Phytosynthesis of titanium dioxide nanoparticles using *Cynodon dactylon* leaf extract and their antibacterial activity

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Abstract. Due to use of nanoparticles, nanotechnology has become an important area of research penetrating in all the fields of science and technology including medicinal chemistry. Titanium dioxide nanoparticles (TiO_2 NPs) play an important role in biotechnology and nanomedicine because of their antimicrobial effect against many pathogens such as bacteria, fungus, viruses and yeast. In this article, we propose an eco-friendly phytosynthesis method of TiO_2 NPs using aqueous leaf extract of *Cynodon dactylon* herbal plant as a reducing agent. The formation of TiO_2 NPs by plant bio-molecules involved in the reduction of metal ions to nanoparticles is demonstrated. The synthesized TiO_2 NPs are analyzed using X-ray diffraction analysis, Fourier transform infrared spectroscopy, laser Raman spectroscopy and field emission scanning electron microscopy. The antibacterial activity of the TiO_2 NPs against gram-positive bacterial pathogens like *Bacillus subtilis* and *Staphylococcus aureus* as well as gram-negative bacterial pathogen like *Escherchia coli* is tested. The obtained results demonstrate potent bactericidal activity of the TiO_2 NPs.

Keywords: TiO_2, phytosynthesis, *Cynodon dactylon*, nanoparticles.

https://doi.org/10.15407/spqeo27.03.287
PACS 61.46.-w, 84.37.+q, 72.80.Jc, 78.67.Bf

Manuscript received 24.10.23; revised version received 12.06.24; accepted for publication 11.09.24; published online 20.09.24.

1. Introduction

Nowadays, synthesis of metal and metal oxide nanoparticles (NPs) receives considerable attention due to applications of such NPs in various fields such as photocatalysis, electronics, sensing, and biotechnology [1–3]. The most important and distinct characteristic of metal oxide nanoparticles is the increased of their surface-to-volume ratio, which is responsible for their fascinating optoelectronic, magnetic, photocatalytic [4, 5] and antimicrobial [6, 7] properties, with the decrease in their size. Earlier [4], antibacterial activity of TiO_2 NPs synthesized by the Muller–Hinton agar method, against *Escherchia coli*, *Bacillus subtilis* and *Staphylococcus aureus* at various concentrations and antimicrobial activity of such NPs produced by the Kirby–Bauer method [6], were investigated. The biological activity of NPs increases with the increase in their specific surface area due to the increase in surface energy. Titanium dioxide (TiO_2) is widely used as an environmentally friendly catalyst because of its very high chemical stability, strong oxidizing power, excellent optical properties and non-toxicity [8–11]. TiO_2 nanoparticles have a broad range of applications for air and water purification, dye-sensitized solar cells, electrochemical devices, pharmaceuticals, sunscreen products particularly to protect skin from the UV rays, white pigments, plastics, paper and inks, and colorants to whiten food and toothpaste [12]. Moreover, the plants use titanium for producing carbohydrates and for the photosynthesis process [13].

Metal oxide NPs with pure and well defined shapes are synthesized by various physical and chemical methods. However, these methods are quite expensive and potentially hazardous to the environment. Moreover, some of the toxic chemicals are absorbed on the surface of the nanoparticles during chemical preparation that may call harmful effects in medical applications [14]. This problem can be overcome by synthesizing NPs by green synthesis methods [15, 16]. Compared to the physicochemical methods, green synthesis has the advantage of being low-cost, environmentally friendly, simple and suitable for medical use. It does not require use of high-quality materials and poisons. Many biological organisms such as plants, algae [17], diatoms [18, 19], bacteria [20–22], enzymes [23, 24], yeast [25], and fungi [26–28] are known to exist. Human cells [29] can convert metal ions extracted from proteins and plant metabolites found in these organisms into metal nanoparticles.

Cynodon dactylon has many names, including *Doob grass, Durva grass, Devil's grass, and Bermuda grass*. *Cynodon dactylon* is a creeping plant 4 to 15 cm long and 1 foot high growing in the tropical climate. Because of its antimicrobial and antiviral properties, it has great significance in Ayurveda in India and is considered as a sacred plant. Phytochemical analysis shows that this plant contains alkaloids, flavonoids, glycosides, tannins, terpenoids, triterpenoids steroids, resins, phytosterols, saponins, carbohydrates, proteins, and glued oils [30]. Formation of TiO_2 NPs by *Cynodon dactylon* leaf metabolites is schematically represented in Fig. 1.

The chemical reactions implicated in the reduction of metal ions (Ti^{4+} & Ti^{3+}) into TiO_2 NPs are discussed in [31]. Ti^{4+} and Ti^{3+} metal ions combine with reduced metabolites and stabilizers to form metal complexes, resulting in the reduction of metal ions. The resulting metal ions and metabolites interact with other complexes *via* van der Waals interactions to form small titanium metal NPs. Then, through the coarsening process, the small particles gradually grow in size and fuse together, ultimately yielding stable TiO_2 NPs. Therefore, the plant metabolites serve as stabilizing agents for formation of metal oxide NPs. This article describes the phyto-synthesis method of TiO_2 NPs from titanium tetra-isopropoxide solution using leaf extract of *Cynodon dactylon* in an aqueous medium and the results of the study of antibacterial activity of such NPs against gram-negative bacterial pathogen like *Escherichia coli* and gram-positive bacterial pathogens like *Bacillus subtilis* and *Staphylococcus aureus*.

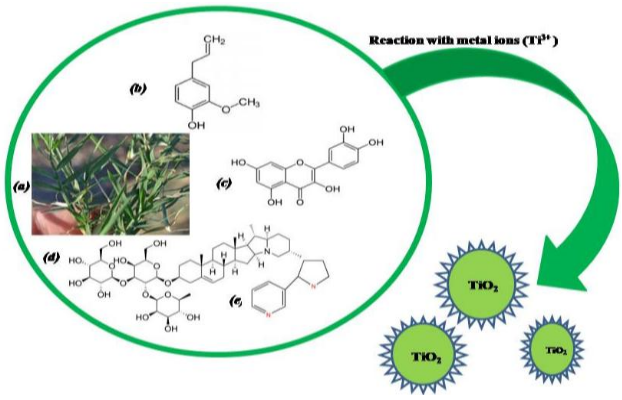


Fig. 1. Schematic representation of the formation process of TiO_2 NPs by *Cynodon dactylon* leaf metabolites. *Cynodon dactylon* leaves (a) and some of its chemical constituents: terpenoids (b), flavanoids (c), alkaloids (d), and saponins (e), which act as reducing as well as stabilizing agents.

2. Experimental

2.1. Synthesis of TiO_2 nanoparticles

Cynodon dactylon leaves (Fig. 1a) were collected in and around the area of Thuraiyur Taluk, Tiruchirapalli District, Tamilnadu. Ten grams of the *Cynodon dactylon* leaves were thoroughly washed with distilled water. Finely cut leaves were dried under the sunlight for three days. The dried leaves were pounded into powder form using an agate motor. An aqueous leaf extract of *Cynodon dactylon* was prepared by boiling the latter in 90 ml of deionized water. The obtained leaf extract was filtered using Whatman No.1 filter paper (pore size 25 µm) and stored at 4 °C for further use. To produce TiO_2 NPs, 0.1 M titanium tetraisopropoxide was added to 10 ml of boiled *Cynodon dactylon* leaf extract solution and stirred at 90 °C for 4 h. The formed TiO_2 NPs were centrifuged at 10k rpm for 15 min, washed with water and centrifuged again at 5000 rpm for 10 min. The separated TiO_2 NPs were dried and ground to calcinate at 75 °C in a muffle furnace for 12 hours. The calcined TiO_2 NPs were used for further experiments.

2.2. Instrumentation

Phase purity and structure of the TiO_2 NPs were analyzed by XPERT-PRO analytical X-ray Diffractometer with X'Pert High Score Plus software operating at a voltage of 45 kV and current of 30 mA with Cu Kα radiation of 1.5405 Å in the scanning range of 2θ configuration from 10° to 80°. The plant leaf extracts and TiO_2 NPs were characterized by Fourier transform infrared (FT-IR) spectroscopy (Thermo Nicolet-380 Madison, USA) at a resolution of 4 cm in the range of 4000 to 400 cm^{-1} at room temperature. Furthermore, TiO_2 NPs synthesized from the *Cynodon dactylon* leaf extracts were studied by Raman spectroscopy using a Princeton Acton SP2500 CS spectrometer with a 0.5 focal length triple grating excitation by an Ar^+ laser (514.5 nm). Surface morphology of TiO_2 NPs was determined using field emission scanning electron microscopy (FESEM) (Leo Supra 50VP field emission SEM) at the accelerating voltage of 10 kV.

2.3. Antibacterial activity

In vitro antibacterial activity against bacterial pathogen *Escherichia coli* was studied by the agar well diffusion method on Mueller–Hinton agar (MHA) [32]. About 10–40 µg of TiO_2 NPs were introduced in the well (6 mm in diameter) of a Mueller Hinton agar plate previously seeded with 50 µl of broth culture of the test organism *E. coli* (18–24 hours single colonies on agar plates torpidly of 0.5 McFarland Standard to 1.5×10^8 colony forming unit (CFU/ml)). The turbidity of the bacterial suspension was measured by an UV-Visible spectro-photometer (Shimadzu UV-3600 PLUS) at 600 nm. The plates seeded with the test organism were incubated at 37 °C for 24 hours. After the incubation periods, the diameter of the growth inhibition zone on the plates was measured. All the tests were performed in triplicates.

3. Results and discussion

3.1. XRD pattern analysis

The X-ray diffraction (XRD) spectra of the synthesized TiO_2 NPs and the *Cynodon dactylon* leaf extract are shown in Fig. 2. For the TiO_2 NPs, sharp diffraction peaks are observed at 25.3°, 37.8°, 48.0°, 53.9°, 55.1°, 62.7°, 68.8°, 70.3° and 75.1° corresponding to (200), (105), (211), (204), (116), (220) and (215) planes, respectively. The XRD patterns agrees with the JCPDS Card No. 89-4921 which confirms that the NPs synthesized using the plant extract are crystalline in nature. The XRD peaks at 25.3° and 48.0° correspond to (101) and (200) crystal planes, proving that the TiO_2 NPs have an anatase form. The increase in the XRD peak width points to the decrease in the NP size, which is in the nanometer range. The average size of the prepared TiO_2 nanocrystals was determined using the Scherrer formula $d = 0.89\lambda/\beta\cdot\cos\theta$, where λ is the X-ray wavelength, and β is the full width at half-maximum of the XRD peak at the angle θ, and was equal to 20 nm.

3.2. Fourier transform infrared spectroscopy

FTIR investigations were used to reveal the compounds responsible for the formation and stabilization of TiO_2 NPs. The FTIR spectra of the prepared TiO_2 NPs and the *Cynodon dactylon* leaf extract are shown in Fig. 3.

The peaks at 3400 and 2923 cm^{-1} correspond to the O–H stretching mode of alcohol and C–H stretching mode of alkanes, respectively. The absorption band at 2255 cm^{-1} is attributed to the C=C stretching mode of alkynes, and the peaks at 1593 and 1404 cm^{-1} may be assigned to the C=C vibrations of aromatic rings. The peaks at 511, 686, and 773 cm^{-1} may be respectively assigned to the metal–oxygen bond stretching and vibration modes. The FTIR results show that O-H bonds and C=C groups are present in the *Cynodon dactylon* leaf extract. This indicates that terpenoids, flavonoids, glycosides, tannins, and protein compounds are effective in reducing metal ions to metal oxide nanoparticles.

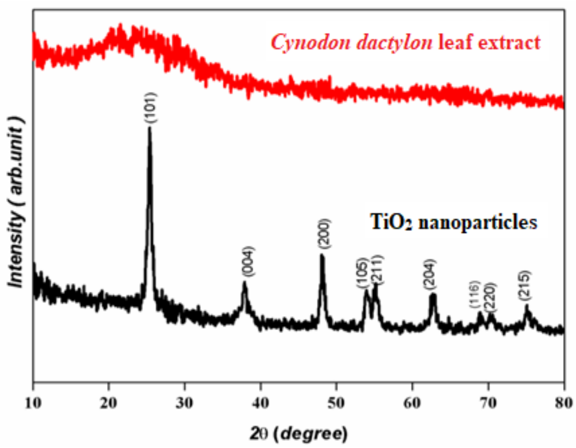


Fig. 2. Powder XRD patterns of TiO_2 NPs and *Cynodon dactylon* leaf extract.

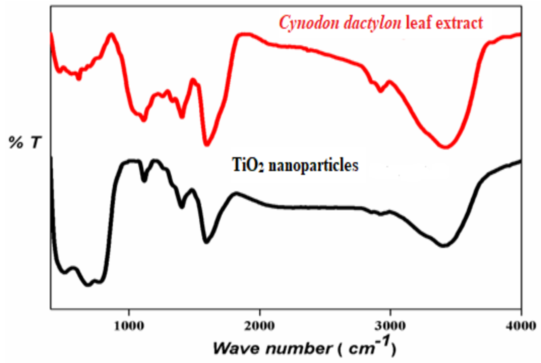


Fig. 3. FTIR spectra of TiO_2 NPs and *Cynodon dactylon* leaf extract.

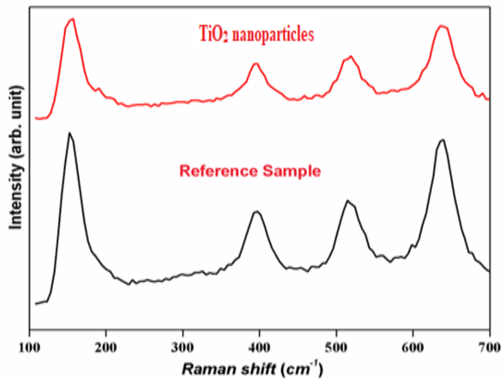


Fig. 4. Raman spectra of reference sample (anatase TiO_2) and synthesized TiO_2 nanoparticles.

In the case of flavonoids, during the tautomeric transformation from the enol form to the ketone form, active hydrogen atoms participate in reducing metal ions to metal nanoparticles.

3.3. Raman spectroscopy

Formation of the TiO_2 NPs due to the metabolites of the *Cynodon dactylon* leaf extract was studied using Raman spectroscopy. Fig. 4 shows Raman spectra of the synthesized TiO_2 NPs and reference anatase TiO_2.

According to [34, 35], anatase TiO_2 exhibits six active Raman modes in the lattice vibrational spectrum: $A_{1g} + 2B_{1g} + 3E_g$. In our study, the prepared TiO_2 NPs show Raman shifts at 145 cm^{-1} (I_g), 399 cm^{-1} (B_{1a}), 516 cm^{-1} (A_{1q} and B_{1q} doublet) and 639 (E_q) cm^{-1}. In its turn, the anatase TiO_2 exhibits characteristic peaks at 145, 399, 516, and 639 cm^{-1} that correspond to the symmetries of I_g, B_{1g}, E_g, and A_{1g}, respectively. The peak at 639 cm^{-1} coincides with that of the reference anatase phase of the TiO_2 NPs indicating a high degree of structural symmetry [35]. This means that the TiO_2 NPs have a long-range order in the anatase phase. However, the shift of the Raman bands towards higher wave numbers with intensity decrease, which confirms that absorption of metabolites of the *Cynodon dactylon* leaf extract takes place on the surface of TiO_2, which results in the formation of TiO_2 NPs. When the particle size decreases to the nanometer scale, two effects on the vibrational properties of the materials might occur. Firstly, the volume of NPs shrinks due to the appearance of the size-induced radial pressure, which leads to the increase in the force constants. Therefore, the Raman bands shift towards higher wave numbers. Secondly, the amplitude of vibrations of the nearest neighbouring bonds decreases due to the increase of the mean square relative displacement. As the particle size decreases, the changes in the vibrational amplitude affect the intensity of the Raman bands.

3.4. Field emission scanning electron microscopy

Fig. 5 shows the surface morphology of the phyto-synthesized TiO_2 NPs obtained by FESEM. The images at different magnifications presented in Fig. 5 visualize the morphology, particle size and aspect ratio of the samples under study. It is found out that some of the prepared TiO_2 NPs are smooth and spherical in shape, while other NPs have no specific shape.

3.5. Antibacterial activity

Antibacterial activity of the TiO_2 NPs against growth of bacteria was studied by agar well diffusion technique by testing zone formation at different dilutions of TiO_2 NPs: 20, 30, 40 and 50 μg. Fig. 6 presents the antibacterial activity of the TiO_2 NPs against gram-negative and gram-positive bacterial pathogens. The zone of inhibition against the test bacterial pathogens such as *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* are presented in Table.

According to the obtained results, high antibacterial activity against gram-negative bacteria *E.coli* (19 mm) and gram-positive bacteria *Bacillus subtilis* (17 mm) and *Staphylococcus aureus* (18 mm) is positively reported by TiO_2 NPs. The antibacterial activity of the TiO_2 NPs was concentration dependent and the inhibition zone increases with increasing the concentration of TiO_2 NPs.

The highest antibacterial activity was exhibited against *E. coli* when compared to other bacteria. *E. coli* is one of the most important causative agents which causes nosocomial infection and is resistant to most of the broad spectrum antibiotics. In this work, an antibiotic chloramphenicol against *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* was also tested as a positive control. The respective inhibition zone diameters of 16 ± 0.0 mm, 20 ± 0.0 and 20 ± 0.1, respectively, were obtained. It was concluded that the TiO_2 NPs exhibited better inhibition as compared to the commercially available antibiotic chloramphenicol. Our study is also supported by the findings of Morteza Haghi *et al.* [34], who evaluated the antibacterial activity of TiO_2 NPs against a pathogenic strain of *E. coli* and found that 0.0%, 0.5% and 1% and 1.5% of TiO_2 NPs were evaluated by optical density reduction and zone of inhibition

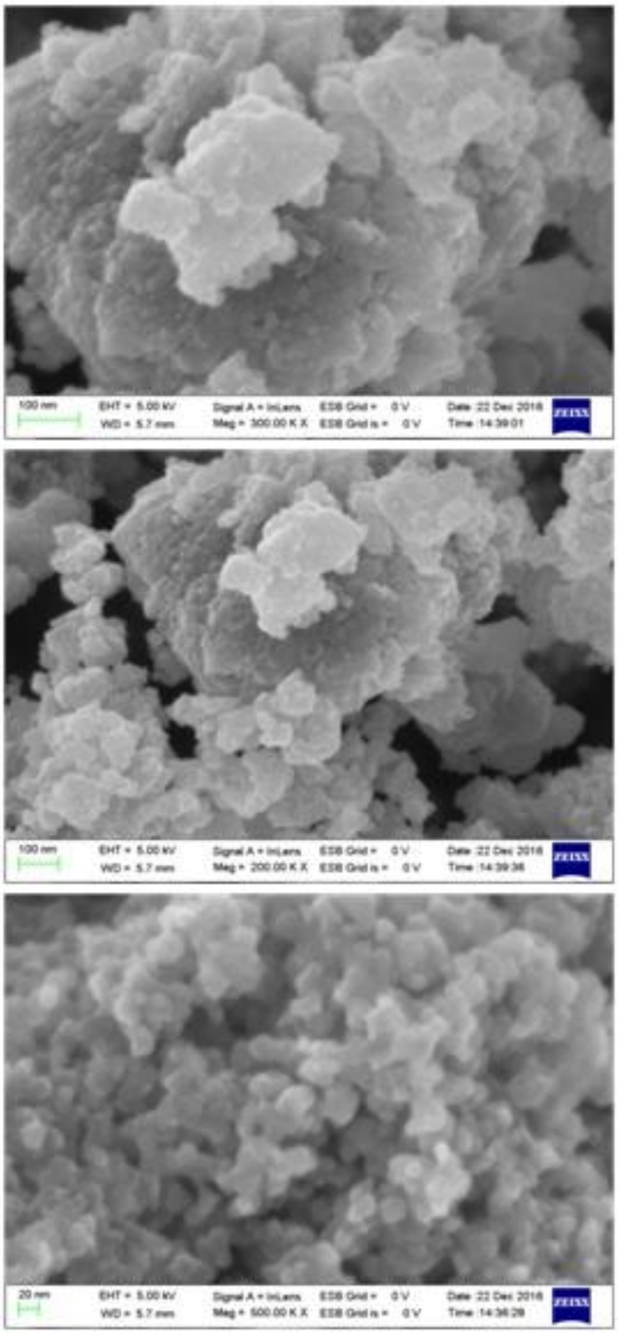
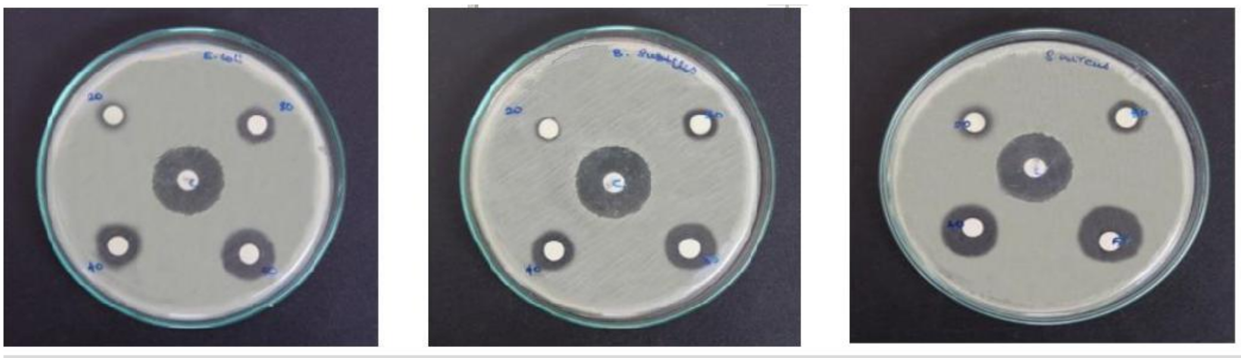


Fig. 5. FESEM image of phytosynthesized TiO_2 NPs.

Table. Antibacterial activity TiO_2 NPs against the bacterial pathogens and its inhibition zones.

Concentration (μg)	Inhibition zone width (mm)		
	Escherichia coli	*Bacillus subtilis*	*Staphylococcus aureus*
20	15 ± 0.0	10 ± 0.3	11 ± 0.4
30	17 ± 1.5	13 ± 1.2	14 ± 0.7
40	16 ± 0.5	15 ± 0.7	16 ± 0.6
50	19 ± 1.0	17 ± 0.8	18 ± 0.8
Control	16 ± 0.0	20 ± 0.0	21 ± 0.1



(a) (b) (c)

Fig. 6. Antibacterial activity of phytosynthesized TiO_2 NPs against bacterial pathogens: (a) *Escherchia coli*, (b) *Bacillus subtilis*, and (c) *Staphylococcus aureus*.

Kirby–Bauer disc diffusion techniques. It was found out that the NPs inactivate cellular enzyme and DNA by binding to electron donating groups such as amides, indos, and hydroxyls. They also create little pores in bacterial cell walls thus killing the bacteria. Therefore, it is evident from the obtained results that the TiO_2 NPs have a potent bactericidal activity.

4. Conclusions

TiO_2 NPs were successfully synthesized by the green synthesis method using *Cynodon dactylon* leaf extract as a raw material. The prepared TiO_2 NPs were characterized by XRD, FTIR and Raman spectroscopy, and their surface morphologies were examined by FESEM. A schematic diagram presents the process of reduction of metal ions to nanoparticles by plant metabolites to produce TiO_2 NPs. The FTIR and Raman spectroscopy showed that the TiO_2 NPs were produced from plant metabolites *via* a bio reduction process. The XRD analysis confirmed that the synthesized particles are TiO_2 NPs in the anatase form. The average particle size calculated from the high-intensity XRD peak (the strongest peak in the XRD pattern) was 20 nm. Antibacterial activity of the TiO_2 NPs against gram-negative bacterial pathogen *Escherichia coli* and gram-positive bacterial pathogen *Bacillus subtilis* was evaluated. The NPs showed better activity against *Escherichia coli* as compared to others bacteria. This activity exceeded that of the commercial antibiotic chloramphenicol.

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Фітосинтез наночастинок діоксиду титану з використанням екстракту листя *Cynodon dactylon* та їх антибактеріальна активність

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Анотація. Завдяки використанню наночастинок нанотехнології стали важливою галуззю досліджень, проникаючи усі області науки та технології, включаючи медичну хімію. Наночастинки діоксиду титану (НЧ TiO_2) відіграють важливу роль у біотехнології та наномедицині внаслідок антимікробної дії проти багатьох патогенів, таких як бактерії, грибки, віруси та дріжджі. У цій статті нами запропоновано екологічний метод фітосинтезу наночастинок TiO_2 з використанням водного екстракту листя трав'яної рослини *Cynodon dactylon* як відновлюючого агента. Продемонстровано, що механізм утворення НЧ TiO_2 біомолекулами рослин полягає у відновленні іонів металів до наночастинок. Синтезовані НЧ TiO_2 проаналізовано з використанням рентгенівської дифрактометрії, інфрачервоної спектроскопії з перетворенням Фур'є, лазерної раманівської спектроскопії та автоемісійної скануючої електронної мікроскопії. Протестовано антибактеріальну активність НЧ TiO_2 проти грам-позитивних бактерій, таких як *Bacillus subtilis* і *Staphylococcus aureus*, а також проти грам-негативних бактерій, таких як *Escherchia coli*. Отримані результати продемонстрували потужну бактерицидну дію НЧ TiO_2.

Ключові слова: TiO_2, фітосинтез, *Cynodon dactylon*, наночастинки.