

Biogenic Synthesis and Characterization of TiO₂ Nanoparticles Using *Lepidagathis* Leaf Extract

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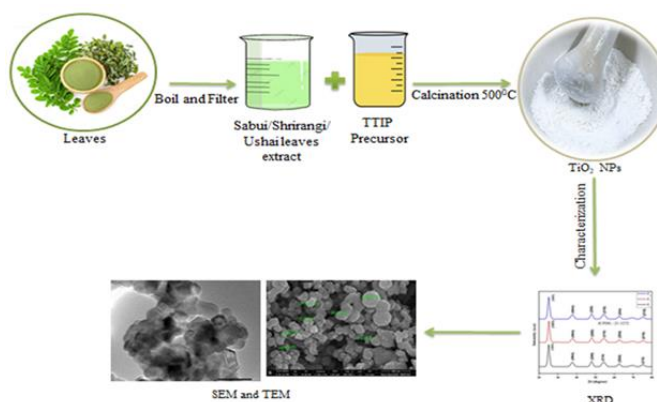
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ABSTRACT

For the first time, titanium dioxide (TiO₂) nanoparticles were synthesized through a green route using leaf extracts from three underexplored species of the Acanthaceae family—*Lepidagathis sabui*, *Lepidagathis Shrirangii*, and *Lepidagathis ushae*. The phytochemicals present in the extracts acted as natural reducing and stabilizing agents, enabling an eco-friendly and cost-effective synthesis without hazardous chemicals. The nanoparticles were thoroughly characterized using UV-Vis, FTIR, SEM, TEM, and EDS analyses. UV-Vis DRS spectroscopy showed an absorption edge with a band gap of ~3.2 eV, typical of TiO₂ semiconductors and indicative of photocatalytic potential. FTIR confirmed Ti–O–Ti vibrations, SEM and TEM revealed uniformly distributed semi-spherical nanoparticles, and EDS verified titanium and oxygen as the primary constituents. These results establish Acanthaceae leaf extracts as sustainable bioresources for TiO₂ nanomaterial fabrication, highlighting their promise in photocatalysis, environmental remediation, and broader nanotechnology applications.

Keywords: Titanium dioxide, green synthesis, plant extract, sustainable materials, Acanthaceae, *Lepidagathis*.



I. INTRODUCTION

Nanotechnology has emerged as a transformative field, where the development of efficient and sustainable nanomaterials is of paramount importance. Among these, titanium dioxide (TiO₂) nanoparticles have attracted considerable attention due to their distinctive structural, optical, and chemical properties.[1] However, conventional methods of TiO₂ synthesis often require hazardous chemicals, involve high energy consumption, and generate toxic by-products, which restrict their eco-friendly applicability.[2],[3],[4],[5]

In this context, green synthesis using plant extracts has gained prominence as an alternative strategy. Phytochemicals such as flavonoids, tannins, phenolics, and glycosides naturally present in plants act as reducing and stabilizing agents during nanoparticle formation. This eliminates the need for toxic reagents while offering improved stability and biocompatibility of nanoparticles.⁵[6] Unlike microbial routes, plant-mediated synthesis is faster, simpler, scalable, and cost-effective, making it highly suitable for sustainable nanotechnology.

Among diverse botanical resources, plants belonging to the Acanthaceae family are particularly promising. These species are phytochemically rich, containing alkaloids, glycosides, phenolics, and terpenoids, which play a crucial role in nanoparticle formation and stabilization.[7] Despite this, the use of Acanthaceae plants in nanoparticle synthesis has been largely unexplored, and their potential remains underutilized. This gap presents an opportunity to discover novel

reducing and capping agents that may impart unique structural and functional features to nanoparticles.

In particular, *Lepidagathis sabui*[8], *Lepidagathis Shrirangii*[9] and *Lepidagathis Ushae*[10] recently reported members of the Acanthaceae family, are noteworthy.[8],[9],[10] These plants are known for their diverse phytochemical profiles and medicinal relevance in traditional systems, yet their role in nanomaterial fabrication has not been investigated. Their rich bioactive composition makes them highly suitable for green synthesis, offering the possibility of generating TiO₂ nanoparticles with unique size, morphology, and stability characteristics.

The novelty of the present study lies in the first-time use of an Acanthaceae plant species for the green synthesis of TiO₂ nanoparticles, providing a sustainable and eco-friendly route that avoids conventional chemical hazards. Furthermore, the synthesized nanoparticles were comprehensively characterized using advanced analytical techniques, including X-ray diffraction (XRD), UV-visible spectroscopy, and high-resolution transmission electron microscopy (HR-TEM), to establish their structural and morphological features. This approach not only broadens the scope of green nanotechnology but also introduces an unexploited plant resource as a new pathway for TiO₂ nanoparticle synthesis. By highlighting the untapped potential of Acanthaceae species, this study opens new directions for the exploration of other underutilized plant families in nanomaterial fabrication, thereby contributing to the advancement of sustainable and bio-inspired nanotechnology.

II. Experimental Methods:

Collection and processing of plant leaves:

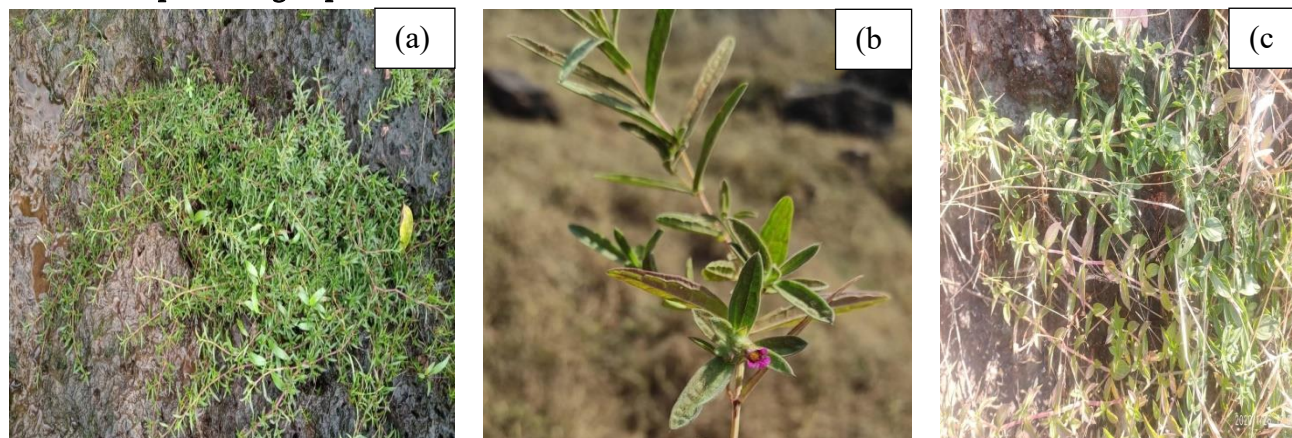


Fig 1. Images of selected plant leaves a) *Lepidagathis Sabui* b) *Lepidagathis Shrirangii* c) *Lepidaathis Ushae*

Fresh leaves of *Lepidagathis sabui*[8], *Lepidagathis Shrirangii*[9], and *Lepidagathis ushae*[10] were collected from Ratnagiri, Maharashtra, India, a region renowned for its rich diversity of medicinal flora.[11]The leaves were thoroughly washed with clean tap water to remove dust and other impurities. They were then air-dried at room temperature for

seven days, following standard green synthesis protocols to preserve the bioactive phytochemicals. After complete drying, the leaves were ground into a fine powder using a mixer grinder. The resulting powdered material was stored in an airtight container to prevent moisture absorption and degradation of the bioactive compounds. [6],[12]

2.2 Biosynthesis of TiO₂ NPs:



Fig 2. a) Addition of Plant extract to Precursor b) TiO₂ NPs c) After Calcination TiO₂ NPs

For the synthesis of titanium dioxide (TiO₂) nanoparticles, Titanium (IV) isopropoxide was obtained from Himedia Laboratories for this study. Titanium isopropoxide was first dissolved in ethanol and stirred for 10 minutes to ensure uniform hydrolysis. An equal-volume (1:1) mixture of 50 mL

aqueous plant extracts from *Lepidagathis sabui*, *Lepidagathis Shrirangii*, and *Lepidagathis Ushae* was then added to 50 mL of 0.5 M titanium isopropoxide solution under continuous stirring. The reaction mixture was maintained at 50°C for 4 hours, during which a visible colour change to yellowish indicated

the preliminary formation of TiO₂ nanoparticles.³ After four hours of continuous stirring, the nanoparticles were collected by centrifugation at 3000 rpm for 10 minutes. The obtained solid product was dried in a hot air oven at 100–120°C for 6 hours to remove moisture. Finally, the dried material was calcined at 500°C for 2 hours in a muffle furnace. [4],[13],[14],[15]

III. Results and Discussion:

X-ray diffraction (XRD) Analysis:

The crystallite size and phase of the synthesized nanoparticles were analyzed using a Rigaku MiniFlex 600 powder X-ray diffractometer with Cu K α radiation ($\lambda = 0.15418$ nm) at 40 kV and 15 mA. XRD measurements were performed in the 2θ range of 20–80° with a scan rate of 0.12°/s and a step size of 0.02°. The diffraction patterns of samples (Lepidagathis Sabui (P₆), Lepidagathis Shrirangii (P₇), and Lepidagathis Ushae (P₈) show sharp peaks at 2θ values of 25.2°, 37.8°, 47.9°, 54.3°, 62.8°, and 75.2°, corresponding to the (101), (004), (200), (210), (204), and (215) planes, which match well with the tetragonal anatase TiO₂ phase (JCPDS 21-1272). The intense (101) reflection confirms high crystallinity, while the absence of extra peaks indicates phase purity. The crystallite sizes, calculated using the Debye–Scherrer equation $D = k\lambda/(\beta\cos\theta)$ Where, λ = wavelength (0.154 nm), β = FWHM (Full Width at Half Maximum of more intense peak), $K = 0.89$ and θ = diffraction angle. The observed crystalline size were found to be 13.52 nm (P₆), 5.85 nm (P₇), and 5.72 nm (P₈), suggesting that (P₈) possesses the largest crystallites with better crystallinity. Overall, the XRD analysis confirms that all three samples are single-phase anatase TiO₂ with slight variations in crystallite size due to differences in growth during synthesis.[5], [16]

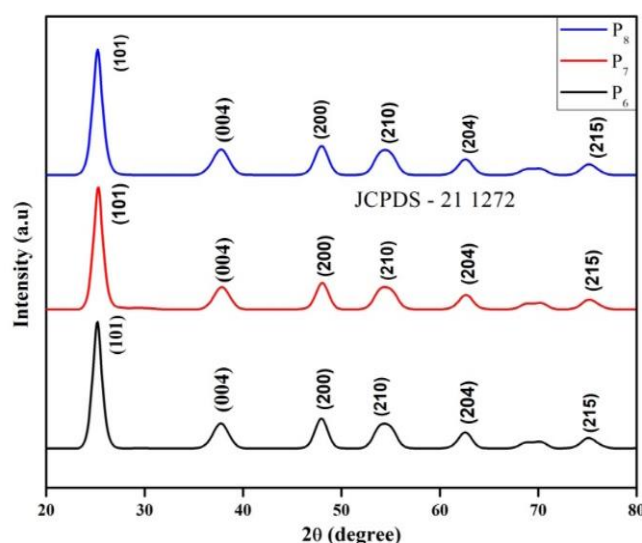


Fig.3 XRD Pattern of TiO₂ GNPs a) *Lepidagathis* Sabui(P₆) b) *Lepidagathis* Shrirangii(P₇) c) *Lepidagathis* Ushae(P₈)

The Fourier-transform infrared (FTIR) of TiO₂ GNPs:

The Fourier-transform infrared (FTIR) spectra of samples (P₆), (P₇), and (P₈) were recorded using a Perkin Elmer spectrometer in the wavenumber range of 400–4000 cm⁻¹ (Fig. 4). A strong absorption band in the low-frequency region (400–700 cm⁻¹) corresponds to the Ti–O–Ti stretching vibrations, which serve as the characteristic fingerprint of titanium dioxide. The distinct peak observed at ~438 cm⁻¹ further confirms Ti–O bonding, verifying the successful formation of TiO₂ nanoparticles. The absence of additional sharp peaks in the higher wavenumber region indicates high purity, while slight shifts in the Ti–O–Ti band position compared to bulk TiO₂ suggest nanoscale dimensions and surface modification. These modifications are likely induced by plant-derived phytochemicals, which play a dual role in the reduction and stabilization of TiO₂ during green synthesis and remain attached to the nanoparticle surface. Such surface functionalization enhances photocatalytic activity, biocompatibility, and stability by influencing the dispersion behaviour, optical response, and interaction of TiO₂ nanoparticles with organic pollutants or biological systems. The FTIR

findings are consistent with the XRD results, where both techniques confirm the formation of phase-pure anatase TiO_2 . Together, these analyses demonstrate that the synthesized nanoparticles are highly crystalline with nanoscale dimensions and surface functionalization imparted by bio-organic molecules.[17], [18]

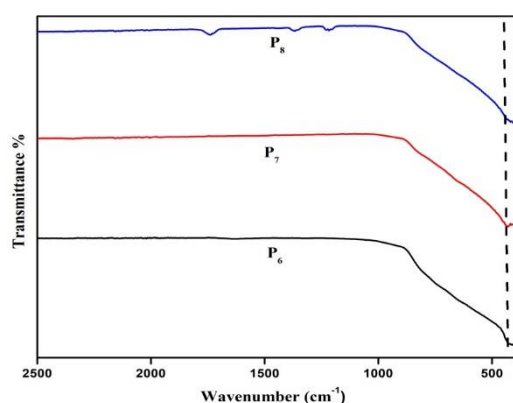


Fig.4 FTIR spectrum of green-synthesized TiO_2 nanoparticles showing characteristic Ti–O–Ti stretching vibrations.

Brunauer–Emmett–Teller (BET) Analysis

BET analysis was performed for sample P_6 , which was selected as a representative owing to its optimum

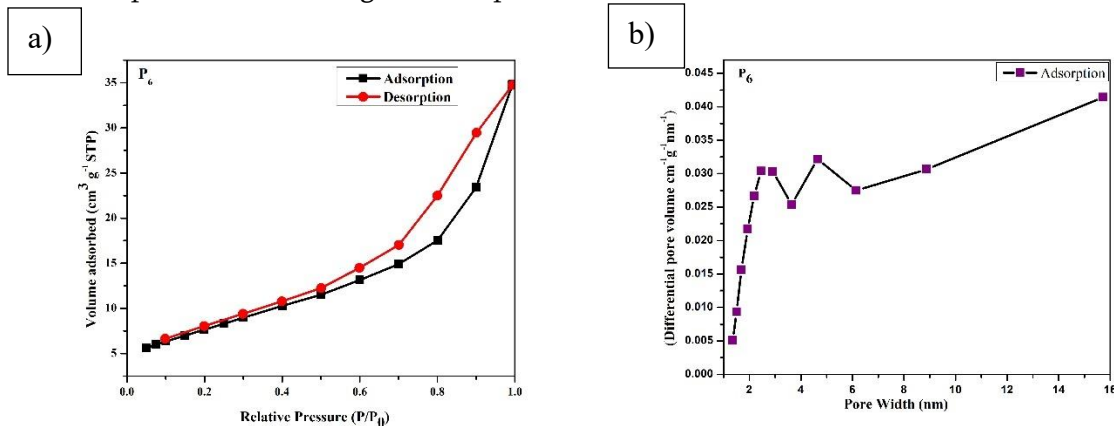


Fig:5. BET Plot of a) N_2 adsorption–desorption isotherm and b) Pore size distribution of TiO_2 GNPs

Ultraviolet–Visible Diffuse Reflectance Spectroscopy (UV–DRS) analysis :

The optical properties of green-synthesized TiO_2 nanoparticles were investigated using UV–DRS analysis (Figure 6). The absorption edge appeared

properties and to avoid repetition, since similar trends were expected across the series. The nitrogen adsorption–desorption isotherm of sample P_6 (Figure 5a) exhibits a **Type IV profile with an H3 hysteresis loop**, confirming its **mesoporous nature**. [19] The gradual uptake at low relative pressures ($P/P_0 < 0.3$) corresponds to monolayer–multilayer adsorption, while the sharp increase at higher pressures ($P/P_0 > 0.8$) indicates **capillary condensation in mesopores**. The total adsorbed volume ($\sim 35 \text{ cm}^3 \text{ g}^{-1} \text{ STP}$) reflects appreciable pore volume. [1], [20]

BET analysis (BELSORP MAX II) gave a **specific surface area of $44.471 \text{ m}^2 \text{ g}^{-1}$** , while BJH analysis (Figure 5b) indicated an **average pore diameter of 7.68 nm**, consistent with mesoporosity. The pore size distribution reveals a dominant contribution at **2–3 nm** with additional pores up to $\sim 16 \text{ nm}$, suggesting a **hierarchical, slit-like mesoporous framework** typical of H3 hysteresis. [21]

Overall, sample P_6 can be classified as **mesoporous with moderate surface area and bimodal pore distribution**, making it suitable for applications in adsorption, catalysis, and diffusion-driven processes.

around **380 nm**, corresponding to electronic transitions from the valence band to the conduction band. This transition arises due to the interaction of **Ti 3d orbitals with O 2p orbitals** of the oxide framework, which suppresses high-valency Ti states and restricts

the recombination of photoexcited electrons with holes. As a result, the **lifetime of charge carriers** is extended, enhancing light absorption efficiency.

The direct bandgap energy value 3.2 eV for the synthesized TiO₂ GNPs calculated from tauc plot method. The optical band gaps of the TiO₂ nanoparticles synthesized using different *Lepidagathis* extracts were found to be **3.19 eV (L. sabui, Figure 6a)**, **3.20 eV (L. Shrirangii, Figure 6b)**, and **3.20 eV (L. Ushae, Figure 6c)**. These values are consistent with the reported band gap of anatase TiO₂ (~3.2 eV), confirming the successful synthesis of semiconducting nanoparticles.

The slight variation among the three samples can be attributed to differences in the phytochemical composition of the plant extracts, which acted as **reducing and stabilizing agents** during nanoparticle

formation. The addition of leaf extracts to titanium isopropoxide resulted in a visible color change from light yellow to whitish yellow, indicating the reduction of Ti⁴⁺ ions and formation of Ti–O bonds. Similar band gap values have been reported earlier for TiO₂ nanoparticles synthesized from Acanthaceae leaf extracts.[22],[23]

The wide band gap of the green-synthesized TiO₂ nanoparticles plays a crucial role in **suppressing charge carrier recombination** and promoting efficient **electron–hole pair generation and separation**. This improved charge separation enhances photocatalytic efficiency by facilitating the production of **reactive oxygen species (ROS)** and hydroxyl radicals, which are highly effective in degrading organic pollutants and microbial contaminants.

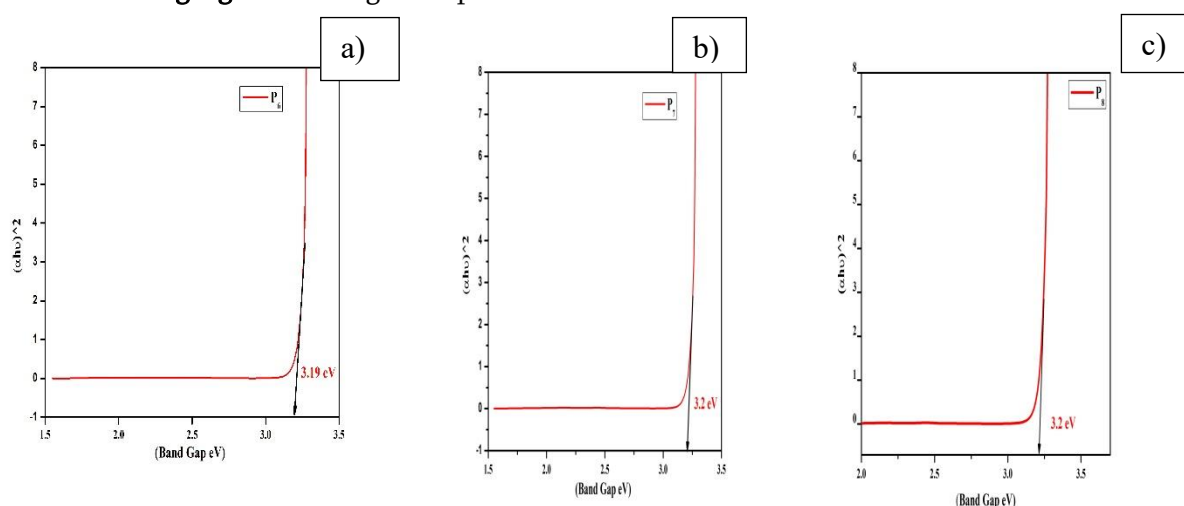


Fig.6 UV DRS spectrum of TiO₂ NPs by using a) *Lepidagathis* Sabui(P_6) b) *Lepidagathis* Shrirangii(P_7) c) *Lepidagathis* Ushae(P_8)

Scanning Electron Microscopy (SEM) analysis

The SEM micrograph displays TiO₂ nanoparticles synthesized via a green route, exhibiting predominantly spherical to slightly irregular morphologies. The nanoparticles are densely packed and exhibit a high degree of agglomeration, which is typical due to their high surface energy and strong interparticle Van der Waals forces. Surface texture analysis reveals the presence of fine nano-granular

structures on the particle surfaces, likely representing smaller crystallites attached to larger aggregates.[24] Particle size measurements, indicated in green, show dimensions that confirming their nanostructured nature. The rough surface morphology and size variation suggest that phytochemical compounds from the plant extract acted as capping and stabilizing agents during synthesis. These agents likely contributed to the prevention of excessive grain growth while allowing partial agglomeration.[25] The

image highlights the fine nanostructural features of photocatalytic and surface-reactive properties. the TiO_2 GNPs, which are crucial for their

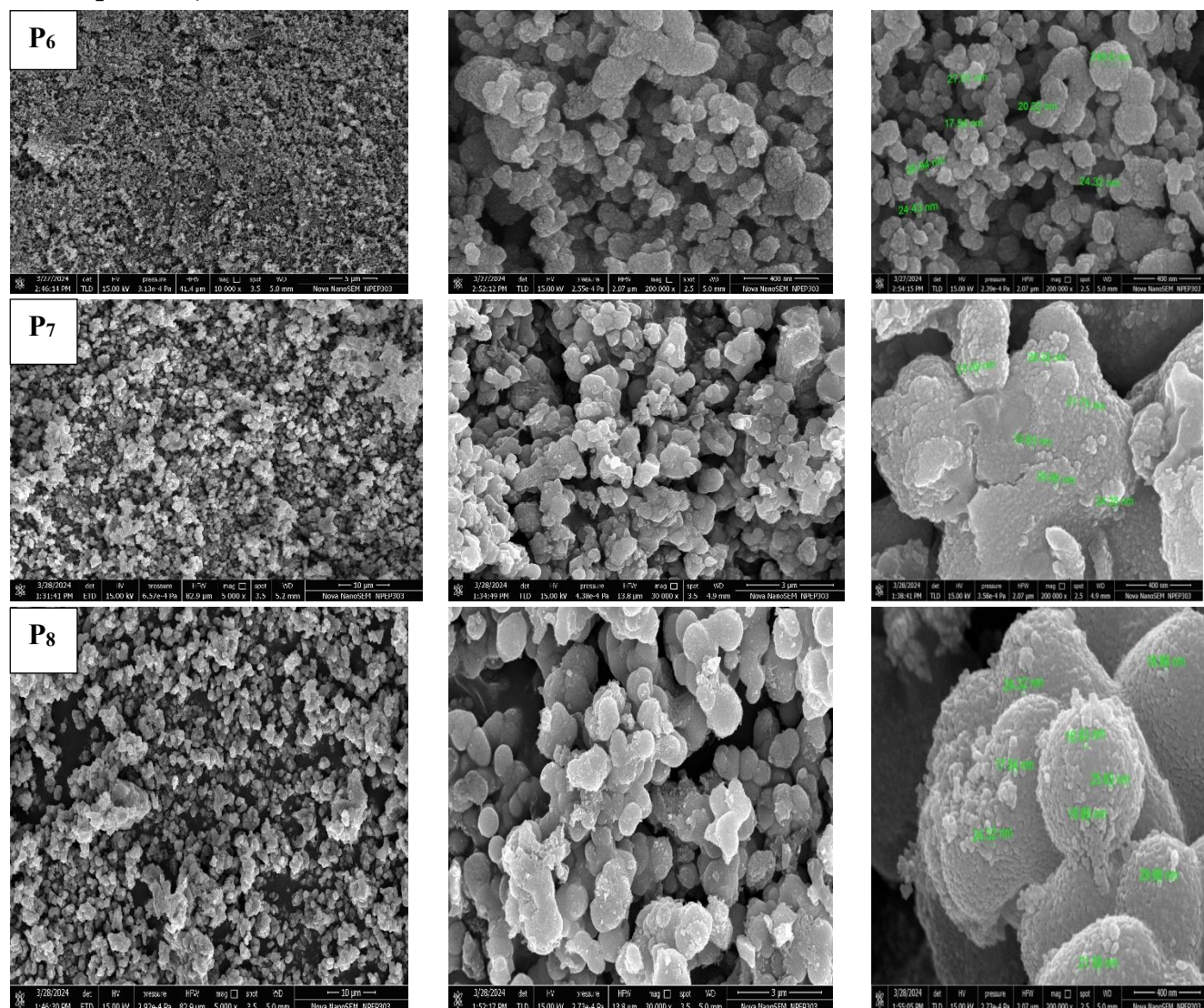


Fig.7 SEM micrographs of TiO_2 nanoparticles synthesized by using *Lepidagathis Sabui*(P_6), *Lepidagathis Shrirangii*(P_7) *Lepidagathis Ushae*(P_8). extract showing Predominantly spherical to slightly irregular morphology.

Energy Dispersive X-ray Spectroscopy (EDS) Analysis:

The chemical composition of the synthesized nanoparticles was examined using elemental mapping EDAX analysis as shown in Fig. 8. The results display well-defined peaks corresponding to Ti and O, confirming the successful formation of TiO_2 nanoparticles. [26][27][28] The EDS spectra of TiO_2 nanoparticles synthesized using *Lepidagathis sabui* (P6), *Lepidagathis Shrirangii* (P7), and *Lepidagathis Ushae*(P8) leaf extracts confirm Ti and O as the major constituents, with compositions of Ti 52.22% and O

47.78% for P6, Ti 54.85% and O 45.15% for P7, and Ti 59.17% and O 40.83% for P8. The nearly stoichiometric Ti:O ratio in P6 indicates a well-formed oxide, whereas the progressively higher titanium content in P7 and P8 suggests increasing oxygen deficiency, possibly due to variations in the phytochemical composition of the extracts. Such oxygen vacancies can introduce defect sites, influencing crystallinity, band gap, and photocatalytic efficiency. These elemental findings from EDS complement the XRD results, where the confirmed

TiO₂ phases (anatase/rutile) are supported by the observed Ti–O composition, with oxygen deficiency correlating to subtle peak broadening or shifts in the diffraction patterns. The EDAX elemental mapping as

displayed in Fig. 9, of the synthesized TiO₂ nanoparticles shows a homogeneous distribution of Ti and O, confirming uniform composition with no significant impurities.[26],[1]

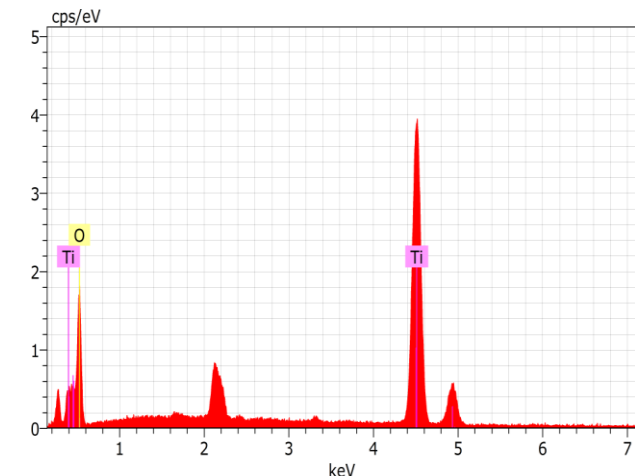
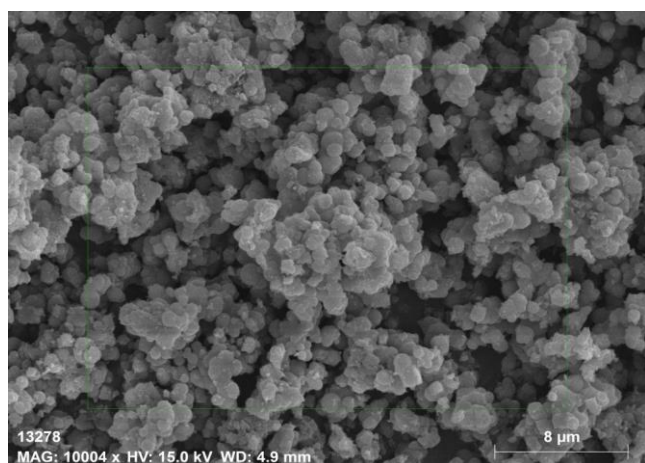
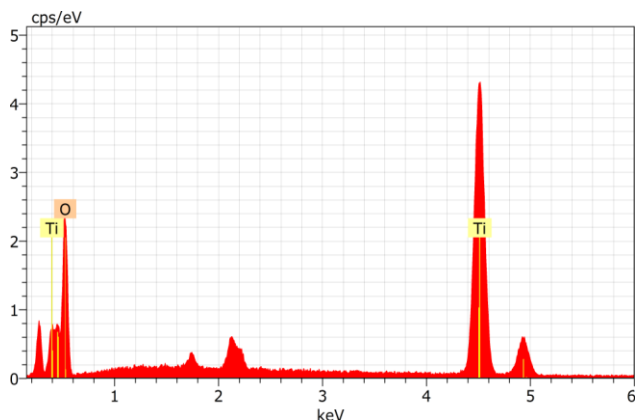
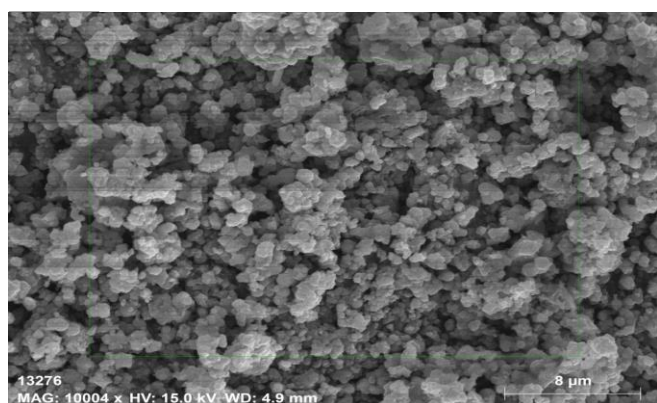
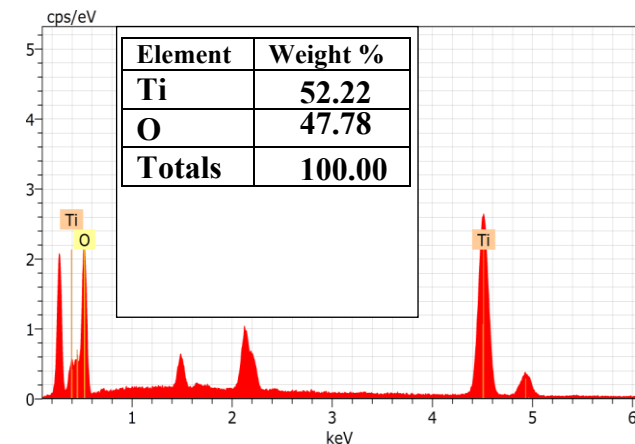
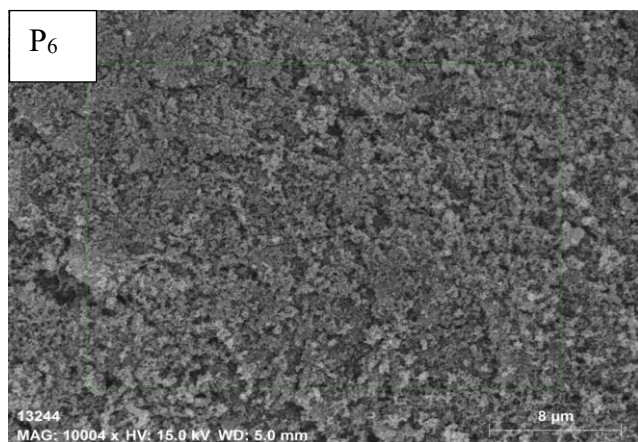


Fig.8 EDS spectrum of TiO₂ nanoparticles synthesized by P₁) *Lepidagathis Sabui* P₂) *Lepidagathis Shrirangii* P₃) *Lepidagathis Ushae* Leaf Extract.

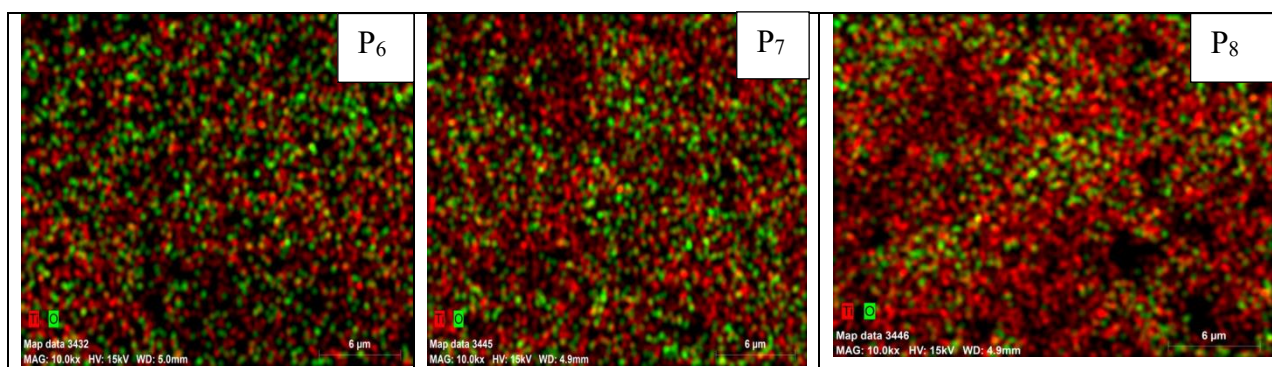


Fig.9 EDAX mapping of Synthesized TiO₂ GNPs.

High-Resolution Transmission Electron Microscopy (HR-TEM) analysis

The morphology of the green synthesized TiO₂ nanoparticles was investigated using Transmission Electron Microscopy (TEM). The TEM micrograph (Fig.10) demonstrates that the nanoparticles are predominantly spherical to quasi-spherical in shape and are assembled into dense agglomerated clusters. [29] Such aggregation is a common phenomenon in

biologically synthesized nanoparticles and may be attributed to the high surface energy of TiO₂ as well as the presence of phytochemicals from the plant extract, which act as both reducing and stabilizing agents. The individual particle size was estimated to be in the range of 10–30 nm, confirming the nanoscale dimensions achieved through this eco-friendly synthesis route. [24],[30]

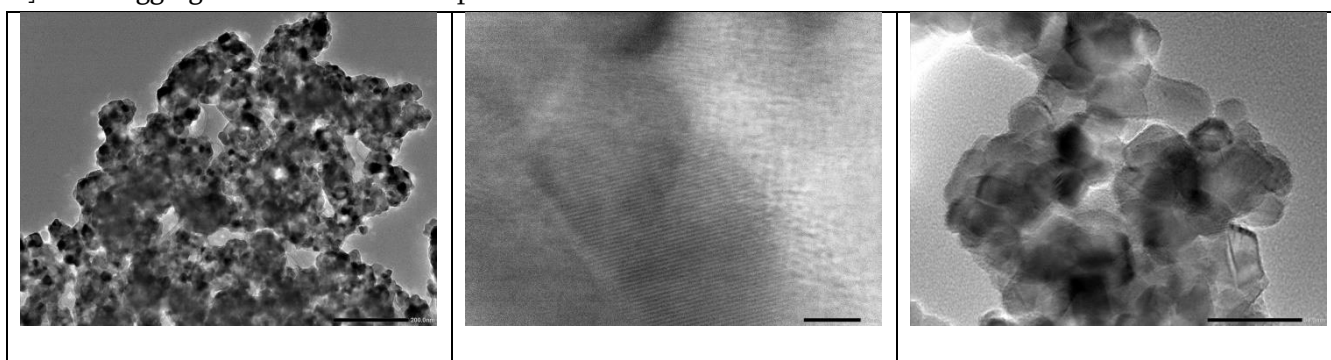


Fig.10 TEM micrographs of TiO₂ nanoparticles synthesized by using Sabui, Shrirangii and UshaeExtract

The darker contrasts in the image correspond to the TiO₂ nanoparticles, while the lighter regions represent the carbon support grid. The relatively sharp particle boundaries and uniform contrast indicate crystalline nature, which is consistent with the expected properties of TiO₂. Importantly, the use of plant-mediated synthesis not only facilitates particle nucleation but also enhances the stability of the nanoparticles by capping them with bioactive compounds, though some degree of agglomeration is still observed. Overall, the TEM analysis validates the successful fabrication of TiO₂ nanoparticles using a green synthesis approach. The nanoscale size,

crystalline features, and bio-mediated stabilization collectively highlight the potential of this sustainable method in producing functional nanomaterials for photocatalytic, biomedical, and environmental applications.

IV. Conclusion

The green synthesis of TiO₂ nanoparticles using leaf extracts from three *Lepidagathis* species—*L. sabui*, *L. Shrirangii*, and *L. Ushae*—was achieved for the first time. The phytochemical constituents of the extracts served as reducing and stabilizing agents, enabling the

eco-friendly formation of nanoparticles. UV-Vis analysis confirmed a direct band gap of 3.2 eV, and FTIR spectra showed Ti-O vibrations. SEM and TEM images revealed semi-spherical morphologies with nanoscale features. BET surface area analysis indicated mesoporosity, while EDS confirmed the elemental purity of titanium and oxygen. These results highlight the suitability of *Lepidagathis* extracts as green reducing and stabilizing agent for TiO₂ nanoparticles, underscoring their potential utility in photocatalytic dye degradation.

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