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Essential Oils as Alternatives for Root-Canal Treatment and Infection Control against *Enterococcus faecalis*—A Preliminary Study

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Featured Application: This interdisciplinary study assesses new potential therapeutic and prophylaxis methods against *Enterococcus faecalis*, one of the microbial species often related with dental pathology, in the "end of antibiotic era", also targeting patients who embrace the non-chemical treatment alternatives.

Abstract: Since natural alternatives are needed in dentistry for the treatment of root canal, where the standard irrigant is NaOCl with significant toxicity, the aim of the study was to assess the antibacterial properties of non-chemical root-canal irrigants (aqueous extracts of oregano, thyme, lemongrass, melaleuca and clove essential oils) against *Enterococcus faecalis*. For this, aqueous extracts of each essential oil (AqEO) were prepared. A solution of sodium hypochlorite (NaOCl) was used as a positive standard against which the antimicrobial effects of AqEO could be reported. The root canals of seven teeth were inoculated with 20 µL of *Enterococcus faecalis* ATCC29212 inoculum and incubated overnight at 37 °C. All the teeth canals were instrumented and were irrigated with the corresponding AqEO, NaOCl and saline solution, then rinsed with saline. Bacteriological samples for each canal post-instrumentation were collected with sterile paper points which were inoculated on culture media. A second processing followed the same methodology but involved only irrigation and no instrumentation. Using instrumentation, thyme and clove completely inhibited *Enterococcus faecalis* growth. Without instrumentation, clove and oregano AqEOs completely reduced the bacterial load as seen in direct inoculation, but bacterial growth was observed in all the samples after enrichment, except for NaOCl. Nevertheless, the turbidity of the enrichment media was lower for the samples irrigated with AqEOs than for control. In conclusion, AqEOs of thyme, oregano and clove showed a promising antibacterial effect, especially when teeth instrumentation was performed.

Keywords: essential oils; *Enterococcus*; antibacterial effect; root-canal infection control

1. Introduction

The infection of the dental root canal system is called an endodontic infection. This infection can cause an apical periodontitis and the progression of different forms of apical

periodontitis is due to some microorganisms [1–3]. The microorganisms reach the pulp by dentinal tubules especially when there is an open cavity after a coronal fracture and the pulp is in contact with the septic oral environment. Another pathway for the microorganism is the periodontal membrane when they use the lateral channel or the apical foramen [4].

In the oral cavity, nearly 700 species of bacteria can be found, most part of Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria [5]. There are two types of intraradicular infections: primary and secondary, each with specific microbiology. The primary infection of the root canal system results from colonization with a heterogenous group of microbes that have entered the pulp tissue, when exposed during caries or traumas; this group is dominated by Gram-negative oral anaerobic bacteria (*Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., *Veillonella* spp.), aside other bacterial species which are also part of the commensal oral microflora: Gram-positive anaerobic bacteria (*Propionibacterium* spp., *Bifidobacterium* spp., anaerobic streptococci), treponemas or *Campylobacter* spp. [5,6]. The secondary intraradicular infection appears inside the root canal system, after the treatment of the affected tooth has been initiated. Secondary intraradicular infections harbor a limited number of bacterial species, with predominance of Gram-positive bacteria, namely *Enterococcus faecalis*, oral streptococci, lactobacilli or *Candida albicans*, while Gram-negative bacteria are involved to a lesser extent [6]. The most common microorganism found in asymptomatic, persistent endodontic infections is *E. faecalis*. Its incidence in this type of infection varies from 24% to 77% [6,7]. *E. faecalis* possesses enzymes and constitutive structures that are able to suppress the action of lymphocytes and promote inflammation, which contributes to progression of endodontic infections [4]. Also, this microorganism has the ability to attach to dentin due to an inner component which is the collagen-binding protein [8].

The essential oils (EO) extracted from plants have been used for centuries as spices and in medicine for their antibacterial and antifungal properties. These properties depend on their chemical composition, the provenance of the plant, the climatic and growth condition and as well as on distillation process [9]. The genus *Origanum* L., (Lamiaceae), comprises 38 species most of which grow in the Mediterranean area, North Africa, Europe and Asia. The major constituents are terpenes, and the principal terpenes are carvacrol, thymol, γ-terpinene and *p*-cymene. There is also present linalool, β-myrcene, *trans*-sabinene hydrate and β-caryophyllene. Oregano EO has antiparasitic, antibacterial, antifungal and antioxidant properties. Also, it is used as culinary herb or preservative [10–12]. The genus *Thymus* consists of about 215 species that grow in hot and dry climates. *Thymus vulgaris* is native to southern Europe and it has been cultivated for its culinary and spice properties. The EO extracted from *Thymus vulgaris* has antimicrobial, antiviral and antiseptic activities and it contains geraniol, linalool, gamma-terpineol, carvacrol, thymol and *trans*-thujan-4-ol/terpinen-4-ol. The chemical composition of the EO depends on the growth region and the environment [13–15]. *Cymbopogon flexuosus* (lemongrass) belongs to the family Poaceae and is a medicinal grass which is used as a food, perfumery and in pharmaceutical industries [16]. Previous studies showed the antimicrobial effect of lemongrass EO. It is mainly formed from monoterpenes such as citral [17–19]. The plant *Melaleuca alternifolia* belongs to the Myrtaceae family which grows throughout South America, Australia and western India. It is used in food, medicine, agriculture and cosmetics. Named also as tea tree, it is reported to be an antimicrobial agent [20–24]. The major constituents are terpinene-4-ol, γ-terpinene, and α-terpinene, 1,8-cineole. Other constituents are ρ-cymene, terpinolene and limonene. the composition of *M. alternifolia* EO is influenced by climatic and local conditions. Terpinen-4-ol is the active ingredient responsible for the therapeutic properties. The terpenes have the ability to change the permeability of the pathogen's cellular membrane [20,22].

Eugenia caryophylata (clove), is a tree which belongs to Myrtaceae family, and grows naturally in East Indonesia, and its cultivated in many countries such as Brazil, Tanzania, and India. The oil extracted from the tree has major components such as eugenol, βcaryophyllene, α-humulene, caryophyllene oxide and eugenyl acetate. Clove EO exert

biological and pharmacological activities. It also has an antibacterial, antioxidant and antimycotic effect [25,26].

Sodium hypochlorite (NaOCl) is commonly used for root canal disinfection. Previous studies confirmed the use of NaOCl against *E. faecalis* as a very potent root canal irrigant and after the reaction with water, it releases chlorine, which can be unpleasant for patients. Also, the organic tissue-dissolving properties of NaOCl are non-selective, meaning that it is capable of indistinguishably dissolving both vital and necrotic remnants of pulp, therefore being toxic to periapical tissues. Cytotoxic and genotoxic effects on human peripheral lymphocytes have been studied when applying sodium hypochlorite on human peripheral lymphocytes in vitro [27–31].

Due to the possible side effects of the synthetic irrigants and to safety concerns the interest in natural alternatives has grown in the last few decades and the most important of these are the products that can be obtained from plants, which have many medicinal and antimicrobial properties [17]. Some previous studies reported the efficacy of different plant extracts against the *E. faecalis* which concluded that the plant compounds can be used as a possible alternative to NaOCl [31].

The aim of the study was to assess the antibacterial properties of non-chemical, natural root-canal irrigants. Being a preliminary study, the antibacterial effects of five aqueous extracts of essential oils were screened against *E. faecalis*, one of the bacteria that are involved in chronic periodontitis and failed root canal treatments.

2. Materials and Methods

2.1. Essential Oils, Working Solutions and Bacterial Strains

The essential oils originated from the same manufacturer (doTERRA International LLC, Pleasant Grove, United States), obtained by distillation, and were purchased from a local retailer. Through the precise traceability system provided by the manufacturer of the oils used in the present study (each EO bottle bears the inscription of a lot number that is fully traceable), every HPLC analysis is publicly available (Supplementary Materials). The essential oils used in this study originated from crops cultured in different countries: Lemongrass—harvested in India; Clove– harvested in Madagascar; Melaleuca—harvested in Kenya and Australia; Thyme—harvested in Germany; Oregano—harvested in Turkey. The purity of the extracted oils is certified by the Certified Pure Therapeutic Grade (CPTG) certification.

Aqueous extracts of each essential oil (AqEO) were prepared. For this, equal amounts of each EO (oregano, thyme, lemongrass, melaleuca and clove) and sterile bi-distilled water (4 mL of each) were gently mixed overnight in 15 mL centrifuge tubes using an orbital mixer. The aqueous phase was recovered and further used as canal irrigant. One ml of the aqueous extract of each essential oil (AqEO) was also sent to the University of Bari for high-performance liquid chromatographic (HPLC) analysis. HPLC analysis was carried out on a chromatograph LC20 Chromatography Enclosure (Thermo Fisher Scientific Inc., Monza, Milan), with a PDA-100 Photodiode Array Detector and a Fluorescent detector Ultimate 3000. Anhydrous powders (sodium acetate, sodium ascorbate, boric acid, ascorbic acid) and all the chemical reagents were purchased, if not specifically reported, from Sigma-Aldrich (Milan, Italy). Distilled water, 96% ethanol, dimethyl-sulfoxide (DMSO), acetonitrile, 99.8% glacial acetic acid, 100% methanol and all solvents used for HPLC and optical density readings were of analytical grade or HPLC grade from Fisher Scientific (Thermo Fisher Scientific Inc., Monza, Italy). Reversed phase columns Hypersep C-18 supplied by Thermo Scientific were used to carry out a solid phase extraction (SPE). HPLC analysis was carried out using a PolarAdvantage II (4.6 × 150 mm; 3 µm) C18 column directly connected to an Adsorbosphere C18 pre-column. The injection volume of analysis samples was of 100 µL. Elution was carried out at a flow rate of 0.80 mL/min for 40 min using, as mobile phase, a mixture of water and acetonitrile (A = acetonitrile, B = distillated water) characterized by the following gradient: 20% A in B for 16 min, from 16 to 30 min the mixture gradually changes to 70% A, remaining stable for 5 min, then returning to 20% A until the end of the analysis. Different chromatographic runs were carried out on

different biological matrices (AqEO). Analytes were monitored with a diode array detector and CHROMELEON v.6.8 software connected to the chromatographing system was used for the acquisition and processing of data.

A solution of 5.25% sodium hypochlorite (NaOCl—commercial name Chloraxid, Cerkamed, Poland) was used as an antimicrobial agent (irrigant with documented antimicrobial properties) (27) and was considered a positive standard to whom the antimicrobial effects of EO could be reported to. Sterile saline solution was used for negative standard (irrigant without antimicrobial properties).

Enterococcus faecalis ATCC29212 (Oxoid Ltd., Basingstoke, United Kingdom) was revitalized from −80 °C glycerol stocks on blood agar. After checking the purity of the culture, a bacterial inoculum of was prepared from in sterile saline solution, at a concentration of 1 McFarland units.

2.2. In Vitro Antibacterial Effects of Aqueous Extracts of Essential Oil (AqEO)

The minimum inhibitory concentration (MIC) of each AqEO was assessed against *E. faecalis* ATCC29212, using the microdilution method. Shortly, binary dilutions of each AqEO were made in the rows of a 96-well plate, with 100 µL per well. One hundred microliters of standard bacterial inoculum in 2x Muller-Hinton broth containing approximately 10^3 CFU/mL *E. faecalis* ATCC29212 were pipetted over the 100 µL of AqEO. The plate was incubated at 35 °C for 18 h. The MIC was assessed by looking for the color change in wells after addition of 3 µL of resazurin and further 1 h incubation. Resazurin (blue) is reduced to resorufin (pink) by metabolically active bacteria by aerobic respiration, proving the bacterial growth.

2.3. Collection and Preparation of Samples

Seven single-canal mandibular premolars were extracted for orthodontic reasons. Each patient signed a consent form due to the ethical requirements. An ultrasonic scaler was used to remove the plaque and the tartar, and each tooth was stored in saline solution. All the teeth were decoronated at the CEJ level using a high-speed diamond fissure bur. The canals were enlarged gradually using K-files up to number 20. Saline solution was used as irrigant. The apex of each tooth was sealed with micro-hybrid composite resin (Super-Cor, Spofa Dental, Jicin, Czech Republic) to prevent the leakage of irrigant, essential oils and microorganisms. The external part of the root of each tooth was sealed by using nail polish (Figure 1). All the teeth were sterilized by autoclaving at 121 °C for 15 min.

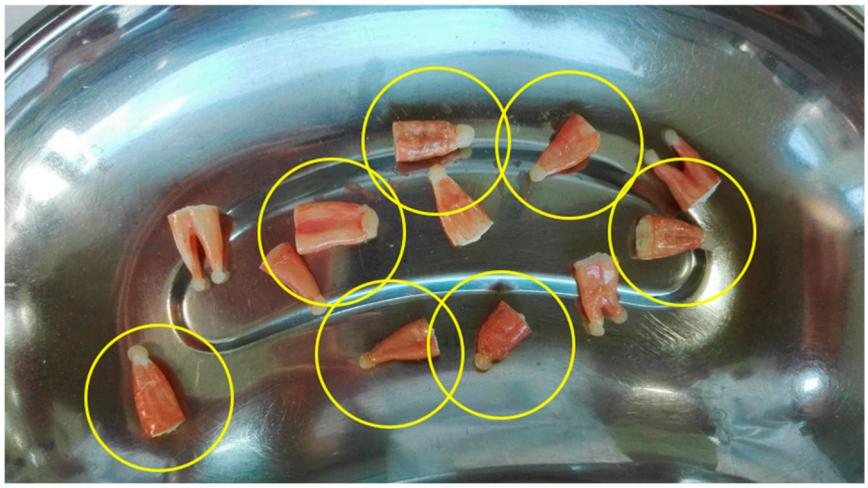

Figure 1. Sealed and sterilized single-canal mandibular premolars.

2.4. Infection of Root Canal

The prepared teeth were individually distributed in sterile 2 mL microcentrifuge tubes. The root canals were inoculated with 20 µL of bacterial inoculum and incubated overnight at 37 °C in order to allow the bacteria to adhere and penetrate the dentin.

2.5. First Processing and Bacteriological Analysis of Infected Teeth

The purpose of the first experiment was to screen the persistence of viable bacteria after mechanical and chemical treatment of the root canals. For this, 7 teeth were randomized as follows: the first five were used for irrigation respectively with AqEO of oregano, thyme, lemongrass, clove, melaleuca; the sixth was used for irrigation with NaOCl and the seventh served as control. All the maneuvers were performed aseptically and using sterile instruments.

Bacterial samples were collected before instrumentation, in order to prove the infection; for this, each tooth was gently irrigated with 2 mL of sterile saline and then bacterial samples were collected with sterile paper points.

All the teeth canals were then instrumented using Hyflex (Coltene, Whaledent Ltd., United Kingdom) rotary files from 20 till 40/04. Between instrumentations, root canals of the teeth were irrigated with 3 mL of the corresponding AqEO, NaOCl, or saline solution for controls. At the end of preparation, each root canal was supplementarily irrigated with 2 mL of sterile saline solution for 1 min to remove the remaining AqEO and NaOCl. Sterile paper points were used for each canal for post instrumentation sampling. The paper points were maintained in the canal for 1 min and then transferred in 2 mL of nutrient broth for enrichment of viable bacteria (after incubation, the broth will remain clear if no bacteria are viable).

2.6. Second Processing and Bacteriological Analysis of Infected Teeth

After assessing the results of the first experiment, we developed a second experiment in order to assess the antibacterial effects of AqEO in the absence of mechanical processing of the root canals. Thus, the teeth were re-sterilized and then infected using the same methodology as described before. This time, the treatment of the root canals was performed only by irrigation, without instrumentation, in the same conditions as in the first processing.

The bacteriological analysis was performed identically as in the first experiment, by enrichment in nutrient broth. Moreover, a semi-quantitative assessment was also performed: 20 microliters from the washing solutions were inoculated on the surface of blood agar plates in order to quantify the remaining viable bacteria. The plates were incubated overnight at 37 °C and the number of developed colonies was counted using the IUL Flash-and-Go automatic colony counter instrument (IUL Instruments SA, Barcelona, Spain).

3. Results

3.1. In Vitro Antibacterial Effects of AqEO

The antibacterial effect of AqEOs was assessed on *E. faecalis*. The lower MIC was observed for thyme, with a value of 12.5% *v/v*. Oregano was the second most effective, with a MIC of 25% *v/v*. Lemongrass, clove and melaleuca presented higher MICs, of 50% *v/v* (Figure 2).

HPLC results confirmed the proportion of the bioactive compounds of the AqEO as for the technical reports of the manufacturers.

3.2. Processing and Bacteriological Analysis of Infected Teeth

After the first processing that included instrumentation, bacterial growth was observed in the enrichment media for oregano, lemongrass and melaleuca. In a synergism with mechanical treatment, thyme and clove completely inhibited *E. faecalis* growth. After the second processing which involved only irrigation and no instrumentation, after enrichment in broth, bacterial growth was observed in all the samples except NaOCl. Nevertheless, the turbidity of the enrichment media was lower for the samples irrigated with AqEOs than

for control (Figure 3) considering the same incubation time, which suggests a degree of inhibitory effect.

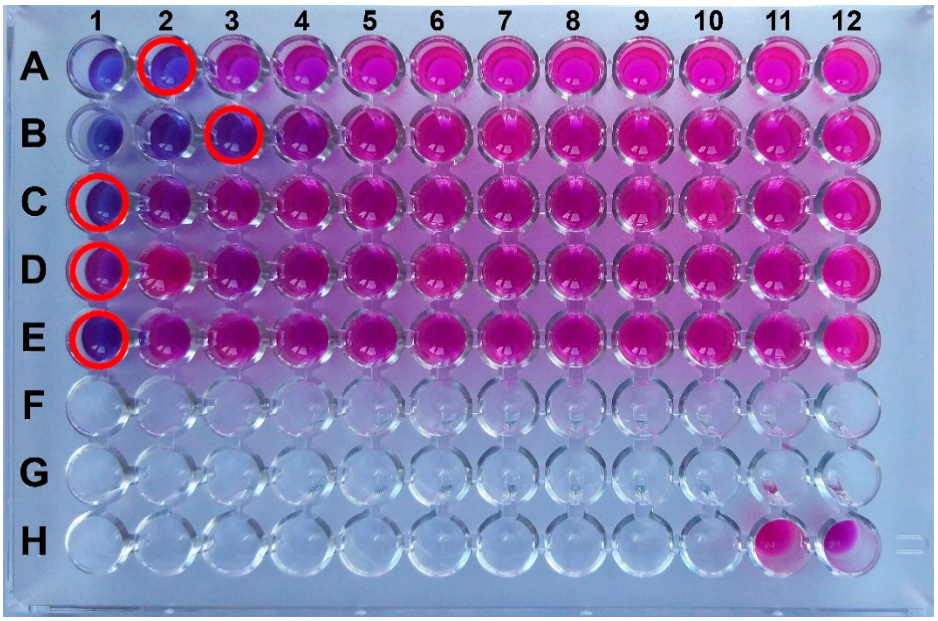

Figure 2. Assessment of minimum inhibitory concentrations (MICs) for aqueous extracts of essential oils (AqEOs) against *E. faecalis*: A—Oregano; B—Thyme; C—Lemongrass; D—Clove; E—Melaleuca; H11- positive control; H12—negative control. The concentration of AqEOs in columns 1–12 (*v*/*v*) are: 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.4%, 0.2%, 0.1%, 0.05% and 0.025%.

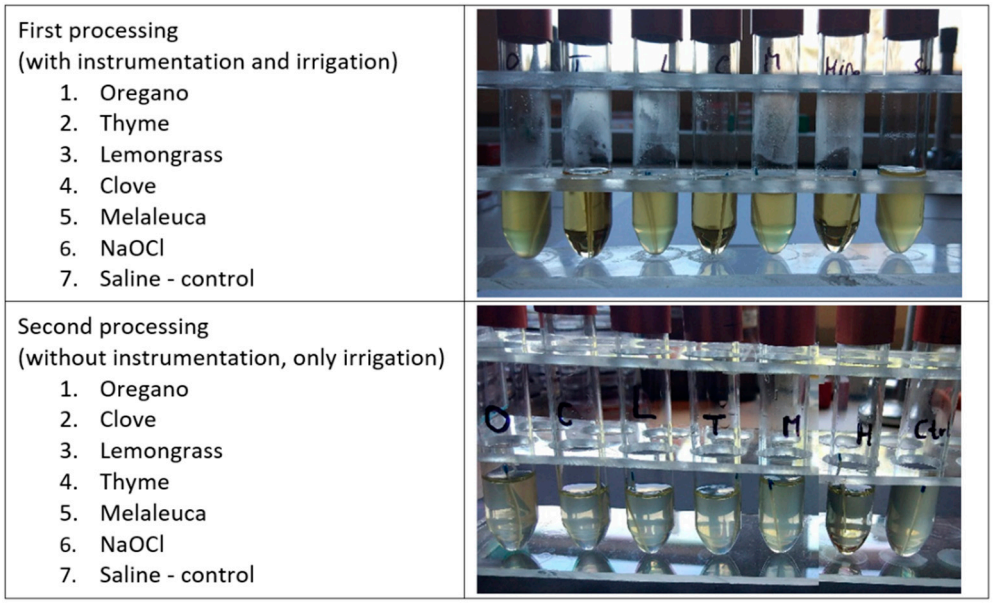

Figure 3. Bacterial growth in enrichment media after irrigation with AqEOs, with and without instrumentation.

During the second processing, the persistence of bacteria in the root canals after irrigation with AqEO, without instrumentation was also evaluated by the semi-quantitative method. The results showed a complete clearance of *E. faecalis* (no bacterial growth on agar plates) by clove and oregano EO, as well as by NaOCl. Thyme was also highly effective, with only a few colonies being observed on the culture medium after irrigation. Lemongrass showed also an important effect, decreasing the bacterial load with more than 75%. Melaleuca AqEO presented an effect similar to that obtained by irrigation only (with

saline solution) (Table 1, Figure 4). Despite complete inhibition in the direct inoculation method on agar plates, the enrichment showed the presence of viable bacteria, as seen in Figure 3.

Table 1. Effect of AqEO on *E. faecalis* growth.

	CFU/mL (Control)	CFU/mL (AqEO)	% of Inhibition
Clove	6.9×10^4	0	100.0%
Thyme	1.2×10^5	10^2	99.9%
Oregano	5.4×10^4	0	100.0%
Lemongrass	7.5×10^4	1.9×10^4	75.1%
Melaleuca	1.4×10^5	9.5×10^4	31.5%
NaOCl	1.5×10^5	0	100.0%
Control	1.2×10^5	7.3×10^4	37.2%

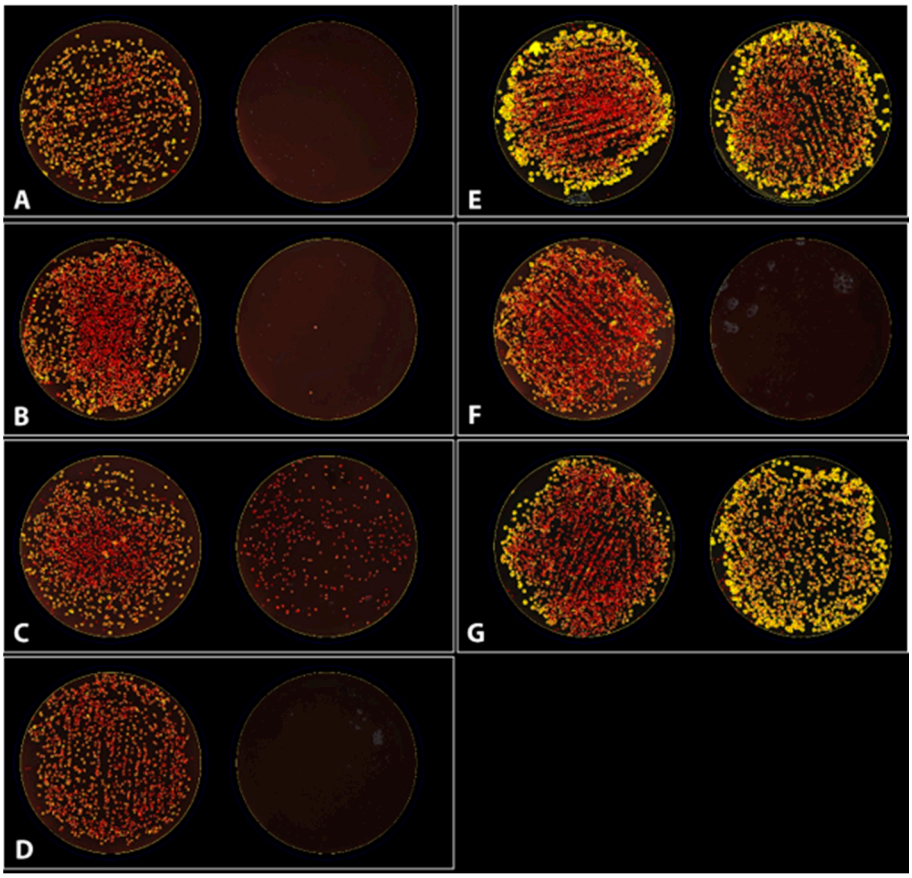

Figure 4. Bacterial growth after canal irrigation with the tested solutions. (**A**) Oregano; (**B**) Thyme; (**C**) Lemongrass; (**D**) Clove; (**E**) Melaleuca; (**F**) NaOCl; (**G**) Saline—control. Each panel presents the plates before and after treatment.

4. Discussion

This study was carried out in order to provide data regarding the antimicrobial potential of essential oils of oregano, thyme, lemongrass, clove or melaleuca, and to assess their role as alternative therapeutic intracanal medication in infected root canals. This preliminary screening research was conducted in order to assess the antibacterial effects of EO in a simulated environment, in a model that resemble a real clinical situation, keeping note of anatomo-physiological details and current root canal treatment protocol.

To increase the success of root canal treatment it is necessary to use a biocompatible intracanal irrigant able to reduce or eliminate the bacteria from the root canal [28]. Despite the qualities of NaOCl, it has clinical disadvantages, especially cytotoxicity. The search for bio-friendly and more biocompatible irrigants made us determined to undertake this study, also considering the rising interest in natural alternatives to chemical antiseptics and antimicrobials, in particular EO and probiotics. Herbal products, due to their biocompatibility are used in many medical fields [32].

Plants are able to defend themselves against microbial aggressors due to their secondary metabolites which are present. Also, the leaves, the bulbs, and the seeds of the plants contain antimicrobial active compounds, which contribute to the antibacterial properties of the plants [13].

E. faecalis has the ability to invade dentinal tubules and resist even in the presence of calcium hydroxide dressings for more than 10 days, as reported by some authors, due to its power to adapt to harsh environmental conditions [9,10,33]. Also, *E. faecalis* is an initiator of endodontic biofilm, being able to form microcolonies on the root canal dentin surface [33].

Antibacterial agents present either bactericidal (act aggressively on key bacterial structures), or inhibitory effects (interfere with bacterial structures or metabolic pathways, being a reversible phenomenon). Current chemical compounds such as NaOCl or chlorhexidine are highly effective, but do not persist at their action site.

Sodium hypochlorite (NaOCl) is commonly used for root canal disinfection, as a root canal irrigant, which in concentration of 1–6% is able to reduce bacterial infection [27,28,30]. Although NaOCl is commonly used for root canal disinfection, it presents disadvantage such as chlorinated odor, which can be unpleasant for both doctor and patient. This odor is a result from the reaction of NaOCl with water [34]. Because of safety concerns, cytotoxic reactions and the potential side effects of the synthetic irrigants, the use of herbal agents has been increased recently; studies reported that the herbal agents have an excellent biocompatibility, anti-inflammatory, antioxidant properties and increased antimicrobial activity [31,34–37]. The most effective method to combat *E. faecalis* is to use 2% chlorhexidine in combination with sodium hypochlorite as endodontic irrigant, which is most often recommended [8]. Nonetheless, NaOCl is known to be highly toxic to periapical tissues [9]. Severe complications were also described following NaOCl extrusion during root canal treatment [38].

Essential oils are complex, concentrated natural bioactive compounds with documented antibacterial activity, acting with similar effectiveness on antibiotic-susceptible or on multidrug-resistant bacteria, even if organized in biofilms [39]. The EO mechanism of action is complex, depending on the content of bioactive compounds; for example, they may interfere with bacterial enzymes, respiratory pathways, membrane integrity, protein synthesis or transmembrane transportation functions [9]. Also, EO have the ability to achieve high concentrations inside the bacterial biofilm, and thus are able to efficiently eradicate *E. faecalis*, even if aggregated and organized in biofilms. Moreover, the review findings of Estrela et al. show that chlorhexidine or NaOCl present a low ability to eliminate *E. faecalis* [40].

In intracanal endodontic biofilms, *E. faecalis* form colonies on the canal walls and in the root canal dentin tubules, either as surface aggregates (in rich nutrient environment) or adherent cell clumps with many dead cells and islands of active bacteria that degrade the dentin (in nutrient-deprived environment). *E. faecalis* has the ability to precipitate the apatite from dentin, and develop a "calcified" biofilm. In addition, other bacteria and fungi are capable of forming colonies on root canal walls. All these contribute to *E. faecalis* persistence in root canals [33,41]. Even if all these are supporting the pathogenicity of *E. faecalis* in root canal infections, other studies question its relevancy as the predominant pathogen in the etiology of secondary endodontic infections, because enterococci are not common colonizers of oral cavity [42]. Thus, the origin of *E. faecalis,* as responsible for

secondary intraradicular infection, is most probably exogenous, rather than the patient's oral microbiome [43].

Our results suggest that both mechanical cleaning, completed by chemical decontamination, are needed in order to properly control the canal microflora and to prevent infection relapse. When comparing the root canal treatment with and without instrumentation, the results show that this procedure significantly contributes to bacterial clearance. Without instrumentation, which is described as part of the root canal treatment protocol, bacteria manage to survive in a more or less latent state in the tubular structure of the dentin, with chance of later reactivation. *Enterococcus* spp., but also other species such as *Streptococcus mutans*, *Streptococcus sanguis* or *Fusobacterium nucleatum* (an important co-aggregator of *E. faecalis*) have the ability to strongly bind underlying tissues and organize in biofilms, which are more resilient to antibacterial agents [33]. Our results are in concordance with other findings [44–46], that showed the importance of instrumentation in clearing the bacterial biohazard in root canals. Following instrumentation and irrigation, the AqEOs of oregano, thyme and clove showed no bacterial growth after processing the samples harvested with sterile paper points from the root canal. We have to mention here the limitation of the paper-point sampling method, where the accuracy of sampling and the absorbance rate may vary despite the controlled experimental conditions; also, contamination is possible during the handling. Without instrumentation, AqEOs decreased the bacterial load (clove and oregano down to no bacterial growth, as seen in the direct paper-points assessment), but viable bacteria still persisted.

Oregano and thyme EO are known as having one of the best antibacterial effects. The antibacterial effect of oregano extract on *E. faecalis*, observed in our study, was also reported in previous studies, where a concentration 1% and NaOCl had similar antibacterial effect, and better effect than ethylenediaminetetraacetic acid. Moreover, at this concentration, oregano EO is less cytotoxic than NaOCl [47,48]. Oregano AqEO content was mostly composed of carvacrol, followed by 5–10% of each para-cymene, gamma-terpinene and linalool. Carvacrol was generally found to be main constituent of oregano EO, with very good antibacterial effect [21,49]. In this study, from all the oils evaluated, thyme EO presented the lowest MIC against *E. faecalis*, as previously reported [49–51]. In concordance with other findings [52], our HPLC analysis showed thymol, para-cymene, gamma-terpinene and linalool as main bioactive constituents of thyme EO.

The other tested AqEOs (lemongrass, clove, melaleuca) presented high MIC values (50% *v/v*), supposedly making them ineffective as antibacterial substances. As a reminder, in our study aqueous solutions of EO were used, so a lower activity is expected than compared with whole EO effect. Nevertheless, clove AqEO (in synergism with instrumentation) was capable of completely neutralizing *E. faecalis* from the root canal. The major bioactive constituent of clove is eugenol and eugenyl-acetate (with broad-spectrum antimicrobial activity against Gram-positive, Gram-negative, fungi and viruses, and widely used as sedative dressing, cement formulations or endodontic sealer), comprising more than 95% of all detected compounds [53,54]. This makes us conclude that even small amounts of these compounds are enough to exert the bioactive effects, and thus may explain the difference between the high MIC versus the good ex vivo efficiency in treating infected root canals. Because of its bioactive potency (good antibacterial effect, but also tissue irritant), clove EO is recommended to be used in small concentration, to protect the vital tissues [55], but still to provide an effective result.

The failure of root canal therapy due to *E. faecalis* infection is associated with microbial-related factors such as proton pump activity, collagen adherence or inter- and intraspecies horizontal gene transfer [56] which makes the treatment difficult; this is the reason of choosing this study to search for more bio-friendly and biocompatible irrigants. Complementary to this, other studies confirmed the usefulness of probiotics for dental health [57,58]. However, other studies suggest new approaches that might be used for the treatment of *E. faecalis* infections, such as transcriptome sequencing for identification of genes that are associated with bacterial survival and adaptability [59]. Another therapeutic approach

is the use of isolated phages for targeting *E. faecalis* in dental infections [60]. These perspectives could offer a more effective therapeutic approach that excludes the use of both natural and chemical substances alike. Rather than by just studying the MIC and MIB of natural substances on *E. faecalis*, another aspect that needs to be further explored in order to precisely find a naturally derived bactericidal compound, is the specific array of characteristics that enables *E. faecalis* to escape chemo-mechanical instrumentation. These mechanisms include activation of some survival genes, the targeting of highly nutritional tooth areas, or the presence of strong bacterial synergism [61].

Given these facts, since the root canal treatment is constantly evolving, we can assert that natural products, particularly essential oils, can be alternative intracanal irrigants against bacteria. Since this is an in vitro study, further studies are needed to investigate the potential of these essential oils, especially if considering that, in vivo, the bacterial colonization of the pulp occurs by numerous bacterial species. Thus, a limitation of this preliminary study is that the antibacterial activity of AqEOs was evaluated against only one species, *E. faecalis*.

5. Conclusions

In this present study we can assert that after the teeth instrumentation, AqEOs of thyme and clove completely inhibited *Enterococcus faecalis* growth. Instead, without instrumentation, none of the treatments had the ability to completely stop the bacterial growth, except NaOCl. Nevertheless, by optimizing the extraction process in aqueous form, essential oils may become good alternatives to chemical disinfectants.

Supplementary Materials: The producer's HPLC analysis are available online at https://www.mdpi.com/2076-3417/11/4/1422/s1.

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