Research article

Chemical Composition of the Essential Oils in Portuguese Daucus Carota Subspecies

Sofia J. Silva[1]*, Jasmine Barata[2], Violeta R. Lopes[3], Ana M. Barata[3], Arlindo Gomes[1,4], Lúcia Silva[1,4], and Ana Luísa Fernando[5]

[1]Departament of Chemistry, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal
[2]Chemical and Engineer Department, Universidad de Lorraine, 54601 Villers Les Nancy Cedex, France
[3]Banco Português de Germoplasma Vegetal, INIAV, I.P., Quinta de S. José, S. Pedro de Merelim, 4700-859, Braga, Portugal
[4]FibEnTech, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal
[5]MEtRICs, Department of Science and Technology of Biomass (DCTB), NOVA School of Science and Technology, New University of Lisbon, Campus de Caparica, 2829-516 Caparica, Portugal

Corresponding Author: Sofia J. Silva; email: sofia.joia.silva@ubi.pt

Published 10 August 2022

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the FibEnTech21 Conference Committee.

Abstract.
This study focused on five different subspecies of Portuguese *Daucus carota* L., to identify the main compounds in the essential oils of umbels from each subspecies. The isolation of the essential oils was performed by hydrodistillation methods followed by further composition analysis by gas chromatography-mass spectrometry, ultimately demonstrating that each subspecies had different major compounds. This study concluded that the main compounds found in *Daucus carota* subsp. *maritimus* were geranyl acetate, followed by trans-methyl-isoeugenol, α-pinene, cis-asarone and elemicin. *Daucus carota* subsp. *hispidus* presented with the major compounds as geranyl acetate, caryophyllene oxide, trans-methyl-isoeugenol and sabinene. For *Daucus carota* subsp. *maximus*, the major compounds were cis-asarone, geranyl acetate and elemicin, which was similar to *Daucus carota* subsp. *carota* which presented with the major compounds as geranyl acetate, cis-asarone, trans-methyl-isoeugenol and α-pinene. Finally, the major components of *Daucus carota* subsp. *sativus* were carotol and daucol.

Keywords: essential oils, chemical composition, *Daucus carota*, hydrodistillation, GC-MS

1. Introduction

Essential oils from aromatic plants are used for hundreds of years ago, as they are composed by volatile secondary metabolites that rapidly are absorbed through the skin and further provide notorious therapeutic benefits of high importance in Human life [1]. Essential oils are complex mixtures and researchers aim to know their composition, to allow practical application and understand which beneficial and adverse effects these oils might have [2]. The Apiaceae family is one of the most studied related to essential oils, not only for being one of the most consumed worldwide, but also because they are used as food, flavoring agents, medical purposes and for being nutraceutical plants [1,2]. One of the species of this family is *Daucus carota*, or as it is commonly called, carrot. *D. carota* is a biannual plant with its flowering seasons from June to August. The umbels appear white and round in full blossom. Before the domestication, the carrot root appeared white, but after intensive breeding, orange carrot became carotenoid-rich existing still other root colours like purple [3]. This specie has some subspecies like *Daucus carota* supbsp. *sativus* and *Daucus carota* subsp. *carota* that are presented worldwide, being the first one the only cultivated and assumed to be originated by selective breeding of the ancestral wild form of the second one [4,5]. According with Heywood study in 1968, there were recognized eleven wild subspecies of *Daucus carota*. In previous studies, a great variety of compounds with different proportions were described in essential oils from umbels. In Tunisia, *Daucus carota* subsp. *maritimus* presented as major components elemicin, geranyl acetate, α-pinene, myrcene and cubenol [1]. *Daucus carota* subsp. *hispidus*, presented as major components α-cadinol, *trans*-methyl-isoeugenol, premnaspirodiene and carotol [6]. As in the chemical analysis of *Daucus carota* subsp. *maximus*, in Italy, it appears that the major compounds are carotol and β-bisabolene, however, a previous Portuguese study presented as major components α-pinene, geranyl acetate, *cis*-asarone, *trans*-methyl-isoeugenol and elemicin [7,5]. For *Daucus carota* subsp. *carota*, a German study compared Polish, French and Moroccan essential oils and it has reported as main component in all of them carotol. The one collected in Poland had higher amounts of verbenol, caryophyllene oxide, α-pinene and *trans*-pinocarveol. French oil presented α-pinene, caryophyllene oxide and caryophyllene. Finally, Moroccan oil presented caryophyllene oxide and β-bisabolene as major components [8]. Accordingly with the same Portuguese study α-pinene and geranyl acetate are the higher percentage components [5], which differs from the ones observed in *Daucus carota* subsp. *sativus*, in India, where the major components are carotol α-pinene, myrcene and limonene [9].

2. Material and methods

2.1. Plant Material

Plant material was provided and maintained in *ex situ* by the *Banco Português de Germoplasma Vegetal* (BPGV). Flowering and mature umbels were preserved at dry place and room temperature.

2.2. Essential oil isolation

All the subspecies were submitted to hydrodistillation, where essential oils from the plant umbels were extracted. First 10 g of plant materials are packed in a distillation flask and then is added 200 mL of water and brought to boil and maintain for 2 h [10]. For this method, we used a Clevenger apparatus.

2.3. Essential oil analysis and chemical composition

Essential oils analysis and chemical composition were proceeded by GC-MS chromatography using an Agilent 7890A GC coupled with an Agilent 5975C inert XL mass selective detector. For the separation of the volatile compounds was used an DB-5 J&W GC capillary column (5% phenylmethylpolysiloxane, 30m length, 0,25mm diameter and 0,25μm film), being helium the mobile phase, at 1mL/min. The components identification were obtained by comparison of the retention times and mass spectra, which were also compared with the ones at the data system library NIST and Wiley.

3. Results and discussion

The yields obtained in our hydro-distillation extractions are present in table 1 and they are very small in all subspecies.

After the oil extraction, we proceeded to analyse the compounds present in our oils. The main compounds found in *Daucus carota* subsp. *maritimus* were geranyl acetate (33.9%), followed by *trans*-methyl-isoeugenol (9.3%), α-pinene (7.2%), *cis*-asarone (6.9%) and elemicin (6.0%). *Daucus carota* subsp. *hispidus* presented as major compounds geranyl acetate (41.1%), caryophyllene oxide (9.1%), *trans*-methyl-isoeugenol (7.0%) and sabinene (4.3%). For *Daucus carota* subsp. *maximus*, the major compounds were *cis*-asarone (47.1%), geranyl acetate (14.8%) and elemicin (13.1%), similar with *Daucus carota* subsp. *carota* that presented as major compounds geranyl acetate (32.2%), *cis*-asarone (29.6%), *trans*-methyl-isoeugenol (9.8%) and α-pinene (6.5%). Finally, *Daucus carota* subsp. *sativus* has presented as major components carotol (53.8%) and daucol (26.7%). All subspecies presented, even though at some small percentage in some of them, geranyl acetate, *trans*-methyl-isoeugenol, *β*-bisabolene, caryophyllene oxide and *cis*-asarone, as shown in **Table 2.**

It has been reported that some variations occurred in comparison with the previous studies of different countries. Focussing on *Daucus carota* subsp. *maritimus* results

Table 1: Yield percentage (v/w)

Subspecies	Yield (%)
D. carota subsp. *maritimus*	0.39
D. carota subsp. *hispidus*	0.33
D. carota subsp. *maximus*	0.45
D. carota subsp. *carota*	0.23
D. carota subsp. *sativus*	0.06

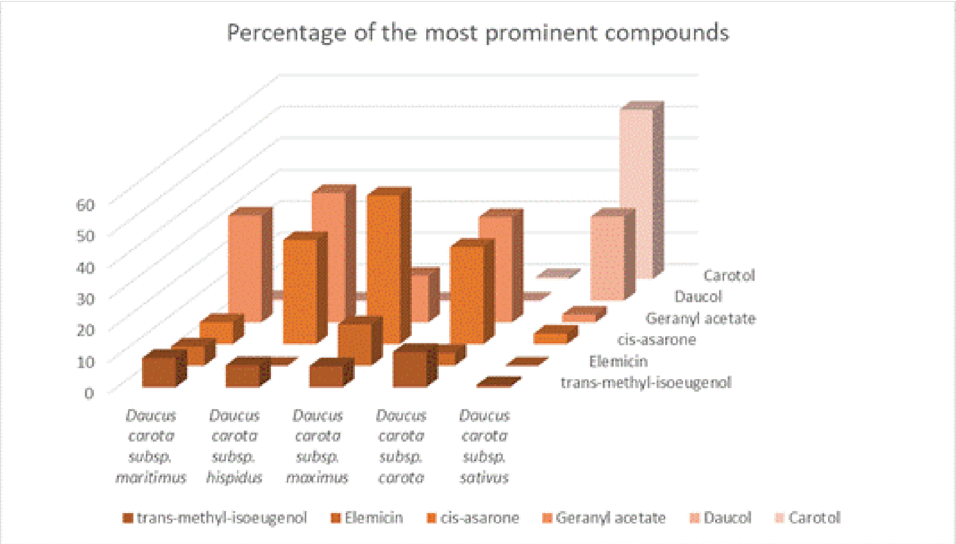

Figure 1: Percentage of the most prominent compounds in *Daucus carota* subspecies.

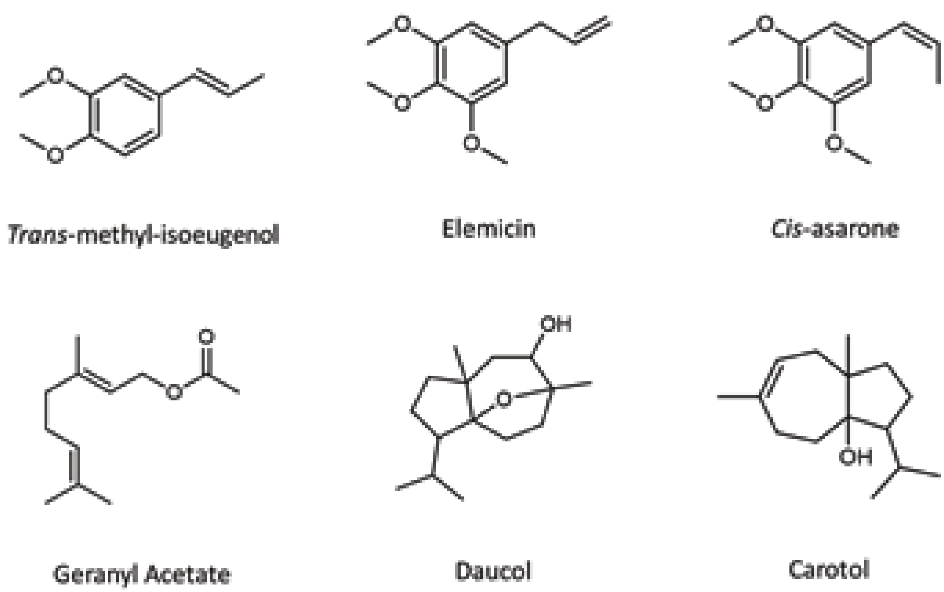

Figure 2: Structures of the most prominent compounds in *Daucus carota* subspecies.

		% *Daucus carota* subspecies				
Compounds	RI	*maritimus*	*hispidus*	*maximus*	*carota*	*sativus*
α - pinene	936	7.2	2.0	1.0	6.5	-
Camphene	950	0.5	-	-	-	-
Sabinene	973	1.5	4.3	2.3	1.2	-
β - pinene	978	5.5	1.7	1.9	4.2	-
β - myrcene	989	1.1	0.5	-	0.7	-
α -terpinene	1017	-	-	-	-	-
p - cymene	1024	0.3	0.4	-	-	-
Limonene	1029	2.0	1.3	0.9	1.1	-
γ-terpinene	1059	-	0.5	-	-	-
cis - Linalool Oxide (furanoid)	1075	0.5	-	-	0.7	-
trans - Linalool Oxide (furanoid)	1083	0.3	-	-	-	-
α - terpinolene	1086	-	-	-	-	-
Linalol	1099	2.2	1.7	-	1.2	-
α - campholenol	1124	0.3	0.7	-	-	-
trans - Pinocarveol	1140	1.2	3.3	0.8	0.9	-
trans - Verbenol	1144	0.3	3.3	-	-	-
cis - Verbenol	1145	1.7	2.3	-	1.3	-
Sabina ketone	1157	-	0.7	-	-	-
Pinocarvone	1160	0.6	1.0	-	-	-
p-mentha-1,5-dien-8-ol	1166	0.5	0.5	-	-	-
4-Terpineol	1177	0.4	2.4	1.5	-	1.3
α - terpineol	1189	0.5	-	-	-	-
Myrtenal	1192	1.3	3.2	-	-	-
Verbenone	1206	0.6	-	-	-	-
Carvone	1242	-	0.4	-	-	-
Linalyl acetate	1255	1.9	-	-	-	-
Bornyl acetate	1283	-	0.9	-	-	-
α - terpinenyl acetate	1347	0.4	-	-	-	-
Geranyl acetate	1379	33.9	41.1	14.8	32.2	2.5
β - selinene	1486	0.3	-	-	0.8	-
trans - methyl - isoeugenol	1495	9.3	7.0	6.8	9.8	0.7
β - bisabolene	1508	3.4	4.0	3.9	2.2	0.5
Elemicin	1553	6.0	-	13.1	4.1	-
Spathulenol	1576	-	0.4	-	-	0.3
Caryophyllene oxide	1581	1.9	9.1	4.5	2.5	1.2
Carotol	1595	2.2	-	-	0.7	53.8
Daucol	1639	0.6	-	-	-	26.7
cis - asarone	1679	6.9	3.3	47.1	29.6	3.3

Table 1: Main components found in the analysed Daucus carota subspecies.

has shown that major components were geranyl acetate, *trans*-methyl-isoeugenol, α-pinene, *cis*-asarone and elemicin. Geranyl acetate and α-pinene as expected represented most of the compounds, such as elemicin, reported by Majdoub in [1], however, the concentrations of *trans*-methyl-isoeugenol and *cis*-asarone were higher than the ones in other countries. For *Daucus carota* subsp. *hispidus*, geranyl acetate was the major component, followed by caryophyllene oxide, *trans*-methyl-isoeugenol and sabinene. From the previous studies, *trans*-methyl-isoeugenol was the one who kept the most similar quantity compared to the Tunisian study. *Daucus carota* subsp. *maximus* appears to have as major components *cis*-asarone, geranyl acetate and elemicin, a very diverse constitution compared to the Italian oil, by Gaglio in [7], that presented as major component carotol, that was not traced in our oil and β-bisabolene, that only presented 3.9% of our oil. Comparing to the Portuguese study, geranyl acetate presented similar percentage, however *cis*-asarone presented a much higher quantity and *trans*-methyl-isoeugenol a smaller percentage than the one referred, like α-pinene. *Daucus carota* subsp. *carota* presented as major components, geranyl acetate, *cis*-asarone, *trans*-methyl-isoeugenol and α-pinene. Comparing to the German study, by Sieniawska in [8], our oil percentage of carotol was significantly lower, not even being in the major components, being only similar the quantity of α-pinene. However, the previous Portuguese study presented a similar percentage of geranyl acetate, being the only concordance among the studies, since α-pinene in our oil presented a much smaller percentage and *cis*-asarone, the second most prominent component in our oil was not traced in the previous study. Finally, *Daucus carota* subsp. *sativus*, also presented different results, being carotol and daucol the most prominent components. According with the Indian study, carotol is the major component, however, it hasn't been described daucol as a major component in previous studies, and α-pinene, myrcene and limonene were not traced in our study.

Many outcomes indicate these variations in the percentage of the major components depend on the location, if they are wild or cultivated and stage of flowering, and for that reason, some diversity was observed to other studies. As stated for Snene [6], due to its nutritional and therapeutic values, the essential oil combination from *Daucus carota* subspecies from different countries all over the world show considerable quantitative and qualitative variability [6]. It is also considerable as a possible variation reason the harvesting stage. The extraction method can also be one of the causes of variety in these components, as we can see in Madjdoub study, different percentage of the components were presented, depending on the extraction method used [1].

The percentage of some of the most prominent compounds and its distribution per each subspecies are present as visual representation in **Figure 1** and in **Figure 2** their structures.

4. Conclusions

Essential oils obtained by hydrodistillation of umbels from five subspecies of Portuguese *Daucus carota* L. were analysed by gas chromatography-mass spectrometry (GC-MS). A total of 38 compounds were identified. For three subspecies (*maritimus*; *hispidus* and *carota*), high levels of geranyl acetate are found. *Daucus carota* subsp. *maximus* presented *cis*-asarone as main compound but geranyl acetate is the second main component and elemicin the third. *Daucus carota* subsp. *sativus* has more than 50% of carotol and daucol as second main compound. Knowing the amount of geranyl acetate present in these oils, and considering the therapeutic value of monoterpenes like it, we could use these oils due to its pharmacological properties, in cases off inflammation, pain-related and antioxidant effects [11]. Previous studies also demonstrated that both carotol and daucol present high antifungal activity, probably due to its polar groups, which can be a good indicator that *Daucus carota* subsp. *sativus* essential oil might be a good antifungal oil [12].

Acknowledgments

We acknowledge *the Banco Português de Germoplasma Vegetal* for providing our plant samples. This research was funded by Fundação para a Ciência e a Tecnologia, FCT, through the funding of the UID Fiber Materials and Environmental Technologies (FibEnTech-UBI), project UIDB/00195/2020.

References

[1] Majdoub S, El Mokni R, Muradalievich AA, Piras A, Porcedda S, Hammami S. Effect of pressure variation on the efficiency of supercritical fluid extraction of wild carrot (*Daucus carota* subsp. maritimus) extracts. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences. 2019;1125:121713;1-6. https://doi.org/10.1016/j.jchromb.2019.121713

[2] Khalil N, Ashour M, Fikry S, Singab AN, Salama O. Chemical composition and antimicrobial activity of the essential oils of selected apiaceous fruits. Future Journal of Pharmaceutical Sciences. 2018;4(1):88–92. https://doi.org/10.1016/j.fjps.2017.10.004

[3] Ng TB, Fang EF, Li X, Lu Q, Wong JH, Guo H. Essential oils in food preservation, flavor and safety. *In Essential Oils in Food Preservation, Flavor and Safety*. Elsevier Inc; 2015;34;303-308 https://doi.org/10.1016/B978-0-12-416641-7.00034-1

[4] Spooner, DM. Daucus : Taxonomy, Phylogeny, Distribution. in *The Carrot Genome* 2019 ; 9–26. https://doi:10.1007/978-3-030-03389-7_2.

[5] Tavares AC, Loureiro J, Castro S et al. Assessment of *Daucus carota* L. (apiaceae) subspecies by chemotaxonomic and DNA content analyses. Biochemical Systematics and Ecology. 2014;55 :222–230. https://doi.org/10.1016/j.bse.2014.03.031

[6] Snene A, Mokni R, El Mahdhi A, Joshi RK, Hammami S. Comparative study of essential oils composition and in vitro antibacterial effects of two subspecies of *Daucus carota* growing in Tunisia. South African Journal of Botany. 2020;130:366–370. https://doi.org/10.1016/j.sajb.2020.01.028

[7] Gaglio R, Barbera M, Aleo A, Lommatzsch I, La Mantia T, Settanni L. Inhibitory activity and chemical characterization of *Daucus carota* subsp. maximus essential oils. Chemistry and Biodiversity. 2017;14(5);1-6. https://doi.org/10.1002/cbdv.201600477

[8] Sieniawska E, Świątek Ł, Rajtar B, Kozioł E, Polz-Dacewicz M, Skalicka-Woźniak K. Carrot seed essential oil—Source of carotol and cytotoxicity study. Industrial Crops and Products. 2016;92:109–115. https://doi.org/10.1016/j.indcrop.2016.08.001

[9] Verma RS, Padalia RC, Chauhan A. Chemical composition variability of essential oil during ontogenesis of *Daucus carota* L. subsp. sativus (Hoffm.) Arcang. Industrial Crops and Products. 2014;52:809–814. https://doi.org/10.1016/j.indcrop.2013.12.012

[10] Azmir J, Zaidul ISM, Rahman MM et al. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering. 2013;117(4):426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

[11] Khayyat SA, Sameeh MY. Bioactive epoxides and hydroperoxides derived from naturally monoterpene geranyl acetate. Saudi Pharmaceutical Journal. 2018;26(1):14–19. https://doi.org/10.1016/j.jsps.2017.11.005

[12] Sharma M, Chahal KK, Kaur R, Singh R, Kataria D. Antifungal potential and structure activity relationship of carrot seed constituents. Journal of Food Biochemistry. 2019;43(9):1–9. https://doi.org/10.1111/jfbc.12971