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GREEN SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITIES OF CERIUM NANOPARTICLES FROM TOMATO FRUIT EXTRACT

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ABSTRACT

The environmental friendly synthesis of nanoparticles process is revolutionary step in the field of nanotechnology. The present study focuses on the simple effective and rapid approach for the synthesis of cerium nanoparticles and investigation of its anticancer activity. On addition of fruit extract to cerium sulphate solution the change in color of the reaction mixture was observed which proved the formation of nanoparticles. The synthesized cerium nanoparticles were characterized by various instrumental methods like UV-Vis spectroscopy, Scanning Electron Microscopy (SEM), and X Ray Diffraction methods. The synthesized cerium nanoparticles were spherical in shape with an average particle size of 5-10nm. The anti-inflammatory activity of cerium nanoparticles was tested and confirmed by invitro method. Hence, the present research aims to open new avenues for the improvement of medicinal uses with the synthesis of cerium nanoparticles by using tomato extract for various ailments and to bring the anti-inflammatory medicinal plants to the scientists notice, to educate awareness and add values to the resources.

KEYWORDS: Nanoparticles, particle size, green synthesis, anti-inflammatory activity.

INTRODUCTION

As the toxicological attributes of the conventional route of synthesizing metal and metaloxide nanoparticles are inevitable, an exponential increase in the search of an alternate route of synthesis exists in the recent scientific research. Plant derived products, such as the extracts of leaf, seed and flower play a vital role in chelating chemical compounds pointing out an

effective, cleaner and eco safe way of synthesizing nanoparticles. Green chemistry allows surface modification which has a strong influence on the physical, chemical, electrical and optical properties of nanoparticles. Application of green chemistry for the synthesis of biocompatible metal nanoparticles (NPs) has gained considerable attention in recent years for potential applications in biomedicine.[1,2] Integration of green chemistry principles to nanotechnology is one of the key issues in nanoscience research. The development of the concept of green nanoparticle preparation has grown the need for environmentally benign metal nanoparticle synthesis protocols to avoid adverse effects in medical applications.[3]

Nanotechnology is unique in that it represents not just one specific area, but a vast variety of disciplines ranging from basic material science to personal care applications.[4] The development of nanoparticles for the delivery of therapeutic agents has introduced new opportunities for the improvement of medical treatment.[5] Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures.[6] They exhibit completely new or improved properties bases on specific characteristics such as size, distribution and morphology[7] than compared to the bulk materials. Nanomaterials are present in some sunscreens, toothpaste, sanitary ware coatings and even food products.[8] Nanoparticles of noble metals, such as gold, silver and platinum, are widely applied in products that directly come in with human body, so there is a growing need to develop environmentally friendly processes of nanoparticles synthesis does not use toxic chemicals.[9] The inorganic nanoparticles are found to be effective in scavenging oxygen based free radicals.[10]

Thanks to unique physicochemical characteristics of CeO_2-NS due to which they possess broad range of biochemical and plant biotechnological applications.[11] Meanwhile, in case of tomato, CeO_2-NS tremendously supplemented the growth factors with positive effect at concentration of less than 10 mg L^{-1}. Our motivation towards synthesizing tomato mediated cerium nanoparticles and testing for anti-inflammatory and anticancer activity stemmed from these findings. Herein, we report the green synthesis of cerium nanoparticles using tomato fruit. The prepared nanoparticles were characterized by UV-Vis, SEM, FTIR and XRD.

Characterization of Cerium nanoparticles

Fresh tomato (20 gms) were washed separately and later smashed inside a grinder. The smashed fruits were then filtered using whattman filter paper No.1 to remove the debris. The filtred juice was centrifuged at 10000 rpm for 10 minutes to obtain the liquid fruit extract and

stored at 4^0C, further the filtrate was treated with 90 ml of aqueous solution of 1mM cerium sulphate and the formed nanoparticles were filtered.

UV-Vis Spectra analysis: Ultraviolet visible spectrophotometer (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. This means it uses light in the visible and adjacent (near UV and near- infrared (NIR) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. Ultraviolet visible spectrophotometer (UV-Vis) is procured from Shimadzu. A small aliquot of the sample was taken for UV-Vis spectrum analysis (200-800nm).

SEM Analysis of Cerium Nanoparticles: Scanning Electron Microscope (SEM) analysis was done using (JEOL Model JSM - 6390LV) SEM machine. The films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

Fourier Transform Infrared: Dried powder of the CeNPs was subjected to analyze the presence of possible functional groups for resulting in formation of CeNPs using Fourier transform infrared (ATR schimadzu Japan) spectroscopy.

EDAX Analysis: EDAX (Energy Dispersive X-ray) analysis of purified CeNPs was carried out using the instrument for confirming the elemental composition of the sample.

Anti-inflammatory activities

In-vitro anti-inflammatory activity of new Cerium nanoparticles with tomato extract by inhibition of albumin denaturation method.

The anti-inflammatory activity of Cerium nanoparticles with tomato extract was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima *et al* and Sakat *et al*[12,13] followed with minor modifications. The reaction mixture was consists of new chemical compound **(**100, 200, 300, 400 and 500 µg/ml**)** and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The new chemical compound mixture was incubated at 37^0C for 20 min and then heated to 51^0C for 20 min, after cooling the sample the turbidity was measured at 660 nm. (UV- Visible Spectrophotometer Model Shimadzu) The experiment was performed in triplicate.

The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

RESULTS AND DISCUSSION

UV-Vis Spectroscopy studies

Fig.1 shows the UV-Vis absorption spectrum of synthesized cerium nanoparticles using Cary 500 in the range of 200 nm to 800 nm. Absorption in the near ultraviolet region arises from electronic transitions associated within the sample. The cerium nanoparticles showed the peak around 314 nm which is very specific of cerium nanoparticles. The spectrum of nanostructure CeO_2 nanoparticles indicates that the pure cerium oxide nanoparticles exhibit maximum absorption peaks at 341 nm. Nanostructured CeNPs exhibit a strong absorption bands at 314 nm due to charge-transfer transactions from oxygen 2p to cerium 4f overturns the well f-f spin orbit slitting of the Ce 4f state. The broadness of the absorption shoulder in the UV region is attributed to the self assembly of the nanoparticles and this is confirmed from SEM study.

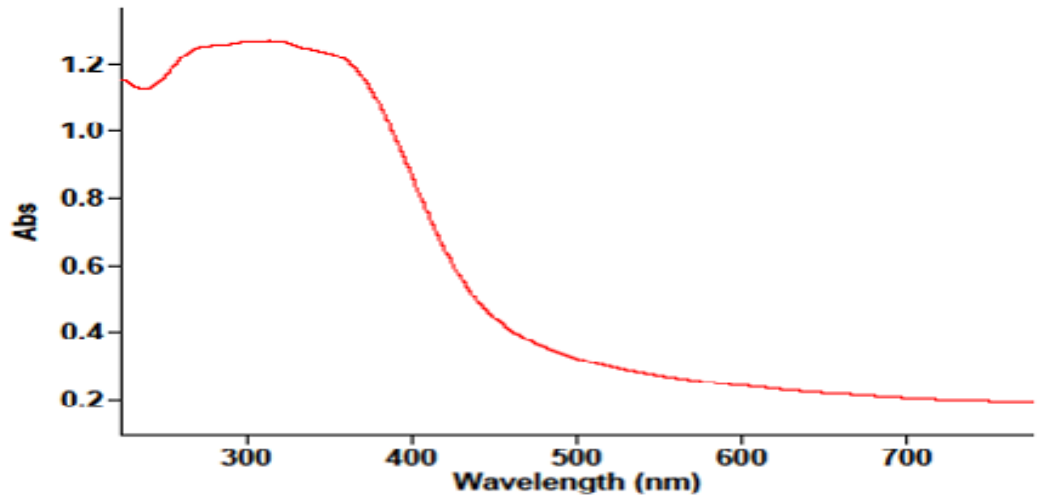

Fig. 1: UV-Vis spectrum of synthesized CeNPs.

SEM Analysis

The SEM image (Fig.2a,b,c,d) shows average CeNPs size of 5.09 and 5.10nm at the accelerating voltage of 25 kV with working distance of the sample at 25mm and it is noticed that most of particles are spherical, flower like and some are elongated in shape and these particles are observed to be agglomerated.

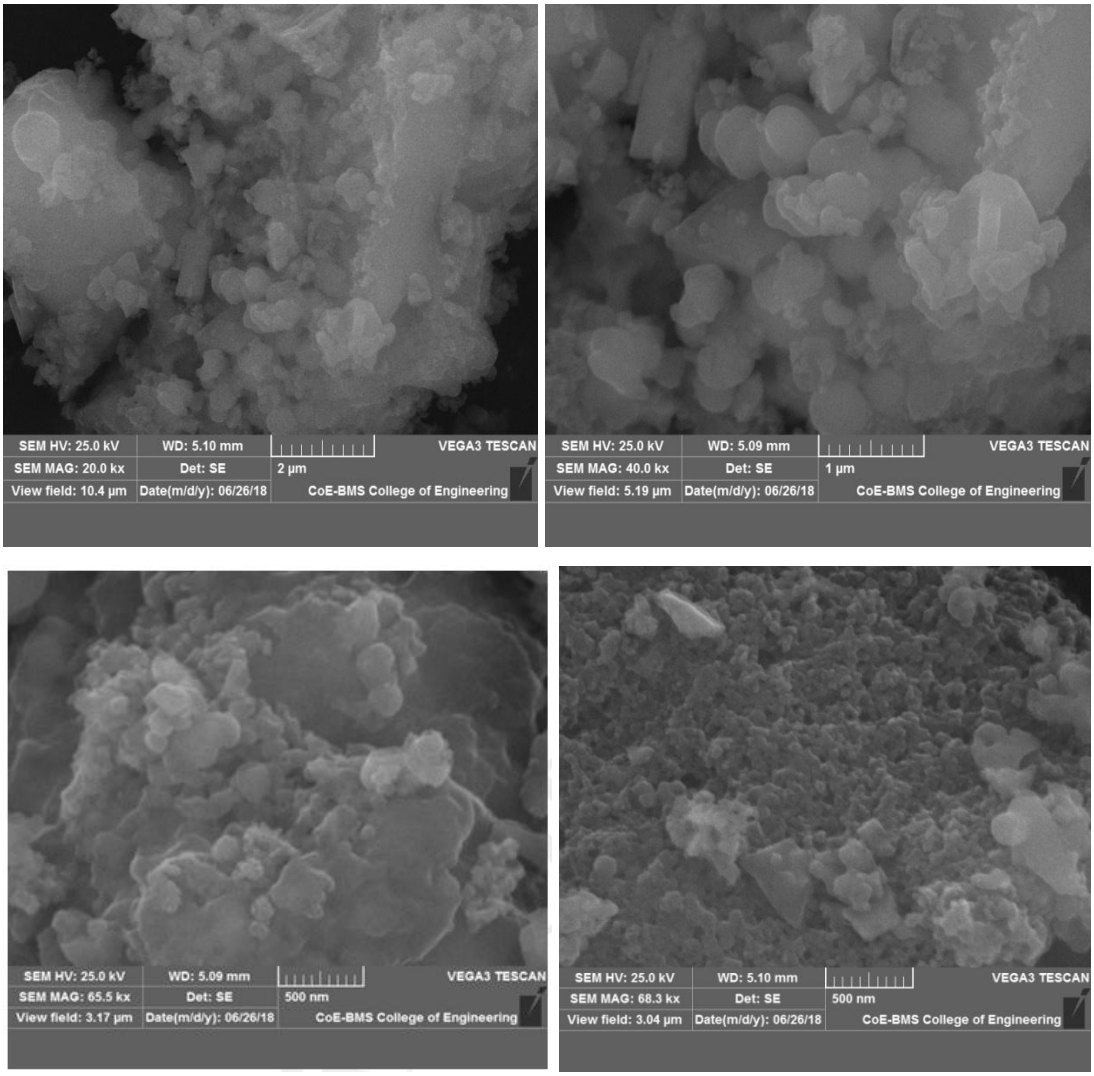

Fig. 2: (a, b, c & d).

Fourier Transform Infrared

Representative FTIR spectra of pure tomato extract and CeNps are shown in figure.3 respectively. The spectrum of tomato extract shows a number of frequencies in the range 2100 to 1000 cm^{-1} corresponding to C=O stretching (1680 cm^{-1}) of organic acid present, secondary amine (1620 cm^{-1}) from the proteins resent in the extract. In comparison with the spectra, it is evident that the peak (1680 cm^{-1}) due to acid groups present in tomato extract is missing in the CeNPs spectrum which confirms that these groups are responsible for reduction. The shifting of bands from 1620 to 1610 cm^{-1}, 1450 to 1445 cm^{-1} and 1060 to 1050 cm^{-1} indicates the direct involvement of proteins in stabilizing the sol particles.[14] The FTIR spectra also showed a broad absorption band at 3380 cm^{-1} mainly due to OH groups on the surface of the nanoparticles. The spectra showed a peak at 555 cm^{-1} which is the

characteristic peak of CeNPs. A new peak at 1050 cm^{-1} was found to have been appeared confirmed the synthesis of nanoparticles.

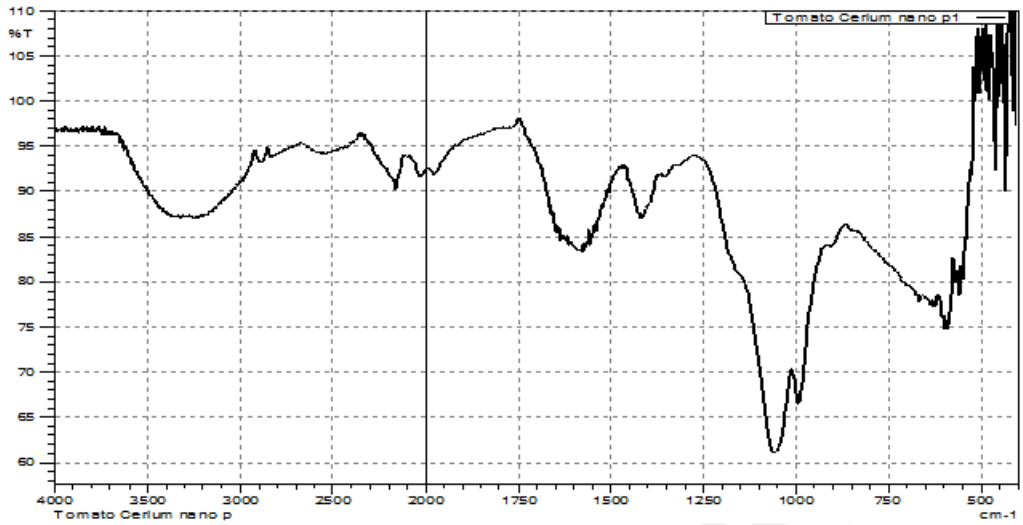

Fig. 3: IR Spectra of CeNPs.

EDAX Analysis: Energy dispersive spectroscopy (EDAX) of CeO_2 prepared by wet synthesis is shown in Fig.4&5 which confirms the existence of Ce and O with weight percent. EDAX shows peaks of cerium and oxygen of as-prepared sample with less impurity such as K, Cl, N and S Table.1. The metallic cerium nanoparticles generally show typical optical absorption peak approximately around 5 KeV due to surface Plasmon resonance.

Table 1.

Element		Weight %	Atomic %
C		25.49	45.43
O		35.28	47.19
P		0.73	0.50
S		1.94	1.29
Ce	36.28	5.59	

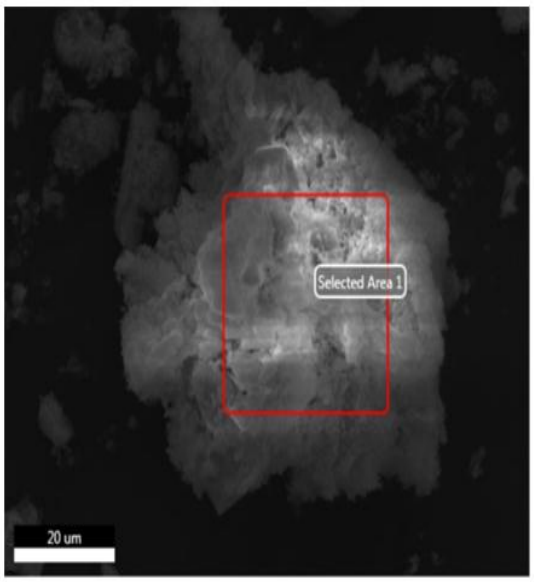

Fig. 4.

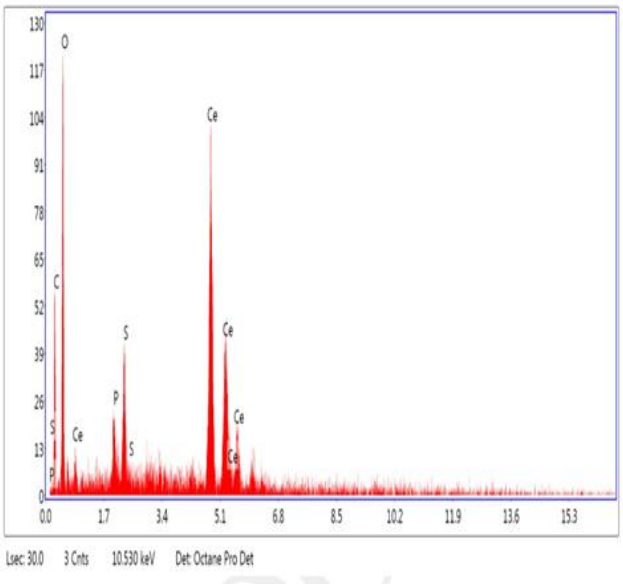

Fig. 5.

Anti-inflammatory activities

Effect of Cerium nanoarticles with tomato extract on Protein Denaturation

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of new chemical compound to inhibit protein denaturation was evaluated. It was effective in inhibiting heat induced albumin denaturation. The percentage inhibition of protein denaturation was found to be 25 – 52. Maximum inhibition of 52% was observed at 500 µg/ml. Aspirin, a standard anti- inflammation drug showed the maximum inhibition 70% at the concentration of 100 µg/ml compared with control. The results are presented in table. 1.

Table 1: Effect of Cerium nanoarticles with tomato extract on heat induced protein denaturation.

Sl. No.	Concentration (µg/ml)	Absorbance at 660 nm	% inhibition of protein denaturation
1	Control	0.40 ± 0.04	---
2	100	0.30 ± 0.02*	25
3	200	0.27 ± 0.01**	32
4	300	0.25 ± 0.03**	37
5	400	0.22 ± 0.01**	45
6	500	0.19 ± 0.03**	52
7	Aspirin 100	0.12 ± 0.02**	70

Values are mean ± SEM, n=3,*Significant values, p<0.01 and p<0.001 compared to control.

Statistical analysis

The data obtained from the above findings were subjected to statistical analysis following one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results using Graph pad prism software.

CONCLUSIONS

The green synthesis of tomato fruit extract was shown to be eco-friendly and produced nanoparticles are fairly uniform in size and shape. The developed nanoparticles showed good anti-inflammatory activity. This rapid synthesis technique can be a promising method for the preparation of nanoparticles. In future enormous number of cerium nanoparticles using various plant extracts should be synthesized and its various medicinal values should be analysed to provide low cost natural products for various ailments. This method shall be valuable in environmental, biotechnological, pharmaceutical and medical applications.

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