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Chemical analysis of the essential oil of *Ferula glauca* L. (Apiaceae) growing in Marche (central Italy)

F. Maggi [a,*], D. Lucarini [a], B. Tirillini [b], G. Sagratini [c], F. Papa [c], S. Vittori [c]

[a] *Department of Environmental Sciences, Section of Botany and Ecology, University of Camerino, Faculty of Pharmacy, Via Pontoni 5, I-62032 Camerino, Macerata, Italy*
[b] *Institute of Botany, University of Urbino, Via Bramante 28, I-61029 Urbino, Italy*
[c] *Department of Chemical Sciences, University of Camerino, Via Sant'Agostino 1, I-62032 Camerino, Italy*

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ABSTRACT

Ferula glauca L. (Apiaceae), formerly believed a subspecies of *Ferula communis* L., but at the present considered a distinguishable species, was studied for the first time for volatiles from leaves, flowers, fruits and roots. The chemical analysis of the essential oil obtained from different populations growing in Marche (central Italy) was performed by GC-FID and GC–MS. The differences in 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. In particular, the oils obtained from leaves and roots, contained as major compounds (E)-caryophyllene, caryophyllene oxide, myristicin and elemicin, that can be useful as marker components. Finally, the oils contained some daucane derivatives, that were detected also in *F. communis* and responsible for important biological properties.

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 (Pimenov and Leonov, 1993). The Italian Flora comprises 3 species: *Ferula arrigonii* Bocchieri, *Ferula communis* L. and *Ferula glauca* L. (Conti et al., 2005); *F. arrigonii* lives in small areas of Sardinia, while *F. communis* and *F. glauca*, with rare exceptions, coexist in practically all the Peninsula and the Islands (Fig. 1), mainly in mediterranean and submediterranean sectors, respectively, with the former more widespread than the latter.

Although *F. communis* and *F. glauca* were formerly considered subspecies (i.e. *F. communis* subsp. *communis* and *F. communis* subsp. *glauca*) (Cannon, 1968; Pignatti, 1982), nowadays botanists believe they are distinguished in two different species (Anzalone et al., 1991; Conti et al., 2005; Kurzyna-Mlynik et al., 2008). In fact, they are distinguishable by several differences in terms of morphology, anatomy, phenology and ecology. These differences are summarized in Table 1.

* Corresponding author. Tel.: +39 0737 404506; fax: +39 0737 404508.
E-mail address: filippo.maggi@unicam.it (F. Maggi).

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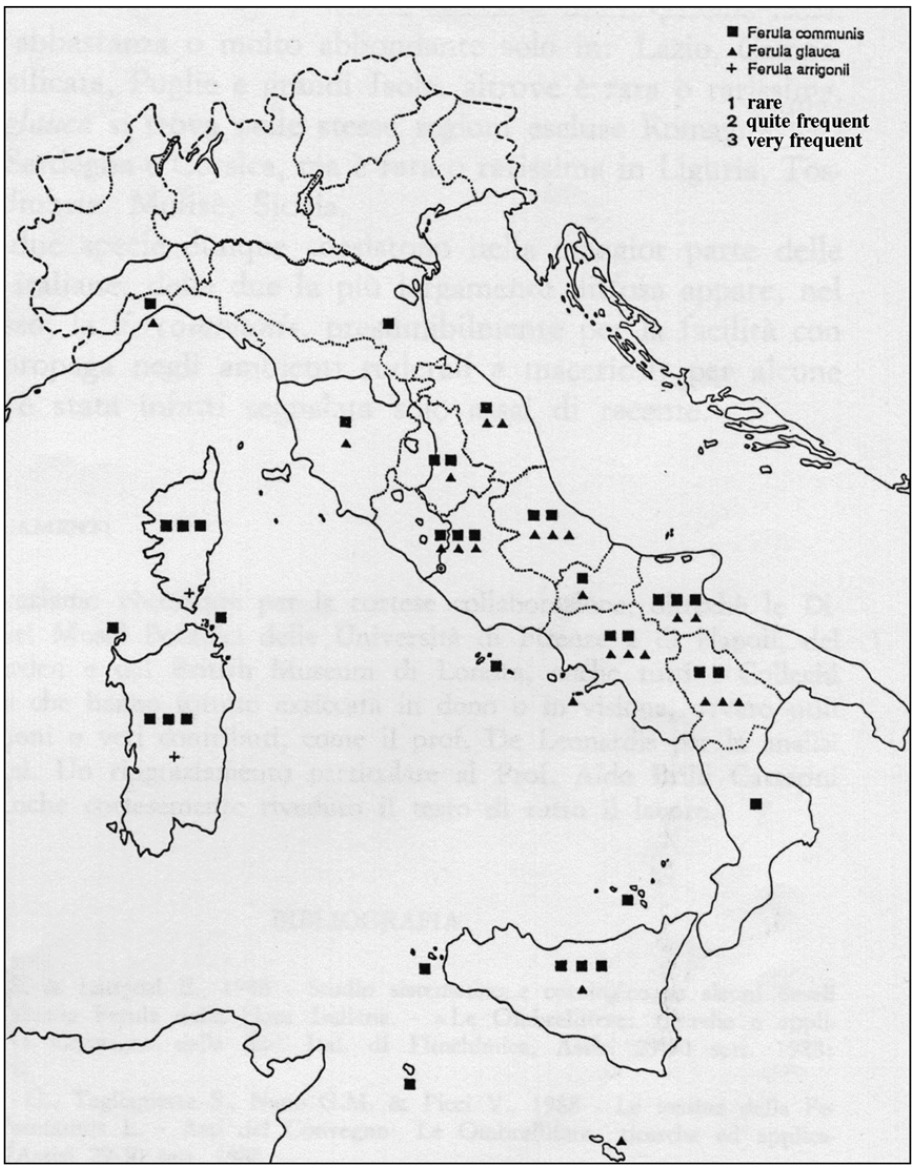

Fig. 1. Distribution in Italy of the *Ferula* species (Anzalone et al., 1991).

F. communis was used in the folk medicine of Sardinia as an antiseptic (Sanna et al., 2006). Several phytochemical studies were performed on this plant, leading to the isolation and characterization of secondary metabolites responsible for biological activity, as coumarins and sesquiterpene esters; most of these are characterized by the daucane skeleton (Appendino, 1997; Appendino et al., 2001).

Further studies have been performed on Sardinian populations of *F. communis*, leading to the discrimination of poisonous and non-poisonous chemotypes characterized by prenylated coumarins and daucane esters, respectively (Marchi et al., 2003; Sacchetti et al., 2003; Arnoldi et al., 2004).

At the same time, because of the unclarified botanical classification, *F. glauca* has not been the subject of many phytochemical investigations as *F. communis*. To the best of our knowledge, Italian populations of *F. glauca* growing in central Italy (Latium) were investigated only by Serafini et al. (1990) who detected in all parts of the plants the sesquiterpene coumarins coladine and coladonine.

As concerning volatile fraction, that can be often an helpful tool to discriminate between different taxa, no papers have been reported on *F. glauca*, while few studies were recently conducted on *F. communis*. Ferrari et al. (2005) studied populations growing in Corsica and found as main components of the leaf oil myrcene (53.5%) and aristolene (8.5%). Marongiu et al. (2005) performed a comparison between hydrodistillation and supercritical fluid extraction of flowerheads from Sardinia: in both extracts they found α- and β-gurjunene as main components. Finally, Rubiolo et al. (2006) studied the volatile fraction obtained from aerial parts of the two chemotypes growing in Sardinia, and detected as major components aristolene (47.1%) and farnesol (21.2%) in the poisonous chemotype, and allohedycaryol (53.7%) in the non-poisonous chemotype, respectively.

Table 1
Main differences between *Ferula communis* and *Ferula glauca* (Anzalone et al., 1991).

Plant features	*Ferula communis*	*Ferula glauca*
Habitus	Stronger, sturdier and stumpier; whorled upper branches	Taller and slender; alternate upper branches
Lamina	Leaf-lobes quite narrow to capillary, green in both sides	Leaf-lobes more breadth, shiny green above and glaucous under
Lamina ramification	Midrib with 4–8 branches for each node	Midrib with only 2 branches for each node
Sheathing bases	Large and more amplexicaul	Narrower and less amplexicaul
Fruit shape	Ovate or obovate	Elliptic
Stoma	Guard cells no different from epidermic cells	Guard cells different from epidermic cells
Phenology	Basal leaves appear in February; flowering in April, fruiting in May	One month later
Ecology	Uncultivated places, ruins, edges of the roads, slopes and scarps, rubbles, hedges, on clayey-calcareous soils, in warm and sunny lowland or hill up to 1000 m	Cliffy environment, old boundaries, edges of the roads, on calcareous or sandy soils; it prefers warm and sunny environments, but occasionally it occurs above 1000 m in central Italy

In order to detect volatile marker compounds suitable to discriminate between *F. communis* and *F. glauca*, we report here for the first time the composition of the essential oils obtained from different parts of *F. glauca* growing in Marche (central Italy).

2. Materials and methods

2.1. Plant material

Leaves, flowers, fruits and roots were separately collected in May–June 2007 at the following cliff locations in Marche (central Italy): Caldarola (N 43°04′33″ E 13°01′35″), Pioraco (N 43°10′38″ E 12°59′53″), Pergola (N 43°30′56″ E 12°49′13″). Plants were botanically confirmed by F. Maggi using available literature (Pignatti, 1982; Anzalone et al., 1991). Voucher specimens were deposited in the Herbarium Camerinensis, Dept. of Environmental Sciences, Sect. of Botany and Ecology, University of Camerino, Italy, under the following accession codes: CAME 13400, CAME 13402, CAME 13441; they are also available at the following website: http://erbariitaliani.unipg.it. Before extraction, the plant material was air dried at room temperature protected from the light for one week.

2.2. Extraction of essential oil

Dried material was hand-cut into small fragments and subjected to hydrodistillation in a Clevenger-type apparatus for 4 h, using *n*-hexane (10 ml) as collector solvent. 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 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 an 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 6890 N–5973 N GC MS system operating in the EI mode at 70 eV, using an 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. 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 analytical grade solvent purchased from Carlo Erba (Milan, Italy); it was successively distilled by a Vigreux column before use. Na_2SO_4 was of analytical reagent grade from J.T. Baker (Deventer, Holland).

Table 2
Constituents of the essential oil from different parts of *F. glauca* L.

Component[a]	RI[b]	Leaves		Flowers		Fruits		Roots		Identification[e]
		Average[c]	STD[d]	Average	STD	Average	STD	Average	STD	
1-Pentanol-4-methyl	840	–	–	–	–	–	–	0.1	0.17	MS,RI
2-Hexanol-5-methyl	866	–	–	–	–	–	–	0.6	1.03	MS,RI
Hexanol	874	–	–	0.1	0.1	–	–	–	–	MS,RI
2-Heptanol	903	–	–	–	–	–	–	0.2	0.26	MS,RI
α-Thujene	928	tr[f]	0.05	tr	0.05	0.1	0.23	–	–	MS,RI
α-Pinene	934	0.3	0.06	6.8	5.05	36.6	11	0.2	0.28	MS,RI,std
Camphene	948	–	–	0.1	0.1	0.2	0.28	–	–	MS,RI
Sabinene	972	–	–	tr	0.05	–	–	–	–	MS,RI
β-Pinene	976	0.1	0.15	3.2	1.95	17.8	2.83	–	–	MS,RI,std
2-Heptanol-5-methyl	977	–	–	–	–	–	–	0.6	0.55	MS,RI
Myrcene	993	3.0	1.53	10.1	6.86	4.1	0.82	–	–	MS,RI,std
α-Phellandrene	1004	–	–	tr	0.05	tr	0.11	–	–	MS,RI,std
Octanal	1006	tr	0.05	0.1	–	–	–	0.2	0.15	MS,RI,std
δ-3-Carene	1011	0.9	0.42	0.7	0.42	–	–	–	–	MS,RI
α-Terpinene	1017	–	–	–	–	–	–	–	–	MS,RI
o-Cymene	1024	tr	0.05	–	–	–	–	–	–	MS,RI
p-Cymene	1029	0.1	–	0.4	0.15	tr	0.05	–	–	MS,RI
Sylvestrene	1032	0.5	0.23	1.2	0.61	–	–	–	–	MS,RI
β-Phellandrene	1032	–	–	–	–	2.5	0.35	–	–	MS,RI
(E)-β-Ocimene	1045	0.3	0.17	tr	0.05	tr	0.11	–	–	MS,RI
(Z)-β-Ocimene	1051	–	–	1.0	0.8	0.5	0.92	–	–	MS,RI
Benzene acetaldehyde	1056	tr	0.05	0.8	1.32	–	–	–	–	MS,RI
γ-Terpinene	1063	tr	0.05	1.6	1.22	0.1	0.17	–	–	MS,RI,std
p-Mentha-2,4(8)-diene	1088	tr	0.05	0.1	0.1	–	–	–	–	MS,RI
6-Camphenone	1090	–	–	–	–	0.5	0.92	–	–	MS,RI
Terpinolene	1090	tr	0.05	tr	0.05	tr	0.11	–	–	MS,RI,std
Perillene	1104	0.2	0.1	tr	0.05	–	–	–	–	MS,RI
Nonanal	1111	tr	0.05	0.1	0.06	–	–	–	–	MS,RI
Octyl acetate	1116	–	–	–	–	–	–	0.9	0.97	MS,RI
α-Campholenal	1125	tr	0.05	–	–	–	–	–	0	MS,RI
Allo-ocimene	1134	–	–	tr	0.05	–	–	–	0	MS,RI,std
(2E)-Nonen-1-al	1168	–	–	0.2	0.28	0.2	0.28	0.1	0.17	MS,RI
Nonanol	1168	0.1	0.17	–	–	–	–	–	–	MS,RI
p-Mentha-1,5-dien-8-ol	1173	0.2	0.06	tr	0.05	–	–	tr	0.11	MS,RI
Terpinen-4-ol	1181	0.1	0.1	0.2	0.1	0.8	0.96	–	–	MS,RI,std
Myrtenal	1198	–	–	–	–	1.0	1.11	–	–	MS,RI,std
α-Terpineol	1198	–	–	0.2	–	0.9	1.55	–	–	MS,RI,std
Ethylguaiacol	1283	–	–	–	–	–	–	0.8	1.44	MS,RI
Bornyl acetate	1289	–	–	tr	0.05	–	–	–	0	MS,RI
4-Vinyl-2-methoxy-phenol	1318	–	–	–	–	tr	–	0.9	1.55	MS,RI
Myrtenyl acetate	1330	–	–	–	–	tr	0.11	–	0	MS,RI
α-Cubebene	1349	tr	0.05	0.1	0.06	–	–	0.2	0.34	MS,RI,std
Eugenol	1364	–	–	–	–	–	–	0.4	0.63	MS,RI
Isoledene	1374	–	–	0.1	–	–	–	–	–	MS,RI
α-Copaene	1374	0.5	0.12	0.5	0.06	tr	0.05	0.4	0.4	MS,RI,std
Daucene	1378	0.4	0.1	0.9	0.21	0.1	0.23	1.0	0.64	MS,RI
β-Bourbonene	1381	0.8	0.31	0.3	0.06	tr	0.11	–	0	MS,RI
β-Cubebene	1387	0.1	0	0.4	0.29	0.1	0.17	0.3	0.46	MS,RI
β-Elemene	1391	0.4	0.37	0.3	0.12	–	–	0.1	0.17	MS,RI
Italicene	1398	0.3	0.06	0.2	–	–	–	–	–	MS,RI
2-epi-β-Funebrene	1406	–	–	0.3	0.28	–	–	1.5	0.7	MS,RI
α-Cedrene	1406	0.5	0.06	0.5	0.06	–	–	–	–	MS,RI,std
Aristolene	1414	–	–	–	–	–	–	3.2	5.59	MS,RI,std
(E)-Caryophyllene	1415	20.5	4.45	9.4	4	1.2	0.31	–	–	MS,RI,std
Cis-Thujopsene	1422	0.3	0.06	0.4	0.1	tr	0.05	0.3	0.46	MS,RI
β-Copaene	1426	0.3	0.06	0.2	0.06	tr	0.05	0.7	1.26	MS,RI
Trans-α-Bergamotene	1432	tr	0.11	0.1	0.11	tr	0.05	0.1	0.17	MS,RI
β-Barbatene	1436	0.3	–	0.6	0.06	–	–	4.0	1.79	MS,RI
Acora-2.4(15)-diene	1443	–	–	–	–	tr	0.05	–	–	MS,RI
Cis-Muurula-3,5-diene	1444	0.2	0.06	tr	0.11	–	–	0.6	0.55	MS,RI
Amorpha-4.11-diene	1445	–	–	–	–	tr	0.05	–	–	MS,RI
α-Humulene	1450	7.6	1.36	3.3	1.53	0.1	0.17	–	–	MS,RI,std
Cis Cadina-1(6),4-diene	1458	0.1	0.17	0.3	0.1	0.3	0.46	0.6	0.58	MS,RI
(E)-β-Farnesene	1460	0.8	0.17	1.9	0.69	tr	0.05	8.4	3.03	MS,RI,std
α-Acoradiene	1465	0.9	0.1	0.7	0.12	–	–	–	–	MS,RI
Cis-Muurola-4(14),5-diene	1467	0.2	0.15	0.7	0.17	0.1	0.23	1.0	0.35	MS,RI
β-Acoradiene	1472	0.5	0.1	0.6	0.06	tr	0.05	–	–	MS,RI
β-Chamigrene	1473	–	–	–	–	–	–	0.9	0.25	MS,RI

(*continued on next page*)

Table 2 (*continued*)

Component[a]	RI[b]	Leaves		Flowers		Fruits		Roots		Identification[e]
		Average[c]	STD[d]	Average	STD	Average	STD	Average	STD	
Trans-Cadina-1-(6),4-diene	1476	–	–	–	–	–	–	0.1	0.23	MS,RI
Germacrene D	1478	6.8	2.48	16.4	3.81	2.1	0.2	–	–	MS,RI,std
β-Selinene	1480	–	–	–	–	–	–	tr	0.11	MS,RI
γ-Curcumene	1481	1.3	0.31	1.7	0.36	0.1	0.23	–	–	MS,RI
γ-Himachalene	1481	0.4	0.63	–	–	–	–	–	–	MS,RI
Ar-curcumene	1483	3.3	0.4	0.1	–	0.5	0.1	0.9	0.21	MS,RI
Trans-muurola-4(14),5-diene	1489	–	–	–	–	–	–	tr	0.11	MS,RI
Bicyclogermacrene	1491	0.6	0.45	1.5	0.4	–	–	–	–	MS,RI
Isodaucene	1495	–	–	–	0	0.1	0.23	0.9	0.57	MS,RI
β-Himachalene	1496	–	–	0.6	0.42	0.1	0.17	0.2	0.28	MS,RI
Epizonarene	1497	–	–	–	–	–	–	3.6	3.55	MS,RI
Cuparene	1498	1.7	0.26	1.9	1.81	–	–	0.4	0.69	MS,RI
α-Zingiberene	1503	1.3	0.4	0.9	0.31	tr	0.11	6.9	2.48	MS,RI
β-Bisabolene	1508	0.2	0.26	2.3	1.51	0.4	0.69	4.0	1.05	MS,RI
β-Curcumene	1510	0.6	1.09	1.8	0.93	0.6	1.09	0.8	1.32	MS,RI
(E.E)-α-Farnesene	1511	1.5	1.41	2.8	0.86	1.5	1.08	–	–	MS,RI
γ-Cadinene	1512	–	–	–	–	–	–	4.1	3.6	MS,RI
(Z)-γ-Bisabolene	1514	–	–	0.1	0.11	–	–	–	–	MS,RI
Trans-calamenene	1521	–	–	tr	0.05	–	–	2.5	2.16	MS,RI
β-Sesquiphellandrene	1521	–	–	0.4	0.06	–	–	–	–	MS,RI
δ-Cadinene	1523	1.3	0.47	1.9	0.51	0.6	0.1	–	–	MS,RI
Myristicin	1527	–	–	–	–	–	–	6.0	3.05	MS,RI,std
γ-Cuprenene	1531	0.3	0.06	0.5	0.06	tr	0.11	0.9	0.29	MS,RI
(E)-γ-Bisabolene	1534	–	–	0.2	–	–	–	–	–	MS,RI
Trans-γ-bisabolene	1537	–	–	–	–	–	–	0.2	0.2	MS,RI
α-Calacorene	1542	0.4	0.21	0.3	0.3	–	–	–	–	MS,RI
α-Copaen-11-ol	1542	–	–	0.5	0.26	–	–	0.7	0.63	MS,RI
Elemicin	1566	–	–	–	–	–	–	9.0	1.95	MS,RI
Isoelemicin	1568	0.1	0.23	tr	0.11	0.2	0.28	–	–	MS,RI
(E)-α-isomethyl-ionol acetate	1569	–	–	–	–	0.5	0.92	–	–	MS,RI
Spathulenol	1577	0.7	0.06	0.4	0.1	–	0	–	–	MS,RI
Caryophyllene oxide	1581	13.9	4.21	1.0	0.26	tr	0.05	–	–	MS,RI,std
Salvial-4(14)-en-1-one	1592	0.5	0.15	0.8	0.68	–	–	–	–	MS,RI
Carotol	1594	–	–	0.4	0.74	–	–	–	–	MS,RI
Guaiol	1597	–	–	–	–	–	–	0.1	0.23	MS,RI,std
Humulene epoxide II	1606	3.1	0.98	0.3	0.06	–	–	–	–	MS,RI
10-Epi-γ-Eudesmol	1618	–	–	–	–	–	–	0.2	0.4	MS,RI
1-Epi-Cubenol	1628	–	–	–	–	–	–	0.4	0.32	MS,RI
Caryophylla-4(12),8(13)-dien-ol	1636	0.3	0.06	–	–	–	–	–	–	MS,RI
Epi-α-Cadinol	1641	–	–	–	–	–	–	2.1	1.14	MS,RI
Epi-α-Muurolol	1643	–	–	0.2	0.28	–	–	–	–	MS,RI
Himachalol	1645	0.4	0.17	0.4	0.51	0.4	0.53	–	–	MS,RI
α-Cadinol	1656	0.6	0.15	1.0	0.32	0.1	0.17	2.7	0.17	MS,RI
β-Atlantone	1666	–	–	0.3	0.3	–	–	–	–	MS,RI
14-Hydroxy-9-epi-trans-caryophyllene	1672	0.9	0.15	–	–	–	–	–	–	MS,RI
Eudesma-4(15),7-dien-1β-ol	1686	0.5	0.15	0.3	0.17	tr	0.11	–	–	MS,RI
Unknown 1[g]	1690	3.6	3.45	2.7	2.55	1.0	1.73	1.3	1.79	
(Z)-α-Trans bergamotol	1693	0.4	0.66	0.8	1.32	0.1	0.23	–	–	MS,RI
10-Nor-calamenen-10-one	1703	–	–	–	–	tr	0.05	–	–	MS,RI
Mint sulfide	1733	tr	0.05	–	–	–	–	–	–	MS,RI
Tetradecanoic acid	1770	–	–	0.1	0.17	–	–	–	–	MS,RI,std
14-Hydroxy-δ-cadinene	1809	–	–	tr	0.11	–	–	–	–	MS,RI
Neophytadiene	1837	1.1	0.26	0.2	0.28	–	–	–	–	MS,RI
Methyl hexadecanoate	1924	–	–	tr	0.05	–	–	–	–	MS,RI
Hexadecanoic acid	1970	1.5	0.61	2.2	1.27	0.5	0.92	0.1	0.23	MS,RI,std
Ethyl hexadecanoate	1996	tr	0.05	tr	0.05	–	–	–	–	MS,RI
Phytol	2113	2.6	0.15	0.2	0.17	–	–	–	–	MS,RI,std
Ethyl linoleate	2163	–	–	tr	0.05	–	–	–	–	MS,RI
Unknown 2[h]	2179	0.4	0.63	0.4	0.63	–	–	0.3	0.43	
Tricosane	2313	–	–	0.1	0.23	–	–	–	–	MS,RI,std
Unknown3[i]	2328	0.7	0.75	–	–	–	–	1.3	1.35	
Teferdine	2391	–	–	tr	0.05	–	–	0.4	0.21	MS,std
Tetracosane	2398	0.4	0.45	–	–	–	–	–	–	MS,RI,std
Pentacosane	2504	0.3	0.17	0.2	0.1	–	–	–	–	MS,RI,std
Unknown 4[j]	2569	–	–	–	–	–	–	1.8	0.76	
Ferutidine	2639	–	–	0.3	0.46	0.7	0.51	0.6	0.15	MS,std
Heptacosane	2662	0.4	0.36	0.3	0.15	–	–	–	–	MS,RI,std
Nonacosane	2900	1.2	0.96	0.5	0.25	–	–	–	–	MS,RI,std

Table 2 (*continued*)

Component[a]	RI[b]	Leaves		Flowers		Fruits		Roots		Identification[e]
		Average[c]	STD[d]	Average	STD	Average	STD	Average	STD	
Total identified (%)		89.8	4.59	92.8	3.52	79.1	14.71	76.3	6.45	
Oil yield (%)		0.05	0.01	0.06	0.02	0.09	0.01	0.03	0.01	
Grouped compounds (%)										
Monoterpene hydrocarbons		5.5	2.62	26.4	14.80	60.8	22.13	0.2	0.29	
Oxygenated monoterpenes		0.5	0.20	0.5	0.15	4.3	5.66	0.1	0.12	
Sesquiterpene hydrocarbons		54.5	1.51	54.6	10.00	10.0	1.13	49.1	15.99	
Oxygenated sesquiterpenes		21.3	5.52	6.6	2.35	2.0	1.06	6.3	0.49	
Diterpenes		3.7	0.12	0.4	0.40	–	–	–	–	
Phenylpropanoids		–	–	–	–	–	–	15.4	5.32	
Daucane esters		–	–	0.4	0.46	0.8	0.71	0.9	0.12	
Aliphatics		4.0	1.53	4.1	2.08	1.1	1.48	2.7	2.60	
Others		0.2	0.32	0.1	0.12	0.3	0.35	1.7	3.00	

[a] Compounds are listed in order of their elution from an HP-5 column.
[b] RI, retention indices as determined on HP-5 column using homologous series of C8-C30 alkanes.
[c] Values represent an average of three determinations.
[d] STD: standard deviation.
[e] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries and Adams (2007); RI, by comparison of RI with those reported from Adams (2007) and NIST05 (2005); std, by injection of an authentic sample.
[f] tr, traces (<0.1%).
[g] Unknown 1, *m*/*z* (10 largest peaks): 173 (999), 145 (961), 201 (891), 131 (409), 216 (326), 159 (213), 128 (212), 115 (208), 174 (208), 129 (193).
[h] Unknown 2, *m*/*z*: 83 (999), 55 (533), 193 (323), 43 (317), 175 (242), 107 (242), 109 (234), 121 (230), 149 (202), 93 (192).
[i] Unknown 3, *m*/*z*: 83 (999), 149 (532), 55 (405), 191 (404), 96 (341), 145 (208), 43 (185), 234 (181), 148 (149), 135 (142).
[j] Unknown 4, *m*/*z*: 175 (999), 83 (927), 132 (751), 55 (444), 119 (225), 133 (210), 126 (208), 43 (173), 105 (170), 157 (157).

2.5. 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 (Adams, 2007; NIST, 2005). In addition, a home-made library, constructed based on the analyses of reference oils and commercially 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 available in author's

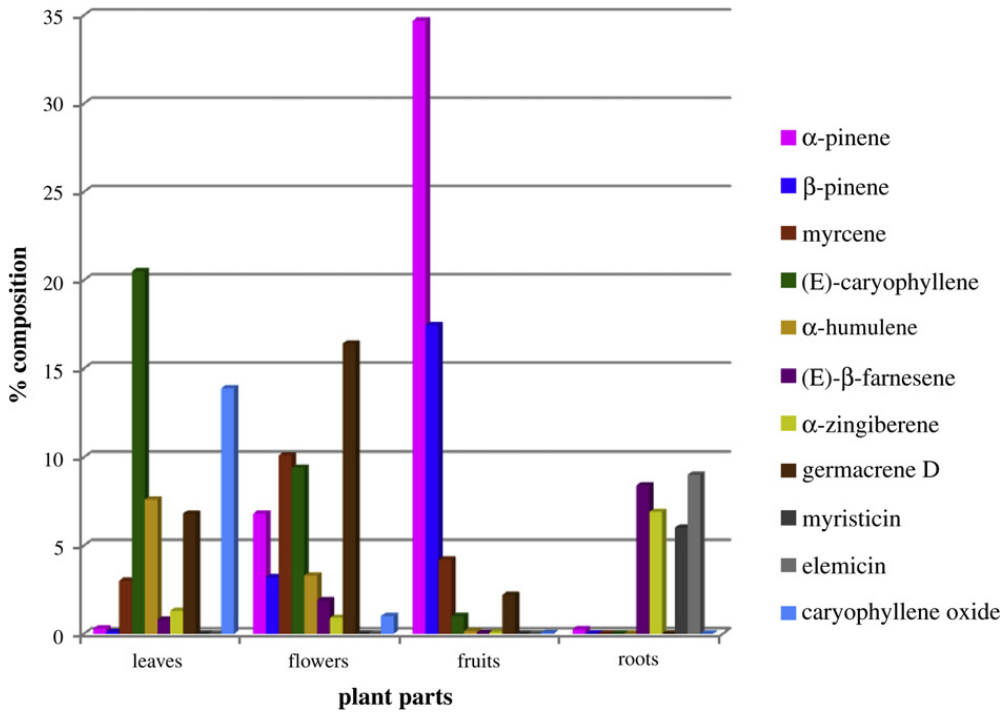

Fig. 2. Percentages (mean values) of the main representative compounds in the essential oil isolated from different parts of *Ferula glauca*.

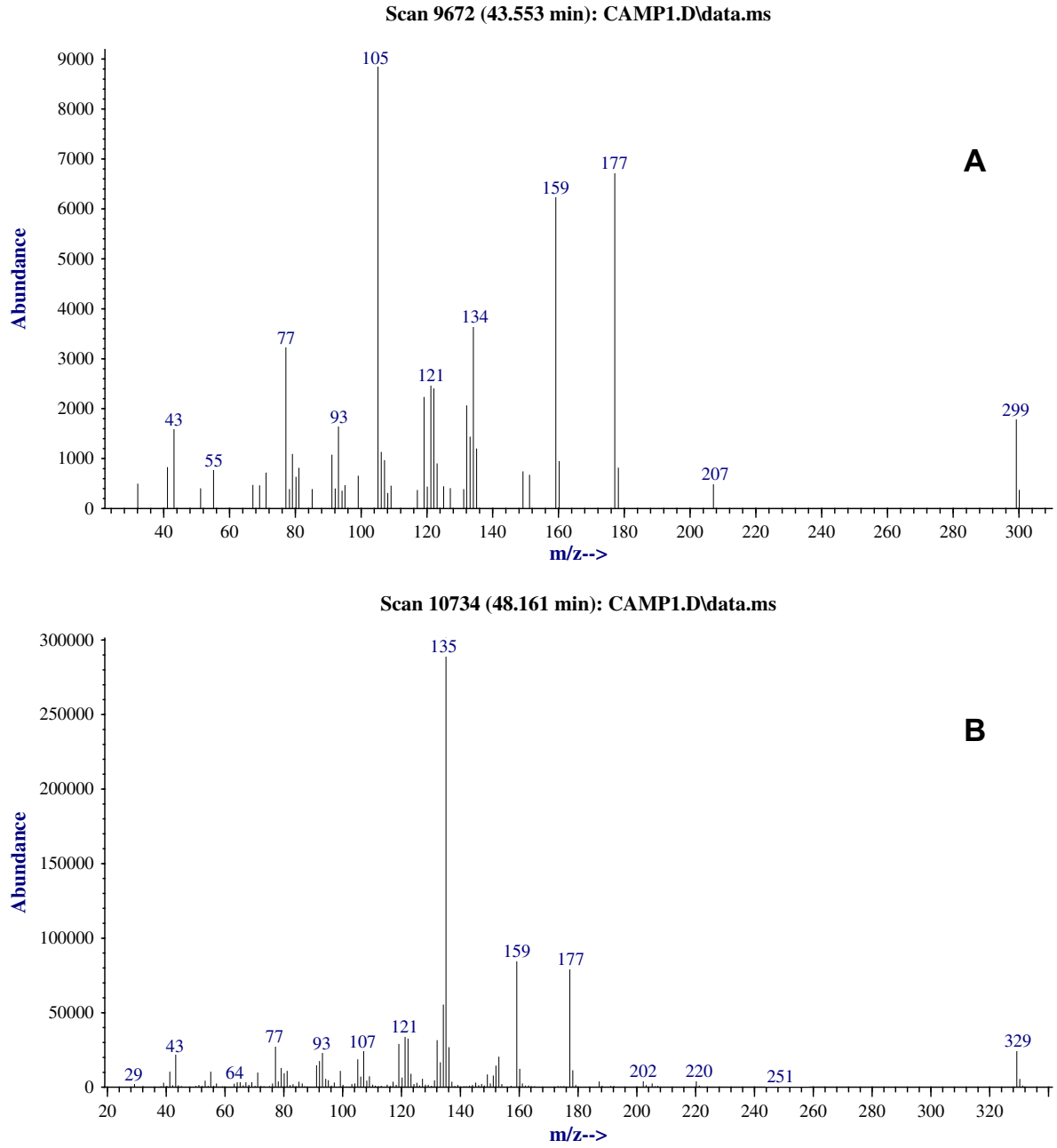

Fig. 3. Mass spectra of two daucane derivatives detected in the essential oil of *Ferula glauca*: (A) teferdine and (B) ferutidine.

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 (Rubiolo et al., 2006). 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.

2.6. Statistical analysis

Analysis of variance (ANOVA) was carried out using the SPSS 13.0 software package for Windows (SPSS Inc. Chicago, Illinois-USA), and the average values have been compared with Tukey's LSD test at $P < 0.05$ (Table 3). The percentages of the compounds recorded for each sample were normalized using the followed formula: (x_j/x_{max}) were x_j is the j-nth percentage of the j-compound and x_{max} is the maximum value for all the j-compounds. The normalized values were submitted to numerical cluster analysis (centroid clustering of mean squared Euclidean distances) from which the dendrogram was derived. Cluster analysis was carried out using SPSS 13.0 software as well.

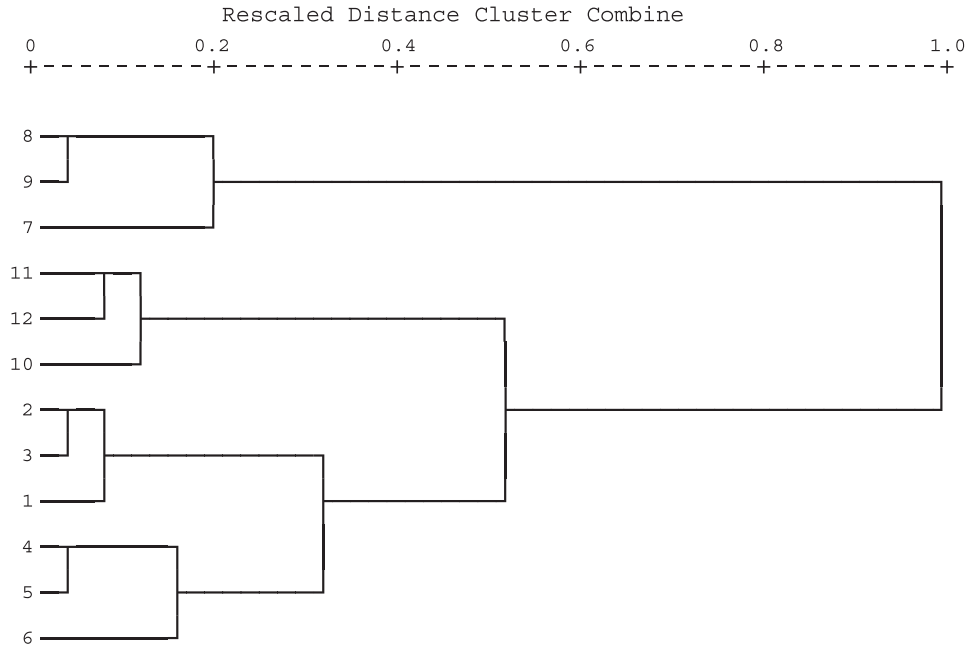

Fig. 4. 1,2,3 = oil composition from leaves; 4,5,6, = oil composition from flowers; 7,8,9 = oil composition from fruits; 10,11,12 = oil composition from roots.

3. Results and discussion

Chemical analysis of essential oils can be an useful tool to discriminate between different taxa, especially when they are hardly distinguishable only on the base of morphological data. This study represents the first screening of volatile fraction reported for *F. glauca*. The aim was to show chemical differences from *F. communis*, in order to add new discriminating elements at the base of the identification of the two taxa.

The composition of the essential oil from different plant parts of *F. glauca*, expressed as average percentages, is summarized in Table 2. A total of 132 volatile components were identified (74 in leaves, 95 in flowers, 55 in fruits, and 54 in roots, respectively) in the oils, with the main representatives reported in Fig. 2. The identified mean percentages of volatiles were in the range of 68.7–96.8%.

3.1. Essential oil from leaves

A total of 74 components were identified in the leaves, accounting for 87.0–95.1% of the total oil (Table 2). The sesquiterpene fraction was the most abundant (71.4–83.0%) with the hydrocarbons accounting for 52.8–55.7% of the total oil; the major components were (E)-caryophyllene (16.0–24.9%), caryophyllene oxide (9.5–17.9%), α-humulene (6.8–9.2%) and germacrene D (5.0–9.6%). The monoterpene fraction was quantitatively poorer (2.8–8.0%), with the hydrocarbon myrcene (1.3–4.2%) being the most abundant. Our results showed qualitative and quantitative differences in leaves oil composition from those reported for *F. communis*. In fact, the oil from Corsica was characterized by a high content of monoterpenes (77.7%), myrcene being the main constituent (53.5%), while the sesquiterpenes aristolene (8.5%) and (E,E)-farnesol (4.3%) were present in appreciable amounts (Ferrari et al., 2005). As concerning Sardinian populations of *F. communis*, two chemotypes were identified by Rubiolo et al. (2006): the first one, corresponding to the poisonous chemotype, characterized by aristolene

Table 3
Chemical variability of the major compounds of *F. glauca* essential oils.

Component	Leaves	Flowers	Fruits	Roots
α-Pinene	0.33a	6.77a	36.6b	0.17a
β-Pinene	0.14a	3.2a	17.83b	0.01a
Myrcene	3.03ab	10.1b	4.1ab	0.01a
(E)-Caryophyllene	20.5c	9.43b	1.17a	0.01a
α-Humulene	7.63c	3.33b	0.11a	0.01a
(E)-β-Farnesene	0.8a	1.9a	0.04a	8.4b
Germacrene D	6.77b	16.4c	2.1ab	0.01a
Caryophyllene oxide	13.9b	1a	0.04a	0.01a
Unknown 1	3.6a	2.67a	1.01a	1.33a

Values within a row for each compound having different letters are significantly different from each other using Tukey's LSD test ($P < 0.05$).

and (E,E)-farnesol as main constituents of the volatile fraction; the second one, corresponding to the non-poisonous chemotype, characterized by the oxygenated sesquiterpene allohedycaryol as the major component, being both chemotypes very rich in sesquiterpenes. The main constituents of the leaf oil of *F. glauca* as (E)-caryophyllene, caryophyllene oxide and α-humulene were completely lacking or occurring in very low amounts in *F. communis*, and for this reason they could be used as marker compounds to discriminate *F. glauca* oil from that of *F.* communis. At the same time, the major volatiles of Sardinian *F. communis* (aristolene, (E,E)-farnesol and allohedycaryol), were completely lacking in *F. glauca* oil. It is interesting to note the presence of daucene (0.3–0.5%), that is a sesquiterpenoid having the same skeleton as daucane esters characterizing the biologically active fraction of *F. communis*, belonging to the nonpoisonous chemotype (Appendino et al., 2001, 2002).

3.2. Essential oil from flowers (umbels)

A total of 95 components were identified in the flowers of *F. glauca*, accounting for 90.1–96.8% of the total oil (Table 2). The oil was dominated by sesquiterpenes, accounting for 53.6–74.8% of the total oil, with (E)-caryophyllene (6.2–13.9%) and germacrene D (14.2–20.8%) as major components, while monoterpenes, with respect to those occurring in leaf oil, were present in higher amounts (10.3–38.7%), with myrcene (2.2–14.5%) and α-pinene (1.6–11.7%) being the main representatives. The composition of flowers oil showed also qualitative and quantitative differences with respect to those reported for *F. communis* from Sardinia (Marongiu et al., 2005; Rubiolo et al., 2006). In fact, aristolene, (E,E)-farnesol, allohedycaryol, α- and β-gurjunene, occurring as major volatiles in Sardinian populations of *F. communis*, were absent in flower oil of *F. glauca*. In addition, germacrene D and (E)-caryophillene were not present in such high content as in *F. glauca*. With the exception of the absence of aristolene, the flower oil was similar, as major components, to that of *F. communis* from Corsica, characterized by a higher content in myrcene (57.4–63.5%) and α–pinene (8.2–8.8%) (Ferrari et al., 2005). Noteworthy is the detection, in addition to that of daucene (0.8–1.1%), of the two daucane esters teferdine (0.1%) and ferutidine (traces to 0.8%) (Fig. 3), that are esters of sesquiterpenic alcohols, with a daucane skeleton, mainly derived from ferutinol, with aromatic acids. They are known for estrogenic properties (Appendino et al., 2002) and antibacterial activity (Al-Yahya et al., 1998), and characterize the nonpoisonous chemotype of *F. communis* growing in Sardinia (Rubiolo et al., 2006).

3.3. Essential oil from fruits

A total of 55 components were identified in the fruits of *F. glauca*, accounting for 68.7–89.5% of the total oil (Table 2). In this case, no data concerning volatiles from fruits of *F. communis* are available from literature. The oil was characterized by a high content of monoterpene hydrocarbons (45.1–76.4%), with α- (24.2–45.2%) and β-pinene (14.7–20.2%) being the major compounds. Sesquiterpenes were present in lower amounts (11.9–12.0%) with respect to those occurring in leaves and flowers. Also in this case the fruit oil was characterized by the presence of daucane derivatives as daucene (0.4%), isodaucene (0.4%) and ferutidine (0.3–1.3%).

3.4. Essential oil from roots

A total of 54 components were identified in the roots of *F. glauca*, accounting for 68.9–80.4% of the total oil (Table 2). Sesquiterpenes constituted the major fraction (37.3–67.0%), being (E)-β-farnesene (4.9–10.3%) and α-zingiberene (4.7–9.6%) the main representative. The second most abundant fraction was represented by phenylpropanoids (9.7–20.2%), with myristicin (2.5%) and elemicin (7.1–11.0%) as the major compounds. In particular, myristicin was the main component, also in other *Ferula* roots (Iranshahi et al., 2006). Owing to the absence of these phenylpropanoids in the roots of *F. communis* (Rubiolo et al., 2006), these volatiles could be used as marker components characterizing *F. glauca* oil. Finally, also the root oil contained the daucane derivatives daucene (0.5–1.7%), isodaucene (0.3–1.4%), teferdine (0.2–0.6%) and ferutidine (0.4–0.7%), all metabolites occurring in the non-poisonous chemotype of *F. communis* (Rubiolo et al., 2006). However, it is interesting to note only in this case the presence of aristolene (9.7%) only in one sample, but we cannot conclude that this sample is toxic. Further phytochemical investigations, in order to detect also prenylated coumarins responsible for toxicity, should be performed.

3.5. Statistical analysis

The hierarchical cluster analysis (Fig. 4) using average linkage showed that oil from leaves and that from flowers join at a distance cluster (ds) = 683; the repetition of the same part of the plants join at a maximal ds = 413. This indicates that the volatile compounds of leaves and flowers are quite similar. Oils from roots and fruits join to the leave and flower oils to more high value (ds = 1103 and 2108, respectively) and we may decide that these oil compositions were very unlike to the others. The value of (E)-β-farnesene in the root oils becomes different to the other values, based on ANOVA analysis, as well as α-pinene and β-pinene in the fruit oils (Table 3).

In conclusion, the differences detected in essential oil composition between *F. communis* and *F. glauca* make the volatile fraction a reliable marker to distinguish between them, and confirm the botanical data at the base of their discrimination (Anzalone et al., 1991; Conti et al., 2005; Kurzyna-Mlynik et al., 2008). *F. glauca* essential oil, obtained in particular from leaves and roots, contained volatile as (E)-caryophyllene, caryophyllene oxide, germacrene D, α-humulene, myristicin and elemicin,

that can be useful as marker components in order to discriminate it from *F. communis*. Finally, the absence of aristolene and the occurrence of daucane derivatives, should make *F. glauca* belonging to nonpoisonous chemotype. Anyway, further phytochemical investigations on this plant are expected in the future.

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