Original Article

Evaluation of the Antimicrobial Efficacy of *Elettaria cardamomum* Oil, *Trachyspermum ammi* Oil and 5% Sodium Hypochlorite Against *Enterococcus faecalis* Biofilm Formed on Tooth Substrate

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Abstract

Context: The usual cause of nonfulfillment of endodontic therapy is the persistence of microorganisms in the root canal system due to ineffective disinfection. *Enterococcus faecalis* is one of the most prevalently isolated microorganisms following a failure in root canal treatments. Sodium hypochlorite is among the most effectively used irrigant solutions but has many shortcomings. Herbal alternatives for sodium hypochlorite might prove to be superior due to their high antimicrobial activity, biocompatibility, and their antioxidant and anti-inflammatory properties. **Aims:** This study is aimed to evaluate the antimicrobial efficacy of *Trachyspermum ammi* oil and *Elettaria cardamomum* oil against 2-week-old and 4-week-old *E. faecalis* biofilms formed on tooth substrate. **Settings and Design:** A pure culture of *E. faecalis* was grown on brain heart infusion agar, inoculated into brain heart infusion broth, and incubated at 37°C overnight. Single rooted human mandibular premolars were sectioned below cementoenamel junction, enlarged, and vertically sectioned along the midsagittal plane. The samples were then placed in tissue culture wells inoculated with 2 ml of the bacterial solution and incubated at 37°C. **Materials and Methods:** Group 1 *E. cardamomum* oil (cardamom), Group 2 *T. ammi* oil (ajwain), Group 3 5% sodium hypochlorite, and Group 4 Saline (control) (n = 10). At the end of the 2nd and 4th weeks, all groups were treated for 10 min with 3 ml of the respective solutions. Quantitative analysis was performed by serial dilution. **Results:** *T. ammi* oil and sodium hypochlorite treated teeth showed complete elimination of both the 2-week-old and 4-week-old *E. faecalis* biofilm. Meanwhile, saline and *E. cardamomum* oil-treated teeth still showed the presence of *E. faecalis*. **Conclusions:** The use of *T. ammi* oil as a root canal irrigant solution can be considered as an alternative to sodium hypochlorite.

Keywords: *Biofilm, Elettaria cardamomum, Enterococcus faecalis, sodium hypochlorite, Trachyspermum ammi*

Introduction

Effective debridement, complete disinfection, and three-dimensional obturation of the entire root canal system are prerequisites for the long-term success of endodontic treatment. Microorganisms' contribution to the initiation and progression of pulpal and periapical diseases has been validated in animal models and human studies by various authors.[1-3]

Persistent intra-radicular infections or secondary infections usually occur due to endodontic treatment procedures that have not met the standard for control and elimination of infection.[4,5] *Enterococcus faecalis*, a facultative bacteria, is one of the most prevalently isolated species from failed/infected root canals of both primary and permanent teeth with its prevalence ranging from 10% to 76%.[6-8] *E. faecalis* can maraud dentinal tubules and attach to collagen in the presence of human serum,[9] can survive extreme alkaline pH levels (9.6)[10] and prolonged periods of starvation[11] and can even resist antimicrobial effects of diverse endodontic irrigants and intracanal medicaments.[6]

The role of irrigant solutions is important since mechanical instrumentation alone cannot eliminate all microorganisms. Sodium hypochlorite is the most popular endodontic irrigant solution as it is a potent antimicrobial and has tissue dissolving properties.[12] However, the inability of sodium hypochlorite to remove the smear layer, its toxic effects on periapical tissues, unpleasant taste, and short shelf life are

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among the common issues, and hence, attempts are made to search for newer irrigant solution.

Herbal alternatives for sodium hypochlorite might prove to be advantageous. They are becoming popular due to their high biocompatibility, antimicrobial activity, and anti-oxidant and anti-inflammatory properties.[13] The knowledge regarding medicinal plants has been assimilated in the due course of many centuries and has been mentioned earliest in *Rigveda*.[14-16] Phytomedicine has been used in dentistry as an analgesic, sedative, anti-inflammatory, antibiotic, and also as an endodontic irrigant solution. Herbal preparations can be obtained from the roots, leaves, seeds, stem, and flowers.[17]

Trachyspermum ammi (ajwain) is a plant which is widely grown in India, Pakistan, the South East, and the Near East of Iran. Its seeds are utilized as an antioxidant, antiseptic, carminative, antifungal, and antibacterial agent. It is also used as a principal source of thymol, which has been proclaimed to be a germicide, antispasmodic, and antifungal agent.[18]

Elettaria cardamomum (cardamom), whose seeds are used as a spice in preparing food, is commonly grown in Sri Lanka, India, Myanmar, and Malaysia. Its seeds are said to have anti-carcinogenic, anti-ulcerogenic, antimicrobial, and anticonvulsant actions.[19,20]

The purpose of this study was to compare the antimicrobial efficacy of *T. ammi* essential oil, *E. cardamomum* essential oil, and 5.25% sodium hypochlorite in 2-week-old and 4-week-old *E. faecalis* biofilms formed on tooth substrate.

The null hypothesis is that there will be no significant difference in the antimicrobial efficacies of the test solutions when compared with sodium hypochlorite.

Materials and Methods

Sample preparation

A total of 40 freshly-extracted single-rooted mandibular premolars were selected for the study. The teeth were immersed in 10% formalin for disinfection and fixation of the organic tissue for a period of 24 h, which was followed by cleaning of the external debris and calculus with an ultrasonic scaler. Teeth were kept in physiologic saline for the rest of the period. Each tooth was radiographed to validate the existence of a single patent canal, following which they were sectioned below cemento-enamel junction to get a standardized tooth length of 8 mm.

The working length of each tooth was determined by using a size 10 K file. Root canals were then instrumented using a step-back technique with hand instruments, and the canals were enlarged to an apical size 40 K file. Canals were irrigated with 3 ml of saline during the procedure to remove any debris. All teeth were divided into two halves by vertically sectioning along the midsagittal plane and then minimally ground to get flat surfaces. The samples were then sterilized by autoclave at 121°C for 20 min.

Contamination of the teeth

A pure culture of *E. faecalis* (ATCC 29212) was grown on brain heart infusion agar inoculated into brain heart infusion broth and incubated at 37°C overnight. Then, a total of 50 µl of inocula were transferred to presterilized individual microcentrifuge tubes containing 1 ml of the respective broths and teeth. All the procedures were carried out in a biosafety cabinet. The purity of the culture was checked by sub-culturing 5 µl of the broth from the incubated teeth in the respective broths, on agar plates. The teeth samples were placed in tissue culture wells following which the wells were inoculated with 2 ml of the bacterial solution and incubated at 37°C. Contamination of the teeth was carried out for 2 weeks for half the total teeth and 4 weeks for the remaining half teeth.

E. cardamomum and *T. ammi* essential oils were purchased from Karothukuzhi Ayurveda Pharmacy (Aluva, Kerala, India). The samples were divided into four groups based on the irrigant solution used:

- Group 1: *E. cardamomum* oil (cardamom)
- Group 2: *T. ammi* oil (ajwain)
- Group 3: 5% sodium hypochlorite
- Group 4: Saline (control).

Antimicrobial assessment

Antimicrobial assessment was done inside class 2 biological safety cabinet at the end of the 2nd and 4th weeks. All groups were treated for 10 min, with 3 ml of the respective solutions. Following this, the tooth sections were placed in a test tube containing 1 ml of Milli-Q water and shaken in a vortex mixer for 60 s. This procedure was followed by serial dilution of the microbiological sample, which was done in Eppendorf tubes and the serially diluted microorganisms were plated onto brain heart infusion agar and incubated at 37°C for 24 h to determine the colony-forming units per milliliter (CFU/ml) using the Miles *et al.* method.[21]

Statistical analysis was performed using IBM SPSS statistics 20 Windows (SPSS Inc., Chicago, Il, USA). For all the continuous variables, the results are given as the mean ± standard deviation, and for categorical variables, the results are given as a percentage.

The numerical variable between the two groups was compared using the Mann–Whitney U test, a nonparametric assessment. A value of $P < 0.05$ was considered for statistical significance.

The ethical clearance for the study was given by the Institution Research Board, Amrita Institute of Medical Sciences, Kerala, India.

Results

Quantitative analysis of 2-week-old *E. faecalis* biofilm saline-treated tooth samples showed an average value of

$114.3 \times 10^9 \pm 12.28 \times 10^9$ CFU/ml followed by *E. cardamomum* oil treated samples at $114.0 \times 10^9 \pm 11.83 \times 10^9$ CFU/ml ($P = 0.879$) [Table 1]. Sodium hypochlorite and *T. ammi* oil showed 100% eradication of *E. faecalis* [Graph 1].

Quantitative analysis of 4-week-old *E. faecalis* biofilm *E. cardamomum* oil-treated tooth samples showed an average value of $120.34 \times 10^9 \pm 16.28 \times 10^9$ CFU/ml followed by saline-treated samples at $114.01 \times 10^9 \pm 15.54 \times 10^9$ CFU/ml ($P = 0.324$) [Table 2]. Sodium hypochlorite and *T. ammi* oil showed 100% eradication of *E. faecalis* [Graph 2].

Therefore, *T. ammi* oil showed equal, if not greater, antibacterial efficacy against 2-week-old and 4-week-old *E. faecalis* biofilms when compared to sodium hypochlorite. By contrast, saline solution and *E. cardamomum* oil were inferior to sodium hypochlorite in exhibiting germicidal activity.

Discussion

Complete elimination of microorganisms from the root canal is not possible by chemo-mechanical preparation alone. Mechanical instrumentation can efficiently shape but not clean the canal, with almost 40% of the canal remaining untouched even after the procedure.[22] Irrigation being complementary to instrumentation helps in enabling the removal of pulp tissue and/or microorganisms from areas not accessible by mechanical instrumentation.[23]

E. faecalis, a facultative anaerobe, was chosen as the microorganism in this study as it is frequently recovered in root canals associated with persistent infections.[6,24] The virulence factor of *E. faecalis* in failed endodontically-treated teeth may be related to its capability to invade dentinal tubules and adhere to collagen in the presence of human serum.[9] It can survive harsh environments like extreme alkaline pH levels (9.6) and the temperature of 60°C for 30 min and even enter a viable but noncultivable state.[25] A functioning proton pump, which drives protons into the cell to acidify the cytoplasm, is critical for its survival under a high pH.[26]

The resistance of these microorganisms is increased by the formation of a biofilm. It has been shown that antibiotic resistance is a thousand times higher when there is a biofilm formation when compared to that of planktonic cells.[24] Biofilm investigations performed on polycarbonate or glass substrate will not provide an accurate indication of the bacteria-substrate interaction.[27] Hence, *E. faecalis* biofilm was established on a tooth substrate in this study.[28]

In this *in vitro* study, the anti-microbial activity of various irrigant solutions was compared to eliminate endodontic pathogens responsible for root canal failure. It was observed that *T. ammi* oil and 5% sodium hypochlorite manifested the most antibacterial effectiveness against both 2-week-old and 4-week-old *E. faecalis* biofilms whereas *E. cardamomum* oil followed by saline were the least effective. 5% sodium hypochlorite exhibited excellent antibacterial activity both in 2-week-old and 4-week-old biofilms with complete elimination of bacteria. It has been previously observed that 5.25% concentration shows higher solvent potential and bactericidal effect with lower surface tension and, consequently, better root canal decontamination.[29]

Among the herbal extracts, *T. ammi* essential oil (ajwain) showed equal, if not greater, antibacterial efficacy than 5% sodium hypochlorite with complete elimination of 2-week-old and 4-week-old biofilms after 10 min of exposure. Ajwain seeds contain phenols (30%–50% thymol, 1%–7% carvacrol) and monoterpenes (20%–35% terpinene

Table 1: Mean and standard deviations of colony-forming units in 2 weeks *Enterococcus faecalis* biofilm

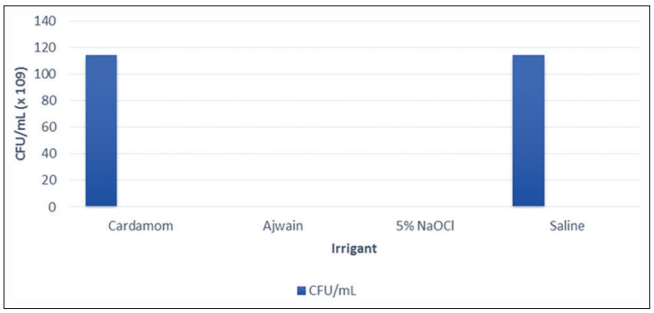
Group	*n*	Mean±SD (CFU/ml)
Cardamom	10	$114.0 \times 10^9 \pm 11.83 \times 10^9$
Ajwain	10	0
5% sodium hypochlorite	10	0
Saline	10	$114.3 \times 10^9 \pm 12.28 \times 10^9$

SD: Standard deviation; CFU: Colony-forming units

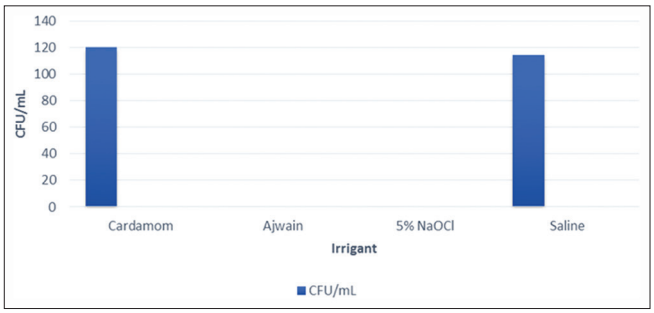
Table 2: Mean and standard deviations of colony-forming units in 4 weeks *Enterococcus faecalis* biofilm

Group	*n*	Mean±SD (CFU/ml)
Cardamom	10	$120.34 \times 10^9 \pm 16.28 \times 10^9$
Ajwain	10	0
5% sodium hypochlorite	10	0
Saline	10	$114.01 \times 10^9 \pm 15.54 \times 10^9$

SD: Standard deviation; CFU: Colony-forming units



Graph 1: Comparison of quantitative analysis of 2 weeks *Enterococcus faecalis* biofilm



Graph 2: Comparison of quantitative analysis of 4 weeks *Enterococcus faecalis* biofilm

and 20%–25% paracymene, pinene, and limonene. The possible explanation of the antibacterial activity of ajwain could be because of the presence of major ingredients thymol and carvacrol. Thymol has been reported to be a germicide, antispasmodic, and antifungal agent.[30] Thymol kills bacteria resistant to even prevalent third-generation antibiotics and multidrug-resistant microbial pathogens[31] and exhibited potent antimicrobial activity with minimum inhibitory concentrations ranging from 0.625 to 10.0 mg/mL.[32] Amanthi *et al*. evaluated the antibacterial activity of Ajwain oil against *E. faecalis* and *Streptococcus mutans* by the agar well diffusion method. It was found that ajwain oil showed a maximum zone of inhibition against *E. faecalis* and *S. mutans* at 100 µl/ml.[33]

However, *E. cardamomum* oil was not found to be very effective against 2-week-old and 4-week-old *E. faecalis* biofilms, as per a study conducted by Sharma Revathi *et al*. where cardamom extract only showed slight sensitivity against *E. faecalis* (7%) and *E. faecium* (1%).[34]

Herbal alternatives are readily available, cost-effective, have an increased shelf life, low toxicity, and lack microbial resistance.[35] Hence, they may be considered as an alternative for the traditional root canal irrigant solutions.

Conclusions

Within the limitations of the current study, *T. ammi* oil and 5% sodium hypochlorite showed complete elimination of *E. faecalis* biofilms formed on tooth substrate over a 2-weeks and 4-weeks assessment period. Therefore, the use of *T. ammi* oil as a root canal irrigant solution can be considered as an alternative to sodium hypochlorite. Further research is needed to conclusively recommend the same as a root canal irrigant solution.

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Conflicts of interest

There are no conflicts of interest.

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