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Volatile Constituents of the Flowers and Leaves-Stems of three *Doronicum* taxa from Turkey

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The composition of the essential oils obtained from fresh flowers and leaves-stems of 3 *Doronicum* taxa, 2 of them are endemic to Turkey, have been studied. The essential oils were separately obtained from hydrodistillation in a clevenger-type apparatus and their analyses were performed by GC and GC-MS. A range of 14-27 compounds were identified, constituting over 89.3 to 97.7 % of total oil composition. Sesquiterpene hydrocarbons were shown to be the main group of constituents of all 3 *Doronicum* taxa. The main component of the flowers and leaves-stems in the essential oils of *Doronicum orientale* and *Doronicum bithynicum* ssp. *sparsipilosum* was (E)-β-farnesene (41.1, 35.7, 47.5 and 55.4 %, respectively); while (E)-caryophyllene (24.2 and 52.7 %) was the major compound in the essential oils of *Doronicum macrolepis*.

Key Words: *Doronicum orientale*, *Doronicum bithynicum* subsp. *sparsipilosum*, *Doronicum macrolepis*, Essential oil, GC, GC-MS.

INTRODUCTION

Doronicum L. (Asteraceae) represented with 15 native taxa of which 14 are in species level in Turkey[1]. They are perennial herbs with short usually horizontal rhizome and 8 of the taxa are endemic to Turkey[1]. Half of the Turkish taxa are distributed in Euxine province from phytogeographical point of view and materials of this study were collected form this province. As well *Doronicum macrolepis* Freyn. & Sint. and *D. bithynicum* J.R. Edmondson subsp. *sparsipilosum* J.R. Edmondson are endemic and 3 of the others are rare in Turkey[1,2]. D. *orientale*, the most common one in Turkey, has been used as a folk medicine to cure infertility[1-3].

A previous phytochemical study on *D. orientale* has shown the presence of flavonoids type natural compounds[4]. To our best of knowledge, there is no previous report on the composition of the essential oil analysis of *D. orientale, D. bithynicum*

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subsp. *sporsipilosum* and *D. macrolepis*. The essential oil constituents of the plants were obtained by the widely used hydrodistillation method in a clevenger-type apparatus[5-9]. The obtained crude essential oils were then investigated by GC and GC-MS technique[5-9]. Identification of the compounds was made by a typical library search (NIST, WILLEY), authentic compounds and literature comparison[5-10]. In the present work, we report the constituents of the essential oils of the flowers and leaves-stems from three *Doronicum* taxa.

EXPERIMENTAL

D. oriental and *D. bithynicum* subsp. *sparsipilosum* were collected from Abant (Çayirbiçki) and Aladag-Bolu (at a height of *ca.* 1100 and 1005 m, respectively) in the northwestern part of Turkey in May 2007. *D. macrolepis* was collected from Uzungöl-Trabzon (at a height of *ca.* 1800 m) in the northeastern part of Turkey in July 2007. The plants were authenticated immediately after collection[1,2]. Voucher specimens were deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 16040, KATO: 16041 and KATO: 12759, respectively), Karadeniz Technical University, Turkey.

Isolation of the essential oils: The fresh plant materials were separated into flowers and leaves-stems parts. Crude essential oils of *D. orientale*, *D. bithynicum* subsp. *sparsipilosum* and *D. macrolepis* were obtained from the fresh flowers and leaves-stems parts (*ca.* 140 g, each) by hydrodistillation in a clevenger-type apparatus[5-9] with cooling bath (-15 ºC) system (3 h) (yields: 0.12-0.15 %, 0.13-0.19 %, 0.15-0.18 % and (v/w), respectively). The obtained oils were dissolved in HPLC grade *n*-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at 4-6 ºC in a sealed brown vial. One mL of the essential oils was directly injected separately into GC and GC-MS instrument.

Gas chromatography (GC) analysis: The capillary GC-FID analysis was perfo-rmed using an Agilent-5973 Network System, equipped with a FID (supplied with air and hydrogen of high purity) and a split inlet. The chromatographic column used for the analysis was HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 µm). Helium was used as carrier gas at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. One 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 240 °C with a 3 °C/min heating ramp. The identity of each compound was supported by comparing their retention indices (RI) to published values. The sample was analyzed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

Gas chromatography-mass spectrometry (GC-MS) analysis: GC-MS analysis of the essential oils was performed using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis

was HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Helium was used as carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. One 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 240 °C with a 3 °C/min heating ramp.

Identification of components: Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_6-C_{32}) as standards. The constituents of the oil were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Willey), authentic compounds (α-pinene, β-pinene, camphor, carvacrol and α-copaene) and with data published in the literature[5-10].

RESULTS AND DISCUSSION

The compositions of the essential oils of the 3 *Doronicum* taxa are listed in Table-1. Altogether, 43 essential compounds were identified by GC and GC-MS with HP-5 column from flowers and leaves-stems parts of *D. orientale, D. bithynicum* subsp. *sparsipilosum* and *D. macrolepis*. Among them, (E)-β-farnesene (41.1/35.7 and 47.5/55.4 %, respectively) was the major compound in *D. orientale* and *D. bithynicum* subsp. *sparsipilosum* and (E)-caryophyllene (24.3/52.7 %) was main constituent in *D. macrolepis*.

The chemical class distributions of the essential oils of the constituents are summarized in Table-2. The compounds were separated into 6 classes, which were monoterpene, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenoids and others (Table-2). The sesquiterpenes were the major constituents of all the 3 species in the ratio of 86.0, 88.7 and 45.7 % from flowers and 80.2, 88.4 and 79.6 % from leaves-stems parts, respectively. The total ratios of identified compounds in all parts (flowers and leaves-stems) of 3 species were found to be similar. α-Copaene, (E)-caryophyllene, germacrene D, (E,E)-α-farnesene, caryophyllene oxide and tricosane were common to *D. orientale, D. bithynicum* subsp. *sparsipilosum* and *D. macrolepis*.

The GC-MS analysis of the essential oils of *D. orientale*, *D. bithynicum* subsp. *sparsipilosum* and *D. macrolepis* allowed the identification of 22, 21 and 27 compounds in the flowers and 14, 17 and 23 compounds in the leaves-stems, respectively (Table-2)[5-10]. The number of volatile compounds present in flowers is greater than in leaves-stems parts of all the 3 species. The results clearly indicate that the major constituents of the essential oil composition of the flowers and leaves-stems of *D. orientale, D. bithynicum* subsp. *sparsipilosum* and *D. macrolepis* were very similar (Table-2). But, some chemical differences on the composition of the essential oils of *D. orientale, D. bithynicum* subsp. *sparsipilosum* and *D. macrolepis* were found and probably related to the different subspecies and/ or to the geographical origin of the plants.

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF
D. orientale, *D. bithynicum* subsp. *sparsipilosum* AND *D. macrolepis*[a,b]

No.	Compounds	*D. orientale*		*D. bithynicum* subsp. *sparsipilosum*		*D. macrolepis*		Exp. RI	Lit. RI
		Flowers	L.S.[c]	Flowers	L.S.[c]	Flowers	L.S.[c]		
		%Area	%Area	%Area	%Area	%Area	%Area		
1	*2E*-Hexenal	-	-	-	0.1	-	-	858	855
2	α-Pinene[d]	-	-	-	0.1	2.6	0.8	941	939
3	β-Pinene[d]	-	-	-	-	2.0	-	980	979
4	2-Pentylfuran	0.4	-	0.4	0.1	-	0.3	989	990
5	α-Phellandrene	-	-	-	-	2.4	-	1005	1003
6	*o*-Cymene	-	-	-	-	-	0.1	1026	1026
7	(*Z*)-β-Ocimene	-	-	-	-	2.4	0.3	1041	1037
8	Nonanal	0.2	-	0.1	-	-	0.1	1099	1101
9	*trans*-Thujone	-	-	-	-	0.1	-	1117	1114
10	Camphor[d]	-	-	-	-	-	0.2	1146	1146
11	*p*-Methyl acetophenone	-	-	-	-	0.5	0.2	1180	1183
12	Decanal	0.3	-	0.1	-	-	-	1199	1202
13	Octanoic acid ethyl ester	-	-	-	-	0.3	-	1199	1195
14	Carvacrol[d]	0.1	0.3	-	0.5	0.6	0.4	1296	1297
15	Undecanal	0.1	-	0.1	-	-	-	1303	1307
16	(2E,4E)-Decadienal	0.1	-	0.2	-	0.5	0.1	1316	1317
17	Presilphiperfol-7-ene	-	-	-	-	0.2	-	1340	1337
18	α-Cubebene	-	-	-	-	0.1	0.1	1349	1351
19	α-Copaene[d]	0.2	0.2	0.3	0.3	3.3	1.2	1376	1377
20	β-Cubebene	-	-	-	-	-	1.2	1387	1388
21	β-Elemene	4.9	11.9	12.4	5.8	-	-	1390	1391
22	(E)-Caryophyllene	8.2	7.8	9.1	7.6	24.3	52.7	1420	1419
23	α- Humulene	-	-	-	-	12.3	10.7	1453	1455
24	(E)-β-Farnesene	41.1	35.7	47.5	55.4	-	-	1457	1457
25	Germacrene D	8.6	20.5	7.5	5.7	11.8	10.8	1486	1485
26	α-Zingiberene	18.7	2.6	11.3	11.7	-	-	1492	1494
27	(E,E)-α-Farnesene	4.0	0.6	0.6	1.4	1.4	1.8	1503	1506
28	Δ-Cadinene	0.3	0.9	-	0.5	1.3	1.1	1522	1523
29	Caryophyllene oxide	0.7	0.5	0.2	0.4	3.5	4.9	1579	1583
30	Oplopenone	-	-	-	-	1.3	-	1604	1608
31	α-Cadinol	0.2	0.3	0.2	0.4	-	2.1	1659	1654
32	Selin-11-en-4-α-ol	0.3	10.4	4.2	5.3	-	-	1656	1660
33	Pentadecanal	0.3	-	0.4	-	-	0.6	1714	1713
34	Methyl tetradecanoate	-	-	-	-	3.7	-	1728	1724
35	Tetradecanoic acid	-	-	-	-	3.3	-	1773	1769
36	Ethyl tetradecanoate	-	-	-	-	1,3	-	1795	1796
37	Hexahydrofarnesyl acetone	0.4	-	0.5	-	0.6	-	1845	1846
38	Methyl hexadecanoate	0.3	-	0.3	-	2.6	0.4	1920	1922
39	Hexadecanoic acid	-	-	0.1	-		-	1982	1983
40	Ethyl hexadecanoate	-	-	-	-	1.4	-	1995	1993
41	Methyl linoleate	-	-	-	-	5.1	0.6	2099	2096
42	*cis*-Phytol	0.3	1.5	1.6	1.0	-	-	2114	2114
43	Tricosane	0.5	0.1	0.6	0.1	0.4	0.2	2299	2300
	Total identification	90.2	93.3	97.7	96.4	89.3	90.9		

[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; [b]Percentages obtained by FID peak-area normalization; [c]L.S.: Leaves and Stems; [d]Identified by authentic samples.

TABLE 2
CHEMICAL CLASS DISTRIBUTION IN THE ESSENTIAL OILS OF
D. orientale, *D. bithynicum* subsp. *sparsipilosum* AND *D. macrolepis*

Compound class	*D. orientale*				*D. bithynicum* subsp. *sparsipilosum*				*D. macrolepis*			
	Flowers		Leaves-Stems		Flowers		Leaves-Stems		Flowers		Leaves-Stems	
	%Area	N[a]	%Area	N[a]	%Area	N[a]	%Area	N[a]	%Area	N[a]	%Area	N[a]
Monoterpens	-	-	-	-	-	-	0.1	1	9.4	4	1.1	2
Monoterpenoids	0.1	1	0.3	1	-	-	0.5	1	0.7	2	0.6	2
Sesquiterpenes	86.0	8	80.2	8	88.7	7	88.4	8	54.7	8	79.6	8
Sesquiterpenoids	1.6	4	11.2	3	5.1	4	6.1	3	5.4	3	7.0	2
Diterpenoid	0.3	1	1.5	1	1.6	1	1.0	1	-	-	-	-
Others	2.2	8	0.1	1	2.3	9	0.3	3	19.1	10	2.6	9
Total	90.2	22	93.3	14	97.7	21	96.4	17	89.3	27	90.9	23

[a] N = Number of compounds.

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