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Antimicrobial Activity and Essential oil Compositions of Two *Ranunculus* Species from Turkey: *R. constantinopolitanus* and *R. arvensis*

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The isolated essential oils of *Ranunculus constantinopolitanus* and *Ranunculus arvensis* were tested for antimicrobial activity against the bacteria *E. coli, K. pneumoniae, P. aeruginosa, E. faecalis, S. aureus, B. cereus* and the fungus *C. albicans*. They showed moderate antimicrobial activity against *P. aeruginosa, E. faecalis, S. aureus* and *C. albicans*. The composition of essential oils obtained from the air-dried *R. constantinopolitanus* and *R. arvensis* were also analyzed by GC-MS. 45 and 36 Components were identified in the essential oils and the main component of these taxa was (Z)-phytol in the ratio 23.6 and 19.5 % from *R. constantinopolitanus* and *R. arvensis*, respectively.

Key Words: *R. constantinopolitanus*, *R. arvensis*, Ranunculaceae, Antimicrobial activity, Essential oil, GC-MS.

INTRODUCTION

Ranunculus L. (Ranunculaceae) represented with 94 native taxa of which 82 are in species level in Turkey. They are annual or perennial herbs and 19 of the taxa are endemic to Turkey[1-4]. All parts of the plant are poisonous when it is fresh. The toxins are destroyed by heat or by drying and the plant also have a strongly acrid juice that can cause blistering to the skin[5,6]. *R. factorial* L. and *R. scabrous* L. have been used pain killer and cure of hemorrhoids and *R. acre* L., *R. asioticus* L. and *R. sceleratus* L. were used in folk medicine to treat blain[7]. *R. constantinopolitanus* (DC.) Urv., is a perennial herb and very common in Turkey and it grows moist places especially in marshy meadows[1]. *R. arvensis* L. is mainly distributed in Mediterranean and Irano-Turanian region of Turkey. It is an annual herb and often grows in segetal habitats, often in cornfields[1].

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To our best knowledge, no previous work has been reported on the essential oil analysis and antimicrobial activity of *R. constantinopolitanus* and *R. arvensis*. But, antimicobacterial activity of *R. constantinopolitanus* is mentioned in the literature[8]. Thus, the systematic research was carried out by the extraction of the essential oil constituents of the plants by hydrodistillation in a Clevenger-type apparatus. The obtained essential oils were then investigated by GC-MS technique. Identification of the compounds was made by a typical library search and literature comparison[9-16].

EXPERIMENTAL

R. constantinopolitanus and *R. arvensis* were collected in Trabzon, Turkey (A7) in May 2005, Voucher specimens (No. Coskunçelebi 632-2005 and 633-2005, KTUB) were deposited in the Herbarium of the Department of Biology, Karadeniz Technical University, Turkey. The species were identified immediately after collection[1,2] and air-dried at room temperature for later analysis.

Isolation of the essential oils: 60 g of air-dried plant material from each taxon were blender into small pieces and the essential oils were obtained by steam distillation in 500 mL distilled H_2O for 3.5 h in a Clevenger-type apparatus with ice-water cooling bath system (4 h) (yields: 0.18 and 0.25 % (v/w), respectively). The oils were taken by HPLC grade *n*-hexane (0.5 mL) and dried over Na_2SO_4 kept at 4 ºC in a sealed brown vial. One mL of the extracts was directly injected into the GC-MS instrument.

Identification of components: The components of the oil were identified by their retention times, retention indices relative to C_6-C_{32} *n*-alkanes by comparison of their mass spectra with those of mass spectral libraries (NIST and Willey) with data published in the literature[9-16].

GC-MS Analysis: GC-MS analyses were as described previously[13].

Antimicrobial activity assessment: All test microorganisms were obtained from the Refik Saydam Hifzissihha Institute (Ankara, Turkey) and are as follows: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas auroginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 709 ROMA, *Candida albicans* ATCC 10231.

Agar well diffusion method: The agar well diffusion method was adopted[17,18]. Overnight cultures of microorganisms were adjusted to *ca.* 10^6 cfu/mL according to McFarland turbidity standards and spread over the appropriate media (Mueller-Hinton agar (Difco, Detroit, MI) for bacteria, Sabouraud Dextrose agar (Difco, Detroit, MI) for yeast) in petri dishes. Wells of 5 mm diameter were punched into the agar medium and filled

with 100 µL of essential oil solutions. The plates were incubated at 37 °C for 18-48 h and the inhibition zones around the wells were measured (data not shown). The antimicrobial effects of solutions that produce 6 mm zones of inhibition were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined.

The antibacterial and antifungal assays were performed in Mueller-Hinton broth at pH 7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively, in 96 well-plates according to National Committee for Clinical Laboratory method. The MIC was defined as the lowest concentration that showed no growth. Ampicillin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. The samples were dissolved in chloroform to prepare sample stock solution. Chloroform with dilution of 1:10 was used as solvent control. The results are shown in Table-1.

TABLE-1
SCREENING RESULTS FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS FROM *R. constantinopolitanus* AND *R. arvensis*

Sample	Stock solution (µg/mL)	Microorganisms and MIC[a] values (µg/mL)						
		Ec	Kp	Pa	Ef	Sa	Bc	Ca
R. constantinopolitanus	400	–	–	–	10	10	–	20
R. arvensis	300	–	–	8	8	8	–	34
Ampicillin		8	32	>128	2	2	2	
Fluconazole								8

[a]MIC = The lowest concentration causing total inhibition of microbial growth in µg/mL.
Ec = *Escherichia coli* ATCC 25922, Kp = *Klebsiella pneumoniae* ATCC 13883, Pa = *Pseudomonas aeruginosa* ATCC 10145, Ef = *Enterococcus faecalis* ATCC 29212, Sa = *Staphylococcus aureus* ATCC 25923, Bc = *Bacillus cereus 702* Roma, Ca = *Candida albicans* ATCC 10231; (–): no activity at stock solution concentration (100 µL).

RESULTS AND DISCUSSION

The antimicrobial activities for the essential oils of *R. constantinopolitanus* and *R. arvensis* were tested in vitro using the agar-well diffusion method with the microorganisms[17,18] as seen in Table-1. The essential oils showed antibacterial activity against three bacteria and against the yeast-like fungus tested. The test extracts showed better antimicrobial activity against Gram-positive bacteria in comparison to the Gram-negative bacteria. The essential oil extracts of *R. constantinopolitanus* showed antimicrobial activity against

Enterococcus faecalis ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231. The essential oil of *R. arvensis* were effective against *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231. But no antimicrobial activity was observed against the bacteria *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 10145, *Bacillus cereus* 702 Roma for *R. constantinopolitanus* and *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883 and *Bacillus cereus* 702 Roma for *R. arvensis*.

The essential oil compositions of *R. constantinopolitanus* and *R. arvensis* were analyzed by GC-MS with HP-5 column. A total of 45 and 36 components were identified on the basis of a typical library search and literature data with selecting only the components showing matches exceeding 81 %, which represented about 97.3 and 93.6 % of total composition of the essential oils in *R. constantinopolitanus* and *R. arvensis*, respectively[9-16]. The general chemical profile of the essential oils, the percentage content and the calculated retention indices of the constituents are summarized in Table-2.

TABLE 2
IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF
R. constantinopolitanus AND *R. arvensis*

No.	Compounds	A[a]		B[a]		Exp. RI	Ident. LRI
		Area (%)	Q (%)	Area (%)	Q (%)		
1	Alyl isovalerate	90	0.7	99	0.8	936	938
2	2-Pentyl-furan	83	0.6		-	986	990
3	*n*-Octanal	91	1.6	-	-	1000	1001
4	*p*-Cymene	91	0.4	-	-	1021	1025
5	1-Octanol	90	0.5		-	1065	1070
6	*n*-Nonanal	91	1.6	90	0.6	1104	1102
7	1-Nonanol	90	0.4		-	1164	1171
8	4-Terpineol	96	1.2		-	1170	1177
9	*n*-Decanal	91	0.7	91	0.2	1204	1204
10	β-Cyclocitral	96	0.5		-	1219	1219
11	Carvacrol methyl ether	93	7.2	-	-	1245	1244
12	Thymol	88	0.3	-	-	1293	1290
13	*n*-Tridecane	-	-	95	0.4	1298	1299
14	Carvacrol	95	3.2		-	1302	1299
15	2,4-Decadienal	90	0.6		-	1315	1314
16	β-Bourbonene	86	0.4	-	-	1386	1388
17	Tetradecane	95	0.2	-	-	1400	1400
18	α-Gurjunene		-	99	1.8	1408	1409
19	*trans*-Caryophyllene	99	1.1	98	0.7	1419	1418

No.	Compounds	A[a]		B[a]		Exp. RI	Ident. LRI
		Area (%)	Q (%)	Area (%)	Q (%)		
20	β-Gurjunene		-	98	1.8	1433	1432
21	*endo*-Arabozol	91	1.2	-	-	1434	1435
22	Aromadendrene		-	98	5.9	1440	1439
23	α-Guaiene	-	-	91	0.8	1445	1440
24	Geranyl acetone	91	0.7	99	3.9	1454	1453
25	Allo-aromadendrene			97	2.9	1465	1461
26	δ-Gurjunene		-	98	1.2	1473	1473
27	Germacrene D	97	1.0		-	1482	1480
28	α-Amorphene	99	0.5	-	-	1484	1485
29	β-Ionone	98	1.3	96	1.6	1486	1485
30	α-Selinene		-	98	2.3	1489	1494
31	Viridiflorene	-	-	99	1.4	1495	1497
32	*n*-Pentadecane	82	0.4	-	-	1500	1500
33	β-Bisabolene	99	1.6	-	-	1509	1506
34	Tridecanal	-	-	86	0.4	1511	1510
35	Δ-Cadinene	97	0.5	-	-	1517	1523
36	*trans*-Calamenene	89	3.2	97	2.9	1526	1529
37	β-Thujaplicinol	85	2.7	-	-	1536	1538
38	α-Calacorene	81	0.7	97	1.3	1545	1546
39	Ledol	-	-	85	1.0	1569	1569
40	Spathulenol	88	0.8	-	-	1579	1578
41	Caryophyllene oxide	81	1.0		-	1584	1581
42	Globulol	99	2.0	99	7.4	1585	1585
43	Viridiflorol		-	95	2.4	1593	1593
44	Tetradecanal	81	0.5	95	1.0	1598	1611
45	β-Eudesmol		-	83	1.1	1624	1649
46	α-Muurolol		-	84	1.2	1643	1645
47	Cadalene	90	3.8	99	3.2	1677	1674
48	*n*-Pentadecanal	91	2.7	91	2.6	1715	1715
49	6,10,14-Trimethyl-2-pentadecanone	97	4.7	99	8.5	1845	1845
50	Farnesyl acetone		-	90	0.9	1919	1917
51	Methyl Hexadecanoate	98	3.5	96	2.5	1925	1922
52	Isophytol	86	0.5	86	0.6	1949	1948
53	Manool	-	-	89	1.0	2058	2057
54	1-Octadeceanol	99	1.7	99	0.6	2084	2082
55	Methyl Linoleate	99	8.7	99	5.4	2094	2096
56	*(Z)*-Phytol	91	23.6	91	19.5	2114	2114
57	*n*-Docosane	98	0.6		-	2200	2200
58	Phytol acetate	88	0.7	-	-	2221	2218
59	Tricosane	97	1.0	95	1.8	2300	2300
60	*n*-Pentacosane	96	4.8	97	2.0	2500	2500
61	*n*-Heptacosane	98	1.7	-	-	2700	2700
Total isolate			97.3		93.6		

Unknown	RI	m/z (%)	A	B
Un-1	1663	220(5), 205(15), 133(60), 119(75), 91(100), 79(77).	1.3	–
Un-2	1769	236(12), 218(22), 147(49), 105(57), 95(60), 81(100).	–	1.9
Un-3	1870	243(6), 223(18), 167(10), 149(100), 57(20).	–	1.0
Total unknown			1.3	2.9
Total isolate			97.3	93.6
Total			98.6	96.5

RI = Retention index; LRI = Literature retention index; Q = Quality; A = *Ranunculus constantinopolitanus*; B = *Ranunculus arvensis*.
[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.

(Z)-Phytol (23.6 %), methyl linoleate (8.7 %), carvacrol methyl ether (7.2 %), *n*-pentacosane (4.8 %) and 6,10,14-trimethyl-2-pentadecanone (4.7 %) were the main constituents of the essential oil of *R. constantinopolitanus*, whereas (Z)-phytol (19.5 %), 6,10,14-trimethyl-2-pentadecanone (8.5 %), globulol (7.4 %), aromadendrene (5.9 %) and methyl linoleate (5.4 %) were the main components of the essential oil of *R. arvensis*. The chemical class distribution of the essential oils of *R. constantinopolitanus* and *R. arvensis* are reported in Table-3. The compounds were separated into six classes, which were monoterpene, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenoids and others (Table-3). Twenty compounds were common to all two species with the similar total ratio of 67.5 and 67.1 % in *R. constantinopolitanus* and *R. arvensis*, respectively. Some chemical differences on the composition of the essential oils of *R. constantinopolitanus* and *R. arvensis* were found and probably related to the different subspecies and/or to the geographical origin of the plants.

TABLE-3
CHEMICAL CLASS DISTRIBUTION OF THE ESSENTIAL OIL COMPONENTS OF *R. constantinopolitanus* AND *R. arvensis*

Compound class	*R. constantinopolitanus*		*R. arvensis*	
	Area (%)	Number of compounds	Area (%)	Number of compounds
Monoterpene	0.4	1	-	-
Monoterpenoids	18.3	9	5.5	2
Sesquiterpenes	12.8	9	26.2	12
Sesquiterpenoids	8.5	4	22.5	7
Diterpenoids	24.8	3	21.1	3
Others	32.5	19	18.3	12
Common compounds	67.5	20	67.1	20

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