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Phenolic compounds, bioactive content and antioxidant capacity of the fruits of mulberry (*Morus* spp.) germplasm in Turkey

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ABSTRACT

The study was carried out in 2014 and 2015, and aimed to determine some important biochemical and antioxidant characteristics of the fruits of mulberry (*Morus* spp.) cultivars and genotypes found in Malatya (Turkey). Phenolic compounds (protocatechuic acid, vanillic acid, ellagic acid, rutin, quercetin, gallic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, *o*-coumaric acid, phloridzin and ferulic acid), organic acids, sugars, vitamin C and antioxidant capacity were analyzed in sampled fruits. The results showed that most of the biochemical content and antioxidant capacities of the cultivars and genotypes were significantly different from one another ($p < 0.05$). Among the phenolic compounds, rutin (118.23 mg 100 g^{-1}), gallic acid (36.85 mg 100 g^{-1}), and chlorogenic acid (92.07 mg 100 g^{-1}) were determined to have the highest values for most of the fruit samples. Malic acid and citric acid were dominant among the organic acids for all the cultivars and genotypes except 44-Nrk-05. Glucose was measured as a more abundant sugar than fructose and sucrose in all samples. Antioxidant capacity, on the other hand, varied between 6.17 and 21.13 µmol TE g^{-1} among the cultivars and genotypes analyzed.

Key words: cultivar, genotype, mulberry, phytochemicals

INTRODUCTION

Fruit growing is one of the important and paying branches of horticulture, and has been practiced in most countries of the world for centuries. It is one of the important income sources of the main fruit-growing countries. Fruit species have been used not only for nutrition purposes but also to meet personal and social needs such as curing diseases, beautifying the planet, etc. (Hegedus et al. 2010, Canan et al. 2016, Sorkheh and Khaleghi 2016, Zorenc et al. 2016).

Mulberry was cultivated especially for sericulture at first, but then became a fruit species with ever-increasing popularity along with the

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increased use of it also in human nutrition, food, and pharmaceutical industries. Mulberry has a wide distribution area in regions with tropical, semi-tropical, or temperate climates, thanks to its high adaptation ability (Ercisli and Orhan 2007, Ercisli and Orhan 2008, Orhan and Ercisli 2010). Four mulberry species, namely *Morus rubra*, *Morus nigra*, *Morus alba* and *Morus laevigata*, have grown naturally in Turkey for many years and show high diversity (Ercisli 2004, Ozgen et al. 2009). In recent years, an increasing number of studies have been conducted on mulberry fruits in relation to morphological, biochemical, phytochemical and antioxidant characteristics, and their contribution to human nutrition and health (Ercisli and Orhan 2007, Koyuncu et al. 2014, Sanchez et al. 2014, Sanchez-Salcedo et al. 2015). Mulberry fruits are generally consumed fresh or dried, and are also used as raw material in numerous branches of industry producing, for example, sorbet, fruit juice, wine, milk, yogurt, ice cream, vinegar, marmalade, jam, molasses, fruit leather, churchkhela (locally named Mulberry Kome), cosmetics, and pharmaceuticals in mulberry-growing countries, including Turkey (Gungor and Sengul 2008, Gundogdu et al. 2011). In addition to fresh consumption, black and red mulberries are extensively used for making jam, juice and marmalade; whereas white mulberries, which constitute 95% of mulberries in Turkey, are consumed as dried fruit (4%), used in making molasses (70%) and kome, a special local mulberry product (10%), or eaten fresh (5%) (Ercisli 2004).

Mulberries, especially the black and purple-coloured ones, are a very rich source of anthocyanins (Ercisli and Orhan 2008). White mulberries, which are rich in flavonoids, are also known as an important nutritional source for protecting the immune system (Butt et al. 2008). Previous studies had revealed that phenolic compounds having a protective effect in coronary heart disease and some types of cancer are also anti-aging owing to their antioxidant characteristics instrumental in eliminating free radicals (Rodriguez-Mateos et al. 2014). Because of its high phytochemical content, the black mulberry fruit has been used in folk medicine from old times against several disorders such as nausea, vomiting, digestive disorders, diabetes, hypertension, coughs, anaemia, arthritis, mouth sores, gingival diseases, fever, and fatigue (Gungor and Sengul 2008). Organic acids and sugars contribute to the taste of product, especially in fresh fruits. In addition to increasing the attractiveness of mulberry fruits for consumption, these components, along with antioxidant substances, have found use in diverse areas of pharmacology (Soyer et al. 2003). Chemical content and antioxidant capacity of fruits are influenced by numerous factors. In particular, environmental conditions and genotype structure have great effects on the formation of these substances (Mikulic-Petkovsek et al. 2012, Sanchez et al. 2014). It has been revealed in several studies that the quality of local products made of particular wild or semi-wild edible fruits is also improved as a result of the high level of chemical components in mulberry species growing naturally in various regions of Turkey (Ercisli and Orhan 2008, Ozgen et al. 2009, Gundogdu et al. 2011, Orhan and Ercisli 2010). Mulberry consumption per capita is also increasing day by day as a result of these characteristics. According to data of the Turkish Statistical Institute, annual mulberry production in Turkey reached 69.334 tons in 2016 (TSI 2016).

Genetic variation is the main prerequisite for a breeding programme for horticultural crop plants in the world. Therefore, investigation of the genetic source of variation among genotypes and commercial cultivars of different fruit species is always critical to the initiation of a breeding programme. Most of the mulberry species found in Turkey consist of wild and old trees. Production of mulberry fruit occurs in almost every region of Anatolia. Limited information exists in the literature about the biochemical status of the mulberry genotypes in Turkey. In Turkey, active mulberry breeding has increased in the last decades and Turkish breeders are facing problems in the use of some novel sources of variation in their breeding programmes due to the lack of information about the biochemical properties of the available genotypes. Therefore, this study can be a starting point to investigate new genotypes with better biochemical characteristics. In this study, certain foreign mulberry cultivars and local genotypes of mulberry growing in Turkey were analyzed. Anti-cancer phenolic compounds, organic acids, and antioxidant capacity are the most important quality criteria of mulberry fruits, especially in terms of human health. Therefore, we believe that this study will serve as a novel source of variation for Turkish and international breeders searching for variations to develop novel commercial cultivars with a high antioxidant capacity and phenolic content.

MATERIAL AND METHODS

Experimental site description

The weather data for both years are given below (Fig. 1). The fertilization practices, pest and disease management, and irrigation were conducted properly in each year. Location of the experimental site: 38° 21' N and 38° 20' E, with an altitude of 973 m above sea level.

Fruit samples

In this study, eight standard foreign mulberry cultivars originated in China, Japan and South Korea, and eleven mulberry genotypes from Turkey were used. The important plant characteristics of the cultivars and genotypes are given in Table 1.

The plants were grown together in the National Fruit Genetics Resources Plot of the Malatya Fruit

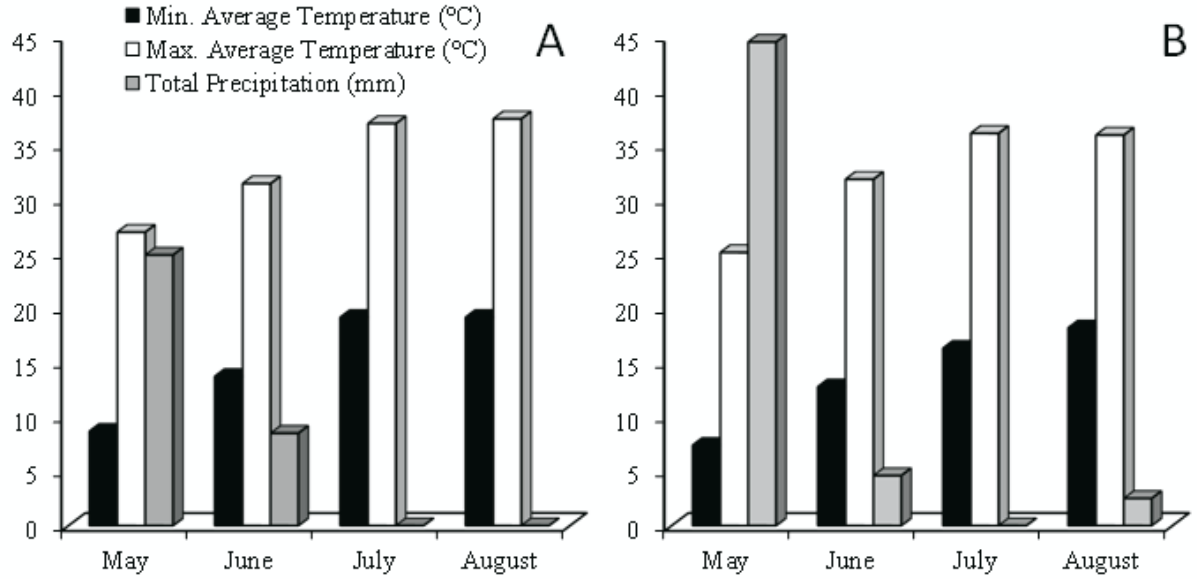

Figure 1. Weather parameters of the experimental mulberry-growing area for 2014 year (A) and 2015 (B) (Malatya province)

Table 1. Some important plant characteristics of mulberry cultivars and genotypes

Cultivar/Genotype	Species	Origin	Fruit colour
Angut-Bayırbağ	*Morus alba*	Erzincan, Turkey	Pink
Elaziğ-Çekirdekli	*Morus alba*	Elaziğ,Turkey	White
Istanbul-dut (24-10)	*Morus alba*	Erzincan, Turkey	White
44-MRK-05	*Morus alba*	Malatya, Turkey	White
Arapgir-0011	*Morus alba*	Malatya, Turkey	White
Arapgir-0012	*Morus alba*	Malatya, Turkey	White
44-KE-10	*Morus alba*	Malatya, Turkey	White
24-MRK-01	*Morus alba*	Erzincan, Turkey	White
24-KE-05	*Morus alba*	Erzincan, Turkey	White
23-MRK-09	*Morus nigra*	Elaziğ, Turkey	Black
44-BA-05	*Morus nigra*	Malatya, Turkey	Black
Ship Yeoung	Not known	South Korea	Black
Suwean Daeyap	Not known	South Korea	Black
Roso	Not known	South Korea	Black
Yong Cheanchoe	Not known	South Korea	Black
Gosho Eromi	Not known	Japan	Black
Thengxiang	*Morus alba*	China	White
Kokusa 20	Not known	Japan	Black
He ye bar	Not known	China	Black

Research Institute. Harvesting was performed in both 2014 and 2015 when the fruits of the investigated cultivars and genotypes had reached the commercial ripe stage. Approximately 1 kg fruit samples were taken from each cultivar and genotype. Fruit samples were collected at the same time and were stored at –80°C until analyses were performed.

Chemicals

Organic acid standards (oxalic, citric, malic, succinic, fumaric, and tartaric acid), phenolic acid standards (gallic, chlorogenic, *o*-coumaric, *p*-coumaric, ferulic, vanillic, syringic, caffeic, ellagic and protocatechuic acid), polyphenols standards (catechin, phloridzin, quercetin, rutin), sugar standards (glucose, fructose, and sucrose), and vitamin C standard (L-ascorbic acid) were obtained from Sigma–Aldrich (St. Louis, MO, 71 USA). The other chemicals were obtained from Merck (Darmstadt, Germany) unless otherwise indicated.

Analysis of phenolic compounds

Protocatechuic, gallic, chlorogenic, ellagic, caffeic, *p*-coumaric, *o*-coumaric, vanillic, syringic and ferulic acids as well as catechin, rutin, quercetin and phloridzin were detected among phenolic compounds in mulberry fruits, with the modified method of Rodriguez-Delgado et al. (2001) and Gundogdu et al. (2011). Fruit extracts were mixed with distilled water in a ratio of 1:1. The mixture was centrifuged for 15 min. at 15,000 rpm. Supernatants were filtrated with a coarse filter paper and twice with a 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), and injected into an HPLC (Agilent, USA). Chromatographic separation was performed with a 250 × 4.6 mm, 4 µm ODS column (HiChrom, USA). Solvent A – methanol : acetic acid : water (10:2:28) and Solvent B – methanol : acetic acid : water (90:2:8) were used as the mobile phase (Tab. 2). Spectral measurements were made at 254 and 280 nm, and the flow rate and injection volume were adjusted to 1 mL min^{-1} and 20 µL, respectively.

Table 2. Gradient elution programme for the determination of phenolic compounds in mulberry fruit

Time (min.)	Dissolvent A (%)	Dissolvent B (%)
0	100	0
15	85	15
25	50	50
35	15	85
45	0	100

Analysis of organic acids

Succinic, oxalic, citric, malic, fumaric, and tartaric acids contents of berries were determined according to Bevilacqua and Califano (1989). Three replicates including 30 fruits per replicate were used. Juice extracts were obtained by mashing the berries in cheesecloth, after which the samples were stored at -20°C until analysed. 5 mL of each sample was mixed with 20 mL of 0.009 N H_2SO_4 (Heidolph Silent Crusher M, Germany), then homogenized for 1 hour with a shaker (Heidolph Unimax 1010, Germany). The mixture was centrifuged for 15 min. at 15,000 rpm, and supernatants were filtrated twice with a 0.45 µm membrane filter following filtration with a coarse filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) and run through a SEP-PAK C18 cartridge. Organic acid readings were performed with HPLC using an Aminex column (HPX-87 H, 300 × 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) at 214 and 280 nm wavelengths, controlled with the Agilent package program (Agilent, USA).

Analysis of vitamin C

Vitamin C content was detected with a modified HPLC procedure suggested by Cemeroglu (2007). 5 mL of the fruit extracts was supplemented with 2.5% (w/v) metaphosphoric acid (Sigma, M6285, 33.5%), then centrifuged at 6,500 rpm for 10 min. at 4°C. 0.5 mL of the mixture was brought to the final volume of 10 mL with 2.5% (w/v) metaphosphoric acid. Three replicates including 30 fruits per replicate were used. Supernatants were filtered with a 0.45 µm PTFE syringe filter (Phenomenex, UK). C_{18} column (Phenomenex Luna C18, 250 × 4.60 mm, 5 µ) was used for the identification of ascorbic acid at a temperature of 25°C. Double distilled water with 1 mL min^{-1} flow rate and pH of 2.2 (acidified with H_2SO_4) was used as a mobile phase. Spectral measurements were made at 254 nm wavelength using DAD detector. Different standards of L-ascorbic acid (Sigma A5960) (50, 100, 500, 1000, and 2000 ppm) were used for the quantification of ascorbic acid readings.

Determination of trolox equivalent antioxidant capacity (TEAC)

Trolox equivalent antioxidant capacity (TEAC) was determined with ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation

by dissolving ABTS in an acetate buffer using potassium persulphate (Ozgen et al. 2006). Three replicates including 30 fruits per replicate were used. For longer stability, the mixture was diluted with 20 mM sodium acetate buffer in an acidic pH of 4.5, and read at 734 nm wavelength, 0.700 ±0.01. For spectrometric assay, 3 mL $ABTS^{\cdot+}$ was mixed with 20 µL fruit extract sample and incubated for 10 min. Absorbance was read at 734 nm wavelength.

Sugar analysis

The modified method of Melgarejo et al. (2000) was used for sugar (fructose, glucose and sucrose) analyses. Three replicates including 30 fruits per replicate were used. 5 mL of fruit extracts was centrifuged at 12,000 rpm for 2 minutes at a temperature of 4°C. Supernatants were passed by SEP-PAK C_{18} cartridge. HPLC readings were made with µbondapak-NH_2 column using 85% acetonitrile as liquid phase with refractive index detector (IR). Fructose and glucose standards were used for sugar calculations.

Statistical analysis

Three replicates including 30 fruits per replicate were used. Descriptive statistics of phenolic compounds, organic acids, sugars, vitamin C, and antioxidant capacity extracted from cultivars and genotypes were represented as the mean ±SE. Experimental data were evaluated using analysis of variance (ANOVA), and significant differences among the means of three replicates ($p < 0.05$) were determined by Duncan's multiple range test using the SPSS 20 for Windows.

RESULTS AND DISCUSSION

Phenolic compounds

Phenolic compounds such as protocatechuic acid, vanillic acid, ellagic acid, rutin, quercetin, gallic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, *o*-coumaric acid, phloridzin, and ferulic acid varied in all the cultivars and genotypes at a statistically significant level, $p < 0.05$ (Tabs 3 and 4). Among the studied phenolic compounds, chlorogenic acid was dominant in the fruits of Ship Yeoung, Suwean Daeyap, Yong Choenchoe, Gosho Eromi, Kokusa-20, 23-MRK-09, Angut Bayırbağı, Elazığ Çekirdekli, İstanbul-dut (24-10), 44-MRK-05, Arapgir-0011, Arapgir-0012, 44-KE-10, 24-MRK-01, 24-KE-05, and rutin dominated in Roso, Thengxiang, He ye bar, 23-MRK-09 and 44 BA-05.

Table 3. Protocatechuic acid, vanillic acid, ellagic acid, rutin, quercetin, gallic acid and catechin contents (mg 100 g^{-1}) of mulberry cultivars and genotypes (mean for 2014 and 2015)

Cultivars and genotypes	Protocatechuic acid	Vanillic acid	Ellagic acid	Rutin	Quercetin	Gallic acid	Catechin
Ship Yeoung	1.33 ±0.02g*	0.24 ±0.00i	4.78 ±0.03c	32.73 ±1.07i	7.73 ±0.04c	13.95 ±0.05o	3.47 ±0.07i
Suwean Daeyap	0.82 ±0.00l	1.13 ±0.03e	2.89 ±0.05f	44.90 ±0.12g	2.16 ±0.01k	36.85 ±0.25a	2.13 ±0.02l
Roso	0.71 ±0.02m	1.76 ±0.03c	4.99 ±0.03b	109.94 ±0.64b	1.89 ±0.01l	22.00 ±0.10i	9.27 ±0.06b
Yong Choenchoe	1.46 ±0.02f	1.08 ±0.01f	2.76 ±0.06g	60.00 ±0.35f	1.09 ±0.02n	24.10 ±0.40g	2.04 ±0.03m
Gosho Eromi	2.71 ±0.04b	0.40 ±0.01h	4.32 ±0.05d	37.78 ±0.45h	1.18 ±0.01m	12.85 ±0.15p	2.14 ±0.06l
Thengxiang	3.78 ±0.08a	1.32 ±0.02d	3.95 ±0.04e	79.64 ±1.35c	2.76 ±0.05j	23.30 ±0.30h	9.85 ±0.06a
Kokusa 20	1.62 ±0.03d	2.03 ±0.02b	2.45 ±0.04h	59.74 ±0.73f	1.03 ±0.02o	28.10 ±0.70e	3.78 ±0.02h
He ye bar	0.87 ±0.02k	0.85 ±0.03g	5.21 ±0.04a	118.23 ±1.37a	6.64 ±0.02e	19.60 ±0.10k	5.21 ±0.08e
23-mrk-09	1.55 ±0.03e	0.24 ±0.001i	2.00 ±0.02i	75.78 ±0.65d	0.98 ±0.01o	36.30 ±0.10b	8.02 ±0.06c
44-ba-05	1.62 ±0.04d	3.86 ±0.05a	1.62 ±0.06j	68.78 ±0.37e	2.15 ±0.02k	14.95 ±0.35n	3.83 ±0.03h
Angut-Bayırbağı	1.72 ±0.02c	0.17 ±0.01j	1.22 ±0.03k	28.37 ±0.45k	6.81 ±0.02d	15.98 ±0.03m	1.78 ±0.04n
Elazığ-Çekirdekli	1.46 ±0.01f	0.88 ±0.02g	0.74 ±0.03n	29.74 ±0.33j	10.42 ±0.02a	31.10 ±0.07c	2.33 ±0.02k
İstanbul-dut (24-10)	1.13 ±0.02i	0.21 ±0.001i	1.16 ±0.01kl	20.81 ±0.21m	5.12 ±0.01h	18.20 ±0.23l	1.13 ±0.02p
44-MRK-05	1.08 ±0.02j	0.09 ±0.00k	1.04 ±0.03m	22.45 ±0.09 l	4.19 ±0.03i	19.67 ±0.24k	1.32 ±0.04o
Arapgir-0011	1.57 ±0.01ed	0.03 ±0.00l	1.17 ±0.01kl	28.38 ±0.47k	6.46 ±0.03f	29.40 ±0.23d	4.83 ±0.07f
Arapgir-0012	1.68 ±0.05c	0.17 ±0.01j	1.22 ±0.06k	27.33 ±0.11k	6.45 ±0.01f	26.27 ±0.27f	4.31 ±0.08g
44-KE-10	1.42 ±0.03f	0.06 ±0.00kl	1.20 ±0.01k	32.85 ±0.20i	6.38 ±0.01g	24.27 ±0.34g	7.05 ±0.11d
24-MRK-01	1.43 ±0.04f	0.08 ±0.01k	1.12 ±0.02l	10.54 ±0.08n	7.93 ±0.11b	21.43 ±0.87j	2.51 ±0.06j
24-KE-05	1.23 ±0.01h	0.05 ±0.00l	1.12 ±0.03l	30.01 ±0.24j	6.81 ±0.05d	30.58 ±0.09c	2.02 ±0.02m

*Difference between means designated with the same letter in the same column is not significant at 0.05 level

Table 4. Chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, o-coumaric acid, phloridzin, and ferulic acid contents (mg 100 g^{-1}) of mulberry cultivars and genotypes (mean for 2014 and 2015)

Cultivars and genotypes	Chlorogenic acid	Caffeic acid	Syringic acid	*p*-coumaric acid	*o*-coumaric acid	Phloridzin	Ferulic acid
Ship Yeoung	40.75 ±0.80h*	9.98 ±0.06f	3.55 ±0.06k	3.76 ± 0.05c	1.68 ±0.08j	0.16 ±0.00j	2.74 ±0.03c
Suwean Daeyap	87.56 ±1.25b	4.66 ±0.02m	1.83 ±0.07m	2.31 ±0.03f	3.22 ±0.01h	0.93 ±0.03c	1.67 ±0.01h
Roso	61.02 ±0.99e	9.74 ±0.01g	7.05 ±0.06de	5.67 ±0.07a	6.17 ±0.09a	0.48 ±0.01g	0.76 ±0.02m
Yong Choenchoe	92.07 ±0.07a	9.67 ±0.04g	6.97 ±0.06e	2.09 ±0.06g	3.66 ±0.03e	0.26 ±0.01i	1.41 ±0.02jk
Gosho Eromi	39.62 ±0.97h	5.90 ±0.06k	3.06 ±0.07l	3.87 ±0.04c	1.71 ±0.03j	0.16 ±0.00j	1.43 ±0.05jk
Thengxiang	73.84 ±0.24d	5.67 ±0.07l	6.10 ±0.03f	3.40 ±0.45d	4.82 ±0.04b	0.66 ±0.03f	1.73 ±0.04gh
Kokusa 20	78.90 ±8.70c	15.82 ±0.02d	7.04 ±0.04de	2.04 ±0.06g	4.48 ±0.05d	0.79 ±0.03d	1.77 ±0.02g
He ye bar	24.84 ±0.79j	6.97 ±0.16j	4.72 ±0.04i	4.79 ±0.03b	4.71 ±0.04c	0.17 ±0.01j	1.48 ±0.02j
23-mrk-09	71.76 ±0.27d	16.11 ±0.04c	10.75 ±0.05b	1.48 ±0.02ij	3.56 ±0.05f	0.42 ±0.04h	1.39 ±0.01k
44-ba-05	85.40 ±2.80b	21.09 ±0.06a	7.11 ±0.13d	1.31 ±0.08j	3.48 ±0.07g	0.11 ±0.00k	1.57 ±0.04i
Angut-Bayırbağı	40.60 ±0.50h	4.34 ±0.02o	1.16 ±0.02n	1.62 ±0.01hi	0.88 ±0.01l	0.75 ±0.02e	0.98 ±0.01l
Elazığ-Çekirdekli	45.96 ±1.68g	15.86 ±0.05d	8.22 ±0.04c	2.68 ±0.06e	0.48 ±0.01n	1.15 ±0.03a	2.67 ±0.04c
İstanbul-dut (24-10)	33.03 ±0.13i	2.44 ±0.01r	11.91 ±0.12a	1.73 ±0.00h	0.38 ±0.00o	0.63 ±0.03f	4.79 ±0.09a
44-MRK-05	32.23 ±0.03i	4.47 ±0.06n	7.13 ±0.13d	2.71 ±0.01e	0.53 ±0.02n	0.91 ±0.02c	2.99 ±0.10b
Arapgir-0011	42.74 ±1.36gh	7.67 ±0.01i	3.92 ±0.09j	0.76 ±0.01k	0.77 ±0.01m	1.09 ±0.05b	2.20 ±0.05e
Arapgir-0012	57.33 ±1.61e	11.27 ±0.04e	3.93 ±0.04j	0.78 ±0.01k	0.40 ±0.01o	0.63 ±0.02f	2.37 ±0.07d
44-KE-10	51.69 ±0.91f	17.28 ±0.13b	5.45 ±0.06h	0.72 ±0.02k	3.10 ±0.04i	0.41 ±0.00h	2.02 ±0.07f
24-MRK-01	41.28 ±1.50h	3.89 ±0.06p	5.77 ±0.04g	0.70 ±0.01k	1.17 ±0.04k	1.13 ±0.02a	1.68 ±0.06h
24-KE-05	30.79 ±0.05i	8.55 ±0.01h	1.16 ±0.03n	0.71 ±0.02k	3.27 ±0.04h	0.17 ±0.00j	1.70 ±0.02gh

*Difference between means designated with the same letter in the same column is not significant at 0.05 level

Memon et al. (2010) had reported that chlorogenic acid was 17.03-24.45 mg 100 g^{-1} in *Morus alba* fruits and 3.79-7.05 mg 100 g^{-1} in *Morus laevigata* fruits. In the studies by Gundogdu et al. (2011) and Eyduran et al. (2015), chlorogenic acid and rutin were determined as the major two phenolic compounds in mulberry fruits, which is in agreement with our study. Gecer et al. (2016) had determined rutin at a level of 1.22 mg g^{-1} in black mulberry fruits and 2.37 mg g^{-1} of chlorogenic acid in white mulberry fruits at the highest level. Chlorogenic acid has been reported to be formed by the esterification of caffeic acid and quinic acid (Çam and Hisil 2004). Zadernowski et al. (2005) determined that phenolic compounds imparting taste in ripening berry fruits were affected by genetic factors and pre-harvest conditions. In addition, genetic factors, ecological factors (moisture, light, temperature, and soil structure), and cultivation practices can also be regarded as factors that affect phenolic compounds in mulberry fruits (Gundogdu et al. 2011).

The Istanbul-dut (24-10) genotype was found to have a higher syringic acid content than the other cultivars and genotypes. The caffeic acid and vanillic acid contents of the 44b-Ba-05 genotype were higher than in the other genotypes and standard varieties. The measured amount of protocatechuic acid was the highest in the Thengxiang cultivar (3.78 mg 100 g^{-1}) and the lowest in the Roso (nosang) cultivar (0.71 mg 100 g^{-1}). Vanillic acid in the fruits of the mulberry cultivars and genotypes was between 0.24 mg 100 g^{-1} and 2.03 mg 100 g^{-1}, with the 44-ba-05 genotype containing the highest amount of 3.86 mg 100 g^{-1}. The amount of ellagic acid was found to have the highest value of 5.21 mg 100 g^{-1} in the He ye bar cultivar and the lowest value of 0.74 mg 100 g^{-1} in the Elazığ-çekirdekli genotype. The cultivar He ye bar had the highest rutin content in its fruit at 118.23 mg 100 g^{-1}, while the 24-MRK-01 genotype had the lowest value of 10.54 mg 100 g^{-1}. The quercetin content was determined to have the highest value of 10.42 mg 100 g^{-1} in the Elazığ-çekirdekli genotype, and the lowest result of 0.98 mg 100 g^{-1} was obtained in 23-Mrk-09. Gallic acid and catechin were measured in the ranges of 12.85-36.85 mg 100 g^{-1} and 1.13-9.85 mg 100 g^{-1}, respectively, among the cultivars and genotypes (Tab. 3). On the other hand, the chlorogenic acid content was determined to be at the highest level of 92.07 mg 100 g^{-1} in the Yong choenchoe cultivar; the lowest level of 24.84 mg 100 g^{-1} was determined in the He ye bar cultivar.

The highest caffeic acid content was 21.09 mg 100 g^{-1} in the 44-BA-05 genotype; its lowest value was 2.44 mg 100 g^{-1} in the İstanbul-dut (24-10) genotype. In turn, the highest syringic acid content was 11.91 mg 100 g^{-1} in the İstanbul-dut genotype; its lowest value was 1.16 mg 100 g^{-1} in the genotypes Angut and 24-KE-05. The *p*-coumaric acid content was measured to be higher in the cultivars than in the genotypes and its highest value was 5.67 mg 100 g^{-1} in the Roso cultivar, whereas the lowest amounts of *p*-coumaric acid were contained in 24-MRK-01, 24-KE-05, 44-KE-10, Arapgir-0011 and Arapgir-0012. The highest *o*-coumaric acid content was determined in Roso, while the lowest value was found in Istanbul-dut (24-10). The phloridzin content was higher in the genotypes than in the cultivars, and its highest value was 1.15 mg 100 g^{-1} in the fruits of the Elazığ-çekirdekli genotype. In terms of ferulic acid content, the Istanbul-dut genotype gave the best result with 4.79 mg 100 g^{-1}. Gundogdu et al. (2011) had measured the amounts of gallic acid, catechin, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, vanillic acid, rutin, and quercetin as 0.15, 0.08, 0.13, 0.10, 0.13, 0.06, 0.13, 0.04, 1.42, and 0.11 mg g^{-1} in black mulberry fruits, and as 0.22, 0.04, 0.12, 0.13, 0.05, 0.05, 0.03, 0.02, 0.01, and 0.02 mg g^{-1} in white mulberry fruits, respectively, which shows similarities with our study.

By using three different extraction methods, i.e. sonication, magnetic stirring and homogenization, Memon et al. (2010) had obtained the reported phenolics from *Morus alba* fruits as follows: gallic acid 3.57-5.81 mg 100 g^{-1}, protocatechuic acid 2.30-3.49 mg 100 g^{-1}, vanillic acid 3.70-4.57 mg 100 g^{-1}, syringic acid 6.31-9.19 mg 100 g^{-1}; and from *Morus laevigata* fruits as follows: gallic acid 9.69-10.88 mg 100 g^{-1}, protocatechuic acid 1.67-5.61 mg 100 g^{-1}, vanillic acid 4.63-8.20, syringic acid 3.94-8.11 mg 100 g^{-1}, and ferulic acid 4.93-8.42 mg 100 g^{-1}. It was thought that the variations in the concentration of the phenolic compounds might have been associated with the use of the different extraction methods.

In this research, it was determined that the genotypes 44-BA-05, Istanbul-dut, 24-MRK-01 and 44-BA-05 showed promising characteristics when compared to standard cultivars in terms of phenolic compounds.

Organic acids

Statistically significant differences ($p < 0.05$) occurred among both cultivars and genotypes in terms of the concentration of organic acids (Tab. 5).

Table 5. Oxalic acid, citric acid, tartaric acid, malic acid, succinic acid, and fumaric acid content (g 100 g^{-1}) of mulberry cultivars and genotypes (mean for 2014 and 2015)

Cultivars and genotypes	Oxalic acid	Citric acid	Tartaric acid	Malic acid	Succinic acid	Fumaric acid
Ship Yeoung	0.98 ±0.04b*	4.20 ±0.02b	0.79 ±0.01a	7.78 ±0.17ef	0.62 ±0.01e	0.01 ±0.00j
Suwean Daeyap	0.60 ±0.02f	2.16 ±0.02g	0.51 ±0.02cd	6.03 ±0.05hi	0.82 ±0.02c	0.07 ±0.00g
Roso	1.00 ±0.04b	3.61 ±0.06c	0.53 ±0.04c	4.93 ±0.05k	0.95 ±0.02b	0.01 ±0.00j
Yong Choenchoe	0.68 ±0.03e	2.67 ±0.03f	0.49 ±0.02d	5.36 ±0.04jk	0.82 ±0.04c	0.04 ±0.00h
Gosho Eromi	1.18 ±0.05a	3.03 ±0.08e	0.65 ±0.01b	6.19 ±0.11h	0.83 ±0.03c	0.01 ±0.00j
Thengxiang	0.58 ±0.02fg	3.23 ±0.06d	0.51 ±0.03cd	6.91 ±0.07g	0.68 ±0.01de	0.01 ±0.01j
Kokusa 20	0.55 ±0.05g	1.96 ±0.06h	0.21 ±0.01h	5.69 ±0.05ij	0.44 ±0.01g	0.03 ±0.01hi
He ye bar	0.73 ±0.01de	1.98 ±0.04h	0.26 ±0.01g	12.70 ±0.10a	0.81 ±0.05c	0.03 ±0.00i
23-mrk-09	0.39 ±0.02hij	2.16 ±0.04g	0.43 ±0.03e	8.82 ±0.04cd	0.70 ±0.02d	0.04 ±0.00h
44-ba-05	0.16 ±0.01l	6.50 ±0.04a	0.00 ±0.00k	5.60 ±0.55ij	0.48 ±0.02g	0.00 ±0.00k
Angut-Bayırbağı	0.35 ±0.01jk	0.82 ±0.01n	0.11 ±0.00j	8.63 ±0.03d	0.68 ±0.01de	0.12 ±0.01e
Elazığ-Çekirdekli	0.57 ±0.02fg	1.05 ±0.03l	0.17 ±0.01i	3.70 ±0.03l	0.55 ±0.04f	0.03 ±0.00i
İstanbul-dut (24-10)	0.71 ±0.05de	0.97 ±0.03m	0.09 ±0.00j	12.45 ±0.96a	0.96 ±0.03ab	0.13 ±0.01d
44-MRK-05	0.34 ±0.02k	0.70 ±0.04o	0.00 ±0.00k	10.77 ±0.11b	1.01 ±0.10a	0.08 ±0.01f
Arapgir-0011	0.42 ±0.02hi	2.16 ±0.06g	0.17 ±0.01i	9.12 ±0.05c	0.66 ±0.05de	0.21 ±0.01a
Arapgir-0012	0.43 ±0.03h	1.85 ±0.03i	0.82 ±0.02a	8.78 ±0.34cd	0.50 ±0.01fg	0.18 ±0.01b
44-KE-10	0.42 ±0.03hi	1.51 ±0.05j	0.36 ±0.01f	7.51 ±0.04f	0.95 ±0.02b	0.07 ±0.01g
24-MRK-01	0.38 ±0.02ijk	2.12 ±0.05g	0.38 ±0.02f	7.77 ±0.04ef	0.94 ±0.03b	0.07 ±0.01g
24-KE-05	0.79 ±0.02c	1.16 ±0.01k	0.36 ±0.01f	8.03 ±0.06e	0.96 ±0.04ab	0.17 ±0.00c

*Difference between means designated with the same letter in the same column is not significant at 0.05 level

Malic acid and citric acid were dominant organic acids in the fruits of all the mulberry cultivars and genotypes. They were followed by oxalic acid, succinic acid, tartaric acid, and fumaric acid. The concentrations of malic acid and citric acid were between 3.70 g 100 g^{-1} (Elaziğ çekirdekli) and 12.70 g 100 g^{-1} (He ye bar and Istanbul dut), and 0.70 g 100 g^{-1} (44-MRK-05) and 6.50 g 100 g^{-1} (44-BA-05), respectively (Tab. 5). In parallel to this study, Ozgen et al. (2009) from Turkey and Sanchez et al. (2014) from Spain determined that malic and citric acid from among the organic acids found in mulberry fruits were the most abundant. Eyduran et al. (2015) reported that malic acid was the dominant organic acid in mulberry fruits, with a concentration between 1.13 and 3.04 g 100 g^{-1}. Gecer et al. (2016) stated that the highest values of malic acid found in black and white mulberries were 3.07 and 2.13 g 100 g^{-1}, respectively. Gundogdu et al. (2011) measured citric acid and malic acid in black mulberries as 1.084 and 1.323 g 100 g^{-1}, and in white mulberries as 0.393 and 3.095 g 100 g^{-1}, respectively.

The highest oxalic acid content was 1.18 g 100 g^{-1} in the Gosho aromi cultivar and its lowest value was 0.16 g^{-1} in the 44-Ba-05 genotype. On the other hand, the 44-Ba-05 genotype had the highest citric acid content, while the 44-nrk-05 genotype had the lowest value. Tartaric acid content was measured between 0.09 g 100 g^{-1} (Istanbul-dut) and 0.82 g 100 g^{-1} (Arapgir-0012). However, the difference in tartaric acid content between the Arapgir-0012 genotype and the cultivar Ship yeoung was not significant. There was also no significant difference between the Istanbul-dut genotype and the Angut genotype. In two samples tartaric acid was not detected. The highest succinic acid content was 1.01 g 100 g^{-1} in 44-MRK-05, and its lowest value was 0.44 g 100 g^{-1} in the Kokusa 20 cultivar. The fumaric acid content was determined to vary among all the cultivars and genotypes in the range of 0.01 g 100 g^{-1} to 0.21 g 100 g^{-1}. Gundogdu et al. (2011) had measured tartaric acid, succinic acid, and fumaric acid in black mulberries as 0.123, 0.342 and 0.011 g 100 g^{-1}, and in white mulberries as 0.223, 0.168, and 0.024 g 100 g^{-1}, respectively. Mikulic-Petkovsek et al. (2012) measured the fumaric acid content in mulberry fruits at the lowest level. They determined the concentrations of citric acid, tartaric acid, succinic acid and fumaric acid in mulberry fruits in the ranges of 0.48 to 1.03 g 100 g^{-1}, 0.15 to 0.43 g 100 g^{-1}, 0.12 to 0.44 g 100 g^{-1}, and 0.01 to 0.12 g 100 g^{-1}, respectively. The differences in the concentration of organic acids might be associated with factors such as genetic factors, cultivation practices, climatic conditions, and soil structure (Ruttanaprasert et al. 2014). The organic acid content is a determinant of fruit taste depending on the acid-sugar balance. Organic acids in fruits and vegetables mostly occur in a free form or are combined as salts, esters or glycosides (Cemeroğlu and Acar 1986). In addition to imparting taste to fruits, organic acids are among the chemicals that also have a vital importance in protecting human health. It has been understood in some studies that organic acids, especially malic acid, citric acid and tartaric acid, make significant contributions to human health in several respects such as enhancing the immune system, preventing the formation of kidney stones, eliminating oral diseases, reducing the risk of poisoning by toxic metals, beautifying and strengthening of the skin, and reducing fibromyalgia symptoms (Abraham and Flechas 1992, Penniston et al. 2007).

Vitamin C

Differences were observed between the cultivars and genotypes in terms of vitamin C content (Tab. 6). The highest vitamin C content was measured as 31.34 mg 100 g^{-1} in the Thengxtang cultivar; it had the lowest values in the Suwean daeyap cultivar and the 24-MRK-01 genotype as 18.20 mg 100 g^{-1} and 18.15 mg 100 g^{-1}, respectively. Lale and Ozcagiran (1996) had measured the vitamin C content in black and purple mulberries as 16.6 and 11.9 mg 100 mL^{-1}, respectively. Ercisli and Orhan (2008) stated that the vitamin C content of fruits taken from black mulberry genotypes grown in the Northeast Anatolia Region of Turkey varied between 14.9 and 18.8 mg 100 mL^{-1}. Ercisli and Orhan (2007) reported the vitamin C content in white, red, and black mulberries as 22.4, 19.4, and 21.8 mg 100 mL^{-1}, respectively. In another study, the vitamin C content of black and purple mulberry fruits was measured as 20.79 and 18.87 mg 100 mL^{-1}, respectively (Ercisli et al. 2010). Imran et al. (2010) reported that white and black mulberries contained vitamin C in the amount of 15.20 and 15.37 mg 100 g^{-1}, respectively. In a study conducted by Eyduran et al. (2015) to analyze the fruits of white and black mulberries, vitamin C content ranged from 10.12 to 18.22 mg 100 g^{-1}. Gecer et al. (2016) found the vitamin C content of white and black mulberries as 12.74 and 16.42 mg 100 g^{-1}, respectively. Karacali (2012) mentioned that fruit types could be classified into three groups: poor, average, or rich in terms of vitamin C content, and in this respect mulberry fruits are generally assigned to the group which is

Table 6. Vitamin C, total antioxidant capacity (TEAC), and sugar content of mulberry cultivars and genotypes (mean for 2014 and 2015)

Cultivars and genotypes	Vitamin C (mg 100 g^{-1})	TEAC (µmol TE* g^{-1})	Glucose (g 100 g^{-1})	Fructose (g 100 g^{-1})	Sucrose (g 100 g^{-1})
Ship Yeoung	22.13 ±0.00f**	15.19 ±0.07f	8.15 ±0.11b	7.11 ±0.04b	1.35 ±0.03c
Suwean Daeyap	18.20 ±0.06n	13.13 ±0.09j	7.22 ±0.03d	5.15 ±0.02g	0.92 ±0.01ghi
Roso	19.38 ±0.03l	11.13 ±0.08k	6.24 ±0.06h	5.07 ±0.05g	0.88 ±0.02ij
Yong Choenchoe	21.35 ±0.03gh	13.57 ±0.09h	8.17 ±0.04b	6.23 ±0.04d	1.34 ±0.04c
Gosho Eromi	29.31 ±0.07b	8.23 ±0.02o	7.70 ±0.09c	6.11 ±0.03d	1.14 ±0.05d
Thengxiang	31.34 ±0.01a	18.35 ±0.11b	7.07 ±0.06e	5.84 ±0.09e	0.96 ±0.01g
Kokusa 20	22.17 ±0.01f	15.18 ±0.04f	6.93 ±0.06f	5.30 ±0.03f	1.08 ±0.04ef
He ye bar	21.14 ±0.00hi	14.17 ±0.06g	6.41 ±0.07g	4.55 ±0.21h	0.90 ±0.04hij
23-mrk-09	25.14 ±0.01d	6.17 ±0.03p	5.20 ±0.07i	4.10 ±0.01i	0.94 ±0.01gh
44-ba-05	18.48 ±0.20m	9.84 ±0.04m	5.30 ±0.06i	5.11 ±0.03g	1.14 ±0.02d
Angut-Bayırbağı	26.26 ±0.38c	15.31 ±0.02e	7.19 ±0.05d	6.23 ±0.06d	1.10 ±0.02de
Elazığ-Çekirdekli	19.47 ±0.22l	13.13 ±0.02j	6.15 ±0.03h	5.17 ±0.04g	1.07 ±0.06ef
İstanbul-dut (24-10)	22.56 ±0.01e	16.25 ±0.04d	8.09 ±0.08b	6.79 ±0.07c	1.32 ±0.04c
44-MRK-05	21.46 ±0.02g	18.07 ±0.06c	5.20 ±0.06i	4.19 ±0.08i	0.85 ±0.02j
Arapgir-0011	20.46 ±0.06j	11.10 ±0.02k	7.19 ±0.06d	5.87 ±0.11e	1.14 ±0.03d
Arapgir-0012	19.41 ±0.30l	13.24 ±0.03i	5.19 ±0.03i	4.12 ±0.01i	0.95 ±0.02gh
44-KE-10	21.03 ±0.03i	9.13 ±0.05n	6.24 ±0.06h	5.15 ±0.11g	1.04 ±0.04f
24-MRK-01	18.15 ±0.03n	10.11 ±0.05l	9.22 ±0.09a	7.90 ±0.04a	1.60 ±0.03b
24-KE-05	19.73 ±0.02k	21.13 ±0.06a	8.18 ±0.07b	6.87 ±0.12c	1.91 ±0.05a

*TE – Trolox equivalent
**Difference between means designated with the same letter in the same column is not significant at 0.05 level

designated as the average group in terms of vitamin C content.

Antioxidant activity

Total antioxidant capacity (TEAC) results for mulberry fruits are given in Table 6. There were statistically significant differences between the cultivars and genotypes ($p < 0.05$). The TEAC content was determined to be between 6.17 µmol TE g^{-1} (23-MRK-09 genotype) and 21.13 µmol TE g^{-1} (24-KE-05 genotype) (Tab. 6). Gundogdu et al. (2011) had reported that black mulberries had higher TEAC values compared to white mulberries. Gungor and Sengul (2008) reported that antioxidant capacity in white mulberries varied between 18.16 and 19.24 µmol TE g^{-1}. Ozgen et al. (2009) measured antioxidant activity in black mulberries in the range of 6.8 to 14.4 µmol TE g^{-1}. Eyduran et al. (2015) indicated that there was variation among mulberry genotypes in terms of total antioxidant capacity, which was measured between 6.17 and 14.40 µmol TE g^{-1}, and that black mulberries had a higher TEAC value compared to white mulberries. In parallel with this, Gecer et al. (2016) also reported that black mulberries had a higher TEAC value (9.17 µmol TE g^{-1}) than white mulberries (6.17 µmol TE g^{-1}). A significant difference in terms of antioxidant capacity has been observed between white and black mulberries grown in Spain (Sanchez et al. 2014). The health importance of mulberry fruits has increased recently because of their potential for high antioxidant activity (Sanchez et al. 2014). Therefore, mulberry genotypes (especially the 24-KE-05 genotype) have been found to be important for high antioxidant content, and we believe that this will help mulberry breeders who are interested in developing elite cultivars with high antioxidant capacity.

Sugars

In this study, the concentrations of glucose, fructose, and sucrose, which are essential sugars in mulberry fruits, were determined and the differences between the cultivars and genotypes were revealed (Tab. 6). The level of sucrose was measured to be lower than that of the other sugars. The highest values in terms of glucose and fructose content were obtained for the 24-MRK-01 genotype as 9.22 g 100 g^{-1} and 7.90 g 100 g^{-1}, respectively. The highest sucrose content was also determined as 1.91 g 100 g^{-1} in the 24-KE-05 genotype (Tab.

6). Previously, great differences had been observed between genotypes and cultivars in terms of sugar content in fruit samples taken from mulberry trees in different countries. In Spain, Sanchez et al. (2014) determined the glucose content and fructose content of fully ripened white mulberries between 4.22 and 5.37 g 100 g^{-1}, and between 6.53 and 8.55 g 100 g^{-1}, respectively, and the glucose content and fructose content of black mulberries between 3.19 and 7.45 g 100 g^{-1}, and between 4.82 and 11.7 g 100 g^{-1}, respectively. Mahmood et al. (2012) measured the glucose and fructose contents of black mulberries harvested when fully ripe in the climatic conditions of Pakistan as 2.50 and 5.36 g 100 g^{-1}, and the glucose and fructose contents of white mulberries as 3.21 and 4.97 g 100 g^{-1}, respectively. Eyduran et al. (2015) determined that the glucose content of fruits taken from all black and white mulberry genotypes was higher than the fructose content, with the highest glucose and fructose concentrations of 9.44 and 7.70 g 100 g^{-1}, respectively, obtained from white mulberries. Gecer et al. (2016) evaluated black and white mulberries and found higher levels of fructose (8.16 and 7.69 g 100 g^{-1}, respectively) and glucose (9.55 and 8.31 g 100 g^{-1}, respectively). In Spain, the determined values were highest for fructose and glucose and lowest for sucrose (Sanchez et al. 2014). Ozgen et al. (2009) stated that the fructose and glucose contents of fourteen black and red mulberry genotypes ranged from 5.50 to 7.12 g 100 mL^{-1} and from 4.86 to 6.41 g 100 mL^{-1}, respectively. In another study, Mikulic-Petkovsek et al. (2012) indicated that glucose and fructose determined in 25 wild and cultivated mulberries were more abundant, and the glucose content of black mulberry fruits growing wild in Slovenia was measured as 3.68 g 100 g^{-1} and the fructose content as 3.99 g 100 g^{-1}. The amounts of sugars determined in the fruits of mulberry cultivars and genotypes vary depending on genetic factors, cultivation practices, and environmental conditions (Gundogdu et al. 2011).

CONCLUSIONS

1. In the presented study, attempt was made to optimize the effects of various factors on the biochemical content of mulberry fruits by growing mulberry cultivars and genotypes under the same environmental conditions and in a place where the same cultivation practices were implemented. Therefore, only the genetic differences among the cultivars and genotypes were effective in determining the biochemical content of fruits, and those differences were found to be statistically significant ($p < 0.05$) when the results obtained for the phytochemical content of the analyzed mulberry fruits were examined.
2. Examined mulberry cultivars and genotypes were found to be rich in phenolic compounds such as chlorogenic acid, caffeic acid, *p*-coumaric acid, and *o*-coumaric acid, which are especially known for anti-cancer, anti-fungal, allelopathic, and anti-microbial characteristics. According to the results of numerous studies, this is thought to provide positive influence for increasing the value and consumption of mulberry fruits, as a source of phytochemicals with important benefits in terms of nutrition and health. In addition to providing benefits for both producers and consumers, this will also contribute to the development of improvement studies and industries related to these fruits.
3. It is thought that the results obtained in this study are important in terms of being a source for further studies and revealing nutritional values of world gene pools. This study has a unique quality in terms of revealing relations of these phytochemicals with their corresponding genes and developing new cultivars by conducting genetic improvement studies. In addition, the paper describes the genotypic response of some mulberry genotypes from Anatolia in respect of some biochemical properties and we believe that it will help international mulberry breeders who are interested in developing elite cultivars with better qualities as these genotypes might be used as parents in mulberry breeding.

AUTHOR CONTRIBUTIONS

M.G., I.C, M.K.G, T.K. and S.E. – contributed equally to this work.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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