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Comparative Antibacterial Capabilities of *Origanum Onites Oil* and Diode Laser against *Enterecoccus faecalis* Contaminated Primary Root Canals

Aylin İslam[1,2], Serdar Susever[2,3], Duygu Yiğit Hanoğlu[4], Kemal Hüsnü Can Başer[4], Serap Çetiner[5]

[1]Department of Pediatric Dentistry, Near East University Faculty of Dentistry, Northern Nicosia, North Cyprus
[2]Research Center of Experimental Health Sciences, Near East University, Northern Nicosia, North Cyprus
[3]Department of Nutrition and Dietetics, Near East University Faculty of Health Sciences, Northern Nicosia, North Cyprus
[4]Department of Pharmacognosy, Near East University Faculty of Pharmacy, Northern Nicosia, Northern Cyprus
[5]Department of Pediatric Dentistry, Kyrenia University Faculty of Dentistry, Kyrenia, North Cyprus

ORCID iDs of the authors: A.İ. 0000-0001-6338-4505; S.S. 0000-0002-4988-5634; D.Y.H. 0000-0003-1345-4768; K.H.C.B. 0000-0003-2710-0231; S.Ç. 0000-0003-1793-1840.

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BACKGROUND/AIMS
Enterecoccus faecalis is the most dominant and most isolated resistant species from infected/failed permanent teeth undergoing root canal therapy or painful primary teeth with periapical radiolucency. The main purpose of the present research was to compare the antimicrobial effectiveness of essential oil of Origanum onites and diode laser irradiation against *E. faecalis* removal from primary root canals.

MATERIAL and METHODS
A total of 60 retained human primary incisors were selected randomly and divided into six experimental groups as follows: Group I: negative control; Group 2: positive control; Group 3: sodium hypochlorite (NaOCl); Group 4: diode laser; Group 5: Origanum onites (OO) oil; Group 6: diode laser + OO oil. The gas chromatography–mass spectrometry (GC–MS) and GC analyses of *OO oil* were carried out with an Agilent 5977B GC-MSD and Agilent 7890B GC systems, respectively. A 2-W diode laser was used in a continuous action mode with a wavelength of 980 nm for 20 seconds. NaOCl and OO oil were applied continuously for 5 minutes to each root canal. Multiple comparisons and the significances between experimental groups were statistically analyzed using Tukey's multiple comparisons test. The level of significance was accepted at .05 for the entire statistical analysis ($P = .05$).

RESULTS
The major reductions were observed in the groups of NaOCl (98.3%) and diode laser + Origanum onites oil combination (92.5%).

CONCLUSION
Combined therapy of diode laser irradiation following Origanum onites oil application in primary root canal disinfection may be used as an ideal effective chemo-mechanical alternative to NaOCl irrigation.

Keywords: Origanum onites oil, *E. faecalis*, diode laser, antibacterial

INTRODUCTION

The pulpectomy procedure seems to be complicated in primary teeth due to the existence of ramifications, microcanals, and root resorption areas that do not allow sufficient bacterial elimination from the root canal system with mechanical instrumentation and irrigation.[1,2]

Various bacterial species colonize root canals, but *Enterecoccus faecalis* is the most dominant and most isolated resistant species from infected/failed permanent teeth undergoing root canal therapy or painful primary teeth with periapical radiolucency.[3-6] The main properties of *E. faecalis* contribute toward its capacity to adapt to severe environmental

Corresponding Author: Aylin İslam
E-mail: aylin.islam89@gmail.com

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conditions, as evidenced by its penetration into dentinal tubules, resistance to extreme alkaline pH values, exhibition of virulence factors, and biofilm formation.[7-11]

Currently, sodium hypochlorite (NaOCl) is still the most used irrigant. However, there are major adverse effects including that it is toxic, functions as an irritant when it reaches the apex because of the physiologic resorption of primary roots, and has harmful effects on dentin elasticity and flexural strength. In addition, it is unsuccessful for smear layer removal with limited dentinal tubule penetration to a depth of 100 μm.[12,13]

Over the last decade, various alternative irrigative methods consisting of lasers/laser-activated irrigation and ultrasonic/sonic irrigation systems have been developed and popularized to increase the effectiveness of root canal disinfection and *E. faecalis* elimination.[14-16]

Besides laser technology, the usage of plant-derived/natural/herbal extracts has increased as new efficient therapies against growing antibacterial resistance and oral bacteria. Various natural extracts or their complexes have been investigated, and it has been shown that herbs can manage oral infections to improve oral health.[17] Oregano is one of these herbs and is a term which describes different subspecies. The genus Origanum is rich in phenolic compounds with their powerful antibacterial and antifungal capabilities.[25] Generally, the main components of Oregano essential oils are composed of the monoterpenic phenols carvacrol and thymol. These main components are biosynthesized from γ-terpinene through p-cymene.[26,27] Oregano essential oils have been reported that exhibit anticarcinogenic, antioxidant, antifungal, antibacterial, antioxidant, analgesic and antispasmodic properties under different growth conditions.[28]

There are limited research investigating some herbal drugs as an intracanal medicament in primary root canals.[22-24] Various studies have discussed the antibacterial activities and cytotoxicity of Origanum species, particularly, its essential oil on oral microorganisms.[18-21] However, no research has focused on the antibacterial efficiency of specific Origanum onites oil (OO oil) as a root canal irrigant in primary teeth.

In the light of current clinical problems (such as complex morphology of primary teeth, toxic and irritant effects of NaOCl), the main objective of the present research was to compare the antimicrobial effectiveness of essential oil of *Origanum onites* and diode laser irradiation in primary root canals and to suggest a more conservative clinical alternative to NaOCl irrigation against *E. faecalis* removal from primary root canals.

Main Points

- Combined therapy of diode laser irradiation following OO oil application in primary root canal disinfection should be recommended as an ideal effective chemo-mechanical alternative to NaOCl irrigation.
- A small difference between the *E. faecalis* reduction percentages of NaOCl (98.3%) and diode laser–Origanum onites oil combination (92.5%) groups was observed.
- Further in vivo investigations with larger sample sizes are required to provide a clinically valuable antibacterial treatment with minimal chair-time as an important point of children s treatment.

MATERIAL and METHODS

The present experimental study was evaluated and approved by the Institutional Review Board of Near East University (NEU / 2018 / 58-609). The commercial essential oil of Origanum onites was acquired from TûRER Inc.

The commercial essential oil of *Origanum onites* was acquired from TÜRER Inc.

Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–MS Analysis: The GC–MS analysis was carried out with an Agilent 5977B GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as the carrier gas (0.8 mL min^{-1}). GC oven temperature was kept at 60°C for 10 minutes and programed to 220°C at a rate of 4°C min^{-1}, and kept constant at 220°C for 10 minutes and then programed to 240°C at a rate of 1°C min^{-1}. The split ratio was adjusted to 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450. The sample was dissolved in 10% *n*-hexane, and 1 μL was injected.

GC Analysis: The GC analysis was carried out using an Agilent 7890B GC system. The flame ionization detector (FID) detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a triplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of Compounds: Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their linear retention index (LRI) to series of *n*-alkanes. Computer matching against commercial databases (Wiley GC/MS Library, NIST Chemistry Volatile oil constituents of *Origanum* species WebBook)[29,30] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data were used for the identification.[31,32]

Antimicrobial Susceptibility of Origanum Onites: *E. faecalis* was incubated at 37°C for 48 hours in blood agar (LAB028, LAB M Limited, Lancashire, United Kingdom). The microdilution method was used for the antimicrobial susceptibility test. Extracts were prepared at the following concentrations: 500, 250, 125, 62.5, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, and 0.03 μg mL^{-1}. The bacterial and *E. faecalis* suspensions were adjusted to 0.5 McFarland standard turbidity. In this method, dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Saint Louis, USA) and water were used as control groups. To each of the dilutions, 100-μL of broth cultures and 100-μL of standard microorganisms were added. The microplates were incubated at 37°C for 48 hours. The turbidity reading was performed by a spectrophotometer.

Preparation of Teeth: A total of 60 retained human primary incisors without physiological or pathological resorption on more than one-third of the apical root were used. First, all superficial debris, tissue tags and calculus were removed, and all

samples were stored in normal saline solution (VACOLITER, Baxter, Turkey). Then, the crowns of the primary teeth were sectioned from the cement enamel junction and, for standardization, the length of the root canals was set at 8 mm. Later, the pulp tissues were extirpated from the root canals, and canals with an apical size of F3 were prepared using rotary instruments (x-smart, DENTSPLY, York, ABD). During the preparing and shaping procedure, 5.25% sodium hypochlorite (NaOCl, Chlorax, Cerkamed, Wola, Poland) in sterile saline was used as an irrigant agent. At the end of the preparation process, the smear layer was removed using 17% Ethylenediaminetetraacetic acid (EDTA) (Endo-Solution, Cerkamed, Wola, Poland), and all samples were irrigated again with sterile saline. Before the experiments, all prepared samples were autoclaved at 121°C for 30 minutes.

***E. faecalis* Contamination of Root Canals:** A 30-µL suspension of pure cultured *E. faecalis* Muller Hinton Broth (LAB114, LAB M Limited, Lancashire, United Kingdom) was used to contaminate each root canal with a sterile insulin syringe. After completing *E. faecalis* contamination, all samples (except negative control group) were incubated *at* 37°C for 48 hours. Following this incubation period, the samples were treated according to the experimental design.

Determination of Experimental Groups: The samples were selected randomly and divided into six equal experimental groups (n = 10) as follows:

Group 1: negative control (no contamination with *E. faecalis*, only normal saline);

Group 2: positive control (ATCC 29212 *E. faecalis* contamination, only normal saline);

Group 3: 5 mL 2.5% NaOCl;

Group 4: diode laser;

Group 5: 5 mL Origanum onites oil; and

Group 6: diode laser + 5 mL Origanum onites oil (overnight).

NaOCl and OO Oil Were Applied Continuously for 5 Minutes to Each Root Canal

Diode Laser Irradiation: Diode laser irradiation was performed using a diode laser (MEDENCY, Primo, Vicenza, Italy). A 2-W diode laser was used in a continuous action mode with a wavelength of 980 nm using a 200 µm diameter optical fiber for 20 seconds. Laser irradiation was initiated at the coronal portion of each root canal with helicoidal optical fiber movements down to 1-mm short of the apical area.

Intracanal bacterial samples were taken before and after canal disinfection to determine the CFU count.

Statistical Analysis

Multiple comparisons and the significances between mean values of the experimental groups were statistically analyzed using *Tukey's multiple comparisons test*. The percentages of reduction in colony counts (%RCC) were evaluated using the following equation:

CFUS (before treatment) – CFUS (after treatment) × 100 = %RCC

CFUS (before treatment)

Table 1. Essential Oil Composition of *Origanum* Onites (Türer Inc.)

LRI	Compound name	Relative percentage amounts (%) A
1020	α-Pinene	0.4
1024	α-Thujene	1.1
1072	Camphene	0.3
1119	β-Terpinene	0.1
1172	Myrcene	0.6
1177	α-Phellandrene	0.2
1191	α-Terpinene	1.3
1213	Limonene	0.2
1222	β-Phellandrene	0.2
1260	γ-Terpinene	6.9
1287	*p*-Cymene	4.1
1298	Terpinolene	0.1
1457	1-Octen-3-ol	0.1
1478	*trans*-Sabinene hydrate	0.4
1555	Linalool	1.4
1564	*cis*-Sabinene hydrate	0.2
1569	Linalyl acetate	0.1
1624	Terpinene-4-ol	0.7
1628	β-Caryophyllene	0.5
1638	Aromadendrene	0.1
1717	α-Terpineol	0.2
1728	Borneol	0.4
1748	β-Bisabolene	1.1
1770	Carvone	tr
1786	δ-Cadinene	tr
1793	γ-Cadinene	0.1
1899	Carvacryl acetate	0.1
2033	Caryophyllene oxide	0.1
2159	Spathunelol	0.1
2205	T2Cadinol	0.2
2210	Thymol	0.2
2243	Carvacrol	78.4
	Total	100.0

A: essential oil of Origanum onites (TÜRER, Inc.); LRI: linear retention indices calculated against n-alkanes; %: calculated from FID data; tr: trace (<0.1%).
Abbreviations: LRI, linear retention indices; FID, flame ionization detector; tr, trace.

RESULTS

The analysis results of commercial *Origanum onites* essential oil composition are given in Table 1. The totally analyzed essential oil was represented with 32 components. The major compounds were determined as carvacrol (78.4%), g-terpinene (6.9%), and *p*-cymene (4.1%), respectively.

According to the study results, bacterial growth was observed both before and after treatment in all 10 samples of the positive control group, whereas no bacterial growth was observed in the negative control group. Briefly, all samples treated with the diode laser and *Origanum onites oil* were positive for bacterial growth both before and after treatment. However, for the sodium hypochlorite (NaOCl) group, no bacterial growth was observed after treatment. After the 48 hours cultivation period, a significant decrease was detected for the combined diode laser and *Origanum onites oil* group.

The percentages of reduction in *E. faecalis* colony counts after irrigation procedures are shown in Table 2. The major reductions were observed in the groups of NaOCl and diode laser-*OO oil* combination. Moreover, the small difference between

Table 2. *E. faecalis* Reduction in Colony Counts of Each Group

Groups	RCC (%)	CFU mL^{-1} (±SD)
Positive control	0	3.04×10^5 ($\pm 6.7 \times 10^3$)
NaOCl	98.3	5.1×10^3 ($\pm 2.7 \times 10^3$)
Diode laser	32	2×10^5 ($\pm 4.5 \times 10^3$)
Origanum onites oil	25.5	2.26×10^5 ($\pm 1.5 \times 10^4$)
Diode laser + *Origanum onites* oil	92.5	2.2×10^4 ($\pm 6 \times 10^3$)

Bacterial base line count was 3×10^5 CFU mL^{-1}, and no bacterial growth was observed in negative control.
Abbreviations: NaOCl, sodium hypochlorite; RCC, reduction in colony counts.

the *E. faecalis* reduction percentages of NaOCl (98.3%) and diode laser–*Origanum onites oil* combination (92.5%) groups was found to be statistically significant. A greater reduction was observed in NaOCl in comparison to the diode laser–*Origanum onites oil* combination ($P = .0317$). Although *Origanum onites oil* RCC was about 25.5% without diode laser irradiation, when *Origanum onites oil* irrigation was combined with diode laser irradiation, the RCC was detected to be about 92.5%. This difference between groups was found to be significant ($P < .0001$). The same trend was detected between diode laser irradiation alone and diode laser–*Origanum onites oil* combination ($P < .0001$). The statistical comparisons between all groups are presented in Table 3.

DISCUSSION

E. faecalis is isolated with greater frequency from permanent root canals but clinically, it is copiously present in primary root canals.[33] In endodontic treatments, because of the bactericidal effects of lasers, various kinds of lasers, such as Er:YAG, Nd:YAG, and diode lasers, have been developed and used to provide infection control. In the present study, the diode laser was preferred because it has a good bactericidal effect and does not cause an unacceptable temperature rise in periodontal/external root tissues.[34-36] Also, the deep penetration capability of diode lasers into the dentinal tubules has been shown to be satisfactory under in vivo conditions.[37] For this reason, the diode laser irradiation was performed with a newly designed endodontic tip that was set to operate in the continuous mode for a regular effect at a power output of 2 W. According to Dai et al.'s[38] study, 2 W power was used to remove the smear layer on primary teeth while avoiding dentinal melting. However, diode laser irradiation alone with 2 W power output did not effectively reduce the *E. faecalis* bacterial count (32%). This ineffectiveness of the diode laser might be explained by biostimulatory effects of laser. The minimal exposure to laser irradiation in the present study may have helped to increase the physiological activities of *E. faecalis* for proliferation instead of reduction.[39] Also, the wavelength could be another reason that explains inadequate efficiency of diode laser. The wavelength of 980 nm in diode lasers has a powerful water absorption capacity, so superficial dentin layers benefit from the majority of antimicrobial effects in comparison to deeper tubule layers.[40] Hence, the disinfection capacity of diode laser is decreased against resistant bacteria like *E. faecalis*, which can penetrate deeper dentinal tubules. Another reason for the lower percentage of bacterial reduction with diode laser irradiation could be justified by the findings of Borges et al.'s[41] study. They reported that gram-positive bacteria such as *E. faecalis* need to be irradiated with additional-repeated modes for disruption of the bacterial cell wall. Although no effective bacterial reduction was determined and no attempts were made to find out main interaction between diode laser and *E. faecalis*, the main bactericidal mechanism of diode laser irradiation was based upon the photothermal effect, which stimulates the bacterial cell death by creating localized heating sides around the bacterial microenvironment.[42]

Essential oils that are rich in phenolic compounds in particular have the capabilities to change the permeability of cell membrane via diffusion into the phospholipids layer of the bacterial cell wall, thus affecting protein synthesis leading to cytoplasmic changes and blocking cellular functions.[43,44]

According to our results, the bactericidal effect of *Origanum onites oil* without diode laser irradiation as an intracanal irrigant agent on *E. faecalis* was inadequate (25.5%). This situation may be explained with more than one action of mechanism. First, *Origanum onites oil* is highly vaporizable, so it may have lost its antibacterial effectiveness during incubation. Second, the primary tooth microstructure may complicate the

Table 3. The Statistical Comparisons between All Experimental Groups. The Major Reductions Were Observed in the Groups of NaOCl and Diode Laser-Origanum Onites Oil Combination

Tukey's multiple comparisons test	Mean diff.	95% CI of diff.	Significant	Summary	*P* value
Positive vs. negative control	3.042	2.877 to 3.207	Yes	$P < .0001$	<.0001
Positive vs. NaOCl	2.991	2.826 to 3.156	Yes	$P < .0001$	<.0001
Positive vs. diode laser	0.9744	0.8093 to 1.139	Yes	$P < .0001$	<.0001
Positive vs. Origanum onites	0.7784	0.6133 to 0.9435	Yes	$P < .0001$	<.0001
Positive vs. laser-Origanum onites	2.816	2.651 to 2.981	Yes	$P < .0001$	<.0001
Negative vs. NaOCl	−0.051	−0.2161 to 0.1141	No	not significant	.9415
Negative vs. diode laser	−2.068	−2.233 to −1.903	Yes	$P < .0001$	<.0001
Negative vs. Origanum onites	−2.264	−2.429 to −2.099	Yes	$P < .0001$	<.0001
Negative vs. diode laser-Origanum onites	−0.226	−0.3911 to −0.06095	Yes	$P < .005$	.0022
NaOCl vs. diode laser	−2.017	−2.182 to −1.852	Yes	$P < .0001$	<.0001
NaOCl vs. Origanum onites	−2.213	−2.378 to −2.048	Yes	$P < .0001$	<.0001
NaOCl vs. diode laser-Origanum onites	−0.175	−0.3401 to −0.009947	Yes	$P < .05$	.0317
Diode laser vs. Origanum onites	−0.196	−0.3611 to −0.03095	Yes	$P < .05$	.0112
Diode laser vs. diode laser-Origanum onites	1.842	1.677 to 2.007	Yes	$P < .0001$	<.0001
Origanum onites vs. diode laser-Origanum onites	2.038	1.873 to 2.203	Yes	$P < .0001$	<.0001

Abbreviation: NaOCl: sodium hypochlorite.

penetration of *Origanum onites oil*, which is viscous and may be unable to penetrate dentinal tubules, especially in the case of smear layer. In agreement with Man et al.'s[45] study, the lower antibacterial effect of *OO oil* on *E. faecalis* may be related to oil's aqueous form which leads to a decrease in its antibacterial activity. Resultantly, DMSO was preferred to decrease the hydrophobic viscous structure of essential oil and also to reduce its side effects in the present study. While DMSO decreases the side effects and viscous structure of *OO oil*, it reduces the purity of essential oils. Therefore, in the present study, the *OO oil* was not used in its pure form, so that *OO oil* alone was detected ineffective against to *E. faecalis*. Another important factor that affects the antibacterial activity is the thymol and carvacrol content of *OO oil*. Higher amounts of thymol exhibit stronger antibacterial activity.[46] In a previous study by Başer, carvacrol was identified as the main component responsible for the biological activities of origanum species including *Origanum onites*.[47] According to GC/MS analysis in the present study, carvacrol (78.4%), γ-terpinene (6.9%), and *p*-cymene (4.1%) were detected as the major components of *OO* oil. Therefore, the *E. faecalis* reduction capacity of *Origanum onites oil* in this study may be explained by its high carvacrol component. In contrast to Ok et al.'s[20,21] studies, the reason for the differences with these studies may be caused by the lower percentage of thymol component (0.2%) in *OO oil* used our study and also the application area of the oregano essential oils. In Ok et al.'s[20,21] studies, oreganum extract solutions were found nontoxic and more effective against to *E. faecalis* than our results. Also thymol component was ranged between 1% and 1.25%, and mature/permanent teeth were used in those studies.

Parallel to our results, the effectiveness of NaOCl was shown by Oliveira et al.,[48] who found that, as an endodontic irrigant, only NaOCl had the capacity to kill the entire bacterial population. Of the considered treatments, we similarly observed that 2.5% NaOCl was the most effective endodontic irrigant for *E. faecalis* removal from primary root canals. However, this effective disinfection of 2.5% NaOCl probably arose due to the larger instrumental size of the primary root canals. In agreement with Kumar et al.'s[49] study, this larger preparation size of the root canals enables the dentinal tubules to be opened and removes the intratubular bacteria by allowing more effective penetration of NaOCl.

In the current study, the antibacterial efficacy of diode laser + OO oil combination could be clarified by Al Shahrani et al.'s[50] study, which claimed that the photonic energy of laser may activate and enhance irrigant agents by providing greater accessibility to unreachable parts of dentinal tubules.[50] Furthermore, the increasing antibacterial effect of *OO oil* in the long-term (overnight) may be explained by the increased smear layer dissolution and dentinal tubule penetration of *OO oil*. In other words, diode laser may act as a synergistic factor when combined with *OO oil*, and thus diode laser may increase the antibacterial effect of *OO oil*.

Furthermore, in vivo experiments with larger sample size need to be tested for a better understanding of the main antibacterial mechanism of diode laser irradiation and *Origanum onites oil*.

Within the limitations of this study, we can conclude that the combined therapy of diode laser irradiation following *OO oil* application in primary root canal disinfection may be used as an ideal effective chemo-mechanical alternative to NaOCl irrigation, especially in larger apical sized primary teeth with physiological resorption. Also, antibiotic resistance due to long-term medicinal usage may be prevented using this method. Prior to using irrigant agents including herbal drugs or laser, the viscosity of an essential oil, complex morphological root-canal structure of primary teeth, smear layer, deep penetration to dentinal tubules, timing properties, and biocompatibility must be taken into consideration and the ideal agent must be selected. Further studies are required to provide a clinically valuable antibacterial treatment choice with minimal chair-time as an important point of children's treatment.

Ethics Committee Approval: Ethical committee approval was received from the Near East University (NEU / 2018 / 58-609).

Informed Consent: Written informed consent was obtained from patients before they participated in the study.

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