

Annealing Temperature Influences the Cytocompatibility, Bactericidal and Bioactive Properties of Green Synthesised TiO₂ Nanocomposites from *Calotropis Gigantea*

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Research Article

Keywords: natase-TiO₂-NPs, *Calotropis gigantea*, bactericidal agent, annealing temperature influence, bioactive green elements, fibroblast cells lines model, cytocompatible

Posted Date: December 6th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1134597/v1>

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Version of Record: A version of this preprint was published at Chemical Papers on May 16th, 2022. See the published version at <https://doi.org/10.1007/s11696-022-02230-z>.

Abstract

Annealing is a crucial functional parameter relevant to the green synthesis and bactericidal properties of TiO₂ nanocomposites (TiO₂-NPs). In this work, the effect of the annealing temperature on the physicochemical and bactericidal properties of TiO₂-NPs obtained from *Calotropis gigantea* was comprehensively studied. The synthesised TiO₂-NPs were characterised using scanning electron microscopy, transmission electron microscopy, X-ray diffraction, energy-dispersive X-ray spectroscopy, UV–Vis spectrophotometer and FTIR spectroscopy. The bactericidal properties were determined via the minimum inhibitory concentration (MIC) and Kirby–Bauer disc diffusion methods. The cytocompatibility of green TiO₂ was further investigated using fibroblast cells lines model. Results indicated that amorphous-phase TiO₂-NPs were transformed into the anatase phase at 500 °C with a crystallite size of 40.9 nm and MIC of 100 mg/mL towards *S. aureus* (colony count reduction from 4.3 log₁₀ to 1.01 log₁₀). Whereas TiO₂-NPs annealed at 400 °C demonstrated no bacterial reduction, TiO₂-NPs annealed at 500 °C showed a moderate zone of inhibition of 6.33–6.83 mm towards *Escherichia coli* and *Pseudomonas aeruginosa*. Findings from this study found that TiO-500C nanocomposites concentration at 100 mg/mL is cytocompatible to the fibroblast cells lines with proliferation rate/activity higher than 116% after 24 h treatment. The plant-mediated nano-sized cubic and spherical anatase TiO₂-NPs encapsulated bioactive green elements, such as carbon, sodium, magnesium, chlorine, potassium, calcium and sulphur, from the *C. gigantea* extract, ultimately leading to versatile and eco-friendly bactericidal agents with wound-healing properties. Further studies are necessary to support the findings of this work.

1. Introduction

Recent progress in research on natural products has resulted in the development of many natural plant-mediated nanoparticles (NPs) with bactericidal properties; amongst these materials, green synthesised titanium dioxide NPs (TiO₂-NPs) have sparked great interest on account of their potential use in wound healing therapy. *Calotropis gigantea* is a traditional medicinal plant with antimicrobial properties that is often used to treat skin diseases [1] and open wounds [2]. Aqueous solutions of *C. gigantea* leaf extract function as excellent reducing and capping agents in the formation of green TiO₂-NPs. Indeed, given their promising bactericidal properties for addressing skin and wound infections due to pathogens, plant-mediated TiO₂-NPs are amongst the most extensively studied bactericidal agents in the biomedical field (Table 1). TiO₂-NPs exist in three phases, namely, anatase, rutile and brookite, under different processing conditions [56].

Table 1
Cytocompatibility, physicochemical and bactericidal properties of green synthesised TiO₂-NPs.

Precursor	Reducing agent	Calcination temperature (°C)	Size (nm)	Shape	Antimicrobial activity	Killing mechanism	Toxicity	Application
Titanium tetra chloride	Orange peel	500	Crytallite: 17.30	Porous angular	ZOI: <i>E. coli</i> : 9 mm, <i>P. aeruginosa</i> : 14 mm and <i>S. aureus</i> : 12 mm	Nanoparticle's attachment and generation of ROS	Nil	Antibacterial, anticancer and humidity sens
TiO(OH) ₂	<i>Euphorbia prostrata</i>	Nil	Grain: 83.22	Circular and irregular	<i>Leishmania</i> parasites	ROS production	Nil	Antileishmani agents
Titanium isopropoxide	Aloe vera	500	Grain: 60 ± 80	Irregular	Nil	Nil	Nil	Semiconductor material
TiO ₂ solution	Lemon-Fruit	Nil	Grain: 20 ± 200	Near-spheroid and irregular	<i>Dickeya dadantii</i> : 60% reduction at 50 µg/mL	Generation of oxidative stress	Nil	Phytopathogene agent
TiCl ₄	<i>Cicer arietinum</i> L.	500	Crytallite: 14	Spherical	Nil	Nil	Nil	Lithium Ion Battery
TiO ₂ solution	<i>Vigna unguiculata</i>	Nil	Nil	Oval	ZOI: <i>E. coli</i> : 2 cm, <i>Salmonella</i> : 4 cm, <i>Enterobacter</i> : 4 cm, <i>P. aeruginosa</i> : 3.5 cm and <i>S. marcescens</i> : 4 cm at 75 µl	Nil	Nil	Anticancer treatments
TiO ₂ solution	<i>Vigna radiata</i> legumes	Nil	Nil	Oval	ZOI: <i>S. aureus</i> : 2.8 cm, <i>E. coli</i> : 3.3 cm, <i>Enterobacter sp.</i> : 3.2 cm, <i>S. marcescens</i> : 3.6 cm, <i>Salmonella sp.</i> : 2.8 cm, <i>P. aeruginosa</i> : 3.2 cm, <i>K. pneumoniae</i> : 3 cm, <i>P. mirabilis</i> : 3.4 cm and <i>Shigella sp.</i> : 3.6 cm 100 µl	Hydroxyl groups	Nil	Antibacterial and anticancer
TiO ₂ solution	<i>Azadirachta indica</i>	60	15 ± 50	Spherical	ZOI: <i>E. coli</i> : 27.67 mm, <i>B. subtilis</i> : 24 mm, <i>S. typhi</i> : 21.63mm, <i>S. aureus</i> : 27 mm and <i>K. pneumoniae</i> : 25.33 mm at 200 µg/ml; MIC: <i>E. coli</i> : 10.42 µg/ml, <i>B. subtilis</i> : 25 µg/ml, <i>S. typhi</i> : 10.42 µg/ml, <i>S. aureus</i> : 20.83 µg/ml and <i>K. pneumoniae</i> : 16.66 µg/ml; MBC: <i>E. coli</i> : 166.67 µg/ml, <i>B. subtilis</i> : 166.67 µg/ml, <i>S. typhi</i> : 100 µg/ml, <i>S. aureus</i> : 133.3 µg/ml and <i>K. pneumoniae</i> : 83.33 µg/ml	Nil	Nil	Antimicrobial agents
TiO ₂ solution	<i>Cinnamomum tamala</i>	Nil	23	Irregular	Nil	Nil	Nil	Anticancer drug treatment
Titanium oxysulphate	Polyvinyl Pyrrolidone	300 ± 500	Grain: 10–20	Irregular	Nil	Nil	Nil	Photocatalytic
TiO ₂ solution	<i>Cinnamon</i>	500	Grain: 70–150	Spherical	Nil	Nil	Nil	Solar cells

Precursor	Reducing agent	Calcination temperature (°C)	Size (nm)	Shape	Antimicrobial activity	Killing mechanism	Toxicity	Application
TiO ₂ solution	<i>Solanum trilobatum</i> L.	Nil	Grain: 70	Spherical and oval	Effective against <i>P. h. capitis</i> , <i>H. a. anatolicum</i> , and <i>A. subpictus</i>	Nil	Nil	Antiparasitic
TiO(OH) ₂	<i>Mangifera indica</i>	Nil	Grain: 30	SEM: Spherical and oval; TEM: round	Effective against <i>R. microplus</i> , <i>Hyalomma anatolicum anatolicum</i> , <i>Haemaphysalis bispinosa</i> , <i>A. subpictus</i> , and <i>Culex quinquefasciatus</i>	Nil	Nil	Antiparasitic
TiO(OH) ₂	<i>Glycosmis cochinchinensis</i>	Nil	Grain: 40	Spherical	ZOI: <i>S. saprophyticus</i> : 21 mm, <i>B. subtilis</i> : 19 mm, <i>E. coli</i> : 23 mm and <i>P. aeruginosa</i> : 25 mm at 100 µL	Nil	Nil	Photocatalytic and antimicrobial
Titanium (IV)-isopropoxide	<i>Carica papaya</i>	400	Grain: 15.6	Cages	Nil	Nil	Nil	Photocatalytic
Titanium (IV) isopropoxide	<i>Ocimum basilicum</i> L.	500	Grain: 50	Hexagonal	Nil	Nil	Nil	Nil
Titanium tetra isopropoxide	<i>Pomegranate Peels</i>	90	Grain: 75–90	Nil	Nil	Nil	Nil	Pharmaceutic
Titanium Chloride	<i>Aloe Vera</i>	500	Grain: 32	Irregular	Nil	Nil	Nil	Nil
Titanium oxy-sulfate	<i>Acacia catechu</i>	700	Grain: 17.90	Spherical and hexagonal-shaped	Effective against <i>S. aureus</i>	Nil	Nil	Photocatalytic and antimicrobial
Titanium oxysulfate	<i>Gum Kondagogu</i>	500 – 900	Grain: 8–13	Spherical	Nil	Nil	Nil	Photocatalytic
Titanyl hydroxide	<i>Euphorbia heteradena</i> Jaub	Nil	Grain: 20	Spherical	Nil	Nil	Nil	Biomedical and pharmaceutical
TiO ₂ solution	<i>Sesbania grandiflora</i>	Nil	Grain: 43–56	Triangular, square and spherical	Nil	Nil	Lethal to zebrafish at dose less than 2.5 mg/L	Drug
TiO(OH) ₂	<i>Artemisia haussknechtii</i>	Nil	Grain: 92.58 ± 56.98	Spheres	ZOI: Lack of growth for <i>S. epidermidis</i> and <i>S. marcescens</i> and no inhibition for <i>E. coli</i> and <i>S. aureus</i>	Nil	Nil	Antibacterial
TiO(OH) ₂	<i>Protoparmeliopsis muralis</i> Lichen	Nil	Grain: 133.32 ± 35.33	Spherical	ZOI: <i>S. aureus</i> : 10.00 mm, <i>E. coli</i> : 11.66 mm and <i>P. aeruginosa</i> : 9.66 mm; MIC: 80 µg/mL; MBC: 100 µg/mL	Nil	Nil	Antibacterial
Titanium tetraisopropoxide	<i>Trianthema portulacastrum</i>	450	Crytallite: 6–8	Different	Effective against wheat Rust (<i>Ustilago tritici</i>)	Nil	Nil	Antifungal
Titanium tetraisopropoxide	<i>Chopodium quinoa</i>	450	Crytallite: 6–8	Round	Effective against wheat Rust (<i>Ustilago tritici</i>)	Nil	Nil	Antifungal
Ti plate	<i>H. rosa-sinensis</i>	550	Nil	Nil	Nil	Nil	Nil	Wastewater treatment

Precursor	Reducing agent	Calcination temperature (°C)	Size (nm)	Shape	Antimicrobial activity	Killing mechanism	Toxicity	Application
Titanium isopropoxide (IV)	<i>Pristine pomegranate peel</i>	200 – 1100	Grain: 1–5 µm	Randomly oriented	ZOI: <i>S. aureus</i> : 22 mm, <i>E. coli</i> : 19 mm and <i>P. aeruginosa</i> : 17 mm at 2 wt.%; MIC: <i>S. aureus</i> : 189.1 µg/mL, <i>E. coli</i> : 304.7 µg/mL and <i>P. aeruginosa</i> : 309.2 µg/mL at 90 µg/mL; MBC: <i>S. aureus</i> : 200 µg/mL, <i>E. coli</i> : <310 µg/mL and <i>P. aeruginosa</i> : <315 µg/mL	Production of reactive oxygen species	Nil	Water disinfection
Titanium-isopropoxide	<i>Syzygium cumini</i>	570	Grain: 11	Spherical and irregular	Nil	Nil	Nil	Wastewater treatment
Titanium (IV) oxide	<i>Withania somnifera</i>	120	Grain: 50–90	Spherical and square	MIC: <i>E. coli</i> : 32 µg/mL, <i>P. aeruginosa</i> : 32 µg/mL, MRSA: 32 µg/mL, <i>L. monocytogenes</i> : 60 µg/mL, <i>S. marcescens</i> : 8 µg/mL, and <i>C. albicans</i> : 60 µg/mL; MBC: <i>E. coli</i> : 32 µg/mL, <i>P. aeruginosa</i> : 60 µg/mL, MRSA: 60 µg/mL, <i>L. monocytogenes</i> : 128 µg/mL, <i>S. marcescens</i> : 16 µg/mL, and <i>C. albicans</i> : 128 µg/mL	Reactive oxygen species production	Nil	Anti-infective agent
Titanium isopropoxide	<i>Sonchus asper</i>	500	Grain: 9–22	Spherical	ZOI: <i>E. coli</i> : 12 mm, <i>S. aureus</i> : 12 mm and <i>K. pneumoniae</i> : 10 mm	ROS generation	Nil	Biomedical
TiO ₂ solution	<i>Allium eriophyllum</i> Boiss	Nil	Grain: 22	Spherical	ZOI disc diffusion: <i>C. guilliermondii</i> : 39.6 mg/mL, <i>C. krusei</i> : 40.6 mg/mL, <i>C. albicans</i> : 36.2 mg/mL, <i>C. glabrata</i> : 36.4 mg/mL, <i>P. aeruginosa</i> : 35 mg/mL, <i>S. typhimurium</i> : 35 mg/mL, <i>E. coli</i> : 36.6 mg/mL, <i>S. aureus</i> : 36.8 mg/mL, <i>S. pneumoniae</i> : 38.8 mg/mL and <i>B. subtilis</i> : 39.8 mg/mL; MIC for Gram (+) and Gram (-): 2-4 mg/mL; MBC for Gram (+) and Gram (-): 4 mg/mL	ROS generation	No cytotoxicity against human umbilical vein endothelial cells	Industrial and remedial

Precursor	Reducing agent	Calcination temperature (°C)	Size (nm)	Shape	Antimicrobial activity	Killing mechanism	Toxicity	Application
TiO ₂ solution	Fruit's peel agro-waste: plum, kiwi and peach	400–500	Grain: plum: 47.1–63.2, kiwi: 54.1–85.1 and peach: 200	Cylindrical	ZOI: ≥2 and ≤20 mm for <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> and <i>B. subtilis</i> at 12.5–100 µg/mL	HO and O ₂ radicals	Nil	Biomedical
TiO(OH) ₂	<i>Cola nitida</i>	Nil	Grain: 25.00–191.41	Near spherical	49.2–73.4% growth inhibition of <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>K. pneumoniae</i> at 80 µg/mL using broth method	Ti ⁴⁺ , generation of reactive oxygen species and hydroxyl radicals	Nil	Catalytic and biomedical
Titanium Oxysulfate	<i>Hibiscus-rosa-sinensis</i> L.	100	Nil	Spherical	ZOI: <i>V. cholerae</i> : 17 mm, <i>P. aeruginosa</i> : 14 mm, <i>S. aureus</i> : 14.5 mm at 20 µg/mL	Nanoparticle's penetration	Nil	Biomedical
Titanium tetra isopropoxide	<i>Terminalia Catappa</i>	450	Crytallite: 10–21	Spherical	Nil	Nil	Nil	Nil
Titanium tetra isopropoxide	<i>Carissa Carandas</i>	450	Crytallite: 10–21	Spherical	Nil	Nil	Nil	Nil
TiO ₂ solution	<i>Echinacea purpurea</i> Herba	Nil	Grain: 120	Spherical clusters	Nil	Nil	Nil	Nil
Titanium isopropoxide	<i>Azadirachta indica</i>	Nil	Grain: 124	Interconnected spherical	Nil	Nil	Nil	Photocatalytic
Titanium isopropoxide	<i>Origanum vulgare</i>	Nil	Grain: 341	Spherical	Nil	Nil	Nil	Therapeutic agent
TiO(OH) ₄ solution	<i>A. altissima</i>	Nil	Grain: 60–100	Spherical	Nil	Nil	Nil	Photocatalytic agent
Titanium (IV) isopropoxide	<i>Phyllanthus niruri</i>	350	Grain: 30–50	Spherical	Nil	Nil	Nil	Photocatalytic material
Titanium oxy sulphate	<i>Trigonella foenum-graecum</i>	700	Grain: 20–90	Spherical	ZOI: <i>S. aureus</i> , <i>E. faecalis</i> , <i>K. pneumoniae</i> , <i>S. faecalis</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>P. vulgaris</i> , <i>B. subtilis</i> , <i>Y. enterocolitica</i> and <i>C. albicans</i> : 8.5–11.6 mm at 10 mg/mL	Reactive Oxygen Species (ROS), mainly hydroxyl radicals (-OH)	Nil	Biomedical
Titanium tetraisopropoxide	Soluble starch	500	Grain: 20–70	Irregular spherical	Nil	Nil	Nil	Photocatalytic
Titanium tetrachloride	<i>M. citrifolia</i>	400	Grain: 15–19	Quasi-spherical	ZOI: <i>S. aureus</i> : 13 mm, <i>E. coli</i> : 10 mm, <i>B. subtilis</i> : 12 mm, <i>P. aeruginosa</i> : 9 mm, <i>C. albicans</i> : 13 mm and <i>A. niger</i> : 6 mm at 150 µg/mL	Ti ⁴⁺ , generation of reactive oxygen species	Nil	Biomedical
Titanium (IV) isopropoxide	<i>Fomes fomentarius</i>	Nil	Grain: 100–120	Irregular	ZOI: <i>E. coli</i> : 15 mm and <i>S. aureus</i> : 11 mm at 100 µg/mL	Nil	Nil	Antibacterial and anticancer
Titanium (IV) isopropoxide	<i>Fomitopsis pinicola</i>	Nil	Nil	Irregular	MIC/MBC: 62.5/125 µg/mL for <i>E. coli</i> and <i>S. aureus</i>	Nil	Nil	Biomedical

Precursor	Reducing agent	Calcination temperature (°C)	Size (nm)	Shape	Antimicrobial activity	Killing mechanism	Toxicity	Application
Titanium chloride	<i>Jatropha curcas</i> L.	450	Grain: 10–20	Spherical	Nil	Nil	Nil	Wastewater
Titanium tetraisopropoxide	<i>Piper betel</i>	400	Grain: 6.6	Round	Nil	Nil	Nil	Photocatalytic
Titanium tetraisopropoxide	<i>Ocimum tenuiflorum</i>	400	Grain: 7.0	Round	Nil	Nil	Nil	Photocatalytic
Titanium tetraisopropoxide	<i>Moringa oleifera</i>	400	Grain: 6.6	Round	Nil	Nil	Nil	Photocatalytic
Titanium tetraisopropoxide	<i>Coriandrum sativum</i>	400	Grain: 6.8	Round	Nil	Nil	Nil	Photocatalytic
TiO(OH) ₂	<i>Psidium guajava</i>	Nil	Grain: 32.58	Spherical	ZOI: <i>S. aureus</i> : 25 mm, <i>E. coli</i> : 23 mm, <i>A. hydrophila</i> : 17 mm, <i>P. mirabilis</i> : 20 mm and <i>P. aeruginosa</i> : 19 mm at 20 µg/mL	ROS generation	Nil	Biomedical
TiO ₂ solution	<i>Moringa oleifera</i>	Nil	Grain: 100	Spherical	Nil	Nil	Nil	Skin infection treatments
Titanium tetraisopropoxide	<i>Moringa Oleifera</i>	500	Crytallite: 12.22	Nil	Nil	Nil	Nil	Nil
Titanium tetraisopropoxide	<i>Mentha arvensis</i>	500	Grain: 20–70	Spherical	ZOI: <i>E. coli</i> : no response, <i>P. vulgaris</i> : 15 mm, <i>S. aureus</i> : no response, <i>A. cuboid</i> : no response, <i>A. fumigatus</i> : no response and <i>A. niger</i> : 15 mm at 30 mg/mL	ROS generation	Nil	Antibacterial & antifungal
Titanium tetraisopropoxide	<i>Acanthophyllum Laxiusculum</i>	400	Grain: 20–25	Nanospheres	Nil	Nil	Nil	Nil
TiO ₂ solution	<i>Cassia fistula</i>	Nil	Nil	Spherical	Effectie against <i>S. aureus</i> and <i>E. coli</i>	Nil	Nil	Nil

Scientists worldwide have sought to establish methods to control the size and morphology of TiO₂-NPs because particle size exerts a massive influence on their bactericidal properties [57]. Even slight variations in the annealing conditions could lead to prominent effects on the phase and morphology of TiO₂-NPs [56]. The present work discusses the formation of bioactive elements of *C. gigantea* leaf extract and the morphology of the resultant TiO₂-NPs under the effect of different annealing temperatures (i.e., 400 and 500°C). The physicochemical properties of the TiO₂-NPs were characterised using scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDAX), UV–Vis spectrophotometry and Fourier transform infrared (FTIR) spectroscopy. Additionally, bactericidal and cytocompatibility properties of green TiO₂-NPs were further investigated.

2. Materials And Methods

2.1. Green synthesis and characterisation of bioactive TiO₂-NPs

2.1.1. Green synthesis

TiO₂-NPs was synthesised via green route by the reaction of *C. gigantea* leaves extract aqueous solution with Titanium (IV) isopropoxide (Sigma-Aldrich). This grounded green synthesis method uses the previous work protocol [58, 59]. Green TiO₂-NPs samples calcined at different low temperatures 400°C and 500°C are designated as TiO₂-400C and TiO₂-500C, respectively.

2.1.2. X-ray diffraction

X-ray diffractometer (XRD; Bruker D8) was used to study crystalline nature and size of green TiO₂-NPs. The X-ray diffraction peaks were captured via Cu K α radiation with wavelength of $\lambda = 0.1541$ nm and step scan mode with step size of 0.030° in the range of 10° to 90°. The equipment was operated at a voltage of 40 kV and current of 30 mA. Scherrer's equation was applied to determine the average crystallite size of green TiO₂ which is as under

$$d = \frac{K\lambda}{\beta \cos\theta'} \quad \text{Equation 1}$$

where d represents the crystallite size, $K = 0.9$ is the shape factor, λ stands for the X-ray wavelength of Cu K α radiation (1.541 Å), θ is used as a Bragg diffraction angle, and β symbolize the full-width at half-maximum (FWHM) of the respective diffraction peak.

2.1.3. SEM observation

The morphology of TiO₂-NPs was further investigated using scanning electron microscopy (SEM Fei Quanta FEG 650). Samples were placed on a stub with carbon tape and coated with Pt for 1-min prior to imaging and elemental analysis. The TiO₂-NPs and bioderived elements composition and their percentage from green TiO₂-NPs was further confirmed with EDAX.

2.1.4. TEM analysis

TEM (FEI TECHNAI F20 G2) is used for analyzing shape and grain size of TiO₂-NPs. At first, TiO₂-NPs was dispersed in absolute ethanol and then sonicated for 30 min. After that, a single drop of TiO₂-NPs solution was added onto a lacey carbon film-coated copper grid (300 mesh) and then dried at room temperature for 30 min. At last, it was kept in desiccator prior to TEM imaging.

2.1.5 FTIR study

The functional groups of green TiO₂-NPs were recorded by FTIR spectroscopy (Perkin Elmer) within the range of 4000-400 cm⁻¹ through the KBr pellet method. The FTIR samples were prepared by dispersing small dosage of TiO₂-NPs uniformly in a KBr matrix which was then compressed to thin transparent disc.

2.1.6 UV-Visible spectroscopy

The absorption spectrum of TiO₂-NPs was determined using UV-Vis spectrophotometer (Varian) in wavelength range between 200-700 nm.

2.2. Antibacterial tests

Kirby-Bauer disc diffusion test and minimum inhibitory concentration (MIC) were determined after 24 h of contact with the prepared green samples according to following methods [58, 59]. Four overnights cultured ATCC bacterial suspensions of *P. aeruginosa* 27853, *K. pneumoniae* 700603, *E. coli* 25922 and *S. aureus* 29213 were employed to study antibacterial effect of TiO₂-NPs. 30 µg of Cefoxitin discs used as a positive control. 10% (v/v) DMSO act as negative control in this study.

2.3. Cytocompatibility assay

The cytocompatibility assay was performed on fibroblast cells lines model, L929 obtained from American Type Culture Collection (ATCC, USA) and is maintained in RPMI-1640 media (Gibco, Life technologies) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) PenStrep (Gibco, USA), sodium bicarbonate, 12.5 g/mL HEPES and 1% (v/v) L-glutamine at 37°C in a 5% CO₂ humidified atmosphere. 10% (v/v) of dimethyl sulfoxide (DMSO) was adapted as strong cytotoxic material (negative control) while fibroblast cells lines without administration of TiO₂ as blank control. The *in vitro* cytocompatibility of green-synthesised TiO₂ on fibroblast cells lines was performed by direct contact method according to the protocol recommendations in ISO 10993-5 (2009) [60, 61].

The cell viability percentage was determined using (1:10) alamarBlue™ Cell viability reagent DAL1025 (Invitrogen, United Kingdom). At first, cells were subcultured, trypsinized and seeded at a density of 1×10^4 cells/well (100 µl/well) on 96 well plate and grown for 24 h in CO₂ incubator at 37°C. After that, different concentrated TiO₂ (0, 25, 50 and 100 mg/mL) were added in the culture media and kept overnight. Then, the serially diluted test sample solution ($n=3$) was incubated with monolayer cells at 37°C for 24 h. The proportion of live and healthy cells after treatment were further estimated quantitatively through color change from blue to pink using Alamar blue assay where the treated cells were incubated as a minimum 20 h before measuring the absorbance at wavelength 570 nm and 600 nm using a microplate reader (Bio-Tek Instruments, USA). The cytocompatibility of test sample was compared with the toxic material (10% (v/v) DMSO) and blank control. The live and dead cells were examined microscopically via Olympus CKX41 optical light microscope at magnification of 10 X and 20 X.

2.4 Statistical analysis

The different calcination temperature treated group of samples were analysed using analysis of two-way ANOVA implemented in the GraphPad Prism software package. Results were considered statistically significant if p value is less than 0.05. Data are presented as mean $\pm$ standard deviation (SD).

3. Results And Discussions

3.1. Physicochemical characterisation of the TiO₂-NPs

The SEM images in Figure 1a and 1b show that the TiO₂-400C and TiO₂-500C NPs are spherical in shape with a nano-sized and agglomerated morphology (Figure 1a and 1b, insets). The EDAX images of the TiO₂-NPs confirm the presence of Ti and O, which make up approximately 29.28wt.% and 36.72wt.%, respectively, of TiO₂-400C and 33.55wt.% and 41.91wt.%, respectively, of TiO₂-500C (Table 2, Figure 1c and 1d). The TEM images in Figure 1e and 1f reveal that the NPs produced at a low calcination temperature are nearly spherical with a large, agglomerated morphology whilst those calcined at a high temperature are spherical and cuboidal in shape with a partly agglomerated morphology.

EDAX analysis successfully identified an abundance of bioactive elements in *C. gigantea*, such as carbon (C), calcium, chlorine, sodium, magnesium, potassium and sulphur, in agreement with a previous research [58, 59] (Table 2). These elements are by-products of the green synthesis of the NPs. The combination of these bioderived elements with high concentrations of C and antimicrobial species, such as hydroxyl radicals, hydrogen peroxide, superoxide anions and titanium (IV), may arrest the growth and development of skin pathogens [62].

Table 2

SEM-EDAX chemical weight composition (%) results for different calcined TiO₂ nanocomposites.

Sample	C	O	Na	Mg	Cl	K	S	Ca	Ti
TiO ₂ -400C	27.13	36.72	0.42	0.26	0.47	4.84	–	0.89	29.28
TiO ₂ -500C	9.41	41.91	0.51	0.47	3.87	9.56	0.71	–	33.55

The XRD patterns of TiO₂-400C and TiO₂-500C (TiO₂ ICDD No. 01-086-1157) are illustrated in Figure 2a. The XRD spectrum of TiO₂-500C showed characteristic peaks at 25.36°, 37.99°, 48.04°, 54.19°, 55.00°, 62.80°, 68.59°, 70.27°, 74.95° and 82.12°, which respectively correspond to the (101), (004), (200), (105), (211), (204), (116), (220), (215) and (224) crystal planes of anatase-phase TiO₂-NPs [63]. The XRD pattern of TiO₂-400C exhibited a single broad peak, which confirms its amorphous character [56].

The green TiO₂-500C NPs had a tetragonal crystalline structure. The crystallite size of green TiO₂-NPs obtained at a high calcination temperature was calculated from the strongest XRD peak at 25.36° using Scherrer's equation and found to be 40.9 nm. The XRD patterns of the TiO₂-NPs confirm the presence of pure TiO₂ along with *C. gigantea* bioderived elements, in agreement with the SEM-EDAX analyses (Figure 1c and 1d).

The functional groups responsible for the formation of green TiO₂-NPs were determined by FTIR analysis. The FTIR spectrum of the green nanocomposites shown in Figure 2b reveals a broad peak at 3438 cm⁻¹, which could be assigned to the O–H stretching vibrations of flavonoids from *C. gigantea* [58, 59]. The peak at 1633 cm⁻¹ could be attributed to C=C (carbonyl group), and the peaks at 1361 and 1119 cm⁻¹ could respectively be attributed to the O–C–O stretching vibrations of esters and the C–O stretching vibrations of bio-derived elements from the *C. gigantea* leaf extract [58, 59]. The peak at 595 cm⁻¹ confirms the presence of TiO₂-NPs with Ti–O–Ti framework bonds [63].

UV-Vis spectrophotometry was applied to measure the bandgap of the green synthesised TiO₂-NPs, and the results obtained are shown in Figure 2c. The absorbance peak at 228 nm is due to the dispersion of natural C in deionised water [58, 59], and the small peak at 327 nm confirms the formation of green synthesised TiO₂-NPs [64]. The UV-Vis spectrum of the control *C. gigantea* leaf extract showed two prominent absorbance peaks; the sharp peak at 206 nm corresponds to the carbonyl group, whilst the low broad peak at 269 nm represents phenolic compounds [58, 59].

3.2. Assessment of minimum inhibitory concentration

A preliminary minimum inhibitory concentration (MIC) study performed in this research focused on *Staphylococcus aureus* because staph skin infections are most commonly observed in open wounds. The MIC of TiO₂-500C for *S. aureus* was 50.0 mg/mL. TiO₂-500C exhibited strong bactericidal activity at a high concentration of 100 mg/mL (Figure 3e). However, the results for TiO₂-400C showed no bacterial reduction despite these NPs having a low MIC of approximately 25.0 mg/mL. A sharp decrease in colony count from 4.3 log₁₀ (control *S. aureus*) to 1.01 log₁₀ was observed for TiO₂-500C at a concentration of 100 mg/mL (Figure 3). Here, * indicates statistically significant differences (****p ≤ 0.0001) between the different calcination groups for each measurement. There is significant different between control strain group and anatase TiO₂-500C group at concentration of 100 mg/mL.

The stronger bactericidal effect of TiO₂-500C compared with that of TiO₂-400C may be attributed to the anatase crystalline structure of the former, as confirmed by the XRD pattern shown in Figure 2a [65]. Inhibition of bacterial colonies was observed only at a high dosage of the NPs. Thus, green TiO₂-500C nanocomposites may be considered an excellent bactericidal agent against *S. aureus*, a gram-positive bacterium [66]. Table 3 and Figure 3a–e illustrates the MICs of the green TiO₂-NPs for *S. aureus*.

Table 3
MIC of green TiO₂-NPs against *S. aureus*.

Samples	MIC (mg/mL)
TiO ₂ -400C	25
TiO ₂ -500C	50

3.3. Determination of zone of inhibition

The bactericidal activity of the TiO₂-NPs was further evaluated against *S. aureus*, the gram-negative bacterium *Escherichia coli* and the antibiotic-resistant bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* via the Kirby–Bauer disc diffusion method. In this study, TiO₂-500C showed a fairly large zone of inhibition (ZOI) for *E. coli* and *P. aeruginosa* (Table 4 and Figure 4). By comparison, commercial P25 and TiO₂-400C showed poorer ability to interfere with

the cell wall of multi-drug resistant (MDR) and non-MDR strains and disrupt their biochemical processes at a concentration of 100 mg/mL. Cefoxitin antimicrobial discs showed a large ZOI against all four bacterial strains. Subhapiya et al. found that green TiO₂-NPs synthesised from *Trigonella foenum-graecum* extract have a ZOI of approximately 11.2 mm against *S. aureus* [43].

Previous studies demonstrated enhancements in the photocatalytic properties of C-decorated TiO₂-NPs for water purification [63, 67, 68, 69] and solid rocket propellants [70]. None of these works, however, have highlighted the bactericidal activity of natural C-encapsulated TiO₂-NPs. The present study is the first to report that TiO₂-500C nanocomposites could encapsulate the bioactive elements of *C. gigantea* leaf extracts and possess strong inhibitory effects against the tested organisms. This plant-mediated anatase TiO₂-based bactericidal agent may be a promising eco-friendly and non-hazardous biomaterial for future pharmaceutical applications.

Table 4
ZOI (mm) of green TiO₂-NPs against skin pathogens. "NA" be a sign of no antimicrobial activity observed for this sample.

Strain	TiO ₂ -400C	TiO ₂ -500C	Commercial P25	Negative control	Positive control
<i>S. aureus</i>	6±0.00	6±0.00	6±0.00	NA	10±0.00
<i>E. coli</i>	6±0.00	6.83±0.29	6±0.00	NA	10±0.00
<i>K. pneumoniae</i>	6±0.00	6±0.00	6±0.00	NA	9±0.00
<i>P. aeruginosa</i>	6±0.00	6.33±0.14	6±0.00	NA	9±0.00

3.4. Cytocompatibility profiles

The cytocompatibility of TiO₂-400C and TiO₂-500C with different concentration (0, 25, 50 and 100 mg/mL) were investigated by fibroblast cells lines model as described in Figure 5 with the blank control group and 10% (v/v) DMSO. Blank control group set as 100% viability. Based on ISO 10993-5 (2009) recommendation it can be concluded that all different-concentrated green TiO₂ nanocomposites is considered as cytocompatible since the cell viability is higher than 70%. Comparatively, DMSO exhibited significant cytotoxicity to the tested cell lines with cell viability percentage of approximately 31%. It strongly indicates statistically significant differences (****p ≤ 0.0001) between different-concentrated green TiO₂ group and DMSO treated group. In addition, more healthy live cells with elongated filopodia (the leg like of the cell) between 95% and 129% were seen in all TiO₂-400C (Figure S1b, c, d, f, g and h) and TiO₂-500C (Figure S1j, k, l, n, o and p) treated nanocomposites, respectively. The outcome showed green TiO₂ had ability in promoting proliferation and viability of fibroblast cells lines.

However, TiO₂-500C caught much more attention in this study due to its strong bactericidal activity against Gram-positive *S. aureus* (Figure 3e) and high proliferation of fibroblast cells lines at concentration level of 100 mg/mL (Figure 5). The sedimentation of agglomerated green TiO₂ on bottom plate was picturized through yellow circle (Figure S1). Previously, Chellappa et al have investigated the effect of TiO₂ nanoparticles concentration related to cytotoxicity and cytocompatibility against MG63 cell lines and discovered that rapid cell proliferation and enhanced viability were seen at different concentrations without any adverse toxicity [61]. Although, proliferation and survival of fibroblast cells lines slightly decreased when the concentration of green TiO₂ is further increased. It is ascribed to the higher level of ROS generation at high concentration level of TiO₂ [66]. Future works on ROS release quantification and long-term cytocompatibility properties of green TiO₂ on fibroblast cells lines model is needed for further understanding of the molecular level.

4. Conclusions

Nano-sized cubic and spherical anatase TiO₂-500C decorated bioactive green elements nanocomposites with cytocompatible behavior was successfully synthesised from *C. gigantea* leaf extract solution. Differences in annealing temperature exerted remarkable impacts on the crystalline phases, morphology, cytocompatibility and bactericidal activities of the green TiO₂-NPs towards *S. aureus*, *E. coli* and *P. aeruginosa*. Green TiO₂-NPs was found to be cytocompatible on fibroblast cells lines with increased cell viability (≥116%). Thus, the TiO₂-NPs developed in this work can address current limitations related to pathogen-induced open wound skin infections and wound healing characteristic. However, further investigation is needed to determine the detailed bactericidal mechanism of bioderived anatase TiO₂-NPs.

Abbreviations

C. gigantea: *Calotropis gigantea*, TiO₂-NPs: TiO₂ nanocomposites, C: carbon, Na: sodium, Mg: magnesium, Cl: chlorine, K: potassium, Ca: calcium, S: sulfur, MIC: minimum inhibitory concentration, XRD: X-ray diffraction, MDR: multi-drug resistant, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *S. aureus*: *Staphylococcus aureus* and *P. aeruginosa*: *Pseudomonas aeruginosa*

Declarations

Acknowledgements

The authors would like to thank Universiti Sains Malaysia for sponsoring this work under Research University Grant (RUI) EKSESAIS TAHUN 2019 (1001/CIPPT/8012338). The support of all the technical staff of Advanced Medical and Dental Institute and School of Materials and Mineral Resources

Engineering, Universiti Sains Malaysia, Pulau Pinang, Malaysia, in the characterization of the sample is also acknowledged.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to the patent application for methods of making and using of titanium dioxide nanocomposites formed by green synthesis but are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

Funding

This research was funded by the Research University Grant (RUI) EKSESAIS TAHUN 2019 (1001/CIPPT/8012338) from Universiti Sains Malaysia.

Authors' contributions

G Ambarasan Govindasamy mainly contributes in writing original draft and carried out the green sample preparation, sample characterization and the antibacterial assays, included bacterial preparation, MIC, Kirby-Bauer disc diffusion method and cell viability assay. Nor Hazliana Harun, Wan Nuramiera Faznie Wan Eddis Effendy, and Srimala Sreekantan assist in the procedures. Rabiatal Basria S. M. N. Mydin is the principal investigator contributes in the concept idea, experimental design, writing process and gave final approval of this paper for publication. All authors have given approval to the final version of the manuscript.

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References

1. G. Kumar, L. Karthik, K.V.B. Rao, ANTIBACTERIAL ACTIVITY OF AQUEOUS, EXTRACT OF *Calotropis gigantea* LEAVES – AN IN VITRO STUDY. International Journal of Pharmaceutical Sciences Review and Research. **4**(2), 141 (2010)
2. K. Sangeetha, P.F. Steffi, B.T. Selvi, S. Priyadarshni, Phytochemical Evaluation, GC-MS Analysis of Phytoactive Compounds and Antibacterial Activity Studies from *Calotropis gigantea*. J. Pharm. Sci. & Res. **12**(6), 789–794 (2020)
3. A.M. Amanulla, R. Sundaram Green synthesis of TiO₂ nanoparticles using orange peel extract for antibacterial, cytotoxicity and humidity sensor applications. Materials Today: Proceedings. 2019; 8: 323-331
4. A.A. Zahir, I.S. Chauhan, A. Bagavan, C. Kamaraj, G. Elango, J. Shankar, N. Arjaria, S.M. Roopan, A.A. Rahuman, N. Singh, Green synthesis of silver and titanium dioxide nanoparticles using *Euphorbia prostrata* extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in *Leishmania donovani*. Antimicrob. Agents Chemother. **59**, 4782–4799 (2015)
5. A. Khadar, D.K. Behara, M.K. Kumar, Synthesis and Characterization of Controlled Size TiO₂ Nanoparticles via Green Route using *Aloe vera* Extract. International Journal of Science and Research (IJSR). **5**(11), 2319–2064 (2016)
6. A. Hossain, Y. Abdallah, M.A. Ali, M.M.I. Masum, B. Li, G. Sun, Y. Meng, Y. Wang, Q. An, Lemon-Fruit-Based Green Synthesis of Zinc Oxide Nanoparticles and Titanium Dioxide Nanoparticles against Soft Rot Bacterial Pathogen *Dickeya dadantii*. Biomolecules **9**, 863 (2019)
7. A.A. Kashale, K.P. Gattu, K. Ghule, V.H. Ingole, S. Dhanayat, R. Sharma, J.-Y. Chang, A.V. Ghule, Biomediated green synthesis of TiO₂ nanoparticles for lithium ion battery application. Composites Part B: Engineering **99**, 297–304 (2016)
8. A. Chatterjee, M. Ajantha, A. Talekar, N. Revathy, J. Abraham, Biosynthesis, Antimicrobial and Cytotoxic Effects of Titanium Dioxide Nanoparticles Using *Vigna unguiculata* Seeds. International Journal of Pharmacognosy and Phytochemical Research **9**(1), 95–99 (2017)
9. A. CHATTERJEE, D. NISHANTHINI, SANDHIYA, N. and J. ABRAHAM, BIOSYNTHESIS OF TITANIUM DIOXIDE NANOPARTICLES USING *VIGNA RADIATA*. Asian. J Pharm Clin Res. **9**(4), 85–88 (2016)
10. B.K. Thakur, A. Kumar, D. Kumar, Green synthesis of titanium dioxide nanoparticles using *Azadirachta indica* leaf extract and evaluation of their antibacterial activity. South African Journal of Botany **124**, 223–227 (2019)

11. F. He, W. Yu, X. Fan, B. Jin, *In vitro* cytotoxicity of biosynthesized titanium dioxide nanoparticles in human prostate cancer cell lines. *Tropical Journal of Pharmaceutical Research* **16**(12), 2793–2799 (2017)
12. G.V. Khade, M.B. Suwarnkar, N.L. Gavade, K.M. Garadkar, Green synthesis of TiO₂ and its photocatalytic activity. *J. Mater. Sci.: Mater. Electron.* **26**(5), 3309–3315 (2015)
13. G. Nabi, W. Raza, M.B. Tahir, Green Synthesis of TiO₂ Nanoparticle Using *Cinnamon* Powder Extract and the Study of Optical Properties. *J. Inorg. Organomet. Polym Mater.* **30**, 1425–1429 (2019)
14. G. Rajakumar, A.A. Rahuman, C. Jayaseelan, T. Santhoshkumar, S. Marimuthu, C. Kamaraj, A. Bagavan, A.A. Zahir, A.V. Kirthi, G. Elango, P. Arora, R. Karthikeyan, S. Manikandan, Jose, S. *Solanum trilobatum* extract-mediated synthesis of titanium dioxide nanoparticles to control *Pediculus humanus capitis*, *Hyalomma anatolicum anatolicum* and *Anopheles subpictus*. *Parasitol Res.* 2013; 113: 469-479
15. G. Rajakumar, A.A. Rahuman, S.M. Roopan, I. Chung, K. Anbarasan, V. Karthikeyan, Efficacy of larvicidal activity of green synthesized titanium dioxide nanoparticles using *Mangifera indica* extract against blood-feeding parasites. *Parasitol. Res.* **114**(2), 571 (2015)
16. H. Rosi, S. Kalyanasundaram, Synthesis, characterization, structural and optical properties of titanium-dioxide nanoparticles using *Glycosmis cochinchinensis* Leaf extract and its photocatalytic evaluation and antimicrobial properties. *World News of Natural Sciences* **17**, 1–15 (2018)
17. H. Kaur, S. Kaur, J. Singh, M. Rawat, S. Kumar, Expanding horizon: green synthesis of TiO₂ nanoparticles using *Carica papaya* leaves for photocatalysis application. *Mater. Res. Express.* **6**, 095034 (2019)
18. H.A. Salam, R. Sivaraj, *Ocimum basilicum* L. var. *purpurascens* Benth. -LAMIACEAE Mediated Green Synthesis and Characterization of Titanium Dioxide Nanoparticles. *Adv. Biores.* **5**(3), 10–16 (2014)
19. J. Dubey, A. Singh, Green, Synthesis of TiO₂ Nanoparticles Using Extracts of *Pomegranate* Peels for Pharmaceutical Application. *International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)* **9**(1), 85–87 (2019)
20. K.G. Rao, C.H. Ashok, K.V. Rao, C.H.S. Chakra, P. Tambur, Green Synthesis of TiO₂ Nanoparticles Using *Aloe Vera* Extract. *International Journal of Advanced Research in Physical Science (IJARPS)* **2**(1A), 28–34 (2015)
21. K. Chand, D. Cao, D.E. Fouad, A.H. Shah, N. Lakhan, M. Dayo, A.Q. Sagar, H.J. Zhu, K. and A.M. Mohamed, A. Photocatalytic and antimicrobial activity of biosynthesized silver and titanium dioxide nanoparticles: A comparative study. *J. Mol. Liq.* **316**, 113821 (2020)
22. K.S. Saranya, V.V.T. Padil, C. Senan, R. Pilankatta, K. Saranya, B. George, S. Wacławek, M. Cerník, Green Synthesis of High Temperature Stable Anatase Titanium Dioxide Nanoparticles Using *Gum Kondagogu*: Characterization and Solar Driven Photocatalytic Degradation of Organic Dye. *Nanomaterials* **8**, 1002 (2018)
23. M. Nasrollahzadeh, S.M. Sajadi, Synthesis and characterization of titanium dioxide nanoparticles using *euphorbia heteradena* Jaub root extract and evaluation of their stability. *Ceram. Int.* (2015). <http://dx.doi.org/10.1016/j.ceramint.2015.07.079>
24. M. Srinivasan, M. Venkatesan, V. Arumugam, G. Natesan, N. Saravanan, S. Murugesan, S. Ramachandran, R. Ayyasamy, A. Pugazhendhi, Green synthesis and characterization of titanium dioxide nanoparticles (TiO₂ NPs) using *Sesbania grandiflora* and evaluation of toxicity in zebrafish embryos. *Process Biochem.* **80**, 197–202 (2019)
25. M. Alavi, N. Karimi, Characterization, antibacterial, total antioxidant, scavenging, reducing power and ion chelating activities of green synthesized silver, copper and titanium dioxide nanoparticles using *Artemisia haussknechtii* leaf extract. *Artificial Cells, Nanomedicine, and Biotechnology* **46**(8), 2066–2081 (2017)
26. M. Alavi, N. Karimi, T. Valadbeigi, Antibacterial, Antibiofilm, Antiquorum Sensing, Antimotility, and Antioxidant Activities of Green Fabricated Ag, Cu, TiO₂, ZnO, and Fe₃O₄ NPs via *Protoparmeliopsis muralis* Lichen Aqueous Extract against Multi-Drug-Resistant Bacteria. *ACS Biomater. Sci. Eng.* **5**, 4228–4243 (2019)
27. M.A. Irshad, R. Nawaz, M. Zia ur Rehman, M. Imran, M.J. Ahmad, S. Ahmad, A. Inaam, A. Razzaq, M. Rizwan, S. Ali, Synthesis and characterization of titanium dioxide nanoparticles by chemical and green methods and their antifungal activities against wheat rust. *Chemosphere* **258**, 127352 (2020)
28. M.S.F.A. Zamri, N. Sapawe Performance studies of electrobiosynthesis of titanium dioxide nanoparticles (TiO₂) for phenol degradation. *Materials Today: Proceedings.* 2018; 5: 21797-21801
29. M. Abu-Dalo, A. Jaradat, B.A. Albiss, N.A.F. Al-Rawashdeh, Green synthesis of TiO₂ NPs/pristine pomegranate peel extract nanocomposite and its antimicrobial activity for water disinfection. *Journal of Environmental Chemical Engineering* **7**, 103370 (2019)
30. N.K. Sethy, Z. Arif, P.K. Mishra, P. Kumar, Green synthesis of TiO₂ nanoparticles from *Syzygium cumini* extract for photo-catalytic removal of lead (Pb) in explosive industrial wastewater. *Green Process Synth* **9**, 171–181 (2020)
31. N.A. Al-Shabib, F.M. Husain, F.A. Qais, N. Ahmad, A. Khan, A.A. Alyousef, M. Arshad, S. Noor, J.M. Khan, P. Alam, T.H. Albalawi, S.A. Shahzad, Phyto-Mediated Synthesis of Porous Titanium Dioxide Nanoparticles From *Withania somnifera* Root Extract: Broad-Spectrum Attenuation of Biofilm and Cytotoxic Properties Against HepG2 Cell Lines. *Front. Microbiol.* **11**, 1680 (2020)
32. N. Babu, V.M. Pathak, A. Singh, Navneet, *Sonchus asper* leaves aqueous extract mediated synthesis of Titanium dioxide nanoparticles. *The Pharma Innovation Journal.* **8**(4), 817–822 (2019)
33. N. Seydi, S. Saneei, A.R. Jalalvand et al., Synthesis of titanium nanoparticles using *Allium eriophyllum* Boiss aqueous extract by green synthesis method and evaluation of their remedial properties. *Appl Organometal Chem* **33**, e5191 (2019)
34. N. Ajmal, K. Saraswat, M.A. Bakht, Y. Riadi, M.J. Ahsan, M. Noushad, Cost-effective and eco-friendly synthesis of titanium dioxide (TiO₂) nanoparticles using fruit's peel agro-waste extracts: characterization, *invitro* antibacterial, antioxidant activities. *Green Chem. Lett. Rev.* **12**(3), 244–254 (2019)

35. P.O. Akinola, A. Lateef, T.B. Asafa, L.S. Beukes, A.S. Hakeem, H.M. Irshad, Multifunctional titanium dioxide nanoparticles biofabricated via phytosynthetic route using extracts of *Cola nitida*: antimicrobial, dye degradation, antioxidant and anticoagulant activities. *Heliyon*. **6**, e04610 (2020)
36. P.S.M. Kumar, A.P. Francis, T. Devasena, Comparative Studies on Green Synthesized and Chemically Synthesized Titanium Oxide Nanoparticles. A Validation for Green Synthesis Protocol using *Hibiscus* Flower. *J. Environ. Nanotechnol.* **3**(4), 78–85 (2014)
37. R. Rajendhiran, V. Deivasigamani, J. Palanisamy, S. Masan, S. Pitchaiya *Terminalia catappa* and *carissa carandas* assisted synthesis of TiO₂ nanoparticles – A green synthesis approach. *Materials Today: Proceedings*. 2020; 45(2): 2232-2238
38. R. Dobrucka, Synthesis of Titanium Dioxide Nanoparticles Using *Echinacea purpurea* Herba. *Iranian Journal of Pharmaceutical Research* **16**(2), 756–762 (2017)
39. R. Sankar, K. Rizwana, K.S. Shivashangari, V. Ravikumar, Ultra-rapid photocatalytic activity of *Azadirachta indica* engineered colloidal titanium dioxide nanoparticles. *Appl Nanosci* **5**, 731–736 (2015)
40. R. Sankar, R. Dhivya, K.S. Shivashangari, V. Ravikumar, Wound healing activity of *Origanum vulgare* engineered titanium dioxide nanoparticles in Wistar Albino rats. *J Mater Sci: Mater Med.* **25**(7), 1701 (2014)
41. S. Ganesan, I.G. Babu, D. Mahendran, P.I. Arulselvi, N. Elangovan, N. Geetha, P. Venkatachalam Green engineering of titanium dioxide nanoparticles using *Ageratina altissima* (L.) King & H.E. *Robines*. medicinal plant aqueous leaf extracts for enhanced photocatalytic activity. *Annals of Phytomedicine*. 2016; 5(2): 69-75
42. S. Shanavas, A. Priyadharsan, S. Karthikeyan, K. Dharmaboopathi, I. Ragavan, C. Vidya, R. Acevedo, P.M. Anbarasan Green synthesis of titanium dioxide nanoparticles using *Phyllanthus niruri* leaf extract and study on its structural, optical and morphological properties. *Materials Today: Proceedings*. 2020; 26(4): 3531-3534
43. S. Subhapiya, P. GomathiPriya, Green synthesis of titanium dioxide (TiO₂) nanoparticles by *Trigonella foenum-graecum* extract and its antimicrobial properties. *Microb. Pathog.* **116**, 215–220 (2018)
44. S.S. Muniandy, N.H.M. Kaus, Z. Jiang, M. Altarawneh, H.L. Lee, Green synthesis of mesoporous anatase TiO₂ nanoparticles and their photocatalytic activities. *RSC Adv.* **7**, 48083 (2017)
45. M. Sundrarajan, K. Bama, M. Bhavani, S. Jegatheeswaran, S. Ambika, A. Sangili, P. Nithya, R. Sumathi, Obtaining titanium dioxide nanoparticles with spherical shape and antimicrobial properties using *M. citrifolia* leaves extract by hydrothermal method. *Journal of Photochemistry & Photobiology, B: Biology* **171**, 117–124 (2017)
46. S. Rehman, R. Farooq, R. Jermy, S.M. Asiri, V. Ravinayagam, R.A. Jindan, Z. Alsalem, M.A. Shah, Z. Reshi, H. Sabit, F. Khan, A. 8A Wild *Fomes fomentarius* for Biomediation of One Pot Synthesis of Titanium Oxide and Silver Nanoparticles for Antibacterial and Anticancer Application. *Biomolecules* **10**, 622 (2020)
47. S. Rehman, R. Jermy, S.M. Asiri, M.A. Shah, R. Farooq, V. Ravinayagam, M.A. Ansari, Z. Alsalem, R.A. Jindan, Z. Reshi, F.A. Khan, Using *Fomitopsis pinicola* for bioinspired synthesis of titanium dioxide and silver nanoparticles, targeting biomedical applications. *RSC Adv* **10**, 32137 (2020)
48. S.P. Goutam, G. Saxena, V. Singh, A.K. Yadav, R.N. Bharagava, K.B. Thapaa, Green synthesis of TiO₂ nanoparticles using leaf extract of *Jatropha curcas* L. for photocatalytic degradation of tannery wastewater. *Chem. Eng. J.* **336**, 386–396 (2018)
49. T. Pushpamalani, M. Keerthana, R. Sangavi, A. Nagaraj, P. Kamaraj. Comparative analysis of green synthesis of TiO₂ nanoparticles using four different leaf extract. *Materials Today: Proceedings*. 2021; 40(1): S180-S184
50. T. Santhoshkumar, A.A. Rahuman, C. Jayaseelan, G. Rajakumar, S. Marimuthu, A.V. Kirthi, K. Velayutham, J. Thomas, J. Venkatesan, S. Kim, Green synthesis of titanium dioxide nanoparticles using *Psidium guajava* extract and its antibacterial and antioxidant properties. *Asian Pacific Journal of Tropical Medicine*. **7**(12), 968–976 (2014)
51. V. Sivaranjani, P. Philominathan, Synthesize of Titanium dioxide nanoparticles using *Moringa oleifera* leaves and evaluation of wound healing activity. *Wound Medicine*. **12**, 1–5 (2015)
52. V. Patidar, P. Jain, Green Synthesis of TiO₂ Nanoparticle Using *Moringa Oleifera* Leaf Extract. *International Research Journal of Engineering and Technology (IRJET)* **04**(3), 2395–0056 (2017)
53. W. Ahmad, K.K. Jaiswal, S. Soni, Green synthesis of titanium dioxide (TiO₂) nanoparticles by using *Menthaarvensis* leaves extract and its antimicrobial properties. *Inorganic and Nano-Metal Chemistry* **50**(10), 1032–1038 (2020)
54. Z. Madadi, T.B. Lotfabad, Aqueous Extract of *Acanthophyllum Laxiusculum* Roots as a Renewable Resource for Green Synthesis of Nano-sized Titanium Dioxide Using the Sol – gel method. *Advanced Ceramics Progress (ACERP)* **2**(1), 26–31 (2016)
55. N. Swathi, D. Sandhiya, S. Rajeshkumar, T. Lakshmi, Green synthesis of titanium dioxide nanoparticles using *cassia fistula* and its antibacterial activity. *Int. J. Res. Pharm. Sci.* **10**(2), 856–860 (2019)
56. S. Sugapriya, R. Sriram, Lakshmi, Effect of annealing on TiO₂ nanoparticles. *Optik* **124**, 4971–4975 (2013)
57. Carol López de Dicastillo, M.G. Correa, F.B. Martínez, Camilo Streitt and Maria José Galotto. Antimicrobial Effect of Titanium Dioxide Nanoparticles, Antimicrobial Resistance - A One Health Perspective, Mihai Mareş, Swee Hua Erin Lim, Kok-Song Lai and Romeo-Teodor Cristina, IntechOpen, 2020
58. G.A. Govindasamy, R.B.S.M.N. Mydin, S. Sreekantan et al., Compositions and antimicrobial properties of binary ZnO–CuO nanocomposites encapsulated calcium and carbon from *Calotropis gigantea* targeted for skin pathogens. *Sci Rep* **11**, 99 (2021)
59. G.A. Govindasamy, R.B.S.M.N. Mydin, S. Sreekantan et al., Effect of calcination temperature on physicochemical and antimicrobial properties of green synthesised ZnO/C/Ca nanocomposites using *Calotropis gigantea* leaves. *Adv. Nat. Sci: Nanosci. Nanotechnol.* **12**, 015013 (2021)

60. N.H. Harun, S.M.N. Rabiatal Basria, Mydin, Srimala Sreekantan, Khairul Arifah Saharuddin & Azman Seenii. *In vitro* bio-interaction responses and hemocompatibility of nano-based linear low-density polyethylene polymer embedded with heterogeneous TiO₂/ZnO nanocomposites for biomedical applications. *J. Biomater. Sci. Polym. Ed.* **32**(10), 1301–1311 (2021)
61. M. Chellappa, U. Anjaneyulu, G. Manivasagam, U. Vijayalakshmi, Preparation and evaluation of the cytotoxic nature of TiO₂ nanoparticles by direct contact method. *International Journal of Nanomedicine*. **10**(1), 31–41 (2015)
62. C. Cheng, D. Sun, W. Chu, Y. Tseng, H. Ho, J. Wang, P. Chung, J. Chen, P. Tsai, N. Lin, M. Yu, H. Chang, The effects of the bacterial interaction with visible-light responsive titania photocatalyst on the bactericidal performance. *J. Biomed. Sci.* **16**, 7 (2009)
63. M. Nasrollahzadeh, M. Atarod, B. Jaleh, M. Gandomirouzbahani, In situ green synthesis of Ag nanoparticles on graphene oxide/TiO₂ nanocomposite and their catalytic activity for the reduction of 4-nitrophenol, congo red and methylene blue. *Ceram. Int.* **42**, 8587–8596 (2016)
64. Novel multi-layer cross-linked TiO₂/C nanosheets and their photocatalytic properties. *New Journal of Chemistry*. **2014**; 38(4): 1647
65. G. Fu, P.S. Vary, C.-T. Lin, Anatase TiO₂ Nanocomposites for Antimicrobial Coatings. *The Journal of Physical Chemistry B*. **109**(18), 8889–8898 (2005)
66. S.S. Behera, U. Das, A. Kumar, A. Bissoyi, A. Singh, Kumar, Chitosan/TiO₂ composite membrane improves proliferation and survival of L929 fibroblast cells: Application in wound dressing and skin regeneration. *Int. J. Biol. Macromol.* **98**, 329–340 (2017)
67. M. Sharma, K. Behl, S. Nigam, M. Joshi, TiO₂-GO nanocomposite for energy and environmental applications: A green synthesis approach. *Vacuum* **156**, 434–439 (2018)
68. M.S.A.S. Shah, A.R. Park, K. Zhang, J.H. Park, P.J. Yoo, Green Synthesis of Biphasic TiO₂-Reduced Graphene Oxide Nanocomposites with Highly Enhanced Photocatalytic Activity. *ACS Appl. Mater. Interfaces*. **4**, 3893–3901 (2012)
69. R. Atchudan, T.N.J.I. Edison, S. Perumal, D. Karthikeyan, Y.R. Lee, Effective photocatalytic degradation of anthropogenic dyes using graphene oxide grafting titanium dioxide nanoparticles under UV-light irradiation. *J. Photochem. Photobiol., A* **333**, 92–104 (2017)
70. A. Dey, V. Nangare, V. More, P. Khan, M.A.S. Khanna, P.K. Sikder, A. K. and Chattopadhyay, S. Graphene Titanium dioxide nanocomposite (GTNC): One Pot Green Synthesis and Its Application in Solid Rocket Propellant. *J. Name.* **00**, 1–3 (2013)

Figures

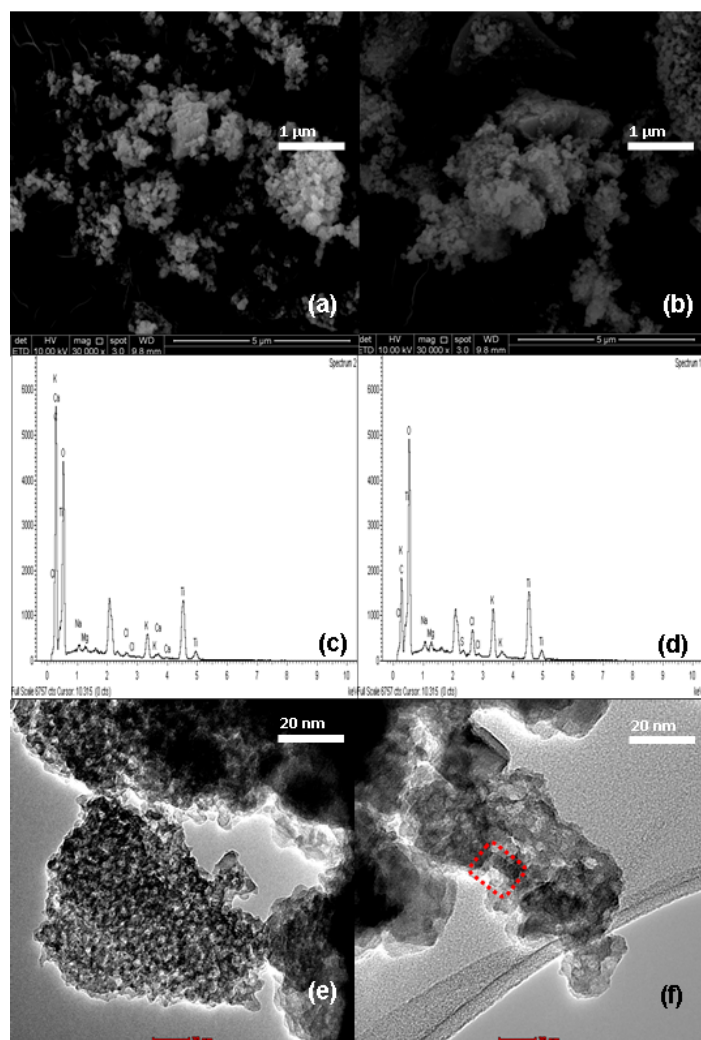


Figure 1

Morphology of bioactive green TiO2 nanocomposites; (a) SEM image of TiO2-400C (5 µm), (b) SEM image of TiO2-500C (5 µm), (c) EDAX spectra of TiO2-400C, (d) EDAX spectra of TiO2-500C, (e) TEM images of TiO2-400C (20 nm) and (f) TEM images of TiO2-500C (20 nm)

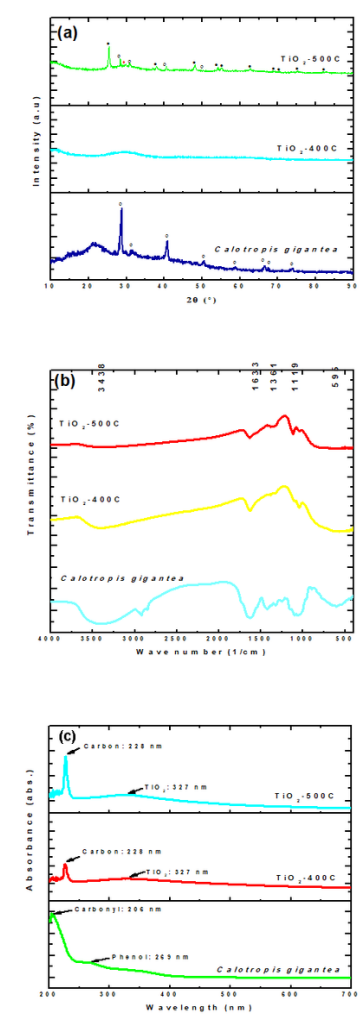


Figure 2
XRD, FTIR and UV-vis spectra (a) XRD peaks of TiO2-NPs [: C. gigantea leaves extract, : Anatase-TiO2 : Additional peaks after green synthesis], (b) FT-IR spectrum of TiO2-NPs and (c) UV-Vis spectrum of TiO2-NPs.

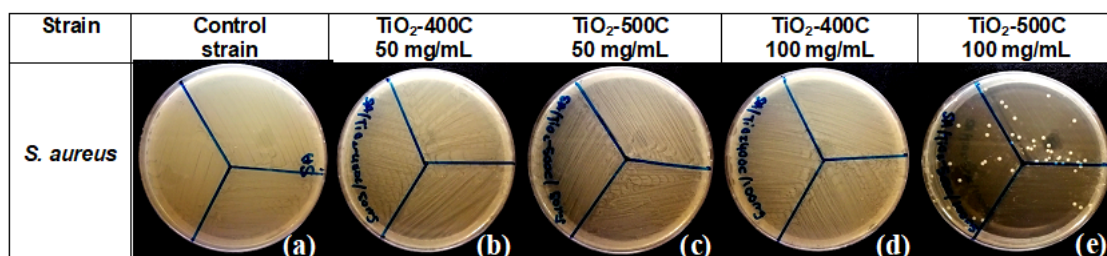
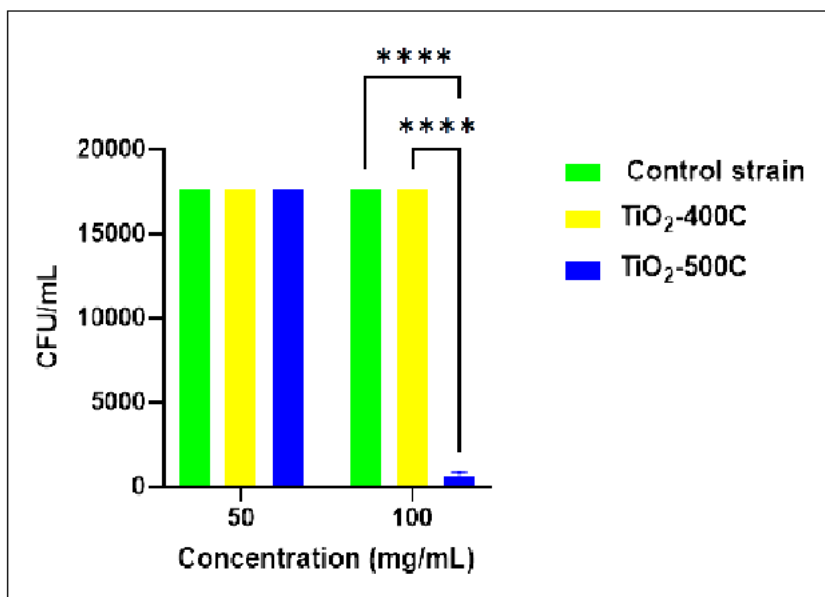


Figure 3

Number of colony-forming units per milliliter (CFU/mL) of *S. aureus* remained after treatment of different concentration of different calcined TiO₂-NPs; (a) Control strain, (b) TiO₂-400C at 50 mg/mL, (c) TiO₂-500C at 50 mg/mL, (d) TiO₂-400C at 100 mg/mL and (e) TiO₂-500C at 100 mg/mL. Petri dish with more than 300 colonies cannot be counted and are designated too many to count (TMTC). Data shown are mean value $\pm$ SD (n=3, ****p-value $\leq$ 0.0001).

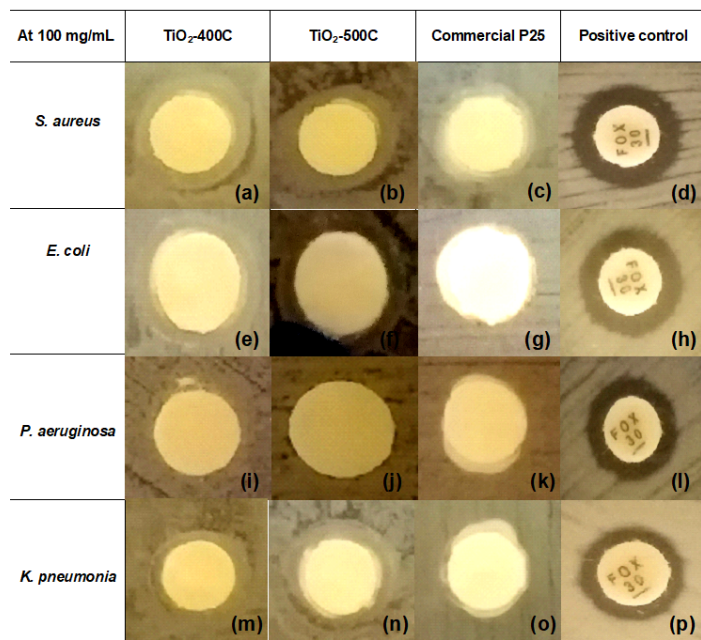
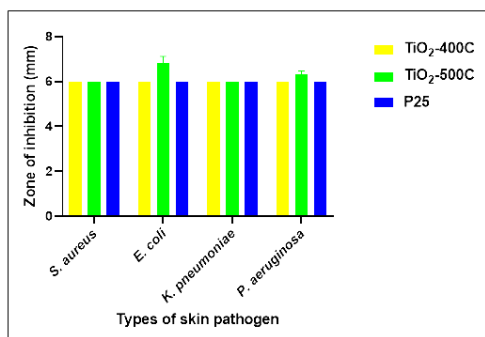


Figure 4

Zone of inhibition (mm) exhibited by 100 mg/mL of TiO₂-NPs against non-MDR and MDR skin pathogens. (a) TiO₂-400C against *S. aureus*, (b) TiO₂-500C against *S. aureus*, (c) Commercial P25 against *S. aureus*, (d) Positive control (cefoxitin-30μg) against *S. aureus*, (e) TiO₂-400C against *E. coli*, (f) TiO₂-500C against *E. coli*, (g) Commercial P25 against *E. coli*, (h) Positive control (cefoxitin-30μg) against *E. coli*, (i) TiO₂-400C against *P. aeruginosa*, (j) TiO₂-500C against *P. aeruginosa*, (k) Commercial P25 against *P. aeruginosa*, (l) Positive control (cefoxitin-30μg) against *P. aeruginosa*, (m) TiO₂-400C against *K. pneumoniae*, (n) TiO₂-500C against *K. pneumoniae*, (o) Commercial P25 against *K. pneumoniae*, and (p) Positive control (cefoxitin-30μg) against *K. pneumoniae*. 10% DMSO as negative control sample did not show any antibacterial activity towards tested pathogens. These data statistically analysed by two-way ANOVA test using GraphPad Prism software. Data shown are mean value ± SD (n=3, ****p-value≤0.0001).

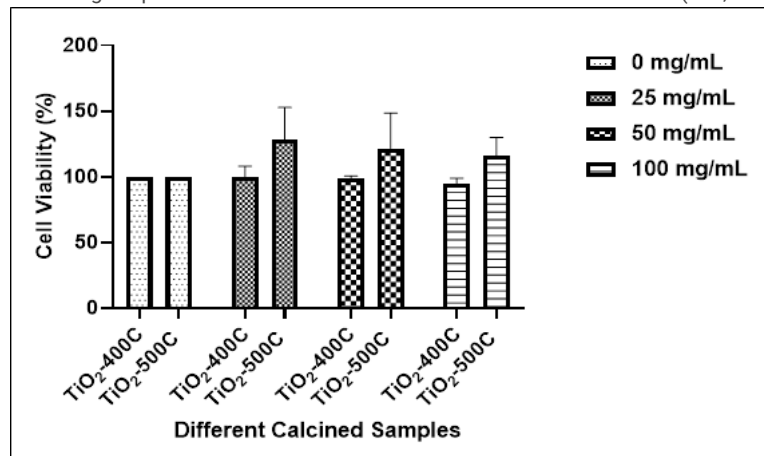


Figure 5

Alamar blue assay results of different-concentrated green TiO₂-400C and TiO₂-500C along with control (n=3).

Supplementary Files

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