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Original article

ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENT OF WATER AND ETHANOL EXTRACTS FROM *ACHILLEA MILLEFOLIUM* L.

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Abstract

Achillea millefolium (Asteraceae, yarrow) has been used in folk medicine against several disturbances including skin inflammations, spasmodic and gastrointestinal disorders. In this study, ethanol and water extracts were prepared from A. millefolium flowers, leaves and seeds. Antioxidant activities were measured by ferric thiocyanate method, and H_2O_2 radical scavenging activity assays and phenolic compounds and flavonoid contents of A. millefolium extracts were also determined. In conclusion, extracts of A. millefolium flowers, leaves and seeds had effective H_2O_2 radical scavenging activity, total antioxidant activity, and these antioxidant activities were compared with BHA and α-tocopherol as reference antioxidants.

Key words: *Achillea millefolium, Yarrow, Antioxidant, Total phenolic, Flavonoid*

Achillea millefolium L. Su ve Etanol Ekstrelerinin Antioksidan Aktivitesi, Total Fenolik ve Flavonoit İçeriği

Achillea millefolium (Asteraceae, civanperçemi) deri iltihaplanmaları, spazmodik ve gastrointestinal bozukluklar gibi çeşitli rahatsızlıklara karşı halk ilacı olarak kullanılmıştır. Bu çalışmada, etanol ve su ekstreleri A. millefolium çiçek, yaprak ve tohumlarından hazırlandı. Antioksidan aktivite, ferik tiyosiyanat metodu ve H_2O_2 radikal temizleme testleriyle ölçüldü ve A. millefolium ekstrelerinin fenolik bileşikleri ve flavonoit içerikleri saptandı. Sonuç olarak, A. millefolium çiçek, yaprak ve tohumları etkili H_2O_2 radikal temizleme aktivitesi ve lipid peroksidasyon inhibisyonu aktivitesi gösterdi ve bu antioksidan aktiviteler referans antioksidanlar BHA ve α-tokoferol ile karşılaştırıldı.

Anahtar kelimeler: *Achillea millefolium, Civanperçemi, Antioksidan, Total fenolik, Flavonoit*

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INTRODUCTION

Oxidative stress plays an important role in the development of aging process and some diseases such as cancer, neurodegenerative and cardiovascular diseases and diabetes in organisms (1). Dietary antioxidants protect the body against free radicals. There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relation to their nutritional incidence and their role in health and disease. Bioactive natural substances having the additive and synergistic effects in plant food are responsible for their potent antioxidant activities (2-6).

The genus *Achillea* (Asteraceae) is represented by about 85 species mostly found in Europe and Asia and a handful in North America (7). Forty species of *Achillea* are widely distributed in Turkey (8). As far as ethnopharmacologic background is concerned, *Achillea millefolium* is a well-known species amongst the members of *Achillea* (9). It is known as "civanperçemi" and used in folk remedies as an appetizer, wound healer, diuretic, carminative or menstrual regulator (10,11). Phenolic compounds, such as flavonoids and phenolcarbonic acids, constitute one of the most important groups of pharmacologically active principles in *Achillea millefolium* (yarrow). It is suggested that anti-inflammatory (12), antimicrobial (13), choleretic (14) and cytotoxic (15) activities of *Achillea* plants are mainly attributed to the flavonoid and phenolcarbonic acid complex (16).

The aim of this research is to determine flavonoid contents, phenolic compounds, H_2O_2 radical scavenging and inhibition of lipid peroxidation of water and ethanol extracts of *Achillea millefolium* of leaves, flowers and seeds.

EXPERIMENTAL

Plant materials and extraction procedures

Achillea millefolium (Asteraceae) leaves, flowers and seeds were obtained from Mus in Turkey. All samples were dried in air and at dark. For extraction (ethanol or water), 25 g sample of *A. millefolium* leaves, flowers and seeds into a fine powder in a mill and were mixed five times with 100 mL solvent. Extraction continued until the extraction solvents became colorless (total solvent volume 500 mL). The obtained extracts were filtered and the filtrate was collected, then solvent was removed by a rotary evaporator (17).

Hydrogen peroxide scavenging capacity

The ability of the *A. millefolium* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (18). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 μg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *A. millefolium* extracts and standard compounds were calculated:

$$\text{Scavenged } H_2O_2\ (\%) = [(A_C - A_S)/A_C] \times 100$$

where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample of *A. millefolium* extracts or standards.

Inhibition of lipid peroxidation-ferric thiocyanate method

The antioxidant activity of *A. millefolium* extracts and standards was determined according to the ferric thiocyanate method in linoleic acid emulsion (19). With this method peroxide formation occurred during the oxidation of linoleic acid oxidation. The latter ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm. The percent inhibition of lipid peroxidation in linoleic acid emulsion was calculated by the following equation:

Inhibition of lipid peroxidation (%) = A_C-A_S/A_C x 100

where A_C is the absorbance of the control reaction and A_S is the absorbance in the presence of the sample of *A. millefolium* extracts. In the control, the sample was replaced with an equal volume of ethanol.

Determination of total phenolic compounds

Total soluble phenolic compounds in the *A. millefolium* extracts were determined with Folin–Ciocalteu reagent according to the method of Slinkard & Singleton (20) using pyrocatechol and quercetin as a standard phenolic compound. Briefly, 1 mL of the *A. millefolium* extracts solution (contains 1000 µg extract) in a volumetric flask diluted with distilled water (46 mL). One milliliter of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min 3 mL of Na_2CO_3 (2%) was added and then was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The total concentration of phenolic compounds in the *A. millefolium* extracts determined as microgram of pyrocatechol and quercetin equivalent by using an equation that was obtained from standard pyrocatechol and quercetin graph.

Chromatographic conditions for flavonoid analysis

Chromatographic analysis was carried out using PREVAIL C 18 reversed-phase column (150x4.6 mm, 5 µm) diameter particles. The mobile phase was methanol/water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid (21). This mobile phase was filtered through a 0.45 µm membrane filter (millipore), then deaerated ultrasonically prior to use. Naringin, rutin, resveratrol, morin, myricetin, naringenin and kaempferol were quantified by DAD following RPHPLC separation at 280 nm for naringin, naringenin, 254 nm for rutin, morin, myricetin, 306 nm for resveratrol and 265 nm for kaempferol. Flow rate and injection volume were 1.05 mL/min and 10 µL, respectively. The chromatographic peaks of the analyses were confirmed by comparing their retention time and UV spectra with those of the reference standards. Quantification was carried out by the integration of the peak using the external standard method. All chromatographic operations were carried out at a temperature of 25 °C.

RESULTS AND DISCUSSION

Hydrogen peroxide scavenging capacity

The scavenging ability of water and ethanol extracts of *A. millefolium* on hydrogen peroxide is shown Table 1 and compared with BHA and α-tocopherol as standards. The *A. millefolium* extracts were capable of scavenging hydrogen peroxide in an amount dependent manner. 100 µg of water and ethanol extracts of *A. millefolium* exhibited 17.75-40.63 % scavenging activity on hydrogen peroxide. In the other hand, at the same dose, α-tocopherol and BHA exhibited 44.58 % and 39.26 % hydrogen peroxide scavenging activity. Those values close to α-tocopherol, but lower than that BHA. The hydrogen peroxide scavenging effect of 100 µg of the extracts of *A. millefolium* standards decreased in the order of α-tocopherol (44.58 %) > yarrow seed ethanol (40.63 %) > yarrow flower ethanol (40.57 %) > BHA (39.26 %) > yarrow leaf water (23.63 %)

> yarrow leaf ethanol (20.07 %) > yarrow flower water (18.19 %) > yarrow seed water (17.75 %). Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells (22). Thus, the removing of H_2O_2 is very important for antioxidant defence in cell or food systems.

Inhibition of lipid peroxidation

The inhibition of lipid peroxidation of *A. millefolium* extracts and the standard compounds was determined by the ferric thiocyanate method in a linoleic acid system. *A. millefolium* extracts had strong antioxidant activity. The effects of *A. millefolium* extracts on lipid peroxidation of linoleic acid emulsion are shown in Table 1. At the 100 µg/mL concentration, *A. millefolium* extracts exhibited 90.31-92.09 % lipid peroxidation of linoleic acid emulsion. On the other hand, at the same concentration, α-tocopherol showed 40.49 % inhibition of peroxidation of linoleic acid emulsion. The results clearly showed that *A. millefolium* extracts had more total antioxidant activity than α-tocopherol at the same concentration (100 µg/mL).

Table 1. Antioxidant activity results of *A. millefolium* extracts.

Extracts	**Inhibition of Lipid Peroxidation (%)** (100 µg/mL)	**H_2O_2 Scavenging Activity (%)** (100 µg/mL)
Flower water	91.53	18.19
Flower ethanol	90.31	40.57
Leaf water	91.43	23.63
Leaf ethanol	90.77	20.07
Seed water	92.09	17.75
Seed ethanol	91.89	40.63
BHA	nt	39.26
α-tocopherol	40.49	44.58

nt: not tested

Total phenolic compounds

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (23). According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in some plant species (24). 74, 134, 78, 128, 70 and 126 mg quercetin equivalent of total phenols (QETP) was detected in 1 g of dried weight of *A. millefolium* flower water, flower ethanol, leaf water, leaf ethanol, seed water and seed ethanol extracts (respectively). 18.82, 19.30, 20.25, 18.34, 19.78 and 18.82 mg pyrocatechol equivalent of total phenols (PETP) was detected in 1 g of dried weight of *A. millefolium* flower water, flower ethanol, leaf water, leaf ethanol, seed water and seed ethanol extracts (respectively) (Table 2).

Flavonoid contents

In this study, it was determined that flavonoid contents of *A. millefolium* leaves extracts were higher than *A. millefolium* flower extracts. It was observed that in both flower and leaf extracts the highest flavonoid is naringin. Flavonoid contents of *A. millefolium* extracts are shown in Table 3. Rutin, resveratrol, morin, naringin, naringenin, myricetin, quercetin and kaempferol were determined in the *A. millefolium* flower and leaf extracts. 52, 24, 2, 54, 529, 12 and 673 µg rutin, resveratrol, morin, myricetin, naringin, naringenin and total (respectively) flavonoid were detected in 1 g of extracts of *A. millefolium* flower. 979, 53, 1797, 11 and 2840 µg rutin, resveratrol, naringin, quercetin and total (respectively) flavonoid were detected in 1 g of extracts of *A. millefolium* leaves.

Table 2. Total phenolic compounds of *A. millefolium* extracts (mg/g DW).

Extracts (1 g)	QETP (mg)	PETP (mg)
Leaf water	78	20.25
Leaf ethanol	128	18.34
Flower water	74	18.82
Flower ethanol	134	19.30
Seed water	70	19.78
Seed ethanol	126	18.82

Table 3. Flavonoids content in *A. millefolium* water extracts (μg/g).

Flavonoids	Flower Water Extract	Leaves Water Extract
Rutin	52	979
Resveratrol	24	53
Morin	2	Trace
Myricetin	54	Trace
Naringin	529	1797
Naringenin	12	Trace
Kaempferol	Trace	Trace
Quercetin	Trace	11
Total	673	2840

Keser et al. (17) have studied water and ethanol extracts of flowers, leaves and seeds of *A. millefolium* by DPPH, ABTS, superoxide radical scavenging and metal chelating activities. In their study, it was observed that ABTS radical scavenging activity is the highest in flower ethanol extract (97.40 %), the lowest in the seed water extract (55.76 %); DPPH radical scavenging activity is the highest in flower ethanol extract (91.03 %), the lowest in the seed ethanol extract (79.94 %); superoxide radical scavenging activity is the highest in the seed water extract (90.67 %), the lowest in the seed ethanol extract (40.00 %); metal chelating activity is the highest in the seed water extract (65.76 %), the lowest in the seed ethanol extract (22.64 %). Our study and their study are shown similar results. Because, in our study, it was observed that H_2O_2 radical scavenging activity is the highest in seed ethanol extract (40.63 %), the lowest in seed water extract (17.75 %); inhibition of lipid peroxidation is the highest in the seed water extract (92.09 %), the lowest in flower ethanol extract (90.31 %).

Adam et al. (25) have reported DPPH radical scavenging activity and total phenolic compounds (as quercetin) of *A. millefolium* leaf of water/acetonitrile (70/30) extracts. These researchers have determined DPPH radical scavenging activity 17.82-18.31 %; total phenolic compounds 58-64.5 mg quercetin/100 gram leaf. Total phenolic compounds results are lower than our study results. Because, in our study, it was determined that total phenolic compounds of *A. millefolium* leaf water extract is 78 μg quercetin/g, leaf ethanol extract is 128 μg quercetin/g.

Candan et al. (11) have investigated antioxidant activity and monoterpens of methanol extracts and essential oils of *A. millefolium*. In their study, it was observed that DPPH and superoxide radical scavenging activities of methanol extracts are lower than essential oils. They have reported that monoterpens are 24.6 % eucalyptol, 16.7 % camphor, 10.2 % α-terpineol, 4.2 β-pinene and 4 % borneol.

Trumbeckaite et al. (16) have studied water/ethanol (60/40) extracts of *A. millefolium*. In their research, DPPH radical scavenging activity was determined by HPLC and they investigated inhibition of H_2O_2 generation in rat heart mitochondria. According to study results, *A. millefolium* extracts are shown to possess 308.8 μmol/g trolox equivalent DPPH radical scavenging activity and inhibition of H_2O_2 generation as 45% in rat heart mitochondria. In our study, H_2O_2 radical scavenging activity is higher in flower ethanol extract (40.57%) and seed ethanol extract (40.63 %) than other extracts.

As a conclusion, the water and ethanol extracts of *A. millefolium* showed hydrogen peroxide scavenging and strong inhibition of lipid peroxidation activities when compared to standards such as BHA and α-tocopherol. The results of this study showed that the water and ethanol extract of *A. millefolium* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.

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