

Cocos nucifera-mediated green synthesis of TiO_2 nanoparticles with enhanced photocatalytic, antibacterial, and antibiofilm activity

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Abstract

This study synthesizes titanium dioxide nanoparticles (TiO₂ NPs) using pollen extract from *Cocos nucifera* in an eco-friendly manner and evaluates their multifunctional potentials, such as photocatalytic performance and antibacterial activity. By adjusting pH, metal ion concentration, and reaction time, the optimization parameters were adjusted. UV-Vis spectroscopy, HRTEM, XRD, and FTIR techniques were used to analyze the physicochemical characteristics of the TiO₂ NPs. The presence of functional groups involved in stabilizing nanoparticles was confirmed by FTIR, whereas XRD confirmed crystallite sizes of ~ 17.4 nm, while HRTEM showed particle sizes ranging from 5–100 nm. 86.57% and 70.34%, biofilm inhibition rates observed, and antibacterial experiments showed significant inhibition against *Staphylococcus aureus* and *Escherichia coli*, respectively, 23.0 mm and 29.5 mm. Photocatalytic degradation of methylene blue achieved 97.8% in the presence of sunlight and 98.5% in the presence of UV radiation for a 180-minute cycle. According to these findings, the green-synthesised TiO₂ NPs have promise for use in conservational and biomedical applications. Their promising photocatalytic effectiveness, good antibacterial and antibiofilm effectiveness, are giving potential applications in infection prevention and wastewater treatment. The adaptability and scalability of using *Cocos nucifera* as a biogenic agent, as studied to bring supportable nanotechnology for practical applications.

1. Introduction

Nanobiotechnology has gained increasing importance in the last several years since it may be used in many areas, such as nanomedicine [1], drug delivery [2], and immunology [3]. Modern nanotechnology tactics have come a long way to provide sustainable and focused biomedical solutions by combining inorganic modification, organic reduction, and framework development. Although there is significant potential, a full examination of nanoparticle toxicity and regulatory issues is still necessary before they can be widely used in clinical settings.

Nanomaterials are important for more than just medicine. They are also important for environmental sustainability, extending the shelf life of food, biosensing, and waste management. For studying the structural and functional features of nanoparticles, techniques including Transmission Electron Microscopy (TEM) [4], Scanning Electron Microscopy (SEM) [5], X-ray Diffraction (XRD) [6], and Dynamic Light Scattering (DLS) [7] are very important. TEM is one of them that provides very detailed information on the shape and size distribution of nanoparticles [8]. Nanobiotechnology becomes a multidisciplinary platform that may be used in health, agriculture, and cleaning up the environment when integrated with molecular biology technologies like PCR and DNA sequencing and computer modelling [9, 10].

Green synthesis offers a sustainable approach to make nanoparticles instead of the usual way. It uses biological agents like plant extracts, enzymes, or microbes as reducing and capping agents. This makes it less hazardous to the environment and more compatible with living things [11–14]. This method has worked especially well for making titanium dioxide (TiO₂) nanoparticles [15, 16], TiO₂NPs made in a green way could be useful for treating water, converting solar energy, and biomedical uses [17]. These

nanoparticles can stop bacteria from growing, break down dyes, and break up biofilms made by germs like *S. aureus* and *E. coli* [18, 19]. Their photocatalytic properties enable the breakdown of synthetic dyes that are otherwise persistent in the environment due to their aromatic complexity. When light hits TiO_2 , it makes reactive oxygen species (ROS) that can break these chemical bonds. This turns harmful dyes into safe end products like water and CO_2 . Not only does this procedure help the environment, but it also helps with modern wastewater treatment methods [20]. Recent studies have shown more and more that green-synthesized nanomaterials can be used in biomedical and environmental domains through experiments and real-world uses, for example, silver nanoparticles derived from neem and aloe vera showed significant ROS-mediated antibacterial activity, establishing the relevance of phytochemicals [21–23]. These results show that more research is needed on biogenic TiO_2 nanoparticles in different roles. The goal of this study is to make and describe TiO_2 nanoparticles using an environmentally safe process, and then test how well they work as antimicrobials, antibiofilms, and photocatalysts in varied lighting circumstances. In this study, *Cocos nucifera* pollen extract is used as a novel and underexplored bio-reducing agent for the green synthesis of TiO_2 nanoparticles. This method improves photocatalytic and antibacterial activity in both UV and sunlight as compared to traditional plant sources. Although green-synthesized TiO_2 exhibits promise in biological and environmental applications, additional toxicity and regulatory assessment are necessary before clinical use can occur. By creating TiO_2 nanoparticles using extract from *Cocos nucifera* and assessing their antibacterial, antibiofilm, and photocatalytic efficacy in both UV and solar light, this work seeks to close that gap.

2. Materials and methods

All reagents used were of analytical grade. Titanium (IV) chloride ($\geq 99\%$, Sigma-Aldrich, USA), methylene blue (HiMedia, India), and nutrient agar (Merck, India) were used without further purification. TiO_2 0.1 molar concentration was made precisely, 0.0798 grams per 100 ml [24]. *Cocos nucifera* pollen[25] and distilled water. To adjust the pH, Hydrochloric acid was used to lower pH, while Sodium hydroxide was needed to increase it. *Staphylococcus aureus* and *Escherichia coli* microorganism strains were obtained from Sree Balaji Dental College, Chennai.

2.1 Refinement of Botanical Extract

First, the plant samples (*Cocos nucifera*) were examined for any contamination, Mold, possible external damage, alteration, etc. As Fig. 1 explains, the entire process involved selecting approximately 120 grams of undamaged *Cocos nucifera* pollen samples, which were then weighed and introduced into a beaker. At the same time, distilled water measuring up to 600 ml was heated to 100°C using a mantle. The boiling water was then collected and *Cocos nucifera* pollens into the boiling water and then covered with aluminium foil. The mixture was reduced to about 400 ml. After reduction, the mixture was filtered to get the required *Cocos nucifera* extract.

2.2 Metal Solution Formulation

To obtain a molarity of 0.1, the essential amount of TiO_2 used in this experiment was accurately calculated by the formula stated earlier as $\text{Weight/molecular weight} \times 100$, while the molecular weight of Titanium is 79.87 g/mol, and the molecular weight of oxygen is 15.999 g/mol. As a result, the requisite quantity of TiO_2 was liquefied in 100 ml of deionised water, which is equivalent to 0.07987 grams which then formulated the metal solution.

2.3 Refinement of Extract

The *Cocos nucifera* extract was transferred to a 100 mL conical flask and stirred continuously. At the same time, the metal solution was gradually transferred into the flask drop by drop from a 50-millilitre burette, and each drop was administered at 5 minutes a drop. The temperature was kept at 50°C throughout the process, with the stirring carefully monitored. The exact time (in minutes) when any noticeable alteration in the visual appearance of the liquid solution was recorded. This process was repeated until a noticeable color change occurred, at which time the exact reading from the burette was recorded.

2.4 Analysis and Refinement

Optimization of the characterization procedures was initiated with scrupulous pH selection for the extract, beginning with the targeted 5.48 value. The pH scale from 4 to 11 was scrupulously assessed with the pH value incrementally altered using NaOH and HCl titration with continuous UV spectrometric assay. Further optimization efforts were implemented to the substrate and metal ion concentrations and the reaction time regimen, with all optimization aspects meticulously scrutinized via UV spectroscopy measurements. The optimal mass ratios and time durations were determined following vigorous experimentation, allowing precise control of the reaction kinetics.

2.5 pH Fine-Tuning

The *Cocos nucifera* pollen substrate was divided into eight separate beakers, each of which held precisely 50 milliliters of substrate that had been carefully adjusted to pH values between 4 and 11. Using UV spectroscopy, thorough examinations were methodically carried out for every pH variation, revealing unique spectral signatures.

2.6 Adjusting Substrate Concentration

An exact quantity of one milliliter of metal ion was added to each of the various substrate quantities, which ranged from nine milliliters to forty-nine milliliters, prior to experimentation. Analyses using UV spectroscopy were carried out for every test tube, carefully examining the spectral data. The optimal substrate volume was determined following rigorous assessment, representing the most advantageous concentration for further procedures.

2.7 Metal Ion Concentration Fine-Tuning

The metal ion concentration underwent systematic adjustments, spanning from 0.5 milliliters to 4.0 milliliters, while maintaining a consistent optimised substrate volume. Each test tube was subjected to

rigorous UV spectroscopy analysis, meticulously scrutinizing the spectral data for distinctive patterns.

2.8 Time Parameter Fine-Tuning

UV spectroscopy was promptly executed immediately following the amalgamation of optimized substrate with optimised metal ions. Sequential readings were meticulously taken at 5-minute intervals, spanning a comprehensive timeline of 30 minutes.

2.9 Mass Production of the Sample

After adjusting factors such, as pH levels, substrate amount, metal ion concentration, and time variations the large-scale production process starts with tuning the pH of the solution obtained from *Cocos nucifera* shells. Next, the necessary quantity of TiO_2 (the chosen metal ion) for producing 2000 ml of *Cocos nucifera* shell pollen extract is calculated. The measured amount of TiO_2 is then mixed into a sterilised IV (Intravenous set). The solution is controlled to release one drop every 1.30 minutes. The mixture is placed on a stirrer until complete dissolution of the metal ion occurs. This detailed procedure guarantees the preparation of the bulk production solution for use.

2.10 Calcination

The liquid nanoparticles go through a change when poured into beakers and heated in an air oven set at 80°C . As time passes the liquid evaporates, leaving crystals behind. These crystals are then crushed using a motor and pestle device. The resulting crushed crystals are cautiously placed in a crucible. Heated in a furnace, at 800°C for 3 hours. After this heating, the crucible is allowed to cool, for around 24 hours. Subsequently, the nanoparticles are delicately collected from the crucible marking the completion of the calcination process [26].

2.11 Characterization of Titanium oxide nanoparticles

The synthesised TiO_2 nanoparticles were analysed for their size, appearance, and texture. The physical characteristics of the TiO_2 nanoparticles were determined using an X-ray diffractometer. Specifically, a Bruker diffractometer with copper-potassium alpha X-rays ($\lambda = 1.5406 \text{ \AA}$) was employed (Bruker, Germany). Using a stage size of 0.1972, data were gathered on a two-theta scale ranging from 10° to 70° . This analysis ensured that the TiO_2 particles were in the form of precipitates sized at approximately 10^{-10} . Additionally, Fourier-transform infrared spectroscopy (FT-IR) was used to verify the involvement of biomolecules and to identify functional groups and OH bonds in the synthesised TiO_2 nanoparticles (Thermo Fisher, Summit Lite, USA). The external features of the nanoparticles were examined by JEOL JEM-2100 HRTEM (JEOL Ltd., Japan) operated at 200 kV to investigate the morphology, size, and crystallinity of the synthesized TiO_2 nanoparticles.

2.12 Anti-Bacterial Activity

We're investigating the antibacterial abilities of titanium dioxide (TiO_2) nanoparticles against *S. aureus* and *E. coli* by agar well diffusion methods. We start by preparing a Mueller-Hinton broth (HiMedia, India) growth medium and pouring it into petri plates for bacterial culture. After inoculating the plates with the

bacteria, we introduce TiO_2 nanoparticles into 8 mm wells at different concentrations, ranging from 50 to 250 μL , while maintaining control wells with just the DMSO solution. Negative controls included wells that contained DMSO alone, with a final concentration less than 1%. To dissolve the TiO_2 nanoparticles, DMSO was used, with its influence on bacterial growth being kept in check. The plates are then placed in a controlled incubator at 37°C to allow interaction between the nanoparticles and bacteria.

2.13 MIC (Minimum Inhibitory Concentration) Assay

Understanding the MIC allows researchers to identify the lowest nanoparticle dosage necessary to control bacterial expansion. Our study uses titanium dioxide (TiO_2) nanoparticles to target *E. coli* and *S. aureus*. Ensure the sterility of all necessary equipment, like 96-well ELISA plates, micropipettes, and micropipette tips. Then, 100 μL of LB broth was filled in the ELISA plate wells, reserving one well for the TiO_2 nanoparticles. The rest receive 2x dilutions of the nanoparticles to test their effectiveness. Next, we introduce *S. aureus* and *E. coli* to each well, each containing varying nanoparticle concentrations. except for the negative control. These ELISA plates are then incubated to allow for bacterial growth. Concentrations ranging from 100 to 0.78 $\mu\text{g/mL}$ were used to test TiO_2 NPs. After incubating at 37°C for 24 hours, MIC was resolutely determined. Tests were conducted in triplicate according to CLSI M07-A9 guidelines.

2.14 Minimum Bactericidal Concentration

After pinpointing the inhibition of nanoparticles against both gram-positive and gram-negative microorganisms, the 50 μL samples from the wells where meticulously extracted bacterial growth had been hindered. These samples were then cautiously placed onto BHI agar medium plates for subsequent analysis. The petri plates were kept at a constant 37°C , for 24hrs in an incubator. The MBC endpoint, indicating the lowest dosage of antibacterial agent to wipe out 99.9% of bacteria, was established. The experiment was closely monitored before and after incubation of the agar plates to observe the presence or absence of bacteria. To verify bacterial eradication, the MIC plates were next carefully examined with an ELISA plate reader [27].

2.15 Anti-Biofilm Assay

In the biofilm inhibition assay, 96-well plates were inoculated with a standardized culture of *S. aureus* and *E. coli*. Following a 24-hour incubation period, the bacteria developed biofilms. To determine how well synthesised TiO_2 NPs suppress the formation of biofilms, they are added to the wells and incubated for a further 24 hours after being diluted in DMSO. Following the removal of the supernatant and well cleaning, the biofilms were stained with crystal violet and then destaining with ethanol. Biofilm inhibition is quantified by measuring the optical density (OD) at 570 nm [28].

2.16 Photocatalytic activity of TiO_2

Throughout the experiment, samples were periodically analyzed using UV–Vis spectroscopy to monitor the degradation of methylene blue by TiO_2 NPs under sunlight and UV irradiation. To start, the dye is mixed into a liquid with extra substances like catalysts or additives to help break it down better and

adjustments are made to the pH if needed. The dye combination is subjected to UV radiation and sunlight, two factors that degrade the dye. It's mixed thoroughly to ensure even treatment. Throughout the process, samples are regularly taken and checked using spectroscopy to measure the breakdown of the dye. The catalytic chemical reaction of the TiO_2 Nanoparticles was calculated by degrading methylene blue dye in sunlight and UV rays over the period. A methylene blue (MB) was prepared for 1 ppm in water. TiO_2 nanoparticles ranging from 10 micrograms to 0.078 micrograms were added to 5 ml of the MB dye, and the control was kept without TiO_2 nanoparticles. The mixture was stirred and exposed to sunlight and UV irradiation for 60, 120, and 180 minutes (1,2,3 Hrs). A control was used to compare any changes in the color of the MB dye solution. Next, the heterogeneous mixture was subjected to continuous churning while being exposed to UV light and sunshine. The sample, which was positioned 10 cm away, was illuminated by a Philips 365 nm UV lamp from the Netherlands. At an ambient temperature of around 40°C and an intensity of about 100,000 lux, sunlight studies were carried out throughout peak hours (11 AM–2 PM). The duration of the radiation was 180 minutes.

The average temperature during the experiment was approximately 40°C , with an average sunshine duration of 3 hours. Various concentrations of TiO_2 with dye were tested for UV spectroscopy absorbance at a fixed wavelength (λ) of 600 nm using the UV-1800, Genesys 180, Thermo Fisher Scientific, USA, to examine the photocatalytic activity in TiO_2 NPs. The degradation of MB dye in sunlight and UV rays as a method to analyse the photocatalytic activity of TiO_2 NPs [29].

2.17 Photocatalyst Regeneration

The reusability of the green-synthesised TiO_2 nanoparticles was measured by a regeneration experiment that involved three successive cycles of methylene blue (MB) degradation under UV and solar radiation. The TiO_2 NPs were separated by centrifugation at 5000 rpm for 10 min, following each 180-minute degradation run. Any remaining dye was then completely cleaned with distilled water, dried in a hot-air oven for two hours at 60°C , and then used again for the subsequent degradation cycle using a new 1 ppm MB solution. After every cycle, degradation efficiency was measured using UV–Vis spectrophotometry ($\lambda = 600 \text{ nm}$).

2.18 Kinetic Analysis of Dye Degradation

To study how quickly methylene blue (MB) broke down, experiments were conducted using the perfect amount of TiO_2 nanoparticles. These experiments were executed under UV and sunlight, in a concentration of 1 ppm. UV-Vis spectrophotometry was used to detect the absorbance of MB at 600 nm after aliquots were discarded at predefined intervals of 60, 120, and 180 minutes. Models of pseudo-first and second order were used to measure the degradation kinetics. Since C_0 is the starting concentration and C is the concentration at time t , the plot of $\ln(C_0/C)$ vs time was utilized for pseudo-first-order kinetics. The linear regression's slope was used to determine the rate constant (k).

2.19 Analysis of Statistics

Every experiment was carried out in triplicate ($n = 3$), and the mean $\pm$ standard deviation (SD) was used to express the results. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to assess group differences and determine statistical significance. P values less than 0.05 were regarded as statistically significant. The mean inhibition zones were compared for MIC and MBC evaluations, and correlation coefficients (R^2) were used in linear regression analysis to evaluate the fit of pseudo-first-order kinetics for dye degradation kinetics. For graphical representations and statistical analyses, OriginPro 2021 and GraphPad Prism 9.0 were utilized.

3. Results and Discussion

3.1 UV–Visible Spectroscopy Analysis

UV-Vis study turns into an active and dependable method for estimating the synthesis and stability of TiO_2 NPs. Figure 2 illustrates that the substrate was sourced from *Cocos nucifera* pollen extract and showed the 2.4 λ (lambda) absorbance, SnCl_2 metal solution, and TiO_2 nanoparticles, respectively, showed 3 λ (lambda) in UV spectroscopy.

3.1.1 pH Fine-Tuning

Cocos nucifera pollen substrate, adjusted to pH levels 4 to 11 in 50 mL beakers, showed a UV spectroscopy peak at pH 11, indicating an optimal condition. Significantly, a prominent peak was discerned consistently at pH 11, denoting the optimal condition for subsequent processes shown in Fig. 3 (a). The absorbance wavelengths of synthesised Titanium oxide nanoparticles between 200 and 800 nm were measured through a UV–Vis spectrophotometer (UV-1800, Genesys 180, Thermo Fisher Scientific, USA) [30].

3.1.2 Substrate Concentration Fine-Tuning

Substrate volumes from 9ml to 49ml were tested with 1ml of metal ion each; UV spectroscopy pinpointed 29ml as optimal after thorough analysis. The optimised substrate concentration is to be 29 millilitres for further experiments, as shown in Fig. 3(b).

3.1.3 Metal Ion Concentration Fine-Tuning

Figure 3(c) shows that the metal ion concentrations ranging from 0.5 to 4.0 ml were tested in 0.5 ml increments using a 29 ml substrate. Following UV spectroscopy analysis, 3.5 ml was identified as the optimal concentration. This careful examination emphasised that 3.5ml was the most effective dosage for future procedures.

3.1.4 Time Parameter Fine-Tuning

UV spectroscopy started promptly after mixing 29ml substrate with 2.5ml metal ion; peak activity was observed at 25 minutes. Remarkably, the peak of significance emerged conspicuously at the 25-minute

juncture (Fig. 3d), marking a pivotal moment in the progression of reaction kinetics.

The optimised parameters for the synthesised TiO₂ nanoparticles are as follows: pH 11, 29 mL of substrate extract, 3.5 mL of precursor solution, and a reaction time of 25 minutes. Nanoparticles with consistent absorbance properties and dependable morphology are produced under these criteria, making them suitable for further phytochemical and application research.

3.2 Characterization of Titanium oxide nanoparticles

3.2.1 HR-TEM Analysis

HR-TEM was employed to characterize the synthesized TiO₂ nanoparticles, giving us a lot of information about their surface shape and crystal structure. Figure 4(a–f) shows a lot of different pictures of the nanoparticles: (a) shows pure TiO₂ with a spherical shape; (b) and (c) exhibit TiO₂ nanoparticles that have been doped with Ag and Zn, respectively, showing how doping changes the structure. When TiO₂ nanoparticles are doped with Ag and Zn, their dispersion and functionality are enhanced, and their morphology changes. Figures 4(d) and 4(e) show low and high magnification views, respectively, that corroborate the uniform dispersion and nanoscale characteristics. The particle size seen through HR-TEM varied from 5 to 100 nm, while XRD analysis indicated a larger average crystallite size of approximately 51 nm, likely due to the presence of aggregated domains. The Selected Area Electron Diffraction (SAED) pattern in Fig. 4(f) shows concentric diffraction rings, which are typical of the anatase crystalline phase of TiO₂. The observed lattice fringes and SAED rings confirm that nanocrystalline TiO₂ was successfully made utilizing a green synthesis approach. This makes them good for use as antimicrobials and photocatalysts [31].

3.2.2 FTIR Analysis

The *Cocos nucifera* extract treated with TiO₂ underwent FT-IR analysis to elucidate the bond linkages and functional groups involved. The FT-IR analysis on the plant extract-based synthesized titanium nanoparticles helped in determining the functional groups. The presence of the phenol group was confirmed by the detection of a clear band at 3430 cm⁻¹, which suggested O-H stretching vibrations (Fig. 5a). The C-N, methyl group, and C-O bond stretching bands are shown by the bands at 2920 cm⁻¹ and 2852 cm⁻¹. The FT-IR spectrum of TiO₂ NPs showed a prominent peak at 1117 cm⁻¹, which indicated alkyl amine vibrations. The peak intensity also increased, suggesting a reaction where the oxygen from titanium ions is replaced by oxygen from hydroxyl groups (OH) in the plant extract, bonding to carbonyl groups. The peaks located at 865 cm⁻¹ and higher are due to bending vibrations of C-H stretching bands. Bending vibrations of aromatics, amines, amides, and acids resulted in absorption peaks at 688 cm⁻¹ and 616 cm⁻¹, as observed. The spectral analysis provides significant information on the surface modifications and interactions of the nanoparticles, which are important for various applications, from catalysis to biomedical engineering [32]. Lastly, the FTIR spectra can be processed utilizing data analysis techniques such peak integration and deconvolution to improve the spectra's comprehension and produce an accurate TiO₂ nanoparticle characterisation.

3.2.3 X-ray diffractometer (XRD) analysis of titanium nanoparticles

The crystal structure of the synthesised TiO_2 NPs was analysed using XRD. The XRD outline, exposed in Fig. 5b, was obtained in the two-theta scale of $20\text{--}80^\circ$ with copper potassium alpha x-ray energy, commonly applied in laboratory-level x-ray apparatuses. The XRD pattern displays seven peaks at $2\theta^\circ = 28.4001, 34.5894, 41.3065, 50.6140, 59.8585, 67.6014, \text{ and } 74.0582$. These detected peaks resemble the anatase crystal-like stage of TiO_2 . The intensity counts are 110, 101, 200, 211, 002, 112, and 301, showing good agreement with the observed peaks. The XRD pattern exhibited peaks at $2\theta = 25.3^\circ$, confirming the existence of anatase TiO_2 nanoparticles (JCPDS No. 21-1272). Lastly, the aggregate crystal size of TiO_2 nanoparticles can be determined from the equation below using the DS (Debye-Scherrer) method.

$$D = K \lambda / (\beta \cos \theta), \dots\dots \text{Eq. 1}$$

where D is the crystallite size, λ is the X-ray radiation wavelength, K is the constant of Scherrer, β is the full width at half-maximum height, and θ is the angle of Bragg's diffraction. approximately 17.4 nm of average crystallite size was determined with a (101) peak, confirming the particles' nanoscale dimensions. Notably, particle sizes ranging from 5 to 100 nm were found by high-resolution TEM investigation, indicating that the detected particles might be composed of several crystallites or show some degree of agglomeration.

3.3 Antimicrobial Evaluation of TiO_2 Nanoparticles

3.3.1 Antibacterial activity

After the designated incubation period, we examine the plates for zones where bacterial growth is absent, indicating the nanoparticles' antibacterial effectiveness. This cautiously controlled experiment aims to uncover the antibacterial properties of TiO_2 nanoparticles. Gram + ve and Gram-ve bacteria might respond contrarily to nanoparticles depending on the appearance of their cell's external layers [33].

Additionally, TiO_2 NPs exhibited increased activity, possibly due to their spherical surface morphology. Figure 6 explains antibacterial activity of TiO_2 nanoparticles (TiO_2 NPs) was significant, and the inhibition increased with concentration. Their process includes producing reactive oxygen species (ROS), which damage DNA, denature proteins, and rupture bacterial cell walls, ultimately resulting in bacterial death. Effective inhibition was achieved against both Gram-positive and Gram-negative bacteria. The biocompatibility and broad-spectrum effectiveness of TiO_2 NPs point to their possible application in biomedical fields such as food preservation, medical device coatings, and wound healing [34, 35].

The zone of inhibition was visually observed and recorded in Fig. 7 (a-d). In Figs. 7(a&b), a strong zone of inhibition was observed for 200 μL and 250 μL in *E. coli*, with measurements of 27.3 mm and 29.5 mm, respectively and Fig. 7(c&d). The antibacterial activity of *S. aureus* was remarkable, with a strong zone

measuring 21.5 mm and 23.0 mm in diameter in 200 and 250 μL and the mean growth inhibition percentages were 100% against the test microorganism. Figure 8 (a) explains the antibacterial activity of TiO_2 NPs against both organisms graphically. The zone of inhibition at different concentrations is summarized in Tables 1 and 2. The inhibition zones for each concentration were carefully measured, and the statistical analysis clearly showed a significant rise ($p < 0.05$) in antibacterial effectiveness as the TiO_2 NP concentration increased. Compared to the lower concentrations, the 250 μL concentration showed much greater inhibition zones in both *E. coli* and *S. aureus*, according to the data in Tables 1 and 2.

3.3.2 Minimum inhibitory concentration

After incubation, we use an ELISA plate reader to determine the MIC values. These values indicate the lowest nanoparticle concentration needed to inhibit bacterial growth effectively. This research aims to understand how well titanium dioxide nanoparticles can halt the growth of *S. aureus* and *E. coli* in agar broth through this methodical approach [36]. The MIC concentration was determined from the results; the MIC of TiO_2 was found to be 0.62 $\mu\text{g/mL}$, and it can minimally inhibit. *E. coli*, while 1.25 $\mu\text{g/mL}$ was needed to inhibit the growth of *S. aureus* (Table 3).

The results revealed that the enhanced bactericidal activity recorded for the TiO_2 is consistent with the MIC, and the difference between the measured and background OD600 was less than 0.01. A representative MIC of TiO_2 on *E. coli* and *S. aureus* is shown in Fig. 8(b)

Table 3

MIC of TiO_2 nanoparticles against *S. aureus* and *E. coli*, showing percentage inhibition at various concentrations. Data represent mean $\pm$ SD ($n = 3$). Statistical differences between concentrations were evaluated using ANOVA with Tukey's post hoc test. $p < 0.05$

S.No	TiO_2 NP Concentration ($\mu\text{g/mL}$)	Inhibition (%) – <i>S. aureus</i>	Inhibition (%) – <i>E. coli</i>
1	0.078	78 $\pm$ 0.2	82.5 $\pm$ 0.32
2	0.156	80.23 $\pm$ 0.4	84.2 $\pm$ 0.12
3	0.312	82.05 $\pm$ 0.35	86 $\pm$ 0.2
4	0.625	84.52 $\pm$ 0.3	88.43 $\pm$ 0.4
5	1.25	86.23 $\pm$ 0.21	90.1 $\pm$ 0.4
6	2.5	88.12 $\pm$ 0.5	92 $\pm$ 0.3
7	5	90 $\pm$ 0.4	94.1 $\pm$ 0.4
8	10	92.12 $\pm$ 0.36	95.02 $\pm$ 0.5

3.3.3 Minimum bactericidal concentration

The *E. coli* and *S. aureus* growth varies with different concentrations of TiO_2 . The lowest concentration of TiO_2 , known as the minimum inhibitory concentration (MIC), was determined by observing agar plates

for any bacterial growth after 20–24 hours [37]. The 10^5 (0.625 µg/ml) dilution of MIC confirmed the minimum bactericidal concentration of *E. coli*, while TiO_2 10^4 (1.25 µg/ml) was determined as the MBC of *S. aureus*. The MIC and MBC values between the two strains exhibited notable differences, with a p-value of 0.041 suggesting statistical significance.

3.4 Visible and UV-Assisted Photocatalytic Activity

Following a comprehensive analysis of the experimental data, which showed a decrease in methylene blue dye concentration and the presence of intermediate byproducts, the photocatalytic degradation process was established. A clear, strong pattern of spectral shifts in the UV-Vis data provided compelling supporting evidence [38]. At 600 nm, the methylene blue solution showed a prominent absorption peak, evidenced by its vibrant blue color and significant light absorption. Throughout the experiment, a steady decrease in absorbance, measured spectrophotometrically, indicated the dye's progressive degradation. As depicted in Fig. 9(a), UV irradiation dramatically enhanced the degradation efficiency of TiO_2 nanoparticles, resulting in near-total breakdown of the target compound within 3 hours. A visible clearing of the solution indicated this effect. In comparison to Fig. 9 (b) displays the degradation trend under sunlight exposure, the dye's slow but noticeable breakdown was visible. Visually, the intense, deep blue of the methylene blue solution gradually faded, becoming almost completely colorless in both instances; the vibrant hue slowly vanished. The control group that was exposed to sunlight without TiO_2 NPs didn't change color much, which shows how the nanoparticles acted as catalysts. These results demonstrate that TiO_2 NPs are more effective as photocatalysts under UV light, making them particularly useful for removing dyes from wastewater [39].

Plant-assisted nanomaterials, such as those synthesized using *Cassia trifolia*, have demonstrated a range of applications, including photocatalytic, sensing, and biomedical uses, highlighting their multifunctionality [40]. The high degradation efficiency could be attributed to the strong dye adsorption on TiO_2 surfaces and radical-based photocatalysis, as indicated by the kinetic analysis results. TiO_2 NPs offer eco-friendly uses through non-toxic production, biocompatibility, and increased ROS production upon exposure to light. Although challenges such as batch variability and residual organics affecting stability are crucial for scaling up. Under both sunlight and UV irradiation, electron-hole pairs are born within TiO_2 , a silent, energetic creation. Figure 10 shows a schematic explanation of the ROS breakdown down methylene blue into CO_2 and H_2O .

3.5 Photocatalyst Regeneration Study

TiO_2 NPs' regeneration capacity was evaluated over 3 successive MB dye degradation cycles. The nanoparticles underwent centrifugation, washing, drying, and reuse without further treatment following each cycle. Table 4 shows that under UV irradiation, the degradation efficiency decreased marginally with each reuse, going from 98.5% in the first cycle to 95.6% in the third. The uncertain aggregation of the nanoparticles or surface fouling could be the reason for this loss. These results confirm the high stability and reusability of the catalyst. The TiO_2 NPs' photocatalytic performance remained consistently high

during three deterioration cycles, with just a slight decrease in efficiency (~ 3–5%), although XRD examination was not performed following the photocatalytic cycles [41]. Under operating conditions, this continuous activity indirectly promotes the nanoparticles' structural stability. To validate crystallographic integrity and evaluate any phase changes following repeated use, future research will include post-treatment XRD examination.

Table 4

Regeneration performance of green-synthesized TiO₂ nanoparticles over three successive methylene blue degradation cycles under UV and sunlight irradiation.

Cycle	Degradation Efficiency (%) – UV	Degradation Efficiency (%) – Sunlight
1st	98.5 ± 0.4	97.8 ± 0.6
2nd	96.9 ± 0.6	96.2 ± 0.5
3rd	95.6 ± 0.5	94.0 ± 0.5

3.6 Pollutant Degradation Kinetics

The kinetics of the pseudo-first-order reaction were detected in the photocatalytic breakdown of methylene blue. With R² values above 0.98 for both sunlight and UV irradiation treatments, the ln(C₀/C) against time plots demonstrated good linearity, confirming this. Under UV irradiation, the computed apparent rate constant (k) was 0.0174 min⁻¹, while under sunlight exposure, it was 0.0156 min⁻¹ [42]. These results show slightly higher under UV than sunlight (Fig. 11). These findings reveal the green-synthesised TiO₂ nanoparticles' efficient and reliable photocatalytic activity. High R² values (UV: 0.991, sunshine: 0.984) were obtained from a linear regression of ln(C₀/C) with time, which validated pseudo-first-order kinetics under both UV and sunshine. in UV irradiation, the rate constant was substantially higher (p < 0.05) than in sunlight, suggesting that the photocatalytic performance was statistically superior.

3.7 Biofilm inhibition

In biofilm inhibition activity, various bacteria form biofilms. Biofilms are common and develop on almost all surfaces submerged in natural water environments. Biofilms give bacteria certain properties that are not present in the planktonic state, which is why dental plaque is considered a biofilm [40]. Titanium dioxide nanoparticles can eradicate pathogens such as *E. coli* and *S. aureus*. Biofilm poses a significant threat to the environment, industry, and human health. Figure 12 illustrates that titanium dioxide nanoparticles are more effective against *E. coli* (70.34%) compared to *S. aureus* (86.57%) in terms of biofilm activity. The nanoparticles have interacted with the cell membranes of both microorganisms, releasing a uniform substance and attaching to the cell chain as part of the biofilmBiofilm inhibition by TiO₂ NPs resulted in notable reductions in OD570 absorbance for both *S. aureus* and *E. coli* compared to the untreated controls, with statistical significance (p < 0.01).). *S. aureus* had a significantly greater inhibition rate (86.57%) than *E. coli* (70.34%), which might be due to variations in the biofilm matrix between the two species.

4. Conclusion

TiO₂ nanoparticles were effectively green-synthesised in this study using pollen extract from *Cocos nucifera* employing an environmentally benign, and biocompatible method at room temperature without the need for chemical additions. With an average crystallite size of roughly 17.4 nm, XRD verified the crystalline nature of the rod-shaped TiO₂ nanoparticles. Furthermore, a size distribution between 5 and 100 nm was revealed by HRTEM examination, suggesting the presence of agglomerated or polycrystalline particles. Their surface area, shape, and structural integrity were all validated by FTIR and other techniques. The produced TiO₂ NPs showed excellent photocatalytic activity, degrading methylene blue by 97.8% in light and 98.5% in UV after 180 minutes. Kinetic analysis and light-induced ROS generation confirmed their radical-based photocatalytic mechanism. Their MICs were 0.62 µg/mL for *E. coli* and 1.25 µg/mL for *S. aureus*, respectively, and they demonstrated strong antibacterial activity as well as biofilm inhibition rates of 86.57% and 70.34%. Significant differences were confirmed through ANOVA for concentration-dependent effects in antimicrobial and photocatalytic studies, supporting the reproducibility and efficacy of the green-synthesized TiO₂ nanoparticles ($p < 0.05$). These results support the biosynthesized TiO₂ NPs' versatility for use in biological and eco-friendly applications. To investigate their potential in drug administration, wound healing, sensing, and real-time diagnostics, more research is necessary.

Declarations

Plant collection and permissions

The *Cocos nucifera* pollen used in this study was collected from Ponnoli Nagar village, Salem district, Tamil Nadu (11°39'N, 78°08'E), from publicly accessible areas without disturbing the local ecosystem. The species is widely cultivated and not listed as endangered under the Indian Biodiversity Act. The collection complied with all relevant local and national regulations.

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Authors' Contributions

Tamilselvi- Writing – original draft, Kanagasabapathy Sivasubramanian-Software & prepared figures, Loganathan Lingeshwaran: Formal analysis, Palanivel Velmurugan-Supervision, Anurag Sureshababu-Methodology, Vanga Dharma Teja-Data curation, Kishore Kumar- Data curation, Jeyanthi Rebecca L- Conceptualisation, S. Anbuselvi- Resource, E. Devasagaya Daisy- editing, Sivanraju Rajkumar: Conceptualization, review and editing.

Conflict of Interest

The authors declare that there is no conflict of interest related to this work

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Funding Declaration

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Ethics, Consent to Participate, and Consent to Publish

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

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Table 1 and 2

Tables 1 and 2 are available in the Supplementary Files section.

Figures

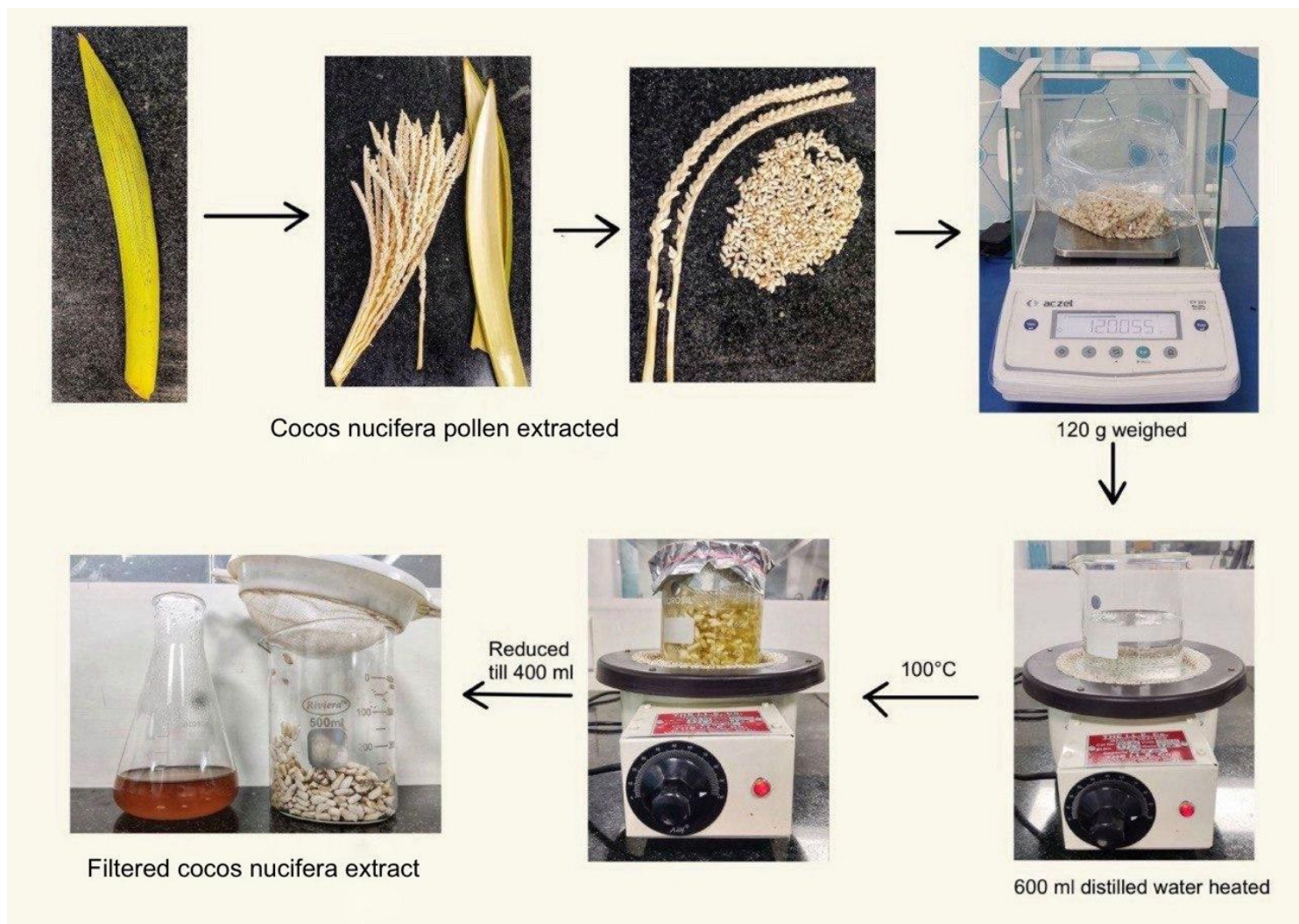


Figure 1

Sequential steps involved in the botanical extraction of *Cocos nucifera* for nanoparticle synthesis.

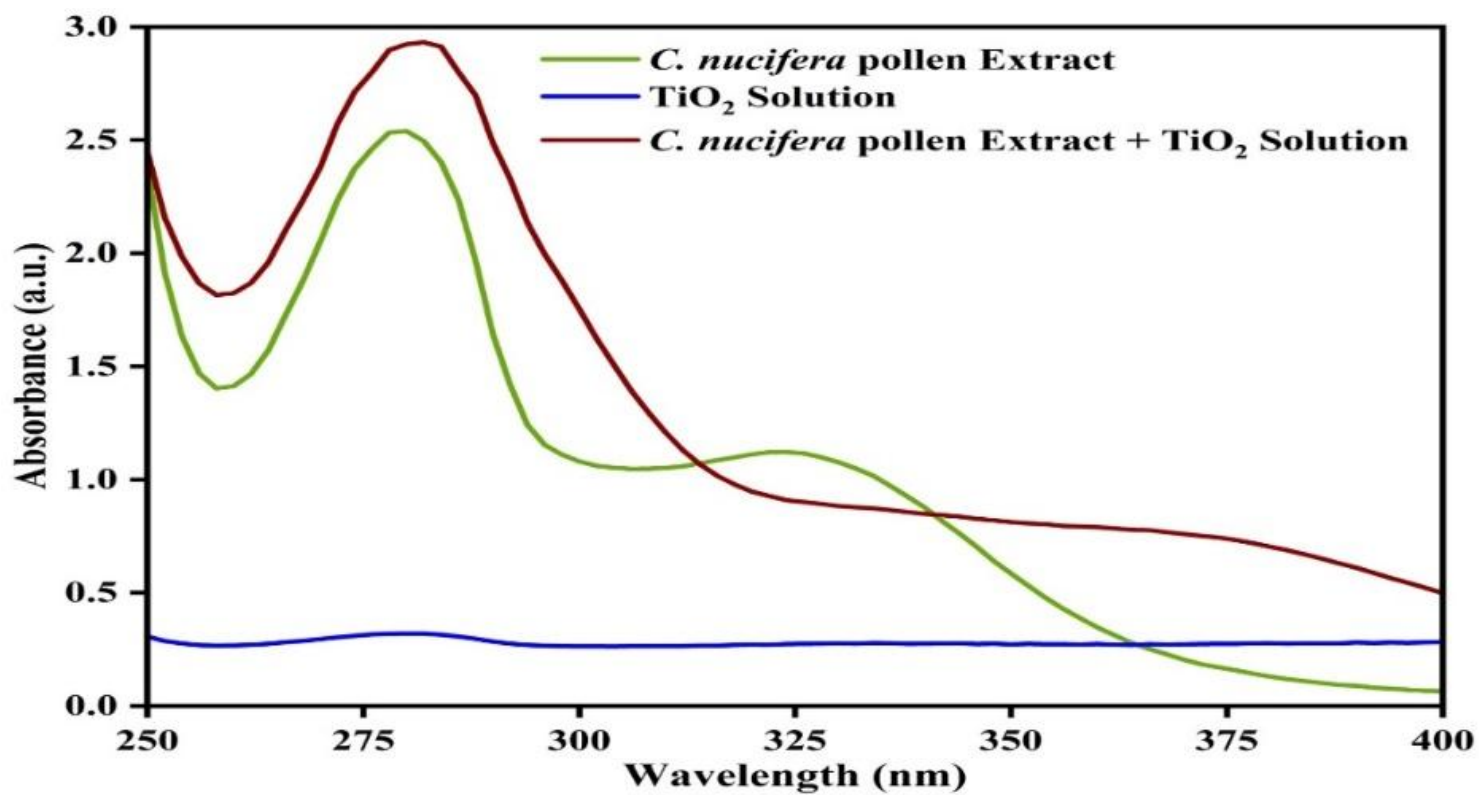


Figure 2

UV-Vis absorption spectra of plant extract, SnCl₂ solution, and TiO₂ nanoparticles, indicating λ_{max} values for synthesis verification

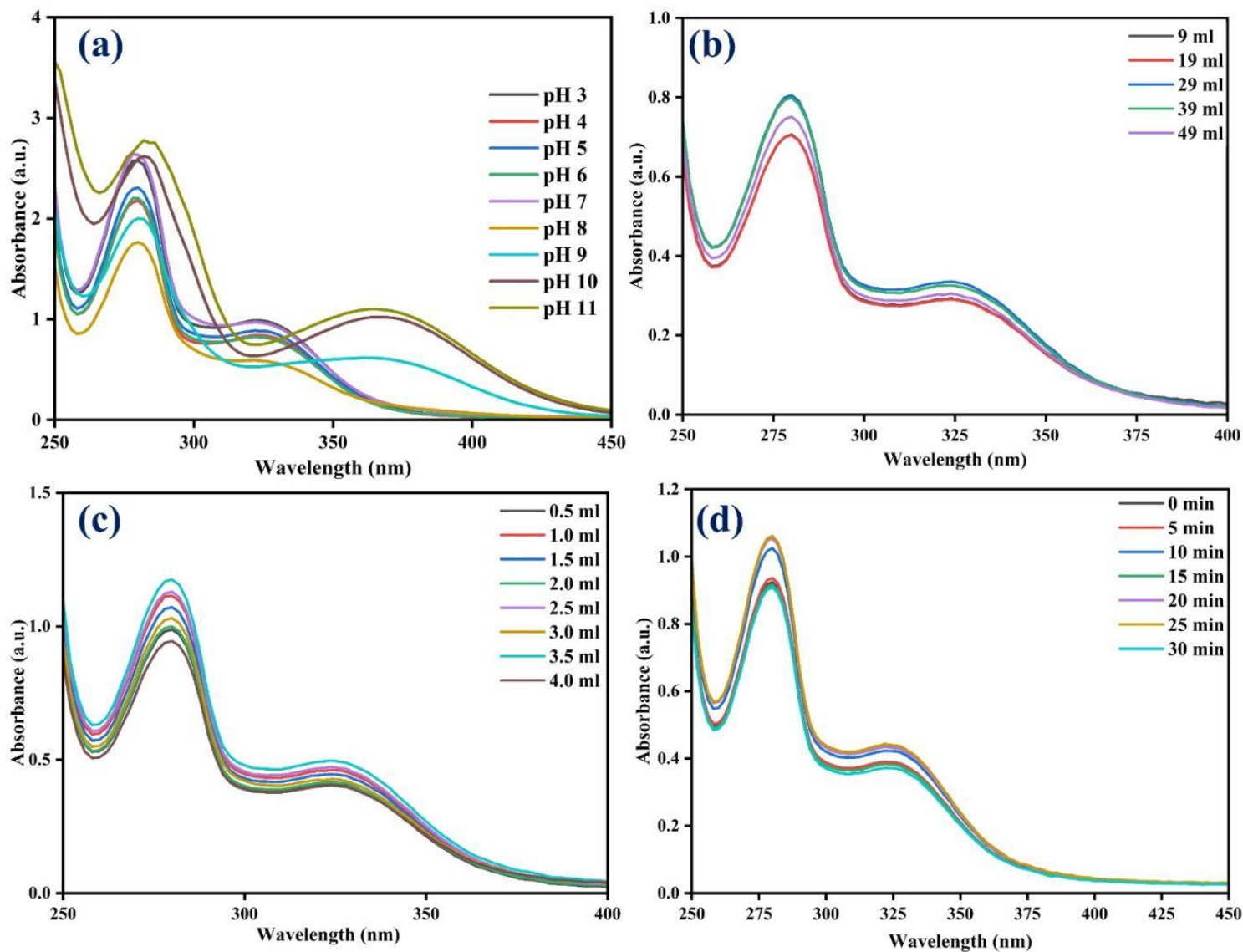


Figure 3

Optimization of TiO_2 nanoparticle synthesis: (a) pH optimization (4–11), (b) substrate volume (9–49 mL), (c) metal ion concentration (0.5–4.0 mL), (d) time optimization (0–30 min).

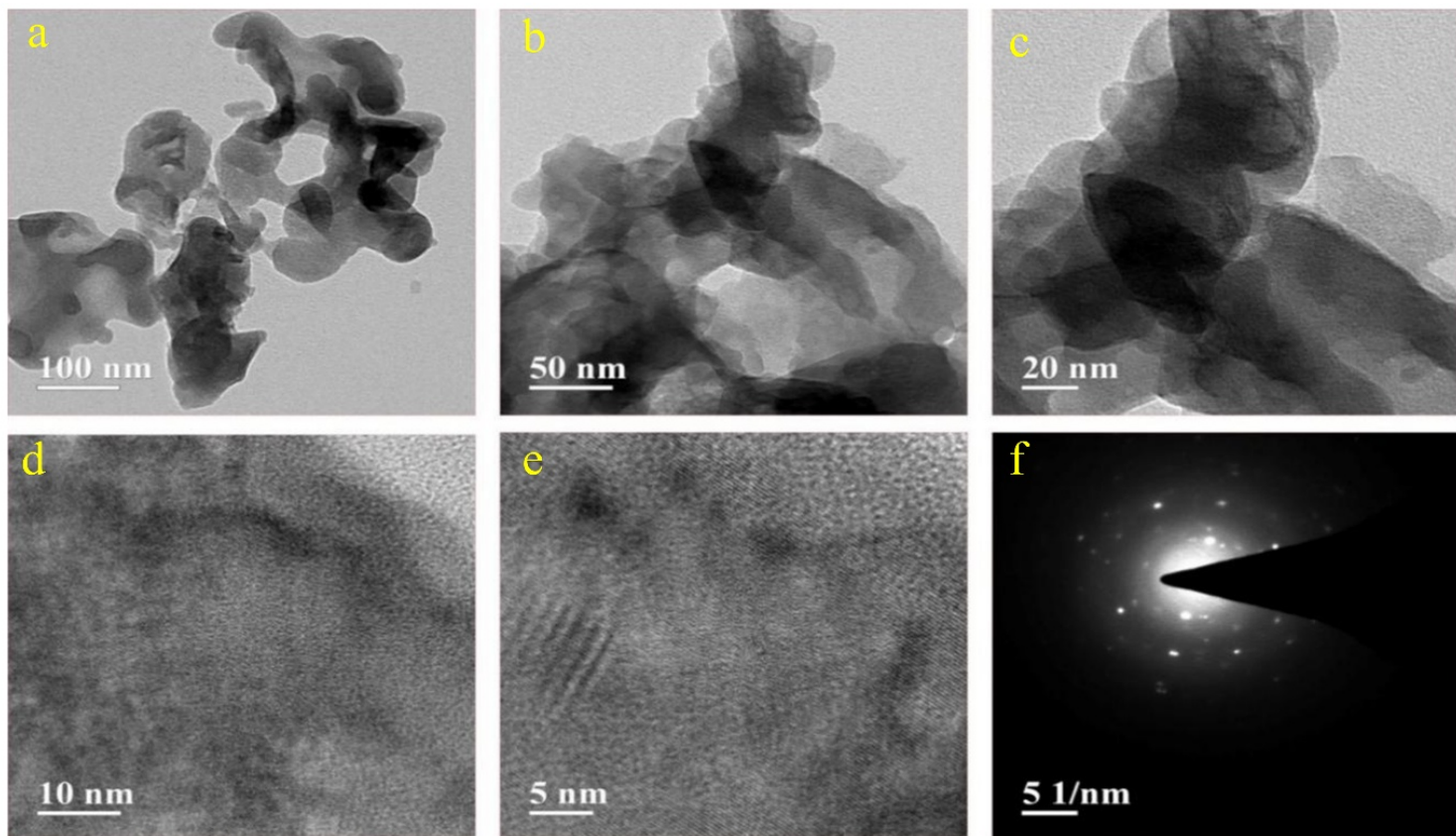


Figure 4

HR-TEM micrographs of synthesized TiO_2 nanoparticles. (a) Pure TiO_2 showing spherical morphology; (b) Ag-doped TiO_2 ; (c) Zn-doped TiO_2 ; (d) TiO_2 at low magnification; (e) TiO_2 at high magnification; (f) SAED pattern confirming crystalline structure of TiO_2 nanoparticles.

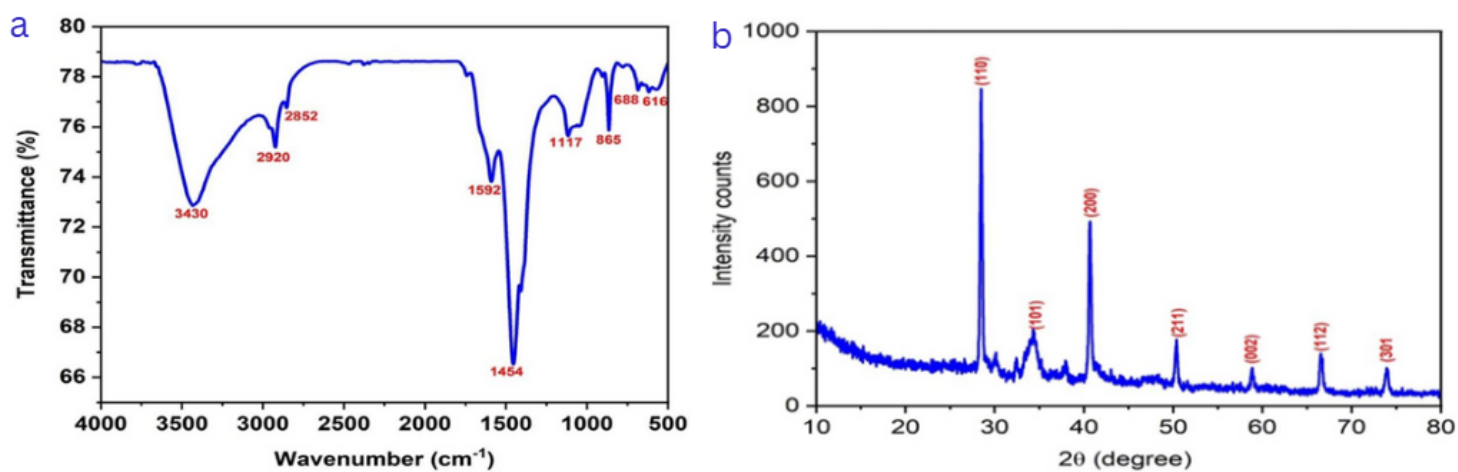


Figure 5

(a) FTIR spectrum of green-synthesized TiO_2 nanoparticles showing functional peaks at 3430, 2920, 2852, 1592, 1454, and 1117 cm^{-1} . (b) XRD pattern showing crystalline structure with indexed planes (110), (101), (200), (211), (002), (112), and (301), matching tetragonal rutile TiO_2 .

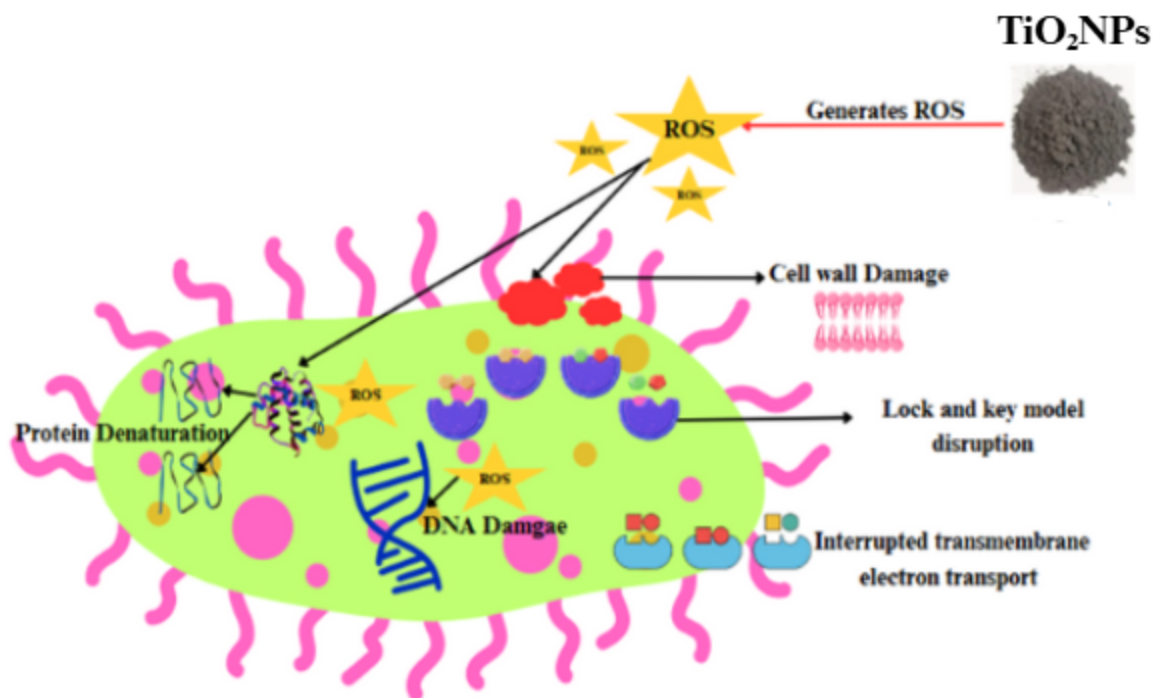


Figure 6

Schematic illustration of the proposed antibacterial mechanism of TiO_2 nanoparticles interacting with bacterial cells.

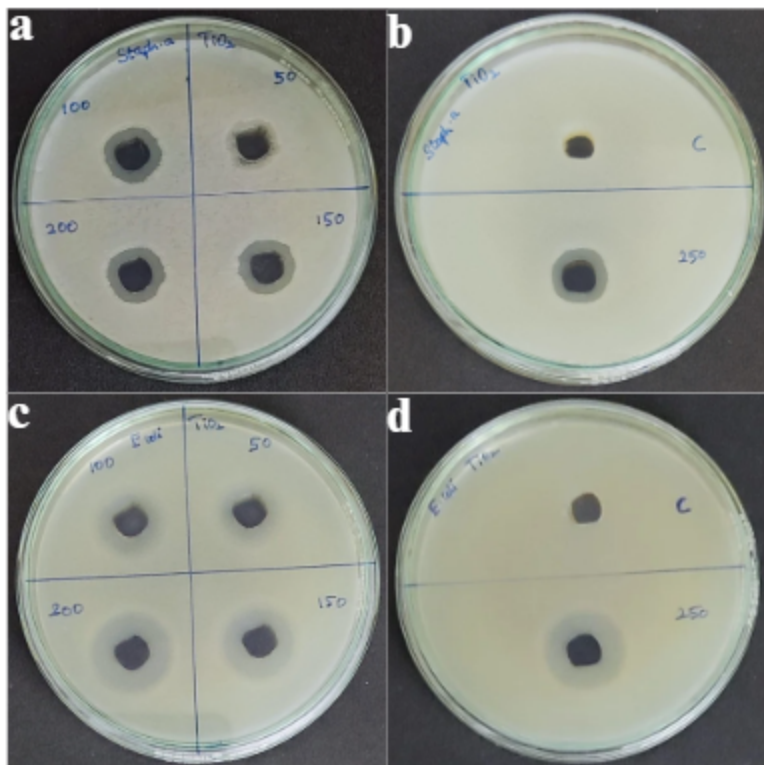


Figure 7

(a&b) Zone of inhibition showing antibacterial activity of TiO_2 nanoparticles against *Staphylococcus aureus*; (c&d) Zone of inhibition against *Escherichia coli*.

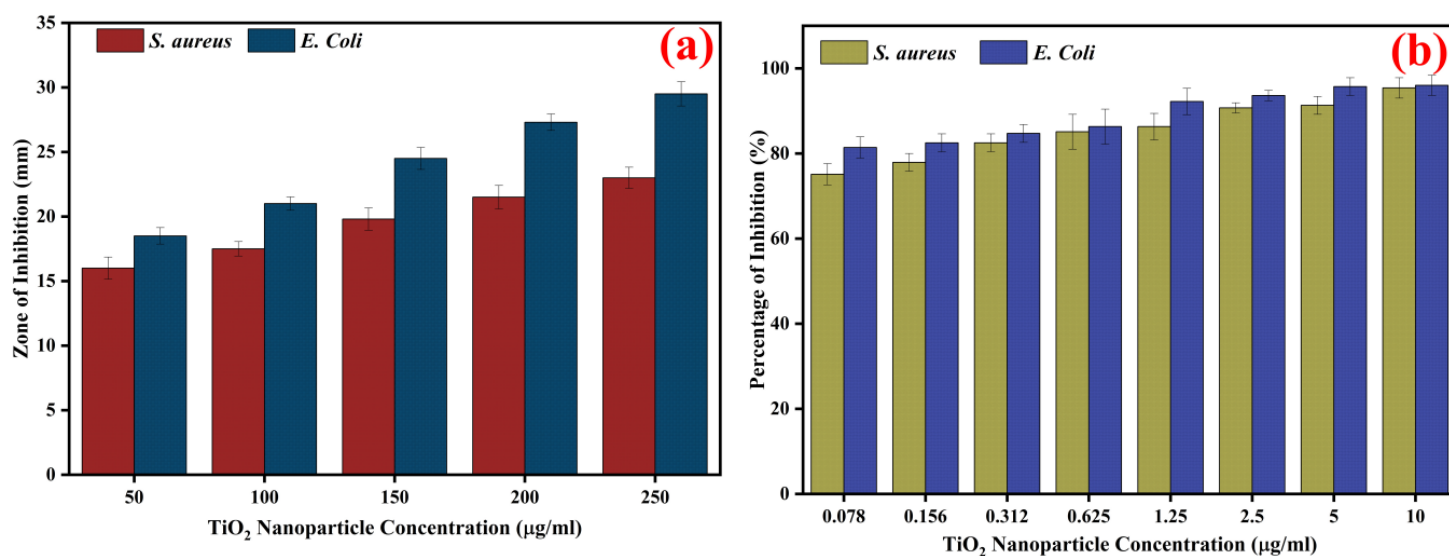


Figure 8

(a) Antibacterial activity zones for *E. coli* and *S. aureus*. (b) MIC determination showing concentration-dependent inhibition. Data expressed as mean $\pm$ SD ($n = 3$). Statistical analysis performed using one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$.

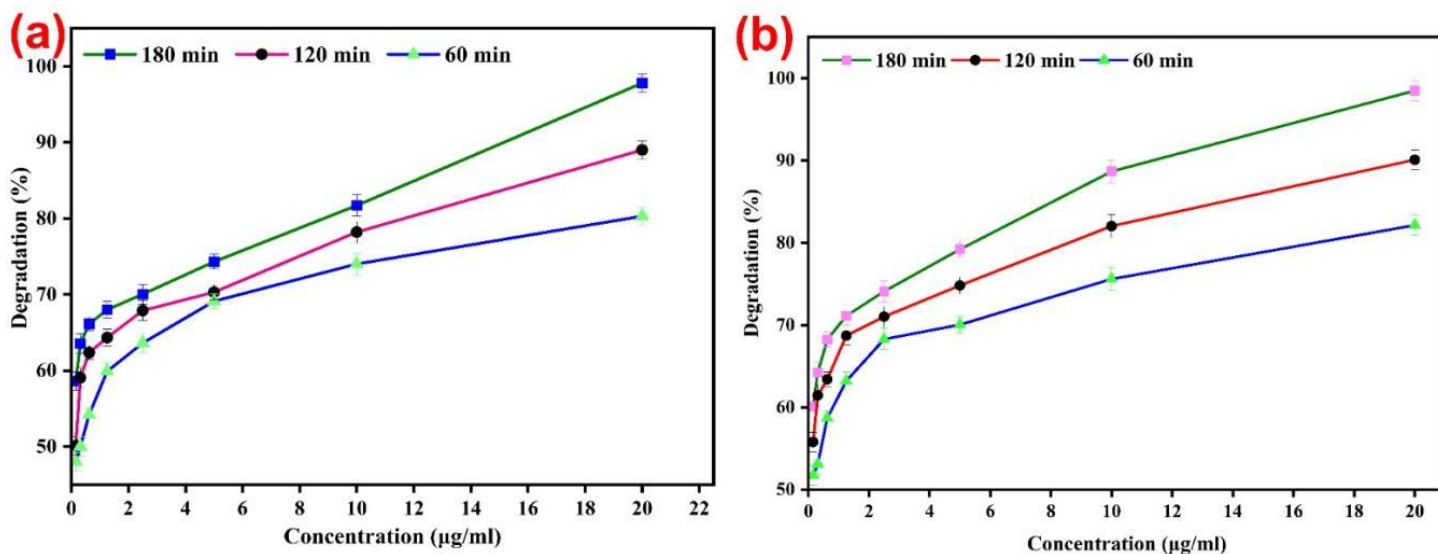


Figure 9

(a, b) Photocatalytic activity of TiO₂ nanoparticles under UV and sunlight, demonstrating methylene blue photocatalytic efficiency. Error bars represent $\pm$ SD of triplicate samples. $p < 0.05$ indicates a statistical difference.

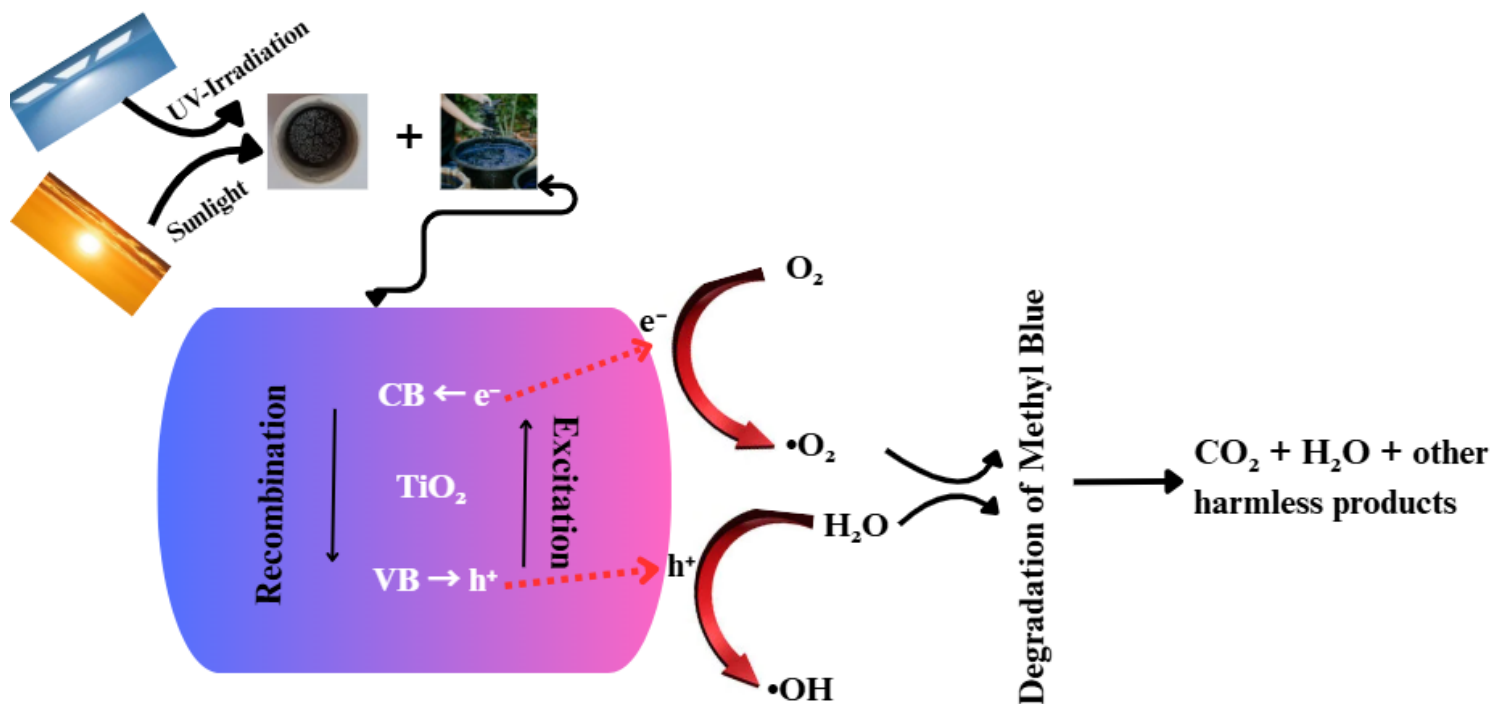


Figure 10

Mechanism of TiO₂ NPs' photocatalytic degradation of methylene blue in the presence of light. The dye breaks down into CO_2 and H_2O when light creates e^-/h^+ couples, where h^+ forms $\cdot OH$ from water and e^- forms $\cdot O_2^-$ from oxygen.

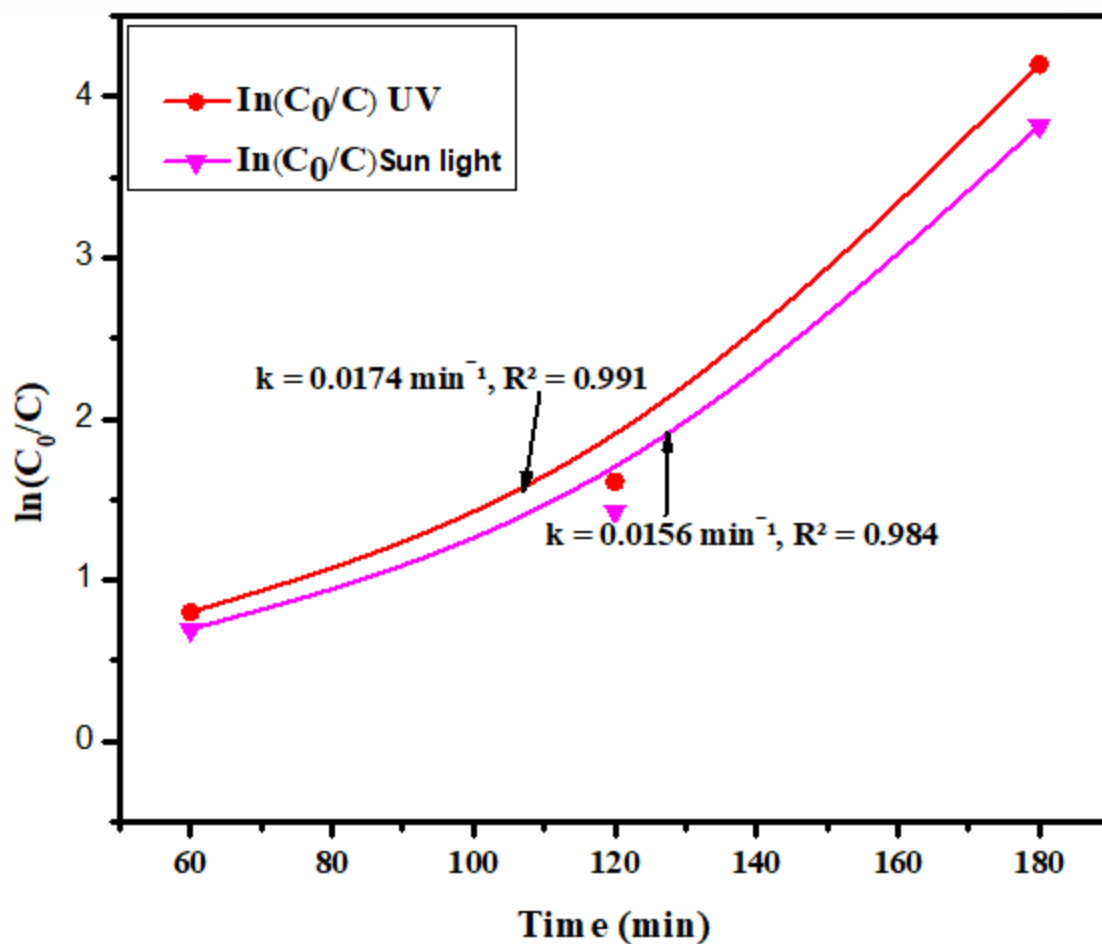


Figure 11

Pseudo-first-order kinetic plot of methylene blue degradation using green-synthesized TiO_2 nanoparticles under UV and sunlight irradiation. Rate constants were $k = 0.0174 \text{ min}^{-1}$ ($R^2 = 0.991$) for UV and $k = 0.0156 \text{ min}^{-1}$ ($R^2 = 0.984$) for sunlight, with a statistically significant difference ($p < 0.05$, $n = 3$).

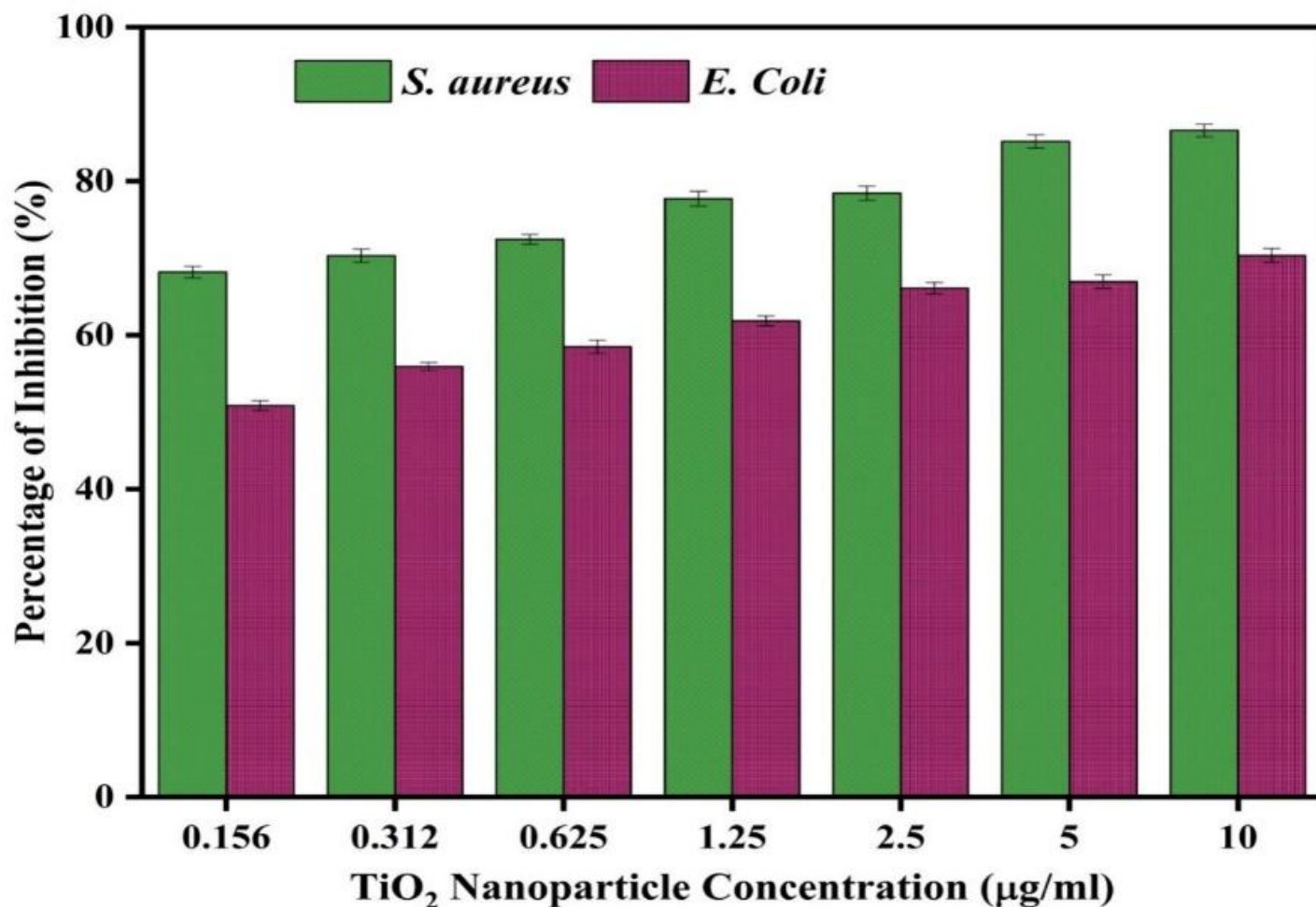


Figure 12

Antibiofilm assay results showing TiO_2 nanoparticle inhibition of *S. aureus* and *E. coli* biofilms. Data expressed as mean $\pm$ SD ($n = 3$). $p < 0.05$

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [image1.jpeg](#)
- [Table1and2.docx](#)