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Antibacterial efficacy: Sodium hypochlorite, chlorhexidine and herbal irrigants against *Enterococcus faecalis*

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Abstract:
Persistent *Enterococcus faecalis* biofilm contributes to post-treatment endodontic disease and remains a useful model for comparing root-canal irrigants. This *in vitro* study inoculated 150 extracted single-rooted human premolars with *E. faecalis* for 21 days and randomized them to 3% sodium hypochlorite, 2% chlorhexidine, 10% Triphala, 10% neem, or saline (n=30 per group). Post-irrigation bacterial reduction was assessed by colony counts, confocal viable-cell percentage and residual dentinal-tubule penetration. Mean post-irrigation log10 CFU/mL was 1.18 ± 0.42, 1.74 ± 0.51, 2.33 ± 0.58, 2.56 ± 0.61 and 5.71 ± 0.45, respectively, with corresponding viable-cell percentages of 6.8 ± 3.1%, 11.9 ± 4.7%, 18.6 ± 6.2%, 22.4 ± 7.0% and 72.5 ± 8.4% ($p<0.001$). Thus, sodium hypochlorite produced high antibacterial effect, followed by chlorhexidine, while Triphala and neem significantly reduced *E. faecalis* compared with saline but remained less effective than conventional irrigants.

Keywords: *Enterococcus faecalis*, sodium hypochlorite, chlorhexidine, Triphala, neem, herbal irrigant, root canal disinfection

Background:
Microbial persistence within the root canal system is a principal biological cause of post-treatment apical periodontitis. Mechanical preparation reduces the intracanal microbial burden, but fins, isthmuses, lateral canals and dentinal tubules remain inaccessible to instruments and require chemical disinfection **[1, 2]**. *Enterococcus faecalis* is frequently used in endodontic disinfection research because it tolerates nutrient deprivation, penetrates dentinal tubules, forms organized biofilm and can survive alkaline stress **[3, 4]**. Although its role in every failed case should not be overstated, its resistance profile makes it a useful challenge organism for comparing irrigants. Sodium hypochlorite remains the principal endodontic irrigant because it combines broad antimicrobial activity with organic tissue dissolution. Its effectiveness depends on concentration, exposure time, temperature, irrigant exchange, biofilm maturity and the buffering effect of dentin. Cytotoxicity and accidental extrusion, however, require careful clinical handling **[5-7]**. Chlorhexidine provides broad antimicrobial activity and dentin substantivity, but it cannot dissolve necrotic tissue. It is therefore generally considered an alternative or supplementary irrigant rather than a complete replacement for sodium hypochlorite. Direct mixing with sodium hypochlorite should be avoided because an undesirable precipitate can form **[8, 9]**. Interest in herbal irrigants has increased because plant-derived compounds may provide antimicrobial and antioxidant actions with favorable biocompatibility. Triphala contains *Terminalia chebula*, *Terminalia bellirica* and *Emblica officinalis*, whereas neem contains limonoids, flavonoids, tannins and other bioactive constituents. Both have showed activity against endodontic organisms in laboratory studies **[10-12]**. Laboratory comparisons often use agar diffusion, but diffusion through agar may not reflect irrigant behavior in dentin or mature biofilm. A dentin-block or whole-root model combined with culture and confocal microscopy provides a more clinically relevant assessment because it measures both recoverable bacteria and spatial viability. The present study compared conventional and herbal irrigants against mature *E. faecalis* biofilm in standardized extracted teeth **[13, 14]**. Therefore, it is of interest to compare the sodium hypochlorite, chlorhexidine, Triphala, neem and saline for reducing mature *E. faecalis* biofilm under standardized root-canal conditions.

Materials and Methods:
Study design and specimen selection:
This controlled *in vitro* study used 150 freshly extracted human mandibular premolars with a single straight canal and mature apex. Teeth with resorption, cracks, previous endodontic treatment, calcification, or more than one canal were excluded.

Standardization:

Crowns were removed to obtain roots 15 mm in length. Working length was established 1 mm short of the apical foramen and canals were prepared to size 40/0.04 using rotary instrumentation. During preparation, canals were irrigated with saline to prevent exposure to the test solutions. Smear layer was removed using 17% EDTA for one minute followed by sterile saline. Apical foramina were sealed with light-cured resin and external root surfaces were coated with nail varnish.

Sterilization and inoculation:
Specimens were autoclaved and randomly selected sterility controls were cultured. A reference strain of *E. faecalis* was adjusted to 0.5 McFarland turbidity and inoculated into each canal. Roots were incubated at 37°C for 21 days, with fresh broth replacement every 48 hours. Biofilm maturation was confirmed in five additional pilot roots by scanning electron microscopy.

Group allocation:
Specimens were randomized into five equal groups (n=30): Group A, 3% sodium hypochlorite; Group B, 2% chlorhexidine gluconate; Group C, 10% Triphala aqueous extract; Group D, 10% neem leaf extract; and Group E, sterile saline. Herbal extracts were filtered, standardized by dry-weight concentration, freshly prepared and tested for sterility before use.

Irrigation protocol:
Each canal received 5 mL of the assigned irrigant delivered through a 30-gauge side-vented needle positioned 1 mm short of working length over 60 seconds. The irrigant remained in the canal for an additional 4 minutes and was manually agitated with a sterile gutta-percha cone. Canals were then flushed with sodium thiosulfate for sodium hypochlorite, neutralizing broth for chlorhexidine, or sterile saline for herbal and control groups.

Microbiological sampling:
Baseline samples were collected with three sterile paper points before irrigation. Post-treatment samples were collected immediately after neutralization. Serial dilutions were plated on brain-heart infusion agar, incubated for 48 hours and expressed as log10 CFU/mL. Laboratory investigators were blinded to group codes.

Confocal analysis:
Ten roots from each group were split longitudinally, stained with LIVE/DEAD BacLight and examined by confocal laser scanning microscopy at coronal, middle and apical thirds. Viable-cell percentage and maximum depth of viable bacterial penetration were measured using standardized image-analysis settings.

Statistical analysis:
Normality was evaluated using the Shapiro-Wilk test. Baseline comparability and post-treatment outcomes were analyzed using one-way ANOVA with Tukey post-hoc testing. Within-group CFU changes were assessed using paired tests. Repeated-measures analysis compared canal thirds. Statistical significance was set at $p<0.05$.

Results:
Baseline bacterial counts were comparable among all groups ($p=0.912$), with mean values ranging from 6.38 to 6.46 log10 CFU/mL. No specimen was excluded after randomization. All active irrigants significantly reduced bacterial counts compared with baseline ($p<0.001$). Sodium hypochlorite showed the greatest reduction, followed by chlorhexidine, Triphala and neem. Saline produced only a limited flushing effect. Tukey analysis demonstrated significant differences between sodium hypochlorite and every other group ($p\leq0.006$). Chlorhexidine was significantly more effective than both herbal irrigants ($p<0.01$). Triphala showed a lower mean residual count than neem, although the difference was modest ($p=0.048$). Confocal findings followed the culture results. The apical third retained more viable biofilm than the coronal and middle thirds in every group ($p<0.001$). Sodium hypochlorite showed the lowest viable-cell percentage and shallowest residual penetration, whereas saline showed dense viable biofilm along canal walls and within dentinal tubules. Culture, overall confocal and canal-third findings are summarized in **Tables 1, 2 and 3** respectively.

Table 1: Baseline and post-irrigation bacterial counts

Group	n	Baseline log10 CFU/mL	Post-treatment log10 CFU/mL	Mean log reduction	p value
3% sodium hypochlorite	30	6.44 ± 0.37	1.18 ± 0.42	5.26 ± 0.49	<0.001
2% chlorhexidine	30	6.41 ± 0.40	1.74 ± 0.51	4.67 ± 0.55	<0.001
10% Triphala	30	6.46 ± 0.35	2.33 ± 0.58	4.13 ± 0.61	<0.001
10% neem	30	6.38 ± 0.42	2.56 ± 0.61	3.82 ± 0.66	<0.001
Saline	30	6.43 ± 0.39	5.71 ± 0.45	0.72 ± 0.31	0.014

Table 2: Confocal laser scanning microscopy outcomes

Group	Viable cells (%)	Residual biofilm area (%)	Maximum viable penetration (μm)	Overall p value
3% sodium hypochlorite	6.8 ± 3.1	8.6 ± 4.0	76 ± 24	<0.001
2% chlorhexidine	11.9 ± 4.7	14.8 ± 5.9	108 ± 31	
10% Triphala	18.6 ± 6.2	22.5 ± 7.1	146 ± 39	
10% neem	22.4 ± 7.0	26.9 ± 8.3	162 ± 44	
Saline	72.5 ± 8.4	78.2 ± 9.1	318 ± 57	

Table 3: Viable-cell percentage according to canal third

Group	Coronal third (%)	Middle third (%)	Apical third (%)	Within-group p value
3% sodium hypochlorite	4.2 ± 2.0	6.1 ± 2.7	10.1 ± 4.0	<0.001
2% chlorhexidine	8.0 ± 3.6	10.7 ± 4.1	17.0 ± 5.8	<0.001
10% Triphala	13.1 ± 4.8	17.2 ± 5.3	25.5 ± 7.2	<0.001
10% neem	16.0 ± 5.6	20.8 ± 6.2	30.4 ± 8.0	<0.001
Saline	65.2 ± 8.1	71.9 ± 7.8	80.4 ± 8.7	<0.001

Discussion:

Sodium hypochlorite produced the greatest reduction in recoverable *E. faecalis* and the lowest residual viability, confirming the importance of its combined antimicrobial and tissue-dissolving actions. Its superiority is consistent with studies showing that adequate concentration and contact time can disrupt mature biofilm, although complete sterilization is rarely achieved **[5-7].** Chlorhexidine ranked second. Its cationic molecules bind to dentin and bacterial cell walls, producing membrane disruption and substantivity. The absence of tissue dissolution remains a major limitation, but the low residual viability observed here supports its use when sodium hypochlorite is contraindicated or as a carefully sequenced supplementary agent **[8, 9]**. Both herbal preparations produced substantial bacterial reduction compared with saline. Triphala showed slightly greater activity than neem, possibly because its polyphenolic and tannin-rich constituents affect membrane permeability, enzyme systems and oxidative balance. Earlier studies have reported antimicrobial effects of Triphala against *E. faecalis* biofilm, although sodium hypochlorite generally remains more effective **[10, 11].** Neem also reduced culture counts and viable-cell percentage. Its azadirachtin, nimbidin, quercetin and related compounds have antimicrobial and anti-inflammatory properties. Variation in plant source, extraction solvent, storage, pH and active-compound concentration may explain inconsistent results among herbal studies **[12]**. Standardization is therefore necessary before routine clinical recommendation. The discrepancy between culture and confocal microscopy is clinically important. Culture can underestimate viable but non-cultivable cells, while confocal imaging shows spatial persistence. The higher apical viability in all groups reinforces the difficulty of irrigant exchange near the apex and supports activation methods that improve fluid replacement **[13, 14].** The present protocol standardized concentration, volume, contact time and needle position, but it did not include sonic or ultrasonic activation. Activation could change the relative ranking by improving penetration and shear forces. Dentin buffering and the age of the biofilm also influence susceptibility and should be incorporated into future models **[15, 16].** Herbal irrigants should not be interpreted as direct substitutes for sodium hypochlorite on the basis of antibacterial activity alone. An ideal irrigant must also dissolve tissue, remove smear layer in combination protocols, remain stable, avoid staining and demonstrate acceptable cytotoxicity. Clinical trials are needed before definitive recommendations can be made. Limitations include use of a single-species biofilm, straight canals and immediate outcomes. Multispecies biofilm, curved anatomy, irrigation activation, cytotoxicity testing and long-term substantivity should be examined. Within these limits, Triphala and neem showed biologically meaningful activity and justify further formulation research. The influence of dentin and biofilm-related factors on antimicrobial performance is also supported by prior work **[17, 18]**. Recent studies from 2024-2026 report antibacterial activity of Triphala, neem and other herbal irrigants against *E. faecalis*, while retaining conventional irrigants as important comparators **[19-21]**.

Conclusion:

Three percent sodium hypochlorite was the most effective irrigant against mature *E. faecalis* biofilm, followed by 2% chlorhexidine. Triphala and neem extracts significantly reduced bacterial counts and viable biofilm compared with saline but were less effective than conventional irrigants. Herbal preparations may serve as adjunctive or investigational alternatives after appropriate standardization, safety testing and clinical validation.

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