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ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *ALCHEMILLA PERSICA* ROTHM.

ALCHEMILLA PERSICA ROTHM. BİTKİSİNİN ANTİOKSİDAN AKTİVİTESİ VE FİTOKİMYASAL ANALİZİ

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ABSTRACT

In present study, the extracts prepared using aerial parts and roots of Alchemilla persica Rothm. were evaluated for their antioxidant activity by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and measurement of malondialdehyde (MDA) levels. HPLC analyses of the extracts were also performed using some phenolic acid and flavonoid standards. The hydro-methanolic extract of the aerial parts was found to possess significant antioxidant activity in both assays. IC_{50} values for the aerial parts and roots are determined as 0.055 M and 0.151 M respectively against DPPH radical. In TBARS assay, the MDA level of the aerial parts was found to be 5.9 nmol/ml, while 19.08 nmol/ml for the root extracts.

Key words: *Alchemilla persica, Rosaceae, Antioxidant activity, DPPH, MDA*

ÖZET

Bu çalışmada Alchemilla persica Rothm.'nın (Rosaceae) toprak üstü ve köklerinden hazırlanan ekstrelerin antioksidan aktivitesi 1,1-difenil-2-pikrilhidrazil (DPPH) radikal süpürücü etki ve malondialdehit

(MDA) seviyelerinin ölçülmesiyle tespit edilmiştir. Bazı fenolik asit ve flavonoit standartları kullanılarak ekstrelerin YBSK analizleri de gerçekleştirilmiştir. Toprak üstü kısımlarından hazırlanan ekstre belirgin bir antioksidan aktivite göstermiştir. DPPH radikaline karşı toprak üstü ve kök ekstreleri için IC_{50} değeri sırasıyla 0.055 M ve 0.151 M olarak belirlenmiştir. Tespit edilen MDA seviyesi toprak üstü ekstresi için 5.9 nmol/ml, iken kök ekstresi için 19.08 nmol/ml'dir..

Anahtar kelimeler: *Alchemilla persica, Rosaceae, Antioxidant activity, DPPH, MDA*

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INTRODUCTION

The genus *Alchemilla* L. is reported to comprise of more than one thousand species (1). According to the records of the Flora of Turkey fifty species of this genus are listed and these are distributed especially at the north-east Anatolia (2), but recently researches have yielded some more species so it is established that this genus is represented by seventy four species eight of which are endemic (3-4).

Alchemilla species is used as a folk medicine especially in north-east region of Turkey (4). According to the phytochemical studies on *Alchemilla* sp., it contains tannins, coumarins as well as flavonoids such as orientin, quercetin, quercitrin, isoquercetin, vitexin, rutin, hyperoside etc. (4-10).

A. vulgaris L. (Syn. *A. xanthochlora* Rothm.) is the most commonly used species and also listed in the European Pharmacopeia 6.0 (11). The medicinal part of the plant is aerial part with flowers. The use of this plant against mild or non-specific diarrhea is approved by Commission E (12). *A vulgaris* which is widely known as lady's mantle, bear's foot or lion's foot is traditionally used due to their tanin content for the treatment of inflammation of the upper digestive tract, diarrhoea internally and as wound healing and astringent externally (5, 13-14). Another use of *Alchemilla* species is for the adaptation to the hormonal levels of the body in case of menopause (13). It is also used as gargle against mouth and throat inflammation (12). *A. vulgaris* is reported to be an important remedy in folk medicine in Bulgaria. It is used to heal inflammations in mouth, bleeding of the nose, furuncules and gynecological diseases. This plant is also considered to regulate the glandular activity of uterine and reduce bleeding. Uses of the infusion prepared with this plant as astringent, antidiarrhetic, antiinflammatory and antisecptic are recorded (15-16). In Canada, it is reported that ruminants are fed with *A. vulgaris* against retained plasenta (17). Aerial parts of this plant are also used as a folk remedy in Montenegro, it is reported that the plant is used

internally to treat mild and nonspecific diarrhea, menopausal complaints and dysmenorrhea as well as ulcers, eczema and skin rashes externally (18). It is also used as antihemorrhagic, antidiarrheal and astringent in France (19). The study of Pawlaczyk et al. (20) have revealed the anticoagulant activity of *A. vulgaris* which is traditionally used of as an antiinflammatory, carminative and antidiarrheal remedy and also against gastiritis and burns in Poland. According to an *in vivo* study, the extract prepared from the aerial part of *A. vulgaris* and containing polyphenolic compounds is found to stimulate synthesis and pheripheral deiodination of thyroid hormones in rats which are subjected to intense cooling (21). The studies for the evaluation of antioxidant activity have shown that the hydro-alcoholic and methanolic extracts as well as the polar fractions of methanolic exracts prepared using aerial parts of *A. vulgaris* have posseses significant activity. This activity has been thought to arise from the phenolic compounds of the extract such as flavonoids and gallic acid (16, 22-26). The study of Ondrejovic et al. (27) showed the significantly higher antioxidant activity of methanolic extract of *A. vulgaris* in comparison with the extracts prepared using *n*-hexane, chloroform, ethylacetate and water as solvents. According to the study of Trouillas et al. (19), Water-soluble fractions of hyro-alcoholic extract prepared using *A. vulgaris* exhibited antiinflammatory and antiproliferative activity as well as antioxidant activity. Aerial part of *A. vulgaris* is also considered to exhibit antimicrobial activity due to its tannin content (28). Studies on the treatment of minor mouth ulcer have shown that a preparation which contains standard 3% extract of *Alchemilla vulgaris* in glycerine (Aphtarine®) exhibited a significant healing. *in vivo* studies have shown the wound healing activity of *A. vulgaris* and this activity is reported to be associated with promitotic activity in epithelial cells and myofibroblasts (29-30). *A. vulgaris* is also reported to show inhibitory activity of pancreatic lipase in the study of Slanc et al. (31).

The decoction prepared using *A. arvensis* (L.) Scop. is reported to be used as diuretic as well as the decoction of *A. vulgaris* leafs and shoots (32). The polar extract prepared using the aerial parts of *A. mollis* (Buser) Rothm. was found to possess free radical scavenging activity (9-33). *A. rizeensis* Pawl. (34) and *A. pedata* A. Rich. (8) are also reported to exhibit antimicrobial activity due to their tannin content. Besides, *A. pedata* which is traditionally used for wound healing in Ethiopia exhibited antiinflammatory activity (8). The *in vitro* study of Türk et al. (10) on *A., erythropoda* Juz*., A. ikizdereensis* Kalheber*, A. oriturcica* B. Pawl. and *A. trabzonica* Hayırlıglı-Ayaz et Beyazoğlu showed that these species had apoptotic and necrotic effects on HeLa cells. In the study of Nikolova et al. (35), *A. jumrukczalica* Pawl. which is an endemic species in Bulgaria showed significant antioxidant activity. In the same study, phenolic content of this species has also been investigated and the results has revealed that the antioxidant activity and phenolic content

were correlated. According to the ethnobotanical studies, infusion prepared from the leaves of *A. pseudocartalinica* Juz. is used as constipant, diuretic and tonic internally at the east Anatolia (36).

In present study, the extracts prepared using aerial parts and roots of *A. persica* were evaluated for their antioxidant activity by using DPPH free radical scavenging assay and measurement of MDA levels. HPLC analyses of the extracts were also performed.

EXPERIMENTAL

Plant Material

Plant material was collected from Erzurum-Kop Passage, Turkey. The taxonomic identification of these plants was confirmed by H. Duman, in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University. Voucher specimens were kept in the herbarium of Ankara University, Faculty of Pharmacy (AEF 25896).

Preparation of Extracts

Aerial parts and roots of the plant was seperated, than dried and powdered plant materials were extracted with methanol:water (80:20) mixture by continuous stirring at room temperature for 8 hours. After filtration, extract was concentrated to dryness under reduced pressure and low temperature (40-50 °C) on a rotary evaporator to give crude extract.

HPLC Analysis

HPLC analyses were carried out according to the method of Küpeli Akkol et al. (37). As described previously, this HPLC method was developed and validated to analyse phenolic acids; chlorogenic acid, caffeic acid, ferulic acid, rosmarinic acid, p-coumaric acid and flavonoids; apigenin, luteolin, quercetin, hyperoside, rutin, hesperidin.

DPPH Radical Scavenging Activity

DPPH scavenging activity tests were carried out according to the method of Brand Williams et al. (38) 0.01 g of sample was dissolved in 10 ml DMSO and seven different concentrations (1 mg/ml to 0.015 mg/ml) were prepared with ½ dilutions. 2.9 ml DPPH solution (10^{-4} M in ethanol) was added into 0.1 ml of sample solutions. The mixture was shaken vigorously and incubated 30 minute in 30°C water bath. Absorbance of the resulting solution was measured at 517 nm UV-visible spectrophotometer (Shimadzu). All the assays were carried out in triplicates with propylgallate as a positive control. Percentage of inhibition (DPPH scavenging activity) determined as follows.

% DPPH radical-scavenging = [(Absorbance of DPPH - Absorbance of sample) / Absorbance of DPPH] x 100

Decreased absorbance of the reaction mixture indicates stronger DPPH radical-scavenging activity. The IC_{50} value of the sample was calculated via linear regression analysis using % inhibition and concentration values.

TBARS Assay

The measurement of MDA levels was performed spectrophotometrically. The plant extracts were solved in dH_2O were incubated with 8.125 mM $CuSO_4$ solution. TCA (%0.1) and TBA (%0.67) solution was added after incubation and the absorbance at 532 nm were recorded. Quantitation of TBARS was performed by comparison with a standard curve of malondialdehyde equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetraetoxypropane and the result was expressed as nmol/ml.

RESULTS AND DISCUSSION

DPPH free radical scavenging activities of the *A. persica* root and aerial part extracts were determined by the measurement of the decrease in absorbance of DPPH after reduction at 515 nm. The extracts were found to exhibit DPPH free radical scavenging activity with IC_{50} values of 0.055 M and 0.151 M for the aerial parts and roots respectively. DPPH radical scavenging activities of the antioxidants are considered to be due to their hydrogen donating abilities. This method is a widely used method to evaluate antioxidant activities relatively short time compared to other methods (39-40).

TBARS assay is a method to evaluate lipid peroxidation. MDA formation is the result of oxidation and enzymatic degredation of polyunsaturated fatty acids. MDA, which is an indicator of lipid peroxidation reveals a reddish color with TBA, thus MDA level, is measures at 532 nm (41). In TBARS assay, the extract of aerial parts significantly reduced MDA level. The MDA level of the aerial parts was found to be 5.9 nmol/ml, where it is 19.08 nmol/ml for the root extracts.

Use of different chemicals, pesticides, pollutant, smoking, alcohol intake and even some synthetic medicine increase the risk of diseases due to free radicals (42). Plants produce a large amount of antioxidants and represent important sources of natural antioxidants (41). Therefore there has been a growing interest to identify antioxidant compounds from plant sources which are pharmacologically potent and have low or no side effects (42).

In current study antioxidant activities of *A. persica* aerial parts and roots were evaluated using two different methods. *A. persica* scavenged DPPH radical and decreased MDA level significantly. Activity of aerial part extract was determined higher than root extract in both test.

Generally phenolic content of the plant materials well correlated with their antioxidant activity (43). The relation between amount of phenolic compounds and antioxidant activity have found to be highly correlated according to the some literatures while the others have found no direct correlation or only a very weak one as the other substances including tocopherols and *β*-carotene raise the antioxidant activity (44). The results of the HPLC analyses showed that none of the tested phenolic acid and/or flavonoids were detected in *A. persica*. However according to the HPLC chromatograms and UV absorbances of the peaks it may be suggested that *A. persica* extracts may contain phenolic compounds. Thus the antioxidant activity of the extracts may be attributed to their phenolic content.

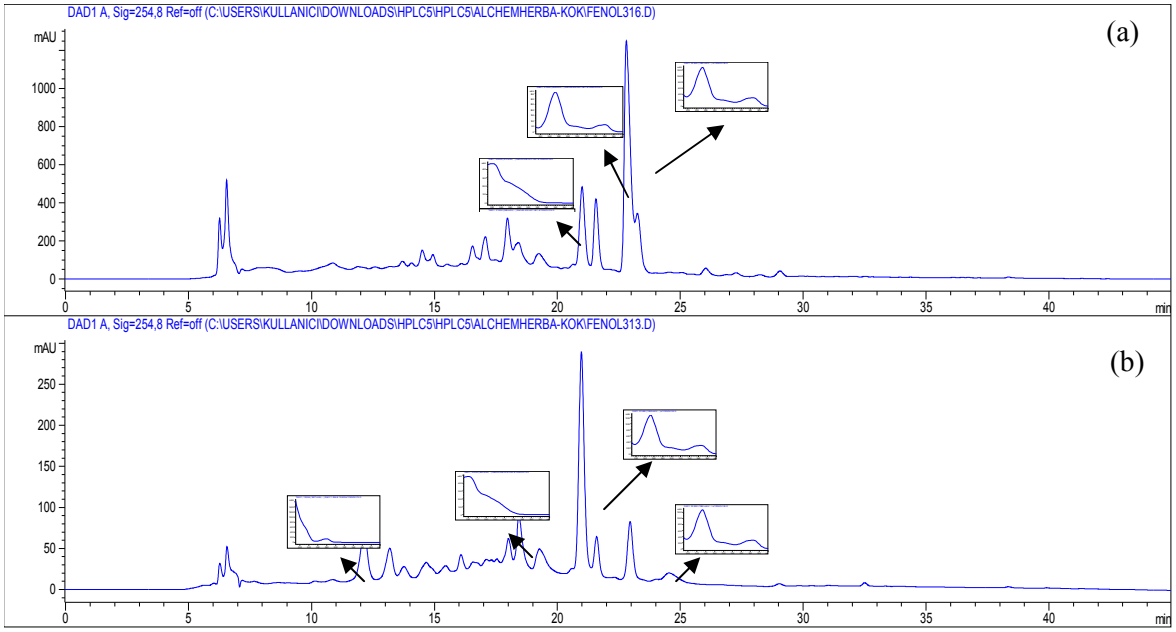

Figure 1. HPLC chromatogram of *A. persica* aerial part (a) and root (b) extract

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