

BIOGENIC-PRODUCTION OF TITANIUM NANOPARTICLES USING PLANT EXTRACT OF *SCOPARIA DULCIS*

PROJECT WORK

Submitted in partial fulfillment of the requirement for the award of
Bachelor of Science degree in Microbiology

By

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

SATHYABAMA

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

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Chandrika S (41300008) who carried out the project entitled "**BIOGENIC-PRODUCTION OF TITANIUM NANOPARTICLES USING PLANT EXTRACT OF SCOPARIA DULCIS**" done under my supervision from January 2024 to April 2024.

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DECLARATION

I, Chandrika S hereby declare that the Project Report entitled "BIOGENIC-PRODUCTION OF TITANIUM NANOPARTICLES USING PLANT EXTRACT OF *SCOPARIA DULCIS*" 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 Microbiology.

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ABSTRACT

Nanotechnology is the manipulation of materials and structures at the nanoscale to create new materials and devices. Nanoparticles have unique properties and behaviours due to their size and shape. Titanium Nanoparticles can be used to deliver drugs directly to cancerous cells. Titanium nanoparticles are used in a variety of fields including imaging and sensing. Nanoparticle characterization is crucial for understanding their properties, ensuring reproducibility in synthesis, and optimizing their applications. The techniques play vital roles in obtaining about the size, shape, composition, and other characteristics of nanoparticles. Characterizes the nanoparticles such as Fourier transform infrared (FTIR) spectroscopy, scanning electron microscope-energy dispersive X-ray spectroscopy (SEM-EDAX), powder X-ray diffraction (XRD), ultraviolet-visible spectroscopy (UV-VIS), are common techniques are used for the titanium nanoparticles. Antimicrobial studies were observed as both gram-positive and gram negative bacteria such as *S.aureus* and *E.coli*. Here, we performed integrated systems of toxicology and anticancerous approach for detailed study to evaluate the dose dependent response of cancer cells against nanoparticles and the possible role of the titanium exposed Renal Carcinoma Cancer cells. For this purpose cell line was treated with titanium nanoparticles of different concentrations. DAPI staining revealed that titanium nanoparticle at higher concentration caused significant damage to the DNA and decreases the cell viability. Apoptosis was assessed using acridine orange staining, compared to the nontreated controls. Furthermore, the acridine orange staining showed an increased apoptosis after treatment. The nanoparticles are involved in the size reducing and stability of kidney stones as well as other diseases and degenerative processes. Nano-based drug delivery devices such as titanium nanoparticles are increasingly used to treat kidney stone size analyzing. Renal stones are the main channel for drug elimination in the kidney and targeting ligand compatibility. Researchers are using nanoparticles to develop new therapeutic drugs that can increase efficacy and reduce side effects. Nanoparticles can also combine multiple functions, such as therapeutic and diagnostic functions, into a single nanoparticle platform.

Keywords: Green synthesis, Titanium dioxide, anticancer activity, apoptosis, nuclear fragmentation, kidney stones size analyse.

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LIST OF ABBREVIATIONS

AO	Acridine orange
AChE	Acetylcholinesterase
AFM	Atomic Force Microscopy
A-T	Adenine-Thymine
CO ₂	Carbon dioxide
CV	Cell Viability
DAPI	4', 6-diamidino-2-phenylindole
DCS	Dynamic light scattering
DNA	Deoxyribonucleic acid
DsDNA	double strand Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's modified Eagle's medium
EDAX	Energy-dispersive X-ray analysis
EDTA	Ethylenediaminetetraacetic acid
ED	Electron diffraction
E.coli	Eschericia Coli
ELISA	Enzyme linked immunosorbent assay
FBS	Fetal Bovine Serum
FE-SEM	Field-Emission Scanning Electron Microscopy
FTIR	Fourier-transform infrared microscopy
HCl	Hydrochloric acid
IC ₅₀	Half maximal inhibitory concentration
MALDI-TOF spectroscopy	Matrix assisted laser desorption/ionization time of flight mass
MIC	Minimal inhibitory concentration
MTT	(3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide

MEM	Modified Eagle's medium
NCCS	National Centre for Cell Science
NPs	Nanoparticles
NMR	Nuclear Magnetic Resonance
NACL	Sodium chloride
O ₂	Oxygen
PBS	Phosphate-buffered saline
PH	Potential of hydrogen
PDT	Populations doubling times
RF	Radio frequency
ROS	Reactive oxygen species
RCC	Renal Carcinoma Cancer cells
RPM	Revolutions per minute
RPMI-1640	Roswll park memorial institute
SEM	Scanning electron microscopy
Si	Silicon oxides
S.aureus	Staphylococcus aureus
TEM	Transmission electron microscopy
Ti	Titanium
TiO ₂ NPs	Titanium dioxide Nanoparticles
TiO ₂	Titanium dioxide
TiOSO ₄	Titanium oxysulfate
UV-vis	Ultraviolet-visible spectroscopy
XRD	X-Ray Diffraction
XPS	X-ray photoelectron spectroscopy
ZOI	Zone of inhibition

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CHAPTER 1

INTRODUCTION

Nanotechnology is the branch of science and engineering development which deals small molecule components and its structure, molecules, physical-chemical properties in depth to its atoms. The evolutionary of this streamline lead us to understand the molecules at the 'Nano' scale level. Nanotechnology is one of the fast driven cores with potential impact to pharmaceuticals, manufacturing and agriculture industries. Nanoscale material features and functionality structure manipulation control are applied in this field to produce various properties of materials with unique features and functionalities. (Ajmal N. *et al.*, 2019).

In Nanotechnology, a term "nanoparticle" is commonly used to refer to particle with dimensions ranging from 1 to 100 nanometers. These nanoparticles are having unique properties and behaviours due to their size, which can differ significantly from larger particles or bulk materials. Nanoclusters are tiny assemblies of atoms or molecules with at least one dimension falling within the range of 1 to 10 nanometers (Begum S.J, *et al.*, 2022).

A nanopowder is collections of ultrafine particles, nanoparticles, or nanoclusters that are often agglomerated or clumped together. Nanocrystals are single crystals or single-domain ultrafine particles that are typically on the nanometer scale. These are well-defined crystalline structures, and it can be made from wide range of materials including metals, semiconductors, and ceramics. Nanoparticles are used to deliver drugs directly to target cells or tissues of cancerous cells, reducing side effects and improving the effectiveness of treatments. This is particularly relevant in cancer therapy, where targeted drug delivery can minimize damage to healthy cells (Kumar V *et al.*, 2009).

Titanium dioxide (TiO₂) nanoparticles are well known for their photocatalytic properties. Exposure to ultraviolet (UV) light can decompose organic matter and produce a self-purifying effect. Titanium dioxide (TiO₂) is not actually a divalent alkali metal. This is not a barium sulfate salt, but a separate compound composed

of titanium and oxygen. TiO_2 is a white crystalline solid, insoluble in water and alcohol, and soluble in concentrated acids. It is odorless and known for high index of refractiveness and ability to diffuse brightness, making it an important component in a variety of products requiring brightness and opacity. Due to its excellent light scattering properties, it is used in variety of industrializes including paints, food coloring, sunscreen. (Santhoshkumar *et al.*, 2019)

Green synthesis typically uses non-toxic plant extracts, reducing the need for hazardous chemicals and minimizing environmental impact. Titanium nanoparticles synthesized using plant extracts often exhibit better biocompatibility, making them potentially for a variety of biomedical applications, including drug delivery for Vero cells and Renal Carcinoma Cancer cells. Nanoparticles produced through green synthesis may have unique properties due to the biomolecules present in plant extracts, which may make them well suited for targeted drug delivery and other applications without the need for additional coatings (Al-Shabib A.V., *et al.*, 2020).

Scoparia dulcis Linn, commonly known as 'sweet broom weed' or 'licorice weed' is actually a perennial plant known for its diverse bioactive compounds and medicinal uses. Belonging to the Scrophulariaceae family, this plant has demonstrated therapeutic potential in treating a range of ailments, including cancer diagnosis, diabetes, diarrhea, stomach pain, hepatitis, bronchitis, fever, hypertension, and ulcers. Additionally, it possesses antibacterial, anti-diabetic, anti-fungal, anti-inflammatory, antiviral, and hypercholesterolemic elements. A diverse array of chemical compounds can be found in the plant components of *Scoparia dulcis* L., but their main characteristic is their pharmacological activity. Diterpenoids, triterpenoids, alkaloids, flavonoids, phenols, and steroids are some of these constituents.

The shape and morphology of titanium oxide nanoparticles were investigated using characterization techniques including UV-Vis, SEM, EDAX, XRD, and FTIR. Ellman's approach was used to study the inhibition of Alzheimer's disease-related enzymes in an acetylcholinesterase (AChE) study on green-generated TiO_2 NPs from *Scoparia dulcis* extract (Sundrarajan M *et al.*, 2011).

Nanoparticles are small size and exhibit unique, size-dependent properties that differ from bulk materials and individual atoms or molecules. Increasing the surface area to volume ratio at the Nano-scale has significant effects on physical, chemical, optical and electronic properties. Because nanoparticles are so small, most nanoparticle atoms are located near the surface.

Compared to bulk materials, they have a higher surface area to volume ratio, which affects their reactivity and surface properties. Depending on the size, shape, and composition of nanoparticles, they can exhibit different optical properties. This could lead to electromagnetic resonance, an interesting phenomenon that could be used in a variety of fields, from imaging and sensing to catalysis. In particular, nanoparticles with increased surface area are more reactive than bulk materials and are widely used in catalysis (Nabi *et al.*, 2019).

The surface atoms of nanoparticles play an important role in determining their chemical, mechanical, electrical, and optical properties. Increased reactivity, altered melting point, altered optical and catalytic properties, and other electronic properties compared to the bulk product are mainly attributed to this phenomenon. Nanoparticles have unique properties because a significant portion of their atoms are located on the surface, increasing their sensitivity to environmental changes, increasing the potential for chemical reactions, and altering their thermal and optical properties. An important area of research to increase solar energy absorption and improve the efficiency of solar panels has been the use of nanoparticles in photovoltaic cells. Nanoparticles have a high surface-to-volume ratio, giving them a large surface area with which they can interact with light. Nanoparticles can scatter and trap light more efficiently, lengthening the interaction path between light and matter and increasing the potential for absorption. The free electron set oscillations on the surface of some nanoparticles interact significantly with incident light, resulting in optical effects. This can increase light absorption by focusing and strengthening the electric field around the nanoparticles. Quantum effects can make the properties of materials different from those of bulk matter on very small scales. Thermal energy at room temperature can be sufficient to spontaneously switch magnetization direction when ferroelectrics are reduced to very small sizes (less than 10 nm). This can lead to accidental transitions to different states, which can result in loss or

corruption of information, making it unsuitable for applications that require persistent storage in memory (Santos *et al.*, 2015).

Nanoparticles can be seriously damaged by collisions with nearby solvent molecules. They remain stationary because their constant movement prevents them from disappearing or floating in the liquid. The interaction of nanoparticles with materials is strongly influenced by their surface properties, especially because of their very small size. Nanoparticles have the ability to form stable suspensions through various processes, including surface charge, steric hindrance, and electrostatic repulsion.

Nanoparticles act as a counter-acting the pull of gravity, these characteristics can prevent settling down the chemical compounds. Because of these quantum effects and their small size, nanoparticles often exhibit unique optical properties. Surface plasmon resonance is a common phenomenon that can occur due to the interaction of light with these small particles and is especially noticeable for nanoparticles of noble metals such as gold and silver. As a result of this phenomenon, nanoparticles come in a variety of colours due to their special way of absorbing and scattering light. For example, due to their optical properties, the colour of gold nanoparticles in solution can range from dark red to black depending on their size and shape (Edmundson *et al.*, 2014)

Clay nanoparticles in polymer matrices are an effective way to improve the mechanical properties of polymer compounds. These particles, often called Nano-fillers, have the ability to increase the stiffness, strength and thermal stability of polymers. The addition of clay nanoparticles to the polymer matrix enhances and strengthens the overall mechanical properties of the resulting composites. Introducing hard clay nanoparticles helps increase the strength of the polymer. This is often measured using tensile, flexural and other mechanical property tests. An important parameter that indicates that an amorphous polymer is transitioning from a hard and brittle state to a more flexible and elastic state is the glass transition temperature of nanoparticles (R Shashikant Kuchekar *et al.*, 2017)

The glass transition temperature can increase thermal stability and improve polymer performance. The properties of clay nanoparticles, such as hardness, are

transferred to the polymer matrix. Nanoparticles have been used for various purposes, such as attaching them to textile fibres. This is often done to create smart, functional fabrics with improved properties such as enhanced strength, conductivity or even the ability to respond to external stimuli (Bao, S.J., *et al.*, 2012).

Surface Plasmon resonances impart unique optical and electromagnetic properties to materials, including catalysis and sensitivity. In addition to metallic, dielectric, and semiconductor nanoparticles, hybrid structures such as core-shell nanoparticles have also been created. These quantum dots are another term for extremely small (specifically less than 10 nm) semiconductor nanoparticle materials used for quantization of electromagnetic energy. Small particles are used as drug carriers or for imaging purposes in biomedical applications.

Soft and semi-solid nanoparticles were synthesized. Liposomes are a type of semi-solid nanoparticle prototype. Currently, various types of liposomal nanoparticles are being used to deliver clinically, including vaccines and anti-cancer drugs.

The nanoparticles are produced several ways including abrasion and thermal decomposition. In the abrasive method, large particles at the macroscopic or microscopic level are reduced to size by grinding in a ball mill or planetary ball mill. To recover nanoparticles, the resulting particles undergo air fractionation. Particles in the air are classified by density and size. In the pyrolysis method, a vapor precursor, which may be in liquid or gaseous form, is passed through an orifice under high pressure and then combusted. The precursor burns to form a solid residue that often resembles soot and contains nanoparticles. After pyrolysis, the solid product undergoes air fractionation to extract oxide particles from the by-product gas. It is important to remember that during pyrolysis, agglomerates and aggregates may form instead of individual elementary particles. Clusters of particles are called aggregates, and groups of aggregates are called agglomerates (Krishnasamy *et al.*, 2015).

Thermal plasma technology plays an important role in generating nanoparticles through processes such as evaporation and cooling. The thermal plasma with a temperature of about 10,000 K can provide the energy needed to

vaporize small micron-sized particles. As the evaporated material cools as it leaves the plasma region, nanoparticles form. Nanoparticles are created using a variety of thermal plasma torches, such as Electrical arc jet plasma, plasma, and radio frequency (RF) induced plasma. This same electric arc that forms between the anode and cathode in an arc plasma reactor provides the energy needed for evaporation and reaction. Arc plasma can be used to evaporate quartz sand at atmospheric pressure. It is possible to create fumed silica by quickly cooling a mixture of silica vapor and plasma gas with oxygen. Energy is transferred to the plasma in high-frequency induction plasma torches via an electromagnetic field produced by an induction coil. Working with a variety of gases, such as inert, reducing, oxidizing, and other aggressive media, is made possible by this technique. The contact between the plasma gas and the HF induction plasma torch is eliminated. the electrode and the plasma gas, minimizing potential contamination sources. A versatile and controlled technique for creating nanoparticles with particular characteristics is the use of thermal plasma in nanoparticle production (Amanulla A. M., *et al.*, 2019). The frequency range covered by this technique is 200 kHz-40 MHz. The use of radio frequencies in plasma processes is included in this scope. Large-scale industrial installations have been tested at power levels of up to 1 MW, while laboratory installations typically operate at 30 to 50 kW. The droplets of raw material injected into the plasma have a very short residence time. This indicates that the process proceeds rapidly and that small droplet sizes are necessary to guarantee full evaporation in a brief amount of time. It takes small droplet sizes to accomplish full evaporation in a brief amount of residence time. Different materials for nanoparticles have been synthesized using RF plasma methods. Ceramic nanoparticles, including carbides/carbides, nitrides, and oxides of silicon (Si) and titanium (Ti), are specifically mentioned. (Chauhan R., *et al.*, 2019).

Inert gas condensation is the common method to produce nanoparticles, especially from low melting point metals. Metals with low melting points are first evaporated in a vacuum chamber. This is typically achieved by heating the metal source to change it from a solid phase to a vapor phase. An inert gas such as argon or helium is introduced into the vacuum chamber. It creates a controlled environment that prevents metal nanoparticles from oxidizing during the process

and transport metal vapours. The metal vapor is then rapidly cooled by a flow of inert gas. This cooling process is often achieved by expanding the gas to lower its temperature. As a result of this rapid cooling, supersaturated vapor is formed. In supersaturated metal vapors, nucleation occurs and nano-sized particles are formed. The particles begin to grow as additional metal atoms condense into the core. A flow of inert gas entrains the nanoparticles and allows them to be removed from the evaporation zone. The method involves exposing the remaining vapor to produce nanoparticles. Nanoparticles can be deposited on a substrate to form a thin film or layer (Shashikant Kuchekar R., *et al.*, 2017).

Nanoparticle characterization is crucial for understanding their properties, ensuring reproducibility in synthesis, and optimizing their applications. The techniques play vital roles in obtaining about the shape, size, and composition are the characterization of nanoparticles. To attain ability and control of nanoparticle production and uses, nanoparticle characterization is important. Many techniques are used for characterization, most of which are derived from nanoparticles of material sciences. Common techniques are Fourier transform infrared spectroscopy (FTIR), scanning electron microscope-energy dispersive X-ray spectroscopy (SEM-EDS), transmission electron microscopy (TEM), atomic force microscopy (AFM), dynamic light scattering (DLS), powder X-ray diffraction (XRD), x-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), ultraviolet-visible spectroscopy(UV-VIS), dual polarization interferometry, and nuclear magnetic resonance (NMR) are common techniques (Irshad *et al.*, 2020).

The agar diffusion method of the synthesized TiO₂NPs against various microorganisms was evaluated by the plate diffusion method. Additional agar diffusion method was evaluated using a microdilution method to estimate the minimal inhibitory concentration (MIC) of the synthesized TiO₂NPs. Titanium nanoparticles (TiO₂NPs) have been used for their unique properties and extensive biomedical applications. TiO₂NPs are recognized as an alternative antimicrobial agent with low toxicity in humans and used in various in vitro and in vivo applications. Antimicrobial substances are used to prevent kidney diseases and slow the formation of kidney stones. Subsequently, the synthesized TiO₂NPs

showed effective antibacterial activity against both Gram-positive and Gram-negative bacteria, namely *Staphylococcus aureus* and *Escherichia coli*. Pathogens were highly sensitive to TiO₂NPs. The synthesized TiO₂NPs showed significant potential in the analysis of kidney stones, 68.58% of kidney diseases were closed. (Martinez-Gutierrez, 2010)

TiNPs can act as carriers for cancer drugs or therapeutic agents. By conjugating or encapsulating drugs into TiNPs, targeted delivery to renal carcinoma cancer cells and Vero cells can be achieved, improving therapeutic efficacy and minimizing side effects. TiNPs can exert their anticancer effects and cytotoxicity through unique mechanisms, such as generating reactive oxygen species (ROS) or disrupting cell signaling pathways. These mechanisms can lead to the development of new treatments. TiNPs can act as carriers for cancer drugs, protecting them from degradation and improving their transport into cancer cells. This can improve the therapeutic efficacy of drugs and at the same time reduce their systemic toxicity (Sethy *et al.*, 2020). Due to its self-sufficiency, non-toxicity, and effective drug delivery, titanium nanoparticles have become more important in therapeutic research and dominate large-scale nanomedicine. Numerous uses for titanium nanoparticles have been identified in biomedical research, including drug administration, biomarkers, tissue/tumor imaging, chemical sensing, optical response, biosensors, and photothermal treatment. Titanium nanoparticles provide great promise in cancer treatment because they may produce oxidative stress, damage to the mitochondrial respiratory chain, and halt the formation of adenosine triphosphate, which damages DNA and triggers apoptosis. Most of the time, a novel class of anticancer drugs has made extensive use of titanium nanoparticle complexes to lower cancer rates. Based on this, one of the most significant developments in cancer treatment research is the synthesis of active titanium nanoparticles (Schuler *et al.*, 2013)

The primary form of cell death that happens in response to cell cytotoxicity is known to be apoptosis. It is a tightly controlled process of cell suicide that is typified by a number of phenotypic and cellular alterations, such as chromatin condensation, DNA fragmentation, membrane blebbing, and significant protein cell lysis. Renal Carcinoma cancer cells treated with titanium nanoparticles were

stained with cytidine orange and seen under a fluorescence microscope to identify both early and late apoptosis and necrosis (Thangam *et al.*, 2014)

Using DAPI labeling, nuclear damage and chromatin condensation—two common indicators of apoptosis—were assessed in renal carcinoma cancer cells (A498) treated with TiO₂NPs. A-T-rich sections of the DNA minor groove are bound by dsDNA stained with DAPI. TiO₂NPs significantly altered the nuclei of treated Renal Carcinoma cancer cells, most likely as a result of DNA condensation and chromatin alteration. They also caused a loss of cell structure, exhibiting blue fluorescence with white condensed regions, while the untreated control cells seemed normal. DAPI staining has confirmed the concept of cell death phenomena, which is predicated on the hypothesis that nanoparticles' lethality leads to increased rates of damage and apoptosis. These results suggested that TiO₂NPs can damage DNA and chromatin (Kowsalya *et al.*, 2020).

Kidney disease is a multistep process involving the build-up of different molecular alterations. The way kidney cells and the surrounding cells operate is impacted by these molecular alterations. Numerous genetic alterations, including aneuploidy, deletions, insertions, loss of heterozygosis, and mutations, are linked to a variety of kidney diseases (Sadikovic *et al.*, 2008; Li *et al.*, 2019).

Abnormal cell self-regulating proliferate of epithelial cystic cells in kidney stones is linked to cell separation and upregulation of many proliferation factors; this process has resemblance to a malignant process. Evidence suggests that kidney stones, polycystic kidney disease, gallbladder inflammation and cholelithiasis, prostatitis, calciphylaxis, and ovarian cancer, among other illnesses and degenerative processes, may be caused by nanoparticles. (Grantham, 1990)

The Kidney stone membrane lacks complement regulators and is vulnerable to attack if the cell layer are compromised, and the kidney, which have prominent complement isolation membranes, exhibit imbalances in complement activation (Ricklin *et al.*, 2018). High complement component concentrations, pH variations, and disruption or fragility of the glycocalyx membrane are some other factors that may make the kidney more vulnerable to complement-induced damage (Thurman, 2015). A variety of pathogenic mechanisms can lead to kidney diseases that are complement-mediated and cause kidney stones (Crews *et al.*, 2019).

Researchers are currently using nanoparticles to create novel therapeutic medications that have the potential to improve efficacy while lowering side effects. Moreover, several purposes, like medicinal and diagnostic ones, can be combined into a single nanoparticle platform (Chin *et al.*, 2020)

The present investigation focused on “**Biogenic production of Synthesis and Characterization of Titanium Nanoparticles from plant extract of *Scorpio dulcis***” and studies on their anti-cancer, anti-microbial, cytotoxic and antitumor effects.

CHAPTER 2

LITERATURE SURVEY

2.1 PROPERTIES OF TITANIUM NANOPARTICLES

In a study use of nanotechnology refers to a collection of procedures and approaches intended to produce materials with unique properties and enhanced functions. Richard Feynman first proposed the idea of nanotechnology in 1959, and it is now regarded as one of the areas of science and technology development with the fastest rate of growth worldwide (Santos *et al.*, 2015). Nanoparticle use and application are primarily concentrated in the fields of cosmetics, energy, healthcare, and chemistry. Nanoparticles can be found in both organic and inorganic modules, such as ferritins, liposomes, micelles, dendrimers, and semiconductor NPs like oxides, nitrides, and sulfides, as well as metal NPs with magnetic properties (Nabi *et al.*, 2019).

For the goal of researching is known that titanium exists as an oxide of titanium and that these nanoparticles of metal oxides have special optical, thermal, electric, and magnetic properties. The use of high energy pressure and temperature, as well as the use of hazardous chemicals during the synthesis process, are the limitations of these synthesis methods. Biological extracts can be used as reducing agents in green synthesis methods to produce TiNPs in an economical and environmentally friendly manner. This method can also result in the large-scale production of NPs. (Edmundson *et al.*, 2014; Chen *et al.*, 1995).

2.2 SYNTHESIS OF TITANIUM NANOPARTICLES

In this study, the freshly created TiO₂NPs used in this investigation were made in accordance with the modifications. TiO₂NPs were created by adding 10 ml of propolis extract (500 mg/ml) to 20 ml of an aqueous solution containing 2 mM titanium oxide and stirring continuously for 24 hours. After 30 minutes of centrifugation at 800 ×g, titanium oxide nanoparticles were preserved for later use (Krishnasamy *et al.*, 2015)

2.3 CHARACTERIZATION OF TITANIUM NANOPARTICLES

For this study, Using JASCO V-670 Uv visible spectrophotometer equipped with a diffuse reflectance attachment for powder samples, absorption spectra of the biosynthesized TiO₂NPs were recorded for the study of titanium nanoparticles between wavelength scan of 200 and 800 nm. Energy dispersive X-ray spectroscopy and field emission scanning electron microscopy were used to characterize the bio-synthesized TiO₂NPs. Using an X-ray diffractometer (XRD-7000, Shimadzu Co., Japan), the average crystal structure and size of the biosynthesized titanium oxide NPs were examined. The radiation operated at 40 kV and 30 mA, and 2 θ was recorded between 10 and 80 using Cu K α (λ = 1.54056 Å). High-resolution transmission electron microscopy (HRTEM, Tecnai F20 G2, and Philips, Netherlands) was used to analyze the morphology at an accelerating voltage of 200 kV (Mukunthan K & Balaji S *et al.*, 2012).

The surface functional groups of the bio-synthesised TiO₂ nanoparticles were analyzed and detected at the scanning range of 4000-400cm⁻¹ using Fourier transform infrared spectroscopy (FTIR, Perkin Elmer 65). Similarly, visible spectroscopy is a commonly used technique in nanoparticle characterization, and it is applied to the optical property of TiO₂NPs (R. Shashikant Kuchekar *et al.*, 2017). For the purpose of characterizing different titanium nanoparticles in the size range of 2-100 nm and beyond, the ultraviolet (UV) and visible (Vis), also known as UV-vis, within the 300-800 nm range are typically employed (Pal *et al.*, 2007; Gugliotti *et al.*, 2005). According to their published results, the UV-visible absorption spectrum (wavelength versus absorbance) shows two distinct peaks at wavelengths of 264 nm and 322 nm, which are specific to TiO₂NPs (Schuler *et al.*, 2013; Yeganeh-Faal *et al.*, 2017).

An investigation into Fourier transform infrared spectroscopy (FTIR) is a characterization method that yields infrared spectra of solid, liquid, and gaseous absorption, emission, and photo conductivity. For all of the synthesized TiO₂NPs, FT-IR spectroscopy was also used, either by green or by other methods. By recording the FTIR spectra in the range of 4000 to 400 cm⁻¹, one could determine the potential functional groups present in the synthesized samples. It was observed that the FTIR spectra of TiO₂NPs produced using green method were

similar. Typically, green samples exhibited an absorption band between 650 and 700 nm, which is indicative of a Ti-O-Ti stretching bond. The characteristic peak of TiNPs anatase is located between these absorption peaks. (Irshad *et al.*, 2020; Sethy *et al.*, 2020)

In this study using a Bruker D8 Advanced X-ray diffractometer (Billerica, MA, USA), a study was conducted in which the prepared nanoparticles in the 2θ range of 20 to 80° were identified as being in the crystalline phase. The apparatus has a nickel filter and a proportional counter that uses Cu K α radiation ($\lambda = 1.5405 \text{ \AA}$). The XRD pattern was frequently used to characterize the crystallinity and size of the crystallites in the synthesized material. In order to compare the X-ray diffraction pattern with the material's standardized plane angle pattern, structural information was acquired. The obtained results are compared with the Joint Committee on Powder Diffraction Standards (XRD, JCPDS Card No. 78-2486) to confirm crystallinity (Abisharani *et al.*, 2019; Goutam *et al.*, 2018).

This value is associated with the anatase type of TiO₂NPs with the phases (anatase, brookite, and rutile) according to the X-Ray Diffraction pattern of crystals. Furthermore, the observation of the anatase form of TiO₂NPs at this particular plane angle is a common trend among researchers. This property of TiO₂NPs is unaffected by the synthesis method; for example, the study found that the 2θ peak 25.6° is present in both TiO₂NPs synthesized using the green method and the sol-gel method. It is established that the synthesis method is independent of the crystallinity of TiO₂NPs by comparing the XRD pattern of TiO₂NPs synthesized by the plant extracts of *T. portulacastrum* and *C. quinoa* with the sol-gel method. (Irshad *et al.*, 2020)

In this study, the size of TiO₂ nanoparticles could be determined in a titanium nanoparticle to the application of scanning electron microscopy (SEM). The TiO₂ nanoparticles made from plants exhibit a high degree of polydispersity, with a size range of approximately 120nm. The 10 μ m and 20 μ m scale bars are used. The points in which the measurement was taken are denoted by the numbers 1, 2, 3, 4, 5, and 6. In order to obtain additional understanding of the characteristics of TiO₂ nanoparticles, EDAX techniques were employed for the sample analysis. The

nanoparticles exhibit agglomeration and spherical clusters with poor dispersion. It is challenging to study individual nanoparticles due to agglomeration.

Using the disc-diffusion method, a study was conducted to assess the antimicrobial activity of synthesized TiO₂NPs against various microorganisms. In order to determine the minimum inhibitory concentration (MIC) for the synthesized TiO₂NPs, additional antimicrobial activity was assessed using the microdilution method. The test is conducted three times. Compared to yeast and Gram-negative bacteria, the newly synthesized nanoparticles exhibited greater antimicrobial activity against Gram-positive bacteria. The Treated microorganisms, *P. vulgaris* exhibited the highest resistance (inhibition zone diameter of 7 mm and MIC value of 128 µg/mL), whereas *S. epidermidis* demonstrated the highest susceptibility (inhibition zone diameter of 35 mm and MIC value of 8 µg/mL) (P. Ristivojevic, 2016).

The cytotoxicity effect of the synthetic TiO₂NPs was evaluated in three human cancer cell lines (colon, liver, and breast cell line) in a different study conducted in the United States. The triple negative breast cancer cell line (MDA-MB 231) and the liver cancer cell line (HepG-2) were cultured in RPMI-1640 (Lonza, USA) supplemented with 10% FBS, while the colon cancer cell line (Caco-2) was grown in DMEM (Lonza, USA) containing 10% FBS. In sterile 96-well plates, 4 × 10³ cancer cells were seeded per well. Following a 24-hour period, three cancer cell lines were cultured at three different concentrations of the tested nanoparticles (40, 20, 10, 5, and 2.5 µg/ml) for a full 72 hours at 37 °C in an incubator with 5% CO₂. The viability of the cancer cells was measured after the incubation period. Following a 24-hour period, each well received 20 µl of 5 mg/ml MTT (Sigma, USA), and the plates were incubated for three hours at 37 °C. After incubating TiO₂NPs for 72 hours, the MTT solution was removed, 100 µl DMSO was added, and the absorbance of each well was measured using a microplate reader (BMG LabTech, Germany) at 570 nm to estimate the inhibition of cancer cell growth. The applications inhibitory concentration (IC₅₀) was calculated. GraphPad Instat was used to calculate the half-maximal and Applications inhibitory concentration (IC₅₀) values. Furthermore, a phase contrast inverted microscope equipped with a digital camera was used to examine morphological changes in cells both before and after

treatment with the synthesized TiO₂NPs (Olympus, Japan) (Martinez-Gutierrez, *et al.*, 2010)

A study that looked into apoptosis was conducted with minor modifications to methodology and assessed the presence of inter-nucleosomal DNA cleavage using an agarose gel electrophoresis DNA fragmentation assay. In short, MCF-7 cells were grown in 25 cm² cell culture flasks over night and exposed for 24 hours to an IC₅₀ concentration of TiO₂NPs. After a gentle scrape, the cells were collected using centrifugation. Following a 15-minute incubation period at 3°C, the cells were again suspended in 0.5 ml of lysis buffer (10 mM Tris-HCl, 0.5 mM EDTA (pH 8.0), 2% SDS, and 1 mM NaCl). To denature the protein content and purify the DNA, proteinase K (20 mg/ml) was added. For 45 minutes, the mixture was incubated at 5°C. This was followed by DNA extraction with a solution of equal amounts of phenol, chloroform and isoamyl alcohol (25:24:1). DNA was treated with 3 M sodium acetate (100 µl) for precipitation, and ice-cold 70% ethanol (200 µl) was used to wash the DNA pellet. DNA was examined electrophoretically in 1.5% agarose gels containing 0.1 µg/ml ethidium bromides and photographed using a gel documentation system (Thangam *et al.*, 2014).

Changes associated with TiO₂NPs-mediated apoptosis in MCF-7 cells were examined by acridine orange/ethidium bromide (AO/EB) double staining. Both viable and nonviable cells absorb AO dye and emit green fluorescence, while EB dye is absorbed only by nonviable cells and emit red fluorescence upon loss of membrane integrity. MCF-7 cells were cultured in a 6-well plate (1 x 10⁵ cells per well) and treated for 24 hours with the IC₅₀ concentration of TiO₂NPs. Untreated Cancer cells were used as a control. Treated cells were washed with PBS and stained with 20 µl AO/EB staining solution (100 µg/ml AO and 100 µg/ml EB) with 4/17 for 5 min. Stained cells were observed under a fluorescence microscope (Invitrogen EVOS FL Cell Imaging; 40x magnification) (Kowsalya *et al.*, 2020).

2.4 ROLE OF TITANIUM NANOPARTICLES IN KIDNEY STONE ANALYSIS

The purpose of the research was that the impact of nanotechnology on humans and animals could open new opportunities for research and change in the field of health science and became an urgent topic for consideration as a therapeutic tool (Sichert *et al.*, 2015). Kidney disease is a global problem, affecting

approximately 750 million people worldwide, and the severity of the disease varies widely. The prevalence of kidney disease and the availability of treatment are largely determined by social, cultural and political variables, all of which contribute to significant imbalances (Crews *et al.*, 2019). Nanoparticles are one-dimensional objects ranging in size from 1 to 100 nm. Nanotechnology refers to the development and production of NP materials of atomic or molecular size (Ma *et al.*, 2020). In medical nanotechnology, NPs are used for description, assembly, control and application of healing drugs and agents (Contera *et al.*, 2020).

The goal of the achievement is to study kidney disease becoming an important epidemiological problem worldwide. Despite significant scientific achievements, the pathophysiological processes involved in the development of many kidney diseases are still unknown. As a result of the rapid development of nanomedicine, a number of materials have been used in clinical therapy. The goal of developing kidney-targeted drugs is to increase drug levels and therapeutic efficacy while reducing drug toxicity and side effects. Changing the properties of the nanoparticle drug delivery system on the physiological or pathological characteristics of kidney stones can help achieve the goal of kidney stone size analysis. Although nanotechnology has shown some advantages in kidney diseases, several challenges need to be addressed and improved, including limited manufacturability, high cost, need for in vivo stability, less selective regulation, and potential damage to non-target organs. Currently, research on kidney-targeted drug delivery systems focuses on finding acceptable carriers and improving targeting efficiency, while the release profile and metabolic processes of drug delivery systems after penetration into target cells are still under investigation. Based on the data, our future research will focus on the creation of kidney-specific delivery systems for particle-based therapeutic drugs (Merlin & Li, *et al.*, 2022).

The size and distribution of the particles are the most important characteristics of nanoparticles because they dictate the delivery mechanisms, in vivo dispersion, biological fate, toxicity, and targeting potential. They also affect the stability of nanoparticles and the loading and release of drugs. The characterization of synthesized NPs is one of the most challenging tasks. The methods that are currently most frequently used to examine biosynthetic NPs are

X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and ultraviolet-visible (UV-vis) spectroscopy.

Nanoparticles are oxidative stress, inflammation, and interactions with the cell membrane, NPs may be harmful to cells. Conversely, Nano-toxicity is dictated by the drug concentrations and molecule purity, in addition to other characteristics like surface form, size, and ionic charge (Voigt *et al.*, 2014). Examples of toxicity that could result from independent drug delivery include inflammatory toxicity, excipient toxicity, particle toxicity, and contaminant toxicity. Toxicology should be studied in vitro and in vivo separately. The literature on the toxicity of therapeutic NPs is lacking. When examining each NP's potential for therapeutic use, nanotoxicity is an important subject to look into, particularly for those that call for multiple doses (De Jong *et al.*, 2013; El-Ansary *et al.*, 2013; De Matteis and Rinaldi, 2018). Prior to obtaining complete regulatory approval, every nanoparticle recommended for therapeutic use needs to have its local and systemic toxicity thoroughly investigated. To effectively deliver medications to the kidney, a great deal of research has been done on the anatomy of the kidneys and the characteristics of drug delivery devices (Kamaly *et al.*, 2016; Chen *et al.*, 2020).

According to upcoming research, after absorption, NPs travel throughout the body and engage in interactions before being eliminated by the kidney or the reticuloendothelial system (RES) are 20-25% of the cardiac output, or 1.1-1.2 L of blood, is received by the kidneys every minute. Each kidney contains an average of one million nephrons, the fundamental functional unit of the kidney. The size, shape, and charge of nanoparticles must be taken into account when creating nanomedicine because these factors will determine whether the kidneys clear the particles or accumulate them (Moss and Siccardi *et al.*, 2014).

Interaction sites and potential uptake sites of NPs that are extracted or used to target the kidney NPs enter the kidney through the renal artery and are transported to the afferent arteriole, where, depending on the characteristics of the particle, they either remain in the bloodstream or undergo renal filtration from the blood in the glomerular capillaries. While active targeting nanoparticles like peptides and antibodies are being researched to improve kidney targeting, characterized NPs may permit passive accumulation in the kidneys (Huang *et al.*, 2021).

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To synthesis, characterize the titanium nanoparticle using plant extract of *Scorpiadulcis* and to study their antimicrobial, cytotoxic and antitumor effects.

3.2 OBJECTIVES

Nanoparticles, depending on certain features like size, distribution, and morphology, show entirely new or enhanced properties. Antimicrobial activity is demonstrated by titanium nanoparticles. Since they don't require toxic chemicals for the synthesis protocol, the use of environmentally benign materials like plant leaf extract, bacteria, fungi, and enzymes for the synthesis of titanium nanoparticles offers numerous advantages of eco-friendliness and compatibility for pharmaceutical and other biomedical applications. Considering each of these perspectives, the current study was created with the following objectives in mind:

- To synthesize titanium nanoparticles using plant extract of *Scorpiadulcis*.
- 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 activity of the synthesized titanium nanoparticles against some of the pathogens like *S.aureus* and *E.coli*.
- To study the cytotoxic effect of the synthesized titanium nanoparticles on Vero cell lines and evaluate the anticancer activity against renal carcinoma cancer cell lines. Access the anti-inflammatory properties of the synthesized titanium nanoparticles, focusing in kidney stone applications and exploring drug delivery applications.

CHAPTER 4

MATERIALS AND METHODS

The materials used and the methods followed in conduct of various experiments and detailed here.

4.1 PREPARATION OF PLANT EXTRACTS OF *SCOPARIA DULCIS*

Preparation of 2.5mg of plant extract of *Scoparia dulcis* is weighed and it was crushed without adding water in a pestle and mortar. The 2.5mg of extract is boiled at 100°C and filtrate was again passing through whatmann no.1 filtering paper (pore size (25µm). The filtration then obtained and then stored at 4°C for further use.

4.2 PREPARATION OF 1MM TITANIUM SOLUTION

Titanium solution of about 1.599mg of TiO_2 weighed and it was dissolving in 100ml of distilled water in 500ml of conical flask. The preparation was mixed and swirled properly. The flask was then covered with aluminium foil in order to prevent the titanium solution from photocatalytic properties.

4.3 SYNTHESIS OF TITANIUM NANOPARTICLES

Synthesis titanium nanoparticles using 1MM of aqueous solution of titanium oxy sulphate (TiO_2) at a concentration of are prepared. 20 ml of *Scoparia dulcis* extract 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 at 24 hrs and white colour was occurred. Then, centrifuge at 6000RPM for 30 minutes. Supernatant is discarded and collected pellet.

4.4 ANALYSIS OF SYNTHESIZED TITANIUM NANOPARTICLES USING UV-VISIBLE SPECTROSCOPY

To measure the absorbance of synthesized Titanium Nanoaprticle under UV-Visible spectrophotometry. Ultraviolet-visible spectral range, absorption or reflectance spectroscopy is referred to as ultraviolet spectroscopy. It uses light in the visible and nearby ranges, according to this. The apparent color of the

compounds involved is directly impacted by the absorption of reflectance in the visible spectrum. Molecules and particles undergo electron mobility transitions in this electromagnetic range. This spectroscopy aids in determining whether nanoparticles are present. The absorption spectra of the nanoparticles are distinct over a range of wavelengths, from 200 nm to 800 nm.

A double beam UV-vis spectrophotometer in the 200-800nm range was used. The pellet phase adsorption for titanium nanoparticles was measured in 1cm quartz cell at 25°C. These dispersions were diluted to keep their measured absorbance values in the 2-5 range throughout the entire wave-length range.

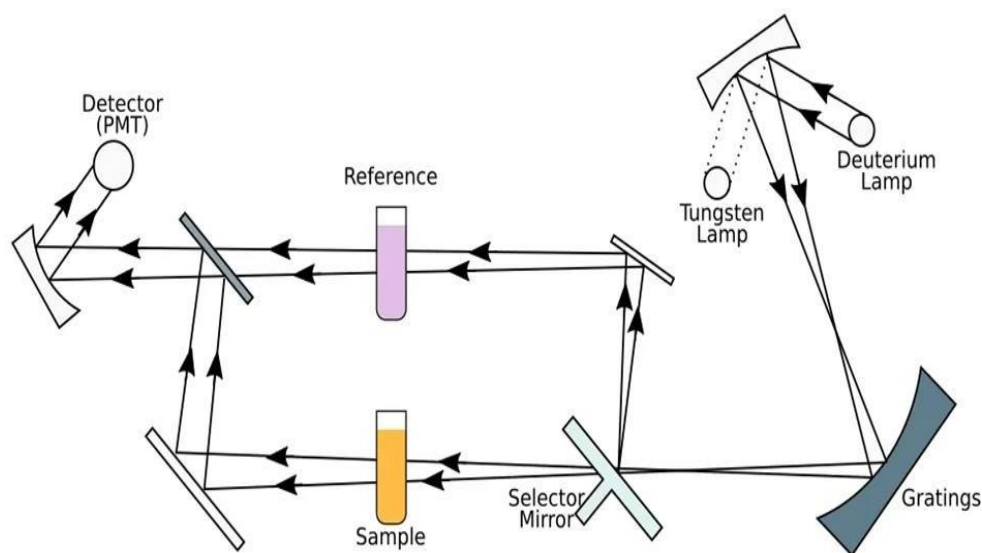


Fig 4.1: Working principle of UV-visible spectrophotometer

The reduction of pure TiO_2 ions is monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. 1ml of the sample solution was mixed with 2ml of sterile distilled water in a glass quartz cuvette. The contents were pipetted in and out to enhance proper mixing. Plant extract of *scoparia dulcis* of 3ml alone was served as blank. After keeping the blanks in the sample compartment, the baseline correction was made. Out of the two blanks, one blank was replaced with the sample solution and it was scanned from 800nm to 200nm. UV-Vis spectral

analysis is done by using double beam UV-Vis spectrophotometer (SpectrascanUV-2700, Thermo Scientific).

4.5 CHARACTERIZATION OF SYNTHESIZED TITANIUM NANOPARTICLES USING SEM

Rutherford elastic scattering is the bending of an electron's path caused by a fast electron coming into contact with a nucleus in the sample. This phenomenon is caused by the Coulomb attraction those results. A portion of these electrons undergo total backscattering and resurface on the sample's incoming surface. Images with both compositional and topological information can be obtained from the primary electrons that arrive at a specific detector location because the scattering angle is highly dependent on the atomic number of the involved nucleus. Loosely bound conduction band electrons in the sample can also interact with high-energy incoming electrons. The interaction provides these secondary electrons with a negligible amount of energy, and As a result, their area in the sample is extremely small—just a few nm.

As a result, the sample only releases secondary electrons that are produced very close to the surface. This indicates that the detection mode—which is the most often used SEM mode—offers topographic images with a high degree of resolution. Through the process of effective ionization, high-energy primary electrons can transfer electrons from the valence band to the conduction band when they strike the semiconductor sample. Either radiative or non-radiative processes can then be used to recombine the generated electron-hole pairs. Using a scanned beam, spectroscopy of the sample's visible and near-visible light allows for the mapping of spatial variations in luminescence and the determination of information about radiation recombination processes.

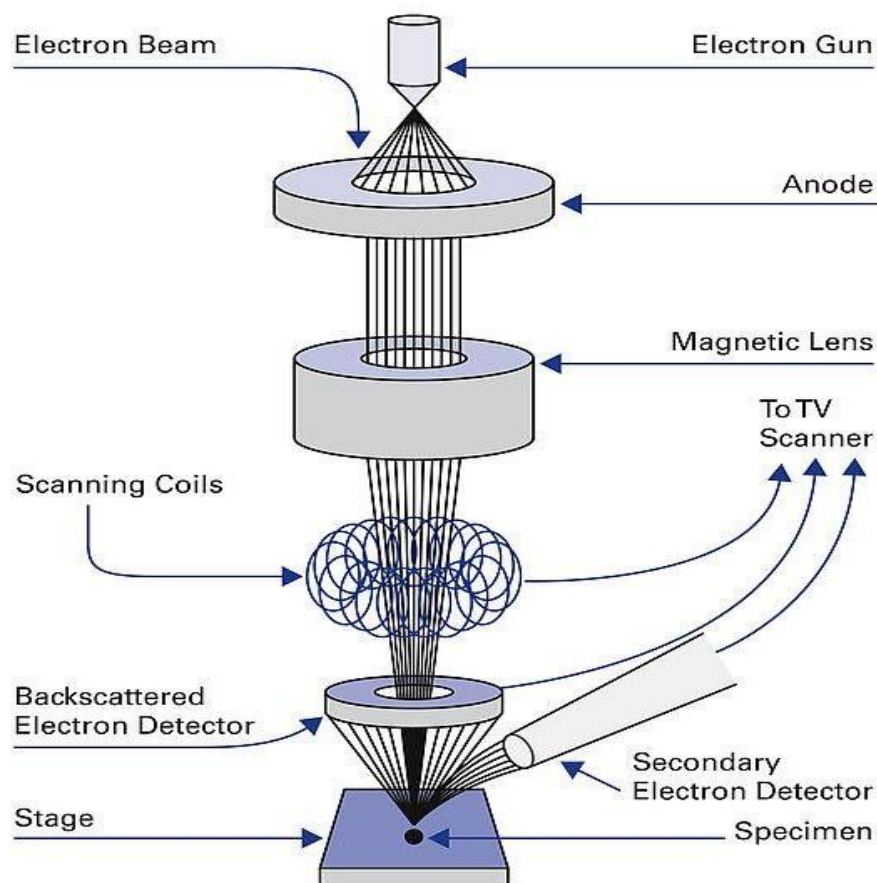


Fig 4.2: Working principle of SEM

The spatial extent of the collected image is determined by the distance between adjacent radio spots on the sample. Fast electronic transmission allows analysis of multiple particle fields without moving the stage; phase shift is reserved for large moves. Because a focused electron beam can only be generated in a relatively high vacuum; the beam, sample and detector are placed in a vacuum cabinet. The useful magnification achievable with SEM is determined by the size of the focal point projected onto the sample and is also ultimately limited by the diffraction of the focused electron beam.

The morphology and size distribution of titanium nanoparticles were analyzed using Scanning Electron Microscopy (SEM) according to the method. Initially, a small aliquot of the synthesized nanoparticles suspension was drop-cast onto a clean silicon wafer substrate and allowed to air-dry. The substrate was then sputter-coated with a thin layer of gold to enhance sample conductivity and

minimize charging effects during imaging. Subsequently, the prepared sample was loaded into the SEM chamber and subjected to vacuum conditions. SEM images were acquired at various magnifications using appropriate accelerating voltages and working distances to optimize resolution and contrast. The nanoparticles morphology, size, and surface features were characterized based on the SEM micrographs obtained.

4.6 CHARACTERIZATION OF EDAX ANALYSIS OF SYNTHESIZED TITANIUM NANOPARTICLES USING EDAX

An inner shell electron can be ionized, which allows an electron beam to interact with an atom. An outer electron fills the resulting vacancy and can release its energy by emitting an X-ray or, more importantly in this case, by using an Auger electron. As a result, the X-ray spectrum displays distinctive lines that represent the relevant electronic transitions. These lines are unique to a particular element, so it is possible to infer the material's composition. This can be used to show the abundance of a specific element as a function of position or to provide quantitative information about the elements present at a given point on the sample.

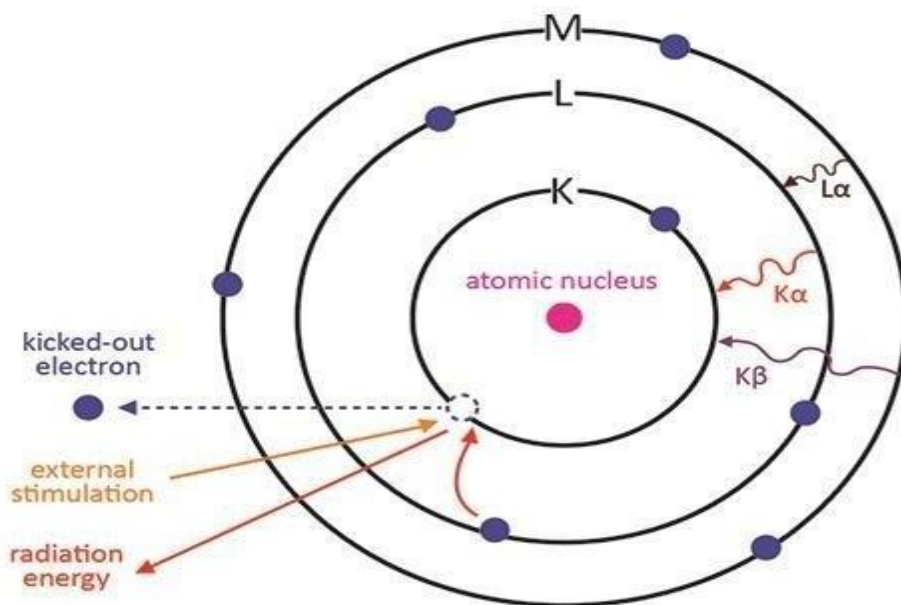


Fig 4.3: Working principle of EDAX

The elemental analysis of titanium nanoparticles, Energy-Dispersive X-ray Spectroscopy (EDS) was employed. Initially, the synthesized titanium

nanoparticles were dispersed onto a conductive substrate and allowed to air-dry. Subsequently, the prepared sample was mounted onto the SEM stage for analysis. The EDS analysis was conducted using appropriate parameters such as accelerating voltage and acquisition time to optimize signal-to-noise ratio and minimize background interference. The X-ray signals emitted by the nanoparticles upon electron beam excitation were collected by the EDS detector, generating spectra indicative of the elemental composition. Titanium content and the presence of any impurities or additional elements were determined based on characteristic X-ray peaks. Spatial mapping capabilities were utilized to assess the distribution of titanium within the nanoparticles, providing valuable insights into their chemical structure and homogeneity.

4.7 CHARACTERIZATION OF SYNTHESIZED TITANIUM NANOPARTICLE USING FTIR

The functional groups on the sample's surface are examined using FTIR. High-spectral resolution data across a broad spectral range is simultaneously collected by an FTIR spectrometer. For the same information, Fourier-transform spectroscopy is a less natural method. With this technique, a multi-frequency light beam is simultaneously shone at the sample in place of a monochromatic one, and the amount of the beam that is absorbed by the sample is measured. Subsequently, an alternative frequency combination is added to the beam, providing a second data point. This procedure is repeating many times. After that, a computer uses all of this information to calculate the absorption at each wavelength by going backwards. FTIR analysis that computer uses all of this information to calculate the absorption at each wavelength by going backwards. FTIR analysis of nanoparticles can be used to determine which chemical groups contributed to their creation and stabilization.

In order to confirm any potential structural characteristics in the samples, FTIR spectrum studies of the titanium nanoparticles are carried out. Using Fourier transform infrared spectroscopy, the surface of titanium nanoparticles in chemical functionalization by oxidation can be analysed. FTIR analysis was carried out on Agilent Cary 630 FTIR in mid-infrared range of 7000-350 cm^{-1} . FTIR spectra of

powdered titanium nanoparticles ground in pellet were captured, with 200 scans averaged at a resolution of 1 cm^{-1} .

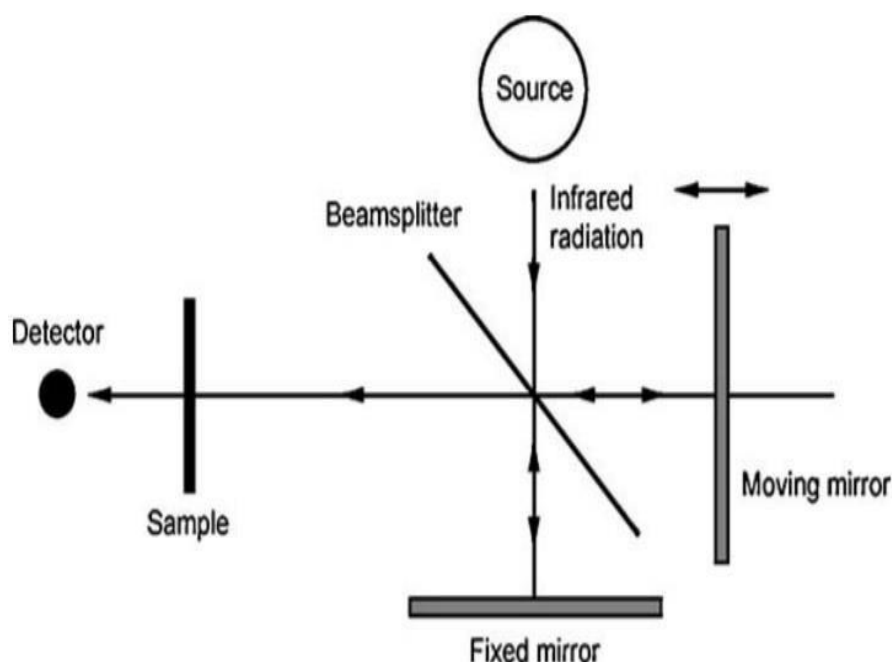


Fig 4.4: Working principle of FTIR

The FTIR analysis of titanium nanoparticles was conducted according to the method. Initially, a small amount of synthesized titanium nanoparticles was dispersed in potassium bromide (KBr) pellets. These pellets were prepared by grinding a mixture of titanium nanoparticles and KBr powder in a mortar and pestle. Subsequently, the pelletized samples were placed in the sample holder of the FTIR spectrometer. Spectral scans were performed over the mid-infrared range ($4000\text{--}400\text{ cm}^{-1}$) with a resolution of 4 cm^{-1} . Background spectra were collected and subtracted from the sample spectra to eliminate any interference. The resulting FTIR spectra were analyzed to identify characteristic peaks corresponding to functional groups present on the surface of titanium nanoparticles, aiding in the elucidation of their chemical composition.

4.8 CHARACTERIZATION OF SYNTHESIZED TITANIUM NANOPARTICLE USING XRD

The X-Ray diffraction (XRD) are used to ascertain crystallographic and amorphous properties of titanium nanoparticles. The radiation used was Cu K α

sources at 1.8 kW power in a theta scan mode with a wavelength of 0.40506nm to determine the amorphous structure and lattice planes in the material. The samples were scanned at diffraction angle ranged from $2\theta = 5^\circ$ to 80° (the step size is 0.025 degree/step) with exposure time 0.1 s/step. There was an applied voltage of 40 kV and an applied current of 40 mA. The Nelson-Riley (NR) approximation was used to correct the 2θ scan data in order to prevent systematic errors caused by sample holder thermal expansions and misalignments. The Nelson-Riley method is an extrapolation technique that corrects the systematic error caused by the sample's eccentricity with respect to the diffractometer's rotation axis. It has been discovered that this eccentricity is proportional to $\cos^2(\theta)$. Plotting the computed lattice parameter "a" from the raw data against $\cos^2(\theta)/\sin(\theta)$, a commonly used function for cubic systems, is how the correction is made. After that, the points are linearly fitted, and the corrected lattice parameter is found at the intersection with the y-axis.

XRD analysis was carried out on ARL EQUINOX 3000. Based on the diffractogram, various lattice planes against particular diffraction angles are computed and utilized to ascertain the intrinsic details of the structure

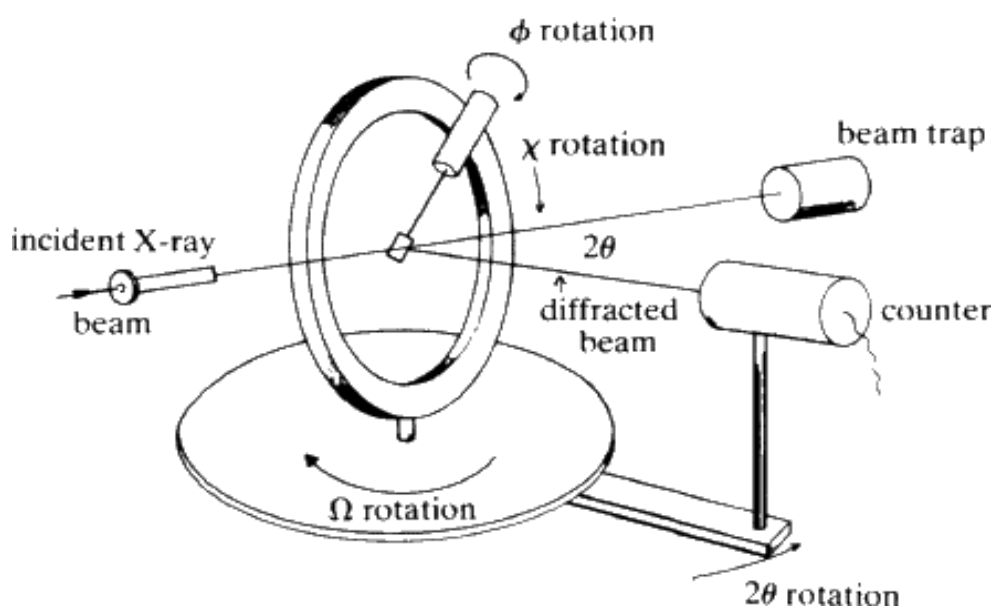


Fig 5.5: Working principle of XRD

The XRD analysis of titanium nanoparticles were finely ground and homogenized to ensure uniformity. Subsequently, the powdered sample was mounted on a glass slide using double-sided adhesive tape, ensuring a flat and smooth surface for analysis. X-ray diffraction patterns were recorded using a PANalytical X'Pert PRO diffractometer equipped with Cu K α radiation ($\lambda = 1.5406$ Å) operated at 40 kV and 30 mA. The scanning range was set from 10° to 80° (2 θ) with a step size of 0.02° and a scanning rate of 2°/min. The obtained diffraction data were analyzed using X'Pert HighScore software to identify the crystalline phases present in the titanium nanoparticles and determine their crystallographic structure.

4.9 ANTIMICROBIAL STUDIES

The agar well diffusion method was used to test the sample's antibacterial activity. *Staphylococcus aureus* and *Escherichia coli* were the pathogens used in this study. The newly cultured pathogens were quickly spread out on nutrient agar medium. 4 mm diameter wells were aseptically punched using a sterile cork borer, and then filled with varying concentrations of sample (25, 50, 75, and 100 µg/ml) and 20 µl of gentamycin (positive control; 10 µg). Following a 24-hour incubation period at 37°C, the zone of inhibition (ZOI; mm) that developed around the wells was measured for each plate.

Escherichia coli and *Staphylococcus aureus* were used in the diffusion method antibacterial assays. To put it briefly, bacteria were cultivated using nutrient broth/agar medium. Nutrient agar plates were inoculated with 100 µl of fresh overnight cultures of each culture. The cork borer was used to punch five wells: one in the center and four surrounding it. A standard gentamycin antibiotic disc weighing 24 µg was inserted into the center well, and the surrounding wells were filled with 25 µl, 50 µl, 75 µl, and 100 µl of the *Scoparia dulcis* synthesized nanoparticle solutions. After a 24-hour incubation period at 37°C, the plates were visually inspected to check for the development of ZOI.

4.10 CYTOTOXICITY AND ANTI-CANCER EFFECTS

Being toxic to cells/ is known as cytotoxicity. Different cell fates can arise from treating cells with a cytotoxic compound. Necrosis is a condition in which the cells

lyse rapidly and lose their membrane integrity. The cells can either initiate apoptosis, a genetic program that causes controlled cell death, or they can cease actively proliferating and dividing (a decrease in cell viability). The MTT can also be used to track cytotoxicity.

It is crucial to ascertain the cytotoxicity concentration of the synthesized titanium nanoparticles before investigating their antitumor activity. The upper limit of the extract concentration that does not harm the cell lines is known as the cytotoxicity test. Cell viability and death were assessed following the addition of the titanium nanoparticles that had been synthesized. Additional metabolic intervention experiments, like the MTT assay, validate the outcome.

MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole)

A cytotoxic drug is applied to cells that are growing exponentially. The amount of time needed for the maximum amount of damage to happen is typically used to calculate the exposure duration, although the drug's stability also plays a role. To separate the cells that are still viable and capable of proliferating from those that are viable but incapable of doing so, the drug is removed from the cells and they are allowed to proliferate for two to three populations doubling times (PDTs). MTT dye reduction is then used to indirectly calculate the number of surviving cells.

Once the MTT-formazan has been dissolved in an appropriate solvent, the amount of MTT-formazan produced can be measured spectrophotometrically. Cell culture assays are primary application that facilitation the evaluation of cell viability and proliferation. Since certain substances would either promote or prevent cell viability and growth, it can also be used to assess the cytotoxicity of possible medicinal agents and hazardous materials.

Materials which are used,

- Cell cultures
- Renal Carcinoma Cancer cell line (A498)
- Vero cell line
- DMEM (Dulbecco's modified Eagle's medium)

- MEM (modified Eagle's medium)
- 10% fetal bovine serum
- 1% penicillin streptomycin
- Cell culture flasks

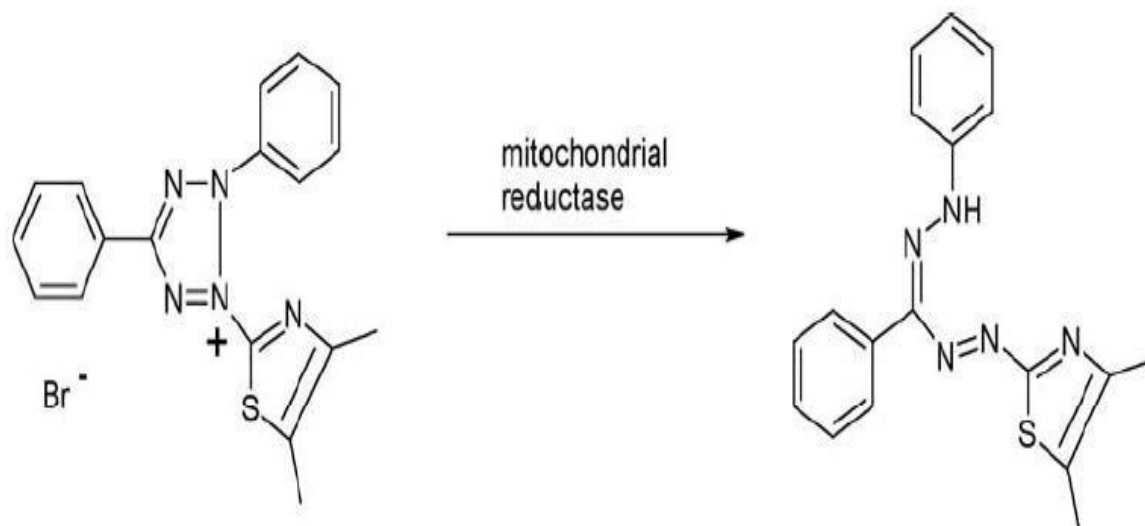


Fig 4.6: Metabolization of MTT by a mitochondrial reductase to a formazan salt by viable cells

Vero cell line and Renal Carcinoma Cancer Cell line (A498) were acquired from NCCS Pune. The cell lines were kept in a CO₂ incubator at 37°C and 5% CO₂. Using an inverted microscope, cultures were examined to determine the level of confluency and to confirm that there were no bacterial or fungal contaminants present. The cell monolayer was rinsed in half the volume of culture medium using PBS free of Ca²⁺ and Mg²⁺. The washed cell monolayer was treated with 1 ml of trypsin/EDTA per 25 cm² of surface area. To cover the monolayer with trypsin, the flask was rotated. After being put back in the CO₂ incubator, the flask was left for two to ten minutes. To make sure that every cell was detached and floating, the cells were inspected under an inverted microscope.

Materials which are used for MTT Assay,

1. Microtiter plate reader with 650- and 570-nm filters
2. Inverted microscope

3. Multi-channel pipette
4. 37°C incubator
5. Laminar flow hood
6. Microtiter plate (flat-bottomed)
7. Sterile tubes (5 ml)
8. Serological pipettes
9. Sterile pipette tips
10. DMSO

MTT Solution: 5 mg/ml MTT in PBS. Solution must be filter sterilized after adding

4.11 MTT ASSAY FOR RENAL CARCINOMA CANCER CELLS

Trypsinization was performed on a T-25 flask, and then 5 ml of full media was added to the trypsinized cells. For five minutes, 15 milliliters of sterile falcon tube were centrifuged at 500 rpm in the swinging bucked rotor (about 400 x g). After removing the media, the cells were resuspended in 1.0 milliliter of full media. We removed the cells in an aseptic manner. After that, they were tallied and noted in milliliters. 75,000 cells per milliliter of the cells (CV=CV) were diluted. The cells were diluted using complete media. Each well received 100µl of cells, totaling 7500 cells, and were left to incubate for the entire night.

New media and artificial titanium nanoparticles in different concentrations were added after the used media was removed. Each well had a final volume of 200µl. The MTT plate was incubated for four hours at 37°C in a CO2 incubator. The media and the artificially created titanium nanoparticles were taken out and placed into a fresh MTT 96-well plate after four hours. Each well received 80µl of media and 20µl of 5 mg/ml MTT. MTT was added to one set of wells, but no cells were added to the control set, which was kept aseptic. After that, the plates were incubated in a culture hood for 3.5 hours at 37°C. The media was taken out of the

picture. There was no disruption to the cells, and PBS was not used to rinse them. Next, 150µl of MTT solvent (DMSO) was added.

The medium was taken out of the six-well plates containing the Vero Cells and the Renal Carcinoma Cancer Cells (A498) using a micropipette. Phosphate buffer salted water was used to wash the cells. After fixing the cells with 1 milliliter of cold ethanol, they were incubated for roughly five minutes. Following fixation, distilled water was used to wash the cells. After that, the fixed cells were examined at a radical 40X magnification using a standard light microscope.

4.12 DAPI STAINING

Fluorescence microscopy of DAPI-stained cells (4', 6-diamidino-2-phenylindole) was used to study changes in the morphology characteristics of apoptosis. Nucleic acid staining with DAPI is another method for measuring nuclear condensation and fragmentation. In a 4-well plate, renal carcinoma cells were grown at a density of 1×10^6 cells per well on a glass cover slip. 10% fetal bovine serum was added to RPMI-1640 medium, which also contained 1% streptomycin and penicillin. The cells were then incubated for 24 hours at 37 °C in a humidified 5% CO₂ environment.

Titanium nanoparticle samples at 40µg/mL were applied to the cells for a full day. The cells cultured on the cover slip underwent three rounds of washing with 1 x phosphate-buffered saline (PBS), were fixed in 4% paraformaldehyde for 20 minutes, and were then stained with DAPI following two more rounds of washing with PBS. Using Vector Labs' vectar shield mounting medium, it was adhered to the glass slide. The Zeiss LSM780NLO confocal microscope system was used to take the pictures. $[\% \text{ of apoptotic cells} / (\text{total number of apoptotic nuclei} / \text{total number of cells counted}) \times 100]$ yielded the percentage of apoptotic nuclei.

4.13 ACRIDINE ORANGE STAINING

The dye acridine orange damages the membrane of the lysosome when exposed to intense blue light. Acridine orange is a weak base metachromatic dye that can stain lysosomes and nucleic acids while passing through plasma membranes. It can distinguish lysosomes (reddish-orange granules) from other cellular constituents (diffuse green) at low concentrations. In acidic environments,

acridine orange molecules get protonated and become trapped inside lysosomes. Acridine orange molecules build up in lysosomes, causing the excitation to change from green (530 nm) to red (620 nm). Acridine orange molecules become deprotonated when the PH of the lysosomes rises or if their membranes are damaged. Afterwards, these molecules can return to the cytoplasm. The emission changes back from red to green as a result. Resultantly, lysosomal membrane damage and a change in the color of the lysosomes from red to green occur when acridine orange-loaded cells are exposed to blue light for an extended period of time (>1 min). Acridine orange staining was employed to evaluate the destabilization of lysosomes. In short, 96-well black plates were seeded with 1 x 10⁶ renal carcinoma cancer cells (A498) per well, which were allowed to grow for two days before being incubated with 40µg/ml of titanium nanoparticles. The cells were incubated with acridine orange after a 24-hour period.

4.14 THE PILOT STUDY OF KIDNEY STONES WITH TITANIUM NANOPARTICLES

The aim of this study was to determine the kidney stone size analysing using titanium nanoparticles. The characterization of the synthetic nanoparticles is one of the most challenging tasks. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and ultraviolet-visible (UV-vis) spectroscopy have already been utilized as the most widely used techniques to examine biosynthetic TiO₂NPs.

Oxidative stress, inflammation, and interactions with the cell membrane are some of the ways that nanoparticles can be cytotoxic. On the other hand, factors that affect nanotoxicity include drug concentration, molecule purity, and other characteristics like surface shape, size, and ionic charge of the nanoparticles. Any NP's therapeutic potential should be considered when examining nanotoxicity, especially for those that need to be taken in multiple doses. To successfully deliver drugs to the kidney, the features of drug delivery devices and the structure of the kidney have been thoroughly studied.

The objective of developing kidney-targeting medications, like titanium, is to lower drug toxicity and side effects while raising drug levels and therapeutic efficacy. Targeting the kidney necessitates adjusting and controlling the properties of the nanoparticles in its delivery system, which are determined by the physiological or pathological features of the body.

Materials required which are used,

- Kidney stones
- Beakers
- Microcentrifuge tubes
- Spatula
- Water
- Magnetic stirrer
- Weighing machine
- Hydrochloric acid (HCl)
- Ethylenediaminetetraacetic acid (EDTA)
- Sodium chloride (NaCl)

The objective of this study was to evaluate the efficacy of titanium nanoparticles in analyzing kidney stone size. The procedure involved several steps to prepare the samples and assess the effects of titanium nanoparticles on the kidney stone specimens. Initially, representative samples of kidney stones, each weighing approximately one gram, were obtained and placed in individual clean and dry breakers. Subsequently, water solutions of varying concentrations were prepared in separate beakers to serve as the reaction medium. Add 1ml of sodium chloride (NaCl) solution was incorporated to maintain the ionic strength of the reaction mixture.

Next, titanium nanoparticles solutions of different concentrations, ranging from 1000 μ l to 22 μ l, were prepared in separate containers. Carefully measured

volume of each Titanium Nanoparticles solutions were then added to the respective beakers containing the prepared reaction medium and Subsequent to the addition of titanium nanoparticles, thorough mixing of the contents in each beaker was achieved using a magnetic stirrer to ensure uniform dispersion of the kidney stone sample and nanoparticles. The reaction mixtures were allowed to incubate for a predetermined period to enable interaction between the kidney stone components and the titanium nanoparticles.

Following the designated reaction time, analytical techniques such as spectrophotometry, microscopy, or particle size analysis were employed to assess any changes in kidney stone size induced by the presence of titanium nanoparticles. Observations and measurements, including alterations in morphology, composition, or size distribution of the kidney stone particles, were meticulously recorded. This comprehensive procedure aimed to elucidate the potential of titanium nanoparticles in kidney stone size analysis, providing valuable insights into novel approaches for kidney stone diagnosis and treatment.

CHAPTER 5

RESULTS AND DISCUSSION

Following are the results obtained from the present investigation.

5.1 SYNTHESIS OF TITANIUM NANOPARTICLES

Plant extract of *Scoparia dulcis* are mixed with 1mM titanium solution (1:9 ratios) and they were incubated under sunlight for 4 mins. As the *Scoparia dulcis* plant extract was mixed in the aqueous solution of titanium ion complex, it started to change colourless to white due to reduction of titanium ion, which indicated the formation of titanium nanoparticles in the reaction mixture. The change of colour is due to the excitation of surface plasmon vibrations in titanium nanoparticles (Krishnasamy *et al.*, 2015).

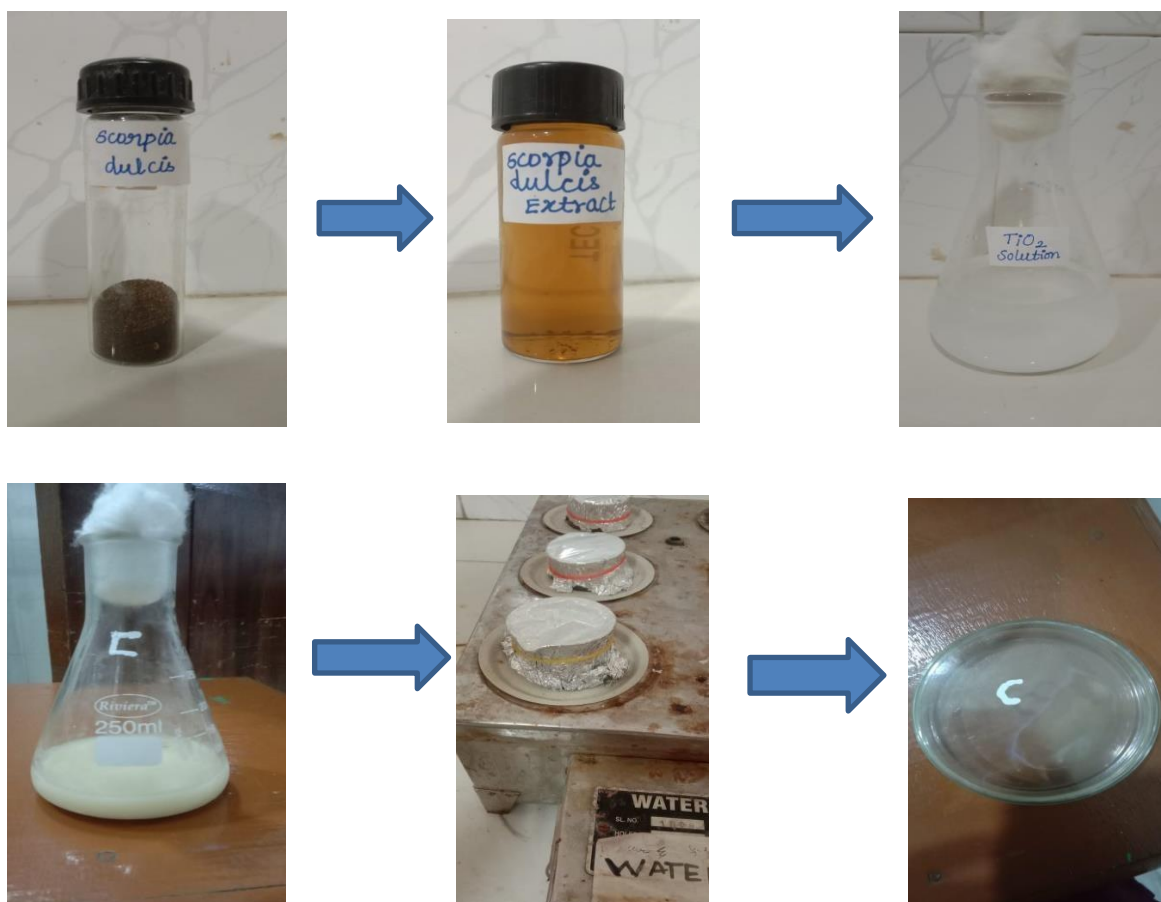
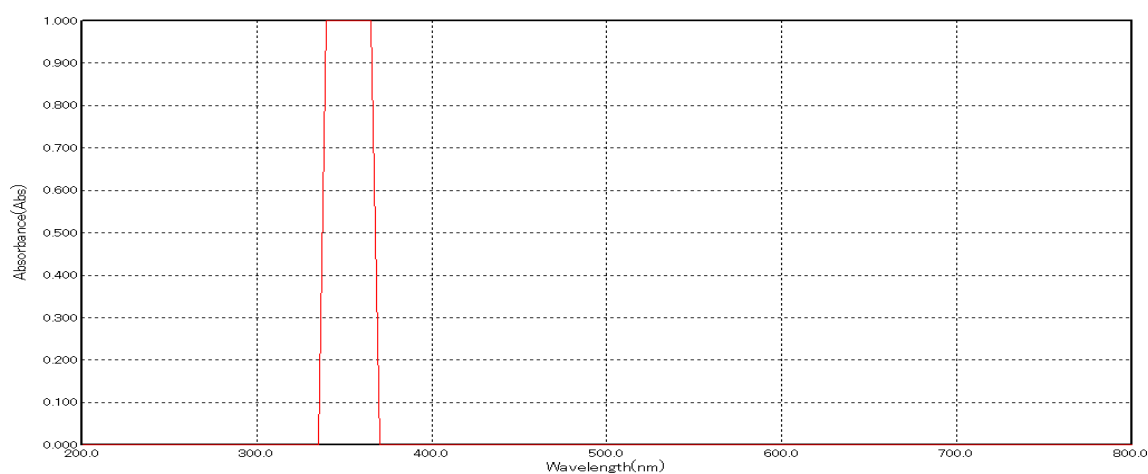


Fig 5.1: Synthesis of titanium nanoparticles

- | | | |
|--------|---|--|
| Tube 1 | - | Extract of <i>Scorparia dulcis</i> |
| Tube 2 | - | Preparation of Extract of <i>Scorparia dulcis</i> |
| Tube 3 | - | 1Mm of titanium oxy sulphate solution |
| Tube 4 | - | 20ml <i>Scorparia dulcis</i> filtrate + 80ml TiO ₂ (after four mins under sunlight) |

5.2 ANALYSIS OF SYNTHESIZED TITANIUM NANOPARTICLES USING UV-VISIBLE SPECTROPHOTOMETER

It is generally recognized that UV-Vis spectroscopy can be used to examine size and shape-controlled nanoparticles in aqueous suspensions. Double beam UV-Vis spectrophotometer (SpectrascanUV-2700, Thermo Scientific) was used to analyze the samples.



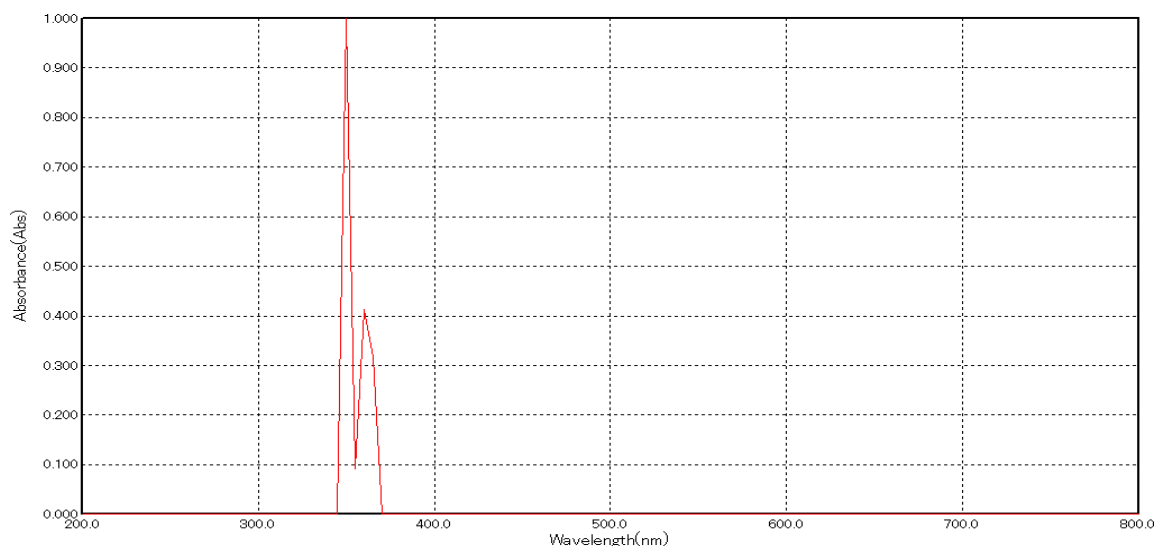


Fig 5.2: Absorption peak of synthesized titanium nanoparticles from *Scoparia dulcis* under UV-Visible double beam spectrophotometer

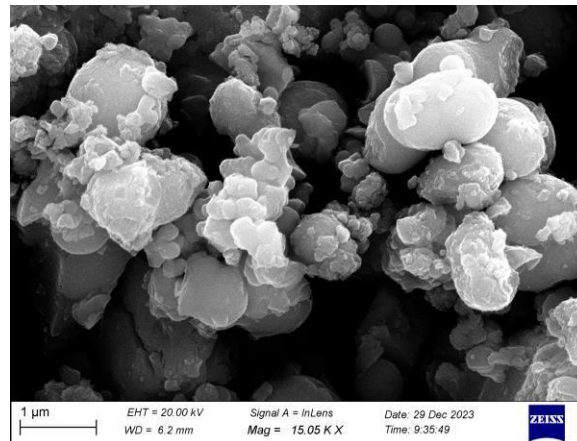
Table 5.1 UV-Visible spectrum peak list for Titanium nanoparticles

Peak List			
No.	Wavelength(nm)	Abs	Trans(%)
No Peak Data!			
Max Record 200.0 nm – 800.0 nm			
No.	Wavelength(nm)	Abs	Trans(%)
1	0.0	0.000	0.0
MinRecord 200.0 nm – 800.0 nm			
No.	Wavelength(nm)	Abs	Trans(%)
1	0.0	0.000	0.0
Average Record 200.0 nm – 800.0 nm			
No.	Wavelength(nm)	Abs	Trans(%)
1	200.0-800.0	0.028	99.3
Record List			
No.	Wavelength(nm)	Abs	Trans(%)
1	800.0	0.000	100.0
6	775.0	0.001	100.1
11	750.0	0.000	100.0
16	725.0	0.000	100.0
21	700.0	0.000	100.0
26	675.0	0.000	100.0
31	650.0	0.000	100.0
36	625.0	0.000	100.0
41	600.0	0.000	100.0
46	575.0	0.000	100.0
51	550.0	0.000	100.0
56	525.0	0.000	100.0

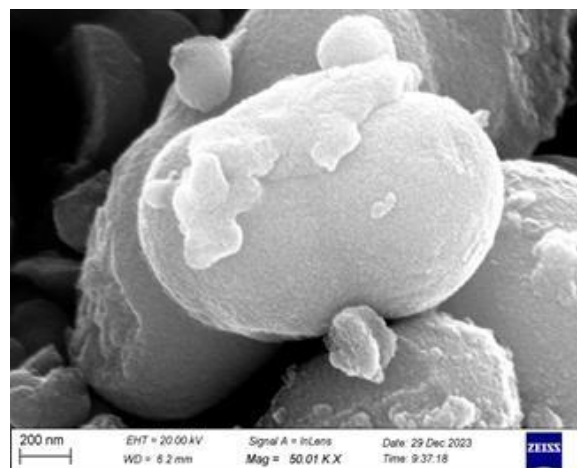
Plant extract of *Scoparia dulcis* acted as blank. Small aliquot was prepared by mixing the sample solution to sterile distilled water. It was then scanned under UV from 800nm to 200nm. It shows the UV-Vis spectra are record from the reaction mixture after 2 hours. Absorption spectra of titanium nanoparticles formed in the reaction medium obtained from *Scoparia dulcis* has absorbance peak at 360 nm. The broadening of the peak indicates the titanium nanoparticles synthesized from *Scoparia dulcis* are polydispersed. (Begum S.J *et al.*, 2022).

5.3 CHARACTERIZATION OF SYNTHESIZED TITANIUM NANOPARTICLES USING SEM

- The biosynthesised titanium nanostructure eby employing plant-extract of *Scoparia dulcis* was further confirmed by size and structure view under the scanning electron microscope. The useful magnification achievable with SEM is determined by the size of the focal point projected onto the sample and is also ultimately limited by the diffraction of the focused electron beam. High resolution imaging of single nanoparticles with size well below 10nm. The titanium nanoparticle was observed at 37nm in 1 μ m and 4 μ m in size (Jia et al., 2019)



(A)



(B)

Fig 5.3: FESEM of images of titanium nanoparticles in *Scoparia dulcis*

5.4 CHARACTERIZATION OF SYNTHESIZED TITANIUM NANOPARTICLES USING EDAX

- The biosynthesised titanium nanostructure by employing plant extract of *Scoparia dulcis* was further confirmed by structural view under the scanning electron microscope and possible compounds in each of the sample was identified using EDAX. The elemental composition such as oxygen, sulfur, titanium and carbon in 10µm presented in the titanium nanoparticles. Using EDAX, the percentage of titanium present in the synthesized titanium nanoparticle solution from *Scoparia dulcis* is 58.12%. (Irshad *et al.*, 2020)

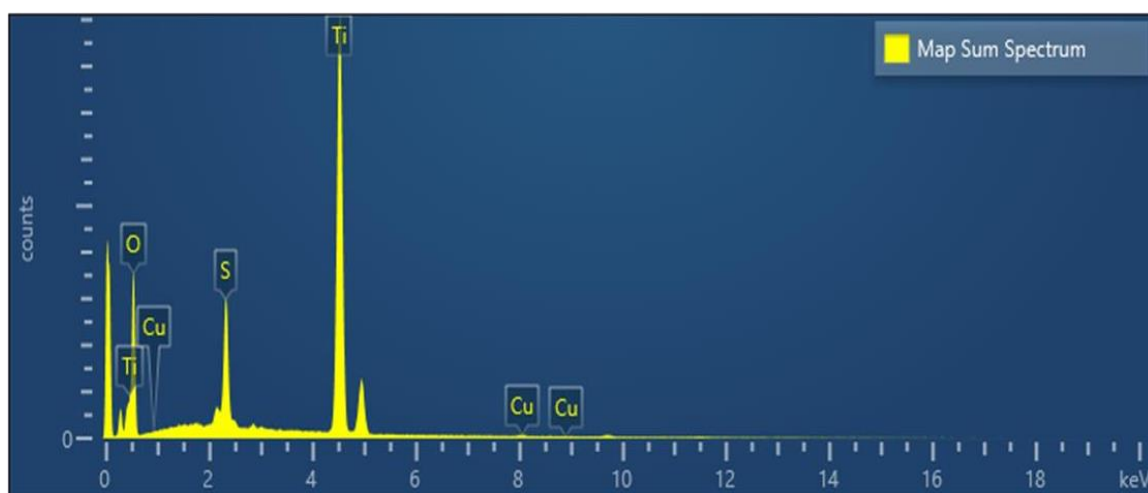


Fig 5.4: EDAX analysis of titanium and other compounds from *Scoparia dulcis*

Table 5.2 – EDAX analysis of chemical compounds present in titanium nanoparticles

Map Sum Spectrum				
Element	Line Type	Weight %	Weight % Sigma	Atomic %
O	K series	52.49	0.32	75.83
S	K series	5.43	0.07	3.91
Ti	K series	41.71	0.29	20.12
Cu	K series	0.38	0.09	0.14
Total		100.00		100.00

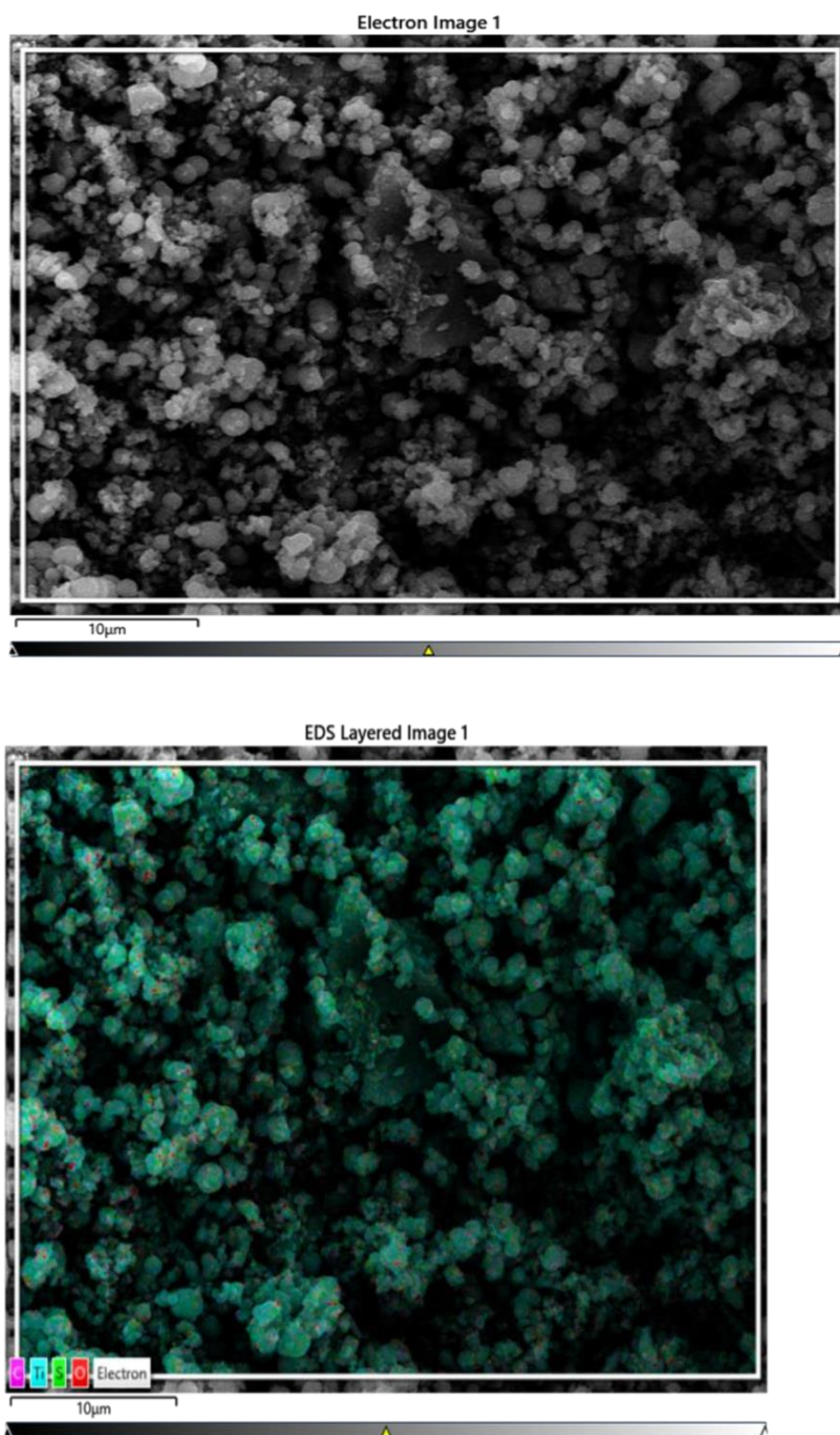


Fig 5.4: EDAX images of titanium nanoparticles using *Scoparia dulcis*

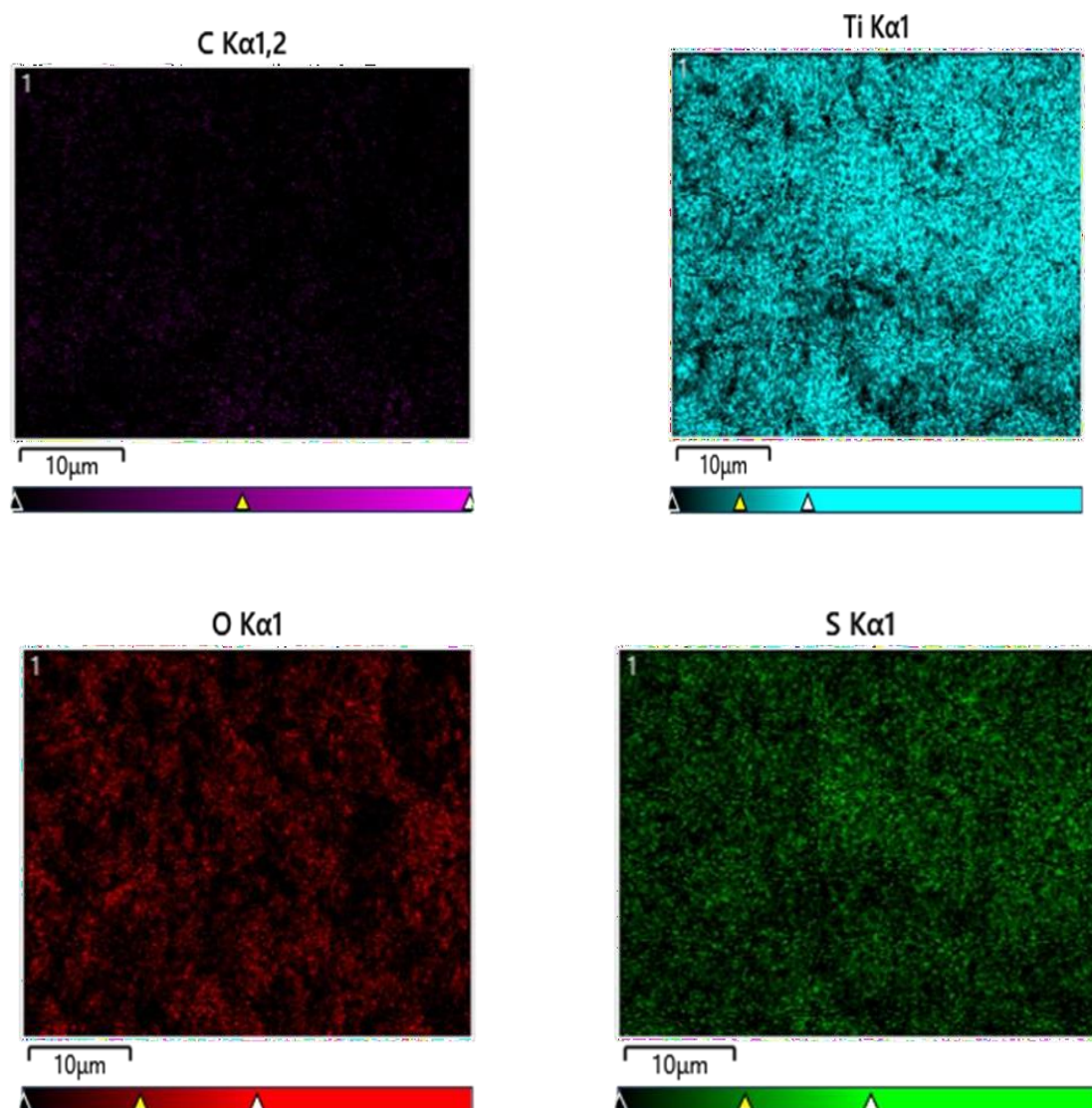


Fig 5.5 EDS- Electron images of titanium nanoparticles using *Scoparia dulcis*

5.5 CHARACTERIZATION OF SYNTHESIZED TITANIUM NANOPARTICLES USING FTIR

To characterize the synthesized titanium nanoparticle by using plant extract of *scoparia dulcis* and confirmed under crystallographic structure of the material and the possible compounds in each of the sample was identified using FTIR. FTIR for titanium nanoparticles range from 400-4000 cm^{-1} at the point of 1122.3 FTIR spectra of titanium nanoparticle using *Scoparia dulcis* exhibited an absorption band between 900 and 1200 cm^{-1} , which is indicative of a O -Ti- O stretching bond (Fig 5.6). (Amanulla,A.Metal.,2019)

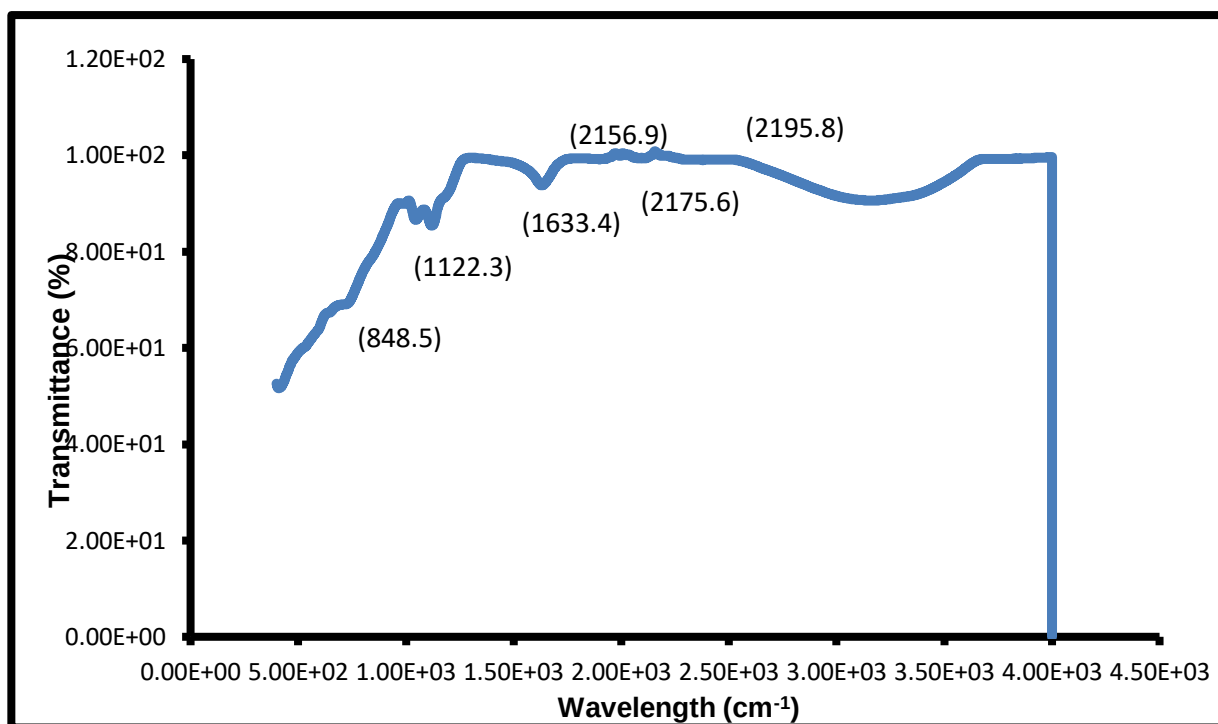


Fig 5.6: FTIR analysis of titanium and other compounds from *Scoparia dulcis*

CHARACTERIZATION OF SYNTHESIED TITANIUM NANOPARTICLES USING XRD

To characterize the synthesized titanium nanoparticle by using plant extract of *scoparia dulcis* and confirmed under crystallographic structure of the material and the possible compounds in each of the sample was identified using XRD. It identifies the composition of titanium nanoparticles and crystalline phase and 2θ range of 20° to 80° was analyzed in X-Ray diffractometer. The titanium nanoparticles peaks at 40.5° which are indexed as 343 as confirmed the titanium nanoparticles. It can provides insights into the particle size and morphology and high crystallinity phase of titanium nanoparticles (Fig5.7). (Irshad et al., 2020)

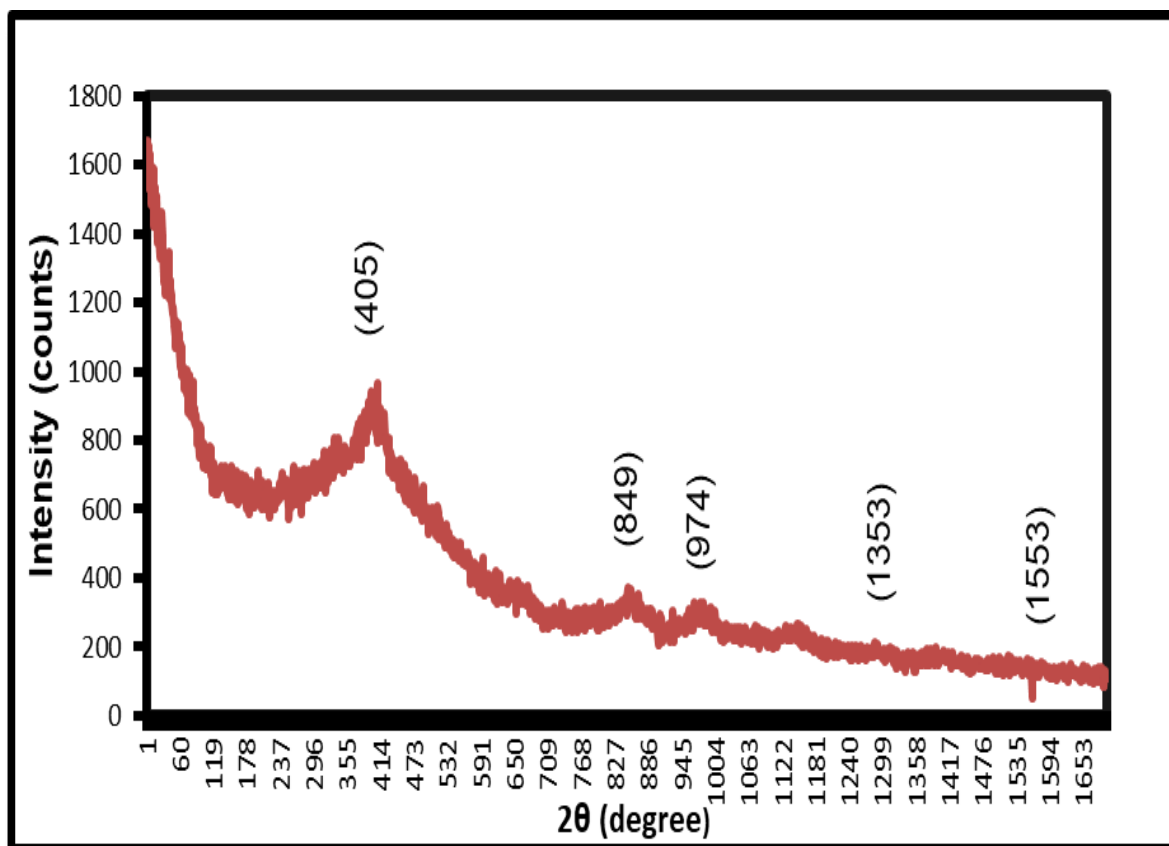


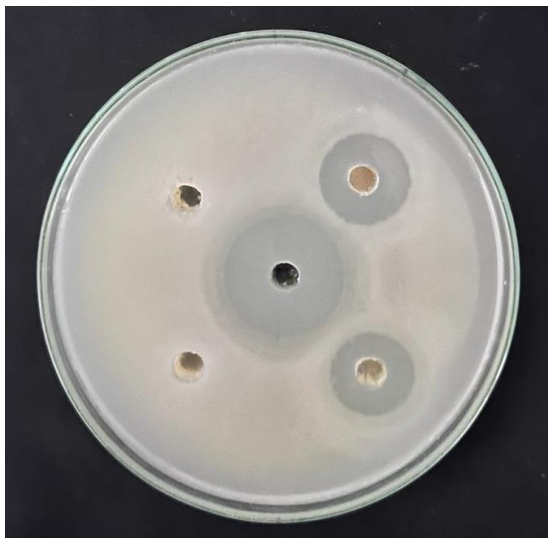
Fig 5.7: XRD analysis of titanium and other compounds from *Scoparia dulcis*

5.6 ANTIMICROBIAL STUDIES

The nanoparticles synthesized by green synthesis are found toxic against *E.coli* and *S.aureus* at the concentration of 100 μ l (Plates 4 and 5). Titanium nanoparticles can exhibit antibacterial activity against *E. coli* and *Staphylococcus aureus* and it shows a clear inhibition zone and they were compared with the standard antibiotics like gentamycin disc of 24 μ g (Mosachristas *et al.*, 2019).

The control, gentamycin disc had formed ZOI of 24 mm diameter against *E.coli*, Whereas the synthesized titanium nanoparticles from *Scoparia dulcis* for 25 μ l, 50 μ l, 75 μ l, and 100 μ l was showing ZOI of 0mm, 0mm, 16mm, and 19mm

respectively. When compared to the control, nanoparticles are showing better zone of inhibition with 75µl and 100µl concentration against *E.coli*.



E. coli

Fig 5.8: Antimicrobial effects of titanium nanoparticles from scoparia dulcis on Escherichia coli

Well c - 24µg of gentamycin antibiotic disc

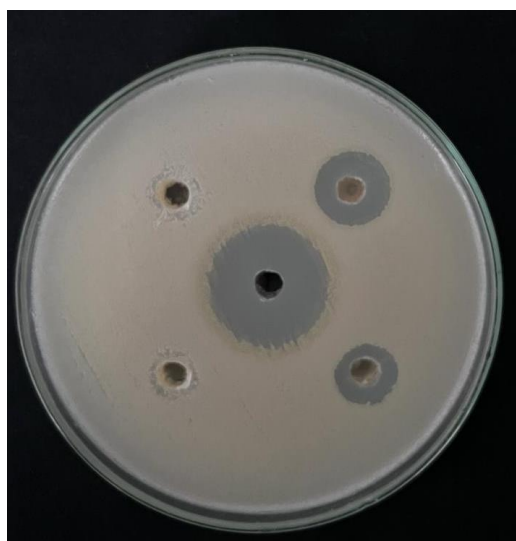
Well 1 - 25µl of synthesized titanium nanoparticle solution (from *Scoparia dulcis*)

Well 2 - 50µl of synthesized titanium nanoparticle solution (from *Scoparia dulcis*)

Well 3 - 75µl of synthesized titanium nanoparticle solution (from *Scoparia dulcis*)

Well 4 - 100µl of synthesized titanium nanoparticle solution (from *Scoparia dulcis*)

The control, gentamycin disc had formed ZOI of 24 mm diameter against *Staphylococcus aureus*, Whereas the synthesized titanium nanoparticles from *Scoparia dulcis* for 25 µl, 50 µl, 75 µl, and 100 µl was showing ZOI of 0mm, 0mm, 12mm, and 15mm respectively. When compared to the control, nanoparticles are showing better zone of inhibition with 75µl and 100µl concentration against *Staphylococcus aureus*.



Staphylococcus aureus

Fig 5.9 – Antimicrobial effects of synthesized titanium nanoparticles from *Scoparia dulcis* on *Staphylococcus aureus*

Well c - 24µg of gentamycin antibiotic disc

Well 1 - 25µl of synthesized titanium nanoparticle solution (from *Scoparia dulcis*)

Well 2 - 50µl of synthesized titanium nanoparticle solution (from *Scoparia dulcis*)

Well 3 - 75µl of synthesized titanium nanoparticle solution (from *Scoparia dulcis*)

Well 4 - 100µl of synthesized titanium nanoparticle solution (from *Scoparia dulcis*)

Table 5.3 – ZOI (mm) formed by synthesized titanium nanoparticles from *Scoparia dulcis* against varying strains of microorganisms

Microorganism	25	50	75	100	+ve
SA	0	0	12	15	24
E.coli	0	0	16	19	24

5.7 CYTOTOXICITY EFFECTS AND ANTICANCER ACTIVITY

The MTT dye assay is one of the colorimetric assays for measuring the activity of enzymes that reduce MTT or close dyes (XTT, MTS, and WSTs) to formazan dyes, giving a purple color.

The synthesized titanium nanoparticles from plant extract of *Scoparia dulcis* was treated with both Vero cells and Renal Carcinoma Cancer Cells (A498) with varying concentration such as 10µg, 20µg, 30µg, 40µg, 50µg, 60µg, 70µg. The untreated Vero Cells and Renal Carcinoma Cancer Cells (A498) served as control. (Aswini *et al.*, 2021)

Table 5.4 – % of Inhibition of Vero cells are treated with varying concentration of the synthesized titanium nanoparticles from *Scoparia dulcis* and their corresponding absorbance in ELISA reader

NAME	CONCENTRATION	OD1	OD2	MEAN	% of viability	% of inhibition
CONTROL		0.049	0.052	0.0505	92.58443465	7.415565345
scorpia dulcis	10	0.218	0.289	0.2535	62.7753304	37.2246696
scorpia dulcis	20	0.217	0.311	0.264	61.23348018	38.76651982
scorpia dulcis	30	0.323	0.364	0.3435	49.55947137	50.44052863
scorpia dulcis	40	0.341	0.386	0.3635	46.6226138	53.3773862
scorpia dulcis	50	0.429	0.413	0.421	38.17914831	61.82085169
scorpia dulcis	60	0.458	0.497	0.4775	29.8825257	70.1174743
scorpia dulcis	70	0.575	0.545	0.56	17.76798825	82.23201175

5.7.1 Cytotoxic and anticancer effects of titanium nanoparticles synthesized from *Scoparia dulcis*

The cytotoxicity of vero cell lines having an IC_{50} value of 1.025 μg when it was treated with titanium nanoparticles from *Scoparia dulcis* whereas the cytotoxicity of Renal Carcinoma Cancer Cell (A498) was showing an IC_{50} of 0.26525 μg and this study says when the titanium nanoparticles treated can cause cytotoxic effect for Renal Carcinoma Cancer Cells (A498) at 0.26525 μg , and the same concentration would not affect the normal vero cells (Table 5.4 and Fig 5.10)

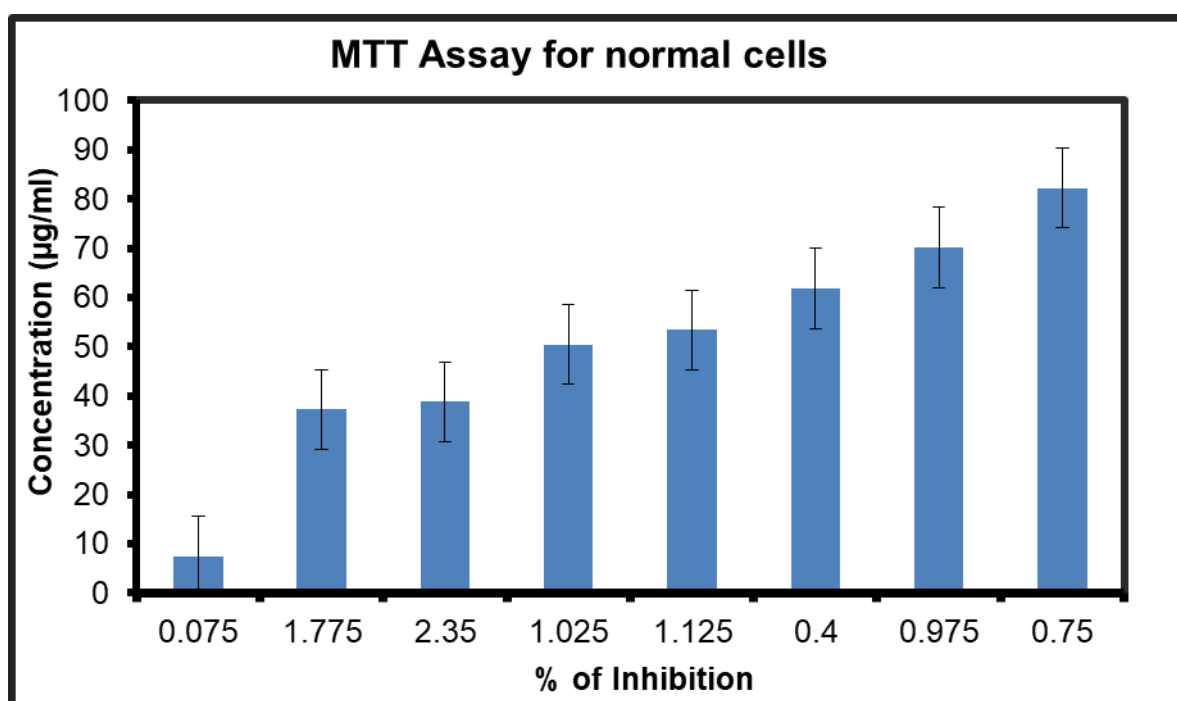


Fig 5.10: % of Inhibition of calculated on MTT absorbance in Vero cells that are treated with varying concentration of the synthesized titanium nanoparticles from *Scoparia dulcis* and IC_{50} dose in response graph, their corresponding absorbance in ELISA reader.

Table 5.5 – % of Inhibition of Renal Carcinoma Cancer cells (A498) are treated with varying concentration of the synthesized titanium nanoparticles from *Scoparia dulcis* and their corresponding absorbance in ELISA reader

NAME	CONCENTRATION	OD1	OD2	MEAN	% OF VIABILITY	% OF INHIBITION
control		0.076	0.222	0.149	91.64329781	8.356702187
Scoparia dulcis	5	0.058	0.544	0.301	83.11833988	16.88166012
Scoparia dulcis	10	0.093	0.878	0.4855	72.77061133	27.22938867
Scoparia dulcis	15	0.112	1.109	0.6105	65.75995513	34.24004487
Scoparia dulcis	20	0.146	1.443	0.7945	55.44026921	44.55973079
Scoparia dulcis	25	0.284	1.345	0.8145	54.31856422	45.68143578
Scoparia dulcis	30	0.247	1.654	0.9505	46.69097027	53.30902973

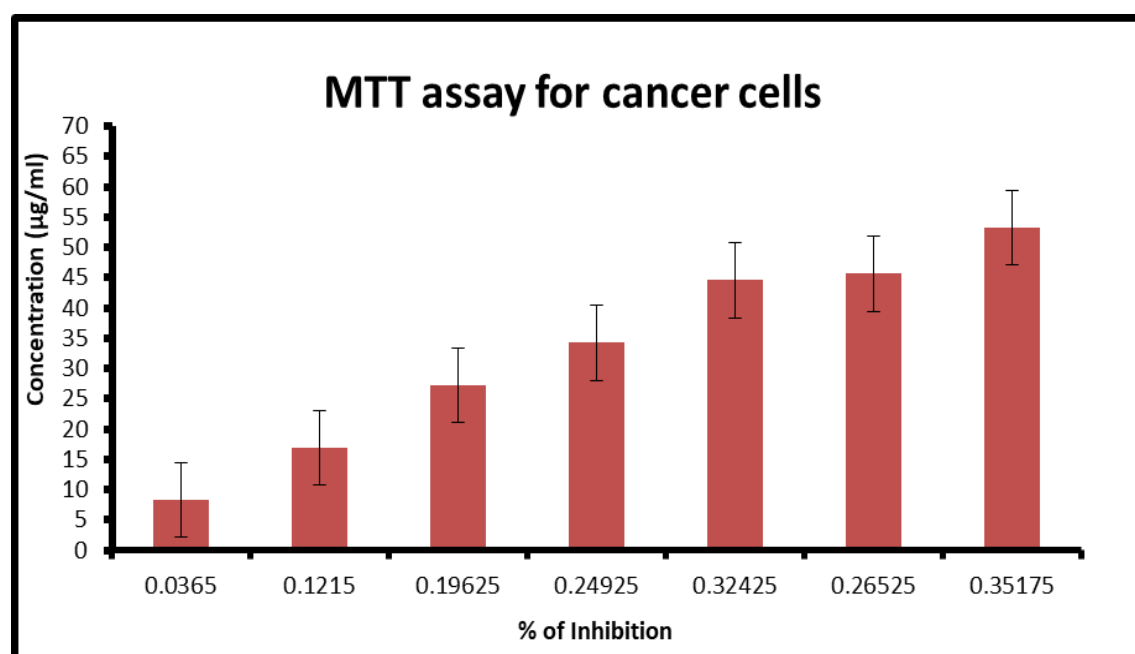


Fig 5.11: % of Inhibition of calculated on MTT absorbance in Renal Carcinoma Cancer cells (A498) that are treated with varying concentration of the synthesized titanium nanoparticles from *Scoparia dulcis* and IC50 dose in response graph, their corresponding absorbance in ELISA reader.

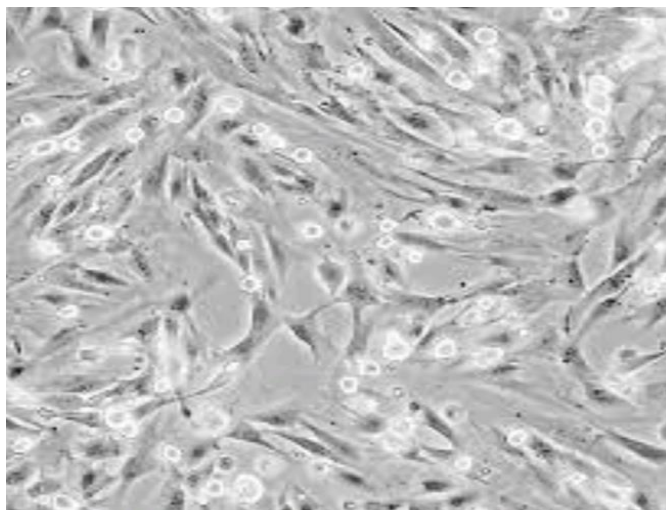
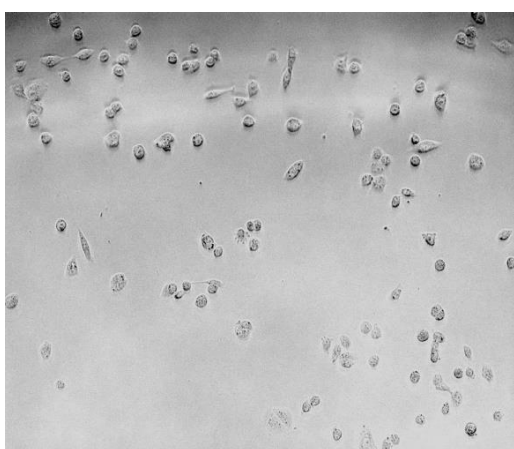
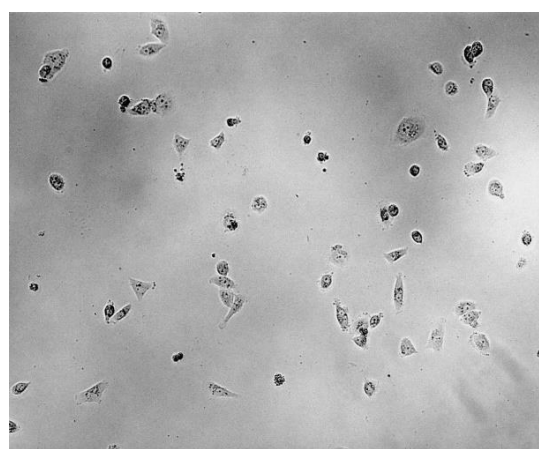


Fig 5.12: Untreated Vero cells and treated Vero cells with synthesized titanium nanoparticles from plant extract of Scoparia dulcis under radical light microscope of 40X magnification



Untreated Renal Cancer Cells

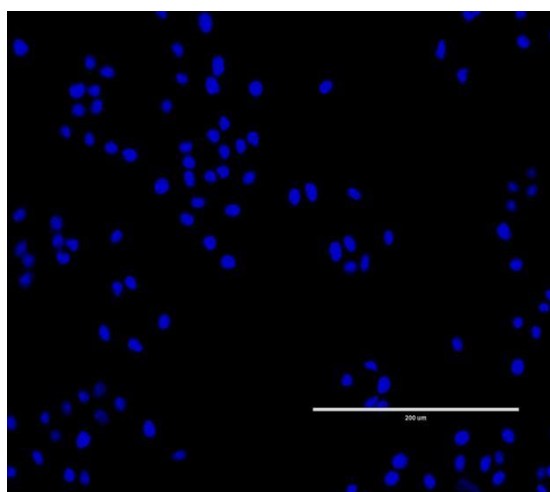


Treated Renal Cancer Cells

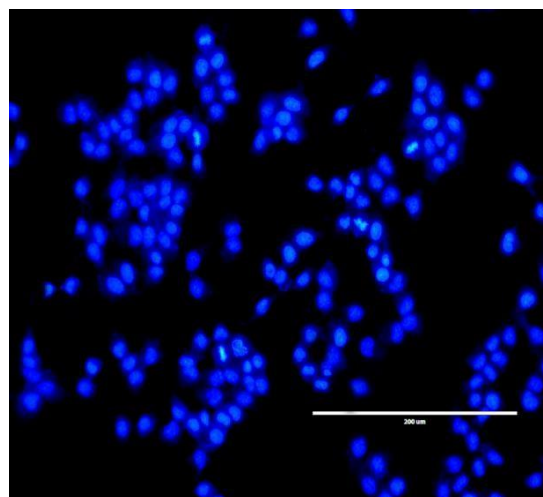
Fig 5.13: Treated Renal Carcinoma cancer cells (A498) with synthesized titanium nanoparticles from plant extract of Scoparia dulcis under radical light microscope of 40X magnification

5.8 DAPI STAINING

DAPI (40-60-diamidino-2-phenyl-indole) staining was used to identify the nuclear morphology alterations (condensed chromatin and fragmented nuclei) in Renal Carcinoma Cancer cells (A498) treated with titanium nanoparticles. DAPI stain colors both live and fixed cells by binding firmly to DNA regions rich in adenine and thymine. One million cells per well in a 6-well plate were cultured and exposed to titanium nanoparticles at an IC_{50} (0.26525) concentration for a duration of 24 hours. Following incubation, the cells were permeabilized with 0.2% Triton X-100 (50 μ l) for 10 min at room temperature, fixed with 3% paraformaldehyde (50 μ l), and cleaned with PBS. Following a 5-minute staining process with 10 μ l of DAPI stain (0.5 μ g/ml), the cells were examined under a fluorescence microscope. (Kowsalya *et al.*, 2020)



CONTROL

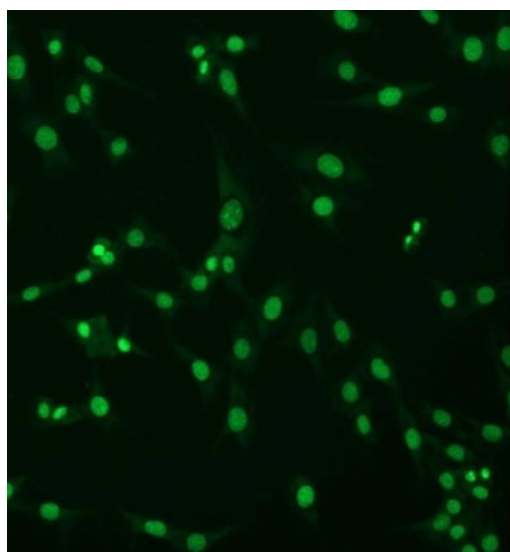


IC_{50} TREATED CELLS

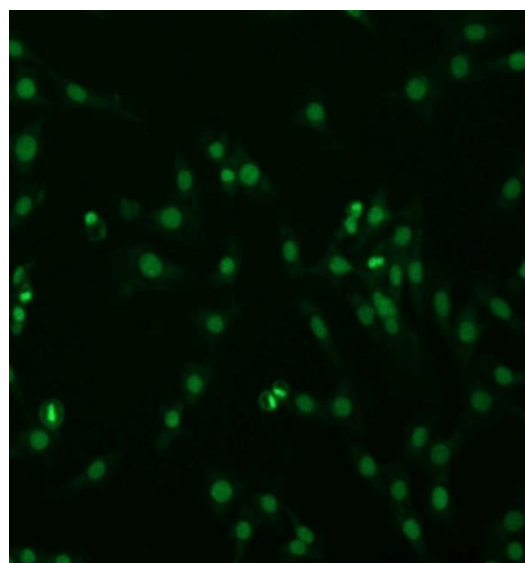
Fig 5.14: Fluorescent images of DAPI for the renal carcinoma cancer cells (A498) exposed to titanium nanoparticles

5.9 ACRIDINE ORANGE STAINING

Acridine orange staining was used to examine the titanium nanoparticles mediated apoptosis-associated alterations in renal carcinoma cancer cells (A498). Cells that are viable or nonviable both absorb AO stain and fluoresce green. After being cultivated in a 6-well plate with 1×10^5 cells per well, the Renal Carcinoma Cancer cells (A498) were exposed to titanium nanoparticles at an IC_{50} (0.26525) concentration for a duration of 24 hours. As a control, the untreated Renal Carcinoma Cancer cells (A498) were used. After treating the cells with PBS, they were stained for five minutes using 20 μ l of an AO staining solution (100 μ g/ml AO). A fluorescence microscope (Invitrogen EVOS FL Cell Imaging; 40 $\times$ magnification) was used to view the stained cells. (Uzma *et al.*, 2020)



CONTROL



IC_{50} TREATED

Fig 5.15: AO staining fluorescent micrographs of titanium nanoparticles treated renal carcinoma cancer cells (A498)

5.10 THE PILOT STUDY OF KIDNEY STONE WITH TITANIUM NANOPARTICLES

A sample of the kidney stone is typically subjected to different conditions to assess its reactivity and solubility. For instance, in the described experiment, a portion of the kidney stone (1g) is added to a beaker with varying concentrations of water. Then, add known volume of sodium chloride (NaCl) solution is added to create an environment conducive to chemical reactions. Additionally, different concentrations of titanium nanoparticles are introduced to the solution. Titanium nanoparticles are known for their potential application in various fields, including medicine and catalysis. The dissolution behavior of the kidney stone is evaluated based on its size. It is observed that if the stone is between 5 and 10 mm in size, approximately 30% of it will dissolve under the experimental conditions. However, if the stone is smaller than 5 mm, it will dissolve a lesser amount.

TABLE 6.6 - KIDNEY STONE SIZE ANALYZING

days	1000μl	500μl	250μl	125μl	63μl	22μl
Control	0.29	0.26	0.16	0.11	0.09	0.07
day 5	0.55	0.47	0.39	0.26	0.21	0.15
day 10	0.39	0.32	0.28	0.23	0.21	0.12
day 15	0.34	0.29	0.24	0.21	0.18	0.13
day 20	0.31	0.27	0.21	0.16	0.12	0.09
day 25	0.29	0.25	0.18	0.11	0.07	0.06
day 30	0.24	0.19	0.13	0.09	0.06	0.04

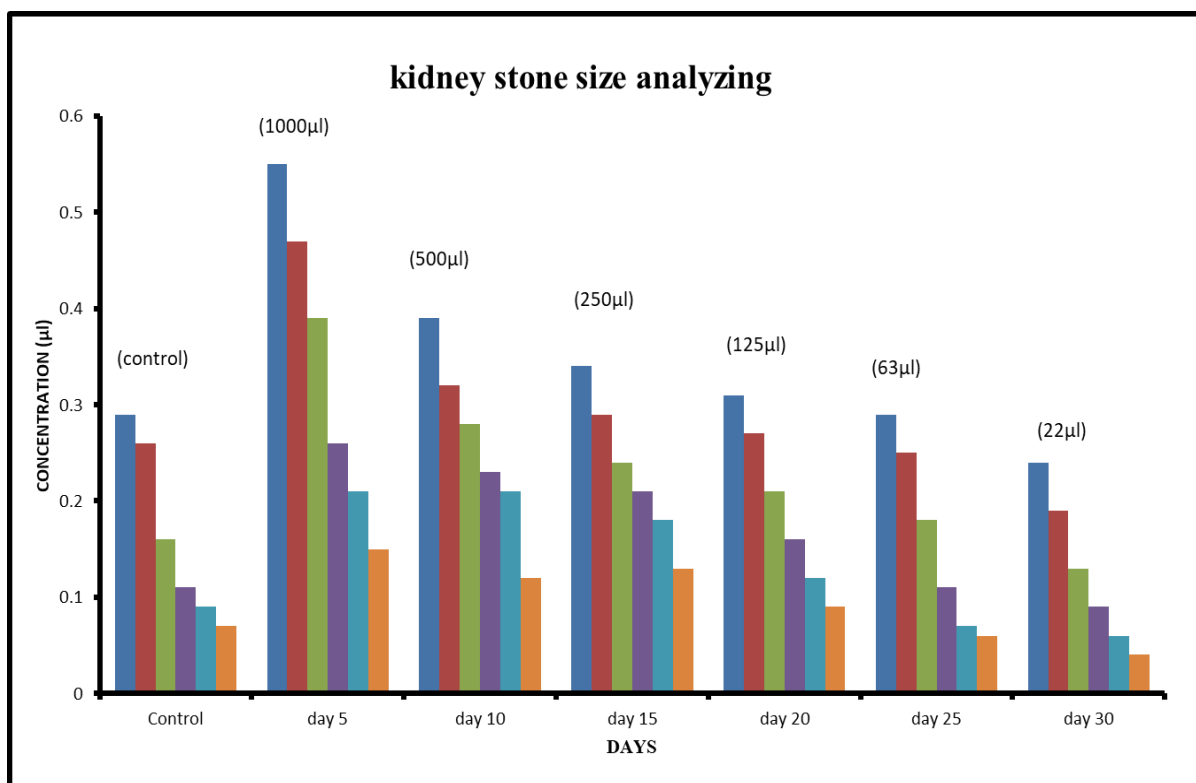


Fig 5.15: Role of titanium nanoparticles in kidney stone size analysing with response graphs



CONTROL



DAY 5



DAY 10



DAY 15



DAY 20



DAY 25



DAY 30

Fig 5.17: Images of different concentration of titanium nanoparticles in kidney stone size analysing

CHAPTER 6

SUMMARY AND CONCLUSION

Following are the highlights emanated from the investigation.

The titanium nanoparticles are synthesizing from the plant extract of *Scoparia dulcis* and synthesized titanium nanoparticles were detected by observing the change of colour from colourless to white colour. While observing the synthesized titanium nanoparticles solutions under UV-Visible double beam Spectrophotometer, there was broadening of the peak for the sample at 360nm. This says that all the synthesized nanoparticles are not of uniform size. They are polydispersed titanium nanoparticles.

The titanium nanoparticles synthesized from *Scoparia dulcis* was characterized using SEM and the size of titanium nanoparticles ranged between 37nm and 41nm. SEM enables detailed examination of the morphology and size distribution of titanium nanoparticles synthesized with plant extracts. It provides quantitative data on elemental ratios, allowing researchers to assess the purity and composition of the synthesized nanoparticles. The percentage of titanium present in the synthesized titanium nanoparticle solution from *Scoparia dulcis* is 53.18%.

FTIR analysis identifies the presence of functional groups on the surface of titanium nanoparticles synthesized with plant extracts. The titanium nanoparticles synthesized from *Scoparia dulcis* was characterized to confirmed under functional group characterized and crystallographic structure of the material and the possible compounds in each of the sample was identified using XRD.

The titanium nanoparticles synthesizing from *Scoparia dulcis* when treated against *E.coli* and *S.aureus* was showing the maximum ZOI as 16mm, 19mm in *E.coli*, 12mm and 15mm in *S. aureus* respectively. The concentration present in 75 μ l and 100 μ l each of the sample loaded is 1.69 μ g. Out of this, the titanium

nanoparticles synthesized was effective against *S.aureus* and *E.coli* are less effective. The antimicrobial studies of the synthesizing titanium nanoparticles are promising against *S.aureus* and *E.coli* and also less effect are observed, when comparing with control, gentamycin.

The titanium nanoparticles derived from *Scoparia dulcis* exhibited cytotoxicity against both the vero and renal carcinoma cancer cell lines, with respective IC_{50} values of 1.125 μg and 0.26525 μg . According to the study, titanium nanoparticles made from *Scoparia dulcis* were effective at 0.26525 μg against cancer cells that cause renal carcinoma. A cytotoxic and the anticancer activity were also investigated with the vero cells and renal carcinoma cancer cell lines respectively. Therefore, the present investigation concludes that the green synthesized titanium nanoparticles are one of the potent tools of anticancer activity. DAPI staining images expressing the emergence of apoptosis in renal carcinoma cancer cells. DAPI staining, which revealed obvious alterations in the nuclear morphology (chromatin condensation and nuclear fragmentation) of the titanium nanoparticle-treated cells, was used to confirm apoptosis. The application of titanium nanoparticles induced cell shrinkage, membrane blebbing, and ruffling, as well as other typical apoptotic features, according to morphological studies using AO staining. When AO intercalate with the cell's DNA, they produce green fluorescence. Under a fluorescent microscope, viable cells will take up the AO stain and appear green. The size and shape of the untreated cells were essentially uniform, and they released AO's green fluorescence.

It is possible to suggest that titanium nanoparticles can function as drug molecules for targeted drug delivery based on current research. Nevertheless, there is currently a lack of a scientific paradigm regarding the potential (adverse) reactivity of nanoparticles, and our knowledge of the fundamentals of how nanoparticles interact with living things is limited. To create and use safe nanoparticles in drug delivery in the future, one must have a conceptual understanding of the biological reactions to nanomaterials. Moreover, close cooperation between individuals involved in particle production and drug delivery is required for the sharing of ideas, strategies, and know-how to advance this problem.

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