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Chemical composition and antimicrobial activity of the essential oil from *Ferula glauca* L. (*F. communis* L. subsp. *glauca*) growing in Marche (central Italy)

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ABSTRACT

The essential oil obtained from different parts of *Ferula glauca* L. (formerly considered as a subspecies of *F. communis*) growing in Marche (central Italy), was analyzed for the first time by GC-FID and GC-MS. The major volatiles were (*E*)-caryophyllene and caryophyllene oxide in leaves, α-pinene, myrcene and germacrene D in flowers, α- and β-pinene in fruits, (*E*)-β-farnesene, myristicin and elemicin in roots, respectively. The differences in composition detected with respect to *F. communis*, made the volatile fraction a reliable marker to distinguish between them, and confirm the botanical data at the base of their discrimination. Furthermore, the oil was assayed for its antimicrobial activity by the broth microdilution method. *B. subtilis* was found to be the most sensitive microorganism, with the lowest MIC values.

1. Introduction

The genus *Ferula*, belonging to the family Apiaceae, includes about 170 species occurring from central Asia westward throughout the Mediterranean region to northern Africa [1]. The Italian Flora comprises of 3 species: *F. arrigonii* Bocchieri, *F. communis* L. and *F. glauca* L. [2].

Ferula glauca was formerly considered to the range of subspecies of *F. communis* (i.e. *F. communis* subsp. *glauca*) [3], but actually is considered a different species, distinguishable by several differences in terms of morphology, anatomy, phenology and ecology [2,4,5].

No ethnobotanical data are reported for *F. glauca*, while the decoction of dried roots of *F. communis* was used in Sardinia as antiseptic [6]. However, the latter was reported to include two chemotypes occurring in Sardinia, one chemotype being highly toxic to animals and humans [7].

To our knowledge, *F. glauca* has never been the subject of deep phytochemical investigations; most of them focused on *F. communis* and lead to the isolation and characterization of prenylated coumarins and daucane esters from poisonous and non-poisonous chemotypes, respectively [7–10]. In particular, daucane esters isolated from several species of the genus *Ferula*, such as ferutinin and its analogues, are known to be potent phytoestrogen molecules, having an affinity for the estrogen receptors, and therefore able to produce positive effects on menopausal-associated disorders [9].

As concerning volatile fraction, that can be often a helpful tool to discriminate between different taxa, no papers have been reported to date on *F. glauca*, while few studies were recently conducted on *F. communis*. As major volatiles the following were reported: myrcene and aristolene in the leaf oil from Corsica [11]; α- and β-gurjunene in flowerheads oil from Sardinia [12]; aristolene and farnesol in the poisonous chemotype, and

Abbreviations: LV, leaves;FL, flowers;FR, fruits;RT, roots.

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allohedycaryol in the nonpoisonous chemotype, respectively, from aerial parts of Sardinian populations [13].

In the present study, we report for the first time the composition and the antimicrobial activity of the essential oils obtained from different parts of *F. glauca* growing in Marche (central Italy).

2. Experimental

2.1. Plant material

Leaves (LV), flowers (FL) fruits (FR) and roots (RT) of the plant were collected in Pioraco (central Italy, GPS coordinates: N 43°10′38″ E 12°59′53″) in May–June 2007. The plant was botanically confirmed using available literature [3,4]. A voucher specimen was deposited in the Herbarium Camerinensis, Dept. of Environmental Sciences, Sect. of Botany and Ecology, University of Camerino, Italy, under the accession code CAME 13402; it is also available at the following website: http://erbariitaliani.unipg.it.

2.2. Extraction of essential oil

Essential oils were obtained by hydrodistillation of dried LV, FL, FR and RT using a Clevenger-type apparatus for 4 h. *n*-Hexane (10 ml) was used as the collector solvent as reported in literature [14]. After evaporation of the solvent under N_2 flow, the oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at −20 °C before analyses. Three oil samples for each collection were obtained by hydrodistillation and subsequently analyzed by GC-FID and GC-MS. The oil yields (0.02–0.07%) were calculated on a dry weight basis.

2.3. GC-FID and GC-MS analysis

GC-FID analysis of the volatile components was carried out using an Agilent 4890D instrument with FID detector and a HP-5 capillary column (5% phenylmethylpolysiloxane, 25 m, 0.32 mm i.d.; 0.17 μm film thickness) (J & W Scientific, Folsom, CA), working with the following temperature program: 5 min at 60 °C, and subsequently at 4 °C/min up to 220 °C, then 11 °C/min up to 280 °C, held for 15 min; injector and detector temperatures, 280 °C; carrier gas, helium (1.4 ml/min); injection volume of 1 μL, split ratio, 1:34. GC-MS analysis was performed using an Agilent 6890N-5973N GC-MS system operating in the EI mode at 70 eV, using a HP-5MS capillary column (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness) (J & W Scientific, Folsom), which was programmed at 60 °C for 5 min, then ramp at 4 °C/min to 220 °C, then 11 °C/min up to 280 °C, held for 15 min, finally 11 °C/min up to 300 °C, held for 5 min; carrier gas: helium; flow rate: 1.0 ml/min; injector and transfer line temperatures: 280 °C; injection volume: 2 μl; split ratio: 1:50; scan time: 75 min; acquisition mass range: 29–400 amu.

2.4. Identification and quantification of volatile components

The identification of volatile components was based on computer matching with the WILEY275, NIST05, and ADAMS libraries, as well as by comparison of the mass spectra and retention indices (RI) with those reported in the literature [15,16]. In addition, a home-made library, constructed based on the analyses of reference oils and commercial available standards, was used as well. Whenever possible, components were identified by comparison of their retention times, mass spectra and retention indices relative to *n*-alkanes with those of authentic standards (obtained from Sigma-Aldrich, Milan, Italy) available in author's laboratory. Daucane esters teferdine and ferutidine were identified by comparison of mass spectra with those of pure compounds furnished by Prof. Rubiolo (Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin, Italy) and with those reported in literature [13]. Percentage compositions of the oil components were obtained from electronic integration using flame ionization detection (FID, 280 °C), dividing the area of each component by the total area of all components isolated under these conditions. The percentage values for volatile components were the mean of three injections of each oil sample.

2.5. Chemicals

Pure commercial essential oil components, used as standards for GC-FID and GC-MS analyses, were obtained from Sigma-Aldrich (Milan, Italy). Teferdine and ferutidine were kindly supplied by Prof. Rubiolo (Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin). All compounds were of analytical standard grade. *n*-Hexane was an analytical grade solvent purchased from Carlo Erba (Milan, Italy); it was distilled by a Vigreux column before use. Na_2SO_4 was of analytical reagent grade from J.T. Baker (Deventer, Holland).

2.6. Microorganism and growth conditions

Staphylococcus aureus (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 13706) and *Candida albicans* (ATCC 14053) were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). *Streptococcus mutans* (DSM 20523) was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen, GmbH (DSM, Braunschweig, Germany). Bacterial strains were cultivated on Müeller Hinton Broth (OXOID, Unipath Limited, Basingstoke, UK) while *C. albicans* was cultivated on Sabouraud liquid Medium (OXOID). Cells cultures were incubated at 37 °C for 24 h and then used. The cells suspension was adjusted with sterile saline solution to obtain a turbidity comparable to that of McFarland n. 0.5 standard (1.5×10^8 cells/ml).

2.7. Minimal inhibitory concentration (MIC)

MIC was determined using a broth microdilution method with a 96-well microtiter plate [17]. One series of 2-fold dilutions of each oil sample in acetone (ranging from 10 to 0.001 mg/ml) for each microbial strain was prepared using the same media as above. Each series was inoculated with 0.8 μl of each microbial strain (1.5×10^8 cells/ml). Determinations were carried out evaluating the microbial growth in the wells using a stereomicroscope (GSZ2, Ascania, Germany). Chloramphenicol for Gram-positive and Gram-negative bacteria, and

Table 1
Constituents of the essential oil from *Ferula glauca* growing in Marche (central Italy)

Component [a]	RI [b]	% [c]				Identification [e]
		LV	FL	FR	RT	
Hexanol	874		0.2			MS,RI
α-Thujene	928	0.1	0.1			MS, RI
α-Pinene	934	0.3	11.7	24.2	0.5	MS, RI, std
Camphene	948		0.1			MS, RI, std
Sabinene	972		0.1			MS, RI, std
β-Pinene	976	0.1	5.1	14.7		MS, RI, std
Myrcene	993	4.2	13.6	3.4		MS, RI, std
α-Phellandrene	1004		0.1			MS, RI, std
Octanal	1006	tr [d]	0.1			MS, RI, std
δ-3-Carene	1011	1.2	0.8			MS, RI, std
o-Cymene	1024	tr	tr			MS, RI
p-Cymene	1029	0.1	0.5			MS, RI, std
Sylvestrene	1032	0.6	1.5			MS, RI
β-Phellandrene	1032			2.8		MS, RI
Benzene acetaldehyde	1044		2.3			MS, RI
(*Z*)-β-Ocimene	1045	0.5				MS, RI
(*E*)-β-Ocimene	1051		0.2			MS, RI
γ-Terpinene	1063		1.9			MS, RI, std
p-Mentha-2,4(8)-diene	1088	0.1	0.1			MS, RI
Terpinolene	1090	0.1	tr			MS, RI, std
6-Camphenone	1090			1.6		MS, RI
Perillene	1104	0.3	0.1			MS, RI
Nonanal	1111	0.1	0.1			MS, RI
α-Campholenal	1125	0.1				MS, RI
allo-Ocimene	1134		0.1			MS, RI, std
2E-Nonen-1-al	1168			0.5		MS, RI
p-Mentha-1,5-dien-8-ol	1173	0.2	0.1			MS, RI
Terpinen-4-ol	1181	0.1	0.1	1.9		MS, RI, std
α-Terpineol	1198	tr	0.2	2.7		MS, RI, std
Myrtenal	1198			2.2		MS, RI, std
α-Cubebene	1349	0.1	0.1			MS, RI, std
α-Copaene	1374	0.4	0.4			MS, RI, std
Isoledene	1374		0.1			MS, RI
Daucene	1378	0.3	0.7		1.7	MS, RI
β-Bourbonene	1381	0.5	0.3			MS, RI
β-Cubebene	1387	0.1	0.2			MS, RI
β-Elemene	1391		0.2			MS, RI
Italicene	1398	0.3	0.2			MS, RI
α-Cedrene	1406	0.4	0.5			MS, RI, std
2-epi-β-Funebrene	1406		0.5		2.2	MS, RI
(*E*)-Caryophyllene	1415	24.9	8.2	1.1		MS, RI, std
cis-Thujopsene	1422	0.3	0.5			MS, RI
β-Copaene	1426	0.2	0.2			MS, RI
trans-α-Bergamotene	1432		0.2			MS, RI
β-Barbatene	1436	0.3	0.6		6.1	MS, RI
cis-Muurula-3,5-diene	1444	0.2	0.2		1.0	MS, RI
α-Humulene	1450	6.8	2.5			MS, RI, std
cis Cadina-1(6),4-diene	1458		0.2		1.3	MS, RI
(*E*)-β-Farnesene	1460	0.7	1.5		10.0	MS, RI
α-Acoradiene	1465	0.8	0.8			MS, RI
cis-Muurola-4(14),5-diene	1467	0.2	0.5		1.3	MS, RI
β-Acoradiene	1472	0.4	0.6			MS, RI
β-Chamigrene	1473				1.2	MS, RI
Germacrene D	1478	5.7	14.2	2.1		MS, RI, std
γ-Curcumene	1481	1.0	1.8			MS, RI
ar-Curcumene	1483	2.9	0.1	0.6	1.0	MS, RI
Bicyclogermacrene	1491	0.6	1.1			MS, RI
Isodaucene	1492				1.1	MS, RI
β-Himachalene	1496		0.7			MS, RI
Epizonarene	1497				7.1	MS, RI
Cuparene	1498	1.4	3.6			MS, RI
α-Zingibirene	1502	1.1	0.6		4.7	MS, RI
β-Bisabolene	1508				5.1	MS, RI
β-Curcumene	1510		1.0	1.9		MS, RI
(*E*,*E*)-α-Farnesene	1511	1.8	3.6	2.7		MS,RI
γ-Cadinene	1512				6.8	MS,RI
β-Bisabolene	1513		2.8			MS, RI
(*Z*)-γ-Bisabolene	1514		0.2			MS, RI
β-Sesquiphellandrene	1521		0.3			MS, RI
trans-Calamenene	1522		0.1		3.9	MS, RI
δ-Cadinene	1523	1.1	1.5	0.7		MS, RI
Myristicin	1527				7.4	MS, RI, std
γ-Cuprenene	1531	0.3	0.5		1.2	MS, RI
(*E*)-γ-Bisabolene	1534		0.2			MS, RI
α-Copaen-11-ol	1541		0.2			MS, RI
α-Calacorene	1542	0.3	0.2			MS, RI
Elemicin	1566				9.0	MS, RI
E-α-Isomethyl-ionol acetate	1569			1.6		MS, RI
Spathulenol	1577	0.7	0.3			MS, RI
Caryophyllene oxide	1581	14.3	0.7			MS, RI, std
Salvial-4(14)-en-1-one	1592	0.4	1.0			MS, RI
Humulene epoxide II	1606	2.8	0.3			MS, RI
Caryophylla-4(12), 8(13)-dien-ol	1636	0.3				MS, RI
epi-α-Cadinol	1641				2.9	MS, RI
Himachalol	1645	0.3	0.3	1.0		MS, RI
α-Cadinol	1655	0.4	0.6		2.8	MS, RI
β-Atlantone	1666		0.4			MS, RI
14-Hydroxy-9-epi-trans-caryophyllene	1672	0.8				MS, RI
Eudesma-4(15),7-dien-1β-ol	1686	0.3	0.4			MS, RI
Neophytadiene	1837	1.2	0.5			MS, RI
Methyl hexadecanoate	1924		0.1			MS, RI
Hexadecanoic acid	1970	1.6	1.1	1.6		MS, RI, std
Ethyl hexadecanoate	1996		0.1			MS, RI
Phytol	2113	2.6	0.3			MS, RI, std
Ethyl linoleate	2163		0.1			MS, RI
Teferdine	2391				0.6	MS, std
Pentacosane	2504	0.2	0.1			MS, RI, std
Ferutidine	2639		tr	1.3	0.4	MS, std
Heptacosane	2662	0.1	0.1			MS, RI, std
Nonacosane	2900	0.3	0.3			MS, RI, std
						MS, RI, std
Total identified (%)		87.3	96.8	68.7	79.7	
Grouped compounds (%)						
Monoterpene hydrocarbons		7.3	38.3	45.1	0.5	
Oxygenated monoterpenes		0.7	0.4	8.3		
Sesquiterpene hydrocarbons		52.8	50.8	9.2	56.1	
Oxygenated sesquiterpenes		20.3	4.2	2.7	5.7	
Diterpenes		3.8	0.8			
Phenylpropanoids					16.4	
Daucane esters			tr	1.3	1.0	
Aliphatics		2.3	2.3	2.1		

[a] Compounds are listed in order of their elution from a HP-5 column.
[b] RI, linear retention indices as determined on HP-5 column using homologous series of C8–C30 alkanes.
[c] Percentages obtained by FID peak-area normalization; values represent an average of three determinations.
[d] tr, traces (<0.1%).
[e] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries and Adams [14]; RI, by comparison of RI with those reported from Adams [14] and NIST05 [15]; std, by injection of an authentic sample. LV: leaves, FL: flowers; FR: fruits; RT: roots.

amphotericin B for yeasts (both from Sigma-Aldrich, Buchs, Switzerland), were used as positive controls. Available standard β-pinene, (*E*)-caryophyllene and caryophyllene oxide (all from Sigma-Aldrich, Milan, Italy) were also tested under identical conditions to compare their activities with that of the investigated oils. All the experiments were conducted in triplicate. A negative control, inoculating acetone without oils, was also included.

Table 2
Antimicrobial activity (MIC) of *Ferula glauca* essential oils and of β-pinene, (*E*)-caryophyllene and caryophyllene oxide

Microorganisms	MIC (μg/ml)[a]							
	Essential oil samples				Pure components			Positive control[b]
	LV[g]	FL[h]	FR[g,i]	RT[i,h]	β-pinene	(*E*)-caryophyllene	caryophyllene oxide	
S. aureus ATCC 25923[c]	625	1250	625	1250	2500	2500	1250	5
S. mutans DSM 20523[d,]	310	1250	310	310	625	1250	2500	10
B. subtilis ATCC 6633[e]	78	38	310	78	310	310	155	10
E. faecalis ATCC 29212[d]	310	625	310	625	2500	2500	2500	10
E. coli ATCC 13706[d,]	625	625	625	625	2500	625	1250	5
C. albicans ATCC 14053[f]	1250	1250	1250	1250	310	155	78	1

[a]Values represent an average of three determinations.
[b]Chloramphenicol for gram-positive and gram-negative bacteria, Amphotericin B for yeast.
[c,d,e,f,g,h,i]Samples and strains having different letters are significantly different from each other using Student's *t*-test ($p \leq 0.05$). LV: leaves, FL: flowers; FR: fruits; RT: roots.

2.8. Statistical analysis

The antimicrobial activity of essential oils was evaluated by the Student's *t*-test, using the SPSS 13.0 software package for Windows. Values of $p \leq 0.05$ were considered as statistically significant.

3. Results and discussion

Chemical composition of the essential oils from different parts of *F. glauca* is reported in Table 1. One hundred components were identified in the volatile fraction of *F. glauca* (60 in leaves, 82 in flowers, 19 in fruits, 23 in roots, respectively), accounting for 68.7–96.8% of the total oil. The major volatiles were (*E*)-caryophyllene (24.9%) and caryophyllene oxide (14.3%) in LV, α-pinene (11.7%), myrcene (13.6%) and germacrene D (14.2%) in FL, α- (24.2%) and β-pinene (14.7%) in FR, (*E*)-β-farnesene (10.0%), myristicin (7.4%) and elemicin (9.0%), in RT, respectively. Noteworthy is the high content in LV of (*E*)-caryophyllene that recently has been found to act as a nonpsychoactive cannabinoid receptor agonist [18].

Sesquiterpenes were the most abundant in LV (73.1%), FL (55%) and RT (61.8%), while monoterpenes predominated in the FR (53.4%). Phenylpropanoids (16.4%) were present only in the RT. It is interesting to note the presence in FR and RT oils of teferdine (0.6%) and ferutidine (0.4–1.3%), that are esters of sesquiterpenic alcohols, with a daucane skeleton, mainly derived from ferutinol, with aromatic acids. They have similar structure to daucane ferutinin and its analogues that are known to possess strong estrogenic properties [9] and antibacterial activity [19], and characterize the nonpoisonous chemotype of *F. communis* growing in Sardinia [13]. In addition neither aristolene nor farnesol, that are volatile markers for the poisonous chemotype, were detected in the oils of *F. glauca*.

In conclusion, the differences in essential oil composition detected between *F. glauca* and *F. communis* made the volatile fraction a reliable marker to distinguish between them, and confirmed the botanical data at the base of their discrimination [2,4,5]. Moreover, on the base of the occurrence of daucane esters in the oil, it could be interesting to perform on this plant further phytochemical studies in order to detect in the non-volatile fraction new daucane-type molecules possessing important biological activities.

The results of antimicrobial activity are reassumed in Table 2. According to the statistical analysis, the gram-positive *B. subtilis* was the most sensitive strain, with MIC values ranging from 38 to 310 μg/ml. A medium inhibitory activity was evidenced against the gram-positive responsible for caries *S. mutans* (MIC 310–1250 μg/ml) [20], and the gram-negative *E. faecalis* (MIC 310–625 μg/ml) and *E. coli* (MIC 625 μg/ml). No remarkable activity was observed against the gram-positive *S. aureus* (MIC 625–1250 μg/ml), and the yeast *C. albicans* (MIC 1250 μg/ml) that resulted the most resistant strain. Results demonstrated also that LV and FR essential oils were the most active on the tested microorganisms. LV oil was significantly more active than FL and RT oils; FR oil was significantly more active than FL oil. In fact, LV and FR essential oils showed the highest amount of (*E*)-caryophyllene, and α- and β-pinene, respectively, that are known to possess antimicrobial potential [21,22]. From comparison of antimicrobial values between oil samples and some pure components (Table 2), we observed that β-pinene, (*E*)-caryophyllene and caryophyllene oxide gave a lower inhibition activity with respect to the *Ferula* oils against all tested bacteria, whilst higher against *C. albicans*. This confirms that other active components play an important role on the synergistic effects of the oils on the inhibition of bacteria. Finally, the inhibition activity exhibited by RT oil may be attributed to the phenylpropanoids myristicin and elemicin [23,24].

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