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Biological activity and chemical composition of the essential oil from the fruits of *Ferula halophila* Peşmen

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ABSTRACT

The hydrodistilled essential oils of the dried fruits of *F. halophila* Peşmen, an endemic species growing near Salt Lake in Central Anatolia, were analyzed by GC and GC-MS systems; 27 and 24 compounds representing 92.1% and 79.8% of the essential oils were characterized, respectively. The main component of the essential oils was identified as β-phellandrene. The antibacterial and anticandidal effects of the essential oils were determined by using partly modified CLSI methods M7-A7 and M27-A2, respectively. The essential oils from two specimens showed weak to moderate inhibitory effects on the tested pathogenic bacteria (MIC, 125-2000 μg/mL) and *Candida* panels (MIC, 156-1250 μg/mL).

Keywords: Antibacterial, anticandidal, essential oil, *Ferula halophila*

INTRODUCTION

The genus *Ferula* (Apiaceae) comprises more than 220 species[1] and is widespread throughout the Mediterranean area and Central Asia. It is represented by 24 species, 13 of which are endemic in the Flora of Turkey[2-4].Several species, such as *Ferula assa-foetida*, *Ferula gummosa* and *Ferula latisecta* have been used in folk medicine to treat stomachache, hysteria, infant colitis, and asthma[5]. The extracts of *Ferula persica*, *Ferula mongolica*, *Ferula ferulago* and *Ferula sinaica* have been used in traditional medicine as antidiabetic[6], abortive[7], an-

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tispasmodic[8] and muscle relaxant[9] respectively. Different parts of *Ferula* have been used in treating various diseases such as neurological disorders, inflammations, dysentery, digestive disorders, rheumatism, headache, arthritis, and dizziness[10]. They also have been reported as antipyretic[11], contraceptive[12,13], and smooth muscle relaxant[14]. *F. assa-foetida* is used traditionally in the treatment of diabetes, asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion, bronchitis, and influenza. It was also believed that this plant has aphrodisiac, sedative and diuretic properties[15].

Recent studies have shown *Ferula assa-foetida* gum extract improved the morphological changes of the diabetic pancreas and stimulated the regeneration of the β cells[16]. Several studies showed hypotensive, neuroprotective, memory-enhancing, anti-oxidant, hepatoprotective, antimicrobial, anticarcinogenic, anti-obesity and anthelmintic effects for various species of *Ferula* and their constituents[17,18]. Pharmacological and biological studies indicate that the extracts and compounds of the genus *Ferula* have various biological activities, such as antibacterial[18], anti-inflammatory[19], antihypertensive[20] and cytotoxic[21]. The main phytochemical components present in the genus *Ferula* are coumarins, coumarin esters, sesquiterpenes, sesquiterpene lactones, monoterpene, monoterpene coumarins, prenylated coumarins, sulfur-containing compounds, phytoestrogen, flavonoids and carbohydrates[19].

Previous studies reported that the aerial parts extracts of *Ferula halophila* exhibited antiviral[22], antioxidant, α-amylase, α-glucosidase, tyrosinase and cholinesterase inhibitory activity and contain phenolic compounds[23,24]. Different bioactivities and uses of the *Ferula* species essential oils have been reported[25]. There are no reports in the literature dealing with the essential oil of *F. halophila*. This work is the first report concerning the chemical composition and antibacterial and antifungal activity of the essential oil obtained from the fruits of *F. halophila*. The hydrodistilled essential oils of the dried fruits of endemic species *Ferula halophila* were analyzed by GC and GC-MS and tested for their antibacterial and antifungal activity using micro-broth dilution methods.

METHODOLOGY

Plant material

The fruit specimens of *Ferula halophila* were collected near Salt Lake in Central Anatolia in June and July 2012. A voucher specimen identified by Prof. Dr. H. Duman (Gazi University, Ankara) was deposited in the Herbarium of Gazi University (GAZI Nr. 9898000001582).

Isolation of the essential oils

Dried and crushed fruits of the plant were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The oil yields of the fruits collected in June and July were 1.3 and 0.6 %, respectively on a moisture-free basis. The oil was dried over anhydrous sodium sulphate and stored in sealed vials in the dark, at 4°C, ready for GC and GC/MS analyses and biological activity.

GC and GC/MS conditions

GC/MS: The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60m x 0.25mm, 0.25μm 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 40:1. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

GC: The GC analysis were done with Agilent 6890N GC system fitted with a FID detector set at a temperature of 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.

Identification of compounds

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, Mass Finder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in calculating relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of the analysis are shown in Table 1.

Table 1. The Composition of the essential oils of *Ferula halophila*

RRI	Compounds	EO1	EO2	IM
1032	α-Pinene	1.0	0.3	t_R, MS
1174	Myrcene	3.3	1.8	t_R, MS
1176	α-Phellandrene	1.7	0.6	t_R, MS
1203	Limonene	1.4	1.1	t_R, MS
1210	β-Phellandrene	71.8	37.8	t_R, MS
1280	*p*-Cymene	0.5	0.5	t_R, MS
1290	Terpinolene	tr	-	t_R, MS
1481	Longipinene	0.3	1.1	MS
1493	α-Ylangene	0.3	2.0	MS
1504	Daucene	0.1	-	MS
1513	Longicyclene	0.1	0.6	t_R, MS
1549	β-Cubebene	0.4	0.9	MS
1550	cis-α-Bergamotene	-	tr	MS
1568	trans-α-Bergamotene	-	0.6	MS
1590	Bornyl acetate	0.3	-	t_R, MS
1612	β-Caryophyllene	-	0.9	t_R, MS
1661	α-Himachalene	0.8	4.5	MS
1687	α-Humulene	0.3	-	t_R, MS
1711	g-Himachalene	1.0	3.8	MS
1729	γ-Himachalene	0.6	2.9	MS
1743	Eremophilene	4.9	7.0	MS
1755	Dauca-8,11-diene	0.2	1.0	MS
1783	β-Sesquiphellandrene	0.1	-	MS
1786	ar-Curcumene	0.2	-	MS
2008	Caryophyllene oxide	0.4	1.2	t_R, MS
2045	β-Himachalene oxide	tr	0.5	MS
2045	Carotol	tr	-	MS
2179	6-Epi-cubenol	-	0.8	MS
2232	α-Bisabolol	0.5	1.9	t_R, MS
2232	2-Himachalen -7-ol	0.8	7.3	MS
2296	Myristicine	1.1	0.7	MS
	Grouped compounds (%)			
	Monoterpene hydrocarbons	79.7	42.1	
	Sesquiterpene hydrocarbons	9.3	25.3	
	Oxygenated sesquiterpenes	1.7	11.7	
	Others	1.4	0.7	
	Total %	92.1	79.8	

EO1 and EO2: Dried fruits essential oils of *F. halophila* collected in June and July. RRI: Relative retention indices calculated against *n*-alkanes; %: calculated from the FID chromatograms; tr: Trace (<0.1 %). Identification method (IM): t_R, identification based on the retention times of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data.

Antimicrobial assay

Antibacterial and anticandidal effects of the samples were evaluated by using partly modified CLSI (formerly NCCLS) micro dilution broth methods M7-A7 and M27-A2 respectively[26,27].

Escherichia coli NRRL B-3008, *P. aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Bacillus cereus* NRRL B-3711, *B. subtilis* NRRL B-4378, *Serratia marcescens* NRRL B-2544, *Staphylococcus epidermidis* ATCC 12228, *E. coli* O157:H7 RSSK 234 (RSSK; RSHM National Type Culture Collection Strains of Bacteria), two different strains of *Candida albicans* (clinically isolated, Osmangazi University, Faculty of Medicine, Department of Microbiology and ATCC 90028), *C. utilis* NRRL Y-12968, *C. krusei* NRRL Y-7179, *C. glabrata* (clinically isolated, Osmangazi University, Faculty of Medicine, Department of Microbiology and ATCC 90028) were used as the test microorganisms. Chloramphenicol (Merck), Ampicillin (Merck), Amphotericin-B (Sigma-Aldrich) and Ketoconazol (Sigma-Aldrich) were used as standard antimicrobial agents.

RESULTS AND DISCUSSION

The essential oils of the dried fruits of *F. halophila* collected in June and July were analysed by GC and GC-MS systems; 27 and 24 compounds representing 92.1% and 79.8% of the essential oils were characterized, respectively. The main component of the essential oils was identified as β-phellandrene (72%) collected in June. The other EO distilled from July samples were consist of several sesquiterpenes and decreased β-phellandrene content (38%). Monoterpene hydrocarbons (June and July; 79.7%, 42.1%), sesquiterpene hydrocarbons (9.3%, 25.3%) and oxygenated sesquiterpenes (1.7%, 11.7%) were the main groups present in the oils respectively. Monoterpene hydrocarbons were the most abundant among these groups representing 79.7% collected in June harvest while 42.1% in the sample of collected in July, followed by sesquiterpenes and oxygenated sesquiterpenes 11.0% and 37.0 % respectively. While monoterpenes were high in the essential oil obtained from the plant material collected in June, monoterpenes were decreased and sesquiterpenes increased in the samples collected in July.

The antibacterial and anticandidal effects of the essential oils were determined by using partly modified CLSI methods M7-A7 and M27-A2, respectively. Tables 2 and 3, show that the essential oils from two specimens exhibited weak to moderate inhibitory effects on the tested pathogenic bacteria (MIC, 125-2000 µg/mL) and *Candida* panels (MIC, 156-1250 µg/mL). Interestingly, essential oils obtained from June and July plant samples were demonstrated different bioactivity.

Table 2. Antibacterial effects of F. halophila essential oils (MIC, µg/mL)

Microorganisms	EO1	EO2	S1	S2
Escherichia coli	2000	2000	3.9	1
Pseudomonas aeruginosa	2000	2000	62.5	15.6
Salmonella typhimurium	500	500	3.9	1
Bacillus cereus	1000	1000	7.8	1
Bacillus subtilis	500	125	1.9	1
Serratia marcescens	1000	500	15.6	15.6
Staphylococcus epidermidis	1000	1000	3.9	1
E. coli O157:H7	1000	2000	3.9	1

EO1 and EO2: Dried fruits essential oils of *F. halophila* collected in June and July, **S1:** Chloramphenicol, **S2:** Ampicillin

Table 3. Anticandidal effects of F. halophila essential oils (MIC, µg/mL)

Microorganisms	EO1	EO2	S1	S2
*Candida albicans**	625	625	0.05	0.1
Candida utilis	625	156	1.6	0.05
Candida tropicalis	625	1250	0.2	0.2
Candida krusei	625	312	1.6	0.2
Candida albicans	1250	625	0.1	0.2
Candida glabrata	1250	1250	3.2	0.2

EO1 and EO2: Dried fruits essential oils of *F. halophila* collected in June and July, **S1:** Ketoconazole, **S2:** Amphotericin-B, *: Clinically isolated strain

Except from *E. coli* O157:H7 and *C. tropicalis*, essential oil of the *F. halophila* collected in July were more active against all test microorganisms having MIC values between 125-2000 µg/mL. July sample was also rich in himachalenes. A previous study was reported that the himachalanes were demonstrated antibacterial activity at various doses 46 to 3000 µg/mL (MIC) [28]. In other study a correlation have been found between the antibacterial activity against MRSA and sesquiterpene compounds in *Ferula akitsckensis* essential oil obtained from leaves at budding stage [29]. β-phellandrene-rich June essential oil were showed weaker effects against all test panel between the concentration of 625 to 2000 µg/mL.

To our knowledge, no previous study has examined the antimicrobial effects of *Ferula halophila* essential oils. Furthermore, it was also showed in this study that the plants collected in different months have different inhibitory effects.

In a previous study, dichloromethane extract of the roots of *Ferula halophila* was evaluated for its antimicrobial activity against 36 different pathogenic bacteria and *Candida* strains. *Stenotrophomonas maltophilia* and *Candida albicans* were inhibited by the extract at lowest concentration (0.3 mg/ml). MIC values have been determined for other test strains ranking from 10 mg/ml to 0.3 mg/ml [30].

In other study, antimicrobial activities of water, methanol extracts and their several fractions of the aerial and underground parts of *F. halophila* were screened by using disc diffusion method. *Staphylococcus aureus, Bacillus cereus* and *B. subtilis* were determined as the most susceptible test strains with 7-10 mm inhibition zones. Chloroform fraction were demonstrated moderate antibacterial effects [31].

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