

# Green Synthesis and Characterization of Titanium Dioxide Nanoparticle (TiO<sub>2</sub>) Synthesized Using the Leaf of Eucalyptus Globulus - An In Vitro Study

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## ABSTRACT

**Aim And Objective:** The aim of the present study was to synthesize and characterize Titanium Dioxide nanoparticles (TiO<sub>2</sub> NP) using the leaf of Eucalyptus Globulus. To evaluate the shape and size of TiO<sub>2</sub> NPs synthesized using the leaf of Eucalyptus globulus.

**Material And Methods:** In 100ml distilled water, 1 g of Eucalyptus globulus was mixed and dissolved. The herbal formulation was heated for 15 minutes at 70°C and filtered. Using an orbital shaker, this filtrate was homogeneously combined with 0.39mg of Titanium dioxide and then used to bio reduce titanium (Ti) ions to titanium dioxide nanoparticles (TiO<sub>2</sub> NPs). The surface characterization of the titanium dioxide nanoparticles was assessed using UV-Visible absorbance spectroscopy, Scanning Electron Microscopy (SEM) and functional group evaluation using Fourier transform infrared spectroscopy (FTIR). **Result:** Scanning electron microscope results revealed that the synthesized Titanium dioxide nanoparticles were spherical in shape and their average size ranged from 20-70 nm.

**Conclusion:** Titanium dioxide nanoparticles can be effectively extracted from Eucalyptus globulus when used as a reducing agent.

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## INTRODUCTION

Nanomedicine is a novel therapeutic approach that has recently gained attention for its applicability in disease pathogenesis. Nanomaterials have unique size-dependent physical and chemical features such as nano-size, optical, catalytic, thermodynamic, high surface area to volume ratios, and super-paramagnetic and electrochemical capabilities that allow them to be used in medical applications (1). The chemical composition and form of nanoparticles also impact their characteristics (2). As a result, a plethora of protocols and methods for the synthesis, functionalization, and application of nanoparticles and nano-carriers have flooded the scientific and clinical communities with new therapeutic approaches ranging from molecular targeting to radio frequency ablation, personalized therapies to minimally invasive techniques (3). However, nanotechnology faces significant challenges in terms of biocompatibility, in-vivo kinetics, tumor targeting efficacy, acute and chronic toxicity, escape from the reticuloendothelial system, and cost-effectiveness. Zinc Oxide (ZnO), Manganese Oxide (MnO<sub>2</sub>),

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Titanium dioxide (TiO<sub>2</sub>), Iron Oxide (Fe<sub>2</sub>O<sub>3</sub>) (4), and their functionalized variants have proved advantageous in biological applications throughout the last few decades (5). Solgel, solid state reaction, microwave irradiation, ultrasonication, sono-electrochemical, and thermal breakdown are some of the synthetic processes utilized to prepare these nanoparticles (5). The hydrothermal procedure has shown to be the most beneficial of the techniques outlined above (5,6). Under regulated temperature or pressure in aqueous circumstances, hydrothermal synthesis is done in autoclaves with or without Teflon liners. The internal pressure created is mostly determined by the temperature and amount of solution injected to the autoclave. This approach is commonly used to produce small-sized particles with low agglomeration (7). Green synthesis, which uses plant-based extracts as a medium, has also shown to be an efficient and cost-effective approach for nanoparticle preparation. A variety of plants and plant extracts with significant antimicrobial activity are being employed in the manufacturing of nanoparticles. One such plant is Eucalyptus globulus, which is a known powerful medicinal herb with anti-inflammatory and anti-microbial properties.

Titanium dioxide (TiO<sub>2</sub>), an inert and durable material, has attracted attention due to its excellent photocatalytic activity, chemical stability, nontoxicity, quick electron transfer to molecular oxygen, and abundant availability at a low price, which is in great demand in industries (7,8). Titanium dioxide is found in three different forms: anatase, brookite, and rutile. It has a wide surface area, good surface morphology, is nontoxic, and has good biological activity, including antibacterial activity (9). The anatase form of nanoTiO<sub>2</sub> and UV light stimulation are required for optimal antibacterial efficacy, since photo-catalysis produces peroxidation of the PUFA phospholipid component of the microbial lipid membrane, resulting in loss of respiratory function and cell death (9,10). However, there are emerging concerns that Titanium NPs may pose health risks by causing cytokine release, which leads to inflammation (9,10). Titanium nanoparticles (NPs) are frequently employed in orthopedics and dentistry because they are a well-known antibacterial and bone-repairing substance with good fracture resistance, ductility, and weight to strength ratio. Because titanium inhibits cell adhesion and proliferation, functionalized and coated forms of titanium can be employed (11). Titanium has also been used in a variety of applications, including antibacterial coatings (12), photocatalytic degradation of organic pollutants (13), self-cleaning surfaces (14), and water and air purifiers (14,15). The present study has focused on the synthesis and characterization of TiO<sub>2</sub> NPs using Eucalyptus globulus as a reducing medium.

## MATERIALS AND METHODS

### preparation of leaf extract:

1 g of Eucalyptus globulus powder was mixed with 100ml of distilled water to form a homogenous mix of the plant extract.

The solution was then heated and boiled in a heating mantle. Using Whatman no:1 filter paper, the boiled plant extract was filtered and the supernatant solution was collected in a beaker (Figure 1).

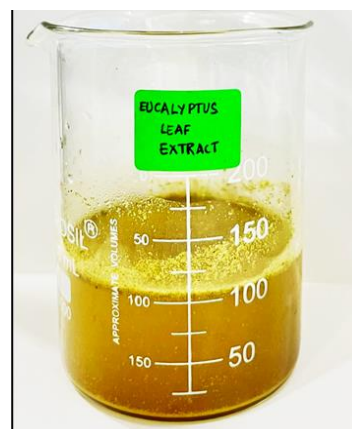


Figure 1: Filtered Eucalyptus globulus leaf extract.

### Preparation Of Titanium Dioxide Nanoparticles

50 ml of distilled water was mixed with 0.39g of titanium dioxide powder. To this, 50ml of the obtained supernatant plant extract solution prepared was mixed (Figure 2).

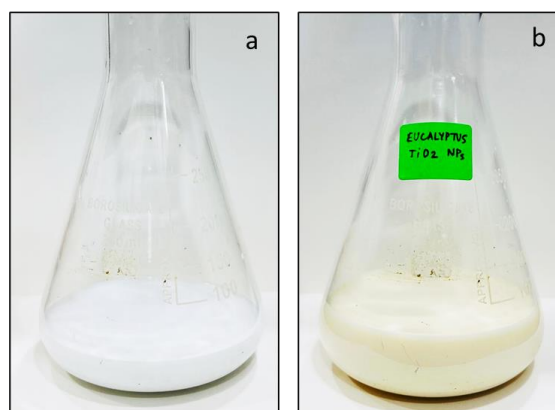
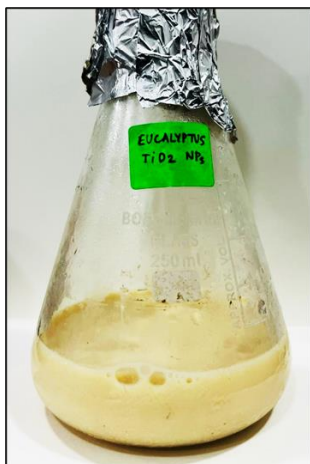


Figure 2: (a)TiO<sub>2</sub> mixture with distilled water, (b) TiO<sub>2</sub> solution mixed with the plant extract.

The obtained solution was then placed on a magnetic stirrer/orbital shaker overnight for visual and chemical changes observed during the synthesis of TiO<sub>2</sub> nanoparticles. A color change from white to whitish brown was observed (figure 3). This was due to the reduction of metal ion (Ti<sup>4+</sup>) indicating the synthesis of TiO<sub>2</sub> NPs. Using hourly UV-vis spectrometric data, titanium dioxide nanoparticle production was evaluated and the same was used to confirm the presence of TiO<sub>2</sub> nanoparticles produced in the solution.

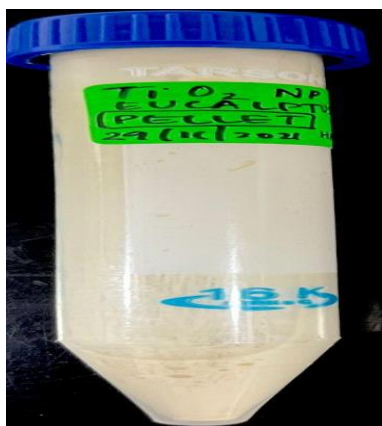


**Figure 3:** Sample after continuous stirring on an orbital shaker. Note the change in colour.

Upon confirmation, the solution was equally divided and poured into centrifuge tubes for centrifugation (figure 4). The pellet obtained was collected in a separate tube and later poured into petri dishes (figure 5). These petri dishes (figure 6) were dried in a hot air oven and the dry substrate containing the TiO<sub>2</sub> nanoparticle was scrapped and the powder was collected and labeled (figure 7).



**Figure 4:** Tubes ready for Centrifugation with TiO<sub>2</sub> nanoparticle mixture.



**Figure 5:** Pellet collected



**Figure 6:** Petri dish with sample post drying in a hot air oven.



**Figure 7:** Powdered sample collected in a single tube.

### Characterization Of Synthesized Titanium Dioxide Nps using Sem AND Ftir

The JSM -IT800 NANO SEM (Scanning electron microscopy) was used to view the obtained nanoparticle and also confirm its shape and size. SEM was used to examine

external specifications of adsorbent morphology. An FTIR spectrophotometer was used to study the functional groups on the biaxial surface.

UV-Vis Spectroscopy was used to record the synthesized titanium dioxide NPs' UV-Vis peak of absorption. The samples' scanning range ranged from 350 to 660 nm. A distilled water standard was used to interpret all UV-Vis absorption spectra.

Fourier transforms infrared spectroscopy (FTIR): FTIR is a good analytical tool to illustrate the binding properties of synthesized nanoparticles. FTIR analysis of our green synthesized TiO<sub>2</sub> nanoparticles was performed by using Bruker Alpha II FTIR spectrometer. The spectrum was recorded in the range of 400-4000 cm<sup>-1</sup>.

## RESULTS

### Characterization Of Tio2 Nanoparticle (Sem, Ftir, Uv-Vis Spectroscopy)

#### Uv- Vis Spectroscopy

The UV-vis absorbance spectroscopy method was used to determine molecule size and the band hole of the synthesized TiO<sub>2</sub> NPs. The absorption spectrum was recorded for the

sample in the range of 250-650 nm. The UV absorption spectrum of synthesized TiO<sub>2</sub> NPs is given in Figure 8 which showed the formation of peak in the wavelength of 400 nm. The results of the presents study are similar to those of previous studies (16).

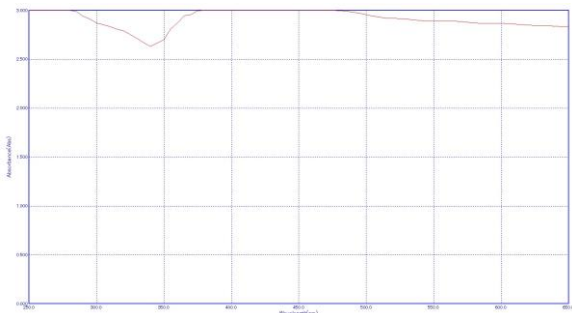


Figure 8: UV- vis spectroscopy analysis of TiO<sub>2</sub> NPs.

### Ftir Spectrum Of Titanium Dioxide Nanoparticles

In the present study, the estimation of the synthesized TiO<sub>2</sub> NPs was done at room temperature between the wavelengths 400-4000 cm<sup>-1</sup>. The FTIR spectra of the synthesized titanium dioxide nanoparticles is given in Figure 9 . From the given FTIR spectrum it can be observed that at 528.68 cm<sup>-1</sup> TiO<sub>2</sub> bending mode can be observed and at 1067.59 cm<sup>-1</sup>. This may be attributed to surface water sorption. TiO<sub>2</sub> stretching mode can be observed. Stretching can be observed upto 3336 cm<sup>-1</sup>

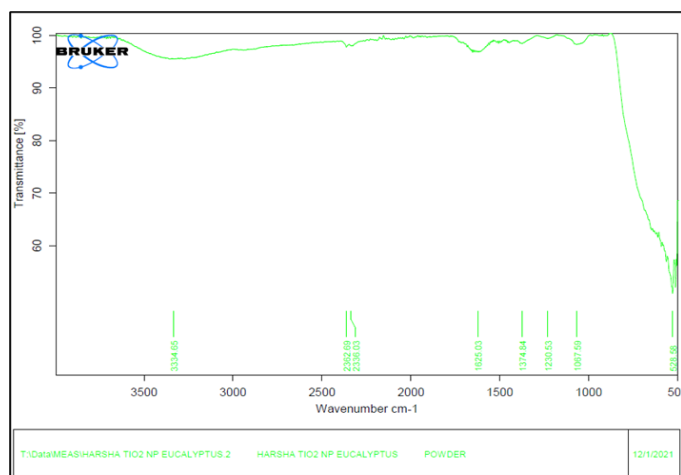


Figure 9: FTIR spectrum of the synthesized TiO<sub>2</sub> nanoparticle.

### Scanning Electron Microscopy (Sem)

The scanning electron microscope was used to evaluate the size, shape and surface properties like morphology of the synthesized nanoparticle. Figure 10 illustrates the SEM picture at 10x magnification of TiO<sub>2</sub> NPs synthesized using the leaf extract of Eucalyptus Globulus. The SEM image shows uniform distribution of the TiO<sub>2</sub> NPs that are smooth and spherical in

shape. The average size of the nanoparticle ranges between 20-70 nm.

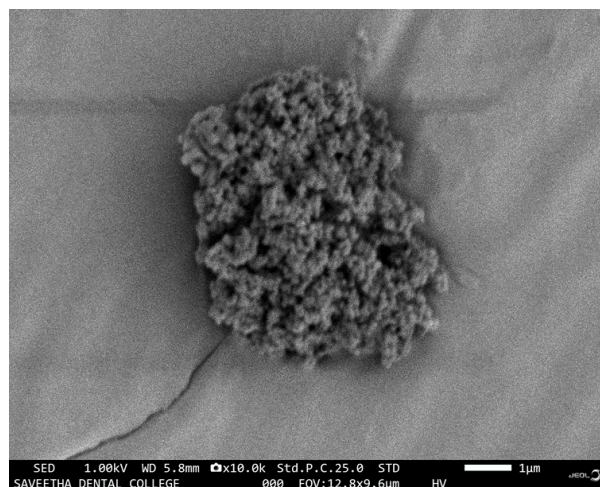


Figure 10: SEM image of TiO<sub>2</sub> NPs.

### DISCUSSION

The creation of efficient antimicrobial agents that can improve the therapeutic profile and efficacy of therapeutic agents is one of the most serious issues facing modern medicine(17). Recent developments in nanoscience and nanotechnology have made it possible to create novel nanomaterials, which has aided in the creation of a number of innovative and potent antimicrobial agents (18).

In comparison to the early years of the century, nanoparticle synthesis has advanced significantly in recent years. Earlier, conventional techniques were used to synthesize the nanoparticles(19). Large-scale nanoparticle synthesis can be accomplished more quickly using conventional physical and chemical techniques, but hazardous compounds are required as capping agents to preserve stability. These methods caused environmental toxicity because dangerous compounds were used. The Green Synthesis approach was proposed and is now widely utilized all over the world to avoid the usage of such dangerous chemicals. It is a method that is both economical and environmentally friendly. The present study utilized this similar method of green synthesis of TiO<sub>2</sub> NPs using Eucalyptus Globulus as a reducing agent.

Studies have demonstrated that eucalyptus leaf extract is a highly effective antioxidant, scavenging reactive oxygen species and shielding antioxidant enzymes which are crucial for cellular defense(20).

Long recognized for its antibacterial qualities is titanium dioxide. By preventing chain reactions, peroxide breakdown, and transition metal oxidation, titanium dioxide nanoparticles act as antioxidants (21). One of the key components of antioxidant activity is the capacity to absorb, neutralize, or quench singlet and triplet oxygen. Keeping these properties in mind, the obtained TiO<sub>2</sub> NP will be further tested for the

antioxidant, antimicrobial properties.

The presence of phytochemicals in the eucalyptus leaf extract itself aids in the creation of metal oxide nanoparticles in the current work by triggering oxidation and reduction reactions(23). Alkynes and alkanes, which are often found in secondary metabolites such as terpenoids, carboxylic acids, alkaloids, and so on, were the functional groups of phytochemicals that induced nanoparticle production (24). It is proposed that proteins can attach to nanoparticles via free amine groups or crystalline residues in the proteins. Carboxylic groups are known to coordinate with metal ions, which may function as a nucleation site for the production of nanoparticles(25). The overall peak from FT-IR analysis supports the presence of protein moiety in Titanium dioxide nanoparticle samples.

Protein molecules or peptide chains are thought to function as template nucleation sites for reducing Titanium ions to create Titanium dioxide nanoparticles. The fast production of NPs was assessed by UV- Visible spectroscopy at a maximal absorbance of 290 nm as preliminary confirmation(20). Extracellular bio-reduction of titanium dioxide nanoparticles offers extensive development of green production of metallic nanoparticles employing plants and plant bark sources(20).

Further research involving extensive evaluation of properties of the synthesized nanoparticle and its potent application in the field of dentistry needs to be done.

## CONCLUSION

Green synthesis of titanium dioxide nanoparticles was achieved successfully using eucalyptus globulus leaf. The synthesized TiO<sub>2</sub> NPs were evaluated for their size, shape and morphology using SEM and FTIR. SEM evaluation confirmed the spherical and smooth surface of the obtained nanoparticle. FTIR demonstrated the role of Eucalyptus globulus leaf in the synthesis of TiO<sub>2</sub> NP. further antimicrobial activity evaluation would pay way for development of effective dental materials.

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