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Chemical Composition and Antimicrobial Activities of the Essential Oil from the Flowers of *Delphinium formosu*m

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The essential oil from the flowers of *Delphinium formosum* Boiss. et Huet. (Ranunculaceae) was obtained by hydrodistillation in a Clevenger-type apparatus and analyzed by GC-MS. 68 Components were identified in the oil. The main components in the essential oil from the flowers of *D. formosum* were found to be tricosane (30.9 %), heneicosane (8.2 %), pentacosane (6.6 %), linalool (2.3 %) docosane (2.2 %) and phytol (2.1 %). The antimicrobial activity of the isolated essential oil from the flowers of the plant was also investigated and it showed moderate antibacterial activity against only *E. faecalis* and *S. aureus*. No antibacterial activity against other 5 bacteria and antifungal activity against 2 yeast-like fungi were observed.

Key Words: *Delphinium formosum*, Ranunculaceae, Essential oils, Antimicrobial activity, GC-MS.

INTRODUCTION

The genus *Delphinium* (Ranunculaceae) is represented with 31 species in Turkey[1,2]. Representatives of the genus *Delphinium* have been used for many years as medicinal plant[3-5]. Some *Delphinium* species have also been used in folk medicine as pediculicides, antirheumatics and insecticides[6].

Previous phytochemical studies on *Delphinium formosum* have shown the presence of many different natural compounds including acylated kaempferol glycoside, kaempferol-3-glucoside-7-rhamnoside, kaempferol-7-rhamnoside[7]; some phenolic acids such as *p*-hydroxy benzoic acid, *p*-coumaric acid[8,9]; benzoxepine derivatives[10]; alkaloids[11]; norditerpenoid alkaloids such as lycoctonine, delsemine A and delsemine B[5,12]. But, to our knowledge, there are no published reports on the chemical composition analysis and antimicrobial activities of essential oil from the flowers of

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D. formosum. As part of this systematic research, the essential oil constituents from the flowers of the plant were extracted by hydrodistillation method in a Clevenger-type apparatus[13,14]. The crude essential oil was then investigated by GC-MS technique and 41 components of various types were identified from the sample. Identification of the compounds was made by a typical library and literature search (NIST, WILLEY)[15-17]. The present work describes the composition of the essential oil and antimicrobial property from the flowers of *D. formosum*.

EXPERIMENTAL

The flowers of *D. formosum* (Ranunculaceae) were collected in August, 2006 from the Long lake of Çaykara-Trabzon in Turkey (at a height of *ca.* 2250 m) A voucher specimen was deposited in the herbarium of the Department of Forestry, Karadeniz Technical University (KATO 11669-2002) in Turkey. The plant was identified immediately after collection[1,2] and air-dried at room temperature for later analysis.

Isolation of the essential oil: The air-dried flowers (55 g) of *D. formosum* were hydrodistilled in a Clevenger-type apparatus using cooling bath (-15ºC) system (3 h). The oil was taken and dissolved in HPLC grade *n*-hexane (0.5 mL) and kept at 4ºC in a sealed brown vial. 1 µL of the extract was directly injected into the GC-MS instrument. The percentage yield of the oil was calculated on a moisture free basis (0.22 ± 0.1, v/w).

Gas chromatography: GC-MS analysis was performed using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan under electron impact ionization (70 eV) was used. The chromatographic column for the analysis was HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 µm). The carrier gas used was helium at a flow rate of 1 mL/min. The injection was performed in splitless mode at 230°C. 1 µL essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 60°C for 2 min and then increased to 260°C with a 5°C/min heating ramp and subsequently kept at 260°C for 13 min. The relative percentage amounts of the separated compounds were calculated from total ion chromatograms by a computerized integrator.

Antimicrobial activity assessment: All test microorganisms were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follows: *Escherichia coli*, *Klebsiella pneumoniae*, *Yersinia pseudotuberculosis*, *Serratia marcescens*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Candida tropicalis*. The essential oil was dissolved in acetone to prepare stock solution of 1000 µg/mL.

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OIL FROM THE FLOWERS OF *Delphinium formosum*

Delphinium formosum					
No.	Compounds	Area (%)	Q (%)	Exp. RI	Ident. LRI
1	*n*-Hexanol	0.2	82	870	871
2	*n*-Octanol	0.4	90	1067	1068
3	Linalool	2.3	91	1095	1097
4	Nonanal	0.1	82	1101	1101
5	Menthone	0.1	98	1152	1153
6	Borneol	0.3	94	1169	1169
7	Terpinen-4-ol	0.1	93	1174	1177
8	α-Terpineol	1.0	87	1189	1189
9	Myrtenal	0.9	97	1195	1196
10	Nerol	0.2	90	1230	1230
11	Pulegone	0.2	96	1241	1237
12	Geraniol	1.0	91	1256	1253
13	Thymol	0.5	95	1293	1290
14	*n*-Tridecane	0.3	97	1300	1300
15	Carvacrol	0.9	95	1302	1299
16	*E*-β-Damascenone	0.2	95	1385	1385
17	*n*-Tetradecane	0.2	92	1400	1400
19	Spathulenol	0.2	83	1580	1578
20	Caryophyllene oxide	0.2	87	1585	1583
22	Benzophenone	0.2	93	1628	1628
23	α-Bisabolol	0.2	90	1686	1686
24	*n*-Heptadecane	0.4	92	1700	1700
25	*n*-Octadecane	0.1	80	1800	1800
26	*n*-Hexadecanol	0.3	95	1881	1876
27	*n*-Nonadecane	0.2	91	1900	1900
28	*(E,E)*-Farnesyl acetone	0.2	94	1920	1918
29	Isophytol	0.2	82	1950	1948
30	Eicosane	0.2	93	2000	2000
31	Manool	0.6	84	2057	2057
32	Methyl linoleate	1.4	99	2095	2096
33	Heneicosane	8.2	96	2101	2100
34	Phytol	2.1	91	2114	2114
35	Docosane	2.2	98	2201	2200
36	Tricosane	30.9	99	2300	2300
37	Tetracosane	1.7	98	2401	2400
38	Pentacosane	6.6	98	2501	2500
39	Hexacosane	0.1	89	2600	2600
40	Heptacosane	1.6	90	2700	2700
41	Nonacosane	0.6	87	2899	2900
Total identified isolate		67.3			

Unknown	RI	m/z (%)	Area (%)
Un-1	1848	268(1), 250(8), 124(12), 109(28), 85(32), 71(60), 58(100).	3.4
Un-2	1870	285(1), 223(8), 149(100), 104(8), 57(20).	1.8
Un-3	1928	270(16), 227(20), 143(24), 87(68),74(100), 55(28).	1.1
Un-4	1964	229(1), 223(4), 149(100), 104(4), 76(4).	1.8
Un-5	2044	313(4), 256(24), 129(48), 78(100), 55(84).	2.7
Un-6	2077	313(4), 278(28), 129(32), 99(88),73(80),55(100).	1.5
Un-7	2085	313(4), 276(1), 256(8), 111(36), 97(68), 83(84), 55(100).	1.7
Un-8	2266	324(1), 309(4), 281(16), 113(12), 85(48), 71(68), 57(100).	5.5
Un-9	2374	338(1), 309(24), 99(16), 85(36), 71(56), 57(100).	1.0
Total Unknown			20.5
Total identified isolate			67.3
Total			87.8

RI, retention index; LRI, literature retention index; Q: Quality;
[a]Compounds are listed in order of elution, RI (retention index) values are calculated from retention times relative to that of *n*-alkanes (C_6-C_{32}) on the non-polar HP-5 column.

Agar well diffusion method: Simple susceptibility screening test using agar-well diffusion method as adapted earlier was used[18-20]. Each microorganism was suspended in Brain Heart Infusion (BHI) (Difco, Detroit, MI) broth and diluted *ca.* 10^6 colony forming unit (cfu) per mL. They were flood-inoculated onto the surface of BHI agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans* and *C. tropicalis*, SDA was used. 5 mm diameter wells were cut from the agar using a sterile cork-borer and 100 µL of the stock solution was delivered into the wells. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganism. Ceftazidime (Fortum) (10 µg) and triflucan (5 µg) were the standard drugs for antibacterial and antifungal activities, respectively. Acetone was used as solvent control. The test was carried out in duplicate. Results were interpreted in terms of diameter of inhibition zone: (-): < 5.5 mm; (+): 5.5-10 mm; (++): 11-15 mm; (+++): ≥ 16 mm. The results are shown in Table-2.

TABLE-2
SCREENING RESULTS FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL COMPONENTS FROM THE FLOWERS OF *D. formosum*

Sample	Stock (µg/mL)	Microorganisms and inhibition zone (mm)								
		Ec	*Yp*	*Kp*	*Sm*	*Ef*	*Sa*	*Bs*	*Ca*	*Ct*
D. formosum	1000	–	–	–	–	++	+	–	–	–
Ceftazidime	10	+++	+++	+++	+++	+++	+++	+++	–	–
Triflucan	5	–	–	–	–	–	–	–	+++	+++

Results were interpreted in terms of the diameter of the inhibition zone: (-): < 5.5 mm; (+): 5.5-10 mm; (++): 11-15 mm; (+++): ≥ 16 mm.
Ec: *Escherichia coli* ATCC 35218, *Yp*: *Yersinia pseudotuberculosis* ATCC 911, *Kp*: *Klebsiella pneumoniae* ATCC 13883, *Sm*: *Serratia marcescens* ATCC 13880, *Ef*: *Enterococcus faecalis* ATCC 29212, *Sa*: *Staphylococcus aureus* ATCC 25923, *Bs*: *Bacillus subtilis* ATCC 6633, *Ca*: *Candida albicans* ATCC 60193, *Ct*: *Candida tropicalis* ATCC 13803.

RESULTS AND DISCUSSION

The essential oil, having pale yellow colour, was obtained by hydrodistillation in a Clevenger-type apparatus[13,14] from the flowers of *D. formosum* and was analyzed by GC-MS with HP-5 column. 41 Components were identified on the basis of a typical library and literature search[15-17] with selecting only the components showing matches exceeding 80 %, which represented about 67.3 % of the total detected constituents (Table-1). Tricosane (30.9 %), heneicosane (8.2 %), pentacosane (6.6 %), linalool (2.3 %) docosane (2.2 %) and phytol (2.1 %) were found as the major compounds in the essential oil of *D. formosum*.

The antimicrobial activity of the essential oil from the flowers of *D. formosum* was tested *in vitro* using the agar-well diffusion method with the microorganisms as seen in Table-2. The essential oil showed antibacterial activity against only *E. faecalis* and *S. aureus*, but no antifungal activity was observed against the two yeast like fungi *C. albicans* and *C. tropicalis*[18-20]. No antibacterial activity was observed against *E. coli*, *Y. pseudotuberculosis*, *K. pneumoniae*, *S. marcescens* and *B. subtilis*.

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