

Morphological Effect of Green Synthesis Cerium Oxide Nanoparticles Enhanced Antibacterial Activity

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Abstract. Cerium oxide nanoparticles (CeO₂NPs) were successfully synthesized using two methods, which are the precipitation and green synthesis. In the green synthesis approach, leaf extracts from *Moringa oleifera* and *Uncaria gambir* Roxb. acted as a natural capping and reducing agents. In contrast, ammonium hydroxide was used in the chemical precipitation method. X-ray diffraction (XRD) analysis confirmed the formation of CeO₂NPs with a cubic fluorite structure. Scanning electron microscopy (SEM) revealed that the nanoparticles had an agglomerate morphology. In contrast, transmission electron microscopy (TEM) showed that the CeO₂NPs were predominantly spherical, with average particle sizes of approximately 5–20 nm for the green synthesis method and 10–40 nm for the precipitation method. The antibacterial assays demonstrated significant activity, particularly against *Staphylococcus aureus*, with the inhibition zones measuring up to 11.05 mm. These findings suggest that green-synthesized CeO₂NPs hold promising potential for applications in the biomedical field due to their biocompatibility and antibacterial properties.

Keywords: Antibacterial, Cerium Oxide, Green Synthesis, *Moringa Oleifera*, Precipitation

INTRODUCTION

Cerium oxide nanoparticles (CeO₂NPs) have attracted considerable attention in

recent years due to their remarkable oxygen storage capacity, making them highly suitable for applications in many fields (Eka Putri *et al.*, 2019). CeO₂NPs have oxygen storage

capacity because cerium has two oxidation numbers, namely trivalent Ce^{3+} and Ce^{4+} tetravalent. Therefore, cerium oxide acts as an oxygen provider in an oxygen-deprived environment. After the oxide surface gives oxygen, Ce^{4+} was reduced to Ce^{3+} because the extra electrons are left behind (Eka Putri *et al.*, 2021; Putri *et al.*, 2022; Eka Putri *et al.*, 2019). When the oxide surface lacks oxygen, Ce^{3+} will tend to receive oxygen again. CeO_2NPs can inactivate hydroxyl radicals of superoxide radicals, nitric oxide, and hydrogen peroxide (Sales *et al.*, 2020; Li *et al.*, 2022). A major issue in healthcare is the rapid development of bacterial resistance to antibiotics. To combat this, various strategies aim to create effective antibacterial agents, with CeO_2NPs gaining attention for their strong antibacterial properties (Arumugam *et al.*, 2015; G. E. Putri *et al.*, 2019). CeO_2NPs exhibit antibacterial activity through the generation of reactive oxygen species (ROS) like superoxide anions and hydroxyl radicals, which damage bacterial cell membranes and cause cell death. The reversible redox cycling between Ce^{3+} and Ce^{4+} enhances catalytic activity and maintains antibacterial effects, making cerium oxide a promising candidate for antibacterial formulations and biomedical applications (Mohammadi *et al.*, 2019; Kalaycioğlu *et al.*, 2020).

The synthesis of metal oxide nanoparticles has been accomplished using various methods. The use of plant extracts is a common approach, largely due to their availability and the wide range of secondary metabolites they possess, which contribute to various biological activities (Nourmohammadi *et al.*, 2020). These secondary metabolites act as natural reducing and capping agents, and hence are sustainable and less toxic (Iconaru *et al.*, 2021; Hao *et al.*, 2020). In addition, this method can

be scaled up to an industry level (Ramachandran *et al.*, 2019). The use of plant extracts in the synthesis has been described in several publications to synthesis of CeO_2NPs , such as *Gloriosa superba* leaf extract (Arumugam *et al.*, 2015), *Acalypha indica* leaf extract (Kannan & Sundrarajan, 2014), *Aloe vera* (Gel *et al.*, 2014), *Hibiscus sabdarifa* flower extract (Thovhogi *et al.*, 2015), *Olei europaea* leaf extract (Abid *et al.*, 2022), *Sida acuta* leaf extract (Senthilkumar *et al.*, 2017), *Prosopis juliflora* leaf extract (Arunachalam *et al.*, 2017), and *Calotropis procera* flower extract (Muthuvel *et al.*, 2020; Chavhan *et al.*, 2020).

In this research, we synthesized CeO_2NPs using three different methods: the precipitation method, and two green synthesis methods employing *Moringa oleifera* and *Uncaria gambir* Roxb. leaf extract. The precipitation method used a mixed solvent system of propanol and water, with ammonium hydroxide (NH_4OH) as the precipitating agent. In contrast, the green synthesis methods using *Moringa oleifera* and *Uncaria gambir* Roxb. extracts utilized only water as the solvent, without any chemical precipitating agents. This highlights the potential advantages of green synthesis in terms of cost-effectiveness and environmental sustainability. Consequently, the study aimed to evaluate the benefits of green synthesis in comparison to the conventional chemical approach.

MATERIALS AND METHODS

Chemicals and Reagents

Precursor used cerium (III) nitrate hexahydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$) 99% trace metal basis from Sigma Aldrich, ammonium hydroxide (NH_4OH) from Sigma Aldrich, used

to control pH of the solution in the precipitation method. Isopropanol from Sigma Aldrich and deionized water as solvents in the precipitation method. *Uncaria gambir* Roxb. and *Moringa oleifera* leaf from the Biological Education and Research Forest of Andalas University, Sumatra Barat, Indonesia.

Synthesis of Cerium Oxide Nanoparticles with the Precipitation Method

Synthesis of CeO₂NPs under typical synthetic conditions, 1.07 g Ce (NO₃)₃.6H₂O was added to a solvent combination of isopropanol and deionized water (3:1), homogenized using magnetic stirring. The pH of the solution was adjusted (around pH ≈ 9–10) using NH₄OH. The stirring of the solution continues for 24 h; then, it has been transferred into a polypropylene (PP) bottle. The PP bottle was then placed in the oven at 100 °C for 72 h, filtered, and calcined at 600 °C for 2 hours.

Synthesis of Cerium Oxide Nanoparticles with *Uncaria gambir* Roxb. Leaf Extract

Cerium oxide nanoparticles were synthesized via the hydrothermal method using NaOH as an oxidizing agent and *Uncaria gambir* Roxb. leaf extract as a capping agent. The synthesis of cerium oxide nanoparticles was started by dissolving 1.74 grams of Ce(NO₃)₃.6H₂O in 75 mL of 6.4M NaOH and 10 mL *Uncaria gambir* Roxb. leaf extract. After that, the mixture was stirred for 30 minutes and homogenized by ultrasonication for 10 minutes. Then the hydrothermal process on the mixture was carried out at 150 °C for 24 hours. The precipitate was then washed with ethanol and distilled water 3 times. Then the precipitate was dried at 105 °C for 12 hours.

Synthesis of Cerium Oxide Nanoparticle with *Moringa oleifera* Leaf Extract

A total of 3.72 g of cerium nitrate hexahydrate was added to 50 mL of *M. oleifera* leaf extract. The solution was stirred using a magnetic stirrer at 80 °C for 2 hours, until the solution changed color from dark brown to light brown. The resulting solid was calcined at 600 °C for 2 hours, to obtain CeO₂NPs.

Antibacterial Activity

The antibacterial activity of CeO₂NPs was evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the disc diffusion method. Mueller Hinton Agar (MHA) was poured into Petri dishes (20 mL each) and allowed to solidify. Then, 1 mL of the overnight bacterial culture was spread on the agar surface. Discs loaded with CeO₂NPs at concentrations of 25%, 50%, 75%, and 100% were placed on the plates, along with amoxicillin as a positive control, DMSO as a negative control. After 24 hours of incubation at 37 °C, inhibition zones were measured. Clear zones around the discs indicated antibacterial activity.

Characterization of CeO₂NPs Nanoparticles

The morphology of CeO₂NPs was analyzed using scanning electron microscope (SEM) (JEOL JSM IT300 microscope) operating at 20 kV and transmission electron microscope (TEM) JEOL JEM-2100 / FEI Tecnai G2 Spirit / Hitachi HT7700. Functional groups contained in the sample were analyzed using Fourier transform infrared spectroscopy (FTIR) (JEOL JSM 6950) in the 400–4000 cm⁻¹ wavenumber range. The crystal structure of the resulting CeO₂NPs was determined using X-ray diffraction (XRD) (XPert Pro Panalytical PW 30/60) with a

graphite monochromator of CuK α radiation. To calculate the crystallite size of CeO₂NPs, Scherrer's equation was used.

RESULTS AND DISCUSSION

XRD Analysis

The XRD pattern of the precipitation method, *Uncaria gambir* Roxb. leaf extract, and *Moringa oleifera* leaf extract mediated synthesized CeO₂NPs are shown in Figure 1. It shows the appearance of specific peaks in 2θ of 28.55°, 33.08°, 47.47°, 56.33°, 59.08°, 69.40°, 76.69°, and 79.07° without any impurities. This pattern refers to cubic CeO₂NPs with hkl of (111), (200), (220), (311), (222), (400), (331), and (420), respectively, based on ICSD standard No 34-0394.

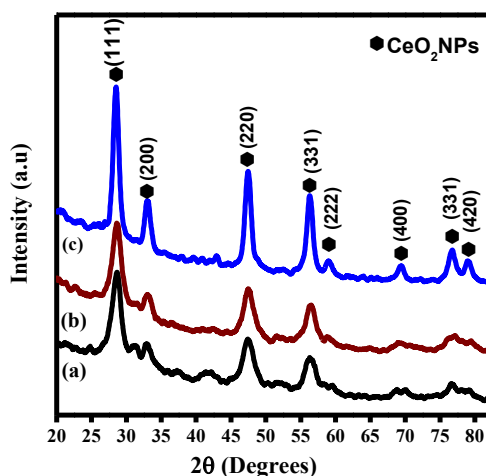


Fig. 1: XRD analysis of CeO₂NPs used (a) Precipitation Method, (b) *Uncaria gambir* Roxb. leaf extract, (c) *Moringa oleifera* leaf extract

The crystalline phase and structure of the synthesized CeO₂NPs were investigated using X-ray diffraction techniques to properly study the position of the atoms in the cubic structure. Figure 1 shows the XRD patterns of CeO₂NPs nanoparticles produced from Ammonium hydroxide, *Uncaria gambir* Roxb., and *Moringa* aqueous extract as a capping

agent. No significant characteristic peaks appear from Ce or other impurities detected on the diffractogram, indicating the high purity of the synthesized CeO₂NPs nanoparticles.

The average diameter of crystalline (D) was measured using Scherrer's formula (Eq. (1)).

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

The crystallite size (D) was calculated using the Scherrer equation of Eq. (1), where K is a dimensionless shape factor that depends on the crystallite geometry (typically 0.9), λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the selected diffraction peak in radians, and θ is the Bragg angle corresponding to that peak.

These findings align with Khaoula Hkiri (Hkiri *et al.*, 2024), where cerium oxide nanoparticles synthesized using *Portulaca oleracea* extract exhibited a cubic crystal system and an average crystallite size of 25–35 nm. The smaller crystallite size in their study may result from specific antioxidants and capping agents in the extract, which limit particle growth during synthesis. This consistency across green synthesis methods suggests that plant-mediated synthesis is an effective approach for producing crystalline CeO₂ nanoparticles with desirable structural characteristics. The results indicate that natural phytochemicals significantly influence nanoparticle formation while ensuring phase purity and crystallinity.

Morphological Analysis

Sample characterization using SEM shows the morphology and topography of the sample. Figure 2 shows SEM images CeO₂NPs synthesized via three methods: (A) precipitation, (B) *Uncaria gambir* Roxb. leaf

extract, and (C) *Moringa oleifera* leaf extract. In Figure 2(A), the nanoparticles from the precipitation method are large, irregular agglomerates with rough surfaces and average sizes over 50 nm, indicating uncontrolled growth. Figure 2(B) displays nanoparticles produced using *Uncaria gambir*, which are smaller and plate-like due to phytochemicals that reduce agglomeration. Figure 2(C) shows nanoparticles from *Moringa oleifera* that are more uniform and faceted, benefiting from better stabilization by flavonoids and polyphenols during growth, resulting in finer, more crystalline structures.

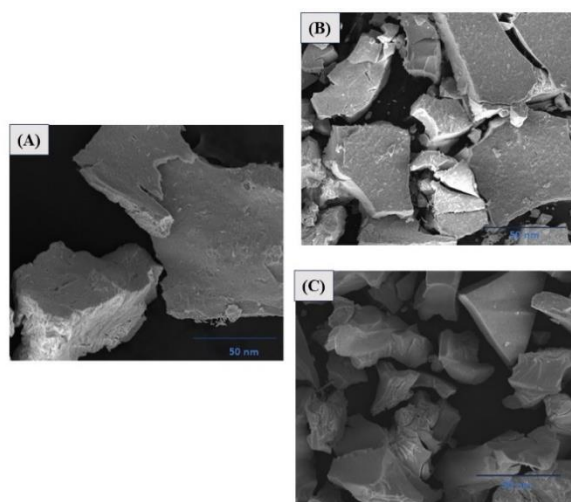


Fig. 2: SEM Morphological analysis of CeO₂NPs used (A) Precipitation method (B) *Uncaria gambir* Roxb. leaf extract (C) *Moringa oleifera* leaf extract

The crystallographic and morphological characteristics of cerium oxide nanoparticles synthesized using three different methods are summarized in Table 1. All samples exhibit a cubic crystal system, which is typical for CeO₂ nanoparticles and confirms the successful formation of the fluorite structure.

In terms of crystallite size (D) in Table 1, the nanoparticles synthesized with other methods range from 10 to 50 nm. The CeO₂

nanoparticles synthesized using *Uncaria gambir* Roxb. leaf extract exhibited slightly larger crystallite sizes (40–50 nm) compared to the *Moringa oleifera*-based sample (30–40 nm). The larger D value in the gambir-based synthesis may result from a slower nucleation rate or weaker capping activity of the phytochemicals compared to *Moringa*. However, both green synthesis methods yielded smaller particle sizes (30–70 nm) than the conventional precipitation approach, indicating better dispersion and stabilization of particles by the plant-derived bioactive compounds.

Table 1. Comparison of the XRD and SEM data of the Synthesis of Cerium Oxide nanoparticles using Precipitation Method, *Moringa oleifera* leaf extract, and *Uncaria gambir* Roxb. leaf extract

	<i>Moringa oleifera</i> leaf extract	<i>Uncaria gambir</i> Roxb. leaf extract	Precipitation Method
Crystal system	Cubic	Cubic	Cubic
Diameter of crystalline (D) (nm)	30–40	40–50	30–40
Diameter of particles (nm) in SEM	30–50	40–70	40–80

Notably, *Moringa oleifera* extract resulted in the smallest and most uniform particle sizes (30–50 nm), suggesting a more effective capping and stabilization process, likely due to its rich content of flavonoids, polyphenols, and antioxidants. This comparative analysis supports the potential of plant-mediated green synthesis for controlling particle size and minimizing agglomeration in CeO₂ nanoparticle production, with *Moringa oleifera* showing the most promising performance among the tested methods. Based on this condition, in Figure 3 in this

manuscript, only the TEM results of the precipitation method and the green synthesis method with *Moringa oleifera* leaf extract are compared.

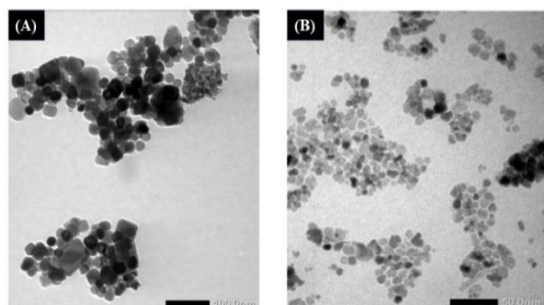


Fig. 3: TEM Image of morphological analysis of CeO₂NPs used (A) precipitation method (B) *Moringa oleifera* leaf extract

Figure 3 shows Transmission Electron Microscopy (TEM) images of CeO₂ nanoparticles (CeO₂NPs) synthesized via the precipitation method and green synthesis using *Moringa oleifera* extract. In Figure 3(A), the nanoparticles from the precipitation method display irregular, agglomerated particles with a size range of 10-40 nm, indicating poor dispersion. In contrast, Figure 3(B) reveals CeO₂NPs synthesized with *Moringa oleifera* extract, which are more uniformly distributed, smaller, and spherical, averaging 5-20 nm. This suggests that the green synthesis method yields better control over particle morphology and distribution due to the stabilizing effects of the phytochemicals in the extract. This research aligned the synthesis of CeO₂NPs using *Portulaca oleracea* extract with sizes ranging from 4 nm to 14 nm (Hkiri *et al.*, 2024).

Antibacterial Activity

The particle size of nanomaterials affects antibacterial activity. The particle size of the cerium oxide nanoparticles is smaller when using *Moringa oleifera* leaf extract as a capping agent. Based on this condition,

cerium oxide was synthesized using *Moringa oleifera* leaf extract as CeO₂NPs for antibacterial activity. These nanoparticles are used to test antibacterial properties against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*).

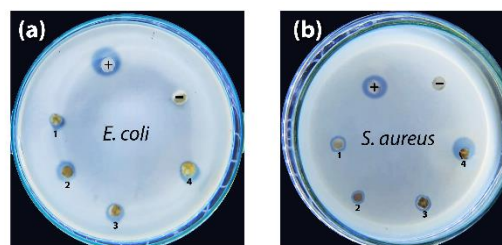


Fig. 4: Antibacterial activity of CeO₂NPs with varying concentrations, 25% (1), 50% (2), 75% (3), and 100% (4) against (a) *E. coli* and (b) *S. aureus*.

The antibacterial activity of synthesized cerium oxide nanoparticles (CeO₂NPs) was assessed against *Escherichia coli* and *Staphylococcus aureus* using the disc diffusion method. Results, as indicated in Table 2, showed a concentration-dependent antibacterial effect, with inhibition zones for *E. coli* ranging from 4.23 ± 0.6 mm at a 25% concentration to 8.46 ± 0.6 mm at a 100% concentration. For *S. aureus*, zones increased from 4.50 ± 0.2 mm to 11.05 ± 0.3 mm as concentration rose, showing that higher concentrations are more effective.

CeO₂NPs exhibited greater antibacterial activity against *S. aureus* compared to *E. coli*, particularly at 100%, likely due to differences in cell wall structures. The positive control (standard antibiotic) produced inhibition zones of 12.45 ± 0.3 mm for *E. coli* and 13.56 ± 0.5 mm for *S. aureus*, respectively.

The antibacterial activity of CeO₂NPs is partly due to having two oxidation states, Ce³⁺ and Ce⁴⁺, so that it can produce hydroxyl radicals, which can damage the bacterial cell wall, disrupt cell metabolism, and inhibit

bacterial cell synthesis. Cerium oxide nanoparticles have antibacterial activity due to their large surface area, which allows for excellent contact with microorganisms. Cerium oxide nanoparticles approach the bacterial cell membrane and penetrate the bacteria. The redox state of the Ce^{3+} and Ce^{4+} nanoparticles diffuses and attacks the bacterial respiratory chain, ultimately leading to cell death. The antibacterial properties of Ce^{3+} and Ce^{4+} nanoparticles will be very helpful in overcoming various health problems (Eka Putri *et al.*, 2018; Gusliani Eka Putri *et al.*, 2019; You *et al.*, 2023).

Table 2. The zone of Inhibition of CeO_2NPs against *Escherichia coli* and *Staphylococcus aureus* bacteria

Samples	Zone of Inhibition (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
Negative Control	-	-
Positive Control	12.45 ± 0.3	13.56 ± 0.5
CeO_2NPs 25% (1)	4.23 ± 0.6	4.50 ± 0.2
CeO_2NPs 50% (2)	6.50 ± 0.5	5.86 ± 0.6
CeO_2NPs 75% (3)	7.28 ± 0.4	7.40 ± 0.5
CeO_2NPs 100% (4)	8.46 ± 0.6	11.05 ± 0.3

CONCLUSIONS

Cerium oxide nanoparticles (CeO_2NPs) were successfully synthesized using aqueous extracts from the leaves of *Moringa oleifera* and *Uncaria gambir* Roxb. as eco-friendly capping and reducing agents. Structural and morphological analyses confirmed the formation of spherical and moderately agglomerated CeO_2NPs . The biosynthesized nanoparticles demonstrated significant antibacterial activity, with inhibition zones measuring up to 11.05 mm against *Staphylococcus aureus* and 8.46 mm against *Escherichia coli*, showcasing their effective antimicrobial properties. The use of dual

plant extracts in this green synthesis approach highlights the novelty of this research, the potential of CeO_2NPs as promising candidates for future biomedical applications, particularly in antimicrobial formulations.

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