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Green synthesis of titanium dioxide nanoparticles using *Psidium guajava* extract and its antibacterial and antioxidant properties

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ABSTRACT

Objective: To determine the efficacies of antibacterial and antioxidant activities of aqueous leaf extract of *Psidium guajava* mediated biosynthesis of titanium dioxide nanoparticles (TiO_2 NPs). **Methods:** Synthesized TiO_2 NPs were tested by disc diffusion method against against human pathogenic bacteria. The total antioxidant activity and phenolic content (Folin-Ciocalteau method) of synthesized TiO_2 NPs and aqueous plant extract were determined. The scavenging radicals were estimated by DPPH method. The synthesized TiO_2 NPs were characterized by XRD, FTIR, FESEM and EDX. **Results:** FTIR spectra of synthesized TiO_2 NPs exhibited prominent peaks at 3 410 cm^{-1} (alkynes), 1 578 cm^{-1}, 1 451 cm^{-1} (alkanes), and 1 123 cm^{-1}(C-O absorption). The morphological characterization of synthesized TiO_2 NPs was analysed by FESEM which showed spherical shape and clusters with an average size of 32.58 nm. The maximum zone of inhibition was observed in the synthesized TiO_2 NPs (20 μ g/mL) against *Staphylococcus aureus* (25 mm) and *Escherichia coli* (23 mm). The synthesized TiO_2 NPs showed more antibacterial activity than the standard antibiotic disk, tetracycline which drastically reduces the chances for the development of antibiotics resistance of bacterial species. The plant aqueous extract and synthesized TiO_2 NPs were found to possess maximum antioxidant activity when compared with ascorbic acid. The content of phenolic compounds (mg/g) in leaf aqueous extract and synthesized TiO_2 NPs were found to be 85.4 and 18.3 mgTA/g, respectively. **Conclusions:** Green synthesized TiO_2 NPs provides a promising approach can satisfy the requirement of large-scale industrial production bearing the advantage of low-cost, eco-friendly and reproducible.

1. Introduction

An important area of research in nanotechnology deals with the synthesis of nanoparticles of different chemical compositions, dimension and controlled monodispersity. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level[1]. Nanotechnology has gained massive applications in the fields of biology and pharmacology[2].

Nanomaterials are part of a commercial revolution that has resulted in an explosion of hundreds of new products due to their diverse physico-chemical properties, enabling their usage in a wide range of innovative applications[3,4]. To avoid the use of toxic organic solvents and severe reaction conditions (temperature, pressure, and long refluxing

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time) for the preparation of nanomaterials, researchers recently have been exploring the possibilities of preparing nanomaterials in aqueous medium with the help of stabilizing or capping agents[5].

In recent years, titanium dioxide (TiO_2) has been extensively used as an environmentally harmonious and clean photocatalyst, because of its optical properties, high chemical stability and nontoxicity[6,7]. Titanium dioxide nanoparticles (TiO_2 NPs) are one of the most important materials for cosmetics, pharmaceuticals[8], skin care wareses, particularly to protect skin from UV rays, whiteness, opacity to products such as paints, plastics, papers, inks, food colorants and toothpastes[9]. The current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains[10] and the TiO_2 NPs have demonstrated significant antibacterial activity[11]. Miller *et al*[12] reported that the TiO_2 generates reactive oxygen species when exposed to ultraviolet radiation, nanoparticulate TiO_2 used in antibacterial coatings and wastewater disinfection has been investigated as an anti-cancer agent. The biocidal polymer-functionalized TiO_2 NPs showed improved inhibition of bacterial growth against *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*) in comparison to the pristine TiO_2 NPs[13]. Zhang and Chen[14] reported that small Ag cluster size and the unique structure of TiO_2 NPs supporting highly dispersed to be the sources of superior bactericidal performance of the room temperature ionic liquids derived Ag/TiO_2. A multifunctional multilayered film containing TiO_2 NPs as contact active antibacterial agent and nanosilver as a release-active antibacterial agent was fabricated via layer-by-layer assembly[15]. Marciano *et al*[16] investigated the bactericidal activity of diamond like carbon films containing TiO_2 NPs and its action by oxidative damage to the bacteria wall, a decrease in the interfacial energy of bacteria adhesion causes an increase in the chemical interaction between *E. coli* and the films, which is an additional factor for increasing bactericidal activity. Rajakumar *et al*[17] reported that the biosynthesis of TiO_2 NPs was achieved using *Aspergillus flavus* extract as a reducing and capping agent which proved to be a good antibacterial material against *E. coli*. Hassan *et al*[18] reported the synthesis and characterization of titania nanorods by sol-gel electrospinning technique and discussed the antibacterial activity and interaction mechanism against *S. aureus*, *E. coli*, *Salmonella typhimurium* and *Klebsiella pneumoniae*.

Antioxidant plays a crucial rule in terminating the oxidative rancidity in food by scavenging the free radical which is generated during oxidation process[19,20]. Generation of free radicals or reactive oxygen species during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress[21]. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process[22]. Oxidative stress is an emerging, general mechanism underlying nanoparticle toxicity[23,24]. Hu *et al*[25] indicated that TiO_2 and zinc oxide nanoparticles could induce significant damage to earthworms due to their antioxidant effects. Crystalline, polyhedral rutile TiO_2 NPs were synthesized and reported for their reduction in cell viability, morphological alterations, compromised antioxidant system, intracellular reactive oxygen species production, significant DNA damage and potential of these NPs to induce cyto-and genotoxicity in cultured human amnion epithelial cells[26]. Nanocarbon black, C60 fullerene, nanoTiO_2 and nanosilica increased the activity of the antioxidant enzyme catalase and also stimulated glutathione transferase in *Mytilus galloprovincialis*[27]. Das *et al*[28] reported the efficient antioxidant and bactericidal effect against *E. coli* and *Pseudomonas aeruginosa* (*P. aeruginosa*) by copper oxide nanoparticles.

In recent years, the biosynthetic method using plant extracts has received more attention than chemical and physical methods and even than the use of microbes, for the nano-scale metal synthesis due to the absence of any requirement to maintain an aseptic environment. New strategies are therefore needed to identify and develop the next generation of drugs or agents to control bacterial infections. Earlier authors reported that the TiO_2 NPs were synthesized from *Annona squamosa* peel extract[29], *Catharanthus roseus* leaf aqueous extract[30] and *Bacillus subtilis*[31].

Guava is the common name of fruits of *Psidium guajava* (*P. guajava*) (Myrtaceae) and spread to various parts of the tropical and subtropical areas. *P. guajava* leaves are commonly used as popular medicine for diarrhoea which is also used for wounds, ulcers, rheumatic pain, and while they are chewed to relieve toothache[32,33]. *P. guajava* has been reported to have antidiarrheal[34], antibacterial[35], anti-inflammatory[36] and anticancer[37] activities. Galactose specific lectin isolated from *P. guajava* fruit ripe was shown to bind with *E. coli*, preventing its adhesion to the intestinal wall and thus preventing infection resulting diarrhea[38]. Quercetin is a major flavonoid present in *P. guajava* leaves and reported to have antidiarrhoeal activity[39].

Green synthesis of silver nanoparticles (Ag NPs) using *P. guajava* leaf extract showed better antibacterial properties than their chemical counterparts even though there was not much difference between their morphologies. FTIR analysis suggested the possible reduction of Ag^+ by the water soluble ingredients like tannins, eugenol and flavonoids in guava leaf[40]. Basha *et al*[41] have reported that the synthesis of gold nanoparticles with guavanoic acid, a phytochemical of *P. guajava* and exhibited remarkable protein tyrosine phosphatase 1B inhibitory activity. Raghunandan *et al*[42,43] observed that the flavonoids isolated from *P. guajava* leaves were responsible for the biosynthesis of gold and Ag NPs. The antimicrobial activity of Ag NPs synthesized from *P. guajava* showed good activity against *E. coli*, *Bacillus cereus* and *Candida tropicalis*[44].

The aim of the present study was to investigate the

antibacterial activity of synthesized TiO_2 NPs using aqueous leaf extract of *P. guajava* against human pathogens. Hence, this process could be suitable for developing a biological process for mass scale production of nanoparticles. The synthesis of TiO_2 NPs was carried via biological reduction which provides suitable capping agent for the stability and viability of the synthesized nanoparticles and reported excellent antibacterial activity.

2. Materials and methods

2.1. Materials

Leaves of *P. guajava* were collected in and around the areas of Melvisharam, Vellore district, Tamil Nadu, India. $TiO(OH)_2$ was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. *Aeromonas hydrophila* (*A. hydrophila*) (MTCC-1739), *Proteus mirabilis* (*P. mirabilis*) (MTCC-442), *E. coli* (MTCC-1677), *S. aureus* (MTCC-3160) and *P. aeruginosa* (MTCC-4030) were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH), propyl gallate, sulphuric acid, sodium phosphate, ammonium molybdate, Folin-ciocalteau reagent, tannic acid, methanol, ascorbic acid were purchased from Merck, Mumbai, India. All the chemicals and reagents were used as received without further purification.

2.2. Synthesis of TiO_2 NPs using leaf aqueous extract of *P. guajava*

Aqueous leaf extract of *P. guajava* was prepared using freshly amassed leaves (20 g). They were surface cleaned with running tap water, followed by distilled water and boiled with 250 mL of double distilled water at 60 ℃ for 15 min. This extract was filtered through nylon mesh (spectrum), followed by millipore hydrophilic filter (0.22 μm) and used for further experiments. For synthesis of TiO_2 NPs, the Erlenmeyer flask containing 100 mL of $TiO(OH)_2$ (0.1 mM) was stirred for 2 h. Twenty mL of the aqueous extract of *P. guajava* was added in 80 mL of $TiO(OH)_2$ at room temperature under stirred condition for 24 h. The pure $TiO(OH)_2$ and aqueous leaf extract of *P. guajava* didn't show any color change and there was no proof for the formation of nanoparticles. After the reaction of *P. guajava* extract with $TiO(OH)_2$, the synthesized nanoparticles turned light green in color.

2.3. Characterization of synthesized TiO_2 NPs

X-ray diffraction (XRD) measurements of the *P. guajava* leaf broth reduced TiO_2 NPs were carried out at 2θ in the range of 20-80 ℃ using Phillips® PW 1830 instrument (CuK α radiation, λ =1.540 6 Å), operated at 40 kV and 30 mA. FTIR analysis of the dried powder of synthesized TiO_2 NPs using Perkin elmer spectrum one spectrometer in attenuated total reflection mode and using spectral range of 4 000-400 cm^{-1} with a resolution of 4 cm^{-1}. For electron microscopic studies, 25 μL of sample was sputter coated on copper stub, and the images of nanoparticles were studied using FESEM (JSM-6700, JEOL, Japan). The fixed samples were coated with carbon and analyzed by energy dispersive X-ray (RONTEC's EDX system, Model QuanTax 200, Germany).

2.4. Antibacterial test

The bacterial culture samples were lyophilized and suspended in nutrient broth with 0.5% sodium chloride at 37 ℃ for 24 h into the viable culture source. All these strains were grown in tryptic soy broth. The strains were grown aerobically at 37 ℃, with 10 mL of medium in 18-150 mm borosilicate glass culture tubes with shaking at 200 rpm under normal laboratory lighting conditions unless specified. Bacterial inoculums were prepared by growing a single colony overnight in nutrient broth and adjusting the turbidity to 0.5 McFarland standards. Mueller-Hinton agar plates were inoculated with this bacterial suspension; synthesized TiO_2 NPs (20 μg/mL) were added to a center well with a diameter of 8 mm. These plates were incubated for 15 min at 4 ℃ (to allow diffusion) and later on at 37 ℃ for 24 h for the bacterial cultures. Positive test results were scored when a zone of inhibition was observed around the well after the incubation period. The zone of inhibition was measured by subtracting the well diameter from the total inhibition zone diameter[45]. Positive test results were scored when a zone of inhibition was observed around the well after the incubation period.

2.5. Total antioxidant activity

Total antioxidant activities of the samples of synthesized TiO_2 NPs and aqueous leaf extract were analysed according to the method of Prieto *et al*[46]. 100 mg of the synthesized TiO_2 NPs were taken into reaction vial and mixed with 0.05% DMSO. In brief, 0.1 mL aliquot of the sample was mixed with 1 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and then incubated at 95 ℃ for 90 min. After samples were cooled to 25 ℃, the absorbance was measured at 695 nm against a blank. The blank contained 1 mL of the reagent solution without the sample. The total antioxidant activity was expressed as the absorbance of the sample. The higher absorbance value indicates the higher antioxidant activity. Ascorbic acid was also assayed for comparison.

2.6. Determination of total phenolics

The total phenolic content was determined by the Folin-Ciocalteau method with some modifications[47]. 1 g/10 mL of sample was filtered with Whatman no.1 paper. 0.5 mL of the sample was added to 2.5 mL of 0.2 N Folin-Ciocalteau reagents and placed for 5 min. 2 mL of 75 g/L of Na_2CO_3 was then added to the total volume made upto 25 mL using distilled water. The above solution was then kept for incubation at room temperature for 2 h. Absorbance was measured at 760 nm using 1 cm cuvette in a perkin-elmer UV-vis lambda spectrophotometer. Tannic acid (0-800 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of tannic acid equivalents (TAE)/g of extract.

2.7. DPPH radical scavenging assay

The DPPH free radical scavenging assay was carried out by the method of Liyana-Pathiranan and Shahidi[48]. 1mL of each of different concentrations (100-500 mg in methanol) of the synthesized TiO_2 NPs and aqueous leaf extract was added to 1 mL of 0.135 mM DPPH in methanol solution. The reaction mixture left in the dark room at 30 min of room temperature. The absorbance of the mixture was measured spectrophotometrically at 517 nm.

3. Results

3.1. X- ray diffraction (XRD)

XRD pattern of the synthesized TiO_2 NPs showed the presence of both anatase and rutile forms which can be denoted at 2 θ peaks at 27.57 °, 36.21 °, 41.37 °, 54.45 °, 56.76 ° and 69.12 °, which were found to be (110), (101), (111), (211), (220) and (112) reflections, respectively and confirmed the nanocrystalline nature of the synthesized particles. The XRD sample showed a dominant peak at 2 θ = 27.57 ° and 41.37 ° which proved the (110) crystallographic plane of anatase and (111) rutile form of TiO_2 NPs, respectively. The particles size estimation was performed by the Scherrer's formula:

$$d=0.9\lambda/\beta \cos\theta$$

where d is the mean diameter of the nanoparticles, λ is wavelength of X-ray radiation source, β is the angular FWHM of the XRD peak at the diffraction angle θ and the data obtained was matched with the data base of Joint Committee on Powder Diffraction Standards file No. 89-4202. The plant synthesized TiO_2 NPs were quite polydisperse calculated average size of 32.58 nm (Figure 1A and B).

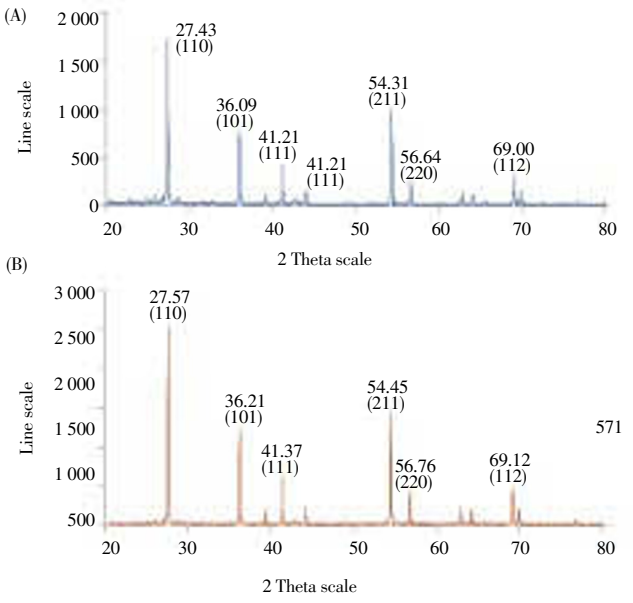

Figure 1. X-ray diffraction patterns of (A) Titanium dioxide, (B) Synthesized TiO_2 NPs.

3.2. Fourier transform infrared spectroscopy (FTIR)

FTIR peaks showed three spectrums namely TiO_2 control, synthesized TiO_2 NPs and *P. guajava* leaf aqueous extract (Figure 2 A, B and C). The peaks were given along with the functional groups responsible for the synthesis of the TiO_2 NPs, which could be depicted to be 3 420^{-1} alcohols (free OH), 3 410^{-1} intramolecular bonded (weak), 3 425^{-1} intramolecular bonded (strong), 2 922^{-1} alkenes, 2 917^{-1} carboxylic acids, 1 659^{-1} nitro compounds (symmetrical stretch), 1 621^{-1} and 1 618^{-1} nitro compounds (asymmetrical stretch), 1 368^{-1} and 1 384^{-1} CH3 umbrella deformation, 1 078^{-1} mononuclear aromatics and 1 065^{-1}-aromatic (Aryl-O-CH_2)[49].

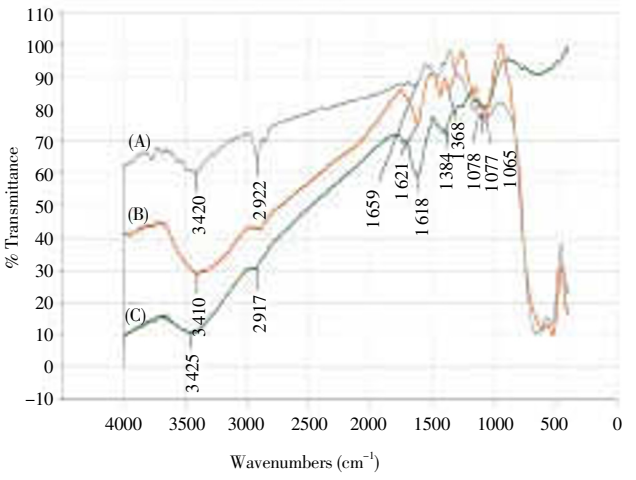

Figure 2. FTIR peaks of (A) Titanium dioxide, (B) Synthesized TiO_2 NPs, (C) *P. guajava* aqueous leaf extract.

Table 5

Effect of KV and CQ on enzymic and non-enzymic antioxidant profiles of *P. berghei*-infected mice.

Treatment	Red cell					Hepatic GSH (μg/g tissue)
	GSH (μg/mL)	SOD (U/mg protein)	CAT (U/mg protein)	GST (U/mg protein)	GPx (U/mg protein)	
Normal	0.68±0.04	1.23±0.15	0.72±0.05	0.88±0.06	0.63±0.05	1.10±0.15
Normal + KV1	0.61±0.05	1.12±0.15	0.68±0.04	0.91±0.05	0.66±0.03	0.96±0.10
Normal + CQ	0.59±0.06	1.25±0.17	0.70±0.04	0.83±0.07	0.54±0.07	0.92±0.08
Infected only	0.33±0.04*	0.71±0.06*	0.48±0.05*	0.36±0.04*	0.28±0.04*	0.63±0.07*
Infected + CQ	0.38±0.05*	0.73±0.04*	0.67±0.03	0.43±0.06*	0.31±0.05*	0.71±0.05*
Infected + KV1	0.56±0.03	0.98±0.15	0.65±0.05	0.79±0.04	0.57±0.03	0.90±0.04
Infected + KV2	0.54±0.06	1.13±0.11	0.69±0.04	0.84±0.06	0.62±0.06	0.95±0.07

Values are given as mean±SD (n=5); * Significantly different from normal (P<0.05); KV1= Kolaviron at a dose of 100 mg/kg, KV2= Kolaviron at a dose of 200 mg/kg,

3.3. Field emission scanning electron microscope (FESEM)

FESEM images were measured and topographical analysis was performed based upon the surface study. Synthesized TiO_2 NPs were smooth and spherical in shape. The images showed the synthesized nanoparticles in various magnifications 15 000×, 30 000× and 50 000× which clearly gives physical morphology, particle size and aspect ratio (Figure 3 A, B and C). The energy dispersive X-ray analysis study (EDX) proves that the particles are crystalline in nature and indeed metallic TiO_2 NPs (Figure 3D). The presence of carbon, oxygen, magnesium and chlorine indicate that the extracellular organic moieties are adsorbed on the surface of the metallic nanoparticles.

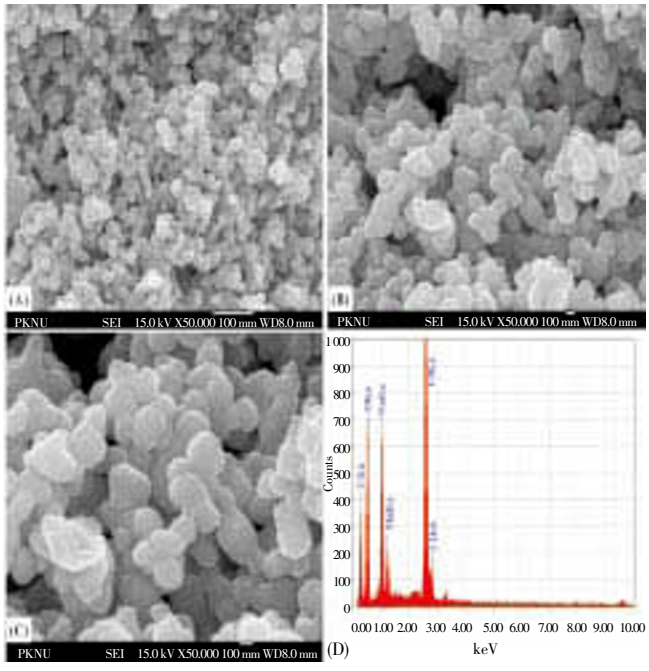

Figure 3. FESEM images of synthesized TiO_2 NPs at different resolution (A) 15 000× (B) 30 000× (C) 50 000× (D) EDX showing the chemical composition.

3.4. Antibacterial activity

The disk diffusion method was performed against the *A. hydrophila*, *P. mirabilis*, *E. coli*, *S. aureus* and *P. aeruginosa*. The synthesized TiO_2 NPs displayed antibacterial activity of pathogenic strains of *A. hydrophila* (17 mm), *P. mirabilis* (20 mm), *E. coli* (23 mm), *S. aureus* (25 mm), *P. aeruginosa* (19 mm) at 20 μg/mL, respectively. The maximum zone of inhibition was observed in the TiO_2 NPs against *S. aureus* and *E. coli* (Figure 4).

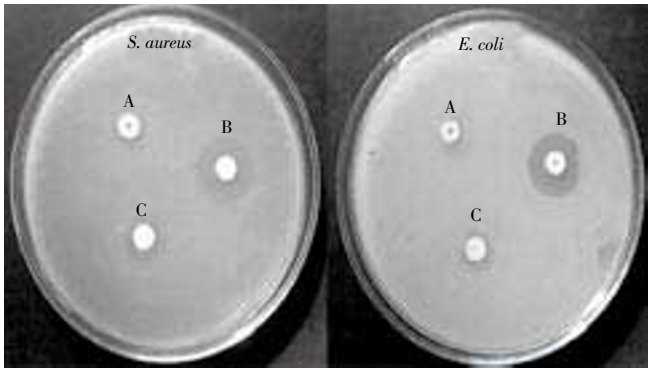

Figure 4. Zone of Inhibition observed against *S. aureus* and *E. coli*. (A) Titanium dioxide-bulk (B) Synthesized TiO_2 NPs (C) Tetracycline.

3.5. Antioxidant activity

In this study, antioxidant activity of the aqueous leaf extract of *P. guajava* and synthesized TiO_2 NPs were investigated and the TiO_2 NPs were found to be effective antioxidants compared with the aqueous leaf extract of *P. guajava*. The antioxidant activity determined using this method differed according to the sample analysed (Figure 5 A). When the absorbance of the extract was compared with that of standard ascorbic acid, the extract was found to possess a higher level of antioxidant activity than ascorbic acid with the highest activity noted in the synthesized TiO_2 NPs. The content of phenolic compounds (mg/g) in leaf aqueous extract and synthesized TiO_2 NPs were found as 85.4 and 18.3 mg TA/g.

The DPPH scavenging assay exhibited effective inhibition activity of both aqueous leaf extract of *P. guajava* and synthesized TiO_2 NPs when compared with the standard, ascorbic acid (Figure 5 B). The DPPH activity of the nanoparticles was found to increase in a dose-dependent manner. However, the synthesized TiO_2 NPs exhibited more inhibition with more than 85% scavenging activity of DPPH than aqueous leaf extract of *P. guajava*. The DPPH free radical scavenging assay showed that synthesized TiO_2 NPs have higher free radical scavenging activity compared to aqueous leaf extract alone.

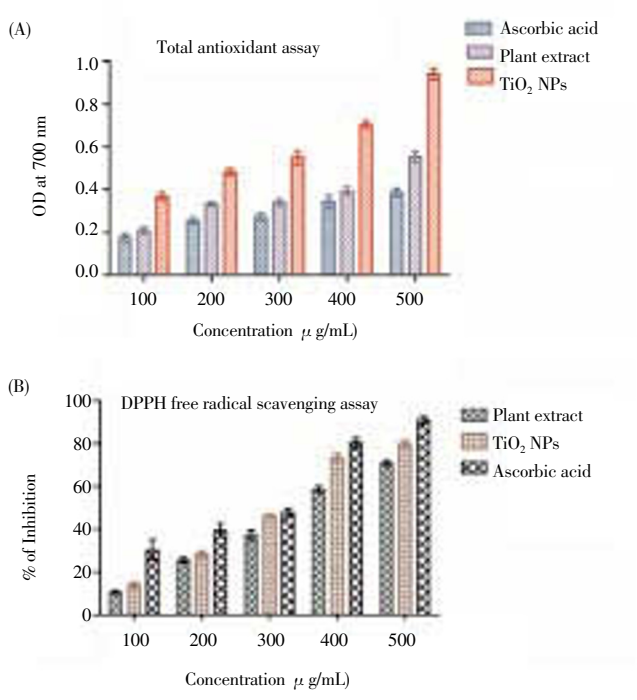

Figure 5. Antioxidant activity of the synthesised TiO_2 NPs.
(A) Total antioxidant activity; (B) DPPH radical scavenging assay.

4. Discussion

The use of the highly structured physical and biosynthetic activities of biological cells for the synthesis of nanosized materials has recently emerged as a novel approach for the synthesis of metal nanoparticles. The positions of principal peaks in XRD were found to be in agreement with the literature[50]. This pattern reflects the shape of the wave functions of the electronic states of the Ti-O-Ti-O chain on the TiO_2 (110)/H_2O interface[51]. Rajakumar *et al*[52] reported the band intensities of the FTIR spectrum for the synthesized TiO_2 NPs from *Eclipta prostrate* leaf aqueous extract are $3\,410^{-1}$, $1\,621^{-1}$, $1\,368^{-1}$, $1\,077^{-1}$ and 1 065 cm^{-1}. These results indicated that alcohols (OH), asymmetrical stretch, primary amines, aromatics and aliphatic amines in *P. guajava* may have been participated in the process of nanoparticle synthesis. Functional groups associated with these were the cause for the bioreduction of $TiO(OH)_2$ to TiO_2 NPs and the FESEM showed poorly dispersed with spherical clusters in shape.

Anas *et al*[53] reported the antibacterial activity of aqueous and organic extracts of *P. guajava* leaves were evaluated against multidrug resistant clinical isolates of *S. aureus* strain. In several studies, the methanolic extract of *P. guajava* showed significant antibacterial activity against *Staphylococcus*, *Shigella*, *Salmonella*, *Bacillus*, *E. coli*, *Clostridium* and *Pseudomonas* and the aqueous extract was more effective against *E. coli* and *P. aeruginosa*[54]. The chemical synthesis may still lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. The antibacterial activity of TiO_2 nanocomposites was investigated qualitatively and quantitatively[55]. Most of the recent researches on the inhibition of bacterial cell growth have been studied by using the suspended-TiO_2 in solution[56-58]. The ability of TiO_2 NPs to produce reactive oxygen species, their toxicity[59], and their applicability[60] has received considerable attention. The antibacterial activity of TiO_2 was related to reactive oxygen species production, especially hydroxyl free radicals and peroxide formed under UV- irradiation via oxidative and reductive pathways, respectively[61]. In suspension, TiO_2 NPs were trapped onto the bacteria surface resulting in the adsorption of TiO_2 particles on the bacteria surface, which could lead to the inactivation of bacteria in couple with the photocatalytic oxidation reaction. There are several possible mechanisms to explain the bactericidal effect of TiO_2 particles. TiO_2 exhibits antimicrobial activity due to its strong oxidizing property when exposed to sunlight or UV-light. The microbial surface was the primary target of the initial oxidative attack when irradiated TiO_2 particles come into contact with microbes[62,63]. The antibacterial test was conducted against *S. aureus* and *E. coli* bacterium using the synthesized sulfated β-cyclodextrin treated fabric TiO_2 NPs[64]. Earlier studies indicated that the antibacterial activity of TiO_2-incorporated polyethylene films should be due to the killing effect property of TiO_2 nanoparticles against *S. aureus* and the TiO_2-incorporated polyethylene film exhibited more effective antibacterial activity[65]. This evidence supports the fact the generation of H_2O_2 at the TiO_2-biofilm interfaces resulting in the destruction of the bacteria within biofilm. The amount of H_2O_2 generated on TiO_2 particles has also been reported to achieve antibacterial activity against various bacterial species[62,66].

Phenols are very important plant constituents because

of their scavenging ability owing to their hydroxyl groups[67]. Phenolic compounds from plants are known to be good natural antioxidants and the activity of synthetic antioxidants was observed to be higher than that of natural antioxidants[68]. The total antioxidant capacity of the aqueous leaf extract of *P. guajava* and synthesized TiO_2 NPs were based on the phosphomolebdnum method where the reduction of Mo (Ⅵ) to Mo (Ⅴ) by the antioxidant compound and the formation of a green phosphate/Mo (Ⅴ) complex[69]. The synthesized TiO_2 NPs were found to have very high total antioxidant capacity as compared to aqueous leaf extract of *P. guajava*.The synthesized TiO_2 NPs showed free radical scavenging activity up to the IC_{50} value of 21.4 μg/mL which is relatively higher in comparison to leaf aqueous extract of *P. guajava*. The TiO_2 and nanosilver have been shown to activate oxidative stress, DNA and mitochondrial damage bio-chemical pathways[70].

In conclusion, as the technological benefits of nanotechnology begin to rapidly move from laboratory to large-scale industrial production, the nanomaterials are used in all biomedical applications. In conclusion, the present novel method is capable of reducing $TiO(OH)_2$ to TiO_2 NPs using *P. guajava* leaf aqueous extract. The synthesized TiO_2 nanoparticles were characterized by using XRD, FTIR, FESEM, EDX and the biological route of synthesis for the TiO_2, provides a fast, purest form of producing nanoparticles. This biological reduction of metal would be boon for the development of clean, nontoxic and environmentally acceptable "green approach" to produce metal nanoparticles, involving organisms even ranging higher plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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