

**THE ANTIOXIDANT ACTIVITY OF SELECTED FRUITS CULTIVATED
IN CANAKKALE, TURKEY**

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Abstract

The aim of this study was to evaluate the antioxidant capacity, total phenolics and flavonoids of selected fruits (apricot, cherry and sour cherry) cultivated in the region of Canakkale, Turkey. For this purpose the methanolic extracts of the fruits were prepared. The free radical scavenging capacity of the fruit extracts was evaluated by using the free radical 1,1'-diphenyl-2-picrylhydrazyl (DPPH) scavenging method. The methanolic extract of sour cherry fruits was found to exhibit higher % inhibition level than the extracts of apricot and cherry ($p < 0.05$). Furthermore, the total phenolic and flavonoid compounds were higher in apricot and cherry when compared with that of sour cherry ($p < 0.05$).

Key words: free radical scavenging capacity, phenolics, flavonoids

1. INTRODUCTION

Oxidative stress in living cells was found to be related to the onset of clinical disorders such as cancer, cardiovascular diseases, diabetes, arthritis, sclerosis, obesity, and Alzheimer's disease (Adly 2010, Aruoma 1998, Block, Patterson & Subar 1992, Dhalla, Temsah & Netticadan 2000). In living cells, free radicals are formed during metabolic reactions or as a result of radiation. These free radicals are compounds with an unpaired electron and are very reactive. The free radicals have been shown to be the precursors of chain reactions leading to the breakdown of the organic compounds such as lipids, proteins and nucleic acids. As the amount of the free radicals within living cells increases, the antioxidant defense system is negatively affected and thus leading to oxidative stress. In living organisms, the oxidative stress is responsible for the aging process of living cells and therefore, oxidative stress has been determined to be related with cancer, cardiovascular diseases, and diabetes (Adly 2010, Block, Patterson & Subar 1992). Research, concerning the relationship between the aforementioned diseases and the type of nutrition, showed out that the intake of food products containing natural antioxidants decreases the incidence of these diseases. Based on these findings, evaluation of the antioxidant potential of food products has recently gained special attention.

In general, food products of plant origin such as, fruits, vegetables, legumes and nuts are considered to be good sources of a number of compounds effective against oxidative stress. These bioactive compounds include phenolics, vitamins, some free amino acids and peptides with antioxidant activities (Block, Patterson & Subar 1992, Kapur & Kapoor 2001, Naczek & Shahidi 2006, Wang et al. 2011).

Nowadays, nutritionists recommend the consumption of fresh and unprocessed food products of plant origin where possible, for the prevention of coronary diseases, cancers, neuropathological diseases, etc. (Block, Patterson & Subar 1992, Kapur and Kapoor 2001).

The chemical composition of plants is mainly affected by the environmental conditions such as climate, altitude and soil conditions. Several researches have revealed that the contents of plants are environment dependent and therefore, samples of different areas should be investigated (Orazem et al. 2011, Ouchemoukh et al. 2012). Furthermore, the chemical composition is greatly affected by the variety type of the plants (Mitic et al. 2012, Mulabagal et al. 2009, Piccolella et al. 2008, Zitka et al. 2011).

Cherry (*Prunus avium*), sour cherry (*Prunus cerasus*) and apricots (*Prunus armeniaca*) belong to the Rosaceae family, *Prunus* genus. The fruits of this genus are grown throughout the world and have been found to contain a number of bioactive compounds including vitamins, organic acids, phenolics, flavonoids, and anthocyanins (Ferretti et al. 2010, Mulabagal et al. 2009, Zitka et al. 2011). These fruits are extensively cultivated in Turkey and thus, comprise an important fresh fruit resource (Ercisli 2009, Ouchemoukh et al. 2012). Fresh produce is mostly preferred, but these fruits serve also as the raw material of dried fruits (apricots) (Ercisli 2009), fruit juices, jams, etc. (sour cherries) (Ferretti et al. 2010, Piccolella et al. 2008).

The aim of the present study was to evaluate the total phenolic and flavonoid content of fresh ripe fruits of cherry, sour cherry and apricot cultivated in the Canakkale region, Turkey. Furthermore, the free radical scavenging capacity of these fruits was determined.

2. MATERIALS AND METHODS

2.1. Chemical reagents

The chemicals used throughout the study were of analytical grade and were purchased from Sigma-Aldrich (USA), SPA (Milan, Italy), Merck (Germany) and Fluka Chemie (Switzerland).

2.2. Plants materials

Fresh ripe fruits of sour cherry (*Prunus cerasus*), cherry (*Prunus avium*), and apricot (*Prunus armeniaca*) cultivated in the region of Canakkale, Turkey were collected and the samples were brought to the laboratory within 2 hours after their collection time. To decrease the effect of high temperatures, the fruits were carried to the laboratory at 4°C.

2.3. Preparation of the fresh fruit sample extracts

The collected fruits were stored at 4°C till analysis (not more than 12 hours) and the fruits in their fresh form were used for the extraction procedure. Each fresh fruit sample (20 g) was extracted according to Maisuthisakul, Suttajit & Pongsawatmanit (2007). Briefly, a fresh fruit sample (20 g) was blended with 60 mL methanol in a blender for 1 minute and the mixture was shaken for 4.5 h. The supernatant was filtered through Whatman filter paper (No. 4). All of the filtrates were evaporated under reduced pressure using a rotary evaporator at 40°C and were weighed in order to determine the yield of the soluble components. Then, the extracts were immediately analyzed for DPPH free radical scavenging activity, total phenolics and flavonoids. Unless otherwise stated, all assays were done in triplicate.

2.4. Analysis of the total phenolic content

The amount of the total phenolics in the methanolic extracts of the fresh fruit samples was measured by using the Folin–Ciocalteu reagent method. This method was applied as described by Djeridane et

al. (2006). The methanol solution of each extract (0.2 mL, 500 mg/mL) was taken into a test tube. 0.5 mL distilled water and 0.5 mL Folin–Ciocalteu reagent were added and the tubes were thoroughly shaken. After 1 min, 0.8 mL of sodium carbonate solution (7.5%) was added and the mixture was allowed to stand for 30 min. with intermittent shaking. The absorbance was measured at 760 nm using a UV–Vis spectrophotometer (Thermo Aquamate). Gallic acid was used as a standard for the calibration curve. The results were calculated according to the calibration curve for gallic acid ($R^2 = 0.961\text{--}0.983$, Table 1). The total phenolic content was expressed as gallic acid equivalents (GAE) in μg per g fresh fruit.

2.5. Analysis of the total flavonoids content

The total flavonoid content was determined according to Quettier-Deleu et al. (2000) using rutin as a standard. The results were calculated according to the calibration curve for rutin ($R^2 = 0.999$, Table 1). The total flavonoid content was expressed as rutin equivalents in μg per g fresh fruit. Three replicates were used for the determination of the mean total flavonoid concentrations of the fresh fruit samples.

2.6. DPPH free radical-scavenging activity assay

The effect of the oxidized fruit extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated as described by Brand-Williams, Cuvelier & Berset (1995). First, every fruit sample was diluted in methanol prior to the analysis (1 mg/mL). Then, the DPPH solution was added to the diluted sample, the mixture was thoroughly mixed, and afterwards left for 30 min. for the reaction to occur. After that, the absorbance of the sample was measured at 515 nm using a UV–Vis spectrophotometer (Thermo Aquamate). The absorbance of the DPPH solution in methanol, without any antioxidant, was also measured as the control. The percentage of DPPH radical scavenging activity was calculated by using the following equation:

$$\% \text{ DPPH scavenging} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

where A_{sample} is the absorbance of sample after the time necessary to reach the plateau (30 min) and A_{control} is the absorbance of DPPH.

The concentrations providing IC_{50} inhibition values (defined as the concentration of the compounds that was able to inhibit 50 % of the total DPPH radicals) were calculated from graph plotting using nonlinear regression and expressed in μg material equivalents/g fresh fruits. Butylated hydroxytoluene (BHT) was used as a positive control. A lower value of IC_{50} indicates a higher antioxidant activity and vice versa.

2.7. Statistical Analysis

To evaluate the differences among the means of the results, one-way ANOVA was applied and the differences among the means were investigated by using Statgraphics Centurion XV software. The results of the analyses were reported as mean $\pm$ SD (standard deviation). The estimated values were considered to be significantly different at $p < 0.05$. Furthermore, the relationship between the free radical scavenging capacity and the phenolic constituents in terms of total phenolics and flavonoids was evaluated by using MS Excel software.

3. RESULTS AND DISCUSSION

The total phenolic and flavonoid content of the fruits analyzed within this study is presented in Table 1. The amount of the total phenolics was found to be in the range of 196.65 - 328.32 μg GAE/g of fresh fruits. The highest phenolic content was detected in cherry. Although the phenolic content of cherries was found to be slightly higher than that of apricots, there was no statistical difference

between the phenolic amounts of cherries and apricots. Among the investigated fruits, the total phenolic content was lowest in sour cherries ($p < 0.05$).

The total flavonoid amount of the analyzed fruits was found to show variation between 2387.68 and 2671.74 μg rutin/g fresh fruits (Table 1). Similar to the results concerning the amount of total phenolics, there was no significant difference between the total flavonoid content of cherry and apricot. On the other hand, sour cherries appear to contain significantly lower flavonoid content when compared with the total flavonoid content of cherries and apricots ($p < 0.05$).

Table 1. Total phenolics and flavonoids of the fruits*

$y = a \cdot x + b$		Sour Cherry**	Cherry	Apricot
	($\mu\text{g/g}$)	196.65 $\pm$ 6.72a	328.32 $\pm$ 9.14b	318.89 $\pm$ 8.02b
Total Phenolics	a	15.832	17.504	17.669
	b	-0.007	-0.149	-0.166
	R ²	0.961	0.983	0.977
$y = a \cdot x + b$				
	($\mu\text{g/g}$)	2387.68 $\pm$ 9.21a	2671.74 $\pm$ 7.65b	2597.23 $\pm$ 11.02b
Total Flavonoids	a	4.295	4.295	4.295
	b	-0.385	-0.385	-0.385
	R ²	0.999	0.999	0.999

*Results are given as mean $\pm$ standard deviations with three replicates.

**Different letters in a row denote a significant difference among the values ($p < 0.05$).

On the other hand, the antioxidant activity of the methanolic extracts of fresh fruits cultivated in the Canakkale region was determined by using the DPPH free radical scavenging method. The results are given in Table 2 as μg / g fresh fruit. 1,1-diphenyl-2-picrylhydrazyl is known to be a stable free radical, which decreases significantly when exposed to proton radical scavengers. Therefore, this method is thoroughly used in studies concerning the evaluation of the antioxidant capacity of various materials (Sanchez-Moreno 2002). The DPPH free radical scavenging activity of the fresh fruits were compared with that of butylated hydroxytoluene, and ascorbic acid. Among the investigated fruits, the lowest IC₅₀ value (indicating high free radical scavenging activity) was determined for sour cherry ($p < 0.05$). Cherry and apricot exhibited similar IC₅₀ and % inhibition levels. Furthermore, it should be pointed out that, for all fruits, the % inhibition level of DPPH was significantly lower than that of BHT and ascorbic acid ($p < 0.05$).

Table 2. Free radical scavenging capacity of the fruits*

		BHT	Ascorbic acid	Sour Cherry	Cherry	Apricot
	IC ₅₀ (μg/g)	1.35±0.09a	1.53±0.08a	44.61±0.21b	68.13±0.12c	66.43±0.30c
DPPH	Inhibition (%)	98.9±0.24a	98.75±0.21a	77.56±0.17b	64.07±0.18c	67.29±0.22c

*Results are given as mean±standard deviations with three replicates.

**Different letters in a row denote a significant difference among the values (p<0.05).

The relationship between the phenolic content and the free radical scavenging capacity of the fruits was evaluated. No correlation was found for the total phenolic and flavonoid contents of the fruits and their free radical scavenging capacity.

In literature, there are various studies concerning the relationship between the phenolic constituents and the antioxidant capacity of various fruits. In some studies it was found out that there was a high correlation between phenolics and antioxidant activity (Egea et al. 2010, Mitic et al. 2012, Ouchemoukh et al. 2012). Nevertheless, there are also studies, reporting that there is either low or no correlation between total phenolic contents and radical scavenging capacity (Turker et al. 2012, Yu et al. 2002).

The high free radical scavenging capacity of plant materials is usually attributed not only to the phenolic composition, but also to the presence of other bioactive compounds such as antioxidant vitamins (e.g.: ascorbic acid, tocopherols), pigments (anthocyanins) and peptides, as well as the structural interaction among these compounds (Djeridane et al. 2006, Wang et al. 2011). Mulagabal et al. (2009) determined the anthocyanin content of cherry and sour cherries, and concluded that these fruits contain appreciable amounts of anthocyanins that are known for their antioxidant, anticancer, antidiabetic and antiobese effects (Feretti et al. 2010, Kapur & Kapoor, 2001, Landete 2012). Therefore, in order to elaborate the free radical scavenging capacity of the analyzed fruits, a more detailed study should be done.

4. CONCLUSION

The fresh ripe fruits of cherry, sour cherry and apricot cultivated in the Canakkale region, Turkey were found to contain appreciable amounts of total phenolics and flavonoids. Furthermore, these fruits have shown substantial free radical scavenging capacity. Nevertheless, in future studies, a comprehensive research of the bioactive compounds such as phenolic acids, anthocyanins, and vitamins should also be done.

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