



Antioxidant Potential of *Heracleum Persicum* Desf. Ex. Fisch. Extracts

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Abstract

Introduction: *Heracleum persicum*, also known as "Golpar" or "suh otu" in Turkey, has long been used as a spice and to treat a variety of illnesses. *H. persicum* is known to contain volatile substances, terpenoids, triterpenes, furanocoumarins, flavonoids, and alkaloids. This study aimed to determine the antioxidant potential with total phenolics and flavonoids contents.

Methods: The ethyl acetate, n-hexane, ethanol, and diethylether extracts of the aerial parts were prepared by maceration. The antioxidant capacity of the extracts were analyzed with DPPH radical scavenging activity. Folin-Ciocalteu and Aluminum chloride methods were used to determine the total phenolics and flavonoid contents.

Results: The highest extraction yield was obtained from ethanol extract whereas the ethylacetate extract with the highest antioxidant potential was found to inhibit DPPH radical by 6.873 ± 0.123 %. The highest phenolic ($247,82 \pm 0,0176$ mg GAE % dw) and flavonoid contents ($155,82 \pm 0,0022$ mg QE % dw) were found in the ethanolic extract of *Heracleum persicum*.

Conclusions: The antioxidant activity, the total phenolics and flavonoid contents of extracts prepared from aerial parts of *H. persicum*, using solvents with different polarities is comparatively analyzed for the first time in this study. The findings suggest its use in food supplements, both as a medicinal plant and as an additive, due to its antioxidant properties and rich phenolic and flavonoid contents.

Keywords: Antioxidant; *Heracleum persicum*; Total Phenolics; Flavonoid

Introduction

The plants are used for basic needs like nutrition, shelter, and defense. The idea of using plants for medicinal purposes has been known since the existence of humanity. It is observed that the

plants are accepted and used by many tribes or civilizations for the purpose of treatment and maintaining existing health. The resistance to drugs and the side effects that emerged after the use of synthetic drugs, along with concerns about the use of these drugs,

and patients' problems with affordability, have made herbal treatments popular again [1]. Considering their traditional use today, researches are being conducted on the pharmacological activities of many plants [2]. In bioactivity studies using plant extracts, antioxidant capacity determination holds the most significant place, and plants exhibiting high antioxidant capacity are generally observed as multifunctional therapeutic agents effective in treating other diseases as well [3,4]. Therefore, the extracts from plants that exhibit the best antioxidant activity are of great importance to the literature.

The Apiaceae family member *Heracleum persicum* Desf. Ex. Fisch is distinguished by its distinct anise aroma. Its base is quite stalky and as wide as 42 cm with a height can vary from 75 cm to 200 cm. The lower leaves, which have five to seven leaflets, are oval in shape and have spear-shaped, hairless tips with a blade that can grow up to 40 by 60 cm height. The compound leaves are lobed while the middle stem leaves contain three to five leaflets, a blade that can grow up to 15 by 45 cm, spear-shaped tips, and sparse hairs which the lower stem leaves are greenish in color, sparsely hairy, and can reach a length of 100 cm. The reddish-green, sparsely hair-covered sheath can reach a length of 10 cm. The white-pale lime green blooms have five petals and five stamens with many brackets. The elliptical fruits with two fused carpels resemble an umbrella and tiny sepals grouped convexly; however, the umbrellas blossoming on these side branches are smaller than those on the main branch [5-9].

Heracleum persicum, not only used in dishes and on vegetables as a spice but also as a flavor enhancer sprinkled on fruits like pomegranates is named as "Golpar" or "suh otu" in Turkey. Golpar, usually used in powdered form, sprinkled over beans, lentils, potatoes, and other legumes, is also mixed with the vinegars in which the lettuce leaves were dipped before consumption. Upon the awareness of the antispasmodic effect of *Heracleum persicum*, it was observed that while cooking, adding a tiny amount (1 or 2 teaspoons) of it to boiling foods like dried beans, provided relief to the stomach. Additionally, the plant's stems are recorded as being used both medicinally and to flavor some fermented vegetables, such as

pickles [10]. The fruits and seeds of *Heracleum persicum* are widely used as a spice due to their impressive fragrance, making them a flavor source in foods [11]. Traditionally used to treat a variety of illnesses, *H. persicum* has been linked to neurological, gastrointestinal, pulmonary, rheumatological, and urinary tract issues [12]. In Iran, the fruit juice from *H. persicum* is traditionally used to treat conditions like memory loss, dementia, dizziness, stroke, unilateral facial paralysis, and hemiplegia. The mixture made from the boiled fruits is also taken as a libido enhancer, appetite stimulant, and tonic for the stomach, liver, kidneys, and digestion. The plant's leaves were dried and powdered to treat leprosy-related venereal sores, while *H. persicum*'s potent aroma is said to be helpful during hiccups [13,14].

H. persicum is known to contain volatile substances, terpenoids, triterpenes, furanocoumarins, flavonoids, and alkaloids. The furanocoumarins, such as sfondin isolated from *H. persicum*, are reported as antioxidant substance that exert their effect by inhibiting the expression of cyclooxygenase-2 enzyme derived from IL-1 beta. Glutathione S-transferase (GST) enzymes play a key role in detoxifying endogenous harmful compounds such as lipid peroxidation or DNA hydroperoxide byproducts and in defense mechanisms against oxidative stress. In addition to antioxidant potential, the effect of the plant on GST enzyme activity was previously investigated [15-17]. In another study, the antioxidant potential of *H. persicum* has been confirmed by its inhibition of lipid peroxidation and DPPH radical scavenging activity [11].

It has been found that plants rich in flavonoids and phenolic compounds are actually natural sources of antioxidants, and therefore, their antioxidant capacity is usually positively correlated with the amount of total phenolics and flavonoid compounds [18-20]. Therefore, this study aimed to reveal the antioxidant potential of this valuable medicinal plant. To the best of our knowledge, in addition to antioxidant activity, the total phenolics and flavonoid contents of extracts prepared from aerial parts of *H. persicum*, using solvents with different polarities is comparatively analyzed for the first time in this study.

Materials and Methods

Plant material and preparation of plant extracts

H. persicum was authenticated and aerial parts of the plants were collected from the Gurpınar district of Van, in 1-8 June 2022 during flowering period, by Fatih Dönmez (Van Yüzüncü Yıl University, Department of Biochemistry, Faculty of Pharmacy). The air dried plant material was thoroughly crushed in a mortar, weighed out in 5 g portions, and subjected to maceration. This process involved sonication in 200 ml of ethyl acetate, *n*-hexane, ethanol, and diethylether solvents for 2 hours in an ultrasonic bath, followed by 8 hours of maceration at room temperature with occasional shaking. After maceration, the residue was filtered through Whatman No. 1 paper, and the residues were re-dissolved in the solvent, and macerated for 8 hours. The two filtrates were combined in a round-bottom flask and then evaporated under reduced pressure using a rotary evaporator. The extracts were then lyophilized and stored at -20°C until analyzed. The extraction yield percentages of the extracts were calculated.

DPPH radical scavenging activity assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) is a radical scavenger that reacts with substances capable of donating hydrogen atoms. For the DPPH stock solution, 4 mg of DPPH was dissolved in 100 ml of methanol. 1 mg/ml extract was mixed with 4 ml of stock DPPH solution and the mixture was left in the dark at room temperature for 30 minutes, shaking occasionally. The absorbance was then measured at 517 nm against methanol. A blank solution without extract was prepared by adding 4 ml of DPPH stock solution to 1 ml of methanol [21]. The DPPH inhibition percentages was calculated with the equation below:

$$\text{DPPH inhibition \%} = [(A_{\text{blank}} - A_{\text{extract}}) / A_{\text{blank}}] \times 100$$

The DPPH inhibition percentages of the extracts were determined in triplicate, and the averages and standard deviations of all three values were calculated. All UV spectrophotometric measurements were performed on an Optima SP 3000 Nano Ultraviolet (UV) spectrophotometer.

Total phenolic content determination

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method. The polyphenols in plant extracts react with specific redox reagent to form a blue complex that can be measured by visible light spectrophotometry. The reaction forms a blue chromophore composed of a phosphotungstic-phosphomolybdic complex, where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds [22,23]. According to the method used, 2.8 ml of deionized water and 2 ml of 2 % sodium carbonate were added to extracts with a concentration of 1 mg/ml, and the mixture was allowed to stand for one minute. Then, 0.1 ml of % 50 Folin Ciocalteu reagent prepared with water was added, and the mixture was allowed to stand in the dark at 25°C for 30 minutes, after which the absorbance of the mixture was measured at a wavelength of 750 nm [22]. The amount of phenolic compounds in the extracts was calculated in triplicate as gallic acid equivalents based on a standard calibration curve prepared using gallic acid (GA). All measurements were performed on an Optima SP 3000 Nano UV spectrophotometer.

Total flavonoid content determination

The total flavonoid content analysis was performed by mixing 0.5 ml from the extracts with 1.5 ml of 96 % ethanol, 0.1 ml of 10% ethanol aluminum chloride solution, and 2.8 ml of deionized water [24]. Absorbances were measured using an Optima SP 3000 Nano UV spectrophotometer at a wavelength of 415 nm after being kept in the dark at 25 °C for 40 minutes. The total flavonoid content equivalent to quercetin was determined based on the standard calibration curve prepared with quercetin (QE).

Statistical analysis

Calibration curve, regression analysis and standard deviations were analyzed using Microsoft Excel 2016.

Results and Discussion

The percentage yield of each extract was shown in Table 1. The highest extraction yield was determined with 20.6 % from ethanol extract of *H. persicum*.

Table 1: The extraction yield percentages of *H. persicum* extracts.

Extracts	Extraction yield %
<i>N</i> -hexane	2.520
Ethanol	20.600
Diethylether	2.003
Ethylacetate	4.256

The percentage inhibitions of DPPH by the extracts are shown in Figure 1. The ethyl acetate extract (1 mg/ml) with the highest antioxidant potential was found to inhibit DPPH radical by 6.873 ± 0.123 %. The ethanol extract, the diethylether extract and the *n*-hexane extract of *H. persicum* at a concentration of 1 mg/ml were found have 5.484 ± 0.163 %, 3.989 ± 0.247 %, and 0.463 ± 0.432 DPPH % inhibitions, respectively.

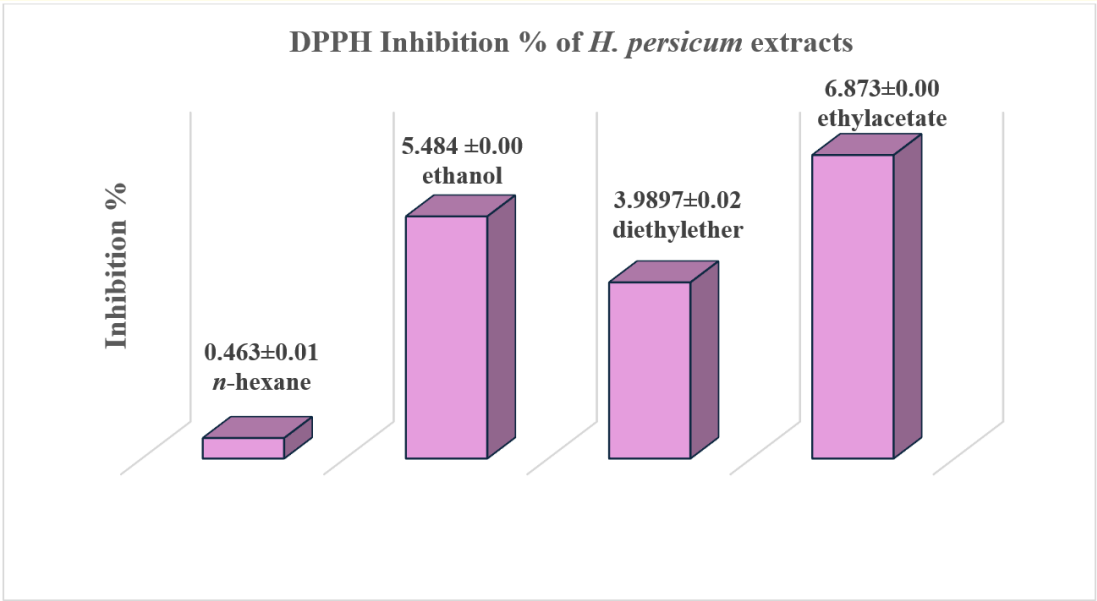


Figure 1: DPPH Inhibition percentages of *H. persicum* extracts.

The regression equation and standart curve for gallic acid and the total phenolic contents of *H. persicum* based on dried weight of the plant material are shown in Figure 2 and Table 2, respectively. Each value was the average of three replicate determinations. The total phenolic contents are given as mean $\pm$ standard deviation. The highest phenolic content was found in the ethanolic extract of *Heracleum persicum*, followed by the ethyl acetate, *n*-hexane and the diethyl ether extract.

A standard curve was first created using Quercetin for the total flavonoid content analysis (Figure 3). The total flavonoid content of *H. persicum* was calculated as quercetin equivalent values. The

data is expressed as gQE/100g of the plant. The highest value is obtained for the ethanol extract, while the lowest amount of flavonoid content is found in the *n*-hexane extract.

In a previous study using linoleic acid peroxidation, four furanocoumarins isolated from the fruits were found to have moderate antioxidant activity. It was also stated that the crude ethyl acetate extract was found to have higher antioxidant activity than the individually isolated components [25]. In a study conducted on *Heracleum persicum*, IC_{50} value for the DPPH inhibition activity of ethanol extracts was found to be 0.438 mg/ml. The total phenolic content of the plant extract was determined as 59.6 ± 2.8 μ g/mg [11]. In

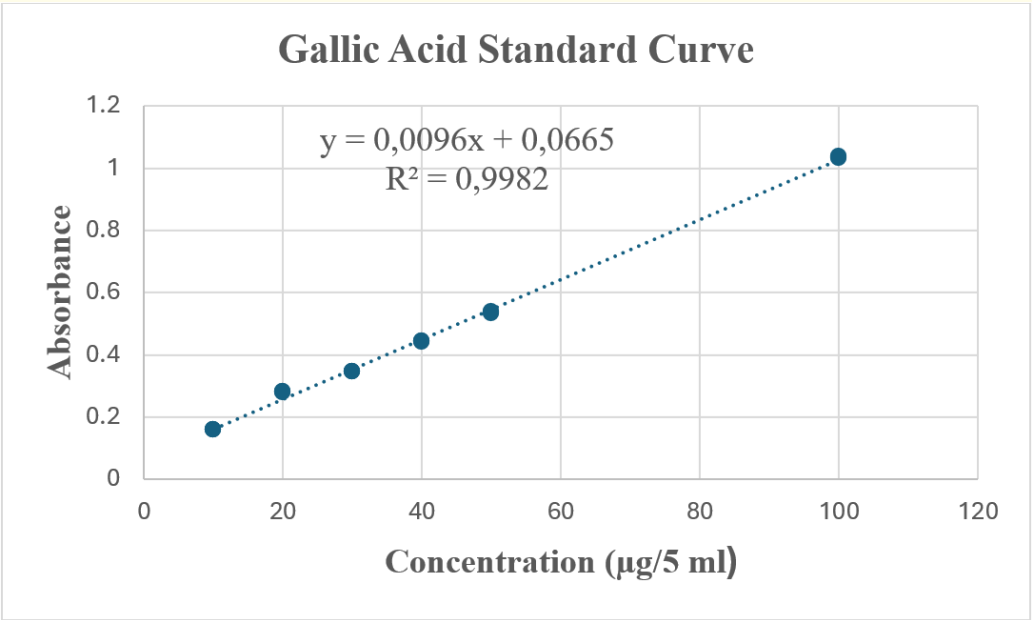


Figure 2: The regression equation and standart curve for gallic acid.

Table 2: The gallic acid equivalent total phenolic contents of *H. persicum*.

Extract	Total Phenolic Contents of <i>H. persicum</i> (mg/100 g dw)
Ethylacetate	62,50 ± 0,0044
Ethanol	247,82 ± 0,0176
<i>N</i> -hexane	60,46 ± 0,003
Diethylether	38,321 ± 0,0019

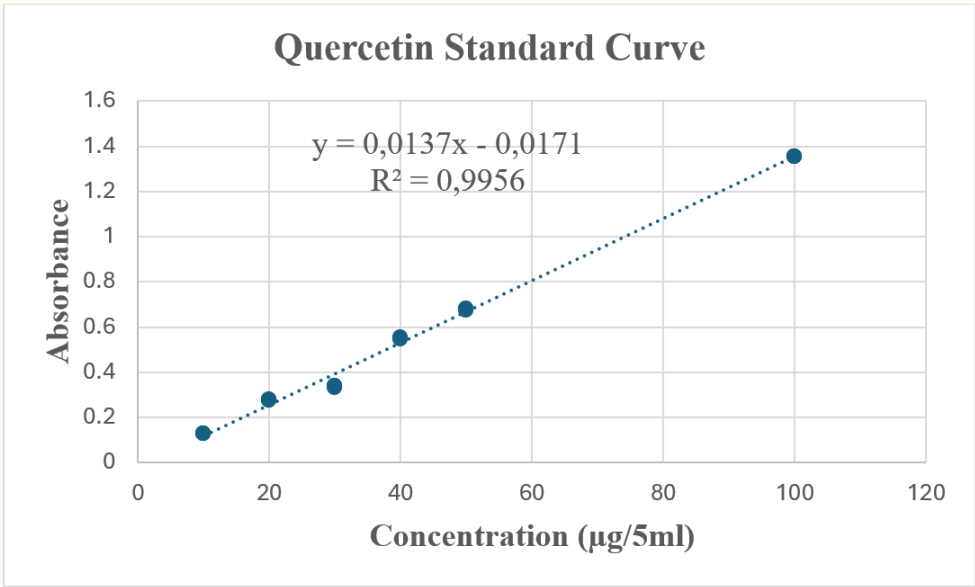


Figure 3: The regression equation and standart curve for quercetin.

another study, *Heracleum persicum* was collected from Shahdezh Mountain in Mazandaran province, Iran and the antioxidant activity of the *n*-hexane extract of roots reached an IC_{50} value of 3.2 mg/ml in the DPPH inhibition activity assay [14]. In a study conducted by Hanachi, *et al.* using gallic acid (GAE) and quercetin (QE) as standards, the total flavonoid concentration of the *H. persicum* root extract was reported as 4.69 mg QE/ml and the total phenolic concentration was determined as 5.45 mg GAE/ml [26]. In another DPPH inhibition assay, the IC_{50} value of the methanolic extract of the fruits were reported as 235 μ g/ml [27]. Firuzi, *et al.* searched for DPPH scavenging activity *Heracleum persicum*'s essential oil and reported IC_{50} as 7.4 ± 1.9 . The essential oil was found to contain 1.353 ± 0.020 mg/ml catechin equivalent total phenolic [28]. In a recent study Okumus and Meydan searched for the antioxidant activity of aqueous ethanolic extract and reported the IC_{50} value for DPPH inhibition as 5.36 mg/ml, and the total phenolic and total flavonoid contents as 20.84 mg GAE/ml and 12.35 mg QE/ml, respectively [29].

Although the ethanol extract included more total phenolic compounds and total flavonoid-structured components in the plant's aerial parts, the ethyl acetate extract of the plant exhibited a higher antioxidant capacity in terms of DPPH radical scavenging activity. In addition to the plant's sterols and coumarins, terpenes and terpenoids, which give the plant its distinctive anise scent, may have had an impact on the ethyl acetate extract's antioxidant activity. When compared to previous research, our findings support *H. persicum*'s moderate antioxidant activity. However, the previous results of total phenolic and flavonoid contents were reported on an extract basis in units such as mg/ml or μ g/ml, but for a more accurate comparison and standardization of the extracts prepared from the herbs, extraction yields should be taking into account as well. It would be more appropriate to explain the amounts in dried plant material. In the present study, the highest extraction yield was obtained from the ethanol extract. The ethanol extract of the plant was found to have higher flavonoids and total phenolic compounds, whereas the ethyl acetate with lower extraction yield exhibited higher antioxidant activity than the ethanol extract of *H. persicum*.

Conclusion

This study compares, for the first time, the antioxidant capacity and total phenolic and flavonoid contents of extracts prepared from the aerial parts of *H. persicum* using solvents with varying polarity. This comparative analysis will serve as an important source for future monographic research on the *H. persicum* plant. The results indicate that this medicinal plant's antioxidant qualities and high phenolic and flavonoid content make it suitable for usage as a dietary supplement and food additive.

Limitations of the Study

The limitations of this study are that the plant was collected from a single location and the antioxidant potential of the extracts was investigated comparatively using only one method.

Funding

The study is non-funded.

Conflict of Interest

The authors don't have conflict of interest.

Author Contributions

F. Donmez collected and authenticated the plant material. Extract preparations and quantitative analysis are conducted by H. Kayalar, G. Ozgun and F. Alaylı. The study is supervised and the manuscript is prepared by H. Kayalar.

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