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Original Research Article

Comparative evaluation of antimicrobial activity of sodium hypochlorite, Triphala, tea tree oil, and green tea associated with manual irrigation against *Enterococcus faecalis (E. Faecalis)* - An in vitro study

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Abstract

Aim: The aim of the study was to compare and evaluate the antimicrobial efficacy of 3 different herbal irrigants using manual irrigation technique through colony forming unit assays against Enterococcus faecalis (E. faecalis) as intracanal irrigants.

Background/Introduction: Enterococcus faecalis is a predominant pathogen associated with persistent endodontic infections and root canal treatment failures. Effective irrigation is crucial for eradicating these bacteria from the complex root canal system. Sodium hypochlorite (NaOCl) is widely used for its potent antimicrobial properties, but its cytotoxicity and potential tissue irritation raise concerns. Herbal alternatives such as Triphala, Tea Tree Oil, and Green Tea have exhibited antibacterial activity, making them potential substitutes for conventional irrigants. However, limited research has directly compared their efficacy in root canal disinfection.

Materials and Methods: Intact, caries-free, single-rooted mandibular 1st premolars extracted for orthodontic purposes were selected. The teeth were decoronated below the cementoenamel junction to a uniform root length of 15mm and were divided into 6 groups based on irrigants: G1 (Positive control: saline irrigation), G2 (Negative control: no inoculation or treatment), G3 (2.5% NaOCl), G4 (Triphala), G5 (Tea Tree Oil), and G6 (Green Tea). First set of samples were collected at 21 days post-inoculation and the second sample was taken post irrigation. Microbiological samples were incubated at 37°C for 18-24 hours to obtain CFU counts. Data were analyzed using Kruskal Wallis and Dunn's post hoc tests for pre-treatment and post-treatment CFU comparison, and Wilcoxon Signed Rank Test for within-group analysis.

Results: Results showed that Groups 3, 4, 5, and 6 significantly reduced CFUs ($p < 0.001$), with 2.5% NaOCl (Group 3) being the most effective, followed by Tea Tree Oil (Group 5), Triphala (Group 4), and Green Tea (Group 6).

Conclusion: In conclusion, Triphala, Tea Tree Oil, and Green Tea showed significant antibacterial activity against E. faecalis, with Tea Tree Oil being the most effective after Sodium Hypochlorite. While herbal alternatives may offer benefits due to the drawbacks of NaOCl, further research is required on their preparation, biocompatibility, and toxicity before clinical use.

Keywords: Sodium hypochlorite, Triphala, Green Tea, Tea Tree Oil, *E. faecalis*, CFU

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1. Introduction

The metabolic by-products of microorganisms play a pivotal role in the initiation and progression of pulp and periapical lesions, ultimately leading to pulp necrosis and triggering inflammatory responses. These microbial metabolites contribute significantly to the pathogenesis of dental infections, highlighting their importance in the development of pulpal and periapical diseases.[1] Root canal infections are typically polymicrobial, with *Enterococcus faecalis* being one of the most significant pathogens involved.[2] Enterococcus faecalis in compromised root canals accounts for 80–90% of infections, significantly causing endodontic therapy failure. Negative bacterial cultures in root canals aid periapical healing. Beyond mechanical cleaning, effective irrigation is crucial for eliminating microorganisms, especially in inaccessible areas.[3]

The successful outcome of endodontic treatment is contingent upon the effective reduction or complete

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eradication of bacteria present in an endodontic infection. Chemo-mechanical debridement plays an important role in root canal disinfection.[1]

While sodium hypochlorite has been the preferred choice for irrigation due to its capacity to dissolve organic matter and strong antimicrobial properties, its usage comes with significant drawbacks. These include irritation to periapical tissues, unpleasant taste, high toxicity, instrument corrosion and burning of surrounding tissues.[4]

Triphala, an Ayurvedic herbal remedy, comprises dried and powdered fruits from three medicinal plants: Terminalia chebula, Terminalia bellerica, and Emblica officinalis. Citric acid, found abundantly in Triphala, plays a role in smear layer removal and functions as a chelating agent. Notably, Triphala possesses remarkable antimicrobial and anti-inflammatory properties making a significant contribution in the cleaning of root canals.[5]

Tea Tree, scientifically called Melaleuca alternifolia, is an indigenous Australian plant renowned for its versatile properties in dentistry. Acting as both antiseptic and antifungal agent, its gentle solvent effect indicates promising prospects in root canal treatment. The primary active compound, Terpinen-4-ol, accounts for the antibacterial and antifungal characteristics of Tea tree oil.[6]

Derived from the young shoots of the tea plant Camellia sinensis, green tea polyphenols demonstrate antimicrobial effects on pyknotic cells of Enterococcus faecalis. Furthermore, the remarkable chelating capabilities demonstrated by green tea implies promising potential for its utilization in root canal treatment.[7]

Nevertheless, limited information or evidence exists regarding the antimicrobial properties of Triphala, Tea Tree Oil, and Green Tea in endodontics. Thus, this in-vitro study was conducted to evaluate and compare the efficacy of herbal root canal irrigants (Triphala, Tea Tree Oil, and Green Tea) with sodium hypochlorite against E. faecalis by assessing the sterility of canal through CFU assay.

2. Materials and Methods

Forty-two intact, caries-free, single-rooted mandibular 1st premolars extracted for orthodontic purposes were used. The extracted teeth were cleared of calculus and periodontal tissue and stored in 10% buffered formalin, then placed in 0.9% saline at 4°C prior to instrumentation.

Teeth were decoronated using a low-speed rotating diamond saw at 20,000 rpm, 15mm from the apex, achieving uniform root length. A coronal access cavity was prepared with a No. 2 access cavity preparation bur. A size #10 K-file was used to determine the working length (1mm short of the apical foramen), confirmed radiographically. A #15 K-file was used to create a glide path. NiTi instruments were operated with an endodontic motor using ethylenediaminetetraacetic acid (EDTA) lubricant. Root canals were instrumented with ProTaper Gold rotary instruments using a crown-down technique:

SX file (300 rpm, 5.10 N*cm torque) up to half of the working length (WL),
S1 file (300 rpm, 5.10 N*cm torque) up to 4mm short of the apex,
S2 files (300 rpm, 1.50 N*cm torque) to full WL,
F1, F2, F3, and F4 files (300 rpm, 3.10 N*cm torque) to full WL.

Each instrumented canal was irrigated with 5ml of 2.5% NaOCl after each file. Final irrigation was performed with 2mL of 17% EDTA followed by saline. Root canals were rinsed with 2mL of 5% sodium thiosulfate to neutralize the residual activity of NaOCl. The apex was filled with light-cured block-out resin, and the external root surface was sealed with two layers of nail polish to create a closed system. Each specimen was fixed with Putty Condensation Silicone in an Eppendorf tube [**Figure 2**] and autoclaved at 121°C for 30 minutes. Sterility was confirmed with a biological indicator.

Enterococcus faecalis ATCC 29212 was propagated in brain heart infusion broth at 37°C for 24 hours. Twenty microliters of bacterial inoculum were added to each canal using a micro-pipette. A sterile size 10 K-file was inserted into the canal and agitated six times to coat the canal wall with the inoculum. The teeth were incubated at 37°C in an aerobic chamber for 21 days to form a biofilm. Fresh inoculum was added on alternate days to prevent saturation.

The 42 teeth were randomly divided into six groups (seven teeth each) [**Figure 2**]. Groups received the following treatments:

1. Group I**:** Positive control (saline irrigation),
2. Group II**:** Negative control (no inoculation, no treatment),
3. Group III**:** 2.5% NaOCl,
4. Group IV: Triphala,
5. Group V**:** Tea Tree Oil,
6. Group VI**:** Green Tea.

Each root canal was irrigated with 10mL of the assigned irrigant for 3 minutes. The irrigation solutions were prepared as follows: For the Triphala solution, Triphala powder (B.V. PUNDIT'S) was dissolved in 10% Dimethyl sulfoxide (DMSO) to achieve a concentration of 5 mg/mL. Tea tree oil was mixed with 85% ethanol to create a 2% concentration. Lastly, 120g of green tea (from K Patel Pvt Ltd) was combined with 5mL of 10% DMSO to prepare the green tea solution.

After treatment, each root canal was filled with sterile saline. A sterile K-file #60 was inserted and agitated for 30 seconds. An absorbent paper point was then inserted into the canal, agitated for 30 seconds, and transferred to a tube with 450µL of sterile saline. The solution was homogenized and diluted to 10^{-3} Aliquots of 100 µL solution and the dilutions were cultivated on the surface of the blood agar. The plates were incubated at 37°C for 18-24 hours, and the colony-forming units (CFUs) were counted to assess the microbial load.

3. Results

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Descriptive analysis of CFUs/ml was performed for each group. The data is expressed as mean and standard deviation (Mean ± SD) for quantitative variables across the groups. The Kruskal Wallis Test followed by Dunn's post hoc Test was used to compare the mean CFUs/ml between 6 groups at Pre and Post Treatment period. The Wilcoxon Signed Rank Test was used to compare the mean CFUs/ml between Pre and Post treatment period in each group. The level of significance was set at $p<0.05$.

While comparing the four irrigating solutions for their ability to reduce bacterial load, it was found that solutions from Groups 3, 4, 5, and 6 showed statistically significant improvements ($p < 0.001$) in reducing CFUs. Group 2, the negative control group, did not show any significant change (**Table 1**).

Among the tested solutions, 2.5% NaOCl (Group 3) performed the best, followed by Group 5(Tea Tree Oil), Group 4(Triphala) and Group 6(Green Tea). The Positive Control group (Group 1) also showed effective results in reducing E. faecalis across all tested groups (**Table 2**).

Table 1: Comparison of mean CFUs (X 10^5 / mL) between pre & post-treatment period in each group using wilcoxon signed rank test

Comparison of mean CFUs (x 105 / ml) between Pre & Post-treatment period in each group using Wilcoxon Signed Rank Test						
Groups	**Time**	**N**	**Mean**	**SD**	**Mean Diff**	**p-value**
Group 1	Pre -Treatment	7	154.43	6.75	22.29	0.02*
	Post Treatment	7	132.14	19.97		
Group 2	Pre -Treatment	7	0.00	0.00	0.00	..
	Post Treatment	7	0.00	0.00		
Group 3	Pre -Treatment	7	151.43	7.41	151.29	<0.001*
	Post Treatment	7	0.14	0.38		
Group 4	Pre- Treatment	7	150.29	8.28	111.43	<0.001*
	Post Treatment	7	38.86	3.72		
Group 5	Pre- Treatment	7	151.86	2.19	135.15	<0.001*
	Post Treatment	7	16.71	5.79		
Group 6	Pre - Treatment	7	148.86	4.63	92.86	<0.001*
	Post Treatment	7	56.00	4.00		

* - Statistically Significant

Group 1: Positive control (Saline), Group 2: Negative Control (No Inoculation, No treatment), Group 3: 2.5% NaOCl, Group 4: Triphala, Group 5: Tea Tree Oil, Group 6: Green Tea

Table 2: Multiple comparison of mean difference in the CFUs/mL between groups during post-treatment

Multiple comparison of mean difference in the CFUs/ml between groups during Post-treatment period using Dunn's Post hoc Test					
(I) Groups	**(J) Groups**	**Mean Diff. (I-J)**	**95% CI for the Diff.**		**p-value**
			Lower	**Upper**	
Group 1	Group 2	132.14	118.03	146.26	0.001*
	Group 3	132.00	117.88	146.12	0.001*
	Group 4	93.29	79.17	107.40	0.002*
	Group 5	115.43	101.31	129.55	0.002*
	Group 6	76.14	62.03	90.26	0.002*
Group 2	Group 3	-0.14	-14.26	13.97	0.32
	Group 4	-38.86	-52.97	-24.74	0.001*
	Group 5	-16.71	-30.83	-2.60	0.001*
	Group 6	-56.00	-70.12	-41.88	0.001*
Group 3	Group 4	-38.71	-52.83	-24.60	0.001*
	Group 5	-16.57	-30.69	-2.45	0.001*

	Group 6	-55.86	-69.97	-41.74	0.001*
Group 4	Group 5	22.14	8.03	36.26	0.002*
	Group 6	-17.14	-31.26	-3.03	0.002*
Group 5	Group 6	-39.29	-53.40	-25.17	0.002*

* - Statistically Significant

Group 1: Positive control (Saline), Group 2: Negative Control (No Inoculation, No treatment), Group 3: 2.5% NaOCl, Group 4: Triphala, Group 5: Tea Tree Oil, Group 6: Green Tea

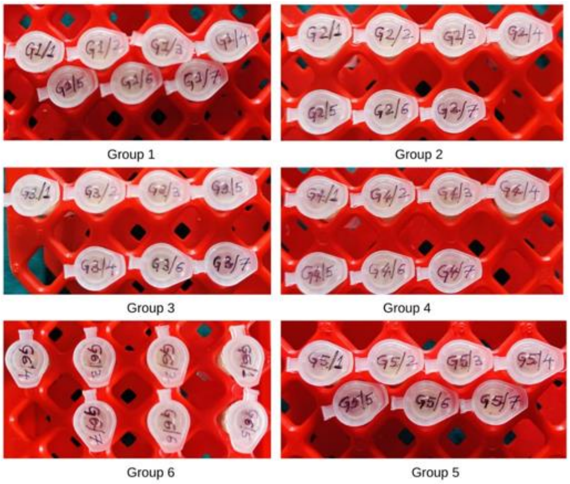

Figure 1: 42 teeth were randomly divided into six groups (seven teeth each)

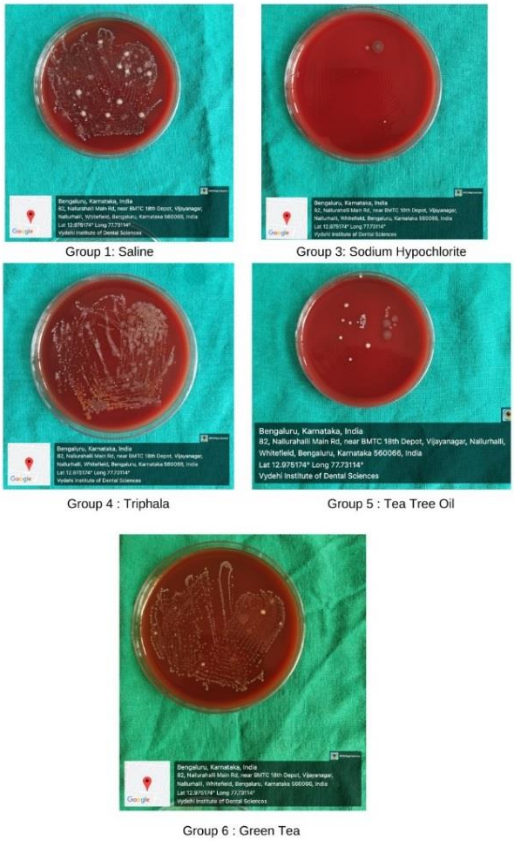

Figure 2: CFU/mL post treatment with irrigants

4. Discussion

Primary endodontic infections result from the accumulation and proliferation of oral microorganisms, particularly opportunistic pathogens, within the root canal. Over time, these microorganisms increase in number, eventually reaching a threshold that triggers infection. Typically harmless in a healthy oral cavity, these pathogens become harmful when they thrive in the enclosed environment of a compromised root canal.[8] In this study, extracted human teeth were utilized as representative samples.

The American Type Culture Collection (ATCC) catalog includes 69 commercially available strains of *Enterococcus faecalis*. Among these, the ATCC 29212 strain stands out for its heightened resistance compared to other strains. This strain demonstrates unique characteristics in its reaction to a range of medications and irrigants commonly used in endodontic treatments. Its increased resistance can be attributed to its origin, as it was initially isolated from the urinary tract, an environment that may have contributed to its ability to withstand changes in pH and resist antimicrobial agents. This distinct behavior makes it an important subject for research, particularly in evaluating the efficacy of various endodontic treatments, as it challenges the effectiveness of conventional therapeutic strategies.[9] Enterococcus faecalis is the most frequently isolated species from the canals of teeth exhibiting post-treatment disease. This Gram-positive, catalase-negative, fermentative, non-sporing facultative anaerobic coccus can invade dentinal tubules, raising the likelihood that microorganisms surviving chemo-mechanical instrumentation and intracanal medications may colonize these tubules and subsequently reinfect the obturated root canal, contributing to a significant proportion of root canal failures.[10] Given the organism's inherent characteristics and its ability to thrive and persist in cases where therapy has failed, it is of significant importance and serves as a critical focus for this study.

Sodium hypochlorite (NaOCl) is the most frequently employed root canal irrigant, primarily due to its strong antimicrobial activity, which is attributed to its highly alkaline pH. The hydroxyl ions released by NaOCl disrupts the structural integrity of bacterial cytoplasmic membranes, leading to irreversible enzyme inactivation and significant interference with biosynthetic pathways critical to the bacteria's cellular metabolism and survival.[11]

The effectiveness of NaOCl on E. faecalis is well documented in literature.[12,13] A study was conducted to compare the efficacy of commonly used medicaments against E. faecalis cultured as a biofilm on dentine substrate and concluded that sodium hypochlorite remains the gold standard for bacterial elimination in root canal therapy. The use of sodium hypochlorite as an endodontic irrigant poses certain challenges due to its high toxicity at elevated concentrations. Its potent chemical properties, while effective in disinfecting the root canal system, can lead to significant tissue irritation upon direct contact. This irritation stems from its caustic nature, which can damage surrounding periapical tissues or oral mucosa if not carefully managed. Consequently, its application requires precise control and appropriate dilution to mitigate risks while maintaining its antimicrobial efficacy.[14] Therefore, in this study, the selected irrigants were compared with sodium hypochlorite to evaluate their effectiveness.

The measurement of CFUs, expressed in log CFU/mL, was employed to evaluate the effects of the proposed treatments in this study.[**Figure 2**] The CFU method has two significant advantages. Firstly, it exclusively counts live bacteria, as it disregards dead cells and other debris. Secondly, it can accommodate counts of any number of bacteria through dilutions if the count is too high, or by using concentrations if the count is too low.[15]

Green tea is made from unfermented leaves and is a rich source of polyphenols, especially flavonoids like catechins, catechin gallates, and proanthocyanidins.[16] A study was conducted to evaluate the antimicrobial efficacy of Triphala, green tea polyphenols (GTP), MTAD, and 5% sodium hypochlorite against E. faecalis biofilm formed on tooth substrate and concluded that Triphala, green tea polyphenols and MTAD showed statistically significant antibacterial activity against E. Faecalis.[8]

Triphala, an Ayurvedic herbal remedy, comprises dried and powdered fruits from three medicinal plants: Terminalia chebula, Terminalia bellerica, and Emblica officinalis.[5] A study was conducted to evaluate the adequacy of smear layer removal and cytotoxicity potential of Triphala in comparison to sodium hypochlorite and concluded that Triphala can be considered as a superior irrigant with good antibacterial efficacy and least cytotoxicity potential compared to conventional hypochlorite irrigating agent.[17]

Tea tree essential oil (TTO) is obtained from the leaves of Melaleuca alternifolia through steam distillation. It possesses broad-spectrum antibacterial properties and is considered bio-safe, frequently used to treat infections caused by fungi, bacteria, or viruses.[18] A study was conducted to compare the antibacterial efficacy of tea tree oil with 3% sodium hypochlorite and 2% chlorhexidine as a root canal irrigant, against E. faecalis. and concluded that Tea tree oil showed statistically significant antimicrobial activity against Enterococcus faecalis, which is very similar to sodium hypochlorite and chlorhexidine.[6] However, few studies have evaluated the comparative antimicrobial efficacy of NaOCl, Tea Tree Oil, Triphala, and Green Tea as irrigants against endodontic pathogens such as E. faecalis. This study aimed to fill this limitation in literature.

In our study NaOCl, Triphala, Tea tree oil and Green Tea were compared for reduction in bacterial load, we found that each irrigating solution had statistically improved results ($p < 0.001$) against *E. faecalis* for all the groups except the negative control group i.e., Group 2. This was in concurrence with the previous studies done using Triphala, green tea polyphenols (GTP), MTAD, 5% sodium hypochlorite,[8] and tea tree oil with 3% sodium hypochlorite and 2% chlorhexidine[6] were tested against E. faecalis and were found to have a significant antibacterial activity.

Result of our study showed that NaOCl group performed the best and the results were similar to the previous studies.[19] In comparison, NaOCl group performed better than Triphala, Green Tea and Tea Tree oil group in reduction of bacterial load and the results were similar to the previous studies where irrigation of NaOCl was found to have better antimicrobial activity than irrigation of Triphala,[8,17,19] Green Tea[8] and Tea Tree oil[6] against *E. faecalis*.

Endodontic infections are polymicrobial and interactions between multiple organisms could potentially have different dynamics than were demonstrated by our study. The limitation of this study is that a single micro-organism was used to infect the root canal.

To evaluate the effectiveness and efficiency of other irrigants, further studies are required with other most prevalent endodontic microorganisms, variable irrigant concentrations, contact times, and volumes in the root canal.

5. Conclusion

Within the limitations of this study, it was concluded that 2.5% NaOCl, Tea Tree Oil, and Triphala all demonstrated significant antibacterial activity against E. faecalis. Among the four tested endodontic irrigants, 2.5% NaOCl (Group 3) performed the best, followed by Tea Tree Oil (Group 5), Triphala (Group 4), and Green Tea (Group 6). The use of herbal alternatives as root canal irrigants offers potential advantages, especially considering the undesirable properties of sodium hypochlorite. However, further studies are required to assess the biocompatibility of these tested irrigants.

6. Ethical No.

VIDS-IEC/UG/APP/2024/119.

7. Source of Funding

None.

8. Conflict of Interest

None.

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