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Full Length Research Paper

Influence of serpentine soils on the flavonoid content of *Hypericum* populations growing in Bulgaria

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The effect of environmental factors on the production of secondary metabolites by plants has attracted a considerable amount of attention. Most species populations growing on serpentine soils are adapted to these special edaphic conditions in different ways. In this study we investigated the flavonoid content in Bulgarian *Hypericum* populations from five *Hypericum* species (*H. cerastoides, H. aucheri*, *H. montbretii*, *H. perforatum* and *H. olympicum*) to evaluate the differences between plants growing on and off serpentine. The results showed that the serpentine substrate influences the amount of flavonoids in *Hypericum* species.

Key words: *Hypericum,* flavonoids, serpentine.

INTRODUCTION

Serpentine habitats are naturally rich in potentially cytotoxic elements like Ni, Fe, Mg, Co, Cr and Cd which exert strong selective pressures on the native vegetation. The effect of the environmental factors on the production of some secondary metabolites including flavonoids by plants has attracted a considerable amount of attention. Most species populations are adapted to environmental conditions in different ways (Lombini et al., 1998). Flavonoids may help plants to live on soils that are rich in toxic metals such as aluminum (Treutter, 2006). Secondary metabolites are present in all higher plants, usually in a high structural diversity. As a rule, a single group of phytochemicals dominates within a given taxon and a few major compounds are often present together with several derivatives and minor components (Wink, 2003). Some UV-B absorbing secondary metabolites with biological effects are established in aerial parts of St. John’s Wort (including flavonoids and hypericin) (Erken et al., 2001) which are influenced by some environmental factors such as altitude of the growing sites and light intensity (Umek et al., 1999), water stress condition (Zobayed et al., 2007), humidity, soil and temperature (Kefeli et al., 2003), genetic variation (Buter et al., 1998), ecotype genetic characteristic and environmental factors (Barnes et al., 2001; Bruni et al., 2009; Filippini et al., 2010; Asadian et al., 2011).

The species of the genus *Hypericum* are widely distributed in Europe and in Bulgaria as well. Some of them grow on the serpentine areas in Rhodope Mountains. The similarity in the morphology of the *Hypericum* species is the main reason for frequent mistakes in their determination especially when growing together in the same locality. Therefore, this study focuses on species occurring on serpentine sites which are potential sources for collection of plant substances (Nedelcheva et al., 2010). *Hypericum perforatum* L. is the most used in traditional medicine from among all *Hypericum* spp. growing in Bulgaria (Nikolov, 2006). The plant has

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spasmolytic, anti-inflammatory, antioxidant, antibacterial and analgesic activities and is used for the treatment of skin wounds, burns, eczema, gastrointestinal tract diseases and psychological disorders. Recently, the species has gained much popularity as an antidepressant and is used in many countries for the treatment of depression. The main groups of biologically active substances in *H. perforatum* are flavonoids, tannins, essential oils and condensed anthraquinones (Asgarpanah, 2012; Nikolov, 2006; Raziq et al., 2011). Previous studies about serpentines have shown that *H. perforatum* is a tolerant species regarding the heavy metal content and in plant material concentration of iron, nickel and chromium (Obratov-Petković et al., 2008).

The aim of this work was to determine primarily the effects of the abiotic stress caused by serpentine habitats on the flavonoid levels in *Hypericum* species. Bearing in mind that the quantity of active substances depends on the ecological factors that affect the vegetative plant organs, we investigated ten populations of five *Hypericum* species: *Hypericum cerastoides* (Spach.) Robson*, Hypericum aucheri* Jaub. et Spach, *Hypericum montbretii* (Spach.) Stoj. et Stef., *Hypericum perforatum L* and *Hypericum olympicum* L. (Hypericaceae) growing on serpentines in Bulgaria to evaluate: 1) flavonoid content in aerial plant parts of natural populations; 2) differences between species and their populations growing on and off serpentine areas.

MATERIALS AND METHODS

Plant materials

Aerial plant parts of 10 populations from five *Hypericum* species were collected during the flowering season from wild habitats of serpentine and non-serpentine areas distributed in Rhodope, Pirin and Rila Mountains. The plants were identified following Tutin (1968), Jordanov and Kozhuharov (1970). The voucher specimens were deposited at The Bulgarian Serpentine Flora Collection (SO-BSF), Faculty of Biology, Sofia University (Nedelcheva et al., 2009) (Table 1).

Quantification of flavonoids

Quantitative determination of flavonoids was performed by spectrophotometric method described in the pharmacopoeia (European Pharmacopeia, 2005). Total flavonoids were determined by formation of a complex with aluminium chloride after acid hydrolysis of the samples. The absorbance of the resulting compounds was measured after 30 min comparing with a compensation liquid at 425 nm. The results were expressed as hyperoside. Flavonoid determination was carried out in triplicate using WPA Lightwave S 2000 Diode Array UV/VIS spectrometer.

Statistical analysis

MedCalc 12.3 (MedCalc Software 2012) was used for statistical calculations. The Kruskal-Wallis one-way analysis of variance was conducted to determine the statistical significance of interspecific differences in the flavonoid content. The results were expressed as mean ± standard error. Probability value of p=0.05 was used as the criteria for significance differences.

RESULTS AND DISCUSSION

Results of quantitative analysis of flavonoids in serpentine populations from *H. montbretii, H. aucheri* and *H. cerastoides* were compared with literature data for non-serpentine populations (Zheleva-Dimitrova et al., 2010). The obtained results from the investigations of *H. perforatum* and *H. olympicum* were compared with literature and with data obtained from the analysis of non-serpentine samples collected from Drangovo.

The flavonoid content in different *Hypericum* populations is shown in Table 2. The total amount of flavonoids ranged in the serpentine samples from 0.76 g /100 to 1.60 g/100 g/dw and in non-serpentine samples from 0.20 g/100 to 1.22 g/100 g/dw. Significant differences (P<0.05) in total flavonoid content were found between the serpentine and non-serpentine *Hypericum* populations. The highest flavonoids content was determined in serpenitine samples of *H. perforatum* (1.60 ± 0.02 g/100 g dw) and *H. montbretii* (1.54 ± 0.05 g/100 g dw) collected from Eastern Rhodope and Rila Mountains. In *H. cerastoides* were observed higher and stable levels of flavonoids (1.29 ± 0.02 g/100 - 1.34 ± 0.03 g/100 g/dw) compared with the non-serpentine sample (Zheleva-Dimitrova et al., 2010).

The amount of flavonoids in all populations of *H. aucheri* varied from 0.74 ± 0.01 g/100 (non-serpentine) to 0.92 ± 0.02 g/100 g dw. Although the results for serpentine and non-serpentine samples were found to be close to each other, there are significant statistical differences (P=0.015). For *H. olympicum* samples from high altitude between minimal and maximal levels was registered in all the investigated samples from Eastern Rhodope Mountains (0.20 ± 0.03 g/100 g dw [non-serpentine sample] to 1.41 ± 0.04 g/100 g dw). Very low flavonoid content was found in the non-serpentine sample from Krumovgrad (0.20 ± 0.03 g/100 g dw) compared with serpentine sample (1.41± 0.04 g/100 g dw -1.39± 0.01 g/100 g dw).

In conclusion the serpentine populations exhibited higher levels of flavonoids than the non-serpentine ones. It could be suggested that these differences are related with the specific stress conditions of the habitat. From the above described results two conclusions could be made: 1) Serpentine habitats as a specific environment offer both stressful and stable conditions for accumulating flavonoids; 2) Flavonoid content is relatively high due to response of the plant defense-reaction. Probably, the observed differences in the flavonoid content are related to the genotypic characteristic of *Hypericum* species. In addition, based on the presented data, only preliminary suggestions could be made supporting the “oxidative pressure hypothesis" as an evolutionary concept for

Table 1. Floristic region and voucher specimen of studied *Hypericum* species.

Species	Population	Locality (Floristic region)	Voucher specimen
H. perforatum	Predela	Pirin/Rila	BSK120612
	Fetler	Eastern Rhodope Mts.	BSK120610
	Chernichevo	Eastern Rhodope Mts.	BSK120609
	Parvenetz	Central Rhodope Mts.	BSK100410
	Dobromirci	Eastern Rhodope Mts.	BSK120611
	Golyamo Kamenyane	Eastern Rhodope Mts.	BSK120616
	##Yagodina	Western Rhodope Mts.	
	#Drangovo	Eastern Rhodope Mts	BSK120614
H. montbretii	Parvenetz	Central Rhodope Mts.	BSK100615
	Fetler	Eastern Rhodope Mts.	BSK120615
	Chichevo	Eastern Rhodope Mts.	BSK120617
	##Trigrad	Western Rhodope Mts.	
H. aucheri	Kazak	Eastern Rhodope Mts.	BSK100611
	Golyamo Kamenyane	Eastern Rhodope Mts.	BSK120618
	Chernichevo	Eastern Rhodope Mts.	BSK120619
	##Momchilgrad	Eastern Rhodope Mts.	
H. cerastoides	Fetler	Eastern Rhodope Mts.	BSK120625
	Dryanova glava	Eastern Rhodope Mts.	BSK120632
	Kazak	Eastern Rhodope Mts.	BSK100618
	##Snezhanka	Western Rhodope Mts.	
H. olympicum	Fetler	Eastern Rhodope Mts.	BSK120626
	Golyamo Kamenyane	Eastern Rhodope Mts.	BSK120629
	Chernichevo	Eastern Rhodope Mts.	BSK120627
	#Drangovo	Eastern Rhodope Mts.	BSK120628
	##Krumovgrad	Eastern Rhodope Mts.	

Mts – Mountains. #Non-serpentine; ##Non-serpentine (Zheleva-Dimitrova et al., 2010).

Table 2. Content of flavonoids (g/100g dw) in populations *of Hypericum* spp.

Species	Population	Mean ± SD	Average rank
*H. perforatum**	Predela	1.60 ± 0.02	22.83
	Fetler	1.57 ± 0.01	20.17
	Chernichevo	1.44 ± 0.03	15.50
	Parvenetz	1.42 ± 0.04	14.00
	Dobromirci	1.40 ± 0.05	12.50
	Golyamo Kamenyane	1.18 ± 0.04	8.00
	#Yagodina	1.12 ± 0.02	5.00
	#Drangovo	0.96 ± 0.04	2.00
*H. montbretii***	Parvenetz	1.54 ± 0.05	11.00
	Fetler	1.41 ± 0.01	8.00
	Chichevo	1.22 ± 0.03	5.00
	#Trigrad	1.04 ± 0.03	2.00
*H. aucheri****	Kazak	0.92 ± 0.02	11.00

Table 2. Contd.

	Golyamo Kamenyane	0.82 ± 0.02	8.00
	Chernichevo	0.76 ± 0.01	5.00
	#Momchilgrad	0.74 ± 0.01	2.00
*H. cerastoides*****	Fetler	1.34 ± 0.03	10.83
	Dryanova glava	1.30 ± 0.02	7.33
	Kazak	1.29 ± 0.02	5.83
	#Snezhanka	1.22 ± 0.02	2.00
*H. olympicum******	Fetler	1.41 ± 0.04	11.67
	Golyamo Kamenyane	1.41 ± 0.03	12.00
	Chernichevo	1.39 ± 0.01	9.33
	#Drangovo	1.12 ± 0.02	5.00
	#Krumovgrad	0.20 ± 0.03	2.00

#Non-serpentine; Results are means ± SD of three different samples; *P = 0.003; ** P = 0.015; ***P = 0.015; ****P = 0.025; *****P = 0.022.

flavonoid distribution and abundance (Close and McArthur, 2002). Further studies on the qualitative composition of flavonoids in more populations of different habitats, dynamics of accumulation, their localization in the organs and tissues of the plant are required for confirming the above mentioned hypothesis.

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