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Some bioactive properties and antimicrobial activity of some *Hypericum* species growing wild in Black Sea region of Turkey

Latife Betül Gül[1*], Nilgün Özdemir[2], Osman Gül[3], Cüneyt Çirak[4] and Ahmet Hilmi Çon[2]

[1] Department of Food Engineering, Engineering Faculty, Giresun University, Samsun, Turkey
[2] Department of Food Engineering, Engineering Faculty, Ondokuz Mayis University, Samsun, Turkey
[3] Department of Food Engineering, Faculty of Engineering and Architecture, Kastamonu University, Kastamonu, Turkey
[4] Bafra Vocational High School, Ondokuz Mayis University, Samsun, Turkey

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ABSTRACT

The present study was conducted to evaluate the biological activity of four *Hypericum* species, including *H. perfoliatum* L., *H. aviculariifolium* Jaup. and Spach subsp. depilatum (Freyn and Bornm.) Robson var. *depilatum*, *H. origanifolium* Wild. and *H. linarioides* Bosse, growing wild in Black Sea Region of Turkey. Ethanolic extractions of both flowers and leaves of *Hypericum* species were performed by maceration method. Total phenolic contents were ranged from 83.89 to 148.04 mg GAE/g for flower and 202.83 to 48.03 mg GAE/g for leaf extracts. Antioxidant activities of the extracts were determined between 8.63 – 39.35 mg trolox equivalent/g and the highest antioxidant activity was observed in *H. linarioides* extracts probably due to high phenolic content. Antimicrobial activities of the extracts were evaluated by minimal inhibitory concentrations (MIC) against seven Gram-positive and two Gram-negative bacteria. MIC results showed that the flower extracts generally exhibited higher antimicrobial activity than the leaf extracts. The most prominent antibacterial activity was displayed by flower extract of *H. perfoliatum* (MIC between 4 – 512 µg/mL) and *Escherichia coli* was the most resistant organisms to all *Hypericum* species.

1. Introduction

In recent years, there is a growing interest in using medicinal and aromatic plants as natural sources in pharmaceutical, food, biotechnology, agricultural and cosmetic industries all over the world (Şerbetçi et al., 2012). The main purpose is to use them or their extracts containing bioactive compounds in foods and pharmaceutical industries to replace synthetic chemicals. In addition, natural antioxidants have the capacity to improve food quality and stability during formulation, manufacture and storage. The plants of the *Hypericum* genus have potential uses in traditional medicine. The genus *Hypericum* L. (family: Hypericaceae) which contains 484 species occurs throughout the world (Cirak, Radušienė, Kurtarc, Marksa, & Ivanauskas, 2020; Mohammed, Şabik, Dogan, Selamoglu, & Sevindik, 2020). The species of this genus have been used in Turkish folk traditional medicine to treat many diseases such as backache, burns, wounds, bacterial and viral infections, hemorrhoids, diarrhea and ulcers due to their antidepressive and wound-healing properties since they are rich natural sources of bioactive pharmaceuticals (Camas, Radusiene, Ivanauskas, Jakstas, & Cirak, 2013; Eroglu Ozkan, Yilmaz Ozden, Ozsoy, & Mat, 2018; Kamila, Ray, Jena, Mohapatra, & Panda, 2018). Also, they have shown other pharmacological activities including antioxidant, antimicrobial, antiviral, anti-inflammatory, hepatoprotective and antitumoral effects (Tusevski et al., 2016). Therefore, there is a growing interest in constituents of *Hypericum* genus. On the other hand, despite the large number of *Hypericum* species, *H. perforatum* L. (Saint John's wort), known by local names such as "sarıkantaron, askerotu, peygamber çiçeği, kanlıot and binbirdelik otu, is certainly the best-known and extensively studied plant all over the world which extracts widely applied for the treatment of mild to moderate depression and skin disorders (Cirak et al., 2016; Ersoy, Eroglu Ozkan, Boga, & Mat, 2020; Tocci et al., 2018). However recent studies shown that other *Hypericum* species have also revealed very interesting functional properties (Ersoy et al., 2020; Napoli et al., 2018; Özdemir, Uzun, Gül, Gül, & Çon, 2020; Saddiqe, Naeem, Hellio, Patel, & Abbas, 2020). Thus, scientific studies

*Corresponding author
E-mail address: latife.betul@giresun.edu.tr

have recently been focused on biological activities of other *Hypericum* species.

Turkey is an important prevalence center of the *Hypericum* genus and a survey by Guner, Aslan, Ekim, Vural, and Babac (2012) has demonstrated the presence of 96 *Hypericum* species in the Turkish flora, of which 46 are endemic. Although numerous studies have been reported about the chemical composition and biological activity of *Hypericum* species and also a few have been conducted on *H. perfoliatum*, there is very limited research about the biological activity of *H. aviculariifolium*, *H. origanifolium* and *H. linarioides*. Therefore, the present study was conducted to evaluate the total phenolic contents, antioxidant and antimicrobial activities of some *Hypericum* species (*H. perfoliatum* L., *H. avicularіifolium Jaup. and Spach* subsp. *depilatum* (Freyn and Bornm.) *Robson* var. *depilatum, H. origanifolium* Wild. and *H. linarioides* Bosse) growing wild in Black Sea Region of Turkey.

2. Materials and methods

2.1. Plant material

The four Hypericum species (*H. perfoliatum* L., *H. avicularіifolium* Jaup. and Spach subsp. depilatum (Freyn and Bornm.) Robson var. depilatum, *H. origanifolium* Wild. and *H. linarioides* Bosse) were collected by Prof. Dr. Cüneyt Çırak from the Western Black Sea Region of Turkey during the summer of 2015 at the flowering stages. The plants were identified by the Department of Biology, Ondokuz Mayis University, Samsun, Turkey, taxonomically.

2.2. Extract preparation

The samples were firstly divided into two parts as flowers and leaves, and after the air-dried at the room temperature under shade, they were milled. The ethanol extracts were obtained by maceration technique using ethanol (Merck, 99.5%, v/v) as solvent. For this, the ground powder was weighted (5 g) and macerated with 200 mL of ethanol for 48 hours at room temperature in the dark with shaking at 220 rpm. The liquid extracts were filtered by Whatman No. 4 filter paper and the organic solvent was evaporated to dryness under vacuum at low temperature (40 ºC) using a rotary evaporator (Buchi Rotavapor, Flawil, switzerland). The dry extract of each plant was further dissolved in 10 mL of methanol and stored in tightly sealed dark glass containers at 4 °C until required for further analysis (Özdemir et al., 2020).

2.3. Total phenolic content

Total phenolic contents of ethanolic extracts of *Hypericum* species were determined by Folin – Ciocalteu method described by Singleton and Rossi (1965), with slight modifications. Briefly, 50 µL of extract was mixed with 450 µL of distilled water and then mix was diluted with 2.5 mL of 0.2 N Folin – Ciocalteu reagents (Sigma Aldrich, Steinheim, Germany). After 5 min, 2 mL of saturated sodium carbonate solution (75 g/L) was added and the mixture was shaken on a shaker for 1.5 h at room temperature for color development. Afterward, the absorbance of solution was measured at 765 nm using UV–visible spectrophotometer (Shimadzu Scientific Instruments, Japan) and compared with gallic acid calibration curve prepared by using different concentrations of Gallic acid. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram of dry material and the values were presented as mean ± standard deviation of triplicate analysis.

2.4. Antioxidant activities

Antioxidant activity of extracts were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. DPPH radical scavenging activity of ethanol extracts was evaluated according to the method described by Brand-Williams, Cuvelier, and Berset (1995) with some modifications. One mL of DPPH solution (0.06 mM) prepared with methanol was mixed with varying concentrations of ethanol extracts and incubated for 30 min in darkness at ambient temperature. The absorbance was measured at 515 nm on a spectrophotometer against a blank and the DPPH radical scavenging activity of extracts was expressed as mg of Trolox equivalents/per gram of sample (mg Trolox equivalent/g dry weight).

2.5. Antimicrobial activity

The antimicrobial effects were evaluated by minimum inhibitory concentration (MIC) according to the Clinical and Laboratory Standards Institute guideline (CLSI, 2006). The estimate of the MIC was carried out by Agar dilution assay against 9 bacterial strains including Gram-positive bacteria: *Staphylococcus aureus* (ATCC 33862), *Bacillus pumilis* (NRRL BD-142), *B. subtilis* (NRRL B-209), *B. licheniformis* (NRRLB-1001), *B. cereus* (NRRL B-3711), *Listeria innocua* (ATCC 33090), *L. monocytogenes* (ATCC 7644) and Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922). All bacteria were kindly provided by Prof. Ahmet Hilmi Çon from the Biotechnology laboratory, Food Engineering Department, Ondokuz Mayıs University (Samsun, Turkey). Microorganisms were stored in glycerol broth at -80 ºC. The plant extracts were prepared in Mueller Hinton Agar medium (Merck, Darmstadt, Germany) at concentration of 1028, 512, 256, 128, 64, 32, 16, 8, 4, 2 µL/mL and the plates incubated at room temperature (22 – 23ºC) for 6 hours in order to dry the agar surface. The test microorganisms were incubated at 30 ºC in Mueller Hinton broth (Merck, Darmstadt, Germany) for 18 h, and the density of each bacterial suspension were adjusted to the turbidity of 0.5 McFarland standards. Then, the bacterial suspensions were inoculated onto the plant extract supplemented Mueller Hinton Agar plates and incubated at 30 °C for 24 – 48 hours. Plates without added extract were inoculated as positive controls. The MIC was considered as the lowest concentration of extract that completely inhibited growth of the organism and expressed in µg/mL. All data represent at least three replicated experiments per microorganism.

2.6. Statistical Analyses

All experiments were carried out in triplicate and all results were presented as mean ± standard deviation. Statistical calculations were carried out using SPSS 20.0 software. The one way analysis of variance (ANOVA) followed by Duncan's multiple range test were applied for determine the differences between *Hypericum* species. The t-test was used to compare the means between plant tissues. Pearson correlation test was also employed to identify the relationship between total phenolic content and antioxidant activity. Values of $p<0.05$ were considered statistically significant ($\alpha = 0.05$).

3. Results and Discussion

3.1. Total phenolic contents

The total phenolic contents values expressed as gallic acid equivalent (mg GAE/g) were given in Table 1. Total phenolic content among all samples varied between 48.03 and 202.83 mg GAE/g for leaf extracts and 83.89 and 148.04 mg GAE/g for flower extracts. Similar results obtained by our previous study (Özdemir et al., 2020) that the total phenolic contents of *H. montbretii*, *H. orientale* and *H. perforatum* species were ranged from 75.22 to 212.49 mg GAE/g . Öztürk, Tunçel, and Potoğlu-Erkara (2009) reported that the total phenolic content of *H. montbrettii*, *H. origanifolium* and *H. perforatum* species are between 104 and 451 mg GAE/g. Among the four studied *Hypericum* species, *H. linarioides* had the highest total phenolic contents as 202.83 mg GAE/g and 148.04 mg GAE/g for leaf and flower extract, respectively. The lowest value was determined in the extract from *H. aviculariifolium*. In the literature, there have been many studies for determining the total phenolic contents of *Hypericum* species (Del Monte et al., 2015; Ersoy et al., 2020; Maltas et al., 2013; Öztürk et al., 2009; Saddiqe et al., 2020), but most of results differ probably due to differences in the extraction conditions (solvent type, solvent concentration and extraction temperature and time) (Seyrekoğlu & Temiz, 2020) and plant parts (Sekeroglu, Urlu, Kulak, Gezici, & Dang, 2017). On the other hand, Kahkonen et al. (1999) stated that the concentration of GAE>20 mg/g dry matter presents remarkably high total phenolic content, that means that the leaf and flower extracts from *Hypericum* species are highly rich sources of these compounds.

Phenolic compounds are mainly responsible for the biological effects including antitumor, antimutagenic, antioxidant and antimicrobial activity, and they also have been shown to possess positive effects on human health, such as decreasing heart disease risk by inhibition of the oxidative of low-density proteins (Del Monte et al., 2015; Ersoy et al., 2020; Saddiqe et al., 2020). Also, Shahidi, Janitha, and Wanasundara (1992) reported that phenolic compounds show anti-inflammatory activity and anti-carcinogenic properties. The ethanolic extracts from the aerial parts of *Hypericum* species are known to contain a number of phenolic compounds, including chlorogenic acid, hyperforin and their derivatives, hyperoside, hypericin, quercetin, rutin, flavonols and flavones (Maskovic et al., 2011). Therefore, the use of medicinal plants including *Hypericum* species containing high phenolic compounds has increased significantly in the world in term of their medicinal and nutritional properties.

3.2. Antioxidant activity

The DPPH radical scavenging activity assay was used to determine the antioxidant properties of the *Hypericum* species and the results were summarized in Table 1. Based on the results, the values ranged from 17.14 to 24.94 and from 8.63 to 39.35 mg trolox equivalent/g for leaf and flower extracts, respectively. Our results were in agreement with Özdemir et al. (2020) who reported the similar antioxidant activity of 12.61 – 29.98 mg trolox equivalent /g for flower extracts and 15.07 – 35.61 mg trolox equivalent/g for leaf extracts from *H. montbretii*, *H. orientale* and *H. perforatum*. There have been many studies conducted to evaluate antioxidant activities of *Hypericum* species that are known to be good sources of antioxidants due to their rich phenolic contents (Eroglu Ozkan et al., 2018; Ersoy et al., 2020; Maltas et al., 2013; Saddiqe et al., 2020).

While leaves and flowers of *H. linarioides* had the highest antioxidant activity, *H. avicularиifolium* extracts showed weak activity among the species studied. However, leaves of *H. linarioides* and *H. perfoliatum* and flowers of *H. avicularиifolium and H. origanifolium* showed high antioxidant activity compared to their other aerial part. This difference is thought to be due to the environment in which the plants grow and the concentration of flavonoids, phenolic acids, and polyphenolics considered to be major contributors to the antioxidant capacity of plants during phenological stages (Mohammed et al., 2020). Similarly, Radulović et al. (2007) stated that the variation of the antioxidant capacity depends on the identity of the species and also the site and date of collection.

It is believed that the phenolic and/or polyphenolic compounds (flavonoid, flavanol, phenolic acid, etc.) biosynthesized in the plant sample might be responsible for the strong antioxidant activities of plant materials (Güzey et al., 2011). Therefore, in this study the antioxidant activity was correlated with total phenolic contents of the different parts of *Hypericum* species. A strong correlation was determined between the total phenolic contents and antioxidant activity of leaf extracts, however, weak correlation was found between the antioxidant activity and phenolic content of flower extracts (Figure 1). There are many studies that reported a positive correlation between total phenolic content and antioxidant activity (Gioti, Fiamegos, Skalkos, & Stalikas, 2009; Saddiqe et al., 2020; Şerbetçi et al., 2012), implying that phenolics are undoubtedly responsible for such inhibition of leaf extracts.

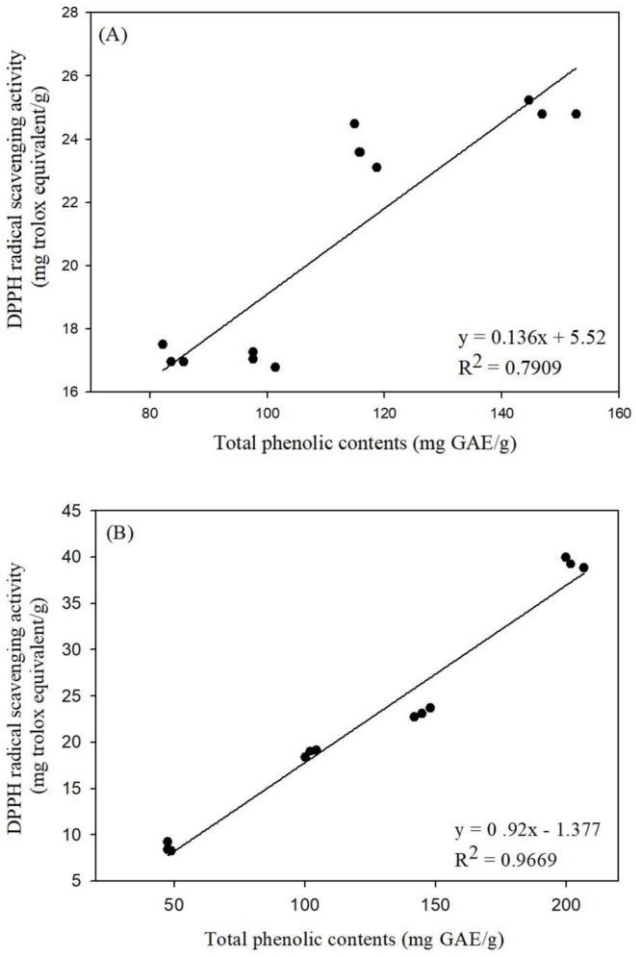

Figure 1. Correlation between total phenolic content and DPPH radical scavenging activity of flower (A) and leaf (B) extracts from four *Hypericum* species.

Table 1. Total phenolic content (mg gallic acid equivalent/g) and antioxidant activity (mg trolox equivalent /g) of ethanolic extracts of *Hypericum* species

Species	Total phenolic content		DPPH assay	
	Flower	Leaf	Flower	Leaf
H. aviculariifolium	83.89±1.83^{dA}	48.03±0.8^{dB}	17.14±0.31^{cA}	8.63±0.52^{dB}
H. perfoliatum	116.47±2.01^{bA}	102.26±2.17^{cB}	23.73±0.71^{bA}	18.83±0.42^{cB}
H. origanifolium	98.89±2.17^{cB}	144.96±2.95^{bA}	17.03±0.24^{cB}	23.16±0.48^{bA}
H. linarioides	148.04±4.13^{aB}	202.83±3.62^{aA}	24.94±0.25^{aB}	39.35±0.56^{aA}

$^{a-d}$ Different letters in same column indicates significant differences at the level of $p<0.05$
$^{A-B}$ Different letters in same line indicates significant differences at the level of $p<0.05$

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

Species	Plant Tissue	Indicator Microorganisms Code*								
		1	2	3	4	5	6	7	8	9
H. aviculariifolium	Flower	128	64	64	64	64	128	-	128	128
	Leaf	512	64	128	128	128	128	1028	128	64
H. perfoliatum	Flower	32	4	4	4	4	8	512	4	4
	Leaf	512	128	128	64	128	512	1028	128	512
H. origanifolium	Flower	32	32	32	32	32	32	1028	32	32
	Leaf	64	64	64	128	64	64	1028	128	64
H. linarioides	Flower	64	32	32	32	32	32	1028	64	32
	Leaf	64	64	64	64	64	32	1028	32	32

*1: *Staphylococcus aureus*, 2: *Pseudomonas aeruginosa*, 3: *Bacillus pumilis*, 4: *Bacillus subtilis*, 5: *Bacillus licheniformis*, 6: *Bacillus cereus*, 7: *Esherichia coli*, 8: *Listeria innocua*, 9: *Listeria monocytogenes*.

3.3. Antimicrobial activity

The antimicrobial activity of leaf and flower extracts was examined by Agar dilution assay against 9 bacterial strains, including seven Gram-positive and two Gram-negative bacteria and the obtained results were presented in Table 2. The minimal inhibitory concentration (MIC) values for all flower and leaf extracts were in the rage of 4-1028 µg/mL, depending on susceptibility of the tested bacteria. Among the test microorganisms, *P. aeruginosa* (Gram-negative) was the most sensitive bacteria to the plant extracts. Whereas, susceptibility was recorded lower in case of *E. coli* (Gram-negative). Similar results obtained by our previous study (Özdemir et al., 2020) reported that the extracts from *Hypericum* species possess the greatest antibacterial activity against *P. aeruginosa* and *Bacillus* with MIC values range from 16 to 32 µg/mL, but for *E. coli* it was insufficient. Our results were also in agreement with Barış et al. (2011) who reported that *P. aeruginosa* are very sensitive to all *Hypericum* extracts. Among the Gram-positive bacteria, *L. monocytogenes* was the most sensitive bacteria to the both of leaf and flower extracts. Similarly, Maltas et al. (2013) found that *H. avicularifolium* subsp. *depilatum var. depilatum* extract is found to be the most effective extract against *L. monocytogenes*. A number of studies are available in the literature regarding the antimicrobial activity of extracts form *Hypericum* species showed different antibacterial activity against tested microorganisms. The extracts from *Hypericum* exhibited more pronounced activities against Gram-positive than Gram-negative bacteria (Mazandarani, Yassaghi, Rezaei, Mansourian, & Ghaemi, 2007; Tusevski et al., 2016). Similarly, Maltas et al. (2013) reported the antibacterial activity of the *H. perforatum* extract is found to be more effective against Gram-positive bacteria, those of Gram-negative bacteria were found to be more resistant to extract. Tian et al. (2009) stated that the absence of lipopolysaccharide membrane surrounding the cell wall of Gram-positive bacteria allowing increase permeability of *Hypericum* antimicrobial metabolites into cells, which is supports the results obtained in the present study. On the other hand, the weak antibacterial activity against the Gram-negative bacteria was ascribed to the presence of outer membrane that may act as an additional barrier (Inouye, Takizawa, & Yamaguchi, 2001). However, Saddiqe et al. (2020) and Veličković, Stankov-Jovanović, Mitić, Kostić, and Palić (2013) stated that the *Hypericum* plants are equally effective against both Gram-positive and Gram-negative bacteria. From the results obtained by Radulović et al. (2007), the antibacterial action of the *Hypericum* extracts was more pronounced on Gram-negative than on Gram-positive bacteria in most cases or is even equal.

MIC results showed that the extracts from flowers have greater antimicrobial activity than the leaf extracts, which is consistent with the previous study (Özdemir et al., 2020). Contrary to our findings, Anusha et al. (2015) reported that the leaf extract from *H. mysorense* displays marked inhibitory activity against test bacteria compared to flower extract. The most prominent antimicrobial activity was exhibited by flower extracts from *H. perfoliatum* against all bacteria except *E. coli*, with MIC values ranged from 4 – 32 µg/mL. This is in agreement with previous studies (Nogueira et al., 2013) which indicated a good antimicrobial activity of *H. perfoliatum* extract. However, the leaf extracts from *H. perfoliatum* had the lowest antimicrobial activities against all the bacterial strains tested. Whereas the leaf extracts from *H. linarioides* had the highest antibacterial activities against all the bacterial strains tested. The previous screening study of nine *Hypericum* species from the Balkans (Radulović et al., 2007) showed that the antimicrobial activity of the crude methanolic extracts of *H. linarioides* shows a broad spectrum of very strong antimicrobial activity.

4. Conclusions

Our results indicated that the ethanolic extracts from *Hypericum* species including *H. perfoliatum* L., *H.*

aviculariifolium Jaup. and Spach subsp. depilatum (Freyn and Bornm.) Robson var. depilatum, *H. origanifolium* Wild. and *H. linarioides* Bosse have potential antioxidant and antimicrobial activities. The plant used in this study particularly *H. perfoliatum* showed strong antibacterial activity against Gram-positive bacterial strains. Also, we could say that undoubtedly *H. linarioides* extract could be used to further investigation for food and pharmaceutical industries, due to its total phenolic content and antioxidant activity.

Declaration of Competing Interest

The authors of the submitted manuscript hereby submit that there is no conflict of interest.

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