Green synthesis of TiO_2 nanoparticles using aqueous extract of the bark of *Ficus religiosa* and investigation of their role in antibacterial activity

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Abstract

In this study, the titanium dioxide was synthesized from the aqueous bark extract of *Ficus religiosa* (Peepal tree). The green-colored nanoparticles were identified by UV-Visible spectroscopy, XRD (X-rays diffraction), TEM (Transmission electron microscopy), SEM-EDX (Scanning Electron Microscopy- Energy Dispersive X-Ray Spectrometry), AFM (Atomic force microscopy), and FT-IR (Fourier transform infrared spectroscopy). The green synthesized titanium dioxide nanoparticles were of varying sizes and shapes. These nanoparticles were analyzed for their antibacterial activity in both the bacteria Gram-negative as well as Gram-positive. Agar disc diffusion has been used to assess antibacterial activity. Against *Escherichia coli* (Gram-negative bacteria), a greater zone of inhibition has been seen in comparison to *Staphylococcus aureus* (Gram-positive bacteria). Overall, this study is focused on the employment of a green approach for the precise nanoparticle preparation of TiO_2 (Titanium dioxide) utilizing an aqueous bark extract of *Ficus religiosa* for the first time along with the investigation of their potential for antibacterial activity.

1. Introduction

The primary focus of nanoscience is the synthesis, characterization, investigation, and exploitation of nanostructured materials. These materials have at least one dimension in the nanoscale range [1]. The major scientific interest of the early 21st century is nanotechnology as researchers make use of the special properties of molecular as well as atomic accumulations created at the nanoscale scale. The capacity to modify these particles' biological, physical, and natural characteristics allows researchers to rationally develop and utilize nanoparticles for target-specific drug delivery, photocatalysis, antimicrobial, microelectronics, anticancer, diagnostic applications, electrochemistry, and agriculture [2]. In current years, there has been a point of interest in creating nanomaterials utilizing eco-friendly and sustainable technology. A technique for using biosynthesized nanoparticles, especially by taking plant extract as a precursor, is gaining popularity in the biomedical field. The process is more affordable and eco-friendly since plant material is natural and easily accessible[3].

Titanium is a hard, shiny metal that resists corrosion. TiO_2 is a solid inorganic white metal oxide that is weak soluble, non-flammable, has thermal stability, and has not been classified as dangerous by the UN (United Nations) GHS (Globally Harmonized System) of Chemical Classification and Labelling [4]. At varying temperatures, it may display three distinct phases in the Nano range, including Brookite, Rutile, and Anatase. Anatase, one of the phases, has been shown to have exceptional physical and chemical capabilities for environmental remediation [5]. Titanium dioxide, its most common constituent, is a photocatalyst and is utilized in the production of pigments. Titanium enhances carbohydrate generation, growth, and photosynthesis rate in plants [6]. Solution Combustion method [7], Solvothermal method [8], Microwave Assisted method [9], Hydrothermal method [10], co-precipitation method [11], CVD method [12], Sol-Gel (wet chemical method) [13], and Green synthesis are the various approaches for the nano-particles of TiO_2 preparation.

In the current paper, the greenly prepared TiO_2 nano-particles utilizing an aqueous extract of *Ficus religiosa* bark is described. The tree is indigenous to India and is said to have originated mostly in Eastern and Northern India. Additionally, it may be found in, Pakistan, China, Bangladesh Sri Lanka, and Nepal, which are neighboring nations [14]. It is the part of Moraceae (mulberry) family and is also called as the Bodhi tree or peepal in India. The tree develops to be rather huge, with broad extending branches and bark of brown color. The leaves are thin and lustrous, while the fruit is compressed and round in form, The color of the new juvenile leaves is crimson-reddish. becoming deep green as they grow. Flowering begins in February, fruits appear in the summer, and ripening is completed just before the beginning season of rain [15]. The average life span of Peepal tree is 1500 years [16]. This plant aids in the treatment of stomatitis, gout, inflammation, ulcers, leucorrhea, and neck glandular swelling. *Ficus religiosa* was shown to have several properties such as healing of wounds [17], antimicrobial activity [18], and acetylcholinesterase enzyme inhibitory action [19]. *Ficus religiosa* been utilized to treat diabetes in the conventional Ayurvedic system [20]. *Ficus religiosa* bark and leaves have also been examined for reducing glucose levels [21]. In normal rabbits, “β-Sitosteryl-D-glucoside” taken from the bark of *Ficus religiosa* demonstrated hypoglycemic action [22]. Because of these extraordinary features of *Ficus religiosa*, their stem bark was selected for the synthesis and antibacterial activity of Titanium dioxide. The overall work is represented in Fig. 1.

2. Material & methods

2.1 Materials Utilized in Experiments

The precursor used for the production of TiO_2 was titanium tetraisopropoxide ($C_{12}H_{28}O_4Ti$), which was bought from Sisco Research Laboratory Pvt. Ltd. in New Mumbai, India. The preparation of TiO_2 nanoparticles used only analytical-grade chemicals and reagents. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) bacterial strain were collected from Department of Microbiology, Institute of Medical Science, BHU (Banaras Hindu University), Varanasi, India.

2.2 Collection and preparation of aqueous extract

Ficus religiosa's fresh bark was gathered from the campus of BHU in Varanasi, Uttar Pradesh, India. To clear the impurities, deionized water was used to wash the bark multiple times. The bark was crushed with mortar and pestle after drying. Then 100ml of deionized water was heated with 4g of obtained bark powder on magnetic stirrer at 70°C for 2 hours. After that, the Whatman filter paper was used to filter the boiling solution. The final aqueous solution was collected and kept at 4° Celsius for additional synthesis process.

2.3 Synthesis of TiO_2 nanoparticles

10ml of Titanium tetraisopropoxide was poured into the beaker and then 20 ml of the final aqueous solution of the bark of *Ficus religiosa* was added to it. The beaker was kept over the stirrer at 50°C for 2 hours for proper mixing of the solutions. The aqueous solution was then centrifuged at 3500rpm for

15minutes. The finished product was rinsed twice after the supernatant was discarded with deionized water and dried in incubator to obtain powdered form of TiO2 which has been shown in Fig. 2.

For the comparison between chemical synthesis and green synthesis, two groups were taken into consideration. One group was designated as control group in which TiO_2 was obtained simply by mixing precursor with deionized water. In the second group, TiO_2 was obtained by reacting precursor with the bark extract. It was found out that the yield of chemical synthesis was 2500mg and that of green synthesis was 3300mg/10ml of TTIP suggesting the presence of strong reducing agents in the bark extract leading to high production of TiO_2 **(**Fig. 3**)**.

2.4 Characterization of Titanium dioxide

Different parameters were used to analyze the produced sample. A Perkin Elmer Ultraviolet (UV)-Visible-Lambda 25 spectrophotometer has been utilized to determine the UV-visible absorbance spectra of Titanium dioxide nanoparticle solutions to calculate the amount of light that a chemical compound absorbs. Perkin Elmer spectrum 65, M A model FT-IR spectrometer was utilized to describe that functional groups existed in TiO_2 nanoparticles. The crystallite structure phase of TiO_2 nanoparticles was determined by X-ray Diffractometer (XRD, Philips TW 1710), with incident radiation of Cu-Kα (40kV and 30mA) in the range of $2\theta = 10–80^{\circ}$. To examine the surface morphological structure and size of greenly produced nanoparticles, SEM (Carl Zeiss, MA) and TEM (Tecnai-G2 20 TWIN, USA) were used. Atomic force microscopy (Park AFM from South Korea) has been utilized to image the topography of green preparation TiO_2 nano-particles.

2.5 *In Vitro* Susceptibility Test

2.5.1 Bacterial culture: 100 mL of distilled water was utilized to dissolve the 2.5 g of broth. A boiling point was reached with the broth and then autoclaved for 15 minutes at 121 degrees Celsius (15 psi pressure). 10 mL of prepared broth has been placed in a test tube, led by the inoculation of *Escherichia coli* and *Staphylococcus aureus* bacteria. Then it was incubated for 24 hours at room temperature. Then again 100 mL of broth was placed in a conical flask and 1 mL of bacteria was inoculated in it and incubated for another 24 hours at room temperature.

2.5.2 Determination of colony forming unit (CFU): A unit of measurement known as is frequently utilized to evaluate the several microorganisms in a sample. Some of the visible colonies on an agar plate were used to calculate CFU. The dilution factor needs to be multiplied to obtain a CFU/ml.

$$\boxed{\textbf{CFU/mL} =} \; \frac{\text{Number of colonies x dilution factor}}{\text{The volume of a culture plate}}$$

$$\text{CFU} = 3.2 \times 10^{5} \text{ cfu/ml}$$

2.5.3 Antibacterial activity is measured using the agar disc diffusion method: -

This method depends on the idea that when a disc with a nanoparticle coating is placed on agar that already has been infected with the bacteria test, the agar medium absorbs moisture and the nanoparticles diffuse radially outward, creating a concentration gradient. After incubation, a clear zone or ring forms around a disc if the chemical prevents bacterial growth. So, an aqueous solution of TiO_2 was placed on the disc and then antibacterial activity was evaluated after 24 hours.

3. Result and Discussion

3.1 UV-Visible analysis

During the formation of greenly synthesis, TiO_2 nanoparticles is visible to the unaided eye. The UV-visible spectrum of absorption of TiO_2 nanoparticles suspended in deionized water is shown in Fig. 4, with the peak of absorption at 263nm. Comparable outcome was found by Hong et al., where, the absorption peak at 265nm was observed [23].

3.2 FT-IR analysis

To further demonstrate in the existence of functional groups of the TiO_2 nano-particles, FT-IR spectral analysis (Fig. 5) was done. FT-IR analysis of greenly prepared nano-particles demonstrates absorption bands at $3420cm^{-1}$ and $1640cm^{-1}$ for stretching along with bending vibration of O-H (water) molecules occurred at the boundary of green synthesized TiO_2 nanoparticles as reported by Amanulla and Sundaran [24]. The significant FTIR absorption band at $2350cm^{-1}$ and $2930cm^{-1}$ were attributed to C = O and N-H respectively which indicate the protein existence, triacylglycerol, and carbohydrate. The absorption band at $1391cm^{-1}$ and $1027cm^{-1}$ have been attributed to C = O and C-N stretching of amines correspondingly as described by Hudlikar et al., in 2012 [25]. The FT-IR absorption peak below $700cm^{-1}$ was attributed to Ti-O as reported by Amanulla and Sundaran [24].

3.3 XRD analysis

The powder XRD pattern of produced TiO_2 nanoparticles demonstrated the presence of a distinct peak (101). The peaks showed that the green synthesized particles were small in crystalline size. Figure 6 showed the X-ray diffraction pattern of TiO_2 nanoparticles scanned at 2 theta range from 10–80 degrees. The diffraction peak at 25.36°, 37.02°, 37.86°, 38.62°, 48.09°, 53.94°, 55.11°, 62.73°, 68.80°, 70.34°, and 75.09° with consistency plans of (101), (103), (004), (112), (200), (105), (211), (204), (110), (220), and (215) respectively represent the TiO_2 particles synthesis which was further “matched with the Joint Committee on Powder Diffraction Standards (XRD, JCPDS Card No. 78-2486) for the confirmation” [26]. Increasing the peak's width, the size of particles declines, like that of the existing material in the nano range.

3.4 AFM analysis

The surface morphology and roughness of greenly synthesized TiO_2 nanoparticles in a 2-dimensional and 3-dimensional form have been picturized in Fig. 7. According to the statistical roughness investigation, the resulting roughness average value was 0.968 nm, the root mean square (RMS) roughness was 0.1713nm, the surface skewness was – 1.037, and the surface kurtosis was 3.720. The topography image of AFM showed unevenness at the surface of greenly synthesized TiO_2 nanoparticles, with more depression than peaks.

3.5 SEM-EDX analysis

The surface view was examined using SEM evaluation of the greenly synthesized TiO_2 nanoparticles which exhibited the angular and irregular nano-structure with a high crystalline network which was shown in **Fig. 8 (a).** Particles are aggregated with bunch sizes less than 100 nm at greater magnification with a bar scale of 200 nm. Particles seem to be deformed spheres in the group. The **EDX spectrum** of TiO_2 in **Fig. 8 (b)** depicted the peaks for the elements carbon (C), titanium (Ti), and oxygen (O), along with their atomic and weight percentages (inset). Carbon peaks were found due to the existence of vitamin K in an aqueous extract of *Ficus religiosa* bark. The main findings of this research are the active involvement of bark components in the greenly prepared TiO_2 nanoparticles, and EDX outcomes further demonstrated the excellent purity of the synthesized sample by demonstrating the lack of additional impurity elements.

3.6 TEM analysis

The size, morphology, and shape of greenly preparedTiO_2 nano-particles have been researched utilizing the TEM technique. The low magnification picture at the 100 nm scale bar was shown in **(**Fig. 9**(a))**, revealing that all of the green synthesized nanoparticles were present in a mono-dispersed stage with some agglomeration. TiO_2 nanoparticles with a wide array of spots revealed their crystalline composition. Additionally, Fig. 9 **(b)** showed the histogram and distribution curve of TiO_2 nano-particles with an average mean size of approximately 78nm.

3.7 Antibacterial activity measured using the agar disc diffusion method: -

5mg/ml and 10mg/ml concentrated TiO_2 nanoparticles-impregnated disc was put on an agar plate previously inoculated with the "*S. aureus* (Gram-positive bacteria)" and "*E. coli* (Gram-negative bacteria)". The TiO_2 nano-particles took up the moisture and spread radially outward through the agar medium. A clear zone or ring of inhibition approximately 10 mm in 5 mg ml-1 and 12 mm zone in 10 mg ml^{-1} formed around a disc in *S. aureus* and an approximately 20 mm zone in 5 mg ml-1 and 22 mm zone in 10 mg ml^{-1} zone formed around the disc in *E. coli* as depicted in Fig. 10 **(a)(b)(c)**, demonstrating that the green synthesized TiO_2 nanoparticles suppressed the bacterial growth excellently.

4. Conclusion

The biogenesis of TiO_2 nanoparticles utilizing an aqueous extract of *Ficus religiosa* bark is an easy, eco-friendly, and very effective approach. The results of UV-Visible spectroscopy showed the absorbance maxima of approximately 263 nm of green synthesized nanoparticles. FT-IR spectral analysis of TiO_2 nanoparticles demonstrates functional groups is present in the Ti-O vibrational band, C = O band, C-H band O-H band, etc. The TEM, SEM, and XRD findings showed that the greenly prepared TiO_2 nano-particles were globular in structure and their dimensions ranged from 50 to 100 nanometers. The greenly prepared nano-particles exhibited good antibacterial activities in contradiction of the Gram-negative (*E. coli*) as well as Gram-positive (*S. aureus*) bacteria. The current study established that the manufacture of TiO_2 nano-particles by utilizing aqueous extract bark of *Ficus religiosa* is a novel method using inexpensive components.

Declarations

Acknowledgment

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Authors contributions

Sujit Yadav contributed to conception, experimental design, and carrying out measurements; Aakriti Prakash contributed to the manuscript composition and carrying out measurements; Punit Tiwari contributed to the manuscript composition; Preeti Suman Saxena contributed to the manuscript composition; Anchal Srivastava contributed to manuscript composition.

Data availability

The processed data is necessary to repeat these results cannot be released at this time since it is part of a larger ongoing investigation.

Conflict of interest

In the study reported in this research, there are no conflicts of interest or competing interests.

References

1. Patel JK, Patel A, Bhatia D (2021) Introduction to nanomaterials and nanotechnology. Emerg Technol Nanoparticle Manuf 3–23. https://doi.org/10.1007/978-3-030-50703-9_1

2. McNeil SE (2005) Nanotechnology for the biologist. J Leukoc Biol 78:585–594. https://doi.org/10.1189/jlb.0205074
3. Ansari A, Siddiqui VU, Rehman WU, et al (2022) Correction: Green Synthesis of TiO2 Nanoparticles Using Acorus calamus Leaf Extract and Evaluating Its Photocatalytic and In Vitro Antimicrobial Activity (Catalysts, (2022), 12, (181), 10.3390/catal12020181). Catalysts 12:. https://doi.org/10.3390/catal12111451
4. Mishra A (2014) Analysis of Titanium Dioxide and Its. Int J Mech Eng Robot Res 3:561–565
5. Mohan R, Drbohlavova J, Hubalek J (2013) Water-dispersible TiO2 nanoparticles via a biphasic solvothermal reaction method. Nanoscale Res Lett 8:1–4. https://doi.org/10.1186/1556-276X-8-503
6. Owolade OF, Ogunleti DO (2008) Effects of Titanium Dioxide on The Diseases, Development and Yield of Edible Cowpea. J Plant Prot Res 48:329–336. https://doi.org/10.2478/v10045-008-0042-5
7. Kitamura Y, Okinaka N, Shibayama T, et al (2007) Combustion synthesis of TiO2 nanoparticles as photocatalyst. Powder Technol 176:93–98. https://doi.org/10.1016/j.powtec.2007.02.009
8. Dinh CT, Nguyen TD, Kleitz F, Do TO (2012) A solvothermal single-step route towards shape-controlled titanium dioxide nanocrystals. Can J Chem Eng 90:8–17. https://doi.org/10.1002/cjce.20574
9. Lee H, Choi M, Kye Y, et al (2012) Control of particle characteristics in the preparation of TiO 2 nano particles assisted by microwave. Bull Korean Chem Soc 33:1699–1702. https://doi.org/10.5012/bkcs.2012.33.5.1699
10. Shivaraju H, Byrappa K, Kumar TMSV, Ranganathaiah C (2010) Hydrothermal Synthesis and Characterization of TiO2 Nanosturctures on the Ceramic Support and their Photo-catalysis Performance. Bull Catal Soc India 9:37–50
11. Rasool K, Usman M, Ahmad M, et al (2011) Effect of modifiers on structural and optical properties of Titania (TiO2) nanoparticles. Saudi Int Electron Commun Photonics Conf 2011, SIECPC 2011 2–6. https://doi.org/10.1109/SIECPC.2011.5876909
12. Benkstein KD, Montgomery CB, Vaudin MD, Semancik S (2005) Synthesis of TiO2 Nanoparticles Using Chemical Vapor Condensation. Mater Res Soc 828:1–6
13. Thangavelu K, Annamalai R, Arulnandhi D (2013) Preparation and Characterization of Nanosized Tio 2 Powder by Sol-Gel Precipitation Route. Int J Emerg Technol Adv Eng 3:636–639
14. Sandeep, Kumar A, Dimple, et al (2018) Ficus religiosa: A wholesome medicinal tree. J Pharmacogn Phytochem 7:32–37
15. Bhalerao SA, Sharma AS (2014) Ethenomedicinal , phytochemical and pharmacological profile of Ficus religiosa Roxb Original Research Article Ethenomedicinal , phytochemical and pharmacological profile of Ficus religiosa Roxb Ficus religiosa is a large perennial tree ,. IntJCurrMicrobiolAppSci 3(11):528–538
16. Pokharel, N., & Adhikari Pokharel B (2021) A Relationship Between Plants and Their Hindu and Buddha Cultures: An Analysis Ficus Religiosa (Pipal). Transcult Dipl Int Law Herit Conserv A Dialogue between Ethics, Law, Cult 143-151. https://doi.org/10.1007/978-981-16-0309-9_2

17. Nayeem N, Rohini R, Asdaq S, Das A (2012) Wound healing activity of the hydro alcoholic extract of <italic>Ficus religiosa</italic> leaves in rats. Internet J Altern Med 6:1–5. https://doi.org/10.5580/696
18. Aqil F, Ahmad I (2003) Broad-spectrum antibacterial and antifungal properties of certain traditionally used indian medicinal plants. World J Microbiol Biotechnol 19:653–657. https://doi.org/10.1023/A:1025128104056
19. Vinutha B, Prashanth D, Salma K, et al (2007) Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J Ethnopharmacol 109:359–363. https://doi.org/10.1016/j.jep.2006.06.014
20. Simmonds, M. S., & Howes MJR (2006) Plants used in the treatment of diabetes., 6th volume. CRC Press Taylor & Francis Group
21. Deshmukh TA, Yadav B V., Badole SL, et al (2008) Antihyperglycaemic activity of alcoholic extract of Aerva lanata (L.) A.L. Juss. ex J.A. Schultes leaves in alloxan induced diabetic mice. J Appl Biomed 6:81–87. https://doi.org/10.32725/jab.2008.011
22. Pandit R, Phadke A, Jagtap A (2010) Antidiabetic effect of Ficus religiosa extract in streptozotocin-induced diabetic rats. J Ethnopharmacol 128:462–466. https://doi.org/10.1016/j.jep.2010.01.025
23. Hong SM, Lee S, Jung HJ, et al (2013) Simple preparation of anatase Tio2 nanoparticles via pulsed laser ablation in liquid. Bull Korean Chem Soc 34:279–282. https://doi.org/10.5012/bkcs.2013.34.1.279
24. Mobeen Amanulla A, Sundaram R (2019) Green synthesis of TiO2 nanoparticles using orange peel extract for antibacterial, cytotoxicity and humidity sensor applications. Mater Today Proc 8:323–331. https://doi.org/10.1016/j.matpr.2019.02.118
25. Hudlikar M, Joglekar S, Dhaygude M, Kodam K (2012) Green synthesis of TiO 2 nanoparticles by using aqueous extract of Jatropha curcas L. latex. Mater Lett 75:196–199. https://doi.org/10.1016/j.matlet.2012.02.018
26. Chen J, Zhang L (2009) NH4Cl-assisted low temperature synthesis of anatase TiO2 nanostructures from Ti powder. Mater Lett 63:1797–1799. https://doi.org/10.1016/j.matlet.2009.05.042

Figures

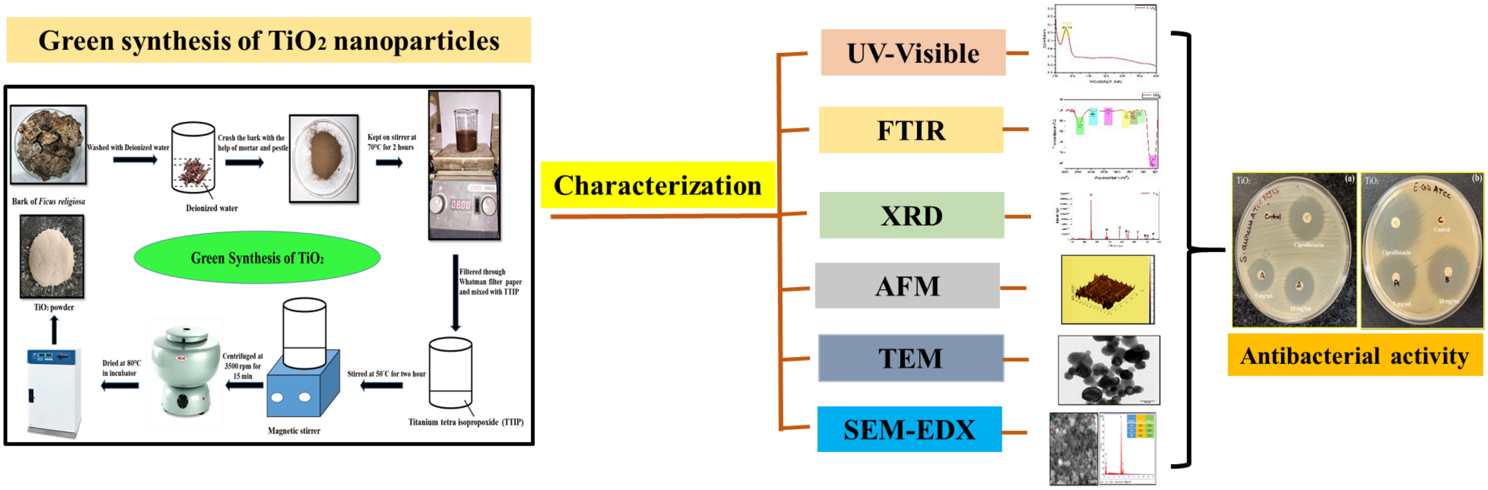

Figure 1

Graphical abstract of the complete work.

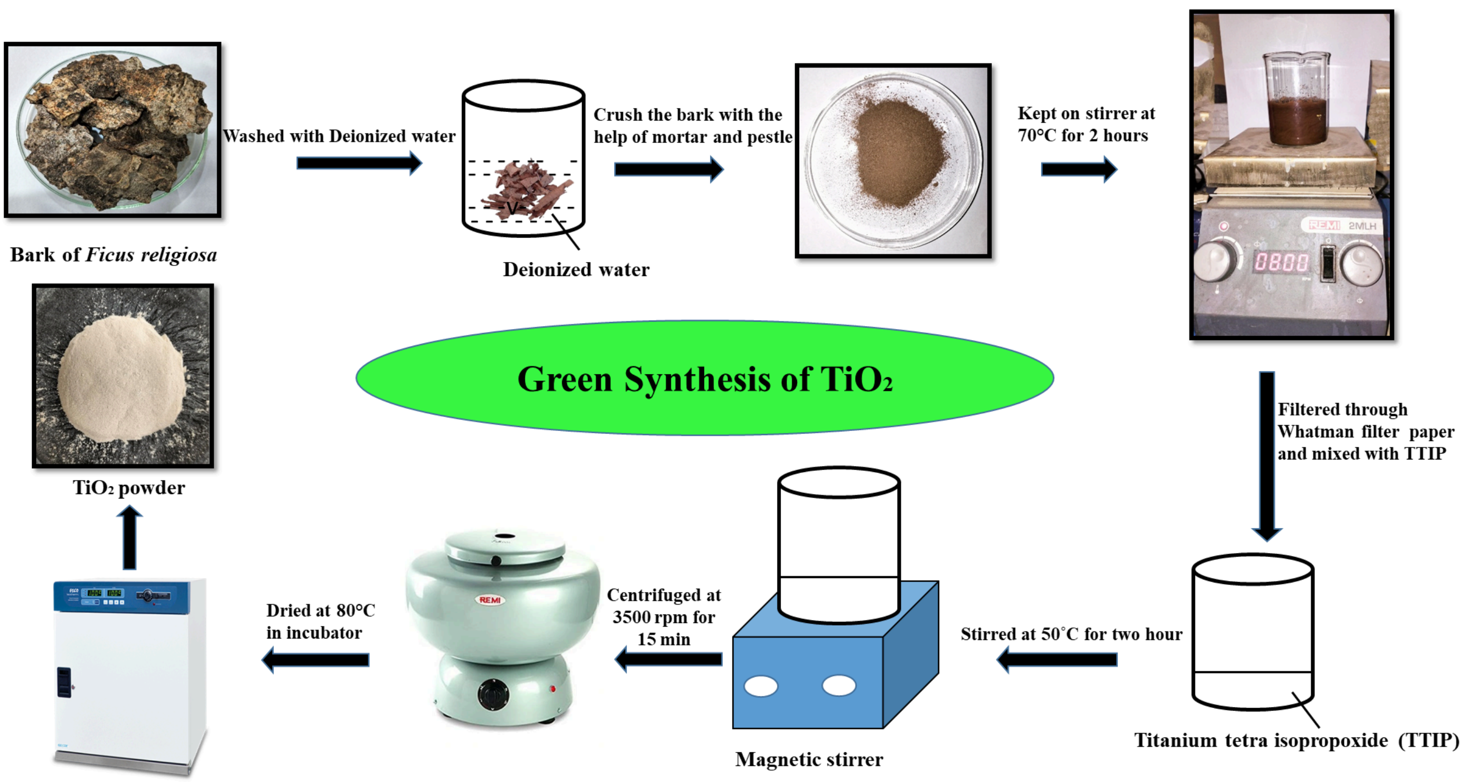

Figure 2

Schematic diagram showing the greenly preparation of TiO_2 nano-particles.

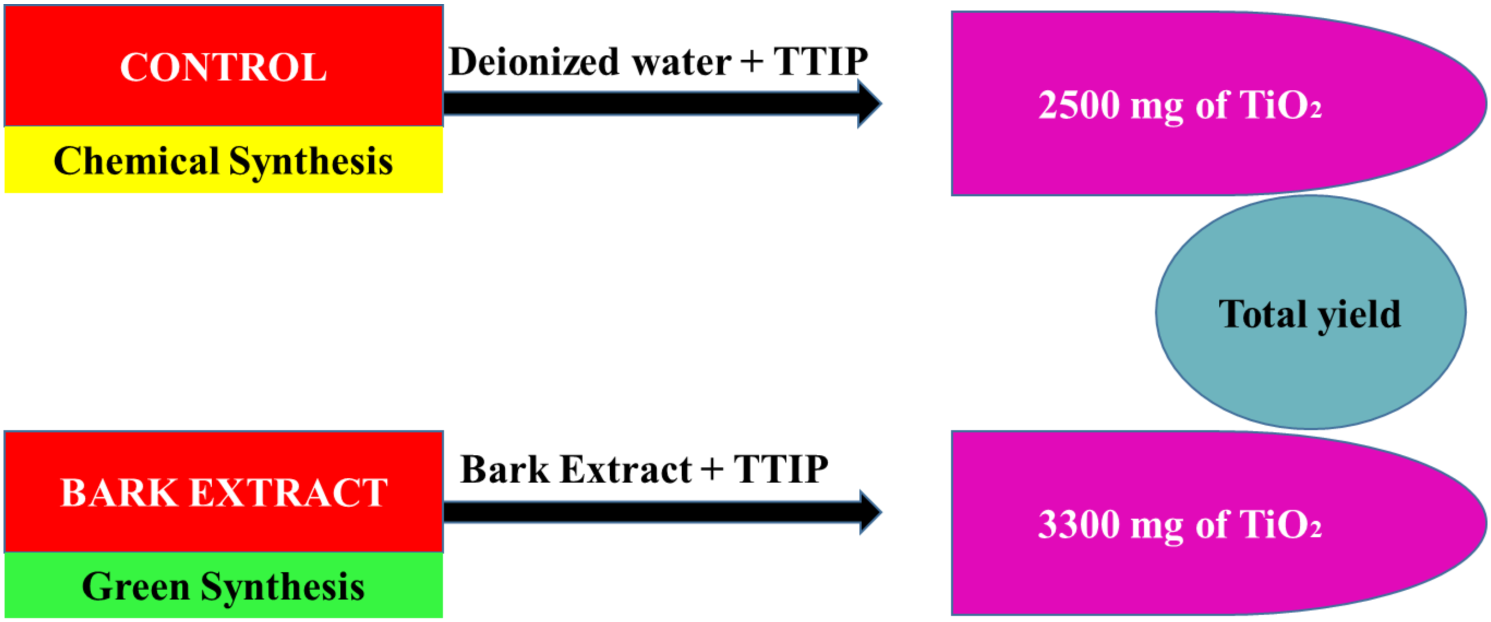

Figure 3

Schematic diagram showing the comparison of chemical synthesis and green preparation of TiO_2 nano-particles.

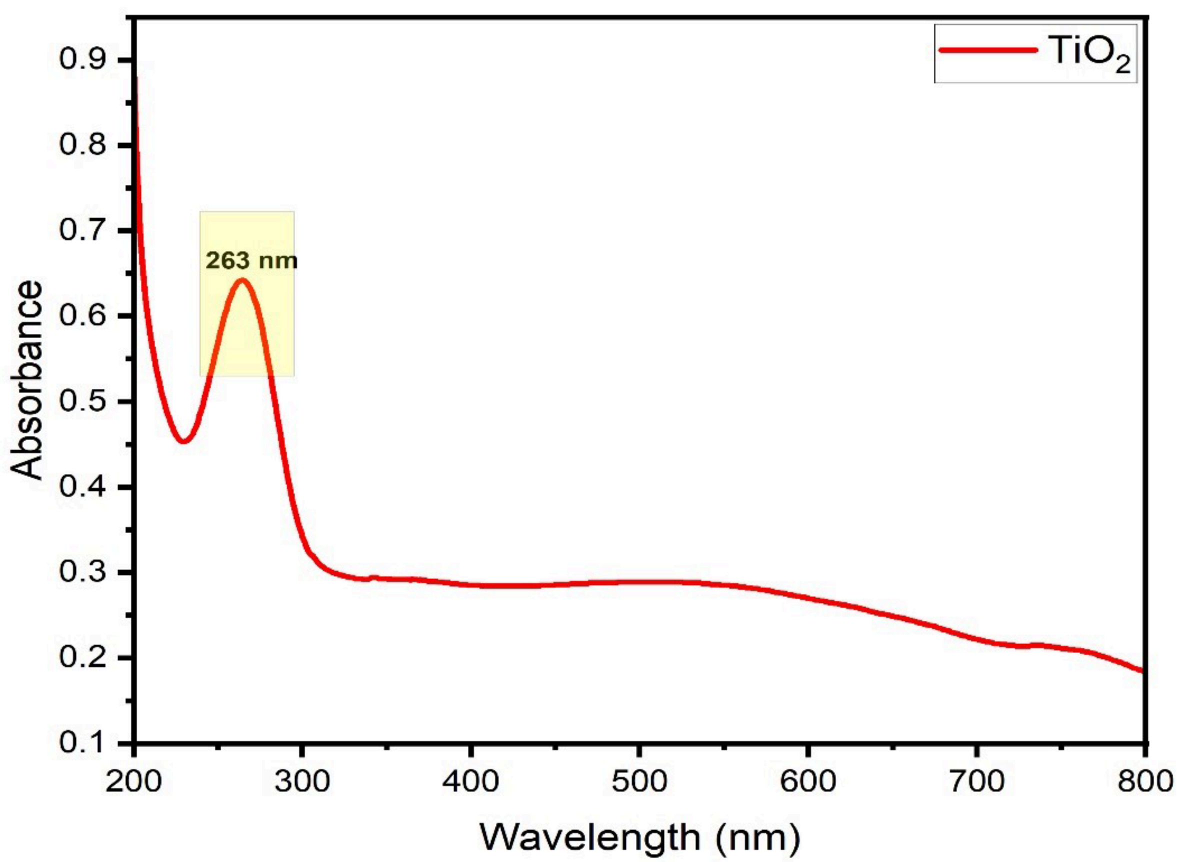

Figure 4

Greenly produced TiO2 nanoparticles have peak of absorption at 263nm in the UV-Visible spectrum.

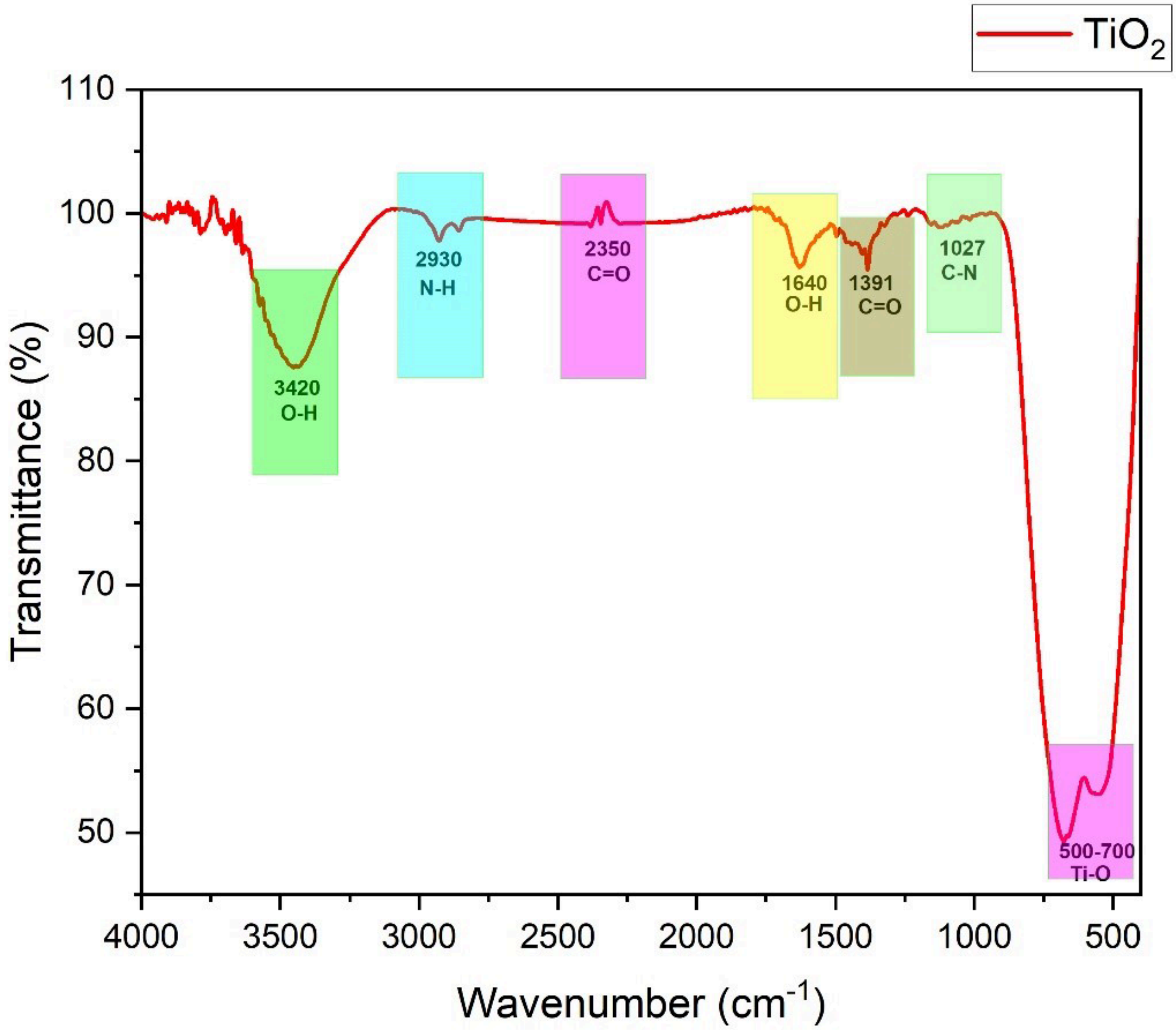

Figure 5

FT-IR spectrum analysis of greenly synthesized TiO_2 nanoparticles through the bark of *Ficus religiosa*

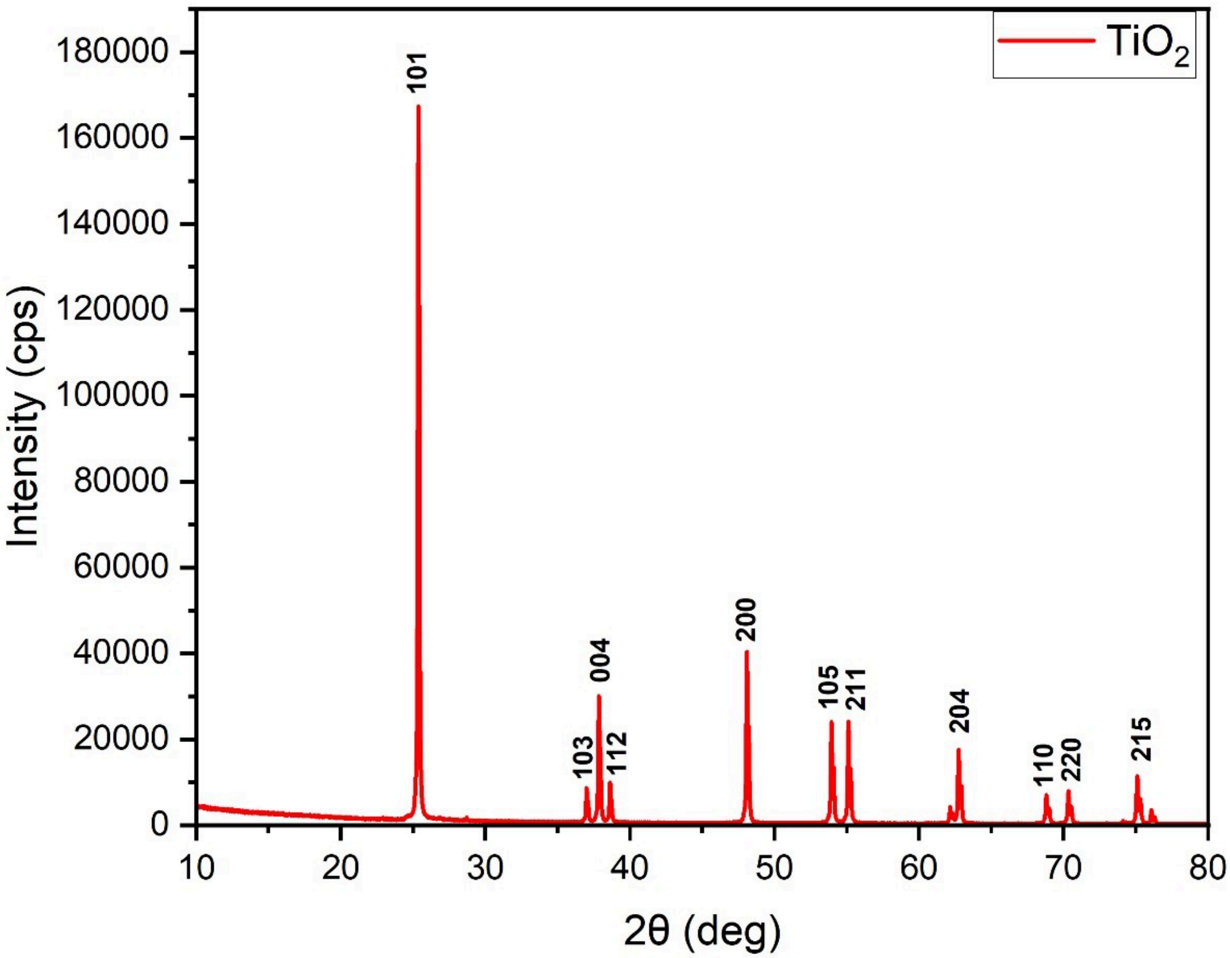

Figure 6

XRD analysis of green synthesized TiO_2 nano-particles by the aqueous extract of bark of *Ficus religiosa.*

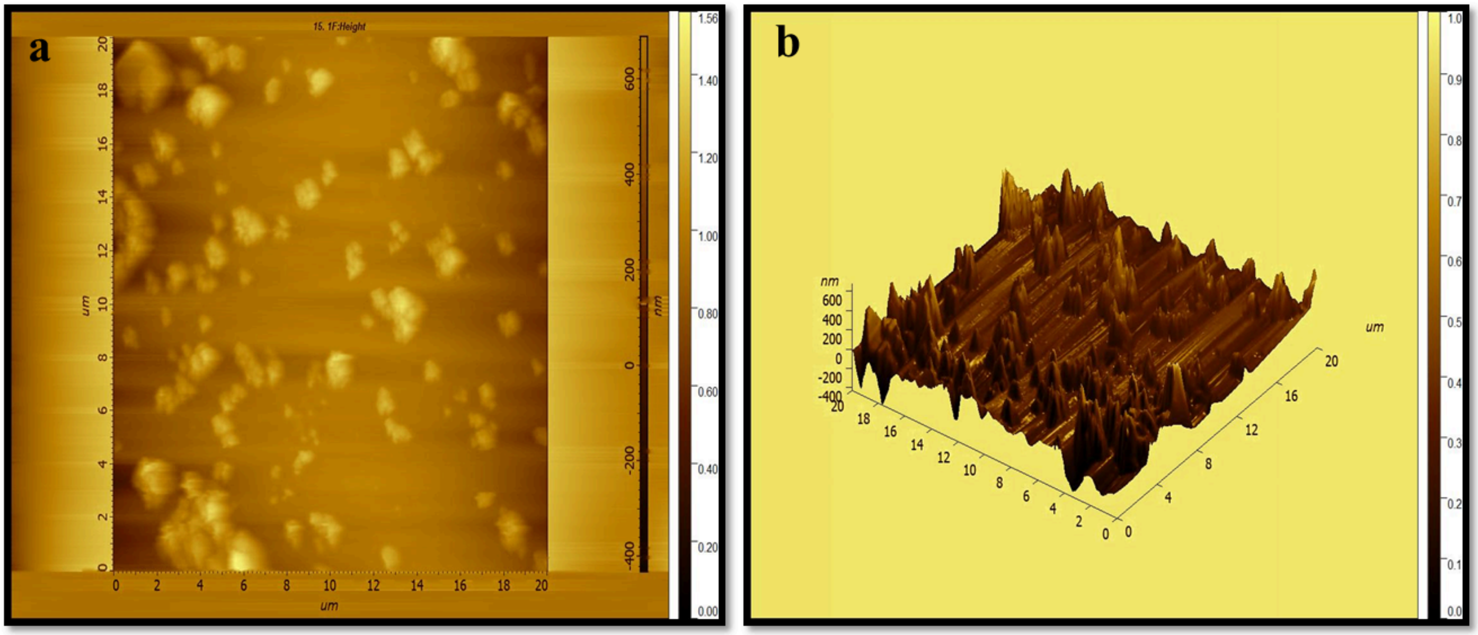

Figure 7

AFM analysis of green synthesized TiO_2 nanoparticles (a) showing a 2D image and (b) a 3D image of the particles.

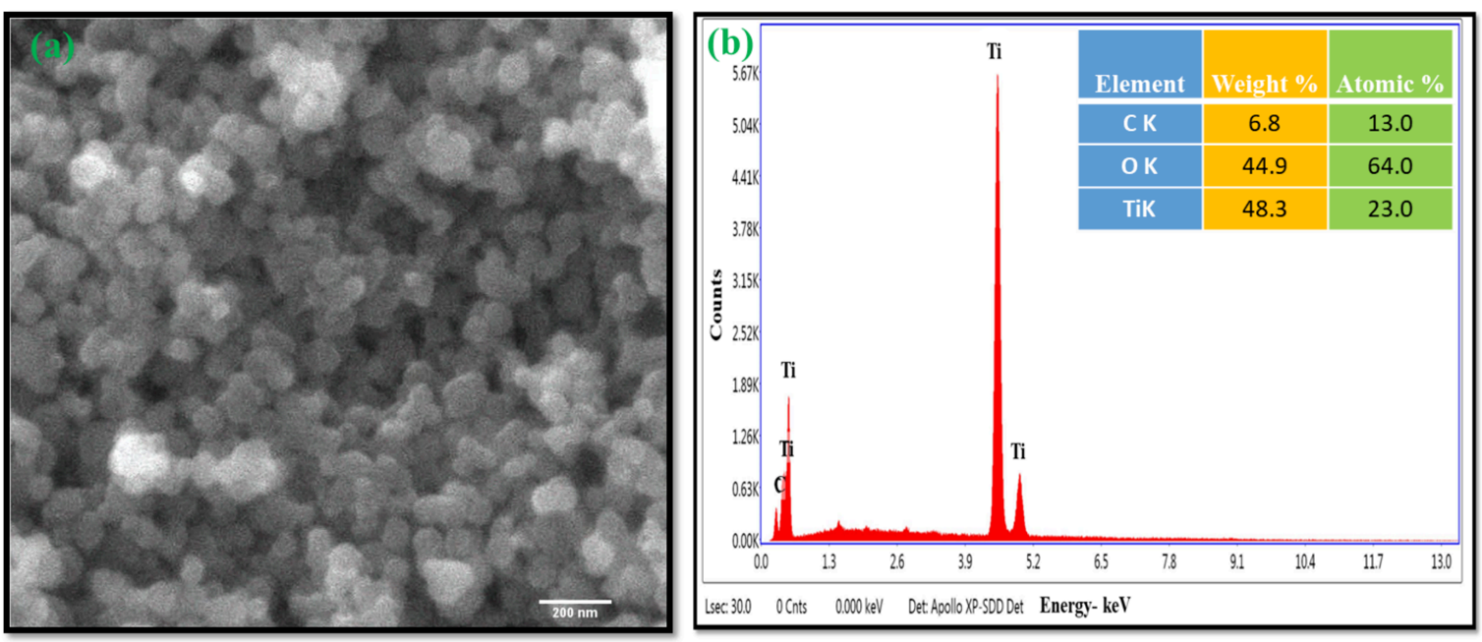

Figure 8

(a) Scanning Electron Microscopic (SEM) analysis of green synthesized TiO_2 nano-particles; **(b)** EDX analysis of TiO_2 nano-particles.

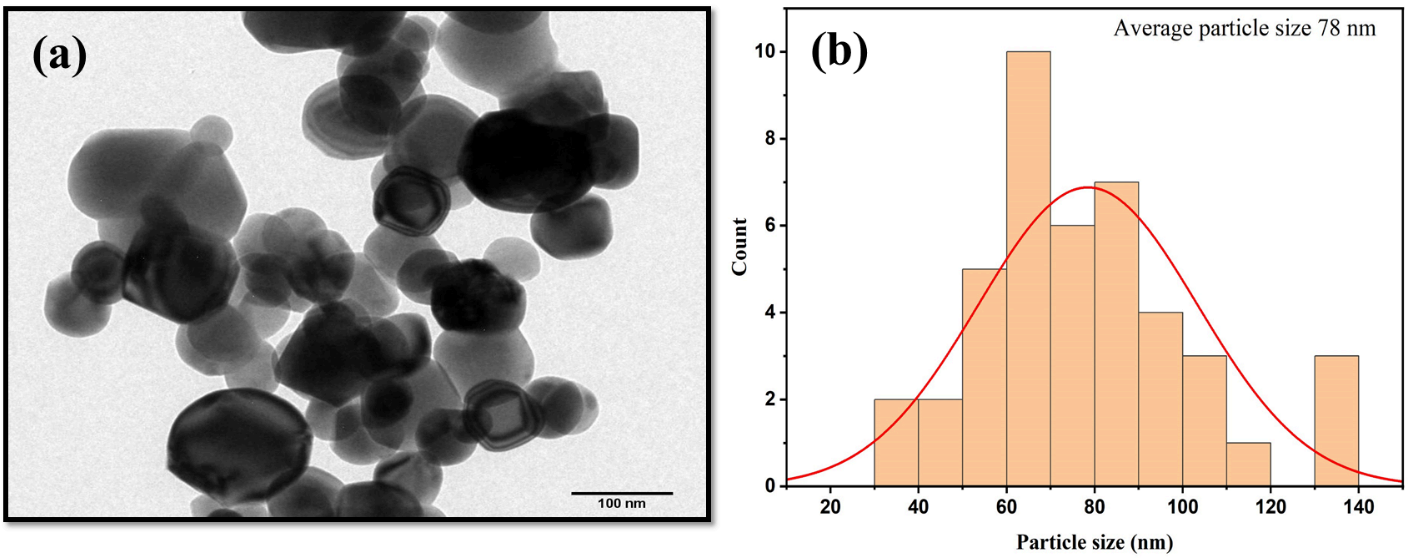

Figure 9

(a) TEM image of greenly prepared TiO_2 nanoparticles; **(b)** Histogram and distribution curve showing average particle size of TiO_2 nanoparticles.

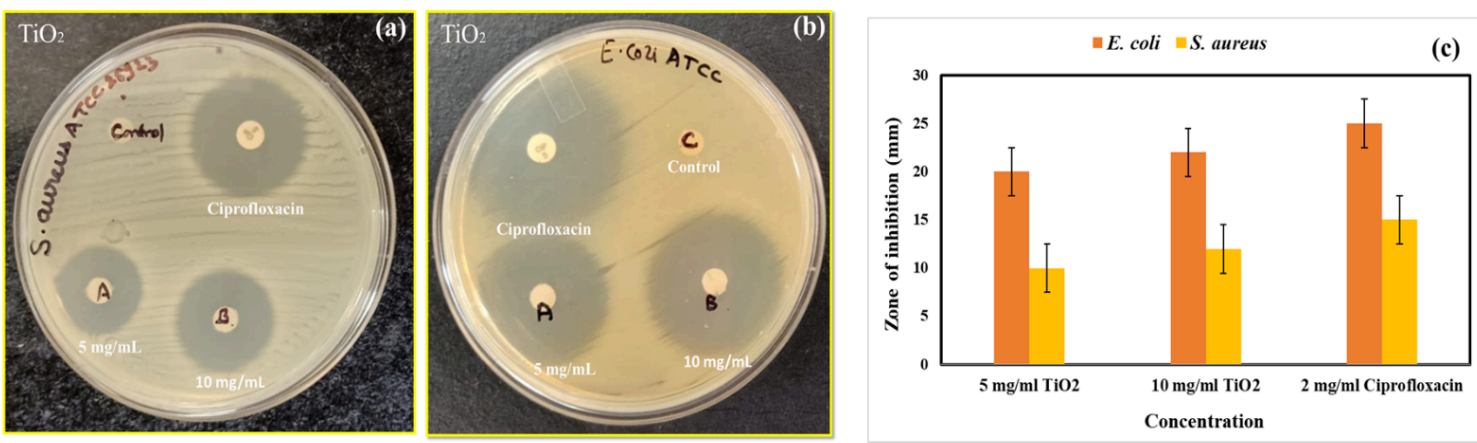

Figure 10

Assessment of antibacterial activity of greenly synthesized TiO_2 nanoparticles against **(a)** *S. aureus* (Gram-positive bacteria), **(b)** *E. coli* (Gram-negative bacteria), and **(c)** Graph shows the zone of inhibition (mm) against different concentrations (mg ml^{-1}) of TiO_2.