Surface flavonoids of *Centaurea stenolepis* and the local endemics *Centaurea davidovii* and *C. parilica* (*Asteraceae*, sect. *Lepteranthus*) from Bulgaria

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Received: July 08, 2013 ▷ Accepted: October 18, 2013

Abstract. The species of *Asteraceae* have been found to be a rich source of externally accumulated flavonoid aglycones. This type of compounds attracts the attention, besides for their importance as chemotaxonomic markers, also for their ecological role. In the present study, two local endemic species, *Centaurea davidovii* and *C. parilica*, and one widespread species from the same group, *C. stenolepis*, are analyzed for their external flavonoid composition. Flavonoid profiles of flower heads and leaves are analyzed separately. The exudates of flower heads have displayed more complex flavonoid profiles. Ten flavonoid aglycones are identified by thin-layer chromatography (TLC), with authentic markers, using three different sorbents (silica gel, polyamide, cellulose) and several combinations of mobile phases. Some differences are observed in the classes of flavonoids accumulating in the examined species: predominantly methylated derivatives of 6-hydroxyflavones in the exudates of *C. davidovii* and *C. parilica,* and methylated derivatives of 6-hydroxyflavonols of *C. stenolepis*. Similar flavonoid profiles are registered of the exudates of *C. davidovii* and *C. parilica*. They yielded apigenin, luteolin, scutellarein 6,4'-dimethyl ethers, 6-hydroxyluteolin 6-methyl ether, and kaempferol 3-methyl ether. Furthermore, in the exudates of *C. davidovii,* scutellarein 6-methyl ether and scutellarein 6,7,4'-trimethyl ethers are detected. Most flavonoids of *C. stenolepis* are methylated flavonols: kaempferol 3-methyl ether, 6-hydroxykaempferol 3,6-dimethyl ethers and quercetagetin 3,6,4'-trimethyl ethers. The species are studied for the first time for their flavonoid aglycone composition.

Key words: *Centaurea*, endemics, external flavonoids, TLC

Introduction

Subtribe *Centaureinae* (Cass.) Dumort. (*Asteraceae* L.) comprises 72 genera, of which only 16 have been investigated for the occurrence of flavonoids. Among them, genus *Centaurea* L. is the most intensively studied. Data on the distribution of flavonoids in 112 *Centaurea* species have been recently summarized (Formisano & al. 2012). Nevertheless, little is known about the external accumulation of flavonoid aglycones in the *Centaurea* species, a widespread phenomenon in the *Asteraceae* family (Wollenweber & Valant-Vetschera 1996; Wollenweber & al. 1997; 2005; Valant-Vetschera & Wollenweber 2007). This type of compounds attracts the attention not only for their usefulness in taxonomic studies (Wollenweber & Schneider 2000; Valant-Vetschera & Wollenweber 2001; Valant-Vetschera & al. 2003), but also for their ecological role as protectors against UV radiation, antimicrobial, insect-deterrent and allelopathic agents (Midiwo & al. 1990; Williams & al. 1997; Cuadra & al. 1997; Chaves & al. 1997, 2001; Onyilagha & Grotewold 2004). When dealing with surface flavonoids,

the greatest advantage is their quick and easy isolation and this is very useful in the comparative studies, or in case one needs a rapid assessment of the quality of plant material.

In the present study, we have analyzed the surface flavonoid profiles of *Centaurea stenolepis* A.Kern., and of the local endemics *Centaurea davidovii* Urum. and *C. parilica* Stoj. & Stef. [*Asteraceae*, sect. *Lepteranthus* (DC.) DC.] from Bulgaria.

Material and methods

Plant material

Preparation of acetone exudates. Air-dried, but not ground (1g), the plant material was briefly (2-3 min) rinsed with acetone at room temperature, so as to dissolve the lipophilic components accumulated on the surface. The obtained acetone filtrate was then dried in a rotary-evaporator to obtain a crude extract, which was suspended in MeOH and then subjected to TLC.

Thin-layer chromatographic analysis: The acetone exudates were screened for surface flavonoids by TLC analysis. Three TLC sorbents and several mobile phases were used for the analysis of flavonoid exudates. Toluene-dioxan-acetic acid (95:25:4, v/v/v) was applied for the development of aglycones mixture on silica gel plates Kiselgel 60 F_{254} (10×20 cm, 0.2 mm layer). Toluene-methylethylketone-methanol (60:25:15, v/v/v); toluene-petrol ether-methylethylketone-methanol (60:30:10:5, v/v/v/v) and toluene-methylethylketone-methanol (30:20:15, v/v/v) were used for the DC-Alufolien Polyamid 11 F_{254} plates (10×20 cm, 0.15 mm layer). Acetic acid–water (30:70, v/v) was used for the cellulose plates DC-Alufolien Cellulose 5552 (10×20 cm, 0.1 mm layer). Chromatograms were viewed under UV light before and after spraying with Natural Product Reagent A, 1% solution of diphenylboric acid 2-aminoethyl ester complex in methanol. The identification of compounds was achieved by co-chromatography with authentic markers obtained from Prof. Eckhard Wollenweber.

Results and discussion

Plant material (flower heads and leaves separately) of *Centaurea stenolepis* and the local endemics, *Centaurea davidovii* and *C. parilica* (*Asteraceae*, sect. *Lepteranthus*), were analyzed for their profiles of surface flavonoid aglycones. Twelve flavonoid aglycones were detected and ten were identified by thin-layer chromatography (TLC). Apigenin (**1**), luteolin (**2**) and their 6-methylated derivatives – scutellarein 6-methyl ether (**3**), scutellarein 6,4'-dimethyl ethers (**4**), scutellarein 6,7,4'-trimethyl ethers (**5**), 6-hydroxyluteolin 6-methyl ether (**6**), 6-hydroxyluteolin 6,3'-dimethyl ethers (**7**), as well as flavonol derivatives – kaempferol 3-methyl ether (**8**), 6-hydroxykaempferol 3,6-dimethyl ethers (**9**) and quercetagetin 3,6,4'-trimethyl ethers (**10**) – were identified by TLC analysis in direct comparison with the authentic compounds obtained by Prof. Eckhard Wollenweber (Fig. 1). The exudates of flower heads displayed more complex flavonoid profiles than the leaf samples. Apigenin (**1**), kaempferol 3-methyl ether (**8**) and 6-hydroxyluteolin-6-methyl ether (**6**) were the common flavonoid aglycones in the flower exudates of all studied species. The exudates of flower heads of *C. davidovii* and *C. parilica* contained additionally common flavones derivatives – scutellarein 6,4'-dimethyl ethers (**4**), luteolin (**2**) and 6-hydroxyluteolin 6,3'-dimethyl ethers (**7**). In contrast of them, the exudate of flower heads of *C. stenolepis* contained flavonol derivatives: 6-hydroxykaempferol 3,6-dimethyl ethers (**9**) and quercetagetin 3,6,4'-trimethyl ethers (**10**).

The leaf exudates displayed simpler flavonoid profiles in comparison to exudates of flower heads. They did not contain the simple flavonoids apgenin and luteolin. The leaf exudates of *C. davidovii* and *C. parilica* exhibited similar flavonoid profiles and they differed only in two flavonoids. *C. davidovii* yielded in addition scutellarein 6-methyl ether and scutellarein 6,7,4'-trimethyl ethers (Table 1).

The identified compounds are in accordance with those reported earlier for other representatives of sect. *Lepteranthus* (Formisano & al. 2012). In contrast to the subfamily *Asteroideae* within subfamily *Cichorioideae*, reports on external flavonoid accumulation are scanty. Only a few taxa have been studied for accumulation of surface flavonoids. The present results confirm the earlier outlined tendency for formation of highly methylated derivatives: 6-substituted flavones and flavonols of representatives of subfamily *Cichorioideae* (Wollenweber & Valant-Vetschera 1996; Valant-Vetschera & Wollenweber 2007). The differences in flavonoid profiles distinguish *C. stenolepis* from the endemics *C. davidovii*

and *C. parilica*. Whereas *C. stenolepis* accumulates methylated derivatives of 6-hydroxyflavonols, the endemics species produce predominantly methylated derivatives of 6-hydroxyflavones. The flavonoid profiles of *C. davidovii* and *C. parilica* were found to be very similar. Besides in their genetic relation, we have looked for an explanation of this similarity also in the fact that they grow in similar habitats – the alpine regions of Stara Planina and Mt. Slavyanka. It has been repeatedly established that surface flavonoids accumulate in plants which grow in arid, semiarid and alpine habitats, which probably is related to the importance of flavonoids as a UV screen and their help in adaptation of plants to environmental stress factors (Cuadra & al. 1997; Williams & al. 1997; Valant-Vetschera & Wollenweber 2001). Further studies are needed to clarify the taxonomic relations between *Centaurea* species and the degree of influence of habitat conditions on the production of surface flavonoids.

Conclusion

The exudates of *Centaurea davidovii* and *C. parilica* were found to produce mainly 6-O-substituted flavones, methyl derivatives of scutellarein and 6-hydroxyluteolin, while the exudates of *C. stenolepis* yielded 6-O-substituted flavonols: methyl derivatives of 6-hydroxykaempferol and quercetagetin, as well as flavonols with 3-methylation. The present work offers for the first time data about composition of surface flavonoids in *Centaurea stenolepis* and the local endemics *Centaurea davidovii* and *C. parilica*.

Acknowledgements. The study was completed within the framework of the project Investigation of the Flora and Vegetation in Bulgaria, financed by Bulgarian Academy of Sciences. For identification of the taxa, support was provided by the SYNTHESYS Project http://www.synthesys.info/ which is financed by the European Community Research Infrastructure Action under the FP7 Capacities Program (FR-TAF-2466 and AT-TAF-2468).

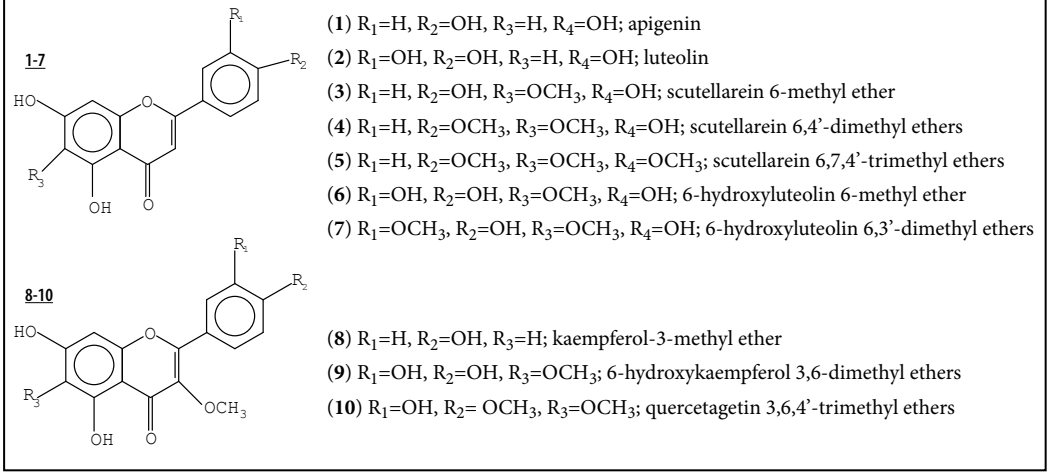

Fig. 1. Structures of the identified flavonoid aglycones (**1-10**).

Table 1. Flavonoid aglycones of *Centaurea* species.

Flavonoid aglycones	*Centaurea* species					
	C. davidovii		*C. parilica*		*C. stenolepis*	
	Flower	Folia	Flower	Folia	Flower	Folia
Apigenin (1)	×		×		**trace**	
Luteolin (**2**)	×		**trace**			
Scutellarein 6-methyl ether(**3**)		×				
Scutellarein 6,4'dimethyl ethers (**4**)	×	×	×	×		
Scutellarein 6,7,4'-trimethyl ethers (**5**)	**trace**	×				
6-hydroxyluteolin 6-methyl ether (**6**)	×	×	×	×	×	×
6-hydroxyluteolin-6,3'-dimethyl ethers (**7**)	**trace**	×	×	×		
Kaempferol 3-methyl ether (**8**)	×		**trace**		**trace**	
6-hydroxykaempferol 3,6-dimethyl ethers (**9**)					×	**trace**
Quercetagetin 3,6,4'-trimethyl ethers (**10**)					×	×

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