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- Review -

Green Synthesis of Nanoceria and the Mechanism Behind Their Antibacterial Activity

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The synthesis of cerium oxide nanoparticles (nanoceria, CeO_2) has received significant attention across scientific and technological disciplines in the last decade. This article explores an overview of the green synthesis method and the antibacterial activity of nanoceria. The utilization of biological materials, such as plants and microorganisms, in the synthesis of nanoceria, has gained attention as an ecofriendly approach. Plants are rich in phytochemicals, including alkaloids, flavonoids, phenols, proteins, and other nutritious components. Likewise, microorganisms generate bioactive metabolites, pigments, enzymes, proteins, acids, and antibiotics. The phytochemicals and metabolites are involved in the reduction of metal salt into nanoceria and provide stability to synthesized nanoparticles. Nanoceria synthesis using plants and microorganisms is facile and ecofriendly, and synthesized nanoceria are biocompatible. Many biomedical applications of nanoceria have been reported, including those that are anticancer, anti-inflammatory, larvicidal, enzyme inhibiting, antibiofilm, and antimicrobial. However, in this review, we focused on and described in detail the antibacterial potential of nanoceria. The antibacterial activity of nanoceria occurs due to excessive reactive oxygen species generation, the impairment of the cell membrane, and the inhibition of cellular mechanisms. Ultimately, this review's primary goal is to provide readers with a logical understanding of the significant achievements of nanoceria as a cutting-edge therapeutic agent for treating a range of microbial pathogens and combating other diseases.

Key words : Antimicrobial, cerium oxide nanoparticles, ecofriendly method, green synthesis, metal oxides

Introduction

Recent years have witnessed a substantial progression in the field of nanotechnology and considered among one of the leading research avenues. Nanoparticles (NPs) have various applications in the different fields, including environmental, industrial, and medical [44, 45, 54, 62]. NPs including a wide range of materials with different physical and chemical properties, including metals (gold (Au), iron (Fe), silver (Ag)), metal oxides (CeO_2, titanium oxide (TiO_2), Zinc oxide (ZnO_2)), quantum dots (CdSe, cadmium selenide), and carbon nanotubes (single walled and multi-walled) [28, 46]. These NPs also present different morphologies such as oval, spherical, cube, triangular, rod, tubes and prisms. NPs are defined as particles that possess a size variation of 1 to 100 nm. NPs are distinguished from bulk materials by their small size, different shapes, higher surface area-to-volume ratio, and their properties [18, 31]. These NPs have unique physicochemical properties and have been utilized in the various fields of biology, chemistry and physics [1, 19, 53].

Among different NPs, nanoceria have been exploited a lot because they are biocompatible, have a unique surface chemistry, and can switch between the oxidation states Ce^{3+} and Ce^{4+}. The relative amount of cerium ions (Ce^{3+}, Ce^{4+}) varies with particle size. In general, when particle size decreases, the percentage of Ce^{3+} ions rise [69]. Ce^{4+} is a potent oxidant, whereas the Ce^{3+} is very resistant to oxidation and will only react with extremely powerful oxidants [42]. Cerium oxide nanoparticles are unique in that they may function as both

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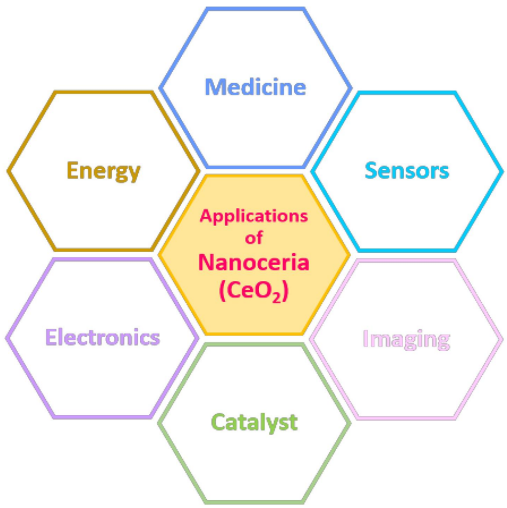

Fig. 1. General applications of nanoceria.

an oxidation and reduction catalyst, depending on the reaction conditions. These actions result from the rapid transition of the oxidation state from Ce^{4+} to Ce^{3+}. The cerium atom has the ability to easily and drastically adjust its electronic configuration to best fit its immediate environment [60]. It is mostly used to make sensors, energy storage cells, catalysts, electronics, and medicines (Fig. 1). NPs are mainly produced in two ways: from the top-down and the bottom-up [2]. Cutting or breaking large objects into small NPs is part of the top-down method. The bottom-up method makes small particles by assembling them atom by atom, molecule by molecule, and cluster by cluster. The different methods have been developed for the NPs synthesis including physical, chemical, and biological [26, 50].

Physical and chemical methods for synthesizing nanoceria including solution precipitation, hydrothermal, sol-gel, spray pyrolysis, ball milling, thermal decomposition, and solvothermal, thermal hydrolysis [17, 23, 24, 51, 70] methods are requiring an extensive amount of energy and toxic chemicals, in addition to producing hazardous byproducts; therefore, researchers are currently concentrating on the biological method as an environmentally friendly alternative. The utilization of plant extracts and microorganisms in the synthesis of nanoceria results in the production of nanoceria that are biocompatible and free of toxic byproducts. It is believed that biomolecules, including enzymes and phytochemicals (phenols, amines, ketones, flavonoids, and terpenoids), play a role in the process of reducing and stabilizing bulk salt into NPs [3, 41, 48, 50]. As of now, numerous approaches for the synthesis of nanoceria and their biomedical implementations have been described (Table 1). Among other biomedical applications, antimicrobial application is certainly the most exploited. In the past, studies have documented the antimicrobial activity of NPs that is dependent on their size and shape [4, 57]. The mechanisms underlying this activity include disruptions of cell wall ion transportation channels, inactivation of enzymes and proteins, DNA damage, and interruption of cell membrane permeability [41, 44]. However, additional research is required to comprehensively clarify the entire mechanism of action. This review focuses on the synthesis of nanoceria using microorganisms and plants, as well as the underlying mechanism that explains nanoceria's antimicrobial activity.

Table 1. Nanoceria synthesis and their biomedical applications

Synthesis method	Synthesis using/route	Shape	Size (nm)	Applications	References
Biological	*Datura metel*	Spherical	5–15	Antioxidant	[68]
	Stachys japonica	Spherical	21	Antioxidant, anti-diabetic	[55]
	Pouteria campechiana	Granular, agglomerated	11.46–15.59	Antioxidant, anticancer, antibacterial, sensor	[43]
	Aquilegia pubiflora	Spherical	28	Biocompatibility, anti-diabetic, anticancer, antioxidant	[27]
Chemical	-	Cuboidal	8–20	Anticancer	[37]
	Sol-gel	Spherical	8–18	Cytotoxicity	[29]
	-	Cubic, triangular	9.52	Antioxidant, anti-genotoxic	[52]
	NH_4OH precipitation	Star, nanorod, polygonal	3–5	Angiogenesis	[16]
	Micro-emulsion	Spherical	7–10	Anti-inflammatory	[8]
	-	-	6, 12	Neuroprotective	[56]
	-	-	<25	Radioprotective	[66]

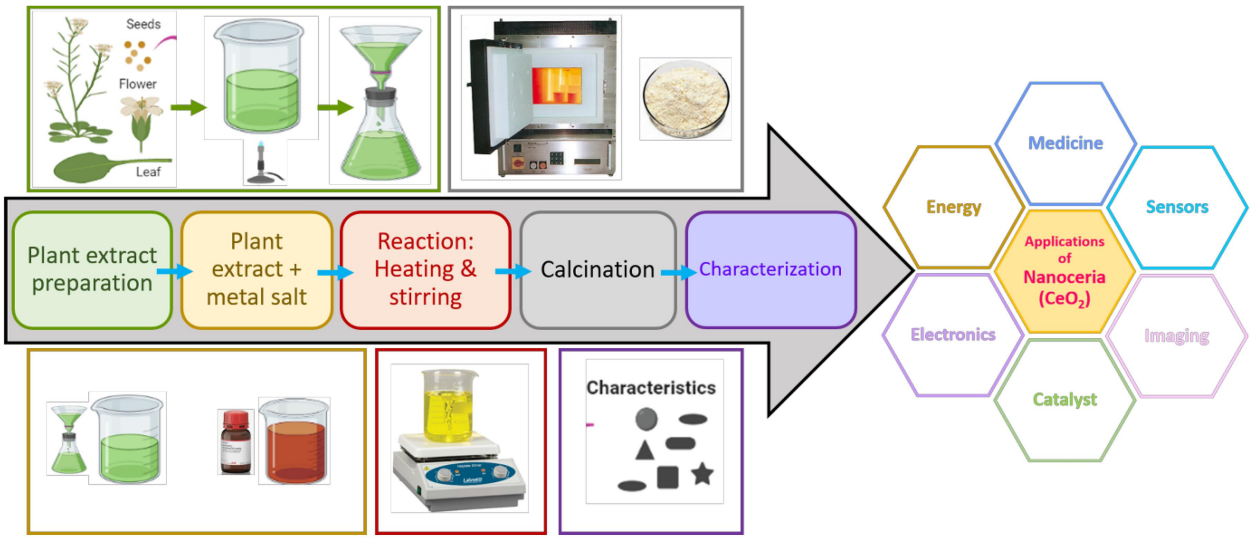

Fig. 2. Schematic diagram of the nanoceria biosynthesis process.

Synthesis of nanoceria

The biological method for synthesis of nanoceria has been used as an alternative to the traditional methods (hydrothermal methods, thermal hydrolysis, spray pyrolysis, ball milling etc.) because it is an ecofriendly, inexpensive, and non-toxic method that uses plant extracts and microorganisms as a natural resource for reducing metal salt into NPs while also providing stability to the synthesized NPs. The schematic representation of the nanoceria production process using a biological method is shown in Fig. 2. This technique involves the preparation of extracts from plants, microbial cells, or microbial cell-free supernatant, which can later be used as a reducing and stabilizing agent. The extract is combined with metal salt and processed to several factors, including temperature, pH, salt or extract ratio, and reaction time, to produce the desired shape, size, and optimum production of nanoceria. Once the reaction completes, the solution needs to be processed by either centrifugation or filtering to separate the nanoceria. Next, the separated nanoceria undergo calcination at various temperatures, which may be carried out in either an oven or a furnace. Ultimately, the calcined nanoceria are analyzed using various analytical methods to understand the characteristics of nanoceria. These manufactured nanoceria are then used for different applications.

The synthesis of nanoceria via a biological approach has been described, using various plant parts extracts such as leaf, seed, flower, rhizome, and fruit, as well as microorganisms including bacteria and fungus (Table 2). Plant extracts contain various phytochemicals that act as reducing agents [41, 46]. This is one of the main reasons why plant extract-mediated nanoceria synthesis is more reported than those synthesized using microorganisms. Additionally, preparing plant extracts is much simpler than using microorganisms [44, 46]. Use of microorganisms for nanoceria requires additional steps such as isolation, identification, growth optimization, and separation of synthesized nanoparticles from microbial cells through cell disruption and centrifugation etc. [21, 39, 45, 49].

Use of plant materials

Aqueous extracts of *Gloriosa superba* and *Azadirachta indica* were found to generate nanoceria with sizes of 5 and 10 to 15 nm, respectively. Transmission Electron Microscopy (TEM) was used to identify their morphology, and X-ray diffraction (XRD) spectroscopy confirmed their surface features [7, 59]. The production of *Hibiscus sabdariffa* flower extract involved boiling the petals in distilled water and then mixing it with cerium (Ⅲ) nitrate hexahydrate for nanoceria synthesis. The obtained nanoceria has been dried and calcined at a high temperature, resulting in the formation of crystalline, spherical nanoceria with a diameter of 3.9 nm. The study using Flourier Transform Infrared Spectroscopy (FTIR) showed that phytochemical functional groups played a role in the formation process of the nanoceria [64]. The formation of metal oxide group, that is CeO_2 were confirmed in multiple studies by using FTIR analysis which indicated by Ce-O bond [10, 14, 59]. Similarly, *Cassia glauca* petals extract were used for nanoceria synthesis; synthesized spherical and irregular shaped nanoceria has been reported for the applications in-

Table 2. Nanoceria synthesis using plant extracts and microorganisms

Biological synthesis		Shape	Size (nm)	Applications	References
Name	Part				
Plants					
Acorus calamus	Rhizome	Spherical, pseudo-spherical	5–40	Antibiofilm	[5]
Azadirachta indica	Leaf	Spherical	10–15	Dye degradation	[59]
Cassia angustifolia	Seed	Spherical	10–12	Dye degradation	[6]
Cassia glauca	Petal	Spherical, irregular	3.20	Enzyme inhibition, antioxidant, antimicrobial	[14]
Calotropis procera	Flower	Spherical	21	Antibacterial, photocatalytic	[40]
Cydonia oblonga	Seed	Agglomerated	9–11	Cytotoxicity, dye degradation	[20]
Gloriosa superba	Leaf	Spherical	5	Antibacterial	[7]
Hibiscus sabdariffa	Flower	Spheres	~3.9	-	[64]
Lemon grass	Grass	Aggregated	10–40	-	[35]
Manilkara zapota	Fruit peel	Spherical	15±2	Photocatalytic, antimicrobial, antidiabetic	[10]
Musa sapientum	Fruit peel	Spherical	4–13	Radioprotective, photocatalytic	[36]
Zingiber officinale	Rhizome	Spherical-agglomerated	3.73, 3.81	Enzyme inhibition, antioxidant, antimicrobial	[9]
Microorganisms					[21]
Aspergillus niger	Fungi	Spherical	5–20	Antibacterial, larvicidal	
Bacillus subtilis	Bacteria	Spherical	8.022	Antioxidant	[49]
Curvularia lunata	Fungi	Spherical	5–20	Antibacterial	[39]
Fusarium solani	Fungi	Spherical	20–30	Antibacterial, antibiofilm	[65]
Humicola sp.	Fungi	Spherical, polydisperse	12–20	-	[32]

cluding enzyme (α-amylase, urease, and lipase) inhibition, antioxidant (free radical scavenging), and antibacterial activity against human pathogens [14]. Spherical and pseudo-spherical nanoceria produced from rhizome extract with sizes ranging from 5 to 40 nm showed antibiofilm efficacy by inhibiting bacterial exopolysaccharide formation [5].

Use of microorganisms

Microorganisms have been shown to produce nanoceria using cell-free supernatants or cell biomass of bacteria and fungus, similar to plant extract synthesis (Table 2). Microorganisms are a significant source of secondary metabolites, and they contribute to producing and stabilizing NPs [45]. Microbial metabolites such as proteins, amino acids, and enzymes contribute significantly to metal salt reduction and nanoceria formation. Cell-free supernatant of bacteria *Bacillus subtilis* yielded spherical nanoceria with a size of 8 nm [49], and cell-free supernatant of fungi *Aspergillus niger* and *Fusarium solani* were reported to yield spherical nanoceria of size 5 to 20 and 20 to 30 nm, respectively, and has antibacterial activity against human pathogens [21, 65]. In contrast, the fungus *Hemicola* sp. cell (mycelia) mass was employed for nanoceria synthesis; mycelial mass suspended with metal salt (cerium (Ⅲ) nitrate hexahydrate) and incubated in shaking incubator, and the resulting extracellularly synthesized nanoceria was spherical, polydisperse, and 12 to 20 nm in size and crystalline nature was confirmed by XRD [32]. Despite all of these uses, the microbial technique of nanoceria synthesis has certain drawbacks, including a significant risk of pathogenicity, contamination, laboratory culture, growth condition management, and so on. However, it has a lot of promise in the realm of nanotechnology and has the potential to be a major route in nanomedicine, but it has yet to be explored. Furthermore, these biogenic NPs may be used for disease management, drug development, and for drug delivery.

Characterization of nanoceria

Currently, several methods are used to characterize nanoceria. Nevertheless, the main sign of nanoceria formation is the change in color of the reaction, which is confirmed by spotting a peak at a certain wavelength using UV-visible spectrophotometry [36, 39, 68]. This shape and size of synthesized nanoceria was observed by scanning electron microscopy [9, 20], and transmission electron microscopy [6, 32]. Raman spectroscopy applied to identify the phases and phase

transition and size determination of nanoceria [39, 48]. The analysis of the interaction between biomolecules from a plant extract or microbial supernatant, which leads to the development of nanoceria and formation of metal oxide bond, is determined using FT-IR [3, 20, 43]. The crystalline nature and elemental composition of nanoceria were determined using XRD analysis [5, 35] and energy dispersive X-ray analysis [48, 65], respectively. These are the few methods listed here that are generally used in the characterization of nanoceria.

Antibacterial effect of nanoceria

The bacteriostatic properties of cerium were first detected at the end of 19th century and started its use into the topical antiseptics in veterinary and human medicine [38]. Scientists conducted research in the mid-twentieth century using cerium Ⅲ) chloride, cerium (Ⅲ) nitrate, and cerium (Ⅳ) sulphate against a panel of 39 bacterial species across 16 genera, including Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa*; they observed bacteriostatic effects of cerium nitrate against all tested bacteria, as well as pH-dependent effects (more effective at slightly acidic pH values). *Pseudomonas* was the most sensitive, followed by *Escherichia* and *Salmonella*, and the least susceptible species was *S. aureus* to cerium nitrate [13]. A further study using cerium nitrate on *E. coli* were conducted and found that cerium uptake into the cell cytoplasm and inhibition of cellular respiration, oxygen uptake and glucose metabolism. The cell wall remains intact but knob-like protrusions were observed, which suggest a disruption of cell wall [61].

Nanotechnology-based therapeutics have recently been used in disease diagnosis, therapy, and the development of new drugs. For example, the antibacterial potential of nanomaterials has been extensively investigated and shown significant results [11, 44-46, 54]. Several studies have demonstrated that the shape, size and composition of nanomaterials surface characterizes their antibacterial properties, similar like other metallic and metal-oxide nanoparticles [11, 44, 46, 47]. Many studies have shown that nanoceria has antibacterial properties against both Gram-positive and Gram-negative bacteria (Table 3), although the exact mechanism of bacteria-killing is not entirely known. In general, nanoceria has the most potent antibacterial effect against Gram-negative bacteria (*E. coli*), which might be attributed to Gram-positive bacteria having a thick layer of peptidoglycan that is difficult to penetrate nanoceria. However, several authors reported opposite findings.

The adsorption of metal oxide nanoparticles onto the bacterial cell wall can takes place due to the electrostatic attraction between the negatively charged cell wall of bacteria and positively charged nanoceria (Ce^{4+}) [58, 63] where Ce^{4+} reduced to Ce^{3+}, resulting in oxidative stress on the membrane lipids and protein [63]. Due to this interactions, nanoceria interact with cell membrane and changes membrane permeability which results in membrane impermeability, protein denaturation, and alteration in cell multiplication, and eventually cause bacterial cell death [7, 12, 22, 65]. The alteration of gene expression due to nanoceria interaction cause impairment of cellular respiration. Compared to nanoceria exposed and unexposed *E. coli*, level of succinate dehydrogenase and cytochrome b terminal oxidase gene expression decreased in nanoceria exposed cells which indicates nanoceria attacks electron flow and bacterial respiration [47]. It is reported that nanoceria mostly kill bacteria by producing a significant amount of ROS such as superoxide (O_2^-), hydroxyl radicals (•OH), and hydrogen peroxides (H_2O_2). ROS are highly reactive and unstable compounds that can strip electrons from cellular macromolecules (nucleic acids, proteins, polysaccharides, lipids and other biological molecules), thereby causing them to become dysfunctional, eventually killing and decomposing bacteria [25, 34, 61, 67]. In addition, Ce (Ⅳ) ions have the potential to catalyze the hydrolysis of a DNA oligomer into fragments, which may lead to death of bacteria [34].

Few factors are affects the antibacterial activity of nanoceria is surrounding pH cause changes in nanoceria surface charges, which can affect the nanoceria particles adsorption affinity towards bacteria [47], shape and size of nanoceria [15, 57], surface chemistry [33], and surface coating [30] concentration [7].

Table 3. Antibacterial effect of nanoceria on pathogenic bacteria

Gram-positive	Gram-negative	References
Bacillus subtilis, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*	*Enterobacter aerogens*, *Escherichia coli*, *Klebshiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella setubal*, *Salmonella typhi*, *Shigella dysenteriae*	[4, 7, 10, 14, 21, 27, 39, 48, 57, 65]

Challenges and future prospects

The biological approach to synthesizing nanoceria is cost-effective, simple, and environmentally benign. Biogenic nanoceria are preferable for biomedical applications because they are free from hazardous chemicals and their shape and size may be altered or regulated throughout the biogenic process of nanoceria production. However, there are still significant issues that need to be addressed in the field of biogenic nanoceria. These challenges include ensuring the stability of synthesized nanoparticles, preventing the development of nanoclusters, addressing the aggregation of nanomaterials, and gaining a thorough understanding of the toxicity of these materials on animals and the environment. Ongoing research is being conducted on the biogenic production of nanoceria. Each day, new studies are being added to the realm of biomedical and medical science, exploring the potential use of NPs for human well-being. The development of nanotechnology and its use in clinical procedures necessitated thorough characterization, regulatory requirements, and appropriate techniques for detecting their toxicity. Nanoceria's antibacterial activities have been recognized for more than a century. To further the translational potential of nanoceria-related antibacterial materials, improved standardization, more systematic investigations, and long-term effects observation are required to increase knowledge of nanoceria's cytotoxicity and processes. This review sheds light on the mechanism of nanoceria as antibacterial agents, which could help to open up new possibilities for their future usage in biomedical fields.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 나노세리아의 친환경 합성과 항균 활성 메커니즘

파틸 마헤슈쿠마르 프라카쉬[1] · 이용석[2] · 조미정[2] · 서용배[2] · 김군도[2,3]*
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세륨 산화물 나노입자(CeO_2), 즉 나노세리아의 합성은 지난 10년 동안 다양한 과학 기술 분야에서 상당한 주목을 받아왔다. 특히 이 논문에서는 환경 친화적 합성 방법과 나노세리아의 항균 활성에 대한 집중적으로 다루고자 한다. 우선, 나노세리아 합성에 있어 식물 및 미생물과 같은 생물학적 소재를 사용하는 방식이 환경 친화적 접근으로 주목을 받고 있다. 예를 들어, 식물은 알칼로이드, 플라보노이드, 페놀, 단백질 및 기타 영양 성분을 포함한 파이토케미컬을 풍부하게 함유하고 있다. 한편, 미생물은 생리 활성 대사산물, 색소, 효소, 단백질, 산 및 항생제를 생성한다. 따라서, 이러한 파이토케미컬과 대사산물은 금속염을 나노세리아로 환원시키는 데 기여할 뿐만 아니라, 합성된 나노 입자에 안정성을 높여준다. 또한, 식물과 미생물을 사용한 나노세리아 합성은 단순하면서도 환경 친화적이라는 장점이 있으며, 결과적으로 합성된 나노세리아는 생체 친화적 특성을 지닌다. 나노세리아는 항암, 항염증, 살충제, 효소 저해, 항생물막 및 항균 작용 등 다양한 생물의학적 응용이 보고되었지만, 이 논문에서는 특히 나노세리아의 항균능력에 중점을 두어 설명하고자 한다. 특히, 나노세리아의 항균 활성은 과도한 반응성 산소종(ROS) 생성, 세포막 손상, 세포 메커니즘 억제를 통해 발현된다. 결국, 이 리뷰의 주요 목적은 다양한 미생물 병원체를 치료하고 다른 질병을 극복하는 데 나노세리아가 중요한 치료제로서 지닌 잠재력에 대해 독자들이 더 깊이 이해할 수 있도록 돕는 것이다.