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ARTICLE

Quantitative flavonoid variations of *Artemisia vulgaris* L. and *Veronica chamaedrys* L. in relation to altitude and polluted environment

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ABSTRACT Influence of altitudinal gradient and polluted environment on externally accumulated flavonoid aglycones were examined. Contents of apigenin in *Veronica chamaedrys* L. and quercetin 3,7,3'-trimethyl ether in *Artemisia vulgaris* L. were determinated. The highest apigenin level was found in populations at alpine regions, whereas no relation was found between quercetin 3,7,3'-trimethyl ether and altitude. The largest amounts of quercetin 3,7,3'-trimethyl ether was found in samples collected from industrial polluted habitats. Ecological significance on external flavonoid aglycones is discussed. **Acta Biol Szeged 49(3-4):29-32 (2005)**

KEY WORDS

flavonoid aglycones
altitudinal gradient
pollution
Artemisia vulgaris
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Flavonoid variations are considered as a phytochemical adaptation to the abiotic and biotic environment by Dixon and Pavia (1995). Lipophilic flavonoid aglycones are accumulated on the plant surface (Wollenweber 1990). It has been assumed that this surface disposition was determined by the chemo-ecological functions of aglycones. There are extensively data that flavonoid synthesis is influenced by different abiotic and biotic factors: UV light radiation, drought, ozone, phytopathogens and insect-deterrent (Tomas-Barberan et al. 1988; Midiwo et al. 1990; Cuadra et al. 1997; Cooper-Driver and Bhattacharya 1998; Lalova 1998; Markham et al. 1998; Simmonds 1998; Chaves et al. 2001; Saleem et al. 2001).

Artemisia vulgaris L. (Asteraceae) and *Veronica chamaedrys* L. (Scrophulariaceae) are perennial herbs, widely distributed in different habitats, from 0 to 1800 meter above sea level (m asl) for *A. vulgaris* populations and from 0 to 2300 m asl for *V. chamaedrys* populations. Simple flavonol methyl ethers have been reported for West-European populations of *A. vulgaris* (Valant-Vetschera et al. 2003) and flavone derivatives of *V. chamaedrys* populations (Nikolova et al. 2003).

Although the intraspecific flavonoid variation has been documented in numerous studies (cited by Bohn1987), only a limited number of reports treated the subject of flavonoid changes along altitudinal gradient (McDougal and Parks 1984) and environmental pollution (Loponen et al. 1998). In the present study we examine external flavonoid aglycones variations of *Veronica chamaedrys* and *Artemisia vulgaris* across altitudinal gradient and polluted environment.

The sampling of populations from habitats with different conditions allowed us to assess the intraspecific variations and main ecological trends of flavonoid accumulation.

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Materials and Methods

Plant material

The plant samples were formed from aerial parts of blossoming plants collected from natural habitats on 24 populations of *A. vulgaris* during 1999, and on 12 populations of *V. chamaedrys* during 2001 in Bulgaria. The sampling sites were chosen to cover regions with different type and degree of environmental pollution as well as at different altitude. Altitudinal gradient includes populations of *V. chamaedrys* and *A. vulgaris* situated at various altitudes from 700 to 2290 m asl at Vitosha mountain (Bulgaria). Pollution gradient includes populations of *A. vulgaris* from habitats with industrial, traffic and background pollution. We examined influence on environmental pollution only on *A. vulgaris* populations because *V. chamaedrys* populations have a rare occurrence in polluted regions. All habitats are situated on similar altitude in the same geographical region. Industrial contamination is mostly by lead (Pb) and iron (Fe) ions and not so much by cooper (Cu) and zinc (Zn). Main toxic emissions are aromatic aerosols, ozone (O_3), sulphur oxide (SOx), hydrogen sulphide (H_2S), carbon oxide (CO), dust (Topalov 2001). Voucher specimens were deposited at the Herbarium in the Institute of Botany, Sofia, Bulgaria.

Sample preparation

Plant exudates were prepared from air-dried, not grounded aerial parts (5 g) by rinsed with acetone 2x20 m1 for 5 min to dissolve the material accumulated on leaf and stem surfaces. After evaporation of acetone, the dried extracts were dissolved in methanol for further TLC analysis.

Table 1. Voucher numbers (SOM) and apigenin content in the samples of *V. chamaedrys* along altitudinal gradient.

SOM	Altitude [m asl]	Apigenin [µg/]±SD*
Co476	750	0.57±0.06
Co669	800	0.80±0.08
Co615	1000	1.60±0.08
Co618	1200	0.42±0.07
Co619	1250	0.47±0.06
Co623	1340	1.00±0.08
Co672	1600	1.56±0.06
Co621	1810	2.08±0.07
Co617	1870	1.80±0.06
Co616	1950	2.12±0.06
Co675	2200	1.61±0.07
Co614	2290	2.05±0.05

*Results are average values of three measurements; m asl: meters above sea level.

Table 2. Voucher numbers (SOM) and quercetin 3,7,3′-trimethyl ether content in the samples of *A. vulgaris* along altitudinal gradient.

SOM	Altitude [m asl]	Quercetin 3,7,3′-trimethyl ether [µg/g]±SD*
Co515	750	0.28±0.06
Co526	800	0.35±0.03
Co517	900	0.34±0.09
Co577	1000	0.32±0.03
Co688	1200	0.29±0.04
Co560	1300	0.35±0.04
Co518	1400	0.32±0.04
Co556	1440	0.29±0.05
Co513	1550	0.30±0.03
Co512	1800	0.28±0.06

*Results are average values of three measurements; m asl: meters above sea level.

Flavonoid quantification

Toluene-dioxan-acetic acid (95:25:4) mixtures were used for the development of plates. Migration distance was 90 mm. 60 µl of *V. chamaedrys* exudates and 40 µl of *A. vulgaris* exudates with unknown concentrations were spotted and developed on Merck aluminum sheets Kiselgel 60 F_{254} (0.2 mm thin layer, 10 x 20 cm) together with standards. Compounds were visualized after spraying with "Naturstoffreagenz A" reagent. The fluorescence emissions of apigenin (Rf = 0.34) and quercetin 3,7,3'-trimethyl ether (Rf = 0.60) were snapped under UV radiation=336 nm with a digital camera, and the images were analyzed by QuantiScan 2.1® Biosoft software (Nikolova et al. 2004). The apigenin and quercetin 3,7,3'-trimethyl ether contents of the exudates were calculated from the densitogram peak areas by comparing to three standards (0.75, 1.5, 3 µg/spot of apigenin and 0.75, 1, 1.5 µg/spot of quercetin 3,7,3'-trimethyl ether) placed on the same plate.

Flavonoid aglycones used as reference compounds in the TLC analysis, namely quercetin 3,7,3'-trimethyl ether and apigenin have been isolated and identified respectively from *A. vulgaris* and *V. chamaedrys* in a previous study (Nikolova 2002).

Table 3. Voucher numbers (SOM) and quercetin 3,7,3′-trimethyl ether content of in the samples of *A. vulgaris* from habitats with different type of pollution.

SOM	Habitat information	Quercetin 3,7,3′-trimethyl ether [µg/g] SD*
industrial polluted zone		
155134	Metal works "Kremikovstsi", 800 m asl	0.48±0.03
Co 573	Chemical works "Verila", 700 m asl	0.46±0.03
Co 510	Railway station "Yana" between metals works and uranium mine, 600 m asl	0.63±0.08
Co663	v. "Ravno pole", there is flying toxic fragments to air, 500 m asl	0.47±0.09
Co511	Uranium mine "Buhovo", 900 m asl	0.52±0.05
traffic polluted zone		
Co662	Sofia, boulevard, 550 m asl	0.40±0.03
Co566	Sofia, main street, 500 m asl	0.35±0.06
Co647	Sofia, highway 550 m asl	0.44±0.03
Co694	Sofia, metro station 500 m asl	0.40±0.08
Co571	Sofia, airport 550 m asl	0.36±0.03
background polluted zone		
Co683	Ljulun mountain, 700 m asl	0.28±0.01
Co515	Vitosha mountain, 700 m asl	0.28±0.06
Co558	Vitosha mountain, 800 m asl	0.22±0.02
Co574	Vitosha mountain, 900 m asl	0.34±0.09
Co606	Lozen mountain, 800 m asl	0.32±0.02

*Results are average values of three measurements; m asl: meters above sea level.

Results and Discussion

Flavonoid aglycones variation along altitudinal gradient

A TLC survey of apigenin variations over altitudinal interval 700-2300 m asl on 12 populations of *V. chamaedrys* was performed and the results are given in Table 1. The apigenin content ranged from 0.47 to 2.17 μg/g of the dried leaf weight. The largest amounts of apigenin was found in the samples at the alpine regions. This pattern could be explained with the xeric alpine habitats and presumable UV-screen function of the leaf surface flavonoids. There are many reports demonstrating that flavonoid synthesis is induced by UV radiation (Lois 1994; Cuadra et al. 1997; Lalova 1998; Markham et al. 1998; Hofmann et al. 2000). Our results are in agreement with previous studies, which report a higher accumulation of exudate flavonoids as a response to more xeric habitats (Wollenweber 1990; Chaves at al. 1997; Willliams et al. 1997; Valant-Vetschera and Wollenweber 2001).

The quantitative analysis on flavonoid content in the 10 populations of *A. vulgaris* over altidutinal inerval 800-1800 m asl showed that the altitude does not have a significant effect on quercetin 3,7,3'-trimethyl ether synthesis (Table 2). We suppose that structural differences on apigenin and quercetin 3,7,3'-trimethyl ether are the reason for their different metabolisms along altitudinal gradient. The O-methylation of the hydroxyl substations inactivates anthioxidant activities of the flavonoids (Cao et al. 1997; Burda and Oleszek 2001). In this way apigenin was more efficient antioxidant than quercetin 3,7,3'-trimethyl ether. The antioxidant activity on aglycones is important for their protective role because UV radiation induces production of free radicals (Foyer et al. 1994).

Flavonoid aglycones variation in dependence of type on environmental pollution

The quantification of quercetin 3,7,3'-trimethyl showed considerable differences among the populations of *A. vulgaris* from the habitats with different type of pollution (Table 3). The populations from the industrial polluted habitats displayed high content on quercetin 3,7,3'-trimethyl ether. Increases of phenolic compounds and flavonoids as a result of pollution impact have been observed in tree species (Loponen et al. 1997, 1998; Giertych et al. 1999), but the reasons for this increase are unclear. Chaves et. al. (1997) concluded that accumulation of methylated flavonoids prevent water loss and increase the general stress tolerance of the plants.

The present study reported infraspecific flavonoid variations of *A. vulgaris* and *V. chamaedrys* in relation to altitudinal gradient and polluted environment. The apigenin accumulation in *V. chamaedrys* was increased at the alpine regions, while the content of quercetin 3,7,3'-trimethyl ether in *A. vulgaris* appeared to be independent of altitude. The synthesis of quercetin 3,7,3'-trimethyl ether was influenced positively by environmental pollution. The data support the postulated ecological significance on external flavonoid aglycones.

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