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Antioxidant Property of *Centaurea solstitialis* L. from Konya, Turkey

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In this study, *Centaurea solstitialis* L. was extracted with petroleum ether using a Soxhlet apparatus. The antioxidant properties of plant extract and synthetic antioxidant compounds were evaluated using different antioxidant tests, including reducing power, DPPH free radical scavenging activity and total phenolic compound. Results were compared with standard butylated hydroxytoluene and butylated hydroxyanisole.

Key Words: *Centaurea soltitialis*, Free radical, DPPH, Folin-Ciocalteu, Reducing power.

INTRODUCTION

Antioxidants are of great importance in terms of preventing oxidative stress that may cause several degenerative diseases[1]. Scientific evidence suggests that antioxidants reduce risk for chronic diseases including cancer and heart disease and many plants are potentially useful for these risks[2-4]. These protective effects have been paticularly attributed to various antioxidant compounds, such as polyphenolic compound, vitamin C and E, β-carotene[5]. *Centaurea* species are medical herb from *Asteraceae* family and 168 species of it are available in Turkey. This herb is known with its Turkish names such as peygamber çiçegi, zerdali dikeni, çoban kaldiran and Timur dikeni. *Centaurea* species are known for their antidiabetic, antidiarrhetic, antirheumatic, antiinflammatory, colagog, choleretic, digestive, stomachic, diuretic, menstrual, astringent, hypotensive, antipyretic, sitotoxic, antibacterial effects by public medicals and are used single or mixed[6]. The present study was undertaken to perform the screening of antioxidant properties of *C. solstitialis*. Taking this into account, the *in vitro* antioxidant activity of the methanol extracts tested, compared to that of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) were assessed by three different tests; total phenolic concentration[7], DPPH free radical scavenging activitiy[8] and reducing power[9].

EXPERIMENTAL

DPPH, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), methanol, Folin-Ciocalteu reagent, potassium ferricyanide trichloroacetic acid, (Sigma-Aldrich), Na_2CO_3, gallic acid, phosphate buffer (Merck).

C. solstitialis L. was collected in central of Konya. The identification and classification of plant was carried out by Dr. Tuna Uysal and all specimens were deposited at the laboratory of Department of Biology, Selcuk University, Konya, Turkey.

Preparation of the methanol extracts: The air-dried and finely ground samples were extracted by using the method described elsewhere[10]. Briefly, the sample, weighing about 100 g, was extracted in a Soxhlet apparatus with methanol at 60 ºC for 6 h. The extract was then filtered and concentrated *in vacuo* at 45 ºC. Finally, the extracts were then lyophilized and kept in the dark at 4 ºC until tested.

Free radical scavenging method: The antioxidant activity of plant extracts was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, $DPPH^{\bullet}$. A methanolic solution of sample of various concentrations was placed in a cuvette and 4 mL of 6×10^{-5} mol/L methanolic solution of DPPH was added. After a 0.5 h incubation period at room temperature, the absorbance was read against a blank at 515 nm. The same procedure was repeated with synthetic antioxidant, BHT and BHA, as positive control and a blank. Inhibition of the free radical $DPPH^{\bullet}$ in per cent (I %) was calculated as follows:

$$I\ \% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate and BHT and BHA were used as positive controls.

Total phenolic compuond assay: The amounts of phenolics in the plant extracts were determined with Folin-Ciocalteu reagent using the method of to 50 mL of each sample, 2.5 mL of 10 % dilution of Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 (7.5 %, w/v) were added and the resulting mixture was incubated at 45 ºC for 15 min. The absorbance of all samples was measured at 765 nm. Gallic acid was used as a standard for the calibration curve. The total amount of phenolic compounds was calculated and expressed as milligrams of gallic acid equivalent.

Reducing power: The reducing power of plant extract was determined by the method of Oyaizu[9]. Different concentrations of *C. solstitialis* extracts in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M,

pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1 %). The mixture was incubated at 50 ºC for 20 min. Aliquots (2.5 mL) of trichloroacetic acid (10 %) were added to the mixture, which was then centrifugated for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

RESULTS AND DISCUSSION

Antioxidant activity of plant extracts according to the DPPH$^{\bullet}$ radical scavenging method. The results showed that the decrease in absorbance of the DPPH$^{\bullet}$ radical was due to its reduction by different antioxidants (Fig. 1). Absorbance decreases as a result of a colour change from purple to yellow as the radical was scavenged by antioxidant through donation of hydrogen to form the stable DPPH-H. Solution was bleached with all the samples tested. However, differences could be observed through different antioxidants used and their concentrations.

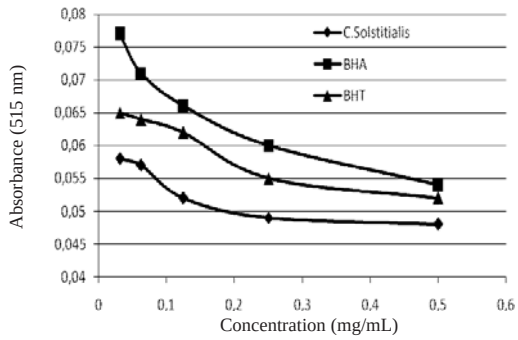

Fig. 1. Reduction of absorbance of plant and controls

Free radical-scavenging capacities of the corresponding extracts were measured by DPPH assay and the results are shown in Fig. 2.

Total phenolic compound assay was measured by Folin-Ciocalteu method[8]. The Folin-Ciocalteu reagent assay was used to determine the total phenolics content. Phenolic contents of plants and standart antioxidant compounds are correlated with the antioxidant activity, as shown in Fig. 3.

Fig. 4 shows the reductive capabilities of plant extract compared with BHA and BHT. For the measurements of the reductive ability, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of *C. solsititalis* extract using the method of Oyaizu[9].

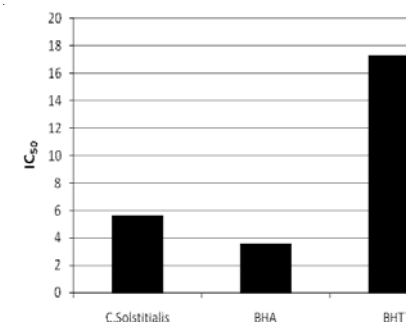

Fig. 2. Value of IC_{50} of plant and controls

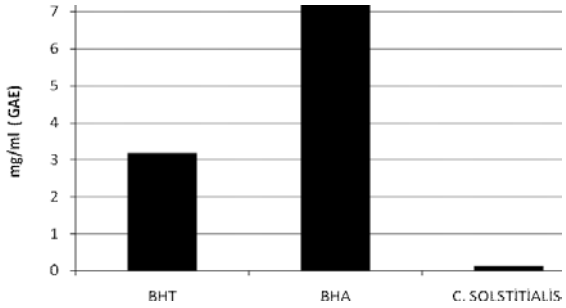

Fig. 3. Total phenolic compound in plant and controls (equivalent mg gallic acid)

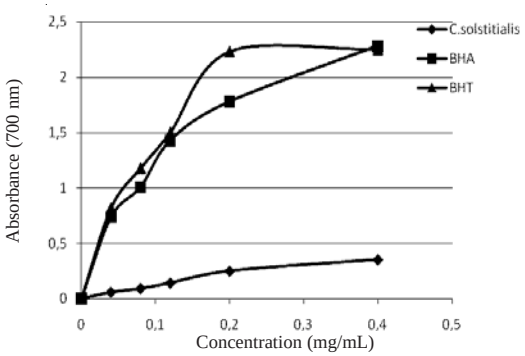

Fig. 4. Reducing Power of *C. solstitalis* and controls

All of the materials in this research exhibited different extent of antioxidant activity. *C. solsititialis* extract showed a higher potency than BHT and lower than BHA in scavenging of DPPH free radical. This may be related to the high amount of flavonoid and phenolic compounds in this

plant extract. The key role of phenolic compounds as scavengers of free radical is emphasized in several reports[11]. The amount of the total phenolics is highest in BHA and phenolic content of plant extract is lower than the others. But according to IC_{50} value and reducing power graphics, *C. solsititalis* exhibited the greatest antioxidant activity. Because lower IC_{50} value indicates higher antioxidant activity[12] and the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

Statistical analysis: All results were obtained in triplicate and data were presented as mean ± standard deviation of three determinations (data were not shown). Statistical analyses were performed using a one-way analysis of variance[13].

Conclusion

The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[14]. As a result present study show that the *C. solstitialis* can be used as accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.

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