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RESEARCH ARTICLE

Assessment of Phenolic Content, Antioxidant Properties and Antimicrobial Activity of Flower and Leaf Extracts from Some *Hypericum* Species Affected by Wild Habitat Altitude

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ABSTRACT

In this study, determination of habitat altitude effect on the total phenolic contents, antioxidant and antimicrobial activities of flower and leaf extracts from *Hypericum montbretii*, *H. orientale* and *H. perforatum* species was aimed. The plants were collected randomly from forages (altitudes were ranged from 430 to 1105 m a.s.l.) located in Western Black Sea Region, Turkey. Antioxidant properties of ethanolic extracts were determined with DPPH and ABTS assay and antimicrobial activities of the extracts on *Bacillus pumilis* NRRL BD-142, *B. subtilis* NRRL B-209, *B. licheniformis* NRRL-B-1001, *B. cereus* NRRL B-3711, *Staphylococcus aureus* ATCC 33862, *Pseudomonas aeruginosa* ATCC 27853, *Listeria inocua* ATCC 33090, *L. monocytogenes* ATCC 7644 and *Escherichia coli* ATCC 25922 were examined. Total phenolic contents and antioxidant activities of *Hypericum* species changed depending on the habitat altitude. The antimicrobial activity of the ethanolic extracts was evaluated by minimal inhibitory concentrations (MIC) method. Flower and leaf extracts exhibited a broad antibacterial spectrum, but they were not effective against *Escherichia coli* (ATCC 25922). Phenolic contents of all *Hypericum* species and antimicrobial activity of only *H. perforatum* extracts were significantly increased by altitude rising, but no positive correlation was detected in antioxidant activity of extracts due to habitat altitude.

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Introduction

In recent years, consumer demand to natural products has increased in the sense of medicine and food all over the world. This current of thought has brought up the use of some natural plant species for the prevention of disease, natural living, and natural feeding. It is determined that some plant extracts have effects such as antimicrobial, antispasmodic, antimutagenic, antioxidant and antiviral according to many active ingredient which they contain in the recent studies (Sudharameshwari and Radhika 2007). Therefore, studies related to the determination of the characteristics of plants and plant active ingredients have become more important. The genus of *Hypericum* is one of the grouped of these plants. *Hypericum* L. is a genus of flowering plants in the family *Hypericaceae* (a subfamily of *Clusiaceae*). This family consists of 46 genera and 1000 species, distributed across tropic and subtropics regions, as

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well as Europe, West Asia, North Africa and North America, and particularly Anatolia (Saroglou et al. 2007; Dadkhah et al. 2014) and it is stated that 46 endemic totally 96 species present in Turkey flora (Çirak et al. 2016). In recent years, *Hypericum* species have gained popularity due to their antidepressant effects (Çirak et al. 2006). According to Bingol et al. (2011) all *Hypericum* species used as sedative, antiseptic and antispasmodic in Turkish folk medicine and have different names like kantaron, peygamber çiçegi, kılıçotu, kuzukıran and binbirdelik otu. Moreover, it is known that some *Hypericum* species have been consumed as tea.

It is known that several environmental factors such as temperature, wind velocity, precipitation, duration of snowpack, soil, temperature extremes, radiation intensities, and length of the vegetation period vary with the altitude of the natural growth locality (Camas et al. 2014). Thus, the chemical composition of *Hypericum* species may significantly alter depending on variation among *Hypericum* species as well as these factors (Xenophontos et al. 2008). Several studies have been conducted to the effects of the habitat altitude of the plants on the chemical content (Xenophontos et al. 2008; Camas et al. 2014) but there is no previous report about the functional properties of *H. perforatum*, *H. orientale* and *H. montbretii*. Therefore, the main aim of this research was to determine the effect of the habitat altitude of the plants on the antimicrobial activity, total phenolic content and antioxidant activity of the ethanol extracts of three *Hypericum* species.

Materials and Methods

Collection and Identification of Plant Materials

Three wild species of *Hypericum* (*H. montbretii* (3), *H. orientale* (4) and *H. perforatum* (5)) were *collected randomly from forages* located (altitudes ranged from 430 to 1105 m a.s.l.) in the Western Black Sea Region, Turkey. Details about the locations where the plants collected were presented in Table 1. The distance between the two collection locations was minimum 15 km. The *plant materials were collected in June* 2015 during flowering stage. *In general, the climate of the* collecting area is classified as warm and temperate. The Köppen-Geiger climate classification is Cfb. At first, the plants were identified taxonomically by the Department of Biology, Ondokuz Mayis University, Samsun, Turkey. The aerial parts were air dried in shadow and fractionized. The leaf and flower parts of the plants were separated and powdered using a laboratory mill. Powdered materials were protected from light until analyzes.

Table 1. Details about the locations of the *Hypericum* species

Species	Location Code	Locality	Altitude (m)	Coordinates
H. montbretii	**A1**	Between Taşköprü and Boyabat	430	41°48' N, 34°11' E
	A2	Between Hanönü and Sinop	496	41°63' N, 34°43' E
	A3	Between Kastamonu and Taşköprü	700	41°62' N, 33°72' E
H. orientale	**B1**	Between Kastomonu and Daday	774	41°43' N, 33°75' E
	B2	Between Ağlı and Seydiler	1012	41°64' N, 33°65' E
	B3	Between Ağlı and Seydiler	1039	41°66' N, 33°71' E
	B4	Seydiler, Tokazlar village	1050	41°62' N, 33°72' E
H. perforatum	**C1**	Between Boyabat and Sinop	339	41°54'N, 33° 47' E
	C2	Hanönü, Çayköy vilage	497	41°63' N, 34°43' E
	C3	Between Ağlı and Kastamonu	803	41°48' N, 33°76' E
	C4	Seydiler, Selmanlı village	994	41°65' N, 33°60' E
	C5	Daday, Karacaören village	1105	41°47' N, 33°21' E

Extraction Procedure of Plant Materials

The maceration technique was used for extraction of plant materials. Five grams of the ground sample was extracted with 200 mL of ethanol (Merck, 99.5%, v/v) at room temperature in the dark with shaking (220 rpm) for 2 days. After maceration, the liquid extract was separated from the solid residue by filtering through Whatman No. 4 filter paper. The solvent was removed with a rotary evaporator at 40°C, at 45 mbar to obtain a dry extract. The crude extracts were dissolved in 10 mL of ethanol and the amount of crude extract in mL was calculated. All the extracts were placed in a glass bottle and stored in the dark at -20°C until use. All assays were made three times and the results were presented as the average of triplicate analyses.

Test Microorganisms

The *in vitro* antimicrobial activities of the plant extracts were analyzed for antimicrobial activity against following of 9 microorganisms: *Staphylococcus aureus* (ATCC 33862), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus pumilis* (NRRL BD-142), *Bacillus subtilis* (NRRL B-209), *Bacillus licheniformis* (NRRL-B-1001), *Listeria inocua* (ATCC 33090), *Listeria monocytogenes* (ATCC 7644), *Escherichia coli* (ATCC 25922), and *Bacillus cereus* (NRRL B-3711), obtained from Food Engineering Department, Ondokuz Mayıs University (Samsun-Turkey). Microorganisms were maintained in glycerol broth at -80°C. These microorganisms were activated 2 times in Mueller Hilton broth (Merck, Darmstadt, Germany) at 30°C overnight before use.

Minimum Inhibitory Concentration (MIC) of Extracts

The minimum inhibitory concentration (MIC) of the extracts was determined according to Agar diffusion assay described by CLSI (2006). Serial dilutions of the plant extracts (1024, 512, 256, 128, 64, 32, 16, 8, 4, 2 µL/mL) were prepared in Muller Hilton Agar medium (Merck, Darmstadt, Germany) according to the standard procedure. After solidification, the plates were incubated at room temperature (22-23°C) for 6 hours to obtain dry the agar surface. Suspensions of the test microorganisms were prepared by matching a McFarland 0.5 turbidity standard. Inoculations were applied to agar surfaces in 1 µL spots, giving approximately 1.5 x 10^5 CFU per spot. Plates without added extract were inoculated as positive controls. All plates were incubated at 30°C for 24-48 hours. The MIC was considered as the lowest concentration of extract which caused a marked inhibition in growth as compared to control and expressed in µg/mL. All data represent at least three replicated experiments per microorganism.

Total Phenolic Content of Extracts

The total phenolic content of the extracts was determined by using the Folin-Ciocalteu (Sigma Aldrich, Steinheim, Germany) phenol reagent method. Gallic acid (Sigma Aldrich, Steinheim, Germany) was used as a standard. The concentration of total phenolic contents in the plants was determined as µg of Gallic acid equivalents (GAE) per 1 mg of extract using the following equation obtained from a standard Gallic acid graph (R^2=0.9973). Briefly, 50 µL (two replicates) of the filtered extracts were mixed with 450 µL of distilled water and 2.5 mL of 0.2 N Folin–Ciocalteu reagents. After 5 min, 2 mL of saturated sodium carbonate (75 g/L) were added on it. The absorbance was measured at 765 nm using a spectrophotometer (Shimadzu Scientific Instruments, Japan) after incubation at 30°C for 1.5 h with discontinuous shaking. Quantitative measurements were performed, based on a standard calibration curve of Gallic acid. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligram per gram of dry material.

Antioxidant Activities of Extracts

Free radical scavenging activity of ethanolic plant extracts was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma Aldrich, Germany) by using the method of Brand-Williams et al. (1995) with some modifications. Briefly, 0.06 mM solution of DPPH in ethanol was prepared. Then, 1 mL of this solution was incubated with varying concentrations of ethanolic plant extracts. After that, the mixtures were shaken well and incubated for 30 min in dark at room temperature. The absorbance of the resulting solution was measured at 515 nm by a spectrophotometer against a blank. The DPPH radical scavenging activity of extracts was expressed as mg of Trolox equivalents/per gram of sample (mg Trolox equivalent/g dry weight).

The improved ABTS assay, described by Thaipong et al. (2006), was used to determine the antioxidant activity of the extracts. 2,2'-azinobis (3-ethlybenzthiazolin-6-sulfonic acid) diammonium salt (ABTS) radical cation was prepared by reacting 7 mmol ABTS stock solution with 2.45 mmol potassium persulfate. ABTS inhibition against Trolox was measured spectrophotometrically. The absorbance was measured at 734 nm by spectrophotometer. Trolox equivalent antioxidant capacity (TEAC) values of samples were calculated from the Trolox standard curve and expressed as Trolox equivalents (mg Trolox equivalent /g dry weight).

Statistical Analyses

Statistical analyses were performed by using SPSS 20.0 software and all values were presented as mean ± standard deviation. To determine the statistical significance between samples, a one-way analysis of variance (ANOVA) was applied then, multiple comparisons was carried out by Scheffe's multiple comparison test. Pearson's bivariate correlation test was also carried out to calculate correlation coefficients (*r*) among antioxidant activity, reducing power and total phenolic content.

Results and Discussion

Antimicrobial Activities of Extracts

In the current study, minimal inhibitory concentration (MIC) of the flowers and leaves of 12 plants extracts were established for a lot of microorganisms and the results were shown in Table 2. This analysis was also applied to determine whether plant extract samples have antimicrobial activity or not. As clearly seen in Table 2, MIC values of the flower and leaf extracts varied in the range of 4 to 1024 µg/mL and 16 to 1024 µg/mL, respectively. Furthermore, the flower extracts exhibited higher antimicrobial activity than the leaf extracts. Being active at dilution level of 100 µg/mL, plant extracts could be considered to have a promising antimicrobial activity. Moreover, MIC values lower than 30µg/mL has higher antimicrobial activity compared to those usual antibiotics (Dall'Agnol et al. 2003). The results of extract samples were individually evaluated according to this information. Firstly, when considering in terms of flower extracts, it is observed that extract samples of *H. montbretii* (A1, A2 and A3) had the most effective antimicrobial activity against *B. pumilus, B. licheniformis* and *Ps. aeruginosa* (MIC values 16 to 32 µg/mL). Antimicrobial activity of extracts (A1, A2 and A3) against *B. cereus* and *B. subtilis* (MIC values 16 to 64 µg/mL) followed this assessment. Antimicrobial activity values shown against *S. aureus* and *Listeria* ssp. were less than the others. Also, the lowest antimicrobial activity of *H. montbretii* samples was shown against *E. coli*. Besides, it was determined that the activity of A3-sample collected from higher locations was lower than the other samples. If the flower extract samples of *H. orientale* were examined, it was seen that they had a similar antimicrobial effect to the flower extract samples of *H. montbretii* except for B3-sample.

Where the antimicrobial activity against *Ps. aeruginosa* and *Bacillus* species was found as high (MIC values 16 to 32 µg/mL), but for *E. coli* it was insufficient. The flower extract of the B3-sample showed a low antimicrobial activity (1024 or >1024 µg/mL) against all tested microorganisms. The flower extracts of *H. perforatum* samples showed higher antimicrobial activity

than the other tested *Hypericum* species. Especially, *Ps. aeruginosa*, *Bacilllus* and *Listeria* species were found as significantly sensitive (MIC values, 4 to 64 µg/mL) to the flower extract samples of this specie. However, like the other *Hypericum* species, the antimicrobial effect of the *H. perforatum* flower extract samples against *E. coli* was insufficient. It was determined that there was no significant relationship between antimicrobial activity of these species and location altitudes. Additionally, it was emphasized that the other microorganisms except for *S. aureus* and *E. coli* showed a quite high susceptibility against the flower extract of C5-sample (MIC values, 4 to 8 µg/mL) and C2-sample (MIC values, 8 to 16 µg/mL), respectively.

Table 2. Minimal inhibitory concentrations (MIC; µg/mL) of ethanolic *Hypericum* extracts

Species	Plant Tissue	Location Code	Indicator Microorganisms Code*								
			1	2	3	4	5	6	7	8	9
H. montbretii	Flower	A1	16	16	16	16	32	16	64	32	1024
		A2	16	16	16	32	32	16	16	16	1024
		A3	32	64	32	32	128	32	128	128	1024
	Leaf	A1	32	64	32	32	256	32	256	64	1024
		A2	128	128	128	128	512	128	128	128	1024
		A3	1024	1024	512	1024	1024	256	512	>1024	>1024
H. orientale	Flower	B1	16	16	16	16	32	16	16	16	1024
		B2	32	32	32	32	64	32	128	128	1024
		B3	1024	1024	>1024	>1024	>1024	1024	>1024	>1024	>1024
		B4	16	16	32	16	256	32	256	256	1024
	Leaf	B1	16	16	16	16	64	16	32	32	1024
		B2	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
		B3	>1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
		B4	128	128	128	128	512	128	256	256	1024
H. perforatum	Flower	C1	16	16	16	16	64	16	16	16	1024
		C2	16	8	8	8	64	8	16	16	1024
		C3	16	16	16	16	64	16	32	32	1024
		C4	64	64	64	64	64	32	64	64	1024
		C5	4	8	4	4	16	8	8	4	1024
	Leaf	C1	128	32	32	128	256	32	128	128	1024
		C2	16	16	16	16	256	16	16	16	1024
		C3	256	256	256	256	512	256	512	512	1024
		C4	512	512	256	256	256	64	64	256	1024
		C5	128	128	64	512	256	128	256	256	1024

*1: *B. pumilus*, 2: *B. subtilis*, 3: *B. licheniformis*, 4: *B. cereus*, 5: *S. aureus*, 6: *Ps. aeruginosa*, 7: *L. innocua*, 8: *L. monocytogenes*, 9: *E. coli*

When viewing all the *Hypericum* species tested, generally, it was observed that flower extracts showed higher antimicrobial activity than the leaf extracts. On the other hand, both flower and leaf extract samples showed an insufficient antimicrobial activity against *E. coli*. Except for *E. coli*, *L. innocua* and *S. aureus*, a significant difference was not determined among the antimicrobial activities of A1-leaf extract against other microorganisms. Besides, the antimicrobial activity of A1-leaf extract was found as greater than the other samples of *H. montbretii*. For *H. montbretii*, it was seen that there was an inverse relationship between location altitudes and antimicrobial activity. Likewise, the leaf extracts of *H. orientale* and *H. perforatum* showed a significant difference against the microorganisms except *E. coli* and *S. aureus*. However, more effective antimicrobial activity was determined against *Bacillus* species and *Ps. aeruginosa* by the leaf extracts of *H. orientale* and *H. perforatum* species, respectively. It was determined that their leaf extracts had less effective than the flower extracts.

According to the results, it was observed that the antimicrobial activity of both flower and leaf extract samples of *H. montbretii* and *H. perforatum* were higher than *H. orientale* species. *H. perforatum* species showed highest antimicrobial activity among the species. Especially, effect of the C5 and C2-flower extract samples were highlighted by MIC values ranging from 4 to 8 µg/mL and from 8 to 16 µg/mL, respectively, except for *E. coli* and *S. aureus*. Besides, it was determined that the leaf extract of C2-sample had a significant activity with a MIC value of 16 µg/mL, and it was determined that the flower extract of A1, A2, B1, C1 and C3-samples had MIC value ≤ 30 µg/mL for most of the tested indicator microorganisms.

Previous reports showed that some *Hypericum* species growing in various regions of the world have remarkably broad spectrum of antimicrobial activities (Rabanal et al. 2002; Dulger et al. 2005). Rabanal et al. (2002) investigated antimicrobial activities on these species of *Hypericum* from the Canary Islands and MIC values were found between 30 and 290 µg/mL. In this study, the most significant activity was observed on the chloroform fraction of *H. canariense*, showing the lower MIC values against *Micrococcus luteus*, *S. aureus* and *S. epidermidis* (with the same MIC value of 30 µg/mL), followed by *Bordetella bronchiseptica* (70 µg/mL) and *B. cereus* (the least affected with a MIC of 290 µg/mL). In a study

by Reichling et al. (2001), hydrous solutions of *H. perforatum* teas were found to be effective against gram-positive bacteria, especially toward methicillin-resistant strains of *S. aureus* (MIC values, 1300 to 2500 µg/mL). Unal et al. (2008) studied on antimicrobial activities of some plants used as remedies in Turkish Medicine. They were stated that the chloroform, acetone, ethanol and water extracts of three *Hypericum* species (*H. heterophyllum, H. hyssopifolium* ssp. *Elogatum var. elongatum* and *H. scabrum*) showed antimicrobial activity against 10 pathogenic bacteria with MIC values ranging between 62.5 and 250 µg/mL. Milosevic et al. (2007) reported that *Ps. glycinea* and *Azotobacter chrococcum* showed extreme sensitivity to the extract of *H. perforatum*, while no effect was observed on *Klebsiella pneumoniae*.

In general, antimicrobial activity of ethanolic *Hypericum* species extracts varied significantly depending on the altitudinal gradient. In both leaf and flower extracts, antimicrobial activity of *H. perforatum* increased with altitude. Contrarily, *H. montbretii and H. orientale* exhibited lower activity with altitude. It was considered that the reason of this result was the effect of the relationship between the amounts of the compounds which exhibit antimicrobial properties in plants and the altitude. The naphthodianthrones, flavonoids, xanthones, tannins, essential oils, phloroglucinols and chlorogenic acid are the major compounds studied of *Hypericum* species as phenolic acid so far and antimicrobial activity has been found to be closely related to them (Radulovic et al. 2007; Çirak et al. 2012). Moreover, some compounds like flavonoids, phenolic acids, proanthocyanidins are overproduced depending on the altitude due to the degree of environmental stress factors like UV-B radiation and temperature etc. (Zidorn et al. 2005; Rieger et al. 2008; Xenophontos et al. 2008; Camas et al. 2014). Thus, we expected increasing of the antimicrobial activity of *Hypericum* extracts in proportion to the altitude of plant growth locations. But, the findings for *H. montbretii and H. orientale* did not correlate with the mentioned assume. However, Martz et al. (2010), one of the study not related to the plant species, reported that the compounds like chlorogenic acid derivatives were produced with lower contents depending on the altitude. Therefore a clear relationship could not been established between the antimicrobial activities of the samples and altitude of the plant growth locations.

Total Phenolic Content of Extracts

The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties. Plant phenols constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers (Bernardi et al. 2008). In this study, it was determined that there is large variation, ranging from 75.22 to 140.72 mg GAE/g dry weight for flower extracts and from 94.89 to 212.49 mg GAE/g dry weight for leaf extract, in total phenolic content of the plant species (Table 3). For flower extracts, while B1-sample showed highest total phenolic content with value of 140.72 GAE/g dry weight ($P<0.05$), leaf extracts C4-sample showed the highest total phenolic content with value of 212.49 mg GAE/g dry weight ($P<0.05$). These results are similar to the amounts (104 to 451 mg GAE/g dry weight) of the total phenolic compound of *H. montbrettii, H. origanifolium* and *H. perforatum* species determined by Özturk et al. (2009) and, likewise, the amounts (125 to 257 mg GAE/g dry weight) of the total phenolic compound in the aerial parts of *H. perforatum* L. determined by Gioti et al (2009). According to literature, total phenolic content of the plant species having GAE >20 mg/g dry weight are remarkably high (Türkan and Demiral 2009). Therefore, it was concluded that the plant species in this study showed as a rich total phenolic source.

Table 3. Bioactive properties of ethanolic *Hypericum* extracts

Species	Location Code	Total Phenolic Content*		ABTS Assay**		DPPH Assay**	
		Flower	Leaf	Flower	Leaf	Flower	Leaf
H. montbretii	A1	122.02±1.50^{d}	94.89±1.01^{h}	304.58±1.03^{b}	291.45±0.52^{h}	21.22±0.77^{cd}	19.92±0.54^{gh}
	A2	118.40±1.11^{e}	158.21±1.25^{de}	304.36±0.79^{e}	416.43±0.42^{d}	17.65±1.37^{def}	27.99±0.44^{cd}
	A3	100.37±1.09^{g}	166.56±0.76^{cde}	310.18±0.88^{d}	377.78±0.42^{f}	18.96±0.43^{de}	26.58±0.54^{f}
H. orientale	B1	140.72±0.44^{a}	141.01±0.62^{f}	324.10±0.85^{a}	337.41±0.42^{g}	27.64±1.07^{a}	32.10±0.52^{abc}
	B2	113.43±0.70^{e}	151.54±0.48^{de}	344.99±1.63^{c}	480.02±0.30^{a}	23.44±0.25^{cd}	35.64±0.62^{a}
	B3	80.66±1.13^{h}	140.03±0.52^{f}	279.11±0.96^{f}	403.29±0.48^{e}	19.76±0.78^{de}	26.99±1.88^{de}
	B4	126.61±1.40^{bc}	149.45±2.00^{e}	371.46±0.88^{b}	430.44±0.44^{c}	24.97±1.79^{ab}	27.92±0.57^{d}
H. perforatum	C1	75.22±0.69^{hi}	128.72±0.96^{g}	254.14±1.13^{g}	387.69±0.62^{f}	16.27±1.73^{def}	30.01±0.37^{bc}
	C2	100.63±1.83^{g}	93.21±1.89^{h}	285.49±0.55^{f}	338.46±0.27^{i}	12.61±2.94^{g}	15.07±0.82^{j}
	C3	104.79±1.35^{g}	163.24±0.88^{cde}	334.37±1.89^{c}	356.81±0.94^{j}	19.24±1.76^{de}	21.66±0.89^{g}
	C4	121.43±0.39^{d}	312.49±0.47^{a}	411.86±0.45^{a}	457.28±0.23^{b}	24.36±0.99^{bcd}	24.67±0.74^{ef}
	C5	123.42±0.77^{cd}	172.31±1.34^{b}	353.86±1.38^{bc}	255.07±0.74^{j}	28.98±1.61^{a}	22.97±1.24^{hi}

*: mg gallic acid equivalent /g dry weight, **: mg trolox equivalent /g dry weight, ***: All values are presented as mean ± S.D and different letters within columns for each sample differ significantly at the level of $P < 0.05$.

The altitude of the *Hypericum* growth locations significantly affected the total phenolic content of the ethanolic extract samples ($P<0.05$). Total phenolic contents of flower extracts of *H. montbretii* and *H. orientale* were decreased with increasing altitude; however a positive correlation was determined between the altitude and total phenolic contents of *H. perforatum*. For leaf extracts of *Hypericum* species except B3 and C5-samples, the higher total phenolic contents accumulation was observed in the higher growing sites. Recent papers reported that several environmental stresses like solar radiation and temperature had proven effect on plant metabolites and generally

increasing solar radiation and reducing temperature at higher altitude resulted in higher phenolic contents of plant (Rieger et al. 2008; Türkan and Demiral 2009; Camas et al. 2014).

Antioxidant Activities of Extracts

Antioxidant activities of the *Hypericum* species, were evaluated by using two methods based on the free radical scavenging capacity (namely the DPPH radical scavenging assay) and the ABTS radical cation decolarization assay, were shown in Table 3. It can be concluded that the plant extracts free radical scavenging activities towards the ABTS assay were quite high antioxidant activity with the values in the range of 255.07-480.02 mg trolox equivalent /g dry weight and free radical scavenging activities towards the DPPH assay were found between 12.61 and 35.64 mg trolox equivalent /g dry weight. Leaf extracts have higher antioxidant activity than the flower extracts. Generally, leaves of the plants carry higher antioxidant activity with regards to the phenolic compounds compared to flowers (Güzey et al. 2011) and there are many studies which reported a positive correlation between total phenolic content and antioxidant activity (Tawaha et al. 2007; Şerbetçi et al. 2012). In spite of that, no correlation was found between the antioxidant activity and phenolic content of leaf extracts. But, especially, the free radical scavenging activities of the flower extract samples towards the ABTS assay are in a very good correlation with the phenolic contents (r = 0.8874). As similar to this result, a moderate correlation with together Pearson's correlation coefficient (r = 0.5718) were determined between free radical scavenging activities towards the DPPH assay of the flower extracts and the phenolic compound values of the same samples. It is considered to be reasons for the difference between the basic principles of the antioxidant activity methods to the difference between correlation forces. Besides, it is known that although the ABTS assay is convenient to determine the antioxidant activity of both hydrophilic and lipophilic compounds (Somogyi et al. 2007), the DPPH assay is convenient to determine that of hydrophilic compounds, since DPPH radical react slowly with peroxyl radicals (Magalhaes et al. 2008). This situation show that chemical structure of the plant extracts is different from each other. In a previous study, determining the antioxidant activity of *Hypericum hircinum* ssp. *Majus* essential oil according to the ABTS and the DPPH assay, it was observed that the oil possessed a more remarkable antioxidant activity in the ABTS assay (90.30 mg trolox equivalent /g dry weight), about 2-fold higher than the activity (47.80 mg trolox equivalent /g dry weight) shown in the DPPH assay (Qassinti et al. 2013). The results of this study are in accordance with our findings.

Antioxidant activity of leaf and flower extracts of the *Hypericum* species studied significantly varied depending on altitudinal variations but there is no correlation with the altitude of the habitat. Marrelli et al. (2014) found that DPPH assay of *H. perforatum* extract no. 1 collected from 370 m altitude showed the best radical scavenging activity but, sample no. 3 collected from 1320 m altitude showed the lowest activity due to containing a minor amount of phenolic. Similarly, Rieger et al. (2008) reported that the plants from higher altitudes cannot contain higher amounts of radical scavenging compounds as a result of their exposure to more climatic conditions.

Conclusion

Total phenolic contents in flowers and leaf extracts of plants increased considerably with the growth altitude and extracts exhibited the best antimicrobial activity together with rising altitude. However, though there is a correlation between the total phenolic contents and antioxidant activity of plant extracts, antioxidant activity is not a positive influence of increasing altitude. *Hypericum* species are used as medicinal plants and today there is growing interest in these plants which are rich in secondary compounds. So that, further studies are necessary to determine the effect of altitudinal changes on secondary compounds of *Hypericum* species and investigate the relationship with antimicrobial/antifungal activity. Additionally, the usage of these plant species as food product, such as tea or food additive, like food coloring, antioxidant etc. should be expanded.

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