Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article DOI: 10.26479/2019.0503.48

GREEN SYNTHESIS AND CHARACTERIZATION OF NANOCERIA FROM *CLITORIA TERNATEA* AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT: The method like green chemistry have more advantageous over physical and chemical methods in the synthesis of nanoparticles because of cost effective and eco-friendly. Biologically synthesized nanoparticles have been widely used in the field of nanomedicine. In the current study Cerium Nanoparticles or Nanoceria have been synthesized using *Clitoria ternatea* leaf extract. The aqueous extract of leaves used a reductant for the synthesis of nanoceria which confirmed by UV- Visible spectroscopy at the different time intervals and FT-IR Vibrational stretching mode. Further, the crystallite size of the nanoparticles was analyzed by Powder XRD about 7nm. The morphology of the nanoparticles determined by microscopic examination of SEM and elemental analysis was carried out by EDAX. Oxidative stress has been linked to heart disease, cancer, arthritis, stroke, respiratory diseases, immune deficiency, emphysema, Parkinson's disease, and other inflammatory or ischemic conditions. Antioxidants are said to help neutralize free radicals in our bodies, and this is thought to boost overall health. So, Antioxidant property was studied in-vitro for the synthesized nanoparticles using standard protocols. Present results also support the advantages of using bio-green method for the production of nanoparticles having the potential of antioxidant activities.

KEYWORDS: Nanomedicine, *Clitoria ternatea*, EDAX, Oxidative Stress, Antioxidant, DPPH.

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1. INTRODUCTION

Nanoscience and nanotechnology are a recent revolutionary development in Science and Engineering that are evolving at a very fast pace [1]. It is driven by the desire to fabricate materials with novel and improved properties that are likely to impact virtually all are of the physical and chemical sciences, biological and health sciences. The superior properties of nanomaterials will promise a revolutionary new approach with a major impact in various innovative applications such as biomedical, environmental, industrial, and food agriculture industries [2-5]. In the recent years, some of the metal oxide nanoparticles like, Fe_3O_4, TiO_2, CuO, ZnO, and CeO_2 are thoroughly been investigated for their various biological activity [6] due to their limited size and a high density of corner or edge surface sites. Among those, Nanoceria has numerous applications in various fields of physics, chemistry, and biology. The rare earth element of Cerium oxide is the cubic-fluorite type of oxide belongs to lanthanide series. Ceria and nanoceria can be found in numerous research technologies and good oxide ion conductors of solid-oxide fuel cells and electrode for gas sensors [7]. Recently, ceria nanoparticles have emerged as a fascinating and lucrative material in biomedical science due to their unique ability to switch oxidation states between III and IV based on environmental conditions [8]. In the field of medical science nanoceria used in endothelia cell protection [9], wound healing [10], anti-cancer applications [11-13], neuroprotection [14, 15], and neuronal regeneration [16]. The ability of nanoceria to switch between oxidation states is comparable to that of biological antioxidants [17]. This ability imparts nanoceria with the very important biological property of radical scavenging. A sustained and combined effort has demonstrated the capability of nanoceria to protect against cellular damage caused by various radicals in different tissues and organ systems as well as biomedical applications. Nanoceria has been shown to impart protection against the ROS [18] and against radiation damage [19]. Different kindsof methods were offered for the synthesis of nanoparticles such as physical, chemical, irradiation, and biological methods. Whereas chemical synthesis can lead to the generation of toxic chemical by-products or require high temperatures and/or high pressures for initiating the reaction, biosynthesis of nanoparticles using plants a simplistic, low cost and eco-friendly. Plants are nature's chemical factories for the synthesis of nanoparticles. Biological synthesis of nanoparticles is a single step bio-reduction method and less energy is used to synthesize eco-friendly. Three main steps are followed for the synthesis of nanoparticles using a biological system: the choice of solvent medium use, the choice of an eco friendly and environmentally benign reducing agent, and the choice of a nontoxic material as a capping agent are to stabilize the synthesized nanoparticles [20]. *Clitoria ternatea L*., belonging to Fabaceae family is a very well-known Ayurvedic medicinal plant used for different ailments. It is commonly called Butterfly pea or Conch flower or 'Aparajita'. The species is believed to be a native of the Caribbean, Central America, and Mexico, but is now naturalized all over the tropical parts of India. The plant bears solitary, axillary, papilionaceous flowers. The fresh root is slightly bitter and acrid in taste. In the Indian systems of medicines particularly in Ayurveda, roots, seeds, and leaves of the species have long been used a brain tonic and is believed to promote memory and intelligence [21]. The leaves and roots are used in the treatment of a number of ailments including body aches, especially infections, urinogenital disorders, and as an anthelmintic and antidote to animal stings [22]. The major phytoconstituents found in *Clitoria ternatea* are the pentacyclic triterpenoids such as taraxerol and taraxerone [23-25]. This medicinally important plant now used as reductants for the synthesis of nanoceria. Due to its exclusive redox properties, cerium oxide (ceria) is finding widespread use in the treatment of medical disorders caused by the reactive oxygen intermediates [26].

2. MATERIALS AND METHODS

Chemicals

Cerium III nitrate hexa hydrate ($Ce(NO_3)_3.6H_2O$), 2, 2-Diphenyl picryl hydrazyl, Ethanol, Ascorbic acid, Sulfuric acid, Ammonium molybdate, Sodium dihydrogen phosphate, Di sodium mono hydrogen phosphate, Potassium ferricyanide, Trichloroacetic acid, Ferric Chloride were procured from standard vendors.

Plant collection

The plants were collected from the surrounding of University area, Sri Paramakalyani Centre for Excellence in Environment Science, Manonmaniam Sundaranar University, Alwarkurichi.

Preparation of Plant extract

5g of healthy leaves of *clitoria ternatea* were weighed and Leaves were initially washed with Twin 80 solution for surface sterilization. Further leaves were washed with running tap water to remove adherents. Then they were cut into fine pieces and boiled for 20 minutes at 70^0C in the distilled water. After 20 minutes the colour of the aqueous solution changes from watery to light yellow. The extract was cooled to room temperature and filtered twice using Whatman No.1 filter paper. The extract was stored in a refrigerator in order to be used for further experiments.

Preparation of cerium oxide nanoparticles

Ten millimolar, aqueous solution of Cerium III nitrate hexa hydrate ($Ce\ (NO_3)_3.6H_2O$) was prepared and aqueous extract of leaves of *Clitoria ternatea* used for the synthesis of cerium oxide nanoparticles. 20 ml of *clitoriaternatea* leaf extract was added drop by drop into 80 ml of aqueous solution of 10mM cerium nitrate. It is kept in magnetic stirrer for 3 hours at room temperature.

Biosynthesis of Cerium Oxide Nanoparticles

The clear aqueous solution will be changed into turbid and pale yellow colour which designates the reduction of Nitrate and formation of oxide particles. After, 24 hours the solution was centrifuged at 8000 rpm for 20 minutes. The collected pellet was air dried in an oven at 80^0C for 4 to 5 hours. The Particles were calcinated at 450^0C for 6hours in a muffle furnace. The particles were collected carefully and stored in an airtight container for characterization studies.

Characterization Techniques

Ultraviolet-Visible Spectroscopy Analysis

UV/Vis spectroscopy is an easily available technique for characterization of metal nanoparticles and also used to determine the shape of plasmonic peaks of the nanoparticles. UV-Vis

Spectrophotometer, Lab man was used to record the optical absorption of the biosynthesized Cerium Oxide Nanoparticles in the spectral range of 200 to 500 nm at different time intervals by taking 2ml of the sample, compared with 2ml of distilled water used as blank respectively initial, 1 hour, 3 hours, 5 Hours and 24 hours at room temperature.

FT-IR Analysis

Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. Fourier Transform infra-red spectroscopy (FT-IR) analysis was carried out in the range of 400 cm^{-1} to 4000 cm^{-1} in Perkin Elmer.

X-Ray Diffraction Studies

Powder diffraction is often easier and more convenient than single crystal diffraction as it does not require individual crystals. A diffraction pattern plots intensity against the angle of the detector, 2θ. The result obtained is called the diffractogram. In a diffraction pattern, the peak position depends upon the wavelength. Interactions between the incident X-ray beam and the sample produce intense reflected X-rays by constructive interference when conditions satisfy Bragg's Law. This law describes the general relationship between the wavelength of the incident X-rays, the incident angle of the beam and the spacing between the crystal lattice planes of atoms. Constructive interference occurs when the differences in the travel path of the incident X-rays is equal to an integer multiple of the wavelength. The biosynthesized CeO_2 NP samples of XRD pattern was recorded on Powder XRD (PAN analytical X'PERT PRO system) using Cu Kα radiation ($\lambda = 1.54060$ Å) in the range of 2Θ from 10^0 to 80^0. The average crystallite size of synthesized CeO_2 NPs as calculated using Debye – Scherrer's formula.

$$[D = 0.9\lambda/\beta\cos\theta]$$

Where, D is the average particle size, k is the shape factor (constant 0.9), λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum of the peak (FWHM) and θ is the diffraction angle.

SEM and EDAX

The **S**canning Electron Microscopic analysis was done using Carl Zeiss Evo 18 Secondary Electron Microscope with EDS machine with the magnification up to 50K – 100K depends on the sample. Thin films of the samples were prepared on a carbon-coated copper grid by just dropping a very small amount of sample on the grid. The excess solution was removed using blotting paper and then the films on the SEM grid were allowed to dry for 1 hour.

The Energy Dispersive X-ray spectroscopy is an analytical technique used for the elemental analysis or chemical characterization of sample analysis. The EDAX octane series was used to carry out semi-quantitative elemental analysis of the samples.

Antioxidant Studies

1. DPPH Free Radical Scavenging Assay

According to our previous findings, the procedure was followed for antioxidant studies. Different concentration of cerium Oxide nanoparticles makes up into 1ml with ethanol. 1ml of 0.3mM 2, 2-Diphenyl picryl hydrazyl (DPPH) in ethanol, was added to the above samples. The reaction mixture was well shaken and incubated in dark for 30 minutes. Absorbance was checked at 517 nm against a blank (ethanol). Ascorbic acid was taken as the standard. Lower the absorbance of the reaction mixture indicates a higher percentage of scavenging activity [27]. The percentage of inhibition or scavenging of free radicals was determined by the formulae; % Inhibition = [(Absorbance Control – Absorbance Sample)/ Absorbance Control] x 100, Where control was prepared as above without sample.

2. Total antioxidant assay

0.01 to 0.05 ml of different concentrations of cerium Oxide nanoparticles were mixed in separate eppendorf with 1 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate; mixed in 1:1:1 ratio) respectively. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity was expressed by increased absorbance [28].

3. Reducing power

Different concentration cerium Oxide nanoparticles were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (10g/l), and then the mixture was incubated at 50º C for 20 minutes. 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used a blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power [29, 30].

Statistical analysis

All antioxidant experiments were done by the analysis of variance and the results are presented as the mean standard error of the mean. Data points were obtained from the mean of at least three triplicates.

3. RESULTS AND DISCUSSION

a) Bio-Synthesis of Nanoceria from *Clitoria ternatea*

Scientific name: *Clitoria ternatea*

Family: Fabaceae

Kingdom: Plantae

Order: Fabales

Higher classification: *Clitoria*

Rank: Species

Fig.1 Leaves of *Clitoria Ternatea* and its classification

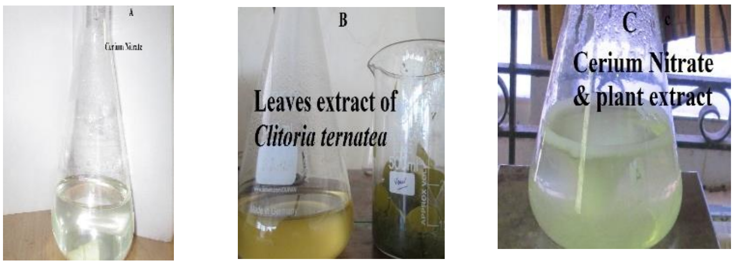

Fig.2 Green Synthesis of Nanoceria – A) Cerium nitrate hexa hydrate aqueous solution B) Leaves extract of *Clitoria ternatea* C) Cerium Nitrate and Plant extract.

Aqueous leaves extract of *Clitoria ternatea* mediated synthesis of nanoceria has more advantageous compared with other bio fabrication techniques using bacteria and fungi. In case of microbes, there will be a lot of drawbacks associated with selection of best bacteria, biocatalyst state, optimal conditions for cell growth and enzyme activity, optimal reaction conditions, culture maintenance, extraction and purification process, issues like contamination and cellular mutations [31]. But plant extract was cheap, non-toxic, biocompatibility and eco-friendly. The nanoparticles synthesized from the plant have more stable and more various in their shape and size [32]. The water extraction, however, is more suitable owing to its simplicity since no contamination from the solvent is expected. The water extraction was satisfactory and organic solvents is an option for the following extraction in order to exploit the recovery of phenolic compounds was suggested by Reis, Rai, and, Abu-Ghannam [33]. The aqueous extract of leaves of *Clitoria ternatea* contains a high quantity of flavonoids and also has Alkaloids, carbohydrates, phenol, tannins, saponins, Terpenoids, and Quinones was reported by [34]. These phytoconstituents was responsible for the reduction of cerium nitrate into cerium oxide Nanoparticles. Use of these natural plant compounds allows the synthesis of metal oxide nanoparticles with narrow distribution [35].

B) Characterization Techniques for Nanoceria

i) UV- Visible Spectrophotometer Studies

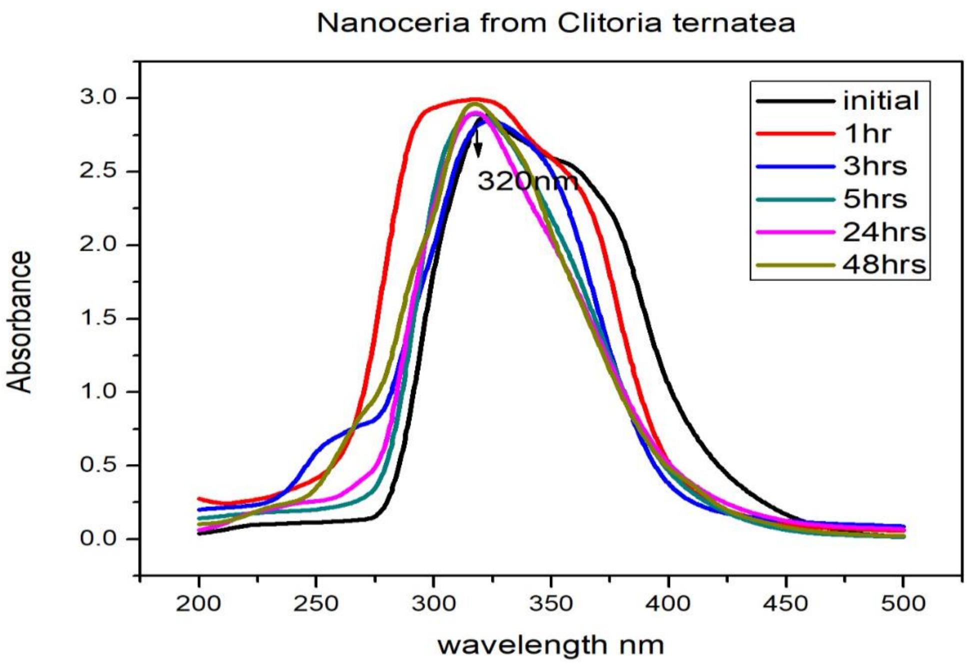

Fig.3 UV-Visible spectroscopy studies at different time intervals-Nanoceria synthesized from *clitoria ternatea*

UV-Vis spectroscopy is a very beneficial and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of Nanoparticle solution [36]. In addition, UV-Vis spectroscopy is fast, easy, simple, sensitive; selective for different types of NPs, needs only a short period time for measurement, and finally, calibration is not required for particle characterization of colloidal suspensions [37]. The constituents of the *clitoria ternatea* extract acting as the reducing and stabilizing agent for the synthesis of Nanoceria. The samples showed the narrow sharp absorbance peak at 320nm in 24 and 48 hours. This can be assigned to the intrinsic band-gap absorption of CeO_2-NPs due to the electron transitions from the valence band to the conduction band. In other words, the absorption of the charge transfer transition from O2p to Ce4f in CeO_2-NPs produces the band at approximately 300nm [38]. From the duration of initial to 5 hours the peak spectrum showed broad which indicated the particle sizes are bulk. When the time increased the particle, size is gradually decreased. The peak height increased as the discharge time increased. An absorbance maximum was obtained in the range of 304-320 nm, a characteristics peak of nanoceria [39]. Nanoceria has two oxidation states Ce (III) was colorless, while Ce (IV) was yellow to red in color [40,41]. Both oxidation states have two different UV adsorption peaks – Ce (III) in the range 230-260nm. Ce (IV) possessed in the range 300-400nm [42]. Here, in our method, the maximum adsorption of the sharp peak found at 320 nm with pale yellow colour [43, 44], which indicates the presence of Ce (IV) oxidation states.

ii) X-Ray diffraction Studies

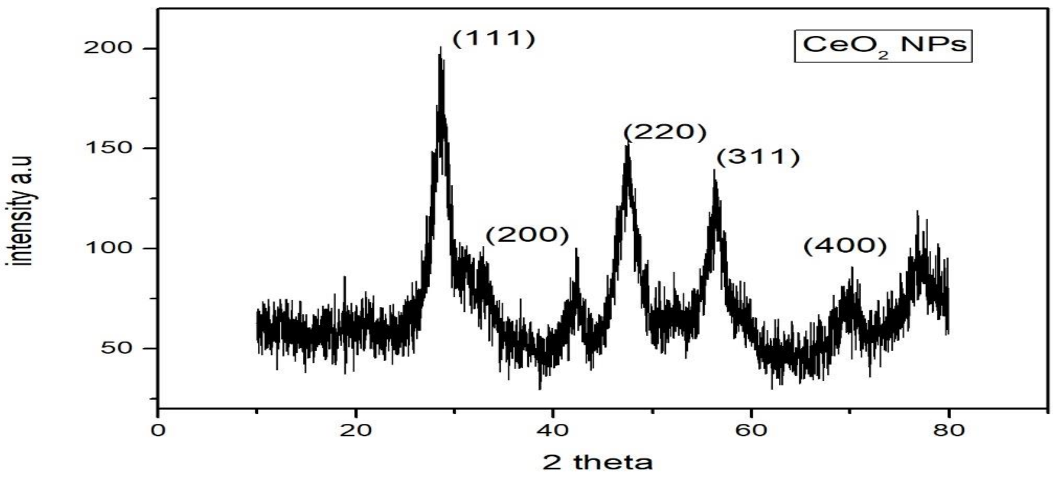

Fig.4 X-ray powder diffraction pattern of CeO_2 using *clitoria ternatea*

The detectable Bragg's peaks with planes of 111, 200, 220, 311, and 400 which correspondence to the angles (2Ɵ) values of 28.68, 42.39, 47.51, 56.59, and 69.81. The standard diffraction peaks show the face-center cubic phase of CeO_2 NPs. The planes that can be indexed to pure cubic fluorite structure for CeO_2. The intensities and positions of the peaks are in agreement with the literature (JCPDS card, No. 4-0593) (45). This crystallographic phase consists of a cubic fluorite-type oxide in which respectively Cerium site is surrounded by 8 Oxygen sites in an f mcc arrangement while each Oxygen site has a tetrahedron Cerium site. From the broadening of the Bragg peaks and using the Debye-Scherrer approximation, the average size of the NPs average ranges within 7.562 nm.

iii) Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

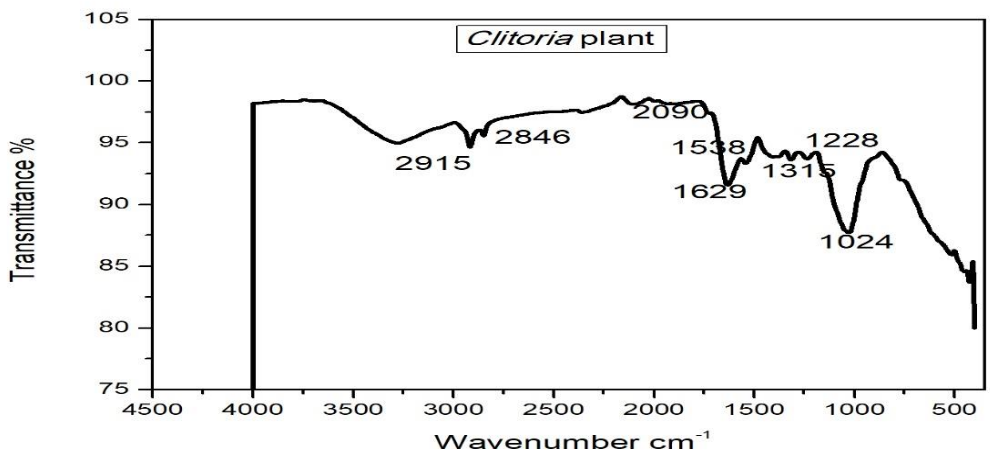

Fig. 5 FT-IR of *clitoria ternatea* leaves

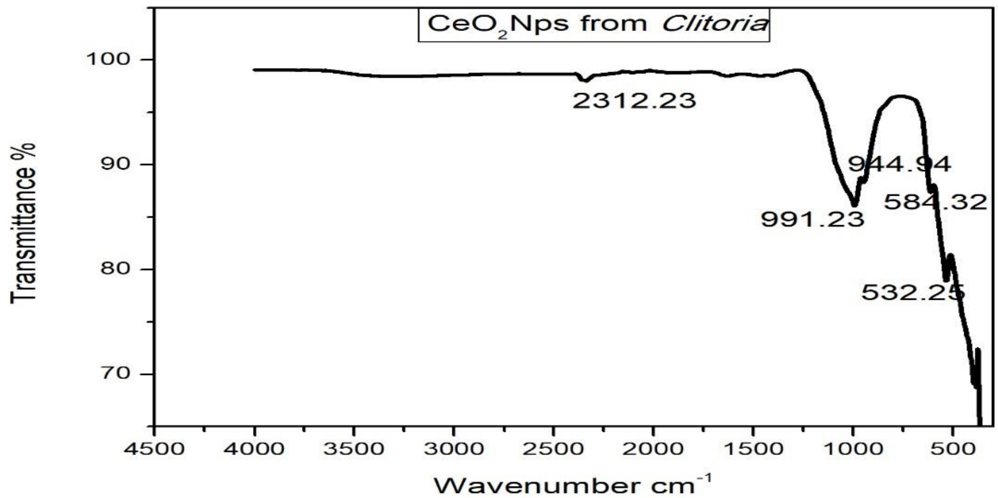

Fig.6 FT-IR of Nanoceria synthesized from *clitoria*

Table.1 The Functional groups of the leaves of *Clitoria ternatea*

Frequency, cm^{-1}	**Bond**	**Functional group**
1024	C-N- Stretch	Aliphatic Amines
1228	C-O Stretch	Alcohols, Carboxylic acids, Esters and Ethers
1315	C-N stretch	Aromatic Amines
1538	C-C stretch (in-ring)	aromatics
1629	N-H bend	Primary amines
2090	C≡C Stretch	Alkynes
2846 & 2915	C-H Stretch	Alkanes

These are the functional groups of the leave extract which were performance as the reductant for the biosynthesis of nanoceria.

In Fig.6 showed the FT-IR spectrum of the CeO2-NPs also exhibits the bands below $700 cm^{-1}$ which is due to the δ (Ce–O–C) mode (46). The chemical nature of CeO_2 was also verified from the FTIR spectrum, which showed a characteristic absorption band at 500 to 550 cm^{-1} due to the Ce-O stretching vibration (47). In our study, Ce-O stretching is observed at 532.25, 584.32, 944.94, and 991.23. So, the absorbance peaks at 429.48, 498.86, 562.54, 619.48, 660.86 and 703.65 cm^{-1} are described to the formation of Ce-O bonds, which are in the IR range (800-400 cm^{-1}) of crystalline cerium oxide active phonon modes. 2200 cm^{-1} to $4000 cm^{-1}$ reflects surface adsorbed H-O-H, C-C, H-H, and C-H bond stretching prints [48-50].

iv) Morphological and Elemental Analysis

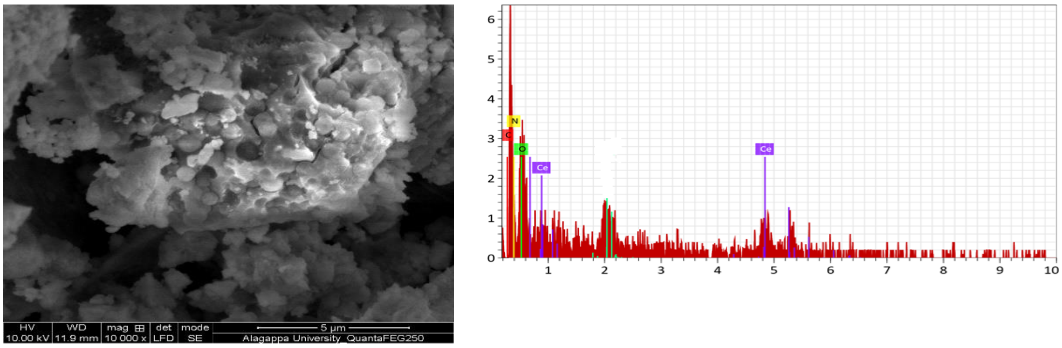

Fig. 7 SEM and Energy dispersive spectrum of Nanoceria

The images show spherical shape and hexagonal nanoparticles, which are in agreement with the XRD. The agglomeration could be encouraged by densification resulting in the slight space between particles. The energy spectrum of the sample exposed the occurrence of Ceria.

C) Antioxidant Studies for Nanoceria from *clitoria ternatea*

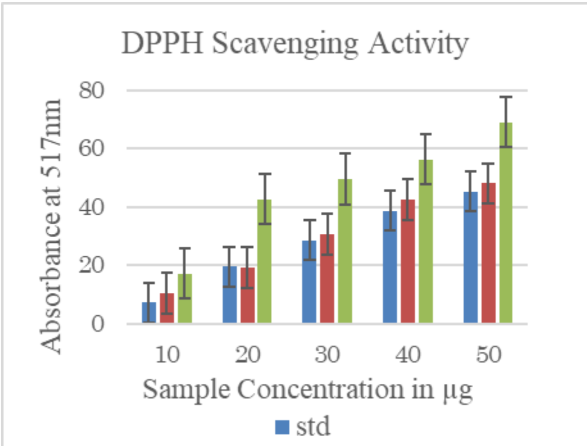

Fig. 8 scavenging activity of DPPH (Di-Phenyl picryl hydrazyl) by Nanoceria

Figure 8 showed the DPPH Free Radical Scavenging Assay of biosynthesized nanoceria (CeO_2) where ascorbic acid (AA) was taken as standard. A freshly prepared DPPH solution showed a deep purple color with maximum absorption at 517 nm. This purple color vanishes when an antioxidant is existing in the medium. Thus, antioxidants molecules can reduce DPPH free radicals and convert them to a colorless product, resulting in a decrease in absorbance at 517 nm [51]. The plant extract showed almost equal or higher activity compared with standard. So, the nanoparticles activity showed a growing way. The DPPH free radical scavenging assay showed 69.18% inhibition efficiency of 50µg/500µl nanoceria. This specified the potent inhibitory capacity of nanoceria when compared with ascorbic acid. The percentage of inhibition of free radicals increased with increase in the concentration of samples.

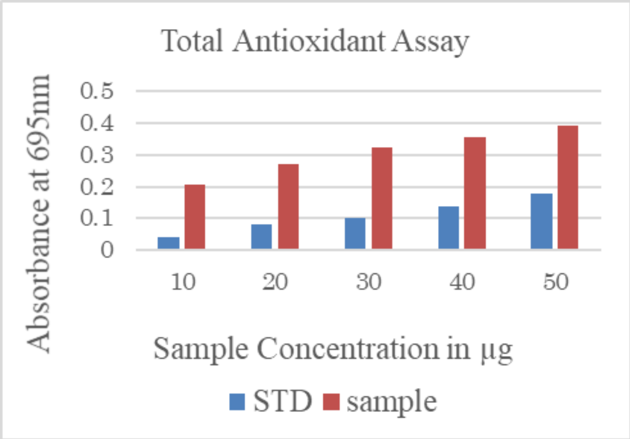

Fig. 9 Total Antioxidant assay for biosynthesized Nanoceria

Fig.9 showed the Total antioxidant capacity of ascorbic acid (AA) and biosynthesized nanoceria. The Total antioxidant capability was valued based on the formation of the phosphomolybdenum complex where the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the development of a green phosphate/Mo (V) complex with the highest absorption at 695 nm [52]. The cerium oxide nanoparticles were found to have very high total antioxidant capacity as compared to the standard.

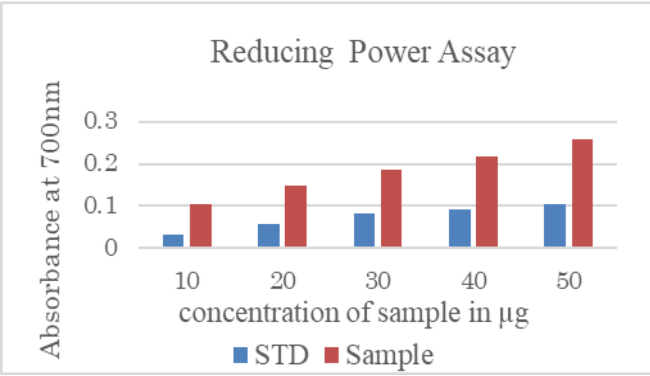

Fig. 10 Reducing Power assay for biosynthesized Nanoceria

Figure 10 showed the Reducing Power Examine of biosynthesized nanoceria where ascorbic acid (AA) was engaged as standard. The reducing ability of a compound depends on the presence of reductants [53] which have been shown to use antioxidant action by breaking the free radical chain by donating a hydrogen atom [54]. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. By measuring the development of Perl's Prussian blue at 700 nm, it is possible to regulate the Fe^{2+} concentration [55]. Nanoceria was found to have very high reducing capacity when compared to the standard and increased with increasing concentration of samples.

4. CONCLUSION

In this process, cerium oxide nanoparticles were successfully synthesized in size of 7.562nm eco-friendly without adding any harmful or hazardous chemical reagents and it was also synthesized at room temperature. Synthesizing with a room temperature of Nanoceria has small, has beneficial biomedical property compared with the nanoparticles produced at high temperature [56]. The synthesized nanoceria confirmed by various characterization techniques and the antioxidant property also proved by chemical assays. The antioxidant is very significant because they are talented in slowing or preventing the oxidation process and ROS activity. Since the synthesized nanoceria could be acting as antioxidant agent for oxidative-stress related diseases like etc.

ACKNOWLEDGEMENT

I am P. Krishnaveni (Register No: 17211232272005) acknowledge the research centre Sri Paramakalyani Centre for Excellence in Environmental Science, Manonmaniam Sundaranar University, Alwarkurichi, providing the support for this research work.

CONFLICT OF INTEREST

Author has no conflict of interest.

REFERENCES

1. Ramsden JJ- What is nanotechnology? Nature. (Lond.). 1990; 344:524-526.
2. Karrina MC, SyedTofail AM- Nanoparticles in biomedical applications. Advances in Physics. 2017; X (2):1:54-88.
3. Dong Fan, Koodali RT, Wang H, Ho W- Nanomaterials for Environmental Applications. Journal of Nanomaterials. 2014; article ID: 276467: 4pages.
4. Santos CSC, Gabriel B, Blanchy M, Menes O, García M, Blanco M, Arconada N, Neto V- Industrial applications of nanoparticles – a prospective overview. Materials Today. 2015; Proceedings 2:456-465.
5. Peters RJB, Bouwmeester H, Gottardo S, Amenta V, Arena M, Brandhoff P, Marvin HJP, Mech A, Moniz FB, Pesudo LQ, Rauscher H, Schoonjans R, Undas AK, Vettori MV, Weigel S, Aschberger K- Nanomaterials for products and application in agriculture, feed and food, Trends in Food Science & Technology. 2016; 54:155-164.
6. Stankic S, Suman S, Haque F, Vidic J- Pure and multi metal oxide nanoparticles: synthesis, antibacterial and cytotoxic properties. Journal of nanobiotechnology. 2016; 14:73.
7. Dahle JT, Arai Y- Environmental geochemistry of cerium: applications and toxicology of nanoceria. Int. J. Environ. Res. Public Health. 2015; 12 (2):1253-1278.
8. Karakoti AS, Kuchibhatla SVNT, Babu KS, Seal S- Direct synthesis of nanoceria in aqueous polyhy-droxyl solutions. J Phys Chem C. 2007; 111(46):1723240.
9. Chen S, Hou Y, Cheng G, Zhang C, Wang S, Zhang J- Cerium oxide nanoparticles protect endothelial cells from apoptosis induced by oxidative stress. Biol. Trace Elem. Res. 2013; 154:156-166.

10. Chigurupati S, Mughal MR, Okun E, Das S, Kumar A, McCaffery M, Seal S, Mattson MP- Effects of cerium oxide nanoparticles on the growth of keratinocytes fibroblasts and vascular endothelial cells in cutaneous wound healing. Biomaterials. 2013; 34:2194-2201.
11. Giri S, Karakoti A, Graham RP, Maguire JL, Reilly CM, Seal S, Rattan R, Shridhar V- Nanoceria: a rare-earth nanoparticle as a novel anti-angiogenic therapeutic agent in ovarian cancer. PLOS One. 2013; 8:54578.
12. Wason MS, Colon J, Das S, Seal S, Turkson J, Zhao J, Baker CH- Sensitization of pancreatic cancer cells to radiation by cerium oxide nanoparticle-induced ROS Production. Nanomedicine. 2013; 9:558-569.
13. Wason MS, Zhao J- Cerium oxide nanoparticles: potential applications for cancer and other diseases. Am. J. Transl. Res. 2013; 5:126-131.
14. Cimini A, D'Angelo B, Das S, Gentile R, Benedetti E, Singh V, Monaco AM, Santucci S, Seal S- Antibody-conjugated PEGylated cerium oxide nanoparticles for specific targeting of Aβ aggregates modulate neuronal survival pathways. Acta Biomater. 2012; 8:2056-2067.
15. Estevez AY, Pritchard S, Harper K, Aston JW, Lynch A, Lucky JJ, Ludington JS, Chatani P, Mosenthal WP, Leiter JC, Andreescu S, Erlichman JS- Neuroprotective mechanisms of cerium oxide nanoparticles in a mouse hippocampal brain slice model of ischemia, Free Radical. Biol. Med. 2011; 51:1155-1163.
16. Bhargava N, Das M, Karakoti AJ, Patil S, Kang JF, Stancescu M, Kindy MS, Seal S, Hickman JJ- Regeneration of adult mice motoneurons utilizing a defined system and anti-oxidant nanoparticles. J. Nanoneurosci. 2009; 1:130-143.
17. Korsvik C, Patil S, Seal S, Self WT- Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles. Chem Commun (Camb). 2007; 10:1056-8.
18. Chen J, Patil S, Seal S, McGinnis JF- Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. Nat Nanotechnol. 2006; 1(2):142-50.
19. Tarnuzzer RW, Colon J, Patil S, Seal S- Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage. Nano Lett. 2005; 5(12):2573-7.
20. Singh M, Manikandan S, Kumaraguru AK- "Nanoparticles: a new technology with wide applications." Research Journal of Nanoscience and Nanotechnology. 2011; 1(1):1-11.
21. Mukherjee P, Kumar V, Kumar NS, Heinrich- The ayurvedic medicine Clitoria ternatea-from traditional use to scientific assessment. Journal of Enthnopharmacology. 2008; 120:291-301.
22. Nirmal S, Bhalke R, Jadhav R, Tambe V- Anthelmintic activity of *Clitoria ternatea*. Pharmacologyonline. 2008; 1:114-119.
23. Banerjee SK, Chakravarti RN- Taraxerol from *Clitoria ternatea*. Bulletin of the Calcutta School of Tropical Medicine. 1963; 11:106-107.

24. Banerjee SK, Chakravarti RN- Taraxerone from Clitoria ternatea. Bulletin of Calcutta School of from Clitoria ternatea. Bulletin of Calcutta School of Tropical Medicine. 1964; 12:23.
25. Uma B, Prabhakar K, Rajendran S- Phytochemical analysis and Antimicrobial Activity of *Clitoria ternatea* Linn. Against extended spectrum beta lactamase producing enteric and urinary pathogens. Asian Journal of Pharmaceutical and Clinical Research. 2009; 2:94-96.
26. Chen S, Hou Y, Cheng G, Zhang C, Wang S, Zhang J- Cerium oxide nanoparticles protect endothelial cells from apoptosis induced by oxidative stress, Biol. Trace Elem. Res. 2013; 154:156-166.
27. McCune LM, Johns T- Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. J. Ethnopharmacol. 2002; 82(2-3):197-205.
28. Banerjee J, Narendhirakannan RT- Phytochemical analyses, antibacterial, in vitro antioxidant and cytotoxic activities of ethanolic extract of *Syzygium cumini* (L.) seed extract. IJPSR. 2011; 2(7):1799-1806.
29. Koleva II, Van Beek TA, Linssen JPH, DeGroot A, Evstatieva LN- Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis. 2002; 13:8-17.
30. Makari HK, Haraprasad N, Patil HS, Ravikumar- In Vitro Antioxidant Activity of the Hexane and Methanolic Extracts of Cordia Wallichii and Celastrus Paniculata. Internet Journal of Aesthetic and Antiaging Medicine. 2008; 1:1-10.
31. Siavsh Iravani- Bacteria in Nanoparticle Synthesis: Current Status and Future Prospects. International Scholarly Research Notices. 2014; Article ID 359316:18 pages.
32. Iravani S- Green synthesis of metal nanoparticles using plants. Green Chemistry. 2011; 13:2638-2650.
33. Reis SF, Rai DK, Abu-Ghannam N- Water at room temperature as a solvent for the extraction of apple pomace phenolic compounds. Food Chemistry. 2012; 135:1991-1998.
34. Chakraborty S, Sahoo S, Bhagat A, Dixit S- Studies on antimicrobial activity, phytochemical screening tests, biochemical evaluation of *Clitorea ternatea* Linn. plant extracts. International journal of research. Granthaalayah 2017; 5(10).
35. Mohamad NAN, Arham NF, Jai J, Hadi A- Plant Extract as Reducing Agent in Synthesis of Metallic Nanoparticles. A Review Advanced Materials Research. 2014; 832:350-355.
36. Ronson CT, Diego S- UV/VIS/IR Spectroscopy Analysis of Nanoparticles, Nanocomposix. 2012; Vol.1.1
37. Tomaszewska E, Soliwoda K, Kadziola K, Celichowski G, Cichomski M, Szmaja W, Grobelny J- Detection limits of DLS and UV-is spectroscopy in characterization of polydisperse nanoparticles colloids. J. Nanomaterials. 2013; Article ID: 313081:10 pages.

38. Orel ZC, Orel B- Optical properties of pure CeO_2 and mixed CeO_2/ SnO_2 thin film coatings. Phys. Status Solidi (b). 1994; 186(1):K33-K36.
39. Davoodbashal M, Park BR, Rhee WJ, Lee SY, Kim JW- Antioxidant potentials of nanoceria synthesized by solution plasma process and its biocompatibility study. Biophysics. 2018; 645:42-49.
40. Kar S, Patel C, Santra S- Direct room temperature synthesis of valence state -engineered ultra-small ceria nanoparticles: investigation on the role of ethylenediamine as a capping agent. J. Phys. Chem. C. 2009; 113:4862-4867.
41. Singh S, Dosan T, Karakoti AS, Kumar A, Seal S, Self WT- A phosphate-dependent shift in redox state of cerium oxide nanoparticles and its effects on catalytic properties. Biomaterials. 2011; 32:6745-6753.
42. Emsley J- Cerium, in: Nature's Building Blocks: An A–Z Guide to the Elements. Oxford University Press, Oxford. 2011; pp.120-125.
43. Maqbool Q, Nazar M, Naz S, Hussain T, Jabeen N, Kausar R, Anwaar S, Abbas F. Jan T- Antimicrobial potential of green synthesized CeO_2 nanoparticles from *Olea europaea* leaf extract. Int. J. Nanomed. 2016; 11:5015.
44. Qaisar Maqbool- Green-synthesised cerium oxide nanostructures (CeO_2-NS) show excellent biocompatibility for phyto-cultures as compared to silver nanostructures (Ag-NS). RSC Adv. 2 017; 7:56575-56585.
45. Maensiri S, Masingboon C, Laokul P, Jareonboon W, Promarak V, Anderson LP, Seraphin S- Egg white synthesis and photoluminescence of plate like clusters of CeO_2 nanoparticles. Crystal Growth Des. 2007; 7:950-956.
46. Deus RC, Cilense M, Foschini CR, Ramirez MA, Longo E, Simões AZ- Influence of mineralizer agent son the growth of crystalline nanosphere by the microwave-hydrothermal method. J. Alloys Compd. 2013; 550:245-251.
47. Das J, Choi YJ, Han JW, Reza AMMT & Kim JH- Nanoceria-mediated delivery of doxorubicin enhances the anti tumour efficiency in ovarian cancer cells via apoptosis. Scientific Reports 7. 2017; Article No. 9513.
48. Arumugam C, Karthikeyan, Hameed ASH, Gopinath K, Gowri S, Karthika V- Synthesis of cerium oxide nanoparticles using Gloriosa superb L. Leaf extract and their structural, optical and antibacterial properties, Mater. Sci. Eng. 2015; 49:408-415.
49. Khan SB, Faisal M, Rahman MM, Jamal A- Exploration of CeO nanoparticles as chemi-sensor and photo-catalyst for environmental applications. Sci Total Environ. 2011; 15:2987-2992.
50. Arunachalam T, Karpagasundaram M, Nithya R- Ultrasound assisted green synthesis of cerium oxide nanoparticles using Prosopis juliflora leaf extract and their structural, optical and antibacterial properties. Materials Science-Poland. 2017; 35(4):791-798.

51. Miladi S and Damak M- In vitro antioxidant activities of Aloe Vera leaf skin extracts. Journal de la Societe Chimique de Tunisie. 2008; 10:101-109.
52. Narendhirakannan RT and Smeera T- In vitro antioxidant studies on ethanolic extracts of leaves and stems of Nyctanthes arbor-tristic(L.) (Night-flowering Jasmine). International Journal of Biological and Medical Research. 2010; 1:188-192.
53. Duh PD, Tu YY and Yen GC- Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum moifolium* Ramat). Lebensmittel–Wissenschaft und Technologie. 1999; 32:269-277.
54. Schimada VL, Fujikawa K, Yahara K and Nakamura T- Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 1992; 40:945-948.
55. Narendhirakannan RT and Limmy TP- In vitro antioxidant studies on ethanolic extracts of leaf, stem and root of Sida rhombifolia (L.). International Journal of Pharma and Biosciences. 2010; 1:1-10.
56. Karakoti AS, Munusamy P, Hostetier K, Kodali V, Kuchibhatla S, Orr G, Pounds JG, Teeguarden JG, Thrall BD, Baer DR- Preparation and Characterization Challenges to understanding environmental and biological impacts of ceria nanoparticles. Surf. Interface Anal. 2012; 44:882-889.