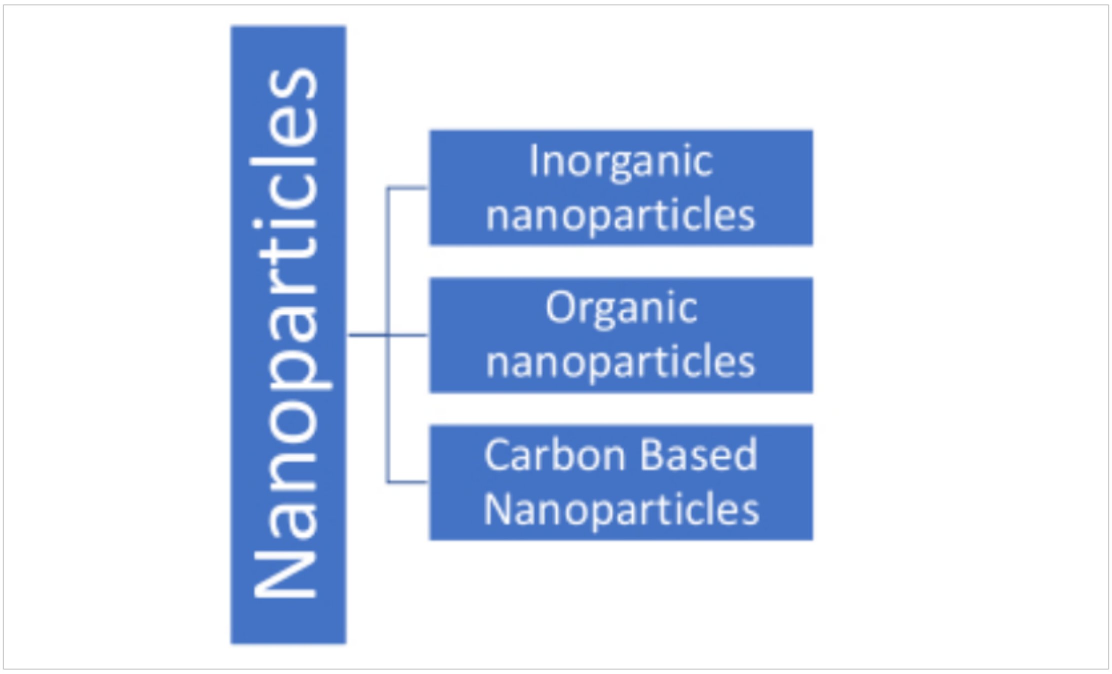


fig 1.1 Different types of Nanoparticle

- AEROGEL

In 1931, Samuel Stephens Kistler invented the aerogel for the first time. It is composed of nm-sized particles which is covalently attached to other. Typically, they are created using non-woven composite paper, resorcinol-formaldehyde aerogel, and pyrolysis. They have a very high porosity. Carbon aerogels can be used as de-ionization electrodes or capacitor electrodes because they are electrically conductive. Additionally, because of large surface area, it helps to make supercapacitors.

Fig 1.2 Aerogel

- CARBON NANOTUBES

Sumio Iijima, a Japanese electron microscopist, made the discovery of CNTs in 1991 while examining the material left behind on the cathode after the manufacture of fullerene via arc-evaporation.CNT is a tube-shaped sheet of rolled graphite. They are carbon atom lattice-based molecular tubes with cylindrical nanostructures and sizes of a few nanometers. Made up of one or more graphene cylinders or sheets with sp^2-hybridised carbon atoms forming a hexagonal network. It consists of hollow or closed ends with lengths varying from a few micrometres to a few millimetres. It exhibits features of low-dimensional materials. At high temperatures, it provides a high yield with good-quality samples. They have unique physical and chemical properties. Three different types exist:

a) Armchair : It has a row of hexagons running perpendicular to the nanotube's axis.

b) Zigzag : This type of nanotube has a linear arranment of carbon bonds running downward the middle of it.

c) Chiral : This type of nanotube has a twist or spiral around it.

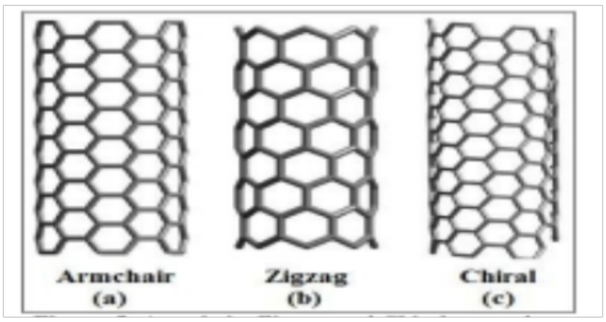


fig 1.3 Types of Carbon Nanotubes

- FULLERENE

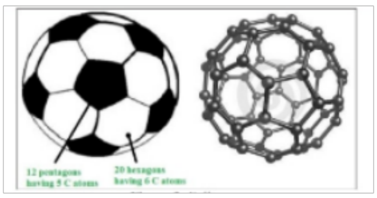
The discovery of the Fullerene or Buckyball molecule, which has 60 carbon atoms (C60), was made in 1985 by the scientists R. F. Curl, H. Kroto, and R. E. Smalley by vaporizing graphite with a laser. These are hollow carbon molecule,typically in the form of spheres or ellipsoids.The most well known fullerene is Buckminister fullerene(C60),which has a soccer ball like structure. fullerene have unique electronic and optical properties.

Fig 1.4 fullerene

- METAL BASED NANOPARTICLE

Metallic nanoparticles are increasingly widely used in engineering and medicinal sciences. A wide range of prospective biotechnology applications are now made possible by the ability to produce and modify these materials with different chemical functional groups that allow them to be combined with useful drugs, ligands, and antibodies. Metallic nanoparticles have an inorganic metal or metal oxide core that is typically surrounded by an organic, inorganic, or metal oxide shell. They have a large surface area compared to their size and are extremely sensitive to things around them. Many other metals, silver (Ag), Titanium (Ti) and zinc (Zn), can be used to make them.

TiO_2 NANOPARTICLE



Due to its broad qualities of domains, including catalysis, photocatalysis, self-cleaning materials, dye-sensitized solar cells, antibacterial agents, as well as water and air purification, titanium dioxides (TiO_2) have been extensively noted. So, for these purposes, TiO2 and materials doped with noble metals are appropriate options. TiO_2 is a non-combustible, colorless, and odorless powder. The crystal phase, particle size, and shape all affect the properties of TiO_2. Titanium dioxide (TiO2) nanoparticles are ideal for a range of purposes because of their high refractive index (n = 2.4), including medicines, coatings, inks, polymers, food, cosmetics, and textiles. TiO2 also has electrical, optical, and morphological properties that make it the ideal material for environmental applications.

There are 3 types: anatase, rutile, and brookite.

Anatase type: It has a crystalline structure similar to the tetragonal framework (with a di pyramidal tendency), and it is mostly used as a photocatalyst when exposed to UV radiation.

Rutile type: A tetragonal precious stone structure (with a tendency toward prisms). This type is mostly used as a white paint color.

Brookite type:It is crystallized as an orthorhombic prism.

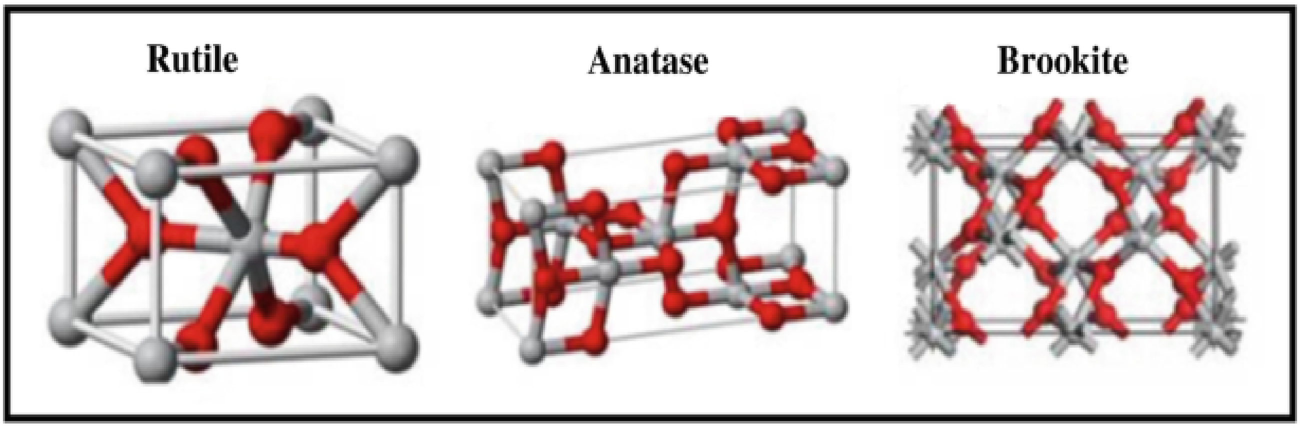


fig 1.5 Mineral forms of TiO_2

METHODS OF SYNTHESIS

There are essentially two ways to create nanoparticles:

1. Bottom-up methods

2. Top-down methods

In the bottom-up method, Atoms, ions, and molecules go through chemical processes which can be used to create nanoparticles.
In top-down methods, To create nanoparticles, bulk materials are broken up into different components.

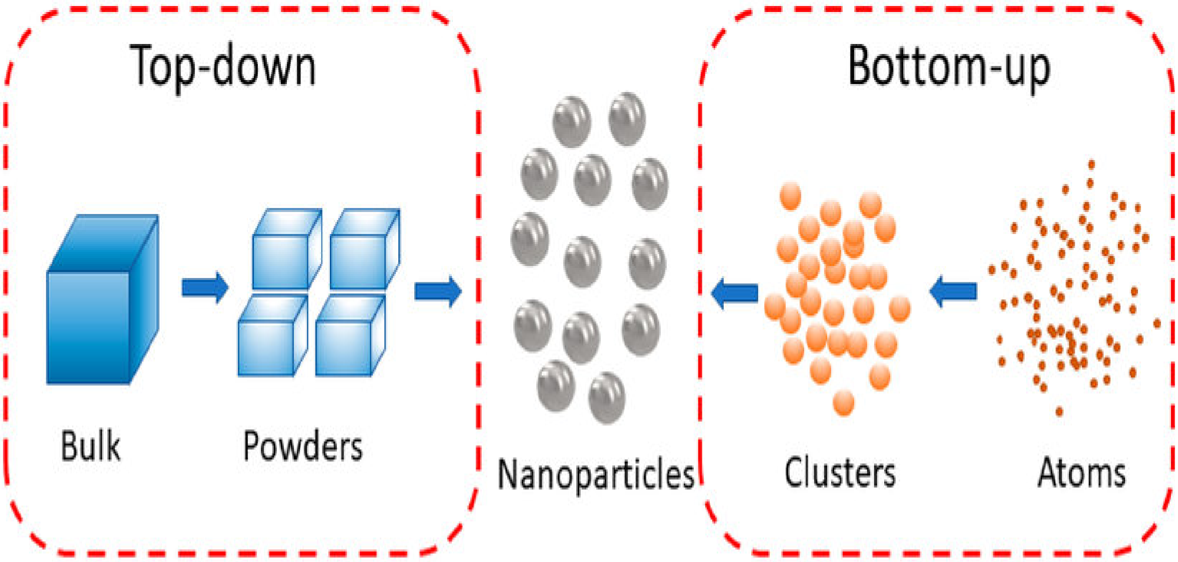


fig 1.6 Methods of synthesis

Physical Techniques

Evaporation-condensation, laser ablation, chemical etching, ball milling, and other physical processes are the basic categories. Physical methods' key benefits include their rapid speed, lack of harmful chemical use, uniform size and shape, etc. On the other hand, the main drawbacks are a high cost, low level of stability, and significant energy consumption.

- Ball-Milling Technique

This approach can be used to create nanoparticles. Benjamin was the first to employ this technique for the synthesis of superalloy. Materials having a macron size undergo a number of modifications in this process. There are several different mechanical mills available for the manufacture of nanoparticles. These mills are categorized depending on their capacity and intended usage. However, because of lengthy process, it is highly challenging to generate ultrafine particles using this method. The quality of products can be impacted by various elements such as milling speed, temperature, mill type, etc.

- Laser Ablation

In pulse laser ablation, a powerful pulsed laser beam is concentrated within the vacuum chamber in order to hit the target that is present in the material. This causes the creation of a plasma, which will later transform into a colloidal solution of nanoparticles. We typically employ a second harmonic generation type laser to prepare nanoparticles.

Chemical Technique

Electrochemical procedures, hydrothermal synthesis, polyol synthesis, sol-gel methods, chemical reduction methods, etc. The chemical process has a higher yield than the physical method, which is its principal advantage. Chemical approaches provide several key benefits, including affordability, heat stability, and size control. The use of hazardous chemicals and solvents, a lack of purity, environmental impact, etc. are some drawbacks, nevertheless.

- Electrochemical Method

In this, current serves as the catalyst for the creation of NPs. By moving an electric current between two electrodes separated by an electrolyte, this approach is accomplished. This indicates that the electrode-electrolyte contact is where the synthesis takes place here. The key benefits of this approach over alternatives include low cost, great flexibility, quick access to equipment, etc. Aside from all of these, this method's numerous other elements are still being researched.

- Sol-Gel Method

Condensation, hydrolysis, and thermal degradation of metal precursors in solution are all included in this process. A sol, a stable solution, forms as a result. High viscosity is created during hydrolysis. However, by adjusting the precursor concentration, temperature, and pH levels, the particle size can be controlled. The following step results in the sol-gel process:

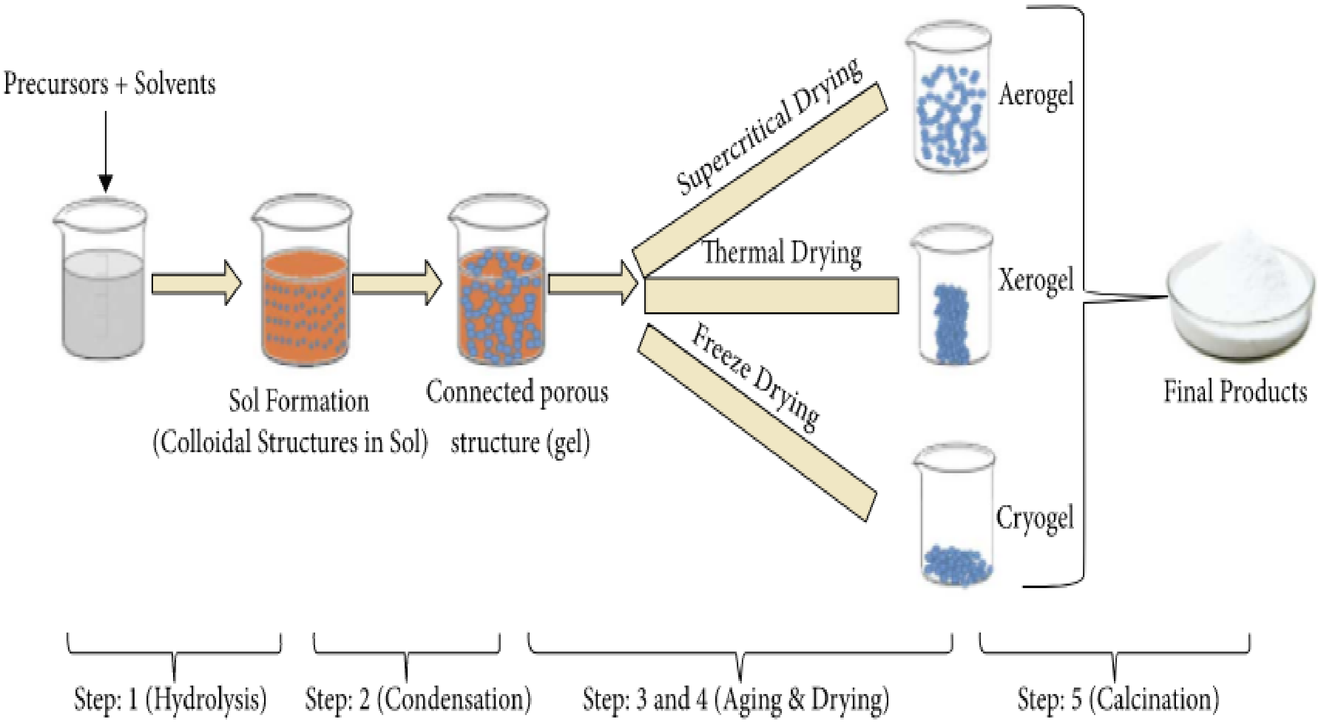


fig 1.7 steps of sol gel method

Biological Methods

We employ the biological approach to get over the drawbacks of the physical and chemical approaches. This technique works best for the creation of nanoparticles. This technique can be used to biological systems like fungi, bacteria, tiny biomolecules, plant extracts, etc. It is feasible to create Ag NPs using this technique. On the other hand, it is also feasible to make a number of other nanoparticles, including graphene and gold.

- Using Plant Extracts

In the creation of nanoparticles, plant extracts are crucial. This procedure is referred known as "green synthesis.". The geranium herb's leaves have been utilized to create gold nanoparticles. To create Ag NPs, 5ml of the extract is mixed with 1ml of aq. $AgNO_3$ solution. The manufacture of the alcoholic extract, however, has also been done using this technique. Silver nitrate and plant extract are stored in a shaker that spins at 150 rpm in the dark.

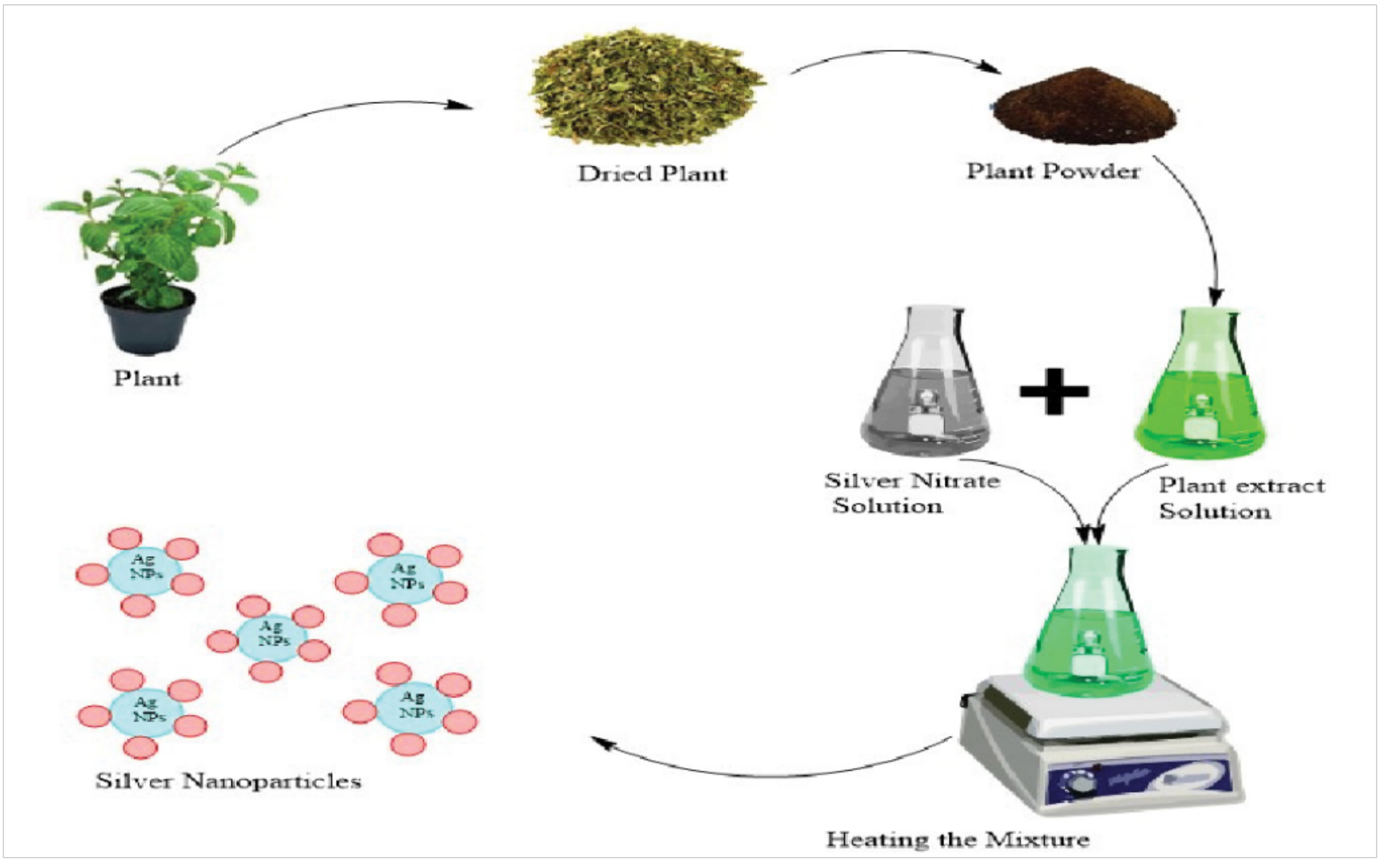


fig 1.8 steps for synthesis of nanoparticle from plant extract

- By Using Microorganism

Due to their environmental friendliness, microbes have attracted for the creation of nanoparticles. Essential processes for producing nanoparticles are extracellular biosynthesis and intracellular biosynthesis. In silver mines, Pseudomonas stuzeriAg295 is regularly discovered. CdS nanoparticles are created by Klebsiella pneumonia.

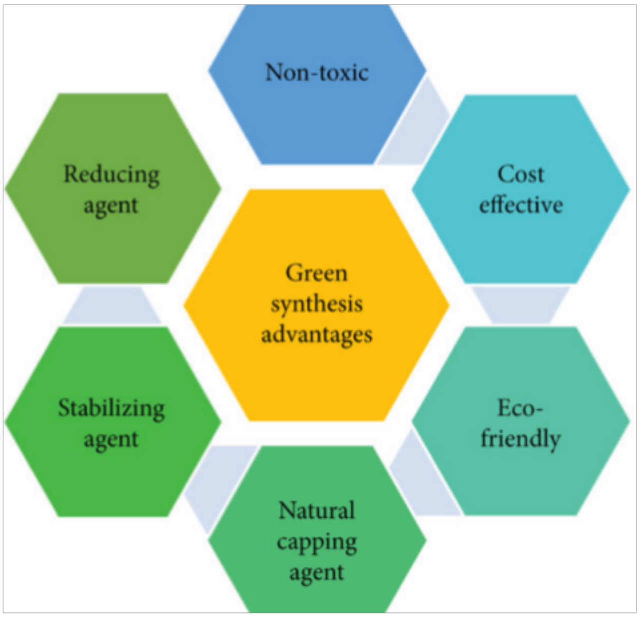


fig 1.9 Advantage of green synthesis

REVIEW OF LITERATURE

Azadirachta indica leaf extract is used for preparation of TiO_2 NP and the use of their antibacterial property propse by B.K. Thakur and A. Kumar. While XRD tests demonstrated the crystalline character of TiO_2 NP. Terpenoids, flavonoids, and proteins that are assumed to be responsible for the creation and stability of titanium dioxide nanoparticles were detected by FTIR studies. The antibacterial activity of the generated TiO2 nanoparticles and TiO2 compound was tested using the test species Escherichia coli, Bacillus subtilis, Salmonella typhi, and Klebsiella pneumoniae. All of examined microorganisms' development was hindered by TiO_2 nanoparticles, according to the study's. In comparison to TiO_2 compound, TiO_2 nanoparticles have a more significant antibacterial activity. SEM, TEM, FT-IR, and XRD are used to characterize produced nanoparticles.Widely effective antibacterial action was shown by synthesized nanoparticles.TiO_2 nanoparticles were discovered to be more efficient than TiO_2 compound

Aloe vera extract is to be used for synthesis of titanium NPS, according to Ganapathi Rao Kandregula and his collegues. Gel contains vitamins ,folic acid, aminoacids. aloe vera extract act as a reducing agent and natural capping agents. Here, the synthesized NPs were examined using UV-Visible spectroscopy, HRTEM, SEM and X-ray diffraction to determine their characteristics. The FCC structure of the synthesized nanoparticles is shown by XRD examination.TGA DTA give the idea about thermal properties. They have tetragonal structure and average partcile size is 32 nm. This technique produces highly stable Ti NPs.

According to J.Rajkumari , Maris Mangdalane and their collegues, titanium nanoparticles were created by green method from Aloe barbadensis mill. The substances utilized in this process are titanium chloride. For the production of Ti NPs, this technique works well. TiO_2 nanoparticle obtained by reduction of $TiCl_4$. The suggested approach is straightforward, environmentally friendly, doesn't call for any expensive equipment, and doesn't require any additional reducing agents or surfactants. This technique is really easy to use, only needs inexpensive tools, and is environmentally friendly.It is feasible to analyze the shape, size, and composition of NPs using UV, TEM and X-ray analyses.The NP have mixture of antase,rutile, and brookite phases.

The creation of titanium nanoparticles from psidium guajava extract and virtual testing for anti-bacterial activity and antioxidant properties have been reported by T Santhoshkumar and his collegues. Using $TiO(OH)_2$ and plant extract for preparing nanoparticle. After the reaction between them,green colored nanoparticle are formed. In this technique, titanium nanoparticles are characterized using XRD and FTIR. $TiO(OH)_2$ is reduced to titanium dioxide nanoparticle using plant extract. comparing the absorbance of extract and ascorbic acid, synthesized nanoparticle possess maximum antioxindant activity. Bacteria such as Staphylococcus aureus and Escherichia coli are used for antibacterial studies.

TiO_2 nanoparticles were produced in 2016 by Chatterjee et al. utilizing Vigna radiata extract. The scientists found that oval-shaped TiO_2 NPs were effective against both Gram-staining bacterias and could be manufactured biologically. O-Ti-O bonding was visible in the FTIR spectra at 1631.78 cm^{-1}.

TiO_2 nanoparticles using agricultural waste material by sathishkumar et al (2020). This research focused on utilizing agricultutal waste materials,such as rice husk and sugarcane bagasse for synthesis of TiO2 NPs. The study demonstrated that these waste material acted as both reducing agent ad stabilizing agent.The synthesized nanoparticle showed photocatalytic activity.

TiO_2 nanoparticles are prepared by green method using *Jatropha curcas* L leaf extract and its photocatalytic degradation of tannery waste water propsed by Surya Pratap Goutam, Gaurav Saxena and their collegues. The aim of these study that, create green titanium dioxide (TiO_2) NPs and assess how well they would work to cure TWW via photocatalysis following secondary (biological) therapy. To test its suitability for treating tannery wastewater (TWW), TiO_2 NPs were made in a single step at room temperature using extract of leaf from Jatropha curcas L biodiesel plant. A UV-Visible spectrophotometer and XRD were also utilized to do further analysis on the environmentally friendly TiO2 NPs. According to the research, leaf extract contains phytochemicals that may have a role in NP capping or stability as well as spherical TiO2 NP production and anatase phase formation. The ecologically benign TiO2 NPs were also initially used to show that they have the ability to simultaneously remove chromium (Cr) and chemical oxygen demand (COD) from secondary treated TWW.

APPLICATON OF NANOPARTICLES

Nanomaterials made of gold, silver, zinc, and other materials have special physicochemical characteristics and are used in biomedicine as a result. Application of platinum nanomaterial in energy storage. Silver nanoparticles may be used as a functional component in a variety of goods and sensors because to their optical qualities. The use of carbon-based nanoparticles in biosensors, bioimaging, drug delivery, enzyme immobilization, and other applications has proved successful.

- Therapeutic and Diagnostic Applications

Inert silica, metal are used to create sphere-shaped nanoparticles that are biocompatible. They are developing into a brand-new class of medicines for the treatment of cancer. They also have the ability to overcome drug resistance, which would result in increased intracellular drug accumulation. Modern molecular diagnostic techniques, such DNA and protein microarray biochips, employ nanotechnologies into their molecular diagnostic applications. Single-cell and single-molecule diagnostics are made possible by nanotechnologies. Recent advancements in cancer nanotechnology provide fascinating possibilities for targeted medicine delivery.

- Treatment of Water

Numerous uses for nanoparticles exist in the treatment of contaminated water and drinking water. Nanomaterials are highly reactive and capable of considerable adsorption because of their small size and large surface area. Utilizing various kinds of nanomaterials, heavy metals, organic pollutants, inorganic anions, and microbes may all be eliminated.. The destruction of contaminants in water and wastewater has been accomplished by photocatalytic degradation using metal oxide nanoparticles, such as titanium dioxide. Because of its non-toxicity, economic viability, chemical stability, strong photoactivity, etc., titanium dioxide is a substance that is heavily explored.

- Energy Reserves

Platinum-based nanoparticles are crucial in the fields of environmental catalysis and energy-related Along with the sequence of catalytic processes, which include organic synthesis, vehicle exhaust gas treatment, hydrogen bonding, and fuel cells, platinum exhibits better catalytic activities and also has great electronic structure . The surface area and number of exposed active sites can be increased by the platinum-based nanoparticle, however.

- Nanomedicine

Silver nanoparticles have a lot of uses in the biomedical industry . Its intriguing size-dependent chemical, physical, and optical characteristics, gold has recently found usage in the field of nanomedicine. For biosensing, labeling, and bioimaging, gold nanoparticles have been employed. In cellular and biomolecular imaging, it is also utilized as a contrast agent. Cancer photodynamic treatment makes considerable use of nanoparticles.

- Catalysis

One of the main uses for nanoparticles is catalysis. Numerous substances, including titanium dioxide, clay, silica, and other metals including iron, aluminum, and others, have been utilized as nanoscale catalysts for many years. Nano-catalysis is regarded as a significant scientific subject because of its high activity, selectivity, and productivity. Metal nanoparticles between 1 and 10 nm in size show exceptional catalytic activity. When compared to equivalent metal complexes, its catalytic activity is superior. Numerous reasons, including, the electrical effect, the quantum size effect, and the surface geometric impact, are attributed to the high activity of nano-catalysts. As a heterogeneous catalyst, the metal nanoparticle suspended in the solution is employed. The catalytic activity of nano-catalysts is significantly influenced by the size of the nanoparticles.Chemistry can make utilization of carbon nanotubes as a catalyst for the partial oxidation of methane, fuel cells, and synthetic ammonia It may be utilized in photocatalytic processes as well.

Sl. No.	Nanoparticles	Major applications
1.	Silver	Antimicrobial activities, garment industries, waste water treatment, cosmetic materials
2	Gold	In chemo immunological studies, DNA fingerprinting, detecting aminoglycosides, antibiotics, cancer diagnosis
3.	Alloy	Imaging, sensors, catalyst, drug delivery and cancer therapy, an antibacterial agent
4.	Magnetic	Cancer treatment, sorting of stem cell and their manipulation, drug delivery, MRI

fig 1.2 Application of Nanoparticle

CHAPTER 2

MATERIALS AND METHODS

CHROMOLAENA ODORATA

Chromolaena odorata, commonly known as Siam weed or chromolaena, is a fast-growing perennial shrub native to the Americas. It belongs to the Asteraceae family and is known for its invasive nature in many parts of the world.The plant has a woody stem and can reach heights of up to 3 meters. Its leaves are oval in shape and have tiny hairs on them. The flowers are small and white, clustered in dense inflorescences.

Chromolaena odorata is considered a problematic weed due to its ability to rapidly spread and outcompete native vegetation. It thrives in disturbed areas such as agricultural fields, roadsides, and abandoned lands. Its prolific seed production and ability to regenerate from stem cuttings contribute to its invasiveness.

Chromolaena odorata also has some useful properties. In traditional medicine, extracts from the plant have been used for various purposes, including treating wounds, skin infections, and respiratory ailments. Some studies have identified bioactive compounds in Chromolaena odorata that exhibit antimicrobial, anti-inflammatory, and antioxidant properties.

fig 2.1 chromolaena Odorata

MATERIALS

- Titanium isopropxide
- Chromolaena odorata leaf
- Distilled water

APPARATUS USED

- Magnetic stirrer
- Tefflon lined stainless- steel autoclave
- Centrifuge

PROCEDURE

- PREPARATION OF PLANT EXTRACT

The plant material, Chromolaena odorata, was gathered from the premises of Ernakulam and subjected to a thorough washing to eliminate any dust or impurities. The washed leaves were then dried in the shade and subsequently ground into a fine powder using a mixer grinder. To prepare the extract,40g of the powdered sample was dissolved in 225 ml of distilled water and heated at 50^{0} C for one hour. Following the heating process, the extract was filtered using whatman No 1 filter paper and stored in the refrigerator for future utilization

- SYNTHESIS OF TiO_2 NANOPARTICLE USING LEAF EXTRACT

To initiate the synthesis process, 5 ml of titanium isopropoxide (TIP) was introduced into 40 ml of the aqueous leaf extract, and the mixture was subjected to magnetic stirring for one hour. Subsequently, the resulting mixture was transferred into a 100 ml autoclave and heated at a temperature of 180 °C in a hot-air oven for 24 hours. After allowing it to cool down, the product was dried at 120 °C for a period of 2 hours. The dried sample was the fine ground using a mortar

and pestle. The obtained powder was then divided into two equal portions. One portion was subjected to annealing, where it was placed in a muffle furnace and heated at a temperature of 300 °C for a period of 2 hours. The annealed sample was designated as TiO2-2.
On the other hand, the remaining portion of the powder was left unannealed. This unannealed sample was named TiO2-1.

- CHARECTERIZATION TECHNIQUES

FTIR SPECTROSCOPY

FTIR (Fourier Transform Infrared) spectroscopy is a powerful analytical technique used to identify and analyse chemical compounds based on their infrared absorption properties. It involves the measurement of the interaction between infrared light and a sample, providing information about its molecular structure and functional groups. In these techniques, a beam of infrared light is passed through a sample, and the resulting spectrum is obtained by measuring the intensity of the transmitted or absorbed light as a function of wavelength. This spectrum represents the unique fingerprint of the sample, allowing for identification and characterization of its chemical composition. Depending on the infrared absorption frequency range 600–4000 cm^{-1}, the specific molecular groups in the sample will be determined through spectrum data in the automated software of spectroscopy. This method is employed to get the infrared spectrum of solid, liquid, and gas absorption, emission, and photoconductivity. It is possible to identify the characteristic functional groups from the spectral bands that allow us to know the conjugation between the nanomaterial and the adsorbed biomolecules. The FTIR spectrum has absorption peaks that are correlated with the nanoparticle's atomic bonds' vibrational frequencies.
FTIR is useful in identifying and characterizing unknown materials, detecting contaminants in a material, finding additives, and identifying decomposition and oxidation.The advantages of FTIR spectroscopy include its sensitivity, speed, and non-destructive nature. It requires minimal sample preparation and can analyse solids, liquids, and gases. Additionally, FTIR spectroscopy can provide valuable information about molecular vibrations, hydrogen bonding, and other structural

features of compounds.

X-RAY DIFFRACTION TECHNIQUE

X-ray diffraction (XRD) is a powerful characterization technique used to analyse the crystal structure and composition of materials. It provides valuable information about the arrangement of atoms within a crystalline sample. In XRD, a beam of X-rays is directed onto a sample, and the resulting diffraction pattern is recorded. This pattern is a unique fingerprint of the crystal lattice and can be used to determine the crystal structure, lattice parameters, and orientation of the sample.

The diffraction pattern that results from the scattering of X-rays by atoms provides details on the crystal's atomic structure. The position of peaks in a diffraction pattern depends on the frequency, and when the sample interacts with the incident X-ray beam under suitable conditions, intense reflected radiation is produced through constructive interference. This relationship between the distance between crystal lattice planes, the incident angle of the X-ray beam, and the wavelength of the incident X-rays is described by Bragg's law.

XRD is widely used in various fields, including materials science, geology, pharmaceuticals, and metallurgy. It can identify different crystalline phases in a sample, determine their relative abundance, and provide quantitative analysis of phase composition.

The advantages of XRD include its non-destructive nature, high accuracy, and ability to analyze both powdered and single-crystal samples. It can also provide information about crystal defects, grain size, and texture of materials.

- ANTIBACTERIAL STUDIES

To screen the antibacterial activity of the synthesised nanoparticles, Agar well diffusion method was adopted. Two bacteria were chosen namely Pseudomonas aeruginosa and E. Coli as test organisms. These test organisms were cultured in nutrient broth and incubated over night for its growth. The synthesised nanoparticles were suspended in Dimethyl Sulfoxide.

For the agar well diffusion method, Petri plates with Mueller –Hinton agar were prepared and autoclaved. The test organisms were swabbed on to the prepared petri plates. Wells were made in each plate with the help of a well-borer for adding 100µL of three different concentrations of nanoparticle (25ug/mL, 50 and 100ug/mL) and a negative control. A positive control (antibiotic) was also added. These plates were then incubated for 18-24 hours at 37 ˚C. After incubation, the zone of inhibition was measured in milli meters

CHAPTER 3

RESULT AND DISCUSSION

ANTIBACTERIAL STUDIES

Sl No:	BACTERIA	ZONE OF INHIBITION	
		Nanoparticle (TiO2 chemically synthesized)	
		1	2
1	Pseudomonas aeruginosa	1.4	1.1
2	Escherichia coli	1.8	1.6

The antibacterial activity of Unannealed TiO_2 and annealed TiO_2 NPs was investigated against two common bacteria Pseudomonas aeruginosa and Escherichia coli by agar well diffusion method. The obtained result were depicted in figures. The result demonstrated that the unannealed TiO_2 exhibited superior antibacterial activity against both bacteria compared to annealed TiO_2 NPs.

Formation of zone of inhibition around nanoparticle depict the antibacterial property. If there is a highest zone of inhibition, they have highest antibacterial property and vice versa. By using Pseudomonas aeruginosa bacteria, a zone of inhibition formed on the unannealed TiO_2 NPs with diameter 1.4 cm and annealed TiO_2 NP with diameter 1.1 cm. Other than Pseudomonas aeruginosa, using gram negative Escherichia coli bacteria, a zone of inhibition formed on the unannealed TiO_2 NPs with diameter 1.8 cm and annealed TiO_2 NP with diameter 1.6 cm. By comparing the results, diameter of zone of inhibition formed around unannealed NP are large so they have more antibacterial property compared with other. Nanoparticle show more antibacterial property towards Escherichia coli which is gram negative bacteria.

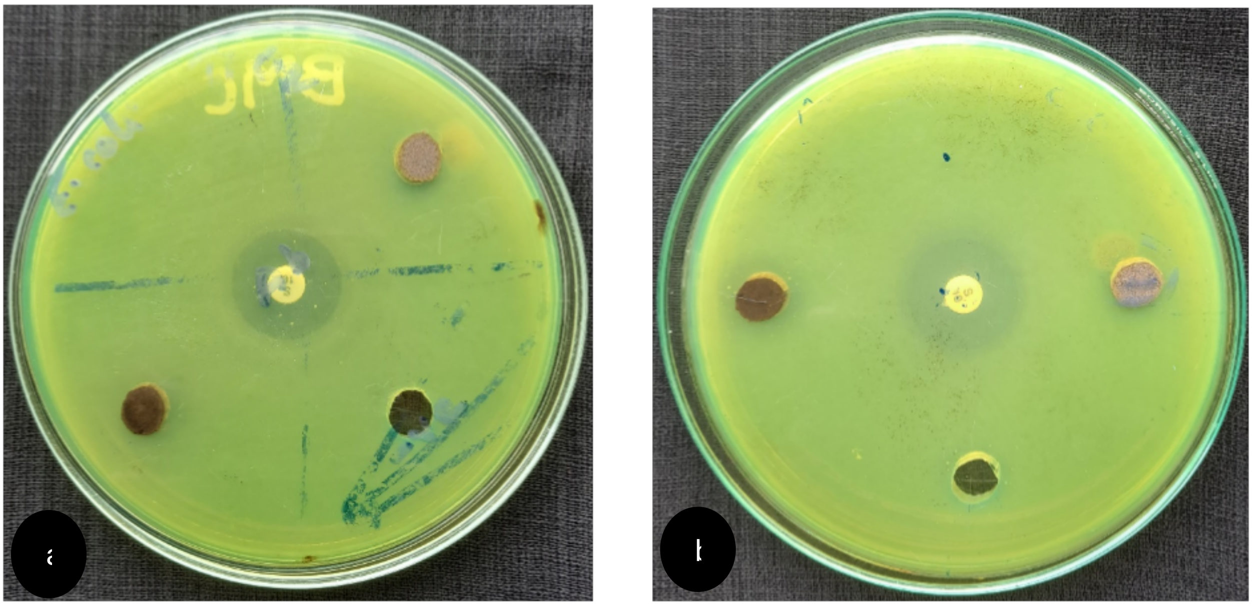
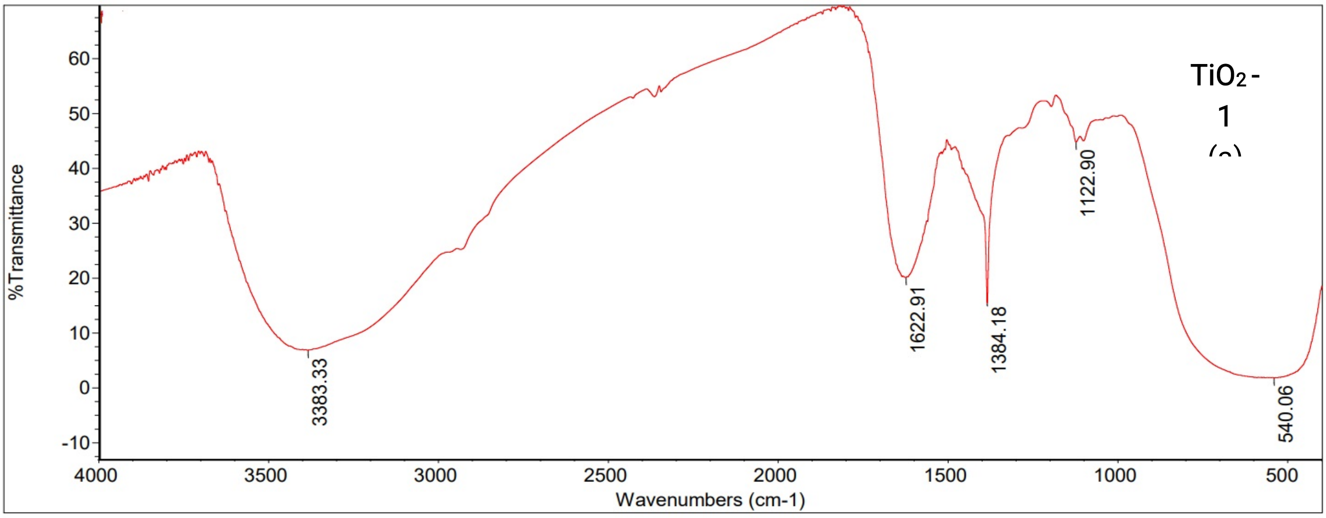


Fig 3.1 (a) E-coli (b) Pseudomonas aeruginosa

FTIR SPECTRUM

The interaction between the plant extract and the nanoparticles is investigated using FT-IR spectra. There are two FTIR spectra for annealed TiO_2 and unannealed TiO_2. Both the TiO_2 NPS having similar region with similar wavelength. By using percentage of transmission with absorbance, we can plot the FTIR spectrum and evaluate the charecteristics of molecule. In the range of 3400-3200 cm-1, a broad band is observed, which corresponds to the stretching vibrations of the hydroxyl group found in various metabolites like flavonoids and polyphenols present in the *chromolaena odorata* plant extract. Peak around at 1600 to 1700 cm^{-1} which is at 1622 cm^{-1}, peak associated with these is bending vibration of water molecule, indicating the presence of adsorbed water on the nanoparticle surface. Peak at 1384.18 cm-1 represent the in plane OH bending. Additionally, a characteristic strong absorption band observed at 1122.9 cm^{-1} and 1102 cm^{-1} for unannealed and annealed TiO_2 respectively, represents the C-O stretching vibrations of alcohols and phenols. With increasing annealing temperature, the plant extract decomposed so the functional groups of plant extract and impurities was disappeared in FTIR spectrum and we get the spectrum of pure TiO_2 nanoparticle. In the FT-IR spectrum of the synthesized TiO_2 nanoparticles, a characteristic broad peak representing the stretching vibrations of Ti-O bond and Ti-O-Ti bending vibration is observed between 450-800 cm-1. The spectrum of compound is a characteristic of it.



TiO_2-2
(b)

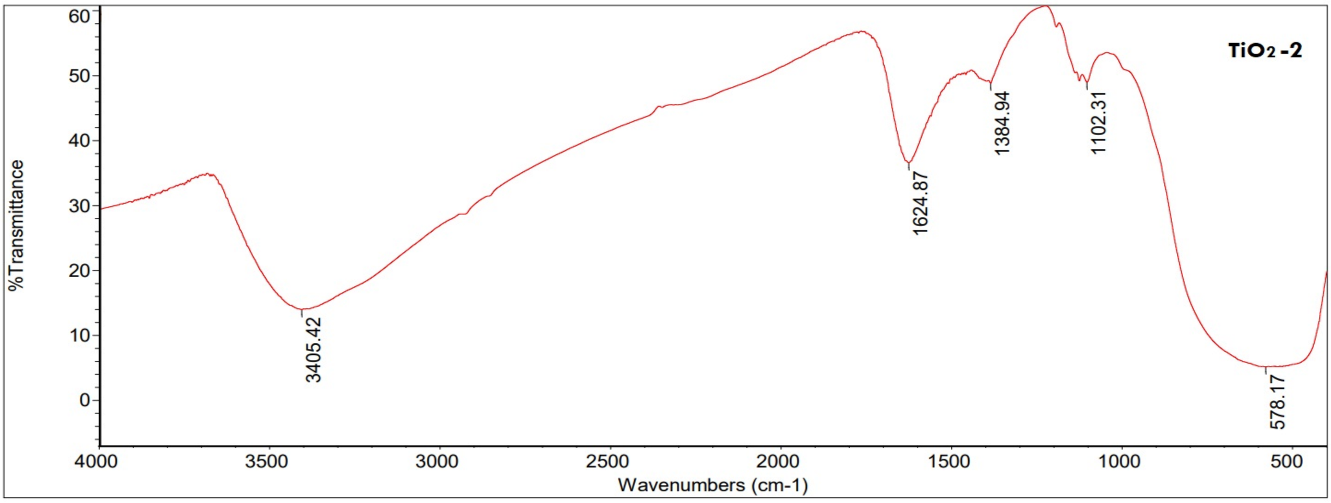


Fig 3.2 FTIR spectrum of (a)unannealed TiO_2 (b)annealed TiO_2

X-RAY DIFFRACTION

2θ	PLANE	PHASE
25.1	1 0 1	A
28.3	1 1 0	R
30	2 1 1	B
37.9	2 0 0	R
47.8	2 0 0	A
54	2 1 1	A
63	2 1 3	A
70	2 2 0	A

The crystallinity and Particle size of the synthesized TiO2 nanoparticles and the phases present in it were revealed by the analysis of X-ray diffraction pattern. Figure shows the XRD pattern of annealed and unannealed TiO2 nanoparticles. The strong and sharp peaks in the XRD pattern indicate the crystalline nature of the samples. The XRD pattern of all the three samples show peaks at 2 θ values 25.1,47.8,54,63 and 70 corresponding to the (101), (200), (211), (213) and (220) planes of anatase phase. Three additional peaks are present at 2 θ values 28.3, 30 and 37.9. The first and third peaks correspond to the lattice plane (110) and (200) of rutile phase and the second peak corresponds to the (211) plane of brookite. particle size is determined by broadening of X-Ray diffraction peaks. This peak broadening is normally caused by finite size effect of crystallites. The particle size can be calculated from the broadening of the diffracted beam using the Debye-Scherrer formula,

$$t = \frac{k\lambda}{\beta \cos\theta}$$

Where t is the crystallite size or particle size, *K* is a dimensionless constant that may range from 0.89 to 1.39, depending on the specific geometry of the scattering objects. λ is the wavelength of X ray (1.5406 Å for Cu K alpha radiation), β is full width at half maximum of X ray peak, and θ is the Bragg angle and it is obtained from the 2 θ value corresponding to the peak with maximum intensity in the XRD pattern.The nano structure was confirmed by this and from the calculations, it was made clear that the size of the particles obtained were less than 100nm. By using 2 θ value corresponding to the peak with maximum intensity in the XRD pattern, we can calculate the particle size using Debye-Scherrer formula. Here 2 θ value of maximum intensity peak is 25.1^0 for annealed and unannealed TiO2. So the θ value is 12.55^0. Substituting the corresponding values of *K,* λ, and cos θ is 0.94, 1.5406Å, and 0.9761067 respectively. B value of annealed and unannealed nanoparticle form xrd data is 1.067 and 0.94 respectively. So the particle size 't' was obtained as 13.904 nm for annealed and 10.51 nm for unannealed TiO2 Nanoparticle. This confirms the nano structure of the TiO_2 particles.

TiO_2- 2 XRD graph Represent the annealed TiO_2 nanoparticle. If annealing temperature improves nanomaterial crystallinity, cause phase transformation and also the particle size. Sharp peaks in the XRD pattern indicate good crystallinity. Here the particle size of annealed tio2 NPs is large as compared to the unannealed tio2. So we can conclude that annealing increases the particle size. Annealing influence both changes in crystallinity as well as occurrence of structural changes and overall, led to an increase in the degree of crystallinity of the materials.

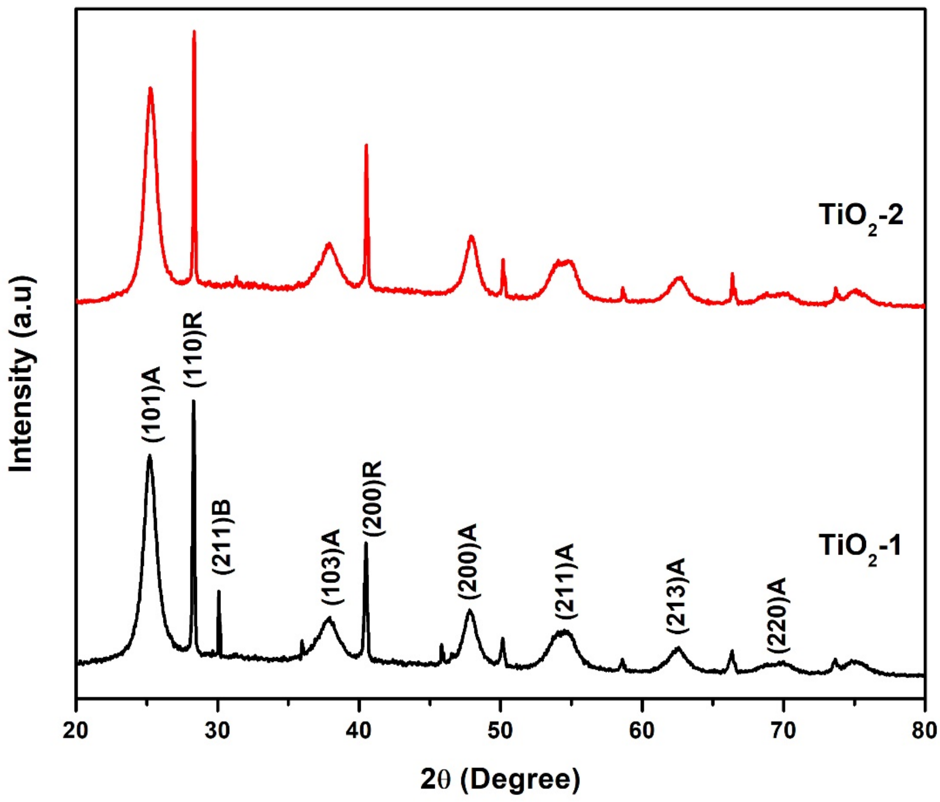


Fig 3.3 XRD Pattern of annealed and unannealed TiO2 nanoparticle

CHAPTER 4

CONCLUSION

TiO_2 nano materials were synthesized from the aqueous leaf extract of the plant *Chromolaena Odorata* using hydrothermal method. The effect of temperature on the properties of the synthesised sample has also been studied. The Characterization of the synthesised materials was done using X-ray diffraction and FTIR spectroscopy. The XRD patterns revealed that the prepared nano particles were crystalline in nature. It also gave information about the crystalline purity of the synthesized materials. Particle size was roughly estimated from XRD patterns with the help of Debye- Scherrer formula. The calculated particle size was below 100 nm. Annealing cause an increase in crystallinity and particle size. FTIR spectra gave an idea about the types of bonds present in the samples which helps to found out the presence of impurities. Annealed sample shows more purity when compared to unannealed one. From the results, it is apparent that the method used for synthesizing TiO_2 nano particles in the current work is an excellent method for preparing nano materials. The successful synthesis of this Nanoparticle using a green and straightforward approach highlights its potential for applications in antibacterial materials and biomedical devices. Unannealed sample shows more antibacterial property when compared to annealed one This method offers a sustainable, simple and cost-effective way of producing TiO_2 nano materials which can be used for a variety of applications.

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