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Antioxidant, Anti-Lipid Peroxidation and Antimicrobial Effect of *Heracleum persicum*

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Abstract: This study was conducted to determine the antioxidant activity, lipid peroxidation inhibitory effect and antimicrobial properties of *Heracleum persicum* plant. DPPH (2, 2-diphenyl, 1-picrylhydrazyl) method was used to determine antioxidant activity, and the Folin method was used to determine total phenolic content. Disc diffusion method was applied to determine antimicrobial activity. In the study, the antioxidant activity value of *H. persicum* was determined as 5.36 mg mL^{-1}, the total phenolic content was 20.84 mg GAE mL^{-1} and the total flavonoid content was 12.35 mg QE mL^{-1}. The plant's inhibition against lipid peroxidation was measured as 3.63 mg mL^{-1}. Its antioxidant activity and inhibitory effect against lipid peroxidation were lower than butylated hydroxyanisole (BHA) in both analyses ($p<0.05$). The plant extract was found to be effective against *Bacillus subtilis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria. As a result, the antioxidant properties, antimicrobial and anti-lipid peroxidation effects of *H. persicum* plant have revealed that this plant has an important potential for use in medicine and pharmacology, as well as for consumption as food.

Keywords: Antimicrobial effect, bioactive compounds, *Heracleum persicum*, lipid peroxidation, flavonoids, phenols

1. Introduction

Reactive oxygen species (ROS) are constantly produced in living things, primarily as a result of aerobic processes and due to many factors, such as oxidative stress, radiation and inflammation. Due to this situation, increased free radicals in the cells are very effective in the pathogenesis of many diseases such as cancer, cardiovascular diseases, Alzheimer's and Parkinson's, and in the aging process (Aruoma, 2003; Doğan and Meydan, 2021). The use of both exogenous and endogenous antioxidants to clear free radicals in the cellular defence system is an important issue. Medicinal plants are important sources of synthetic and herbal medicines used in the prevention or treatment of many diseases from ancient times to the present (Rahimi et al., 2019; Seçkin and Meydan, 2022). These positive effects on health of medicinal plants, which are widely used for their therapeutic properties against various diseases, are due to the phenolic and antioxidant compounds found in their structures.

Phenolic compounds are secondary metabolites found in the structure of plants. They contain one or more hydroxy substituent rings in their structure and quench the free radical by donating a hydrogen atom or an electron (Rodríguez-Rojo et al., 2012). These ingredients have the ability to heal oxidative damage by preventing the formation of free radicals. They can also inhibit lipid peroxidation in cells and exhibit various physiological activities as antioxidants. From a human health perspective, phenolic compounds have an important potential for use in the prevention and treatment of some chronic diseases such as cardiovascular diseases, diabetes and cancer (Del Rio et al., 2013).

Oxidative stress is effective in the emergence and progression of many diseases, and lipid peroxidation (LPO) is one of the markers of

oxidative stress. Lipid peroxidation is a process in which oxidants such as ROS attack unsaturated lipids, producing a wide variety of oxidation products. In this process, oxidants can interact with circulating lipoproteins, alter target nucleophilic sites within biomolecules such as DNA, lipids and proteins, and cause numerous biological effects (Al-Menhali et al., 2020; Karimi et al., 2022).

Heracleum persicum L. is a perennial herbaceous plant belonging to the Umbelliferae family (Apiaceae), known as "Suh" in Türkiye and "Golpar" in Iran. Its homeland is Iran, Iraq and Türkiye and it grows in humid mountainous regions. This plant is often used as a spice in pickles and foods to increase flavour (Shariatifar et al., 2017). The structure of *H. persicum* contains various bioactive components, including volatile (aliphatic esters, carbonyls and terpenes) and non-volatile (flavonoids, furanocoumarins, alkaloids and tannins) components, as well as different minerals (Dehghan et al., 2016). In addition to its consumption as food, the *H. persicum* plant also shows various biological properties such as potential antioxidant, antidiabetic, analgesic, anti-inflammatory, anticonvulsant and cardioprotective (Dehghan et al., 2016; Shariatifar et al., 2017). The potential for use of *H. persicum* in the biomedicine and pharmaceutical industries has recently attracted much attention. This medicinal plant is used in traditional medicine for many different treatments, especially cancer, urinary tract infection, gastrointestinal disorders, neurological and respiratory disorders (Majidi and Lamardi, 2018).

Today, interest in natural products in human nutrition has increased due to the determination of the possible negative effects of artificial antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) on health and the increasing perception of consumers about this problem in recent years. As effective natural antioxidant sources, plants contain phenolics, flavonoids and stilbenes. These plants have the opportunity to be used as potential antioxidants thanks to their safety, effectiveness, biological effects and low cost. Studies have shown that *H. persicum* is rich in phenolic compounds and exhibits high biological activity (Majidi and Lamardi, 2018). This makes them worthy of study as medicinal plants. Considering its increasing potential for use, this study was conducted to determine the antioxidant activity, total phenolic and total flavonoid content, inhibition effect against lipid peroxidation and antimicrobial properties of *H. persicum* plant.

2. Materials and Methods

2.1. Chemical material

Ethanol, methanol, gallic acid (GA), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), and aluminium chloride ($AlCl_3$) were supplied from Merck (Darmstadt, Germany). 2-Thiobarbituric acid (TBA), BHA, Folin-Ciocalteau phenol reagent, and DPPH (2, 2-diphenyl, 1-picrylhydrazyl) free radical were obtained from Sigma Aldrich (Inc. St. Louis, MO. USA).

2.2. Plant material and extraction

The *Heracleum persicum* plant (Figure 1), which constitutes the material of this study, was collected from the Altıncık village of Bingöl Province. The species identification of the plant was made by Prof. Dr. Lütfi BEHÇET at Bingöl University, Faculty of Science, Department of Biology (NB01; 6333). The plant was collected in the first week of May during the flowering phase and the entire plant (leaf, stem, trunk) was used in the study. The plant was first washed, then dried and ground into powder using a laboratory mill. For extraction, 5 g of sample was treated with 100 mL of ethanol-water mixture (80:20) in a magnetic stirrer at 50 °C for 24 h. Then, the extract filtered through coarse filter paper was centrifuged at 8000 rpm for 15 min, and the resulting supernatant was stored at 4 °C for analysis.

Figure 1. *Heracleum persicum* plant

2.3. Antioxidant activity analysis

DPPH radical quenching ability of plant extracts was measured according to Pyo et al. (2004). 100 µL of plant extract prepared at different concentrations was added to 3.9 mL DPPH (100 µM) solution. Samples were incubated at room temperature and in the dark for 60 minutes. Absorbances were then read at 515 nm by a UV-Vis

Shimadzu mini 1800 spectrophotometer (Shimadzu-Japan). Inhibition values (%) were calculated using the formula below (Equation 1), and antiradical activity results were expressed as the graphically calculated IC_{50} (minimum dose required to produce 50% inhibition). BHA was used as a positive control.

$$\% \text{ Inhibition} = [(A_0 - A_s) / A_0] \times 100 \quad (1)$$

This A_0 is the absorbance of the control at 60 minutes and A_s is the absorbance of the sample at 60 minutes.

2.4. Total phenolic content (TPC)

The TPC concentration of the extracts was determined by preparing gallic acid standard solution using the Folin-Ciocalteu method (Singleton and Rossi, 1965). 0.4 mL of extract was mixed with 1.6 mL of Na_2CO_3 solution prepared at 7.5% concentration and vortexed. The same procedure was applied to standard solutions of GA at different concentrations (1-100 mg L^{-1}). After 3 minutes, 2 mL of 10% Folin-Ciocalteu phenol reagent was added and mixture was vortexed again. After 60 min incubation at room temperature, the absorbance was read at 765 nm and the results were calculated as gallic acid equivalents (mg GAE mL^{-1}).

2.5. Total flavonoid content (TFC)

The method suggested by Zhishen et al. (1999) was modified and used. For analysis, 5 mL of water and 0.3 mL of 5% $NaNO_2$ solution were added to 1 mL of extract, then mixed with vortex and incubated for 5 min. Then, 0.6 mL of 10% $AlCl_3$ was added, mixed and incubated for another 5 min. At the end of the period, 2 mL of 1 M NaOH was added to the mixture and made up to 10 mL with pure water, and the absorbance was read at a wavelength of 510 nm. Results were expressed as mg quercetin equivalents (mg QE) mL^{-1}.

2.6. Lipid peroxidation inhibitory activity

LPO analysis was performed according to the previously reported TBA method (Meydan et al., 2020). BHA was used as a positive control in the analysis. Solutions of different concentrates prepared for plant extract were incubated for 1.5 hours at 37 °C in the dark. At the end of the period, 28% trichloroacetic acid (TCA) was added cold and the mixture was centrifuged at 5000 rpm for 20 minutes. After centrifugation, 1.2 mL TBA was added to the supernatants and the reaction was terminated by boiling the mixture at 100 °C for 10 minutes. Absorbance measurements were made at 532 nm and % inhibition values were calculated and the results were evaluated over IC_{50}.

2.7. Determination of antimicrobial activity

Antimicrobial activity of *H. persicum* against gram-negative bacteria (Escherichia coli, *Pseudomonas aeruginosa*) and gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*) was performed using Mueller Hinton agar plates by the disc diffusion method. Briefly, 100 µL of each bacterial strain containing 1×10^6 CFU mL^{-1} was evenly distributed on the prepared nutrient agar Petri plates. Each disc was loaded separately with 10 µL of distilled water (negative control), aqueous plant extract, and 100 µg mL^{-1} rifamycin (positive control). Then, the prepared petri dishes were incubated at 37 °C for 24-48 hours. At the end of the incubation period, the results were recorded and the inhibition zone was expressed as mm in diameter.

2.8. Statistical analysis

All analyses were performed in triplicate and results are presented as mean ± standard deviation. Differences between the obtained data were made using the Statistical Package for the Social Sciences (SPSS) statistical program (version 18.0). The *t-test* was performed and the results were correlated.

3. Results and Discussion

3.1. Antioxidant activity

Free radicals and oxidants are atoms that are unstable due to the presence of unpaired electrons that cause serious cellular damage such as gene mutations, uncontrolled cell proliferation due to cancer, apoptosis dysfunction and inflammation (Maddu, 2019; Koçak et al., 2020). Bioactive compounds found in plant extracts are powerful antioxidants and are important in preventing many diseases related to oxidative stress, including diabetes, arteriosclerosis, cardiovascular diseases, cancer and various neurodegenerative disorders (Dehghan et al., 2016; Özyazıcı, 2022, 2023). Inhibition values of *H. persicum* against DPPH radical at different concentrations are given in Figure 2. The IC_{50} antioxidant activity value of the aerial parts of *H. persicum* used in our study was determined as 5.36 mg mL^{-1} (Table 1). This value was lower than BHA used to compare antioxidant activity ($p<0.05$). However, considering that BHA is a very strong antioxidant, the value obtained shows that the *H. persicum* plant has a significant antioxidant activity. Some furanocoumarins found in the structure of *H. persicum* show antioxidant properties (Souri et al., 2004). Çoruh et al. (2007) examined the antioxidant potential of *H. persicum* using the DPPH radical scavenging activity method. The researchers reported the IC_{50} value of the DPPH radical scavenging activity of *H. persicum* extract

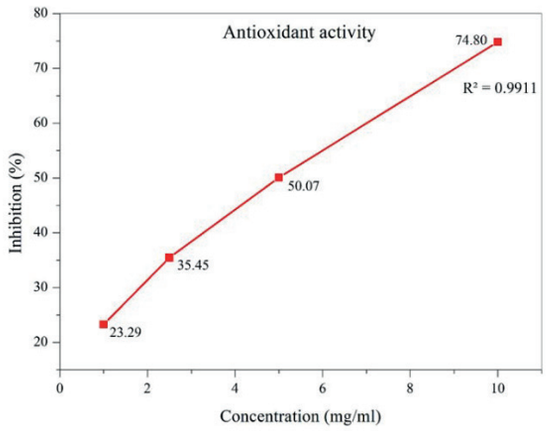

Figure 2. Inhibition values of *H. persicum* in different concentrations

as 0.438 mg mL^{-1}, which was much lower than that of quercetin used for comparison in the same study (IC_{50} value= 0.006 mg mL^{-1}). In a study where the antioxidant activity of the *Heracleum humile* plant was measured using different solvents, IC_{50} values for ethyl acetate, methanol and water were determined as >5, 3.61 and 2.33 mg mL^{-1}, respectively (Ocal et al., 2022). Karan and Genc (2018) examined the extract of the *H. persicum* plant for antioxidant properties using the DPPH method and compared the results with artificial antioxidants such as BHA and BHT. The results from the study determined that the antioxidant properties of artificial antioxidants were higher than the plant extract and that there was a significant connection between phenolic content and antioxidant properties. On the results obtained, the type of plant used, the geography where it grows, climate characteristics, the solvent used, the extraction method, harvest time and storage conditions are effective on antioxidant activity (Okumuş, 2023).

3.2. Total phenolic and total flavonoid content

The TPC content of the *H. persicum* plant species used in our study was determined as 20.84 mg GAE mL^{-1} (Table 1). The TPC of the *H. persicum* plant was determined as 49.12 mg by Akbaribazm et al. (2021). In a different study, the total phenolic content of the Golpar plant extract obtained using microwave was determined as 167.25 mg GAE g^{-1} (Kenari et al., 2020). Dehghan et al. (2016), in their study using hexane as a solvent, reported that the TPC of the aerial parts of *H. persicum* was 167.2 mg GAE g^{-1}. The TPC content of the *Heracleum humile* plant was determined by Ocal et al. (2022) as 20.12, 16.81 and 20.59 mg GAE g^{-1} for ethyl acetate, methanol and water, respectively.

Table 1. Antioxidant activity, TPC, TFC and LPO inhibition activity of the *H. persicum*

Sample	DPPH (IC_{50} mg mL^{-1})	TPC (mg GAE mL^{-1})	TFC (mg QE mL^{-1})	Lipid peroxidation (IC_{50} mg mL^{-1})
H. persicum	5.36±0.01[b]	20.84±0.16	12.35±0.72	3.63±0.26[b]
BHA	0.06±0.01[a]			0.01±0.00[a]

[a, b]: Different superscript lowercase letters indicate differences between samples ($p<0.05$)

The TFC amount of *H. persicum* was determined as 12.35 mg QE mL^{-1} (Table 1). Hanachi et al. (2022) measured the TFC value of *H. persicum* as 4.69 mg QE mL^{-1}. TFC was found to be 22.23 µg mg^{-1} by Nickavar and Abolhasani (2009). In a different study, it was reported that the TFC value of the *Heracleum gorganicum* plant ranged from 2 to 84.84 mg QE g^{-1} (Mazandarani et al., 2011). *Heracleum persicum* is medicinally important plant containing a source of countless pharmaceutically active compounds such as phenolic acids and flavonoid compounds, which could play a major role in the development of new drugs for cancer therapy (Hanachi et al., 2022). It is thought that the difference between the results may be due to the extraction method, the solvent used in the extraction, the period when the plant was collected, geography and environmental factors (Okumuş, 2023).

3.3. Lipid peroxidation inhibition

Figure 3 shows the inhibition values of *H. persicum* against lipid peroxidation at different concentrations. In parallel with the increasing plant concentration, the inhibition percentage also increased. The LPO inhibition activity of *H. persicum* plant was determined as 3.63 mg mL^{-1} (Table 1). This value was found to be lower than the LPO inhibition shown by BHA (0.01 mg mL^{-1}), similar to the antioxidant activity results ($p<0.05$). *H. persicum* plant has an inhibitory effect on lipid peroxidation depending on its total phenolic content (Çoruh et al., 2007). Çoruh et al. (2007) reported that the IC_{50} value of *H. persicum* plant for lipid peroxidation inhibition was 0.503 mg mL^{-1}. *H. persicum* plant contains a variety of antioxidant phytochemicals, including many phenolics and flavonoids (Razzaghi-Abyaneh et al., 2014). These phytochemicals have been reported to counteract

the harmful consequences of oxidative stress, including lipid peroxidation and low-density lipoprotein (LDL) modification, endothelial dysfunction, and platelet aggregation (Grassi et al., 2010). A study by Majidi et al. (2020) determined that *H. persicum* extract could exert significant protective effects on lipid peroxidation and significantly reduce plasma LPO levels, which is associated with its antioxidant activity.

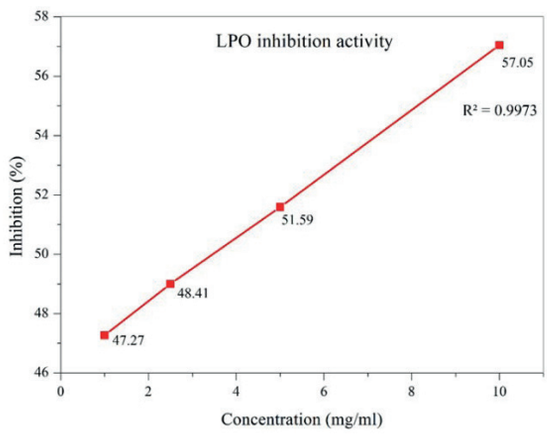

Figure 3. Inhibition values of *H. persicum* against lipid peroxidation in different concentrations

3.4. Antimicrobial activity

The emergence of multidrug resistance in pathogenic microorganisms has initiated various researches to obtain new antimicrobial substances from plant sources. Phenolic compounds and their derivatives are compounds that have an important function in the defence system of plants and have an effect on stopping the development of many pathogenic microorganisms (Araújo et al., 2018). Table 2 shows the zone diameters formed by the *H. persicum* plant against gram-positive and gram-negative bacteria. It was determined that the plant extract had no antibacterial effect against *B. cereus* and *E. coli*. However, it showed the highest antibacterial activity among the test strains against *S. aureus* with a zone diameter of 20 mm. Its effect against *E. faecalis* was found to be the same as rifamycin. Nazemi et al. (2005) reported that the aqueous extract of *H. persicum* did not have any antibacterial effects, but the methanol extract had significant effects on *Bacillus polymyxa*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Staphylococcus aureus* bacteria. Kousha and Bayat (2012) found the zone diameters of *H. persicum* leaf and flower extracts for *B. subtilis* to be between 11.04 and 24.05 mm in different extract concentrations. In the same study, the values found for *B. cereus* ranged from 15 to 23.34 mm. Frey and Meyers (2010) reported that the antimicrobial properties of plants are effective in the therapeutic effect of plants used in traditional treatment. Differences between the results obtained and literature studies may be due to differences in the growth ecosystem of *H. persicum* used (habitat, temperature, altitude), extraction method and solvent. Various factors affecting the growth and bioactive components of plants in an ecosystem, including species, habitat, soil, elevation and geographical location, show remarkable effects on the results obtained.

Table 2. Antimicrobial activity of *H. persicum* against selected microorganisms

Test microorganisms	Extract	Rifamycin
Bacillus cereus ATCC 10876	-	11.25±0.35
Bacillus subtilis ATCC 6633	10.00±0.00	12.50±0.71
Escherichia coli ATCC 25952	-	-
Enterococcus faecalis ATCC 29212	9.50±0.71	9.50±0.00
Pseudomonas aeruginosa ATCC 27853	8.75±0.35	10.00±0.00
Staphylococcus aureus ATTC 29213	20.00±0.00	25.75±0.35

(–) indicates absence of zone of inhibition

4. Conclusions

Bioactive components found in plants are very important in scavenging free radicals, showing antimicrobial effects, and also stabilizing lipid peroxidation. Thanks to these compounds, protection against diseases is provided by reducing free radicals produced in cells due to metabolic processes and oxidative stress. In cases where antioxidant defence systems are insufficient, natural antioxidants must be taken from the diet. Therefore, determining the antioxidant capacity of plants becomes important. In our study, it was determined that *H. persicum* has high antioxidant activity, a significant inhibition against lipid peroxidation and an antibacterial effect. Thus, it has been revealed that this plant, which has health benefits and is easily accessible, is a natural source of antioxidants and has a potential effect on pathogenic bacteria. It is thought that studies on this subject should be further increased in order to evaluate the consumption of this plant as food as well as its use in medicine and pharmacology.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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This research received no external funding.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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