Acta Physiol Plant (2012) 34:1313–1320
DOI 10.1007/s11738-012-0928-8

ORIGINAL PAPER

Secondary metabolites of *Hypericum orientale* L. growing in Turkey: variation among populations and plant parts

Cuneyt Cirak · Jolita Radusiene · Zydrunas Stanius · Necdet Camas · Omer Caliskan · Mehmet Serhat Odabas

Received: 5 August 2011 / Revised: 3 January 2012 / Accepted: 5 January 2012 / Published online: 21 January 2012

Abstract The present study was conducted to determine the variation in the content of several plant chemicals, namely hyperforin, hypericin, pseudohypericin, chlorogenic acid, rutin, hyperoside, isoquercetine, kaempferol, quercitrine and quercetine among ten *Hypericum orientale* L. populations from Northern Turkey. The aerial parts representing a total of 30 individuals were collected at full flowering and dissected into floral, leaf and stem tissues. After dried at room temperature, the plant materials were assayed for chemical contents by HPLC. The populations varied significantly in chemical contents. Among different plant parts, the flowers were found to be the principle organ for hyperforin, hypericin, pseudohypericin and rutin accumulations while the rest of the chemicals were accumulated mainly in leaves in all growing localities. The chemical variation among the populations and plant parts is discussed as being possibly the result of different genetic, environmental and morphological factors.

Keywords Flavonoids · Hyperforin · Hypericins · HPLC · *Hypericum orientale* · Population variability

Introduction

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 (Robson 2001). The species of this genus have been used as healing agents due to their various medicinal properties for hundred of years. *Hypericum* species are also used as sedatives, antiseptics, and antispasmodics in Turkish folk medicine. The *Hypericum* genus is represented in Turkey by 89 species of which 43 are endemic (Davis 1988). *Hypericum orientale* L. is one of the Turkish species of *Hypericum*. The stems are 7–45 cm in long, erect or decumbent and sometimes rooting with adventitious roots. Leaves 10–40 mm, oblong or elliptic-oblong to oblanceolate. Sepals unequal, narrowly oblong and ovate or broadly elliptic to obovate. Petals 10–18 mm, entire, without black dotes. Capsule 7–14 mm, ovoid to ovoid-cyclindric (Davis 1988) (Fig. 1).

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 pharmacological activities of *Hypericum* extracts namely, photodynamic, antidepressive and antiviral activities are mainly attributed to the naphthodianthrones, hypericin and pseudohypericin (Bombardelli and Morazzoni 1995). Hyperforin is a prenylated phloroglucinol derivative that consists of a phloroglucinol skeleton with lipophilic isoprene chains. Results from recent studies have indicated hyperforin as the main chemical, responsible for antidepressant effects of *Hypericum* extracts (Medina et al. 2006). Flavonoids are a group of bioactive compounds present in *Hypericum* plants. They play an important role in preventing cardiovascular diseases and several kinds of cancer (Chu et al. 2000).

Communicated by M. J. Reigosa.

C. Cirak (✉) · N. Camas · O. Caliskan · M. S. Odabas
The Vocational High School of Bafra, Ondokuz Mayıs University, 55040 Bafra, Samsun, Turkey
e-mail: cuneytc@omu.edu.tr

J. Radusiene · Z. Stanius
Institute of Botany, Nature Research Centre, Zaliuju ezeru 49, 08406 Vilnius, LT, Lithuania

Fig. 1 A view from *H. orientale* plant at flowering in rocky slope habitat

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 previous studies, *H. orientale* was reported to contain hyperforin, hypericin, psedohypericin, rutin, hyperoside, quercitrin, quercetin, and chlorogenic acid (Cirak et al. 2007a; Smelcerovic et al. 2008). However, no report is available on the chemical variation among wild populations of this species. In the present study, we report our phytochemical investigations of *H. orientale* populations sampled from ten growing localities in Northern Turkey. Besides, isoquercetine has not yet been detected in this species. Here, we also describe the first occurrence of the chemical in *H. orientale*.

Materials and methods

Plant materials

The plant materials were described in our previous studies (Cirak et al. 2007a). The species were identified by Dr. Hasan Korkmaz, Faculty of Science and Art, Department of Biology, University of 19 Mayis, Samsun, Turkey.

Experimental procedures

The aerial parts of *H. orientale* plants representing a total of 30 shoots were collected at full flowering from ten sites of Northern Turkey (Table 1). The sampling sites were not grazed or mown during the plant gathering period. The top of 2/3 plants was harvested between 11:00 a.m. and 01:00 p.m. Conditions on the day of collection were clear and sunny at all sites. Temperatures ranged from 26 to 32°C. After collected, ten individuals were kept as whole plants and the rest were dissected into floral, leaf and stem tissues. The plant materials were dried at room temperature (20 ± 2°C), and subsequently assayed for chemical contents by HPLC.

Preparation of plant extracts

Air-dried plant material was mechanically ground with a laboratory mill to obtain a homogenous drug powder. Samples of about 0.5 g (weighed with 0.0001 g precision) were extracted in 50 mL of 100% methanol by ultrasonicatation at 40°C for 30 min in a Sonorex Super model RK 225H ultrasonic bath. The prepared extracts were filtered through a membrane filter with pore size of 0.22 μm (Carl Roth GmbH, Karlsruhe, Germany) and kept in a refrigerator until analysis no longer than 3 h. The extracts for naphthodianthrones analysis after ultrasonication were exposure to light for 30 min due to the photoconversion of protohypericin into hypericin and protopseudohypericin into pseudohypericin.

HPLC analysis

A Shimadzu Prominence LC-20A (Shimadzu Europa GmbH, Duisburg, Germany) chromatographic system equipped with two LC-20AD model pumps, a SIL-20AC auto-injector, a thermostat CTO-20AC and a SPD-M20A detector was used for HPLC analysis. Separation of all compounds was carried out using an YMC Pack Pro-C18 (YMC Europe GmbH, Dinslaken, Germany) column (150 mm × 4 mm i.d.; 3 μm particle sizes) with 10 mm guard-precolumn. The mobile phase consists of solvent A [water containing 0.1% trifluoroacetic acid (TFA)] and solvent B (acetonitrile containing 0.1% TFA). The following binary gradient elution program was used: 0–1 min (B 5 → 5%), 1–14 min (B 5 → 20%), 14–20 min (B 20 → 80%), 20–30 min (B 80 → 100%), 30–39 min (B 100 → 100%), 39–39.5 min (B 100 → 5%), 39.5–45 min (B 5–5%). The mobile phase was delivered with a flow rate of 1.0 mL min^{-1}; volume of extract injected was 10 μL. Detection was performed at 210–790 nm wave length range with a constant column temperature at 40°C. The eluted compounds were identified on the basis of their retention time by comparison with retention time of reference standards and also confirmed with UV spectra's of reference standards in the wavelength ranging from 210 to 790 nm.

The hypericin and pseudohypericin elution program was isocratic. The mobile phase consists of acetonitrile containing 0.1% TFA. Flow rate of mobile phase was

Table 1 Geographical data and seasonal climatic conditions of *H. orientale* growing localities from Northern Turkey

Sites	Collection date	Voucher no.	Latitude (N)	Longitude (E)	Elevation (m)	Mean temperature (°C)	Precipitation (mm)	Habitat
Kizlan	July 14, 2010	BMYO # 3/1	40°52′	36°11′	750	10.01	850	*Quercus* woodland
Cakalli	July 18, 2010	BMYO # 3/2	41°04′	36°01′	970	11.10	735	Arid pasturelands
Ladik 1	July 19, 2010	BMYO # 3/3	40°51′	36°09′	727	11.00	750	Rocky and open slopes
Nusratlı	July 14, 2010	BMYO # 3/4	40°51′	36°02′	910	10.17	625	Arid pasturelands
Karapinar 1	July 11, 2010	BMYO # 3/5	40°59′	36°04′	902	11.02	520	Rocky and open slopes
Alacam	July 12, 2010	BMYO # 3/6	40°53′	36°05′	720	13.20	800	*Quercus* woodland
Karapinar 2	July 11, 2010	BMYO # 3/7	40°61′	36°05′	1,250	09.12	530	Arid pasturelands
Ispir 1	July 14, 2010	BMYO # 3/8	39°62′	37°04′	2,020	09.01	900	Rocky and open slopes
Ispir 2	July 14, 2010	BMYO # 3/9	39°61′	37°05′	2,450	09.50	920	Rocky and open slopes
Ladik 2	July 19, 2010	BMYO # 3/10	40°50′	36°08′	700	11.27	640	Stony riverside

1.1 mL min^{-1}. Ten micro liters of extracts were injected. Detection was recorded at 210–790 nm wave length range with a constant column temperature at 40°C.

The quantification of detected compounds was achieved by external standard method at the maximal absorption on the UV spectra of corresponding compounds: chlorogenic acid, 325 nm; rutin, 353 nm; hyperoside, 353 nm; isoquercetrine, 353; kaempferol, 346 nm; quercetrine, 347 nm; quercetine, 368 nm; hyperforin, 270 nm; hypericin and pseudohypericin, 580 nm wavelength. A six-point calibration curves were obtained with pure standards dissolved in MeOH in the concentration range of 0.2–110 μg/mL. All calibration cures showed good linear regression ($r^2 > 0.999$) within the test range. Typical HPLC chromatograms of *H. orientale* extract are shown in Fig. 2. All solvents and standards of reference substances were of HPLC grade and purchased from Roth Chemical Company (Karlsruhe, Germany).

Data analysis

Data for hyperforin, hypericin, pseudohypericin, chlorogenic acid, rutin, hyperoside, isoquercetine, kaempferol, quercitrine and quercetine contents of plant material including whole plant, stem, leaf and flower were objected to ANOVA and significant differences among mean values were tested with the Duncan Multiple Range Test ($P < 0.01$) by MSTAT statistical software. Mean values of the chemical contents were normalized using $x' = \sqrt{x} + 1$ transformation before conducting ANOVA, when

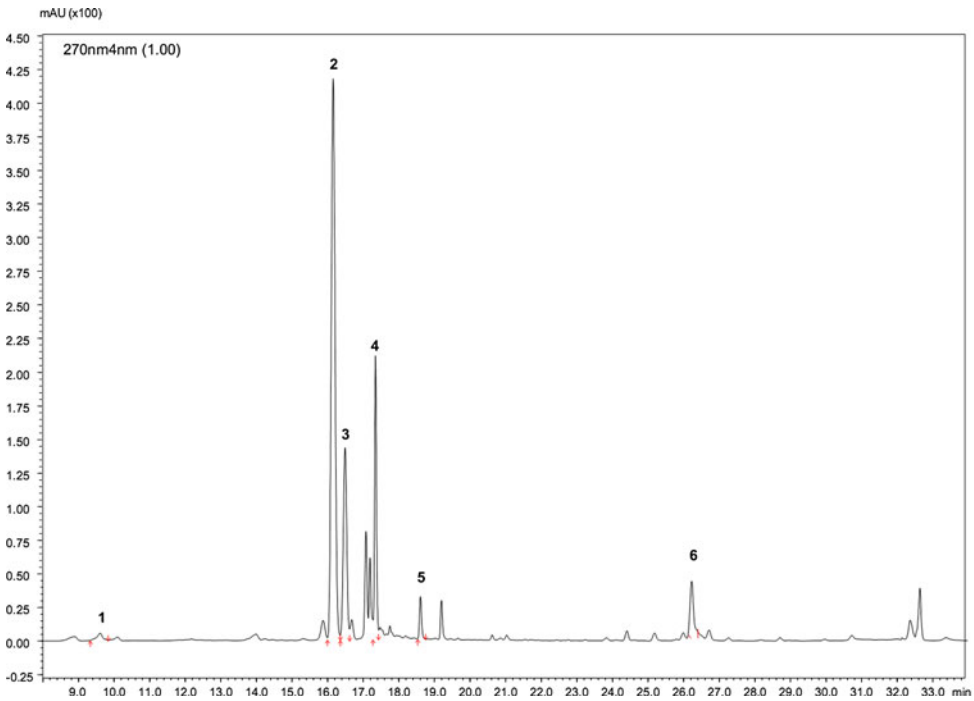

Fig. 2 Typical HPLC chromatogram of *H. orientale* flowers extract detected by UV at 270 nm wave length. Peak identified: *1* chlorogenic acid [retation time (t_R) 9.64 min], *2* hyperoside (t_R 16.18 min), *3* isoquercetine (t_R 16.52 min), *4* quercetrine (t_R 7.37 min), *5* quercetine (t_R 18.63 min), *6* hyperforin (t_R 29.99 min)

necessary, because some chemicals were not detected in several cases.

Results

The content of hyperforin, hypericin, pseudohypericin, chlorogenic acid, rutin, hyperoside, isoquercetine, and quercetine in plant materials including whole shoots, stem, leaf and flower tissues varied significantly among populations ($P < 0.01$) (Table 2). Whereas quercitrin was accumulated in similar levels only in plants from Alacam and Ispir-2 populations and none of analyzed plant materials in the present study produced kaempferol. Hyperforin was detected only in plants from Cakalli, Ispir-2 and Ladik-2 populations and hyperforin content was significantly higher in Ispir-2 population (1.21 mg/g DW). Hypericin and pseudohypericin accumulations occurred only in Alacam, Karapinar, Ispir-1 and Ispir-2 populations. Plants from Ispir-2 population produced the highest amount of both chemicals (1.64 and 3.24 mg/g DW hypericin and pseudohypericin, respectively). Similarly rutin was detectable in the populations of Kizlan, Ladik-1, Nusratli, Karapinar-1, Alacam, and Ispir-2 among which Karapinar-1 produced the highest accumulation level (3.32 mg/g DW). On the contrary, chlorogenic acid, hyperoside, isoquercetine, and quercetine were accumulated in all populations at different levels. Chlorogenic acid accumulation was the highest in Cakalli and Nusratli populations (11.71 and 10.09 mg/g DW) and plants from Ispir-2 population produced the highest amount of hyperoside (16.35 mg/g DW). For isoquercetine, the highest accumulation levels were observed in Nusratli and Ispir-1 populations (4.29 and 3.61 mg/g DW). Similarly, Ispir-2 and Ladik-2 populations produced significantly higher amount of quercetine when compared to the rest of the populations (3.68 and 3.61 mg/g DW).

Stems, leaves and reproductive tissues differed significantly with regard to chemical accumulation (Figs. 3, 4). The highest accumulation level of hyperforin, hypericin, pseudohypericin and rutin was detected in flowers while the rest of the chemicals were accumulated mainly in leaves in all growing localities. As for stems, significantly lower accumulation levels of the secondary metabolites were observed in the organs and they did not produce hyperforin and hypericin.

Discussion

Variations in the levels of bioactive secondary metabolites in populations of *Hypericum* plants have an important impact on the pharmacological activity of tested extracts

Table 2 Hyperforin, hypericin, pseudohypericin, chlorogenic acid, rutin, hyperoside, isoquercetine, kaempferol, quercitrine and quercetine contents of *H. orientale* whole shoots from wild populations located in Northern Turkey (mg/g DW)

Populations	Hyperforin	Hypericin	Psedohypericin	Chlorogenic acid	Rutin	Hyperoside	Isoquercetine	Kaempferol	Quercitrine	Quercetine
Kizlan	0.00b*	0.00c	0.00c	5.67b	1.97b	7.86c	1.69b	0.00	0.00	1.81c
Cakalli	0.23b	0.00c	0.00c	11.71a	0.00c	11.09b	1.68b	0.00	0.00	1.71c
Ladik 1	0.00b	0.00c	0.00c	1.95d	0.83c	1.94d	0.28c	0.00	0.00	1.68c
Nusratlı	0.00b	0.00c	0.00c	10.09a	0.86c	12.22b	4.29a	0.00	0.00	2.17b
Karapinar 1	0.00b	0.00c	0.00c	7.91b	3.32a	10.45b	1.18b	0.00	0.00	1.81c
Alacam	0.00b	0.23b	0.55b	3.97c	0.44c	4.23d	0.51c	0.00	0.24	1.76c
Karapinar 2	0.00b	0.27b	0.54b	6.12b	0.00c	9.16b	1.27b	0.00	0.00	2.43b
Ispir 1	0.00b	0.37b	0.50b	6.31b	0.00c	10.79b	3.61a	0.00	0.00	1.73c
Ispir 2	1.21a	1.64a	3.24a	0.61d	0.40c	16.35a	2.07b	0.00	0.31	3.68a
Ladik 2	0.59a	0.00c	0.00c	6.69b	0.00c	10.21b	1.58b	0.00	0.00	3.61a

* Values followed by different small letters in each column are significantly different ($P < 0.01$) according to Duncan Multiple Range test

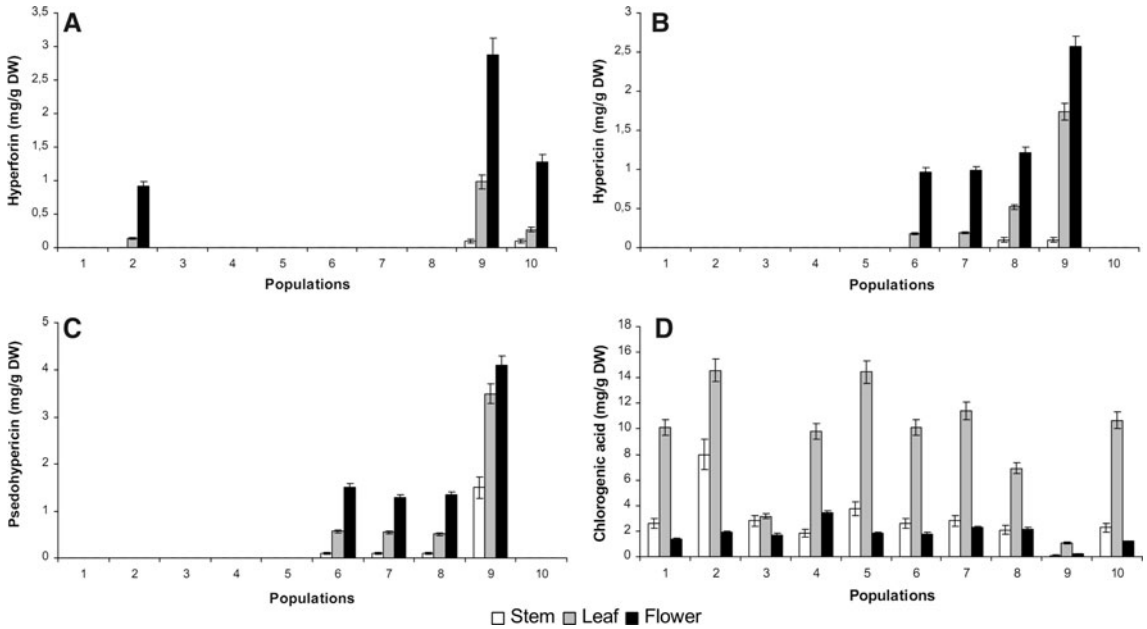

Fig. 3 Hyperforin (**a**), hypericin (**b**), pseudohypericin (**c**) and chlorogenic acid (**d**) contents in stem, leaf and flower of *H. orientale* plants from wild populations located at Northern Turkey. (Populations: *1* Kizlan; *2* Cakalli; *3* Ladik 1; *4* Nusratli; *5* Karapinar 1; *6* Alacam; *7* Karapinar 2; *8* Ispir 1; *9* Ispir 2; *10* Ladik 2) (*Bars* are ±SE)

(Bergonzi et al. 2001). Hence, investigations of population variability in the content of secondary metabolites from different species of *Hypericum* have been made over several decades. In *Hypericum perforatum,* hypericin and pseudohypericin levels in plants from different localities of Australia (Southwell and Bourke 2001), Switzerland (Büter and Büter 2002), USA (Sirvent et al. 2002), Turkey (Cirak et al. 2007b) and Lithuania (Bagdonaite et al. 2010) were investigated. Wild populations of the same species from different growing localities of the world were also investigated for variation in the content of hyperforin (Couceiro et al. 2006), rutin, hyperoside, quercitrin, quercetin, chlorogenic acid and apigenin-7-O-glucoside (Cirak et al. 2007b). In terms of population variability of bioactive substances in other *Hypericum* species, essential oil components in *Hypericum perfoliatum, Hypericum humifusum, Hypericum linarifolium* and *Hypericum pulchrum* were reported to vary significantly with geographic distribution of wild plants (Nogueira et al. 2008). In *Hypericum triquetrifolium,* significant variations were detected in the content of hypericin, pseudohypericin, hyperforin and several flavonoids as rutin, hyperoside, apigenin-7-O-glucoside, kaempferol, quercitrin, quercetin and amentoflavone among four wild populations from Turkey (Cirak et al. 2011). The previous results indicated the regional distribution of *Hypericum* plants as an important source of chemical variability. In the present study, similarly, significant differences were detected in chemical accumulations among wild populations of *H. orientale*. The populations were located in different parts of Northern Turkey and growing sites of them were differed from each other by climatic and geographic factors (Table 1). As a result of these environmental differences, each site has specific ecological conditions and habitat. The chemical variability in the content of evaluated chemicals among populations may be attributed to the different environmental conditions of sampling sites. Especially, it is noteworthy to note that Ispir-2 population produced the highest level of hyperforin, hypericin, pseudohypericin, hyperoside and quercetine and this collection site had the highest elevation (2,450 m) indicating elevation may have a positive effect on secondary metabolite accumulation in *H. orientale.* However, the present findings also indicate a significant genetic difference/similarity among the populations. For example, Ispir-2 and Ladik-2 populations are separated by a distance of 400 km and have substantially different environment (mean temperature, precipitation, elevation etc.). Hence, they should represent distinct populations. However, both populations produced similar amount of hyperforin, quercetine and isoquercetine. On the contrary, although Kizlan and Ladik-1 are separated by only 10 km and have very similar environment, three- and fourfold differences were detected between the populations in chlorogenic acid and hyperoside contents, respectively. Generally, the observed accumulation levels of hypericins, hyperforin and rutin, hyperoside, quercitrine and quercetine were similar those reported by Smelcerovic et al. (2008) who studied the chemistry of several *Hypericum* species from Turkey including *H. orientale*. Similar variations in the content of different phenolics for some other

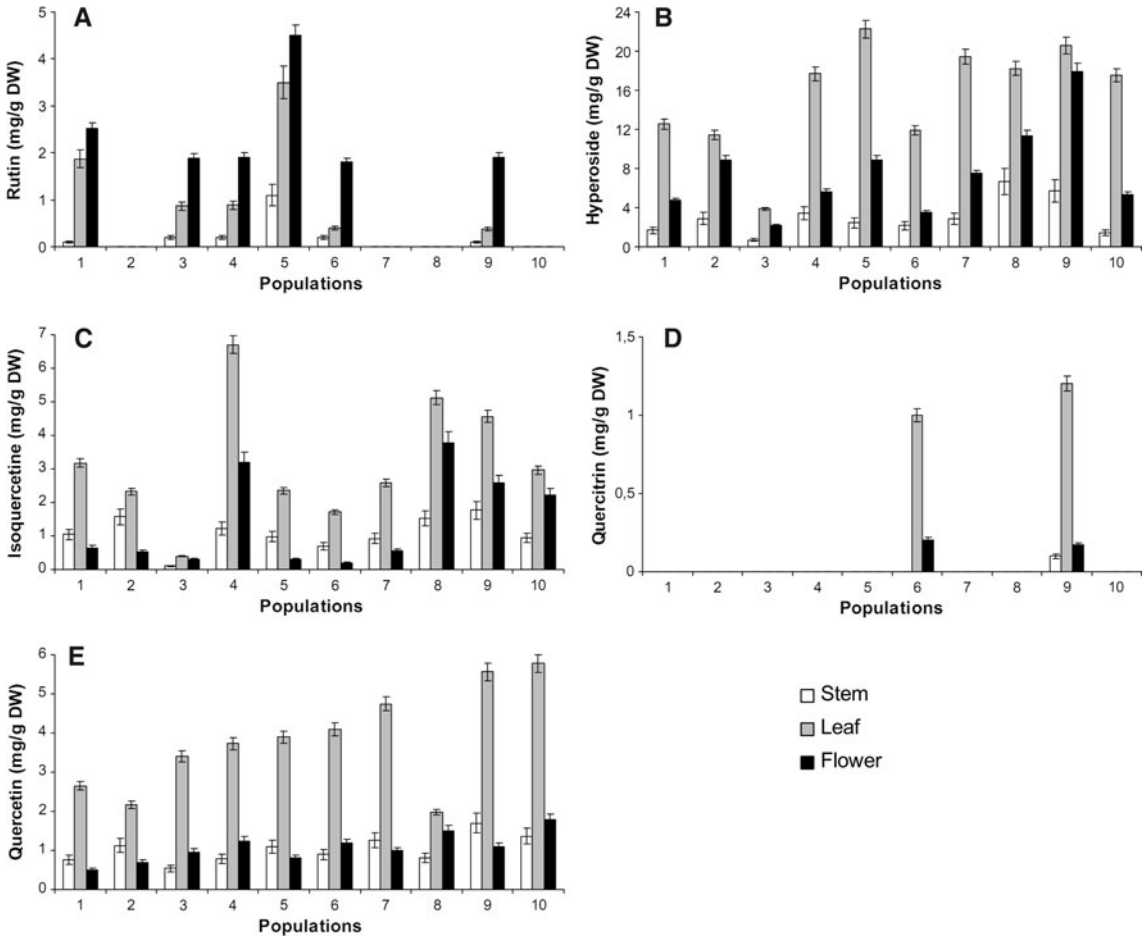

Fig. 4 Rutin (**a**), hyperoside (**b**), isoquercetine (**c**), quercitrin (**d**) and quercetin (**e**) contents in stem, leaf and flower of *H. orientale* plants from wild populations located at Northern Turkey (Populations: *1* Kizlan; *2* Cakalli; *3* Ladik 1; *4* Nusratli; *5* Karapinar 1; *6* Alacam; *7* Karapinar 2; *8* Ispir 1; *9* Ispir 2; *10* Ladik 2) (*Bars* are ±SE)

plant species were also reported (Amaral et al. 2010; Dossett et al. 2010). However, it should be noted that it is necessary to perform detailed analyses using biochemical (i.e. allozymes) and molecular (i.e. RFLP, RAPD, AFLP, ISSR) markers to establish the whole range of genetic diversity of populations. Thus, it would be possible to discriminate exactly the effects of genetic and environmental factors on the observed chemical variability among populations. For example, the genetic diversity within and among seven Tunisian natural populations of *H. humifusum* L., from different geographic regions and bioclimates, was assessed using 11 isozymic polymorphic loci, and 166 RAPD markers by Afef et al. (2012). The authors reported a high genetic variation among Tunisian *H. humifusum* populations and pointed out the necessity for further studies including the variation of the chemical composition among populations and its relationship with the genetic diversity. Combined phytochemical, morphological and molecular studies are also in progress to evaluate the diversity within and among species of *Hypericum* (Nürk and Crockett 2011).

The localization of the various types of secretory structures in which biologically active substances are synthesized and/or accumulated varies among plant tissues, and the levels of phytomedicinal compounds present in a particular *Hypericum* tissue depends on the relative abundance of these secretory structures on the harvested material. In the present study, flowers were found to be main organs for the accumulations of hyperforin, hypericin, pseudohypericin and rutin while chlorogenic acid, hyperoside, isoquercetine, kaempferol, quercitrin and quercetin were accumulated mainly in leaves. Similarly, floral parts had the highest level of hyperforin, hypericin and pseudohypericin in *H. perforatum* (Sirvent et al. 2002) and leaves were superior over generative tissues with respect to chlorogenic adic and hyperoside accumulation in *H. origanifolium* and *H. perfoliatum* (Cirak et al. 2007c, d).

Results from the present study indicate that *H. orientale* accumulates lower concentrations of hyperforin, hypericin, rutin, quercetin and quercitrine, comparable concentrations of pseudohypericin and higher concentrations of chlorogenic acid and hyperoside when compared to

Table 3 Comparison of the chemical content (mg/g DW) in *H. orientale* and *H. perforatum*

Compounds	*H. orientale*	*H. perforatum*	References
Hyperforin	0.00–1.21	8.35–150	Greeson et al. (2001); Maggi et al. (2004); Couceiro et al. (2006)
Hypericin	0–1.64	0.01–2.77	Sirvent et al. (2002); Southwell and Bourke (2001); Bagdonaite et al. (2010)
Psedohypericin	0–3.24	0.05–6.75	Ayan and Cirak (2008); Bagdonaite et al. (2010); Büter and Büter (2002)
Chlorogenic acid	0.61–11.27	1.11–2.19	Maggi et al. (2004); Cirak et al. (2007a)
Rutin	0.00–3.22	0.19–34.71	Martonfi and Repcak (1994); Radusiene et al. (2004)
Hyperoside	1.94–12.22	2.07–7.69	Maggi et al. (2004); Bagdonaite et al. (2012)
Isoquercetine	0.28–4.29	–	No previous report
Kaempferol	0.00	0.22–0.26	Silva et al. (2005)
Quercitrine	0.00–0.31	0.05–4.77	Martonfi and Repcak (1994); Radusiene et al. (2004)
Quercetine	1.68–3.68	0.05–24.12	Martonfi and Repcak (1994); Radusiene et al. (2004)

H. perforatum, a well known and commercial source of the compounds examined (Table 3). In taxonomic point of view, besides, *H. orientale* belongs to the section Crossophyllum Spach. (Robson 2001). To author's knowledge only one other member of the section, *H. adenotrichum* Spach. had already been investigated for the presence of main secondary metabolites (Cirak et al. 2009). The comparison of our results with our previously published report revealed that both members of the section have similar array of constituents and contain hyperforin, hypericin, pseudohypericin, chlorogenic acid, rutin, hyperoside, quercitrine and quercetine. Among the chemicals, hypericins have a proven taxonomic value for the infrageneric classification of the genus *Hypericum*. Because they are not found in species from the primitive sections and seem to be specific only for the taxa of phylogenetically more advanced sections (Robson 2001). Hence, detection of hypericin as well as hyperforin and the phenolics examided in *H. orientale* in the present study supports the taxonomic position of the section Crossophyllum within the genus *Hypericum*.

Conclusions

Results of the present study indicate a significant variation in the chemical contents of *H. orientale* from Turkish populations. It is interesting to note that we did not detect hypericin in this species, represented by only one wild population in our previous study (Cirak et al. 2007a). Hence, regional distribution of this medicinal plant may be an important source of chemical variability and should be considered while optimizing the processing methodology of wild-harvested plant material. Further studies including molecular studies on the wild populations and the relationship between their genetic profiles and secondary metabolite contents are necessary to make more substantial conclusions. Besides, in the present study, the accumulation of isoquercetine in this species was reported by us for the first time. Such kind of data could also be useful for elucidation of the chemotaxonomical significance of the corresponding compounds and phytochemical evaluation of *H. orientale*.

Conflict of interest We declare that we have no conflict of interest.

References

Afef B, Chokri M, Mohamed B (2012) Genetic structure of natural Tunisian *Hypericum humifusum* L. (Hypericacae) populations as assessed by allozymes and RAPDs. Ind Crop Prod 35:217–223

Amaral JS, Valentao P, Andrade PB, Martins RC, Seabra RM (2010) Phenolic composition of hazelnut leaves: influence of cultivar, geographical origin and ripening stage. Sci Hortic 126:306–313

Ayan AK, Cirak C (2008) Hypericin and pseudohypericin contents in some *Hypericum* species growing in Turkey. Pharm Biol 46:191–288

Bagdonaite E, Martonfi P, Repcak M, Labokas J (2010) Variation in the contents of pseudohypericin and hypericin in *Hypericum perforatum* from Lithuania. Biochem Syst Ecol 38:634–640

Bagdonaite E, Martonfi P, Repcak M, Labokas J (2012) Variation in concentrations of major bioactive compounds in *Hypericum perforatum* L. from Lithuania. Ind Crop Prod 35:302–308

Bergonzi MC, Bilia AR, Gallori S, Guerrini D, Vincieri FF (2001) Variability in the content of the constituents of *Hypericum perforatum* L. and some commercial extracts. Drug Dev Ind Pharm 27:491–497

Bombardelli E, Morazzoni P (1995) *Hypericum perforatum*. Fitoterapia 66:43–68

Büter KB, Büter B (2002) Ontogenetic variation regarding hypericin and hyperforin levels in four accessions of *Hypericum perforatum* L. J Herbs Spices Med Plants 9:95–100

Chu YH, Chang CL, Hsu HF (2000) Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agric 80:561–566

Cirak C, Radusiene J, Janulis V, Ivanauskas L (2007a) Chemical constituents of some *Hypericum* species growing in Turkey. J Plant Biol 50:632–635

Cirak C, Radusiene J, Sağlam B, Janulis V (2007b) Variation of bioactive substances and morphological traits in *Hypericum perforatum* populations from Northern Turkey. Biochem Syst Ecol 35:403–409

Cirak C, Radusiene J, Janulis V, Ivanauskas L (2007c) Variation of bioactive secondary metabolites in *Hypericum origanifolium* during its phenological cycle. Acta Physiol Plant 29:197–203

Cirak C, Radusiene J, Janulis V, Ivanauskas L (2007d) Secondary metabolites in *Hypericum perfoliatum*: variation among plant parts and phenological stages. Bot Helv 117:29–36

Cirak C, Radusiene J, Janulis V, Ivanauskas L (2009) Chemical constituents of *Hypericum adenotrichum* Spach, an endemic Turkish species. Nat Prod Res 23:1189–1195

Cirak C, Radusiene J, Janulis V, Ivanauskas L, Camas N, Ayan AK (2011) Phenolic constituents of *Hypericum triquetrifolium* Turra (Guttiferae) growing in Turkey: variation among populations and plant parts. Turkish J Biol 35:449–457

Couceiro MA, Afreen F, Zobayed SMA, Kozai T (2006) Variation in concentrations of major bioactive compounds of St. John's wort: effects of harvesting time, temperature and germplasm. Plant Sci 170:128–134

Davis PH (1988) Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh

Dossett M, Lee J, Finn CE (2010) Variation in anthocyanins and total phenolics of black raspberry populations. J Funct Food 2:292–297

Greeson J, Sanford B, Monti DA (2001) St. John's wort (*Hypericum perforatum* L.): a review of the current pharmacological, toxicological and clinical literature. Psychopharmacol 153:402–414

Maggi F, Ferretti G, Pocceschi N, Menghini L, Ricciutelli M (2004) Morphological, histochemical and phytochemical investigation of the genus *Hypericum* of the Central Italy. Fitoterapia 75:702–711

Martonfi P, Repcak M (1994) Secondary metabolites during flower ontogenesis of *Hypericum perforatum* L. Zahradnictvi 21:37–44

Medina MA, Martinez-Poveda B, Amores-Sanchez MI, Quesada AR (2006) Hyperforin, more than an antidepressant bioactive compound? Life Sci 79:105–111

Nogueira TMJ, Marcelo-Curto A, Figueiredo C, Barroso JG, Pedro LG, Rubiolo P, Bicchi C (2008) Chemotaxonomy of *Hypericum* genus from Portugal: Geographical distribution and essential oils composition of *Hypericum perfoliatum, Hypericum humifusum, Hypericum linarifolium* and *Hypericum pulchrum*. Biochem Syst Ecol 36:40–50

Nürk NM, Crockett SL (2011) Morphological and phytochemical diversity among *Hypericum* species of the Mediterranean Basin. In: Odabas MS, Cirak C (Eds.), *Hypericum*. Med Aromat Plant Sci Biotechnol 5(special issue):14–28

Patocka J (2003) The chemistry, pharmacology, and toxicology of the biologically active constituents of the herb *Hypericum perforatum* L. J App Biomed 1:61–73

Radusiene J, Bagdonaite E, Kazlauskas S (2004) Morphological and chemical evaluation on *Hypericum perforatum* and *H. maculatum* in Lithuania. Acta Hort (ISHS) 629:55–62

Robson NKB (2001) Studies in the genus *Hypericum* L. (Guttiferae). Bull Br Mus (Nat Hist) Bot 8:55–226

Silva BA, Ferreres F, Malva JO, Dias ACP (2005) Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. Food Chem 90:157–167

Sirvent T, Walker L, Vance N, Donna G (2002) Variation in hypericins from wild populations of *Hypericum perforatum* L. in the Pasific Northwest of the U.S.A. Econ Bot 56:41–49

Smelcerovic A, Zuehlke S, Spiteller M, Raabe N, Özen T (2008) Phenolic constituents of 17 *Hypericum* species from Turkey. Biochem Syst Ecol 36:316–319

Southwell IA, Bourke CA (2001) Seasonal variation in hypericin content of *Hypericum perforatum* L. (St. John's wort). Phytochemistry 56:437–441