Institute of Environmental Research, University of Dortmund, Germany

Phytochemical analysis of nine *Hypericum* L. species from Serbia and the F.Y.R. Macedonia

A. Smelcerovic, M. Spiteller

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Michael Spiteller, Institute of Environmental Research, University of Dortmund, Otto-Hahn-Str. 6, 44221 Dortmund, Germany

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The methanol extracts of the aerial parts of nine *Hypericum* species (*H. barbatum*, *H. hirsutum*, *H. linarioides*, *H. maculatum*, *H. olympicum*, *H. perforatum*, *H. richeri*, *H. rumeliacum* and *H. tetrapterum*), collected on different locations in Serbia and the F.Y.R. Macedonia, were obtained by accelerated solvent extraction (ASE) and analyzed for the content of four constituents (hyperoside, quercitrin, hyperforin and hypericin) by LC-MS/MS. All studied extracts contained the characteristic four constituents, but their contents varied between different species and locations. The content of hypericin in *H. barbatum* was significantly higher (3.9 times) than that in *H. perforatum*.

Plants of the genus *Hypericum* have been used as traditional medicinal plants in various parts of the world (Yazaki and Okada 1994). Their main constituents are naphthodianthrones, primarily represented by hypericin and pseudohypericin, flavonoids, such as hyperoside, rutin or quercitrin, and as the third group phloroglucinol derivatives, especially hyperforin and adhyperforin (Nahrstedt and Butterweck 1997). With regards to the active component concentrations in genus *Hypericum*, significant differences are evident between species (Umek et al. 1999; Kitanov 2001), within populations of the same species from different locations (Buter et al. 1998; Kartnig et al. 1989), between the different ontogenesis phases (Tekelova et al. 2000), in cell cultures (Kartnig et al. 1996) and even in individual plants derived from the same clone and grown under the same conditions (Cellarova et al. 1994).

The aim of the present study was to determine and compare the methanol extract compositions of nine wild-growing *Hypericum* species (*H. barbatum*, *H. hirsutum*, *H. linarioides*, *H. maculatum*, *H. olympicum*, *H. perforatum*, *H. richeri*, *H. rumeliacum* and *H. tetrapterum*) from Serbia and the F.Y.R. Macedonia. Data on the phytochemical analyses of nine investigated species already exist (Kartnig et al. 1996; Kitanov 2001; Maffi et al. 2001; Maggi et al. 2004; Umek et al. 1999), while no HPLC analyses of active compounds from *H. linarioides* and *H. rumeliacum* have hitherto been published. The optimal conditions for ASE extraction are 40 °C and 100 bar, with methanol as extraction solvent (Morf et al. 1998) and in the present investigation the extracts were obtained the same way. The compositions of extracts was analysed by a sensitive LC-MS/MS method, modified from Ganzera et al. (2002).

All studied extracts contained hyperoside, quercitrin, hypericin and hyperforin, but their content varied in samples from different species and locations (Table). For the first time, hyperforin was found in *H. barbatum*, *H. hirsutum*, *H. linarioides*, *H. maculatum*, *H. olympicum* and *H. rumeliacum*. There exists a strong correlation of secondary metabolite contents and the infrageneric classification of Robson (1977) among the nine *Hypericum* species from Serbia and the F.Y.R. Macedonia. The highest content of hypericin was observed in the extracts of *H. barbatum, H. richeri* and *H. rumeliacum* (section *Drosocaprium*). The highest content of hyperforin was observed in the extracts of *H. maculatum*, *H. perforatum* and *H. tetrapterum* (section *Hypericum*). The extracts of *H. hirsutum* and *H. linarioides* (section *Taeniocarpium*) were characterised by a low content of hyperforin. The extract of *H. olympicum* (section *Olympia*) has a low content of the investigated active compounds.

The content of hypericin in *H. barbatum* (Fig.) was significantly higher (3.9 times) than in *H. perforatum*, suggesting that *H. barbatum* may represent an alternative to *H. perforatum* in phytotherapeutical preparations. Our results are in agreement with the study of Kitanov (2001), who reported that the total hypericin content in *H. barbatum* is 2.4 times higher than in *H. perforatum*.

Table: Contents of secondary metabolites (mg/g dry weight) in the *Hypericum* species from Serbia and the F.Y.R. Macedonia

Section		*Drosocarpium*			*Hypericum*			*Taeniocarpium*		*Olympia*
Species (No. of samples)		*H. barbatum* (3)	*H. richeri* (1)	*H. rumeliacum* (4)	*H. maculatum* (3)	*H. perforatum* (1)	*H. tetrapterum* (3)	*H. hirsutum* (2)	*H. linarioides* (1)	*H. olympicum* (1)
Hyperoside	avr.	1.32	1.75	0.35	6.71	3.04	4.16	1.24	1.95	0.25
	min.	0.21		0.24	6.37		2.97	0.55		
	max.	2.23		0.44	7.30		6.52	1.92		
Quercitrin	avr.	1.42	0.28	1.22	0.24	0.80	1.24	0.30	0.10	0.39
	min.	0.29		1.05	0.22		0.68	0.25		
	max.	3.65		1.44	0.26		1.58	0.35		
Hyperforin	avr.	0.07	0.36	0.15	0.18	3.55	0.27	0.05	t	0.02
	min.	t		0.03	t		t	t		
	max.	0.22		0.30	0.43		0.49	0.09		
Hypericin	avr.	0.66	0.45	0.23	0.07	0.17	0.15	0.25	0.04	0.05
	min.	0.21		0.14	0.04		0.13	0.08		
	max.	0.97		0.29	0.12		0.19	0.42		

avr. = average content of the compound; min. = minimal content of the compound; max. = maximal content of the compound; t = less than 0.01 mg/g

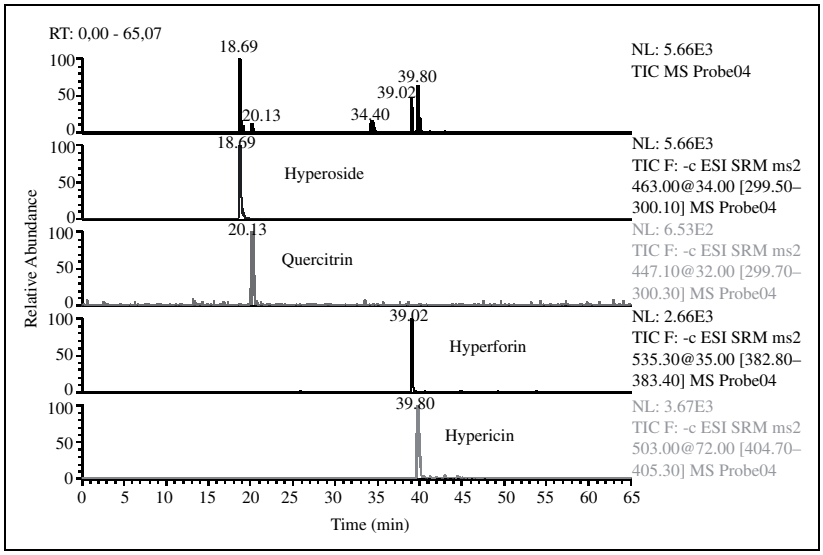

Fig.:
LC-MS/MS chromatogram of *Hypericum barbatum* Jacq. extract

Experimental

The plants were collected at bloom stage from different localities in Serbia and the F.Y.R. Macedonia. The voucher specimens were deposited in the Herbarium Moesicum Doljevac (Serbia).

ASE was performed using a Dionex (Sunnyvale, CA) ASE 200 accelerated solvent extractor. All extractions were performed using 0.5 g dry plant material. ASE conditions were as follows: extraction solvent, methanol; temperature, 40 °C; pressure, 100 bar; 4 cycles with static extraction time of 5 min; flush volume, 60%; final solvent volume 30 mL. The extracts were stored in the dark at 4 °C.

HPLC analysis of the extracts was performed using a Dionex HPLC system, equipped with a UVD 340 s photodiode array detector. Separations were performed on a Luna 3 μ C18 100 Å column (150 × 2 mm, 3 μm particle size) from Phenomenex (Torrance, CA). The mobile phase consisted of 10 mM ammonium acetate buffer adjusted to pH 5.0 with glacial acetic acid (A) and a 9 : 1 mixture of acetonitrile and methanol (B). Gradient elution was performed using the following solvent gradient: from 87A/13B in 10 min to 83A/17B, then in 25 min to 10A/90B and in 5 min to 100B; each run was followed by an equilbration period of 10 min. The eluent flow rate was 0.25 mL/min and the injection volume was 10 μL. All separations were performed at 24 °C. Mass spectra were obtained using a TSQ 7000 mass spectrometer (Thermo Finnigan, Bremen, Germany) equipped with an ESI source operating in negative mode. External calibration was performed in the range of 1 to 20 μg/mL for hyperoside and quercitrin; 0.5 to 20 μg/mL for hyperforin; and 0.5 to 10 μg/mL for hypericin. Standard solutions were stored in the dark at 4 °C. All procedures were carried out under light protection. Within the range of concentrations injected the detector response (peak area) was linear.

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