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RESEARCH ARTICLE

Chemical and antimicrobial characterization of essential oils obtained from aerial part, root and fruit of *Ferulago longistylis* Boiss., an endemic species

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Abstract

The aerial part, root and fruits of *Ferulago longistylis* Boiss. an endemic species, were subjected to the evaluation of essential oil compositions and antimicrobial activity. The essential oils were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The main components of the aerial part were identified as α-pinene (18.7%), bornyl acetate (11.8%), 2,3,6-trimethyl benzaldehyde (9.3%), *p*-cymene (7.7%), for the roots, α-pinene (91.7%); and for the fruits, 2,3,6-trimethyl benzaldehyde (26.5%), α-pinene (14.9%), *(Z)*-β-ocimene (14.1%), myrcene (7.5%), sabinene (7.3%), respectively. The essential oils were evaluated for their *in vitro* antimicrobial activity against a panel of some human pathogens using a broth microdilution technique resulting in relatively weak antimicrobial and antifungal activity (MIC 1.25-10 mg/mL).

Keywords: *Ferulago longistylis*, Apiaceae, essential oil chemistry, antimicrobial activity.

Introduction

Apiaceae, which is one of the largest plant families in the world, is represented by about 160 endemic species in Turkey (Şenol et al., 2018). New species of Apiaceae family in Turkey are still being discovered (Şenol et al., 2018; Menemen et al., 2018). The *Ferulago* W. Koch genus is a member of Apiaceae family and 35 taxa grow naturally in Turkey (Güner et al., 2012). The genus *Ferulago* is an Anatolian-based genus that is very similar to *Ferula* L. (Özhatay & Akalın, 2000). One of the 19 endemic *Ferulago* species in Turkey, *F. longistylis* Boiss., is among the rare endemics (Güner et al., 2012; Gençler Özkan et al., 2007; Başer & Kırımer, 2014). *Ferulago* species are used in Turkish folk medicine for treatment of intestinal worms, hemorrhoids and also used as a tonic, sedative, digestive, however, one of its most significant traditional usage is due to its aphrodisiac activity (Baytop, 1999; Demetzos et al., 2000). Various phytochemical studies revealed that *Ferulago* species contain mainly coumarins such as bergapten, 8-(1,1-dimethylallyl)bergaptol, bergamotin, isoimperatorin, oxypeucedanin, (−)-prantschimgin and (−)-*iso*valerylmarmesin (Jimenez et al., 2000), essential oil (major components 2,3,6-trimethylbenzaldehyde, α-pinene, (*Z*)-β-ocimene, myrcene, *p*-cymene) (Erdurak et al., 2006; Kılıç et al., 2010; Başer & Kırımer, 2014), flavonoids such as quercetin and rutin (Khanahmadi et al., 2011) and quinone (1-acetylhydroquinone 4-galactoside) (Doğanca et al., 1991). Additionally, essential oils of different *Ferulago* species showed antimicrobial activity on various pathogens (Taran et al 2010; Khalighi-Sigarodi et al., 2005; Demirci et al., 2000).

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In this present study, essential oils obtained from 3 different parts (aerial parts, roots and fruits) of *F. longistylis* collected from Refahiye-Erzincan (Turkey) were analyzed for their chemical composition by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). In addition, *in vitro* antimicrobial activity of all essential oils were evaluated by broth microdilution against various human pathogenic microorganisms.

Materials and Methods

Plant material and distillation

F. longistylis was collected from Erzincan-Refahiye, Sakaltutan locality, high mountain steppe, 2030 m (Turkey) on July 13, 2016. The voucher specimen was identified by one of us HD, and deposited in the Herbarium of Ankara University, Faculty of Pharmacy, Ankara, Turkey (AEF 28777).

The air dried *F. longistylis* aerial parts, roots and fruits were initially separated. Each plant material was crushed and grinded prior to hydrodistillation which was distilled for 3 h separately using a Clevenger-type apparatus to produce the essential oils.

Analysis of the essential oils

GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done as a duplicate on the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analysis are given in Table 1.

GC-MS analysis

An Agilent 5975 GC-MSD system was used, where Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set to 250°C. Mass spectra were recorded at 70 eV, where the mass range was from *m/z* 35 to 450.

Identification of the components

Essential oils were analyzed by comparison of their relative retention times (RRT) with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0) (McLafferty & Stauffer,1989; Hochmuth, 2008; ESO, 2000) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils was used for the identification.

Antimicrobial activity

Microbial strains

Standard human pathogenic strains *Escherichia coli* NRRL B-3008 (Gr -), *Salmonella typhimurium* ATCC 13311 (Gr -), *Staphylococcus aureus* ATCC 6538 (Gr +), *Bacillus subtilis* NRRL B-4378 (Gr +); Yeasts: *Candida parapsilosis* NRRL Y-12696, *Candida tropicalis* NRRL Y-12968 strains.

Antimicrobial activity

The broth microdilution protocol was used (CLSI, 2006, Gençler Özkan et al., 2008). Serial dilutions were performed yielding essential oils concentrations ranging from 0.078 - 10 mg/mL, for the standard antibacterial and antifungal agents 0.005 to 0.32 mg/mL were used. DMSO (10%) and distilled water was used to dissolve essential oils and antibiotics respectively. Microbial suspensions prepared according to McFarland No: 0.5 to adjust to $1x10^5$ CFU/mL by using tubidometry (Bioland). 10 µL microbial suspension were added to each well. All experiments were repeated in duplicates. To prevent the antibacterial activity of neighboring wells from affecting each other, they were covered with a sticky film on the plate. The plates were incubated at 37 °C, for 24 h. Then 20 µL resazurin was added to all wells and reproductive wells were stained pink indicating the minimum inhibitory concentrations (MIS), where the average MIC values were calculated based on coloration as shown in Table 3.

Results and Discussion

Essential oils of aerial parts, roots and fruits of *F. longistylis* were obtained by Clevenger apparatus and essential oil yields were calculated to be 0.27%, 0.86% and 6.1% (v/w), respectively. The chemical compositions of all the essential oils obtained were analyzed by GC and GC-MS. As shown in Table 1, seventy-three components were identified for the aerial parts, representing 86.7% of the sample, with α-pinene (18.7%), bornyl acetate (11.8%), 2,3,6-trimethyl benzaldehyde (9.3%), *p*-cymene (7.7%) as major constituents. For essential oil of the roots, forty-eight components were identified representing 99.3% of the sample, with α-pinene (91.7%), β-pinene (2.0%), myrcene (1.1%) as major constituents. For the essential oil of the fruits, thirty-three components were identified, representing 99.1% of the sample, with 2,3,6-trimethyl benzaldehyde (26.5%), α-pinene (14.9%), (*Z*)-β-ocimene (14.1%), myrcene (7.5%), sabinene (7.3%) as main constituents. The chemical composition of all essential oils obtained from different parts of *F. longistylis* are given in Table 1.

Table 1. GC-MS analysis results of essential oil obtained separately from 3 different parts of the *F. longistylis.*

RRI	Compound	FL-AP %	FL-R %	FL-F %	IM
1014	Tricyclene	*tr*	-	-	MS
1032	α-Pinene	18.7	91.7	14.9	RRI, MS
1035	α-Thujene	0.2	0.1	0.2	RRI, MS
1072	α-Fenchene	-	*tr*	-	MS
1076	Camphene	0.7	0.5	0.6	RRI, MS
1093	Hexanal	0.1	*tr*	-	RRI, MS
1118	β-Pinene	0.3	2.0	0.6	RRI, MS
1132	Sabinene	0.2	0.4	7.3	RRI, MS
1135	Thuja-2,4(10)-diene	0.8	0.1	0.4	MS
1174	Myrcene	0.2	1.1	7.5	MS
1188	α-Terpinene	*tr*	-	0.2	RRI, MS
1195	Dehydro-1,8-cineole	0.1	-	-	MS
1203	Limonene	0.3	0.5	0.9	RRI, MS
1218	β-Phellandrene	*tr*	*tr*	0.1	RRI, MS
1244	2-Pentyl furan	*tr*	0.1	-	MS
1246	*(Z)*-β-Ocimene	0.5	0.1	14.1	MS

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1255	*γ*-Terpinene	0.9	0.1	4.0	RRI, MS
1266	*(E)*-β-Ocimene	0.3	*tr*	0.4	MS
1280	*p*-Cymene	7.7	0.4	0.8	RRI, MS
1290	Terpinolene	0.1	*tr*	0.1	RRI, MS
1294	1,2,4-Trimethylbenzene	0.9	*tr*	0.6	MS
1355	1,2,3-Trimethylbenzene	0.3	*tr*	-	MS
1429	Perillene	-	*tr*	-	MS
1439	*γ*- Campholene aldehyde	0.1	*tr*	-	MS
1441	*(E)-2*-Octenal	-	*tr*	-	MS
1452	α,*p*-Dimethylstyrene	0.2	*tr*	-	MS
1477	4,8-Epoxy terpinolene	*tr*	-	-	MS
1499	α-Campholene aldehyde	0.1	0.2	-	MS
1522	Chrysanthenone	-	-	1.5	MS
1532	Camphor	-	*tr*	-	RRI, MS
1535	β-Bourbonone	0.4	-	-	MS
1553	Linalool	0.2	-	0.1	RRI, MS
1582	*cis*-Chrysanthenyl acetate	7.5	0.2	4.2	MS
1586	Pinocarvone	-	*tr*	-	RRI, MS
1590	Bornyl acetate	11.8	0.6	3.3	RRI, MS
1611	Terpinen-4-ol	-	*tr*	0.7	RRI, MS
1612	β-Caryophyllene	2.1	-	0.3	RRI, MS
1614	Carvacrol methyl ether	-	0.1	-	RRI, MS
1616	Hotrienol	0.1	-	-	MS
1645	*cis*-Verbenyl acetate	-	-	0.7	MS
1648	Myrtenal	0.8	0.1	0.1	MS
1655	*(E)*-2-Decenal	-	*tr*	-	MS
1661	*trans*-Pinocarvl acetate	-	-	0.3	MS
1663	*cis*-Verbenol	1.8	*tr*	0.8	RRI, MS
1670	*trans*-Pinocarveol	-	0.1	-	RRI, MS
1683	*trans*-Verbenol	5.0	0.1	3.1	RRI, MS
1704	*γ*-Curcumene	0.4	-	-	MS
1719	Borneol	0.4	*tr*	-	RRI, MS
1725	Verbenone	0.3	*tr*	-	RRI, MS
1726	Germacrene D	1.7	-	0.4	MS
1738	*p*-Mentha-1,5-dien-8-ol	-	-	1.5	MS
1740	α-Muurolene	0.2	-	-	MS
1760	Chrysanthenyl isovalerate II	-	-	-	MS
1764	*cis*-Chrysanthenol	0.2	*tr*	-	MS
1764	*(E)*-2-Undecenal	-	*tr*	-	MS
1779	(*E,Z*)-2,4-Decadienal	-	*tr*	-	MS
1786	*ar*-Curcumene	1.3	-	-	MS
1797	*p*-Methyl acetophenone	*tr*	-	-	MS

1804	Myrtenol	0.1	-	-	MS
1827	(*E,E*)-2,4-Decadienal	0.1	0.1	-	MS
1845	*trans*-Carveol	0.3	*tr*	-	RRI, MS
1849	Calamenene	*tr*	-	-	MS
1849	Cuparene	0.1	-	-	MS
1854	Germacrene B	-	-	0.2	MS
1864	*p*-Cymen-8-ol	0.4	*tr*	0.1	MS
1868	*(E)*-Geranyl acetone	*tr*	-	-	MS
1878	2,5-Dimethoxy-*p*-cymene	0.8	0.1	-	MS
1925	2,3,4-Trimethyl benzaldehyde	1.8	*tr*	2.6	MS
1945	1,5-Epoxy-salvial-4(14)-ene	0.1	-	-	MS
1958	*(E)*-β-Ionone	0.1	-	-	MS
2008	Caryophyllene oxide	2.7	-	-	RRI, MS
2019	2,3,6-Trimethyl benzaldehyde	9.3	0.2	26.5	MS
2037	Salvial-4(14)-en-1-one	0.1	-	-	MS
2050	*(E)*-Nerolidol	0.1	-	-	MS
2071	Humulen epoxide-II	0.3	-	-	MS
2084	Octanoic acid	0.1	-	-	RRI, MS
2131	Hexahydro farnesyl acetone	*tr*	-	-	MS
2144	Spathulanol	0.3	-	-	MS
2148	*(Z)*-3-Hexen-1-ol benzoate	0.3	-	-	MS
2232	α-Bisabolol	*tr*	-	-	RRI, MS
2255	α-Cadinol	*tr*	-	-	MS
2278	Torilenol	0.2	-	-	MS
2324	Caryophylladienol II	0.2	-	-	MS
2369	Eudesma-4(15)7-dien-1-β-ol	0.4	-	-	MS
2392	Caryophyllenol II	0.3	-	-	MS
2500	Pentacosane	0.1	-	-	RRI, MS
2622	Phytol	0.5	-	-	MS
2655	Benzyl benzoate	0.4	-	-	RRI, MS
2670	Tetradecanoic acid	-	*tr*	-	RRI, MS
2900	Nonacosane	0.3	-	-	RRI, MS
2931	Hexadecanoic acid	0.4	0.4	-	RRI, MS
	Yield (%)	**0.27**	**0.86**	**6.1**	
	Total	**86.7**	**99.3**	**99.1**	

RRI: Relative retention indices calculated against *n*-alkanes; %: calculated from FID data; *tr:* Trace (< 0.1 %); MS: identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data, FL-AP: *F. longistylis* aerial part; FL-R: *F. longistylis* roots; FL-F: *F. longistylis* fruits.

After analyzing the composition of the obtained essential oils, potential antibacterial and antifungal activities were also evaluated against four bacteria and 2 yeast by broth microdilution method. The minimum inhibitor concentration of the essential oils is given in Table 2. As shown in Table 2, all essential oil samples showed

anticandidal activity at 1.25-10 mg/mL MIC values. However, root essential oil showed antibacterial or antifungal activity at 1.25-5 mg/mL MIC values against all tested microorganism.

α-pinene, one of the major components of essential oils from *F. longistylis*, might be responsible for this activity. Although there are papers stating that *α*-pinene has antibacterial effect (Aligiannis et al., 2001; Leite et al., 2007), there are also some papers claiming the opposite (Koutsoudaki et al., 2005; Zore et al., 2011). This is explained by the fact that only the (−)-α-pinene of the enantiomers has this effect. Furthermore, *p*-cymene affects Gram (-) bacteria by disrupting the membrane structure (Ultee et al., 2002).

There are 2 studies on the essential oil of *F. longistylis* in the literature. In these studies, fruits (Gençler Özkan et al., 2008) and aerial parts (Kılıç et al., 2010) of *F. longistylis* were collected from the same locality (on July, 2006) and the essential oil composition was determined by GC and GC/MS. Essential oil yield obtained from the fruits of the *F. longistylis* was reported to be 6.4% (Gençler Özkan et al., 2008). Fifty-nine components were identified representing 96.7% of the essential oil of the fruits and major components were determined to be 2,3,6-trimethylbenzaldehyde (29.4%), α-pinene (16.7%), (*Z*)-β-ocimene (15.9%), sabinene (6.2%), myrcene (5.7%) and bornyl acetate (4.4%). These values are quite consistent with our findings for the essential oil of the fruits (2,3,6-trimethyl benzaldehyde 26.5%, α-pinene 14.9%, *(Z)*-β-ocimene 14.1%, myrcene 7.5%, sabinene 7.3%, bornyl acetate 3.3%). Furthermore, thuja-2,4(10)diene (0.4%), chrysanthenone (1.5%) and some other components (0.1% or less) were found in the present study.

Gençler Özkan et al. (2008) also studied the antibacterial and antifungal activity of the essential oil obtained from *F. longistylis* fruits and stated that it had a weak activity (0.5-1.0 mg/mL, MIC values) similar to our results (Table 2). However, there are also minor differences between the composition and antimicrobial effects of essential oils in both studies. The minor changes in the composition of essential oils can be considered to cause minor changes in the antimicrobial effect, as reported here.

For aerial parts, essential oil yield was 0.16% and fifty-nine components representing 92.5% of the essential oil were identified (Kılıç et al., 2010). Major components were 2,3,6-trimethyl benzaldehyde (32.7%), bornyl acetate (12.6%), *p*-cymene (11.9%), *cis*-chrysanthenyl acetate (4.2%), α-pinene (3.5), 2,3,4-trimethyl benzaldehyde (3.1%), *(Z)*-β-ocimene (2.3%). It is observed that the amounts of α-pinene (18.7%) and 2,3,6-trimethyl benzaldehyde (9.3%) are quite different from the present study. In addition, thuja-2,4(10)diene (0.8%), linalool (0.2%), *trans*-verbenol (5.0%), germacrene D (1.7%), α-muurolene (0.2%), 2,5-dimethoxy-*p*-cymene (0.8%), spathulanol (0.3%), torilenol (0.2%) were additionaly determined in our present study. It is thought that possible change of some factors (environmental conditions, climatic conditions, pollution etc.) may result in differences in the chemical composition of essential oils (Figueiredo et al., 2008). The literature data and the results of the present study show that major compounds of the essential oil obtained from *F. longistylis* are α-pinene, 2,3,6-trimethylbenzaldehyde, (Z)-β-ocimene, myrcene, bornyl acetate, sabinene, *p*-cymene (Gençler Özkan et al., 2008; Kılıç et al., 2010).

When the previous studies on *F. longistylis* were reviewed, it was seen that the essential oils obtained from only aerial parts or fruits were analyzed in these studies (Gençler Özkan et al., 2008; Kılıç et al., 2010). Again, it was seen that the only antimicrobial effect of the essential oil obtained only from the fruits was studied.

Table 2. Antimicrobial activity results (MIC, mg/mL)

Microorganisms	Strain Numbers	FL-AP[a]	FL-R[b]	FL-F[c]	AMOX[d]	CLO[e]	FLC[f]
Escherichia coli	NRRL B-3008	-	5	10	0.01	0.01	-
Staphylococcus aureus	ATCC 6538	-	5	-	-	0.01	-
Salmonella typhimurium	ATCC 13311	10	5	-	-	0.4	-
Bacillus subtilis	NRRL B-4378	-	2.5	-	-	0.01	-
Candida tropicalis	NRRL Y-12968	10	1.25	10	-	-	0.08
Candida parapsilosis	NRRLY-12696	5	1.25	5	-	-	0.005

[a] *F. longistylis* aerial part essential oil; [b] *F. longistylis* root essential oil; [c] *F. longistylis* fruit essential oil; [d] amoxicillin; [e] chloramphenicol; [f] fluconazole.

In the present study, the volatile oils were obtained from 3 different parts (aerial parts, fruits and roots) of *F. longistylis* and antimicrobial effects were studied against various human pathogens. To the best of our knowledge, composition of the essential oil obtained from the roots of the *F. longistylis* and its antimicrobial effect have been evaluated for the first time. Furthermore, antimicrobial effect of the essential oil obtained from the aerial parts was determined for the first time.

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