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CHEMICAL COMPOSITION OF *ASTER ALBANICUS* DEG. (ASTERACEAE) ESSENTIAL OIL: TAXONOMICAL IMPLICATIONS

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Abstract: The composition of essential oil isolated from the areal parts of *Aster albanicus* Deg, an endemic species of the central Balkans, was analyzed. In total, 111 compounds were identified, representing 98% of the essential oil. The essential oil was dominated by sesquiterpene (69.3%) and monoterpene hydrocarbons (15.9%), with germacrene D as the most abundant compound (34.7%). Several multivariant statistical methods (HCA, NJ, PCoA) were deployed to infer the relation between *A. albanicus* and other species belonging to this genus. Taxonomical implications are discussed.

Key words: *Aster albanicus;* essential oil composition; chemotaxonomy; sesquiterpenes; monoterpenes

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INTRODUCTION

The genus *Aster* L. (Asteraceae) is comprised of ca.180 Eurasian species, 17 in SE Africa and 1 in N America (Mabberley, 2008). In the flora of Europe, ca. 30 species are recognized. *Aster albanicus* Deg. is an endemic species growing wild in the mountain border between Serbia and Albania (Tutin et al., 1976). Traditional treatments place most species in the genus *Aster* L. According to the latest analyses of morphology, chloroplast DNA RFLP and ITS sequence data, as well as karyotype studies, species of *Aster* are polyphyletic and members of a number of very distinct phylads within the tribe (Lane, 1982; Guy L. Nesom, 1994; Semple et al., 1996; Semple et al., 2001; Noyes and Rieseberg, 1999; Brouillet et al., 2001).

Essential oils have been previously used in the chemotaxonomy of conifers (Šarac et al., 2013; Rajčević et al., 2013) and angiosperms (Harborne and Turner, 1984; Pérez et al., 2000; Skaltsa et al., 2001; Maggio et al., 2012). Thus, further chemical investigation of *Aster* including essential oil (terpenoids) composition and distribution (Tsankova and Bohlmann, 1983; Bohlmann et al., 1985; Chung et al., 1993) show potential chemotaxonomical significance. The composition of the essential oil of *Aster albanicus* Deg. was not previously investigated. Our results in combination with the available data might prove helpful in future infrageneric classificatory schemes of this genus.

MATERIALS AND METHODS

Plant material

Aerial parts were collected from the population of *Aster albanicus* Deg. (Asteraceae) growing wild on Mt. Rogozna, north of Kosovska Mitrovica, during the autumn 2007-2008. A voucher specimen has been deposited at the Herbarium of the Institute of Botany, University of Belgrade, Faculty of Biology (BEOU). Crude essential oil was obtained by 2-h simultaneous distillation-extraction (SDE) in a Likens-Nickerson-type apparatus (Likens and Nickerson, 1964; Chaintreau, 2001) from dried areal parts of the plant (50 g). The volatiles were collected in CH_2Cl_2.

GC/GC-MS (gas chromatography-mass spectrometry)

Analysis was performed on an Agilent 7890A GC system equipped with 5975C MSD and FID, using DB-5 MS column (30 m × 0.25 mm × 0.25 µm). Injection volume was 1 µL and injector temperature was 220°C with a 10:1 split ratio. Carrier gas (He) flow rate was 1.0 ml/min at 210°C (constant pressure mode). Column temperature was linearly programmed in a range of 60-240°C at a rate of 3°C/min. The transfer line was heated at 240°C. The FID detector temperature was 300°C. EI mass spectra (70 eV) were acquired in m/z range of 30-550. A library search and mass spectral deconvolution and extraction were performed using NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software version 2.64.113.71, using retention index (RI) calibration data analysis parameters with a "strong" level and 10% penalty for compounds without an RI. The retention indices were experimentally determined using the standard method involving retention times of *n*-alkanes, injected after the essential oil under the same chromatographic conditions. The search was performed against our own library, containing 4972 spectra. Percentage (relative) of the identified compounds was computed from GC peak area.

Statistical procedures

These are indicated in the results and figure captions article.

Table 1. Relative abundances of compounds in essential oil of *Aster albanicus* Deg. obtained by SDE.

No	KI[a)]	Compound	[%][b)]
1	773	Hexanal	0.1
2	836	Furfural	0.2
3	840	Isovaleric acid	*tr*
4	855	2(*E*)-Hexanal	0.7
5	859	3(*Z*)-Hexenol	0.1
6	871	*n*-Hexanol	0.3
7	879	4(*Z*)-Heptanal	*tr*
8	912	2-Acetylfuran	*tr*
9	915	(2*E*,4*E*)-Hexadienal	*tr*
10	920	Tricyclene	*tr*
11	923	α-Thujene	*tr*
12	939	α-Pinene	1.3
13	945	Camphene	*tr*
14	961	Benzylaldehyde	*tr*
15	967	5-methyl-Furfural	*tr*
16	975	Sabinene	0.2
17	979	β-Pinene	**11.9**
18	986	6-methyl-5-Hepten-2-one	*tr*
19	991	Myrcene	0.7
20	993	(*EE*)-2,5-heptafurnal	0.2
21	1005	α-Phellandrene	*tr*
22	1012	(2*E*,4*E*)-heptadienal	0.3
23	1020	δ-Car-2-ene	*tr*
24	1022	*o*-Cymene	*tr*
25	1030	β-Phellandrene	0.6
26	1039	Benzyl alcohol	*tr*
27	1037	(*Z*)-β-Ocimene	0.5
28	1487	Benzene acetaldehyde	0.1
29	1050	(*E*)-β-Ocimene	0.4
30	1059	γ-Terpinene	*tr*
31	1087	*cis*-Linalool oxide	0.1
32	1089	Terpinolene	0.1
33	1078	1-Nonen-4-ol	0.2
34	1097	Linalool	0.6
35	1101	*n*-Nonanal	0.2
36	1116	Penthyl ethyl alcohol	*tr*
37	1123	*trans*-*p*-Mentha-2,8-dien-1-o	*tr*
38	1137	Pseudo-cyclocitral	0.2
39	1141	*trans*-Pinocarveol	0.3
40	1150	*trans*-Verbenol	*tr*
41	1155	*trans*-Nonen-2-al	*tr*
42	1165	Pinocarvone	0.2
43	1170	*p*-Mentha-1,5-dien-8-ol	*tr*
44	1176	Terpinen-4-ol	0.2
45	1183	*p*-Cymen-8-ol	*tr*
46	1189	α-Terpineol	0.7
47	1196	Myrtenal	0.4
48	1250	*n*-Decenal	*tr*

Table 2 continued:

No	KI[a)]	Compound	[%][b)]
49	1251	ß-Cytrocitral	*tr*
50	1252	Nerol	*tr*
51	1253	Geraniol	0.1
52	1262	*cis*-Chrysanthenyl acetate	*tr*
53	1288	Indole	*tr*
54	1310	Unidentified	0.4
55	1318	(2*E*,4*E*)-Decadienal	*tr*
56	1338	δ-Elemene	0.3
57	1345	α-Cubebene	0.1
58	1351	Eugenol	*tr*
59	1353	α-Copaene	0.1
60	1385	(*E*)-β-Damascenone	0.5
61	1388	β-Cububene	0.6
62	1391	β-Elemene	0.7
63	1394	(*Z*)-Jasmone	*tr*
64	1409	α-Gurjunene	0.1
65	1419	Caryophyllene E	**10.9**
66	1432	β-Copaene	0.2
67	1433	γ-Elemene	0.2
68	1439	α-Guaiene	*tr*
69	1455	α-Humulene	2.5
70	1460	*allo*-Aromadendrene	0.7
71	1485	Germacrene D	**34.7**
72	1485	ß-Selinene	0.3
73	1494	*trans*-Muurola–4(14),5–diene	0.2
74	1500	Bicyclogermacrene	**3.2**
75	1500	α-Muurolene	0.6
76	1509	Germacrene A	0.9
77	1512	γ-Cadinene	0.5
78	1524	δ-Cadinene	2.1
79	1532	*trans*-Cadina-1,4-diene	0.1
80	1534	α-Cadinene	0.1
81	1548	α-Calacorene	*tr*
82	1561	β-Germacrene	**4.8**
83	1564	(*E*)-Nerolidol	*tr*
84	1570	Aromadendrene oxide (2)	0.2
85	1578	Spathulenol	1.3
86	1583	Caryophyllene oxide	1.3
87	1590	6-isopropenyl-4,8a-dimethyl-1,2,3,5, 6,7,8,8a-octahydro-Naphtalen-2-ol	0.2
88	1593	Viridiflorol	0.2
89	1594	Ledol	0.2
90	1608	Humulene epoxide II	0.3
91	1615	1,10-di-*epi*-Cubenol	0.2
92	1618	Juneol	0.2
93	1625	Muurola-4,10(14)-dien-1-beta-ol	1.0
94	1642	*epi*-α-Murrolol	1.3
95	1646	α-Muurolol	0.4
96	1651	β-Eudesmol	0.3
97	1652	α-Cadinol	1.5
98	1657	10,10-dimethyl4-acetil-tricy-clo[5,2,1,0(1,5)]decane	0.2
99	1661	Aristolene epoxide	0.3
100	1677	Germacra-4(15),5,10(14)-trien-1-alpha-ol	0.3
101	1680	Eudesma-4(15),7-dien-1-beta-ol	0.2
102	1693	Junicedranol	0.2
103	1711	Myristaldehyde	0.3
104	1741	Mint sulfide	*tr*
105	1760	Benzyl benzoate	0.2
106	1811	ß-Costol	0.3
107	1879	14-hydroxy-δ-Cadinene	*tr*
108	1949	Phytol	1.0
109	1967	Phytone	*tr*
110	1970	*m*-Tolyl isothiocyanate	*tr*
111	1972	Hexadecanoic acid	0.5
		Monoterpenes	18.5
		Monoterpene hydrocarbons	15.9
		Monoterpenes oxygenated	2.6
		Sesquiterpenes	79.3
		Sesquiterpene hydrocarbons	69.3
		Sesquiterpenes oxygenated	10.1
		Diterpenes	1.0
		Diterpenes oxygenated	1.0
		Others [c)]	3.7
		Unknown	0.4
		TOTAL	98.0

[a)] Kovats indices obtained experimentally using the standard method involving retention times of *n*-alkanes, injected after the essential oil under the same chromatographic conditions. [b)] Contents are given as percentages of the total essential oil composition; *tr*: trace (0.05<*tr*<0.10%); compounds with contents <0.05% are not listed; [c)] Others: aliphatic hydrocarbons, aliphatic aldehydes and alcohols, aliphatic acids and their esters and aldehydes, aromatic ester + aliphatic acid, alkyl aromatic alcohols, aryl esters of aromatic acid.

RESULTS AND DISCUSSION

The analysis of essential oil of *A. albanicus* Deg. (Table 1) showed 111 compounds, representing 98.0% of the oil. The essential oil consisted mainly of sesquiterpene hydrocarbons (69.3%) followed by monoterpene hydrocarbons (15.9%) and oxygenated sesquiterpenes (10.1%). The predominant compound was germacrene D (34.7%). Other representative compounds were β-pinene (11.9%), (*E*)-caryophyllene (10.9%), β-germacrene (4.8%) and bicyclogermacrene (3.2%).

The essential oils of only a few species of *Aster* have been previously investigated. The essential oils of *Aster poliothamnus, A. ageratoides* and *A. subulatus* are dominated by monoterpenes (M. Miyazawa and Kameoka, 1977; Tu et al., 2006; Mitsuo Miyazawa et al., 2008). The essential oil of *A. lanceolatus, A. handelii, A. tataricus* and *A. koraiensis* were dominated by sesquiterpenes (Table 2) (Xiao-ping and Xiaoping, 2006; Dias et al., 2009; Choi, 2012). Several other species, now belonging to other genera, were also previously studied. The species *A. hesperius* Gray (*Symphyotrichum lanceolatum* ssp. *hesperium* (Gray) Nesom – accepted name) had essential oil dominated by non-terpene components (alkanes, alcohols, aldehydes, etc) and monoterpenes (Tabanca et al., 2007), and *A. subulatus* Michx. (*Symphyotrichum subulatum* (Michx.) G.L. Nesom – accepted name) had essential oil dominated by monoterpenes (M. Miyazawa and Kameoka, 1977). *A. scaber* Thunb. (*Doellingeria scabra* (Thunb.) Nees. – accepted name), now excluded from the genus *Aster* and belonging to *Doellingeria* established by Nees (1832), had essential oil dominated by monoterpenes (Chung et al., 1993; Lee et al., 2012). *A. indicus* (*Kalimeris indica* (L.) Sch.Bip. – accepted name) had essential oil dominated by sesquiterpenes.

The genus *Symphyotrichum* belongs to the subtribe Symphyotrichinae G. L. Nesom (1994) and *Doellingeria* is an unplaced genus. On the other hand, the genus *Aster* belongs to the subtribe Asterinae (Cass.) Dumort (1827) of the tribe Astereae Cass. (1819) (G. L. Nesom and Robinson, 2007). In relation to other investigated species, the essential oils of species belonging to *Aster s.l.* varied, from those dominated by monoterpenes to those dominated by sesquiterpenes. There was no evident genus-related pattern in this domination. Furthermore, the composition of the essential oil of *A. albanicus* has the most similarities with *A. ageratoides*, even though sesquiterpenes dominated the former and monoterpenes the latter.

To infer similarities between samples, several statistical methods were deployed using the presence/absence of compounds in the essential oil (without taking into account the average abundances of the components). Cluster analyses using dif-

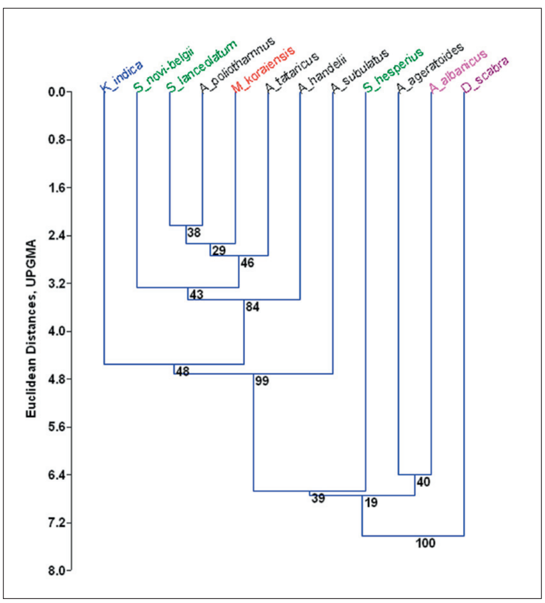

Fig 1. Cluster analysis based on presence/absence of components in essential oil of *Aster s.l.* species (Euclidean distances, UPGMA).

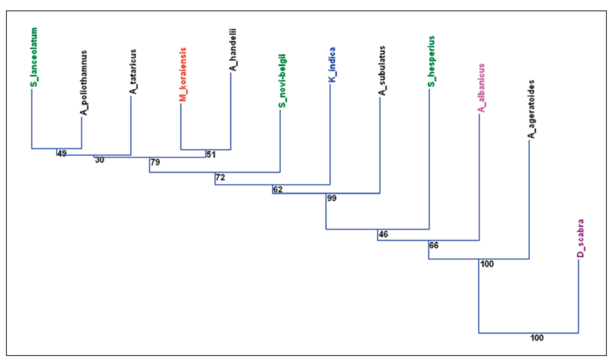

Fig 2. Neighbor joining tree based on presence/absence of components in essential oil of *Aster s.l.* species.

ferent distance measures (Euclidean, Jukes-Cantor, Gower) and UPGMA resulted in the same trees, with high bootstrap support (Fig. 1). The neighborhood joining tree with *D. scabra* used as an outgroup gave similar results, grouping *A. albanicus* and *A. ageratoides* close together and the rest of the species in a related subclade (Fig. 2). Parsimony analysis using the branch-and-bound algorithm and Wagner optimization resulted in several trees, from which the optimal one was chosen (i.e. the one with the highest bootstrap support) (Fig. 3). All cluster analyses gave similar results. *D. scabra* was always in a separate clade from the other species. Furthermore, *A. albanicus* and *A. ageratoides* were always grouped close together, next to all other *Aster* species, including species from other genera (e.g. *Symphyotrichum, Kalimeris* etc.). Species from genus *Symphyotrichum* never formed a separate group.

Principal coordinates analysis (PCoA) was also performed and it showed similar results. Four groups can be separated: (i) *D. scabra* and (ii) *S. hesperium* that formed separate groups, (iii) *A. albanicus* and *A. ageratoides* formed a third group, and (iv) all other species clustered close together into the fourth (Fig. 4).

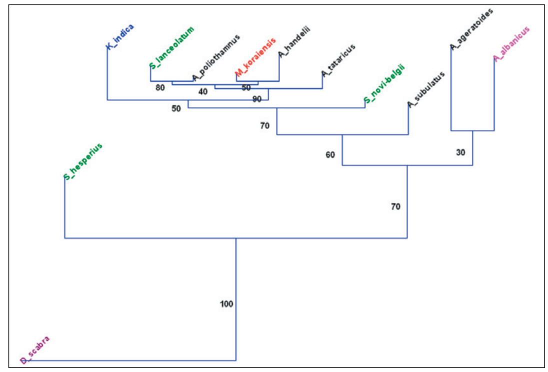

Fig 3. Parsimony analysis based on presence/absence of components in essential oil of *Aster s.l.* species.

According to all available data and the present results, we concluded that the relationship of *Aster s.str.* and other genera belonging to Astereae is unclear, and the systematics within the subtribe Asterinae in the sense of Nesom (2007) is still uncertain. Even though *D. scabra* was always separated, *Aster s.str.* and the species belonging to other genera, i.e. *Kalimeris, Miyamayomena, Symphyotrichum*, always grouped together, forming mixed subclades. However, more extensive study of the essential oil of *Aster* species, which should take into account both compounds and their relative abundances in essential oils, is necessary to enhance resolution and improve systematics within the subtribe Asterinae.

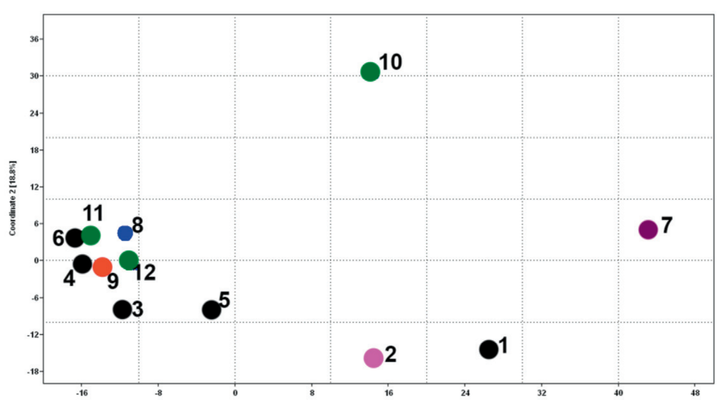

Fig 4. Principle Coordinate Analysis based on presence/absence of components in essential oil of *Aster* s.l. species. 1 – *Aster ageratoides,* 2 – *A. albanicus,* 3 – *A. handelii,* 4 – *A. poliothamnus,* 5 – *A. subulatus,* 6 – *A. tataricus,* 7 – *Doellingeria scabra,* 8 – *Kalimeris indica,* 9 – *Miyamayomena koraiensis,* 10 – *Symphyotrichum hesperium,* 11 –*S. lanceolatum,* 12 – *S. novi-beligii.*

Table 2. Dominant groups of compounds in essential oils of *Aster* s.l.

No.	Taxon	Dominant group	Reference
1	*Aster ageratoides* Turcz.	monoterpenes	(Mitsuo Miyazawa et al., 2008)
2	*Aster albanicus* Deg.	sesquiterpenes	Present work
3	*Aster handelii* Onno	sesquiterpenes	(Xiao-ping and Xiaoping, 2006)
4	*Aster poliothamnus* Diels.	monoterpenes	(Tu et al., 2006)
5	*Aster subulatus* (Michx.) Hort. ex Michx.	monoterpenes	(M. Miyazawa and Kameoka, 1977)
6	*Aster tataricus* L.f.	sesquiterpenes	(Choi, 2012)
7	*Doellingeria scabra* (Thunb.) Nees. *(=syn. Aster scaber* Thunb.*)*	monoterpenes	(Chung et al., 1993; Lee et al., 2012)
8	*Kalimeris indica* (L.) Sch.Bip. (=*syn. Aster indicus* L.)	sesquiterpenes	(Tsubaki et al., 1966)
9	*Miyamayomena koraiensis* (Nakai) Kitam. (=*syn. Aster koraiensis* Nakai)	sesquiterpenes	(Choi, 2012)
10	*Symphyotrichum hesperium (=syn. Aster hesperius)*	other[a)]	(Tabanca et al., 2007)
11	*Symphyotrichum lanceolatum* (Willd) G.L. Nesom *(=syn. Aster lanceolatus* Wild.)	sesquiterpenes	(Semple et al., 1996)
12	*Symphyotrichum novi-beligii (=syn. Aster novi-belgii)*	monoterpenes	(Ibrahim et al., 2006)

[a)] Other: aliphatic hydrocarbons, aliphatic aldehydes and alcohols, aliphatic acids and their esters and aldehydes, aromatic ester + aliphatic acid, alkyl aromatic alcohols, aryl esters of aromatic acid.

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Authors' contributions: NR is the main author, contributed with original data, designed all of the research, organized and analyzed data and wrote the manuscript. VV and LJV contributed to research and data analyses. ZK carried out the field research. PD was the main supervisor of the research project and reviewed several drafts of the manuscript. PJ contributed with original data and with design of all the research, was the main supervisor of the research project and reviewed several drafts of the manuscript. All authors read and approved the final manuscript.

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