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A GC/MS Profile of the Volatile Constituents of the Aerial Parts of *Artemisia abrotanum* L. (Asteraceae) from Serbia

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ABSTRACT

The diethyl ether extract of the aerial parts of *Artemisia abrotanum* L. was analyzed by GC and GC/MS. The main identified constituents were silphiperfol-5-en-3-one A (14.6 %), ascaridole (13.1 %), 1,8-cineole (10.5 %), α-bisabolol oxide A acetate (8.7 %), germacrene D (6.5 %) and borneol (6.0 %).

KEYWORDS

***Artemisia abrotanum* L. (Asteraceae), diethyl ether extract, volatiles, silphiperfol-5-en-3-one A, ascaridole, triquinanes.**

Artemisia abrotanum L. (southernwood) is a highly fragrant species, frequently used as spice and for medicinal purposes.[1] Up to now *A. abrotanum* was mainly investigated for the presence of the following secondary metabolites: coumarins, flavonols, sesquiterpene derivatives and phenolic acids, many of which are of pharmacological significance. For example, spasmolytic flavonols have been isolated from the methanol extract of southernwood as the principals primarily responsible for the observed smooth muscle relaxing activity of this plant.[2] Polyphenolcarboxylic acids could be considered responsible for the choleretic and chalogogic activity of *A. abrotanum* herba.[3,4] A nasal spray formulation containing a mixture of *A. abrotanum* essential oil and flavonols appears to be clinically useful and suitable for treating allergic rhinitis and other upper airway disorders.[5] It has been confirmed that the extracts of *A. abrotanum* are active against different pathogens: *Malassezia* spp., *Candida albicans* and *Staphylococcus aureus,*[6] as well as against *Naegleria fowleri*.[7] In addition, the toluene extract of southernwood exerted strong arthropod repellency, and it was found that coumarin derivatives and thujyl alcohol were the most potent repellant constituents.[8] Although *A. abrotanum* is rich in essential oils, its volatile components have been the subject of only a limited number of studies.[1,9,10] Thus, the aim of this work was to analyze, using GC and GC/MS, the diethyl ether extract of the aerial parts of *A. abrotanum* collected in Serbia. These results would be the first step in determining whether any of the southernwood volatile constituents could be related to its popular ethnopharmacological usage.

GC and GC/MS analyses of the diethyl ether extract of the aerial parts of *A. abrotanum* enabled the identification of 39 components representing 91.0 % of the total GC peak areas (Table 1 and Fig. 1). Terpenoids, the most abundant class of compounds (more than four-fifths of volatiles in the extract), were almost evenly distributed among mono- and sesquiterpenoids. The oxygenated derivatives, in both terpenoid fractions, were predominant (oxygenated to hydrocarbon component ratios, within the mono- and sesquiterpenoids, were 8.8:1 and 4.1:1, respectively). The major contributors were silphiperfol-5-en-3-one A (14.6 %), ascaridole (13.1 %), 1,8-cineole (10.5 %), α-bisabolol oxide A acetate (8.7 %), borneol (6.0 %) and germacrene D (6.5 %). Among the sesquiterpenoids, the structures with triquinane (32.8 %), bisabolane (8.7 %) and germacrene (6.7 %) skeletons were repeatedly encountered, while p-menthane (31.5 %) and bornane-type (11.0 %) compounds were the most abundant among the identified monoterpenoids.

Comparison of the chemical composition of the extracts and essential oils obtained from *A. abrotanum* collected in different countries suggests that the southernwood cultivated in Serbia could be considered as a new chemotype.[1,9,10] Although most of the compounds listed in Table 1 were already identified as southernwood volatiles, their identity or distribution did not match the volatiles' profile of any previously analyzed *A. abrotanum*. For example, there is much similarity between the chemical compositions of the oil and extract of Italian and Serbian *A. abrotanum* (high levels of ascaridole, 1,8-cineole and bisabolane-type sesquiterpenoids). However, volatile triquinanes, one of the dominant compound classes in the Serbian sample (32.8 %), were not even found in trace amounts by Mucciarelli *et al.*[10] In contrast, while the triquinanes were present in high percentage in the sample from Poland, along with 1,8-cineole, no ascaridole was detected.[1]

A typical GC/MS chromatogram of the diethyl ether extract is shown in Fig. 1.

In addition, while the davanone-type sesquiterpenoids were among the major constituents of the essential oil and both solvent extracts (hexane and methanol) obtained from *A. abrotanum* from Poland, they were absent from the present (Serbian) extract.[1] In the oil investigated by Vostrowsky *et al.* no triquinanes nor ascaridole were found, but the davanones as well as a series of geranyl esters were present.[9] Environmental, ecological (e.g. collection site, season, etc.) and genetic factors could be responsible for the observed differences in the volatile profiles of *A. abrotanum* samples originating from different countries.

For a number of identified extract constituents, biological or pharmacological activity has been previously confirmed. It was shown that monoterpene peroxide ascaridole possesses strong anthelmintic properties.[11] 1,8-Cineole, camphor, borneol and eugenol are well-known antimicrobial agents, active against a number of human pathogens.[12] Crude extracts of southernwood, as well as some of its pure constituents were previously tested *in vitro* for antimalarial activity against *Plasmodium*

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Table 1 Composition of the diethyl ether extract of *Artemisia abrotanum* L.

RI[1]	Compound	%	Class	Identification
852	(*E*)-3-Hexenol[2]	0.2	O	*a,b*
952	Camphene	0.9	MB	*a,b,c*
1019	α-Terpinene	0.7	MM	*a,b*
1026	p-Cymene	2.9	MM	*a,b,c*
1034	1,8-Cineole	10.5	MM	*a,b,c*
1046	Phenylacetaldehyde	0.2	O	*a,b,c*
1069	*cis*-Sabinene hydrate	0.5	MT	*a,b*
1103	*trans*-Sabinene hydrate	0.8	MT	*a,b*
1117	2-Phenyl-1-ethanol	0.3	O	*a,b,c*
1149	Camphor	3.5	MB	*a,b,c*
1170	Borneol	6.0	MB	*a,b,c*
1181	Terpinen-4-ol	tr	MM	*a,b,c*
1194	α-Terpineol	0.1	MM	*a,b,c*
1211	*trans*-Piperitol	0.6	MM	*a,b*
1221	*cis*-Sabinene hydrate acetate[2]	0.3	MT	*a,b*
1226	2α-Hydroxy-1,8-cineole[2]	tr	MM	*a,b*
1243	Ascaridole	13.1	MM	*a,b,c*
1259	*cis*-Piperitone epoxide[2]	0.6	MM	*a,b*
1261	*trans*-Piperitone epoxide[2]	0.3	MM	*a,b*
1289	Isobornyl acetate[2]	0.6	MB	*a,b*
1303	Carvacrol	0.6	MM	*a,b,c*
1309	Isoascaridole (*syn.* 1,2:3,4-diepoxy-p-menthane)	2.1	MM	*a,b*
1329	Silphiperfol-5-ene	0.5	STQ	*a,b*
1348	7-*epi*-Silphiperfol-5-ene[2]	0.2	STQ	*a,b*
1360	Eugenol	0.2	O	*a,b,c*
1425	β-Caryophyllene	0.6	SCAR	*a,b,c*
1486	Germacrene D	6.5	SGER	*a,b*
1501	Bicyclogermacrene	0.2	SGER	*a,b*
1511	Silphiperfolan-6α-ol[2]	0.5	STQ	*a,b*
1528	Presilphiperfolan-9α-ol[2]	4.8	STQ	*a,b*
1554	Silphiperfol-5-en-3-one B	1.6	STQ	*a,b*
1561	Silphiperfol-5-en-3-ol A	1.0	STQ	*a,b,c*
1578	Silphiperfol-5-en-3-one A	14.6	STQ	*a,b*
1591	Caryophyllene oxide	1.6	SCAR	*a,b,c*
1751	Bisabolol oxide A (pyranoid)[2]	tr	SB	*a,b*
1825	Unidentified I[3]	0.2		
1962	Scopoletin (*syn.* 6-metoxy-7-hydroxycoumarin)	1.6	CD	*a,b*
2041	Unidentified II[4]	2.9		
2052	α-Bisabolol oxide A acetate[5]	8.7	SB	*a*
2096	Isofraxidin (*syn.* 6,8-dimethoxy-7-hydroxycoumarin)	3.3	CD	*a,b*
2187	Unidentified IV[6]	0.6		
2900	Nonacosane	0.3	O	*a,b,c*
	Total	94.7		
	Monoterpenoids	44.1		
	Oxygenated	39.6		
	Hydrocarbons	4.5		
	p-Menthane (MB)	31.5		
	Bornane and camphane (B)	11.0		
	Thujane (MT)	1.6		
	Sesquiterpenoids	40.8		
	Oxygenated	26.7		
	Hydrocarbons	8.0		
	Triquinane (STQ)	32.8		
	Bisabolane (SB)	8.7		
	Caryophyllene (SCAR)	2.2		
	Germacrane and related (SGER)	6.7		
	Coumarin derivatives (CD)	4.9		
	Others (O)	1.2		

[1]Compounds listed in order of elution on HP-5MS column (RI – experimentally determined retention indices on the mentioned column by co-injection of a homologous series of *n*-alkanes C_8–C_{29}).
[2]Reported as *A. abrotanum* constituent for the first time.
[3]MS (EI, 70 eV), m/z (Ir, %): 248(10), 220(22), 205(38), 191(33), 177(35), 163(21), 159(17), 151(36), 149(33), 135(26), 121(24), 109(21), 107(21), 105(21), 98(27), 91(36), 79(23), 77(28), 67(15), 65(16), 55(22), 43(100).
[4]MS (EI, 70 eV), m/z (Ir, %): 218(1), 203(1), 185(41), 143(39), 137(12), 125(52), 109(31), 95(9), 81(13), 71(16), 55(15), 43(100).
[5]MS (EI, 70 eV), m/z (Ir, %): 218(1), 203(3), 185(48), 143(56), 137(7), 134(10), 125(76), 119(13), 109(16), 107(20), 93(14), 91(23), 81(12), 79(12), 77(14), 55(11), 43(100).
[6]MS (EI, 70 eV), m/z (Ir, %): 218(1), 185(59), 143(45), 125(64), 109(29), 81(15), 71(22), 55(20), 43(100).
tr – trace (<0.05 %); *syn* – synonym; *a* – constituent identified by mass spectra comparison; *b* – constituent identified by retention index matching; *c* – constituent identity confirmed by co-injection of an authentic sample.

falciparum. Two pure compounds were found to be active: the sesquiterpenoid α-bisabolol A oxide acetate and coumarin isofraxidin.[13] α-Bisabolol oxide A acetate was one of the main constituents of Serbian southernwood observed in this paper. Related bisabolene-type sesquiterpenes, bound as esters or coumarin-conjugates were also previously reported as *A. abrotanum* constituents,[14] but were not detected in this study, probably due to the lack of volatility under the experimental conditions used. Isofraxidin, along with another coumarin derivative, scopoletin, represented a significant volatile portion of the analyzed extract. These constituents are among those repeatedly identified in the genus *Artemisia,*[15] and they are recognized as the active choleretic principals of *A. abrotanum.*[16] This suggests an additional confirmation that a great deal of the recognized southernwood pharmacological activity is due to the presence of its volatile constituents.

As already mentioned, volatile triquinanes were the most abundant compound class of the sesquiterpenoid fraction of *A. abrotanum* extract. Constituents with this skeleton-type were previously reported from more then 60 plant genera, mainly from the Asteraceae (Compositae) family, with a few rare exceptions outside this family.[17] It is noteworthy that in all cases where the triquinanes were reported for species not belonging to the Compositae (just 4 of more than 100 investigated taxa), they were not isolated in a pure state from the plant material and were present only as minor or trace constituents, so the possibility of their misidentification cannot be excluded. Thus, it seems that the angular and propellane triquinane sesquiterpenoids could be restricted in higher plants to the Asteraceae family alone (supported yet again by this study).[17] Among the taxa belonging to the genus *Artemisia,* besides in *A. abrotanum,*[1] up to now, the triquinanes were reported as constituents of *A. laciniata,*[18,19] *A. vulgaris,*[12] *A. alba,*[20] *A. iwayomogi,*[21] *A. cantabrica*[22] and *A. chamaemelifolia.*[23] According to the available literature data,[1,12,18–23] it seems that, within the genus *Artemisia,* the triquinanes are produced by the species belonging to the subgenus *Artemisia.*

Experimental

Aerial parts of the plant were collected from a cultivated population of *A. abrotanum,* in the vicinity of the Soko Banja Spa (southeastern Serbia), in July 2008. Voucher specimens were deposited in the Herbarium of the Institute of Botany and Botanical Garden 'Jevremovac', University of Belgrade (BEOU) under the acquisition number 16272.

Fresh above-ground parts of *A. abrotanum* (18 g) were cut into small pieces and extracted in a sealed vessel with 100 mL of diethyl ether in an ultrasonic bath (Bandelin Electronics, Berlin, Germany) for 1 h at room temperature. The extract was gravity filtered through a small column packed with 1 g of Celite® (Merck, Darmstadt, Germany), to remove all insoluble material, and then concentrated to 10 mL at room temperature using a stream of nitrogen before GC and GC/MS analysis. The yield of dry extract, obtained by complete evaporation of the solvent *in vacuo,* was 430 mg (2.4 % m/m).

The GC/MS analyses (three repetitions) were carried out using a Hewlett-Packard 6890N gas chromatograph (Palo Alto, CA, USA) equipped with a fused silica capillary column HP-5MS (5 % phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm) (Agilent Technologies, Palo Alto, CA, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 °C and 300 °C, respectively. The oven temperature was raised from 70 to 290 °C at a heating rate of 5 °C min^{-1} and then held isothermally for 10 min. Helium was used as a carrier gas at 1.0 mL min^{-1}. The sample,

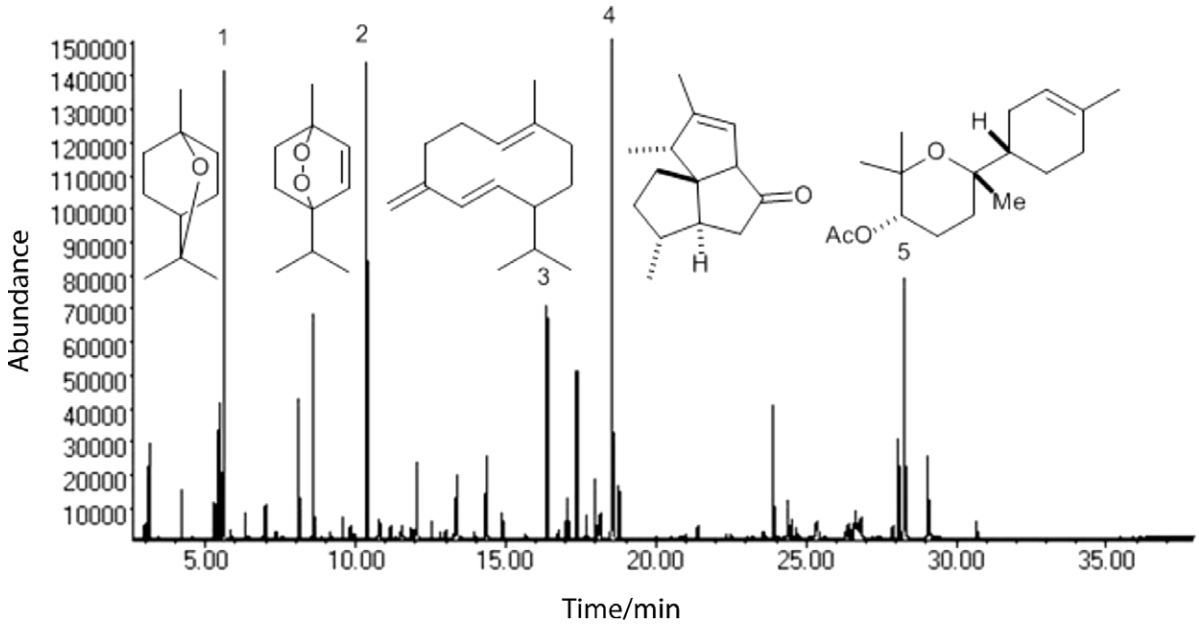

Figure 1 Total ion chromatogram of the diethyl ether extract of the aerial parts of *Artemisia abrotanum* from Serbia with the structural formulae of the main components (labels 1–5 correspond to 1,8-cineole, ascaridole, germacrene D, silphiperfol-3-en-5-one A and α-bisabolol oxide A acetate, respectively).

prepared as previously mentioned, was injected in a pulsed split mode (the flow was 1.5 mL min^{-1} for the first 0.5 min and then set to 1.0 mL min^{-1} throughout the remainder of the analysis; split ratio 40:1). MS (electron ionization, EI) conditions were as follows: ionization voltage 70 eV, acquisition mass range 35–500, scan time 0.32 s. Extract constituents were identified by comparison of their linear retention indices (relative to C_8–C_{29} alkane on the HP-5MS column) with literature values and their mass spectra with those of authentic standards, as well as those from Wiley 6/NIST02,[24] MassFinder 2.3[25] and a homemade MS library with the spectra corresponding to pure substances, and wherever possible, by co-injection with an authentic sample.[26,27] GC (FID) analysis was carried out under the same experimental conditions using the same column as described for the GC/MS experiments. The percentage composition of the extract was computed from the GC peak areas without any corrections.

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References

1 R. Kowalski, J. Wawrzykowski and G. Zawislak, *Herba Pol.,* 2007, **53**, 246–254.
2 O. Bergendorff and O. Sterner, *Planta Med.,* 1995, **61**, 370–371.
3 L. Swiatek, B. Grabias and D. Kalemba, *Pharmaceut. Pharmacol. Lett.,* 1998, **8**, 158–160.
4 C. Gird, R. Flutur and R. Sandu, *Farmacia,* 2006, **54**, 26–34.
5 P. Remberg, L. Bjork, T. Hedner and O. Sterner, *Phytomedicine,* 2004, **11**, 36–42.
6 K. Brodin, H. Alahayar, T. Hedner, O. Sterner and J. Faergemann, *Acta Derm.-Venereol.,* 2007, **87**, 540–542.
7 J. Mendiola, M. Bosa, N. Perez, H. Hernandez and D. Torres, *Trans. Roy. Soc. Trop. Med. Hyg.,* 1991, **85**, 78–79.
8 H. Tunon, W. Thorsell, A. Mikiver and I. Malander, *Fitoterapia,* 2006, **77**, 257–261.
9 O. Vostrowsky, K. Michaelis, H. Ihm and K. Knobloch, *Z. Lebensm. Unters. For.,* 1984, **179**, 125–128.
10 M. Mucciarelli, R. Caramiello, F. Chialva and M. Maffei, *Flavour Frag. J.,* 1995, **10**, 25–32.
11 R. Donkers and M. Workentin, *Chem. Eur. J.,* 2001, **7**, 4012–4020.
12 P. Blagojevic, N. Radulovic, R. Palic and G. Stojanovic, *J. Agric. Food Chem.,* 2006, **54**, 4780–4789.
13 B. Cubukcu, D. Bray, D. Warhurst, A. Mericli, N. Ozhatay and G. Sariyar, *Phytother. Res.*, 1990, **4**, 203–204.
14 A. Mericli, B. Cubukcu, J. Jakupovic and N. Ozhatay, *Planta Med.,* 1988, **54**, 463–464.
15 M. Mucciarelli and M. Maffei, in *Artemisia, Medicinal and Aromatic Plants – Industrial Profiles,* (C.W. Wright, ed.), Taylor and Francis, London, UK, 2002, pp. 24–28.
16 O. Nieschulz and P. Schmersahl, *Arzneim.-Forsch.,* 1968, **18**, 1330–1336.
17 N. Radulovic, M. Misic, G. Stojanovic and R. Palic, *36th International Symposium on the Essential Oils, Book of Abstracts,* L-2-07, Budapest, Hungary, 2005, p. 18.
18 P. Wayerstahl, H. Marschall, M. Schroder, H. Wahlburg and V. Kaul, *Flavour Frag. J.,* 1977, **12**, 315–325.
19 P. Wayerstahl, H. Marschall-Wayerstahl, M. Schroder, J. Brendel and V. Kaul, *Phytochemistry,* 1991, **30**, 3349–3352.
20 P. Blagojevic, M. Jovanovic, R. Palic and G. Stojanovic, Changes in the volatile profile of the *Artemisia lobelii* All. during prolonged plant material storage, *J. Essent. Oil Res.,* RN-2654, accepted for publication.
21 H. Yu, Y. Kim, B. Kil, K. Kim, S. Jeong and Y. You, *Planta Med.* 2003, **69**, 1159–1162.
22 A. San Feliciano, J.M. Del Corral, E. Caballero, A. Alvarez and M. Medarde, *J. Nat. Prod.,* 1986, **49**, 845–853.
23 A. Trendafilova-Savkova, M. Todorova and C. Gussev, *Z. Naturforsch. C,* 2003, **55**, 817–819.
24 F.W. McLafferty and D.B. Stauffer, *Wiley Registry of Mass Spectral Data,* 6th electronic edition with NIST02, John Wiley & Sons, New York, NY, USA, 2004.
25 D.H. Hochmut, W.A. Konig and D. Joulain, *Mass Finder 2.3,* Software and Data Bank, Hamburg, Germany. Available at http://www.massfinder.com
26 H. Van Den Dool and P. Kratz, *J. Chromatogr.,* 1963, **11**, 463–471.
27 R. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry,* 4th edn., Allured Publishing Corporation, Carol Stream, IL, USA, 2007.