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Antimicrobial Activity of the Essential Oil Obtained from the Seed and Oleo-Gum-Resin of *Ferula assa-foetida* Against Oral Pathogens

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A B S T R A C T

Objectives: The objective of this study was to evaluate the antimicrobial activity of the essential oil obtained from oleo-gum-resin and seeds of Ferula assa-foetida.

Materials and Methods: Ferula assa-foetida plants were collected from Tabas, Yazd Province, Iran, during summer 2017. Then, essential oils were obtained from its seeds and oleo-gum-resin using hydrodistillation. Gas chromatography-mass spectrometry (GC-MS) test was performed to determine the contents of the essential oils. Four different concentrations of each oil were prepared (2.5, 5, 10, and 20 µg/ml), and the antimicrobial activity of each dose against four oral bacteria (*Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguis, Streptococcus salivarius, and Lactobacillus rhamnosus*) was evaluated using the disk diffusion method. The data were analyzed using analysis of variance (ANOVA) and Kruskal-Wallis test in SPSS 17 software.

Results: The GC-MS findings exhibited that the main compounds found in essential oils yielded from the seeds and oleo gum resin were (Z) -1-propenyl sec-butyl disulfide and (E) -1-propenyl sec-butyl disulfide. Ferula assa-foetida plant showed a significant antimicrobial effect ($P<0.05$). The essential oil from Ferula assa-foetida oleo-gum-resin had significantly stronger antibacterial properties compared to the essential oil from Ferula assa-foetida seeds ($P<0.001$). Both essential oils showed antibacterial properties similar to that of Chlorhexidine. The growth inhibition zone was significantly dependent on the essential oil concentration for all bacteria ($P<0.05$).

Conclusion: Our study revealed that essential oils from seeds and oleo-gum-resin of Ferula assa-foetida have antimicrobial properties. More laboratory studies are required to reach a definitive conclusion.

Keywords: Ferula foetida; *Streptococcus mutans*; *Streptococcus sanguis*; *Streptococcus salivarius*; *Streptococcus sobrinus*; *Lactobacillus rhamnosus*

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INTRODUCTION

Dental caries is the main reason for tooth loss and dental infections. This disease, which is caused by several factors [1], begins with the accumulation of plaque on dental surfaces [2]. Poor oral health leads to an increase in the

accumulation of microorganisms and their derivatives. *Streptococcus mutans* (*S. mutans)* is the main bacterium found in dental plaque, which is colonized at the age of 6 to 9 months in the human's mouth [2,3] and causes damage to hard dental tissues through the fermentation of sucrose and the production of lactic acid [4]. This bacterium exploits sucrose to form dental plaque. The produced acid demineralizes hard dental tissues, while other bacteria in the *Streptococcaceae* family, such as *S. sobrinus, S. sanguis, and S. salivarius*, as well as *Lactobacilli*, can grow in an environment made by *S. mutans* and contribute to the development of tooth decay [5-7]. The nature of dental plaque is such that the plaque must be controlled regularly and effectively [8,9]. One of the methods for controlling the plaque is the use of mouthwashes [10]. Mouthwashes are particularly beneficial for patients with particular diseases, the elderly with diseases leading to weakening of the hand muscles, or people at risk of microbial infection, such as those susceptible to endocarditis or immune system weakness [11,12]. Mouthwashes can be taken daily and weekly [13]. Considering the increasing use of mouthwashes, it is essential to have access to substances with minimum side effects (pigment formation on dental surfaces, infections, and tissue toxicity) and maximum beneficial effects (plaque control) [14,15].

The scientific implementation of medicinal herbs has become possible with the advancement of chemistry and pharmacy. Nowadays, scientists prefer herbal remedies in many cases. Ferula assa-foetida is one of the plants belonging to the *Apiaceae* family [16,17]. This plant is under cultivation in different parts of the world, including the central and southern regions of Iran, and has been used in different countries [18]. Various studies have pointed to the antioxidant [19], antifungal [20,21], antiviral [22,23], anti-diabetic, and antihypertensive effects of this plant [24,25]. One of the important properties of this plant is its antimicrobial effect, which is a controversial issue. Fani et al [26] suggested that this plant is not effective against *S. mutans*, while studies by Rahman et al [27], Kavoosi et al [19], Kavoosi and Rowshan [28], and Haghighati et al [29] have reported the antimicrobial role of this plant against other bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. The discrepancies among previous studies encouraged us to design a study aiming at investigating the antimicrobial effect of essential oils obtained from seeds and oleo-gum-resin of Ferula assa-foetida on oral pathogens including *S. mutans, S. sanguis, S. salivarius, S. sobrinus,* and *Lactobacillus rhamnosus* (*L. rhamnosus*).

MATERIALS AND METHODS

Preparation of oleo-gum-resin essential oil:

Ferula assa-foetida species, gathered from Tabas, Yazd Province, Iran during summer, was identified botanically by the Yazd Agricultural Research Center. The voucher number of the specimen was 2365. Then, 100 g of oleo-gum-resin was dissolved in one liter of distilled water to extract assa-foetida essential oil using hydrodistillation in the Clevenger device for 3 hours. After drying over anhydrous sodium sulfate, the attained essential oil was kept at 4°C until experimentation [30]. Finally, four different concentrations (2.5, 5, 10, and 20 µg/ml) of essential oil obtained from oleo-gum-resin were prepared.

Preparation of essential oil from Ferula assa-foetida seeds:

First, 200 g of Ferula assa-foetida seeds were powdered and poured into 500 ml of double-distilled water. The hydrodistillation technique using the Clevenger device was employed to extract the seed essential oil which was protected from the light by being kept in glass containers hermetically sealed with rubber lids and covered with aluminum foil at 4ºC.

Disk diffusion method for antimicrobial screening:

The antimicrobial screening by seed and oleo-gum-resin essential oils of Ferula assa-foetida was conducted on standard strains of *S. mutans* (PTCC1683), *S. sanguis* (PTCC1449), *S. sobrinus* (PTCC1601), *S. salivarius* (PTCC1448), and *L. rhamnosus* (PTCC1637) which were prepared from the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The antimicrobial screening was performed using the standard disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2017 protocols [30]. Fresh *Streptococcus* colonies were cultured on blood agar supplemented with 5% defibrinated sheep blood agar medium (Merck KGaA, Darmstadt, Germany), while De Man, Rogosa, and Sharpe (MRS) agar medium (Merck KGaA, Darmstadt, Germany) was used to culture *L.*

rhamnosus for inoculum. A turbidity equivalent to that of 0.5 McFarland turbidity standard, holding 1.5×10^8 colony-forming units per milliliter (CFU/ml), was adjusted by inoculating fresh colonies into 5 ml of Mueller-Hinton broth (Merck KGaA, Darmstadt, Germany) followed by inoculation of *Streptococci* and *L. rhamnosus* using a sterile swab on Mueller-Hinton agar enriched with 5% defibrinated sheep blood agar medium (Merck KGaA, Darmstadt, Germany) and on MRS agar, respectively. Sterilized 6-mm blank paper disks (Padtan Teb Co., Tehran, Iran) were individually impregnated with 10 µl of different concentrations (2.5, 5, 10, and 20 µg/ml) of the essential oils, which were then cultured aseptically on a Mueller-Hinton agar and were incubated at 35ºC with 5% carbon dioxide (CO_2) for 48 hours. Afterward, the diameter (mm) of the non-growth zone was measured triplicate by a ruler to calculate the mean diameter. The controls consisted of disks impregnated with 0.2% Chlorhexidine and sterilized distilled water.

Gas chromatography-mass spectrometry (GC-MS) analysis:

The Hewlett-Packard 5971 GC-MS device (Avondale, PA, USA), available at the Islamic Azad University, Isfahan (Khorasgan) Branch, was used for analyzing the chemical composition of the essential oils with the following settings: 0.25 mm × 30 m poly-dimethylsiloxane DB-1 fused silica capillary column, 0.10-µm film thickness, 1-ml/minute carrier gas of helium, injector temperature of 250ºC, and detector temperature of 200ºC. The column temperature was set variable from 35°C/minute to 180°C/minute at 4°C V/minute followed by 180°C/minute to 280°C/minute at 20°C V/minute. The electronic impact of 70 eV was considered for the mass spectra. A library computer search introduced the ingredients with their retention indices and visual interpretation of the mass spectra [31].

Statistical Analysis:

The data were analyzed using analysis of variance (ANOVA) and Kruskal-Wallis test in SPSS 17 software (SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered as statistically significant.

RESULTS

Chemical compositions:

The GC-MS findings exhibited that the main compounds found in the essential oil yielded from seeds of Ferula assa-foetida are alpha-D-Xylofuranoside and methyl 2, 5-di-O-methyl- (30.2%), (E) -1-propenyl sec-butyl disulfide (13.13%), and (Z) -1-propenyl sec-butyl disulfide (11.34%; Table 1). In addition, the essential oil obtained from Ferula assa-foetida oleo-gum-resin mainly contained (E) -1-propenyl sec-butyl disulfide (36.15%) and (Z) -1-propenyl sec-butyl disulfide (27.93%; Table 2).

Table 1: Chemical composition of essential oil obtained from seeds of Ferula assa-foetida

No.	Composition	%
1	Alpha.-D-Xylofuranoside, methyl 2,5-di-O-methyl-	30.2
2	E-1-propenyl *sec*-butyl disulfide	13.13
3	Z-1-propenyl *sec*-butyl disulfide	11.34
4	Trifluoromethyl t-butyl disulfide	6.33
5	Disulfide, bis(1-methylpropyl)	5.47
6	10-epi-.gamma.-eudesmol	4.37
7	3-Mercapto propionitrile	3. 28
8	Agarospirol	3.50
9	Ethanethioamide	2.90
10	Methyl sec-butyl disulphide	2.75
11	5-epi-7-epi-α-Eudesmol	2.62
12	1-(Methylthio) propyl propyl disulfide	2.53
13	(-)-Aristolene	1.41
14	(Z)-1-(But-2-en-1-yl)-2-(sec-butyl)disulfane	1.30
15	.alpha.-Pinene	1.80
16	3-thione-1,2-Dithiole	1.90
17	N-propyl sec-butyl disulfide	0.44
18	4-(hexadecyloxy)-3-nitrobenzenesulfonyl fluoride	0.39
19	Thiopivalic acid	0.36
20	Benzenepropanoic acid, pentyl ester	0.33
21	2-Thiazolidinethione	0.27
22	Morpholine, 2,6-dimethyl-	0.25
23	Elemol	0.17
24	Dimethyl trisulfide	0.17
Total		94.03

Antibacterial activity:

The antibacterial activity of essential oils yielded from seeds and oleo-gum-resin of Ferula assa-foetida plant was assessed by measuring the average growth inhibition zone. In the current study, the antimicrobial effects of four different concentrations (2.5, 5, 10, and 20 µg/ml) of essential oils obtained from seeds and oleo-gum-resin were evaluated. The results indicated that both essential oils had significant antimicrobial activity at all concentrations ($P<0.05$). Both essential oils showed

Table 2: Chemical composition of essential oil obtained from oleo-gum-resin of Ferula assa-foetida

No.	Composition	%
1	E-1-propenyl *sec*-butyl disulfide	36.15
2	Z-1-propenyl *sec*-butyl disulfide	27.93
3	Guaiol	5.50
4	Carotol	5.14
5	bis (1-methyl propyl) disulfide	3.17
6	α-gurjunene	2.49
7	bis [(1-methylthio) propyl] disulfide	1.12
8	α- longipinene	1.86
9	Methyl penthyl tetrasulfide	1.16
10	Eudesmol(10-epi-gama)	0.98
11	Propyl *n*-butyl disulfide	0.97
12	δ- cadinene	0.95
13	Germacrene B	0.90
14	Methyl 1-(methylthio) ethyl disulfide	0.87
15	E-β-ocimene	0.70
16	Methyl 1-(methylthio) propyl disulfide	0.66
17	β- humulene	0.47
18	β- himachalene	0.44
19	Valencene	0.36
20	Methyl sec-butyl disulfide	0.33
21	Eudesmol(7-epi-alpha)	0.33
22	Patchouli alcohol	0.29
23	α- humulene	0.28
24	Cedrene	0.24
25	α- ylangene	0.22
26	Longifolene	0.20
27	α- copaene	0.19
28	E-α- bisabolene	0.18
29	Cuparene	0.17
30	Neryl acetate	0.16
31	Spathulenol	0.15
32	allo-aromadendrene	0.13
32	β-caryophyllene	0.12
34	Limonene	0.12
35	Z- β-ocimene	0.12
36	p-cymene	0.10
37	Germacrene D	0.07
Total		95.22

antibacterial properties similar to that of Chlorhexidine (Tables 3 and 4).

The average growth inhibition zone was significantly dependent on the essential oil concentration for all bacteria ($P<0.05$) as the 20 µg/ml concentration of both essential oils formed the largest average growth inhibition zone. Furthermore, this study showed that the essential oil from Ferula assa-foetida oleo-gum-resin had significantly stronger antibacterial properties compared to the essential oil from Ferula assa-foetida seeds ($P<0.001$; Tables 3 and 4).

DISCUSSION

Pathogenic microorganisms are one of the main health concerns for human beings. These microorganisms can be controlled easily by several antimicrobial chemicals; however, these chemicals can pose unwanted adverse complications [32]. Accordingly, there is a serious need for a safer therapeutic approach. Medicinal herbs are remarkable candidates and suitable alternatives to allopathic drugs. With the use of medicinal herbs, disadvantages of chemical drugs, such as high treatment costs, side effects, and development of resistance against antibiotics, can be avoided. Green medications have attracted a lot of attention, and the era of synthetic drugs is almost over. Effective ingredients derived from plants have been incorporated into official health care systems [32,33].

Iran is a country with various medicinal plants because of its unique and diverse geographical and climatic conditions; many of these herbs have shown positive therapeutic impacts such as antibacterial activity [34,35].

Chemical compositions:

In the current study, the GC-MS results indicated that the main contents of seed essential oil are Alpha.-D-Xylofuranoside, methyl 2,5-di-O-methyl (30.2%), (E) -1-propenyl sec-butyl disulfide (13.13%), and (Z) -1-propenyl sec-butyl disulfide (11.34%). The contents of the essential oil obtained from oleo-gum-resin are (E) -1-propenyl sec-butyl disulfide (36.15%) and (Z) -1-propenyl sec-butyl disulfide (27.93%; Table 1).

These results are similar to those reported by Kavoosi et al [19], Khajeh et al [36], and Yousefi et al [37] although the levels of the compounds are slightly different. In the study by Kavoosi et al [19], the major components included (E) -1-propenyl sec-butyl disulfide (23.9%), 10-epi-c-eudesmol (15.1%), (Z) -1-propenyl sec-butyl disulfide (8.0%), (Z) -b-ocimene (5.6%), and a-eudesmol (4.5%). In the study by Yousefi et al [37], the main compounds consisted of (E) -1-propenyl sec-butyl disulfide (50%), (Z) -1-propenyl sec-butyl disulfide (9.4%), glubolol (12.5%), and a-pinene (8.8%). In the study by Khajeh et al [36], the major components were (E) -1-propenyl sec-butyl disulfide (40%), (Z) -

Table 3: Antibacterial effect of Ferula assa-foetida oleo-gum-resin essential oil on different bacteria based on inhibition zone diameter (mm), using the disk diffusion method

Species	Concentrations (µg/ml)*				Chlorhexidine 0.2%	P-value
	2.5	5	10	20		
S. mutans	13.5±0.10	13.03±0.58	14.07±0.58	15.1±0.10	15±0.00	0.009
S. sanguis	7.07±0.98	12.0±0.00	12.17±0.15	13.0±0.00	15.53±0.57	0.009
S. sobrinus	9.10±0.10	11.2±0.20	11.6±0.62	12.13±0.58	15.07±0.12	0.011
S. salivarius	13.07±0.57	12.08±0.58	13.14±0.15	14.03±0.20	15.07±0.57	0.009
L. rhamnosus	12.3±0.10	13.16±0.15	16.03±0.57	18.1±0.10	14.1±0.10	0.009

*The inhibition zone diameters (mm) were measured triplicate to report the mean values. Chlorhexidine was the positive control in this test as the reference antimicrobial agent.

Table 4: Antibacterial effect of essential oil obtained from seeds of Ferula assa-foetida on different bacteria based on inhibition zone diameter (mm), using the disk diffusion method

Species	Concentrations (µg/ml)*				Chlorhexidine 0.2%	P-value
	2.5	5	10	20		
S. mutans	8.87±0.21	10.87±0.21	12.07±0.11	13.17±0.15	15±0.00	0.009
S. sanguis	6.94±0.11	7.93±0.30	9.90±0.2	11.94±0.20	15.53±0.57	0.009
S. sobrinus	7.93±0.15	10.16±0.15	11.03±0.2	10.06±0.11	15.07±0.12	0.013
S. salivarius	7.97±0.38	9.0±0.10	10.04±.058	12.0±0.17	15.07±0.57	0.012
L. rhamnosus	6.10±0.10	7.13±0.15	8.43±0.20	8.8±0.10	14.1±0.10	0.011

*The inhibition zone diameters (mm) were measured triplicate to report the mean values. Chlorhexidine was the positive control in this test as the reference antimicrobial agent

1-propenyl sec-butyl disulfide (8.7%), germacrene B (7.8%), and a-pinene (5.9%). It should be noted that the current study was the first to evaluate the contents of Ferula assa-foetida seeds using the GC-MS, while other studies have only assessed the contents of oleo-gum-resin using the GC-MS.

This difference in the major compounds of Ferula assa-foetida essential oils can be related to the climatic conditions, the species used, the harvest time, and the method of processing.

Antibacterial activity:

In the current study, antimicrobial effects of four different concentrations (2.5, 5, 10, and 20 µg/ml) of essential oils obtained from seeds and oleo-gum-resin of Ferula assa-foetida on four oral bacteria were evaluated. The results indicated that all the concentrations had significant antimicrobial effects (Tables 3 and 4). Also, this study showed that this effect can be similar to that of Chlorhexidine. These results are similar to the results reported by Kavoosi and Rowshan [28] and Siddiqui et al [38] although these two studies have evaluated the antimicrobial effect of essential oil obtained from Ferula oleo-gum-resin on *Staphylococcus aureus*, *Bacillus Subtilis,* and *Escherichia Coli*. Both studies used the minimum inhibitory concentration (MIC) method, whereas the disk diffusion method was used in the current study.

The results of the current study are not similar to the results of the study by Fani et al [26], which showed that Ferula assa-foetida does not have any antimicrobial effect on *S. mutans* and *S. sanguis*.

The cited study evaluated aqueous and ethanolic extracts of Ferula, while the current study evaluated essential oils obtained from Ferula assa-foetida seeds and oleo-gum-resin. The mentioned study used the well diffusion method and MIC, while the current study implemented the disk diffusion method. The cited study did not consider any of the concentrations effective on these two bacteria, while in the current study, the lowest assessed concentration (2.5 µg/ml) resulted in a growth inhibition zone with a diameter of 8.87±0.21 mm with seed essential oil and a diameter of 13.5±0.1 mm with oleo-gum-resin essential oil against *S. mutans*. It seems that the difference in the results is caused by different extracts of Ferula assa-foetida and the method used for measuring the antimicrobial effect. Kavoosi and Rowshan [28] investigated the antioxidant and antibacterial activities of Ferula assa-foetida

and showed that this plant has inhibitory effects on the growth of gram-positive bacteria ($P<0.05$). Siddiqui et al [38] reported dose-dependent antimicrobial effects of Ferula assa-foetida essential oil on gram-positive bacteria; the 50 µg/ml concentration had no significant antibacterial effect compared to standard antibiotics, whereas a concentration of 100 µg/ml was more effective compared to standard antibiotics. This result is in line with the findings of the study by Haghighati et al [29] and our study. Based on the reports by Fani et al [26], there was no significant difference in the antibacterial effects of Ferula assa-foetida and Quercus infectoria extracts when comparing *S. mutans* and *S. sanguis*. According to the antimicrobial properties of essential oils obtained from medicinal plants, phenolic monoterpenes are the most important effective substances that can inhibit bacterial growth due to increased permeability and polarizability of the cell membrane, resulting in microleakage of protons from the cells, electric potential imbalance in the membrane, proton motive force reduction, and decreased adenosine triphosphate (ATP) formation [39]. It should be noted that the consequence of decreased membrane potential is the loss of ions, ATP, amino acids, and proteins following the leakage from the cell [40]. One of the indications of membrane damage and cell death is the ion leakage out of the cell [40]. Extracts of several Ferula species have been shown to have moderate antibacterial activity, probably due to the presence of phenols, flavonoids, and sesquiterpenes. High levels of phenolic and flavonoid compounds are associated with the antibacterial activity [41,42].

The hydrophobicity and solubility of the lipid in the compounds can be the reason for their antibacterial activity. However, there are various mechanisms for the antibacterial features of different essential oils. Extensive diffusion of compounds into the lipid bilayer and increased membrane permeability induced by some substances have revealed potent antibacterial activity. Acyclic sulfur-containing compounds are mainly found in both essential oils [43]. Kavoosi et al [19] reported a more effective antimicrobial activity for cyclic compounds compared to acyclic sulfur-containing compounds. The diffusion into the lipid bilayer and membrane permeability are significantly elevated by cyclic compounds through their higher spatial volume, resulting in high membrane permeability and cell death [43].

Nevertheless, more comprehensive studies are required to draw a definitive conclusion regarding the antibacterial activity of medicinal plants and their effective concentrations. Active collaboration of research institutes in different parts of the world is needed to accelerate the investigations in this regard.

CONCLUSION

The present study revealed that essential oils obtained from seeds and oleo-gum-resin of Ferula assa-foetida show antimicrobial effects against oral pathogens.

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