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Green Synthesis Of Titanium Dioxide Nanoparticles By Using Ethanolic Leaf Extract Of *Jatropha Curcas* L.

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ABSTRACT
The current study presents a green, eco-friendly approach for the synthesis of titanium dioxide (TiO_2) nanoparticles using ethanolic leaf extract of *Jatropha curcas* L., a medicinal plant rich in bioactive compounds. Traditional chemical and physical synthesis methods often involve hazardous reagents and energy-intensive processes; in contrast, this biological method offers a sustainable alternative. The leaves were extracted via Soxhlet apparatus using ethanol as solvent, and the resulting extract was employed in the reduction and stabilization of TiO_2 nanoparticles. The synthesized nanoparticles were characterized using GC-MS, DLS, zeta potential, SEM, and XRD techniques. GC-MS analysis confirmed the presence of twelve major phytochemicals, such as (+)-2-Bornanone, caryophyllene, alpha-pinene, and flavonoids, which are known for their antioxidant and antimicrobial properties. DLS analysis revealed an average particle size of 125 nm with a PDI of 0.410, indicating moderate monodispersity. Zeta potential analysis showed a negative surface charge of -35.2 mV, confirming high colloidal stability. SEM images revealed rough, wrinkled surfaces suggesting high surface reactivity, while XRD analysis confirmed the crystalline anatase phase of TiO_2. Antioxidant activity assessed via DPPH assay demonstrated a significant dose-dependent response, with a maximum inhibition of 73.57% at 100 µg/mL and an IC_{50} value of 75.08 µg/mL, comparable to ascorbic acid.

Keywords: Nanotechnology, *Jatropha curcas,* DLS, GCMS

INTRODUCTION

Nanotechnology is one of the most prominent areas of modern scientific research, focusing on the manipulation of matter at the nanoscale. It is an inherently multidisciplinary field with wide-ranging applications across sectors such as agriculture, energy, textiles, medicine, and the automotive industry [1]. Nanoparticles are typically defined as particles ranging in size from 1 to 100 nm, although particles larger than 100 nm may also be classified as nanomaterials depending on their intended use. Recent studies have indicated that various metallic nanoparticles such as those composed of copper (Cu), titanium dioxide (TiO_2), silver (Ag), zinc (Zn), and iron (Fe) can exhibit both beneficial and adverse effects on plant systems. However, comprehensive research is still needed to better understand their interactions and impacts [2]. As primary producers in food chains, plants serve as critical entry points for the bioaccumulation of engineered nanomaterials (ENMs). When applied excessively or without regulation, ENMs can adversely affect air, water, and soil quality, leading to the formation of long-term environmental reservoirs [3]. Among the most widely used nanomaterials in commercial applications today are titanium dioxide (TiO_2) nanoparticles [4]. India possesses abundant reserves of key titanium-bearing minerals, primarily ilmenite ($FeO.TiO_2$) and rutile (TiO_2). TiO_2 naturally occurs in three crystalline forms rutile, anatase, and brookite of which brookite is rare and typically a secondary product of other titanium minerals. The distribution of TiO_2 deposits across various Indian states is summarized in Table 1. TiO_2 nanoparticles offer several advantages, including a high specific surface area, favorable electronic band structure, high quantum efficiency, chemical inertness, and remarkable stability [5]. Consequently, there is growing interest in the cost-effective, large-scale biosynthesis of TiO_2 nanoparticles using eco-friendly biological methods.

Various methods are available for the synthesis of TiO_2 nanoparticles, including sol–gel, solvothermal, hydrothermal, electrochemical, and precipitation techniques. However, each of these methods has certain limitations. For instance, the sol–gel process is time-consuming, often requiring several hours to days for nanoparticle formation. The hydrothermal method involves complex chemical reactions and is primarily synthetic in nature. The precipitation method poses challenges in controlling particle size, as rapid precipitation can lead to the formation of larger, less uniform particles. In contrast, microbial synthesis offers a cost-effective and environmentally friendly alternative. This biological approach operates under mild conditions and avoids the use of hazardous chemicals, making it a sustainable method for nanoparticle production [6].

Table 1 Reservoirs of TiO2. Source

States of India	Resources of ilmenite minerals (million tonnes)	Resources of rutile minerals (million tonnes)
Odisha	96.44	4.47
Tamil Nadu	179.02	8.00
West Bengal	2.05	0.19
Andhra Pradesh	163.05	10.25
Jharkhand/Bihar	0.73	0.01
Gujarat	2.77	0.02
Kerala	145.70	8.41
Maharashtra	3.74	23.00

The extraction of Leaf of *Jatropha curcas* L. brings commercial importance to the plant [7,8]. Jatropha latex has some ethnomedical uses like wound healing and blood coagulation activities [9]. Jatropha curcas L. (Euphorbiaceae), also known as '*Mafengshu*', '*Xiaotongzi*' in Chinese, is cultivated for the medical purpose and widely spread in tropical regions over the world. *Guangxi Herbal Medicine* recorded that it was astringent, slightly cold and toxic, and has the effect of dissipating blood stasis and swelling, stanch bleeding, relieving pain, and preventing itching. Previous studies showed that the chemical components from *J. curcas* were diterpenoids, sesquiterpenes, lignans, and flavonoids [10,11].

MATERIAL AND METHODS

Material

Collection and Preparation of Sample

Leaf of the Jatropha curcas plant was collected from surrounding of local area of Kasegaon, Maharashtra, India, and was authenticated at the Department of Botany, Sadguru Ghadge Maharaj College, Karad, India. Then it has been washed with distilled water to avoid any microbial growth.

Chemicals

TiO (OH)2 analytical grade was purchased from Sigma-Aldrich (USA). All the aqueous solutions were prepared in triple distilled de-ionized water. All other chemicals and reagents were from standard commercial sources and of highest quality available.

Extraction and Preparation of Plant Extracts

A 25 g powdered sample of the Jatropha curcas leaves was placed into a thimble, which was then loaded inside the Soxhlet extractor. A 500 mL round-bottom flask with a condenser was attached to the Soxhlet extractor and filled with the ethanol. The extractor was then set up on a heating mantle. The solvent started to evaporate as it moved through the Soxhlet extractor to the condenser after being heated by the heating mantle. Next, drips of the condensed solvent started appearing in the Soxhlet extractor holding the thimble that contained the plant sample. The solvent with extract was recycled back to the round-bottom flask once the solvent level reached the siphon. This process continued until the designed period for the extraction had finished, and then the solution of the extract was given time to cool down at ambient temperature [12]. Following that, the extract was concentrated via a rotary evaporator after being filtered using Whatman No.1 filter paper. The dried extract was then kept chilled at 4 °C for further testing.

GCMS

GC-MS analysis was carried out with an SHIMADZU QP 2010T which composed of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument employing the following condition: capillary column −624 ms (30 m×0.32 mm×1.8 m) operating in an electronic modeat70eV;helium(99.99%) was used as the carrier gas at a constant flow of 1.491 mL/min and injection volume of 1.0 mL, injector temperature of 140 °C, and ion source temperature of 200 °C. The oven temperature was programmed from 45 °C. Mass spectra were taken at 70 eV [13].

Synthesis of TiO2 Nanoparticles

1 mM aqueous solution of titanium dioxide (TiO_2) was stirred at room temperature (25 °C) for 2 hours to achieve uniform dispersion. Following this, 10 mL of the prepared plant extract was slowly added to 20 mL of the TiO_2 solution under continuous stirring. The reaction mixture was maintained at 25 °C and stirred for an additional 4 hours. A visible color

change to green or dark purple indicated the biosynthesis of TiO_2 nanoparticles via reduction by phytochemicals present in the extract [14].

Characterization of Nanoparticles

Dynamic Light Scattering (DLS)

The spectroscatterer RiNA, GmbH class3B was used to determine DLS measurements (the main size) of the TiO2 NPs. The dried powder was scattered in distilled water and all analyses were performed at 20°C for ten cycles. The experiments for DLS were repeated three times. The non-destructive morphological analysis technique of dynamic light scattering had been applied in phytocomponent analysis. It is highly useful for the accurate sizing of chemical and physical materials that are synthesized using plant phytochemicals [15].

Zeta Potential

The Zeta Potential of Nanoparticles is a typical method for determining the surface charge property of Nanoparticles. It reflects a particle's electrical potential and is influenced by the particle's composition as well as the medium in which it is scattered. The Zeta Potential was analyzed using Malvern Zeta analyzer. The sample was placed in cuvette with necessary dilutions and it was then kept in analyzer to determine the Zeta potential [16].

SEM

SEM was used to characterize the morphology and particle size of AgNPs. A thin film of oven-dried GT AgNP sample was prepared and used over a carbon-coated copper grid via a TESCAN MIRA-3 instrument operated at an accelerated voltage of 20 kV [17].

XRD

XRD technique is used to study the crystalline or amorphous nature and the structure of the synthesized TiO2 NPs. Then the powdered sample was placed on a Shimadzu XRD-6000 and set in the range of $5\text{-}50^{0}$ at a 2ɵ angle [18].

Antioxidant Activity of Nanoparticle:

The antioxidant activity of the synthesized nanoparticles was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. In this method, 1 mL of nanoparticle samples at varying concentrations (20, 40, 60, 80, and 100 µg/mL) was transferred into separate test tubes. To each sample, 1.5 mL of 0.1% methanolic DPPH solution was added, and the mixture was incubated in the dark for 30 minutes to allow the reaction to occur. Following incubation, the color change from purple to yellow was observed, indicating the reduction of DPPH radicals by antioxidants present in the sample. The absorbance of each mixture was measured at 510 nm using a colorimeter.

RESULT AND DISCUSSIONS

GCMS

The GC-MS chromatogram of the ethanolic leaf extract of *Jatropha curcas* revealed the presence of multiple bioactive compounds, each identified by their specific retention times and peak areas. A total of twelve significant phytoconstituents were detected, indicating the chemical complexity and therapeutic potential of the extract.

Among the identified compounds, (+)-2-Bornanone was the major constituent, showing the highest peak area (835,989) at a retention time of 13.426 minutes. This compound, commonly known as camphor, is well-known for its antimicrobial and anti-inflammatory properties. Caryophyllene, a sesquiterpene with notable anti-inflammatory and anticancer potential, was observed at a retention time of 20.998 minutes with a significant peak area of 129,317. Other notable constituents included Alpha-pinene (RT 6.841, peak area 6,583) and Camphene (RT 7.314, peak area 19,449), both of which are monoterpenes known for their antioxidant and antimicrobial effects. Additionally, the presence of flavonoids such as 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one and 3,3',4',5,7-pentahydroxyflavone at retention times 21.287 and 24.464 minutes, respectively, highlights the strong antioxidant potential of the extract. Fatty acid derivatives including Hexadecanoic acid, methyl ester (RT 33.386) and Methyl stearate (RT 37.702) were also detected, contributing to the anti-inflammatory and emollient properties of the extract. Other minor constituents such as Naphthalene, Neophytadiene, and a tetracyclic diterpene at RT 24.800 further support the pharmacological richness of the plant.

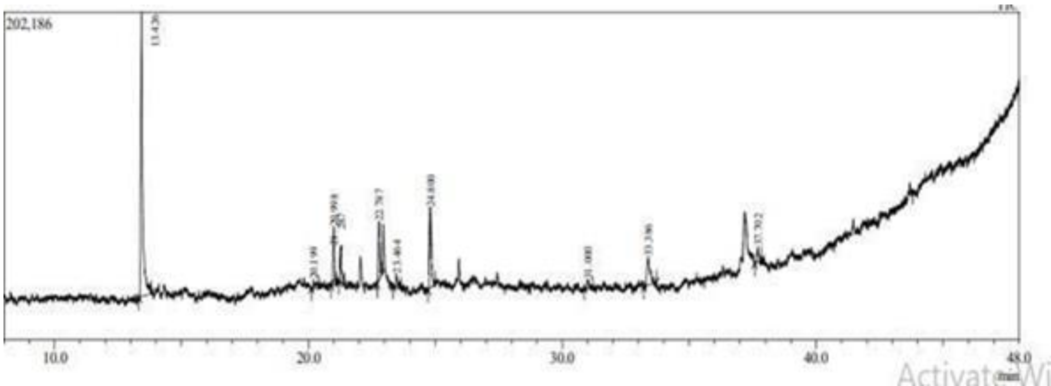

Figure1: GCMS of Jatropha curcas

Table2: GCMS analysis of Jatropha curcas.

Retention time	Peak area	Name
6.841	6583	Alpha pinene
7.314	19449	Camphene
13.426	835989	(+)-2-Bornanone
20.199	25280	Naphthalene
20.998	129317	Caryphyllene
21.287	90383	3,5,7 trihydroxy-2-(4hydroxyphenyl)-4H-chromen-4one
24.464	44750	3,3',4',5,7 pentahydroxyflavone
24.800	206707	(1aR,3aS,7S,7aS,7bR)-1,1,3a,7 Tetramethylde
31.000	20527	Neophytadiene
33.386	13484	Hexadecanoic acid, methyl ester
37.702	33417	Methyl stearate

Particle size

Dynamic light scattering (DLS) revealed a Z-average size of 125 nm with a polydispersity index (PDI) of 0.410, indicating moderate uniformity in particle size. The narrow, symmetric distribution curve suggests a monodisperse population with limited aggregation. This nanoscale dimension confirms the efficiency of the green synthesis method using *Jatropha curcas* extract and is suitable for drug delivery, photocatalysis, and sunscreen due to the high surface area-to-volume ratio.

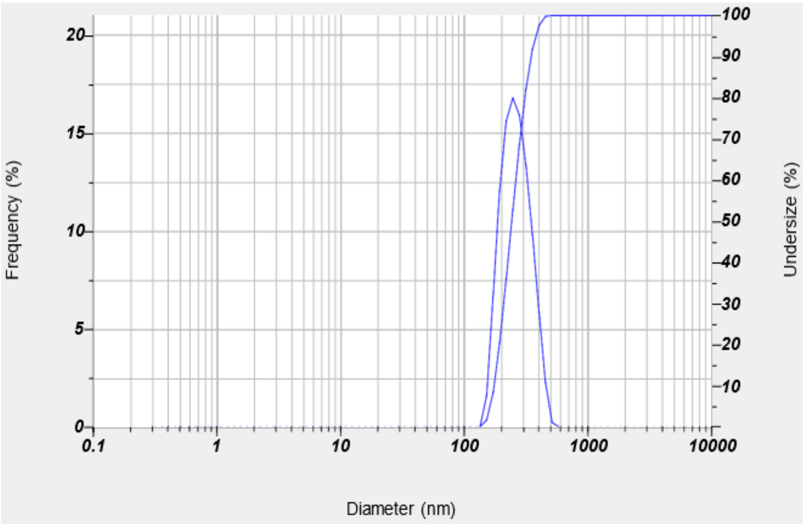

Figure2: Particle size of Nanoparticle

Zeta potential

The synthesized TiO_2 nanoparticles showed a zeta potential of -35.2 mV, indicating strong electrostatic repulsion between particles. This value suggests a highly stable colloidal suspension with minimal risk of aggregation. The negative charge is likely due to phytochemicals in the extract, which act as natural capping and stabilizing agents.

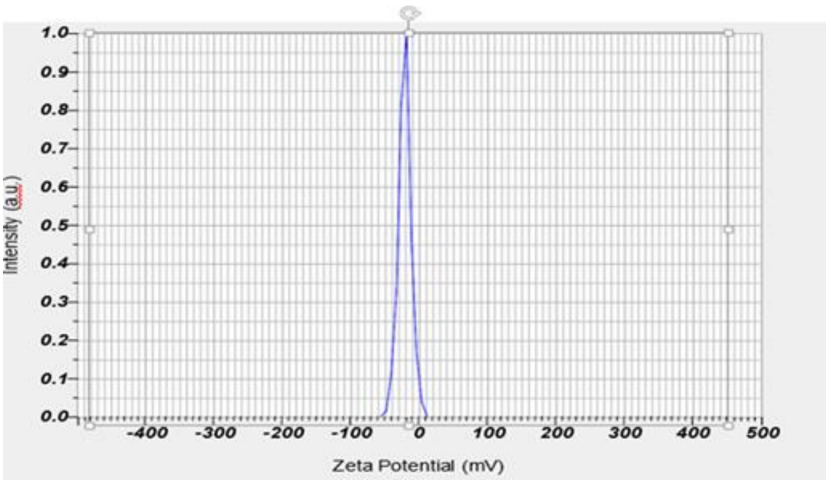

Figure 3: Zeta Potential of Nanoparticle

SEM

Surface morphology of prepared nanoparticle was analyzed by scanning electron microscope (SEM) characterization. Superficial morphology of prepared TiO2 nanoparticles was explained by using scanning electron microscope (SEM), and results are reputed as Figure. The images display microspheres with a rough, wrinkled texture and shrinkage, suggesting successful polymer coating. Visible surface pores and residues imply an even distribution of the plant-extract-capped nanoparticles within the matrix. The rough external appearance may result from the drying or coating process, indicating non-spherical morphology and possibly high surface reactivity. Such morphology is favorable for enhanced interaction with target molecules in catalytic or biomedical applications.

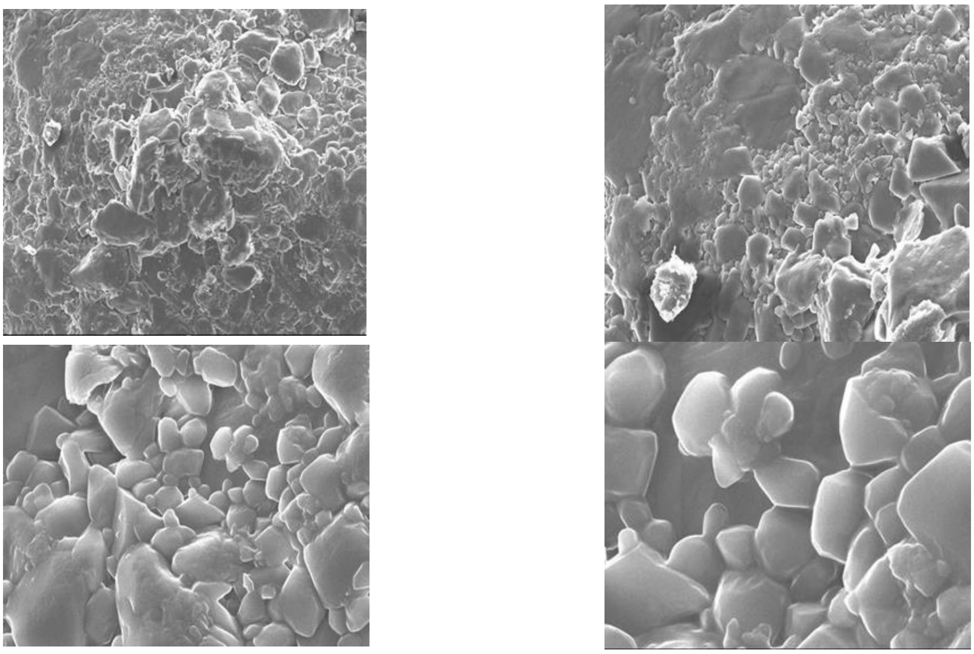

Figure 4: SEM of Particle Size

X-ray Diffraction (XRD) Analysis

XRD analysis confirmed the crystalline nature of the green-synthesized TiO_2 nanoparticles. Peaks observed at 2θ = 25.3°, 48.0°, 54.0°, and 62.6° correspond to the anatase phase of TiO_2 (with Miller indices (101), (200), (105), and (204) respectively). The absence of additional peaks indicates high phase purity and no contamination from other TiO_2 polymorphs like rutile or brookite. This anatase form is particularly desirable due to its superior photocatalytic and antimicrobial properties.

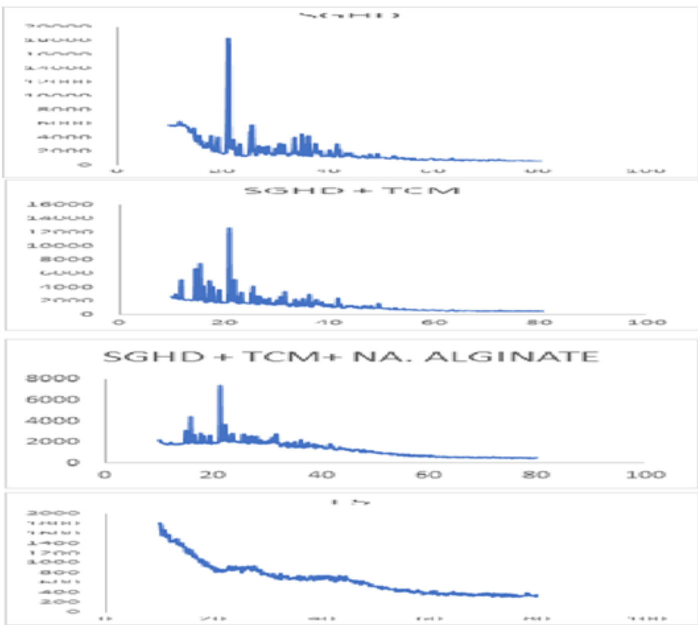

Figure 5: XRD

Antioxidant Activity

The antioxidant activity of the synthesized nanoparticles was evaluated using the DPPH assay, which revealed a notable dose-dependent inhibition of free radicals. At a concentration of 100 µg/mL, the nanoparticles demonstrated a 73.57% inhibition, which is comparable to the standard antioxidant, ascorbic acid, showing 82.90% inhibition. The IC_{50} value was determined to be 75.08 µg/mL, indicating a strong antioxidant capacity. These findings suggest that the bioactive phytochemicals from *Jatropha curcas* are effectively retained on the surface of the titanium dioxide nanoparticles, thereby enhancing their free radical scavenging activity.

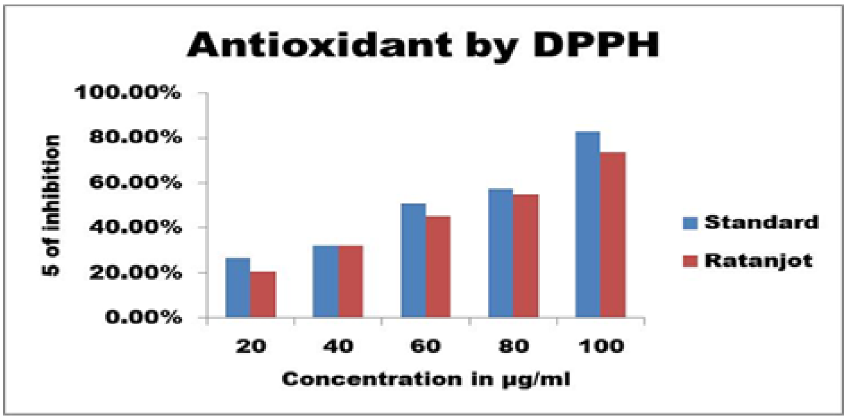

Figure 6: Antioxidant activity of Nanoparticle

Figure 7: Antioxidant assay by DPPH

CONCLUSION

The study successfully demonstrates a cost-effective, sustainable, and green synthesis route for titanium dioxide nanoparticles using *Jatropha curcas* ethanolic leaf extract. The nanoparticles showed favorable physicochemical properties, significant antioxidant potential, and promising characteristics for biomedical and environmental applications.

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