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Biosynthesis of Cerium Oxide Nanoparticles by Fungus *Trichoderma viridae*

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Abstract

Fungi have emerged as an innovative method synthesis of nanoparticles. In the present study, we have reported the synthesis of Cerium oxide nanoparticles (CeO_2 NPs) using *Trichoderma viridae* culture filtrate. The nanoparticles were characterized by UV–Visible spectrometry, X-ray diffraction (XRD), Scanning electron Microscopy (SEM), Fourier transform Infrared Spectrometry (FT-IR) and dynamic light scattering (DLS) analyses. UV–Vis spectral analysis reveals the formation of CeO_2 NPs and the functional groups present in the fungal filtrate responsible for the synthesis of NPs were analysed by FT-IR. The XRD, SEM and DLS analysis confirmed the cubic structure of CeO_2 NPs and spherical shape, with the particle size of around 68nm. The high negative zeta potential value and its free radical scavenging activity indicate the stability and potent antioxidant property of the synthesized nanoparticles. The synthesis of CeO_2 NPs utilising fungi is a simple, cost-effective and green approach and further studies have to carry out to understand its efficacy in preventing oxidative stress related diseases.

Keywords

Antioxidant, cerium oxide nanoparticles, XRD, *Trichoderma viridae*.

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INTRODUCTION

Cerium oxide is a rare earth oxide which belongs to the lanthanide series of the periodic table. Cerium oxide nanoparticles (CeO_2 NPs) has two oxidation states (+3 and +4) which can be transformed to each other depending on the surrounding pH [1]. It is used in a wide range of applications such as, sensor, solid oxide fuel cells, catalyst, sun-screen cosmetics, O_2 storage capacity applications due to their exclusive ability to switch oxidation states [2]. Numerous studies have demonstrated the antioxidant, anticancer and neuroprotective properties of CeO_2 NPs [3-5].

The recent trend in the synthesis of nanoparticles which have improved therapeutic properties as well as being environment-friendly.

Nanoparticles synthesis using biological sources eliminates the need of employing toxic chemicals as reducing and capping agents and also offers cost-effective method of nanoparticle synthesis [6]. Nanoparticles are synthesized using numerous biological sources including plant, bacteria, fungus, yeast, and viruses [7]. Fungus mediated synthesis of nanoparticles is a preferred method compared to other sources due to capable of extracellular

synthesis of nanoparticles, which helps in easy downstream processing and biomass handling [8]. Studies have focused on the synthesis of CeO2 NPs using Aspergillus niger, [9] which are pathogenic to humans and plants. The handling and disposal of the biomass is problematic and is not advisable [10]. Hence, non-pathogenic fungus was selected for the biosynthesis of CeO2 NPs.

Numerous non-pathogenic fungi of the Trichoderma species are established biocontrol agents and their biomass is exploited in different commercial formulations for different agricultural applications [11]. Based on the above facts, the present study was aimed to synthesize and characterize biocompatible CeO2 NPs using Trichoderma viridae and their antioxidant activity.

MATERIALS AND METHODS

Chemicals and reagents

Cerium nitrate hexahydrate [$Ce(NO_3)_3.6H_2O$], 2, 2 - Di (4-tert-octylphenyl)-1-Picryl Hydrazyl (DPPH) were obtained from Sigma (St. Louis, MO, USA.). Dulbecco's modified Eagle's medium (DMEM), Fetal bovine serum (FBS), Antibiotic-antimycotic solution, 2, 7 –dichlorofluorescin diacetate (DCFDA), 3(4, 5-Dimethylthiazol-2)-2, 5-Diphenyl tetrazolium bromide (MTT), were procured from Hi-media, Mumbai. All organic solvents were of spectral grade and general chemicals were of analytical grade and were purchased from local companies.

Biosynthesis of cerium oxide nanoparticles

The fungal strain *Trichoderma viridae* was obtained from Department of Microbiology and Biotechnology, Bangalore University, Bengaluru. The fungal strain was grown in PDB media and incubated at 37^0C under continuous agitation at 120 rpm in an orbital shaker for three days. The fungal culture medium was filtered using Whatmann filter paper and the fungal filtrate was collected and stored at -4^0C until further use. Thereafter, 6 g of $Ce(NO_3)_3.6H_2O$ was added in 250ml of fungal supernatant under vigorous stirring. The stirring was continued for 5 hrs to get homogenous mixing. A white precipitate was formed and then it became a yellowish brown in color upon continuous stirring, finally, the precipitate was calcined for 15 min in muffle furnace at 350^0Cto obtain CeO_2 NPs.

Characterization of CeO_2 NPs

The synthesized CeO_2 NPs were characterized by UV–vis spectral analysis (Multi skan GO, Thermo scientific) in the wavelength ranging and 200 and 800 nm at the resolution of 1 nm. XRD studies were carried out on Shimadzu XRD 7000 maxima, Japan X-ray diffractometer using Ni-filtered CuKa radiation (35kv, 15mA). The morphology of the CeO_2 NPs was examined by scanning electron microscopy (Hitachi S-3400N SEM). The samples were prepared by sprinkling the powder oxides onto double-sided sticky tape and mounting them on a microscope stub. The functional group vibrations were analyzed using FTIR Perkin Elmer spectrometer (spectrum 1000) on KBr plates in the scanning range of 400-4000 cm^{-1}.Dynamic light scattering experiments and zeta potential measurements were carried using Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK).

Statistical analyses

Values are expressed as mean ± SE. significant changes between different concentrations were analysed by one-way analysis of variance. Followed by Tukey's test. Probability values of $p<0.05$ were considered significant. All statistical analysis was performed using SPSS 20 software package for windows.

RESULTS AND DISCUSSION

Uv-vis Spectral spectroscopy

The UV spectrum of the Std. and synthesized cerium oxide nanoparticles is shown in **Fig. 1**. The spectrum shows maximum absorption for wave-length above 300 nm and there after it decreases exponentially with increase of wavelength. The absorption spectra of Std and synthesized CeO_2 NPs showed intense peak around 308 nm indicating the formation of CeO_2 NPs. The observed peak corresponds to the fluorite cubic structure of Ceo_2NPs due to the quantum size effect of the blue shift in UV–Vis spectrum and confirms the charge between the O_2p and Ce_4f states in O^{2-} and Ce^{4+} [12]. The results are in concordance with the studies of [9].

X-ray diffraction (XRD) measurement

The crystalline nature of CeO_2 NPs was confirmed from X-ray diffraction (XRD) analysis **Fig. 2** represents the XRD pattern of CeO_2 NPs synthesized from *Trichoderma viridae*. Bragg reflection peaks centred at 28.52, 33.04, 47.46, 56.30, 59.09, 69.00, 79.07 Deg in the 2θ corresponds to the (111), (200), (220), (311), (222), (400), (331) and (420) planes of CeO_2 NPs. The intense peaks observed in the spectrum are in agreement with the Braggs's reflection of CeO_2 NPs [13]. The standard diffraction peaks show the face-centered cubic phase of CeO_2 NPs. (JCPDS 34-0394).

Scanning electron microscopy (SEM) analysis

The surface morphology of the synthesized was determined by SEM analysis. The structure of the nanoparticles plays an important role in determining their adhesion, interaction and absorption with the body cells. The CeO_2 NPs were mostly spherical in

shape with aggregation **(Fig. 3**). The larger nanoparticles may be due to the aggregation of the smaller ones. The aggregation may take place due to the nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. Our results are in agreement with the studies of [9; 14].

Fourier Transform Infra-red (FT-IR) spectral analysis

FTIR spectrum of Std and green synthesized CeO_2 NPs are shown in **Fig. 4** to explain the structural information. The group of strong, intense bands was observed at 3146, 2360, and 1327 cm^{-1}. The intense band at 3146 cm^{-1} corresponds to (O–H) mode of hydroxyl molecules. The bands observed at 2360 and 1327 cm^{-1} correspond to CeO_2 NPs. The band at 1642 cm^{-1} corresponds to the bending of H–O–H which is partly overlapping the O–C–O stretching band. FT-IR spectra showed the potential biomolecules responsible for the reduction of $CeN0_3$ ions for the synthesis of CeO_2NPs while reacting with T. *Virdae* culture filtrate. The spectra obtained for CeO_2 NPs showed characteristic bands which are in good agreement with the value reported in the previous reports [14-15].

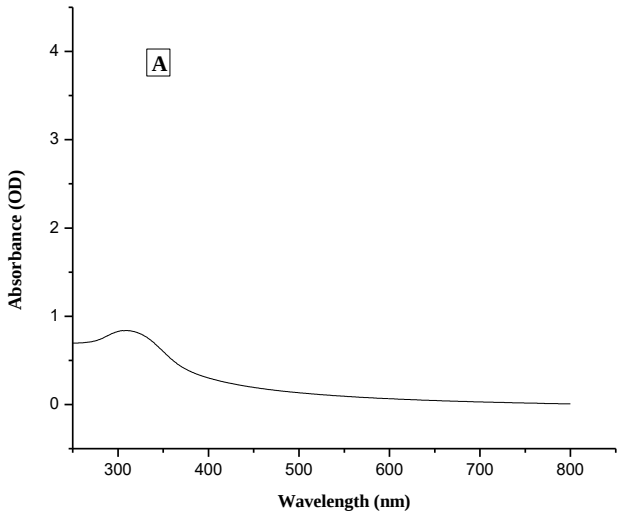
Particles size and zeta potential

The average size of the nanoparticles particles was measured by DLS instrument. The size distribution versus intensity graph is represented in **Fig. 5.** The average size of the Std. and synthesized nanoceria were found to be 18.42 and 67.95 nm respectively. The particle size was larger in green synthesized CeO_2 NPs compared to the Std. CeO_2 NPs is due to the fact that the measured size also includes the bio-organic compounds enveloping the core of the CeO_2 NPs.

Zeta potential values reveal information regarding the surface charge and stability of the synthesized CeO_2 NPs. The zeta potential of the Std. and synthesized nanoceria were found to be -19.8 and -21.3 mV respectively **(Fig. 6).** The higher Zeta potential value of synthesized CeO_2 NPs over the Std. CeO_2 NPs indicates the stability of nanoparticles and cellular uptake behaviour. The rich source of enzymes and secondary metabolites in the fungus filtrate may possibly responsible for reduction of metal ions and efficient stabilization of synthesized nanoparticles.

Free radical scavenging assay

The DPPH assay was used to study the free-radical scavenging capacity of green synthesized CeO_2 NPs in comparison to Std. $Ce0_2$NPs and BHT. The activity is measured spectrophotometrically with DPPH, a stable free radical, which produce a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to no colour [16]. The nano ceria exhibited concentration dependent anti radical activity as shown in **Fig. 7.** The higher activity of the bio synthesized $Ce0_2$NPs may be due to the capping of secondary metabolites synthesized by the fungus.



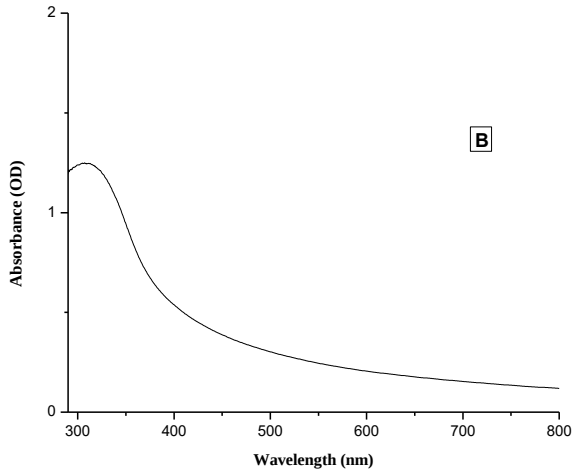
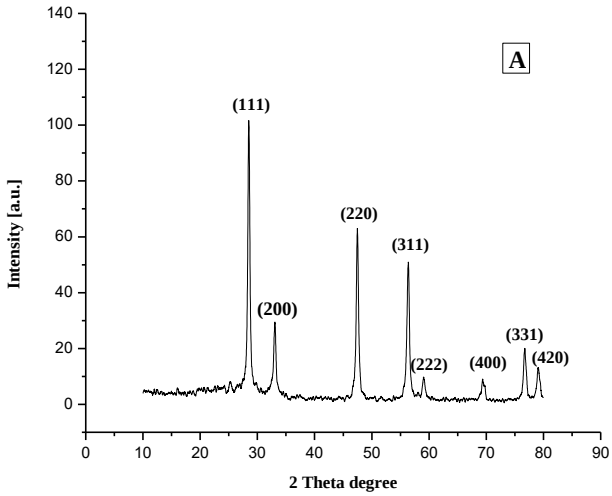


Fig 1: UV Spectra of (A) Standard CeO_2 and (B) Synthesized CeO_2Nps



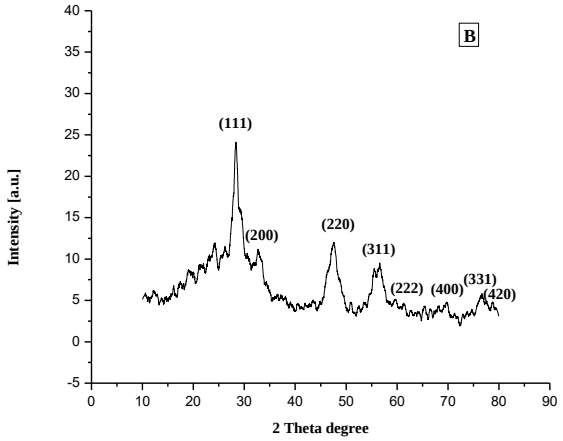


Fig 2: XRD Spectra of (A) standard CeO_2 and (B) synthesized CeO_2 Nps

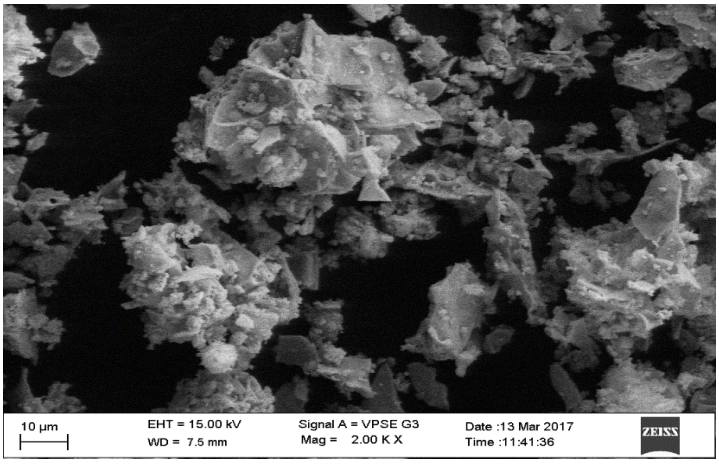


Fig 3: SEM micrograph of synthesized CeO_2 Nps

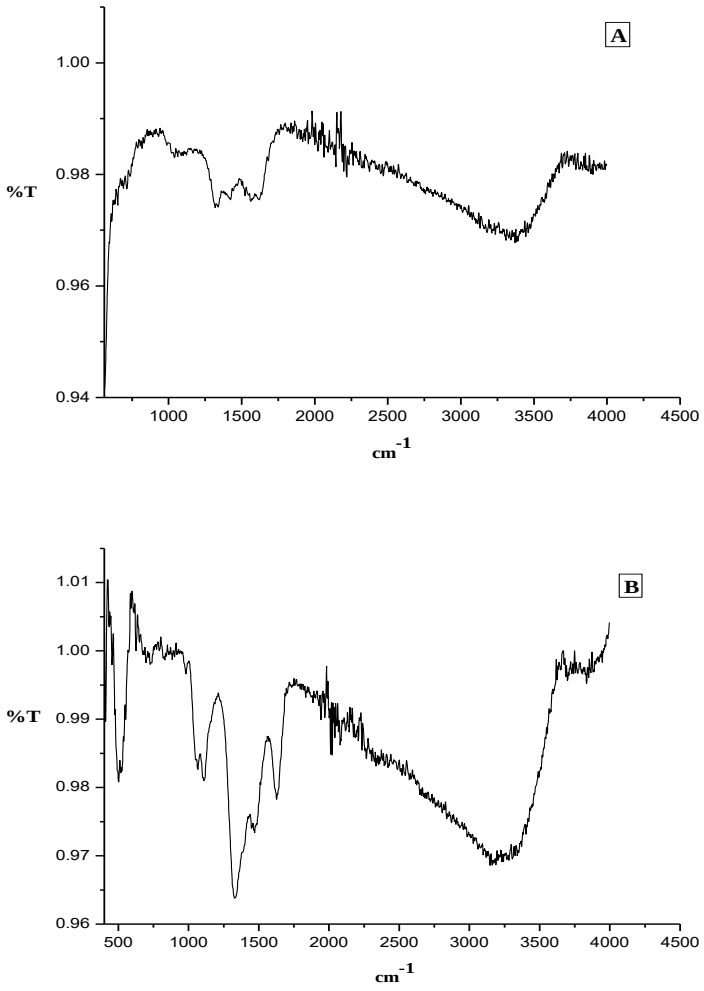


Fig 4: FTIR Spectra of (A) standard CeO_2 and (B) synthesized CeO_2 Nps

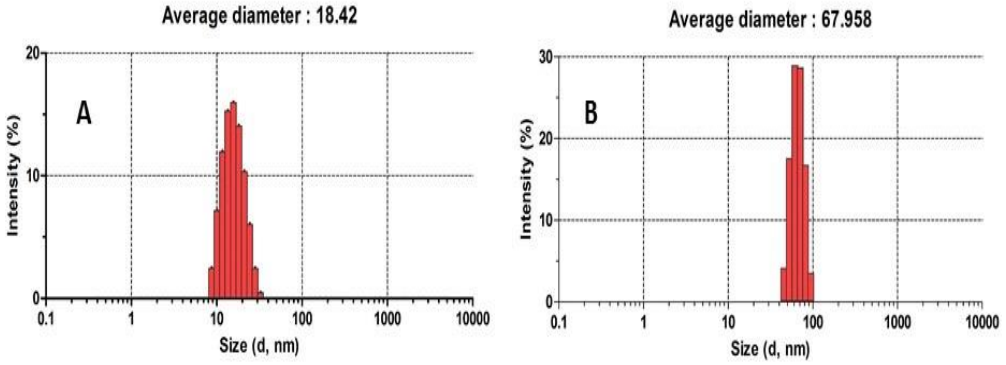


Fig 5: DLS – Particle size distribution analysis (A) Standard CeO_2 and (B) Synthesized CeO_2 Nps

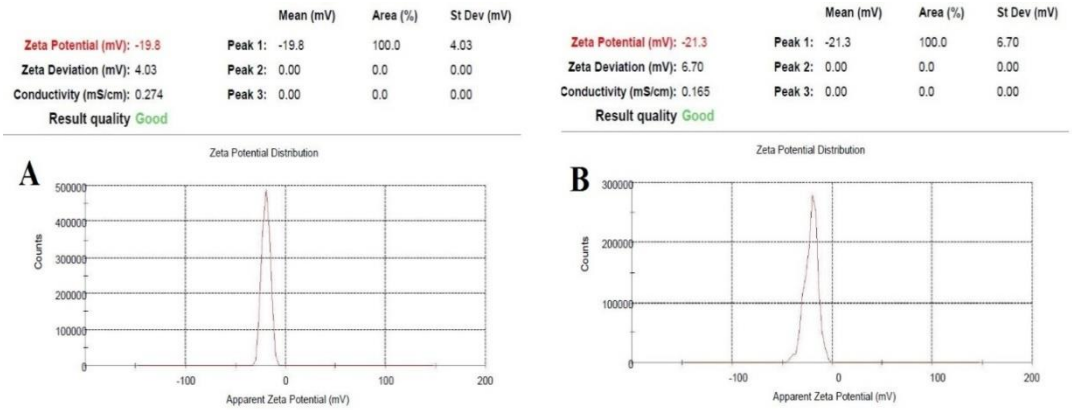


Fig 6: Zeta potential graph (A) Standard and (B) Synthesized CeO_2 Nps

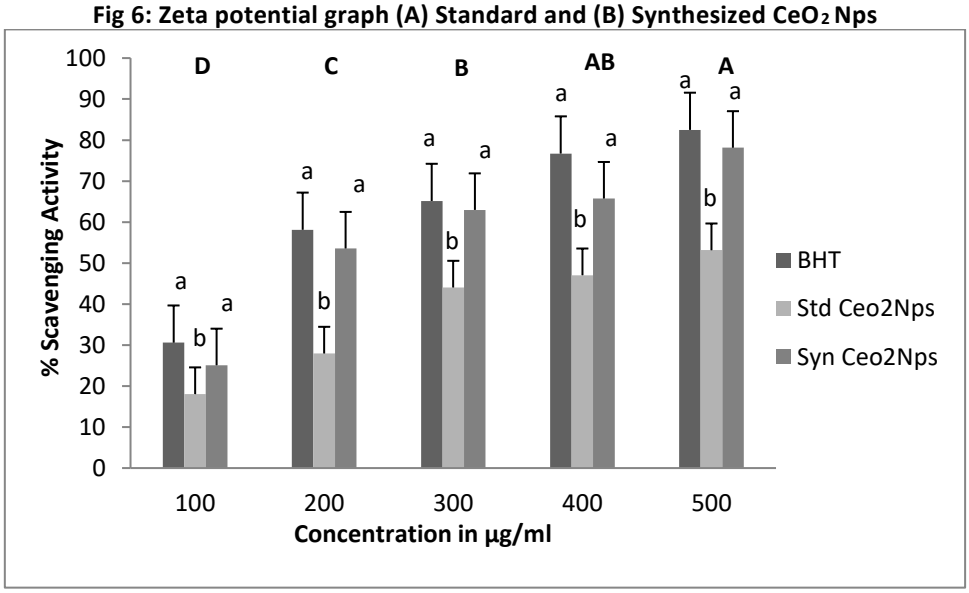


Fig 7: DPPH radical scavenging activity. Values are represented as mean ± S.E (n=3). Significance between the group means of different concentration is represented in upper case and between samples in lower case. Those not sharing the same letters are significantly different at $p<0.05$.

CONCLUSION

CeO_2NPs have been effectively synthesized using T. *Virdae* culture filtrate. The UV–vis spectrum intense peak at 308 nm indicated the formation of cerium oxide nanoparticles. The FTIR spectrum of CeO_2NPs revealed the presence of Ce-O bonds. XRD, SEM and DLS analysis revealed that they are spherical in shape having cubic fluorite structure with average particles size of 68 nm. The green synthesized revealed potent antioxidant activity as demonstrated by its ability to quench free radicals. The method is simple, consistent, cost effective and eco-friendly approach and it can be applied to synthesise other metal oxide NPs.

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