ORIGINAL ARTICLE

Antimicrobial Effect of Australian Antibacterial Essential Oils as Alternative to Common Antiseptic Solutions against Clinically Relevant Oral Pathogens

J. Karbach¹, S. Ebenezer², P. H. Warnke³, E. Behrens⁴, B. Al-Nawas¹

¹ Department of Oral, Maxillofacial and Plastic Surgery University Medical Centre of the Johannes Gutenberg-University Mainz, Mainz, Germany

² Department of Periodontics, M. R. Ambedkar Dental College & Hospital, Bangalore, India

³ Department for Facio-Maxillary and Regenerative Surgery, Griffith University, Gold Coast, Australia

⁴ Department of Oral and Maxillofacial Surgery, University of Kiel, Kiel, Germany

SUMMARY

Background: The aim of the study was to examine the *in vitro* antibacterial activity of different oils in comparison to antiseptics against oral microorganisms.

Methods: The antimicrobial effect of tea tree oil (TTO), eucalyptus oil (EO), lemon grass oil (LGO), and a eucalyptus-based oil mixture (MXT) were tested in comparison to chlorhexidine digluconate (CHX), povidone-iodine (BTA), and octenidine dihydrochloride (OCT). Oral bacterial strains and candida species using the agar diffusion test were used for the antimicrobial study.

Results: All tested oils showed antimicrobial potency against the tested biological indicators. In comparison of all tested substances the largest effective zones were measured for LGO, followed from MXT and CHX. TTO and EO were less effective against the tested micro-organisms followed from BTA.

Conclusions: The results of this study show that some essential oils have better antimicrobial properties than standard oral antiseptics. In a follow-up step, the ideal concentrations, the composition of essential oils, and the mode of application will be evaluated. The antibacterial efficacy of essential oils might be promising for use in clinical and oral hygiene applications. The cost reduction and availability particularly in rural areas with easy access to the originating plants might be advantageous factors to be considered.

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Correspondence:

Julia Karbach DMD, MD, DDS
Department of Oral, Maxillofacial and Plastic Surgery
University Medical Centre of the
Johannes Gutenberg-University Mainz
Mainz, Germany
Phone: +49/6131/173191
Fax: +49/6131/17662
Email: jkarbach@uni-mainz.de

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INTRODUCTION

The oral cavity contains several species of micro-organisms which include bacteria, viruses, and fungi organized into dental plaque. Dental plaque is of importance as an etiologic factor for dental caries, periodontitis, and peri-implantitis. Certain micro-organisms from the dental plaque such as *Streptococcus gordonii* and *Enterococcus faecalis* are the most frequent etiologic agents of infective endocarditis and hence exert their effects distant to the oral cavity as well [1]. Over the past years

several mechanical and chemical methods have been employed to prevent the formation of dental plaque and to reduce the oral bacterial load. Antimicrobials such as povidone iodine, chlorhexidine, and alcohol based mouth rinses have been employed widely against oral bacteria. They are conventional standard antimicrobials used in daily practice. Some side effects from their prolonged usage include staining, xerostomia, taste alterations, burning, and even oral cancer, can reduce the long-term benefits for the patients [2]. Resistance of bacteria, yeasts, and fungi against antibiotics and chemotherapeutics are a growing problem in medicine [3]. As well, it was shown that multi-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) are less susceptible to antiseptics like chlorhexidine and cetrimide than methicillin-sensitive *Staphylococcus aureus* (MSSA) [4]. The emergence of resistant strains with the consistent usage of antimicrobials has lead to the use of essential oils against micro-organisms *in vitro* [5] and *in vivo* [6]. They are safe and effective as antibacterials, antifungals, and antivirals [7]. Essential oils are used in dermatology, aromatherapy, and cosmetology to treat a variety of conditions such as acne, dandruff, hair lice, recurrent herpes labialis, and oropharyngeal candidiasis.

Essential oils are a group of secondary metabolites from plant materials. They are volatile aromatic liquids and the main components are derived from terpenes and their oxidative derivatives, the terpenoids. The exact compositions of essential oils vary depending on the species of plants it originates from. They include hydrocarbons, alcohols, esters, ethers, ketones, and aldehydes. Essential oils are hydrophobic in nature. This character enables them to partition the lipids of the bacterial cell membrane and mitochondria, making them permeable and eventually disturbing the cell structure. It leads to leaching out of critical molecules and ions. The result is the death of the bacterial cell [8].

To date several studies have analysed the effect of pure essential oils and antiseptics [9] to dental bacteria and yeasts [10]. Using essential oils as a mouth rinse, the total amount of oral bacteria as well as mutans streptococci can be reduced [6]. Furthermore, an antibacterial effect against biofilm formations was detected *in vitro* [11]. In the literature, the comparison of the antimicrobial effect of the combination of essential oils and single applications to standard antiseptics has not been evaluated with the oils and the antiseptics used in our study. Micro-organisms from the oral cavity associated with dental caries, periodontitis, peri-implantitis, and lesions of oral soft tissues have been selected for testing. The tested microbes are listed as follows: *Lactobacillus buchneri* (*L. buchneri*), *Lactobacillus brevis* (*L. brevis*), *Streptococcus gordonii* (*S. gordonii*), *Streptococcus mutans* (*S. mutans*), *Parvimonas micra* (*P. micra*), *Aggregatibacter actinomycetemcomitans* (*A. a.*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Enterococcus faecalis* (*E. faecalis*), *Enterococcus faecium* (*E. faecium*), *Candida albicans* (*C.*

albicans), and *Candida glabrata* (*C. glabrata*).

L. brevis and *L. buchneri* are gram-positive bacilli present in dental carious lesions [14]. *S. gordonii* and *S. mutans* are gram-positive cocci and are commensals of the oral cavity; the former is associated with periodontitis, while the latter causes dental caries. *P. micra* and *A. a.* are periodontal pathogens [9]. *S. aureus* and *S. epidermidis* are gram-positive cocci which cause angular cheilitis, parotitis, and mucositis of the oral cavity [12]. *E. faecalis* and *E. faecium* are gram-positive cocci which are associated with endodontic lesions [13]. *E. faecalis* and *S. gordonii* are isolated frequently from patients with infective endocarditis [1]. *C. albicans* and *C. glabrata* are dominant in oropharyngeal candidiasis [15]. The aim of the present study was to evaluate the *in vitro* antimicrobial effect of a combination of oils and single applications of essential oils as compared to standard antiseptics on oral micro-organisms.

MATERIALS AND METHODS

Essential oils

Tea tree oil (TTO; Australia) with terpinen (30%), eucalyptus oil (EO; Australia) with cineol (60 - 85%), lemon grass oil (LGO; Australia) with citral (75%), and eucalyptus-based oil mixture (KM-PT 70, Klonemax®, Central Tilba, NSW, Australia, MXT) were tested for their effect on oral bacteria. Klonemax is a custom made mixture consisting of EO 136 mg/mL, TTO 131 mg/mL, LGO 86 mg/mL, lemon oil (LO) 71 mg/mL, clove oil (CO) 73mg/mL, and thyme oil (TO) 26 mg/mL dissolved in ethanol.

Antiseptics

Povidone-iodine (BTA, Betaisodona® 10 g/100 mL, Mundipharma GmbH, Limburg, Germany), chlorhexidine digluconate (CHX, Paroex® 0.12%, Sunstar GmbH, Kriftel, Germany), and octenidine dihydrochloride (OCT, Octenisept® 0.1 g/100 mL, Schülke and Mayr GmbH, Norderstedt, Germany) were the standard antiseptics.

Bacterial and Candida strains

We assessed the susceptibility of 10 different cultured aerobic and facultative anaerobic bacteria and two yeasts to essential oils and antiseptics (Table 1). The organisms selected were causative for oral, dental, and cutaneous infections and were frequently isolated from dental plaque.

Agar diffusion test

The efficacy of different oils was tested with agar diffusion test. After overnight incubation of the bacterial/candida strains on brain heart infusion agar (BHI; Difco Laboratories, Detroit, MI, USA) at 37°C, one or two well-isolated colonies of each bacterial/candida strain were suspended in 0.9% sodium chloride solution and vortexed until the same density as the McFarland stan-

dard of 0.5 was reached. Then, 0.1 mL dilution aliquots were spread with a sterile bent-glass rod onto the entire surface of the medium rotating the plate after each application to ensure an even distribution of the inoculum. Test discs (6 mm in diameter) loaded with 10 μ L of essential oil or standard antiseptics were then applied with a sterile forceps on the agar plates. The agar plates were incubated at 37°C for 24 - 48 hours [5].

The streptococci (*S. gordonii* and *S. mutans*), *P. micra* and *A. a.* were incubated in the presence of 5% CO₂. After 24 hours the inhibition zone was checked on each plate. In areas where the inhibition zone was less than 6 mm in diameter, the inhibitory effects were classified as "zero". The diameter of the inhibition zones was measured in millimetres (mm).

Evaluation

Each agar diffusion test was repeated three times. When the coefficient of variation was over 5% the test was repeated five times (marked with * in Table 2). The average values and standard deviations of the effective zone of inhibition were calculated and presented with respect to essential oil and bacterial strain, similar to the study of Warnke et al. [5].

The Mann-Whitney U-test was used to identify statistically significant differences between the effectiveness of the essential oils and the standard antiseptics.

RESULTS

Essential oils

The antimicrobial effects of the tested essential oils exhibited a high variation in their efficacy against bacteria and candida strains. However, all tested oils showed antimicrobial potency against the tested biological indicators. The largest effective zones measured were for LGO (9 - 48 mm) and MXT (11 - 38 mm) followed by TTO (9 - 30 mm), and EO (9 - 22 mm) (Table 2).

LGO was most effective against *A. a.* (47.0 ± 1.0), *C. albicans* (36.7 ± 1.2), *C. glabrata* (31.7 ± 1.5), *S. aureus* (28.0 ± 2.6), and *S. gordonii* (27.3 ± 1.5). It also had a moderate effect on *S. epidermidis* (19.3 ± 1.5), the lactobacilli (*L. buchneri* (19.3 ± 0.6), *L. brevis* (16.3 ± 1.5)), and *P. micra* (19.0 ± 1.0). It proved to be less effective against *E. faecalis* (9.3 ± 2.3). The essential oil mixture MXT was highly effective against *A. a.* (38.3 ± 4.0) and *C. albicans* (24.7 ± 1.2), moderately effective against *S. gordonii* (16.3 ± 0.6), *S. epidermidis* (16.3 ± 3.0), and least effective against *E. faecium* (10.6 ± 0.6). TTO was most effective against *A. a.* (34.0 ± 11.3), moderately effective against *S. gordonii* (17.6 ± 5.8), and least against *P. micra* (9.3 ± 1.5). EO was most effective against *A. a.* (22.2 ± 0.3), moderately effective against *S. gordonii* (16.3 ± 0.6) and least against *P. micra* (8.5 ± 0.5). *A. a.* had the highest susceptibility to all four essential oils LGO (47.0 ± 1.0), MXT (38.3 ± 4.0), TTO (34.0 ± 11.3), and EO (22.2 ± 0.3). The enterococci and *P. micra* had the lowest susceptibility to the four

essential oils (Table 2).

Antiseptics

In the common antiseptic group CHX showed the highest antimicrobial effect. The standard antiseptics showed inhibition zones from CHX (11 - 19 mm), OCT (8 - 15 mm), and BET (6 - 9 mm). CHX had a moderate effect against *S. mutans* (19.7 ± 0), *A. a.* (17.0 ± 0), *P. micra* (17.8 ± 0), *S. gordonii* (16.0 ± 0), and was least effective against *E. faecium* (11.2 ± 0) and *E. faecalis* (11.2 ± 0). OCT showed the highest, but only moderate antimicrobial effectivity against *C. glabrata* (15.0 ± 1.0) and the least against *E. faecalis* (8.3 ± 2.1).

BTA showed the highest antimicrobial effectiveness against *S. aureus* (12.2 ± 0.3) and the least against the streptococci, *A. a.*, *E. faecium*, and the candida strains (6.0 ± 0) (Table 2).

Comparison of the antimicrobial effects between essential oils and antiseptics

LGO showed the highest inhibitory effect of all tested products. No standard antiseptic in the control group demonstrated such dominant inhibition effects against *S. gordonii*, *S. aureus*, *A. a.*, *C. albicans*, and *C. glabrata*. Testing LGO in five of the twelve micro-organisms an inhibition zone > 20 mm could be seen (4/12 $\leq$ 20 mm; 1/12 $\leq$ 15 mm; 2/12 $\leq$ 10 mm). The next highest antimicrobial effect against the tested micro-organisms were found in MXT (2/12 > 20 mm; 2/12 $\leq$ 20 mm; 8/12 $\leq$ 15 mm) and CHX (4/12 $\leq$ 20 mm; 8/12 $\leq$ 15 mm). The effectiveness of TTO (1/12 > 20 mm; 1/12 $\leq$ 20 mm; 6/12 $\leq$ 15 mm; 4/12 $\leq$ 10 mm), EO (1/12 > 20 mm; 1/12 $\leq$ 20 mm; 5/12 $\leq$ 15 mm; 5/12 $\leq$ 10 mm), and OCT (11/12 $\leq$ 15 mm; 1/12 $\leq$ 10 mm) was lower compared to LGO, MXT, and CHX. The lowest effect was seen in BTA (1/12 $\leq$ 15 mm; 5/12 $\leq$ 10 mm; 6/12 no inhibition zone) (Figure 1).

All tested oils demonstrated a significantly higher susceptibility compared to BTA ($p = 0.001$). LGO ($p = 0.005$), and the mixture of all oils MXT ($p = 0.039$) had also a higher antimicrobial effect than OCT. LGO ($p = 0.021$) revealed an even higher effect than CHX. On the other hand EO ($p = 0.018$) was less effective than CHX.

DISCUSSION

Essential oils showed a higher or similar effective inhibition of bacteria and fungi compared to the standard antiseptics. The largest inhibition zones measured were found for LGO, followed by the comparable high antimicrobial effect of MXT and CHX. TTO, EO, and OCT showed less effectiveness against the tested micro-organisms. The lowest inhibition zones were measured for BET.

The effectiveness of the essential oils of the study are comparable to the results of a previous study where the susceptibility of several oral and skin pathogens to essential oils *in vitro* were tested with the same method

Table 1. Microbial strains used for *in vitro* susceptibility tests.

Microbial strains	
<i>Lactobacillus buchneri</i>	DSM No.: 20057 *
<i>Lactobacillus brevis</i>	DSM No.: 6235
<i>Streptococcus gordonii</i>	DSM No.: 6777 *
<i>Streptococcus mutans</i>	DSM No.: 20523 *
<i>Staphylococcus aureus</i>	DSM No.: 20231 *
<i>Staphylococcus epidermidis</i>	DSM No.: 20044 *
<i>Enterococcus faecium</i>	DSM No.: 20477 *
<i>Enterococcus faecalis</i>	DSM No.: 20478 *
<i>Parvimonas micra</i>	DSM No.: 20468 *
<i>Aggregatibacter actinomycetemcomitans</i>	DSM No.: 11122
<i>Candida albicans</i>	DSM No.: 1386
<i>Candida glabrata</i>	DSM No.: 11226

Microbial strains used for testing of their susceptibility against the different essential oils and standard antiseptics (Leibnitz Institute DSMZ-German Collection of Microorganisms and Cell Cultures - DSM).

Table 2. Inhibition zones of tested micro-organisms to the tested oils and standard antiseptics.

	<i>L. buchneri</i>	<i>L. brevis</i>	<i>S. g.</i>	<i>S. m.</i>	<i>S. a.</i>	<i>S. e.</i>	<i>E. faecium</i>	<i>E. faecalis</i>	<i>P. m.</i>	<i>A. a.</i>	<i>C. a.</i>	<i>C. g.</i>
TTO	13.0 (± 0.0)	13.0 (± 0.0)	16.6 (± 5.8) *	9.6 (± 0.6) *	10.0 (± 2.0) *	9.8 (± 0.8) *	10.3 (± 0.6) *	13.0 (± 1.0) *	9.3 (± 1.5)	34.0 (± 11.3) *	10.7 (± 0.6)	12.0 (± 0.0)
EO	13.3 (± 2.0) *	13.0 (± 1.7) *	16.3 (± 0.6)	9.5 (± 0.9) *	9.0 (± 1.0) *	8.7 (± 0.6) *	10.3 (± 0.6) *	11.3 (± 0.6) *	8.5 (± 0.5) *	22.2 (± 0.3)	9.0 (± 0.0)	11.7 (± 0.6)
LGO	19.3 (± 0.6)	16.3 (± 1.5) *	27.3 (± 1.5) *	13.7 (± 0.6)	28.0 (± 2.6) *	19.3 (± 1.5) *	10.0 (± 0.1) *	9.3 (± 2.3) *	19.0 (± 1.0) *	47.0 (± 1.0)	36.7 (± 1.2)	31.7 (± 1.5)
MTX	14.0 (± 1.0) *	11.7 (± 0.6)	16.3 (± 0.6)	12.7 (± 1.2) *	15.8 (± 0.8)	16.3 (± 3.0)	10.6 (± 0.6) *	15.3 (± 2.3) *	11.5 (± 0.5)	38.3 (± 4.0)	24.7 (± 1.2)	15.0 (± 1.0) *
CHX	14.0 (± 0.0) *	14.0 (± 0.0) *	16.0 (± 0.0)	19.7 (± 0.0)	15.2 (± 0.0) *	15.0 (± 0.0)	11.2 (± 0.0)	11.2 (± 0.0) *	17.8 (± 0.0) *	17.0 (± 0.0) *	13.3 (± 0.6)	15.0 (± 0.0)
OCT	13.3 (± 0.6)	14.0 (± 1.7) *	11.7 (± 0.6)	12.3 (± 0.3)	14.7 (± 4.2) *	12.0 (± 1.7) *	9.7 (± 2.1) *	8.3 (± 2.1) *	12.2 (± 0.3)	11.0 (± 0.0)	10.5 (± 0.5)	15.0 (± 0.0)
BTA	8.3 (± 0.6) *	8.3 (± 2.1) *	zero	zero	18.2 (± 0.3)	8.7 (± 0.6) *	zero	7.3 (± 0.6) *	7.7 (± 1.2) *	zero	zero	zero

Mean values of inhibition zones (in mm) and standard deviations (in mm) by test substances and test isolates on microbial strains. The fields where the means of the diameter is marked with * the coefficient of variation was over 5% and the tests were done five times. Oils - tea tree oil (TTO), eucalyptus oil (EO), lemon grass oil (LGO), eucalyptus-based oil mixture (MTX). Standard antiseptics - chlorhexidine digluconate (CHX), povidone-iodine (BTA) and octenidine dihydrochloride (OCT). Bacteria - strain types are marked with *. *Lactobacillus brevis* (* *L. brevis*), *Lactobacillus buchneri* (* *L. buchneri*), *Streptococcus gordonii* (* *S. g.*), *Streptococcus mutans* (* *S. m.*), *Staphylococcus aureus* (* *S. a.*), *Staphylococcus epidermidis* (* *S. e.*), *Enterococcus faecium* (* *E. faecium*), *Enterococcus faecalis* (* *E. faecalis*), *Parvimonas micra* (* *P. m.*), *Aggregatibacter actinomycetemcomitans* (* *A. a.*), *Candida albicans* (* *C. a.*), *Candida glabrata* (* *C. g.*). The inhibition zones are measured in millimeters (mm). For areas smaller than 7 mm in diameter, the inhibitory effects were classified as "zero". (Red = inhibition zone: "zero" - < 5 mm, yellow = inhibition zone: 5 - < 10 mm, light green = inhibition zone: 10 - < 20 mm, dark green = inhibition zone: > 20 mm).

Figure 1. a.) Inhibition zones (mm) of supragingival bacteria (*Lactobacillus buchneri*, *Lactobacillus brevis*, *Streptococcus mutans*, and *Streptococcus gordonii*), b.) oral bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Enterococcus faecium*), c.) subgingival bacteria (*Parvimonas micra* and *Aggregatibacter actinomycetemcomitans*), d.) candida species (*Candida albicans* and *Candida glabrata*) to the essential oils (tea tree oil - (TTO), eucalyptus oil - (EO), lemon grass oil - (LGO), eucalyptus-based oil mixture - (MTX) and the standard antiseptics chlorhexidine digluconate - (CHX), povidone-iodine - (BTA), and octenidine dihydrochloride - (OCT).

used in the present study. The antimicrobial activities of thirteen essential oils were tested against bacterial (streptococci, staphylococci) and *Candida* strains, whereas thyme white oil, lemon oil, lemongrass oil, and cinnamon oil showed the highest effect [5]. In the study of Warnke et al., the oils were neither diluted nor chemically altered and no mixture of oils were used like MXT in the present study, which showed similar high effects against the tested bacteria to the standard antiseptic CHX [5].

Takarada et al. [7] tested various essential oils for their

efficacy against oral micro-organisms with a serial dilution method without using standard antiseptics as controls. It was seen that manuka oil and TTO had strong antibacterial activity against periodontopathic bacteria such as *A. a.*, *P. gingivalis*, *F. nucleatum*, and *S. mutans* [7]. However, Takarada et al. did not test LGO, the oil with the highest effectiveness in the present study, and the different methods used do not allow a direct comparison of the study of Takarada et al. with the results of the present study and the study of Warnke et al. [5,7]. One disadvantage in using the agar diffusion test might

be that the effective inhibitory zone depends on the solubility as well as the diffusion characteristics of the substance tested; hence it is difficult to compare oils along with the controls [5]. However, in our study the effective zones are clearly visible and this proves their antimicrobial activity (Figure 1).

It was not part of this study to examine if the *in vitro* results of the study are comparable to *in vivo* antibacterial activity. The biological effect mechanisms of the tested essential oils differ as well as the active ingredients of these oils [16]. The high antimicrobial activity of LGO against *A. a.*, *S. gordonii*, *S. aureus*, and the two *Candida* strains might be caused by the active ingredients myrcene, an antibacterial pain reliever, citronellal, citronellol, and geraniol [17]. Using LGO (*Cymbopogon citratus*) good antifungal activity was shown against biofilm formations of *C. albicans* *in vitro* and against oral candidiasis *in vivo* [18]. Yet, no studies are available for the effect of LGO against *A. a.* and *S. gordonii*. However, several studies proving its antimicrobial potential to various other pathogens have shown the inhibitory effect of LGO [5,17]. TTO (*Melaleuca alternifolia*) has a broad spectrum of activity. It is effective against gram-positive, gram-negative bacteria, acid fast bacilli, yeasts, and fungi [19]. A similar high effect on *A. a.* as in this study was seen in the study of Takarade et al. where different periodontopathic bacterial strains were killed completely by exposure for 30 s to 0.2% EO, manuka oil, and TTO [7]. Although when used topically against streptococci for wound disinfection a good effect was seen [19]. However, in the present study, a moderate effectiveness against *S. gordonii* and only a mild effectiveness against the other tested microorganisms including *S. mutans* was found. If the essential oils cause an inhibition of the adhesion of *S. mutans* was not tested in this study. For EO (*Eucalyptus radiata*), large inhibition zones were seen against *A. a.* and a moderate zone against *S. gordonii*. The effectiveness of EO against *A. a.*, *F. nucleatum*, and *P. gingivalis* was already demonstrated and was confirmed in this study for *A. a.* [7]. The high antimicrobial activity against *S. aureus* and *C. albicans*, detected by Safaei-Ghomi and Ahd, was not confirmed [20]. Interestingly, they showed that the effect of EO against the tested bacteria and yeasts was even higher than that of the main component, 1,8-cineole (eucalyptol) [20]. In a further study of Warnke et al., where clinical observations of 30 cancer patients treated with the same mixture of essential oils as in the present study are reported, no allergic reaction was seen [21]. However, in other studies, cases of allergic reactions using EO have been reported [22]. Allergies might depend on high contents of cineoles [22] within the essential oil solution and concentrations therefore need to be considered. The mixture of essential oils MXT had high antimicrobial activity against *C. albicans* and *A. a.* and the least to *E. faecium* similar to LGO. But LGO had better antimicrobial activity than MXT to *S. gordonii*, *S. aureus*, and *C. glabrata*, which indicates that the mixture of essential oils is not neces-

sarily superior to the individual oils. The same Australian essential oil mixture has been used to effectively treat malodorous cancer wounds [21,23]. The essential oil regimen has successfully reduced tumour malodour, enabled healing, and had anti-inflammatory effects on superinfected ulceration [21], but no *in vitro* data showed these antimicrobial effects for the combination of the essential oils (MTX) until now. The antimicrobial activity of the combinations of the essential oils (MTX) was not quantified by the checkerboard method. Therefore, no information is available whether synergistic or additive antimicrobial effects of the different oils can be used to reduce their minimum effective dose.

In a concentration of 0.2%, CHX is considered to be the "gold standard" for mouthwashes [24]. However, standard antiseptics tested had smaller measured zones of inhibition as compared to the essential oils in the present study. Compared to the results of Warnke et al., the values are likely to vary depending on the tested standard antiseptics [5]. The present results showed larger inhibition zones against bacteria and fungi with CHX as compared to that with BET and OCT. CHX showed moderate inhibition zones for streptococcus species, staphylococcus species, *P. micra*, *C. glabrata*, and *A. a.* and mild effect against all other organisms tested.

Essential oils are generally diluted with alcohol for solubility and for enhancing the volatility of the oil. Alcohol possesses preservability and it was considered that the antimicrobial effect of the essential oils was due to the ethanol content [5]. In this experiment we used 40% ethanol as a control for comparison with the conventional antiseptics and essential oils and no effective zone was found. Most essential oils demonstrated inhibition zones larger than that of 40% ethanol; hence the antimicrobial effect seems to be mainly due to the essential oil rather than the solvent. Warnke et al. also tested the effect of alcohol on the antimicrobial effects of the essential oils, but found that the pure essential oils themselves already showed high antimicrobial effects without any addition of alcohol [5].

Although a good alternative to standard oral antimicrobials, there are several drawbacks to the routine usage of essential oils in general clinical practice. Standardization is difficult as the chemical constituents of plant-derived products often vary [19]. Moreover, the content of active constituents in essential oils seem to vary. Not just the producer of the essential oil matter but also the plant family, and the country of origin play an important role in the antimicrobial activity of the essential oil [5]. As a result of composition changes, essential oils may not be suitable as a sole medication in serious infections; therefore, more studies with focus on the composition of essential oils and the function of the active constituents are required. There is also no accepted standard for testing the effect of these products and therefore it is difficult to establish *in vitro* efficacy in accordance with FDA guidelines [25].

When applying the results of the effectiveness of the essential oils against oral pathogens to a possible use of

these oils against oral diseases, one possibility might be the treatment of periodontitis and mucositis where *A. a.* and *P. micra* can be isolated from periodontal lesions [9]. Rams et al. found that in patients with chronic periodontitis in the United States, frequently subgingival periodontal pathogens were found, such as *A. a.* resistant *in vitro* to therapeutic concentrations of antibiotics commonly utilized in clinical periodontal practice, which do not show resistance to the essential oils [26]. When testing *A. a.* and *P. micra*, all three oils and the mixture of oils showed a high effectiveness comparable to CHX. A reduction of *A. a.* among other tested periodontal pathogens could be seen also in an *in vitro* study using an essential oil mouthwash compared to scaling and root planing in patients with chronic periodontitis by He et al. [27]. On the other hand, in the therapy against caries inducing pathogens like *S. mutans*, *S. gordonii*, and the lactobacillus species as well as in the inducing pathogens found in pulpitis like *E. faecium* and *E. faecalis*, no advantage using essential oils in comparison with standard antiseptics like CHX could be seen. In contrast to the results of this study, Fisher and Phillips found different citrus oils not tested in this study as effective for inhibiting the growth and survival of *E. faecalis*, *E. faecium*, and vancomycin-resistant enterococci. They suggested the antimicrobial activity for them under a range of conditions to reduce healthcare-acquired infections and the costs of the healthcare service [28]. In tumour patients where superinfections with *S. aureus* or *S. epidermidis* as well as *Candida* species are frequently found, only the use of LGO and MTX seems to bring an advantage in comparison with CHX or OCT. Warnke et al. used MTX in 30 cancer patients with tumour necrosis associated with malodour [23]. They saw a reduction of signs of superinfection and pus secretion in the necrotic areas as well as a reduction of the foul smell associated with the necrotic ulcers after 2 - 3 days of treatment with MTX. This allowed the patients to return to the regular ward, and again, enjoy social interactions with relatives, friends, and other patients. Karbach et al. found *Candida* colonization in 83% of the patients after radiation therapy of the head and neck region. They indicated the existing resistance behaviour of *C. albicans* strains to voriconazole and fluconazole and non-*albicans* strains to fluconazole [29]. Therefore, the use of essential oils as topical or intraoral therapy for *Candida* infections might be of therapeutic value especially in view of patients with immunosuppression. The increased resistance of oral bacteria to antibiotics and adverse effects of some antibacterial agents currently used in dentistry indicates a need for alternative products that are safe and effective for prevention and treatment of oral diseases [26]. TTO, LGO, and EO are effective against multi resistant bacteria like vancomycin-resistant *Enterococcus*, methicillin-resistant *Staphylococcus aureus*, multi-resistant *Pseudomonas aeruginosa*, extend-spectrum-beta-lactamase-producing *Escherichia coli*, and *Klebsiella pneumonia* [30]. Essential oils considered

traditional medicines are viewed as good alternatives [7, 30]. The potential benefits of the use of essential oils are that they are inexpensive and easily available in developing countries compared to standard antiseptics. As demonstrated in this study, the essential oils have a good range of action against oral microbes. The results of this study demonstrate that some essential oils have better anti-infective properties than standard oral antiseptics. In a follow-up study, the ideal concentration, composition of various essential oils, and mode of application will be evaluated.

Declaration of Interest:

None of the authors has any conflict of interest to report.

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