Asian Journal of Chemistry; Vol. 35, No. 6 (2023), 1315-1319

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.27570

# Green Fabrication of Titanium Dioxide Nanoparticles and their Antimcrobial and Anticancer Activities

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*Received*: 11 January 2023; *Accepted*: 6 April 2023; *Published online*: 27 May 2023; AJC-21245

In this study, a plant-mediated method for the synthesis of titanium dioxide ($TiO_2$) nanoparticles is reported. Using the titanium oxide and a plant extract of medicinal herb *Leucas cephalotes*, the $TiO_2$ nanoparticles were effectively synthesized. The reaction temperature was kept between 75 ºC and 80 ºC, while 1 M of $TiO_2$ and the plant extract were being processed. The absorption peak for titanium dioxide nanoparticles in the UV-Vis spectrometer was observed at 212 and 345 nm. The typical dimension of the nanoparticles was determined to be 38.99 nm from the XRD pattern. According to the findings, titanium and oxygen were composed with high energy signals of 61.27% and 23.16%, respectively. According to FT-IR spectrum, the Ti-O bonding absorption peak is appeared at 586 $cm^{-1}$. The scanning electron microscopy (SEM) was employed to confirm the spherical shape of the synthesized $TiO_2$ nanoparticles. The green synthesized $TiO_2$ nanoparticles could be used to treat a variety of malignancies. The MTS and MTT assays were used to assess the anticancer activity of biogenic $TiO_2$ nanoparticles. The $IC_{50}$ values were 1.89, 2.00, 1.98 and 4.00 µM, respectively, against the MCF-7, HeLa, PC-3 and A549 nanoparticles cancer cell lines. Additionally, this study has also shown that synthesized $TiO_2$ nanoparticles are highly active against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* as well as against yeast (*Candida albicans*).

**Keywords: Ttitanium dioxide nanoparticles, Anticancer activity, Antimicrobial activity.**

## INTRODUCTION

Metallic nanoparticles have been made using a variety of techniques, but in general, they can be made using chemical, physical and biological techniques [1,2]. Most of the chemical and physical methods are widely recognised for being extremely expensive and delivering adverse effects for the surrounding environment [3,4].

Titanium dioxide nanoparticles are used in a wide variety of items, including toothpastes, toothpaste tubes, paints, plastics, papers, inks and food colouring. These nanoparticles are also used in cosmetic products, medications and skin care products [5,6]. The synthesis of nanoparticles using plants has expanded significantly in recent years [7-9]. However, the physical and electromagnetic properties of ZnO and $TiO_2$ nanoparticles make them perfect for applications in photo and sonodynamic processes [10].

Still a large number of potential plants that could be used as a biosynthetic reducing agent for the synthesis of metal nanoparticles are yet to be explored. Some of the driving motivations behind the biological synthesis of metal nanoparticles include the requirement for producing nanoparticles on a large scale, the need to scale up laboratory production to the point where it can be scaled up to the point of large-scale production and the need to understand their functional mechanism against pathogenic organisms [11,12].

In this work, plant *Leucas cephalotes*, also referred to as dronapushpi in Hindi, was used as the reducing agent for the synthesis of titanium dioxide ($TiO_2$) nanoparticles. The presence of various kinds of phenolic contents of this leaf extract was specifically selected for its usage as a capping or lowering agent in nanoparticle synthesis. The synthesized $TiO_2$ nanoparticles were characterized with UV-visible, FTIR, SEM and XRD techniques. It has also been reported that $TiO_2$ nanoparticles are effective towards cancer cells [13-24]. Thus, the anticancer efficacy of biogenic $TiO_2$ nanoparticles synthesized from *L. cephalotes* were also analyzed using MTS and MTT assays.

## EXPERIMENTAL

The plant material was collected from the hills located at Pauri Garhwal district, India (29º45′30º15′ North and 78º 24′79º23′E). Dr. Neelu Singh, Taxonomist, National Tropical Research Institute of Forest, Jabalpur, authenticated the plant and the plant belongs to the *Leucas cephalotes* (Dronapushpi) family of lamiaceae. The speciment of this plant is preserved in the harbanium of Motherhood University as batch No. UK2023.

**Plant extract:** *L. cephalotes* were washed thoroughly with distilled water to eliminate the dust and foreign materials. After washing, the leaves were dried in shade for 3 to 4 days before being ground into a powder. A grinder was used to reduce the dried leaves to a fine powder. Then, in 1000 mL round bottom flask, 8 g of *L. cephalotes* leaves powder was mixed with 300 mL of deionized simmered for 1 h in water at 85-90 ºC. Once the extract has formed, the aqueous extract solution will turn yellow after 1 h. The extract was filtered through Whatman no. 1 filter paper after being allowed to cool to room temperature.

**Synthesis of $TiO_2$ nanoparticles:** In brief, 20 mL of plant extract (*L. cephatoltes*) was added slowly to 1 M $TiO_2$ solution with continous stirred working with a magnetic stirrer for 2 h at 80 ºC. The colour of the solution went from brown to white colour charge during the process. After incubating the synthesized $TiO_2$ nanoparticles in a solution for 24 h, the nanoparticles were separated by centrifuging the mixture for 15 min at 1000 rpm.

### *In vitro* cytotoxicity study

**Cell line culture:** The PC-3 (prostate cancer cell line), Hela (human endometrial cancer cell line), A459 (human lung cancer cell line) and MCF-7 (human breast tumor) cells were used in this assay and procured from Sigma-Aldrich, USA.

**Cell growth inhibition MTS assay for PC-3 (prostate cancer):**The cell titer 96 aqueous non-radioactive cell growth assay was used to quantify cell numbers, as per the manufacturer's instructions (Promega, WI, USA). The number of viable cells can be determined in a proliferation or cytotoxicity experiment using calorimetric analysis utilizing the cell titer 96 aqueous one-solution test. The electron-coupling reagent PES and the MTS chemical are both present in this solution. When cells bio-reduce the MTS compound (3-(4,5-dimethyl thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphonyl)-2H-tetrazolium), a formed colourful formazan derivative is formed, which is soluble in tissue culture conditions. Cells were placed in 96-well plates at a density of 5,000 cells per well in growth media and left to stand by for 24 h. The cells were then treated with different dosages of chalcones and their derivatives (100, 33.33, 11.11, 3.70, 1.23, and 0.41 μg $mL^{-1}$). After 72 h of treatment, 20 μL of cell titer 96 aqueous solution was added to each well. After 2 h, the lights were switched off and the plates were returned to the incubator. The Spectra Max 340 microplate reader (Molecular Devices, USA) was used to measure absorbance at 490 nm and a reference at 690 nm. The experiments were conducted three times to ensure the accuracy. The cell viability was determined using the following formula:

$$\text{Cell viability } (\%) = \frac{A_S}{A_{control}} \times 100$$

where $A_S$ denotes the absorbance of cells plates that were inoculated with nanoparticle; $A_{control}$ is the absorbance of cells plates, which were inoculated only with broth culture.

**MTS assay for HeLa cancer cell lines:** Rather than treatment, HeLa breast cancer cells were moved to 96-well tissue culture seeded at a density of 5000 cell lines for 24 h. After that the medium was changed to a new medium containing different ratio of PTAE or metallic nanoparticles (100, 33.33, 11.11, 3.70, 1.23 and 0.41 μg/mL). The control was culture medium with no formulation development at all. The samples were removed after 72 h at 37 ºC and 5% $CO_2$ and the rinsed thoroughly twice with sterile PBS. Each well was given 20 μL of MTS solution (0.5 mg/mL) and incubated at 37 ºC for 4 h. Immediately after the removal of the medium, each well was mixed with 100 mL of DMSO to dissolve the formazan crystal formed from MTS. The cell viability was measured using a Spectra Max 340 microplate reader (Molecular Devices, USA) at 490 nm and a reference at 690 nm.

**MTS assay against A459 (human lung cancer) cell line:** The MTS assay is often used to evaluate the cell proliferation, viability and cytotoxicity. The cell growth inhibition assay was performed according to the manufacturer's instructions by using cell titer 96 aqueous non-radioactive cell proliferation assay (Promega, WI, USA). The MTS compound and the electron coupling reagent phenazine ethosuphate are both present in one solution (PES). Cells bioreduce the MTS compound (3-(4,5-dimethylthiazol-2-yl)-5-(-3-carboxymethoxyphenyl)-2(4-sulphonyl)-2*H*-tetrazolium) to a coloured formazan product which is soluble in tissue culture medium. A459 (human lung cancer) cell line was transferred 24 h before treatment to 96-well tissue culture plates at a density of 5000 cells/well.

The medium was then replaced with a new medium containing metallic nanoparticles at different doses (100, 33.33, 11.11, 3.70, 1.23 and 0.41 μg mL). The control group received culture medium that was devoid of any drug formulation. Following 72 h of incubation at 37 ºC and 5% $CO_2$, the media was removed and the rinsed thoroughly twice with sterile PBS. Each well received 20 μL of MTS solution (0.5 μg/mL) and was incubated at 37 ºC for 4 h. Following the removal of the medium, each well received 100 mL of DMSO to solubilize the formazan crystal created from MTS. The cell viability was ascertained by recording the absorbance at 490 nm and a reference at 690 nm using Max 340 microplate reader (Molecular Devices, USA).

**MTT assay for MCF-7, HeLa cells:** The MTT assay was conducted the cytotoxic nature of $TiO_2$ nanoparticles in MCF-7 (human breast cancer) cell lines. In 96-well plate, 5,000 MCF-7 cells were seeded and incubated for 24 h. The cells were also treated with $TiO_2$ nanoparticles at various doses (100, 33.33, 11.11, 3.70, 1.23 and 0.41 μg) and then incubated with 72 h. After that the cells were exposed for 4 h to 10 L of freshly prepared yellow MTT reagents (0.5 μg/mL). Finally, 100 μL of DMSO was introduced and the violet formazan solution's UV absorbance at 570 nm was measured using Multimode reader, Tecan, Austria.

**Antimicrobial assay:** Bacterial strains (*Staphylococcus aureus*, *Pseudomons aeruginosa*, *Escherichia coli*, *Candida albicans* and *Bacillus subtilis*) were cultured overnight at 37 ºC in Mueller-Hinton Agar medium. The inculum as adjusted according to CLSI (clinical and labouratory standard institute) using a sterile sline to the final density of 0.5 McFarland standard ($1.5 \times 10^8$ CFU/mL). The microbial inoculum were spread over the entire surface of plates with growth medium and left for 15 min at room temperature. After that agar plates containing bacteria and 50 µL of nanoparticles solution were incubated at 37 ºC for 24 h. The antimicrobial activities of synthesized nanoparticles was evaluated based on diameter (mm) of inhibition zones that occurs as the results of schiff bases diffusion in the medium and inibition zones of the microbial growth.

## RESULTS AND DISCUSSION

**UV-vis studies:** In order to determine the surface plasmon resonance (SPR) band, the optical characteristics were recorded using Shimadzu UV-3900 UV-Vis spectrometer. The surface plasmon resonance-induced absorption spectra of *L. cephalotes* are shown in Fig. 1 at wavelengths of 212, 238, 305, 366, 379 and 553 nm.

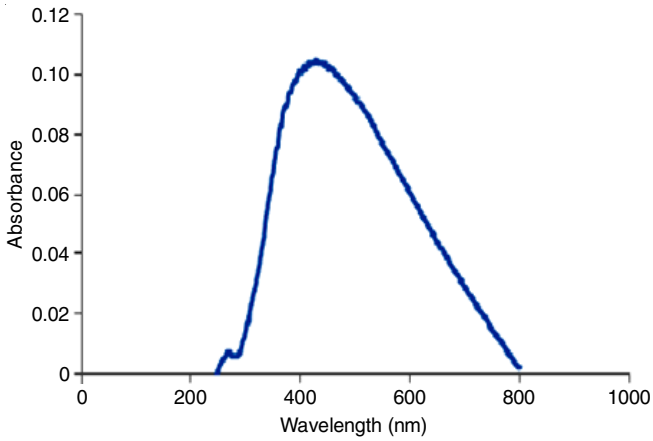


Fig. 1. The UV-visible spectra of the synthesized $TiO_2$ nanoparticles

**FTIR studies:** FTIR studies was conducted on the biogenic $TiO_2$ nanoparticle synthesized from the extract of *L. cephalotes* in order to detect any potential shifts in functional group bonds during the reduction process. Fig. 2 illustrates the FTIR spectrum for extracts of *L. cephalotes*, which showed the multiple distinct peaks of O-H and C=O groups. Others peaks at 3880 and 2895 $cm^{-1}$ are related to the C-H and 1506 $cm^{-1}$ related to show the C=C band and other peaks at 887 and 586 $cm^{-1}$ shows the Ti-O bond.

**XRD studies:** The biogenic $TiO_2$ nanoparticles synthesized from the extract of *L. cephalotes* have crystalline anatase structures, which was confirmed from the XRD peaks appeared at 25º, 36º, 37º, 74º, 47º, 97º, 55º, 12º and 62º (Fig. 3) These results were confirmed in conformity with JCPDS card No. 21-1272 [25]. Moreover, the Debye-Scherrer's equation was used to determine the size of the nanoparticles and the size of $TiO_2$ nanoparticle crystal was found to be 38.99 nm.

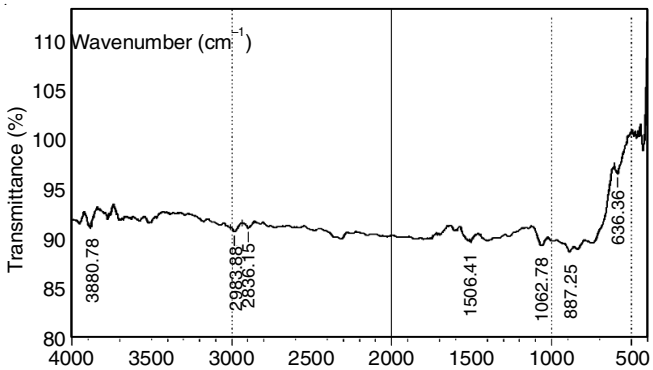


Fig. 2. FTIR spectra of the synthesized titanium dioxide nanoparticles

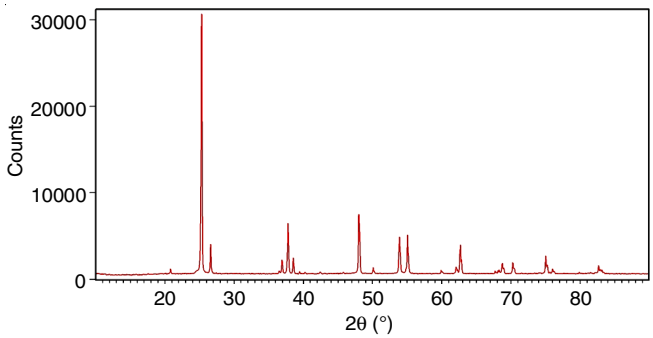


Fig. 3. The XRD spectrum of the synthesized titanium dioxide nanoparticles

**SEM-EDX studies:** Based on the surface investigation, the measurements from SEM (JEOL JEM 2100, Japan) images were used to perform the topographical analysis. The images of the synthesized $TiO_2$ nanoparticle at different magnifications (Fig. 4) clearly demonstrate the smooth and spherical $TiO_2$ nanoparticles, which are in good shape. The EDX investigation confirmed that the nanoparticles are metallic and crystalline in character. According to EDX analysis, the amount of Ti (61.27%) and oxygen (23.16%) and other elements (16.00%) was estimated.

**Size distribution analysis:** Fig. 5 depicts the size distribution of $TiO_2$ nanoparticles powder. The nanoparticles had an average diameter of 1.62 nm and 0.210 PDI value. Fig. 5 depicts a size distribution that appears to be more narrow than the one depicted in Fig. 4. The reason for the disparity is that a Zetasizer 3000HS size analyzer has a hard time picking out a few of smaller particles among many larger particles. The PDI values suggest that the synthesized $TiO_2$ nanoparticles are monodispersed in nature [26].

**Zeta potential studies:** The charge stability of $TiO_2$ nanoparticles can be assessed using the zeta potential. The zeta potential represents the charge density on the surface. $TiO_2$ nanoparticles from *L. cephalotes* extract measured the zeta potential of nanoparticles as -17.8 mV (Fig. 6). This data is consistent with earlier studies [15].

**Anticancer activities:** A preliminary screening by MTT assay with PC-3 (prostrate cancer cell line), Hela, A459 (human lung cancer cell line) and MCF-7 cells (human breast cancer) showed that all the complexes could suppress the cell viability with $IC_{50}$ values at micromolar range cisplatin 13 µM and doxorubicin 4.1 µM used as a standard. The $IC_{50}$ of synthesized

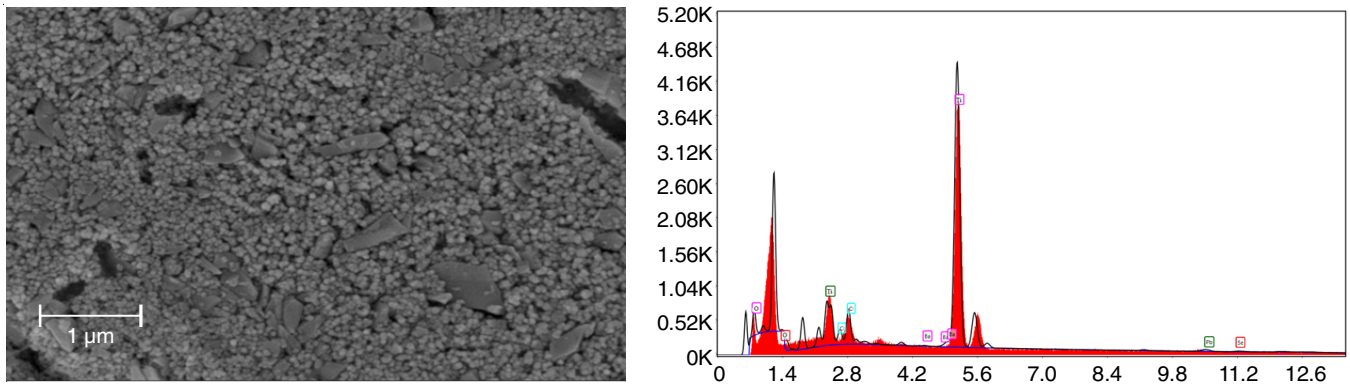


Fig. 4. The SEM and EDAX images of the synthesized $TiO_2$ nanoparticles

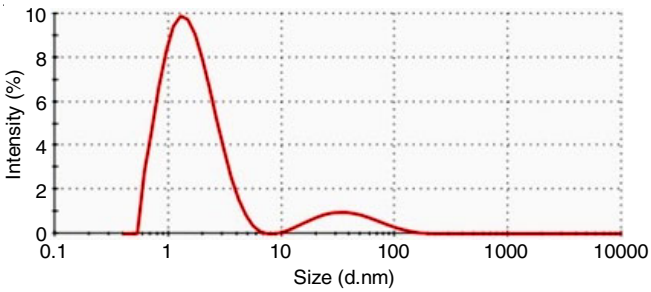


Fig. 5. Particles size distribution of the synthesized $TiO_2$ nanoparticles

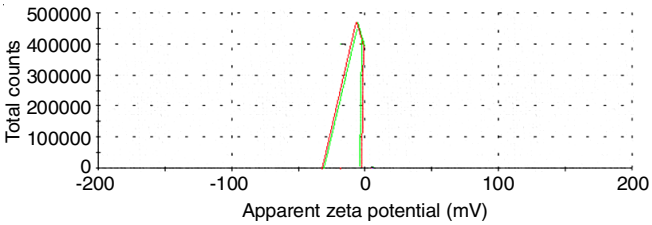


Fig. 6. Zeta potential curve of $TiO_2$ nanoparticles

$TiO_2$ nanoparticles against MCF-7 (breast cancer), HeLa, PC-3 (prostate cancer), A549 (lungs cancer) cell lines were 1.89, 2.00, 1.98 and 4.00 µM, respectively.

**Antimicrobial studies:** The $TiO_2$ nanoparticles synthesized from leaf extract of *L. cephalotes* were also tested for antimicrobial activities against Gram-postive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*) and one yeast *C. albicans*. Results of the inhibition zones values for $TiO_2$ nanoparticles are shown in Table-1. It was observed that synthesized $TiO_2$ nanoparticles had high efficacy against *S. aureus*, *E. coli* and *P. aeruginosa*, however it had low activity on *B. subtilis* and no effect against *C. albicans*.

## Conclusion

Utilizing plant leaf extract from *Leucas cephalotes* to synthesize titanium dioxide nanoparticles was succesfully carried out. EDX analyses identified the size and content of $TiO_2$ structure, were validated by SEM analysis. The size and shape of the synthesized nanoparticles were determined *via* XRD analysis. Moreover, $TiO_2$ nanoparticles showed enhanced cytotoxic effect against MCF-7 (breast cancer) and prostate cancer in comparision to Hela and A549 lungs cancer. It has also studied that synthesized $TiO_2$ nanoparticles have strong activity towards

TABLE-1
INHIBITION ZONES FOR $TiO_2$ NPs NANOPARTICLES GENERATED THROUGH THE AGAR WELL DIFFUSION METHODS

| Bacteria name | Zone of inhibition (mm) |
|---|---|
| *Staphylococs aureus* | 16 |
| *Bacillus subtilis* | NI |
| *Escherichia coli* | 15 |
| *Pseudomons aeruginosa* | 19 |
| *Candida albicans* (MTCC) | NI |

*S. aureus*, *P. aeruginosa* and *E. coli* and low to no activity showed against *B. subtilis* and *C. albicans*.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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