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Composition and Antibacterial Activity of the Essential Oil of *Ferulago confusa* Velen.

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Abstract

Water-distilled essential oil from the crushed fruits of *Ferulago confusa* Velen, collected from Bursa: Keles-Orhaneli in Turkey, was analyzed by GC and GC/MS. Ninety-one components were identified representing 99.5% of the oil. Cis-chrysanthenyl acetate (37.7%) and α-pinene (36.7%) were characterized as the main constituents.

The antibacterial effects of the *Ferulago confusa* oil were evaluated by using microdilution broth method. The oil showed moderate inhibitory effects on the selected human pathogenic bacteria having MIC values ranging 0.6–2.5 mg/mL.

Key Word Index

Ferulago confusa, Umbelliferae, essential oil composition, antibacterial activity, cis-chrysanthenyl acetate, α-pinene

Introduction

The genus *Ferulago* (Umbelliferae) is widely distributed in Anatolia and comprises thirty species, sixteen of them being endemic. As forty-five *Ferulago* species are described in the world, it is a high probability that the gene center for this genus is Anatolia. *Ferulago* species are known as kişniş, kuzu başı, kuzu kemirdi, çakşır and resemble *Ferula* and *Prangos* species, also widely abundant in Turkey (1-5).

Essential oils of *Ferulago asparagifolia* Boiss., *F.galbanifera* (Miller) W. Koch., *F. humilis* Boiss., *F. trachycarpa* Boiss., *Ferulago isaurica* Peşmen and *F. syriaca* Boiss. have previously been investigated by the authors (5-8).

Micro-distilled volatile compounds from *Ferulago* Species (*Ferulago asparagifolia* Boiss., *F.aucheri* Boiss., *F. confusa* Velen., *F.galbanifera* (Miller) W. Koch., *F. humilis* Boiss., *F. idaea* N. Özhatay et E. Akalın, *F. macrosciadia* Boiss., et Ball., *F. mughlae* Peşmen, *F. sandrasica* Peşmen et Quezel., *F. silaifolia* (Boiss.) Boiss., *F. sylvatica* (Beser) Reichb. and *F. trachycarpa* Boiss. growing in Western Turkey have also been investigated (9).

A new monoterpene ester (Ferulagone) was isolated from *Ferulago thirkeana* (Boiss.) Boiss. essential oil (10).

The oil composition of *F. contracta* Boiss. et Hausskn. from Iran has been reported to contain α- and β-phellandrene as major constituents of the flower oil and p-cymene and α-phellandrene as the major components of the stem oil (11).

The authors have previously investigated the antimicrobial activity of *Ferulago asparagifolia* Boiss., *F.galbanifera* (Miller) W. Koch., *F. humilis* Boiss., *F. trachycarpa* Boiss. (5).

Here the authors report findings on the chemistry and antibacterial activities of *F. confusa* from Turkey.

Experimental

Plant material: Fruits of *Ferulago confusa* were collected from Bursa: Keles-Orhaneli village in Turkey in July 2006. Voucher specimens are kept at the Herbarium of the Faculty of Science and Letters, Uludag University in Bursa, Turkey (BULU 30000).

Isolation of the essential oils: Crushed fruits (35 g) of the plant were water-distilled for 3 h using a Clevenger-type apparatus (yield 2.4 %).

GC/MS: 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 He 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

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Table I. Percentage composition of the essential oil of *Ferulago confusa*

RRI	Compound	%	RRI	Compound	%
1032	α-pinene	36.7	1668	(Z)-β-farnesene	0.1
1035	α-thujene	tr	1671	ipsdienol	tr
1072	α-fenchene	tr	1683	*trans*-verbenol	1.2
1076	camphene	0.2	1700	p-mentha-1,8-dien-4-ol (=limonen-4-ol)	tr
1093	hexanal	tr	1704	γ-curcumene	0.2
1118	β-pinene	1.6	1725	verbenone	tr
1132	sabinene	0.4	1726	germacrene D	1.7
1136	thuja-2,4 (10)-diene	tr	1738	p-mentha-1,5-dien-8-ol	0.2
1159	δ-3-carene	tr	1741	β-bisabolene	tr
1174	myrcene	4.5	1755	bicyclogermacrene	0.2
1176	α-phellandrene	2.7	1764	*cis*-chrysanthenol	1.7
1183	p-mentha-1,7(8)-diene (=*pseudolimonene*)	tr	1773	δ-cadinene	tr
1188	α-terpinene	tr	1786	ar-curcumene	0.1
1195	dehydro-1,8-cineole	tr	1804	myrtenol	0.1
1203	limonene	1.5	1823	p-mentha-1(7),5-dien-2-ol	tr
1210	β-phellandrene	1.7	1845	*trans*-carveol	0.1
1215	p-mentha-1, 3, 6-triene	tr	1854	germacrene-B	0.2
1244	amylfuran (=2-pentylfuran)	tr	1864	p-cymen-8-ol	tr
1246	(Z)-β-ocimene	3.6	1900	epi-cubebol	tr
1255	γ-terpinene	tr	1921	α-phellandrene epoxide	tr
1266	(E)-β-ocimene	0.3	1925	2, 3, 4-trimethyl benzaldehyde	tr
1280	p-cymene	0.8	2008	caryophyllene oxide	tr
1290	terpinolene	0.4	2037	salvial-4(14)-en-1-one	tr
1382	allo-ocimene*	tr	2057	ledol	tr
1413	rose furan	0.1	2073	1, 10-diepi-cubenol	tr
1429	perillen	tr	2080	cubenol	tr
1466	α-cubebene	tr	2098	globulol	tr
1476	(Z)-β-ocimene epoxide	tr	2104	viridiflorol	tr
1478	6-campholenone*	tr	2100	heneicosane	tr
1496	α-campholene aldehyde	0.1	2123	salviadienol	tr
1497	α-copaene	0.1	2144	spathulenol	0.3
1535	β-bourbonene	tr	2198	thymol	tr
1544	α-gurjunene	tr	2209	T-muurolol	tr
1549	β-cubebene	tr	2214	ar-turmerol	tr
1571	*trans*-p-menth-2-en-1-ol	tr	2219	carvacrol	tr
1582	*cis*-chrysanthenyl acetate	37.7	2237	*trans*-α-bergamotol	tr
1590	bornyl acetate	0.1	2243	torilenol	0.1
1594	*trans*-β-bergamotene	tr	2255	α-cadinol	tr
1600	β-elemene	tr	2369	eudesma-4(15), 7-dien-1β-ol	0.1
1611	terpinen-4-ol	0.1	2373	germacra-4(15), 5,10(14)-trien-1α-ol*	0.1
1617	acora-3,5-diene	0.1	2503	dodecanoic acid (=*lauric acid*)	tr
1648	myrtenal	0.1	2565	14-hydroxy-α-muurolene	tr
1650	γ-elemene	tr	2607	14-hydroxy-δ-cadinene	tr
1661	*trans*-pinocarvyl acetate	0.2	2655	benzyl benzoate	tr
1670	*trans*-pinocarveol	0.1			

RRI: Relative retention indices tr: trace <0.1% *tentative

Table II. MIC (mg/mL) values of *Ferulago confusa* oil (EssOil) against human pathogenic bacteria

Pathogen	Strain	EssOil	Chloramphenicol
Bacillus cereus, (Gr +)	NRRL B-3711	0.625	0.03125
Enterobacter aerogenes, (Gr -)	NRRL 3567	2.5	0.125
Escherichia coli, (Gr -)	NRRL B-3008	2.5	0.0156
Pseudomonas aeruginosa, (Gr -)	ATCC 27853	2.5	0.125
Salmonella typhimurium, (Gr -)	ATCC 13311	1.25	0.03125
Serratia marcescens, (Gr -)	NRRL B-2544	1.25	0.0625
Staphylococcus aureus, (Gr +)	ATCC 6538	1.25	0.0625
S. aureus (MRSA),* (Gr +)	Clinical isolate	1.25	0.03125

** Methycilline resistant strain*

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: GC analysis was carried out using an Agilent 6890N GC system. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions. FID temperature was 300°C. The essential oil components were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of 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 I.

Microorganisms: All tested microorganisms were acquired from ATCC, NRRL and clinical isolates (Faculty of Medicine, Eskisehir Osmangazi University, Turkey) and were stored at -85°C (New Brunswick, Ultralowfreezer) in micro test tubes (Eppendorf) containing 15% glycerol. Gram-positive (G+) and Gram-negative (G-) microorganisms were refreshed by inoculation on Mueller Hinton Agar (MHA, Acumedia) at 37°C for 24 h. After suffient growth all the microorganisms were transfered in to a liquid medium (Mueller Hinton Broth MHB, Merck) and were incubated at the same conditions

Antibacterial microdilution assay: Antibacterial activities of the essential oil were evaluated in vitro using a microdilution broth method (12,13). Stock solutions of the test samples were prepared in 25% (v/v) dimethylsulfoxide (DMSO, Carlo Erba). Serial dilutions of samples were prepared up to 0.001 mg/mL by using sterile MHB in a 96-well microtiter plate format. Previously grown bacterial suspensions as above were adjusted to $1x10^8$ CFU/mL (visually using McFarland No: 0.5 standard solution) in Mueller-Hinton broth (MHB, Merck, Germany). Finally 100 µL of each bacterial suspension was then added to the appropriate well. The last row, which contained only the serial dilutions of the oil without microorganism, was used as a negative control. To eliminate the solvent effects DMSO dilutions were considered as another control. After incubation at 37ºC for 24 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC, mg/mL). Chloramphenicol was used as internal standard and antibacterial agent. All experiments were repeated in triplicate and average MICs are given in Table II.

Results and Discussion

Previously, microdistilled oil composition of *Ferulago confusa* was reported. The volatiles were obtained from crushed fruits of *F. confusa* by micro-distillation. The main constituent was found as 2,5-dimethoxy-p-cymene (63.4%) (9).

In this present study, crushed fruits of *F. confusa* yielded 2.4% oil by water-distillation. The oil was analyzed by GC and GC/MS. Ninety three compounds were characterized representing 99.5% of the oil with cis-chrysanthenyl acetate (37.7%) and α-pinene (36.7%) as main constituents.

The antibacterial effect of the *F. confusa* oil was evaluated by a microdilution broth method. The oil showed weak to moderate inhibitory effects on selected Gram-positive and Gram-negative human pathogenic bacteria (MIC = 0.6–2.5 mg/mL). Best inhibition was observed against the common food pathogen *Bacillus cereus* with a concentration of 0.625 mg/mL. It was also noticed that the oil was moderately effective (MIC = 1.25 mg/mL) against *Salmonella typhimurium*, *Serratia marcescens, Staphylococcus aureus* and MRSA.

In a previous report the authors found that the oil of *F. bernardii* Tomk. et M. Pimen. contained 2,4,5-trimethyl-benzaldehyde (21.2%) and α-pinene (17.0%) as main constituents. It also showed a weak to moderate activity against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* having MIC values of >1000, 125, 250 µg/mL, respectively (14).

In another study *F. thyrsiflora*, *F. sylvatica* and *F. nodosa* oils had shown moderate activity against *S. aureus* and *S. epidermidis* having same MIC values 350, 400 and 450 µg/mL, respectively (15).

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