



Study of Antimicrobial Performance of green synthesized TiO₂ Nanostructures against *Clostridium perfringens* and *Salmonella typhimurium*

Janaki Kajuluri^{1,2}, S. Saravanan² and N. Sankara Rao^{3*}

¹AU-TDR Hub, Andhra University, Vishakhapatnam, AP, India

²Department of Science and Humanities, Swarnandhra College of Engineering and Technology (A), Narsapur, West Godavari, AP, India

³Department of Chemistry, Dr. V.S. Krishna GDC(A), Vishakhapatnam, AP, India

Received: 12.11.2025 Accepted: 26.11.2025 Published: 30.12.2025

*sankarangp@gmail.com



ABSTRACT

This study explores the green synthesis of titanium dioxide (TiO₂) nanostructures using *Calotropis gigantea* leaf extracts as a reducing and stabilizing agent. The synthesized TiO₂ nanostructures were thoroughly characterized using XRD, UV-visible spectroscopy, FTIR, SEM, and EDX. The XRD analysis confirmed the anatase phase of TiO₂ with an average crystallite size of 6.4 nm. The FTIR spectra identified functional groups associated with the plant extract and TiO₂ nanoparticles, while SEM revealed rectangular-shaped nanostructures with some agglomeration. The EDX analysis confirmed the elemental composition, predominantly titanium and oxygen, with minimal impurities. Antimicrobial assays demonstrated the effectiveness of green-synthesized TiO₂ against *Clostridium perfringens* (Gram-positive) and *Salmonella typhimurium* (Gram-negative). The nanoparticles exhibited the highest zone of inhibition against *Salmonella typhimurium*. These results underline the potential of *C. gigantea* as a green synthesis agent for TiO₂ nanostructures, offering a sustainable and efficient approach to antimicrobial applications.

Keywords: Titanium dioxide nanoparticles; Antimicrobial activity; *Calotropis gigantea*; *Clostridium perfringens*; *Salmonella typhimurium*.

1. INTRODUCTION

Eco-friendly nanotechnology is one of the fastest-growing research areas, driven by high production efficiency, stability, and superior yield. Green-synthesized nanomaterials and structures are gaining widespread recognition for their applications in natural remediation and antimicrobial treatments (Ying *et al.* 2022). Disciplines such as biology, chemistry, physics, food science, medicine, electronics, and aerospace are deeply engaged in advancing the study and application of nanostructures. Nanomaterials exhibit an exceptionally high surface-to-volume ratio. Additionally, these materials demonstrate unique physical and chemical properties that are absent in bulk materials. This is attributed to factors such as their smaller size, larger surface area, enhanced interfaces, and quantum size effects. The size, shape, and surface chemistry of nanoparticles may render them hazardous, even as nanoscale metals are extensively used in diverse fields such as engineering and medicine. Chemically, the synthesis of nanoscale metal oxides is significant and widely practised. However, this may lead to unforeseen consequences, such as environmental contamination and high energy consumption. The green synthesis process is cost-effective, low in pollution, and promotes

environmental and human safety. These advantages make green synthesis preferable to conventional chemical methods. Biological techniques provide an environmentally friendly and sustainable approach to synthesizing nanoparticles and nanostructures. As per (Jayaprakash *et al.* 2024), proteins and polyphenols in green materials can reduce metal ions to a lower valence state without requiring chemical reagents. Metal nanoparticles can be synthesized using green materials, provided suitable conditions, such as temperature, concentration, and air quality, are met. In certain cases, green-synthesized metal nanoparticles exhibit higher quality than chemically synthesized ones. Compared to chemical and physical approaches, green synthesis provides several advantages, such as non-toxicity, pollution-free processes, environmental friendliness, cost efficiency, and enhanced sustainability (Ying *et al.* 2022). However, challenges such as raw material extraction, prolonged reaction times, and product quality issues persist. For example, while the product's particle size may be highly uniform, the synthesis process is time-consuming, and raw materials are often scarce (Anasari *et al.* 2023; Jayanta *et al.* 2014; Singh *et al.* 2023). The general procedure for green synthesis of nanoscale metals involves extracting plant material, mixing it with a metal salt solution under specific conditions, reducing

the metal particle size, filtering, and performing subsequent steps. *Calotropis gigantea* is a commonly used medicinal herb with a long history of traditional applications. Native to Cambodia, Indonesia, Malaysia, the Philippines, Thailand, Sri Lanka, India, and China, *C. gigantea* is traditionally used to treat ailments such as leprosy, leucoderma, ulcers, tumors, piles, and diseases affecting the spleen, liver, and abdomen. The plant also exhibits purgative, alexipharmic, anthelmintic, analgesic, and anxiolytic properties. *Calotropis gigantea* contains diverse phytochemicals, including terpenoids, flavonoids, tannins, phenol derivatives, plant enzymes, proteins, and reducing sugars, which have been extracted and identified from various parts of the plant (Kaur *et al.* 2021). The plant belonging to the family Asclepiadaceae has been a part of traditional medicine for centuries. Currently, the stem, bark, roots, leaves, latex, and flowers of *C. gigantea* are commonly used as reducing and stabilizing agents in nanoparticle synthesis. This study focuses on synthesizing TiO₂ nanostructures using *C. gigantea* leaf extract as a capping and reducing agent.

2. MATERIALS AND METHODS

2.1 Plant Collection and Chemicals

The fresh leaves of *Calotropis gigantea* were collected from Swarnandhra College of Engineering and Technology campus, Narsapur, West Godavari 16.4186° N, 81.6580° E. This plant was identified as weed plant by (Negi and Bisht, 2021). In Asian countries like India, this plant has been using for various health issues (diseases) such as toothache, ear ache, wound healing etc. These plant species were identified and authenticated by taxonomist Dr. A. Balasubramanian. Also, the corresponding voucher specimen has been retained in the department for the future reference.

Analytical grade titanium isopropoxide (TTIP, C₁₂H₂₈O₄Ti) ethanol (ETH, C₂H₆O) and NaOH were used for the preparation of TiO₂ NPs.

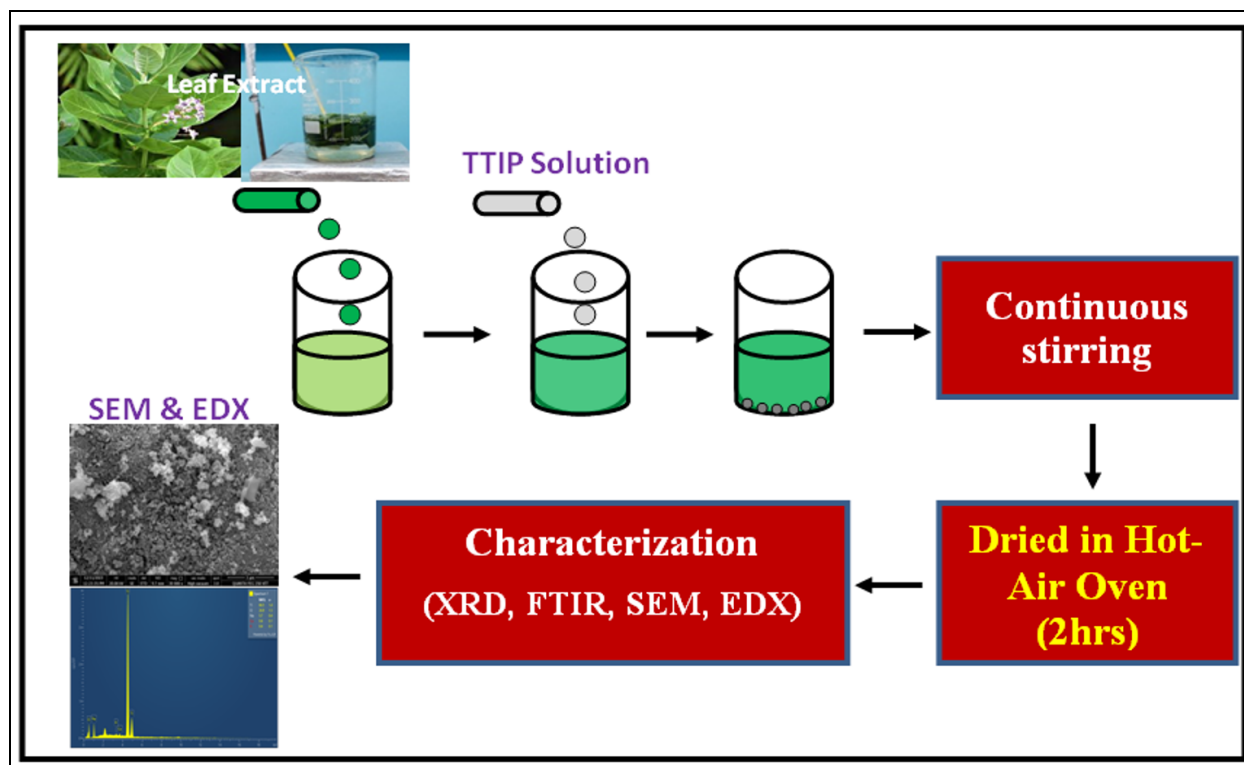


Fig. 1: Steps for the green synthesis of TiO₂ nanostructures

2.2 Preparation of Leaf Extract

The fresh leaves of *Calotropis gigantea* collected were washed thoroughly using distilled water and finely cut into pieces, which were weighed (10 g). These finely cut pieces were boiled in 200 mL of sterile distilled water for an hour at 60°C. Subsequently, the collected leaf extract was filtered using Whatman filter paper to obtain clear filtrate. This extract was stored for nanoparticle synthesis.

2.3 Green Synthesis of TiO₂ Nanoparticles

TiO₂ nanoparticles were synthesized with TTIP by bottom-up approach. At first, 15 mL of the filtered leaf extract was added to 2.1 mL of TTIP directly and stirred for 15 minutes. About 10 mL of 1 M NaOH was added drop by drop in about 15 minutes to the solution until precipitation occurred. The prepared solution was washed by using Whatman filter paper several times with distilled water to remove excess NaOH. The filtrate was placed in

hot air oven for 2 hours at 100°C. After drying, the sample was ground into a fine powder and placed in the oven and sintered at 700°C for 3 hours.

2.4 Assay of Antimicrobial Activity: Agar-well Plate Method

The bacterial cultures of *C. perfringens* and *S. typhimurium* were grown overnight for testing. These cultures were procured from Microbial Type Culture Collection (MTCC) and maintained in freshly prepared nutrient agar slants. These microorganisms were stored at -20°C in glycerol for long-term preservation. Initially, 1 L of nutrient broth was prepared by dissolving 13 g of nutrient agar medium in distilled water, followed by heating to ensure complete dissolution. Further, this medium was sterilized for 15 minutes in autoclave at 15 lbs pressure (121°C). The TiO₂ nanoparticles were dissolved in dimethyl sulfoxide (DMSO) with aliquots of the prepared samples and filled into wells which were already inoculated with specific bacterial species.. The Petri plates with the bacterial cultures along with the nanoparticles were incubated over a period of 24 hours at 37°C. The diameter(mm) of the clear inhibitory zone formed around the discs was measured. Amoxicillin is taken as positive control.

2.5 Characterization

The optical absorption (UV-Visible spectroscopy, Perkin Elmer-Lambda 35, USA), functional group analysis (FTIR, Model: IRAffinity-1, Shimadzu, Japan), structural analysis (XRD, Bruker D8 Advance, Germany), morphological studies (SEM, FEI Quanta 250 FEG, USA), and elemental studies (EDX, APEX, Germany) were carried out.

3. RESULTS AND DISCUSSION

3.1 UV- visible Spectroscopy

The optical properties of the nanostructures were studied at room temperature by using UV-visible absorbance spectroscopy. The absorbance spectrum is presented in Fig. 2. The absorption intensity is affected by particle size, molar concentration, solvents, calcination temperature, etc (Rafique *et al.* 2019). The TiO₂ nanoparticles showed a strong band at 232 nm. Here, the green leaf extract acts as a capping agent and controls the growth. The absorption gradually decreases from the ultraviolet to infrared region.

Bian *et al.* (2008) and Priyanka *et al.* (2019) reported that the absorption increased in longer wavelengths due to the scattering of light irradiation. This observation provides an insight that the increasing the absorbance of green synthesized TiO₂ nanostructures would be useful for opto-electronic response applications.

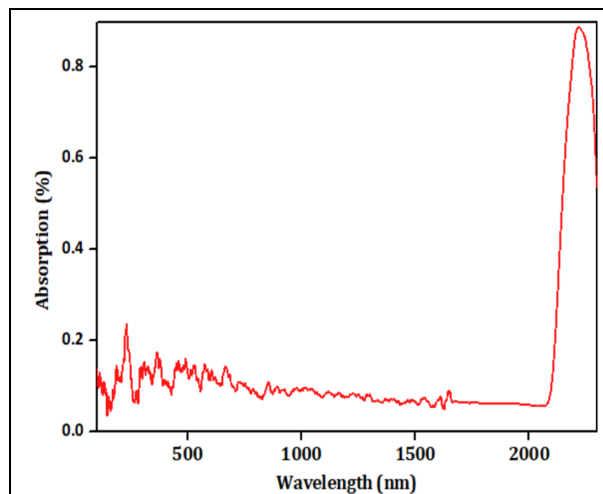


Fig. 2: UV-visible spectrum of green synthesized TiO₂ nanostructures

3.2 Fourier Transform Infrared (FTIR) Spectroscopy: Transmittance

The vibrational modes of green synthesized TiO₂ nanostructures with *Calotropis gigantea* leaf extract is shown in the Fig. 3. This FTIR transmittance spectrum was recorded from 400 to 4000 cm⁻¹ which reveals the characteristic peaks at 863, 1442, 1672, 2170, and 3260 cm⁻¹. The peak appeared at 863 cm⁻¹ reveals Ti-O stretching vibrational modes (Ramzan *et al.* 2021; Helen *et al.* 2023).

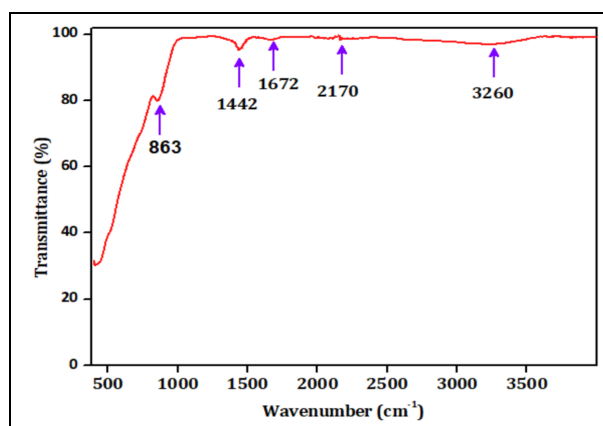


Fig. 3: FTIR spectrum of green synthesized TiO₂ nanostructures

The peak at 1672 cm⁻¹ corresponds to C=O group in flavanones which were reported to be active reducing agents from *Calotropis gigantea* leaf extract (Dubey and Saravanan, 2021). The peak at 2170 cm⁻¹ corresponds to the O-H vibrations and the one at 3260 cm⁻¹ corresponds to hydrogen bonding (Rafique *et al.* 2019). Therefore, FTIR transmittance spectrum confirms the presence of TiO₂ and the phytochemicals present in the leaf extract.

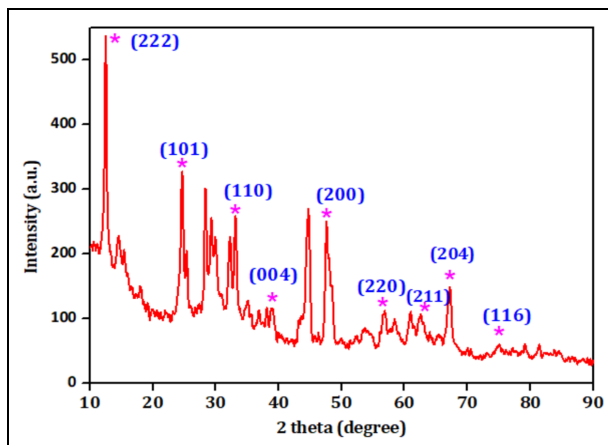


Fig. 4: XRD pattern of green synthesized TiO₂ nanostructures

3.3 X-ray Diffraction Analysis

Fig. 4 shows the XRD pattern of synthesized TiO₂ nanostructures at room temperature. The established XRD diffraction peaks observed at 2θ values of 12.57° (222), 24.54° (101), 33.2° (110), 38.9° (004), 47.7° (200), 62.6° (211), 67.38° (204), and 75.17° (116) correspond to the brookite and anatase phase of TiO₂ and were compared to the standard JCPDS No. 89-4202, 21-1272, and 79-0207 (PCPDF) databases (Helen *et al.* 2023; Saravanan 2021; Manda *et al.* 2022; Azizi *et al.* 2019). In the XRD pattern, diffraction peaks were shifted to the lower 2θ degrees was due to the involvement of plant extract. The crystallite size was calculated using Debye-Scherrer's formula

$$D = \frac{K\lambda}{\beta \cos \theta} \quad \dots (1)$$

where, D is the crystallite size of nanostructures (nm), K is the Scherrer constant (0.94), λ - wavelength (0.15406), β -angular full-width half maximum (FWHM, radians), θ - It was 6.4° for which $2\theta = 12.57$ (Saravanan and Dubey, 2021). The calculated crystallite size and their parameters (α , β and ν) are interrelated with the SEM nanostructures. The lattice strain decreases with the decrement of crystallite size of the sample.

3.4. Scanning Electron Microscopy

The SEM image of green synthesized TiO₂ nanostructures is shown in Fig. 5. The nanostructures are rectangular in shape with some agglomerations (Sallem *et al.* 2020; Ansari *et al.* 2023). With the SEM micrograph, the width and length of nanostructure sizes were found to be 64 and 100 nm, respectively. When the nanostructure size reduced at the nanoscale level, their antibacterial activity also increased considerably (Rafique, *et al.* 2019). Some agglomerations (adhesion particles) were noticed in the nanostructures due to the strong attractive forces. Furthermore, distinct environmental factors play a major role in the

nanostructures, such as van der Waals force and local forces (chemical bonds, weak force-micron sized entities) (Manda *et al.* 2022).

3.5 Elemental Study: Energy Dispersive X-ray Spectroscopy (EDX)

The element mapping of the sample was studied by EDX spectrum. It confirms the presence of Ti and O (Fig. 6). The majority of weight (wt%-Ti=66.46%, O = 26.9%) and atomic (Ti=41.53%, O=50.32%) percentages were noticed for titanium and oxygen. Other elements (Na, K, and Ca) were not in the considerable levels (Table 1). These impurities might have intruded from the muffle furnace during the calcination atmosphere or testing equipment.

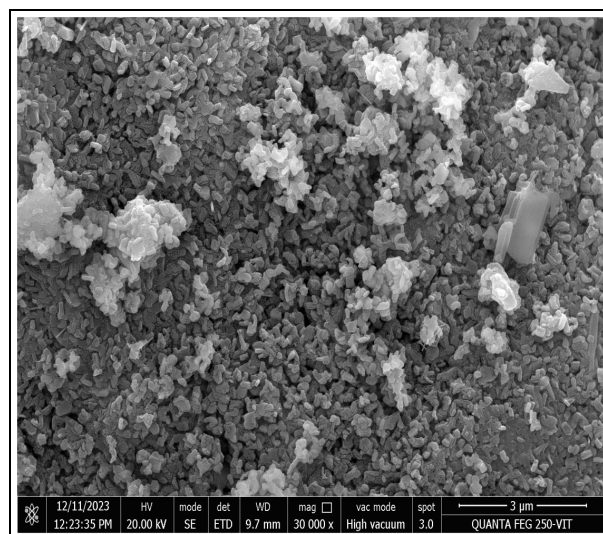


Fig. 5: FESEM morphological image of green synthesized TiO₂ nanostructures

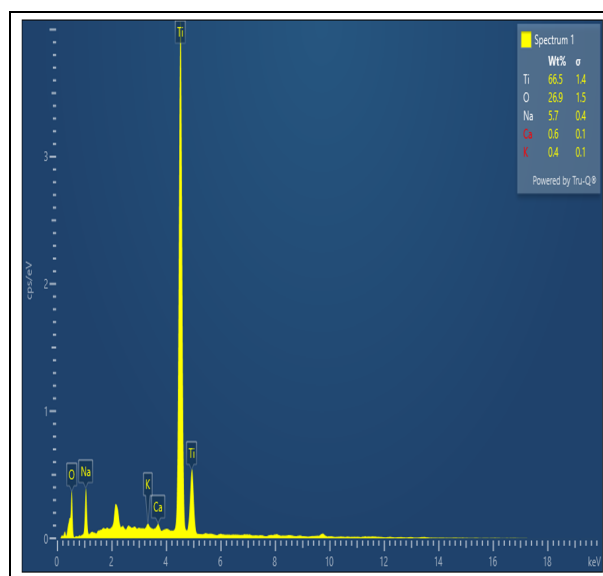


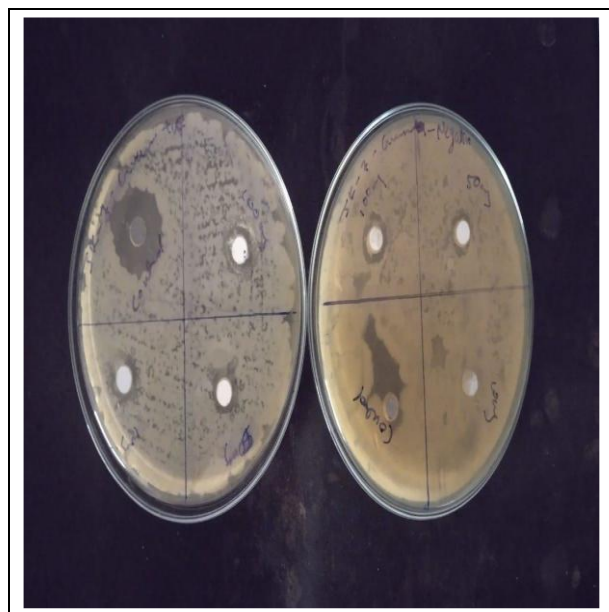
Fig. 6: EDX spectrum of green synthesized TiO₂ nanostructures

Table 1. Elements, atomic and weight percentages of the sample

Element	Wt %	Atomic%
O	26.90	50.32
Na	5.73	7.46
K	0.36	0.28
Ca	0.55	0.41
Ti	66.46	41.53
Total:	100	100

3.6 Antimicrobial Activity

The metal oxide nanoparticles (TiO_2 , ZnO and TiO_2/ZnO) have been reported earlier for their excellent antimicrobial activity in the efficient removal of pathogens (Azizi *et al.* 2019). The antibacterial activity of *Calotropis gigantea*-based TiO_2 nanostructures against two bacterial strains was determined using the well plate method by measuring the inhibition zones.

**Fig. 7: In vitro antimicrobial activity of TiO_2 nanoparticles****Table 2. Antibacterial activity performance based on *Calotropis gigantea* plant**

S. No.	Plant	Antibacterial Activity On	Inhibition Zone	Ref.
1	<i>C. gigantea</i> Leaves	<i>Streptococcus mutans</i> and <i>Lactobacilli acidophilus</i>	16 and 14 mm	(Meenakshi <i>et al.</i> 2015)
2	<i>C. gigantea</i> Leaves	<i>Proteus vulgaris</i>	18 mm	(Waseem, 2020)
		<i>Escherichia coli</i>	21 mm	
		<i>Clostridium botulinum</i>	25 mm	
		<i>Aspergillus niger</i>	14 mm	
		<i>Arthrographis cuboid</i>	26 mm	
		<i>Aspergillus fumigatus</i>	22 mm	
3	<i>C. gigantea</i> Flowers	<i>Staphylococcus aureus</i>	21 mm	(Shopna <i>et al.</i> 2014)
4	<i>Calotropis procera</i> leaf	<i>E. Coli</i>	<14.9 mm	(Nejhad <i>et al.</i> 2023)
		<i>Salmonella typhimurium</i>		

Antimicrobial activities depend on different parameters such as concentration and microorganism, extracts (chloroform, ethyl acetate, and methanol) (Shopna *et al.* 2014). Fig. 7 shows the *E. coli* colonies on agar plates. The minimum inhibitory concentration (10 mg), maximum inhibitory concentrations and disc diffusion were determined up to 24 hours with the prepared nanomaterials. The Gram-positive microorganism (*Clostridium perfringens*) and Gram-negative strain (*Salmonella typhimurium*) exhibited maximum inhibition zones of 15 mm and 19 mm, respectively. Azizi *et al.* (2019) reported comparable antibacterial activity for the TiO_2 and ZnO nanostructures synthesized using 4A zeolite. Antimicrobial activity of

Calotropis gigantea plant parts has been reported by various researchers as shown in Table 2.

4. CONCLUSION

This study highlights the potential of *Calotropis gigantea* leaf extract in the green synthesis of TiO_2 nanostructures, presenting a sustainable alternative to conventional chemical methods. The synthesized TiO_2 exhibited excellent structural, optical, and morphological properties, as evidenced by advanced characterization techniques (SEM, XRD, EDX and FTIR). The antimicrobial activity of the nanostructures was evaluated against *Clostridium perfringens* and

Salmonella typhimurium. These findings emphasize the role of *C. gigantea* as an efficient and eco-friendly capping and reducing agent for TiO₂ synthesis, paving the way for future applications in antimicrobial treatments. This work contributes to the broader field of nanotechnology by integrating green chemistry principles with practical biomedical applications, underscoring the potential for scalable and sustainable antimicrobial solutions.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the Department of Mechanical Engineering, Jayalakshmi Institute of Technology, for providing the necessary resources and infrastructure to conduct this research.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

COPYRIGHT

This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).



REFERENCES

- Anasari, M., Ahmed, S., Abbasi, A., Tajammal, K. M., Subhan, M., Bukhri, N. A., Hatameh, A. A. and Abdelsalam, N. R., Plant mediated fabrication of silver nanoparticle, process optimization and impact on tomato plant, *Sci. Rep.*, 18048 (2023). <https://doi.org/10.1038/s41598-023-45038-x>
- Azizi, L. M., Ehsani, A., Divband, B. and Alizadeh, S. M., Antimicrobial activity of Titanium dioxide and Zinc oxide nanoparticles supported in 4A zeolite and evaluation the morphological characteristic, *Sci. Rep.*, 9, 17439 (2019). <https://doi.org/10.1038/s41598-019-54025-0>
- Bian, F., Zhang, X. Z., Wang, Z. H., Wu, Q., Hu, H. and Xu, J. J., Preparation and size characterization of silver nanoparticles produced by femtosecond laser ablation in water, *Chin. Phys. Lett.*, 25 (12), 4463-4465 (2008). <https://doi.org/10.1007/s10904-019-01173-5>
- Dubey, R. S. and Saravanan, S., Ultraviolet and visible reflective TiO₂/SiO₂ thin films on silicon using sol-gel spin coater, *Nanosys. Phy. Chem. Math.*, 12 (3), 311-316 (2021). <https://doi.org/10.17586/2220-8054-2021-12-3-311-316>
- Helen, R. V. and Ambrose R. J., Green fabrication of titanium dioxide nanoparticles and their applications in photocatalytic dye degradation and microbial activities, *Chem. Phys. Impact.*, 6, 100197(2023). <https://doi.org/10.1016/j.chphi.2023.100197>
- Jayanta, K. and Kwang, H. B., Green Nanobiotechnology: Factors affecting synthesis and Characterization Techniques, *J. Nanomater.*, 1, 417305 (2014). <https://doi.org/10.1155/2014/417305>
- Jayaprakash, M., Sivasubramaniam, S. S., David, E. and Santhakumar, K., Green supercapacitors: Review and Perspective on sustainable template-free synthesis of metal and metal oxide nanoparticles, *RSC Sustain.*, 1224-1245 (2024). <https://doi.org/10.1039/D4SU00009A>
- Kaur, A., Batish, D. R., Kaur, S. and Chauhan, B. S., An overview of the characteristics and potential of calotropisprocera from botanical, ecological and economic perspective, *Front. Plant Sci.*, 12, 690806 (2021). <https://doi.org/10.36721/PJPS.2020.33.6.sup.2801-2807.1>
- Manda, A. A., Drmash, Q. A., Elsayed, K. A., Al-Alotaibi, A. L., Olanrewaju A. I., Onaizi, S. A., Dafalla, H. D. M. and Elhassan, A., Highly efficient UV-visible absorption of TiO₂/Y₂O₃ nanocomposite prepared by nanosecond pulsed laser ablation technique, *Arabian J. Chem.*, 15, 104004 (2022). <https://doi.org/10.1016/j.arabjc.2022.104004>
- Meenakshi, S., Tandon, S., Aggarwal, V., Bhat, K. G., Kappadi, D., Chandrasekhar, P. and Dorwal, R., Evaluation of antibacterial activity of calotropisgigantica against streptococcus mutans and lactobacillus acidophilus: An in vitro comparative study, *J. Conserv. Dent.*, 18(6), 4577-460 (2015). <https://doi.org/10.4103/0972-0707.168809>
- Negi, D. and Bisht, A. S., A review on brief study of calotropis gigantean Linn., *J. Drug Delivery therapeutics*, 11(5), 224-228 (2022). <http://dx.doi.org/10.22270/jddt.v11i5.5008>

- Nejhad, A. A., Behbahani, B. A., Hojjati, M., Vasiee, A. and Mehrnia, M. A., Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of Calotropis procera leaf aqueous extract, *Sci. Rep.*, 13, 14716 (2023).
<https://doi.org/10.1038/s41598-023-42086-1>
- Priyanka, A., Ganesh, K., Suresh, G. and Anjali, R., Synthesis of TiO₂ nanoparticles by electrochemical method and their antibacterial application, *Arabian J. Chem.*, 12, 1815-1822 (2019).
<https://doi.org/10.1016/j.arabjc.2014.12.015>
- Rafique, M., Jahangir, J., Ahmad, Z. A. B., Bilal, T. M., Nabi, G., Isa, K. M., Khalid, N. R., Gillani, S. S. A. and Sadaf, I., Investigation of photocatalytic and seed germination effects of TiO₂ Nanoparticles synthesized by Melia azadarach L. Leaf extract, *J. Inorg. Organomet. Polym. Mater.*, 29, 2133-2144(2019).
<https://doi.org/10.1007/s10904-019-01173-5>
- Ramzan, M., Obodo, R. M., Shahzad, M. I., Mukhtar, S., Ilyas, S. Z. and Mahmood, T., Green synthesis of Cu@TiO₂ via cedrus deodara leaf extract: A novel composite with high photocatalytic and antibacterial activity”, *Curr. Res. Green Sustain. Chem.*, 4, 100137 (2021).
<https://doi.org/10.1016/j.crgsc.2021.100137>
- Saif, M. H. Q., Hamid, M. G., Huda, S. B., Abrar, F. B. A., Khulod, K. A. and Abdullah, S. A., Successful growth of TiO₂ nanocrystals with {001} facets for solar cells, *Nanomater.*, 13(5), 928(2023).
<https://doi.org/10.3390/nano13050928>
- Sallem, F., Villatte, L., Geffroy, P., Goglio, G. and Pagnoux, C., Surface modification of titania nanoparticles by catechol derivative molecules: preparation of concentrated suspensions, *Colloids Surf., A*, 602, 125167(2020).
<https://doi.org/10.1016/j.colsurfa.2020.125167>
- Saravanan, S. and Dubey, R. S., Optical and morphological studies of TiO₂ nanoparticles prepared by sol-gel method, *Mater. Today Proc.*, 47(9), 1811-1814 (2021).
<https://doi.org/10.1016/j.matpr.2021.03.207>
- Saravanan, S., Functional, structural and morphological property of green synthesized silver nanoparticles using azadirachta indica leaf extract, *Mater. Today Proc.*, 47(9), 1815-1818 (2021).
<https://doi.org/10.1016/j.matpr.2021.03.227>
- Shopna, R., Kalaivanan, P., Sivagnanam, I. and Rajamanickam, M., Antioxidant, antimicrobial activities and GC-MS analysis of Calotropis gigantea white flowers, *J. Phytopharma.*, 3(6), 405-409 (2014).
https://www.phytopharmajournal.com/Vol3_Issue6_06.pdf
- Singh, H., Desimone, M. F., Pandya, S., Jasani, S., George, N., Adnan, M., Aldarhami, A., Bazaid, A. S., and Alderhami, S. A., Revisiting the green synthesis of Nanoparticles: Uncovering influences of plant extracts as reducing agents for enhanced synthesis efficiency and its biomedical applications, *Int. J. Nanomed.*, 18, 4727-4750 (2023).
<https://doi.org/10.2147/IJN.S419369>
- Waseem, A., Preliminary phytochemical, antimicrobial and photochemical study of calotrop gigantea leaf extract, *Chem. Lett.*, 9, 105-112 (2020).
<https://doi.org/10.5267/j.ccl.2019.10.001>
- Ying, S., Guan, Z., Ofoegbu, P.C., Clubb, P., Guan, C., He, F. and Hong, J., Green synthesis of nanoparticles: Current developments and limitations, *Environ. Technol. Innov.*, 26, 102336 (2022).
<https://doi.org/10.1016/j.eti.2022.102336>