Review Article

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A Review of Biogenic Synthesis of TiO_2 Nanoparticles Using a Plant-mediated Green Approach for Biomedical Applications

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ABSTRACT

This paper reviews the green synthesis of titanium dioxide nanoparticles (TiO_2 NPs) from different plant parts as sustainable and eco-friendly reducing and stabilizing agents, in comparison to conventional physical and chemical synthesis. The plant-mediated synthesis route is cost-effective and non-toxic for the development of TiO_2 NPs with controlled physicochemical properties. Plants can aid the synthesis of crystalline nanoparticles within an average size range of 10-40 nm more stable, possessing uniform morphology according to XRD/SEM analyses, which reported enhanced stability. Zone diameters are mostly in the range from 12-20 mm on common pathogens like *E. coli* and *S. aureus*. Green synthesized TiO_2 NPs showed high degradation efficiencies up to 90-95% for dyes like methylene blue under UV or visible light irradiation. The results confirm that eliminating the use of hazardous chemicals and enhance the biocompatibility and environmental friendliness of TiO_2 nanomaterials, also plant-derived reducing agents. The review shows that various plant parts (leaves, flowers, stems, seeds, bark) and extraction conditions influence nanoparticle size, morphology, and functional performance. In conclusion, the results highlight the potential of plant-mediated synthesis to provide TiO_2 NPs with tunable properties appropriate for biomedical, photocatalytic, and environmental remediation applications as well as the need for future research for process optimization and scale-up.

Keywords: TiO_2 nano particles; Green synthesis; Photocatalytic degradation; Plant-mediated synthesis.

1. INTRODUCTION

The art of manipulating materials at sizes ranging from 1 to 100 nanometers is known as nanotechnology. It is, in theory, the capacity to produce and employ materials, components, or systems at incredibly small scales (Alhiti and Ali, 2025). Since atomic and molecular-level manipulations enable modifications that are not possible in conventional bulk materials, they can impart enhanced or entirely new properties to the resulting materials, such as improved electrical conductivity, increased strength, and antibacterial functionality (Shastri and Gandhi, 2024; Jasim *et al.* 2025). Norio Taniguchi coined the term "nanotechnology" in 1974, and it is currently the field of scientific study with the fastest rate of growth worldwide (Kumar *et al.* 2024). Energy, chemistry, health, and beauty are the main areas of interest in the synthesis and applications of nanoparticles (Marouzi *et al.* 2021). It is possible to distinguish nanomaterials into organic and inorganic systems. It has been established that organic nanostructures like ferritins, micelles, liposomes and dendrimers are highly utilized biomedically as carriers whereas inorganic nanomaterials, especially those based on metal oxides like TiO_2 have gained much interest because of their environmental and biomedical uses (Shabatina *et al.* 2020). Sulfides, nitrides, and oxides make up metallic and semi-conductive nanoparticles (Ashraf *et al.* 2020). The thermal, optical, electrical, and magnetic properties of TiO_2 nanoparticles are distinct (Sungur, 2021). Titanium could be utilized as a white pigment because of its very high refractive index (n = 2.4) despite the fact that it is insoluble in water under normal conditions and exceedingly stable. The majority of the polymorphic crystalline forms of TiO_2 found in nature are rutile, anatase, and brookite (Žerjav *et al.* 2022). Although rutile is less dense and has a corrosive application resistance, anatase and rutile often share similar properties with regard to the three polymorphic structures (Sharma *et al.* 2019). Furthermore, because it is metastable at higher temperatures and gradually changes into the rutile form, brookite is not frequently discovered.

Fig. 1 shows a steady interest in titanium dioxide nanoparticle (TiO_2 NPs) studies from 2015 to 2025 with publication numbers between 625 and 774 articles each year. On the other hand, Fig. 2 shows slowly rising number of works dealing with the green synthesis of TiO_2 NPs that reached 46 articles in both 2023 and 2024. This proves that there is an ever-increasing requirement for ecologically sound synthesis routes

within nanotechnology. Thus, this data clearly signals the final move away from standard methods toward greener options inside TiO_2 NP studies.

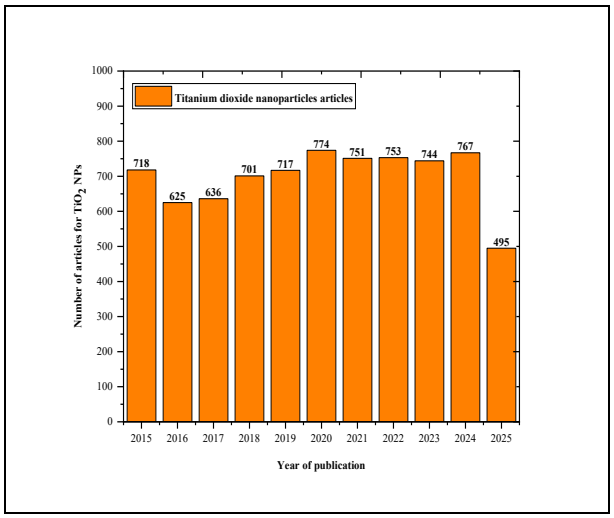


Fig. 1: Chart showing the occurrence of papers published on titanium dioxide nanoparticles from 2015 to 2025 (https://pubmed.ncbi.nlm.nih.gov/ accessed on 20 July 2025)

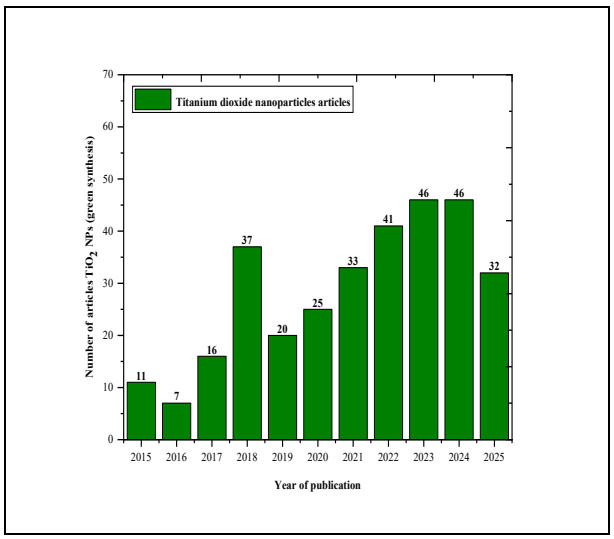


Fig. 2: Graph showing the occurrence of published papers on the green synthesis of titanium dioxide nanoparticles from 2015 to 2025 (https://pubmed.ncbi.nlm.nih.gov/ accessed on 20 July 2025)

The synthesis of TiO_2 nanoparticles has received more and more attention and significance due to various uses of TiO_2 in the nanometer regime, including photodegradation and cleavage applications, photovoltaic devices, electrochemistry, and other electronic sensing methods (Ma *et al.* 2014). Typical techniques for synthesizing TiO_2 NPs include sol-gel, chemical precipitation, chemical vapor deposition, and microhydrothermal techniques (Wang *et al.* 2022). TiO_2 nanoparticles have traditionally been developed in chemical and physical synthesis routes under high pressure, temperature, and energy conditions making the route not only expensive but also environmentally burdensome. Hazardous reduction- and capping agents result in toxic byproducts presenting a great danger to human health and the environment. Plant-mediated green synthesis uses natural phytochemicals (flavonoids, terpenoids, phenolic compounds) as reducing and stabilizing agents. Such green routes(-developing) provide a promising(eco-friendly) alternative route on a large scale TiO_2 nanoparticle production (Irshad *et al.* 2021). Plant extracts act as a reducing agent in the synthesis of titanium dioxide nanoparticles. This makes green synthesis methods very effective (Singh Jassal *et al.* 2022). This review presents a detailed comparative analysis of biosynthesized titanium dioxide nanoparticles with specific attention to plant-mediated synthesis. In addition to general green synthesis routes, which previous reviews have mostly summarized or discussed, this paper particularly and systematically examines the effects of different plant parts (leaves, stems, roots, flowers, and seeds) on the physicochemical characteristics and functional properties of TiO_2 NPs. The review gives a close scientific investigation of the factors that affect the production of titanium dioxide nanoparticles using plant extracts. Particularly, it looks into how the composition and synthesis conditions of extracts influence the size, morphology, stability and reactivity of the synthesized nanoparticles. The correlation between these parameters and the photocatalytic activities and antibacterial activities of TiO_2 nanoparticles is also discussed in the review. This article only reviews literature published during the past ten years, between the years 2015 and 2025, on the synthesis of titanium dioxide nanoparticles using plants. It discusses the mechanisms of synthesis, characterization techniques applied, and major applications in the fields of biomedicine and environmental remediation. Therefore, this work should serve as a structured critique to guide researchers toward an optimal sustainable synthesis strategy for further study development.

2. PROPERTIES OF TITANIUM DIOXIDE NANOPARTICLES

Titanium dioxide nanoparticles have three crystallographic forms naturally occurring in nature, with rutile being most stable at bulk conditions and anatase stable at particle sizes less than 14 nm (Ayorinde and Sayes, 2023). Titanium dioxide has been utilized extensively as a food dye (E171), usually in pigment-grade form, in various crystalline forms (rutile or mixed crystalline). Nevertheless, over the last few years, the application of Ti(Brookite)O_2 in food has been under increased regulatory scrutiny (Teichman *et al.* 2024). Nano- and pigmentary TiO_2 are chemically similar substances; both may exist in more than one crystal structure (Pfaff, 2021). Nano-TiO_2 has the possibility of

eliciting effects at a cellular level while pigmentary TiO_2 is construed to be less hazardous. The high reactivity of nano TiO_2 is primarily attributed to the larger proportion of atoms located on its surface compared to those within the bulk material (Luo *et al.* 2020).

Because of their applicability in many dimensions, TiO_2 nanoparticles have intriguingly demonstrated immense promise. For example, one of the main reasons they are used in building energy devices is their excellent light-conversion efficiency (Bai *et al.* 2014). Because of their low production costs, combined chemical stability, and thin film transparency, they are used as photocatalysts in the majority of environmental remediation procedures for treatments such as wastewater, air pollution, and soil viability enhancement (Ainali *et al.* 2021). Using nanoparticles for photothermal cancer therapy has just recently been feasible. At first, this seemed like the worst course of action due to the absence of radioactive recombination. Generally, TiO_2 nanoparticles are regarded significant for medicinal purposes (Ziental *et al.* 2020a).

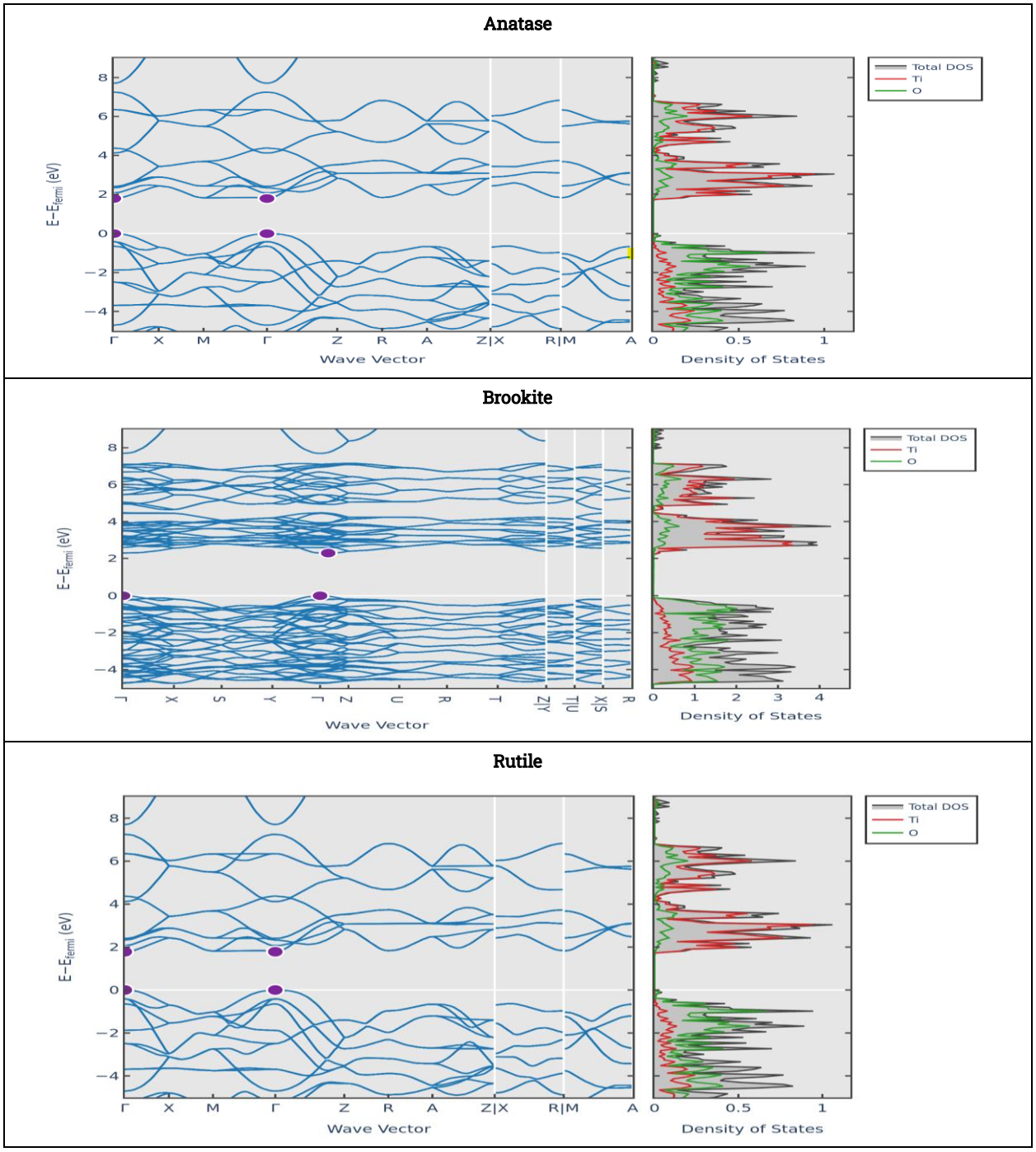


Fig. 3: Electronic band structure and DOS for three TiO_2, namely anatase, brookite and rutile (Landmann *et al.* 2012)

Fig. 3 presents the band structures and respective density of states (DOS) for the three major polymorphs TiO_2: anatase, brookite, and rutile. This work also shows a simplified band diagram which is used to show the location of the bandgap maximum of the valence band of TiO_2 (VBM) and the conduction band minimum (CBM). The diagram is meant to indicate the band gap energy and is not the entire electronic band structure dispersion. The electronic band structure of TiO_2 is determined by a crystalline phase of the substance. Anatase TiO_2 is widely regarded as an indirect bandgap semiconductor material with possible direct transitions at some of the symmetry points. The comparison between anatase, brookite, and rutile will clearly indicate that their band gap values differ; thus, it is this difference that forms a structure-function relationship by controlling electronic structure together with charge carrier dynamics and therefore absorption properties of light in three phases. Thus, the difference in bandgap energies is what results in the different photocatalytic efficiencies and electronic behaviors exhibited by titanium dioxide polymorphs. These distinctions are significant when titanium dioxide-based materials are employed in processes like photocatalysis processes, solar cells and environmental clean-up (Sagadevan *et al.* 2022).

2.1 Crystal Properties

Titanium dioxide (TiO_2) is a very diverse material whose applications can be seen in paints and sunscreens, as well as photocatalysts in purification of the environment and biology (Zietal *et al.* 2020b). Every type of TiO_2 is composed of the same basic structure, known as TiO_6 octahedra and thus, anatase, rutile, and brookite have a comparable structural basis. The only major difference between these forms is through small variations in the manner such units are positioned and distorted in the crystal structure that makes each form possess its particular properties. The octahedra are more distorted in anatase and form a tetragonal structure, but rutile has more compact and less distorted arrangement of the octahedra. Brookite, however, is more complicated in structure (distorted TiO_6 octahedra). Such a difference in the octahedral distortion and connection results in the variability of structural and electronic characteristics of the polymorphs of TiO_2. The properties of such crystals are examined in depth in Table 1.

2.2 Optical Properties of Titanium Dioxide Nanoparticles

Titanium dioxide nanoparticles are helpful in different applications like sunscreen, coatings, and optical devices because of their unique optical properties. Their crystalline structure, particle size, and the media surrounding them largely influence these properties (Ghareeb *et al.* 2024). A detailed explanation of the optical properties of TiO_2 NPs is given in Table 2.

Table 1. It has been modified (Akakuru *et al.* 2020)

No.	Character -istics	Rutile	Anatase	Brookite
1.	Structure	tetragonal	tetragonal - octahedral arrangement	Orthorhom -bic
2.	Lattice Parameters	a = 4.593 Å c = 2.959 Å.	a = 3.784 Å c = 9.514 Å	a = 5.436 Å, b = 9.166 Å, c = 5.135 Å.
3.	Density	4.23 g/cm^3	3.89 g/cm^3	4.13 g/cm^3.
4.	Space group	$P4_2/mnm$	$I4_1/amd$	Pbca
5.	Ti–O band angle (Å)	1.94 (4) 1.98 (2)	1.93 (4) 1.96 (2)	1.87 - 2.04
6.	O–Ti–O bond angle	81.2° 90.1°	77.7° 92.6°	77° - 105°
7.	Volume/m olecule (Å^3)	31.216	34.061	32.172

Table 2. Optical properties of titanium dioxide nanoparticles (Mo and Ching, 1995)

NO.	Property	Anatase	Rutile	Brookite
1.	Refractive Index (n)	2.52 - 2.55	2.71 - 2.73	2.58 - 2.68
2.	Bandgap Energy (eV)	3.2	3.0	3.2 - 3.4
3.	Absorption Edge (nm)	~390	~410	~385 - 390
4.	Optical Density	Lower	Higher	Intermediate

3. SYNTHESIS OF TITANIUM DIOXIDE NANOPARTICLES USING PLANT EXTRACTS

Green synthesis of titanium dioxide nanoparticles by plants is an alternative model safer for the environment and human health. Phytochemicals derived from the plant material used as reducing and stabilizing agents enable nanoparticle formation without the use of toxic reducing or stabilizing agents (Mamdooh and Naeem, 2021). The method is not only environmentally safer but also imparts higher biocompatibility to its resulting nanoparticles toward catalytic applications, photocatalysis, biomedicine, or environmental remediation (Mamdooh and Naeem, 2021). It starts with the careful selection and preparation of plant material (Sunny *et al.* 2022; Swathi *et al.* 2019).

The material is washed, dried, and ground into a fine powder. Bioactive compounds are then extracted from this powder using appropriate solvent, and then the extract is filtered to remove any kind of impurities, making a clear solution. This plant extract will react with a titanium precursor, either titanium tetraisopropoxide (TTIP) or titanium tetrabutoxide. Then hydrolysis at room temperature with proper stirring yields nanoparticles. The obtained mixture is filtered and washed properly to remove any impurities present in it. The dried precipitate is finally subjected to proper calcination under controlled temperatures so as to enable crystallization, ultimately forming TiO_2 nanoparticles possessing good structural and morphological properties as, shown in Fig. 4 (Vijitha *et al.* 2019; Patil and Chaudhari, 2021). When compared to conventional chemical synthesis methods, the biological synthesis of TiO_2 NPs utilizing plant extracts is a less harmful way to the environment (Aslam *et al.* 2021). According to the guidelines of green chemistry, plant extracts such as lemon grass, neem, Indian gooseberry, aloe vera, and *Cinnamomum* sp. could be employed as stabilizing and reducing agents in the environmentally friendly synthesis of TiO_2 NPs (Sunny *et al*. 2022). Reports have emerged on the synthesis of crystalline and pure TiO_2 NPs utilizing plant extracts from *Cassia fistula*, *Cinnamomum* sp., *Cajanus cajan* (pigeon pea), and *Nyctanthes* leaves (Swathi *et al.* 2019) (Patil and Chaudhari, 2021). Apart from their use as reducing agents, the plant extracts might be capping and stabilizing agents throughout the synthesis of metal oxide NPs, like TiO_2 (Vijitha *et al.* 2019). Preparation of TiO_2 nanoparticles by using plant extracts is a low cost, easy, and non-toxic process that can be easily scaled as compared to most methods of synthesis of this chemical compound used traditionally (Singh Jassal *et al.* 2022). The produced NPs are investigated using many techniques, such as UV-vis spectroscopy, scanning electron microscopy (SEM) (Alhiti and Ali, 2025), particle size analysis, and X-ray diffraction (XRD). The morphology and particle size are studied by SEM analysis, and the crystallinity and purity of phase are confirmed by XRD analysis and optical properties are analyzed by UV-Vis spectroscopy (Singh and Dutta, 2018). Strong antibacterial characteristics of the produced TiO_2 NPs against pathogens including *Escherichia coli, Staphylococcus aureus*, and *Acinetobacter baumannii* have shown biomedical uses (Younis *et al*. 2023a).

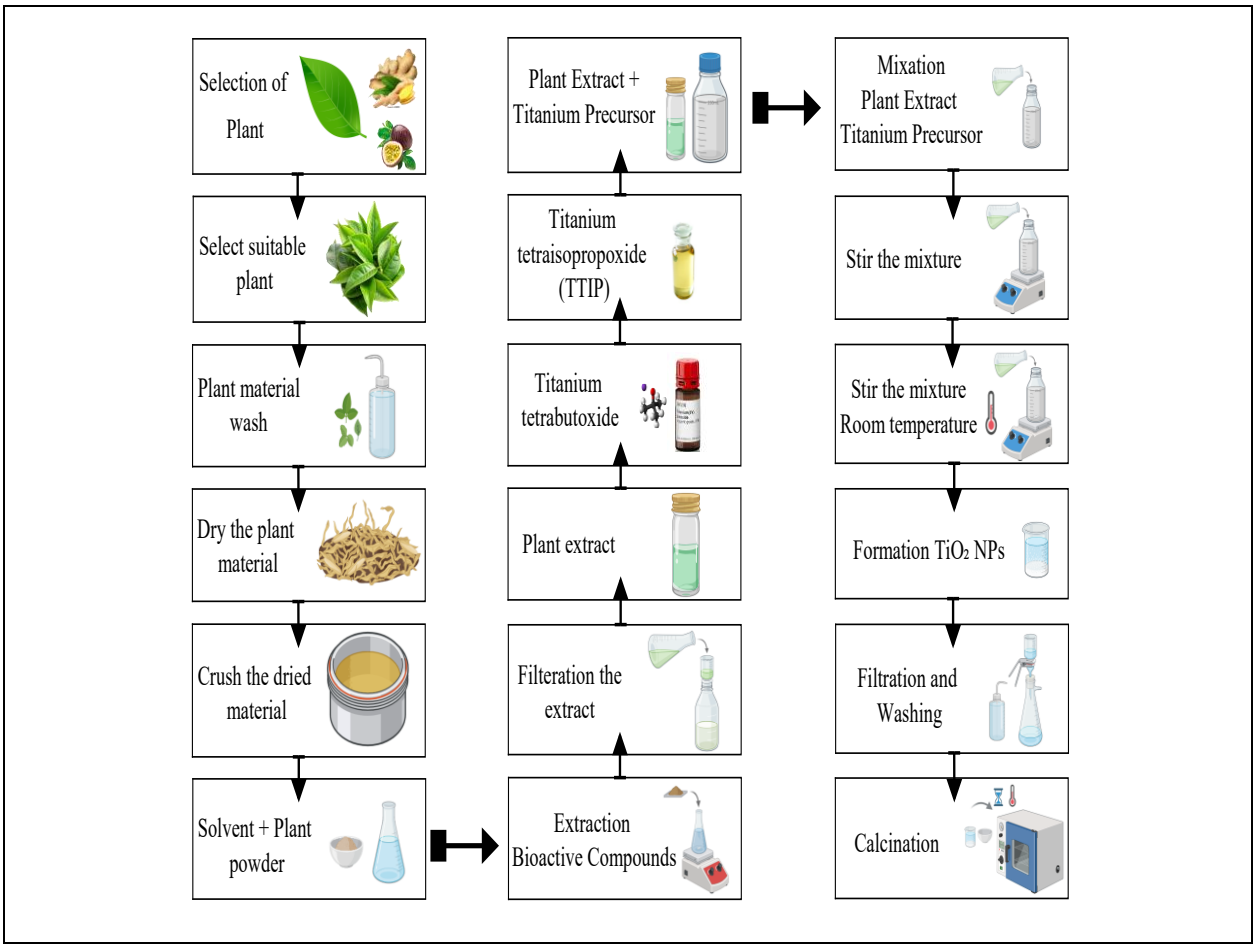


Fig. 4: Green method for synthesizing titanium dioxide nanoparticles using plant extracts

Generally, the utilization of plant extracts in the biological synthesis of TiO_2 NPs is an affordable (Verma *et al.* 2022), environmentally friendly, and scalable approach with possible applications in several sectors, including environmental remediation, nanotechnology, and biomedicine (Singh Jassal *et al.* 2022).

4.CHARACTERIZATION OF TITANIUM DIOXIDE NANOPARTICLES

The main purpose characterization is the determination in a systematic way of some basic physicochemical parameters, such as surface charge, particle size distribution and morphology, degree of aggregation, by analytical techniques.

Molecular vibrations and surface functional groups are studied by Fourier transform infrared spectroscopy (FTIR). More detailed information regarding elemental composition and chemical states is provided through X-ray photoelectron spectroscopy (XPS). Commonly used for elemental analysis is energy-dispersive X-ray spectroscopy (EDS), typically employed jointly with scanning electron microscopy (SEM) which studies surface morphology with good resolution. Internal structure studies on the nanoscale are made possible by transmission electron microscopy (TEM), hydrodynamic size distribution in suspension, together with colloidal stability measured by dynamic light scattering (DLS) (Titus *et al.* 2019). Fig. 5 indicates some of the popular ways of characterizing TiO_2 nanoparticles. Once synthesized, the nanomaterials should be studied thoroughly and their properties compared with the values expected to predetermine that the process of their preparation was effective.

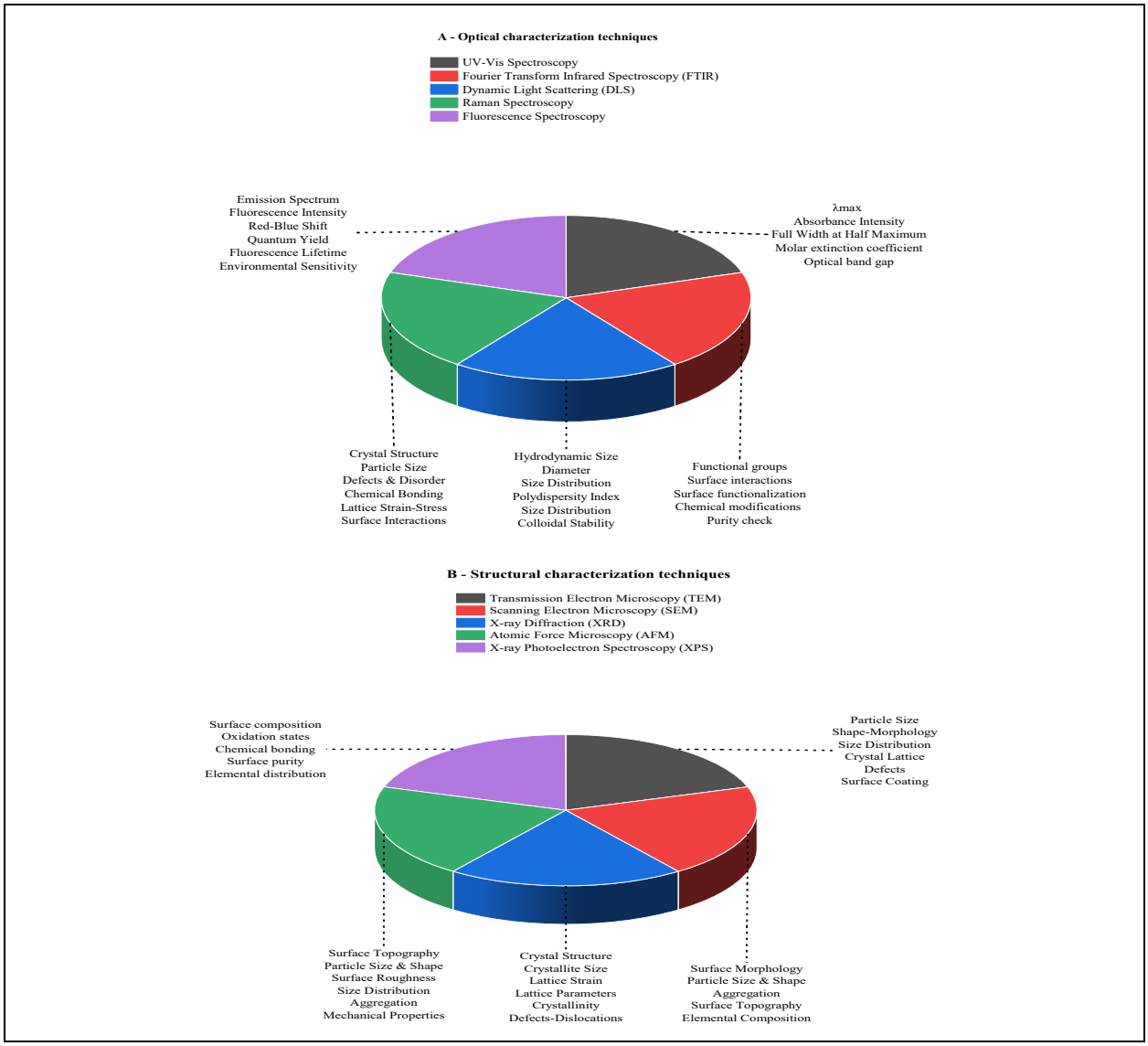


Fig. 5: Nanoparticle characterization techniques (A) Optical characterization and (B) Structural characterization

4.1 Morphology of Titanium Dioxide Nanoparticles

It is possible to investigate the surface and shape of TiO_2 nanoparticles synthesized with the help of plant extracts with the varied number of different methods, which include SEM, TEM with EDX elemental analysis, and particle size analysis (PSA) (Saka *et al*. 2024). According to SEM photos, the majority of developed TiO_2 nanoparticles are spherical (Dey and Srivastava, 2022). Though there are some tiny cracks arising because of the slight surface alterations, the particles are round and are commonly tangled and bundle-like in shape. These photos are also used to show minute pinholes on the surfaces of the particles. The shape and the surface characteristics of the nanoparticles are significant since they have a powerful impact on a large number of their physical and chemical characteristics (Chen *et al.* 2020). TiO_2 is available in a variety of forms such as anatase and rutile that possess tetragonal crystal structure and brookite with an orthorhombic structure. Even in the case of TiO_2 nanoparticles, these crystal structures tend to be identical. These green nanoparticles are very porous and therefore unique in surface shape and texture (Borisov *et al.* 2019). In recent years, nano TiO_2 has made significant strides in environmental applications. Its porous nature makes it highly beneficial for applications in air and water purification as well as environmental chemical reaction catalysis (Gonçalves *et al.* 2021). When TiO_2 NPs are synthesized using plant extracts, the phytochemicals are responsible for the special porosity structure. Terpenoids, alkaloids, steroids, polyphenols, flavonoids, free amino acids, antioxidants, and tannins are a few of them. These substances aid in the development of these special advantageous structures (Aravind *et al.* 2021a). Utilizing plant-derived chemicals for environmental applications, inorganic materials with crystalline TiO_2 NPs and nanotubes develop distinctive surface properties through green synthesis techniques (Gatou *et al.* 2024). The structural properties of nanocrystals are lost when heated to temperatures above 400 °C. The growth of crystals and agglomeration of particles will increase with a rise in the calcination temperature, which will cause an increase in crystallite size and a decrease in specific surface area. Moreover, the phase conversion between anatase and rutile can be performed at a later stage in the temperature, and it depends on the conditions of synthesis and the material used. These heating effects affect the stability and functional behavior of TiO_2 nanoparticles but do not lead to an easy loss of shape or structure. The roughness and, thus, the area accessible for interaction on the particle surface are reduced, as are the borders between the small units that comprise these crystals (i.e., the distances between the small parts) (Mino *et al.* 2020). When synthesized using a microwave-based technique, the TiO_2 NPs were spherical (Renuka *et al.* 2023).

4.2 X-ray Diffraction of Titanium Dioxide Nanoparticles

Measuring crystal size and crystallinity in synthesized materials can be accomplished with the use of XRD (Alhiti *et al.* 2024). By comparing the sample's characteristics with the standard reference data, compositional information is obtained (Ali *et al.* 2022).

The crystallinity of the data obtained is confirmed by means of a comparison between them with the Joint Committee on Powder Diffraction Standards (XRD, JCPDS Card No. 78-2486). The XRD patterns complied with reference patterns thereby confirming the phase of TiO_2. Egerton, (2014) compared and discussed the crystal structure with those reported in the literature, one being similar to a known crystalline form of TiO_2, anatase. Structural analyses performed by different researchers using the green synthesis have found that the synthesized TiO_2 nanoparticles have well-defined crystalline phases, which are usually anatase, rutile, or brookite. The crystal structure of these polymorphs can be tetragonal (anatase and rutile) or orthorhombic (brookite) with each unit being made of a TiO_6 octahedron with various configurations. The appearance of these crystalline phases gives important information on their crystallography and stability (Pugachevskii, 2013). Yadav *et al.* (2024) reported green synthesized TiO_2 NPs with an average crystal size between 30-40 nm. Accurate assessment of the small size of the produced crystals allows the measurements of the physical and chemical characteristics of NPs to be interpreted in their possible usage in several sectors, including environmental and biological application, enhanced.

4.3 UV–vis and FTIR Analysis of Titanium Dioxide Nanoparticles

The optical properties of TiO_2 nanoparticles are studied by UV-Vis spectroscopy. The response of the particles to ultraviolet and visible light is normally measured between 300 and 800 nm to determine the nature of the particles (Mickymaray, 2019). Nanoparticles with a size ranging between 2 and 100 nm can be applied to this method because their size influences the absorption and scattering of light. The findings can help obtain data concerning the size of particles, the aggregation behavior of a particle, and the optical bandgap, which is utilized in various processes, including photocatalysis and purification of the environment (Ahmed *et al.* 2023). Typical titanium dioxide nanoparticle UV-visible absorption spectra clearly show two distinct peaks at 264 nm and 322 nm. These peaks are usually in agreement with most findings for TO_2 NPs (Younis *et al.* 2023a). While the peak at 264 nm is generally normal for electronic transitions of TO_2, the peak at 322 nm indicates the presence of bigger particles or possible agglomeration. Those results validate the special optical characteristics of TiO_2 NPs in

keeping with published data showing same absorption characteristics in such materials. This clarifies the behavior of TiO_2 NPs under applied light exposure, which is essential for their implementation in solar energy and photocatalytic devices (Pugachevskii, 2013). The characteristics of titanium dioxide nanoparticles are determined in aqueous solution using UV–Vis spectroscopy. As indicated by various research, the process of TiO_2 nanoparticles formation is usually followed by hydrolysis and condensation of titanium compounds that results in the generation of Ti–O–Ti networks and nucleation of nanoparticles within a few hours at room temperature. In the process, alteration of the color of reaction mixture might be observed as a result of interactions between biomolecules produced by plants and titanium precursors. Nevertheless, TiO_2 nanoparticles are normally white or milky in color which implies that there is the formation of a colloidal nanoparticle suspension as opposed to actual color change indicating reduction reaction. In particular, if plant extracts are utilized to promote the formation of these nanoparticles, the absorbance spectra for the TiO_2 NPs formed in the solution fall within the range of roughly 245–265 nm. The presence of TiO_2 NPs is shown by these peaks, which also show that their synthesis is successful. This method helps track the development of nanoparticles and is crucial for verifying the stability and production of TiO_2 NPs, particularly in green synthesis techniques that employ plant extracts (Yadav *et al*. 2024).

FTIR spectra of the prepared nanoparticles demonstrate typical Ti–O–Ti stretching vibrations within 400–700 cm^{-1}, which proves the synthesis of TiO_2 NPs framework. Besides, the absorption bands related to the phytochemical functional groups are found, including –OH, –COOH, and $–NH_2$, which could be considered to maintain the reduction and stability of the nanoparticles in the process of its production. These plant extract functional groups serve as capping agents, which inhibit aggregation to improve particle stability (Nair *et al.* 2022).

5. APPLICATION OF TITANIUM DIOXIDE NANOPARTICLES

5.1 Antimicrobial Activity

One traditional method of managing microbial diseases is using several medicinal plants as a source of medication. It is the first-line treatment for a number of deadly Gram-positive and Gram-negative microbial infections since the invention of numerous antibiotics in the twenty centuries (Mickymaray, 2019). Masoudi *et al.* (2023) reported the antimicrobial effect of TiO_2 nanoparticles by the disc diffusion technique at standardized conditions of the experiment where a fixed amount of nanoparticle suspension was put in each disc. The test microorganisms had different diameters of the inhibition zone which ranged between 15.7 ± 0.45 mm and 30.3 ± 0.25 mm. *A. niger* showed an inhibition zone of 30.3 ± 0.25 mm, *B. subtilis* 25.5 ± 0.31 mm, *S. cerevisiae* 25.4 ± 0.36 mm and *P. digitatum* 25.2 ± 0.27 mm, which imply strong antimicrobial activity. Intermediate effects of inhibition were documented against *S. aureus* (23.3 ±0.27 mm) and *E. coli* (20.9 ±0.28 mm) with the lowest values obtained against *K. pneumoniae* (15.7 ±0.45 mm). The difference in the diameter of inhibition zones indicates variations in sensitivity of microbes to TiO_2 nanoparticles in the same experimental conditions. The MIC values were 20-80 µL/mL, MBC values were 40-140 µL/mL and confirmed the concentration dependence of the antimicrobial activity of the produced nanoparticles, as shown in Fig. 6.

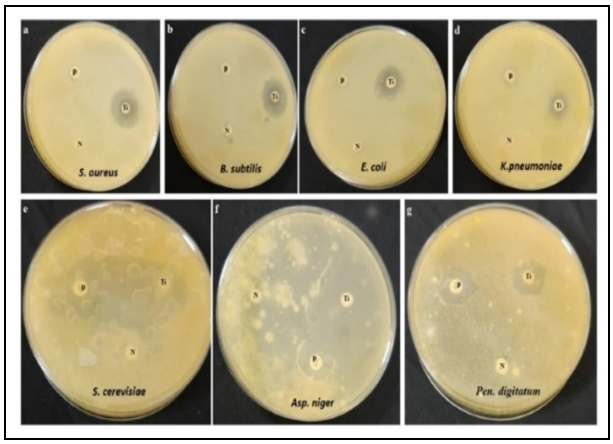


Fig. 6: Antimicrobial activity of titanium dioxide nanoparticles preparation by green method against different microbial strains (Masoudi *et al.* 2023)

When exposed to UV or solar light, titanium dioxide nanoparticles become excited and start the formation of electron–hole pairs on their surface. These carriers then react with molecular oxygen and water molecules to give highly reactive oxygen species (ROS) that include superoxide anion ($O_2^{-}\cdot$), hydroxyl radical (·OH), and hydrogen peroxide. It is basically these ROS that are responsible for the antimicrobial activity of TiO_2 NPs. Excessive build-up of ROS initiates oxidative stress in microbial cells which damages them in several ways. The initial step includes attacking by ROS the lipid part of the cell membrane thus causing permeability changes as well as structural disruption and finally total loss of integrity. Other intracellular biomolecules attacked by ROS include proteins and nucleic acids which undergo oxidation resulting in enzyme inactivation as well as DNA strand breaks (Younis *et al.* 2023b). DNA damage leads to cell death since it inhibits replication and transcription, as shown in Fig. 7.

In addition to being able to be coated on cotton fabric, TiO_2 NPs have shown antifungal efficacy against *Aspergillus niger* and *Trichoderma reesei*, with a

maximal zone of inhibition of 65 mm against *T. reesei* (Awad *et al.* 2022). When added to alginate impression materials used in dentistry, TiO_2 NPs have demonstrated enhanced antibacterial action against *Candida albicans* and *Streptococcus mutans*. Higher concentrations of TiO_2 NPs result in increased antimicrobial activity (Özyıldız *et al.* 2010). Additionally, antimicrobial paper packaging materials were developed using TiO_2 NPs.

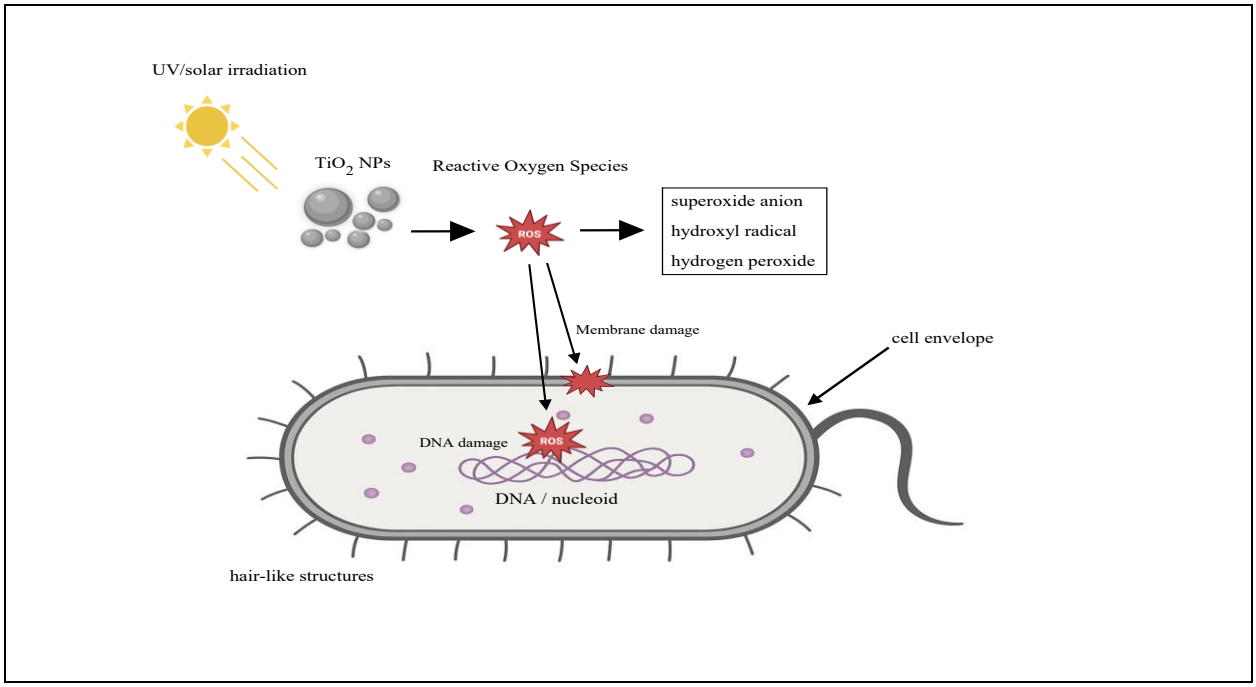


Fig. 7: Schematic of the mechanism of action of titanium dioxide nanoparticles as antibacterial agents

Titanium dioxide nanoparticles develop on the surface of the paper treated from the synthesis of NPs derived from hydrolyzed titanium tetrachloride in an alkaline environment thereafter applied to the paper. Those improved packing materials (Taussarova *et al.* 2019) have shown great resistance to microbial activity. TiO_2 NPs were employed in a range of sectors, including food packaging, textiles, and dentistry; overall, they have shown strong antibacterial characteristics against a range of microorganisms, including fungus and bacteria (Serov *et al*. 2024).

5.2 Anticancer Activity

Titanium dioxide nanoparticles have their anticancer action primarily by means of oxidative stress. TiO_2 nanoparticles have the ability to produce ROS especially in light irradiation after cellular internalization and through the formation of electron-hole pairs. These ROS have the potential to cause oxidative damage to cellular constituents that results into the death of the cell. In spite of the fact that the slightly acidic conditions peculiar to the tumor microenvironment can affect surface reactivity of nanoparticles and cellular interactions, the generation of ROS by TiO_2 is mostly related to photoactivation rather than low pH itself. An enormous quantity of ROS will break redox cellular homeostasis leading to general molecular and organelle damage. The effect of TiO_2 NPs on the induction of toxicity in cancer cells through multiple signaling mechanisms has been discussed below: ROS-based stress may initiate different intracellular events. First, mitochondrial damage shall cause the loss of potential across their membrane and subsequent release of factors involved in apoptosis which further enhances the generation of more ROS that shall activate different signaling cascades leading to apoptosis. Second, ER stress induced by ROS inhibits proper polypeptide folding and calcium homeostasis within cells thereby activating unfolded protein response pathways as well. Both dysfunctions lead to the induction of apoptosis. Genomic level ROS attack nucleic acids, resulting in strand breakage, fragmentation and mutagenic lesions, which result in genomic instability that in turn suppresses proliferation and survival of cancerous cells. Also, ROS induced by TiO_2 NPs regulate multiple cancer progression signaling pathways: NF-κB, activating anti-apoptotically and pro-survival gene transcription, PI3K/Akt/mTOR, activating cell growth and anti-apoptotic responses, β-catenin regulating cell proliferation and fate control through stabilized -catenin and target gene transcription, and MAPK/Erk which activates responses to growth factors and cell proliferation and differentiation. The disturbance of these signals tips the balance toward cell cycle arrest and cell death (Younis *et al*. 2023a). In addition, TiO_2 NPs interfere in cell cycle phases by inhibition of transition from G2 to M phase which blocks unchecked cell division. All these activities bring about apoptosis as well as necrosis of cancer cells, as shown in Fig. 8 (Murugan *et al.* 2016).

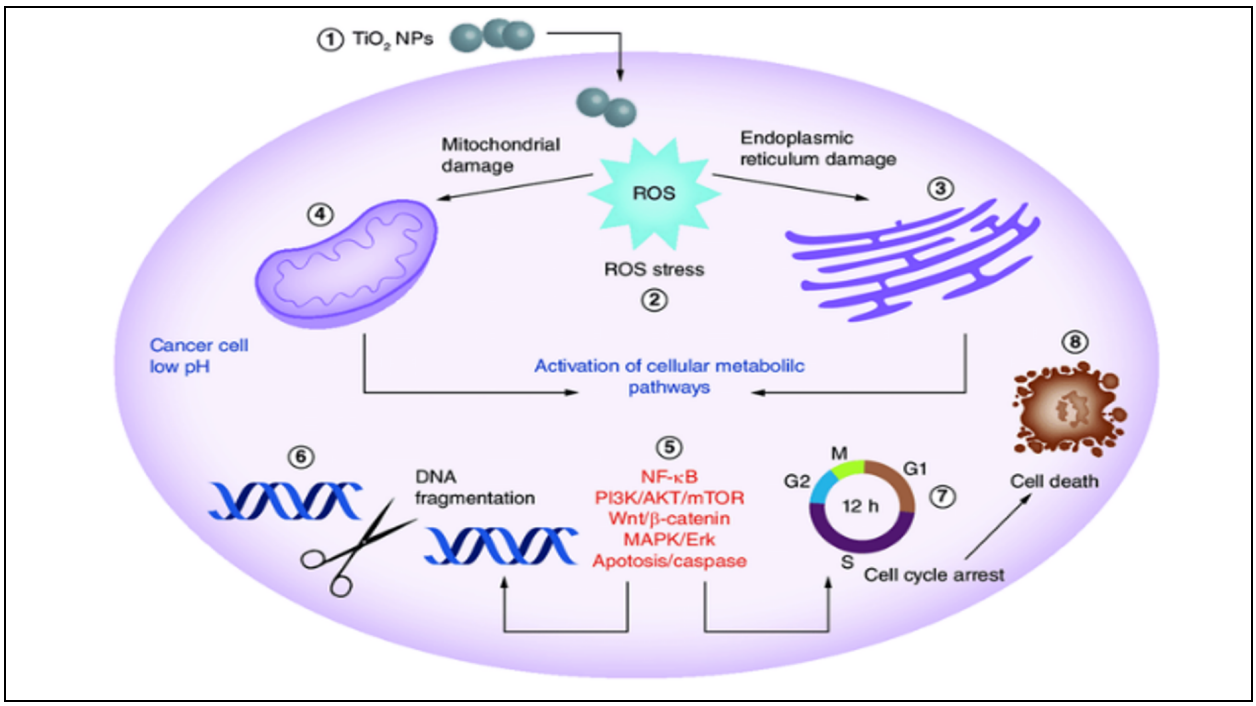


Fig. 8: Schematic of the mechanism of action of titanium dioxide nanoparticles as anticancer Activity

Murugan *et al.* (2016) described the possible mechanism of synthesized TiO_2-NPs acting as anticancer against breast cancer cell MCF-7. Results from MTT assay revealed a cytotoxic effect against MCF-7 cells in a dose-dependent manner with an IC_{50} value of 60 $\mu g \cdot mL^{-1}$, clearly supporting previous findings. The study has further established through the application of AO/EtBr staining under fluorescence microscopy that apoptosis is induced by treatment containing TiO_2-NPs. Morphological data obtained through 4′,6-diamidino-2-phenylindole staining expressed nuclear fragmentation, which is considered one of the morphology markers for identification of apoptosis. Cell treatment with TiO_2 NPs analyzed by Western blotting gave information on cytochrome c expression, which indicates that cell death occurs *via* mitochondrial pathway (Shanthi *et al.* 2015).

The surface engineering of nanocarriers with homing ligands has been reported to improve the specificity of drug delivery (Desai and Hubbell, 1992). Venkatasubbu *et al.* (2013) PEGylated TiO_2-NPs and then attached folic acid before finally conjugating paclitaxel (PAC) (a standard anticancer drug) on it. The anticancer effects of all nanocomposites were tested in animal models for hepatocellular cancer. Surface-modified paclitaxel attached to TiO_2-NPs expressed much higher anti-cancer activity than PAC alone, because surface modification of TiO_2 with FA (TiO_2-PEG-FA-PAC) provides a way to target overexpression with FA receptors in liver cancer cells leading to targeted accumulation of PAC-NPs at the site of cancer cells that reduce toxicity for the drug. Treatment of HepG2 cells with TiO_2-PEG-FA-PAC resulted in a dose-dependent decrease in cell viability. Biochemical and hematological assessments, including alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total and direct bilirubin, albumin, gamma-glutamyl transferase, α-fetoprotein, and titanium dioxide nanoparticle levels, alongside hematological indices such as packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, mean platelet volume, and platelet distribution width, further supported the enhanced anticancer efficacy of TiO_2-PEG-FA-PAC.

5.3 Antifungal Activity

TiO_2 nanoparticles initiate their antifungal efficacy *via* several molecular and cellular pathways. The high amount of ROS and reactive nitrogen species produced due to the noted activity caused by TiO_2 NPs against fungal cells, leads to the disruption of cell wall and membrane integrity. Consequently, oxidative stress initiates metal ion release and signaling for stress pathway as well as direct damage to the membrane which increases its permeability adding on structural instability (Moradpoor *et al.* 2021). Likewise, within the cell, these nanoparticles can disturb essential physiological functions primarily through protein denaturation and oxidation that hamper enzyme activity and associated systems. TiO_2 NPs also induce genetic mutation by DNA damage through interrupting replication accuracy. The net result inhibition of mitochondrial function and hance intracellular ATP synthesis, thus disturbing energy metabolism resulting in cell death. These provide enough validation towards proving potent antifungal activity

triggered by such nanoparticles not only on structural but also metabolic and genetic disturbance as shown in Fig. 9 (Garg *et al.* 2024).

TiO_2-NPs have proved antifungal efficacy against several strains of fungi (Ahmad *et al.* 2018). The sol-gel and green synthesis route adopted by Irshad *et al.* (2020), for TiO_2-NPs were evaluated for their antifungal activity against pathogenic plant fungi associated with wheat rust and *Ustilago tritici*, the major killer of wheat crops. This fungus is inhibited at different concentrations by TiO_2 NPs. Another healthier application for the NPs is due to lesser side effects index compared to fungicides and pesticides which pose quite some hazards to health. More reports on *Fusarium oxysporum* (Mukherjee *et al.* 2020), *Fusarium graminearum*, *Candida albicans* (Ahmad *et al.* 2019), *Macrophomina phaseolina, Coniophora puteana*, and *Poria placenta* are found positive about antifungal activity from TiO_2-NPs (Garg *et al*. 2024). For example, one type has also been suggested as a protective material acting as a coating on buildings having cultural heritage value so as to prevent biodeterioration over mortars. Laboratory results and on-site heritage building prove that the application of TiO_2 is an effective biocidal (lichens and phototropic microorganisms) and preventive material in building deterioration (Mahr *et al.* 2013). TiO_2 NPs show much weaker antifungal activity because of differences in cell envelopes comparing mechanisms against bacteria (Seven *et al.* 2004). Chen *et al.* (2009) tested the effects of TiO_2 NPs on fungal mold *A. niger* growing on the surface of Paulownia wood and found that this fungus was inhibited by treatment with these nanoparticles. De Filpo *et al.* (2013) also reported antifungal activity for wood-decaying fungi, brown (*Mucor circinelloides*) and white rot (*Hypocrea lixii*). Polyvinyl alcohol (PVA) and TiO_2 nanocomposites were synthesized and tested on two fungal strains *Candida albicans* (ATCC 14053) and *Penicillium chrysogenum* (MTCC 5108) (Chougale *et al*. 2020).

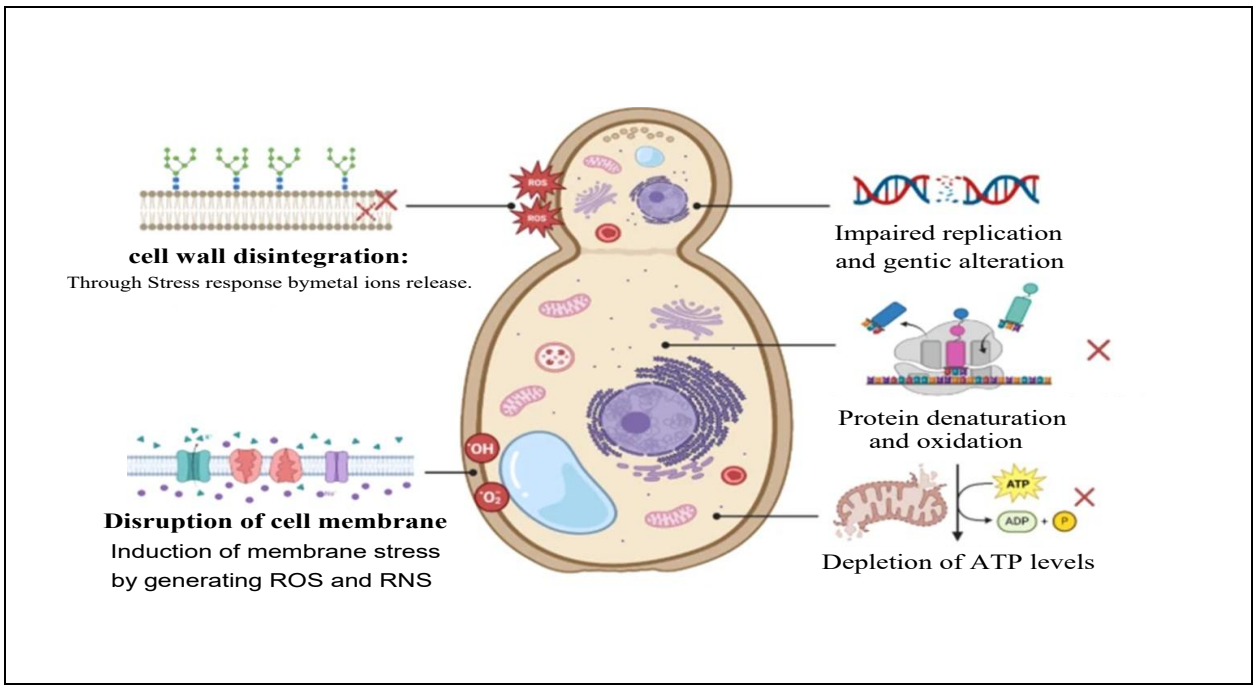


Fig. 9: Schematic of the mechanism of action of titanium dioxide nanoparticles as antifungal Activity

Table 3. Synthesis of titanium oxide nanoparticles from roots, leaves, flowers, seeds and fruit peel extracts

S. No.	Plant Species	Part	Ave. Size (nm)	Shape	Application	Ref.
1.	*Piper betel*	Leaves	5.5	Spherical	▪ Antibacterial activity ▪ Antifungal activity ▪ Antioxidant activity	(Hunagund *et al.* 2017)
2.	*Acacia catechu*	Leaves	25	Spherical, Agglomerated	▪ Antibacterial activity	(Chand *et al.* 2020)
3.	*Artemisia haussknechtii*	Leaves	44	Spherical	▪ Antibacterial activity ▪ Antioxidant activity	(Alavi and Karimi, 2017)
4.	*Syzygium aromaticum*	Twigs	19	Tetragonal	▪ Antibacterial activity ▪ Antibiofilm activity	(Achudhan *et al.* 2020)
5.	*Trigonella foenum-graecum*	Leaves	55	Spherical	▪ Antibacterial activity	(Subhapriya and Gomathipriya, 2018a)
6.	*Morinda citrifolia*	Leaves	10	Spherical	▪ Antibacterial activity ▪ Antifungal activity	(M. *et al*. 2017a)
7.	*Cola nitida*	Leaves, Fruits	107	Spherical	▪ Antioxidant activity ▪ Antifungal activity	(Akinola *et al.* 2020)
8.	*Parthenium hysterophorus*	Leaves	35	Spherical	▪ Antibacterial activity	(Thandapani *et al.* 2018)
9.	*Psidium guajava*	Leaves	33	Spherical	▪ Antibacterial activity ▪ Antioxidant	(Santhoshkumar *et al.* 2014a)
10.	*Azadirachta indica*	Leaves	32.5	Spherical	▪ Antibacterial activity	(Thakur *et al.* 2019)
11.	*Moringa oleifera*	Leaves	100	Spherical	▪ Wound healing	(Sivaranjani and Philominathan, 2016)
12.	*Catharanthus roseus*	Leaves	67.5	Irregular	▪ Antibacterial activity	(Velayutham *et al*. 2012a)
13.	*Acacia nilotica,*	Leaves	12	Spherical	▪ Antibacterial activity ▪ Anticancer activity	(Rao *et al.* 2019)
14.	*Withania somnifera*	Roots	26	Square, Spherical	▪ Antibiofilm activity ▪ Cytotoxic activity	(Shabib *et al.* 2020)
15.	*Withania somnifera, Eclipta prostrata, Glycyrrhiza glabra*	Leaves	7.5 – 11.5	Spherical	▪ Anticancer activity ▪ Antibacterial activity	(Maheswari *et al.* 2020)
16.	*Hibiscus rosa sinensis*	Flowers	7	Spherical, dispersed	▪ Antibacterial activity	(Devasena *et al.* 2014)
17.	*Gloriosa superba*	Leaves	20 - 100	spherical	▪ Antibacterial/biofilm activity ▪ Anticancer activity ▪ Photocatalytic activity	(Mahendran *et al.* 2021)
18.	*Aloe barbadensis mill*	Leaves	25	Spherical	▪ Antibacterial activity ▪ Biofilm activity	(Rajkumari *et al*. 2019)
19.	*Jatropha curcas*	Leaves	13	Spherical	▪ Wastewater treatment ▪ Photocatalytic activity	(Goutam *et al.* 2018)
20.	*Tinospora cordifolia*	Leaves	9	Irregular	▪ Antibacterial activity	(Maurya *et al.* 2012)

					▪ Photocatalytic activity	
21.	*Morinda citrifolia*	Leaves	10	Spherical	▪ Antimicrobial activity	(Syamsol Bahri *et al.* 2021)
22.	*Kniphofia foliosa*	Roots	8.2 - 8.5	Spherical	▪ Antibacterial activity	(Bekele *et al.* 2020)
23.	*Calotropis gigantean*	Flowers	10.5	Spherical, Ovel	▪ Acaricidal	(Marimuthu *et al.* 2013a)
24.	*Solanum trilobatum*	Leaves	70	Spherical	▪ Antiparasitic efficacies	(Rajakumar *et al.* 2014a)
25.	*Euphorbia prostrata*	Leaves	83	Spherical	▪ Antileishmanial activity	(Zahir *et al.* 2015)
26.	*Jatropha curcas*	latex	50	Spherical	▪ Biotechnology ▪ Environmental ▪ Biomedical	(Hudlikar *et al.* 2012a)
27.	*Mangifera indica*	Leaves	30	Spherical	▪ Larvicidal	(Rajakumar *et al.* 2015)
28.	*Psidium guajava*	Leaves	32.5	Spherical, cluster	▪ Antibacterial activity ▪ Antioxidant activity	(Santhoshkumar *et al.* 2014b)
29.	*M. citrifolia*	leaves	10	Spherical	▪ Antimicrobial activity	(M. *et al.* 2017b)
30.	*Annona squamosa*	Peels	23	Polydisperse, Spherical	▪ Bio therapeutic ▪ Bioengineering	(Roopan *et al.* 2012a)
31.	*Luffa acutangula*	Leaves	10 - 49	hexagonal	▪ Antimicrobial activity	(Anbumani *et al.* 2022)
32.	*Myristica fragrans*	Seeds	24.58	Spherical	▪ Photocatalytic activity ▪ Antibacterial activity	(Sagadevan *et al.* 2021)
33.	*Catharanthus roseus*	Leaves	25 - 110	Uneven and clustered	▪ Antiparasitic activity	(Velayutham *et al.* 2012b)
34.	*Annona squamosa*	Peels	23±2	Polydisperse and spherical	▪ Bio therapeutics ▪ bioengineering	(Roopan *et al.* 2012b)
35.	*Jatropha curcas L.*	Leaves	25 - 100	Mostly spherical and uneven	▪ Biotechnology ▪ Environmental ▪ Biomedical ▪ Electronic	(Hudlikar *et al.* 2012b)
36.	*Trigonella foenum-graecum*	Leaves	20 - 90	spherical	▪ Antimicrobial activity	(Subhapriya and Gomathipriya, 2018b)
37.	*Calotropis gigantea*	Flowers	160 - 220	Spherical and aggregated	▪ Acaricidal activity	(Marimuthu *et al*. 2013b)
38.	*Solanum trilobatum*	Leaves	70	Uneven spherical and oval	▪ Larvicidal activity ▪ Pediculicidal activity	(Rajakumar *et al*. 2014b)
39.	*Tinospora cordifolia*	Roots	15.02	triangular	▪ Antibacterial activity ▪ Photocatalytic activity	(Saini and Kumar, 2023)
40.	*Azadirachta indica*	leaves	124	Spherical and interconnect-ted	▪ Photodegradation	(Sankar *et al.* 2015)
41.	*A. cepa (onions), C. annum L*	Leaves	20 - 170, 90.07 - 103.6	Spherical	▪ Antimicrobial activity	(Abdul Jalill, 2018)
42.	*Tulbhagia violacea*	Leaves	3.0 – 3.4	rectangular, spherical	▪ Antioxidant activity ▪ Anticancer activity	(Mbenga *et al.* 2023)
43.	*Ageratina altissima L.*	Leaves	60 - 100	Spherical	▪ Photocatalytic activity	(Ganesan *et al.* 2016)
44.	*Mentha arvensis*	leaves	20 - 70	Smooth and spherical	▪ Antimicrobial activity	(Ahmad *et al.* 2020)

45.	*Laurus nobilis*	Leaves	~ 100	Spherical	▪ Antioxidant activity ▪ Antimicrobial activity	(Rajeswari *et al.* 2023)
46.	*Aegle marmelos*	Leaves	150	spherical	▪ Removed ornidazole of Wastewater	(Ahmad *et al.* 2021)
47.	*Carica papaya*	Leaves	20	Spherical	▪ Photocatalytic activity	(Kaur *et al.* 2019)
48.	*Hibiscus*	Flowers	7	Monodispersed and spherical	▪ Antibacterial activity	(Santhoshkumar *et al.* 2014c)
49.	*jasmine*	Flowers	31 - 42	Spherical	▪ Photodegradation	(Aravind *et al.* 2021b)
50.	*Carum copticum*	Seeds	5.58 ± 12.01	Spherical	▪ Biofilms activity	(Altaf *et al.* 2021)

6. DISCUSSION

It is very important to use the right plant extract for the green synthesis of TiO_2 NPs. Plant extracts containing proteins, organic acids, and higher secondary metabolites operate as stabilizing and reducing agents. These biomolecules support both the stability of nanoparticles and the reduction of metal ions. The surface properties of the nanoparticles, as well as their size and geometry, are largely determined by the individual compounds in the plant extract. This necessitates the use of appropriate plant extracts for the effective and successful synthesis of TiO_2 NPs (Ansari *et al.* 2022). Another important consideration for green synthesis using plant extracts is temperature. The rate of reaction increases with temperature. Better uniformity and stability of the nanoparticles in size can be achieved by creating a suitable environment for controlled nucleation. On the other hand, excessively high temperatures may cause unchecked development, resulting in fluctuating size or aggregation. On the other hand, since energy is scarce at low temperatures, the reaction would be slowed down again due to larger particles and fewer nucleation sites. In light of this, temperature tuning is crucial for nanoparticle stability and size (M *et al.* 2017c). Another crucial factor influencing the synthesis of nanoparticles with plant extracts is pH. Since the biomolecules that catalyze reduction are more active at higher pH values, smaller, more uniform, and stable nanoparticles are produced. The reduction will be slower under acidic conditions, resulting in larger nanoparticles in size. For the production of effective nanoparticles, it is therefore crucial to identify the optimum pH value (Subhapriya and Gomathipriya, 2018c). A study by a team of researchers employing *Aloe barbadensis* (L.) Burm peel extract as a green coating agent could be used to summarize the link between the type of plant extract and the size and form of the generated nanoparticles. While keeping the spherical shape of the nanoparticles, it was discovered that adding the investigated extract up to 10% enhanced the surface area and pore volume (Wellia *et al.* 2024). The size and form of the generated TiO_2 particles could be influenced by the presence of certain secondary metabolites or reducing agents in the plant extracts (Jiménez *et al.* 2021). Fig. 10 shows the correlation between the size of the nanoparticles and the type of plant extract used in their synthesis. The mean size of the particles, as demonstrated in the graph, differs widely among the samples and it measures between around 10 nm and 190 nm. The majority of samples are between 20 and 80 nm, which implies that smaller particles sizes are more likely to be obtained at the synthesis conditions that are under study. Nevertheless, some of the samples have significantly increased particle sizes (more than 120 nm), which indicates that changes in synthesis parameters like plant extract composition or reaction conditions may have a strong impact on the growth of the particle. The graph makes it evident that the majority of nanoparticles are spherical in shape.

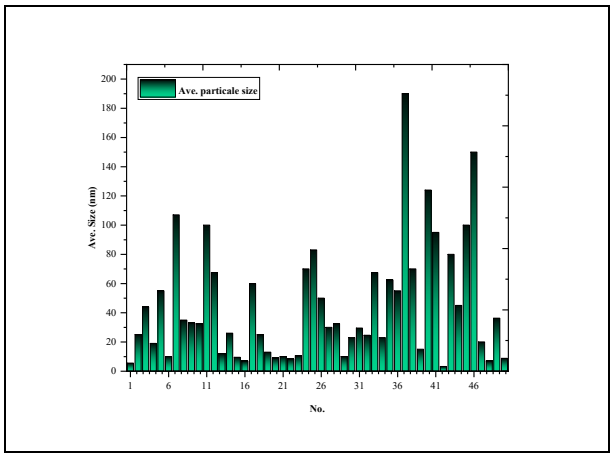


Fig. 10: Relationship between leaf, seed, flower, root and fruit extracts and titanium dioxide particle size

The comparison of various works also indicates that the nature of plant extract and the plant part to be used significantly influence the size and shape of TiO_2 nanoparticles. This also brings out a significant aspect of nanoparticle synthesis which is the ability to select the appropriate plant material and importantly to regulate the conditions of preparation with the aim of realizing nanoparticles with the target structure and properties to be used in various applications.

In short, biosynthesized TiO_2 nanoparticles have great potential in biological uses, particularly in antimicrobial, anticancer and antifungal uses. Synthesis conditions and plant-derived compounds have a strong

influence on their properties influencing particle size, stability, and biological activity. In general, green synthesis offers a sustainable strategy in the creation of TiO_2 nanoparticles with possible biomedical applications.

7. CONCLUSION

Plant-mediated synthesis is one of the efficient and environmentally friendly methods of the synthesis of TiO_2 nanoparticle with a wide array of plant parts that are natural reducing and stabilizing agents. The review has indicated that the selection of a synthetic part of the plant and the characteristics of the synthesis determine the aspects of nanoparticles such as size, morphology, structural properties and optical properties. These differences have a direct impact on the functional behavior of TiO_2 nanoparticles in biomedical and environmental applications.

The review highlights that TiO_2 nanoparticles are increasingly synthesized using green approaches and are widely applied in antibacterial, anticancer, and environmental applications, particularly in pollutant degradation and water purification. Plant source-nanoparticle relationship is a valuable model that enables customization of nanoparticles to suit a given application.

Plant-mediated nanoparticle synthesis is a sustainable alternative to traditional procedures, which provides ecofriendly and cost-efficient production pathways. Future research must be aimed at determining optimal preparation conditions and experimenting with more plants to enhance uniformity and performance that will assist in the generation of TiO_2 nanomaterials for specific applications.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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