American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AkiNik

AJEONP

American Journal of Essential Oils and Natural Products

ISSN: 2321-9114
AJEONP 2017; 5(4): 09-13

Received: 14-08-2017
Accepted: 15-08-2017

DT Asilbekova
Acad. S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan

KhM Bobakulov
Acad. S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan

SA Sasmakov
Acad. S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan

JM Abdurakhmanov
Acad. S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan

ShS Azimova
Acad. S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan

ND Abdullaev
Acad. S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan

Sh Sagdullaev
Acad. S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan

Correspondence:
KhM Bobakulov
Acad. S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan

Composition and antimicrobial activity of essential oils from *Daucus carota* L. subsp. *carota*, growing in Uzbekistan

DT Asilbekova, KhM Bobakulov, SA Sasmakov, JM Abdurakhmanov, ShS Azimova, ND Abdullaev and Sh Sagdullaev

Abstract
The study describes the component composition and antimicrobial activity of essential oils from different part of wild carrot (*Daucus carota* L. subsp. *carota*, family Apiaceae), growing in Uzbekistan. The essential oils from fruits and aerial parts enriched with oxygenated sesquiterpenes (70.0-88.0%), with high content of carotol (68.3-78.3%). The extracted from fruits essential oils possess expressed activity against opportunistic pathogenic fungus of *Candida albicans*.

Keywords: *Daucus carota* L. *subsp.* carota, essential oils, hydro-distillation, GC/MS, antimicrobial activity

1. Introduction
The wild carrot *Daucus carota* L. subsp. *carota*, belonging to the family (Apiaceae), originally cultivated as a medicinal plant, and then cultured (cultivated form - *Daucus carota* var *sativus* Holfm.). In the wild form it is widespread in Central Asia, Western Europe, in the Caucasus, and is very diverse on variety and chemical composition [1, 2]. Different parts of carrot contain essential oils (EO), flavonoids, coumarins, anthocyanins, lipids, falcarindiol and other biologically active compounds [2-13].
In previous scientific studies noted significant differences in EO chemical composition of wild carrots from different regions [5-10, 13]. Component composition of seeds EO of wild carrots depends on the geographical origin of the taxon [7]. Differences in the composition of EO of plants may be due to many factors such as differences in the environmental conditions of the region, season of the collection, the development stage and used extract part of the plant and the method of extraction.
In folk medicine, the fruits are used as anthelmintic and diuretic, at nephrolithiasis and flatulence. Whole or parts of wild carrot possess antibacterial, diuretic, choleretic and salt-soluble effect [2].
Depending on the chemical composition of the EO exhibits antioxidant [10], cytotoxic [9], antibacterial and antifungal activities [5, 8]. The most significant activity was observed in the essential oils of ripe and unripe fruits of wild carrots, containing as major components α-pinene, sabinene and α-muurolene [5]. It should be noted that was investigated the composition and the biological activity of EOs from seeds of cultivated species of *Daucus carota ssp. sativa* which commercially available in Uzbekistan. The essential oil from this taxon, containing as main components β-bisabolene (80.49%), β-asarone (8.82%) and β-bergamotene (5.51%) exhibited antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* [14]. To the best of our knowledge the EO composition and antimicrobial activities of wild carrot from our region has not been reported.
The aim of the present study was to study of the chemical composition and antimicrobial activity of EO of wild carrot (*Daucus carota* L. subsp. *carota*), growing in Uzbekistan. The objects of the research were EOs isolated from the ripe fruit, leaves, flowers and petals.

2. Materials and Methods
2.1 Plant material
The aerial parts of wild carrot were collected in flowering period (May, 2015) and in the fruiting period (June, 2015) in the Tashkent region of Uzbekistan. The objects were dried for a

week to air-dry.

2.2 Isolation
Isolation of EO from ripe fruits, leaves, flowers and petals were carried out by hydro-distillation method at Clevenger apparatus for 5-6 hours.

2.3 Gas chromatography-mass spectrometry (GC-MS) analysis
GC-MS analyses of EOs for all samples were carried on a 7890A GC /5975C Inert MSD (Agilent Technologies, USA) fitted with an HP-5 MS (30.0m x 250.0µm x 0.25µm) capillary column. The carrier gas was helium at a flow rate of 1.3 mL/min. The oven temperature was held at 60 °C for 1 min, increased to 250 °C at 4 °C/min, held for 6 min and then increased to 290 °C at 25 °C/min, held for 5 min. The samples were prepared in *n*-hexane and 1.0 мL injected in splitting mode (75:1). MS conditions were as follows: ionization energy 70 eV, source temperature 180° C, quadrupole temperature 150º C, mass range 10-500 amu. The identification of the components was performed on the basis of chromatographic retention indices (RI) and by comparison of the recorded spectra with stored MS library (W8N05ST and NIST08) and their mass spectral fragmentation patterns with those reported in the literature [15]. Retention indices were calculated by means of a mixture of homologue *n*-alkanes (C8-C20) analyzed under the same chromatographic conditions used for the analysis of EOs.

2.4 Antimicrobial activity
The antibacterial and antifungal activities were determined by using a modified agar diffusion method [16, 17]. Micro-dilution method was used to determine of MIC as described by CLSI [18-19]. Only samples that showed remarkable antimicrobial activity from agar-diffusion were tested for MIC. The microorganism strains *Bacillus subtilis* (RKMUz - 5), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27879), *Escherichia coli* (RKMUz - 221), and fungal strain *Candida albicans* (RKMUz - 247) were used as test cultures. The strains RKMUz were obtained from the collection of the Institute of Microbiology, Uzbekistan Academy of Sciences.

3. Results & Discussion
3.1 The essential oil content in different parts of *Daucus carota*
Air dried aerial part of the plant was divided into flowers (flower head), leaves and stems. Petals were selected from the flower heads. Yield of petals from the flower heads was 11.0% in terms of dry weight of aerial parts of the plant (Table. 1). The results presented in Table 1 shows that the petals from the aerial parts had least mass concentration of EOs (0.5%) in terms of dry weight in comparison with other plant parts. The EO more accumulates in flowers and ripe fruits.

Table 1: The essential oil content in different parts of wild carrot *Daucus carota* L. subsp. *carota*

No	Organ or part of the plant (Vegetation period)	Portion, % from the weight of the aerial part	Humidity, %	Yield of essential oil, %
1.	Aerial part (flowering period)	100	12.1	1.60
1.1.	Flowers	43.0	9.8	1.90
1.2.	Leaves	36.0	12.5	1.53
1.3.	Stems	21.0	10.8	-
1.4.	Petals	11.0*	8.5	0.50
2.	Ripe fruits (fruiting period)	100	8.2	1.56

*The petals were separated from the flower heads.

3.2 Essential oil chemical composition by GC-MS
EO chemical composition from fruits, leaves, flowers and petals were determined by GC-MS. The identified EO components and their RI values are given in Table 2 in the order of their elution on the HP-5MS column. As a result of analysis of wild carrot EO were identified 54 components, among which the largest amount of compounds (48), accounting for 98.5% were observed in EO from the leaves. In EO composition from the fruits, we found only 13 components with total content of 99.0%.

Table 2: The chemical composition of essential oils from various parts of *Daucus carota* L. subsp. carota.

No	Compound	RI	Leaves	Flowers	Petals	Fruits
1	Hexanal	802	<0.1	<0.1	-	-
2	α-Pinene	932	3.1	2.0	1.6	1.3
3	Camphene	950	0.2	0.1	0.1	-
4	Thuja-2,4(10)-diene	954	<0.1	<0.1	<0.1	-
5	Sabinene	972	0.2	0.2	0.1	-
6	β-Pinene	976	0.6	0.5	0.2	1.5
7	β-Myrcene	991	0.7	0.5	0.3	-
8	α-Terpine	1015	<0.1	-	-	-
9	*p*-Cymene	1024	<0.1	<0.1	0.1	-
10	α-Limonene	1028	1.3	1.2	1.5	0.4
11	γ-Terpinene	1058	<0.1	<0.1	-	-
12	Terpinolene	1088	<0.1	0.1	-	-
13	Linalool	1101	0.1	0.2	<0.1	-
14	α-Campholenal	1127	<0.1	-	0.1	-
16	*trans*-Pinocarveol	1139	<0.1	-	0.1	-
17	*trans*-Verbenol	1145	0.1	<0.1	0.2	-
18	Pinocarvone	1160	<0.1	-	<0.1	-
19	α-Phellandren-8-ol	1169	0.1	0.1	<0.1	-

20	Terpinen-4-ol	1178	0.1	0.1	-	-
21	α-Terpineol	1191	<0.1	0.1	<0.1	-
22	Verbenone	1210	<0.1	-	0.1	-
23	*trans*-Carveol	1218	-	-	0.1	-
25	Carvone	1242	-	-	0.1	-
26	α-Longipinene	1350	0.8	1.8	<0.1	0.6
27	α-Copaene	1373	<0.1	<0.1	<0.1	-
28	Daucene	1381	5.0	4.7	0.2	9.0
29	β-Cubebene	1388	<0.1	-	<0.1	-
30	β-Longipinene	1401	<0.1	0.1	-	-
31	Dodecanal	1406	-	-	<0.1	-
32	*cis*-α-Bergamotene	1412	0.1	-	-	-
33	β-Caryophyllene	1417	1.2	0.8	0.5	1.4
34	*trans*-α-Bergamotene	1431	2.1	1.4	0.4	4.7
35	*cis*-β-Farnesene	1444	0.2	0.2	-	-
36	α-Himachalene	1451	0.1	0.1	-	-
37	α-Humulene	1454	0.1	0.1	-	-
38	*trans*-β-Farnesene	1456	3.7	3.3	0.9	3.7
39	*cis*-Muurola-4(14),5-diene	1465	1.7	1.7	<0.1	2.5
40	γ-Muurolene	1475	0.3	-	-	-
41	γ-Curcumene	1477	0.4	0.8	0.1	-
42	β-Himachalene	1499	2.0	2.6	0.1	1.9
43	β-Bisabolene	1505	3.3	2.2	0.5	1.8
44	*cis*-γ-Bisabolene	1510	0.1	-	-	-
45	β-Cadinene	1513	-	-	<0.1	-
46	β-Sesquiphellandrene	1522	0.3	0.5	-	-
47	*cis*-Calamenene	1526	0.1	0.1	0.1	-
48	*trans*-γ-Bisabolene	1532	0.1	0.1	-	-
49	Copaen-15-ol	1550	0.2	-	2.0	-
50	Caryophyllene oxide	1576	0.2	0.2	1.1	-
51	Carotol	1589	68.3	68.8	78.3	69.8
52	Daucol	1638	1.5	3.4	5.7	-
53	cis-α-Copaene-8-ol	1608	-	-	-	0.5
54	β-Eudesmol	1648	0.1	0.2	0.7	-
55	β-Bisabolol	1671	-	-	0.1	-
56	α-Asarone	1674	0.1	-	0.1	-
Number of identified compounds			48	37	39	13
Sum of identified compounds			98.5	98.2	95.2	99.0
Monoterpenes			5.6	4.3	3.6	3.2
Oxygenated monoterpenes			1.0	0.8	0.8	-
Sesquiterpenes			21.6	20.6	2.8	25.8
Oxygenated sesquiterpenes			70.3	72.5	88.0	70.0

*RI – retention indices on HP-5 MS capillary column.

According to results from Table 2, EOs of all studied organs of plants enriched with oxygenated sesquiterpenes with high content of carotol (68.3-78.3%). Besides carotol in the all samples were observed daucene (0.2-9.0 %), *trans*-α-bergamotene (0.4-4.7 %), *trans*-β-farnesene (0.9-3.7 %), β-bisabolene (0.5-3.3 %), α-pinene (1.3-3.1 %), and β-himachalene (0.1-2.6 %). Different organs of the aerial parts of the studied plants are quite similar component composition of essential oiL. However, the fruits accumulate a few numbers of components that almost dominate or are present in significant amounts in the aerial part of plant organs. It should be noted that in the fruit EO more content of daucene in comparison with aerial part of the plant.

In accordance with our results, the major compound of essential oils from flowering and mature umbrellas with seeds of wild carrot from Tunisia in Tunisia was carotol (48.0-55.7%)[13]. On the contrary, the oil from same countries (Sejnane in Tunisia) was rather differed and consisted mainly of 11-α-(H)-himachal-4-en-1-β-ol (12.7–17.4%), sabinene (12.0-14.5%), eudesm-7(11)-en-4-ol (8.2–8.5%), α-selinene (7.4-8.6), and carotol (3.5-5.2%). Carotol (19-33%) also was the main component in wild carrot essential oils from Poland and commercially available oil from Moroccan and French [9]. By Maxia *et al*. [8] have found that in the Sardinian essential oil of flowering and mature umbels with seeds dominated β-bisabolene (17.6-51.0%) and 11-α-(H)-himachal-4-en-1-β-ol (9.0-21.6%). However the oils from Portuguese samples were predominantly composed of geranyl acetate (5.2-65.0%) and α-pinene (3.5–37.9%).

The main components in the essential oils from Algerian *Daucus carota* L. subsp. *carota* aerial part in full flowering were α-pinene (21.3 %) and asarone (18.4 %) [10]. Soković *et al*. [5] reported that oil**s** from the ripe fruits, unripe fruits, flowers, root, leaves, and stem of wild *Daucus carota* L. collected in Serbia contained α-pinene (7.05-51.23%) and sabinene (2.68-36.69%) as major constituents. These major compounds besides geranyl acetate are found in small amounts in our studied *Daucus carota* L. subsp.*carota* oils. It is likely that the composition of essential oil from wild carrots essentially depends on geographic origin. As have been reported by [10] the variations were sufficient to allow the distinction of different chemotypes that were the results of an adaptive process to particular ecologic conditions. Based on our results, we can conclude that wild *Daucus carota* that grows in Uzbekistan refers to a carotol containing chemotype.

3.3 Antimicrobial activity

Antimicrobial test results showed (Table 3), that Gram-positive bacteria - *Bacillus subtilis* and *Staphylococcus aureus* are sensitive to the effect of EOs from all parts of *Daucus carota* L. Essential oils from the fruits possess an expressed activity against fungi *C. albicans* (diameter of inhibition zone is 13 mm and the MIC value of 12 µl/ml). Revealed activity of EO from the fruit in comparison with inactive oil samples from aerial parts of the plant may be due to a high content of *trans*-α-bergamotene and daucene. The essential oils present well known antifungal activity. The antifungal properties of essential oil of various plants (thyme, rosemary, mugwort and various species of mint) which were tested against 39 mold strains were reported by Benjilali *et al*. [20]. Clove essential oil and the main component of clove oil, eugenol, has a strong antifungal effect against different strains of *Candida* and *Aspergillus* [21-24]. Different reported MIC in this study may be the result of using different fungi species and media and amount of main components of the essential oiL.

Table 3: Antimicrobial activity of essential oils from various organs of *Daucus carota* L. subsp. *carota* evaluated by the diameter of inhibition zone (mm) and MIC (µl/ml)

Samples	Gram-positive bacteria		Gram-negative bacteria		Fungi	MIC
	B. subtilis	***S. aureus***	***P. aeruginosa***	***E. coli***	***C. albicans***	***C. albicans***
EO from leaves	6	6	NA	NA	NA	NT
EO from flowers	7	7	NA	NA	NA	NT
EO from petals	6	7	NA	NA	NA	NT
EO from fruits	7	7	NA	NA	13	12
Ampicillin (20 µg/disc)	26	27	NT	26	NT	NT
Ceftriaxone (20 µg/disc)	NT	NT	25	NT	NT	NT
Nystatin (20 µg /disc)	NT	NT	NT	NT	20	4

NA – not active; NT – not tested

4. Conclusions

In conclusion of investigations of the chemical composition and antimicrobial activity of essential oils from wild carrot growing in the conditions of Uzbekistan revealed that carotol dominated in EOs from fruits and aerial parts of the plant. Essential oils from fruits, along with moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*, were exhibited expressed activity against *C. albicans*.

5. Acknowledgments

This work was financially supported by the Program for Applied Scientific Research of Uzbekistan Academy of Sciences (No. FA-A11-TO24)

6. References

1. Flora of Uzbekistan. Publisher of Uzbek SSR Academy of Sciences, Tashkent. 1959; 4:466-470 (in Russ.).
2. Plant Resources of the USSR. Flowering plants, their chemical composition, use. Nauka, Leningrad. 1993; 4:103-104.
3. Azimova ShS, Glushenkova AI. Lipids, Lipophilic Components and Essential Oils from Plant Sources. Springer, London. 2012; 1:24.
4. Asilbekova DT, Khidoyatova ShK, Siddikov DR, Melikuziev FA, Nabiev AA, Sagdullaev Sh.Sh. Abstracts of All-Russian Youth Conference «Problems and Achievements of Chemistry of Oxygen- and Nitrogen-Containing Biologically Active Compounds», Ufa, Russia. 2016; 73-74.
5. Soković M, Stojković D, Glamočlija J, Ćirić A, Ristić M, Grubišić D. Susceptibility of pathogenic bacteria and fungi to essential oils of wild *Daucus carota.* Pharmaceutical Biology. 2009; 47(1):38-43.
6. Gonny M, Bradesi P, Casanova J. Identification of the components of the essential oil from wild Corsican *Daucus carota* L. using ^{13}C-NMR spectroscopy. Flavour & Fragrance JournaL. 2004; 19(5):424-433.
7. Acimovic M, Stankovic J, Cvetkovic M, Ignjatov M, Nikolic Lj. Chemical characterization of essential oil from seeds of wild and cultivated carrots from Serbia. Botanica Serbica. 2016; 40(1):55-60.
8. Maxia A, Marongiu B, Piras A, Porcedda S, Tuveri E, Gonɜalves MJ, *et al*. 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. 2009; 80(1):57-61.
9. 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 & Products. 2016; 92:109-115.
10. Mohammedi H, Mecherara-Idjeri S, Foudil-Cherif Ya, Hassani A. Chemical Composition and Antioxidant Activity of Essential Oils from Algerian *Daucus carota* L. subsp. *carota* Aerial Parts. Journal of Essential Oil-Bearing Plants. 2015; 18(4):873-883.
11. Mahboubi M, Kazempour N, Mahboubi A. The Efficacy of Essential Oils as Natural Preservatives in Vegetable OiL. Journal of Dietary Supplements. 2014; 11(4):334-346.
12. Staniszewska M, Kula J, Wieczorkiewicz M, Kusewicz D. Essential Oils of Wild and Cultivated Carrots - the Chemical Composition and Antimicrobial Activity. Journal of Essential Oil Research. 2005; 17(5):579-583.
13. Marzouki H, Khaldi A, Falconieri D, Piras A, Marongiu B, Molicotti P, Zanetti S. Essential oils of *Daucus carota* subsp. *carota* of Tunisia obtained by supercritical carbon dioxide extraction. Natural Products Communication. 2010; 5:1955-1958.
14. Imamu X, Yili A, Aisa HA, Maksimov VV, Veshkurova ON, Salikhov ShI. Chemical composition and antimicrobial activity of essential oil from *Daucus carota* sativa seeds. Chemistry of Natural Compounds. 2007; 43(4):495-496.
15. Adams RP. Identification of essential oil components by gas chromatography/ mass spectrometry, 4th ed. Allured Publishing Corporation, Illinois. 2007; 401.
16. Wayne P. Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk diffusion susceptibility tests. 19th ed. approved standard. CLSI document. 2009, M100-S19.
17. Lindl T, Bauer J. Zell und Gewebekultur, Gustav-Fischer-Verlag Jena, Berlin. 1989; 181.

18. Clinical and Laboratory Standards Institute (CLSI), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard, National Committee for Clinical Laboratory Standards, Wayne, Pa, USA, 9th edition, 2014.
19. Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, National Committee for Clinical Laboratory Standards, Wayne, Pa, USA, 11th edition, 2014.
20. Benjilali B, Tantaoui-Elaraki A, Ayadi A, Ihlal M. Method to study antimicrobial effects of essential oils: application to the antifungal activity of six Moroccan essences. J Food Protec. 1984; 47:748-752.
21. Ahmad N, Alam MK, Shehbaz A, Khan A, Mannan A, Rashid HS, *et al*. Antimicrobial activity of clove oil and its potential in the treatment of vaginal candidiasis. J Drug Targeting. 2005; 13:555-561.
22. Nunez L, D'Aquino M, Chirife J. Antifungal properties of clove oil (*Eugenia caryophylata*) in sugar solution. Brazilian J MicrobioL. 2001; 32:123-126.
23. Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* (*Eugenia caryophyllus*) on *Candida*, *Aspergillus* and dermatophyte species. J Med. MicrobioL. 2009; 58:1454-1462.
24. Inder Singh Rana, Aarti Singh Rana, Ram Charan Rajak. Evaluation of antifungal activity in essential oil of the *Syzygium aromaticum* (L.) by extraction, purification and analysis of its main component eugenoL. Brazilian J MicrobioL. 2011; 42:1269-1277.