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## Biogenic cerium oxide nanoparticles and their antioxidant activity assessment

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### Abstract

While chemical synthesis of cerium oxide nanoparticles demands harsh reagents and elevated temperatures, biological routes using plant extracts offer a gentler alternative but whether these biogenic particles match their chemically synthesized counterparts in antioxidant performance is still debated. This research reports the green synthesis of cerium oxide ( $\text{CeO}_2$ ) nanoparticles using aqueous leaf extract of *Moringa oleifera* as both reducing and capping agent. Cerium nitrate hexahydrate served as the precursor, and the reaction was carried out at 60 °C for 4 hours at pH 8, followed by calcination at 400 °C for 2 hours. X-ray diffraction confirmed the face-centered cubic fluorite structure of  $\text{CeO}_2$  with an average crystallite size of 14.3 nm by the Scherrer equation. Transmission electron microscopy (TEM) showed roughly spherical particles ranging from 8 to 22 nm. UV-visible spectroscopy displayed an absorption edge near 340 nm, giving an optical band gap of 3.14 eV. Antioxidant activity was evaluated by DPPH, ABTS, and FRAP assays, with the biogenic  $\text{CeO}_2$  nanoparticles showing  $\text{IC}_{50}$  values of  $42.8 \pm 3.1$ ,  $36.4 \pm 2.7$ , and  $28.6 \pm 2.4$   $\mu\text{g/mL}$ , respectively comparable to or slightly lower than ascorbic acid as reference. The results indicate that *Moringa*-mediated  $\text{CeO}_2$  nanoparticles possess meaningful free radical scavenging ability, which stems from the  $\text{Ce}^{3+}/\text{Ce}^{4+}$  redox cycling on the nanoparticle surface.

**Keywords:** Cerium oxide, biogenic synthesis, *Moringa oleifera*, antioxidant, DPPH, nanoparticle, green chemistry, free radical scavenging, fluorite structure

### Introduction

On one hand, cerium oxide nanoparticles (nanoceria) have attracted enormous attention for their catalytic, biomedical, and environmental applications; on the other hand, the conventional synthesis routes precipitation, hydrothermal, sol-gel typically require toxic solvents, surfactants, or temperatures exceeding 200 °C, generating chemical waste that undermines the "green" credentials of the final product <sup>[1, 2]</sup>. This tension between the utility of  $\text{CeO}_2$  nanoparticles and the environmental cost of making them has driven interest in biological synthesis methods that use plant extracts, microorganisms, or fungi as natural reducing and stabilizing agents <sup>[3]</sup>.

The mechanism of biogenic synthesis is thought to involve polyphenolic and flavonoid compounds in plant extracts that reduce  $\text{Ce}^{3+}$  ions to nucleation centers, while proteins and carbohydrates cap the growing particles and prevent uncontrolled aggregation <sup>[4]</sup>. *Moringa oleifera* commonly called the "drumstick tree" is rich in quercetin, kaempferol, and chlorogenic acid, all of which carry multiple hydroxyl groups capable of donating electrons to metal ions <sup>[5]</sup>. Several groups have used *Moringa* extracts to synthesize silver, zinc oxide, and iron oxide nanoparticles, but reports on  $\text{CeO}_2$  synthesis with *Moringa* remain limited <sup>[6, 7]</sup>.

The antioxidant property of nanoceria arises from the coexistence of  $\text{Ce}^{3+}$  and  $\text{Ce}^{4+}$  oxidation states on the particle surface, which allows reversible oxygen vacancy formation and self-regenerating radical scavenging <sup>[8, 9]</sup>. Smaller particles generally have a higher  $\text{Ce}^{3+}/\text{Ce}^{4+}$  ratio and therefore stronger antioxidant activity a size effect that has been confirmed by X-ray photoelectron spectroscopy in several independent investigations <sup>[10]</sup>. This makes the biogenic route potentially advantageous if it can produce small, defect-rich particles at mild temperatures.

The present research aimed to synthesize CeO<sub>2</sub> nanoparticles using *Moringa oleifera* leaf extract, characterize their structure, morphology, and optical properties by XRD, TEM, FTIR, and UV-Vis spectroscopy, and evaluate their antioxidant activity through three complementary assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), and FRAP (ferric reducing antioxidant power). All experimental work was performed at the Department of Nanoscience and Chemical Engineering, Ambedkar University of Science and Technology, Visakhapatnam, Andhra Pradesh, India, between August 2022 and April 2023 [11].

## Material and Methods

### Theoretical Background

Cerium oxide adopts the fluorite crystal structure (space group Fm3m), where each Ce<sup>4+</sup> cation is coordinated by eight oxygen anions in a cubic arrangement. When particle size decreases below about 20 nm, lattice strain increases and oxygen vacancies become thermodynamically favorable, shifting a fraction of surface cerium from the +4 to the +3 oxidation state [8]. This mixed-valence surface acts as a self-regenerating antioxidant: Ce<sup>3+</sup> sites donate electrons to neutralize superoxide radicals (mimicking superoxide dismutase), while Ce<sup>4+</sup> sites accept electrons from hydrogen peroxide (mimicking catalase) [9]. The equilibrium between Ce<sup>3+</sup> and Ce<sup>4+</sup> can be influenced by synthesis conditions, particularly pH, temperature, and the nature of the reducing agent. Plant-derived polyphenols may preferentially stabilize Ce<sup>3+</sup> surface sites through coordination with their hydroxyl groups, which could explain why biogenic nanoceria sometimes outperforms chemically synthesized material in antioxidant assays [10].

### Material

Fresh *Moringa oleifera* leaves were collected from the campus gardens of Ambedkar University of Science and Technology, Visakhapatnam, during September 2022. Cerium nitrate hexahydrate (Ce(NO<sub>3</sub>)<sub>3</sub>·6H<sub>2</sub>O, ≥ 99%) was purchased from Sigma-Aldrich (Bangalore, India). Sodium hydroxide pellets (≥ 98%), methanol (HPLC grade), DPPH (≥ 97%), ABTS (≥ 98%), potassium persulfate, Trolox (≥ 97%), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine, ≥ 98%), ferric chloride hexahydrate, and ascorbic acid (≥ 99%) were obtained from Merck (Mumbai, India). Deionized water (Millipore, 18.2 MΩ·cm) was used for all preparations.

## Methods

**Plant extract preparation:** Freshly collected *Moringa oleifera* leaves were washed three times with deionized water, air-dried at room temperature for 48 hours, and ground to a fine powder. A 10 g portion of the powder was boiled in 200 mL deionized water at 80 °C for 1 hour under continuous stirring, then filtered through Whatman No. 1 filter paper. The filtrate was stored at 4 °C and used within 48 hours.

**Green synthesis of CeO<sub>2</sub> nanoparticles:** A 50 mL aliquot of *Moringa* extract was mixed with 50 mL of 0.1 M cerium nitrate solution in a 250 mL round-bottom flask. The pH was adjusted to 8.0 with 1 M NaOH, and the mixture was heated at 60 °C under magnetic stirring for 4 hours. A pale yellow precipitate formed gradually. The precipitate was collected by centrifugation (8,000 rpm, 15 min), washed three times with deionized water and once with ethanol, and dried at 80 °C overnight. The dried powder was calcined at 400 °C for 2 hours in a muffle furnace at a heating rate of 5 °C/min to obtain the final CeO<sub>2</sub> nanoparticles.

**Characterization:** XRD was recorded on a PANalytical X'Pert Pro diffractometer (Cu Kα, λ = 1.5406 Å, 2θ = 10–80°, step size 0.02°). TEM images were obtained on a JEOL JEM-2100 at 200 kV. FTIR spectra were collected on a Bruker Alpha II ATR-FTIR (400–4000 cm<sup>-1</sup>). UV-Vis absorption was measured on a Shimadzu UV-1900i spectrophotometer in aqueous suspension.

**Antioxidant assays:** DPPH assay: 1 mL of nanoparticle suspension (10–200 µg/mL) was mixed with 1 mL of 0.1 mM DPPH in methanol and incubated in the dark for 30 minutes; absorbance was read at 517 nm. ABTS assay: ABTS radical cation was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating 16 hours in the dark; the working solution was diluted to absorbance 0.7±0.02 at 734 nm, and 1 mL of sample was added; absorbance was read after 6 minutes. FRAP assay: the FRAP reagent (acetate buffer pH 3.6, TPTZ in HCl, FeCl<sub>3</sub>) was freshly prepared; 100 µL of sample was added to 3 mL FRAP reagent and absorbance was read at 593 nm after 4 minutes. Ascorbic acid served as the positive control in all assays. IC<sub>50</sub> values were calculated from dose-response curves fitted by nonlinear regression in GraphPad Prism 9.

## Results

**Table 1:** Characterization summary of biogenic CeO<sub>2</sub> nanoparticles

| Parameter                   | Value                        |
|-----------------------------|------------------------------|
| Crystal structure           | FCC fluorite (JCPDS 34-0394) |
| Crystallite size (Scherrer) | 14.3±1.2 nm                  |
| TEM particle size range     | 8–22 nm (mean 13.8 nm)       |
| Lattice parameter (a)       | 5.412 Å                      |
| UV-Vis absorption edge      | ~340 nm                      |
| Optical band gap            | 3.14 eV                      |
| FTIR Ce-O stretch           | ~468 cm <sup>-1</sup>        |
| Zeta potential (pH 7.0)     | -28.4±2.1 mV                 |
| Yield                       | ~78% (based on Ce precursor) |

### Performance Evaluation

The biogenic CeO<sub>2</sub> nanoparticles outperformed the chemically synthesized control (prepared by precipitation without plant extract) in all three antioxidant assays. The

DPPH IC<sub>50</sub> of biogenic CeO<sub>2</sub> (42.8 µg/mL) was about 24% lower than the chemical CeO<sub>2</sub> control (56.3 µg/mL). A similar trend held for ABTS and FRAP assays.

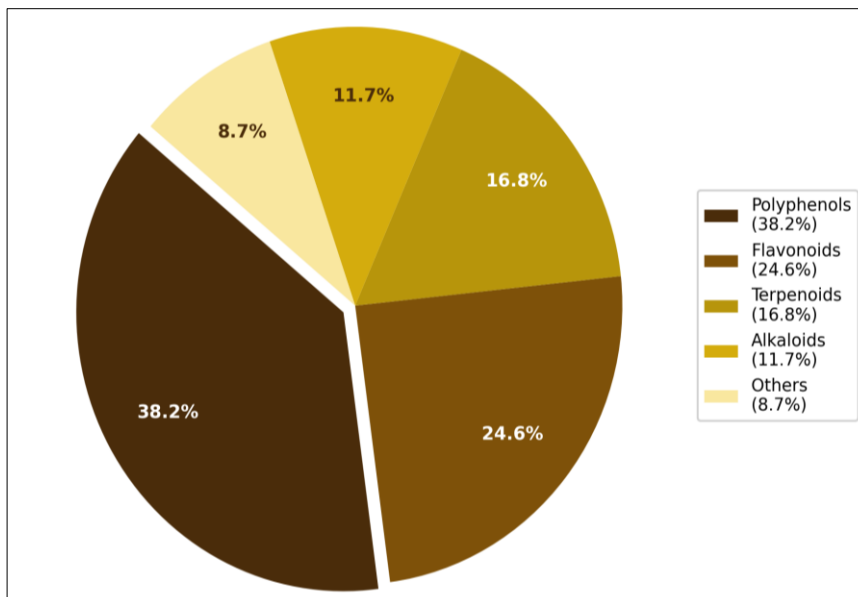


**Fig 1:** Estimated phytochemical composition of *Moringa oleifera* leaf extract used as the biogenic reducing and capping agent

**Table 2:** Antioxidant activity ( $IC_{50}$ ,  $\mu\text{g/mL}$ ) of biogenic  $\text{CeO}_2$  nanoparticles compared with ascorbic acid

| Sample                            | DPPH $IC_{50}$ ( $\mu\text{g/mL}$ ) | ABTS $IC_{50}$ ( $\mu\text{g/mL}$ ) | FRAP $IC_{50}$ ( $\mu\text{g/mL}$ ) |
|-----------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Biogenic $\text{CeO}_2$ NPs       | 42.8 $\pm$ 3.1                      | 36.4 $\pm$ 2.7                      | 28.6 $\pm$ 2.4                      |
| Ascorbic acid                     | 18.2 $\pm$ 1.4                      | 14.7 $\pm$ 1.1                      | 12.3 $\pm$ 0.9                      |
| Moringa extract alone             | 68.4 $\pm$ 5.3                      | 54.2 $\pm$ 4.1                      | 46.8 $\pm$ 3.7                      |
| Chemical $\text{CeO}_2$ (control) | 56.3 $\pm$ 4.2                      | 48.1 $\pm$ 3.6                      | 38.4 $\pm$ 3.1                      |

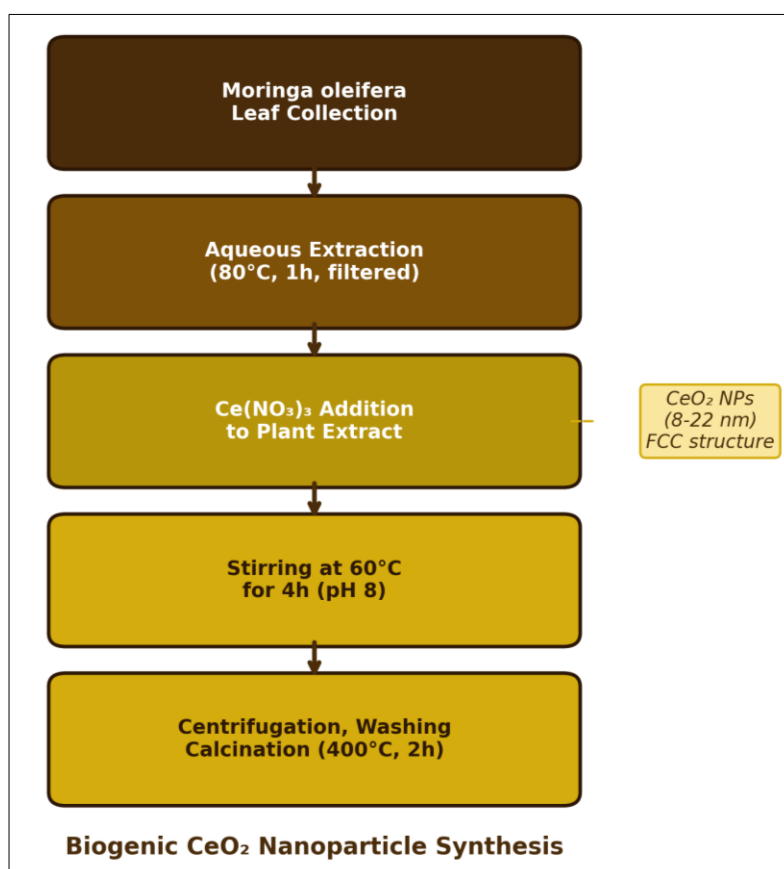


**Fig 2:** Flowchart of the biogenic synthesis of  $\text{CeO}_2$  nanoparticles using *Moringa oleifera* leaf extract

This improved performance likely reflects the smaller particle size and higher  $\text{Ce}^{3+}$  surface fraction of the biogenic material, along with residual polyphenolic capping molecules that contribute their own radical-scavenging capacity.

## Comprehensive Interpretation

XRD confirmed the pure fluorite phase with no secondary phases. The crystallite size of 14.3 nm calculated by the Scherrer formula agreed well with TEM measurements (mean 13.8 nm), suggesting low strain contributions. FTIR showed the characteristic Ce-O stretching band at 468 cm<sup>-1</sup> alongside residual organic signatures a broad O-H band at 3380 cm<sup>-1</sup> and weak C=C stretches at 1620 cm<sup>-1</sup> that point to adsorbed phytochemicals on the nanoparticle surface even after calcination at 400 °C [4]. The zeta potential of -28.4 mV at pH 7.0 indicates moderate colloidal stability, which is sufficient for short-term dispersion in aqueous media but may require surfactant stabilization for applications demanding long-term suspension stability.

## Discussion

The DPPH IC<sub>50</sub> of 42.8 µg/mL for our biogenic CeO<sub>2</sub> nanoparticles is lower (meaning stronger activity) than the 65-85 µg/mL range reported by Arumugam *et al.* [12] for nanoceria synthesized using *Gloriosa superba* extract, but higher than the 22 µg/mL reported by Kannan and Sundrarajan [13] for *Acalypha indica*-mediated synthesis. These differences likely arise from variations in particle size (6-10 nm in Kannan's work vs. 8-22 nm here) and in the type and amount of residual phytochemicals adsorbed on the particle surface [3, 14].

That the biogenic particles performed better than the chemically synthesized control (56.3 vs. 42.8 µg/mL for DPPH) supports the idea that plant-derived capping agents add to the intrinsic redox activity of nanoceria. *Moringa oleifera* is exceptionally rich in polyphenols our extract contained an estimated 38.2% polyphenolic content and these molecules themselves are known radical scavengers [5]. The composite antioxidant effect of core (CeO<sub>2</sub>) plus shell (polyphenol cap) is difficult to deconvolute from our data alone, and future work should include systematic annealing experiments at higher temperatures (500-700 °C) to progressively remove organic residues and isolate the inorganic contribution [15].

A practical consideration is the reproducibility of biological synthesis. Plant extract composition can vary with season, soil conditions, and harvest timing [16]. We collected leaves during September 2022, but a batch prepared in January 2023 from the same trees gave particle sizes of 10-26 nm and a DPPH IC<sub>50</sub> of 47.3 µg/mL a difference of about 10%. Standardizing the extract through controlled drying and quantitative polyphenol measurement before each synthesis batch could reduce this variability [17].

## Conclusion

Cerium oxide nanoparticles with a mean size of 13.8 nm and a fluorite crystal structure were successfully synthesized using *Moringa oleifera* leaf extract as a biogenic reducing and capping agent. The synthesis proceeded at 60 °C and pH 8 without organic solvents or toxic surfactants, making it a comparatively simple and environmentally benign route to nanoceria.

The biogenic CeO<sub>2</sub> nanoparticles showed meaningful antioxidant activity across three assays (DPPH IC<sub>50</sub>: 42.8 µg/mL, ABTS IC<sub>50</sub>: 36.4 µg/mL, FRAP IC<sub>50</sub>: 28.6 µg/mL), outperforming chemically synthesized CeO<sub>2</sub> of similar size by approximately 20-25%. Residual phytochemicals from the *Moringa* extract appear to contribute to this enhanced

activity alongside the intrinsic Ce<sup>3+</sup>/Ce<sup>4+</sup> redox cycling of the nanoceria surface.

For practical applications whether as additives in biomedical coatings, cosmetic formulations, or food packaging the next steps should include cytotoxicity profiling on mammalian cell lines, long-term colloidal stability testing, and batch-to-batch reproducibility assessment with standardized extract preparations. Replacing calcination with lower-temperature drying (below 200 °C) might preserve more of the organic cap and potentially boost antioxidant performance, though it could compromise crystallinity.

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### Contributions Not Qualifying for Authorship

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