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Biosynthesis and Biological Applications of Cerium Oxide Nanoparticles

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Abstract

Nanobiotechnology generates tools from nanotechnology which are used to study biological processes. Nowadays, nanoceria or cerium oxide nanoparticles havebeen employed in varied medicinal applications including antibacterial, antioxidant, antidiabetic, anticancer, drug delivery systems etc. They have unusual property to switch between trivalent and tetravalent oxidation states which consider them excellent candidates for other commercial applications also. The present investigation was focused ongreen synthesis of nanoceria or cerium oxide nanoparticles (CeO_2 NPs) from Pomegranate(*Punica granatum*)peel extract which were also characterized by UV Visible, FT-IR spectroscopy, SEM analysis, EDX and X-ray Diffraction Studies. The antioxidant and antibacterial potential of synthesized CeO_2 NPswere also evaluated. The results displayed that they expressed good antimicrobial activity against *E. coli* and also showedimmense antioxidant activity.

Keywords:*Punica granatum*, Cerium oxide nanoparticles, Nanoceria, Antioxidant, Antibacterial activity.

1. Introduction

Nanotechnology is leading research discipline of the present era with multiple applications in the healthcare sector, industries, imaging etc. It utilizes nanoscale structures of size 1-100 nm. These structures due to their unique properties are of remarkable interest. Among different NPs such as AuNPs, Ag NPs, Cu NPs, Cerium oxide nanoparticles have gained importance due to their high stability, surface chemistry and biocompatibility. Actually, Cerium, a lanthanide series rare earth metal having atomic number 58. It exhibits attractive catalytic properties due to its electronic configuration, unique surface structures and redox activity[1]. It is able to exist in both +3 and +4 stages which make it capable of forming nanoscale cerium oxide particles such as CeO_2NPsand Ce_2O_3NPs [2,3]. These nanoparticles are gaining importance due to their biomedical applications these days. They exhibit antimicrobial, anticancer, anti-inflammatory and antidiabetic potential. They also display defensive role against harmful radiations, toxicants and also play protective role during various pathological conditions [4].

Presently, CeO_2NPs are synthesized by physical, chemical and biological methods. Physical and chemical methods utilize toxic solvents which are hazardous to the biodiversity and ecosystem. Besides that, NPs

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synthesized via these methods are toxic and unstable. In opposite to these, biological method utilizes various biological resources and produce stable NPs. Biological method is also termed as green synthesis. Earlier several scientists, have synthesized successfully from plant materials[5,6]In view of that, in the present work fruit peel was utilized to synthesize NPs. Fruit peels are the pollution controlling agencies and majorly used in fruit processing industries. They contain various phytochemicals having antibacterial activity that can be used by the same food industries as food preservatives [7]. Several food wastes and agro residues such as grape seeds, lemon peels, pomegranate peels, citrus peels and green walnut husks containing different bioactive compounds have been tested for their antimicrobial and antioxidant potential [8,9]. Thus, efforts have been made by various researchers to develop the possibility of reusing plant wastes as the main source of organics. The Pomegranate peels are utilized as a common remedy throughout the world in traditional medicines because of their higher mordancy properties. Pomegranate peel primarily contains tannins, flavonoids, polyphenols and anthocyanins such as delphinidins and cyanidins [10-13].

The present study was focused on synthesis and characterization of cerium oxide nanoparticles from Pomegranate(*Punica granatum*) peel extract. Their antioxidant and antibacterial potential were also evaluated.

2. MATERIALS AND METHODS

The Cerium (III) nitrate hexahydrate used in the present study was procured from Sigma–Aldrich Chemical Co., USA. All the other chemicals used were of analytical grade.

2.1. Plant Material

The Pomegranate (*Punica granatum*) was purchased from the Tirupattur vegetables market and its rind was used as sample. It was grinded to fine powder as per the standard approved protocol for further use.

2.2. Bio-synthesis of CeO_2 nanoparticles

Powder of Cerium oxide was mixed in with 100 mL of deionized water and worked up for 24 h. The came about extraction was assembled in a funnel shaped flagon. From that point, 10 mL Cerium (III) nitrate hexahydrate ($CeN_3O_9.6H_2O$) was poured to 100 mL of *Punica granatum* strip remove. This blend was stimulated continually at a temperature of 80°C for 4–6 hrs. A white quickens confined. The procured extraction was sieved with the help of Whatman No. 1 channel paper[11 µm, medium flow filter paper], the cerium oxide nanoparticles were obtained further the dried material was shown as CeO_2NPs.

2.3.CHARACTERIZATION OF CERIUM OXIDE NANOPARTICLES

2.3.1 UV-Visible spectroscopy analysis

The prepared CeO_2 NPs were seen under UV-Vis spectrophotometer (Model Shimadzu-2700) with appropriate standard and control and it recognized by its trademark absorbance and recurrence.

2.3.2 Fourier-Transform Infrared Spectroscopy (FT-IR)

The FTIR of the incorporated CeO_2 NPs was taken by Nicolet Impact 400 utilizing 500 mg of KBr pellet.

2.3.3Scanning electron microscopy analysis (SEM)

The isolated powdered model was taken with gold particles using a molecule coater under: 0.1 torr pressure, 20 mA present and 70s covering time. Subsurface structure was envisioned by SEM using a 15-

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kVanimating voltage. SEM estimations were investigated out on a Leica sound system check 440 examining electron magnifying instruments. The SEM information helps to shape out the structure and size of the readied nanoparticles.

2.3.4Energy Dispersive X-ray Spectrometer Analysis (EDX):

The energy dispersive X-ray (EDX) spectrometer has confirmed the presence of elemental cerium and the oxygen signal of the cerium oxide nanoparticles.

2.3.5X-ray Diffraction Studies(XRD):

Cerium oxide Nanoparticles were confirmed in the form of crystallite size determined by the Scherrer equation.

2.4. ANTIOXIDANT ASSAY

2.4.1 1,1-diphenyl-2-picrylhydrazyl assessment (DPPH)

The cell reinforcement capacity of the CeO_2NPs were decided through DPPH (2,2-diphenyl-1-picrylhydrazyl) examination. The 1mg/mL arrangement of DPPH in ethanol was sorted out and connected with cerium oxide nanoparticle (40 µg) was determined at 517 nm utilizing spectrometrically.The supportive control was utilized as butylhydroxyanisole (BHA). All of the samples were tested in triplets.

2.5. Antibacterial activity

Mueller Hinton Agar (MHA) plates were fit and inoculated with trial bacterial isolates by dispersing the bacterialinoculate on the surface of the media. Wells with6 mm diameter were punched in the MHA. Cerium oxide nanoparticles with 25µg/ml concentrations were prepared with 1 ml of Dimethyl sulfoxide (DMSO), mixed well and poured into the well. Well comprising DMSO alone acts as a negative control and streptomycin acts as a supportive control. The plates were separated at 37°C for one day. The antibacterial analysis was checked by assessing the expansiveness of the zone in mm as a triplet regard.

2.6 Statistical Analysis

The experimentation was done in triplicate for each component. The results were declared as percentage decrease with regard to control values and compared by one-way ANOVA and Tukey's test. A deviation was considered factually critical if $p \leq 0.05$.

3.RESULTS & DISCUSSION

During the past few years, important research has been carried out in the tract of Nanobiotechnology with the aim of raising its activity. In the current study, CeO_2 NPs was synthesized from *Punicagranatum* peel extract as reducing as well as capping agent and it was characterized by UV and FTIR spectroscopy analysis, SEM, E-DAX followed by X-ray Diffraction Studies. The SEM data assist to shape out the structure and size of the prepared nanoparticles. The antioxidant and antibacterial activity were performed to determine the efficiency of the CeO_2 NPs. The results indicate the improved efficacy was seen in the process and the findings as follows.

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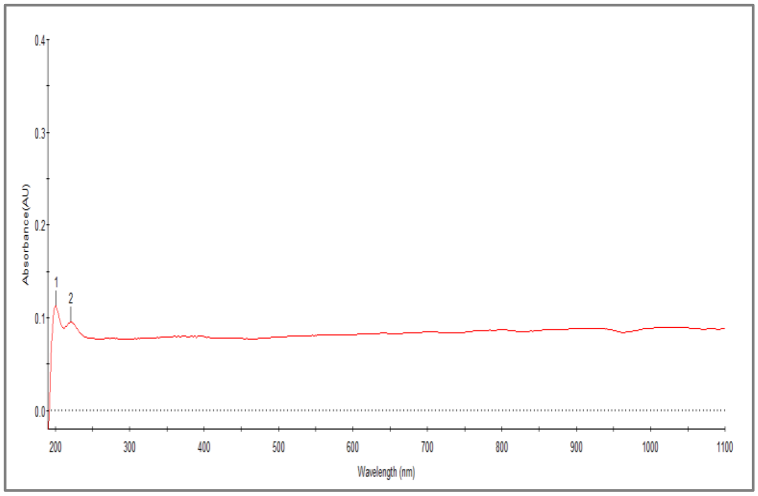

Figure 1: UV- Visible Spectroscopy of CeO_2 NPs from *Punicagranatum* peel extract

The figure 1, shows that the characteristic peak for CeO_2 NPs was 220 nm and 250nm with an absorbance of 0.11. The results are in agreement with available literature [11]. From figure 2, the FTIR spectrum showed different absorption peaks and functional groups of the sample.

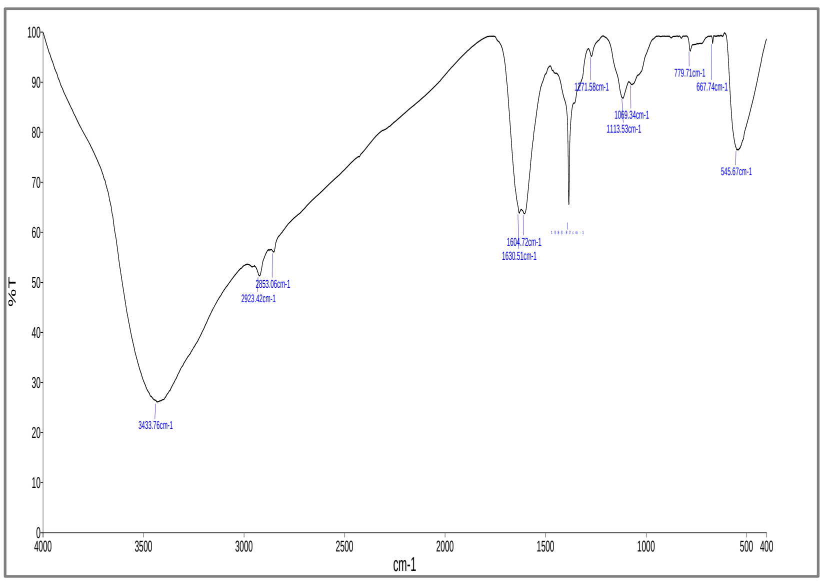

Figure 2: FTIR spectrum of CeO_2 NPsnanoparticles

In 3424cm^{-1} indicates OH stretching, 1625cm^{-1} depict C-O expansion of acetyl group, 1568cm^{-1} shows =C-H stretching, below 1054cm^{-1} fingerprint region, 1388, 1132 cm^{-1}depict C-H bending. Here, a nanoceria was synthesized using aqueous medium by the chemical method. The unique mark district and the plan of groups underneath 832 cm^{-1} affirm the presence of the CeO_2 NPs. In this way the readied CeO_2 NPs were characterized

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utilizing UV and FTIR investigations. The characteristic indication peak in the spectral studies specifies the formation of CeO_2 nanoparticles[14].

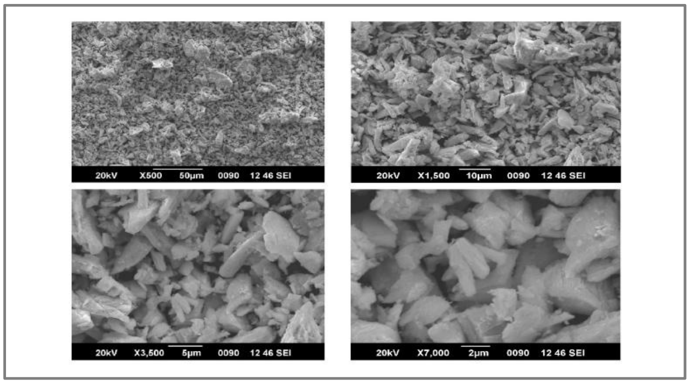

Figure 3: Scanning Electron micrographs of different CeO_2 nanoparticles

The geomorphology of CeO_2 NPs was focused by using the SEM which found a drop like shape. The SEM picture of CeO_2 NPs pieces came about in view of association of $CeN_3O_9.6H_2O$ with strip concentrate of P*unica granatum* for 6 h. are delineated in Figures-3. It was unmistakably outlining that the ordinary SEM pictures of nanoceria pieces have enormous measure of drop shape with a thin size conveyance. The low-objective picture (Figure 3) asserts that the pieces were coordinated in incredibly tremendous entirety and the individual nanoparticles size is around 60 nm and the size of absolute nanoparticles is around 160 nm. These outcomes are concurrent with prior studies[15].

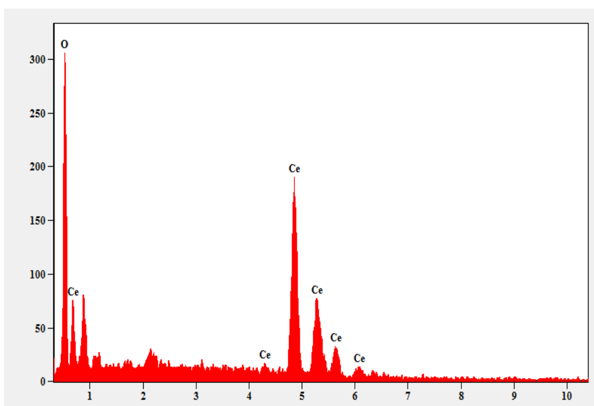

Figure 4: EDX of CeO_2 nanoparticles

Figure 4 Shows that the characteristic peak of CeO_2NPs, that they are Analysis through energy dispersive X-ray spectrometer has confirmed the presence of elemental cerium and the oxygen signal of the cerium oxide nanoparticles.Figure 5, XRD Analysis Shows the Crystallite size for CeO_2 NPs. XRD patterns were measured on an X'Pert Pro diffractometer from analytical Instruments with an acceleration voltage of 40 kV, an emission current of 30 mA and using Cu Kα radiation. The crystallite size was determined by the Scherrer equation.

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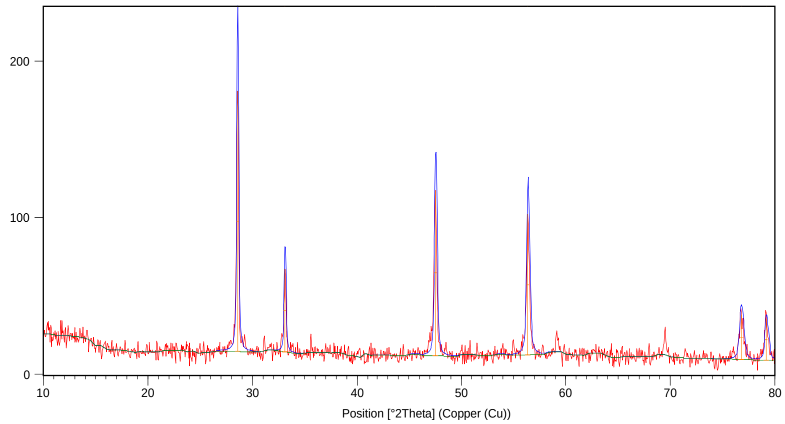

Figure 5: XRD Pattern of Synthesized CeO_2 NPs from *P.granatum* peel extract

3.1. DPPH Scavenging Assay of CeO_2-NPs

In up-to date, CeO_2NPs has revealedthe free radicals scavenging activity which provides defense. CeO_2 NPs with better scavenging activity was successfully isolated and characterized. This work has to be sure that CeO_2 with Ce^{3+}/Ce^{4+} surface ratios nature. The DPPH is extensively utilized in testing scavenging activity seeing as the reaction is an easy and simple method. The DPPH radical absorption activity outcome seems the green extracted CeO_2NPs activity compared with butylhydroxyanisole as standard of positive. As revealed in Figure- 4, nanoceria are capable to get rid of DPPH free radicals in a depends upon the concentration, so various CeO_2NPs quantity (1 – 5 mg/ml) its antioxidant property (±52.5 to ±70 %) is more appears. Also, the consequences show CeO_2NPs are more effective with butylhydroxyanisole. According to the pictorial representation (figure 4), a significant difference was found there is only 9.8 percentages ($p<0.05$). The DPPH assay is well thought-out consistent and regenerate since all harvest the coefficient of difference is lesser.

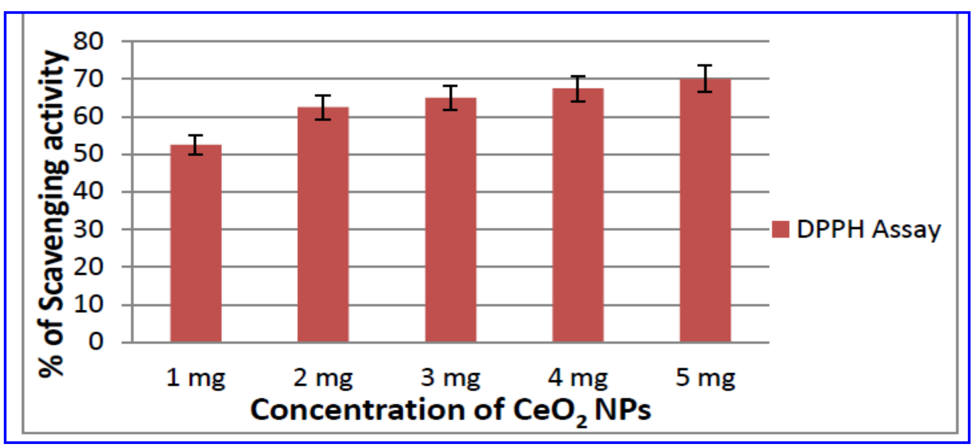

Figure. 4.The inhibitory impact of the bio-blended CeO_2 NPs on DPPH free radicalscontrasted with BHA (Considered as 100% Scavenging action)

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3.2. Antibacterial Activity

The bactericidal movement identified for CeO_2 NPs negligible inhibitory focus known as MIC scope of 10 - 50 µg/mL utilizing Escherichia coli and Staphylococcus aureus as spoken to in figure 5-8. CeO_2 NPs was sonicated before cooperation for 1 h. The inhibitory level of Escherichia coli and Staphylococcus aureus against orchestrated CeO_2 NPs was found to be 40 µg/ml and 10 µg/ml individually (Figure-6&8). In light of the estimations of MIC, the convergence of CeO_2 NPs was characterized for the antibacterial movement.

Figure. 5. Minimum Inhibitory Concentration of CeO_2NPs Against *E. coli*

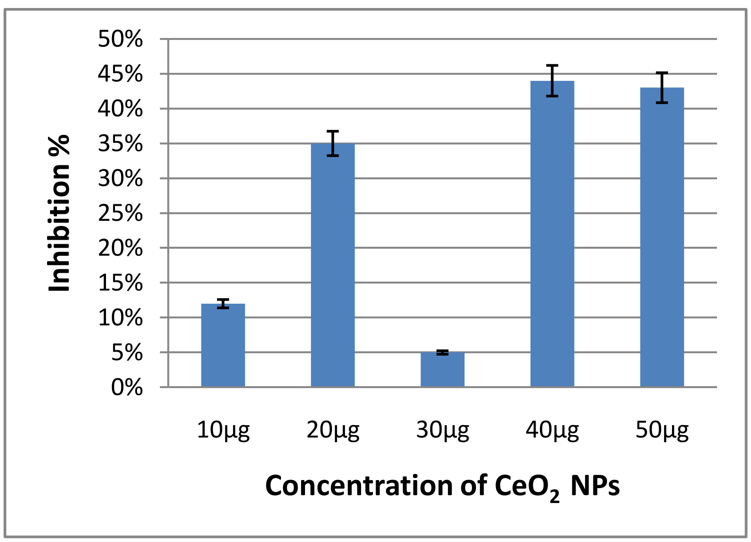

Figure. 6 The inhibitory effect (MIC) of the biosynthesized CeO_2 NPs against *E. coli*

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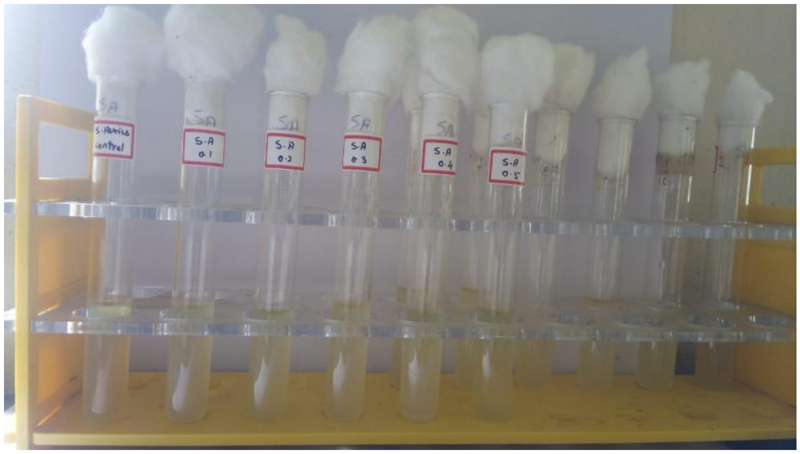

Figure. 7. Minimum Inhibitory Concentration of CeO_2NPs Against *Staphylococcus aureus*

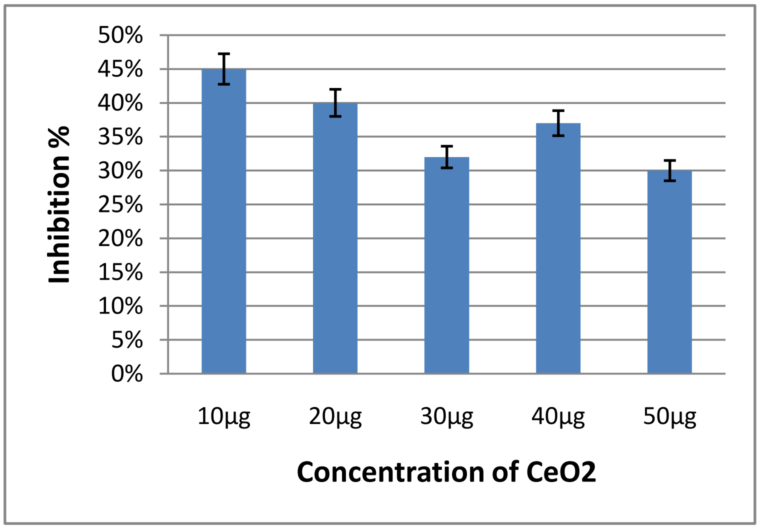

Figure. 8. The inhibitory effect (MIC) of the biosynthesized CeO_2 NPs against *S. aureus*

The zone of restraint of arranged nanoparticles against two distinctive human pathogenic bacterial strains is appears in Table-1. The watched zones for Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus going from 14 and 12 mm independently. To sum things up, antibacterial action was assessed against Staphylococcus aureus and Escherichia coli in negligible inhibitory fixation (MIC) and agar plate dispersion strategy and the subtleties are appeared in figures 9, 10 and table-1. There was no inhibition of growth with DMSO, which indicates that the dissolving solvent itself has no antimicrobial activity. But positive control streptomycin and CeO_2 NPs showed a significant amount of zone of inhibition. On account of E. coli, the antibacterial zone of CeO_2 NPs is tantamount to that of streptomycin alone. More or less related results were observed in the case of *Staphylococcus aureus*. This confirmed that the antibacterial action of green synthesized CeO_2 NPs.

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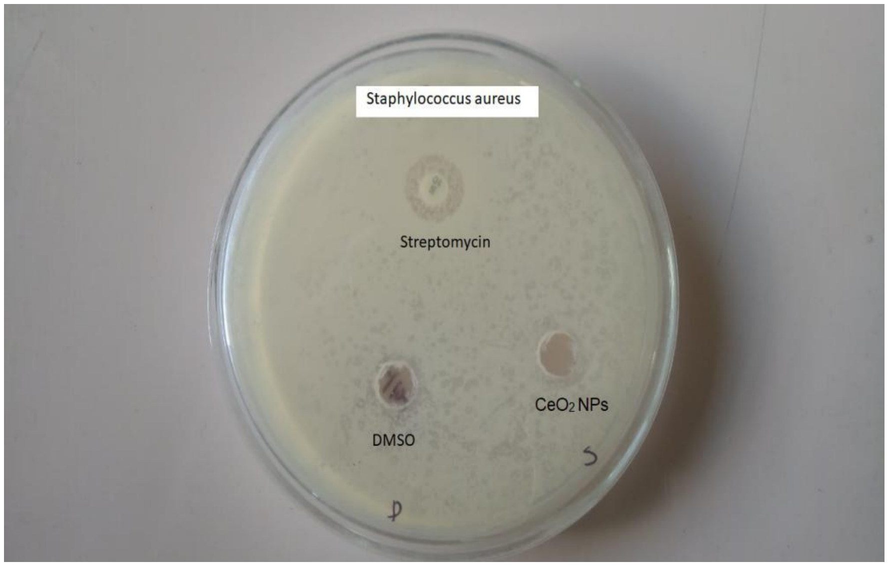

Figure.9. Zone of Inhibition for *Staphylococcus aureus*

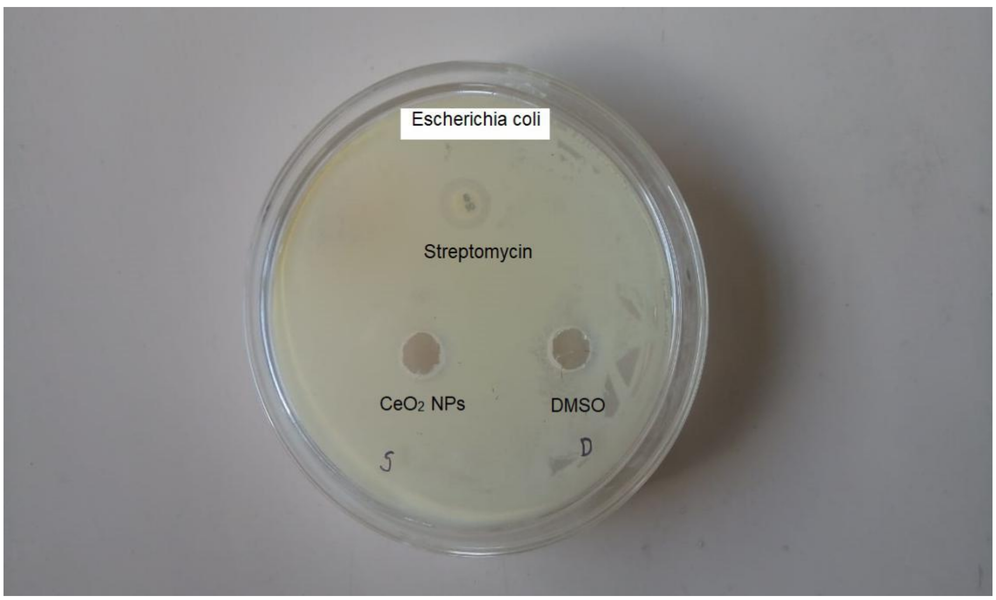

Figure.10. Zone of Inhibition for *Escherichia coli*

Table 1 – Antibacterial activity of CeO_2 NPs

Antibacterial Agents	Zone of inhibition (mm)		
	DMSO	**Streptomycin**	**40 µg of CeO_2 NPs**
Escherichia coli	NIL	12	14

Staphylococcus aureus	NIL	10	12

4. Conclusion

It has been bolstered that the Punica granatum strip remove can orchestrating CeO_2 NPs. The concentrate is answerable for the union of CeO_2 NPs and its size. In view of the outcomes, green interceded CeO_2 NPs displayed better spectroscopic properties when contrasted with its mass partner according to accessible writing. SEM examinations have affirmed the nanosized nature and diminished example of CeO_2 nanoparticles. The Crystal size of the CeO_2 Nanoparticles was studied by XRD Pattern and it was pure and free of impurities.The portrayed CeO_2 NPs have demonstrated better antimicrobial exercises when contrasted with industrially accessible anti-toxins. The outcomes were exciting and equivalent to announced writing. In light of the evaluation considerations, it was presumed that pomegranate skin interceded CeO_2 NPs have incredible cell reinforcement and antibacterial exercises. Thus, it might be a best decision as a strong cell reinforcement and antibacterial specialist in the not-so-distant future. Further the possibility of this plan can be examined to a regularly expanding degree, in order to make an elective treatment for the leading body of various illnesses. The current examination likewise suggested that the utilization of this therapeutic plant might be utilized as a decreasing and topping operator. Therefore, vindicate that strip implantation of conventional plants has colossal applications.

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Conflict of interest

The creators reported no irreconcilable situation. This report doesn't contain any investigations with human or creature subjects experienced by any of the authors.

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