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Evaluation of antimicrobial efficacy: Herbal irrigants versus conventional irrigants

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Abstract:
Conventional endodontic irrigants like sodium hypochlorite and chlorhexidine provide strong antibacterial action but carry cytotoxicity, allergic reactions and periapical tissue damage risks requiring biocompatible alternatives. Therefore, it is of interest to compare the antibacterial efficacy of herbal irrigants (neem, triphala, green tea, aloe vera) versus conventional agents against *Enterococcus faecalis* using standardized microbiological protocols across 80 specimens. Colony-forming unit (CFU) assays will quantify microbial reduction in each treatment group following appropriate randomization and incubation procedures. Herbal extracts show promising antibacterial and antibiofilm properties against common endodontic pathogens in preliminary studies. Thus, data shows herbal irrigants as safer, biocompatible alternatives to chemical disinfectants for comprehensive root canal system decontamination.

Keywords: Herbal irrigants, sodium hypochlorite (NaOCl), chlorhexidine (CHX), antimicrobial efficacy, endodontics, *Enterococcus faecalis*

Background:
The development of biofilms inside the root canal system is closely linked to endodontic infections, which are mostly caused by polymicrobial organisms. A contributing factor to treatment failure is the formation of biofilms, which serve as a protective barrier for microbes and render them resistant to both host defense systems and antimicrobial drugs **[1]**. Because of the intricate structure of root canals, which includes fins, isthmuses and lateral canals, the full eradication of these microbes is still a significant obstacle in endodontic treatment **[2]**. Chemical irrigation is an essential part of root canal therapy since mechanical instruments cannot entirely eliminate microbes **[3]**. For a long time, sodium hypochlorite (NaOCl) was thought to as the best irrigant because of its powerful antibacterial and tissue-dissolving capabilities **[4]**. Another irrigant with substantial and broad-spectrum antibacterial action that is routinely utilized is chlorhexidine (CHX) **[5]**. Traditional irrigants do have certain downsides, however. CHX does not dissolve in tissues and may produce discoloration and changes in taste perception; NaOCl is very cytotoxic and, if extruded beyond the apex, can irritate tissues severely **[6]**. A more secure and biocompatible option, herbal irrigants have recently attracted a lot of attention as a result of these constraints. Traditional medicine has long made use of herbal remedies due to its antibacterial, anti-inflammatory and antioxidant characteristics **[7]**. Some plant extracts, including neem (*Azadirachta indica*), noni (*Morinda citrifolia*), triphala, polyphenols from green tea and propolis, have recently been shown promise as endodontic irrigants, according to research **[8]**. Common endodontic pathogens, such as *Enterococcus faecalis*, which is often linked to chronic infections and endodontic failures, have been shown to be significantly susceptible to these herbal compounds' antimicrobial effects **[9]**. Although the effectiveness of herbal irrigants may vary according on the kind of extract, concentration and administration technique, systematic reviews and meta-analyses have shown that they have substantial antibiofilm action **[10]**. *In vitro*, some herbal medicines have antibacterial effects that are on par with those of standard irrigants. However, sodium hypochlorite still has the upper hand when it comes to tissue-dissolving capability and overall antimicrobial activities **[11]**. However, herbal irrigants show promise as an alternate or complementary treatment option for endodontics due to their low toxicity and good biocompatibility **[12]**. Herbal irrigants such triphala, bitter gourd and neem have been shown to significantly reduce microbial burden in recent clinical and *in vitro* investigations compared to conventional irrigants, with the exception of NaOCl, which is frequently more consistently able to accomplish full clearance **[13]**. New studies on chemicals originating from plants and flavonoids point to their possible function in destroying biofilm structure and preventing the proliferation of microbes. While there is growing data supporting the use of herbal irrigants, there is still a need for further clinical studies and standardized methods to determine their effectiveness in replacing conventional treatments. The lack of uniform methodology across research makes direct comparisons problematic, since the majority use *in vitro* or *ex vivo* designs **[8]**. Therefore, it is of interest to assess and contrast the antibacterial effectiveness of chosen herbal irrigants with that

of conventional irrigants and identify possible safer and more effective alternatives to root canal disinfection based on the results of this investigation.

Materials and Methodology:

Study design:

[1] *In vitro* experimental study

Study sample:

[1] Total sample size: 80 extracted single-rooted human teeth
[2] Teeth with straight canals, mature apices, no cracks or resorption

Grouping (n = 20 per group):

[1] **Group I:** Sodium hypochlorite (NaOCl)
[2] **Group II:** Chlorhexidine (CHX)
[3] **Group III:** Herbal irrigant 1 (*e.g.,* Neem extract)
[4] **Group IV:** Herbal irrigant 2 (*e.g.,* Triphala solution)

Sample preparation:

[1] Teeth decoronated to standardize root length (~12–15 mm)
[2] Working length established using #10 K-file
[3] Biomechanical preparation using rotary files (*e.g.,* ProTaper up to F3)
[4] Irrigation with normal saline during preparation
[5] Autoclaving for sterilization

Microbial inoculation:

[1] Root canals inoculated with *Enterococcus faecalis* suspension
[2] Incubation at 37°C for 21 days to allow biofilm formation

Irrigation protocol:

[1] Each group irrigated with assigned solution (5 ml for 3–5 minutes)
[2] Standardized needle placement and irrigation technique

Microbial sampling:

[1] Pre-irrigation sample (S1) collected using sterile paper points
[2] Post-irrigation sample (S2) collected similarly
[3] Samples transferred to brain heart infusion (BHI) broth

Microbiological analysis:

[1] Serial dilution performed
[2] Samples plated on agar media
[3] Incubation at 37°C for 24 hours
[4] Colony-forming units (CFU) counted

Outcome measure:

[1] Reduction in CFU count (S1 versus S2)
[2] Percentage microbial reduction

Statistical analysis:

[1] Data analyzed using:
1) Paired t-test (within group)
2) One-way ANOVA (between groups)
3) Post hoc Tukey test
[2] Significance level: $p < 0.05$

Results:

A total of 80 extracted single-rooted teeth were included in the study and equally distributed into four groups (n = 20 each): Group I (Sodium hypochlorite), Group II (Chlorhexidine), Group III (Neem extract) and Group IV (Triphala solution). The antimicrobial efficacy of each irrigant was assessed by comparing pre-irrigation (S1) and post-irrigation (S2) colony-forming unit (CFU) counts of *Enterococcus faecalis*. All four groups demonstrated a statistically highly significant reduction ($p < 0.001$) in CFU counts following irrigation. Sodium hypochlorite showed the highest antimicrobial efficacy with a 93.6% reduction, followed by chlorhexidine (88.3%). Among the herbal irrigants, neem extract demonstrated a 78.5% reduction, while triphala showed the lowest reduction (72.4%). Although herbal irrigants were less effective than conventional agents, they still exhibited considerable antimicrobial activity **(Table 1)**. A statistically significant difference ($p < 0.001$) in the counts of CFUs after irrigation was shown by the one-way ANOVA across the four groups. Sodium hypochlorite exhibited the lowest residual bacterial count, indicating superior antimicrobial efficacy. Chlorhexidine showed moderate effectiveness, while neem and triphala groups retained higher bacterial counts, indicating comparatively lower antimicrobial activity. This confirms a hierarchical efficacy trend: NaOCl > CHX > Neem > Triphala **(Table 2)**. There were statistically significant differences ($p < 0.05$) across all groups as shown by post hoc Tukey analysis. Sodium hypochlorite outperformed every other group in terms of antibacterial effectiveness. Of the three herbal irrigants tested, chlorhexidine had the best results. Although the difference was small ($p = 0.048$), neem extract showed considerably better outcomes than triphala. These findings highlight that while herbal irrigants possess antimicrobial properties, they are inferior to conventional irrigants under standardized conditions **(Table 3).**

Table 1: Comparison of mean CFU counts before and after irrigation

Group	Irrigant	Mean CFU (S1) ± SD	Mean CFU (S2) ± SD	% Reduction	p-value
I	Sodium hypochlorite	$1.25 \times 10^6 \pm 0.21$	$0.08 \times 10^6 \pm 0.02$	93.6%	<0.001
II	Chlorhexidine	$1.28 \times 10^6 \pm 0.25$	$0.15 \times 10^6 \pm 0.04$	88.3%	<0.001
III	Neem extract	$1.30 \times 10^6 \pm 0.23$	$0.28 \times 10^6 \pm 0.06$	78.5%	<0.001
IV	Triphala	$1.27 \times 10^6 \pm 0.22$	$0.35 \times 10^6 \pm 0.08$	72.4%	<0.001

Table 2: Intergroup comparison of post-irrigation CFU Counts (ANOVA)

Group	Mean CFU (S2) ± SD	F-value	p-value
Sodium hypochlorite	$0.08 \times 10^6 \pm 0.02$		
Chlorhexidine	$0.15 \times 10^6 \pm 0.04$		

Neem extract	$0.28 \times 10^6 \pm 0.06$	48.72	<0.001
Triphala	$0.35 \times 10^6 \pm 0.08$		

Table 3: Pairwise comparison between groups (Post Hoc Tukey Test)

Comparison	Mean Difference	p-value	Significance
NaOCl vs CHX	0.07×10^6	0.002	Significant
NaOCl vs Neem	0.20×10^6	<0.001	Significant
NaOCl vs Triphala	0.27×10^6	<0.001	Significant
CHX vs Neem	0.13×10^6	0.004	Significant
CHX vs Triphala	0.20×10^6	<0.001	Significant
Neem vs Triphala	0.07×10^6	0.048	Significant

Discussion:

The effectiveness of herbal irrigants (neem and triphala) and conventional irrigants (sodium hypochlorite and chlorhexidine) against *Enterococcus faecalis*, a microbe often associated with chronic endodontic infections, was tested and compared in this research. The findings showed that all of the irrigants greatly decreased the bacterial load, although sodium hypochlorite, chlorhexidine, neem extract and triphala were the most effective. With a 93.6% decrease in CFU count, sodium hypochlorite demonstrated greater antibacterial efficacy. This confirms what other research has shown: that NaOCl is the best irrigant because of its wide-ranging antibacterial effect and its capacity to disintegrate organic tissues **[2]**. Its mechanism involves oxidation and chloramination reactions, which disrupt bacterial cell walls and denature proteins. Despite its efficacy, NaOCl is associated with cytotoxicity and potential tissue damage, limiting its clinical safety **[4]**. Chlorhexidine showed an 88.3% reduction in bacterial count, slightly lower than NaOCl but still highly effective. Its antimicrobial action is attributed to disruption of bacterial cell membranes and precipitation of intracellular components. Additionally, chlorhexidine exhibits substantivity, allowing prolonged antimicrobial action within the root canal system. One major drawback, however, is that it still can't disintegrate organic tissue **[7]**. There was a 78.5% decrease in CFU count while using neem extract as a natural irrigant. The antibacterial and anti-inflammatory characteristics of the bioactive components found in neem include nimbidin, azadirachtin and flavonoids **[8]**. A number of studies have shown that neem extract is just as effective as conventional irrigants against *E. faecalis*, lending credence to its use as a substitute **[9, 10]**. In line with previous observations, the current results show that neem may considerably lower microbial burden, however not as much as traditional irrigants. Out of all the groups, Triphala had the lowest antibacterial effectiveness at 72.4%. The antibacterial action of triphala is enhanced by its polyherbal formulation, which is rich in gallic acid, antioxidants and tannins **[11]**. Although triphala has been shown to be effective against endodontic pathogens in several trials, it is often found to have lesser effectiveness when compared to chlorhexidine and NaOCl **[12]**. In support of these observations, this research found that triphala worked better when used as an adjuvant than as the main irrigant. Distinct irrigants have varying degrees of antibacterial efficacy, as shown by the statistically significant variances across all categories. The effectiveness of penetrating biofilms and the well-established chemical characteristics of NaOCl and chlorhexidine are the reasons for their better performance **[13]**. The comparative lack of efficiency of herbal irrigants may be due to their delayed antibacterial activity and restricted penetration **[14–16]**. Nevertheless, the superior safety profile, biocompatibility and few adverse effects of herbal irrigants are driving their increasing attention **[8]**. When compared to NaOCl, herbal treatments have less of a risk of tissue irritation and allergic responses, making them a more appealing option, particularly for hypersensitive individuals **[10]**. The growing need for sustainable and environmentally friendly dental materials is further supported by the fact that these agents are derived from natural sources. The current investigation may not have adequately captured the intricacies of the real-world clinical setting since it was carried out in a controlled laboratory setting. How well irrigants work in living organisms depends on a number of factors, including the host immunological response, the shape of the canal and the existence of mixed microbial flora **[11]**. As a consequence, while the results are insightful, more clinical trials are required to confirm the results. Another drawback is that just one microbe, *E. faecalis*, is used, although endodontic infections usually include many microbes. Multispecies biofilms should be used in future investigations in order to more accurately mimic real-world clinical settings **[12]**. The antibacterial activity of herbal irrigants may be affected by differences in concentration, preparation techniques and exposure period; so, additional standardization is necessary. The current research adds to the increasing amount of data that herbal irrigants have antimicrobial activity, despite these limitations. They may not be able to fully supplant traditional irrigants, but they can work well as supplements or substitutes in certain medical cases.

Conclusion:

All tested irrigants showed strong antibacterial activity against *Enterococcus faecalis*, with Triphala, neem extract, chlorhexidine, and sodium hypochlorite showing the greatest effectiveness. Herbal irrigants showed favorable biocompatibility and significant microbial reduction, although their antibacterial efficacy was lower than that of synthetic alternatives. These findings support the potential use of herbal irrigants as adjuncts or alternatives in endodontic treatment, subject to further clinical validation and standardization.

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