RESEARCH PAPER

Comparative Evaluation of Echinacea Angustifolia, Aloe Vera, And Chlorhexidine Incorporated Silver Nanoparticle against Enterococcus Faecalis Biofilm - An In-Vitro Study

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ABSTRACT

Purpose: Persistent Enterococcus faecalis biofilms remain one of the major causes of endodontic treatment failure. Nanoparticle-mediated drug delivery systems have emerged as promising approaches to improve antimicrobial efficacy and biofilm penetration. This study evaluated the antibiofilm efficacy of chlorhexidine-incorporated silver nanoparticles (CHX-AgNPs), Aloe vera, Echinacea angustifolia, and sodium hypochlorite against E. faecalis biofilm.

Methods: Forty-five extracted single-rooted mandibular premolars were included. Following biomechanical preparation, smear layer removal, sterilization, and inoculation with Enterococcus faecalis ATCC 51299, biofilm formation was established. Five specimens were initially evaluated spectrophotometrically to confirm biofilm formation. The remaining specimens were randomly divided into five groups (n = 8): Group I – 3% Echinacea angustifolia, Group II – 12.5% Aloe vera, Group III – 1% chlorhexidine-incorporated silver nanoparticles, Group IV – 3% sodium hypochlorite, and Group V – 0.9% saline. Following irrigation, dentinal shavings were collected and analyzed using nanospectrophotometry. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test.

Results: Statistically significant differences were observed among groups ($F = 46.6626$; $p < 0.0001$). Sodium hypochlorite demonstrated the lowest absorbance values indicating maximum antibacterial efficacy. CHX-AgNPs and Aloe vera exhibited antibacterial activity comparable to sodium hypochlorite. Echinacea angustifolia demonstrated moderate efficacy, whereas saline showed the least antibacterial effect.

Conclusion: Both 1% chlorhexidine-incorporated silver nanoparticles and 12.5% Aloe vera demonstrated significant antibiofilm activity against *Enterococcus faecalis*, with efficacy comparable to 3% sodium hypochlorite. These findings suggest their potential as effective adjunctive root canal irrigants and support the use of nanoparticle-mediated drug delivery systems for enhanced endodontic disinfection.

Keywords: *Drug delivery, silver nanoparticles, chlorhexidine, Enterococcus faecalis, biofilm, endodontics*
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INTRODUCTION

Successful endodontic treatment depends on complete elimination of microorganisms from the root canal system and prevention of reinfection[1,2]. However, the anatomical complexity of root canal systems and the presence of resistant microbial biofilms often compromise treatment outcomes[1,2].

Enterococcus faecalis is one of the most frequently isolated microorganisms from failed root canal treatments. Its ability to penetrate dentinal tubules, survive prolonged periods of nutrient deprivation, tolerate alkaline environments, and form resilient biofilms contributes significantly to endodontic treatment failure[3,5].

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Sodium hypochlorite remains the gold standard irrigating solution because of its broad-spectrum antimicrobial activity and tissue-dissolving properties. Nevertheless, concerns regarding cytotoxicity, unpleasant taste, and accidental extrusion have prompted investigations into alternative antimicrobial agents[6].

Natural products such as Aloe vera and Echinacea angustifolia have demonstrated antimicrobial and anti-inflammatory properties. Simultaneously, nanotechnology has emerged as a promising approach for improving antimicrobial delivery within the root canal system[7,10].

Echinacea angustifolia is a medicinal herb known for its antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties. The antibacterial activity of Echinacea is attributed to bioactive constituents such as alkamides, caffeic acid derivatives, echinacoside, polysaccharides, and flavonoids, which have been reported to inhibit microbial growth and biofilm formation[9,10].

Silver nanoparticles possess unique physicochemical characteristics including increased surface area, enhanced penetration capability, and potent antimicrobial activity[11,13]. Incorporation of chlorhexidine into silver nanoparticles may provide synergistic antibacterial action while functioning as a nanoparticle-mediated drug delivery platform capable of improving biofilm disruption[14,16].

Therefore, the present study aimed to evaluate and compare the antimicrobial efficacy of Echinacea angustifolia, Aloe vera, chlorhexidine-incorporated silver nanoparticles, and sodium hypochlorite against Enterococcus faecalis biofilm.

MATERIALS AND METHODS

Study Design

This in vitro experimental study was conducted on extracted human mandibular premolar teeth.

Sample Preparation

Forty-five extracted single-rooted mandibular premolars were selected. Teeth exhibiting fractures, resorption, calcification, or previous endodontic treatment were excluded.

The crowns were removed to standardize root length**(Fig.1).** Working length was established and biomechanical preparation was performed using ProTaper Gold instruments up to size S1. Irrigation during instrumentation was performed using 3% sodium hypochlorite.

The smear layer was removed using 17% EDTA for one minute followed by saline irrigation. Specimens were sterilized by autoclaving at 121°C under 15 psi pressure for 15–20 minutes.

Fig.1:Decoronation of tooth

Preparation of Chlorhexidine-Incorporated Silver Nanoparticles

Fifty milliliters of 1 mM aqueous silver nitrate solution was prepared. Ten milliliters of freshly prepared 2 mM sodium borohydride solution was added under continuous stirring. Trisodium citrate was incorporated as a stabilizing agent.

Subsequently, 2% chlorhexidine gluconate solution was added and the reaction mixture was stirred for an additional 30 minutes **(fig:2a-c)** . Formation of silver nanoparticles was confirmed using ultraviolet-visible spectroscopy, which demonstrated a characteristic peak between 300 and 400 nm.

Fig 2a-c Preparation of Chlorhexidine-Incorporated Silver Nanoparticles (a)chlorhexidine solution (b) silver nanoparticle (c)magnetic stirring

Preparation of 3% Echinacea angustifolia Irrigant
A commercially available Echinacea angustifolia mother tincture was obtained from a licensed homeopathic pharmacy. The mother tincture was diluted with sterile distilled water to obtain a final concentration of 3%**.** The prepared solution was filtered through a sterile 0.22 µm Millipore membrane filter to ensure sterility and stored in sterile amber-colored containers until use.

Preparation of 12.5% Aloe vera Irrigant
Fresh Aloe vera leaves were collected and thoroughly washed with distilled water. The outer rind was removed aseptically, and the inner pulp was extracted. The pulp was disinfected using 70% ethyl alcohol and strained through double-layered filter paper to remove fibrous material and debris. The filtrate was centrifuged at 8,000 rpm for 40 minutes, and the supernatant was collected. To prepare a 12.5% Aloe vera irrigant solution, 12.5 mL of the pure Aloe vera extract was diluted with 87.5 mL of sterile distilled water. The final solution was passed through a 0.22 µm Millipore filter to ensure sterility and stored in sterile containers until use.

Microbial Culture and Biofilm Formation
Enterococcus faecalis ATCC 51299 was cultured (fig:3a)in Brain Heart Infusion broth (fig:3b) and inoculated into prepared root canals. Biofilm formation was established under controlled laboratory conditions. Five specimens were analyzed spectrophotometrically to confirm biofilm development.

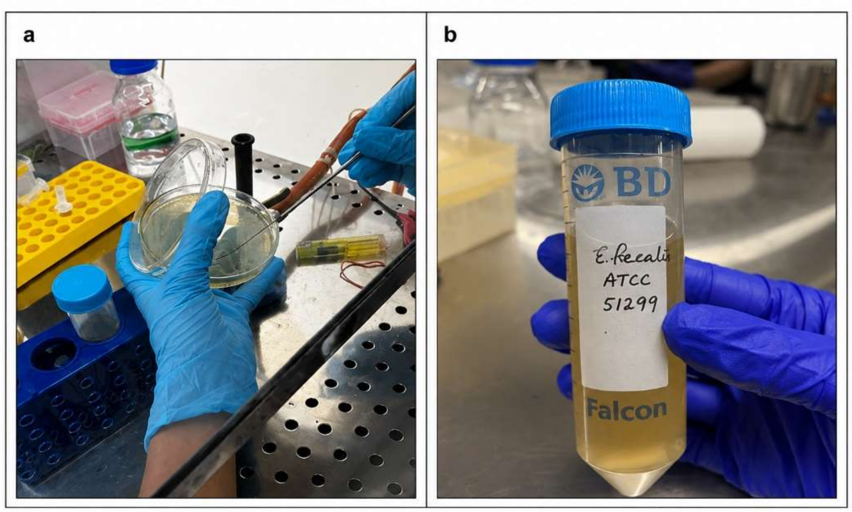


Fig. 3 (a,b): (a) *E. faecalis* ATCC 51299. (b) BHI broth incubation.

Experimental Groups

Group I: Irrigation done using 3% Echinacea angustifolia (n = 8)

Group II: Irrigation done using 12.5% Aloe vera (n = 8)

Group III: Irrigation done using 1% chlorhexidine-incorporated silver nanoparticles (n = 8)

Group IV: Irrigation done using 3% sodium hypochlorite (n = 8)

Group V: Irrigation done using 0.9% sterile saline (n = 8)

Each root canal was irrigated with 5 mL of the assigned irrigant using a 30-gauge side-vented irrigation needle positioned 1 mm short of the working length (fig.4)over a period of 5 minutes.

Irrigation was performed after each file change upto F2 using the assigned irrigant .

Fig.4: Irrigation done using standard protocol

Antimicrobial Assessment
Following irrigation, dentinal shavings were collected using H file and analyzed using a nanospectrophotometer. Absorbance values were recorded as indicators of residual bacterial growth.

Statistical Analysis

Data analysis was performed using SPSS version 26.0. One-way ANOVA was used to compare mean absorbance values among groups, followed by Tukey's post hoc test. Statistical significance was established at $p < 0.05$.

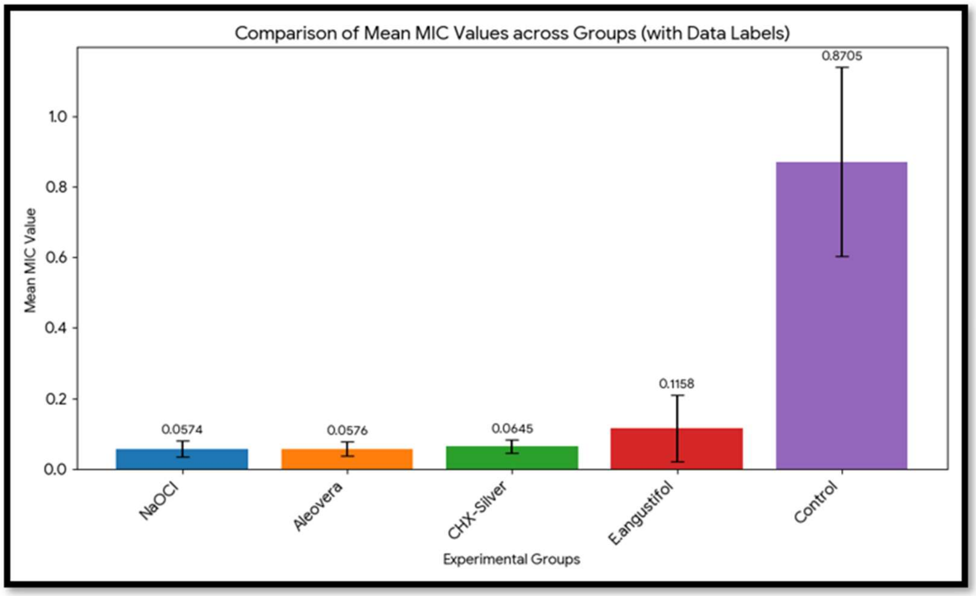
RESULTS

Comparative MIC between groups

Group	Mean ± SD	F-value	p-value
Control	0.8705±0.2677	46.6626	<0.0001*
NaOCl	0.0574±0.0231		
E. angustifolia	0.1158±0.0950		
CHX-Silver	0.0645±0.0189		
Aloe vera	0.0576±0.0208		

Table 1; presents the comparative antimicrobial efficacy of the tested irrigants.

One-way ANOVA demonstrated a highly significant difference among groups ($F = 46.6626$, $p < 0.0001$).



Comparison	Difference in Means	Adjusted p-value	Significance
Control vs. NaOCl	-0.8131	<0.0001*	Significant
Control vs. E. angustifol	-0.7547	<0.0001*	Significant
Control vs. CHX-Silver	-0.806	<0.0001*	Significant
Control vs. Aleovera	-0.8129	<0.0001*	Significant

Table:2 Posthoc table (MIC)

The lowest absorbance values were observed in the sodium hypochlorite group, followed closely by CHX-AgNPs and Aloe vera(Table:2).

Saline demonstrated the highest absorbance values, indicating maximum bacterial survival.

DISCUSSION

The present study evaluated the antimicrobial efficacy of herbal irrigants and a nanoparticle-mediated drug delivery system against Enterococcus faecalis biofilm.

Sodium hypochlorite demonstrated the highest antibacterial efficacy. This finding is consistent with previous reports attributing its antimicrobial effect to irreversible oxidation of sulfhydryl groups of bacterial enzymes, leading to disruption of cellular metabolism[6].

Aloe vera demonstrated antimicrobial efficacy comparable to sodium hypochlorite. Its antibacterial activity may be attributed to bioactive compounds including anthraquinones, phenolic compounds, and saponins that inhibit microbial growth.

The most significant finding of the present study was the substantial antibacterial efficacy exhibited by chlorhexidine-incorporated silver nanoparticles. Similar observations have been reported previously, where chlorhexidine- incorporated silver nanoparticles demonstrated synergistic antibacterial activity against Enterococcus faecalis biofilm.

The enhanced efficacy of CHX-AgNPs may be explained by multiple mechanisms. Chlorhexidine disrupts bacterial cell membranes and increases permeability, facilitating intracellular penetration of silver ions[11,16]. Silver nanoparticles subsequently interfere with cellular proteins, enzymes, and nucleic acids, resulting in bacterial death. Additionally, nanoparticle dimensions facilitate improved penetration into biofilm matrices and dentinal tubules.

From a translational perspective, nanoparticle-mediated delivery systems may provide sustained antimicrobial activity, enhanced biofilm penetration, and improved drug localization within the root canal system. These characteristics support the growing interest in nanotechnology-based endodontic disinfection strategies.

Echinacea angustifolia demonstrated moderate antibacterial efficacy. Its antimicrobial activity has been attributed to phytochemical constituents including alkamides and caffeic acid derivatives[9,10]. However, its efficacy was inferior to sodium hypochlorite and CHX-AgNPs in the present investigation.

The limitations of this study include its in vitro design and lack of advanced nanoparticle characterization, including particle-size distribution, zeta potential analysis, drug-loading efficiency, and release kinetics. Further in vivo investigations are required to validate the clinical applicability of CHX-AgNPs.

CONCLUSION

Within the limitations of this in vitro study, 3% sodium hypochlorite demonstrated the highest antibiofilm efficacy against Enterococcus faecalis.

One percent chlorhexidine-incorporated silver nanoparticles and 12.5% Aloe vera exhibited antibacterial activity comparable to sodium hypochlorite, while 3% Echinacea angustifolia demonstrated moderate efficacy.

The findings suggest that chlorhexidine-incorporated silver nanoparticles and Aloe vera may serve as promising alternative or adjunctive root canal irrigants. Furthermore, the results highlight the potential of nanoparticle-mediated drug delivery systems to enhance endodontic disinfection and improve the management of persistent endodontic infections. However, further in vivo studies and long-term clinical trials are required to validate their safety, efficacy, and clinical applicability.

REFERENCE

1. Berman LH, Hargreaves KM, editors. **Cohen's Pathways of the Pulp.** 12th ed. St Louis: Elsevier; 2021.
2. Ingle JI, Bakland LK, Baumgartner JC. **Ingle's Endodontics.** 7th ed. Hamilton: BC Decker; 2019.
3. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. *J Endod.* 2006;32(2):93-98.
4. Love RM. Enterococcus faecalis—a mechanism for its role in endodontic failure. *Int Endod J.* 2001;34:399-405.
5. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. *J Endod.* 2008;34:1291-1301.
6. Zehnder M. Root canal irrigants. *J Endod.* 2006;32:389-398.
7. Karkare SR, Ahire NP, Khedkar SU. Comparative evaluation of antimicrobial activity of hydroalcoholic extract of Aloe vera, garlic, and 5% sodium hypochlorite as root canal irrigants against *Enterococcus faecalis*: An in vitro study. *J Indian Soc Pedod Prev Dent.* 2015;33(4):274-278.
8. Babadi F, et al. Comparison of the antibacterial effect of sodium hypochlorite and Aloe vera solutions as root canal irrigants in human extracted teeth contaminated with *Enterococcus faecalis*. *J Dent (Shiraz).* 2014.
9. Barnes J, Anderson LA, Gibbons S, Phillipson JD. Echinacea species (*Echinacea angustifolia*, *E. purpurea*, *E. pallida*): A review of their chemistry, pharmacology and clinical properties. *J Pharm Pharmacol.* 2005;57:929-954.
10. Hudson JB. Applications of the phytomedicine Echinacea. *Phytomedicine.* 2012.
11. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv.* 2009;27:76-83.
12. Rodrigues CT, de Andrade FB, de Vasconcelos LRSM, et al. Antibacterial properties of silver nanoparticles as a root canal irrigant against *Enterococcus faecalis* biofilm and infected dentinal tubules. *Int Endod J.* 2018;51:901-911.
13. Morones JR, Elechiguerra JL, Camacho A, et al. The bactericidal effect of silver nanoparticles. *Nanotechnology.* 2005;16:2346-2353.
14. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. *Int Endod J.* 2009;42:288-302.
15. Al-Madi EM, et al. Effect of endodontic irrigants and medicaments mixed with silver nanoparticles against biofilm formation of *Enterococcus faecalis*. *Iran Endod J.* 2023.
16. Srisomboon T, et al. Synergistic bactericidal activity of chlorhexidine loaded on positively charged ionic liquid-protected silver nanoparticles as a root canal disinfectant against *Enterococcus faecalis*: An ex vivo study. *Journal of Ionic Liquids.* 2024.