Clinical, Cosmetic and Investigational Dentistry

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ORIGINAL RESEARCH

# Antibacterial Effect of *Matricaria chamomilla* L. Extract Against *Enterococcus faecalis*

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**Purpose:** With the demand for better efficiency during endodontic therapy, the purpose of this in vitro study was to evaluate the efficiency of ethyl acetate (EthOAc) extract of *Matricaria chamomilla* (L.) against *Enterococcus faecalis* and compare with standard root canal irrigation solutions.

**Material and Methods:** The antibacterial effect against *Enterococcus faecalis* of the EthOAc extract of *Matricaria chamomilla* was assessed without, or with dentine powder, using agar disk diffusion method. The inhibition zones induced by the EthOAc extract were observed after 5 minutes, 60 minutes, and 24 hours and compared with standard irrigation solutions (2% chlorhexidine and 2% sodium hypochlorite) without, or after mixing with dentine powder. Statistical analysis of the results were analysed with the Kruskal–Wallis test and one-way ANOVA.

**Results:** There was no inhibition zone in samples with and without dentine powder after combination of ethyl acetate extract of *Matricaria chamomilla* with sodium hypochlorite. When the extract was used alone in samples without dentine powder, the inhibition zone was 9.7 mm after 24 h, but this zone was decreased after adding dentine powder (7.7 mm). When the extract was used in combination with CHX, the inhibition zone in samples with dentine powder was 25.3 mm, but it was decreased after adding dentine powder (21.7 mm).

**Conclusion:** The EthOAc extract of *Matricaria chamomilla* as alternative root canal irrigant may be a useful natural agent with antibacterial activity against *Enterococcus faecalis*. Also, it showed promising results in endodontic therapy after combination with chlorhexidine in *Enterococcus faecalis* eradication.

**Keywords:** root canal, herbal plants, diffusion method, chlorhexidine, sodium hypochlorite

## Introduction

Root canal debridement is important for successful endodontic treatment.[1] Root canal irrigants should have the following attributes: low toxicity; antimicrobial effect; non-caustic to periodontal tissues; non-allergenic; and the ability to dissolve tissue and root canal debris, inactivate endotoxins, lubricate the canal, and remove the smear layer.[2]

Sodium hypochlorite, a root canal irrigant that is widely used in endodontic therapy, has antibacterial qualities and assists in mechanical root canal debridement and pulp dissolution.[3] In combination with ethylenediaminetetraacetic acid (EDTA) or citric acid, sodium hypochlorite can remove the smear layer; however, it also has toxic effects, allergic potential, and a bad taste.[4,5]

Chlorhexidine gluconate can also be used as an endodontic disinfectant, because it can penetrate the bacterial wall and attack the cell cytoplasm. It has an antimicrobial effect, does not irritate the periapical tissue, and does not have a bad taste, but it does not disintegrate pulp tissue.[6]

Despite the use of various instruments and irrigators during endodontic treatment, complete debridement is not possible due to the anatomical complexity of the root canal system.[7]

Received: 19 December 2022
Accepted: 2 February 2023
Published: 14 February 2023

Research has proven that *Enterococcus faecalis* can be isolated in 24–77% of endodontically treated canals,[8] because of its resistance to intracanal drugs and its ability to form biofilm, invade dentinal canals, and survive in the absence of nutrients for long durations.[9,10]

The antibacterial effect of root canal disinfectants may be reduced in vivo by the presence of organic compounds (eg, albumin) in the inflammatory exudate and hydroxyapatite (the main inorganic component of dentin).[11]

The problems of drug-resistant microorganisms and drug side effects have encouraged investigators to find alternative solutions that increase the antimicrobial effect with no toxicity.

Nowadays herbal products are gaining popularity in dental and medical practice.

*Matricaria chamomilla* is a well-known medicinal plant from the Asteraceae family. The phytochemical composition of *M. chamomilla* essential oil and extracts has been identified as containing more than 120 constituents.[12] *M. chamomilla* has been shown to have strong antibacterial potential against Gram-positive and Gram-negative bacteria.[13] The chamomile plant is known to have antibacterial, anti-inflammatory, antiviral, and antioxidant effects, due to the presence of α-bisabolol, luteolin, quercetin, and apigenin. The clinical efficacy of chamomile has also been reported in selectively removing the root canal smear layer.[14]

Therefore, the aim of this in vitro study was to evaluate the *M. chamomilla* ethyl acetate extracts as a potential antibacterial agent in the inhibition of *E. faecalis* in comparison with sodium hypochlorite and chlorhexidine (CHX).

## Materials and Methods

This paper has been approved by the Ethics Commission of Medical Faculty, University of Prishtina "Hasan Prishtina" (Nr. 2417). For this study, we used 100 extracted human single-rooted teeth in order to collect dentine powder. Dentine powder was used to assess inhibitory effect in medications. Teeth were extracted for periodontal and orthodontic reasons. Patients have given written consent that their teeth after extraction can be used for research purposes in the future. This consent has been approved by the same Ethics Commission. Before the experiment, the teeth were stored in 1% sodium hypochlorite solution and then rinsed and autoclaved at 121°C for 15 min. The crowns of the teeth were cut at the enamel–cementum junction with a diamond saw (Smart Cut 4002, UKAM, Valencia, CA, USA). The root canal was accessed and the pulp tissue was removed. We then used the root canal instruments Endostar E5 (Poldent Co., Warsaw, Poland) to enlarge the root canal and to collect dentin powder. The collected dentin powder was suspended in distilled water at a concentration of 28 mg per 50-µL aliquot.

Materials used in this study were as follows: 2% sodium hypochlorite (ChloraXD, Cerkamed, StatowaWola, Poland), 2% CHX (Gluco-Chex, Cerkamed), ethyl acetate (EthOAc; Sigma Aldrich, Switzerland), and dimethyl formamide (DMF; Sigma Aldrich). Dimethyl formamide was used as extract solvent with no inhibition effect.

The *M. chamomilla* plants were collected in a Kosovo field in 2018. The plant was preserved in the herbarium of the Biology Department of University of Prishtina (no. 324/2018). The aerial part of the plant was air-dried and ground to a powder with a blender. The dried powder was vacuum-packed and stored at –20°C until use. Dried *M.* chamomilla powder (10 g) was extracted twice with 100 mL of 80% ethyl acetate (EthOAc) with a Soxhlet apparatus at boiling point, and the extract was concentrated with a rotary evaporator at 40°C (Figure 1). This extract A4(5) was stored in a deep

**Figure 1** Plant extracts-extracted with a Soxhlet apparatus.

freeze until the time of the experiment. For the antibacterial activity assays, the extracts were dissolved in N, N-dimethylformamide (DMF) at a concentration of 100 mg/mL and stored at 4°C as a stock solution. *Enterococcus faecalis* (ATCC 29212 Thermo Fisher Scientific) was used to evaluate the antibacterial potency of the plant extract, the irrigants, a mixture of the plant extract with the endodontic irrigants, and all of the above mentioned materials with dentin powder. The antibacterial activity of the Soxhlet plant extracts and the endodontic irrigants was analyzed using a disk diffusion assay. A suspension of *E. faecalis* was cultured for 48 h in 1 mL of sterile brain heart infusion (BHI, Liofilchem, Roseto degli Abruzzi, Italy) at 37°C, then adjusted to a turbidity of 0.5 on the McFarland scale (1.5 × 108 cells/mL). This culture was used throughout the experiments. Mueller–Hinton agar (10 mL) was poured into Petri dishes according to manufacturer instructions, which were then inoculated with strains of bacteria by the addition of 0.1 mL of cell culture medium. Sterile filter paper disks impregnated with the endodontic irrigants, plant extracts, and the combination of endodontic irrigants and plant extracts, and all of the above mixed with dentin powder (10 mg/mL), were placed on top of Mueller–Hilton agar plates. The plates were incubated at 5°C for 2 h to permit diffusion of the plant extract, and then incubated at 35°C for 24 h. Disks loaded with the extract solvent (EthOAc) were used as a control group. Vernier calipers were used to record the inhibition zones.

To evaluate the antibacterial effect of the medicaments on dentin powder, 50 µL aliquots of the dentin powder suspended in water were mixed and incubated with 50 µL of the medicaments in sealed test tubes at 37°C for 1 h or 24 h, before the addition of bacteria. Control groups consisted of 50 µL of sterile water instead of dentin powder. The total volume of the test and control mixtures was 150 µL. All mixtures were incubated at 37°C. The dentin powder/endodontic irrigant/bacteria suspension was mixed with a sterile pipette twice (before the 1-h sample) or three times (before the 24-h sample) during the incubation time. Samples for bacterial culturing (10 µL per sample) were taken from the experimental and control suspensions at 5 min, 1 h, and 24 h after the addition of *E. faecalis*. The Petri dishes with agar were observed after 5 min, 60 min, and 24 h (Figure 2). The results were recorded based on the standard disk method according to the US Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antibiotic Sensitivity Testing (EUCAST).

The recorded data were analyzed with SPSS Statistics 22.0. One-way ANOVA was used to test for normal distribution of the tested groups, and the Kruskal–Wallis test was used for non-normal group distribution. A level of $P<0.05$ was considered to be statistically significant.

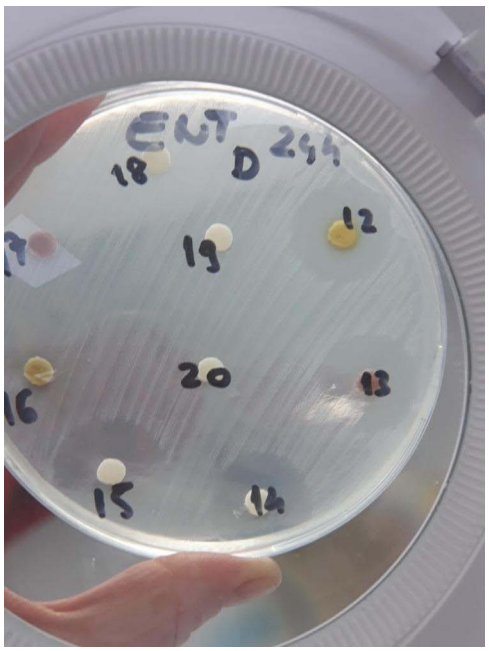


**Figure 2** Inhibition zone (mm) of used agents.

## Results

The growth of *E. faecalis* colonies was recorded at 5 min, 60 min and 24 h after the addition of an ethyl acetate extract of *M. chamomilla* - A4(5) to all test samples with endodontic irrigants without dentin powder, and to all samples of the same mixture with dentin powder. At 5 min after the combination of A4(5) with sodium hypochlorite without dentin powder there was no inhibition zone. When the extract was used alone, the inhibition zone was 9.7 mm, and in combination with CHX it was 30.3 mm. When the *M. chamomilla* ethyl acetate-extract was used alone, the inhibition zone was 31.7 mm. There was a significant difference at 5 min after the culture (*E. faecalis*) was inoculated in the samples without dentin powder according to the tested irrigants (P=0.0015) (Table 1).

At 5 min after culture inoculation, the samples with dentin powder exhibited no inhibition zone in the A4(5)/sodium hypochlorite group. However, when the plant extract was used alone, the inhibition zone measured 7.3 mm, and after combination with CHX it measured 25.3 mm. When sodium hypochlorite was used alone, the inhibition zone was 26.0 mm. Thus, there was a significant difference after the addition of dentin powder in all groups, depending on the medicament used (P=0.0015) (Table 2).

At 60 min after culture inoculation, the samples without dentin powder exhibited no inhibition zone in the extract/sodium hypochlorite group. However, when the extract was used alone, the inhibition zone was 9.3 mm and in combination with CHX, it was 25.7 mm. Additionally, when sodium hypochlorite was used alone, the inhibition zone was 34.7 mm. There was a significant difference at 60 min after inoculation of the culture (*E. faecalis*) in samples without dentin powder according to the tested irrigants (P=0.0015) (Table 1).

**Table 1** Effect of EthOAc Extract of *Matricaria chamomilla* with and without Irrigant in *Enterococcus faecalis* Growth without Dentine Powder

| | 5 min | | | | 60 min | | | | 24 h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Min** | **Max** | **Mean** | **SD** | **Min** | **Max** | **Mean** | **SD** | **Min** | **Max** |
| EthOAc | 9.7 | 0.6 | 9 | 10 | 9.3 | 0.6 | 9 | 10 | 9.7 | 0.6 | 9 | 10 |
| NaOCl/EthOAc | 0.0 | 0.0 | 0 | 0 | 0.0 | 0.0 | 0 | 0 | 0.0 | 0.0 | 0 | 0 |
| CHX/EthOAc | 30.3 | 0.6 | 30 | 31 | 25.7 | 0.6 | 25 | 26 | 25.3 | 0.6 | 25 | 26 |
| NaOCl | 31.7 | 0.6 | 31 | 32 | 34.7 | 0.6 | 34 | 35 | 38.3 | 0.6 | 38 | 39 |
| CHX | 24.7 | 1.5 | 23 | 26 | 24.3 | 0.6 | 24 | 25 | 23.0 | 1.0 | 22 | 24 |
| DMF | 0.0 | 0.0 | 0 | 0 | 0.0 | 0.0 | 0 | 0 | 0.0 | 0.0 | 0 | 0 |
| Kruskal–Wallis | p=0.0015 | | | | p=0.0015 | | | | p=0.0015 | | | |

**Table 2** Effect of EthOAc Extract of *Matricaria chamomilla* with and without Irrigant in *Enterococcus faecalis* Growth with Dentine Powder

| | 5 min | | | | 60 min | | | | 24 h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Min** | **Max** | **Mean** | **SD** | **Min** | **Max** | **Mean** | **SD** | **Min** | **Max** |
| EthOAc | 7.3 | 0.6 | 7 | 8 | 7.7 | 0.6 | 7 | 8 | 7.7 | 0.6 | 7 | 8 |
| NaOCl/EthOAc | 0.0 | 0.0 | 0 | 0 | 0.0 | 0.0 | 0 | 0 | 0.0 | 0.0 | 0 | 0 |
| CHX/EthOAc | 25.3 | 0.6 | 25 | 26 | 21.0 | 1.0 | 20 | 22 | 21.7 | 2.9 | 20 | 21 |
| NaOCl | 26.0 | 1.0 | 25 | 27 | 18.0 | 1.0 | 17 | 19 | 14.3 | 0.6 | 14 | 15 |
| CHX | 22.3 | 0.6 | 22 | 23 | 20.3 | 0.6 | 20 | 21 | 19.7 | 0.6 | 19 | 20 |
| DMF | 0.0 | 0.0 | 0 | 0 | 0.0 | 0.0 | 0 | 0 | 0.0 | 0.0 | 0 | 0 |
| Kruskal–Wallis | p=0.0015 | | | | p=0.0015 | | | | p=0.0015 | | | |

**Abbreviations**: EthOAc, ethyl acetate; CHX, chlorhexidine; NAOCl, sodium hypochlorite; DMF, dimethyl formamide; KW, Kruskal–Wallis.

At 60 min after culture inoculation, the samples with dentin powder exhibited no inhibition zone in the extract/sodium hypochlorite group. When the extract was used alone, the inhibition zone was 7.7 mm, after combination with CHX it was 21.0 mm, and when sodium hypochlorite was used alone it was 18.0 mm. Therefore, there were significant differences after the addition of dentin powder after 60 min in all groups, depending on the medicament used (P=0.0015) (Table 2).

At 24 h after culture inoculation, the samples without dentin powder exhibited no inhibition zone in the extract/sodium hypochlorite group. However, when the extract was used alone, the inhibition zone was 9.7 mm, and in combination with CHX, it was 25.3 mm. Additionally, when sodium hypochlorite was used alone, the inhibition zone was 38.3 mm. There was a significant difference at 24 h after culture inoculation (*E. faecalis*) in samples without dentin powder according to the tested irrigants (P=0.0015) (Table 1).

At 24 h after culture inoculation, the samples with dentin powder exhibited no inhibition zone in the extract/sodium hypochlorite group. When the extract was used alone, the inhibition zone was 7.7 mm, after combination with CHX it was 21.7 mm, and when sodium hypochlorite was used alone it was 14.3 mm. There was a significant difference after the addition of dentin powder after 24 h in all groups depending on the medicament used (P=0.0015) (Table 2).

One-way Anova was used to analyse intergroup comparison. There was no statistical difference between group treated with EthOAc extract of Matricaria Chamomilla and group treated after mixing EthOAc extract with dentine powder ($F$=17.682, p=0.014).

But, there was statistical difference between the group treated with EthOAc/CHX and the group treated with EthOAc/CHX after mixing with dentine powder ($F$=82.14, p<0.001).

## Discussion

*E. faecalis* has several virulence factors that influence its survival in the hostile root canal environment: secretory factors, adhesins, surface structures such as capsular polysaccharides, and antibiotic resistance.[15] As an endopathogenic microorganism, *E. faecalis* has the capacity to penetrate into the dentin tubules and to adhere to the surface of the dentin.[16] For these reasons, this microorganism was selected for testing in our research.

The disk diffusion method for evaluating the antimicrobial activity of the experimental materials was selected because it is a quick and simple procedure that allows for direct contact of the material with specific microorganisms.[17]

Much research has been undertaken under in vitro and in vivo conditions to evaluate and compare the antimicrobial activity of endodontic irrigants at different concentrations. These studies most often used agar diffusion in addition to the direct contact method.[18] Owing to differences in the experimental methodology and in the concentration of the test materials, conflicting results have been reported in the selection of ideal endodontic irrigates.[19]

Because some endodontic medicaments have undesirable side effects, the use of herbal compounds has become more common due to their anti-inflammatory, anti-oxidative, antibacterial, and antiviral effects. They are considered to be healthier and more environmentally acceptable.[20] They are easy to obtain and are well accepted by the host with many useful pharmacological actions comparable with synthetic drugs.

*M. chamomilla*, one of the most popular herbal extracts, is an annual plant from the Asteracea family.[21]

Goes et al[22] studied the antimicrobial and anti-inflammatory properties of *M. chamomilla*, and found that it reduced biofilm accumulation in patients with gingival inflammation.

Venkataram et al[23] reported that application of a hydroalcoholic extract of chamomile resulted in effective and significant removal of the smear layer when compared with 2.5% sodium hypochlorite and effectively cleaned the coronal and middle thirds of the root canal.

Several studies have confirmed the antibacterial activity of *M. chamomilla* ethanolic extracts,[24–26] with the results varying depending on the tissue involved.[27–29] Shakya et al[30] found that *M. chamomilla* flowers significantly reduced *E. faecalis* growth. Plant extracts are well known as intracanal medicaments and irrigants for disinfection, and as agents for removal of the smear layer.[31]

Based on these previous investigations of various plant extracts, we selected a plant growing in our fields for antimicrobial evaluation.

In our study, the ethyl acetate extract of *M. chamomilla* applied alone was found to exert an inhibitory effect on *E. faecalis*, producing an average inhibitory zone of 9 mm. After combination with endodontic preparations, *M. chamomilla* was shown to be effective only after mixing with CHX, and the zone of inhibition was greater than that observed when CHX was used alone. After combination with sodium hypochlorite, *M. chamomilla* exerted no antibacterial effect against *E. faecalis*; no inhibition zone was observed.

Similar to our results, Shah et al[32] showed that although Chamomile oil had more antimicrobial effect on *Enterococcus faecalis* than 2% CHX, both irrigators still had an observable effectiveness against *E. faecalis*.

According to the study of Jafari et al,[33] antibacterial effect of CHX and chamomile rapidly increased, thus indicating materials' abilities to be adsorbed to dentine hydroxyapatite with prolonged gradual releases at therapeutic levels.

Previous research has proven that dentin powder has significantly lower inhibitory activity on medications, than human serum albumin,[34] even at high concentration.[35]

The intensity of this inhibition is based on the drug concentration, the time of contact and the time of exposure of the bacteria to the mixture.[36]

The Haapasalo model[37] is used to efficiently evaluate the interactions between root canal drugs, dentin, and microorganisms. Consistent with the results of Haapasalo et al, our study demonstrated that when endodontic solutions were mixed with dentin powder, the inhibitory areas decreased after contact with *E. faecalis* in comparison with samples without dentin powder at three time intervals. This can be explained by the fact that the components of dentin are responsible for the inhibitory effect in medications caused by moderation of the pH increase by the buffering effect of dentin.

Micro-organisms can use dentin as a source of nutrition using its collagen and calcium, and both of them may have a role in adherence capacity of micro-organisms to extracellular matrix proteins.[38]

In our study, the results were recorded after 5 min, 60 min, and 24 h in order to evaluate inhibition over time. This change was more evident after the 24-h incubation period in samples treated with *M. chamomilla* after combination with chlorhexidine. Herbal extracts represent alternative endodontic therapy, so the results of this study may be considered as novelty.

Since, they are the few studies related to antimicrobial effect of *M. chamomilla* hydroalcoholic extracts in endodontic therapy, further studies should be conducted to evaluate the performance and antimicrobial activity of the above extract for clinical use as an intracanal medicament alone and in combination with other medicaments.

## Conclusion

On the basis of the results of this study, we conclude that ethyl acetate extracts of *M. chamomilla* plant can be used as an alternative disinfectant for root canal disinfection alone, or in combination with CHX. *M. chamomilla* in combination with sodium hypochlorite showed no antibacterial effect.

## Acknowledgments

We thank Helen Jeays, BDSc AE, from Edanz (www.edanz.com/ac) for editing a draft of this manuscript.

## Disclosure

The authors report no conflicts of interest related to this study.

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