International Journal of Recent Trends in Natural Sciences and Research (IJRTNSR)
ISSN: XXXX-XXXX (Online)

GREEN SYNTHESIS OF TITANIUM NANO PARTICLES FROM MARINE ALGAE, CHARACTERIZATION AND IT'S ANTIMICROBIAL ACTIVITY

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ABSTRACT

TiO_2 nanoparticles have a wide range of applications in fields such as photocatalysis, environmental purification, solar energy conversion, cosmetics, sensors, and antimicrobial coatings. The present study investigated the green synthesis of titanium dioxide (TiO_2) nanoparticles using marine brown algae and evaluated their antimicrobial activity against bacterial pathogens. Marine brown algal extract served as a natural reducing and stabilizing agent in an eco-friendly sol–gel synthesis process, followed by drying and calcination to obtain crystalline nanoparticles. UV–Visible spectroscopy confirmed successful nanoparticle formation by showing characteristic absorption in the UV region below 250 nm, while XRD analysis verified the crystalline anatase phase of TiO_2 nanoparticles. The synthesized nanoparticles appeared as a fine white crystalline powder, indicating successful calcination and improved crystallinity. Antimicrobial evaluation using the agar disc diffusion method demonstrated that TiO_2 nanoparticles produced a zone of inhibition of approximately 3 mm, whereas drug-doped nanoparticles exhibited a 6 mm inhibition zone, and the negative control showed no inhibition. Minimum inhibitory concentration (MIC) analysis against Pseudomonas *sp. indicated that bacterial growth was inhibited up to the second dilution (C/2 concentration), confirming effective antibacterial activity. The antibacterial effect was attributed to reactive oxygen species generation and the synergistic interaction between TiO_2 nanoparticles and ciprofloxacin, which enhanced bacterial membrane disruption. Overall, the findings demonstrate that marine brown algae-mediated green synthesis is a simple, sustainable, and effective approach for producing biologically active TiO_2 nanoparticles with promising antimicrobial potential for biomedical and environmental applications.*

Keywords: Green synthesis; Titanium dioxide nanoparticles (TiO_2); Antimicrobial activity; Minimum inhibitory concentration (MIC)

INTRODUCTION

Nanomaterials are increasingly used for targeted drug delivery, imaging, and therapeutic purposes. Their ability to interact at the cellular and molecular levels makes them highly suitable for various biomedical applications (Rai *et al.,* 2018). Titanium dioxide (TiO_2) nanoparticles are among the most extensively studied metal oxide nanoparticles due to their remarkable physicochemical properties including high chemical stability, strong photocatalytic activity, non-toxicity, and low cost. TiO_2 nanoparticles exist mainly in three crystalline forms: anatase, rutile, and brookite. Among these, anatase is considered the most photocatalytically active form due to its higher surface area and better electron mobility (Chen & Mao, 2007).

TiO_2 nanoparticles are widely used in sunscreen products, paints, and food packaging materials due to their UV-blocking ability and chemical stability. Their strong oxidative properties also enable them to generate reactive oxygen species that can effectively destroy microbial cells (Zhao *et al.,* 2015). Green synthesis of nanoparticles has emerged as an eco-friendly and sustainable alternative to conventional physical and chemical synthesis methods.

Biological systems such as plants, bacteria, fungi, and algae contain a variety of biomolecules including proteins, enzymes, polysaccharides, phenolic compounds, and pigments that can act as natural reducing and stabilizing agents during nanoparticle synthesis (Mukherjee *et al.,* 2021). The mechanism of action of TiO_2 nanoparticles involves the generation of reactive oxygen species such as hydroxyl radicals and superoxide ions. These ROS cause oxidative stress, leading to damage of cell membranes, proteins, and DNA. In addition, the small size and high surface area of nanoparticles enhance their interaction with microbial cells, increasing their antimicrobial efficiency. The ability of nanoparticles to penetrate cell membranes further contributes to their effectiveness.

Although several studies have reported the synthesis of nanoparticles using algae, there are still gaps in understanding the optimization of synthesis parameters and detailed characterization of nanoparticles. Further research is required to evaluate antimicrobial activity using different algal species and to explore potential applications in biomedical and environmental fields. The present study focuses on the green synthesis of titanium dioxide nanoparticles, to assess the effectiveness of green synthesis as an eco-friendly and sustainable methods for nanoparticles production using marine brown algae and to evaluate the antimicrobial activity of synthesized TiO_2 nanoparticles against selected bacterial strains using standard microbiological methods. Hence, the present study focuses on the green synthesis of TiO_2 nanoparticles using marine algae and evaluation of their antimicrobial potential

MATERIALS AND METHODS

Marine brown algae were collected from Pitchai Moopan Valasai coastal region near erwadi (Lat 9.2272°N & Lon 78.7845°E), Tamil Nadu, India. The collected samples were used for the synthesis of TiO_2 nanoparticles based on their bioactive potential (Mukherjee *et al.,* 2021). Titanium (IV) Isopropoxide (TTIP) was used as the precursor for TiO_2 nanoparticle synthesis due to its high reactivity in sol–gel processes (Chen & Mao, 2007).

Synthesis of TiO_2 Nanoparticles

Dewatering of Algal Sample

The algal samples were shade dried at room temperature to remove moisture content. Shade drying helps in preserving bioactive compounds without thermal degradation (Rajeshkumar *et al.,* 2013).

Green Synthesis (Preparation of Algal Extract)

Dried algal samples were ground into fine powder to increase surface area for extraction (Mukherjee *et al.,* 2021).About 10 g of algal powder was mixed with 50 mL distilled water and heated at 60–70°C to extract bioactive compounds (Rajeshkumar *et al.,* 2013).The mixture was filtered using Whatman No.1 filter paper to obtain a clear extract (Subhapriya *et al.,* 2018).The filtrate was stored at 4°C to preserve bioactive components (Mukherjee *et al.,* 2021).

Modified Green Sol–Gel Synthesis of TiO_2 Nanoparticles

A sol–gel method was adopted for nanoparticles synthesis due to its efficiency and control over particle size (Chen & Mao, 2007).Ethanol was used as solvent and maintained in an ice bath to control hydrolysis rate. TTIP was added slowly under stirring to ensure homogeneous mixing and controlled reaction (Chen & Mao, 2007). Algal extract was added dropwise, acting as a reducing and stabilizing agent (Mukherjee *et al.,* 2021).Continuous stirring facilitated hydrolysis and condensation reactions leading to nanoparticles formation (Iravani, 2011).The formation of sol indicates initiation of TiO_2 nanoparticles synthesis (Chen & Mao, 2007).Aging for 12–24 hours promote nucleation and growth of nanoparticles.

Drying and Calcination

- The synthesized nanoparticles were dried at 80–100°C to remove moisture content (Subhapriya *et al.*, 2018).
- Calcination at 450–500°C enhances crystallinity and removes organic residues (Chen & Mao, 2007).
- This process results in the formation of anatase phase TiO_2 nanoparticles, which are known for high photocatalytic activity (Chen & Mao, 2007).

Characterization of TiO_2 nanoparticles

UV–Visible Spectroscopy

The synthesized TiO_2 nanoparticles were characterized using a UV–Visible spectrophotometer to confirm nanoparticles formation and evaluate their optical properties. Approximately 1 mg of TiO_2 nanoparticles powder was dispersed in 5 mL of ethanol, and the absorbance spectrum was recorded over the wavelength range of 200–800 nm using ethanol as the blank. The characteristic absorption peak observed in the UV region confirmed the successful synthesis of TiO_2 nanoparticles and indicated their nanoscale optical properties. The analysis was performed following standard UV–Visible spectroscopic procedures described by Li *et al.* (2013) and Vasanth, Murugesh, and Susikaran (2022).

X-Ray Diffraction (XRD)

X-Ray Diffraction (XRD) is a powerful analytical technique used to determine the crystalline structure and phase composition of nanoparticles. It is based on the diffraction of X-rays by the atomic planes in a crystalline material.When X-rays are directed onto a crystalline sample, they are scattered in specific directions, producing a diffraction pattern. This pattern is characteristic of the crystal structure and can be used to identify different phases of materials. In the case of TiO_2 nanoparticles, XRD helps to identify phases such as anatase, rutile, and brookite by comparing the obtained peaks with standard JCPDS data.

Antimicrobial Activity

The antimicrobial activity of the synthesized TiO_2 nanoparticles was evaluated using the agar well diffusion method against *Pseudomonas* sp. on nutrient agar plates. Different concentrations of TiO_2 nanoparticle suspension were tested, with antibiotic-loaded nanoparticles as the positive control and solvent as the negative control. The plates were incubated at 37°C for 24 hours, and the **zone of inhibition** was measured to assess antibacterial activity (Muthusamy *et al.*, 2020).

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of TiO_2 nanoparticles in combination with ciprofloxacin was determined against *Pseudomonas* sp. using the broth dilution method. A two-fold serial dilution was prepared in sterile nutrient broth, inoculated with bacterial culture, and incubated at 37°C for 24 hours.

RESULT AND DISCUSSION

Marine brown algae were successfully collected from Pitchai Moopan valasaicoastal region (Lat 9.2272˚N & Lon 78.7845˚E), near Ervadi Tamil Nadu. The collected samples were morphologically identified based on their brown color, thallus structure, and texture.The brown coloration is due to fucoxanthin pigment, which is characteristic of brown algae. The collected samples were fresh and free from contamination, confirming their suitability for nanoparticle synthesis. Decrease in sample weight. These observations confirm successful dewatering, which is necessary for efficient extraction of bioactive compounds. Similar preprocessing steps have been reported in algae-mediated nanoparticle synthesis (Mukherjee *et al.,* 2021).The aqueous extraction of dried algal powder resulted in a brown-colored filtrate.

The colour change suggests the presence of bioactive compounds, including polyphenols, proteins, and polysaccharides, which serve as reducing and stabilizing agents. This finding aligns with previous studies reporting the role of algal extracts in facilitating nanoparticle synthesis. (Mukherjee *et al.,* 2021; Holdt & Kraan, 2011). This observation indicates the occurrence of hydrolysis and condensation reactions, resulting in nanoparticle formation. Similar visual confirmation in green synthesis approaches has been documented in previous studies. (Iravani, 2011).

Figure-1: Extracted algal sample   Figure-2: Sol-Gel formation

After continuous stirring and aging (12–24 hours), resulting in formation of gel-like structure due to increased viscosity. This observation confirms the advancement of sol–gel reactions and the onset of nanoparticle nucleation. Comparable sol–gel behaviour in TiO_2 synthesis has been documented in previous studies (Chen & Mao, 2007). Calcination within the temperature range of 450–500 °C led to the formation of crystalline TiO_2 nanoparticles, predominantly exhibiting the anatase phase. This observation aligns with previously reported studies. (Chen & Mao, 2007).

UV–Visible Spectroscopy Analysis

UV–Visible spectroscopy confirmed the successful green synthesis of TiO_2 nanoparticles using marine brown algal extract. The absorbance spectrum (200–800 nm) showed a strong absorption peak in the 200–230 nm region, characteristic of TiO_2 nanoparticles. The sharp UV absorption indicated nanoscale particle formation with high surface energy and good optical properties.

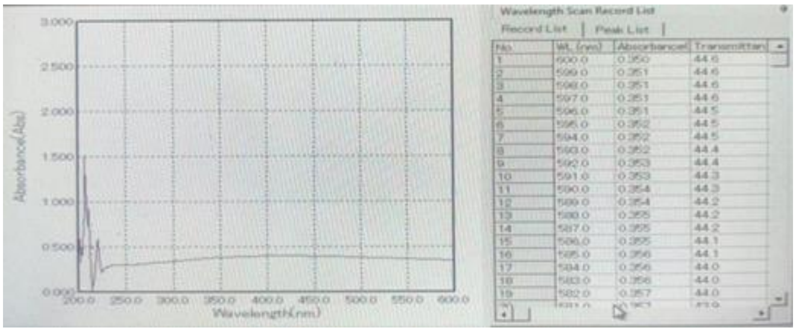

Figure-3:UV spectra of TiO_2 Nanoparticles

A gradual decrease in absorbance beyond 250 nm suggested good dispersion and minimal nanoparticles aggregation. The absence of broad peaks in the visible region confirmed high purity and effective removal of impurities during calcination. The observed absorption pattern was consistent with previous reports on anatase TiO_2 nanoparticles and algae-mediated green synthesis. Overall, the UV–Visible analysis verified the successful formation of stable, pure, and nanosized TiO_2 nanoparticles (Chen & Mao, 2007; Li *et al.*, 2013; Vasanth *et al.*, 2022; Mukherjee *et al.*, 2021).

X-Ray Diffraction

X-ray diffraction (XRD) analysis was carried out to determine the crystalline nature of the synthesized TiO_2 nanoparticles. The analysis was performed at Kalasalingam Academy of Research and Education, Srivilliputhur, using a Bruker D8 Advance diffractometer with Cu-Kα radiation (λ = 1.5406 Å).The diffraction pattern was recorded in the 2θ range of 10° to 80° using Cu-Kα radiation (λ = 1.5406 Å).

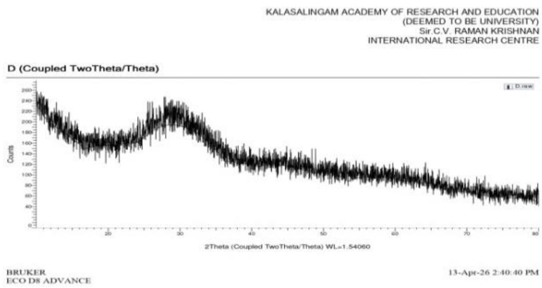

Figure – 4: XRD pattern of TiO_2 nanoparticles

The obtained XRD pattern exhibited distinct diffraction peaks at approximately 2θ values around 25°, 37°, 48°, 54°, 55°, and 62°, which correspond to the characteristic planes of TiO_2 nanoparticles. The prominent peak observed at around 25° is indexed to the (101) plane, which confirms the presence of the anatase phase of TiO_2 (Chen & Mao, 2007). The sharp and well-resolved diffraction peaks observed in the XRD pattern confirm the crystalline nature of the synthesized nanoparticles (Cullity, 2001). Furthermore, the absence of extraneous peaks indicates that the TiO_2 nanoparticles are highly pure and devoid of secondary phases (Diebold, 2003). The diffraction peaks observed in the present study are in good agreement with standard JCPDS data (JCPDS No. 21-1272) for anatase TiO_2, confirming the successful formation of crystalline TiO_2 nanoparticles (Fujishima *et al.*, 2008).

Antimicrobial Activity

The well containing TiO_2 nanoparticles showed a moderate zone of inhibition (~3 mm), confirming antibacterial activity (Muthusamy *et al.,* 2020). The positive control (drug dopes nanoparticles) exhibited a larger zone of inhibition (~6 mm), indicating higher antibacterial efficiency compared to nanoparticles (Balouiri *et al.,* 2016).

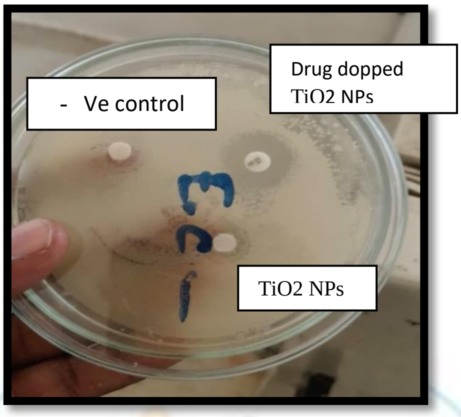

Figure -5: Antimicrobial activity of TiO_2 nanoparticles

The negative control (Ethanol) showed no zone of inhibition (0 mm), confirming that the observed activity is due to TiO_2 nanoparticles and not the solvent (Balouiri *et al.,* 2016, Chen & Mao, 2007). Similar antimicrobial activity has been reported for green synthesized TiO_2 nanoparticles using biological sources, confirming their potential application as alternative antimicrobial agents (Mukherjee *et al.,* 2021 and Muthusamy *et al.,* 2020).

Minimal Inhibitory Concentration

Based on visual observation (Figure - 10), Tube 1 and Tube 2 appeared clear, indicating complete inhibition of bacterial growth, whereas Tubes 3, 4, and 5 showed visible turbidity, indicating bacterial growth. The positive control exhibited turbidity confirming bacterial viability, while the negative control remained clear, confirming sterility of the medium. The MIC was determined as the lowest concentration that prevented visible bacterial growth, which was observed at Tube 2 (C/2 concentration).The MIC was recorded as the lowest concentration showing no visible turbidity, indicating complete inhibition of bacterial growth (Andrews, 2001; Balouiri *et al.*, 2016; Cappuccino & Sherman, 2014). TiO_2 nanoparticles are known to generate reactive oxygen species (ROS), such as hydroxyl radicals and superoxide ions, which induce oxidative stress and damage bacterial cell membranes and intracellular components (Hajipour *et al.,* 2012; Zhao *et al.,* 2015).

CONCLUSION

Overall, the study demonstrates that marine brown algae-mediated synthesis of TiO_2 nanoparticles is an effective green approach for producing biologically active nanoparticles. The synthesized nanoparticles showed promising antimicrobial properties, suggesting their potential applications in medical, pharmaceutical, and environmental fields.In conclusion, green synthesized TiO_2 nanoparticles can serve as a potential alternative to conventional antimicrobial agents. Further studies focusing on optimization, toxicity evaluation, and large-scale production can enhance their applicability in various industrial and biomedical applications.

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