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# Biological synthesis and characterization of titanium dioxide nanoparticle from *Cynodon dactylon*

**R.E. Renitta [a], T.J. Jebaseeli [b,*], A. Dhanaraj [c], S. Paul [d]**

[a] Department of Food Processing Technology, Karunya Institute of Technology and Sciences, Coimbatore, Tamilnadu, India
[b] Department of Computer Science and Engineering, Karunya Institute of Technology and Sciences, Coimbatore, Tamilnadu, India
[c] Department of English, Karunya Institute of Technology and Sciences, Coimbatore, Tamilnadu, India
[d] Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore, Tamilnadu, India
* Corresponding e-mail address: jemima_jeba@karunya.edu
ORCID identifier: https://orcid.org/0000-0003-1418-7323 (R.E.R.);
https://orcid.org/0000-0002-2032-3304 (T.J.J.)

## ABSTRACT

**Purpose:** There are several advantages of using a biological technique to produce nanoparticles versus a chemical method. The primary goal of this work is to characterize and biologically synthesize titanium dioxide ($TiO_2$) nanoparticles from *Cynodon dactylon*. The characterization has experimented with UV-Vis Spectroscopy, EDX analysis, SEM, XRD, and FTIR.

**Design/methodology/approach:** The suggested study uses a simple biological technique to accomplish the systematic biological synthesis of $TiO_2$ nanoparticles utilizing *Cynodon dactylon* plant extract and titanium tetra isopropoxide as a precursor. UV-Vis spectroscopy, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), and X-Ray Diffraction (XRD) are used to confirm the fabrication of the $TiO_2$ nanoparticles. The plant extract as well as titanium-based nanoparticles of the herb, *Cynodon dactylon* will be tested for its antibacterial activity against human pathogens. This eco-friendly technique for nanoparticle synthesis is straightforward and adaptable to major commercial manufacturing and technological applications.

**Findings:** *Cynodon dactylon* biosynthesis of $TiO_2$ nanoparticles is efficient, nutrition dependent, does not employ hazardous compounds, and happens at neutral pH levels. The antibacterial study results show that $TiO_2$ nanoparticles synthesized using *Cynodon dactylon* have good antibacterial properties. $TiO_2$ nanoparticle method of action against bacteria is unknown. This is an alternative process for synthesising $TiO_2$ nanoparticles, apart from other chemical protocols, since this is quick and non-toxic. The antimicrobial property of biologically synthesized $TiO_2$ nanoparticles against *Escherichia coli*, *Staphylococcus aureus*, and *Acinetobacter baumannii* was tested at four different doses of 15 μl/mg, 25 μl/mg, 50 μl/mg, and 75 μl/mg. The present results revealed the 75 μl/mg concentration got the highest zone of inhibition (15, 13, 15 mm) for *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Escherichia coli*.

**Research limitations/implications:** Many nanoparticles smaller than 100 nm are firmly agglomerated with each other in the study. $TiO_2$ nanoparticles absorb in the UV region of 200 to 400 nm. XRD measurements confirmed the presence of $TiO_2$ nanoparticles in the biologically produced sample. In our work, EDX was used to confirm the existence of Ti after its synthesis by *Cynodon dactylon*.

**Practical implications:** The biosynthesized $TiO_2$ nanoparticles utilizing *Cynodon dactylon* plant extracts exhibit a good potent antibacterial activity. The proposed results showed that the $TiO_2$ nanoparticles are well suited for biomedical applications.

**Originality/value:** The suggested research identifies several eco-friendly, biological, and cost-effective procedures for manufacturing nano-coated herbal products. The agar well diffusion technique was used to assess antibacterial activities toward test pathogens such as *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Escherichia coli*.

**Keywords:** Nanoparticle, *Cynodon dactylon*, FTIR, SEM, XRD, Titanium dioxide, Biological synthesis

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BIOMEDICAL AND DENTAL ENGINEERING AND MATERIALS

## 1. Introduction

A nanoparticle is defined as in the following range of $10^{-9}$ nm size range. A human hair has a diameter of 70000 nm, a red blood cell has a diameter of about 5000 nm, and certain simple chemical substances have a diameter of between 0.5 and 5 nm [1]. Nanobiotechnology is one of the fastest developing sectors of nanotechnologies of nano-sized systems. It relates to humans' use of nano-sized components for industrial and medicinal reasons [2]. Nanotechnology is defined as a multi-disciplinary field that covers all branches of science, medicine, physics, engineering, and chemistry, with its defining trait being its scale. Whenever the size of all such materials is minimized, the characteristics of such elements are substantially enhanced. Materials in the nano size, including carbon or silicon, exhibit unique characteristics like increased strength, high conductivity, chemical stability, and other qualities. Metallic nanoparticles showcase unusual properties that have not been shown in a large form [3].

Nanotechnology is employed for a variety of purposes across a wide range of fields due to its enormous potential and capabilities [4]. In biochemistry, particles are superior catalysts as well as biological and chemical sensors [5]. In other prominent sectors, the size and electromagnetic synthesis of nanoparticles are employed for the manufacture of storage devices, where downsizing is a major problem [6]. The primary chemical techniques for nanoparticle production are sol-gel and gas phases. Nanoparticles range in diameter from 1 to 10 nm and have a homogenous crystal structure. To achieve better confinement, the technique created a high level of monodispersity with 20% size fluctuations. Murray et al. [7] recommended that this number be lowered to 5% or less. The chemical synthesis process largely relies on the availability of suitable metal or organic precursors. The primary drawback of this process is that it requires extremely high and severe temperatures and pressures, as well as the use of highly volatile organic solvents. The method is unable to be scaled, and control over crystalline dispersion is restricted [8]. Sol processing is a chemical precipitation synthesis method that differs from other chemical processes. In comparison to previous high-temperature techniques, the nanomaterial is created at a lower temperature. Precursors are employed in sol-gel processing, which takes one of two ways. In the inorganic approach, metal salts in aqueous solutions are utilized as raw materials, whereas metal-organic precursors occur in organic solvents and use metal alkoxides as starting materials. The process begins with the formation of a sol, which is followed by gelling, shape development, drying, and densification. A dopant [9] or altering the nanoparticles regulates the size distribution of the nanoparticles generated by this method.

To produce metal nanopowders, gas-phase synthesis is utilized, which consists of a pressure chamber with a heat source, the precursor material to be transformed into nanoparticles or nanopowder, vacuum infrastructure, and the powder to be collected [10]. To generate spherical nanoparticles, an inert gas is utilized at high pressure. Simultaneously, the lower pressure enables the production of spherical nanoparticles. The precursor metal is then put on a hot element, quickly melting it. A constant stream of gas is introduced into the chamber at this point, and the overflow is evacuated by pumping in such a way that the gas flow removes the evaporated metal from the heat source. In a fixed environment, liquid phase nanoparticles collide and fuse, causing the nanoparticles to grow in a certain pattern and, consequently, the surface to remain spherical and smooth. Because liquid particles are very sensitive, cooling them continues to limit their development, and covering them is critical to preventing agglomeration with other materials [11]. So no chemical processing, cavitation processing [12], micro emulation processing, and high-energy ball milling are some of the additional chemicals utilized in the creation of nanoparticles [13].

A biological method for producing nanoparticles offers several advantages over chemical techniques. Chemical methods include using toxic solvents, substantial energy consumption, and creating hazardous compounds. As a result, it poses a significant risk to the environment. Similarly, the expense of manufacture and the restricted forms of nanoparticles are spherical, reducing their efficient characteristics [14]. There is a pressing need to discover and develop novel synthetic approaches that address the aforementioned problems while still achieving the desired results in the biological method. Many biosynthetic techniques are used in the creation of metallic nanoparticles that are currently present in syntheses that are highly stable. Some of the other metals are being synthesized successfully by the Actinomycetes, bacteria, fungi, viruses, and yeasts kind of microorganisms [15]. Previously, the usage of microorganisms in the area of bioremediation has been documented, and their exceptional capacity to cleanse the ecological environment metal is regarded as eco-friendly nano factories.

The review of related studies reveals many biomimetic efforts have been carried out for gold and silver nanoparticle production. Hence, it is essential to synthesise other important elements, such as magnesium, zinc, phosphorus, titanium, etc., for their various demands and applications. The large quantities of $TiO_2$ nanoparticles are under production process for various applications. It possesses a variety of physicochemical properties compared to other analogues, which exhibit alteration in its bioactivity.

Other single-celled organisms have been discovered to build crystalline structures from inorganic materials, either intercellularly or extracellularly. The nanoparticles synchronized by microorganisms are extremely stable, and investigations have shown that they are not monodispersed. The optimal elements are included in the synthesis of agriculture and harvesting methods [15,16]. The biological and molecular level provides insight into how to enhance the speed, purity, and inherent properties of nanoparticles [31-33]. Plant biomass or extractions are shown to be an additional biological aid in producing metal nanoparticles. These routes do not sufficiently solve the problem. The protein shell regulates particle development and prevents nanoparticle aggregation. Cavities can be seen in the cores of proteins like cowpea, ferritin-like proteins, chaperones, and viruses [17]. Protein-encapsulated nanoparticles have been proven to be efficient transporters of food and medications to particular locations in biological systems. The shape of the nanoparticle is determined by the size and diameter of the inner cavity.

## 1.1. Nanoparticles characterization

Nanoparticles are produced either biologically or chemically and are characterized by monodispersity, size, and water stability, as well as adsorption to biomolecules. The net charge and flocculation in diverse mediums provide critical evidence for the use of nanoparticles. It determines if a particle could be applied in biological and biomedical applications or whether it may be improved in terms of synthetic processes and/or chemical modification. A wide range of characterization techniques are currently accessible, some of which predate technology and are primarily derived from material science.

Table 1.
Techniques used in nanoparticle characterization

| Characterization Technique | Properties analysed |
|---|---|
| UV-Visible spectroscopy | Interface polarizable of nanoparticles, particularly those of metal source |
| TEM, SEM, and EDX | Particle size, morphology, monodispersity, and composition |
| FTIR | Characterization of functional groups on the surface of nanoparticles |

Ultraviolet-Visible (UV) spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning/Transmission Electron Microscopy (SEM/TEM), Dynamic Light Scattering (DLS), Atomic Force Microscopy (AFM), and Energy Dispersion and Analysis of X-rays (EDAX) are the most popular methods used for the characterization of nanoparticles, as shown in Table 1.

### 1.2. *Cynodon dactylon*

*Cynodon dactylon,* often known as arugampul, is just a perennial plant that forms dense mats from stolen rhizomes and is seen in Figure 1. It is a significant medicinal herb used in the Ayurveda healthcare system to cure a variety of illnesses. *Cynodon dactylon* has a bitter, strong, spicy taste and a pleasant odour; it is a digestive, a brain, and heart stimulant, a convulsive, an emmenagogue, a carminative, and is beneficial for soreness, inflammation, and toothache. In India, it is commonly used as a diabetes-controlling drug. The juices obtained from its leaf are internally used against blood vomiting and externally used against chronic wounds. It is native to Europe, Africa, Australia, and much of Asia. It has been introduced to America and the Middle East. The blades are grey-green in colour and short, ranging from 2 to 15 cm in length, with rough edges. The erect stems can reach a height of 1-30 cm. The stems are somewhat flattened and frequently purple-tinged. The seed heads appear in a cluster of two to six spikes at the apex of the stem each spike about 2-5 cm long. Growth begins at temperatures over 150 degrees Celsius, with optimal growth occurring between 24 and 370 degrees Celsius; during the winter, the grass becomes dormant and turns brown. Complete light promotes growth whereas full darkness retards it.

Fig. 1. *Cynodon dactylon*

### 1.3. Nanoparticles of $TiO_2$

$TiO_2$ is a white solid inorganic material that is thermostable, non-flammable, insoluble, and not toxic. It is the metal titanium oxide that naturally occurs in a variety of rocks and mineral sands. It is the ninth most prevalent element in the earth's crust and is chemically inactive. It has a molecular weight of 79.9 g/mol, a boiling point of 2972°C, a melting point of 1843°C, and a relative density of 4.26 g/cm$^3$ at 250°C [18]. $TiO_2$ has two major crystal structures: anatase and rutile; anatase is more chemically active. When exposed to UV radiation, anatase produces reactive oxygen species (ROS). $TiO_2$ anatase has been shown to have a higher hazardous capability than $TiO_2$ rutile. $TiO_2$ nanoparticles are notable among metal oxide nanoparticles. They have been widely utilized in air and water cleansing as well as decolorization solar cells due to their capacity to oxidize, high photostability, and non-toxicity. It has antimicrobial properties when exposed to UV light.

### 1.4. International review status

Biosynthesis of the $TiO_2$ was carried out by using a bacterial strain of *Bacillus mycoides* (Gram-positive bacteria). This resulted in the synthesis of $TiO_2$ nanoparticles at 37°C in a titanyl hydroxide-containing environment. The biologically synthesized nanoparticles have a spherical shape. It also showed an organic shell when characterized using UV-Vis spectroscopy, DLS, FTIR, and TEM. It did not display photo-toxicity as compared to chemically synthesized nanoparticles. Biologically synthesized nanoparticles were integrated into Quantum Dot Solar Cells, especially in comparison to chemically produced $TiO_2$ nanoparticles to develop low-cost and environmentally friendly solar cells. Microbial resistance is a continual issue for the scientific community in terms of developing novel biochemicals and treatments. This study revealed the antibacterial efficacy of $TiO_2$ nanoparticles (25 nm) against an *Escherichia coli* strain. For *E.coli*, the MIC50 value of the $TiO_2$ nanoparticles was 200 g/ml. The zone of inhibition demonstrated the $TiO_2$ nanoparticles' possible microbial load. Furthermore, a proliferation assessment of *E.coli* was studied in the presence and absence of $TiO_2$ nanoparticles, which revealed an impact of obstructive and sub-inhibitory $TiO_2$ levels until 20 hours of *E.coli* incubation. It was also shown that nanoparticles inactivate the cellular enzymes and DNA by binding to amides, hydroxyls, thiols, indoles, carboxylates, etc. This leads to causing little pores on the bacterial cell wall, which increases permeability and cell death [19]. Excellent antibacterial properties are exhibited by metal oxide nanoparticles. Such properties are applied to remove harmful organic materials and bacteria from air and water. More nanosize, X-ray diffraction and scanning electron microscopy experiments were utilized to analyse the $TiO_2$ nanoparticles that were generated. The antibacterial activity of the produced nanoparticles against *Pseudomonas aeruginosa* (ATCC 9027), *B. subtilis* (ATCC 6633), *E. coli* (ATCC 8739), and *Staphylococcus aureus* was examined (ATCC 6538). It was found that the $TiO_2$ nanoparticles were effective against these bacterial strains. Based on the study it was concluded that $TiO_2$ nanoparticles synthesized by the ultrasound method were a good inorganic antimicrobial agent. Nanoparticles of diameter less than 100 nm such as titanium nanoparticles have brilliant and interesting photo-catalytic, dielectric and optical characteristics. The *E.coli* strain was shown to be resistant to every antibiotic employed in this study, and optical density was found to be inversely related to $TiO_2$ nanoparticle concentrations of 0.225, 0.218, 0.158, 0.075, and 0.031 respectively. The greatest zone of inhibition was found to be at the nanoparticle concentration (5 mm). This was due to the inactivation of cellular enzymes in the binding of DNA to electron-donating groups like hydroxyls, amides, thiols, carboxylates, and indoles. This causes small holes in bacterial cell walls, resulting in greater cell death. This study revealed that $TiO_2$ nanoparticles are an effective antibacterial agent and may be employed in a variety of applications [20]. The study was conducted to evaluate the antibacterial activity of silver nanoparticles against MRSA and MSSA isolates obtained from cutaneous infections. In clinical studies, there has been an increasing number of encounters, with soft-tissue and skin infections, due to multi-resistant pathogens. Resistant pathogenic bacteria have been causing a serious outbreak of infections and diseases, which has led researchers and pharmaceutical industries to find other efficient antibacterial agents. This is where metallic nanoparticles present a grand entry. Metallic nanoparticles possessing antimicrobial properties represent an innovative and modern approach coming up with new formulations. The form and size of biologically synthesized nanoparticles are well documented, but the yields must be quantified. Various eco-friendly, biological, and cost-effective procedures for producing nano-coated herbal products must be found.

## 2. Materials and methodology

### 2.1. Materials

To produce $TiO_2$ nanoparticles, all chemicals and reagents must be utilized (Petrochem Performance Chemicals Ltd. – UAE, and Hi-Media – Mumbai). The standard strains (*Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*) used for antibacterial studies were obtained from KMCH, Coimbatore, Tamil Nadu. The plant material was collected from the Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu. Botanists at the Botanical Survey of India at Tamil Nadu Agriculture University campus in Coimbatore successfully identified it. *Cynodon dactylon* was recognized as the organic material. The entire plant was carefully cleaned under tap water, followed by washing in distilled water, and then shade-dried for 14 days. For future usage, the dried plant material was pulverized and kept in airtight containers. The extraction process was carried out by the Soxhlet apparatus for 48 hours. 25 g of the plant sample was extracted using 250 ml of ethanol to obtain the ethanolic extract. The extracts were concentrated to dryness in a hot water bath. Then the ethanolic extract was subjected to antibacterial studies.

### 2.2. Preparation of $TiO_2$ nanoparticles

5 mM of Titanium tetraisopropoxide was added to 70% ethanol in the ratio of 1:100 and kept for continuous stirring for 1 hour to obtain the precursor for $TiO_2$ nanoparticles synthesis. Ethanolic extract was added to the precursor in the ratio of 1:9 and kept overnight, stirring at 50°C. The sample was centrifuged at 10,000 rpm for 15 minutes, followed by an ethanol wash. The sample was centrifuged once again for 10 minutes at 5000 rpm. The supernatant was discarded, and the separated particles were dried and ground. The powder was subjected to calcination at 500°C for 3 hours.

### 2.3. Characterization of $TiO_2$ nanoparticles

The absorbing range of $TiO_2$ nanoparticles was determined using ultraviolet-visible spectroscopy. The sample's chemical composition was evaluated using Energy Dispersive X-ray (EDX) analysis. The specification details of the characterization instruments used in the research are: UV-Vis-NIR Spectroscopy SHIMADZU, SEM – JEOL-JSM-6390, XRD – SHIMADZU XRD-6000, EDAX – OXFORD INCA PENTA FET-X3, and FTIR – Thermofischer Scientific. To investigate the type and crystallite size of $TiO_2$ nanoparticles, X-ray diffraction (XRD) (Rigaku) was conducted using CuK radiation (1.5406) in the 20-800 range. SEM (Tescan Vega3) and a 20 kV acceleration voltage were used to evaluate the surface morphology and particle size. The $TiO_2$ nanoparticles' Fourier Transform Infrared (FTIR) spectroscopy was obtained between 4000 and 500 $cm^{-1}$.

### 2.4. Evaluation of antibacterial activity

The antimicrobial property of biologically synthesized $TiO_2$ nanoparticles against *Escherichia coli*, *Staphylococcus aureus*, and *Acinetobacter baumannii* was tested at four different doses of 15 µl/mg, 25 µl/mg, 50 µl/mg, and 75 µl/mg of biosynthesized $TiO_2$ nanoparticles. Bacteria were grown overnight on Nutrient broth and swabbed over Mueller Hinton agar plates. Agar wells were punctured of

Table 2.
Biosynthesized $TiO_2$ nanoparticles create a zone of inhibition

| Varying concentrations of $TiO_2$ nanoparticles, µg/ml | Zone of inhibition, mm | | |
|---|---|---|---|
| | *Acinetobacter baumannii* | *Staphylococcus aureus* | *Escherichia coli* |
| 15 | 9 | 9.5 | 11 |
| 25 | 12 | 12 | 11 |
| 50 | 13 | 12 | 13 |
| 75 | 15 | 13 | 15 |
| Positive control (Streptomycin) | - | 14 | 14 |
| Negative control (DMSO) | - | - | - |

8 mm size, where the $TiO_2$ nanoparticles were added in the determined varying concentrations, and the antibacterial activity was assessed. After 24 hours of incubation at 37°C, the inhibition zone was measured and computed (Tab. 2). As positive and negative controls, streptomycin and Dimethyl sulfoxide (DMSO) were employed.

## 3. Result and discussion

$TiO_2$ nanoparticles were synthesized by biological means, using *Cynodon dactylon* herb extract. EDX, UV-Vis spectroscopy, SEM, XRD, and FTIR were used to evaluate the biosynthesized $TiO_2$ nanoparticles. EDX is a precise method for estimating titanium's qualitative and quantitative properties. EDX was used in this study to validate the presence of Ti following its synthesis using *Cynodon dactylon*. The presence of Ti in the sample was concluded by the EDX spectrum, which exhibited peaks of Ti as shown in Figure 2. $TiO_2$ nanoparticles absorb light in the UV region of 200 to 400 nm. UV-Visible spectroscopy absorbance at 220 nm was found in this investigation, as shown in Figure 3, confirming the existence of $TiO_2$ nanoparticles in the sample. Figure 4 is an SEM picture of a biostabilized $TiO_2$ nanoparticle. It describes the surface morphology of $TiO_2$ nanoparticles. The result shows many nanoparticles less than 100 nm. Many blocks of $TiO_2$ nanoparticles were observed. Magnified images showed agglomeration of nanoparticles leading to blocks of $TiO_2$ nanoparticles.

It has been previously reported that the antibacterial activity of *Allium sativum L.* against the most emerging multidrug-resistant bacteria and its synergy with antibiotics, FPE was significantly more effective against *S. aureus, E. coli,* and *E. faecalis* VRE + HLAR than the control antibiotic used (gentamicin) ($p < 0.005$) [34]. Similar results were noted by Su et al. 2015 [35] in the evaluation of the antibacterial activity of *Polygonum cupsidatum* extracts against nosocomial drug-resistant pathogens molecules.

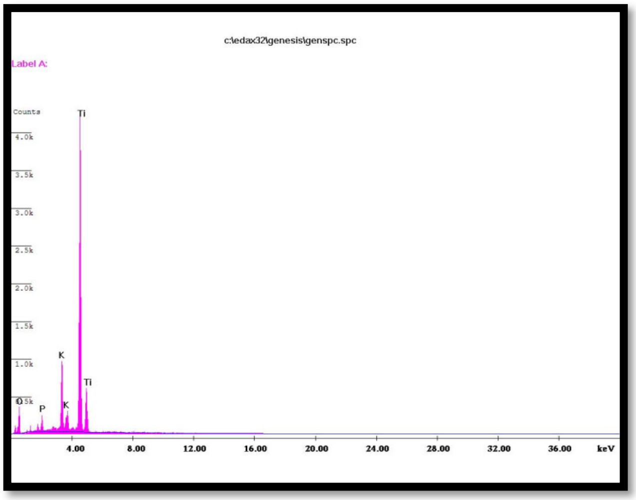

Fig. 2. EDX spectra of Ti and O in biosynthesized $TiO_2$ nanoparticles

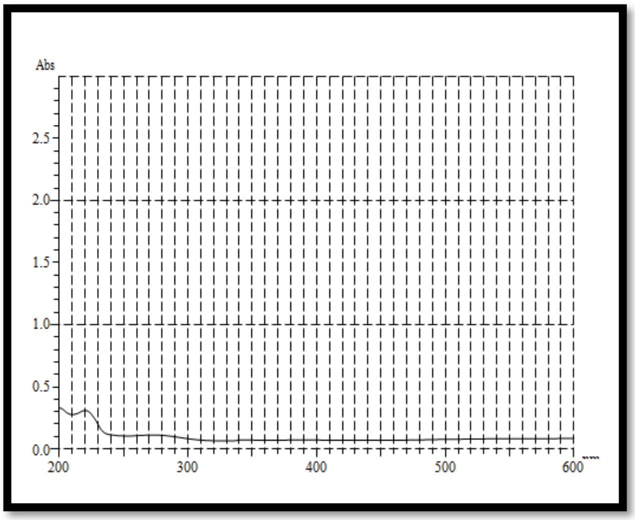

Fig. 3. The UV-Visible absorption spectrum of $TiO_2$ nanoparticles

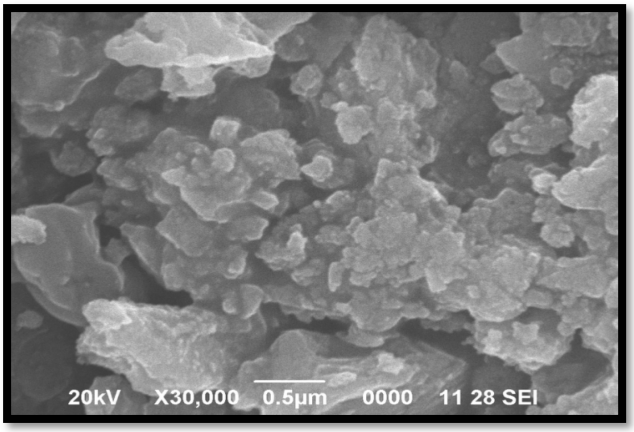

Fig. 4. The biosynthesized $TiO_2$ nanoparticles were imaged using a Scanning Electron Microscope

The XRD pattern of $TiO_2$ nanoparticles using herb extract of *Cynodon dactylon*, which has slight broadening, where the intensity of the diffraction patterns of the peaks was less. The average crystal size of titanium nanoparticles was calculated using DebieScherrer's formula $\left(D = \frac{0.94\lambda}{\beta \cos \Theta}\right)$ was 11.46 nm. The existence of $TiO_2$ nanoparticles in the biosynthesized sample was verified by XRD analysis.

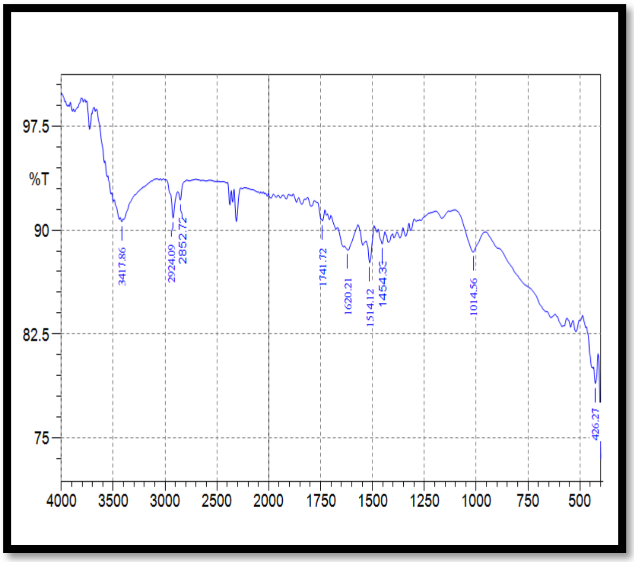

Fig. 5. Biosynthesized $TiO_2$ nanoparticles' FTIR spectrum

As illustrated in Figure 5, the functional group that crowns the nanoparticle surface was studied using FTIR. Due to Hydroxyl -OH stretching and moisture content in the sample, biosynthesized $TiO_2$ nanoparticles demonstrated broadband at 3417.86 $cm^{-1}$, 3800 to 3000 $cm^{-1}$. The peaks at 1741.21 and 1620.21 $cm^{-1}$ are caused by C=O stretching, which shows the existence of carboxyl chemicals in the plant extract, such as an aldehyde, a ketone, an ester, and other carboxylic acids. The presence of aromatic compounds is indicated by the existence of peaks at 1514.12 and 1454.33 $cm^{-1}$ within the range of 1615 to 1495 $cm^{-1}$. The C-H bending was found at the peak of 1014.56 $cm^{-1}$. This might be related to aromatic compound vibration. The stretching vibrations of Ti-O-Ti bonds were detected by the peak at 426.27 $cm^{-1}$. The symmetric and asymmetric stretching vibrations of secondary amines and carbonyl groups cause the peaks at 2924.09 and 2852.72 $cm^{-1}$.

The SEM images are the biostabilized $TiO_2$ nanoparticles obtained from the plant extract. It describes the nanoparticle's surface shape and structure. There are fewer nanoparticles in the image of 100 nm in size to be strongly agglomerated with each other. Primary particles of $TiO_2$ nanoparticles are fused or strongly bound due to the chemical bonds, hence forming aggregates. Due to van der Waal's attractive forces, these aggregates subsequently agglomerate to create micron-sized particles [21]. Hence, in this study also, $TiO_2$ nanoparticles of size less than 100 nm could be agglomerated to form blocks of $TiO_2$.

In earlier publications, the XRD analysis was utilized to confirm the existence of $TiO_2$ nanoparticles [13]. The green synthesis produced an XRD peak pattern of $TiO_2$ nanoparticles using *Cynodon dactylon*, showing a broadening in the diffraction peak and less intensity. The obtained lattice parameters were consistent and close compared to standard titanium nanoparticle data (JCPDS 21-1272). There is a correlation between the size reduction and XRD peak broadening during synthesis by the green route, as mentioned by Ahmad et al. [19]. As a result, the widened peaks in the XRD pattern confirm the reduction in the biosynthesized size of $TiO_2$ nanoparticles. It has been documented the inverse relationship between the surface functionalization of $TiO_2$ nanoparticles and peak intensity, in the earlier reports. Coating of the surface of the nanoparticles by functional groups causes the occurrence of an internal strain among the nanoparticles, resulting in the decrease of the XRD peak [22]. As a consequence, the phytochemicals in the *Cynodon dactylon* extract are believed to have coated the nanoparticles' surfaces, decreasing the strength of the XRD peaks. This phytochemical coating improves the dispensability and stability of nanoparticles, which may, in turn, enhance bioavailability, making it suitable for biological applications. As a result, the XRD profile demonstrates that the green route production of $TiO_2$ nanoparticles utilizing *Cynodon dactylon* is suitable for generating biostabilized and biofunctionalized nanoparticles with effective biomedical applications.

The FTIR results showed shifts from 1125 and 635 $cm^{-1}$ to 1053 and 618 $cm^{-1}$correlates to the phenolic and amine groups, which caps the $TiO_2$ nanoparticles. Nanoparticles coated with amides and phenols are known to have more biomedical activities than compared uncapped nanoparticles. Hence, $TiO_2$ nanoparticles synthesized by the green route may contain biomedical boons. The presence of carbonyl groups, secondary amines, and carboxylate groups in the biosynthesized $TiO_2$ nanoparticles was also shown by the FTIR spectrum. The strong Ti-O-Ti stretching vibration is related to the intense peak observed between 800 and 450 $cm^{-1}$, which shows Ti-O stretching bands. The wide absorption peak at 3417.86 $cm^{-1}$ was detected, which is attributed to the –OH group's stretching vibrations. The FTIR spectrum revealed that the $TiO_2$ nanoparticles produced from the plant extract were coated or surrounded by amines, polyphenols, and proteins. Based on the FTIR findings, the extract's amines and phenolic groups may function as a capping agent on the $TiO_2$ nanoparticles. Hence, here we can easily correlate the FTIR and XRD results confirming the stability of the $TiO_2$ nanoparticles.

As shown in Figure 6 to Figure 14, the findings of the antibacterial investigation demonstrate that $TiO_2$ nanoparticles produced using *Cynodon dactylon* have excellent antibacterial activity. The mechanism of action of $TiO_2$ nanoparticles against bacteria has yet to be determined. It was reported that nanomaterials exhibit the biocidal activity of a broader spectrum against fungi, bacteria, and viruses [23]. The nanoparticles inactivate the membrane protein, which reduces the cellular permeability, eventually resulting in cellular death as discussed by Mohanpuria et al. [24]. Nanomaterials can easily destroy the microbes since it produces retardation in the development and bacterial adhesion of biofilm [25]. Polyphenols have positive properties such as antiviral, anticancer, antifungal, and antibacterial properties. It also inhibits the exotoxins present in the bacteria [26-27]. Similarly, it is suggested that the biosynthesized $TiO_2$ nanoparticles possess good antibacterial activity causing cell damage and cell death to *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Escherichia coli*.

The agar-well diffusion test is used to assess an antimicrobial agent's efficacy against bacteria cultivated in culture. The bacteria of interest are evenly swabbed throughout the culture plates. If a chemical is effective against bacteria at a certain concentration, no colonies will form on agar at a concentration greater than or equal to the effective concentration. This is the inhibitory zone. As a result, the extent of the zone of inhibition is a measure of the compound's efficacy: the bigger the clear region surrounding the well, the more effective the drug [28].

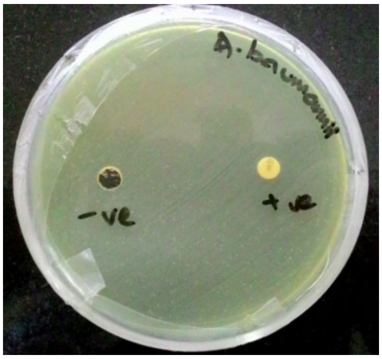

Fig. 6. Plate 1: Positive and negative controls against *Acinetobacter baumannii*

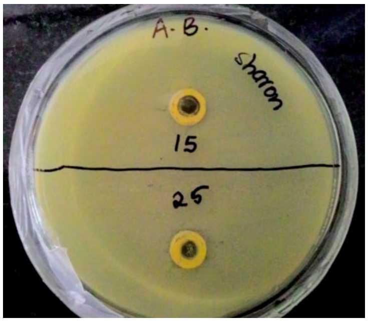

Fig. 7. Plate 2: Biosynthesized $TiO_2$ nanoparticles have antibacterial action against *A. baumannii* (15 µg/ml, 25 µg/ml)

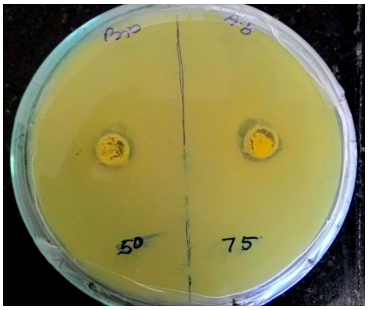

Fig. 8. Plate 3: Biosynthesized $TiO_2$ nanoparticles have antibacterial action against *A. baumannii* (50 µg/ml, 75 µg/ml)

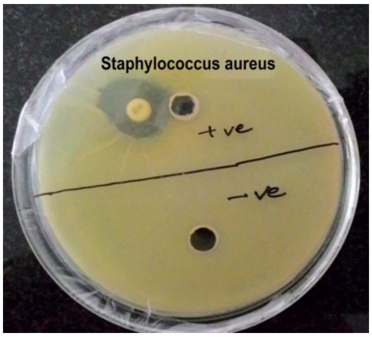

Fig. 9. Plate 4: Positive and negative controls against *Staphylococcus aureus*

Fig. 10. Plate 5: Antibacterial activity of biosynthesized $TiO_2$ nanoparticles against *S. aureus* (15 µg/ml, 25 µg/ml)

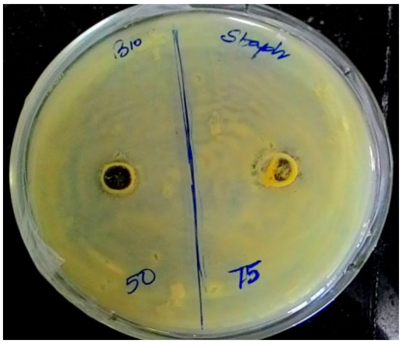

Fig. 11. Plate 6: Antibacterial activity of biosynthesized $TiO_2$ nanoparticles against *S. aureus* (50 µg/ml, 75 µg/ml)

Fig. 12. Plate 7: Positive and negative controls against *E. coli*

Fig. 13. Plate 8: Biosynthesized $TiO_2$ nanoparticles have antibacterial action against *E. coli* (15 µg/ml, 25 µg/ml)

Fig. 14. Plate 9: Antibacterial activity of biosynthesized $TiO_2$ nanoparticles against *E.coli* (50 µg/ml, 75 µg/ml)

The zone of inhibition caused by $TiO_2$ nanoparticles was shown to be quite effective against *S. aureus*, *A. baumannii*, and *E. coli*. After 24 hours of incubation, a maximal zone of inhibition of 15 mm in *A. baumannii* and *E.coli* and 13 mm in *S. aureus* was found at the highest dose tested (75 µg/ml), which was equivalent to the positive control. *A. baumannii* and *S. aureus* had a minimal zone of inhibition of 0.9 mm and 0.95 mm, respectively, whereas *E.coli* had a zone of inhibition of 11 mm for the (15 µg/ml) minimum concentration. Similar results reported that the antibacterial activity of biosynthesized $TiO_2$ (15 µg/ml) nanoparticles from *Hibiscus rosasinensis* showed potential antibacterial activity of 13.5 mm against *S. aureus* [29]. $TiO_2$ derived from *Bauhinia variegata* aqueous extract showed good antibacterial action against *E. coli* and *E. faecalis* [30].

## 4. Conclusions

*Cynodon dactylon* biosynthesis of $TiO_2$ nanoparticles is efficient, nutrition dependent, does not employ hazardous compound and occurs at neutral pH levels. This might be an alternative method for the synthesis of $TiO_2$ nanoparticles, apart from other chemical protocols, since this is quick and non-toxic. This safer $TiO_2$ nanoparticle is further incorporated in various applications such as sunscreens, shampoos, food-packaging material, etc. The disclosed technique of production of $TiO_2$ nanoparticles using herb extract has the potential benefit of being highly stable, which is a significant advantage over other methods now in use. Antibacterial activity against human infections testing can lead to its application in the creation of anti-pathogenic medicines in the pharmaceutical industry. The technique for the production of $TiO_2$ nanoparticles using *Cynodon dactylon* plant extracts, according to the findings of this study. It is an appealing green procedure that is both cost-effective and environmentally benign and it is beneficial for obtaining a high yield of $TiO_2$ nanoparticles.

## Additional information

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