

Phenolic Composition and Antioxidant Activity of Myrtle Fruits and Leaves Grown in Antalya (Türkiye)

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Abstract

Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the family of Myrtaceae that grows spontaneously throughout the Mediterranean area. In Türkiye, myrtle tree is grown in pine forests and riversides, particularly in the Taurus mountains, from sea level to 500–600 m. Their antioxidant activity has been attributed to the presence of phenolic compounds and essential oils. The purpose of this work is to characterize myrtle plants through its physical and chemical characteristics such as phenolic and flavonoid content, antioxidant activity for selecting the promising genotypes in Antalya coastal region of Turkey. Phenolic compounds were extracted from leaves and berries. Phenolic composition was determined by LC-MS-MS. Antioxidant activity was measured with 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Phenolic substance was higher in the leaf samples collected from Kumluca (BM15 and BM16) and in the fruit samples collected from Kemer (BM13) and Kaş (BM19) when compared to other regions. High antioxidant activity was detected in the leaf sample BM16 collected from Kumluca (0.13 µl) and the fruit sample (BM19) collected from Kaş (0.45 µl). It was determined that significantly differences in the phenolic compositions and antioxidant activities of myrtle leaves and fruits were to be found among genotypes grown in different locations.

1. Introduction

Myrtle (*Myrtus communis* L.) is a medicinal and aromatic plant which belongs to the Myrtaceae family has a perennial, evergreen, shrub-shaped and sized up to 1-3 m plants. Wild forms of the myrtle plant grown in the coastal sides of Tunisia, Morocco, Turkey and France while it is cultured in Iran, Spain, Italy and Corsica (Jamoussi et al., 2005). Myrtle, which is one of the typical natural plants of the Mediterranean Basin, is found in natural forms in Adana, Antalya, İçel, Çanakkale, İstanbul, Zonguldak, Sinop, Ordu, Trabzon, İzmir, Samsun, Muğla and Hatay provinces in Turkey. *Myrtus communis* is generally called as mersin in Turkey but also known as “murt”, “hambeles” and

“adi mersin” in the Mediterranean coastal sides. In addition, its leaves are named “bahar” in some places (Oğur, 1994). In Turkey, myrtle plant grows between the coastal strip and pine forests, particularly in heights of up to 500-600 meters from the sea in the Taurus Mountains (Kaya and Aladağ, 2009).

Because of its medicinal and aromatic properties, the myrtle plant has been used as a medicinal herb in the folk medicine for many years in Turkey. Myrtle plants with white colored fruits were started to cultivate prior to the black ones because their larger fruits but they have very short shelf-life and low antioxidant capacity. White myrtle plants are cultivate by grafting on the wild plants grown in the forests or in the border of orchards.

Recently, It is getting more interest to dark colored and organically grown fruits in the world. It is reported that there is a high correlation between antioxidant activity and the amount of phenolic substance in the fruits and leaves. It is stated that the basic phenolics in myrtle are flavonoids and anthocyanins. The amount of myricetin-3-O-galactoside, myricetin-3-O-rhamnoside and quercetin-3-O-glucoside were found to be high among flavonoids (Montoro et al., 2006). Therefore, recently the demand for black myrtle has increased, as in other black colored fruits, due to its high antioxidant content (Martin et al., 1999). In addition to being consumed fresh, the fruits can also be consumed dried fruit or used in liquor production.

It was found that myrtle leaves contain a modest amount of phenolic acid (caffeic, ellagic acid and gallic acid) and quercetin derivatives (quercetin-3-O-galactoside and quercetin-3-O-rhamnoside) while the amount of catechin derivatives (Epigallocatechin, Epigallocatechin-3-O-gallate) and myricetin derivatives (myricetin-3-O-galactoside, myricetin-3-O-rhamnoside) were found to be high (Reynertson et al., 2008). In addition, it is reported that the leaves have strong antioxidant activity due to their chemical structure (Romani et al., 2004). The leaves and fruits are used due to their constipating, disinfectant, appetizing and hemostatic features (İliçim et al., 1998). It is stated that the myricetin extracted from the leaves contributes to the treatment of a wide range of health problems such as rheumatism, cardiovascular diseases, bronchitis and colds. It was found that myricetin extracted from the leaves was effective in the correction of renal dysfunction in experimental animals (Özcan, 2009). A number of studies have been conducted to examine various therapeutic effects and anti-oxidant, anti-

carcinogenic and antiaggregant properties of myricetin (Tzeng et al., 1991; Ong and Khoo, 1997).

Myrtle has a wide range of usage due to high therapeutic phenolic content of leaves and fruits. Identification of the substances constituting the content of leaves and fruits is important to get the highest benefit from the plant. Research shows that the collection time, origin and extraction method of the material significantly affect the content of the plant, and certain substances that are considered an indispensable compound in some places can not be detected by other researchers (Akgül and Bayrak, 1989). In this respect, identification of the content of myrtle plants that grow naturally in Antalya conditions, selection of the promising types and cultivation hereof is important in terms of providing materials for future research.

2. Material and Method

2.1. Plant material and locations

The leaves and fruits of the black myrtle plant grown in the natural flora of Antalya were used as material. Leaves and fruits 19 genotypes were collected from 10 different districts of Antalya and the location information of the collected samples is given in Table 1. Myrtle fruit and leaves were collected from elevations between 50 and 470 m. Myrtle plant is widely available up to 400-500 m altitudes. As a result of the surveys conducted, myrtle plants were also found at altitudes above 500 m (up to 850 m). However, the plant density was observed to be quite low. Fruit size was taken as the major selection criteria. Fruits and leaves are coded as BM (Black Myrtle) and numbered from 1 to 19 (Table 1).

Table 1. Location information.

| Genotype | Location | GPS record |
|----------|----------|----------------------------------|
| BM1* | Gazipaşa | 160 m, 36° 21.600K, 032° 22.766D |
| BM2 | Gazipaşa | 283 m, 40° 28.203K, 36° 44.7365D |
| BM3 | Alanya | 455 m, 36° 34.902K, 031° 59.604D |
| BM4 | Alanya | 370 m, 36° 30.162K, 032° 09.090D |
| BM5 | Manavgat | 67 m, 36° 56.756K, 031° 13.067D |
| BM6 | Serik | 50 m, 37° 07.184K, 030° 54.693D |
| BM7 | Serik | 50 m, 37° 07.184K, 030° 54.693D |
| BM8 | Serik | 290 m, 37° 13.222K, 030° 57.435D |
| BM9 | Serik | 330 m, 37° 14.156K, 030° 58.422D |
| BM10 | Merkez | 230 m, 36° 53.943K, 030° 30.697D |
| BM11 | Merkez | 470 m, 36° 54.003K, 030° 29.907D |
| BM12 | Merkez | 220 m, 36° 52.694K, 030° 30.749D |
| BM13 | Kemer | 205 m, 36° 32.399K, 029° 31.701D |
| BM14 | Kemer | 424 m, 36° 29.298K, 030° 27.151D |
| BM15 | Kumluca | 200 m, 36° 19.447K, 030° 25.021D |
| BM16 | Kumluca | 288 m, 36° 24.207K, 029° 53.456D |
| BM17 | Finike | 343 m, 36° 24.197K, 029° 53.425D |
| BM18 | Demre | 166 m, 36° 15.047K, 029° 57.121D |
| BM19 | Kaş | 254 m, 36° 23.263K, 029° 52.030D |

*BM: Black Myrtle

2.2. Methods

In order to determine the quality criteria in the samples, 10 piece of fruit weight (g), 10 piece of seed weight (g), fruit flesh / seed ratio (%), fruit and leaf size (cm), number of seeds in the fruit (pieces), pH and titratable acidity in fruits (malic acid %), brix (%), total phenolic and flavonoid substance, antioxidant activity and phenolic component analyzes were analysed.

2.2.1. Extraction

In the extraction of phenolic substances from the samples, the method suggested by [Cai et al. \(2004\)](#) was used. Leaves and berries were collected in each area at the time of industrial ripeness, when the berries were fully dark-violet pigmented. After the harvest, the samples were carried to the laboratory, cleaned from impurities and dried at room temperature. After, the leaves and berries were separated and ground in a grinder. 2 g of dried fruit and leaf samples were extracted with 20 ml of 80% methanol for 3 times at room temperature, centrifuged at 4500 rpm (1585 g) for 10 min, and then supernatant passed through a 0.45 µm syringe filter. The extracts were stored at -18°C until analysis.

2.2.2. Total phenolic content

In the colorimetric determination of total phenolic content, the spectrophotometric method defined by [Spanos and Wrolstad \(1990\)](#) was used. For this purpose, 100 µl of extract were taken into a tube and 900 µl of distilled water was added on it. Then, 5 ml of 0.2 N Folin-Ciocalteu solution and 4 ml of saturated sodium carbonate solution (75 g l⁻¹) were added, the tubes were thoroughly stirred by vortex and left in the dark for 2 hours. The total amount of phenolic compound was calculated by utilizing the absorbance value read on the spectrophotometer at the 765 nm wavelength and the curve prepared with gallic acid (GA). Results were expressed as g of gallic acid equivalent (GAE) g⁻¹ dry matter of plant material (g GAE g⁻¹ DM)

2.2.3. Total flavonoid content

The method described by [Karadeniz et al. \(2005\)](#) was used in the colorimetric determination of the total amount of flavonoids by aluminum chloride. Four ml of distilled water and 0.3 ml of 5% NaNO₂ was added on 1 ml of sample and stirred. 0.6 ml of 10% AlCl₃.6H₂O was added after 5 minutes, and 2 ml of 1 mol l⁻¹ NaOH was added 5 minutes after that, and the total volume was filled with 10 ml of distilled water. After stirred thoroughly, the total amount of flavonoid was calculated as the equivalent to mg catechin grams of fruit by using the absorbance value read at 510 nm on spectrophotometer and the catechin curve prepared. The results were

expressed as g of catechin equivalent (CTE) g⁻¹ dry matter (g CTE g⁻¹ DM).

2.2.4. DPPH radical scavenging activity

Stable radical DPPH solution was used in the measurements. The volume was completed to 6 ml by adding various amounts of (10, 20, 40, 60, 80, 100 µl) extracts on 600 µl of DPPH solution prepared with methanol. The tubes were stirred with vortex and kept at room temperature in the dark for 15 minutes. 600 µl of DPPH was added with 5.4 ml of methanol to be used as witness, and allowed to stand for 15 minutes under the same conditions as the samples. After incubation, the absorbance value of the tube contents at 515 nm was read using the spectrophotometer. The % inhibition values of the sample extracts were calculated using the following formula.

$$\text{Inhibition (\%)} = \left[\frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}} \right] \times 100$$

A_{DPPH}: Absorbance value of DPPH witness sample

A_{sample}: Absorbance value of the sample extract

The % inhibition values obtained from different amounts of sample extracts and the concentration values were graphed and the concentration (EC₅₀) reducing the effect of DPPH by 50% for each sample was calculated ([Cemeroğlu, 2010](#)).

2.2.5. Analyses of phenolic compounds through LC-MS-MS

The method described by [Fischer et al. \(2011\)](#) was used with some modification. Phenolic compounds of myrtle samples were identified through LC-MS / MS (High-performance Liquid Chromatography Coupled With Tandem Mass Spectrometry). Agilent 6430 Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA) brand electrospray ion-derived mass spectrometry and Agilent-1290 Infinity (Agilent Technologies, Waldbronn, Germany) brand liquid chromatography were used in the analyses. The study was conducted in positive and negative ion mode. The study was carried out at a flow rate of 0.25 ml min⁻¹ in the Zorbax SB-C18 (150x2.1 mm, 1.8 CAm) (Agilent Technologies, Palo Alto, CA) column. Solutions at different concentrations from the standards were prepared and calibration curves were formed for quantitation. Myrtle samples were injected by passing through a 0.45 µm diameter PVDF (polyvinylidene fluoride) filter. The injection volume was 10 µl. The mobile phase used in the study was Solvent A = (5/95) Methanol: Water (containing 0.01% formic acid and 5 mM ammonium formate) and Solvent B = Methanol (containing 0.01% formic acid and 5 mM ammonium formate). The elution profile used is as follows: 0-1 min 5% solvent B (constant flow), 1-3 min 30% solvent B, 3-

4 min 60% solvent B, 4-5 min 60% solvent B (constant flow), 5-6 min 70% solvent B, 6-8 min 80% solvent B, 8.01 min 5% solvent B, 8.01-10 min 5% solvent B (constant flow).

2.2.6. Statistical analysis

All analyses were performed with 3 replications and the mean and standard deviation (\pm SD) values were calculated (Düzgüneş et al., 1987).

3. Results and Discussion

3.1. General characteristics of fruits, leaves and seeds

Some physical and biochemical properties of the myrtle samples collected are given in Tables 2 and 3. Fruit weight was between 0.538-0.993 g, fruit width between 0.90 -1.24 cm, fruit length between 1.03-1.43 cm, pH between 5.04-5.94, titratable acidity between 0.23-0.97%, and brix between 11.5-21.5%. The width, length and stem length of the myrtle leaves vary between 1.12-1.68 cm, 3.05-4.16 cm and 0.20-0.32 cm, respectively.

The average number of seeds in the fruits ranged from 8 to 26 pieces/fruit and the weight of seeds ranged from 0.06 to 0.17 g in all tested genotypes. Seed / fruit ratio was found to be between 10.45 and 23.66% (Table 3).

Our findings show similarities with the literature. Indeed, Traveset et al. (2001) found that the fruit weight of the wild black myrtle growing in Italy was 0.54 g, seed weight was 7.16 mg, fruit length was 10.87 mm, fruit width was 10.21 mm and the number of seeds per fruit was 12.06. Tuberoso et al. (2007) reported the size of the berries grains to be between 0.19-0.41 g in their study conducted in

Italy. The number of seeds per berry varied between 4 and 16. Fadda and Mulas (2010) found that the weight of fresh fruit was about 400 mg after 150 days from blooming in the Barbara cultivar and about 800 mg after 180 days from blooming in the Daniela cultivar, in a study they conducted in Corsica.

3.2. Total phenolic and flavonoid substance and antioxidant activity of myrtle

The total amount of phenolic and flavonoid substance and antioxidant activities of myrtle fruits and leaves are given in Table 4. The total amount of phenolic substance in the leaves was found to be 69.05 mg GAE g⁻¹ on average. The maximum amount of phenolic substance was found in the BM16 and BM15 genotypes collected from Kumluca with 92.12 and 90.14 mg GAE g⁻¹ while the least amount was found in the leaves of the BM3 genotype collected from Alanya with 50.33 mg GAE g⁻¹.

The total amount of flavonoid in the leaves was found to be 3.82 mg CTE g⁻¹ on average, where the highest amount was found in the BM15 genotype with 6.38 mg CTE g⁻¹ while the lowest amount was found in the leaves of the BM3 genotype with 2.53 mg CTE g⁻¹.

The antioxidant activity was detected to be EC₅₀. A low value indicates high antioxidant activity. The average antioxidant activity of the extracts obtained from the leaves was found to be 0.32 μ l. While the highest activity was seen in the leaves of the BM16 genotype with a total phenolic substance of 0.13 μ l, the lowest activity was observed in the leaves of the BM11 genotype with 0.46 μ l. The correlation between antioxidant activity and the total amount of phenolic substance ($r = -0.74526$) and flavonoid

Table 2. Some physical and biochemical properties of myrtle fruit (\pm SD).

| Genotype | 10 piece of fruit weight (g) | Fruit width (cm) | Fruit length (cm) | pH | Titratable acidity (malic acid %) | Brix (%) |
|----------|------------------------------|------------------|-------------------|-----------------|-----------------------------------|------------------|
| BM1* | 9.93 \pm 0.75 | 1.24 \pm 0.05 | 1.32 \pm 0.08 | 5.44 \pm 0.05 | 0.23 \pm 0.06 | 15.50 \pm 0.71 |
| BM2 | 6.66 \pm 0.34 | 0.97 \pm 0.07 | 1.40 \pm 0.04 | 5.78 \pm 0.06 | 0.40 \pm 0.05 | 14.30 \pm 0.42 |
| BM3 | 6.66 \pm 0.50 | 1.15 \pm 0.06 | 1.12 \pm 0.04 | 5.33 \pm 0.06 | 0.67 \pm 0.09 | 16.00 \pm 1.41 |
| BM4 | 7.05 \pm 0.17 | 1.06 \pm 0.06 | 1.29 \pm 0.07 | 5.40 \pm 0.11 | 0.37 \pm 0.05 | 16.50 \pm 0.71 |
| BM5 | 8.59 \pm 0.22 | 1.16 \pm 0.06 | 1.36 \pm 0.07 | 5.11 \pm 0.01 | 0.69 \pm 0.02 | 11.50 \pm 0.71 |
| BM6 | 7.28 \pm 0.81 | 1.08 \pm 0.06 | 1.29 \pm 0.05 | 5.86 \pm 0.05 | 0.62 \pm 0.07 | 20.90 \pm 0.14 |
| BM7 | 6.72 \pm 0.32 | 1.13 \pm 0.07 | 1.20 \pm 0.03 | 5.91 \pm 0.07 | 0.42 \pm 0.02 | 18.50 \pm 0.71 |
| BM8 | 7.48 \pm 0.37 | 1.12 \pm 0.05 | 1.43 \pm 0.08 | 5.68 \pm 0.09 | 0.47 \pm 0.09 | 13.60 \pm 0.57 |
| BM9 | 7.20 \pm 0.59 | 1.11 \pm 0.06 | 1.32 \pm 0.06 | 5.75 \pm 0.04 | 0.39 \pm 0.02 | 16.90 \pm 0.14 |
| BM10 | 5.97 \pm 0.67 | 1.05 \pm 0.07 | 1.16 \pm 0.06 | 5.42 \pm 0.21 | 0.57 \pm 0.09 | 18.40 \pm 0.85 |
| BM11 | 5.79 \pm 0.65 | 1.03 \pm 0.06 | 1.22 \pm 0.04 | 5.64 \pm 0.09 | 1.22 \pm 0.12 | 21.50 \pm 0.71 |
| BM12 | 7.06 \pm 0.46 | 1.05 \pm 0.05 | 1.36 \pm 0.09 | 5.69 \pm 0.19 | 0.95 \pm 0.07 | 16.40 \pm 0.85 |
| BM13 | 5.53 \pm 0.23 | 1.00 \pm 0.05 | 1.36 \pm 0.05 | 5.62 \pm 0.01 | 0.42 \pm 0.02 | 18.75 \pm 0.35 |
| BM14 | 6.26 \pm 0.68 | 1.08 \pm 0.05 | 1.15 \pm 0.05 | 5.56 \pm 0.02 | 0.39 \pm 0.02 | 16.85 \pm 0.21 |
| BM15 | 5.51 \pm 0.09 | 1.03 \pm 0.06 | 1.14 \pm 0.07 | 5.54 \pm 0.12 | 0.45 \pm 0.02 | 15.50 \pm 0.71 |
| BM16 | 8.65 \pm 0.92 | 1.19 \pm 0.04 | 1.47 \pm 0.10 | 5.62 \pm 0.02 | 0.35 \pm 0.02 | 14.75 \pm 0.35 |
| BM17 | 5.65 \pm 0.31 | 0.95 \pm 0.07 | 1.13 \pm 0.04 | 5.50 \pm 0.05 | 0.42 \pm 0.07 | 11.50 \pm 0.71 |
| BM18 | 5.38 \pm 0.25 | 0.90 \pm 0.04 | 1.03 \pm 0.04 | 5.04 \pm 0.06 | 0.97 \pm 0.19 | 19.50 \pm 0.71 |
| BM19 | 6.17 \pm 0.84 | 1.07 \pm 0.08 | 1.23 \pm 0.06 | 5.45 \pm 0.08 | 0.54 \pm 0.05 | 19.60 \pm 0.57 |

*BM: Black Myrtle

Table 3. Some physical properties of leaf and seed of myrtle (\pm SD).

| Genotype | Leaf width (cm) | Leaf length (cm) | Petiole length (cm) | Number of seeds in the fruit (piece) | 10 piece of seed weight (g) | Seed/fruit ratio (%) |
|----------|-----------------|------------------|---------------------|--------------------------------------|-----------------------------|----------------------|
| BM1* | 1.65 \pm 0.28 | 4.07 \pm 0.23 | 0.27 \pm 0.05 | 26 \pm 4 | 0.08 \pm 0.01 | 18.59 \pm 2.70 |
| BM2 | 1.22 \pm 0.16 | 3.41 \pm 0.28 | 0.29 \pm 0.02 | 11 \pm 3 | 0.06 \pm 0.01 | 11.07 \pm 0.54 |
| BM3 | 1.19 \pm 0.07 | 3.34 \pm 0.19 | 0.20 \pm 0.05 | 25 \pm 3 | 0.06 \pm 0.01 | 20.15 \pm 0.53 |
| BM4 | 1.60 \pm 0.14 | 4.07 \pm 0.36 | 0.22 \pm 0.01 | 21 \pm 3 | 0.06 \pm 0.01 | 18.45 \pm 2.78 |
| BM5 | 1.50 \pm 0.25 | 2.87 \pm 0.20 | 0.25 \pm 0.04 | 8 \pm 3 | 0.17 \pm 0.01 | 15.62 \pm 4.26 |
| BM6 | 1.49 \pm 0.28 | 3.38 \pm 0.38 | 0.28 \pm 0.04 | 16 \pm 2 | 0.09 \pm 0.01 | 18.97 \pm 0.78 |
| BM7 | 1.64 \pm 0.23 | 4.09 \pm 0.46 | 0.29 \pm 0.04 | 9 \pm 2 | 0.11 \pm 0.01 | 13.20 \pm 2.59 |
| BM8 | 1.19 \pm 0.12 | 3.38 \pm 0.29 | 0.30 \pm 0.02 | 13 \pm 3 | 0.13 \pm 0.01 | 20.81 \pm 1.97 |
| BM9 | 1.18 \pm 0.11 | 3.02 \pm 0.32 | 0.24 \pm 0.02 | 10 \pm 2 | 0.09 \pm 0.01 | 14.44 \pm 3.96 |
| BM10 | 1.53 \pm 0.47 | 3.49 \pm 0.60 | 0.25 \pm 0.06 | 9 \pm 2 | 0.17 \pm 0.01 | 23.66 \pm 2.40 |
| BM11 | 1.31 \pm 0.07 | 3.55 \pm 0.16 | 0.24 \pm 0.03 | 9 \pm 2 | 0.10 \pm 0.01 | 12.30 \pm 0.68 |
| BM12 | 1.45 \pm 0.09 | 3.67 \pm 0.22 | 0.28 \pm 0.04 | 19 \pm 2 | 0.08 \pm 0.01 | 21.53 \pm 2.71 |
| BM13 | 1.68 \pm 0.10 | 4.16 \pm 0.35 | 0.32 \pm 0.05 | 9 \pm 1 | 0.13 \pm 0.03 | 18.68 \pm 0.01 |
| BM14 | 1.31 \pm 0.06 | 3.37 \pm 0.60 | 0.25 \pm 0.02 | 17 \pm 3 | 0.10 \pm 0.01 | 23.64 \pm 3.06 |
| BM15 | 1.22 \pm 0.11 | 3.05 \pm 0.24 | 0.25 \pm 0.02 | 8 \pm 1 | 0.06 \pm 0.01 | 10.45 \pm 3.28 |
| BM16 | 1.16 \pm 0.08 | 4.16 \pm 0.12 | 0.24 \pm 0.02 | 16 \pm 4 | 0.09 \pm 0.01 | 14.47 \pm 2.37 |
| BM17 | 1.42 \pm 0.10 | 3.11 \pm 0.24 | 0.27 \pm 0.02 | 14 \pm 1 | 0.07 \pm 0.01 | 22.78 \pm 2.82 |
| BM18 | 1.56 \pm 0.14 | 3.63 \pm 0.17 | 0.25 \pm 0.05 | 12 \pm 1 | 0.11 \pm 0.02 | 14.35 \pm 0.60 |
| BM19 | 1.12 \pm 0.18 | 3.27 \pm 0.35 | 0.24 \pm 0.02 | 15 \pm 1 | 0.07 \pm 0.01 | 19.07 \pm 0.18 |

*BM: Black Myrtle

Table 4. Total phenolic (TPC), flavonoid (TFC) content and antioxidant activity (AA) of fruit and leaf (\pm SD).

| Genotype | TPC of leaf (mg GAE.g ⁻¹) | TFC of leaf (mg CTE.g ⁻¹) | AA of leaf extract (EC50, μ l) | TPC of fruit (mg GAE.g ⁻¹) | TFC of fruit (mg CTE.g ⁻¹) | AA of fruit extract (EC50, μ l) |
|----------|---------------------------------------|---------------------------------------|------------------------------------|--|--|-------------------------------------|
| BM1* | 65.03 \pm 2.32 | 3.25 \pm 0.11 | 0.30 \pm 0.02 | 21.60 \pm 0.23 | 1.30 \pm 0.01 | 0.96 \pm 0.08 |
| BM2 | 72.84 \pm 6.67 | 3.79 \pm 0.26 | 0.32 \pm 0.03 | 13.17 \pm 1.01 | 1.04 \pm 0.04 | 1.12 \pm 0.14 |
| BM3 | 50.33 \pm 3.33 | 2.53 \pm 0.08 | 0.44 \pm 0.02 | 23.78 \pm 3.59 | 1.31 \pm 0.06 | 0.83 \pm 0.02 |
| BM4 | 74.94 \pm 3.20 | 3.80 \pm 0.05 | 0.39 \pm 0.01 | 18.81 \pm 2.48 | 0.71 \pm 0.10 | 1.05 \pm 0.11 |
| BM5 | 60.96 \pm 8.62 | 3.33 \pm 0.23 | 0.29 \pm 0.01 | 17.96 \pm 1.43 | 1.19 \pm 0.07 | 1.28 \pm 0.11 |
| BM6 | 54.72 \pm 0.60 | 2.97 \pm 0.15 | 0.39 \pm 0.01 | 22.62 \pm 0.24 | 1.39 \pm 0.08 | 0.94 \pm 0.11 |
| BM7 | 62.59 \pm 3.95 | 3.47 \pm 0.12 | 0.33 \pm 0.01 | 27.64 \pm 1.43 | 1.72 \pm 0.03 | 0.86 \pm 0.18 |
| BM8 | 74.87 \pm 2.07 | 4.11 \pm 0.03 | 0.24 \pm 0.04 | 30.08 \pm 1.40 | 2.57 \pm 0.02 | 0.69 \pm 0.02 |
| BM9 | 77.09 \pm 3.79 | 4.34 \pm 0.09 | 0.33 \pm 0.07 | 30.58 \pm 2.37 | 2.59 \pm 0.06 | 0.80 \pm 0.17 |
| BM10 | 71.79 \pm 1.41 | 3.70 \pm 0.08 | 0.33 \pm 0.03 | 23.39 \pm 0.76 | 1.62 \pm 0.05 | 1.10 \pm 0.05 |
| BM11 | 61.80 \pm 6.48 | 3.20 \pm 0.67 | 0.46 \pm 0.02 | 21.23 \pm 1.95 | 1.25 \pm 0.06 | 1.47 \pm 0.01 |
| BM12 | 68.94 \pm 1.53 | 3.87 \pm 0.04 | 0.35 \pm 0.06 | 22.94 \pm 1.62 | 1.69 \pm 0.06 | 1.23 \pm 0.06 |
| BM13 | 63.34 \pm 7.27 | 3.66 \pm 0.07 | 0.39 \pm 0.08 | 35.01 \pm 1.77 | 2.12 \pm 0.06 | 0.68 \pm 0.13 |
| BM14 | 64.80 \pm 6.28 | 3.49 \pm 0.26 | 0.37 \pm 0.01 | 23.98 \pm 1.04 | 1.22 \pm 0.04 | 1.21 \pm 0.03 |
| BM15 | 90.14 \pm 3.03 | 6.38 \pm 0.01 | 0.19 \pm 0.02 | 27.01 \pm 0.37 | 1.69 \pm 0.02 | 0.66 \pm 0.03 |
| BM16 | 92.12 \pm 2.68 | 5.53 \pm 0.32 | 0.13 \pm 0.03 | 21.42 \pm 2.42 | 1.15 \pm 0.07 | 0.95 \pm 0.04 |
| BM17 | 76.62 \pm 5.96 | 4.16 \pm 0.05 | 0.14 \pm 0.03 | 32.69 \pm 2.68 | 2.06 \pm 0.05 | 0.60 \pm 0.08 |
| BM18 | 64.24 \pm 6.18 | 2.88 \pm 0.60 | 0.35 \pm 0.10 | 25.47 \pm 1.40 | 1.74 \pm 0.05 | 0.91 \pm 0.04 |
| BM19 | 64.70 \pm 0.96 | 4.07 \pm 0.08 | 0.26 \pm 0.01 | 39.16 \pm 0.92 | 3.42 \pm 0.13 | 0.45 \pm 0.03 |
| Average | 69.05 | 3.82 | 0.32 | 25.19 | 1.67 | 0.71 |

*BM: Black Myrtle

substance ($r = -0.73817$) in the leaves were found to be significant.

A total of 25.19 mg GAE g⁻¹ of phenolic compounds were found in myrtle fruits. The highest total phenolic content was found in the fruits of BM19 genotype collected from Kaş with 39.16 mg GAE g⁻¹, while the lowest amount was found in the fruits of the BM2 genotype from Gazipaşa with 13.17 mg GAE g⁻¹.

The total amount of flavonoids in the fruits of myrtle was found to be 1.67 mg CTE g⁻¹ on average. The highest amount of flavonoid was found in BM19 fruits with 3.42 mg CTE g⁻¹ and the lowest amount was found in BM4 genotype with 0.71 mg CTE g⁻¹.

The average antioxidant activity value of the fruits was found to be 0.71 μ l. The highest activity was found in the BM19 genotype (0.45 μ l) which has

the highest amount of phenolic and flavonoid substances, while the lowest amount was found in BM11 fruits (1.47). [Barboni et al. \(2010\)](#) found that the total phenolic content of the fruits of myrtle collected from different ecologies in the Corsica island ranged between 22.20 and 67.43 mg GAE g⁻¹. [Amensour et al. \(2010\)](#) prepared extracts using different solvents from the leaves and fruits of the myrtles they collected from Morocco and found the total amount of phenolic substance in the methanol extract to be 31.25 and 14.68 mg GAE g⁻¹ for leaves and fruit respectively. [Reynertson et al. \(2008\)](#) reported that the total phenolic content of the leaves of the myrtle fruit ranged between 3.57 and 101 mg g⁻¹. According to our findings, it is seen that the total phenolic substance in myrtle leaves range between 50.33 and 92.12 mg GAE g⁻¹ and that in the fruits

between 13.17 and 39.16 mg GAE g⁻¹. Kanoun et al. (2014) found that the total amount of phenol and flavonoids in the leaves of the myrtle they collected from Algeria were 119.23 mg GAE g⁻¹ and 6.56 mg CTE g⁻¹ respectively, while the amounts in the fruits were 70.26 mg GAE g⁻¹ and 3.87 mg CTE g⁻¹ respectively. The amounts of phenolic and flavonoid substances in the myrtle fruits and leaves are seen to be highly variable. Given the fact that the genotype, the climate and soil conditions of the sampled region and year affects the amount of phenolic substance (Revilla et al., 1997; Ribereau-Gayon et al., 2000; Montealegre et al., 2006). This variation can be considered normal.

Myrtle leaves were found to contain more phenolic and flavonoid substances, and higher antioxidant activity as a consequence of this, when compared with the fruits. In this regard, our findings comply with those of Kanoun et al. (2014) and Amensour et al. (2010).

3.3. Phenolic compounds of myrtle fruits and leaves

The majority of the phenolic compounds found in the fruits of myrtle are composed of flavonol group compounds. The amounts of the flavonol-group components examined are given in Table 5 and 6 for the fruits and leaves respectively. When Table 5 is examined, average amounts of myricetin, myricetin-3-glucoside, myricitrin, quercetin-3-B-D glucoside and quercitrin in the fruits were found to be 13.22, 134.91, 478.79, 829.48 and 50.38 mg kg⁻¹ respectively. No myricetin was detected in the BM13 genotype. The highest amount of myricetin (32.85 mg kg⁻¹) was found in fruits of the SM16 genotype. The lowest amount of myricetin-3-glucoside was found in SM4 (34.87 mg kg⁻¹)

whereas the highest was found in BM9 fruits with 233.17 mg kg⁻¹. The lowest amount of myricitrin was found in the fruits of SM4 with 133.22 mg kg⁻¹ whereas the highest was found in the fruits of BM17 with 664.38 mg kg⁻¹. The amount of quercetin-3-B-D glucoside in the fruits is rather high and the highest amount of the substance was found in the BM17 genotype with 1117.79 mg kg⁻¹. The amount of quercitrin was found to be higher in the fruits of BM13 with 83.62 mg kg⁻¹. Our findings comply with those of Montoro et al. (2016). The researchers reported that the main polyphenols in the myrtle fruits and extracts were flavonoids and anthocyanins and found that amounts of myricetin-3-O-galactoside, myricetin-3-O-rhamnoside (myricitrin) and quercetin-3-O-glucoside among the flavonoids were higher.

When Table 6 is examined, the average amount of myricetin in the leaf was found to be 3.75 mg kg⁻¹, myricetin-3-glucoside to be 220.79 mg kg⁻¹, myricitrin to be 763.93 mg kg⁻¹, quercetin-3-B-D glucoside to be 1177.12 mg kg⁻¹, and quercitrin to be 31.07 mg kg⁻¹. While the highest amount of myricetin was mostly found in the leaves of the SM1 genotype (12.08 mg kg⁻¹), no myricetin could be detected in the leaves of BM8. The lowest amount of myricetin-3-glucoside was found in the leaves of BM13 with 142.10 mg kg⁻¹ whereas the highest amount was found in BM9 with 338.29 mg kg⁻¹. The highest amount of myricitrin in the leaves was found in BM9 with 965.70 mg kg⁻¹. As in the fruit, the most common compound in the leaf was quercetin-3-B-D glucoside, and the highest amount was detected in the leaves of the BM9 genotype with 1662.81 mg kg⁻¹. The highest amount of quercitrin was found in the BM19 genotype with 49.30 mg kg⁻¹.

Romani et al. (1999) found that myrtle leaves contain low amounts of quercetin derivatives

Table 5. Phenolic compounds of myrtle fruits (mg kg⁻¹) (±SD).

| Genotype | Myricetin | Myricetin-3-glucoside | Myricitrin | Quercetin-3-B-D glucoside | Quercitrin | Total |
|----------|-----------------|-----------------------|--------------|---------------------------|------------|---------|
| BM1* | 4.94±0.04 | 176.85±10.73 | 471.61±7.92 | 763.75±55.13 | 51.69±1.57 | 1468.84 |
| BM2 | 3.55±0.39 | 69.95±1.18 | 506.32±9.59 | 908.01±4.58 | 79.92±3.91 | 1567.75 |
| BM3 | 15.77±2.02 | 117.53±4.24 | 405.08±22.08 | 692.96±53.80 | 41.38±3.51 | 1273.17 |
| BM4 | 1.47±0.25 | 34.87±3.77 | 133.22±14.28 | 231.39±25.31 | 24.98±1.61 | 425.92 |
| BM5 | 28.27±1.00 | 98.88±3.53 | 618.31±19.22 | 1041.40±46.63 | 54.05±3.79 | 1840.91 |
| BM6 | 6.20±0.15 | 130.06±3.15 | 523.13±4.10 | 915.36±4.35 | 44.21±2.01 | 1618.96 |
| BM7 | 26.28±4.88 | 218.04±5.42 | 436.86±6.00 | 770.23±38.31 | 34.33±3.88 | 1485.74 |
| BM8 | 3.25±0.54 | 182.12±5.09 | 422.96±12.71 | 771.93±28.45 | 56.84±1.23 | 1437.09 |
| BM9 | 22.12±2.92 | 233.17±1.35 | 500.12±4.04 | 922.04±15.58 | 45.05±2.40 | 1722.49 |
| BM10 | 6.75±0.15 | 119.29±13.10 | 540.43±5.14 | 921.42±33.94 | 49.40±2.16 | 1637.30 |
| BM11 | 20.40±2.74 | 194.90±2.04 | 362.95±6.64 | 645.96±36.42 | 26.17±1.24 | 1250.38 |
| BM12 | 12.11±0.65 | 127.51±5.22 | 581.63±1.96 | 1017.57±10.24 | 41.14±1.44 | 1779.96 |
| BM13 | nd [‡] | 100.62±5.09 | 615.97±35.10 | 949.14±53.39 | 83.62±3.93 | 1749.35 |
| BM14 | 14.67±0.33 | 66.98±1.26 | 474.08±28.40 | 829.15±34.93 | 47.43±0.02 | 1432.32 |
| BM15 | 8.99±0.69 | 155.22±7.19 | 358.11±7.04 | 672.24±1.84 | 51.44±1.73 | 1246.00 |
| BM16 | 32.85±2.32 | 119.65±7.26 | 415.50±5.37 | 724.51±13.41 | 42.09±1.38 | 1334.60 |
| BM17 | 30.60±1.51 | 224.58±10.89 | 664.38±25.46 | 1163.79±23.74 | 37.74±5.26 | 2122.09 |
| BM18 | 5.82±0.54 | 95.19±4.19 | 534.29±27.61 | 1090.56±40.32 | 72.40±2.21 | 1798.26 |
| BM19 | 7.13±0.18 | 97.97±2.41 | 532.07±31.04 | 727.64±18.82 | 72.97±3.75 | 1437.79 |
| Average | 13.22 | 134.91 | 478.79 | 829.48 | 50.38 | |

*BM: Black Myrtle, nd: not determined

Table 6. Phenolic compounds of myrtle leaves (mg kg⁻¹) (±SD).

| Genotype | Myricetin | Myricetin-3-glucoside | Myricitrin | Quercetin-3-B-D glucoside | Quercitrin | Total |
|----------|-----------------|-----------------------|--------------|---------------------------|------------|---------|
| BM1* | 12.08±0.71 | 225.28±4.22 | 756.56±17.66 | 1109.11±57.26 | 32.46±2.59 | 2135.49 |
| BM2 | 3.53±0.04 | 146.99±9.72 | 838.10±62.01 | 1368.34±5.97 | 48.75±0.76 | 2405.71 |
| BM3 | 3.39±0.55 | 167.07±2.76 | 657.95±13.93 | 1080.52±19.10 | 21.39±0.86 | 1930.32 |
| BM4 | 2.96±0.02 | 222.42±1.04 | 667.09±9.11 | 1093.89±35.28 | 19.30±0.26 | 2005.66 |
| BM5 | 2.43±0.68 | 223.86±0.79 | 882.92±35.03 | 942.05±20.14 | 41.87±2.14 | 2093.14 |
| BM6 | 2.14±0.20 | 206.69±32.04 | 624.18±19.73 | 1084.46±31.63 | 29.28±2.14 | 1946.76 |
| BM7 | 3.38±0.54 | 193.70±0.48 | 679.93±4.59 | 1132.69±6.30 | 19.39±1.98 | 2029.09 |
| BM8 | nd [†] | 273.11±5.22 | 772.44±16.63 | 1126.97±138.11 | 20.38±0.41 | 2192.90 |
| BM9 | 5.75±0.36 | 338.29±12.56 | 965.70±15.96 | 1662.81±24.80 | 28.68±1.20 | 3001.23 |
| BM10 | 2.26±0.37 | 155.46±13.55 | 683.14±10.89 | 1168.34±8.62 | 31.05±1.49 | 2040.27 |
| BM11 | 6.01±0.48 | 269.45±4.01 | 661.38±4.68 | 1173.14±24.81 | 22.25±1.11 | 2132.24 |
| BM12 | 0.88±0.02 | 237.70±3.10 | 895.03±14.27 | 1098.14±1.32 | 24.41±0.17 | 2256.16 |
| BM13 | 2.10±0.22 | 142.10±0.41 | 762.13±53.37 | 1298.73±32.18 | 29.88±1.91 | 2234.93 |
| BM14 | 0.93±0.10 | 161.53±2.47 | 661.90±7.82 | 1145.35±76.89 | 32.44±0.95 | 2002.14 |
| BM15 | 0.45±0.07 | 243.18±0.59 | 826.23±27.05 | 1049.82±58.22 | 33.50±3.28 | 2153.17 |
| BM16 | 8.05±0.58 | 231.13±2.13 | 843.65±8.30 | 1349.03±28.67 | 29.32±2.04 | 2461.17 |
| BM17 | 5.31±0.33 | 255.38±5.14 | 774.81±8.97 | 1394.11±8.84 | 39.68±1.27 | 2469.29 |
| BM18 | 1.90±0.14 | 197.22±15.88 | 639.59±32.81 | 1231.22±73.15 | 37.04±2.03 | 2106.96 |
| BM19 | 7.59±0.58 | 304.43±24.06 | 922.00±50.71 | 856.61±70.14 | 49.30±1.57 | 2139.93 |
| Average | 3.75 | 220.79 | 763.93 | 1177.12 | 31.07 | |

*BM: Black Myrtle, nd: not determined

(quercetin-3-O-galactoside and quercetin-3-O-rhamnoside (quercitrin)) and high amounts of myricetin derivatives (myricetin-3-O-rhamnoside (myricitrin)). In our study, the amount of quercetin derivatives was found to be higher. In this study, the amounts of myricetin and quercetin glucoside derivatives were detected. This difference may have occurred because the amount of galactoside derivatives could not be detected.

4. Conclusions

- Phenolic substance was found to be high in the leaves of the samples collected from Kumluca (BM15 and BM16) and in the fruits of those collected from Kemer (BM13) and Kaş (BM19).
- High antioxidant activity was detected in the leaf sample BM16 collected from Kumluca (0.13 µl) and the fruit sample (BM19) collected from Kaş (0.45 µl).
- The seed / fruit ratio ranged between 10.45% and 23.66%. This rate was 19.07% in BM19 genotype while it was 10.45% and 18.68% in BM15 and BM13 genotypes. Although the number of seeds was less in these two genotypes in comparison to the BM19 genotype, their antioxidant activity values (0.66 and 0.68 µl) were found to be higher.
- The correlations between total phenolic substance and antioxidant activity ($r = -0.74526$ for leaves, $r = -0.78952$ for fruit) and between total amount of flavonoid substance and antioxidant activity ($r = -0.73817$ for leaves, $r = -0.71061$ for fruit) were found to be significant.
- Considerable differences between genotypes were observed in terms of amounts of phenolic compounds in both leaves and fruits. The highest amount of flavonol-group compounds examined in myrtle fruits and leaves were found to be quercetin-

3-B-D glucoside, followed by myricitrin, myricetin-3-glucoside, quercitrin, and myricitrin.

- The phenolic flavonoid substance contents and antioxidant capacities of the fruits and leaves of the myrtle plant growing in the natural Antalya flora has been revealed and the promising genotypes have been identified. These genotypes are important in providing material for future research.
- The amount of phenol and tannin in the fruit is largely due to the seeds and is directly related to the antioxidant activity. However, the selection of dark-colored and large types with fruit flesh that contain high amounts of phenol will allow the emergence of new genotypes with little acerbity without reducing the antioxidant effect in black myrtle.
- It can be recommended that the genotypes with high values in terms of the measured parameters are utilized as natural antioxidant sources in the food and pharmaceutical industry because they contain substances that are both beneficial for human health and are natural antioxidants. There is a transition from synthetic products to natural products for a healthier life in world societies. Therefore, utilization of the fruit species known as the most important natural antioxidant sources is gaining importance.

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