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Five wild-growing *Artemisia* (Asteraceae) species from Serbia and Montenegro: Essential oil composition and its chemophenetic significance

MAJA RADULOVIĆ[1]*, NEMANJA RAJČEVIĆ[1], MILAN GAVRILOVIĆ[1], JELICA NOVAKOVIĆ[1], DANIJELA STEŠEVIĆ[2], PETAR D. MARIN[1] and PEĐA JANAĆKOVIĆ[1]

[1]*Department of Morphology and Systematics of Plants, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000, Belgrade, Serbia and* [2]*Department of Biology, Faculty of Natural Sciences and Mathematics, University of Montenegro, Džordža Vašingtona bb, 20000, Podgorica, Montenegro*

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Abstract: In this work, the essential oils (EOs) obtained by hydrodistillation from the aerial parts of five *Artemisia* species*: A. alba* Turra, *A. pontica* L., *A. scoparia* Waldst. & Kitam., *A. vulgaris* L., originating from Serbia and *A. umbelliformis* Lam. subsp. *eriantha* (Ten.) Vallès-Xirau & Oliva Brañas, originating from Montenegro were analyzed by gas chromatography coupled with mass spectrometry (GC/MS). In total, 91 compounds were detected, and 78 were identified. Even though a high number of compounds were detected, each sample had only 18 to 35, attesting to a great diversity of compounds within these taxa. Depending on the species and the locality (geographical origin), the EO was dominated by either monoterpenes or sesquiterpenes, with artemisia ketone, 1,8-cineole (eucalyptol), fragranol, *α*-thujone, *β*-thujone and myrcene being the dominant compounds. The obtained results were coupled with extensive literature data and used in multivariate chemometric approach to assess the chemophenetic significance of the EO.

Keywords: hydrodestilation; GC/MS; chemometrics.

INTRODUCTION

Artemisia L. (Artemisiinae, Anthemidae, Asteraceae) is a large genus that contains nearly 500 mostly perennial taxa. However, there is still no universal agreement on the number of taxa within the genus.[1] *Artemisia* taxa mainly grow in different ecosystems of the northern hemisphere in Asia, Europe, and North America[2], while a broad area of Central Asia is the center of their diversity.[3]

* Corresponding author. E-mail: maja.radulovic@bio.bg.ac.rs
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Artemisia taxa show large variability in morphological and phytochemical characters.[4] The systematics and nomenclature of the genus is complex, and it is a challenge for taxonomists. Although there are some conflicts between classical and molecular datasets, *Artemisia* has been traditionally divided into five subgenera: *Artemisia*, *Absinthium* (Miller) Less., *Dracunculus* (Besser) Rydb., *Seriphidium* Besser ex Less. and *Tridentatae* (Rydb.) McArthur[5], to which one more, *Pacifica* Hobbs & Baldwin, has been added.[6]

Literature data about the phytochemistry of different *Artemisia* species showed large structural diversity of specialized metabolites. These plants are very aromatic, with a characteristic pungent smell.[7] The specific aromatic odor is a consequence of a high quantity of volatile terpenes, primarily present in the flowers and leaves.[8] Monoterpenes and sesquiterpenes are dominant compounds in the essential oil (EO) of many species.[9–11]

Plant metabolic profile is genetically determined, thus similarity in metabolite content is applicable in assessing the phylogenetic relationship of higher plants.[12] It was shown that plant metabolites (*e.g.*, EOs) could be helpful in assessing the taxonomic relationship among some *Artemisia* taxa[8]. In this regard, continuing chemophenetic studies (description of the diversity of specialized metabolites in any given plant taxon)[13] contribute to the phenetic description of its taxa, and, in combination with other tools (*e.g.*, morphology, anatomy, molecular methods), could help in establishing natural classification of the genus *Artemisia*.

The objectives of the present study were to investigate and determine composition of the EOs of five wild-growing *Artemisia* species: four species from Serbia (*A. alba* Turra, *A. pontica* L., *A. scoparia* Waldst. & Kitam. and *A. vulgaris* L.), and one species from Montenegro (*A. umbelliformis* Lam. subsp. *Eriantha* (Ten.) Vallès-Xirau & Oliva Brañas); and to evaluate their significance in chemophenetics using a chemometric multivariate approach.

EXPERIMENTAL

Plant material

Plant material (aerial parts) of four species from Serbia and one species from Montenegro was collected during the growing season (Table S-I of the Supplementary material to this paper). The collected plant material in full bloom was identified using floras of Serbia and Europe.[14,15] Voucher specimens were deposited at the Herbarium (BEOU) of the University of Belgrade – Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac." Standard herbarium acronym follows Index herbariorum.[16]

Isolation of EO

The aerial parts of each plant species were dried at room temperature and then chopped. Between 26 and 340 g of plant material was placed in a round-bottomed flask and 800–2000 mL of distilled water was added. Hydrodistillation was performed for 3 h using a Clevenger-type apparatus, according to the procedure described in Ph. Eur. 6.[16] The obtained oils were

stored at 4 °C before GC analysis. The extraction yield of oil was calculated according to an earlier described equation.[8] For GC analysis, 10 μL of crude essential oil was dissolved in 1 mL of dichloromethane.

GC-FID and GC/MS analyses

The GC-FID and GC/MS analyses were performed with an Agilent 7890 A apparatus equipped with a 5975 C mass-selective detector (MSD), a flame ionization detector (FID), and an HP-5 MSI fused-silica cap (column length 30 m, diameter 0.25 mm, film thickness 0.25 mm). The oven temperature was programmed linearly, rising from 60 to 240° at 3° min^{-1}; the injector temperature was 220°; the detector temperature was 300°, and the transfer-line temperature was 240°. The carrier gas was He (1.0 mL min^{-1} at 210°, constant pressure mode) with an injection volume of 1 μL and a split ratio of 10:1. Electron impact mass spectra (EI-MS; 70 eV) were acquired over the *m*/*z* range 40–550. Library search and mass spectral deconvolution and extraction were performed using the NIST AMDIS (automated mass spectral deconvolution and identification system) software, version 2.64.113.71, with the retention index (*RI*) calibration data analysis parameters set to the strong level and a 10 % penalty for compounds without an *RI*. The *RI*s were experimentally determined using the standard method involving retention times (t_R) of *n*-alkanes injected after the EO under the same chromatographic conditions. The search was performed against our homemade library, containing 4972 spectra. The relative contents of identified compounds were computed from the GC peak areas.

Statistical analysis

Statistical analysis was performed on 3875 numerical data. Standard statistics (mean, standard deviation, distribution) were used to study the data prior to Discriminant Analysis (DA). All statistical analyses were performed using PAST 4.06b.[17]

RESULTS AND DISCUSSION

Artemisia *EO composition and yield*

The yield and organoleptic characteristics of the EOs of the studied *Artemisia* species are given in Table I.

TABLE I. Yield and organoleptic characteristics for essential oils of the investigated *Artemisia* species

Sample	*m* / g		Yield wt. %	Organoleptic characteristics
	Dry plant material	Obtained oil		
A. alba	31.8	0.0138	0.043	Transparent yellow, mild herbaceous sweet odor
A. pontica	32.2	0.0816	0.253	Transparent yellow, strong sharp bitter odor
A. scoparia	140.0	0.3181	0.227	Transparent yellow, strong, sour greasy odor
A. umbelliformis subsp. *eriantha*	26.6	0.1493	0.561	Transparent yellow, strong herbaceous sharp odor
A. vulgaris	340.0	0.1363	0.040	Bright yellow, strong unpleasant moldy odor

The conducted GC-FID and GC/MS analyses resulted in the detection of 91 compounds, making on average 96.7% of the total oil. All compounds are listed in Table II.

TABLE II. Chemical constituents of the essential oils of investigated *Artemisia* species; *RI*, retention indices relative to *n*-alkanes on HP-5 MS; %, Relative percentage obtained from the peak area; SO, sesquiterpene oxygenated; #, tentatively identified; Ni, not identified, Ni_1, *m/z* 41, 55, 70, 83, 97, Ni_2, *m/z* 81, 43, 109, 71 53, Ni_3, *m/z* 81, 41, 69, 135, 107, Ni_4, *m/z* 95, 147, 162, Ni_5, *m/z* 68, 43, 93, 108, 121, Ni_6, *m/z* 91, 43, 93, 119, 134, Ni_7, *m/z* 71, 43, 107, 93, 79, Ni_8, *m/z* 123, 71, 107, 81, 41, Ni_9 SO, *m/z* 157, 143, 218, 129, 91, Ni_{10} SO, *m/z* 216, 178, 159, 147, 95, Ni_{11}, *m/z* 109, 148, 175, 43, 193, Ni_{12} SO, *m/z* 217, 232, 171, 91, 105, Ni_{13} SO 214, 156, 115, 55, 171

No.	*RI*	Compound	Content, %				
			A. alba	*A. pontica*	*A. scoparia*	*A. umbelliformis* subsp. *eriantha*	*A. vulgaris*
1	865	(*Z*)-Salvene	–	–	–	0.4	–
2	903	Santolina Triene	–	–	–	–	0.3
3	922	α-Thujene	–	–	–	0.2	0.5
4	929	α-Pinene	–	–	0.7	–	0.5
5	944	Camphene	–	–	0.1	–	–
6	945	α-Fenchene	–	–	–	0.4	–
7	969	Sabinene	–	–	0.1	0.2	6.2
8	973	Artemiseole	0.3	–	–	–	–
9	973	β-Pinene	–	0.4	0.1	0.4	0.7
10	987	Myrcene	–	–	–	–	22.0
11	999	Yomogi alcohol	2.0	–	–	–	–
12	1015	α-Terpinene	–	–	–	–	0.4
13	1024	*p*-Cymene	0.8	1.3	0.9	0.2	1.3
14	1032	1,8-Cineole	12.2	58.2	57.2	0.3	6.2
15	1058	γ-Terpinene	–	0.4	0.1	0.1	–
16	1060	Artemisia ketone	45.3	–	–	–	17.6
17	1066	*cis*- Sabinene hydrate	–	0.2	–	0.2	–
18	1084	Artemisia alcohol	2.3	–	–	–	0.4
19	1094	Ni_1	–	–	–	0.2	–
20	1095	6,7-Epoxymyrcene	–	–	–	–	0.8
21	1101	*cis*-Sabinene hydrate (*cis* for IPP vs OH)	–	0.2	–	–	–
22	1107	Ni_2	0.7	–	–	–	–
23	1108	β-Thujone	–	6.8	34.5	73.7	–
24	1117	α-Thujone	–	0.7	3.8	15.8	–
25	1122	*cis*-*p*-Menth-2-en-1-ol	–	0.2	0.2	–	–
26	1130	Ni_3	7.7	–	–	–	–
27	1138	*trans*-Pinocarveol	–	0.2	0.4	–	–
28	1139	*iso*-3-Thujanol	–	–	–	0.1	–
29	1139	Monoterpenol	0.7	–	–	–	–
30	1139	*trans*-Sabinol	–	–	–	0.4	–
31	1143	*trans*-Verbenol	–	0.4	0.2	0.1	–

TABLE II. Continued

No.	*RI*	Compound	Content, %				
			A. alba	*A. pontica*	*A. scoparia*	*A. umbelliformis* subsp. *eriantha*	*A. vulgaris*
32	1148	Camphor	3.7	–	–	–	–
33	1149	*neo*-3-Thujanol	–	–	–	0.1	–
34	1155	Sabina ketone	–	–	–	0.2	–
35	1155	Isoborneol	–	–	0.1	–	–
36	1162	Pinocarvone	–	–	0.3	0.1	–
37	1165	*δ*-Terpineol	–	0.4	–	–	–
38	1166	Borneol	0.7	–	0.2	–	–
39	1171	Artemisyl acetate	0.4	–	–	–	–
40	1175	Terpinen-4-ol	1.0	1.0	0.3	0.3	1.3
41	1182	*cis*-3-Hexenyl butyrate	0.6	–	–	–	–
42	1193	Myrtenal	–	0.3	0.2	–	–
43	1197	Myrtenol	–	–	–	0.4	–
44	1202	*γ*-Terpineol	–	–	–	0.1	–
45	1213	Fragranol	–	14.7	–	–	–
46	1277	Ni_4	–	0.4	–	–	–
47	1291	*trans*-Sabinyl acetate	–	–	–	0.1	–
48	1337	*δ*-Elemene	–	–	–	0.1	–
49	1342	Ni_5	–	4.1	–	–	–
50	1375	*α*-Copaene	–	–	–	–	0.5
51	1384	*β*-Bourbonene	–	0.7	–	–	0.7
52	1391	*β*-Elemene	–	–	–	–	2.2
53	1414	Ni_6	–	–	–	0.2	–
54	1415	*trans*-*α*-Bergamotene	–	–	–	–	0.6
55	1419	(*E*)-Caryophyllene	–	–	–	–	3.1
56	1435	*cis*-α-Bergamotene	–	–	–	–	0.4
57	1453	*α*-Humulene	–	–	–	–	1.3
58	1456	(*Z*)-β-Farnesene	–	–	–	–	0.8
59	1460	Cabreuva oxide B	–	0.5	–	–	–
60	1477	Cabreuva oxide D	–	0.3	–	–	–
61	1476	*γ*- Muurolene	–	–	–	0.1	0.6
62	1481	Germacrene D	–	–	–	–	5.0
63	1484	*β*-Selinene	–	–	–	–	9.9
64	1495	*α*-Selinene	–	0.4	–	–	1.2
65	1496	Bicyclogermacrene	–	–	–	–	1.0
66	1523	*δ*-Cadinene	–	–	–	–	0.7
67	1553	$C_{15}H_{24}$ isomer#	–	–	–	–	0.6
68	1555	Ni_7	–	0.8	–	–	–
69	1563	(*Z*)-Nerolidol	–	0.7	–	–	–
70	1576	Spathulenol	–	1.1	–	–	3.5
71	1581	Caryophyllene oxide	–	0.6	0.9	–	4.6
72	1593	Ni_8	–	0.2	–	–	–
73	1608	Humulene epoxide II	–	–	–	–	1.3
74	1610	$C_{15}H_{24}O$ isomer#	–	–	–	–	0.7
75	1623	$C_{15}H_{22}O$ isomer#	0.8	–	–	–	–

TABLE II. Continued

No.	*RI*	Compound	Content, %				
			A. alba	*A. pontica*	*A. scoparia*	*A. umbelliformi* subsp. *eriantha*	*A. vulgaris*
76	1628	1-*epi*-Cubenol	–	–	–	0.1	–
77	1654	Pogostol	–	–	–	–	1.0
78	1670	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	–	–	–	–	0.8
79	1672	Valeranone	–	–	–	0.1	–
80	1675	Cadalene	0.3	–	–	–	–
81	1684	α-Bisabolol	1.0	–	–	–	–
82	1685	Germacra-4(15),5,10(14)-trien--1-α-ol	0.8	–	–	–	0.8
83	1691	Solavetivone	7.9	–	–	–	–
84	1713	Ni_9 SO	0.5				
85	1720	(8*S*,8*aS*)-3,8-Dimethyl-4-propan--2-ylidene-1,2,6,7,8,8*a*-hexahydroazulen5-one	1.1	–	–	–	–
86	1749	Cyclocolorenone	0.9	–	–	–	–
87	1788	Ni_{10} SO	0.4	–	–	–	–
88	1847	6,10,14-trimethyl-2-pentadecanone	0.3	–	–	–	–
89	1849	Ni_{11}	0.7	–	–	–	–
90	1945	Ni_{12} SO	–	–	–	1.4	–
91	1954	Ni_{13} SO	–	–	–	0.4	–
		Total monoterpenes	**69.2**	**85.2**	**98.9**	**93.7**	**58.3**
		Monoterpene hydrocarbons	3.0	1.9	1.8	1.9	31.9
		Monoterpenes oxygenated	66.1	83.2	97.0	91.7	26.4
		Total sesquiterpenes	**13.7**	**8.3**	**0.9**	**2.1**	**41.3**
		Sesquiterpene hydrocarbons	0.0	5.1	0.0	0.1	28.6
		Sesquiterpene oxygenated	13.7	3.2	0.9	1.9	12.6
		Other	0.9	0.0	0.0	0.0	0.0
		Unknown	9.0	1.3	0.0	0.4	0.0
		Total	**92.9**	**94.9**	**99.8**	**96.2**	**99.6**
		Total (No. of identified compounds)	**25**	**26**	**18**	**29**	**35**

Depending on the species and locality, different groups of volatile terpenes were present in the EOs. The results showed that monoterpenes are the dominant constituents in the EOs of all investigated species. The EOs of *Artemisia scoparia*, *A. umbelliformis* subsp. *eriantha* and *A. pontica* were strongly dominated by oxygenated monoterpenes (97.1, 91.7, and 83.2 %, respectively). In *A. vulgaris*, however, monoterpene hydrocarbons were found in a similar amount as oxygenated monoterpenes (31.9 and 26.4%, respectively).

Additionally, *A. vulgaris* was the only analyzed species with a high abundance of sesquiterpenes, with sesquiterpene hydrocarbons representing the greatest part (28.64 %). In the other species, sesquiterpenes were moderately present in the EOs,

and oxygenated sesquiterpenes were more common: *A. alba* – 13.8 %, and *A. vulgaris* – 12.7 %, the exception being *A. scoparia,* which had less than 1 % of sesquiterpenes present. The most dominant compound of *A. alba* was artemisia ketone (45.317 %), followed by 1,8-cineole (12.174 %), while in the oil of *A. pontica*, the most prevalent was 1,8-cineole (58.2 %), followed by fragranol (14.7 %). The EO of *A. scoparia* was characterized by an extremely high percentage of 1,8-cineole (57.2 %), followed by *β*-thujone (34.5%). In contrast, the EO of *A. umbelliformis subsp. eriantha* was characterized by *β*-thujone (73.7 %) and *α*-thujone (15.8 %). Myrcene (22.0 %) and artemisia ketone (17.6 %) were the most dominant constituents in the EO of *A. vulgaris*. The present data are in concordance with literature data.[1,8] Based on the obtained results, the EOs of *Artemisia* species can be put into two groups – those highly dominated with a single compound and those with two to three co-dominant compounds. All EOs of the studied species belong to the formergroup, except for *A. vulgaris* that belongs to the latter.

Chemophenetics of Artemisia *based on EO*

To check the chemophenetic significance, discriminant analysis, including 120 literature data,[8,18–24] was performed. Only compounds present on average in mid-to-high amounts (above 0.5%) of the essential oil profile were used. Species represented with only one or two data points were not assigned to a group but marked for the discriminant linear classifier and placed on the scatter plot after discriminant analysis (marked with an asterisk), Fig. 1. Three components were

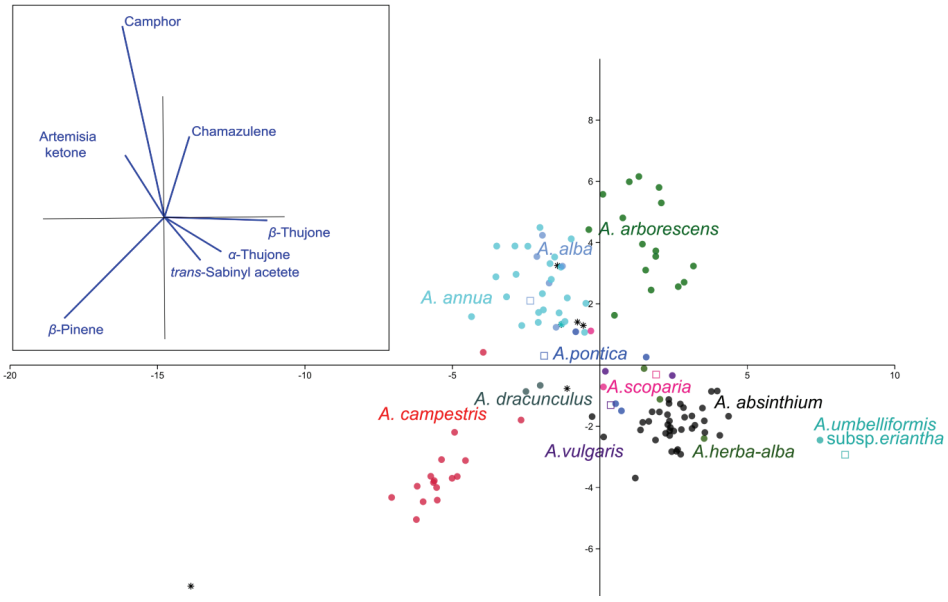

Fig. 1. Discriminant analysis (DA) scatter plot: ● – literature data, □ – present data, * –plotted after DA according to the results of discriminant linear classifier.

responsible for most of the separation species – camphor, *β*-pinene, and *β*-thujone. An additional four compounds contributed the most to the separation (artemisia ketone, chamazulene, *α*-thujone, and *trans*-sabinyl acetate). The present samples grouped according to the species and not the locality or the phenophase, with the exception of *A. pontica* that showed more similarity with *A. annua* and *A. absinthium* essential oil than with other *A. pontica* samples, though only several data points were available for this species, so that it can be an artifact caused by a low number of samples in the discriminant analysis.

CONCLUSIONS

The results are in agreement with a previous detailed chemometric analysis of *Artemisia* EO, where the variability detected in the EO composition of different taxa, attributed to both genetical and environmental factors, correspond to evolutionary trends and molecular data. The present results, although obtained from a rather limited number of investigated taxa, indicate that the EO composition could be a useful chemophenetic character within this genus.

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ИЗВОД

ПЕТ САМОНИКЛИХ ВРСТА *Artemisia* (ASTERACEAE) ИЗ СРБИЈЕ И ЦРНЕ ГОРЕ: САСТАВ ЕТАРСКОГ УЉА И ЊЕГОВ ХЕМОФЕНЕТИЧКИ ЗНАЧАЈ

МАЈА РАДУЛОВИЋ[1], НЕМАЊА РАЈЧЕВИЋ[1], МИЛАН ГАВРИЛОВИЋ[1], ЈЕЛИЦА НОВАКОВИЋ[1], ДАНИЈЕЛА СТЕШЕВИЋ[2], ПЕТАР Д. МАРИН[1] и ПЕЂА ЈАНАЋКОВИЋ[1]

[1]*Универзитет у Београду – Биолошки Факултет, Катедра за морфологију и систематику биљака, Студентски трг 16, 11000, Београд и* [2]*Департман за биологију, Универзитет Црне Горе – Природно–математички факултет, Џорџа Вашингтона бб, 20000, Подгорица, Црна Гора*

У овом раду анализирана су етарска уља добијена хидродестилацијом из надземних делова пет врста рода *Artemisia*: *A. alba* Turra, *A. pontica* L., *A. scoparia* Waldst. & Kitam., *A. vulgaris* L., из Србије и *A. umbelliformis* Lam. subsp. *eriantha* (Ten.) Vallès-Xirau & Oliva Brañas, пореклом из Црне Горе, коришћењем гасне хроматографије комбиноване са масеном спектрометријом (GC/MS). Укупно је детектовано 91 једињење, од чега је 78 идентификовано. Иако је укупно детектован велики број једињења, у сваком узорку је детектовано између 18 и 35 једињења, што сведочи о великом хемијском диверзитету етаских уља испитиваних таксона. У зависности од врсте и локалитета (географског порекла) етарским уљима су доминирали монотерпени или сесквитерпени, где су артемизија кетон, 1,8-цинеол (еукалиптол), фрагранол, *цис*-тујон, *транс*-тујон и мирцен били доминантна једињења. Добијени резултати су упарени са литературним и искоришћени у мултиваријантном хемометријском приступу како би се проценио хемофенетички значај етарског уља.

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