Original Article

Comparative antimicrobial efficacy of oregano oil, chlorhexidine, and sodium hypochlorite against *Enterococcus faecalis*: An *in vitro* study

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ABSTRACT

Objective: The purpose of this study was to evaluate the comparative antibacterial efficacy of 0.2% oregano oil, 2% chlorhexidine (CHX), and 3% sodium hypochlorite (NaOCl) in disinfection of dentin tubules contaminated with *Enterococcus faecalis*.

Materials and Method: One hundred and twenty human extracted anterior teeth were infected with *E. faecalis* for 21 days. They were assigned into three groups ($n = 40$) – Group 1: 3% NaOCl, Group 2: 2% CHX, and Group 3: 0.2% oregano oil. Disinfection protocol was followed using 31-gauge side-vented needle. After chemomechanical preparation, the apical 5 mm of the roots was removed frozen in liquid nitrogen and pulverized to expose *E. faecalis* in dentinal tubules. The number of colony-forming units of *E. faecalis* per mg dentin was determined. The data were analyzed statistically.

Results: Zone of inhibition of oregano oil was found to be 15 mm, and the values were significant in Group III ($P < 0.05$) as compared to the other groups assessed.

Conclusion: Within the study's limitation, 0.2% oregano oil showed better disinfection property against *E. faecalis* among tested irrigants. Therefore, it can be used as an effective alternative root canal irrigant.

Keywords: Chlorhexidine, *Enterococcus faecalis*, essential oil, sodium hypochlorite

INTRODUCTION

The endodontic treatment's success is based on the elimination of microorganism from root canal.[1] In primary endodontic infections *Enterococcus faecalis*, a Gram-positive facultative anaerobe was found 4%–40%.[2] Compared to conditions with primary endodontic infection, *E. faecalis* was reported to show nine times more prevalent in a failed root canal treatment.[2] In such infection, the prevalence ranges from 24% to 77%.[3] *E. faecalis* can survive in a harsh environment with extreme alkaline pH of 9.6, with scarce nutrients and at a temperature of 60°c for 30 min.[4]

E. faecalis possesses certain virulence factors such as lytic enzymes, cytolysin, pheromones, and lipotiechoic acid, suppressing lymphocytes' action, potentially contributing to endodontic failure.[2,5] It is relatively minute to invade

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and live within dentinal tubules and can endure prolonged periods of starvation.[3]

Sodium hypochlorite (NaOCl) is highly efficient in eliminating *E. faecalis* biofilm. The disadvantage of NaOCl is its tissue toxicity, unpleasant taste and odor, corrosion of instruments, and inability to remove the inorganic part of the smear layer.[6] The other disadvantage is that it reduces the elastic modulus and flexural strength of dentin.[7]

Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent. Its antimicrobial action is related to its cationic bisbiguanide molecular structure. It is bacteriostatic at low concentration while at higher concentration, it is bactericidal as it brings about coagulation and precipitation. It bears a property of substantivity and low-grade toxicity. Therefore, CHX gluconate can be used both as an irrigant and intracanal medicament.[8]

Many species and herbs exert antimicrobial activity due to their essential oil fractions. For 1000 of years, clove oil (eugenol) has been used in dentistry. Creosote, which contains several phenolic compounds such as cresol, is also used to sedate inflamed dental pulp.[9] The antimicrobial activity of essential oil is due to several small terpenoids and phenol compounds.

Origanum vulgare (*Lamiaceae* family) is an endemic plant found in India, the southern part of Iran and Mediterranean region, which has been used traditionally used for the antiseptic purpose.

Origanum essential oil is an edible plant oil used in food products. Reports have shown that oregano, thyme, clove, and cinnamon possess antimicrobial property.[10]

To the best of our knowledge, there have been no previous studies on assessing the antibacterial activity of origanum essential oil by agar diffusion method and comparing it with NaOCl and CHX. Based on our preliminary pilot study, the minimal inhibitory concentration of oregano oil was 0.2%. According to the recent report, 0.25% of oregano oil showed effective antibacterial activity against *E. faecalis*.[11] Therefore, the study aimed to evaluate and compare the antimicrobial efficacy of 0.2% oregano essential oil, 3% NaOCl, and 2% CHX using agar diffusion method.

MATERIALS AND METHOD

Bacterial strains

Bacterial strains, Gram-positive (*E. faecalis*) ATCC 29212, were chosen based on their clinical and pharmacological importance. The bacterial microorganisms were cultured on nutrient agar by spread plate technique and were incubated for 24 h at 37°C. The bacterial strains were grown in Mueller-Hinton agar plates at 37°C (the bacteria were grown in the nutrient broth at 37°C) and maintained on nutrient agar slants at 4°C. The stock cultures were maintained at 4°C. Sterile spreader was used for inoculation of these organisms across respective media.

Collection of plant material

The fresh leaves of origanum vulgare were collected from the southern region of India (Tamil Nadu).

Isolation of essential oil

Origanum leaves were dried in a hot air oven at 60°C till a constant weight was obtained. The dried leaves were ground and placed inside a Soxhlet apparatus. Petroleum ether was used as a solvent for the extraction of oil. Soxhlet apparatus was run at 60°C for 8 h. After which the solvent (40°C–60°C) was evaporated in a rotary evaporator to isolate the essential oil.

One hundred and twenty extracted single-rooted teeth with mature apices were obtained and stored in saline. The teeth were soaked in 5.25% NaOCl for 30 min to remove the residual tissues and debris from the root surface. Each tooth was radiographed to confirm the presence of a single canal. Haapasalo and Orstavik proposed the infected dentin model, which was later modified by Kho and Baumgartner used in the present study.

The incisal surface was reduced and decoronated to make the length as 14 mm. An access preparation was performed with a high-speed round bur, and patency was confirmed with a 10K file. The working length was determined, and the canals were subsequently enlarged to 20K file at the working length. Polyvinyl siloxane impression material was expressed into 2 ml scintillation vials. The teeth were embedded in impression material up to the cementoenamel junction. After the material gets set, the cementoenamel junction was sealed with cyanoacrylate. The vials were then placed into 10 ml scintillation vials, and the caps were fitted to create an individual chamber. The teeth customized models and scintillation vials were subjected to autoclaving at 121°C for 30 min.

A 24-h pure culture suspension of *E. faecalis* ATCC 29212 was cultivated in brain heart infusion (BHI) broth. The experimental groups were inoculated with *E. faecalis* by placing the suspension in each tooth's access with micropipette. The fresh inoculum was added every 48

h and cultured for 6 weeks under the aerobic condition at 37°C. At the end of the 6-week incubation period, the experimental group's teeth were randomly assigned to one of the three groups. The teeth were instrumented to master apical file size, and each tooth received irrigation for the same amount of time. The coronal flaring was performed with gates Glidden 2–4 drills, and canal preparation was made with ProTaper (Dentsply, USA) rotary instruments in a crown-down technique. Standard irrigation regimen was followed during cleaning and shaping to maintain aseptic condition. To endure patency recapitulation was done using 10 K file, and instrumentation was done to a maximum size of 50/06 at the working length.

Experimental groups were as follows:

- Group 1: 3% NaOCl
- Group 2: 2% CHX
- Group 3: 0.2% oregano oil.

Irrigation protocol (Group 1, 2, 3)

Five milliliter disposable 31-gauge side-vented syringe needle irrigation was carried out, respectively, with 1 mm short of apex for 15 min. After that, the canal was rinsed with saline to flush the debris. Finally, 5 ml of 17% ethylenediaminetetraacetic acid (EDTA) was used as the final agent for 1 min and then flushed with saline. Using EDTA as a final rinse can penetrate dentinal tubules and remove the smear layer formed following biomechanical preparation. Therefore, usage of EDTA liquid as final rinse helps in better penetration of sealer during endodontic treatment.

To test for bacterial survival in the apical 5 mm of the root canal system and dentinal tubules, surgical handpiece (Impact Air 45) was used to remove apical 5 mm. The samples were pulverized for 30 s in liquid nitrogen. The samples were suspended in 1 ml of sterile BHI. Ten-fold dilutions were prepared, and 0.1 ml aliquots of the suspensions were spread on BHI agar media. They were incubated at 37°C for 48 h, and colony-forming units were determined. The specimens from the teeth were sampled and cultured using the same techniques.

RESULTS

The results were recorded on based on the diameter of zones. The data were analyzed using IBM SPSS (IBM SPSS predictive analytics community, Armonk, New York) statistics software 23.0 version. To find the significant difference between the groups, one-way ANOVA was used. For multivariate analysis, a *post hoc* Tukey's test was used. All three groups showed substantial reduction of viable bacteria. It was observed that oregano oil showed more of microbial inhibition (15 mm) than CHX (13 mm) and NaOCl (13 mm) [Graph 1]. When all the three groups were compared, Group III showed statistically significant ($P < 0.05$) result as compared to others.

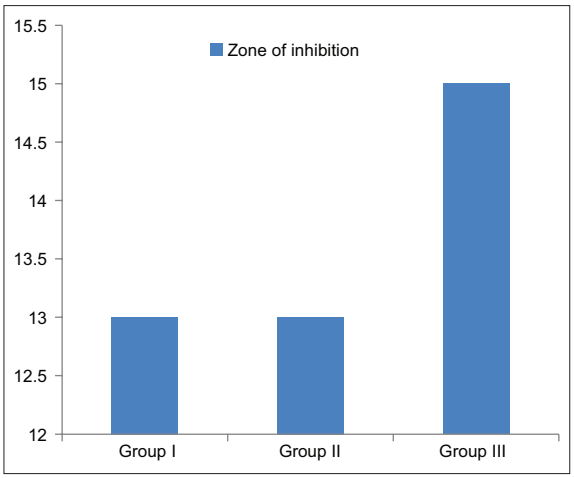
DISCUSSION

The challenges encountered during root canal treatment include inherent limitation of endodontic materials for chemomechanical preparation, microbiological challenges, and root canal morphological challenges. However, the three-dimensional disinfection of the root canal system is not achievable.[12] In routine endodontic practice, the commonly employed synthetic irrigants for the disinfection of the root canal system include NaOCl, CHX, and MTAD. Researches have found that due to demerit of the conventional used agents, such as reduction in dentin properties regarding NaOCl and reduced antimicrobial efficiency of CHX, the need for alternative herbal agents has become crucial.[13] This study is the first study to report antimicrobial efficiency of oregano essential oil against *E. faecalis* and its comparison with the conventional synthetic agents for root canal disinfection.

Previous studies have reported that higher NaOCl concentration (4–5.25%) was more effective in eliminating *E. faecalis* than CHX (1%–2%).[14,15] On the contrary, Vianna and Gomes reported that, using the same agar diffusion method, the 5.25% NaOCl showed low antibacterial activity than 2% CHX against *E. faecalis*.[16] The reason for contradictory results for the studies mentioned above may due to the use of hollow stainless steel tubes for the placement of test irrigant rather than paper disks onto the agar plate. The present study shows a similar inhibition zone in both 3% NaOCl and 2% CHX.

It is well known in the literature that the bactericidal property exists when NaOCl is added to water, strong oxidizing agent, hypochlorous acids are formed, which contains active chlorine. Evidence reports that chlorine's antibacterial effect is by the irreversible oxidation of essential enzymes and disruption of bacterial cell.[17]

The essential oil from aromatic and medicinal plants has known to possess antimicrobial and antioxidant property. Origanum vulgare (*Lamiaceae* family) is an endemic plant found in India, Southern part of Iran and Mediterranean region, which has been used traditionally used for the antiseptic purpose. Origanum essential oil showed a higher zone of inhibition in eliminating *E. faecalis*. This is due to inhibition of ATPase activity and increasing the nonselective permeability of bacterial cell membrane. It inhibits the microbial colonization



Graph 1: Zones of inhibition in different groups compared

and also makes the microbes more sensitive to antibacterial agents.[18] Moreover, the pH of oregano essential oil was found to be 6, which also substantiates the antibacterial activity, as the lower pH of the compound is said to have bactericidal property.[11] Furthermore, when tissue inflammatory response was evaluated on a rat model, it showed mild inflammatory response.[19]

In case of secondary or persistent endodontic infections, Gram-positive organisms such as enterococci are mostly predominant. Previous study reports that Gram-negative microorganisms are found to be more sensitive to herbal compounds than Gram-negative organisms. The reason is attributed due to a difference in the cell wall structure. A study by Mellencamp *et al*.[20] demonstrated the antimicrobial activity of oregano extract on various pathogens enterococcus species and concluded that extract had a maximal antibacterial activity on both Gram-positive and Gram-negative organisms with highest antioxidant activity. Therefore, oregano essential oil can be used as an adjunct in root canal disinfection.

There are few limitations in the present study. Root canal system is polymicrobial, therefore, assessing the antibacterial activity single species (*E. faecalis*) alone cannot be applicable in routine endodontic practice. Therefore, future studies are needed to prove the same.

Implications of future research should focus on the contact time of oregano essential oil required to eliminate the microorganisms from the root canal system. Although it is in oil form, its ability to act as a lubricant during instrumentation of root canal should be assessed. Furthermore, its ability to remove the dentinal debris and tissue dissolution need to be examined. Primary root canal debridement depends on the usage of root canal irrigants for complete chemical debridement and disinfection. Ideally, the newer formulated irrigants should show the pulpal dissolution and bacterial inorganic smear removal property. Hence, future studies have to concentrate on an irrigant's ability to remove the inorganic smear and dissolve the vital, inflamed, or necrotic pulp tissues.

CONCLUSION

The present study concludes that 0.2% origanum essential oil reported to show better antibacterial activity against *E. faecalis* when compared to 3% NaOCl and 2% CHX. Further clinical trials are required to highlight the antimicrobial activity of plant extracts against root canal pathogen.

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Conflicts of interest
There are no conflicts of interest.

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