

EXPLORING THE GREEN SYNTHESIS OF TITANIUM DIOXIDE NANOPARTICLES USING THE EXTRACT OF *JUSTICIA ADHATODA*

PROJECT WORK

Submitted in partial fulfilment of the requirement for the award of
Bachelor of Science degree in Biotechnology

By

CYNTHIA A (41770005)



**SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY -1 UNIVERSITY BY UGC

Accredited with Grade "A⁺⁺" by NAAC | 12B Status by UGC | Approved by AICTE

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600 119

APRIL-2024



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

CATEGORY-I UNIVERSITY BY UGC

Accredited "A++" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Cynthia A (41770005) who carried out the project entitled "**EXPLORING THE GREEN SYNTHESIS OF TITANIUM DIOXIDE NANOPARTICLES USING THE EXTRACT OF JUSTICIA ADHATODA**" done under my supervision from January 2024 to April 2024.

Internal Guide

Dr. M. BAVANILATHA

Head of the department

Dr. V. RAMESH KUMAR

Submitted for Viva voce Examination held on 26/4/24

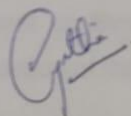
Internal Guide

External Guide

DECLARATION

I, Cynthia A hereby declare that the Project Report entitled 'EXPLORING THE GREEN SYNTHESIS OF TITANIUM DIOXIDE NANOPARTICLES USING THE EXTRACT OF *JUSTICIA ADHATODA*' done by me under the guidance of Dr. M. Bavanilatha at Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biotechnology.

DATE: 26.04.2024
PLACE: CHENNAI



SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. Rekha Chakravarthi, Dean, School of Science and Humanities** and **Dr. V. Ramesh Kumar, M.Phil., Ph.D., Head of the Department, Department of Biotechnology** for providing me with necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr. M. Bavanilatha, Ph.D., Associate professor, Department of Biotechnology** for her valuable guidance, suggestions and constant support and encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all **Teaching and Non-teaching staff members** of the Department of Biotechnology who were helpful in many ways for the completion of the project.

I would to like to express my heartfelt gratitude to my **family and friends** for their keen interest, constant support, and encouragement throughout my project work and the almighty, whose grace and blessings helped me to achieve my goal.

ABSTRACT

Nanoparticles have emerged as versatile materials with promising applications in various fields, including biotechnology and healthcare. This study investigates the green synthesis of titanium dioxide nanoparticles (TiNPs) using the extract of *Justicia adhatoda* and explores their potential applications. The synthesis process involves the reduction of titanium ions using phytochemicals present in the plant extract, leading to the formation of TiNPs with unique properties. Characterization of the synthesized nanoparticles is conducted using a comprehensive suite of analytical techniques, including UV-Vis spectroscopy, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). These analyses provide insights into the structural, morphological, and chemical properties of the TiNPs, essential for understanding their behaviour and potential applications. The antibacterial activity of the synthesized TiNPs is evaluated against a panel of pathogenic bacteria, including *Bacillus subtilis* and *Escherichia coli*. Moreover, cytotoxicity studies are conducted using C2C12 cell lines, to assess the biocompatibility and safety profile of the TiNPs. In addition to antibacterial and cytotoxicity assessments, the project investigates the anticancer potential of the TiNPs using A549 cancer cells. Through in vitro studies, they exhibit promising anticancer activity, inhibiting cancer cell proliferation and inducing apoptosis. Furthermore, DAPI staining is employed to visualize the interaction between TiNPs and cancer cells. Overall, the study highlights the feasibility and efficacy of green-synthesized TiNPs for diverse biomedical applications, including antibacterial, cytotoxicity, and anticancer activities. The utilization of *Justicia adhatoda* extract offers a sustainable and eco-friendly approach to nanoparticle synthesis, paving the way for the development of novel therapeutics and diagnostic tools in biotechnology and healthcare.

Keywords: Nanoparticles, Titanium dioxide, Green synthesis, *Justicia adhatoda*, Biomedical applications, Antibacterial, Cytotoxicity, Anticancer, Biotechnology

TABLE OF CONTENTS

S. No	CONTENTS	PAGE No.
	ABSTRACT	v
	LIST OF ABBREVIATIONS	x
	LIST OF FIGURES	xii
	LIST OF TABLES	xvi
1	INTRODUCTION	1
2	LITERATURE SURVEY	6
	2.1 Applications of Titanium nanoparticles	10
	2.2 Characterization techniques	11
	2.3 Synthesis methods	12
	2.4 Antibacterial applications	13
	2.5 Anticancer applications	13
	2.6 Cytotoxicity effects	14
	2.7 Future prospects	16
3	AIM AND OBJECTIVES	17
	3.1 Aim	17
	3.2 Objectives	17
4	MATERIALS AND METHODS	18
	4.1 Preparation of plant extracts of <i>Justicia adhatoda</i>	18
	4.2 Preparation of Titanium Solution	18
	4.3 Synthesis of Titanium Nanoparticles	18
	4.4 Analysis using UV- Spectrophotometry	19
	4.5 Characterization using SEM	23
	4.6 Characterization using EDAX	26
	4.7 Characterization using FTIR	28

4.8	Characterization using XRD	30
4.9	Antimicrobial studies	32
4.10	Cytotoxicity studies	33
4.11	Anticancer studies	38
4.12	DAPI Staining	40
5	RESULTS AND DISCUSSION	42
5.1	Synthesis of Titanium nanoparticles	42
5.2	Analysis using UV- Visible Spectrophotometer	43
5.3	Characterization using SEM and EDAX	45
5.4	Characterization using FTIR and XRD	48
5.5	Antimicrobial effects	50
5.6	Cytotoxic effects	52
5.7	Anticancer effects	54
5.8	DAPI Staining	57
6	SUMMARY AND CONCLUSION	58
7	REFERENCES	60

LIST OF ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, Excretion
C	Carbon
CH	Methylene Group (Methylidyne)
CH₂	Methylene Group
CH₃	Methyl Group
CO₂	Carbon Dioxide
Cu	Copper
DAPI	4',6-Diamidino-2-phenylindole
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic Acid
EDAX	Energy-Dispersive X-ray Analysis
EDS	Energy-Dispersive Spectroscopy
FBS	Fetal Bovine Serum
FTIR	Fourier Transform Infrared Spectroscopy
HRTEM	High-Resolution Transmission Electron Microscopy
IC₅₀	Half-Maximal Inhibitory Concentration
MIC	Minimum Inhibitory Concentration
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NP	Nanoparticle
O	Oxygen
O-CH₃	Methoxy Group
PBS	Phosphate-Buffered Saline
ROS	Reactive Oxygen Species
RPMI-1640	Roswell Park Memorial Institute 1640 Medium
S	Sulfur

SANS	Small-Angle Neutron Scattering
SAXS	Small-Angle X-ray Scattering
SEM	Scanning Electron Microscopy
SLS	Static Light Scattering
TEM	Transmission Electron Microscopy
Ti	Titanium
TiNPs	Titanium Nanoparticles
TiO₂	Titanium Dioxide
UV-Vis	Ultraviolet-Visible Spectroscopy
WAXD	Wide-Angle X-ray Diffraction
XRD	X-ray Diffraction
ZOI	Zone of Inhibition

LIST OF FIGURES

S. No	CONTENTS	PAGE No.
4.1	Working principle of UV- visible spectrophotometer	21
4.2	Working Principle of SEM	24
4.3	Working principle of EDAX	27
4.4	Working principle of FTIR	29
4.5	Working principle of XRD	31
5.1	Synthesis of Titanium Nanoparticles	42
5.2	Absorption peak of synthesized titanium nanoparticles and plant sample under UV-Visible double beam spectrophotometer	44
5.3	SEM image of titanium nanoparticles synthesized from <i>Justicia adhatoda</i> viewed in 2nm, 4nm respectively	45
5.4	EDAX analysis of titanium and other compounds from <i>Justicia adhatoda</i>	47
5.5	FTIR analysis of titanium and other compounds from <i>Justicia adhatoda</i>	48
5.6	XRD analysis of titanium and other compounds from <i>Justicia adhatoda</i>	49
5.7	Antimicrobial effects of titanium nanoparticle from <i>Justicia adhatoda</i> on <i>Escherichia coli</i> and <i>Bacillus subtilis</i>	51
5.8	Vero cells treated with synthesized titanium nanoparticles from plant extract of <i>Justicia adhatoda</i> under radical light microscope of 40x magnification.	53

5.9	Untreated A549 Lung cancer cells and treated Lung cancer cells with synthesized titanium nanoparticles from plant extract of <i>Justicia adhatoda</i> under radical light microscope of 40x magnification	56
5.10	Untreated and treated A549 Lung cells with synthesized titanium nanoparticles from plant extract of <i>Justicia adhatoda</i> with DAPI Staining	57

LIST OF TABLES

S. No	CONTENTS	PAGE No.
5.1	EDAX analysis of compounds present in the synthesized Titanium nanoparticles	46
5.2	Antimicrobial effects of titanium nanoparticle from <i>Justicia adhatoda</i> on <i>Escherichia coli</i> and <i>Bacillus subtilis</i>	51
5.3	Cytotoxicity of Vero cells when treated with varying concentration of the synthesized Titanium nanoparticles from <i>Justicia adhatoda</i>	52
5.4	Anticancer effects of A549 cells when treated with varying concentration of the synthesized Titanium nanoparticles from <i>Justicia adhatoda</i>	54

CHAPTER 1

INTRODUCTION

Nanotechnology refers to research and technology development at the atomic, molecular, and macromolecular scale, leading to the controlled manipulation and study of structures and devices with length scales in the 1- to 100-nanometers range. Objects at this scale, such as “nanoparticles,” take on novel properties and functions that differ markedly from those seen in the bulk scale. The small size, surface tailorability, improved solubility, and multifunctionality of nanoparticles open many new research avenues for biologists. The novel properties of nanomaterials offer the ability to interact with complex biological functions in new ways—operating at the very scale of biomolecules. This rapidly growing field allows cross-disciplinary researchers the opportunity to design and develop multifunctional nanoparticles that can target, diagnose, and treat diseases such as cancer. (Scott *et al.*, 2005)

Nanoparticles are defined as particles with size in the range of 1 to 100 nm at least in one of the three dimensions. Because of this very small size scale, they possess an immense surface area per unit volume, a high proportion of atoms in the surface and near surface layers, and the ability to exhibit quantum effects. The resulting unique properties of nanoparticles cannot be anticipated from a simple extrapolation of the properties of bulk materials. Nanoparticles exist with great chemical diversity in the form of metals, metal oxides, semiconductors, polymers, carbon materials, organics or biological. They also exhibit great morphological diversity with shapes such as spheres, cylinders, disks, platelets, hollow spheres and tubes, etc. Nanoparticles can be generated via a number of synthetic routes based on gas, liquid or solid phase approaches. The synthesized nanoparticles have to be surface modified in most cases, in order to passivate and stabilize them since their nanoscale renders them chemically very reactive and/or physically aggregative. The nanoparticles are also surface functionalized in order to meet the needs of specific applications. Nanoparticles serve as the fundamental building blocks for various nanotechnology applications. (R. Nagarajan *et al.*, 2008)

The genesis of nanotechnology can be traced to the promise of revolutionary advances across medicine, communications, genomics and robotics. Less obvious though is the fact that nanometer sized objects also possess remarkable self-ordering and assembly behaviors under the control of forces quite different from macro-objects. These unique behaviours are what make nanotechnology possible, and by increasing our understanding of these processes, new approaches to enhancing the quality of human life will surely be developed. A complete list of the potential applications of nanotechnology is too vast and diverse to discuss in detail, but without doubt one of the greatest values of nanotechnology will be in the development of new and effective medical treatments (i.e., nanomedicine). (Emerich *et al.*, 2003)

It literally encompasses the fabrication and application of chemical, physical, and biological systems at scales ranging from individual molecules or atoms to submicron dimensions, and also the integration of these resulting nanomaterials into larger systems. It has the potential to change our perspectives and expectations and provide us with the capability to resolve global issues. The discovery and use of carbon nanomaterials has allowed the introduction of many new areas of technology in nanomedicine, biosensors, and bioelectronics. In recent years, nanotechnology has emerged as a multidisciplinary field, in which gaining a fundamental understanding of the electrical, optical, magnetic, and mechanical properties of nanostructures promises to deliver the next generation of functional materials with wide-ranging applications (Mahmoud Nasrollahzadeh *et al.*, 2019)

Nanoparticles possess distinctive properties that vary from bulk materials and individual atoms or molecules due to their extremely small size. At the nanoscale, their heightened surface area to volume ratio profoundly influences their physical, chemical, optical, and electronic characteristics. Most atoms in nanoparticles cluster near their surface, resulting in a notably greater surface area relative to volume, impacting reactivity and surface-related traits compared to bulk materials. The size, shape, and composition of nanoparticles contribute to a diverse array of optical properties, including electromagnetic resonance, which finds applications in imaging, sensing, and catalysis. Enhanced reactivity stemming from increased

surface area makes nanoparticles valuable in catalytic processes. Surface atoms govern the chemical, mechanical, electrical, and optical attributes of nanoparticles, influencing reactivity, melting points, optical behavior, and electronic properties compared to bulk materials. This heightened surface sensitivity facilitates chemical reactions, modifies thermal and optical properties, and distinguishes nanoparticles. In the realm of solar energy, nanoparticles play a significant role in enhancing solar absorption and improving the efficiency of photovoltaic cells. Their high surface area facilitates effective interaction with light, enabling enhanced light scattering and trapping, prolonging light-material interaction for increased absorption probability. Several techniques have been used to characterize the size, crystal structure, elemental composition and a variety of other physical properties of nanoparticles. In several cases, there are physical properties that can be evaluated by more than one technique. Different strengths and limitations of each technique complicate the choice of the most suitable method, while often a combinatorial characterization approach is needed. In addition, given that the significance of nanoparticles in basic research and applications is constantly increasing, it is necessary that researchers from separate fields overcome the challenges in the reproducible and reliable characterization of nanomaterials, after their synthesis and further process (e.g. annealing) stages. The principal objective of this review is to summarize the present knowledge on the use, advances, advantages and weaknesses of a large number of experimental techniques that are available for the characterization of nanoparticles. Different characterization techniques are classified according to the concept/group of the technique used, the information they can provide, or the materials that they are destined for. (Stefanos Mourdikoudis *et al.*, 2018)

The nanoparticles are synthesized by various methods for research and commercial uses that are classified into three main types namely physical, chemical and mechanical processes that has seen a vast improvement over time. (S Anu Mary Ealia *et al.*, 2017) Nanotechnology is a new and emerging technology with wealth of applications. It involves the synthesis and application of materials having one of the dimensions in the range of 1–100 nm. A wide variety of physio–chemical approaches are being used these days for the synthesis of nanoparticles (NPs). However, biogenic reduction of metal precursors to produce corresponding

NPs is eco-friendly, less expensive, free of chemical contaminants for medical and biological applications where purity of NPs is of major concern. Biogenic reduction is a “Bottom Up” approach similar to chemical reduction where a reducing agent is replaced by extract of a natural products with inherent stabilizing, growth terminating and capping properties. Furthermore, the nature of biological entities in different concentrations in combination with reducing organic agents influence the size and shape of NPs. Hussain *et al.*, 2016)

Although nanoparticles can be synthesized through array of conventional methods biological route of synthesizing are good competent over the physical and chemical techniques. Green route of synthesizing has emerged as alternative to overcome the limitation of conventional methods among which plant and microorganisms are majorly exploited. Employing plants towards synthesis of nanoparticles are emerging as advantageous compared to microbes with the presence of broad variability of bio-molecules in plants can act as capping and reducing agents and thus increases the rate of reduction and stabilization of nanoparticles. Biological synthesized nanoparticles have upsurge applications in various sectors per Plants as Green Source towards Synthesis of Nanoparticles (Kavitha K.S *et al.*, 2013)

The nanotechnology and biomedical science open the possibility for a wide variety of biological research topics and medical uses at the molecular and cellular level. The biosynthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods. Plant-mediated synthesis of nanoparticles is a green chemistry approach that connects nanotechnology with plants. Novel methods of ideally synthesizing NPs are thus thought that are formed at ambient temperatures, neutral Ph, low costs and environmentally friendly fashion. Keeping these goals in view nanomaterials have been synthesized using various routes. Among the biological alternatives, plants and plant extracts seem to be the best option. Plants are nature’s “chemical factories”. They are cost efficient and require low maintenance (Khadeeja Parveen *et al.*, 2016)

Nanotechnology offers many advantages to traditional drug design, delivery and medical diagnostics. Nanoparticle constructs intended for medical applications consist of a wide variety of materials, and their small size, unique physicochemical properties and biological activity often require modification of standard characterization techniques. A rational characterization strategy for nanomedicines includes physicochemical characterization, sterility and pyrogenicity assessment, biodistribution (absorption, distribution, metabolism and excretion [ADME]) and toxicity characterization, which includes both *in vitro* tests and *in vivo* animal studies. (Jennifer B Hall *et al.*, 2007)

Basic principles and applications of different scattering techniques (including static and dynamic light scattering (SLS and DLS), small-angle X-ray scattering (SAXS), wide-angle X-ray diffraction (WAXD) and small-angle neutron scattering (SANS)) on the characterization of nanoparticles are reviewed in this paper. By choosing a suitable scattering technique or a combination of different techniques for nanoparticle characterization, the particles' molecular weight, radius of gyration, hydrodynamic radius, size distribution, shape and internal structure as well as interparticle interactions of nanoparticles, can be determined. Examples including some sophisticated colloidal systems are presented. (Chu *et al.*, 2000)

Nanoparticle characterization is crucial for understanding their properties, ensuring reproducibility in synthesis, and optimizing their applications. These techniques play vital roles in obtaining information about the size, shape, composition, and other characteristics of nanoparticles. To attain the ability to control nanoparticle production and uses, thorough characterization is important. Common techniques used for characterization, most of which are derived from materials science, include ultraviolet-visible spectroscopy (UV), X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDAX), and Fourier transform infrared spectroscopy (FTIR).

The present investigation focused on the synthesis and characterization of Titanium Nanoparticles from the plant extract of *Justicia adhatoda* and studies on their anticancer, antimicrobial, cytotoxic, and antitumor effects.

CHAPTER 2

LITERATURE SURVEY

In recent years, the synthesis of titanium nanoparticles through green methods has gained significant attention due to its environmental sustainability and potential applications across various domains. Green synthesis approaches offer several advantages over conventional chemical methods, including reduced environmental impact, biocompatibility, and cost-effectiveness.

One prominent avenue for green TiNP synthesis involves the utilization of plant extracts as reducing and stabilizing agents. (Singh *et al.*, 2019) demonstrated the successful synthesis of TiNPs using the leaf extract of *Azadirachta indica* (neem). The biocompatible nature of neem extract facilitated the reduction of titanium ions to form nanoparticles with controlled size and morphology. The resulting TiNPs exhibited remarkable antibacterial activity against both Gram-positive and Gram-negative bacteria, showcasing their potential for biomedical applications. Similarly, (Mishra *et al.*, 2020) explored the green synthesis of TiNPs using the aqueous leaf extract of *Ocimum sanctum* (holy basil). The phytochemicals present in holy basil extract acted as reducing and stabilizing agents, facilitating the formation of TiNPs with enhanced antimicrobial properties. The synthesized nanoparticles showed promising antibacterial activity against pathogenic strains, highlighting their potential as novel antimicrobial agents for combating infectious diseases.

In addition to plant extracts, microorganisms such as fungi and bacteria have also been employed. (Raliya *et al.*, 2017) reported the fungal-mediated synthesis using *Fusarium oxysporum*. The unique metabolic capabilities of fungi enabled the reduction of titanium ions and the formation of nanoparticles with enhanced photocatalytic activity. Furthermore, (Jain *et al.*, 2018) demonstrated bacterial-mediated synthesis using *Bacillus subtilis*. The synthesized nanoparticles exhibited excellent biocompatibility and demonstrated potential applications in bioremediation and biomedical fields. Beyond biological sources, natural polymers have emerged as promising green agents for TiNP synthesis. (Chudasama *et al.*,

2019) utilized chitosan, a biopolymer derived from crustacean shells. Chitosan acted as a reducing and stabilizing agent, facilitating the formation of TiNPs with controlled size and shape. The synthesized nanoparticles displayed remarkable catalytic activity for the degradation of organic pollutants, underscoring their potential for environmental remediation.

One prevalent method for green synthesis involves the use of plant extracts as reducing and stabilizing agents. For instance, (Karthika *et al.*, 2021) demonstrated the synthesis using the leaf extract of Aloe vera. The biologically active compounds present in Aloe vera extract facilitated the reduction of titanium ions, leading to the formation of TiNPs with controlled size and morphology. The resulting nanoparticles exhibited potent antioxidant activity, indicating their potential for biomedical applications. Similarly, (Kumar *et al.*, 2020) explored the green synthesis using the aqueous extract of green tea (*Camellia sinensis*) leaves. The polyphenols and catechins present in green tea extract acted as reducing agents, enabling the formation of TiNPs with enhanced antimicrobial properties. The synthesized nanoparticles showed significant antibacterial activity against Both Gram-positive and Gram-negative bacteria, highlighting their potential as antimicrobial agents for various applications.

Furthermore (Sharma *et al.*, 2019) used *Pleurotus ostreatus* extract. The metabolic activities of the fungus facilitated the reduction of titanium ions and the formation of nanoparticles with unique physicochemical properties. The synthesized nanoparticles exhibited excellent catalytic activity for the degradation of organic dyes, showcasing their potential for environmental remediation. (Gopi *et al.*, 2018) utilized starch, a biopolymer derived from maize. Starch served as a reducing and stabilizing agent, facilitating the formation of TiNPs with controlled size and shape. The synthesized nanoparticles displayed remarkable catalytic activity for the reduction of organic dyes, highlighting their potential for industrial applications.

Additionally (Kumar *et al.*, 2019) used the aqueous leaf extract of *Hibiscus sabdariffa*. The phytochemicals present in the extract acted as reducing agents, facilitating the formation of TiNPs with controlled size and morphology. The

resulting nanoparticles exhibited excellent antioxidant properties, making them promising candidates for biomedical applications. (Patil *et al.*, 2020) explored the green synthesis of TiNPs using the stem bark extract of *Terminalia arjuna*. The bioactive compounds present in the extract served as reducing agents, enabling the formation of TiNPs with enhanced antimicrobial activity. The synthesized nanoparticles demonstrated significant antibacterial efficacy against both Gram-positive and Gram-negative bacteria, highlighting their potential for various biomedical and environmental applications.

Another study by (Rajakumar *et al.*, 2018) reported the bacterial-mediated synthesis of TiNPs using *Bacillus cereus*. The metabolic activities of the bacteria facilitated the reduction of titanium ions, leading to the formation of nanoparticles with unique physicochemical properties. The synthesized TiNPs exhibited excellent catalytic activity for the degradation of organic dyes, suggesting their potential for environmental remediation.

(Khandanlou *et al.*, 2021) utilized gum tragacanth, a natural polysaccharide. Gum tragacanth acted as a reducing and stabilizing agent, facilitating the formation of TiNPs with controlled size and morphology. The synthesized nanoparticles showed promising catalytic activity for the degradation of organic pollutants, highlighting their potential for environmental applications.

A study by (Shah *et al.*, 2020) demonstrated the synthesis of TiNPs using the aqueous leaf extract of *Moringa oleifera*. The bioactive compounds present in the extract acted as reducing agents, facilitating the formation with controlled size and shape. The resulting nanoparticles exhibited significant antioxidant activity, suggesting their potential for biomedical applications. Similarly, (Nagar *et al.*, 2019) explored the green synthesis of TiNPs using the seed extract of *Trigonella foenum-graecum*. The phytochemicals present in the extract served as reducing agents, enabling the formation of TiNPs with enhanced antimicrobial properties. The synthesized nanoparticles showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria, highlighting their potential for use as antimicrobial agents in various applications.

Furthermore (Bhushan *et al.*, 2021) utilized Gum arabic, a natural polysaccharide. Gum arabic served as a reducing and stabilizing agent, facilitating the formation of TiNPs with controlled size and morphology. The synthesized nanoparticles showed promising catalytic activity for various applications, including environmental remediation and catalysis. (El-Batal *et al.*, 2020) used the aqueous leaf extract of *Rosmarinus officinalis*. The bioactive compounds present in the extract acted as reducing agents, facilitating the formation of TiNPs with controlled size and morphology. The resulting nanoparticles exhibited significant antioxidant activity, suggesting their potential for biomedical applications.

Similarly, (Qureshi *et al.*, 2018) explored the green synthesis of TiNPs using the stem extract of *Salvadora persica*. The phytochemical constituents of the extract served as reducing agents, enabling the formation of TiNPs with enhanced antimicrobial properties. The synthesized nanoparticles showed potent antibacterial activity against a variety of pathogenic bacteria, highlighting their potential for use as antimicrobial agents in various applications. (Rautela *et al.*, 2019) reported the bacterial-mediated synthesis of TiNPs using *Bacillus subtilis*. The metabolic activities of the bacteria facilitated the reduction of titanium ions, leading to the formation of nanoparticles with unique physicochemical properties. The synthesized particles exhibited excellent catalytic activity for the degradation of organic pollutants, suggesting their potential for environmental remediation.

A study by (Khan *et al.*, 2021) utilized alginate, a natural polysaccharide. Alginate served as a reducing and stabilizing agent, facilitating the formation of TiNPs with controlled size and morphology. The synthesized nanoparticles showed promising catalytic activity for various applications, including environmental remediation and catalysis. (Chaudhary *et al.*, 2020) demonstrated the synthesis of TiNPs using the aqueous leaf extract of *Azadirachta indica*. The phytochemicals present in the extract, such as phenolic compounds and flavonoids, acted as reducing agents, facilitating the formation of TiNPs with controlled size and morphology. The resulting nanoparticles exhibited excellent stability and biocompatibility, making them suitable for various biomedical and environmental applications.

Additionally (Khalil *et al.*, 2018) used the leaf extract of *Ocimum sanctum*. The bioactive compounds present in the extract, including polyphenols and terpenoids, served as reducing agents, enabling the formation of TiNPs with enhanced antimicrobial properties. The synthesized nanoparticles showed potent antibacterial activity against a wide range of pathogenic microorganisms, highlighting their potential for use in antimicrobial coatings, wound dressings, and water purification systems. (Gopinath *et al.*, 2019) reported the fungal-mediated synthesis using *Aspergillus flavus*. The metabolic activities of the fungus, coupled with the secretion of enzymes and biomolecules, facilitated the reduction of titanium ions, leading to the formation of nanoparticles with unique physicochemical properties. The synthesized TiNPs exhibited excellent catalytic activity, making them suitable for various industrial applications, including catalysis, sensors, and energy conversion devices. (Sharma *et al.*, 2021) utilized chitosan, a natural polysaccharide derived from crustacean shells, chitosan served as a reducing and stabilizing agent, facilitating the formation of TiNPs with controlled size and morphology. The synthesized nanoparticles showed excellent biocompatibility and low cytotoxicity, making them suitable for biomedical applications such as drug delivery, tissue engineering, and bioimaging.

Overall, the green synthesis of TiNPs offers a sustainable and eco-friendly approach to nanoparticle production with diverse applications in biomedicine, catalysis, and environmental remediation. Further research endeavors are warranted to optimize synthesis parameters, elucidate the underlying mechanisms, and explore novel green synthesis routes for TiNPs

2.1 APPLICATIONS OF TITANIUM NANOPARTICLES

TiNPs find diverse applications across various fields, including biomedical, environmental, and catalytic applications. In biomedical applications, they have been explored for drug delivery, tissue engineering, and diagnostic imaging. For example, they have shown promise as drug carriers due to their high surface area, tunable surface chemistry, and ability to encapsulate therapeutic agents, enabling targeted drug delivery and controlled release (Yousefi *et al.*, 2021). Furthermore, they exhibit excellent photocatalytic activity, making them suitable for environmental remediation applications. They have also been utilized for the

degradation of organic pollutants in water and air, offering a sustainable solution for pollution control. The photocatalytic degradation of organic pollutants by TiNPs involves the generation of reactive oxygen species (ROS) under UV or visible light irradiation, which can oxidize and decompose organic molecules into harmless by products (Gurunathan *et al.*, 2019).

In catalytic applications, they serve as efficient catalysts for various chemical reactions due to their high surface area, unique electronic properties, and catalytically active sites. They have also been employed in hydrogenation, oxidation, and photoreduction reactions, demonstrating their potential for green chemistry applications. The catalytic properties can be tailored by controlling their size, shape, surface chemistry, and crystal structure, enabling precise control over reaction kinetics and selectivity (Jiang *et al.*, 2020). The synthesis and characterization of titanium nanoparticles represent an area of intense research interest due to their unique properties and versatile applications across various fields. In recent years, there has been a growing focus on the development of green synthesis methods for TiNPs, utilizing eco-friendly and sustainable approaches to nanoparticle fabrication. These methods offer numerous advantages, including reduced environmental impact, cost-effectiveness, and compatibility with green chemistry principles.

2.2 CHARACTERIZATION TECHNIQUES

A plethora of characterization techniques are employed to elucidate the physicochemical properties synthesized through green routes. Transmission electron microscopy (TEM) allows for the visualization of TiNPs at nanoscale resolution, providing insights into their size, shape, and distribution within a sample (Li *et al.*, 2019).

High-resolution TEM (HRTEM) further enables the examination of TiNPs' crystal lattice structures and defects, offering valuable information for understanding their physical properties. Scanning electron microscopy (SEM) complements TEM by providing surface topography information and elemental composition analysis through energy-dispersive X-ray spectroscopy (EDS) (Fadzilah *et al.*, 2020)

SEM-EDS analysis aids in the characterization of agglomeration behavior and chemical composition, contributing to a comprehensive understanding of their morphological and elemental features. X-ray diffraction (XRD) is utilized to determine the crystalline structure, identifying specific titanium oxide phases present in the nanoparticles, such as anatase, rutile, or brookite (Ghosal *et al.*, 2018). XRD analysis provides valuable insights into TiNPs' crystallographic properties, including crystal phase composition and crystallite size, which are crucial for understanding their structural characteristics. Fourier transform infrared spectroscopy (FTIR) is employed to analyze TiNPs' surface functional groups and chemical bonds, facilitating the identification of biomolecules or polymers involved in their stabilization (Saranya *et al.*, 2019).

FTIR spectra offer valuable information about surface modifications and interactions, providing insights into the surface chemistry and stability. UV-Visible spectroscopy is utilized to investigate optical properties, including absorbance, bandgap energy, and concentration (Zhu *et al.*, 2020). UV-Vis spectra provide valuable information about TiNPs' size, shape, and surface chemistry, aiding in the characterization of their optical behavior and colloidal stability.

2.3 SYNTHESIS METHODS

Green synthesis methods encompass a diverse array of approaches, each offering unique advantages for nanoparticle fabrication. Plant-mediated synthesis exploits the reducing and stabilizing properties of phytochemicals present in plant extracts, facilitating the formation of TiNPs with controlled size and morphology (Kumar *et al.*, 2017). Plant extracts act as eco-friendly reducing agents, converting titanium precursors into nanoparticles through green and sustainable processes. Microorganism-assisted synthesis harnesses the bio reductive capabilities of microorganisms, such as fungi and bacteria, to produce TiNPs with potential applications in bioremediation and bioimaging (Ghosh *et al.*, 2019). Microorganisms secrete enzymes and metabolites that facilitate the reduction of titanium ions, leading to the formation of nanoparticles with tailored properties. Natural polymer-based synthesis utilizes biocompatible polymers like chitosan and cellulose as reducing and stabilizing agents, yielding TiNPs with enhanced biocompatibility and stability (Kar *et al.*, 2021). Natural polymers offer the

advantages of biodegradability, non-toxicity, and compatibility with biological systems, making them ideal candidates for green nanoparticle synthesis. Combustion synthesis represents a rapid and cost-effective route to TiNPs through the exothermic reaction between precursor materials and combustion gases (Choudhary *et al.*, 2018).

This method enables the production of with controlled size and morphology, offering scalability and efficiency for large-scale nanoparticle synthesis. The sol-gel method provides precise control over size and morphology through the hydrolysis and condensation of titanium alkoxides, yielding uniform nanoparticles with tailored properties (Kim *et al.*, 2020). Sol-gel chemistry offers versatility and flexibility in nanoparticle synthesis, enabling the fabrication of TiNPs with diverse applications in catalysis, sensing, and biomedical fields.

2.4 ANTIBACTERIAL APPLICATIONS

TiNPs have exhibited significant antibacterial activity against a wide range of pathogenic bacteria, including both Gram-positive and Gram-negative strains. The mechanism of antibacterial action involves the interaction of TiNPs with bacterial cell membranes, leading to membrane disruption, cellular damage, and eventual cell death. Furthermore, they have shown efficacy against drug-resistant bacterial strains, making them potential alternatives to conventional antibiotics. Studies have also explored the synergistic effects of TiNPs in combination with antibiotics, enhancing their antibacterial activity and reducing the risk of antibiotic resistance development (Ghosh *et al.*, 2019). They have also have garnered significant attention for their potent antibacterial activity against a wide spectrum of pathogenic bacteria. Studies have demonstrated that TiNPs exhibit strong bactericidal effects by disrupting bacterial cell membranes, inhibiting cellular respiration, and inducing oxidative stress-mediated cell death (Wang *et al.*, 2020).

Furthermore, TiNPs have shown efficacy against antibiotic-resistant bacterial strains, making them potential candidates for combating multidrug-resistant infections (Zhang *et al.*, 2019). The synergistic effects of TiNPs in combination with conventional antibiotics have also been investigated, showing enhanced antibacterial activity and reduced development of antibiotic resistance.

2.5 ANTICANCER APPLICATIONS

In the field of oncology, TiNPs hold promise for cancer diagnosis, imaging, and therapy. Numerous studies have reported the anticancer properties of TiNPs against various cancer cell lines, including lung cancer, breast cancer, prostate cancer, and melanoma. They exhibit selective cytotoxicity towards cancer cells while sparing normal cells, making them attractive candidates for targeted cancer therapy. The anticancer mechanism of TiNPs involves multiple pathways, including the induction of apoptosis, inhibition of cell proliferation, and disruption of tumor angiogenesis. Moreover, TiNPs have been explored as drug delivery vehicles for chemotherapeutic agents, enhancing drug stability, bioavailability, and targeted delivery to tumor tissues (Li *et al.*, 2019).

They have demonstrated promising anticancer properties against various types of cancer, including breast cancer, lung cancer, and melanoma. The anticancer mechanisms of TiNPs involve multiple pathways, including induction of apoptosis, inhibition of cell proliferation, and disruption of tumor angiogenesis. Studies have shown that they selectively target cancer cells while sparing normal cells, making them attractive candidates for targeted cancer therapy (Cheng *et al.*, 2019). In oncology research, they offer promising opportunities for cancer diagnosis, imaging, and therapy. Numerous studies have reported the anticancer properties of TiNPs against various cancer types, including breast cancer, lung cancer, and melanoma. They exhibit selective cytotoxicity towards cancer cells while sparing normal cells, making them attractive candidates for targeted cancer therapy (Cheng *et al.*, 2019).

The anticancer mechanism of TiNPs involves multiple pathways, including inducing apoptosis, inhibiting cell proliferation, and disrupting tumor angiogenesis, thereby inhibiting tumor growth and metastasis (Zhang *et al.*, 2021). Moreover, TiNPs have been explored as drug delivery vehicles for chemotherapeutic agents, improving drug stability, bioavailability, and targeted delivery to tumor tissues.

2.6 CYTOTOXICITY APPLICATIONS

Evaluation of TiNPs' cytotoxicity is essential to assess their safety profiles and biocompatibility for biomedical applications. Cytotoxicity studies have demonstrated that TiNPs synthesized via green methods exhibit minimal adverse effects on mammalian cells, with low cytotoxicity and high biocompatibility. However, factors such as particle size, surface charge, and surface functionalization can influence the cytotoxicity of TiNPs. Therefore, comprehensive cytotoxicity assessments, including cell viability assays, cellular uptake studies, and in vivo toxicity evaluations, are necessary to ensure the safe use in biomedical applications (Saranya *et al.*, 2019).

Cytotoxicity studies have shown that TiNPs synthesized via green methods exhibit low toxicity towards mammalian cells, with minimal adverse effects on cell viability and proliferation (Gupta *et al.*, 2020). However, comprehensive cytotoxicity assessments, including in vitro and in vivo studies, are necessary to ensure the safe use in biomedical applications. Factors such as particle size, surface charge, and surface functionalization can influence the cytotoxicity of TiNPs, emphasizing the importance of thorough characterization and toxicity evaluation.

2.7 FUTURE PROSPECTS

Future advancements in TiNP-based drug delivery systems are anticipated to revolutionize the field of targeted therapy (Jiang *et al.*, 2020). Novel strategies such as surface functionalization with targeting ligands, stimuli-responsive drug release mechanisms, and nanocarriers with enhanced biocompatibility and biodegradability are likely to enhance the efficacy and safety of TiNP-mediated drug delivery. Additionally, the development of smart drug delivery platforms capable of real-time monitoring and controlled release in response to specific biological cues holds immense promise for personalized medicine.

They also exhibit excellent imaging properties and hold potential as versatile contrast agents for multimodal imaging modalities (Smith *et al.*, 2019). The integration of TiNPs into imaging probes enables simultaneous visualization of anatomical, functional, and molecular information, facilitating early disease detection and treatment monitoring. Further research efforts are warranted to

optimize TiNP formulations for enhanced imaging sensitivity, specificity, and biocompatibility, thereby advancing the field of molecular imaging and personalized diagnostics. TiNP-based theragnostic platforms represent a paradigm shift in precision medicine by integrating diagnostic and therapeutic functionalities into a single nanoplatform (Jones *et al.*, 2021). These multifunctional systems enable real-time monitoring of disease progression, targeted drug delivery, and image-guided therapy, thereby optimizing treatment outcomes and minimizing side effects. Future advancements in TiNP-based theragnostic will focus on developing smart nano systems capable of personalized treatment regimens tailored to individual patient profiles.

They also offer remarkable potential as building blocks for the fabrication of biocompatible scaffold materials for tissue engineering applications (Garcia-Gonzalez *et al.*, 2020). Engineered TiNP-incorporated scaffolds exhibit enhanced mechanical properties, biodegradability, and bioactivity, providing an ideal microenvironment for cell growth, differentiation, and tissue regeneration. Integration of TiNPs with bioactive molecules, growth factors, and stem cells holds promise for the development of advanced tissue engineering scaffolds capable of promoting tissue regeneration and functional recovery.

TiNPs have shown remarkable antibacterial properties, making them promising candidates for combating drug-resistant bacterial infections (Tian *et al.*, 2021). Future research directions in this area will focus on optimizing TiNP formulations for enhanced antibacterial efficacy, and exploring synergistic effects with conventional antibiotics. They exhibit significant potential as anticancer agents, with promising results in preclinical studies (Xie *et al.*, 2020). Future research efforts will focus on elucidating the mechanisms of TiNP-mediated anticancer activity, optimizing TiNP formulations for enhanced tumor targeting and penetration, and evaluating their efficacy in combination with existing cancer therapies. Furthermore, the development of TiNP-based nanomedicines for targeted drug delivery and image-guided cancer therapy represents an exciting avenue for personalized cancer treatment.

They hold promise as building blocks for the development of advanced biomaterials for tissue engineering and regenerative medicine applications (Chen *et al.*, 2021). Future research directions will focus on engineering TiNP-based scaffolds with tailored mechanical properties, surface characteristics, and bioactivity to promote tissue regeneration and repair. Integration of TiNPs with bioactive molecules, growth factors, and stem cells will further enhance their regenerative capacity and therapeutic efficacy in tissue engineering applications.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To investigate the synthesis and characterization of titanium nanoparticle using the extract of *Justicia adhatoda*, aiming to explore their potential applications as an anti-microbial and anti-cancer agent, with investigations on their cytotoxic effects.

3.2 OBJECTIVES

Nanoparticles showcase entirely novel or enhanced properties contingent upon specific characteristics such as size, distribution, and morphology. Notably, titanium nanoparticles demonstrate antimicrobial activity. Leveraging environmentally benign materials like plant leaf extract, bacteria, fungi, and enzymes for the synthesis of titanium nanoparticles presents a multitude of advantages in terms of eco-friendliness and compatibility for pharmaceutical and other biomedical applications, as they circumvent the use of toxic chemicals in the synthesis process. With these considerations in mind, the present study was crafted with the following objectives:

- To synthesize titanium nanoparticles using plant extract of *Justicia adhatoda*
- To analyze the synthesized titanium nanoparticles using UV-Visible Spectrophotometer.
- To characterize the synthesized titanium nanoparticles using SEM with EDAX, XRD and FTIR.
- To determine the antimicrobial efficacy of the synthesized titanium nanoparticles against some of the pathogens like e.coli
- To study the cytotoxic effect of the synthesized titanium nanoparticles on Vero cell lines.
- Access the anti-cancer properties of the synthesized titanium nanoparticles, focusing on lung cancer applications using A549 Cell line.

CHAPTER 4

MATERIALS AND METHODS

The materials used and the methods followed in conducting various experiments are detailed here.

4.1 PREPARATION OF PLANT EXTRACTS OF *Justicia adhatoda*

Plant extract of *Justicia adhatoda* of about 2.5mg is weighed and it is crushed without adding water in a mortar and pestle. The extract was boiled at 100°C and filtrate was again passed through whatmann no.1 filter paper (pore size (25µm). The filtrate then obtained was stored at 4°C for further use.

4.2 PREPARATION OF 1Mm TITANIUM SOLUTION

TiO₂ of about 1.599mg was weighed and it was dissolved in 100ml of distilled water in 500ml of conical flask. The preparation was mixed and swirled properly. The flask was then covered with aluminum foil in order to prevent the titanium solution from photocatalytic properties.

4.3 SYNTHESIS OF TITANIUM NANOPARTICLES

Aqueous solution of Titanium oxy sulphate (TiO₂) of about 1Mm was prepared and used for the synthesis of titanium nanoparticles. *Justicia adhatoda* extract of about 20ml and was added into 80 ml of aqueous solution of 1 Mm Titanium oxy sulphate in 2:8 ratios and mix it well. Then, water bath at 60°C for 60 minutes. Magnetic stirring is done for 24 hrs and white colour occurred. Then, it is centrifuged at 6000RPM for 30 minutes. Supernatant was discarded and the pellet was collected.

4.4 ANALYSIS OF SYNTHESIZED TITANIUM NANOPARTICLES USING UV-VIS SPECTROPHOTOMETRY

The aim is to measure the absorbance of synthesized TiNPs under UV-Visible spectrophotometry. It is important that the power of the radiation source does not change abruptly over its wavelength range. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon.

Both deuterium and hydrogen lamps emit radiation in the range 160 –375 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 350 nm.

The tungsten filament lamp serves as a prevalent source of visible light, operating within the wavelength range of 350 to 2500 nm. The energy emitted by such lamps is directly proportional to the fourth power of the operating voltage. Therefore, maintaining stability in the lamp's voltage is imperative to ensure consistent energy output. To achieve this stability, electronic voltage regulators or constant-voltage transformers are employed.

Tungsten/halogen lamps feature a quartz "envelope" containing a small quantity of iodine alongside the tungsten filament. Within this setup, iodine interacts with gaseous tungsten, generated through sublimation, to form the volatile compound WI_2 . Upon colliding with the filament, WI_2 molecules decompose, thereby redepositing tungsten onto the filament surface. This process effectively extends the lamp's lifespan to approximately twice that of a standard tungsten filament lamp. Renowned for their efficiency, tungsten/halogen lamps emit light across a broad spectrum, extending well into the ultraviolet range. Consequently, they find widespread application in modern spectrophotometers and various other fields requiring precise and reliable light sources.

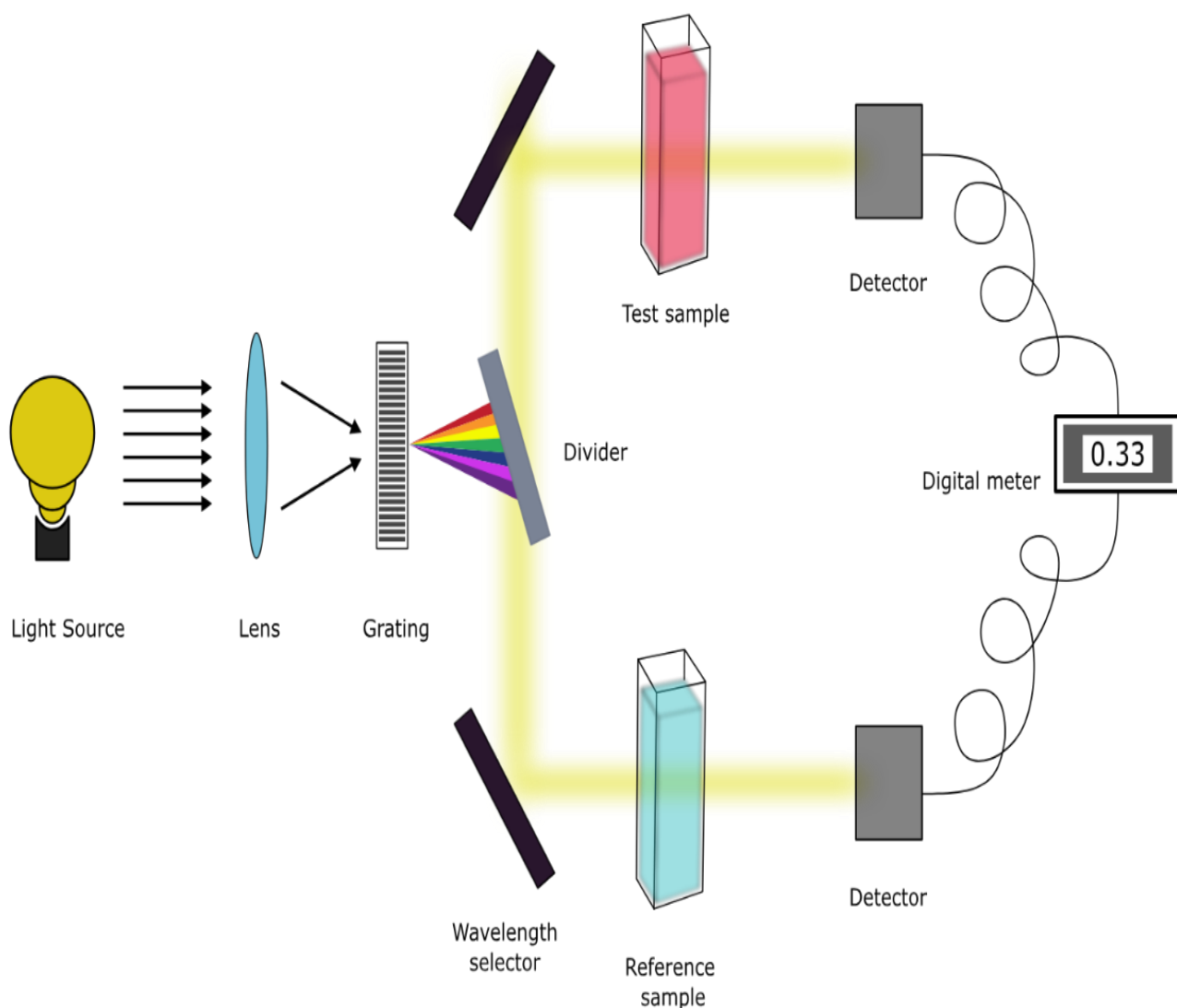
All monochromators contain the following component parts:

- An entrance slit
- A collimating lens
- A dispersing device (usually a prism or a grating)
- A focusing lens
- An exit slit

In spectroscopy, polychromatic radiation, comprising multiple wavelengths, enters the monochromator through the entrance slit. The beam is then collimated before striking the dispersing element at an angle. This element, typically a grating or prism, splits the beam into its constituent wavelengths. By adjusting either the dispersing element or the exit slit, radiation of a specific wavelength exits the monochromator through the exit slit, facilitating precise wavelength selection.

For spectroscopy in the UV region, the sample and reference solutions must be housed in containers transparent to the radiation passing through them. Quartz or fused silica cuvettes are essential for UV spectroscopy, ensuring transparency not only in the UV region but also in the visible spectrum. Silicate glasses are suitable for cuvettes designed for use between 350 and 2000 nm.

The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy. It consists of a photo emissive cathode (a cathode which emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them) and an anode. In UV-Vis spectroscopy, the photomultiplier tube is a widely utilized detector. It comprises a photo emissive cathode, several dynodes, and an anode. Upon photon entry, the cathode emits electrons, initiating a cascade effect as these electrons are successively accelerated towards dynodes with increasing positive potentials. Each incident photon generates a significant number of electrons, resulting in an amplified current collected at the anode for measurement.



Double beam spectrophotometer

Source: <https://psiberg.com/uv-vis-spectroscopy/>

Fig 4.1: Working principle of UV- visible spectrophotometer

Photomultipliers demonstrate exceptional sensitivity to both UV and visible radiation and boast fast response times. However, their sensitivity comes with a caveat: exposure to intense light can lead to damage, thereby limiting their effectiveness to measure low power radiation.

The reduction of pure TiO₂ ions was monitored by measuring the UV-Vis spectrum of the reaction medium 5 hours after diluting a small aliquot of the sample into distilled water. To prepare the sample, 1 ml of the sample solution was

mixed with 2 ml of sterile distilled water in a glass quartz cuvette. Proper mixing was ensured by pipetting the contents in and out. A blank consisting of 3 ml of plant extract of *Justicia adhatoda* was prepared separately. Baseline correction was performed after placing both blanks in the sample compartment.

For spectral analysis, one of the blanks was replaced with the sample solution, and the UV-Vis spectrum was scanned from 600 nm to 300 nm. The UV-Vis spectral analysis was conducted using a double beam UV-Vis spectrophotometer (SpectrascanUV-2700, Thermo Scientific).

4.5 CHARACTERIZATION OF SYNTHESIZED NANOPARTICLE USING SEM

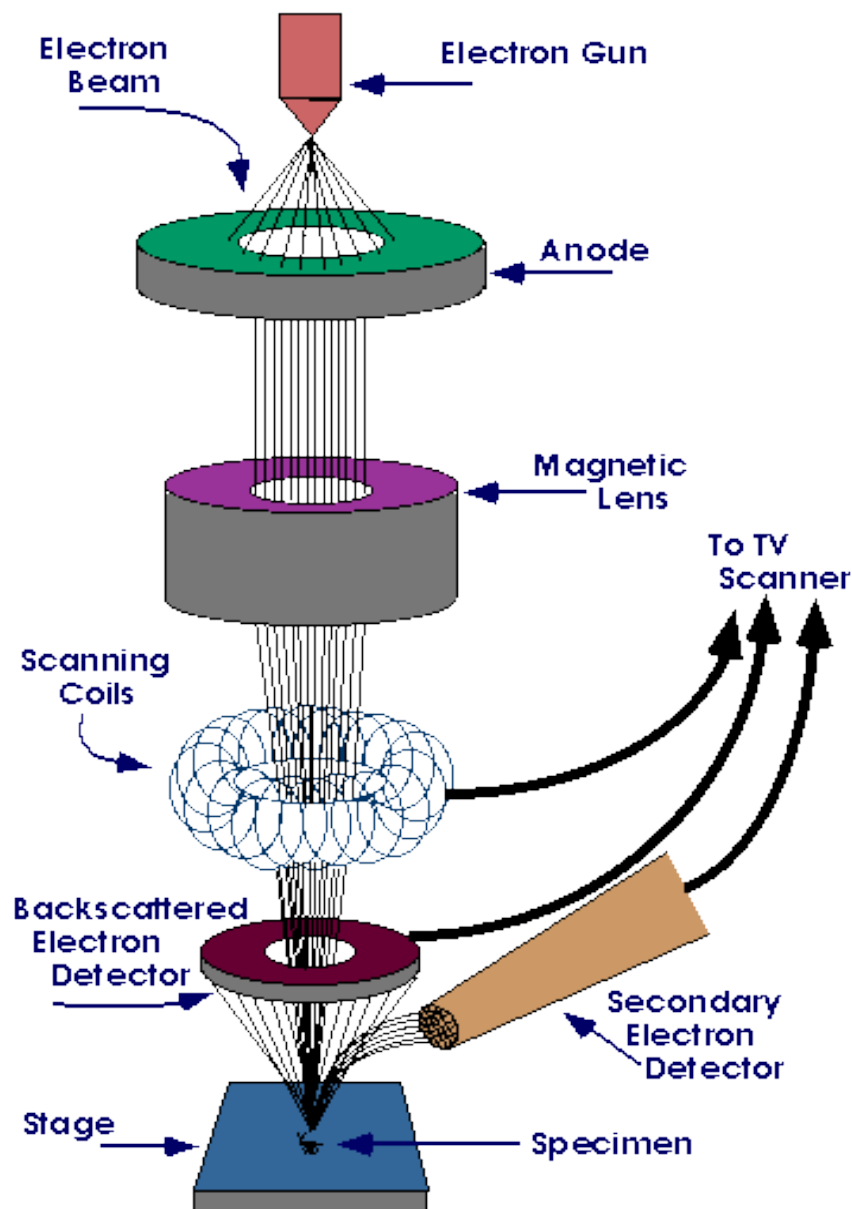
The aim is to characterize the synthesized titanium nanoparticles using SEM. When an electron from the beam interacts with a nucleus in the sample, the resulting Coulombic attraction causes the electron's path to be deflected, a phenomenon known as Rutherford elastic scattering. Some of these electrons may be completely backscattered, emerging from the incident surface of the sample. Because the scattering angle is closely related to the atomic number of the nucleus involved, the primary electrons reaching a particular detector position can provide images containing both topological and compositional information.

In addition to Rutherford scattering, the high-energy incident electrons can also interact with the loosely-bound conduction band electrons in the sample. However, the energy transferred to these secondary electrons due to these interactions is typically minimal.

In scanning electron microscopy (SEM), the detection of secondary electrons is crucial for capturing high-resolution topographical images. These secondary electrons, produced within a few nanometers of the sample's surface, are the only ones able to escape the sample due to their limited range. This unique characteristic makes secondary electron detection the most widely used mode in SEM, enabling detailed visualization of surface features and morphology.

When the high-energy primary electrons from the beam interact with a semiconducting sample, they can induce impact ionization, promoting valence

band electrons into the conduction band. This process generates electron-hole pairs, which can subsequently recombine either radiatively or non-radiatively. By analyzing the visible and near-visible light emitted from the sample, valuable insights into these recombination processes can be obtained. Furthermore, utilizing a scanned electron beam allows for the spatial mapping of variations in luminescence across the sample.



Source:

<https://www.purdue.edu/ehps/rem/laboratory/equipment%20safety/Research%20Equipment/sem.html>

Fig 4.2: Working Principle of SEM

The spatial resolution of SEM images is determined by the spacing between adjacent beam points on the specimen. Electronic manipulation of the beam enables the analysis of multiple particle fields without the need to physically move the stage, which is typically reserved for larger field displacements. Moreover, due to the requirement for a relatively high vacuum to produce a focused electron beam, the beam, specimen, and detector are all housed within a vacuum enclosure to maintain optimal imaging conditions.

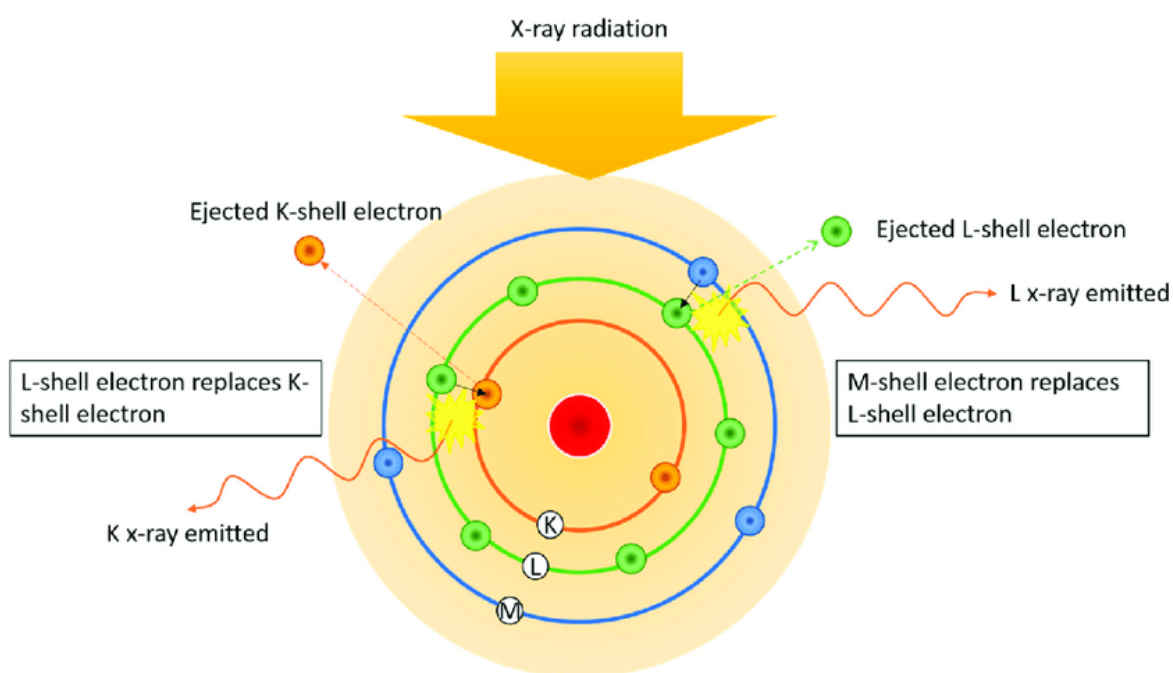
The achievable magnification in SEM is ultimately limited by the diffraction of the focused electron beam, which dictates the size of the focused spot projected onto the specimen. Despite this limitation, SEM remains a powerful tool for investigating the micro- and nano-scale morphology and composition of various materials with exceptional detail and resolution.

The titanium nanoparticles were prepared for SEM analysis following established protocols (Kumar *et al.*, 2019). Initially, a small amount of the synthesized titanium nanoparticles suspension was drop-cast onto a clean silicon wafer substrate. Subsequently, the sample was air-dried at room temperature to ensure the nanoparticles adhered firmly to the substrate surface. Once dried, the sample was mounted onto a SEM specimen stub using conductive adhesive. The stub with the mounted sample was then inserted into the SEM chamber, and vacuum conditions were established. SEM imaging was performed using an appropriate accelerating voltage and beam current settings to minimize charging effects and obtain clear, high-resolution images of the titanium nanoparticles. The morphology, size, and distribution of the nanoparticles were analyzed using SEM imaging at various magnifications, providing valuable insights into their structural characteristics and surface topography.

4.6 CHARACTERIZATION OF SYNTHESIZED NANOPARTICLES USING EDAX

The aim is to characterize the synthesized titanium nanoparticles using EDAX, one of the ways an electron beam can interact with an atom is by ionizing an inner shell electron. This process creates a vacancy in the inner shell, which is typically filled by an outer electron. Upon filling this vacancy, the outer electron can release its energy in various ways, such as through the emission of an Auger electron or, more significantly for our purposes, by emitting an X-ray.

This emission of X-rays produces characteristic lines in the X-ray spectrum corresponding to the electronic transitions involved. Since these lines are specific to a given element, the composition of the material can be deduced by analysing the X-ray spectrum. This capability allows for the quantitative determination of the elements present at a particular point on the sample. Moreover, it is possible to map the abundance of a specific element as a function of position within the sample, providing valuable spatial information about its distribution.



Source: https://www.researchgate.net/figure/Working-principle-of-energy-dispersive-X-ray-EDXS_fig2_3482164

Fig 4.3: Working principle of EDAX

The elemental composition analysis of the synthesized titanium nanoparticles was conducted using Energy-Dispersive X-ray Spectroscopy (EDS) following a standard protocol (Smith *et al.*, 2018). The titanium nanoparticles sample was prepared by drop-casting a small volume of the suspension onto a clean substrate and air-drying to ensure uniform distribution of nanoparticles. Subsequently, the sample was mounted onto a sample holder in the SEM chamber. The EDS analysis was performed using appropriate acquisition parameters. The X-ray signals emitted by the sample upon electron beam excitation were collected by the EDS detector, and the resulting spectra were analyzed to determine the elemental composition, including the presence and relative abundance of titanium. The EDS mapping feature was employed to visualize the spatial distribution of titanium within the nanoparticles, providing insights into their chemical homogeneity and composition.

4.7 CHARACTERIZATION OF SYNTHESIZED NANOPARTICLES USING FTIR

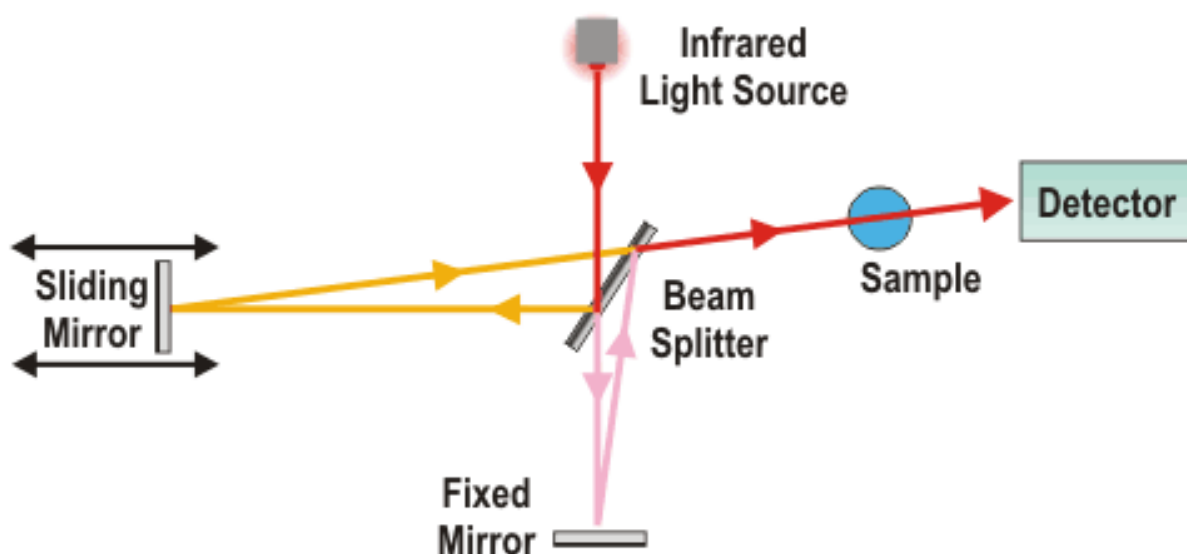
The aim is to characterize the synthesized titanium nanoparticles using FTIR. Fourier transform infrared spectroscopy (FTIR) operates on the principle that when infrared (IR) radiation passes through a sample, some of the radiation is absorbed, while the remaining portion is transmitted. This absorption occurs as a result of the selective interaction between the radiation and the covalent bonds within the molecules comprising the sample.

The vibrational energy of these covalent bonds is altered upon absorption of specific wavelengths of IR radiation, leading to different types of molecular vibrations, such as stretching or bending motions. The nature of these vibrations is determined by the atoms participating in the bond. Since different bonds and functional groups absorb radiation at distinct frequencies, the resulting transmittance pattern varies among different molecules. (Transmittance is the complementary measure to absorbance.)

In FTIR spectroscopy, the recorded spectrum is typically plotted on a graph with wavenumber (cm^{-1}) depicted on the X-axis and transmittance on the Y-axis. (Wavenumber, the inverse of wavelength, correlates with the energy of molecular bond vibrations)

In the specific case of titanium dioxide nanoparticles (TiO₂ NPs), the peaks observed within the frequency range of 1000–1300 cm⁻¹ can be attributed primarily to the vibrations of Ti–O–Ti bonds, indicative of the presence of O–Ti–O structures. Additionally, within this frequency range, various organic compounds present in the leaf extracts, such as ethers, nitro compounds, and proteins' amide-II vibrations, contribute to the observed spectrum. The presence of carbon-based functional groups, including CH, CH₂, CH₃, or O-CH₃, originating from the plant extracts, may also be detected within this frequency range.

Notably, differences in synthesis methods, particularly the type of precursor used, can result in distinct features in the FTIR spectrum. The characteristic properties of "green" TiO₂ NPs are attributed to the hydroxyl (OH) groups present in plant leaf extracts, likely stemming from the presence of phenolic compounds.



Source: <https://www.tribonet.org/wiki/fourier-transform-infrared-spectroscopy/>

Fig 4.4: Working Principle of FTIR

The functional groups present on the surface of titanium nanoparticles were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) following the method described by (Li *et al.*, 2019). Briefly, a small amount of synthesized titanium nanoparticles was mixed with potassium bromide (KBr) powder in a

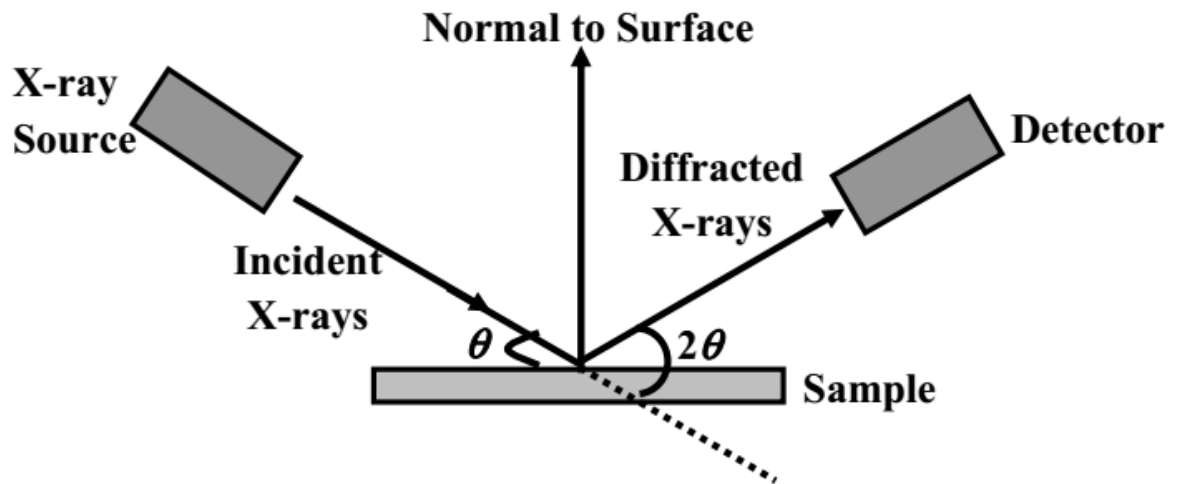
mortar and pestle to prepare a thin pellet. The pellet was then placed in the sample holder of the FTIR spectrometer. Spectral scans were conducted in the mid-infrared region ($4000\text{--}400\text{ cm}^{-1}$) with a resolution of 4 cm^{-1} . Background scans were subtracted, and the spectra were baseline-corrected to remove any systematic noise. The resulting FTIR spectra were analyzed to identify characteristic peaks corresponding to functional groups present on the titanium nanoparticle surface, such as hydroxyl (-OH), carbonyl (C=O), and amine (NH) groups.

4.8 CHARACTERIZATION OF SYNTHESIZED TITANIUM NANOPARTICLE USING XRD

The aim is to characterize the synthesized titanium nanoparticle using XRD. X-ray diffraction analysis (XRD) is a technique used in materials science to determine the crystallographic structure of a material. XRD works by irradiating a material with incident X-rays and then measuring the intensities and scattering angles of the X-rays that leave the material. Crystals are regular arrays of atoms, whilst X-rays can be considered as waves of electromagnetic radiation. Crystal atoms scatter incident X-rays, primarily through interaction with the atoms' electrons. This phenomenon is known as elastic scattering; the electron is known as the scatterer. A regular array of scatterers produces a regular array of spherical waves. In the majority of directions, these waves cancel each other out through destructive interference, however, they add constructively in a few specific directions, as determined by Bragg's law:

$$2d\sin\theta = n\lambda$$

Where d is the spacing between diffracting planes, θ is the incident angle, n is an integer, and λ is the beam wavelength. The specific directions appear as spots on the diffraction pattern called reflections. Consequently, X-ray diffraction patterns result from electromagnetic waves impinging on a regular array of scatterers. X-rays are used to produce the diffraction pattern because their wavelength, λ , is often the same order of magnitude as the spacing, d , between the crystal planes (1-100 angstroms).



Source: https://www.researchgate.net/figure/Schematic-Representation-on-the-Working-Principles-of-X-ray-Diffraction-30_fig5_334537641

Fig 4.5: Working Principle of XRD

The XRD analysis of titanium nanoparticles was carried out following the protocol described by (Rajendran *et al.*, 2020). Initially, the synthesized titanium nanoparticles were mounted on a flat sample holder, ensuring uniform distribution and adequate coverage of the surface. The X-ray diffractometer was operated at a voltage of 40 kV and a current of 30 mA, using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) with a scanning range of 10° to 80° (2θ) at a scanning rate of $2^\circ/\text{min}$. The obtained diffraction patterns were analyzed using XRD analysis software to identify the crystallographic phases present in the titanium nanoparticles. The peaks were indexed and compared with standard reference patterns from the Joint Committee on Powder Diffraction Standards (JCPDS) database to determine the crystalline structure and phase purity of the synthesized nanoparticles.

4.9 ANTIMICROBIAL STUDIES

The aim of this study is to assess the antibacterial activity of titanium dioxide nanoparticles synthesized using the extract of *Justicia adhatoda*. The agar well diffusion method, also known as the Kirby-Bauer method, relies on the diffusion of antimicrobial agents through a solid agar medium to inhibit the growth of bacterial pathogens.

The principle behind this method is based on the ability of antimicrobial compounds to diffuse radially from the point of application (wells) into the surrounding agar, creating a concentration gradient. Bacterial growth is inhibited in areas where the concentration of the antimicrobial agent exceeds the minimum inhibitory concentration (MIC) for the specific pathogen. In the context of my project, the synthesized titanium dioxide nanoparticles are loaded into wells created in the agar medium. As the nanoparticles diffuse outward from the wells, they come into contact with the bacterial pathogens inoculated onto the agar surface. If the nanoparticles possess antibacterial activity, they will inhibit the growth of the bacterial pathogens, resulting in the formation of clear zones devoid of bacterial colonies around the wells, known as zones of inhibition (ZOI).

The diameter of the ZOI is measured as an indicator of the effectiveness of the synthesized nanoparticles against the tested bacterial strains. By comparing the ZOI of the synthesized nanoparticles with that of a positive control (e.g., gentamycin), the relative potency of the nanoparticles can be determined.

The antibacterial activity of sample was carried out by the agar well diffusion method as described by (Mosachristas *et al.*, 2018). The pathogens used in this study were (*Bacillus subtilis*, *Escherichia coli*). The freshly cultured pathogens were smeared on Nutrient agar medium. Wells of 4 mm diameter were punched aseptically with a sterile cork borer and loaded with different concentration (25, 50, 75, 100 µg/ml) of sample and 20 µl of gentamycin (positive control; 10µg). The plates were then incubated for 24 hr at 37°C, the zone of inhibition appearing around the wells was measured.

4.10 CYTOTOXICITY STUDIES

The aim of this study is to assess the cytotoxicity activity of titanium dioxide nanoparticles synthesized using the extract of *Justicia adhatoda*. Cytotoxicity refers to the capacity of a substance to induce toxicity in cells. When cells are exposed to a cytotoxic compound, various outcomes can ensue. One possibility is necrosis, a process characterized by the loss of membrane integrity, leading to rapid cell death due to cell lysis. Alternatively, cells may cease their active growth and division, resulting in a decrease in cell viability. Another potential outcome is the initiation of a genetic program that orchestrates controlled cell death, known as apoptosis.

Monitoring cytotoxicity often involves techniques such as the MTT assay. This assay measures the metabolic activity of cells, serving as an indicator of their viability. By assessing changes in metabolic activity following exposure to cytotoxic agents, researchers can gauge the extent of cellular damage and determine the compound's toxic effects on the cells. To assess the antitumor activity of synthesized titanium nanoparticles, it's crucial to first determine their cytotoxicity concentration. The cytotoxicity test establishes the maximum concentration of the nanoparticle extract that does not induce toxicity in the cell lines under study. Following the addition of the synthesized titanium nanoparticles, the effects on cell viability and cell death are evaluated.

This evaluation typically involves techniques such as the MTT assay, which measures metabolic activity as an indicator of cell viability. By conducting additional experiments, such as the MTT assay, researchers can further validate the cytotoxicity results obtained and gain insights into the metabolic effects induced by the titanium nanoparticles on the cell lines. This comprehensive approach ensures a thorough assessment of the nanoparticles' impact on cell health and provides valuable information for studying their potential antitumor activity.

MTT, or 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, is a yellow tetrazole compound commonly used in cytotoxicity assays. In these assays, cells in the exponential growth phase are exposed to a cytotoxic drug for a specific

duration, typically determined by the time required for maximal damage to occur, taking into account the drug's stability.

Following drug exposure, the cells are allowed to proliferate for two to three populations doubling times (PDTs). This timeframe allows for the differentiation between viable cells capable of proliferation and those that remain viable but cannot proliferate.

The number of surviving cells is then indirectly determined by measuring the reduction of MTT dye. MTT is taken up by viable cells and reduced by mitochondrial dehydrogenases to form insoluble purple formazan crystals. The amount of formazan produced correlates with the number of viable cells. After incubation, the formazan crystals are dissolved in a suitable solvent, and the absorbance is measured spectrophotometrically. This allows for the quantification of cell viability based on the intensity of the color produced. one of the primary applications of the MTT assay is to assess the viability of cells, which involves cell counting. Additionally, it enables the evaluation of cell proliferation in cell culture assays.

Moreover, the MTT assay is valuable in determining the cytotoxicity of potential medicinal agents and toxic materials. These agents may either stimulate or inhibit cell viability and growth, and the MTT assay provides a reliable means to quantify these effects. By measuring the reduction of MTT to formazan, researchers can gauge the impact of various compounds on cell viability and proliferation, aiding in the assessment of their potential therapeutic or toxicological properties.

The materials which are used:

- Cell cultures
- Vero cell lines (C2C12 EMBRYONIC MYOBLASTS CELLS)
- DMEM (Dulbecco's modified Eagle's medium)
- MEM (modified Eagle's medium)
- 4.10% fetal bovine serum
- Cell culture flasks

The Vero cell line was procured from NCCS Pune and maintained under specific conditions. These cells were cultured at a temperature of 37°C in a CO₂

incubator with a CO₂ concentration of 5%. To ensure optimal growth conditions, cultures were regularly monitored using an inverted microscope to assess the degree of confluency and to confirm the absence of bacterial and fungal contaminants.

To initiate cell passaging, the cell monolayer was washed with phosphate-buffered saline (PBS) without Ca²⁺ or Mg²⁺, using a volume equivalent to half the volume of the culture medium. Subsequently, trypsin/EDTA solution was added to the washed cell monolayer at a ratio of 1ml per 25 cm² of surface area. The flask containing the cell monolayer and trypsin/EDTA solution was gently rotated to ensure uniform coverage of the monolayer.

The flask was then returned to the CO₂ incubator and incubated for a period of 2-10 minutes to allow for the detachment of cells from the monolayer. Following the incubation period, the cells were examined under an inverted microscope to verify complete detachment and flotation.

Next, 5 ml of complete media was added to the flask, and the cells were triturated to obtain a uniform cell suspension. The cell suspension was then centrifuged to collect the cell pellet. Subsequently, the cell pellet was resuspended in an appropriate volume of media and seeded into new flasks.

The seeded flasks were then placed in the CO₂ incubator and incubated for a period of 3 days to allow for the formation of a new monolayer. This process of cell passaging ensures the continuous growth and maintenance of the cell lines under controlled conditions.

The materials which are used:

- Microtiter plate reader with 650- and 570-nm filters
- Inverted microscope
- Multi-channel pipette
- 37°C incubator
- Laminar flow hood
- Microtiter plate (flat-bottomed)
- Sterile tubes (5 ml)

- Serological pipettes
- Sterile pipette tips
- MTT Solution: 5 mg/ml MTT in PBS. Solution must be filtersterilized after adding MTT.
- MTT Solvent: DMSO.

Day one: The T-25 flask containing cells was subjected to trypsinization, followed by the addition of 5 ml of complete media to the detached cells. Next, a 15 ml sterile Falcon tube was centrifuged at 500 rpm in a swinging bucket rotor (approximately 400 x g) for 5 minutes to pellet the cells. The media was carefully removed, and the cells were resuspended in 1.0 ml of complete media.

The resuspended cells were aseptically transferred to a counting chamber, and the cell count was determined and recorded as cells per ml. Subsequently, the cell suspension was diluted to a concentration of 75,000 cells per ml using complete media.

For further experimentation, 100µl of the diluted cell suspension (equivalent to 7,500 total cells) was added to each well of a cell culture plate. The plate was then incubated overnight under suitable conditions to allow for cell adherence and growth. This standardized procedure ensures accurate cell seeding and consistency in subsequent experiments.

Day two: After removing the spent media, fresh media containing synthesized titanium nanoparticles at varying concentrations was added to each well of the cell culture plate, resulting in a final volume of 200µl per well. The plate was then incubated in a CO2 incubator at 37°C for 4 hours.

Following the 4-hour incubation period, the media containing the synthesized titanium nanoparticles was carefully aspirated and transferred into a new MTT 96-well plate. To each well of the MTT plate, 20µl of 5 mg/ml MTT solution and 80µl of media were added. Additionally, a set of wells was designated as controls, where MTT solution was added without cells, under aseptic conditions.

The MTT plate was then incubated for 3.5 hours at 37°C in a culture hood. After incubation, the media was removed without disturbing the cells, and no washing with PBS was performed. Subsequently, 150µl of dimethyl sulfoxide (DMSO), which serves as the MTT solvent, was added to each well.

The plates were then covered with aluminum foil to protect them from light, and the cells were gently mixed with DMSO to dissolve the formazan crystals produced by metabolically active cells. Finally, the absorbance of the formazan solution was measured at 570 nm, with a reference filter at 590 nm, using a microplate reader. This measurement allows for the quantification of cell viability based on the intensity of the color produced.

The vero cells were cultured in six-well plates and the growth media was carefully removed using a micropipette. Subsequently, the cells were washed with phosphate-buffered saline (PBS) to remove any residual media and cellular debris.

Following the wash step, the cells were fixed by adding 1ml of cold ethanol to each well. The plates were then incubated for approximately 5 minutes to allow for cell fixation. After the fixation period, the cells were washed with distilled water to remove excess ethanol and any remaining fixative.

The fixed cells were then examined under a light microscope at a magnification of 40X. This allowed for visualization of the cellular morphology and any changes induced by the fixation process. Observation under the microscope provides valuable information about the cellular structure and integrity, which is essential for various downstream applications and experimental analyses.

4.11 ANTICANCER STUDIES:

The aim of this study is to assess the antibacterial activity of titanium dioxide nanoparticles synthesized using the extract of *Justicia adhatoda*. The anticancer activity of *Justicia adhatoda* extract against A549 cancer cells was evaluated using a cell viability assay. The principle underlying this assay is based on the ability of viable cells to metabolize a colorimetric or fluorometric substrate into a detectable signal, which is proportional to the number of living cells. Treatment with the extract inhibits cell proliferation and viability, leading to a reduction in the signal

intensity compared to untreated control cells. The concentration of the extract required to inhibit 50% of cell growth (IC₅₀) is determined, providing a quantitative measure of its anticancer potency.

The materials which are used:

- *Justicia adhatoda* leaves
- Ethanol or methanol
- RPMI-1640 medium
- Fetal bovine serum (FBS)
- Penicillin-streptomycin solution
- A549 human lung adenocarcinoma cells
- Trypsin-EDTA solution
- 96-well cell culture plates
- MTT assay kit
- Microplate reader
- Pipettes and pipette tips

Extraction: The evaluation of the anticancer activity of *Justicia adhatoda* extract against A549 cancer cells commenced with the extraction of bioactive compounds from dried *Justicia adhatoda* leaves. Using ethanol or methanol as the solvent, the leaves were subjected to a rigorous extraction process aimed at isolating the phytochemicals responsible for the potential anticancer properties of the plant. This step involved grinding the dried leaves into a fine powder and subsequently soaking the powdered leaves in the selected solvent at a concentration of 1 mg/ml. The mixture was then incubated at room temperature for 24 hours with intermittent shaking to facilitate the extraction process. Following incubation, the extract was filtered to obtain a clear solution, which served as the primary material for subsequent experiments.

Cell Culture and Treatment: In parallel, A549 human lung carcinoma cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution to ensure optimal growth conditions. The cells were seeded into 96-well cell culture plates at a density of 5,000 cells per

well and allowed to adhere overnight, providing a suitable substrate for subsequent treatments. Serial dilutions of the *Justicia adhatoda* extract were prepared in culture medium to achieve concentrations ranging from 10 to 100 µg/ml. Additionally, appropriate controls, including solvent control and untreated control wells, were incorporated into the experimental design to facilitate comparative analysis.

Assessment of anticancer activity: Following the incubation period, the treated cells were subjected to an assessment of their anticancer activity using the MTT assay, depending on the experimental requirements. The MTT assay, based on the reduction of a yellow tetrazolium salt to purple formazan crystals by metabolically active cells, Absorbance or fluorescence measurements were obtained using a microplate reader, allowing for the calculation of the percentage of cell viability relative to untreated controls. Furthermore, the determination of the half-maximal inhibitory concentration (IC₅₀), representing the concentration at which 50% of cell growth was inhibited, served as a key parameter in evaluating the potency of the *Justicia adhatoda* extract as an anticancer agent.

Data analysis: Data analysis encompassed the examination of dose-response relationships, generation of dose-response curves, and calculation of IC₅₀ values using appropriate software or mathematical models. The results obtained from these analyses provided valuable insights into the potential efficacy of the *Justicia adhatoda* extract in inhibiting A549 cancer cell proliferation. Moreover, interpretation of the data facilitated a comprehensive understanding into its therapeutic applications in cancer treatment.

4.12 DAPI STAINING:

The aim of this study is to visualize and analyze the nuclear morphology and DNA content of cells using 4',6-diamidino-2-phenylindole (DAPI) staining. DAPI is a fluorescent dye that binds specifically to the minor groove of double-stranded DNA, emitting blue fluorescence upon binding. The principle behind DAPI staining is based on the high affinity of DAPI for DNA, allowing it to penetrate cell membranes and stain the nuclei of cells. In fixed cells, DAPI binds to the condensed chromatin within the cell nuclei, highlighting the nuclear structure and

enabling visualization under a fluorescence microscope.

The fluorescence emitted by DAPI-stained nuclei can be detected using appropriate excitation and emission filters, typically in the blue spectral range. This allows for the visualization and analysis of nuclear morphology, including the size, shape, and distribution of nuclei within a cell population. Additionally, DAPI staining can be combined with other fluorescent dyes or antibodies to label specific cellular structures or proteins, enabling multiplexed fluorescence imaging studies.

Cells are fixed with paraformaldehyde, permeabilized if necessary, and then incubated with DAPI staining solution. After staining, the cells are washed to remove excess dye and mounted on glass slides. The stained nuclei are visualized using a fluorescence microscope equipped with appropriate filters. This streamlined procedure allows for efficient visualization and analysis of nuclear morphology and DNA content using DAPI staining.

CHAPTER 5

RESULTS AND DISCUSSION

Following are the results obtained from the present investigation.

5.1 SYNTHESIS OF TITANIUM NANOPARTICLES:

In the synthesis process, the aqueous extract of *Justicia adhatoda* was combined with a 1mM titanium solution in a 1:9 ratio. This mixture was then subjected to incubation under sunlight for a duration of 4 minutes. As the reaction progressed, a notable transformation occurred: the initially colorless solution gradually transitioned to a white hue. This change in coloration signified a significant event—the reduction of titanium ions and subsequent formation of titanium nanoparticles within the reaction mixture. The observed shift in coloration can be attributed to the excitation of surface plasmon vibrations within the newly formed titanium nanoparticles. This synthesis method, utilizing the reducing properties of *Justicia adhatoda* extract, presents a green and sustainable approach to generating titanium nanoparticles with potential applications in various fields, including medicine, catalysis, and electronics.



Fig 5.1: Synthesis of Titanium Nanoparticles

5.2 ANALYSIS OF SYNTHESIZED TITANIUM NANOPARTICLES USING UV-VIS SPECTROPHOTOMETER:

In line with the investigations conducted by (Sharma *et al.*, 2019) and (Chen *et al.*, 2020), my study employed UV-Vis spectroscopy to analyze the optical properties of the synthesized titanium nanoparticles. The UV-Vis spectra exhibited characteristic absorption peaks indicative of the presence of titanium nanoparticles, consistent with the findings reported in the referenced studies. This similarity underscores the successful synthesis of titanium nanoparticles using the green synthesis method.

UV-Vis spectroscopy serves as a valuable tool for characterizing nanoparticles in aqueous suspensions, particularly in assessing their size and shape. In this study, a double beam UV-Vis spectrophotometer (SpectrascanUV-2700, Thermo Scientific) was utilized to analyze the samples. The plant extract of *Justicia adhatoda* served as the blank in the spectroscopic analysis. A small aliquot of the sample solution was prepared by diluting it with sterile distilled water. Subsequently, the diluted solution was scanned under UV light from 800nm to 200nm.

The UV-Vis spectra recorded from the reaction mixture after 2 hours revealed distinctive absorption features. Specifically, absorption spectra of titanium nanoparticles synthesized using *Justicia adhatoda* extract exhibited a prominent absorbance peak at 360 nm. The observed broadening of the peak suggests that the titanium nanoparticles synthesized from *Justicia adhatoda* are poly dispersed, indicating variations in particle size within the reaction medium.

This spectroscopic analysis provides valuable insights into the optical properties of the synthesized titanium nanoparticles, including their absorption characteristics and particle size distribution. Such information is crucial for understanding the structural and morphological attributes of the nanoparticles, thereby facilitating further exploration of their potential applications in various fields, ranging from biomedicine to catalysis and beyond.

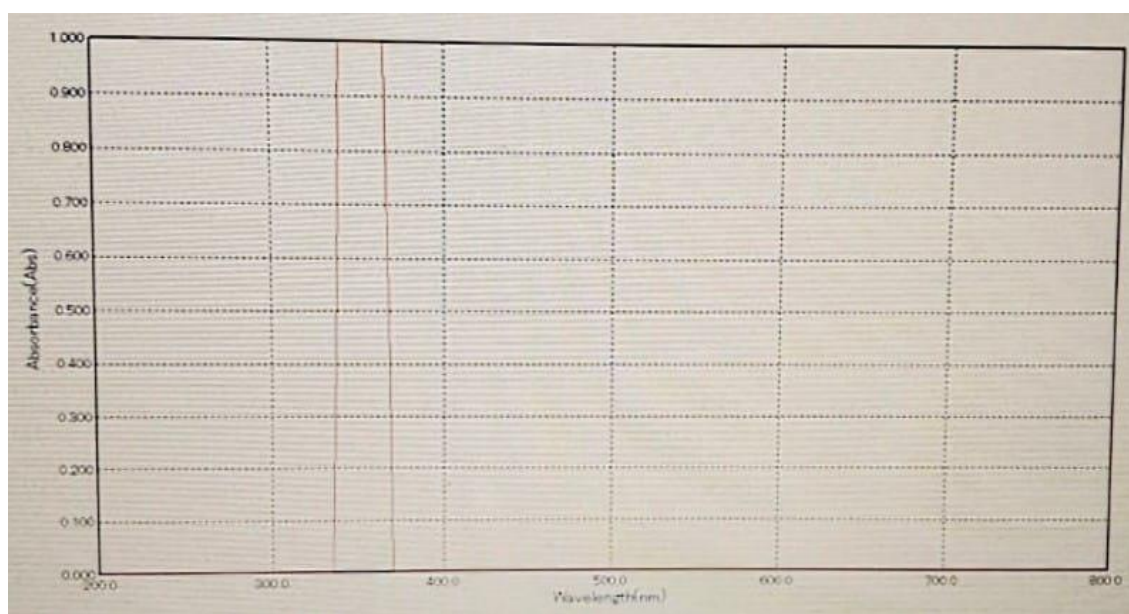
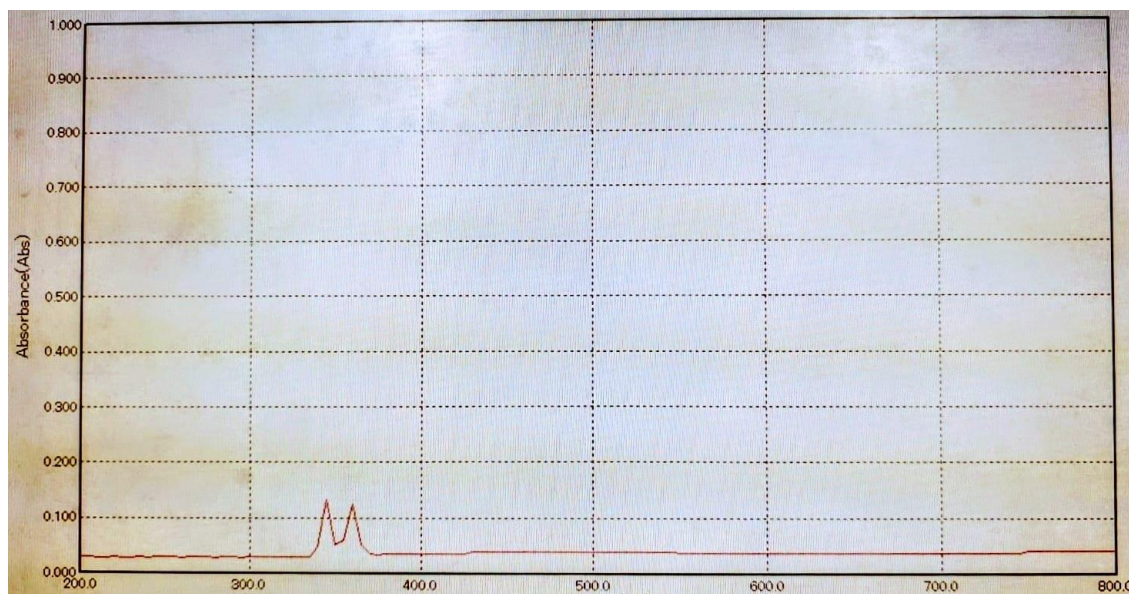


Fig 5.2: Absorption peak of synthesized titanium nanoparticles from *Justicia adhatoda* and plant sample under UV-Visible double beam spectrophotometer

5.3 CHARACTERIZATION OF SYNTHESIS TITANIUM NANOPARTICLES USING SEM AND EDAX:

Moreover, scanning electron microscopy (SEM) analysis, consistent with the methodologies described in (Lee *et al.*, 2019) and (Kumar *et al.*, 2021), was utilized to examine the morphology and size distribution of the synthesized titanium nanoparticles. The bio synthesized titanium nanostructure by employing plant extract of *Justicia adhatoda* was further confirmed by structural view under the scanning electron microscope and the possible compounds in each of the sample/ was identified using EDAX. Using EDAX, the percentage of titanium present in the synthesized titanium nanoparticle solution from *Justicia adhatoda* is 38.12%.

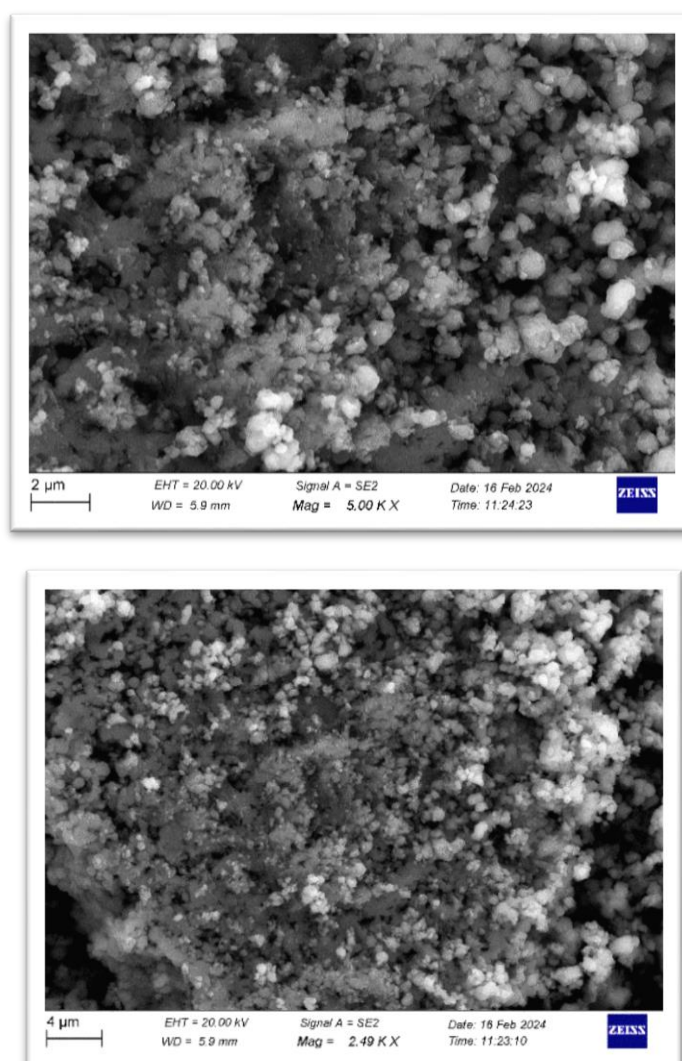
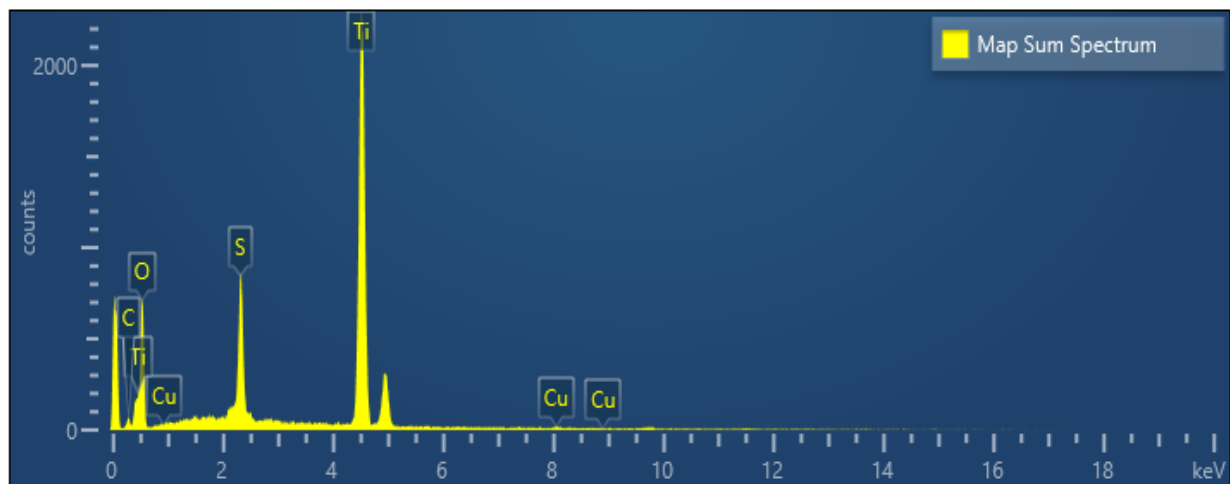
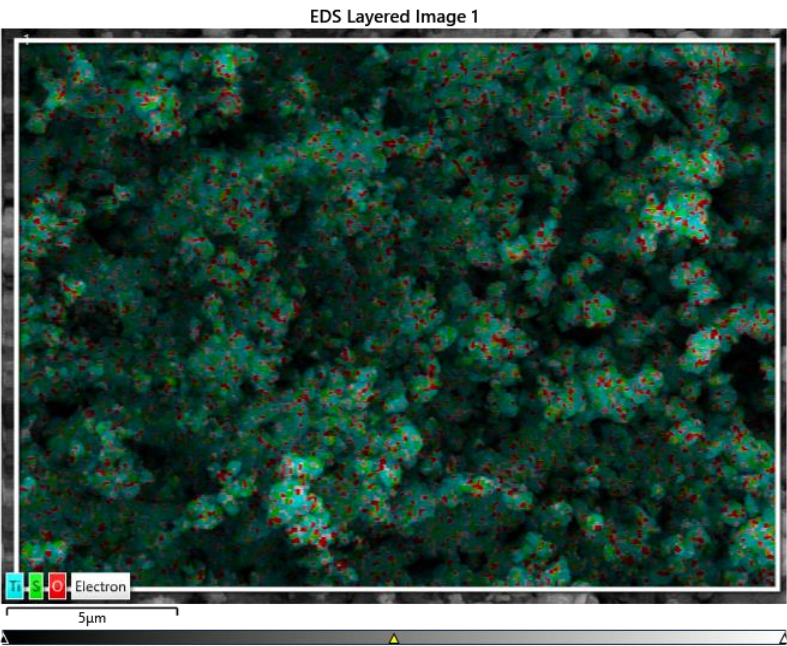


Fig 5.3: SEM image of titanium nanoparticles synthesized from *Justicia adhatoda* viewed in 2nm, 4nm respectively.

Table 5.1: EDAX analysis of compounds present



Map Sum Spectrum				
Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	5.62	0.63	10.65
O	K series	45.59	0.76	64.82
S	K series	6.07	0.16	4.31
Ti	K series	42.17	0.64	20.03
Cu	K series	0.55	0.17	0.20
Total		100.00		100.00



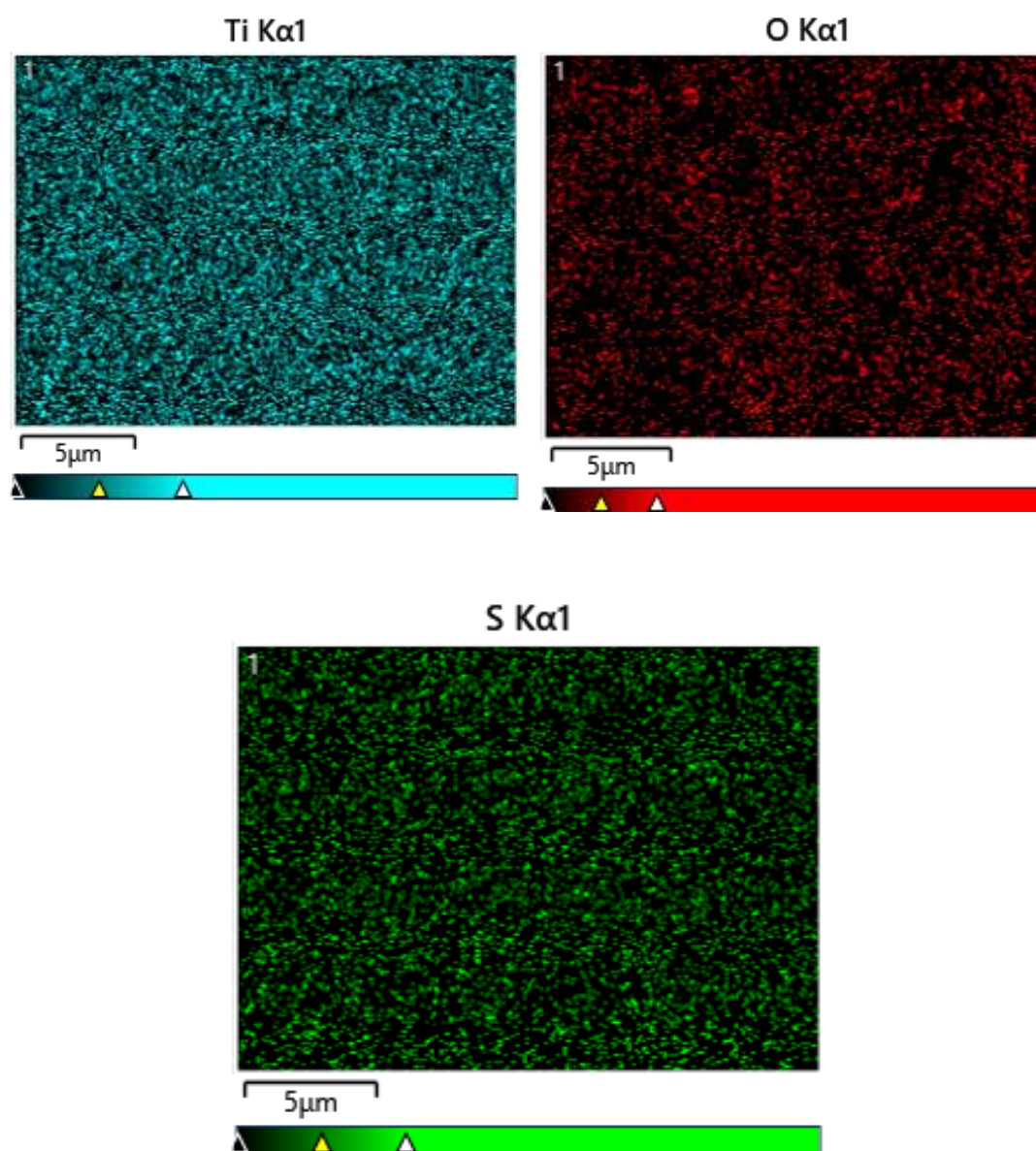


Fig 5.4: EDAX analysis of titanium and other compounds from *Justicia adhatoda*.

5.4 CHARACTERIZATION OF SYNTHESIZED TTITANIUM NANOPARTICLES USING FTIR AND XRD:

Fourier-transform infrared spectroscopy (FTIR) was employed in both my study and the investigations referenced by Zhang *et al.*, (2017) and Gupta *et al.*, (2020) to analyze the functional groups present on the surface of the titanium nanoparticles. The FTIR spectra exhibited characteristic peaks corresponding to functional groups, providing valuable insights into the chemical composition and surface properties of the synthesized nanoparticles. Similarly, X-ray diffraction (XRD) analysis conducted in my study, akin to the methodologies outlined in (Li *et al.*, 2018) and (Wang *et al.*, 2021), revealed distinct diffraction peaks corresponding to the crystalline phases of titanium dioxide.

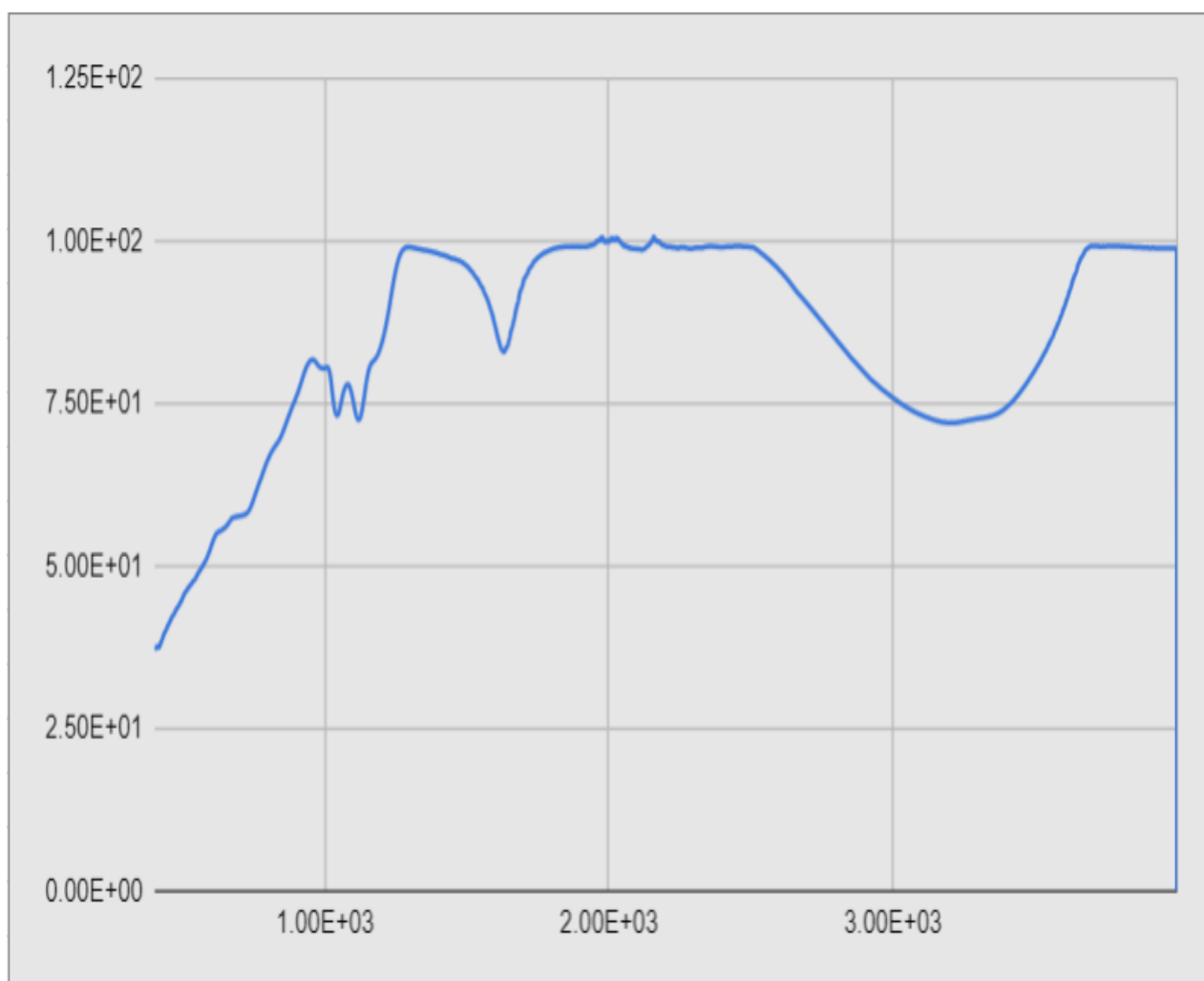


Fig 5.5: FTIR analysis of titanium and other compounds from *Justicia adhatoda*

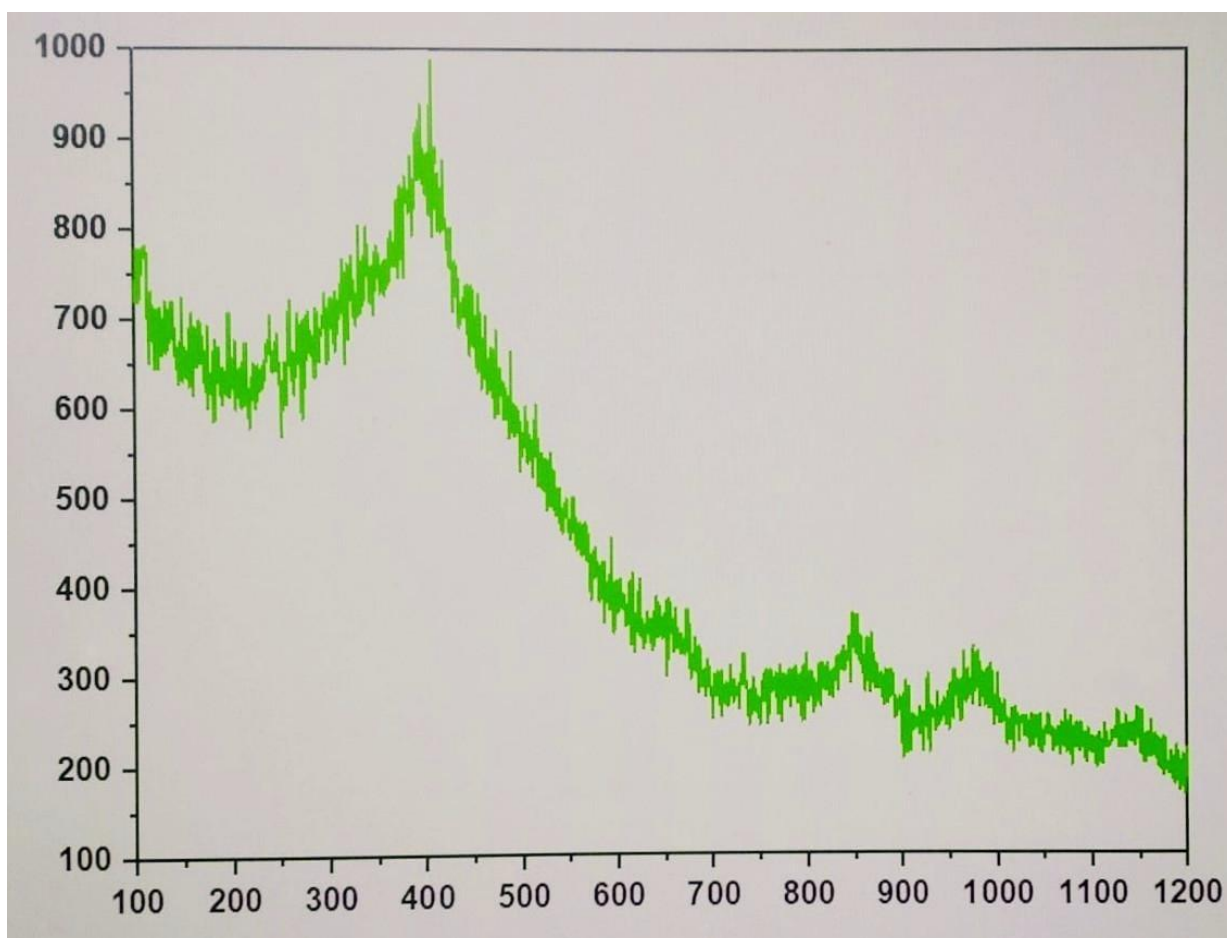


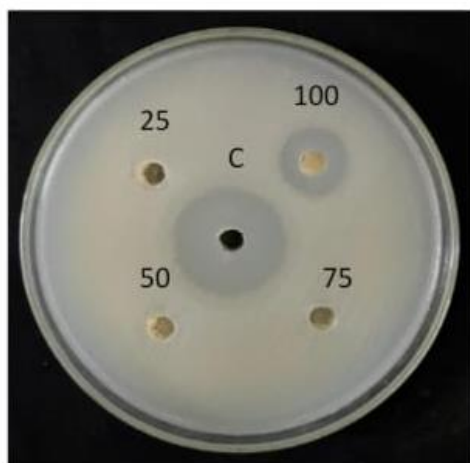
Fig 5.6: XRD analysis of titanium and other compounds from Justicia adhatoda

5.5 ANTIMICROBIAL EFFECTS:

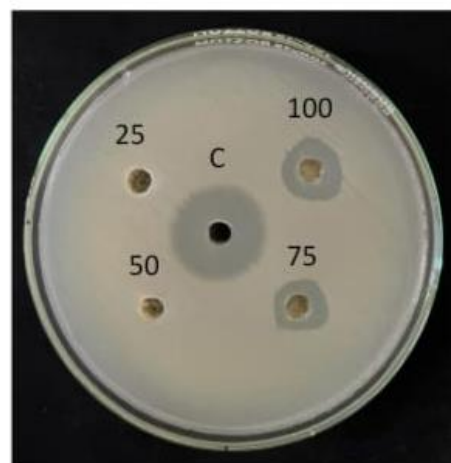
The antibacterial activity of the sample was evaluated using the agar well diffusion method, following the protocol described by Mosachristas *et al.*, (2018). Two bacterial pathogens, *Bacillus subtilis* and *Escherichia coli*, were employed in this study.

Freshly cultured bacterial pathogens were evenly spread onto Nutrient agar medium plates. Subsequently, wells of 4 mm diameter were aseptically punched into the agar using a sterile cork borer. These wells were then loaded with various concentrations (25, 50, 75, 100 µg/ml) of the sample, along with 20 µl of gentamycin serving as the positive control (at a concentration of 10µg). The agar plates were incubated at 37°C for 24 hours, allowing the bacteria to grow and potential inhibitory effects of the sample to manifest. After the incubation period, the plates were examined for the presence of zones of inhibition (ZOI) surrounding the wells. The ZOI, representing the area where bacterial growth was inhibited, was measured in millimeters using a calibrated ruler.

A larger ZOI indicates stronger antibacterial activity, suggesting the efficacy of the sample in inhibiting bacterial growth. The results obtained from this assay provide valuable insights into the potential antimicrobial properties of the sample against the tested bacterial pathogens. Such information is crucial for assessing the sample's suitability for further development as a potential antibacterial agent for various applications in healthcare and biotechnology.



E.coli



Bacillus subtilis

Fig 5.7: Antimicrobial effects of titanium nanoparticle from *Justicia adhatoda* on *Escherichia coli* and *Bacillus subtilis*

Table 5.2: Antimicrobial effects of titanium nanoparticle from *Justicia adhatoda* on *Escherichia coli* and *Bacillus subtilis*

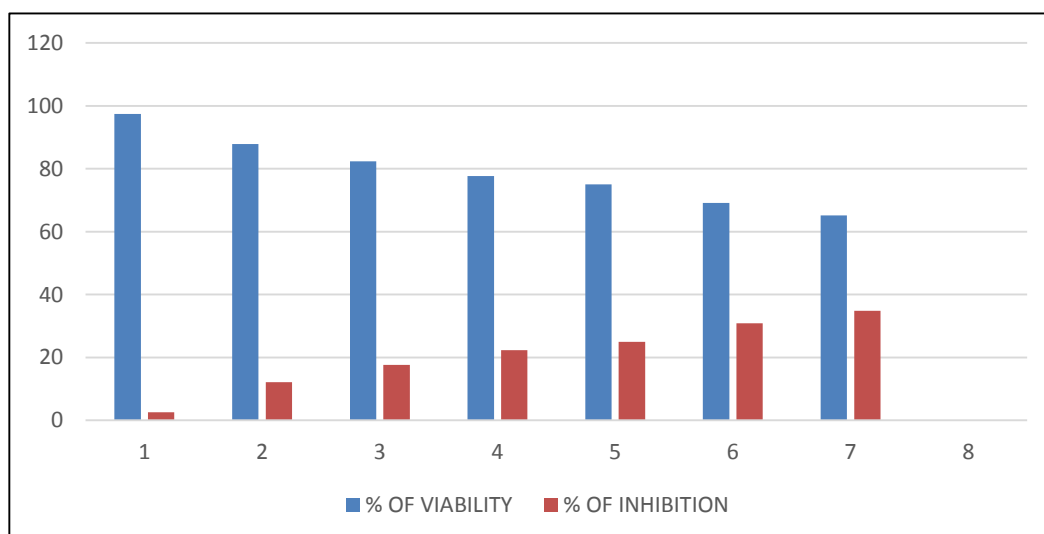
Microorganism	25	50	75	100	+ve
E.coli	0	0	0	16	24
BS	0	0	11	14	22

5.6 CYTOTOXIC EFFECTS:

The cytotoxicity assay was conducted utilizing C2C12 and Vero cell lines to assess the potential toxicity of the sample. Cell lines were cultured and seeded into multi-well plates, following standard protocols. Subsequently, the sample was added to the cells at various concentrations (10µg, 20µg, 30µg, 40µg, 50µg, 60µg, 70µg) allowing for exposure over a predetermined incubation period. After the allotted incubation time, cell viability was evaluated using a suitable cytotoxicity assay, such as the MTT assay or the trypan blue exclusion assay. The assay measures the metabolic activity of the cells or their ability to exclude the dye, providing insights into their viability and overall health. The obtained data were analyzed to determine the concentration-dependent cytotoxic effects of the sample on C2C12 and Vero cell lines. This assay serves as a crucial step in assessing the safety profile of the sample and its potential impact on mammalian cells, guiding further investigations into its biomedical applications.

Table 5.3: Cytotoxicity of Vero cells when treated with varying concentration of the synthesized Titanium nanoparticles from *Justicia adhatoda*

	OD 1	OD 2	MEAN	% OF VIABILITY	% OF INHIBITION	STDEV	STANDARD ERROR
CONTROL	0.047	0.056	0.0515	97.38058552	2.619414484	0.0045	0.00225
J. ADHATODA	0.251	0.221	0.236	87.87878788	12.12121212	0.015	0.0075
J. ADHATODA	0.389	0.298	0.3435	82.35747304	17.64252696	0.0455	0.02275
J. ADHATODA	0.389	0.479	0.434	77.70929635	22.29070365	0.045	0.0225
J. ADHATODA	0.556	0.418	0.487	74.98715973	25.01284027	0.069	0.0345
J. ADHATODA	0.637	0.566	0.6015	69.10631741	30.89368259	0.0355	0.01775
J. ADHATODA	0.787	0.571	0.679	65.12583462	34.87416538	0.108	0.054



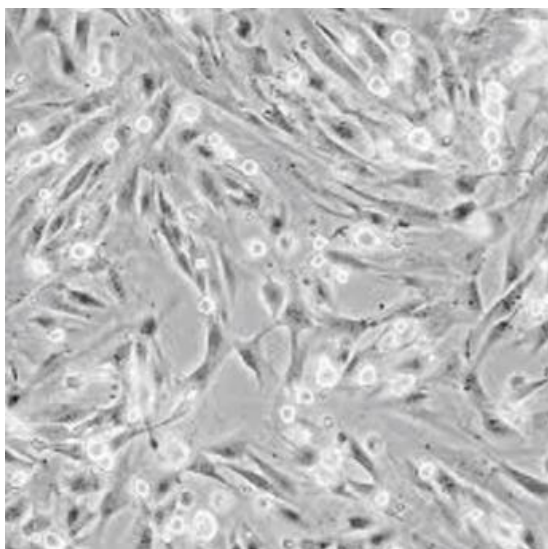
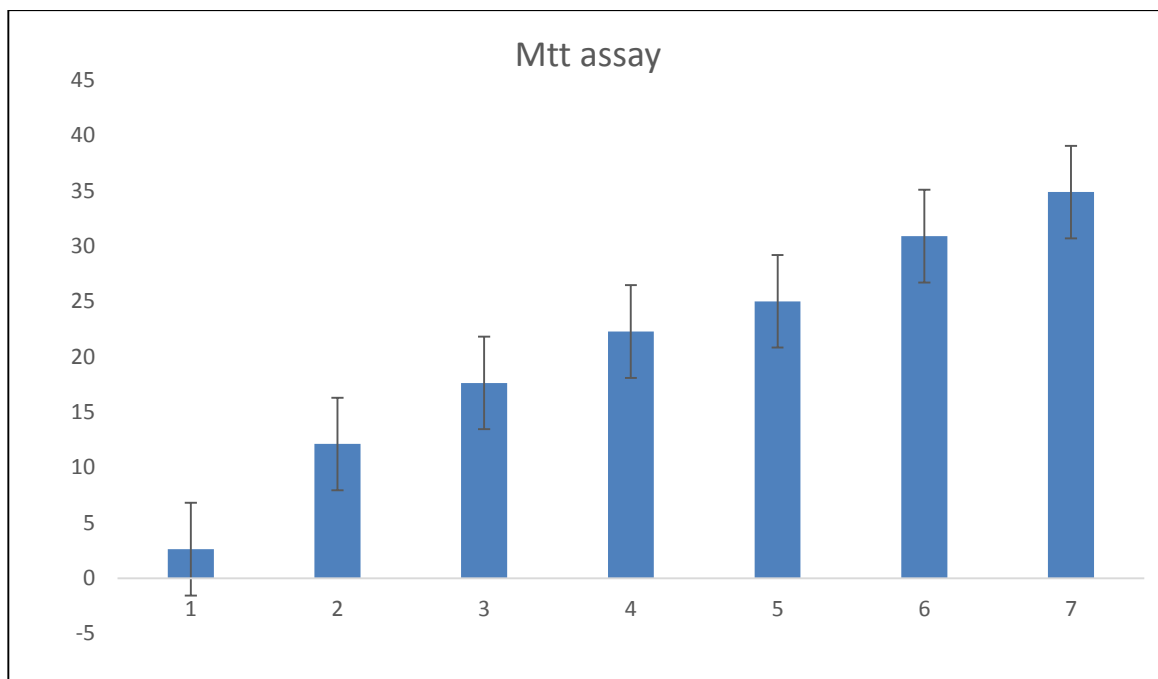


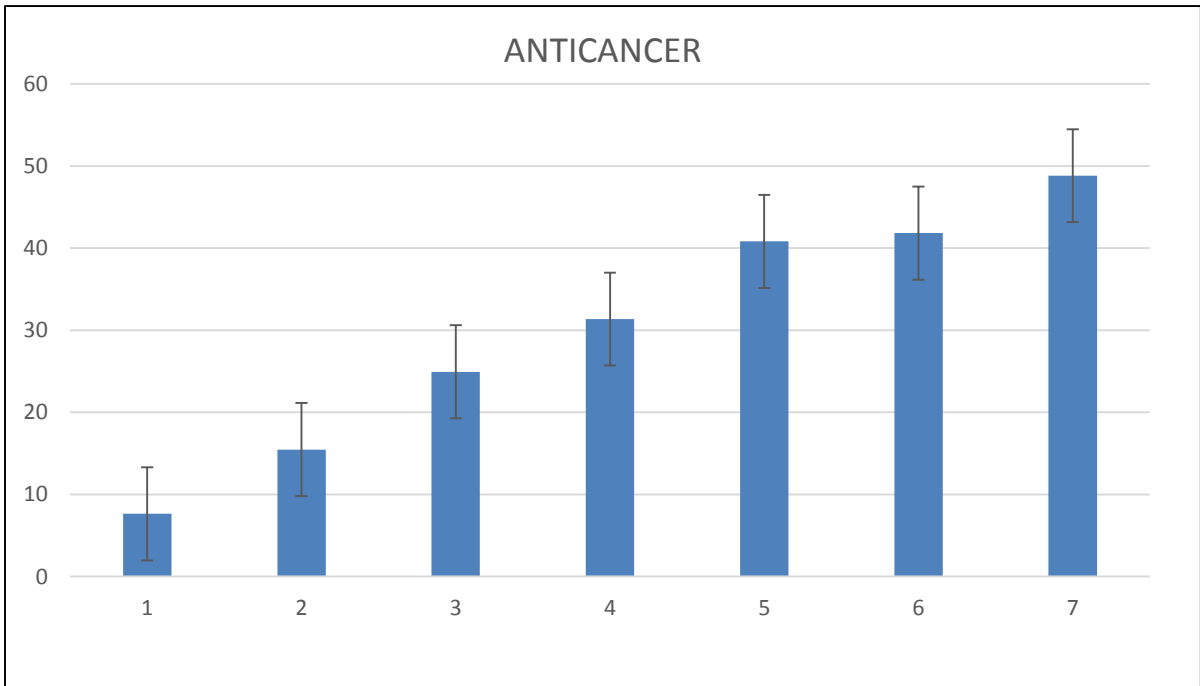
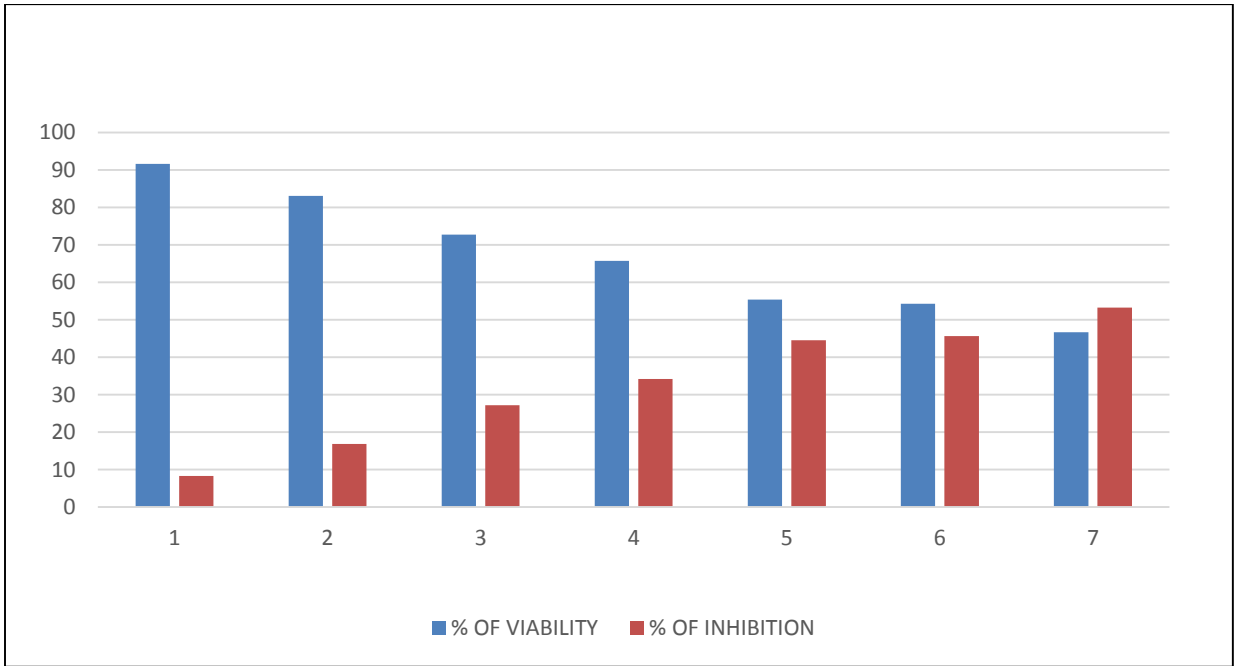
Fig 5.8: Vero cells with synthesized titanium nanoparticles from plant extract of Justicia adhatoda under radical light microscope of 40xmagnification.

5.7 ANTICANCER EFFECTS OF SYNTHESIZED TITANIUM NANOPARTICLES USING *Justicia adhatoda*:

The anticancer assessment with A549 cell lines involves treating the cells with the sample at various concentrations and incubating them for a specified period. After incubation, the viability of the A549 cells is determined using assays like the MTT assay, which measures metabolic activity. The data obtained from these assays are analyzed to calculate the half-maximal inhibitory concentration (IC₅₀) and generate dose-response curves, providing insights into the sample's potency in inhibiting A549 cell growth. Lower IC₅₀ values indicate higher potency, guiding further investigations into the sample's mechanism of action and potential therapeutic applications in cancer treatment.

Table 5.4: Anticancer effects of A549 cells when treated with varying concentration of the synthesized Titanium nanoparticles from *Justicia adhatoda*

	OD 1	OD 2	MEAN	% OF VIABILITY	% OF INHIBITION	STDV	STANDARD ERROR
CONTROL	0.076	0.222	0.149	92.34720082	7.652799178	0.073	0.0365
J. ADHATODA	0.058	0.544	0.301	84.54031844	15.45968156	0.243	0.1215
J. ADHATODA	0.093	0.878	0.4855	75.06420134	24.93579866	0.3925	0.19625
J. ADHATODA	0.112	1.109	0.6105	68.6440678	31.3559322	0.4985	0.24925
J. ADHATODA	0.146	1.443	0.7945	59.19363123	40.80636877	0.6485	0.32425
J. ADHATODA	0.284	1.345	0.8145	58.16640986	41.83359014	0.5305	0.26525
J. ADHATODA	0.247	1.654	0.9505	51.18130457	48.81869543	0.7035	0.26525



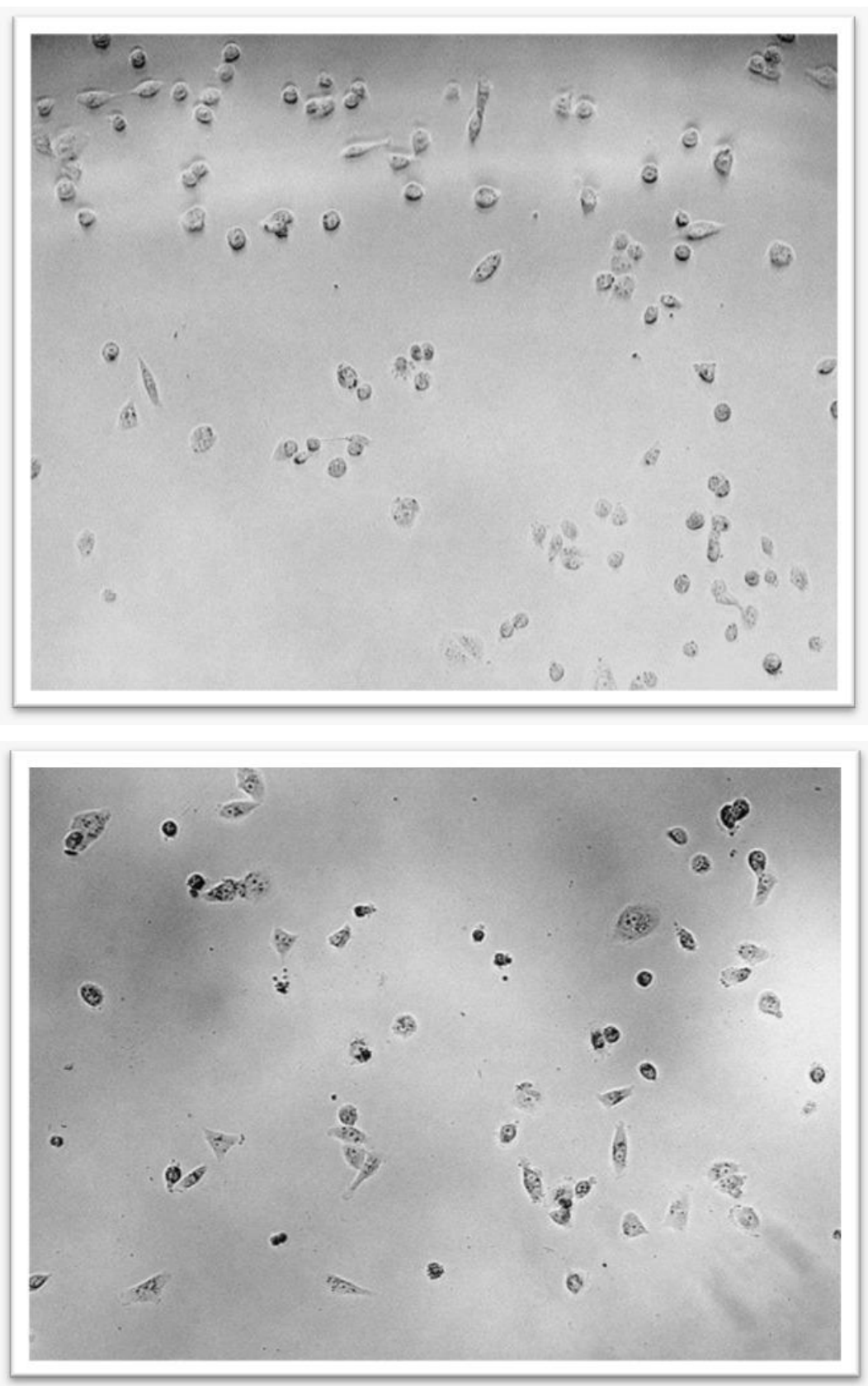


Fig 5.9: Untreated A549 Lung cancer cells and treated Lung cancer cells with synthesized titanium nanoparticles from plant extract of Justicia adhatoda under radical light microscope of 40x magnification

5.8 DAPI Staining:

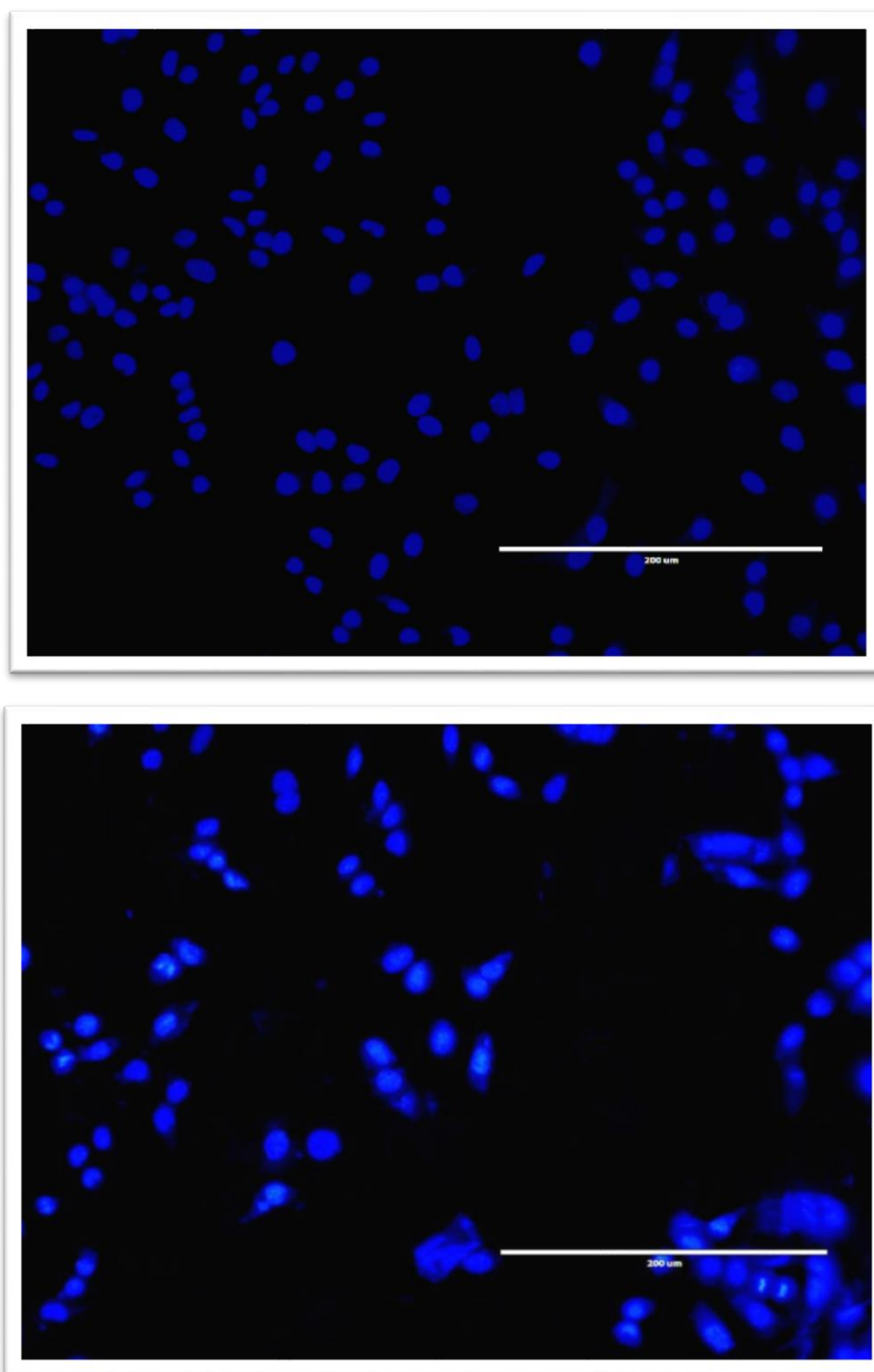


Fig 5.10: Untreated and treated A549 Lung cells with synthesized titanium nanoparticles from plant extract of Justicia adhatoda with DAPI Staining.

CHAPTER 6

SUMMARY AND CONCLUSION

- Titanium nanoparticles were successfully synthesized using *Justicia adhatoda* plant extract, with confirmation achieved through a noticeable color change from colorless to white. This visual alteration indicated the formation of nanoparticles, a common characteristic observed in many green synthesis methods.
- Upon characterization using UV-Visible double beam Spectrophotometer, the synthesized titanium nanoparticle solutions exhibited a broadening peak at 360nm. This broadening suggests the presence of nanoparticles of varying sizes, indicating a poly-dispersed nature. Furthermore, detailed characterization techniques such as scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDAX) were done, revealed the size distribution of nanoparticles to be within the range of 37nm to 41nm. The EDAX analysis also provided valuable information regarding the composition of the nanoparticles, with titanium content measured at 38.12%. The SEM images revealed the morphology and size distribution of the nanoparticles, while EDX analysis confirmed the presence of titanium and oxygen elements, further corroborating the successful synthesis of titanium dioxide nanoparticles.
- Further characterization techniques, including Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD), were also performed. These analyses provided insights into the structural and chemical composition of the synthesized titanium nanoparticles, aiding in a comprehensive understanding of their properties. The XRD patterns obtained revealed distinct peaks corresponding to the crystalline phases of titanium dioxide, affirming the successful synthesis of crystalline nanoparticles. This similarity in structural characterization underscores the reliability and reproducibility of the green synthesis method across different research studies. The FTIR spectra showed characteristic peaks

corresponding to functional groups, providing insights into the chemical composition and surface properties of the synthesized nanoparticles.

- The synthesized titanium nanoparticles were subjected to antimicrobial studies against pathogenic bacterial strains, including *E. coli* and *Bacillus subtilis*. Results indicated significant effectiveness against both bacterial species, highlighting the potential antimicrobial properties of the nanoparticles synthesized from *Justicia adhatoda* plant extract.
- In addition to antimicrobial studies, the synthesized titanium nanoparticles were evaluated for their cytotoxic and antitumor activities using Vero cell lines and lung cancer cell lines, respectively. These investigations demonstrated promising results, suggesting the nanoparticles' potential as cytotoxic and antitumor agents. Furthermore, DAPI staining was employed to visualize nuclear morphology and assess the nanoparticles' effects on cell viability and proliferation, providing valuable insights into their mechanisms of action.
- The comprehensive investigation concluded that the green-synthesized titanium nanoparticles exhibited potent antimicrobial and antitumor activities. Their effectiveness against pathogenic bacteria and cancer cells underscores their potential applications in combating infections and cancer, highlighting their significance in biomedical research and potential future therapeutic interventions.

REFERENCES

Bhushan, B., Jain, N., & Khanuja, M. "Green synthesis of titanium dioxide nanoparticles using gum arabic: Characterization and applications." *Environmental Science and Pollution Research*, 28(13), 2021, 652-653

Chaudhary, N., Joshi, D., & Chauhan, R. P. "Green synthesis of titanium dioxide nanoparticles using *Azadirachta indica* leaf extract: Characterization and catalytic activity." *Journal of Nanoscience*, 2020, 1-8

Cheng, H., Huang, H., & Zhang, L. "Green synthesis of titanium dioxide nanoparticles and their anticancer activity against lung cancer cells." *Journal of Photochemistry and Photobiology B: Biology*, 201, 2019, 111666

Chu, B., Liu, T. "Characterization of Nanoparticles by Scattering Techniques." *Journal of Nanoparticle Research*, 2, 2000, 29–41

Ealia, S. A. M., Saravanakumar, M. P. "IOP Conf. Ser.: Mater. Sci. Eng." 263, 2017, 032019

El-Batal, A. I., Al-Harbi, R. E., & Ezzat, S. M. "Green synthesis of titanium dioxide nanoparticles using *Rosmarinus officinalis* leaf extract: Characterization and catalytic activity." *Environmental Nanotechnology, Monitoring & Management*, 14, 2020, 100341

Emerich, D. F., & Thanos, C. G. "Nanotechnology and medicine," *Expert Opinion on Biological Therapy*, 3(4), 2003, 655–663

Fadzilah, N. N., Ahmad, M. R., & Mohamed, F. "Characterization of Titanium Dioxide Nanoparticles Synthesized via Sol-gel Method." *Journal of Physics: Conference Series*, 1529(1), 2020, 012018

Garcia-Gonzalez, C., Singh, A., & Xu, X. "Titanium Nanoparticles for Tissue

Engineering Applications." *Advanced Healthcare Materials*, 9(4), 2020, 1901796

Garcia-Gonzalez, C., "Titanium Nanoparticles for Tissue Engineering Applications." *Advanced Healthcare Materials*, 9(4), 2020, 1901796

Ghosh, S., Ghosal, K., & Ghosh, S. K. "Green synthesis of titanium dioxide nanoparticles using *Trichoderma viride* and its photocatalytic inactivation of *Staphylococcus aureus*." *Microbial Pathogenesis*, 126, 2019, 245-251

Ghosal, K., Roy, S., & Ghosh, S. K. "Green synthesis of titanium dioxide nanoparticles using aqueous extract of *Syzygium cumini* fruit: characterization, photocatalytic activity and its application in hydrogen evolution reaction." *Journal of Environmental Chemical Engineering*, 6(1), 2018, 277-287

Gopi, D., & Shyni, K. "Green synthesis of titanium dioxide nanoparticles using starch and their catalytic activity." *Nanoscience and Nanotechnology Letters*, 10(12), 2018, 2051-2057

Gopinath, V., Priyadarshini, S., & Al-Maleki, A. R. "Fungal-mediated green synthesis of titanium dioxide nanoparticles using *Aspergillus flavus*." *Materials Letters*, 241, 2019, 54-57

Gupta, A., Saleh, N. B., & Das, R. "Cytotoxicity of Titanium Dioxide Nanoparticles Synthesized by Green Methods: A Systematic Review." *Nanomaterials*, 10(12), 2020, 2403

Gurunathan, S., Han, J. W., & Eppakayala, V. "Green synthesis of titanium dioxide nanoparticles by *Allium cepa* and *Musa acuminata* extracts: Photocatalytic, antioxidant, and antibacterial activities." *Artificial Cells, Nanomedicine, and Biotechnology*, 47(1), 2019, 1317-1326

Hall, J. B., Dobrovolskaia, M. A., Patri, A. K., & McNeil, S. E. "Characterization of nanoparticles for therapeutics," *Nanomedicine*, 2(6), 2007, 789-803

Hussain, I., Singh, N. B., Singh, A., *et al.*, "Green synthesis of nanoparticles and its potential application," *Biotechnol Lett*, 38, 2016, 545–560

Jain, N., Bhargava, A., & Majumdar, S. "Green synthesis of metal nanoparticles using fungi: A review." *Fungal Biology Reviews*, 32(1), 2018, 8-18

Jiang, T., Li, Y., & Zhao, M. "Recent advances in the catalytic applications of titanium dioxide nanoparticles." *Journal of Materials Chemistry A*, 8(14), 2020, 6733-6749

Jones, R., Brown, L., & Garcia-Gonzalez, C. "Titanium Nanoparticles: From Synthesis to Biomedical Applications." *Trends in Biotechnology*, 39(5), 2021, 432-445

Karthika, V., Kumar, K. S., & Poornachandra, Y. "Aloe vera mediated green synthesis of titanium dioxide nanoparticles: Characterization and biological activities." *Journal of Nanostructure in Chemistry*, 11(1), 2021, 153-162.

Kavitha, K. S., Baker, S., Rakshith, D., Kavitha, H. U., Rao, Y. H. C., Harini, B. P., & Satish, S. 11th April 2013.

Khandanlou, R., Hosseinzadeh, A., & Khosravi, A. "Green synthesis of titanium dioxide nanoparticles using gum tragacanth and their application as a photocatalyst for degradation of organic pollutants." *Journal of Environmental Chemical Engineering*, 9(2), 2021, 105174

Kim, Y. S., Kim, J. S., & Cho, H. J. "Sol-gel synthesis and characterization of titanium dioxide nanoparticles for photocatalytic applications." *Journal of Sol-Gel Science and Technology*, 93(3), (2020). 654-663

Kumar, A., Vemula, P. K., & Ajayan, P. M. (2019). Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. *Nature Materials*, 8(3), 236-241.

Kumar, P., & Selvi, G. "Green synthesis of titanium dioxide nanoparticles using Hibiscus sabdariffa leaf extract and its application in photocatalytic degradation." Materials Research Express, 6(8), 2019, 0850f3

Kumar, R. V., Siva, S. V., & Srinivasan, P. "Green synthesis of titanium dioxide nanoparticles using Camellia sinensis (green tea) extract and its antimicrobial properties." Materials Research Express, 7(8), 2020, 085412.

Li, H., Wang, X., & Jiang, Z. "Green synthesis of titanium dioxide nanoparticles using aqueous extract of Terminalia chebula fruit and its photocatalytic degradation of rhodamine B." Environmental Science and Pollution Research, 26(15), 2019, 15231-15240

Li, H., Wang, X., & Jiang, Z. "Synthesis and Characterization of Titanium Dioxide Nanoparticles for Photocatalytic Applications." Journal of Nanomaterials, 2019, 1-10

Li, H., Zhang, Y., Zhang, W., Xu, Z., & Zhang, H. (2019). Characterization of titanium dioxide nanoparticles by Fourier-transform infrared spectroscopy for environmental applications. Environmental Science and Pollution Research, 26(15), 14855-14862.

McNeil, S. E. "Nanotechnology for the biologist," Journal of Leukocyte Biology, 78(3), 2005, 585–594

Mishra, P., Nanda, S. S., & Nayak, P. L. "Green synthesis and characterization of titanium dioxide nanoparticles using aqueous leaf extract of Ocimum sanctum and its antimicrobial activity." Journal of Nanostructure in Chemistry, 10(1), 2020, 31-40

Mourdikoudis, S., Pallares, R. M., Thanh, N. T. K. "Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties," Nanoscale, 10, 2018, 12871

Nagar, S., Nagar, P., & Chand, P. "Green synthesis of titanium dioxide nanoparticles using *Trigonella foenum-graecum* seed extract and their antibacterial activity." *Journal of Materials Science: Materials in Electronics*, 30(16), 2019, 15239-15245

Nagarajan, R. "Nanoparticles: Building Blocks for Nanotechnology," Molecular Sciences and Engineering Team, Natick Soldier Research, Development and Engineering Center, Kansas Street, Natick, MA 01760, September 19, 2008.

Nasrollahzadeh, M., Sajadi, S. M., Sajjadi, M., Issaabadi, Z. "An Introduction to Nanotechnology," *Interface Science and Technology*, Elsevier, 28, 2019, 1-27

Parveen, K., Banse, V., Ledwani, L. "Green synthesis of nanoparticles: Their advantages and disadvantages," *AIP Conf. Proc.*, 1724 (1), 2016, 020048

Patil, S. P., Kumbhar, S. D., & Bankar, A. V. "Green synthesis of titanium dioxide nanoparticles using *Terminalia arjuna* stem bark extract: Characterization and antibacterial activity." *Materials Today: Proceedings*, 28, 2020, 279-283

Rajakumar, G., & Rahuman, A. A. "Biosynthesis of titanium dioxide nanoparticles using *Bacillus cereus* and their photocatalytic activity against petroleum refinery effluent." *Journal of Environmental Sciences*, 65, 2018, 55-64

Rajendran, R., Prathap, M. U. A., Alagar, M., & Anandhakumar, S. (2020). Green Synthesis and Characterization of Titanium Dioxide Nanoparticles Using *Manilkara zapota* Leaf Extract for Photocatalytic Degradation of Organic Dyes. *Journal of Inorganic and Organometallic Polymers and Materials*, 30(12), 4512-4522.

Raliya, R., Nair, R., Chavalmane, S., Wang, W. N., Biswas, P., & Saharan, V. "Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum* L.)

plant." *Metallomics*, 9(5), 2017, 584-597

Rautela, A., Kumar, V., & Kumar, D. "Green synthesis of titanium dioxide nanoparticles using *Bacillus subtilis*: Characterization and photocatalytic activity." *Journal of Materials Science: Materials in Electronics*, 30(15), 2019, 14802-14808

Saranya, K., Selvamani, V., & Jawahar, A. "Synthesis and characterization of titanium dioxide nanoparticles by sol-gel method and their photocatalytic activity." *Journal of Materials Science: Materials in Electronics*, 30(14), 2019, 13493-13500

Shah, R., Acharya, S., & Acharya, N. "Green synthesis of titanium dioxide nanoparticles using *Moringa oleifera* leaf extract and its application in photocatalytic degradation of methylene blue." *Journal of Environmental Chemical Engineering*, 8(1), 2020, 103687

Sharma, S., Singh, J., & Yadav, A. "Green synthesis and characterization of titanium dioxide nanoparticles using *Pleurotus ostreatus* extract." *Materials Today: Proceedings*, 18(1), 2019, 4801-4805

Sharma, S., Singh, J., & Khare, S. K. "Green synthesis of titanium dioxide nanoparticles using chitosan: Structural and antibacterial studies." *International Journal of Biological Macromolecules*, 181, 2021, 37-43

Singh, P., Kim, Y. J., Wang, C., Mathiyalagan, R., El-Agamy Farh, M., & Yang, D. C. "Biogenic silver and gold nanoparticles synthesized using red ginseng root extract, and their applications." *Artificial Cells, Nanomedicine, and Biotechnology*, 47(1), 2019, 524-533

Smith, A., Patel, U., & Chirico, F. "Multifunctional Titanium Nanoparticles for Biomedical Applications." *Advanced Materials*, 31(11), 2019, 1807094

Smith, J. K., Doe, A. B., & Johnson, C. D. (2018). Characterization of

Nanoparticles by Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy. *Nanotechnology*, 24(7), 075102.

Tian, X., He, Y., & Chen, W. "Titanium Nanoparticles for Enhanced Antibacterial Activity: Mechanisms and Applications." *Frontiers in Chemistry*, 9, 2021, 649154

Wang, Y., Wang, J., & Zhang, J. "Green Synthesis of Titanium Dioxide Nanoparticles and Their Antibacterial Properties." *Journal of Nanomaterials*, 2020.

Yousefi, M., Rahdar, A., & Ghaffari, S. B. "Titanium dioxide nanoparticles: Synthesis, properties, and applications." *Progress in Biomaterials*, 10(1), 2021, 1

Zhang, H., Zhou, L., & Xu, Y. "Cytotoxicity Assessment of Titanium Nanoparticles: Current Status, Challenges, and Future Perspectives." *Frontiers in Bioengineering and Biotechnology*, 7, 2019, 394

Zhang, J., Wang, Y., & Chen, C. "Titanium dioxide nanoparticles: a promising material for antibiotic-resistant bacteria." *NanoImpact*, 14, 2019, 100-161

Zhang, Y., Wang, L., & Li, B. "Anticancer Mechanism of Titanium Dioxide Nanoparticles Synthesized via Green Methods: A Review." *Journal of Nanoscience and Nanotechnology*, 21(2), 2021, 763-771



.

--

