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Detection of Hyperforin in Turkish Species of *Hypericum* (Guttiferae)

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ABSTRACT

In the present study, six *Hypericum* species from the Turkish flora were investigated for the presence of hyperforin namely *H. heterophyllum* Vent, *H. hyssopifolium* L., *H. linarioides* Bosse, *H. orientale* L., *H. scabrum* L. and *H. triquetrifolium* Turra. For this purpose, the aerial parts were collected at full flowering, dissected into floral, leaf and stem tissues, air-dried at room temperature and then assayed for hyperforin by HPLC. Hyperforin was detected only in flower tissues of *H. hyssopifolium* (29.2 mg/g DW) and *H. linarioides* (6.28 mg/g DW). This data could be useful for elucidation of the chemotaxonomical significance of hyperforin and for the phytochemical evaluation of *H. hyssopifolium* and *H. linarioides*.

Keywords: HPLC, *Hypericum hyssopifolium*, *Hypericum linarioides*

INTRODUCTION

Hypericum is a large genus of herbs or shrubs which grow in temperate regions of the world and the species belonging to this genus have been used as traditional medicinal plants due to their various medicinal properties for hundred of years (Demirci *et al.* 2005). In particular, extracts of *Hypericum perforatum* L. are now widely used in Europe as a drug for the treatment of depression (Patocka 2003). The *Hypericum* genus of *Guttiferae* is represented in Turkey by 89 species of which 43 are endemic (Davis 1988).

Hypericum plants have been reported to contain many bioactive secondary metabolites from different classes namely naphthodianthrones, phloroglucinols, flavonoids, phenylpropanes, essential oils, amino acids, xanthones, tannins, procyanidins and other water-soluble components which possess a wide array of biological properties (Greeson *et al.* 2001; Kitanov 2001; Çırak *et al.* 2006; Tanaka and Takaishi 2006).

Many pharmacological activities of *Hypericum* extracts appear to be attributable to their hypericins and hyperforin content (Barnes *et al.* 2001). Results from recent studies have indicated hyperforin as the main chemical, responsible for antidepressant effects of *Hypericum* extracts (Roz and Rehavi 2004). It also exhibits anti-inflammatory (Feisst and Werz 2004), antitumoral (Schwarz *et al.* 2003) and antiangiogenic (Dona *et al.* 2004) effects. It has been recommended as a marker compound for the routine standardization of *Hypericum* products (Gerlie and Koda 2001). Due to these reasons, many species of *Hypericum* have been investigated for the presence of hyperforin, but only a few of them were reported to contain this compound (Kirakosyan *et al.* 2003; Maggi *et al.* 2004; Piovan *et al.* 2004; Klingauf *et al.* 2005; Martonfi *et al.* 2006; Smelcerovic *et al.* 2006).

In the present study, the aim was to determine hyperforin content in stems, leaves and flowers of some *Hypericum* species growing wild in Turkey namely *H. heterophyllum* Vent, *H. hyssopifolium* L., *H. linarioides* Bosse, *H. orientale* L., *H. scabrum* L. and *H. triquetrifolium* Turra.

MATERIALS AND METHODS

Plant material

The aerial parts of the aforesaid *Hypericum* plants were collected at full flowering in June, 2005 from three sites of 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 (OMUZF # 127-*H. heterophyllum*, OMUZF #128-*H. hyssopifolium*, OMUZF # 129-*H. linarioides*, OMUZF #131-*H. orientale*, OMUZF #133-*H. scabrum* and OMUZF#134-*H. triquetrifolium*). The plant materials were dissected into floral, leaf and stem tissues, air-dried at room temperature and grounded to powder using a laboratory mill, then assayed for hyperforin by HPLC.

Chemicals

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

Extraction and High Performance Liquid Chromatography (HPLC) analysis of hyperforin

The isolation and analysis of hyperforin were done according to protocols reported by Murch *et al.* (2002). About 100 mg sample was transferred into an amber-colored 20 ml vial with 5 ml acetone:methanol (50:50, v:v) and sonicated for 30 min (Ultra-sonic FS-14 Sonicator; Fisher Scientific, Nepean, ON, Canada). The sample was centrifuged at 3000 rpm for 10 min (GS-6 series centrifuge, Beckman Instruments Inc, Palo Alto, CA, USA) and the supernatant was filtered using 0.2 µm nylon syringe filter (Waters Chromatography Inc., Mississauga, ON, Canada). Aliquots of each sample (500 µl) were transferred into a clear glass auto-sampler

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vial, which were sealed with Teflon coated aluminum lids. Amber glass vials were immediately analyzed for hyperforin (within 7 h of extraction). A 20 µl aliquot of sample was injected into a Shimadzu 10AD HPLC system consisting of an SCL-10A system controller, SIL-10A auto injector, SPD-M 10AV photodiode array detector at 270 nm and a CTO-10A column oven (Shimadzu, Canada) 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) (Phenomenex, Torrance, CA, USA). The analyses were separated from the extracts with isocratic flow of 0.1 M triethylammonium acetate and acetonitrile (33:67, v:v) at 1 ml/min. Significant calibration curves ($r^2 > 0.989$) were used for quantification of this compound. The limit of detection of hyperforin was 1.0 µg/ml. Three determinations were done for each sample and the mean value was calculated.

RESULTS AND DISCUSSION

Hyperforin was detected only in flower tissues of *H. hyssopifolium* (29.2 mg/g DW) and *H. linarioides* (6.28 mg/g DW). Previous literature reported hyperforin concentrations in *H. perforatum*, well known and commercial source of hyperforin, between 8.35-150 mg/g DW from different accessions of the world (Kirakosyan *et al.* 2003; Maggi *et al.* 2004). Comparing the results obtained for our plant material with those reported by named authors it may be concluded that lower quantities of this compound were established in *H. yssopifolium* and *H. linarioides* as reported some other authors. Maggi *et al.* (2004) reported hyperforin content of *H. hyssopifolium* as 0.13 mg/g DW and Smelcerovic *et al.* (2006) determined hyperforin accumulation in *H. linarioides* at 0.02 mg/g DW level. The authors used methanolic extract of flowering tops as adopted by us.

Hypericum plants are characterized by the presence of secretory structures, including light glands, dark glands and secretory canals, in which biologically active substances are synthesized and/or accumulated (Ciccarelli *et al.* 2001). The localization of various types of secretory structures varies among plant tissues and flowers with leaves are the main organs for the secretory structures. The level of phytomedicinal compounds present in a particular *Hypericum* tissue depends on the relative abundance of these secretory structures on the harvested material. Hence, high level of hyperforin accumulation in flowers of *H. hyssopifolium* and *H. linarioides* observed in the present study may be attributed to relative abundance of the secretory structures on this tissue (Çırak *et al.* 2007a, 2007b, 2007c; Çırak and Radusiene 2007).

Considering the pharmacological significance of hyperforin and its possible use in therapeutics, it is important to find new sources of its location. Hence, the species of *Hypericum* reported here to contain hyperforin may find important application in medicinal treatment. Such kind of data may also be useful for chemotaxonomical evaluation of this compound.

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