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Chemical Constituents of Some *Hypericum* Species Growing in Turkey

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The present study was conducted to determine the content of pharmacologically important constituents hypericin, pseudohypericin, chlorogenic acid, rutin, hyperoside, apigenin-7-O-glucoside, quercitrin, quercetin and vitexin in eight *Hypericum* species namely, *H. aviculariifolium* Jaup. and Spach subsp. *depilatum* (Freyn and Bornm.) Robson var. *depilatum* (endemic), *H. lydium* Boiss., *H. montbretii* Spach, *H. orientale* L., *H. origanifolium* Willd, *H. perfoliatum* L., *H. perforatum* L. and *H. pruinatum* Boiss. and Bal. growing in different locations of Turkey. Wild growing plants were harvested at flowering and after dried subsequently assayed for the constituents by HPLC method. Hyperoside, quercitrin and pseudohypericin were found in all species. Hypericin, quercetin and chlorogenic acid were also detected in all species with the exceptions of *H. orientale* for hypericin, *H. montbretii* for quercetin and *H. lydium* for chlorogenic acid. Apigenin-7-O-glucoside accumulation was observed in all examined species at various levels, except for *H. orientale* and *H. origanifolium*. Rutin was detected in *H. avicularifolium, H. lydium, H. orientale, H. perfoliatum* and *H. perforatum*. On the contrary of the other species, vitexin was found in only *H. motbretii*. The presence of flavonoid vitexin in the genus *Hypericum* was reported for the first time.

Keywords: chlorogenic acid, flavonoids, HPLC, hypericin, pseudohypericin, turkish *Hypericum*

The genus *Hypericum* contains approximately 400 different species of annuals, perennials, shrubs and small trees, ranging from very small perennials to trees belonging to *Guttiferae* family. *Hypericum* species are used as sedatives, antiseptics, and antispasmodics in Turkish folk medicine (Baytop, 1999). The *Hypericum* genus 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 major phytomedicinal compounds of *Hypericum* plants are phloroglucinol derivatives hyperforin and adhyperforin, the naphthodianthrones hypericin and pseudohypericin, the flavonoids hyperoside, rutin, quercitrin, quercetin and biapigenin and the phenylpropanes caffeic acid and chlorogenic acid which possess a wide array of biological properties (Patocka, 2003).

The naphthodianthrones are considered as marker compounds for identification of *Hypericum* species and the pharmacological activities of *Hypericum* extracts namely, photodynamic, artidepressive and antiviral activities are mainly attributed to their hypericin and pseudohypericin contents (Bombardelli and Morazzoni, 1995). Flavonoids, the main components present in *Hypericum* plants, have attracted considerable interest as dietary constituents. Recently results from clinical studies indicate their possible role in preventing cardiovascular diseases and several kinds of cancer (Chu et al., 2000). Although hypericin and hyperforin have been reported to mainly contribute to the pharmacological effects of *Hypericum* extracts, flavonoids have also made an important contribution to the antidepressant activity (Gastpar and Zeller, 2005).

Increased market demand for *Hyperici herba* has led to intensive studies on the chemistry and biological activities of *Hypericum* plants. This is especially true for *H. perforatum*, which is the most common and well known species. Although numerous investigations have been carried out on the chemical composition of *H. perforatum*, comparatively few compounds have been reported from other members of *Hypericum* genus. In the present study, we investigated eight Turkish *Hypericum* species namely, *H. avicularifolium* Jaup. and Spach subsp. *depilatum* (Freyn and Bornm.) Robson var. *depilatum* (endemic), *H. lydium* Boiss., *H. montbretii* Spach, *H. orientale* L., *H. origanifolium* Willd, *H. perfoliatum* L., *H. perforatum* L. and *H. pruinatum* Boiss. and Bal. (Figure 1) for the presence of nine compounds, including naphthodianthrones hypericin and pseudohypericin, phenylpropane chlorogenic acid and flavonoids, as rutin, hyperoside, apigenin-7-O-glucoside, quercitrin, quercetin and vitexin.

MATERIALS AND METHODS

Plant Material

The aerial parts of eight wild species were collected at full flowering from various localities of Northern Turkey. Species were 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 and the numbers of the voucher specimens are given in Table 1. Sampling was done in these wild populations by a randomized collection of 30 individuals. The top 1/3 of the crown was harvested between 12:00 AM and 13:00 PM. Conditions on the day of collection were clear and sunny at the all sites and the temperature ranged from 24 to 35°C. The plant material was dried at room temperature (20±2°C) and subsequently assayed

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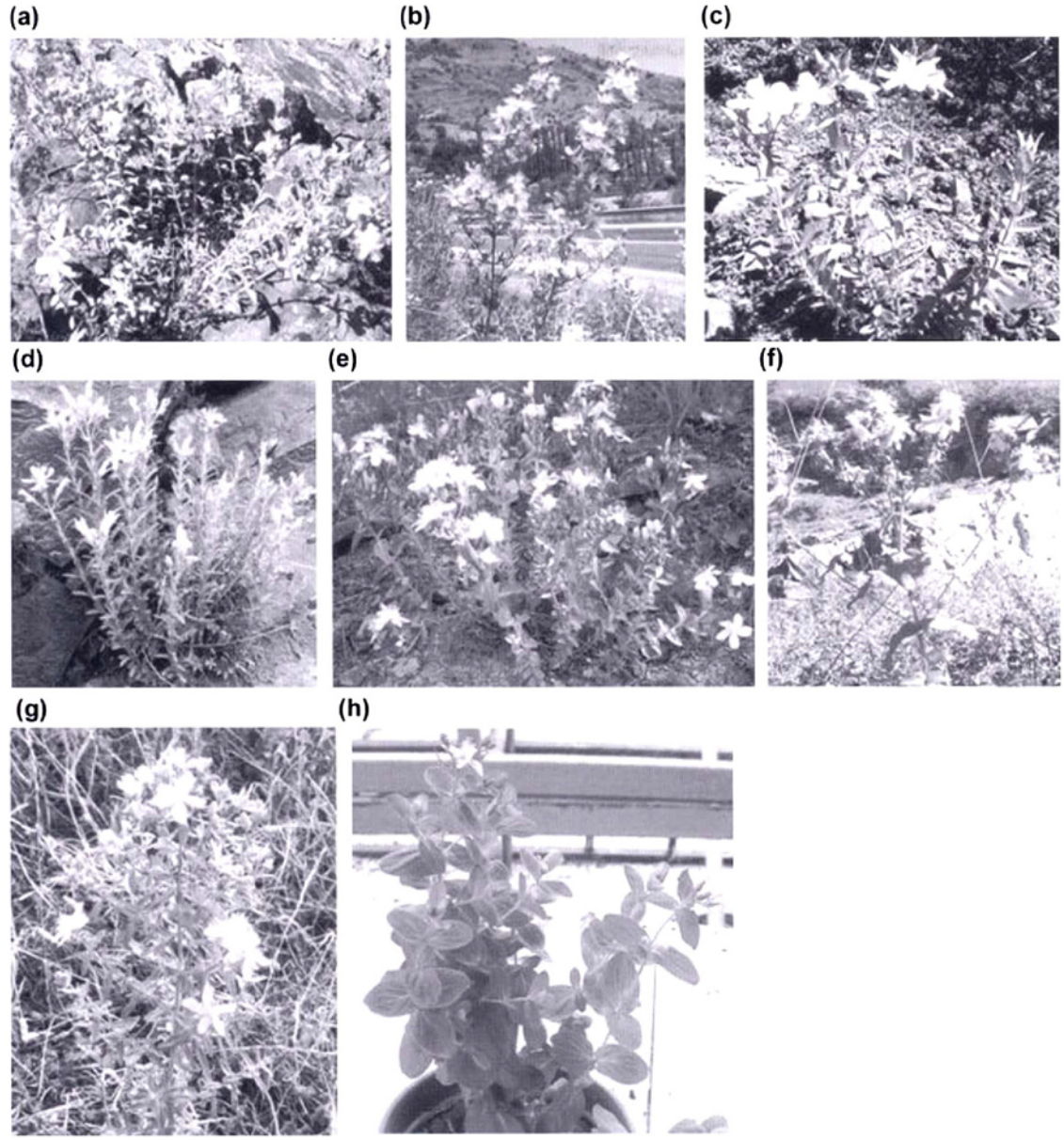

Figure 1. Flowering plants of *H. aviculariifolium* (a), *H. lydium* (b), *H. montbretii* (c), *H. orientale* (d), *H. origanifolium* (e), *H. perfoliatum* (f), *H. perforatum* (g) and *H. pruinatum* (h) growing in Turkey.

for chemical constituents by HPLC.

Preparation of Plant Extracts

Samples of 0.5-1.0 g each of air-dried plant material with moisture content of 10.0% were mechanically ground to obtain a homogenous drug powder and extracted with 96 % EtOH (50 mL) for 72 h, at room temperature. The prepared extracts were kept in dark in a refrigerator until used. Conversion of protohypericin is performed by exposure to light for 30 min. before analysis by HPLC (Kurth and Spreemann, 1998; Michelitsch et al., 2000). Portion of 1 mL of the fresh drug extracts was taken up for HPLC analyses of hypericin. Each of 1-mL aliquot of the extracts was diluted with 19 mL of EtOH for flavonoid analyses. All solvents and standards were of HPLC grade and purchased from Roth (Karlsruhe, Germany).

HPLC Analysis

HPLC analysis of phenolic acid and flavonoids was performed using a Waters model 2690 gradient pump with Waters 2487 UV-detector and XTerra RP18 column (150 × 3.9 mm, 3,5 µm). Compounds on the column were separated with 5% 0.1% trifluoracetic acid $C_2HF_3O_2$ in water (solvent A) and 95% 0.1% $C_2HF_3O_2$ in acetonitrile (solvent B), using a following gradient elution program: 0-45 min. 95-55% A, 5-45% B; 45-50 min. 55% A, 45% B; 50-55 min. 55-95% A, 45-5% B. Flow rate: 0.4 mL/min. Injection volume: 10 µL. The column temperature was at 20°C. The elution was monitored at 360 nm and obtained data were compared with authentic samples of the respective compounds (Kovacs et al., 2004).

Hypericins were analyzed according to Pierluigi and Piergiorgio (2000) and a modified HPLC method described in

Pharmeuropa (2004). A Waters 2690 gradient pump with UV detector Waters 2487 and Hypersil ODS C18 column (150 × 4.6 mm, 5 μm) were used. The elution program was isocratic. The mobile phase consisted of ethyl acetate/15.6 g/L sodiumdihydrogen phosphate-phosphoric acid NaH_2PO_4/methanol (16:17:67). The flow rate: 1.0 mL/min. Injection volume: 10 μL. The column temperature was at 20°C. The elution was monitored at 590 nm and the obtained data were compared with standard samples of hypericin and pseudohypericin.

The quantity of compound was calculated from an external standard calibration in the concentration range of 0.5-100.0 μg/mL (r^2 = 0.997). Each sample was analyzed twice and the mean value was used for calculation.

RESULTS AND DISCUSSION

Collection sites and habitat of the *Hypericum* species examined are shown in Table 1 where the species listed alphabetically. Hyperoside, quercitrin and pseudohypericin were found in all species. The highest accumulation of hyperoside was observed in *H. perfoliatum* and *H. perforatum* (16.98 and 16.58 mg/g DW, respectively) while *H. aviculariifolium* and *H. lydium* produced the highest amount of quercitrin (4.93 and 4.31 mg/g DW, respectively).

Hypericin, quercetin and chlorogenic acid were also detected in all species with the exceptions of *H. orientale* for hypericin, *H. montbretii* for quercetin and *H. lydium* for chlorogenic acid. *H. perforatum* and *H. aviculariifolium* produced the highest amount of hypericin (2.82 and 2.14 mg/g DW, respectively) whereas the highest pseudohypericin accumulation was observed in *H. perfoliatum* (2.11 mg/g DW) which is in agreement with our previous study (Çırak et al., 2006). Various level of hypericins accumulation was also observed for the species examined as reported by Kitanov (2001) and Çırak (2006). Interestingly, *H. orientale* produced pseudohypericin but not hypericin in the present study. Kitanov (2001) and Ayan et al., (2004) detected neither hypericin nor pseudohypericin in *H. orientale* whereas Ayan and Çırak (2007) reported *H. orientale* to contain both hypericin forms. Kitanov (2001) and Ayan et al., (2004) used spectrophotometric methods for hypericin determination while Ayan and Çırak (2007) performed an HPLC method as adopted by us in the present study. Hence, the conflict regarding pseudohypericin and hypericin content of *H. orientale* among the aforesaid studies can be attributed to different methods adopted for chemical analysis.

Quercetin content was found to be higher in *H. perforatum* and *H. lydium* (1.40 mg/g DW) while *H. perfoliatum* produced much higher amount of chlorogenic acid (20.35 mg/g DW) when compared to the other species examined. Apigenin-7-O-glucoside accumulation was observed in all examined species at various levels with the exception of *H. orientale* as reported in our previous study (Çırak et al., 2007). *H. montbretii* was found to be superior to other species with respect to apigenin-7-O-glucoside content. Rutin was detected in *H. aviculariifolium, H. lydium, H. orientale, H. perfoliatum* and *H. perforatum*. This compound was produced by those species at similar level. On the contrary of

Table 1. Collection sites and habitat of the *Hypericum* species examined.

Species	Voucher numbers	Collection site	Latitude (N)	Longitude (E)	Elevation (m)	Habitat
*H. aviculariifolium**	OMUZF # 108	Gümüş	40° 52′	35° 14′	785	Rocky and open slopes
H. lydium	OMUZF # 109	Havza	40° 55′	35° 37′	580	*Pinus* woodland
H. montbretii	OMUZF # 100	Çakallı	41° 04′	36° 01′	570	Damp and shady places among rocks
H. orientale	OMUZF # 131	Merzifon	40° 51′	35° 29′	2300	*Pinus* woodland
H. origanifolium	OMUZF # 109	Çakallı	41° 04′	36° 01′	570	Arid pasturelands
H. perfoliatum	OMUZF # 101	Çakallı	41° 04′	36° 01′	570	Damp and shady places among rocks
H. perforatum	OMUZF # 61	Samsun	41° 35′	35° 56′	195	Pasturelands
H. pruinatum	OMUZF # 107	Gümüş	40° 52′	35° 14′	785	Igneous slopes and rock ledges

*endemic

Table 2. Mean values for chlorogenic acid, rutin, hyperoside, apigenin-7-O-glucoside, quercitrin, quercetin, vitexin, hypericin and pseudohypericin contents of *Hypericum* species examined (mg/g DW).

Species	Chlorogenic acid	Rutin	Hyperoside	Apigenin-7-O-glucoside	Quercitrin	Quercetin	Vitexin	Hypericin	Psedohypericin
*H. aviculariifolium**	0.02	0.04	12.83	0.04	4.93	0.10	-	2.14	0.59
H. lydium	-	0.26	4.37	0.68	4.31	1.40	-	0.18	0.34
H. montbretii	2.48	-	12.64	2.25	1.21	-	0.96	1.39	1.17
H. orientale	2.12	0.08	8.94	-	2.39	0.74	-	-	0.09
H. origanifolium	2.34	-	15.89	-	3.90	0.05	-	1.43	1.11
H. perfoliatum	20.35	0.02	16.98	0.03	2.11	0.05	-	1.06	2.11
H. perforatum	0.66	0.66	16.58	0.21	2.85	1.40	-	2.82	1.86
H. pruinatum	0.68	-	2.82	0.18	0.56	0.51	-	0.79	0.52

*endem

the other compounds which were detectable in many of the species examined, the flavonoid vitexin was found in only *H. motbretii* and the presence of this flavonoid in the genus *Hypericum* was reported for the first time (Table 2).

CONCLUSIONS

The main conclusions from the above data are that 1. *H. aviculariifolium, H. perfoliatum* and *H. perforatum* contain the similar array of constituents and their chemical profile is very similar. 2. The *Hypericum* species examined are different evidently in concentration of analysed compounds. *H. perfoliatum* produce major amount of chlorogenic acid, whereas *H. montberetii* is the only species producing vitexin and higher amount of apigenin-7-0-glucoside. *H. avicularii-folium, H. lydium* and *H. origanifolium* are rich in quercitrin. 3. Considering the pharmacological significance of bioactive compounds present in *Hypericum* species and their possible use in therapeutics it is important to find new sources of their location. Hence, the species examined may find important application in medicinal treatment. 4. To date, vitexin has never been detected in the genus *Hypericum*. Therefore, the present findings could be useful for chemotaxonomical evaluation of corresponding compounds. 5. The results also indicate HPLC method as more sensitive and reliable analytical technique for phytochemical analysis of complex plant extracts.

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