Araştırma Makalesi
(Research Article)

Ege Üniv. Ziraat Fak. Derg., 2018, 55 (1):89-94

DOI: 10.20289/zfdergi.391005

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GC/MS Analysis and Antimicrobial Activity of The Volatile Compounds From *Dianthus carmelitarum Reut. ex Boiss* and *Dianthus calocephalus Boiss.* Grown in Turkey

Türkiye'de Yetişen *Dianthus carmelitarum* Reut. ex Boiss ve *Dianthus calocephalus* Boiss. Bitkilerinin Uçucu Bileşenlerinin GC/MS Analizleri ve Antimikrobiyal Aktiviteleri

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Alınış (Received): 15.05.2017 Kabul tarihi (Accepted): 21.08.2017

Key Words:

Dianthus carmelitarum, *Dianthus calocephalus*, GC-MS, GC-FID, Antimicrobial activity

ABSTRACT

The chemical composition of the essential oils of *Dianthus carmelitarum* Reut. ex Boiss. and *Dianthus calocephalus* Boiss. were identified by GC-FID and GC-MS. Essential oils of *D. carmelitarum* and *D. calocephalus* were obtained from the whole part of fresh plant by Clevenger-type distillation. Analyses of the essential oils gave 40 and 35 compounds, constituting over 86.58% and 76.45% in the total, respectively. Main components in *D. carmelitarum* were heneicosane (11.69%), docosane (10.52%), tetracosane (9.20%) and pyhtol (4.62%). Whereas, 4,4-dimethyl-2-pentene (17.65%), phytol (15.47%), pentacosane (4.38%) and hexahydrofarnesyl acetone (3.08%) were the major constituents in *D. calocephalus*. Antimicrobial activity of the essential oils obtained from *D. carmelitarum* and *D. calocephalus* were evaluated against nine gram positive, gram negative bacteria and the yeast-like fungi using disc diffusion and broth microdilution methods. The essential oils of *D. carmelitarum* and *D. calocephalus* showed good antimycotic activity against *Candida albicans* having the MIC values of 668 µg/mL and 1041 µg/mL, respectively.

Anahtar Sözcükler:

Dianthus carmelitarum, *Dianthus calocephalus*, *GC-MS*, *GC-FID*, antimikrobial aktivite

ÖZET

***Dianthus carmelitarum* Reut. ex Boiss. ve *Dianthus calocephalus* Boiss. bitkilerinin uçucu yağlarının kimyasal bileşimleri GC-FID ve GC-MS ile aydınlatılmıştır. Taze bitkilerin uçucu yağları Clevenger tip destilasyon ile elde edilmiştir. Bitkilere ait uçucu yağ analizinde 40 ve 35 bileşik aydınlatılmış olup, bitkilerin toplam uçucu yağlarının sırasıyla %86.58 ve %76.45 kısmı aydınlatılmıştır. *D. carmelitarum* . bitkisinin ana bileşikleri, heneikosan (%11.69), dokosan (%10.52), tetrakosan (%9.20) ve Fitol (%4.62) olarak bulunmuştur. *D. calocephalus* bitkisinin ana bileşenleri ise; (4,4)-dimetil-2-penten (%17.65), fitol (%15.47), pentakosan (%4.38) ve hekzahidrofarnesil aseton (%3.08) bileşikleri bulundu. Elde edilen uçucu yağların dokuz adet gram pozitif, gram negatif bakteriler ve mayalara karşı antimikrobial aktivite tayinleri yapılmıştır. *D. carmelitarum* ve *D. calocephalus* bitkilerinin uçucu yağlarının *Candida albicans* bakterisine karşı antimikotik aktivite değerleri MIK olarak sırasıyla 668 µg/mL ve 1041 µg/mL gösterdiği tespit edilmiştir.**

INTRODUCTION

Dianthus L., belongs to a member of the Caryophyllaceae family, which was the second largest genus of the family in Turkey (Hamzaoğlu et al., 2015a). There are approximately 86 genera and 2200 species in the world, (İlçim et al., 2013). The genus *Dianthus* L. was represented by 76 species, 2 subspecies and 12 varieties and 45% of these taxa are endemic to Turkey (Tel, 2012; Menemen and Hamzaoğlu, 2000; Davis et al., 1988: Reeve, 1967). *D. carmelitarum* is an endemic to Turkey but *D. calocephalus* is native in Balkans and Transcaucasia

as well (Hamzaoğlu, 2012b; Hazar and Baktir, 2012; Uzun and Terzioğlu, 2008; Reeve, 1967).

Dianthus L. is common mainly in Europe and Asia approximately 300 species, with a few species in North-South Africa and one species in the North America (*Dianthus repens*) and Mediterrean region (Erst et al., 2014; Shaulo and Erst, 2011; Vural, 2008). It crops breed as efficient in coastal area and cultivated area (Hsieh et al., 2004). Generally, *Dianthus* genus is known carnation (*D. caryophyllus*), pink (*D. plumarius*) and sweet william (*D. barbatus*) (Ali et al., 2008; Cristea et al., 2013a). It has been commonly used for the ornamention arrangement, but also it has importance in folk medicine as well agricultural purposes (Turan et al., 2017b; Turan, 2015a; Pirbalouti et al., 2013). Carnation flowers used as expectorant syrup. The essential oil of *Dianthus* L. genus were commonly used in serums, creams and massage oil and they have various biological activities such as; antispasmodic, cardiotonic, diaphoretic, nervine, anxiety, antidepression, analgesic, diuretic, antihepatotoxic, and dermatologic (Ibrahim, 2016; Tisserand and Young, 2014; Chevallier, 1996; Chopra et al., 1986; Durucasu et al., 2009).

Previous works, about some of the *Dianthus* L. species; *Dianthus vanensis, Dianthus aticii, Dianthus aytachii* and *Dianthus gokayii* have been mentioned (Hamzaoğlu et al., 2015a; Erst et al., 2014; İlçim et al., 2013; Vural, 2008; Yılmaz et al., 2011; Cristea et al., 2010b). Literature survive reveled chemical composition and biological activity of *Dianthus acicularis, D. caryophyllus*, and *D. carmelitarum* (Turan et al., 2017b; Azadi et al., 2016; Ibrahim, 2016a; Kirillov et al., 2017; Turan, 2015a; Mohammed and Al-Bayati, 2009; El-Ghorab et al., 2006; Bonjar, 2004). Floral fragrance compounds of seven Dianthus species (*D. arenarius, D. armeria, D. barbatus, D. deltoides, D. monspessulanus, D. superbus,* and *D. sylvestris*) have also mentioned (Jurgens et al., 2003). Hexane extract of *Dianthus elegans* d'Urv. var. *elegans*, *D. erinaceus* Boiss. var. *erinaceus*, *D. lydus*, and *D. zonatus* Fenzl. var. *zonatus* (Durucasu et al., 2009) and essential oil of *Dianthus superbus* var. *longicalycinus* have been studied by GC-MS (Kishimoto et al., 2013). To our knowledge, there are no reports on the chemical composition and antimicrobial activity of the essential oils of *D. carmelitarum* and *D. calocephalus*. In this work, percentages of the essential oil composition *D. carmelitarum* and *D. calocephalus* were investigated by GC-FID and GC-MS technique and antimicrobial activity of essential oils were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined (Bayram et al., 2013; Ünlü and Erkek, 2013; Basmacıoğlu-Mayaloğlu et al., 2011).

MATERIAL and METHODS

Plant material: *D. carmelitarum* and *D. calocephalus* were collected from Karaçam, Çaykara, Trabzon, Turkey (at a height of ~1350 m and ~1250 m, respectively) at 13.00-14.00 p.m. on July 28th, 2015. Both of them were collected on the grassy places and were authenticated by Prof.Dr.Salih Terzioglu. Harvesting time for the *D. carmelitarum* are from June to August and spadix time for the *D.carmelitarum* are from May to September during the year (Reeve, 1967). Voucher specimens have been deposited at the Herbarium of the Faculty of Forestry, Karadeniz Technical University, Turkey.

Isolation of the essential oils: The fresh whole plant materials (120g and 135g, respectively) were divided into small pieces and placed in to flask with 1L water. Then essential oils of *D. carmelitarum* and *D. calocephalus* were subjected to hydrodistillation by using a Clevenger-type apparatus for 4h with cooling bath (12°C) system (yield 0.15% and 0.10% (v/w), respectively). The essential oils were dried over anhydrous sodium sulfate and stored +4 °C until the GC-MS analysis. All of these works were done at the Department of Chemistry, Faculty of Science in KTU.

Gas chromatography mass spectrometry (GC- MS) analysis: GC-MS analysis was performed Agilent-5973 Network System, equipped with a FID (supplied with air and hydrogen of high purity) and a split inlet. HP-5 capillary column (30m×0.32 mm i.d., film thickness 0.25 µm). Helium was used as carrier gas, at a flow rate of 1mL/min. The injections were performed in splitless mode at 230 °C. Two microliters of essential oils solutions in *n*-hexane (HPLC grade) was injected and analysed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp. The injection was done in duplicate. Identification of each compound was supported by comparing their indices (RI) with published values (Cansu et al., 2013; Adams, 2004). The percentage compositions of the oils were computed from GC peak areas without using correction factors. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic

column is same as GC/FID. Helium was used as carrier gas, at a flow rate of 1mL/min. The injections were performed in splitless mode at 230°C. Two microliters of essential oils solutions in hexane (HPLC grade) was injected and analysed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp.

The GC peak area values from the HP-5 column separation were evaluated to find the ratio of the components of each of essential oils and matching mass spectral data were done compare of retention indices of using reference compounds (*n*-tetradecane, *n*-pentadecane, *n*-hexadecane, *n*-octadecane, *n*-undecane, *n*-nonadecane, *n*-docosane, *n*-tricosane, *n*-tetracosane and *n*-pentacosane), NIST and WILEY library and literature comparison.

Antimicrobial Activity: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607, and *Candida albicans* ATCC. All extracts were weighed and dissolved in hexane to prepare extract stock solution.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution test method and the minimal inhibition concentration (MIC) values (µg/mL) were determined (Woods et al., 2003; Yücel et al., 2017). The antibacterial and antifungal assays were performed in Mueller-Hinton, Brain Heart Infusion and Potato Dextrose mediums. Ampicillin (10.000 µg/mL), streptomycin (10.000 µg/mL) and fluconazole (2.000 µg/mL) were used as standard antibacterial and antifungal drugs, respectively. Each test was done in duplicate and repeated twice.

RESUL Tand DISCUSSION

Composition of essential oils: Hydrodistillation of fresh all parts of *D. carmelitarum* and *D. calocephalus* yielded 0.15% and 0.10% (v/w) of essential oils, respectively. Forty and thirty-five compounds, representing 86.58% and 76.45% of the oils, were identified using GC-MS and quantitative and qualitative analytical results are listed in Table 1. The main components of the essential oil of *D. carmelitarum were* heneicosane (11.69%), docosane (10.52%), tetracosane (9.20%), phytol (4.62%), and β-ionone (4.05%). The major compounds identified in the essential oil of *D. calocephalus* were 4,4-dimethyl-2-pentene (17.65%), phytol (15.47%), pentacosane (4.38%), hexahydrofarnesyl acetone (3.08%), and β-ionone (2.51%).

The chemical classes distributions of the volatile constituents of the essential oils of *D. carmelitarum* and *D. calocephalus* are summarized in Table 1. The compounds are separated into six classes, which were terpene, terpenoids, aldehydes, hydrocarbons, esters and others (Table 1). Mainly, 14 terpenes (12.45%), 8 terpenoids (16.63%), 4 aldehydes (8.01%), 10 hydrocarbons (38.76%) and 4 esters compounds (5.52%) from the essential oil of *D. carmelitarum* and 4 terpenes (3.18%), 9 terpenoids (30.12%), 5 aldehydes (5.74%) and 8 hydrocarbons compounds (30.26%) from the essential oil of *D. calocephalus* were identified. Analyses of the essential oil from *D. carmelitarum* and *D. calocephalus* showed that hydrocarbons (38.40%) and terpenoids (30.38%) were predominant, respectively.

Dianthus superbus var. *longicalycinus* had high amts. of β-ocimene and β-caryophyllene (Kishimoto et al., 2013). In our case we did not observed them. Floral fragrance constituents of seven Dianthus species (*D. arenarius, D. armeria, D. barbatus, D. deltoides, D. monspessulanus, D. superbus,* and *D. sylvestris*) have been studied by GC-MS and fatty acid derivatives, benzenoids, phenyl propanoids, isoprenoids, and nitrogen containing compounds were reported (Jurgens et al., 2003). And also, GC-MS studies for the hexane extract of *Dianthus elegans* d'Urv. var. *elegans*, *D. erinaceus* Boiss. var. *erinaceus*, *D. lydus*, and *D. zonatus* Fenzl. var. *zonatus* were also mentioned that they mainly consist of terpenes, essential fatty acids, and volatile compounds (Durucasu et al., 2009). Comparing the present data (Table 1) with those previously reported in literature, the study of essential oils shown different chemical profiles in each Dianthus species. Many reports have shown that plant growth and development are affected by genetic and environmental factors, which contributes to differences in chemical variation of essential oils of the plants. And also, ecological factors light and temperature have also been known to influences the production of essential oils.

Table 1. Volatile components in the essential oils of *D. carmelitarum* and *D. calocephalus*

Compounds		A		B		
		[a]Area%	Exp. RI[b]	[a]Area%	Exp. RI[b]	Lit. RI
1	2-Pentylfuran	4.36	986	2.02	988	990
2	4,4-Dimethyl-2-pentene	-	-	17.65	970	972
3	p-Mentha -(7)-8 diene	0.92	1003	-	-	1004
4	p-Cymene	0.66	1020	-	-	1025
5	δ-Limonene	2.70	1025	-	-	1029
6	Benzen acetaldehyde	-	-	0.78	1039	1042
7	Octanol	-	-	0.66	1062	1042
8	Nonanal	3.95	1105	2.55	1105	1101
9	Decenal	1.25	1202	0.97	1199	1202
10	1-Carboxaldehyde cyclohexene	-	1216	0.49	-	1220
11	β-Cyclocitral	2.25	1215	0.36	1216	1221
12	Decanol	-	-	0.78	1267	1270
13	Tridecane	0.64	1301	-	-	1300
14	(*2E,4E*)-Decadienal	2.16	1318	0.95	1316	1317
15	3-Hexenyl cis tiglate	0.95	1327	1.1	1328	1333
16	Dehydro-ar-ionone	-	-	0.26	1348	1354
17	Undecanol	-	-	0.44	1369	1370
18	Farnesene	0.40	1379	-	-	1380
19	β-Damascenone	0.78	1380	1.73	1379	1385
20	Trans-β caryophyllene	2.04	1410	1.14	1408	1409
21	β-Funebrene	0.43	1420	-	-	1415
22	α-Humulene	0.48	1449	-	-	1455
23	Geranyl acetone	-	-	1.49	1449	1455
24	Farnesane	1.91	1453	-	-	1455
25	cis-Muurola-4(14)-5 diene	1.66	1470	-	-	1467
26	(*E*) -β-Ionone	4.05	1480	2.51	1486	1485
27	δ-Gurjunene	0.49	1482	-	-	1477
28	Pentadecane	0.37	1501	0.42	1500	1500
29	Tridecanal	0.65	1512	-	-	1510
30	δ-Amorphene	0.76	1514	-	-	1512
31	(*E*)-Nerolidol	-	-	0.34	1562	1563
32	3-Hexen-1-ol benzoate	-	-	0.64	1569	1571
33	Caryophyllene oxide	0.52	1577	0.36	1580	1583
34	Heptadecane	0.75	1697	0.98	1703	1700
35	(*2E*)-Tetradecanol	0.46	1710	0.42	1713	1715
36	Octadecane	-	-	0.38	1803	1800
37	Neophytadiene	-	1.44	-	1834	1837
38	Hexahydrofarnesyl Acetone	1.68	1845	3.08	1843	1845
39	Isobuthyl phthalate	0.67	1868	1.20	1865	1868
40	Hexadecanol	0.39	1871	0.64	1872	1876
41	Nonadecane	0.51	1898	-	-	1900
42	Farnesyl Acetone	1.78	1912	2.47	1911	1915
43	Methyl hexadecanoate	0.91	1944	0.35	1941	1938
44	Isophytol	-	-	1.91	1950	1947
45	Eicosane	1.20	1995	-	-	2000
46	Ethyl hexadecanoate	0.46	2000	-	-	1993
47	Methyl linoleate	3.48	2097	-	-	2095
48	Heneicosane[c]	11.69	2101	-	-	2100
49	Phytol	4.62	2115	15.47	2111	2117[c]
50	Docosane	10.52	2205	0.42	2198	2200[c]
51	Tricosan	1.90	2300	2.72	2301	2300[c]
52	Tetracosane	9.20	2398	1.87	2402	2400[c]
53	Pentacosane	1.98	2496	4.38	2498	2500[c]
	Terpenes	12.45		3.18		
	Terpenoids	16.63		30.12		
	Aldehydes	8.01		5.74		
	Hydrocarbons	38.76		30.26		
	Ester	5.52		2.19		
	Others	5.21		4.96		
	Total	86.58%		76.45%		

A: *Dianthus carmelitarum*, B: *Dianthus calocephalus*.
[a]Area% obtained by FID peak-area normalization. [b]RI calculated from retention times relative to that of *n*-alkanes (C_5-C_{32}) on the non-polar HP-5 column. [c]Identified by authentic samples.

Antimicrobial Activity: The antimicrobial activities of the essential oils of *D. carmelitarum* and *D. calocephalus* were tested *in vitro* using the agar-well diffusion method (Woods et al., 2003; Yücel et al., 2017) with the microorganisms *E. coli, Y. pseudotuberculosis, P. aeruginosa, S. aureus, E. faecalis, M. smegmatis*, *B. cereus*, and *C. albicans* which are listed in Table 2. The essential oils of *D. carmelitarum* and *D. calocephalus* showed only good antimycotic activity against the fungus *C. albicans* with the minimum inhibitory concentration (MIC) values of 668 µg/mL and 1041 µg/mL, respectively. The essential oils did not show antimicrobial activity against other the tested bacteria (*E. coli, Y. pseudotuberculosis, P. aeruginosa, S. aureus, E. faecalis, M. smegmatis* and *B. cereus*).

ACKNOWLEDGEMENTS

The author thanks Prof. Dr. Salih Terzioğlu for characterization of plant materials and Prof. Dr. Şengül Alpay Karaoğlu for test the antimicrobial activity. This work supported by a grants from Karadeniz Technical University Research Fund.

Table 2. Screening for Antimicrobial Activity of Essential Oils of *D. carmelitarum* and *D. calocephalus* .

Samples	Stock Sol. (µg/mL)	Microorganisms and Minimal Inhibition Concentration (µg/mL)							
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca
D. carmelitorum	106.800	-	-	-	-	-	-	-	668
D. calocepholus	83.300	-	-	-	-	-	-	-	1041
Ampicillin	10.000	2	32	>128	2	2	<1		
Streptomycin	10.000							4	
Fluconazole	2.000								<8

Ec: *Escherichia coli*, Yp: *Yersinia pseudotuberculosis*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Bc: *Bacillus cereus*, Ms: *Mycobacterium smegmatis*, Ca: *Candida albicans*, (-): no activity.

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