

Full Length Research Paper

# Antioxidant properties of 12 cornelian cherry fruit types (*Cornus mas* L.) selected from Turkey

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***Cornus mas* L. is a naturally growing dogwood species in Anatolia. In present study, the antioxidant activity of 50% aqueous methanol extracts of 12 cornelian cherry types were evaluated by various antioxidant assay, including free radical scavenging, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging and metal (Fe<sup>+2</sup>) chelating activities. The methanolic fruit extracts of the *Cornus mas* L. fruit types (H<sub>2</sub>O<sub>2</sub> and diphenylpicrylhydrazyl (DPPH) scavenging activities, Fe<sup>+2</sup> chelating activity) examined in the assay showed the strongest activities. The highest antiradical activity was found 44-05 type (0.943) followed by 44-04 (0.860), 77-09 (0.810) and 77-05 (0.723) types respectively. The lowest value was found 44-01 (0.537), 77-06 (0.580), 77-11 (0.567) and 77-02 (0.563) types. The highest chelating activity was found; 44-05 (54.213%), 44-04 (49.890%), 77-09 (47.287%) and 77-05 (45.180%) cornelian cherry type respectively. The lowest chelating activity was found 77-06 (33.883%), 44-01 (34.106%) and 77-11 (34.510%) types. In terms of H<sub>2</sub>O<sub>2</sub> inhibition, the highest rate was obtained from 44-05 (79.103%), the lowest one was 44-01 type to be 37.720%.**

**Key words:** Cornelian cherry (*Cornus mas* L.), antioxidant properties, fruit.

## INTRODUCTION

Food-based fruit and vegetables probably reduces the lower risk of heart disease and some neurological diseases and there is evidence that some types of vegetables and fruits in general, protect against some cancer types. Since fruits and vegetables happen to be good sources of antioxidants, this suggested that antioxidants might prevent some types of diseases (Stanner et al., 2004).

Cornelian cherry (*Cornus mas* L.) belongs to the family *Cornaceae* (Rop et al., 2010). It is a wild plant that grows in Asia and Europe, as well as being recently cultivated in Turkey (Kalyoncu et al., 2009, Gulcin et al., 2005). Turkey is an important producer of cornelian cherries (*Cornus mas* L.), especially in northern Anatolia (Yaltirik, 1981; Ercisli et al., 2007). In Turkey, approximately 12,800 tons of cornelian cherry fruit is produced per annum (Kalyoncu et al., 2009; Celik et al., 2006). This

species grows in the temperate zone of Eurasia on calcareous, well-drained forest soils. It ranges from a shrub to a small tree of about 7-8 m in height and can be cultivated under the shade of tall trees (Ercisli et al., 2007; Yaltirik, 1981). The species is highly tolerant to diverse abiotic and biotic conditions. The fruits are very valuable for fresh consumption and for processing to produce syrups, juices, jams, spirits and other traditional products (Rop et al., 2010).

Recently, some studies have been published about antioxidant activity of cornelian cherry fruits (Hashempour et al., 2010, Rop et al. 2010, Yilmaz et al. 2009, Tural and Koca, 2008, Pantelidis et al., 2007; Gulcin et al., 2005). On the other hand, the bioactive content of fruits varies from genotype to genotype (Ercisli and Orhan, 2007). Therefore, attention has more recently been focused on assessing the distribution on biologically active compounds among different cultivars/genotypes (Pantelidis et al., 2007). In the present study, the antioxidant activity of 12 cornelian cherry types was examined in the different antioxidant assays including

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free radical scavenging activity, metal ( $\text{Fe}^{2+}$ ) chelating activities (%) and inhibition activity of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ).

## MATERIALS AND METHODS

### Materials

Materials were obtained from cornelian cherry garden of Ataturk Horticultural Research Institute-Yalova, Turkey. Cornelian cherry garden is eight years old and samples consist of from 12 cornelian cherry types from East Anatolia (Malatya) (44-01, 44-04, 44-05, 44-07) and South Marmara (Yalova) (77-01, 77-02, 77-03, 77-05, 77-09, 77-10, 77-11, 77-12) region bring by selection. Materials were brought in PVC containers. Samples were kept in sealed containers at  $-18^\circ\text{C}$  during the study.

### Preparation of extracts

About 2.5 g fresh fruit samples were extracted by homogeny in mixer (Ultra turrax) with 50 ml solvent (50% water-methanol). The extracts were centrifuged at  $4,000 \times g$  for 3 min at  $4^\circ\text{C}$  after draining on coarse filter paper. And then the filtrate was drained by blue band filter paper (no: 391).

### Methods

#### Free radical scavenging effect

The radical scavenging activity against the diphenylpicrylhydrazyl (DPPH) radical was evaluated according to the method of Serteser et al. (2008), with some minor modifications. The assay mixture contained 1.5 ml of  $0.09 \text{ mg ml}^{-1}$  DPPH (Sigma Chemical Co., St Louis, MO, USA) in methanol, 1 ml acetate buffer solution (100 mM, pH 5.5). The dilutions between 0.4 and  $4 \text{ mg ml}^{-1}$  were prepared with methanol. Then 3.9 ml DPPH solution prepared with  $6 \times 10^{-5} \text{ M}$  methanol was added to each 0.1 ml dilution and shaken well. The mixture was prepared and incubated for 60 min at room temperature in the dark. The absorbance of the remaining DPPH was determined at 517 nm against a blank. The scavenging activity was expressed as the  $\text{IC}_{50}$  value ( $\text{mg ml}^{-1}$ ). All analyses were carried out in duplicate.

Linear regression equations of absorbance against concentrations were determined by measuring the absorbances of seven different concentrations of DPPH ( $6 \times 10^{-5} \text{ M}$ ) stock solution:

$$A (517 \text{ nm}) = 15,465 (C \text{ DPPH}) - 0.0187 (R^2 = 0.987)$$

The remaining DPPH concentrations against absorbance values of sample series of different concentrations were calculated and then the remaining DPPH percentage was calculated:

$$\% \text{ Remaining DPPH} = \frac{[\text{DDPH}] \text{ sample}}{[\text{DPPH}] \text{ control}}$$

Exponential regression equation was obtained between the rate of the remaining DPPH percentage and the DDPH amount of sample *in vitro*, and the sample concentrations of plants that decrease the initial DPPH concentrations by 50% (efficient concentration  $[\text{EC}_{50}]$ ). The antiradical activity (AE) was calculated by dividing  $\text{EC}_{50}$  values into 1.

#### $\text{Fe}^{2+}$ chelating activity

The modified methods of Lim and Murtijaya (2007) were used for determination of the  $\text{Fe}^{2+}$  chelating activities of samples. One

milliliter of extracts with different concentrations between 6 and 45  $\text{mg ml}^{-1}$  and 3.7 ml deionizer water were mixed. 0.1 ml of 2 mol  $\text{FeCl}_2$  solution was added and shaken and kept at dark and room temperature for 70 min. Then, 0.2 ml of 5 mM ferrozine was added and shaken again, and the absorbance of the obtained  $\text{Fe}^{2+}$ -ferrozine complex after 10 min was measured at 562 nm. One millilitre of water was used instead of sample for the control. The equation is as follows (Yen and Wu, 1999):

$$\text{Chelating activity (\%)} = [1 - (\text{absorbance of sample} / \text{absorbance of control})] \times 100$$

#### $\text{H}_2\text{O}_2$ inhibition effect

The  $\text{H}_2\text{O}_2$  inhibition effect of spice and plant extracts was determined by spectrophotometer (Ruch et al., 1989). One millilitre (2.6 and 10  $\text{mg/ml}$ ) of sample, 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and 0.6 ml of 43 mM  $\text{H}_2\text{O}_2$  were mixed and after 60 min the absorbance of mixture was measured at 230 nm. Control solutions without  $\text{H}_2\text{O}_2$  were prepared for each sample concentration. To determine the  $\text{H}_2\text{O}_2$  concentration that was not involved in the reaction, a linear regression equation was used. Phosphate buffer (3.4 ml) was added to 0.6 ml 10.15, 25.43 at 230 nm. Linear equation formulas were obtained by the graphic of standard curve of absorbance vs. different concentrations of (+)- Catechin.

$A (230) = 0.0125 \times C (\text{H}_2\text{O}_2, \text{ mM}) + 0.0873 (R^2 = 0.9783)$ (+)-Catechin was used as the reference antioxidant. The equation used is as follows:

$$\text{H}_2\text{O}_2 \text{ inhibition capacity (\%)} = [1 - (\text{H}_2\text{O}_2 \text{ conc. of sample} / \text{H}_2\text{O}_2 \text{ conc. of control})] \times 100$$

#### Statistical analyses

Statistical analysis was done using the JAMP. Differences between means were analysed by ANOVA test ( $p < 0.05$ )(Puskulcu and Ikiz, 1989). This research was performed by three duplicates with a replicate.

## RESULTS AND DISCUSSION

### Free radical scavenging activity

DPPH, as a partially organic radical, is used to determine the antioxidant activities of many plant extracts and compounds (Brand-Williams et al., 1995). This method is based on a decrease in alcoholic DPPH solution in the presence of H binding antioxidant b ( $\text{DPPH} + \text{AH} \rightarrow \text{DPPH} - \text{H} + \text{A}$ ). A DPPH solution is dark violet colored and has a strong absorption range at 517 nm. It loses its color when transformed to DPPH-H and the absorption level decreases. This decrease in absorption shows the cytochrometric decrease in DPPH (Serteser et al., 2008, 2009a, b). The DPPH radical scavenging effects of cornelian cherry fruit extracts are given in Table 1. The DPPH radical scavenging assay is commonly employed to evaluate the ability of antioxidant to scavenge free radicals. The use of the DPPH free radical is advantageous in evaluating antioxidant effectiveness because it is more stable than the hydroxyl and super oxide radicals (Layina-Pathirana et al., 2006). Radical

**Table 1.** DPPH radical scavenging effects of fruit extracts\*

Cornelian cherry type	EC <sub>50</sub>	AE
44-05	1.060	0.943 a
44-04	1.163	0.860 b
77-09	1.240	0.810 c
77-05	1.380	0.723 d
44-07	1.450	0.690 e
77-01	1.513	0.660 f
77-12	1.540	0.657 fg
77-10	1.583	0.633 g
77-06	1.730	0.580 h
77-11	1.770	0.567 h
77-02	1.780	0.563 h
44-01	1.863	0.537 i
LSD value	-	0.046

<sup>a</sup>Efficiency coefficient (EC<sub>50</sub>) (mg sample/ mg DPPH): sample amount needed to decrease the DPPH concentration at the beginning by 50%, <sup>b</sup>Antiradical activity (AE): 1 / EC<sub>50</sub>. Levels not connected by same letter are significantly different (p<0.05).

**Table 2.** Fe<sup>+2</sup> chelating activity (%) of fruit extracts.

Cornelian cherry type	Chelating activity (%)
44-05	54.213 a
44-04	49.890 b
77-09	47.287 c
77-05	45.180 d
44-07	41.440 e
77-01	41.180 e
77-12	39.983 f
77-02	36.246 g
77-10	36.233 g
77-11	34.510 h
44-01	34.106 h
77-06	33.883 h
LSD value	2.071

Levels not connected by same letter are significantly different (p<0.05).

scavenging activity, expressed as EC<sub>50</sub>, ranged from 1.060 to 1.863 mg ml<sup>-1</sup>. The inverse relationship was found between antiradical activity and EC<sub>50</sub> values in the cornelian cherry fruit types. Because of a lower EC<sub>50</sub> value indicates greater antioxidant activity. Among 12 cornelian cherry types have different results. According to Table 1, the highest antiradical activity was found 44-05 type (0.943) followed by 44-04 (0.860), 77-09 (0.810) and 77-05 (0.723) types respectively. The lowest value was found 44-01 (10.537), 77-06 (0.580), 77-11 (0.567) and 77-02 (0.563) types and these values were in the same group to be statistically and these were the second

lowest group. Although, grown under the same conditions, statistically significant differences were observed among types in terms of antiradical activity. The reason for this is the bioactive content of fruits varies from genotype to genotype (Ercisli and Orhan, 2007). Tural and Koca (2008) obtained that EC<sub>50</sub> values were in the range of 0.29-0.69 mg.ml<sup>-1</sup> in different natural cornelian cherry types in their work.

Serteser et al. (2008) researched on some medicinal and aromatic plants obtained from several locations of the Afyonkarahisar province in Turkey and they found that cornelian cherry fruit had the highest antiradical activity (0.928) among their experimental plants. The same researchers found that the highest DPPH radical scavenging effects were in the fruit extracts of *Cornus* and *Morus* species with values which varied from 1.078-1.212 (EC<sub>50</sub>). Tural and Koca (2008) reported that the methanolic extracts of the Cornelian cherry (*Cornus mas* L.) fruits showed EC<sub>50</sub> (mg ml<sup>-1</sup>) (DPPH reduction) values as 0.52. EC<sub>50</sub> values were lower than the results of Tural and Koca (2008). Dragovic-Uzelac et al. (2007) determined that DPPH values in two different cornelian cherry types were between 33.41 and 39.89 mmol Trolox equivalent/kg f.w. among some fruit species in their work. Paulovicsova et al. (2009), found that the antiradical activities were 77.59- 84.56 and 88.85% in three cornelian cherry types. However, these contradictory results are most likely due to differences in methodology and experimental conditions used in the different studies. Due to the wide variety of potential antioxidant compounds, such as vitamins, flavonoids, phenolic acids and sulphur compounds present in plants, differences in the method of sample extraction can results in a wide variation in the antioxidant activity of the extract (Nuutila et al., 2003). The DPPH method is based on reduction of stable DPPH nitrogen radicals in presence of antioxidants (Huang et al., 2005). Moreover, even for values obtained from the same assay, a comparison with the data in the literature was problematic due to the large variability within the food item and to the lack of standardization of the assays.

### Fe<sup>+2</sup> chelating activity

Chelating agents may have great importance for rancidity of oily foods; even though they are not antioxidant materials. Because iron catalyzes this reaction during lipid peroxidation, Ferrozin forms a complex with Fe<sup>+2</sup>. The amounts of complex and red color decrease in the presence of the other chelating agents. A decrease in absorption values can be determined by changes in the color. The decrease in absorption shows the effectiveness of chelating agent added with the exception of ferrozin (Serteser et al., 2008, 2009a, b). Table 2 shows the chelating activities of cornelian cherry types fruit extracts.

According to the Table 2, the highest chelating activity

**Table 3.** H<sub>2</sub>O<sub>2</sub> inhibition activity of fruit extracts.

Cornelian cherry type	Inhibition (%)
44-05	79.103 a
44-04	60.753 b
77-09	58.117 c
77-05	54.710 d
44-07	50.743 e
77-12	49.067 f
77-01	49.013 f
77-02	43.943 g
77-10	42.863 g
77-11	41.320 h
77-06	39.870 i
44-01	37.720 j
LSD value	2.228

Levels not connected by same letter are significantly different ( $p < 0.05$ ).

was found; 44-05 (54.213%), 44-04 (49.890%), 77-09 (47.287%) and 77-05 (45.180%) cornelian cherry type respectively. These four values were statistically different groups. The lowest chelating activity was found 77-11 (33.883%), 44-01 (34.106%) and 77-11 (34.510%) type and these values were statistically the same group. The other types of values has taken place among these values.

Serteser et al. (2009a) reported that the highest chelating activity was found in *Cornus mas* fruit extracts. The chelating activities of *Cornus* spp. Fruit extracts were found higher compared with other fruit extracts. The highest chelating activity was observed in the *Cornus* species ranging from 44.64-45.72%. Pantelidis et al. (2007) found that *Cornus mas* cv. Vermio cultivar had 83.9  $\mu\text{mol g}^{-1}$  dw Ferric Reducing Antioxidant Power (FRAP) value. Chelating agents may have a great importance for rancidity of oily foods, even if they are not antioxidant materials. Because iron catalyzes this reaction during lipid peroxidation (Yen and Duh, 1994). The FRAP assays evaluate the chain-breaking antioxidant potential (Ghiselli et al., 1995) and the reducing power of the sample (Benzie and Strain, 1996), respectively.

FRAP assay works employing metals ions for oxidation, measuring the formed ferrous ions by increased absorbance, instead of the use of organic radical producers (Schlesier et al., 2002). The same authors strongly suggested the application of at least two methods for determination of antioxidant activity, due to differences between the test systems.

### H<sub>2</sub>O<sub>2</sub> inhibition activity

This method is used to eliminate O<sub>2</sub>•<sup>-</sup>, even though the superoxide radical anion (O<sub>2</sub>•<sup>-</sup>) does not initiate lipid Ersoy et al. 101

oxidation directly. Super reactive hydroxyl radical (.OH) may be formed from the Fenton Reaction ( $\text{Fe}^{+2} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{+3} + \text{OH}^- + \text{.OH}$ ) in the presence of metal ions. For this reason, H<sub>2</sub>O<sub>2</sub> inhibition activity is an important method for the determination of antioxidant characteristics (Serteser et al., 2008, 2009a, b). The H<sub>2</sub>O<sub>2</sub> inhibition activities of cornelian cherry fruit extracts are given in Table 3.

Cornelian cherry types have different rates in terms of H<sub>2</sub>O<sub>2</sub> inhibition. The highest rates were found in 44-05 (79.103%), 44-04 (60.753%), 77-09 (58.117%) and 77-05(54.710%) types as well as in the other two methods. In addition, they were obtained from the type that was between these values and as such, they were statistically different. The lowest value was found 44-01 type to be 37.720%. Serteser et al. (2009a) reported that, *Cornus* species showed the highest H<sub>2</sub>O<sub>2</sub> inhibition values (54.32-65.42%) in their research. The H<sub>2</sub>O<sub>2</sub> inhibition value found from 44-05 type in our research was higher than cornus types which were in Serteser et al. (2009a) research.

In conclusion, the information obtained in this article might be useful for the development of food products and appropriate additives with antioxidant properties. Having the highest antioxidant content types have been 44-05, 44-04, 77-09 and 77-05 respectively. Therefore, these types are primarily offered as a source of antioxidants.

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