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Ferulago angulata and *Tetrataenium lasiopetalum*: Essential oils composition and antibacterial activity of the oils and extracts

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ABSTRACT

In the present research, the chemical composition of the essential oils from *Ferulago angulata* and *Tetrataenium lasiopetalum* were analyzed by the gas chromatography-mass spectrometry (GC-MS). The antibacterial activity of the extracts and essential oils of both species was also evaluated against *Staphylococcus aureus* and *Enterococcus faecalis* (Gram-positive bacteria) and *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria), using agar disk diffusion and micro-dilution methods. Our results showed that essential oil from *F. angulata* was a rich source of *cis*-β-ocimene, α-pinene and α-phellandrene and can be used in industries such as pharmaceutical, cosmetic and food. Additionally, *T. lasiopetalum* essential oil had high percentage of camphene, germacrene-D, and 1,8-cineole. Gram positive bacteria were more susceptible than Gram-negative bacteria to all oils and extracts. The results of our study showed that in both species, especially *T. lasiopetalum,* the extract and essential oils exhibited reliable inhibitory activity against both Gram-positive and Gram-negative bacteria and have the potential to be considered as natural alternatives for food preservatives.

1. Introduction

The genus *Ferulago* from the Apiaceae consists of 40 species, eight of which are found in Iran (Davis, 1972; Ghasemi Pirbalouti et al., 2016; Razavi et al., 2015). *Ferulago angulata* (Schlecht) Boiss (known as "Chavir" in Persian) is a perennial shrub with thick rootstock, erect stems, small flowers, and yellow fruits (Mozafarian, 1983). It grows naturally in the high altitudes of the west and central parts of Iran, north of Iraq, and southeast of Turkey. In traditional medicine, *Ferulago* is used as a sedative, tonic, anti-parasitic, remedy for digestive panics and hemorrhoids and aphoristic properties (Oztürk et al., 2004; Sefidkon and Omidbaigi, 2004). Antibacterial and antifungal activities of *Ferulago* spp. have been previously investigated, and their significant inhibitory effects against microorganisms have been reported (Ghasemi Pirbalouti et al., 2016; Shahbazi et al., 2015). The chemical characteristics of essential oils in *F. angulata* have been the subjects of some earlier studies. α-Pinene, *Z*-β-ocimene, bornyl acetate, γ-terpinolene, and sabinene were found to be the main components of volatile oils (Ghasemi Pirbalouti et al., 2016; Razavi et al., 2015; Sefidkon and Omidbaigi, 2004).

Tetrataenium lasiopetalum (Boiss.) Manden (Syn: *Heracleum lasiopetalum*) with the Iranian name of "Golpar-e barfi" or "Karsum'', belonging to the Apiaceae, is widely distributed in the Middle East (Mozafarian, 1983). In the Iranian traditional medicine, leaves and fruits of *T. lasiopetalum* are used for various purposes e.g. antiseptic, carminative, digestive and flavoring agent with applications as food spices as well (Masoudi and Kakavand, 2017; Sonboli et al., 2007). The biological properties and composition of *T. lasiopetalum* essential oil have been reported previously (Sonboli et al., 2007).

Antimicrobial activities of plants have been documented in ancient literature, and continued to the present time (Ghasemi Pirbalouti et al., 2013). Recently, food preservation via natural products, especially with plant-base origins, has become a worldwide trend. This is for two reasons: 1) concerns regarding the safety of synthetic compounds and 2) the increase in microbial resistance against conventional food preservatives (Ozkan et al., 2010; Shetty and Labbe, 1998). This particular attention

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on plants essential oil and extract is mainly a result of a wide customer satisfaction, presence of structurally different active components, and a relatively higher safety for human consumption (Bajpai et al., 2007; Cavar et al., 2008).

As far as we found in the literature, there has been no comprehensive study on the chemical compositions and antimicrobial activities in *F. angulata* and *T. lasiopetalum* grown in the west of Iran. Hence, here we report the volatile oil compositions of *F. angulata* and *T. lasiopetalum* from the west of Iran. Additionally, we evaluate their in-vitro inhibitory effects on four Gram-positive and Gram-negative bacteria.

2. Material and methods

2.1. Plant materials

Shoots of *F. angulata* and *T. lasiopetalum* were gathered from the wild growing plants at the full flowering stage in June 2015 from the west of the country. Voucher specimens (Herbarium numbers: 4175 and 3375 for *F. angulata* and *T. lasiopetalum,* respectively) were deposited at the Herbarium at the Sanandaj's Research Center for Agriculture and Natural Resources. Aerial parts of plants were air-dried at room temperature (20–25 °C) in dark for two weeks.

2.2. Essential oils isolation

Essential oils of each species were separately isolated by hydrodistillation of the air-dried plants material, according to the method of the European Pharmacopeia (Council of Europe, 1997). Afterwards, the extracted oils were dried over anhydrous sodium sulfate and kept in tightly sealed dark vials at 4 °C before analysis. Based on the dry weight of samples, the essential oil content (w/w) of each species was determined.

2.3. Preparation of extracts

Aerial parts of the air-dried plants were chopped up into a fine powder. Then, ethanolic extract of each species were prepared. For each species, 30 g of dried plants were soaked with 300 mL of ethanol. The sample was shaken at room temperature for 72 h, then filtered throughout filter paper (filter paper GF/A, 110 mm; Whatman, Maidstone, UK). The obtained extract was concentrated by evaporation of alcohol using a rotary evaporator at 40 °C. Finally, the residual extract was stored at −20 °C prior to antimicrobial assay (Barros et al., 2007). The extraction process was performed in triplicate for each species.

2.4. Oil analysis procedure

Gas chromatography (GC) analysis of the volatile oils was conducted using an Agilent Technologies-7890 A apparatus equipped with a HP-5MS capillary column (30 m × 0.25 mm i. d., film thickness 0.25 μm) and flame ionization detector (FID). Oven temperature was programmed to increase from 60 to 280 °C at a rate of 4 °C/min and finally held isothermally for 10 min. Injector and detector (FID) temperatures were 250 and 300 °C, respectively. Helium was used as carrier gas at a linear flow rate of 1.1 ml/min. The split ratio was equal to 1/20.

Gas chromatography–mass spectrometry (GC–MS) analysis was performed in an Agilent Technologies-5977 A gas chromatograph equipped with above mentioned column and coupled with an Agilent Technologies-5977 Analyzer. Oven temperature was 60–280 °C at a rate of 4 °C/min; Ion source and interface temperatures were 200 and 250 °C, respectively; carrier gas: He, with an ionization voltage of 70 eV; split ratio was 1/20; Mass range from m/z 43–456. All samples and standards were injected in three independent replicates.

The major components of oils were identified by comparing their mass spectra with those of a computer library (NIST08 and Wiley 9.0) or with those of authentic compounds. Additionally, they were confirmed by comparing their retention indices with those of authentic compounds or with data reported in the literature (Adams, 2007). The retention indices of each compound was calculated under temperature programmed conditions for n-alkanes (C_5–C_{24}) and the oil on a HP-5MS column in the same conditions. For quantification, relative area percentages obtained by GC–FID were used without consideration of the calibration factor.

2.5. In-vitro antibacterial assays

2.5.1. Preparation of bacteria

The in-vitro antibacterial activities of ethanolic extracts and essential oils were assessed on two different Gram-positive bacterial strains: *Staphylococcus aureus* (PTCC1431) and *Enterococcus faecalis* (PTCC 1015) and two different Gram-negative bacterial strains: *Escherichia coli* (PTCC1399) and *Pseudomonas aeruginosa* (PTCC1430). The bacterial strains were purchased as lyophilized vials from the Biotechnology department, Industrial and infectious fungi and bacteria collection center, Scientific research and industry organization, Iran.

2.5.2. Evaluation of the antibacterial activities

The in-vitro antimicrobial activities of ethanolic extracts and essential oils were examined by the disc diffusion test with some modification. Mueller–Hinton agar (Merck, Germany) was used to prepare the culture medium. An initial bacterial suspension culture required for the test was adjusted to 0.5 McFarland standards (containing 107 CFU/ml). Plates were inoculated using swabs soaked by inoculated normal saline. The extracts and essential oils were dissolved in acetone before being tested for antimicrobial activity and series of two-fold dilutions were made in a concentration range from 3.125 to 400 mg/ml for extract, and a range from 0.25 to 64 mg/ml for essential oils. Sterile paper discs of 6 mm diameter were impregnated with 5 mg of each sample (final volume of 20 mL) and then placed onto the inoculated agar surface. After cold treatment for 1.5 h at 4 °C, plates were incubated for 24 h at 37 °C. The diameter of growth inhibition zones was measured using caliper at three directions (Murray et al., 2007). All assays were done three times.

The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of both extracts and essential oils were determined using the broth micro-dilution susceptibility tests according to the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Micro-broth dilution assays were performed using plastic micro-dilution trays. The inoculants of the bacterial strains were provided from freshly cultured bacteria (adjusted to 0.5 McFarland standard turbidity) and then were further diluted (1:100) just before being added to the trays. Dilution series of ethanolic extracts were prepared from 3.125 to 400 mg/ml and dilution series of essential oils were prepared in a concentration range of 0.5–128 mg/ml. Acetone and medium were used as a negative control. Micro-dilution trays were incubated at 37 °C for 24 h (NCCLS, 2000). MBCs were ascertained by sub-culturing of 100 ml from each negative well and from the positive control onto a nutrient agar plate. The lowest concentration of the extracts or essential oils at which bacteria failed to grow visibly in liquid media, was recorded as MICs. The MBCs were described as the minimum concentration that could kill 99.9% of the bacteria. All experiments were done three times.

3. Result and discussion

3.1. Chemical composition of essential oil

Hydrodistillation of the aerial parts of *F. angulata* gave a pale yellow oil with a content of 0.87% (w/w), based on the dry weight of samples. GC–FID and GC–MS analyses of *F. angulata* essential oil resulted in the identification of 14 compounds corresponding to 99.14% of the oil. The essential oil consisted mainly of monoterpene hydrocarbons (91.92%), and oxygenated monoterpenes (6.58%), with a low portion of

sesquiterpenes hydrocarbons (0.64%). The major constituents were *cis*-*β*-ocimene (42.75%), *α*-pinene (13.4%), *α*-phellandrene (10.32%), and *p*-cymene (6.66%) (Table 1).

Our results along with the previous reports (Akhlaghi, 2008; Ghasemi Pirbalouti et al., 2016; Razavi et al., 2015; Sefidkon and Omidbaigi, 2004; Shahbazi et al., 2015), indicate that the volatile oil content in *F. angulata* aerial parts collected from different regions of Iran at flowering stage, is between 0.25% and 1.85%. Similar to what we found, Akhlaghi (2008) reported a high percentage of monoterpene hydrocarbons in the essential oil of *F. angulata.* Earlier studies have also identified *α*-pinene, *cis*-*β*-ocimene, sabinene, *trans*-*β*-ocimene, *α*-phellandrene, *β*-phellandrene, *p*-cymene, bornyl acetate, *γ*-terpinolene, linalool, *γ*-terpinene, and myrcene as the dominant components of oil from the aerial parts in *F. angulata,* from Iran (Akhlaghi, 2008; Ghasemi Pirbalouti et al., 2016; Razavi et al., 2015; Sefidkon and Omidbaigi, 2004; Shahbazi et al., 2015).

The volatile oil extracted from the aerial parts of *T. lasiopetalum* was found to be a yellow liquid with a content of 0.55% (w/w), based on the dry weight of sample. In total, nineteen volatile constituents were identified in the essential oil of *T. lasiopetalum*, representing about 94.54% of the oil, of which the major components were found to be camphene (41.73%), germacrene-D (13.11%), 1,8-cineole (8.1%), octanol acetate (6.22%), and *cis*-*β*-ocimene (5.59%). The main components belonged to monoterpene hydrocarbons (camphene, 1,8-cineole, *Cis*-*β*-ocimene, *α*-pinene, *β*-pinene, and limonene), sesquiterpenes hydrocarbons (germacrene-D and *β*-sesquiphellandrene), and other classes of metabolites (octanol acetate and hexanol) (Table 2).

Previous studies on the volatile oil of the aerial parts in *T. lasiopetalum* found varying compositions of oils with an oil content ranging from 0.03% to 0.35% (Ghasemi Pirbalouti et al., 2013; Sonboli et al., 2007). Concerning the essential oil composition, it is noteworthy that Sonboli et al. (2007) identified germacrene-D, *β*-bisabolene, *α*-zingiberene, *β*-sesquiphellandrene, and trans *α*-bergamotene as the principal components. 2-ethylhexyl acetate, *n*-octanol, and hexanol were also found as the major volatiles of *T. lasiopetalum* by Ghasemi Pirbalouti et al. (2016). While, Masoudi and Kakavand (2017) found germacrene-D and (*E*,*Z*)-farnesol to be the major components of oil in this species. According to the literature, there is a great diversity and variability in the essential oil composition of the both studied species. Genetic factors, seasonal variations, geographical locations and climatic conditions could all be responsible for this variability (Hadian et al., 2011; Shahbazi et al., 2015). Therefore, antimicrobial activity of both species essential oil is subjected to a high degree of variation which is mostly because of the chemical composition diversity (Chorianopoulos et al., 2004).

Table 1
Essential oil constituents of *Ferulago angulata*.

No.	Components	RI [a]	KI[b]	Percentage[c]
1	α -Thujen	931	930	0.71
2	α-Pinene	939	938	13.4
3	Camphene	953	952	2.95
4	Sabinene	976	980	3.37
5	β–myrcene	991	993	4.45
6	α-phellandrene	1005	1009	10.32
7	*p*-cymene	1026	1029	6.26
8	β-Phellandrene	1031	1036	3.76
9	*Cis*-β-Ocimene	1040	1038	42.75
10	γ-terpinene	1062	1066	3.95
11	Terpinolene	1088	1090	3.74
12	Linalool	1098	1102	1.01
13	Bornyl acetate	1285	1288	1.83
14	β -Caryophyllene	1418	1423	0.64
Monoterpenes hydrocarbons				91.92
Oxygenated monoterpenes				6.58
Sesquiterpenes hydrocarbons				0.64
Total identified				**99.14**

[a] Retention indices determined on HP-5MS capillary column.
[b] Kovats index.
[c] The percentage composition was computed from the GC peak areas.

Table 2
Essential oil constituents of *Tetrataenium lasiopetalum.*

No.	Components	RI[a]	KI[b]	Percentage[c]
1	α-Thujene	931	930	0.12
2	α-Pinene	**939**	937	3.45
3	Camphene	953	951	41.73
4	β-Pinene	979	981	2.57
5	1,8-cineol	1030	1032	8.10
6	β-Phellandrene	1031	1034	0.19
7	Limonene	1032	1035	2.78
8	*Cis*-β-Ocimene	1040	1041	5.59
9	γ-Terpinene	1062	1059	0.92
10	α-Terpineol	1188	1086	1.27
11	Hexanol	1197	1099	3.03
12	Octanol acetate	1216	1220	6.22
13	Thymol	1286	1288	0.57
14	Carvacrol	1295	1299	0.62
15	Germacrene-D	1474	1478	13.11
16	Cuparene	1498	1502	0.56
17	β-Bisabolene	1502	1503	0.47
18	β-Sesquiphellandrene	1516	1519	3.08
19	Caryophyllene oxide	1573	1578	0.16
Monoterpenes hydrocarbons				57.35
Oxygenated monoterpenes				10.56
Sesquiterpenes hydrocarbons				17.22
Oxygenated sesquiterpenes				0.16
Others				9.25
Total identified				**94.54**

[a] Retention indices determined on HP-5MS capillary column.
[b] Kovats index.
[c] The percentage composition was computed from the GC peak areas.

3.2. Antimicrobial activity

The antibacterial activities of the volatile oils and extracts in *F. angulata* and *T. lasiopetalum* on two Gram-positive pathogenic strains and two Gram-negative pathogenic strains of bacteria are presented in Table 3. As can be seen, the effectiveness of oils and extracts against tested bacteria was variable (Table 3). The ability of the oils from both species, in preventing the microorganisms was higher than those of their extracts. The minimum inhibitory concentration (MIC) values of the volatile oils differed from 0.5 to 4 mg/ml and the respective minimum bactericidal concentration (MBC) values differed from 1 to 8 mg/ml. On the other hand, the corresponding MIC values of the extracts ranged of 6.25–25 mg/ml with the respective MBC values from 12.5 to 50 mg/ml (Table 3).

Generally, Gram-positive bacteria were more susceptible to all tested oils and extracts. The maximum activity of extracts was observed on *S. aureus* with MIC values of 6.25 mg/ml for *T. lasiopetalum* and 12.5 mg/ml for *F. angulata*, and a MBC value of 12.5 mg/ml. On the contrary, *P. aeruginosa* with a MIC value of 25 mg/ml and MBC values of 25 mg/ml for *T. lasiopetalum* and 50 mg/ml for *F. angulata* was found to be the most resistant strain. The maximum antimicrobial activity of both species essential oil was also observed against *S. aureus* with MIC values of 2 and 0.5 mg/ml and MBC values of 4 and 1 for *F. angulata* and *T. lasiopetalum*, respectively, followed by *E. faecalis* with MIC values of 2 and 1 mg/ml and MBC values of 4 and 2 for *F. angulata* and *T. lasiopetalum*, respectively. While, *P. aeruginosa* appear to be the most resistance microorganism to both species essential oil with MIC and MBC values of 8 for *F. angulata* and MIC and MBC values of 4 for *T. lasiopetalum*. Overall, the results showed that the oil and extract of *T. lasiopetalum* appeared to be more active than those of *F. angulata.* The growth of *S. aureus* was prevented only by the application of 0.5 mg/ml of *T. lasiopetalum* oil (Table 3).

Antimicrobial activities of the volatile oils and extracts from both plants have previously been reported, although in different values and

Table 3
Antibacterial activities of essential oils and extracts from *Ferulago angulata* and *Tetrataenium lasiopetalum*.

Microorganism	Extract						Essential oil						Chloramphenicol (30 μg/disc)		
	F. angulata			T. lasiopetalum			F. angulata			T. lasiopetalum					
	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC
S. aureus	12.8 ± 0.25	12.5	12.5	20.6 ± 0.28	6.25	12.5	27.2 ± 0.5	2	4	42.5 ± 0.08	0.5	1	20.25 ± 0.4	0.5	2
E. faecalis	10.3 ± 0.28	25	12.5	17.3 ± 0.25	12.5	12.5	24.5 ± 0.25	2	4	30.2 ± 0.29	1	2	17.11 ± 07	2	8
E.coli	8.3 ± 0.23	25	50	12.8 ± 0.5	12.5	25	18.4 ± 0.27	4	8	18.5 ± 0.77	2	4	18.22 ± 0.9	1	4
P. aeruginosa	9.1 ± 0.08	25	50	10.6 ± 0.19	25	25	21.5 ± 0.58	8	8	23.7 ± 0.55	4	4	16.5 ± 0.6	2	8

IZ: Inhibition zones diameter (mm) including diameter of sterile disc (6 mm), values are given as mean ± SD (3 replicates); **MIC**: minimum inhibitory concentration values are given as mg/ml; **MBC**: minimal bactericidal concentration values given as mg/ml; Essential oil tested at 8 mg/disc in diffusion tests and extract tested at 25 mg/disc.

spectra of activity regarding their composition. Results from a study by Ghasemi Pirbalouti et al. (2016) indicated that the volatile oil from *F. angulata* had weak to moderate inhibitory activities on *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus*, and *Salmonella typhimurium*. On the other hand, Shahbazi et al. (2015) reported that the essential oil from *F. angulata* could be a potentially rich source of antibacterial compounds on food-borne bacteria. Different reports on antimicrobial activities of the *F. angulata* essential oil among earlier researches could be attributed to differences in bacterial strains used, culture media, volume of the essential oils and the thickness of the agar layer (Ait-Ouazzou et al., 2012; Elizaquível et al., 2013; Tajkarimi and Ibrahim, 2011). The results of Sonboli et al. (2007) showed that the volatile oil of *T. lasiopetalum* exhibited moderate to high antimicrobial activities on *Saccharomyces cerevisiae*, *Candida albicans, E. coli*, *Bacillus subtilis* and *Staphylococcus epidermidis*. While, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were found to be resistant.

In this study, Gram-positive bacteria were more susceptible to all volatile oils and extracts tested. Previously, the lower susceptibility of Gram-negative bacteria to the inhibitory effects of volatile oils and extracts has been attributed to the hydrophilic cell wall structures of these microorganisms (Hadian et al., 2011). In the present study, the high antibacterial activities of volatile oils from *F. angulata* and *T. lasiopetalum* on Gram-positive bacteria could probably be attributed to the relatively high percentage of monoterpene hydrocarbons (Lis--Balchin et al., 1998). In addition, antimicrobial activity of essential oils may be due to a complex interaction between the major compounds and other components of the oils (Ghasemi Pirbalouti et al., 2016; Hadian et al., 2011). Overall, variation and diversity in chemical composition of medicinal plants essential oil and extract, observed due to the genetic factors and the environmental conditions, results in high degree of variation in antimicrobial activity of essential oils and extracts (Hadian et al., 2011).

4. Conclusion

According to our findings, the essential oil of *F. angulata* could be serving as a potential source of *cis*-*β*-ocimene, *α*-pinene, and *α*-phellandrene for applications in the food, cosmetic, and pharmaceutical industries. Furthermore, *T. lasiopetalum* had highest percentages of camphene and Germacrene-D, and its essential oil presented high percentages of 1,8-cineole, *Cis*-*β*-ocimene, and Octanol acetate. Our study showed that the essential oils and extracts of *F. angulata* and *T. lasiopetalum* present antimicrobial activities on four bacterial species. But, essential oil and extract from *T. lasiopetalum* appeared to be more active against four bacterial strains tested than those from *F. angulata*.

Author contributions

Study conception and design: Mumivand, Asrin Aghemiri, Avin Aghemiri, Morshedloo, and Nikoumanesh. Data acquisition: Mumivand, Asrin Aghemiri, and Avin Aghemiri. Analysis and interpretation of data: Morshedloo, Asrin Aghemiri, and Mumivand. Drafting of manuscript: Mumivand, Asrin Aghemiri. Critical revision: Mumivand, Morshedloo, and Nikoumanesh. All authors have read and approved the final version of manuscript, and will be accountable for all aspects of the work.

Declaration of competing interest

The authors declare that they do not have any conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101407.

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