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Hypericin and Pseudohypericin Contents in Some *Hypericum* Species Growing in Turkey

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Abstract

Hypericin and pseudohypericin content in stems, leaves, and flowers of some *Hypericum* species growing in Turkey, namely, *H. heterophyllum* Vent, *H. hyssopifolium* L., *H. linarioides* Bosse, *H. monbretii* Spach, *H. orientale* L., *H. origanifolium* Willd., *H. perforatum* L., *H. scabrum* L., and *H. triquetrifolium* Turra, was determined by HPLC. Hypericin and pseudohypericin were detected in all species tested except for *H. heterophyllum*, and the presence in *H. orientale* and *H. scabrum* was reported by us for the first time in the current study. Hypericin and pseudohypericin contents observed in the study varied greatly depending on species and plant tissues. The lowest levels of hypericin and pseudohypericin were detected in leaves of *H. hyssopifolium* [0.030 and 0.051 mg/g dry weight (DW), respectively] whereas flowers of *H. montbretii* produced the highest levels of both hypericin forms (2.52 mg/g DW hypericin and 3.58 mg/g DW pseudohypericin). *H. montbretti* and *H. triquetrifolium* were found to be superior over *H. perforatum* with regard to hypericin and pseudohypericin content, encouraging the cultivation and biological evaluation of these species in Turkey.

Keywords: HPLC, hypericin, *Hypericum* species, pseudohypericin.

Introduction

The genus *Hypericum* contains approximately 400 different species of annuals, perennials, shrubs, and small trees, ranging from very small perennials to trees. The species of this genus have been used as healing agents for hundreds of years due to their various medicinal properties (Dias et al., 1998). *Hypericum* species are also used as sedatives, antiseptics, and antispasmodics in Turkish folk medicine (Baytop, 1999). The *Hypericum* genus (Guttiferae) is represented in Turkey by 89 species of which 43 are endemic. The most abundant and well-known species is *H. perforatum* (Davis, 1988).

The methanol extract from the aerial parts of several *Hypericum* species has been reported to contain at least 10 classes of bioactive compounds, namely, the naphthodianthrones hypericin and pseudohypericin (Kitanov, 2001), the phloroglucinol derivatives hyperforin and adhyperforin (Maggi et al., 2004; Smelcerovic et al., 2006), flavonoids (Radusiene et al., 2004), phenylpropanes (Chandrasekera et al., 2005), essential oils (Bertoli et al., 2003), amino acids (Karryev & Komissarenko, 1980), xanthones (Hong et al., 2004; Tanaka & Takaishi, 2006), tannins (Dall'Agnol et al., 2003), procyanidins and other water-soluble components (Greeson et al., 2001), which possess a wide array of biological properties (Patocka, 2003).

Many pharmacological activities of *Hypericum* extracts appear to be attributable to their hypericins and hyperforin content (Barnes et al., 2001). The naturally occurring red pigments, hypericin and pseudohypericin, have been reported to exhibit important biological activities, namely photodynamic, antiviral, antiretroviral, antibacterial, antipsoriatic, antidepressant, and antitumoral activities (Bombaradelli & Morazzoni, 1995; Gadzovska et al., 2005; Guedes & Eriksson, 2005). The photodynamic and photocytotoxic properties of hypericins allow them to acts as antiviral agents, indicating their possible use in the treatment of human immunodeficiency virus type 1 (HIV-1) (Meruelo et al., 1988) and cancer (Agostinis et al., 2002). However, it should be noted that results from recent studies have indicated hyperforin, rather than hypericins, as the main chemical responsible for antidepressant effects of *Hypericum* extracts (Chatterjee et al., 2001; Roz

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& Rehavi, 2004). Hypericins have been found only in *Hypericum* species, thus are chemotaxonomically important for the infrageneric classification of *Hypericum* genus (Kitanov, 2001). Although hyperforin is a major component occurring in concentrations of 2–4% of the total extract of *H. perforatum*, hypericins remain the popular marker substances for the standardization of the herbal product because of its instability in the presence of oxygen and light (Medina et al., 2006; Nahrsedt & Butterweck, 1997). But still, hyperforin has been recommended as one of the marker compounds for the routine standardization of St. John's wort products (Gerlie & Koda, 2001). Thus, hypericins, together with hyperforin, have importance from a quality control point-of-view. Because of these reasons, many individual or groups of species of *Hypericum* have been investigated for the presence of hypericins (Alali et al., 2004; Ayan et al., 2004; Çırak, 2006; Çırak et al., 2006; Ferraz et al., 2002; Martonfi & Repcak, 2004; Piovan et al., 2004; Radusiene et al., 2004).

In the current study, the aim was to determine hypericin and pseudohypericin content in stems, leaves, and flowers of some *Hypericum* species growing in Turkey, namely, *H. heterophyllum* Vent, *H. hyssopifolium* L., *H. linarioides* Bosse, *H. monbretii* Spach, *H. orientale* L., *H. origanifolium* Willd., *H. perforatum* L., *H. scabrum* L., and *H. triquetrifolium* Turra.

Materials and Methods

Experimental procedures

Hypericum plants were collected between August and September 2004 from three sites in northern Turkey: Maçka (40°49′ N; 39°37′E; 270 m sea level), Erbaa (40°41′N; 36°34′E; 230 m sea level), and Kastamonu (41°24′N; 33°45′E; 790 m sea level), and identified by Dr. Hasan Korkmaz, Department of Biology, University of 19 Mayis, Samsun, Turkey. Voucher specimens were deposited in the herbarium of Ondokuz Mayis University Agricultural Faculty. Sampling was randomized from plant crowns that had at least three stems. The top one-third of the crown was harvested between 12:00 AM and 13:00 PM at full flowering stage. Conditions on the day of collection were clear and sunny at all the sites. Temperatures ranged from 24°C to 35°C. The plant materials were dried at room temperature (20 ± 2°C). After being air-dried, samples were dissected into tissue parts and subsequently assayed for hypericin and pseudohypericin contents.

Hypericin and pseudo hypericin analysis

Chemicals

Reference standards of hypericin and pseudohypericin were purchased from ChromaDex, Inc. (Laguna Hills, CA, USA). The high-performance liquid chromatography (HPLC)-grade acetonitrile, acetone, and methanol were purchased from Caledon (Mississauga, ON, Canada). Triethylammonium acetate is a product of Sigma-Aldrich Canada (Oakville, ON, Canada).

Extraction and HPLC analysis of hypericin and pseudohypericin

The isolation and analysis method for hypericin and pseudohypericin was done according to previously published protocols (Much et al., 2002). Briefly, the plant tissues were ground into fine powder with a laboratory mill, and approximately 100 mg of powdered plant sample was transferred into an amber-colored 20-mL vial. Extracts were prepared in 5 mL acetone:methanol (50:50, v/v) with 30-min sonification. Samples were centrifuged at 3000 rpm for 10 min, filtered using a 0.2-μm nylon syringe filter, and 500-μL aliquots of each sample were transferred into a clear glass autosampler vial. Clear glass vials were exposed to a light source for 30 min to complete the conversation of the proto forms of hypericin and pseudohypericin before analysis. A 20-μL sample of the extract was injected into a Shimadzu 10AD HPLC system (Japan) consisting of an SCL-10A system controller, SIL-10A autoinjector, SPD-M 10AV photodiode array detector at 588 nm, and a CTO-10A column oven with separation on a Phenomenex Hypersil C_{18} column (3.0 μm; 4.6 × 100 mm) with a C_{18} guard column (4 × 3 mm). The analyses were separated isocritically using a mobile phase of 0.1 M triethylammonium acetate and acetonitrile (33:67, v/v) at a flow rate of 1 mL min^{-1}. Calibration curves ($r^2 > 0.989$) were used for quantification of each compound. The limit of detection of hypericin and pseudohypericin was 0.1 μg/mL. Recovery of hypericin was above 91%, and pseudohypericin was consistently recovered at greater than 65%.

Results and Discussion

Hypericin and pseudohypericin were detected in all species tested except for *H. heterophyllum*, Abundance of these compounds varied among species and tissues (Table 1). Higher quantities of pseudohypericin were observed in both leaves and flowers of *H. montbretii* and *H. triquetrifolium*, whereas hypericin content was higher than that of pseudohypericin in both tissues of *H. orientale*, *H. origanifolium*, and *H. scabrum*. In addition, leaves accumulated higher amounts of pseudohypericin than hypericin although, in general, flowers were found to have higher levels of hypericin in *H. hyssopifolium* and *H. perforatum*. Among different plant parts, flowers were the main storage for both compounds, and hypericin and pseudohypericin were detected only in flowers in the case of *H. linarioides*. Exceptionally, the highest accumulation of hypericin and pseudohypericin was observed in leaves of *H. triquetrifolium* and *H. perforatum*. Likewise, in all earlier reports, the floral parts have the highest hypericin concentrations in *H. perforatum* L. (Büter et al., 1998; Sirvent et al., 2002), *H. maculatum* Crantz

Table 1. Hypericin and pseudohypericin content in stems, leaves, and flowers of some *Hypericum* species growing in Turkey.

Species	Voucher numbers	Hypericin (mg/g DW)			Pseudohypericin (mg/g DW)		
		Stem	Leaf	Flower	Stem	Leaf	Flower
H. heterophyllum	OMUZF #127	—	—	—	—	—	—
H. hyssopifolium	OMUZF #128	—	0.030	0.139	—	0.051	0.075
H. linarioides	OMUZF #129	—	—	0.101	—	—	0.109
H. montbretii	OMUZF #130	—	0.439	2.52	—	0.708	3.58
H. orientale	OMUZF #131	—	0.142	0.645	—	0.076	0.226
H. origanifolium	OMUZF #132	—	0.096	0.394	—	0.072	0.079
H. perforatum	OMUZF #61/2	0.050	2.03	0.830	0.050	1.24	0.51
H. scabrum	OMUZF #133	—	0.041	0.081	—	0.034	0.074
H. triquetrifolium	OMUZF#134	0.020	2.07	0.822	0.046	2.47	1.65

(Radusiene et al., 2004), *H. pruinatum* Boiss, and Bal. and *H. aviculariifolium* Jaup. and Spach subsp. depilatum (Freyn and Bornm.) Robson var. depilatum (Çırak et al., 2006). Also, as observed in the current study, leaves were reported to have higher amount of hypericin than floral parts in *H. triquetrifolium* Turra (Alali et al., 2004). Generally, stems produced neither hypericin nor pseudohypericin, and these compounds were detected only in stem tissues of *H. triquetrifolium* and *H. perforatum*.

Hypericin and pseudohypericin contents observed in the current study varied greatly depending on species and plant tissues. The lowest levels of hypericin and pseudohypericin were detected in the leaves of *H. hyssopifolium* (0.030 and 0.051 mg/g DW, respectively), whereas flowers of *H. montbretii* produced the highest levels of both hypericin forms (2.52 mg/g DW hypericin and 3.580 mg/g DW pseudohypericin). When compared with *H. perforatum*, a well-known and commercial source of hypericins, moderate quantities of these compounds were established in *H. linarioides*, *H. orientale*, and *H. origanifolium*, whereas the lowest quantities were observed in *H. hyssopifolium* and *H. scabrum*. It is important to note that *H. montbretti* and *H. triquetrifolium* were found to be superior over *H. perforatum* with regard to hypericin and pseudohypericin content.

In the current study, it is the first time we have reported the presence of hypericin and pseudohypericin in *H. orientale* and *H. scabrum*. However, it should be noted that our results for some reinvestigated species did not confirm the results from previous studies. For example, no hypericin or pseudohypericin was detected in *H. scabrum* by Kitanov (2001) or in *H. orientale* by Ayan et al. (2004). Hypericin content in flowering plants of *H. triquetrifolium* was reported as 0.9 (Kitanov, 2001) and 3.6 mg/g DW (Alali et al., 2004). Maggi et al. (2004) reported *H. hyssopifolium* to contain hypericin at 10.29 mg/g DW level. Our results for hypericin content of the same species are either higher or lower than those of this preceding work. The differences between the results from current and previous studies on the same species of *Hypericum* in terms of hypericin content may be due to the different methods used for chemical analysis, geographical and ecological factors, climatic conditions prevailing on sampling sites, and plant growth stages in which plants are harvested.

Conclusions

Considering the pharmacological significance of hypericins, their possible use in therapeutics, and taxonomic value for infrageneric classification of *Hypericum*, it is important to find other sources of these natural compounds. At this point, the high hypericin and pseudohypericin content of *H. montbretii* and *H. triquetrifolium*, as well as *H. perforatum*, encourages the cultivation and biological evaluation of these species in Turkey. The results also indicated that the quantitative variation in hypericin and pseudohypericin content of Turkish species of *Hypericum* evaluated here allows the selection of the best plant samples for their cultivation and conservation in field collections. Therefore, wild populations of these *Hypericum* species are potentially important sources for breeding and improvement of the cultivated varieties.

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