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ESSENTIAL OIL COMPOSITION OF FOUR *Achillea* SPECIES FROM THE BALKANS AND ITS CHEMOTAXONOMIC SIGNIFICANCE

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The chemical composition of the essential oils of Achillea clavennae *L.,* Achillea holosericea *Sibth. & Sm.,* Achillea lingulata *W. & K., and* Achillea millefolium *L. from the Balkans was determined by GC and GC/MS analyses. The main components were 1,8-cineole in* A. holosericea, *camphor in* A. clavennae, β*-pinene in A. millefolium, and* τ*-cadinol in* A. lingulata. *A detailed chemotaxonomic discussion is presented.*

Key words: *Achillea millefolium, A. holosericea, A. clavennae, A. lingulata,* essential oil composition, GC/MS, Compositae, chemotaxonomy.

The *Achillea* genus is one of the most important genera in the Compositae family (tribe Anthemideae, subtribe Achilleinae) due to its use in traditional and modern medicine [1, 2]. Members of *Achillea* genus are distributed mainly in the northern hemisphere, with a preference for xeric and alpine habitats [3].

The genus *Achillea* has a complex taxonomy, and studies on the composition of the essential oils have been used as an additional characteristic of inter- and infraspecific differentiation [4]. The presence of infraspecific chemical taxa among these species is well documented and is thought to be genetically determined but also greatly influenced by environmental and ontogenetic factors [5]. Here we report on the chemical composition of the oils obtained from *Achillea clavennae* L., *Achillea holosericea* Sibth. & Sm., *Achillea lingulata* W. & K., and *Achillea millefolium* L. (Table 1) with the aim of demonstrating its implications on the chemotaxonomy of the genus.

The yields of the acquired essential oils were low compared to the oils previously isolated from the plants of the same genus [4, 6]: 0.17% for *A. clavennae,* 0.09% for *A. holosericea,* 0.07% for *A. lingulata,* and 0.08% for *A. millefolium.*

The essential oil composition of the *Achillea* species considered for this study was, as expected, characterized by the presence of mono- and sesquiterpenoids. The oil almost entirely consisted of monoterpenoids in *A. clavennae* and *A. holosericea* while *A. lingulata* had a significantly high percentage of sesquiterpenoids (72.39%). In the oil isolated from *A. millefolium* neither mono- nor sesquiterpenoids dominated considerably. These facts could be related to the maturation of the plant. A greater quantity of monoterpenoids compared to sesquiterpenoids is anticipated before the flowering phase and vice versa [7]. The monoterpenoid fraction of the essential oil of all four species was dominated by the oxygenated compounds, the only exceptions being β-pinene and sabinene in *A. millefolium.* The most abundant oxygenated sesquiterpenoid in all four species was caryophyllene oxide.

The main constituents of *A. millefolium* (Millefolium section, Millefoliatae group) essential oil were β-pinene (32.63%), β-caryophyllene (16.52%), sabinene (11.48%), and chamazulene (5.86%), and these four compounds constituted 66.49% of the oil. To the best of our knowledge β-pinene has never before been reported as the most abundant constituent of *A. millefolium* oil although Kalinkina [8] found that the fraction consisting of sabinene and β-pinene formed the major portion of the oil. Compared to the oil investigated by Chalchat [9] (Table 2) isolated from *A. millefolium* plants originating from the same area, the oil investigated herein showed significant differences. This suggests that environmental conditions, i.e., the mountainous setting (2139 m altitude) contrasting the river bank, have a profound effect on the composition of the oil.

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TABLE 1. The Composition of the Essential Oils of Four Balkan *Achillea* Species, %

Component*	*A. millefolium*	*A. lingulata*	*A. clavennae*	*A. holosericea*	Retention indices	
					RI 1**	RI 2***
α-Thujene	0.51	-	-	-	938	928
α-Pinene	2.52	-	0.60	0.72	946	937
Camphene	-	-	3.10	2.05	962	950
Sabinene	11.48	-	-	-	992	976
β-Pinene	32.63	-	0.62	0.57	995	974
β-Myrcene	-	-	0.30	-	1013	990
α-Phellandrene	0.76	-	-	-	1041	1000
α-Terpinene	0.47	-	-	-	1044	1013
p-Cymene	-	-	-	0.11	1047	1015
1,8-Cineole	4.57	0.18	43.92	47.42	1050	1025
γ-Terpinene	0.39	-	-	-	1082	1056
Terpinolene	0.42	-	-	-	1084	1089
Pinocarvone	-	-	-	0.34	1113	1144
Camphor	-	0.35	46.87	23.51	1139	1126
cis-Chrysanthenol	-	-	-	0.20	1150	1102
Linalool	-	1.00	0.30	0.55	1153	1087
Borneol	1.68	7.99	0.73	17.11	1162	1155
Isoborneol	-	1.66	0.10	2.41	1175	1148
4-Terpineol	0.36	-	-	-	1181	1168
Geraniol	-	4.22	-	-	1237	1265
Bornyl acetate	2.42	3.96	-	-	1259	1270
2-Dodecanone	-	0.29	-	-	1308	1348
δ-Elemene	-	0.47	-	-	1319	1388
α-Copaene	0.75	0.21	0.18	-	1338	1376
Geranyl acetate	-	1.32	-	-	1365	1379
β-Bourbonene	0.41	1.01	-	-	1376	1386
β-Caryophyllene	16.52	0.84	-	-	1409	1423
β-Longipinene	0.20	-	-	-	1430	1359
α-Cedrene	-	0.57	-	-	1434	1422
α-Humulene	2.13	-	-	-	1439	1437
Valencene	-	3.21	-	-	1446	1489
Germacrene D	2.12	1.53	-	-	1464	1480
Bicyclogermacrene	2.70	1.18	0.25	0.26	1480	1497
γ-Cadinene	-	2.98	-	-	1491	1513
δ-Cadinene	-	1.10	-	-	1498	1526
(*Z*)-Nerolidol	-	3.23	-	-	1504	1535
(*E*)-Nerolodol	-	-	-	1.16	1536	1557
Spathulenol	-	-	-	0.32	1564	1575
Caryophyllene oxide	3.74	16.61	2.25	2.03	1566	1581
α-Bisabolol	-	1.44	-	-	1596	1686
τ-Cadinol	-	22.48	-	-	1638	1642
α-Bisabolene oxide	-	12.79	-	-	1670	1682
Chamazulene	5.86	-	-	0.86	1739	1725
Total	92.64	90.62	99.22	99.62		
Monoterpenoids	58.21	20.68	96.54	94.99		
Oxygenated	4.46	20.68	91.92	91.54		
(Iso)camphane type	4.10	13.96	50.80	45.08		
Thujane type	11.99	0	0	0		
Pinane type	35.15	0	1.22	1.83		
Menthane type	6.97	1.18	44.22	48.08		
Sesquiterpenoids	34.43	69.65	2.68	4.63		

*The components are listed in order of elution on an SPB1 column described in the experimental section.

**Retention indices on an SPB1 column.

***Retention indices on a DB-5 column.

- The compound was not detected in the essential oil.

Thujane type compounds, particularly β-thujone and sabinene, precursors of thujyl ketones [10], were reported as the main components of *A. millefolium* from Canada [11]. The camphane type compounds, common major constituents of the *Achillea* oils, are present as minor constituents. The reduced amount of these compounds is usually related to the higher concentrations of β-pinene and sabinene. The menthane type compounds constitute 6.97% of the oil, and 1,8-cineole has the highest participation (4.57%).

The sesquiterpenoid fraction of the essential oil of *A. millefolium* was dominated by β-caryophyllene. *A. millefolium* oils rich in β-caryophyllene were isolated from plants growing wild in Germany [12]. Since caryophyllene oxide succeeds β-caryophyllene in the biosynthetic pathway, their amounts seem to be correlated. These two compounds sum up to 20.26%, which is close to 25.2%, the value reported for the total amount of these two compounds in the essential oil of *A. millefolium* from Cuba [13]. Unlike those findings, where the ratio of β-caryophyllene to caryophyllene oxide was 4:1, the enzymatic epoxidation in our case led to an approximately reverse ratio. α-Humulene, the compound formed in a concurrent metabolic pathway to β-caryophyllene, is found in the amount of 2.13%. Besides these compounds germacrene D and bicyclogermacrene were found in significant quantities.

Chamazulene, a sesquiterpene-generated compound probably responsible for the physiological activity of *A. millefolium* oil, has also been identified (5.86%). The occurrence of chamazulene in the essential oil discriminates between *A. millefolium* species of different levels of ploidity [14]. According to those results the prerequisite for the considerable proazulene content is closely related to tetraploid plants. A comparison of our *A. millefolium* essential oil with the one from Siberia, Tomsk region [8], surprisingly indicates the deviation from the generally observed variability of the essential oil composition (Table 2). This unexpected similarity linking plants from distinctly different geographic origins suggests that certain *A. millefolium* chemotypes can be scattered at several disconnected localities, and it appears that the genetic resemblance of *A. millefolium* collected at these two localities outweighs the climate and the environmental influences.

A. holosericea (Millefoliatae section, Filipendulinae group) is an endemic species distributed throughout the southern Balkan Peninsula. The most prominent component of the essential oil was 1,8-cineole (47.42%). The occurrence of 1,8-cineole in the essential oil of *A. holosericea* has never before been accounted for to such a high extent [1, 15, 16].

The oil was also rich in camphor and borneol, amounting to 23.51% and 17.11%, respectively. The sum of the quantities of these two compounds was 40.62%, comparable to the previously published results for the species growing spontaneously in the same environmental background in which this sum was 55.00% [15]. The overall conclusion appears to be that the production of (iso)camphane type monoterpenoids (camphene, camphor, borneol) was under genetic control while the overproduction of 1,8-cineole probably arose by virtue of various simultaneous influences from the surroundings. Noteworthy seems to be the fact that all menthane type monoterpenoids previously detected in *A. holosericea* [1, 16] have been replaced by 1,8-cineole in our case.

The chemical composition of the *A. millefolium* and *A. holosericea* essential oils presented here clearly reflect the two different groups (Millefoliatae and Filipendulinae) within the Millefolium section.

Two components of the essential oil of *A. clavennae* (Ptarmicae section, *A. clavennae* group), 1,8-cineole and camphor, made up 90.79% of the oil. The high content of camphane type compounds was previously described [15, 17, 18]. The high camphor level in *A. clavennae* was probably due to the efficient oxidation of borneol whose concentration was expectedly low [19]. In addition to these marker compounds the oil consisted of camphene (3.10 %) and caryophyllene oxide (2.25%).

The oil of *A. clavennae* has shown remarkable similarity to the oil of *A. holosericea*, even though they belong to different sections of the *Achillea* genus, the main difference being the high percentage of borneol (17.11%) in the later. In view of the fact that these two species were collected at the same locality, the production of the terpenes seems to be governed exclusively by environmental factors, as suggested by Maffei [19].

A pronouncedly different distribution of essential oil constituents was found in *A. lingulata* (Ptarmicae section, Ptarmica group), the central features being the high contents of τ-cadinol (22.48%), which has never before been reported as the main component in *A. lingulata* essential oil, caryophyllene oxide (16.61%), and α-bisabolene oxide (12.79%) which are noticeably opposed to previously published results [18, 20]. This proves that *A. lingulata* can be classified in the "low camphor" group of yarrows, together with *A. ptarmica, A. chrysocoma,* and *A. corabensis* [16].

Since the Balkan *Achillea* species can be clearly separated from the Central and Western European species (Table 2) [24], it is of considerable interest to assess the inter- and infraspecific diversity of the *Achillea* species from the Balkans, bearing in mind that no conclusive arguments can be drawn solely by consideration of the essential oil composition.

TABLE 2. Variation of *A. millefolium* Essential Oil Composition with Location

Component	YU*	CA*	GR1*	CU*	RU*	KZ*	IR*	GR2*	EE*	DE*
α-Pinene	1.8	1.6	0.4	0.4	2.67	0.6	2.4	0.06	**8.4**	1.6
Sabinene	0.2	**8.7**	0.4	**5.4**	**28.35****	1.3	-	0.24	**8.3**	**15.2**
β-Pinene	**3.7**	**8.6**	0.3	-	**28.35****	2.1	2.9	0.18	**21.8**	**7.3**
α-Terpinene	-	0.9	**7.0**	-	2.33	0.5	0.4	0.15	0.3	0.6
p-Cymene	1.4	2.2	**7.4**	0.6	2.06	1.1	1.0	0.76	0.6	0.1
1,8-Cineole	**23.3**	**13.8**	**10.5**	**5.7**	**12.78**	**8.7**	**6.1**	**11.87**	**6.9**	1.0
Linalool	-	-	0.5	1.0	-	0.3	**4.1**	**5.85**	-	-
α-Thujone	**4.5**	1.1	1.5	-	-	0.4	-	0.07	-	-
β-Thujone	0.8	**16.5**	0.2	-	-	0.1	-	0.06	-	Tr.
Camphor	**12.7**	**11.6**	**8.1**	1.2	0.18	**16.0**	**5.9**	**22.23**	1.2	0.1
Borneol	**15.0**	**3.5**	-	**9.8**	2.13	**6.2**	-	**8.5**	0.6	1.4
cis-Carveol	0.3	-	-	-	-	0.4	0.1	0.12	-	-
cis-Chrysanthenyl acetate	-	-	Tr.	-	-	0.6	0.2	-	-	-
Terpinen-4-ol	2.5	**6.2**	1.1	2.8	-	3.1	2.6	1.91	0.7	1.8
Ascaridole	-	-	**47.2**	-	-	-	-	-	-	-
(*E/Z*)-Nerolidol	1.0	-	Tr.	-	-	2.4	**9.5**	-	**4.7**	-
Spathulenol	0.1	-	-	-	-	0.7	-	0.54	-	-
β-Caryophyllene	0.4	1.9	0.4	**5.2**	**6.06**	2.1	2.1	1.95	**5.4**	**22.2**
Germacrene D	-	-	Tr.	0.8	**8.4**	2.4	-	**8.50**	2.7	**11.2**
Caryophyllene oxide	1.6	1.4	0.2	**20.0**	-	2.3	**4.7**	**3.80**	1.2	0.8
Chamazulene	-	1.6	-	-	**5.14**	-	-	-	**12.7**	**24.1**
α-Bisabolol	-	-	0.1	-	-	**5.5**	-	-	0.7	-
α-Copaene	-	-	-	-	-	-	**11.1**	-	0.3	-

*YU - Yugoslavia [9], CA - Canada [11], GR1 - Greece [27],GR2 - Greece [6], CU - Cuba [13], RU - Russia [8], KZ - Kazakhstan [26], IR - Iran [24], EE - Estonia [25], DE - Germany [12].

**The value represents the sum of sabinene and pinene content.

Tr.: traces.

- The compound was not detected in the essential oil.

EXPERIMENTAL

Plant Material. The aerial parts of *A. clavennae* and *A. holosericea* were collected in July 2002 from Galicica mountain, Ohrid, Macedonia, at an altitude of 1500 m and 1600 m, respectively, and deposited in the BEOU Herbarium, Faculty of Biology, University of Belgrade, under voucher numbers 16026 and 16027. The aerial parts of *A. lingulata* were collected in June 2003 at the highland pastures of the mountain Stara Planina, eastern Serbia, at an altitude of 1600 m and deposited in the BEOU Herbarium under voucher number 16031. The aerial parts of *A. millefolium* were collected in July 2003 at the banks of the river Nisava, urban surroundings, eastern Serbia, and deposited in the BEOU Herbarium under voucher number 16033. All species were identified by Dr. Vladimir Randelovic, Faculty of Sciences and Mathematics, Department of Biology, University of Nis.

Isolation of Essential Oils. The above-ground parts of the species were used for the analysis of the essential oil composition. 1 kg of dried ground plant material was subjected to hydrodistillation for 2.5 h using a Clevenger-type apparatus, and the oils were dried over anhydrous $MgSO_4$ and kept at 4°C until analysis.

GC/MS. The GC/MS analysis of the oils was performed using a Hewlett-Packard 5890 series II gas chromatograph equipped with an SPB1 (30 m × 0.25 mm i.d., 0.25 μm film thickness) or DB-5 (30 m × 0.2 mm i.d., 0.25 μm film thickness) fused silica capillary column directly coupled to a mass selective detector MSD 5971A of the same company which was operated in the EI mode (70 eV). Helium was the carrier gas at a flow rate of 1 mL/min. The injector was operated at 250°C and the oven temperature was programmed as follows: isothermal 50°C for 3 min, then gradually increased to 250°C at 5°C/min and finally isothermal at 250°C for 15 min. GC analysis was performed under the same conditions as GC/MS. The volume injected was 0.1 μL of 10% solution (diluted in diethyl ether).

Identification Procedure. The linear retention indices on both capillary columns (SPB1 and DB-5) for all of the compounds were determined by co-injection of the sample with a solution containing the homologous series of C_8–C_{25} *n*-alkanes. Individual identification of components was based on comparison of their mass spectra with those of the Wiley 275.1 MS library and those described by Adams [21], as well as on comparison of their retention indices according to Van Den Dool [22] with literature values [21, 23] and, wherever possible, by co-injection with an authentic sample. The area percentage was obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

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