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Antibacterial efficacy of herbal irrigants against *Enterococcus faecalis*: An *in vitro* study

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Abstract:
Enterococcus faecalis persists in root canals after chemo mechanical preparation and is a major cause of endodontic *Enterococcus faecalis* is a major cause of persistent root canal infection and endodontic treatment failure. Therefore, it is of interest to compare the antibacterial efficacy of Triphala, Neem, Propolis and Turmeric extracts against *E. faecalis* using *in vitro* assays. Ninety root canal specimens were assigned to six groups and antibacterial activity was evaluated by agar well diffusion, MIC and MBC assays. NaOCl showed the largest inhibition zone (22.4±1.8 mm), while Propolis showed the greatest activity among herbal irrigants, followed by Triphala, Neem and Turmeric ($p<0.001$). Thus, propolis showed superior antibacterial efficacy among herbal irrigants, supporting its potential as a biocompatible alternative for root canal irrigation.

Keywords: *Enterococcus faecalis*, herbal irrigants, root canal irrigation, propolis, triphala, neem, turmeric, antibacterial efficacy, zone of inhibition, MIC, endodontics

Background:
Root canal treatment aims to eliminate microorganisms from the root canal system through chemo mechanical preparation and irrigation, thereby preventing reinfection and ensuring long-term periapical health **[1].** Despite advances in instrumentation techniques, the complex anatomy of the root canal system including lateral canals, isthmuses, fins and dentinal tubules renders complete microbial elimination a formidable challenge **[2].** Among the diverse polymicrobial flora implicated in endodontic infections and treatment failures, *Enterococcus faecalis* has emerged as the most clinically significant pathogen, being recovered in 24–77% of failed root canal treatments **[3].** Its persistence is attributed to a constellation of virulence factors including the ability to form biofilms, invade dentinal tubules to depths of 150–300 µm, tolerate alkaline environments, survive in nutrient-depleted conditions and resist the action of commonly used intracanal medicaments **[4].** Sodium hypochlorite (NaOCl) remains the irrigant of choice in contemporary endodontic practice due to its broad-spectrum antimicrobial activity, tissue-dissolving capacity and ability to penetrate biofilms **[5].** However, NaOCl is associated with significant drawbacks including cytotoxicity to periapical tissues when inadvertently extruded, bleaching of clothing, unpleasant odour, alteration of dentinal microhardness and elastic modulus and the potential for severe hypochlorite accidents **[6].** These limitations have driven increasing interest in plant-derived alternatives that may offer comparable antibacterial efficacy with superior biocompatibility and reduced adverse effects**.** Several herbal preparations have been investigated as potential endodontic irrigants. Triphala, a polyherbal formulation comprising equal proportions of *Terminalia chebula*, *Terminalia belerica* and *Phyllanthus emblica*, possesses potent antioxidant, antimicrobial and anti-inflammatory properties attributable to its high content of gallic acid, ellagic acid and chebulinic acid **[7].** Neem (*Azadirachta indica*) contains nimbidin, nimbin and quercetin, which disrupt bacterial cell walls and inhibit biofilm formation**.** Propolis, a resinous substance produced by honeybees, is rich in flavonoids, phenolic acids and terpenoids that exert bactericidal effects by inhibiting bacterial cell division and membrane function **[8]**. *Curcuma longa* (turmeric), whose active constituent curcumin inhibits quorum sensing and bacterial adhesion, has also showed promising antibacterial activity in several *in vitro* studies**.** Despite individual investigations of these herbal irrigants, a systematic comparative evaluation of their antibacterial efficacy against *E. faecalis* using standardised methodology remains limited **[9]**. The present *in vitro* study was therefore designed to compare the antibacterial activity of Triphala, Neem, Propolis and Turmeric extracts against a standard strain of *E. faecalis* (ATCC 29212) using agar well diffusion assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination, with 5.25% NaOCl as the positive control and normal saline as the negative control **[10]**. Therefore, it is of interest to report the comparative antibacterial efficacy of Triphala, Neem, Propolis and Turmeric extracts against *Enterococcus faecalis* using standardized *in vitro* assays.

Materials and Methods:

Study design and ethical clearance:
This was an *in vitro* comparative microbiological study conducted in the Department of Conservative Dentistry and Endodontics in collaboration with the Department of Microbiology. Ethical clearance was obtained from the Institutional Ethics Committee prior to commencement of the study. As no human subjects were directly involved, informed consent was not applicable.

Test organism:
A standard reference strain of *Enterococcus faecalis* (ATCC 29212) was obtained from the microbiology department and maintained on Brain Heart Infusion (BHI) agar. For experimental inoculation, a bacterial suspension was prepared in sterile normal saline and adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 colony-forming units per mL) using a spectrophotometer at 625 nm.

Preparation of herbal extracts:
All plant materials were authenticated by a botanist before use. Crude extracts were prepared by the cold maceration method. Dried powders of Triphala (equal parts *Terminalia chebula*, *Terminalia belerica* and *Phyllanthus emblica*), Neem leaves (*Azadirachta indica*) and Propolis (ethanol-extracted resin) and Turmeric rhizome (*Curcuma longa*) were individually macerated in 70% ethanol for 72 hours with intermittent shaking. The extracts were filtered through what man No.1 filter paper concentrated using a rotary evaporator at 40°C under reduced pressure and lyophilised to yield dry powders. Working solutions at a concentration of 25 mg per mL were prepared in sterile distilled water immediately before use. All extracts were filter-sterilised through 0.22 µm membrane filters.

Study groups:
A total of 90 experimental units were divided into six groups of 15 each as described in **Table 1**

Agar well diffusion assay:
Mueller–Hinton agar (MHA) plates were prepared and allowed to solidify. The standardised *E. faecalis* suspension was uniformly swabbed onto the agar surface using sterile cotton swabs in three directions to produce a confluent lawn of growth. Wells of 6 mm diameter were punched aseptically using a sterile cork borer. Each well received 100 µL of the respective test irrigant. Plates were pre-incubated at 4°C for 1 hour to allow pre-diffusion of the irrigants before being incubated at 37°C for 24 hours under aerobic conditions. The zone of inhibition (ZOI) around each well was measured in millimetres using a digital Vernier calliper by a blinded observer and the mean of three readings was recorded.

Minimum inhibitory concentration (MIC):
MIC was determined by the broth microdilution method in sterile 96-well microtitre plates using Mueller–Hinton broth (MHB). Serial two-fold dilutions of each herbal extract (ranging from 100 mg per mL to 0.195 mg per mL) were prepared in MHB. Each well received 100 µL of diluted extract and 100 µL of the bacterial suspension (final inoculum: ~5 x 10^5 CFU per mL). Plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of extract that produced no visible turbidity.

Minimum bactericidal concentration (MBC):
From all clear wells at and above the MIC, 10 µL aliquots were sub-cultured onto BHI agar plates and incubated at 37°C for 48 hours. The MBC was defined as the lowest concentration of extract that resulted in a 99.9% reduction in the original inoculum (no visible colony growth on sub-culture).

Statistical analysis:
Data were entered in Microsoft Excel and analysed using SPSS version 26.0. Descriptive statistics included mean and standard deviation for ZOI. Normality of data distribution was confirmed by the Shapiro–Wilk test. One-way ANOVA was used to assess overall differences in ZOI across all six groups. Pairwise post-hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test. A p-value < 0.05 was considered statistically significant and p < 0.001 was considered highly significant.

Results:
Table 1 shows experimental groups, irrigants, concentrations and study parameters. **Table 2** presents the mean zone of inhibition (ZOI) values for all six irrigant groups. The positive control, 5.25% NaOCl, produced the largest mean ZOI of 22.4 ± 1.8 mm, confirming the potency of the reference standard against *E. faecalis*. Among the herbal irrigants, Propolis extract demonstrated the highest antibacterial activity with a mean ZOI of 18.3 ± 1.5 mm (range 16–21 mm), followed by Triphala (16.7 ± 1.4 mm), Neem (14.9 ± 1.6 mm) and Turmeric (13.2 ± 1.3 mm). The negative control (normal saline) produced no zone of inhibition (0.0 mm) in any replicate, confirming the absence of non-specific inhibition. **Table 3** summarises the MIC and MBC values for each herbal extract. Propolis and Triphala demonstrated the lowest MIC (6.25 mg per mL) and MBC (12.5 mg per mL) values, indicating superior bactericidal potency. Neem and Turmeric required higher concentrations to achieve bactericidal effect (MIC 12.5 mg per mL; MBC 25.0 mg per mL). The MBC and MIC ratio was 2.0 for all herbal extracts, confirming bactericidal rather than bacteriostatic activity at clinically relevant concentrations. Normal saline showed no inhibitory activity at any concentration tested. Table 4 One-way ANOVA revealed a highly significant overall difference in ZOI across all six groups ($F = 48.37$, $p < 0.001$), as shown in Table 4. Post-hoc Tukey's HSD analysis demonstrated that NaOCl produced a significantly larger ZOI than all herbal irrigants ($p < 0.001$ for all pairwise comparisons). Among herbal groups, Propolis was significantly superior to Triphala (mean difference 1.6 mm, $p = 0.021$), Neem (3.4 mm, $p < 0.001$) and Turmeric (5.1 mm, $p < 0.001$). Triphala was significantly superior to Neem (1.8 mm, $p = 0.011$) and Turmeric ($p < 0.001$). All active irrigants produced significantly larger ZOI than normal saline ($p < 0.001$).

Table 1: Experimental groups, irrigants, concentrations and study parameters (n=15 per group)

Group	Irrigant	Concentration	Volume (mL)	Contact Time	n
I	5.25% NaOCl (positive control)	5.25%	5	5 min	15
II	Triphala extract	25 mg/mL	5	5 min	15
III	Neem (*Azadirachta indica*) extract	25 mg/mL	5	5 min	15
IV	Propolis extract	25 mg/mL	5	5 min	15
V	Turmeric (*Curcuma longa*) extract	25 mg/mL	5	5 min	15
VI	Normal saline (negative control)	–	5	5 min	15

Table 2: Zone of inhibition (mm) of all irrigant groups against *E. faecalis* (ATCC 29212)

Irrigant / Group	Mean ZOI (mm)	SD	Min (mm)	Max (mm)
5.25% NaOCl (Group I)	22.4	1.8	20	25
Triphala extract (Group II)	16.7	1.4	14	19
Neem extract (Group III)	14.9	1.6	12	17

Propolis extract (Group IV)	18.3	1.5	16	21
Turmeric extract (Group V)	13.2	1.3	11	15
Normal saline (Group VI)	0.0	0.0	0	0

Values expressed as Mean ± SD (n=15 per group).

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of herbal irrigants against *E. faecalis*

Irrigant	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC Ratio	Bactericidal Activity
5.25% NaOCl	–	–	–	Reference standard
Triphala extract	6.25	12.5	2.0	Bactericidal
Neem extract	12.5	25.0	2.0	Bactericidal
Propolis extract	6.25	12.5	2.0	Bactericidal
Turmeric extract	12.5	25.0	2.0	Bactericidal
Normal saline	No inhibition	No inhibition	–	No activity

Table 4: One-way ANOVA and Tukey's post-hoc comparison of zone of inhibition across irrigant groups

Comparison	Mean Diff. (mm)	SE	df	F / t-value	p-value
One-way ANOVA (overall)	–	–	5	48.37	<0.001**
NaOCl vs Triphala	5.7	0.62	–	–	<0.001**
NaOCl vs Neem	7.5	0.64	–	–	<0.001**
NaOCl vs Propolis	4.1	0.61	–	–	<0.001**
NaOCl vs Turmeric	9.2	0.63	–	–	<0.001**
Propolis vs Triphala	1.6	0.59	–	–	0.021*
Propolis vs Neem	3.4	0.61	–	–	<0.001**
Propolis vs Turmeric	5.1	0.62	–	–	<0.001**
Triphala vs Neem	1.8	0.60	–	–	0.011*
All groups vs saline	–	–	–	–	<0.001**

**p < 0.05; **p < 0.001*

Discussion:

The present study compared the antibacterial efficacy of Triphala, Neem, Propolis and Turmeric against *Enterococcus faecalis* using agar well diffusion, MIC and MBC assays. All herbal irrigants demonstrated significant antibacterial activity, with Propolis showing the highest efficacy, followed by Triphala, Neem and Turmeric. However, 5.25% sodium hypochlorite (NaOCl) remained the most effective irrigant overall. The superior antibacterial activity of NaOCl observed in the present study is comparable with the findings of Tariq *et al.* **[11]**, who reported inhibition zones ranging from 20–24 mm against *E. faecalis*. Their results confirmed that NaOCl remains the most effective endodontic irrigant because of its excellent antimicrobial activity and tissue-dissolving ability, although concerns regarding cytotoxicity continue to encourage the development of safer alternatives. Among the herbal irrigants, Propolis demonstrated the greatest antibacterial activity. This finding agrees with Moreno-Florez *et al.* **[12],** who reported that ethanolic Propolis extract exhibited antibacterial activity comparable to conventional intracanal medicaments against *E. faecalis*. The flavonoids and phenolic compounds present in Propolis are believed to disrupt bacterial cell membranes and inhibit bacterial growth, explaining its superior performance in the present study. Triphala ranked second in antibacterial efficacy, which is consistent with the findings of Chavan *et al.* **[13].** They demonstrated that Triphala produced antibacterial activity comparable to sodium hypochlorite in infected root dentin. The antimicrobial effect of Triphala has been attributed to its polyphenolic constituents, including gallic acid and ellagic acid, which inhibit bacterial adhesion and biofilm formation. Neem extract showed moderate antibacterial activity, similar to the observations of Ghonmode *et al.* **[14]**, who reported comparable inhibition zones against *E. faecalis*. Neem contains active compounds such as nimbidin, nimbolide and quercetin that interfere with bacterial cell wall integrity and reduce biofilm formation. Although less effective than Propolis and Triphala, Neem remains a promising herbal irrigant because of its additional anti-inflammatory properties. Turmeric demonstrated the lowest antibacterial activity among the herbal irrigants. This result is in agreement with Bhavani *et al.* **[18]**, who also observed relatively lower inhibition zones for curcumin against *E. faecalis*. The reduced antibacterial activity may be related to the poor aqueous solubility of curcumin, which limits its diffusion and bioavailability during *in vitro* testing. The present findings are further supported by Ferreira *et al.* **[15],** who showed that Neem extract significantly reduced *E. faecalis* biofilm biomass, suggesting that herbal irrigants may provide additional anti-biofilm benefits beyond simple antibacterial action. Similarly, Parolia *et al.* **[16]** reported that Propolis effectively inhibited *E. faecalis* biofilms even at relatively low concentrations, reinforcing the strong antimicrobial potential observed in the present study. Although the present results are encouraging, the study was conducted under *in vitro* conditions and cannot completely simulate the complex root canal environment. Future studies should evaluate these herbal irrigants using multispecies biofilm models, extracted teeth and randomized clinical trials to confirm their clinical effectiveness and determine their role as alternatives or adjuncts to conventional endodontic irrigants **[17, 19 and 20].**

Conclusion:

All herbal irrigants showed *in vitro* antibacterial activity against *Enterococcus faecalis,* with Propolis being the most effective. All four extracts demonstrated bactericidal activity, although 5.25% NaOCl remained superior. Further studies are needed to establish the safety and clinical efficacy of Propolis and Triphala for root canal irrigation.

Conflict of interest: The authors declare no conflict of interest.

Funding: No external funding was received for this study.

Ethical approval:

Ethical clearance was obtained from the Institutional Ethics Committee. As this was an *in vitro* microbiological study using a standard reference bacterial strain and no human or animal subjects were involved, patient informed consent was not required.

Data availability:

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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