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Green Synthesis of Titanium Dioxide Nanoparticles Using Eucalyptus and Piper Longum Extract and its Antibacterial Activity

Dr. Rahul Koppaka
Postgraduate student
Saveetha Dental College and Hospital

Dr. Amrutha Shenoy
Assistant Professor
Saveetha Dental College and Hospital
(Corresponding author)

Dr. Rajesh Kumar
Professor
Saveetha Dental College and Hospital

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KEYWORDS
Ti Nanoparticles, Eucalyptus, Antimicrobial activity, Oral Pathogens, Amoxicillin.

ABSTRACT

Background:

This study aimed to synthesize Titanium dioxide nanoparticles using eucalyptus and piper longum extract and evaluate their antibacterial activity against Staphylococcus aureus, Streptococcus mutans, and Enterococcus faecalis .[1]]

Materials and Methods:

Dried leaves of Eucalyptus and Piper longum were used.
These leaves were mixed in 20 ml distilled water, the solution was then brought to a boil and allowed to boil for 15 mins. This solution was then passed through a hydrophilic filter paper to extract the plant solution.[2]
For synthesis of TiO_2 Nanoparticles, the Erlenmeyer flask containing 100 mL of $TiO(OH)_2$ (0.1 mM) was stirred for 48 hrs. Initially no colour change was noticed.
20 ml of the plant extract was added to 80 ml titanium oxide solution.
The plant extract was then added to the Titanium solution and the solution was again left to stir for 24 hrs. After 24 hours a colour change was noticed, indicating the formation of nanoparticles.
The solution was then placed in a tube, and made to run through the centrifugal machine to extract the titanium oxide nanoparticles.
UV-visible spectroscopy, Fourier transform infrared (FTIR) analysis, scanning electron microscopy (SEM), and energy-dispersive electron microscopy (EDX) were used to characterise the NPs. The agar disc diffusion technique was used to test antimicrobial activity against S. aureus, S. mutans, and E. faecalis.

RESULTS

The antibacterial activity of TF-TiO_2NPs and their dependence on the selected microbial species, which were S. aureus, S. faecalis, E. coli, P. vulgaris, E. faecalis, P. aeruginosa, Y. enterocolitica, B. subtilis, and the fungus C. albicans.
Plant extract-mediated TF-TiO_2NPs have antimicrobial action against S. aureus (10 mm), E. faecalis (2 mm), S. mutans (2 mm). Zones of inhibition (ZOI) of 2-15 mm were measured using TF-TiO_2NPs.
The maximum inhibition zone of S.mutans (18 mm) was found in 1.5% TiO_2NP. The standard antibiotic chloramphenicol/nystatin was used as a positive control.

CONCLUSION

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Synthesized titanium dioxide nanoparticles exhibited broad spectrum antimicrobial activity against a vast range of pathogens. In the current scenario, keeping in view the problem of multi drug resistance in bacteria, one of the most promising and novel antimicrobial agents could be the nanoparticles.

INTRODUCTION

The production of nanoparticles with various chemical compositions, dimensions, and regulated monodispersity is an important topic of nanotechnology study. Nanotechnology is emerging as a rapidly growing discipline with applications in science and technology for the purpose of producing new nanoscale materials.[3] In the domains of biology and pharmacology, nanotechnology has a wide range of applications.[4] Nanomaterials are part of a commercial revolution that has spawned hundreds of new products as a result of their various physico-chemical properties, which allow them to be used in a wide range of novel applications. To prevent the use of hazardous organic solvents and harsh reaction conditions (temperature, pressure, and long refluxing time)[4,5].

Researchers have recently started looking into the possibility of creating nanomaterials in an aqueous medium with the use of stabilizing or capping agents for nanomaterial synthesis.[4–6] Because of its optical characteristics, high chemical stability, and nontoxicity, titanium dioxide (TiO2) has been widely used as an environmentally friendly and clean photocatalyst in recent years. Titanium dioxide nanoparticles (TiO2 NPs) are one of the most important elements in cosmetics, pharmaceuticals, and skin care products, especially for UV protection, whiteness, and opacity in paints, plastics, papers, inks, food colorants, and toothpaste.[7]

The increased microbial resistance to metal ions, antibiotics, and the creation of resistant strains has piqued researchers' curiosity, and TiO2 NPs have shown strong antibacterial activity. When exposed to ultraviolet radiation, TiO2 produces reactive oxygen species, according to Miller et al. Nanoparticulate TiO2 used in antibacterial coatings and wastewater treatment has been studied as an anti-cancer agent. In comparison to pristine TiO2 NPs, biocidal polymer-functionalized TiO2 NPs showed better bacterial growth suppression against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus). [8]

Small Ag cluster size and the unique structure of TiO2 Nanoparticles supporting highly disseminated are the origins of enhanced bactericidal activity of the room temperature ionic liquids generated.

Since there is no need to maintain an aseptic environment, the biosynthetic technique utilizing plant extracts has attracted more attention in recent years than chemical and physical methods, and even the employment of microbes, for nano-scale metal synthesis. As a result, new approaches are required to identify and develop the next generation of antibacterial medicines or agents.[9]

MATERIALS AND METHODS

<u>Materials</u>:

Preparation of plant extract:

Dried leaves of Eucalyptus and Piper longum were used. These leaves were mixed in 20 ml distilled water, the solution was then brought to a boil and allowed to boil for 15 mins. This solution was then passed through a hydrophilic filter paper to extract the plant solution.

Fig1. Piperlongum leaves

Fig2. Eucalyptus leaves and powder

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-Synthesis of Ti Naonoparticles:

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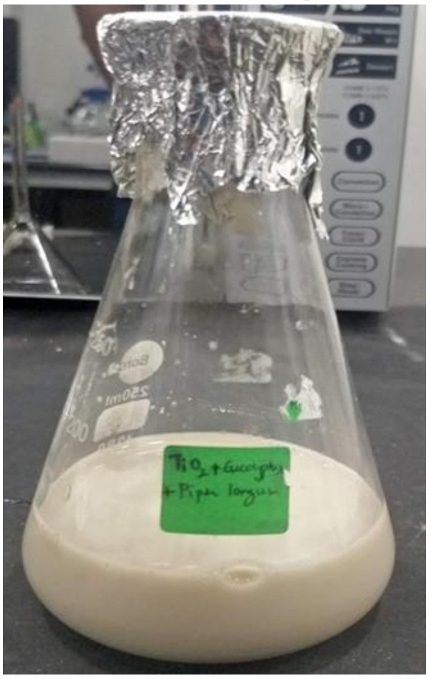
The plant extract was then added to the Titanium solution and the solution was again left to stir for 24 hrs. After 24 hours a colour change was noticed, indicating the formation of nanoparticles.

The solution was then placed in a tube, and made to run through the centrifugal machine to extract the titanium oxide nanoparticles.

-Characterisation of Titanium Oxide nanoparticles:

The characterization of synthesized green mediated nanoparticles was analyzed by different techniques such as, the surface plasmon resonance (SPR) band for nano Titanium was identified by ultraviolet- visible (UV-Vis) spectroscopic analysis.

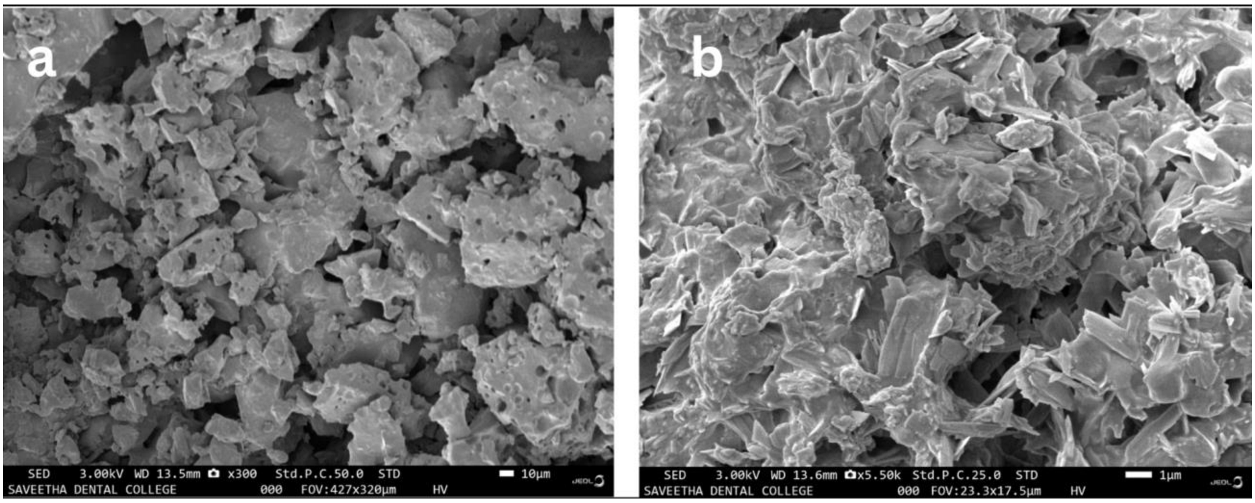
Shape and size were analyzed using



RESULTS

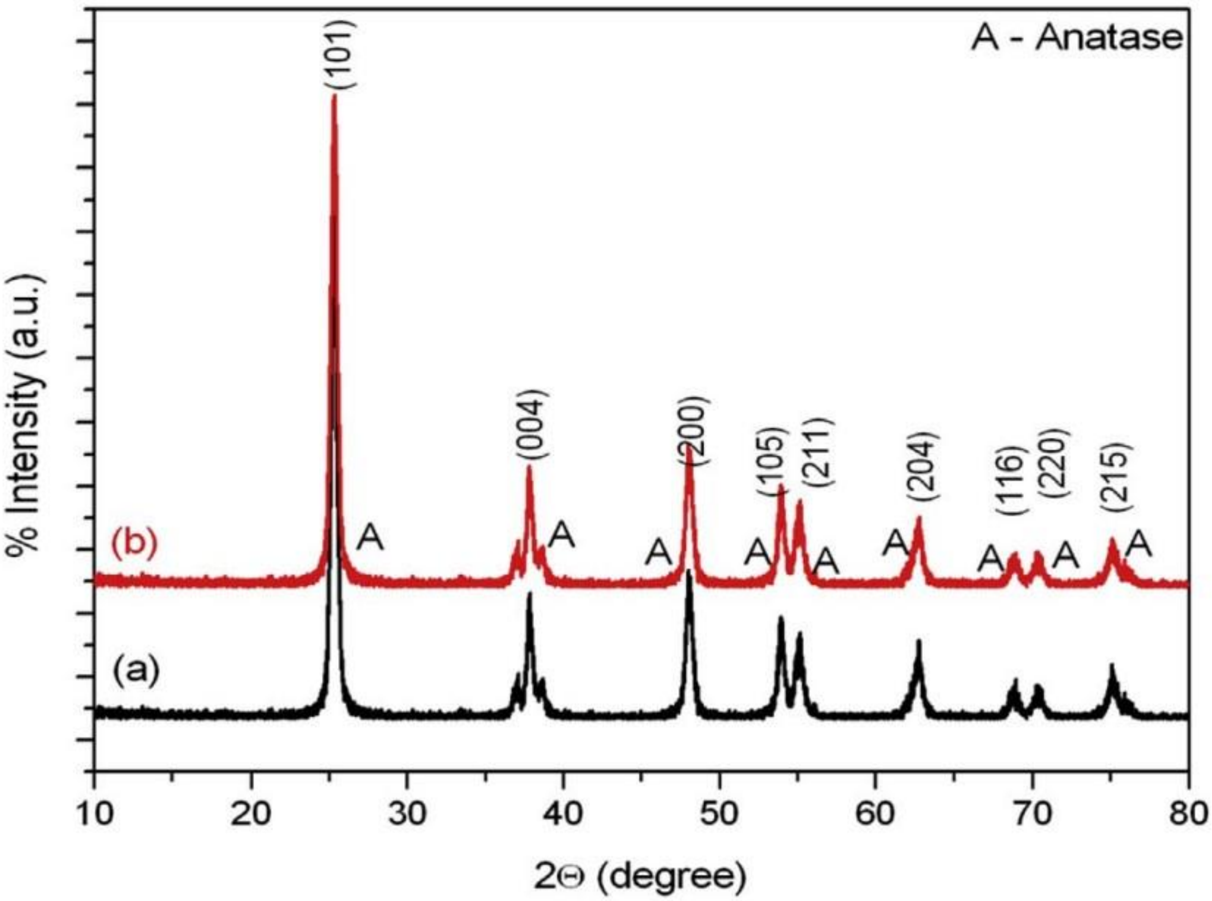
Scanning electron microscope (SEM)-

High Resolution Scanning Electron Microscopy (HR-SEM) was used to characterise the TiO2NPs and TF-TiO2NPs that were produced. The sample was air distributed across carbon tape before being coated or sputtered with gold and mounted for analysis. The Hitachi S-3400N SEM equipment was then used to perform energy dispersive X-ray spectroscopy with a thermal energy dispersive x-ray spectroscopy attachment.



X-ray diffraction method (XRD)-

The X-Ray Diffraction (XRD) method is used to determine the phase formation and crystallinity of TiO2NPs. The PW 1148/89-based X-ray diffractometer was used for the study, which used nickel filtered Cu radiation (k = 1.54056) at 298 K. The instrument was outfitted with a graphite monochromator and then operated at 30 mA and 40 kV. The diffractogram was obtained in the 10-80° range of 2. The raw data was processed with Origin 8 software and compared to the standard JCPDS database attachment.



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The Fourier transform infrared spectroscopy (FT-IR)-used to analyze phytochemicals responsible for nanoparticle synthesis.FTIR measurements were taken on Bruker vertex 70. Powdered and dried TiO2NPs were pelleted with Potassium bromide (KBr) (1:3 ratio). The spectra were chronicled in the wavenumber range of 400 cm−1 - 4000 cm−1 and analysed by deducting the pure KBr spectrum.

Elemental composition and chemical states of synthesized Titanium nanoparticles were characterized by X-ray photoelectron spectroscopy (XPS). Transmission electron microscopy (TEM) techniques utilized for the visualization of structural characteristics of green nano Titanium nanoparticles and topographical structure of the synthesized nano Titanium analyzed by using atomic force microscopy (AFM).

AntiMicrobial Test-

The usual disc diffusion technique was used to assess the antimicrobial activity of the TF-TiO2NPs. Overnight grown bacterial suspensions of Staphylococcus aureus, Enterococcus faecalis, Candida albicans and Streptococcus mutans were daubed on separate NA plates. Individually, Whatman filter paper (No. 1) discs of 6 mm diameter were infused with 20 L of a 10 mg/mL solution of TF-TiO2NPs in dimethylsulfoxide (DMSO). The discs were vaporised before being imprinted on the plates. Positive controls for fungus and bacteria were Nystatin and Chloramphenicol (30 g/mL, respectively). As a negative control, DMSO was used.Triplicates were maintained, and bacterial cultures were kept at 37 °C for 24 hours, whereas fungal cultures were cultured at 25 °C. The diameter zones of inhibition were measured using a ruler, and the mean value for each organism was recorded and expressed in millimetres.

TiO2				
Organism	25 ul	50ul	100 ul	AB
S. aureus	10	11	11	15
S. mutans	9	9	9	18
E. faecalis	9	9	9	12
Zone of Inhibition (in mm)				

The antibacterial activity of TF-TiO2NPs and their dependence on the selected microbial species, which were S. aureus, S. faecalis, E. coli, P. vulgaris, E. faecalis, P. aeruginosa, Y. enterocolitica, B. subtilis, and the fungus C. albicans.

Plant extract-mediated TF-TiO2NPs have antimicrobial action against S. aureus (10 mm), E. faecalis (2 mm), S. mutans (2 mm), and C.albicans (2 mm). Zones of inhibition (ZOI) of 2-15 mm were measured using TF-TiO2NPs.

The maximum inhibition zone of S.mutans (18 mm) was found in 1.5% TiO2NP. The standard antibiotic chloramphenicol/nystatin was used as a positive control.

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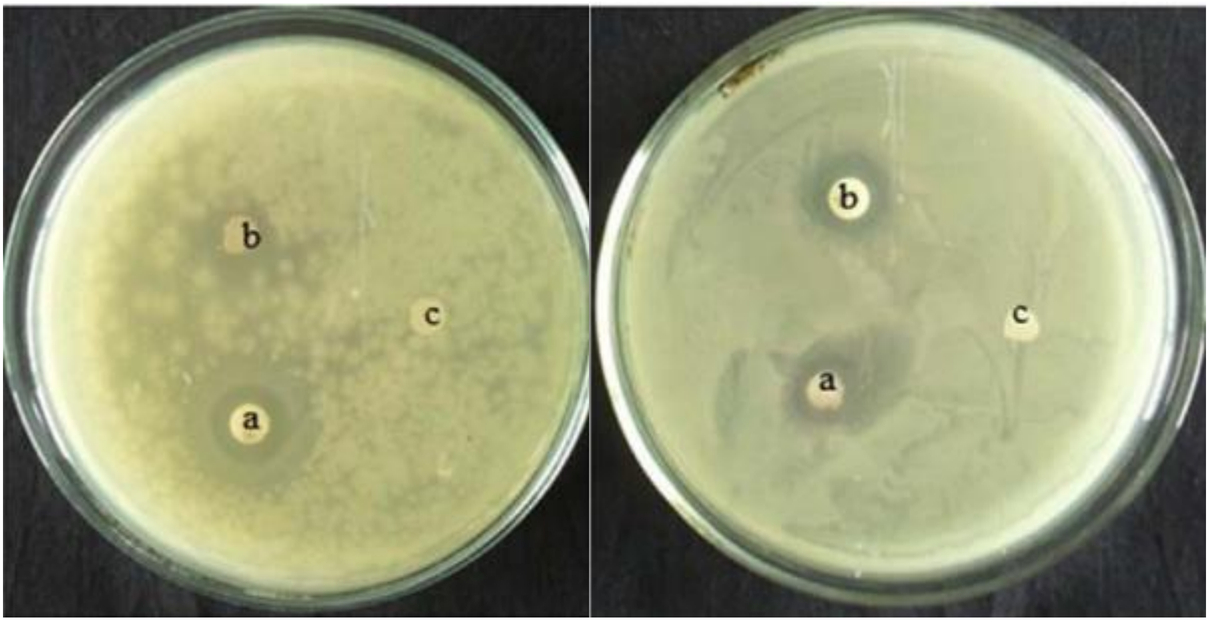
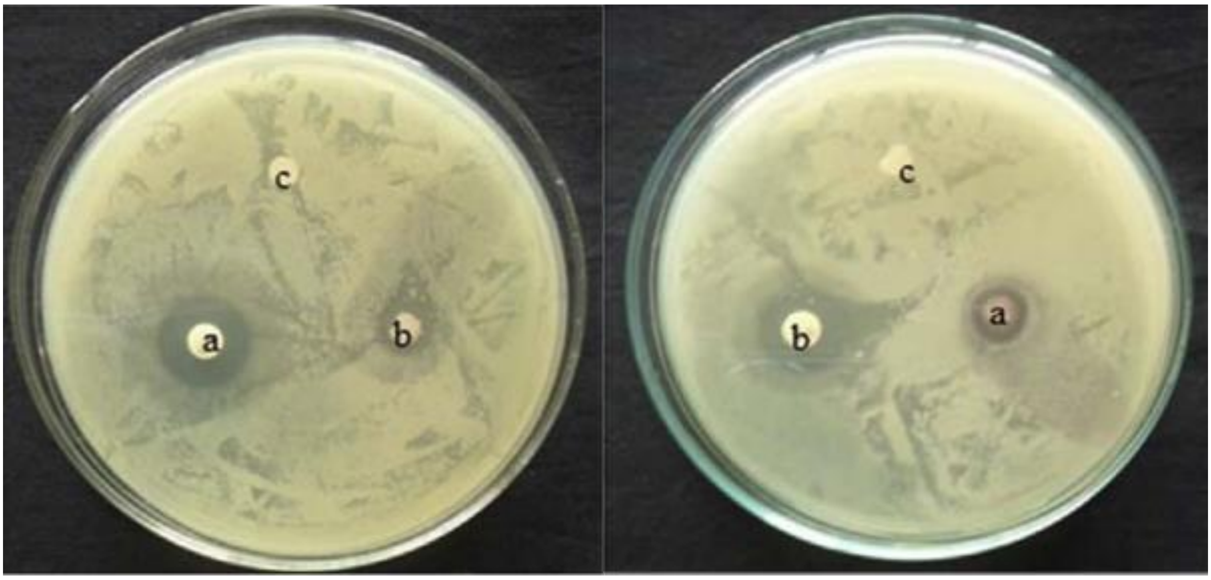


S.aureus E.faaecalis



C.albicans S.mutans

Antimicrobial activity of TiO_2 nanoparticles against different microorganisms depicting zones of inhibition of (a) Positive control (b) TiO_2 nanoparticles and (c) Dimethyl sulfoxide control.

It was observed that the Titanium nanoparticles synthesised and charaterised showed some enhanced antimicrobial activity against specific organisms.

Positive effect was seen most against S.aureus followed by E.faecialis

The viability of S. aureus, MRSA, and E. faecialis was dramatically decreased following treatment with TiO_2/anatase/GER and TiO_2/rutile/GER, according to both live and dead tests. Dead cells (stained in red) and living cells (stained in green) were scarcely visible at doses of 0.25 mg/ml and 0.5 mg/ml, showing that the therapy was bactericidal against all strains, including MRSA. Control sample images revealed an increase in living cells (stained green) and a reduction in dead cells (stained red).

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DISCUSSION

The purpose of this study was to evaluate the antibacterial activity of synthesised TF-TiO_2NPs and its reliance on the chosen microbial species, namely S. aureus, S. faecalis, E. coli, P. vulgaris, E. faecalis, P. aeruginosa, Y. enterocolitica, B. subtilis, and fungus C. albicans. Antimicrobial activity of plant extract-mediated TF-TiO_2NPs against S. aureus (10 mm), E. faecalis (2 mm), S. mutans (2 mm), and C.albicans (2 mm). With TF-TiO_2NPs, zones of inhibition (ZOI) of 2-15 mm were measured. In 1.5% TiO_2NP, the highest inhibition zone of S.mutans (18 mm) was detected.[10] As a positive control, the conventional antibiotic chloramphenicol/nystatin was utilized. [11]

Because of the presence of hydroxyl groups, titanium nanoparticles are capable of disintegrating the outer membranes of bacteria, resulting in the organisms' death . Gram-positive bacteria were more resistant to the antibacterial impact than Gram-negative bacteria.[10,12] The presence of Gram-negative bacteria's thin cell wall, which contains several layers of lipopolysaccharide and a few layers of peptidoglycan, was linked to their low antibacterial activity. Gram-positive bacteria, on the other hand, have a comparatively thick cell wall made up of many layers of peptidoglycan. There is no accurate description of TF-TiO_2NPs' antibacterial mechanism. TiO_2 has been utilised in the treatment of burn injuries for over a century.[13]

The present study's findings demonstrated significant antibacterial properties. TiO_2 nanoparticles synthesised from Eucalyptus and Piper longum leaf extracts can be utilised to treat human illnesses caused by the microorganisms employed in this study. Titanium dioxide nanoparticles were shown to be an effective antibacterial medicine in this study, which might pave the way for the development of novel antimicrobial treatments.[14]

CONCLUSION

Green synthesis of titanium dioxide nanoparticles was achieved because of the

presence of terpenoids, flavonoids and proteins in Eucalyptus and Piper longum as these bioactive compounds were responsible for the synthesis of these nanoparticles. Synthesis using a green approach is a simple, inexpensive and eco-friendly process which reduces the use of toxic chemicals. Synthesized titanium dioxide nanoparticles exhibited broad spectrum antimicrobial activity against a vast range of pathogens. In the current scenario, keeping in view the problem of multi drug resistance in bacteria, one of the most promising and novel antimicrobial agents could be the nanoparticles.

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