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# Preparation of Dental Varnish Using Ziziphus Oenoplia Mediated Selenium Nanoparticles And It's Antimicrobial Activity Against Oral Pathogens

**Nitika Ruthlyn R[1], S.Sangeetha[2]**
[1,2]Department of Anatomy, Saveetha dental college and hospital, Saveetha institute of Medical and Technical Sciences (SIMATS),Saveetha University, Chennai – 600077, India.
*Corresponding author Email: sangeethas.sdc@saveetha.com

***ABSTRACT***
***Background:****Nanotechnology plays a major role in the field of dentistry and medicine.Dental caries affect the aesthetics and the quality of life of an individual.The plant extract was used in order to stabilise the antimicrobial activity of the nanoparticles. Creating a dental varnish by integrating nanoparticles with plant extract can be a solution to minimise the side effects of normal dental varnishes.The antimicrobial activity of the varnish was assessed by the Agar well diffusion method.*
***Methods:****The antimicrobial activity of the varnish was assessed by agar well diffusion method by adding to the wells at different concentrations(25, 50, and 100 μg/mL).Time kill kinetic assay was performed to assess the efficacy of the varnish.The standard for bacteria and fungi is Amoxyrite and Fluconazole respect.*
***Results****:It was observed that as the concentration of the varnish increases,the zone of inhibition also increases. The prepared dental varnish exhibits a very strong antimicrobial activity against the oral pathogens like Streptococcus mutans,Staphylococcus aureus,Enterococcus faecalis,Lactobacillus,Candida albicans.*
***Conclusion:****As the prepared dental varnish exhibits excellent antimicrobial activity,further studies can be carried in vivo followed by human trials in order to pave the way for wide usage.*
***Keywords:*** *Dental varnish,selenium nanoparticles,antimicrobial activity,Ziziphus oenoplia,oral microbes*

## INTRODUCTION

A dental varnish is a thin film of liquid which is applied on all the surfaces of the tooth.It is later converted into a solid film which is consistent by physical and chemical processes in the oral cavity. The main significance of dental varnishes is to prevent the development of caries on the surfaces of the teeth by the release of active substances like fluoride. It also improves the esthetics of the shade of the tooth.Most of the dental professionals recommend that fluoride varnishes must be applied at least twice a year after a child reaches the age of three years (1). Developments of caries in the tooth affects the quality of life of an individual.Fluoride varnishes are expected to reduce the cavities of the tooth by 40% (2). It improves the mineralization of the enamel and saves all the money which will be required for restorative procedures if the varnish is not applied.It controls the oral bacteria and it is considered to be safe (3).

The Rhamnaceae family medicinal plant Ziziphus oenoplia (L.) Mill is used in traditional South Indian cuisine.Z. oenoplia has been utilised for its wound-healing,gastrointestinal, hypotensive, diuretic, antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties in rural areas (4). It is an essential plant, often called as Jackal jujube in English, is frequently discovered in the scorching areas of northern Australia and tropical Asia. It is a traditional herbal remedy used to relieve pain in the abdomen (5). The vegetative tissues contain cyclopeptide alkaloids.Cyclopeptide alkaloids constitute macrocyclic compounds having a ring structure that is replaced using a couple of extra units that are composed of an amino acid, a β-hydroxy amino acid, with a hydroxystyryl amine component (6). Because Ziziphus oenoplia can grow in semi-arid and arid environments, it is occasionally planted for soil conservation. Because of its deep roots, it is useful in agroforestry operations as they aid to avoid soil deterioration.

Nanotechnology is one of the growing fields in dental sciences which requires more exploration to determine the properties of nanoparticles.Nanomaterials are fortunate nanocarriers that have remarkable qualities that can replace conventional medication, such as excellent stability, solubility behaviours, and thermal characteristics. They also have controlled shape and surface modification. Among these, selenium nanoparticles have gained increased attention because of their special antioxidant qualities.Selenium is a crucial micronutrient trace element which is vital for individuals as well as animals. It may also have significant health advantages (7). Numerous reports have demonstrated the biocompatibility of SeNps to humans. In one such study, participants received daily modest doses of antioxidant vitamins and minerals, such as zinc, β-carotene, vitamin C, vitamin E, and selenium, to investigate the function of selenium as a protective agent (8). Nanoparticles, with their smaller size and cell lysis capabilities, are utilised in dental applications like local anaesthesia, hypersensitivity, cancer treatment, and diagnostics. They have antibacterial, anti-adhesive, cationic, and biocidal properties, making them useful in wound dressings.(9)It seems that using selenium nanoparticles (SeNPs) as a delivery system might work well for getting drugs to the right places. The therapeutic potential of selenium has been extensively researched and well studied. Its effectiveness over a range of cancerous cells, pathogenic microbes, infections caused by viruses, neuroprotective qualities, diabetic management, oxidative stress, and inflammation-mediated diseases including rheumatoid arthritis have all been studied. Notably, SeNPs are superior to other nanoparticles because of selenium's remarkable role in immune system control. SeNPs the synthesis, which has ecological sustainability and biological compatibility, is an attractive substitute for conventional chemical and physical procedures.(10)

In just one phase of green synthesis, metal ions can be reduced to nanoparticles by means of biomolecules found in plant extracts. At room temperature and pressure, this natural reduction of metal ion to base metal can be easily scaled up and is extremely quick. Plant extract-mediated synthesis is safe for the environment. (11)It is possible to carry out this synthesis using bacteria, plants, algae, fungi, and other materials. Certain plant components, including the leaves, fruits, roots, stems, and seeds, are being utilised for the production of different nanoparticles as a result of phytochemicals found in the extract, which function as reducing and stabilising agents.(12) Furthermore, it has been observed that leaf extracts from a number of plants, including Aloe vera, tamarind, neem, lemongrass, and geranium, have the ability to reduce Au (III) ions into gold nanoparticles and transform silver ions into silver nanoparticles as well.(12,13)The fundamental idea behind green synthesis techniques is that the phytochemicals found in plant parts have two functions: they stabilise nanoparticles and act as a natural reductant.(14)

From an aqueous sodium selenosulfate precursor, a straightforward wet chemical technique has been designed to produce glucose stabilised selenium nanoparticles.To prepare selenium nanoparticles, either reduction or oxidation method can be used, starting with a suitable precursor. There are a few published papers on the process of making selenium nanoparticles. Among these, wet chemical techniques, vapour phase diffusion, and physical vapour deposition are noteworthy and well-liked thus far. It is still difficult to manufacture stable selenium nanoparticles with good catalytic activity in a repeatable yet straightforward manner. Chemical reduction, which calls for a reducing agent and stabiliser, is the primary synthetic method for creating selenium nanoparticles.(15)Greenly produced metal nanoparticles are cheaper, easier to use, and a superior option than those made by physical or chemical processes.According to FT-IR investigations, extract from the leaves of Withania somnifera contains effective plant-based constituents that function as reducing agents during the synthesis of Se NPs and during the capping process, which gives Se NPs durability.(16)

Because of their potential antioxidant and antibacterial properties, SeONPs will be used to combat resistant microorganisms.(17)The amazing antimicrobial activity of green synthesised selenium nanoparticles made from clove and lemongrass can be put to dental materials to improve their qualities.(18)Clove and cardamom have a synergistic impact when added to selenium nanoparticles, and they can be used in place of over-the-counter antioxidant and anti-inflammatory medications.(19).As of now no research is done based on integrating selenium nanoparticles with Ziziphus oenoplia.This, together with their antimicrobial properties, is the most popular justification for the use of nanoparticles in dentistry. Another characteristic of nanoparticles is their opposition to friction.(20)

In order to develop a novel dental varnish formulation and assess its antibacterial effectiveness against oral infections, the study will integrate nanotechnology (selenium nanoparticles) with natural products (Ziziphus oenoplia). This could lead to breakthroughs in dental care and oral health therapies.

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## MATERIALS AND METHODS

### Preparation of plant extract

For the preparation of plant extract,1g of plant powder is added to distilled water which measures up to 100 ml and it is mixed thoroughly.For 15 to 20 minutes,the solution was heated in a heating mantle for getting the required components.After boiling the mixture,it is filtered using Whartman's filter paper no.1.This plant extract is collected and employed to synthesise nanoparticles

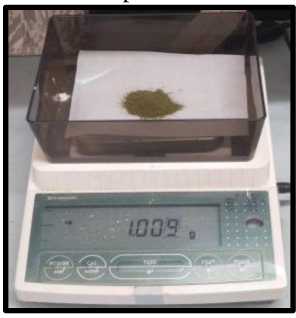
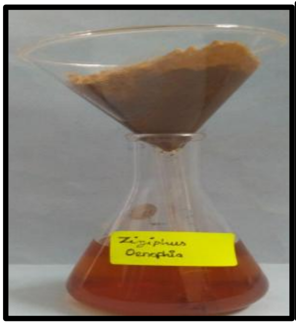



**Fig.1.1.1g of ziziphus**



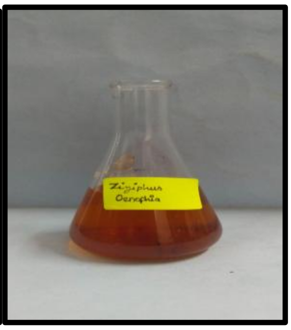
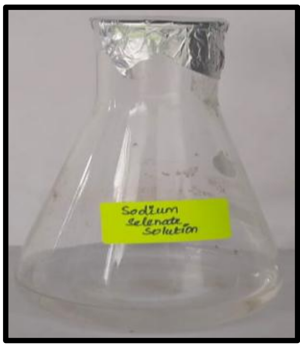


**Fig.3. filtered using glass funnel** **Fig.4.Prepared plant extract**

### Preparation of nanoparticles

The selenium nanoparticles were synthesised using biological methods because it is non toxic and ecofriendly.The selenium nanoparticles are synthesised by the mixing of 0.034 g of sodium selenate with distilled water which measures up to 70 ml.After mixing,the sodium selenate solution is mixed with 30 ml of Ziziphus oenoplia extract and it is kept in the orbital shaker for 48 hrs.The synthesised nanoparticles were subjected to UV readings for testing the presence of nanoparticles.After 2 days,the nanoparticles are synthesised in centrifuge in 8000 rpm for 10 minutes.It was collected in the form of pellets.This mixture can be used for various dental and medical applications.



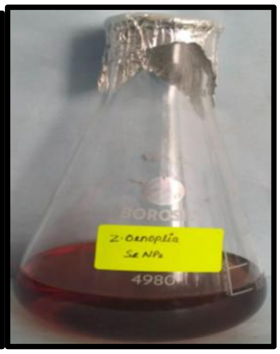




**Fig.5. Sodium selenate solution** **Fig.6.Plant extract with sodium selenate Solution** **Fig.7.Ziziphus oenoplia With selenium nanoparticles**

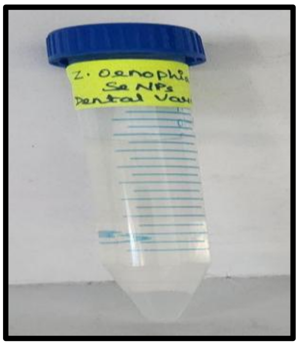


**Fig.8.Dental varnish**

**Antimicrobial activity of dental varnish**

Using the agar well diffusion method, the antibacterial activity of the green produced selenium nanoparticles was assessed. Mueller Hinton agar plates were made and autoclaved for 15–20 minutes at 121°C to sterilise them. The medium was sterilised, then put onto sterile Petri plates and left to cool to room temperature. Using sterile cotton swabs, the bacterial suspension (Streptococcus mutans, Lactobacillus sp., Staphylococcus aureus, Candida albicans, and E. Faecalis) was equally distributed onto the agar plates. With the use of a sterile polystyrene tip, wells of 9 mm in diameter were made in the agar plates. Next, varying quantities of selenium nanoparticles (25, 50, and 100 µg/mL) were added to the wells. As a standard, an antibiotic (such as Bacteria-Amoxyrite or Fungi-Fluconazole) was employed.

**Fig.9.Agar plate1 -Lactobacillus**

**Fig.10.Agar plate 2 -Enterococcus faecalis**

**Fig.11.Agar plate 3 -Streptococcus mutans**

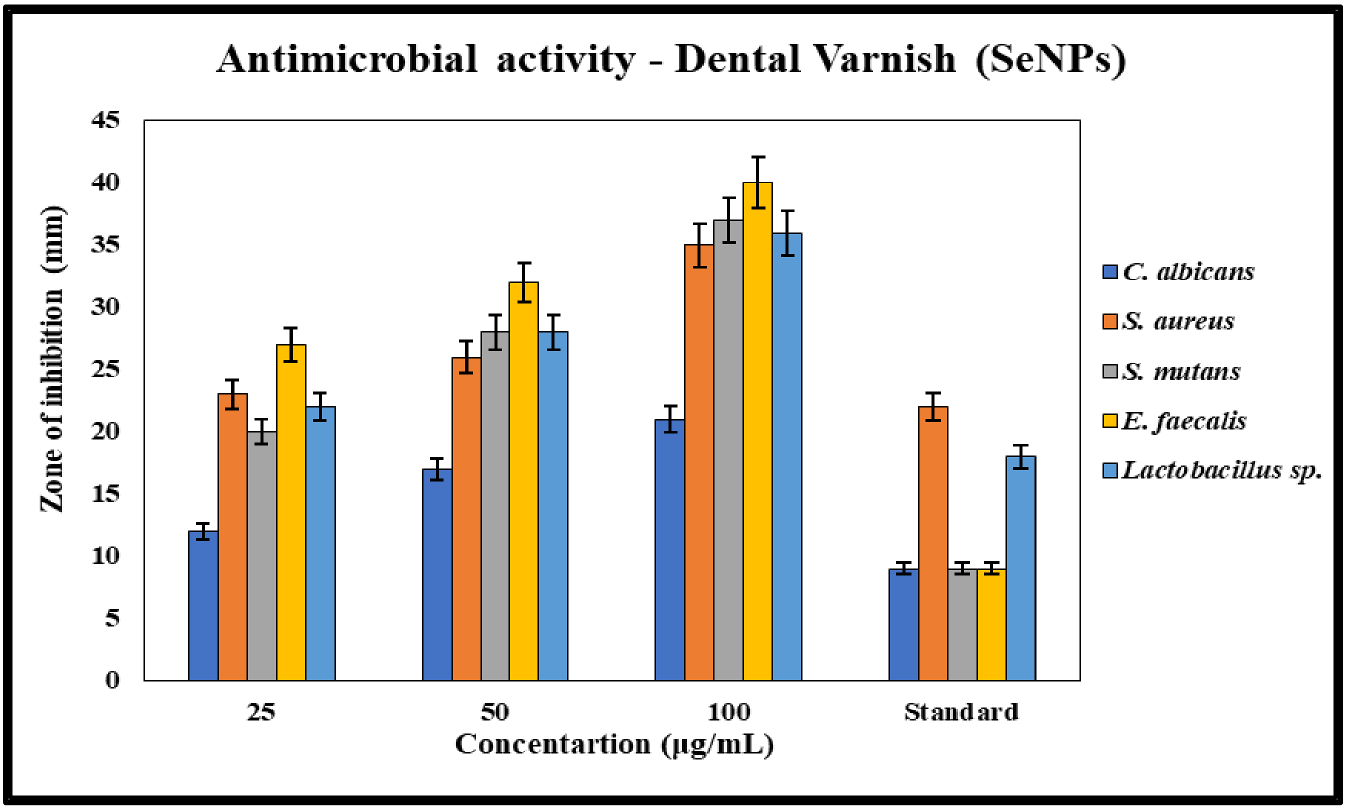
**Fig.12.Agar plate 4 -Staphylococcus aureus**

**Results**

**Table 1.1**

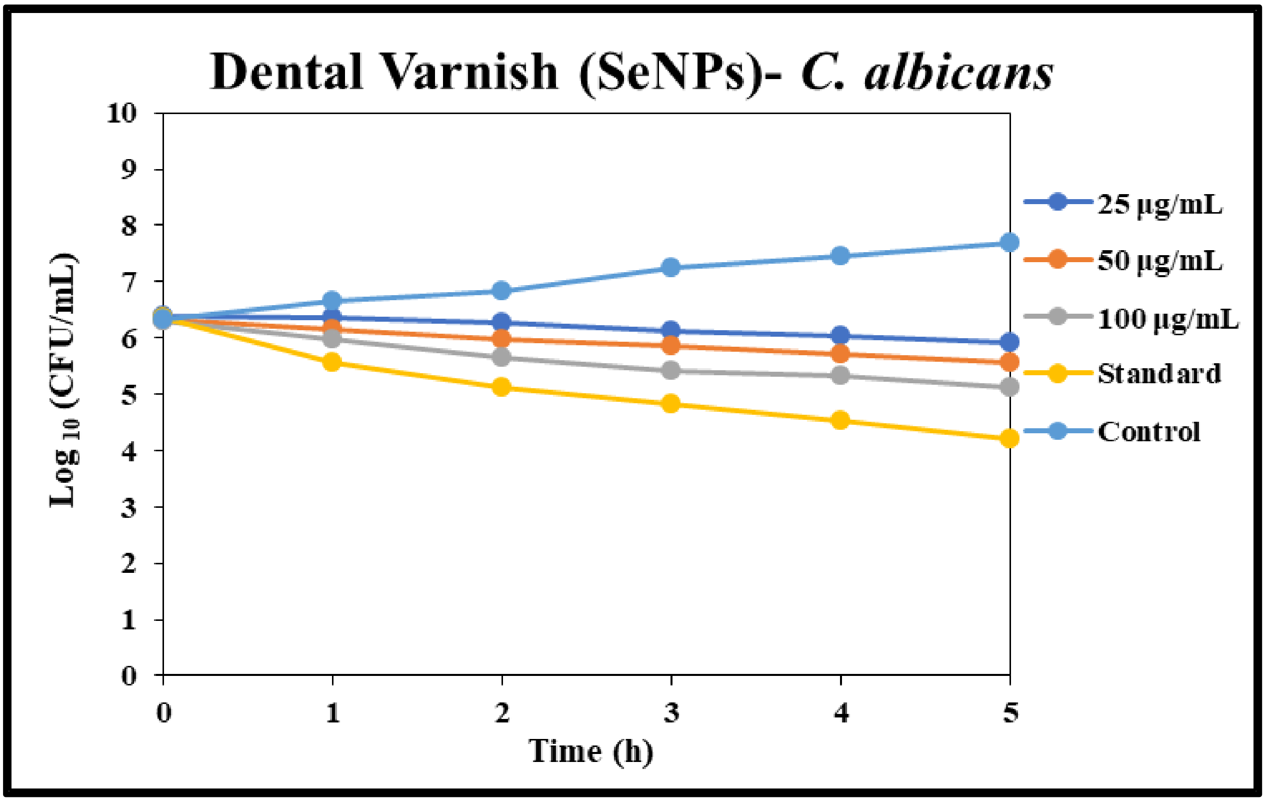
| Concentration | 25 | 50 | 100 | Standard |
|---|---|---|---|---|
| C. albicans | 12 | 17 | 21 | 9 |
| S. aureus | 23 | 26 | 35 | 22 |
| S. mutans | 20 | 28 | 37 | 9 |
| E. faecalis | 27 | 32 | 40 | 9 |
| Lactobacillus sp. | 22 | 28 | 36 | 18 |

The table 1.1 depicts the zone of inhibition of various oral pathogens when the dental varnish is added at different concentrations



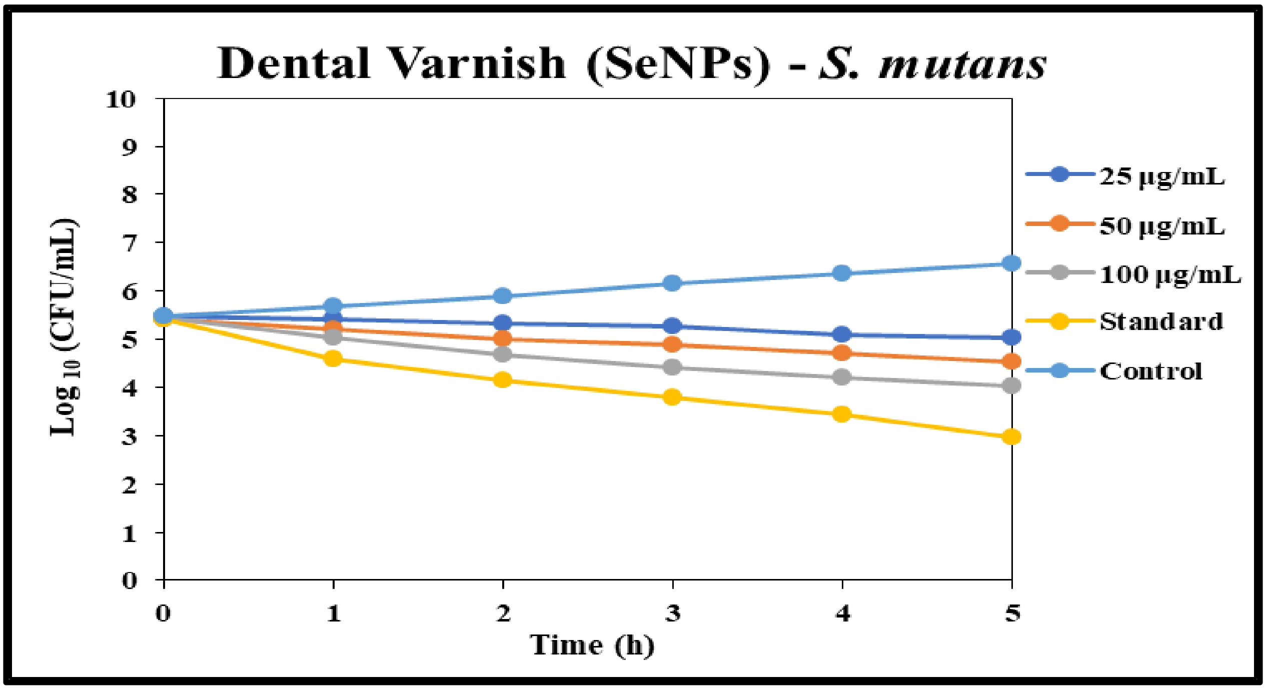

GRAPH 1.1

In the above graph 1.1,the X axis indicates the concentration and the Y axis indicates the zone of inhibition in mm.The graph depicts the zone of inhibition of various oral pathogens when the dental varnish is added at different concentrations



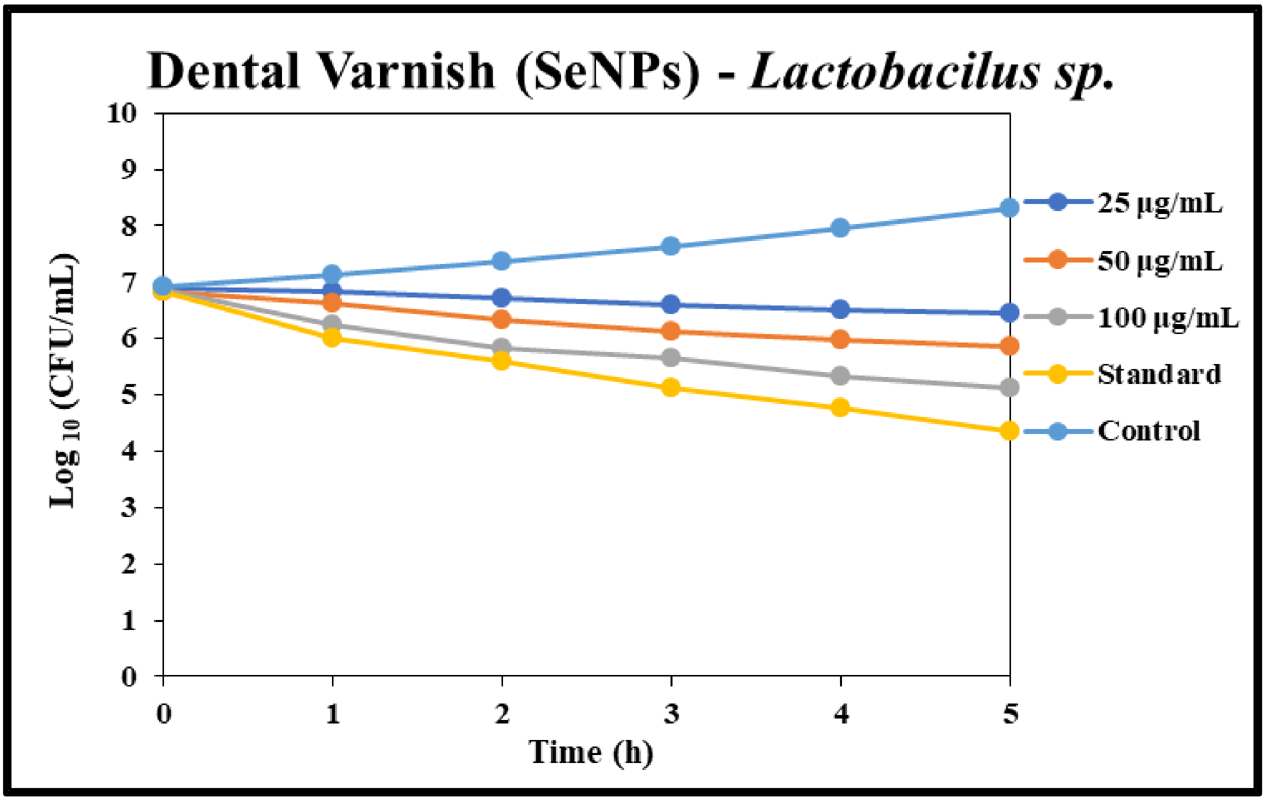

GRAPH 1.2

In the graph 1.2,the X axis indicates the time in hours and the Y axis indicates the viable cell population in a colony which is measured by CFU/mL.The graph depicts cell viability(CFU/mL) of Candida albicans after the dental varnish is added at different concentrations which is recorded from 0 to 5 hours.



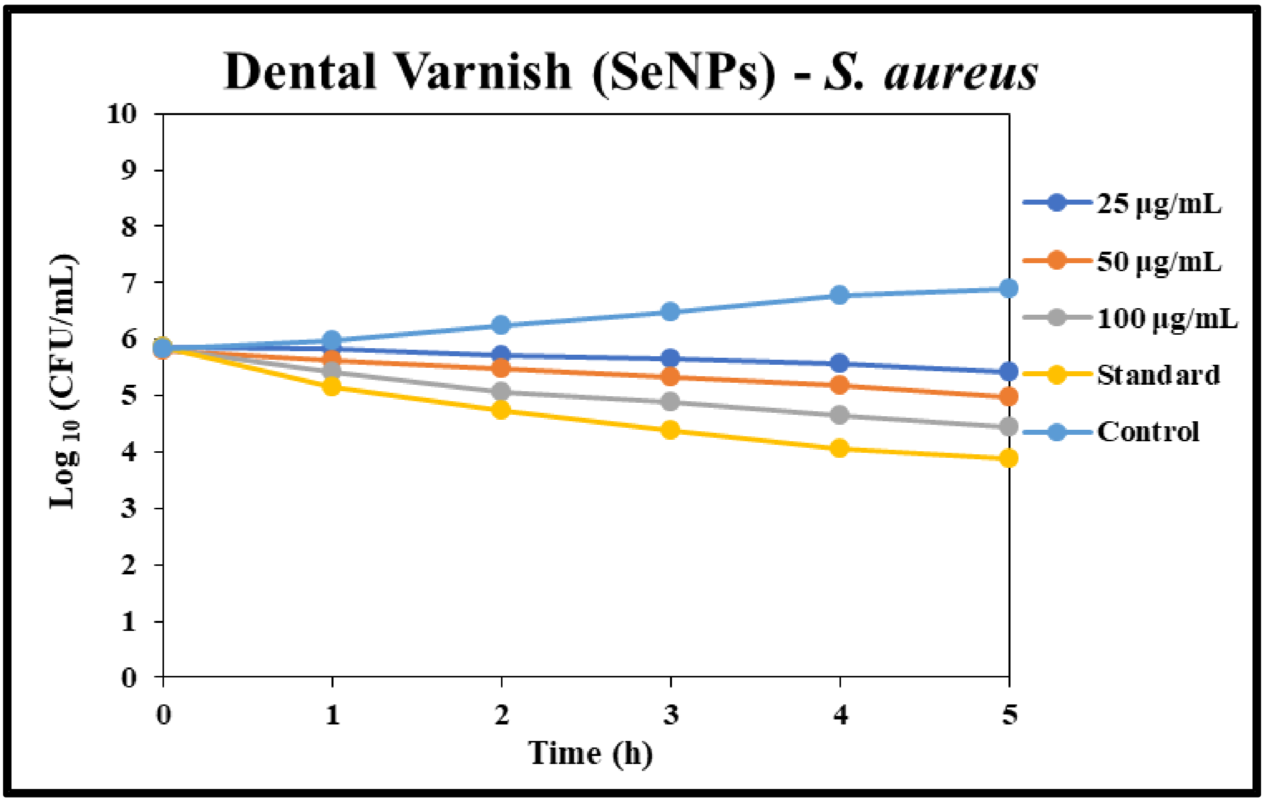

GRAPH 1.3

In the graph 1.3,the X axis indicates the time in hours and the Y axis indicates the viable cell population in a colony which is measured by CFU/mL.The graph depicts cell viability(CFU/mL) of Streptococcus mutans after the dental varnish is added at different concentrations which is recorded from 0 to 5 hours.



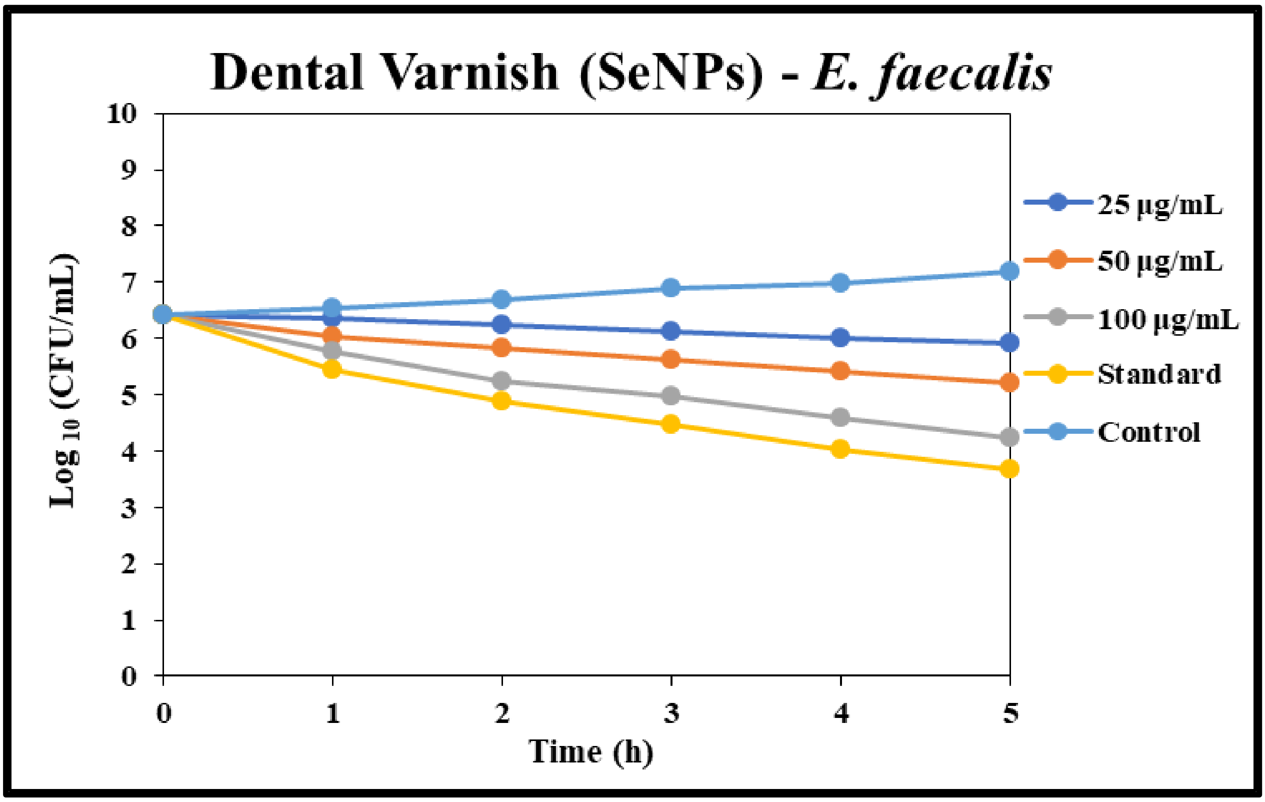

GRAPH 1.4

In the graph 1.4,the X axis indicates the time in hours and the Y axis indicates the viable cell population in a colony which is measured by CFU/mL.The graph depicts cell viability(CFU/mL) of Lactobacillus sp. after the dental varnish is added at different concentrations which is recorded from 0 to 5 hours.




GRAPH 1.5

In the graph 1.5,the X axis indicates the time in hours and the Y axis indicates the viable cell population in a colony which is measured by CFU/mL.The graph depicts cell viability(CFU/mL) of Staphylococcus aureus after the dental varnish is added at different concentrations which is recorded from 0 to 5 hours.




GRAPH 1.6

In the graph 1.6,the X axis indicates the time in hours and the Y axis indicates the viable cell population in a colony which is measured by CFU/mL.The graph depicts cell viability(CFU/mL) of Enterococcus

**faecalis after the dental varnish is added at different concentrations which is recorded from 0 to 5 hours.**

## DISCUSSION

According to the table 1.1,at the concentration of 25 μg/mL,the zone of inhibition of candida albicans is recorded to be 12 mm;at the concentration of 50 μg/mL,the zone of inhibition of candida albicans is recorded to be 17mm and at the concentration of 100μg/mL,the zone of inhibition of candida albicans is recorded to be 21 mm.These values are observed to be more compared to the zone of inhibition of control which is 9 mm.At the concentration of 25 μg/mL,the zone of inhibition of the microbe S.aureus is 23 mm;at the concentration of 50μg/mL,the zone of inhibition is said to be 26mm and at the concentration of 100μg/mL,the zone of inhibition is said to be 35 mm which is more compared to the control which is actually 22mm.At the concentration of 25 μg/mL,the zone of inhibition of S.mutans is said to be 20mm.At the concentration of 50 μg/mL,the zone of inhibition is 28 mm and at the concentration of 100μg/mL,the zone of inhibition of 37 mm.It is greater than the control.At the concentration of 25 μg/mL,the zone of inhibition of e.faecalis is 27mm: at the concentration of 50 μg/mL,the zone of inhibition of e.faecalis is 32mm and at the concentration of 100 μg/mL,the zone of inhibition is 40 mm which is more compared to the standard which is 9mm.At the concentration of 25 μg/mL,the zone of inhibition of lactobacillus sp. is 22 mm:at the concentration of 50 μg/mL,the zone of inhibition is 28mm and the concentration of 100μg/mL,it is recorded to be 36 mm which is more than the standard.We can conclude that as the concentration of nanoparticles increases,the zone of inhibition also increases.

In graph 1.1,the x axis indicates the concentration of the dental varnish in μg/mL and the y axis indicates the zone of inhibition in mm.The zone of inhibition of the oral pathogens Candida albicans,Staphylococcus aureus,Streptococcus mutans,Enterococcus faecalis and Lactobacillus is plotted for different concentrations( μg/mL) of the synthesised dental varnish with Ziziphus oenoplia and selenium nanoparticles.It can be inferred that the concentration of the dental varnish is directly proportional to the zone of inhibition.

The graph 1.2 represents the time kill kinetic analysis of the effect of the prepared dental varnish on Candida albicans,a type of yeast.The x axis indicates the time which ranges from 0 to 5 hours and the y axis indicates the cell viability that can be determined by counting the number of colonies per ml;which can be indicated by the unit CFU/mL.It can be inferred that as the time increases from 0 to 5 hours,the number of colonies per ml decreases,i.e.the cell viability decreases which is better compared to the standard which is fluconazole.

The graph 1.3 represents the time kill kinetic analysis of the effect of the prepared dental varnish on Staphylococcus aureus,a gram positive bacteria.The X axis indicates the time which ranges from 0 to 5 hours and the Y axis indicates the cell viability that can be determined by counting the number of colonies per ml which is indicated by CFU/mL.It can be inferred as the time increases from 0 to 5 hours,the number of colonies per ml decreases;i.e.the cell viability decreases which is better compared to the standard amoxyrite.

The graph 1.4 represents the time kill kinetic analysis of the effect of the prepared dental varnish on Streptococcus mutans,an anaerobic gram positive bacteria.The X axis indicates the time which ranges from 0 to 5 hours and the Y axis indicates the cell viability that can be determined by counting the number of colonies per ml which is indicated by CFU/mL.It can be inferred as the time increases from 0 to 5 hours,the number of colonies per ml decreases;i.e.the cell viability decreases which is better compared to the standard amoxyrite.

The graph 1.5 represents the time kill kinetic analysis of the effect of the prepared dental varnish on Enterococcus faecalis,a facultative anaerobic gram positive bacteria.The X axis indicates the time which ranges from 0 to 5 hours and the Y axis indicates the cell viability that can be determined by counting the number of colonies per ml which is indicated by CFU/mL.It can be inferred as the time increases from 0 to 5 hours,the number of colonies per ml decreases;i.e.the cell viability decreases which is better compared to the standard amoxyrite.

The graph 1.6 represents the time-kill kinetic analysis of the effect of the prepared dental varnish on Lactobacillus sp. ,an aerotolerant anaerobic gram positive bacteria.The X axis indicates the time which ranges from 0 to 5 hours and the Y axis indicates the cell viability that can be determined by counting the number of colonies per ml which is indicated by CFU/mL.It can be inferred as the time increases from 0 to 5 hours,the number of colonies per ml decreases;i.e.the cell viability decreases which is better compared to the standard amoxyrite.
In a similar study, the antibacterial activity of the synthesised CS- and CL-produced CuONPs, the zone of inhibition against S. mutans, S. aureus, E. faecalis, and C. albicans was measured. Although the observed antibacterial effect against the studied pathogens was not equivalent to the antibiotic's zone of inhibition, it was nonetheless substantial. As the concentration of nanoparticles increased, so did the zone of inhibition. According to reports, S. aureus has the biggest zone of inhibition.(21)

According to FT-IR investigations, extract from the leaves of Withania somnifera contains active phytoconstituents that function as reducing agents during the synthesis of Se NPs and during the capping process, which gives Se NPs stability.Compared to physical and chemical approaches, the green produced metal nanoparticle is easier, less expensive, and a better option. Synthesised green With a particle size ranging from 40 to 90 nm, selenium nanoparticles (Se NPs) were discovered to be nearly spherical. Using EDX and XRD, it was determined that the mineral is highly pure and crystalline. The amorphous Se NPs' DPPH scavenging study reveals strong antioxidant activity and suggests that they could be employed as an antibacterial agent to treat bacterial infections.The results of the antiproliferative activities indicate that Se NPs have greater growth control against A549 lung cancer. According to the photocatalytic investigation, these Se NPs are effective at breaking down MB when exposed to sunshine. They can therefore be used in textile and water treatment facilities.(16)

UV-vis, FTIR, XRD, SEM, TEM, and thermal analysis techniques were used to characterise ZnONPs and SeONPs. The results showed that the generated ZnONPs and SeONPs are crystalline in nanoscale, with particle sizes ranging from 20 to 45 nm. The antimicrobial activity of SeONPs and ZnONPs was assessed; the findings showed that SeO-NPs had greater antimicrobial activity than ZnONPs and that the two have an opportunity to be effective against prevalent human pathogens like Gram-positive and Gram-negative bacteria as well as single-celled and multicellular fungal organisms. Furthermore, ZnONPs and SeONPs exhibit little toxicity on 1-BJ1 normal cells and a potential antioxidant activity. Last but not least, promising green biosynthesized ZnONPs and SeONPs have the potential to be both antioxidants and antimicrobials, which will be used to control antibiotic-resistant bacteria.(17)

## CONCLUSION
The study conducted in vitro can conclude that incorporating the green synthesised nanoparticles into dental varnish could provide promising results.The effect of the prepared dental varnish on E.faecalis is notable because it is the predominant cause of various dental health issues.The viable cell population decreases with increase in time thereby proving the effectiveness of the varnish.Further studies should focus on performing it in vivo to explore its impact in the society and improve the quality of life.Comparing the effect of dental varnish in a large sample size in necessary to bring out the effectiveness of the dental varnish and to replace the fluoride varnishes.

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