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Research Article

Phenological Variations in the Surface Flavonoids of *Artemisia vulgaris* L. and *Artemisia absinthium* L.

Milena NIKOLOVA[1,*], Dragan VELIČKOVIĆ[2]

[1]Institute of Botany, Bulgarian Academy of Sciences, Acad. G. Bonchev str., 23, 1113 Sofia, BULGARIA

[2]Zdravlje-Actavis, 199 Vlajkova St., 16000 Leskovac, SERBIA AND MONTENEGRO

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Abstract: Qualitative and quantitative variations in the surface flavonoids in relation to phenological development of *Artemisia vulgaris* L. and *Artemisia absinthium* L. were examined. Plant material was harvested at different phenological stages (vegetative, before budding, floral budding, flowering, and fruiting) of the life cycle of the species. In *A. vulgaris* and *A. absinthium* acetone exudates, 6 and 4 flavonoid aglycones were identified, respectively, by TLC analysis. Quercetin 3,7,3'-trimethyl ether is the main flavonoid in the exudates of *A. vulgaris* while quercetagetin 3,6,7,3',4'-pentamethyl ether is the predominant flavonoid in *A. absinthium*. Qualitative variations in the flavonoid composition of *A. vulgaris* were established. Quantification of the flavonoid aglycones shows that their content was highest in the flowering stage. During the phenological development all of the detected flavonoids in *A. absinthium* were synthesised but differences in their relative amounts were observed. The main flavonoid aglycones were most abundant at the budding stage.

Key Words: *Artemisia vulgaris* L., *Artemisia abisinthium* L., surface flavonoid, phenological variation

Introduction

Studies on surface flavonoids have focused mainly on their distribution in plant species (Wollenweber & Dietz, 1981; Wollenweber, 1990, Valant-Vetschera et al., 2003a; Wollenweber et al., 2005). Phenological variations in the flavonoid composition have been examined only for a few species (Bohm, 1987; Voirin & Bayet, 1992; Vogt & Gülz, 1994). Wollenweber & Valant-Vetschera (1996) have stressed already that such data could be of use for elucidation of the biological and chemotaxonomical significance of exudate flavonoids. They also observed considerable flavonoid variation during the phenological cycle of *A. pontica* L. External flavonoids of *Artemisia vulgaris* L. and *Artemisia absinthium* L. have been examined already. Methyl derivatives of the flavonoids quercetin and kaempferol have been found (Wollenweber et al., 1989; Nikolova, 2002; Valant-Vetschera et al., 2003b) but, to the best of our knowledge, there are no data about variations in the surface flavonoids of *Artemisia vulgaris* and *Artemisia absinthium* during their phenological development.

The present paper concerns phenological variations in surface flavonoid aglycones from the aerial parts of *A. vulgaris* and *A. absinthium.* It is a part of our studies on variability in *Artemisia* flavonoids and their chemotaxonomical significance (Nikolova, 2002, 2006).

Materials and Methods

Plant material. The aerial parts of *A. vulgaris* and *A. absinthium* were collected during the vegetative season in 2003 at different stages of development from wild populations in south-east Serbia (Bogojevce village, surroundings of Leskovac town). Dr Vlastimir Stamenkovic identified the plants.

Sample preparation. Plant exudates were prepared from air-dried, unground aerial parts (2 g) rinsed with 20 ml of acetone for 5 min to dissolve the material accumulated on leaf and stem surfaces. After evaporation of acetone, the dried extracts were dissolved in 200 µl and 600 µl of methanol, respectively, for *A. vulgaris* and *A. absinthium* samples.

* E-mail: milena_n@bio.bas.bg

Flavonoid analysis. Eight flavonoid aglycones were used as reference compounds in the TLC screening, namely kaempferol 3,7-dimethyl ether, quercetin, quercetin 3,3'-dimethyl ether, quercetin 3,7-dimethyl ether, quercetin 3,7,3'-trimethyl ether, quercetagetin 3,6,7-trimethyl ether, quercetagetin 3,6,7,3'-tetramethyl ether, and quercetagetin 3,6,7,3',4'-pentamethyl ether. The compounds were isolated and identified from *A. vulgaris* and *A. absinthium* previously (Nikolova, 2002).

Two TLC systems were used for the identification of the flavonoid aglycones. A toluene-dioxan-acetic acid mixture (95:25:4, v/v/v) was used for the development of silica gel sheets Kieselgel 60 F_{254} (10 × 20 cm, 0.2 mm layer). A toluene-methylethylketone-methanol mixture (60:25:15, v/v/v) was used for the development on polyamide DC-11 sheets (10 × 20 cm, 0.15 mm layer). The 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).

Flavonoid quantification. Some 20 µl of *A. vulgaris* exudates and 5 µl of *A. absinthium* exudates with unknown concentrations were spotted and developed on Merck aluminium sheets Kieselgel 60 F_{254} (0.2 mm thin layer, 10 ¥ 20 cm) together with standards. A toluene-dioxan-acetic acid (95:25:4) mixture was used for the development of TLC sheets. Migration distance was 90 mm. The compounds were visualised after spraying with 3% methanol solution of iron(III) chloride ($FeCl_3$) reagent. The coloured spots of compounds were scanned and the images were analysed by QuantiScan 2.1® Biosoft software (Nikolova et al., 2004). The flavonoid content of the exudates was calculated from the densitogram peak areas by comparing to 3 standards (2.5, 5, and 10 µg/spot of quercetin) placed on the same sheet.

Variation in the flavonoid compounds during phenological development was estimated by analysis of 5 acetone exudates from each stage of both species.

Results and Discussion

In this study we report the flavonoid profile and the dynamic of flavonoid content in 2 *Artemisia* species at 5 stages of vegetation. The TLC analysis revealed the presence of kaempferol 3,7-dimethyl ether, quercetin, quercetin 3,3'-dimethyl ether, quercetin 3,7-dimethyl ether, quercetin 3,7,3'-trimethyl ether, and quercetagetin 3,6,7,3',4'-pentamethyl ether in the *A. vulgaris* exudates. Quercetin 3,7,3'-trimethyl ether was the main flavonoid. The dynamic of its content during the vegetative period is given in the Figure. Quercetin 3,7,3'-trimethyl ether was the most abundant at the flowering stage.

During the vegetative stage of development only kaempherol 3,7-dimethyl ether and quercetin 3,7,3'-trimethyl ether were detected in trace amounts. In addition to these flavonoids, accumulation of quercetagetin 3,6,7,3',4'-pentamethyl ether was observed at the before budding stage. Quercetin 3,3'-dimethyl ether, quercetin 3,7-dimethyl ether, and quercetin were yielded additionally during the budding stage. At the flowering stage the highest content of flavonoid aglycones was observed. Further aging of the plant led to the disappearance of quercetagetin 3,6,7,3',4'-pentamethyl ether. In all phenological stages kaempherol 3,7-dimethyl ether was synthesised in trace amounts (Table 1).

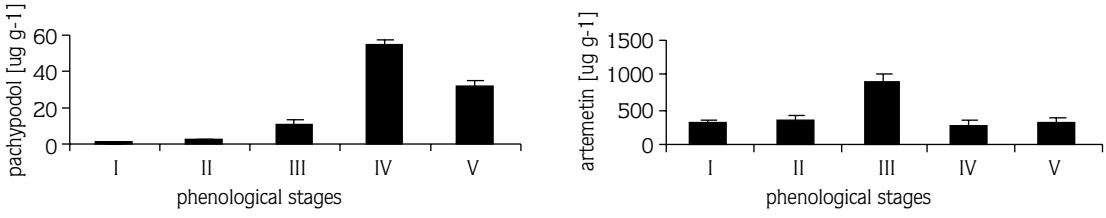

Figure. Contents of quercetin 3,7,3'-trimethyl ether (*pachypodol*) of *A. vulgaris* and quercetagetin 3,6,7,3',4'-pentamethyl ether (*artemetin*) of *A. absinthium*. Each value represents the mean of 5 independent determinations ± S.D.
Phenological stages: I - vegetative, II - before budding, III - floral budding, IV - flowering V – fruiting.

Table 1. Changes in flavonoid aglycones composition in *A. vulgaris* aerial parts during phenological cycle.

Flavonoid aglycones	Phenological stages					Plant parts		
	vegetative	before budding	budding	flowering	fruiting	leaves	stems	flowers
Kae-3,7-diMe	+	+	+	+	+	+	+	+
Quercetin			••	+	+	•		
Que-3,3'-diMe			+	•	•	+		+
Que-3,7-diMe			+	••	••	+		••
Que-3,7,3'-triMe	+	+	•	•••	••	••	+	•••
Queg-3,6,7,3',4'-pentaMe		•	+	••	+	•		•

Legend: Kae-3,7-diMe - kaempferol 3,7-dimethyl ether; Que-3,3'-diMe - quercetin 3,3'-dimethyl ether; Que-3,7-diMe - quercetin 3,7-dimethyl ether; Que-3,7,3'-triMe - quercetin 3,7,3'-trimethyl ether; Queg-3,6,7,3',4'-pentaMe - quercetagetin 3,6,7,3',4'-pentamethyl ether
Approximate flavonoid amount: + - traces - under 2 μg g^{-1} • 2-20 μg g^{-1} •• 20-40 μg g^{-1} ••• 40-60 μg g^{-1}

Table 2. Changes in flavonoid aglycones composition in *A. absinthium* aerial parts during phenological cycle.

Flavonoid aglycones	Phenological stages					Plant parts		
	vegetative	before budding	budding	flowering	fruiting	leaves	stems	flowers
Kae-3,7-diMe	+	+	+	+	+	+		+
Queg-3,6,7-triMe	+	+	+	+	+	+	+	+
Queg-3,6,7,3'-tetraMe	•	••	•	•	+	•	+	••
Queg-3,6,7,3',4'-pentaMe	•	••	•••	••	•	+	+	•••

Legend: Kae-3,7-diMe - kaempferol 3,7-dimethyl ether; Queg-3,6,7-triMe - quercetagetin 3,6,7 -trimethyl ether; Queg-3,6,7,3'-tetraMe - quercetagetin 3,6,7,3'-tetramethyl ether; Queg-3,6,7,3',4'-pentaMe - quercetagetin 3,6,7,3',4'-pentamethyl ether
Approximate flavonoid amount:
+ - traces - under 50 μg g^{-1} • 50-200 μg g^{-1} •• 200-400 μg g^{-1} ••• 400-800 μg g^{-1}

The flavonoid profiles of the separate plant parts (stem, leaves, flowers) at the flowering stage were characterised. A very simple flavonoid pattern was found in the stems. This plant part contains only trace amounts of quercetin 3,7,3'-trimethyl ether and kaempherol 3,7-dimethyl ether. The leaves of *A. vulgaris* are the organ with enhanced synthesis of quercetagetin 3,6,7,3',4'-pentamethyl ether. The highest content of quercetin 3,7,3'-trimethyl ether was found in the flowers (Table 1).

A TLC survey of flavonoid profiles of *A. absinthium* during phenological development was performed and the results are given in Table 2. Kaempferol 3,7-dimethyl ether, quercetagetin 3,6,7-trimethyl ether, quercetagetin 3,6,7,3'-tetramethyl ether, and quercetagetin 3,6,7,3',4'-pentamethyl ether were identified. Quercetagetin 3,6,7,3',4'-pentamethyl ether (*artemetin*) was the most abundant flavonoid in the exudates. Quantification of this compound shows that its content was the highest at the budding stage (Figure).

The qualitative flavonoid composition of *A. absinthium* was unvaried during the phenological cycle. Differences were detected only in relative amounts. The level of flavonoids was highest at the budding stage (Table 2). Very low flavonoid content was found in the stems. The flowers are richest in polymethoxylated derivatives of quercetagetin (Table 2).

Phenological variations in the surface flavonoids of *A. vulgaris* and *A. absinthium* may be important in the adaptation of the plant to the local environment. It has been suggested that external flavonoids enhance plant survival and reproduction due to their protective and allelopathic properties (Chaves et al., 2001; Onyilagha & Grotewold, 2004). Polymethoxylated flavonoids have been determined as insect deterrents and compounds with antifungal properties (Tomas-Barberan et al., 1988; Midiwo et al., 1990; Grayer & Harborne, 1994). We suppose that the high flavonoid content at the budding and flowering stages of both species could be connected with a protective role on surface flavonoids.

In conclusion, the examples discussed above show that external flavonoids of *A. absinthium* are stable in their qualitative composition during phenological development while the flavonoid profiles of *A. vulgaris* exhibited qualitative and quantitative variability, and these data would be useful in chemotaxonomic and comparative studies.

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