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Antioxidant and Phenolic Profile of Mahaleb Plant as a Functional Food

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Abstract: *Prunus mahaleb* L. (syn. *Cerasus mahaleb* L. Mill.) (mahaleb) is a member of medicinal plants and grows naturally in Tokat and in some other regions of Turkey. Seed and flesh of mahaleb fruit are used as mahaleb vine, mahaleb flour, mahaleb oil and as puree.Wild fruits contain many bioactive compounds, such as phenolic compounds, anthocyanins, flavonoids, and vitamins C and tocopherols. Depending on its health promoting effects it is usually used in folk medicine, such as for diabetes. It is shown that wild fruits and other parts of the mahaleb tree possess free radical scavenging, antioxidant, anti-inflammatory, and anticancer activities, and are classified as functional foods in preventing several chronic diseases. The present study evaluates the phenolic contents and antioxidant activities of the methanolic extracts of the fruits, leaves and barks of mahaleb plant that is collected from Tokat. The total phenolic content (170.21 mg gallic acid GA/g) in barks and the total flavonoid (260.5 mg quercetin QE/g) and anthocyanin (38.54 mg catechin CA/g) contents in fruits were greater than the other parts of the plants. Antioxidant activity of the samples was determined using the 2,2-diphenil-1picrylhydrazyl radical (DPPH). The antioxidant activity was the highest with the fruit, which showed 90.2% in DPPH assays. This study showed that all parts of mahaleb have high phenolic, flavonoid, and anthocyanin contents, potentially. These findings suggest that mahaleb plant (fruits can be consumed; the other parts not consumed but can be used in other ways) could serve as a source of bioactive compounds and as being rich in anthocyanin pigments, it could be used as a natural food colorant and antioxidant component in the utilization formula of functional foods.

Key words: *Prunus mahaleb* L., phenoliccompounds, anthocyanins, flavonoids, antioxidant, functional foods.

1. Introduction

Mahlab or mahaleb cherry (*Prunus mahaleb* L.) is a perennial shrub or tree of the Rosaceae family. Its homeland is Anatolia and West Asia; also found in Central Europe [1]. It has white flowers that bloom in April-May, has toothed edged leaves that are wide and round. In May-June, 6-12 mm in length fruits are juicy and single-core, as it ripens, it turns from yellow to red and then to black [2] (Fig. 1). Fruits are sour and acrid with bitter taste and strong aroma. The genus *Prunus* has several plant species that produce a great deal of raw materials for the horticulture, ornamental, food, and pharmaceutical industries [2, 3]. Crops of this genus, are mainly used as fresh fruits, processed sweets, jams, juices, candies, and natural sweeteners in pharmaceutical products [3].

In Turkey, fruits and seeds of the mahaleb trees have been used as a tonic for the heart problems. It is also a traditional medicine for diabetes and gastrointestinal problems [4]. The oil of the kernels contains little amounts of cyanogenic glycosides and coumarin derivatives [5]. The seed kernels have been used to treat pediatric diarrhea, and used as sedative and vasodilator in some countries [6].

A considerable interest has been developed over the years in fruits and vegetables due to their potential biological and health promoting effects. Due to the health benefits attributed to various fruits, numerous studies have been conducted in recent years to evaluate their properties in terms of bioactivity [7]. The protective effect of fruits has been attributed to their bioactive antioxidant constituents, including vitamins, lycopin, carotenoids, polyphenols [8]. Polyphenols are secondary metabolites of plants involved in antioxidative

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Fig. 1 General view of *Prunus mahaleb* L. [2].

defence against biotic and abiotic stresses. Among various antioxidant compounds present in fruits and vegetables, polyphenols (including anthocyanins) have received much attention since it was reported to have a positive influence on human health [7, 8].

Small coloured fruits, such as mahaleb cherries are important source of polyphenols. They are potentially raw material for functional foods because of substantial content of polyphenols, especially anthocyanins. In these fruits, polyphenols may contribute to the bitterness, astringency, colour, flavour, odour and oxidative stability [9].

Colour is the most important indicator of maturity in many fruit species. Fruit colouration is influenced by the concentration and distribution of various anthocyanins in the skin. Red fruits, including mahaleb and sour cherries, are rich in these types of compounds [10]. Anthocyanins, have been reported to possess anti-inflammatory, anti-neurodegenerative, and anti-oxidative activities [11], which are considered as a natural source of antioxidants that can reduce the risk of degenerative diseases caused by oxidative stress, such as cardiovascular diseases and cancer [12]. They are identified as anti-inflammatory and anti-carcinogenic agents [13].

The presence of phenolic substances, including anthocyanins, cyaniding 3-glycosides, O-coumaric acids, and quercetin derivatives in mahaleb has been previously reported [14]. Total phenolic content and antioxidant activity of natural extracts are of particular interest to food and drug industry which are looking for plant extracts with significant medicinal properties to be used as food and for treatment of certain health problems [15].

There is a growing interest in searching antioxidants from plant sources. Mahaleb is a medicinal plant, different parts of this plant such as leaf, bark and fruits have strong antioxidative properties and are used in medicinal drug preparation. Fruits of this plant are also consumed as functional food. On the other hands, mahaleb is not recognized as cherry or sour cherry which are the other members of the same genus. Therefore, the aim of this work is to evaluate various phenolic contents and antioxidant activities of the methanolic extracts of the fruits, leaves and barks of mahaleb plant that is collected from Tokat.

2. Materials and Methods

2.1 Plant Materials

The black mahlab (*P. mahaleb* L.) samples were collected in June season in Tokat Turkey. The fruit and seeds were separated by hand, the fruit part was freeze-dried using a freeze-drier and stored at 4 °C and dark conditions until analyses.

2.2 Preparation of Extracts

Dried leaves and barks were ground to powder using a mortar and pestle. Thirty grams of each sample were extracted with 300 mL of 95% methanol for 48 h at room temperature. The extracts were filtered and dried with rotary evaporator, and then freeze-dried. For the fruits, all the extraction steps were the same as above except for adding of (400 mL of 1 M sodium acetate, 240 mL of 1 M HCl and 360 mL of water) to fresh fruits. Plant extracts were kept in refrigerator prior to the assays. The mixture was homogenized and centrifuged twice at 4 °C at 5,000 *g* for 15 min. The supernatant was collected and used for analysis.

2.3 Measurement of Anthocyanin Content

Pigments were extracted with 5 mL acidified (1% HCl, *w/v*) methanol by shaking the extracts for 48 h at 4 °C. The absorbance of the extracts, clarified by filtration, was measured at 530 and 657 nm. The Formula A530-0.25 A657 was used to compensate for the contribution of Chlandits degradation products to the absorbtion at 530 nm [16].

2.4 Determination of TFC (Total Flavonoid Content)

The TFC was determined using the method suggested by Huang et al. [17] with minor modifications. Five mL of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract (0.4 mg/mL). Absorption of the resulting solution was read at 367 nm using UV-visible spectrophotometer (Cecil, UK) against a blank sample containing 5 mL extract solution with 5 mL methanol without $AlCl_3$. The TFC was determined using a standard curve with quercetin as the standard. TFC was expressed as mg of quercetin equivalents (QE) per gram of dry extract (mg QE/g DE). All experiments were performed in triplicate.

2.5 Determination of Total Phenolic Content

Total phenolic content of rocket extracts was spectrophotometrically determined by Folin Ciocalteureagentassay according to Singleton and Rossi [18], using gallic acid as a standard compound for the preparation of calibration curve (20-120 mg/L). Total phenolic content of samples was measured at 670 nm and expressed as mg gallic acid equivalents (GAE)/g freshweight.

2.6 Determination of Antiradical Efficiency of Samples

Antiradical efficiency of the samples was determined using 2,2-diphenyl-1-icrylhydrazyl (DPPH) free radical. Firstly, percent inhibition values of samples were determined. For this aim, 0.1 mL of extract was mixed with 3.9 mL of DPPH solution (0.1 mM in methanol), and the mixtures were incubated for 30 min in a dark place at room temperature. Absorbance of the samples was measured at 517 nm (Agilent 8453 spectrophotometer, USA) at the end of the incubation period.

Table 1 Anthocyanin, total phenolic, total flavonoid contents and antioxidant (DPPH) activity of methanolic extracts of leaves, bark andfruits of mahalebtree.

	Anthocyanin content (mg/g fresh weight (FW))	Total phenolics (mg GAE/g FW)	Total flavonoids (mg QE/mg FW)	DPPH activity (%)
Leaf	24.03 ± 0.06	126.04 ± 0.33	138 ± 0.63	79.35 ± 0.29
Bark	13.43 ± 0.07	170.21 ± 0.12	192 ± 0.82	88.23 ± 0.34
Fruit	38.54 ± 0.04	162.52 ± 0.25	260.5 ± 0.68	90.20 ± 0.38
Ascorbicacid	-	-	-	78.12 ± 0.24
Butylated Hydroxytoluene (BHT)	-	-	-	76.05 ± 0.26
Butylated hydroxyanisole (BHA)	-	-	-	76.35 ± 0.34
α-tocopherol	-	-	-	71.56 ± 0.12

2.7 Statistical Analyses

Experimental data were evaluated by using analysis of variance (ANOVA) and the significant differences amongst the means of the three replicates. The level of statistical significance was set at $p \leq 0.05$.

3. Results and Discussion

Regarding the radical scavenging activity of mahaleb, the highest antioxidant activity (90.20%) was found in fruit, followed by bark (88.23%) and leaf (79.35%). The reducing powers of positive controls (vitamin C, BHT, vitamin E), exhibited slightly lower reducing power (78.12%, 76.05%, 71.56) respectively, that can be attributed to mahalebs high phenolic content (Table 1). According to these results, the antioxidant activity was the highest with the fruit, which showed 90.2% in DPPH assays (Table 1).

The antioxidant activity of phenolic is due to their capacity to scavenge free radicals, donate hydrogen atoms or electrons, and chelate metal cations [19]. The results obtained in this study showed that the highest antioxidant activity was observed in the parts that have the highest phenolic content. These results are in agreement with previous studies [20, 21] which reported a correlation between free radical scavenging activity and TFC.

Flavonoids are a very diverse group of polyphenolic compounds in plants possessing antioxidant activity [22]. It has been reported that the flavonoids of mahaleb vary among genotypes and different parts of the mahaleb tree [23]. In this study, the highest total flavonoid content was found in fruits compared with leaves and barks (Table 1). Total phenolic content (170.21 mg GA/g) in barks and total flavonoid (260.5 mg QE/g) and anthocyanin (38.54 mg CA/g) contents in fruits were greater than the other parts of the plants. Anthocyanins are natural phenolic compounds that give color to the plants, vegetables, and fruits. They protect plants against a variety of oxidants through a number of mechanisms [24]. Anthocyanins possess known pharmacological activities and are used by humans for therapeutic purposes. In this study fruits have higher anthocyanins significantly, compared to leaves and bark (Table 1). These results indicated that mahaleb fruits can serve as a rich source of anthocyanins.

4. Conclusion

The findings of the present study showed that mahaleb plant contains high levels of total phenolic, flavonoid, anthocyanin compounds in different plant parts, especially in riped black fruits, suggesting the importance of mahaleb as a natural source of bioactive compounds that could be applicable in food, pharmaceutical, and cosmetic industries. These findings may be crucial in future research concerning mahaleb-based functional food products, a promising component of designed food with enhanced antioxidant potential.

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