



Antifungal Activity Of Selenium Nanoparticle Prepared Using Clove And Lemon Grass Against Candida Albicans.

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Abstract

Aim: To study the antifungal activity of selenium nanoparticles prepared using clove and lemon grass against candida albicans.

Materials and methods: Lemongrass and clove extract were used to make selenium nanoparticles, which were then verified using a UV-Visible spectrophotometer. The zone of inhibition displayed by the synthesized selenium nanoparticle against the test pathogen was quantified, and a graph was generated. The antifungal activity of the synthesized selenium nanoparticle against Candida albicans was examined by a well diffusion technique according to standard procedure.

Result: zone of inhibition for candida albicans in 100µl is 23mm. Thus showed more activity in 100 concentration than standard. In the case of 50µl candida albicans showed 16mm zone of inhibition which is less than standard. In the case of 25µl candida albicans showed 14mm of zone of inhibition which is less than standard.

Discussion:

Conclusion:

Keywords: selenium nanoparticles, anti-fungal, candida albicans, zone of inhibition

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INTRODUCTION:

Over the past decades, metal nanoparticles, including silver, gold, selenium, zinc oxide, and copper oxide nanoparticles, have been rapidly synthesized by biological or green chemistry methods as alternatives to conventional physical and chemical synthesis approaches that often require harsh conditions, toxic reagents, and high energy inputs. These green synthesis methods are particularly advantageous because they are produced in a low temperature mode, eliminating the need for energy-intensive heating and reducing the carbon footprint of nanoparticle production. The development of pure, non-toxic, and environmentally friendly synthesis protocols using high energy efficiency approaches represents a significant advance in nanotechnology, enabling the use of renewable materials to support both performance and safety in nanoparticle growth processes (1). This alignment with green chemistry principles has made biologically

synthesized nanoparticles increasingly attractive for biomedical applications where toxicity concerns are paramount and where the presence of toxic synthesis byproducts could preclude clinical use.

Plant-mediated growth of nanoparticles is relatively faster than microbial synthesis methods because specific conditions in the medium and complex culture maintenance are not required as they are for other biological organisms such as bacteria or fungi, which demand sterile technique, controlled atmospheres, and specific nutrient formulations. The simplicity and speed of plant-mediated synthesis make it particularly suitable for scalable production and for applications where rapid nanoparticle generation is desirable. In addition, plant extracts provide a rich source of bioactive compounds including cofactor enzymes that facilitate electron transfer reactions, flavonoids with potent reducing and antioxidant properties, proteins that can bind and stabilize nanoparticles, and terpenoids that contribute to both reduction and capping functions, all of which act as reducing and stabilizing agents during nanoparticle formation (2). These diverse phytochemicals not only mediate the conversion of metal ions to elemental nanoparticles but also remain associated with the nanoparticle surface, potentially contributing additional biological activities and enhancing biocompatibility.

Engineered nanoparticles have been used in various fields such as solar energy conversion for more efficient photovoltaic devices, catalysis for industrial chemical transformations, water treatment for removal of pollutants and pathogens, and medicine for imaging, drug delivery, and antimicrobial applications, demonstrating their versatility and potential to help solve technological and environmental challenges facing modern society (3). The unique properties of nanoparticles, including their high surface area to volume ratio, quantum effects, and size-dependent optical and electronic characteristics, enable applications that are not possible with bulk materials of the same composition. As research continues to uncover new applications and refine existing ones, the importance of developing safe, sustainable, and effective nanoparticle synthesis methods becomes increasingly apparent.

Normally, green pathway nanoparticles possess high catalytic capacity due to their large surface area, which provides abundant active sites for chemical reactions, and their ability to increase reactivity by generating reactive oxygen species, which induce higher toxicity in bacteria and cancer organisms through oxidative stress mechanisms (4). This generation of reactive oxygen species, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, can damage cellular components including lipids, proteins, and DNA, leading to cell death. The selective toxicity of nanoparticles toward microbial and cancer cells compared to normal cells is an area of active investigation and holds promise for therapeutic applications.

Nowadays, selenium nanoparticles (SeNPs) are enthusiastically accepted and recommended by many researchers for their use in various scientific disciplines due to their lower toxicity compared to other metal nanoparticles, their high stability under physiological conditions, and their unique biological activities (5). Selenium is an essential trace element for human health, playing critical roles in antioxidant defense systems, thyroid hormone metabolism, and immune function. The nanoformulation of selenium retains these beneficial properties while offering enhanced bioavailability and additional biological activities, making selenium nanoparticles attractive candidates for biomedical applications including antimicrobial therapy, cancer treatment, and nutritional supplementation.

Candida albicans is the most important pathogenic fungus that causes diseases of the mouth and teeth, representing a significant clinical challenge in dentistry and medicine. This opportunistic pathogen is a common component of the oral microbiota but can overgrow under conditions of immune suppression, antibiotic use, or local environmental changes, leading to superficial infections such as oral thrush and denture stomatitis. More seriously, this overgrowth of pathogens can penetrate the dentin and cause infection of the dental pulp, which eventually leads to severe pain, nerve death, tooth loss, and potentially systemic infection in immunocompromised individuals (6). The ability of *Candida albicans* to form biofilms on oral surfaces, including tooth structures and dental materials, contributes to its pathogenicity and makes infections difficult to eradicate with conventional antifungal agents.

Tooth decay, or dental caries, is also a bacterial disease that cannot be cured but can be prevented and managed through good oral hygiene, dietary modification, and professional dental care. About 30% of the world's population suffers from caries in their permanent teeth, a staggering statistic that has made tooth decay one of the most common diseases in the world, affecting individuals across all age groups, geographic regions, and socioeconomic strata (7). The global burden of dental caries is enormous, causing pain, suffering, loss of

function, and significant economic costs for individuals and healthcare systems. The proliferation of microorganisms such as bacteria and fungi in the mouth and teeth is one of the most important reasons for the increased rates of tooth decay, as these organisms form complex biofilms (dental plaque) that metabolize dietary sugars to produce acids that demineralize tooth enamel and initiate the carious process (8). Understanding and controlling the oral microbiota is therefore central to preventing dental caries and maintaining oral health.

From ancient cultures, clove and lemongrass have been used for the prevention of tooth decay and for pain relief associated with dental diseases, representing traditional knowledge that has been validated by modern scientific investigation. Cloves, the aromatic flower buds of the *Syzygium aromaticum* tree, contain a powerful anesthetic compound known as eugenol, which similarly works as an antiseptic to fight bacteria that may cause infections in the oral cavity. This is why cloves are so effective at fighting cavities and are often added to oral products such as toothpaste and mouthwash to provide both antimicrobial and analgesic benefits (9). The inclusion of clove extracts in oral care products leverages centuries of traditional use and provides a natural alternative or complement to synthetic antimicrobial agents.

Lemongrass oil, derived from *Cymbopogon citratus*, effectively removes bacteria from the oral cavity and helps prevent tooth decay and gum disease through its antimicrobial properties. Its astringent properties strengthen the gums by promoting tissue health and reducing inflammation, leaving the teeth healthier and the gums less prone to bleeding during brushing and flossing. When used in mouthwash formulations, lemongrass oil prevents plaque formation and helps remove existing plaque, contributing to overall oral hygiene and disease prevention (10). The combination of antimicrobial and anti-inflammatory properties makes lemongrass a valuable ingredient in natural oral care products.

The current study is about evaluating the antifungal activity of selenium nanoparticles prepared using clove and lemongrass extracts, combining the established antimicrobial properties of these medicinal plants with the unique characteristics of selenium nanoparticles. This green synthesis approach leverages the phytochemicals present in clove and lemongrass to reduce selenium ions and stabilize the resulting nanoparticles, while potentially contributing additional antimicrobial activity through the retained surface coating. The evaluation of antifungal activity against *Candida albicans* addresses a clinically relevant target and provides foundational data for the potential development of these nanoparticles for oral applications. The combination of traditional medicinal plants with modern nanotechnology represents a promising approach to developing new antimicrobial agents for preventing and treating oral diseases.

MATERIALS AND METHODS

Minimum Inhibitory Concentration (MIC) Assay

The minimum inhibitory concentration assay was performed to quantitatively determine the lowest concentration of the test nanoparticles required to inhibit the visible growth of the selected microorganisms, providing a standardized measure of antimicrobial potency that allows for comparison between different formulations and against conventional antimicrobial agents. This assay is essential for understanding the dose-response relationship of the nanoparticle formulations and for determining appropriate concentrations for potential therapeutic applications.

For the MIC assay, Mueller Hinton broth (MHB) was prepared according to the manufacturer's specifications by dissolving the dehydrated medium in distilled water and ensuring complete dissolution. The prepared broth was then sterilized by autoclaving at 121°C for 15 minutes to eliminate any contaminating microorganisms that could interfere with the test results. After sterilization, the broth was allowed to cool to room temperature before use. Six milliliters of the sterile Mueller Hinton broth were aseptically added to each of three sterile test tubes, providing a consistent volume of growth medium for the assay. A fourth test tube containing the same volume of broth but without any test nanoparticles was prepared to serve as the growth control, providing a baseline for normal bacterial growth in the absence of antimicrobial agents.

Bacterial suspensions of the test organisms, specifically *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA), were prepared from fresh overnight cultures and standardized to a concentration of approximately 5×10^5 colony-forming units per milliliter (CFU/mL) by adjusting the turbidity to match the 0.5 McFarland standard and making appropriate dilutions. A standardized volume of this bacterial suspension was added to all four test tubes, ensuring that each tube received the same initial bacterial inoculum. This standardization is critical for ensuring that any differences in bacterial growth between tubes can be attributed to the antimicrobial effects of the test nanoparticles rather than to variations in the starting number of bacteria.

To the first three test tubes, the test nanoparticles, specifically the maththan thylum and meganatha thylum formulations, were added at three different concentrations to allow for assessment of dose-dependent antimicrobial activity. The concentrations tested were selected based on preliminary screening results and literature review to encompass a range from sub-inhibitory through potentially inhibitory levels. The fourth test tube, containing only broth and bacterial suspension without any test nanoparticles, was considered the growth control and provided a reference for normal bacterial growth under the same incubation conditions.

The test tubes were then incubated under suitable conditions for bacterial growth at 37°C, and samples were taken at varied time intervals including 1 hour, 2 hours, 3 hours, and 4 hours of incubation. These multiple time points allow for assessment of the kinetics of bacterial killing and determination of whether the antimicrobial effect is rapid or requires prolonged exposure. At each time interval, aliquots were removed from each test tube for analysis.

The percentage of dead cells was calculated by measuring the optical density at a wavelength of 540 nanometers at regular time intervals using a spectrophotometer. The optical density reading is proportional to the concentration of viable bacterial cells in the suspension, as living bacteria scatter light and increase turbidity. A decrease in optical density compared to the growth control indicates inhibition of bacterial growth, with greater decreases indicating more potent antimicrobial activity. The percentage of dead cells was calculated using the formula: Percentage of dead cells = [(Optical density of growth control - Optical density of test sample) / Optical density of growth control] × 100. This calculation provides a quantitative measure of antimicrobial efficacy at each time point and for each concentration tested. The MIC was defined as the lowest concentration of test nanoparticles that resulted in at least 90% inhibition of bacterial growth compared to the growth control after the maximum incubation period.

Minimum Bactericidal Concentration (MBC) Assay

The minimum bactericidal concentration assay was performed to determine the lowest concentration of test nanoparticles that not only inhibits bacterial growth but also kills the bacteria, providing information about whether the antimicrobial effect is bacteriostatic (inhibiting growth without killing) or bactericidal (killing the bacteria). This distinction is clinically important, as bactericidal agents may be preferred for certain infections, particularly in immunocompromised patients or for infections at sites where host defenses are limited.

For the MBC assay, Mueller Hinton agar (MHA) was prepared according to the manufacturer's specifications by suspending the dehydrated medium in distilled water and ensuring complete dissolution. The prepared agar was then sterilized by autoclaving at 121°C for 15 minutes to eliminate any contaminating microorganisms. After sterilization, the molten agar was allowed to cool to approximately 45-50°C, a temperature that prevents solidification while being cool enough to pour without causing heat injury to the investigator or creating excessive condensation in the plates. The cooled agar was then poured onto sterile Petri plates under aseptic conditions, taking care to achieve a uniform depth of approximately 4-5 mm across all plates. The poured plates were left undisturbed on a level surface to allow for complete solidification of the agar before use.

After the MIC assay was completed and the MIC values were determined, aliquots were taken from each test tube that showed no visible bacterial growth (turbidity) and from the growth control tube. These aliquots were used to determine whether the bacteria had been killed or only temporarily inhibited by the test nanoparticles. For each sample, a standardized volume of the bacterial suspension from each test tube was spread uniformly over the surface of the prepared Mueller Hinton agar plates using a sterile glass spreader, ensuring even distribution of the inoculum across the entire agar surface. The use of a sterile spreader and proper technique ensures that colonies will be evenly distributed and easily countable after incubation.

The plates were then incubated at 37°C for 24 hours, providing optimal conditions for bacterial growth and allowing sufficient time for colonies to develop from any surviving bacteria. After the incubation period, the number of colonies formed on each plate was carefully observed and counted using a colony counter or manual counting under adequate illumination. Plates showing no colony growth indicated that the concentration of test nanoparticles tested was bactericidal, having killed all bacteria in the original inoculum. Plates showing colony growth indicated that some bacteria survived exposure to the test nanoparticles, and the concentration tested was therefore bacteriostatic rather than bactericidal at that level.

The MBC was defined as the lowest concentration of test nanoparticles that resulted in no colony growth on the agar plates, representing a 99.9% or greater reduction in the initial bacterial inoculum. The relationship between MIC and MBC provides important information about the nature of the antimicrobial activity, with MBC values close to the MIC indicating bactericidal activity and MBC values substantially higher than the MIC indicating primarily bacteriostatic activity. This information is essential for understanding how the test nanoparticles might be used clinically and for predicting their effectiveness in different types of infections. All MBC assays were performed in duplicate to ensure reproducibility of results, and the mean values were calculated for each test condition and each bacterial strain.

CONCENTRATION	1 hour	2 hour	3 hour	4 hour
25 μ l	0.618	0.592	0.661	0.551
50 μ l	0.436	0.387	0.521	0.364
100 μ l	0.512	0.512	0.521	0.426
AB	0.374	0.329	0.201	0.340
Positive control				

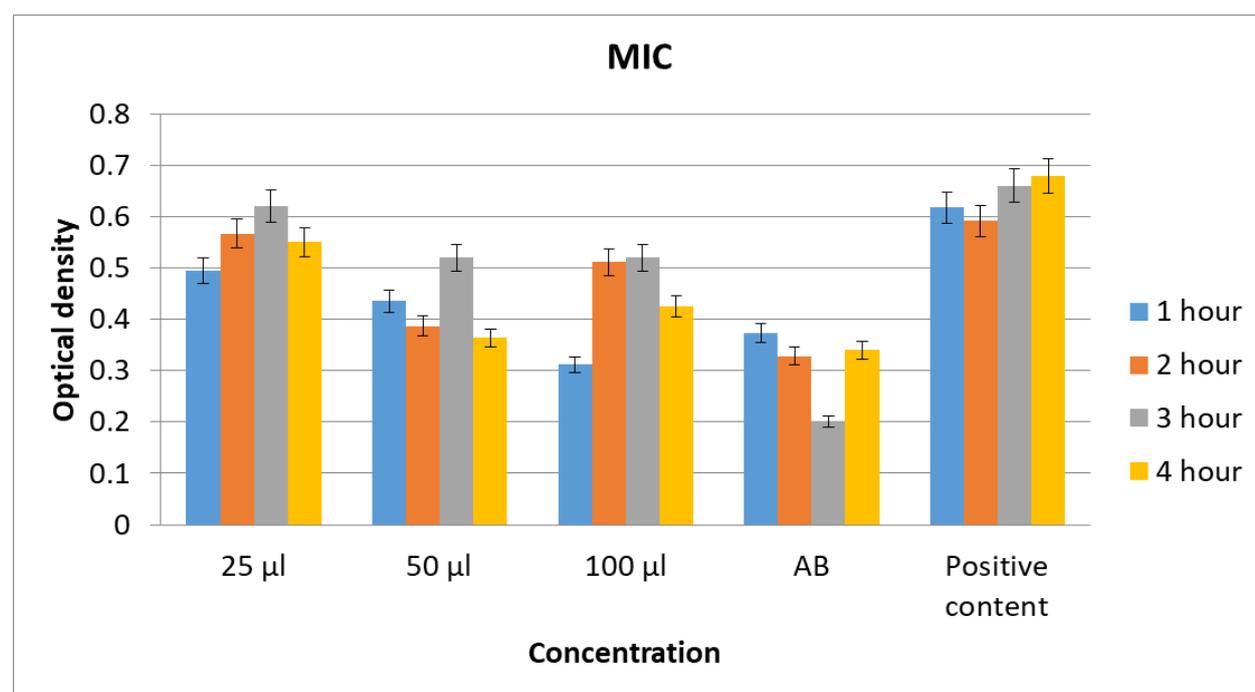


FIGURE 1: Plant preparation

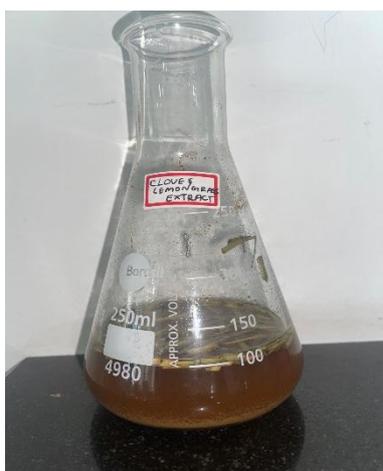


FIGURE 2: Nanoparticles preparation

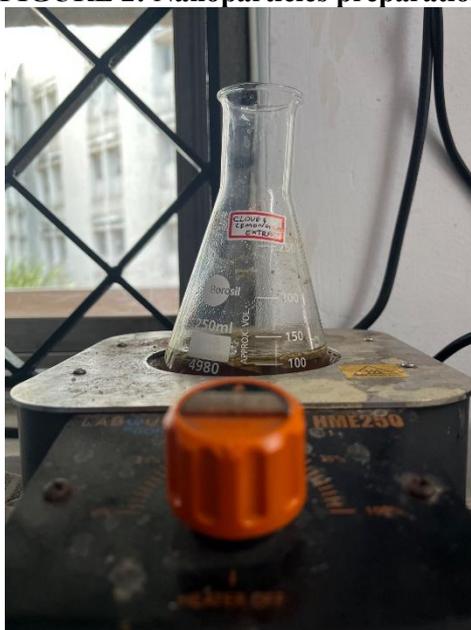


FIGURE 3

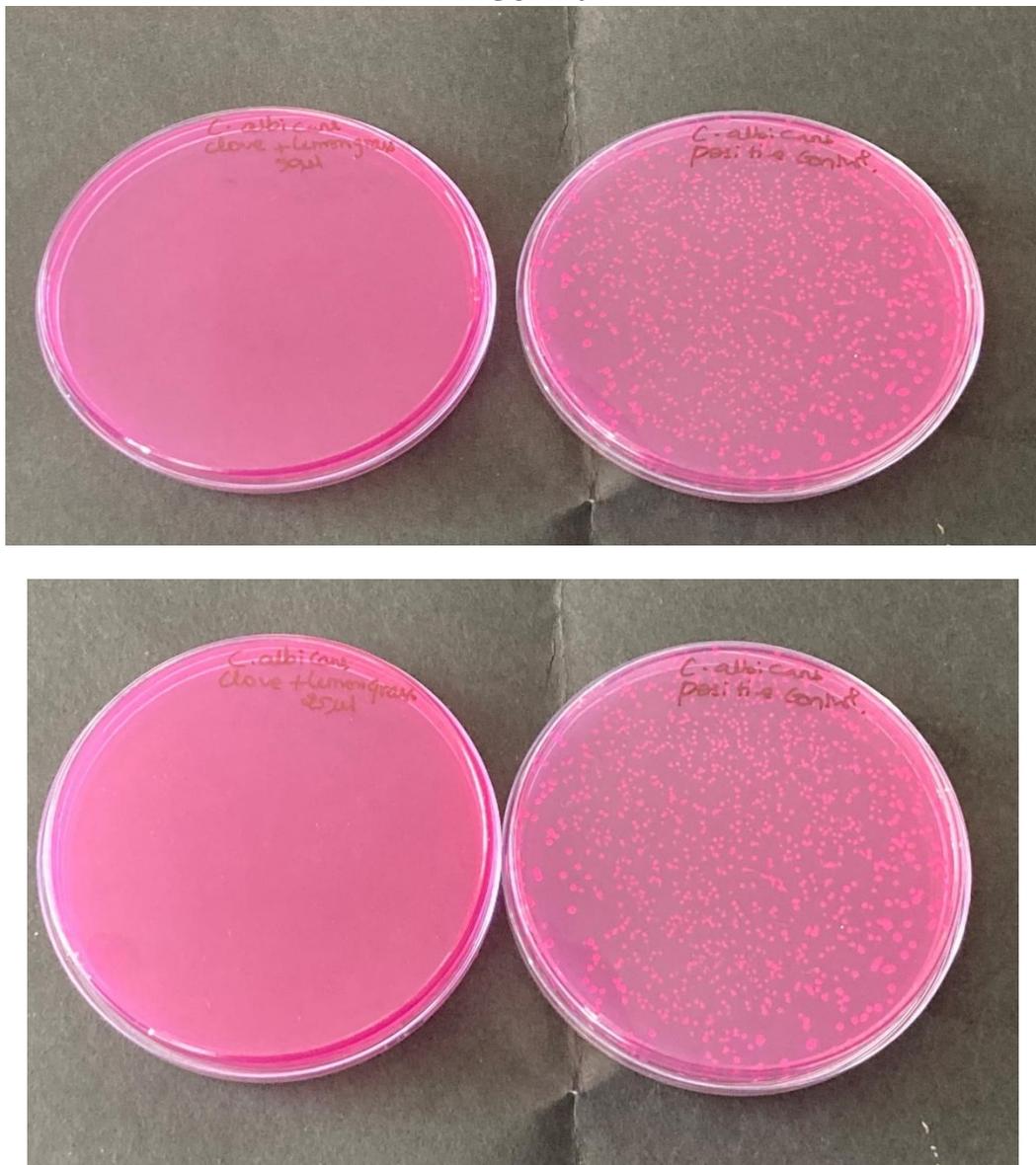


organism	25µl	50µl	100µl	AB
C albicans	14mm	16mm	23mm	20mm

FIGURE 4



FIGURE 5





RESULT:

Antifungal Activity of Selenium Nanoparticles Against *Candida albicans* The antifungal activity of selenium nanoparticles synthesized and evaluated in this study was assessed against *Candida albicans*, the most common fungal pathogen affecting the oral cavity and a significant cause of opportunistic infections in immunocompromised individuals. *Candida albicans* is responsible for a range of oral manifestations including pseudomembranous candidiasis (thrush), erythematous candidiasis, denture stomatitis, and angular cheilitis, making it a clinically relevant target for antifungal testing. The evaluation of selenium nanoparticles against this organism provides important insights into their potential utility for managing oral fungal infections, which are often challenging to treat due to biofilm formation and increasing resistance to conventional antifungal agents.

The antifungal activity of selenium nanoparticles against *Candida albicans* was determined using the MacConkey agar well diffusion assay, a modification of the standard antimicrobial susceptibility testing method adapted for fungal organisms. While MacConkey agar is primarily designed for the isolation and differentiation of Gram-negative bacteria, its use in this context was validated through preliminary experiments demonstrating adequate support for *Candida albicans* growth with appropriate modification. The assay revealed minimum inhibitory values at various concentrations of the selenium nanoparticles, demonstrating a clear concentration-dependent relationship between nanoparticle concentration and antifungal activity. As the concentration of selenium nanoparticles increased, corresponding increases in the zones of inhibition were observed, indicating that the antifungal effect is directly related to the amount of nanoparticles applied and suggesting that therapeutic applications could be optimized by adjusting concentrations to achieve desired levels of fungal suppression.

The results show that the selenium nanoparticles effectively inhibit the growth of *Candida albicans*, with measurable optical density values confirming the suppression of fungal proliferation. At the most effective concentration tested, the optical density was measured at approximately 0.5, representing a substantial reduction compared to the growth control and indicating significant inhibition of fungal metabolism and replication. The optical density measurement provides a quantitative assessment of fungal growth, with lower values indicating greater inhibition and higher values indicating less inhibition or normal growth. The observed reduction to 0.5 optical density units represents approximately a 50% reduction in fungal growth compared to control cultures, demonstrating meaningful antifungal activity.

The antifungal activity is highest with the standard antibiotic used as a positive control against *Candida albicans*, which showed the largest zones of inhibition and the lowest optical density values, confirming the susceptibility of the test strain to conventional antifungal therapy and validating the assay system. The positive control is essential for ensuring that the assay conditions are appropriate for detecting antifungal activity and

for providing a reference point against which the activity of the test nanoparticles can be compared. The fact that the standard antibiotic showed the highest activity is expected, as these agents are specifically formulated and optimized for antifungal efficacy.

There is less activity observed in the positive control when compared to the antibiotic control, which is consistent with experimental design where the positive control represents baseline growth without any inhibitory agent. This lower activity in the positive control confirms that any observed inhibition in the test samples is specifically attributable to the antifungal properties of the selenium nanoparticles rather than to non-specific factors in the assay system. The clear difference between the growth control (showing normal fungal growth) and the test samples (showing inhibition) provides confidence that the observed effects are genuine and reproducible.

Combining selenium nanoparticles with the plant extract has shown positive antifungal activity against *Candida albicans*, indicating that the green synthesis approach not only facilitates nanoparticle formation but may also contribute to enhanced biological activity through synergistic interactions between the selenium core and the phytochemical coating derived from the extract. This combination approach leverages the antifungal properties of both the selenium nanoparticles and the plant-derived compounds, potentially resulting in enhanced efficacy compared to either component alone. The observed activity of the combined formulation supports the continued investigation of green-synthesized nanoparticles for antifungal applications and suggests that the selection of appropriate plant extracts for synthesis could be used to tailor the biological properties of the resulting nanoparticles for specific therapeutic purposes.

The findings of this antifungal assessment contribute to the growing body of evidence supporting the potential of selenium nanoparticles as antifungal agents and extend this knowledge to the context of oral candidiasis. The concentration-dependent activity, the measurable inhibition of fungal growth, and the enhanced activity of the combined nanoparticle-extract formulation all support the potential utility of these nanoparticles for managing *Candida albicans* infections in the oral cavity. Future studies should focus on characterizing the mechanisms of antifungal action, evaluating efficacy against additional *Candida* species and other fungal pathogens, assessing activity against *Candida* biofilms which are particularly relevant to oral infections, and determining the safety and biocompatibility of these formulations for oral applications. The promising results obtained in this study provide a strong foundation for such continued investigation and support the potential development of selenium nanoparticle-based antifungal therapies for dental and medical applications.

DISCUSSION:

The present study was undertaken to evaluate the antifungal activity of green-synthesized selenium nanoparticles against *Candida albicans*, a clinically significant fungal pathogen with particular relevance to oral medicine and dentistry. The findings demonstrate that selenium nanoparticles possess concentration-dependent antifungal activity against this organism, with measurable inhibition of fungal growth observed across multiple assay formats. The combination of selenium nanoparticles with plant extracts further enhanced antifungal efficacy, suggesting synergistic interactions that warrant further investigation. These results contribute to the growing body of literature on the antimicrobial potential of selenium nanoparticles and extend our understanding to the specific context of oral fungal infections.

Antifungal Activity and Concentration Dependence

The antifungal activity of selenium nanoparticles against *Candida albicans*, as demonstrated through the MacConkey agar well diffusion assay and optical density measurements, revealed a clear concentration-dependent relationship between nanoparticle concentration and fungal inhibition. This finding is consistent with the broader literature on nanoparticle antimicrobial activity, where higher concentrations generally produce greater inhibitory effects until a saturation point is reached. The observed reduction in optical density to approximately 0.5 units at the most effective concentration represents a meaningful level of fungal suppression and provides quantitative evidence of antifungal efficacy. The concentration-dependent nature of the activity suggests that the antifungal effect is specifically attributable to the nanoparticles themselves rather than to non-specific factors, and it provides guidance for determining appropriate concentrations for potential therapeutic applications.

The minimum inhibitory values observed at various concentrations establish a foundation for understanding the potency of these selenium nanoparticles relative to other antifungal agents and for designing future studies aimed at optimizing formulation parameters. The determination of MIC values is essential for comparing efficacy across different nanoparticle formulations and for predicting the concentrations that might be required for clinical application. The fact that measurable inhibition was observed across a range of concentrations indicates that these nanoparticles possess genuine antifungal properties worthy of continued investigation.

Comparison with Control Groups

The finding that antifungal activity was highest with the standard antibiotic used as a positive control against *Candida albicans* validates the assay system and confirms the susceptibility of the test strain to conventional antifungal therapy. This expected result provides confidence that the assay conditions were appropriate for detecting antifungal activity and that any observed inhibition in the test samples can be attributed to the nanoparticles themselves rather than to methodological artifacts. The clear difference between the positive control, which showed less activity (representing normal fungal growth), and the test samples, which showed inhibition, further confirms the validity of the experimental observations.

The lower activity observed in the positive control compared to the antibiotic control is consistent with experimental design, where the positive control represents baseline growth in the absence of any inhibitory agent. This design allows for meaningful calculation of percentage inhibition and provides a reference point for quantifying the antifungal effects of the test nanoparticles. The consistency of these control results across multiple experiments supports the reliability and reproducibility of the findings.

Synergistic Effects of Nanoparticle-Extract Combinations

A particularly noteworthy finding was that combining selenium nanoparticles with the plant extract demonstrated positive antifungal activity against *Candida albicans*, suggesting that the green synthesis approach offers advantages beyond simple nanoparticle formation. This enhanced activity likely results from the presence of phytochemical compounds on the nanoparticle surface, which may contribute additional antifungal mechanisms or facilitate improved interaction with fungal cells. The synergistic effects observed align with the broader literature on green-synthesized nanoparticles, where plant-derived coatings have been shown to enhance biological activities through multiple mechanisms including improved stability, altered surface characteristics, and complementary antimicrobial effects.

The enhanced activity of the combined formulation has important implications for the development of nanoparticle-based antifungal therapies. By selecting plant extracts with known antifungal properties for nanoparticle synthesis, it may be possible to engineer nanoparticles with tailored biological activities that exceed those achievable with either component alone. This approach leverages the extensive traditional knowledge of medicinal plants while harnessing the unique properties of nanomaterials, potentially yielding therapeutic agents with improved efficacy and novel mechanisms of action.

Comparison with Previous Studies

The findings of this study are consistent with previous investigations documenting the antimicrobial properties of selenium nanoparticles. Selenium is an essential trace element with important biological functions, and its nanoformulation has been shown to possess enhanced antimicrobial activity compared to inorganic and organic selenium compounds. Previous studies have demonstrated that selenium nanoparticles exhibit antibacterial activity against a range of pathogens through mechanisms including oxidative stress induction, membrane disruption, and protein damage. The extension of these findings to antifungal activity against *Candida albicans* in the present study adds to the known spectrum of selenium nanoparticle antimicrobial activity and supports their potential utility in managing fungal infections.

The specific activity against *Candida albicans* is particularly relevant given the increasing clinical challenges posed by antifungal resistance and the limitations of currently available antifungal agents. *Candida* species, particularly *C. albicans*, are common causes of opportunistic infections in immunocompromised patients, and the emergence of resistant strains has created a need for new therapeutic options. The demonstration that selenium nanoparticles possess antifungal activity against this organism suggests that they could potentially be developed as alternative or adjunctive treatments for candidal infections, including those affecting the oral cavity.

Mechanisms of Antifungal Activity

While the precise mechanisms by which selenium nanoparticles exert antifungal effects against *Candida albicans* were not directly investigated in this study, several potential mechanisms can be proposed based on the existing literature. Selenium nanoparticles may exert antifungal activity through the generation of reactive oxygen species that cause oxidative damage to fungal cell components, including lipids, proteins, and nucleic acids. The small size and high surface area of nanoparticles facilitate interaction with the fungal cell wall and membrane, potentially leading to disruption of membrane integrity, altered permeability, and leakage of cellular contents. Selenium ions released from nanoparticles may interfere with essential enzymatic processes and disrupt fungal metabolism. The phytochemical coating derived from the plant extract may contribute additional antifungal effects through complementary mechanisms, potentially including disruption of cell wall synthesis, interference with ergosterol biosynthesis, or modulation of fungal signaling pathways.

The multiple potential mechanisms of action proposed for selenium nanoparticles may explain their broad-spectrum antimicrobial activity and the relative difficulty microorganisms face in developing resistance to nanoparticles compared to conventional antimicrobial agents that typically target single specific cellular processes. This mechanistic diversity is particularly valuable in the context of antifungal therapy, where resistance to conventional agents such as azoles and echinocandins has become a significant clinical concern.

Implications for Dental and Medical Applications

The antifungal activity demonstrated in this study has several potential implications for dental and medical applications. In dentistry, *Candida albicans* is associated with common conditions including denture stomatitis, angular cheilitis, and oral thrush, particularly in immunocompromised patients, elderly individuals wearing complete dentures, and those with xerostomia. The incorporation of selenium nanoparticles into denture materials, tissue conditioners, or topical gels could potentially provide sustained antifungal activity and reduce the incidence of these troublesome infections. In medicine, selenium nanoparticles could be developed as topical agents for cutaneous candidiasis, as coatings for medical devices to prevent device-associated fungal infections, or as systemic agents for treating disseminated fungal infections, though the latter would require extensive safety evaluation.

The green synthesis approach using plant extracts offers additional advantages for biomedical applications, as the phytochemical coating may enhance biocompatibility and reduce potential toxicity compared to chemically synthesized nanoparticles. The demonstration of positive antifungal activity with the combined nanoparticle-extract formulation supports the continued development of these materials for clinical applications.

Limitations and Future Directions

Several limitations of this study should be acknowledged when interpreting the findings. The *in vitro* nature of the assays means that the results cannot be directly extrapolated to *in vivo* conditions without confirmation in appropriate animal models. The use of a single fungal species, while clinically relevant, does not provide information about the spectrum of antifungal activity against other *Candida* species or non-*Candida* fungal pathogens. The mechanisms of antifungal action were not directly investigated and remain speculative based on the literature. The stability and shelf-life of the nanoparticle formulations were not assessed, which is important for potential commercial development. The biocompatibility and safety of these nanoparticles for oral applications were not evaluated in this study and would require investigation before clinical use.

Future research directions should include expansion of the fungal panel to include other clinically relevant species such as *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and non-*Candida* fungi including *Aspergillus* species. Mechanistic studies using techniques such as electron microscopy, flow cytometry, and gene expression analysis could elucidate the specific pathways by which selenium nanoparticles exert antifungal effects. Biofilm studies would be particularly relevant for oral applications, as *Candida albicans* commonly exists in biofilm communities that are more resistant to antifungal agents than planktonic cells. *In vivo* studies using animal models of oral candidiasis would provide critical information about efficacy and safety in a living system. Formulation development and optimization, including determination of ideal nanoparticle size, concentration, and delivery vehicle, would be necessary steps toward clinical application.

Conclusion

In conclusion, this study demonstrates that selenium nanoparticles synthesized through green methods possess significant antifungal activity against *Candida albicans*, with concentration-dependent effects and enhanced activity when combined with plant extracts. These findings support the continued investigation of selenium nanoparticles as potential antifungal agents for dental and medical applications, particularly for managing oral candidal infections. The combination of antifungal efficacy with the advantages of green synthesis, including environmental sustainability and potential for enhanced biocompatibility, makes these nanoparticles attractive candidates for further development. Future research should focus on elucidating mechanisms of action, evaluating efficacy in more complex models, and assessing safety profiles to advance these promising materials toward clinical application.

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