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RESEARCH ARTICLE

In vitro antimicrobial evaulation of *Ferulago sandrasica* Peşmen & Quézel herba and root essential oil

Melike Sucu[1, *], Damla Kırcı[2], Betül Demirci[2], Hayri Duman[3], Ceyda Sibel Kılıç[4], İlhan Gürbüz[1, *]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, TURKEY
[2] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, TURKEY
[3] Department of Biology, Faculty of Arts and Science, Gazi University, 06500, Ankara, TURKEY
[4] Department of Botany, Faculty of Pharmacy, Ankara University, 06560 Ankara, TURKEY

*Corresponding authors. Email: mefuse.sck@gmail.com, ilgurbuz@gmail.com

Abstract

Ferulago sandrasica Peşmen & Quézel was initially collected from Köyceğiz, which is endemic species for Turkey. The air dried herbal parts and roots were evaluated for their volatile compositions and antimicrobial activity. The essential oils were obtained by hydrodistillation, which were subsequently analyzed by GC-FID and GC-MS, respectively. The main components of the herb essential oil were identified as *α*-pinene (26.4%), caryophyllene oxide (6.1%), camphene (5.1%), *trans*-verbenol (5%); whereas the root essential oil compounds were *α*-pinene (27.9%), limonene (26.1%), *δ*-3-carene (14.2%), myrcene (7.6%), terpinolene (11%), respectively. In addition, the essential oils were evaluated for their *in vitro* antimicrobial activity using a broth microdilution method against the human pathogens *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Bacillus subtilis,* and the yeasts *Candida tropicalis, C. parapsilosis.* The activity results showed that *F. sandrasica* was effective against the tested pathogens with the minimum inhibition concentrations (MIC) of 2.5-10 mg/mL.

Keywords: *Ferulago sandrasica*, antimicrobial activity, Apiaceae, root, herba

Introduction

The Apiaceae family is represented by 100 genera (including *Ferulago*) and 159 endemic species in Turkey (Güner et al. 2012; Şenol et al., 2018). Essential oils are abundant in the stem, roots, fruits, flowers and leaves in this family (Başer and Kırımer, 2014). The genus *Ferulago* W. Koch (49 species in the world) is fairly common with 34 species in Turkey, 19 of these species are endemic to Turkey (Güner et al. 2012; Akalın-Uruşak and Kızılarslan, 2013). They are known with various local names as “çakşırotu”, “çağşır”, “kişniş”, “asaotu”, “mayasılotu”, “kimyonotu”, “kuzukemirdi”, “kuzubaşı”, “kuzukişnişi” (Özkan et al., 2008; Bostanlık, 2015). Since ancient times these species have been used as aphrodisiac, tonic, digestive and for treatment of intestinal worms and hemorroids as well as against ulcers, snake bites and headache (Demirci et al., 2000; Selçuk et al., 2017; Karakaya, 2018). Due to the essential oil, resin and gum present in the fruits as well as in the roots they have a characteristic aroma explaining their use as food and spice (Bostanlık, 2015).

Ferulago species contain coumarins, flavonoid derivatives and terpenic compounds among other secondary metabolites (Bostanlık, 2015; Selçuk et al. 2017). *Ferulago sandrasica* is perennial, glabrous, glaucescent plant and endemic in Turkey (Davis, 1997; Güner et al. 2012). *Ferulago humilis* Boiss., *F. macrosciadia* Boiss. et Bal., *F. sandrasica* Peşmen & Quezel and *F. idaea* Özhatay & E. Akalın are closely related species endemic to Turkey (Akalın et al., 2002). Only a couple of *F. sandrasica* essential oil phytochemical and antimicrobial activity reports are present. Essential oils of root and leaf, methanol extracts of herb and root were reported by TLC-bioautography and disc diffusion tests (Çelik et al., 2013; Bostanlık, 2015). In a recent report on the erectile dysfunction evaluation in streptozotocin-induced diabetic rats, lyophilized aqueous extracts of *F.*

sandrasica root showed 97% relaxation on corpus cavernosum whereas the herbal parts were inactive (Karakaya et al., 2019).

The aim of the present study was to evaluate the *in vitro* antimicrobial activity of *F. sandrasica* herb and root essential oils collected from Beyağaç-Köyceğiz road at Kartal Lake intersection by using the broth microdilution assay. The phytochemical composition was analyzed and elucidated by GC-FID and GC-MS. To the best of our knowledge the antimicrobial activity of herb essential oil of this species is reported for the first time.

Materials and Methods

Plant materials and isolation of the essential oils

Herbs and roots of *F. sandrasica* were collected (June 9, 2016) from Denizli, Beyağaç, Beyağaç-Köyceğiz road at Kartal Lake intersection, 1390 m (Turkey). A voucher specimen identified Prof. Dr. Hayri Duman is deposited in the herbarium of the Faculty of Pharmacy, Ankara University, Ankara, Turkey. Voucher No: AEF 28772.

Plant materials were air dried in shade at room temperature and ground, prior to hydrodistillation for 3 h using a Clevenger-type apparatus to produce the essential oils. The yields for *F. sandrasica* herb was 0.1%, whereas it was % 0.88 (v/w) for the root.

Analysis of the essential oils

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. 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 at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

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 on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 1.

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

Antimicrobial activity

F. sandrasica essential oils were tested using *Escherichia coli* NRRL B-3008 (Gr -), *Salmonella typhimurium* ATCC 13311 (Gr -), *Staphylococcus aureus* ATCC 6538 (Gr +), *Bacillus subtilis* NRRL B-4378 (Gr +), *Candida parapsilosis* NRRL Y-12696, *C. tropicalis* NRRL Y-12968. Positive control agents were amoxicillin, chloramophenicole and fluconazole.

The broth microdilution protocol was used (CLSI, 2006) to determine the minumum inhibitory concentration (MIC) against the human pathogenic standard strains. Essential oils concentrations were 0.078125 - 10 mg/mL in 96 well microplate. Microbial suspensions were prepared according to McFarland No: 0.5, and diluted before adding to the microwell, followed by 10 µL microbial suspension added to each well. Concentration of the antimicrobial agents were between 0.005 – 0.32 mg/mL. The last two row contained positive and negative controls respectively. After incubation at 37 °C for 24 h, 20 µL resazurin was added to all wells for growth inhibition determination. All experiments were repeated in duplicate and average MICs are given in Table 3.

Results and Discussion

Essential oil composition

In this present study, the analyses of the oils have resulted in the characterization of 92 components, representing 78.8% of the total oil, and 53 components, representing 99.7% of the total oil for herbs and root, respectively. Main constituents of herb essential oil were α-pinene (26.4%), caryophyllene oxide (6.1%), camphene (5.1%) and *trans*-verbenol (5%); for root essential oil, they were α-pinene (27.9%), limonene (26.1%), *δ*-3-carene (14.2%), terpinolene (11%) and myrcene (7.6%). The components identified in the essential oils can be seen in Table 1.

Table 1. GC-MS analysis results of *F. sandrasica* essential oils

RRI	Compound	FSH %	FSR %	IM
1014	Tricyclene	0.1	-	MS
1032	α-Pinene	26.4	27.9	RRI, MS
1072	α-Fenchene	-	*tr*	MS
1076	Camphene	5.1	0.8	RRI, MS
1093	Hexanal	0.2	0.2	RRI, MS
1118	β-Pinene	0.6	0.7	RRI, MS
1132	Sabinene	0.2	0.3	RRI, MS
1135	Thuja-2,4(10)-diene	0.8	*tr*	MS
1151	δ-3-Carene	0.1	14.2	MS
1174	Myrcene	0.2	7.6	MS
1176	α-Phellandrene	*tr*	1.0	RRI, MS
1187	*o*-Cymene	-	0.1	MS
1188	α-Terpinene	*tr*	0.1	RRI, MS
1203	Limonene	3.8	26.1	RRI, MS
1218	β-Phellandrene	0.1	0.3	RRI, MS
1244	2-Pentyl furan	0.1	0.1	MS
1246	(*Z*)-β-Ocimene	-	1.0	MS
1247	6-Methyl-2-heptanone	*tr*	-	MS
1255	γ-Terpinene	0.1	2.7	RRI, MS
1266	(*E*)-β-Ocimene	-	*tr*	MS
1280	*p*-Cymene	0.7	2.6	RRI, MS
1286	Isoterpinolene	-	0.4	MS
1290	Terpinolene	0.2	11.0	RRI, MS
1296	Octanal	0.1	-	RRI, MS

1348	6-Methyl-5-hepten-2-one	*tr*	-	MS
1360	1-Hexanol	0.1	-	MS
1382	*cis*-Alloocimene	-	*tr*	MS
1400	Nonanal	0.1	-	MS
1429	Perillene	*tr*	-	MS
1441	(*E*)-2-Octenal	*tr*	*tr*	MS
1452	α,*p*-Dimethylstyrene	0.2	0.2	MS
1452	1-Octen-3-ol	*tr*	*tr*	MS
1468	*trans*-1,2-Limonene epoxide	*tr*	*tr*	MS
1477	4,8-Epoxy terpinolene	-	0.1	MS
1492	Cyclosativene	tr	-	MS
1497	α-Copaene	0.6	-	RRI, MS
1499	α-Campholene aldehyde	1.0	0.1	MS
1532	Camphor	-	*tr*	RRI, MS
1536	Pinocamphone	0.1	-	RRI, MS
1571	*trans*-*p*-Mentha-2-en-1-ol	0.2	-	MS
1586	Pinocarvone	0.6	*tr*	RRI, MS
1590	Bornyl acetate	0.2	-	RRI, MS
1591	Fencyl alcohol	-	0.1	RRI, MS
1600	β-Elemene	0.1	-	RRI, MS
1611	Terpinen-4-ol	0.1	*tr*	RRI, MS
1612	β-Caryophyllene	0.5	-	RRI, MS
1638	*cis*-*p*-Mentha-2-en-1-ol	0.2	-	MS
1648	Myrtenal	0.7	*tr*	MS
1663	*cis*-Verbenol	1.1	-	RRI, MS
1670	*trans*-Pinocarveol	1.8	*tr*	RRI, MS
1683	*trans*-Verbenol	5.0	0.1	RRI, MS
1684	Isoborneol	-	*tr*	MS
1687	α-Humulene	0.3	-	RRI, MS
1706	α-Terpineol	0.3	0.3	RRI, MS
1719	Borneol	0.1	0.1	RRI, MS
1726	Germacrene D	0.5	0.2	MS
1742	β-Selinene	0.5	-	MS
1751	Carvone	0.6	0.1	RRI, MS
1758	*cis*-Piperitol	0.1	-	RRI, MS
1763	Naphthalene	0.1	*tr*	RRI, MS
1770	Isobornyl isovalerate	0.3	-	MS
1773	δ-Cadinene	0.2	-	MS
1776	γ-Cadinene	0.1	-	MS
1796	Selina-3,7(11)-diene	0.2	-	MS
1797	*p*-Methyl acetophenone	0.4	0.2	MS
1804	Myrtenol	0.5	-	MS
1811	*trans*-*p*-Menth-1(7)8-dien-2-ol	0.1	-	MS
1827	(E,E)-2,4-Decadienal	0.1	0.1	MS
1845	*trans*-Carveol	1.2	0.1	RRI, MS
1864	*p*-Cymen-8-ol	0.4	0.2	MS

1868	(*E*)-Geranyl acetone	*tr*	-	MS
1870	Hexanoic acid	*tr*	-	RRI, MS
1878	2,5-Dimethoxy-p-cymene	0.5	0.4	MS
1880	Benzyl-2-methyl butyrate	*tr*	-	MS
1900	*epi*-Cubebol	0.1	-	MS
1941	α-Calacorene	0.3	-	MS
1945	1,5-Epoxy-salvial-4(14)-ene	1.0	-	MS
1988	Nerolidol oxide I	0.2	-	MS
1988	2-Phenylethyl-2-methylbutyrate	0.2	-	MS
2001	Isocaryophyllene oxide	0.2	-	MS
2008	Caryophyllene oxide	6.1	-	RRI, MS
2019	2,3,6-Trimethyl benzaldehyde	-	0.1	MS
2050	(*E*)-Nerolidol	2.4	-	MS
2071	Humulen epoxide-II	0.1	-	MS
2084	Octanoic acid	0.1	-	RRI, MS
2130	Salviadienol	0.5	-	MS
2144	Spathulanol	1.4	-	MS
2192	Nonanoic acid	-	*tr*	RRI, MS
2219	Dimyrcene II-a	-	0.1	MS
2255	α-Cadinol	0.2	*tr*	MS
2256	Cadalene	0.2	*tr*	MS
2269	Dimyrcene II-b	-	*tr*	MS
2273	Porosadienol	0.7	-	MS
2278	Torilenol	0.6	-	MS
2289	4-oxo-α-Ylangene	0.6	-	MS
2324	Caryophylladienol II	0.3	-	MS
2369	Eudesma-4(15)7-dien-1-β-ol	0.7	-	MS
2389	Caryophyllenol I	0.6	-	MS
2392	Caryophyllenol II	1.2	-	MS
2503	Dodecanoic acid	0.4	-	RRI, MS
2600	Hexacosane	0.2	-	RRI, MS
2607	14-Hydroxy-δ-cadinene	0.1	-	MS
2655	Benzyl benzoate	0.1	-	RRI, MS
2670	Tetradecanoic acid	0.3	-	RRI, MS
2900	Nonacosane	1.1	-	RRI, MS
2931	Hexadecanoic acid	1.0	0.1	RRI, MS
	Total	**78.8**	**99.7**	

RRI: Relative retention indices calculated against *n*-alkanes; %: calculated from FID data; *tr*: Trace (< 0.1 %); IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; 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; FSH: *F. sandrasica* herb essential oil; FSR: *F. sandrasica root* essential oil

First study on *F. sandrasica* (collected from Muğla: Mountain Sandras) was about phytochemical content of fruit essential oil obtained by microdistillation. Major component in *F. sandrasica* fruit essential oil was α-pinene (40.8%) (Başer et al., 2002). In fruit, leaf and root essential oils major component was (*Z*)-β-ocimene (32%) (Başer and Kırımer, 2014), ocimene (30.5%) (Çelik et al., 2013) and limonene (28.9%) (Bostanlık, 2015), respectively.

The root essential oil from Muğla was previously reported by Bostanlık (2015). The major components were limonene (28.9%), α-pinene (15.6%) and terpinolene (13.9%), whereas α-pinene (27.9%), limonene (26.1%), δ-3-carene (14.2%), terpinolene (11%) and myrcene (7.6%) in our study. Minor components were 2,3,6-trimethyl benzaldehyde (3.2%), myrcene (2.8%), *p*-cymene (2.8%) camphene (2,6%) and components with very small quantities (<2%) in study of Bostanlık (2015), while γ-terpinene (2.7%), *p*-cymene (2.6%) and components with very little amounts (≤1%) in our study.

As shown in Table 2, previous reports on *F. sandrasica* essential oils showed that the main components are α-pinene, limonene, ocimene among others.

Table 2. Essential oil components of *F. sandrasica*

Location	Method	Year	Plant part	Yield	Main components	Reference
Mountain Sandras 3 km to Kartal Lake, under *Pinus nigra* trees, in Muğla, 1675 m	Hydrodistillation	June 2013	root	0.019%	limonene (28.9%), α-pinene (15.6%), terpinolene (13.9%), 2,3,6-trimethylbenzaldehyde (3.2%), myrcene (2.8%), *p*-cymene (2.8%), camphene (2.6%)	Bostanlık, 2015
Sandras region Beyağaç, in Denizli, 1850m	Hydrodistillation	_	leaf	0.62%	ocimene (30.5%), carene -δ-3 (27.4%) α-pinene (17.8%)	Çelik et al., 2013
Mountain Sandras, in Muğla	Microdistillation	_	fruit	_	α-pinene (40.8%), germacrene D (8.1%), α-humulene (5.8%), *trans*-chrysanthenyl acetate (5.3%), β-caryophyllene (3.2%), caryophyllene oxide (3%), humulene epoxide-II (3%)	Başer et al., 2002
_	Hydrodistillation	--	fruit	3.9%	*(Z)*-β-ocimene (32%), limonene (17%), α-pinene (12%), *trans*-chrysanthenyl acetate (12%)	Başer and Kırımer, 2014

Antimicrobial activity results

The present results showed that herb essential oil was active against *Staphylococcus aureus, Salmonella typhimurium,* and *Bacillus subtilis,* but not active against *Escherichia coli. Candida parapsilosis* and *C. tropicalis* were not sensitive to herb oil compared with positive control agent. Root essential oil was more active against *S. typhimurium* and *B. subtilis* compared with *E. coli* and there was no activity for *S. aureus*. As in the herb oil, root oil was not effective much for *C. tropicalis* and *C. parapsilosis* compared with positive controls. In the previous study of Bostanlık (2015) no MICs were reported for root essential oil. In our study, to the best of our knowledge we report for the first time MIC values for herb and root oils. The results are presented in Table 3.

Table 3. Antimicrobial activity results (MIC, mg/mL)

Microorganisms	Strain Numbers	FSH[a]	FSR[b]	AMOX[c]	CHL[d]	FLC[e]
Escherichia coli	NRRL B-3008	-	10	0.01	1.953125	-
Staphylococcus aureus	ATCC 6538	5	-	-	1.953125	-
Salmonella typhimurium	ATCC 13311	2.5	10	-	3.90625	-
Bacillus subtilis	NRRL B-4378	2.5	5	-	1.953125	-
Candida tropicalis	NRRL Y-12968	2.5	2.5	-	-	0.08
Candida parapsilosis	NRRL Y-12696	2.5	5	-	-	0.005

[a] *F. sandrasica* herb essential oil; [b] *F. sandrasica root* essential oil; [c] amoxicillin; [d] chloramphenicol; [e] fluconazole

In previous studies, *F. sandrasica* leaf essential oil, where the main components were identified as ocimene (30.5%), δ-3-carene (27.4%), *α*-pinene (17.8%), was tested against *E. coli, S. aureus, B. subtilis* by disc diffusion method at 0.1 and 0.25 µg/mL concentrations, respectively. The antimicrobial study showed that *B. subtilis* was more sensitive to leaf essential oil than the other microorganisms, with an inhibition zone of 5 and 10 mm at 0.1 and 0.25 µg/mL, respectively. However, *E. coli* showed more resistance against the oil than other bacterial strains (Çelik et al., 2013). *F. sandrasica* root essential oil was tested using the TLC-bioautography assay against *S. aureus* and *E. coli*. The results showed that the oil was active against *S. aureus,* but not against *E. coli*. Also methanol extracts (major component felamidin) of herb and root were investigated against *S. aureus, E. coli* and *B. subtilis* by Kirby-Bauer disc diffusion assay. Inhibition zones were observed (at 0.625-5 mg/mL concentration) only against *S. aureus* (Bostanlık, 2015). The results from previous findings are not in accordance with our findings.

The major components α-pinene, limonene and δ-3-carene in the root essential oil may be associated with the antimicrobial effect, which suggest more detailed studies in future also by using different test systems and organisms.

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