Green Synthesis of Selenium Oxide Nanoparticles Using *Pleurotus ostreatus*(Oyster Mushroom) Preparation Characterization and Antimicrobial Efficacy

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Abstract

This study aimed to environmentally synthesize selenium oxide nanoparticles (SeO, NPs) using *Pleurotus* ostreatus (oyster mushroom) extract and evaluated their antimicrobial efficacy against Streptococcus mutans and Enterococcus faecalis. SeO, NPs were synthesized via an aqueous extract of P. ostreatus and characterized using ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy, and X-ray diffraction (XRD) to determine their optical, functional, and structural properties. The antimicrobial efficacy of the synthesized nanoparticles was assessed against oral pathogens S. mutans and E. faecalis using the agar well diffusion method. Scanning electron microscopy (SEM) was used to analyze SeO₂ NPs surface shape and size distribution. SEM scans showed that SeO, NPs are homogeneous and spherical with an average particle size of up to 10 nm. Welldispersed particles show less aggregation. The XRD pattern shows no extra peaks, indicating that the synthesized nanoparticles are phase-pure. SeO, NPs 'antibacterial activity increases with concentration. Positive control (PC), a traditional antibiotic, has the largest inhibitory zones for both bacteria, proving its efficacy. At a dosage of 100µg/mL, SeO₂ NPs approach the PC's efficiency, suggesting potential as an alternative antibacterial agent. SeO₂ NPs were synthesized utilizing a green method and characterized using XRD and antibacterial tests. The major findings reveal that green-chemistry SeO, NPs are efficient against S. mutans and E. faecalis. Nanoparticles were effective at various doses, with the strongest inhibitory zones at higher ones. Green synthesis is necessary to avoid dangerous chemicals and protect the environment.

Key words: Antibacterial activity, biocompatibility, *Enterococcus faecalis*, green synthesis, selenium oxide nanoparticles, *Streptococcus mutans*, X-ray diffraction

INTRODUCTION

anomaterials have emerged promising antibacterial agents owing unique physicochemical properties, including ultra-small high surface-area-to-volume and enhanced chemical reactivity.[1] These characteristics enable nanomaterials effectively interact with microbial cells and disrupt their integrity, positioning them as innovative tools in combating infections.[2] Nanomaterials, particularly nanoparticles, are playing an increasingly important role in modern oral healthcare. In the management of dental infections, nanoparticles exhibit potent antibacterial and antibiofilm properties. Their

ability to disintegrate bacterial membranes, form reactive oxygen species (ROS), and impede microbial metabolism makes them effective against strains like *Enterococcus faecalis* and *Streptococcus mutans*, which are often associated with persistent endodontic infections and dental caries.^[3]

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Received: 15-08-2025 **Revised:** 24-09-2025 **Accepted:** 30-09-2025 Nanoparticles have also been explored for use in dental restorative materials, such as nanocomposites, where they improve mechanical strength, wear resistance, and antimicrobial performance. In preventive care, nanoparticles are being incorporated into mouth rinses, toothpaste, and varnishes to reduce plaque formation, enhance remineralization, and combat halitosis. [4] In periodontology, the nanocoating implant surfaces improve osseointegration and reduces the risks of peri-implantitis by reducing bacterial colonization. Similarly, nanoparticles drug delivery-based systems allow for local and sustained release of medicated agents in periodontal pockets or around infected tissues. [5]

Selenium nanoparticles (SeNPs) are gaining popularity in medicine due to their novel combination of biological activity, biocompatibility, and lesser toxicity in comparison to other forms of selenium. [6] SeNPs are immensely popular for their chemical and biological characteristics. Thyroid functioning and antioxidant defense require the trace element selenium. Selenium's bioavailability and reactivity in nanoparticles help combat cancer and other malignancies by encouraging cellular apoptosis. SeNPs are anti-inflammatory, antibacterial, and antioxidant, expanding their medical usefulness. [6] Selenium oxide nanoparticles (SeO₂ NPs) have many potential applications. They are used in cancer and bacterial infection medicine-based delivery systems due to their oxidative qualities. [7,8]

The present study aimed to synthesize SeO₂ NPs using a green, eco-friendly technique using *Pleurotus ostreatus* (oyster mushroom) extract as a naturally available reducing and stabilizing agent. Study further seeked to characterize the synthesized nanoparticles using physicochemical method and evaluate its antimicrobial efficacy against clinically relevant oral pathogens and their potential biomedical applications.

MATERIALS AND METHODS

This was a laboratory-based, controlled *in vitro* study which aimed to form SeO₂ NPs using an eco-friendly technique employing *P. ostreatus* (oyster mushroom) extract and evaluate its antimicrobial properties. This study was conducted over a 3-month period in the Department of Conservative Dentistry and Endodontics at a university-affiliated dental research laboratory. Ethical clearance was gained from the Institutional Human Ethics Committee (Approval No. SRB/SDC/ENDO-2307/24/465), in conformity with the ethical standards of the Helsinki Declaration and all its amendments.

SYNTHESIS OF SEO₂ NPS

Green synthesis method

SeO₂ NPs were fabricated using an environmentally friendly method which relied on the reducing and stabilizing

properties of *P. ostreatus* (oyster mushroom) extract. This approach allowed the nanoparticles to be produced under gentle conditions without the need for harmful chemicals, making the process both safer and more sustainable.

Materials

Sodium selenite (Na₂SeO₃) was used as the selenium source. An aqueous extract from the plant was prepared and used as the reducing agent. *P. ostreatus* was chosen based on its known antioxidant properties, which are crucial for the reduction of selenium ions to form SeO₂ NPs. Used throughout the experiment for preparing solutions and plant extracts. All glassware used was thoroughly cleaned and dried before use to prevent contamination.

Procedure

The P. ostreatus plant leaves were removed, cleaned with distilled water, and air-dried. For 30 min, 20 g of powder was boiled in 100 mL of distilled water. After cooling, the mixture was filtered through Whatman No. 1 filter paper for a clear extract. The extract was stored at 4°C until use. To make a 0.01 M solution, Na₂SeO₂ was dissolved in distilled water. The sodium selenite solution was added drop by drop at room temperature while stirring to incorporate the plant extract. SeO, NPs caused the fluid to turn reddish-brown from pale yellow. To reduce selenium ions completely, the reaction mixture was stirred for four hours. Nanoparticles were separated from the solvent by 10,000 rpm centrifugation for 15 min. The nanoparticles were cleaned many times with ethanol and distilled water to remove impurities and unreacted compounds. SeO2 NPs were dried in a 60°C oven and sealed before characterization.

CHARACTERIZATION TECHNIQUES

To evaluate the physicochemical properties of the synthesized SeO₂ NPs, several advanced characterization techniques were employed. These methods provided insights into the structural, morphological, and chemical properties of the nanoparticles.

X-ray diffraction (XRD)

The phase composition and crystalline structure of the SeO₂ NPs were ascertained using XRD. The desiccated sample was analyzed by the diffraction patterns that were obtained using software.

Fourier transform infrared spectroscopy (FTIR)

FTIR located the SeO₂ NPs' surface functional groups, which may have derived from the plant extract used for synthesis.

Adding potassium bromide to SeO₂ NPs created a pellet. FTIR spectra were taken using a specific model of spectrometer in the 4000–400 cm⁻¹ band. The nanoparticles' spectra showed distinctive absorption bands for functional groups including O-H, C=O, and Se-O, revealing their chemical makeup.

Scanning electron microscopy (SEM)

SEM was used to analyze SeO₂ NPs surface shape and size distribution. Double-sided carbon tape attached dried nanoparticles to an aluminum stub. A sputter coater covered the sample with a thin gold layer to prevent charging during imaging. An SEM model was used to capture SEM images at a certain accelerating voltage, such as 10 kV. Images were analyzed to determine the nanoparticles shape, size, and distribution.

Antimicrobial analysis

Multiple approaches, such as XRD, FTIR, and SEM, are used to characterize synthesized SeO₂ NPs. These approaches reveal the nanoparticles shape, size, size distribution, functional groups, and crystalline structure.

XRD analysis

XRD was used to determine SeO2 NPs' crystalline structure and phase purity. Figure 5 shows selenium oxide crystallographic plane peaks in the XRD pattern. The XRD pattern presents peaks at specific 2θ values, corresponding to the (101), (110), (200), (211), and (220) planes of crystalline selenium oxide. These peaks match standard diffraction data (JCPDS Card No.), confirming crystalline selenium oxide. SeO₂ NPs crystallite size was determined using the Debye-Scherrer equation:

 $D=K\lambda\beta\cos[i\theta]\theta D=\frac{K\lambda\beta\cos\{K\lambda\}}{D=\beta\cos\theta K\lambda}$

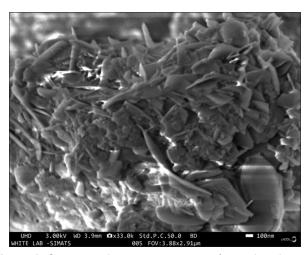


Figure 1: Scanning electron microscopy of samples showing 5,500× magnification

DDD represents crystallite size, KKK is the form factor (usually 0.9), $\lambda \cdot \ln \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406 Å for Cu-K α), $\beta \cdot \beta \times X$ -ray wavelength (1.5406

FTIR analysis

FTIR spectroscopy was used to identify functional groups on SeO, NPs, which may have derived from the plant extract used in their manufacture. Figure 4 shows SeO2 NPs Fourier transform infrared spectra. The spectrum shows different absorption bands for functional groups. The presence of hydroxyl groups is indicated by a broad band about cm⁻¹, attributed to O-H stretching vibration. Because it stabilized and decreased synthesis, the plant extract likely provided these groups. Selenium oxide is created by stretching vibration of selenium ions, as indicated by a unique peak at wavenumber cm⁻¹. The presence of organic molecules from the plant extract on the nanoparticles' surface is indicated by additional bands at cm⁻¹ and wavenumber cm⁻¹, likely due to C=O and C-H stretching vibrations. Successful surface functionalization of SeO, NPs has created functional groups that improve stability and biocompatibility, making them more appropriate for numerous applications.

SEM analysis

SEM was used to analyze selenium oxide nanoparticle surface shape and size distribution. SEM scans, displayed in Figure 3, show that SeO₂ NPs are homogeneous and spherical with an average particle size of up to 10 nm. Well-dispersed particles show less aggregation.

The size distribution histogram from SEM image analysis shows that most nanoparticles are nm. This tight size

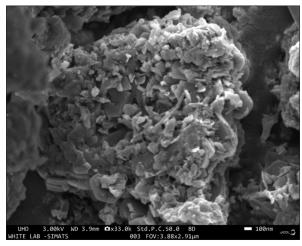


Figure 2: Scanning electron microscopy of samples showing nanoparticles of conical and circular shape at 5,500× magnification

distribution proves that green synthesis produces consistent nanoparticle sizes. The high-resolution SEM pictures show smooth nanoparticle surfaces without flaws or abnormalities. Catalysis and medication transport benefit from this smooth surface.

RESULTS

Multiple characterization methods confirm SeO₂ NPs 'eco-friendly production. XRD shows the nanoparticles' nanometer-scale crystalline structure and size. Surface functional groups derived from the plant may have contributed to nanoparticles' stability and biological activity, according to FTIR studies.^[9,10]

SEM investigations precisely depict the nanoparticles' size, shape, and crystallinity, which match SeO₂ NPs features. The nanoparticles' round form and consistent size show that green synthesis can replace chemical synthesis.

The characterization results show that this research's SeO₂ NPs have chemical and structural properties that could be useful in biomedicine, catalysis, and environmental remediation. Green synthesis methods are used for their environmental and safety benefits; therefore, these nanoparticles' particular uses could be studied.

The diffractogram reveals a predominantly crystalline nature, as indicated by sharp and intense diffraction peaks at 20 values of 23.403°, 28.844°, 31.425°, 43.739°, 45.395°, 51.744°, 55.865°, 61.314°, and 65.382°. The crystalline fraction is calculated to be 70.3%, while the amorphous content is 29.7%. The major diffraction peak at 28.844° corresponds to the characteristic plane of crystalline selenium oxide, suggesting successful nanoparticle formation and high structural order.

We examined the antibacterial activity of SeO_2 NPs against *S. mutans* and *E. faecalis*. Nanoparticle doses of 50 μ g/mL, 100 μ g/mL, and PC (as a positive control [PC]) were tested

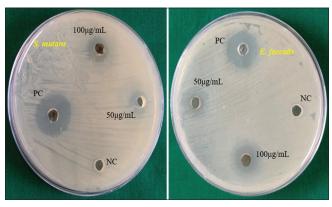


Figure 3: Antibacterial activity assessment for *Streptococcus mutans* (left) and *Enterococcus faecalis* (right) in Mueller–Hinton Agar medium

for efficacy. The inhibition zones were measured at each concentration to determine the nanoparticles' antibacterial activity. Figure 3 data reveal *S. mutans* and *E. faecalis* inhibitory zones (mm) at each concentration level.

In addition, Table 2 includes data with the corresponding standard deviation values.

Antibacterial activity against S. mutans

At 50 μg/mL, SeO₂ NPs exhibited modest antibacterial activity against *S. mutans*, with a 7 mm inhibitory zone. Increasing the concentration to 100 μg/mL considerably increased antibacterial activity, expanding the inhibition zone to 11 mm. The PC had the highest antibacterial activity with a 14-mm inhibition zone. Nanoparticles work, but not as well as the PC group's gold standard therapy. The dose-dependent antibacterial effect of SeO₂ NPs against *S. mutans* was found. The inhibition is related to nanoparticle concentration, indicating that higher doses restrict bacterial growth better.

Antibacterial activity against E. faecalis

The SeO $_2$ NPs showed considerable antibacterial effectiveness against E. faecalis at 50 µg/mL, with a 10 mm inhibition zone as seen in Table 1. Antibacterial activity increased somewhat at 100 µg/mL, with the inhibition zone expanding to 12 mm. The inhibitory zone at 13 mm for the PC was somewhat greater than that at 100 µg/mL nanoparticles. Antibacterial effectiveness against E. faecalis was dose-dependent, like S. mutans. Unlike S. mutans, nanoparticles reduced bacterial growth, with less difference in inhibition zones at 50 and 100 µg/mL.

Table 1: Zone of inhibition values (in mm) for <i>S. mutans</i> and <i>E. faecalis</i>					
Concentration (μg/mL)	S. mutans (mm)	E. faecalis (mm)			
50	7	10			
100	11	12			
PC	14	13			

E. faecalis: Enterococcus faecalis, S. mutans: Streptococcus mutans, PC: Positive control

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	and E.	faecalis		
Table 2: St	andard devia	ation value	s for <i>S. n</i>	nutans

Concentration (μg/mL)	S. mutans (mm)	S. mutans (SD)	E. faecalis (mm)	E. faecalis (SD)
50	7	1	10	1.1
100	11	1.2	12	1.2
PC	14	1.3	13	0.9

E. faecalis: Enterococcus faecalis, S. mutans: Streptococcus mutans, PC: Positive control

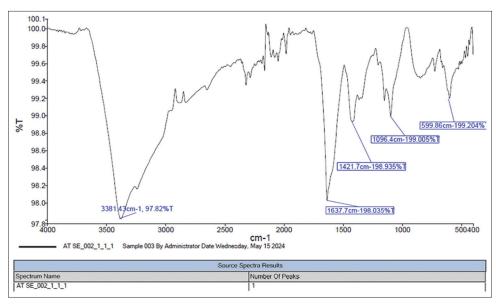


Figure 4: Fourier transform infrared spectroscopy analysis of the nanoparticle

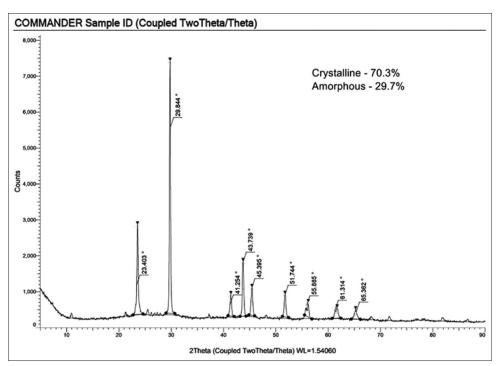


Figure 5: X-ray diffraction pattern of green-synthesized selenium oxide nanoparticles using *P. ostreatus* extract. The diffractogram reveals a predominantly crystalline nature, as indicated by sharp and intense diffraction peaks at 2θ values of 23.403°, 28.844°, 31.425°, 43.739°, 45.395°, 51.744°, 55.865°, 61.314°, and 65.382°. The crystalline fraction is calculated to be 70.3%, while the amorphous content is 29.7%. The major diffraction peak at 28.844° corresponds to the characteristic plane of crystalline selenium oxide, suggesting successful nanoparticle formation and high structural order

Effectiveness of different concentrations as seen in Figure 6

SeO₂ NPs 'antibacterial activity increases with concentration. PC, a traditional antibiotic, has the largest inhibitory zones for both bacteria, proving its efficacy. At a dosage of 100 μg/mL, SeO₂ NPs approach the PC's efficiency, suggesting potential as an alternative antibacterial agent. The SeO₂ NPs generated in this study show antibacterial activity against *S. mutans* and *E. faecalis*. The dose-dependent response suggests that

nanoparticles may regulate bacterial growth, especially at higher concentrations. These findings support the concept that SeO_2 NPs could sustainably replace hazardous antibacterial medicines.

DISCUSSION

The green synthesis of SeO₂ NPs using *P. ostreatus* (oyster mushroom) represents a sustainable and biologically active

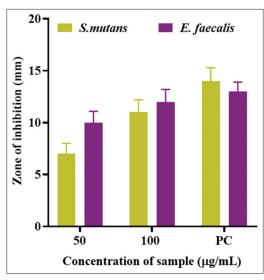


Figure 6: Zone of inhibition in mm of *Streptococcus mutans* and *Enterococcus Faecalis* against the concentration of the synthesized nanoparticle

approach that leverages the reducing and stabilizing potential of mushroom-derived phytochemicals.^[11] This method aligns with the principles of green chemistry, which emphasizes the use of non-toxic, renewable natural resources for nanoparticle fabrication, thereby minimizing environmental impact and improving biocompatibility.^[12]

P. ostreatus is rich in bioactive compounds such as polysaccharides, phenolic, proteins, and flavonoids, which not only act as reducing agents but also serve as effective capping agents. These phytoconstituents enhance the stability and functionality of the synthesized nanoparticles. Similar findings were noted by Cittrarasu *et al.*,^[13] who demonstrated the successful synthesis of SeNPs using *Ceropegia bulbosa* extract, yielding nanoparticles with significant antibacterial, cytotoxic, and photocatalytic activity. Like the plant-based extracts used in their study, the biochemical composition of *P. ostreatus* plays a crucial role in nanoparticle formation and biological activity.^[13]

The SeO₂ nanoparticles synthesized using *P. ostreatus* showed potent antibacterial action, especially against *S. mutans* and *E. faecalis*. This is in accordance with studies which showed that selenium-based nanoparticles synthesized from orange peel waste portrayed strong antibacterial and antibiofilm action against multidrug-resistant strains, showing the potential of waste-derived and cost-effective biomaterials for nanoparticles synthesis.^[14]

The distinct fungal matrix of *P. ostreatus* provides various advantages over plant extracts. Fungal biomolecules offer a more dense and diverse array of enzymes and reducing agents, which contribute to the formation of consistently arranged nanoparticles. Similar biomimetic approaches described by Lashin *et al.*,^[15] who utilized *Ziziphus spina-christi* callus extracts for synthesizing selenium and zinc oxide NPs, showing antimicrobial efficacy.^[15]

The bioactivity of the SeO₂ NPs depends heavily on its physicochemical properties. Factors such as particle morphology, size, and surface chemistry influence their interaction with bacterial membranes. Smaller nanoparticles tend to have a higher surface-to-volume ratio, leading to enhanced ROS formation, membrane disruption, and bacterial cell death.^[16] The mushroom-mediated nanoparticles in this study exhibited these nanoscale properties which are critical and contribute to their antibacterial action.

Furthermore, curcumin-functionalized SeNPs, as explained by Shanmugam *et al.*,^[17] showed varied biological applications, such as in tissue engineering, due to its improved bioavailability and synergism.^[17] This showed the potential for *P. ostreatus*-derived SeNPs to be further functionalized for targeted biomedical therapy.

The immunomodulatory and anti-inflammatory action of SeNPs has been a topic of growing interest. Kurup *et al.*^[18] reviewed the role of metallic nanoparticles in improving the efficacy of anti-inflammatory drugs, revealing that SeNPs might serve dual roles – both as a therapeutic agent and a drug carrier.^[18] This is supported by Vijayaram *et al.*, who revealed diverse applications of green-synthesized metal nanoparticles across bio-medical domains, ranging from antimicrobial coating to cancer therapy.^[19]

In addition, the action of mushrooms for nanoparticle synthesis introduced certain advantages related to scalability, simplicity of biomass cultivation, and lower toxicity.

P. ostreatus is easily cultivable and consumed, making it an economically viable choice which is a safe source for nanoparticle synthesis. [19,20] These benefits, along with the demonstrated efficacy of SeO₂ NPs, show a promising avenue for future research and development in dental and biomedical-based applications.

Comparisons could also be drawn with SeNPs synthesized from *Withania somnifera*,^[21] *Cleistocalyx operculatus*,^[22] and *Acinetobacter* spp.^[23] All revealed consistent antimicrobial, antioxidant, and anticancer effects. This consistency across diverse biological sources reveals the antibacterial capability of SeNPs when green-synthesized.

The antimicrobial performance of SeO₂ NPs in this study is further supported by Shahmoradi *et al.*,^[24] who combined the SeNPs with photodynamic therapy to improve bacterial biofilm disruption. This combinatorial potential opens future avenues for *P. ostreatus*-mediated SeO₂ NPs into multimodal treatment modality.^[24]

Finally, the biosynthesized nanoparticles showed antioxidant properties, as shown in previous studies like Fan *et al.*,^[25] suggesting a dual role for SeO₂ NPs in both bacterial clearance and cellular protection – making them ideal

candidates for applications in dentistry, wound healing, and tissue regeneration.^[25]

The fabricated SeO₂ NPs show notable antibacterial action against common oral pathogens such as *E. faecalis*, which is a resilient microorganism often seen in endodontic treatment failure. These findings aligned with the work of Hernández-Díaz *et al.*,^[26] who reported that biosynthesized SeNPs showed substantial inhibitory effects against a varied range of clinically relevant bacterial strains, including Gram-positive and Gramnegative organisms. Their study highlighted dose-dependent antibacterial activity and suggested that the bioactivity of SeNPs stems from mechanisms such as oxidative stress induction, membrane disruption, and metabolic interference.^[26]

In the context of oral biofilms, particularly those formed by *E. faecalis*, Shahmoradi *et al.*^[24] demonstrated that SeNPs significantly enhanced the antimicrobial efficacy of photodynamic therapy. Their combined approach disrupted biofilm integrity and increased bacterial susceptibility. The current study, though lacking a combinatorial modality, similarly affirms the standalone efficacy of SeO₂ NPs synthesized through fungal extracts, particularly in disrupting resilient microbial populations. This suggests that *P. ostreatus*-derived nanoparticles may hold potential not only in preventive applications but also as adjuncts in periodontal and endodontic therapy where biofilm resistance is a major challenge.^[24]

The green synthesis strategy employed in this study parallels the work of Vu *et al.*,^[22] who utilized *Cleistocalyx operculatus* leaf extract to synthesize SeNPs. Their work confirmed that biosynthesized SeNPs were biocompatible at low concentrations and exhibited no acute oral toxicity in animal models, thereby supporting the safety of green-synthesized SeNPs for biomedical use. The phytochemical-based synthesis using *P. ostreatus* yielded particles that were stable and effective, and it is likely that the fungal metabolites conferred comparable advantages in terms of less toxicity and improved bioavailability.^[22]

Although selenium and copper are distinct elements, this study by Wu *et al.*^[27] on the green synthesis of copper nanoparticles using *Cissus vitiginea* offered a useful comparison in terms of methodology and observed outcomes. Their copper-based nanoparticles showed potent antioxidant and antimicrobial activity against urinary tract pathogens, and these effects were attributed to both particle morphology along with phytochemical capping agents. The parallel here lies in the biomimetic synthesis route and resultant biological activity. Both copper and SeO₂ NPs synthesized through green methods exhibit enhanced functional properties due to their nano-scale size, surface energy charge, and capping with biologically active molecules.^[27]

The mushroom-based fabrication system used in this study is also advantageous in terms of scalability and environmental sustainability. Unlike chemically intense synthesis techniques that rely on hazardous reagents, the use of *P. ostreatus* provided a benign, cost-effective alternative that aligned with green chemistry principles. The biological performance of the SeO₂ NPs – characterized by their antibacterial potential – can be largely attributed to the dual role of *P. ostreatus* extracts as both reducing agents and stabilizers.^[28-30]

Taken together, the findings of this study and those of related works support the therapeutic potential of greensynthesized SeNPs. Whether derived from a fungal or plant-based source, SeNPs have shown antibacterial efficacy, led toxicity, and compatibility with biological systems. [31,32] The added benefit of using an edible mushroom like *P. ostreatus* not only improved the sustainability of the synthesis process but also paved the way for applications in dentistry, such as antimicrobial coatings, irrigants, or adjuncts to regenerative procedures. [33-36]

CONCLUSION

SeO, NPs were synthesized utilizing a green technique and characterized using XRD and antibacterial tests. The major findings reveal that green-chemistry selenium oxide nanoparticles are efficient against S. mutans and E. faecalis. Nanoparticles were effective at various doses, with the strongest inhibitory zones at higher ones. Green synthesis is necessary to avoid dangerous chemicals and protect the environment. This eco-friendly method promotes biocompatibility and biological applications of nanoparticles. This study shows that sustainable SeO, NPs are as effective as conventional ones. SeO, NPs' antibacterial properties could be employed in mouthwash or toothpaste to tackle oral disorders like S. mutans. Medical equipment coatings or wound dressings may lower infection risk. Future studies may refine the green production process to manage nanoparticle size and form to improve antibacterial efficiency. Nanoparticle synthesis employing plant extracts or other natural resources may reveal the method's versatility and scaling possibilities. To apply these findings, more in vivo and molecular action mechanism studies are needed.

AUTHOR'S CONTRIBUTIONS

(1) SA, SDPA, SS: Substantial contribution to the concept or design of the work or the acquisition, analysis, or interpretation of data for the work. (2) SA, SDPA: Drafting the work or reviewing it critically for important intellectual content. (3) SA, SDPA, SS: Final approval of the version to be published. (4) SA, SDPA, SS: Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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