Evaluation of chemical composition and antimicrobial activities of *Scrophularia striata* essential oil on dental caries pathogens

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Abstract

Introduction: Oral diseases are among the most important worldwide infectious diseases. Due to drug resistance and the side effects of chemical drugs, the use of herbal medicines has increased. *Scrophularia striata* (*S. striata*) *is* a herbal flowering plant that is used in microbial infections. Therefore, this study aimed to evaluate the antimicrobial activity of *S. striata* essential oil on dental carrier's pathogens.
Materials and Methods: In this study, *S. striata* essential oil was prepared and its antimicrobial activity was evaluated by disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) methods on dental caries pathogens *Streptococcus mutans* (*S. mutans*), *Lactobacillus rhamnosus* (*L. rhamnosus*), *Actinomyces viscosus* (*A. viscosus*), and *Candida albicans* (*C. albicans*). Moreover, the chemical composition of *S. striata* essential oil was evaluated by gas chromatography-mass spectrometry (GC-MS) method.
Results: Our results showed that the most antibacterial activity of *S. striata* essential oil was related to *A. viscosus* (22.9 mm), *L. rhamnosus* (21.7 mm), and *S. mutans* (16.9 mm) essential oil showed a low antifungal activity against *C. albicans*. The dominant chemical composition of *S. striata* essential oil was terpens (39.8%).
Conclusion: In general, *S. striata* essential oil has an appropriate 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: Dental caries Antimicrobial, *Scrophularia striata*, Essential oil

Introduction

On the report of the World Health Organization (WHO), oral diseases are regarded as a widespread and non-communicable disease (1). Different kinds of microorganisms have a role in causing oral diseases like dental caries and periodontal disease (2). Dental caries is the most common oral disease in industrialized countries which affects 60-90% of children and the majority of adult and streptococci, lactobacilli, and Actinomyces are the main causes of dental caries (3). A variety of chemical compounds are commercially available to prevent and treat dental caries, but these cause changes in the oral microbiome and cause undesirable side effects such as diarrhea, vomiting, and discoloration of the teeth (4). The use of natural compounds for the prevention, control, and treatment of dental caries has been considered (5). The side effects of herbal medicine are considerably lower than chemical drugs and extreme use of antibiotics and chemical antibacterial compounds lead to antibiotic resistance (6). In recent years, the extract and essential oil of a large number of natural compounds have been used in cosmetic and hygienic industries dues to their antimicrobial and antioxidant properties (7).

Scrophularia striata (*S. striata*) is a member of the Scrophulariaceae family and mostly used as a medicinal herb. Scrophulariaceae family are nearly 220 genera and 3000 species which grow in most parts of Iran, Turkey, and Azerbaijan (8). The member of this family has been shown many biological effects including antimicrobial, antiviral, and anti-inflammatory properties (9). The *S. striata* contain cinnamic acid, flavonoids (quercetin), isorhamnetin-3-O-rutinoside, nepitrin, and phenylpropanoid glycoside which found in various parts of the plant (10). The various forms of *S. striata* have been used for the treatment of allergies, rheumatics, and chronic inflammatory diseases for a long time. Its extract can be used as an antibacterial against and has a role in decreasing edema, cell infiltration, and proliferation of activated T-lymphocytes in joint tissues (11). Also, it acts as an inhibitor of some inflammatory factors, and several studies have shown that their anti-inflammatory properties are due to the presence of iridoids and phenylpropanoids (12, 13).

According to the antimicrobial properties of *S. striata*, it can be effective in the treatment of oral infections. Therefore, this study aimed to investigate the antimicrobial effects of *S. striata* essential oil on dental carrier's pathogens *Streptococcus mutans* (*S. mutans*), *Lactobacillus rhamnosus* (*L. rhamnosus*), *Actinomyces viscosus* (*A. viscosus*), and *Candida albicans* (*C. albicans*) and evaluate its chemical composition.

Materials and Methods

Preparation of Essential Oil

The *S. striata* was collected from medical plants centers in Tabriz city and identified and approved bythe Herbarium of the Islamic Azad University, Tabriz Branch. The 100 gr *S. striata* was dried in a dark place and then powdered. The obtained powder was added to a balloon containing 600 ml of distilled water. The essential oil was obtained using a Clevenger apparatus (Zarin Pyrex, Iran) and sterilized using a 0.4 μg syringe filter. The prepared essential oil was stored at 4°C until required.

Preparation of Bacterial Strains

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

Evaluation of Antibacterial Activity

Agar Disk Diffusion: The agar disk diffusion method is used to evaluate the antibacterial activity of *S. striata* essential oil. First, the standard 0.5 McFarland microbial suspension was prepared and cultured on blood agar medium (Merck, Germany). The antimicrobial susceptibility disks containing different concentrations of *S. striata* 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 considered as positive control and a disk containing the solvent of essential oil (distilled water) were used as a negative control. In the end, the inhibition zone diameter was assessed after 48 hours' incubation at 37°C.

Broth Micro-Dilution: The antibacterial activity of *S. striata* essential oil was also evaluated using broth microdilution method. The different concentrations of *S. striata* essential oil (50%, 25%, 12.5%, 6.25%, 3.12%, and 1.56%) were prepared using sterile Brain Heart Infusion (BHI) medium (Merck, Germany) in sterile tubes. The microbial suspensions with standard 0.5 McFarland concentration were prepared and then cultured in the BHI medium. The cultured bacterial strains without *S. striata* essential oil was considered as positive controls and BHI medium was considered as negative controls. The prepared tubes were incubated at 37°C for 24 hours and the least concentration of *S. striata* essential oil without opacity was considered as the Minimum Inhibitory Concentration (MIC). After treated strains were cultured on blood agar medium, the minimum concentration without bacterial growth was assumed as the Minimum Bactericidal Concentration (MBC).

Evaluation of Essential Oil Compounds

The gas chromatograph (Shimadzu-QP2010, Japan) with the ZB-WAX column (length 20 m, inner diameter 0.18 mm, thickness 18.1 µm) was used to identify the compounds of the *S. striata* essential oil. The essential oil of *S. striata* 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/splitless (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 *S. striata*. The spectral values were compared with Kovatz index values in the standard tables and the compounds of the essential oil of *S. striata* were identified according to data and information available in the GC-MS library.

Results

Antibacterial Activity

Agar Disk Diffusion: The obtained results showed that the largest growth inhibition zone was related to the *A. viscosus* (22.9 mm), *L. rhamnosus* (21.7 mm), and *S. mutans* (16.9 mm). Moreover, the growth inhibition zone of *C. albicans* was 5.1 mm. The *S. striata* essential oil showed a larger growth inhibition zone than the penicillin, enrofloxacin, florfenicol. The Amoxicillin created the largest inhibition zone in the studied bacterial strains (Table 1).

Broth Micro-dilution: The obtained results showed that the *A. viscosus*, *L. rhamnosus*, and *S. mutans* showed a high sensitivity (MIC=1.56% and MBC=3.12%) to *S. striata* essential oil. Also, the *C. albicans* showed a high resistance to *S. striata* essential oil.

Chemical Composition

According to the obtained results, 26 compound were identified in the *S. striata* essential oil, which was 88.4% of the essential oil. The dominant chemical composition found in the *S. striata* essential oil was terpens (39.8%) (Table 2).

Table 1. Inhibition zone diameter of *S. striata* essential oil on dental caries pathogens.

Bacterial strains	Inhibition zone diameter (mm)				
	Essential oil	**Penicillin**	**Enrofloxacin**	**Amoxicillin**	**Florfenicol**
A. viscosus	22.9	19.9	19.2	24.3	18.7
L. rhamnosus	21.7	17.8	19.0	18.8	16.6
S. mutans	16.9	16.9	17.1	18.5	16.4
C. albicans	5.1	2.7	5.4	3.8	3.5

Table 2. The obtained compounds of *S. striata* essential oil using GC/MS.

No.	Compounds	Frequency	No.	Compounds	Frequency
1	Terpens	39.8	14	Tetradecanedioic	0.8
2	p-Cymene	14.5	15	α-Thujene	0.8
3	Palmitic acid	9.4	16	α-Phellandrene	0.7
4	Saturated fatty	6.7	17	β-Myrcene	0.7
5	Thymol	1.7	18	α-Pinene	0.6
6	Carvacrol	1.2	19	2-Decanol	0.5
7	Linalool	1.6	20	Benzyl benzoate	0.5
8	B-caryophyllene	1.6	21	trans-Nerolidol	0.5
9	β-Elemene	1.3	22	Phytol	0.5
10	p-Xylene	1.1	23	Isopulegol	0.4
11	2-Undecanone	1.0	24	Z-β-Damascenone	0.3
12	δ-Terpinene	0.9	25	Camphene	0.3
13	α-Terpineol	0.8	26	E,Z-Farnesol	0.2

Discussion

Recently, secondary metabolites of medicinal plants have been studied for their antimicrobial effects (14), and it has been reported that most of the herbs have antifungal, antiphrastic, antibacterial and antiviral properties (15). Therefore, plant extracts have been widely used in the pharmacology, herbal pharmacology, medical and clinical microbiology, phytopathology and food preservation (16). 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 (17). In this study, antibacterial and antifungal activity of *S. striata* 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* (22.9 mm diameter) and *C. albicans* (5.1 mm diameter). In addition, the *S. striata* essential oil created a larger inhibition zone than the Enrofloxacin, Penicillin, and Florfenicol 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 Safavi et al. reported that the largest diameter of inhibition zone (12 mm) due to *S. striata* essential oil was related to gram positive *S. aureus* (18). In another study by Moori Bakhtiari et al. reported that the largest diameter of inhibition zone (26 mm) due to *S. striata* essential oil related to *S. aureus* (19). According to the obtained results in present study and also mentioned studies it can be said that the essential oil of *S. striata* 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 *S. striata* essential oil was terpens (39.8%). Previous studies reported that terpens has an antimicrobial activity on various pathogenic bacterial strains. The presence of this compound in the phytochemicals of *S. striata* essential oil can be a reason for the inhibitory potency of this essential oil on different bacterial strains (20). The presence of terpens in the phytochemicals of the essential oils of various medicinal plants can cause its antimicrobial activity against different bacterial strains (21). Phytochemical studies on essential oils of medicinal plants indicate the presence of terpens, and its antimicrobial activity indicates their inhibitory effect on Gram-positive and Gram-negative bacteria (22). 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 *S. striata* 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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