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Original Research Article

In vitro cytotoxicity of biosynthesized titanium dioxide nanoparticles in human prostate cancer cell lines

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Abstract

Purpose: *To establish a green method for production of titanium dioxide (TiO_2) nanoparticles (NPs) using Cinnamomum tamala (C. tamala) leaf extract, and examine the in vitro cytotoxicity of the product in a human prostate cancer (D145) cell line.*
Methods: *TiO_2 NPs were synthesized by mixing 20 mL of C. tamala leaf extract with 0.1 M titanium dioxide ($Ti(OH)_2$) (80 mL) in aqueous solution with stirring for 2 h at room temperature. The TiO_2 NPs were characterized using x-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), x-ray photoelectron spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM), selected-area electron diffraction, and energy dispersive x-ray spectroscopy. The in vitro cytotoxicity against D145 cells was determined using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay.*
Results: *TEM and DLS analyses showed that the NPs were irregularly shaped, with an average particle size of 23 nm. The FT-IR spectrum of C. tamala leaf extract showed that the biomolecules were potentially involved in reduction processes. The negative zeta potential of -14 mV indicated that the NPs were stable and discrete while their crystalline nature was confirmed by XRD. Cytotoxicity analysis showed that the TiO_2 NPs exhibit a dose-dependent toxic effect on D145 cells.*
Conclusion: *A facile and less expensive approach for the production of TiO_2 NPs using C. tamala leaf extract has been developed. The TiO_2 NPs showed dose-dependent cytotoxicity towards D145 cells.*

Keywords: *Anticancer activity, Cinnamomum tamala, Green synthesis, Prostate cancer, TiO_2 nanoparticles*

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INTRODUCTION

Cancer is one of the leading causes of death worldwide, and is characterized by the proliferation of abnormal cells. There are a range of methods used to treat cancer, including surgery, chemotherapy, and radiotherapy. However, the use of these standard methods is limited because they are expensive and have many side effects. Hence, effective, low-cost nontoxic treatments with fewer side effects are required. Nanomaterials with small diameters (1–100 nm) exhibit unique physicochemical characteristics, including high surface area,

enhanced reactivity, the ability to enter cells easily, and the ability to affect different biological systems. These unique interactions between nanoparticles (NPs) and biomolecules may have applications for cancer diagnosis and treatment [1-4]. Titanium oxide nanoparticles (TiO_2 NPs) have been used in a wide range of commercial applications, particularly in consumer products such as textiles, laundry additives, room sprays, water purifiers, and food storage containers [5-10].

TiO_2 is a good photocatalyst, and is widely used in surface coatings as a self-cleaning and self-disinfecting material. Moreover, it plays a key role in environmental purification due to its specific nontoxicity, photoaccelerated super hydrophobicity, and antifogging effects [11,12]. There are a range of advantages to using an inorganic nanomaterial such as TiO_2 rather than an organic material in biomedical applications. These advantages include stability, easy of fabrication, less toxicity, heat-resistance, and sufficient durability in the absence of light exposure [13]. TiO_2 NPs can be synthesized using a variety of methods such as hydrolysis, thermolysis, the sol/gel method [14], hydrothermal methods [15], and flame synthesis [16]. However, all of these methods require expensive and toxic chemicals. In this respect, green methods, which involve simple eco-friendly routes [17-19] for synthesizing nanomaterials have several advantages over chemical and physical methods.

In this study, we present an ecologically friendly method to synthesize TiO_2 NPs from aqueous leaf extracts of *Cinnamomum tamala* (*C. tamala*). The prepared NPs were characterized using a range of techniques, and then investigated for anticancer activity in a human prostate carcinoma (D145) cell line.

EXPERIMENTAL

Materials

$Ti(OH)_2$ powder was obtained from Sigma-Aldrich (St. Louis, MO, USA). The human prostate carcinoma (D145) cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). Milli-Q® water was used in all experiments.

Preparation of plant extracts

The leaves of the *C. tamala* plant were obtained from plants located nearby Zhejiang University in Hangzhou city in the month of June 2016, and later dried under sunlight and cross verified by Xianchun Zhang, taxonomist at Chinese National Herbarium, Beijing, China. The voucher specimen for *C. tamala* leaves was kept for reference at the Chinese National Herbarium, Beijing, and the corresponding voucher number is 223. Fresh *C. tamala* leaves were thoroughly washed, initially with tap water, followed by Milli-Q® water. The leaves (20 g) were boiled in 250 mL Milli-Q® water at 60°C for 15 min. Unwanted solid particles were removed from the extract by filtering through Whatman No-1 filter paper (pore size: 11 µm).

Green synthesis of nanoparticles

To synthesize TiO_2 NPs, approximately 80 mL of 0.1 M titanium dioxide (TiO_2) aqueous solution was stirred for 2 h at room temperature. Then, 20 mL of leaf extract was added and the solution was stirred for an additional 24 h. The particles were then repeatedly centrifuged and washed with ethanol and chloroform, and dried at room temperature for 24 h.

Characterization of nanoparticles

Transmission electron microscopy (TEM) imaging, selected-area electron diffraction (SAED) patterning, and electron dispersive X-ray spectroscopy (EDS) analyses were carried out using a JEM 2100 high-resolution TEM system (JEOL, Tokyo, Japan) with an accelerating voltage of 200 kV. The X-ray diffraction (XRD) spectrum of the centrifuged and dried sample of TiO_2 NPs was obtained using a Bruker D8 Advanced X-ray diffractometer with a copper potassium-alpha (Cu-Kα) source (λ = 1.5406 Å). The zeta potential of the synthesized NPs was measured using a Malvern Zetasizer Nano ZS90 counter (Malvern, UK) at 25 °C. X-ray photoelectron spectroscopy (XPS) data were obtained using a Kratos Axis Ultra 165 spectrometer (Manchester, UK) with a monochromatic aluminum potassium-alpha (Al-Kα) X-ray source ($h\alpha$ = 1486.6 eV). The Fourier transform infrared (FT-IR) spectrum was recorded using a Shimadzu IRAffinity 1 spectrometer (Tokyo, Japan).

In vitro cytotoxicity assessment

An *in vitro* cytotoxicity assessment of the prepared TiO_2 NPs was conducted to investigate the viability and cellular fate of the D145 cells. The toxicity and cell viability of the D145 cells were investigated after exposing them to increasing concentrations of TiO_2 NPs (0.05, 0.1, and 0.5 µg/mL). A cell counter was used to determine the percentage of viable cells after an incubation period of 48 h. The control experiment

was without TiO_2 NPs. The cells remained 100 % viable.

Statistical analysis

All of the cytotoxicity experiments were performed three times for each concentration. Absolute values of each experiment were converted into percentages. All data points corresponding to the concentration versus cytotoxicity plot are shown as the arithmetic mean percent inhibition compared to the control standard error. A one-way analysis of variance (ANOVA) was used to determine the statistically significant differences between the mean values. A value of $p < 0.05$ was considered as significant.

RESULTS

Characteristics of the TiO_2 nanoparticles

Figure 1 shows the XRD peaks of the TiO_2 NPs synthesized using the leaf extracts. The anatase phase was confirmed by the presence of *2θ* values corresponding to 25.49°, 37.82°, 48.05°, 55.06°, and 62.60°. All of the observed peaks were in good agreement with the anatase phase (Joint Committee on Powder Diffraction Standards (*JCPDS*) CPDS no: 99-201-6205).

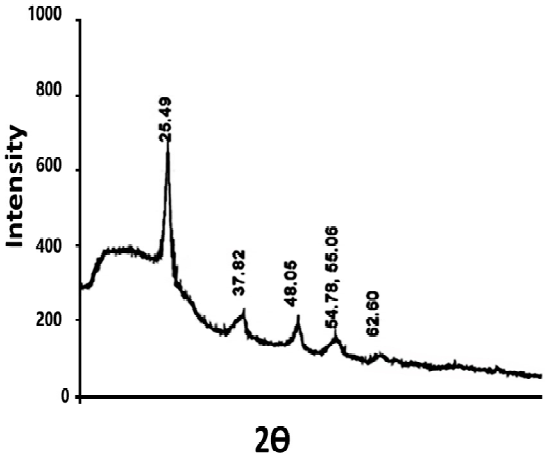

Figure 1: X-ray diffraction (XRD) peaks of green-synthesized titanium oxide nanoparticles

The synthesized NPs were ~23 nm in size, as indicated by TEM images (Figures 2 a, b and Figure 3 b). The stability of the NPs was determined based on their zeta potential value of -14.0 ± 0.52 mV (Figure 3a). Thus, the TiO_2 NPs were stable and discrete in the aqueous medium used. No loss of stability was observed even after 6 months at room temperature. The NPs had an irregular morphology with little agglomeration.

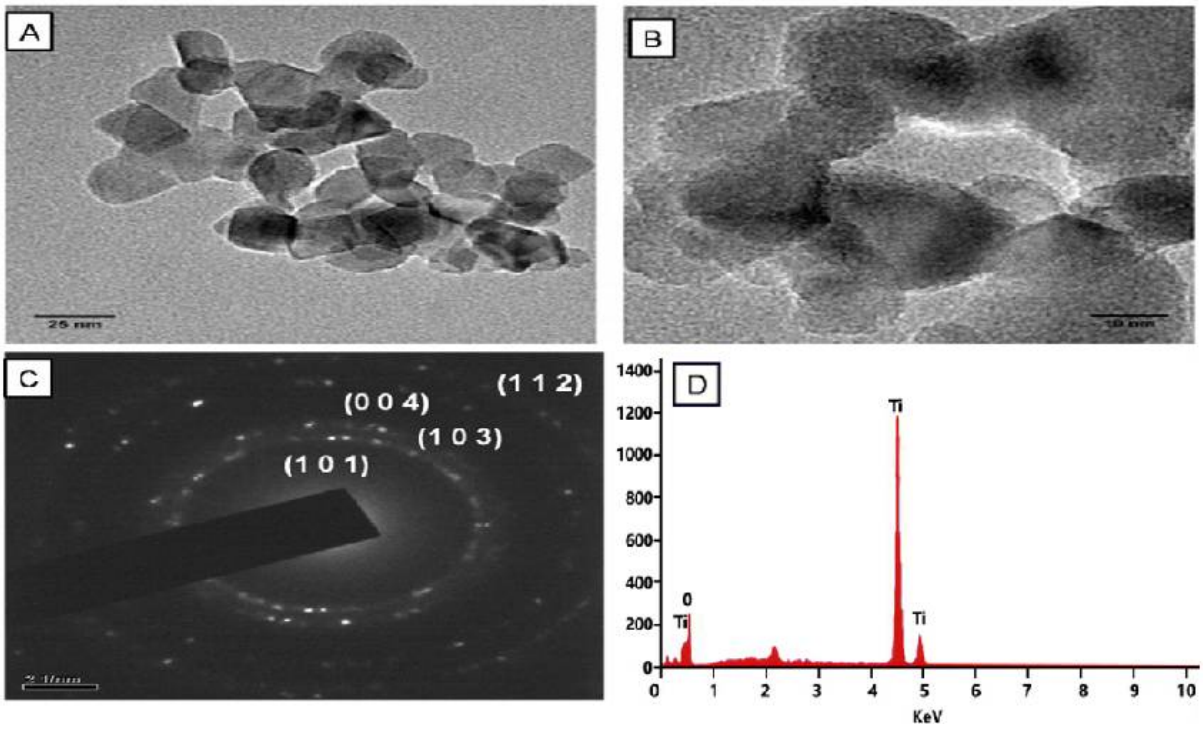

Figure 2: (A, B) Transmission electron microscopy (TEM) images of TiO_2 NPs, (C) selected-area electron diffraction (SAED) pattern of the NPs, and (D) energy dispersive X-ray spectroscopy (EDS) profile showing the elements contained in the NPs

The SAED pattern of the TiO_2 NPs is shown in Figure 2 C. The dark rings correspond to the standard polycrystalline diffraction rings associated with the anatase phase (indexed). No other phases were observed in the diffraction rings. The EDS mapping of the NPs, shown in Figure 2 D, revealed the expected elements; no peaks other than the TiO_2 peaks were observed

XPS analyses were carried out to determine the oxidation state of the TiO_2 NPs. High-resolution XPS spectra (Figure 4) showed Ti 2p and O 1s peaks. The binding energy at 459.2 eV corresponds to Ti $2p_{3/2}$ spin-orbital splitting photoelectrons (Figure 4A), and the binding energy signal at 530.5 eV is associated with O 1s photoelectrons.

Fourier transform infrared spectroscopy (FT-IR) was performed to identify the possible reducing groups present in the leaf extract; the results are shown in Figure 5. In this spectrum, the peak at 3336.4 cm^{-1} represents the strong stretching vibration frequency of the hydroxyl (-OH) group, and the peak at 1612.8 cm^{-1} corresponds to the stretching frequency of the -C=C bonds. The bands at 1383.6 and 1520.4 cm^{-1} represent the N–H bending mode. The band at 1022.2 cm^{-1} was assigned to the C-N stretching vibration of the primary amines (Figure 6).

In vitro cytotoxicity

The *in vitro* cytotoxicity of the synthesized TiO_2 NPs was also studied in an aqueous medium using D145 cells exposed to various concentrations of TiO_2 NPs (0.05, 0.1, and 0.5 µg/mL). The TiO_2 NPs were toxic to the D145 cells. The viability was 88 % when the cells were treated with a TiO_2 NP concentration of 0.05 µg/mL, and 38 % when a concentration of 0.5 µg/mL was used, as shown in Figure 7.

The cytotoxicity of TiO_2 NPs increased with NP concentration. From these results, we concluded that the cytotoxicity of the bio-fabricated TiO_2 NPs was concentration dependent.

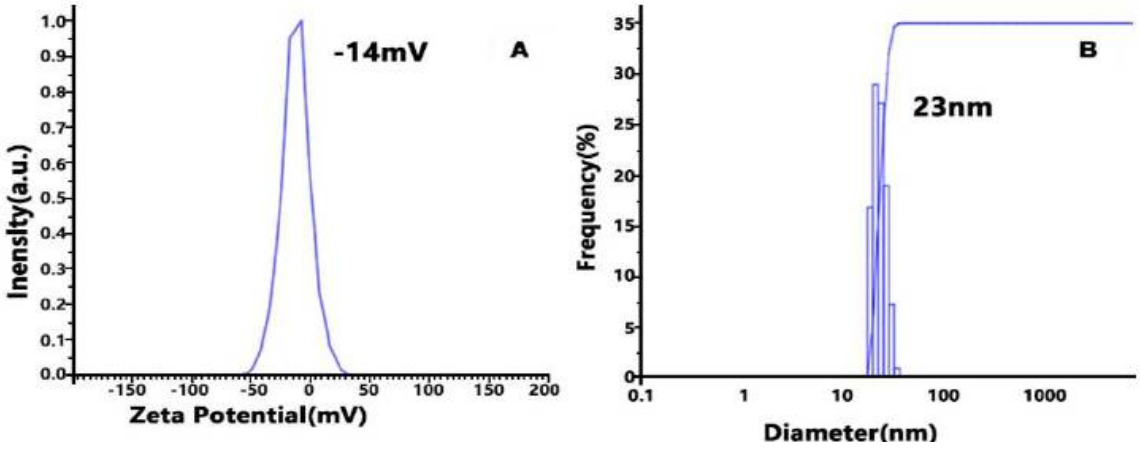

Figure 3: (A) Zeta potential value of TiO_2 NPs and (B) average size of the prepared NPs.

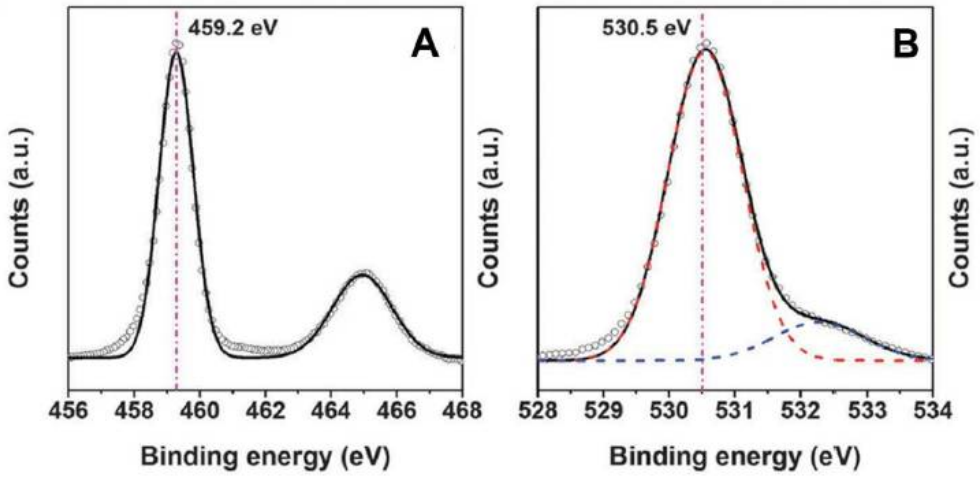

Figure 4: High-resolution x-ray photoelectron spectroscopy (XPS) spectra of (A) the titanium 2p peak and (B) the oxygen 1s peak of TiO_2 NPs

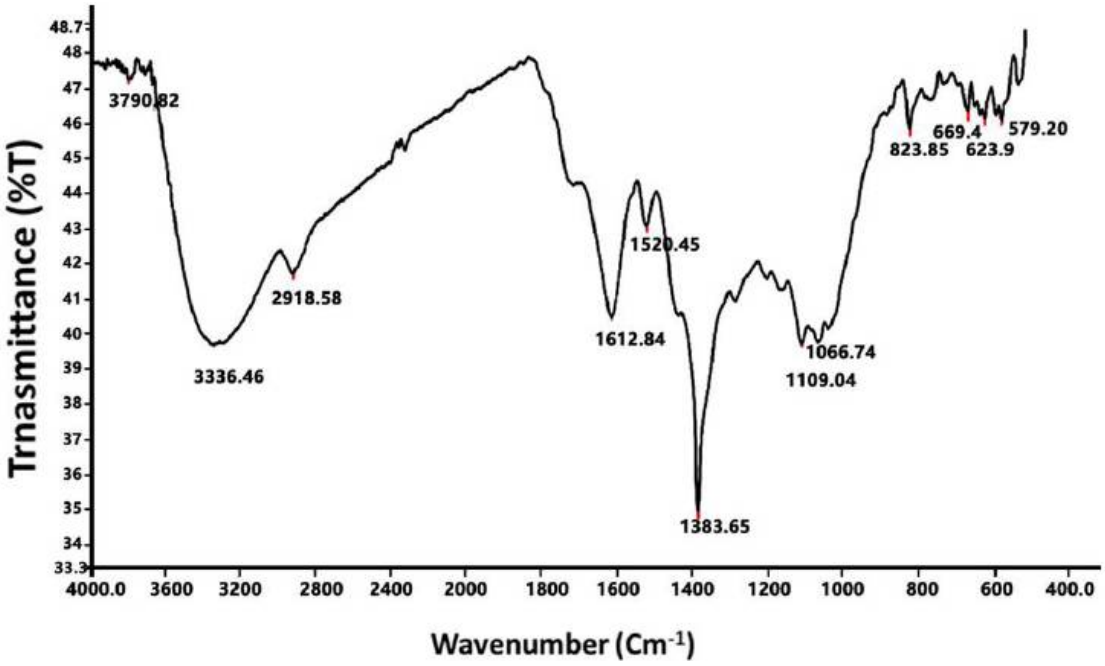

Figure 5: Fourier transform infrared (FT-IR) spectrum of TiO_2 NPs prepared using *Cinnamomum tamala* leaf extract

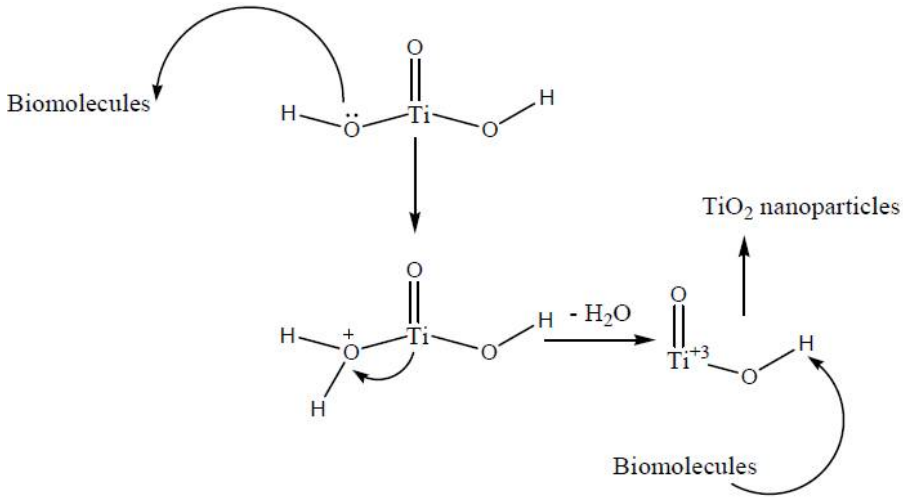

Figure 6: Plausible mechanism for the formation of TiO_2 NPs

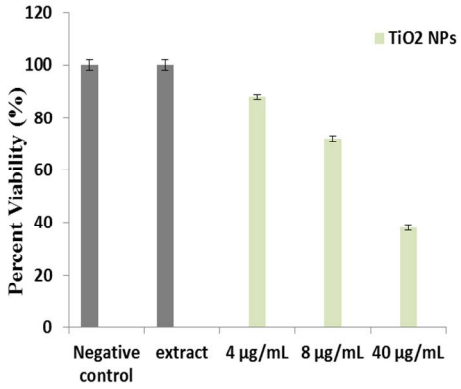

Figure 7: Viable D145 cells after exposure to TiO_2 NPs

DISCUSSION

The biomolecules present in the leaf extract formed a thin organic layer on the NPs. This layer may act as a capping agent, as evidenced by the uniform distribution of NPs inside the bio-reduced aqueous solution, even at the nanoscale level. Based on XPS data, TiO_2 NPs and titanium metal ions were present in their highest oxidation state (+4). All of these results are in good agreement with previous reports [24,25], which confirmed the formation of pure, single-phase TiO_2 NPs.

FTIR spectra suggest that the secondary metabolites of the leaf extract contain a hydroxyl group that may possibly reduce the NPs. It is also believed that these groups are bound to TiO_2 NPs via free carboxylate ions from amino acid residues containing hydroxyl functional groups.

The cytotoxicity of the bio-fabricated TiO_2 NPs was concentration dependent. The concentration-dependence of the cytotoxicity of diastase-stabilized AgNPs towards mouse fibroblast (3T3) cell lines has already been reported [20]. Thus, TiO_2 NP synthesis using the method proposed could provide an important anticancer drug treatment.

CONCLUSION

TiO_2 NPs have been successfully synthesized by an eco-friendly method using *C. tamala* leaf extracts. The NPs exhibited *in vitro* anticancer activity against D145 cells in a dose-dependent manner. Thus, the findings of this study provide evidence that useful anticancer compounds can be synthesized using plant-derived NPs.

DECLARATIONS

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest associated with this work.

Author contributions

We declare that this work was done by the author(s) named in the article and all liability pertaining to claims related to the content of the article will be borne by the authors. Feiping He and Weixing Yu played a key role in the preparation and characterization of TiO_2 NPs. Xiaosong Fan and Baiye Jin conducted the cytotoxicity experiments for the prepared TiO_2 NPs and helped in writing the manuscript.

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