Research Article

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Preparation of Dental Varnish using Nanosilver-chitosan Composite and its Antibacterial Activity Against *Streptococcus mutans* and *Lactobacillus spp.*

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ABSTRACT

Dental caries, caused by *Streptococcus mutans* and *Lactobacillus spp*., are a major oral health issue. Nanosilver-chitosan composite varnishes offer antibacterial protection, inhibit biofilm formation, and support enamel remineralization, making them a promising solution for managing dental decay. Orange and grape peel extracts were combined with chitosan and silver nitrate to synthesize chitosan-silver nanoparticles (CS-AgNPs). Antimicrobial activity was evaluated using agar well diffusion and time-kill assays. Data were analyzed *via* one-way ANOVA. The varnish demonstrated dose-dependent antibacterial activity, with increasing inhibition zones for higher concentrations and consistent reductions in bacterial growth. Time-kill assays revealed significantly lower optical density at 25 µg/mL, 50 µg/mL, and 100 µg/mL compared to the control, indicating effective bacterial inhibition. The efficacy of this dental varnish was comparable to a standard antibacterial agent. Strong activity was observed against *Lactobacillus spp*. Statistical analysis confirmed significant differences among the groups ($p < 0.05$). These findings highlight the potential of nanosilver-chitosan varnish as an advanced preventive material for dental caries. Further research on its long-term safety and efficacy could facilitate its integration into routine dental care practices.

Keywords: Nanosilver-chitosan; Dental varnish; Antibacterial activity; *Streptococcus mutans; Lactobacillus spp.*

1. INTRODUCTION

Dental caries, often known as tooth decay, continue to be a major oral health issue around the world. *Streptococcus mutans* and *Lactobacillus spp*. are two important bacterial species implicated in the onset and progression of dental caries (Girija and Ganesh, 2022). Oral hygiene methods have proven helpful to some extent, although they are not always adequate for controlling bacterial colonization and biofilm formation. As a result, novel techniques are needed to combat dental caries and improve oral health. Nanoparticles with linear polysaccharides from shrimp and other crab chitin shells are used in dental varnish preparation (Ganesh *et al*. 2024). These features make dental varnish an appropriate and effective delivery mechanism for the nanosilver-chitosan composite, helping to promote oral health and prevent dental cavities (Qu *et al*. 2023). Varnish attaches effectively to tooth surfaces, forming a protective layer that allows for extended contact between the nanosilver-chitosan composite and bacteria (Harini *et al*. 2022). This encourages direct interaction with cariogenic bacteria while inhibiting bacterial adhesion and biofilm formation. Then, it permits regulated release of the nanosilver-chitosan combination, ensuring a long-lasting antibacterial action against *Streptococcus mutans* and *Lactobacillus spp*. Furthermore, dental varnishes are simple to apply clinically, requiring little time and effort, making them useful for preventive and therapeutic interventions. They also have an aesthetically pleasant appearance, which improves patient acceptability and compliance with treatment (Chokkattu *et al.* 2023).

The type and concentration of the varnish base, solvent selection, compatibility with additives (such as fluoride compounds), as well as viscosity and pH optimization, are key factors in formulation (Baik *et al.* 2021). Biocompatibility and safety of the nanosilver-chitosan composite varnish before being employed in clinical settings are assessed. Obstacles may be encountered during cytotoxicity trials, quantification of effects of varnish on oral tissues, and regulatory compliance and ethical considerations (Alsharbaty *et al.* 2024). Scalability, cost-effectiveness, convenience of administration, and long-term clinical efficacy are important considerations for the eventual use of varnish in dental practice (Alam *et al*. 2023).

The use of silver nanoparticles in the nanosilver-chitosan composite varnish has various advantages. Silver nanoparticles have powerful and broad-spectrum antibacterial characteristics, effectively preventing the growth of many pathogenic microorganisms, including cariogenic bacteria. The regulated release of varnish ensures a sustained and persistent antibacterial impact over time (Bruna *et al.* 2021). The smaller size and increased surface area of nanoparticles enhance their reactivity and dispersion within the varnish. This improves their interaction with bacteria, providing stability and durability to silver nanoparticles, and maintaining antimicrobial efficacy throughout the shelf life of varnish (Shree *et al.* 2023).

Chitosan was chosen for its antibacterial properties, biocompatibility, adhesive properties, controlled release capabilities, and film-forming properties (Harugade *et al.* 2023). These attributes contribute to the antibacterial activity of varnish and overall oral health. Chitosan has the ability to encapsulate and deliver various bioactive agents, such as nanoparticles or drugs, due to its unique physicochemical properties. In the case of a nanosilver-chitosan composite varnish, chitosan can serve as a carrier for the nanosilver particles, allowing controlled release of the antimicrobial agent and enhancing its effectiveness against cariogenic bacteria (Mascarenhas *et al.* 2024). Varnish plays a crucial role in preventing dental caries, facilitates the remineralization of weakened enamel, effectively repairing early signs of decay and enhancing resistance to acid erosion. Additionally, fluoride varnish is valuable in treating dentin hypersensitivity by occluding open dentinal tubules, reducing the transmission of external stimuli to the tooth nerve (Cai *et al.* 2021).

Orange and grape peel extracts exhibit antibacterial activity due to the presence of bioactive compounds such as flavonoids, polyphenols, and other secondary metabolites (Zaki *et al.* 2024). Incorporation of chitosan into silver in the formation of a nanocomposite for dental varnish offers multifaceted advantages. Acting as a stabilizing agent, chitosan prevents the agglomeration of silver nanoparticles (AgNPs), ensuring a uniform distribution in the varnish and maintaining their effectiveness in antibacterial activity (Parvekar *et al.* 2020). The unique ability of chitosan to release silver ions in a controlled manner contributes to a sustained antibacterial effect, enhancing long-lasting efficacy against bacterial growth. Additionally, the combination of its inherent antibacterial properties with silver results in a synergistic antibacterial effect, boosting overall activity against a broad spectrum of bacteria, including those associated with dental caries. Moreover, its bioadhesive properties promote superior retention on dental surfaces, facilitating prolonged contact between the nanocomposite and bacteria, thereby further improving its antibacterial efficacy (Farasati *et al.* 2024). This comprehensive approach exploits the potential of chitosan-silver nanocomposites for effective dental applications.

2. MATERIALS AND METHODS

2.1 Extract Preparation

Orange and grape peels were dehydrated (for 24 hours) in a hot air oven. Fig. 1 shows the dehydrated samples. A mortar and pestle were used to grind 2 g of orange and grape peels. About 100 mL of distilled water was added to the ground powder and then the mixture was filtered through a sterile cotton cloth. The filtrate was placed in a heating mantle and maintained at 50 to 60 ℃ until it condensed to a volume of 10 mL (Fig. 2).

Fig. 1: Orange and grape peel

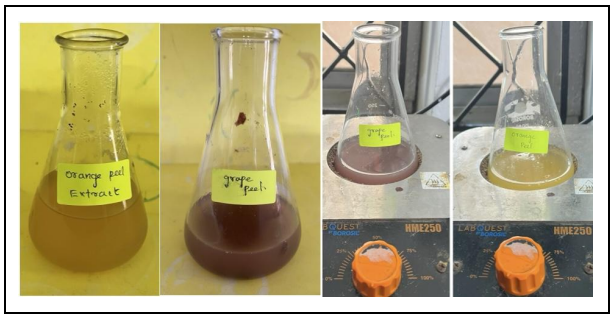

Fig. 2: Preparation of orange and grape peel extract

3. PREPARATION OF CS-Ag NPs

Chitosan powder was dissolved in glacial acetic acid to obtain chitosan solution. In another container, $AgNO_3$ solution was prepared using distilled water. Chitosan solution was added dropwise to the silver nitrate solution under constant stirring. This initiated formation of chitosan-silver nanoparticles (CS-Ag NPs). The pH of the solution was adjusted by adding NaOH. A slightly basic pH was maintained to aid in nanoparticle stabilization. Stirring of solution was continued while heating it on a hotplate.

Heating facilitated the reduction of silver ions and the formation of nanoparticles. Stirring was continued until a uniform dispersion was achieved. The

solution was allowed to cool naturally for 12-24 hours at 37°C. Aging influences the size and stability of the nanoparticles. The solution was subjected to ultrasonication to enhance the dispersion and stability of the nanoparticles. This step also prevented agglomeration. Centrifugation of the solution was done to separate any unreacted materials or large particles. The supernatant contained stabilized chitosan-silver nanoparticles. The pH of the solution was adjusted to 4.5-5.5 using 1% of acetic acid. Chitosan-silver nanoparticle solution was thoroughly stirred to ensure a homogeneous mixture (Fig. 3 and Fig. 4).

Fig. 3: Preparation of chitosan-AgNPs

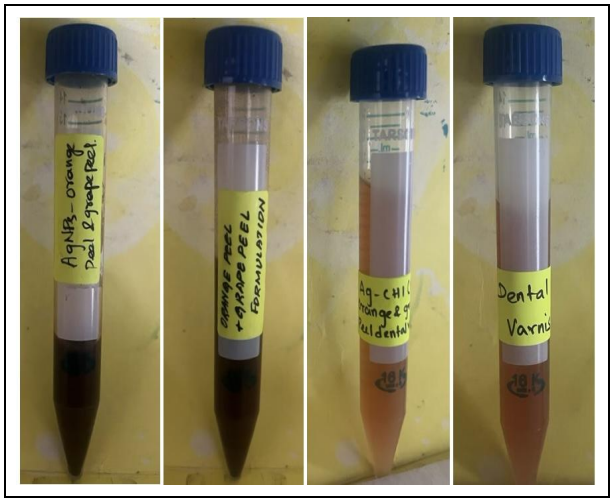

Fig. 4: Synthesis of nanosilver chitosan incorporated dental varnish

4. ANTIBACTERIAL ACTIVITY

The antibacterial activity of the green synthesized silver nanoparticles was evaluated using the agar well diffusion technique. Mueller-Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15-20 minutes. After sterilization, the medium was poured on to the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9-mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations (25 µg, 50 µg, 100 µg) of Ag NPs. Amoxyrite was used as a standard. The plates were incubated at 37°C for 24 hours. The antimicrobial activity was evaluated by measuring the diameter (mm) of the inhibition zone surrounding the wells using a ruler (Fig. 5).

5. TIME-KILL CURVE ASSAY

A 1-mL aliquot of the bacterial was added to 9 mL of Mueller-Hinton broth containing the Ag NPs at a concentration of 25 µg, 50 µg, 100 µg. The final microbial concentration was approximately 10^6 CFU/mL. The mixture was then incubated at 37°C with shaking at 200 rpm for varied time intervals (1 hour, 2 hours, 3 hours, 4 hours). Then the percentage of dead cells was calculated at a wavelength of 600 nm at regular time intervals. The data were analyzed (one-way ANOVA) using SPSS.

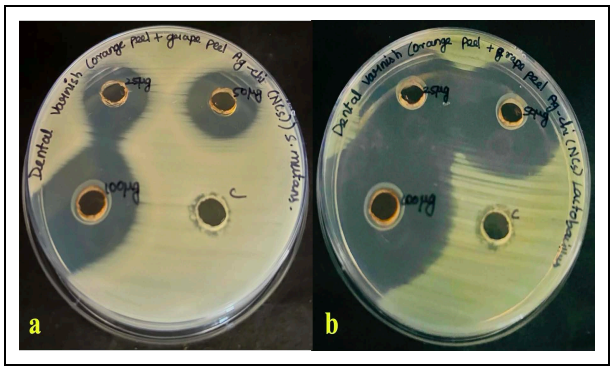

Fig. 5: Antibacterial activity of dental varnish containing orange peel and grape peel extracts against (a) *Streptococcus mutans* and (b) *Lactobacillus spp*.

6. RESULTS

6.1 Antibacterial Activity

The zone of inhibition was measured for *S. mutans* and *Lactobacillus spp*. In the control group, the calculated zone of inhibition was 11 mm for *S. mutans* and 12 mm for *Lactobacillus spp*. For S. mutans, the zone of inhibition was 25 mm at 25 µg/mL, 20 mm at 50 µg/mL, and 24 mm at 100µg/mL. For *Lactobacillus spp*., the zone of inhibition was 15 mm at 25 µg/mL, 16 mm at 50 µg/mL, and 18 mm at 100µg/mL. Table 1 summarizes the results of One-way ANOVA. It presents the results of an analysis conducted to assess whether there are significant differences in antimicrobial activity among the groups. The F-ratio (2.442) and p-value (0.204) indicate that the differences are not statistically significant at the 0.05 level (Graph 1).

Table 1. One-way ANOVA for antimicrobial activity

Source of Variation	Sum of squares	df	Mean Square	F	Significance
Between Groups	682.375	3	227.458333	2.442	0.204
Within Groups	372.5	4	93.125		
Total	1054.875	7			

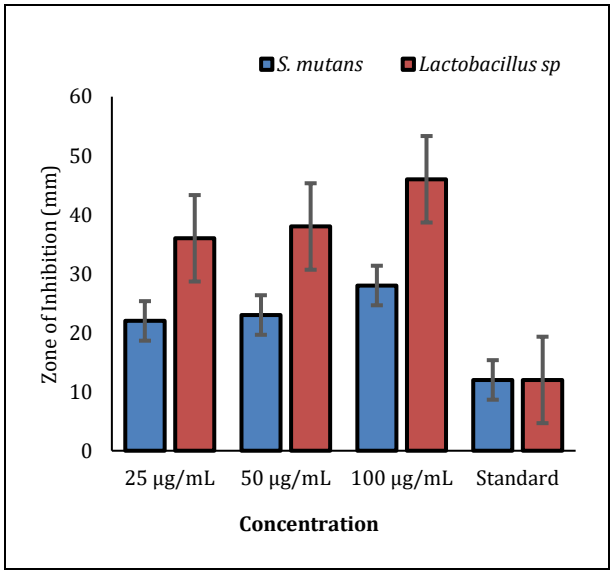

Fig. 6: Zone of inhibition of nanosilver chitosan incorporated dental varnish against *S. mutans* and *Lactobacillus spp.*

The antibacterial activity increases with concentration for both the bacteria, with *Lactobacillus spp*. showing a greater sensitivity at all concentrations. The experimental varnish demonstrated significantly higher inhibition zones compared to Amoxyrite. The varnish showed a dose-dependent antibacterial effect. This suggests the potential of the varnish as an effective antimicrobial agent against oral bacteria (Fig. 6). Table 2 illustrates the analysis of variance for the time-kill curve of *Lactobacillus spp.* across different groups. The results show a statistically significant difference between the groups ($F = 65535$, $p < 0.05$), indicating that group means vary significantly. The absence of within-group variability suggests homogeneity within each group.

Table 2. One-way ANOVA results for time-kill curve analysis of *Lactobacilli*

Source of Variation	Sum of Square	df	Mean Square	F	Significance
Between Groups	0.00165275	3	0.00055092	65535	0.00
Within Groups	0	0	65535		
Total	0.00165275	3			

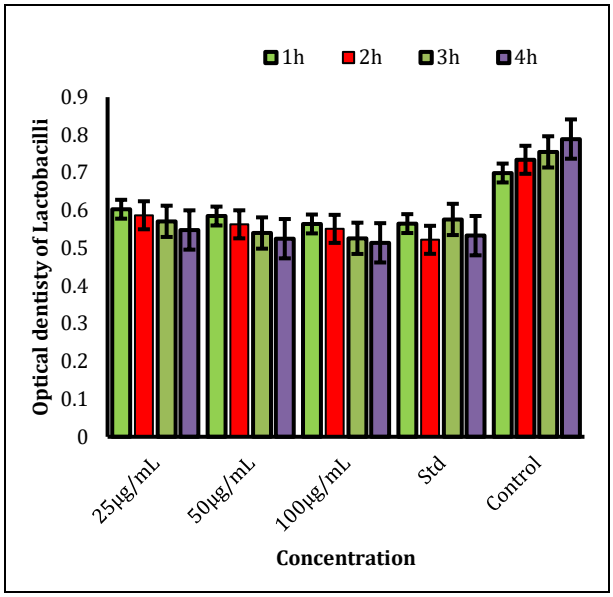

Fig. 7: The graph represents the optical density (OD) measured at different time intervals (1 h, 2 h, 3 h, and 4 h) for varying concentrations of a dental varnish (25 µg/mL, 50 µg/mL, and 100 µg/mL), a standard (Std), and a control

At 25 µg/mL, 50 µg/mL, and 100 µg/mL, the optical density values remain relatively stable over time (1 hour to 4 hour), suggesting a consistent inhibitory effect on bacterial growth. Higher concentrations (100 µg/mL) appear to show slightly reduced OD values compared to lower concentrations, indicating stronger antibacterial activity. The standard group shows OD values comparable to or slightly higher than those of the varnish-treated groups. This suggests the efficacy of the varnish is similar to the Amoxyrite. The control group shows significantly higher OD values, which increase over time, indicating uninhibited bacterial growth (Fig. 7).

The dental varnish containing orange peel and grape peel extracts exhibits dose-dependent antibacterial effects. The varnish is effective at maintaining low bacterial growth, comparable to Amoxyrite. In contrast, the control group demonstrates significant bacterial proliferation over time. This supports the potential use of the varnish as an antimicrobial agent.

Table 3. Time-kill curve analysis of *Streptococcus mutans*

Source of Variation	Sum of Square	df	Mean Square	F	Significance
Between Groups	0.0013632	3	0.0004544	0.049	0.00
Within Groups	0.1477408	16	0.0092338		
Total	0.149104	19			

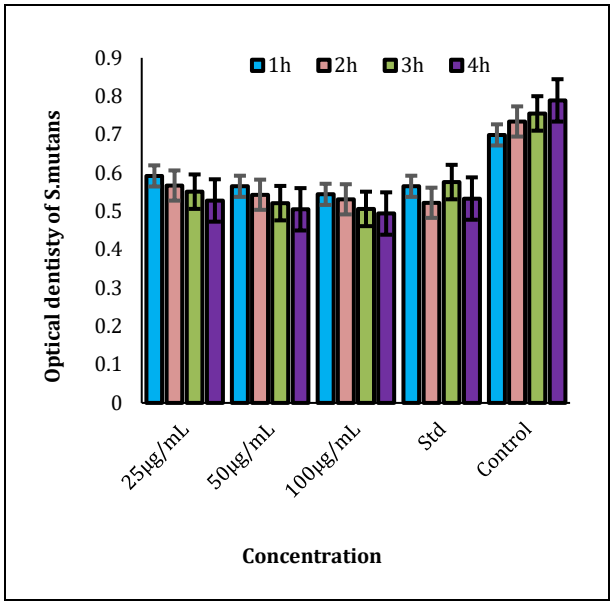

Fig. 8: Optical density readings measured at different concentrations (25 µg/mL, 50 µg/mL, 100 µg/mL), over 1 hour, 2 hours, 3 hours, and 4 hours.

The OD values for 25 µg/mL, 50 µg/mL, and 100 µg/mL remain relatively stable over time. There is no significant variation between the time points (1 hour, 2 hours, 3 hours, 4 hours) for these concentrations. The control and standard showed consistently higher OD readings compared to the test concentrations (25, 50, and 100 µg/mL). Among all groups, the control group exhibited the highest OD values across all time points. For each group (concentration, Std, and control), the OD did not alter significantly over time (from 1 hour to 4 hours) (Fig. 8). Table 3 summarizes the sources of variance (between groups and within groups) with their respective sum of squares (SS), degrees of freedom (df), mean squares (MS), F-statistic, and *P*-value. The significant *P*-value (< 0.05) indicates a statistically significant difference among the groups in the time-kill analysis.

7. DISCUSSION

The presented research findings demonstrate the antibacterial activity of orange and grape peel extract, in combination with nanosilver-chitosan dental varnish, against *S. mutans and Lactobacillus spp.*

The zone of inhibition measured in the current research for *S. mutans* and *Lactobacillus spp.* is consistent with previous research that has highlighted the antimicrobial potential of natural extracts and nanomaterials (Kishore *et al*. 2021). Our study illustrates that the zone of inhibition was higher at elevated concentrations compared to the standard, reinforcing dose-dependent effect. The time-kill assays in our study offer insights into the dynamic impact of orange and grape peel extract, as well as Amoxicillin, on microbial growth. The decrease in optical density over time, particularly in higher concentrations, suggests a pronounced inhibitory effect on both *S. mutans* and *Lactobacillus spp.* This aligns with previous research that has explored the antibacterial agents and their influence on microbial populations (Rajeshkumar *et al.* 2022).

Comparing the optical density values at various time points with the control group in this study indicates a significant reduction in microbial density, emphasizing the bactericidal potential of the tested agents. The concentration-dependent trend observed further findings from existing literature, where higher concentrations are often associated with increased antimicrobial efficacy (Maheshwaran *et al.* 2021). Similar to a previous study (Rilah *et al*.2023) on *Lactobacillus spp.*, our study results present a concentration-dependent trend.

Further studies exploring a wider range of concentrations are needed to establish optimal and safe concentrations for practical use. Future investigations should consider potential interactions with human cells, ensuring that the proposed agents do not cause unintended harm to the host tissues.

8. CONCLUSION

Dental varnish using a nanosilver-chitosan composite showcases a promising avenue for the development of advanced dental care materials. The observed antibacterial activity against *S. mutans* and *Lactobacillus spp.* highlights the potential clinical significance of this nanocomposite in preventing and managing dental caries. As research in nanotechnology and dental materials progresses, further investigations into the long-term safety, efficacy, and compatibility of the nanosilver-chitosan composite dental varnish will undoubtedly contribute to its successful integration into routine dental care practices. The development of such innovative materials underscores the continuous effort to enhance preventive strategies in oral healthcare and address the persistent challenges associated with oral bacterial infections.

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CONFLICT OF INTEREST

The authors would like to declare no conflict of interest in the present study.

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