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Research article

The effects of extraction conditions on the antioxidant activities, total polyphenol and monomer anthocyanin contents of six edible fruits growing wild in Hungary

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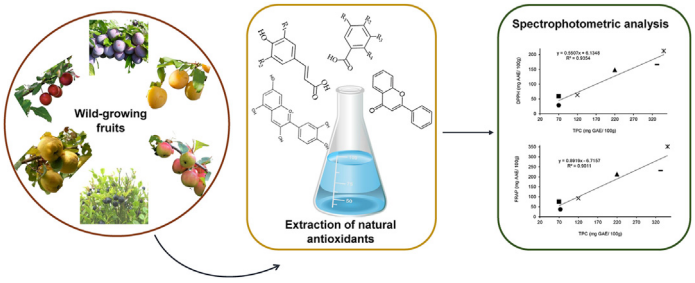
HIGHLIGHTS

- Three independent variables were examined to extract natural antioxidants from wild fruits.
- The solvent acidity and solvent type were the most significant factors.
- The spectrophotometric analysis of the extracts revealed good antioxidant properties.
- Antioxidant activities showed high correlation with total polyphenol.

GRAPHICAL ABSTRACT



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ABSTRACT

Although wild fruits are significantly underutilized in most countries, they could be good sources of valuable bioactive compounds with antioxidant properties. Therefore the present study focused on the study of a conventional extraction technique (maceration with shaking; MAC_S) to extract natural antioxidants and anthocyanin colorants from six edible wild-growing fruits (European crab apple, bilberry, yellow-, red-, and purple-skinned greengage, and quince). One-factor-at-a-time (OFAT) methodology was chosen to investigate the influences of three different parameters (solvent type, extraction time and solvent acidity) on the total polyphenol contents (TPCs), total monomeric anthocyanin (TMA) contents, and antioxidant capacities, specifically ferric reducing power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity (DPPH). After optimization, the recorded TPCs and antioxidant activities proved to be significantly higher for all analyzed fruits when compared to differing extraction conditions. For European crab apple and purple-skinned greengage, the best extraction conditions were a ratio of 80:20 (v/v) EtOH–H_2O, 1% (v/v) of HCOOH, and an extraction time of 90 min. In the case of red-skinned greengage, the extraction parameters were the same as the above except for the acid concentration (0.5%; v/v) used. For quince, the optimized conditions required a 50:50 (v/v) EtOH–H_2O mixture, an extraction time of 90 min, and 0.5% (v/v) HCOOH concentration. The best conditions for the extraction of bilberry and yellow-skinned greengage were an EtOH–H_2O combination of 50:50 (v/v), extraction time of 60 min, and HCOOH concentration of 0.5% (v/v). The highest TPC and antioxidant activity were observed in quince (281–510 mg GAE/100g and 109–395 mg AAE/100g) whereas the lowest were measured in European crab apple (55.9–70.0 mg GAE/100g and 20.1–43.2 mg AAE/100g). Bilberry exhibited the highest TMA content (346 mg CGE/100g). Overall, our results showed that these wild fruits could be a good source of natural antioxidants for the local residents.

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1. Introduction

In recent decades, several studies have confirmed that polyphenols may have a beneficial effect on human health. Polyphenols are secondary plant metabolites that help prevent or delay various diseases such as type 2 diabetes, cancer, or cardiovascular diseases [1, 2]. These bioactive compounds are naturally found in fruits, vegetables, herbs, and cereals [3, 4]. Nowadays, anthocyanins are some of the most widely studied polyphenolic plant compounds. These water-soluble pigments are responsible for blue, red, or purple colors in fruits, vegetables, or flowers [5]. In the food industry, anthocyanins are used as natural food colorants. They also exhibit beneficial effects on human health due to their antioxidant, anti-inflammatory, and anticarcinogenic properties [6].

The Hungarian region of Kisalföld is home to many wild plants, including fleshy fruits and medicinal herbs. These species are readily available, and possess strong nutritional and therapeutic properties [7]. Wild edible fruits are one of the alternative sources of healthy and nutritious food. However, these plants are often underutilized or lesser known [8]. European crab apple (*Malus sylvestris* (L.) Mill.) or wild apple is a small fruit tree of the Rosaceae family. They are round fruits of about 3 cm in diameter with a bright red color on the sunny side [9]. Bilberry (*Vaccinium myrtillus* L.) is a low-growing shrub and belongs to the Ericaceae family. It is also known as the European blueberry. Bilberry fruits are small (approximately 4–6 mm in diameter) and fleshy, containing tiny, brownish moon-shaped seeds. They have a slightly acidic but sweet flavor. The fruits ripen between July and September [10]. *Prunus domestica* ssp. *italica*, better known as the greengage, belongs to the family Rosaceae, which is composed of 100 genera and about 3 000 species [11]. Greengage fruits are identified by their round shape and smooth texture. The skin ranges in color from yellow to red or purple. They have a sweet, subtly acidic flavor [12]. In Hungary, greengage fruit may be consumed as fully ripe fruits in the late summer through early fall. The quince (*Cydonia oblonga* Mill.) is the sole member of the genus *Cydonia* in the family Rosaceae. Its fruits ripen in mid to late fall; they have very tough flesh and astringent flavor [13]. In Hungary, it often grows wild near creek banks.

Nowadays, wild fruits have received increased attention because of their significant antioxidant properties. Measurements have shown that these fruits may be a good source of vitamin C, as well as other vitamins, flavonoids, and phenolic acids [14, 15, 16, 17]. To take advantage of the benefits of such compounds in fruits, researchers must use appropriate extraction techniques. Among the conventional techniques, maceration with shaking is the most extensively used method for the extraction of polyphenols from fruits due to its simplicity, speed, and cost-effectiveness [18]. The extraction efficiency of phytochemicals depends on several parameters such as solvent type, extraction time, solvent composition, pH, etc. Therefore, the aim of the present study is to investigate the effect of extraction conditions on the total polyphenol content, monomeric anthocyanin, and antioxidant properties of six fruits growing wild in Hungary.

2. Methods

2.1. Chemicals

The Folin-Ciocalteu reagent, gallic acid, ferric chloride hexahydrate, sodium acetate, absolute ethanol, potassium chloride, and formic acid were purchased from Merck (Germany). 2,2-diphenyl-1-picrylhydrazyl and L-ascorbic acid were from Sigma-Aldrich (Hungary). Furthermore, for the experiments, we used high purity deionized water (18MΩ cm), 2,4,6-tripyridyl-s-triazine (Acros Organics, Germany), hydrochloric acid (Biolab, Hungary), and anhydrous sodium carbonate (Riel-de Haën, Germany).

2.2. Equipment

An FSG 2502 vacuum sealer machine (Kitchenware, Austria) was used for the vacuum packing. The high purity deionized water was generated with a Zeneer Power I system (Human Corporation, Korea). All fruits were homogenized with a 2222 hand blender (Momert, Hungary). The extracts were prepared with a 358S laboratory shaker (Elpan, Poland). The samples were centrifuged using a Z206A laboratory centrifuge (Hermle, Germany). For the spectrophotometric analysis, a Spectroquant Pharo 100 (Merck, Germany) spectrophotometer was used.

2.3. Plant material

The completely ripened wild-growing fruits (Figure S1), namely European crab apple (*Malus sylvestris* (L.) Mill.), bilberry (*Vaccinium myrtillus* L.), yellow-, red-, and purple-skinned greengage (*Prunus domestica subsp. italica*), and quince (*Cydonia oblonga* Mill.) were picked during the 2021 harvest season (coordinates: 47°54′54.07″ N, 17°9′17.6″ E). Thus, greengages and blueberry were harvested in August, while European crab apple and quince were in September and October, respectively. The Kisalföld region is located in northwestern Hungary, and is characterized by a continental climate, with hot dry summers and mildly cold winters. The vacuum-packed samples were stored at -18 °C until analysis.

2.4. Extraction method development for extractable polyphenols from fruits

A one-factor-at-a-time methodology was used to define the best extraction condition considering three factors (independent variables). In initial screening experiments, various ethanol-water (EtOH:H_2O) mixtures (0:100, 20:80, 50:50, 80:20, and 100:0, v/v) were tested to investigate the effect solvent type on the total polyphenol content, monomeric anthocyanin, and antioxidant properties. All samples were extracted by maceration with shaking (180 rpm) at room temperature for 90 min. Then, the optimal solvent type was further used to determine the influence of extraction time (30, 60, 90, 120, and 150 min). Finally, we examined the impact of HCl and formic acid (HCOOH) at different percentages (0.1, 0.5, and 1.0%; v/v) on antioxidant properties as well as the total phenolic content and monomeric anthocyanin of fruit extracts. Preliminary experiments with purple-skinned greengage were used to determine the appropriate sample weight: solvent ratio (1:5 g/mL); therefore, we used 3.0 g of fruit and 15 mL of solvent in each case. After extraction, samples were centrifuged at 6000 rpm for 15 min, and then supernatants were collected and directly used for further analysis. If necessary, the supernatants of fruit extracts were diluted with high purity deionized water to an appropriate concentration to obtain an absorbance value in the linear range of the calibration curve.

2.5. Determination of total phenolic content

The total phenolic content (TPC) of wild fruit extracts was measured using Folin-Ciocalteu reagent [19]. Briefly, the fruit extracts (50 μL) were reacted with 1.5 mL of water, 2.5 mL of Folin-Ciocalteu reagent (1:10 diluted with water), and 2 mL of sodium carbonate solution (7.5 g/100 mL). After 90 min of incubation at room temperature, the absorbance reading was measured against the blank solution at 725 nm. Gallic acid was used as a standard, and the results were expressed as milligram gallic acid equivalents per 100 g of fruit weight (mg GAE/100 g).

2.6. Antioxidant capacity methods

For the ferric ion reducing antioxidant power assay (FRAP), the procedure followed the method of Benzie and Strain [20] with some modifications. The stock solutions included 300 mM of acetate buffer (pH

3.6), 10 mM of TPTZ solution in 40 mM HCl, and 20 mM of $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 100 mL acetate buffer, 10 mL TPTZ solution, and 10 mL $FeCl_3.6H_2O$ solution and then warmed to 37 ± 1 °C before using. Fruit extracts (aliquot of 100 μL) were allowed to react with 3 mL of the working solution at 37 ± 1 °C for 4 min. The absorbance values were measured at 593 nm versus the blank. Results were expressed as mg of L-ascorbic acid equivalents per 100 g of fruit weight (mg AAE/100 g).

The DPPH radical scavenging activity was measured according to a modified method described by DiNardo et al. [21]. Briefly, fruit extracts (aliquot of 100 μL) were reacted with 3 mL of freshly prepared methanolic DPPH solution (0.1 mM). The mixture was incubated in the dark at room temperature for 30 min and the absorbance was read at 517 nm. The inhibition percentage (IP) was calculated as follows $[(A_B - A_S)/A_B] \times 100$, where A_B is the absorbance of the blank, and A_S is the absorbance of the sample. According to IP, the antioxidant activity of each sample was calculated and expressed as L-ascorbic acid equivalents per 100 g of fruit weight (mg AAE/100 g).

2.7. Total monomeric anthocyanin

The total monomeric anthocyanin (TMA) content of bilberry, purple-skinned greengage, red-skinned greengage, and European crab apple was measured using a pH-differential protocol [22]. Briefly, two dilutions of the same sample were prepared by adding 0.5 mL of fruit extract to 4.5 mL of potassium chloride buffer (0.025 M, pH 1.0) and to 4.5 mL of

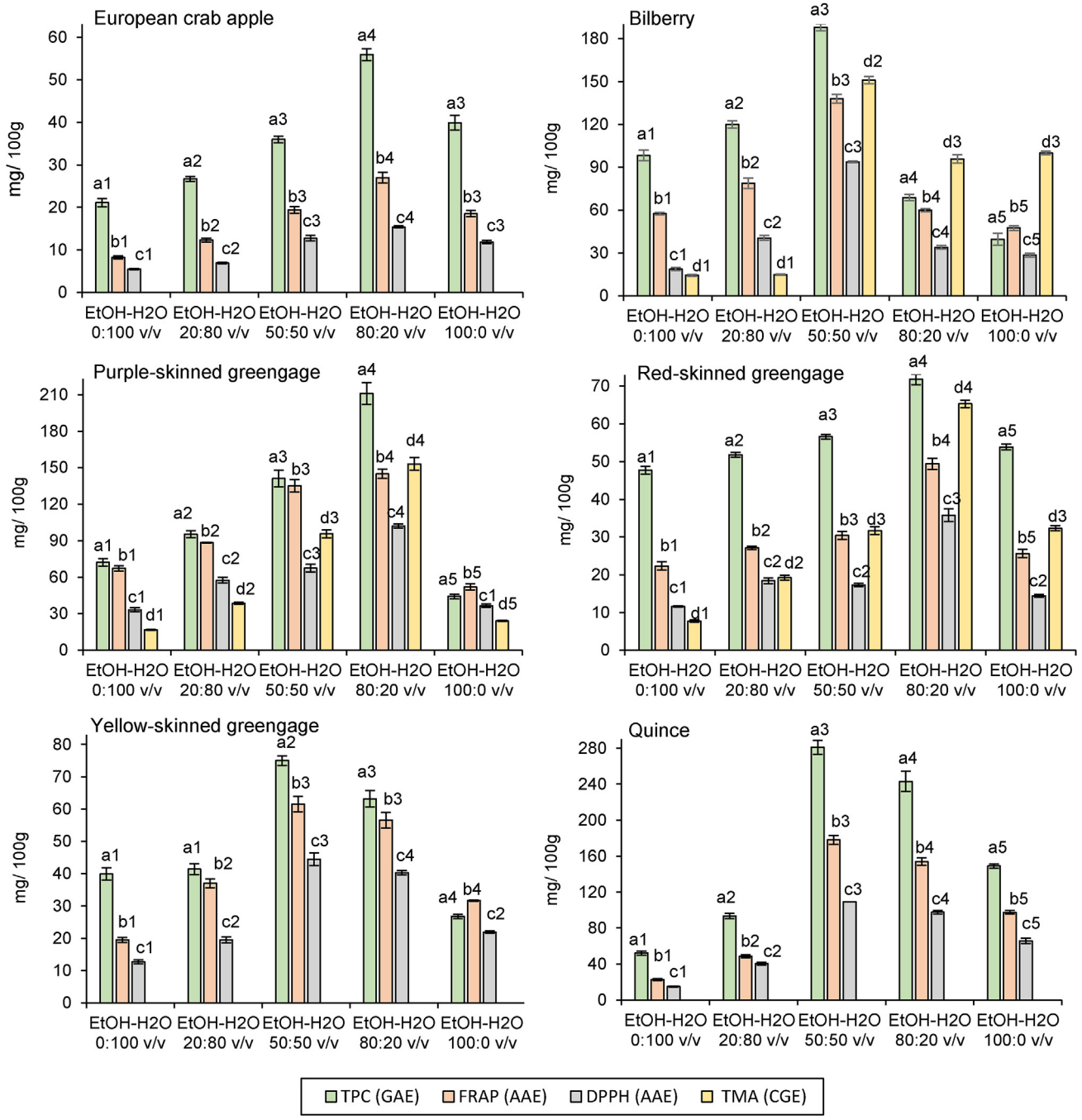

Figure 1. Effect of ethanol content on the final total phenolic content, antioxidant activities, and total monomeric anthocyanin content of six different wild-growing fruits. Data presented are mean values ±SD (n = 3). Different letters denote significant differences ($p \leq 0.05$). GAE: gallic acid equivalent, AAE: ascorbic acid equivalent, CGE: cyanidin 3-glucoside equivalent.

Table 1. Effect of extraction time on the final total phenolic content, antioxidant activities, and total monomeric anthocyanin content of six different wild-growing fruits.

Fruit	Extraction time (min)	TPC (mg GAE/100 g)	FRAP (mg AAE/100 g)	DPPH (mg AAE/100 g)	TMA (mg CGE/100 g)
European crab apple	30	39.5 ± 1.38^{a}	18.3 ± 0.49^{a}	10.1 ± 0.83^{a}	nd
	60	43.8 ± 1.47^{b}	21.1 ± 0.76^{b}	15.5 ± 0.24^{b}	nd
	90	55.9 ± 1.36^{c}	27.0 ± 1.25^{c}	20.1 ± 0.42^{c}	nd
	120	54.0 ± 1.56^{c}	25.2 ± 1.03^{c}	18.8 ± 0.25^{c}	nd
	150	53.5 ± 1.56^{c}	24.8 ± 0.91^{c}	18.6 ± 0.57^{c}	nd
Bilberry	30	138 ± 6.57^{d}	104 ± 3.65^{d}	76.2 ± 3.71^{d}	79.5 ± 2.31^{a}
	60	188 ± 3.89^{e}	142 ± 1.73^{e}	99.4 ± 1.30^{e}	175 ± 1.98^{b}
	90	183 ± 6.97^{e}	138 ± 3.09^{e}	93.7 ± 0.69^{f}	151 ± 2.62^{c}
	120	180 ± 1.97^{e}	133 ± 2.63^{ef}	90.0 ± 1.30^{f}	149 ± 2.98^{c}
	150	175 ± 2.49^{ef}	129 ± 3.45^{f}	84.7 ± 1.63^{fg}	118 ± 4.25^{d}
Purple-skinned greengage	30	151 ± 7.22^{d}	139 ± 0.47^{e}	95.4 ± 3.12^{ef}	153 ± 5.30^{c}
	60	203 ± 6.71^{g}	141 ± 2.52^{e}	101 ± 2.78^{e}	193 ± 0.33^{e}
	90	211 ± 9.00^{g}	145 ± 3.86^{f}	102 ± 1.66^{e}	225 ± 1.99^{f}
	120	218 ± 4.50^{g}	127 ± 2.28^{g}	98.0 ± 0.83^{e}	181 ± 6.20^{g}
	150	207 ± 4.58^{g}	107 ± 1.64^{d}	86.0 ± 1.25^{h}	136 ± 5.28^{c}
Red-skinned greengage	30	56.6 ± 1.24^{c}	43.8 ± 1.35^{h}	26.2 ± 0.45^{i}	56.8 ± 0.49^{h}
	60	59.9 ± 2.30^{c}	43.2 ± 0.89^{h}	31.8 ± 1.26^{j}	63.0 ± 2.62^{h}
	90	71.8 ± 1.40^{h}	49.4 ± 1.46^{i}	35.8 ± 1.69^{k}	65.3 ± 0.99^{hi}
	120	72.4 ± 3.15^{h}	42.5 ± 1.11^{h}	35.5 ± 0.50^{k}	60.6 ± 2.30^{h}
	150	63.9 ± 2.99^{i}	39.5 ± 1.08^{hj}	33.1 ± 0.95^{jk}	54.6 ± 0.33^{hj}
Yellow-skinned greengage	30	73.3 ± 2.01^{h}	51.5 ± 2.39^{i}	36.2 ± 1.25^{k}	nd
	60	84.4 ± 2.16^{j}	63.5 ± 1.54^{k}	45.6 ± 0.74^{l}	nd
	90	75.0 ± 1.47^{h}	61.5 ± 2.42^{k}	44.5 ± 1.95^{l}	nd
	120	68.1 ± 2.43^{hi}	49.8 ± 0.48^{i}	34.2 ± 0.17^{l}	nd
	150	65.7 ± 0.58^{i}	43.4 ± 1.88^{h}	30.2 ± 0.90^{km}	nd
Quince	30	259 ± 12.6^{k}	132 ± 4.41^{eg}	106 ± 3.28^{e}	nd
	60	267 ± 8.93^{k}	146 ± 5.96^{el}	104 ± 3.36^{e}	nd
	90	281 ± 7.62^{k}	178 ± 4.93^{m}	109 ± 1.38^{e}	nd
	120	263 ± 6.65^{k}	171 ± 2.16^{m}	101 ± 1.97^{e}	nd
	150	248 ± 4.63^{kl}	158 ± 5.87^{l}	98.7 ± 1.16^{e}	nd

Different letters denote significant differences ($p \leq 0.05$), nd: not detected. Data presented are mean values ±SD ($n = 3$).

sodium acetate buffer (0.4 M, pH 4.5), respectively. After 15 min incubation at room temperature, the absorbance was measured at 520 and 700 nm versus the high purity deionized water. Results were expressed as mg cyanidin 3-glucoside equivalents per 100 g of fruit weight (mg CGE/100 g). A molar absorption coefficient of 26.900 L/mol/cm and a molecular weight of 449.3 g/mol were used to calculate the total anthocyanin concentration of the extract.

2.8. Statistical analysis

All measurements were performed in triplicate, and the results are expressed as the mean ± standard deviation (SD). Analyses of variance (ANOVA) followed by the Tukey post hoc test were used to compare the significant difference in the data. Differences were considered statistically significant when $p < 0.05$. The statistical analyses were carried out using Microsoft Excel 2013 software.

3. Results and discussion

3.1. Extraction solvent evaluation

Although methanolic extraction may obtain good phenolic recovery [23, 24, 25], it endangers human health. Since ethanol is categorized as GRAS (generally recognized as safe) solvent, it may be a good choice for extracting natural ingredients from fruits [21]. Hence, in this work, extraction was performed on the fruits using different EtOH–H_2O mixtures. The investigation of the effect of ethanol content was carried out through TPC, antioxidant properties (FRAP, DPPH), and TMA contents (Figure 1).

As shown in Figure 1 all fruit extracts contained phenolic compounds, and the content of these ingredients varied according to ethanol content. For quince, a 438% (from 52.2 to 281 mg GAE/100g) increase in the TPC is indicated when the ethanol concentration was increased from 0 to 50%. A similar tendency was observed for yellow skinned-greengage (88.0%, from 39.9 to 75.0 mg GAE/100 g) and bilberry (91.3%, from 98.3 to 188 mg GAE/100 g). Moreover, a significant TPC increase was recorded for purple-skinned greengage (193%, from 72.1 to 211 mg GAE/100 g), European crab apple (165%, from 21.1 to 55.9 mg GAE/100g), and red-skinned greengage (50.5%, from 47.7 to 71.8 mg GAE/100 g) when the ethanol concentration was increased from 0 to 80%. Our results are partly consistent with those obtained by Dumitraşcu [26], who found that 50–70 EtOH–H_2O mixtures were the best solvent systems for phenolics from cornelian cherry fruits.

The antioxidant properties of the six fruits were evaluated using FRAP and DPPH assay. Our study has shown that EtOH–H_2O mixtures were significantly ($p < 0.05$) more effective than the water or pure ethanol solvent system used for extraction (Figure 1). In general, the antioxidant activity of fruit extracts increased with the increasing concentration of ethanol. For FRAP assay, the highest antioxidant activity levels were measured when using 80:20 (v/v) EtOH–H_2O mixture for European crab apple (27.0 ± 1.25 mg GAE/100g) and red-skinned greengage (49.4 ± 1.46 mg GAE/100g). Furthermore, the yellow-skinned greengage extracts obtained by 50:50 (v/v) and 80:20 (v/v) EtOH–H_2O mixtures did not show a significant difference ($p < 0.05$). At the same time, the antioxidant properties of bilberry and quince decreased when the ethanol concentration was higher than 50%. Similar tendencies were also observed for the DPPH assay (Figure 1). This research was also consistent with Insang et al. [27], who reported that when ethanol concentration

Table 2. Effect of acidity on the final total phenolic content, antioxidant activities, and total monomeric anthocyanin content of six different wild-growing fruit.

Fruit	Acid content (v/v%)	TPC (mg GAE/100g)		FRAP (mg AAE/100g)		DPPH (mg AAE/100g)		TMA (mg CGE/100g)	
		HCl	HCOOH	HCl	HCOOH	HCl	HCOOH	HCl	HCOOH
European crab apple	0.0	55.9 ± 1.36^{a}		27.0 ± 1.25^{a}		20.1 ± 0.42^{a}		nd	
	0.1	59.2 ± 2.79^{a}	57.3 ± 1.19^{a}	32.0 ± 1.28^{b}	28.9 ± 0.52^{a}	21.0 ± 0.98^{a}	22.5 ± 0.57^{a}	19.6 ± 0.66^{a}	12.0 ± 0.33^{c}
	0.5	68.4 ± 1.25^{b}	61.2 ± 2.64^{ac}	40.2 ± 1.37^{c}	30.5 ± 0.32^{ab}	nr	26.9 ± 1.28^{b}	22.0 ± 0.66^{a}	13.1 ± 0.66^{c}
	1.0	63.4 ± 1.62^{c}	70.0 ± 2.91^{b}	43.2 ± 0.81^{c}	37.3 ± 1.44^{cd}	nr	28.8 ± 1.05^{b}	29.6 ± 1.33^{b}	13.8 ± 0.33^{c}
Bilberry	**0.0**	**188 ± 3.89^{d}**		**142 ± 1.73^{e}**		**99.4 ± 1.30^{c}**		**175 ± 1.98^{d}**	
	0.1	203 ± 2.96^{e}	208 ± 1.94^{e}	220 ± 8.46^{f}	177 ± 4.99^{i}	181 ± 1.89^{d}	134 ± 5.78^{e}	317 ± 5.29^{e}	203 ± 2.19^{g}
	0.5	252 ± 2.97^{f}	220 ± 2.94^{h}	257 ± 8.07^{g}	213 ± 5.06^{h}	nr	148 ± 3.50^{f}	346 ± 5.65^{e}	245 ± 1.64^{f}
	1.0	193 ± 4.04^{g}	218 ± 3.33^{h}	197 ± 4.21^{h}	197 ± 3.49^{hj}	nr	169 ± 4.68^{g}	246 ± 5.62^{f}	261 ± 2.62^{f}
Purple-skinned greengage	**0.0**	**211 ± 9.00^{eh}**		**145 ± 3.86^{e}**		**102 ± 1.66^{c}**		**225 ± 1.99^{h}**	
	0.1	302 ± 2.92^{i}	216 ± 3.15^{eh}	155 ± 0.67^{e}	143 ± 5.62^{eg}	144 ± 4.03^{f}	112 ± 2.81^{h}	237 ± 3.92^{h}	231 ± 3.29^{h}
	0.5	377 ± 9.43^{j}	284 ± 8.91^{k}	170 ± 0.38^{f}	198 ± 4.56^{h}	nr	154 ± 2.91^{f}	267 ± 0.33^{i}	245 ± 7.92^{fh}
	1.0	370 ± 7.24^{j}	333 ± 2.41^{l}	167 ± 0.68^{f}	231 ± 4.59^{k}	nr	166 ± 6.31^{g}	246 ± 1.31^{fh}	248 ± 5.94^{fh}
Red-skinned greengage	**0.0**	**71.8 ± 1.40^{b}**		**49.4 ± 1.46^{l}**		**35.8 ± 1.69^{i}**		**65.3 ± 0.99^{j}**	
	0.1	81.2 ± 0.98^{m}	73.0 ± 1.68^{b}	50.9 ± 1.70^{l}	51.7 ± 1.74^{l}	39.6 ± 0.91^{i}	43.9 ± 2.84^{j}	97.9 ± 0.33^{k}	67.1 ± 3.30^{j}
	0.5	88.3 ± 1.90^{n}	77.5 ± 0.77°	51.2 ± 1.91^{l}	62.7 ± 2.56^{m}	nr	50.6 ± 0.95^{k}	96.1 ± 1.31^{k}	81.5 ± 2.97^{l}
	1.0	91.1 ± 2.35^{n}	69.7 ± 1.56^{b}	54.3 ± 0.68^{l}	75.9 ± 2.14^{n}	nr	59.5 ± 0.60^{l}	72.6 ± 3.95^{j}	86.8 ± 3.62^{l}
Yellow-skinned greengage	**0.0**	**84.4 ± 2.16^{m}**		**63.5 ± 1.54^{m}**		**45.6 ± 0.74^{jk}**		**nd**	
	0.1	89.6 ± 1.78^{n}	87.3 ± 1.95^{n}	64.8 ± 2.81^{m}	57.6 ± 2.72^{p}	47.7 ± 0.80^{jk}	40.0 ± 1.27^{ij}	nd	nd
	0.5	95.3 ± 0.81^{qn}	120 ± 2.12^{r}	67.4 ± 1.61^{m}	92.4 ± 1.22^{q}	nr	63.4 ± 2.25^{m}	nd	nd
	1.0	121 ± 3.75^{r}	93.2 ± 3.89^{nq}	80.3 ± 3.19°	67.9 ± 2.23^{m}	nr	45.8 ± 1.34^{j}	nd	nd
Quince	**0.0**	**281 ± 7.62^{k}**		**178 ± 4.93^{i}**		**109 ± 1.38^{h}**		**nd**	
	0.1	445 ± 16.1^{s}	349 ± 6.94^{l}	349 ± 7.49^{r}	333 ± 10.6^{r}	329 ± 10.4^{n}	210 ± 6.04°	nd	nd
	0.5	475 ± 17.5^{s}	351 ± 8.92^{l}	384 ± 11.2^{s}	351 ± 4.03^{r}	nr	212 ± 5.93°	nd	nd
	1.0	510 ± 4.87^{s}	332 ± 6.83^{l}	395 ± 11.5^{s}	343 ± 14.1^{r}	nr	220 ± 2.67°	nd	nd

Different letters denote significant differences ($p \leq 0.05$), nd: not detected, nr: not relevant. Data presented are mean values ±SD (n = 3).

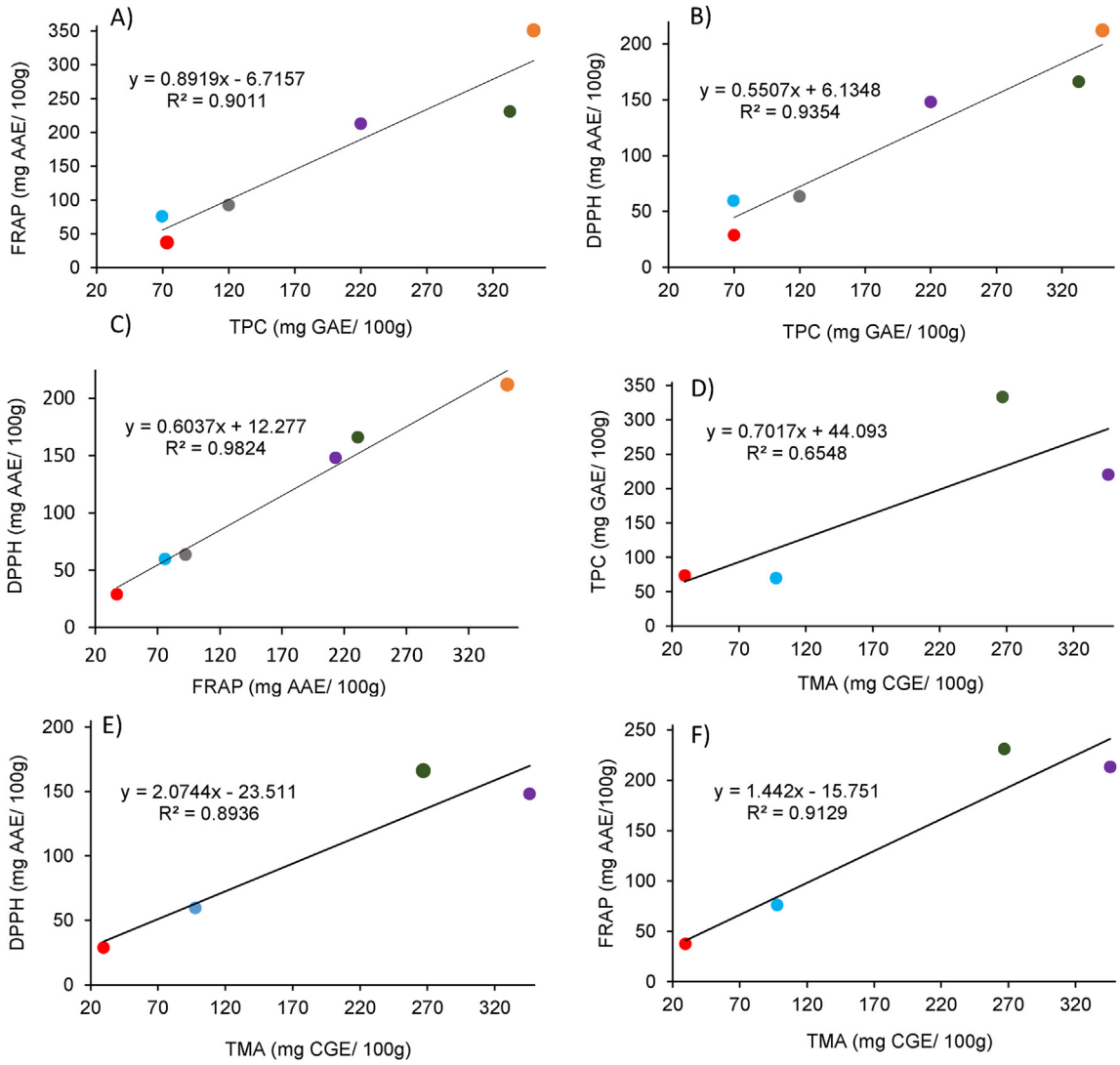

Figure 2. Correlation analysis (A) Correlation between TPC and FRAP values of wild fruits (B) Correlation between TPC and DPPH values of wild fruits (C) Correlation between FRAP and DPPH values of wild fruits (D) Correlation between TMA and TPC values of wild fruits (E) Correlation between TMA and DPPH values of wild fruits (F) Correlation between TMA and FRAP values of wild fruits. Different colors denote European crab apple (red), bilberry (purple), yellow-skinned greengage (grey), red-skinned greengage (blue), purple-skinned greengage (dark green), and quince (orange).

was increased above 60%, the antioxidant activity of mulberry tended to decrease.

The effect of ethanol concentration on the TMA extraction efficiency was evaluated, as well. 80:20 (v/v) EtOH–H_2O mixture exhibited the significant capability for extracting TMA for greengages (Figure 1), while TMA yield from bilberry (151 ± 2.62 mg CGE/100g) was the highest with 50:50 (v/v) mixture of ethanol. Similar results were reported by Oancea, Stoia, and Coman [28], where 50:50 (v/v) EtOH–H_2O mixture showed more efficiency (149 mg CGE/100g) than other aqueous ethanolic mixtures and water for extraction of TMAs from blueberry.

The solvent polarity plays a crucial role in the appropriate recovery of phenolic compounds. It is well known that phenolics are often most soluble in organic solvents less polar than water [29]. As shown in Figure 1, our study also supported this general fact. Solvent systems with a lower (EtOH) or excessively high (H_2O) polarity index were not appropriate for the higher response of natural antioxidants recovery from wild fruits. From the results shown in Figure 1, it is evident that a certain degree of increase in the solvent polarity enhances the solubility of antioxidant compounds from fruits. All in all, these observations suggest that wild fruits generally contain phenolic compounds of intermediate polarity.

3.2. Extraction time evaluation

The choice of the best extraction time is crucial to reducing energy consumption and the costs of the extraction process. Extraction time also had a significant effect ($p < 0.05$) on the extraction efficiency (Table 1). For instance, the TPC of European crab apple increased by 41.5% (from 39.5 mg GAE/100g to 55.9 mg GAE/100g) when the extraction time was increased from 30 min to 90 min. Our results are in accordance with those of Reis et al. [30], who demonstrated that 90 min was the best time for extracting phenolic compounds from apple pomace. A similar trend was seen for purple-skinned greengage (39.7%, from 151 mg GAE/100g to 211 mg GAE/100g), red-skinned greengage (2.69%, from 56.6 mg GAE/100g to 71.8 mg GAE/100g), and quince (8.49%, from 259 mg GAE/100g to 281 mg GAE/100g). Rather et al. [31] maximized the recovery of phenolic compounds from quince using surface response methodology (RSM). They found that the phenolic compounds may extract in a shorter time (20 min) at higher temperatures (65 °C). It is well known that longer extraction times may increase the possibility of phenolic degradation [32]. In this study, this phenomenon was also observed (Table 1).

The extraction time affected the antioxidant activity determined by the two different methods differently. Overall, in both antioxidant capacity assays, increased antioxidant activity were observed when the extraction time was increased. In the case of European crab apple, purple-skinned greengage, red-skinned greengage, and quince, the highest antioxidant activities for both DPPH and FRAP were found at 90 min. A similar tendency was observed for bilberry and yellow-skinned greengage at 60 min of extraction time. Also, the measured values for both DPPH and FRAP decreased significantly ($p < 0.05$) for most fruits after 120 min of extraction time, which indicated that longer extraction time decreases antioxidant activity (Table 1). Our results also show that TMA contents of purple- and red-skinned fruits were higher with increasing extraction time. The highest change was seen for bilberry (120%, from 79.5 ± 2.31 mg CGE/100g to175 ± 1.98 mg CGE/100g), followed by purple-skinned greengage (47.0%, from 153 ± 5.30 mg CGE/100g to 225 ± 1.99 mg CGE/100g), and red-skinned greengage (15.0%, from 56.8 ± 0.49 mg CGE/100g to 65.3 ± 0.99 mg CGE/100g). In contrast to our findings, Bamba et al. [33] found that TMA contents of blueberry pomace after sonication for 90 min was significantly higher than after 30 and 60 min.

3.3. Acidified solvent evaluation

We further investigated the effect of acidity on extraction efficiency because several studies showed that the solvent acidification may play a crucial role in increasing phenolic solubility [34, 35]. TPC, FRAP, DPPH, and TMA values are presented in Table 2 for the two acids tested.

Changing the acid concentration did cause a significant difference ($p < 0.05$) in the TPC for each of the fruits. In the case of European crab apple (69.7 ± 3.47 mg GAE/100g) and bilberry (252 ± 2.97 mg GAE/100g), the highest TPC was obtained for EtOH–H_2O mixtures with a content of 0.5% HCl. At the same time, the addition of 1.0% HCl to 80:20 (v/v) and 50:50 (v/v) EtOH–H_2O mixtures resulted in better TPC for red-skinned greengage (91.1 ± 2.35 mg GAE/100g) and quince (510 ± 4.87 mg GAE/100g), respectively. In addition, we did not find a significant difference ($p < 0.05$) for purple-skinned greengage when the HCl content was increased from 0.5% (377 ± 9.43 mg GAE/100g) to 1.0% (370 ± 7.24 mg GAE/100g). Our results also revealed that the acidification with HCl had a significant effect ($p < 0.05$) on the antioxidant properties of fruit extracts. However, it is also crucial to note that the acidity of the medium can affect the result, regardless of the actual composition of the sample [36]. This tendency may be particularly noticeable when extracts are used directly for measurement after extraction. A similar observation was also made during the application of the DPPH method, when HCl was used at a higher (0.5 and 1.0%) concentration (data not shown). It was clear that a strong pH skewed the results obtained by DPPH. Although a similar phenomenon was not seen for the FRAP and Folin-Ciocalteu assay, we repeated our studies with an organic acid (HCOOH) weaker than HCl. The DPPH radical scavenging activity for fruit extracts varied according to the acid concentration used. Except for yellow-skinned greengage and European crab apple, the highest DPPH radical scavenging activity was measured with 1.0% HCOOH. Further, similar results were observed with FRAP assay for European crab apple, purple-skinned greengage, and red-skinned greengage (Table 2).

When comparing the results obtained with two different acids, it can be seen that HCl gives better TPC and TMA recoveries. Our results showed that the addition of HCl to extraction solution at lower concentrations (0.1 and 0.5%) allows better TMA recovery than HCOOH in the highest concentration (1.0%). This is likely due to the fact that a stronger acid hydrolysis step may help the release of these compounds from plant cell walls [37]. However, acid concentration may cause a partial degradation of TMA [38]. We found that 1.0% HCl addition significantly decreased the TMA concentrations of bilberry, red-skinned greengage, and purple-skinned greengage (Table 2). A similar observation was also seen for the results of TPC. As shown in Table 2, 0.1% HCl resulted in higher TPC recoveries than 0.1% or 0.5% HCOOH for red-skinned greengage and quince. At the same time, we did not find a significant difference ($p < 0.05$) between 1.0% HCl (121 ± 3.75 mg GAE/100g) and 0.5% HCOOH (120 ± 2.12 mg GAE/100g) when measuring TPC of yellow-skinned greengage. A similar observation was shown for European crab apple. However, in this case, the yields obtained with 1.0% HCOOH (70.0 ± 2.91 mg GAE/100g) and 0.5% HCl (68.4 ± 1.25 mg GAE/100g) showed no significant difference ($p < 0.05$). Additionally, the results of the antioxidant activity measurements show that HCOOH is safer to use during extraction - even at higher concentration (up to 1.0%) - than HCl at a lower concentration (suggested use up to 0.1%).

3.4. Correlation of TPC and TMA to antioxidant properties

As shown in Figure 2C, the FRAP values of the six wild fruit extracts were highly correlated with the DPPH values ($R^2 = 0.98$). This observation indicates that components responsible for reducing oxidants were consistent with those scavenging free radicals in fruit extracts. In addition, a positive correlation was observed between TPC and antioxidant values (Figure 2A, B). At the same time, the determination coefficient (R^2) was higher between TPC and DPPH activity ($R^2 = 0.94$) than that of TPC and FRAP activity ($R^2 = 0.90$). Overall, the results of Figure 2A, B suggest that approximately 90% of the antioxidant activity in fruit extracts is due to the contribution of phenolic constituents. A similar trend in the correlation between TPC and FRAP values ($R^2 = 0.96$) of fourteen wild edible fruits was also presented by Lamien-Meda et al. [39]. Also, high correlations between the TPC and DPPH capacity ($R^2 = 0.86–0.92$) of nectarine, peach, and plum were found by Gil et al. [40].

We also examined the correlation of TMA to TPC and antioxidant activities (Figure 2D). As shown in Figure 2E and F, TMA was in significant correlation with FRAP ($R^2 = 0.91$) and DPPH ($R^2 = 0.89$). A considerable correlation was not detected between TMA and TPC ($R^2 = 0.65$).

4. Conclusion

An environmentally friendly MAC_S method has been established to extract natural antioxidants and anthocyanin colorants from edible wild fruits. In the case of European crab apple and purple-skinned greengage, the best conditions for TPC, FRAP, and DPPH were as follows: ethanol concentration, 80% (v/v); extraction time, 90 min; and the HCOOH concentration, 1% (v/v). For red-skinned greengage, the extraction parameters were the same as the above except for the acid concentration (0.5%; v/v) used. Moreover, our study suggested the following optimal extraction parameters for the other fruits: 50% ethanol concentration, 90 min extraction time, and 0.5% HCOOH concentration for quince; 50% ethanol concentration, 60 min extraction time, and 0.5% HCOOH concentration for bilberry and yellow-skinned greengage. Further, the colorimetric assay confirmed the beneficial effect of the increase of HCl concentration on the yield of TMAs from red and purple-skinned fruits. However, our study has some drawbacks that must be acknowledged. First, the chosen optimization methodology (OFAT) does not address interactions among product and process variables (e.g., extraction solvent mixture composition, temperature, time, and so on). Second, colorimetric procedures cannot identify the individual active ingredients — only the total level of phenolics or their groups can be determined. Besides, several factors may also cause interference during analysis. Third, the analyzed wild fruits are mainly found in European countries.

Declarations

Author contribution statement

Beatrix Sik: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zsolt Ajtony, Erika Lakatos, Rita Székelyhidi: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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