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Antioxidant Capacity and Antimicrobial Activity of Paeonia Peregrina L. [Uşak-Itecik Tulip] Extracts and Its Phenolic and Flavonoid Compounds

Ayşe Özdemir
Department of Biochemistry, Faculty of Medicine, Uşak University, Uşak, Turkey

Introduction: The objective of this study is to investigate the chemical characteristics of *Paeonia* - Itecik tulip and to evaluate the antioxidant and antimicrobial activities of an extract obtained from its petals and leaves.

Materials and Methods: Itecik tulip is native to Uşak region and its extracts were prepared to determine the antioxidant activity by using total flavonoid content analysis and phenolic content assay methods.

Results: It was detected that radical scavenging activity was higher in the petals than in the leaves, total phenolic content was 23.75 mg GAE/gr in the extract taken from the petals and flavonoid content was higher in the leaves than in the petals. Furthermore, in terms of antimicrobial activity, it was found that it was approximately 3 times more effective against K. Pneumoniae compared to Erithromycin and 2.5 times more effective compared to Penicillin and Vancomycin.

Conclusions: It can be concluded that our study subject Itecik tulip leaves may be effective in terms of antioxidant activity and radical scavenging activity It may be useful to compare *Paeonia* petals with more antibiotics to assess its efficacy. In our research, we would like to emphasize that we will have played a role especially in the recognition of itecik tulip.

Keywords: *Paeonia*, Antioxidant, Antimicrobial, Phenolic and Flavonoid

Introduction

Scientists use plenty of plants as research subjects particularly not only because of the efficient chemical substances in their content, but also because the plants used for medical purposes can be functional and offer treatment alternatives.

Paeonia pregrina is a plant belonging to the *Paeoniaceae* family publicly known as peony, with various species, and with leaves and large, beautiful, impressive petals. It is reported that twelve species grow in Turkey and it is more commonly known as peony or paeony. *Paeoniaceae* family which consists of diverse

Corresponding: Ayşe Özdemir, MD; Dept. of Biochemistry, Uşak University, Faculty of Medicine, Uşak, Turkey
ORCID ID: 0000-0003-2639-7344
E-mail: ayse.ozdemir@usak.edu.tr
E-mail2: ayse.ozdemir@hotmail.com
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species grow in many regions around the world especially including Mediterranean Region, Himalayas and Asia (1-3). P.Arietina, P.Daurica, P.Kesrouanensis, P.Masculasubsp. Bodurii, P.cf. Mascula subsp. Mascula, P.Mascula subsp., P.cf. Arasicola officinalis, P.Peregrina, P.Tenuifolia, P. Wittmanniana, P.Xkayae, the known 12 species found in Anatolia, are reported to be under preservation in the herbarium of Istanbul University, Faculty of Pharmaceutical Science (3).

It is demonstrated in various studies that, galloyl-paeoni-florin, the biological content of *Paeonia* which is highly intensively used in Chinese medicine, has an even stronger antioxidant activity than α-tocopherol and that *Paeonia* has antiaggregant, anticonvulsant, anxiolytic, hypoglycemic, analgesic, sedative, anti-inflammatory, antiosteoporotic effects and especially its roots are used as antiallergic and antipyretic (3-7). In addition, it is reported that it used to be utilized against sicknesses such as coughing and ulcer in Anatolian medicine. New-generation studies conducted in Turkey also emphasize its salicylic acid content and many biological activities (4-7).

The subject of our study, *Paeonia Peregrina*, locally called "Uşak-Itecik Tulip" (Figure-1), is known to grow by itself at an altitude of 1200m on Itecik Hill near Kayağıl Village of Uşak between May and June and is currently preserved by the Ministry of Environment and Forestry. Belonging to *Paeoniaceae* family and shaped like a potato, it has tubers 25-75 cm buried under the ground. It is also called the "cannonball tulip" in the region because, according to a local story, it took its color from the blood of martyrs who stayed in the region during the Turkish War of Independence and managed to live for 15 days and that is why it lives for about 2 weeks (8, 9). As reported in another study, there are plenty of plants growing in Anatolia that are currently examined for their phytochemical properties and Itecik tulip is one of them (9).

Figure-1. Uşak-Itecik Tulip (from Uşak-Itecik Highlands)

Antioxidant activity against diseases has been the subject of researches for years. It is reported that chemical phenolics have strong radical scavenging activity but may cause some side effects, thus, there is a tendency toward the use of plant-based natural resources with fewer side effects (7, 9).

Figure-2. Uşak - Itecik Tulip in Bud and Fully-Bloomed

Figure-3. Drying Petals and Leaves of Uşak- Itecik Tulip

The objective of this study is to detect the free radical scavenging activities of "Itecik Tulip" and its leaves as well as the phenolics contained in the plant which is currently preserved by the government and known to grow by itself, to determine its antioxidant and antimicrobial properties and to contribute to the promotion of this plant native to Uşak region.

Materials And Methods

Acquisition of the Plant

Plant Collection

Drying *Paeonia Peregrina* L. plants and their leaves (Figure 1-2), which grow on Itecik Hill near Kayağıl Village of Uşak, was collected by the Directorate of Nature Conservation and National Parks in mid-May. 2019. The species was characterized by Prof. Dr. Serdar Gökhan Şenol from Deparment of Biology, University of Ege. EGE43185 was given as a herbarium voucher specimen at the University of Ege (Izmir). Herbarium specimen was prepared and deposited at the Department of Biology, Faculty of Science, University of Ege. The plant is currently preserved in the herbarium of Ege University.

Plant Material Extraction

They were dried in the biochemistry laboratory of Uşak University, Medical Faculty under suitable circumstances and the petals, leaves and branches were separated from each other (Figure-3). The petals, leaves and branches minced from this plant were powdered by blender and dried in air. The petals, leaves and branches of the plant were extracted separately by using 2,2-difenil-1-pixil-hidrazil as solvent as described in previous report (10). Extracts from each were prepared and antioxidant activity, total flavonoid content analysis and phenolic content assay were determined using the following methods.

Experimental Design

Determination of Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The DPPH test was used to measure the free radical scavenging activity in each sample. The 0.100 g of petal and 0.008 g of leaf samples were obtained from 1 gram dried petal and leaf extracts and dissolved in 100 µl DMSO (Dimethyl sulfoxide). Antioxidant activity (Free Radical Scavenging Activity) of the samples was determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, as described in some prior modifications (10). 150µL of each sample was mixed with 5850 µL DPPH solution and incubated in 27°C for an hour. Absorbance was measured in Shimadzu UV-1800 spectro photometer at 515 nm. Ascorbic acid was used as positive control.

DPPH radical scavenging ability of the sample is calculated using the following formula:

Inhibition % = $[[A_{blank} - Sample] / A_{blank}] \times 100$

Blank; Absorbance of Control,

Sample; Absorbance of Test Compound.

Total Flavonoid Content Analysis

Total flavonoid content was quantified by using aluminum chloride colorimetric method (11). 50 µl of extracts was poured into 10 ml test tubes, 950 µl of methanol was added, 6400 µl of deionized water and then 300 µl of 5% $NaNO_2$ solution was added and mixed. 300 µl of 10% $AlCl_3$ solution was added into the mixture and mixed again. Following 5 minutes of incubation, 2000 µl of 1 M NaOH solution was added and total volume was made up to 10 mL. The mixture was left to rest for 15 minutes and its absorbance was measured in Shumadzu UV-1800 spectro photometer at 510 nm. Catechin was used as standard and total flavonoid content was expressed as mg catechin acid equivalent (CAE)/1 g per dry weight.

Total Phenolic Content Assay

Phenolic contents in the extracts were determined by using the Folin-Ciocalteu method modified by Elzaawely and Tawata (12). 7250 µl of deionized water, 500 µl of extracts and 250 µl of Folin-Ciocalteu reagent were added into 10 mL tubes, mixed and left to rest in a dark room for 5 minutes. 2000 µl (7.5%) of Na_2CO_3 was added thereon, total volume was made up to 10 mL and the mixture was incubated at room temperature in a dark place for 30 minutes. After incubation, absorbance values of samples were measured in Shumadzu UV-1800 spectrophotometer at 765 nm wave length. Gallic acid was used as standard and results were expressed as mg gallic acid equivalent (GAE)/1 g dry weight (DW).

Antimicrobial Activity Test

Preparation of Test Substances and Disk Diffusion Method

0.100 g petal and 0.008 g leaf extracts were prepared from 1 g of dried petals and leaves and dissolved in 100 µl DMSO. Resulting samples were used in antimicrobial activity experiment. Disk diffusion method was utilized for antimicrobial activity. 30 µl of petal and 30 µl leaf extract as well as solvent DMSO were absorbed into empty disks. Bacteria cultivated in Nutrient Broth medium were recultivated in petri dishes containing Nutrient Agar and the disks into which the test substances had been absorbed were placed thereon. Vancomycin, Chloramphenicol, Penicillin, Tetracycline and Erythromycin antibiotics were used as positive control and DMSO was used as negative control. Petri dishes were incubated at 37ºC for 24 hours. Following the incubation process, obtained zone diameters were measured and results were assessed.

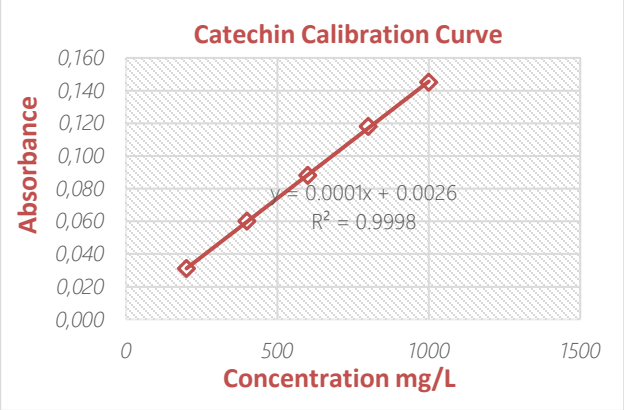
Results

Antioxidant activity of Itecik Tulip is presented in Table-1, Total Flavonoid Content Analysis Calibration Curve of Itecik Tulip in Graph 1, Total Flavonoid Content Analysis of Itecik Tulip in Table-2. The % Inhibition of DPPH (Free Radical Scavenging Activity) were 88.09 % and 24.35 % for petals and leaves.

Table-1. Antioxidant Activity of Itecik Tulip

Samples	DPPH % Inhibition
Petals	88.09
Leaves	24.35
Ascorbic Acid	96.96

Abbreviations. DPPH: Free Radical Scavenging Activity



Graph-1. Total Flavonoid Content Analysis Calibration Curve of Itecik Tulip

Total content flavonoid was detected to be 26.22 mg CAE/g in leaves and 10.32 mg CAE/g in petals. Total phenolic content in the leaf extract was 3.16 mg GAE/g while total phenolic content in the petal extract was 23.7 mg GAE/g.

Table-2. Total Phenolic and Flavonoid Content Analysis of Itecik Tulip

Samples	Total Phenolic (mg GAE/g Sample)	Total Flavonoid (mg CAE/g Sample)
Petals	23.75	10.32
Leaves	3.16	26.22

Antimicrobial Activity of Itecik Tulip is given in Table-3. Moreover, the effect of Itecik Tulip in terms of Antimicrobial Activity against Pseudo monas (P.) Aerouginosa is shown in Figure-4 and its effect against Klebsiella (K.) Pneumoniae and Bacillus (B.) Subtilis is given in Figure 5 and 6, respectively.

Figure-4. Antimicrobial Activity of Itecik Tulip - P. Aerouginosa

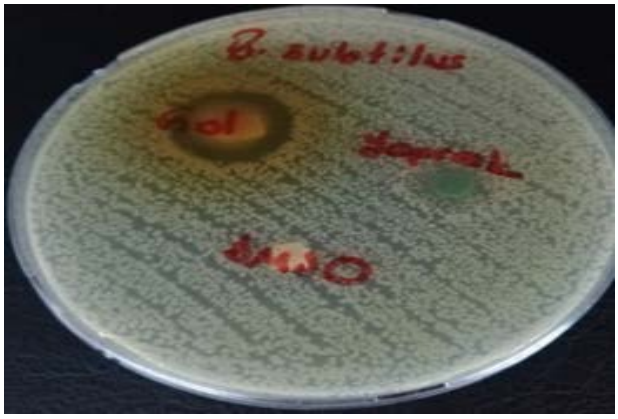


Figure-5. Antimicrobial Activity of Itecik Tulip- B.subtilis

Figure-6. Antimicrobial Activity of Itecik Tulip - K. Pneumoniae

Discussion

Plant-based natural products which are produced naturally and considered to have very few side effects are highly popular today due to the side effects of synthetic drugs used for oxidative stress and diseases caused by oxidative stress. These products receive attention not only because they are a trend, it is also because of their chemical activity (9).

Substances called reactive oxygen particles cause great harm to living beings, therefore, activity against the elimination of free radicals is important in terms of the efficacy of plant extracts used (7, 13, 14). A great number of plants and naturally-produced materials are used today because they are a treatment alternative for oxidative free radicals which are widely known and thought to cause numerous diseases.

Table-3. Antimicrobial Activity of Itecik Tulip

Microorganism	Positive Control					Test Substances		Negative Control
	Va (30)	P (10)	C (30)	E (15)	Te (30)	Petals	Leaves	DMSO
P. aerouginosa	6	6	12	6	9	18	-	-
K. pneumoniae	9	8	25	6	17	20	-	-
B. subtilis	22	31	36	29	16	19	-	-

Abbreviations: Vancomycin:V; Chloramphenicol:C; Penicilline:P; Tetracycline: Te; Erythromycine:E

In a study conducted with neuroblastic cell lines derived from pheochromocytoma, it was observed that *Paeonia* lactiflora can cure the oxidative stress and apoptosis induced by H_2O_2 and can be used in treatment (7). In the present study, although the free radical scavenging activity of Itecik tulip as oxidative stress indicator was found lower than the positive control group, higher activity was seen in its petals than in the leaves. It is also reported in another study that dried leaves of *Paeonia suffruticosa*, a plant frequently used in Chinese medicine, have been used in various treatments for centuries and contain high quantities of phenols, glycosides and monoterpene and its pharmacological properties should be investigated (15). According to the results of a comprehensive study, total phenolic content of *Paeonia* extracts found in Western Anatolia was 4.2 mg GAE/ g while total phenolic content of *Paeonia* extracts found in northwestern Anatolia was 10.5 mg GAE/ g (3). In our study, total phenolic content in the petal extract was 23.75 mg GAE/ g while total phenolic content in the leaf extract was 3.16 mg GAE/ g. In a study conducted by Orhan et al., the roots of 12 *Paeonia* species were used and phenolic content in all species was found lower than the phenolic content in petal extract used in our study (3). Total content flavonoid, on the other hand, was detected to be 10.32 mg CAE/ g in petals and 26.22 mg CAE/ g in leaves. Although phenolic contents of dried leaves were observed to be low in the present study, flavonoid content in leaves was higher than in petals. In another study, flavonoid contents and antioxidant activities of plants are correlated (13). Thus, it can be concluded that our study subject Itecik tulip leaves may be effective in terms of antioxidant activity and radical scavenging activity.

It is reported in a study that paeoniflorin found in the roots of *Paeonia*, in particular, has anticonvulsant and sense-enhancing character, is used in dementia treatment and induces vasodilator effect and its metanol extract reduces cholesterol in hypercholesterolemic rats (16). Several studies also report that there are high quantities of unsaturated fatty acids in Paeonia (13). We have not investigated the fatty acid ratio of the plant, thus, we have not detected yet whether or not it contains unsaturated fatty acid. It is also stated that knowledge of chromosome counts of plants is important for observing relationship between plants and examining the plants agriculturally (3). Assessment of Itecik tulip in terms of these aspects is also significant for us.

When we assessed the plant's antimicrobial activity against P. Aerouginosa microorganism according to the zone diameters, petal extract of the plant was 3 times more effective compared to Vancomycin, Penicillin and Erythromycin and 2 times more effective compared to Tetracycline. P. Aeruginosa is the most common micro organism in nosocomial infections and is resistant to many antibiotics. Studies also suggest that individuals with genetic resistance to certain drugs have higher resistance to antipseudomonal antibiotics (aminoglycosides, penicillins, cephalosporins, fluoroquinolone and carbapenems) and the distribution of such genes must be monitored. It may also be life-saving to start receiving an effective antipseudomonal drug although this infectious agent prolongs the hospitalization period and has high mortality rate (17,18). K. Pneumoniae is another microorganism known to cause noso comial infections and infections in individuals with suppressed immune system very frequent (19,20,21,22). In the present study,

the petal extract of Paeonia was 3 times more effective against Pneumonia compared to Erythromycin and about 2.5 times effective compared to Penicillin and Vancomycin. It is suggested that there is a tendency toward narrow-spectrum antibiotics to prevent developing resistance to P. Aeruginosa and K. Pneumoniae which are still two of the feared nosocomial microorganisms today (18). From this point of view, it may be useful to compare Paeonia petals with more antibiotics to assess its efficacy and consider it as a treatment alternative.

The objective of our study was to examine the antioxidant and antimicrobial characteristics of Itecik tulip, native to Uşak region, by using flavonoid and phenolic assays. It was observed that Itecik tulip leaves exhibited antioxidant activity and radical scavenging activity, there was a tendency toward narrow-spectrum antibiotics to prevent developing resistance to P. Aeruginosa and K. Pneumoniae and a more comprehensive study should be conducted to assess the efficacy of this plant. In addition, it will be our next goal to expand the scope of our study to look into the characteristics of this plant in more detail and examine its effects on cancerous tissues. We also believe that we achieved our second goal by contributing to the promotion of this plant which is currently under preservation.

Author' contributions

Asye Ozdemir contributed to microbiological, biochemical analysis and writing the present manuscript. And so contributed in selection of the plant material, preparation of plant extracts.

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Conflict of Interests

The author declares that he has no competing interests.

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Contact Details

Ayşe Özdemir, MD; Biochemistry, Uşak University, Faculty of Medicine, Uşak, Turkey; E-mail: ayse.ozdemir@usak.edu.tr; ayse.ozdemir@hotmail.com; ORCID: 0000-0003-2639-7344

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