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Pseudohypericin and hyperforin in two Turkish *Hypericum* species: Variation among plant parts and phenological stages

Cüneyt Çırak [a,*], Jolita Radušienė [b], Necdet Çamas [c]

[a] *Faculty of Agriculture, Department of Agronomy, Ondokuz Mayıs University, 55139 Kurupelit, Samsun, Turkey*
[b] *Institute of Botany, Zaliuju ezeru 49, Vilnius LT-08406, Lithuania*
[c] *Ondokuz Mayıs University, Vocational School of Bafra, 55040 Samsun, Turkey*

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Abstract

The genus *Hypericum* has received considerable interest from scientists, as it contains the variety of structurally diverse natural products which possess a wide array of biological properties, mainly hypericins and hyperforin. In the present study, variations of pseudohypericin and hyperforin were investigated in two Turkish species of *Hypericum*, namely *Hypericum perfoliatum* and *Hypericum origanifolium*. Wild growing plants were harvested at vegetative, floral budding, flowering, fresh fruiting and mature fruiting stages, and dissected into stem, leaf and reproductive tissues and assayed for chemical contents by high performance liquid chromatography method. Content of pseudohypericin and hyperforin in samples of the whole plant increased during the course of ontogenesis in both species. The highest levels of the chemicals were reached at full flowering (2.62 mg/g dry weight (DW) pseudohypericin and 1.84 mg/g DW hyperforin for *H. perfoliatum*; 0.93 mg/g DW pseudohypericin and 1.63 mg/g DW hyperforin for *H. origanifolium*). Among different reproductive parts, full opened flowers produced the highest amount of pseudohypericin (1.18 mg/g DW) and hyperforin (4.36 mg/g DW) in *H. origanifolium*. Similarly, the highest pseudohypericin accumulation was observed in full opened flowers in *H. perfoliatum* (7.41 mg/g DW) while floral buds of this species produced the highest amount of hyperforin (7.80 mg/g DW). These data can be useful when elucidating the medicinal properties of the species and the chemosystematic significance of hyperforin and pseudohypericin in the relationships among species of *Hypericum*.

Keywords: HPLC; Hyperforin; *Hypericum origanifolium*; *Hypericum perfoliatum*; Ontogenetic and morphogenetic variation; Pseudohypericin

1. Introduction

Hypericum is a genus of about 400 species of flowering plants belonging to the family Guttiferae (Robson, 1981). Several *Hypericum* species have been used as traditional medicinal plants for hundred of years due to their wound-healing, bactericide, anti-inflammatory, diuretic and sedative properties (Demirci and Baser, 2005). In particular, extracts of *Hypericum perforatum* L. are now widely used in Europe as a drug for the treatment of depression (Patocka, 2003). In Turkey, the genus is represented by 89 species of which 43 are endemic (Davis, 1988).

* Corresponding author. Tel.: +90 362 4576020x1369; fax: +90 362 4576034.
E-mail address: cuneytc@omu.edu.tr (C. Çırak).

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Hypericum species have been reported to contain many bioactive compounds namely naphthodianthrones, phloroglucinols, flavonoids, phenylpropanes, essential oils, amino acids, xanthones, tannins, procyanidins and other water-soluble components that possess a wide array of biological properties (Greeson et al., 2001; Kitanov, 2001; Tanaka and Takaishi, 2006; Smelcerovic et al., 2006).

Many pharmacological activities of *Hypericum* extracts appear to be attributable to their hypericins and hyperforin contents (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 (Guedes and Eriksson, 2005). Hypericins have been found only in *Hypericum* species, thus, are chemotaxonomically important for the infrageneric classification of *Hypericum* genus (Kitanov, 2001). Although most attention has been paid to the pharmacological properties of hypericin, the principal naphthodianthrone in *Hypericum* extracts is pseudohypericin. It was found two to three times more abundant than hypericin in the species of *Hypericum* containing both hypericin forms (Cameron and Raverty, 1976; Çırak et al., 2007a). Hyperforin is a prenylated phloroglucinol derivative that consists of a phloroglucinol skeleton with lipophilic isoprene chains (Medina et al., 2006). 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.

Hypericum perfoliatum L. is a perennial herbaceous plant, usually growing in shady places among rocks. Although it has not been domesticated yet, *H. perfoliatum* is widely distributed in Northern Turkey. The plant has already been used in naturopathic treatment for ear pain in children (Sarrell et al., 2003) and results of current studies have indicated *H. perfoliatum* as a promising medicinal plant for cancer treatment (Couladis et al., 2001). *Hypericum origanifolium* Willd is another member of *Hypericum* genus from Turkish flora. This herbaceous perennial grows in grassy communities of rocky slopes or steppe in Northern Turkey and has great pharmaceutical potential, with its well-documented phenolic and hypericin contents (Çırak et al., 2007b). Both species were reported to contain pseudohypericin by Kitanov (2001). However, hyperforin had not been detected in *H. perfoliatum* and *H. origanifolium*. In our previous studies, morphogenetic and phenological changes in the content of hypericin and several flavonoids in these species were documented (Çırak et al., 2007b,c). In the present study, variations of pseudohypericin and hyperforin were investigated in *H. perfoliatum* and *H. origanifolium*.

2. Materials and methods

2.1. Brief description of plant materials

The plant materials were described in our previous studies (Çırak et al., 2007b,c). The species were identified by Dr. Hasan Korkmaz, Faculty of Science and Art, Department of Biology, Ondokuz Mayis University, Samsun, Turkey. Voucher specimens were deposited in the herbarium of Ondokuz Mayis University, Agricultural Faculty (OMUZF #101 for *H. perfoliatum* and OMUZF #109 for *H. origanifolium*).

2.2. Experimental procedures

The plant material of *H. perfoliatum* and *H. origanifolium* was collected in a dry grassland within the Çakallı district of Samsun province, Turkey (41°04′N; 36°01′E; 470 m above sea level) from April till September 2005. The mean temperature during the sampling period was 18.5 °C, and the total precipitation was 450 mm. The sampling site was not grazed or mown during the plant-gathering period. The material represented 20 randomly gathered plants in five phenological stages: vegetative, floral budding, full flowering, fresh fruiting and mature fruiting. Newly emerged shoots (4–6 weeks old-age) with leaves were harvested at the vegetative stage (April 27, 2005 for both species). For the floral budding stage, only shoots with floral buds were selected (May 20 for *H. origanifolium*; June 10 for *H. perfoliatum*). At the full flowering stage, only shoots with full opened flowers were harvested (June 14 for *H. origanifolium*; June 24 for *H. perfoliatum*). At the fresh fruiting stage, the shoots which had green capsules were harvested (July 5 for *H. origanifolium*; July 25 for *H. perfoliatum*). At the mature fruiting stage, the shoots which had dark brown capsules were harvested (August 10 for *H. origanifolium*; September 10 for *H. perfoliatum*). The top of 2/3 of the plant was harvested between 12:00 am and 13:00 pm. After collected, 10 individuals were kept as whole plants and