SUPPLEMENTARY MATERIAL

Chemical composition of the essential oil from carnation coniferous (*Dianthus acicularis* Fisch. ex Ledeb) growing wild in Northern Kazakhstan

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ABSTRACT

The aim of the study was to investigate volatile compounds from the aerial parts of *Dianthus acicularis* of the genus *Dianthus* of the family *Caryophyllaceae* grown wild in Northern Kazakhstan for the first time. *D. acicularis* is a typical Trans-Volga-Kazakhstani endemic. *D. acicularis* has high resistance to the bacterial wilt, a serious disease caused by *Burkholderia caryophylli*. The qualitative and quantitative compositions of the specimens of the essential oils were analyzed by the method of GC-MS. The main constituents of *D. acicularis* essential oil were methyl ketones - 2-pentadecanone (26.9-32.2%) and 2-tridecanone (4.7-17.7%), identified for the first time in the *Dianthus* genus. The methyl ketone activity provides protection of the plants from herbivores and fungal pathogens. One can suppose that the presence of 2-pentadecanone and 2-tridecanone in the essential oil of carnation coniferous provides its resistance to different insects and pathogens, including the resistance to the bacterial wilt.

Key words: carnation coniferous (*Dianthus acicularis* Fisch. ex Ledeb); *Caryophyllaceae*; essential oil composition; methyl ketones; 2-pentadecanone; 2-tridecanone.

Experimental

1. Plant material

Collection of the material was carried out in places of natural growth of *Dianthus acicularis* Fisch. ex Ledeb in Northern Kazakhstan in Akmola and in North-Kazakhstan regions. The specimens for the study were collected on 23-25 June, 2015 in the stage of full blooming (**Figure S1**). Identification and documentation (certificates of the specimens) of the plant species were made by Dr Tamara Stikhareva with the use of the key to the plants “Kazakhstan Flora” (Pavlov 1960). The herbarium of the identified plants was placed at the Department of Breeding of Kazakh Research Institute of Forestry and Agroforestry in Shchuchinsk. Drying to the air-dry condition of the raw material was done in a well-ventilated room, spread out on a paper by smooth thin layer (3-4 cm) and often it's stirring. Characteristic of the places of collection is given in **Table S1** (hereinafter the studied specimens are named according to their characteristics and conditions of the habitat). The climate of Northern Kazakhstan is mainly sharply continental. Winter in the north of the country is cold and long. Summer is moderately warm. An average temperature of the coldest month (January) varies from -25°C to -35°C and the temperature of the hottest month (July) varies from $+25^{\circ}\text{C}$ to $+35^{\circ}\text{C}$. An average amount of precipitation is 300-600 mm.

Figure S1. Carnation coniferous (*Dianthus acicularis* Fisch. ex Ledeb)

Table S1. The places of collection of the plant material

Number of the specimen	Characteristic of the habitat	Growing conditions
The place of collection - the Republic of Kazakhstan, Akmola Region, State National Natural Park “Burabay”		
I	<p>A glade</p> <p>The stipa community with domination of the representatives of the genus <i>Stipa</i> (L.), <i>Filipendula hexapetala</i> Gilib. and others.</p> <p>The total projecting cover of the grass stand – 70-80%.</p> <p>The projecting cover of <i>Dianthus acicularis</i> – 5-7%.</p> <p>The height of <i>Dianthus acicularis</i> – 20-25 cm</p>	Dry growing conditions
The place of collection – the Republic of Kazakhstan, North–Kazakhstan Region, State National Natural Park “Kokshetau”, Shalkarskiy branch		
II	<p>The pine forest, composition -10 pine trees, age – 110 years, site class V, density – 0.4.</p> <p>The cereal-forb community rich in herbs with domination of <i>Sedum hybridum</i> L., <i>Dianthus acicularis</i> Fisch., <i>Calamagrostis epigeios</i> (L.) Roth. and others.</p> <p>The total projecting cover of the grass stand – 20-30% (in most cases– in rock clefts and micro surface valley).</p> <p>The total projecting cover of <i>Dianthus acicularis</i> – 3-5%.</p> <p>The height of <i>Dianthus acicularis</i> - 10-15 cm</p>	Very dry growing conditions
The place of collection – the Republic of Kazakhstan, Akmola Region, State National Natural Park “Kokshetau”, Zerendinskiy branch		
III	<p>The forest cultures of the pine: age - 34 years, site class V, density – 0.4, there rarely are found the pine trees of the age of 100 years, site class V.</p> <p>The forb-stipa community rich in herbs with domination of the representatives of the genus <i>Stipa</i> (L.), <i>Festuca sulcata</i> Hack., <i>Seseli ledebourii</i> G. Don., <i>Galium verum</i> L. and others.</p> <p>The total projecting cover of the grass stand – 50-60%.</p> <p>The projecting cover of <i>Dianthus acicularis</i> – 5-7%.</p> <p>The height of <i>Dianthus acicularis</i> – 20-25 cm</p>	Dry growing conditions

2 Isolation of the essential oil

The essential oil of all the air-dried samples (flowers and leaves) (100 g) was isolated by hydrodistillation for 2 hours with the use of the Clevenger-type apparatus. Hexane was used as a trap. The essential oil had a slightly yellowish colour.

The essential oil sample (25 mg) was placed into a 25-mL volumetric flask, dissolved in hexane (15 mL), adjusted to the mark, and stirred until the oil was fully mixed.

3 Qualitative and quantitative analyses

The qualitative and quantitative compositions of the specimens of essential oils were analyzed by the GC/MS method on a gas chromatograph Clarus 580 with the mass selective detector Clarus-SQ 8 (PerkinElmer). The RestekRxi®-1ms capillary column (Crossbond® 100% dimethylpolysiloxane, 0.25 mm × 30 m × 0.25 µm) at the flow rate of gas-carrier of helium 1 mL/min was used. The temperature of the vaporizer was 280°C. The temperature of the column was 40°C, with the programming of the temperature up to 280°C at the rate of changing the temperature 2°C/minute. The mode of injection was the flow division 1:25. The volume of the sample was 1.0 microliters. The temperature of the mass-spectrometric detector was 240°C with EI⁺ 70 eV, scan time 4-120 minutes, scan range was 39-500 m/z. The percent contents of constituents were calculated automatically from peak areas in the total ion chromatogram (**Figures S2-S4, Table S2**). Constituents were identified from mass spectra using the NIST library and retention times calculated relative to saturated hydrocarbons. The injections were carried out in three replications.

Table S2. Percentage composition of the essential oil from aerial parts of *Dianthus acicularis* Fisch. ex Ledeb according to the data of GC/MS

No.	Compound	RI	RI _{lit}	%		
				I	II	III
1	hexanal	781	776±4	0.2	0.3	0.3
2	(E)-2-hexenal	819	827±3	0.1	-	-
3	(Z)-3-hexen-1-ol	830	839±5	-	0.2	-
4	1-hexanol	845	854±5	-	0.4	0.2
5	5-methyl-2-furanmethanol	854	-	-	0.3	-
6	α-pinene	921	933±4	-	-	1.1
7	6-methyl-5-hepten-2-one	958	964±3	-	-	0.2
8	nonanal	1078	1083±3	0.2	-	0.2
9	decanal	1176	1185±2	0.8	0.7	0.5
10	thymol	1267	1270±5	0.9	-	-
11	2-undecanone	1269	1273±3	0.2	-	-
12	(E,E)-2,4-decadienal	1280	1289±3	0.1	-	-
13	2-undecanol	1285	1287±2	0.2	-	-
14	4Z-hexenyl tiglate	1299	-	0.3	0.7	1.1
15	eugenol	1319	1335±8	-	0.9	-
16	caryophyllene	1395	1419±5	0.2	-	0.2
17	cis-geranylacetone	1421	1429±4	0.3	0.4	0.4
18	unidentified component 1	1434	-	0.7	0.3	0.4
19	β-ionone	1445	-	-	0.3	0.2
20	methyl isoeugenol	1451	1467±1	0.3	-	-
21	2-tridecanone	1469	1477±3	17.7	4.7	7.6
22	unidentified component 2	1470	-	-	-	0.3
23	2-tridecanol	1480	1500±10	3.2	2.2	2.1
24	pentadecane	1500	1500	0.4	0.2	0.3

25	1,2,3-trimethoxy-5-(2-propenyl)-benzene	1506	1522±6	0.3	-	-
26	(Z)-3-hexen-1-ol benzoate	1523	1550±6		0.3	0.3
27	caryophyllene oxide	1535	1574±7	0.4	0.4	0.4
28	2-tetradecanone	1540	1579±9	1.1	0.6	0.7
29	(3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene	1559	-	0.4	-	0.3
30	guaiol	1562	1589±5	0.2	-	-
31	isoelemicin	1608	-	1.0	-	0.7
32	bulnesol	1632	1655±4	0.1	0.3	-
33	unidentified component 3	1645	-	5.8	6.4	5.4
34	2-hexadecanol	1658	-	-	0.4	0.2
35	Z-10-pentadecen-1-ol	1666	-	-	-	0.2
36	2,3-dihydro-6-trans-farnesol	1672	-	0.2	-	-
37	2-pentadecanone	1688	1681±4	32.2	26.9	27.5
38	2-pentadecanol	1697	-	1.9	2.6	2.3
39	1-pentadecanal	1700	1694±3	-	0.2	-
40	farnesol	1702	1681±9	1.2	0.6	0.8
41	2-hexadecanone	1758	1782±4	1.7	2.7	2.2
42	hexadecanal	1802	1795±2	-	-	0.2
43	6,10,14-trimethyl-2-pentadecanone	1835	1842±3	-	0.4	0.2
44	unidentified component 4	1851	-	-	0.8	0.6
45	1-hexadecanol	1871	1863±5	2.4	3.6	5.4
46	2-heptadecanone	1888	1884±5	1.0	2.7	2.1
47	2-heptadecanol	1898	-	-	0.3	0.2
48	nonadecane	1900	1900	0.2	0.2	0.2
49	n-hexadecanoic acid	1954	1954±11	-	0.7	2.6
50	geranyllinalool	2001	2020±10	1.8	2.1	3.4
51	9-eicosene	2013	-	-	2.6	-
52	(Z,Z)-9,12-octadecadien-1-ol	2023	-	0.4	0.6	1.5
53	10-heneicosene	2068	2060±N/A	1.0	1.6	1.4
54	phytol	2096	2102±5	0.1	4.7	3.4
55	heneicosane	2100	2100	1.2	2.8	1.8
56	docosene	2310	-	1.2	1.8	2.3
57	2-methyltricosane	2345	2364±1	1.1	2.1	2.3
58	tetracosane	2400	2400	-	1.0	-
59	1-docosanol	2479	2488±N/A	-	0.4	0.7
60	pentacosane	2500	2500	0.3	0.5	0.9
61	heptacosane	2700	2700	0.2	0.4	0.4
Total				83.2	82.3	85.7

Note: RI, relative retention indices to C6 – C40 n-alkanes on RestekRxi®-1ms column;

I, II, III - The number of the specimens (see Table S1)

Figure S2. Gas chromatogram of the essential oil of the aerial parts of *D. acicularis* from Sample I.

Figure S3. Gas chromatogram of the essential oil of the aerial parts of *D. acicularis* from Sample II.

Figure S4. Gas chromatogram of the essential oil of the aerial parts of *D. acicularis* from Sample III.

Table S3. The main components of essential oil, floral scent of different *Dianthus* species

Species	Source	Main components	References
<i>D. superbus</i> L.	Fresh flowers	6,10,14-trimethyl-2-pentadecanone (28.39%), (Z)-phytol (6.80%), geranyl acetone (4.65%), n-hexanol (4.32%), farnesyl acetone (3.01%)	(Yu et al. 2008)
	Floral scent	(Z)- β -ocimene (49.8%), β -caryophyllene (23.5%)	(Jürgens et al. 2003)
<i>D. caryophyllus</i> L.	Fresh flowers	hexadecanoic acid (28.67%), 9,12-octadecadienoic-acid (z,Z) (21.20%), eugenol (18.22%), benzyl benzoate (12.62%), eicosene (7.70%), benzyl salicylate (6.85%), phenyl ethyl alcohol (1.36%)	(El-Ghorab et al. 2006)
	Air-dried flower buds	eugenol (74.0 %), β -caryophyllene (22.4 %), α -caryophyllene (1.9 %), eugenol acetate (1.1 %), methyl salicylate (0.2 %)	(Kimbaris et al. 2012)
<i>D. rupicola</i> Biv.	Fresh flowers	thymyl 2-methylbutyrate (27.8%), thymyl isobutyrate (13.4%), α -pinene (3.7%), p-methoxyacetophenone (3.3%), 6,10,14-trimethyl-2-pentadecanone (2.9%), heptacosane (2.9%), nonacosane (2.8%), p-cymene (2.7%)	(Casiglia et al. 2014a)
<i>D. jacobssii</i> Rech. f.	Dried flowers	methyl benzoate (13.5%), (Z)- β -ocimene (10.1%), (Z)-3-hexenyl acetate (8.3%), β -caryophyllene (6.6%), (Z)-3-hexenyl benzoate (5.4%), n-octanal (5.3%), hexadecanoic acid (4.5%), methyl salicylate (4.1%), benzaldehyde (4.1%), (Z)-3-hexenol (3.7%), (E)- β -farnesene (3.2%), α -bisabolol (3.1%)	(Azadi et al. 2016)
<i>D. sylvestris</i> Wulfen	Floral scent	methylbenzoate (85.7%)	
<i>D. arenarius</i> L.	Floral scent	methylbenzoate (42.1%), methyl salicylate (14.5%), dimethyl salicylate (8.9%)	
<i>D. armeria</i> L.	Floral scent	(Z)-3-hexenyl-1-acetate (29.8%), D-limonene (11.6%), n-nonanal (11.0%)	
<i>D. deltoides</i> L.	Floral scent	(Z)-3-hexenyl-1-acetate (27.1%), benzaldehyde (15%), benzeneacetaldehyde (10.6%)	(Jürgens et al. 2003)
<i>D. barbatus</i> L.	Floral scent	n-nonanal (14.7%), β -caryophyllene (10.3%)	
<i>D. monspessulanus</i> L.	Floral scent	(Z)- β -ocimene (52.4%), β -caryophyllene (18.5%)	

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