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RESEARCH ARTICLE

Bio-Synthesis of Cerium Oxide Nanoparticles from *Coriandrum sativum L.* Leaf Extract and their Antibacterial Activity

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ABSTRACT: The present work focuses on the green synthesis of Cerium Oxide nanoparticles using aqueous solution of *Coriandrum sativum* leaf extract. Cerium nitrate is used as precursor for the preparation and *Coriandrum sativum* leaf extract is used as an effective reducing agent. The optical properties of biosynthesized CeO_2 nanoparticles are characterized by UV-Vis spectroscopy which shows the surface plasmon resonance at 276 nm. The Fourier transform infrared spectroscopy (FT-IR) is used to identify the functional groups of bio-molecules present in the samples. The results of X-ray diffraction (XRD) study revealed that most of the nanoparticles are cubic fluorite structure and grains are crystalline in nature. The study of SEM images shows that as-prepared CeO_2 nanoparticles are spherical and fluorite in shape with slight agglomeration and EDAX spectroscopy confirms the presence of Ce and O in the samples with high purity. The antibacterial activity was studied with gram-negative and gram-positive bacteria such as *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. The gram-positive *Bacillus subtilis* shows the minimum zone of inhibition when compared with gram-negative.

KEYWORDS: Cerium Oxide nanoparticles, *Coriandrum sativum*, Antibacterial activity

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1. INTRODUCTION

Cerium oxide (CeO_2) is an important rare-earth metal oxide processes interesting applications in various areas of research [1-5]. Ceria (CeO_2) has a fluorite-like cubic structure in which each cerium site is surrounded by eight oxygen sites in face-centered cubic (FCC) arrangement and each oxygen site has a tetrahedron cerium site. In the nanoparticles research, various chemical methods have been employed to synthesize CeO_2 nanoparticles such as hydrothermal, mechanochemical, sonochemical, combustion synthesis, sol-gel, semi-batch reactor, microemulsion and spray-pyrolysis [6, 7]. The present study deals with biosynthesis of CeO_2 nanoparticles using *Coriandrum sativum* leaves, as the method of preparation is simple, environmentally friendly and cost effective.

Several research works have been reported on biosynthesis of nanoparticles using *Coriandrum sativum* as a reducing agent. In this, Asma Ashraf et al [8] reported the synthesize of silver nanoparticles using *Coriandrum sativum* and studied the antibacterial properties in a couple of gram negative and a couple of gram positive bacteria in the presence of antibiotic viz. gentamicin to judge their impact. Khan et al [9] reported the green synthesis and characterization of silver nanoparticles using *Coriandrum sativum* leaf extract. Balamurugan and Saminathan [10] studied the characterization of *Coriandrum sativum* mediated silver nanoparticles and evaluation of its antimicrobial and wound healing activity. Moreover, Badrinarayanan and Sakthivel [11] extracted the extracellular biosynthesis of gold nanoparticles from coriander leaf for large scale commercial production. Surya Pratap Goutam et al [12] synthesized Coriander extract mediated Zinc oxide nanoparticles and studied their structural, optical and antibacterial properties. Vaudeo Kulkarni et al [13] recommended that Coriander leaf extract is an efficient biocatalyst for synthesis of copper nanoparticles. Alya O. AlQuraidi et al [14] confirmed the Phytotoxic and Genotoxic Effects of Copper Nanoparticles in Coriander using RAPD. Rathore et al, [15] evaluated the extracellular one pot green synthesis of Palladium nanoparticles seed extract of *Coriandrum sativum* for antimicrobial activity.

In our laboratory, we have successfully synthesized the silver nanoparticles using leaf extracts of plants like *Carica papaya* [16], *Psidium guajava* [17], *Pongamia pinnata* [18] as a reducing agent. Based on our experience, we have synthesized CeO_2 nanoparticles using *Coriandrum sativum* leaves extract as reducing agent. *Coriandrum sativum* is an annual plant that belongs to family *Apiaceae*, Genus-*Coriandrum L.*, Species-*Coriandrum sativum L.* Coriander originates from Southern Europe and Northern Africa to Southwestern Asia. All parts of the Coriander are edible. The presence of protein level is found to be higher in leaves of *Coriandrum sativum* than in seeds and also possess high amount

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of calcium, iron, magnesium, phosphorus, sodium, zinc, vitamin–C, thiamine, riboflavin, niacin in leaves [19]. In spite of the importance, the present study has been undertaken to synthesis Cerium oxide nanoparticles by a rapid and eco-friendly biosynthesis method from fresh leaves of *Coriandrum sativum* and to examine the potential role of *Coriandrum sativum* leaf extract as antimicrobial agent for Gram positive bacteria such as *Bacillus subtilis* and *Bacillus cereus* and Gram negative bacteria such as *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

2. MATERIALS AND METHODS

2.1. Materials

The chemicals used in this present study are of AR grade with 99% purity and used without further purification. To synthesize Cerium oxide nanoparticles (CeO_2-NPs), the Cerium nitrate hexahydrate ($Ce(NO_3)_3.6H_2O$) and acetone were obtained from Merck. All the glasswares were used in this experiment was washed with acid followed by distilled water.

2.2 Preparation of leaf extract

The fresh leaf of *Coriadrum sativam* as shown in figure 1 was procured from local market and washed several times with double distilled water in order to remove the dust particles. 10 g of leaves was taken in a 250 ml Erlenmeyer flask and boiled in 100 ml double distilled water for 20 min. The leaf extract was filtered with Whatmann No.1 filter paper and stored at -4° C for further use.

2.3. Biosynthesis of CeO_2 nanoparticles

In this method, 0.5 M of Cerium nitrate hexahydrate was taken in 30 ml of deionized water and stirred constantly for 1 hr and then 0.25 % of NaOH was dissolved with 20 ml of deionized water and added drop by drop to the stirring solution. After 2 hrs, gel solution will be formed and then 20 ml of leaf extract was added into the solution and stirred at 80 °C for 2 hrs to get the brownish precipitate. The precipitate was kept for 24 hrs and washed 2 to 3 times using ethanol and annealed for 3 hrs at 700° C to get the final product.

2.4 Antibacterial assay

The antibacterial activities of leaf extract were carried out by well diffusion method. The concentrations of the test compounds were taken in DMSO and used in the concentration of 25, 50, 75 and 100 μg. The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain. Well made of diameter 6 mm was pre-sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile well papers. Then the prepared wells were placed on the culture medium. Standard drug streptomycin (10μg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 ºC for 24 h. The diameter of the clear zone around the well was measured and expressed in millimeters as its anti-bacterial activity.

2.5. Characterization Methods

The optical absorption spectra of the samples were recorded by UV-visible spectrometer. The functional groups were identified by FT-IR spectroscopy. Using X pert PRO diffractometer with a CuKα radiation (Kα = 1.5406 A°) the X-ray diffraction (XRD) patterns of the prepared samples was recorded. The size and morphology of the nanoparticles were analyzed using scanning electron microscopy (SEM) and EDAX for elemental analysis of the prepared sample.

3. RESULTS AND DISCUSSION

3.1 UV-Vis spectroscopy

UV-visible absorption spectral study is helpful in understanding electronic structure of the optical band gap of the material. Absorption in the near ultraviolet region arises from electronic transitions associated within the sample. The results of UV–Vis absorption spectra of CeO_2 nanoparticles are shown in Figure 2. From the figure, it can be seen that there is a strong absorption band at low wavelength near 276 nm that corresponds to band gap energy of 3.26 eV. The UV absorption ability of CeO_2 is related to band gap energy. The UV-absorption edge provides a reliable estimate of the band gap of any system.

3.2 Fourier-transform infrared spectroscopy

FT-IR spectroscopy is used to identify the functional groups of the samples and to study the molecular vibration motion of atoms and molecules. The FT-IR spectra of CeO_2 nanoparticles using *Coriander sativum* leaf extract is reported in Figure 3. The spectra have several significant absorption peaks recorded in the range from 4000-400 cm^{-1}. The absorption broad peak at 3465 cm^{-1} is assigned to OH stretching vibrations of H_2O [6]. 2887cm-1 is attributed to CH_2 stretching**.** The peak observed near 2330 cm^{-1} is due to the C-H stretching [20]. The absorption band at 1635 cm^{-1} is due to the scissor bending mode of associated water [7]. The absorption is peak around 1450 cm^{-1} is assigned to the bending vibration of C-H stretching. The band at 848 cm-1 corresponds to metal–oxygen bond [7]. The spectra observed at 2979, 1565, 1388, 1255, 1152, 1071 cm^{-1} is a type of functional group absorption of organic compounds that indicate the presence of impurities. Impurities are nothing but unwanted residues of leaf extract [21].

3.3 X - ray Diffraction (XRD)

Figure 4 shows the XRD pattern of biosynthesized CeO_2-NPs using *Coriandrum sativum* leaf extract. The exhibited peaks correspond to the hkl values of (111), (200), (220), (311), (222), (400), (331), (420), (422) and (511) with cubic fluorite structure. These diffraction peaks are identified as Cerium oxide nanoparticles, which is consistent with the JCPDS card no. 89-8436. The mean size of the CeO_2 nanoparticles has been estimated from Debye-Sherrer's formula according to following equation:

D=0.89λ/β cosθ, Where, 0.89 is the shape factor, λ is the x-ray wavelength, β is the line broadening at Full width at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle. The grain size of as-prepared CeO_2 nanoparticles is 13.56 nm from Debye-Sherrer's equation.

The reduction in the grain size of CeO_2-NPs is mainly due to the presence of biomolecules in the leaf extract involved in the formation of nanoparticles which decrease the nucleation and subsequent growth rate of the CeO_2 nanoparticles.

Figure 1 Coriandrum sativum leaves

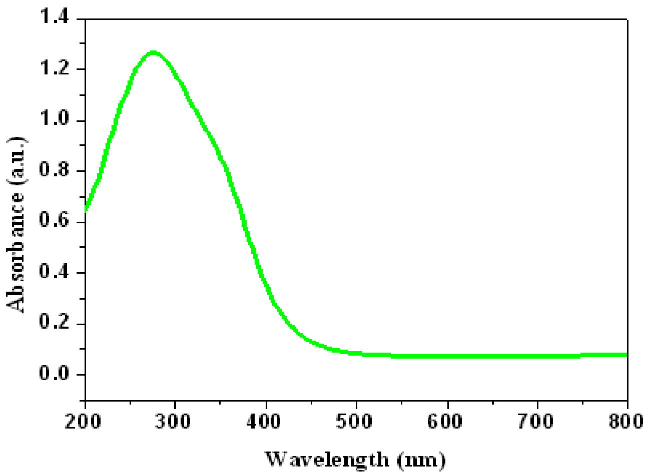

Figure 2 UV-Vis spectra of the biosynthesized CeO_2 nanoparticles using *Coriander Sativum* leaf extract.

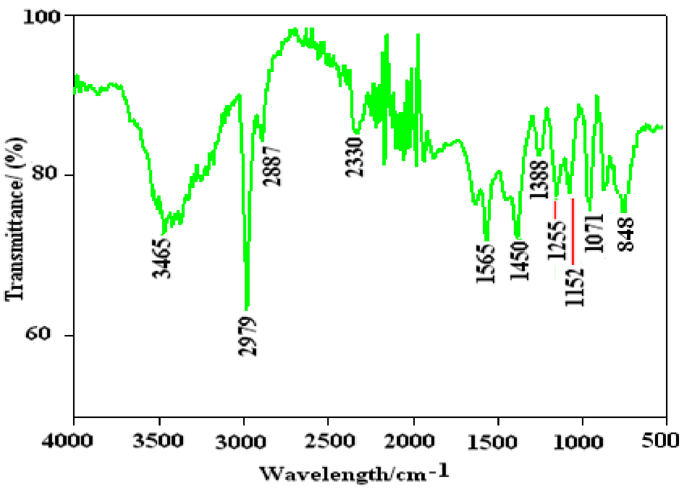

Figure 3 FT-IR spectra of the biosynthesized CeO_2 nanoparticles using *Coriander sativum* leaf extract.

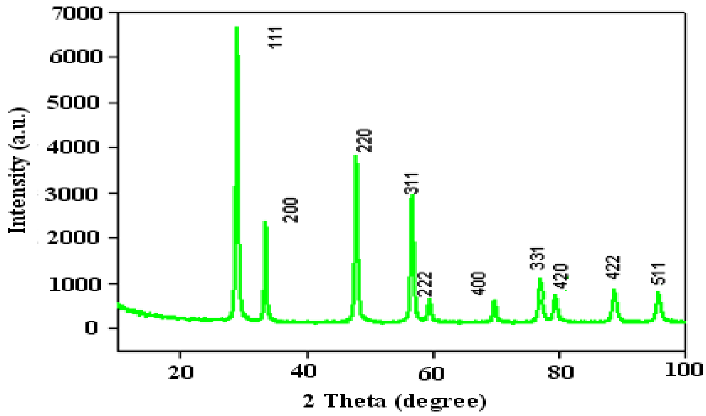

Figure 4 XRD patterns of the biosynthesized CeO_2 nanoparticles using *Coriander Sativum* leaf extract.

Table 1 Elemental analysis of CeO_2 Nanoparticles

Element	**Line**	**Mass%**	**Atom%**
O	K	5.37±0.07	33.18±0.41
Ce	L	94.63±0.46	66.82±0.32
Total		100.00	100.00

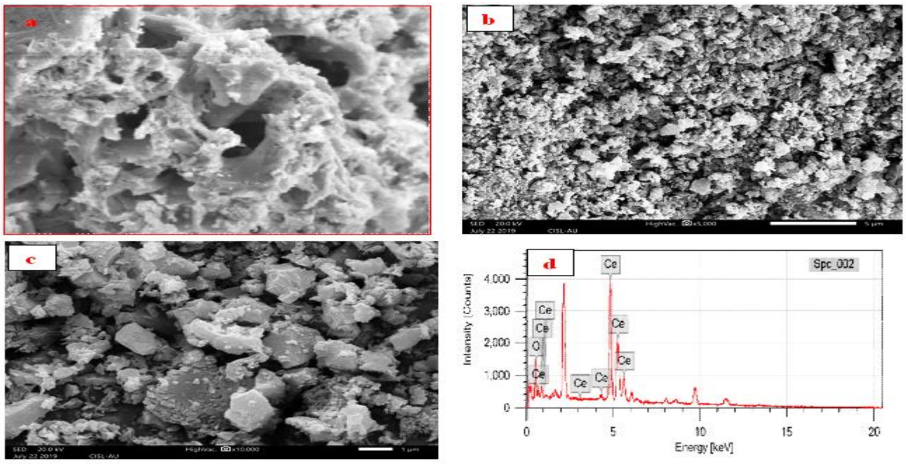

Figure 5 SEM with EDX image of the Biosynthesized CeO_2 nanoparticles using *Coriander Sativum* leaf extract.

3.2 SEM with EDS

Figure 5 shows the SEM images of as-prepared CeO_2 nanoparticles with slight agglomeration. It can be seen that the size of CeO_2 decreases with increasing leaf extract concentration and the uniformity of size increases with increasing temperature. Further elemental analysis was carried out using EDAX spectroscopy as shown in Figure 5(d) which shows the presence of Ce and O in the samples with high purity which is consistent with the starting material composition.

3.5. Antibacterial activity of CeO_2 nanoparticles

The CeO2 nanoparticles showed inhibitory effect against the growth of both Gram negative and Gram positive bacteria. The inhibition zone for the Gram positive bacteria of *Bacillus subtilis* showed 6 and Bacillus *cereus* showed 5 mm, when compared with that of streptomycin, the positive control, which shows 27 mm and 25 mm. The inhibition zone for the Gram negative bacteria of *Klebsiella pneumonia* showed 9 mm and Pseudomonas *aeruginosa* showed 12 mm, whereas the standard drug, streptomycin, exhibited zone of inhibition is 28 mm and 32 mm. The gram-positive bacteria show minimum zone of inhibition when compared to gram-negative. This is because of the cell wall in gram positive bacteria is composed of a thick peptidoglycon layer consisting of linear polysaccharide chain cross linked by short peptides forming more rigid structure as a result; it was difficult for the nanoparticles to penetrate the cell wall. However, in gram-negative bacteria, the cell wall possesses thinner peptidoglycon layer, where the nanoparticle can easily penerate. The CeO_2 nanoparticles penetrate into the cell membrane and bound with the electron donor functional groups present in DNA and disrupt the function of DNA which cannot replicate and leads to the cell dead [22].

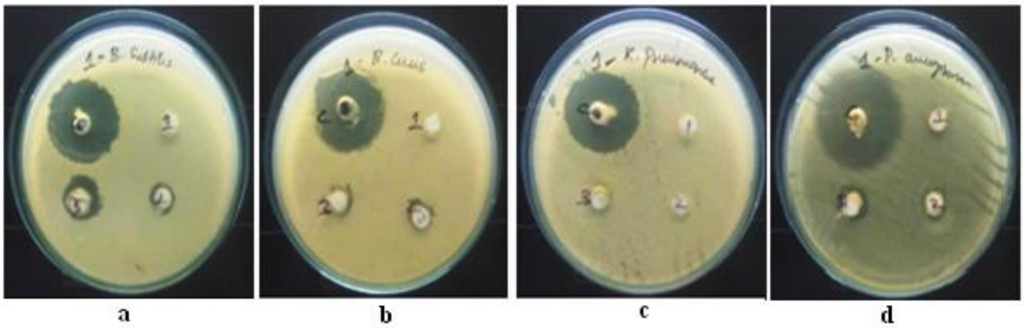

Figure 6 Well diffusion method containing CeO_2 nanoparticles for a) *Bacillus subtilis* b) *Bacillus cereus* c) *Klebsiella pneumonia* d) *Pseudomonas aeruginosa* using *Coriander sativum* leaf extract for Antibacterial activities

Table 2 Zone of inhibition of biosynthesized CeO_2 Nanoparticles for Gram positive and Gram negative bacteria

Sample	Concentration	Zone of Inhibition (mm)			
		Gram positive bacteria		Gram negative bacteria	
		Bacillus subtilis	*Bacillus cereus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
1	10µl (50 µg)	1	-	1	-
1	25 µl (125 µg)	3	2	6	5
1	50 µl (250 µlg)	6	5	9	12
Control (Streptomycin)	25 µl (25 µg)	27	25	28	32

4. CONCLUSION

In summary, Cerium oxide nanoparticles have been successfully synthesized via green synthesis in aqueous solution of *Coriandrum sativum* leaf extract. The Ceria nanoparticles show a strong UV-vis absorption below 400 nm with a well-defined absorption peak at 276 nm. FTIR data reveals the presence of Ce-O stretching mode of CeO_2 which is in correlation with XRD datas. XRD spectra shows cubic fluorite structure of CeO_2 is crystalline and the grain size is observed at 13.56 nm. SEM images show the prepared CeO_2 nanoparticles with slight agglomeration due to biomass of leaf. EDAX shows the presence of Ce and O in the samples with high purity. The antibacterial activity of CeO_2 shows the highest zone of inhibition for *Bacillus cereus* with 12 mm. This work aimed to report the green synthesis of CeO_2 NPs for the economic way in reducing environmental pollution.

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