Evaluation of chemical composition and antimicrobial activities of *Eucalyptus camaldulensis* essential oil on dental caries pathogens

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Abstract

Introduction: Various microorganisms are involving in oral infections. Antimicrobial agents used against oral pathogens have side effects and can caused drug resistance. Eucalyptus is one of the most widely used plant with antimicrobial activity. Therefore, the aim of this study was to investigate the chemical composition and antimicrobial activity of *Eucalyptus camaldulensis* (*E. camaldulensis*) essential oil on dental caries pathogens includes *Streptococcus mutans* (*S. mutans*), *Lactobacillus rhamnosus* (*L. rhamnosus*), *Actinomyces viscosus* (*A. viscosus*), and *Candida albicans* (*C. albicans*).

Materials and methods: In this study, *E. camaldulensis* essential oil was prepared and its antimicrobial activity was evaluated by disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) methods on stablished terminology strains of *C. albicans*, *S. mutans*, *L. rhamnosus* and *A. viscosus*. Moreover, chemical composition of *E. camaldulensis* essential oil was evaluated by gas chromatography-mass spectrometry (GC-MS) method.

Results: Our results showed that the most antibacterial activity of *E. camaldulensis* essential oil was related to *L. rhamnosus*, *A. viscosus*, and *S. mutans*. Also, *E. camaldulensis* essential oil showed an appropriate antifungal activity against *C. albicans*. The dominant chemical composition of *E. camaldulensis* essential oil was 1,8-cineole (36.62%).

Conclusion: In general, *E. camaldulensis* essential oil has an appropriate antifungal and antibacterial activity against oral pathogens. Therefore, it can be use in pharmaceutical industry to produce antimicrobial agents against dental caries and oral infectious diseases.

Keywords: Oral infection, Oral pathogens, Antimicrobial activity, Essential oil, Eucalyptus

Introduction

Oral infections are the most common human infections in the worldwide (1). Many types of aerobic and anaerobic bacteria are involved in progress of the oral and adjacent structures infections (2). Such infections are often multi-microbial, affecting more than one pathogenic bacteria (3). Oral fungal infections are also one of the most common opportunistic infections in patients with immune deficiency (4).

Due to the side effects of chemical drugs, the tendency to herbal medicine is increasing in the worldwide today (5). The unnecessary use of antibiotics and other chemical drugs has also led to a dramatic increase in the drug resistance bacteria. Therefore, natural resources, especially medicinal and edible plants, are considered

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as ecological reservoirs (6). Moreover, in recent years, the manufacturer of health products has aimed to the substitution of natural compounds in their products, and much research performed on the antimicrobial effects of plants essential oils and extracts (7). Recent studies have shown that extract and essential oil of a large number of traditional herbs have inhibitory effects on various pathogenic microorganisms (8, 9). Therefore, due to antimicrobial and anti-oxidant properties, many plants have been used in cosmetic and hygienic products (6, 7).

Eucalyptus is a member of the Myrtaceae family, and is one of the most important medicinal plants. There are more than 400 species of eucalyptus, which only a few species have been imported into Iran over the last century, and have been planted in various areas, such as the north and south regions of Iran. *Eucalyptus camaldulensis* (*E. camaldulensis*) is a rich source of polyphenols and terpenoids, and its main composition is eucalyptol or cineol (70 to 80%). Members of this family are an important source of essential oils with extensive biological activities including antioxidant, antibacterial and antifungal properties (10). Recently, Eucalyptus is widely used in the pharmaceutical, food, and cosmetics industries. The essential oil of Eucalyptus leaves is widely used worldwide as a disinfectant and reduces symptoms of cough, sore throat, flu, clotting, fever, bloating, congestion and other infections (11). Also, extract of this plant has antimicrobial activity against a wide range of gram positive and gram negative bacteria such as *Staphylococcus aureus* (*S. aureus*), *Shigella dysenteriae* (*S. dysenteriae*), *Salmonella paratyphi* (*S. paratyphi*), *Escherichia coli* (*E. coli*), *Bacillus cereus* (*B.s cereus*), and *Candida albicans* (*C. albicans*) (12, 13).

Considering that dental caries is one of the most important problems in the worldwide and also the Eucalyptus is one of the inexpensive and indigenous Iranian herbs, the aim of this study was to evaluate the antimicrobial activity of *E. camaldulensis* essential oil on oral pathogenic strains includes *Streptococcus mutans* (*S. mutans*), *Lactobacillus rhamnosus* (*L. rhamnosus*) and *Actinomyces viscosus* (*A. viscosus*) as well as *C. albicans*.

Materials and methods

Preparation of Eucalyptus essential oil

The *E. camaldulensis* collected from medical plants centers in Urmia city, and approved by the Herbarium of the Islamic Azad University, Urmia Branch. 100 gr of *E. camaldulensis* was dried in a dark place and then powdered. 600 ml distilled water was added on the obtained powder. The essential oil was extracted using a Clevenger apparatus (Zarin Pyrex, Iran) and sterilized using a 0.4 µm syringe filter. The prepared essential oil was stored in the 4°C until used.

Preparation of bacterial and fungal strains

The studied dental caries pathogens in present study were include *A. viscosus* (PTCC 1202), *L. rhamnosus* (PTCC 1637), *S. mutans* (PTCC 1683), and *C. albicans*. These established terminology bacterial strains were purchased from the Iranian Biological Resource Center-Persian Type Culture Collection (IBRC-PTCC).

Antibacterial activity of Eucalyptus essential oil

Agar disk diffusion: The antibacterial activity of *E. camaldulensis* essential oil was evaluated using agar disk diffusion method. The standard 0.5 McFarland microbial suspension was prepared and cultured on the blood agar medium. The antimicrobial susceptibility disks with different concentrations of *E. camaldulensis* essential oil (50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%) were used. The antibiotic disks include florfenicol (30 µg), enrofloxacin (5 µg), amoxicillin (25 µg), and penicillin (6 µg) were used as positive controls. Also, a disk containing

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the solvent of essential oil (distilled water) were considered as negative control. The inhibition zone diameter was evaluated after 48 hours incubation at 37°C.

Broth micro-dilution: The antibacterial activity of *E. camaldulensis* essential oil was also evaluated using broth micro-dilution method. The different concentrations of *E. camaldulensis* essential oil (50%, 25%, 12.5%, 6.25%, 3.12%, and 1.56%) were prepared using sterile Brain Heart Infusion medium. The standard 0.5 McFarland microbial suspension was prepared and cultured in the Brain Heart Infusion medium. The cultured bacterial strains without *E. camaldulensis* essential oil was considered as positive controls. Also, Brain Heart Infusion medium without the bacterial strains were considered as negative controls. After 24 hours incubation at 37°C, the least concentration of *E. camaldulensis* essential oil without opacity was considered as the Minimum Inhibitory Concentration (MIC). After culture treated strains on blood agar medium, the minimum concentration without bacterial growth was considered as the Minimum Bactericidal Concentration (MBC).

Study of Eucalyptus essential oil constituents

The gas chromatograph (Shimadzu-QP2010, Japan) with ZB-WAX column (length 20 m, inner diameter 0.18 mm, thickness 18.1 µm) were used to identify the compounds of the Sumac fruit essential oil. The essential oil of Sumac fruit was diluted with normal hexane and 1 µl was injected into gas chromatography/mass spectrometry (GC/MS). The initial temperature of the oven was 50°C, maintained at this temperature for 5 minutes (thermal gradient: 3°C per minute) and then the temperature was increased to 240°C. The final temperature of the oven was 300°C and maintained at this temperature for 3 minutes (thermal gradient: 3°C per minute). The temperature of the injector was 300°C and split/split less (1 to 50). Helium (99.9999%) was used as the carrier gas at a flow rate of 1ml/min. Then, mass spectrometry (Agilent 5973, USA) (length 20 m, inner diameter 0.25 µm, thickness 0.25 mm) was used. The temperature of the ionization chamber was 150°C, the temperature of the detector was 230°C, the ionization energy was 70 eV and the mass analyzer was Quadrupole. The scan mass range was 40 m/z to 550 m/z. The mass spectrometry was used to determine the compounds of the essential oil of Sumac fruit. The spectral values were compared with Kovatz index values in the standard tables and the compounds of the essential oil of Sumac fruit were identified according to data and information available in the GC-MS library.

Results

Antimicrobial activity of Eucalyptus essential oil

Agar disk diffusion: The obtained results showed that the largest growth inhibition zone was related to the *L. rhamnosus* (21.3 mm), *A. viscosus* (19.7 mm) and *S. mutans* (18.8 mm). Moreover, the growth inhibition zone of *C. albicans* was 21.5 mm. The *E. camaldulensis* essential oil showed a larger growth inhibition zone than the amoxicillin. The enrofloxacin created the largest inhibition zone in the studied bacterial strains (Table 1).

Broth micro-dilution: According to the obtained results, the L. rhamnosus, A. viscosus and S. mutans showed a high sensitivity (MIC=1.56% and MBC=3.12%) to E. camaldulensis essential oil. Also, the C. albicans showed a same sensitivity to E. camaldulensis essential oil.

Compounds of Eucalyptus essential oil

According to the obtained results, 34 compound were identified in the E. camaldulensis essential oil, which was 84.67% of the essential oil. The dominant chemical composition found in the E.

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camaldulensis essential oil was 1,8-cineole (36.62%) (Table 2).

Table 1. Inhibition zone diameter of *E. camaldulensis* essential oil on dental caries pathogens.

Bacterial strain	Inhibition zone diameter (mm)				
	Essential oil	Penicillin	Enrofloxacin	Amoxicillin	Florfenicol
Actinomyces viscosus	21.3	18.3	17.0	17.3	22.6
Lactobacillus rhamnosus	19.7	16.0	15.6	15.6	17.6
Streptococcus mutans	18.8	21.6	23.3	18.3	27.3
Candida albicans	21.5	5.5	7.2	4.3	6.8

Table 2. The obtained compounds of *E. camaldulensis* essential oil using GC/MS.

No.	Compounds	Frequency	No.	Compounds	Frequency
1	1,8-cineole	36.62%	18	Myrtenol	0.37%
2	α-pinene	11.17%	19	Spathulenol	0.33%
3	γ-terpinene	9.76%	20	Bicylogemacrene	0.32%
4	β-pinene	7.76%	21	Borneol	0.27%
5	Trans-pinocarveol	7.12%	22	3-methylbutanal	0.22%
6	Allo-aromadendrene	2.07%	23	Terpinolene	0.22%
7	P-cymene	1.55%	24	Linalool	0.21%
8	Epiglobulol	1.03%	25	α-fenchyl alcohol	0.20%
9	Ledol	0.78%	26	Caryophyllene oxide	0.19%
10	Viridiflorol	0.69%	27	Epi-globulol	0.18%
11	Myrtenal	0.67%	28	Cis-carveol	0.18%
12	Camphene	0.63%	29	Cryptone	0.17%
13	δ-terpinene	0.54%	30	Piperitone	0.15%
14	Isoamyl isovalerate	0.43%	31	Trans-carveol	0.14%
15	Eremophilene	0.42%	32	T-muurolol	0.10%
16	γ-cadinene	0.38%	33	Carvacrol	0.10%
17	α-gurjunene	0.38%	34	α-cadinol	0.10%

Discussion

Recently, secondary metabolites of medicinal plants have been studied for their antimicrobial effects (15), and it has been reported that most of the herbs have antifungal, antiphrastic, antibacterial and antiviral properties (16). Therefore, plant extracts have been widely used in the pharmacology, herbal pharmacology, medical and clinical microbiology, phytopathology and food preservation (17). Traditional herbal medicine has been used for treatment of various diseases for several centuries in many parts of the world, and these antibacterial agents have revolutionized the treatment of various bacterial infections (18). In this study, antibacterial and antifungal activity of *E. camaldulensis* essential oil on the most important oral pathogens such as *S. mutans*, *L. rhamnosus* and *A. viscosus* as well as *C. albicans* fungi were investigated by agar disk diffusion and broth micro-dilution methods.

The obtained results showed that the largest inhibition zone was related to *A. viscosus* (21.3 mm diameter) and *C. albicans* (21.5 mm diameter). In addition, the *E. camaldulensis* essential oil created a larger inhibition zone than the amoxicillin antibiotic in the studied strains. Many studies have investigated the antimicrobial activity of medicinal plants on various gram-negative and gram-positive bacteria and fungi. In a study by Mota et al. reported that the largest diameter of inhibition zone (90 mm) due to Eucalyptus essential oil was related to gram positive *S. aureus* (19). In another study by Nahaei et al. reported that the largest diameter of inhibition zone (22 mm) due to Eucalyptus essential oil related to *S. aureus* (20). According to the obtained results in present study and also mentioned studies it can be said that the essential oil of

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Eucalyptus has an appropriate antibacterial activity against Gram positive and Gram negative bacteria as well as pathogenic fungi.

The obtained results from GC-MS assay showed that the dominant chemical composition of *E. camaldulensis* essential oil was 1,8-cineole (36.62%). Previous studies reported that 1,8-cineole has an antimicrobial activity on various pathogenic bacterial strains. The presence of this compound in the phytochemicals of Eucalyptus essential oil can be a reason for the inhibitory potency of this essential oil on different bacterial strains (24). The presence of 1,8-cineole in the phytochemicals of the essential oils of various medicinal plants can cause its antimicrobial activity against different bacterial strains (25). Phytochemical studies on essential oils of medicinal plants such as *Salvia leriifolia* (*S. leriifolia*) indicate the presence of 1,8-cineole, and its antimicrobial activity indicates their inhibitory effect on Gram-positive and Gram-negative bacteria (26). Therefore, it can be said that this inhibitory activity can be involved with this chemical compound.

Conclusion

The results of this study showed that the essential oil of Eucalyptus can have inhibitory effects on bacterial strains (*S. mutans*, *A. viscosus* and *L. rhamnosus*) and fungi (*C. albicans*). Therefore, according to the herbal and native origin of this drug, and less side effects in compared to other chemical compounds and antibiotic, it can be used in pharmaceutical industry to production of antibacterial, disinfectants and mouthwashes drugs to control of infectious diseases and dental caries.

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