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RESEARCH ARTICLE

Secondary metabolites of seven *Hypericum* species growing in Turkey

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ABSTRACT

Context The genus *Hypericum* (Hypericaceae) has attracted remarkable scientific interest as its members have yielded many bioactive compounds.

Objective The current study presents investigations on the accumulation of hypericin, pseudohypericin, hyperforin, adhyperforin, chlorogenic acid, neochlorogenic acid, caffeic acid, 2,4-dihydroxybenzoic acid, 13,118-biapigenin, hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, (+)-catechin and (−)-epicatechin in seven *Hypericum* (Hypericaceae) species growing wild in Turkey, namely, *H. aviculariifolium* Jaup. and Spach subsp. *aviculariifolium* (Freyn and Bornm.) Robson var. *albiflorum* (endemic), *H. bithynicum* Boiss., *H. calycinum* L., *H. cardiophyllum* Boiss., *H. elongatum* L. subsp. *microcalycinum* (Boiss. and Heldr.) Robson, *H. hirsutum* L. and *H. xylosteifolium* (Spach) N. Robson.

Materials and methods The plant materials were collected at flowering period and dissected in different tissues. Air-dried plant material including stems, leaves and flowers was mechanically powdered with a laboratory mill and samples (0.1 g) were extracted in 10 mL of 100% methanol by ultrasonication at 40 °C for 30 min for HPLC-PDA analyses.

Results Accumulation levels of the investigated compounds varied greatly depending on species and plant part.

Discussion For the first time, the detailed chemical profiles of corresponding Turkish *Hypericum* species were reported and the results were discussed from a phytochemical point of view.

Conclusions The present data have importance in evaluation of plant resources of *Hypericum* genus in selecting the new potential sources of bioactive compounds.

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Chemical characterization; flavonoids; HPLC-PDA; naphthodianthrones; phenolic acids; phloroglucinols

Introduction

Hypericum (Hypericaceae) is one of the 100 largest genera that comprise an estimated 22% of angiosperm diversity (Carine & Christenhusz 2010) with the presence of 484 species from 36 taxonomic sections (Crockett & Robson 2011). *Hypericum* species are well-known healing agents in traditional medicine due to various medicinal properties. Despite the large number of *Hypericum* species, only *Hypericum perforatum* L. has been studied in depth as pharmaceutically important medicinal crop plant which extracts widely used in Europe as a drug for the treatment of mild and moderate depression (Fiebich et al. 2011). Turkey is an important prevalence centre of *Hypericum* species. According to the most recent count by Güner et al. (2012), there are a total 96 *Hypericum* species in the flora of Turkey, 46 of which are endemic. All *Hypericum* species have been used in Turkish folk medicine under the names "kantaron, peygamber çiçeği, kılıçotu, kanotu, kuzukıran and binbirdelik otu" as sedatives, antiseptics and antispasmodics (Bingol et al. 2011).

Curative value of *Hypericum* plants have been mainly attributed to the phytochemicals, namely phloroglucinol derivatives, naphthodianthrones, different phenolic compounds and essential oils which possess a great variety of bioactivities (Kasper et al. 2010). Among the bioactive compounds, hypericins and hyperforins were reported to be synergistically responsible for the proven antidepressant activity of *Hypericum* extracts (Du et al. 2006). Hyperforins were also reported to exhibit antitumour (Schwarz et al. 2003), anti-inflammatory (Feisst & Werz 2004) and antiangiogenic (Schiavone et al. 2014) effects. Hypericins are well-recognized compounds with their

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proved antiviral, antiretroviral, antibacterial, photodynamic, antitumour and antidepressant activities (Guedes & Eriksson 2005). Although hypericins and hyperforin have been reported to principally support to the pharmacological effects of *Hypericum* extracts, phenolic acids and flavonoids have made also an important contribution to the antimicrobial (Zhao et al. 2010) and antidepressant (Gastpar & Zeller 2005) activities, respectively. Despite high evaluation and numerous published papers, *Hypericum* species are of high-priority research due to the increasing pharmacological significance and commercial value of *Hyperici herba*. In this regard, many of *Hypericum* species from different regions of the world (Ferraz et al. 2002; Khan et al. 2011; Marrelli et al. 2014; Pirbalouti et al. 2014) as well as from Turkish flora (Oztürk et al. 2009; Uzun 2009; Toker 2009; Cirak et al. 2010; Bertoli et al. 2011; Cirak et al. 2013) have been investigated in respect to bioactive compound accumulation. However, it should be noted that only *H. perforatum* has been investigated in depth throughout the world both chemically and pharmacologically and only a few studies have been undertaken on other *Hypericum* species although their proven pharmacological importance (Stojanovic et al. 2013). According to Karioti and Bilia (2010), the chemical profile of only one-quarter of *Hypericum* species has been surveyed at the present time. Considering the pharmacological potential of *Hypericum* genus and lack of chemical information on corresponding species, we aimed to present chemical evaluation of seven *Hypericum* species from Turkish flora, namely *H. aviculariifolium* Jaup. and Spach subsp. *avicullariifolium* (Freyn and Bornm.) Robson var. *albiflorum* (endemic), *H. bithynicum* Boiss., *H. calycinum* L., *H. cardiophyllum* Boiss., *H. elongatum* L. subsp. *microcalycinum* (Boiss. and Heldr.) N. Robson, *H. hirsutum* L. and *H. xylosteifolium* (Spach) N. Robson, according to the content of naphthodianthrones hypericin and pseudohypericin, phloroglucinol derivatives hyperforin and adhyperforin, phenolic acids such as chlorogenic acid, neochlorogenic acid, caffeic acid, 2,4-dihydroxybenzoic acid, flavonoids like, hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, (+)-catechin, (−)-epicatechin and 13,118-biapigenin.

Materials and methods

Plant material

The aerial parts of 30 accessions of each seven wild species were randomized at full flowering from various localities of Northern and Southern parts of Turkey. The plant material consisted of one to three shoot from the top and was harvested between 12:00 a.m. and 13:00 p.m. 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 air-dried at room temperature (20 ± 2 °C) and then dissected into different tissues

The botanical identification of the species was made by Dr. Fatih Yayla from Gaziantep University, Faculty of Arts and Sciences, Department of Biology, Turkey. Voucher specimens were deposited in the herbarium of Ondokuz Mayis University Agricultural Faculty, the numbers of voucher specimens are given in Table 1.

Preparation of plant extracts

Air-dried plant material was mechanically ground with a laboratory mill to obtain a homogenous drug powder. Samples of approximately 0.1 g (weighed with 0.0001 g precision) were extracted in 10 mL of 100% methanol by ultrasonication at 40 °C for 30 min. The prepared extracts were filtered through a 0.22 µm membrane filter and stored at 4 °C until analysis. The extracts for naphthodianthrones analysis were exposure to light under xenon lamp (765 W/m^2) for 8 min. due to the photoconversion of protohypericins into hypericins.

HPLC analysis and quantification

A Waters Alliance 2695 (Waters, Milford, MA) separation module system equipped with Waters 996 PDA

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

Species/section	Voucher numbers	Collection site	Latitude (N)	Longitude (E)	Elevation (m)	Habitat
H. avicullariifolium subsp. *avicullariifolium* var. *albiflorum* (endemic)[a]	BMYO # 127	Gümü	40°52′	35°14′	785	Rocky and open slopes
H. bithynicum	BMYO # 128	Abant	40°44′	31°36′	1350	*Pinus* woodland
H. calycinum	BMYO # 130	Akçakoca	40°50′	31°09′	570	*Pinus* woodland
H. cardiophyllum	BMYO # 135	Yeniyazi	37°04′	37°42′	2250	Rocky and open slopes
H. elongatum subsp. *microcalycinum*	BMYO # 136	Nizip	37°00′	37°52′	440	Igneous slopes and rock ledges
H. hirsutum	BMYO # 137	Bayburt	40°15′	40°13′	1250	Rocky and open slopes
H. xylosteifolium	BMYO # 145	Pazar	41°10′	40°53′	100	Igneous slopes and rock ledges

[a]Species are listed in alphabetically.

diode-array detector was used for HPLC analysis. Data were analyzed using Empover Software chromatographic manager system (Waters Corporation, Milford, MA). The separation of flavonoids, epicatechin and hyperforin was carried out using a SunFire C18 column (3.5 µm, 150 mm × 3.0 mm i.d.; Waters, Milford, MA) with 10 mm guard-precolumn. The binary gradient elution method was used for detection of corresponding compounds. The mobile phase consisted of eluent A (water acidified with 0.3% phosphoric acid) and eluent B (acetonitrile containing 0.3% phosphoric acid). The elution program was used as follows: 16% B at 0–12 min, from 16 53% B at 12–18 min, from 53 97% B at 18–18.1 min, 97% B at 18.1–29 min; at 30 min elution conditions returned to initial profile and were kept at least 15 min before next injection. The flow rate was 0.6 mL/min at 0–18.1 min and was changed to 0.8 mL/min at 19–29 min. The column temperature was constant 25 °C. The volume of extract injected was 10 µL. Peaks were detected at a wavelength range of 270–360 nm according to absorption maxima for hyperforin at 270 nm, for epicatechin at 277 and for flavonoids at 360 nm. The ACE C18 column (5.0 µm, 250 × 4.6 mm i.d.; MAC-MOD Analytical, Inc., Chadds Ford, PA) with guard precolumn was used for separation of phenolic acids and catechin. The mobile phase of gradient elution was consisted of eluent A (water acidified with 0.5% glacial acetic acid) and eluent B (acetonitrile was used for separation of dihydroxybenzoic acid and catechin while methanol for elution of caffeic acid derivatives). The elution program was fixed as follows: 5 35% B at 0–30 min, 35 90% B at 30–36 min and 90 5% B at 36–37 min. The flow rate was 1.0 mL/min at 25 °C column temperature, injected volume 10 µl. Detection was monitored at a wavelength range of 277–324 nm according to absorption maxima of catechin at 277 nm, 2,4-dihydroxybenzoic acid at 290 nm, caffeic and caffeoylquinic acids at 324 nm.

Naphthodiantrones were analyzed according to the modified pharmacopeial HPLC method (Anon. 2012) using the ACE C18 column (5.0 µm, 150 mm × 4.6 mm i.d. (MAC-MOD Analytical, Inc., Chadds Ford, PA) with guard precolumn. The mobile phase of isocratic elution consisted of ethyl acetate, aqueous 0.1 M sodium dihydrogen phosphate solution adjusted to pH 2.0 by using phosphoric acid and methanol (39:41:160, v/v). The flow rate was 1.0 mL/min at 40 °C column temperature. The volume of extract injected was 20 µL. Detection was performed at 560 nm wave length. Chromatographic peaks were identified by comparing retention times and spectral characteristics of the eluting peaks to those of authentic reference standards using HPLC-PDA.

The quantification of the compounds was carried out by the external standard method. Standards stock solutions were prepared freshly in methanol and diluted to six different concentrations to obtain a set of concentration ranges. Three injections per concentration were performed to determine linearity. The calibration curve for each of the analyte was constructed by plotting chromatogram peak area at absorption maxima versus the known concentration of standard solution. A linear regression equation was calculated by the least squares method. The regression coefficients of all calibration curves were $R^2 \geq 0.999$ confirming the linearity of the concentration ranges. The precision of the method was demonstrated for all analytes, since all the obtained relative standard deviations (RSD) values were lower than 5.0%. The concentration of compounds was expressed as mg/g dry mass (DM). Solvents used were of HPLC grade and purchased from Sigma-Aldrich (Saint Louis, MO). Water was filtered through the Millipore HPLC grade water preparation cartridge (Millipore, Bedford, MA). Reference substances were purchased from ChromaDex (Santa Ana, CA), Sigma-Aldrich (Saint Louis, MO), HWI ANALYTIK GmbH and Roth GmbH (Karlsruhe, Germany).

Data analysis

Principal component analysis (PCA) was carried out using the statistical software package SPSS Version 20.0 (SPSS Inc., Chicago, IL). PCA analysis is the two-dimensional visualization of the position of investigated accessions relative to each other. The principal components represent the axes which are the orthogonal projections for the values representing the highest possible variances in the case of PC1 and PC2. The obtained data were used to create scatter plot diagrams (Backhaus et al. 1989). Therefore, a factor analysis was performed, whereby each variable was used to calculate relationships between variable and investigated factors. Based on the obtained data, the cluster dendogram was created showing the relationship of investigated accessions regarding to their chemical composition.

Results

In the present study, seven *Hypericum* species native to Northern and Southern Turkey were analyzed for the presence and quantity of 17 bioactive compounds. Identified compounds were detected in all accessions at various levels depending on species and plant parts, except for hypericins, hyperforins, caffeic acid, avicularin and rutin which have not been detected in some cases. Mostly, lower accumulation level of the chemicals was

observed in stems and accumulation pattern of the tested compounds in flowers and leaves varied depending on the species. Flowers were found to be superior over leaves in the quantities of hypericin, pseudohypericin, hyperforin, adhyperforin, hyperoside and 13,118-biapigenin accumulations while the chlorogenic, neochlorogenic, caffeic and 2,4-dihydroxybenzoic acids were mainly accumulated in leaves. The highest accumulation level of the rest compounds were observed in either leaves or flowers depending on the species. For example, isoquercitrin accumulation was the highest in flowers of *H. aviculariifolium* subsp. *aviculariifolium* var. *albiflorum*, *H. cardiophyllum* and *H. hirsutum* and in leaves of *H. bithynicum*, *H. calycinum, H. elongatum* subsp. *microcalycinum* and *H. xylosteifolium* (Table 2).

Results of PCA illuminated the accumulation pattern of the investigated compounds in different plant parts more deeply. The score plots for the first two PCs explained 37.854 and 28.303% (totally 66.157%) of the total variance of the chemical data in flowers, 42.479 and 25.418 (totally 67.897%) in leaves and 36.288 and 27.072 (totally 63.36%) in stem parts of different *Hypericum* species (Figures 1–3). The results indicated that whole parts of *H. hirsitum* display different chemical profile when compared with the investigated *Hypericum* species.

It was found that three species, *H. calycinum, H. cardiophyllum* and *H. xylosteifolium*, had not accumulated hypercin and pseudohypericin (Table 2). The total concentration of naphthodianthrones varied from 0.01 to 0.58 mg/g DM in stems and from 0.11 to 1.65 mg/g DM in leaves of *H. elongatum* subsp. *microcalycinum* and *H. hirsutum,* respectively. The highest amounts of these compounds was found in flowers of *H. bithynicum* (6.7 mg/g DM), while flowers of *H. elongatum* subsp. *microcalycinum* yielded the lowest quantities of hypericins (0.65 mg/g DM). Accumulation levels of hyperforin and adhyperforin were the highest in all parts of *H. hirsutum* (15.84 and 1.61 mg/g DM/flowers, 10.28 and 0.67 mg/g DM/leaves, 1.54 and 0.34 mg/g DM/stems of hyperforin and adhyperforin, respectively). Low amounts of phloroglucinols were detected in *H. bithynicum, H. calycinum* and *H. xylosteifolium* while *H. aviculariifolium* subsp. *aviculariifolium* var. *albiflorum* and *H. elongatum* subsp. *microcalycinum* did not accumulate corresponding compounds. Phenolic acids varied greatly among the species and parts of the plant. Amounts of corresponding compounds ranged from 0.03 mg/g DM in flowers and stems of *H. elongatum* subsp. *microcalycinum* to 24.80 mg/g DM in leaves of *H. xylosteifolium* for chlorogenic acid, from 0.02 mg/g DM in stems of *H. calycinum* to 6.39 mg/g DM in leaves of *H. bithynicum* for neochlorogenic acid and from 0.01 mg/g DM in stems of *H. calycinum* to 0.19 mg/g DM in leaves of *H. bithynicum* for 2,4-dihydroxybenzoic acid. Caffeic acid was detected at quite low amounts in all tested species, with the exception of *H. bithynicum* and *H. calycinum.* The amount of caffeic acid varied from 0.01 to 0.36 mg/g DM depending on species and plant parts. Hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, 13, II8-biapigenin, (+)-catechin and (−)-epicatechin in most cases were detected in all the studied *Hypericum* species, with the exception of rutin that was not found in all tissues of *H. xylosteifolium* and *H. aviculariifolium* subsp. *aviculariifolium* var. *albiflorum*. Leaves of *H. cardiophyllum* and *H. calycinum* produced the highest amounts of hyperoside and isoquercitrin (7.77 and 17.56 mg/g DM, respectively), while both compounds were not detected in stems of *H. xylosteifolium*. Leaves of *H. aviculariifolium* subsp. *aviculariifolium* var. *albiflorum* and *H. bithynicum* produced highest amounts of quercitrin (5.29 and 8.75 mg/g DM, respectively), while leaves of *H. hirsutum* yielded the highest level of rutin (43.34 mg/g DM). Quercitrin was not detected in leaves and stems of *H. xylosteifolium*. The highest level of 13,118-biapigenin was detected in flowers of *H. hirsutum* and *H. cardiophyllum* (3.61 and 4.84 mg/g DM, respectively). Flowers of *H. bithynicum* were superior in accumulation of (−)-epicatechin (7.57 mg/g DM). All species accumulated from the lowest to average amounts of quercetin, avicularin and (+)-catechin, with the exception of avicularin which was not detected in all parts of *H. xylosteifolium* (Table 2).

Discussion

According to Maggi et al. (2004), three kinds of secretory structures including black nodules, translucent glands and secretory canals have been evidenced in plant tissues from the anatomical and morphological standpoints. Secretory structures are sites of accumulation and/or synthesis of different secondary metabolites. For example, hypericins are thought to be present only in of *Hypericum* species whose aerial parts bear dark glands (Lu et al. 2001). Positive correlation was reported in dark glands density and hypericin content in leaf of *H. perforatum, Hypericum pruinatum* Boiss. & Balansa and *Hypericum aviculariifolium* subsp. *depilatum* var. *depilatum* (Freyn and Bornm.) Robson var. *depilatum* (Cirak et al. 2006). The localization of the secretory structures varies greatly among plant tissues and, therefore, the level of phytochemicals in a particular *Hypericum* tissue depend on the relative abundance of secretory structures in the harvested material (Zobayed et al. 2006). As a result, organ-dependence of secondary metabolites is common among *Hypericum* species. In the

Table 2. Hypericin (**1**), pseudohypericin (**2**), hyperforin (**3**), adhyperforin (**4**), chlorogenic acid (**5**), neochlorogenic acid (**6**), caffeic acid (**7**), 2,4-dihydroxybenzoic acid (**8**), hyperoside (**9**), isoquercitrin (**10**), quercitrin (**11**), quercetin (**12**), avicularin (**13**), rutin (**14**), 13,118-biapigenin (**15**), (+)-catechin (**16**) and (−)-epicatechin (**17**) contents (mg/g DM) in different tissues of *Hypericum* plants growing wild in Turkey.

			Compounds																
Species	Section	Plant part	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
H. aviculariifolium subsp. *aviculariifolium* var. *albiflorum*	Origanifolia Stef.	Flower	0.39	0.70	0.00	0.00	1.66	0.13	0.03	0.04	0.57	0.47	7.63	0.09	0.00	0.00	1.12	0.11	0.79
		Leaf	0.31	0.64	0.00	0.00	4.19	0.18	0.04	0.05	0.47	0.20	5.29	0.02	0.04	0.00	0.25	0.00	0.16
		Stem	0.05	0.12	0.00	0.00	1.45	0.11	0.01	0.01	0.29	0.12	2.03	0.02	0.00	0.00	0.01	0.00	0.00
H. bithynicum	Drosocarpium Spach.	Flower	0.99	5.71	0.01	0.01	0.10	1.43	0.00	0.14	4.29	5.87	1.04	0.06	0.12	1.26	2.65	1.65	7.57
		Leaf	0.10	0.44	0.00	0.00	0.39	6.39	0.00	0.19	3.26	13.63	8.75	0.94	0.12	0.68	0.25	0.18	3.36
		Stem	0.02	0.07	0.00	0.00	0.07	0.27	0.00	0.09	0.96	1.30	0.15	0.02	0.08	0.22	0.02	0.19	4.14
H. calycinum	Eremanthe (Spach) Endl.	Flower	0.00	0.00	0.01	0.01	1.59	0.04	0.00	0.06	0.32	5.01	0.26	0.31	0.07	0.34	0.65	1.64	2.17
		Leaf	0.00	0.00	0.00	0.00	17.31	2.02	0.00	0.13	1.61	17.56	4.94	0.68	0.36	0.86	0.02	0.41	2.16
		Stem	0.00	0.00	0.00	0.00	0.28	0.02	0.00	0.01	0.18	5.30	0.10	0.13	0.06	0.16	0.01	0.40	0.75
H. cardiophyllum	Arthrophyllum Jaub. & Spach.	Flower	0.00	0.00	0.49	0.49	6.16	3.56	0.03	0.12	7.84	3.48	0.19	1.35	0.69	0.03	4.84	0.07	0.28
		Leaf	0.00	0.00	0.10	0.10	9.98	5.98	0.03	0.16	7.87	3.15	0.03	0.34	0.36	0.26	0.05	0.31	3.14
		Stem	0.00	0.00	0.00	0.00	0.08	0.03	0.00	0.07	1.78	2.11	0.00	0.06	0.09	0.00	0.00	0.13	0.03
H. elongatum subsp. *microcalycinum*	Drosanthe Spach.	Flower	0.24	0.41	0.00	0.00	0.03	4.72	0.12	0.01	7.01	1.63	2.87	0.46	0.56	0.64	2.21	0.00	0.46
		Leaf	0.04	0.07	0.00	0.00	0.11	4.94	0.13	0.07	7.61	4.27	5.01	0.14	0.24	0.00	0.02	0.04	1.48
		Stem	0.00	0.01	0.00	0.00	0.03	0.46	0.01	0.06	1.69	0.81	1.17	0.03	0.08	0.00	0.02	0.00	0.68
H. hirsutum	Taeniocarpium Jaub. & Spach.	Flower	1.09	3.42	15.84	1.61	0.06	3.41	0.01	0.01	5.26	7.38	6.82	0.24	0.57	17.17	3.61	1.38	3.54
		Leaf	0.38	1.27	10.28	0.67	0.16	5.55	0.01	0.09	3.03	7.17	2.75	0.03	1.28	43.34	0.31	0.83	3.41
		Stem	0.29	0.29	1.54	0.34	0.03	1.15	0.00	0.02	1.00	1.91	0.76	0.00	0.00	0.00	0.03	0.27	0.00
H. xylosteifolium	Inodorum Stef.	Flower	0.00	0.00	0.38	0.33	0.07	0.04	0.08	0.09	0.14	0.41	0.21	0.02	0.00	0.00	0.07	0.00	1.35
		Leaf	0.00	0.00	0.09	0.01	24.80	1.43	0.36	0.11	0.70	3.49	0.00	0.12	0.00	0.00	0.00	0.14	2.11
		Stem	0.00	0.00	0.00	0.00	1.69	0.10	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03

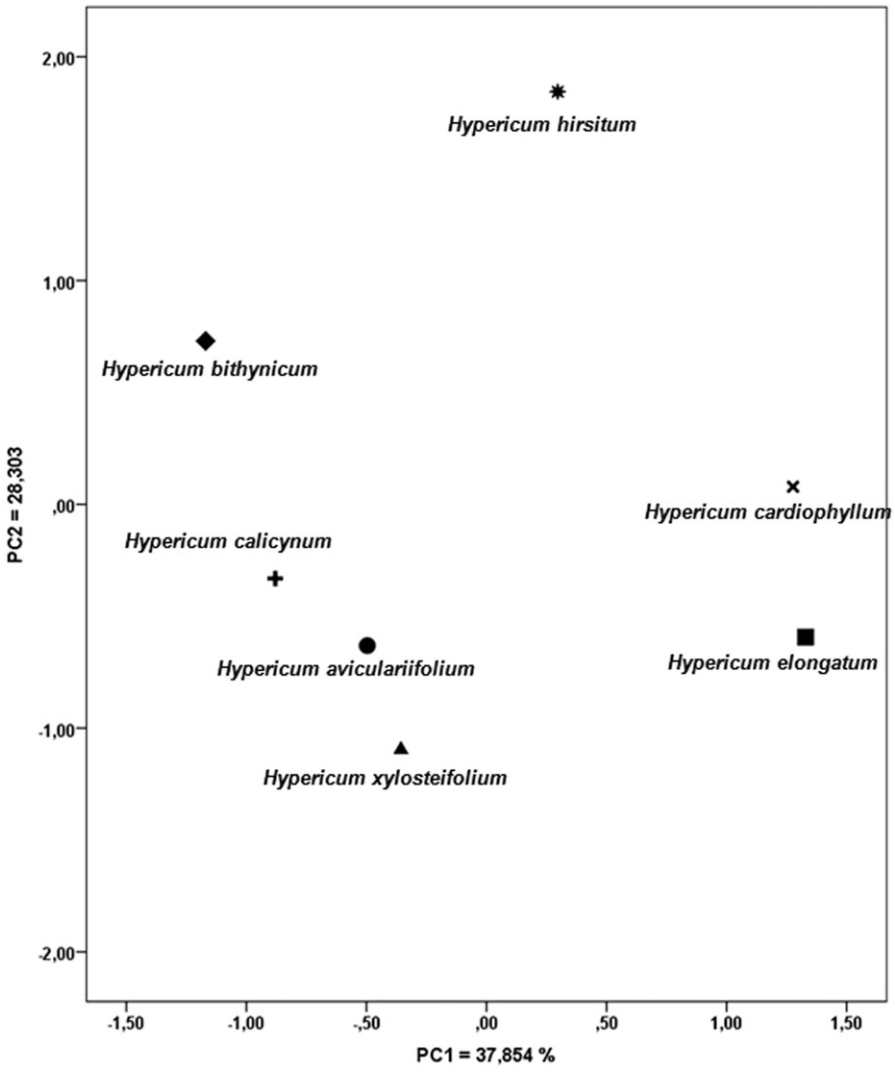

Figure 1. PCA of leaf parts of different *Hypericum* species.

present research, the investigated species differ in flowers and leaves morphology, thereby in the dimension and distribution of secretory structures through the plant parts (Figure 4). This phenomenon could explain the great variation observed in content of compounds among the species as well as their different tissues.

Among the *Hypericum* species investigated in the present study, *H. calycinum* is a well-known medicinal plant with its fixed antimicrobial (Nogueira et al. 2013), antifungal, antimalarial (Decosterd et al. 1991) and antioxidant (Kirmizibekmez et al. 2009) activities. Especially, its antidepressant effect could be of interest which was detected as potent as that of *H. perforatum*, the officinal species used in the treatment of mild to moderate depression (Oztürk et al. 1996). *Hypericum hirsutum* was also exhibited antiinflammatory (Katarina et al. 2007), antimicrobial and antioxidant activities (Radulovic et al. 2007). Comparing the results obtained for our plant material of *Hypericum* species with those reported from other localities, similar results were obtained for the composition of some species. In accordance to our results, *H. calycinum* was reported to contain chlorogenic acid, quercetin, quercitrin, hyperoside, isoquercitrin, rutin, 13,118-biapigenin, (+)-catechin, (−)-epicatechin (Kirmizibekmez et al. 2009), hyperforin and adhyperforin (Klingauf et al. 2005) but not to accumulate hypericin and pseudohypericin (Kitanov 2001). Furthermore, we detected neochlorogenic acid, 2,4-dihydroxybenzoic acid and avicularin in *H. calycinum*. Smelcerovic et al. (2008) reported that *H. hirsutum* and *H. bithynicum* accumulated hypericin, pseudohypericin, hyperforin, rutin, hyperoside, quercitrin and quercetin, which corresponds to our data, however additionally we detected dihydroxybenzoic acid and caffeic acid derivatives, 13,118-biapigenin, (+)-catechin and (−)-epicatechin,

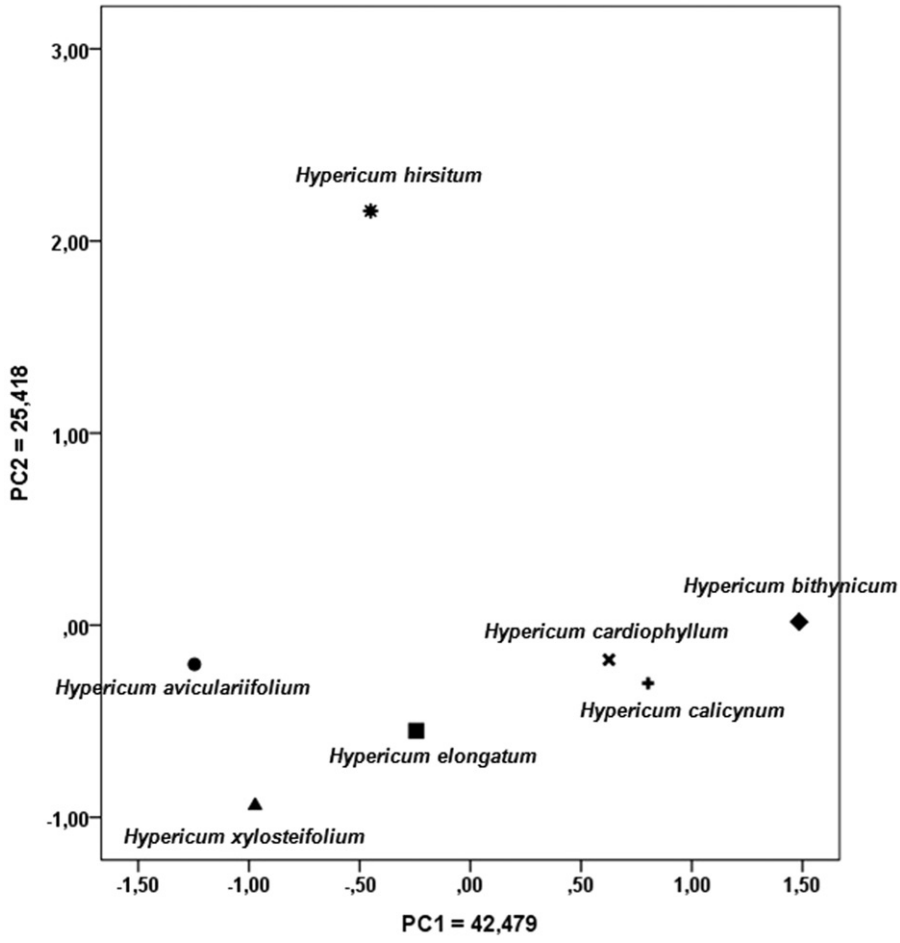

Figure 2. PCA of flower parts of different *Hypericum* species.

isoquercitrin and avicularin. Our study confirmed that *H. xylosteifolium* did not contain hypericin and pseudohypericin as indicated by Kitanov (2001). Despite the quantitative differences which probably derived from geographic origins of plants, harvesting time, sampling procedures, analytical techniques used, etc., our present findings on the abovementioned *Hypericum* species in principle are consistent with those of the previous investigations. Moreover, in the corresponding species, we detected more compounds that can indicate new characteristics of plant materials. This confirms that even investigated species could assume as potential sources for further assay of bioactive compounds. On the contrary, to our knowledge, no data are available in the current literature on *H. avicullariifolium* subsp. *avicullariifolium* var. *albiflorum*, *H. cardiophyllum* and *H. elongatum* subsp. *microcalycinum* secondary metabolites. Thus, we reported the detailed chemical profiles of these *Hypericum* species for the first time. As shown in Table 2 previously investigated species, especially *H. hirsutum* and *H. bithynicum* produced higher levels of the tested compounds generally when compared with others. However, it is interesting to note that among the first investigated species, flowers of *H. cardiophyllum* accumulated the highest hyperoside and quercetin levels, observed in the present study (7. 87 and 1.35 mg/g DM, respectively, Table 2). This phenomenon indicates that new species are good candidates as new sources of bioactive compounds and deserve to be investigated more deeply.

Screening of secondary metabolites on *Hypericum* species, besides the importance in discovery of new sources of active compounds, can be useful devices in taxonomic survey of *Hypericum* genus (Crockett & Robson 2011). Morphological characters in many cases are not sufficient correctly to distinguish taxonomic sections and identify the individual plants. In this regard, chemotaxonomic significance is attributed to some of *Hypericum* metabolites such as naphthodianthrones hypericins (Kitanov 2001), dimeric phloroglucinol uliginosin B (Ferraz et al. 2002), flavonoids as hyperoside, quercetin, quercitrin (Cirak et al. 2010), rutin and mangiferin (Nunes et al. 2010). The current study presented chemical composition of species

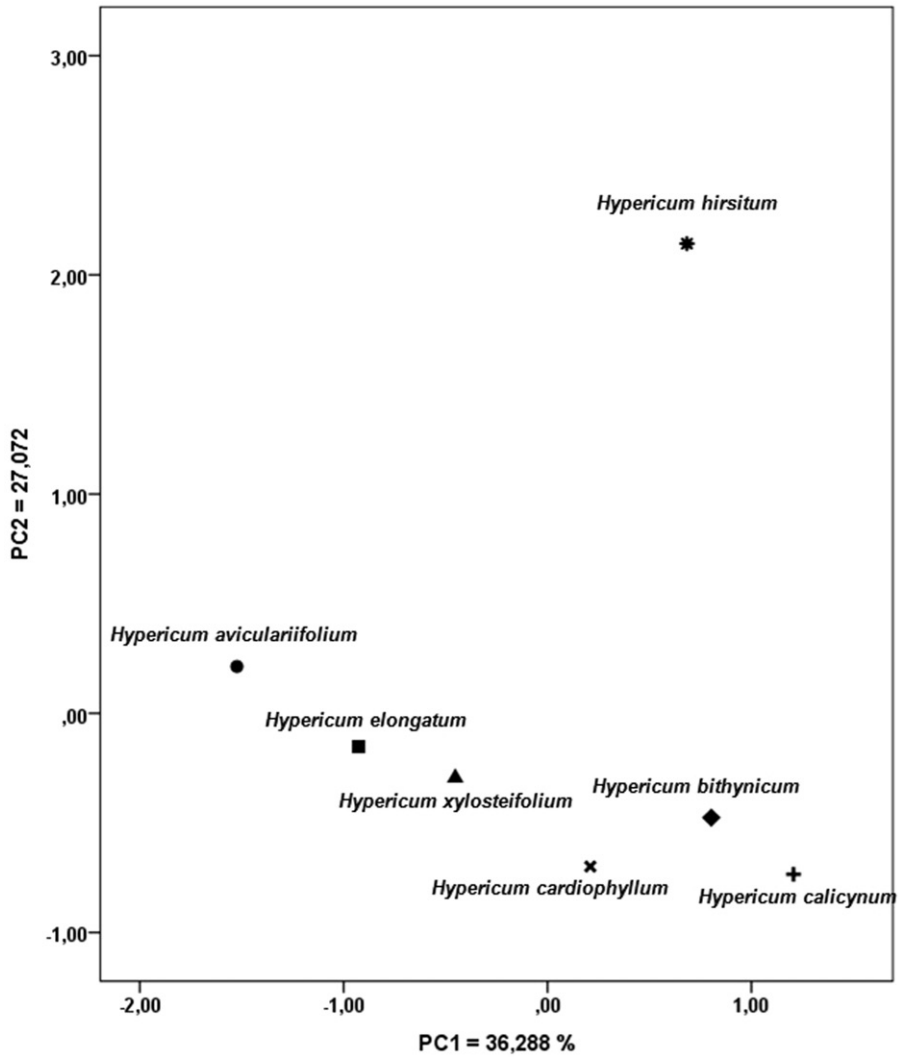

Figure 3. PCA of stem parts of different *Hypericum* species.

from different sections within *Hypericum* genus (Table 2).

PCA is useful statistical analysis for the differentiation of plant materials and its results can give information about differences and similarities of various species regarding their chemical composition (Smelcerovic et al. 2008; Bertoli et al. 2011). Results of PCA in the present study revealed that *Hypericum* species investigated on the basis of their chemical composition were highly different. The results may be helpful in further morphological and chemical investigations on the corresponding species as well as in evaluating the plants for medicinal purposes.

Conclusions

Increasing interest in recent years for using medicinal and aromatic plants as natural sources in pharmaceutical, food, biotechnology, agricultural and cosmetic industries all over the world has resulted in extensive efforts to discover new sources of potential bioactive phytochemicals. In this sense, the present screening data could be helpful in selecting the future targets of new sources of bioactive compounds of *Hypericum* species for phytochemical and biological studies as well as enriching our current knowledge about *Hypericum* genus chemistry. Besides, this is the first report describing the chemical profile of *H. aviculariifolium* subsp. *aviculariifolium* var. *albiflorum*, *H. cardiophyllum* and *H. elongatum* subsp. *microcalycinum* as well as the occurrence of several new compounds in *H. bithynicum, H. calycinum, H. hirsutum* and *H. xylosteifolium*. The present data are of great interest to reveal new sources of raw material as potential pharmaceuticals. The chemical evaluation of *Hypericum* species could be used as additional tool in completing the taxonomy of genus and for understanding the evolution of its diversity.

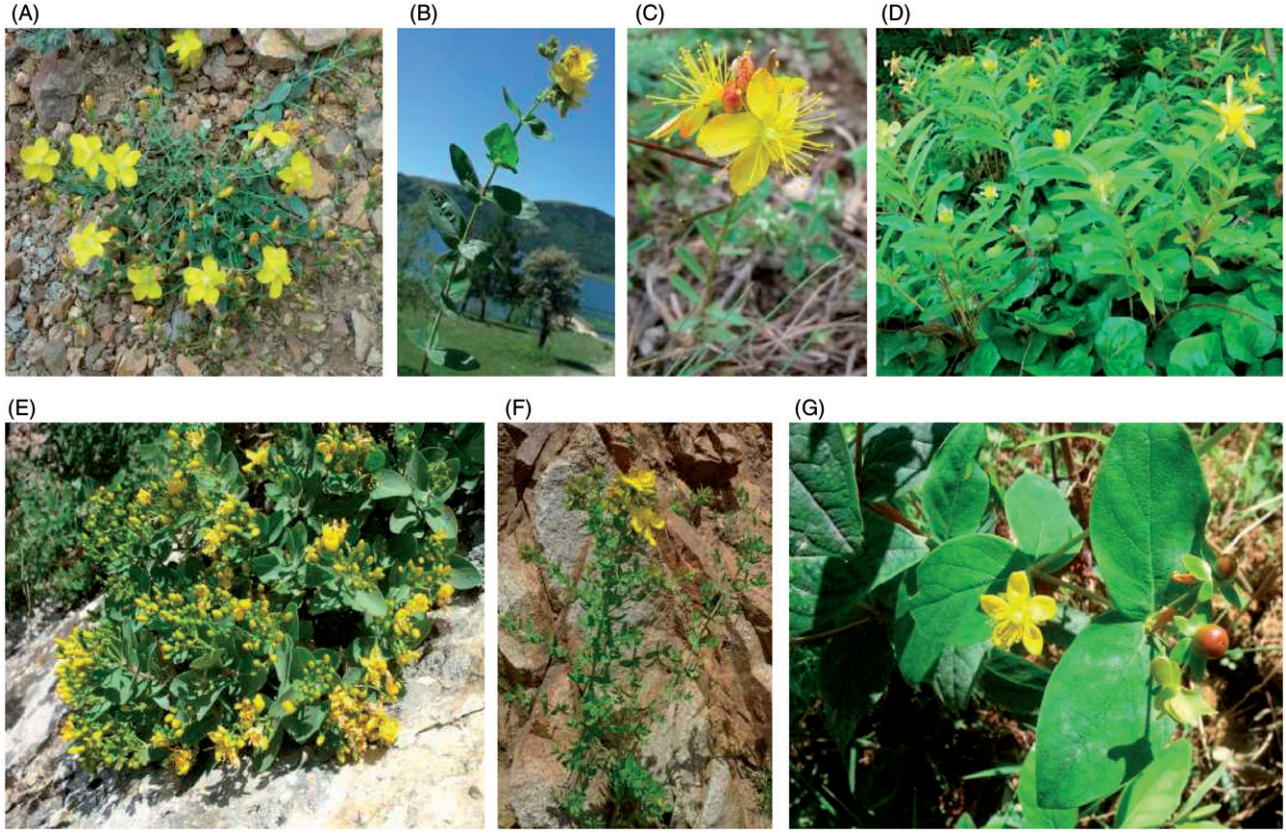

Figure 4. A view from *H. aviculariifolium* subsp. *avicullariifolium* var. *albiflorum* (A), *H. bithynicum* (B), *H. elongatum* subsp. *microcalycinum* (C), *H. calycinum* (D), *H. cardiophyllum* (E), *H. hirsutum* (F) and *H. xylosteifolium* (G) plants at flowering in their native habitats.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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