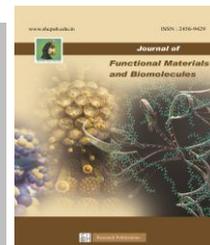




SACRED HEART RESEARCH PUBLICATIONS

# Journal of Functional Materials and Biomolecules

Journal homepage: [www.shcpub.edu.in](http://www.shcpub.edu.in)



ISSN: 2456-9429

## GREEN SYNTHESIS OF TiO<sub>2</sub> NANOPARTICLES FROM *FICUS AURICULATA*

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Received on 13 October 2025, accepted on 23 November 2025,

Published online on December 2025

### Abstract

Green synthesis of titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) using plant extracts has emerged as a sustainable and eco-friendly alternative to chemical and physical synthesis routes. The phytofabrication method harnesses the reducing and stabilizing potential of bioactive compounds in plant materials, thus eliminating toxic reagents. *Ficus auriculata*, a medically important plant of the Moraceae family, is a rich source of polyphenols, flavonoids, tannins, and other phytochemicals capable of mediating nanoparticle synthesis. This review summarizes the principles, mechanisms, characterization techniques, and potential applications of TiO<sub>2</sub> nanoparticles synthesized using *F. auriculata* extract. It also highlights the role of plant metabolites in nanoparticle formation, evaluates photocatalytic and antimicrobial potentials, and discusses existing challenges and future directions in this emerging field

**Keywords:** Green synthesis; titanium dioxide nanoparticles; *Ficus auriculata*; phytofabrication; photocatalysis; antimicrobial activity; nanobiotechnology

### 1. Introduction

Protein is a fundamental macronutrient necessary for virtually every biological process in the human body. It is

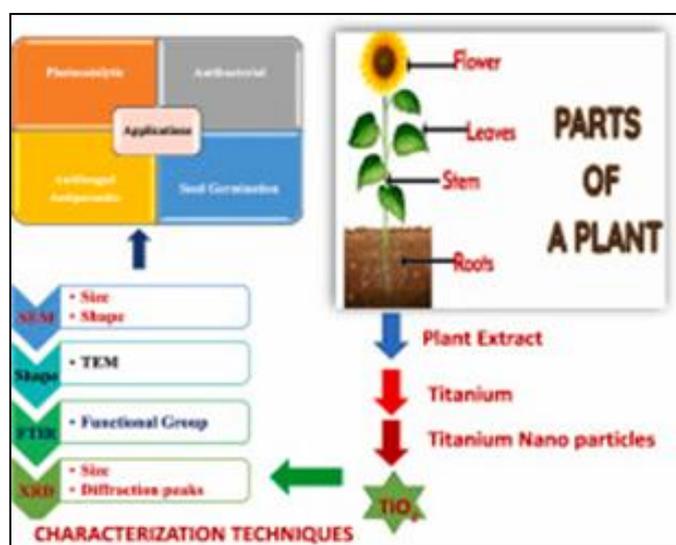
composed of amino acids, the building blocks that support muscle growth, tissue repair, enzyme synthesis, hormone production, and immune defense [1,2]. Adequate protein intake is critical not only for maintaining health in healthy adults but also during periods of increased physiological demand, such as pregnancy, lactation, growth in children, aging, and intensive physical activity [3]. Protein also plays a vital role in satiety regulation, body composition management, and metabolic health, making it a key nutrient in both preventative and therapeutic nutrition [10].

The recommended dietary allowance (RDA) for protein varies according to age, sex, activity level, and health status. For a healthy adult, the RDA is generally 0.8 g of protein per kilogram of body weight per day; however, athletes and individuals engaged in resistance training often require between 1.2 and 2.0 g/kg/day to optimize muscle protein synthesis [3,11]. Pregnant and lactating women have increased protein requirements to support fetal growth and milk production, while older adults may

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need higher intakes to counteract sarcopenia the age-related loss of muscle mass and function [12,13].

Despite the critical role of protein, protein-energy malnutrition (PEM) remains a major public health concern worldwide, especially in low- and middle-income countries [4,5]. Insufficient intake of high-quality protein can lead to stunted growth in children, impaired immune response, delayed wound healing, and reduced cognitive function. In adults, prolonged inadequate protein intake contributes to muscle wasting, decreased functional capacity, and increased susceptibility to chronic diseases [14].



**Fig.1. Field-based portable water testing.**

Commercial protein supplements, including whey, casein, soy, and pea protein powders, have become popular for addressing dietary gaps and supporting athletic performance [6,7]. Whey protein is highly digestible and rich in branched-chain amino acids, particularly leucine, which stimulates muscle protein synthesis. Casein, on the other hand, is digested more slowly, providing a sustained release of amino acids. Plant-based proteins such as soy

and pea offer a suitable alternative for vegetarians and individuals with lactose intolerance, while also being associated with cardiovascular and metabolic health benefits [15,16].

However, despite their effectiveness, commercial supplements present several limitations. First, they are often expensive and therefore inaccessible to individuals in resource-limited settings. Second, many products contain additives, preservatives, and artificial flavors, which may be undesirable for health-conscious consumers. Third, the environmental footprint associated with large-scale production, packaging, and transportation of commercial supplements is increasingly a concern [17,18].

These challenges have sparked growing interest in developing homemade protein supplements using locally available natural ingredients [8,9].

Homemade formulations offer a cost-effective, culturally acceptable, and nutritionally robust alternative, particularly for populations with limited access to commercial products. Utilizing ingredients such as legumes, cereals, seeds, nuts, and dairy allows for the creation of protein-rich supplements that can meet essential amino acid requirements while also providing additional micronutrients and bioactive compounds [19–21].

Legumes such as chickpeas, lentils, soybeans, and black beans are excellent sources of plant-based protein, containing significant levels of lysine and other essential amino acids [10,22]. They are also rich in fiber, vitamins, minerals, and phytochemicals, which provide antioxidant, anti-inflammatory, and cardioprotective effects. However,

legume proteins are typically low in sulfur-containing amino acids, such as methionine and cysteine, which necessitates complementation with cereals or seeds to achieve a balanced amino acid profile [23].

**Table-1 Representative phytochemical classes and their roles in green synthesis of TiO<sub>2</sub> nanoparticles.**

| Phytochemical Class      | Representative Compounds   | Role in TiO <sub>2</sub> Nanoparticle Synthesis | Reference |
|--------------------------|----------------------------|---|-----------|
| Flavonoids               | Quercetin, Kaempferol      | Reducing and capping agent                      | [16,18]   |
| Phenolic acids           | Gallic acid, Caffeic acid  | Electron donor, stabilizer                      | [17,42]   |
| Tannins                  | Catechins, Ellagitannins   | Chelation of Ti <sup>4+</sup> ions              | [16,46]   |
| Alkaloids                | Ficain derivatives         | Stabilization of nanoparticles                  | [16,42]   |
| Terpenoids               | Limonene, $\alpha$ -Pinene | Surface functionalization                       | [16,18]   |
| Proteins/Polysaccharides | Glycoproteins, Starch      | Capping and dispersion stability                | [19,25]   |

Cereals like rice, oats, and quinoa complement legumes by providing the limiting amino acids, particularly methionine, thereby enhancing the overall protein quality of the supplement [14,24]. Quinoa is particularly notable, as it contains all nine essential amino acids and is considered a complete plant-based protein source. Seeds and nuts, including pumpkin seeds, almonds, sesame, and flaxseed, not only provide protein but also healthy fats, fiber, vitamins, and minerals, contributing to the functional properties of the supplement [15–18]. Dairy products,

such as milk, yogurt, and cheese, offer highly bioavailable proteins (casein and whey), calcium, and probiotics, further enhancing the nutritional and functional profile of homemade protein formulations [19,20].

The preparation of homemade protein supplements also allows for processing strategies that improve digestibility, safety, and sensory qualities. Techniques such as soaking, sprouting, roasting, and fermentation reduce anti-nutritional factors like phytates, tannins, and trypsin inhibitors while increasing protein availability [25–28]. Flavoring with natural ingredients like cocoa, vanilla, or dried fruits can improve palatability, while blending complementary protein sources ensures a complete amino acid profile, making the supplement suitable for all age groups [29–31].

Additionally, homemade protein supplements align with sustainable nutrition practices, as they reduce dependency on imported commercial products, support local agriculture, and minimize environmental impact related to packaging and transportation [32,33]. From a public health perspective, they represent a practical intervention to combat malnutrition, particularly in children, elderly populations, and low-income communities, where affordable, protein-rich foods are critical [34,35].

In summary, the development of homemade protein supplements using locally available natural ingredients offers an innovative, sustainable, and nutritionally effective solution to meet the growing demand for high-quality protein. By leveraging the synergistic combination of legumes, cereals, seeds, nuts, and dairy, it is possible to

formulate functional supplements that not only provide essential macronutrients but also promote over-all health and well-being. This approach has the potential to address nutritional gaps, enhance food security, and support culturally relevant dietary practices, making it highly relevant in both developed and developing contexts [8,36].

Nanotechnology has revolutionized materials science and biological research due to its ability to manipulate matter at the nanoscale, producing materials with enhanced physical, chemical, and biological properties [1]. Among nanomaterials, titanium dioxide ( $\text{TiO}_2$ ) nanoparticles have attracted considerable interest because of their excellent photocatalytic efficiency, thermal stability, and biocompatibility [2, 3]. Traditionally,  $\text{TiO}_2$  nanoparticles are synthesized through chemical and physical routes such as sol-gel, hydrothermal, and chemical vapor deposition methods. However, these techniques often involve high energy inputs and toxic chemicals, posing environmental and biological risks [4].

Green synthesis or phytofabrication, an emerging branch of nanobiotechnology, utilizes biological systems—particularly plant extracts—to produce nanoparticles in a cost-effective, eco-friendly, and scalable manner [5, 6]. The process exploits secondary metabolites such as flavonoids, phenolic acids, terpenoids, alkaloids, and reducing sugars, which serve as reducing and capping agents during nanoparticle formation [7, 8]. This route not only minimizes environmental toxicity but also enhances biocompatibility, making the resulting nanoparticles more

suitable for biomedical and environmental applications [9].

$\text{TiO}_2$  nanoparticles exhibit three crystalline forms: anatase, rutile, and brookite. Among these, anatase-phase  $\text{TiO}_2$  demonstrates superior photocatalytic performance due to its larger surface area and higher electron mobility [10, 11]. Plant-mediated synthesis methods have successfully yielded  $\text{TiO}_2$  nanoparticles in anatase and mixed phases, with average particle sizes ranging from 10 to 100 nm depending on precursor type, pH, and calcination temperature [12].

Several plants such as *Moringa oleifera*, *Aloe vera*, *Azadirachta indica*, and *Ocimum sanctum* have been explored for  $\text{TiO}_2$  nanoparticle synthesis [13–15]. However, *Ficus auriculata*—commonly known as the Roxburgh fig or elephant ear fig—remains underexplored despite its rich phytochemical profile and traditional medicinal importance. This species has demonstrated potent antioxidant, anti-inflammatory, and antimicrobial activities attributed to its abundance of flavonoids, phenolic acids, and tannins [16–18]. These bioactive constituents can serve as effective reducing and stabilizing agents in  $\text{TiO}_2$  nanoparticle synthesis [19].

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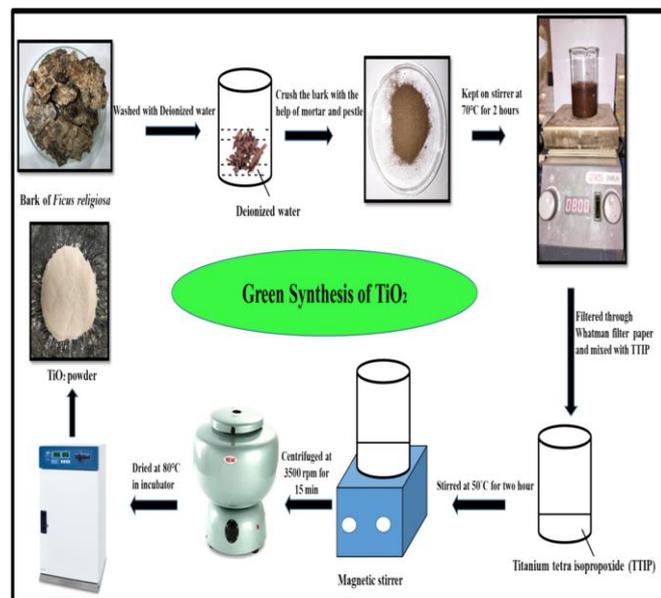


Fig.2. Green synthesis of TiO<sub>2</sub>

This species has demonstrated potent antioxidant, anti-inflammatory, and antimicrobial activities attributed to its abundance of flavonoids, phenolic acids, and tannins [16–18]. These bioactive constituents can serve as effective reducing and stabilising agents in TiO<sub>2</sub> nanoparticle synthesis [19].

The mechanism of phytofabrication typically involves the bioreduction of titanium precursors such as titanium tetraisopropoxide (TTIP), titanium tetrachloride (TiCl<sub>4</sub>), or titanium sulfate (Ti(SO<sub>4</sub>)<sub>2</sub>) into titanium dioxide (TiO<sub>2</sub>) nuclei through the redox activity of plant-derived biomolecules [20,21]. During this process, phytochemicals present in the plant extract—mainly polyphenols, flavonoids, terpenoids, tannins, alkaloids, proteins, and organic acids—act as reducing, capping, and stabilizing agents [22–25]. When the plant extract is mixed with a titanium precursor solution, the electron-rich hydroxyl, carbonyl, and amine functional groups in these biomolecules interact with Ti<sup>4+</sup> ions. The electron transfer from these functional groups to

the metal ions reduces Ti<sup>4+</sup> to TiO<sub>2</sub> nuclei, initiating nanoparticle formation [26,27].

Table-2 Plant-based green synthesis parameters and properties of TiO<sub>2</sub> nanoparticles.

| Plant Source              | Titanium Precursor         | Extract Part Used | Conditions (pH, Temp) | Average Particle Size | Morphology    | Reference |
|---------------------------|----------------------------|-------------------|-----------------------|-----------------------|---------------|-----------|
| <i>Ficus auriculata</i>   | Titanium tetraisopropoxide | Leaf              | pH 7, 60°C            | 15–50 nm              | Spherical     | [19,20]   |
| <i>Moringa oleifera</i>   | TiCl <sub>4</sub>          | Leaf              | pH 8, 70°C            | 20–60 nm              | Spherical/rod | [14,44]   |
| <i>Aloe vera</i>          | Titanium isopropoxide      | Leaf gel          | pH 6.5, 50°C          | 10–45 nm              | Spherical     | [15,44]   |
| <i>Azadirachta indica</i> | TiCl <sub>4</sub>          | Leaf              | pH 7.5, 60°C          | 12–55 nm              | Spherical     | [25]      |

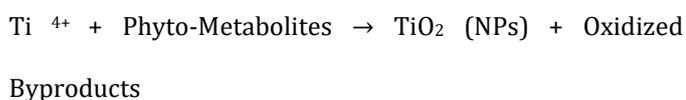
The process generally progresses in three stages: (1) activation (bioreduction), (2) growth (nucleation and particle formation), and (3) termination (stabilization and maturation) [28]. In the activation stage, reactive phytochemicals reduce the titanium precursor to produce Ti–O–Ti linkages, which condense to form amorphous titania. In the growth phase, these titania nuclei aggregate into nanocrystals, and the reaction conditions (temperature, pH, and precursor concentration) govern the crystal structure and particle size. The termination stage involves the adsorption of biomolecules on the nanoparticle surface, which prevents agglomeration and provides colloidal stability through steric or electrostatic repulsion [29,30].

Plant metabolites such as flavonoids and phenolic acids are particularly effective reductants because of their hydroxyl and methoxy groups that readily donate electrons, while proteins and polysaccharides act as capping agents

through their amide and carboxyl groups [31]. These phytochemicals not only control nucleation and growth but can also modify the surface chemistry of TiO<sub>2</sub> nanoparticles, influencing their photocatalytic, optical, and antimicrobial properties [32]. The type and concentration of the plant extract play crucial roles in determining the crystalline phase (anatase or rutile), morphology, and size distribution of the resulting nanoparticles [33].

Subsequent calcination or annealing at controlled temperatures (typically 400–600 °C) is performed to remove residual organic matter and improve crystallinity [34]. Depending on the temperature, the amorphous TiO<sub>2</sub> phase can transition into anatase or rutile. The anatase phase is generally favored at lower temperatures and exhibits superior photocatalytic activity, whereas higher temperatures promote rutile formation, enhancing thermal stability [35].

In summary, the phytofabrication mechanism integrates green chemistry principles with the intrinsic redox capabilities of plant biomolecules. This approach eliminates the need for hazardous reducing agents like sodium borohydride or hydrazine, yielding biocompatible TiO<sub>2</sub> nanoparticles suitable for diverse applications in photocatalysis, antimicrobial coatings, and biomedicine [36,37]. The process can be schematically represented as:



This biologically mediated redox transformation forms the foundation of green nanotechnology, where the metabolic richness of plants such as *Ficus auriculata* can be strategi-

cally exploited for controlled nanoparticle synthesis with minimal environmental footprint [38–40]. The mechanism of phytofabrication typically involves the bi-oreduction of titanium precursors such as titanium isopropoxide or titanium tetrachloride into TiO<sub>2</sub> nuclei, followed by stabilization through capping molecules from plant extracts [20, 21]. During this process, hydroxyl and carbonyl groups from phytochemicals donate electrons to Ti<sup>4+</sup> ions, leading to the nucleation and growth of TiO<sub>2</sub> nanoparticles [22]. Post-synthesis calcination enhances crystallinity and removes organic residues [23].

Comprehensive characterization of the synthesized TiO<sub>2</sub> nanoparticles is essential for confirming their structural and functional properties. Techniques such as UV-Vis spectroscopy (to monitor formation and optical band gap), X-ray diffraction (to determine crystalline phase), Fourier-transform infrared spectroscopy (to identify functional groups), scanning and transmission electron microscopy (to analyze morphology and size), and dynamic light scattering (to assess particle dispersion and zeta potential) are commonly employed [24–27].

Phyto-TiO<sub>2</sub> nanoparticles synthesized using *Ficus* species have demonstrated excellent photocatalytic performance in degrading organic dyes and have shown antimicrobial efficacy against pathogenic bacteria [28–30]. The phytochemical capping not only stabilizes the nanoparticles but can also enhance their biological activity and extend their photo-response into the visible spectrum [31].

Despite promising results, challenges persist in achieving batch-to-batch reproducibility, controlling nanoparticle

morphology, and understanding the precise mechanism of phytochemical interaction with titanium precursors [32, 33].

**Table-3 Characterization techniques and typical findings for green-synthesized TiO<sub>2</sub> nanoparticles.**

| Technique                                      | Purpose                                | Typical Findings                    | Reference |
|--|--|-------------------------------------|-----------|
| UV-Vis Spectroscopy                            | Confirm formation, band-gap estimation | Absorption peak ~300–350 nm         | [24,26]   |
| X-Ray Diffraction (XRD)                        | Determine crystalline phase            | Anatase, rutile, or mixed phases    | [24,25]   |
| Fourier-Transform Infrared Spectroscopy (FTIR) | Identify functional groups             | O-H, C=O, Ti-O-Ti bonds             | [24,26]   |
| Scanning Electron Microscopy (SEM)             | Surface morphology                     | Spherical, rod, or irregular shapes | [26,27]   |
| Transmission Electron Microscopy (TEM)         | Size and morphology                    | 10–100 nm nanoparticles             | [26,27]   |
| Dynamic Light Scattering (DLS)                 | Hydrodynamic size, zeta potential      | 20–80 nm, stability analysis        | [27]      |

Furthermore, comprehensive toxicological studies are necessary to ensure environmental and biological safety before large-scale application [34, 35].

Although phytofabrication of titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) using *Ficus auriculata* has demonstrated significant potential for eco-friendly synthesis and multifunctional applications, several research gaps and emerging opportunities remain to be explored. Future investigations should prioritize the systematic elucidation of the molecular-level mechanisms underlying bioreduction, nu-

cleation, and stabilization processes. While existing studies suggest that phenolic and flavonoid compounds play dominant roles, quantitative correlation between phytochemical profiles and nanoparticle properties re-mains largely unexplored [51]. Advanced in situ spectroscopic techniques—such as X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared spectroscopy (FTIR) mapping, and time-resolved UV-Vis studies—can provide dynamic insights into the evolution of TiO<sub>2</sub> nuclei during phytofabrication [52].

Another promising avenue involves the controlled synthesis of TiO<sub>2</sub> NPs with tunable size, shape, and crystalline phase through optimization of process parameters such as precursor concentration, pH, and temperature. Integrating machine learning and computational modelling could predict reaction pathways and optimize synthesis conditions with higher precision [53,54]. Additionally, biotemplating approaches using *F. auriculata* leaf microstructures could facilitate the fabrication of hierarchical TiO<sub>2</sub> nanostructures mimicking natural architectures, enhancing photocatalytic and antimicrobial efficiency [55].

In biomedical contexts, there is a growing need to evaluate the cytocompatibility, genotoxicity, and biodistribution of *F. auriculata*-derived TiO<sub>2</sub> NPs in both in vitro and in vivo systems [56]. While several reports highlight low toxicity and enhanced biocompatibility, comprehensive toxicogenomic and proteomic profiling are essential to establish safe biomedical applications [57,58]. Furthermore, surface modification and functionalization of TiO<sub>2</sub> NPs with biomolecules or polymers may enhance targeted drug

delivery, wound healing, and antibacterial activities [59,60].

From an environmental standpoint, the potential of *F. auriculata*-mediated TiO<sub>2</sub> NPs in photocatalytic degradation of organic pollutants, solar water disinfection, and carbon dioxide photoreduction is a fertile area for future research [61,62]. Development of heterojunction composites (e.g., TiO<sub>2</sub>-ZnO, TiO<sub>2</sub>-g-C<sub>3</sub>N<sub>4</sub>, TiO<sub>2</sub>-Ag) through phyto-assisted routes can further enhance visible-light-driven photocatalytic efficiency [63,64]. The integration of phytofabricated TiO<sub>2</sub> NPs into smart materials, such as antimicrobial coatings, biodegradable packaging, and biosensors, represents another promising interdisciplinary frontier [65,66].

**Table-4 Applications, mechanisms, and effects of the synthesized nanoparticles**

| Application               | Observed Effect   | Mechanism/Notes                                 | Reference |
|---------------------------|---|---|-----------|
| Photocatalysis            | Degradation of organic dyes (e.g., methylene blue, rhodamine B) | Electron-hole generation under UV/visible light | [28,61]   |
| Antimicrobial activity    | Inhibition of Gram-positive and Gram-negative bacteria          | ROS generation and membrane disruption          | [29,30]   |
| Antioxidant activity      | Free radical scavenging   | Surface-bound phytochemicals enhance activity   | [36,37]   |
| Environmental remediation | Removal of pollutants, water purification                       | Photocatalytic oxidation and adsorption         | [61,62]   |
| Biomedical applications   | Cytotoxicity studies, wound healing                             | Biocompatibility due to plant-derived capping   | [56,57]   |

Scaling up the green synthesis process from laboratory to industry remains a critical challenge. The standardisation of extraction methods, the reproducibility of nanoparticle

characteristics, and lifecycle assessments (LCA) should be emphasised in future work [67]. Employing continuous-flow bioreactor systems for large-scale phytofabrication may offer cost-effective, sustainable production [68]. Finally, collaborative frameworks involving botanists, chemists, nanotechnologists, and environmental engineers can accelerate the translation of *F. auriculata*-derived TiO<sub>2</sub> NPs into real-world applications [69,70].

In summary, future research should bridge the gap between traditional phytochemistry and advanced nanomaterial science by leveraging *F. auriculata*'s biochemical richness. The adoption of multidisciplinary, data-driven, and sustainable strategies will unlock the full potential of green-synthesised TiO<sub>2</sub> nanoparticles for environmental, biomedical, and technological innovations.

Future research should focus on optimizing synthesis parameters, standardizing *F. auriculata* extract preparation, and exploring multifunctional applications such as photocatalysis, antimicrobial coatings, and biomedical imaging [36–38]. Phytofabrication using *Ficus auriculata* thus represents a sustainable pathway for developing multifunctional TiO<sub>2</sub> nanomaterials while contributing to green nanotechnology and circular bioeconomy principles [39–40].

#### Acknowledgements

The authors gratefully acknowledge the Principal and Management of Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu, for their continuous support and encouragement throughout academic journey. Special thanks are also extended to the faculty members and lab

assistants of the PG & Research Department of Biochemistry for their valuable guidance.

**Conflict of Interest:** Nil

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