Original Article

Comparative evaluation of the antimicrobial efficacy of QMix, electrochemically activated water, ozonated water, and triphala against *Enterococcus faecalis*: An *in vitro* study

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Abstract

Background: The Antimicrobial Efficacy of 4 New Irrigants against *Enterococcus Faecalis*.

Aim: This research aimed to assess the effectiveness of various irrigants in combating *E. faecalis*.

Materials and Methods: A total of 75 teeth were decoronated. Root canals were prepared up to size 25. Roots were inoculated with *E. faecalis* and incubated at 37°C for 14 days: Group I – Sodium hypochlorite (5.25% Prime Dental), Group II – Q mix (Dentsply Sirona), Group III – Electrochemically activated water (Custom-made apparatus), Group IV – Ozonated water (Groots Ozone Generator), Group V – Triphala (Dabur). After 14 days, the colony-forming unit (CFU) count was done. Further enlargement up to 40 file size was performed. 2 ml of all five irrigants was used for 2 min each. The teeth were placed back in a sterile Eppendorf tube with physiologic saline. CFU counts were calculated on the 1st, 2nd, and 5th day.

Statistical Analysis: Shapiro–Wilk test, one-way ANOVA, and Bonferroni test.

Results: Group I showed the highest efficacy, followed by Group II, Group III, Group IV, and Group V.

Conclusion: Qmix showed good promise as an effective irrigant.

Keywords: Colony-forming unit; chlorhexidine; electrochemically activated; *Enterococcus faecalis*; NaOCl

INTRODUCTION

Endodontic diseases are primarily caused by bacteria and their metabolic byproducts.[1] *Enterococcus faecalis* is the most common organism in failed root canals and persistent periapical lesions. It survives in nutrient-poor environments and high alkalinity (up to pH 11.5) and forms biofilms that enhance resistance, complicating eradication during root canal therapy. Because mechanical instrumentation cannot reach all canal areas, irrigation is essential for the reduction of microbial load and to eliminate the chance for reinfection.[2,3]

Sodium hypochlorite (NaOCl) is widely used for its strong antimicrobial activity, tissue-dissolving ability, and cost-effectiveness. Its efficacy increases with higher concentration, volume, and warming. However, NaOCl is

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cytotoxic, has an unpleasant odor and taste, can bleach clothing, corrode metals, does not eliminate all bacteria, fails to fully remove the smear layer, and may alter dentin structure. Consequently, alternative irrigants are recommended.[3-5]

This study evaluated the antibacterial efficacy of four newer irrigants – Triphala, ozonated water, electrochemically activated (ECA) water, and QMix – against *E. faecalis*. Triphala, an herbal formulation of Amalaki, Bibhitaki, and Haritaki, contains tannic and citric acids that confer antimicrobial and chelating properties, along with antioxidant and anti-inflammatory effects.[6,7] Ozonated water demonstrates strong bactericidal activity, which can be enhanced when combined with NaOCl, though its effectiveness depends on concentration, delivery, and microbial specificity.[8-11]

ECA water, produced via electrolysis to yield anolyte and catholyte solutions, exhibits broad antimicrobial activity with low cytotoxicity and performs comparably to NaOCl in debris removal while improving smear layer removal.[12-14] QMix, containing chlorhexidine (CHX), Ethylenediaminetetraacetic acid (EDTA), and a surfactant, efficiently removes the smear layer and reduces *E. faecalis* load. The surfactant lowers surface tension, enhancing flow, wettability, and contact with dentin.[15-17]

Null hypothesis

The null hypothesis tested was that there would be no significant difference in the antimicrobial efficacy of NaOCl, QMix, ECA water, ozonated water, and Triphala solution against *E. faecalis* at different time intervals.

DATA COLLECTION

Sample size

I. Seventy-five extracted permanent single-rooted teeth were collected
II. The 75 samples were divided into 5 groups with 15 samples in each group based on the type of irrigant used.
    - Group I – NaOCl (5.25% Prime Dental)
    - Group II – Q mix (Dentsply Sirona)
    - Group III – ECA water (Custom-made apparatus)
    - Group IV – Ozonated water (Groots Ozone Generator)
    - Group V – Triphala (Dabur).

Type of study/study design

In vitro experimental.

Feasibility

Yes.

Inclusion criteria

- Sound extracted single-rooted teeth with a single apical foramen
- The degree of curvature of the root should be within 0°–10°
- Teeth with evident root formation have been selected.

Exclusion criteria

- Extensive caries
- Canals that were calcified were avoided
- There should not be any resorption
- Multi-rooted teeth or teeth with more than one canal
- Sharp bent or extensively curved roots.

Methodology

A total of 75 single-rooted teeth that had been freshly extracted were used in this study. The obtained samples were then cleaned and stored in normal saline before use.

Specimen preparation

- The extracted teeth were decoronated at the Cemento-enamel junction (CEJ) with a diamond cutting disc
- The root canals were biomechanically prepared initially up to 25 K-file (till the tip of the roots), and the debris was removed by irrigating with normal saline
- The apices were sealed with composite resin and followed by coating with nail varnish from the outside, then autoclaved.

Bacterial species preparation

- Pure strains of *E. faecalis* were seeded in a petri dish containing Brain Heart Infusion (BHI) agar
- Incubation of agar plates was done for 48 h
- A standard suspension of *E. faecalis* in distilled water was prepared with a dilution of 109 cells/mL to get a similar turbidity as the McFarland 3 standard.

Preparation of species inoculation

- The root canals were separately inoculated in a sterile Eppendorf tube until they were completely submerged in the solution
- The whole setup was put inside the incubator for 14 days
- 37°C was the temperature that was maintained.

Confirming the collection of species from the root canals of teeth

- After 14 days of growth, samples were collected from root canals by inserting sterile paper points for 1 min into them
- Paper points were then agitated in distilled water, and the species was diluted to 103 times to obtain a total dilution of 1012 times
- 10 µL from the above suspension was streaked in 75 petri dishes with BHI agar with an "L" spreader, and 37°C was the temperature at which it was incubated

- After that, the colony-forming unit (CFU) was counted with the help of a colony counter and recorded individually for each agar plate.

Preparation of irrigating solutions

Sodium Hypochlorite
In this study, 5.25% NaOCl was used.

Qmix
In this study, Qmix from Dentsply Sirona was used. It is composed of 2% CHX, 17% EDTA. It comes in a premixed, ready-to-use format.

Electrochemically activated water
This study utilized a custom-designed apparatus comprising two chambers, each capable of holding 50 ml of solution, separated by a polymer membrane with 0.45 μm pores. Within each chamber, two graphite rods measuring 6 cm in length and 1 cm in diameter were affixed to the top and connected to a power supply capable of delivering up to 12.5 volts and 3.5 amps. Tap water was added to both chambers, and electrolysis was initiated using a power supply set to 12 amps and 2 volts. The resulting solution from the anodic compartment was collected and employed for the study.

Ozonated water
In this study, Ozonated water produced by the Groots ozone generator was used. The probe of the ozone generator was immersed in a beaker containing 100 ml of water for 1 h. The water was treated with an ozone generator for 1 h for use in this study.

Triphala
In this study, a Triphala solution was prepared by dissolving 0.5 g of commercially available Triphala powder in 1000 mL of dimethyl sulfoxide to obtain a concentration of 5 mg/dL.

Irrigation

The samples were distributed randomly into five groups for irrigation:

Out of the groups, one was the control group and the rest four were experimental groups:

- Group I – NaOCl (5.25% Prime Dental)
- Group II – Q mix (Dentsply Sirona)
- Group III – ECA water (Custom-made apparatus)
- Group IV – Ozonated water (Groots Ozone Generator)
- Group V – Triphala (Dabur).

The samples were then irrigated according to their respective allocated irrigants with a quantity of 2 mL each for 2 min.

The samples were placed in a sterile Eppendorf tube with saline.

Final collection of species

- To check for the growth of bacteria on the 1st, 2nd, and 5th day, sterile paper points were inserted into root canals and kept for 1 min
- Paper points were agitated in 100 μL of distilled water, and the dilution was taken into account and later on was adjusted while colony counting after 24 h of incubation from the 25 petri dishes that were streaked with the final collection of species, by paper points, with the help of "L" spreader
- The same process was repeated on the 2nd and 5th day
- CFU was counted on all three 3 respective days
- Final colony count reading for Group I (NaOCl) after 1st day [Figure 1a], 2nd day [Figure 1b], and 5th day [Figure 1c] of bacterial growth
- Final colony count reading for Group II (Qmixtm Dentsply Sirona) after 1st day [Figure 1d], 2nd [Figure 1e], and 5th [Figure 1f] day of bacterial growth
- Final colony count reading for Group III (ECA Water) after 1st day[Figure 1g], 2nd [Figure 1h], and 5th [Figure 1i] day of bacterial growth
- Final colony count reading for Group II (Ozonated Water) after 1st day [Figure 1j], 2nd [Figure 1k], and 5th [Figure 1l] day of bacterial growth
- Final colony count reading for Group II (Triphala solution) after 1st day [Figure 1m, 2nd [Figure 1n], and 5th [Figure 1o] day of bacterial growth.

Statistical analysis

The statistical software SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) is used for the analysis of data. The descriptive statistics, like mean, standard deviation, and standard error of mean of the data, were calculated. The normality of data was tested by the Shapiro–Wilk test and found to be normally distributed. The significance of means of CFU, Inter-group comparison between irrigants at a time interval by one-way ANOVA followed by the Bonferroni test, and intra-group comparison between time intervals was done paired *t*-test. The 95% confidence interval and 5% level of significance were used for the analysis of data.

RESULTS

NaOCl (5. 25%) and QMix both showed remarkable antibacterial activity against *E. faecalis*, with no discernible difference between them. QMix was significantly more effective than ECA water, ozonated water, and Triphala, while ECA water and ozonated water showed moderate activity. Triphala exhibited the least bacterial reduction, indicating that NaOCl remains the most potent of the tested irrigants. Mean CFU values from lowest to highest were: NaOCl (0.98), QMix (1.10), ozonated water (2.10), ECA water (2.20), and Triphala (2.60) [Table 1 and Figure 2].

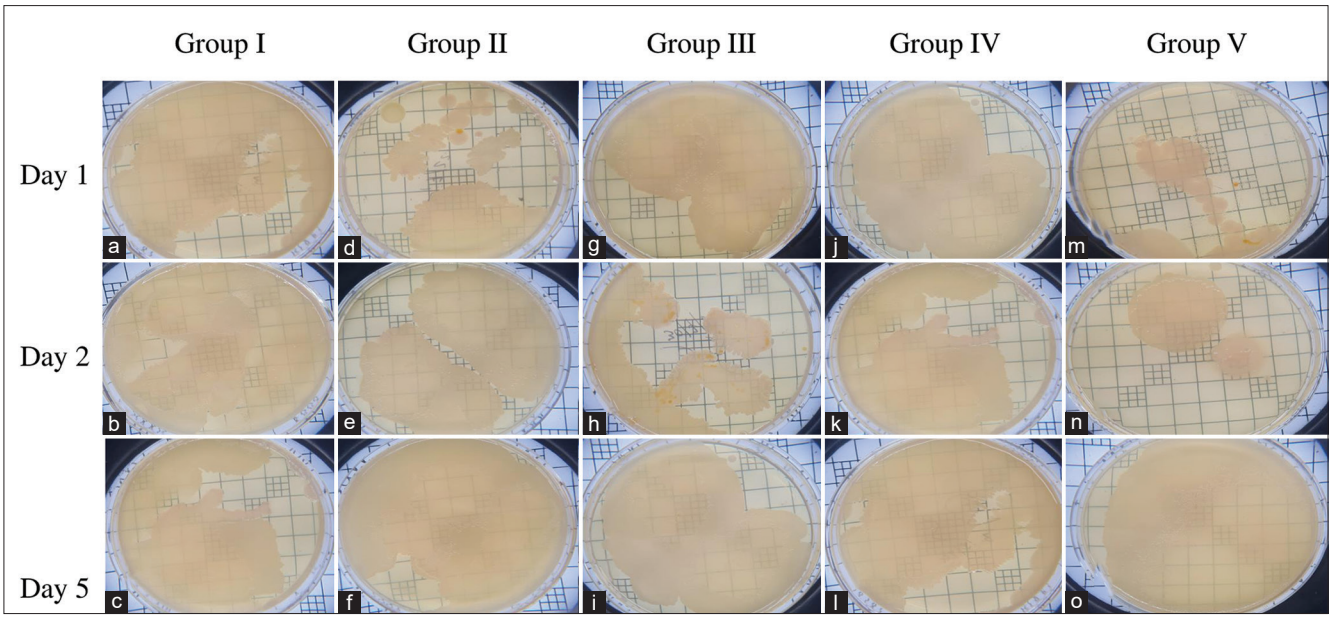


Figure 1: (a) Colony-forming unit (CFU) Counts for Sodium Hypochlorite (NaOCl) group on Day 1, (b) CFU Counts for NaOCl group on Day 2, (c) CFU Counts for NaOCl group on Day 5, (d) CFU Counts for Qmix group on Day 1, (e) CFU Counts for Qmix group on Day 2, (f) CFU Counts for Qmix group on Day 5, (g) CFU Counts for electrochemically activated (ECA). Water group on Day 1, (h) CFU Counts for ECA. Water group on Day 2, (i) CFU Counts for ECA. Water group on Day 5, (j) CFU Counts for Ozonated water group on Day 1, (k) CFU Counts for Ozonated water group on Day 2, (l) CFU Counts for Ozonated water group on Day 5, (m) CFU Counts for Triphala Solution group on Day 1, (n) CFU Counts for Triphala Solution group on Day 2, (o) CFU Counts for Triphala Solution group on Day 5

Table 1: Intra-group comparison of means CFU (×10⁷) of an irrigant between two time intervals by Paired t-test

Irrigant	Paired differences					*t*	df	*P*
	Mean	SD	SEM	95% CI of the difference				
				Lower	Upper			
Sodium hypochlorite								
Day 1–day 2	−0.2667	0.0577	0.0333	−0.4101	−0.1232	−80.000	2	0.015*
Day 2–day 5	−0.1000	0.1000	0.0577	−0.3484	0.1484	−10.732	2	0.225 (NS)
Day 1–day 5	−0.3667	0.0577	0.0333	−0.5101	−0.2232	−11.000	2	0.008**
Q MIX								
Day 1–day 2	−0.2333	0.1155	0.0667	−0.5202	0.0535	−3.500	2	0.073 (NS)
Day 2–day 5	−0.0667	0.0577	0.0333	−0.2101	0.0768	−2.000	2	0.184 (NS)
Day 1–day 5	−0.3000	0.1732	0.1000	−0.7303	0.1303	−3.000	2	0.095 (NS)
ECA water								
Day 1–day 2	−0.4000	0.2646	0.1528	−1.0572	0.2572	−2.619	2	0.120 (NS)
Day 2–day 5	−0.3667	0.1528	0.0882	−0.7461	0.0128	−4.158	2	0.053 (NS)
Day 1–day 5	−0.7667	0.2082	0.1202	−1.2838	−0.2496	−6.379	2	0.024*
Ozonated water								
Day 1–day 2	−0.5000	0.1000	0.0577	−0.7484	−0.2516	−8.660	2	0.013*
Day 2–day 5	−0.3667	0.2082	0.1202	−0.8838	0.1504	−3.051	2	0.093 (NS)
Day 1–day 5	−0.8667	0.1528	0.0882	−1.2461	−0.4872	−9.827	2	0.010*
Triphala								
Day 1–day 2	−0.1333	0.1155	0.0667	−0.4202	0.1535	−2.000	2	0.184 (NS)
Day 2–day 5	−0.1000	0.1732	0.1000	−0.5303	0.3303	−1.000	2	0.423 (NS)
Day 1–day 5	−0.2333	0.1528	0.0882	−0.6128	0.1461	−2.646	2	0.118 (NS)

*Significant $P<0.05$, **Highly significant $P<0.01$. NS: Not significant, $P>0.05$, SEM: Standard error of mean, CI: Confidence interval, SD: Standard deviation

DISCUSSION

The success of root canal treatment depends largely on effective chemomechanical preparation, in which irrigants provide flushing action, tissue dissolution, antimicrobial activity, and low toxicity.[18] *E. faecalis* was chosen for this study due to its high prevalence in endodontic infections, reported up to 90%.[19] Based on the statistically significant differences observed among the tested irrigants at all evaluated time intervals, the null hypothesis was rejected.

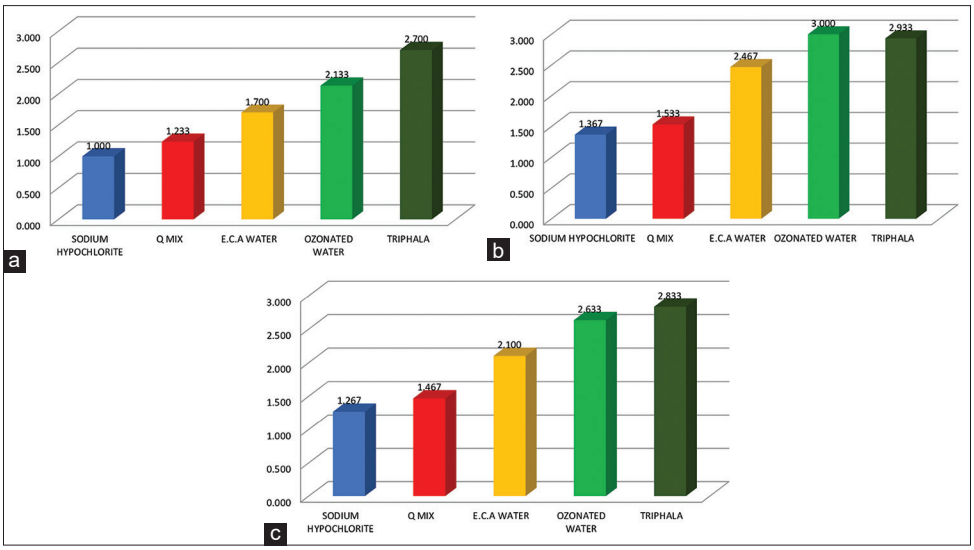


Figure 2: Represents Graphs Comparing mean Colony-forming unit (CFU) Counts of all five irrigants across Days 1, 2 and 5. (a) Comparison of mean CFU count ($\times 10^7$) of five irrigants on Day 1, (b) Comparison of mean CFU count ($\times 10^7$) of five irrigants on Day 2, (c) Comparison of mean CFU count ($\times 10^7$) of five irrigants on Day 5

NaOCl is widely used for its broad antibacterial spectrum, tissue-dissolving ability, and debris removal. CHX, a cationic molecule, disrupts bacterial membranes and exhibits bacteriostatic or bactericidal effects depending on concentration.[4] However, CHX cannot dissolve necrotic tissue, and NaOCl has cytotoxicity, odor, allergenic potential, and limited smear layer removal. Consequently, CHX is considered an adjunct rather than a replacement for NaOCl, highlighting the need for safer, effective alternative irrigants.[20]

QMix, combining CHX, EDTA, a surfactant, and saline, has shown smear-layer removal comparable to 17% EDTA and strong antibacterial activity against *E. faecalis*. Studies report QMix outperforms CHX, MTAD, and EDTA/NaOCl, particularly when used with agitation, producing the highest number of bacteria-free samples. Its surfactant reduces surface tension, improving penetration into dentinal tubules, while maintaining biocompatibility.[15,16] In this study, QMix showed antimicrobial efficacy most comparable to NaOCl.

ECA water, specifically the anolyte solution, demonstrates broad antimicrobial activity with low cytotoxicity and reduced allergenic response. It has comparable debris removal to NaOCl and good smear-layer elimination. In this study, ECA achieved moderate microbial reduction, although clinical validation is needed.[14]

Ozonated water exhibits potent bactericidal activity at low concentrations. While some studies show similar efficacy to NaOCl under sonication, others report inferior performance against *E. faecalis* biofilms.[10,11] Our findings align with the latter, with both ozonated water and ECA showing initial microbial reduction but notable regrowth by day 5, likely due to instability and rapid loss of activity in the presence of organic material.[14,21]

Triphala, prepared in DMSO to optimize its purest properties, demonstrated the lowest antimicrobial activity among the tested groups. Another study by Choudhary *et al*. showed that CHX performed better than herbal irrigants, which is in accordance with the results of our study. Its higher viscosity and particulate content likely reduced flushing efficiency compared to clear solutions, consistent with previous reports.[7]

Limitations of the study

This study was conducted under *in vitro* conditions, which may not accurately replicate the complex clinical environment of the root canal system. Factors such as host immune response, tissue buffering capacity, presence of organic matter, and intracanal fluid dynamics could influence the antimicrobial efficacy of irrigants *in vivo*.

Only a single bacterial species (*E. faecalis*) was evaluated, whereas endodontic infections are typically polymicrobial in nature. The use of planktonic cultures and a standardized biofilm model may not fully represent mature, multispecies biofilms encountered clinically.

The antimicrobial efficacy of the irrigants was assessed using CFU counts alone; additional methods, such as confocal laser scanning microscopy or molecular techniques, could provide more detailed insights into biofilm viability and depth of penetration.

Furthermore, a single concentration, volume, and contact time were used for each irrigant, which may not reflect variations in clinical irrigation protocols. The effects of

irrigant activation techniques and interactions with dentin were not evaluated in this study.

CONCLUSION

Within the limitations of this study, it can be concluded that Qmix showed an indistinguishable reduction in CFU counts compared to NaOCl. There are several factors that determine the success of your endodontic treatment. Irrigation is one such factor that alters the outcome of your treatment, and it serves as an effective way of inhibiting the pathogen *E. faecalis*. Apart from antimicrobial efficacy, other properties such as flushing action, removal of smear layer, etc., should be evaluated for new irrigants. Hence, this recommends that further studies should be conducted to check the potency of these irrigants along with their effect *in vivo*.

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Conflicts of interest
There are no conflicts of interest.

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