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In Vitro Antioxidant Capacity of Acetone Extracts from Leaves and Flowers of *Achillea grandifolia*

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ABSTRACT

The aim of this study was to examine for the first time the antioxidant capacity of acetone extracts from Achillea grandifolia flowers and leaves using cupric reducing antioxidant capacity (CUPRAC), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity methods. Also, total phenolic contents of acetone extracts were determined as catechin equivalent. The acetone extract from A. grandifolia flowers showed higher amount of total phenolic contents (12.78 ± 0.60 µg CE 100 µg extract^{-1}) than leaves extract (5.50 ± 0.14 µg CE 100 µg extract^{-1}). The flowers extract exhibited stronger DPPH radical scavenging activity (IC_{50} = 0.23 ± 0.02 mg/mL) than BHT (IC_{50} = 0.37 ± 0.01 mg/mL) and leaves extract (IC_{50} = 0.97 ± 0.02 mg mL^{-1}). Therefore, the flowers extract of A. grandifolia may be a worthy natural antioxidant source and to be applicable in both medicine and the food industry.

Keywords: Antioxidant capacity, *A. grandifolia*, CUPRAC, DPPH.

INTRODUCTION

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural [1]. Synthetic antioxidants such as BHA (butylated hydroxyanisole) or BHT (butylated hydroxytoluene) have been widely used for many years to preserve freshness, flavour, and colour of foods and animal feeds as well as to improve the stability of pharmaceuticals and cosmetics. But, currently used synthetic antioxidants have been suspected to cause negative health effects. Some experimental studies have reported that both BHA and BHT have tumour-promoting activity [2]. Hence, there is a trend to substitute them with naturally occurring antioxidants. Besides the well-known and traditionally used natural antioxidants from tea, fruits, vegetables and spices, many other plant species have been investigated in the search for novel antioxidant [3].

Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids and some other endogenous metabolites, which are rich in antioxidant activity [4].

The genus *Achillea* (family: Asteraceae) is represented by about 85 species mostly found in Europe and Asia and a handful in North America. Forty species of *Achillea* are widely distributed in Turkey [5]. The aerial parts of different species of the genus *Achillea* L. (Asteraceae) are widely used in folk medicine due to pharmacological properties, such as anti-inflammatory, antioxidant, antispasmodic, antihemorrhoidal, stomatiche, antiseptic and emmenagogue [6]. *A. grandifolia* is a flowering plant in the family Asteraceae and distributed Balkan Peninsula and Turkey. To the best of our knowledge, information concerning the *in vitro* antioxidant features of *A. grandifolia* has not been found in the literature.

The aim of this study was to evaluate for the first time the antioxidant effects of acetone extracts from flowers and leaves using various antioxidant capacity methods, including total phenolic compounds contents by Folin-Ciocalteu Reagent (FCR), DPPH free radical scavenging activity and cupric reducing antioxidant capacity (CUPRAC).

MATERIALS AND METHODS

Plant material: *A. grandifolia* was collected from Kartepe (altitude of 1520-1625 m) in Kocaeli, Turkey, in May 2011. The plant was authenticated by Prof. Ertan Tuzlacı. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy (MARE 14583), Marmara University, Istanbul, Turkey.

Preparation of the extracts: The fresh plant materials were separated into flowers and leaves. The plant material was dried in shadow and then ground to small pieces using a grinder. Each of the dried plant samples (1.0 g) were Soxhlet extracted with acetone at controlled temperature. The collected extracts were concentrated under reduced pressure below 45 °C using rotary evaporator ensuring complete removal of the solvent. Dried extracts of the samples kept at 4–6 °C until used.

Determination of total phenolic compounds: The amount of total phenolic compounds in the *A. grandifolia* extracts were determined according to the colorimetric assay including a blue molybdenum-tungsten complex in the presence of phenolics by using Folin-Ciocalteu Reagent (FCR) [7]. 0.1 mL of extract solution was diluted with distilled water (4.6 mL) and 0.1 mL of FCR (diluted 1:3, v/v) was added. Then, 3 mL of Na_2CO_3 (2.0%) was added and the mixture was left standing at ambient temperature for 2 h. The absorbance value was read at 760 nm against a reagent blank. The total phenolic content was determined using a standard curve with catechin (5.0 - 50 μg mL^{-1}) as the standard. Total phenolic content is expressed as μg of catechin equivalents (CE) 100 μg of $extract^{-1}$. All the determinations were carried out three times. The calibration equation for catechin was absorbance = 0.0081 CE (μg) – 0.0042 (R^2= 0.9993).

DPPH free radical scavenging activity assay: The antioxidant activity of plant was tested by the DPPH (2,2'-diphenyl-1-picrylhydrazyl) free radical scavenging method [9]. Stock solution (5.0 mg mL^{-1}) of the acetone extracts of the flowers and leaves of *A. grandifolia* was prepared in pure ethanol from which serial dilutions were carried out to obtain the concentrations 0.1 - 0.8 mg mL^{-1}. In this assay, 4 mL of 0.1 mM ethanolic DPPH solution was added to 1 mL of extract solution. The mixture were shaken in a vortex for 10 seconds and then placed in a dark place. The decrease in absorbance at 517 nm was determined using spectrophotometer after 30 min for all samples. The percentage of DPPH free radical-scavenging activity of plant extracts was calculated as:

$$\text{DPPH free-radical scavenging activity (I \%)} = [(A_0 - A) / A_0] \times 100$$

Where, A_0 is the absorbance of the control solution (containing all reagents except plant extracts); A is the absorbance of the DPPH solution containing plant extracts. The parameter IC_{50} value is used for the explication of the results from the DPPH. It is defined as the concentration of substrate that causes 50% loss of the DPPH activity. The IC_{50} value of the extracts was estimated using Graphpad Prism 5 Demo. All

determinations were performed in triplicate. Ascorbic acid and BHT were used as positive control standard.

Cupric reducing antioxidant capacity (CUPRAC) assay: Cupric reducing antioxidant capacity (CUPRAC) method were used for evaluate the antioxidant capacity of plant extracts [10]. 1 mL each of Cu(II) (1.10^{-2} M), neocuproine ethanolic solution ($7.3.10^{-3}$ M) and 1 M NH_4Ac buffer solution were mixed in a test tube. Extracts (x mL) and pure EtOH ((1.1 − x) mL) were added to the initial mixture so as to make the final volume: 4.1 mL. The mixture was vortexed for 10 sec. and absorbance measurement was performed exactly after 30 min at 450 nm against a reagent blank. The calibration curve was prepared with trolox (25 – 250 μmol L^{-1}). The results are expressed as the total antioxidant capacity in μM trolox of different concentration of the extracts.

RESULTS AND DISCUSSION

Determination of total phenolic content: The total phenolic compounds in the extracts were determined from the regression equation of calibration curve (Y = 0.0081x – 0.0042 (R^2 = 0.9993) and expressed in catechin equivalents (CE). The total phenolic compounds in the flowers and leaves extracts showed the following order: flowers extract (12.78 ± 0.60 μg CE 100 μg $extract^{-1}$)> leaves extract (5.50 ± 0.14 μg CE 100 μg $extract^{-1}$). According to obtained results, the flowers extract of plant showed higher amount of total phenolic compounds than leaves extract.

DPPH free radical scavenging activity assay: The free radical scavenging capacity of acetone extracts of the plant was measured by DPPH assay. Butylated hydroxytoluene and ascorbic acid were used as standards. The DPPH radical scavenging activity of acetone extracts from the *A.grandifolia* flowers and leaves are shown figure 1. The DPPH radical scavenging capacity of the acetone extracts and standards showed the following order: ascorbic acid (IC_{50} = 0.13 ± 0.01 mg mL^{-1})> flowers extract (IC_{50} = 0.23 ± 0.02 mg mL^{-1})> BHT (IC_{50} = 0.37 ± 0.01 mg mL^{-1})> leaves extract (IC_{50} = 0.97 ± 0.02 mg mL^{-1}). The acetone extract from the *A.grandifolia* flowers showed higher free radical scavenging activity than BHT and leaves extract.

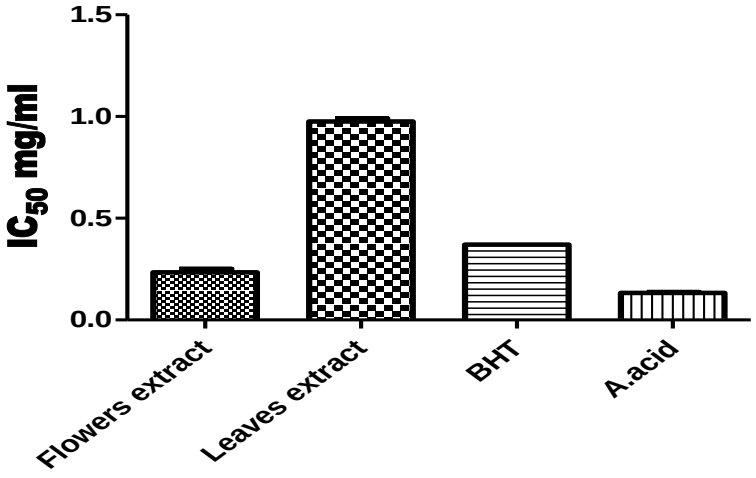

Fig.1 IC_{50} values of extracts and standards

Cupric reducing antioxidant capacity (CUPRAC) assay: The cupric reducing antioxidant capacity of acetone extracts of the plant was measured by CUPRAC assay. The cupric reducing antioxidant capacity

of acetone extracts from *A.grandifolia* flowers and leaves were expressed as µM trolox and are shown table 1.

Table1. CUPRAC values (µM trolox) of flowers and leaves extracts

	CUPRAC values (µM trolox)		
Extracts	0.4 mg mL^{-1}	0.2 mg mL^{-1}	0.1 mg mL^{-1}
Flowers extract	0.500 ± 0.003	0.271 ± 0.013	0.151 ± 0.003
Leaves extract	0.179 ± 0.004	0.096 ± 0.002	0.046 ± 0.004

The acetone extract from the *A.grandifolia* flowers showed higher cupric reducing antioxidant capacity than leaves extract throughout all the concentration.

To the best of our belief, there have been no studies of the antioxidant effects of acetone extracts from *A. grandifolia* flowers and leaves. Hence, in the present studies the flowers and leaves extract from *A. grandifolia* were tested with various antioxidant capacity methods. Preliminary phytochemical screening of acetone extract showed the existence of flavonoids and phenolic acids. In further studies, this phenolic antioxidant compounds will be characterized by Q-TOF LC/MS system from this extracts.

APPLICATIONS

The acetone extract from *A. grandifolia* flowers is applicable in both medicine and the food industry as a natural antioxidant source.

CONCLUSIONS

The flowers extract showed higher DPPH radical scavenging, cupric reducing antioxidant capacity and amount of total phenolic compounds than the leaves extract. Also, this extract exhibited stronger free radical scavenging activity than BHT. In addition, in this study, the significant linear correlation was confirmed between the values of the total phenolic contents with antioxidant capacity of plant extracts. As a result of this study, the flowers extract from *A. grandifolia* may be a worthy natural antioxidant source and to be applicable in both medicine and the food industry.

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