

## Green synthesis of nano CeO<sub>2</sub> using *Cleistocalyx operculatus* leaf extract

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### ABSTRACT

*This study investigates the green synthesis of CeO<sub>2</sub> nanoparticles using an extract from Cleistocalyx operculatus leaves, a traditional medicinal plant rich in antioxidants. A detailed solvent optimization process identified ethanol-water (1:1) as the optimal medium for extracting the highest levels of polyphenols and flavanols, essential for reducing and stabilizing nanoparticles. The morphology, structure, and physicochemical properties of the synthesized nanoparticles were analyzed using advanced techniques such as FE-SEM, EDX, XRD, FT-IR, UV-Vis, BET, and Zeta potential analysis. XRD analysis confirmed that the CeO<sub>2</sub> nanoparticles featured a fluorite cubic crystal structure with a crystallite size of 12.04 nm. The nanoparticles had a high surface area (49.71 m<sup>2</sup>/g), stable zeta potential (-42 mV), and optical and functional properties characteristic of CeO<sub>2</sub>. These findings highlight the viability of green synthesis as an eco-friendly, cost-effective approach for CeO<sub>2</sub> nanoparticle production.*

**Keywords:** Green synthesis; CeO<sub>2</sub> nanoparticles; Cleistocalyx operculatus; Polyphenol; Flavanol.

### 1. INTRODUCTION

Green synthesis is an emerging trend in chemistry aimed at minimizing or eliminating the use and generation of hazardous substances during chemical production. This approach provides significant economic and environmental advantages, serving a sustainable alternative to traditional chemical and physical methods [1]. Unlike conventional approaches, green synthesis methods operate under mild conditions without the need for high pressure, energy, temperature, or toxic chemicals. As a result, many researchers have increasingly focused on synthesizing nanomaterials using plant extracts [2].

The solvents commonly employed in green synthesis are water or ethanol. These solvents are preferred due to their low toxicity and ability to dissolve reducing agents and metal salts effectively. The reducing agents are extracted from plant parts (such as leaves, flowers, or roots) by grinding and extracting them in the selected solvent. Compounds such as phenolics, alkaloids, flavonoids, and terpenoids in the plant extracts act as reducing agents, converting metal ions into metallic nanoparticles. Biocompatible stabilizing agents, often natural compounds found in the plant extract, help maintain nanoparticle stability and prevent aggregation or precipitation [3].

Leaves of *Cleistocalyx operculatus* Roxb., a member of the *Myrtaceae* family, have traditionally been consumed as a beverage in Vietnam for a long time [4]. The plant is rich in polyphenols, which serve as key reducing agents, facilitating the conversion of metal ions into nanoparticles [5]. Several studies have synthesized nanoparticles using extracts from *Cleistocalyx operculatus* leaves, such as selenium nanoparticles [5], iron nanoparticles [6], and Fe/Graphene composite nanoparticles [7].

Cerium dioxide (CeO<sub>2</sub>) is a rare earth oxide widely used in thermocatalysis, electrocatalysis,

and photocatalysis due to its reversible  $Ce^{4+}/Ce^{3+}$  redox couple and adjustable oxygen vacancy sites. Conventional methods for synthesizing  $CeO_2$  nanoparticles include chemical co-precipitation, hydrothermal processes, sol-gel methods, spray pyrolysis, and microemulsion techniques [8-12]. However, these methods often require expensive equipment, lengthy synthesis durations, and the use of large quantities of toxic chemicals, leading to environmental pollution. Consequently, researchers have shifted toward using plant extracts for synthesizing  $CeO_2$  nanoparticles, providing an environmentally friendly, rapid, and cost-effective alternative.

This study aims to identify the optimal solvent for extracting *Cleistocalyx operculatus* leaves to achieve the highest total polyphenol and flavonoid content. Subsequently,  $CeO_2$  nanoparticles were synthesized using the leaf extract. The synthesized material was characterized using various techniques, including Field-Emission Scanning Electron Microscopy (FE-SEM), Energy Dispersive X-ray Spectroscopy (EDX), X-ray Diffraction (XRD), UV-visible spectroscopy, and Zeta potential analysis.

## 2. EXPERIMENT PREPARATION

### 2.1. Materials

Dried *Cleistocalyx operculatus* leaves were sourced from a traditional herbal medicine store in Hanoi, Vietnam. The leaves were cleaned, dried at 70 °C for 24 hours, and pulverized into fine particles (<1 mm). Chemicals used in the study included ethanol (96%), deionized water,  $Ce(NO_3)_3 \cdot 6H_2O$ , gallic acid, quercetin, and reagents for total polyphenol and flavanol determination.

### 2.2. Sample Preparation Procedure

#### 2.2.1. Extraction of leaf compounds

Three solvents (water, ethanol, and ethanol-water in a 1:1 ratio) were evaluated for their efficiency in extracting polyphenols and flavanols. Five grams of pulverized leaves were mixed with 100 mL of solvent and stirred at 100 rpm at 80 °C for 1 hour. After centrifugation (4500 rpm, 5 minutes) and filtration, the extracts were analyzed for their bioactive compound content.

#### 2.2.2. Synthesis of $CeO_2$ Nanoparticles

The optimal extract was mixed with  $Ce(NO_3)_3 \cdot 6H_2O$  (8.68 g dissolved in 20 mL of extract). The solution was stirred at 80 °C for 5 hours, dried at 80 °C for 8 hours, and calcined at 500 °C for 3 hours to obtain  $CeO_2$  nanoparticles [13].

### 2.3. Characterization Techniques

The total polyphenol content was determined following the method of Singleton (1999) [14], with some modifications. The Folin-Ciocalteu reagent was used for the quantification, and a standard curve was established using gallic acid at the following concentrations: 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL. The steps were as follows: i) using a micropipette, 0.2 mL of each standard solution was mixed with 1 mL of 10% Folin-Ciocalteu reagent; ii) the mixture was allowed to react for 3 to 8 minutes before adding 0.8 mL of 7.5% sodium carbonate solution; iii) each tube was capped and mixed thoroughly; iv) the reaction mixtures were left at room temperature for 60 minutes; v) absorbance was measured at 765 nm using a UV-Vis spectrophotometer.

The standard curve equation was determined as  $y = ax + b$ . The extract samples were diluted to appropriate concentrations, and the same procedure was followed to measure their absorbance. The total polyphenol content of the *Cleistocalyx operculatus* leaf extract was calculated using the standard curve equation and the following formula:

$$P = \frac{C \times V}{m} \text{ (mg GAE/g dry leaf)}$$

Where: P: Total polyphenol content (mg GAE/g dry leaf);  
C: Polyphenol concentration derived from the standard curve (µg/mL);

V: Volume of the extract (mL);  
m: Mass of the leaf sample (g).

The total flavonoid content was determined using the aluminum chloride (AlCl<sub>3</sub>) colorimetric method in an alkaline medium, following the protocol described by Marinova et al. (2005) [15], with specific modifications. A standard curve was constructed using quercetin solutions prepared at concentrations of 50 µg/mL, 100 µg/mL, 200 µg/mL, 250 µg/mL, 400 µg/mL, and 500 µg/mL in methanol. The *Cleistocalyx operculatus* leaf extract was diluted with double-distilled water to an appropriate concentration. For the reaction, 1 mL of the diluted extract (or standard solution) was transferred into a test tube, followed by the addition of 0.3 mL of 5% NaNO<sub>2</sub>. The mixture was allowed to stand for 5 minutes before adding 0.3 mL of 10% AlCl<sub>3</sub>. After standing for another 6 minutes, 2 mL of 1 M NaOH was added to the mixture, which was then mixed thoroughly and diluted to a final volume of 10 mL using double-distilled water. The absorbance of the final solution was measured at 510 nm using a UV-Vis spectrophotometer. This method provides a reliable and quantitative measurement of flavonoid content, expressed as quercetin equivalents (QE). The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry leaf sample (mg QE/g) and calculated using the following formula:

$$F = \frac{C \times V}{m}, \text{ mg QE/g dry leaf}$$

Where: F: Total flavonoid content (mg QE/g dry leaf);  
C: Flavonoid concentration obtained from the standard curve (µg/mL);  
V: Volume of the extract (mL);  
m: Mass of the dry leaf sample (g).

The total polyphenol and flavonoid contents were measured using a UV-Vis S80 Biochrom spectrophotometer (UK). The morphology and elemental composition of the CeO<sub>2</sub> nanoparticles were analyzed with a JSM-IT800 scanning electron microscope (FE-SEM, Japan) equipped with an Ultim Max 65 energy-dispersive X-ray (EDX) detector (Oxford Instruments). The phase structure of the CeO<sub>2</sub> nanoparticles was examined using an X-ray diffraction (XRD) instrument (D8 Advance, Bruker, Germany).

The characteristic functional groups of the CeO<sub>2</sub> nanoparticles were identified using a Nicolet IS 10 Fourier-transform infrared (FT-IR) spectrometer (USA) with KBr pellet techniques. The analysis was conducted with 32 scans at a resolution of 4 cm<sup>-1</sup>, covering a spectral range from 4000 cm<sup>-1</sup> to 400 cm<sup>-1</sup>. The stability of the nanoparticle dispersion in water was assessed through zeta potential measurements using the SZ-100Z2 zeta analyzer (Horiba, Japan).

The characteristic absorption spectrum of the CeO<sub>2</sub> nanoparticles was analyzed using an Agilent UV-Vis NIR Cary 6000i spectrophotometer, scanning a wavelength range of 600 nm to 200 nm at a rate of 600 nm/min. The specific surface area was determined using the BET method with a TriStar II instrument (Micromeritics, USA). Before adsorption analysis, the sample was vacuum-dried at 150 °C for 3 hours under a pressure of 5 µm Hg.

### 3. RESULTS AND DISCUSSION

The highest polyphenol content was observed in the 96% ethanol/water (1:1) solvent, reaching 107.08 ± 0.78 mg GAE/g, as shown in table 1. This solvent ratio offers an optimal balance between the polarity of water and the solubility of ethanol, facilitating the effective extraction of a wide range of bioactive compounds, including polyphenols, from *Cleistocalyx operculatus* leaves. In contrast, the polyphenol content in the 96% ethanol extract was slightly lower at 67.00 ± 0.78 mg GAE/g, reflecting the reduced efficiency of pure ethanol in extracting polar compounds. The water extract exhibited a moderate polyphenol content of 72.3 ± 1.7 mg GAE/g, underscoring its polarity-dependent limitations in dissolving less polar components.

Similarly, the highest flavanol content was recorded in the 96% ethanol/water (1:1) solvent, with a value of  $108.38 \pm 2.96$  mg QE/g. The flavanol content in the 96% ethanol extract was notably lower at  $94.49 \pm 2.22$  mg QE/g, while the water extract had the lowest flavanol content, measuring  $52.83 \pm 2.22$  mg QE/g. These results highlight the superior performance of the 96% ethanol/water mixture in extracting both polyphenols and flavanols, attributed to its ability to dissolve compounds across a broader polarity spectrum.

Polyphenols and flavonols are recognized for their robust antioxidant capabilities, which are critical in green synthesis processes. Their potential to neutralize free radicals and interact with oxidative species not only mitigates harmful oxidative reactions but also contributes to the sustainable production of environmentally friendly materials [16, 17]. Based on these findings, the 96% ethanol/water (1:1) solvent was confirmed as the optimal medium for extracting bioactive compounds from *Cleistocalyx operculatus* leaves.

**Table 1.** Summary of total polyphenol and flavanol content.

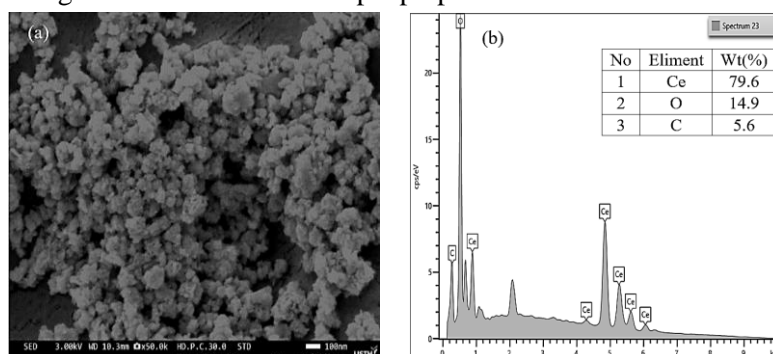
No	Extraction solvent	Polyphenol content (mg GAE/g) <sup>(1)</sup>	Flavanol content (mg QE/g) <sup>(2)</sup>
1	Water	$72.3 \pm 1.7$	$52.83 \pm 2.22$
2	96% Ethanol	$67.00 \pm 0.78$	$94.49 \pm 2.22$
3	96% Ethanol/Water (1:1)	$107.08 \pm 0.78$	$108.38 \pm 2.96$

<sup>(1)</sup>: The values were calculated based on the standard curve equation of gallic acid ( $y = 0.008x + 0.002$ ;  $R^2 = 0.994$ );

<sup>(2)</sup>: The values were calculated based on the standard curve equation of quercetin ( $y = 0.0006x + 0.0063$ ;  $R^2 = 0.9913$ ).

The FE-SEM image (figure 1a) illustrates the morphology of CeO<sub>2</sub> nanoparticles synthesized using *Cleistocalyx operculatus* leaf extract. The nanoparticles exhibit a spherical shape with sizes ranging from 10–20 nm. A tendency for the particles to aggregate into clusters is observed, which could be attributed to van der Waals forces or electrostatic interactions occurring during the synthesis process.

The EDX spectrum (figure 1b) confirms the elemental composition of the nanoparticles, showing the presence of cerium (Ce) and oxygen (O) as the primary components of CeO<sub>2</sub>. Additionally, the spectrum reveals a carbon (C) content of 5.6%, likely originating from the carbon substrate used during FE-SEM and EDX sample preparation.



**Figure 1.** a) FESEM images of CeO<sub>2</sub> nanoparticles; b) EDX spectrum of CeO<sub>2</sub> nanoparticles.

The XRD pattern (figure 2a) of the CeO<sub>2</sub> nanoparticles displays eight distinct peaks at  $2\theta$  values of  $28.54^\circ$ ,  $33.08^\circ$ ,  $47.49^\circ$ ,  $56.35^\circ$ ,  $59.10^\circ$ ,  $69.41^\circ$ ,  $76.71^\circ$ , and  $79.21^\circ$ . These peaks correspond to the (111), (200), (220), (311), (222), (400), (331), and (420) planes, characteristic of the fluorite cubic crystal structure (space group Fm-3m). The lattice parameter,  $a = b = c = 5.411$  Å, matches the standard reference JCPDS card number 00-034-0394, confirming the successful synthesis of

CeO<sub>2</sub> nanoparticles using the leaf extract [18]. The average crystal size (ddd) was calculated using the Debye-Scherrer equation based on the (111) plane, yielding a size of 12.04 nm. This result aligns with the size range observed in the SEM analysis. Figure 2b shows the UV-Vis diffuse reflectance spectrum (UV-Vis DRS) of the CeO<sub>2</sub> nanoparticles synthesized using *Cleistocalyx operculatus* leaf extract. The spectrum exhibits a characteristic absorption peak at 345 nm, which is consistent with the optical properties of CeO<sub>2</sub> nanoparticles. This result aligns with previous findings reported by Nadjia L. *et al.* [19], confirming the successful synthesis and optical behavior of CeO<sub>2</sub> nanoparticles produced through this green synthesis method.

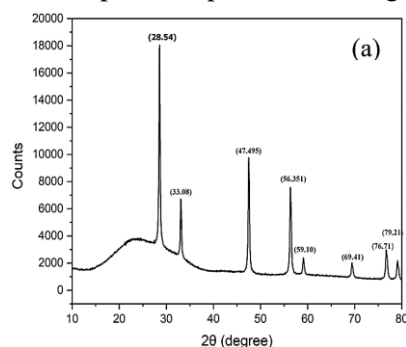


Figure 2a. XRD pattern of CeO<sub>2</sub> nanoparticles.

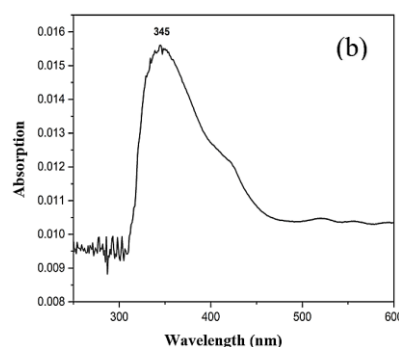


Figure 2b. UV-Vis DRS spectrum of CeO<sub>2</sub> nanoparticles.

The FT-IR spectrum of the CeO<sub>2</sub> nanoparticles is presented in figure 3. The absorption peaks at 3424 cm<sup>-1</sup> and 1628 cm<sup>-1</sup> correspond to the stretching and bending vibrations of O-H groups, indicating the presence of adsorbed water on the nanoparticle surface. The peak at 1384 cm<sup>-1</sup> is attributed to the vibrations of C=O groups, potentially arising from the adsorption of CO<sub>2</sub> from the air onto the metal cations of the CeO<sub>2</sub> nanoparticles [20]. Furthermore, the Ce=O bonds are evident in the region between 400 cm<sup>-1</sup> and 700 cm<sup>-1</sup>, with a specific peak at 456 cm<sup>-1</sup> confirming the characteristic vibrations of Ce=O bonds. This analysis highlights the functional groups present on the nanoparticle surface and supports the successful synthesis of CeO<sub>2</sub> nanoparticles.

The zeta potential of the CeO<sub>2</sub> nanoparticles, synthesized using *Cleistocalyx operculatus* leaf extract, is shown in figure 3b. For zeta analysis, the nanoparticles were dispersed in deionized water and sonicated for one minute. The measured zeta potential of -42 mV indicates good electrostatic stability of the CeO<sub>2</sub> nanoparticle suspension, suggesting minimal aggregation and stable dispersion in the solution.

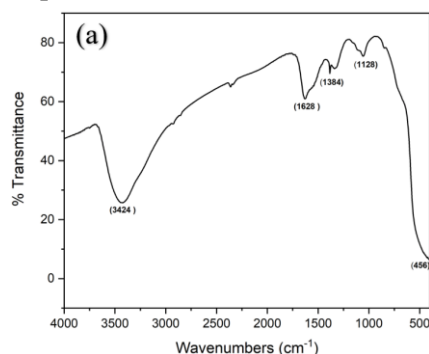


Figure 3a. FT-IR spectrum of CeO<sub>2</sub> nanoparticles.

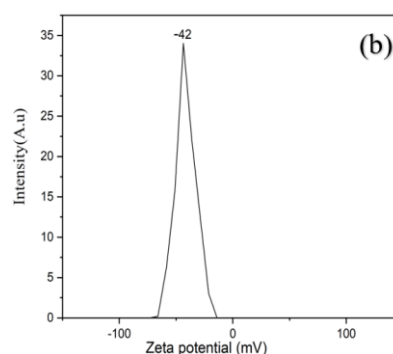
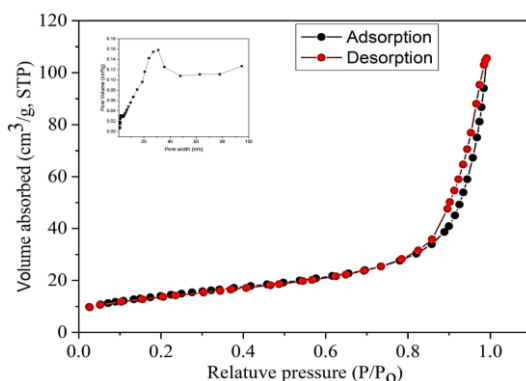


Figure 3b. Zeta potential of CeO<sub>2</sub> nanoparticles.

Figure 4 illustrates the nitrogen adsorption/desorption isotherms of the synthesized CeO<sub>2</sub> nanoparticles. Based on the IUPAC classification, the isotherm corresponds to Type III with an H3-type hysteresis loop. This indicates that the CeO<sub>2</sub> nanoparticles consist of aggregated or

agglomerated particles. The sample indicates a specific surface area with suitable pore volume and small pore diameter. BET analysis revealed that the CeO<sub>2</sub> nanoparticles possess a specific surface area of 49.71 m<sup>2</sup>/g, a pore volume of 0.163 cm<sup>3</sup>/g, and an average pore diameter of 16.36 nm. These characteristics confirm the suitability of the material for applications requiring high surface area and controlled pore structures, such as catalysis and adsorption processes. These findings align with similar studies on CeO<sub>2</sub> nanomaterials, [21, 22] supporting the reliability of the observed characteristics in this study.



**Figure 4.** Adsorption/Desorption isotherms of CeO<sub>2</sub> nanoparticles.

#### 4. CONCLUSIONS

The synthesis of CeO<sub>2</sub> nanoparticles from the leaf extract has been successfully synthesized. The average particle size, determined by XRD, was 12.04 nm, confirming the formation of nanoscale CeO<sub>2</sub>. The Zeta potential of -42 mV indicated good stability and dispersion of the CeO<sub>2</sub> nanoparticle system. Additionally, BET analysis revealed a high surface area of 49.71 m<sup>2</sup>/g, beneficial for applications such as adsorption and photocatalysis that require efficient material-environment interaction. The ethanol (96%)/water mixture in a 1:1 volume ratio can be used to extract polyphenols and flavonoids from *Cleistocalyx operculatus* leaves, yielding a higher concentration of these bioactive compounds compared to using water or ethanol alone.

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## TÓM TẮT

### Nghiên cứu tổng hợp xanh vật liệu nano $CeO_2$ từ dịch chiết lá vòi

Nghiên cứu này tập trung vào tổng hợp xanh hạt nano  $CeO_2$  từ dịch chiết lá vòi (*Cleistocalyx operculatus*), một dược liệu giàu hoạt chất chống oxy hóa. Nghiên cứu đã tối ưu hóa được tỷ lệ dung môi chiết ethanol/nước là (1:1), cho hàm lượng tổng polyphenol và tổng flavanol cao, chất đóng vai trò quan trọng trong quá trình khử và ổn định hạt nano. Đặc trưng hình thái, cấu trúc và các tính chất lý-hóa của hạt nano tổng hợp được phân tích bằng các kỹ thuật hiện đại như FE-SEM, EDX, XRD, FT-IR, UV-Vis, BET và thế Zeta. Kết quả phân tích nhiễu xạ tia X (XRD) cho thấy các hạt nano  $CeO_2$  có cấu trúc tinh thể lập phương kiểu fluorite, với kích thước tinh thể 12,04 nm. Ngoài ra, các hạt nano có diện tích bề mặt riêng cao (49,71  $m^2/g$ ), thế Zeta ổn định (-42 mV) và thể hiện các đặc tính quang học cũng như tính chất đặc trưng của  $CeO_2$ . Kết quả này khẳng định tiềm năng của tổng hợp xanh như một phương pháp thân thiện với môi trường, tiết kiệm chi phí và hiệu quả trong tổng hợp hạt nano  $CeO_2$ .

**Từ khóa:** Tổng hợp xanh; Hạt nano  $CeO_2$ ; Dịch chiết lá vòi; Polyphenol; Flavanol.