

Eco-friendly Synthesis and Characterization of CeO₂-doped TiO₂ Nanoparticles Using Biowaste: Unveiling Potent Anti-inflammatory and Antioxidant Properties

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Abstract - In this study, CeO₂-doped TiO₂ nanoparticles were synthesized using an eco-friendly approach, utilizing an aqueous extract from the calyx leaves (outer covering) of *Physalis peruviana* fruits. The morphology of the green-synthesized nanoparticles was characterized using UV-Vis spectroscopy, FT-IR, SEM, and X-ray diffraction. The anti-inflammatory activity of the biosynthesized NPs was evaluated through a denaturation test. The use of the fruit's outer calyx, commonly discarded as food waste, presents a sustainable strategy for repurposing food and biological waste in nanotechnology. The NPs exhibited favorable magnetic properties, a well-defined crystalline structure, and notable anti-inflammatory effects, highlighting their potential for therapeutic and medical applications, particularly under green synthesis protocols.

Keywords: Eco-friendly synthesis, CeO₂-doped TiO₂ nanoparticles, biowaste, anti-inflammatory activity, antioxidant activity.

I. INTRODUCTION

With the emergence of nanoparticles (NPs), offering notable benefits in a variety of scientific and technological applications, nanotechnology has become a ground-breaking topic [1,2]. Due to their numerous enormous and unique properties, particularly their extraordinary biological attributes have attracted irreversible interest and are being employed in a variety of industries, including medication delivery, electronics, bioimaging, pharmaceuticals, healthcare, diagnostics, and antimicrobial treatments [3-5]. With its diverse approach, nanomedicine serves as a vital link that closes the significant gap between biological systems and the intricacies of nanostructures. In particular, there are numerous therapeutic potentials for metal oxide NPs. The current scientific community is focusing on green assisted synthesis of metal oxide nanoparticles, emphasizing their superior material characteristics and transport capacities [6, 7]. Because of their remarkable biological qualities, plants and their derivatives are frequently employed in the synthesis of NPs. Sustainable processes are preferable to expensive, hazardous, inefficient

physical and chemical processes when developing new materials with improved characteristics. The above issues are resolved by the biosynthesis of nanoparticles, which also makes effective utilization of valuable materials from waste possible [8].

Among metal oxides, TiO₂ is unique in that it is an inexpensive semiconductor with a band gap of 3.2 eV that finds extensive application in drug delivery, photocatalysis, biotechnology, and water treatment [9–11]. Because of their distinct surface chemistry, stability, and lack of toxicity, its nanoparticles are widely prized in biological applications for their antibacterial, anticoagulant, anti-inflammatory, antiviral, and anticancer qualities [12–14]. TiO₂ has several applications, but there hasn't been much research done on mixing it with rare earth elements like cerium oxide (CeO₂). TiO₂'s performance is improved by CeO₂, which is well-known for its biocompatibility, redox potential, and antioxidant qualities. This results in intriguing nanoparticles with potential uses in medicine and therapy. Due to its multiple oxidation states (Ce³⁺ and Ce⁴⁺), cerium is well-known for its redox potential and is therefore frequently employed as an efficient oxidation system [15, 16]. In light of this, the biological fabrication of these two remarkable metal oxide in one NPs has become essential for enhancing therapeutic treatments' efficacy and acting as potent agents for the advancement of medicine. Recently, *cleome celidonii* plant leaf extract was used to create environmentally benign CeO₂-doped TiO₂ nanoparticles, which demonstrated outstanding photocatalytic activity [17].

The eco-friendly synthesis of nanoparticles using plant-based waste offers significant advantages, including simplicity, cost-effectiveness, and the availability of abundant raw materials. With growing concerns over the management of byproducts from food processing, this approach provides an efficient alternative to costly disposal methods by transforming food waste into valuable nanomaterials [18]. Agricultural and food waste materials have gained substantial attention as natural reducing agents in nanoparticle synthesis, offering a sustainable solution for large-scale production. This

method not only addresses waste management challenges but also facilitates the synthesis of nanoparticles with high yield and efficiency in an environmentally friendly manner. Utilizing plant waste, such as the calyx of *Physalis peruviana*, introduces biocompatible and non-toxic agents that enhance nanoparticle properties, making them highly desirable for biomedical, environmental, and industrial applications.

Capsule berries, or *Physalis peruviana*, have antiviral, antioxidant, and antibacterial qualities. Flavonols, phenolics, and quercetin are among the bioactive substances found in its outer calyx, which is frequently thrown away as trash yet has important medicinal potential [19]. During the creation of nanoparticles, these calyx leaves can act as reducing and capping agents, strengthening the particles' anticancer, antibacterial, antipyretic, diuretic, immunomodulatory, and anti-inflammatory properties [20].

The study's innovation is in the waste-to-wealth initiative, which turns waste into something valuable and uses less energy than the conventional heating method. It also examines the anti-inflammatory properties of biosynthesized CeO₂-doped TiO₂NPs made from biowaste. This novel process for creating CeO₂-doped TiO₂NPs from the biowaste of *Physalis peruviana* fruits waste offers a robust, environmentally friendly, and all-encompassing combination of waste management and nanotechnology.

II. MATERIAL AND METHODS

2.1 Chemicals and reagents

Titanium isopropoxide, cerium nitrate hexahydrate, ethanol, phosphate-buffered saline, diclofenac sodium diphenyl picryl hydroxyl (DPPH), butylated hydroxytoluene (BHT).

2.2 Preparation of the green aqueous calyx leaves extract

Fruits of *Physalis peruviana* were bought from a local Jaipur, Rajasthan, India market. After removing the outer layer of fruiting calyx leaves, the fruits were chopped into small pieces, cleaned with water, and allowed to air dry. The dried calyx leaves were then crushed and further ground into finer particles. In a 500 mL conical flask, about 20g of crushed leaves were submerged in 100 mL of deionized water and heated for 30 to 35 minutes at 60 °C while stirring constantly. Following room temperature cooling, it was filtered using Whatman No. 1 filter paper and stored at 4 °C for later use.

2.3 Green Synthesis of CeO₂-doped TiO₂nanoparticles

CeO₂-doped TiO₂ nanoparticles were biosynthesized by dissolving 0.1 M titanium isopropoxide in 100 mL deionized water under agitation for 10 minutes under magnetic stirrer at

room temperature. A separate solution of 0.01 M cerium nitrate hexahydrate in 50 mL deionized water was gradually added and stirred for 10 minutes. Then, 20 mL of *Physalis peruviana* calyx extract was introduced in steps. The mixture was heated upto 80°C and stirred until a paste formed, which was washed, centrifuged at 4000 rpm for 25 minutes, and dried at 80°C for 24 hours. Finally, the paste was calcined at 500°C for 2 hours, yielding fine crystalline CeO₂-doped TiO₂ nanoparticles.

2.4 Anti-inflammatory Assay

Green-synthesized CeO₂-doped TiO₂ NPs, derived from the calyx leaves extract of *Physalis peruviana* fruits, were utilized to treat inflammation via the protein denaturation method [21]. The reaction mixture consisted of 0.2 ml egg albumin, 2.8 ml phosphate-buffered saline, and 2 ml of the test synthesised NPs extracts, with different concentrations of 100, 200, 300, and 400 µg/ml. Double-distilled water was used as a control in equivalent volumes. The mixtures were incubated at 37 °C for 15 minutes, then heated at 70 °C for 5 minutes. After cooling, the absorbance was measured at 660 nm using a spectrophotometer. Diclofenac sodium was used as a reference drug, undergoing the same procedure to determine its absorbance.

2.5 Antioxidant Activity

The free radical scavenging potential of green synthesized CeO₂-doped TiO₂NPs was evaluated by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and BHT taken as reference antioxidant compound following standard procedure [22]. 0.1mM concentrated DPPH was dissolved in methanol to achieve various concentrations of the sample CeO₂-doped TiO₂NPs. The reaction mixture was agitated and left to incubate in a dark environment for 30 minutes at room temperature. An alteration in color was noted, and the absorbance was measured at 517 nm using a UV-Visible spectrophotometer.

III. RESULTS AND DISCUSSION

3.1 Ultraviolet-Visible Spectrophotometry

UV-Vis spectroscopy was used to assess the optical properties of CeO₂-doped TiO₂ NPs. The absorbance spectrum (Fig. 1) shows a TiO₂ absorption band around 400 nm, with an additional peak at 350 nm attributed to CeO₂. This blue shift suggests that CeO₂ enhances UV and visible light absorption by TiO₂ [23]. The introduction of CeO₂ reduces the bandgap due to increased oxygen from ceria blocking the valence band. As the molar concentration of CeO₂ increases, peak intensity decreases, indicating greater ceria presence on the TiO₂ surface and reduced absorbance.

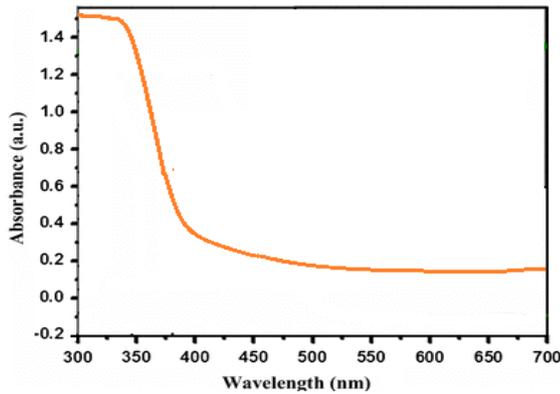


Figure 1: UV-Vis absorbance spectrum of green synthesized CeO₂-doped TiO₂NPs

3.2 Fourier Transformer-infrared (FT-IR)

To determine the biological components and reducing or capping agents in the extract that might be essential to the synthesis of NPs, the FTIR spectrum was examined. Figure 2 displays the FTIR spectrum of biosynthesized CeO₂-doped TiO₂NPs. Ti-O-Ti bond is associated with a sharp vibration peak that was recorded at <700 cm⁻¹ [24]. The Ce-O-Ce bond is linked to the vibration peaks detected at 1065 and 1062 cm⁻¹. Stretching vibrations of Ce-O were attributed to another peak at around 552 cm⁻¹ [25]. The information above leads us to the conclusion that cerium oxide and TiO₂ nanoparticles have a coupling effect. The ester bonds in polyphenolic compounds, the OH-stretching vibrations of phenolic components, and the stretching vibrations of carbonyl groups are the peaks that are seen when examining the reducing agents that operate as functional groups in the creation of CeO₂-doped TiO₂NPs [26].

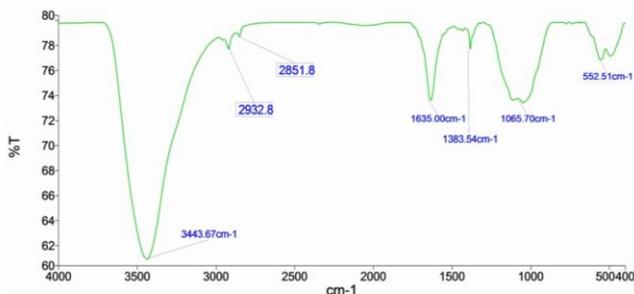


Figure 2: FT-IR spectrum of CeO₂-doped TiO₂ NPs

3.3 X-ray Diffraction (XRD)

The XRD pattern of green synthetic CeO₂-doped TiO₂NPs is shown in Figure 3. To confirm that the composited components had an effect on the overall crystal structure, the XRD pattern of the green produced NPs was studied. The diffraction peaks at 2θ = 28.35, 36.82, 44.10, 54.70, 56.31, and 64.7 are assigned to the tetragonal anatase phase of TiO₂

nanoparticles and correspond to the (101), (004), (200), (105), (211), and (204) miller planes, respectively [27]. Furthermore, the face centered cubic structure of cerium oxide was assigned to the significant diffraction peaks seen at 2θ = 28.25, 33.04, 47.52, 56.14, 58.96, and 69.15 with miller planes (111), (200), (220), (311), (222), and (400) correspondingly. The appearance of cerium oxide on the surface of the synthesized nanoparticles indicated that the crystal arrangement of the nanoparticles did not change during the doping process.

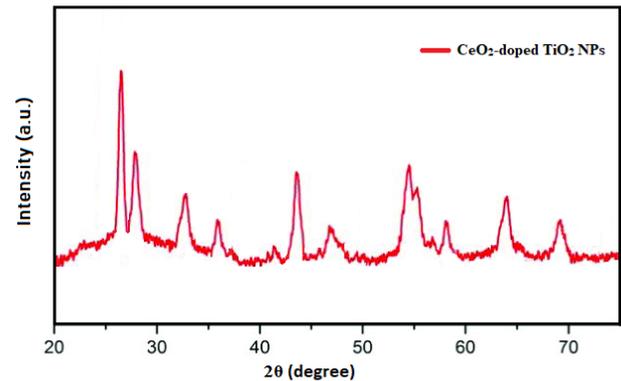


Figure 3: XRD pattern of CeO₂-doped TiO₂ NPs

3.4 Scanning Electron Microscopy (SEM)

The size and surface morphology of the synthesized CeO₂-doped TiO₂ NPs were analyzed using SEM. Image shown in Fig. 4 at scales of 500 nm reveal that the TiO₂ nanoparticles exhibit a nearly spherical morphology with uniform distribution and minimal agglomeration. The TiO₂ surfaces appear rough and textured due to the deposition of CeO₂ nanoparticles. The spherical shape is likely influenced by phytochemicals in the aqueous extract of *Physalis peruviana* calyx leaves, which act as capping agents and regulate the growth rates of the metal oxide [28]. We might have concluded that the biofabrication of CeO₂-doped TiO₂NPs was successful based on all of the previously noted results. We could also have validated the doping of cerium oxide on the surface of TiO₂ nanoparticles.

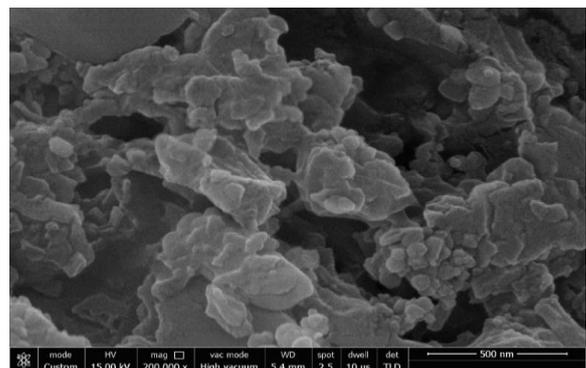


Figure 4: SEM image of CeO₂-doped TiO₂ NPs at 500 nm

3.5 Evaluation of Anti-inflammatory Activity

Inhibition of protein denaturation activity of greenly synthesized CeO₂-doped TiO₂NPs are investigated using egg albumin. Synthesised NPs made from calyx leaves extracts of *Physalisperuviana* fruits prevented the heat-induced denaturation of egg albumin. All of the measured concentrations significantly decreased the denaturation of egg albumin in a concentration-dependent manner. At the highest measured concentration (400 µg/ml), the maximum percentage of inhibition was observed as shown in figure 5.

Inflammation is the body's defence mechanism against infections. Reactive oxygen species (ROS) play a crucial role in inflammation, and their overproduction can lead to chronic inflammation by affecting intracellular signalling. Plants with anti-inflammatory properties are traditionally used to treat inflammatory diseases [29].

In this context, the combination of plant-based molecules with nanomaterial development has been investigated for various biological activities. Recently, attention has shifted to bioprocessing and green chemistry, which utilize economical, biocompatible, and environmentally friendly reducing agents to create new nano-textured materials. As per reported studies, plant extract mediated cerium and titanium oxide nanoparticles have shown significant potential as anti-inflammatory and antioxidant in biological systems [30].

The calyx is the primary by-product generated during the harvesting and selling of *Physalisperuviana* fruits. It contains numerous chemical constituents with diverse biological activities, including anti-inflammatory, antioxidant, anti-diabetic, nephroprotective effect and anticancer properties. Studies have clearly demonstrated that the calyx leaf extract of *Physalisperuviana* possesses significant biological activities, attributed to the presence of flavonoids, terpenoids, and glycosides in this biowaste. The extract obtained from the calyx leaves of *Physalisperuviana* exhibited higher toxicity compared to leaf extracts reported in other studies [31]. This increased toxicity aligns with the protective role of the calyx in safeguarding the fruit, suggesting the presence of potent bioactive metabolites [32].

It is suggested that the biological applications of cerium and titanium, combined with the remarkable anti-inflammatory properties of *Physalisperuviana* calyx, represent a promising therapeutic approach to attenuate inflammation.

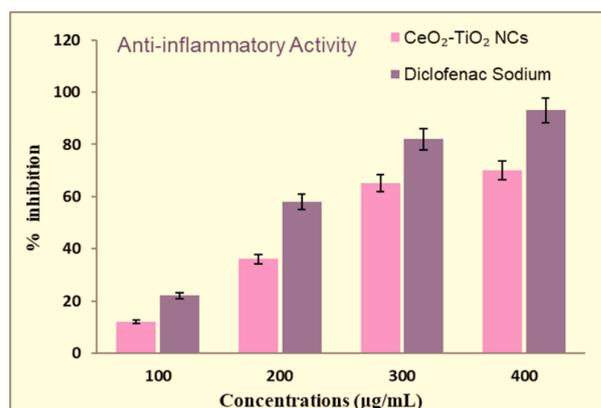


Figure 5: Anti-inflammatory activity of CeO₂-doped TiO₂ NPs

3.6 Evaluation of Antioxidant Activity

In order to decrease DPPH, a stable free radical, one must take hydrogen or an electron from a donor in order to form yellowish diphenyl picrylhydrazine molecule. The spectrophotometric approach relies on the ability of the antioxidant sample to scavenge stable colored radicals of DPPH by quenching. The antioxidant activity of green synthesized nanoparticles was investigated against DPPH at different concentrations display in fig 7. The radical scavenging properties observed in CeO₂-doped TiO₂ nanoparticles was compared to standard. The bar graph clearly demonstrates the dose-dependency of nanoparticles and found to be potent scavenger. The results show that TiO₂ nanoparticles doped with CeO₂ significantly improved their ability to scavenge radicals, mainly because the cerium oxide treatment amplified the surface oxygen vacancies [33]. These effects have significant biological process relevance and are comparable to the actions of biological antioxidants.

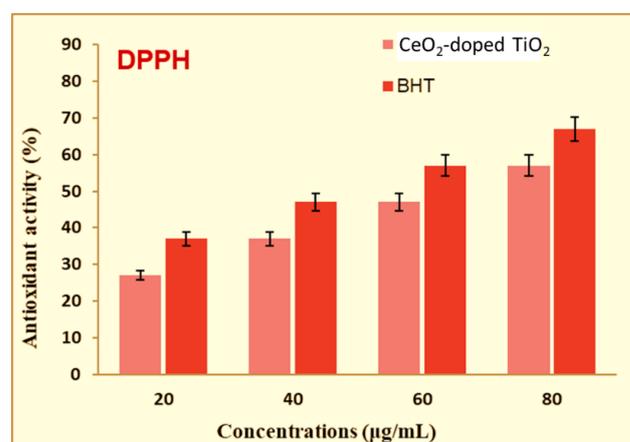


Figure 6: DPPH assay of CeO₂-doped TiO₂ NPs

IV. CONCLUSION

In this study, a green approach was employed to successfully synthesize CeO₂-doped TiO₂ nanoparticles using aqueous calyx leaves extract of fruits of *Physalis Peruviana*

plant. The process of synthesis is purely environmental friendly and economically efficient, utilizing a biowaste material. The synthesized nanoparticles exhibited promising anti-inflammatory and radical scavenging activities and this enhancement are due to synergistic effect (TiO_2 and CeO_2) which contingent upon the overall count of surface oxygen vacancies and the alternation in band gap and surface area within the nanoparticle. Hence, the green synthesized of CeO_2 -doped TiO_2 nanoparticles from biowaste material emerges as a straightforward, remarkably convenient, and promising approach with significant potential for utilization in biomedical domains.

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