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Green Synthesis Of Titanium Nanoparticle From Leaf Extract Of Prosopis Cineraria And Cytotoxicity Analysis

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Abstract
The current research goal was to successfully bio-synthesize titanium NPs from the leaf extracts of Prosopis cineraria. Alkaloids, saponins, flavonoids, tannins, steroids, phenols, carbohydrates, and proteins were found to be the phytochemicals in the leaf at quantities of 268.5, 26.5, 61.7, 27.7, 1.393, 32.1, 7.9, and 1.98 mg/ml during the study. The development of titanium nanoparticle was validated by utilizing UV-Visible spectroscopy showing a peak range of 200-400 nm. The morphology of nanoparticle was studied utilizing TEM analysis which showed a size range of titanium NPs from 13.49 to 23.07 nm at a scale of 200 nm. The FTIR spectrum showed multiple peaks at 3401.46 cm^{1} (aromatic primary amine), 3326.69 cm^{1} (imino compound, =N-H stretch), 1634.81 cm^{1} (amide group) and 686.83 cm^{1} (aromatic C-H out-of-plane bend), while the peaks between 666.37cm^{1} to 619.26 cm^{1} represents the alkyne C-H band, respectively. During the MTT assay, the lowest concentration tested, i.e. 0.125 mg/mL) yielded the highest cell viability at 95.65%. Based on the findings, it can be inferred that the created NPs have the potential to demonstrate various antimicrobial, antioxidant, and pharmacological properties, making them applicable in the biomedicine, environmental pollution, remediation, and healthcare industries. Prosopis cineraria leaf extract is more

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[1] . INTRODUCTION

The engineering of matter at the nanoscale, or nanotechnology, has become a topic that has the potential to revolutionize many different industries. The phrase "nanotechnology" was first termed by Prof. Norio Taniguchi, highlighting its focus on manipulating and controlling structures and phenomena at the nanometre scale.[1] Nanomaterials have demonstrated potential in applications extending from targeted drug delivery and bio-imaging in medicine to high-performance electronics, energy conversion and storage, and environmental remediation.[2]Several techniques have been developed and utilized regarding the (NPs) synthesis, such as approaches for chemical and physical synthesis. The significance of green synthesis in nanoparticle production are multi-fold, such as that it minimizes or eliminates the need for such harmful substances, thereby reducing the environmental impact and health risks associated with nanoparticle synthesis.[3] Additionally, green synthesis techniques often involve simple and cost-effective procedures, making them economically viable for large-scale nanoparticle production. Secondly, plant extracts and other renewable materials are utilized in green production, a technique, that decreases the reliance on non-renewable resources, making it a more sustainable approach.[4]Metal NPs have originated from the exploration of materials at the nanoscale and have gained substantial interest because of their distinctive attributes as well as widespread instances of use.[5] Several metals, such as gold and silver, are biocompatible and can be used in biomedical applications. They exhibit low toxicity and can be functionalized for targeted drug delivery, imaging, and bio-sensing.[6] (TiO_2 NPs) have accumulated substantial focus because of their distinctive qualities and extensive-ranging applications. The various characteristics of titanium turn it into an advantageous substance for a variety of sectors, including aerospace, automotive, medical, and chemical processing. [7]

suitable for nanoparticle synthesis due to its richer phytochemical content, greater efficiency in nanoparticle stabilization, and enhanced biological activity, making it superior to pods, bark, or stem.
Keywords: *Titanium Nanoparticle, Prosopis cineraria, phytochemicals, FTIR, TEM, cytotoxiocity*

Titanium, as a transition metal, exhibits multiple oxidation states or reduction states in various industrial applications. The most common oxidation states of titanium are +2, +3, and +4. Titanium dioxide (TiO_2) is widely employed in its +4-oxidation state as a catalyst, pigment, and photocatalyst. It is utilized in various chemical processes, such as the production of plastics, coatings, and ceramics.[8] The implication of TiO_2 NPs lies in their capability to improve the performance of various materials and systems. In drug delivery systems, TiO_2 NPs can enhance drug loading and release profiles, leading to improved therapeutic outcomes.[9] Titanium NPs, particularly TiO_2 NPs (Titanium Dioxide NPs), possess a substantial amount of study focus on their potential use in solving sustainability issues.[10]In terms of applications, TiO_2 NPs find extensive use in medicine, especially in scaffolds for tissue engineering, medication delivery systems, implant materials, and bioimaging. Their distinct qualities distinguish them suitable for orthopaedic implants due to their excellent biocompatibility, strength, and corrosion resistance.[11] TiO_2 NPs are also utilised in electronics, catalysts, energy storage devices, and cleanup of the environment.[12] The importance of TiO_2 NPs lies in their capacity to enhance effectiveness of various materials and systems. Their incorporation into orthopedic implants enhances bone integration, reduces inflammation, and improves mechanical stability.[13] In drug delivery systems, TiO_2 NPs can enhance drug loading and release profiles, leading to improved therapeutic outcomes.[14] Moreover, TiO_2 NPs have been investigated as catalysts for a range of chemical processes because of their distinct reactivity and surface characteristics.[15]Titanium NPs have shown promising potential as environmental remediation agents due to their unique properties and reactivity. In particular, TiO_2 NPs have been thoroughly researched for their potential to alleviate environmental issues. TiO_2 NPs have been used in wastewater treatment, heavy metal removal from water, and photocatalytic destruction of organic contaminants and air purification.[16] Their large surface area, photocatalytic activity, in addition to the capacity to produce reactive oxygen species make them effective in the breakdown of organic pollutants and the transformation of harmful substances into less toxic forms. The use of TiO_2 NPs in environmental remediation offers benefits like minimal cost, high efficiency, and environmental sustainability. These NPs offer a viable method for eliminating contaminants from various environmental matrices, contributing to the mitigation of environmental contamination.[17]*Prosopis cineraria* is a medicinal plant species that is economically significant and found in semi-arid as well as in arid areas of India, Pakistan, Afghanistan, Arabia, and Iran, belongs to the Fabaceae family. A phytochemical exploration of the leaves exposed the presence of derivatives of phenolic acid and hydrocarbons.[18] *Prosopis cineraria* has various applications across different fields of industry, growth, and development. Various parts of *Prosopis cineraria*, including leaves, bark, and gum, have traditional medicinal uses. They are believed to possess therapeutic properties and are used in remedies for ailments such as respiratory disorders, skin conditions, digestive issues, and as a general tonic.[19, 20] The applications of *Prosopis cineraria* across various fields highlight its versatility, economic importance, and contributions to sustainable development in arid regions.[21]Numerous phytochemicals, including phenols, flavonoids, tannins, alcohols, ethers, and amines, are abundant in *Prosopis cineraria* leaf extract and are necessary for the reduction and capping of metal ions during the nanoparticle-making process. These materials enable the ecologically friendly production of stable, bioactive copper and silver NPs with enhanced antibacterial and anticancer capabilities.[22]

Significance of (TiO_2) NPs

(TiO_2) NPs are among the most the most acclaimed and studied NPs due to their remarkable physical, chemical, and optical properties.[23] The three primary crystalline forms of TiO_2, a naturally occurring oxide of titanium, are anatase, rutile, and brookite. The anatase form, in particular, is highly active in photocatalysis due to its large bandgap, which makes it useful in a variety of environmental and energyrelated applications.[24]The medical field is another important area where TiO_2 NPs are used. TiO_2 is biocompatible, which makes it sustainable for use in drug delivery systems and medical implants. Its high surface area enhances its performance in bio-sensing applications, while its UV-blocking ability makes it a common ingredient in sunscreens and cosmetic products. Additionally, TiO_2 NPs are being researched for their potential in cancer therapy, where they could be applied to targeted medication delivery and as photosensitizers in photodynamic therapy.[25]Similarly, environmental toxicity concerns regarding TiO_2 NPs are addressed by who studied the impact of the impact of these NPs on rainbow trout sperm cells' kinematics and biochemical quality. The study revealed that exposure to TiO_2 NPs significantly decreased sperm cell velocity and induced oxidative stress, marked by an increase in superoxide dismutase (SOD) activity and total glutathione levels. These results suggest that while TiO_2 NPs have valuable industrial and medical applications, their environmental toxicity, especially in aquatic systems, warrants careful consideration due to potential negative impacts on aquatic life.[26]For example, a 2025 study demonstrated the green synthesis of (TiO_2) NPs using *Ulva lactuca*, a marine macroalgae. These NPs showed significant antibacterial, anti-inflammatory, and anticancer activity, with strong cytotoxic effects against oral carcinoma cells. This study reports the green synthesis of (TiO_2 NPs) using *Ulva lactuca* extract. The ULTiO_2 NPs were crystalline (20–50 nm), spherical, and rich in functional groups. They showed strong antibacterial, anti-inflammatory (22%–78% protein denaturation reduction), and anticancer activity (IC_{50} = 28.74 μg/ml). These properties highlight their potential for use in medical coatings and drug delivery.[27]This study reports the greenly produced TiO_2 NPs using *Chrysanthemum indicum* leaf extract. The cubical, evenly distributed NPs were characterized by SEM and FTIR. They showed an 8% diesel degradation over 10 days and exhibited hemolytic, antidiabetic, anti-inflammatory, and antioxidant activities. These results demonstrate how biosynthesized TiO_2 NPs can be used as environmentally friendly agents for bioremediation and biomedical applications.[28]Another study synthesized TiO_2 NPs using *Piper longum* leaf extract, confirming the formation of anatase-phase TiO_2 with notable antibacterial properties., the study confirmed the NPs' biological activity.[29] Similarly, TiO_2 NPs synthesized using *Psidium Guajava* were incorporated into chitosan-based films for antimicrobial packaging applications. These NPs demonstrated strong antibacterial efficacy.[30]Another study explores the green synthesis of (TiO_2) NPs using *Spirulina* extract and their antibacterial activity against multidrug-resistant bacteria. The synthesized NPs showed a 322 nm absorbance peak, 3.85 eV band gap, spherical shape, and 61.4% crystallinity (anatase phase). They effectively inhibited MRSA, *P. aeruginosa*, *E. coli*, and *E. faecalis*, with strong activity at 80 μg/ml. Minimum bactericidal concentrations ranged from 7.812 to 62.5 μg/ml. These findings support the potential of Spirulina-mediated TiO_2 NPs as bactericidal agents against resistant pathogens.[31] **Green Synthesis**

Green synthesis has become a more ecologically sustainable alternative for researchers as worries about the effects of traditional nanoparticle synthesis techniques on the environment have grown. In order to create NPs in an environmentally friendly way, green synthesis uses biological resources like plant extracts, bacteria, fungi, and algae. This method of producing NPs is more economical and environmentally friendly since it eliminates the need for dangerous chemicals and high-energy inputs.The basic principle of green synthesis is green chemistry, which seeks to reduce the usage of harmful chemicals, reduce energy consumption, and prevent waste generation.[32] By using natural reducing agents, such as phytochemicals found in plant extracts, green synthesis methods can produce NPs without the need for harmful chemicals or extreme reaction conditions. Additionally, green synthesis is typically carried out at ambient temperatures and pressures, further reducing energy consumption and environmental impact.[33]

PROSOPIS CINERARIA

A perennial tree native to arid parts of South Asia and the Middle East, *Prosopis cineraria* is also referred to as the khejri tree or the ghaf tree. In recent years, the phytochemicals found in *P. cineraria* have garnered attention for their potential in nanoparticle synthesis due to their biological activity and eco-friendly properties.[34]

Botanical Significance of Prosopis cineraria

The leguminous tree *Prosopis cineraria* is a member of the Fabaceae family. It is a drought-tolerant species that thrives in desert environments and is widely distributed in India, Pakistan, and the Middle East. The tree is highly valued for its medicinal properties, as its leaves, bark, and pods contain a variety of bioactive compounds, including flavonoids, tannins, and alkaloids.[35]

Phytochemicals in Prosopis cineraria

The naturally occurring substances called phytochemicals give plants their color, flavor, and resistance to disease. In *Prosopis cineraria*, a diverse array of phytochemicals has been identified, including alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, and glycosides. It has been demonstrated that these substances have important therapeutic qualities, including antidiabetic, anti-inflammatory, antioxidant, and antibacterial effects .[36] **Alkaloids**

A class of organic molecules with nitrogen atoms found in nature are called alkaloids. They are known for their pharmacological properties and have been widely studied for their role in plant defense mechanisms. In *Prosopis cineraria*, alkaloids such as prosopine, prosopinine, and prosopidine have been identified. As reducing agents in the environmentally friendly creation of NPs, these substances have demonstrated the ability to reduce metal ions to create NPs. Alkaloids are known for their high reactivity, which makes them effective in reducing metal salts into their respective NPs.[37] **Flavonoids**

Flavonoids are a group of polyphenolic compounds found in many plants, including *P. cineraria*. These compounds are responsible for the vivid coloration of plants and are known for their antioxidant properties. Flavonoids such as quercetin, kaempferol, and rutin have been isolated from *P. cineraria*. These substances are essential for stabilizing and reducing NPs. Flavonoids' antioxidant properties allow them to transfer electrons to metal ions, which speeds up the reduction process and produces stable NPs. Additionally, flavonoids serve as capping agents, regulating the size and shape of NPs and preventing their aggregation.[38] **Tannins**

A class of polyphenolic chemicals called tannins is well-known for its astringent qualities and capacity to precipitate proteins. *P. cineraria* contains significant amounts of tannins, which contribute to its medicinal and ecological significance. It has been discovered that tannins serve two functions in the creation of NPs: they donate electrons to metal ions to function as reducing agents and as stabilizing agents by forming a protective layer around the NPs, thus preventing their aggregation. The ability of tannins to bind to metal ions makes them particularly effective in controlling the morphology of NPs.[39]

Phenols and Phenolic Acids

Phenolic compounds are abundant in *P. cineraria* and are recognized for their strong antioxidant activity. These compounds include gallic acid, ferulic acid, and caffeic acid, which have been documented to contribute to the environmentally friendly production of metal NPs. Phenolic compounds are capable of donating hydrogen atoms or electrons to produce NPs from metal ions. Their antioxidant properties also make them effective capping agents, stabilizing the NPs and enhancing their biocompatibility.[40] **Saponins**

Saponins are glycosides with foaming characteristics, and they have been identified in the leaves and pods of *P. cineraria*. These compounds possess surface-active properties and have been shown to assist in the stabilization of NPs. By enclosing the NPs in a protective shell, saponins can improve their dispersion in aqueous solutions and stop them from aggregating. The biocompatibility of NPs, which makes them appropriate for biomedical applications, is also enhanced by saponins.[41] **Terpenoids**

Many different types of plants produce terpenoids, also known as isoprenoids, which are a broad and varied class of chemical substances. Numerous terpenoids found in P. cineraria have been demonstrated to have antioxidant and antibacterial qualities. Terpenoids serve as stabilizing and reducing agents during the manufacture of NPs. They help metal ions be reduced to create NPs and guarantee that the particles stay stable in solution, which stops them from aggregating.[42]Studies on *Prosopis cineraria*, highlight its medicinal, nutritional, and ecological significance. A study provided an ethnopharmacological review of *P. cineraria*, focusing on its extensive phytochemical profile, which includes compounds such as spicigerine, stigmasterol, and prosogerin derivatives. These substances support a range of pharmacological actions, such as anti-inflammatory, anti-cancer, and antioxidant properties. The study reinforces the plant's longstanding use in traditional Ayurvedic treatments for ailments like leprosy, bronchitis, and leucoderma, suggesting further exploration of its medicinal potential.[43]In the context of environmental significance, *P. cineraria* plays a crucial role in arid ecosystems, particularly in the Thar Desert. Malik studied the nutraceutical properties of *P. cineraria* pods (sangri) and found them rich in proteins, flavonoids, tannins, and essential minerals like calcium and potassium. The study showed significant antioxidant activity, indicating potential for dietary and health applications as a dietary source and a natural means to combat oxidative stress. This research highlights the pods' value as a nutraceutical food, offering health-promoting effects that align with local dietary practices.[44]

Role of Phytochemicals in Nanoparticle Synthesis

The phytochemicals present in *Prosopis cineraria* contribute significantly to the environmentally friendly production of NPs. The process of green synthesis, which uses biological entities like plant extracts, microorganisms, or biomolecules, offers an eco-friendly alternative to conventional chemical methods. A study by Haider et al. (2020) focused on the green synthesis of nickel oxide (NiO) NPs using extracts from *Zingiber officinale* (ginger) and *Allium sativum* (garlic). They demonstrated that phytochemically reduced NiO NPs demonstrated improved antibacterial efficacy against *Staphylococcus aureus* strains that are resistant to multiple drugs and effective catalytic activity for dye degradation, presenting a dual function as an eco-friendly bactericidal agent and industrial catalyst. This approach exemplifies the potential of phytochemical-assisted NP synthesis for applications in in ecological restoration and healthcare sectors.[45] The synthesis of gold and silver NPs has also benefited from phytochemicals, as reviewed by Zuhrotun et al. (2023). Their research highlighted that polyphenols, reducing sugars, and proteins in plant extracts not only drive the reduction of metal ions but also impact NP size, stability, and bioactivity. Specifically, smaller, well-defined NPs with greater stability and activity were achieved due to the precise influence of these phytochemicals, supporting their application across biomedical and industrial sectors.[46]

Additionally, the environmental applications of zinc oxide (ZnO) NPs synthesized via phytochemical routes were explored. Their study showed that ZnO NPs exhibit significant antimicrobial activity and pollutant-degrading capabilities, suggesting their utility in environmental cleanup, water purification, and eco-friendly antibacterial products. The versatility and biosafety of phytogenic ZnO NPs underscore the potential of phytochemicals to support sustainable, large-scale NP production.[47]

Importance of Prosopis cineraria in Green Synthesis

Prosopis cineraria bark extract is used in the study to create silver oxide (AgO) NPs in an environmentally responsible manner. The extract has two functions: capping and decreasing. The face-centered cubic structure of the NPs, which had an average size of 69.95 nm, was verified. SEM, which reveals square forms, and UV-Vis spectroscopy, which shows an SPR peak at 601 nm, were used as characterization techniques. The AgO NPs had strong antibacterial activity against gram-positive (like *S. aureus*) and gramnegative (like *E. coli*) bacteria, indicating their potential for use in biomedical applications.[48]Another study describes the environmentally friendly manufacture of copper oxide NPs utilizing *Prosopis cineraria* leaf extract, a plant indigenous to the hot deserts of India. Using copper acetate, the process yielded hexagonal and spherical NPs sized 11–43 nm, confirmed via TEM and AFM. SEM-EDX and UV-Vis spectroscopy (peak at 380 nm) verified the presence of copper oxide, while FTIR analysis showed that flavonoids and phenolic compounds in the extract facilitated the reduction. The findings confirm an eco-friendly method for producing copper oxide NPs from *Prosopis cineraria*.[49]A broad review of green synthesis using various plant extracts, highlighting their ability to create NPs and minimize metal ions (e.g., gold, silver, and zinc oxide). Utilizing biological resources, such plant extracts, lowers the environmental footprint compared to traditional chemical synthesis, as it avoids the release of hazardous by-products and minimizes energy consumption.[50]

COMPARISON OF PROSOPIS CINERARIA WITH OTHER PLANT EXTRACTS

Green synthesis methods for TiO_2 NPs utilize various plant extracts as reducing and stabilizing agents. *Prosopis cineraria* is one such plant It has been thoroughly investigated for its potential in nanoparticle synthesis. In this section, we will compare the benefits and limitations of *P. cineraria* with other commonly used plants such as *Moringa oleifera*, *Azadirachta indica* (neem), and *Ocimum sanctum* (holy basil).

1. **Phytochemical Composition and Reducing Ability**

The ability of plant extracts to lower metal ions and stabilize the resultant NPs is largely dependent on their phytochemical makeup. Flavonoids, tannins, alkaloids, and phenolic acids are among the many bioactive substances found in *Prosopis cineraria* that act as reducing agents in the environmentally friendly manufacture of TiO_2 NPs. These compounds not only reduce the titanium ions but also stabilize the NPs, preventing agglomeration and improving their dispersibility.[35] **2.**

Efficiency and Yield

Moringa oleifera extracts, on the other hand, are known for producing TiO_2 NPs with excellent stability and uniformity. The use of *M. oleifera* in green synthesis has been shown to produce NPs with high surface areas, which are advantageous for photocatalytic and energy storage applications. However, variations in the plant extract's composition can have an impact on the yield of NPs.[51]

3. Applications and Performance

The choice of plant extract for TiO_2 nanoparticle synthesis can significantly impact the performance of the NPs in various applications. TiO_2 NPs synthesized using *Prosopis cineraria* have demonstrated excellent performance in environmental applications like pollutant degradation and water purification. Because of their high stability and reactivity, TiO_2 NPs produced from *Moringa oleifera* have demonstrated considerable promise in environmental applications. These NPs can be used in wastewater treatment systems because they effectively break down dyes, insecticides, and other organic contaminants.[51] **Toxicity Studies on NPs**

Cytotoxicity of Green-Synthesized NPs

Green synthesis has been pitched as a less hazardous substitute for chemical processes, but studies show that greenly synthesized NPs can still pose risks of cytotoxicity depending on factors such as size, concentration, surface charge, and type of reducing agents used in the synthesis Cytotoxicity refers to the ability of NPs to damage or kill cells, which can have negative consequences for the environment and human health. Studies on cytotoxicity have shown that while NPs produced using eco-friendly techniques tend to have lower toxicity than those synthesized via chemical routes, they can still induce adverse effects at higher concentrations. For example, TiO_2 NPs synthesized using *Azadirachta indica* (neem) extract exhibited cytotoxic effects on human fibroblast cells at high doses, but lower concentrations were found to be biocompatible and did not cause significant harm.[52]While green synthesis methods often reduce the cytotoxicity of NPs by stabilizing them with biocompatible agents, the risk of ROS generation and subsequent cellular damage remains, particularly at higher nanoparticle concentrations. Additionally, the charge and other surface characteristics of NPs and functionalization with plant-derived biomolecules, can influence their interaction with cells, thereby modulating their cytotoxicity.[14]

Ecotoxicity and Environmental Impact

However, even greenly synthesized NPs can cause environmental harm if they accumulate in ecosystems at high concentrations. For example, zinc oxide (ZnO) NPs synthesized using *Prosopis cineraria* extract showed low toxicity towards soil microorganisms at low concentrations, but higher doses disrupted microbial activity, which could impact soil health and nutrient cycling. Therefore, it is essential to carefully assess the environmental risks of green-synthesized NPs, especially when they are used in applications that involve direct release into the environment, such as water treatment and agricultural products.[53]The present study aims at bio-synthesizing TiO_2 NPs utilizing leaf extracts of *Prosopis cineraria.* Various phytochemicals of the sample plant were analysed along with the study of different characterization's such as TEM, FTIR, and UV-Vis spectroscopy of the produced NPs for their better understanding. Additionally, the NPs' cytotoxicity, was also examined.

2. MATERIAL AND METHODS 2.1) Collection of Plant Material

The plant specimen used in the experiment, i.e. *Prosopis cineraria* was collected from the Sohan nagar area located in Rajasthan, India. The authentication of the sample plant was done by the University of Rajasthan, Jaipur, on December, 27, 2022. Accession no. RUBL21310 was provided by the Botany Department for the plant sample.

a) b) Figure 1: Collection of Prosopis cineraria leaves 2.2) Phytochemical Screening

a. Alkaloids:

A millilitre of extract was used. After adding 5.0 ml of the phosphate buffer as well as 5.0 ml of Bromocresol Green (BCG) to the extract, 4.0 ml of chloroform was incorporated, and the mix was thoroughly rattled. A layer of chloroform was collected and added to a 10.0 ml flask to fill the level. At 470 nm, the absorbance was measured. The standard dosage for atropine was 100 mg/mL.[54] **b.**

Saponins:

The method taken was as described by Obdoni and Ochuko.[55] ml of the extract was placed in a flask containing 100 milliliters of 20% ethanol. The sample had been exposed to heat to approximately 55 °C for four hours, with frequent agitation over a bath of hot water. Further extraction of the residue was done using 200 ml of 20% ethyl alcohol after the mixture had been filtered. Inside a water bath that is around 90 °C, the blended extracts were reduced to 40 millilitres. After that, the concentration was added to a 250-ml separation funnel, and the extract was given a good shake after diethyl ether (20 ml) was integrated. After repeating the purification process, the foremost layer of diethyl ether was discarded, and only the aqueous layer was left. Following the addition of n-butanol (60 ml), 5%

NaCl (10 ml) was used twice to wash the mixed n-butanol extracts. After evaporation of the solution, the samples were kept to be dried in a furnace to a consistent weight, and the values were reported as milligrams per gram of extract.

c. Flavonoids:

Chang evaluated the sample's total flavonoid content using their approach.[56] A slightly modified colorimetry approach was used to determine the flavonoid concentration. With the addition of distilled water (2 ml) and a sample solution (0.5 ml), 0.15 ml of a 5% $NaNO_2$ solution was added. Subsequently, 2 millilitres of NaOH solution (4%) were further incorporated into the mix and kept for 6 minutes. Afterwards, 0.15 millilitres of $AlCl_3$ (10%) solution was incorporated and allowed to settle for another six minutes. Water was promptly added on to make the final volume of 5 millilitres. The mixture was then well combined and left to stand for another fifteen minutes. At 510 nm, the mixture's absorbance has been determined using a water blank as a reference. As a standard, 100 mg/mL of quercetin was taken.

d. Tannins:

Tannin content was determined using the techniques of Peri and Pompei.[57] In a test tube, 1 millilitre of the sample extracts was collected. 1 ml of distilled water was put to use to make up the volume, and 1 ml of water was used as the blank. Following the addition of 5 ml of 35% sodium carbonate and 0.5 ml of Folin's phenol reagent (1:2), this was allowed to settle at room temperature for five minutes. At 640 nm, a blue colour developed, and the colour's intensity was measured. The tannin content of the extract was calculated by plotting a standard graph of gallic acid at 10 mg/mL. **e. Steroids:**

One millilitre of the steroid soution test extract was poured into ten millilitre flasks. H_2SO_4 (4N, 2 ml) and $FeCl_3$ (H_2O) x (0.5% w/v, 2 ml) were added to the extract, then K3. $Fe(CN)_6$ (0.5% w/v, 0.5 ml) was also added to it. After 30 minutes of constant shaking at 70±20 °C in a water bath, the mix was diluted to the desired level using distilled water. In comparison to the reagent blank, the intensity of absorption was measured at 780 nm[58].

f. Total Phenolic Content (TPC):

Using the Folin-Ciocalteau method, the TPC content of the extract was determined.[59]1 ml of FolinCiocalteau's reagent, 1.9 ml of distilled water, 0.1 ml of extract, and 1 ml of sodium carbonate were added to a tube. After incubation for 2 hours at 25 °C, the reaction mixture's absorbance was measured at 765 nm. Gallic acid at a concentration of 10 mg per millilitre was plotted on a standard graph to find the extract's phenolic concentration.

g. Carbohydrates:

For three hours, 10 millilitres of the extract and five millilitres of HCl (2.5 N) were hydrolysed in a steaming water bath. After bringing it to RT, solid Na_2CO_3 was added and stirred until the effervescence subsided. After centrifuging the contents, deionised water was added to the supernatant to make 100 milliliters. This allowed for the pipetting of 0.2 ml of sample to make 1 ml of volume using deionised water. Next, 1.0 millilitres of phenol reagent and 5.0 millilitres of sulphuric acid will be added. For twenty minutes, the tubes were placed at 25–30 °C. Using glucose (10 mg/ml) as a standard and an absorbance measurement made at 490 nm, the concentration was computed.[60] **h. Proteins:**

After extracting 0.2 millilitres, 1.0 millilitres were obtained using distilled water. After adding alkaline copper reagent (5.0 ml) to each tube, they were left to stand for ten minutes. After adding Folin's Ciocalteau reagent (0.5 ml), the mix was permitted to incubate for 30 minutes without the presence of light. At 660 nm, the colour's developed intensity was measured, and BSA (mg/mL)) was taken as standard, from which the concentration of proteins was calculated.[61]

2.3) Green Synthesis of Titanium Oxide NPs

10 grams of dried *Prosopis cineraria* leaf were weighed separately and ground into a paste by slowly adding

50 ml of distilled water to a mortar and pestle. After centrifuging the ground paste for 15 minutes at 15,000 rpm, the supernatant was gathered in an Erlenmeyer flask. 150 millilitres of distilled water were added and boiled to lukewarm in another flask. In the heated water, 0.1 M of TTIP (titanium tetra isopropoxide) was incorporated and subjected to sonication for duration of 20 minutes. After sonication, the plant leaf extract was gradually incorporated into the TTIP solution. The magnetic stirrer was utilised to agitate the mixture, and colour changes in the solution were noted. After 24 hours, the prepared nanoparticle was centrifuged, the pellet was collected, and it was air-dried at RT. The separated titanium oxide nanoparticle was calcinated at a temperature of 550° C for 4 hours in a muffle furnace. Finally, the nanoparticle was obtained, and the weight was measured. FTIR, UV-Vis, and TEM spectra were used to characterize the produced TiO_2 NPs.

2.4) Characterisation of nanomaterials

Titanium NPs were subjected to a physical-chemical characterisation using standard techniques. Following the "green chemistry" production of PC-TiO_2 NPs, a critical step involves characterising the particles' form, size, surface area, and dispersity. Several strategies were employed to achieve this. **a. Fourier transform infrared (FTIR) spectroscopy**

The sample's FTIR was examined using the IR Affinity-1 FTIR Shimadzu Spectrometer at a resolution of 4 cm1 in the diffuse reflectance working mode. A tiny quantity of dried material ground with KBr pellets is needed for this instrument. The purpose of this activity was to discover the functional groups that are present on the material surfaces.

b. UV-Vis spectra analysis

To track the progress of the bio-reduction of Ag^+ in aqueous solution, 1 ml suspension samples were taken on a regular basis. The samples were then diluted with deionised water (2 ml) and scanned in UV-visible (vis) spectra using a spectrophotometer with a resolution of 1 nm, covering wave lengths of 200 to 700 nm. UV-vis spectra were taken every 0, 15, 30, 45, 60 minutes, and 24 hours.

c. Transmission Electron Microscopy (TEM)

Using a TEM, the analysis was executed to produce high-resolution 2-dimensional pictures at a power of 100 kV for the identification of the morphological traits as well as the size dissemination pattern of the biogenic TiO_2 NPs. After rinsing the sample three times with distilled water, 5 µL of the sonicated sample solution was dropped over a copper (Cu) grid covered with carbon. The sample was then left to dry for 48 hours at RT.

2.5) Cytotoxicity analysis

a) Growing the HCT116 cell line:

A cell line had been bought from Protein Design Private Limited, located in Mathikere, Bangalore. The cells were grown for 48 hours up to 80% confluency in an environment that is more humid with 95% air and 5% CO_2 in a 25 mm flask using Dulbecco's modified eagle mixture (DMEM, Sigma), 10% foetal bovine serum (Thermo), penicillin/streptomycin, and glutamine. **b) Plating the cells:**

Trypsin/EDTA (Thermo) was used to collect the cells, and DPBS (Sigma) was used to wash them. A 5000well plate had cells inserted into each well and left overnight to culture. **c) Treatment of the cells:**

Different concentrations of treatment fractions were added in 100-ul media without serum and incubated for 24 hours. A crucial component for determining cell viability, cytotoxicity, and proliferation is the MTT test, which assesses a cell's metabolic activity. This colorimetric test was designed based on the reduction process by metabolically active cells. Yellow tetrazolium salt (3-(4, 5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide, or MTT) is reduced to purple formazan. The living cell's mitochondrial reductase enzyme transforms MTT into formazan. Using a multi-well spectrophotometer, absorbance at 500–600 nanometres is measured to provide the colour of the resultant solution. A solution of solubilisation is applied to dissolve the formazan crystals that are initially intractable. The number of live, metabolically active cells increases with increasing solution darkness. **d) Reagent preparation for MTT salt**

5 mg/mL of culture media and buffer salt solutions, 20 mg/mL of ethanol, and 10 mg/mL of water are the concentrations at which MTT dissolves. 5 mg/mL of phosphate buffered saline solution should be used. Use sonication or vortexing for mixing.

After adding MTT, the solution is sterilised by the filter. MTT solution should be kept cold (-20 °C) for at least six months. Keep out of storage for longer than a few days at 4°C.

e) Assay protocol

1. Firstly, discard the media from the cell cultures. With caution, aspirate the medium for adhering cells. In a centrifuge that is compatible with microplates, spin the 96-well plates at 1,000 x for five minutes at 4 °C. Carefully take out the media.
2. Put 50 µL in each well of MTT solution and 50 µL of serum-free medium.
3. The plate should be incubated for three hours at 37 °C.
4. After the incubation, 150 µL of MTT solvent should be present in each well.
5. Use an orbital shaker to shake the plate for fifteen minutes after wrapping it in foil. Sometimes, for full dissolution of the MTT formazan, the liquid must be pipetted.
6. At OD = 590 nm, find the absorbance. In 1 hour, read the plate. **f) Data analysis**

Cell proliferation assays

1. For every sample, average the duplicate reading.
2. Deduct the background of the cultural media from the test reading. This represents the scaled absorbance. The number of cells directly correlates with the absorbance.

Cell cytotoxicity assays

1. Take an average of each sample's duplicate reading.
2. Deduct the assay readings from the background of the cultural media. The rectified absorbance is shown here. With rectified absorbance, compute the percentage cytotoxicity using the following formula:

% cytotoxicity = (100 x (control - sample))

2.6) Statistical Analysis: 3 duplicates of each treatment were carried out, and the mean ± standard error. The SE of the results was given.

SE = SD / √N

Here, SD = standard deviation, and N = number of observations or sample sizes.

3) RESULT AND DISCUSSION

3.1) Phytochemical Analysis

Quantitative phytochemical analysis holds substantial importance as it grants precise measurements of the concentration or content of specific bioactive compounds in plant samples. The quantitative study was also performed along with the qualitative study in the current research on the leaves of *P. cineraria*. During the study, it was found that alkaloids, saponins, flavonoids, tannins, steroids, total phenolic content, carbohydrates, and proteins were present in concentrations of 268.5, 26.4, 61.7, 27.7, 1.393, 32.1, 7.9, and 1.98 mg/mL. A study was conducted where it was demonstrated the phytochemical presence in *Prosopis cineraria* leaves, and the results revealed that in ethyl acetate extract, the total flavonoid concentration was 22.18 mg/100 mg of dry extract.[62] The whole tannin concentration was determined to be 3.38 mg/100 mg of the dried extract, whereas the TPC was 39.21 mg/100 mg, respectively, in the ethanolic extract. Similarly, Pandey investigated the phytochemical compounds in three different extracts (methanolic, hydroalcoholic, and aqueous) of the bark of *P. cineraria.* [63] The total flavonoid content, phenolic content, and tannin content were found to be highest in the hydroalcoholic extract, i.e., 66.95 mg RE/g, 98.01 mg GAE/g, and 56.30 mg TAE/g, followed by methanolic and aqueous extracts. Till date, very little work has been conducted on the quantitative analysis of phytochemicals in *Prosopis cineraria* leaves with ethanolic extract. Therefore, in the present study, the quantitative screening of different phytochemical compounds has been conducted and reported.

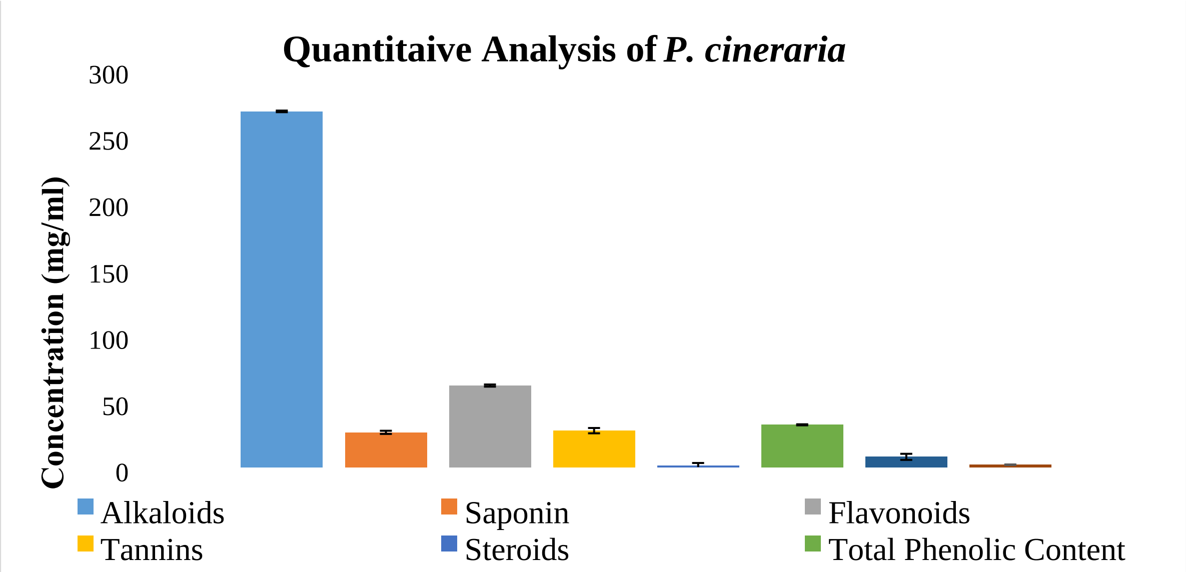
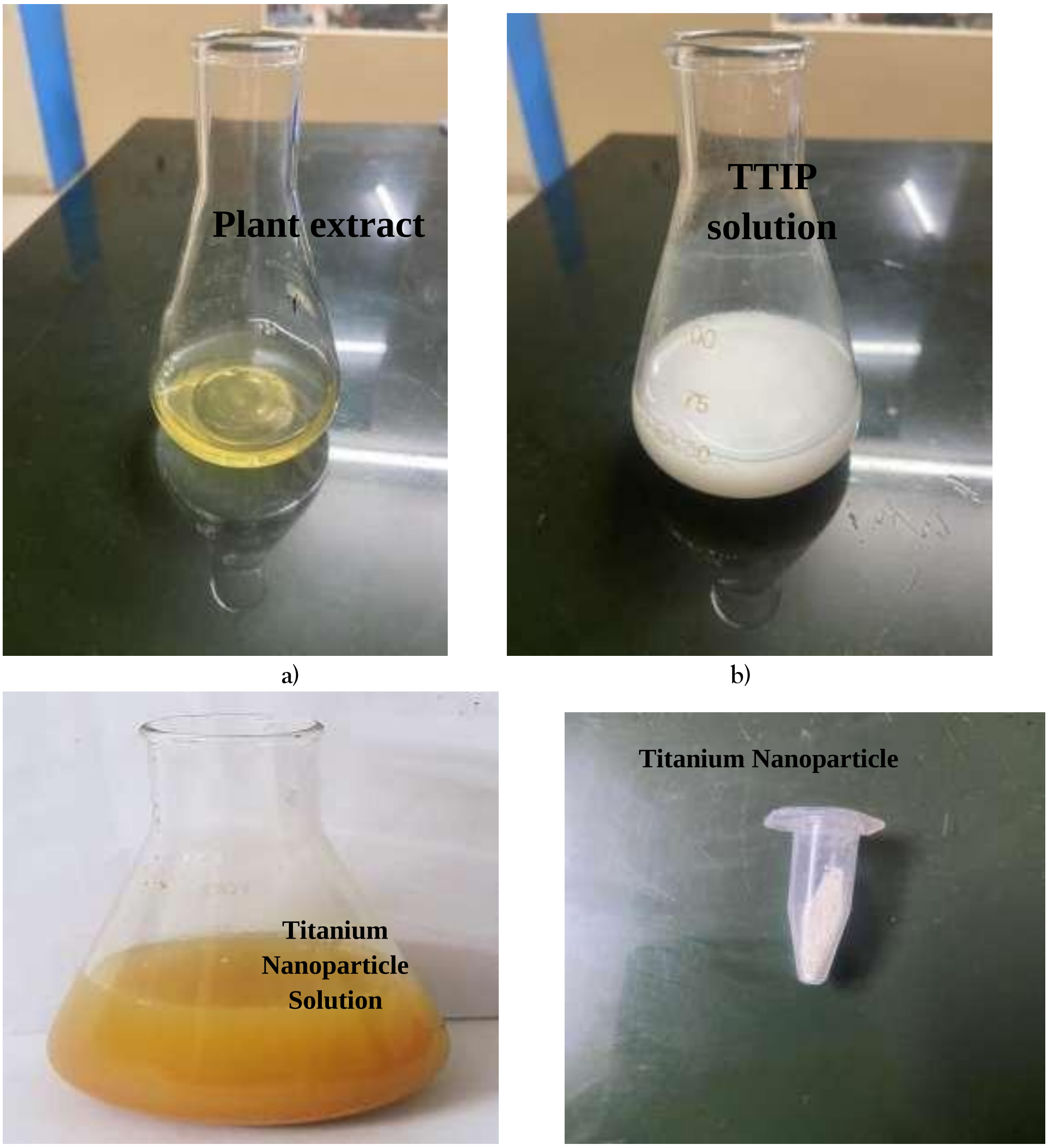


Figure 2: Graphical representation of quantitative analysis of P. cineraria leaf using ethyl acetate extract

3.2) Green Synthesis of Titanium Oxide NPs

The research aims at the formation of TiO_2 NPs with a sample taken from the leaves of Prosopis cineraria. The leaf extract was prepared using water as the solvent. The leaf extract being added to a solution of TTIP prompted a distinct variance in the colouration of the solution, transforming it from a yellow to a sandal hue (Figure 3). The appearance of an orange colour confirmed the successful reduction of titanium iso-propoxide, prompting the development of the development of titanium NPs. The calcination of synthesized NPs using a muffle furnace resulted in the white coloration of titanium NPs. The subsequent characterization of the synthesized NPs involved comprehensive analyses using FTIR, TEM, and UV-Vis spectrophotometry.



d) **Figure 3: Various stages of TiO_2 nanoparticle synthesis from Prosopis cineraria leaf extract using TTIP solution**

3.3) Characterization of Nanomaterials i) FTIR spectroscopy

FTIR is used for nanoparticle characterisation, providing information about the chemical composition, functional groups, surface modifications, adsorbates, chemical reactions, and molecular structure of NPs. In the current study, manufactured titanium NPs' FTIR spectra were noted on the scale of 400 cm1 to 4000 cm^{1}. The absorbance peak at 3401.46 cm^{1} corresponds to aromatic primary amine, NH stretch, whereas the peak at 3326.69 cm^{1} represents imino compound, =N-H stretch. The absorbance peak at 1634.81 cm^{1} represents the presence of an amide group, whereas the peak at 1615.40 cm^{1} relates to an open-chain imino (-C≡N-). The peak at 686.83 cm^{1} relates to the aromatic C-H out-of-plane bend, while the peaks between 666.37 cm^{1} and 619.26 cm^{1} represent the alkyne C-H band, respectively. The presence of functional groups along the surface of TiO_2 NPs was examined using FTIR spectral analysis on green

TiO_2 produced from the aqueous extract of *Erythrina variegata* leaves.[64] The spectrum, which was documented in the range of 4000 to 400 cm^{-1}, shows that the peaks at 3418 and 1628 cm^{-1} are caused by the O-H stretching and bending vibration of the surface-adsorbed water, while other bands were seen at 2836 cm^{-1}, 2932 cm^{-1}, 1589 cm^{-1}, and 1071 cm^{-1} (ethers), representing the secondary amines, hydrogenbonded alcohols, and aliphatic of the nitro compound with stretching of N-O. Also, in another study, TiO_2 NPs were created from *Luffa acutangula* leaf extract, and the presence of a functional group was determined using the FTIR technique. The analysis resulted in a range of peaks at 3393 cm^{-1} (phenol), 1611 cm^{-1} (amines), 1381 cm^{-1} (nitro group), 1132 cm^{-1} (carboxylic acid), and 598 cm^{-1} (alkyl halides), respectively.[65]

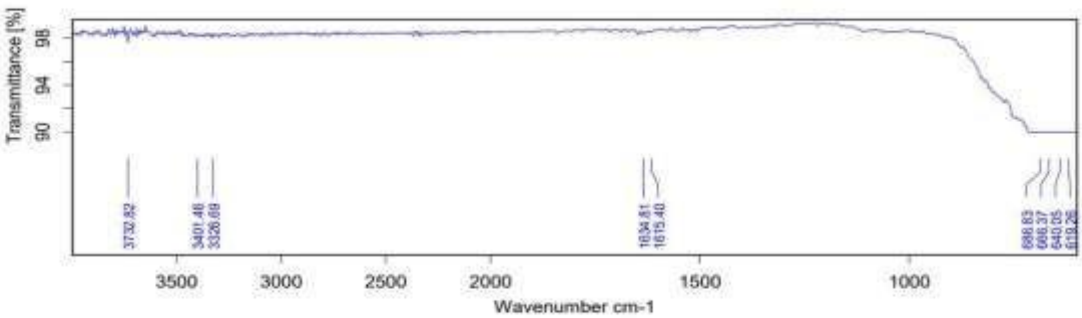


Figure 4: FTIR graph of synthesized TiO_2 NPs

ii) UV-Vis spectra Analysis

UV-Vis spectroscopy is a versatile technique for nanoparticle characterisation, providing insights into nanoparticle size, shape, concentration, stability, surface plasmon resonance, surface functionalisation, synthesis kinetics, optical properties, and band gap. The UV-Vis spectrum of the biosynthesized TiO_2 NPs was noted in the range of 200–400 nm. The absorption maximum at 371 nm confirms the development of TiO_2 NPs. Likewise, greenly produced TiO_2 NPs from the aqueous extract of *Erythrina variegata* leaf were produced, and their formation was verified using UV-Vis spectra using a double-beam spectrophotometer in the range 200–900 nm. The absorption maximum is at 317.6 nm, which establishes the production of titanium NPs.[64] *Prosopis campechiana* leaf extracts were used to create the titanium oxide nanoparticle, which was confirmed using a UV-Vis spectrophotometer. The absorption spectrum was recorded, showing a peak at 230 and 320 nm, respectively, and a range of 200 to 800 nm.[66]

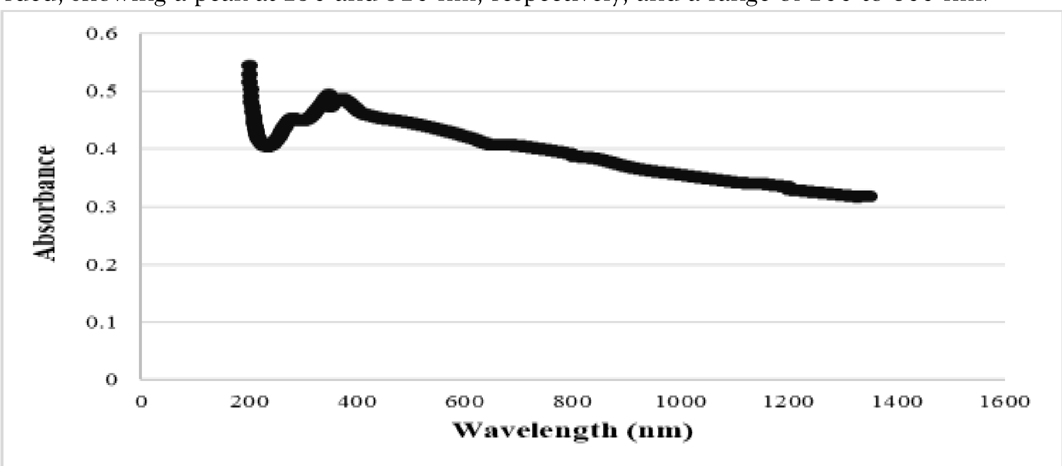


Figure 5: UV-Vis spectra of greenly synthesized TiO_2 NPs iii)
Transmission Electron Microscopy (TEM)

TEM enables the imaging of NPs at higher magnifications and resolutions than SEM. It is well-suited for studying nanoparticle size, shape, crystallography, defects, and interfacial properties. In the research, the morphology of the produced TiO_2 NPs from *P. cineraria* leaf extract was studied by TEM. The synthesized nanoparticle was calcined at 550° C which illustrates its bigger size and the agglomeration becoming significant. The particle size ranged from 13.49–23.07 nm at a scale of 200 nm and 9.727–44.50 nm at a micrograph scale of 1 µm (Figure 6). Also, a study was done to synthesise TiO_2 NPs using leaf extract from *Luffa acutangula*.[65] Through TEM investigation, NPs' dimensions and shapes were studied, and the findings revealed that the created TiO_2 NPs were hexagon-shaped and ranged in size from 10 to 49 nm. In a comparable manner, a study on the TEM investigation of white-coloured TiO_2 NPs synthesized with *Azadirachta indica* extract demonstrated that the particles varied in size from 15 to 45 nm, with spherical shapes and smooth surfaces.[67]

Figure 6: TEM micrographs of TiO_2 NPs at various micro-scales- a) 200 nm, b) 1 µm

3.4) Cytotoxicity analysis

In this study, MTT assay data revealed titanium dioxide (TiO_2) nanoparticle cytotoxicity at concentrations ranging from 0.125 to 2.5 mg/ml. At 2.5 mg/ml, cell viability dropped to 41.09%, indicating significant cytotoxicity. Reducing the concentration to 2 mg/mL slightly improved viability to 46.0%. At 1 mg/ml, viability increased to 56.67%, and at 0.5 mg/ml, it rose to 76.12%, indicating safer levels for cell contact. At 0.25 mg/ml, cell viability was 93.54%, and the lowest concentration, 0.125 mg/ml, showed minimal cytotoxicity with 95.65% viability, making it optimal for long-term biomedical use. The effects of the cytotoxicity of TiO_2 NPs have been widely studied, revealing a complex interplay between nanoparticle characteristics and cellular responses. TiO_2 NPs' autophagic effects on human keratinocytes (HaCaT cells) at non-cytotoxic concentrations were investigated. It was observed that there is an increase in autophagic activity that is dose-dependent without significant cytotoxicity at lower concentrations, suggesting that TiO_2 NPs might affect cellular processes such as autophagy prior to the onset of observable cytotoxic effects.[68]

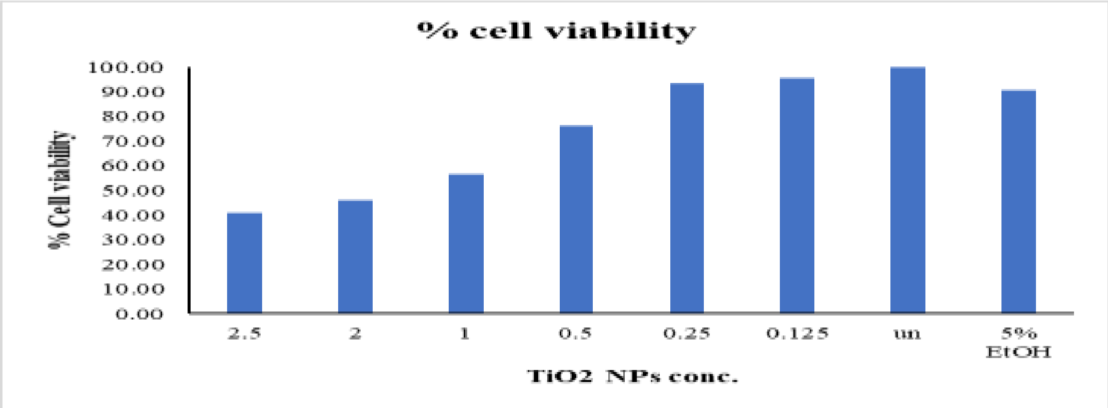


Figure 7: % Cell viability

CONCLUSION

In the study, the phytochemical evaluation of *Prosopis cineraria* was analyzed. Along with it the biosynthesis of titanium NPs from leaf extract of *Prosopis cineraria* was also done. A variety of techniques, including FTIR, TEM, and UV-Vis spectroscopy, were used to analyze the generated nanoparticle. The investigation indicated the prevalence of various phytochemical compounds in the leaf of *Prosopis cineraria* such as proteins, phenol, carbohydrates, lipids, saponin and flavonoids. Also, the titanium NPs were successfully synthesized and formation of titanium NPs was authorized using UV-Vis spectroscopy. FTIR study revealed presence of different functional groups. The TEM study confirmed the nano-sized of NPs with a size of 13.49-23.07 nm at a scale of 200 nm. Cytotoxicity analysis using the MTT assay revealed that TiO_2 NPs exhibit significant cytotoxic effects at higher concentrations, indicating their potential application in cancer treatment due to selective cytotoxicity towards cancer cells and are optimal for long-term biomedical use. As the outcomes obtained, it can be concluded that the green synthesis of TiO_2 NPs may exhibit different anti-microbial, anti-oxidant and pharmacological activities and can be utilized in the field of biomedicine, pollution treatment and health care sectors.

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Data Availability: All data analyzed during this study are included in the manuscript.

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