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Essential Oil Analysis and Antimicrobial Activities of *Anthemis marschalliana* ssp. *pectinata* and *Anthemis cretica* ssp. *argaea* from Turkey

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The present work describes chemical composition and antimicrobial activity of the essential oils of *Anthemis marschalliana* Wild ssp. *pectinata* (Boiss) Grierson and *Anthemis cretica* L ssp. *argaea* (Boiss & Bal) Grierson. The essential oils were obtained from all the parts of the plant by hydrodistillation and analyzed by GC-FID and GC-MS. Forty two and forty four components representing 88.6 and 89.6 % of the total oils were characterized and the main components of these species were found to be spathulenol (21.7 %), humulene epoxide II (5.9 %), β-pinene (4.8 %) and α-bisabolol (4.6 %) from *A. marschalliana* ssp. *pectinata* and β-pinene (14.6 %), α-pinene (14.3 %), borneol (10.6 %) and β-acorenol (6.5 %) from *A. cretica* ssp. *argae*, respectively. The antimicrobial activities of the isolated essential oils of the plants were also investigated and they showed moderate antibacterial activity against *Yersinia pseudotuberculosis* and *Bacillus cereus*.

Key Words: *A. marschalliana* ssp. *pectinata*, *A. cretica* ssp. *argaea*, Essential oil, Antimicrobial activities, GC-MS.

INTRODUCTION

The genus *Anthemis* L. (Asteraceae) is represented with 50 native species including 80 intraspecific taxa, 27 of them is endemics, in Turkey[1,2]. Many species such as *A. nobilis* L. *A. arvensis* L., *A. altissima* L. and *A. auriculata* Boiss. are used in Anatolian folk medicines[3]. This genus with the rate of 54 is among the richest genera in terms of endemic species[1]. *A. cretica* L. subsp. *argeae* (Boiss. & Bal) Grierson is an endemic taxa for Turkey and distributes mainly in North-East Anatolia. They are herbaceous perennial herbs grown in rocky mountain slopes and dry lands[4].

To our best of knowledge, there is no published report on the essential oil analysis and antimicrobial activities of *A. marschalliana* ssp. *pectinata* and *A. cretica* ssp. *argaea*. But antimicrobial activity of *A. cretica* ssp. *argaea* for some different

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microorganisms was mentioned in the literature[5]. As part of this systematic research, the essential oil constituents of the plants were obtained by the widely used hydrodistillation method in a Clevenger-type apparatus. The obtained crude essential oils were then investigated by GC-FID and GC-MS techniques. Identification of individual components was made by comparison of their retention times with those of analytical standards of available terpenes and terpenoids and by computer searching, matching mass spectral data with those held in NIST and Willey library of mass spectra and literature comparison[6-14].

EXPERIMENTAL

Anthemis marschalliana Wild ssp. *pectinata* (Boiss) Grierson and *Anthemis cretica* L ssp. *argaea* (Boiss & Bal) Grierson were collected from Çaykara-Trabzon (at a height of *ca.* 2900 m, A7) in the northeastern part of Turkey in July 2005. Voucher specimens (No. Coskunçelebi 554a and 554b-2005 KTUB) were deposited in the Herbarium of the Department of Biology, Karadeniz Technical University, Turkey. The plants were identified immediately after collection[1,2,4] and air-dried at room temperature for later analysis.

Isolation of the essential oils: Crude essential oils of *A. marschalliana* ssp. *pectinata* and *A. cretica* ssp. *argaea* were obtained from the air-dried whole plants (~50 g, each) by hydrodistillation in a Clevenger-type apparatus with cooling bath (-15 ºC) system (3 h) (yields: 0.17 and 0.22 % (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 µL of the extracts for each was directly injected into the GC and GC-MS instrument.

Gas chromatography: The capillary GC-FID analysis was performed 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 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 samples were analyzed twice and the percentage compositions of oils were computed from the GC peak areas without using correction factors.

Gas chromatography-mass spectrometry: GC-MS analysis 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 carrier gas, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. One µL essential oils solutions in hexane (HPLC grade) were 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.

Antimicrobial activity assessment: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 10145, *Bacillus cereus* 709 ROMA, *Listeria monositogenes* ATCC 43251, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Candida tropicalis* ATCC 13803. Dimethyl sulphoxide (DMSO) was used solved solution.

Agar well diffusion method: Simple susceptibility screening test using agar-well diffusion method was used[15,16]. Each microorganism was suspended in Mueller Hinton (Difco, Detroit, MI) broth and diluted *ca.* 10^6 colony forming unit (cfu) per mL. They were 'flood-inoculated' onto the surface of Mueller Hinton agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. tropicalis*, SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 50 µL of the extract substances were 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 organism. Ampicillin (10 µg) and triflucan (5 µg) were standard drugs. Dimethyl sulphoxide was used as solved control. The results are shown in Table-3.

RESULTS AND DISCUSSION

The obtained crude essential oils of *A. marschalliana* ssp. *pectinata* and *A. cretica* ssp. *argaea* were investigated by GC-FID and GC-MS technique with HP-5 column. A total of 42 and 44 components were characterized on the basis of authentic compounds (α-pinene, camphene, β-pinene, α-terpinene, limonene, γ-terpinene, borneol and α-terpineol), literature data[6-14] and a typical library search (NIST, WILLEY) with selecting only the components showing matches exceeding 80 %, which represented about 88.6 and 89.6 % of total composition of the essential oils in *A. marschalliana* ssp. *pectinata* and *A. cretica* ssp. *argaea*, respectively. The general chemical profile of the essential oils, the percentage content and the retention indices of the constituents are summarized in Table-1.

Spathulenol (21.7%), humulene epoxide II (5.9 %), β-pinene (4.8 %) and α-bisabolol (4.6 %) were the main constituents of the essential oil of *A. marschalliana* ssp. *pectinata*; whereas β-pinene (14.6 %), α-pinene (14.3 %), borneol (10.6 %) and β-acorenol (6.5 %) were the main components of the essential oil of *A. cretica* ssp. *argaea*.

The chemical class distribution and the main components in each class of the essential oils of *A. marschalliana* ssp. *pectinata* and *A. cretica* ssp. *argaea* are reported in Table-2. The compounds were separated into 6 classes, which were monoterpene, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenoids and others (Table-2). The dominant constituents were sesquiterpenoids and monoterpene

TABLE 1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF
A. marschalliana ssp. *pectinata* AND *A. cretica* ssp. *argaea*

No.	Compounds	A[a,b]		B[a,b]		Exp. RI	Lit. RI
		% Area	Q (%)	% Area	Q (%)		
1	α-Pinene[c]	0.9	96	14.3	97	941	939
2	Camphene[c]	-	-	4.7	98	956	954
3	Benzaldehyde	-	-	4.0	96	961	960
4	β-Pinene[c]	4.8	96	14.6	96	980	979
5	*n*-Octanal	0.8	64	-	-	1000	999
6	α-Phellandrene	-	-	0.5	94	1005	1003
7	β-Terpinene[c]	-	-	0.5	96	1019	1017
8	Limonene[c]	0.9	96	-	-	1030	1029
9	1,8-Cineole	-	-	3.5	90	1033	1031
10	(Z)-β-Ocimene	0.9	86	-	-	1038	1037
11	γ-Terpinene[c]	0.8	92	0.9	97	1061	1060
12	Terpinolene	0.7	91	0.5	96	1089	1089
13	*n*-Nonanal	1.4	86	0.5	80	1102	1101
14	Camphor	-	-	5.8	96	1147	1146
15	Pinocarvone	1.0	96	0.5	98	1164	1165
16	Borneol[c]	-	-	10.6	92	1171	1169
17	Terpinen-4-ol	-	-	0.5	96	1178	1177
18	Naphthalene	0.9	97	-	-	1180	1181
19	α-Terpineol[c]	-	-	0.8	84	1190	1189
20	Myrtenal	0.9	96	-	-	1194	1196
21	*n*-Decanal	0.8	87	-	-	1203	1202
22	*trans*-Carveol	1.5	80	-	-	1219	1217
23	Carvone	-	-	0.4	90	1244	1243
24	(E)-2-Decenal	0.9	90	-	-	1265	1264
25	(2E-4E)-Decadienal	1.3	95	1.0	93	1317	1317
26	δ-Elemene	-	-	0.7	98	1337	1338
27	α-Terpinyl acetate	0.7	82	-	-	1351	1349
28	Eugenol	-	-	0.5	96	1360	1359
29	(*E*)-β-Damascenone	1.9	97	-	-	1383	1385
30	Cyperene	-	-	0.5	98	1398	1399
31	Tetradecane	3.9	96	0.5	95	1400	1400
32	α-Gurjunene	-	-	0.5	96	1408	1410
33	Dodecanal	0.6	84	-	-	1409	1409
34	β-Funebrene	1.1	90	-	-	1414	1415
35	(*E*)-Caryophyllene	-	-	0.5	90	1418	1419
36	2,5-Dimethoxy-*p*-cymene	-	-	0.7	96	1426	1427
37	α-*trans*-Bergamotene	0.9	91	-	-	1434	1435
38	α-Guaiene	-	-	0.4	92	1441	1440
39	α-Humulene	1.3	85	-	-	1454	1455
40	(E)-β-Farnesene	0.8	81	-	-	1459	1457
41	Ar-Curcumene	-	-	0.5	97	1482	1481
42	γ-Himachalene	0.8	81	-	-	1484	1483
43	β-Selinene	-	-	0.7	85	1492	1490
44	Viridiflorene	2.5	90	-	-	1495	1497
45	α-Cuprenene	-	-	0.5	82	1505	1506

No.	Compounds	A[a,b]		B[a,b]		Exp. RI	Lit. RI
		% Area	Q (%)	% Area	Q (%)		
46	Tridecanal	1.3	83	-	-	1511	1510
47	Myristicin	1.9	97	0.5	97	1521	1519
48	α-Calacorene	-	-	0.5	82	1545	1546
49	Elemicin	-	-	0.5	94	1558	1557
50	(E)-Nerolidol	1.9	82	-	-	1562	1563
51	Spathulenol	21.7	95	1.5	86	1578	1578
52	Helifolen-12-al A	-	-	0.7	80	1592	1593
53	Humulene epoxide II	5.9	83	-	-	1607	1608
54	*trans*-Arteannuic alcohol	-	-	0.8	85	1614	1613
55	β-Acorenol	-	-	6.5	84	1635	1637
56	Cubenol	3.1	82	-	-	1648	1647
57	(E)-14-hydroxy-9-epi-caryophyllene	-	-	1.2	80	1671	1670
58	α-Bisabolol	4.6	85	1.9	84	1686	1686
59	10-nor-Calamenen-10-one	2.9	85	-	-	1701	1702
60	(Z)-Nuciferal	-	-	0.5	80	1715	1715
61	(E)-Sesquilavandulyl acetate	1.5	80	1.4	83	1742	1741
62	(Z)-Lanceol	0.9	80	-	-	1759	1761
63	Cyclocolorenone	-	-	1.1	80	1760	1761
64	γ-Curcumen-15-al	0.6	80	-	-	1766	1768
65	β-Bisabolenol	-	-	0.8	81	1791	1790
66	Hexahydrofarnesyl acetone	3.8	99	1.1	99	1846	1846
67	Oplapanonyl acetate	-	-	0.5	80	1890	1888
68	Pimaradiene	1.2	92	-	-	1951	1950
69	Eicosane	0.9	89	-	-	1999	2000
70	Heneicosane	0.7	81	-	-	2098	2100
71	Tricosane	0.9	91	0.5	98	2300	2300
72	Pentacosane	1.8	95	-	-	2499	2500
	Total isolate	88.6		89.6			

Unknown	RI	m/z (%)	A	B
Un-1	1608	254(4), 222(16), 164(44), 121(100), 105(20), 93(28), 79(24)	-	0.8
Un-2	1650	246(5), 172(100), 141(28), 113(24), 101(60), 87(52), 63(24)	-	3.8
Un-3	1673	246(6), 217(8), 197(10), 133(28), 119(100), 105(44), 91(56)	2.6	-
Un-4	1714	246(2), 212(24), 197(48), 96(40), 82(72), 57(100)	1.7	-
Un-5	1866	223(8), 149 (100), 104(8), 76 (4), 57(18)	1.1	0.6
Un-6	1978	446(3), 256(36), 213(32), 129(44), 73(100), 60(88)	-	1.9
Un-7	2019	302(2), 256(4), 220(100), 205(40), 190(36), 115(32), 71(44)	-	0.9
Un-8	2053	302(2), 256(4), 153(32), 135(60), 69 (100), 57(60)	-	1.3
Un-9	2133	302(6), 280(8), 135(16), 95(55), 81(76), 67(92), 55(100)	2.7	-
Total unknown			8.1	9.3
Total isolate			88.6	89.6
Total			96.7	98.9

RI = Retention index; Q = Quality; A = *A. marschalliana* ssp. *pectinata*, B = *A. cretica* ssp. *Argaea*; [a]RI 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]Identified by authentic samples.

TABLE-2
CHEMICAL CLASS DISTRIBUTION AND THE MAIN COMPONENTS IN EACH CLASS OF THE ESSENTIAL OILS OF *A. marschalliana* ssp. *pectinata* AND *A. cretica* ssp. *argaea*

Compound class	*A. marschalliana* ssp. *pectinata*			*A. cretica* ssp. *argaea*		
	% Area	N[a]	Major component	% Area	N[a]	Major component
Monoterpene	9,0	6	β-Pinene	36,0	7	β-Pinene
Monoterpenoids	6,0	5	*trans*-Carveol	23,3	9	Borneol
Sesquiterpenes	7,4	6	Viridiflorene	4,8	9	β-Selinene
Sesquiterpenoids	40,2	8	Spathulenol	16,9	11	β-Acorenol
Diterpenoids	1,2	1	Pimaradiene	-	-	-
Others	24,8	16	Tetradecane	8,6	8	Benzaldehyde

[a]N = Number of compounds.

in the quantity of 40.2 and 36.0 % in *A. marschalliana* ssp. *pectinata* and *A. cretica* ssp. *argaea*, respectively. Fourteen compounds were common to all 2 species with the total ratio of 49.2 % in *A. marschalliana* ssp. *pectinata* and 39.7 % in *A. cretica* ssp. *argaea*. Some chemical differences on the composition of the essential oils of *A. marschalliana* ssp. *pectinata* and *A. cretica* ssp. *argaea* were found and probably related to the different subspecies and/or to the geographical origin of the plants.

The antimicrobial activity for the essential oils of *A. marschalliana* ssp. *pectinata* and *A. cretica* ssp. *argaea* were tested *in vitro* using the agar-well diffusion method[15,16] with the microorganisms as seen in Table-3. The essential oils showed weak antibacterial activity against *Y. pseudotuberculosis*, *B. cereus* in *A. marschalliana* ssp. *pectinata* and *Y. pseudotuberculosis*, *B. cereus*, *S. aureus* and *C. tropicali* in *A. cretica* ssp. *argaea* but no activity was observed against the bacteria *E. coli*, *P. aeruginosa*, *L. monocitogene*s, *E. faecalis* and yeast like fungi *Candida tropicali*, respectively.

TABLE-3
SCREENING RESULT FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS FROM *A. marschalliana* ssp. *pectinata* AND *A. cretica* ssp. *argaea*

Sample	Stock (μg/50 μL)	Microorganisms and inhibition zone (mm)							
		Ec	Yp	Pa	Bc	Li	Sa	Ef	Ct
A. marschalliana ssp. *pectinata*	440	-	7	-	7	-	-	-	-
A. cretica ssp. *argaea*	590	-	6	-	8	-	7	-	8
Ampicillin	10	10	18	18	15	10	35	10	
Triflucan	5								25

Ec = *Escherichia coli* ATCC 25922, Yp = *Yersinia pseudotuberculosis* ATCC 911, Pa = *Pseudomonas aeruginosa* ATCC 10145, Bc = *Bacillus cereus 702 Roma*, Li = *Listeria monocitogenes* ATCC 43251, Sa = *Staphylococcus aureus* ATCC 25923, Ef = *Enterococcus faecalis* ATCC 29212, Ct = *Candida. tropicali* ATCC 13803. Amp. = Ampicillin, Flu. = Fluconazole, (-) = no activity.

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