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# Eco-Friendly Synthesis of Titanium Dioxide Nanoparticles Using Stem Extract of Basella Alba and Its Wound Pathogen Control

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Nanomaterials are mostly hazardous due to their small size and increased surface activity, which makes it very easy for them to penetrate and enter biological systems. Therefore, the primary goal of the study is the eco-friendly synthesis of titanium dioxide nanoparticles using Basella alba stem extract. Basella alba is considered as a useful medicinal plant, and it tends to have some titanium nanoparticles within them. The agar well plate diffusion method and time kill curve assays were studied to evaluate the antimicrobial activity. As a result, the titanium dioxide tends to have less antimicrobial activity, where there is no zone of inhibitions and considered to be less effective. But in the time-kill kinetics analysis, the prepared nanoparticles showed mild bacteriostatic and bacteriostatic activity. To conclude titanium dioxide comparatively shows less activity in wound pathogen control. So, the future prospect of this study is comparatively less.

**Keywords:** Anti-inflammatory property, Basella alba, nanotechnology, titanium dioxide nanoparticle, wound pathogen.

## 1. Introduction

Nanotechnology is the scientific process of creating, assembling, analyzing, and utilizing materials and devices whose smallest functional organization, in at least one dimension, is on the nanoscale, or one billionth of a meter (Silva, 2004; Kurian et al., 2022). The swift advancement of nanoscience suggests that nanoscale production will soon be integrated into nearly all fields of science and technology (Malik et al., 2023). Nanomaterials can enhance the performance of composite objects by contributing their unique properties, and they can also be utilized directly to produce sophisticated and potent devices due to their distinct characteristics (Sim & Wong, 2021). In the future, nanomedicine is expected to play a crucial role in both improving normal human physiology and curing human disorders.

Large volumes of titanium dioxide (TiO2) nanoparticles are produced globally for various applications. The physicochemical characteristics of titanium dioxide nanoparticles differ from those of their fine particle analogs, potentially modifying their bioactivity (Shi et al., 2013). The application of titanium dioxide nanoparticles accelerates chlorophyll production, photosynthetic activity, and the activities of antioxidant and rubisco enzymes, boosting agricultural productivity. Titanium dioxide acts as a plant stimulant, activating numerous defense mechanisms that help plants tolerate a variety of abiotic stressors (Gohari et al., 2020; He et al., 2023). Despite their widespread use, there is limited knowledge about the biological effects and cellular response mechanisms of TiO2-NPs (Peters et al., 2014; He et al., 2023).

The primary focus of current research is on the environmentally friendly production of nanoparticles to enhance their biological efficacy (Saquib et al., 2020). The Basellaceae family includes the plant species Basella alba, known for its strong antioxidant capabilities and high mucilage content (Rifaath et al., 2023). Aqueous extracts of Basella alba exhibit antibacterial activity (Yazhini et al., 2021). The findings of this study demonstrate that the fruit extract from Basella alba possesses significant antibacterial properties, supporting its use in folk medicine to treat various infectious diseases. This study aims to explore the eco-friendly synthesis of titanium dioxide nanoparticles using Basella alba stem extract and to analyze their effectiveness in controlling wound pathogens. By leveraging the natural bioactive compounds in Basella alba, the study seeks to develop a sustainable approach to nanoparticle synthesis and evaluate the potential biomedical applications of these nanoparticles in treating infections.

## 2. Materials and Methods

### 2.1 Preparation of titanium dioxide

Two grams of powdered Basella alba were obtained from the herbal garden at Saveetha Medical College. The sample was heated at 50°C and 60°C using a heating mantle for ten minutes. The boiling extract was then filtered through muslin cloth to obtain a clear solution. To synthesize the titanium dioxide nanoparticles, 50 milliliters of the prepared plant extract were combined with 50 milliliters of a precursor solution of titanium oxide. The mixture was kept on a magnetic stirrer and continuously stirred for 48 hours. As a reducing and stabilizing agent, 20 milliliters of titanium dioxide were added to 50 milliliters of distilled water for each two grams of Basella alba. This method ensures the effective synthesis of titanium dioxide nanoparticles using Basella alba extract, leveraging its natural bioactive compounds for an eco-friendly production process.

### 2.2 Agar well diffusion method

The antimicrobial activity of the green-synthesized copper oxide nanoparticles was assessed using the agar well diffusion method. Mueller Hinton agar plates were prepared and sterilized in an autoclave at 121°C for 15–20 minutes. Once sterilized, the medium was poured onto sterile Petri dishes and allowed to cool to room temperature. Bacterial suspensions (Escherchia coli, Enterococcus faecalis, Pseudomonas aeruginosa, and Staphyloccus aureus) were evenly spread over the agar plates using sterile cotton swabs. Wells, each 9 mm in diameter, were created in the agar using a sterile polystyrene tip. These wells were then filled with varying concentrations (25, 50, 100 µg/mL) of titanium dioxide nanoparticles. A standard antibiotic (Amoxyrite) was included for comparison. The plates were incubated at 37°C for 24 hours for bacterial cultures. Antimicrobial activity was determined by measuring the diameter of the inhibition zones around the wells. The zone of inhibition was measured with a ruler in millimeters (mm) and recorded.

2.3 Time-kill kinetics analysis:

A time-kill curve assay was performed to evaluate the bactericidal properties and concentration-dependent effects of titanium dioxide nanoparticles on the growth of Escherchia coli, Enterococcus faecalis, Pseudomonas aeruginosa, and Staphyloccus aureus over regular time intervals. The assay involved culturing these wound pathogens in Mueller Hinton Broth with varying concentrations of silver nanoparticles (25, 50, and 100 µg/mL) and conducting time-kill curve analysis. For comparison, an antibiotic standard Amoxyrite was used. Prior to the assay, a four-hour pre-incubation period in a medium without antimicrobial agents was conducted to ensure that all pathogens had reached a stable early-to-mid log phase. An inoculum of 0.5 McFarland standard for each pathogen was prepared in sterile phosphate-buffered saline from cultures grown on Mueller Hinton agar plates at 37°C for 18–20 hours. Subsequently, 30 µL of this inoculum was diluted in 15 mL of antimicrobial-free Mueller Hinton Broth preheated to 37°C, and 90 µL of the diluted mixture was evenly distributed across each well of a 96-well ELISA plate. To each well containing 90 µL of pre-incubated wound pathogens, 10 µL of TiO2NPs at five different concentrations, as well as an untreated control, were added.

## 3. Results and Discussion

1.1 Preparation of titanium dioxide nanoparticles


Figure 1. Preparation of titanium dioxide nanoparticles (a) Weighing of B. alba leaves, (b)

mixing of B. alba leaf with distilled water, (c) heating of B. alba, (d) Filtration of leaf extract using muslin cloth, (e) 30 millimolar of titanium oxide solution, (f) Mixing of leaf extract and titanium oxide solution, (g) Synthesis of titanium dioxide nanoparticles

3.2 Antibacterial activity:

Figures 2 and 3 demonstrate that there is no zone of inhibition against any of the wound pathogens, indicating that the B. alba-mediated titanium dioxide nanoparticles exhibit minimal antimicrobial activity in the agar well diffusion method.

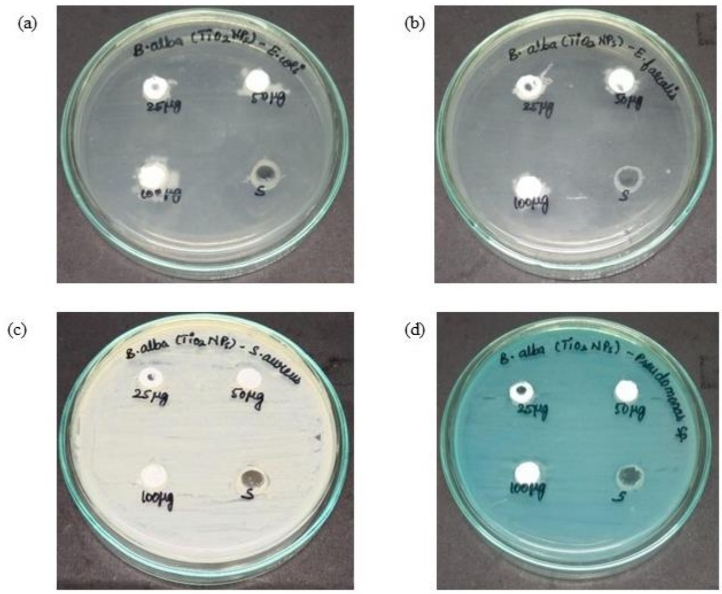


Figure 2. Antimicrobial activity of B. alba mediated titanium dioxide nanoparticles a) E. coli b) E. faecalis c) S. aureus d) Pseudomonas sp.

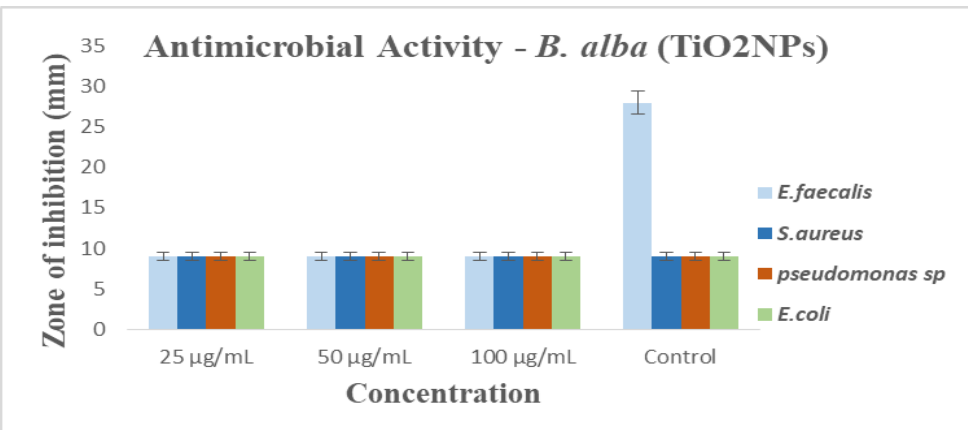


Figure 3. Graph displaying the Antimicrobial potential of B. alba mediated titanium dioxide nanoparticles

a. Time-kill kinetics analysis

The prepared TiO2 nanoparticles were responsible for a decrease in viable cells for the investigated wound pathogens, according to the time kill curve assay results. The results of time kill curve assay shows that TiO2 NPs exhibit minimal bactericidal action against the microorganisms, with all three concentrations (25, 50, 100 µg/mL) demonstrating bacteriostatic activity. Specifically, even at these concentrations, the nanoparticles only showed mild bacteriostatic activity as shown in figure 4. For S. aureus, all three concentrations and the standard exhibited mild bactericidal activity. In the case of E. coli, the 25 µg/mL concentration showed mild bacteriostatic activity, whereas higher concentrations exhibited slight bactericidal activity. For E. faecalis, the standard showed the highest bactericidal activity.

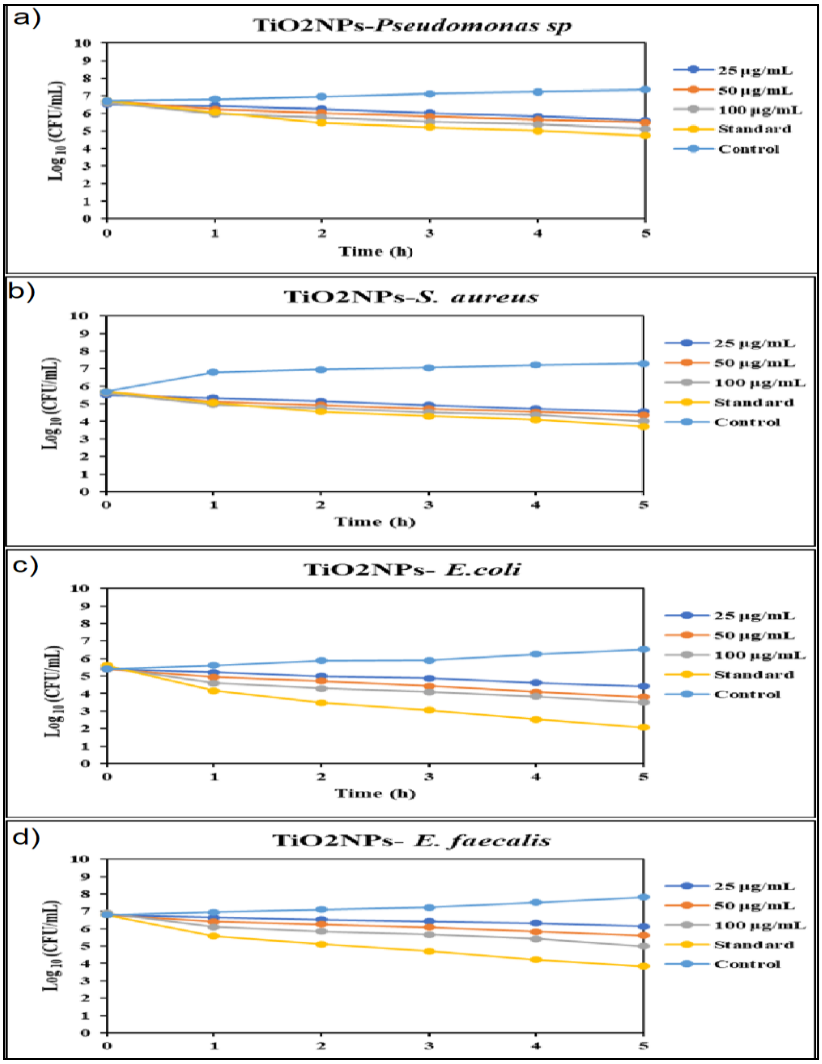


Figure 4: Time-kill curve assay of titanium dioxide against wound pathogens

### 3.3 Discussion

The increasing use of antibiotics has led to the necessity for developing new drugs due to the growing resistance of microorganisms. Green synthesis offers numerous benefits, including long-term usage and biomedical applications without the presence of toxic chemicals. The present study indicates that titanium dioxide nanoparticles mediated by Basella alba exhibit limited antibacterial/microbial activity against the tested species (Sharma et al., 2022; Irshad et al., 2021). The effectiveness of plants against microbes is often due to secondary metabolites, which are abundant in herbs and serve as the active components. The study demonstrates that Basella alba-mediated titanium dioxide nanoparticles may be less effective in treating bacterial diseases. For example, the methanolic extract of Basella alba showed very low antimicrobial activity against both gram-positive and gram-negative bacteria, with inhibition zones less than 10 mm (Munusamy & Shanmugam, 2023; Weir et al., 2012).

Several studies have confirmed the antibacterial properties of titanium dioxide nanoparticles. One such study examined the development of various microbiological cultures in the presence of these nanoparticles (Rifaath et al., 2023; Sankar et al., 2024). The findings suggest that Basella alba-mediated titanium dioxide nanoparticles show very little or no antibacterial efficacy against S. aureus, B. subtilis, and E. coli. In contrast, titanium dioxide nanoparticles combined with lemongrass and ginger exhibit potent antimicrobial activity (Yazhini et al., 2021; Sungur, 2021).

In the case of E. faecalis, the antibacterial activity of titanium dioxide nanoparticles was visible in small zones, with an inhibitory zone up to 30 mm. However, moderate antibacterial activity was demonstrated using copper nanoparticles for the same species (Ziental et al., 2020; Wang et al., 2022). The time kill curve study demonstrated bactericidal action against the organisms, with the standard exhibiting maximal bactericidal activity. Despite the antimicrobial assay showing less zone of inhibition against cariogenic pathogens using Basella alba-mediated titanium dioxide nanoparticles, detectable antibacterial activity was present in the remaining Lactobacillus species.

## 4. Conclusion

The time kill curve and agar well diffusion studies reveal that titanium dioxide nanoparticles exhibited negligible antibacterial activity against P. aeruginosa and E. coli wound infections. Furthermore, the nanoparticles demonstrated reduced cytotoxicity across both high and low concentrations.

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