

Antimicrobial Activity of Seven Essential Oils From Iranian Aromatic Plants Against Common Causes of Oral Infections

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Background: Over the past two decades, there has been a growing trend in using oral hygienic products originating from natural resources such as essential oils (EOs) and plant extracts. Seven aromatic plants used in this study are among popular traditional Iranian medicinal plants with potential application in modern medicine as anti-oral infectious diseases.

Objectives: This study was conducted to determine the chemical composition and antimicrobial activities of essential oils from seven medicinal plants against pathogens causing oral infections.

Materials and Methods: The chemical compositions of EOs distilled from seven plants were analyzed by gas chromatography/mass spectrometry (GC/MS). These plants included *Satureja khuzestanica*, *S. bachtiarica*, *Ocimum sanctum*, *Artemisia sieberi*, *Zataria multiflora*, *Carum copticum* and *Oliveria decumbens*. The antimicrobial activity of the essential oils was evaluated by broth micro-dilution in 96 well plates as recommended by the Clinical and Laboratory Standards Institute (CLSI) methods.

Results: The tested EOs inhibited the growth of examined oral pathogens at concentrations of 0.015-16 µL/mL. Among the examined oral pathogens, *Enterococcus faecalis* had the highest Minimum Inhibitory Concentrations (MICs) and Minimum Microbicidal Concentrations (MMCs). Of the examined EOs, *S. khuzestanica*, *Z. multiflora* and *S. bachtiarica*, showed the highest antimicrobial activities, respectively, while *Artemisia sieberi* exhibited the lowest antimicrobial activity.

Conclusions: The excellent antimicrobial activities of the tested EOs might be due to their major phenolic or alcoholic monoterpenes with known antimicrobial activities. Hence, these EOs can be possibly used as an antimicrobial agent in treatment and control of oral pathogens.

Keywords: Essential Oil; infections; Medicinal Plants

1. Background

Dental caries is one of the main global public health problems. Based on a recent systematic analysis, oral conditions affected about 3.9 billion people worldwide and untreated caries in permanent teeth was the most prevalent condition with “a global prevalence of 35% for all ages combined” (1). Accumulation of microbial plaque on dental surfaces is the first step of dental caries and periodontal diseases. These microbial plaques composed of native oral flora and cariogenic bacteria. The dental caries is then progressed by further destruction of teeth by acids produced by these bacteria (2). About twenty-five species of streptococci live in the oral cavity. Of these, oral streptococci such as *Streptococcus mutants* and *S. sobrinus* have direct association with tooth decay (3), while others

such as *S. sanguis* and *S. salivarius* are less harmful and considered as normal microbial population of the oral cavity. *Staphylococcus aureus* is another Gram-positive cocci responsible for oral infections (4, 5). This species can be isolated from oral cavity of specific groups such as elderly and children (6, 7). *Enterococcus faecalis* is another Gram-positive cocci commonly isolated from endodontic infections (8, 9).

Yeasts including *Candida* species are also found in oral cavity as normal flora. Under certain circumstances such as avitaminosis, using broad spectrum antibiotics and immunosuppressive agents, they might colonize and adhere to soft and hard tissue surfaces such as dentures and form a biofilm (10). Proper oral hygiene and using

oral mouthwashes with antimicrobial activity are considered as the main approaches in prevention of oral infections. Within the past two decades, emergence of resistance to various antimicrobial compounds has accelerated dramatically. Of these, methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) species and azole-resistant *Candida* species are among the main pathogens responsible for oral infections (11-13). On the other hand, most of these synthetic antimicrobial products have some adverse effects, which make them less popular.

An alternative approach to overcome these issues might be using natural antimicrobial products and phytochemicals. The Middle East has unique niches for medicinal plants, which have been used for treating diseases and infections for thousands of years in traditional medicine. It has been shown previously that plants and their aromatic products have potential antimicrobial activities (14). Essential oils distilled from these aromatic plants have known medicinal properties and widely used to treat a variety of diseases (Table 1). As mentioned in Table 1, these EOs are rich in terpenoid compounds in particular monoterpenes with known antimicrobial properties (15). The previous studies demonstrated the successful usage of essential oil (EO) based mouthwashes in preventing and controlling formation of plaque and gingivitis as well as reducing bad breath and odor-causing bacteria (16).

2. Objectives

In the previous studies, the EOs distilled from seven local medicinal plants of southern parts of Iran were prepared and their main constituents were identified by gas chromatography/mass spectrometry (Table 1). Concern-

ing the emergence of resistance to antibiotics in the past decades, their unavoidable adverse effects and regarding the global tendency towards using natural products and phytochemicals in medicine, the present study was conducted to evaluate antimicrobial activity of the seven common EOs, as listed in Table 1, against the common causes of oral infections.

3. Materials and Methods

3.1. Plant Materials

The areas of plant collection were reported in the Table 1. *Salvia mirzayanii* was deposited at the Herbarium of Medical Science of Department of Pharmacognosy, Faculty of Pharmacy, Shiraz University of Medical Sciences, Iran (Voucher No. 663). The *Satureja bachtiarica* was deposited at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH), Shahid Beheshti University, Tehran, Iran (Voucher No. MPH-1577). The other plants were deposited at the Herbarium of Faculty of Agriculture, Shiraz University, Shiraz, Iran with voucher numbers of SUHA-111 for *Artemisia sieberi*, and SUHA-110 for *Oliveria decumbens*, as well as at Herbarium of Shiraz University, Shiraz, Iran with voucher numbers of SUH-24989 for *Ocimum sanctum*, SUH-24985 for *Carum copticum* and SUH-24985 for *Zataria multiflora*.

3.2. Essential Oil Extraction

The aerial parts of the mentioned medicinal plants were harvested at proper growth stage and then air-dried. EOs were prepared by hydrodistillation using a glass Clevenger-type apparatus according to the method

Table 1. Major Components and Biological Activities of Essential Oils of Medicinal Plants

No.	Plant Name	Collection Area	Major Components	Biological Activities	References
1	<i>Satureja khuzestanica</i>	Mazhin, Lorestan, Iran	Carvacrol (87.7%)	Antifungal and antibacterial activities, Treatment of common cold and bronchitis	(17)
2	<i>Satureja bachtiarica</i>	Semirom, Isfahan, Iran	Caryophyllene oxide (17.0%), Thymol (28.0%), Carvacrol (13.2%)		
3	<i>Ocimum sanctum</i>	Borazjan, Bushehr, Iran	1,8-Cineole (20.78%), β -bisabolene (20.99%), Eugenol (15.70%),	anti-oxidative, antibacterial, antifungal, antimalaria, antihelminthic, antidiabetic, treatment of hypertension and skin disease	(18)
4	<i>Artemisia sieberi</i>	Fasa, Fars, Iran	1,8-cineole (21.1%), Camphor (16.53%), α -thujone (13.08%)	antidiabetic, antiviral, antispasmodic, vermicide, poison antidote and antimicrobial effects, Antifungal	(19)
5	<i>Zataria multiflora</i>	Darab, Fars, Iran	Thymol (37.88%), Carvacrol (27.16%)	stimulates innate immunity, antibacterial and antifungal activities, antiseptic, analgesic, and carminative	(20)
6	<i>Carum copticum</i>	Kazereun, Fars, Iran	Thymol (36.7%), γ -terpinene (36.5%), p-cymene (21.1%)	diuretic, carminative, analgesic, anti-dyspnea and anti-inflammatory, antiseptic	(21)
7	<i>Salvia mirzayanii</i>	Bandar Abbas, Hormozgan, Iran	Cineol (41.20%), linalyl acetate (10.72%)	Antibacterial, free radical scavenging, antioxidant, neuroprotective	(22)

recommended by the European Pharmacopoeia (23). The extracted EO samples were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature (4°C).

3.3. GC and GC/MS Analysis

The analysis of EOs was performed using a Thermoquest-Finnigan Trace GC-MS instrument equipped with a DB-5 fused silica capillary column (60 m × 0.25 mm i.d., film thickness 0.25 mm). The oven temperature was programmed to increase from 60 to 250°C at a rate of 4°C per minute and finally held for 10 min; transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1 mL/min with a split ratio of 1/50. The quadrupole mass spectrometer was scanned over the 35-465 amu (atomic mass units) with an ionizing voltage of 70 eV and an ionization current of 150 mA. The GC/FID analysis of the oils was conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 mm). Nitrogen was used as the carrier gas at the constant flow rate of 1.1 mL/min; the split ratio was the same as that used for GC/MS. The oven temperature was raised from 60 to 250°C at a rate of 4°C per min and held for 10 minutes. The injector and detector (FID) temperatures were kept at 250 and 280°C, respectively. Semi-quantitative data was obtained from FID area percentages without using correction factors.

3.4. Identification of Essential Oil Components

Retention indices (RI) were calculated using retention times of n-alkanes (C6-C24) that were injected after the oil at the same temperature and conditions. The compounds were identified by comparison of their RI with those reported in the literature (24) and their mass spectrum was compared with the Wiley Library.

3.5. Determination of Antimicrobial Activities

3.5.1. Microorganisms

The antimicrobial activities of the EOs against some oral pathogens including standard species of *S. mutants* (ATCC 35668), *S. sanguis* (ATCC 10556), *S. salivarius* (ATCC 9222), *S. sobrinus* (ATCC 27607), *E. faecalis* (ATCC11700), *S. aureus* (ATCC 25923, 29213 and ATCC 700698), *C. albicans* (ATCC 10261), *C. dubliniensis* (CBS 8501), *C. tropicalis* (ATCC 750), *C. krusei* (ATCC 6258) and *C. glabrata* (ATCC 90030) and four clinical isolates of *S. mutants* were determined in this study.

3.5.2. Determination of Minimum Inhibitory Concentration

Minimum Inhibitory Concentrations (MICs) were determined using broth microdilution method rec-

ommended by the CLSI with some modifications (25, 26). Briefly, for determination of antifungal activities against yeasts, serial dilutions of the EOs (0.015 to 16.0 µL/mL) were prepared in 96-well microtiterplates using RPMI-1640 media (Sigma, St. Louis, USA) buffered with MOPS (Sigma, St. Louis, The USA). To determine the antibacterial activities, serial dilutions of the EOs (0.062 to 32.0 µL/mL) were prepared in Muller-Hinton Broth media (Merck, Darmstadt, Germany). Test fungi or bacteria strains were suspended in the media and the cell densities were adjusted to 0.5 McFarland standards at 530 nm wavelength using a spectrophotometric method (this yields stock suspension of 1.5×10^6 cells/mL for yeast and 1.5×10^8 cells/mL for bacteria). 100 µL of the working inoculums was added to the microtiter plates and the plates (treated wells and untreated controls) were incubated in a humid atmosphere at 30°C for 24-48 hours (fungi) or at 37°C for 24 hours (bacteria). 200 µL of the uninoculated medium was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without essential oil) were included.

The growth in each well was compared with that of the growth control well. MICs were visually determined and defined as the lowest concentration of the essential oil producing no visible growth. Each experiment was performed in triplicate. In addition, media from wells with fungi showing no visible growth were further cultured on Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) and from wells with bacteria showing no visible growth on Muller-Hinton agar (Merck, Darmstadt, Germany) to determine the minimum microbicidal concentration (MMC). MMCs were determined as the lowest concentration yielding no more than four colonies, which corresponds to a mortality of 99.9% of the microbes in the initial inoculums.

4. Results

The antibacterial activities of the EOs against the common causes of oral infections are shown in Table 2. The EOs inhibited the growth of examined bacteria at concentrations of 0.062-4 µL/mL, except *E. faecalis*, which was inhibited at concentration of 0.125-16 µL/mL. Furthermore, all of the EOs exhibited the minimal microbicidal activity (MMC) for the tested bacteria at concentrations ranging from 0.25 to 16 µL/mL, except EO *A. sieberi*, which showed bactericidal activities at the range of 1 - 32 µL/mL. Of the tested bacteria, *E. faecalis* had the highest MICs and MBCs. For the standard yeasts tested, the MICs for the EOs were in the range of 0.015 - 2 µL/mL (Table 2). All the tested *Candida* spp. were killed by the EOs at about the same or twice the concentration of their corresponding MICs. Of the examined EOs, *S. khuzestanica*, *Z. multiflora* and *S. bachtiarica* showed the highest antimicrobial activities, respectively, while *A. sieberi* exhibited the lowest antimicrobial properties.

Table 2. Antimicrobial Activity (Minimum Inhibitory Concentration, MIC; Minimum Microbicidal Concentration, MMC) of the Essential Oils Against Oral Pathogens ^{a,b}

Microorganisms	1		2		3		4		5		6		7	
	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
Streptococcus mutants ATCC 35668	0.062	0.125	0.25	0.5	0.5	1	2	4	0.25	0.5	0.5	1	0.062	1
S. mutants 1	0.125	0.25	0.5	0.5	1	4	2	4	0.5	1	0.5	1	0.125	1
S. mutants 2	0.25	0.5	0.5	1	0.5	1	1	2	0.25	0.25	1	2	0.062	1
S. mutants 3	0.25	0.5	0.5	1	0.5	1	1	4	0.25	0.5	0.5	2	0.062	1
S. mutants 4	0.125	0.25	0.5	0.5	0.25	1	0.5	1	0.125	0.25	0.5	1	0.062	1
S. sanguis ATCC 10556	0.125	0.25	0.5	1	0.25	0.5	1	2	0.25	0.5	0.25	1	0.125	1
S. salivarius ATCC 9222	0.125	0.25	0.25	0.5	0.5	1	4	>32	0.125	0.125	0.25	0.5	0.5	4
S. sobrinus ATCC 27607	0.062	0.5	0.25	0.5	0.5	2	2	16	0.125	0.125	0.25	0.5	0.125	4
Enterococcus faecalis ATCC11700	0.125	0.5	0.5	2	1	4	16	>32	0.5	1	2	8	0.25	16
S. aureus ATCC 25923	0.25	0.5	0.5	1	1	8	2	4	0.125	0.25	0.5	1	0.062	4
S. aureus ATCC 29213	0.125	0.25	0.25	0.5	1	8	2	4	0.125	0.5	1	2	0.125	2
S. aureus ATCC 700698	0.125	0.25	0.25	0.5	2	16	1	2	0.25	0.5	1	4	0.062	4
Candida albicans ATCC 10261	0.031	0.031	0.031	0.062	0.125	0.25	1	2	0.062	0.125	0.125	0.125	1	2
C. dubliniensis CBS 8501	< 0.015	0.031	0.062	0.125	0.125	0.125	2	4	0.062	0.125	0.125	0.125	0.5	2
C. glabrata ATCC 90030	< 0.015	0.031	0.031	0.125	0.062	0.125	1	2	0.062	0.125	0.031	0.062	0.25	0.5
C. tropicalis ATCC 750	0.062	0.125	0.125	0.25	0.25	1	2	4	0.25	1	0.25	0.5	0.25	1
C. krusei ATCC 6258	0.031	0.031	0.125	0.125	0.25	0.5	2	4	0.25	0.5	0.25	0.25	1	4

^a Abbreviations: MIC, Minimum Inhibitory Concentration; MMC, Minimum Microbicidal Concentration.

^b 1, *Satureja khuzestanica*; 2, *Satureja bachtiarica*; 3, *Ocimum sanctum*; 4, *Artemisia sieberi*; 5, *Zataria multiflora*; 6, *Carum copticum*; 7, *Salvia mirzayanii*.

5. Discussion

Microbial flora accumulated on the mucosal and dental surfaces of the oral cavity are responsible for dental caries and biofilm formation (27). It has been reported that EOs are capable of inhibiting the growth of these microorganisms and the formation of biofilms (28). These aromatic oils were used successfully in the management of recurrent aphthous stomatitis, plaque formation and gingivitis (29, 30). In this regard, lower potency of chlorhexidine in comparison to EOs has been reported previously (31). Organisms predominant in plaque formation and tooth decay are of the genus *Streptococcus*. Similar to the study of Adiguzel et al. (32), the growth of the standard and clinical isolates of the streptococci was inhibited by tested EOs at concentrations of 0.062 to 1 µL/mL, except EOs of *A. sieberi*, which exhibited higher MICs (0.5-2 µL/mL).

The ability of *S. aureus* to develop methicillin resistance is becoming a matter of great concern. MICs of the tested EOs against *S. aureus* were in the range of 0.06-2 µL/mL, which is concordant with most previous studies (33, 34). In addition, EOs successfully inhibited the growth of *E. faecalis* recognized as the commonly isolated bacteria from endodontic infections (8, 9). These results are in agreement with the report of Sonboli et al. who showed significant antimicrobial activities of three *Salvia* species EOs against *E. faecalis* with MICs of 10 µL/mL (33). *Candida*

spp. are another resident of the oral cavity associated with oral candidiasis and biofilm formation (10). Similar to the previous studies (28, 32, 35), all of the EOs exhibited fungicidal activities against the standard species of *Candida* at concentrations ranged <0.015-0.5 µL/mL, except *A. sieberi* and *S. mirzayanii* which inhibited the growth of tested yeasts at concentrations of 0.25-2 µL/mL.

Hydrophobicity is one of the main characteristics of EOs, which enables their incorporation into the cell membrane (15). As shown in Table 1, *S. khuzestanica*, *S. bachtiarica*, *Z. multiflora* and *C. copticum* were rich in phenolic monoterpenes, including carvacrol and thymol. It has been shown that antimicrobial activity of these phenolic monoterpenes is due to hydroxyl groups at different positions around the phenolic ring through disruption of the cytoplasmic membrane and leakage of ions and ATP. Although no strong antibacterial activity was reported for terpinene as the second main constituent of *C. copticum* (15), this activity might be attributed to the high thymol concentration of this plant. p-Cymene is another major component identified in the EO of *Carum copticum*, which is a hydrophobic molecule and causes swelling of the cytoplasmic membrane (36). It is not an effective antibacterial when used alone (37, 38). However, in combination with other phenolic compounds such as thymol, it has shown a greater antimicrobial activity by

incorporating cymene in the lipid bilayer of bacteria and facilitating transport of thymol across the cytoplasmic membrane (39).

EOs of *O. sanctum*, *A. seiberi*, and *S. mirzayanii* were rich in 1,8-cineol and exhibited strong antimicrobial activity against tested microbes. It has been shown previously that 1,8-cineol has significant antimicrobial activities alone or in combination with other monoterpenes or drugs. Of the tested EOs, *S. mirzayanii* had the highest 1,8-cineol concentration and the lowest MICs against Gram-positive cocci, which is in accordance to the results of above study (40). These results supported the idea of using EOs in mouthwashes and denture cleansers, since they show high efficacy in inhibiting microbial strains, even in the plant form. Furthermore, anti-inflammatory activity (41, 42) and pleasant odor and flavor of these EOs are additional advantages to their antimicrobial activities to be used as a mouth rinse and other oral hygienic products.

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Authors' Contributions

Study concept and design: Kamiar Zomorodian, Mohammad Jamal Saharkhiz and Mohammad Reza Moein. Acquisition of data: Tahereh Golzar and Pooria Ghadiri. Analysis and interpretation of data: Farideh Bahrani, Keyvan Pakshir and Mohammad Mehdi Fani. Drafting of the manuscript: Kamiar Zomorodian, Pooria Ghadiri and Peiman Mehriar. Critical revision of the manuscript for important intellectual content: Peiman Mehriar. Administrative, technical and material support: Mohammad Mehdi Fani. Study supervision: Kamiar Zomorodian.

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