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Positive correlations between hypericin and putative precursors detected in the quantitative secondary metabolite spectrum of *Hypericum*

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ABSTRACT

A spectrum of eight pharmacologically important secondary compounds, all putatively belonging to the polyketide pathway (hypericin, pseudohypericin, emodin, hyperforin, hyperoside, rutin, quercetin, and quercitrin) were analyzed in several hypericin-producing species of *Hypericum* by LC–MS/MS. Different organs such as leaves, stems and roots of wild-grown plants of *Hypericum hirsutum* L., *Hypericum maculatum* Crantz s. l., *Hypericum montanum* L., *Hypericum tetrapterum* Fr. collected in Slovakia and of *Hypericum perforatum* L. collected in India were examined individually. Highest contents of hypericin, pseudohypericin, and emodin were found in *H. montanum*, suggesting that there are alternative species to *H. perforatum* with high pharmaceutical value. Amounts of hyperforin and quercetin were highest in *H. perforatum*, whereas highest contents of hyperoside and quercitrin were found in *H. maculatum*. A significant positive correlation between hypericin and pseudohypericin as well as between hypericin and emodin was observed by Kruskal's multidimensional scaling (MDS), indicating a parallel enhancement of emodin as a common precursor in the biosynthetic pathways of hypericin and pseudohypericin. Furthermore, MDS combined with principal component analysis (PCA) revealed strong correlations in the occurrence of pseudohypericin and emodin, pseudohypericin and quercitrin, hypericin and quercitrin, emodin and quercitrin, hyperoside and quercitrin, rutin and quercetin, and, hyperforin and quercetin. On the other hand, rutin showed a negative correlation with emodin as well as with quercitrin. Furthermore, hierarchical agglomerative cluster analysis (HACA) clustered hypericin and pseudohypericin, grouping emodin at equal distance from both. Considerable infraspecific variability in secondary compound spectrum and load of different populations of *H. maculatum* from Slovakia underscores the need for detailed studies of genotypic variation and environmental factors in relation to polyketide biosynthesis and accumulation.

1. Introduction

Various species of the genus *Hypericum* have long been used as medicinal plants in various parts of the world due to their therapeutic efficacy (Yazaki and Okada, 1994). Their main constituents are napthodianthrones, primarily represented by hypericin and pseudohypericin, flavonoids such as hyperoside, rutin, quercetin and quercitrin, and a third group of phloroglucinol derivatives such as hyperforin and adhyperforin (Nahrstedt and Butterweck, 1997). With regard to concentrations of the active components, significant differences are evident between different species of *Hypericum* (Umek et al., 1999; Kitanov, 2001), between different populations of the same species from different localities (Buter et al., 1998; Kartnig et al., 1989), between different ontogenetic phases of the same individual (Tekelova et al., 2000), in cell cultures (Kartnig et al., 1996), and even between different plants regenerated from the same *in vitro* cultivated clone and grown under the same conditions (Cellarova et al., 1994). The genus *Hypericum* is monophyletic and belongs to the family Hypericaceae that was shown to be distinct from Clusiaceae in molecular phylogenetic analysis (Korotkova et al., in press). *Hypericum* comprises more than 400 species of herbs, shrubs and even trees (Robson, 2006).

More recently, a series of detailed studies on the secondary metabolite contents of different *Hypericum* species were performed that included a number of species from Serbia (Smelcerovic et al., 2004, 2006a,b, 2007; Gudzic et al., 2007; Glisic et al., 2008), Macedonia (Smelcerovic and Spiteller, 2006), Tenerife (Bonkanka et al., 2008), and Turkey (Spiteller et al., 2008; Smelcerovic et al., 2008). Furthermore, studies have been made on the very important and commercially recognized *Hypericum perforatum* L. from India (Buter et al., 1998; Verma et al., 2008). Nevertheless, the exact patterns of distribution of the main phytochemicals are not fully understood. It is not clear in how far the spectrum and the

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concentration of hypericin and related secondary compounds relates to certain genotypes within species, whether there are pronounced differences of this spectrum between different species, and whether there are clear differences in secondary compound accumulation in different aerial (leaves, stems, etc.) and non-aerial (roots) organs. It is also not understood in how far environmental factors influence the spectrum of secondary compounds and their expression. A major challenge for such comparative studies is to ensure that identical and reliable methods of secondary compound analysis are employed that ensure comparability of chemical data. Robust comparative data are needed to understand the production of secondary compounds by the plant in both a phylogenetic (evolution of biosynthetic pathways) and an environmental (factors influencing expression of metabolites) context. From a pharmaceutical point of view, a characterization of the spectrum of secondary compounds in *Hypericum* is needed, as several different molecules in fact constitute bioactive compounds.

The objective of the present study was to determine a broad spectrum of phytochemical constituents of wild-grown *Hypericum* species in different plant organs and to evaluate possible distribution patterns as well as correlations in the accumulation of certain compounds. Compounds for chemical analyses were selected both accounting for their presumed presence in the polyketide pathway like hypericin (Brockmann et al., 1950; Birch, 1967; Thomson, 1957; Schröder, 1997) and due to their pharmacological importance, namely, hypericin, pseudohypericin, emodin, hyperforin, hyperoside, rutin, quercetin, and quercitrin. For each plant sample, extractions were performed with the leaves, stems and roots separately, in organic and aqueous phases, in parallel using highly selective and sensitive LC–MS/MS. Living plants were collected in Slovakia from wild populations of *Hypericum hirsutum* L., *Hypericum montanum* L., *Hypericum tetrapterum* Fr., and of *Hypericum maculatum* Crantz, the latter from four different natural populations, and of *Hypericum perforatum* L., representing a natural population in Jammu and Kashmir, India. The data obtained were subjected to various statistical evaluations, including multivariate analysis (MVA), Kruskal's multidimensional scaling (MDS), principal component analysis (PCA), linear discriminant analysis (LDA), and hierarchical agglomerative cluster analysis (HACA). This study reveals several new correlations in the appearance of certain compounds that appear to be relevant in further elucidating the biosynthetic pathway of especially hypericin and hyperforin in *Hypericum*. Whereas accumulation patterns in different plant organs were previously only known for hypericin in *H. perforatum* (Zobayed et al., 2006), we provide data for a broader spectrum of *Hypericum* species and compounds. Our results are relevant for pharmacological and phytotherapeutic studies utilizing *Hypericum*.

2. Results and discussion

2.1. Phytochemical profiling by multivariate analysis (MVA)

All together, nine different organs and extraction methods were analyzed for eight different plants representing five species of *Hypericum* (Table 1). The concentrations of emodin, rutin, hyperoside, quercetin, quercitrin, hyperforin and hypericin in leaves, stems and roots, both in organic, aqueous and total (organic and aqueous) phases are shown in Table 2. Based on LC–MS/MS analyses, it was revealed that *H. montanum* had the highest contents of hypericin, pseudohypericin, and their probable precursor emodin. Therefore, *H. montanum* might be an alternative to *H. perforatum* that currently is the most widely used species of *Hypericum* world-wide in phytotherapeutic preparations. Nevertheless, it is worth mentioning that any phytomedicine produced from *H. montanum* would evidently have to pass through the necessary regulatory frameworks including detailed toxicological and clinical studies. Hypericin was found in all species, and from all localities, which is in agreement with the study of hypericins in *Hypericum* species from Bulgaria (Kitanov, 2001). Pseudohypericin and emodin were also observed in all the species studied. The highest content of hyperforin was found in *H. perforatum* followed by *H. maculatum* (specimen 3907). This corroborates the previously published data on the content of hyperforin in *H. maculatum* (Smelcerovic and Spiteller, 2006). All the species studied contained hyperforin which is in agreement with the data obtained before (Smelcerovic and Spiteller, 2006). However, not all *Hypericum* species contain hyperforin (Umek et al., 1999; Maggi et al., 2004). *H. maculatum* (specimen 3907) also contained the highest amount of rutin. The highest contents of hyperoside and quercitrin were found in individuals of *H. maculatum* from two Slovakian populations located in close proximity (specimens 3907 and 3908). The greatest amount of quercetin was found in *H. perforatum*, which is in line with a previous investigation (Smelcerovic et al., 2008).

Multivariate analysis (MVA) of the LC–MS/MS data was carried out to evaluate the individual and holistic phytochemical variability due to differences between categories, namely, the different plant species, the different plant parts (leaves, stem and roots), as well as the organic and aqueous phases (Fig. 1). From the MVA, it was evident that the highest contents of hypericin, pseudohypericin, emodin, hyperforin, and quercitrin in the respective species of *Hypericum* were present only in the organic extracts of the leaves. However, for the stronger polar molecules of rutin, hyperoside, and quercetin, the highest contents were found in the combined organic and aqueous extracts of the leaves. Computation of the individual averages for each category revealed the

Table 1
Locality and voucher information of the *Hypericum* species studied. Specimens of the Slovakian plants are deposited in the herbarium of the Botanical Garden Berlin-Dahlem and of the Indian material in the herbarium of the Indian Institute of Integrative Medicine (IIIM), Canal Road, Jammu 180 001, India.

Plant taxon	Voucher number	Collection site
Hypericum perforatum L.	112/IIIM-S	*India*, Harwan, Jammu & Kashmir, (34°07′ N, 74°52′ E, 1587 m altitude), 10 km from Srinagar, rocky vegetation
Hypericum maculatum Crantz s. l.	T. Borsch & J. Kosuth 3900	*Slovakia*, Spisska Tomašovca N of Kosice, (48°56′ N, 20°27′ E, 620 m NN), meadow vegetation
Hypericum hirsutum L.	T. Borsch & J. Kosuth 3901	*Slovakia*, Spisska Tomašovca, N of Kosice, (48°54′ N, 20°27′ E, 620 m NN), Abies-Fagus forest
Hypericum maculatum Crantz s. l.	T. Borsch & J. Kosuth 3902	*Slovakia*, Tatra Mountains, Térry Cottage (49°11′ N, 20°12′ E, 1680 m NN), tall herb vegetation close to stream
Hypericum montanum L.	T. Borsch & J. Kosuth 3903	*Slovakia*, hillside N of Kosice (48°44′ N, 21°13′ E, 327 m NN), Querco-Carpinetum forest
Hypericum tetrapterum Fr.	T. Borsch & J. Kosuth 3913	*Slovakia*, valley close to Nová Sedlica (49°01′ N, 22°20′ E, 306 m NN), Scirpus-dominated swampy tall herb vegetation
Hypericum maculatum Crantz s. l.	T. Borsch & J. Kosuth 3908	*Slovakia*, Hačava, (48°40′ N, 20°49′ E, 440 m NN), meadow vegetation
Hypericum maculatum Crantz s. l.	T. Borsch & J. Kosuth 3907	*Slovakia*, Hačava, (48°40′ N, 20°52′ E, 460 m NN), meadow vegetation