ORIGINAL RESEARCH

Comparative Evaluation of Antimicrobial Efficacy of Sodium Hypochlorite, Triphala, *Eucalyptus*, and Carvacrol against *Enterococcus faecalis*: An *In Vitro* study

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Abstract

Objective: This study aimed to evaluate the antimicrobial effect of sodium hypochlorite (NaOCl), Triphala, *Eucalyptus*, and carvacrol on *Enterococcus faecalis* as the most common microorganism isolated from infected root canals.

Materials and methods: Seventy-five mandibular premolar teeth were randomly distributed into five study groups: 5.25% NaOCl, 10% Triphala, 1.25% *Eucalyptus*, 0.6% carvacrol, and negative control (saline) group. Samples were taken using paper points from the canal spaces and using Gates–Glidden (GG) drills from dentinal tubules; after sample culturing, the colony forming unit (CFU) were counted, which was analyzed by the Wilcoxon signed-rank test.

Results: All irrigants have shown a reduction of microorganisms in the root canal space. After the use of NaOCl and *Eucalyptus*, the bacterial count was significantly reduced in the canal, as well as dentin sampling, as compared to Triphala and carvacrol. The antimicrobial efficiency of all irrigants against *E. faecalis* revealed a significant difference ($p < 0.05$).

Conclusion: All irrigants exerted significant antimicrobial activity against *E. faecalis*. Around 1.25% of *Eucalyptus* was the most effective irrigant than 5.25% of NaOCl, Triphala, and carvacrol.

Keywords: Antimicrobial agent, Carvacrol, *E. faecalis*, *Eucalyptus*, Triphala.

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Introduction

The aim of endodontic management is the disinfection of the root canals along with dentinal tubules from bacteria, which is the main cause of endodontic diseases. Root canal is an intricate system with multiple portals, including fins, anastomoses, and lateral canals, that help to harbor and grow bacteria. These kinds of canals are complicated to clean biomechanically, where an irrigant solution does not reach them easily. The development of persistent root canal infections may occur in these complex anatomical structures.[1] *E. faecalis* is the key etiological bacteria for persistent periapical pathology, which is gram-positive, facultative, and anaerobic cocci. Physicochemical characteristics of *E. faecalis*, like the biofilm formation, inherent bacterial resistance, and capability to invade dentine, help in its growth and endurance in starvation conditions.[2–4]

Biomechanical preparation can help to remove most bacteria in pulp space, though antibacterial solutions/irrigants are crucial for successful endodontic treatment.[5] Numerous antibacterial irrigant solutions are used along with biomechanical preparation. Currently, NaOCl is the most accepted and commonly used root canal irrigant with better intracanal efficacy against *E. faecalis*.[5] Though it has some limitations and drawbacks like corroding to devices, a decrease in flexural strength, and elastic modulus of dentine.[6] Effect on surrounding tissues due to cytotoxicity, unlikable taste, chemical effects prompted by intracanal irrigants, and increasing antibiotic resistance by microbes, newer intracanal irrigant solutions need to be searched and considered.

The usage of herbal medicines as root canal irrigants in endodontic therapy has gained increased interest. Some studies in the past have shown the antibacterial efficiency of *Eucalyptus* leaf extract.[7] Essential oil extract from *Eucalyptus globules* leaves has shown antibacterial effectivity on gram-negative bacteria (*Escherichia coli*) and gram-positive bacteria (*Streptococcus aureus*).[7] Triphala is a herbal medicine that primarily contains "amulaki" (*Emblica officinalis*), "halituki" (*Terminalia chebula*), and "bibhitaki" (*Terminalia bellirica*). The citric acid of the fruits works as a chelating agent, which helps in the elimination of the smear layer from the canal wall.[8–15] Prabhakar et al. evaluated the antibacterial efficacy of Triphala as an irrigant against *E. faecalis*.[15] Carvacrol is one of the ingredients of organum and essential oil that is synthesized by the fusion of cymol sulfonic acid and caustic potash. It has broad-spectrum antimicrobial activity and increases the nonselective permeability of bacterial cell membranes.[9]

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Considering the drawbacks or shortcomings of currently used root canal irrigants, the need for the search for antimicrobial solutions as root canal irrigants with more efficacies of disinfection and better biological as well as chemical properties continues. The rationale of this research is to search and evaluate root canal irrigants that are equally or more effective than NaOCl against a selective endodontic pathogen. No study has been found in the literature which compares the antimicrobial efficiency of NaOCl, *Eucalyptus*, Triphala, and carvacrol as an irrigant in endodontic treatment. Therefore, *in vitro* study is proposed to compare the antimicrobial effect of these test irrigants against *E. faecalis*.

Materials and Methods

The research study was conducted in the Department of Pediatric and Preventive Dentistry, Department of Microbiology, and Microbiology Laboratory.

Ethical Approval

The study protocol was reviewed and approved by the Regional Ethical review board (MUHS/PG-T/E1/2593).

Preparations of Specimens

Seventy-five human mandibular premolars without any caries were chosen for the research. The single-rooted teeth without any cracks, grooves, resorption, and canal obliteration were selected. Periodontal curettes were used to clean the outer tooth surface; after that, they were kept in 2.5% NaOCl solution (Nimai Dento, India) for decontamination and kept in the saline solution till used. The crown portions of all premolars were cut, and standardized root lengths of 15 mm were achieved. The biomechanical preparation was done using K-files till #20 (Dentsply-Maillefer, Ballaigues, Switzerland using tap water irrigation. An ultrasonic bath with 17% ethylenediaminetetraacetic acid (Prime Dental Products Pvt. Ltd., Thane, India) for 10 minutes was used to remove the smear layer, followed by 5.25% NaOCl irrigation for 10 minutes and 1 hour under tap water to remove chemicals. The root apices and surface of teeth samples were enclosed with resin and nail polish, respectively, to prevent microbial leakage. The samples were autoclaved at 121°C for 15 minutes after being transferred into glass tubes containing brain heart infusion (BHI) broth medium (Merck, Darmstadt, Germany) and then kept in an incubator at 37°C for 48 hours.

E. faecalis (ATCC 29212) was acquired from Central Scientific Instruments Organisation, Chandigarh, India, and full-grown overnight in BHI until 0.5 McFarland standard (1.5 × 108 CFU/mL) turbidity was achieved. The glass tubes with samples were opened to replace 2 mL of sterile BHI with 2 mL of the bacterial inoculum and incubated at 37°C for 21 days. The glass tubes were revived every 2 days to verify the growth of bacteria. The contamination was verified by gram staining, colony morphology assessment on BHI, *E. faecalis* broth, and bile esculin tests after 21 days. The teeth would be excluded after contamination.

Preparation of Irrigants

Triphala

A ready-made available Triphala (IMPCOPS Ltd., Chennai, India) churn was used. Triphala irrigant of 10% was made by dissolving Triphala powder in dimethyl sulfoxide (DMSO 10%) (S.D. Fine Chem Pvt. Ltd., India).

Eucalyptus Leaf Extract

The fresh leaves of *Eucalyptus* plants were collected. Leaves were washed with distilled water and then kept for drying at room temperature and powdered. The powder was then extracted with ethanol by using the Soxhlet apparatus for 24 hours. The extract was concentrated using a rotary evaporator. The extracts were again dissolved in DMSO to get a concentration of 1.25%.

Carvacrol

A ready-made available carvacrol preparation was used. To adjust the required concentration, carvacrol was dissolved in DMSO, and a concentration of 0.6% was achieved.

Antimicrobial Assessment

The study samples were irrigated using sterile saline water and dried clean with gauze, and randomly placed into five groups (n = 15), according to root canal irrigant solutions, as follows: group I, 5.25% NaOCl; group II, 10% Triphala; group III, 1.25% *Eucalyptus* extract; group IV, 0.6% carvacrol; and group V, saline (negative control). After dividing the teeth samples into five groups to determine and compare the antimicrobial effect of test irrigants, the sampling of each canal before the use of test irrigant was done using sterile paper points and transferred to a tube having 1 mL of BHI and left for bacteriological evaluation. Subsequently, the crown-down technique was used for cleaning and shaping root canals by using Protaper universal rotary files till F2 with a one-length technique as per the manufacturer. After that, 2 mL of each irrigant was used for canal irrigation (5.25% NaOCl, 1.25% *Eucalyptus* extract, 10% Triphala, 0.6% carvacrol, and saline) for 5 minutes using a 29-gauge needle before using new instruments. The canals were irrigated with 4 mL of saline solution after the use of new instruments.

Bacterial Sampling

Root Canal Sample

Sample collection was done using sterile paper points (Meta Dental Co., Seoul, Korea) from each root canal after instrumentation. The paper points soaked the moisture inside the canal, which were then placed in tubes having 1 mL of BHI broth. The glass tubes with paper points were held on a vortex mixer so that the microorganisms on the paper points were dispersed uniformly. After that, streaking was done using a microstreaker (inoculation loop). The plates were then kept in an incubator at 37°C overnight, and CFU was observed and counted (Fig. 1).

Dentin Sample

After canal sampling, dentin samples from canal walls were collected after instrumentation was done by using #3, 4, and 5 sterile GG drills (Dentsply-Maillefer, Ballaigues, Switzerland). The GG drill #3, 4, and 5 removes dentine flakes from the canal wall in depths of 200, 400, and 600 µm, respectively. The spiral flutes on the GG drill were collected from the dentinal shavings, which were directly collected in separate test tubes with the help of a micro brush, and the streaking was done on plates. The plates were then kept in an incubator at 37°C overnight, and CFU was counted. The total number of bacterial colonies that appeared multiplied by the dilution factor gives the total number of CFU per tooth. All the experiment was performed in triplicates under strict aseptic condition. The purity of the infection was checked to confirm the purity of bacterial growth.

Statistical Analysis

Colony forming unit obtained data were imported from Excel into the software Statistical Package for the Social Sciences (SPSS 20.0; IBM, Armonk, New York, USA) for analysis. Probability $p < 0.05$ is considered as significant, as alpha error set at 5% with a confidence interval of 95% set in the study. Data were found to be nonparametric in nature, as they could not follow normality principles. Hence, a nonparametric test was used. Wilcoxon signed-rank test was used to find out statistical significance in relation to the antimicrobial efficacy of each study group against *E. faecalis* before and after irrigation. Kruskal–Wallis *H* test was applied to compare antimicrobial efficacy among the five groups.

Results

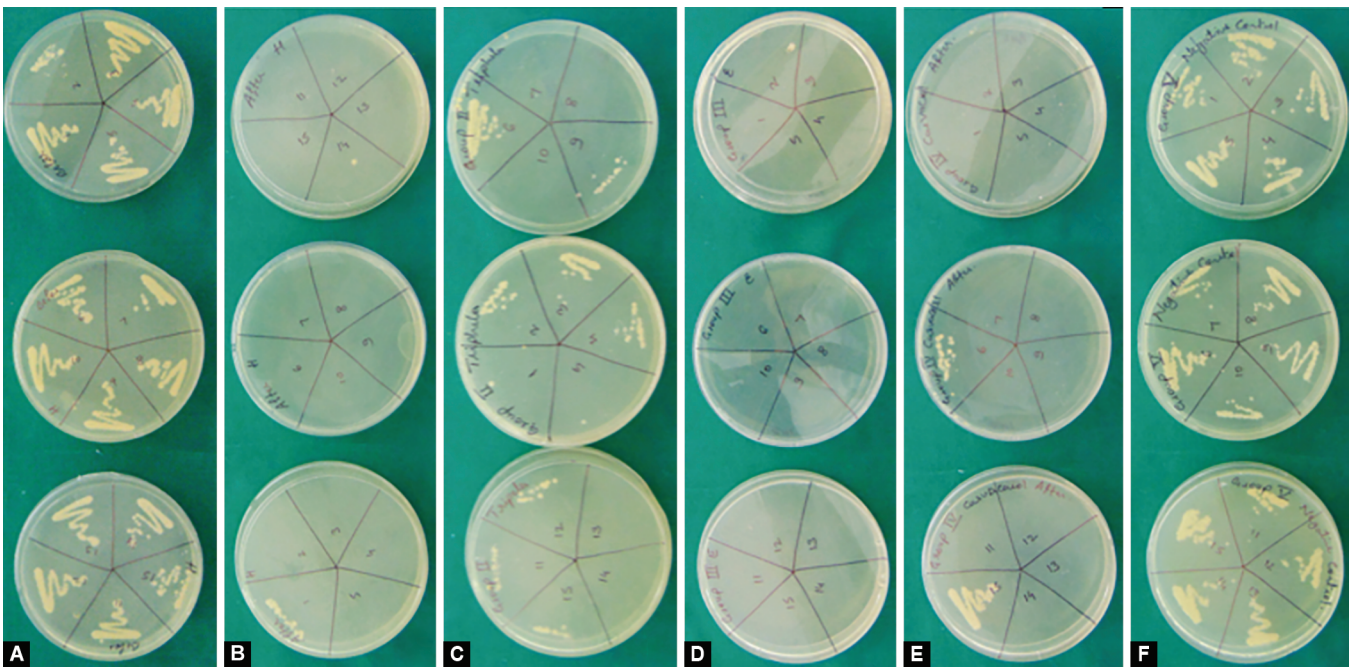
The overall antimicrobial efficiency of all test irrigants against E. *faecalis* before and after biomechanical preparation and irrigation is represented in Table 1 and Figure 2. All the test irrigants have significantly reduced bacteria when compared with the negative control group ($p < 0.05$). There was a significant difference found in CFU count for all test groups ($p < 0.05$). The reduction of bacterial CFU count after the use of *Eucalyptus* extract and NaOCl was more, and they have shown efficient results than Triphala and carvacrol.

According to the results obtained in our study, the *Eucalyptus* extract has shown complete inhibition of *E. faecalis*. NaOCl has shown a significant reduction in bacteria after *Eucalyptus*. The overall reduction in microorganisms after the use of Triphala and carvacrol was significantly less when compared with other groups.

The mean CFU count obtained from dentin samples for all study groups in all depths was significantly less compared to the negative control group (saline group) ($p < 0.05$) (Table 2 and Fig. 3). Antimicrobial effect of *Eucalyptus*, NaOCl, carvacrol, and Triphala was observed in all depths, that is, 200, 400, and 600 µm. The efficacy of 10% Triphala was noticeably <1.25% *Eucalyptus*, 5.25% NaOCl, and carvacrol. *Eucalyptus* has shown the highest antimicrobial effect against *E. faecalis* followed by NaOCl, carvacrol, and Triphala. Moreover, the analysis points out a significant increase in log CFU counts by increasing dentin depth.

Discussion

The prime objective of endodontic treatment is the complete cleaning and disinfection of the root canal system.[10] Ability of bacteria to penetrate into the dentine wall, inadequate diffusion of irrigants into dentinal tubules, and inactivation of these irrigants by dentin complicate the complete disinfection.[2] *E. faecalis* is observed



Figs 1A to F: CFU obtained before use of irrigants (A); CFU obtained after use of irrigants in respective groups as (B) group I, (C) group II, (D) group III, (E) group IV, and (F) group V

Table 1: Intragroup comparison of antimicrobial efficacy of all test irrigants against *E. faecalis* before and after irrigation in terms of change in CFU count

	Mean CFU ± standard deviation (SD)			
Groups	*Before irrigation*	*After irrigation*	*Wilcoxon signed-rank test*	*p-value, significance*
Group I (hypochlorite)	103.16 (18.12)	1.08 (3.15)*	W = −5.857	$p < 0.001$**
Group II (Triphala)	103.16 (18.12)	13.40 (14.74)*	W = −5.843	$p < 0.001$**
Group III (*Eucalyptus*)	103.16 (18.12)	0.0 (0.0)*	W = −5.867	$p < 0.001$**
Group IV (carvacrol)	103.16 (18.12)	6.24 (16.12)*	W = −5.446	$p < 0.001$**
Group V (negative control)	103.16 (18.12)	58.77 (15.67)	W = −5.589	$p < 0.001$**

*$p < 0.05$, significant difference; **$p < 0.001$, highly significant

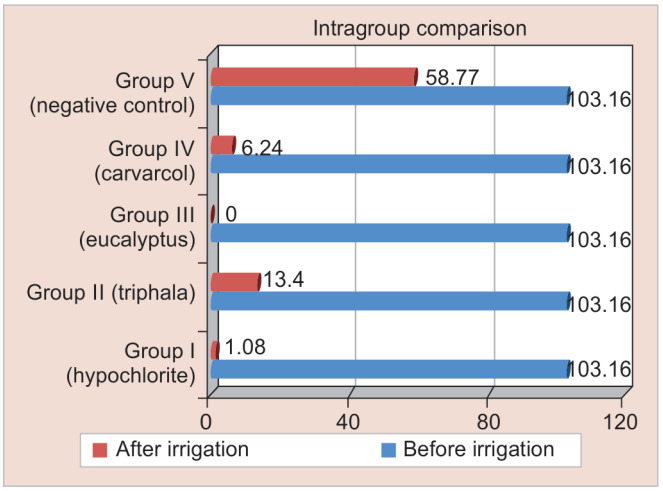


Fig. 2: Intragroup comparison of before and after irrigation

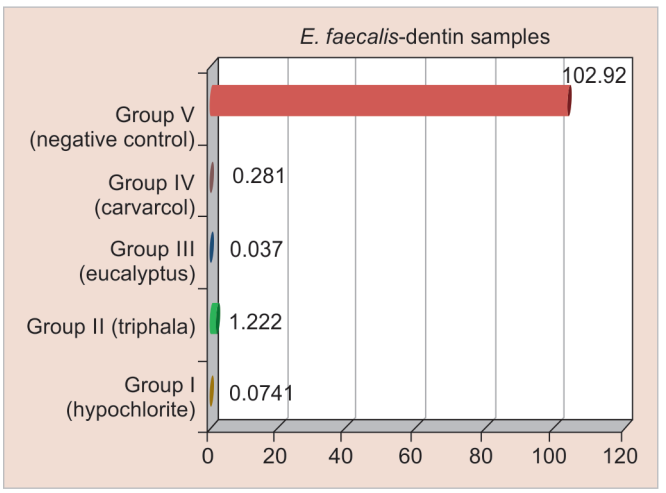


Fig. 3: Antimicrobial efficacy against *E. faecalis* in dentin samples

Table 2: Comparison of CFU count obtained from dentin samples using #3, 4, and 5 GG drills among all test irrigants after irrigation

Groups	*Mean CFU*	*SD*	*Kruskal–Wallis H test*	*p-value, significance*
Group I (hypochlorite)	0.0741*	0.33	H = 1202.3	$p < 0.001$**
Group II (Triphala)	1.222*	4.23		
Group III (*Eucalyptus*)	0.037*	0.18		
Group IV (carvacrol)	0.281*	1.06		
Group V (negative control)	102.92	34.07		

*$p < 0.05$, significant difference; **$p < 0.001$, highly significant

as a major microorganism in the failure of endodontically treated teeth; therefore, a standard strain of *E. faecalis* was chosen in this research. Mechanical instrumentation during endodontic treatment can eliminate most of the bacteria along with debris, while antimicrobial irrigants help to eradicate the remaining bacteria, especially in unreachable areas where mechanical instrumentation does not help.[11] For successful endodontic treatment, an ideal irrigating agent/solution should exhibit maximum antimicrobial efficacy along with tissue dissolving properties with minimal or no toxic effect on surrounding tissue.[2] NaOCl has been the most commonly used and accepted root canal irrigant, which has shown better antimicrobial effect and tissue dissolving property during the endodontic procedure. Although its popularity, it is incapable of the complete elimination of microbial colonies from canal space.[12] Also, it has some major disadvantages, including allergic potential[13] and cytotoxic effects on surrounding tissues.[14]

Looking at the incidence of bacterial resistance to the drug and associated drawbacks of root canal irrigants, it is advisable to make use of root canal irrigants from natural extracts.[15] The natural extract substitutes have shown some major advantages like more shelf life, lower toxicity, availability, cost-effectiveness, and mainly the lack of microbial resistance.[16] Since plant-derived natural extracts represent a rich source of antimicrobial compounds, certain of these derivatives have been incorporated into oral hygiene products. Considering these advantages of herbal extracts, some of them were used in our studies, such as Triphala, *Eucalyptus*, and carvacrol. It has been reported that these test irrigants possess inherent antimicrobial properties. However, studies about the antimicrobial efficacy of these extracts against endodontic pathogens are lacking in the literature, and their effects in a biofilm tooth model have not been studied deeply.

The concentration of the herbal extract was taken from the previous studies.[17–19] These test agents have shown superior antimicrobial activities at certain concentrations in respective studies so that the herbal irrigants with the most potent antimicrobial activity at that concentration can be determined and used against *E. faecalis*, which was incorporated in the tooth model.

The CFU was counted under the electronic colony counter and statistically analyzed. The mean CFU count obtained from low to high with all irrigants tested was as follows: group III, 1.25% *Eucalyptus* (0.00 CFU); group I, 5.25% NaOCl (1.08 CFU); group IV, carvacrol (6.24 CFU); group II, Triphala (13.40 CFU); and group V, normal saline (58.77 CFU). The CFU count obtained before and after irrigation using root canal irrigants was analyzed, and it showed a significant difference ($p < 0.05$).

Herbal extracts, Triphala, *Eucalyptus*, and carvacrol, were found to be efficient against *E. faecalis*. Among them, *Eucalyptus* was more effective than Triphala and carvacrol. Nourzadeh et al. also studied the antimicrobial efficacy of *Eucalyptus* along with NaOCl, and in his study, he found that NaOCl has shown a greater antimicrobial effect against *E. faecalis* as compared to *Eucalyptus*,[20] but in the present study, *Eucalyptus* has shown greater antimicrobial efficacy against *E. faecalis* than NaOCl. This result could be attributed to the concentration of *Eucalyptus* used in our study, which has shown the highest antimicrobial activity. Also, in the previous studies of Paz et al.,[21] Kudi et al.,[22] and Vlietinck et al.,[23] it is found that *Eucalyptus* has shown its antimicrobial activity more on gram-positive bacteria like *E. faecalis*. Studies have shown the antimicrobial effect of various *Eucalyptus* species extracts on *Streptococcus mutans*, *Lactobacillus*, *E. faecalis*, and *Candida albicans*.[24,25] It is also found that components of *Eucalyptus* species cause an effect on *E. faecalis* by interfering with enzymes which helps in the fatty acid synthesis pathway.

Pujar et al.[8] stated after a comparison of the antimicrobial efficacy of Triphala, green tea polyphenols, and 3% of NaOCl on *E. faecalis* biofilms formed on tooth substrate. Triphala has shown significantly better antibacterial activity but was not effective as NaOCl. Nosrat et al.[19] studied the effect of carvacrol as a final endodontic irrigant against *E. faecalis*. They confirmed that 0.6% of carvacrol could effectively disinfect the root canals. Gill and Holley.[26] and Helander et al.[27] studied the adenosine triphosphate (ATP) changes at the cellular level in bacteria induced by carvacrol.

In this research, dentine flakes of depths 200, 400, and 600 µm from the dentine wall were evaluated and studied. As found earlier in the literature, the number of *E. faecalis* in the most superficial layer (200 µm) of dentin was very low as compared to the dentine layer of 400 and 600 µm depths, indicating effective antibacterial action of irrigant solutions on dentine wall.[11] Antimicrobial effect of all test irrigants in dentinal tubules was considerably more than a normal saline solution (negative control). The result obtained from dentin samples was analogous to the results of canal sampling. The test irrigants 1.25% *Eucalyptus*, 5.25% NaOCl, 0.6% carvacrol, and 10% Triphala have shown their antimicrobial effect in all depths, that is, 200, 400, and 600 µm with variable antimicrobial efficacy. The *Eucalyptus* had shown complete inhibition at 200 and 400 µm depth. The effectiveness of 10% Triphala was considerably <1.25% *Eucalyptus* and 5.25% NaOCl. The inhibitory effect of 10% Triphala was less in all depths. The efficacy is dependent on the penetration ability of antimicrobial agents into dentinal tubules. In our study, the intratubular effect of NaOCl, *Eucalyptus*, Triphala, and carvacrol had significant differences with the negative control group. Nourzadeh et al.[20] found that NaOCl has shown a significant difference in antimicrobial efficacy in comparison with *Eucalyptus galbie*, where NaOCl has shown deeper penetration into dentinal tubules.

However, literature shows that by increasing the concentration of irrigants and increasing the diffusion for antibacterial effect, complete bacterial elimination from the dentinal tubules can barely be achieved by irrigants, so dentinal tubules show the presence of microorganisms.[11] Multiple reasons should be considered for this incomplete bacterial elimination, like concentration of irrigants, time, and penetration into dentinal tubules. A 5-minute irrigation time was considered for test irrigants to show their antimicrobial effect on *E. faecalis*. Whereas literature shows no confirmation about the exact time needed for irrigants to have a complete antimicrobial effect. Buffering effect of dentine might reduce the antimicrobial efficacy of herbal extracts. Also, the inadequate diffusion of root canal irrigants and their inactivation by dentin and microbial biofilms are considered a reason for the incomplete elimination of bacteria.

Conclusion

In light of the problems associated with the usage of high NaOCl concentrations and promising results obtained in the present study with *Eucalyptus*, Triphala and carvacrol assure a long and successful use in the endodontic field. *Eucalyptus* showed better results in this study than NaOCl. Further clinical and *in vitro* studies determine its tissue dissolving efficacy and establish these herbal extracts' usage as endodontic irrigant is the need of the hour.

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Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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