The Pharmaceutical and Chemical Journal, **2019, 6(5):112-118**

Available online ***www.tpcj.org***

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Composition of the Essential Oils of *Daucus carota* L. and *D. guttatus* Sm. from Greece and their Chemotaxonomy

George Ladopoulos, Aikaterini Koutsaviti, Olga Tzakou*

Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 157 71 Athens, Greece
* E mail: tzakou@pharm.uoa.gr

Abstract Stems and leaves, inflorescences and roots from two *Daucus* species (*D. carota* and *D. guttatus*) from Greece were subjected to hydrodistillation. The composition of the obtained essential oils was analyzed by means of GC-FID and GC-MS. This is the first report on the essential oil of *D. carota* and *D. guttatus* collected in Greece. In both species, the essential oils from different plant parts were similar in quality, showing mainly quantitative differences. Between the two species, however, not only quantitative, but also significant qualitative differences were observed. The monoterpenes, sabinene (2.3-32.2%), α-pinene (tr-23.9%), terpinen-4-ol (tr-7.7%), and limonene (tr-6.6%), characterized *D. carota* essential oils, whereas the sesquiterpene hydrocarbon acora-3,7(14)-diene 9.2-60.1%, and the phenylpropane derivatives, epoxy-pseudoisoeugenyl isobutyrate (18.6-64.9%), and epoxy-pseudoisoeugenyl 2-methylbutyrate (0.6-33.0%) were the main constituents of *D. guttatus* essential oil. According to our results, the chemical profile of the studied taxa can serve as additional evidence for distinguishing these two species.

Keywords *Daucus carrota*, *Daucus guttatus*, essential oils, phenylpropane derivatives

Introduction

The genus *Daucus* (Umbelliferae) is a cosmopolitan genus of herbaceous plants and comprises 22 species, 10 of which are found in Europe [1]. Most widely known is *Daucus carota*, which is found in Europe, Asia and N. Africa. *D. carota* is extremely polymorphic, including many subspecies such as *D. carota* subsp. *carota* which is also known as wild carrot and *D. carota* subsp. *sativus*, a naturally occurring species, selectively bred to produce the vegetable commonly known as carrot [2-4]. Additionally, *D. guttatus* is found in C. and S. Italy, the Balkan Peninsula, the Aegean region and Romania.

D. carota has had many uses in traditional medicine. The essential oil from different plant parts of *D. carota* is used in cosmetics, as a functional ingredient in food industry, while it is also known to exert a plethora of bioactivities such as antioxidant, anti-inflammatory, hepatoprotective, and anti-parasitic properties [5-8]. The volatiles of *D. carota* have been already studied [9, 10-11], while the literature data about the essential oil composition of *D. guttatus* are only scarce [3, 8]. In the framework of our ongoing research on the chemistry of aromatic and medicinal plants from the Balkan Peninsula and the Mediterranean area we studied the volatile constituents of *D. carota* and *D. guttatus* grown wild in Greece in an effort to evaluate if their chemical profile can serve as an additional element to distinguish the two species.

Materials and Methods

Plant material: Aerial parts along with roots of *D. carota* and *D. guttatus* were collected in June 2015 from Erythres, Prefecture of Attiki. Identification was performed by Dr. Theophanis Constantinidis (Associate Professor, Faculty of Biology, National and Kapodistrian University of Athens).

Essential oil extraction: Stems & leaves *(*DCVSL, DAESL), inflorescences (DCVINF, DAEINF) and roots (DCVR, DAER) of *D. carota* and *D. guttatus* respectively, were subjected separately to hydrodistillation for 3h using a modified Clevenger-type apparatus with a water-cooled receiver, in order to reduce overheating artifacts.

The isolated essential oils of pale yellow color were dried over anhydrous sodium sulfate and stored at 4 ^{0}C until further analysis. Respective yields (% w/w) of the essential oils were as follows: *D. carota* stems and leaves (DCVSL) 0.14%, *D. carota* inflorescences (DCVINF) 0.23%, *D. carota* roots (DCVR) 0.01%, *D. guttatus* stems and leaves (DAESL) 0.05%, *D. guttatus* inflorescences (DAEINF) 0.29% and *D. guttatus* roots (DAER) 0.04%.

Gas chromatography and gas chromatography-mass spectrometry analysis: GC analysis of components was carried out on a SRI (Brooks, Hatfield, PA, USA) 8610C gas chromatograph equipped with a DB-5 fused silica capillary column (30 mx0.32 mm; film thickness 0.25 μm; J&W, CA, USA), a split-splitless injector and a FID detector. Injection and detection were performed at 280 ^{0}C in a split ratio 1:10. The oven temperature was 60 ^{0}C at the time of the injection, raised to 280 ^{0}C at a rate of 3 ^{0}C/min. GC–MS analysis was carried out using a Hewlett-Packard(Hewlett-Packard GmbH, Walbronn, Germany) 6890 gas chromatograph equipped with a HP-5MS fused silica capillary column (30 mx 0.25 mm; film thickness 0.25 μm, Agilent, Palo Alto, CA, USA), a split-splitless injector and a Hewlett- Packard 5973 MS detector operating in electron ionization mode at 70 eV. Injection was performed at 220 ^{0}C in a split ratio 1:10, while detection was performed at 300 ^{0}C. The oven temperature was 60 ^{0}C at the time of the injection, raised to 300 ^{0}C at a rate of 3 ^{0}C/min. In both cases the carrier gas was helium at a flow rate of 1.2 mL/min and the injected volume was 1μL of essential oil-pentane solution (10% v/v).

Identification of compounds: The identification of the chemical constituents was based on comparison of their relative retention indices and mass spectra with those obtained from authentic standards (Sigma Chemical Co., St. Louis, USA, PhytoLab GmbH and Co., Germany and in-house isolated and identified metabolites) and/or reported in the NIST/NBS and Wiley libraries and the literature [12].

Results and Discussion

In total 76 metabolites were identified in the essential oils of both species. The essential oils from the stems-leaves (DCVSL) and the inflorescences of *D. carota* (DCVINF) showed a chemical similarity, while the essential oil from the roots (DCVR) had more significant differences, both qualitative and quantitave, compared to the samples of the aerial parts. The percentage of the identified compounds reached 92.5% and 85.0% for DCVSL and DCVFL samples respectively, while for sample DCVR was only 42.1%, showing a large number of unidentified constituents. Samples DCVSL and DCVINF were both dominated by monoterpenes (89.4% and 59.3%, respectively), with sabinene (32.2% and 22.2%), α-pinene (23.9% and 13.9%), terpinen-4-ol (7.7% and 4.1%), limonene (6.6% and 3.9%), and β-pinene (4.9% and 2.4%), being the most important representatives. However, sesquiterpenes comprised only 2.7% of the DCVSL sample, while their percentage in DCVINF sample reached 24.3%. Specifically, *trans*-asarone, α-acoradiene, and germacrene D were the most abundant sesquiterpenes (4.9%, 4.1%, 3.4%) in *D. carota* inflorescences essential oil, while they were found only in traces in the stems-leaves essential oil, apart from germacrene D, which was detected in 1.3%. The sesquiterpenes sesquisabinene (1.6%), *cis*-α-bergamotene (0.7%), and β-sesquiphellandrene (0.5%) were present only in the DCVINF sample.

A variety of factors affect the composition of *D. carota* essential oils such as the plant part, the vegetative stage and the geographical origin. In general, monoterpenes and/or sesquiterpenes dominate the leaves, stem and blooming umbel (inflorescences) essential oils, while seed and root essential oils are dominated by sesquiterpenes and phenylpropanoids [7].

As evidenced by published studies the volatiles of *D. carota* vary among its subspecies. Most studies investigate the essential oil of *D. carota* subsp. *carota*, where monoterpenes constitute a typical class of components [4-6, 8, 13, 14]. In most cases, the constituents α-pinene, sabinene, myrcene, limonene, terpinen-4-ol, are found in significant amounts, regardless of the plant part or origin. Geranyl acetate is also an important compound that often appears in *D. carota* seed essential oil [4, 6-8, 14, 15]. According to other studies, less common metabolites of *D. carota* subsp. *carota* oil are carotol, β-bisabolene and α-terpinolene [7, 14, 15]. Other *Daucus* taxa either show similar chemical profile to our samples such as *D. halophilus* essential oils, which are dominated by sabinene, α-pinene, limonene, or quite different chemical constitution, for instance *D. carota* subsp. *hispanicus* essential oils, which are dominated by myristicin [9, 16]. The chemical composition of DCVSL and DCVINF samples is similar to the typical chemical profile of the *D. carota* subsp. *carota* essential oils.

In the essential oil of *D. carota* root (DCVR) monoterpenes and sesquiterpenes were present in almost equal percentage (21.2% and 20.9%). The most abundant constituents from the monoterpene class were terpinolene (11.6%), isobornyl acetate (4.0%), and γ-terpinene (3.3%), while β-chimachalene (7.1%), α-acoradiene (4.5%), and α-bisabolol (3.3%) were the most abundant sesquiterpene derivatives, thus the root essential oil of *D. carota* was significantly different compared with the essential oil from stems and leaves (DCVSL), and inflorescences (DCVINF).

Literature data regarding the composition of *D. carota* root essential oil are scarce; however Chizzola (2010) also identified terpinolene as the most prevailing compound of root essential oil [14].

The analyzed *D. guttatus* essential oils showed similar chemical profiles, exhibiting only quantitative differences. The chemical composition of the studied plant parts (stems and leaves-DAESL, inflorescences-DAEINF and roots-DAER) was quite different compared with the corresponding *D. carota* essential oils. Samples DAESL, DAEINF and DAER contained mainly sesquiterpenes and phenylpropane derivatives. The most abundant constituent in the stems-leaves essential oil was the sesquiterpene hydrocarbon acora-3,7(14)-diene (60.1%), while phenylpropane derivatives characterized the inflorescences (67.5%) and root (70.4%) essential oils, with epoxypseudoisoeugenyl isobutyrate (64.9%, 31.8% respectively), and epoxypseudoisoeugenyl 2-methylbutyrate (0.6%, 33.0% respectively), being major components.

Radulović et al. [3] and Jawdat et al. [8], who investigated *D. guttatus* essential oils from Serbia and Syria respectively, revealed important variations in the essential oil constitution. Jawdat et al. [8] identified apiole (43.3%), β-bisabolene (34.2%) and myristicin (15.4%) as the main compounds, while Radulović et al. [3] identified β-pinene (18.8%), α-pinene (17.3%), geraniol (8.6%) and limonene (6.0%). Therefore, both studies showed completely different chemical profiles compared to our samples. This could be attributed to a variety of factors that affect the composition of *D. carota* and *D. guttatus* essential oils such as the plant part, the vegetative stage and the geographical origin as well as the investigated subspecies.

Overall, *D. guttatus* essential oils were rich in phenylpropane derivatives with epoxy-pseudoisoeugenyl isobutyrate (18.6-64.9%) and epoxy-pseudoisoeugenyl 2-methylbutyrate (0.6-33.0%) being the main constituents, whereas this class of compounds was absent in *D. carota* samples. Sabinene (2.3-32.2%), α-pinene (tr-23.9%), terpinen-4-ol (tr-7.7%) and limonene (tr-6.6%) characterized *D. carota* essential oils, while in *D. guttatus* samples monoterpenes were scarcely present. To the best of our knowledge this is the first report on the essential oil of *D. carota* and *D. guttatus* collected in Greece.

Table 1: Composition of essential oils from stems & leaves (DCVSL, DAESL), inflorescences (DCVINL, DAEINF) and roots (DCVR, DAER) of *D. carota* and *D. guttatus*, respectively

			Daucus carota			*Daucus guttatus*		
No.	Compound	RI[a]	DCVSL	DCVINF	DCVR	DAESL	DAEINF	DAER
1	α-Thujene	930	1.1	0.9	-	-	-	-
2	α-Pinene	939	23.9	13.9	tr	-	-	-
3	Camphene	954	0.3	0.5	-	-	-	-
4	Sabinene	975	32.2	22.2	2.3	-	-	-
5	β-Pinene	979	4.9	2.4	tr	-	-	-
6	Myrcene	990	3.5	2.5	tr	-	-	-

			Daucus carota			*Daucus guttatus*		
No.	Compound	RI[a]	DCVSL	DCVINF	DCVR	DAESL	DAEINF	DAER
7	α-Terpinene	1017	2.0	1.3	tr	-	-	-
8	*p*-Cymene	1024	tr	tr	tr	-	-	tr
9	Limonene	1029	6.6	3.9	tr	-	-	-
10	*cis*-β-Ocimene	1037	0.4	1.5	-	-	-	-
11	*trans*-β-Ocimene	1050	tr	0.5	-	-	-	-
12	γ-Terpinene	1059	3.1	2.1	3.3	-	-	tr
13	*cis*-Sabinene hydrate	1070	0.9	0.8	-	-	-	-
14	1-Nonen-3-ol	1086	-	0.1	-	-	-	-
15	Terpinolene	1088	0.8	0.6	11.6	-	-	-
16	4-Nonanol	1091	tr	tr	-	-	-	-
17	3-Nonanol	1096	tr	tr	-	-	-	-
18	Linalool	1096	0.3	0.4	-	-	-	-
19	*trans*-Sabinene hydrate	1098	0.6	0.5	-	-	-	-
20	1,3,8-*p*-Menthatriene	1110	tr	tr	-	-	-	-
21	*cis*-*p*-Menth-2-en-1-ol	1121	-	0.4	-	-	-	-
22	*trans*-*p*-Mentha-2,8-dien-1-ol	1122	0.5	-	-	-	-	-
23	Geijerene	1143	-	-	-	-	tr	0.6
24	Terpinen-4-ol	1177	7.7	4.1	tr	-	-	-
25	α-Terpineol	1188	0.6	0.5	-	tr	-	-
26	Isobornyl acetate	1285	tr	0.2	4.0	-	-	-
27	Pregeijerene	1287	-	-	-	-	-	0.3
28	Thymol	1290	-	-	-	tr	-	-
29	δ-Elemene	1338	-	-	-	0.2	0.2	-
30	α-Terpinyl acetate	1349	-	0.1	-	-	-	-
31	β-Elemene	1390	0.3	0.4	tr	tr	tr	-
32	Acora-3,7(14)-diene	1408	tr	-	-	60.1	19.6	9.2
33	*cis*-α-Bergamotene	1412	-	0.7	-	-	-	-
34	β-Caryophyllene	1419	tr	1.5	-	-	-	-
35	β-Gurjunene	1433	-	-	-	0.6	0.4	tr
36	*trans*-α-Bergamotene	1434	tr	1.0	-	-	-	-
37	*cis*-β-Farnesene	1442	tr	0.4	tr	-	-	-
38	*trans*-β-Farnesene	1456	tr	2.3	tr	0.3	tr	7.4
39	Sesquisabinene	1459	-	1.6	-	-	-	-
40	α-Acoradiene	1466	tr	4.1	4.5	-	-	-
41	β-Acoradiene	1470	-	tr	-	-	0.2	-
42	10-*epi*-β-Acoradiene	1475	-	-	-	0.5	-	-
43	Germacrene D	1485	1.3	3.4	1.8	0.6	-	tr
44	δ-Selinene	1492	-	-	tr	0.8	1.0	-
45	Bicyclogermacrene	1500	tr	1.4	-	0.3	0.8	-
46	β-Himachalene	1500	-	-	7.1	-	-	-

			Daucus carota			*Daucus guttatus*		
No.	Compound	RI[a]	DCVSL	DCVINF	DCVR	DAESL	DAEINF	DAER
47	α-Chamigrene	1503	-	-	-	-	1.5	-
48	α-Alaskene	1512	-	-	-	4.8	-	0.4
49	δ-Amorphene	1512	tr	0.5	-	-	-	-
50	β-Sesquiphellandrene	1522	-	0.5	-	tr	tr	-
51	Kessane	1530	-	-	2.2	-	-	-
52	Germacrene B	1561	-	tr	2.0	-	tr	-
53	Junenol	1619	-	-	-	-	0.6	-
54	*cis*-Cadin-4-en-7-ol	1636	-	-	-	-	0.7	-
55	β-Acorenol	1637	-	-	-	0.6	0.4	-
56	*epi*-α-Cadinol	1640	tr	0.3	-	-	-	-
57	*epi*-α-Muurolol	1642	tr	tr	tr	-	-	-
58	Cubenol	1646	-	-	-	1.5	-	-
59	β-Eudesmol	1650	0.5	-	-	-	-	-
60	α-Cadinol	1654	-	0.3	tr	-	-	-
61	*epi*-β-Bisabolol	1671	-	0.2	-	-	-	-
62	*trans*-Asarone	1676	tr	4.9	-	-	-	-
63	*epi*-α-Bisabolol	1684	-	0.4	-	0.6	-	-
64	α-Bisabolol	1685	0.6	0.4	3.3	-	-	-
65	β-Sinensal	1699	-	-	-	0.3	0.6	tr
66	(*E*)-Pseudoisoeugenyl isobutyrate	1742	-	-	-	0.5	2.0	3.2
67	α-Sinensal	1756	-	-	-	tr	tr	-
68	Epoxy-pseudoisoeugenyl isobutyrate	1793	tr	-	-	18.6	64.9	31.8
69	(*E*)-Pseudoisoeugenyl 2-methylbutyrate	1842	-	-	-	tr	-	2.4
70	Epoxy-pseudoisoeugenyl 2-methylbutyrate	1895	-	-	-	0.6	0.6	33.0
71	13-*epi*-Manool oxide	2010	-	0.2	-	-	-	-
72	Tricosane	2300	-	0.2	-	tr	0.2	-
73	Tetracosane	2400	-	tr	-	tr	tr	-
74	Pentacosane	2500	-	0.2	-	0.3	0.3	-
75	Heptacosane	2700	0.4	0.3	-	0.3	tr	-
76	Nonacosane	2900	tr	0.4	-	1.9	0.2	-
	Total identified		92.5	85.0	42.1	93.4	94.2	88.3
	Grouped components							
	TERPENES		92.1	83.8	42.1	71.2	26.0	17.9
	monoterpene hydrocarbons		80.3	53.6	17.2	-	-	tr
	oxygenated monoterpenes		9.1	5.7	4.0	tr	-	-
	sesquiterpene hydrocarbons		1.6	17.8	17.6	68.2	23.7	17.9
	oxygenated sesquiterpenes		1.1	6.5	3.3	3.0	2.3	tr
	oxygenated diterpenes		-	0.2	-	-	-	-
	HYDROCARBONS		0.4	1.1	-	2.5	0.7	-
	aliphatic alcohols		tr	0.1	-	-	-	-

			Daucus carota			*Daucus guttatus*		
No.	Compound	RI[a]	DCVSL	DCVINF	DCVR	DAESL	DAEINF	DAER
	PHENYLPROPANE DERIVATIVES		tr	-	-	19.7	67.5	70.4
	oil yield % w/w		0.14	0.23	0.01	0.05	0.29	0.04

Constituents listed in order of elution from an HP-5 MS column.
[a]RI, retention indices on the HP-5 MS column relative to C_9-C_{23} *n*-alkanes.
tr, trace (<0.1%), -, not detected.

Conclusions

The distinct chemical profiles of the two *Daucus* essential oils, analyzed in our study, are noteworthy and presumably of chemotaxonomic importance. Chemical information can be used as complementary evidence useful in distinguishing between *Daucus carota* and *D. guttatus*, particularly when morphological or molecular evidence is lacking.

References

[1]. Mabberley, D. J. (1997). *The Plant-Book. A portable dictionary of the vascular plants*, Cambridge University Press, Cambridge, UK, 2nd Ed., 736.
[2]. Heywood, V. H. (1968). *Daucus* L. in *Flora Europaea*. Vol. 2, Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M. and Webb D.A. (eds). Cambridge University Press, Cambridge, UK, 373.
[3]. Radulović, N., Dordević, N., Stojanović-Radić, Z. (2011). Volatiles of the Balkan endemic *Daucus guttatus* ssp. *zahariadii* and cultivated and wild-growing *D. carota* - A comparison study. *Food Chemistry*, 125(1):35-43.
[4]. Flamini, G., Cosimi, E., Cioni, P. L., Molfetta, I., Braca, A. (2014). Essential-oil composition of *Daucus carota* ssp. *major* (Pastinocello Carrot) and nine different commercial varieties of *Daucus carota* ssp. *sativus* fruits. *Chemistry & Biodiversity*, 11(7):1022-1033.
[5]. Mohammedi, H., Mecherara-Idjeri, S., Foudil-Cherif, Y., Hassani, A. (2015). Chemical composition and antioxidant activity of essential oils from Algerian *Daucus carota* L. subsp. *carota* aerial parts. *Journal of Essential Oil Bearing Plants*, 18(4):873-883.
[6]. Staniszewska, M., Kula J., Wieczorkiewicz, M., Kusewicz, D. (2005). Essential oils of wild and cultivated carrots - the chemical composition and antimicrobial activity. *Journal of Essential Oil Research*, 17(5):579-583.
[7]. Maxia, A., Marongiu, B., Piras, A., Porcedda, S., Tuveri, E., Gonçalves, M. J., Cavaleiro C., Salgueiro, L. (2009). Chemical characterization and biological activity of essential oils from *Daucus carota* L. subsp. *carota* growing wild on the Mediterranean coast and on the Atlantic coast. *Fitoterapia*, 80(1):57-61.
[8]. Jawdat, D., Al-Faoury, H., Odeh, A., Al-Safadi, B. (2015). Essential oils in seeds of *Daucus* spp.: Insights into the development of potential new cultivars. *Industrial Crops and Products*, 74:867-874.
[9]. Bendiabdellah, A., Dib, M. E. A., Djabou, N., Hassani, F., Paolini, J., Tabti, B., Costa, J., Muselli, A. (2014) *Daucus carota* ssp. *hispanicus* Gouan. essential oils: chemical variability and fungitoxic activity. *Journal of Essential Oil Research*, 26(6):427-440.
[10]. Ma, T., Luo, J., Tian, C., Sun, X., Quan, M., Zheng, C., Kang, L., Zhan, J. (2015). Influence of technical processing units on chemical composition and antimicrobial activity of carrot (*Daucus carrot* L.) juice essential oil. *Food Chemistry*, 170:394-400.
[11]. El Idrissi, M., Elhourri, M., Amechrouq, A. (2013). Identification by chromatographic and spectroscopic techniques of chemical compounds of essential oils extracted from *Daucus carota* L. ssp. *Physical and Chemical News*, 69:83-88.

[12]. Adams, RP. (2007). *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. Allured Publishing Corporation, Carol Stream IL, 4th Ed.

[13]. Staniszewska, M., Kula, J. (2001). Composition of the essential oil from wild carrot umbels (*Daucus carota* L. ssp. *carota*) growing in Poland. *Journal of Essential Oil Research*, 13(6):439-441.

[14]. Chizzola, R. (2010). Composition of the essential oil from *Daucus carota* ssp. *carota* growing wild in Vienna. *Journal of Essential Oil Bearing Plants*, 13(1):12-19.

[15]. Rokbeni, N., M'rabet, Y., Dziri, S., Chaabane, H., Jemli, M., Fernandez, X., Boulila, A. (2013). Variation of the chemical composition and antimicrobial activity of the essential oils of natural populations of Tunisian *Daucus carota* L. (Apiaceae). *Chemistry & Biodiversity*, 10(12):2278-2290.

[16]. Tavares, A. C., Goncalves, M. J., Cavaleiro, C., Cruz, M. T., Lopes, M. C., Canhoto, J., Salgueiro, L. R. (2008). Essential oil of *Daucus carota* subsp. *halophilus*: composition, antifungal activity and cytotoxicity. *Journal of Ethnopharmacology*, 119(1):129-134.