



GREEN SYNTHESIS OF TITANIUM DIOXIDE NANOPARTICLES (TiO₂ NPS) USING LEAF EXTRACT OF *DATURA INNOXIA*

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ABSTRACT

Introduction: A cost effective and environment benign technique is developed for the synthesis of Titanium dioxide nanoparticles (TiO₂ NPs) using the aqueous leaf extract of *Datura innoxia* which act as a reducing and stabilizing agent. **Materials and Method:** The prepared Titanium dioxide nanoparticles were characterized using dynamic light scattering (DLS), ultraviolet-visible spectroscopy (UV-Vis), transmission electron microscopy (TEM) and Fourier-transform infrared spectroscopy (FTIR). The synthesized nanoparticles were tested against four different bacterial species. 2, 2-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) assay was carried out to evaluate the antioxidant potential of the synthesized TiO₂ NPs. **Results:** TEM study showed the formation of hexagonal shaped TiO₂ NPs with average size ranging from 36-42 nm. The FTIR spectrum indicated the role of nitro compounds and O-H group in the synthesis of TiO₂ NPs. The synthesized TiO₂ NPs were found to be maximum effective against *Bacillus cereus* and *Proteus vulgaris*. Maximum of 81% DPPH radical scavenging activity was exhibited by the TiO₂ NPs. **Conclusion:** This simple, low cost, eco-friendly method for synthesizing stable TiO₂ NPs provides a valuable alternative approach for large scale synthesis.

KEY WORDS

Antibacterial activity, Antioxidant activity, *Datura innoxia*, DLS, FTIR, TEM, Titanium dioxide nanoparticles, UV-Vis spectroscopy.

INTRODUCTION:

Nanotechnology is trending 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 enormous applications in the fields of biology and pharmacology [2]. With gradual advancements such as the invention of techniques like transmission electron microscopy, atomic force microscopy, dynamic light scattering etc., nanotechnology today has reached a stage where it is considered as the future to all. Nanotechnology is likely to have a profound influence on our economy and society in the early 21st

century, comparable to that of semiconductor technology, information technology, or cellular and molecular biology. Science and technology research in nanotechnology promise breakthroughs in areas such as materials and manufacturing, nanoelectronics, medicine and healthcare, energy, biotechnology, information technology, and national security [3].

In practice, the physical, chemical and biological approaches are employed for the synthesis of nanomaterials. Particularly, the biological methods are showing significant interest because of their less environmental consequences and energy intensive

process. In recent years, the plant-mediated biological synthesis of nanoparticles is gaining importance due to its cost effectiveness and eco-friendliness. In recent years, TiO₂ NPs has been extensively used as an environmentally harmonious and clean photocatalyst, because of its optical properties, high chemical stability and nontoxicity [4, 5]. TiO₂ NPs are one of the most important materials for cosmetics, pharmaceuticals [6], skincare wares, particularly to protect skin from UV rays, whiteness, and opacity to products such as paints, plastics, papers, inks, food colorants and toothpastes [7].

In this study, the plant *Datura innoxia* belonging to *Solanaceae* family is used for the synthesis of TiO₂ NPs.

MATERIALS AND METHODS

TiO₂ was used directly in powdered form as obtained from the Sigma Chemicals, India. For preparing the aqueous solutions and washing purpose, double distilled water was used throughout the experiment. Healthy leaves *Datura innoxia* was collected from the local areas of Surat (Gujarat, India).

Preparation of leaf extract: Fresh leaves were collected from plants and washed with distilled water so that the dust particles present on the plant were removed so that they do not interfere with the binding of TiO₂. Aqueous extract of leaves of different plants were prepared using 10 gm fresh leaves boiled with 50 mL of double distilled water at 90 °C for 20 min. The extracts were filtered through a medium filter. The filtrate is then used as the reducing and stabilizing agent for TiO₂ solution.

Biosynthesis of TiO₂ nanoparticles using leaf extract: 20mL of the prepared leaf extract was added to 80mL of 1mM of TiO₂. The experiment was conducted at 25°C, kept under agitation for 2hrs at 120 rpm. A change in colour from faint to dark was observed.

Characterization of synthesized TiO₂ nanoparticles: UV-Visible spectra of TiO₂ NPs were recorded with a UV-vis Spectrophotometer (DR-6000) procured from HACH (Germany). The size of TiO₂ NPs were measured using dynamic light scattering by Zetasizer Ver. 6.00 (Malvern, UK). FTIR spectra of TiO₂ NPs were analysed by FTIR spectrometer (Shimadzu, Japan) in attenuated total reflection mode and using spectral range of 4 000-400 cm⁻¹ with a resolution of 4 cm⁻¹. Transmission electron microscopy (TEM) analysis (Philips Technai-20) was performed for characterizing the size and shape of the

biosynthesized TiO₂ NPs. A drop of the TiO₂ NPs solution was loaded on carbon-coated copper grids, and dried in vacuum before analysis.

Antibacterial activity of TiO₂ nanoparticles: Antibacterial activity of TiO₂ NPs were evaluated by the agar well diffusion method. For agar well diffusion method [9], antimicrobial susceptibility was tested on solid (Agar-agar) media in petri plates. For bacterial assay, nutrient agar (NA) (40 gm/L) was used for developing surface colony growth. About 100 µl of TiO₂ NPs solution was added with sterile pipette into the wells and allowed to diffuse at room temperature. Negative Control experiments comprising inoculums with sterile double distilled water were set up, while as Positive control 1mg/ml streptomycin sulphate solution was taken. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured [9].

(2, 2-Diphenyl-1-picryl-hydrazyl-hydrate [DPPH] assay):

The DPPH antioxidant assay was determined [10]. Briefly, 1mM DPPH in 99.5% Ethanol. To 0.5ml of DPPH radical solution, 2 ml of the prepared solution of TiO₂ NPs was added (whole leaf derived nanoparticle), and the reaction mixture was vortexed for 10s and allowed to stand at room temperature for 30 min. The absorbance was recorded at 517 nm by using UV- Spectrophotometer and sample without test extract as control solution. Ascorbic acid was used as reference antioxidant compound.

The percentage of DPPH radical scavenging activity is expressed as:

$$\text{DPPH scavenging effect (\%)} = [1 - (\text{Test sample absorbance} / \text{blank sample absorbance})] \times 100(\%)$$

RESULTS AND DISCUSSION

Characterization of synthesized TiO₂ nanoparticles

UV-Vis Spectrophotometric analysis

The UV-Vis spectrum was recorded with a UV-Vis Spectrophotometer (DR-6000). Maximum absorption of TiO₂ NPs were found to be in the UV region with a sharp peak at 332 nm (Figure 1).

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of biosynthesized TiO₂ NPs from *Datura innoxia*. The peak around 3000/cm appeared due to the -OH stretching and the Ti-O stretching vibration is confirmed by the peak at the region of 1400-1460/cm. The

peaks present at the range of 1020-1250/cm depicts the existence of aliphatic amines [12].

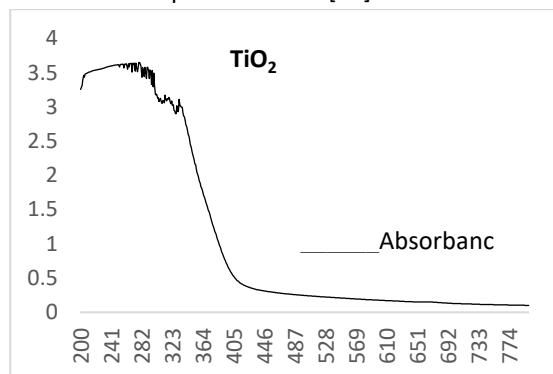


Figure 1: UV-Vis Spectroscopy of synthesized TiO_2 nanoparticles.

The FTIR spectrum showed characteristic band at 1621^{-1} corresponds to nitro compounds (asymmetrical stretch) [14]. Peaks observed at $1631.78/\text{cm}$ which is prominent in figure 3 indicates O-Ti-O bond [11]. The band intensities in different regions of the spectrum for the *Datura innoxia* extract and synthesized TiO_2 NPs test samples were analysed. There was a shift in the following peaks: 3672.59 , 1503.56 and 1129.36 shifted to 3673.55 , 1504.53 and 1132.25 respectively indicates the synthesis of nanoparticles as the peaks have shifted due to the interaction of TiO_2 with plant extract and formation of nanoparticles (Figure 2, 3).

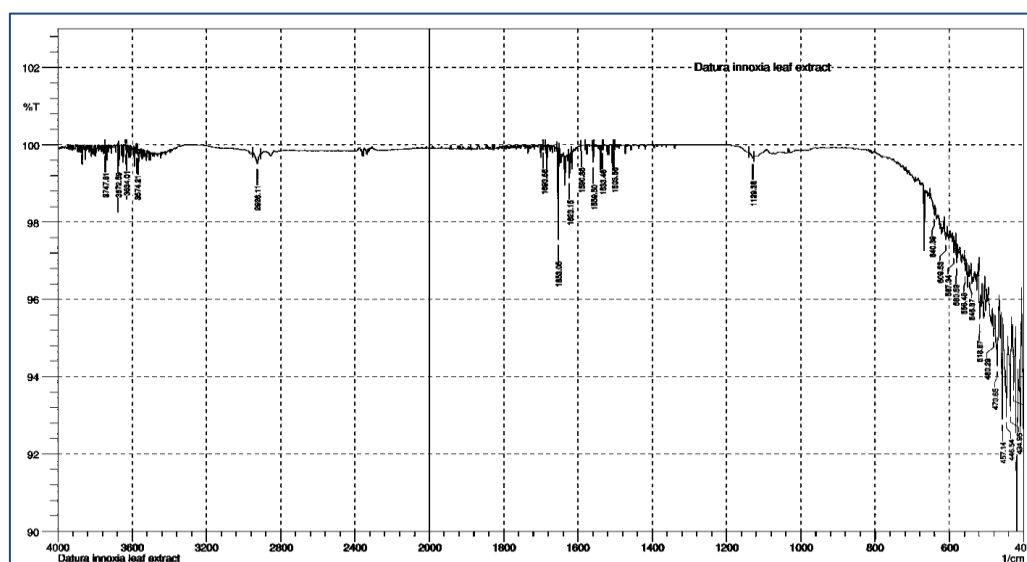


Figure 2: FTIR Analysis of *Datura innoxia* leaf extract

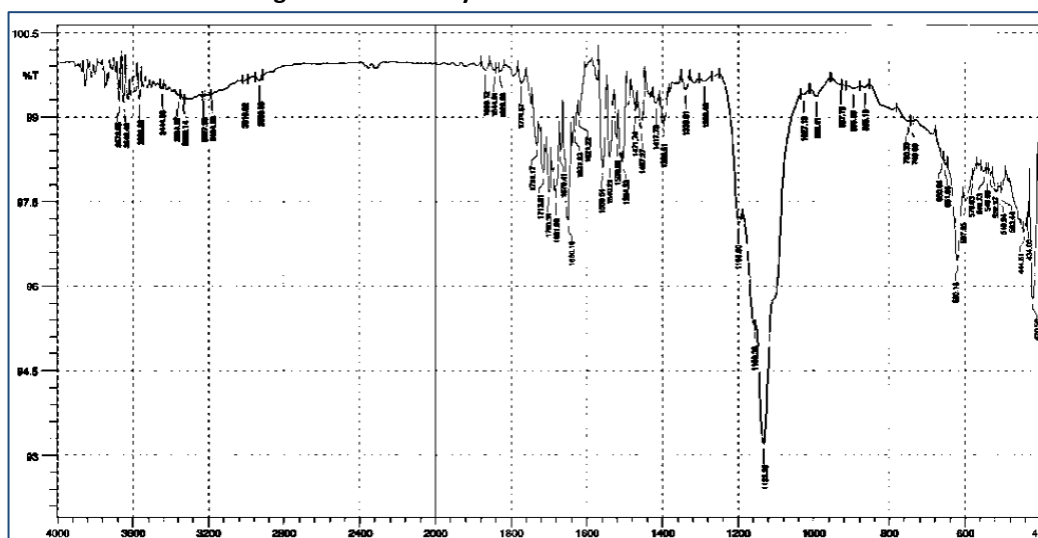


Figure 3: FTIR Analysis of TiO_2 nanoparticles from *Datura innoxia* leaf extract

Particle size measurement

The average particle size of TiO₂ NPs was found to be 78.8 nm with a peak intensity of 100% (Figure 4 & 5).

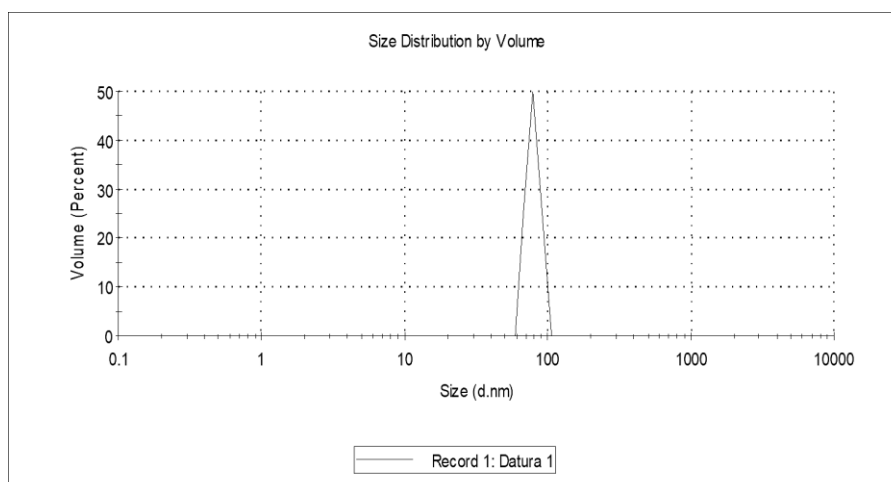


Figure 4: Particle size distribution based on volume

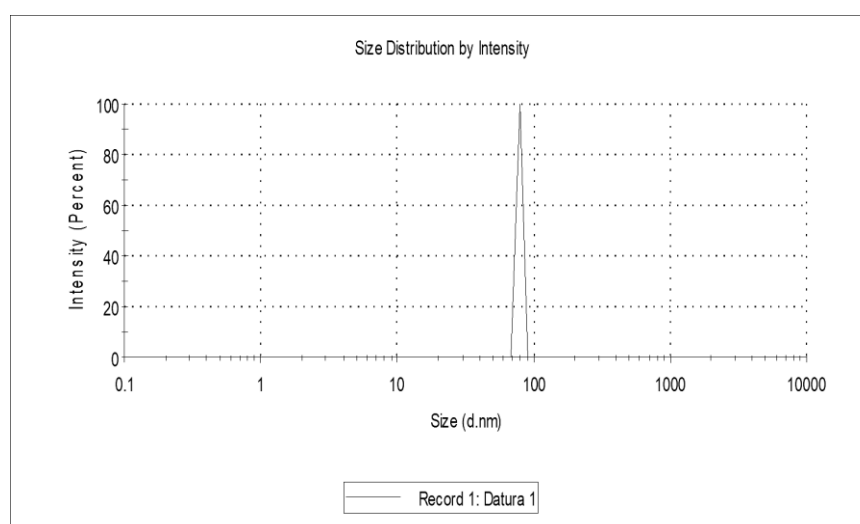


Figure 5: Particle size distribution based on intensity

TEM microscopy:

The morphology and size of the green synthesized TiO₂ NPs were also determined by TEM images. The

nanoparticles were hexagonal in shape with moderate variation in size (Figure 6). The size was in the range of 36 – 42 nm.

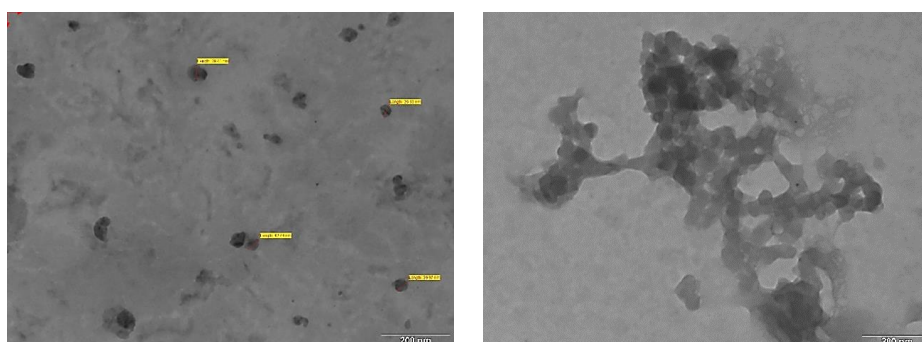


Figure 6: TEM images of titanium nanoparticles at 200nm scale

Antibacterial activity of TiO₂ nanoparticles

The TiO₂ NPs were effective against all the four tested organisms used for the antibacterial tests. Zone of inhibition were observed in the plates and recorded in

table 1 and figure 7. TiO₂ NPs are capable of dissolving the outer membranes of bacteria due to the presence of hydroxyl groups leading to the death of the organisms [7].

Table 1: Zone of inhibition in mm for each tested organism

Organisms	Zone of Inhibition(mm)		
	TiO ₂ salt	PC	TiO ₂ NPs
<i>Pseudomonas aeruginosa</i>	0	22	25
<i>Bacillus cereus</i>	0	30	35
<i>Proteus vulgaris</i>	0	20	26
<i>Enterobacter aerogenes</i>	0	20	22

*PC-Positive Control

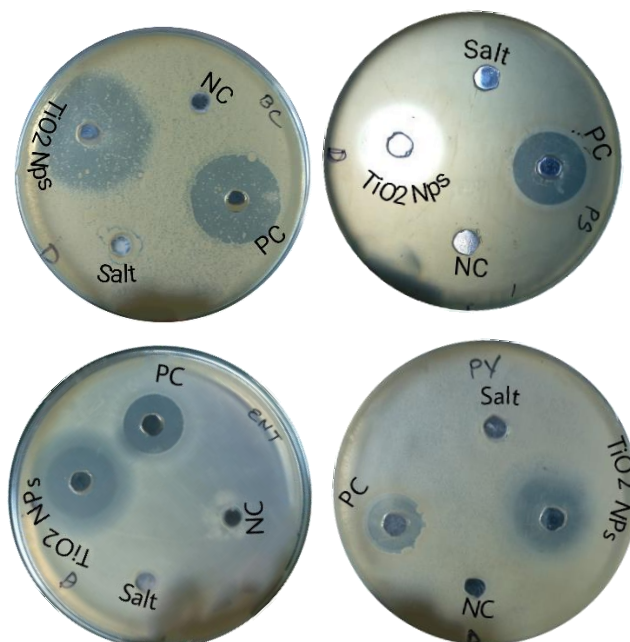


Figure 7: Antibacterial analysis of TiO₂ nanoparticles

The synthesized TiO₂ NPs were found to be maximum effective against *Bacillus cereus* and *Proteus vulgaris*.

(2, 2-Diphenyl-1-picryl-hydrazyl-hydrate [DPPH] assay)

Free radicals are unstable atoms which are always a hassle for the formation of stable bonds by receiving or

donating an unpaired electron. The stable compound DPPH gets reduced by gaining a hydrogen or electron. The change in colour in the test sample after 30 minutes incubation indicates the nature of the nanoparticles to be antioxidant and hence the reducing activity of the nanoparticles [13].

Table 2: DPPH free radical scavenging activity

Name of the sample	% DPPH scavenging activity
TiO ₂ Nps derived from <i>Datura innoxia</i> leaf extract	81%
<i>Datura innoxia</i> leaf extract	68%
TiO ₂ salt	35%

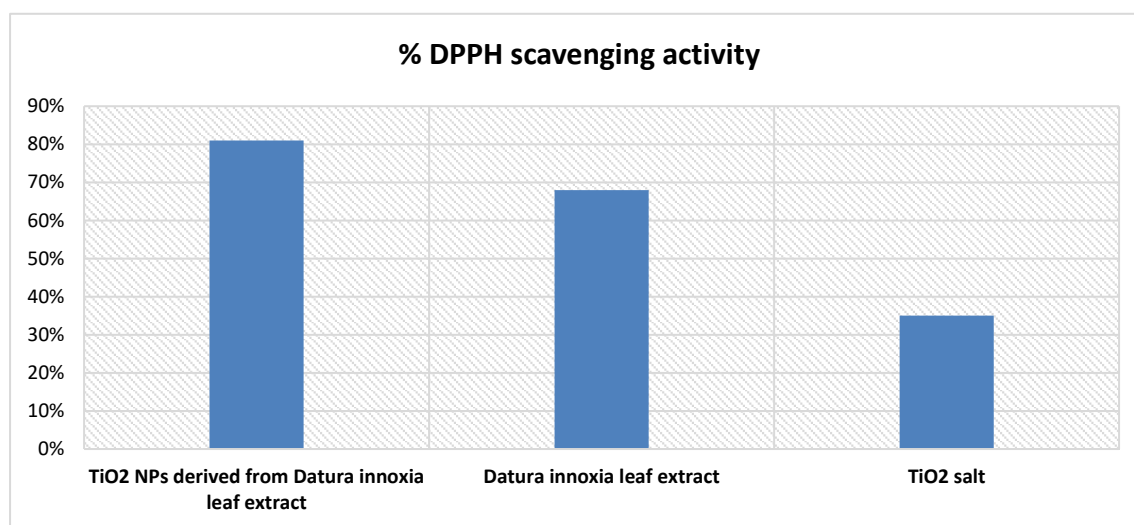


Figure 8: DPPH radical scavenging assay

The synthesized TiO₂ NPs exhibited more inhibition with 81% scavenging activity of DPPH than aqueous leaf extract of *Datura innoxia*. The DPPH free radical scavenging assay showed that synthesized TiO₂ NPs have higher free radical scavenging activity compared to aqueous leaf extract alone.

Few previous authors have reported the biosynthesis of TiO₂ NPs using plants for like *Catharanthus roseus* [15], *Eclipta prostrata* [16] and *Nyctanthes Arbor-Tristis* [17]. The current study for the first time is reporting the synthesis of

TiO₂ NPs using leaf extract of *Datura innoxia*.

This ecofriendly approach of synthesis of TiO₂ NPs can prove to be beneficial in biomedical and other biotechnological applications.

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