

RESEARCH ARTICLE

Green Synthesis and Biological Evaluation of Cerium Oxide Nanoparticles Using *Saccharomyces boulardii* with Potent Anti-Candida Activity

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ABSTRACT

The synthesis of metallic and semiconductor nanoparticles through physical and chemical methods is well established; however, biological synthesis approaches are increasingly gaining attention due to their simplicity, cost-effectiveness, and eco-friendly nature. In the present study, cerium oxide nanoparticles (CeO₂-NPs) were synthesized using an aqueous solution of cerium salts mediated by the yeast *Saccharomyces boulardii*. This probiotic microorganism grows rapidly, is easy to cultivate on an industrial scale, and serves as an efficient biological system for nanoparticle biosynthesis. The obtained CeO₂-NPs exhibited an average particle size of approximately 62.54 nm, a zeta potential of roughly -45 mV, and inhibition zone diameters ranging from (24.6 ± 1.1) to (11.2 ± 0.8) mm at 1000 and 62.5 mg/mL against tested *Candida spp.*

The synthesized CeO₂-NPs were characterized using various analytical techniques, including Scanning Electron Microscopy (SEM), Field Emission Scanning Electron Microscopy (FESEM), Energy-Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD), to determine their size, morphology, and structural properties. The use of *S. boulardii* as a biogenic mediator demonstrates a sustainable and scalable approach for producing metal oxide nanoparticles. Furthermore, CeO₂-NPs exhibit promising antibacterial and antifungal properties, making them suitable for biomedical and tissue engineering applications requiring infection control. Overall, the study demonstrates that biologically synthesized CeO₂-NPs possess significant antimicrobial potential suitable for biomedical applications.

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INTRODUCTION

The synthesis of metallic and semiconductor nanoparticles forms a cornerstone of nanotechnology, allowing precise control over their size, shape, and morphology to tailor physicochemical properties for specific applications [1]. Nanoparticles contain a high proportion of surface atoms, resulting in a large surface-to-volume ratio that enhances their electronic, optical, and catalytic properties compared with bulk materials [2]. In nanotechnology, nanomaterials have been extensively explored across various scientific disciplines, including physics, chemistry, biology, medicine, pharmacy, and materials science.

Their unique optical and electronic properties, which differ significantly from those of their bulk counterparts, make them particularly valuable for diverse applications. Nanomaterials are generally defined as materials with dimensions ranging from 1 to 100 nanometers [3]. The synthesis process can be carried out using both biological and inorganic materials. In biological synthesis, living organisms such as bacteria, fungi, algae, yeasts, molds, viruses, and, most commonly, plants are employed as bio-factories for nanoparticle production [4]. The green synthesis method offers several advantages over conventional chemical and physical techniques, including environmental friendliness, cost-effectiveness, biocompatibility,

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and safety. Furthermore, numerous studies have demonstrated that cerium oxide nanoparticles (CeO_2 NPs) synthesized through green approaches exhibit remarkable antibacterial and photocatalytic activities [5]. Cerium oxide nanoparticles (CeO_2 NPs) have emerged as promising therapeutic agents in the fields of biology and medical science. Their physicochemical properties—such as particle size, degree of agglomeration in suspension, and surface charge—are critical determinants of how they interact with target cells. In recent years, CeO_2 NPs have been successfully synthesized through various bio-mediated methods employing natural and organic matrices as stabilizing and reducing agents. These green approaches not only enhance nanoparticle biocompatibility and stability but also address major safety concerns, thereby creating favorable conditions for their effective application in biomedical systems [6]. This approach provides a novel and environmentally friendly method that mitigates ecological concerns while expanding the potential clinical applications of metallic nanoparticles. In response to increasing awareness of pollution and toxicity associated with conventional synthesis routes, numerous studies have emphasized the adoption of green synthesis strategies for producing Cerium oxide nanoparticles (Z CeO_2 NPs) using cost-effective biological sources. In this regard, a variety of microorganisms—including bacteria, yeasts, and fungi—have been extensively explored as efficient bio-factories for nanoparticle synthesis [7]. Based on their proven safety and health benefits, well-known probiotic microorganisms such as lactic acid bacteria and *Saccharomyces cerevisiae* var. *boulardii* represent promising candidates for use as host expression systems. Lactic acid bacteria and other intestinal microorganisms can serve as microbial cell factories for nanoparticle synthesis or therapeutic compound production. However, certain limitations exist. From a pharmacokinetic perspective, factors such as metabolic stability, bioavailability, and interaction with host intestinal environments may influence their overall efficiency and applicability [8].

Saccharomyces boulardii is well known for its ability to survive within the human gastrointestinal tract due to its remarkable tolerance to low pH and elevated temperatures. Previous studies have explored the metabolic engineering of *S. boulardii* and its application as a potential oral vaccine delivery vehicle in mouse models. However, to

the best of our knowledge, no studies have yet investigated the use of engineered *S. boulardii* for the production of prebiotics that exert beneficial effects on host health through the selective stimulation of the growth or activity of probiotic-like bacteria in the colon [9].

Among these microorganisms, yeasts have received considerable attention as promising candidates for nanoparticle production. This is largely attributed to their remarkable tolerance to metal ions, high enzyme production, substantial biomass yield, and strong capacity for metal bioaccumulation. In particular, the probiotic yeast *Saccharomyces cerevisiae*, belonging to the phylum Ascomycota and the class Saccharomycetes, has been widely studied for its potential in green nanoparticle synthesis [10]. *Saccharomyces boulardii*, a nonpathogenic microorganism, exhibits several advantageous traits, including widespread availability and the presence of diverse bioactive compounds such as proteins and enzymes. These characteristics make *S. boulardii* a promising candidate for environmentally friendly nanoparticle synthesis. Similarly, *Saccharomyces cerevisiae* holds considerable commercial significance due to its exceptional physiological and genetic properties, rendering it a highly suitable organism for nanoparticle production [11]. Examples of nanoparticles currently under investigation include those composed of cerium, cellulose, silver, titanium, iron, aluminum, manganese, tantalum, gold, or combinations of these elements. Among them, cerium is of particular interest, as it is the first element in the lanthanide series and a member of the rare earth family [12]. During the formation of cerium oxide nanoparticles (CeO_2 NPs), cerium atoms integrate with oxygen, resulting in a fluorite crystal structure. Cerium oxide can exist in both the +3 and +4 oxidation states, and the redox cycling between these states underlies its notable antioxidant properties. The presence of oxygen vacancies in CeO_2 also enables its application as a solid electrolyte in fuel cells. Cerium oxide nanoparticles have attracted significant attention in nanotechnology due to their diverse applications, including catalytic converters, fuel additives, and self-regenerating antioxidants [13]. The present study aims to synthesize cerium oxide nanoparticles (CeO_2 -NPs) using the probiotic yeast *Saccharomyces boulardii* via a green, environmentally friendly approach and to evaluate their physicochemical characteristics, biocompatibility, and biological activities.

MATERIAL

Freshly cultured S. boulardii

To prepare the *Saccharomyces boulardii* as OD₆₀₀ and CFU/mL extract solution (1 g/L), 0.1 g of the dry yeast extract powder was dissolved in 100 mL of deionized (DI) water. Cerium nitrate hexahydrate (Ce(NO₃)₃·6H₂O) was dissolved in 100 mL of DI water to prepare a 20 mM stock solution of cerium nitrate. For the green synthesis of Cerium oxide nanoparticles (CeO₂NPs), the cerium ions (Ce³⁺) present in the cerium nitrate solution were reduced using the *S. boulardii* extract show Fig. 3. a. in the synthesis NPs. The 100 mL yeast extract solution was added dropwise to 100 mL of the cerium nitrate solution, resulting in a half-diluted mixture (10 mM) exposed to the fungal extract. The formation of cerium oxide nanoparticles at room temperature was initially indicated by a gradual color change in the reaction mixture, which was later confirmed by UV-Vis spectroscopy. After completion of the reaction (24 hours), the mixture was centrifuged at 10,000 rpm for 30 minutes to separate the colloidal nanoparticles. The supernatant was carefully decanted, and the obtained pellet was resuspended in 20 mL of DI water and centrifuged again at 5,000 rpm for 10 minutes to remove residual impurities [14].

Characterization of Cerium oxide nanoparticles

The morphology and structural characteristics of the synthesized nanoparticles were analyzed using various advanced techniques. Field Emission Scanning Electron Microscopy (FE-SEM) was employed to visualize and evaluate the shape, surface morphology, particle size distribution, degree of aggregation, and surface functionalization of individual nanoparticles. Atomic Force Microscopy (AFM) was utilized to measure the diameter and height of the nanoparticles, providing detailed three-dimensional surface profiles. The optical properties of the nanoparticles were investigated using UV-Visible (UV-Vis) spectroscopy, while X-ray Diffraction (XRD) analysis was conducted to determine the crystallographic structure of the material. The functional groups associated with the biosynthesized nanostructures were identified using Fourier Transform Infrared (FT-IR) spectroscopy (RXI, PerkinElmer, Inc., USA) within the range of 4000–400 cm⁻¹ at a resolution of 4 cm⁻¹. Additionally, Zeta Potential (ZP) analysis was performed to assess the surface charge and stability

of the nanoparticles, and Energy Dispersive X-ray (EDX) spectroscopy was employed to determine their elemental composition [15].

Anti-Candida Activity of CeO₂ Nanoparticles

The produced cerium oxide nanoparticles (CeO₂-NPs) were tested for their antifungal properties against a variety of *Candida* species, such as *Candida glabrata*, *Candida tropicalis*, and *Candida albicans*. With minor adjustments, the assays were carried out using the broth microdilution and agar well diffusion techniques in accordance with the Clinical and Laboratory Standards Institute's (CLSI, M27-A3) criteria.

Agar Well Diffusion Assay

On Sabouraud Dextrose Agar (SDA), fresh fungal cultures were created, and they were cultured for 24 hours at 37 °C. Using a sterile cotton swab, a fungal suspension equal to 0.5 McFarland standard ($\approx 1 \times 10^6$ CFU/mL) was evenly applied to the surface of SDA plates. Using a sterile cork borer, wells of 6 mm in diameter were made in the agar and filled with 100 µL of CeO₂-NPs at concentrations varying from 62.5 to 1000 µg/mL.

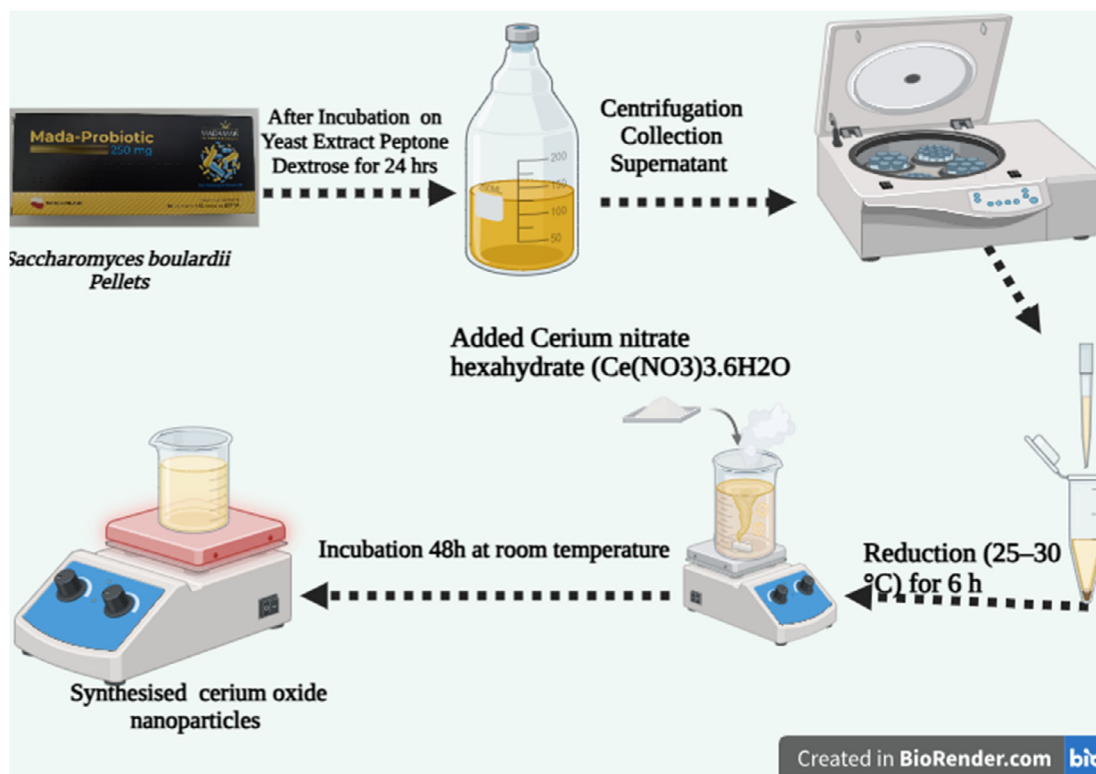
The zone of inhibition (mm) surrounding each well was measured after the plates were incubated for 24 to 48 hours at 37 °C. The negative control was sterile distilled water, while the positive control was fluconazole (30 µg/mL) [16].

Minimum Inhibitory Concentration (MIC) Determination

The MIC of CeO₂-NPs against *Candida* spp. was ascertained on sterile 96-well microtiter plates utilizing the microbroth dilution procedure. In Sabouraud Dextrose Broth (SDB), the nanoparticles were serially diluted twice to reach final concentrations of 1000, 500, 250, 125, and 62.5 µg/mL. 100 µL of fungal suspension (1×10^6 CFU/mL) was added to each well. Plates were incubated at 37 °C for 24 h, and the optical density was measured at 600 nm using a microplate reader. The MIC was defined as the lowest concentration at which no discernible growth occurred, number of replicates (n), the control strains employed, and the criteria used to determine the minimum inhibitory concentration (MIC) to ensure clarity and reproducibility. [17].

Statistical analysis

Using Graph Pad Prism 6, statistical analysis

Fig. 1. Synthesis CeO₂-NPs preparation

was performed on the collected data. The unpaired t-test was used in this study. The findings are expressed using the mean $\pm$ standard deviation (SD) of three tests [18].

RESULT

Synthesis of CeO₂-NPs

The physicochemical properties of the synthesized cerium oxide nanoparticles (CeO₂ NPs), which can be inferred from their optical characteristics, play a crucial role in determining their effectiveness, functional behavior, and morphological features. During the synthesis process, slight color changes were observed in the aqueous solution, indicating the formation of nanoparticles. The color gradually shifted from milky white to a pale yellowish hue, confirming the successful synthesis of CeO₂ NPs. show Fig 2. in the Synthesis CeriumNPs. The chemical reduction of metal salts is one of the most viable and promising synthetic approaches for the preparation of metallic nanoparticles, offering a simple, cost-effective, and efficient process that requires relatively minimal experimental setup. Cerium oxide nanoparticles (CeO₂ NPs) possess a transitional oxidation state

between Ce⁴⁺ and Ce³⁺. These nanoparticles can readily adjust their electronic configuration depending on the surrounding medium. The surface of CeO₂ NPs exhibits oxygen vacancies, which arise due to the loss of oxygen atoms or surface electrons. This unique property enables reversible redox cycling between Ce⁴⁺ and Ce³⁺ states, often represented as CeO₂ $\leftrightarrow$ CeO_{2-x}, and is primarily responsible for their remarkable catalytic and antioxidant activities.

Characterization

FTIR analysis

The FTIR spectrum of cerium nitrate hexahydrate (Ce(NO₃)₃.6H₂O), show Fig. 3. a. in the FTIR analysis characteristic bands of coordinated water and nitrate groups. A broad band at ~ 3400 cm⁻¹ and a peak at 1635 cm⁻¹ correspond to O–H stretching and H–O–H bending vibrations of water molecules, confirming the hydrated structure. Distinct nitrate vibrations are observed at 1385 cm⁻¹ (asymmetric stretching, ν_3), 1045 cm⁻¹ (symmetric stretching, ν_1), 825 cm⁻¹ (in-plane deformation, ν_2), and 720 cm⁻¹ (out-of-plane deformation, ν_4). The band at ~ 460 cm⁻¹ is

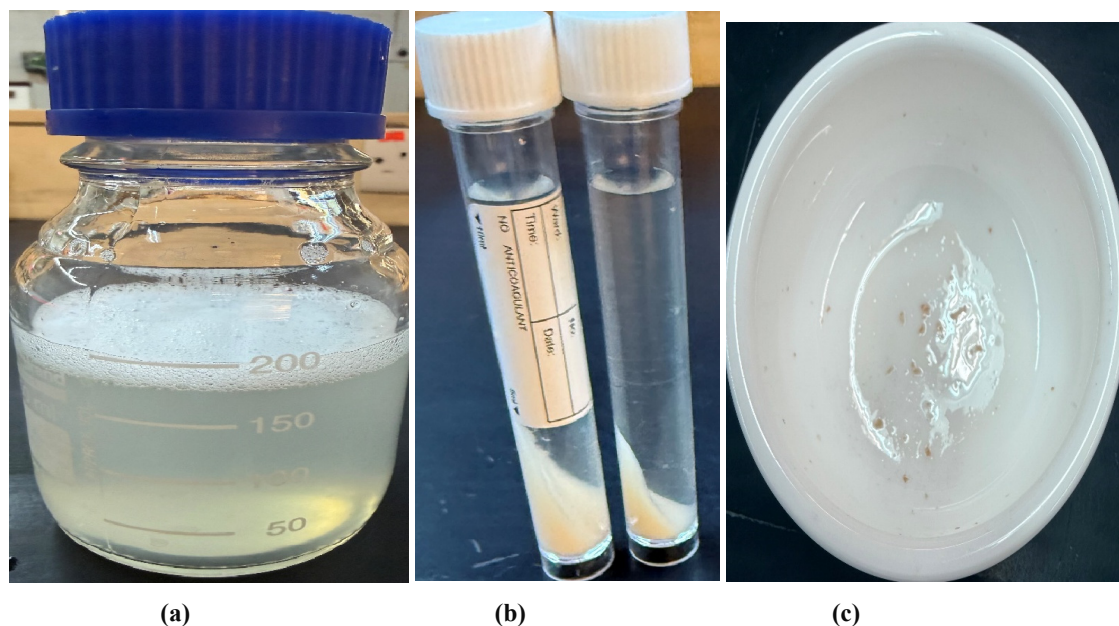


Fig. 2. Synthesis CeO₂-NPs by biological method. a) Mixture of CeO₂-NPs after incubation for two days in the dark, b) Centrifugation c) Powder nanoparticle after microwave drying.

attributed to Ce–O stretching, verifying metal–oxygen coordination in the compound.

The FTIR spectrum of cerium nitrate hexahydrate (Ce(NO₃)₃·6H₂O) (Fig 3-b) displays distinct absorption bands confirming the presence of coordinated water molecules and nitrate ions. The broad band at ~3400 cm⁻¹ and the peak at 1635 cm⁻¹ correspond to O–H stretching and H–O–H bending vibrations of water, indicating its hydrated nature. Characteristic nitrate bands appear at 1385 cm⁻¹ (asymmetric stretching, ν_3), 1045 cm⁻¹ (symmetric stretching, ν_1), 825 cm⁻¹ (in-plane deformation, ν_2), and 720 cm⁻¹ (out-of-plane deformation, ν_4). A distinct band at 460 cm⁻¹ corresponds to Ce–O stretching, confirming coordination between cerium and oxygen atoms. These features collectively verify the structure of Ce(NO₃)₃·6H₂O.

Ultraviolet-visible absorption spectroscopy

One essential spectroscopic approach for evaluating metal nanoparticles is UV-vis absorption spectroscopy. The results of the UV-vis analysis as shown Cerium nitrate hexahydrate's (Ce(NO₃)₃·6H₂O) UV-visible absorption spectra showed clear absorption characteristics typical of Ce³⁺ ions. The O²⁻ → Ce³⁺ charge transfer transition and, to a lesser extent, the 4f → 5d electronic

transitions of cerium ions are responsible for the noticeable absorption band that was seen in the ultraviolet area at around 290–300 nm. The molecule stays in the Ce³⁺ oxidation state with no Ce⁴⁺ contribution, as indicated by the lack of further peaks in the visible region. The high electronic excitation energy of Ce²⁺ ions is confirmed by the strong absorption in the UV region, which is in line with data from the literature for cerium-based salts. These findings validate Ce(NO₃)₃·6H₂O's optical purity and offer a baseline for additional optical analysis and comparison with cerium oxide nanoparticles made from this precursor in show Fig.4-a and The O²⁻ → Ce⁴⁺ charge transfer transition within the CeO₂ lattice is responsible for the strong and distinct absorption band in the ultraviolet region about 320–340 nm that was observed in the UV-visible absorption spectrum of the produced cerium oxide nanoparticles (CeO₂-NPs). In contrast to the absorption peak near 290 nm of the precursor cerium nitrate hexahydrate (Ce(NO₃)₃·6H₂O), the observed red shift in the absorption edge signifies the successful synthesis of CeO₂ nanoparticles with quantum confinement effects. Fig. 4- b

Respectively

Zeta Potential analysis

Zeta potential measurements were used to

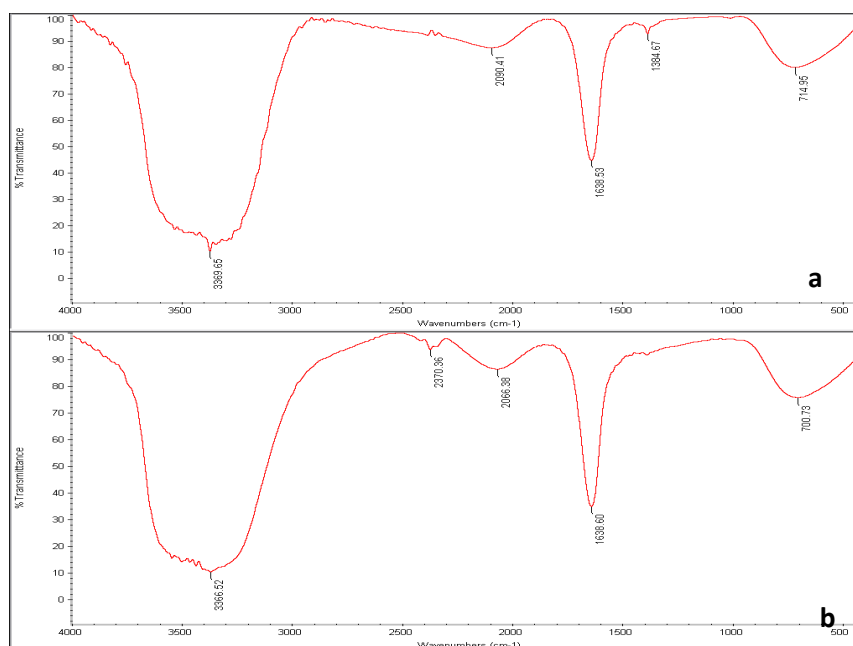


Fig. 3. FTIR spectra of a) Cerium nitrate hexahydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), b) $\text{CeO}_2\text{-NP}$

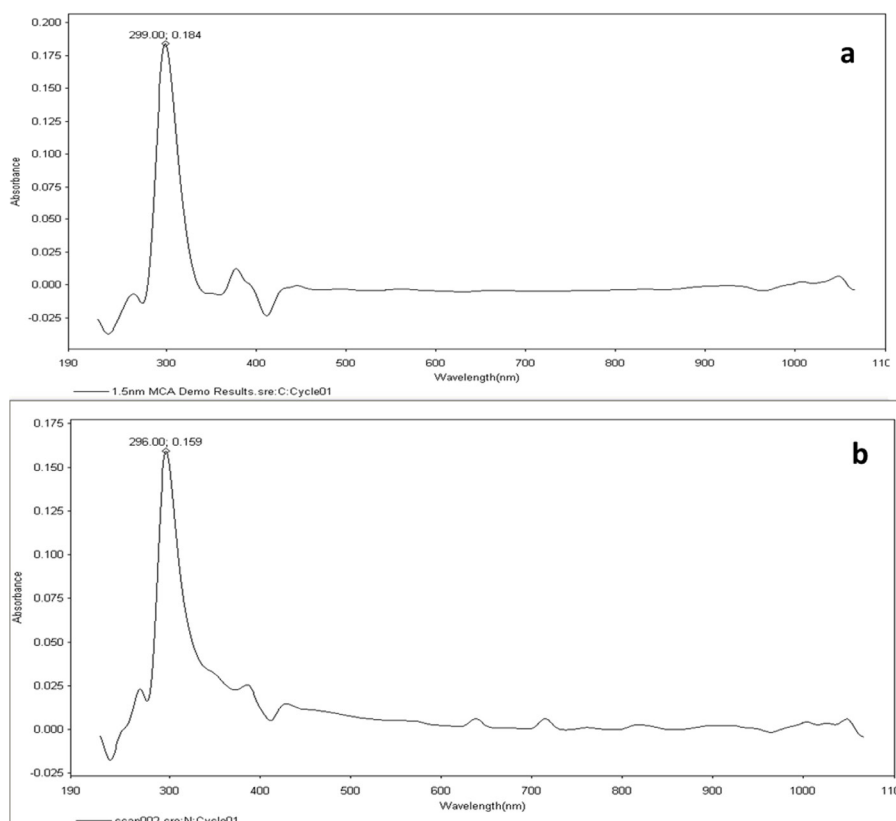
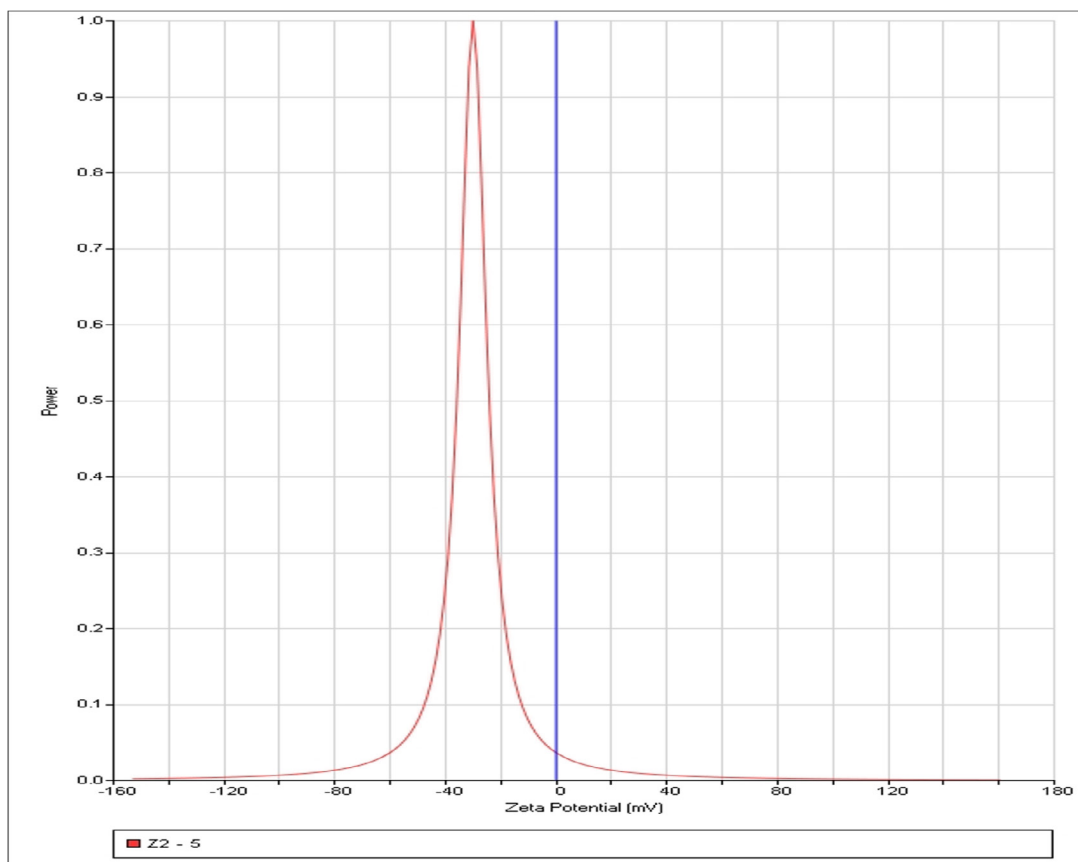


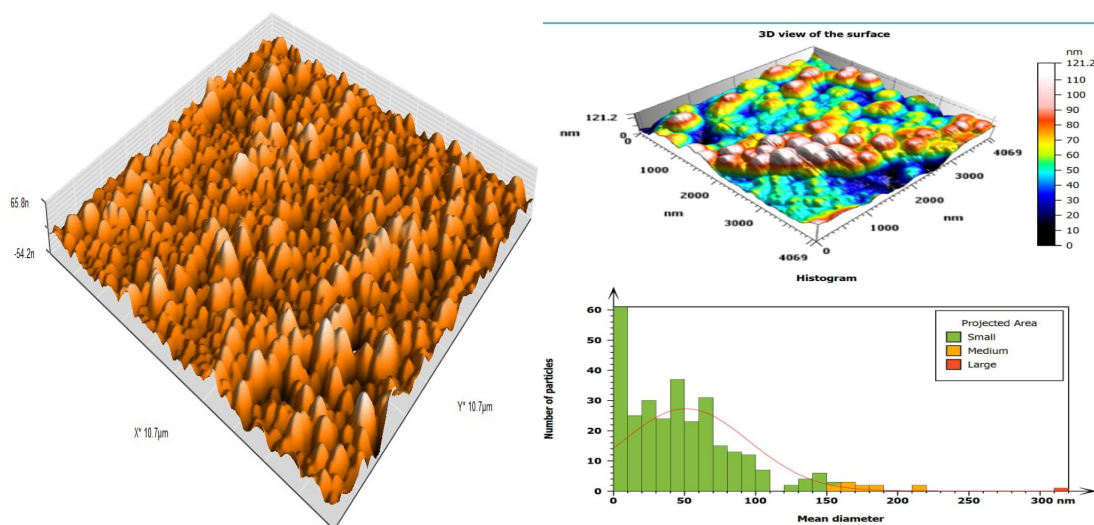
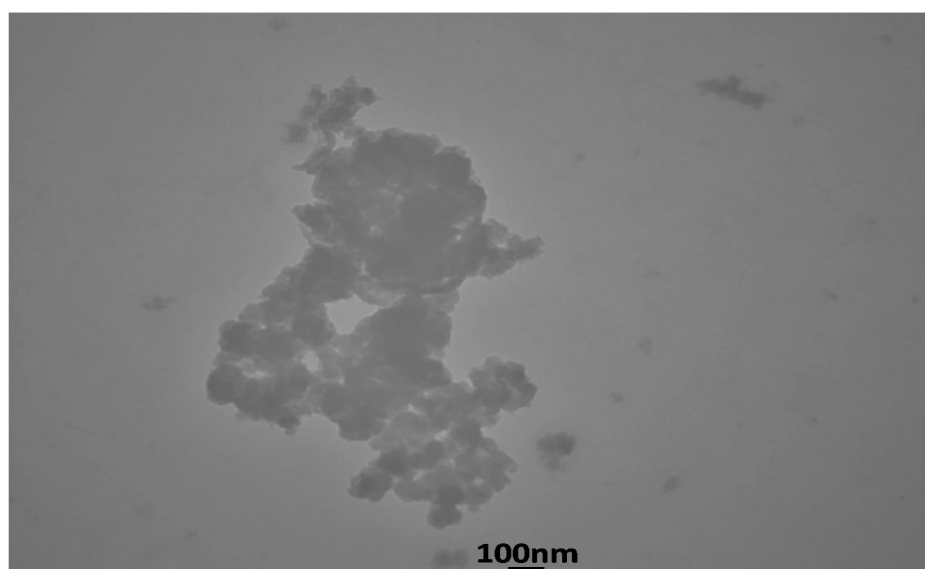
Fig. 4. UV-visible spectra of a) Cerium nitrate hexahydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), b) $\text{CeO}_2\text{-NPs}$

Fig. 5. Zeta Potential analysis of CeO₂-NPs

assess the surface charge and colloidal stability of the produced cerium oxide nanoparticles (CeO₂-NPs). The CeO₂-NPs demonstrated a substantially negative surface charge, shown in Fig. 5, with a zeta potential value of roughly -45 mV. The nanoparticles' strong electrostatic repulsion, which inhibits aggregation and guarantees good colloidal stability in suspension, is suggested by their high negative zeta potential. The presence of oxygen-containing functional moieties and surface hydroxyl groups, which came from the plant extract employed in the green synthesis method, is responsible for the negatively charged surface. Nanoparticles having zeta potential values higher than ± 30 mV are regarded as highly stable in aqueous dispersions based on stability criteria. The biosynthesized CeO₂-NPs are therefore stable and evenly distributed, which makes them appropriate for use in biological and environmental applications, as confirmed by the observed zeta potential.

Atomic force microscopy (AFM) analysis

The prepared CeO₂ nanoparticles (CeO₂-NPs) were examined using Atomic Force Microscopy (AFM) with a CSPM system to analyze and describe their surface morphology and distribution. The AFM micrographs revealed that the CeO₂-NPs exhibited irregular and triangular cluster morphologies. According to the three-dimensional AFM analysis, the nanoparticles displayed an average height and diameter of approximately 62.54 nm, which was consistent with the results obtained from the particle size analyzer, as shown in Fig. 6. Using Atomic Force Microscopy (AFM) with threshold detection, the size distribution and surface morphology of the produced cerium oxide nanoparticles (CeO₂-NPs) were examined. Well-dispersed nanoparticles with uneven and clustered surface topography were visible in the 2D and 3D AFM micrographs. The particles had a variety of shapes, including triangular and spherical ones. About 9.03% of the scanned surface was covered by the 303 nanoparticles that were

Fig. 6. Atomic force microscopy analysis of CeO₂-NPsFig. 7. TEM images of CeO₂-NP

found, with a particle density of 1.83×10^7 particles/mm². With diameters ranging from 5.91 to 310.2 nm and an average Z-max height of 563.4 nm, the mean particle diameter was calculated to be 50.92 nm. The histogram confirmed the nanoscale dimensions of the generated CeO₂-NPs by demonstrating that the majority of the particles lie inside the small-sized region (below 100 nm). These findings support the effective synthesis of nanosized CeO₂ particles and are in good agreement with data from the particle size analyzer.

Transmission Electron microscope (TEM)

Using Transmission Electron Microscopy (TEM), the size distribution and shape of the produced cerium oxide nanoparticles (CeO₂-NPs) were investigated. Since CeO₂ has a high surface energy and strong interparticle interactions, the TEM picture show in the Fig.7 shows that the CeO₂-NPs are primarily irregularly shaped and have a tendency to form aggregated clusters. The individual nanoparticles are in the nanometer range, with a diameter of roughly 10–30 nm, and they

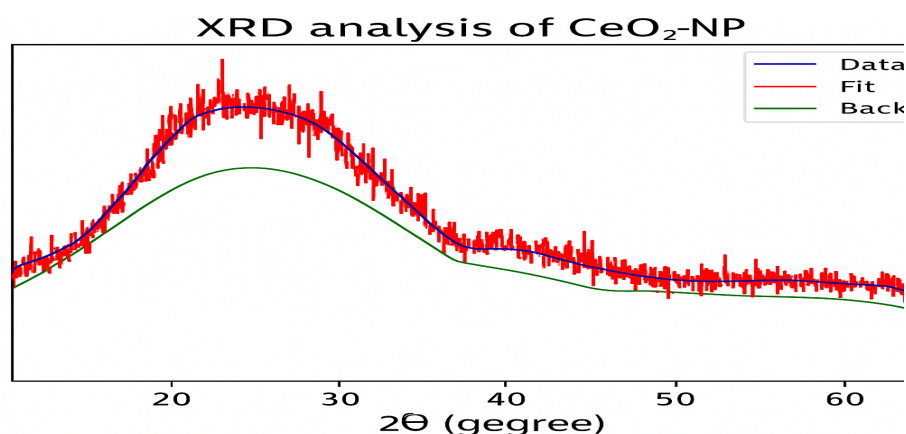
Fig. 8. XRD analysis of CeO₂-N

Table 1. A comparative table summarizing the key physicochemical properties (particle size, morphology, zeta potential)

Characterization	Parameter	CeO ₂ -NPs Results	Notes / Observations
Particle Size	TEM	10–30 nm	Nearly spherical, some aggregation
	AFM	Avg. diameter: 50.92 nm, height: 563.4 nm	Triangular and spherical clusters
Morphology	TEM / AFM	Irregular, clustered, triangular & spherical	Aggregation due to high surface energy
Zeta Potential	Surface charge	–45 mV	High stability, strong electrostatic repulsion
UV–Vis Absorption	λ _{max}	320–340 nm	Red shift vs. precursor (290–300 nm), indicates CeO ₂ formation
FTIR	Functional groups	O–H, Ce–O, nitrate	Confirms coordination and nanoparticle formation
XRD	Crystallinity	Cubic fluorite, 2θ = 28.5°, 33.1°, 47.5°, 56.3°	Nanocrystalline, high phase purity

seem to be almost spherical. In keeping with other findings on biosynthesized CeO₂ nanoparticles, the observed aggregation suggests the creation of polycrystalline structures. The drying procedure used to prepare the sample may also cause this kind of aggregation. Overall, the successful fabrication of CeO₂ nanoparticles with nanoscale dimensions is confirmed by the TEM micrograph.

X-ray diffraction XRD

The XRD pattern of the synthesized cerium oxide nanoparticles show in the Fig. 8 confirms the formation of a crystalline cubic fluorite structure, characteristic of CeO₂. Distinct diffraction peaks were observed at 2θ values of approximately 28.5°, 33.1°, 47.5°, and 56.3°, corresponding to the (111), (200), (220), and (311) planes, respectively (JCPDS No. 34-0394). The broadness of the peaks indicates the nanocrystalline nature of the material. In the figure, the red line represents the experimental data, the blue line the fitted curve, and the green line the background signal. The average crystallite size, calculated using the Debye–Scherrer equation, is consistent with the TEM observations. These results

confirm the successful synthesis of nanocrystalline CeO₂ with high phase purity. A comparative table summarizing the key physicochemical properties show Table (1).

Anti-Candida Activity of CeO₂ Nanoparticles

The results shown in the graph indicate that the anti-Candida activity of CeO₂ nanoparticles against *C. glabrata* is dose-dependent. As the concentration of CeO₂ nanoparticles increases, the zone of inhibition diameter also increases, demonstrating a stronger antifungal effect.

The agar well diffusion method was used to evaluate the biosynthesized cerium oxide nanoparticles' (CeO₂-NPs) antifungal effectiveness against *Candida glabrata*. The diameter of the inhibitory zones in show in the Fig. 9 shows that the CeO₂-NPs increased their antifungal activity in a concentration-dependent manner. The maximum inhibition zone at the highest concentration (1000 mg/mL) was 24.6 ± 1.1 mm, whereas the inhibition zone at the lowest tested dose (62.5 mg/mL) was roughly 11.2 ± 0.8 mm. Inhibition zones at

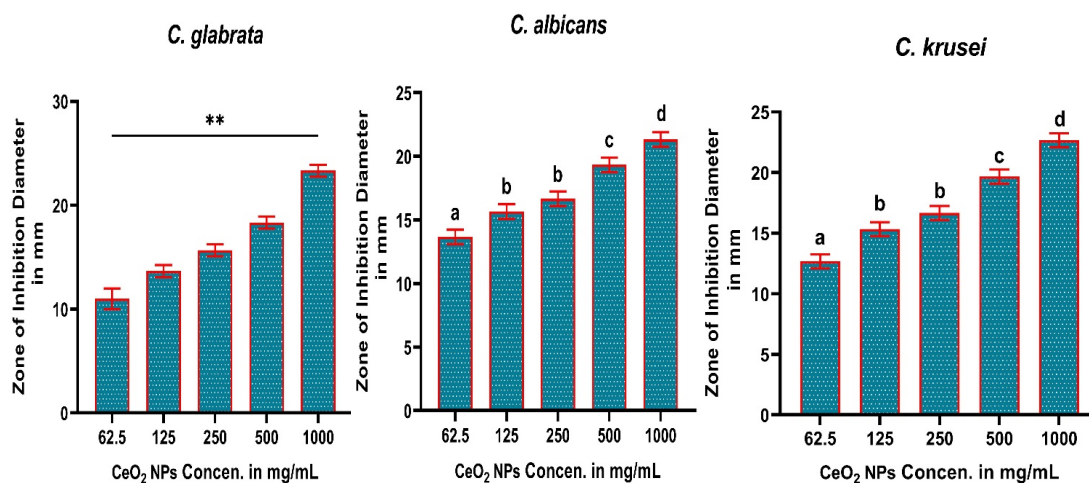


Fig. 9. Mean $\pm$ SD Zone of Inhibition in mm treated with of CeO₂-NP against candida spp at Different Concentrations between 1000, 500, 250, 125, and 62.5 mg/mL. Standard Deviation, ($n = 3$)

intermediate values (125, 250, and 500 mg/mL) were roughly 14.3 ± 0.9 , 17.5 ± 1.0 , and 20.2 ± 0.9 mm, respectively. A robust dose-response association was indicated by the statistically significant increase in inhibitory zone diameter with increasing nanoparticle concentration ($p < 0.01$). The increased antifungal activity at greater concentrations might be explained by CeO₂-NPs' wide surface area and tiny particle size, which allow them to interface with fungal cell membranes and cause oxidative stress, membrane breakdown, and cellular component leakage. Further supporting the observed antifungal effect is the possibility that the negatively charged surface (as verified by zeta potential analysis) encourages electrostatic interactions with the positively charged fungal cell surface. All things considered, these findings show that biosynthesized CeO₂-NPs have strong anti-Candida activity. CeO₂ nanoparticle concentration and its antifungal action on *C. glabrata* are directly correlated, according to the findings shown in the bar chart. Higher concentrations of CeO₂ NPs are more effective at preventing the growth of the fungus, as seen by the zone of inhibition diameter steadily increasing as the concentration rises. This result is in line with recent studies on CeO₂ nanoparticles' antibacterial capabilities, indicating that they may be used as a successful antifungal agent, especially against *Candida glabrata*, which is notorious for being resistant to traditional antifungal medications.

DISCUSSION

Probiotic microorganisms have attracted considerable attention in food technology due to their positive impact on human health and well-being, particularly in preventing chronic degenerative diseases and gastrointestinal disorders. While lactic acid bacteria remain the most commonly employed probiotics in dairy-based products, their use is restricted by the high prevalence of lactose intolerance affecting more than 75% of the global population. Moreover, no prior reports have documented the antifungal activity of the synthesized *S. boulardii*-mediated CeO₂ NPs against *Candida* species. Our results show that these biogenically produced nanoparticles have strong anti-Candida efficacy, indicating a novel therapeutic potential, whereas previous research have primarily focused on antibacterial or antioxidant capabilities. Thus, this study highlights the originality and biomedical significance of CeO₂ NPs by expanding their applicability to fungal diseases and introducing *S. boulardii* as a novel and effective biological system for nanoparticle synthesis.

This challenge has driven interest in developing non-dairy probiotic alternatives, such as cereal-based fermented beverages, which offer a suitable and nutritious medium for probiotic growth and consumption [18]. Nanoparticles synthesized by yeast predominantly accumulate within the intracellular environment. Owing to their surface plasmon resonance (SPR) properties, these nanoparticles exhibit strong absorption in the

ultraviolet and visible regions of the electromagnetic spectrum, which facilitates their easy detection and optical characterization [19]. The biomass obtained from *Saccharomyces cerevisiae* yeast, commonly used in beer production, was once considered a waste byproduct after fermentation. However, it is now recognized for its valuable bioactive components, including β -glucans, proteins, and functional peptides. This yeast biomass has demonstrated significant potential for reuse in fermentation processes with regenerated wort and for the synthesis of organic minerals and other biotechnological applications [20]. Another study demonstrated [21]. The use of biogenic yeasts offers additional advantages, particularly through their ability to supply essential minerals that promote the formation of complex nanoparticles (NPs). The size of selenium nanoparticles (SeNPs), as determined by dynamic light scattering (DLS), was influenced by both the metallic cores and the biomolecular layers coating their surfaces, which serve as natural stabilizing agents. Similarly, natural resources such as plants, bacteria, fungi, and yeasts are capable of biologically synthesizing cerium oxide nanoparticles (CeO_2 NPs) at both intracellular and extracellular levels. These organisms utilize diverse biomolecules as reducing and stabilizing agents, allowing the eco-friendly synthesis of CeO_2 NPs without the need for toxic or hazardous chemical reagents. In the present study, *Saccharomyces boulardii* was utilized for the green synthesis of cerium oxide nanoparticles under *in vitro* conditions using YPD broth as the growth medium to initiate cerium ion reduction and nanoparticle formation. Due to its remarkable physicochemical properties, cerium oxide (CeO_2) has attracted considerable attention in recent years. Cerium oxide nanomaterials (nanocerium) have found widespread applications across various fields; however, their increasing use has led to potential environmental release and human exposure, primarily through inhalation, which raises significant safety and toxicological concerns [22]. Green-synthesized cerium oxide nanoparticles (CeO_2 -NPs) exhibit enzyme-mimetic activities similar to several natural antioxidant enzymes such as catalase, superoxide dismutase, and peroxidase. These catalytic properties enable CeO_2 -NPs to scavenge intracellular reactive oxygen species (ROS), thereby reducing oxidative stress and preventing cellular damage. An interesting feature of CeO_2 -NPs is their ability to act as both oxidation and reduction catalysts. This dual

redox behavior provides the nanoparticles with regenerative antioxidant properties, which are strongly influenced by the surrounding medium and environmental conditions in which the reactions occur [23]. Study agree with [24]. Dried yeast cultures synthesized only a small quantity of nanoparticles (NPs), whereas freshly cultured *Saccharomyces boulardii* produced a substantially higher yield. The resulting nanoparticles were predominantly monodispersed, with some exhibiting slight aggregation outside the cells. This study provides the first evidence that nanoparticle synthesis by *S. boulardii* occurs primarily through an intracellular mechanism, consistent with previous findings reported for bacteria and fungi. To confirm the synthesis and evaluate the catalytic activity of CeO_2 nanoparticles (CeONPs), several key parameters were assessed, including average particle size, radius, chemical composition, and surface properties. Scanning electron microscopy (SEM) analysis revealed that the CeONPs had an average particle size ranging from roughly 10–30 nm [25]. On other hand anovel, rapid, highly sensitive, and selective non-enzymatic, label-free method was developed for morphine detection based on the fluorescence emission of surface-modified cerium oxide nanoparticles (CeO_2 NPs) [26]. The synthesized Fe_2O_3 nanoparticles were evaluated for their antimicrobial activity against selected microorganisms. At a concentration of 100 mg/L, the nanoparticles exhibited inhibitory effects against *Escherichia coli* bacteria as well as *Aspergillus flavus* and *Trichophyton mentagrophytes* fungi, as determined by the Zone of Inhibition (ZOI) and Minimum Inhibitory Concentration (MIC) assays. Moreover, the Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC) were found to completely inactivate *Salmonella typhi* at the same dose (100 mg/L) [27]. Nickel nanoparticles (NiNPs) were successfully synthesized using *Khaya senegalensis* stem bark extract, resulting in stable, spherical nanoparticles with notable antimicrobial potential. This green synthesis approach provides an eco-friendly alternative to conventional chemical methods and underscores the significance of plant-mediated nanotechnology in biomedical applications [28].

A recent advancement in the field of functional beverages is the development of probiotic beer—a novel formulation incorporating probiotic microorganisms such as *Saccharomyces boulardii* to biosynthesize organic cerium. The presence

and quantification of cerium (Ce) in biogenic *S. bouldarii* have been confirmed through various instrumental analyses, validating the successful incorporation of this mineral into the fermented beverage. However, such analyses pose significant challenges, as detecting and characterizing trace elements within complex biological matrices require highly precise and sensitive methodologies. With the growing interest in cerium-enriched functional foods, there is an increasing demand for the establishment of rapid, efficient, cost-effective, and environmentally friendly analytical techniques to accurately identify and quantify trace elements synthesized by microorganisms like *S. bouldarii*.

CONCLUSIONS

This study demonstrates that *Saccharomyces bouldarii* extract can be used as a green, cost-effective, and environmentally friendly reducing and stabilizing agent to synthesize cerium oxide nanoparticles (CeO_2 -NPs). The biosynthesized nanoparticles exhibited favorable physicochemical properties, including nanoscale size, stability, and distinctive $\text{Ce}^{4+}/\text{Ce}^{3+}$ redox activity. Biological evaluation revealed significant antifungal activity against *Candida* species, likely mediated by reactive oxygen species (ROS) generation and disruption of cell membrane integrity. Importantly, the use of *S. bouldarii* minimized the need for hazardous chemicals and enhanced the biocompatibility of CeO_2 -NPs. However, further studies, including cytotoxicity assays and in vivo evaluations, are needed to fully validate their safety and therapeutic potential.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest related to the research, authorship, or publication of this manuscript. All authors have disclosed any financial or personal relationships that could potentially influence or bias the work presented.

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