

# Green synthesis of selenium nanoparticles using clove and lemon grass and its antibacterial activity against *E. faecalis*

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## ABSTRACT

**Introduction:** Nanotechnology is the branch of science and engineering which involves designing and using devices and systems by manipulating atoms and molecules at nanoscale. Nanotechnology has a wide application in the field of medicine and dentistry which resulted in the emergence of a new field known as Nanomedicine. In this study Clove and lemongrass are preferred for Green synthesis of Selenium because to check the anti-bacterial activity of the clove and lemongrass extracted selenium nanoparticle against enterococcus faecalis. Selenium has anti-cancer and anti-microbial properties, Gold which has DNA labeling, biosensor, drug delivery, cancer therapy and anti-microbial properties.

**Aim:** The aim of this study is to evaluate the antibacterial activity of selenium nanoparticles prepared using clove and lemongrass against *E. faecalis*.

**Materials and methods:** Antibacterial activity of Selenium nanoparticles acts against the strain *E. faecalis*. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller Hinton Agar were prepared and sterilized for 15 minutes at 121°C. Media poured into the sterilized plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The selenium nanoparticles with different concentrations (25µL, 50µL, 100µL) were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hours at 37°C. After the incubation time the zone of inhibition was measured.

**Result:** The results of the minimum inhibitory concentration values of the selenium nanoparticles is shown in table 1. In 100 µL concentration the MIC value decreased. The minimum bactericidal concentration of the selenium nanoparticles is shown in figure 4. In 100 µL concentration there is an increased MBC effect seen. The MIC of *E. faecalis* is shown in Graph 1. The X axis represents the different concentrations of selenium nanoparticles (25µL, 50µL, 100µL) and standard antibiotic amoxyrite. The Y axis represents the absorbents. As the concentration of the nanoparticle increased the MIC also increased.

**Discussion:** In the present study MIC and MBC values have increased with respect to 100 µL concentration of the selenium nanoparticles. In a study conducted by Rangrazi et al have concluded with the same results that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the selenium nanoparticles against gram-negative and gram-positive bacteria showed MIC values of 0.068, 0.137, and 0.274 mg ml<sup>-1</sup>. The results proved that the concentration of 0.274 mg ml<sup>-1</sup> had a higher bactericidal effect than the other concentrations. As the concentration of selenium nanoparticles increased to 0.274 mg ml<sup>-1</sup> the bacteria were completely killed.

**Conclusion:** Within the limits of the study it can be concluded that the green synthesized selenium nanoparticles using clove and lemongrass have remarkable antibacterial potentiality and can be added to dental materials to enhance their properties. Further animal studies are required for the evaluation of the efficacy of the nanoparticles.

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## INTRODUCTION

Nanotechnology is the branch of science and engineering which involves designing and using devices and systems by manipulating atoms and molecules at nanoscale. Nanotechnology has a wide application in the field of medicine and dentistry which resulted in the emergence of a new field known as Nanomedicine. It is very helpful in understanding the pathophysiology basis of disease, diagnosis and prevention of disease. Nanoparticles which are involved in Nanotechnology are nothing but the small particles that range between 1 to 100 nanometers in size.

## KEYWORDS:

Clove, lemongrass, selenium nanoparticles, anti-bacterial activity, MIC, MBC

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These undetectable particles can exhibit a wide physical and chemical properties to their larger material counterparts. Nanoparticles are used in the areas of biomedicine, drug delivery systems, genetics, pharmaceuticals, chemical industries, optics, mechanics, etc. The most commonly involved metal nanoparticles are selenium which has anti-cancer and anti- microbial property, Gold which has DNA labeling, biosensor, drug delivery, cancer therapy and anti-microbial property, Palladium which is a biocatalyst, Copper which has anti- microbial property, Iron which has a anti-cancer, molecular imaging and cancer therapy activities, Zinc oxide which is used in cosmetics and coatings, Silver which has anti- cancer, anti- microbial and anti- viral properties and platinum has an anti- cancer property (Urnuksaikhani et al. 2021) (Swain 2016).

Green synthesis of metallic nanoparticles has gained much attraction among researchers in recent years because it has several benefits. It is cost effective, good stability, less time consuming, release of non toxic by-products, eco friendly and most importantly they are very good for large scale synthesis (Malhotra and Alghuthaymi 2022). There are several plant species involved in green synthesis of nanoparticles. For example Silver nanoparticles can be synthesized from Geranium indicum, Cyperus sp etc. Gold nanoparticles from Aloe vera, Garcinia mangostana etc. Likewise Selenium can be synthesized from Hawthorn, Dried raisin, Lemon plant, Bougainvillea spectabilis, Ginger fruit, Ashwagandha (*Withania somnifera*) (Alagesan and Venugopal 2019), Fenugreek (*Trigonella foenum- graecum*), Avaram (*Cassia auriculata*), Arauna (*Terminalia arjuna*), Java tea, Lavender leucas (*Leucas lavandulifolia*), Aloe vera, Garlic (*Allium sativum*), Walnut (*Juglans regia*), Horseshoe geranium (*Pelargonium Zonale*), Asteriscus graveolens, catharanthus roseus, Orange peel, Clausea dentata, mountain persimmon, Emblica officinalis, Ficus benghalensis, Drumstick (*Moringa oleifera*) Guava (*Psidium guajava*) and Cacao bean shell (*Theobroma cacao L.*) (Pyrzynska and Sentkowska 2021). Selenium nanoparticles can also be synthesized from clove and cinnamon extract and lemongrass (*cymbopogon*). In this study Clove and lemongrass are preferred for Green synthesis of Selenium because to check the anti- bacterial activity of the clove and lemongrass extracted selenium nanoparticle against enterococcus faecalis.

*Enterococcus faecalis* is a normal commensal of the human oral cavity, GIT and vagina because it finds it as a good environment for its growth since these areas are with rich nutrients and low oxygen levels and intricate ecology. Due to their *E faecalis* is a gram negative aerobic bacteria. *E faecalis* are a source of many nosocomial infections. They are now third most common among the nosocomial pathogens. They are known to cause urinary tract infections, bacteremia, intra- abdominal infections and endocarditis. In a previous study the author stated that the selenium nanoparticle mediated antibacterial activity over *e faecalis* had a good effect and was able to combat biofilm formation. Therefore, it has a significant role in dentistry particularly in root canal disinfection (Miglani and Tani-Ishii 2021). Some of the nanoparticles which are having antibacterial potential against *e faecalis* are listed. Silver

nanoparticles have an antimicrobial activity against biofilm forming *e faecalis* on root dentin (Halkai et al. 2018).  $Fe_2O_3$  has an antibacterial activity against *e faecalis* when combined with antibiotics (Shahbazi, Morshedzadeh, and Zaeifi 2019). Chitosan - propolis nanoparticle CPN 250 and CPN 100 had antibacterial activity and can be used as an intracanal medicaments in patients wherein the root canal treatment have failed (Parolia et al. 2020). The aim of this study is to evaluate the antibacterial activity of selenium nanoparticles prepared using clove and lemongrass against *e faecalis*.

## MATERIALS AND METHODS

### Preparation of extract

Cloves and lemongrass were obtained from an organic store, chennai. The leaves of the lemongrass were washed thoroughly under the tap water. For seven days, the leaves were dried in the shade at room temperature. The leaves of the lemongrass and dry cloves were separately grounded using a mixer grinder into fine powder. 1 gm of powdered leaves of lemongrass and cloves was added to 100 mL of distilled water and heated at a temperature of 60- 70°C using a heating mantle. Finally, using whatman no. 1 filter paper, the mixture was filtered and extract was stored for further use ( Figure 1).

### Synthesis of Selenium Nanoparticles

Sodium selenite ( $Na_2SeO_3$ ), 30mM dissolved in 50mL of distilled water. To that, 50mL of clove and lemongrass extract was slowly added. Then, the reaction mixture was kept on a magnetic stirrer at 650- 700 rpm for 48- 72 hours (Figure 3).

### Antibacterial activity

Antibacterial activity of Selenium nanoparticles acts against the strain *e faecalis*. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller hinton Agar were prepared and sterilized for 15 minutes at 121°C. Media poured into the sterilized plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The selenium nanoparticles with different concentrations (25µL, 50µL, 100µL) were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hours at 37°C. After the incubation time the zone of inhibition was measured.

## RESULTS

The results of the minimum inhibitory concentration values of the selenium nanoparticles is shown in table 1. In 100 µL concentration the MIC value decreased. The minimum bactericidal concentration of the selenium nanoparticles is shown in figure 4. In 100 µL concentration there is an increased MBC effect seen. The MIC of *e faecalis* is shown in Graph 1. The X axis represents the different concentrations of selenium

nanoparticles (25 $\mu$ L, 50 $\mu$ L, 100 $\mu$ L) and standard antibiotic amoxyrite. The Y axis represents the absorbents. As the concentration of the nanoparticle increased the MIC also increased.

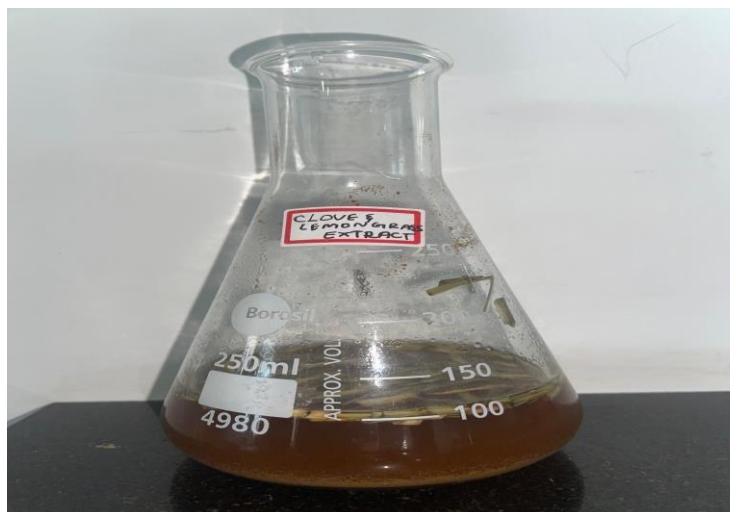


Figure 1: representing the preparation of clove and lemongrass extract.

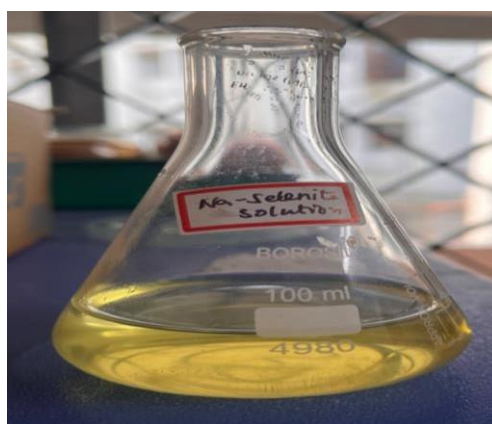
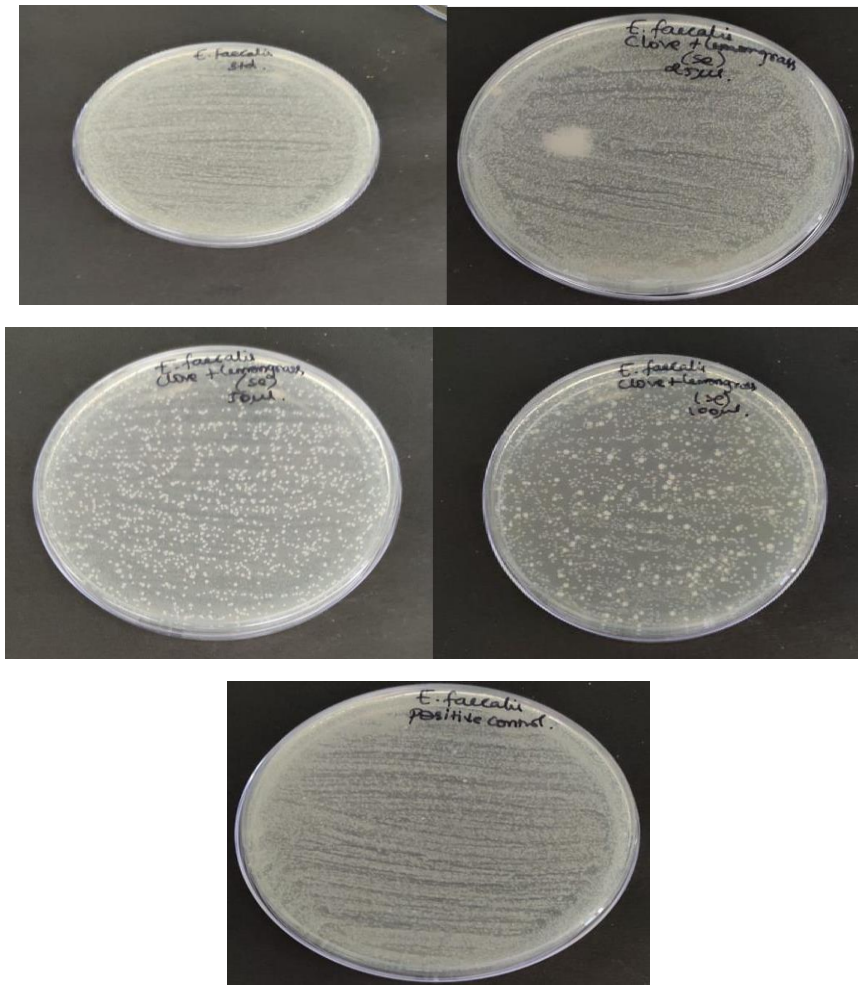


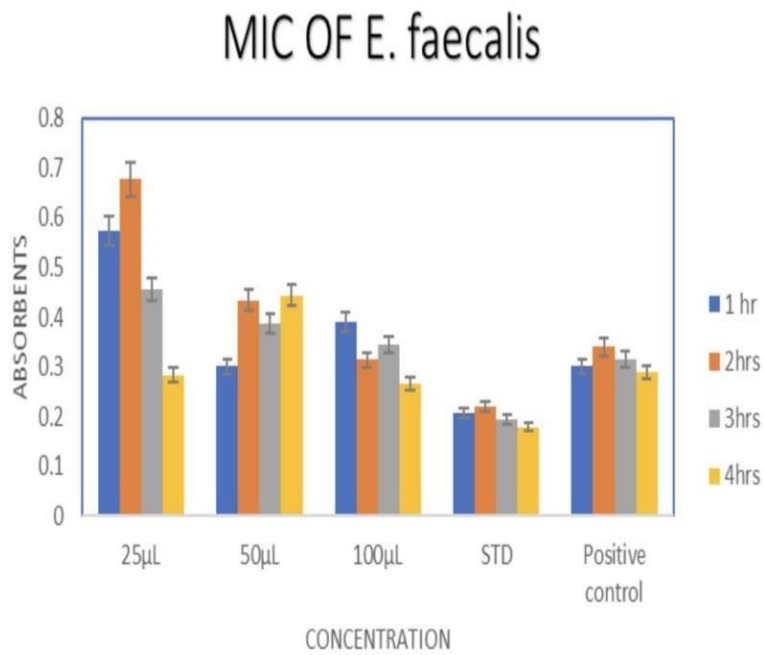
Figure 2: representing the preparation of the nanoparticles.



Figure 3: representing the synthesis of Selenium Nanoparticles.



**Figure 4:** Representing Minimum bactericidal concentration for Selenium nanoparticles.



**Fig 5 :** Minimum inhibitory concentration and time kill assay of green synthesized selenium nanoparticles



The above graph 1 depicts the MIC of *E. faecalis*. The X axis represents the different concentrations of selenium nanoparticles (25µL, 50µL, 100µL) and standard antibiotic amoxyrite. The Y axis represents the absorbance. Amoxyrite was used as standard. The minimum inhibitory dose for *E. faecalis* was found to 50 µL concentration. At 50 µL concentration, the growth inhibition was initiated at 2 hours which signifies the sensitive nature of the pathogen to the green synthesized selenium nanoparticles.

## DISCUSSION

Antibiotic resistance by the bacteria is one of the main issues. At present, infections and associated diseases became quite common despite the intense effort made by the medical fraternity. Many researchers made study on the development of nanostructured inorganic compounds with anti-bacterial activity. The use of selenium nanoparticles is a replacement to conventional products as they have significant anti-bacterial activity (Hernández-Díaz et al. 2021, Rajeshkumar et al., 2016, Sowmya et al., 2018).

Clove and lemongrass are growing interest among the researchers to use as antibacterial agents. They have the power to kill even the MRSA. So, they can be a good alternative for the routinely followed protocols. In a study the author stated that the minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC) values of clove oil against planktonic cells ranged from 2.0 % to 3.0 % and 2.4 % to 5.0 %. The data show that combined clove and lemongrass essential oils efficiently kills *S. aureus* and is therefore an alternative way for staphylococcus aureus eradication (Amalia et al. 2019).

Selenium nanoparticles have a great anti-bacterial activity compared to the other nanoparticles. It can be combined with other available treatment protocols or biosynthesized from herbal products. Selenium nanoparticles combined with photodynamic therapy against enterococcus faecalis biofilm could be an alternative disinfectant and for root canal treatment (Shahmoradi et al. 2021, Jayapriya et al., 2022). Many researchers suggest that the nanoparticles mediated treatment protocol would be beneficial in the field of dentistry. Since selenium has potent anti-bacterial activity it can be used in the formulation of root canal disinfectant, oral gel, mouth wash, toothpaste and even in suture materials (Miglani and Tani-Ishii 2021, Agarwal et al., 2018; Iyer et al. 2021).

In the present study MIC and MBC values have increased with respect to 100 µL concentration of the selenium nanoparticles. In a study conducted by Rangrazi et al have concluded with the same results that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the selenium nanoparticles against gram-negative and gram-positive bacteria showed MIC values of 0.068, 0.137, and 0.274 mg ml<sup>-1</sup>. The results proved that the concentration of 0.274 mg ml<sup>-1</sup> had a higher bactericidal effect than the other concentrations. As the concentration of selenium nanoparticles

increased to 0.274 mg ml<sup>-1</sup> the bacteria were completely killed (Rangrazi et al. 2020) (Priyadarshini et al. 2021).

In a similar study conducted by Beladi et al, concluded that selenium nanoparticles have more antibacterial activity compared to the use of selenium. In their study to determine MIC and MBC, disc diffusion and serial broth method were used and the results turned out to be the antibacterial activity of the selenium and selenium nano-particles demonstrated to be effective on 32% and 56% of the strains studied.

## CONCLUSION

Within the limits of the study it can be concluded that the green synthesized selenium nanoparticles using clove and lemongrass have remarkable antibacterial potentiality and can be added to dental materials to enhance their properties. Further animal studies are required for the evaluation of the efficacy of the nanoparticles.

Ethical approval-NA

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Informed Consent- None declared

## Authorship contribution

PS compiled the manuscript RKS conducted the study TL designed the study MT Performed Proof reading of the Manuscript

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