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The Antioxidant Activity of the Leaves of *Cydonia vulgaris*

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Abstract : The antioxidant activities of water, ethanol, and ether extracts of the leaves of *Cydonia vulgaris* Pers. were determined by the thiocyanate method. The antioxidant activity of water extract was increased with the increasing amount of extract (200 µg-1000µg) added to the linoleic acid emulsion. Ether extract was the most effective antioxidant among the extracts. Like antioxidant activity, the reducing power of water extract was concentration dependent. However, ethanol extract was the highest in reducing power, and ether extract was the lowest. The results obtained in the present study indicate that the leaves of *Cydonia vulgaris* are a potential source of natural antioxidants. In addition, we could suggest that although the reducing power of a substance may be an indicator of its potential antioxidant activity, there is not necessarily a linear correlation between these two activities.

Key Words: Antioxidant activity, reducing power, *Cydonia vulgaris*

Introduction

Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O_2^-) and hydroxyl radicals (OH'), as well as non-free-radical species such as hydrogen peroxide (H_2O_2) (1,2). In living organisms various ROSs can form in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionising radiation, certain pollutants, organic solvents, and pesticides (3-5). Free radicals can cause lipid peroxidation in foods, which leads to their deterioration (6,7). In addition, reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer (8-11). When produced in excess, ROSs can cause tissue injury. However, tissue injury can itself cause ROS generation (12). Nevertheless, all aerobic organisms, including human beings, have antioxidant defences that protect against oxidative damages, and numerous damage removal and repair enzymes to remove or repair damaged molecules (4,13-15). However, this natural antioxidant mechanism can be inefficient, and hence dietary intake of antioxidant compounds is important (11,16,17). There are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. However, it has been suggested that these compounds have some side effects (18,19). In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease (20). Therefore, research for the determination of the natural antioxidants source is important.

The quince (*Cydonia vulgaris* Pers.) has been cultivated from prehistoric periods in countries extending from Iran to India. The ancient Greeks and Romans grew the quince for its attractive pinkish flowers and fragrant fruit (21,22). It is cultivated throughout Turkey but especially in western Anatolia (23). The quince is a low spreading type tree which attains a height of up to 8 m; twigs sparsely tomentose when young, becoming glabrous. Leaves ovate to oblong or occasionally suborbicular, up to 10x7 cm, entire, bilaterally white-tomentose at first, becoming glabrous above and densely villous beneath, petiole 1-2 cm. Flowers 4-6 cm diam,

sepals glandular, toothed, reflexed. Fruit pear-shaped or subglobose, (3-)-5-12 cm, yellowish, fragrant (3). Some cultivars are Pineapple, Orange, Van Deman, Champion, Rea, Meech, Angers, and Smyrna (21, 24).

It has been reported that the leaves and fruits of the quince have some positive effects in the medical treatment of various conditions including cardiovascular diseases, haemorrhoids, bronchial asthma, and cough (25-27). In addition, the leaves of *Cydonia vulgaris* Pers. have been reported to have a tranquilizing effect (28). Nevertheless, there is as yet no report concerning the antioxidant effect of this plant. Our main objective is the determination of potential natural antioxidant sources. However, the purpose of this particular study is the determination of antioxidant activities of various extracts of *Cydonia vulgaris* Pers. As some effects of this plant have been reported, this plant was chosen.

Materials and Methods

Preparation of Extracts

Leaves of *Cydonia vulgaris* Pers. collected in Muğla, Turkey, and were left on a bench to dry. A 15-gram dried sample was chopped into small parts in a blender and then extracted with 450 mL of boiled water by stirring for 30 min followed by filtration. Afterwards, the filtrate was freeze-dried.

Ethanol extract was obtained as follows: 15g of dried and chopped leaves was extracted with 450 ml ethanol by stirring for 6 hours. In the ether extraction, the same amount of sample was extracted with ether in a soxhlet apparatus until extraction solvents become colourless. Both of the extractions were followed by filtration and evaporation of the filtrate to dryness in vacuum.

Antioxidant activity

Antioxidant activity was determined by the thiocyanate method. Each sample (containing 200-1000µg extract) in 0.5 mL of distilled water was mixed with 2.5 mL of linoleic acid (Sigma) emulsion (0.02M, in 0.04M pH 7.0 phosphate buffer) and 2 mL phosphate buffer (0.04M, pH 7.0) in a test tube and incubated in darkness at 37°C. The amount of peroxide was determined by reading absorbance at 500 nm after colouring with $FeCl_2$ and thiocyanate at intervals during incubation (29). α-Tocopherol (Sigma) was used as standard antioxidant.

Reducing Power

Extracts (100-1000 µg) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2M, pH 6.6) and 2.5 mL potassium ferricyanide [$K_3Fe(CN)_6$] (1%), and then the mixture was incubated at 50°C for 30 min. Afterwards, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of upper layer solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm (29). Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical calculations were done by using Statistica for Windows 4.3 and SPSS 9.0. Values of $p<0.05$ were considered to be significant and values of $p<0.01$ very significant.

Results and Discussion

In the present study, antioxidant activity was determined by the thiocyanate method in that the amount of peroxides formed in emulsion during incubation was determined spectrophotometrically by measuring absorbance at 500 nm. High absorbance is an indication of high concentrations of formed peroxides.

The antioxidant activity of water extract of *Cydonia vulgaris* leaves increased with an increasing amount of extract. A similar property was determined with ether or ethanol extract (data not shown). As can be seen in Figure 1, there is no clear difference between the control and the sample containing 200 µg extract. However, peroxidation is suppressed about 6 hours in the presence of 400 µg or 500 µg extract, and after that it begins to increase. In the presence of 1000 µg extract or 500 µg α-tocopherol, the peroxidation process is delayed for about 12 hours.

In order to determine the statistical significance of the above results, SPSS 9.0 software was used. After two-way variance analysis, which showed that there was a statistically significant difference (P=0.000), multiple comparison was carried out by LSD. There were statistically highly significant differences between the control and 1000 µg extract or control and 500 µg α-tocopherol (P=0.000 for both). It was also interesting to determine that 1000 µg extract or and 500 µg α-tocopherol were significantly different from 500 µg

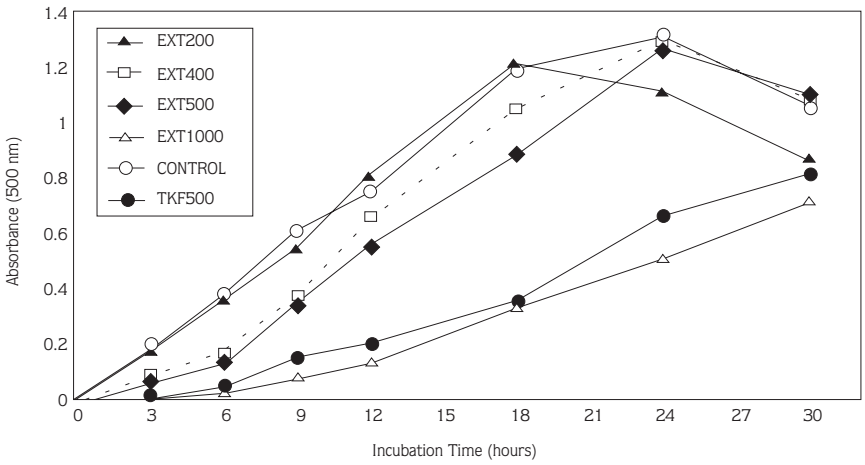

Figure 1. Antioxidant activity of lyophilized water extracts of the leaves of *Cydonia vulgaris* (Numbers following ext indicates the µg of extract added to the emulsion and tkf500 = 500 µg α-tocopherol).

extract (P=0.000 and P=0.002 respectively). However, the difference between 1000 µg extract and 500 µg α-tocopherol was not significant (P=0.503).

Ethanol extract had higher activity than water extract, but the difference was not statistically significant (P=0.883). Although they were able to suppress oxidation for about 6 hours, their antioxidant activities were not statistically different from that of the control (P=0.606, for control and water extract; P=0.507 for control and ethanol extract). Nevertheless, the most effective antioxidant activity was shown by ether extract (Figure 2) (P=0.000 for control and ether extract). It was also interesting to find that ether extract had even higher antioxidant activity than α-tocopherol (P=0.006). Hence it was able to delay peroxidation for 30 hours.

Like antioxidant activity, the reducing power of water extract was also concentration dependent. Hence the reducing power of extract is increased as amount of extract increased (Figure 3). Even in the presence of 100 µg extract, the reducing power was significantly higher than that of the control (p=0.02), in which there was no extract.

Unlike antioxidant activity, the reducing power of ether extract was the lowest one. However, even this extract had significant reducing power activity (p=0.04 between the control and ether extract). Among the extracts, ethanol extract had the highest reducing power activity (Figure 4). Although α-tocopherol was more effective than ethanol extract, this difference was not statistically significant (p=0.074).

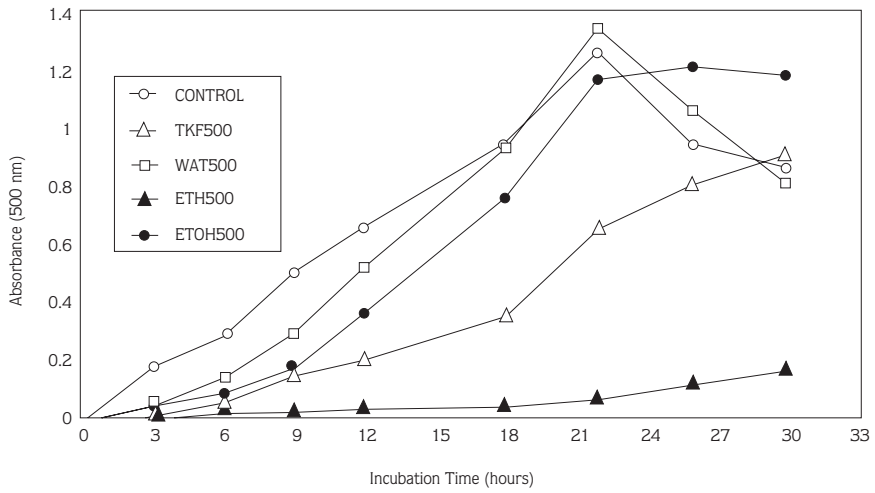

Figure 2. Antioxidant activities of dried ether, ethanol extracts, and lyophilized water extract of the leaves of *Cydonia vulgaris*. In each there was 500 µg of indicated dried extract or α-tocopherol while in the control there was no extract. (tkf=α-tocopherol; wat=water; eth=ether; etoh=ethanol).

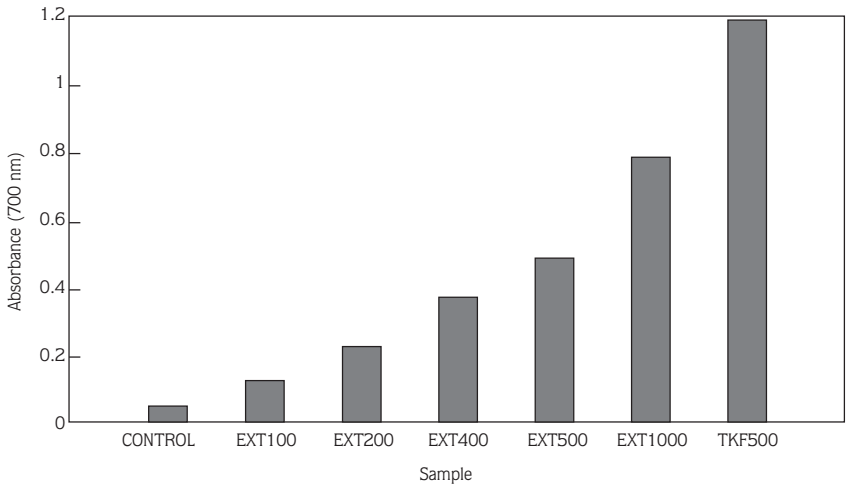

Figure 3. Reducing power of lyophilized water extract of the leaves of *Cydonia vulgaris* (Numbers following ext indicates the µg of extract added to the emulsion and tkf500 = 500 µg α-tocopherol).

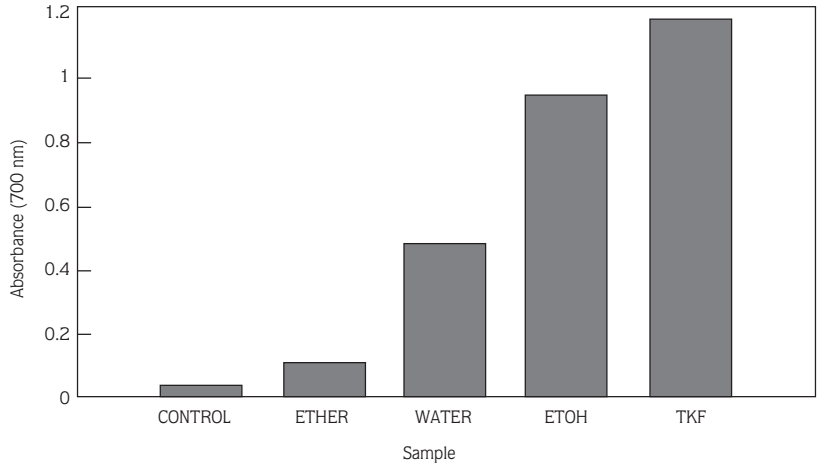

Figure 4. Reducing power of dried ether extract, ethanol extract, and lyophilized water extract of the leaves of *Cydonia vulgaris*. In the each of that there was 500 µg of indicated dried extract or α-tocopherol (TKF) while in the control there was no extract.

It was interesting to find that although ether extract had the highest antioxidant activity, it was less effective in reducing power. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (30). However, the antioxidant activities 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, and radical scavenging (31). Hence we can suggest that there is always no linear correlation between total antioxidant activity and reducing power activity. Thus, although ether extract has low reducing power, it could have high total antioxidant activity.

The present study suggests that the leaves of *Cydonia vulgaris* Pers. might be a potential source of natural antioxidant.

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