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Secondary metabolites of *Hypericum* species from the *Drosanthe* and *Olympia* sections

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ABSTRACT

Eight *Hypericum* species native to Southern Turkey from *Drosanthe* and *Olympia* sections were investigated for the presence of several bioactive compounds, namely, hypericin, pseudohypericin, hyperforin, adhyperforin, the chlorogenic, neochlorogenic, caffeic and 2,4-dihydroxybenzoic acids, hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, (+)-catechin, (−)-epicatechin, mangiferin, I3, II8-biapigenin, and amentoflavone for the first time. Plants were harvested at flowering, dried at room temperature, dissected into different tissues, and assayed for chemical contents. HPLC analysis of methanolic fractions displayed similar chemical profile and significant quantitative differences among the investigated taxa. The present results support the taxonomic value of hypericins, rutin, and mangiferin at the sectional level and make an important contribution to our current knowledge about *Hypericum* chemistry. Such kind of data could also be beneficial for explanation of the chemotaxonomic utility of the corresponding compounds as well as phytochemical evaluation of the species tested.

1. Introduction

Hypericum is a genus, included by the plant family Hypericaceae and consists of 484 species in forms of small trees, shrubs, and herbs, distributed in 36 taxonomic sections (Crockett and Robson 2011). These species occur naturally in all temperate parts of the world but are absent in habitats having extreme environmental conditions such as deserts and poles. Turkey is an important center for the genus *Hypericum*, and according to the most recent count by Güner et al. (2012), there are a total of 96 *Hypericum* species in the flora of Turkey from 19 sections and 46 of them are endemic. All *Hypericum* species have been traditionally 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). A number of *Hypericum* species native to southern part of Anatolia assigned to the sections Drosanthe Spach. and Olympia Spach. with 20 and 2 representatives in flora of Turkey, respectively (Davis 1988).

Phytochemical investigations on the species from sect. *Olympia*, such as *H. polyphyllum* Boiss. et Balansa and *H. olympicum* L. (Kitanov 2001), and the species from sect. *Drosanthe*, such as *H. olivieri* (Spach) Boiss., *H. scabrum* L., *H. lydium* Boiss. (Cirak 2006; Cirak et al. 2007a; Ayan et al. 2008; Camas et al. 2014), *H. helianthemoides* (Spach) Boiss. (Moein et al. 2011), and *H. hyssopifolium* Vill. (Smelcerovic et al. 2008), have revealed that these species are valuable sources of naphthodianthrones, phloroglucinol derivatives, phenolic acids, flavonols, and biflavonoids. In addition, alkanes, fatty acids, and essential oils were identified in some species of corresponding sections such as *H. olympicum* (Stojanovic et al. 2003), *H. salsolifolium* Hand.-Mazz., *H. retusum* Aucher (Bagci and Yuce 2011a), *H. lydium* (Şerbetçi et al. 2012), *H. hyssopifolium*, *H. lysimachioides* Boiss. & Noe (Toker et al. 2006), *H. scabrum* (Morteza-Semnani et al. 2006), *H. capitatum* Choisy var. *capitatum* and var. *luteum* Robson (Bagci and Yuce 2011b). The occurrence of these phytochemicals in *Hypericum* plants is associated with the antidepressant (Stein et al. 2012), anti-inflammatory (Crockett et al. 2008), antiproliferative (Schmidt et al. 2012), and antibacterial (Saddiqe et al. 2010) activities of *Hypericum* extracts. On the other hand, some chemotaxonomic significance has also been attributed to flavonoids hyperoside, quercetin, quercitrin (Cirak et al. 2010), naphthodianthrones hypericins (Kitanov 2001), dimeric phloroglucinol uliginosin B (Ferraz et al. 2002a), xanthone mangiferin (Nunes et al. 2010), and to several volatile constituents as non-terpenes and sesquiterpenes (Smelcerovic et al. 2007).

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Despite the large number of *Hypericum* species, only *H. perforatum* L. has been investigated intensively throughout the world both chemically and pharmacologically. It is a commercialized species, and its extracts are now widely used in Europe as a drug for the treatment of mild to moderate depression (Fiebich et al. 2011). When compared to *H. perforatum*, few studies have been undertaken on other members of this genus although their proven pharmacological importance (Stojanovic et al. 2013) and the chemical profile of approximately three-quarters of *Hypericum* species has not yet been surveyed (Karioti and Bilia 2010). Considering the pharmacological potential of *Hypericum* species and the lack of chemical information on *Hypericum* genus, we aimed to present chemical evaluation of eight *Hypericum* species from *Drosanthe* and *Olympia* sections according to the content of naphthodianthrones hypericin and pseudohypericin, phloroglucinol derivatives hyperforin and adhyperforin, phenolic acids as chlorogenic, neochlorogenic, caffeic and 2,4-dihydroxybenzoic acids, flavonol glycosides hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, flavanols (+)-catechin and (−)-epicatechin, xanthone mangiferin, and biflavonoids I3,II8-biapigenin and amentoflavone.

2. Materials and method

2.1. Brief description of plant materials

Sect. *Drosanthe* includes herbaceous, perennial, sometimes woody at the base plants; flowers with petals and stamens, petals sometimes red-veined or red tinged, usually unguiculate, black glands located along to sepal and petal margins, rarely superficial, or at leaf apices. Flowering plants of evaluated 6 species from this section are shown in Fig. 1.

The species from sect. *Olympia* are perennial herbs, often shrubby at the base, glabrous, stems usually without axillary shoots, petals, and stamens are persistent; black glands present on anthers and sometimes on leaves, sepals and petals, and petals on times with superficial black glands (Davis 1988). Flowering plants of evaluated two species from sect. *Olympia* are shown in Fig. 2.

The aerial parts of *Hypericum* plants from both sections representing a total of 30 individuals for each species were collected at full flowering from Southern Turkey in June 2013. The species names, their voucher numbers, and geographical data of collection sites are shown in Table 1. Species were identified by Dr. Fatih Yayla, 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 top of 2/3 plants was harvested between 11:00 am and 13:00 pm. Conditions on the day of collection were clear and sunny at all sites and temperatures ranged from 28 °C to 35 °C. The plant materials were dried at room temperature (20 ± 2 °C) and, after separated into different tissues, were subsequently assayed for chemical contents by HPLC.

2.2. 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 were extracted in 10 μL of 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.

2.3. HPLC conditions, analysis, and quantification

A Waters Alliance 2695 (Waters, Milford, USA) separation module system equipped with Waters 2487 UV/Vis and Waters 996 PDA diode-array detectors was used for HPLC analysis. Data were analyzed using Empower Software chromatographic manager system (Waters Corporation, Milford, USA).

The separation of flavonoids, epicatechin, and hyperforin was carried out according to the pharmacopoeial method (Pharm. Eur., 2012) on an ODS hypersil column (3 μm, 150 mm × 4.6 mm i.d., Thermo Fisher scientific Inc. USA) with 10 mm guard-precolumn. The binary gradient elution method was used for detection of corresponding

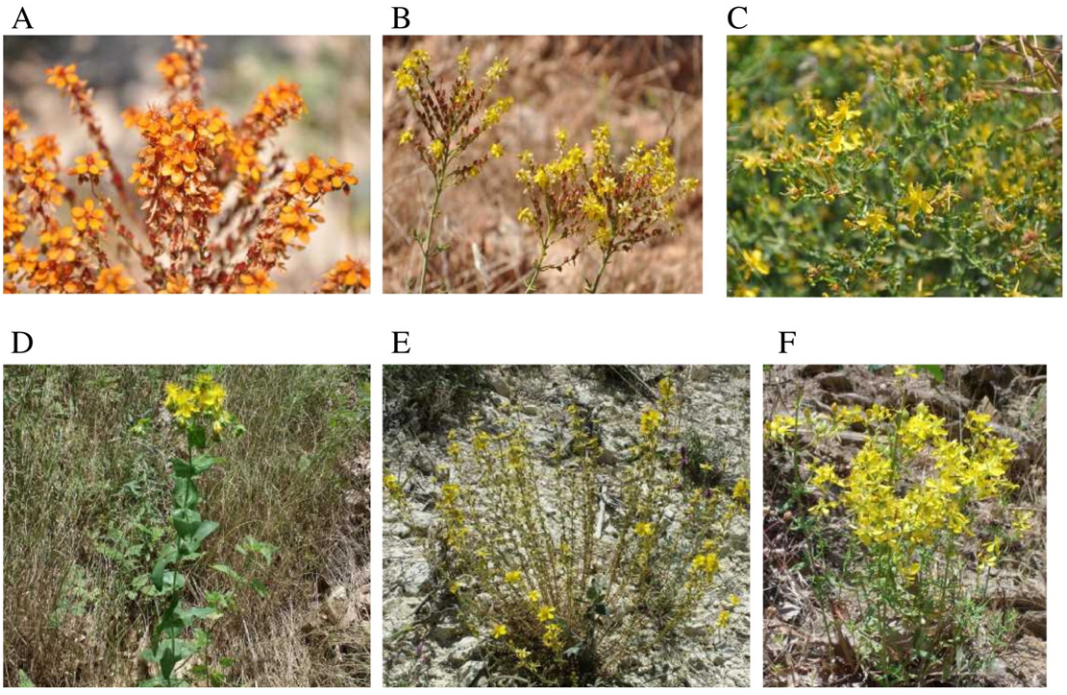

Fig. 1. Flowering plants of *H. capitatum* var. *capitatum* (A), *H. capitatum* var. *luteum* (B), *H. retusum* (C), *H. spectabile* (D), *H. elongatum* var. *elongatum* (E), and *H. salsolifolium* (F) from sect. *Drosanthe*.

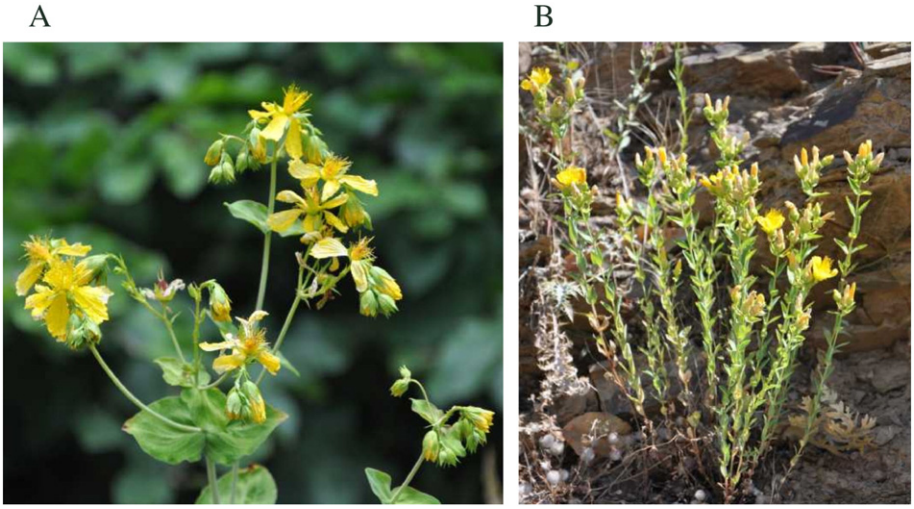

Fig. 2. Flowering plants of *H. olympicum* (A) and *H. polyphyllum* (B) from sect. *Olympia*.

compounds. The mobile phase consisted of water Milli-Q acidified with 0.3% phosphoric acid as eluent A and acetonitrile containing 0.3% phosphoric acid as eluent B. The elution program was used as follows: 16% B at 0–12 min, from 16% to 53% B at 12–18 min, from 53% to 97% B at 18–18.1 min, 97% at 18.1–29 min, and from 97% to 16% at 29–30 min. Flow rate was 0.6 mL/min at 0–19 min and was changed to 0.8 μL/min at 19–30 min. The column temperature was 25 °C. The volume of extract injected was 10 μL. Peaks were detected at a wavelength range of 270–360 nm.

The ACE C18 column (5.0 μm, 250 × 4.6 mm i.d.; MAC-MOD Analytical, Inc) with guard-precolumn was used for separation of phenolic acids, catechin, and mangiferin. The binary gradient elution of eluent A—water acidified with 0.5% glacial acetic acid and eluent B—100% methanol was used as a mobile phase for the detection of the compounds. The separation was fixed as following program: from 5% to 35% B at 0–30 min, from 35% to 90% B at 30–36 min, and from 90% to 5% B at 36–37 min. The flow rate was 1.0 μL/min, and the column temperature was 25 °C. Detection was monitored at a wavelength of 275–360 nm.

Naphthodiantrones were analyzed according to a little modified pharmacopoeial HPLC method (Pharm. Eur., 2012) using the ACE C18 column (5.0 μm, 150 mm × 4.6 mm i.d. (MAC-MOD Analytical, Inc) 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 using phosphoric acid and methanol (16:17:67% *v*/v). The flow rate was 1.0 μL/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. A calibration curve for each of the compounds was constructed by plotting peak area against 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 results are reported in terms of RSD. The retention time, linear range, regression equation, correlation coefficient, and RSD values of each analyte are summarized in Table 2. The concentration of compounds was expressed as mg/g dry mass (DM).

Solvents used were of HPLC grade and purchased from Roth GmbH (Karlsruhe, Germany). Water was filtered through the Millipore HPLC grade water preparation cartridge (Millipore, Bedford, USA). Reference substances were purchased from ChromaDex (Santa Ana, USA), Sigma-Aldrich (Saint Louis, USA), HWI ANALYTIK GmbH, and Roth GmbH (Karlsruhe, Germany).

2.4. Data analysis

Principal component analysis (PCA) was carried out using the statistical software package SPSS Version 20.0. This analysis is the two-dimensional visualization of the position of investigated exemplars relative to each other. The principal components represent the axes which are the orthogonal projections for the values representing the highest possible variances in this 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, also a dendogram (cluster) was created (Backhaus et al. 1989) showing the

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

Species[a]	Voucher numbers	Collection site	Latitude (N)	Longitude (E)	Elevation (m)	Habitat
H. capitatum var. *capitatum*	OMUZF # 122	Yeniyazı	37° 04′ N	37° 42′ E	620	Arid pasturelands
H. capitatum var. *luteum*	OMUZF # 123	Yeniyazı	37° 04′ N	37° 42′ E	620	*Pinus* woodland
H. elongatum var. *elongatum*	OMUZF # 114	Nizip	37° 00′ N	37° 52′ E	440	Rocky and open slopes
H. olympicum	OMUZF # 135	Nizip	37° 00′ N	37° 52′ E	440	Igneous slopes and rock ledges
H. polyphyllum	OMUZF # 136	Kocatepe village	37° 02′ N	37° 41′ E	780	Arid pasturelands
H. retusum	OMUZF # 141	Hamo hill/İslahiye	36°57′ N	36° 30′ E	1200	Igneous slopes and rock ledges
H. salsolifolium	OMUZF # 122	Huzurlu Plateau/İslahiye	36° 59′ N	36° 26′ E	1400	Igneous slopes and rock ledges
H. spectabile	OMUZF # 144	Hamo hill/İslahiye	36° 57′ N	36° 30′ E	800	Rocky and open slopes

[a] Species are listed in alphabetically.

Table 2
The retention time, linear range, regression equation, correlation coefficient, and precision of each detected analytes of HPLC analysis on examined *Hypericum* species.

Analytes	Processing wavelength, nm	Retention time, min	Linearity range, μg/μL	R^2	Regression equation	RSD (%)
2,4-Dihydroxybenzoic acid	290	13.3	0.31–9.80	0.9995	$Y = 2.01 \cdot 10^4 X + 1.56 \cdot 10^3$	3.08
Neochlorogenic acid	324	15.0	0.61–196.00	0.9999	$Y = 3.43 \cdot 10^4 X - 5.32 \cdot 10^3$	0.51
(+)-Catechin	275	19.3	0.30–95.00	0.9999	$Y = 1.20 \cdot 10^4 X + 3.96 \cdot 10^3$	3.19
Chlorogenic acid	324	21.4	0.30–194.00	0.9999	$Y = 3.05 \cdot 10^4 X + 4.43 \cdot 10^3$	0.31
Caffeic acid	324	24.4	0.31–49.00	0.9999	$Y = 5.25 \cdot 10^4 X + 7.17 \cdot 10^3$	0.18
Mangiferin	360	29.0	2.19–280.80	0.9997	$Y = 1.82 \cdot 10^4 X$	1.00
(−)-Epicatechin	277	7.2	0.30–195.60	0.9999	$Y = 1.08 \cdot 10^4 X$	2.50
Rutin	360	14.8	0.28–178.42	0.9990	$Y = 2.73 \cdot 10^4 X$	2.73
Hyperoside	360	15.2	0.29–187.02	0.9996	$Y = 4.75 \cdot 10^4 X$	4.67
Isoquercitrin	360	15.5	0.29–188.30	0.9984	$Y = 3.30 \cdot 10^4 X$	8.60
Avicularin	360	16.2	0.15–19.16	0.9999	$Y = 4.53 \cdot 10^4 X$	1.58
Quercitrin	360	16.6	0.31–196.76	0.9991	$Y = 3.53 \cdot 10^4 X$	6.92
Quercetin	360	19.2	0.31–196.00	0.9990	$Y = 4.59 \cdot 10^4 X$	7.07
13,II8-Biapigenin[a]	360	20.5	0.28–181.80	0.9990	$Y = 4.26 \cdot 10^4 X$	7.09
Amentoflavone	360	20.9	0.28–181.80	0.9990	$Y = 4.26 \cdot 10^4 X$	7.09
Hyperforin	270	25.5	3.11–199.00	0.9999	$Y = 2.42 \cdot 10^4 X$	0.83
Adhyperforin	270	26.0	1.02–65.00	0.9999	$Y = 2.42 \cdot 10^4 X$	0.51
Pseudohypericin	590	2.9	0.38–96.20	0.9998	$Y = 6.86 \cdot 10^4 X$	2.13
Hypericin	590	8.4	0.37–95.10	0.9997	$Y = 1.00 \cdot 10^5 X$	2.52

[a] Processing of 13,II8-biapigenin peaks was performed by using calibration curve of amentoflavone reference substance.

relationship of investigated samples regarding their chemical composition.

3. Results

In the present study, eight species of *Hypericum* native to Southern Turkey were analyzed for the presence and quantity of 19 bioactive compounds. HPLC analysis of methanolic fractions displayed similar chemical profile and significant quantitative differences among the investigated taxa. No caffeic acid accumulation was observed in plants from sect. *Drosanthe* while plants of sect. *Olympia* did not produce hyperforin and adhyperforin. Generally, lower accumulation level of the chemicals was observed in stems. Flowers were found to be superior over leaves with respect to hypericin, pseudohypericin, hyperforin, adhyperforin, caffeic acid, quercetin, I3,II8-biapigenin, amentoflavone, mangiferin, and (+)-catechin accumulations while chlorogenic acid, neochlorogenic acid, and isoquercitrin were mainly accumulated in leaves in both sections. The accumulation pattern of the tested compounds in flowers and leaves varied with sections. For example, hyperoside, quercitrin, rutin, and (−)-epicatechin accumulations were the highest in flowers of the species from sect. *Drosanthe* but in leaves of the species from sect. *Olympia*. Accordingly, leaves of the species from sect. *Drosanthe* accumulated the highest level of 2,4-dihydroxybenzoic acid and avicularin while flowers of species from the sect. *Olympia* dominated with the highest content of corresponding compounds (Tables 3 and 4).

Results of PCA illuminated the accumulation pattern of the investigated compounds in different plant parts more deeply. The calculated principal component (PC) values for the tested compounds are shown in Table 5.

The score plots for the first two PCs explained 26.72% and 20.85% (totally 40.81%) of the total variance of the chemical data. The obtained scatter plot using PC1 and PC2 is shown in Figs. 3 and 4. The results indicated that the stems of all the investigated *Hypericum* species display nearly similar chemical profile, while flowers of *H. capitatum* var. *luteum*, *H. capitatum* var. *capitatum*, *H. elongatum* var. *elongatum*, *H. spectabile*, *H. polyphyllum*, *H. retusum*, and the leaves of *H. spectabile* differed significantly according to their chemical composition.

Table 3
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), I3, II8-biapigenin (15), amentoflavone (16), mangiferin (17), (+)-catechin (18), and (−)-epicatechin (19) contents (mg/g DM) in different plant parts of some *Hypericum* species from sect. *Drosanthe*.

Species[a]	Plant parts	Compounds																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
H. capitatum var. *capitatum*[b]	Leaf	0.03	0.01	–	–	0.12	9.68	–	0.10	1.98	2.07	9.91	0.35	1.69	0.98	0.07	–	–	–	0.89
	Flower	0.11	0.12	0.01	0.01	0.08	2.71	–	0.07	2.25	5.22	12.03	1.01	0.95	2.06	0.73	0.02	–	0.32	2.69
	Stem	–	–	–	–	0.06	0.21	–	0.05	0.69	1.41	1.84	0.04	0.42	–	–	–	–	–	0.46
H. capitatum var. *luteum*[b]	Leaf	0.02	0.01	0.02	0.02	0.11	11.23	–	0.16	3.12	3.18	9.49	0.21	3.46	0.64	0.08	–	–	0.03	1.12
	Flower	0.04	0.06	0.04	0.04	0.05	4.30	–	0.06	6.39	4.03	10.88	0.76	1.36	3.23	0.78	0.02	–	0.09	3.34
	Stem	–	–	–	–	–	0.45	–	0.04	1.79	2.35	2.50	0.03	0.92	–	–	–	–	0.04	1.43
H. retusum	Leaf	1.04	1.32	–	–	12.39	8.72	–	0.17	6.61	12.22	0.96	0.25	0.36	5.11	0.21	–	0.04	–	1.49
	Flower	1.18	2.41	0.03	0.03	10.62	4.71	–	0.07	12.02	10.14	1.45	0.74	0.18	15.66	2.16	0.07	0.09	0.11	2.02
	Stem	0.02	0.03	–	–	0.40	0.17	–	0.03	0.63	1.51	0.06	0.06	0.02	0.75	0.01	–	–	–	1.31
H. spectabile[b]	Leaf	0.60	1.60	–	–	88.37	2.46	–	0.08	–	0.80	1.27	0.01	0.07	23.73	0.01	–	–	0.04	0.31
	Flower	1.35	2.33	–	–	16.73	1.02	–	0.03	0.34	0.27	5.52	0.07	–	63.94	2.48	0.12	2.18	0.41	1.50
	Stem	–	0.01	–	–	3.25	0.03	–	0.04	0.03	0.15	0.54	–	–	2.03	–	–	0.08	0.18	0.66
H. elongatum var. *elongatum*	Leaf	0.02	–	0.01	0.01	0.04	0.12	–	0.19	1.14	1.91	8.52	0.11	0.14	23.54	0.12	–	–	–	0.44
	Flower	0.03	–	0.04	0.04	0.03	0.04	–	0.07	1.29	0.91	10.81	0.60	0.06	27.51	1.07	0.04	0.05	–	1.03
	Stem	–	–	–	–	0.03	0.02	–	0.09	0.33	0.87	7.40	0.10	–	2.78	0.01	–	–	–	0.27
H. salsolifolium[b]	Leaf	0.01	0.02	–	–	0.69	5.01	–	0.09	1.98	7.74	0.40	0.13	0.94	0.33	0.07	–	–	0.18	3.73
	Flower	0.14	0.39	–	–	0.44	1.61	–	0.05	6.11	1.80	0.53	0.46	0.52	1.56	0.75	0.05	–	0.25	3.95
	Stem	–	–	–	–	0.40	0.26	–	0.05	1.56	0.92	0.42	0.09	0.09	0.24	0.01	–	–	0.01	2.17

[a] Species are listed in accordance with the classification by Davis (1988).
[b] Endemic.

Table 4
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), I3, II8-biapigenin (15), amentoflavone (16), mangiferin (17), (+)-catechin (18), and (−)-epicatechin (19) contents (mg/g DM) in different plant parts of some *Hypericum* species from sect. *Olympia*.

Species[a]	Plant parts	Compounds																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
H. olympicum	Leaf	0.24	1.56	–	–	35.16	9.94	0.01	0.11	0.45	0.21	0.17	0.03	0.01	24.74	0.05	–	–	0.09	0.81
	Flower	0.31	1.86	–	–	11.80	3.01	0.03	0.14	0.32	0.09	0.13	0.21	0.05	5.63	0.81	0.04	–	0.19	0.09
	Stem	–	0.04	–	–	3.76	0.77	–	0.06	0.09	0.01	0.06	0.03	0.03	2.63	0.01	–	–	–	0.40
H. polyphyllum	Leaf	0.47	1.79	–	–	16.21	6.31	0.03	0.03	0.45	0.43	0.69	0.02	0.01	52.57	0.06	–	–	0.01	0.70
	Flower	0.56	2.01	–	–	15.78	5.01	0.06	0.06	0.04	0.23	0.50	0.13	0.05	17.91	1.09	0.07	–	0.28	0.25
	Stem	0.01	0.02	–	–	2.79	0.57	–	0.03	0.17	0.74	0.23	0.05	0.01	6.23	0.01	–	–	0.01	0.01

[a] Species are listed in accordance with the classification by Davis (1988).

Regarding the quantitative amount of tested compounds, hypercin, and pseudohypericin concentrations varied from 0.01 mg/g DM in leaves of *H. salsolifolium* to 1.35 mg/g DM in flowers of *H. spectabile* and from 0.01 in leaves of *H. capitatum* var. *capitatum* and var. *luteum* to 2.41 mg/g DM in flowers of *H. retusum*. *H. spectabile*, *H. salsolifolium*, *H. olympicum*, and *H. polyphylum* did not accumulate hyperforin and adhyperforin and those compounds were detected only in flowers and/or leaves of the rest species at low amounts (0.01–0.04 mg/g DM). As for the phenolic acids, an extreme variation was noticed in accumulation levels of chlorogenic and neochlorogenic acids. Yields for corresponding compounds ranged from 0.03 mg/g DM in flowers and stems of *H. elongatum* var. *elongatum* to 88.37 mg/g DM in leaves of *H. spectabile* for chlorogenic acid and from 0.02 mg/g DM in stems of *H. elongatum* var. *elongatum* to 11.23 mg/g DM in leaves of *H. capitatum* var. *luteum* for neochlorogenic acid. 2,4-Dihydroxybenzoic acid was accumulated at quite low amounts in all tested species and its accumulation levels varied between 0.03 and 0.19 mg/g DM, depending on species and plant parts. Caffeic acid was detected only in species from sect. *Olympia* and the highest content was accumulated in flowers of *H. polyphyllum* (0.06 mg/g DM). Hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, 13,II8-biapigenin, and (−)-epicatechin were detected generally in all parts of *Hypericum* plants studied. Flowers and leaves of *H. retusum* produced the highest level of hyperoside and isoquercitrin (12.02 and 12.22 mg/g DM, respectively), while the lowest amounts of the corresponding compounds were observed in stems of *H. spectabile* and *H. olympicum* (0.03 and 0.01 mg/g DM, respectively). Flowers of *H. spectabile* yielded the highest level of rutin and 13,II8-biapigenin (63.94 and 2.48 mg/g DM, respectively), and these compounds were absent in stems of *H. capitatum* var. *capitatum* and var. *luteum*. The highest level of quercitrin and avicularin was detected in flowers and leaves of *H. capitatum* var. *capitatum* (12.03 and 1.69 mg/g DM, respectively), and stems of *H. retusum* yielded the lowest amount of both compounds (0.06 and 0.02 mg/g DM, respectively). (−)-Epicatechin contents ranged between 3.95 mg/g DM in *H. salsolifolium* flowers and 0.01 mg/g DM in *H. polyphyllum* stems. Quercetin was accumulated at moderate levels (1.01–0.02 mg/g DM depending on species and plant parts) when compared to the other tested flavonols. *H. elongatum* var. *elongatum* did not produce (+)-catechin while this compound was detected in different tissues of the rest species at low amounts. No mangiferin accumulation was observed in species from sect. *Olympia*. This compound was detected only in different parts of *H. retusum*, *H. spectabile*, and *H. elongatum* var. *elongatum* and reached its highest accumulation level in flowers of *H. spectabile* (2.18 mg/g DM). Amentoflavone was detectable in all species but only in flowers. Its accumulation levels varied between 0.02 and 0.12 mg/g DM depending on the species (Tables 3 and 4).

Table 5
Cumulative values of calculated principal components for 8 *Hypericum* species.

Principal component	Total	Cumulative
1	25.672	25.672
2	20.846	46.518
3	12.682	59.200
4	9.125	68.325
5	7.861	76.186
6	7.472	83.659
7	4.493	88.152
8	3.477	91.629
9	3.727	94.356
10	1.719	96.074
11	1.607	97.681
12	0.906	98.586
13	0.556	99.143
14	0.416	99.559
15	0.216	99.774
13	0.123	99.897
17	0.074	99.971
18	0.022	99.993
19	0.070	100.00

4. Discussion

The taxonomy of the genus *Hypericum* is largely based on morphology (Crockett and Robson 2011). However, using only the morphological characteristics has caused uncertainties in taxonomical division of this genus. First, some sections closely resemble to each other and few morphological characteristics serve to differentiate and identify them. Besides, identifying the separate plants merely based on morphological characters is hard and can lead to some mistake as mentioned by Davis (1988) to indicate the morphological parallelism among members of the sections of *Adenosepalum* Spach. and *Origanifolia* Stef. Thus, studies on qualifying chemical profile of species can serve as an additional tool in taxonomic analysis of *Hypericum* genus (Camas et al. 2014).

In a previous paper, Kitanov (2001) reported *H. olympicum* and *H. polyphyllum* to contain hypericin and pseudohypericin, but these species were not investigated so far for the presence of other *Hypericum* chemicals. Besides, to the best of our knowledge, there is no previous report on polar chemistry of the investigated species of sect. *Drosanthe*. Thus, it is the first time we have reported the presence of 19 compounds in *H. capitatum* var. *capitatum* and var. *luteum*, *H. retusum*, *H. spectabile*, *H. elongatum* var. *elongatum*, and *H. salsolifolium* as well as in *H. olympicum* and *H. polyphyllum*. As shown in Tables 3 and 4, chemical profiles of the species from the same section closely resemble each other despite the distinct quantitative variation in chemical content of plant material. Six species from sect. *Drosanthe* yielded all the tested compounds at various levels excluding caffeic acid, absent in all species; hyperforins, absent in *H. spectabile* and *H. salsolifolium*; and mangiferin, absent in *H. salsolifolium* and both varieties of *H. capitatum*. In our previous study, we described chemical constituents of three other *Hypericum* species from sect. *Drosanthe*, namely, *H. olivieri*, *H. scabrum*, and *H. lydium* (Camas et al. 2014). The comparison between present and previous results revealed that all members of sect. *Drosanthe* include

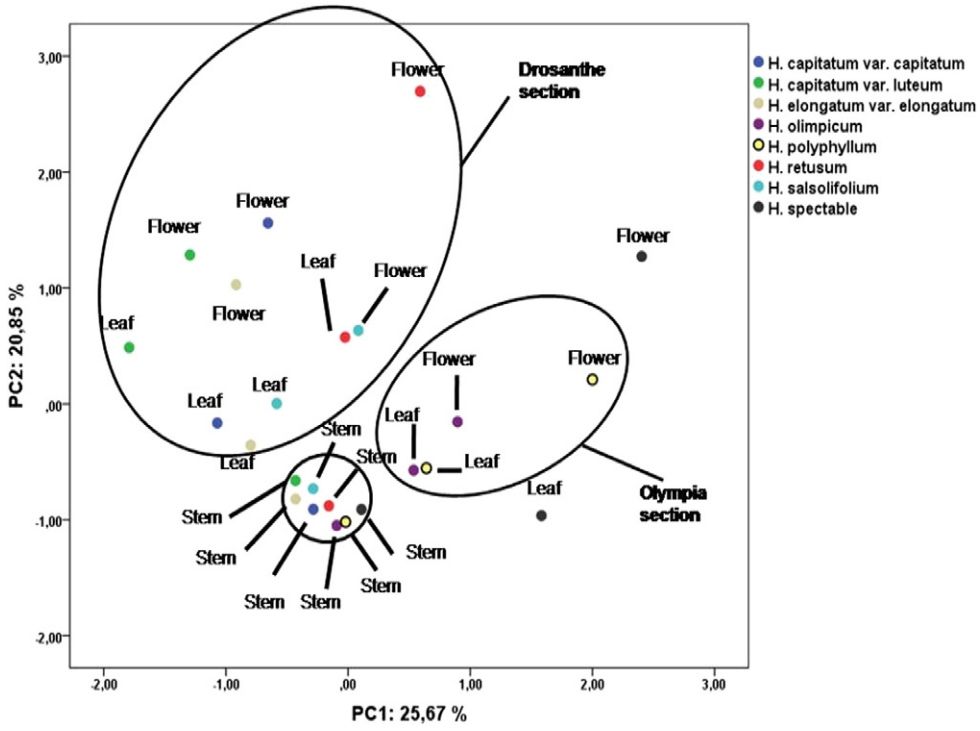

Fig. 3. Scatter plot of investigated *Hypericum* species.

hypericin, pseudohypericin, chlorogenic acid, neochlorogenic acid, 2,4-dihydroxybenzoic acid, amentoflavone, hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, and (+)-catechin and (−)-epicatechin and have similar chemical profile. In analogy to sect. *Drosanthe*, all the tested chemicals were detected in the two presented species of sect. *Olympia* except for hyperforins and mangiferin.

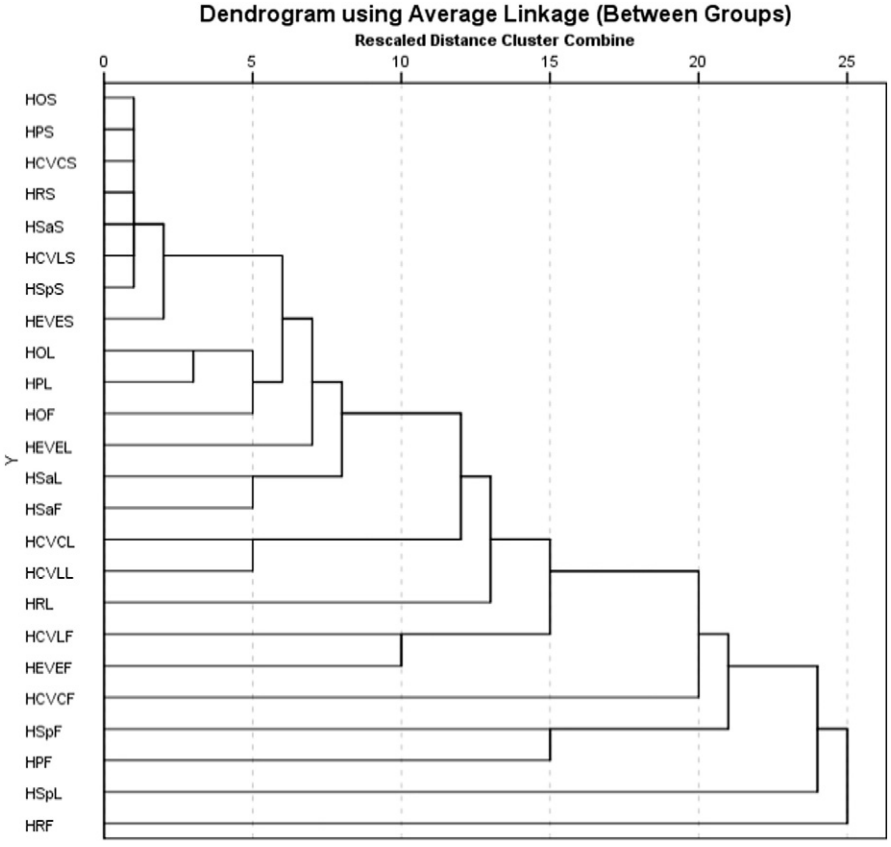

Fig. 4. Dendogram of investigated plant parts in eight *Hypericum* species (HCVC = *H. capitatum* var. *capitatum*, HCVL = *H. capitatum* var. *luteum*, HEVE = *H. elongatum* var. *elongatum*, HO = *H. olimpicum*, HP = *H. polphyllum*; HR = *H. retusum*, Has = *H. salsilifolium*, HSp = *H. spectabile*, S = Stem, F = flower, L = leaf).

Among the bioactive compounds, hypericins were reported to have an authenticated taxonomic value for the infrageneric classification of the genus *Hypericum* (Robson 1977). Kitanov (2001) did not detect hypericin and pseudohypericin in the taxa of the primitive sections but identified these compounds only in the species of the most phylogenetically advanced sections. Moreover, hypericins were notified to be included only in the species of *Hypericum* whose aerial parts bear dark glands (Lu et al. 2001). In our previous studies, we reported a positive correlation between dark gland number and hypericin content of leaf in *H. perforatum* (Cirak et al. 2007b), *H. avicullariifolium* Jaup. and Spach subsp. *depilatum* (Freyn and Bornm.) Robson var. *depilatum* and *H. pruinatum* Boiss. and Bal. (Cirak et al. 2006). Several authors also reported that the absence of dark glands in *Hypericum* plants is concerned with the lack of both hypricin forms in these species (Ferraz et al. 2002b; Nor et al. 2008; Crockett and Robson 2011). In the present study, we observed that detection of hypericins is consistent with the presence of dark glands in all aerial parts of the investigated species as shown in Fig. (5).

Rutin, a flavonol making an important contribution to the antidepressant activity of *Hypericum* extract (Noldner and Schotz 2002) was detected in all investigated species in the present study. This compound was also detected in *H. olivieri*, *H. scabrum*, and *H. lydium* from sect. *Drosanthe*; *H. confertum* Choisy, *H. thymifolium* Banks and Sol., *H. linarioides* Bosse, and *H. pruinatum* from sect. *Taeniocarpium* (Camas et al. 2014); *H. origanifolium* Willd. (Cirak et al. 2007c) and *H. avicullariifolium* subsp. *depilatum* var. *depilatum* (Cirak. et al. 2013) from sect. *Origanifolia*; *H. perforatum* (Dogrukol et al. 2001) and *H. triquetrifolium* (Hosni et al. 2011) from sect. *Hypericum*; *H. adenotrichum* Spach (Cirak et al. 2009) and *H. orientale* L. (Cirak et al. 2012) from sect. *Crossophyllum*, but not detected in 13 *Hypericum* species, native to Southern Brazil from sect. *Trigynobrathys* (Nunes et al. 2010) and some chemotypes of *H. perforatum* (Martonfi et al. 2001). The results indicate that the compound may have some chemotaxonomic utility at the sectional or subsectional level.

Mangiferin is a widely distributed xanthone in the species of *Hypericum* (Bennett and Lee 1989). Kitanov and Nedialkov (1998) found this compound in 26 of 36 analyzed taxa and reported that it seems to be specific only for the taxa of two tribes *Hypericeae* and *Cratoxyleae* and thus has little chemotaxonomic significance for infrageneric classification of the genus. We did not detect mangiferin in species of sect. *Olympia* as Kitanov and Nedialkov (1998) but detected in three species of sect. *Drosanthe*. This compound was not also detected in 19 Brazilian species of *Hypericum* from sect. *Trigynobrathys* (Nunes et al. 2010).

The monomeric phloroglucinol derivatives like hyperforin was reported to accumulate in several species of *Hypericum* from different sections such as *H. perforatum* (Smelcerovic et al. 2008), *H. montbretii* (Cirak and Radusiene 2007), *H. lydium*, *H. pruinatum*, *H. confertum* (Camas et al. 2014), *H. avicullariifolium* subsp. *depilatum* var. *depilatum*, and *H. orientale* (Cirak. et al. 2013). There is no evidence that monomeric phloroglucinol derivatives have chemotaxonomic value unlike to dimeric ones, which were reported to exhibit taxonomic evidence at the sectional level for the species from sect. *Brathys* and *Trigynobrathys* (Barros et al. 2013). Hence, detection of hypericins, rutin, mangiferin as well as hyperforins, and the other tested compounds in *Hypericum* species investigated in the present study supports the taxonomic position of the sect. *Drosanthe* and *Olympia* within the genus *Hypericum*. However, it should be noted that several species of *Hypericum* from other sections were previously reported to have the above mentioned compounds. Thus, it may not be reasonable to assign them as a clear taxonomic pattern at the infrageneric level (Barros et al. 2013). It is interesting to note that no caffeic acid accumulation was observed in sect. *Drosanthe*, but both species of sect. *Olympia* yielded this compound which was also detected previously in four species from sect. *Taeinocarpium* (Camas et al. 2014). Similarly, mangiferin was not accumulated in sect. *Olympia* but occurred in three species from sect. *Drosanthe*, suggesting that the pattern for occurrence of caffeic acid and mangiferin may be related to the evolution of the different taxonomical groups of *Hypericum*.

Regarding the proven bioactivities of *Hypericum* chemicals, especially the antimicrobial (Nogueira et al. 2013), antiviral (Schmitt et al. 2001), hepatoprotective (Wang et al. 2008), and antidepressant (Stein et al. 2012) ones, the results presented here have also a pharmacological value and may be helpful in the evaluation and selection of species as new sources of valuable bioactive chemicals.

As shown in Fig. 3, results of PCA, an useful statistical analysis for the differentiation of plant material regarding their chemical profile (Smelcerovic et al. 2008; Bertoli et al. 2011), indicated a considerable variation in chemical accumulation among different plant parts of the tested species. In the present paper, we observed that all the detected chemicals were deposited in the same organs of species from the same section. However, in some instances, the same compound was accumulated mainly in leaves in sect. *Olympia* but in flowers in sect. *Drosanthe* or vice versa. Light glands, dark glands, and secretory canals were reported to be secretory structures of *Hypericum* plants and main sites of synthesis and/or accumulation of *Hypericum* chemicals (Ciccarelli et al. 2001). The localization of these secretory structures in plant tissues varies depending on species (Nürk et al. 2013; Maggi

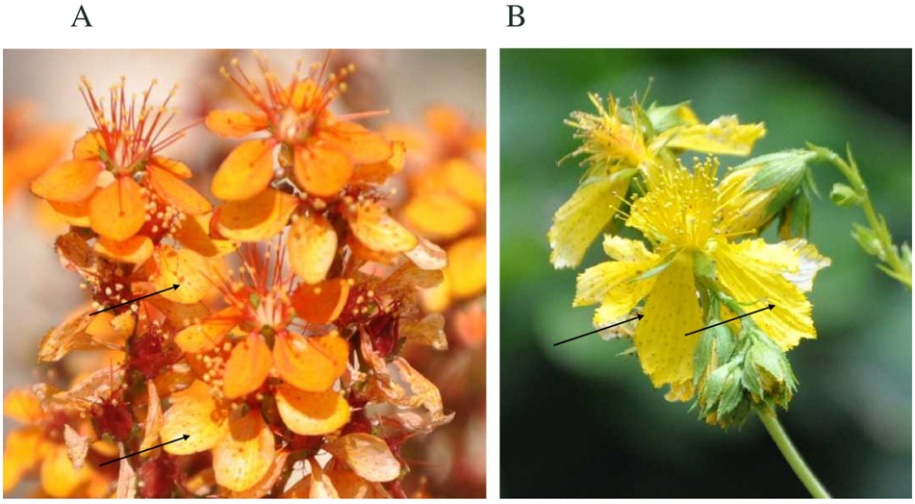

Fig. 5. Dark glands as an example in flowers of *H. capitatum* var. *capitatum* (A) and *H. olympicum* (B). Arrows indicate the glands.

et al. 2004). The distinct chemical diversity among different plant parts of the tested species can be attributed to the variation in localization of secretory structures among the species from different sections.

5. Conclusions

Characterization of naphthodianthrones, monomeric phloroglucinol derivatives, phenolic acids, flavonols, flavanols, biflavonoids, and xanthones in the *Hypericum* species, native to Southern parts of Turkey, has reconfirmed the value of *Hypericum* genus as a source of bioactive compounds. In a chemotaxonomical point of view, the resemblance in chemical profile of the species from the same section as well as the occurrence of hypericin, pseudohypericin, rutin, and absence of caffeic acid and mangiferin in some tested species has indicated some taxonomic value for the corresponding compounds with the requirement of further studies to make more substantial inferences. Considering the fact that secondary chemistry of an estimated 60% of *Hypericum* species is still largely unknown (Crockett and Robson 2011), the present data have also made an important contribution to our current knowledge about chemistry of *Hypericum* genus.

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