ANTIOXIDANT ACTIVITIES OF BLACK MULBERRY (*Morus nigra*)

KARA DUTUN (*Morus nigra*) ANTİOKSİDANT AKTİVİTESİ

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ABSTRACT

In this study, the antioxidant properties of black mullberry (*Morus nigra*) fruits and leaves were evaluated by determining DPPH radical scavenging ability and lipid peroxidation inhibition activity. The total phenolic contents of the extracts were also assessed by Folin method. The water and methanol extracts of both fruits and leaves have antioxidant potential. The highest antioxidant activity was obtained from methanol extract of black mulberry leaves with 33.1 %. This was followed by methanol extract of the fruit with 28.7 % inhibition. The lowest activities were shown in the both of water extracts of leaves and fruits with 12.1% and 24.3% inhibition, respectively. There is a statistically significant correlation between DPPH radical scavenging and total phenolic compounds ($r = 0.855$, $p < 0.01$).

Key words: Black mulberry, Antioxidant activity, Total phenolic compounds.

ÖZET

Bu çalışmada, Kara dutun (*Morus nigra*) meyve ve yapraklarındaki antioksidant aktiviteler araştırılmıştır. Meyve ve yapraklardaki antioksidant özellikler, DPPH radikal giderme aktivitesi ve lipit peroksidasyon inhibisyon aktivitesiyle belirlenmiştir. Yine, örneklerdeki toplam fenolik madde içerikleri Folin metoduyla tespit edilmiştir. Hem meyve hem de yaprakların su ve metanol ekstraktlarının antioksidant aktiviteye sahip olduğu görülmüştür. En yüksek antioksidant aktivite (%33.1) yaprakların metanol ekstraktlarından elde edilirken bunu meyvelerin metanol ekstraktları (% 28.7) takip etmiştir. En düşük aktiviteler ise yaprak ve

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meyvelerin su ekstraksiyonlarında sırasıyla % 12.1 ve % 24.3 değerleriyle tespit edilmiştir. DPPH radikal giderme aktivitesi ve toplam fenolik madde içerkleri arasında istatistiki olarak çok önemli ($r = 0.855$, $p < 0.01$) korelasyon belirlenmiştir.

Anahtar Kelimeler: Kara dut, Antioksidan aktivite, Toplam fenolik madde.

1. INTRODUCTION

High intakes of fruits and vegetables have been associated with lower incidences of chronic diseases such as cancer and hearth disease. In addition to the vitamins and minerals known to be present in fruits and vegetables, phytochemicals such as flavonoids and other phenolics may contribute to this protective effect. Many of these phytochemicals have antioxidant activity and may help protect cells against the oxidative damage caused by free radicals (Wada and Ou, 2002).

Fruits and vegetables contain many different antioxidant compenents. These antioxidants include carotenoids, vitamins, phenols, dietary glutathionine, and endogenous metabolits (Hanasaki *et al.*, 1994).

The mulberry belongs to the genus *Morus* of the family *Moraceae*. Mulberry is widely spread through-out all regions from the tropics to the sub-arctic and from sea level to altitudes as high as 4000m. It is native to southwestern Asia, where it has been cultivated for so long that its precise natural range is unknown. It is a small deciduous tree growing to 10-13 m tall. The leaves are 10-20 cm long and 6-10 cm broad (up to 23 cm long on vigorous shoots), downy on the underside, the upper surface rough with very short, stiff hairs. The edible fruit is dark purple, almost black, when ripe, 2-3 cm long, a compound cluster of several small drupes; it is richly flavoured, similar to the red mulberry (*Morus rubra*) but unlike the more insipid fruit of the white mulberry (*Morus alba*) (Davis, 1982).

In Turkey, and in other countries black mulberries are not only consumed fresh but also used to produce jam, sweeten fruit marmalade (a locally dried fruit pulp product), syrup several types of soft drinks and traditional products such as mulberry molasse, mulberry pestil (dried layers of fruit pulp) (Şengül *et al.*, 2005).

Mulberries are a good source of vitamins and minerals, especially contain a high amount of anthocyanin (Gerasopoulos and Stavorulakis, 1997). Besides its use as food, the plant fruits, leaves and barks are well known as traditional medicine in Turkey and have been used for many years as an anti-fever, as a laxative, anthelmetic, expectorant and facilitate discharge of urine and lower blood pressure (Baytop, 1999).

Recently, it has gained an important position in the local soft drink market, although its biological and pharmacological effects are still poorly defined. However, little information is avaible on antioxidant capasities in leaves of black mulberry in Turkey. The investigation presented here in was undertaken for antioxidant capacity of fruit and leaves of black mulberry (*Morus nigra*).

2. MATERIALS AND METHODS

Preparation of extracts

The black mulberry (*Morus nigra*) fruits were collected at their optimum commercial maturity in Uzumlü Town, Erzincan, Turkey. The fresh fruit samples were packed on ice while being transported to the laboratory. Fruits samples were frozen at –20^{o}C until extraction. The leaves of black mulberry were dried in shade and powdered with a blender. The fruits and powdered leaves (20mg) were extracted with methanol in a Soxhlet apparatus for 24 h. Then methanol was evaporated with rotary evaporator. Water extracts were also prepared by adding boiling water (200ml) to 20 g of powdered material in a glass flask and incubated at room temperature for 2 hours on a rotating shaker (200 rpm). Mixture was filtered using Whatman (No.1) filter paper and then filtrate was lyophilized. All extracts were stored in freezer at –24^{o}C until use.

Antioxidant activity

The antioxidant activity was determined according to the thiocyanate method (Yıldırım *et al.,* 2003). Briefly, stock extracts solutions were prepared at 2 mg/ml concentration. Required stock solutions were mixed with 2.5 ml of 0.02 M linoleic acid (Fluka) emulsion [contains an equal weight of Tween-20 (Sigma) in pH 7.4

phosphate-buffered saline (Sigma)], and the final volume was adjusted to 5 ml with phosphate-buffered saline (0.02 M, pH 7.4) in a test tube and incubated in darkness at 40 °C. Final concentrations of the extracts were 100 µg/ml. BHT (Sigma) was used as positive control (100 µg/ml). The amount of peroxide was determined by measuring the absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate after 24 h incubation. Lower absorbance indicates higher antioxidant activity. To eliminate the solvent effect, the same amount of solvent used to prepare the solutions of test samples was added into the control test sample, which contains the linoleic acid emulsion. Measurements of antioxidant activity were carried out for three sample replications, and values are the average of three replicates. This activity is given as percent Lipid Peroxidation Inhibition which is calculated with the equation

$$\text{Lipid peroxidation inhibition (\%)} = \left[\frac{ControlAbs. - SampleAbs.}{ControlAbs.}\right] x100$$

DPPH radical scavenging activity

Experiments were carried out as described previously (Yıldırım *et al*., 2001). Briefly, 0.5 mM DPPH (Fluka) radical solution in methanol was prepared, and then 1 ml of this solution was mixed with 3 ml of the sample solution. Final concentrations of extracts were 100 and 300 µg/ml. BHT was used as a positive control at the same concentrations. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as percent DPPH radical scavenging, which is calculated with the equation

$$Activity\% = \left[\frac{ControlAbs. - SampleAbs.}{ControlAbs.}\right] x100$$

Control contains 1 ml of DPPH solution mixed with 3 ml of ethanol. The measurements of DPPH radical scavenging activity were carried out for two sample replications, and values are an average of two replicates.

Determination of total phenolic compounds

Antioxidant compounds generally contain phenolic group(s). Because of this, amounts of phenolic compounds in each of the extract were compared to obtain more information about the extract(s) which posses(s) antioxidant potential. This was carried out as described previously (Yıldırım *et al*., 2003). Briefly, extract solution was transferred into a tube and then final volume was adjusted to 4 ml by addition of distilled water. Afterward, 0.25 ml of Folin-Ciocalteu Reactive (FCR) (Fluka) was added into this mixture and after 3 min 0.75 ml of Na_2CO_3 solution was added. Subsequently, mixture was shaken on a shaker for 2h at room temperature and then absorbance was measured at 760 nm. Amount of total phenolic compounds were carried out for two sample replications, and values are an average of two replicates. Gallic acid was used as the standard for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent using the following equation based on the calibration curve:

$$Y=0,2582X$$

Where Y is the absorbance of the sample and X is the gallic acid the equivalent ($\mu g ml^{-1}$)

Statistical analysis

Statistical analyses were carried by using SPSS 13.0. Values at $p<0.05$ were considered to be significant and $P< 0.01$ very significant.

3. RESULTS AND DISCUSSION

In the present study the antioxidant activity of water and methanol extracts of black mulberry fruits and leaves were determined by thiocyanate method in which the amount of peroxides formed in the linoleic acid emulsion during incubation is determined spectrophotometrically by measuring the absorbance at 500 nm. The presence of lyophilized water and methanol extract in the linoleic acid emulsion at the concentration of 100 µg/ml was able to reduce the formation of peroxides. The highest percent inhibition of lipid peroxidation was measured in methanol extract of black mulberry leaves with 33.1%. This was followed by methanol extract of the fruit

with 28.7% inhibition. The lowest activities were shown in the both of water extracts of leaves and fruits with 12.1% and 24.3% inhibition, respectively (Figure 1).

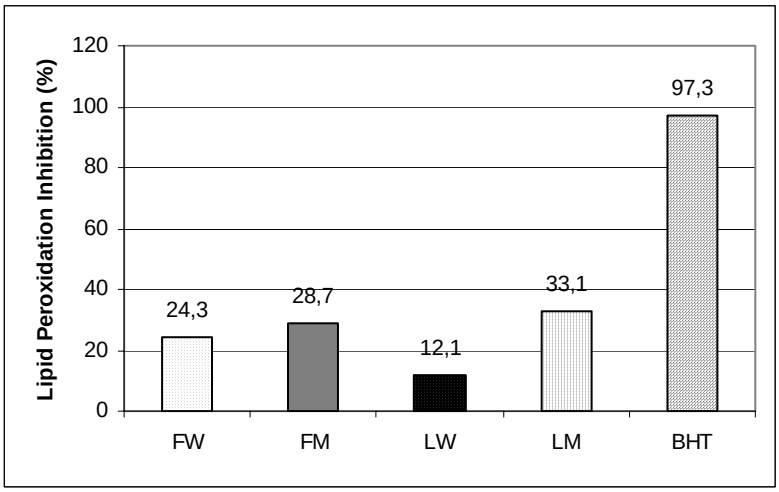

Figure 1. The inhibition of lipid peroxidation by 100 µg/ml extract and BHT. Measurements were carried out after 24 hours of incubation at 37°C (F: Black mulberry fruit, L: Black mulberry leaf, M: Methanol extract, W: Water extract, BHT: Butylated hydroxytoluene).

Mulberries are good source of sugars, acids and anthocyanin content, which are also responsible for their color, and taste and presumably also their antioxidant properties. Bae and Suh, (2007) reported that the ethanolic extract from mulberry fruit showed moderate inhibitory ability on lipid oxidation (23.7-47.6%) at 76 µg and high inhibitory ability (52.7-73.3%) at 255 µg. In present study, all extract have antioxidant potential, and our findings agree with these data. The antioxidant activity of red and nigra mulberry fruits has been reported in some studies (Kim *et. al.*, 1998; Özgen *et al.*, 2009). This activity was due to polyphenol compenents including anthocyanins in mulberry fruits. Anthocyanin pigments are of prominent importance in mulberry fruits because of their dual role; first they constitute an integral part of the sensory attributes because their levels, various forms and derivatives pertain directly to the colouration of the final product; Second, they have been claimed to possess diverse biological properties and therefore are considered as

secondary metabolites with potential nutritional value (Bae and Shu, 2007).

The DPPH radical scavenging activities of all of the extracts were concentration dependent. DPPH radical scavenging activities of water and methanol extracts of black mulberry fruits and leaves were shown in Figure 2.

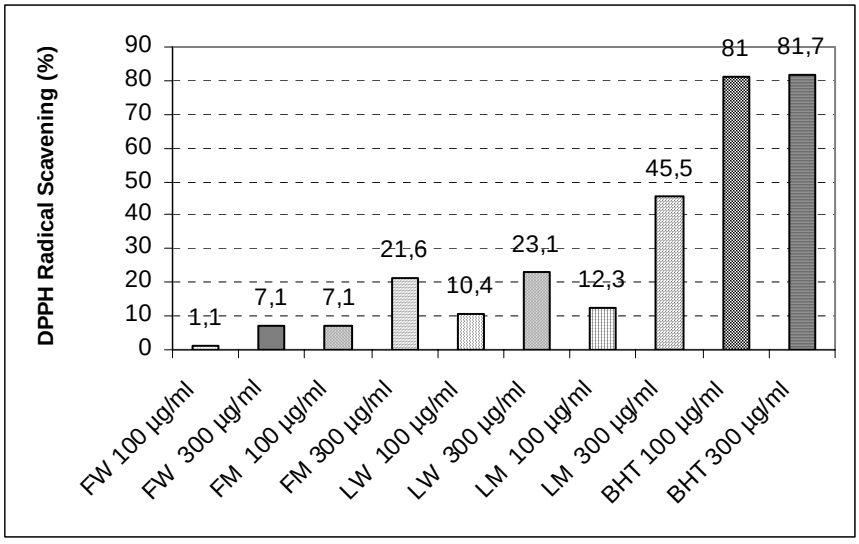

Figure 2. DDPH Radical Scavenging Activity (F: Black mulberry fruit, L: Black mulberry leaf, M: Methanol extract, W: Water extract, BHT: Butylated hydroxytoluene).

The radical scavenging activity in both of methanol extracts of fruits and leaves was higher than water. As a concentration of 100 µg/ml water and methanol extract of black mulberry fruits, the scavenging activities were 1.1% and 7.1%, while at a concentration of 300 µg/ml the respective activities were 7.1% and 21.6%. There was a noticeable extract concentration effect in these extracts. Like fruit extracts, there was a detectable extract concentration activity in the leaves at the studied (100-300 µg/ml) concentration. Thus, at the concentration of 300 µg/ml water extract DPPH radical scavenging activity was 23.9% while it was 45.5% at 300 µg/ml methanol extract of mulberry leaves.

The total phenolic compounds present in extracts were determined using the Folin-ciocalteu phenol reagent. Total phenolic

compounds were higher in 300 µg/ml than 100 µg/ml (Fig. 3). Unlike DPPH radical scavenging activity, there was no detectable activity in the black mulberry fruit and leaf extracts at the studied (100-300 µg/ml) concentration. The highest phenolic compounds was present as 2.6 µg/ml gallic acid equivalent in 300 µg/ml methanol extract of mulberry leaves, whereas the lowest content was obtained from 100 µg/ml methanol extract of mulberry fruits with 0.5 µg/ml gallic acid equivalent.

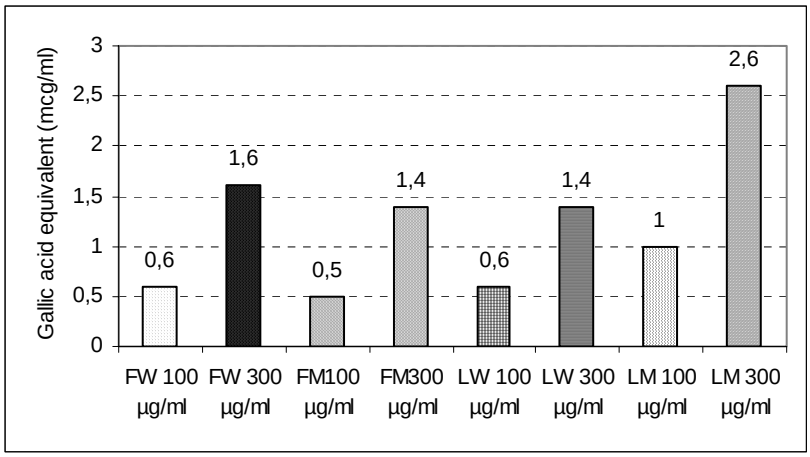

Figure 3. Amount of total phenolic compounds (F: Black mulberry fruit, L: Black mulberry leaf, M: Methanol extract, W: Water extract).

In earlier studies, high levels of total phenolic compounds were detected in wild mulberries (Kim *et al*., 1999). In contrast, Park *et al*. (1997) reported low levels of anthocyanin contents of matured mulberry fruits. In our study the amount of total phenolic compounds are ranged 0.5-2.6 µg/ml. Thus, phenolic compounds of mulberries can be somewhat variable, depending on cultivar and maturation stage.

In fact according to Pearson correlation test there is statistically significant correlation between DPPH radical scavenging and total phenolic compounds ($r = 0.855$, $p < 0.01$); between lipid peroxide inhibition and DPPH radical scavenging ($r = 0.604$, $p < 0.05$). In varies studies total phenolic compounds and DPPH radical scavenging activities was measured in order to estimate the contribution of those substances to antioxidant activity (Hwang et.

al., 2001). According to our results, there is a significant correlation among these parameters that our results compatible above studies. The high potential of phenolic compounds to scavenge radicals may be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups (Sawa *et. al*., 1999).

In conclusion, mulberries have recently been received much attention as potential sources of functional foods due to several biological effects. Our findings on antioxidant activity of mulberry fruits and leaves could justify some biological uses of this plant. Isolation and identification of phenolic compounds existed mulberry fruits and leaves will be the subject of further research projects.

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