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Extraction of Bioactive Compounds in Wild Bilberry (*Vaccinium Myrtillus* L.) in The Eastern Black Sea Region With Different Techniques

Evren ALTIOK [1], Sibel KACMAZ [2*], Duygu ALTIOK [2]

[1] Giresun Üniversitesi, Mühendislik Fakültesi, Genetik ve Biyomühendislik Bölümü, Giresun, Türkiye
[2] Giresun Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Giresun, Türkiye
Evren ALTIOK ORCID No: 0000-0002-6437-5990
Sibel KACMAZ ORCID No: 0000-0003-3642-4940
Duygu ALTIOK ORCID No: 0000-0002-8503-2145

**Sorumlu yazar: sibel.kacmaz@giresun.edu.tr*

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Bilberry, Bioactive Compounds, Ultrasound Extraction, Microwave Extraction, Maceration

Abstract: Classical solvent extraction (CSE), solvent maceration (SM), ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE) techniques were applied for the extraction of bioactive compounds of wild bilberry (*Vaccinium myrtillus* L.) fruit collected from the Eastern Black Sea Region in Turkey. Among these techniques, MAE is the most prominent in terms of total phenolics, total flavonoids and total anthocyanins yield in the extract. Total phenolics, total anthocyanin and total flavonoid content in MAE extract were determined as 1035 ± 16 mg gallic acid/100 g fresh bilberry weight (fw), 963 ± 7 mg cyanidin 3-glucoside equivalents/100 g fw and 150 ± 4 mg quercetin equivalent/100 g fw, respectively. Seven different anthocyanin compounds were identified in bilberry extracts. The effect of extraction techniques on the anthocyanin's distribution was revealed. Accordingly, malvidin-3-O-glucoside was found to be the most dominant anthocyanin in wild bilberry fruit.

Doğu Karadeniz Bölgesindeki Yabani Çalıçileği (*Vaccinium Myrtillus* L.) Meyvelerinde Biyoaktif Bileşiklerin Farklı Tekniklerle Ekstraksiyonu

Anahtar Kelimeler
Yabani Çalıçileği, Biyoaktif Bileşikler, Ultrason Ekstraksiyonu, Mikrodalga Ekstraksiyonu, Maserasyon

Öz: Türkiye'de Doğu Karadeniz Bölgesi' nden toplanan yabani çalıçileği (*Vaccinium myrtillus* L.) meyvesinin biyoaktif bileşiklerinin ekstraksiyonu için klasik solvent ekstraksiyonu (CSE), solvent maserasyonu (SM), ultrason destekli ekstraksiyon (UAE) ve mikrodalga destekli ekstraksiyon (MAE) teknikleri uygulandı. Bu teknikler arasında ekstrakttaki toplam fenolik, toplam flavonoid ve toplam antosiyanin verimi açısından en iyi tekniğin MAE olduğu görüldü. MAE ekstraktındaki toplam fenolikler, toplam antosiyanin ve toplam flavonoid içeriği sırasıyla 1035 ± 16 mg gallik asit/100 g fw, 963 ± 7 mg siyanidin 3-glukozit eşdeğeri/100 g fw ve 150 ± 4 mg kuersetin eşdeğeri/100 g fw olarak belirlendi. Yabani çalıçileği ekstraktlarında yedi farklı antosiyanin bileşiği tanımlandı. Ekstraksiyon tekniklerinin antosiyanin dağılımı üzerindeki etkisi ortaya çıkarıldı. Buna göre, yabani çalıçileği meyvesinde en baskın antosiyaninin malvidin-3-O-glukozit olduğu belirlendi.

1. INTRODUCTION

Bilberry (*Vaccinium myrtillus* L.) is a wild fruit that can grow in temperate, Mediterranean or subtropical climate. With a water content of more than 80%, bilberry fruit is a good source of fiber, vitamins and minerals, and contains high levels of polyphenols, flavonoids, anthocyanins and other components that exhibit significant bioactivities. Associated with the antioxidant capacity of such different bioactive compounds that can prevent or slow oxidative processes, a diet rich in fruits and vegetables is reported to improve overall health and prevent cardiovascular diseases, neurodegenerative diseases, and different types of cancer [1]. Bilberry is one of the richest natural sources of anthocyanins, a large group of water-soluble flavonoids that give fruits, flowers and vegetables their characteristic blue/red color. Bilberry fruits contain 15 major anthocyanins, consisting of 5 anthocyanidin aglycones delphinidin, cyanidin, peonidin, petunidin, and malvidin, which are formed as 3- O-glycosides attached by galactose, glucose, and

arabinose [2-3]. In addition, other phenolic compounds it contains are molecules with at least one hydroxyl group attached to an aromatic ring and play a major role in plant metabolism [4]. However, all these compounds found in bilberry differ in content and composition depending on type of species, cultivation process, soil and climate conditions that affect plant growth. Although the composition and antioxidant activities of anthocyanins and phenolic compounds of bilberries grown in Europe, North America and Colombia have been investigated [5], there is lack of studies on the quantification and bioactivity of anthocyanins, phenolics and flavonoids of bilberry grown in Turkey.

Conventional phenolic compound extraction techniques include maceration and solvent extraction, which are highly efficient but require high solvent consumption and result in possible solvent toxicity [6]. In recent years, studies have focused on the discovery and design of extraction processes that will provide a safe and high-quality natural extract by higher yields in shorter extraction time, lower energy consumption and using renewable sources [4]. For this reason, anthocyanin and other phenolic compounds extractions from different fruits and plant materials such as sour cherry, grape seed, flax seed, potato peel, lemon peels, coffee, cherry laurel fruits and leaves and blueberry have been widely investigated by using environmentally friendly techniques such as ultrasound assisted extraction (UAE) [4] and microwave assisted extraction (MAE) [7] as an alternative to conventional solvent extraction and maceration methods. UAE is one of the modern methods used to extract bioactive compounds found in vacuoles of plant cells by the application of sound waves to a plant material/solvent mixture. It increases mass transfer by affecting cell permeability by different mechanisms. However, high-frequency ultrasound can tear the surface of the fruit, causing further leakage and pigment loss during process. Due to the possible changes in chemical structures and the high reactivity of the components, it may cause the formation of colorless or brown compounds [4]. MAE is an environmentally friendly technique with very few CO2 emissions and less solvent and time requirement. It works with two energy transfer mechanisms as dipole rotation and ionic conduction. Polar solvents that can effectively absorb electrical energy are more effective in microwave extraction. Besides, a high solvent performance due to high dielectric constant and dispersion factor ensures the effective impact of microwave in microwave extraction [7]. Considering all these, bilberry extraction techniques can significantly affect the amount and profile of phenolics, anthocyanins and flavonoids to be recovered.
The aim of this study is to evaluate the extraction of bioactive compounds from bilberry fruits grown in the Eastern Black Sea Region of Turkey. In this context, the effect of different extraction techniques such as classical solvent extraction (CSE), solvent maceration (SM), ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE) on the yield of total phenolics, total monomeric anthocyanins and total flavonoids was determined. The anthocyanin profiles of the extracts were also assessed.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Ethanol and orthophosphoric acid (85% purity, HPLC grade) were purchased from Sigma Aldrich GmbH (Buch's, Switzerland). Water was deionized and filtered through a Millipore filter system (Millipore, USA) before use. All solvents, reagents and standards used were of analytical grade. The Folin-Ciocalteu reagent, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, sodium acetate, gallic acid, hydrochloric acid-potassium chloride buffer and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (95%) were purchased from Merck Company (Steinheim, Germany). Rutin and quercetin were purchased from Sigma Aldrich GmbH (Germany). Acetonitrile (HPLC grade) and acetic acid (99.8 %) were obtained from Merck (Darmstad, Germany). Formic acid was obtained from Fluka (Deisenhofen, Germany). Anthocyanin standards of delphinidin-3-O-glucoside chloride, delphinidin-3-O-galactoside chloride, petunidin-3-O-glucoside chloride, cyanidin-3-O-arabinoside chloride, cyanidin-3-O-glucoside chloride, malvidin-3-O-glucoside chloride and malvidin-3-O-arabinoside chloride were obtained from Merck (Darmstad, Germany).

2.2. Plant Material

Fresh, ripe wild bilberry (*Vaccinium myrtillus* L.) fruits were picked randomly from native habitat of the species in Giresun in Eastern Black Sea Region of Turkey in July, 2020. Whole fresh and ripe fruits were frozen and stored at -20 ± 1°C until use.

2.3. Extraction

Fresh bilberry fruits were crushed in a mortar to produce a thick puree which was subjected to four different extraction processes; classical solvent extraction (CSE), solvent maceration (SM), ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE). For each extraction procedure, a 10 ±0.1 g puree was placed into a 50 ml flat-bottom flask and aqueous ethanol was added to the flask to a final ethanol concentration of 70% stoichiometrically to a final solid:liquid ratio as 1:20. The mixture was homogenized for a few minutes and used in the extraction process.

For CSE, the homogenate (sample-solvent homogeneous mixture) was extracted for 2 h by orbital shaking in the dark at room temperature. The samples were taken for the analysis at 10 min, 30 min, 1 h and 2 h. For SM, the homogenate was kept in the dark at +4 °C for 6 weeks and the sample was taken at the end of the extraction process. UAE procedure was carried out by treating the homogenate with ultrasound for the period of 5, 10- and 20-min using lab scale ultrasound water bath (53kHz-180-Watt, Kudos SK3310HP) at 40 ºC. MAE was performed in an ordinary house-hold microwave oven

(Inoksan GDM239DAH-S, Microwave output 800 W with the power of 2450 MHz). A Pyrex double-walled vessel where ice flakes were placed between the walls was used to keep the temperature below 60 °C during the application and to eliminate the risk of solvent loss by evaporation and any compound degradation due to rapid temperature rise. Container having homogenate was placed on a rotational plate in the middle of the oven and was treated by microwave irradiation for within three different times (2, 4, 6 minutes).

Three replicates of samples were prepared for each extraction procedure. Once the extraction process was completed, the suspensions were filtered through paper filter for removing the pulp and centrifuged at 3000 rpm for 5 minutes. The supernatants as crude extract were preserved at +4 °C in the dark until analysis.

2.4. Total Phenolics Quantification

The amount of total phenolic compounds in the bilberry extracts was determined by the Folin-Ciocalteu method described by Slinkard et al. [8] using gallic acid as a standard. The Folin-Ciocalteu reagent was prepared by diluting with distilled water (1:10, v/v). 0.5 mL crude extract sample was mixed with daily prepared 2.5 mL folin reagent and left to stand for 2.5 min at room temperature. Then, 1.25 mL of sodium carbonate solution (7% in deionized water) was added. This mixture was left for reaction for 40 minutes in the dark. The absorbance of the sample was measured at a wavelength of 725 nm with a UV-Visible spectrophotometer (MAPADA UV-6100PCS, China) with three replications. A standard curve of absorbance with respect to different concentrations of gallic acid standard (0–80 mg/L) was generated and used for calculation of concentration of total phenolics in the samples. The total phenol content was calculated as mg gallic acid (GA)/100 g fresh bilberry weight (fw) based on the standard curve of gallic acid.

2.5. Total Anthocyanins Quantification

A spectrophotometric pH differential method described by AOAC [9] was used for quantification of total anthocyanins content of bilberry extracts.

500 µL of extract solution was diluted with 2 mL of buffer solutions (potassium chloride buffer with pH 1.0 and sodium acetate buffer with pH 4.5) to prepare 2 different dilutions with different pH levels. The appropriate dilution factor was determined as a 1-part test sample and 4 parts buffer dilution with the absorbance at 520 nm and 700 nm within the linear range of the spectrophotometer (between 0.2 and 1.4 AU). The dilutions were kept in the dark at room temperature for 20 minutes and absorption of the solutions was measured at 520 nm and 700 nm against blank taken as water using a UV-Visible spectrophotometer. Total monomeric anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents (C3GE) and calculated using Eq. (1).

$$MA = \frac{[(A_{520}-A_{700})_{pH1.0}-(A_{520}-A_{700})_{pH4.5}]\times MW\times DF\times 10^5}{\varepsilon\times l}\times\frac{V}{m} \quad (1)$$

where MA is the total monomeric anthocyanin (C3GE, mg/100 g fw); A is the absorbance at different wavelengths for different pH buffers; MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF is the dilution factor; ε is the molar absorptivity (L. mol-1.cm-1; 26900) of cyanidin-3-glucoside; l is the pathlength (cm); V is the total volume of solvent used in the extraction (L); m is the amount of fresh bilberry (g) and 105 is the conversion factor.

2.6. Total Flavonoid Quantification

Total flavonoid content was investigated by two different spectrophotometric methods in order to quantify the total flavonoid content and to estimate the major flavonoids based on the formation of aluminum-flavonoid complexes that exhibits the differences in type of flavonoid compounds [10].

In the first procedure, 1 mL of test extract was put into a 10-ml glass vial. After adding of 0.5 mL of each AlCl3 (2%, w/v), water, HCl (1 M), CH3COONa (1 M), the mixture was waited for 10 min at room temperature. The absorbance of all samples was measured at 425 nm using the UV-Vis spectrophotometer.

In the second procedure, 250 µl extract was taken into a 10-ml glass vial. 1.25 ml distilled water and 75 µl (5%) NaNO2 were added. The glass vial was waited for 6 min. Then 150 µl 10% (AlCl3) and 500 µl of NaOH (1M) and 275 µl distilled water was added. The absorbance of samples was measured at 510 nm using the UV-Vis spectrophotometer.

The content of flavonoid (mean of three determinations) was expressed as mg quercetin equivalent/100 g fw.

2.7. HPLC Analysis

Stock solutions of anthocyanin standards were prepared by weighing 5 mg of each standard into a 50 mL volumetric flask and adding 2 mL of HCl/methanol (2:98, v/v) followed by 10% phosphoric acid to a total volume of 50 mL and mixed. Stock solutions were stored at -40 ºC in glass vials until needed.

The bilberry crude extract obtained by different extraction techniques was concentrated by evaporating the solvent using a rotary evaporator (Hei-VAP, Heidolph Instruments, Germany) until as final solution amount of 2 grams was obtained. The solutions were stored in the dark at -40 ºC in glass bottles until analysis. It was thawed, brought to room temperature and vortexed before to HPLC analysis. A small amount of sample was taken from each solution, diluted 10 times with 5.5% acetic acid solution (Mobile phase A), passed through PTFE filter and injected to the HPLC.

The HPLC analysis was performed by Shimadzu SCL-10AVP HPLC system equipped with querternary pump, an autosampler, a column thermostat, UV/VIS detector

and system controller. Lab Solutions (LC Solution-Version 1.25) computer software was used throughout the analysis. Separation was achieved on a BISCHOFF ProntoSIL C18 column (4.6 mmx250 mm, 5 □m; Germany). The detection wavelength was set at 520 nm and flow rate was 1 mL/min. The injection volume was 20 µL. Mobile phase A was 5.5% aqueous acetic acid solution and mobile phase B was acetonitrile/water/formic acid=50:40:10 (v/v/v).

The gradient elution program was as follows: between 0 and 20 min, 2%–14% B; 20–40 min held at 14% B; 40–50 min increased to 15% B; 50–55 min increased to 19% B; 55–65 min raised to 20% B, 65–110 min washed and re-equilibrated with 2% mobile phase B.

2.8. Statistical Analysis

In this work, all experiments were carried out in triplicate and quantitative data are expressed as mean values of at least three analytical determinations with the respective standard deviation.

3. RESULTS AND DISCUSSION

The recovery of bioactive compounds of wild bilberry fruit grown in Giresun province was investigated by different extraction techniques. To analyze the effects of extraction method and time of extraction on the amount of total phenolics, total flavonoids and total monomeric anthocyanins in bilberry extracts different sets of experiments were designed and analyzed.

3.1. Total Phenol, Total Flavonoid and Total Monomeric Anthocyanin Contents

Four different extraction methods as microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), classical solvent extraction (CSE), and solvent maceration (SM) methods were used to compare the total phenolic contents of bilberry extracts. Depending on the nature of the extraction method, total phenol content of the samples taken at different time of extractions is compared in Figure 1.

Extraction yield of phenolic compounds through MAE, CSE, UAE and SM differed accordingly the extraction technique and time of extraction where the highest total phenol content of the bilberry extracts were in the range of 750-1035 mg GA/100 g fw. Among these extraction methods, MAE was the most efficient method which yields highest amount of total phenols (1035 ± 16 mg GA/100 g fw) in just 2 minutes of extraction. There was no statistically significant difference in total phenol contents extracted over the longer MAE period. However, MAE method has some disadvantages. The major problem arises in MAE is to keep the temperature under controlled during the application.

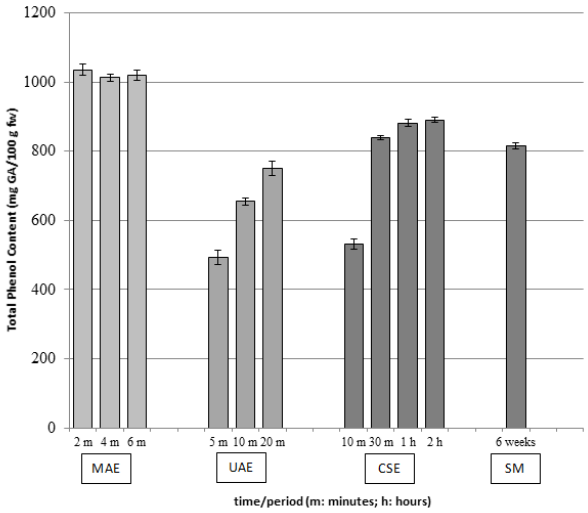

Figure 1. Total phenol content of the samples taken in MAE, UAE, CSE and SM extractions

In this study, although it was tried to be controlled by ice flakes at the double-wall vessel, the temperature was sometimes measured above 65 °C during the extraction process. This can be explained by the ability of the ethanol-water molecules to absorb microwave energy and rapidly pass it on in the form of heat to other molecules in solvents that can accelerate the extraction process in a way that no other technique can match. This mechanism is explained by the dissipation factor (tanδ), which is defined as the ratio of the dielectric loss (ε″) to the dielectric constant (ε′) of the solvent [11]. Accordingly, the rapid temperature rise in the bilberry matrix yields faster diffusion rate and provides faster extraction kinetics. However, compound degradation should be considered due to both the rapid temperature increase and possible chemical property changes occurred during the process. Closed vessel application requirement, high instrumental cost and safety are other disadvantages of the MAE. Total phenol content value of extract obtained by UAE was determined as 749 ± 20 mg GA/100 g fw. UAE is an alternative extraction method with some advantages such as moderate use of solvent and reproducibility. The mechanism underlying of the UAE is the formation of tiny bubbles in the solvent that collapse, causing the pressure and temperature changes and therefore enhancing the mass transfer of the natural components to the solvent. However, the UAE process can promote higher degradation rates of the compounds obtained by extraction, especially when it reaches temperatures above 75 ºC. This disadvantage can also reduce the extraction rate constant as a result of lower surface tension and increased vapor pressure of cavitation bubbles [6]. In the SM method performed at 4°C, however, the total phenol content in the solution was determined as 815 ± 10 mg GA/100 g fw, similar to the values obtained UAE method. Similar total phenol content was determined as 890 ± 8 mg GA/100 g fw for CSE. CSE is a well-known and highly efficient method that is easy to use at atmospheric pressure and ambient temperature, does not require sophisticated equipment and widely employed in a variety of industries. Total phenol content of the extracts in present study was higher compared with those reported for CSE method

which was applied under similar extraction condition for bilberries from Montenegro [12] where it ranged from 392 to 520, from Macedonia [13] where it was 393-706, from [14-15] where it was 890 ± 9, from North East Anatolia (Turkey) [16] where it was 679 ± 5 and also for bog bilberry from Finland [17] where it was 161 ± 1. When these values are compared, it can be said that the results vary according to the type of the bilberry, the region where it grows, the extraction technique used and application conditions. It has been reported that light intensity, photoperiod, temperature and growing location influence the level of total phenolics in berries and at altitude higher than 1500 m, higher amounts of total phenolics were observed [12].

In current study, total monomeric anthocyanin amount of MAE, UAE, CSE and SM extracts was determined as 903 ± 9, 832 ± 9, 762 ± 8 and 667 ± 5 mg C3GE/100 g fw, respectively. Total monomeric anthocyanin content of the extracts in other studies was reported for bilberries from Macedonia [13] where it was 151-507 and from North East Anatolia (Turkey) [16] where it was 164. Može et al. [18] reported the anthocyanin content of Slovenian bilberries as 1210 mg C3GE/100 g fw.

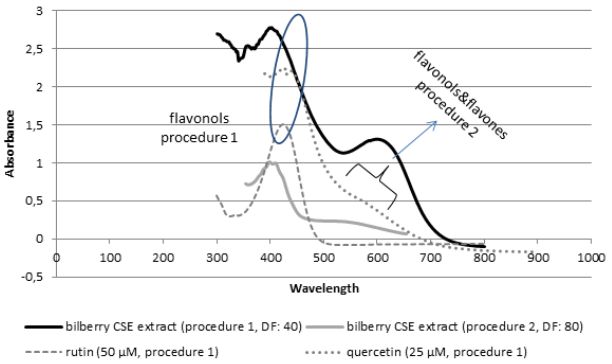

Figure 2. Absorption spectrum of Al-flavonoid complex of bilberry extracts, Al-rutin and Al-quercetin complexes of standards.

Total flavonoid contents were also determined in MAE, UAE, CSE and SM extracts as 150±6, 146 ± 5, 144 ± 3 and 114 ± 3 mg quercetin equivalent/100 g fw, respectively. The total flavonols of bilberry and bog bilberry from Macedonia was reported as 12.1 and 51 mg quercetin equivalent/100 g fw, respectively [13]. Neamtu et al. [19] evaluated the effect of solvent used during extraction of the flavonoids from wild bilberry fruit from South Carpathian Mountains in Romania and reported the flavonoids content in the range of 3.88-8.41 mg quercetin equivalent / g dry weight. Li et al. [20] found that the total flavonoid content of bilberry fruit grown in Nanjing as 13.5 mg quercetin equivalent / g dry weight. A recent study was performed to determine both anthocyanin and flavonoid contents of the different blueberry cultures (both cultivated and wild types) grown in North and South Black Sea region in Turkey [21]. It was reported that anthocyanin and flavonoid contents were determined in the ranges of 43.03-295.06 mg C3GE /100 g fw and 30.44-99.69 mg quercetin equivalent/100 g fw, respectively. The present study reveals that wild bilberry which grows native on high mountain pastures in Giresun almost 1.5 times higher flavonoid content and 2.5 times higher anthocyanin content than blueberries grows in same region. Two different procedures were applied to determine the flavonoid content of the bilberry extract according to Pekal et al. [10]. These procedures were based on the formation of aluminum-flavonoid complexes and exhibited significant differences depending on the type of flavonoids. From the flavonols group, quercetin and rutin were selected as model compounds to check the effect of both procedures' reaction environments. As seen from Figure 2, formed Al-quercetin and Al-rutin complexes with acetate salts (used in procedure 1), showed strong absorption band at 420 nm. But, only Al-quercetin complex exhibited weak absorption band at 560 nm. On the other hand, while flavone, luteolin-aluminum complexes showed absorption peak at 405-420 nm in the procedure 1, it shifted to 530-560 nm but to a lower absorption value when the procedure 2 was applied. For the flavonols group, strong absorption peak was seen at 420- 440 nm in procedure 1 [10], but differentiative absorbance peak at 510 nm was reported only for rutin in procedure 2. According to this information, absorption band was detected when procedure 2 applied to the bilberry extract, which indicates the presence of the rutin and/or luteolin. Stanoeva et al. [13] pointed out to high content of rutin, which constitutes 18% of the total flavonols present in bilberry.

3.2. Profiles of Anthocyanins

The anthocyanin profile of bilberry extracts varied according to the extraction technique applied. Typical anthocyanin profile of bilberry extract was given by HPLC chromatogram (Figure 3). The ratio of the peak area of anthocyanins, identified based on the retention time of available standards, to the sum of all anthocyanin peak areas was used to compare the effect of extraction methods on individual anthocyanin recovery (Table 1).

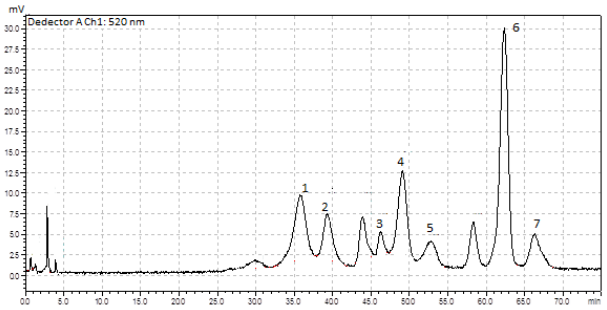

Figure 3. HPLC anthocyanin profile of bilberry extracts. 1. Delphinidin-3-O-glucoside; 2. Delphinidin-3-O-galactoside; 3. Petunidin-3-O-glucoside; 4. Cyanidin-3-O-arabinoside; 5. Cyanidin-3-O-glucoside; 6. Malvidin-3-O-glucoside; 7. Malvidin-3-O-arabinoside

Although the overall anthocyanin profiles are similar for all extracts, changes in individual anthocyanins are inconsistent and show different variability by the extraction method. Malvidin-3-O-glucoside has the highest proportion in all extracts but mostly in the CSE and SM extracts. It was reported that, malvidin possesses great antioxidant capacity [22], antihypertensive activity [23], and plays a role in controlling cellular activities

[24]. In these two methods, the bilberries are kept in direct contact with the solvent. As ultrasound and microwave effects were used in UAE and MAE, respectively, it increased the recovery of other anthocyanins in bilberry extract, and thus different distributions were observed in anthocyanin profiles. However, malvidin stands out with its high percentage distribution in these extracts as well. This can be attributed to microwave energy heating the solvent, increasing its interaction with the sample and accelerating the extraction of the extractable components. Other studies also support that microwave extraction reduces processing time compared to conventional extraction and provides higher yields with less solvent and energy requirements [25-26]. Apparently, delphinidin-3-O-glucoside and cyanidin-3-O-glucoside are also affected considerably. Heffels et al. [3] reported that individual anthocyanins in bilberry are arranged according to their polarity as delphinidin > cyanidin > petunidin > peonidin > malvidin. It seems that more polar anthocyanins show significant variances. Consequently, different extraction techniques lead to the different and variable anthocyanin proportions in the extracts.

Table 1. Anthocyanin proportions* in bilberry extracts

	Extraction technique			
Anthocyanin	**UAE**	**MAE**	**CSE**	**SM**
Delphinidin-3-O-glucoside	11.30	7.36	14.64	9.64
Delphinidn-3-O-galactoside	7.13	6.80	7.80	8.63
Petunidin-3-O-glucoside	1.48	1.55	2.15	1.48
Cyanidin-3-O-arabinoside	12.10	8.77	13.67	10.44
Cyanidin-3-O-glucoside	8.18	4.99	4.84	5.68
Malvidin-3-O-glucoside	27.63	24.29	35.97	37.64
Malvidin-3-O-arabinoside	5.26	4.10	6.27	5.60

* Values are expressed as % of total anthocyanins

4. CONCLUSION

The results we obtained have shown that the bilberry fruit, which spreads naturally in Giresun province, is a very rich source of natural bioactive compounds. Extraction methods were evaluated for the efficient recovery of these bioactive compounds and the effect of the extraction technique on anthocyanin distribution was revealed. MAE method was found to be very suitable extraction processes regarding the amount of total phenolics (1035 ± 16 mg gallic acid/100 g fresh bilberry weight (fw), flavonoids (150±6 mg quercetin equivalent/100 g fw) and anthocyanins (903 ± 9 mg C3GE/100 g fw) and the extraction time. Different anthocyanin proportions indicate that profile changes are due to different extraction techniques. Depending on the applied extraction technique applied, it was determined that different mechanisms affect the extraction performance of bilberry anthocyanins during the extraction process.

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