

Some Biochemical Characteristics of Fruit Samples of *Sambucus nigra* Genotypes from Northeastern Turkey

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Abstract

There are numerous wild edible fruit species in the world and they are important components of dietary utilization, local pharmacopoeia, cultural and religious practices of indigenous people. In this study, some important berry characteristics of seven seed propagated black elderberry (*Sambucus nigra*) genotypes sampled from Rize province, Turkey, were assessed. The genotypes exhibited a great diversity on most of the biochemical characteristics. Berries of genotypes showed moderate Vitamin C content ranged from 26 to 38 mg per 100 g fresh weight. Total phenolic and total anthocyanin content in fruits of the *S. nigra* genotypes has also high value ranged from 360 to 447 mg gallic acid equivalent (GAE) per 100 g fresh weight and 307 to 412 mg cyanidin-3-glucoside equivalent per 100 g fresh berry, respectively. Antioxidant activity was found between 5.28 and 7.11 mmol trolox equivalent per 100 g fresh weight basis. Citric acid and fructose determined as major organic acid and sugar for all genotypes. For its high vitamin C, total anthocyanin and total phenolic content and high antioxidant activity of fruits of wild grown *S. nigra* may have provide many health benefits and can be domesticated to establish orchards in Turkey.

Keywords: biochemical diversity; content; elderberry; less known fruits

Introduction

Horticultural plants present numerous types of species, cultivars, genotypes and accessions, occurring worldwide. The food industry is currently searching for new functional foods and nutraceuticals to help meet the demand presented by the consumers of natural, immunity-boosting and health-promoting plant based food product (Ercisli *et al.*, 2011; Sengul *et al.*, 2011; Zia-Ul-Haq *et al.*, 2014).

Turkey, located between junctions of three important plant gene centers, exhibited a rich diversity of wild edible fruits. Wild edible fruits constitute major component of food sources and livelihood in particular for rural peoples for employment and income generation in inner, eastern and northern part of Turkey. Most of the wild edible fruits have also unique aroma and taste, which affects product quality (Ercisli *et al.*, 2008; Vijayan *et al.*, 2008).

Almost all wild edible fruits are naturally grown and gathered by rural people during maturation period from

varied habitats including natural forests, pastures, agricultural fields, human disturbed areas such as roadsides and wastelands and utilization of certain wild plant foods is integral to their socio-cultural life (Cuce and Sokmen, 2017). They are consumed as substitute for staple food. In particular inner, northern and eastern part of Turkey, many rural families sells wild food plants in local market to generate cash income (Ercisli *et al.*, 2008).

Sambucus nigra (black elderberry) is native to most of European and North American countries and generally known as medicinal plant. Because the flavour of the raw elderberry fruit is not acceptable to all tastes, though when cooked it makes delicious jams, preserves, pies etc. The dried elderberry fruit are less bitter. Elderberry fruit is about 8 mm in diameter and is borne in large clusters in late summer to early autumn. They are often used to make a sparkling wine. Sweet tea is made from the dried elderberry flowers (Veberic *et al.*, 2009; Maja-Mikulic *et al.*, 2016).

In literature, limited information is available about biochemical diversity of *Sambucus nigra* genotypes. Thus, in this study, we aimed to determine and compare some important biochemical features of *Sambucus nigra* genotypes naturally growing in Rize province located in Northeastern Turkey.

Materials and Methods

Plant material

Commercially ripe fruits of *Sambucus nigra*, naturally grown in Rize province situated Northeastern parts of Turkey were harvested. In commercial harvest period, black colored fruits were collected in autumn during the year 2017 from 7 pre-selected genotypes that show higher yield, pest and diseases free and more attractive bigger fruits.

Biochemical and bioactive composition

Sample preparation and extraction

For the organic acids, specific sugars, total phenolic, total anthocyanin contents and total antioxidant capacity analyses, harvested fruits immediately brought to the cooler state and stored at -20 °C until further analyses. During analysis, the cold fruits were taken and thawed to room temperature. A laboratory blender used to homogenise fruit samples (100 g lots of fruits per genotypes) and a single extraction procedure (taking 3 g aliquots and transferring it inside tubes and extracted for one hour with 20 mL buffer including acetone, water (deionized), and acetic acid (70:29.5:0.5 v/v) (Singleton and Rossi, 1965).

Extraction of sugars and organic acids

Five grams samples slurries were mixed with deionized water or metaphosphoric acid (2.5%) for the analysis of individual sugar and organic acid, respectively. The obtained homogenates were centrifuged at 10000 rpm for 10 min. The samples were filtered into HPLC vials using 0.45 µm PTFE membrane filter for analysis. All HPLC solvents were sonicated. All samples and corresponding standard injection were repeated three times and the mean values were calculated.

Chromatographic conditions

The Perkin Elmer HPLC system controlled by software Totalchrom navigator (version 6.2.1), consists of a pump and UV detector was used for analysis of the samples. Organic acids separation and determination were performed as per the method reported by Shui and Leong (2002). The sugars were determined using the method of Bartolome *et al.* (1995) with help of HPLC with refractive index (RI) detector. The separation was carried out on SGE SS Exsil amino column (250 × 4.6 mm ID). The isocratic elution was performed using acetonitrile (80%) and deionised water (20%) with a flow rate of 0.9 mL/min. The column was operated at 30 °C and the sample injection volume was at 20 µL. Quantification of organic acids and sugars were performed against the reference standards.

Total phenolic contents

Total phenolic contents (TPC) of the samples were evaluated using the method of Singleton and Rossi (1965). In this procedure, each extract (1 mL) was mixed with Folin-Ciocalteu's reagent and water 1:1:20 (v/v). The samples were incubated for 8 min. Then the addition of sodium carbonate (10 mL) having a concentration of 7% (w/v) was performed. After incubation for 2 h, the absorbance at 750 nm was measured. Total phenolic contents were calculated against the reference standard

calibration curve of gallic acid. The TPC was expressed as mg of gallic acid equivalents (GAE) per 100 g of sample (fresh weight (FW) basis).

Total anthocyanin content

Total anthocyanins contents were measured using a pH differential method of Giusti and Wrolstad (2001) with help of UV-visible spectrophotometer. The absorbance was measured both at 533 and 700 nm in buffers solution at pH 1.0 and 4.5. The total anthocyanins were calculated from the absorbance values and molar extinction coefficient value of 29,600. Total anthocyanins contents were expressed as mg of cyanidin-3-glucoside equivalent in per 100 g of fresh sample.

Ferric reducing antioxidant power assay

FRAP (Ferric reducing antioxidant power) assay was used for antioxidant capacity analysis. For this purpose, acetic fruit extract (50 µL), FRAP reagent (2.95 mL), acetate buffer (0.1 mol/L), TPTZ (10 mmol/L), and ferric chloride of 20 mmol/L (10:1:1 v/v/v) were used. The values of samples absorbance were compared with those of the reference standard calibration curves in the range of 10-100 µmol/L of Trolox was used to determine FRAP values of samples. The FRAP was expressed as mmol per 100 g of Trolox equivalent on the basis of the fresh weight of fruits (Benzie and Strain, 1996).

Statistical analysis

All samples were five times replications for each experiment. For analysis of variance, the obtained data were used for means calculation. Duncan multiple range tests were performed at the significant level of $P < 0.05$.

Results and Discussion

Ascorbic acid (Vitamin C), total phenolic content, total anthocyanin and total antioxidant capacity

Vitamin C (ascorbic acid), total phenolic content, the total anthocyanin, and total antioxidant capacity of fruit samples of *Sambucus nigra* genotypes are given in Table 1. The genotypes showed vitamin C content between 26 and 38 mg per 100 g fresh fruit weight and statistically significant differences ($p < 0.05$) are evident (Table 1). Previous studies indicated genotype/cultivar dependent vitamin C content of elderberry fruits. For example Mratinic and Fotiric (2007) reported vitamin C in elderberry fruit samples between 20-35 mg/100 ml in Serbia. In Denmark, vitamin C content in 13 elderberry cultivars was reported between 6-25 mg/100 ml (Kaack and Austed, 1998). In Czech Republic vitamin C was found average 26 mg per 100 g (Porpacz and Laszlo, 1984). Our values presented in Table 1 on vitamin C are between these limits.

The black elderberry genotypes are shown a wide variation on total phenolic content. Total phenolic content varied from 360 mg GAE per 100 g to 447 mg GAE per 100 g. Previous studies were also confirmed high total phenolic content of elderberry fruits. For example Lee and Finn (2007) reported 327-582 mg GAE/g total phenolic content in fresh *Sambucus nigra* fruits. Mikulic-Petkovsek *et al.*

(2016) reported total phenolic content of elderberry fruit ranged from 268 to 683 mg gallic acid equivalent (GAE) per 100 g fresh fruit in fruits of wild grown elderberry in Slovenia and they also found that total phenolic content is tightly connected with the contents of single phenolics. Our total phenolic content results are also in accordance with Lee and Finn (2007). Perkins-Veazie *et al.* (2015) reported total phenolics between 421-719 mg GAE per 100 g elderberry fresh fruits in USA.

Total anthocyanin content of elderberry genotypes statistically differed each other ($p < 0.05$) and varied from 307 mg to 412 mg cyanidin-3-glucoside equivalent per 100 g fresh fruits (Table 1). The major anthocyanins in black elderberry fruits were reported as cyanidin-3-glucoside and cyanidin-3-sambubioside (Veberic *et al.*, 2009). Veberic *et al.* (2009) reported total anthocyanin between 602-1265 mg per 100 g in fruits of elderberry genotypes, which indicated higher values than our samples. However, Bronnum-Hansen and Hansen (1983) found total anthocyanin content in 26 elderberry cultivars and genotypes ranged from 200 to 1000 mg cy-3-glu/100 g fresh fruits and Lee and Finn (2007) reported between 140 to 280 mg cy-3-glu/100 g fresh fruits. Perkins-Veazie *et al.* (2015) reported total anthocyanins between 85-385 mg cy-3-glu/100 g elderberry fresh fruits in USA.

The differences could be results of cultivars/genotypes used, genetic background, harvest period, different extraction methods, ecological conditions and sampling years. *Sambucus* is a diverse genus and there were incredible variation among species on most of the morphological and biochemical traits. These variations are more obvious in particular on total phenolic and anthocyanin content (Mikulic-Petkovsek *et al.*, 2016). These results indicate total anthocyanin richness of black elderberry fruits and high polyphenol content of *Sambucus nigra* comparable with high anthocyanin included fruits such as black mulberry, cranberry, blueberry, black currant, blackberry and blue honeysuckle.

FRAP values of genotypes are shown in Table 1 and results indicated statistically significant differences ($p < 0.05$) among genotypes in the total antioxidant capacities. Total antioxidant capacity was between 5.28 and 7.11 mmol/100 g. Halvorsen *et al.* (2002) reported average FRAP value in elderberry genotype grown in Norway was 5.24 mmol/100 g. This value was 3.64 mmol/100 g for blueberry, 5.03 mmol/100 g for cranberry, 3.06 mmol/100

g for raspberry and 2.17 mmol/100 g for strawberry, respectively (Halvorsen *et al.*, 2002). The antioxidant capacity of *Sambucus nigra* fruit was quantified by a several methods, and all the results showed a very high antioxidant at very low concentrations (Veberic *et al.*, 2009).

Sugars

Sugar content of *Sambucus nigra* genotypes are shown in Table 2. We found significant differences ($p < 0.05$) in specific sugar contents among black elderberry genotypes, as presented in Table 2. The major sugar for all black elderberry genotypes was fructose and followed by glucose. The sucrose values of all genotypes were found to be the lowest (Table 2). Fructose content of genotypes ranged from 40.11 to 50.42 g per kg fresh fruit samples. Glucose and sucrose content were between 38.24-48.36 and 9.54-11.22 g per kg fresh fruit samples (Table 2). The sugar content of the elderberry fruits used in this study is comparable to data reported for *S. nigra* fruits (Veberic *et al.*, 2009; Thomas *et al.*, 2015; Maja-Mikolic *et al.*, 2016). A favorable fructose to glucose ratio (1:1) has been determined in elderberry fruits, which is in accordance to the report of Sadilova *et al.* (2009). The nutritional and health perspective, fruit with increased fructose levels should be favored by the consumers as this sugar tastes sweeter than glucose or sucrose and has a much lower glycemic index compared to other analyzed sugars (Atkinson *et al.*, 2008). Elderberry fruit accumulates comparably low levels of total sugars (47.19-109.16 g/kg FW) than frequently consumed fruit such as apples (128.2-191.55 g/kg FW) (Mikulic-Petkovsek *et al.*, 2007) and sweet cherry (177.91-242.87 g/kg FW) (Gundogdu and Bilge, 2012). Therefore, elderberry products (juice, syrups, jams etc.) represent an interesting supplement to human diet.

Organic acids

The organic acid contents of black elderberry fruits are shown in Table 3. As indicated in Table 3, black elderberry fruits had five major organic acids (citric, malic, tartaric, shikimic and fumaric acid). The concentration of citric, malic, tartaric, shikimic and fumaric acid ranged from 7.38 to 8.02 g/kg FW; 5.12 to 6.44 g/kg FW; 1.63 to 2.04 g/kg FW; 0.22 to 0.39 g/kg FW and 0.14 to 0.31 g/kg FW, respectively (Table 3).

Table 1. Vitamin C, total phenolics, total anthocyanins and antioxidant capacity in fruits of seven *Sambucus nigra* genotypes

Genotypes	Vitamin C (mg/100 g)	Total phenol (mg GAE/100 g)	Total anthocyanin (mg cy-3-glu/100 g)	FRAP (mmol/100 g)
R1	30c	447a	366bc	7.11a
R2	34b	424b	412a	6.84b
R3	26d	391cd	345c	5.86d
R4	29cd	410bc	328cd	6.65bc
R5	34b	360d	383b	5.28e
R6	38a	383cd	358bc	5.83d
R7	28cd	401c	307d	6.50c

Different letters indicate the statistical difference within the same column among genotypes at 5% level.

Table 2. Specific sugar content of *Sambucus nigra* genotypes (g/kg FW)

Genotypes	Sucrose	Fructose	Glucose
R1	10.05ab	42.37e	40.22bc
R2	10.76ab	40.11f	38.24c
R3	9.54b	44.34d	39.65bc
R4	9.86ab	45.36cd	43.41b
R5	10.41ab	50.42a	48.36a
R6	11.22a	48.73b	45.22ab
R7	9.73ab	46.44c	43.88ab

Different letters indicate the statistical difference within the same column among genotypes at 5% level.

Table 3. Organic acid content of *Sambucus nigra* genotypes (g/kg FW)

Genotypes	Citric	Malic	Tartaric	Shikimic	Fumaric
R1	7.69ab	5.44bc	1.77cd	0.22b	0.20ab
R2	7.81ab	5.12c	1.65d	0.39a	0.27ab
R3	7.90ab	5.51bc	1.80c	0.30ab	0.14b
R4	7.38b	5.70b	2.04a	0.33ab	0.22ab
R5	7.44ab	6.06ab	1.86bc	0.26ab	0.27ab
R6	8.02a	6.21ab	1.98ab	0.33ab	0.19ab
R7	7.59ab	6.44a	1.94b	0.37a	0.31a

Different letters indicate the statistical difference within the same column among genotypes at 5% level.

Mikulic-Petkovsek *et al.* (2016) determined four organic acids in the elderberry berries as citric acid, malic acid, shikimic acid and fumaric acid. They found that citric acid (3.11 to 4.81 g/kg FW) was the most abundant organic acid in all cultivars/selections, followed by malic acid and smaller concentrations of shikimic and fumaric acid. Our results are indicating that elderberry fruit is exceptionally rich in citric acid. This is an important parameter for processing purposes. Compared to apple, (Hofer *et al.*, 2005), sweet cherry (Usenik *et al.*, 2008) and sour cherry (Bonertz *et al.*, 2006), and justifies the leading role of the elderberry fruits for richness citric acid.

Conclusions

The results indicated that variation occurs for biochemical and bioactive characteristics in *Sambucus nigra* genotypes. To determine the superior genotypes of *Sambucus nigra* based on high bioactive content could increase the intake of health promoting compounds. These genotypes may be highly desirable in germplasm breeding programs to breed quality varieties of *Sambucus nigra* with high fruit size and antioxidant potential.

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