

Antibacterial and smear layer removal capability of oregano extract solution

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ABSTRACT

Objective: The aim of this *in vitro* study was to evaluate the antimicrobial effect of oregano extract solution (OES) against *Enterococcus faecalis* within root canals and dentin tubules, and its effect on smear layer. **Materials and Methods:** A total of 180 human maxillary central incisors was selected. After removal of coronal part of the teeth, root canals were prepared using ProTaper rotary files (Dentsply, Tulsa Endodontics, OK, USA) to #F3 with the crown-down manner. The roots were randomly assigned to 15 groups ($n = 12$ for each). In the first seven groups, the antimicrobial effects of the test groups were evaluated. Suspensions of *E. faecalis* cultures were adjusted to 1.0 McFarland (1×10^8 colony-forming unit [CFU]/ml), and sterilized teeth were placed in Eppendorf tubes and kept at 37°C for 4 weeks. Samples were then taken from the root canals before irrigation using three sterile paper points. Dentin samples were taken from root canals with ProTaper #F4 and #F5 series rotary instruments after irrigation. The aliquots of samples were placed into the brain heart infusion and incubated at 37°C for 48 h and then the CFUs were counted. In the other eight groups, the efficacy of the irrigation solutions on removing the smear layer was evaluated using scanning electron microscope (Leo 440, Oxford Microscopy Ltd., Cambridge, England) analysis. Statistical evaluation of the microbiological data was performed using the Kruskal–Wallis and Mann–Witney U-test ($P < 0.05$). **Results:** There was a statistically difference between the groups ($P < 0.05$). Chlorhexidine gluconate (CHX), 5% and 2% OES wasn't found to be statistically significant regarding their antibacterial activities against *E. faecalis* ($P > 0.05$). 1% OES and NaOCl showed similar antimicrobial effect ($P > 0.05$), and 1% OES and NaOCl were better than ethylenediaminetetraacetic acid (EDTA) and saline ($P < 0.05$) but not as successful as CHX. According to the results obtained from dentin, CHX is the most effective solution within dentinal tubules. Different concentrations of OES were not achieved smear layer removal alone but OES in conjunction with 17% EDTA was the final irrigating solution achieved the smear layer removal without dentin erosion. **Conclusions:** Within the limitations of this study, OES appears to be a possible alternative to NaOCl as a root canal irrigant on the eradication of *E. faecalis* and removal of smear layer.

Key words: Antimicrobial effect, chlorhexidine gluconate, *Enterococcus faecalis*, irrigation solution, NaOCl, oregano extract solution, smear layer

INTRODUCTION

The success of a root canal treatment relies on the proper removal of pulpal remnants, bacteria, and their products from the root canal system.^[1] Mechanical preparation alone is not able to remove the pulpal remnants and bacteria effectively from root canals due to the fact that microorganisms are present in all parts of the root canal system, especially in anastomoses, lateral canals, and

dentin tubules.^[2] Effectively used irrigation in the root canal system is one of the most essential steps for the elimination of microorganisms from these locations.^[3]

An ideal irrigation solution has to have a proper antibacterial and tissue dissolving effect on the necrotic pulp remnant and minimum toxic effect on the periapical tissue. NaOCl is the most commonly used root canal irrigation solution up to date due to its antimicrobial

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effect and tissue dissolving properties,^[4] but it is harmful when put in contact with periapical tissue.^[5-7] Chlorhexidine gluconate (CHX) is an alternative irrigation solution to NaOCl that has a wide range of antimicrobial activity against both Gram-positive and Gram-negative microorganisms, especially against *Enterococcus faecalis*.^[8] However, a main disadvantage of CHX is its lack of organic tissue dissolution capabilities. Hence, other solutions have been experimented as alternative irrigation solutions to NaOCl and CHX.

Origanum minutiflorum is a plant found throughout South-Western Anatolia, especially in Isparta, Turkey. The plant's extract, especially its oil, has an antimicrobial effect on many microorganisms. Dadalioglu and Evrendilek^[9] reported that *O. minutiflorum* has an antimicrobial effect on *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus*. Similarly, Baydar *et al.*^[10] found that 1–2% origanum oil has an antimicrobial effect on *E. faecalis*.

Up to date, there were no *in vivo* or *in vitro* studies of oregano extract solution (OES) as a root canal irrigant in the literature. Therefore, the aim of this *in vitro* study was to evaluate the antimicrobial effect of OES as an intracanal irrigant against *E. faecalis* using bacteria culturing method, and its effect to the smear layer using a scanning electron microscope (SEM) (Leo 440, Oxford Microscopy Ltd., Cambridge, England).

MATERIALS AND METHODS

The method followed was a modification of the technique described previously by Berber *et al.*^[11] A total of 180 extracted human mature maxillary central incisor teeth with a single root and without root resorption was selected. The crowns were removed with a water-cooled diamond saw (Buehler Ltd., Lake Bluff, IL, USA) to leave uniform 15 mm apical sections of root.

The root canal instrumentation was done using an endodontic X-Smart micro-motor (Dentsply-Maillerfer, Ballaigues, Switzerland) at a speed of 350 rpm with ProTaper NiTi rotary files (Dentsply, Tulsa Endodontics, OK, USA) to size #F3 with the crown-down manner. The 180 specimens were randomly assigned to 15 groups ($n = 12$ for each), antimicrobial evaluation was performed in the first seven groups. In the other eight groups, effect of the solutions on the smear layer was studied.

The smear layer was removed in the first seven groups with 3 ml 17% ethylenediaminetetraacetic

acid (EDTA) (Merck KGaA, Darmstadt, Germany) for 1-min, followed by 3 ml 5.25% NaOCl (Wizard, Ankara, Turkey) for 1-min, and then 5 ml distillate water for 1-min as outlined by Teixeira *et al.*^[12] Samples were after that autoclaved at 121°C at 1 atm for 20 min, and then placed into glass tubes containing 5 ml of brain heart infusion (BHI) broth medium (BHI, Oxoid, Basingstoke, UK).

Cultivation of the *Enterococcus faecalis* and contamination of samples

Pure cultures of *E. faecalis* (ATCC 29212) cultures were previously cultivated in a BHI broth for 24 h and then cultured in 5% defibrinated sheep blood and BHI agar plates. Suspensions of this bacterium were adjusted to 1.0 McFarland (1×10^8 colony-forming units [CFU]/ml) and sterilized teeth were placed in Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and kept at 37°C for 4 weeks.

Sterile pipettes were used to remove 5 ml of sterile BHI and to replace it with 5 ml of the bacterial inoculum. The medium was changed every 7 days to avoid saturation and confirm the growth of *E. faecalis*.^[13] Four weeks later, the purity of the cultures was confirmed by Gram staining, catalase production.

Microbial sampling of the root canals

Samples were taken from the root canals before irrigation using three sterile paper points. After that, 6 ml of each solution (Group 1: EDTA; Group 2: CHX; Groups 3-5: 1%, 2%, 5% EOS, respectively; Group 6: 5.25% NaOCl; and Group 7: Sterile saline) was used for 2 min in each canal.^[13] After irrigation procures sterile paper points were placed into root canals for 1-min, and then transferred to Eppendorf tubes containing 1 ml BHI broth, and then vortexed for 1-min. After three times 10-fold serial dilutions were done for each sample, aliquots of 0.1 ml were plated onto the BHI and blood agar plates combination and incubated at 37°C for 48 h. Growing colonies were counted and recorded as CFU.

Dentin samples of root canals

Dentin samples were taken from root canals with ProTaper #F4 and #F5 series rotary instruments after irrigation. First, #F4 rotary instruments were rotated into the dentin for 15 s, and the collected dentin samples were transferred to the Eppendorf tubes containing 1 ml BHI broth. This was then repeated with the ProTaper #F5 series. All samples were vortexed for 1-min, and then the Eppendorf tubes were incubated at 37°C for 48 h. After two times 10-fold serial dilutions were done for each sample, aliquots of 0.1 ml were

plated onto the BHI and blood agar plates combination and incubated at 37°C for 48 h and recorded as CFU.

Scanning electron microscope evaluation

Smear layer removal procedure was done as described by Teixeira *et al.*^[12]

- Group 8: 3 ml 5.25% NaOCl for 1-min + 3 ml 17% EDTA for 1-min + 5 ml distilled water for 1-min
- Group 9: 3 ml 1% OES for 1-min + 5 ml distilled water for 1-min
- Group 10: 3 ml 2% OES for 1-min + 5 ml distilled water for 1-min
- Group 11: 3 ml 5% OES for 1-min + 5 ml distilled water for 1-min
- Group 12: 3 ml 1% OES for 1-min + 3 ml 17% EDTA for 1-min + 5 ml distilled water for 1-min
- Group 13: 3 ml 2% OES for 1-min + 3 ml 17% EDTA for 1-min + 5 ml distilled water for 1-min
- Group 14: 3 ml 5% OES for 1-min + 3 ml 17% EDTA for 1-min + 5 ml distilled water for 1-min
- Group 15: 3 ml sterile saline for 1-min + 3 ml 17% EDTA for 1-min + 5 ml distilled water for 1-min.

A longitudinal groove on the buccal and lingual surfaces on each specimen was prepared on each specimen using a diamond disc (SS White, Lakewood, New Jersey, USA) at low speed and split into two halves. The specimens were dehydrated, held in a 50%, 75%, 85%, and 96.5% ethyl alcohol solution, dried, mounted on metallic stubs, sputter-coated with a gold alloy (Polaron SC7620 Sputter Coater; VG Microtech Inc., Japan), and examined with a SEM (Leo 440, Oxford Microscopy Ltd., Cambridge, England) ×8,000 magnification.

The data were statistically analyzed using the SAS (SAS Institute, Raleigh, NC, USA) computer program. Each CFU count was transformed to Log_{10} to normalize the data before statistical evaluation. Statistical evaluation was done with the Kruskal–Wallis and Mann–Whitney U-test. The level of significance was set at $P < 0.05$.

RESULTS

Statistical evaluation of the groups concerning the antimicrobial effectiveness of the irrigation solutions is shown in Table 1. There was a statistically difference among the groups ($P < 0.05$). According to the results obtained in the root canal after irrigation, CHX, 5% and 2% OES were not found to be statistically significant regarding their antibacterial activities against *E. faecalis*. 1% OES and NaOCl showed a similar antimicrobial effect ($P > 0.05$) and were not as successful as CHX ($P < 0.05$).

Table 1: Mean of the Log_{10} CFU and SD (\pm SD) of *Enterococcus faecalis* in experimental groups within root canals and dentin tubules

| Irrigation solutions | Before irrigation | After irrigation | Dentin samples #F4 | Dentin samples #F5 |
|----------------------|--------------------------------|---------------------------------|--------------------------------|--------------------------------|
| EDTA | 6.567 \pm 0.494 ^a | 4.309 \pm 0.491 ^a | 4.046 \pm 0.189 ^a | 4.047 \pm 0.224 ^a |
| CHX | 6.508 \pm 0.403 ^a | 0 \pm 0 ^b | 0 \pm 0 ^b | 0 \pm 0 ^b |
| 1% OES | 6.287 \pm 0.976 ^a | 1.404 \pm 1.803 ^{cd} | 4.205 \pm 0.071 ^c | 3.817 \pm 1.235 ^a |
| 2% OES | 6.551 \pm 0.556 ^a | 0.391 \pm 0.926 ^{bc} | 4.159 \pm 0.094 ^c | 3.81 \pm 1.206 ^a |
| 5% OES | 6.375 \pm 0.578 ^a | 0.383 \pm 0.904 ^{bc} | 4.068 \pm 0.266 ^c | 4.092 \pm 0.290 ^a |
| NaOCl | 6.440 \pm 0.461 ^a | 2.308 \pm 1.739 ^d | 4.113 \pm 0.077 ^c | 4.137 \pm 0.079 ^a |
| Sterile saline | 6.591 \pm 0.536 ^a | 3.756 \pm 1.349 ^a | 4.174 \pm 0.080 ^c | 4.147 \pm 0.075 ^a |

*There is no statistically significant difference between shown in the same column and the values with the same superscript ($P > 0.05$). SD: Standard deviation, CFU: Colony-forming unit, CHX: Chlorhexidine gluconate, OES: Oregano extract solution

According to the results obtained from the dentin samples, CHX was the most effective solution compared to other groups ($P < 0.05$). CHX penetrated into the dentin tubules and eradicated all of the *E. faecalis*. No statistical difference was found between all OES groups and 5.25% NaOCl, which both could not penetrate into the dentin tubules.

According to the results obtained from the SEM evaluation, 1% OES, 2% OES, or 5% OES followed by 17% EDTA completely removed the smear layer without dentin erosion [Figure 1a-c]. However, % OES, 2% OES, or 5% OES without 17% EDTA failed to remove the smear layer alone [Figure 2a-c]. Irrigation with 5.25% NaOCl and 17% EDTA combination achieved the removal of the smear layer [Figure 3].

DISCUSSION

Enterococcus faecalis, a very persistent microorganism isolated from in infected teeth that invades deep into dentin tubules, was isolated in cases of failed root canal treatment.^[14,15] In the present study, *E. faecalis* was used to evaluate the antimicrobial efficiency of solutions. The results obtained from this study showed that 5.25% NaOCl was effective against *E. faecalis* within root canals. There was no statistical difference was found between 1% OES and 5.25% NaOCl ($P > 0.05$) in terms of their effectiveness against *E. faecalis* in this study. But there was a statistically significant difference between the antimicrobial effect of 2% OES and 5% OES and 5.25% NaOCl ($P < 0.05$). The present findings also indicate that in the eradication of *E. faecalis*, NaOCl was not as effective as CHX. Onçag *et al.*^[8] found that 2% CHX was significantly more effective against *E. faecalis* than 5.25% NaOCl. In our

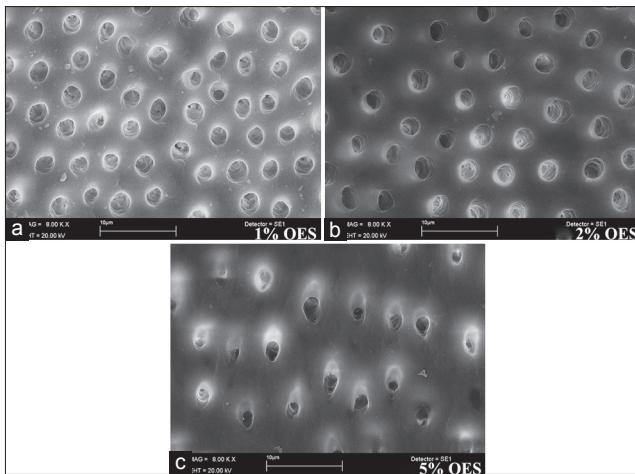


Figure 1: Scanning electron micrographs (a) 1% oregano extract solution (OES) + 17% ethylenediaminetetraacetic acid (EDTA) (b) 2% OES + 17% EDTA (c) 5% OES + 17% EDTA

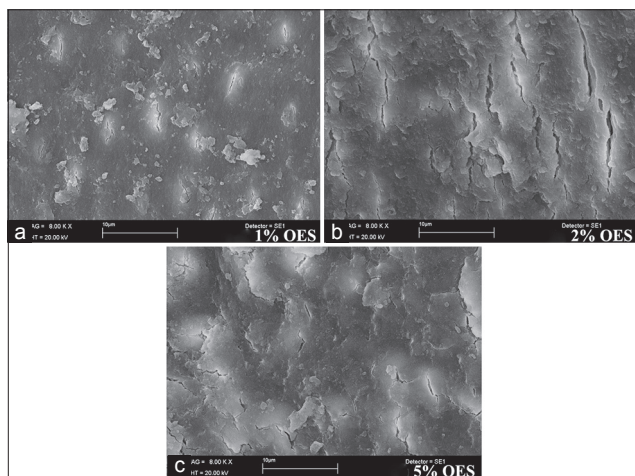


Figure 2: Scanning electron micrographs (a) 1% oregano extract solution (OES) (b) 2% OES (c) 5% OES

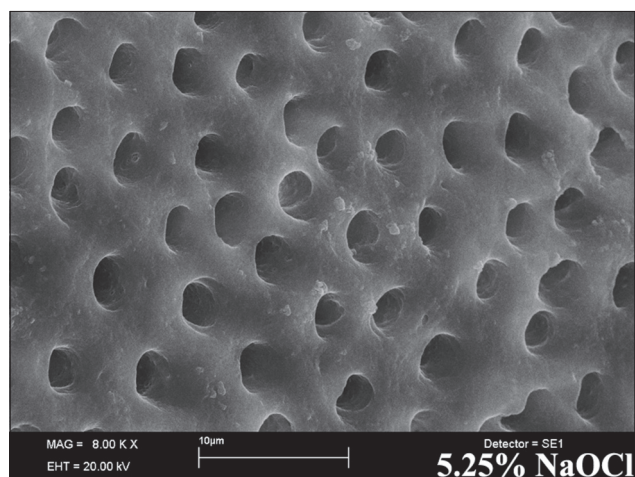


Figure 3: Scanning electron micrographs of 5.25 NaOCl + 17% ethylenediaminetetraacetic acid

than 5.25% NaOCl ($P < 0.05$). This result shows that our findings support the results of Onçag *et al.*^[8]

Microorganisms may invade to the dentin tubules of both vital and necrotic pulp, but vital teeth may limit the invasion of the microorganism with a pulp defense system.^[16] Microorganisms that live in dentin tubules are an important risk factor in the success of the root canal treatment.^[11,17] Berber *et al.*^[11] indicated that no statistical difference was found between 0.5% and 5.25% concentrations of NaOCl as a root canal irrigant. However, in regard to its antimicrobial effect on dentin tubules, 5.25% NaOCl was evaluated as highly effective. Vahdaty *et al.*^[18] stated that NaOCl had an antimicrobial effect against *E. faecalis* in 500 μ m deep dentin tubules, and Gomes *et al.*^[19] found the same in 10 μ m deep dentin tubules. Mohammadi and Shahriari^[20] found that NaOCl had no antimicrobial effect when CHX had not penetrated deep into the dentin tubules, and Basrani *et al.*^[21] and Gomes *et al.*^[19] have stated that 2% CHX completely eradicated *E. faecalis* from root canals and dentin tubules. Similarly, in our findings, NaOCl and OES could not penetrate into the dentin tubules, and CHX was highly effective against *E. faecalis* in the dentin tubules ($P < 0.05$). However, 2% and 5% OES more effectively eradicate *E. faecalis* than 5.25% NaOCl in the root canals ($P < 0.05$).

In the present study, NaOCl and EDTA, combination was used to remove the smear layer. Although NaOCl was unsuccessful in removing the smear layer alone, it has been used for many years due to its capacity to act as a solvent of organic tissue. Beltz *et al.*^[22] stated that 5.25% NaOCl dissolved over 90% of organic components, and that 17% EDTA dissolved above 70% of inorganic components. Several studies^[23-25] have stated that an EDTA and NaOCl combination has the ability to remove the smear layer without damaging dentin walls. In the present study 5.25% NaOCl and 17% EDTA combination was used as a positive control group, it was found that the smear layer was completely removed, all dentin tubules were opened, and the root canals were clean in all parts. Contrary to the findings of Serper and Calt,^[26] no intertubular and peritubular erosion was established. The results of our findings were similar to many articles in literature.^[12,23,25,27] This difference may have arisen from the use of a 1-min 10 ml 17% EDTA solution in their study.^[26] Similarly, Ballal *et al.*^[28] reported that using 5 ml 17% EDTA did not cause erosion in peritubular dentin.

study, CHX eradicated all of the *E. faecalis* from the root canal system and was significantly more effective

Alternatively to NaOCl, different OES concentrations were also used alone as well as combined with EDTA

to remove the smear layer, and found that OES could not remove the smear layer alone [Figure 2]. However, when the OES and EDTA combination was used, it removed the smear layer and opened dentin tubules without erosion. The combination of 1%, 2% or 5% OES and 17% EDTA removed surface smear layer, dentin tubules were opened in the coronal, middle, and apical part [Figure 1]. Torabinejad *et al.*^[29] reported that the opening of the dentin tubules was greater in the coronal third than the apical third in connection to insufficient irrigation due to the small diameter of the apical part. Similarly, in this study, all solutions that removed the smear layer led to dentin tubules that were opened more in the coronal part than the apical part.

CONCLUSION

Within the limitations of this study, 1% OES and 5.25% NaOCl have the same antimicrobial activity against *E. faecalis* and 2% or 5% OES had more effective antibacterial action than 5.25% NaOCl. OES and NaOCl have the same effect on the removing of the organic compound of the smear layer. Therefore, OES appears a possible alternative to 5.25% NaOCl as a root canal irrigant.

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