Green Synthesis of Orange Peel and Grape Peel Formulation Assisted Silver Nanoparticles Mouth Rinse and Antibacterial Activity Against Streptococcus Mutans and Staphylococcus Aureus in Tooth Samples

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**Abstract:** Orange peel and grape peels being employed as a part of folklore and traditional medicine, it is utilized for the treatment of various ailments, including stomachaches and cancer.Silver nanoparticles are widely known for their broad-spectrum activity against microbes and various pathogens, including oral bacteria. Their unique physical and chemical properties, coupled with their nanoscale size, allow for enhanced antimicrobial efficacy. *S. mutans* serves as a key causative factor in the development of dental caries, whereas S. aureus is responsible for a range of oral and systemic infections.The objective of this research is to examine or explore the green synthesis of a mouth rinse formulation using silver nanoparticles (AgNPs) assisted by orange peel and grape peel extracts.2 grams of orange and grape peel were taken and grounded with the help of mortar and pestle. 100 ml of distilled water was added, and the mixture was filtered using a sterile cotton cloth. One millimole of silver nitrate solution was taken with a micropipette and mixed with 10 ml of orange and grape peel extract in the proportions of 80:10:10 ratio and the entire solution was kept in the heating mantle and condensed to 10 ml.The tooth samples were exposed to S.mutans and S.aureus petri plates. Tap water was taken as control media. Tooth samples were then kept in orange and grape peel extract. Number of colonies was counted.Orange peel and grape peel extracts actively consist flavonoids., phenols, terpenes, etc, that possess antimicrobial properties. These bioactive compounds have the ability to act as reducing and stabilizing agents during the formation of AgNPs, which enhances the plant extracts antibacterial activity. The zone of inhibition was also calculated (in mm) to assess the antibacterial potential of orange and grape peel synthesized silver nanoparticle extract.The green synthesis of a mouth rinse formulation using silver nanoparticles (AgNPs) assisted by orange and grape peel extracts shows significant potential as a sustainable and effective approach for combating oral bacterial infections.The mouthwash with AgNPs has effectively demonstrated its antimicrobial efficacy against the bacterial activities of Streptococcus mutans (S. mutans) and Staphylococcus aureus (S. aureus) using tooth samples as a representative oral environment.

**Keywords:** Orange peel, grape peel, antibacterial activity, mouthwash, zone of inhibition

# Introduction

The maintenance of general health is largely dependent on maintaining good oral health. Of the various dental illnesses, caries and periodontal diseases are the most prevalent and are primarily caused by Streptococcus mutans and Staphylococcus aureus [(Alias & Yusof, 2020; Harsha & Subramanian, 2022)](https://paperpile.com/c/PJVUtq/Lmqq+bzvs). S. mutans, which is well-known for its capacity to generate acid and create biofilms, is essential for the development of the plaque that leads to tooth decay [(Zhang et al., 2023)](https://paperpile.com/c/PJVUtq/9NYJ). S. mutans is identified as a significant contributor to dental caries in humans, and one of its crucial virulent characteristics involves the formation of a biofilm on tooth surfaces known as plaque. This bacterium manufactures glucan from sucrose with the assistance of glycosyltransferases (GTF). Subsequently, glycans facilitate the secure attachment of these cells to tooth surfaces. Additionally, S. mutans produces various glycan-binding proteins (Gbp) believed to enhance adhesion. Moreover, the cell surface protein antigen c (PAc), the primary surface protein of S. mutans, is linked to its cariogenic potential, as it might play a role in bacterial attachment to tooth surfaces through interaction with saliva. These surface proteins of the bacteria collaborate to create plaque, ultimately leading to the development of dental caries. [(Deepika et al., 2022; Solanki et al., 2022; Yoo et al., 2019)](https://paperpile.com/c/PJVUtq/xjeY+RyYK+VE9V).

In this research, we use nanobiotechnology and green synthesis to formulate a mouthwash to highlight its antibacterial activity. Mouthwash should be versatile in decreasing germ population, helps in reducing halitosis, and in anesthetizing oral wounds without discoloring the tooth. Mechanism involves oxidative stress induced membrane disruption, by inferring in metabolism of protein and carbohydrate. Mouth rinse thereby infer in oral biofilm formation on tooth surfaces [(Ajay, Rakshagan, et al., 2022; Chidambaram et al., 2022; Takenaka et al., 2022)](https://paperpile.com/c/PJVUtq/htB5+JU86+S3uD). An optimal mouthrinse which can inhibit microorganisms with a decreased toxicity load to the oral tissues with better results aimed at dragging herbal extract based mouthwash which can have a better outcome with less toxicity. Numerous bioactive substances with potential therapeutic benefits have been found in grape skin and orange peel extracts. This makes it unnecessary to use risky stabilizers and reducing agents, resulting in a safer synthesis procedure [(Ajay, Sasikala, et al., 2022; Saquib et al., 2020)](https://paperpile.com/c/PJVUtq/9BHw+KQA7).Orange peel and grape peel extracts consist of bioactive compounds such as flavonoids, phenols, and terpenes, that possess antimicrobial properties. These bioactive compounds which have the ability to act as reducing and stabilizing agents during the synthesis of AgNPs [(Ajay, Suma, et al., 2022; Praipipat et al., 2023)](https://paperpile.com/c/PJVUtq/jScX+snTW) Orange and grape peels are common agricultural wastes that are frequently discarded. They do, however, contain a number of phytochemicals with antimicrobial characteristics, including limonene, hesperidin, quercetin, and resveratrol [(Maiti 2021; Maqbool et al., 2023)](https://paperpile.com/c/PJVUtq/hVn5+rCld).Silver nanoparticles (AgNPs) have emerged as promising candidates due to their antimicrobial properties. Their diminutive size and expansive surface-to-volume ratio enhance their engagement with bacterial cells, causing disruptions in their membrane integrity and impeding their growth. Furthermore, the utilization of natural plant extracts in the production of nanoparticles has become popular and presents numerous benefits compared to conventional chemical approaches. [(Katyal et al., 2021; Prasher & Sharma, 2022)](https://paperpile.com/c/PJVUtq/VzNw+MyCi). Additionally, it has been demonstrated that AgNPs enhance the antibacterial activity of natural plant extracts when mixed with them. The two main bacterial species linked to dental caries and periodontal disorders are Streptococcus mutans and Staphylococcus aureus.Very little research has been done before to pinpoint these caries causing organisms based on the bioactive secondary metabolites to measure their antimicrobial efficacy. To maintain oral cleanliness, conventional mouthwashes with synthetic ingredients have been used extensively. Henceforth the main aim of this research is to use green synthesis methods to prepare orange peel and grape skin extract assisted AgNPs which can be incorporated into oral formulations. The antibacterial activity of the prepared extract is tested against S. mutans and S. aureus using tooth samples as the test substrate.

# Materials and method

## Study design

The peel of orange and grape were taken and dried in an autoclave for 24 hours and used for the study.

## Extract preparation

On the following day, 2 grams of orange and grape peel were taken, dried and grounded using a mortar and pestle (Figure 1). 100 ml of distilled water was added, and the mixture was filtered using a sterile cotton cloth. The filtered liquid was then placed in a heating mantle and the temperature was maintained at 50 to 60 degrees and allowed to condense until the extract reaches a volume of 10 ml (Figure 2).

## Silver nitrate solution preparation

One millimole of silver nitrate was taken with a micropipette and mixed with 10 ml of orange and grape peel extract in the proportions of 80:10:10 ratio and the entire solution was kept in the heating mantle and condensed to 10 ml.The tooth samples were placed in hydrogen peroxide for 24 hours to remove all the existing microbes in the extracted teeth. The tooth samples were then placed in sterile water. Grouping was done to check the antibacterial activity. Group 1 was chosen to be -Tooth exposed to S.aureus infected media. Group 2 was chosen to be - Tooth sample placed in S.mutans infected media and Control group was chosen to be Tooth sample placed in tap water (Figure 3).The tooth samples were exposed to S.mutans and S.aureus petri plates. Tap water was taken as control media. Tooth samples were then kept in orange and grape peel extract. Number of colonies was counted. Another set of tooth samples were placed in just tap water and the growth of microbes of the control group was observed by placing it on the petriplate. And the zone of inhibition was calculated in mm for tooth samples placed in both S. aureus and S. mutans (Figure 4).

## Antibacterial activity

The antibacterial properties of silver nanoparticles were evaluated using the agar well diffusion technique. The extract underwent preparation and sterilization, and Mueller Hinton agar plates were autoclaved at 121°C for 15–20 minutes. Once sterilized, the medium was applied to the surface of sterile Petri plates and allowed to cool to room temperature. Bacterial suspension (*Streptococcus mutans, Staphylococcus aureus*) was evenly distributed on the agar plates using sterile cotton swabs. Subsequently, 9mm-diameter wells were created in the agar plates using a sterile polystyrene tip. Different concentrations of Ag NPs (25 g, 50 g, and 100 g) were then added to the wells. An antibiotic (such as Bacteria-Amoxyrite) was employed as the standard for comparison. The plates were incubated at 37°C for 48 hours for bacterial cultures. The antibacterial activity was assessed by measuring the diameter of the inhibitory zone around the wells. A ruler was used to measure the zone of inhibition's diameter in millimeters (mm), and the data were recorded (Figure 5).

To determine the amount of mouthwash in the tooth sample, the formula below was utilized, with sterile water serving as the control

Bacterial growth =Number of colonies of bacteria \*10[⁵](https://www.hotsymbol.com/symbol/superscript-five) CFU/mL

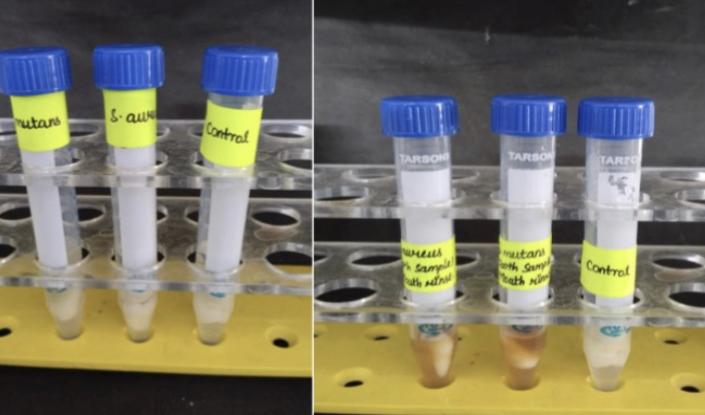
This formula was applied to calculate the colony-forming units per milliliter in orange and grape peel extracts containing AgNPs.



**Figure 1:** Dried orange and grape peel samples

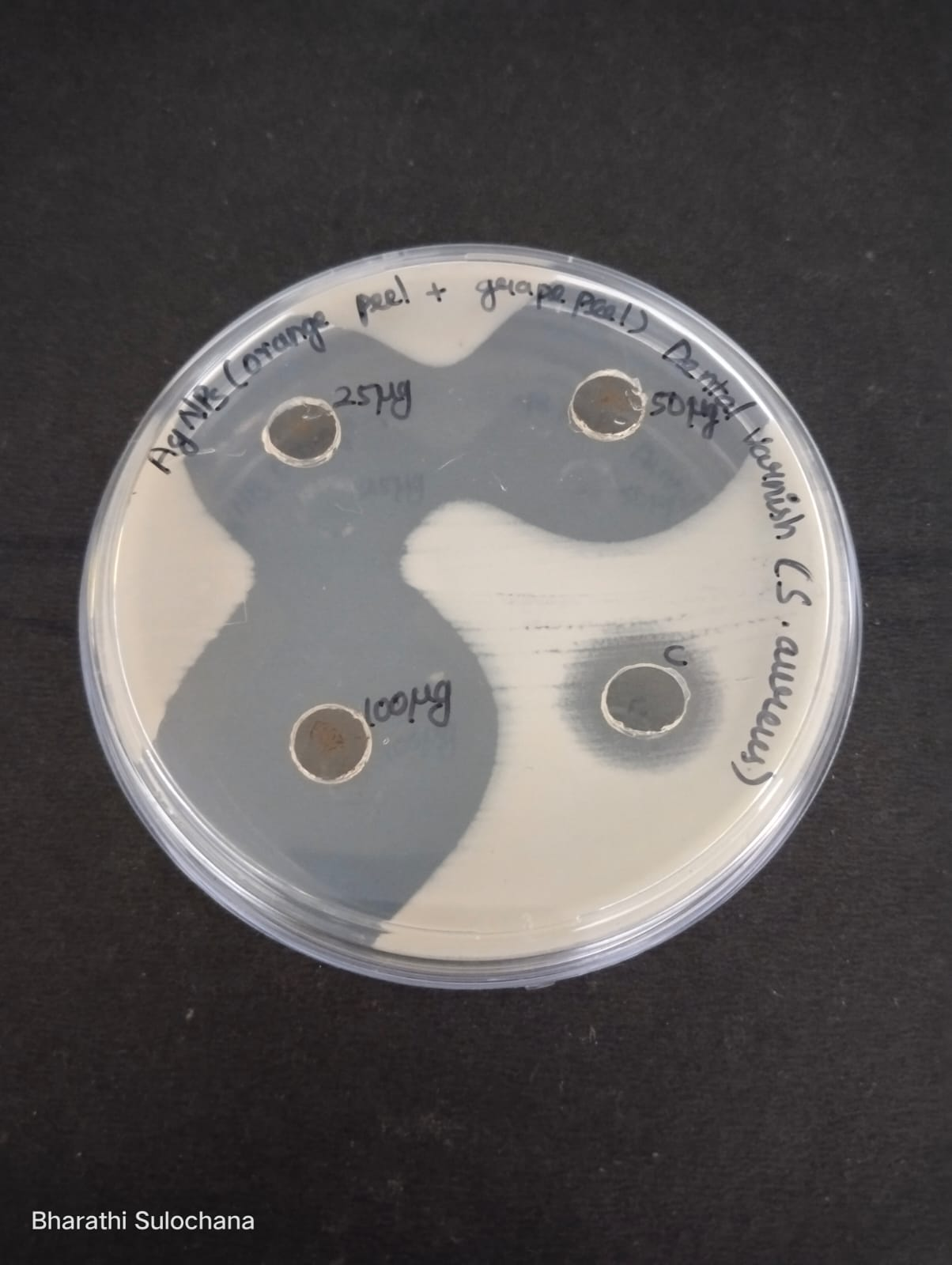


**Figure 2:** Orange and grape peel extract with silver nanoparticles



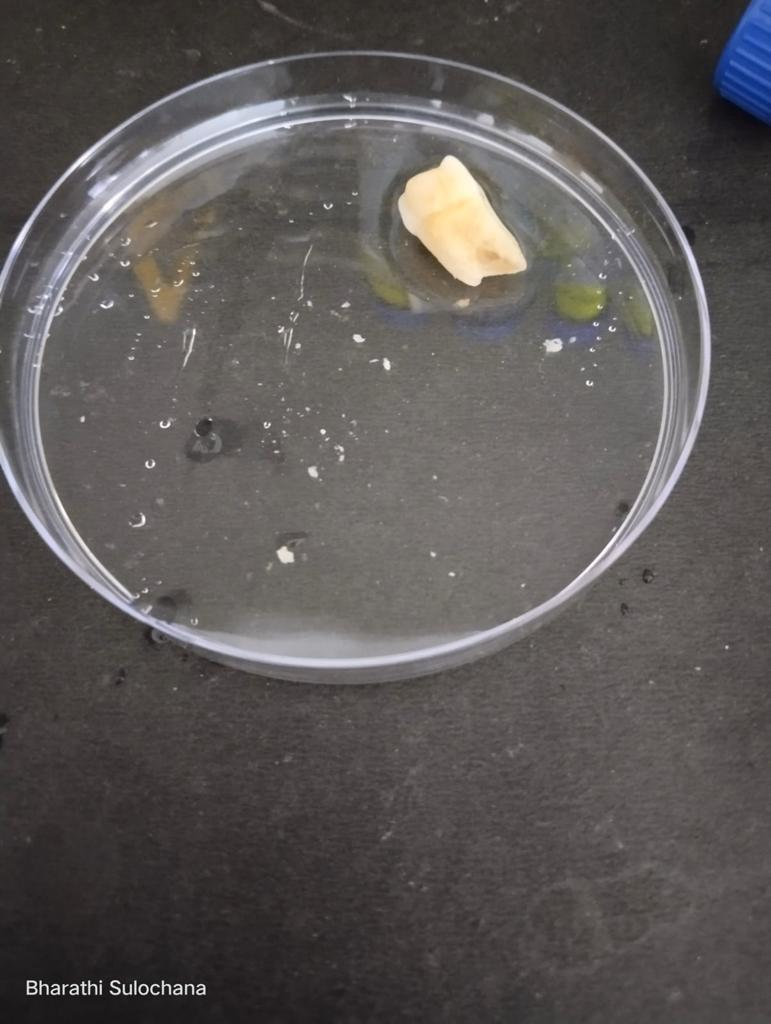
1. (b)

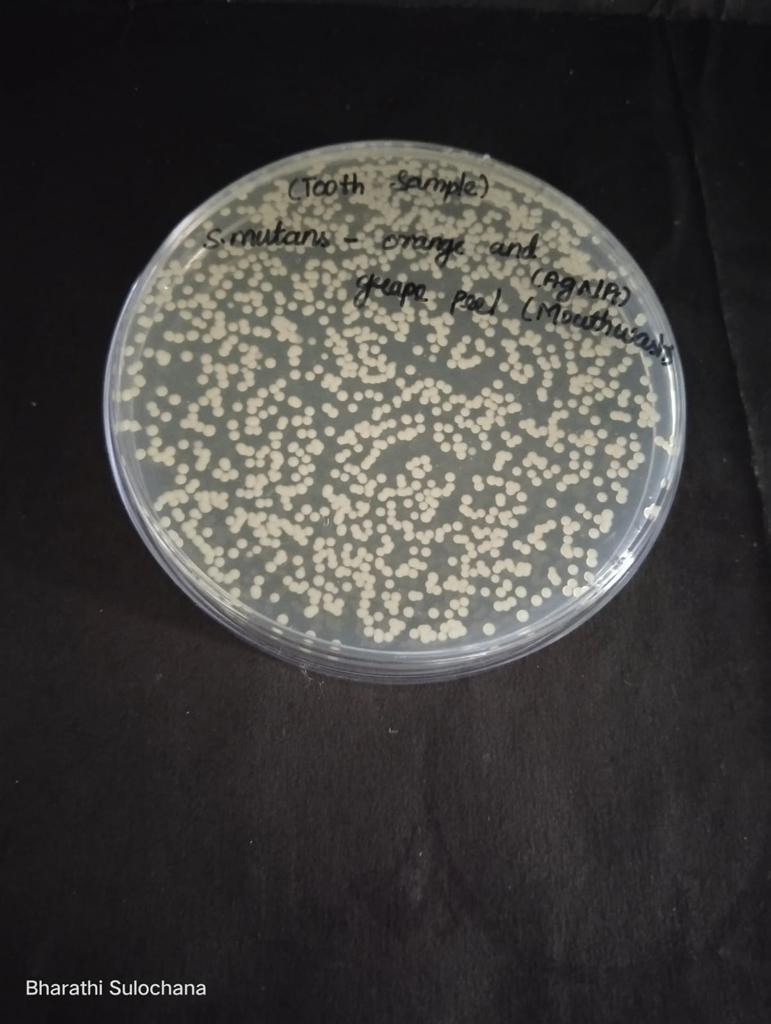
**Figure 3: (a) (b)** Tooth samples placed in the orange and grape peel extract with silver nanoparticles

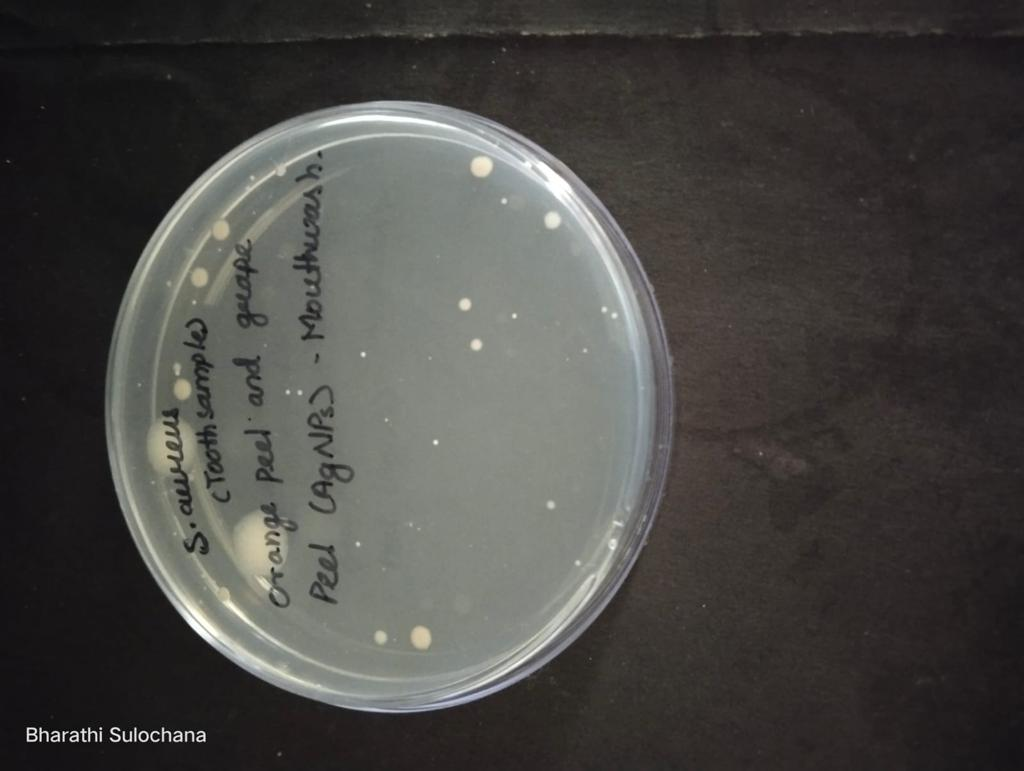


1. (b)

**Figure 4:** Zone of inhibition of *S aureus* and *S mutans* where the zone of inhibitionfor *S aureus* was observed to be larger than *S mutans*



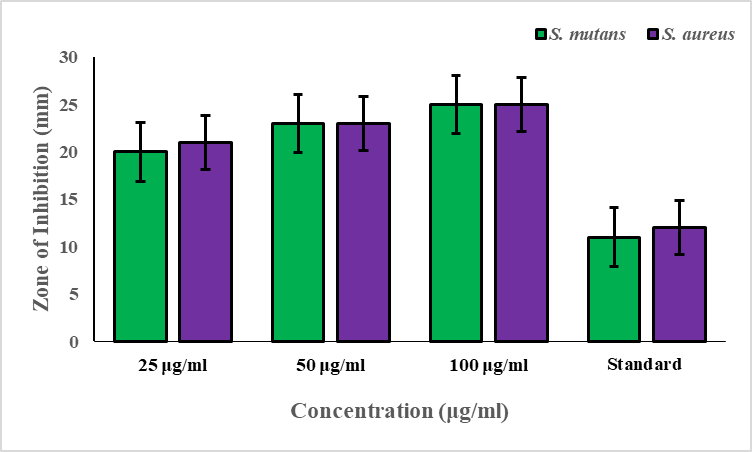




**Figure 5:** Growth of bacterial colonies demonstrated in petri plates after the tooth sample is soaked in different microorganism affected mouthrinse

# Results

The control group consisted of tooth samples placed in tap water. Group 1 comprised tooth samples in a culture medium affected by S. aureus, while Group 2 involved tooth samples in a culture medium affected by S. mutans. The zone of inhibition, determined by bacterial growth on a petri plate, was measured for each group. In the control group, the calculated zone of inhibition was 11mm for S. mutans and 12mm for S. aureus. For Group 1 (S. mutans), the zone of inhibition was 20mm at 25µg/ml, 23mm at 50µg/ml, and 25mm at 100µg/ml. For Group 2 (S. aureus), the zone of inhibition was 21mm at 25µg/ml, 23mm at 50µg/ml, and 25mm at 100µg/ml.



**Figure 6SFigure 6S:** Antibacterial activity of orange and grape peel extract + Ag NPs against *S aureus* and *S mutans*

Graph 1 was plotted for the data obtained above and the sample of orange and grape peel extract was found to have significant antibacterial activity against the pathogens S. mutans and S.aureus. It’s activity was found to be much better than that of normal tap water hence it would be very effective as a mouthwash(Rafi et al., 2024). When the antibacterial activity against S. mutans and S. aureus are compared with each other, the sample was found to work better against S. aureus than S. mutans.

Bacterial growth colonies of S.mutans is 1440\*10[⁵](https://www.hotsymbol.com/symbol/superscript-five) CFU/mL while S. aureus showed growth less than S.mutans with 40\*10[⁵](https://www.hotsymbol.com/symbol/superscript-five) CFU/mL. Hence we can say that the plant extract does show significant antibacterial activity against both S. aureus and S. mutans but the activity shown against growth S. aureus is more than that against S. mutans.

# Discussion

Orange peel and grape peel are wealthy sources of bioactive compounds, counting polyphenols and flavonoids, which can act as diminishing and capping operators within the synthesis of AgNPs. The method of green blend includes the diminishment of silver particles by these bioactive compounds, driving to the arrangement of AgNPs.The extricates from orange and grape peel contain different phytochemicals that not as it were to advance the decrease of silver particles but moreover upgrade the soundness and antimicrobial properties of the synthesized AgNPs [(Balaji Ganesh S & Sugumar, 2021; Jabin et al., 2021; Rajiv Gandhi et al., 2023)](https://paperpile.com/c/PJVUtq/vjsn+kC0E+tWBn). Once the AgNPs are synthesized, they can be joined into a mouth wash detailing for verbal wellbeing applications (Tuluwengjiang et al., 2024). Mouth flushes are commonly utilized for their antimicrobial properties, helping within the avoidance of dental caries and periodontal illnesses. S. mutans and S. aureus are two imperative verbal pathogens related with dental caries and different verbal contaminations.[(3D Metal Printing in Orthodontics: Current Trends, Biomaterials, Workflows and Clinical Implications, n.d.)](https://paperpile.com/c/PJVUtq/YycO) Testing the antibacterial movement of the silver nanoparticles mouth flush against these microscopic organisms can give important bits of knowledge into its potential as an verbal healthcare item [(Manzur-Valdespino et al., 2022; 2021)](https://paperpile.com/c/PJVUtq/tVQO+vGkS).

Several parameters can be evaluated to determine the antibacterial effectiveness, including the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the AgNPs mouthwash against S. mutans and S. aureus[(Rajeshkumar et al., 2021; Sushanthi 2021)](https://paperpile.com/c/PJVUtq/Paoc+ELhq). Furthermore, the adequacy of the mouth wash in lessening bacterial grip and biofilm arrangement on the tooth surface can too be explored [(Rajeshkumar & Malarkodi, 2014; Shathviha et al., 2021; Tharani et al., 2023; Tiwari & Jain, 2023)](https://paperpile.com/c/PJVUtq/WD5J+Ta0I+avJM+UYcb).This can be accomplished using techniques such as scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) to visualize and quantify the arrangement of the biofilm[(Bourgou et al., 2012; Dharman 2021)](https://paperpile.com/c/PJVUtq/NsYz+mPBR). Employing the natural combination of AgNPs, along with orange and grape peel, as a mouthwash, represents a promising approach to developing an effective and natural antimicrobial product for oral healthcare[(Cao, 2017; Nguyen et al., 2023; Ramamurthy 2021)](https://paperpile.com/c/PJVUtq/Sc47+GTWW+C2Ya). The investigation into the environmentally friendly production of mouthwash utilizing silver nanoparticles, with the aid of formulations derived from orange and grape peel, possesses specific limitations. Notably, the study has been confined to in vitro experimentation, and the mouthwash formulation has not been subjected to testing on human subjects. These limitations may affect the broader applicability and thorough comprehension of the research outcomes, particularly concerning the antibacterial efficacy against S. mutans and S. aureus in tooth samples.The study constraints, including the restricted sample size and the necessity for additional research to assess factors such as the stability, shelf-life, in vivo effectiveness, and safety of the mouthwash rinse. Conducting comparative studies with established commercial mouthwashes or antibacterial agents would yield valuable insights into the potential advantages offered by the silver nanoparticle-based mouthwash rinse.

# Conclusion

In conclusion, the synthesis of silver nanoparticles was successfully achieved through environmentally friendly methodologies employing extracts derived from orange and grape peels. The efficacy of these nanoparticles in combating bacterial pathogens, specifically *S. aureus and S. mutans*, was systematically assessed using tooth samples. The results of the study demonstrated promising antibacterial properties of the silver nanoparticles, highlighting their potential for applications in dental hygiene. Furthermore, the investigation extended to explore the feasibility of incorporating these nanoparticles into a mouth rinse formulation. The findings suggest that these Silver nanoparticles have the capacity to function as a potent ingredient in mouth rinses, opening avenues for the development of novel oral care products with enhanced antibacterial capabilities and eco-friendly origins. This research contributes to the growing field of sustainable nanotechnology in dentistry, showcasing the versatility and promising antimicrobial properties of nanoparticles derived from natural sources.

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