

Intraspecific variability in the flavonoid composition of *Artemisia vulgaris* L.

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Surface flavonoid profiles in forty populations of *Artemisia vulgaris* L. (Asteraceae) were analyzed. The major constituents observed in the leaf exudates were methylated flavonoid aglycones based mainly on quercetin. Three intraspecific flavonoid chemotypes were determined, the chryso-splenetin (quercetagenin 3,6,7,3'-tetramethyl ether) chemotype, the artemetin (quercetagenin 3,6,7,3',4'-pentamethyl ether) chemotype and chemotype without these two compounds. Most of the populations corresponded to these chemotypes.

Key words: *Artemisia vulgaris*, Asteraceae, flavonoid aglycones, chryso-splenetin, artemetin, chemotype

Introduction

Surface flavonoid aglycones are often used in chemotaxonomic studies on Asteraceae at the generic and species level (VALANT-VETSCHERA and WOLLENWEBER 1996, WOLLENWEBER et al. 1997, STEVENS et al. 1999). Recent articles showed that a flavonoid pattern could also be specific for a chemotype (REPČÁK et al. 1999, WILLIAMS et al. 2000, MARTONFI et al. 2001, VIEIRA et al. 2003).

The species of the genus *Artemisia* (Asteraceae) have been extensively surveyed for their surface flavonoid constituents (WOLLENWEBER et al. 1989, VALANT-VETSCHERA and WOLLENWEBER 1995, WOLLENWEBER and VALANT-VETSCHERA 1996). *Artemisia vulgaris* L. (Asteraceae) is a perennial polymorphic species, widespread in temperate areas (South Europe, North Africa, North America and Asia). Simple flavonol methyl ethers have been reported for West-European populations (VALANT-VETSCHERA et al. 2003) and flavone derivatives for Asian populations (LEE et al. 1998) of *A. vulgaris*.

The present paper is concerning with the surface flavonoid aglycones on 40 populations of *Artemisia vulgaris*. The sampling of populations from habitats with different conditions allowed us to assess the influence of environmental conditions on flavonoid synthesis and accumulation.

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Material and methods

Plant material was collected from natural habitats on 40 populations of *A. vulgaris* from Bulgaria, details of which are given in Table 1. The sampling sites were chosen to cover re-

Tab. 1. Voucher numbers (SOM) and collection sites of *A. vulgaris* populations. No – numbers of samples, m asl – meters above sea level

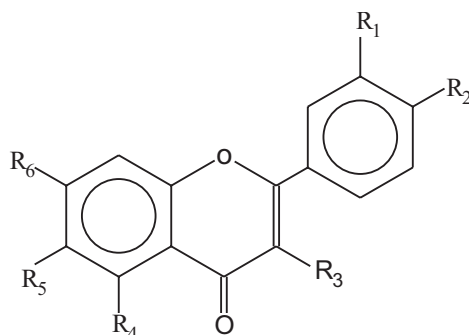
No	SOM	Altitude m asl	Origin of material, pollution impact
1	155134	800	Sofia region, metal work, industrial polluted
2	Co573	500	Sofia region, chemical work, industrial polluted
3	Co663	500	Sofia region, there is flying toxic fragments to air, industrial polluted
4	Co515	750	Vitosha mountain, background polluted
5	155136	500	Sofia, center, traffic crossing, traffic polluted
6	Co557	600	Sofia, housing district, traffic polluted
7	Co578	800	Vitosha mountain, background polluted
8	Co606	750	Lozen mountain, background polluted
9	Co577	1000	Vitosha mountain, background polluted
10	Co571	600	Sofia, airport, traffic polluted
11	Co662	600	Sofia, boulevard main street, traffic polluted
12	Co510	600	Railway station between metal work and uranium mine, industrial polluted
13	155138	800	Pernic, metal work, industrial polluted
14	Co566	600	Sofia, traffic crossing, traffic polluted
15	Co570	600	Sofia, bus station »East«, traffic polluted
16	Co608	50	Varna, traffic polluted
17	Co574	900	Vitosha mountain, background polluted
18	Co520	300	Vratza, chemical work, industrial polluted
19	Co511	800	Uranium mine, industrial polluted
20	Co518	1400	Vitosha mountain, background polluted
21	Co519	30	Bourgas, petrol work, industrial polluted
22	Co508	550	Sofia, center, traffic polluted
23	Co569	600	Sofia, bus station, »South«, traffic polluted
24	Co561	600	Sofia, bus station »North«, traffic polluted
25	Co568	600	Sofia bus station »West«, traffic polluted
26	Co652	800	Trunk-road around Sofia, traffic polluted
27	Co565	500	Sofia region, traffic polluted
28	Co682	600	Sofia housing district, traffic polluted
29	Co694	600	Sofia, Metro station, traffic polluted
30	Co562	650	Ljuin mountain, background polluted
31	Co558	800	Vitosha mountain, background polluted
32	Co609	700	Ljulim mountain, background polluted
33	Co556	1440	Vitosha mountain, background polluted
34	Co642	1470	Vitosha mountain, background polluted
35	155141	1555	Vitosha mountain, background polluted
36	Co512	1800	Vitosha mountain, background polluted
37	Co560	1300	Vitosha mountain, background polluted
38	Co661	350	Strandzha mountain, background polluted
39	Co647	550	Sofia region, installation of centralized heating system, industrial polluted
40	Co648	500	Sofia region, housing distinct

gions with different types and degrees of environmental pollution as well as at different altitudes. Voucher specimens were deposited at the Herbarium of the Institute of Botany, Sofia (SOM).

Plant exudates were prepared from air-dried, not ground aerial parts (2g) by rinsed with acetone 20 mL for 5 min to dissolve the material accumulated on leaf and stem surfaces. After evaporation of acetone, the dried extracts were dissolved in 250 μ L methanol. 40 μ L from each *A. vulgaris* exudate were applied in triplicate on TLC plates for analysis. Two TLC systems were used for identification and quantification of flavonoid aglycones. Toluene-dioxan-acetic acid (95:25:4, v/v/v) was used for the development of silica gel plates Kieselgel 60 F₂₅₄ (10x20 cm, 0.2 mm layer). Toluene-methylethylketone-methanol (60:25:15, v/v/v) was used for development on polyamide DC-11 plates (10x20 cm, 0.15 mm layer). Chromatograms were viewed under UV radiation=336 nm before and after spraying with »Naturstoffreagenz A« (a 1% methanolic solution of diphenyl-boric acid –ethanolamine complex).

Seven flavonoid aglycones were used as reference compounds in the TLC screening, namely, kaempferol 3,7-dimethyl ether (**A**), quercetin (**B**), quercetin 3,3'-dimethyl ether (**C**), quercetin 3,7-dimethyl ether (**D**), quercetin 3,7,3'-trimethyl ether (**E**), quercetagenin 3,6,7,3'-tetramethyl ether (**F**), quercetagenin 3,6,7,3',4'-pentamethyl ether (**G**) (Fig. 1). Compounds have been isolated and identified from *Artemisia vulgaris* in a previous study (NIKOLOVA 2002).

Variation of flavonoid compounds within populations of the taxa involved was estimated by TLC analysis of 3 individuals from populations.



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| R ₁ =R ₅ =H, R ₂ =R ₄ =OH, R ₃ =R ₆ =OCH ₃ | kaempferol 3,7-dimethyl ether (A) |
| R ₁ =R ₂ =R ₃ =R ₄ =R ₆ =OH, R ₅ =H | quercetin (B) |
| R ₁ =R ₃ =OCH ₃ , R ₂ =R ₄ =R ₆ =OH, R ₅ =H | quercetin 3,3'-dimethyl ether (C) |
| R ₁ =R ₄ =R ₆ =OH, R ₃ =R ₆ =OCH ₃ , R ₅ =H | quercetin 3,7-dimethyl ether (D) |
| R ₁ =R ₃ =R ₆ =OCH ₃ , R ₁ =R ₄ =OH, R ₅ =H | quercetin 3,7,3'-trimethyl ether (E) |
| R ₁ =R ₃ =R ₅ =R ₆ =OCH ₃ , R ₂ =R ₄ =OH | quercetagenin 3,6,7,3'-tetramethyl ether (F) |
| R ₁ =R ₂ =R ₃ =R ₅ =R ₆ =OCH ₃ , R ₄ =OH | quercetagenin 3,6,7,3',4'-pentamethyl ether (G) |

Fig. 1. Structures of the flavonoid aglycones found in *Artemisia vulgaris*

Results and discussion

A TLC survey of flavonoid profiles on 40 populations of *A. vulgaris* was performed and the results are given in Table 2. Methylated free flavonoid aglycones based mainly on quercetin were accumulated in the leaf exudates of the studied populations. Quercetin 3,7,3'-trimethyl ether appeared to be the main flavonoid aglycone. The aglycone kaempferol 3,7-dimethyl ether was found only in trace amounts. Quercetin, quercetin 3,7-dimethyl ether and quercetin 3,3'-dimethyl ether were also detected. The differences in the qualitative flavonoid composition among populations related to highly methylated quercetagenin derivatives – quercetagenin 3,6,7,3'-tetramethyl ether and quercetagenin 3,6,7,3',4'-pentamethyl ether. These compounds were proved only in some populations. The populations (1–9) yielded quercetagenin 3,6,7,3'-tetramethyl ether (chrysosplenetin chemotype). The latter compound was not observed in the exudates of populations (10–20) where quercetagenin 3,6,7,3',4'-pentamethyl ether was established (artemetin chemotype). In the exudates of populations (21–40) neither one of these two compounds was (chemotypes without quercetagenin derivatives). The latter chemotype appeared to be the most widespread, in approximately 50% of all studied populations. This is in agreement with a recent publication on the flavonoid content in three populations of *A. vulgaris* from Austria and Germany (VALANT-VETSCHERA et al., 2003), where only chemotypes without quercetagenin derivatives have been established.

The presence of chemotypes of the plants in relation to altitudinal gradient has been described (SEIGLER and WOLLENWEBER 1983, MCDUGAL and PARKS 1984). Therefore, we looked for a correlation between altitude or environmental pollution and flavonoid composition of *A. vulgaris*. Only a variability in the quantitative flavonoid composition of *A. vulgaris* was observed whereas no relation was found between qualitative flavonoid composition and altitude or type of environmental pollution. This result supports the view that qualitative flavonoid composition is a suitable marker for chemotaxonomic researches in *A. vulgaris*.

The present results are another example of flavonoid chemotypes in plants. Comparative analysis of flavonoid content and other signs of populations (ploidy level) of *A. vulgaris* will be subject of future investigation.

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Tab. 2. Distribution of flavonoid aglycones in *Artemisia vulgaris* populations. A: kaempferol 3,7-dimethyl ether, **B:** quercetin, **C:** quercetin 3,3'-dimethyl ether, **D:** quercetin 3,7-dimethyl ether, **E:** quercetin 3,7,3'-trimethyl ether, **F:** quercetagenin 3,6,7,3'-tetramethyl ether, **G:** quercetagenin 3,6,7,3',4'-pentamethyl ether; (–) absent; (tr.) present in trace amounts (●) present in small amounts; (●●) present in normal amounts (●●●) present in large amounts

Sample No	Flavonoid aglycones						
	A	B	C	D	E	F	G
1	tr.	●●	●	●	●●●	●●●	–
2	tr.	●●	●	●	●●●	●●	–
3	tr.	●	●	●	●●●	●	–
4	tr.	●●	●	●	●	●●	–
5	tr.	tr.	●	●	●●	tr.	–
6	tr.	●	●		●	tr.	–
7	tr.	●	●	●	●	tr.	–
8	tr.	●●	●	●	●	tr.	–
9	tr.	●	●	●	●●	●	–
10	tr.	●	tr.	tr.	●●	–	●●
11	tr.	●●●	●	●	●●	–	●
12	tr.	●●	●	●	●●●	–	●
13	tr.	●	●	●	●●●	–	●
14	tr.	●	●	●	●●	–	●
15	tr.	●	●	●	●●●	–	●
16	tr.	●	●	●	●●●	–	●●●
17	tr.	●●	●	●	●●	–	●
18	tr.	●●	●	●	●●●	–	tr.
19	tr.	●	●	●	●●●	–	tr.
20	tr.	●●	●	●	●●	–	●
21	tr.	●●●	●	●	●	–	–
22	tr.	●	●	●	●	–	–
23	tr.	●	●	●	●●	–	–
24	tr.	●	●	●	●	–	–
25	tr.	●●	●	●	●●	–	–
26	tr.	●	●	●	●	–	–
27	tr.	●	●	●	●●	–	–
28	tr.	●	●	●	●	–	–
29	tr.	●	●	●	●●	–	–
30	tr.	●	●	tr.	●	–	–
31	tr.	●	●	●	●	–	–
32	tr.	●●	●	●	●●	–	–
33	tr.	●●●	●	●	tr.	–	–
34	tr.	●	●	●	●	–	–
35	–	●	tr.	tr.	●	–	–
36	tr.	●●●	tr.	●	●	–	–
37	●	●	tr.	tr.	●	–	–
38	tr.	●●	●	●	●●●	–	–
39	tr.	●	●	●	●●●	–	–
40	tr.	●	●	●	●●●	–	–

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