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Evaluation of Antioxidant Properties of Wild and Cultivated Blueberry Leaves and Their Phenolic Compound

Onur Tolga OKAN[*1], Esra ULUSOY[2], Mehmet ÖZ[3], Ilhan DENİZ[4]

[1]Karadeniz Technical University, Maçka Vocational School, Department of Chemistry and Chemical Processing Technologies, Trabzon, TÜRKİYE
[2]Karadeniz Technical University, Maçka Vocational School, Department Food Processing, Trabzon, TÜRKİYE
[3]Gümüşhane University, Department of Forestry and Environment Sciences, Graduate Education Institute, Gümüşhane, TÜRKİYE
[4]Karadeniz Technical University, Faculty of Forestry, Department of Forest Industrial Engineering, Trabzon, TÜRKİYE
*Corresponding Author: onurtolgaokan@ktu.edu.tr

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Abstract

Aim of study: This study aimed to investigate the antioxidant activity and polyphenol and flavonoid content of blueberry leaves, which could have positive effects on human health.

Area of study: The research was conducted in three cities of Türkiye's Karadeniz region, where blueberry cultivation is prevalent: Giresun, Trabzon, and Rize.

Material and method: In this study, 29 blueberry leaves (27 cultivated and two wild) were selected as sample materials. The total phenolic content (Folin-Ciocalteu method), total flavonoid content (aluminum (III) chloride method), and total antioxidant capacity (DPPH and FRAP) of the samples were analyzed. Phenolic characterization was performed using HPLC.

Main results: The total phenolic content varied between 8.36 and 121.61 mg GAE/g, whereas the total flavonoid content ranged from 0.91 to 3.07 mg QE/g. The results revealed that blueberry leaves had high antioxidant activity and contained considerable amounts of polyphenols and flavonoids. Chlorogenic acid was identified as the dominant compound in all leaves samples.

Research highlights: These findings indicate that blueberry leaves could be a valuable source of antioxidants in the food and cosmetic industries, comparable to the fruit itself.

Keywords: Bioactive Compounds, Ericaceae, Non-wood Forest Products

Doğal ve Kültür Maviyemiş Yapraklarının Antioksidan Özellikleri ve Fenolik Bileşenlerinin Değerlendirilmesi

Öz

Çalışmanın amacı: Bu çalışma, insan sağlığı üzerinde olumlu etkileri olabilecek maviyemiş yapraklarının antioksidan aktivitesini, polifenol ve flavonoid içeriğini araştırmayı amaçlanmıştır.

Çalışma alanı: Araştırma, Türkiye'nin Karadeniz bölgesinde maviyemiş yetiştiriciliğinin yaygın olduğu üç ilde gerçekleştirildi: Giresun, Trabzon ve Rize.

Materyal ve yöntem: Bu çalışmada, örnek materyal olarak 29 maviyemiş yaprağı (27 yetiştirilen ve 2 yabani) seçildi. Ayrıca örneklerin toplam fenolik içeriği (Folin Ciocalteu Yöntemi), toplam flavonoid içeriği (Alüminyum (III) klorür yöntemi) ve toplam antioksidan kapasitesi (DPPH ve FRAP) analiz edildi. Fenolik karakterizasyon HPLC kullanılarak gerçekleştirildi.

Temel sonuçlar: Toplam fenolik içeriği 8.36 ile 121.61 mg GAE/g arasında değişirken, toplam flavonoid içeriği 0.91 ile 3.07 mg QE/g arasında değişmekteydi. Sonuçlar, maviyemiş yapraklarının yüksek antioksidan aktiviteye sahip olduğunu ve önemli miktarda polifenol ve flavonoid içerdiğini ortaya koymuştur. Klorojenik asit, tüm yaprak örneklerinde baskın bileşik olarak tanımlanmıştır.

Araştırma vurguları: Bu bulgular, maviyemiş yapraklarının meyve ile karşılaştırılabilir şekilde gıda ve kozmetik endüstrilerinde antioksidanlar açısından değerli bir kaynak olabileceğini göstermektedir.

Anahtar kelimeler: Biyoaktif Bileşikler, Ericaceae, Odun Dışı Orman Ürünleri

Introduction

Fruits, leaves, wild herbs, and fungi, known as non-wood forest products (NWFPs), constitute globally consumed sources of essential biological nutrients. These products are often utilized in the production of functional and premium food items, as well as nutraceuticals. NWFPs play a crucial role in foreign trade in many countries, serving as a major revenue source and contributing to the reduction of rural poverty and local economic development (Öz et al., 2015). Blueberries are recognized globally as one of the most commercially important NWFPs. The commercial cultivation of blueberries has expanded to the Southern Hemisphere, with significant production in countries such as Australia, New Zealand, and South America. Originally native to North America, blueberries were mainly cultivated as highbush varieties until the 1930s when they were introduced to Europe (Gao and Draper, 2010). Naturally acidic soils ideal for blueberry cultivation (Ochmian et al., 2015) were found in the Karadeniz region of Türkiye. The area in question is also known as the traditional habitat of the native lowbush blueberry species, which has thrived there for centuries. Although highbush blueberry cultivation in Turkey started in 2000, the cultivation of this fruit has already been established in various parts of the world. Blueberries are highly valued not only for their delicious taste, but also for their health-promoting compounds, which greatly contribute to their popularity. Blueberries are well known for their rich content of biologically active compounds, such as phenolic acids, tannins, anthocyanins, and flavonoids. These compounds are believed to have protective effects against various chronic diseases, including cardiovascular diseases, cancer, inflammation, obesity, and diabetes (Yang et al., 2014). Although blueberry berries are harvested for their edible qualities, the leaves of the plant are often dried and used in the preparation of tea blends or potpourri mixtures. When studying the literature, it becomes apparent that a significant number of studies conducted on blueberries typically focus on the fruit of the plant. Although experimental studies have reported antimicrobial, antioxidant, anti-inflammatory, neuroactive, and anti-obesity properties of blueberry leaves extracts (Pilijac-Zegarac et al., 2009; Değirmencioğlu et al., 2017), some studies have been conducted specifically on blueberry leaves. Several studies have evaluated the levels of antioxidants, phenolics, tannins, and anthocyanins in the leaves of wild and cultivated blueberry plants. Within this framework, the phenolic content of blueberry leaves from the Lanfeng cultivar was analyzed. Examination of blueberry leaves has identified various phenolic compounds, such as kaempferol, quercetin, gallic acid, syringic acid, vanillic acid, caffeic acid, and *p*-coumaric acid (Yang et al., 2014). Another research team investigated phenolic profile and antioxidant activity of blueberry leaves (*Vaccinium formosum*) using various solvents at different maturity levels. In their study on commercially mature Vaccinium leaves extracted with different solvents, Deng et al. (2014) identified vanillic acid at concentrations of 61.10%, 70.00%, and 69.10%, ferulic acid at 7.90%, 7.80%, and 6.30%, and gallic acid at 6.40%, 4.40%, and 6.60% in ethanol, acetone, and methanol, respectively. The DPPH, ORAC, and reducing power values were significantly affected by the ripeness of the leaves and solvent used ($p<0.05$). In another research, it was found that the phenolic compounds and total monomeric anthocyanin levels in blueberry leaves changed according to the drying method employed. Notably, leaves dried at 60°C using microwave-assisted hot-air drying had a comparable total monomeric anthocyanin content and antioxidant activity to those that were freeze-dried. These results indicate that microwave-assisted drying at this temperature can serve as an efficient alternative to maintain the phenolic compounds and antioxidant capacity of blueberry leaves (Routray et al., 2014). The goal of this study was to conduct a comprehensive analysis of the phenolic profiles, antioxidant properties, and total phenolic content of leaves from both wild and cultivated blueberry varieties in Türkiye. Understanding the link between phenolic content and agronomic factors, as well as identifying specific blueberry leaf genotypes,

is the key to enhancing the nutritional quality of processed blueberry leaf products.

Material and Methods

Study Area

Twenty-nine blueberry leaf samples were collected from the Karadeniz region in northeastern Türkiye, specifically from the Trabzon, Rize, and Giresun divisions. After harvesting, the leaves were frozen and stored at -18°C (Table 1).

Table 1. Locations of blueberry leaves sample collection

No	Cultivar	Place
1	Berkeley	Trabzon
2	Bluecrop	Giresun
3	Bluegold	Giresun
4	Bluejay	Giresun
5	Blueray	Trabzon
6	Brigitta	Giresun
7	Chandler	Giresun
8	Darrow	Giresun
9	Duke	Trabzon
10	Earlyblue	Rize
11	El-Crop	Trabzon
12	Elliot	Trabzon
13	Herbert	Trabzon
14	Jersey	Trabzon
15	Jubile	Giresun
16	Legassi	Trabzon
17	Misty	Giresun
18	Northcountry	Trabzon
19	Northland	Giresun
20	Oneil	Giresun
21	Ozarkblue	Giresun
22	Patriot	Trabzon
23	Putte	Trabzon
24	Spartan	Trabzon
25	Sunrise	Giresun
26	Sunshine	Giresun
27	Torro	Trabzon
28	*V. corymbousum*	Rize
29	*V. myrtillus*	Rize

Preparation of Extracts

Around 1 g of each blueberry leaves sample was mixed with 40 mL 99% methanol and blended for three minutes. The resulting mixture was continuously shaken at room temperature for 24 h using a Heidolph Promax 2020 shaker (Schwabach, Germany). To remove particulates, the mixture was filtered through filter paper, and the final volume was brought to the desired level using methanol. The methanolic extract was then split into two equal portions: one for assessing antioxidant properties, and the other for analyzing phenolic compounds via HPLC. The second portion, intended for phenolic compound analysis, was subjected to liquid-liquid extraction. Each methanolic extract was treated with 100 mg of the sample in a solution adjusted to pH 2.0 ± 0.1, and mixed thoroughly. The solution was subjected to three extractions using 5 mL diethyl ether and ethyl acetate. The combined organic phases were filtered through a 45 μm filter and concentrated. The remaining residue was dissolved in 2.0 mL of methanol for HPLC analysis (Okan et al., 2018).

Total Phenolic Contents (TPC)

Singleton et al. (1999) described a method for determining TPC of methanolic extracts. In this method, 50 μL of the sample was mixed with 750 μL of Folin-Ciocalteu reagent diluted 1:14 with water. After a 3-minute incubation, 250 μL of 20% Na_2CO_3 solution was added. The mixture was left to react in the dark for 30 min. The absorbance was read at 760 nm using a spectrophotometer (UV-2100, Unicam), with methanol serving as the blank. Gallic acid was used as the standard, and the TPC of the extracts was reported in milligrams of gallic acid equivalents per gram of dry plant material (mg GAE/g).

Total Flavonoid Content (TFC)

TFC was determined using a spectrophotometric method, with measurements taken at a wavelength of 430 nm in accordance with the method outlined by Hatipoğlu et al. (2013). Each extract was mixed with methanol to obtain a stock solution at a concentration of 4 mg/ml. Subsequently, 0.5 ml of the sample was combined with 1.5 ml of 2% $AlCl_3.6H_2O$ solution in methanol. The resulting mixture was transferred to sealed containers and stored in the dark room. Subsequent absorbance measurements were performed at 430 nm wavelength. An $AlCl_3$ solution in methanol was used as a blank. All measurements were performed in triplicate. In addition, various concentrations of quercetin in methanol were prepared and examined. The amount of flavonoids in the extract was determined and

reported as milligrams of quercetin equivalent flavonoids per gram of dry matter (mg QE/g).

Assessment of Antioxidant Properties

The method described by Kartal et al. (2007) was used to quantify the DPPH free radical scavenging capacity of the blueberry leaves extracts. The purple-colored DPPH radical exhibited a decrease in intensity upon exposure to substances possessing antioxidant properties. Antioxidant efficacy was evaluated by measuring the absorbance at 517 nm. Each experiment was conducted in triplicate, and the results are presented as SC_{50} values (mg/mL). The antioxidant capacity of the extract was determined by examining its ability to reduce the concentration of the ferric tripyridyl-triazine (Fe^{3+}-TPTZ) complex. The experimental methods were based on the protocol described by Benzie and Strain (1999), with certain adjustments implemented. The evaluation examined how the antioxidant compounds in the extracts transformed the ferric tripyridyl-triazine complex (Fe^{3+}-TPTZ) into a blue Fe (II) TPTZ compound.

The ferric reducing antioxidant power (FRAP) reagent was prepared by mixing three components in a 10:1:1 ratio. The mixture consisted of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ dissolved in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$. Each sample was then mixed with 3 mL freshly prepared FRAP reagent and incubated at 37°C for 4 min. Absorbance was measured at 595 nm using a spectrophotometer, with distilled water used as the blank for reference.

Phenolic Profile Analysis by HPLC

A total of 18 phenolic compound standards were used for HPLC analysis (Fig. 1).

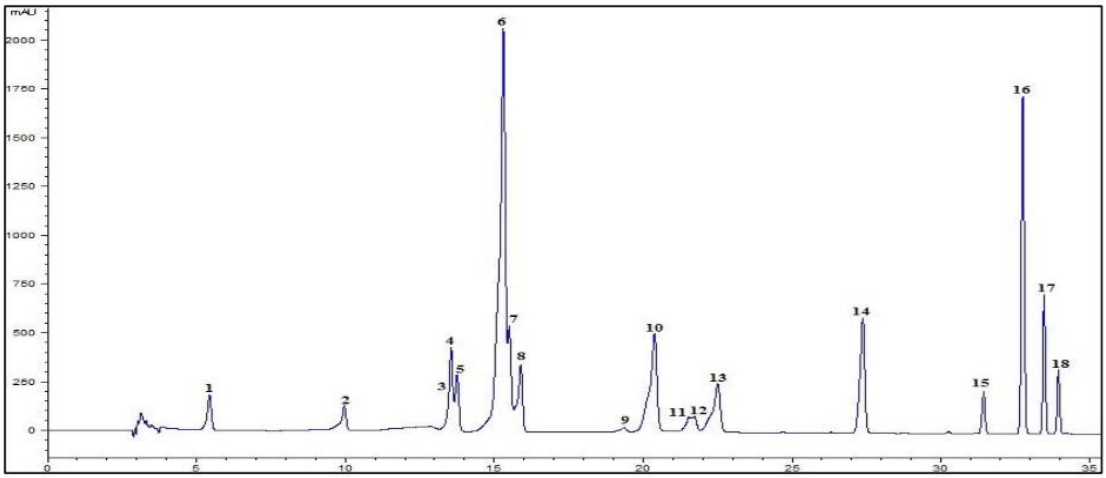

Figure 1. Chromatograms of phenolic reference standards

1: Gallic acid, 2: Protocatechuic acid, 3: Chlorogenic acid, 4: p-OH benzoic acid, 5: Vanillic acid, 6: Caffeic acid, 7: Syringic acid, 8: Ferulic acid, 9: Ellagic acid, 10: Rutin, 11: p-Coumaric acid, 12: Benzoic acid, 13: Rosmarinic acid, 14: o-coumaric acid 15: quercetin, 16: t-cinnamic acid, 17: Curcumin, and 18: Kaempferol

The methanol extract obtained from the biomass was analyzed using HPLC-DAD following the methodology provided by Okan et al. (2018). The analytical system utilized was an Agilent Technology 1260 Infinity HPLC-DAD, featuring quaternary pumps and an automatic injector. This setup was enhanced using a diode array detector (DAD) (model 1260 DAD VL). In the analysis, a reverse-phase AC-18 column (250 mm × 4.6 mm ID, 5 µm particle size, HICHROM, UK)was mounted on a column oven (1260 TCC), and a 1260 QUAT pump VL was used in the system. The HPLC mobile phase consisted of solvent A (water with 2% acetonitrile) and solvent B (a 70:30 mixture of acetonitrile and water). This mixture was sonicated, stirred, and continuously degassed using an integrated system within the HPLC setup. The injection volume was adjusted to 20 µL and the column temperature was maintained at 30°C. Reference standards were used to generate calibration curves for quantification, ranging from 1.5 to 25 ppm,

with a regression coefficient of ≥0.999 for all phenolic compounds. Gradient programming was employed to ensure a steady flow rate of 1 ml/min throughout the process. Additionally, the flow rate of mobile phase B (5% of the mixture) was set for the first three minutes. The mobile phase percentage was gradually increased to 15%, 20%, 25%, 40%, and 80% at the 8th, 10th, 18th, 25th, and 35th minutes, respectively, before being reduced to 5% at the 40th minute, followed by a 10-minute equilibration phase. The eluent was continuously monitored throughout the process at three wavelengths (280 nm, 315 nm, and 350 nm) using a PDA detector.

Statistical Analysis

A one-way analysis of variance (ANOVA) was utilized to examine the potential statistical differences in the properties of blueberry leaves. TPC, TFC, FRAP, and DPPH were measured. After conducting ANOVA, Duncan's post-hoc test was applied to identify specific differences between the groups.

Results and Discussion

Total Phenolic and Flavonoid Content

Table 2 shows a comparison of TPC and TFC in wild and cultivated blueberry leaves.

Table 2. Total phenolic, total flavonoids and antioxidant activities of blueberry leaves

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH-SC_{50} (mg/ml)	FRAP (µmol Trolox/g)
Berkeley	78.83±1.46^{lmno}	1.82±0.08^{de}	3.08±0.13^{cd}	413.83±0.17^{z}
Bluecrop	70.15±0.97^{ij}	1.83±0.02^{def}	6.93±0.31^{j}	150.74±0,17^{i}
Bluegold	78.78±1.93^{lmno}	1.87±0.07^{efghi}	6.97±0.41^{j}	213.37±0.43^{r}
Bluejay	72.15±1.81^{jk}	1.83±0.01^{def}	12.73±0.12^{n}	72.28±0.35^{d}
Blueray	39.99±1.09^{c}	1.87±0.020^{efghi}	9.85±0.63^{l}	87.61±0.17^{f}
Brigitta	73.03±0.36^{k}	1.90±0.01^{ghi}	3.78±0.4^{1ef}	270.28±0.60^{q}
Chandler	78.86±2.44^{lmno}	1.90±0.01^{ghi}	3.13±0.50^{cd}	397.77±0.35^{v}
Darrow	88.11±0.49^{p}	1.85±0.01^{defg}	4.06±0.60^{f}	275.47±1.29^{w}
Duke	79.83±0.36^{mno}	1.91±0.04^{hi}	4.11±0.04^{f}	215.05±1.12^{s}
Earlyblue	77.72±1.94^{lmn}	1.83±0.03^{def}	6.74±0.07^{j}	203.69±0.17^{n}
El-Crop	121.61±1.43^{s}	3.07±0.02^{l}	2.03±0.24^{a}	548.39±0.29^{e}
Elliot	81.03±0.73^{o}	1.75±0.01^{bc}	3.44±0.09^{de}	356.05±0.43^{x}
Herbert	30.12±0.12^{b}	1.83±0.02^{def}	14.10±0.47^{o}	66.47±0.26^{c}
Jersey	44.036±0.49^{d}	1.91±0.05^{hi}	8.42±0.15^{k}	184.81±2.59^{l}
Jubile	69.28±0.12^{i}	1.82±0.01^{def}	5.84±0.07^{i}	205.62±0.43^{o}
Legassi	77.74±1.82^{lmn}	1.73±0.01^{b}	3.18±0.08^{cd}	355.56±0.17^{x}
Misty	65.93±1.94^{h}	1.79±0.05^{cd}	8.67±0.75^{k}	161.93±0.43^{j}
Northcountry	76.51±1.81^{l}	2.03±0.01^{j}	5.25±0.08^{h}	212.06±0.34^{p}
Northland	46.79±0.37^{e}	1.86±0.03^{efgh}	9.43±0.38^{l}	184.61±0.86^{l}
Oneil	77.12.±1.45^{lm}	1.84±0.01^{defg}	12.20±0.12^{m}	116.93±0.26^{g}
Ozarkblue	49.20±0.75^{f}	1.87±0.02^{efghi}	8.52±0.61^{k}	134.47±0.17^{h}
Patriot	31.24±0.49^{b}	1.92±0.05^{i}	13.77±0.41^{o}	64.27±0.40^{b}
Putte	78.75±1.83^{lmno}	1.86±0.01^{efgh}	3.32±0.32^{cd}	242.92±0.43^{u}
Spartan	64.89±0.97^{h}	1.84±0.03^{efgh}	9.55±0.13^{l}	177.79±0.23^{k}
Sunrise	64.01±0.69^{h}	1.86±0.01^{efgh}	12.65±0.13^{n}	77.29±0.26^{e}
Sunshine	73.17±2.06^{k}	1.83±0.01^{def}	5.73±0.95^{i}	214.20±0.43^{rs}
Torro	8.36±0.06^{a}	0.91±0.02^{a}	19.28±0.3^{p}	56.04±0.06^{a}
V. arctostaphylos	92.78±0.73^{r}	2.11±0.003^{k}	2.56±0.43^{b}	398.52±040^{v}
V. myrtillus	79.92±0.85^{no}	1.84±0.01^{defg}	2.95±0.12^{bc}	402.18±0.15^{y}

Different letters (a–z) in the same columns indicate significant differences at the 5% level ($p < 0.05$)

The TPC of the leaves exhibited significant variation, ranging from 8.36±0.06 121.61±1.43 mg GAE/g. El-Crop exhibited the maximum TPC at 121±1.43 mg GAE/g, whereas Torro showed the minimum at 8.36±0.06 mg GAE/g. Among the wild species, *V. arctostaphlos* and *V. myrtillus* exhibited the highest TPC, with values of 92.78±0.73 and 79.92±0.85 mg GAE/g, respectively. The cultivated species Darrow, Eliot, Duke, Chandler, Berkeley, Bluegold, and Putte exhibited high total phenolic content

(TPC), with values ranging from 88.11±0.49 78.75±1.83 mg GAE/g. According to the TPC results in this study, Brightwell and Rabbiteye blueberry (*V. ashei*) leaves possess a substantial amount of 81.82 ± 0.75 mg GAE/g TPC, while its fruits contain a notable 26.94 ± 0.29 mg GAE/g TPC (Li et al. 2012). According to Piljac-Žegarac et al. (2009) and Lee et al. (2014), the leaves of blueberry plants have been found to possess significantly higher levels of antioxidants than their fruit counterparts (Piljac-Žegarac et al. 2009; Lee et al. 2014). A study conducted by Hasanloo et al. (2011) found that the total phenolic content (TPC) of blueberry leaves collected from various regions of Iran, including Masuleh, Hoor, Kelardasht, and Asalem, varied significantly between 11.48 and 42.69 mg GAE/g, depending on the time of collection (May and August). In a previous study, the TPC of 87 blueberry leaves varieties was reported to have a mean value of 44.8 mg/g. Additionally, six varieties (Blueray, Bluejay, Darrow, Herbert, Jersey, and Jubile) showed similar results, while twelve blueberry leaves varieties (Brigitta, Duke, Elliot, Misty, Northland, Patriot, Spartan, Sunrise, Sunshine, Berkeley, Bluegold, Earlyblue, Chandler, Legassi, Puru, Toro) exhibited higher TPC values than our study (Ehlenfeldt and Prior 2001). Other research has likewise found variations in the TPC of different blueberry leaves, ranging from 111.71±4.59-184.99± 4.31 mg per gram of sample fresh weight (Debnath-Canning et al., 2020).

Flavonoids comprise the most significant group of polyphenols, comprising over 8000 members. These compounds contribute to antioxidant capacity (Pietta et al. 2003). In analyzing the TFC levels of blueberry leaves (as shown in Table 2), it was concluded that El-Crop had the highest value at 3.07±0.02 mg QE/g, while the Torro variety had the lowest value at 0.91±0.02 mg QE/g. The TFC of the leaves of the natural varieties of *V. arctostaphylos* and *V. myrtillus* were found to be 2.11±0.003 and 1.84±0.01 mg/g, respectively. Vućic et al. (2013) found that the TFC ratio of dried leaves from *V. myrtillus* was 43.08 mg RUE/g in aqueous solutions, 81.98 mg RUE/g in ethanol solutions, and 94.49 mg RUE/g in ethyl acetate solutions. According to a study conducted by Li et al. (2012), the TFC content of *V. ashei* leaves was found to be 47.80 mg QE/g. In a different study, the amount of total phenolic content (TFC) in the leaves of *V. arctostaphylos* from the regions of Masuleh, Hoor, Kelardash, and Asalem was examined for the months of May and August. These findings revealed variations in the TFC levels of naturally grown blueberries from different regions of Iran. These differences can be explained by a number of factors, including the influence of both genotypes and the varying environmental conditions. Flavonoid synthesis is known to be affected not only by genetic makeup but also by external biotic and abiotic factors, such as soil composition, climate, and exposure to stressors (Kalt et al., 2000; Koca and Karadeniz, 2009). This combination of genetic and environmental influences likely contributes to the observed fluctuations in flavonoid content across different regions.

Antioxidant Activities

The ability of bioactive compounds to inhibit or decelerate oxidative processes in various substances, including DNA and lipids, is known as antioxidant activity. This protective function is applicable to both living systems such as food and human items. (Shahidi, 2000; Naczk and Shahidi, 2004). This protective function operates in biological organisms as well as in consumables. Several techniques are available for assessing the antioxidant capabilities of blueberry leaves. In this study, we employed FRAP and DPPH to evaluate the antioxidant potential of blueberry leaves. The results shown in Table 2 demonstrate that V. *arctostaphylos* and *V. myrtillus* possess the most significant antioxidant activity based on both methods. Berkeley and El-Crop demonstrated the highest antioxidant activity among the cultivated leaves. The antioxidant capacity of blueberry leaves, as measured by DPPH and FRAP assays, displayed a range of values between 2.03±0.24 to 19.28±0.3 mg/ml and 56.04±0.06 to 548.39±0.29 μmol Trolox/g, respectively. Pervin et al. (2013) reported that

the DPPH value for *V. corymbosum* was 0.12 mg/mL, and the leaves extracts were capable of scavenging 93.07% of the radicals (Pervin et al., 2013). Yuan (2011) examined the antioxidant activity of leaves of various *Vaccinium* species in a study conducted in 2009 and 2010. In particular, the FRAP antioxidant analysis of *V. darrowi* was found to be 582.59 µmol/g in 2009 and 694.24 µmol/g in 2010; *V. arboretum* was determined to be 401.53 µmol/g in 2009 and 549.90 µmol/g in 2010; and *V. fuscarum* was determined to be 353.32 µmol/g in 2009 and 352.77 µmol/g in 2010. According to a study that analyzed the antioxidant activity of 87 varieties of *V. corymbosum* using the ORAC method, the results revealed a range of antioxidant activity between 245.3 and 971.3 µmol TE/g (Ehlenfeldt and Prior, 2001). A study was carried out in four locations in Iran (Masuleh, Hoor, Kelardasht, and Asalem) during two distinct time periods (May and August) with the aim of evaluating the antioxidant properties of *V. arctostaphylos* leaves using FRAP and DPPH techniques. The FRAP activity of the blueberry leaves obtained from Asalem and Kelardasht was reported to be 39.09 and 21.96 mmol/g respectively in August. Additionally, it was reported that these values were determined to be 10.70 and 49.41 mmol/g respectively in May. Conversely, the DPPH activity of the blueberry leaves obtained from Masuleh and Hoor was reported to be 0.29 and 0.79 mg/ml respectively in May, and 0.28 and 0.61 mg/ml respectively in August (Hasanloo et al. 2011). Previous studies on the antioxidant capacity of blueberry fruits have shown inconsistent results. The observed differences may be attributed to a range of factors, including biological variations, organ growth, cycles of pollinator activity, nature of plant components (such as leaves and flowers), types of secretory structures, changes across seasons, physical or chemical damage, environmental influences, climate conditions, pollution levels, presence of diseases and pests, soil-related factors, geographical differences, genetic influences and evolutionary processes, storage methods, sociopolitical circumstances, availability of plant material or space, and the need for manual labor (Okan et al. 2018).

Determination of Phenolic Compounds

The significance of phenolic compounds in leaves lies in their impact on human health, their influence on the formation of taste and odor, their role in color changes, their antimicrobial properties, and their antioxidative effects. In this study, the phenolic components present in the leaves were determined by HPLC-DAD analysis. Eighteen phenolic standards were identified and quantified; the results are listed in Table 3.

Table 3. The phenolic compounds composition of studied blueberry leaves with HPLC-DAD expressed in mg/100 g and shown as mean ± SD (n = 3)

Sample Name	Gallic Acid	Protocatechuic Acid	Catechin	Chlorogenic Acid	Caffeic Acid	Epicatechin	Ferulic Acid	Ellagic Acid	Rutin	*o*-coumaric Acid	Quercetin	Kaempferol
Berkeley	0.63±0.06	n.m.	n.m.	284.59±7.06	2.0±0.07	n.m.	0.67±0.16	n.m.	61.79±2.55	n.m.	59.18±1.96	2.09±0.21
Bluecrop	0.52±0.04	n.m.	n.m.	326.10±4.35	1.37±0.09	8.96±0.38	n.m.	8.08±0.58	15.96±0.87	n.m.	1.49±0.05	n.a
Bluegold	0.48±0.06	0.32±0.03	n.m.	107.30±0.96	16.06±0.47	n.m.	n.m.	n.m.	10.45±0.96	0.76±0.04	22.18±3.47	0.54±0.05
Bluejay	1.32±0.08	n.m.	n.m.	244.05±0.98	35.19±3.70	n.m.	0.92±0.05	n.m.	26.06±2.87	n.m.	10.05±0.87	0.92±0.05
Blueray	0.46±0.04	n.m.	3.84±0.2	32.08±0.52	2.46±0.16	n.m.	0.81±0.07	n.m.	n.m.	n.m.	6.40±0.44	0.36±0.04
Brigitta	0.99±0.12	0.42±0.08	n.m.	267.43±10.09	50.50±4.82	n.m.	n.m.	n.m.	195.20±3.16	1.09±0.10	12.81±2.43	1.23±0.12
Chandler	0.41±0.05	n.m.	n.m.	348.48±5.63	2.58±0.14	n.m.	n.m.	n.m.	32.48±0.90	n.m.	7.25±0.26	n.a
Darrow	0.84±0.02	0.28±0.05	n.m.	248.57±3.51	4.90±1.75	n.m.	n.m.	n.m.	21.21±2.01	n.m.	29.97±2.11	0.60±0.05
Duke	n.m.	n.m.	n.m.	38.09±0.94	3.43±0.84	6.97±1.01	n.m.	n.m.	67.38±1.76	n.m.	2.90±0.13	n.a
Earlyblue	3.00±0.50	n.m.	n.m.	231.60±10.42	77.66±10.17	n.m.	n.m.	n.m.	175.19±8.19	n.m.	26.16±3.00	3.77±0.56
Eln.m.Crop	n.m.	n.m.	n.m.	395.94±11.36	1.61±0.20	2.74±0.18	n.m.	n.m.	44.25±0.88	n.m.	13.46±0.90	n.m.
Elliot	0.69±0.06	n.m.	n.m.	408.56±12.83	7.89±0.77	n.m.	n.m.	n.m.	159.38±10.06	n.m.	2.74±0.85	n.m.
Herbert	0.48±0.04	n.m.	n.m.	169.99±10.99	9.57±0.27	n.m.	0.96±0.09	n.m.	3.35±0.40	n.m.	0.47±2.51	n.m.
Jersey	n.m.	n.m.	n.m.	30.73±0.55	9.33±1.38	19.41±0.21	1.02±0.07	n.m.	5.23±0.48	n.m.	0.95±0.08	n.m.
Jubile	0.62±0.18	n.m.	n.m.	173.58±3.18	82.99±3.31	n.m.	1.07±0.12	8.86±0.62	87.86±6.95	0.27±0.07	6.63±0.51	n.m.
Legassi	0.54±0.06	n.m.	n.m.	332.03±39.02	15.41±0.54	28.61±0.49	n.m.	n.m.	55.58±0.73	n.m.	2.71±0.06	n.m.
Misty	0.49±0.02	n.m.	n.m.	217.61±6.95	73.25±4.91	7.85±00.6	0.85±0.11	n.m.	n.m.	n.m.	3.98±0.74	n.m.
Northcountry	n.m.	n.m.	n.m.	323.52±14.00	174.08±7.93	1.78±0.11	1.17±0.06	n.m.	80.68±3.40	n.m.	3.66±0.16	n.m.
Northland	0.47±0.05	n.m.	n.m.	186.76±2.23	30.95±1.50	2.01±0.12	1.03±0.08	n.m.	42.74±3.37	n.m.	3.65±0.62	n.m.
Oneil	0.87±0.99	n.m.	n.m.	167.57±2.04	15.89±0.18	6.45±0.45	0.84±0.06	n.m.	3.78±0.40	n.m.	4.87±0.09	n.m.
Ozarkblue	n.m.	0.78±0.20	n.m.	381.37±3.09	35.12±1.01	55.31±0.61	1.25±0.31	n.m.	21.64±1.04	n.m.	4.33±0.57	n.m.
Patriot	n.m.	n.m.	n.m.	83.44±2.35	7.20±0.72	n.m.	1.45±0.07	n.m.	17.38±1.40	n.m.	4.34±002	n.m.
Putte	0.88±0.06	n.m.	n.m.	205.21±7.1	23.99±2.08	n.m.	0.85±0.09	n.m.	5.36±0.22	n.m.	13.88±1.09	n.m.
Spartan	n.m.	n.m.	n.m.	202.41±7.97	2.27±0.56	16.17±0.48	n.m.	n.m.	10.37±11.82	n.m.	6.60±0.37	n.m.
Sunrise	n.m.	n.m.	n.m.	172.94±7.17	34.95±3.59	n.m.	0.77±0.12	2.79±0.14	n.m.	n.m.	7.96±0.69	0.32±0.05
Sunshine	n.m.	0.35±0.10	n.m.	454.56±30.50	32.11±2.62	n.m.	0.74±0.05	n.m.	n.m.	n.m.	25.60±3.71	0.083±0.001
Torro	0.29±0.05	n.m.	n.m.	14.35±1.12	1.83±0.06	n.m.	0.46±0.07	12.74±1.40	61.71±5.31	n.m.	0.76±0.04	0.03±0.002
V. arctostaphylos	0.51±0.11	n.m.	n.m.	362.09±13.01	2.70±0.48	n.m.	175.83±5.88	13.80±0.48	n.m.	n.m.	4.68±0.76	n.m.
V. myrtillus	0.62±0.06	0.35±0.08	n.m.	391.66±33.37	5.98±0.98	162.43±6.01	n.m.	n.m.	11.36±1.48	n.m.	3.17±0.49	n.m.

p-OH benzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, *t*-cinnamic acid and apigenin couldn't be determined. n.m: not measured

In all blueberry leaves sample, chlorogenic acid was the dominant compound (Table 3). Caffeic acid and quercetin were detected at varying levels across all leaves samples. Twenty of the twenty-nine leaves species whose phenolic content was analyzed included gallic acid; six of these leaf species were found to include protocatechuic acid, 12 of these leaf species included epicatechin, 17 of which had ferulic acid, five of these leaf species had ellagic acid, rutin was found in twenty-four of these leaf species, and kaempferol was found in ten of these leaf species. None of the leaves contained detectable levels of vanillic acid, syringic acid, *p*-hydroxybenzoic acid, *t*-cinnamic acid, *p*-coumaric acid, or apigenin. A qualitative analysis of five different blueberry leaves varieties by Oszmiański et al. (2011) revealed that chlorogenic acid is the predominant compound in *V. myrtillus*. A study in Poland analyzed HPLC and phenolic compounds in Bluecrop blueberry leaves. It found that caffeic acid was the most abundant phenol, with a concentration of 67.4 mg/100 g (dry weight). Quercetin was the second most abundant, at 21.6 mg/100 g. The study also identified ellagic acid (15.0 mg/100 g), gallic acid (1.6 mg/100 g), and kaempferol (5.9 mg/100 g), but did not measure the dry weight of p-coumaric acid (Skupień et al., 2006). The literature largely parallels this study. In a study conducted on *V. formosum* leaves using different solvents, nine phenolic compounds were identified. Caffeic acid (9.991 mg/g) was the dominant compound in the methanolic extract. In addition, gallic acid (0.542 mg/g), protocatechuic acid (0.718 mg/g), vanillic acid (0.5320 mg/g), syringic acid (0.399 mg/g), p-coumaric acid (0.034 mg/g), ferulic acid (1.081 mg/g), quercetin (0.246 mg/g), and kaempferol (0.009 mg/g) have been detected (Deng et al. 2014). In this study, syringic, vanillic, and p-coumaric acids were not detected in blueberry leaves samples, whereas ferulic acid and kaempferol were detected in some blueberry leaves samples. Caffeic acid and quercetin were found in all blueberry leaves samples. Häkkinen and Torronen (2000) suggested that this was due to variations in phenolic compound synthesis observed among hybrids of the same plant species (Häkkinen and Torronen, 2000). Phenolic compounds obtained from the leaves of *V. ashei* collected in different months (May, September, and November) were analyzed using HPLC-UV. The dominant compounds detected were chlorogenic acid, rutin, and caffeic acid. Among the leaves collected in May, *V. ashei* leaves had the highest concentrations of chlorogenic acid, caffeic acid, and rutin, measuring 33.61 mg/g, 0.54 mg/g, and 6.50 mg/g, respectively. In contrast, chlorogenic acid levels dropped to 8.84 mg/g in November, while the lowest levels of caffeic acid and rutin were observed in September, at 0.09 mg/g and 2.30 mg/g, respectively. The findings of this study are largely aligned with those reported by Zhu et al. (2013) regarding the identified compounds. However, discrepancies were observed in the quantitative measurements of these substances.

Conclusions

The evaluation of antioxidant properties, polyphenolic content, and phenolic profiles of both wild and cultivated blueberry leaves revealed significant variation across different samples. Wild blueberry leaves demonstrated higher levels of total polyphenols, flavonoids, and antioxidant activity when compared to cultivated varieties. Chlorogenic acid emerged as the dominant phenolic compound in all samples, while several other phenolic acids and flavonoids, such as caffeic acid and quercetin, were present at varying levels. These findings highlight the potential of blueberry leaves, particularly from wild varieties, as valuable raw materials for applications in the functional food and beverage industries, including the cold beverage and tea markets. The high antioxidant content, coupled with the diverse range of bioactive compounds, suggests that blueberry leaves could serve as an important source of natural antioxidants with potential health benefits. Furthermore, the identification of key phenolic compounds may support the selection of suitable genotypes for commercial production, aiding in the creation of blueberry leaf-based products with improved nutritional benefits.

Ethics Committee Approval

N/A

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Author Contributions

Conceptualization: O.T.O., İ.D.; İnvestigation: O.T.O., M.Ö.; Material and Methodology: O.T.O, E.U.; Supervision: O.T.O., İ.D.; Writing-Orginal Draft: O.T.O., E.U.; Writing-review & Editing: O.T.O., M.Ö., E.U. All authors have reviewed and approved the final version of the manuscript for publication.

Conflict of Interest

The authors has no conflicts of interest to declare.

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References

Benzie, I.F.F. and Strain, J.J. (1999). Ferric reducing/antioxidant power assay:Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, 299, 15-27.

Debnath-Canning, M., Unruh, S., Vyas, P., Daneshtalab, N., Igamberdiev, A.U., Weber, J.T. (2020). Fruits and leaves from wild blueberry plants contain diverse polyphenols and decrease neuroinflammatory responses in microglia. *Journal of Functional Foods*. 68, 103906.

Değirmencioğlu, N., Gürbüz, O., Karatepe, G. E., Irkin, R. (2017). Influence of hot air drying on phenolic compounds and antioxidant capacity of blueberry (*Vaccinium myrtillus*) fruit and leaf. *Journal of Applied Botany and Food Quality*, 90, 115-125.

Deng, Y., Yang, G., Yue, J., Qian, B., Liu, Z., Wang, D., Zhong, W., Zhao, Y. (2014). Influences of ripening stages and extracting solvents on the polyphenolic compounds, antimicrobial and antioxidant activities of blueberry leaf extracts. *Food Control*, 38, 184-191.

Ehlenfeldt, M.K. and Prior, R.L. (2001). Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *Journal of Agricultural Food Chemistry*, 49, 2222-2227.

Gao, G., and Draper, E. (2010). Growing blueberries in the home garden. *Agriculture and Natural Resources*, 1-8.

Häkkinen, S.H., and Törrönen R.A. (2000). content of flavonols and selected phenolic acids in strawberries and vaccinium species: Influence of cultivar, cultivation site and technique. *Food Research International*, 33, 517-524.

Hasanloo, T., Sepehrifar, R., Hajimehdipoor, H., (2011). Levels of phenolic compounds and their effects on antioxidant capacity of wild *Vaccinium arctostaphylos* L. (Qare-Qat) collected from different regions of Iran. *Turkish Journal of Biology*, 35, 371-377.

Hatipoğlu, G., Sökmen, M., Bektaş, E., Daferera, D., Sökmen, A., Demir, E., Şahin, H. (2013). Automated and standard extraction of antioxidant phenolic compounds *of Hyssopus Officinalis L. Ssp. Angustifolius*, *Industrial Crops and Products* 43, 427–433.

Kalt, W., Mcdonald, J.E. and Donner, H. (2000). Anthocyanins, phenolics and antioxidant capacity of processed lowbush blueberry products. *Journal of Agricultural and Food Chemistry.* 65, 390-393.

Kartal, N., Sökmen, M., Tepe, B., Daferera, D., Polissiou, M. & Sökmen, A. (2007). Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. *Food Chemistry* 100, 584-589.

Koca, I. and Karadeniz, B. (2009). Antioxidant properties of blackberry and blueberry fruits grown in the Blacksea region of Turkey. *Scientia Horticulturae*, 121, 447-450.

Lee, I.C., Kim, D.Y., Choi, B.Y. (2014). Antioxidative activity of blueberry leaf extract prevents high-fat diet-induced obesity in C57BL/6 mice. *Journal of Cancer Prevention*, 19(3), 209-215.

Li, C., Feng, J., Huang, W.Y., An., X.T. (2012). Composition of polyphenols and antioxidant activity of rabbiteye blueberry (*Vaccinium ashei)* in Nanjing. *Journal of Agricultural and Food Chemistry* 61, 523-531.

Naczk, M., and Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, 1054, 95-111.

Ochmian, I., Kozos, K., Chelpinski, P., Szczepanek, M. (2015). Comparison of berry quality in highbush blueberry cultivars grown according to conventional and organic methods. *Turkish Journal of Agriculture and Forestry 3*9, 174-181.

Okan, O.T., Deniz, İ., Yaylı, N., Şat, İ.G., Öz, M., Hatipoğlu Serdar, G. (2018). Antioxidant activity, sugar content and phenolic profiling of blueberries cultivars: a comprehensive comparison. *Notulae Botanicae Horti Agrobotanici*, 46(2), 639-652.

Oszmiański, J., Wojdylo, A., Gorzelany, J., Kapusta, I. (2011). Identification and characterization of low molecular weight polyphenols in berry leaf extracts by HPLC-DAD and LC-ESI/MS. *Journal of Agricultural and Food Chemistry*. 59, 12830-12835.

Öz, M., Deniz, İ., Okan, O. T., Fidan, M. S. (2015). Chemical composition of oleoresin and larvae gallery resin of *Pinus Brutia* attacked by *Dioryctria sylvestrella* ratz. *Drvna industrija*, 66(3), 179-188.

Pervin, M., Hasnat, A., Lim, B.O. (2013). Antibacterial and antioxidant activities of *Vaccinium corymbosum* L. leaf extract. *Asian Pacific Journal of Tropical Disease*, 3(6), 444-453.

Pietta, P., Gardana, C., Pietta, A. (2003). Flavonoids in Herb. *Flavonoids in Health and Disease* (First Press), pp. 43-69. New York: Marcel Dekker Inc.

Piljac-Žegarac, J., Belščak, A., Piljac, A. (2009). Antioxidant capacity and polyphenolic content of blueberry (*Vaccinium corymbosum* L.) leaf infusions. *Journal of Medicinal Food*, 12(3), 608-614.

Routray, W., Orsat, V., Gariepy, Y. (2014). Effect of different drying methods on the microwave extraction of phenolic components and antioxidant activity of highbush blueberry leaves. *Drying Technology*, 32(16), 1888-1904.

Shahidi, F. (2000). Antioxidants in food and food antioxidants. *Molecular Nutrition & Food Research*, 44, 158-163.

Singleton, V. L., Orthofer, R., Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, 299, 152-178.

Skupień, K., Oszmiański, J., Kostrzewa-Nowak, D., Tarasiuk, J. (2006). In vitro antileukaemic activity of extracts from berry plant leaves against sensitive and multidrug resistant HL60 Cells. *Cancer Letters* 236(2), 282-291.

Vućic, D.M., Petković, M.R., Grabovac, R.B.B., Stefanović, O.D., Vasić, S.M., Comić, R.L. (2013). Antibacterial and antioxidant activities of bilberry (*Vaccinium myrtillus* L.) in vitro. *African Journal of Microbiology Research*, 7(45), 5130-5136.

Yang, G., Yue, J., Gong, X., Qian, B., Wang, H., Deng, Y., Zhao, Y. (2014). Blueberry leaf extracts incorporated chitosan coatings for preserving postharvest quality of fresh blueberries. *Postharvest Biology and Technology*, 92, 46–53.

Yuan W., (2011). Anthocyanins, phenolics, and antioxidant capacity of *Vaccinium* L. in Texas, USA. *Pharmaceutical Crops*, 2, 11-23.

Zhu, L., Liu X., Tan, J., Wang, B. (2013). Influence of harvest season on antioxidant activity and constituents of Rabbiteye Blueberry (*Vaccinium ashei*) leaves. *Journal of Agricultural and Food Chemistry*, 61, 11477-11483.