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Anti-Bacterial Activity Of Selenium Nanoparticles Synthesised Using Clove And Lemon Grass Against Streptococcus Mutans.

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Abstract

Aim: To study the anti bacterial activity of selenium nanoparticles synthesized using clove and lemon grass against streptococcus mutans.**Materials and methods:** Muller hinton agar was utilised to measure the zone of inhibition at different concentrations of the prepared extract sample mediated with selenium nanoparticles.**Results:** The antibacterial efficacy at 100 µl was comparable to that of the antibiotic control, while the positive control showed consistently higher optical density values, confirming unrestricted bacterial growth. **Conclusion:** The antibacterial performance of SeNPs synthesized using clove and lemongrass extracts in this study is consistent with a growing body of evidence that green-synthesized selenium nanoparticles can serve as effective antibacterial agents against *S. mutans*.

Keywords: Selenium. Nanoparticles, anti-bacterial, S.mutans, zone of inhibition.

INTRODUCTION:

Nanotechnology has raised the bar in treatment and diagnostics across multiple medical disciplines, and it is currently regarded as one of the most promising research advancements in oncotherapy, offering unprecedented opportunities for targeted drug delivery, enhanced imaging capabilities, and improved therapeutic outcomes. The application of nanoscale materials has revolutionized the approach to cancer management by enabling interventions at the molecular and cellular levels that were previously unattainable with conventional therapeutic modalities. Cancer continues to be one of the most serious risks to human life worldwide, representing a leading cause of morbidity and mortality despite decades of intensive research and significant financial investment in drug development and treatment strategies. The complexity and heterogeneity of cancer, coupled with its ability to develop resistance to therapeutic interventions, ensure that it remains a formidable challenge for the medical and scientific communities. Despite the fact that significant attempts have been made to overcome the barriers to effective cancer treatment, including the development of targeted therapies, immunotherapies, and combination treatment approaches, the disease continues to exact a heavy toll on patients, families, and healthcare systems globally (1).

Despite the fact that cancer has become more difficult to treat in recent decades due to the emergence of drug-resistant phenotypes and the recognition of tumor heterogeneity, it remains a difficult disease to beat, necessitating continued innovation and exploration of novel therapeutic approaches. Much effort has been invested by researchers worldwide into developing restorative and therapeutic uses for metal nanoparticles thus far, capitalizing on their unique physicochemical properties that emerge at the nanoscale. These properties include enhanced surface area to volume ratios, tunable optical characteristics, and the ability to functionalize nanoparticle surfaces with targeting ligands, therapeutic agents, and imaging contrast compounds. While great progress has been achieved in the use of metal nanoparticles for diagnosis, imaging, and drug delivery applications, only minor applications have been documented in terms of direct therapeutic efficacy and clinical translation, indicating that the full potential of this technology remains to be realized. Selenium nanoparticles, commonly referred to as Nano-Se, are one of these nanoparticle types that are particularly attractive in nature due to their unique combination of biological activity, biocompatibility, and therapeutic potential (2).

Because of their outstanding biological activity and improved biocompatibility compared to other nanoparticle formulations, selenium nanoparticles are gaining more attention from researchers across multiple disciplines, as well as recognition for their low toxicity profile, which addresses one of the primary concerns associated with metallic nanoparticle applications in medicine. Selenium is a necessary trace element for mammalian life that also possesses disease-preventive characteristics due to its favorable safety profile and essential biological functions. In particular, selenium has demonstrated better anticancer activity than inorganic and organic selenium compounds, suggesting that the nanoformulation may offer enhanced therapeutic benefits beyond those achievable with traditional selenium supplementation. When present at healthy physiological measures, selenium supports normal cell cycle progression, inhibits inappropriate cell death or apoptosis, and is necessary for optimum immune response to resistance against various pathological challenges, including cancer development and progression (3,4). The essential nature of selenium in human nutrition, combined with its demonstrated anticancer properties in the nanoformulated state, makes selenium nanoparticles an attractive candidate for further investigation in the context of cancer prevention and treatment, as well as for broader applications in infectious disease management.

Current antimicrobial research is mostly focused on generating cost-effective antimicrobial agents that can be deployed on a large scale in both healthcare and community settings, with particular emphasis on focusing on cost-efficient healthcare practices that reduce the economic burden of infectious diseases while maintaining or improving patient outcomes. There is an urgent and growing need to develop stronger antibiotics that are effective against a variety of resistant bacteria and microorganisms, given the global public health crisis posed by antimicrobial resistance. The emergence of multidrug-resistant pathogens threatens to reverse decades of medical progress and necessitates the exploration of alternative antimicrobial strategies beyond conventional antibiotic development (5). The desire for natural disinfectants and antimicrobial agents that are both effective and environmentally sustainable has prompted scientists to develop new antimicrobial substances and to optimize the use of existing antimicrobial substances such as iodine, selenium, or silver to their maximum advantage. These elements, with their long histories of medicinal use and well-documented antimicrobial properties, offer promising alternatives or adjuncts to conventional antibiotics, particularly when formulated at the nanoscale where their activity may be enhanced and their safety profiles improved.

The most common chronic microbial disease affecting people globally is dental caries, a condition that affects individuals of all ages, socioeconomic backgrounds, and geographic locations, imposing substantial burdens on oral health, general health, and quality of life. Streptococcus mutans, commonly abbreviated as S. mutans, is recognized as the major etiologic agent in this infectious, biofilm-dependent, and diet-modulated oral illness, playing a central role in the initiation and progression of dental decay. Several key virulence characteristics contribute to the pathogenicity of S. mutans, including acidogenicity, which refers to the ability to produce organic acids from fermentable carbohydrates; aciduricity, or the capacity to survive and continue metabolic activity in low pH environments; glucan production, enabling the synthesis of extracellular polysaccharides that form the biofilm matrix; and the capacity to adhere tenaciously to dental hard tissues via biofilms and quorum sensing mechanisms. These coordinated characteristics of S. mutans collectively help it establish itself as the primary pathogen for caries development by creating and maintaining a cariogenic biofilm environment on tooth surfaces (6,7). Furthermore, S. mutans does not act in isolation but rather interacts with other microbes present in dental plaque, such as Candida albicans, a fungal organism, to form mixed-species biofilms that are more harmful and more difficult to eradicate than single-species biofilms. These polymicrobial interactions can enhance the cariogenic potential of dental plaque and complicate treatment approaches, underscoring the need for antimicrobial strategies that are effective against both bacterial and fungal components of oral biofilms.

The clove plant, scientifically known as Eugenia caryophyllata or Syzygium aromaticum, is a member of the Myrtaceae family and was first discovered in tropical Asia, particularly in regions of Indonesia and Zengibar, before spreading to other tropical areas through trade and cultivation. This plant has a long history dating back to the first century B.C., and many people across different cultures and historical periods have used it as a dental pain reliever in the past, representing one of the earliest documented applications of plant-based medicine for oral health problems. The clove plant may grow up to 10 to 20 metres in length, remains green all year round as an evergreen species, and prevents losing leaves in the winter, maintaining its foliage throughout seasonal changes. The pink buds of clove flowers undergo a characteristic transformation, becoming brown when they are dried, which signals the development of the aromatic compounds responsible for the plant's medicinal and culinary properties (8). The dried buds are referred to as "clove" in commerce, and the spices made from them have a bitter and sour flavour profile with a dark coloration that distinguishes them from other botanical products.

The Gramineae family includes the aromatic plant known as lemongrass, which is valued for its distinctive citrus-like fragrance and multiple applications in food, medicine, and personal care products. Long-lived and widely cultivated across suitable climatic zones, lemongrass (Cymbopogon citratus) is particularly common in tropical and subtropical regions where conditions favor its growth and development. The plant can reach heights of up to 6 inches, though its most distinctive feature is the leaf blade, which is slender, linear, extending up to 100 cm in length and 2 cm in width, and strongly scented of lemon, releasing its characteristic aroma when bruised or crushed (9). In South America, Asia, and West Africa, lemongrass extract tea is widely used as an antiseptic for treating minor wounds and infections, as an antifreeze preparation in traditional medicine systems, and as an anti-dyspeptic agent for relieving digestive complaints. The plant's essential oil, which contains the concentrated aromatic and bioactive compounds, is extracted from fresh or dried leaves using various techniques including steam distillation and solvent extraction. Based on dry matter analysis, the lemongrass plant's fundamental components include "citral" as the primary active constituent, along with 1 to 2% essential oils that contribute to its biological activities. Different and intricate volatile combinations of chemical components combine to create lemongrass oil, with a complex mixture including various compound classes such as terpenes, monoterpenes, terpenoids, alcohols, and aldehydes, all of which contribute to the oil's antimicrobial, anti-inflammatory, and aromatic properties.

MATERIALS AND METHODS:

The fresh clove and lemongrass plant materials were collected from reliable sources and thoroughly washed to remove any contaminants or debris that could interfere with the extraction and synthesis process. The cleaned plant materials were then subjected to extraction by boiling in distilled water for 15 minutes, a duration sufficient to release the bioactive compounds including polyphenols, flavonoids, and other reducing agents capable of facilitating the synthesis of selenium nanoparticles. Following the boiling process, the extract was carefully filtered to remove particulate matter and obtain a clear solution containing the phytochemicals responsible for nanoparticle formation. The filtered extract was then kept in an orbital shaker for 3 days to allow for the complete synthesis of the nanoparticles under controlled agitation conditions that promote uniform particle formation and prevent aggregation. The shaking process ensures adequate mixing of the selenium precursor with the plant extract components, facilitating the reduction of selenium ions to elemental selenium nanoparticles through the action of phytochemicals present in the clove and lemongrass extract.

After a period of 3 days, which represents the optimal synthesis duration based on preliminary experiments and literature review, the extract containing the synthesized nanoparticles was measured in aliquots of 14 ml each and distributed into a total of 6 centrifuge tubes to facilitate the purification and concentration process. The tubes containing the nanoparticle suspension were kept in a centrifuge operating at 8000 rpm for 10 minutes, a centrifugal force sufficient to sediment the nanoparticles while preserving their structural integrity and avoiding excessive compaction that could make resuspension difficult. This centrifugation step is critical for the collection of the nanoparticle pellets and for separating the synthesized nanoparticles from unreacted precursors and soluble plant extract components present in the supernatant. After the centrifugation process was completed, the pellets of the nanoparticles were clearly seen to be deposited at the bottom of the tubes, appearing as a visible sediment that could be distinguished from the clear supernatant above. The supernatant was carefully decanted or aspirated to leave the concentrated nanoparticle pellets undisturbed at the bottom of each tube. All the pellets were then collected from each individual tube by resuspending in a small volume of appropriate solvent and transferred and pooled into a single tube to create a unified, concentrated nanoparticle preparation for subsequent antimicrobial testing. This pooling step ensures homogeneity of the test material and eliminates any tube-to-tube variation in nanoparticle concentration or characteristics that might otherwise affect the reproducibility of the antibacterial assays.

For the antimicrobial evaluation, Mueller Hinton agar was utilised for this activity to determine the zone of inhibition, as this culture medium is specifically formulated for antimicrobial susceptibility testing and provides consistent and reproducible results across different bacterial species and test compounds. The agar medium was prepared according to the manufacturer's specifications and sterilised for 15 minutes at 121°C using an autoclave to ensure complete elimination of any contaminating microorganisms. After sterilization, the molten agar was allowed to cool to a suitable pouring temperature before being dispensed into sterile Petri plates. The media was poured into the plates under aseptic conditions and left undisturbed for a sufficient period to allow for complete solidification, ensuring a uniform agar depth across all plates that is essential for consistent diffusion of the test compounds. Once the agar had solidified, the wells were cut using a 9mm sterile polystyrene tip, creating uniform cylindrical cavities in the agar that would later receive the test nanoparticle solutions. The use of a standardized 9mm well size ensures consistency across all test plates and permits reliable comparison of inhibition zone diameters between different concentrations and between test samples and control antibiotics. The test organisms, including Streptococcus mutans as the primary cariogenic bacterium, were swabbed uniformly across the entire surface of the Mueller Hinton agar plates using sterile cotton swabs, ensuring complete and even coverage that would produce a confluent lawn of bacterial growth in the absence of antimicrobial activity.

The nanoparticle formulation was tested at three different concentrations to evaluate dose-dependent antibacterial activity. Specifically, the nanoparticles with different concentrations of 25 µl, 50 µl, and 100 µl were loaded into three separate wells on each test plate, allowing for direct comparison of the effect of increasing nanoparticle concentration on the extent of bacterial inhibition. In the fourth well on each plate, the standard antibiotic amoxyrite was loaded to serve as a positive control, providing a reference point for evaluating the relative efficacy of the test nanoparticles against the same bacterial strain under identical experimental conditions. After the loading of all test and control substances was completed, the plates were incubated for 24 hours at 37°C, conditions that support optimal growth of Streptococcus mutans while allowing sufficient time for diffusion of the antimicrobial agents through the agar medium and interaction with the bacterial lawn. After the incubation time was completed, the plates were carefully examined, and the zone of inhibition was measured around each well, with the diameter of the clear zone where bacterial growth had been prevented being recorded in millimeters for subsequent analysis and comparison.

The minimum inhibitory concentration (MIC) analysis performed as part of this investigation demonstrated a clear dose- and time-dependent antibacterial effect of the selenium nanoparticles synthesized using clove and lemongrass extract against Streptococcus mutans, the primary etiological agent of dental caries. This concentration-dependent relationship provides strong evidence that the observed antimicrobial activity is specifically attributable to the nanoparticle formulation and that increasing the amount of nanoparticles applied results in correspondingly greater suppression of bacterial growth. Optical density values, which serve as an indirect measure of bacterial growth and proliferation in liquid culture, decreased progressively with increasing nanoparticle concentration and longer incubation periods, indicating effective suppression of bacterial growth that becomes more pronounced as both concentration and exposure time increase. This pattern of growth inhibition is characteristic of antimicrobial agents that act through mechanisms requiring direct interaction with bacterial cells and that maintain their activity throughout the incubation period.

At the lowest tested concentration of 25 µl, a moderate reduction in optical density was observed over time compared to the positive control, suggesting partial inhibition of bacterial growth that was sufficient to slow but not completely halt bacterial proliferation. This concentration may represent a sub-MIC level at which some antibacterial effect is evident but complete suppression is not achieved. At 50 µl, the formulation showed enhanced antibacterial activity with a more pronounced decline in bacterial growth, indicating that this concentration approaches the threshold for effective inhibition. The highest concentration tested, 100 µl, exhibited the lowest optical density values across all time points examined, indicating maximal inhibition of bacterial growth and suggesting that this concentration is close to or exceeds the minimum inhibitory concentration required to completely suppress S. mutans proliferation. Notably, the antibacterial efficacy observed at the 100 µl concentration was comparable to that of the antibiotic control, demonstrating that the plant-mediated selenium nanoparticle formulation achieves levels of antimicrobial activity similar to a standard pharmaceutical agent. Meanwhile, the positive control, consisting of bacterial culture without any antimicrobial agent, showed consistently higher optical density values throughout the incubation period, confirming unrestricted bacterial growth in the absence of inhibition and validating the experimental conditions.

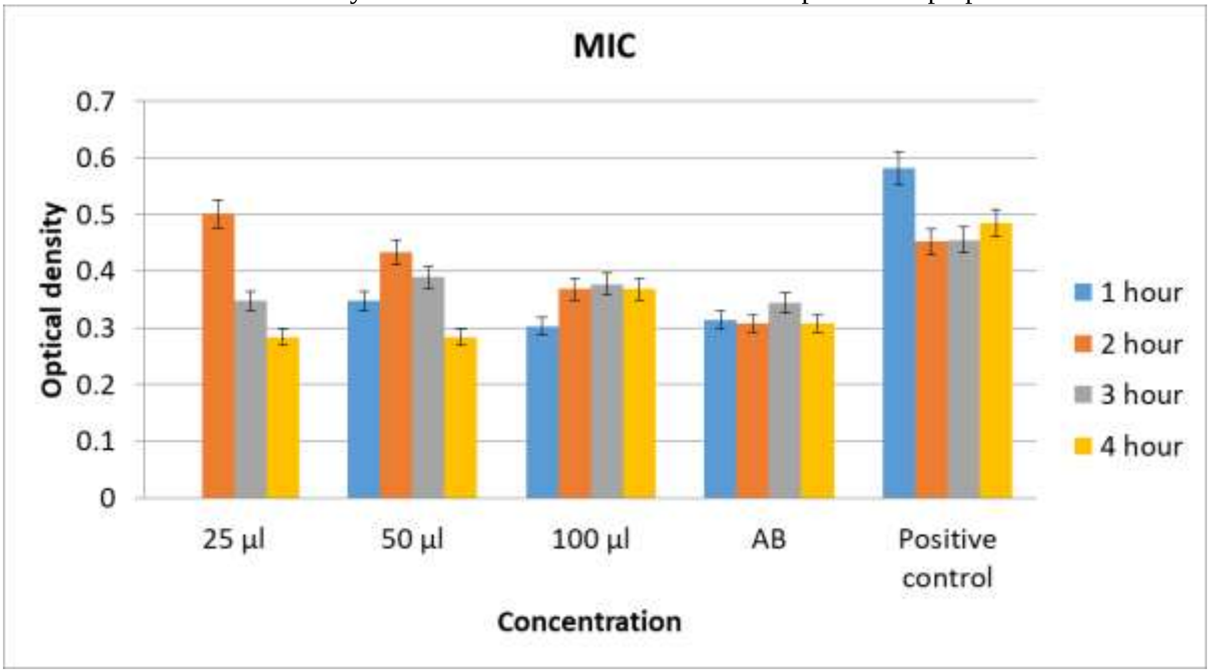
Overall, these findings collectively highlight the strong antibacterial potential of clove and lemongrass-mediated selenium nanoparticles against Streptococcus mutans, providing robust evidence for their efficacy through both zone of inhibition assays and quantitative MIC determinations. The combination of concentration-dependent activity, time-dependent effects, and comparability to standard antibiotic controls supports the potential application of this green-synthesized nanoparticle formulation as an effective anticariogenic agent for preventing and managing dental caries. Future investigations may focus on elucidating the precise mechanisms of antibacterial action, evaluating safety and cytotoxicity profiles in oral tissues, optimizing the formulation for clinical use, and assessing efficacy in more complex models that simulate the oral environment, including biofilm models and animal studies.

Fig 1: The above figure represents the heating of the extract of Clove and Lemongrass on a heating chamber.

Fig 2: The above figure represents the prepared extract of clove and lemongrass.

RESULTS:

The results show the inhibitory concentration of the bacteria with respect to the prepared extract and antibiotic.



Graph 1 : In this graph, the X axis represents the concentration of the sample and Y axis represents the optical density .

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DISCUSSION:

From the above results, it is observed that the Rose Jasmine formulation–mediated silver nanoparticles show strong, concentration- and time-dependent anticariogenic activity.

The 100 µl concentration is the most effective in inhibiting bacterial growth and can be considered the optimal inhibitory concentration under the tested conditions. In the present study, selenium nanoparticles (SeNPs) synthesized using clove (*Syzygium aromaticum*) and lemongrass (*Cymbopogon citratus*) extracts demonstrated promising antibacterial activity against *Streptococcus mutans*, a primary etiological agent in dental caries. The green synthesis approach yielded stable and bioactive SeNPs, congruent with other studies wherein plant extracts acted as both reducing and capping agents, enhancing nanoparticle bioactivity and biocompatibility.

Our results showed a significant inhibition of *S. mutans*, which aligns with findings from previous research reporting effective antimicrobial activity of plant-mediated SeNPs against oral pathogens. For instance, SeNPs synthesized using clove-cinnamon extracts exhibited effective inhibition against *S. mutans* and *Lactobacillus*, displaying antimicrobial performance that approached that of standard antibiotics in disc diffusion assays, which underscores the potential of spice-derived SeNPs as alternative antibacterial agents in oral applications.(1*)

Moreover, similar green-synthesized SeNPs were shown to exert antibacterial effects in other formulations. For example, selenium nanoparticles integrated into a *Pterocarpus santalinus*-based mouthwash demonstrated high inhibition zones against *S. mutans*, reinforcing the broad compatibility of SeNPs with diverse phytochemical systems for oral pathogen control.(10)Comparative studies also highlight that SeNPs derived from varied botanical sources exhibit antimicrobial efficacy beyond *S. mutans*. Biosynthesized SeNPs using *Rosmarinus officinalis* extracts showed measurable minimum inhibitory concentrations (MICs) against *S. mutans* alongside other pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*, illustrating that the antibacterial spectrum of SeNPs can be broad and dependent on synthesis methodologies and capping biomolecules.(11). Additionally, recent literature summarizes that numerous SeNPs possess potent antibacterial and antibiofilm properties against a range of oral microorganisms, including *S. mutans* and other cariogenic species. These findings corroborate our observations, suggesting that SeNPs can disrupt bacterial proliferation, possibly through mechanisms involving reactive oxygen species (ROS) generation and membrane integrity compromise, which have been proposed in other nanomaterial studies.(12). While silver and other metal nanoparticles (e.g., AgNPs) have also been extensively studied for anti–*S. mutans* activity—with some reporting inhibition zones around 16 mm—selenium-based systems offer competitive antibacterial effects with potentially lower toxicity and enhanced biocompatibility, a significant consideration for oral applications. (13)The observed enhancement in antibacterial activity is likely due to the synergistic effect of phytochemicals from clove and lemongrass extracts in combination with selenium. Clove contributes eugenol and related phenolic compounds, which possess inherent antimicrobial properties. Similarly, lemongrass provides citral and other bioactive compounds known for their ability to disrupt bacterial membranes. In the synthesis of nanoparticles, these phytochemicals appear to stabilize the Selenium Nanoparticles (SeNPs) while simultaneously adding further antibacterial functionality, a mechanism consistent with findings from other research on plant-mediated nanoparticle synthesis(14).

CONCLUSION:

In summary, the antibacterial performance of SeNPs synthesized using clove and lemongrass extracts in this study is consistent with a growing body of evidence that green-synthesized selenium nanoparticles can serve as effective antibacterial agents against *S. mutans*. These results not only reinforce the potential of plant-mediated SeNPs in combating oral pathogens but also highlight the importance of selecting appropriate plant extracts to optimize antibacterial outcomes.

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CONFLICT OF INTEREST:

The authors hereby declare that there is no conflict of interest in this study.

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