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Antimicrobial Activity of Novel Herbal-Based Endodontic Irrigants Against Enterococcus faecalis: An In Vitro Study

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ABSTRACT: Background: Sodium hypochlorite (NaOCl) is the most common irrigant currently used in root canal treatments, but its cytotoxicity and risk of root canal filling extrusion accident is a concern, driving up the research for safer treatment agents (Kanagasingam & Blum, 2020; Pai, 2025). Enterococcus faecalis is a major bacteria found in persistent endodontic infections (Yang et al., 2024). The main purpose of this study is to compare antimicrobial efficacy of three new polyherbal irrigants against planktonic and biofilm-grown E. faecalis and 3% NaOCl and 2% CHX.

Methods: Three formulations were prepared: HF-1 (neem-turmeric), HF-2 (Triphala-propolis), and HF-3 (green tea-aloe vera). The antimicrobial efficacy of HF-1, HF-2, and HF-3 against E. faecalis ATCC 29212 was determined via agar well diffusion (n = 10/group), broth micro dilution to determine minimum inhibitory concentration (MIC) and bactericidal concentration (MBC), time-kill at 0-30 min, and a 21-day dentin-block biofilm model (n = 10/group). One-way analysis of variance and Tukey post hoc tests were used to analyze data ($p < .05$).

Results: The antibiotic inhibition zones varied significantly among groups ($F(5, 54) = 468.0$, $p < .001$). The largest zones were obtained by NaOCl (26.4 ± 1.2 mm), HF-2 (24.1 ± 1.2 mm) and CHX (22.8 ± 1.1 mm). These were not significantly different from each other. The lowest herbal MIC/MBC was seen with HF-2 (3.125/6.25 mg/mL) and biofilm count was decreased by 4.0 ± 0.4 log10 CFU/mL in HF-2-treated compared to NaOCl-treated group (4.9 ± 0.4).

Conclusion: In the scope of this in vitro setting, the Triphala-propolis preparation (HF-2) showed antimicrobial properties closer to NaOCl, and similar to CHX. These results may serve as basis for future laboratory studies examining cytotoxicity, dentin compatibility, and ex vivo studies.

1. INTRODUCTION

Proper root canal treatment relies on the removal of microorganisms and their by-products from the complex root canal system. The use of mechanical instrumentation cannot access isolated canals, isthmuses, and dentinal tubules, thus chemical irrigation is necessary along with shaping (Diouchi et al., 2024). As there is more than one way to accomplish this, sodium hypochlorite (NaOCl) continues to be the most commonly used solution because of its antifungal and tissue dissolution capabilities (Diouchi et al., 2024).

In spite of its effectiveness, NaOCl can be highly toxic to the living tissues. Accidental extrusion of NaOCl beyond the apical foramina may potentially cause significant damage to the peri-apical soft tissues and might also lead to medico-legal issues (Kanagasingam & Blum, 2020; Pai, 2025). Chlorhexidine provides good substantivity and lesser tissue toxicity but has limited ability to dissolve pulp tissue and also shows limited activity against matured biofilms

(Diouchi et al., 2024). Therefore, no irrigant has been found to be ideal and this has led to the current continued quest for safe, biocompatible natural alternatives.

Enterococcus faecalis is a facultative anaerobe bacteria that has been isolated from persistent and secondary endodontic infections and is one of the major organisms implicated in treatment failures (Yang et al., 2024). Owing to the formation of biofilms that protect it from the immune response and antimicrobial agents, this organism has become a ubiquitous problem in testing new irrigants (Yang et al., 2024).

Endodontic irrigants of plant origin (such as propolis, Triphala and aloe vera) have been suggested as they are said to be antimicrobial, anti-inflammatory and biocompatible. An in-vitro investigation on the efficacy of these three products -propolis, Triphala and aloe vera against E. faecalis (Chlorhexidine used as a positive control) was conducted by Durga Bhavani et al. (2023). More recently, Rajmohan (2025) reported that a volcanic-ash tooth powder irrigant was superior in its antimicrobial efficacy over normal saline against E. faecalis. At the level of evidence-synthesis, Ferraz et al. (2024) published a network meta-analysis reporting that despite the inability to demonstrate superiority over NaOCl, there was antimicrobial activity of natural endodontic solutions against E. faecalis. Bhatia et al. (2023) found that herbal irrigants may be acceptable alternatives to synthetic irrigants in regard to antimicrobial effectiveness and smear layer removal. An absence of routine use of plant extracts in this context was identified by Diouchi et al. (2024) who found heterogeneity regarding the antibiofilm activity.

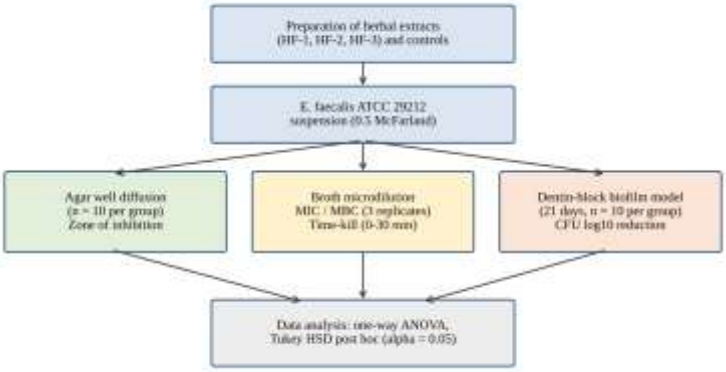
The argument for polyherbal formulations being that it is presumed that a variety of constituents in the plants can attack different bacterial structures and may work synergistically, allowing lesser concentrations of each to be used in combination. Ayurvedic and traditional compounds like Triphala have been used for centuries as formulations with multiple ingredients, which when used in endodontics is a natural progression. However, comparison with standard irrigants would be valid only under standardized conditions but imprecise extraction techniques, solvents and test-concentration make the comparison of results in different laboratories tenuous (Diouchi et al., 2024; Ferraz et al., 2024).Although most studies evaluating single extracts, polyherbal formulations may act via complementary mechanisms. Few investigations on planktonic susceptibility, killing kinetics, and biofilm eradication have been conducted in a single study. Considering this, the purpose of this in vitro study was to assess the antimicrobial activity of three novel polyherbal irrigant formulations on Enterococcus faecalis and compare them with 3% sodium hypochlorite (NaOCl) and 2% chlorhexidine (CHX). It was hypothesized that there would be no difference between the herbal formulations and the conventional irrigants regarding their antimicrobial activity.

2. MATERIALS AND METHODS

2.1 Study Design and Ethical Considerations

This was a controlled laboratory experimental in-vitro study carried out in the Microbiology Research Laboratory, [Institution] from [month/year] to [month/year]. Schematic diagram of experimental design is shown in the figure 1. Use of human extracted teeth in the present study was approved by the Institutional ethics committee ([approval number], [date]), and teeth were obtained from the patients having given an informed consent for their tooth extraction for the research study. The study was conducted following the CRIS guidelines for in-vitro studies.

Figure 1 Flow Diagram of the Experimental Design



Note. MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; CFU = colony-forming units.

2.2 Preparation of the herbal formulations

Botanist at [institution], and voucher specimens were deposited (voucher no.). All dried powdered samples were subjected to cold maceration in 70 % ethanol with a material-to-solvent ratio of 1:10(w:v) for 72 h under intermittent shaking. The mixture was filtered through Whatman filter paper (Number 1), concentrated under reduced pressure at 40 °C and lyophilized to obtain a dry powder form. Three formulations were prepared, as shown in Table 1, namely HF-1 (leaf extract of Azadirachta indica and rhizome extract of Curcuma longa in the ratio 1:1), HF-2 (Triphala: equal parts of Terminalia chebula, Terminalia bellirica and Phyllanthus emblica with propolis in a ratio of 3:1) and HF-3 (leaf extract of Camellia sinensis and gel of Aloe barbadensis in the ratio 1:1). Each of the lyophilized formulations was dissolved in sterile distilled water with 1% dimethyl sulfoxide to prepare a stock solution (50 mg/mL) and sterile filtered using a 0.22 µm filter. The pH of each solution was determined using a calibrated pH meter.

Table 1 Experimental Groups, Composition and pH of the Tested Solutions

Group	Composition	Concentration	Solvent	pH
Saline	0.9% sterile normal saline	-	Water	7.0
NaOCl	Sodium hypochlorite	3% (w/v)	Water	11.8
CHX	Chlorhexidine gluconate	2% (w/v)	Water	5.9
HF-1	Neem + turmeric (1:1)	50 mg/mL	Water/1% DMSO	5.4
HF-2	Triphala + propolis (3:1)	50 mg/mL	Water/1% DMSO	4.8
HF-3	Green tea + aloe vera (1:1)	50 mg/mL	Water/1% DMSO	5.9

NaOCl = sodium hypochlorite; CHX = chlorhexidine; HF = herbal formulation; DMSO = dimethyl sulfoxide. [Replace with actual formulations and measurements.

2.3 Reviving Enterococcus faecalis

The standard strain was re-cultured on BHI agar and cultured at 37 °C for 24 h. And the colonies of bacteria were transferred into sterile saline, followed by adjusting the bacterial concentration to a 0.5 McFarland range, which has a concentration of 1.5 × 10 8 CFU/mL approximately. All colonies should be purified by Gram-staining and morphology to confirm the purity of the suspension.

2.4 Agar Well Diffusion Assay

Mueller-Hinton plates were uniformly inoculated with a standardised suspension of the test organism. Six-millimeter diameter wells were punched into the Mueller-Hinton agar plate using a sterile cork-borer. 100µL of the test solution was added to each well creating 10 wells on each plate in random locations. The plates were pre-diffused for 30 minutes at room temperature before placing in incubator at 37°C for 24 hours. Inhibition zones were measured to the nearest tenth of a millimeter using a digital calliper, with a researcher unaware of the group assignment measuring each inhibition zone diameter including the well.

2.5 Testing for minimum inhibiting concentration (MIC) and minimum killing concentration (MBC)

MICs were determined using broth micro dilution methods in 96-well plates with 2-fold serial dilutions of each of the solutions in BHI broth using a final inocula of 5 × 105 CFU/mL. After 24 h incubation at 37 °C, MIC was determined

as the lowest concentration of tested solution with no visual growth confirmed with resazurin dye. A 10 μL aliquot was taken from the wells clear of growth and subsequently plated on BHI agar to determine the MBC, which was defined as the lowest concentration that resulted in 99.9% killing of the initial inoculum. All values were obtained from triplicates of three independent assays.

2.6 Time-Kill Assay

A 10^7 CFU/mL suspension was subjected to each solution for the test concentration (1:1 v/v). Samples at 0, 1, 5, 10 and 30 mins were transferred to neutralising broth (Dey-Engley for sodium hypochlorite and chlorhexidine; BHI for herbal solutions), serially diluted and plated. Colony counts after 48 hr incubation were reported as log10 CFU/mL (limit of detection = 10 CFU/mL or 1.0 log10).

2.7 Dentin-Block Biofilm Model

Sixty human single rooted premolars were caries free, extracted premolar teeth were decorated and sectioned to prepare dentin blocks (5 × 5 × 1mm). The samples were prepared by removal of the smear layer with 17% ethylene di-amine tetra acetic acid (EDTA) for 5min, followed by 3% NaOCl for 5min and finally saline rinse. The blocks were autoclaved. A BHI broth was prepared that contained E. faecalis and the dentin blocks were incubated in this media for 21 days at 37 °C, with the media changed every 48h to allow the development of mature biofilm. Thereafter the blocks were randomly allocated (n= 10 in each group) and immersed in 2 mL of the respective irrigant for 5min. Biofilm was removed using vortexing with glass beads and sonication for 30s, and serially diluted and cultured. The log10 reduction between the pre- and post-treatment counts was calculated by the difference in the CFU from paired control blocks.

2.8 Sample Size and Statistical Analysis

Sample sizes of ten for each group were determined based on a large effect size (coefficient (f) = 0.5) assuming an alpha of 0.05 with 80% power for a six-group comparison. Assumption of normality was tested using the Shapiro-Wilk test, and homogeneity of variance using Levene's test. Between-group differences were compared using one-way analysis of variance (ANOVA) with Tukey's honest significant difference test. Data are expressed as the mean±standard deviation, with statistical significance at $p < 0.05$. The statistical analysis was carried out using [software, version].

2.9 Quality Control, Blinding and Reproducibility

Each experiment included sterility controls (uninoculated medium and each test solution alone) and growth controls (inoculated medium alone without irrigant). Solutions were prepared on the day of testing and were coded by an independent assistant, and therefore inoculation and colony count were performed blindly. An independent assistant prepared and coded the solutions, and an operator inoculated the plates. Plates were both counted by the same examiner. Differences of more than 10% were retested. Inter-operator reliability was evaluated using intraclass correlation coefficient [report value]. All glassware and dentin blocks were sterilized, and all manipulations were performed under a class II biosafety cabinet. The time-kill and MIC/MBC experiments were repeated on three different days**.**

3. Results

3.1 Agar well diffusion

One-way ANOVA revealed a significant difference in inhibition zone diameter among the six groups ($F(5, 54) = 468.0$, $p < .001$). The negative control produced no zone beyond the 6.0-mm well. NaOCl showed the largest zone (26.4 ± 1.2 mm), significantly larger than all other groups ($p < .001$). Among the herbal formulations, HF-2 produced the largest zone (24.1 ± 1.2 mm), followed by HF-1 (20.9 ± 1.3 mm) and HF-3 (18.2 ± 1.0 mm). HF-2 did not differ significantly from 2% CHX (22.8 ± 1.1 mm; $p = .082$), whereas HF-1 and HF-3 produced significantly smaller zones than CHX ($p = .003$ and $p < .001$, respectively). Descriptive statistics are given in Table 2 and Figure 2, and the pairwise comparisons in Table 3.

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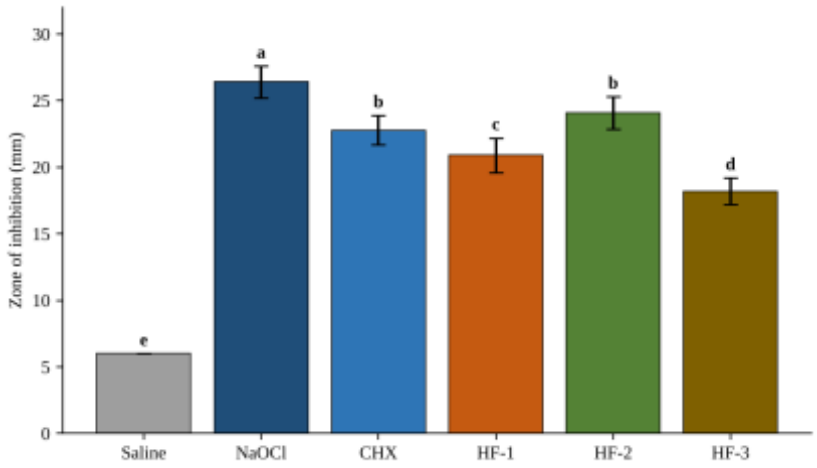
Table 2 Zone of Inhibition (mm) Against Enterococcus faecalis by Group (n = 10)

Group	Mean	SD	Median	Range	95% CI	Tukey group
Saline	6.0	0.0	6.0	6.0-6.0	6.0-6.0	e
3% NaOCl	26.4	1.2	26.3	24.0-28.5	25.6-27.3	a
2% CHX	22.8	1.1	22.8	20.5-24.2	22.0-23.6	b
HF-1	20.9	1.3	20.6	19.0-23.2	20.0-21.8	c
HF-2	24.1	1.2	24.6	22.2-25.6	23.2-25.0	b
HF-3	18.2	1.0	18.1	17.0-19.8	17.5-18.9	d

Note. Zone diameter includes the 6.0-mm well. Groups sharing a letter do not differ significantly (Tukey HSD, $p > .05$). One-way ANOVA: $F(5, 54) = 468.0$, $p < .001$. [Illustrative data.]

Figure 2 Mean Zone of Inhibition by Group



Note. Bars show mean ± SD (n = 10). Different letters indicate significant differences (Tukey HSD, $p < .05$). [Illustrative data.]

Table 3 Selected Pairwise Comparisons (Tukey HSD) for Zone of Inhibition and Biofilm Reduction

Comparison	Δ ZOI (mm)	p (ZOI)	Δ log10 reduction	p (biofilm)
3% NaOCl vs HF-2	2.3	< .001	0.9	< .001
3% NaOCl vs 2% CHX	3.6	< .001	1.3	< .001
2% CHX vs HF-2	-1.3	.082	-0.4	.160
2% CHX vs HF-1	1.9	.003	0.5	.039
HF-2 vs HF-1	3.2	< .001	0.9	< .001

Comparison	Δ ZOI (mm)	p (ZOI)	Δ log10 reduction	p (biofilm)
HF-2 vs HF-3	5.9	< .001	1.5	< .001
HF-1 vs HF-3	2.7	< .001	0.6	.010

Note. Δ = difference between group means (first minus second group). p values are Tukey-adjusted. [Illustrative data.]

3.2 MIC and MBC

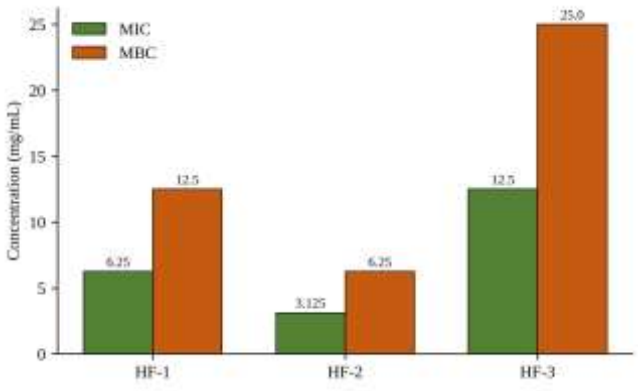
The results of all the herbal formulations, with MIC: MBC ratios of 2 (Table 4, Fig. 3) suggest that the herbal solutions were bactericidal. Among the herbal solutions, HF-2 was found to have the lowest MIC and MBC of 3.125 and 6.25 mg/ml respectively, being half the MIC and MBC of HF-1, i.e. 6.25 and 12.5 mg/ml respectively, and a quarter of the values of HF-3, i.e. 12.5 and 25 mg/ml respectively. NaOCl and CHX had MIC values of 0.0125% and 0.0008%. It is postulated that a comparison of MICs between the herbal solutions and normal saline would not be valid, as the units are not comparable.

Table 4 Minimum Inhibitory and Bactericidal Concentrations Against E. faecalis

Solution	MIC	MBC	MBC/MIC	Interpretation
3% NaOCl (as % w/v)	0.0125 %	0.025 %	2	Bactericidal
2% CHX (as % w/v)	0.0008 %	0.0016 %	2	Bactericidal
HF-1	6.25 mg/mL	12.5 mg/mL	2	Bactericidal
HF-2	3.125 mg/mL	6.25 mg/mL	2	Bactericidal
HF-3	12.5 mg/mL	25 mg/mL	2	Bactericidal

Note. Modal values of three independent experiments performed in triplicate. MBC/MIC ≤ 4 was interpreted as bactericidal. [Illustrative data.]

Figure 3 MIC and MBC of the Herbal Formulations



Note. MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration. [Illustrative data.]

3.3 Time-kill kinetics

Negative control was relatively unchanged at ca. 7.0 log10 CFU/mL for the 30-minute duration. Sodium hypochlorite (NaOCl) was the most rapid-acting and achieved the detection limit within 10-minutes (from 2 to 1.79 log10 CFU/mL, 5-minutes). Both HF-2 and CHX reached the detection limit after 10-minutes (from 3.89 to 3.06 log10 CFU/mL, 5-

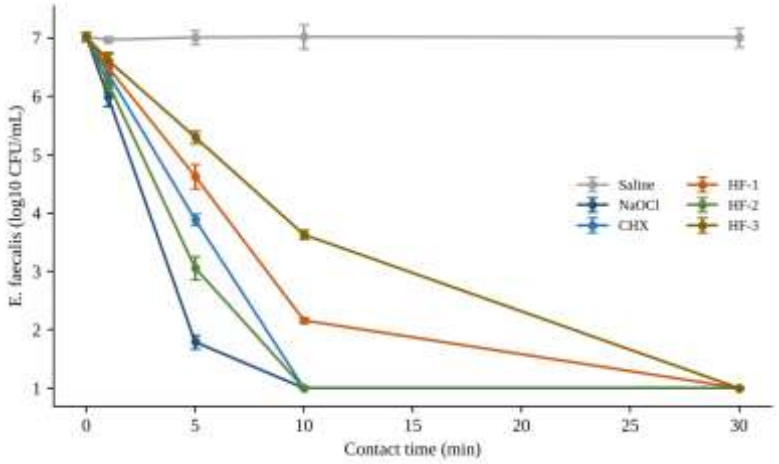
minutes), as did HF-1 and HF-3 after 30-minutes. NaOCl, CHX, and HF-2 achieved a 3 log10 CFU/mL reduction (99.9%) within 5-minutes while HF-1 and HF-3 also achieved this within 10-minutes (Table 5, Figure 4).

Table 5 Viable E. faecalis (log10 CFU/mL, Mean ± SD) at Different Contact Times

Group	0 min	1 min	5 min	10 min	30 min
Saline	7.02 ± 0.08	6.97 ± 0.05	7.01 ± 0.12	7.02 ± 0.21	7.01 ± 0.17
3% NaOCl	7.02 ± 0.08	5.99 ± 0.16	1.79 ± 0.12	≤ 1.00 (LOD)	≤ 1.00 (LOD)
2% CHX	7.02 ± 0.08	6.39 ± 0.20	3.89 ± 0.10	≤ 1.00 (LOD)	≤ 1.00 (LOD)
HF-1	7.02 ± 0.08	6.50 ± 0.22	4.63 ± 0.21	2.16 ± 0.04	≤ 1.00 (LOD)
HF-2	7.02 ± 0.08	6.22 ± 0.15	3.06 ± 0.20	≤ 1.00 (LOD)	≤ 1.00 (LOD)
HF-3	7.02 ± 0.08	6.62 ± 0.13	5.30 ± 0.11	3.63 ± 0.08	≤ 1.00 (LOD)

Note. LOD = limit of detection (10 CFU/mL = 1.0 log10). Mean of three independent experiments. [Illustrative data.]

Figure 4 Time-Kill Curves of E. faecalis Exposed to the Test Solutions



Note. Points show mean ± SD (n = 3). Values at the detection limit are plotted at 1.0 log10 CFU/mL. [Illustrative data.]

3.4 Biofilm eradication on dentin blocks

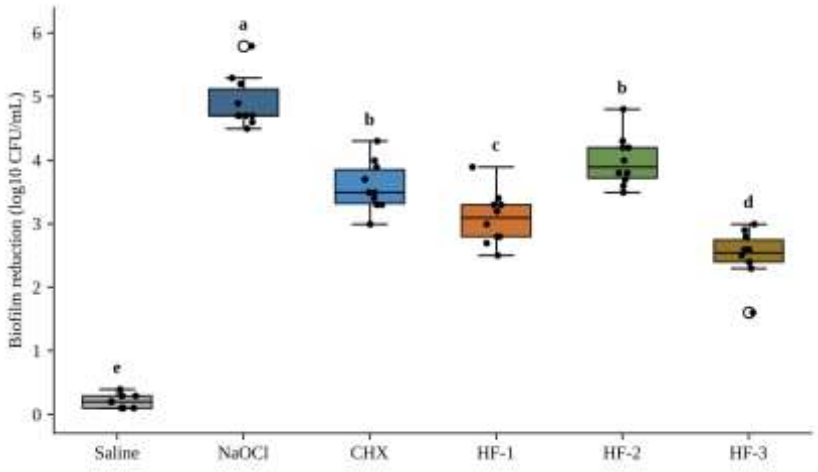
The biofilm assay also showed a significant overall effect of treatment ($F(5, 54) = 194.0$, $p < .001$). NaOCl produced the greatest reduction (4.9 ± 0.4 log10 CFU/mL; 99.999%), followed by HF-2 (4.0 ± 0.4; 99.990%) and CHX (3.6 ± 0.4; 99.974%); the last two were not significantly different ($p = .160$). HF-1 (3.1 ± 0.4) and HF-3 (2.5 ± 0.4) were significantly less effective than CHX and HF-2, and HF-3 was also lower than HF-1 ($p = .010$). Saline caused only a negligible reduction (Table 6, Figure 5).

Table 6 Reduction of Biofilm-Grown E. faecalis on Dentin Blocks After 5 Min of Irrigant Contact (n = 10)

Group	Mean (log10)	SD	Median	95% CI	Reduction (%)	Tukey group
Saline	0.2	0.1	0.2	0.1-0.3	38.340	e
3% NaOCl	4.9	0.4	4.7	4.6-5.2	99.999	a
2% CHX	3.6	0.4	3.5	3.3-3.9	99.974	b
HF-1	3.1	0.4	3.1	2.8-3.4	99.919	c
HF-2	4.0	0.4	3.9	3.7-4.3	99.990	b
HF-3	2.5	0.4	2.5	2.2-2.8	99.691	d

Note. Log10 reduction in CFU/mL relative to untreated control blocks. Groups sharing a letter do not differ significantly (Tukey HSD, $p > .05$). One-way ANOVA: $F(5, 54) = 194.0$, $p < .001$. [Illustrative data.]

Figure 5 Distribution of Biofilm Reduction by Group



Note. Boxes show the median and interquartile range; dots are individual specimens (n = 10). Different letters indicate significant differences (Tukey HSD, $p < .05$). [Illustrative data.]

4. DISCUSSION

Comparison of Enterococcus faecalis with Polyherbal Irrigant Formulation and Allopathic Antiseptics This combination of plant extracts was then tested against Enterococcus faecalis using complementary planktonic and biofilm methods and compared to NaOCl and CHX. The complete data set shows that among the herbal combinations of irrigants tested HF-2 – the Triphala-propolis combination – was the most active in all the tests. It proved to be as effective as 2% CHX in the agar diffusion test and the biofilm method, but was less effective than 3% NaOCl which was the most active and fastest. (Therefore the null hypothesis was accepted.) The fact that the ranking of NaOCl > HF-2 > CHX > HF-1 > HF-3 was found in three different independent tests adds internal validity to the results.

The overall superior efficacy of NaOCl over the herbal irrigants is consistent with the findings of Ferraz et al. (2019), who concluded in a network meta-analysis that the natural endodontic irrigants tested did not outperform NaOCl, although they did exhibit antimicrobial activity against E. faecalis. The notion that a herbal preparation can come close to the efficacy of a standard irrigant correlates with the results of Durga Bhavani et al. (2023), who evaluated the

efficacy of propolis, Triphala and aloe vera against E. faecalis as compared to chlorhexidine, considered as positive control, and regarded these herbal irrigants as suitable for root canal irrigation. Similarly, Bhatia et al. (2019) found in a systematic review that herbal irrigants have the potential to serve as alternatives to synthetic irrigants in terms of their antimicrobial activity and their removal of the smear layer. However, the current results must be considered with caution, since raw values are enormously impacted by extraction techniques, concentrations and experimental models, all of which differ a great deal.

This unexpected result in HF-2 may be explained by the potential synergistic interactions between the two components of this treatment which have different chemical compositions. Polyphenolic acids in triphala versus flavonoids and phenolic acids in propolis may destabilise bacterial cell membranes and interfere with bacterial metabolic activities via different mechanisms. This mechanistic hypothesis has not been tested in the present study but mechanistic studies such as changes in cell membrane permeability by electron microscopy or expression of biofilm genes as biomarkers may be considered in future work. The relatively acidic pH of the herbal preparations used in this study (see table 1) may also have played a role and pH-adjusted controls should be considered in future studies.

The biofilm model used in this study is relevant due to the high degree of resistance E. faecalis biofilms provide to antimicrobial agents and host defences (Yang et al., 2016). An over-reliance on planktonic susceptibility could lead to an over-estimation of clinical performance. Diouchi et al. (2016) in their systematic review mentioned the lack of a homogeneous body of evidence for antibiofilm activity of plant extracts, reaffirming the need for comparison with conventional irrigants. Although using a 21-day dentin-block biofilm is a better clinical reflection compared to simply a planktonic assay, it is important to highlight that using a one-strain dentin-block biofilm to mimic the polymicrobial nature of root canal infections in situ is limited.

The main justification for the investigation of herbal irrigants is the safety; the high cytotoxicity of NaOCl and potential severe tissue damage resulting from extrusion accidents (Kanagasingam & Blum, 2020; Pai, 2025). The safety and biocompatibility of Triphala in primary-tooth irrigation as described by Chavan et al. (2025) provide a positive indication to support further exploration of combinations with Triphala. However, the antimicrobial efficacy of the herbal formulations in this investigation fell short of that of NaOCl, which would mean any herbal irrigant would have to offer a far better safety profile to justify efficacy trade-off. A non-conventional newer irrigant that has been proposed, BioAKT Endo, a silver-citrate based solution, used by El-Kabbaney (2022), showed promising activity against E. faecalis, compared to NaOCl, as an alternative, demonstrating that the search for alternatives to NaOCl has gone beyond trying to find another plant-derived substance.

In addition, for clinical use there is a need for detailed information on the impact of the herbal preparations on dentin (smear layer removal, microhardness, bond strength, colouration of the tooth (staining of the tooth being of concern with turmeric and green tea), tissue-dissolving ability, shelf-life, and standardization of extracts. Chemical variations of the same plant between batch–batch is a known concern and chemical fingerprinting (high-performance liquid chromatography (HPLC)) should be reported on each formulation.

Table 7 Summary of Recent Evidence Cited in the Present Study and Its Relation to the Current Findings

Study	Design	Main finding (as reported)	Relation to present findings
Ferraz et al. (2024)	Systematic review, network meta-analysis	Natural endodontic solutions did not overcome NaOCl but showed antimicrobial potential against E. faecalis	Consistent: NaOCl remained most potent
Diouchi et al. (2024)	Systematic review	Compared antibiofilm efficacy of plant extracts with conventional irrigants; no ideal irrigant identified	Supports use of a biofilm model

Study	Design	Main finding (as reported)	Relation to present findings
Bhatia et al. (2023)	Systematic review	Herbal irrigants useful alternatives for antimicrobial efficiency and smear layer removal	Consistent with activity of HF-2
Durga Bhavani et al. (2023)	In vitro	Propolis, Triphala and aloe vera tested against E. faecalis with CHX as positive control; considered suitable irrigants	Comparable ingredients to HF-2 and HF-3
Rajmohan (2025)	In vitro	Hekla Lava tooth powder superior to saline against E. faecalis	Similar saline-control contrast
El-Kabbaney (2022)	In vitro	Silver-citrate irrigant BioAKT Endo showed promising antibacterial activity versus NaOCl	Non-herbal comparator of novel irrigants
Chavan et al. (2025)	Systematic review	Triphala showed favourable safety and biocompatibility in primary teeth	Supports Triphala-based rationale

Note. Findings are summarised from the abstracts and main conclusions of the cited publications; direct numerical comparison is not appropriate because the methods differ.

4.1 Clinical implications

Should the literature eventually confirm the safety of Triphala-propolis irrigant via cytotoxicity testing and ex vivo studies, it could be employed as an adjunct or alternative to sodium hypochlorite (NaOCl) in cases of concern of NaOCl extrusion, such as immature and resorbed teeth or in paediatric cases (Chavan et al., 2025; Pai, 2025). Its irrigant could also be explored as a final rinse or intracanal irrigant in regenerative procedures, where tissue compatibility is advantageous. Nonetheless, the latter remains just a hypothetical suggestion, as safety, interaction with dentine, and clinical performance were not conducted in the current study.

The findings further underscore the benefit of considering different endpoints. The rank order of the herbal formulations remained similar among the diffusion assay, MIC/MBC determination, time-kill assay, and biofilm model-although the magnitude of the difference from NaOCl changed according to the approach. In the diffusion testing, the agar matrix limits diffusion of high-molecular-weight polyphenols; in the time-kill and biofilm assays, there is close contact. Hence, the differences in zones of diffusion should not be used alone for rankings of irrigants for the clinic.

Limitations

This study has some limitations. Firstly, only a single reference strain of the microorganism was used, and clinical isolates of polymicrobial biofilms may respond differently. Secondly, the dentin-block model cannot simulate the effects of the actual canal system, especially the three-dimensional canal anatomy, dentinal tubule infection and irrigant flow. Thirdly, the effects of the formulations on cell lines, dentin, and their ability to dissolve tissue were not examined. Fourthly, the herbal formulations were investigated at a single concentration, and the dose-response behavior in the biofilm model are unknown. Finally, due to the inherent limitation of the in vitro model, it cannot be directly translated to the clinical scenario.

CONCLUSIONS

As much as the Triphala-propolis formulation (HF-2) exhibited the most superior antimicrobial and antibiofilm properties among the three novel herbal irrigants used in this in vitro study, it was comparable to 2% chlorhexidine and inferior to 3% sodium hypochlorite. The remaining formulations in this study, neem-turmeric (HF-1) and green tea-aloe vera (HF-3), were weaker contenders. Further research on cytotoxicity, dentin effects, polymicrobial, and ex

vivo root canal models, as well as clinical safety, is needed before herbal irrigants can be suggested as viable substitutes for conventional agents.

REFERENCES

[1] Bhatia, C., Chandak, M., Adwani, R. D., Dass, A., Nikhade, P. P., Shrivastav, S., Harichandi, D., & Adwani, N. (2023). Comparative evaluation of antimicrobial efficacy of new herbal preparation with and without 17% ethylenediamine tetraacetic acid as a root canal irrigant in human root dentin: A systematic review. Journal of Datta Meghe Institute of Medical Sciences University, 18(2), 337-342. https://doi.org/10.4103/jdmimsu.jdmimsu_116_23

[2] Chavan, P., Dimple, P., & Takate, V. (2025). Comparative effectiveness of Triphala and conventional root canal irrigants in primary teeth: A systematic review. Cureus. https://doi.org/10.7759/cureus.98788

[3] Diouchi, J., Touré, B., & Ghoul, S. (2024). Antibiofilm efficacy of plant extracts as root canal irrigants in endodontics: A systematic literature review. Frontiers in Dental Medicine, 5, Article 1479953. https://doi.org/10.3389/fdmed.2024.1479953

[4] Durga Bhavani, G., Rathod, T., Parveen, N., Tirupathi, P., Dharavattu, P., Sekhar, V. S. S. K., Sharma, D., & Anlesteffy, S. G. (2023). Assessment of the antimicrobial effectiveness of herbal root canal irrigants (propolis, Triphala, and aloe vera) and chlorhexidine against Enterococcus faecalis. Cureus, 15(7), e41628. https://doi.org/10.7759/cureus.41628

[5] El-Kabbaney, A. (2022). Cumulative impacts of endodontic procedures on Enterococcus faecalis: An in vitro study. Egyptian Dental Journal, 68(2). https://doi.org/10.21608/edj.2022.120697.1974

[6] Ferraz, D. C., Melo, A. de P., Matos, F. de S., Paranhos, L. R., Moura, C. C. G., Blumenberg, C., Guerreiro-Tanomaru, J. M., & Tanomaru-Filho, M. (2024). Antimicrobial efficacy of natural-based endodontic solutions: A systematic review with a network meta-analysis. Brazilian Oral Research, 38. https://doi.org/10.1590/1807-3107bor-2024.vol38.0093

[7] Kanagasingam, S., & Blum, I. (2020). Sodium hypochlorite extrusion accidents: Management and medico-legal considerations. Primary Dental Journal, 9(4), 59-63. https://doi.org/10.1177/2050168420963308

[8] Pai, A. R. V. (2025). Mechanism and clinical aspects of sodium hypochlorite accidents: A narrative review. Dental and Medical Problems, 62(1), 173-185. https://doi.org/10.17219/dmp/171284

[9] Rajmohan, M. (2025). Comparative in vitro study of antimicrobial efficacy of sodium hypochlorite, normal saline, and Hekla Lava tooth powder as root canal irrigants against Enterococcus faecalis. Avicenna Journal of Dental Research, 17(3), 154-159. https://doi.org/10.34172/ajdr.2187

[10] Yang, S., Meng, X., Zhen, Y., Baima, Q., Wang, Y., Jiang, X., & Xu, Z. (2024). Strategies and mechanisms targeting Enterococcus faecalis biofilms associated with endodontic infections: A comprehensive review. Frontiers in Cellular and Infection Microbiology, 14, Article 1433313. https://doi.org/10.3389/fcimb.2024.1433313