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Original Article

TOTAL PHENOLIC, FLAVONOID CONTENTS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *HESPERIS PENDULA*

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ABSTRACT

Many natural products are used in complementary medicine. Plants are widely used among these natural products. In this study, it was aimed to determine the total phenolic and flavonoid contents, total antioxidant status and antimicrobial activity of *Hesperis pendula* DC. In this context, the above-ground parts of the plant were extracted with ethanol and methanol. The total antioxidant level of the plant was determined using Rel Assay Diagnostics kits (Megatıp/Türkiye). The total phenolic content was assessed using the Folin-Ciocalteu reagent. Aluminum chloride assay was used to estimate the total flavonoid content. Antimicrobial activity was tested against bacterial and fungal strains by agar dilution method. As a result of the studies, it was observed that the ethanol extract of the plant had higher TAS (Total antioxidant status) (5.707±0.194 mmol/L), TOS (Total oxidant status) (21.646±0.239 µmol/L) and OSI (Oxidative stress index) (0.380±0.017) values. Total phenolic content was higher in ethanol extract (116.78±2.51 mg/g) while total flavonoid content was higher in methanol extract (93.64±2.16 mg/g). It was observed that the ethanol and methanol extracts of the plant inhibited the growth of bacteria at 100-200 µg/mL concentrations. It was determined that ethanol extract inhibited the growth of fungi at 200 µg/mL concentration and methanol extract at 200-400 µg/mL concentrations. In this context, it was determined that *H. pendula* could be a natural antioxidant and antimicrobial source.

KEYWORDS: Antimicrobial, Antioxidant, Hesperis, Oxidant, Total Phenolic, Flavonoid

1. Introduction

In contrast to conventional medicine, complementary and alternative medicine practices are among the first forms of healthcare used by human societies to combat illness [1]. Many fungi, plants, and animals utilized in the supplementary and alternative medicine of many civilizations have proven effective in the prevention and treatment of illness. In addition to conventional medical care, many individuals nowadays are also seeking out alternative treatments for their health problems [2,3]. Food, shelter, warmth, equipment, spice, and medicine are just few of the many uses for plants across the globe. Plants have been reported by many researchers to have many different activities such as antioxidant, anticancer, antiproliferative, hepatoprotective, anti-inflammatory, anti-aging, antiallergic, DNA protective, antimicrobial [4- 11]. The total phenolic and flavonoid contents, as well as the biological activities, of *Hesperis pendula* D.C. were assessed in this research. In addition, due to the lack of sufficient studies on *H. pendula*, it was aimed to determine the potential of the plant.

The Brassicaceae family includes annual and perennial plants. It is a cosmopolitan family with mostly herbaceous and shrub forms. It is represented by 337 genera and 3350 species worldwide. The *Hesperis* L. (Brassicaceae) species is widespread in southern and central Europe, southwestern Asia, the Caucasus, the mountainous regions of western China, and Mongolia. This genus is represented by 56 species worldwide. Flowers of various purple and white tones may be seen on plants belonging to the genus *Hesperis*. *Hespera* is the Greek word for evening because of the unique aromas it produces in the late afternoon and evening [12].

2. Matherials and Methods

Plant samples were collected from Gaziantep (Turkey)

and herbarium samples of the plant were preserved in the Biology department of Gaziantep University. Flora of Turkey [13] was used in order to identify the plants found. Distilled water was used to clean dust or muddy parts of the plant parts. To make powder, the plant material was first dried in a laboratory environment out of direct sunlight. The powder samples weighed 30 g, and then 250 mL of ethanol was used to extract them in a Soxhlet device for 6 hours at 50 °C. The same steps were used to extraction with methanol. During the testing phase, extracts made with ethanol and methanol were stored at +4 °C.

2.1. Antimicrobial activity tests

The effects of plant extracts against the reference bacterial and fungal strains were examined using the agar dilution technique.

Standard Gram-positive bacteria: *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212.

Standard Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606.

Standard Fungi: *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135, *C. glabrata* ATCC 90030.

Muller Hinton Broth medium was used to cultivate Gram-negative and Gram-positive bacteria. RPMI 1640 Broth medium was used to cultivate the fungi strains. Distilled water was used to fine-tune the plant concentrations. MIC (Minimum Inhibitory Concentration) values were calculated to represent the concentrations of extract needed to significantly slow the growth of bacteria and fungi in the lab. Plant extracts were adjusted at 800-12.5 µg/mL concentrations [14-16].

2.2. Total phenolic and flavonoid tests

The plant extracts were used to make 1 mL stock solutions. Dried distillers' grains were used to fine-tune the concen-trations. The stock solutions were diluted with Folin-Ciocalteu reagent (1 mL, 1:9, *v/v*). Next, 0.75 mL of 1% sodium car-bonate was added and the mixture was vortexed. It was then incubated for 2 hours and absorbance was measured at 760 nm. Based on the gallic acid standard solution calibration curve [17], total phenolic content was calculated and reported as mg.GAE/g.

The aluminium chloride assay was used to quantify the total flavonoid content of the plant extracts [18]. 0.5 mL of Quercetin, 0.5 mL of plant extract, 4.3 mL of methanol, 0.1 mL of 10% aluminum nitrate and 0.1 mL of 1 M ammonium acetate were mixed and incubated for 40 minutes. The absorbance was read at 415 nm and results were then recorded as a function of mg.QE/g.

2.3. Antioxidant activity tests

The Rel Assay Diagnostics (Megatıp/Türkiye) TAS (Total antioxidant status) and TOS (Total oxidant status) kits were used to calculate the total antioxidant and oxidant potentials of plant extracts. TAS tests of plant extracts were calibrated with trolox, whereas TOS tests were calibrated with hydrogen peroxide. TAS and TOS were reported in units of mmol Trolox equiv./L and µmol hydrogen peroxide equiv./L, respectively [19,20]. The oxidative stress index (OSI) was calculated by dividing the total oxidant status by the total antioxidant status [21].

3. Results and Discussion

3.1. Antimicrobial activity

Many illnesses have microorganisms as a root cause. Many microbial infections are treated with synthetic antimicrobial medicines [22]. The current medications employed are inadequate because of the potential negative effects of synthetic pharmaceuticals and the rise in the number of resistant microbes owing to antibiotic abuse [23,24]. To address this issue, scientists have begun looking for novel antimicrobials. Numerous scientists have recently shown the potential of plants as natural antibacterial medicines. The antimicrobial properties of *H. pendula* were tested on many common bacterial and fungus strains. The findings are shown in Table 1.

Table 1. MIC values of ethanol and methanol extracts of *Hesperis pendula*

Extract	*S. aureus*	*S. aureus* MRSA	*E. faecalis*	*E. coli*	*P. aeruginosa*	*A. baumannii*	*C. albicans*	*C. glabrata*	*C. krusei*
Ethanol	100	100	100	200	100	100	200	200	200
Methanol	100	200	200	200	100	200	200	400	400

100, 200 and 400 µg/mL represents the lowest concentration that inhibits the growth of microorganisms (n=3)

The antimicrobial activity of *H. pendula* has not been reported in the literature. In studies on different *Hesperis* species, it has been reported that the methanol extract of *Hesperis matronalis* subsp. *matronalis* (Current name: *Hesperis matronalis* L.) is effective against *Escherichia coli*, *Yersinia pseudotuberculosis, Klebsiella pneumonia* subp. *pneumonia*, *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis*, *Bacillus cereus, Mycobacterium smegmatis*, *Candida albicans* and *Saccharomyces cerevisiae* at different concentrations [25]. In another study, it was reported that the ethanol extract of *Hesperis matronalis* L. was effective against *Staphylococcus aureus* [26]. In our study, it was determined that ethanol extract of *H. pendula* showed higher activity than methanol extract. In addition, it was determined that ethanol extract of the plant inhibited the growth of *S. aureus, S. aureus* MRSA, *E. faecalis, A. baumannii and P. aeruginosa* at 100 µg/mL concentration. Also, it was determined that the ethanol extract of the plant inhibited the growth of *E. coli, C. albicans, C. glabrata* and *C. krusei* at 200 µg/mL concentration. It was determined that the methanol extract of the plant inhibited the growth of *S. aureus* and *P. aeruginosa* at 100 µg/mL concentration. In addition, it was determined that the methanol extract of the plant inhibited the growth of *S. aureus* MRSA, *E. faecalis, E. coli, A. baumannii* and *C. albicans* at 200 µg/mL concentration. It was found that the methanol extract of the plant inhibited the growth of *C. glabrata* and *C. krusei* at 400 µg/mL concentration. As a result, it was observed that the plant extracts prevented bacterial and fungal strains from forming colonies at 100-400 µg/mL

concentration. In this context, it was determined that the plant has antimicrobial potentials against standard bacterial and fungal strains.

3.2. Total phenolic and flavonoid contents

Plants may generate secondary metabolites that have a wide range of biological effects. Because of these characteristics, they are involved in a wide variety of biological processes [27]. We analysed H. pendula to calculate its total phenolic and flavonoid content. The results are shown in Table 2.

Table 2. Total Phenolic, flavonoid, antioxidant and oxidant values of *Hesperis pendula*

	Etanol	Methanol
TAS (mmol/L)	5.71±0.20	5.08±0.11
TOS (µmol/L)	21.65±0.24	18.69±0.21
OSI	0.380±0.017	0.368±0.011
TPC (mg/g)	116.8±2.5	104.6±2.5
TFC (mg/g)	83.8±3.0	93.6±2.2

Values are given as mean ± standard deviation. (n=3)

Total phenolic and flavonoid contents of *H. pendula* have not been reported in the literature. It was detected for the first time in our study. The experiments showed that the total phenolic content of the plant ethanol extract was higher (116.8±2.5). Methanol extract of the plant had a higher total flavonoid content (93.6±2.2). The total phenolic content of various *Hesperis isatidea* D.A. German & AlShehbaz is reported to be 109.66 mg/g, while the total flavonoid content is reported to be 15.42 mg/g [28]. We found that *H. pendula*, the species employed in this research, has higher total phenolic and flavonoid content than *H. isatidea*. Because of its high levels of both phenolic and flavonoid antioxidants, *H. pendula* has been identified as a potential natural source.

3.3. Antioxidant potential

As a normal byproduct of metabolic processes, living things constantly release free radicals. These free radicals are generally safe in small doses, but in higher concentrations they may damage cells [29]. When free radical levels grow, the antioxidant defence mechanism takes effect to bring them back down. The antioxidant defence system is designed to neutralise free radicals, however in certain situations their levels are too high for this to be effective [30]. When this happens, oxidative stress sets in. Multiple sclerosis, Alzheimer's disease, Parkinson's disease, cardiovascular disease, neurological illnesses, and cancer are all linked to oxidative stress in humans [31]. Antioxidant supplements may be used to mitigate or prevent oxidative stress. We investigated the antioxidant and oxidant levels of *H. pendula* to get a full picture of the plant's health. The potential of the plant as a source of natural antioxidants has been evaluated in this setting. Table 2 displays the data collected.

No study has been found in the literature to determine the TAS, TOS and OSI values of *H. pendula*. It was determined for the first time in our study. Antioxidant potentials of other *Hesperis* species such as *Hesperis matronalis*, *H. matronalis*, *H. isatidea* have been reported in the literature [25, 28, 32]. In our study, the antioxidant potential of *H. pendula* was determined. TAS, TOS and OSI values of various plant species have been reported in the literature. In these studies, TAS, TOS and OSI values of *Mentha longifolia* ssp. *longifolia* (current name: *Mentha longifolia* (L.) L.) (TAS: 3.628, TOS: 4.046, OSI: 0.112), *Rumex scutatus* L. (TAS: 8.656, TOS: 4.951, OSI: 0.057), *Rhus coriaria* var. *zebaria* Shahbaz (TAS: 7.342, TOS: 5.170, OSI: 0.071), *Allium calocephalum* Wendelbo (TAS: 5.853, TOS: 16.288, OSI: 0.278), *Helianthemum salicifolium* (L.) Mill. (TAS: 9.490 mmol/L, TOS: 14.839 µmol/L, OSI: 0.157) were reported [33-37]. Compared to these studies, the TAS values of the ethanol and methanol extracts of *H. pendula* used in our study were higher than for *M. longifolia* ssp. *longifolia* and lower than for *R. scutatus, R. coriaria* var. *zebaria*, *A. calocephalum* and *H. salicifolium*. TAS value is an indicator of all endogenous antioxidant compounds found in natural products [38]. In this context, it has been determined that *H. pendula* used in our study has antioxidant potential. TOS value is an indicator of all endogenous oxidant compounds produced within natural products. It is recommended to limit the consumption of natural products with high TOS values [38]. OSI value shows how much the oxidant compounds produced in natural products are suppressed by antioxidant compounds [38]. It was determined that the ethanol and methanol extracts of *H. pendula* used in our study had higher TOS and OSI values than extracts from *M. longifolia* ssp. *longifolia*, *R. scutatus, R. coriaria* var. *zebaria*, *A. calocephalum*, and *H. salicifolium*. In this context, although the antioxidant levels are high in the plant, the oxidative stress index was determined to be high due to the high oxidant levels. As a result, although the plant has antioxidant potential, it was determined that the plant has a high potential to produce oxidant compounds.

4. Conclusions

Total phenolic, flavonoid contents, antioxidant and oxidant potentials of *H. pendula* were determined in this research. The plant extracts were also tested for their ability to inhibit the growth of common bacterial and fungal species. The findings showed that the plant may have antioxidant potential. It has also been shown that plant extracts are effective against bacteria. As a result, it was determined that the plant has antioxidant and antimicrobial potential.

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