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Comparative analysis of essential oil components of two *Daucus* species from Algeria and their antimicrobial activity

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Abstract

The essential oils obtained by hydrodistillation from leaves and stems of Algeria native, Daucus carota L. subsp. carota and Daucus carota L. subsp. gummifer were analyzed by GC and GC–MS. In total, 67 compounds, which accounted for more than 90.0 g/100g of the total composition of the oils, have been identified. α-pinene (26.0-34.1 g/100g), sabinene (1.5-14.0 g/100g), limonene (0.5-13.0 g/100g), β-pinene (0.6-11.2 g/100g), myrcene (10.0-13.1 g/100g), terpinene-4-ol (2.4-4.9 g/100g), caryophyllene oxyde (0.8-6.0 g/100g), spathulenol (0.6-4.3 g/100g), p-cymene (3.3-4.4 g/100g) and isiospathulenol (0.2-3.8 g/100g) have been identified as the main components of both essential oils. The comparison of essential oil components of Daucus species between the present and previous work indicated that the composition of oils vary greatly with respect to the plant parts used, geographical locations and stage of development, mainly for the proportion of terpenoids and phenylpropanoids. In addition, we reported for the first time, the antibacterial activity of D. carota L. subsp. gummifer essential oil against eight bacteria. The most prominent inhibitory action of this essential oil was observed against Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus and Escherichia coli strains at a concentration of 2.5 mg/ml. The essential oil compositions of two Daucus samples can therefore be proposed as new potential sources natural for pharmaceutical industries.

Keywords*: D. carota* L. subsp. *gummifer; D. carota* L. subsp. *Carota;* Essential oils; Antimicrobial activity; Comparative analysis.

Introduction

Essential oils as antimicrobial agents are recognized as safe natural substances to their users and for the environment and they have been considered at low risk for resistance development by pathogenic microorganisms[1,2]. Nowadays, the interests for essential oil extracted from aromatic plants are multiple and diversified. Based on their therapeutic properties and the chemical substances isolated from their volatile parts, it may allow further application in particular biological activities[3]. Apiaceae represent one of the best-known plant families, widely distributed in temperate climate regions where they are often used as spices, vegetables or drugs owing to the presence of useful secondary metabolites such as essential oils[4]. Essential oils of Apiaceae family have been widely used throughout history for their pharmacological activities, such as antibacterial, antifungal, antiviral, antiparasitic, insecticidal and antispasmodic[5,6]. Today they are being used in pharmaceutical, sanitary, cosmetic, agricultural and food industries. *D. carota* L. is an Apiaceae native from Europe, Asia, Africa and Macaronesia. Traditional medicine uses *D. carota* extracts for hepatic and renal insufficiency as well as for skin disorders, e.g. burns and furunculous[7]. Various compositions of *D. carota* L. subsp. *carota* and *D. carota* L. subsp. *gummifer* essential oils have been reported, characterized by the occurrence of a main component, α-pinene (up to 51%), sabinene (up to 60%), geranyl cetate (up to 76%), elemicin (up to 35%), E-methylisoeugenol (up 30%) and carotol (up to 66%)[8-18] (table 2), but they didn't give homogeneous results. These may be due to the genetic, plant parts used, geographical locations, season at the time of collection and analytical methods However, only a few studies have been reported on the antimicrobial activity of the investigated essential oils[10,12,16]. As far as we were concerned, the chemical composition of *D. carota L.* subsp. *carota* and *D. carota* subsp. *gummifer* essential oils was never reported in Algeria. The aim of the present study was to investigate the qualitative and quantitative chemical composition (compared with the results reported in the previous studies) and to assess the in vitro antimicrobial activities of essential oils obtained from aerial parts of *D. carota L.* subsp. *carota* and *D. carota* L. subsp. *gummifer*. To the best of our knowledge, this is the first study to compare the chemical composition of these both subspecies and evaluate the antimicrobial activity of *D. carota* L. subsp. *gummifer* from Algeria.

Material and Methods

Plant material and Oil isolation: The aerial parts (stems and leaves) of *D. carota* L. subsp. *carota* (DCSC) and *D. carota* L. subsp. *gummifer* (DCSG) were harvested from Bensekrane forest area (at about 30 km north west of Tlemcen - Algeria) in

July, 2009, and from Saf-Saf village at about 5 km north-Est of Tlemcen – Algeria, respectively. The plant material was botanically identified by Pr. Noury Benabadji (Laboratory of Ecology and Ecosystem Management of University of Tlemcen Algeria). The fresh aerial parts were submitted to hydrodistillation from 5 h using a Clevenger-type apparatus according to the European Pharmacopoeia[19].

Gas chromatography: GC analyses were carried out using a Perkin Elmer Clarus 600 GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The apparatus was used for simultaneous sampling to two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 μm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Temperature program: 60 to 230°C at 2°C.min-1 and then held isothermal 230°C (30 min). Carrier gas: helium (1 mL.min-1). Injector and detector temperatures were held at 280°C. Split injection was conducted with a ratio split of 1:80. Injected volume: 0.1 μL.

Gas chromatography-mass spectrometry: The oils and the fractions obtained by CC were investigated using a Perkin Elmer TurboMass quadrupole analyzer, directly coupled to a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions 146 were the same as described above. Ion source temperature: 150°C; energy ionization: 70 eV; electron ionization mass spectra were acquired with a mass range of 35-350 Da; scan mass: 1s. Oil injected volume: 0.1 μL, fraction injected volume: 0.2 μL.

Component identification and quantification: Identification of the components was based i. on the comparison of their GC retention indices (RI) on non polar and polar columns, determined relative to the retention time of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data[20]; and ii. on computer matching with commercial mass spectral libraries[20,21] and comparison of spectra with those of our personal library. The quantification of the collective essential oil components was carried out using peak normalization including response factors (RFs) with internal standard[22]. Component quantification was expressed in g/100g. Tridecane was introduced in all sample oils at same concentration (0.7 g/100 g) as internal standard.

Test microorganisms: Essential oils were tested against eight microorganisms, including Gram positive *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Bacillus cereus* ATCC 9634, Gram negative *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 10031.

Biological screening: Antimicrobial activity was evaluated using a broth microdilution method. A dilution agar method was used to determine the Minimum Inhibitory Concentrations (MIC). Stock solutions were obtained by dissolving extracts in dimethylsulfoxide (DMSO 1%). Serial dilutions were made to obtain concentrations ranging from 0.2 to 10 mg/ml of the essential oil. Each mixture was added to Mueller–Hinton agar for bacteria[23,24]. The Petri dishes contained a sterile solution of DMSO and the culture medium, respectively and incubated at 37°C. Tetracycline served as positive control, while the solvent (10% aqueous DMSO) was used as a negative control. The experiments were performed in triplicate. According to the MIC values expressed in mg/mL, results were appreciated as follows: not sensitive (-) for value higher to 6 mg/mL, moderately sensitive (+) for value between 3.0 and 5.0 mg/mL and sensitive (++) for value to 2.5 mg/mL.

Results and Discussion

Essential oil composition: GC and GC–MS analysis of *DCSC* and *DCSG* essential oils allowed to identify 67 components, 40 of which were common to both the two essential oils, which accounted for more than 90.0 g/100g of the oils. All components were identified by comparison of their EI–MS and GC-retention indices and mass spectra with those of our laboratory produced "Arômes" library, except three compounds which were identified by comparison with spectral data and retention indices from the literature (table -1). The essential oil yields, calculated from fresh material for *DCSC* and *DCSG* were 1.52 and 1.64 %, respectively (Table -1). The oil of *DCSC* made up the higher contribution of monoterpene hydrocarbons (83.2 g/100g), α-pinene (26.0 g/100g), sabinene (14.0 g/100g), limonene (13.0 g/100g), β-pinene (11.2 g/100g), myrcene (10.0 g/100g), p-cymene (4.4 g/100g) and terpinene-4-ol (4.9 g/100g) were found as the characteristic components of this fraction in *DCSC* essential oils. However, this fraction was also the main one in the oil of *DCSG* (54.0 g/100g) with α-pinene (34.1 g/100g), myrcene (13.1 g/100g) and p-cymene (3.3 g/100g) as the most abundant compounds. The oxygenated monoterpenes represented 8.6 g/100g of the total oil of *DCSC*. This fraction had a similar percentage (8.2 g/100g) in the essential oil of *DCSG*. In both cases, this fraction was dominated by terpinene-4-ol, which was more abundant in *DCSC* (4.9 g/100g) than that in *DCSG* (2.4 g/100g). The amount of oxygenated sesquiterpenes of *DCSG* (20.5 g/100g) was approximately tenfold as much as that of *DCSC* (2.0 g/100g). Caryophyllene oxyde (6.0 g/100g), spathulenol (4.3 g/100g) and isospathulenol (3.8 g/100g) were the main oxygenated sesquiterpenes in *DCSG*. The sesquiterpene hydrocarbons are present in small amounts and constitute about 6.4 and 1.2 g/100g of the oil composition in *DCSC* and *DCSG*, respectively. The content of the non-terpenic compounds of *DCSG* essential oil (1.0 g/100g) was more than that of *DCSC* (0.5 g/100g). This fraction was dominated by nonane (*DCSG* 0.5 g/100g) and 2-methyl butyl isovalerate (*DCSG* 0.3 g/100g, *DCSC* 0.2 g/100g). The phenylpropanoids compounds were the lowest component in

DCSC (0.2 g/100g). In, trans-methyl isoeugenol (0.1 g/100g) and elemicin (0.1 g/100g) were the only phenylpropanoids compounds. However, *DCSC* oil is characterized by the absence of phenylpropanoids compounds (table -1).

Compared with previous studies: Compared the results obtained from our plant material of *DCSC* and *DCSG* with those previous reports on the same species from other localities[8-18], it is interesting to point out that there are some qualitative and quantitative difference between the present work and those studies (table -2). Relative to the literature, it appears that Algerian *DCSG* essential oil exhibited chemical composition that differed from that of Spain origin, while was rather similar in comparison with that reported in the literature from Italy. The geranyl acetate (51.7-76.9%) was identified as major component in the spanish *DCSG* essential oil[9]. Sabinene (26.8-60.6%) and α-pinene (10.8-12.2%) were the major components of *DCSG* essential oil from Italy[8]. However, α-pinene (34.1 g/100g) and sabinene (13.1 g/100g) were the most important features of our essential oils. Other hand, the composition of *DCSC* essential oils (monoterpene hydrocarbons) was rather similar in comparison with those reported in the literature from Serbia, Poland and Lithuania[16-18]. Contrarily, the composition of DCSC essential oils was rather differed in comparison with those reported in the literature from Corsican, Turkey, Portugal, Italy and Tunisia[10-14]. Contrarily, the composition of *DCSC* essential oils was rather differed in comparison with those reported in the literature from Corsican, Turkey, Portugal, Italy and Tunisia[10-14]. Indeed, *DCSC* essential oils from Corse was largely dominated by (E)-methylisoeugenol (21.8-33.0%) and elemicin (11.4-16.3%). However, carotol (48.0-55.7%) and elemicin (31.5-35.3%) were the major components of *DCSC* essential oil from Tunisia. On one hand, some compounds, mainly including carotol, 11α-H-himachal-4-en-1-β-ol, daucene and eudesm-7(11)-en-4-ol were present with higher amount (up 66%, 21%, 8% and 8%, respectively) in the essential oils of *DCSC* from Italy, Tunisia and Portugal[11,12], whereas they were not identified in our essential oils (table -2). Generally, the observed differences in chemical composition of the various oils, when compared with those reported in previous studies, mainly for the proportion of terpenoids and phenylpropanoids could be due to a number of factors. Such factors may include differences in climatic conditions, plant parts used, geographical locations, season at the time of collection, stage of development and processing of plant materials before extraction of the oils. The phenomenon existed in many plants, and was reported by many previous studies, such as the genus Hypericum[25]. These showed that variations were sufficient to allow the distinction of different chemotypes that were the results of an adaptative process to particular ecologic conditions.

Antibacterial activity: The antimicrobial activity of *DCSC* and *DCSG* essential oil was evaluated against 8 bacterial strains. The antibacterial effect is presented in table-3. The essential oil of *DCSG* shown higher activity against bacteria than the *DCSC* essential oil. Essential oil obtained from *DCSG*, exhibited activity against six bacterial strains, with MIC values ranging from 2.5 to 5 mg/ml. The most prominent inhibitory action of this essential oil was observed against three gram-positive bacterium (*L. monocytogenes*, *B. cereus* and *S. aureus*) and one gram-positive bacterium (*E. coli*) at 2.5 mg/ml. However, no inhibition was showed against *B. subtilis* and *E. faecalis* up to the value of 6 mg/mL. *DCSC* essential oil had, in most cases, moderate antibacterial activity, showing inhibitory effect at 3.8-5.1 mg/ml against *B. cereus*, *L. monocytogenes* and *S. aureus* (MIC = 3.8, 4.3 and 5.1 mg/ml, respectively) (table 3). It has been reported that the flowering umbel, and mature umbel oils from *DCSC* (collected in Poland), previously tested for inhibitory effect on microorganisms (*S. aureus*, *B. subtilis*, and *C. albicans*) were dominated by α-pinene (17-42%) and sabinene (19-40%)[16]. On the other hand, oil sample, obtained from aerial parts of *DCSC* growing wild in Corsica, at the end of flowering stage, containing mainly (E)-methylisoeugenol (21.8%), β-bisabolene (21.3%), elemicin (16.3%) and α-pinene (15.9%) were reported as antimicrobial against the human enteropathogen Campylobacter[10]. The acquired results confirm and supplement present findings about antibacterial characteristics of Apiaceae.

Conclusion

The findings showed that both *Daucus* had a considerable variation in the essential oil composition and this study also demonstrated the occurrence of α-pinene chemotype in *D. carota* ssp *gummifer* from Algeria. Both oils were the complex mixture of volatile compounds, most of which had antiseptic, anti-inflammatory and antimicrobial properties. Other hand, according to the antimicrobial results gram negative bacteria were the most resistant and the gram positive bacteria exhibited good sensitivity. However, further work is necessary to explore the efficacy of these essential oils as an antibacterial agents.

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Table -1
Essential oils of *D. carota L.* Subsp. *carota (DCSC)* and *D. carota L.* Subsp. *gummifer (DCSG)* from Algeria

N°	Components [a]	*l*RI$_a$ [b]	Ri$_a$ [c]	Ri$_p$ [d]	*DCSC* [e]	*DCSG* [e]	Identification [f]
1	Nonane	906	908	907	-	0,5	RI, MS
2	Nonene	883	879	930	-	0,1	RI, MS
3	α-Thujene	922	920	1019	0,9	-	RI, MS
4	α-Pinene	936	931	1016	26.0	34,1	RI, MS
5	Camphene	943	945	1062	1,8	0,8	RI, MS
6	Thuja-2,4(10)-diene	946	948	1117	0,2	-	RI, MS
7	Sabinene	964	968	1117	14.0	1,5	RI, MS
8	β-Pinene	978	970	1102	11,2	0,6	RI, MS
9	Myrcene	987	980	1152	10.0	13,1	RI, MS
10	α-Phellandrene	997	999	1159	0,2	-	RI, MS
11	3-methyl butyl isobutyrate	1002	1002	1170	0,1	-	RI, MS
12	2-methyl butyl isobutyrate	1004	1007	1170	0,1	-	RI, MS
13	α-Terpinene	1008	1011	1267	0,9	-	RI, MS
14	p-Cymene	1011	1015	1256	4,4	3,3	RI, MS
15	Limonene	1025	1022	1195	13.0	0,5	RI, MS
16	(Z)-β-Ocimene	1024	1027	1221	0,1	-	RI, MS
17	(E)-β-Ocimene	1034	1037	1237	0,2	-	RI, MS
18	γ-Terpinene	1047	1051	1233	0,1	0,1	RI, MS
19	Nonan-2-one	1070	1073	1392	0,1	0,1	RI, MS
20	Terpinolene	1078	1080	1274	0,2	-	RI, MS
21	Linalool	1082	1085	1392	0,8	-	RI, MS
22	2-methyl butyl isovalerate	1098	1090	1274	0,2	0,3	RI, MS
23	α-Campholenal	1105	1093	1470	0,2	0,2	RI, MS
24	trans-Pinocarveol	1125	1124	1648	0,3	0,7	RI, MS
25	Pinocarvone	1136	1130	1547	0,3	1,3	RI, MS
26	Cryptone	1157	1158	1658	0,1	0,3	RI, MS
27	Terpinene-4-ol	1161	1165	1586	4,9	2,4	RI, MS
28	Myrtenal	1172	1171	1619	0,2	-	RI, MS
29	α-Terpineol	1176	1177	1685	0,4	0,2	RI, MS
30	Verbenone	1184	1182	1694	0,1	0,7	RI, MS
31	Carvone	1214	1216	1749	0,1	0,2	RI, MS
32	Cuminaldehyde	1217	1213	1778	0,2	0,2	RI, MS
33	Bornyl acetate	1269	1270	1515	0,9	1,8	RI, MS
34	α-Terpenyl acetate	1334	1333	1676	-	0,1	RI, MS
35	Geranyl acetate	1362	1360	1749	0,1	0,1	RI, MS
36	α-Ylangene	1375	1374	1470	0,1	0,2	RI, MS
37	α-Copaene	1379	1371	1457	0,1	0,3	RI, MS
38	β-Elemene	1389	1385	1550	tr	-	RI, MS
39	α-Cedrene	1417	1410	1563	-	0,2	RI, MS
40	β-Ylangene	1420	1413	1530	-	-	RI, MS
41	trans-Caryophyllene	1424	1426	1592	0,4	1,1	RI, MS
42	β-Copaene	1430	1438	1602	-	-	RI, MS
43	trans-α-Bergamotene	1432	1431	1573	0,1	0,4	RI, MS
44	α-Sesquisabinene	1435	1435	1637	0,1	1,2	RI, MS
45	(E)-β-Farnesene	1448	1449	1659	0,2	1,3	RI, MS
46	trans-methyl Isoeugenol	1463	1465	2170	0,1	-	RI, MS
47	γ-muurolene	1473	1471	1667	0,1	0,2	RI, MS
48	Germacrene D	1480	1483	1663	-	0,2	RI, MS
49	γ-Humulene	1483	1487	1682	0,1	0,1	RI, MS

50	β-Selinene	1483	1495	1703	-	-	RI, MS
51	Bicyclogermacrene	1494	1499	1683	-	0,1	RI, MS
52	β-Bisabolene	1500	1494	1720	0,1	0,2	RI, MS
53	Cuparene	1504	1500	1824	-	0,6	RI, MS
54	δ-Cadinene	1515	1519	1762	0,1	0,1	RI, MS
55	Elemicin	1518	1527	2232	0,1	-	RI, MS, Ref
56	E-α-Bisabolene	1531	1526	1733	-	0,2	RI, MS
57	β-Caryophyllene oxide	1546	1550	2156	-	0,1	RI, MS, Ref
58	1,5 epoxy-Salvial-4(14)ene	1561	1563	1912	0,1	0,1	RI, MS
59	Spathulenol	1572	1568	2095	0,6	4,3	RI, MS
60	Caryophyllene oxyde	1576	1574	1937	0,8	6.0	RI, MS
61	Copaborneol	1592	1588	2154	-	1,9	RI, MS
62	Humulene epoxyde II	1601	1595	2009	0,1	2,1	RI, MS
63	Isospathulenol	1625	1615	2386	0,2	3,8	RI, MS, Ref
64	T-Muurolol	1634	1624	2138	0,1	0,4	RI, MS
65	α-Cadinol	1645	1638	2227	0,1	0,7	RI, MS
66	(Z)-α-Santalol	1669	1667	2306	-	0,3	RI, MS
67	(E,Z)-Farnesol	1685	1680	2313	-	0,8	RI, MS
Oil yield (%, w/w)					1,52	1,64	
Total identification (g/100g)					95,9	90,1	
Monoterpene hydrocarbons					83,2	54.0	
Oxygenated monoterpenes					8,6	8,2	
Sesquiterpene hydrocarbons					1,4	6,4	
Oxygenated sesquiterpenes					2.0	20,5	
Phenylpropanoids					0,2	-	
Non-terpenic compounds					0,5	1.0	

[a]Order of elution is given on apolar column (Rtx-1)., [b] Retention indices of literature on the apolar column (lRI$_A$) reported from Konig et al., (2001).[c]Retention indices on the apolar Rtx-1 column (RI$_a$). [d]Retention indices on the polar Rtx-Wax column (RI$_P$). [e]Algerien *Daucus* (concentrations in g/100g): *D. carota* L. subsp. *carota* (**DCSC**), *D. carota* L. subsp; gummifer *(***DCSG**). [f] RI: Retention Indices; MS: Mass Spectra in electronic impact mode; Ref.: compounds identified from literature data: Koning et al., 2001 (**55, 57, 63**)

Table -2
Main components of the essential oils of *D. carota L.* Subsp. *carota* and *D. carota L.* Subsp. *gummifer* from different origins previously reported

Plant origin		Italy	Spain	Tunisia (Sejnane)	Tunisia	Italy	Portugal
Subspecies		*Gummifer*	*gummifer*	*carota*	*carota*	*carota*	*Carota*
References		(A)	(B)	(C)	(C)	(D)	(D)
Extraction modes		HD	HD	CO_2	CO_2	HD	HD
	Major Components	%	%	%	%	%	%
No	Plant parts	Leaves; Fruits	Fruits	Flowering umbels and umbels	Flowering umbels and umbels	Flowering umbels and umbels	Flowering umbels and umbels
4	α-Pinene	10.8-12.2	-	-	-	-	13.0-37,9
7	Sabinene	26.8-60.6	4.42-11.13	12.0-14.5	-	-	
8	β-Pinene	1.4-3.4	-	-	-	-	2.3-3.5
9	Myrcene	-	-	-	-	-	-
15	Limonene	2.1-5.7	-	-	-	-	-
16	(Z)-Ocimene	0.4-5.0	-	-	-	-	-
18	γ-Terpinene	-	-	-	-	-	-
20	Terpinolene	0.6-4,7	-	-	-	-	-
21	Linalool	-	3.97-5.18	-	-	-	-
27	Terpinen-4-ol	4.8-5.4	-	-	-	-	-
-	Daucene	-	-	-	-	-	-
35	Geranyl acetate	-	51,7-76,9	-	-	-	15.0-65.0
-	(Z,Z)-α-farnesene	-	-	-	-	-	-
-	α-selinene	-	-	7.4-8.6	-	-	-
48	Germacrene D	0.2-6.9	-	-	-	-	-
46	E-Methylisoeugenol	-	-	-	-	1.3-10.0	-
52	β-Bisabolene	-	-	-	-	17.6-51.0	-
55	Elemicin	-	-	-	31.5-35.3	5.2-6.4	0.3-6.4
-	Carotol	-	-	3.5-5.2	48.0-55.7	2.4-25.1	-
-	11α-H-himachal-4-en-1-β-ol	-	-	12.7-17.4	-	9.0-21.6	21.6
-	Eudesm-7(11)-en-4-ol	-	-	8.2-8.5	-	-	-
Plant origin		Turkey	Corsican	Corsican	Serbia	Poland	Lithuania
Subspecies		*carota*	*Carota*	*carota*	*carota*	*carota*	*carota*
References		(E)	(F)	(G)	(H)	(I)	(J)
Extraction modes		HD	HD	HD	HD	HD	HD
	Major Components	%	%	%	%	%	%
No	Plant parts	Seed	Aerial parts	Aerial Parts	All Organs	Umbels	Fruits
4	α-Pinene	-	15.9	24.9	7.05–51.23	41.0	16.0–24.5
7	Sabinene	-	-	3.7	2.68–36.69	18.0	28.2–37.5
8	β-Pinene	-	-	-	-	-	-
9	Myrcene	-	-	3.5	3.04-7.18	7.0	2,5
15	Limonene	-	-	-	1.79-9.59	5.0	2.3–4.0
16	(Z)-Ocimene	-	-	-	-	-	
18	γ-Terpinene	-	-	-	-	-	2.9–6.0
20	Terpinolene	-	-	-	-	-	-
21	Linalool	-	-	-	-	-	-
27	Terpinen-4-ol	-	-	-	1.22-3.48	5.0	4.6–7.5
-	Daucene	8.74	-	-	-	-	-
35	Geranyl acetate	-	-	-	-	-	-
-	(Z,Z)-α-farnesene	5.86	-	-	-	-	-
-	α-selinene	-	-	-	-	-	-
48	Germacrene D	-	-	-	-	-	-
46	E-Methylisoeugenol	-	21.8	33.0	-	-	-
52	β-Bisabolene	-	-	-	-	-	-
55	Elemicin	-	16.3	11.4	-	-	-
-	Carotol	66.78	-	-	-	-	-
-	11α-H-himachal-4-en-1-β-ol	-	-	-	-	-	-
-	Eudesm-7(11)-en-4-ol	-	-	-	-	-	-

Only the main components were reported; main components are classified by number corresponding to the table-1; Extraction mode: HD: Hydrodistillation; CO_2: Supercritic Carbone dioxide extract[8,9,11,12,14,16,18].

Table -3
MIC (mg/mL) of *D. carota L.* Subsp. *carota (DCSC)* and *D. carota L.* Subsp. *gummifer (DCSG)* essential oils on bacterial strains

Bacterial strains	***DCSC***	***DCSG***
Gram-positive bacterium		
B. subtilis	$> 6.0^{(-)}$	$> 6.0^{(-)}$
L. monocytogenes	$2.5^{(++)}$	4.3
B. cereus	$2.5^{(++)}$	$3.8^{(+)}$
S. aureus	$2.5^{(++)}$	$5.1^{(+)}$
P. aeruginosa	$5.0^{(+)}$	$> 6.0^{(-)}$
E. faecalis	$> 6.0^{(-)}$	$> 6.0^{(-)}$
Gram-negative bacterium		
K. pneumoniae	$5.0^{(+)}$	$> 6.0^{(-)}$
E. coli	$2.5^{(++)}$	$> 6.0^{(-)}$

Values are means of triplicate determinations, Essential oils are classified as (-) not active, (+) moderately active and (++) active.