Green Synthesis of *Spirulina* Mediated Titanium Dioxide Nanoparticles and Their Characterization

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Abstract
In recent times, green synthesis of nanoparticles had received significant consideration due to the requirement of non-hazardous chemicals, low-cost approaches, renewable materials and eco-friendly alternative to the traditional production processes because of its growing industrial applications. The present study focused on synthesis of *Spirulina* mediated Titanium dioxide nanoparticles (TiO2 NPs) was used to study its effects on economic traits of silkworm (*Bombyx mori* L.). The green synthesis of TiO2 NPs was characterized by UV, PSA, XRD, FTIR, SEM and TEM. In this study the UV-Visible absorbance spectroscopy result was found to be nanoparticles were formed at the range of 300 nm. SEM images shows that the nanoparticles are in spherical shape and TEM images shows that the nanoparticles are spherical in shape and their size range from 50 - 60 nm.

Keywords: *Spirulina,* Titanium dioxide, Green synthesis and Characterization

Introduction

Sericulture is one of the most remarkable industries, which includes the exploitation of mulberry (*Morus* sp.) leaves for rearing of silkworm (*B. mori*) to produce silk. The deprivation of nutrients in mulberry leaves leads to reduce the growth and development of silkworms such a situation, fortification of mulberry leaves helps to improve the cocoon production. There are many protein sources available to enrich the mulberry leaves, *viz.*, proteins, amino acids, botanicals, probiotics and etc.

Recently, some reports revealed that blue green algae *S. platensis* contains various minerals, 18 amino acids such as glycine, glutamine, histidine, lysine, methionine, cysteine, creatine, phenylalanine, serine, proline, tryptophan, asparagine, pyruvic acid, and pivotal vitamins like tocopherol, biotin, thiamine, niacin, riboflavin, pyrodozoic acid, folic acids, beta-carotene and vitamin B12, etc. (Kumar *et al.,* 2009; Ganeshprabhu *et al.*, 2011; Thangappandiya and Dharanipriya, 2019).

Nanomaterials belongs to the range of below 100 nm has unique chemical, physical, electrical, and mechanical properties and also diversely utilized in the field of medical, biotechnology, microbiology, pharmaceutics and chemistry, engineering, inexpensive catalyst, cytotoxicity study, etc.

Researchers are going for a green synthesis route to prepare nanomaterials because the green synthesis approach is simple, ecofriendly, and cost-effective. Green synthesis is a fascinating method for material science.In the past few decades, metal oxide semiconductors such as ZnO, MgO, CuO, CdO, NiO, etc. were widely used, and it is prepared via physical, chemical, and biological methods. Among them, TiO2 NPs are a well-known semiconductor with a wide bandgap of 3.2 eV for anatase and 3.0 eV for rutile phase, but the brookite phase is rare to obtain. The Anatase and rutile phase of TiO2 exhibits a tetragonal crystal structure, but the brookite phase is an orthorhombic structure. The transition metal oxide, mainly TiO2, is widely used in cosmetics, photocatalysts, medicines, sensors, and solar cell applications because of its peculiar properties like interconnected pores and large surface area.

Nowadays, the metal and metal oxide nanoparticles are synthesized by chemical as well as physical methods such as the microwave, hydrothermal, solid-state, solution route method, sol–gelchemical phase decomposition vapour, solvothermal crystallization, ultrasonic irradiation and green synthesis method. Nevertheless, these methods generate heterogeneous NPs with high energy consumption and also the chemicals process involves synthetic capping, reducing, and stabilizing agents which results in the creation of anti-environmentally safe by-products. In recent years researchers are focused on the green synthesis route to the synthesis of metal and metal oxide nanoparticles. The bio-mediated metal and metal oxide NP's shows potential application on drug delivery, nanocatalyst, nano-medicine, biosensor, biotechnology, and microbiology. The green synthesis method is similar to the chemical reduction process, where the costly chemical reagents are replaced by plant extracts and microorganisms and also reduces the toxicity, which enhances its biomedical applications.

The bio-mediated TiO2 NPs exhibit excellent antibacterial, anti-inflammatory, anti-fungal, anti-microbial, and several biological activities. The decomposition of microorganisms by its photo-semiconductor properties results in the enhancement of biological activities. There are numerous reports on the preparation of TiO2 NPs from Cinnamon Powder, *Mangifera indica, Citrus reticulate,Azadirachtaindica leaf, Murayakoenigii, Curcuma longa, Cynodondactylon, Annona squamosa, Morindacitrifolia, Psidium guajava*and *Jatropha curcas*. Fungus-mediated towards the biological applications. Moreover, the morphology, size, shape, porosity, and crystallinity depend upon the concentration of precursor and temperature.

The aim of the present study was the green synthesis of *Spirulina* mediated Titanium dioxide nanoparticles and their characterization.

Materials and method

Algae materials

The dried powder of *Spirulina* was procured from R.K. Algae Centre, Mandapam, Tamil Nadu, India. The species of the *Spirulina* taxonomically documented and certified at Botanical Survey of India, Coimbatore, Tamil Nadu, India.

Preparation of aqueous extraction of *S. platensis*

The aqueous extract of *S. platensis* was obtained by heating, Around 10g of finely ground *S. platensis* powder in 100 ml of deionized water at 90℃ for 45 mins, then the solution was filtered through Whatman filter paper No.1 to remove debris. The resultant clear green coloured solution obtained was stored at 4-8℃ (Some *et al.,*2019).

Preparation of titanium dioxide solution

Titanium dioxide was prepared by 0.08g TiO2 powder was dissoloved in 1000 ml of distilled water. The resulted solution was 0.01mM TiO2 solution, which is stored for further studies.

Synthesis of titanium dioxide nanoparticles

Spirulina mediated TiO2NPs were synthesised utilising 0.01 mM titanium dioxide and aqueous extract of *Spirulina platensis* as bio-reductant and capping agent in a green synthesis. 20 mL of aqueous extract was added to 80 mL of 0.01 M TiO2 solution, which was maintained at room temperature for 6 hours with continuous stirring in a hotplate magnetic stirrer. A change in colour confirmed the production of TiO2NPs (pale yellow).

Characterization

UV, PSA, FTIR, XRD, SEM, and TEM with EDAX were used to characterized the green synthesis of *Spirulina*-mediated TiO2NPs.

UV-vis absorbance spectroscopy

The green synthesis of *Spirulina* mediated titanium dioxide nanoparticles were confirmed by optical measurement using UV-visible spectroscopy in the wavelength ranging between 200 and 800 nm at the resolution of 1 nm (Subhapriya *et al.,* 2018).

Particle size analyzer

The green synthesis of *Spirulina* mediated Titanium dioxide nanoparticles size distribution and shape parameters were analyzed by the diffraction of a laser light source (Sankar *et al.,* 2014).

Fourier transform infrared spectroscopy

FT-IR analysis was used to investigate the functional groups of *Spirulina*-mediated TiO2NPs. The Nicolet TM spectrometer was used to examine the FT-IR spectroscopy of green synthesis of *Spirulina*-mediated TiO2 NPs. The sample was stored in an FT-IR sample chamber, and the spectra was collected with 16 scans in the mid-IR range 4000-400 cm-1 at a resolution of 4 cm-1. To eliminate spectrum interference caused by ambient carbon dioxide and water vapour, the interferometer and detection chamber were purged with dry nitrogen. Before each sample was analysed, the air background spectrum was recorded (Dev *et al.,* 2017).

X-ray diffraction

The phase development and crystallinity of *Spirulina* mediated TiO2NPs are studied by using X-ray diffraction, an analytical technique. Smart Lab was used to record the XRD pattern of produced *Spirulina*-mediated TiO2 NPs. Powder

X software was used to compute the lattice parameters. The Scherrer's equation was used to compute the particle size (d spacing value) of the sample (Aravind *et al.*, 2021).

Scanning electron microscope

Size of nanoparticles measured with precision nanoscale using a scanning electron microscope (SEM). SEM was used to examine the exterior morphology shape and size of *Spirulina* mediated TiO2 NPs (Yedurkar *et al*., 2016).

Transmission electron microscopy with energy dispersive X-ray spectroscopy

Transmission electron microscopy was utilised to characterise the individual particles of the material, which has a 10,000-fold higher resolution than scanning electron microscopy. For TEM analysis, a little amount of sample was dispersed in dry ethanol and sonicated for 10 minutes. A single drop of Spirulina-mediated TiO2NPs was carefully placed on a copper-coated grid in the prepared sample. A Jeol JEM-2100 electron microscope was used to capture TEM pictures (Japan). The elemental analysis of the produced TiO2NPs was also carried out using EDAX.

Result and Discussion

UV-Vis Absorbance Spectroscopy

The molecule size and band hole of integrated green synthesis of titanium dioxide nanoparticles are determined using UV–vis absorbance spectroscopy. Fig. 1 displays the UV absorption spectra of produced TiO2, which shows a prominent absorption band at 300 nm. Our findings are consistent with the published literature, indicating the presence of a titanium dioxide band at 380–400 nm (Ahmad et al., 2020)

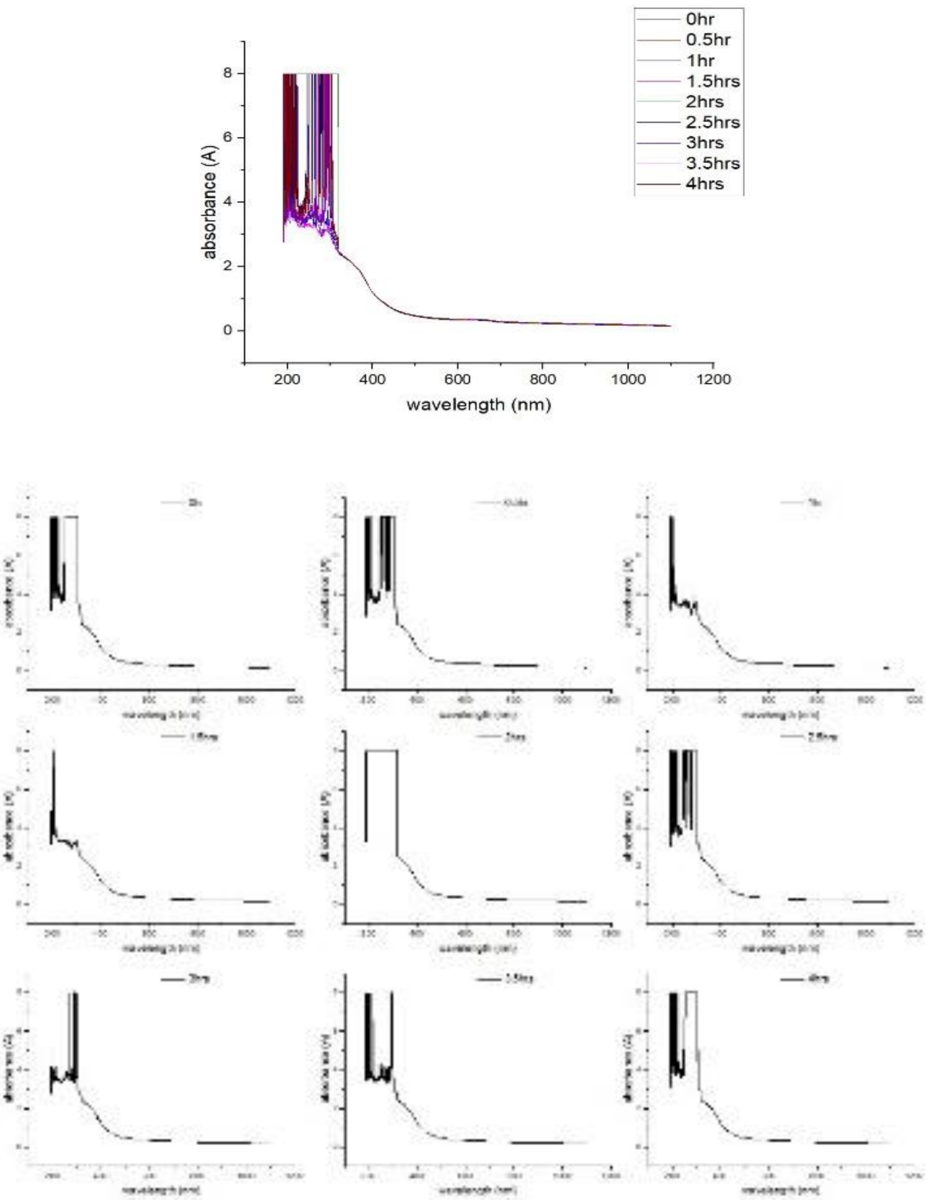

Fig. 1. UV-Vis Absorbance Spectroscopy of Spirulina-mediated TiO_2 NPs

Particle Size Analyzer

The size distribution and form properties of *Spirulina*-mediated Titanium dioxide nanoparticles were studied using the diffraction of a laser light source. For the green synthesis of titanium dioxide nanoparticles, the zeta potential is -20.08 mV (Fig. 2) and the refractive index is 2.155. Previous research on the ultra-rapid photocatalytic activity of *Azadirachta indica* manufactured colloidal titanium dioxide nanoparticles revealed a zeta potential of – 24 mV (Sankar *et al.*, 2014).

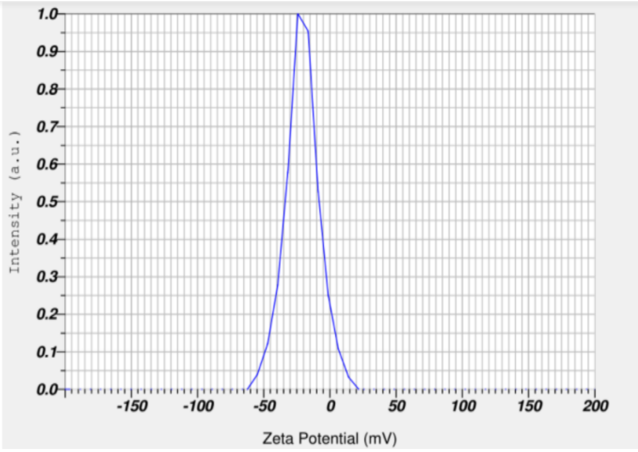

Fig. 2 Particle Size analyzer of Spirulina-mediated TiO_2 NPs

FTIR

The FTIR spectra of Spirulina-mediated TiO2NPs are displayed in Fig. 3, which was previously published (Gundasundari et al., 2017). The concentrated values observed at 3339 cm^{-1} and 2971 cm^{-1} in Spirulina mediated TiO2NPs confirm the presence of the stretching vibrations of the Alcohol and hydroxyl molecule, which are present in Normal "polymeric" OH stretch and Saturated aliphatic (C-H) bonds. In the green synthesis of Spirulina mediated TiO2NPs, the bending vibrations were observed at 2949, 2846, 2294, 2118, 1740, 1636, 1441, 1368, 1232, 1059, and 539 cm^{-1}. Aliphatic amine stretching vibrations were found at a peak of 1059 cm^{-1}. The amide group was discovered near the apex of 1636 cm^{-1} in Fig. Also available are carboxyl or carbonyl groups. C=C stretching is responsible for the peak at 1740 cm^{-1} (non-conjugated). The peak at 1059 cm^{-1} corresponds to aliphatic amine stretching, while the new peak at 539 cm^{-1} could be attributable to O-Ti-O bond stretching. This indicates that the TiO2 NPs were successfully implanted. The stretching of alkene groups could be attributed to two peaks seen at 2949 and 2846 cm^{-1}. C=O stretching of the amide group was detected at the peak 1636 cm^{-1}, N–H bending of the amide group was observed at the peak 1441 cm^{-1}, and C–N stretching of the (alkyl) amide group was observed at the peak 1441 cm^{-1}. This FTIR examination of Spirulina demonstrates that TiO2 NPs were successfully coated on the surface.

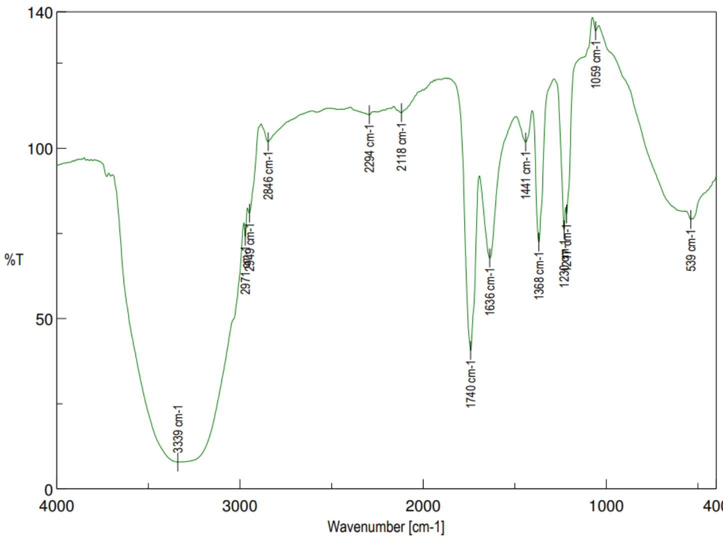

Fig. 3 FTIR analysis of Spirulina-mediated TiO_2 NPs

XRD

The XRD pattern of green synthesis of TiO2 nanoparticles is shown in Fig. 4. With diffraction angles of 27.79, 36.45, 41.57, 44.41, 54.65, 57.01, 63.11, 64.39, 69.22, 70.09, 76.88, 82.61, 84.47, and 88.01, it indicates the formation of good crystalline titanium dioxide with anatase phase shape. The prominent peak at 27.79 in the XRD pattern of green synthesis of TiO2 nanoparticles is only connected with the crystallographic plane of TiO2 anatase. The final material's stoichiometry is highly dependent on the partial pressure used during the synthesis. As a result, our synthesised TiO2 nanoparticles could exhibit a variety of stoichiometries. Trigonella foenum-graecum extract was recently samples were validated a high crystallinity level with diffraction angles of 25.28, 37.5, 47.5, 55, 62.5, 70 and 76.5 which confirms the formation of well crystalline titanium with anatase phase and it is well evident with the standard JCPDS database (# 21-1272). The diffraction angle perceived at 25.28 is associated to the (101) crystallographic plane of TiO2 anatase only (Subapriya et al., 2018).

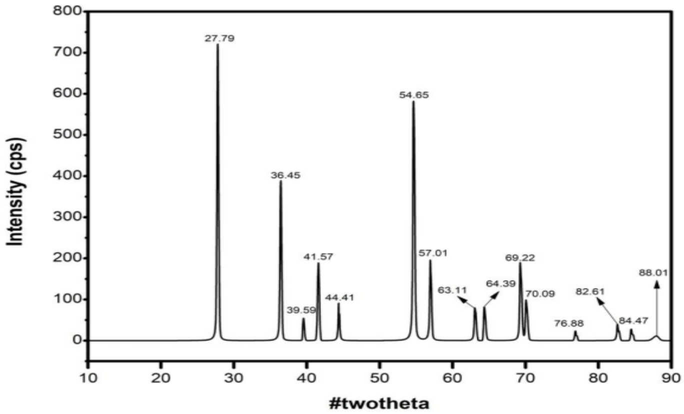

Fig. 4 XRD analysis of Spirulina-mediated TiO_2 NPs

SEM

Scanning Electronic Microscopy was used to investigate the form, size, and surface features such as morphology. Fig. 5 and 6 shows a SEM image of TiO2 nanoparticles made with S. platensis aqueous extract. The particles are uniformly dispersed, smooth, and spherical in shape, according to the SEM image of produced nanoparticles. We determined the average size of the produced nanoparticles using SEM images, which was found to be in the range of 90 to 150 nm. Fig. 5 denotes a 5nm scale range, while Fig. 6 denotes a 4nm scale range. They recently used Mentha arvensis leaves extract to synthesise spherical TiO2-NPs with a size range of 20.0–70.0 nm in a green synthesis of titanium dioxide (TiO2) nanoparticles (Ahmad et al., 2020)

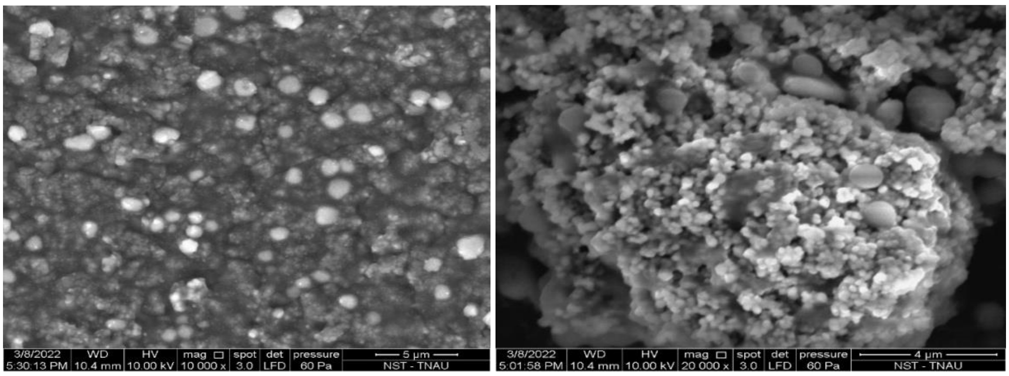

Fig. 5 and 6 SEM analysis of Spirulina-mediated TiO_2 NPs

TEM with EDAX

Energy-Dispersive Spectroscopy (EDAX) and Transmission Electron Microscopy (TEM) Analysis The shape, size, and size distribution of TiO2-NPs synthesized by S. platensis are shown in the figure. Without any aggregation, the TEM picture shows an excellent distribution of spherical TiO2-NPs (Fig. 7 and 8). The irregular and regular spores of green synthesis of TiO2 NPs are clearly shown. Furthermore, imaging examination revealed that the produced TiO2-NPs were between 50.0 and 60.0 nm in size. Trigonella foenum-graecum extract was recently used to make spherical TiO2-NPs with a size range of 20.0–25.0 nm (Subapriya et al., 2018). Previously, the effectiveness of metabolites found in algal cell extract in the fabrication of spherical TiO2-NPs was examined (Prasad et al., 2007). EDAX analysis was used to analyse the quantitative elemental structure of green produced TiO2-NPs (Fig. 9). The manufactured TiO2-NPs comprise Ti, Cu, Zn, O, Na, C and Al, according to data analysis. The EDAX result indicates the successful synthesis of TiO2-NPs using *S. platensis* filtrate metabolites; additionally, EDAX analysis confirms that Ti and Fe occupied the principal elements in the nanostructure. Other peaks like C, Na, and Al could be connected to the degradation of capping agents such polysaccharides, proteins, amino acids, and sugars due to X-ray emissions (Tristram *et al.*, 2007). The presence of Ti and Fe as a substantial component of TiO2-NPs generated by S. platensis was confirmed in a recent investigation.

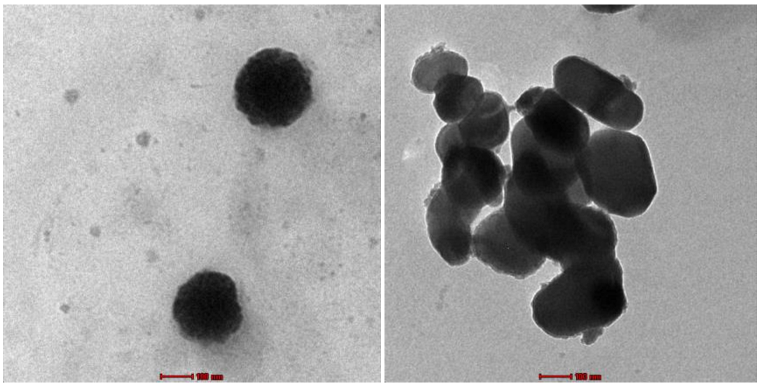

Fig. 7and 8 TEM analysis of Spirulina-mediated TiO_2 NPs

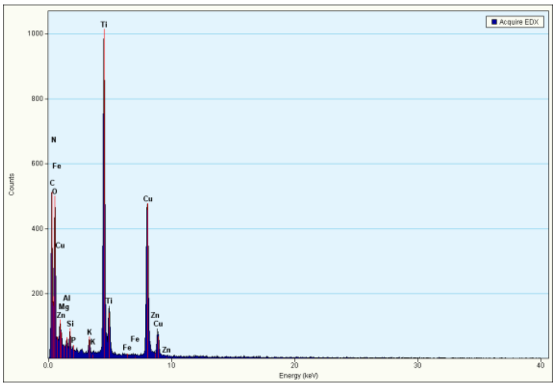

Fig. 9 TEM with EDAX analysis of Spirulina-mediated TiO_2 NPs

Conclusions

The green synthesis approach was utilised to create metaloids nanoparticles utilising spirulina algae extract as a reducing agent. It is a cost-effective, non-toxic, and environmentally friendly method. SEM and TEM were used to confirm the surface morphology. EDAX analysis validates the presence of the major components in the nanostructure. XRD predicts that the particles are crystalline, and FTIR confirms the functional group. UV-Visible absorbance spectroscopy analysis was used to assess the maximum absorbance and their respective band gap energy of green synthesis of spirulina mediated titanium dioxide nanoparticles, which validated the creation of nanoparticles.

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