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Synthesis, characterization and evaluation of biosynthesized cerium oxide nanoparticle for its anticancer activity on breast cancer cell(mcf 7)

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Abstract

Biosynthesis of metal oxide nanoparticles using plant is more desirable than physical and chemical methods, due to its eco friendliness. Therefore, in the present study involves the synthesis of cerium oxide nanoparticle by using *Brophyllamdaigremontianum* plant ethanolic extract. The obtained powder was subsequently calcinatedat 600^0C. Synthesized cerium oxide nanoparticles was characterized by X-ray diffraction, Scanning electron microscopy, High resolution transmission electron microscopy, UV visible spectroscopy, Fourier transform infrared spectroscopy and Brunauer Emmett-teller (BET) measurement. To evaluate the anticancer activity of the spherical structured CeO_2-Nps,it was tested towards the normal (L6) and breast cancer cell (MCF 7). It was found that Biosynthesized CeO_2-Nps showed better cytotoxicity against cancerous MCF 7 cell line comparedtonormal L6 cell line due to its smaller size and high surface area.

Keywords

Biosynthesis, CeO2-Nps, calcination,Bryophyllamdaigremontianum, Breast cancer(MCF 7)

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Contents

1. Introduction

Cancer is an abnormal or uncontrolled growth of a cell that can spread to other healthy tissues of the body, although cancer can destroy many organs. But breast canceris currently one of themajorcauses of death among women in developed countries. Among the various advances in radiotherapy and chemotherapy for Cancer treatment [1],chemotherapy treatment takes hours, days, weeks or even several months. Radiotherapy sometimes damage close to the other organs.Both this treatments has some side effects, are cost effective and haverisk.Therefore cancer therapy needs for easily accessible

and safe treatment. The development of Nano science has attracted considerable attention for Cancer treatment.

The chemistry of cerium is differs from alkaline earth, post transition and transition elements due to its screening effect and nature of metricconverterProductID4f4f orbitals.These orbital arrangements gives rare earth unique catalytic, magnetic, photo catalytic and electronic properties[2,3], These different properties give new type of applications which are not possible with transition elements. Cerium oxide nanoparticle otherwise called nanoceria. Cerium exhibits two main oxidation states namely Ce^{4+} and Ce^{3+}.Both oxidation states are auto regenerative. Spherical shaped $Ce_2O_3$was synthesized byRenu et.al using hydrolysis method [4].$Ce_2O_3$is thermally less stable than CeO_2-Nps, but the face centered cubic fluorite type crystal structure of CeO_2-Nps highly stable than main group and other rare earth metal oxides. Nano ceria possess many unusual properties such as high conductivity, large magnetic moment, high complexation property, oxygen storage capacity, nonstoichiometric, and reduction behaviour.Thenanometer scale ceria could exhibit exceptional properties like larger band gap than bulk ceria[5]. High concentration of Ce(III)ions inceria strongly absorb UV light which leads to red shift in the absorbance spectrum between300-400nm.Cerium oxide is one of the inexpensive materials it has been used in various applications such as catalysis[6], gas sensor[7], polishing reagent[8], luminescent materials[9], solid oxide fuel cells[10], under UV irradiation pure ceria used to splitting of water to form oxygen gas[11]As a good catalytic converter that convertsharmful carbon monoxide to safe carbon dioxide[12],the scavenging effect is attributed to the presence of Ce^{3+} ions in ceria that reduce the free radical species[13], theredox property of ceria qualify itself to function as an anticancer vehicle for prostate cancer cell[3]. Nano ceria thin film has unusual properties like high refractive index, high dielectric constant and lattice constant which is similar to Si thin film [14,15]. The performance in the above mentioned application strongly depends on structural features. High oxygen storage and transport capacity of CeO_2-Npswidely depend on number of oxygen vacancies and at thesametime, there are two possibilities to maintain fluorite type of crystal structure, one is reduction of Ce^{4+} into $Ce^{3+}$and other is chemically doped ceria with other transition metal oxide, cerium doped zinc oxide used in solar cell and photo catalytic applications[16-19]. Several methods such as sol gel [20],hydrothermal [21,22,23], precipitation[24-26], solvothermal[27,28],emulsion method[29], combustion synthesis[30,31], microwave method[32] and thermal decomposition[33,34]are used for synthesis of CeO_2-Nps. Inchemical methods, synthesis procedure employs capping agentoradditives, non-polar solvents and toxic chemicals thus not suitable for biomedical fields. Therefore, many researchers are forced to go the biocompatible and clean process to synthesize nanoparticle and develop biosynthesis. Biosynthesis process plays very important role in nanotechnology; it eliminates toxic chemicals which are formed as by products in certain chemical reactions and ensures organic solvent free synthetic protocol. It is easily scaled up for large scale synthesis and cost effective. Plant extracts may act both as reducing and capping agents in Nano particle synthesis[35,36,37].*Bryophyllamdaigremontianum*is otherwise called air alligator plant, succulent herb, devil's backbone, andmother-of-thousands. It has been used in some biological applications such asantidiabetic, anti-inflammatory, antinociceptive, and antimicrobial activity. *Bryophyllamdaigremontianum* leaves contain organic acids, tannins, p-hydroxybenzal dehyde, alkaloids and group of chemicals also known as bufadienolides. They are Bryophyllin, bersaldegenin-1,3,5-orthoacetate, bersaidgenin-3-O-acetate and bersaldegenin-1–O-acetatedaig remontianion,[38].U.Supratman et.al reported that anticancer activity of bufadienolides from plant extract. Natural bufadienolides have strong anticancer activity towards several cell line such as human lung carcinoma A-549, walker intramuscular-256 carcinoma, and human carcinoma of nasopharynx[39].

In this study, we explore, for the first time, the green synthesis of CeO_2-Nps using bufadienolides from ethanolic extract of *Bryophyllamdaigremontianum* and examine the cytotoxity of cerium oxide against human breast cancer MCF7 cell line.

2. Experimental

2.1 Materials

Cerium Nitrate Hexahydrate(99.9%), 3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazoliumbromide(MTT), FetalBovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco′s Modified Eagle′s Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co,St Louis, USA. EDTA, Glucose and antibiotics purchased from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO), propanol and Ethanol were procured from E.Merk Ltd., Mumbai, India. Fresh and green leaves of *Bryophyllamdaigremontianum*plant were collected from Mottur village,Salem district (Tamil Nadu, India).

2.2 Synthesis of Cerium oxide nanoparticle

The leaves of *Bryophyllamdaigremontianum*plant were dried at room temperature and powdered well. Then 10 g of dried powder was mixed with 100 ml of ethanol and the mixture

was heated at 60^{o}C for 1 hours. It was filtered by using Whatman filter paper (No.1) and the filtrate was collected. This ethanolic extract was used as stock solution for the study. 40ml of 0.1M of Cerium Nitrate Hexahydrate was taken in a beaker and 40ml of distilled water was added. This solution was stirred using a magnetic stirrer in order to arrive at a homogeneous solution was formed. To this aqueous solution*Bryophyllamdaigremontianum* leaf extract of 20 ml was added. The reaction mixture was stirred and heated on hot plate at 80^{o}C till the supernantgot evaporated. The obtained product was grounded into a fine powder, washed with ethanol and tried at room temperature. This powder was calcinated at 600^{o}C for 1 hour.

2.3 Characterization

The CeO_2-Nps synthesized by using *Bryophyllamdaigremontianum*leaves extract were subjected to the X-ray diffraction. This was carried out using Cu-$\alpha(\lambda$ =1.5406 Å) radiation source in PANalyticalX'pert PRO model X-ray powder diffractometer.Morphology of Ceria was examined by using scanning electron microscopy(FEI Quanta FEG200) and EDAX (FEI Nova NanoSEM 450), The nanostructure was obtained(HRTEM) operated at 300KeV. FTIR spectra were obtained using BRUKER Optik GmbH FTIR spectrometer model TENSOR 27 in the diffuse reflection mode in the spectral range 500-4000cm^{-1} with 1cm^{-1} resolution. The optical band gap was measured by using UV Visible spectrometer (Aalytic Jena specord 200 plus) ad surface area measured by BET (Micromeritics'Tristar II).

2.4 Cell culture

MCF-7 (Human Breast cancer cell line) and (Normal cell line) cell cultures were procured from National Centre for Cell sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was supplemented with 10% inactivated felt Bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B(5μg/ml) in an humidified atmosphere of 5%CO_2 at 37^{o}C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 micro-titre plates (Tarsons India Pvt. LTD., Kolkata, India)

2.5 Cytotoxicity of CeO_2-Nps by MTT assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using medium containing 10% FBS and were the cell viability by MTT assays. The absorbance was measured using a micro-plates reader at a wavelength of 540nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose response curves for each line.

$$\%\text{Growth inhibition } = 100 - \frac{\text{Mean Of of individual test group}}{\text{Mean OD of control group}} \times 100$$

3. Results and discussion

3.1 X-ray diffraction

Phase purity of the CeO_2-Nps wasmeasured using powder X-ray diffraction. X-ray diffraction patterns of prepared and 600^{o}C calcinted CeO_2 sample are shown in Fig.1. All peaks for each sample were significantly got broadened indicating small crystallite size. It is especially true for as obtained powder which exhibits very diffuse diffraction in such way that some atomic plane are low intensity due to their smaller size. The strong and sharp diffraction peaks indicate the good crystallization of the CeO_2-Nps calcinated 600^{o}C. No peak of any other phase was detected indicate the high purity and face centered cubic structure according to JCPDF789-0694.

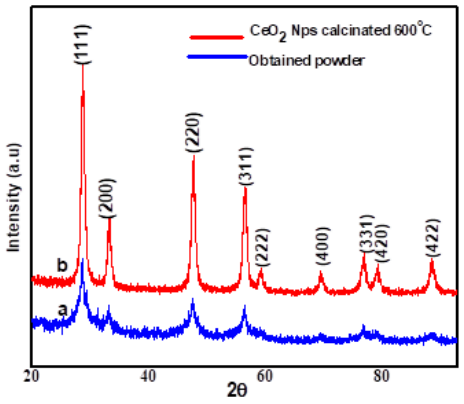

Figure 1. (a)XRD patternof powder before calcination, (b) XRD pattern of powder after calcination

4. SEM and TEM analysis

SEM and TEM images are shown in fig 2a and 2b. Sample calcinated at 600^{o}Cshows clear image and the particles are almost spherical in nature which is free from agglomeration.

4.1 EDX analysis

EDX spectrum of biosynthesized CeO_2-Nps isfig .2d.This analysis confirmed the presence of elemental Cerium and Oxygen of the CeO_2Nps. X_axis displays energy in keV, y axis displays the number of X-ray counts. Cerium and Oxygen ratio helps to find out the stoichiometry of Cerium. Increase in the elemental composition of Cerium is due to oxidation state change from 3 to 4. Energy disperse X-ray spectrometer (EDAS) mapping of Ce(fig.2e) and O (fig.2f) correspond the shape of a single CeO_2-Nps(fig.2c) nanosphere which further testifies the homogeneousity of CeO_2-Nps.This shows that CeO_2-Nps synthesis using *Bryophyllamdaigremontianum*leaf extract is a reliable method.

4.2 BET

The nitrogen adsorption-desorption isotherms are shown in fig.3. According to the IUPAC classification the isotherm

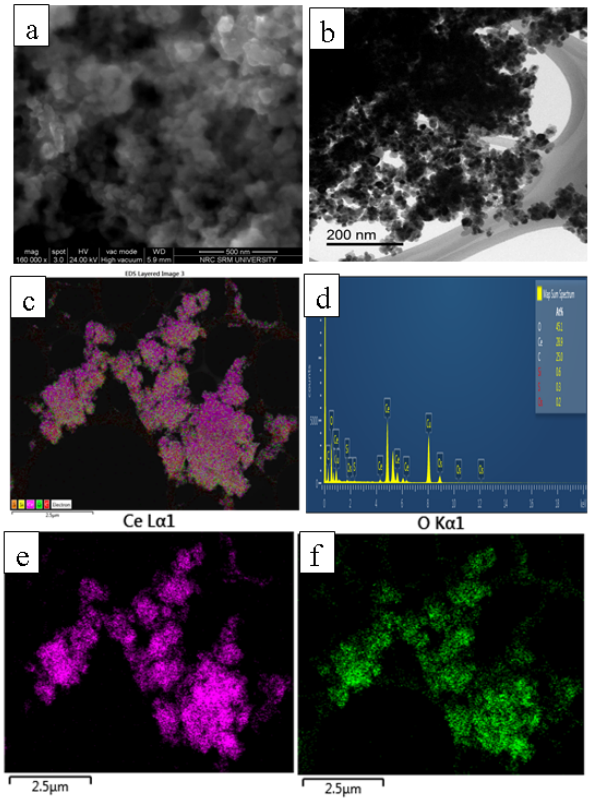

Figure 2. a)SEM imageof CeO_2-Npscalcinated at 600^{o}C b)TEM imageof CeO_2-Npscalcinated at 600^{o}C c)EDSmapping of CeO_2-Npscalcinated at 600^{o}C d)EDS spectrum of CeO_2-Nps;e)EDS mapping of Ce f)EDS mapping of O

of type IV and with hysteresis loop associated with mesoporous materials [40].TheBET surface area of green synthesized 600^{o}C calcinatedCeO_2-Nps is 30.19m^2/g, which is that of higher than bulk ceria. In comparison, a recent report of P.Tamizhurai et.al[41]on CeO_2-Nps particle synthesised using alovera extract revealed that the surface area of the particle was 19.2m^2/g. The present work provides high surface area and smaller size for the 600^{o}C calcinated CeO_2-Nps.

4.3 UV visible spectra

The UV Visible spectra of CeO_2-Nps are shown in fig.4.a. The spectra show that strong absorption occurs at 330 nm. It acts as a good UV blocker [42]. Due to charge transfer transition occurs between O4p orbital to Ce4f orbital. UV visible absorption data used to calculate the direct band gap of the CeO_2-Nps samplecalcinated at 600^{o}C from this mathematical formula

$$\alpha h\nu = E_D(h - E_g)^{1/2}$$

Where α is the optical absorption coefficient, hν is the photon energy, ED is constant and E_g is the direct band gap. The derivative spectrum of the calcinated sample illustrate in the inset of fig 4.b. The estimated band gap value 3.0eV is

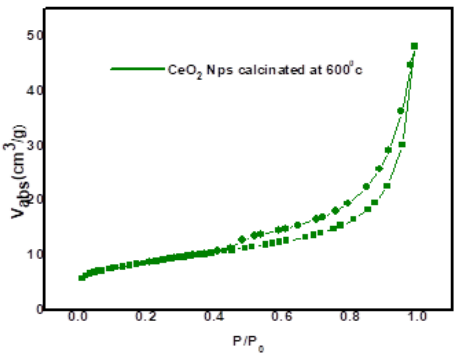

Figure 3. Adsorption–desorption isotherms of CeO_2-Npscalcinated at 600^{o}C

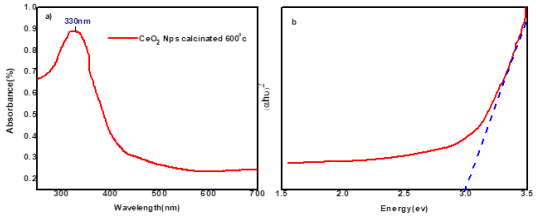

Figure 4. (a) UV visible spectraof CeO_2-Npscalcinated at 600^{o}C,b) Band gap estimationof CeO_2-Npscalcinated at 600^{o}C

higher than that of bulk Ceria. This means that CeO_2-Nps capped by some organic compound and it is increased the band gap. The absorption spectrum revealed a good absorption occurring at 330nm, This proves that their product could be useful for some medicinal applications.

4.4 FTIR

FTIR Spectra (Fig.5) is used to determine the capping and stabilization of the CeO_2-Npsby bufadienolids, organic acids, tannin and alkaloids from *Bryophyllamdaigremontianum*plant extract. A very broad and strong absorption peak appear at3200-3650 cm^{-1} which can be assigned to the –OH stretching of bufadienolids, tannin and water, The peak value is 2360 cm^{-1}.The peak value at 1117cm^{-1} which means that having some CO are present.

The peak value at 2925.41 cm^{-1} and 2856.66cm^{-1}are due to the presence of alkene, the peak value at 1602cm^{-1}C-H stretching in polyphenol, the bands 1350cm^{-1} are due to Ce-O-Ce vibration the peak value 516cm^{-1} and 420cm^{-1} of Ce-O stretching occurs only 600^{o}C calcinated sample, which means sample have better crystalinity than obtained powder. FTIR result clearly demonstrate the antitumor active bufadienolids like Bryophyllin, daigremontianin, bersaldege-nin-1,3,5-orthoacetate, bersaldege-nin-3-orthoacetate, and bersaldege-nin-1-orthoacetate are can cap the nanoparticles.

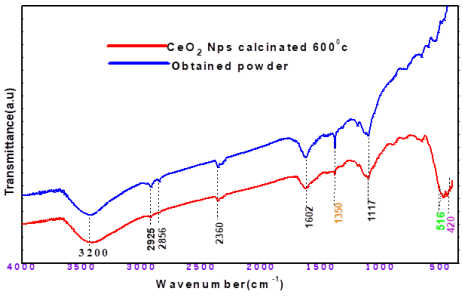

Figure 5. FTIR of synthesizedpowderbefore calcination, (b)FTIR of CeO_2-Npsafter calcination

4.5 Evaluation of vitro cytotoxicity of CeO_2-Nps in normal and MCF cell Line

To evaluate the anticancer effect of the 600^{o}C calcinated Cerium oxide nanoparticle, MTT assay was performed in MCF-7 (Human Breast cancer cell line) compared normal L-6 cellLine (Rat muscle). The result demonstrated CeO_2-Nps are least toxic to normal L-6 cell, where its IC_{50} value is 298.17. The results are cell Viability and IC_{50} values are summarized in Fig.6. Increase the CeO_2-Nps concentration with decreasing the cell viability of MCF-7 was absorbed Fig.7. The 50% 0f cell death occur at lower concentration 175.04, therefore, this nanoparticle had better cytotoxicity against MCF-7 cell line, due tothis nanoparticle had smaller size and larger surface area.

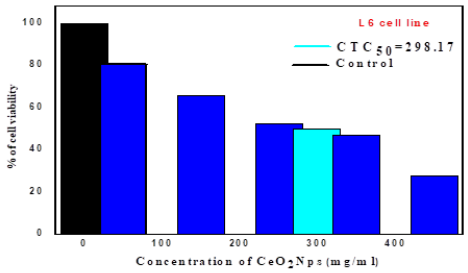

Figure 6. MTT assay results conforming the Vitro cytotoxicity of green synthesized CeO_2-Npsagainst L6 cell line

4.6 Morphological changes of normal and MCF7 cell via interaction with CeO_2-Nps

The vivo cytotoxicity of cerium oxide nanoparticles measured by microscopy images are show in Fig.8 This result demonstrated increase the concentration of nanoparticle with reduction of MCF7 cell line. This nanoparticle treated morphology change compare with untreated control group and normal L6 cell line.

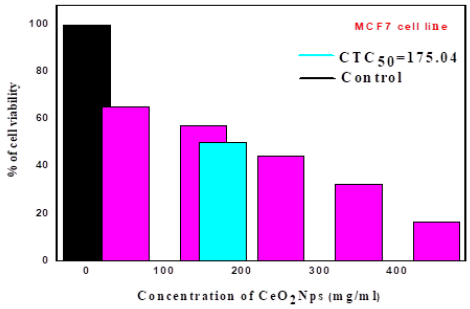

Figure 7. MTT assay results conforming the Vitro cytotoxicity of green synthesized CeO_2 nanoparticle against MCF7 cell lin

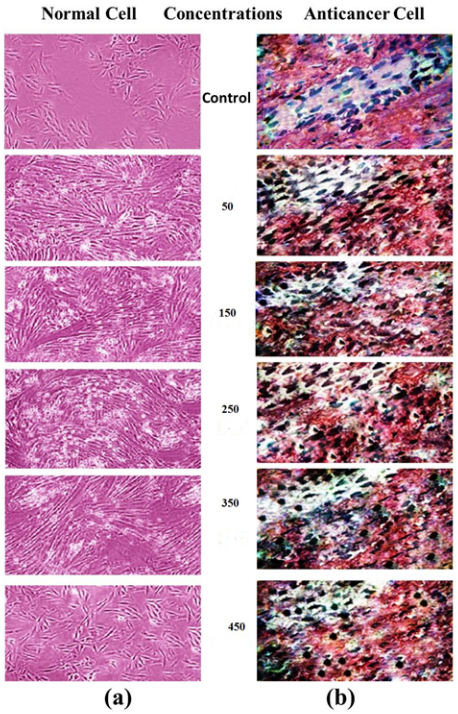

Figure 8. (a) Apoptosis morphological changes in normal cell,(b)Apoptosis morphological changes in MCF7 cells following the exposure treated with different concentration of CeO_2-Npsfor 24h.

5. Conclusion

In this research, first time CeO_2-Nps successfully synthesized by one step biosynthesis methods using *Bryophyllamdaigremontianum*. XRD spectrum shows face centred cubic CeO_2-Nps had high purity and good crystalline nature. SEM and TEM images show spherical shape of ceria nanoparticle. The strong absorption occurs at 330nm on UV-visible spectrum due to their smaller size and higher band gap.BET results show high surface area. An MTT assay showed that CeO_2-Nps concentration increase with decrease the MCF7 cell concentration. Here Ceria nanoparticle majorly attack MCF7 cell compare

to normal cell. Therefore this biosynthesis method producing Ceria nanoparticle is biocompatible and development of cancer treatment.

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