

# Antioxidant Capacity, Sugar Content, and Tandem HPLC–DAD–ESI/MS Profiling of Phenolic Compounds from *Aronia melanocarpa* Fruits and Leaves (Nero and Viking Cultivars)

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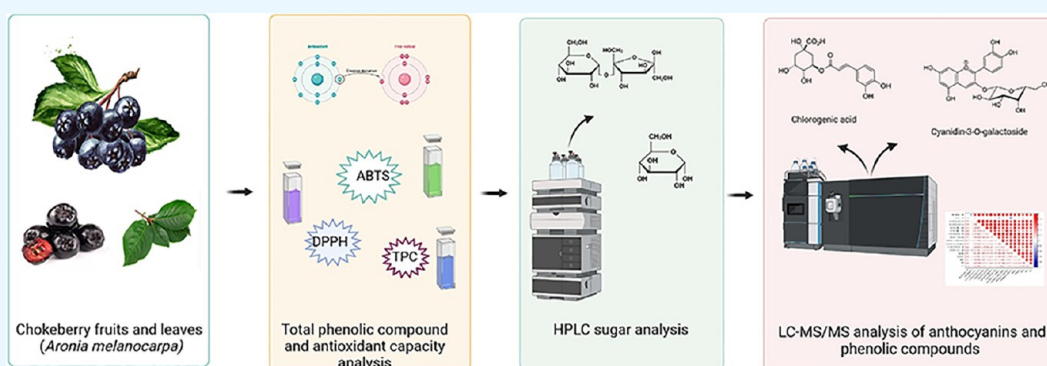


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**ABSTRACT:** This study examined the phenolic profile, sugar composition, and antioxidant capacities of chokeberry fruits and leaves obtained from four different Turkish provinces. A total of 21 phenolics including phenolic acid and its derivatives, flavanols, anthocyanins, and flavonols were determined in the fruits, while the leaves had 19 phenolics, including phenolic acid and its derivatives, flavanols, and flavonols. The total amount of phenolic compounds was the highest in both fruits and leaves in the samples from the Bursa province. *Cis 5-O-p-coumaroylquinic acid* and *secoxyloganin* were quantified for the first time in both fruits and leaves. In summary, it was found that different geographical locations significantly affected the phenolics, sugar contents, and antioxidant activities of the fruits and leaves.

## 1. INTRODUCTION

*Aronia* is a shrubby plant with berry fruits from the *Aronia* genus of the *Rosaceae* family. This plant, known also as chokeberry, has two main species: black-colored *Aronia melanocarpa* (Michaux) Elliot and red-colored *Aronia arbutifolia* (Linnaeus) Elliot. A third species, the purple-colored *Aronia prunifolia* (Marshall) Rehder, is considered a hybrid of the former two species.<sup>1</sup> Different varieties of aronia can be grown all over the world. Nero (Czech Republic) and Viking (Finland) are the two most widely known varieties of this species. In addition, Rubina (Russia), Kurkumäcki (Finland), Hugin (Sweden), Fertödi (Hungary), Albigowa, Dabrowice, Serina, Galicjanka (Poland), and Aron (Denmark) are the other important varieties.<sup>2,3</sup> The aronia plant, native to North America, does not have special soil and climate demands. It spread to Western Europe and Russia in the 1900s and is now commercially grown, especially in Eastern and Central Europe.<sup>4–7</sup>

Although aronia fruit can be consumed fresh, it is usually processed into alternative products such as dried fruit, fruit puree, fruit juice, jam, tea, liquor, and wine due to its sour and

bitter taste.<sup>2,6,7</sup> It is also used as an ingredient in functional foods and dietary supplements due to its high anthocyanin content and as a natural colorant in the food and pharmaceutical industry.<sup>8</sup> Aronia fruit has a unique bioactive composition along with phenolic groups such as anthocyanins, procyanidins, phenolic acids, flavonols, dietary fiber (~5%), reducing sugar (16–18%), organic acids (mainly malic and citric acids), vitamins (B and C groups), and minerals.<sup>6,9</sup> The most important and abundant phenolic groups in aronia fruits are anthocyanins and procyanidins. Anthocyanins are the pigments that give the fruits their dark red, blue, and purple colors.<sup>2</sup> Procyanidins, on the other hand, are oligomeric (2–10 units) and polymeric (>10 units) (epi)catechins, which are formed by the combination of a certain number of monomeric

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units. In addition to these, aronia fruit is also very rich in phenolic acids such as chlorogenic and neochlorogenic acid. Flavonols, on the other hand, are generally composed of quercetin derivatives and are less abundant than the other phenolic groups mentioned above.<sup>3,10</sup> Aronia berries contain a large amount of phenolics compared to other berry fruits such as blackberries, cranberries, and strawberries, making them a unique fruit.<sup>11</sup> The reason for this is that it has a long ripening and harvesting period compared to other berry fruits, enriching it in terms of types and amounts of phenolics.<sup>12</sup> Aronia fruit has been reported to have antioxidant, antibacterial, anti-mutagenic, anticancer, anti-inflammatory, and antidiabetic activities thanks to its high polyphenol and anthocyanin content.<sup>13,14</sup>

Aronia plants have significant amounts of leaves, but they are usually considered as residues.<sup>15</sup> However, in South Korea, the first fresh leaves of the aronia plant, which are slightly sour-sweet, are consumed. Aronia leaves, which have high contents of polyphenol, flavonoid, and chlorophyll, mainly contain phenolic groups such as flavonoids and hydroxycinnamic acids and exhibit high antioxidant, anti-inflammatory, and antibacterial activity thanks to their phenolic compounds.<sup>16</sup> In addition to these activities, aronia leaves have been reported as effective in the prevention and treatment of cancer, leukemia, and other chronic diseases.<sup>15,17</sup> Although several studies have been conducted on the aronia plant leaves, data are still limited to its potential beneficial effects. The high diversity of biophenols may support the utilization of these components to enhance the nutritional and functional value of food products.<sup>18,19</sup>

The total phenolic content of fruits and leaves may vary depending on the variety, location, climatic conditions, ripening, and harvest time.<sup>6,20</sup> Aronia plant grown in different regions is exposed to differing climatic conditions (temperature, precipitation, number of sunny days, etc.), resulting in significant differences in the amount of phenolic compounds.<sup>21</sup>

In the literature, the effects of various factors including ripening, harvest time, variety, product types (fresh fruit, juice, and fruit pulp), and extraction methods have been studied on the product quality, phenolic content, and antioxidant activity.<sup>3,8,10–12,15,22–24</sup> However, studies examining the effects of growing locations and regions on the phenolic content and composition of aronia fruits and leaves are limited. Thus, the aims of this study were to determine the phenolic compounds, antioxidant activities, and sugar content of aronia fruits and leaves from two cultivars (Nero and Viking) grown in four different provinces in Turkey.

## 2. RESULTS AND DISCUSSION

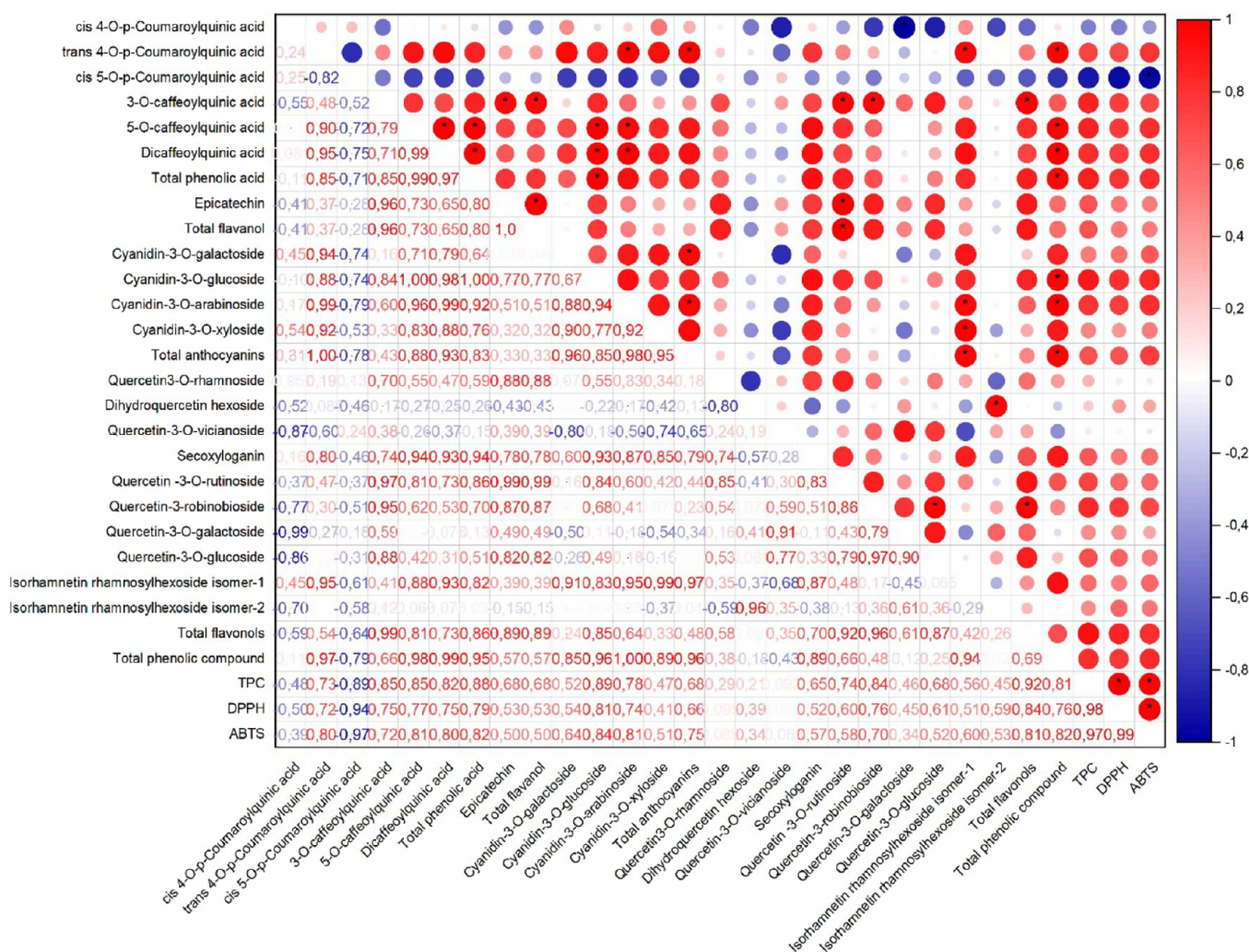
### 2.1. Antioxidant Activity, Total Phenolic Content, and Sugar Composition.

**2.1.1. Fruits.** The antioxidant capacities of the fresh chokeberry fruits and leaves obtained from different provinces (Bursa, Kirklareli, Kirsehir, and Trabzon) were determined by the DPPH and ABTS methods and are presented in Table 1. Statistically significant differences were found in the antioxidant activities of the chokeberry fruits obtained from different regions ( $P < 0.05$ ). The highest DPPH antioxidant capacity value (11,758.3  $\mu\text{mol Trolox}/100\text{ g}$ ) and ABTS antioxidant capacity value (18,363.9  $\mu\text{mol Trolox}/100\text{ g}$ ) were determined in Bursa fruit samples, while the lowest DPPH antioxidant capacity value (8975.0  $\mu\text{mol Trolox}/100\text{ g}$ ) and ABTS antioxidant capacity value (13,950.0  $\mu\text{mol Trolox}/100\text{ g}$ ) were observed in Trabzon fruit samples. Jakobek et al.<sup>14</sup>

Table 1. Antioxidant Capacity of Chokeberry Fruit and Leaf Samples from Four Different Locations<sup>a</sup>

	Bursa		Kirklareli		Kirsehir		Trabzon	
	fruit	leaf	fruit	leaf	fruit	leaf	fruit	leaf
total phenolics (mg GAE/100 g)	1701.7 $\pm$ 14.6 <sup>c</sup>	1088.3 $\pm$ 26.3 <sup>d</sup>	1551.9 $\pm$ 14.6 <sup>b</sup>	687.2 $\pm$ 8.1 <sup>b</sup>	1491.0 $\pm$ 137.1 <sup>b</sup>	744.5 $\pm$ 33.9 <sup>c</sup>	1300.9 $\pm$ 71.7 <sup>a</sup>	568.6 $\pm$ 4.4 <sup>a</sup>
antioxidant activity								
DPPH ( $\mu\text{mol trolox}/100\text{ g}$ )	11758.3 $\pm$ 326.9 <sup>d</sup>	5258.9 $\pm$ 122.3 <sup>d</sup>	11083.3 $\pm$ 225.5 <sup>c</sup>	2673.3 $\pm$ 163.9 <sup>b</sup>	10089.0 $\pm$ 84.3 <sup>b</sup>	3871.1 $\pm$ 84.7 <sup>c</sup>	8975 $\pm$ 252.9 <sup>a</sup>	2173.3 $\pm$ 178.9 <sup>a</sup>
ABTS ( $\mu\text{mol trolox}/100\text{ g}$ )	18363.9 $\pm$ 162.5 <sup>d</sup>	7588.9 $\pm$ 302.1 <sup>d</sup>	16947.2 $\pm$ 468.3 <sup>c</sup>	4927.8 $\pm$ 76.8 <sup>b</sup>	15272.2 $\pm$ 546.0 <sup>b</sup>	5502.2 $\pm$ 74.3 <sup>c</sup>	13950 $\pm$ 385.8 <sup>a</sup>	3993.3 $\pm$ 37.9 <sup>a</sup>

<sup>a–d</sup>Different letters in the rows represent statistically significant differences ( $P < 0.05$ ). Varieties: Bursa, Trabzon, Kirsehir Viking, and Kirklareli Nero.



\*  $p < 0.05$

**Figure 1.** Correlation matrix of the antioxidant activity and phenolic profile of the chokeberry fruit samples.

determined the antioxidant activity of fresh Nero variety chokeberry fruits as  $156.27 \mu\text{mol Trolox/g}$  fresh weight (FW) according to the DPPH method as in our study. In other previous studies, the antioxidant activities of chokeberry fruit were determined as  $158 \mu\text{mol Trolox/g}$  FW and  $109,191 \mu\text{mol Trolox/g}$  FW.<sup>25,26</sup> The current study's findings ( $89.7$ – $183.6 \mu\text{mol Trolox/g}$ ) were similar to these reported data.

The total phenolic content of chokeberry fruits was determined according to the method of Kelebek et al.<sup>3</sup> The total phenolic contents of the chokeberry fruits varied between  $1300.9$  and  $1701.74 \text{ mg GAE}/100 \text{ g}$ , and significant differences were determined among the regions ( $P < 0.05$ ) (Table 1). The highest total phenolic content was also observed in the samples from Bursa. Similar findings as of the present study were reported in some other previous studies as  $1455.2 \text{ mg GAE}/100 \text{ g}$  and  $1388 \text{ mg GAE}/100 \text{ g}$ .<sup>6,27</sup> However, Ochmian et al.<sup>28</sup> determined higher total phenolic contents of chokeberry fruits from four cultivars as follows: Nero:  $1950$  and Viking:  $1845 \text{ mg GAE}/100 \text{ g}$  compared to the current study. On the other hand, Jakobek et al.<sup>11</sup> reported phenolic contents in chokeberry fruits of four cultivars (Nero, Viking, Galicianka, and wild type) with the Viking cultivar having the highest during two consecutive

years ( $10804.1 \text{ mg GAE}/\text{kg}$  in 2010 and  $12055.7 \text{ mg GAE}/\text{kg}$  in 2011). Denev et al.<sup>26</sup> reported that the total polyphenol content of 23 different chokeberry samples grown in the climatic conditions of Bulgaria ranged from  $1022$  to  $1795 \text{ mg}/100 \text{ g}$  pointing to a high variation. It was observed that there are slight differences between the findings of this study and the previous studies. These differences can be tied to different fruit varieties, different growing regions, and different growing conditions such as climate, fertilization, and soil structure.<sup>6</sup>

The phenolic compounds are responsible for the antioxidant activity of the plant, and therefore, there is a good relationship between the phenolic content and antioxidant activity of plant material.<sup>26</sup> A similar relationship was observed between the phenolic content and antioxidant activity (DPPH and ABTS) of the chokeberry fruit and leaf samples in this study (Table 1).

Correlation analysis was performed to determine the relationships between antioxidant analysis (DPPH, ABTS), total phenolic content (TPC), and phenolic compounds in chokeberry fruits obtained from different provinces (Bursa, Trabzon, Kirsehir, and Kirlareli) (Figure 1). A strong positive correlation was found between TPC and total antioxidant tests

Table 2. Sugar Content of Chokeberry Fruit and Leaf Samples from Four Different Locations<sup>a</sup>

	Bursa		Kırklareli		Kırşehir		Trabzon	
	fruit	leaf	fruit	leaf	fruit	leaf	fruit	leaf
sugar composition (g/100 g)								
sucrose	0.48 ± 0.01 <sup>b</sup>	1.17 ± 0.01 <sup>b</sup>	0.35 ± 0.01 <sup>a</sup>	0.88 ± 0.01 <sup>a</sup>	0.44 ± 0.03 <sup>b</sup>	1.28 ± 0.01 <sup>c</sup>	0.32 ± 0.01 <sup>a</sup>	0.87 ± 0.01 <sup>a</sup>
glucose	5.61 ± 0.0 <sup>c</sup>	0.71 ± 0.0 <sup>c</sup>	4.86 ± 0.01	0.63 ± 0.03 <sup>b</sup>	6.21 ± 0.01 <sup>d</sup>	0.47 ± 0.02 <sup>a</sup>	2.37 ± 0.00 <sup>a</sup>	0.63 ± 0.0 <sup>b</sup>
fructose	5.23 ± 0.13 <sup>c</sup>	0.70 ± 0.01 <sup>b</sup>	3.90 ± 0.01 <sup>b</sup>	0.77 ± 0.05 <sup>b</sup>	5.22 ± 0.02 <sup>c</sup>	0.60 ± 0.01 <sup>a</sup>	3.11 ± 0.02 <sup>a</sup>	0.98 ± 0.03 <sup>c</sup>
sorbitol	7.59 ± 0.04 <sup>d</sup>	3.16 ± 0.00 <sup>d</sup>	5.82 ± 0.06 <sup>b</sup>	2.29 ± 0.02 <sup>c</sup>	7.09 ± 0.04 <sup>c</sup>	1.55 ± 0.04 <sup>a</sup>	4.09 ± 0.03 <sup>a</sup>	1.62 ± 0.0 <sup>b</sup>
<b>total sugar</b>	<b>18.92 ± 0.08<sup>c</sup></b>	<b>5.73 ± 0.02<sup>d</sup></b>	<b>14.92 ± 0.05<sup>b</sup></b>	<b>4.57 ± 0.03<sup>c</sup></b>	<b>18.96 ± 0.05<sup>c</sup></b>	<b>3.91 ± 0.06<sup>a</sup></b>	<b>9.89 ± 0.07<sup>a</sup></b>	<b>4.10 ± 0.02<sup>b</sup></b>

<sup>a</sup>–<sup>d</sup>Different letters in the rows represent statistically significant differences ( $P < 0.05$ ). Varieties: Bursa, Trabzon, Kırşehir Viking, and Kırklareli Nero.

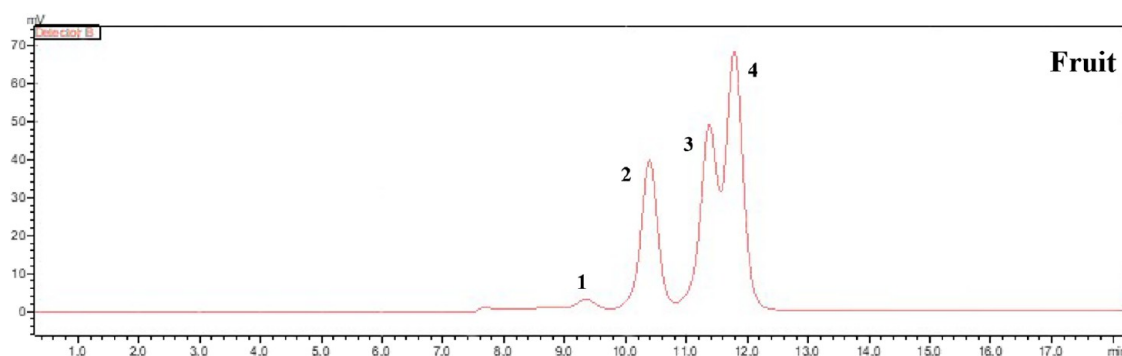


Figure 2. HPLC chromatogram of chokeberry fruit content (1; sucrose, 2; glucose, 3; fructose; 4; sorbitol).

( $r = 0.98$  for TPC vs DPPH;  $r = 0.97$  for TPC vs ABTS) (Figure 1).

The sugar compositions of the chokeberry fruits were also examined (Table 2 and Figure 2). Significant differences in the sugar contents were found according to the sampling locations. The total sugar amounts were determined as 18.92, 14.92, 18.96, and 9.89 g/100 g in the samples from Bursa, Kırklareli, Kırşehir, and Trabzon, respectively. The highest sugar content was found in the Bursa and Kırşehir samples, while the lowest was detected in the Trabzon sample (Table 1). In a former study, Ochmian et al.<sup>28</sup> reported that the total sugar contents of chokeberry fruit from four different cultivars (Galicjanka, Hugin, Nero, and Viking) were between 9.16 and 13.79 g/100 g. The total sugar content of the Viking cultivar (9.16 g/100 g) in the study is comparable to the sugar content of the Trabzon sample in the current study (9.89 g/100 g). The amounts of sucrose, glucose, fructose, and sorbitol in the present study ranged from 0.32 to 0.48, 2.37 to 6.21, 3.11 to 5.23, and 4.09 to 7.59 g/100 g, respectively (Table 2). Sorbitol was detected as the main sugar in all chokeberry fruits, is a sugar alcohol, and can be used as a sugar substitute. Sorbitol actually was reported to have a lower glycemic index compared to sucrose (65) and glucose (100).<sup>9,16</sup> Denev et al.<sup>26</sup> reported that sorbitol was the main sugar with 6.6 to 13.0 g/100 g in 23 chokeberry fruits of Nero cultivar.

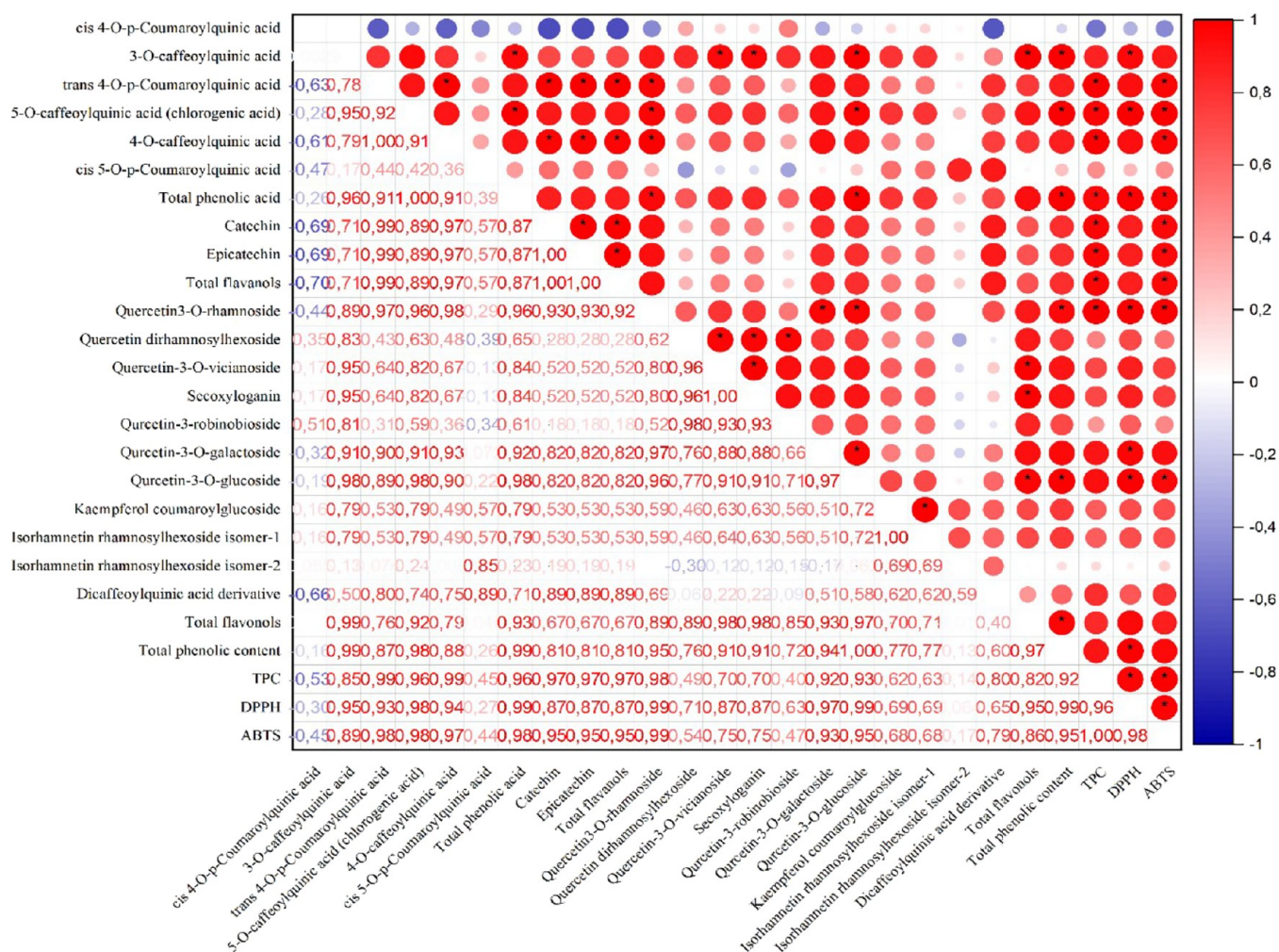
**2.1.2. Leaves.** The antioxidant activities of the chokeberry leaves from four regions (Bursa, Kırklareli, Kırşehir, and Trabzon) are listed in Table 1. Significant differences were found between the antioxidant activities obtained from different regions ( $P < 0.05$ ). Similar to the fruit samples, the highest antioxidant activities (DPPH: 5258.9 and ABTS: 7588.9  $\mu\text{mol Trolox}/100\text{ g}$ ) were determined in the samples from Bursa, while the lowest values (DPPH: 2173.3 and ABTS: 3993.3  $\mu\text{mol Trolox}/100\text{ g}$ ) were found in the samples from Trabzon (Table 1).

The total phenolic contents of all chokeberry leaves varied between 568.6 and 1088.3 mg GAE/100 g, and significant differences were determined according to region ( $P < 0.05$ ) (Table 1). The highest total phenolic content was also determined in the sample from Bursa. Thi and Hwang<sup>15</sup> studied the total phenolic contents of chokeberry leaves with different extraction solvents (water and 80% ethanol) and at two maturity stages (young and old). They found total phenolics between 69.5 and 250.8 mg/g DM, and the highest value was in the sample extracted with ethanol. They also reported that young leaves contained more polyphenols than those of older leaves. The total phenolic content of chokeberry (Nero) leaves from southeast Serbia was reported as 1947 mg/100 g under optimum extraction conditions similar to the findings of the present study.<sup>27</sup>

The correlation analysis found a strong positive correlation between TPC and total antioxidant tests ( $r = 0.98$  for TPC vs DPPH;  $r = 0.94$  for TPC vs ABTS) (Figure 3).

Total sugar contents of the chokeberry leaf samples were also found as statistically significant ( $P < 0.05$ ) (Table 2 and Figure 4). The highest total sugar content was found in the Bursa sample, and the lowest was in the Kırşehir sample. The amounts of sucrose, glucose, fructose, and sorbitol ranged from 0.87 to 1.17, 0.47 to 0.71, 0.60 to 0.98, and 1.55 to 3.16 g/100 g, respectively. Sorbitol was found as the main sugar in all leaves, similar to the fruits. Similar to the present study, sorbitol had the highest sugar content in chokeberry leaves in an earlier study.<sup>16</sup>

**2.2. Phenolic Profiles of the Chokeberry Fruits and Leaves.** **2.2.1. Fruits.** The phenolic profiles of the chokeberry fruits identified by LC-DAD-ESI-MS/MS are displayed in Table 3 and Figure 5. All phenolics were specified in negative and positive ionization mode. A total of 21 phenolic compounds were identified and quantified in chokeberry fruits from each of the four sampling provinces. The phenolic groups



\*  $p < 0.05$

Figure 3. Correlation matrix of the antioxidant activity and phenolic profile of the chokeberry leaf samples.

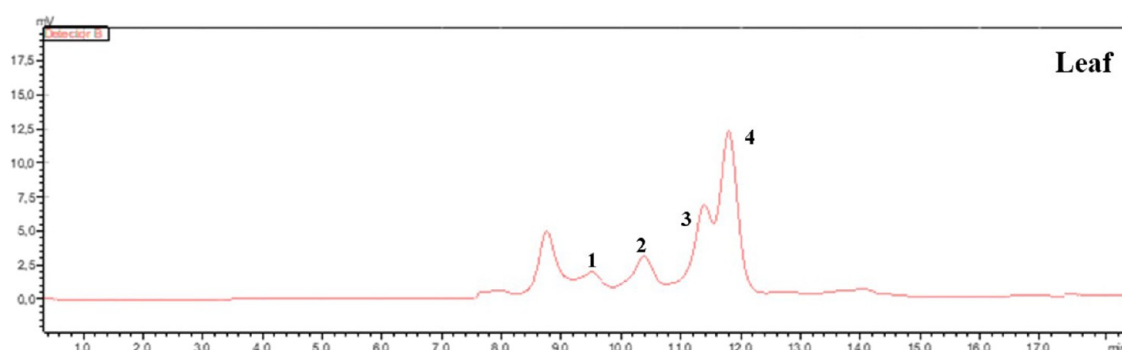


Figure 4. HPLC chromatogram of chokeberry leaf sugar content (1; sucrose, 2; glucose, 3; fructose; 4; sorbitol).

found in all fruit samples were flavonols (10), phenolic acid and its derivatives (6), anthocyanins (4), and a flavanol (Table 3).

The total amounts of the phenolic compounds were determined as 1590.6 mg/kg in the Bursa, while it was 1058.9 mg/kg in the Kırklareli, 1023.7 mg/kg in the Kırşehir, and 997.7 mg/kg in the Trabzon samples (Table 3). When the total phenolics were compared, the highest values were found in the Bursa samples, and the lowest values were in Trabzon samples (Table 3). It was found that locations had a

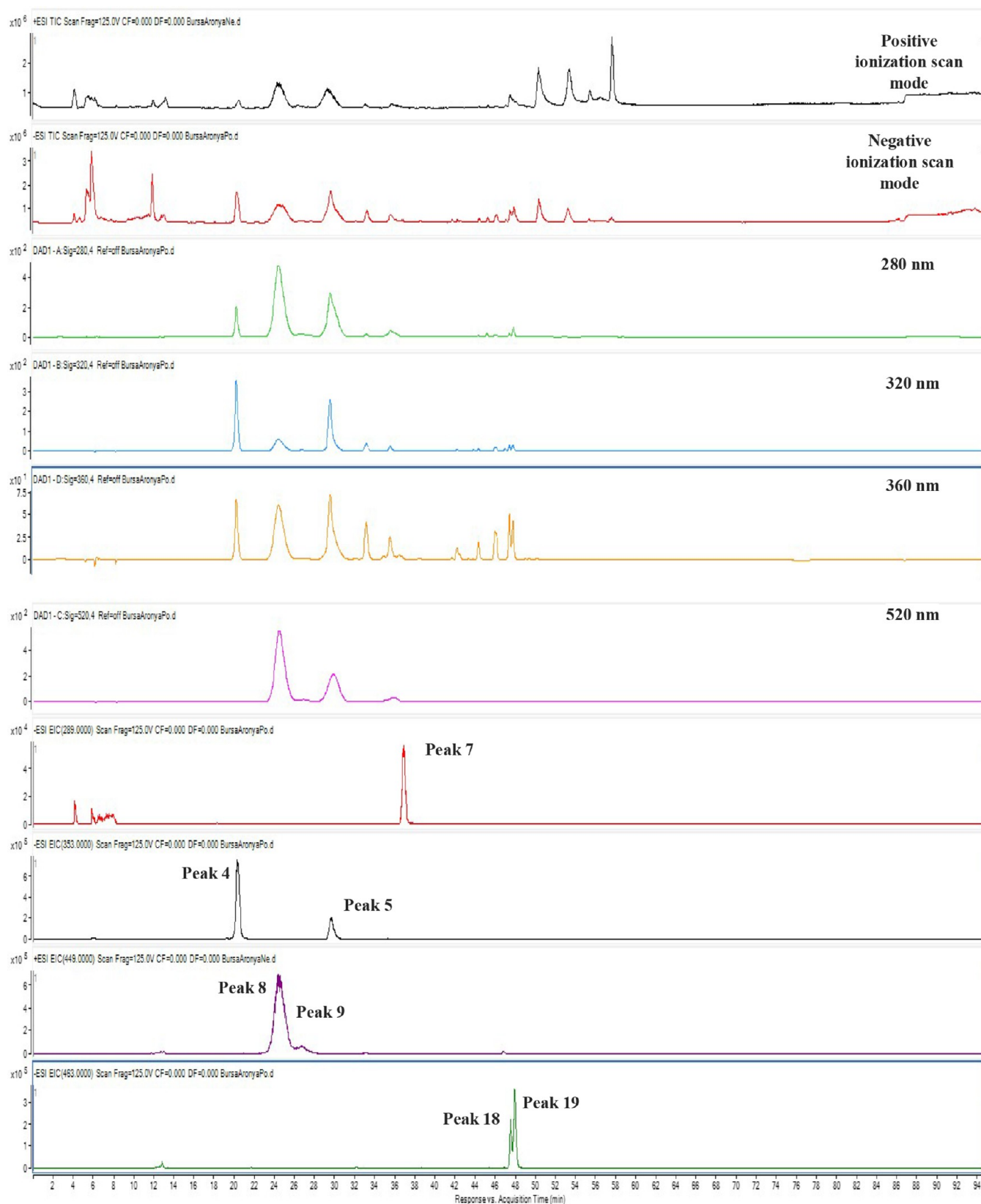
statistically significant effect ( $P < 0.05$ ) on the amount of phenolic compounds in chokeberry fruits.

The most abundant phenolic group was anthocyanins in the chokeberry fruits (628.7–1022.8 mg/kg) (Table 3). This result is in agreement with the data from the earlier studies in which anthocyanins were reported as the major phenolics (cyanidin-3-galactoside, cyanidin-3-arabinoside) in chokeberry fruits.<sup>11</sup> Anthocyanins are mainly responsible for deep red, blue, and purple colors in fruits.<sup>2</sup> In the present study, a total of four typical anthocyanin compounds (cyanidin-3-O-galacto-

Table 3. Phenolic Compounds of the Chokeberry Fruit Samples (Means  $\pm$  Standard Deviations) (mg/kg)<sup>a</sup>

	phenolic compounds	molecular formula	RT* (min)	$\lambda_{\max}$ (nm)	$[M - H]^{-2}/[M - H]^+$	MS/MS ions	Bursa	Kırklareli	Kırşehir	Trabzon
phenolic acids and derivatives										
1	<i>cis</i> 4- <i>O-p</i> -coumaroylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	13,06	312	337 <sup>1</sup>	173, 163, 93	0.42 $\pm$ 0.02 <sup>a</sup>	0.25 $\pm$ 0.02 <sup>a</sup>	0.26 $\pm$ 0.02 <sup>a</sup>	0.58 $\pm$ 0.04 <sup>a</sup>
2	<i>trans</i> 4- <i>O-p</i> -coumaroylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	26,88	312	337 <sup>1</sup>	173, 163, 93	0.26 $\pm$ 0.00 <sup>d</sup>	0.14 $\pm$ 0.00 <sup>c</sup>	0.10 $\pm$ 0.00 <sup>a</sup>	0.13 $\pm$ 0.00 <sup>b</sup>
3	<i>cis</i> 5- <i>O-p</i> -Coumaroylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	39,20	312	337 <sup>1</sup>	191, 173, 163, 119	1.11 $\pm$ 0.00 <sup>a</sup>	1.32 $\pm$ 0.01 <sup>b</sup>	1.87 $\pm$ 0.03 <sup>c</sup>	1.92 $\pm$ 0.01 <sup>d</sup>
4	3- <i>O</i> -caffeoylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	20,53	326	353 <sup>1</sup>	191, 179, 127, 173, 85	91.77 $\pm$ 0.21 <sup>d</sup>	67.59 $\pm$ 0.57 <sup>b</sup>	85.43 $\pm$ 0.11 <sup>c</sup>	48.00 $\pm$ 0.74 <sup>a</sup>
5	5- <i>O</i> -caffeoylquinic acid (chlorogenic acid)	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	29,59	320	353 <sup>1</sup>	191, 173, 127, 111, 93, 109	389.42 $\pm$ 0.06 <sup>d</sup>	205.56 $\pm$ 0.49 <sup>b</sup>	233.77 $\pm$ 0.69 <sup>c</sup>	179.62 $\pm$ 1.66 <sup>a</sup>
6	dicafeoylquinic acid	C <sub>25</sub> H <sub>34</sub> O <sub>12</sub>	35,71	245, 326	515 <sup>1</sup>	353, 191, 179, 173	4.25 $\pm$ 0.09 <sup>c</sup>	2.30 $\pm$ 0.01 <sup>b</sup>	2.41 $\pm$ 0.00 <sup>b</sup>	2.14 $\pm$ 0.04 <sup>a</sup>
<b>total phenolic acids</b>										
flavanol										
7	epicatechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	36,57	278	289 <sup>1</sup>	245, 175	3.49 $\pm$ 0.06 <sup>c</sup>	2.45 $\pm$ 0.02 <sup>b</sup>	3.50 $\pm$ 0.01 <sup>c</sup>	2.18 $\pm$ 0.02 <sup>a</sup>
<b>total flavanol</b>										
anthocyanins										
8	cyanidin-3- <i>O</i> -galactoside	C <sub>21</sub> H <sub>21</sub> O <sub>11</sub>	24,62	279, 514	449 <sup>2</sup>	287	605.29 $\pm$ 1.23 <sup>d</sup>	509.53 $\pm$ 1.29 <sup>b</sup>	438.25 $\pm$ 0.27 <sup>a</sup>	519.26 $\pm$ 2.96 <sup>c</sup>
9	cyanidin-3- <i>O</i> -glucoside	C <sub>21</sub> H <sub>21</sub> ClO <sub>11</sub>	26,65	279, 514	449 <sup>2</sup>	287	22.91 $\pm$ 1.77 <sup>b</sup>	17.86 $\pm$ 2.34 <sup>ab</sup>	18.68 $\pm$ 2.12 <sup>ab</sup>	16.63 $\pm$ 0.03 <sup>a</sup>
10	cyanidin-3- <i>O</i> -arabinoside	C <sub>20</sub> H <sub>19</sub> ClO <sub>10</sub>	29,24	279, 514	419 <sup>2</sup>	287	345.27 $\pm$ 0.51 <sup>d</sup>	173.54 $\pm$ 0.11 <sup>c</sup>	151.49 $\pm$ 0.41 <sup>a</sup>	160.53 $\pm$ 2.16 <sup>b</sup>
11	cyanidin-3- <i>O</i> -xyloside	C <sub>20</sub> H <sub>19</sub> ClO <sub>10</sub>	35,81	279, 514	419 <sup>2</sup>	287	49.33 $\pm$ 0.50 <sup>c</sup>	19.94 $\pm$ 0.10 <sup>a</sup>	20.26 $\pm$ 0.25 <sup>b</sup>	31.15 $\pm$ 0.07 <sup>b</sup>
<b>total anthocyanins</b>										
flavonols										
12	quercetin-3- <i>O</i> -rhamnoside	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	32,02	350	447 <sup>1</sup>	315	5.24 $\pm$ 0.22 <sup>c</sup>	2.36 $\pm$ 0.01 <sup>a</sup>	5.83 $\pm$ 0.09 <sup>d</sup>	3.56 $\pm$ 0.02 <sup>b</sup>
13	dihydroquercetin hexoside	C <sub>21</sub> H <sub>32</sub> O <sub>12</sub>	33,22	266, 355	465 <sup>1</sup>	303, 285	1.42 $\pm$ 0.03 <sup>b</sup>	7.23 $\pm$ 0.00 <sup>c</sup>	0.80 $\pm$ 0.00 <sup>a</sup>	1.05 $\pm$ 0.34 <sup>ab</sup>
14	quercetin-3- <i>O</i> -vicianoside	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	44,51	266, 355	595 <sup>1</sup>	463, 301	1.69 $\pm$ 0.00 <sup>a</sup>	4.61 $\pm$ 0.33 <sup>b</sup>	6.89 $\pm$ 0.18 <sup>c</sup>	1.17 $\pm$ 0.12 <sup>a</sup>
15	secoxylloganin	C <sub>17</sub> H <sub>24</sub> O <sub>11</sub>	45,25	235	403 <sup>1</sup>	223, 371, 191, 149	10.46 $\pm$ 0.50 <sup>c</sup>	5.51 $\pm$ 0.73 <sup>a</sup>	7.61 $\pm$ 0.21 <sup>b</sup>	6.48 $\pm$ 0.51 <sup>b</sup>
1	quercetin-3- <i>O</i> -rutinoside	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	46,03	355	609 <sup>1</sup>	301	2.61 $\pm$ 0.01 <sup>d</sup>	1.77 $\pm$ 0.02 <sup>b</sup>	2.47 $\pm$ 0.04 <sup>c</sup>	1.56 $\pm$ 0.01 <sup>a</sup>
17	quercetin-3- <i>O</i> -robinobioside	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	46,23	255, 360	609 <sup>1</sup>	301, 447, 373, 271,	5.47 $\pm$ 0.55 <sup>b</sup>	4.92 $\pm$ 0.49 <sup>b</sup>	5.53 $\pm$ 0.13 <sup>b</sup>	3.36 $\pm$ 0.36 <sup>a</sup>
18	quercetin-3- <i>O</i> -galactoside	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	47,51	353	463 <sup>1</sup>	301, 300, 179	5.86 $\pm$ 0.06 <sup>b</sup>	8.50 $\pm$ 0.24 <sup>f</sup>	9.14 $\pm$ 0.10 <sup>e</sup>	3.02 $\pm$ 0.42 <sup>a</sup>
19	quercetin-3- <i>O</i> -glucoside	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	47,93	355	463 <sup>1</sup>	301	21.58 $\pm$ 0.10 <sup>b</sup>	20.28 $\pm$ 0.36 <sup>b</sup>	26.97 $\pm$ 0.18 <sup>c</sup>	7.63 $\pm$ 0.93 <sup>a</sup>
20	isorhamnetin rhamnosylhexoside isomer-1	C <sub>28</sub> H <sub>31</sub> O <sub>16</sub>	48,99	270, 356	623 <sup>1</sup>	314	21.68 $\pm$ 0.49 <sup>d</sup>	1.61 $\pm$ 0.15 <sup>b</sup>	1.36 $\pm$ 0.04 <sup>a</sup>	7.08 $\pm$ 0.00 <sup>c</sup>
21	isorhamnetin rhamnosylhexoside isomer-2	C <sub>28</sub> H <sub>31</sub> O <sub>16</sub>	49,34	270, 356	623 <sup>1</sup>	315, 300	1.08 $\pm$ 0.11 <sup>b</sup>	1.67 $\pm$ 0.16 <sup>b</sup>	1.01 $\pm$ 0.01 <sup>a</sup>	0.81 $\pm$ 0.16 <sup>a</sup>
<b>total flavonols</b>										
<b>grand total</b>										
							77.1 $\pm$ 0.55 <sup>d</sup>	58.5 $\pm$ 2.45 <sup>b</sup>	67.6 $\pm$ 0.50 <sup>c</sup>	35.7 $\pm$ 0.95 <sup>a</sup>
							1590.6 $\pm$ 2.55 <sup>d</sup>	1058.9 $\pm$ 6.28 <sup>c</sup>	1023.7 $\pm$ 2.35 <sup>b</sup>	997.7 $\pm$ 6.78 <sup>a</sup>

<sup>a</sup>–<sup>d</sup>Different letters in the rows represent statistically significant differences ( $P < 0.05$ ). RT: retention time; <sup>1</sup>negative ionization mode; <sup>2</sup>positive ionization mode. Varieties: Bursa, Trabzon, Kırşehir Viking, and Kırklareli Nero.



**Figure 5.** LC-DAD-ESI-MS/MS chromatograms of some phenolic compounds identified in negative and positive ionization modes in chokeberry fruit samples. Peaks correspond to the compounds in Table 3.

side, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and cyanidin-3-*O*-xyloside) were detected and identified in all chokeberry fruits (Table 3). Cyanidin-3-*O*-galactoside

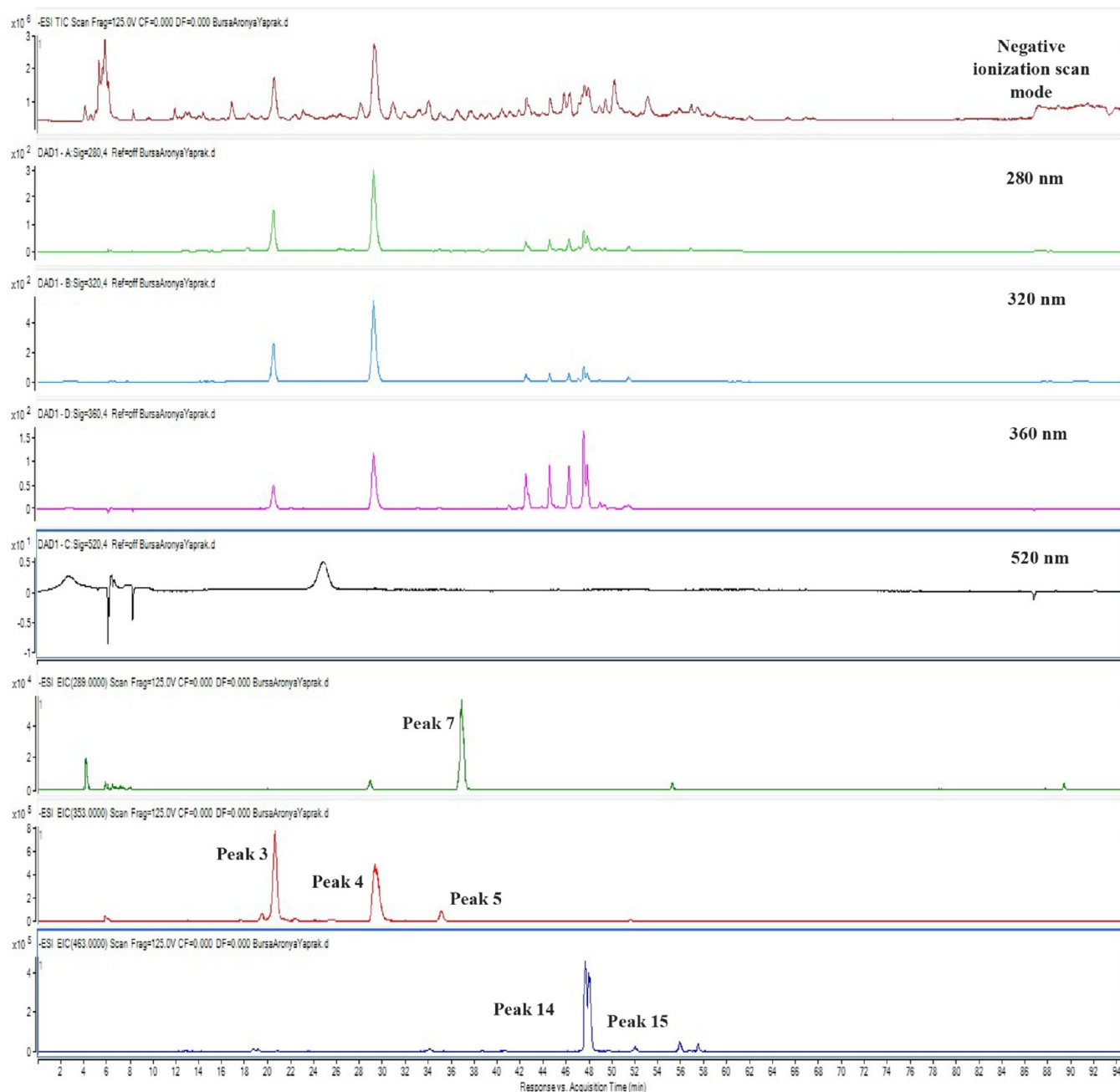
(438.25–605.29 mg/kg) and cyanidin-3-*O*-arabinoside (151.49–345.27 mg/kg) were the main anthocyanins with greater amounts in all fruit samples. The two anthocyanin

Table 4. Phenolic Compounds of the Chokeberry Leaf Samples (Means  $\pm$  Standard Deviations) (mg/kg)<sup>a,c</sup>

	phenolic compounds	molecular formula	RT (min)	$\lambda_{max}$ (nm)	[M - H] <sup>-</sup>	MS/MS	Bursa	Kirklareli	Kirsehir	Trabzon
phenolic acids and derivatives										
1	<i>cis</i> 4- <i>O-p</i> -coumaroylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	13,06	312	337 <sup>1</sup>	173, 163, 93	0.65 $\pm$ 0.01 <sup>a</sup>	0.84 $\pm$ 0.01 <sup>b</sup>	1.08 $\pm$ 0.01 <sup>c</sup>	0.82 $\pm$ 0.01 <sup>b</sup>
2	3- <i>O</i> -caffeoylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	20,54	326	353 <sup>1</sup>	191, 179, 127, 173, 85	42.36 $\pm$ 0.11 <sup>d</sup>	18.67 $\pm$ 0.02 <sup>b</sup>	37.78 $\pm$ 0.07 <sup>c</sup>	10.94 $\pm$ 0.04 <sup>a</sup>
3	<i>trans</i> 4- <i>O-p</i> -coumaroylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	26,88	312	337 <sup>1</sup>	173, 163, 93	8.86 $\pm$ 0.02 <sup>d</sup>	6.22 $\pm$ 0.02 <sup>b</sup>	6.40 $\pm$ 0.04 <sup>c</sup>	5.72 $\pm$ 0.05 <sup>a</sup>
4	5- <i>O</i> -caffeoylquinic acid (chlorogenic acid)	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	29,29	320	353 <sup>1</sup>	191, 173, 127, 111, 93, 109	144.95 $\pm$ 0.09 <sup>d</sup>	58.13 $\pm$ 0.09 <sup>b</sup>	91.10 $\pm$ 0.05 <sup>c</sup>	14.23 $\pm$ 0.03 <sup>a</sup>
5	4- <i>O</i> -caffeoylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	35,03	326	353 <sup>1</sup>	191, 191, 179, 173, 135	9.01 $\pm$ 0.02 <sup>d</sup>	2.41 $\pm$ 0.03 <sup>b</sup>	3.39 $\pm$ 0.01 <sup>c</sup>	1.86 $\pm$ 0.00 <sup>a</sup>
6	<i>cis</i> 5- <i>O-p</i> -coumaroylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	39,20	312	337 <sup>1</sup>	191, 173, 163, 119	9.04 $\pm$ 0.02 <sup>c</sup>	10.09 $\pm$ 0.04 <sup>d</sup>	6.72 $\pm$ 0.02 <sup>b</sup>	6.13 $\pm$ 0.05 <sup>a</sup>
	<b>total phenolic acid</b>						<b>214.9 <math>\pm</math> 0.05<sup>d</sup></b>	<b>96.6 <math>\pm</math> 0.21<sup>b</sup></b>	<b>146.5 <math>\pm</math> 0.03<sup>c</sup></b>	<b>39.7 <math>\pm</math> 0.08<sup>a</sup></b>
flavanols										
7	catechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	28,99	280	289 <sup>1</sup>	245, 205	4.47 $\pm$ 0.05 <sup>d</sup>	1.74 $\pm$ 0.01 <sup>c</sup>	1.28 $\pm$ 0.01 <sup>b</sup>	0.68 $\pm$ 0.01 <sup>a</sup>
8	epicatechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	36,87	270	289 <sup>1</sup>	245, 205, 179	2.91 $\pm$ 0.01 <sup>d</sup>	1.13 $\pm$ 0.02 <sup>c</sup>	0.83 $\pm$ 0.01 <sup>b</sup>	0.44 $\pm$ 0.00 <sup>a</sup>
	<b>total flavanols</b>						<b>7.38 <math>\pm</math> 0.05<sup>d</sup></b>	<b>2.87 <math>\pm</math> 0.03<sup>c</sup></b>	<b>2.10 <math>\pm</math> 0.01<sup>b</sup></b>	<b>1.12 <math>\pm</math> 0.01<sup>a</sup></b>
flavonols										
9	quercetin-3- <i>O</i> -rhamnoside	C <sub>21</sub> H <sub>30</sub> O <sub>11</sub>	32,02	350	447 <sup>1</sup>	315	3.47 $\pm$ 0.00 <sup>d</sup>	1.15 $\pm$ 0.00 <sup>b</sup>	1.92 $\pm$ 0.00 <sup>c</sup>	0.90 $\pm$ 0.00 <sup>a</sup>
10	quercetin dirhamnosylhexoside	C <sub>26</sub> H <sub>38</sub> O <sub>16</sub>	42,55	445	755 <sup>1</sup>	609, 489, 301, 271, 255, 179	26.63 $\pm$ 0.05 <sup>c</sup>	8.37 $\pm$ 0.03 <sup>a</sup>	33.72 $\pm$ 0.03 <sup>d</sup>	14.81 $\pm$ 0.04 <sup>b</sup>
11	quercetin-3- <i>O</i> -vicianoside	C <sub>17</sub> H <sub>24</sub> O <sub>11</sub>	44,51	266, 355	595 <sup>1</sup>	463, 301	23.63 $\pm$ 0.01 <sup>c</sup>	10.04 $\pm$ 0.03 <sup>a</sup>	24.68 $\pm$ 0.01 <sup>d</sup>	10.79 $\pm$ 0.05 <sup>b</sup>
12	secxyloganin	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	45,25	235	403 <sup>1</sup>	223, 371, 191, 149	2.36 $\pm$ 0.00 <sup>c</sup>	1.00 $\pm$ 0.02 <sup>a</sup>	2.47 $\pm$ 0.00 <sup>d</sup>	1.08 $\pm$ 0.00 <sup>b</sup>
13	quercetin-3- <i>O</i> -rabinobioside	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	46,23	354	609 <sup>1</sup>	463,301	25.54 $\pm$ 0.03 <sup>c</sup>	12.76 $\pm$ 0.01 <sup>a</sup>	36.51 $\pm$ 0.04 <sup>d</sup>	14.99 $\pm$ 0.02 <sup>b</sup>
14	quercetin-3- <i>O</i> -galactoside	C <sub>21</sub> H <sub>30</sub> O <sub>12</sub>	47,51	355	463 <sup>1</sup>	301, 300, 179	41.33 $\pm$ 0.04 <sup>d</sup>	6.82 $\pm$ 0.01 <sup>a</sup>	25.17 $\pm$ 0.05 <sup>c</sup>	10.17 $\pm$ 0.03 <sup>b</sup>
15	quercetin-3- <i>O</i> -glucoside	C <sub>21</sub> H <sub>30</sub> O <sub>12</sub>	47,93	355	463 <sup>1</sup>	301, 300, 255, 179	24.71 $\pm$ 0.04 <sup>d</sup>	11.55 $\pm$ 0.00 <sup>b</sup>	19.23 $\pm$ 0.03 <sup>c</sup>	8.95 $\pm$ 0.02 <sup>a</sup>
16	kaempferol coumaroylglucoside	C <sub>30</sub> H <sub>36</sub> O <sub>13</sub>	48,92	366, 350	593 <sup>1</sup>	285	9.23 $\pm$ 0.01 <sup>c</sup>	8.98 $\pm$ 0.01 <sup>b</sup>	9.41 $\pm$ 0.03 <sup>d</sup>	6.24 $\pm$ 0.01 <sup>a</sup>
17	isorhamnetin rhamnosylhexoside isomer-1	C <sub>28</sub> H <sub>31</sub> O <sub>16</sub>	48,99	623	623 <sup>1</sup>	314	8.55 $\pm$ 0.01 <sup>c</sup>	8.31 $\pm$ 0.01 <sup>b</sup>	8.72 $\pm$ 0.03 <sup>d</sup>	5.78 $\pm$ 0.01 <sup>a</sup>
18	isorhamnetin rhamnosylhexoside isomer-2	C <sub>28</sub> H <sub>31</sub> O <sub>16</sub>	49,34	315, 300	623 <sup>1</sup>	315, 300	6.04 $\pm$ 0.01 <sup>b</sup>	9.00 $\pm$ 0.02 <sup>d</sup>	6.14 $\pm$ 0.01 <sup>c</sup>	3.80 $\pm$ 0.03 <sup>a</sup>
19	dicafeoylquinic acid derivative		51,98	515	515 <sup>1</sup>	353, 191, 179	14.54 $\pm$ 0.05 <sup>d</sup>	10.82 $\pm$ 0.04 <sup>c</sup>	3.73 $\pm$ 0.03 <sup>b</sup>	1.48 $\pm$ 0.02 <sup>a</sup>
	<b>total flavonols</b>						<b>186.0 <math>\pm</math> 0.02<sup>d</sup></b>	<b>88.8 <math>\pm</math> 0.09<sup>b</sup></b>	<b>171.7 <math>\pm</math> 0.21<sup>c</sup></b>	<b>78.9 <math>\pm</math> 0.15<sup>a</sup></b>
	<b>grand total</b>						<b>408.3 <math>\pm</math> 0.03<sup>d</sup></b>	<b>188.0 <math>\pm</math> 0.30<sup>b</sup></b>	<b>320.3 <math>\pm</math> 0.19<sup>c</sup></b>	<b>119.8 <math>\pm</math> 0.24<sup>a</sup></b>

<sup>a</sup>–<sup>d</sup>Different letters in the rows represent statistically significant differences ( $P < 0.05$ ). RT: retention time; <sup>1</sup>negative ionization mode; <sup>2</sup>positive ionization mode. Varieties: Bursa, Trabzon, Kirsehir Viking, and Kirklareli Nero.





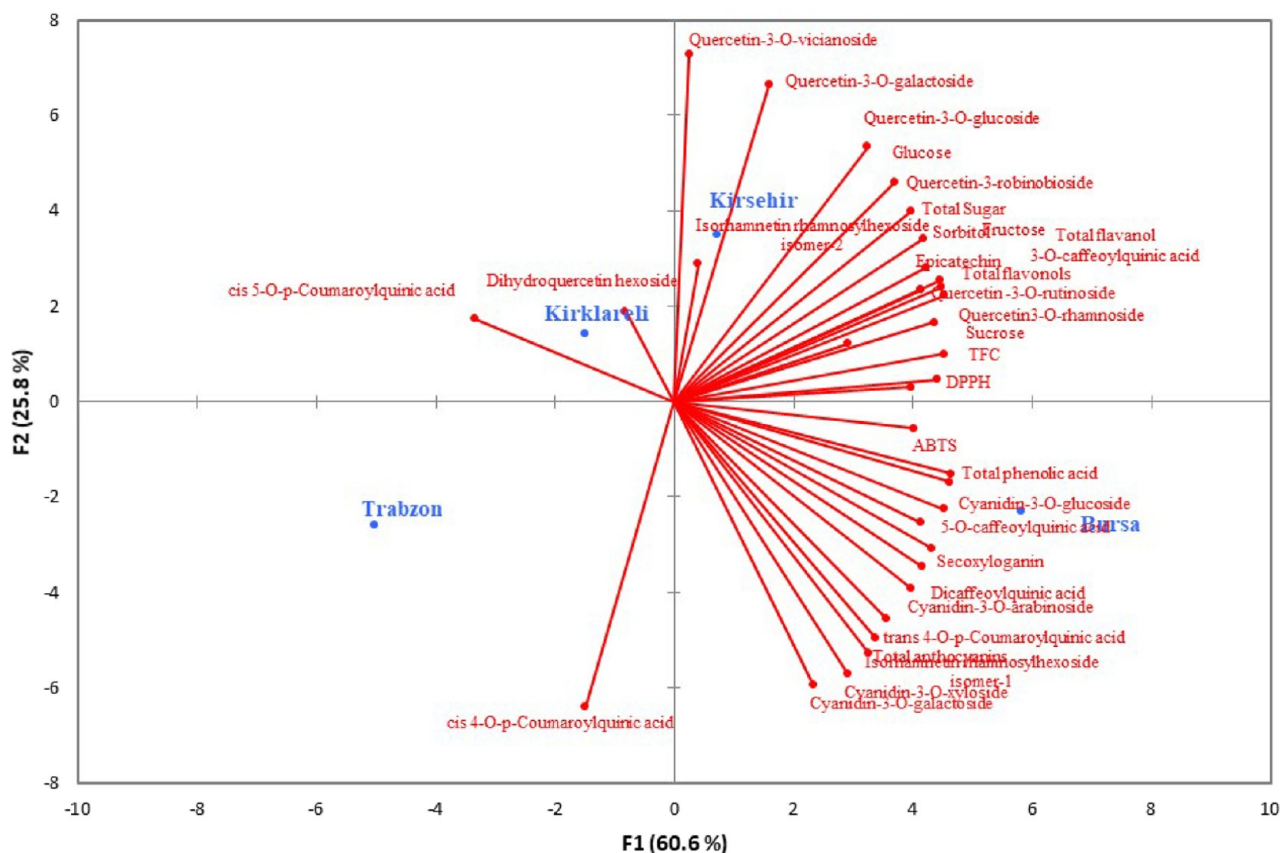
**Figure 6.** LC-DAD-ESI-MS/MS chromatograms of some phenolic compounds identified in negative ionization mode in chokeberry leaf samples. Peaks correspond to the compounds in Table 4.

compounds mentioned above were also detected in the highest amounts in previous studies.<sup>1,10,26,29</sup> The contents of cyanidin-3-*O*-glucoside (16.63–22.91 mg/kg) and cyanidin-3-*O*-xyloside (19.94–49.33 mg/kg) were found to be lower in the present study. The geographical regions had a significant effect on anthocyanin contents, and four anthocyanin compounds were detected with the highest amount in the Bursa samples. Similarly, Szopa et al.<sup>1</sup> found significant differences between the anthocyanin contents of chokeberry fruits from different origins.

5-*O*-Caffeoylquinic acid (chlorogenic acid, 179.62–389.42 mg/kg) followed by 3-*O*-caffeoylquinic acid (48.00–91.77 mg/kg) was the most dominant phenolic acid in all fruit samples after cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside (Table 3). This is in agreement with the

literature.<sup>14</sup> Chlorogenic acid is a common phenolic acid found in various vegetables, fruits, and medicinal plants. Its concentration varies depending on cultivars, environmental factors, and postharvest applications.<sup>3</sup> As with other compounds, chlorogenic acid was quantitated mostly in the Bursa samples (389.42 mg/kg). Similarly, it was reported that the most dominant phenolic acid in chokeberry fruits obtained from different places in Poland was chlorogenic acid, and there were differences in their amounts.<sup>1</sup> Dicafeoylquinic acid was detected only in fruit samples in the present study (Table 3). *Cis* 5-*O*-*p*-coumaroylquinic acid has not been reported in chokeberry fruits in previous studies, but this compound was detected for the first time (1.11–1.92 mg/kg) in chokeberry fruits in the present study with its highest amount in the samples from Trabzon.

## PCA biplot for chokeberry fruits (Explained variance; F1 and F2: 86.4 %)



**Figure 7.** PCA biplot of the phenolic compounds, antioxidant activities, and sugar composition of chokeberry fruit samples from four different locations (Bursa, Kirsehir, Trabzon (Viking), and Kırklareli (Nero)).

A total of 10 flavonols and one flavanol (epicatechin) were detected in all chokeberry fruits in the current study (Table 3). The total contents of flavonol (35.70–77.07 mg/kg) and flavanol (2.18–3.50 mg/kg) were lower than those of the other phenolic groups, similar to a former study.<sup>10</sup> Two of the flavonols (dihydroquercetin hexoside and quercetin-3-*O*-rutinoside) were determined only in fruit samples in the present study (Table 3). Secoxyloganin and dihydroquercetin hexoside compounds were not reported in previous studies but were detected for the first time in this study in chokeberry fruits. Also, the highest amount of secoxyloganin was detected in the samples from Bursa. A positive and high correlation ( $r = 0.84$ ) was found between cyanidin-3-*O*-galactoside, the dominant phenolic compound in chokeberry fruit samples, and total phenolic compound content. In addition, a positive and moderate correlation was found between antioxidant analyses (DPPH,  $r = 0.54$ ; ABTS,  $r = 0.64$ ).

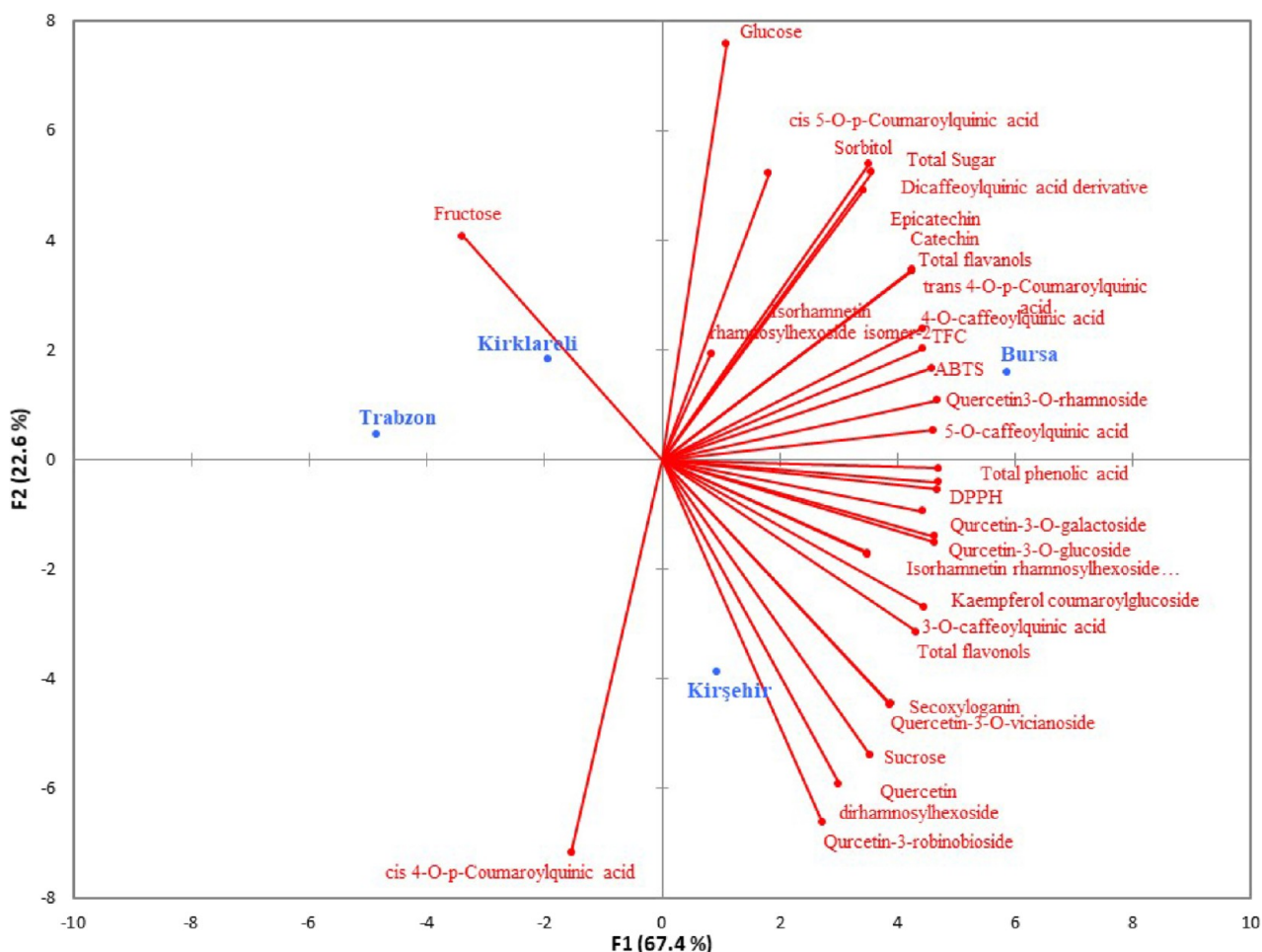
**2.2.2. Leaves.** The phenolic profiles of the chokeberry leaves were determined by LC-DAD-ESI-MS/MS and are tabulated in Table 4 and Figure 6. A total of 19 phenolic compounds were detected in all chokeberry leaf samples. The phenolic groups found in the leaves were flavonols (11), phenolic acid and derivatives (6), and flavanols (2). Unlike the chokeberry fruits, anthocyanins were not detected in chokeberry leaves. Similarly, earlier studies reported no anthocyanin compounds in chokeberry leaves from Poland.<sup>1,30</sup> The most abundant phenolic group in the Bursa and Kirsehir samples was phenolic

acid and derivatives, while it was flavonols in the Kırklareli and Trabzon samples in the current study (Table 4).

It was also seen that the differences in the total phenolic contents of the leaves obtained from different locations were statistically significant ( $P < 0.05$ ). The highest value was found in the Bursa samples, while the lowest was in the Trabzon samples in the chokeberry leaves, similar to that in chokeberry fruits. The highest total amount of the phenolic compounds was quantified as 408.3 mg/kg in the chokeberry leaf samples from Bursa followed by Kirsehir (320.3 mg/kg), Kırklareli (188.0 mg/kg), and Trabzon (119.8 mg/kg) (Table 4).

In the current study, six phenolic acids and derivatives were identified (LC-DAD-ESI-MS/MS) in all chokeberry leaves (Table 4). However, unlike the fruit samples, 4-*O*-caffeoylquinic acid was detected only in leaves. 5-*O*-Caffeoylquinic acid (chlorogenic acid, 28.46–289.89 mg/kg) was detected in the highest amount in the Bursa samples compared to other phenolic compounds as similar in the fruit samples. Our findings were supported by the previous reports that chlorogenic acid was identified as the most abundant phenolic in the chokeberry leaves.<sup>1,16,27,32</sup> *Cis* 5-*O*-*p*-coumaroylquinic acid was not detected in previous studies in chokeberry leaves, while it was identified for the first time in chokeberry leaves in the current study with the highest amount in the Kırklareli sample (Table 4). *Cis* 4-*O*-*p*-coumaroylquinic and *trans* 4-*O*-*p*-coumaroylquinic acids were identified in all leaf samples. It was reported in a previous study that a phenolic acid identified as

PCA biplot for chokeberry leaves (Explained variance; F1 and F2: 90 %)



**Figure 8.** PCA biplot of the phenolic compounds, antioxidant activities, and sugar composition of chokeberry leaf samples from four different locations (Bursa, Kirsehir, Trabzon (Viking), and Kirklareli (Nero)).

*p*-coumaroylquinic acid isomer 1 was detected in chokeberry leaves.<sup>32</sup>

Two flavanol compounds, catechin and epicatechin, were identified in all chokeberry leaves, and both of them were again detected in the highest amount in the Bursa sample (Table 4). Catechin and epicatechin have been reported in some previous studies, but they were not found together in the same study.<sup>30,33</sup>

A total of 11 flavonols were detected in all chokeberry leaves, three of which (quercetin dirhamnosylhexoside, kaempferol coumaroylglucoside, and dicaffeoylquinic acid derivative) were determined only in leaf samples (Table 4). In a former study, the dicaffeoylquinic acid isomer was also detected in the leaves.<sup>3</sup> Another flavonol (secoxyloganin) was detected for the first time in chokeberry leaves in the present study. Unlike the fruits, the highest amount of secoxyloganin was determined in the Kirsehir sample. The highest total flavonol amount was found in the Bursa sample, along with all phenolic compounds. A positive and strong correlation was found between 5-*O*-caffeoylquinic acid, the predominant phenolic compound in chokeberry leaf samples, total phenolic compound content ( $r = 0.98$ ) and antioxidant analyses (DPPH,  $r = 0.98$ ; ABTS,  $r = 0.98$ ).

The variations in the phenolic contents of the chokeberry leaves obtained from different locations can be attributed to

the differences in the geographical areas, climatic parameters (temperature, precipitation, etc.) and growing conditions.<sup>14,27,31</sup>

**2.3. PCA Results.** Principal component analysis (PCA) was applied with phenolic compounds, antioxidant activities, and sugar composition of the chokeberry fruits to evaluate the relationship among four sampling locations (Figure 7). The first two principal components explained about 86.4% of the total variance (Figure 7). F1 accounts for 60.6%, while F2 explains about 25.8% of the variance. It was noticed that the chokeberry fruit samples were well categorized around the origin. All phenolic compounds except *cis* 5-*O*-*p*-coumaroylquinic acid, dihydroquercetin hexoside, and *cis* 4-*O*-*p*-coumaroylquinic acid were positioned very close to each other in the PCA biplot. Total phenolic content, antioxidant activity, and total sugar were effective in separating samples from Kirsehir and Bursa. Accordingly, the samples from these two locations were positioned on the right side of the F1 axis (Figure 7). The high level of *cis* 4-*O*-*p*-coumaroylquinic acid was the phenolic compound that separated the Trabzon sample from the other samples. And also, the high levels of *cis* 5-*O*-*p*-coumaroylquinic acid and dihydroquercetin hexoside phenolic compounds seemed to differentiate the Kirklareli sample from others. Therefore, these samples (Kirklareli and

Trabzon) were located on the negative side of the F1 axis in the PCA plot (Figure 7).

The PCA biplot showing the relationships among phenolic compounds, antioxidant activities, and sugar composition in chokeberry leaves obtained from different locations is presented in Figure 8. The first two principal components defined about 90% of the total variance. F1 and F2 components account for about 67.4% and 22.6% of total variance, respectively. It was found that the chokeberry leaf samples were well categorized around the origin. The samples from Kirklareli and Trabzon were found to be very similar to each other (Figure 8). It was determined that all phenolic compounds except *cis* 4-*O*-*p*-coumaroylquinic acid were positioned very close to each other in the PCA biplot. As with fruits, the total phenolic content, antioxidant activities, and total sugar were effective in separating Kirsehir and Bursa samples from the other two samples according to the biplot diagram drawn with F1 and F2 components. Kirsehir and Bursa samples were located on the right side of the F1 axis, while Kirklareli and Trabzon samples were positioned on the left side of the F1 axis.

In summary, PCA showed that chokeberry fruits and leaves obtained from four different locations showed a strong correlation between the sample origin and phenolic compounds, antioxidant activities, and total sugar (Figures 7 and 8).

### 3. MATERIALS AND METHODS

**3.1. Raw Materials: Chokeberry Fruit and Leaf Samples.** Chokeberry is cultivated widely in four different regions in Turkey. Among these regions, the Viking cultivar is grown in Bursa, Trabzon, and Kirsehir, and the Nero cultivar is grown in Kirklareli. The Viking cultivar could not be cultivated in Kirklareli, and instead of this, the Nero cultivar, which has economic and commercial importance, is produced. Chokeberry fruits and leaves were obtained from four provinces of Türkiye, Bursa (40°00'35"N 29°24'45"E, 100 m altitude), Kirklareli (41°40'35"N 27°39'34"E, 203 m altitude), Kirsehir (39°31'53"N 34°20'27"E, 991 m altitude), and Trabzon (41°01'07"N 39°31'22"E, 0 m altitude) in August and September of 2021. The samples were obtained from local producers. The chokeberry cultivar from Bursa, Trabzon, and Kirsehir was Viking, while it was Nero from Kirklareli. Fresh chokeberry samples were used in the analyses. The analyses were started immediately, and the samples were kept in a refrigerator at 4 °C during the analyses.

**3.2. Chemicals.** Millipore-Q water purification equipment was used to obtain distilled water (Millipore Corp., Saint-Quentin, France). Sucrose, glucose, fructose, sorbitol, and formic acid (HPLC grade) and phenolic standards (epicatechin, cyanidin-3-*o*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, 5-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid) were procured from the Merck Company (Darmstadt, Germany). Methanol, ethanol, acetonitrile, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, Folin-Ciocalteu phenol reagent, sodium carbonate, and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, ABD). All of the chemicals were of HPLC or analytical grade.

**3.3. Analysis of Sugars.** The sugar extraction of the chokeberry fruits and leaves was carried out according to the method reported by Lee and Coates.<sup>34</sup> Samples of 2 g were weighed and extracted with 20 mL of pure water for 30 min.

After this, the mixture was centrifuged at 4 °C and 5500 rpm for 15 min. Then, the supernatant was filtered through a 0.45 μm pore size membrane filter before injection.

Analysis of sugar was carried out according to the method reported by Kelebek et al.<sup>35</sup> An HPLC system (Shimadzu LC-10A HPLC Series, Kyoto, Japan) with a pump, a refractive index detector (RID-10A; Shimadzu), and an Aminex HPX-87H column (300 7.8 mm) (Bio-Rad, CA, USA) was utilized at 55 °C under the following analytical conditions: 0.4 mL/min flow rate, 0.09 mol/L H<sub>2</sub>SO<sub>4</sub>, eluent with 6% acetonitrile (v/v). By comparison of the retention time of each sugar to that of a standard, the chromatographic peak corresponding to each sugar was identified. A calibration curve was created using standards (sucrose, glucose, fructose, and sorbitol) to evaluate the relationship between the peak area and concentration.

**3.4. Analysis of Phenolic Compounds.** **3.4.1. Extraction of Phenolic Compounds.** Samples of 2 g were weighed into a centrifuge tube and added with 20 mL of methanol/water (80:20, v/v). The extraction was carried out for 60 min with a magnetic stirrer. Then, the mixture was centrifuged at 4 °C and 5500 rpm for 15 min. The supernatant was filtered through a 0.45 μm pore size membrane filter before the injection.<sup>36</sup>

**3.4.2. LC-MS/MS Analysis of Anthocyanins and Phenolic Compounds.** The analysis of phenolic compounds was carried out using the LC-DAD-ESI-MS/MS with negative and positive ionization mode as described by Keskin et al.<sup>37</sup> An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) employed ChemStation software was used. The analysis was executed on a Beckman Ultrasphere ODS column (Roissy CDG, France; 4.6 × 250 mm). The mobile phase comprised two solvents: solvent A: water/formic acid (99:1; v/v) and solvent B: acetonitrile/solvent A (60:40; v/v). Phenolic compounds in the samples were eluted using 0.5 mL/min flow rate at 25 °C according to the method used by Kelebek and Selli.<sup>38</sup> Peaks in the UV–VIS spectra (200–600 nm) were obtained and examined. Phenolic compounds were identified using the retention times as UV spectra were compared to authentic standards and then confirmed by using an LC-MS/MS spectrometer (Agilent 6430) with a source of electrospray ionization (ESI) by using the following parameters: drying gas of N<sub>2</sub> at 12 l/min, capillary temperature of 400 °C, and nebulizer pressure of 45 psi of ESI/MS. For quantification, standard phenolic calibration curves were used.<sup>35</sup> The limit of detection and quantification were calculated using signal-to-noise ratio (S/N) values of 10 and 3, respectively.

**3.5. Analysis of Antioxidant Activity.** The antioxidant activities of the chokeberry fruits and leaves were determined using the two methods of ABTS and DPPH assays. The antioxidant analyses were performed in accordance with the method by Kelebek et al.<sup>36</sup> A UV–vis spectrophotometer was used to measure the absorbance of the ABTS and DPPH solutions (UV-1601, Shimadzu, Kyoto, Japan) at 734 and 517 nm, respectively. When the sample extracts were mixed with a DPPH solution, the color of the solution changed from violet to yellow depending on the corresponding hydrazine. The reducing ability of the antioxidants against DPPH was determined by monitoring the absorbance decrease at 515 nm. For the ABTS assay, a solution of 7 mM ABTS and 2.45 mM potassium bisulfate was incubated in the dark for 12–16 h. Subsequently, the solution was diluted with sodium acetate buffer (pH 4.5) to achieve an absorbance of 0.70 ± 0.01 at 734 nm. Upon addition of 20 μL of the sample extract to 2.98 mL of the prepared buffer, the mixture was incubated at room

temperature in the dark. The absorbance value was then measured at 734 nm using a UV–vis spectrophotometer. In both antioxidant capacity analyses (DPPH and ABTS analysis), a trolox standard solution was used at various concentrations to obtain the standard curve (3125–200  $\mu\text{mol}$ ,  $R^2 = 0.99$ ).

**3.6. Analysis of Total Phenolic Content.** The total phenolic contents of the samples were measured using Folin Ciocalteu's method.<sup>36</sup> 200  $\mu\text{L}$  of sample extract/standard solution and 1.5 mL of Folin-Ciocalteu reagent (1:10) were added to the spectrophotometer cuvette. After 5 min, 1.5 mL of 6% sodium carbonate solution was added to the tubes. The absorbance values were measured at 765 nm with a UV–VIS spectrophotometer (Shimadzu UV1201, Kyoto, Japan). Absorbance values were calculated with the gallic acid standard curve (3125–200 ppm,  $R^2 = 0.99$ ). The data were calculated as milligrams of gallic acid equivalent per 100 g (mg GAE/100 g).

**3.7. Statistical Data Analysis.** The data were subjected to one-way variance analysis (ANOVA), and the differences in mean values were analyzed by Duncan's multiple comparison test by utilizing SPSS software (Version 24.0; SPSS Inc., Chicago, IL, USA). In addition, the XLSTAT statistical software program (Addinsoft, New York, NY, USA) was also used for PCA.

## 4. CONCLUSIONS

The fruits and leaves of chokeberry obtained from four provinces (Bursa, Kırklareli, Kırşehir, and Trabzon) in Türkiye were analyzed to investigate the phenolic composition, antioxidant properties, and sugar contents in detail. Differences were found in the data of the chokeberry samples (fruit and leaf) of the same species grown in different locations. In general, the phenolic contents of the samples from Bursa were higher than those of the samples from the other provinces. The most dominant phenolic group was anthocyanins in the fruits, but they could not be detected in the leaves. In the leaves of chokeberry samples obtained from Bursa and Kırşehir, the dominant phenolic group was identified as phenolic acids. In contrast, the samples from Kırklareli and Trabzon exhibited flavonols as the dominant phenolic group. This difference in phenolic composition between different regions reveals the effect of the growing region on the phenolic profile of chokeberry leaves. While cyanidin-3-*O*-galactoside was the most prominent colored compound in the fruits, chlorogenic acid was the dominant compound in the leaves. The most important finding in the study was that *cis* 5-*O*-*p*-coumaroylquinic acid and secoxyloganin were detected in both fruits and leaves of chokeberry for the first time. In addition, dihydroquercetin hexoside was also identified for the first time but only in the fruits. Again, the leaves and fruits from the Bursa province were found to have the highest total phenolic content and antioxidant activity. Sorbitol constituted about 50% of the total sugar in fruits and leaves.

In conclusion, chokeberry fruits and leaves are recognized as important crops with high phenolic contents and antioxidant properties. It is also used in the health field due to its hypoglycemia, anti-inflammatory, antibacterial, hepatoprotective, and anticancer effects. This valuable fruit is used in a wide range of products, from fruit juices to wines, jams, teas, effervescent tablets, and dietary supplements. This study revealed that chokeberry fruits and leaves have valuable phenolic compounds and high antioxidant activities. The findings emphasize that leaves are also valuable products that

can be used in food, pharmaceutical, and cosmetic industries together with fruits due to their valuable bioactive components.

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### Notes

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