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Anthocyanins and phenolic compounds of *Mahonia aquifolium* berries and their contributions to antioxidant activity

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ABSTRACT

Antioxidant activities, total phenolic and total monomeric anthocyanin contents were determined in mahonia berry extracts in different solvents. The methanol:water extract was purified to obtain anthocyanin and non-anthocyanin phenolic fractions, one at a time, and antioxidant activities of these fractions were also analyzed. Total phenolic and monomeric anthocyanin contents in mahonia berries were ranged from 1.30 (chloroform extract) to 1049.40 (methanol extract) mg GAE/100 g FW, and 40.68 (water extract) to 380.99 (ethanol extract) mg/100 g FW, respectively. The main phenolic and anthocyanin in mahonia berries were chlorogenic acid (373.12 mg/100 g FW), and cyanidin-3-*O*-glucoside (253.40 mg/100 g FW), respectively. The antioxidant activity of the anthocyanin fraction of the berries was approximately twofold higher than that of the phenolic fraction. The contribution of phenolic and anthocyanin compounds to the antioxidant activity of mahonia berries was greater than 83%. Phenolic compounds, especially anthocyanins, are the major antioxidant compounds found in mahonia berries.

1. Introduction

Mahonia aquifolium is an invasive woody evergreen shrub with sweet smelling bright yellow flowers that bloom in April. It has red-colored fruits that are often referred to as Oregon grapes. It is native to western North America, but it has spread to other areas of America, Australia and Europe and is used for landscaping and medicinal purposes. Extracts and ointments prepared from the root, stems and branches of *Mahonia aquifolium* are used to treat psoriasis. Mahonia is planted in parks and gardens as an ornamental plant in Turkey. Almost in all the humid soil in every region of Turkey, it is planted for ornamental purposes. There are many species of Mahonia plant, but the most grown and planted species in Turkey is Mahonia aquifolium (Marakoglu, Akbulut, & Calisir, 2010).

Pharmacological studies have demonstrated that the alkaloid content of the shrub gives rise to its antidiarrheal, antimicrobial, anti-psoriasis and anti-inflammatory properties (He & Mu, 2015; Volleková, Košťálová, Kettmann, & Tóth, 2003). Magnoflorine, berberine, palmatine, jatrorrhizine, and columbamine are alkaloids that can be isolated from the roots of the shrub. However, the aerial part of the plant only contains berberine, palmatine and jatrorrhizine (Košťálová, Brázdovièová, & Tomko, 1981; Volleková, Košťálová, Kettmann, & Tóth, 2003).

Mahonia aquifolium has been studied to identify the alkaloids and pharmacological properties of its bark of root, stems, branches and leaves. However, there are very few studies on *Mahonia*

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aquifolium fruits. Marakoglu et al. (2010) and Gunduz (2013) reported the morphological, technological and phytochemical properties of *Mahonia aquifolium* berries. The phenolic and anthocyanin contents of mahonia berries are considerably high and are responsible for the bright red color of their berries (Gunduz, 2013; Marakoglu et al., 2010).

Phenolics are one of the most important phytochemicals found in berries due to their contributions to fruit qualities, such as color and taste. Anthocyanins, glycosides of anthocyanidins, are a prominent phenolic, particularly in red and dark colored berries. Anthocyanins are natural plant pigments that are responsible for colors ranging from red to violet (Nicoue, Savard, & Belkacemi, 2007). There are 23 anthocyanidins found in nature; however, only six (cyanidin, peonidin, petunidin, malvidin, pelargonidin, delphinidin) are commonly found in edible plants. There have been more than 500 different types of anthocyanins identified in nature. Anthocyanins are differentiated by variations in their chemical structure, such as the number of hydroxyl groups; number, nature and position of the sugars attached; and acylation of sugars with acids (Castaneda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Giusti & Wrolstad, 2003; Kong, Chia, Goh, Chia, & Brouillard, 2003; Senica, Stampar, Veberic, & Mikulic-Petkovsek, 2016; Wrolstad, Durst, & Lee, 2005). These structural variations contribute to the variations of color and antioxidant activity among the different anthocyanins (Kähkönen & Heinonen, 2003). Cyanidin and peonidin are responsible for orange-red colors in plants, malvidin, delphinidin, and petunidin produce blue-red colors in plants (Kong et al., 2003).

Anthocyanins extracted from plants are commonly used in food coloring such as drinks, medicinal plants and Turkish delight (Kong et al., 2003; Ozen, Akbulut, & Artik, 2011). Anthocyanins and other phenolics are also biologically active compounds. They act as antioxidants by chelating metal ions and scavenging hydroxyl radicals (.OH) and superoxide anion radicals ($.O_2-$) (Kong et al., 2003). Antioxidant capacity varies across different anthocyanins and anthocyanidins. For instance, the antioxidant capacity of peonidin is higher than malvidin, petunidin, pelargonidin, peonidin-3-glucoside and peonidin-3-arabinoside (Kähkönen & Heinonen, 2003).

The polyphenol concentration in berries varies depending on plant genotype (cultivar) and environmental factors, such as cultivation, weather conditions, ripeness, harvest time, storage duration and conditions (Kähkönen, Hopia, & Heinonen, 2001; Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012, 2015; Szajdek & Borowska, 2008; Zorenc, Veberic, Stampar, Koron, & Mikulic-Petkovsek, 2016). Generally, the anthocyanin content is higher in black-colored berries than in red-colored berries (Feng et al., 2016; Mikulic-Petkovsek et al., 2014). The phenolic profile also varies across different berries (Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2012; Szajdek & Borowska, 2008). The anthocyanin profile of blueberries is more complex than those of other berries (Cabrita & Andersen, 1999; Zorenc, Veberic, Stampar, Koron, & Mikulic-Petkovsek, 2017).

Extraction is the first and one of the most important steps of studies when focusing on the phytochemical properties of plant materials. The extraction efficiency directly affects the results and conclusions of these studies (Azmir et al., 2013). Therefore, researchers should carefully take into consideration the right method and condition to be used in a particular extraction. Various extraction methods, such as microwave-assisted extraction, ultrasound-assisted extraction and supercritical fluid extraction have been developed to extract phytochemicals from materials (Wang & Weller, 2006). However, conventional solvent extraction is still widely used to chemically extract phytochemicals from materials (Dai & Mumper, 2010). Extraction duration and temperature, solvent type and polarity, sample to solvent ratios, physical properties and chemical composition of sample can all affect the extraction yield (Azmir et al., 2013; Dai & Mumper, 2010). Water, methanol, acetone and ethanol are polar organic solvents, widely used to extract polar molecules from the food matrix. Ethyl acetate and chloroform are generally used to extract semi-polar molecules. The order of solvents according to their polarity is water > methanol > ethanol > acetone > ethyl acetate > chloroform.

Recent studies examining *Mahonia aquifolium* have focused on the alkaloid content of the stem bark of shrubs. Although the fruit of *M. aquifolium* is a rich source of polyphenolic compounds, it has not received adequate attention from researchers. The aims of our research were to (1) identify the polyphenolic compounds of *Mahonia aquifolium*, (2) determine the best solvent for extraction of polyphenols and other antioxidants from the berry and (3) find out the antioxidant contributions of anthocyanin and non-anthocyanin phenolic compounds extracted from the fruit.

2. Material and methods

2.1. Chemicals

Protocatechuic acid, (+)-catechin, syringic acid, 2,5-dihydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, rutin, isorhamnetin-3-*O*-glucoside, myricetin, luteolin, delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside were purchased from Extrasynthese (Genay, France). Acetonitrile, chloroform, ethanol, acetone, ethyl acetate, methanol, acetic acid, Folin-Ciocalteu's reagent, sodium carbonate, sodium hydroxide, sodium acetate trihydrate, hydrochloric acid, potassium peroxodisulfate were acquired from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzo thiazoline-6-sulphonic acid) (ABTS) and 6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (Steinheim, Germany). Ultrapure water used in extraction and analyses was obtained through Millipore Direct-Q 3 UV ultrapure water system (Millipore, USA).

2.2. Materials

The berries used in this study were collected from *Mahonia aquifolium* shrubs planted in a garden at the Selcuk University Alaeddin Keykubat Campus in the middle of July 2015. The fruits were transported to the laboratory in a polyethylene bag and stored at +4 °C until the extraction.

2.3. Extraction method

Extracts of the mahonia berries were prepared using water, ethanol, methanol, ethyl acetate, chloroform, acetone and methanol:water mixture (80:20) individually. The antioxidant activity, total phenolic content and monomeric anthocyanin content of the different berry extracts were assessed to determine the extraction efficiencies of the solvents. Briefly, fresh fruit (5 g) was extracted with 25 ml of different type of solvents (water, ethanol, methanol, ethyl acetate, chloroform, acetone and methanol:water (80:20) using a homogenizer at 1422*g* (WiseMixTM HG-15D; Daihan Scientific, Korea) for 2 min and centrifuged at 3000*g* for 10 min at 4 °C (NF 800R, Nuve, Turkey). After removing the supernatant, the residue was re-extracted with the same amount of fresh ultrapure water. Extraction was repeated three times. All supernatants were collected in a glass jar. All extracts were stored at −18 °C until tested.

2.4. Extraction and purification of phenolics and anthocyanins

Fruits were extracted with an acidified methanol:water mixture (80:20) as described above to obtain a crude extract. The purification process was performed on crude methanol:water extracts using a C18 Sep-Pac cartridge to remove the sugars and organic acids from the extract and to obtain anthocyanins and other phenolics one at a time. Two ml of crude extract was uploaded into C18 SPE cartridges (Agilent, USA) conditioned by passing water, ethyl acetate and methanol. To remove the organic acids and sugars, first water was passed from the cartridge and then the phenolics and anthocyanins were eluted with ethyl acetate and methanol, respectively. Ethyl acetate and methanol eluents were evaporated at 35 °C and then resuspended in 1 ml of methanol.

2.5. Total phenolic content

The total phenolic content of the extracts was determined using the Folin-Ciocalteu colorimetric method (Akbulut & Coklar, 2015). Briefly, 250 μl of extract, adequately diluted with its solvent, was mixed with 1250 μl of Folin-Ciocalteu reagent (0.2 N) and 1000 μl of sodium carbonate solution (75 g/L). After 2 h of incubation at room temperature in the dark, the absorbance was measured at 765 nm using a spectrophotometer (U-1800, Hitachi, Japan). Standard solutions of gallic acid at concentrations of 12.5–200 ppm were used to construct the calibration curve. The results were expressed as mg of gallic acid equivalents (GAE)/100 g fresh weight (FW).

2.6. Total monomeric anthocyanin content

The total monomeric anthocyanin content in extracts was determined using the pH differential method described previously by Akbulut and Coklar (2015). Two flasks were filled with 1 ml of extract each. The first flask was diluted with 4 ml of pH 1.0 buffer (potassium chloride, 0.025 M) and the second one diluted with pH 4.5 buffer (sodium acetate, 0.4 M), separately. After 30 min wavelengths at 515 and 700 nm were measured and the absorbance difference was calculated according to Eq. (1). The total monomeric anthocyanin content of the extracts was calculated according to Eq. (2); the results were expressed in mg of cyanidin-3-glucoside/100 g FW.

$$A = (A_{520nm} - A_{700nm})_{pH1.0+} - (A_{520nm} - A_{700nm})_{pH4.5} \quad (1)$$

$$\text{Total monomeric anthocyanin content (mg/100 g)} = (A \times MW \times DF \times 100)/(\varepsilon \times l) \quad (2)$$

where A is the absorbance difference, DF is the dilution factor, MW is molecular weight of cyanidin-3-glucoside (449.20 g/mol), ε is the molar absorptivity of cyanidin-3-*O*-glucoside (26,900 L mol^{-1} cm^{-1}) and l is a pathlength of 1 cm.

2.7. Antioxidant activity

2.7.1. DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH·) antioxidant activity of the extracts and anthocyanin and non-anthocyanin phenolic fractions were determined according to methods described by Brand-Williams, Cuvelier, and Berset (1995). Briefly, 0.1 ml aliquots of the berry extracts were added to 3.9 ml of DPPH (6×10^{-5} M) methanolic solution. After a 30 min incubation at room temperature in the dark, the absorbance was measured at 515 nm. The results were expressed as mmol trolox equivalent/kg FW.

2.7.2. ABTS assay

Using methods described by Re et al. (1999), we determined the ABTS antioxidant activity of extracts and phenolic fractions. To generate the ABTS· radical, 2.5 ml of potassium persulfate solution (2.45 Mm) were added to 5 ml of ABTS solution (7 mM). The mixture was incubated at room temperature for approximately 16 h. The stock solution was diluted with ethanol until an absorbance of 0.700 ± 0.02 at 734 nm was reached. Ten microliters of extract were added to 1000 μl of ABTS· solution. The absorbance at 734 nm was measured after 6 min and the reduction in absorbance was determined. Trolox was used as the standard; the results were expressed as mmol trolox equivalent/kg FW.

2.7.3. FRAP assay

Ferric reducing antioxidant powers (FRAP) of the extracts and phenolic fractions were determined according to the methods described by Benzie and Strain (1999). Fifty μL of extract and 150 μl of deionized water were added to 1.5 ml of the freshly prepared FRAP reagent (300 M acetate buffer [pH 3.6]:10 M TPTZ:20 M $FeCl_3 \cdot 6H_2O$; 10:1:1). The reaction mixture was incubated at 37.0 °C for 4 min. The absorbance was measured at 593 nm. The results were calculated according to the $FeSO_4 \cdot 7H_2O$ calibration curve prepared in the range of 100–1000 μmol/L. The results were expressed as μmol Fe^{+2}/g FW.

2.8. Determination of phenolic and anthocyanin profiles

Analyses of the phenolic compounds in the purified anthocyanin and non-anthocyanin phenolic fraction were carried out using an Agilent 1260 Infinity Series HPLC system equipped with a G1329B autosampler, G1311C a pump, a G1316A column oven and a G1315D diode array detector. Before the injection, the fractions were filtered through a 0.45 μm pore size syringe filter (Sartorius AG, Goettingen, Germany). Separation was achieved using a reverse phase C18 column (5 μm, 250 × 4.6 mm i.d.). The mobile phase consisted of acetic acid:water (A) and water:acetonitril: acetic acid (B). The flow rate was 0.75 ml/min and the gradient was as follows: 10–14% B (5 min), 14–23% B (11 min), 23–35% B (5 min), 35–40% B (14 min), 40–100% B (3 min), 100% B isocratic (3 min), 100–10% B (3 min) and 10% B isocratic (4 min). The detector was set to 280, 320 and 360 nm for non-anthocyanin phenolics and 520 nm for anthocyanins. Phenolic identification was confirmed by comparing the retention times and the UV spectra. The data were analyzed using Chemstation software (Demir, Yildiz, Alpaslan, & Hayaloglu, 2014).

2.9. Statistical analysis

The results were expressed as the mean ± standard deviation (SD) and subjected to one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant. The statistical analyses were performed using MINITAB (Release 14, Minitab Inc. USA).

3. Results and discussion

3.1. Total phenolic and monomeric anthocyanin content

Table 1 shows the results of total phenolic and monomeric anthocyanin contents of *Mahonia aquifolium* berries which were extracted using different solvents. The total phenolic content of the berries extracted with the methanol:water solvent was found to be 1007.50 mg/100 g FW. Our findings on the total phenolic content of mahonia berries differ considerably from previous results reported in the literature. Gunduz (2013) and Marakoglu et al.

Table 1
Total Phenolic content, monomeric anthocyanin content, DPPH, ABTS and FRAP antioxidant activities of *Mahonia aquifolium* berries in different solvents.

Extracts	Total phenolic content (mg GAE/100 g FW)	Total Monomeric Anthocyanin Content (mg/100 g FW)	DPPH (mmol TE/kg FW)	ABTS (mmol TE/kg FW)	FRAP (μmol Fe^{+2}/g)
Water	853.00 ± 21.2c	40.68 ± 1.08c	13.03 ± 0.25d	27.36 ± 6.94c	58.67 ± 1.11d
Ethanol	1014.60 ± 4.32ab	380.99 ± 1.55a	27.36 ± 1.47c	45.67 ± 2.60ab	138.93 ± 0.98b
Methanol	1049.40 ± 62.10a	360.99 ± 4.81a	35.26 ± 1.88b	49.95 ± 1.06a	136.34 ± 8.07b
Ethyl acetate	157.70 ± 7.17d	nd	4.36 ± 0.17e	7.02 ± 1.37d	17.72 ± 1.18e
Chloroform	1.30 ± 0.22e	nd	0.36 ± 0.04e	0.43 ± 0.03d	3.85 ± 0.10f
Acetone	914.40 ± 8.83bc	159.38 ± 11.10b	14.41 ± 0.68d	35.71 ± 5.85bc	108.39 ± 2.31c
Methanol:water	1007.50 ± 43.74ab	354.56 ± 4.08a	44.47 ± 1.79a	54.97 ± 0.02a	188.56 ± 0.92a

Results were given as mean ± standard deviation (n = 3) and different letters in the same column indicate statistically significant differences.

(2010) determined the total phenolic content of the fruit to be 500.93–664.68 and 457.46 mg/100 g, respectively. Differences in phenolic content could be attributed to differences in the solvents used for extraction, except for many other factors such as ripeness and environmental conditions (e.g., water deficit, temperature, UV radiation exposure).

According to our results, the total phenolic content of the mahonia fruit was higher than those of *Vaccinium uliginosum* (Su et al., 2016), bilberry (Bakowska-Barczak, Marianchuk, & Kolodziejczyk, 2007; Mikulic-Petkovsek et al., 2012), elderberry (Lee & Finn, 2007; Mikulic-Petkovsek, Ivancic, Schmitzer, Veberic, & Stampar, 2016), barberry (Akbulut, Calışır, Marakoğlu, & Coklar, 2009), European cranberry bush (Akbulut, Calisir, Marakoglu, & Coklar, 2008), highbush cranberry (Dudonné et al., 2015), chokeberry (Dudonné et al., 2015), *Prunus mahaleb* (Mikulic-Petkovsek, Stampar, Veberic, & Sircelj, 2016), lingonberry (Mikulic-Petkovsek et al., 2012), red and black currants (Mikulic-Petkovsek et al., 2012), red and white gooseberries (Mikulic-Petkovsek et al., 2012).

The total phenolic content of the mahonia fruit in the different extraction solvents ranged from 1.30 to 1049.40 mg GAE/100 g of FW. Phenolic extraction of the fruit was highly affected by the solvent used. The methanol extracts had the highest phenolic content. The lowest phenolic contents were observed in chloroform (1.30 mg/100 g) and ethyl acetate (157.70 mg/100 g) extracts. The phenolic extraction of the Mahonia fruit was poor when chloroform was used as the solvent. Methanol, ethanol and the methanol:water mixture were found to be the most effective extraction solvents.

Similar to our results, Onivogui, Letsididi, Diaby, Wang, and Song (2016) demonstrated that methanol and ethanol were more effective than ethyl acetate and water in extracting phenolic and antioxidant compounds from *Anisophyllea laurina* fruits.

Hayouni, Abedrabba, Bouix, and Hamdi (2007) studied the effects of solvents on phenolic compound extraction of *Tunisian Quercus coccifera L.* and *Juniperus phoenicea L.* fruits using chloroform, water, acetone, acetone:water:acetic acid and ethyl acetate: methanol:water. They found that chloroform was the least effective solvent for phenolic compound extraction.

As seen in Table 1, mahonia is one of the important sources of anthocyanin among the other anthocyanin containing fruits. The total monomeric anthocyanin content of the mahonia fruit extracted with the methanol:water solvent was determined to be 354.56 mg/100 g FW. Its anthocyanin content was approximately 10 times higher than strawberries, raspberries, cranberries, Japanese wineberry and cloudberries (Dudonné et al., 2015; Szajdek & Borowska, 2008; Veberic, Slatnar, Bizjak, Stampar, & Mikulic-Petkovsek, 2015).

Anthocyanin content of black berries is higher than in red berries (Feng et al., 2016; Zorenc et al., 2016). Feng et al. (2016) and Mikulic-Petkovsek et al. (2015) reported that anthocyanin contents in wild berries ranging from 10 to 1058 mg/100 g and 350 to 1500 mg/kg, respectively.

Similar to the total phenolic content, the monomeric anthocyanin levels of the mahonia fruit varied depending on the extraction solvent used. While there was no monomeric anthocyanin detected in the chloroform and ethyl acetate extracts, the levels measured in the ethanol, methanol and methanol:water extracts were high. There was no significant difference between the monomeric anthocyanin content of mahonia berries in methanol, ethanol and methanol:water extracts. Extraction of anthocyanins using acetone and water was also found to be insufficient when compared to ethanol and methanol.

Lapornik, Prošek, and Wondra (2005) used water, ethanol (70%) and methanol (70%) to extract anthocyanins from black currant, red currant and grape by-products. They found that methanol and ethanol extracts of the by-products contained more anthocyanin than the water extract. Similarly, Wang, Jung, Tomasino, and Zhao (2016) extracted anthocyanins from blueberries, cherries and red pear peel using methanol, ethanol and acetone. They determined that methanol and ethanol were more effective than acetone in extracting anthocyanins from cherries and pear peel. Celant, Braga, Vorpagel, and Salibe (2016) also reported that ethanol extracts of five blackberry cultivars had a higher anthocyanin content than the water extracts.

While water had the highest polarity index amongst the solvents used in our study, the total phenolic and monomeric anthocyanin content of the methanol:water, methanol and ethanol extracts were higher than the water extract. In addition to polarity, the viscosity and density of the solvent also affected the extraction yield. The yield and speed of extraction may be increased by using solvents with low viscosity and low density (Hemwimol, Pavasant, & Shotipruk, 2006). The higher phenolic contents in the methanol: water, ethanol and methanol extracts may have been due to the low densities and high polarities of these solvents.

3.2. Anthocyanins and phenolic compounds in mahonia berries

Fig. 1 shows the LC-DAD chromatogram of phenolics in *Mahonia aquifolium* berries. Six phenolic acids were identified in the berries, namely protocatechuic acid, syringic acid, *p*-coumaric acid, caffeic acid, 2,5-dihydroxybenzoic acid, and chlorogenic acid. Chlorogenic acid was the primary phenolic compound found in mahonia berries and constituted approximately 54% of the total phenolic content of the berries.

Consistent with this finding, others have reported that chlorogenic acid is the primary phenolic in white crowberries (León-González et al., 2013), bilberries (Ancillotti et al., 2016), cranberry bush (Velioglu, Ekici, & Poyrazoglu, 2006), and blueberries (Gao & Mazza, 1994). On the other hand, according to our results, mahonia berries had higher chlorogenic acid levels (373.12 mg/100 g) than many of the other berries reported in previous studies (Gao & Mazza, 1994; Može et al., 2011; Taruscio, Barney, & Exon, 2004).

Other phenolic acids in the mahonia berries have been found in smaller amounts (Table 2). While 2,5-dihydroxybenzoic and

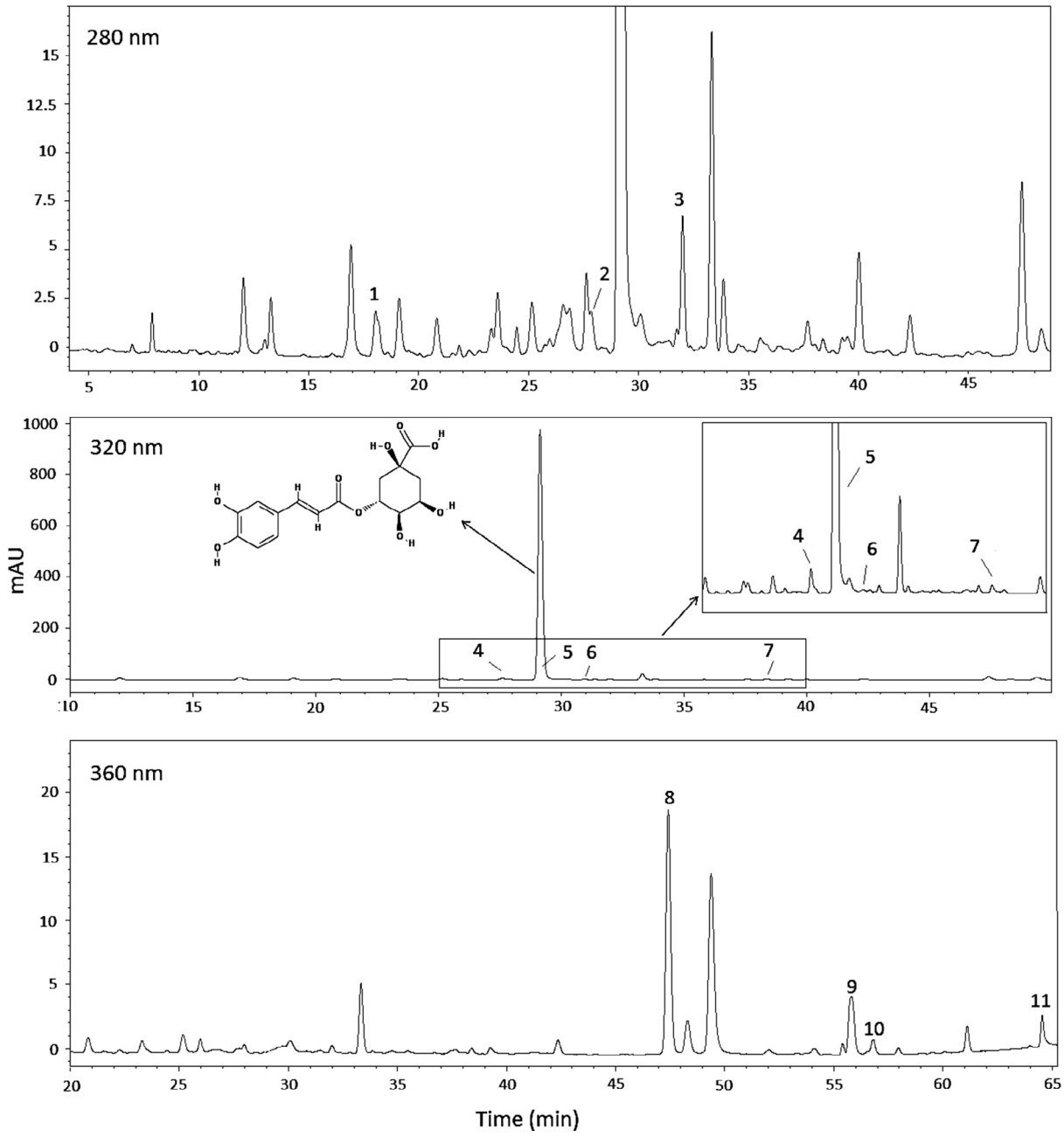

Fig. 1. Chromatogram of phenolic compounds in mahonia fruit (1:Protocatechuic acid, 2: (+)-Catechin, 3: Syringic acid, 4: 2,5-dihydroxybenzoic acid, 5: Chlorogenic acid, 6: Caffeic acid, 7: *p*-coumaric acid, 8: Rutin, 9: isorhamnetin-3-*O*-glucoside, 10: Myricetin, 11: Luteolin).

Table 2
Phenolic compounds in *Mahonia aquifolium* berries.

Phenolic Compounds	Amounts (mg/100 g FW)
Protocatechuic acid	1.40 ± 0.20
(+)-Catechin	5.80 ± 0.61
Syringic acid	2.76 ± 0.60
2,5-Dihydroxybenzoic acid	7.28 ± 0.47
Chlorogenic acid	373.12 ± 11.94
Caffeic acid	0.36 ± 0.06
p-Coumaric acid	0.88 ± 0.34
Rutin	25.15 ± 2.01
Isorhamnetin-3-*O*-glucoside	5.39 ± 0.47
Myricetin	1.96 ± 0.03
Luteolin	0.46 ± 0.06

syringic acids were found at the levels of 7.28 and 2.76 mg/100 g, respectively, the concentrations of other phenolic acids were relatively low (<1.5 mg/100 g).

Caffeic and *p*-coumaric acids are present in almost all berries. Whereas, protocatechuic acid, syringic acids and 2,5-dihydroxybenzoic acids have only been detected in bilberry, cranberry, red currant, blueberry, rose hip and crowberry (Mattila, Hellström, & Törrönen, 2006; Zadernowski, Naczk, & Nesterowicz, 2005).

The flavonol content of mahonia berries was higher than that of flavan-3-ols, consistent with a previous study on bilberries (Može et al., 2011). Catechin was the only detectable flavan-3-ol found in mahonia berries (5.80 mg/100 g). Može et al. (2011) found that there was low concentrations of catechin in blueberries and bilberries to be 1.8 mg/100 g and 0.2 mg/100 g, respectively.

We also detected rutin (25.15 mg/100 g), isorhamnetin-3-*O*-glucoside (5.39 mg/100 g), myricetin (1.96 mg/100 g), and luteolin (0.46 mg/100 g) in mahonia berries. Häkkinen, Kärenlampi, Heinonen, Mykkänen, and Törrönen (1999) measured the concentrations of myricetin in bog whortleberry, cranberry, bilberry, blueberry, crowberry, black currant berries in the range of 14–142 mg/kg.

In a previous study, the rutin contents of Saskatoon berry, alpine bearberry, chokeberry, black crowberry, and honeysuckle varied from 0.05 to 12.5 mg/100 g (Dudonné et al., 2015). Our findings demonstrate that the amount of rutin found in mahonia berries is higher than in the berries studied previously.

Berries are considered to be a major anthocyanin source. The anthocyanin content of berries is highly variable. Like many other fruits, berries possess distinctive anthocyanin profiles.

Fig. 2 shows the chromatogram of mahonia berries anthocyanins detected at 520 nm. Cyanidin-3-*O*-glucoside, as a primary anthocyanin, comprised 70% of the total anthocyanin content in mahonia berries. In descending order, cyanidin-3-*O*-rutinoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and delphinidin-3-*O*-rutinoside were also identified in the fruit (Table 3).

Similar to our results, cyanidin-3-glucoside has been found to be a prominent anthocyanin in *Lonicera caerulea* (Dudonné et al., 2015; Oszmiański, Wojdyło, & Lachowicz, 2016), *Fuchsia magellanica* (Ruiz et al., 2013), *Phillyrea latifolia* (Longo, Scardino, & Vasapollo, 2007) and *Vaccinium myrtillus* (Veberic et al., 2015) berries.

Table 3
Anthocyanins in *Mahonia aquifolium* berries.

Anthocyanins	mg/100 g FW
Delphinidin-3-*O*-glucoside	6.71 ± 1.84
Delphinidin-3-*O*-rutinoside	3.38 ± 0.79
Cyanidin-3-*O*-glucoside	253.40 ± 7.27
Cyanidin-3-*O*-rutinoside	45.57 ± 2.03
Pelargonidin-3-*O*-glucoside	12.56 ± 0.71
Peonidin-3-*O*-glucoside	12.89 ± 1.41
Malvidin-3-*O*-glucoside	25.64 ± 1.66

3.3. Antioxidant activity of mahonia berries

3.3.1. Antioxidant activity of berry extracts using different solvents

The antioxidant activity of fruits and vegetables is attributed to their phytochemical compounds such as carotenoids, ascorbic acid and betalains. However, recent studies have demonstrated that polyphenols are the primary antioxidative compounds of various fruits and vegetables. As in many fruits and vegetables, the high antioxidant capacity of berries is based on the phenolic compounds and their concentrations. Phenolics can neutralize radicals via hydrogen atom (HAT) and/or single-electron (SET) transfer (Craft, Kerrihard, Amarowicz, & Pegg, 2012). $ABTS^{\bullet}$ and $DPPH^{\cdot}$ radicals can be deactivated by both the HAT and SET mechanisms. Therefore, in this study two mixed-mode methods (SET/HAT) (DPPH and ABTS) and one SET method (FRAP) were used to estimate the antioxidant activity of the mahonia fruit.

The antioxidant activity of the berry as determined by DPPH, ABTS and FRAP assays in methanol:water extract were found to be 44.47 mmol TE/100 g, 54.97 mmol TE/100 g and 188.56 μmol Fe^{+2}/g, respectively (Table 1).

Like other berries, the antioxidant activity of the mahonia fruit was high. Prior and Cao (2000) reported that the antioxidant activities in blueberries, cranberries and blackberries exceeded 20 μmol TE/g. Bakowska-Barczak et al., 2007 reported that the antioxidant capacity of 14 Canadian berries ranged from 1.60 to 9.55 mmol TE/100 g fresh mass. They found that honeysuckle berries had the highest TEAC value and red Nanking cherries had the lowest.

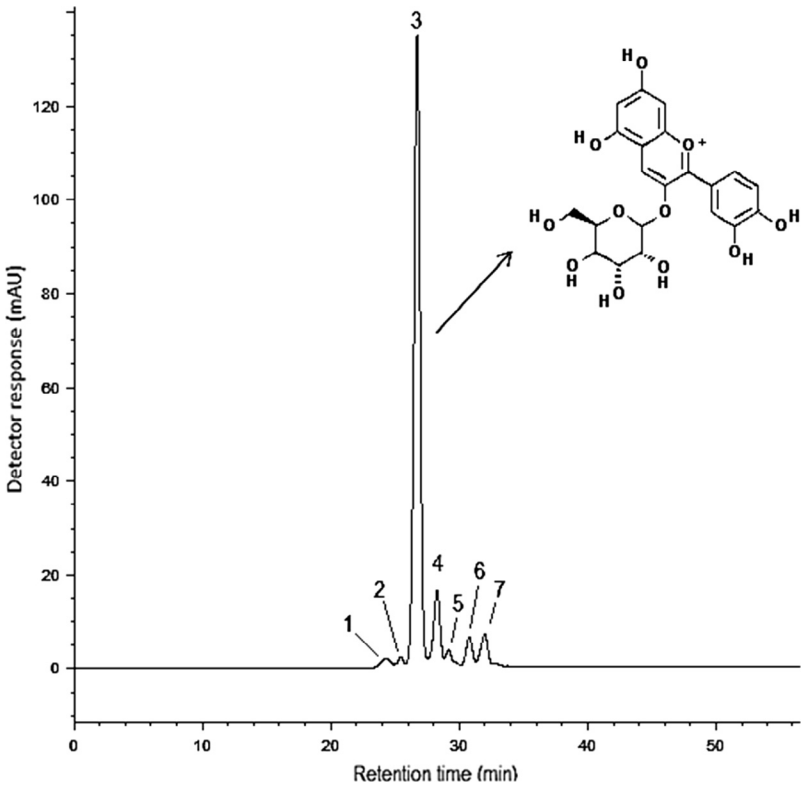

Fig. 2. Chromatogram of anthocyanin in mahonia berries (1:Delphinidin-3-*O*-glucoside, 2: Delphinidin-3-*O*-rutinoside, 3: Cyanidin-3-*O*-glucoside, 4: Cyanidin-3-*O*-rutinoside, 5: Pelargonidin-3-*O*-glucoside, 6: Peonidin-3-*O*-glucoside, 7: Malvidin-3-*O*-glucoside).

The DPPH antioxidant activity of *Vaccinium uliginosum* berries was reported to range from 44.22 to 113.40 mg/100 g FW (Su et al., 2016). It could be said that mahonia fruit exhibits equal and/or higher antioxidant activity than that of some other fruits such as elderberry (Mikulic-Petkovsek et al., 2016), white cherry (Mikulic-Petkovsek et al., 2016) and blackthorn (Mikulic-Petkovsek et al., 2016). As was the case for the phenolic and anthocyanin contents, the solvent type also significantly affected the antioxidant activity. The antioxidant capacity and phenolic content of mahonia extracts were correlated (Table 1). Extracts that had higher phenolic concentrations exhibited higher antioxidant activity. Fruits extracted with the methanol:water solvent mixture had the highest antioxidant activity as measured by the three methods (DPPH, ABTS and FRAP).

The highest DPPH antioxidant activity was measured in the methanol:water extract, while the chloroform extract had the lowest DPPH antioxidant activity. Similar results were observed in the FRAP measurements of antioxidant capacity. The order of antioxidant activity of each extract from lowest to highest was: chloroform, ethyl acetate, water, acetone, ethanol, methanol and methanol:water. However, the FRAP antioxidant activity of the methanol and ethanol extracts did not differ. The antioxidant activities measured using the ABTS assay were consistent with those obtained with FRAP and DPPH.

The order of ABTS antioxidant activity levels measured in the mahonia berry extracts was as follows: methanol:water = methanol > ethanol > acetone > water > ethyl acetate = chloroform. The antioxidant activities of the extracts measured with DPPH, ABTS and FRAP increased as the solvent polarity increased. This finding indicates that the antioxidant activity of mahonia berries results from its polar compounds.

3.3.2. Antioxidant capacities of the phenolic and anthocyanin fractions

Crude extracts may contain non-phenolic compounds such as organic acids, sugars and chlorophylls. These compounds can reduce or increase the antioxidant activity of extracts by interfering with the phenolics. In some cases, additional purification is applied to the crude extract to remove these compounds to obtain purified phenolic extracts (Kähkönen et al., 2001).

We purified the methanol:water crude extract to obtain pure anthocyanin and non-anthocyanin phenolic extracts and to determine the antioxidant activities of mahonia anthocyanins and other phenolics, in particular. The quantity of compounds in the phenolic fraction was higher than in the anthocyanin fraction (Tables 2 and 3). Whereas, the antioxidant activity of the anthocyanin fraction was much more than non-anthocyanin phenolic fraction. As shown in Fig. 3, the antioxidant activities of anthocyanin fractions determined with DPPH, ABTS and FRAP were nearly 2 times higher than those measured in the phenolic fractions of berries. These results may be due to varying antioxidant capacities across the individual phenolics.

Approximately 29.22 and 56.72% of the DPPH antioxidant activity of the crude extract was contributed by the phenolic compounds and anthocyanins, respectively. However, the combined contribution of both of the fractions to the DPPH antioxidant activity was nearly 85.94%. Similar results were observed in the FRAP antioxidant activity in the berries. The antioxidant activity of the anthocyanin fraction was higher than the phenolic fraction as

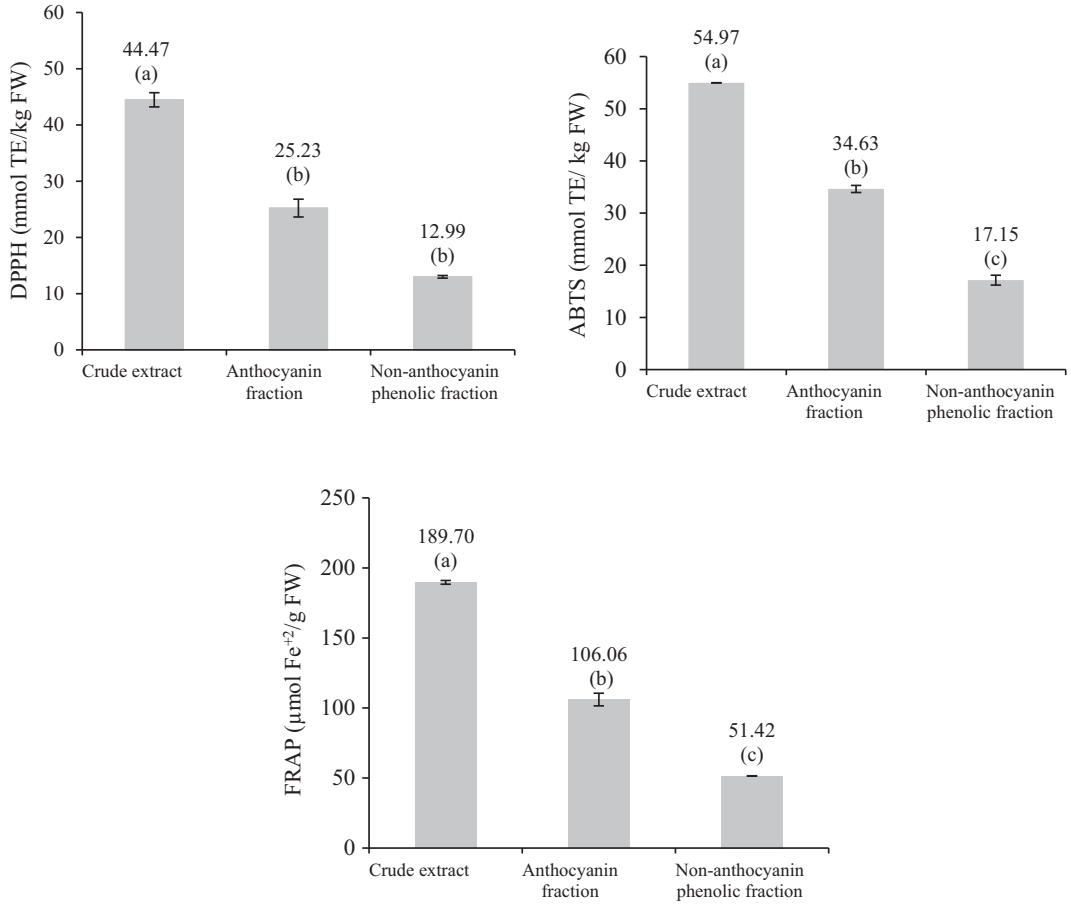

Fig. 3. DPPH, ABTS and FRAP antioxidant capacities in crude extract, anthocyanin and non-anthocyanin phenolic fractions of mahonia berries. Different letters in each graphic indicate statistically significant difference.

determined by the FRAP assay. Phenolic compounds and anthocyanins accounted for 55.91 and 27.11% of the FRAP antioxidant activity, respectively. While the combined contribution of phenolics and anthocyanins to the antioxidant activity determined by the ABTS assay was 94.20%; 63.00% of the ABTS values were contributed by anthocyanins.

The results of the three different antioxidant assays demonstrated that the antioxidant activity of the crude mahonia extract was higher than that of the phenolic and anthocyanin fractions. However, the contribution of the phenolics and anthocyanins to antioxidant activity was considerably higher. Based on these findings, it can be said that polyphenols, particularly anthocyanins, are the main antioxidative compounds of mahonia berries. Higher antioxidant activity measured in the crude extract could have resulted from other antioxidant compounds such as alkaloids and vitamin C that are found in the extract. Similarly, synergistic interactions between the polyphenols and other antioxidants may be among the causes of the higher antioxidant activity of the crude extract.

The antioxidant capacity of individual phenolics may vary as a result of its chemical structure. Rice-Evans, Miller, and Paganga (1997) and Heo, Kim, Chung, and Kim (2007) reported that cyanidin possess nearly 3 times more antioxidant activity than chlorogenic acid. Similarly, antioxidant activity of cyanidin-3-glucoside is higher than that of cyanidin-3-rutinoside and lower than that of cyanidin (Heo et al., 2007). In contrast, Wang, Cao, and Prior (1997) found that cyanidin-3-glucoside had highest ORAC value among cyanidin-3-rhamnoglucoside, cyanidin, and cyanidin-3-galactoside.

As reported above, chlorogenic acid was the primary phenolic compound in the phenolic fraction of the mahonia berries. Previous studies have demonstrated that chlorogenic acid possesses antioxidant activity (Puupponen-Pimiä, Nohynek, Alakomi, & Oksman-Caldentey, 2005; Rice-Evans et al., 1997). High antioxidant activity in the phenolic fraction of Mahonia berries may have resulted from its considerably high chlorogenic acid content.

In terms of quantity, the majority of the phenolic content was comprised of chlorogenic acid. However, anthocyanins, particularly cyanidin-3-glucoside, were the primary contributor to the antioxidant power of the extract.

4. Conclusions

This work intended to determine the best effective extraction solvent for high total phenolic content, total monomeric anthocyanin content and antioxidant efficacy of *Mahonia aquifolium* berries by using different solvents, and phenolic and anthocyanin compounds of extract obtained from the most effective solvent. In the same time, the contribution of phenolic and anthocyanin extracts to the antioxidant capacity of the mahonia berries was separately revealed. The best extracting solvent for total phenolic content, total monomeric anthocyanin content and antioxidant capacity were pure methanol, pure ethanol and methanol:water (80:20 v/v) mixture, respectively. However, taking safety into consideration, ethanol can be used instead of methanol when preparing anthocyanin-based mahonia berries extracts for consumption. The evidence from this study suggests that polyphenolic compounds are among the primary antioxidants in mahonia berries. The main phenolic compound in mahonia berries was found to be chlorogenic acid and the main anthocyanin compound was cyanidin-3-*O*-glucoside. The anthocyanin contribution to the antioxidant activity of mahonia berries was nearly twice that of non-anthocyanin phenolics.

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