Phytofabrication of TiO_2 nanoparticles and their antibacterial efficacy: a study using *Cucumis sativus* and *Raphanus sativus*

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Green synthesis has attained a great importance in the field of science due to its enormous advantages over conventional methods. The synthesis of metal nanoparticles from plants is a convenient and ecofriendly process with its applications in various fields like drug delivery, environmental remediation and agriculture sector. The present study describes the synthesis of titanium nanoparticles by *Cucumis sativus*, and *Raphanus sativus* using simple method. The nanoparticles synthesized using plant extracts were calcinated at 400 °C to obtain the dried nanoparticles and characterized through various characterization techniques like X-ray diffraction (XRD), Fourier-transformed infrared spectroscopy (FTIR), Energy dispersive X-ray spectroscopy (EDX), and Scanning electron microscope (SEM). The characteristic peak for the synthesis of TiO_2 nanoparticles was appeared in FTIR. The phytochemicals present in the plant extract act as capping as well as reducing agents and are responsible for the synthesis of metallic nanoparticles. The antibacterial activity of green synthesized nanoparticles was assessed against various bacterial strains. The good antibacterial properties of titanium nanoparticles suggest their useful applications in the field of medicine.

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1. Introduction

Plant extracts are better biological mediators for the synthesis of nanoparticles than microorganisms [1]. They have a wide range of metabolic functions and are plentiful, stable, safe to use, cost-effective and kind to the environment. The synthesis of the nanoparticles from the biological (green) systems has been the subject of numerous studies for use in the pharmaceutical, environmental, cosmetic, biomedical, and agricultural fields [2]. Moreover, plant extracts are more scalable and provide quick metal ion reduction [3]. Phytochemicals are the primary agents thought to be responsible for synthesizing nanoparticles facilitated by plants [4]. The main phytochemicals that cause the ions to reduce spontaneously are quinones, carboxylic acids, terpenoids, and flavonoids. It is possible to stabilize and reduce the nanoparticles using various plants [5]. Generally, different physical and chemical techniques have been employed for synthesizing NPs, depending on how well the protocols worked to accomplish the desired applications. Physical techniques like ball milling, flame pyrolysis, laser ablation, and electric arc discharge, so forth, typically call for pricey equipment as well as high pressure and temperature [6]. On the other hand, NPs are made chemically by reducing or breaking down metal complexes in the solutions with different chemical reductants, like hydrazine, sodium borohydride, or at high temperatures [7]. Despite the widespread use of these

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techniques, the reductants, stabilizers, reactants, and different organic solvents employed in them are poisonous and may pose a risk to the environment [8]. Green chemistry, which is currently a developing phenomenon for synthesizing different chemical products, comprising NPs, significantly lessens the environmental threat by removing potentially harmful substances from methods of preparation that are harmful to human health [9].

Titanium dioxide nanoparticles, or TiO_2 NPs, are expected to have a variety of important effects on the morphophysiological and biochemical characteristics of some plants [10]. Additionally, it has been reported that the application of titanium dioxide nanoparticles enhanced the production of chlorophyll, the potential activity of antioxidant enzymes and rubisco, and the photosynthetic rate by a large amount, all of which have a major positive impact on crop yield [11]. Another study found that nano-TiO_2 had a beneficial effect on plant growth, soluble sugars, proline contents, and antioxidant defense system. It also showed a decrease in hydrogen peroxide and malondialdehyde contents [12]. Additionally, a different study showed that exogenous applications of the titanium dioxide nanoparticles could mitigate the effects of salinity stress on tomato crops by enhancing yield, phenolics, agronomic parameters, chlorophyll contents and antioxidant capacity [13]. TiO_2 NPs can be produced using a variation of top-down and bottom-up techniques, including solvothermal, hydrothermal, co-precipitation, sol-gel, combustion, biological methods, and microwave-assisted processes [14]. Biological method can be divided into three primary groups: a) Using microorganisms i.e. fungi and bacteria b) Making use of plant parts and their extracts or powder; and c) Making use of templates such as viruses and DNA [15]. Plants extracts and plants have become more and more popular over time. Numerous uses resulting from the effective biosynthesis of TiO_2 NPs are investigated. Titanium nanoparticles have many uses, one of which is photocatalysis [16].

Since titanium dioxide nanoparticles (TiO_2 NPs) have a high degree of chemical stability, low toxicity, and photocatalytic properties, they are widely accepted in the environmental arena. These particles have been applied as food colorants, cosmetics, dyes, toothpaste and pharmaceuticals, in addition to providing UV protection for the skin [17]. In addition, TiO_2 NPs have stronger antioxidant and antibacterial properties than other metal nanoparticles (such as Au, ZnO, and Ag, NPs) because of their photocatalytic feature [18]. TiO_2 nanoparticles find extensive application in drug delivery. Particles assume different forms, such as tablets and capsules. Drug's controlled release action is carried out by the combination of the nanoparticles and the drug. Drugs such as daunorubicin and valproic acid employ TiO_2 NPs as carriers [19]. TiO_2 nanoparticles exhibit photocatalytic antimicrobial activity upon exposure to UV light (<385 nm) [20]. The microbial cell surface thickness determines how effective TiO_2 NPs' antimicrobial activity is, and have an order of virus > bacterial wall > bacterial spore. The bacterial membrane is harmed by the photocatalytic activity of the TiO_2 NPs, which increases the peroxidation of the unsaturated phospholipids in the plasma membrane [18]. Additionally, it obstructs important biological processes such as semi-permeability, oxidative phosphorylation reaction, and respiration [21].

The current research study has been accomplished by synthesizing titanium oxide nanoparticles using plant extract of *Cucumis sativus*, and *Raphanus sativus* and their characterization done by FTIR, XRD, SEM and EDX. However, antibacterial potential of these particles checked against six bacteria although streptomycin used as standard drug.

2. Experimental work

2.1. Collection of plant and preparation of green extract

All plants were collected from local market of District Sialkot, Pakistan and were thoroughly washed, dried and then extract was obtained. Fresh *Cucumis sativus*, and *Raphanus sativus* were washed and dried under shade after which they were cut in small pieces and added in distilled water in a large beaker and kept on heating plate and heated for 2-3 hours at 60°C. The extract was filtered and stored in refrigerator for further use [22]. Both plant extracts were employed for synthesis of nanoparticles.

2.2. Synthesis of TiO_2 nanoparticles

The 0.1M titanium butoxide solution was prepared in 20 mL distilled water. 20 mL of aqueous plant extract which was taken in an Erlenmeyer flask and titanium solution was added into it, the color of leaf extract turned milky. The resulting solution was kept on shaker and set on 1200 rpm for 1 hour [23]. The solutions were transferred in china dish and dried in oven at 100°C for 12 hours and nanoparticles were calcinated in furnace for 4 hours at 400 °C after which solid nanoparticles were crushed into a fine powder using mortar. The white powdered titanium dioxide nanoparticles were obtained which were then preserved for further analysis and characterization. The overall scheme for synthesis is shown in figure **1**.

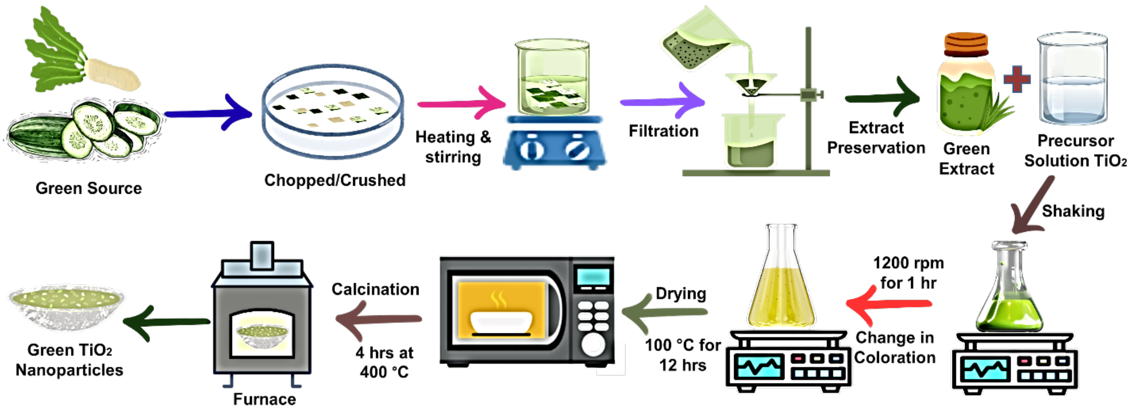

Fig. 1. Green synthesis of TiO_2 NPs using extracts of Cucumis sativus and Raphanus sativus.

2.3. Antibacterial activity

Antibacterial activity of the synthesized nanoparticles was performed against different strains bacteria using disk diffusion method considering already reported with few modifications [24]. A complete process for antibacterial activity was shown in figure **2**. Sample solution was prepared by adding 0.02 g of the synthesized nanoparticle in 4 mL distilled water. 7.5 g of agar-agar and 7.3 g of nutrient broth were dissolved in 200 mL of distilled water by continuous heating. The mixture was autoclaved for 1 hour. After the solution had been sterilized, 20 mL of the nutrient agar medium was added on a petri plate and was allowed to solidify into a gel-like consistency. The disks were placed at suitable distance in the culture medium and 30 µL of all the samples were loaded on these disks using micropipette. Laminar flow cabinet was used for whole study in order to avoid any contamination. The petri plates were then kept in incubator for 24 hours at 37 °C. Zone of inhibition of bacteria by different samples were estimated by measuring the diameter in millimeters.

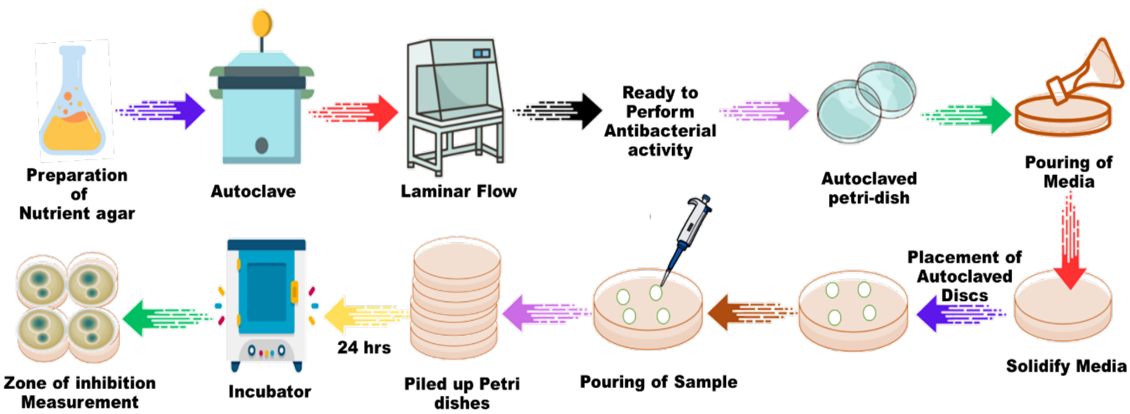

Fig. 2. Flowsheet of antibacterial activity process.

3. Results and discussion

3.1. Fourier transform infrared spectroscopy

Fourier Transform-Infrared Spectroscopy is used for determining the functional groups of the chemicals in range of 400-4000 cm^{-1} [25]. The FTIR spectrum of *Cucumis sativus* derived titanium nanoparticles showed prominent peaks at 3239.34, 103.083 and 606.50 cm^{-1}. The broad peak at 3239.34 cm^{-1} is due to the O-H stretching of alcoholic group [26]. The sharp peak at 606.50 cm^{-1} corresponds to the Ti-O-Ti stretching which is the characteristic peak of titanium nanoparticles and indicates the formation of titanium nanoparticles. The sharp peak at 1103.08 cm^{-1} denotes C=O stretching vibrations of the carboxylic acids [27]. FTIR spectrum of titanium nanoparticles synthesized from *Raphanus sativus* showed major peaks at 3328.53, 2976.10, 1584.71, 1393.08, and 1145.06 cm^{-1}. The peak at 3328.53 cm^{-1} is due to the stretching vibration of OH band which is the indication of presence of phenolic compounds and alcohol, while the peak at 1393.8 cm^{-1} corresponds to N-O bending vibration [28]. The peak at 565.04cm^{-1} is due to the Ti-O stretching confirming the formation of titanium nanoparticles. The peak at 2976.10 cm^{-1} corresponds to the C-H bonds presence [29]. The peak at 1584.71 cm^{-1} is due to the C=O stretching vibrations of the amide which is characteristic of –COOH functional group as shown in figure **3** [30].

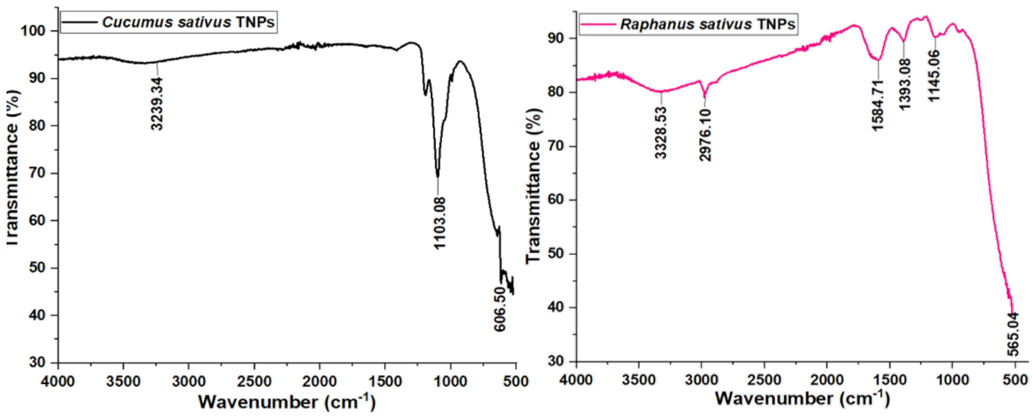

Fig. 3. FTIR spectrum of Cucumis sativus, and Raphanus sativus derived titanium nanoparticles.

3.2. Scanning electron microscope

SEM analysis was done to evaluate the size and morphology of the *Cucumis sativus* titanium dioxide nanoparticles. The synthesized nanoparticles showed a rough, irregular and heterogenous structure with size ranging from 10 to 55 nm. The scanning electron microscope (SEM) analysis done for the evaluation of size and morphology of TiO_2 nanoparticles synthesized from the *Raphanus sativus* leaves showed spherical, smooth and uniformly distributed TiO_2 nanoparticles (figure 4). The uniform nanoparticles were gathered in the form of clusters and their uniform distribution may be due to the phytochemical constituents of the *Raphanus sativus* leaves extract.

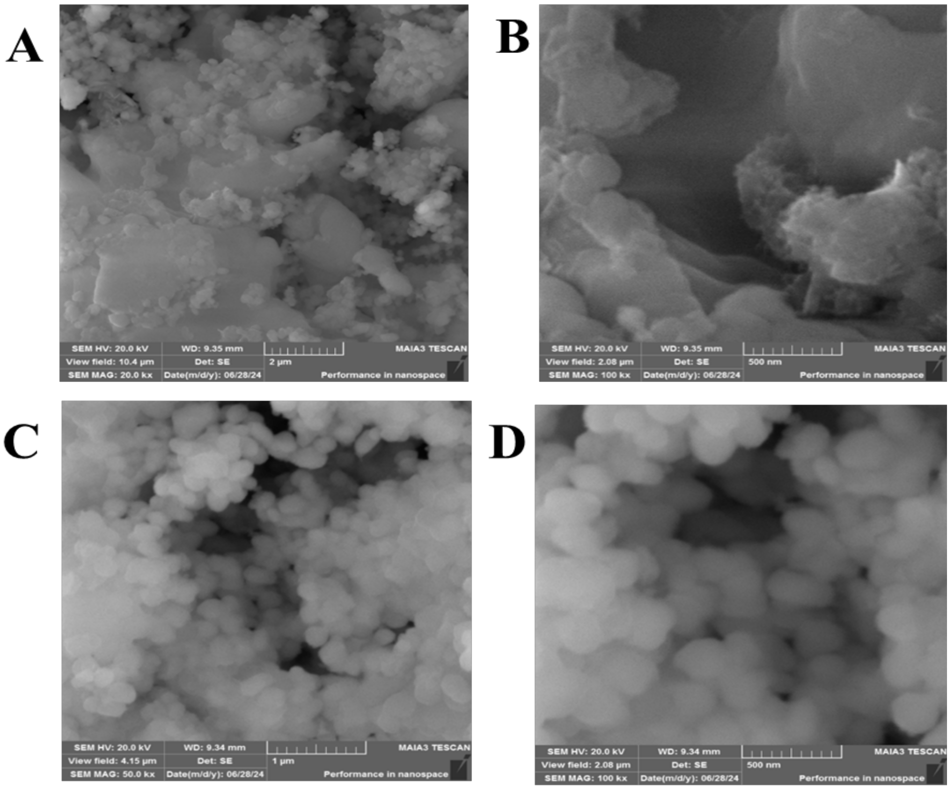

Fig. 4. SEM images of Cucumis sativus, and Raphanus sativus, based titanium dioxide nanoparticles.

3.3. Energy dispersive X-ray

EDX analysis was used for quantification and identification of elements present in respective samples [31]. EDS spectrum of *Cucumis sativus* TNPs exhibited a titanium peak at 4.7 keV while other elements also appeared like K, Zr, Cl, and Na mainly because of the phytochemicals present in the plant extract. The EDS spectrum of *Raphanus sativus* TNPs showed a strong Titanium peak at 4.7keV. The presence of O and Ti indicates the formation of titanium dioxide nanoparticles. A feeble peak of Al may arise due to the traces of aluminum foil used for covering flasks and china dish during synthesis process. Very short peaks of K indicate the phytochemicals present in the plant extract. EDS spectrum of *Raphanus sativus* TNPs exhibited a strong titanium peak at 4.7keV.O is due to the titanium dioxide NPs and Al appeared may be from the use of aluminum foil while other elements like K, Cl, Mo, Mg and Na can be attributed to the biomolecules in the plant extract (figure 5).

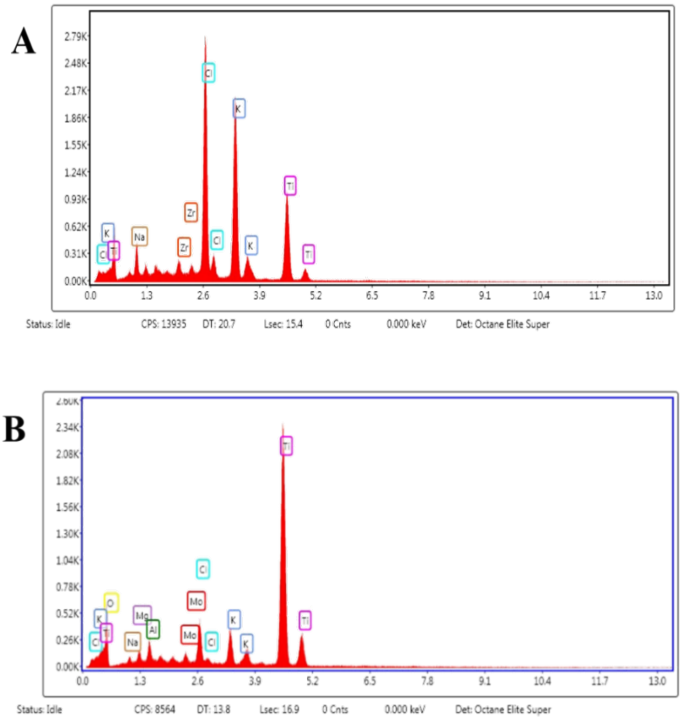

Fig. 5. EDX spectrum of A) Cucumis sativus, B) Raphanus sativus TNPs.

3.4. X-ray diffraction studies

The XRD spectrum exhibited peaks with 2θ at 27.50°, 36.15°, 41.33°, 54.45°, 56.78° and 69.50°. These peaks relate to the rutile TiO_2 values of (110), (101), (200), (111), (211), (220), (112), (301), (302), (212) and (022). The obtained XRD spectrum revealed the tetragonal structure and all the values were compared to standard JCPDS No. No. 00-001-1292 [32] for *Cucumis sativus*, and 00-004-0551 for *Raphanus sativus* extract [33]. All the peaks are matched with the rutile phase of titanium dioxide structure (figure 6).

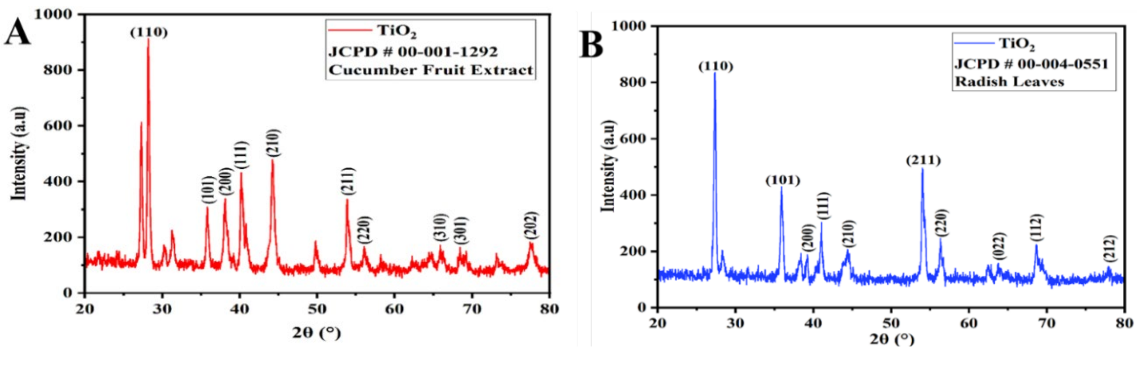

Fig. 6. XRD spectrum of Cucumis sativus, and Raphanus sativus based titanium dioxide NPs.

3.5 Antibacterial activity

The antibacterial activity of the plant extracts and their titanium-based nanoparticles was studied against bacterial strains i.e. *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Chromohalobacter israelensis* and *Halomonas halophila*. The results in the form of inhibition zone are shown in table **1**. It is observed that plant extracts are less active as compared to their nanoparticles. *Cucumis sativus* TNPs, *Raphanus sativus* TNPs both exhibited almost excellent results against *H.halophila* with inhibition zones of 22.6 mm and 27.3 mm, respectively, in comparison to standard streptomycin 37.5 mm. However, against *N. gonorrhoeae* exhibited about 20 mm inhibition zone while there is low response exhibited *against E. coli* and *S. typhimurium.* Antibacterial activity results in graphical presentation are presented in figure 7.

Table 1. Antibacterial activity of synthesized nanoparticles.

Samples	**Inhibition Zone (mm)**					
	E. coli	***S. typhimurium***	***S. aureus***	***N. gonorrhoeae***	***C. israelensis***	***H. halophila***
C. sativus	8.7	9.1	8.3	10.4	7.9	8.3
C- TNPs	10.2	12.7	16.5	20.1	15.6	22.6
R. sativus	6.2	6.9	8.3	8.6	9.2	11.3
R- TNPs	9.7	8.6	16.1	21.8	16.5	27.3
Streptomycin	31.2	33.7	22.7	28.7	33.4	37.5

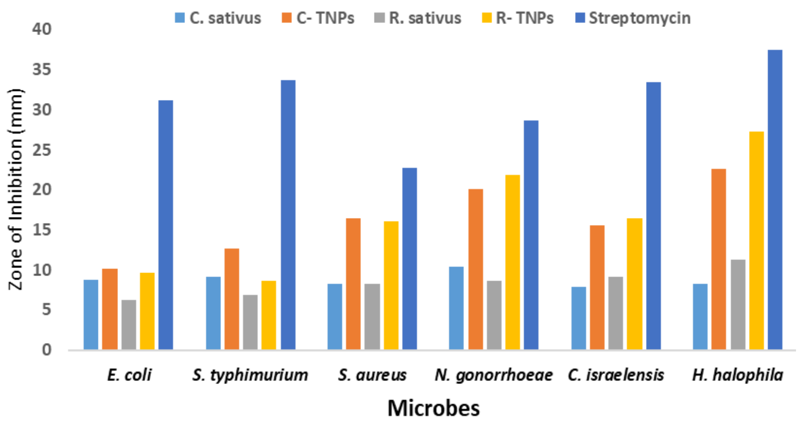

Fig. 7. Antibacterial activity of synthesized nanoparticles.

4. Conclusion

In this study, plant mediated synthesis of nanoparticles was carried out. TiO_2 nanoparticles were synthesized from two different plants extracts as reducing agents which were then characterized using various techniques e.g. XRD, FTIR, SEM, and EDX. FTIR spectra suggested the existence of various functional groups that might be responsible for the nanoparticle synthesis. SEM micrographs suggested that the majority of nanoparticles had smooth, spherical structure with uniform distribution throughout the nanoparticle while one nanoparticle sample had irregular shape. EDX analysis confirmed the presence of titanium, and oxygen elements in the nanoparticles. The synthesized nanoparticles were tested against six different bacterial strains for their antibacterial activity, nanoparticles inhibited bacterial growth and showed good antibacterial activity which highly recommend the use of these green synthesized nanoparticles for antibacterial drugs in medical field. The synthesis of nanoparticles using plants is an emerging work in various fields and is gaining enormous popularity. This study revealed that these nanoparticles have the potency to cure the infectious diseases spreading throughout the world with high spin.

Data Availability Statement

No additional new data were created.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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