

ANTIBACTERIAL ACTIVITY OF A NOVEL GREEN-SYNTHESIZED NANO-SILVER FLUORIDE AGAINST STREPTOCOCCUS MUTANS: A COMPARATIVE IN VITRO STUDY

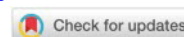
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Abstract: Background: Silver diamine fluoride is an effective non-invasive material for caries arrest, but its clinical use is limited due to the black discoloration it causes. Green synthesis of silver nanoparticles has emerged as a promising non-discoloring alternative with antimicrobial potential. Purpose: To evaluate the antibacterial activity of a novel green-synthesized nano-silver fluoride prepared with *Camellia sinensis* extract and compare its efficacy with silver diamine fluoride and silver diamine fluoride combined with potassium iodide against *Streptococcus mutans*. Methodology: Antibacterial activity was assessed using the agar well diffusion method. Three concentrations (100%, 10% and 1%) of each material were tested and inhibition zone diameters were measured. The data were analyzed using non-parametric statistical methods with a significance level of $p < 0.05$. Results: Silver diamine fluoride demonstrated the strongest antibacterial activity at all tested concentrations. The green-synthesized nano-silver fluoride exhibited concentration-dependent antimicrobial activity. No statistically significant differences were observed between the highest concentrations of nano-silver fluoride, silver diamine fluoride and silver diamine fluoride combined with potassium iodide. Potassium iodide alone showed no antibacterial effect. Conclusions: The green-synthesized nano-silver fluoride demonstrated significant concentration-dependent antibacterial activity against *Streptococcus mutans*. Although its inhibition zones were smaller than those of silver diamine fluoride, its activity at higher concentrations suggests promising potential as an alternative silver-based agent for non-operative caries management.

Keywords: silver diamine fluoride; nano-silver fluoride; green synthesis; *Streptococcus mutans*; antibacterial activity

Field: Medical Sciences and Health

1. INTRODUCTION

Silver diamine fluoride (SDF) is a well-established non-invasive material for arresting dental caries. First introduced in Japan by Nishino and Yamaga, it has regained considerable attention in recent years because of its proven clinical effectiveness. (Gao et al., 2021) Its therapeutic action is based on three principal mechanisms: remineralization, antiproteolytic and antibacterial effects. (Greenwall-Cohen et al., 2020) However, the permanent black discoloration associated with SDF remains its major clinical limitation. (Nguyen et al., 2017)

The application of potassium iodide (KI) following SDF has been proposed to reduce tooth discoloration. (Costa RO et al., 2013) Nevertheless, this effect is only temporary and the reduction of free silver ions may compromise the antibacterial efficacy of the material. (Sayed et al., 2018)

Silver nanoparticles have emerged as a promising alternative because of their high surface-area-to-volume ratio, enhanced antimicrobial activity and chemical stability. (Yin et al., 2020) Unlike SDF, materials that contain nanoparticles like nano-silver fluoride (NSF) do not cause tooth discoloration. (Liao et al., 2019)

Furthermore, green synthesis using plant extracts provides an environmentally friendly approach while avoiding toxic reducing agents. Therefore, the aim of the present study was to evaluate the antibacterial activity of a novel green-synthesized nano-silver fluoride against *Streptococcus mutans* and compare it with silver diamine fluoride and silver diamine fluoride combined with potassium iodide.

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2. MATERIALS AND METHODS

Bacterial strain

- *Streptococcus mutans* (reference strain ATCC® 35668™, Ridacom Ltd.). The strains were provided in the form of lyophilized cultures from a microbiological collection and stored at -20°C until the time of experimental work.

Culture media

- Modified LAPTg10 - broth

Composition (g/dm³): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10; Tween 80 - 1cm³/dm³; pH=6.6-6.8. The medium was sterilized for 20 min at 121°C.

- Modified LAPTg10 - agar

Composition (g/dm³): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10; Tween 80 - 1cm³/dm³; agar - 15; pH=6.6-6.8. The medium was sterilized for 20 min at 121°C.

- Brain Heart Infusion (BHI) - broth

Composition (g/dm³): Infusion from Brain Heart - 6.0; peptic digest of animal tissue (peptone a) - 6.0; Pancreatic Digest of Gelatin (Peptone G) - 14.5; NaCl - 5.0; glucose (dextrose) - 3.0; Na₂HPO₄ - 2.5. pH 7.4 ± 0.2. Sterilized for 15 min at 121°C.

Chemicals and solutions:

- Saline solution

Composition (g/dm³): NaCl - 5. Sterilized for 20min at 121°C.

- Ultrapure water - Ultrapure water - DNASE/RNASE - Free (Canvax Reagents SL)

Composition: water, deionized, reverse osmosis, ultraviolet light treatment, distillation and 0.1µm membrane filtration; pH - 5.4 - 7.0 at 25° C.

- Riva Star - capsules kit (SDI Dental Limited, Australia)

Composition: 38% silver diamine fluoride (SDF) - Ag - 253,870 ppm (25.3%), F - 44,800 ppm (4.48%), NH₃ - 8%, KI - 58.3%; pH - about 10.

- Nano-silver fluoride (NSF)

Composition: AgNO₃ - 0.01 mol/L, K₂CO₃, NaF - 0.01 g/mL, aqueous extract of *Camellia sinensis*; pH - 9.0. The NSF formulation was synthesized using a previously established green synthesis protocol. (Palankalieva et al., 2025)

Rehydration and cultivation of bacterial strain

The lyophilized *S. mutans* strain was rehydrated according to the manufacturer's instructions by adding 1 mL of sterile BHI broth to the ampoule. To achieve the required standardized cell concentration, an optimized cultivation protocol was applied. Briefly, 0.5 mL of the rehydrated suspension was transferred into 5 mL of modified LAPTg10 broth supplemented with 0.2 mL of 20% glucose. The cultures were incubated under anaerobic conditions using an anaerostat system (Anaerocult®, MERCK) at 37°C for at least 48 hours.

Preparation of bacterial inoculum

Bacterial suspensions were prepared to a concentration equivalent to 0.5 McFarland standard (approximately 1×10⁸ CFU/mL).

Determination of antimicrobial activity

The antimicrobial activity of the tested agents was evaluated using the agar well diffusion method. Petri dishes containing modified LAPTg10 agar were inoculated with the prepared bacterial suspensions. After agar solidification, wells with a diameter of 6 mm were aseptically created and evenly distributed to avoid overlapping inhibition zones.

Experimental groups and test solutions

The antimicrobial activity of the following agents was studied: silver diamine fluoride (SDF), silver diamine fluoride in combination with potassium iodide (SDF+KI), green-synthesized nano-silver fluoride with *Camellia sinensis* extract (NSF) and potassium iodide (KI). The test substances were diluted with ultrapure water in 3 decreasing concentrations - 100%, 10% and 1%. The created groups for each concentration of each compound are represented in Table 1.

Table 1. Experimental groups and concentrations of the tested silver compounds.

Group	Test agent	Concentration
Gr-NSF_100	Nano-silver fluoride (NSF)	100%
Gr-NSF_10	Nano-silver fluoride (NSF)	10%
Gr-NSF_1	Nano-silver fluoride (NSF)	1%
Gr-SDF_100	Silver diamine fluoride (SDF)	100%
Gr-SDF_10	Silver diamine fluoride (SDF)	10%
Gr-SDF_1	Silver diamine fluoride (SDF)	1%
Gr-RS_100	Silver diamine fluoride and potassium iodide (SDF+KI)	100%
Gr-RS_10	Silver diamine fluoride and potassium iodide (SDF+KI)	10%
Gr-RS_1	Silver diamine fluoride and potassium iodide (SDF+KI)	1%
Gr-KI_100	Potassium iodide (KI)	100%
Gr-KI_10	Potassium iodide (KI)	10%
Gr-KI_1	Potassium iodide (KI)	1%

Source: Authors' own research

The designation "100%" refers to the undiluted primary solution of the respective compound - 38% silver diamine fluoride and 5% nano-silver fluoride.

A volume of 0.06 mL from each test solution was introduced into the wells using a sterile micropipette. Ultrapure water was used as a negative control.

Incubation and measurement

The plates were incubated at 37°C under anaerobic conditions. Antimicrobial activity was assessed by measuring the diameter of the inhibition zones (in mm). All experiments were performed in quadruplicate.

Statistical methods

For data processing and graphical presentation, the IBM SPSS Statistics for Windows program (version 30.0, 2024) was used. The normality of the distribution of numerical variables (zones of inhibition in mm) was checked with the Shapiro-Wilk test. The variables were described with medians (Median) and interquartile ranges (IQR). For intergroup analysis Kruskal-Wallis test, followed by Mann-Whitney U test and Dunn test with Bonferroni correction were applied. All tests were performed at a significance level of $p < 0.05$.

3. RESULTS

After carrying out the agar-diffusion method for determining antibacterial activity, the zone of inhibition was observed. The measurement of its diameter in mm correlated with the antimicrobial activity of the tested solution.

The Shapiro-Wilk test for normality of distribution showed that the values of the studied groups were not normally distributed ($p < 0.05$). The Kruskal-Wallis test showed a statistically significant difference between the groups ($p < 0.05$). To determine between which groups a statistically significant difference was observed, Dunn's multiple comparisons test with Bonferroni correction was used.

Table 2 summarizes the medians (Median), interquartile ranges (IQR), as well as the results of the comparative statistical analysis.

The median values of the inhibition zones of *S. mutans* show that the highest antimicrobial activity is possessed by all concentrations of silver diamine fluoride - Gr-SDF_100 (Median - 40, IQR - 0), Gr-SDF_10 (Median - 40, IQR - 0), Gr-SDF_1 (Median - 40, IQR - 0), as well as the 100% solution of silver diamine fluoride with potassium iodide - Gr-RS_100 (Median - 40, IQR - 0). Laboratory-synthesized nano-silver fluoride demonstrated smaller diameter zones of inhibition at concentrations of 100% and 10% (Gr-NSF_100 - Median - 20, IQR - 0; Gr-NSF_10 - Median - 10, IQR - 0), but there was no statistically significant difference between these two groups and the groups of silver diamine fluoride and the 100- and 10-percent solutions of SDF+KI ($p > 0.05$). At 1% of the nano-silver fluoride solution, no bacterial inhibition was observed (Gr-NSF_1 - Median - 0, IQR - 0). All concentrations of potassium iodide also had no antibacterial effect against strains of *S. mutans* (Gr-KI_100 - Median - 0, IQR - 0; Gr-KI_10 - Median - 0, IQR - 0; Gr-KI_1 - Median - 0, IQR - 0).

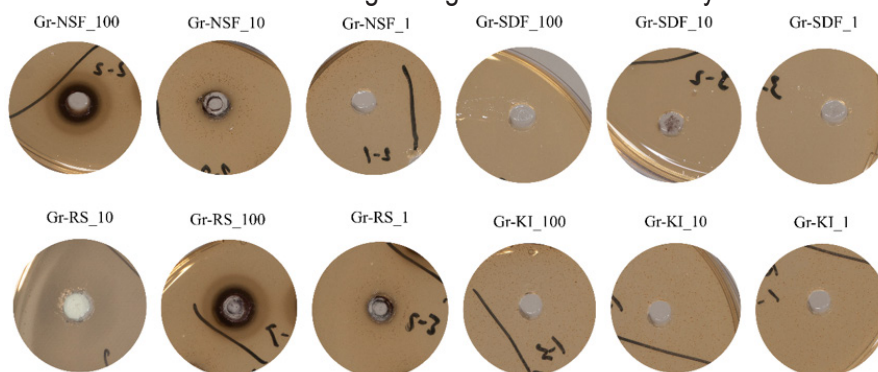
Table 2. Comparison of the inhibition zone diameters (mm) against *S. mutans* among the tested groups.

	Gr-NSF_100 (1) Median (IQR)	Gr-NSF_10 (2) Median (IQR)	Gr-NSF_1 (3) Median (IQR)	Gr-SDF_100 (4) Median (IQR)	Gr-SDF_10 (5) Median (IQR)	Gr-SDF_1 (6) Median (IQR)	Gr-RS_100 (7) Median (IQR)	Gr-RS_10 (8) Median (IQR)	Gr-RS_1 (9) Median (IQR)	Gr-KI_100 (10) Median (IQR)	Gr-KI_10 (11) Median (IQR)	Gr-KI_1 (12) Median (IQR)	Dunn's multiple comparisons test with Bonferroni correction
<i>S. mutans</i>	20 (0)	10 (0)	0 (0)	40 (0)	40 (0)	40 (0)	40 (0)	21,50 (5)	10,50 (1)	0 (0)	0 (0)	0 (0)	3↔4: $p < 0.05$ 3↔5: $p < 0.05$ 3↔6: $p < 0.05$ 4↔10: $p < 0.05$ 4↔11: $p < 0.05$ 4↔12: $p < 0.05$ 5↔10: $p < 0.05$ 5↔11: $p < 0.05$ 5↔12: $p < 0.05$ 6↔10: $p < 0.05$ 6↔11: $p < 0.05$ 6↔12: $p < 0.05$ 7↔10: $p < 0.05$ 7↔11: $p < 0.05$ 7↔12: $p < 0.05$

Source: Authors' own research

Figure 1 shows the inhibitory zones around each tested substance.

Figure 1. Representative inhibition zones produced by the tested silver compounds against *S. mutans* following the agar well diffusion assay



Source: Authors' own research

In Gr-NSF_100, a well-defined inhibitory zone with sharp and defined borders was observed. In Gr-NSF_10, such zone was also observed, but in a smaller size. This confirms the inhibitory effect of these two concentrations against *S. mutans*. In contrast, Gr-NSF_1 did not demonstrate an antibacterial effect, since there was no inhibitory halo around the well in the petri dish.

All groups of silver diamine fluoride - Gr-SDF_100, Gr-SDF_10 and Gr-SDF_1, showed wide homogeneous zones without bacterial growth, which is an indicator of the strong antibacterial effect at all concentrations of this compound.

In the commercial product Riva star, a decrease in the diameter of the inhibitory zone was observed with a decrease in the concentration of the solutions. Gr-RS_100 showed the widest halo, which decreased with Gr-RS_10 and was the smallest with Gr-RS_1. At all three concentrations, single streptococci were observed in the inhibitory halo.

At all concentrations of potassium iodide, no zone of inhibition was observed and bacterial growth was visible to the edge of all wells containing the different concentrations of this solution.

4. DISCUSSIONS

A dose-dependent antibacterial effect was observed for all tested silver compounds except SDF, with higher concentrations of SDF+KI and NSF producing larger inhibition zones. This suggests that the antimicrobial activity of SDF+KI and the laboratory-synthesized NSF is closely related to the availability of free silver ions in the solution. Silver ions are well known for their broad-spectrum antibacterial activity, while *S. mutans* is one of the primary microorganisms involved in dental caries development. (Lansdown, 2006) The consistently strong antibacterial activity of SDF observed in the present study is in agreement

with previous reports. (Wu et al., 2019)

The largest inhibition zones were produced by SDF, whereas lower concentrations of SDF+KI showed reduced antibacterial activity. Similar findings were reported by Alvarez-Marín et al. (2024), who also observed bacterial colonies within the inhibition zones of SDF+KI. This reduction is likely explained by the formation of insoluble silver iodide (AgI), which decreases the concentration of free silver ions responsible for the antibacterial effect. (Sayed et al., 2018)

Green-synthesized silver nanoparticles have attracted increasing attention because of their antimicrobial potential. Previous studies demonstrated that silver nanoparticles effectively inhibit *S. mutans* growth and biofilm formation. For example, Kumar et al. (2018) evaluated the antibacterial activity of green-synthesized silver nanoparticles prepared using *Viola serpens* leaf extract as a reducing and stabilizing agent. Using an agar diffusion assay, the authors demonstrated significant antibacterial activity against *S. mutans* and confirmed the potential of silver nanoparticles to inhibit biofilm formation.

However, to the best of our knowledge, there are no studies that have analyzed the antimicrobial effect of green-synthesized nano-silver fluoride prepared with *Camellia sinensis* (green tea) extract against *S. mutans*.

Silver nanoparticles tested alone and without the addition of other components, have been shown to successfully inhibit the growth of cariogenic bacteria. (Pérez-Díaz et al., 2015) The antimicrobial activity of fluoride is also well established. (Soleimani et al., 2021) Green tea possesses antibacterial properties against cariogenic bacteria that support its potential as an effective reducing and stabilizing agent in the synthesis of NSF. (Anita et al., 2015) In this context, the synergistic action of silver nanoparticles, fluoride and green tea extract could potentially enhance the antimicrobial efficacy and justify the application of such a combination for the prevention and stabilization of caries lesions.

According to the available literature, no comparative studies have been published comparing the antibacterial effect of SDF, SDF+KI and green-synthesized NSF against cariogenic bacteria.

The present findings are consistent with those of Karaduran et al. (2024), who reported greater antibacterial activity of SDF and SDF+KI than NSF. In contrast, Sharma et al. (2023) found NSF to be the most effective silver-based material. Such discrepancies are likely related to differences in NSF synthesis protocols, which can substantially influence the nanoparticle characteristics and biological activity. (Chahardoli et al., 2022)

The limitations of the study should be taken into consideration. Although the agar diffusion method is a standard approach for evaluating antibacterial activity, it cannot fully reproduce the complexity of oral biofilms and in vivo conditions. (Sissons CH et al., 1991) Therefore, these findings should be considered preliminary and further laboratory and clinical studies are required to confirm the antibacterial efficacy of green-synthesized nano-silver fluoride.

5. CONCLUSIONS

The novel nano-silver fluoride demonstrated promising antibacterial activity against *Streptococcus mutans* and may represent a sustainable alternative for non-operative caries management. Further in vitro and in vivo studies are needed to confirm its long-term antimicrobial performance and clinical applicability.

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