Dental Research Journal

Original Article

Comparison of antimicrobial effect of *Ziziphora tenuior, Dracocephalum moldavica, Ferula gummosa,* and *Prangos ferulacea* essential oil with chlorhexidine on *Enterococcus faecalis*: An *in vitro* study

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ABSTRACT

Background: Different irrigating solutions with high antimicrobial activity have been introduced for cleaning of the root canal system. However, effects of *Prangos ferulacea* (PF), *Ziziphora tenuior* (ZT), *Dracocephalum moldavica* (DM), and *Ferula gummosa* (FG) on oral and dental pathogens have not been extensively evaluated due to their optimal biocompatibility and insignificant side effects. The aim of this study was to evaluate the antibacterial effects of essential oils of mentioned plants on *Enterococcus faecalis*.

Materials and Methods: In this *in vitro* study the plants were collected from Zanjan Province, Iran. Analysis of the essential oil was carried out by gas chromatography/mass chromatography. Micro-broth dilution and disc diffusion methods were used for assessment of the antimicrobial activity, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated.

Results: All the four essential oils had antibacterial effects on *E. faecalis*, and ZT had the greatest antibacterial activity. Assessment of the mean diameter of the growth inhibition zone showed higher antibacterial activity of PF and ZT than that of chlorhexidine. The MIC and MBC of ZT showed that the antimicrobial activity of ZT against *E. faecalis* was greater than that of other plants evaluated in this study.

Conclusion: The results of this study indicated significant antibacterial effects of the mentioned plants on *E. faecalis*. The greatest antimicrobial activity belonged to ZT. The current study suggests extraction of effective compounds in these medicinal plants to use them in the clinical setting.

Key Words: Chlorhexidine, *Dracocephalum moldavica, Enterococcus faecalis, Ferula*

Received: June 2017
Accepted: September 2017

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INTRODUCTION

The main goal of modern dentistry is to maintain the function of the masticatory system throughout one's life. This goal is hard to achieve without endodontic therapy in most patients.[1] *Enterococci* are responsible for most of the primary infections of the root canal system. They have been isolated from many treated root canals with chronic apical periodontitis and failed root canal treatment.[2] These bacteria are

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How to cite this article: Nazemisalman B, Vahabi S, Yazdinejad A, Haghghi F, Jam MS, Heydari F. Comparison of antimicrobial effect of *Ziziphora tenuior*, *Dracocephalum moldavica*, *Ferula gummosa*, and *Prangos ferulacea* essential oil with chlorhexidine on *Enterococcus faecalis*: An *in vitro* study. Dent Res J 2018;15:111-6.

capable of invading the dentinal tubules. They can tolerate different ecological conditions, well adapt to unfavorable intracanal environment, and are multidrug resistant. *Enterococci* are highly sensitive to sodium hypochlorite and also chlorhexidine (CHX). However, their sensitivity decreases with a reduction in the concentration of these antimicrobial agents.[3] Since inadequate cleaning and shaping as well as debris pushed into the root canal are among the factors responsible for root canal treatment failure, different irrigating solutions are used for cleaning and disinfection of the root canal system.[4] Irrigating solutions must have low cytotoxicity and surface tension, adequate flow, and sustained antimicrobial activity. Moreover, they must be easily accessible and affordable and have a pleasant odor. An irrigating solution with high antimicrobial activity and insignificant adverse effects is obviously preferred.[5]

Due to extensive antimicrobial activity, CHX has several applications in dentistry, and due to strong antimicrobial activity, relatively long substantivity, and lack of toxicity, CHX is considered a favorable irrigating solution for root canals. However, its frequent use results in dental stains, burning sensation of the mucosa, xerostomia, gingival desquamation, impaired taste, and adverse systemic complications if swallowed accidentally.[6] Since there are numerous side effects of chemical agents and emerging resistance of bacteria to these agents, researchers have been in search of herbal products with antimicrobial, antifungal, and anticancer properties since herbal medications often have minimal side effects and are affordable. However, studies on the properties of organic compounds for dental applications are not many. Four medicinal plants harvested in Iran with significant antibacterial properties, which have been traditionally used for the treatment of oral and dental conditions, are evaluated in this study.

Prangos ferulacea (PF) is native to Iran and possesses significant pharmaceutical, nutritional, and antioxidant properties. Strong antibacterial properties of this plant against several Gram-positive and Gram-negative bacteria have been previously confirmed.[7-9] *Ziziphora tenuior* (ZT) grows on mountain slopes.[10] The extract of some species of this plant such as *Ziziphora clinopodioides* and *Ziziphora persica* is capable of preventing the growth of a wide range of Gram-positive and Gram-negative pathogenic microorganisms.[11-13] *Ferula gummosa* (FG) has extensive antibacterial properties as well.[14] Essential oil of the seeds of this plant has strong antimicrobial activity against Gram-positive bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus*.[15] *Dracocephalum moldavica* (DM) has a wide spectrum of antimicrobial activity against pathogens such as *Streptococcus mutans, Micrococcus luteus*, *S. aureus*, *Candida albicans*, *Escherichia coli*, and *Bacillus subtilis*.[16]

To the best of authors' knowledge, no previous study has evaluated the antibacterial activity of the aforementioned four medicinal plants against oral and dental pathogens. Thus, this study aimed to compare the antibacterial effects of PF, ZT, FG, and DM essential oils in comparison with CHX on *E. faecalis*.

MATERIALS AND METHODS

In this *in vitro* study the plants were collected from Zanjan City, Iran, identified in the herbarium of Zanjan University of Medical Sciences and dried in shade. Essential oils were extracted using the Clevenger apparatus via the distillation method. Analysis of the essential oil was carried out using gas chromatography/mass chromatography.

Tested microorganisms

S. mutans, Streptococcus sobrinus, Streptococcus salivarius, and *Lactobacillus acidophilus* strains were obtained and microbiologically approved. Microorganisms were stored in brain–heart infusion broth containing 15% glycerol (v/v) at −80°C. Before the experiment, microbial suspension was transferred to nutrient broth and incubated overnight at 37°C. The turbidity of the inoculum suspension was adjusted at 0.5 McFarland standard concentration to achieve 1.5×10^8 colony-forming units/ml. Double-seriated dilution in 0.1% (w/v) suspension of peptan in distilled water in nutrient agar was used to ensure the viability of microorganisms.

Disc diffusion method

Assessment of the antimicrobial activity of essential oils was done using the well-plate method. Standard strain *E. faecalis* (ATCC51299) was used for this purpose. After culturing *E. faecalis* in blood agar medium, 0.5 McFarland microbial suspension in saline was prepared. For this purpose, 0.5 mL of 1.175% barium chloride solution was added to 99.5 mL of 1% sulfuric acid. Next, bacterial suspension was streak cultured on Mueller-Hinton agar using a cotton swab. Wells were created in the plate using a sterile Pasteur pipette, and 50 µL of each essential oil was inoculated into the wells. After 15 min, the plates were incubated at 35°C–37°C for 18–24 h. The diameter of the growth inhibition zone was measured by a ruler and reported in millimeters. Gentamycin was used as control in disc diffusion method.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

To determine the minimum inhibitory concentration (MIC) of essential oils, micro-broth dilution method was used; 5% dimethyl sulfoxide was used to dissolve the essential oils. In addition, 100 μL of fresh culture medium was added to nine wells of each row. These materials were added to the first well to reach a final concentration of 1:200 (v/v) (1 μL of essential oil + 10 μL of dimethyl sulfoxide + 89 μL of the culture medium). Next, 100 μL of the first well was removed and added to the second well and mixed by pipetting up and down ten times. The same was repeated for the next wells and 100 μL of the 7^{th} well was discarded (not added to the 8^{th} well). The 8^{th} well served as the positive control (contained bacteria without essential oils) and the 9^{th} well served as the negative control (without essential oils or bacteria to control for sterility). Next, 100 μL of microbial suspension was added to the 8^{th} well and 100 μL of sterile culture medium was added to the 9^{th} well. Serial dilutions of essential oils (1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, and 1:12800 v/v) were prepared as follows; 100 μL of 0.5 McFarland standard concentration of the microbial suspension was added to each well and the plate was incubated at 37°C for 18–24 h. Minimum concentration of essential oil with no turbidity was considered as the MIC. For MBCs with this culture (subculture) of transparent tubes (no growth), MIC test was performed and the minimum bactericidal concentration (MBC) as the concentration that kills 99.9% or more of the initial inoculation is considered.

RESULTS

This study assessed the antibacterial effect of PF, ZT, DM, and FG on *E. faecalis* using broth micro dilution and disc diffusion methods. Tables 1 and 2 show the antimicrobial activity of the four essential oils against *E. faecalis*. As observed, all the four essential oils had antibacterial effects on *E. faecalis*.

Results of disc diffusion test

The mean diameter of the growth inhibition zone of *E. faecalis* in millimeter (based on the results of disc diffusion test) for PF, ZT, DM, FG, CHX %0.02, and Gentamycin was found to be 23, 25, 12, 18, 21, and 8 mm, respectively. The mean diameter of the growth inhibition zones in the well plate method for ZT showed that it had greater antimicrobial activity against *E. faecalis* than others. Furthermore, ZT and PF had higher antimicrobial activity than CHX.

Table 1: List of plants evaluated in this study

English name of plants	Vernacular name (source)	Source
ZT	Kakuti	Zanjan, Iran
PF	Jashir	Zanjan, Iran
FG	Barije	Zanjan, Iran
DM	Baderashboo	Zanjan, Iran

ZT: *Ziziphora tenuior*, PF: *Prangos ferulacea*; FG: *Ferula gummosa*; DM: *Dracocephalum moldavica*

Table 2: The mean diameter of the growth inhibition zones (mm) of *Enterococcus faecalis* based on the results of disc diffusion test for chlorhexidine and essential oils

Plants, CHX and Gentamycin	Chlorhexidine %0.02	PF	ZT	DM	FG	Gentamycin
Mean diameter of growth inhibition zone of *Enterococcus faecalis*	21	23	25	12	18	8

ZT: *Ziziphora tenuior*, PF: *Prangos ferulacea*; FG: *Ferula gummosa*; DM: *Dracocephalum moldavica*

Results of micro-broth dilution

The MIC of PF, ZT, DM, FG, and CHX for *E. faecalis* was 2.275, 0.2625, 1.0125, 1.1625 μg/mL, and 1/512, respectively. The MBC of PF, ZT, DM, FG, and CHX for *E. faecalis* was resistant (0), 0.525, resistant (0), 1.1625 μg/mL, and 1/128, respectively. The results of MIC showed that ZT had the highest antibacterial activity among the tested essential oils. However, all the four essential oils had lower antibacterial activity than CHX because the essential oils were insoluble in water, so the solution was nonuniform, and PF and DM just inhibited the growth of *E. faecalis* and not to kill the bacteria.

DISCUSSION

Different and meaningful antimicrobial activity was found for many of the tested herbal extracts. Despite the available studies on the antibacterial activity of PF, ZT, DM, and FG, their effects on oral and dental pathogens have not been extensively evaluated. This study assessed the antibacterial effects of essential oils of the aforementioned four medicinal plants on *E. faecalis* using micro-broth dilution and disc diffusion methods. As reported in Tables 2 and 3, all the four essential oils had antibacterial effects on *E. faecalis,* and ZT had the greatest antibacterial activity. Assessment of the mean diameter of the growth inhibition zone using disc diffusion method showed higher antibacterial activity of PF and ZT than that of CHX. The mean diameter of the growth inhibition zone of *E. faecalis* for PF had a small difference with that of ZT. Similarly, Ozturk and Ercisli indicated that *Z clinopodioides* and *Z. persica* extracts inhibited the growth of a wide range of Gram-positive and Gram-negative pathogenic microorganisms.[11,12] Since all these species belong to the same family of plants, their findings were in agreement with ours. Another study showed that *Z. clinopodioides* extract prevented the growth of *Klebsiella pneumoniae* and *E. coli* Gram-negative bacteria. They also found that *Z. clinopodioides* had no significant antimicrobial activity against *P. aeruginosa,* which was different from our study. This difference may be due to differences in plant species and bacterial strains used. Durmaz *et al*. in 2006 and Massumi *et al*. in 2007 evaluated the antibacterial activity of PF fruit essential oil against Gram-positive and Gram-negative bacteria such as *Bacillus cereus, P. aeruginosa, Staphylococcus epidermidis*, *E. coli*, *and S. aureus* and reported results similar to those of the current study. The antimicrobial effects of PF on other oral pathogens also confirmed our findings.[8,9]

The MIC and MBC of ZT showed that the antimicrobial activity of ZT against *E. faecalis* was greater than that of other plants evaluated in this study. Chitsa *et al*. evaluated the antibacterial activity of *Z. clinopodioides* essential oil against several bacterial strains and reported that it prevented the growth and proliferation of all Gram-positive and Gram-negative bacteria evaluated in their study. It had the greatest antibacterial activity against *Salmonella typhimurium* (with MIC of 25 μg/mL), which was in agreement with our results.[13] Other studies indicated that *Z. clinopodioides* extract inhibited the growth of *B. subtilis* and *S. epidermidis,* which was in line with our findings.[17-19] Behravan *et al*. showed that *Z. clinopodioides* extract had no effect on *E. coli.* Another study reported significant inhibitory effect of ZT on *B. cereus* and *S. aureus* Gram-positive bacteria. Its effect on *S. aureus* oral pathogen was in accordance with our results.[20] Ghasemi *et al*. evaluated the antimicrobial effects of FG and reported significant antibacterial activity of this medicinal plant against *S. aureus* and *C. albicans*.[21] Haghighati *et al*. found that DM extract had no antimicrobial activity against *S. mutans, C. albicans*, or *Actinobacillus actinomycetemcomitans,* which was in contrast to the findings of the current study, and DM just inhibited the growth of *E. faecalis* and not to kill the bacteria. This difference may be attributed to different bacterial strains evaluated in the two studies.[22] Abdolbaki *et al*. demonstrated that DM essential oil had similar antimicrobial effects (MIC = 0.07 mg/mL) *on S. aureus* and *M. luteus* Gram-positive and *Serratia marcescens* Gram-negative bacteria, which highlighted the dose-dependent effect of this essential oil. In their study, the mean diameter of the growth inhibition zone *for S. aureus*, *S. marcescens, M. luteus*, and *B. cereus* due to exposure to DM essential oil in 0.8 mg/disc was 3.8, 3.8, 3.4, and 2.8 mm, which were all smaller than the value obtained in our study for *E. faecalis* (1.2 mm).

Table 3: Minimum bactericidal concentration and minimum inhibitory concentration of chlorhexidine and essential oils evaluated in this study

Plants, CHX and Gentamycin	PF	ZT	DM	FG	Chlorhexidine
Enterococcus faecalis					
MIC	2.275	0.2625	1.0125	1.1625	1/512
MBC	0	0.525	0	1.1625	1/128

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; ZT: *Ziziphora tenuior*; PF: *Prangos ferulacea*; FG: *Ferula gummosa*; DM: *Dracocephalum moldavica*

Phenolic compounds (thymol) and the terpenoids present in plant essential oils have a wide spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria.[23,24] Compounds such as alpha-terminal, terpinen-4-ol, and linalool (which have a low concentration in the formulation of essential oils and extracts) have high antimicrobial activity if used in their pure form.[24] Several studies have reported optimal antibacterial activity of terpinen-4-ol,[25-27] 1, 8-cineol,[28] and alpha-terpineol.[29] Strong inhibitory effects of linalool on 12 bacteria have been documented.[30] In addition, the antiseptic and antibacterial activities of DM have been confirmed and are attributed to the presence of citral, geranial, and neural in its composition.[23]

This study is believed to be the first to specifically assess the effects of essential oils of PF, ZT, FG, and DM on *E. faecalis*. However, sodium hypochlorite is the standard, most commonly used root canal irrigating solution, and one limitation of this study was that sodium hypochlorite and its MIC and MBC were not evaluated in this study. However, we instead evaluated CHX as the gold standard antimicrobial mouth rinse to somehow compensate for this limitation.

Evaluation of essential oils of plants in our study instead of aqueous or alcoholic extracts evaluated in previous studies was due to the fact that effective compounds (which have antimicrobial activity) have higher concentrations in essential oils than in extracts. Furthermore, it appears that heat negatively affects the antimicrobial efficacy of effective substances in extracts and decreases their antimicrobial properties. Thus, the use of essential oils of plants instead of their extract was a strength of this study.[31]

CONCLUSION

The results of this study indicated significant antibacterial effects of the essential oils of PF, ZT, FG, and DM on *E. faecalis*. The greatest antimicrobial activity belonged to ZT. PF and DM just inhibited the growth of *E. faecalis* and not to kill the bacteria. Considering the optimal biocompatibility and insignificant side effects of these medicinal plants as well as the increased resistance of microorganisms to antibiotics, the current study suggests extraction of effective compounds present in the composition of these medicinal plants to further assess their antibacterial activity in the clinical setting.

Clinical relevance

Scientific rationale for the study was to find a nonchemical product for the disinfection since, to the best of authors' knowledge, no previous study has evaluated the antibacterial activity of the afore-mentioned four medicinal plants against oral and dental pathogens.

Principal findings - These herbal essences (Ziziphora) were quite beneficial in prohibiting the selected bacteria *in vitro*. The antibacterial effects of Ziziphora are similar to the most commercially available mouth rinse (CHX), while CHX has got some side effects such as stating and supra calculus formation, Ziziphora product may have no adverse effects.

Practical implications - At the second step, a natural product, something as a herbal mouth rinse or endodontic irrigator, can be made up.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial, in this article.

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