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Evaluation of the Antibiofilm Efficacy of Sodium Hypochlorite Combined with Green-Synthesized Silver Nanoparticles Against Enterococcus faecalis - An Invitro Study

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ABSTRACT

Background: *Enterococcus faecalis* biofilms contribute significantly to endodontic treatment failure. Sodium hypochlorite (NaOCl), though effective, presents cytotoxicity at higher concentrations. Green-synthesized silver nanoparticles (AgNPs) offer a promising adjunct for safer and more effective disinfection. The aim of the study is to assess and compare the antibiofilm efficacy of NaOCl alone and in combination with AgNPs synthesized using *Ficus religiosa* bark extract against *E. faecalis* biofilms.

Methods: *F. religiosa* extract was prepared and used for the green synthesis of AgNPs using 1 mM $AgNO_3$. Synthesized nanoparticles were characterized and incorporated with NaOCl. A microtiter plate assay assessed antibiofilm activity against *E. faecalis* across serial concentrations (3% to 0.09%). Biofilm inhibition was quantified using crystal violet staining and optical density at 570 nm. Statistical significance was evaluated using one-way ANOVA with Tukey's post-hoc test ($p < 0.05$).

Results: The NaOCl + AgNP formulation demonstrated significantly higher antibiofilm activity than NaOCl alone at all tested concentrations ($p < 0.05$). At 0.09%, the combination achieved 42.44% inhibition compared to 9.91% with NaOCl alone, indicating enhanced efficacy even at minimal concentrations.

Conclusion: Combining NaOCl with *F. religiosa*-mediated AgNPs markedly improves antibiofilm performance against *E. faecalis*. This biogenic nanoparticle approach offers a safer, more effective strategy for root canal disinfection and could reduce reliance on higher concentrations of NaOCl.

Keywords: *Green synthesis; Ficus religiosa; silver nanoparticles; sodium hypochlorite; Enterococcus faecalis; biofilm inhibition; root canal irrigation*

1. INTRODUCTION

Irrigation plays a crucial role in endodontic therapy, primarily by facilitating the removal of debris, necrotic tissue, and bacterial biofilm from the root canal system.[1] The efficacy of irrigation is significantly influenced by the choice of irrigant, its concentration, and the method of delivery. Sodium hypochlorite is a vital solution formed through the chemical fusion of chlorine with sodium hydroxide solution. It is a broad spectrum disinfectant. Sodium hypochlorite serves as a cornerstone irrigant in the realm of root canal treatment. [2, 3] Its remarkable efficacy lies in its capacity to dissolve pulpal tissue while concurrently acting as a potent disinfectant. By targeting and dissolving organic matter, it effectively sterilizes the canal, thereby eliminating potential pathogens and facilitating optimal endodontic outcomes. This dual action of dissolving organic content and disinfecting the canal underscores sodium hypochlorite's indispensable role in modern endodontic practice. [4–6] However, despite its versatility, caution is warranted due to its acidic nature, particularly when used at higher concentrations. [7–10] Moreover sodium hypochlorite offers limited retention and lacks controlled release capabilities. In contrast, hydrogel-based systems offer sustained drug release, high biocompatibility, and better adherence to dentin surfaces, making them promising alternatives in endodontic drug delivery.[11] Herbs and herbal medicines have traversed through millennia, offering therapeutic benefits deeply rooted in tradition and folklore. Among these, *Ficus religiosa (F. religiosa)*, commonly known as the sacred fig or Arasampattai in Tamil, occupies a revered place. [12] This botanical marvel has long been used for its ability to detoxify the blood, cleansing it of impurities. Additionally, traditional wisdom attributes to it the capacity to alleviate burns and soothe skin irritations in powdered form. [13, 14] In this study, the effects of both sodium hypochlorite and *F. Religiosa* combined with nanotechnology were explored. The burgeoning field of nanotechnology has become a focal point in various realms of dentistry worldwide. This technological advancement leverages the unique properties of materials at the nanoscale, which often exhibit enhanced physical, chemical, and biological behaviours compared to their larger counterparts. In medicine, nanotechnology is revolutionizing drug delivery, diagnostics, and tissue engineering. Nanoparticles, due to their size, can navigate biological systems with high precision, enabling targeted therapy and reducing side effects. The versatility and adaptability of nanomaterials make them suitable for various innovative treatments. [15]

Silver is an antimicrobial material and disinfectant that is relatively free of adverse effects. Silver nanoparticles exhibit superior antimicrobial activity against *Enterococcus faecalis*, particularly in biofilm form, owing to their nanoscale size and enhanced surface area that allows deeper penetration and stronger membrane interaction.[16] Silver nanoparticles (AgNPs) possess a broad spectrum of antibacterial, antifungal, and antiviral properties, making them invaluable in medical applications. These nanoparticles can penetrate bacterial cell walls, altering cell membrane structures and leading to cell death. Their efficacy stems not only from their nanoscale size but also from their large surface area to volume ratio. This attribute enhances their interaction with microbial cells, increasing membrane permeability, generating reactive oxygen species, and disrupting DNA replication through the release of silver ions. [17]

Numerous methodologies, both physical and chemical, have been explored for the synthesis of AgNps. However, many of these approaches necessitate stringent protocols involving high pressure, temperature, toxic chemicals, and specialized technical expertise. Recognizing the need for alternatives that mitigate these drawbacks, there is a growing imperative for greener, more sustainable methods. In the context of root canal disinfection, where the cytotoxicity of sodium hypochlorite is a concern, integrating nanoparticles with sodium hypochlorite presents a promising avenue. Embracing a greener approach not only addresses environmental concerns but also enhances cost-effectiveness and accessibility. Therefore, the pursuit of natural product-based synthesis of nanoparticles emerges as a timely and welcomed strategy, offering a harmonious blend of innovation and environmental stewardship in dental nanotechnology. Thus, this study aimed to compare the anti-biofilm efficacy of the crude extract of *F. religiosa*with the AgNps and sodium hypochlorite as an alternative irrigating solution encountering the chief dental pathogen *Enterococcus faecalis* (*E. faecalis)* for an effective root canal treatment.

2. MATERIALS AND METHODS

Collection and preparation of plant extract

Arasampattai bark (*F. religiosa*) was collected from the Tamil Nadu horticulture farm and subjected to a meticulous preparation process for further study. Initially, the bark was rinsed with double distilled water and shade-dried for five to ten days to preserve its phytochemical integrity, then finely powdered and weighed. For the experimental procedures, one per cent sodium hypochlorite was prepared by dissolving one gram of the compound in 100 ml of distilled water. Similarly, a one per cent plant powder solution was made by mixing the powdered bark with sterile distilled water, maintained under stirring conditions to ensure complete dissolution. This solution was then filtered using Whatman No.1 filter paper, and the extract obtained was stored at 4°C to preserve its bioactive components until further use.

Characterization of nanoparticles

To facilitate the synthesis of AgNps, a one-millimolar silver nitrate solution was prepared and protected from light with aluminium foil to prevent photolysis. The filtered plant extract was added dropwise to the silver nitrate solution under continuous stirring. This methodical addition continued until a visible colour change indicated the formation of silver nanoparticles. The specific time and concentration required for the reaction were carefully recorded. Subsequently, the reaction mixture underwent centrifugation at 6000 rpm for 10 minutes at 4°C to separate the nanoparticles. The supernatant was then retained for further analysis, ensuring the process yielded a clean and concentrated nanoparticle solution.

Biofilm assay

The experiment was carried out in two groups: Group 1 used sodium hypochlorite, while Group 2 combined sodium hypochlorite with AgNps. The Microtiter well plate method was adopted to determine the antibiofilm efficacy of the samples. Initially, a 96-well plate was prepared, and 100 microliters of Brain Heart Infusion (BHI) broth was added to all the wells. The prepared 1% sodium hypochlorite was added to the first well and serially diluted to achieve concentrations of 3%, 1.5%, 0.75%, 0.37%, 0.18%, and 0.09%. Similarly, the plant-mediated silver nanoparticles (AgNPs) solution was also serially diluted in the plate. Subsequently, 50 microliters of an overnight-grown culture of *E. faecalis* (OD600 = 0.1) were added to all the wells. The plate was maintained with media and cell controls and incubated at 37°C for 48 hours [Figure 1].

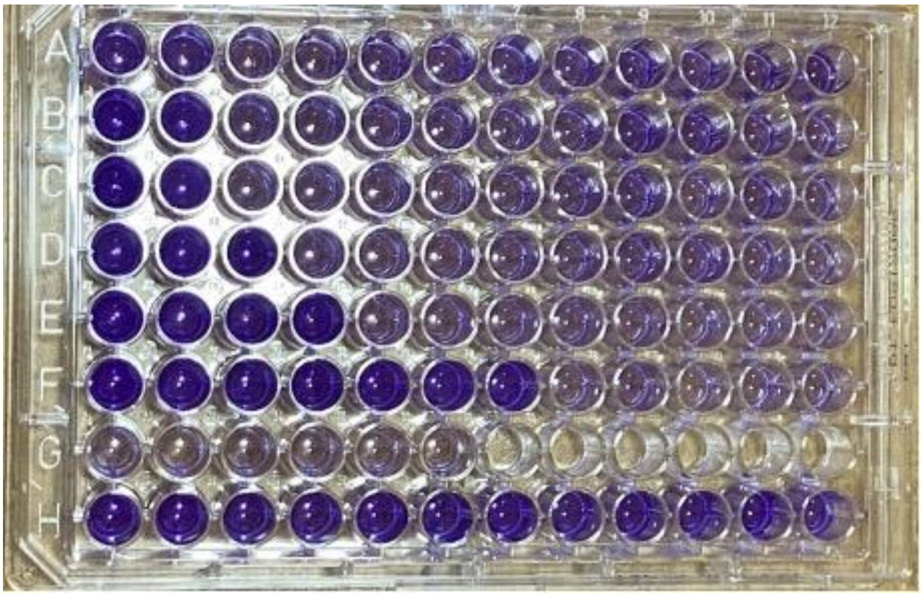

Figure 1. Represents the microtiter plate showing different intensities of 1% ,3%, 1.5%, 0.75%, 0.37%, 0.18%, and 0.09 % dye correlating with the biofilm formation / inhibition as influenced by the sodium hypochlorite and sodium hypochlorite with silver nanoparticles.

After the incubation period, the contents of the plate were emptied and washed with phosphate-buffered saline (PBS, pH 7.0). To each well, 150 µL of 0.2% crystal violet was added and left undisturbed for 20 to 30 minutes to stain the biofilm. The excess and unbound stain was removed by washing the wells again with PBS. Then, 125 µL of 30% glacial acetic acid was added to each well to dissolve the crystal violet, ensuring thorough mixing until complete dissolution of the dye. The absorbance value was measured at 570 nm using an ELISA plate reader. All experiments, including the synthesis of silver nanoparticles (AgNPs), biofilm formation, and antibiofilm efficacy testing, were carried out in triplicates. Specifically, for the antibiofilm assay, each concentration of sodium hypochlorite, sodium hypochlorite combined with AgNPs, and the control group (*E. faecalis* culture without treatment) was tested in three independent experimental setups. This allowed us to account for variability and ensure the reproducibility of the results. The data obtained from the biofilm assays were collected as optical density (OD) readings at 570 nm, corresponding to the biofilm quantification using crystal violet staining. The OD readings for each experimental condition were averaged across the three replicates to obtain mean values. These mean OD values were then used to calculate the percentage of biofilm inhibition for each concentration of sodium hypochlorite and the AgNP-mediated sodium hypochlorite solution.

Statistical analysis:

Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test to compare the antibiofilm efficacy between the different groups (sodium hypochlorite alone vs. sodium hypochlorite combined with AgNPs) across the varying concentrations. Statistical significance was set at $p < 0.05$. The results were expressed as mean ± standard deviation (SD), and error bars were included in the graphical representation of the data to reflect the variability among replicates. Additionally, the statistical analysis software SPSS (version 25.0) was used to perform the calculations.

3. RESULTS

This study demonstrated the profound impact of combining sodium hypochlorite and *F. religiosa* with silver nanoparticles in inhibiting biofilm formation compared to sodium hypochlorite treatment alone. The antibiofilm efficacy of sodium hypochlorite and sodium hypochlorite combined with AgNPs at various concentrations (3%, 1.5%, 0.75%, 0.37%, 0.18%, 0.09%) was analyzed using one-way ANOVA. This analysis was followed by Tukey's post-hoc test to determine the statistical significance of differences between the groups. Analysis of the graphical data revealed that the green synthesis of AgNps combined with sodium hypochlorite exhibited remarkable biofilm inhibition against *E. faecalis*, particularly at higher concentrations. Specifically, at a 3% concentration, the sodium hypochlorite group showed an efficacy of 64.86%, while the experimental group with the combined treatment exhibited an increased efficacy of 72.06%. This notable difference highlights the enhanced antibiofilm capabilities of the combined treatment [Table 1].

Table 1. Percentage of biofilm inhibition when sodium hypochlorite and silver nanoparticles mediated sodium hypochlorite came in contact with *E. faecalis*.

Sodium Hypochlorite	Sodium hypochlorite + silver nanoparticles	Concentration %
64.86	72.03	3
59.16	66.97	1.5
54.95	62.46	0.75
40.84	60.06	0.37
29.73	57.36	0.18
9.91	41.44	0.09

When comparing both groups at a minimal concentration of 0.75%, the combined treatment still achieved an impressive biofilm inhibition efficacy of 62.46%, closely rivalling the efficacy of sodium hypochlorite used at a higher concentration of 3%. These findings align with Mukundan et al. (2024)[18], who reported that 1% sodium hypochlorite was as effective as 3% in reducing microbial load in primary root canals, highlighting the clinical feasibility of reduced concentrations for safer disinfection. These findings were visually corroborated by the comprehensive analysis presented, which clearly illustrates the superior performance of the sodium hypochlorite and AgNps combination [Figure 2].

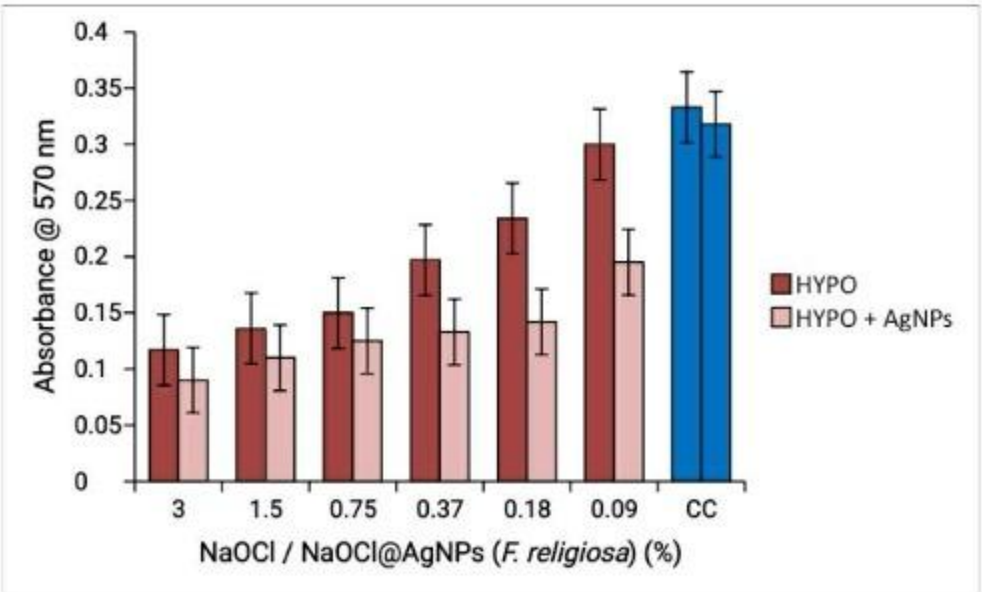

Figure 2. Biofilm inhibition as measured by absorbance at 570 nm in the presence of sodium hypochlorite (HYPO) and sodium hypochlorite combined with AgNPs (NaOCl@AgNPs) at different concentrations (3%, 1.5%, 0.75%, 0.37%, 0.18%, 0.09%). Error bars represent the standard deviation (SD) of three independent experiments. The blue bars represent the control group (CC).

The data suggests that incorporating AgNps synthesized from *F. religiosa* significantly enhances the antimicrobial and antibiofilm efficacy of sodium hypochlorite, offering a potentially more effective solution for root canal disinfection. This study underscores the potential benefits of nanotechnology in improving traditional antimicrobial treatments. The statistical analysis revealed a significant difference ($p < 0.05$) in biofilm inhibition between sodium hypochlorite alone and sodium hypochlorite combined with AgNPs at all tested concentrations. The combined treatment consistently demonstrated higher antibiofilm efficacy across the concentrations tested, with the most notable differences observed at lower concentrations (e.g., 0.75%, 0.09%). It underscores the superior performance of naturally mediated AgNps integrated with sodium hypochlorite. This approach not only enhances efficacy but also offers a viable alternative by reducing concentration requirements and mitigating adverse effects, thus holding significant promise for improving root canal disinfection protocols. In a similar context, Sharma et al. (2025) observed that turmeric-synthesized copper nanoparticles exerted selective cytotoxic effects on oral cancer cells while preserving the viability of normal cells, suggesting their biosafety.

4. DISCUSSION

Sodium hypochlorite stands out as the predominant irrigation solution in endodontic procedures. Clinically recommended at a concentration of 3%, Sodium hypochlorite offers a delicate balance between efficacy and safety, mitigating potential drawbacks associated with higher concentrations. [13] Renowned for its potent antimicrobial properties and remarkable tissue solubility, Sodium hypochlorite effectively dissolves both organic and inorganic components within the root canal system. [14] This dual capability makes it indispensable for achieving a clean and disinfected canal, crucial for successful endodontic therapy. However, the aggressive interaction of Sodium hypochlorite with dentin poses challenges, as it can lead to excessive dissolution and resorption of dentinal tissue, potentially impacting the structural integrity of the tooth.

Despite its extensive use and benefits, Sodium hypochlorite has certain limitations, including its inability to eradicate the smear layer and lack of substantivity, which means it does not continue to exert antimicrobial effects after application. These shortcomings can compromise the thorough disinfection of the root canal system, leaving residual bacteria that may cause reinfection. As a cornerstone of endodontic therapy, Sodium hypochlorite's limitations drive ongoing research efforts to enhance its properties and develop complementary solutions that can address these gaps. Innovations such as combining Sodium hypochlorite with other agents, like AgNps, aim to optimize its antimicrobial efficacy and ensure more comprehensive root canal disinfection, ultimately improving clinical outcomes and patient care.

Furthermore, the consequences of sodium hypochlorite accidents extend beyond immediate discomfort. In addition to severe pain, irritation, rapid tissue swelling, and bleeding from the periapical area, tissue necrosis can ensue if the solution extravagates beyond the apex. Sodium hypochlorite, while highly effective as an endodontic irrigant, poses inherent risks due to its cytotoxic nature. Accidental exposure of periapical tissues to undiluted or improperly administered sodium hypochlorite can lead to serious complications. [19–21] These complications underscore the importance of implementing safer and more effective alternatives, such as incorporating AgNps derived from herbal formulations, to minimize the risks associated with root canal irrigation and improve patient safety during endodontic procedures. Recent developments in bioinspired nanocomposites, particularly those integrating graphene oxide, AgNPs, and phytochemicals like allicin, have demonstrated synergistic antimicrobial effects, reduced cytotoxicity, and structural preservation of dentin by mimicking natural immune defense mechanisms. [22] The rising popularity of herbal formulations reflects a growing appreciation for their diverse medicinal properties. In a study conducted by Farasat *et al*. it was observed that nanosilver (NS) solution exhibits notable efficacy as an endodontic irrigation solution for primary teeth infected with *E. faecalis*. This finding highlights the potential of nanosilver as a promising alternative for addressing microbial challenges in endodontic treatments, underscoring the ongoing exploration of novel therapeutic options. In a similar study Wu et al, observed similar effects of silver nanoparticles with 0.02 % on *E. Faecalis.* [23] Additionally, the adverse effects of sodium hypochlorite are mitigated by the use of herbal formulations, which serve to minimize its concentration and enhance its therapeutic efficacy. Singh et al. (2025)[24] showed that silver nanoparticles synthesized from neem and turmeric extracts exhibited potent antibacterial activity against *E. faecalis* and *S. mutans*, supporting their relevance in endodontic disinfection. The *F. Religiosa* was selected for the study due to its vivid pharmacological applications. The *F. Religiosa* bark is known to inhibit the burning sensation, irritability and provides better antibacterial activity. Meghal et al. (2024)[25] additionally reported that *Ficus religiosa* bark was effective in synthesizing carbon quantum dots, demonstrating notable fluorescence and biosensing capabilities, which highlight the broader functional potential of botanically derived nanomaterials. In a study by Sharma et al, the *F. religiosa* showed increased inhibiting activity against the primary periodontal pathogens and plaque colonizers.[26] Also, in the other study by Baliyan *et al.* it was suggested that flavonoids and multiplepolyphenols present in *F. Religiosa* are responsible for antioxidant and free-radical scavenging activity.[27] In inflammatory conditions the number of free radicals is increased therefore it can be suggested that theantioxidant property of *F. Religiosa* may aid in neutralizing the free radicals and curing the disease. In Qing Y *et al.* study, they have investigated the potential of silver nanoparticles as an antibacterial agent and theirantibiofilm effect. The study showed the 99% bactericidal effects of *S. mutans* and *S. aureus* in the oral cavity. [28] In a similar study by Wu et al, AgNP solution resulted in 79.6% antibacterial efficiency on *E. faecalis* compared with the control group and sodium hypochlorite groups.[23] Thus, the combination of sodiumhypochlorite and *F. Religiosa* adds an advantage to the formulation by leveraging the synergistic effects of both components.

The AgNps extracted from the herbal formulation exhibits superior efficacy in irrigation compared to sodium hypochlorite, primarily by reducing side effects. Recent in-silico investigations have demonstrated that imperatorin, a bioactive compound isolated from Aeglemarmelos, exhibits strong binding affinity toward the VanA protein of E. faecalis, indicating its potential to target vancomycin-resistant strains.[29] This supports the growing paradigm of integrating phytochemicals into antimicrobial strategies, complementing our findings on the synergistic efficacy of green-synthesized AgNPs with sodium hypochlorite. This innovative approach can help prevent the accidents and adverse reactions often associated with sodium hypochlorite in clinical settings. By identifying the synergistic effect of green synthesis when incorporated into sodium hypochlorite, this study suggests a more effective and safer alternative for root canal irrigation. The integration of AgNps enhances antimicrobial activity and biofilm disruption while potentially minimizing the cytotoxicity and tissue damage linked to traditional sodium hypochlorite use. Furthermore, the potential of *F. Religiosa* to contribute active ingredients that enhance the efficacy of irrigation solutions warrants further exploration. Detailed investigations into the bioactive compounds present in *F. Religiosa* could lead to the development of more refined and potent formulations. Future clinical studies are essential to validate the clinical utility of this combined treatment, ensuring its safety, effectiveness, and practicality in endodontic procedures. These studies will help confirm the therapeutic benefits and pave the way for the adoption of this innovative irrigant in routine dental practice, potentially revolutionizing root canal disinfection methods.

5. CONCLUSIONS

Within the limitations of the study, it can be concluded that the experimental group, comprising sodium hypochlorite combined with AgNps prepared using the crude extract of F. religiosa, demonstrated superior antimicrobial properties. This combination was more effective in disrupting biofilms compared to the conventional 3% sodium hypochlorite used as a root canal irrigant. The enhanced efficacy is likely attributed to the synergistic effect of AgNps and the bioactive compounds in *F. Religiosa* extract. These nanoparticles exhibit a high surface area to volume ratio, facilitating better interaction with microbial cells. The crude extract possibly contributed additional antimicrobial properties. The study suggests that this novel combination could significantly improve endodontic disinfection protocols. However, further research is necessary to fully understand the mechanisms involved and to evaluate the long-term outcomes.

Additionally, the safety and biocompatibility of these new irrigants need thorough investigation before clinical application.

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COMPETING INTEREST

None

DECLARATION OF INTEREST

The authors claim to have no competing financial interests.

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