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Physico-chemical characteristics of some wild grown European elderberry (*Sambucus nigra* L.) genotypes

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ABSTRACT

Wild grown European elderberry (*Sambucus nigra*) plants are widespread in different parts of Turkey and have been used in folk medicine so a long time. Some selected physico-chemical characteristics such as berry weight, protein, pH, total acidity, soluble solid, reducing sugar, vitamin C, total antioxidant capacity (FRAP assay), total phenolic and total anthocyanins of four pre-selected wild grown European elderberry fruits were investigated. Significant differences on most of the chemical content were detected among the genotypes used. The genotype AR2 had the highest protein content (2.91%), while AR4 had the lowest protein content (2.68%). The genotypes with the highest total antioxidant capacity, total phenolic and total anthocyanin content were AR2 (6.37 mmol/100 g fw; 432 mg GAE/100 g fw and 283 mg cyaniding-3-glucoside/100 g fw). The results showed that European elderberry very rich in terms of health components.

KEYWORDS: anthocyanins, elderberry, total antioxidant, vitamin C

INTRODUCTION

A lot of epidemiological studies showed that consumption of diet rich horticultural plants including fruits, grapes and vegetables reduced risk of heart disease, cancer, and other chronic diseases (1, 2). A major benefit from such a diet may be increased consumption of antioxidants (3), including carotenoids, ascorbate, tocopherols, and red, blue, and purple pigments known as anthocyanins (4). Among horticultural plants, berries and small fruits such as blueberries (*Vaccinium* L. species), blackberries (*Rubus* L. hybrids), black currants (*Ribes nigrum* L.), sea buckthorn (*Hippophae rhamnoides* L.) and cornelian cherries (*Cornus mas* L.) are rich sources of dietary anthocyanins and antioxidants (5,6,7,8,9). Therefore these deep colored berries and small fruits are recognized as more healthy to human body (10).

Black or common elder (*Sambucus nigra*), also called elderberry, is a widespread species, that grows on sunlight-exposed locations in most parts of Europe, Asia, North Africa and the USA. (11). Elderberries are general grown wild in several countries in Europe and are cultivated on a small scale in some northern European countries. Elderberry fruits are seldom used for fresh consumption; mostly it is processed to concentrates and juices.

Recently, elderberries have received increased attention due to their high contents of anthocyanins, that are widely used as colour ingredients in various beverages, and which may also provide nutritional benefits. Anthocyanins, as well as other flavonoids, exhibit antioxidant, anticarcinogenic, immune-stimulating, antibacterial, antiallergic and antiviral properties; therefore, their consumption may contribute to prevention of several degenerative diseases such as

cardiovascular disease, cancer, inflammatory disease and diabetes (12). Caused by potential health benefits, the anthocyanin content in juices and extracts from elderberry fruits (*Sambucus nigra*) has also received attentions, which have been used in clinical studies (13-16).

The juice pressed from black elderberry fruit contains various bioactive, including antioxidants, anthocyanins, total phenolics, Vitamin A and C, protein, sugars, organic acids etc. (17,18). The elderberry fruits can also be made into jam, pies and sauces. All green parts are poisonous, containing cyanogening glycosides. The flowers may be used to make an herbal tea, which is believed to be remedy for colds and fever. In Turkey, elderberry bark, roots, stems, and fruits have been used particularly by local peoples living in rural areas as medicine and foods (19).

There is considerable amount of literature that examines the bioactive compounds in berry fruits such as blackberry, raspberry, strawberry, etc. However, there have been limited data on bioactive compounds of European elderberry fruit. Therefore, the purpose of this study was to determine the bioactive compounds in wild grown elderberry in Turkey, and also to investigate the possibilities of utilizing it as a valuable functional food ingredient or a resource for nutraceutical products in the future.

MATERIALS AND METHODS

Collection and preparation of elderberry fruit samples

A total four pre selected European elderberry (*Sambucus nigra* L.) genotypes naturally grown in Artvin district were used in investigation. Approximately 1 kg full mature fresh fruits per genotype were hand picked and quickly transport to laboratory in cold chain and fruit samples were analyzed immediately after harvesting. The fruits were mashed in a homegeniser and prepared for further analysis. Four replicates were used per analysis. The parameters were analysed were: Protein, soluble solid content (SSC), total acidity (TAc), pH, reducing sugar, ascorbic acid (AsA), total phenolics (TP), total anthocyanins (TA) and total antioxidant capacity (TAC).

Berry weight was determined by a digital balance. Soluble solid content expressed as % were determined by a digital refractometer (Kyoto Electronics, Model RA-250HE) at 22 °C. Total acidity (%) as citric acid, protein (%) and pH was determined by AOAC (20) method. Ascorbic acid and reducing sugar was quantified with the reflectometer set of Merck Co (Merck RQflex). Results were expressed as mg ascorbic acid (AsA) 100 ml and %, respectively.

Total anthocyanin (TA) analysis was determined by using pH differential method (21) using a UV spectrophotometer. Absorbance was measured at 520 and 700 nm in buffers at pH 1.0 and 4.5 using $A = (A_{535}-A_{700})_{pH\ 1.0} - (A_{535}-A_{700})_{pH\ 4.5}$ with a molar extinction coefficient of 29,600. Results were expressed as mg of cyanidin-3-glucoside equivalent in 100 g fresh weight (mg cy-3-glu/g fw) basis.

Total phenolic content (TP) was estimated using the Folin-Ciocalteu colorimetric method described by Ough and Amerine (22). Fruit phenolics were extracted from 10 g of fresh samples using 40 mL of 80 % (by volume) aqueous ethanol. The mixture was extracted (in water bath at 80 °C), kept for 20 min in inert atmosphere, and filtered through a Whatman filter paper using a Büchner funnel. Extraction of the residue was repeated under the same conditions. The filtrates were combined and diluted to 100 mL in volumetric flask with 80 % aqueous ethanol, and the obtained extract was used for determination of TP. The content of TP was measured as follows: 0.5 mL of diluted extract or standard solutions of gallic acid (20–500 mg/L) was added to a 50-mL volumetric flask containing 30 mL of ddH_2O, then 2.5 mL of Folin-Ciocalteu reagent were added to the mixture and shaken. After 5 min, 7.5 mL of 7 % Na_2CO_3 solution were added with mixing and the solution was immediately diluted to 50 mL with ddH_2O. After incubation at room temperature for 2 h the absorbance of the solution was measured at 750 nm. TP was expressed as mg GAE/100 g fw. The extract of total phenolics was also used for FRAP assay.

Total antioxidant activity (AA) was estimated by using ferric reducing ability of plasma (FRAP) assay (23). The assay was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid, and 20 mmol/L ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1, v/v/v) to form the FRAP reagent just prior to analysis. For each assay, laboratory duplicates from each replicate plus 2.97 mL of FRAP reagent and 30 mL of sample extract were mixed. After 30 min the absorbance of the reaction mixture at 593 nm was determined on a spectrophotometer. Results were expressed mmol Trolox equivalents per 100 g fresh weight basis.

Statistical analysis

The experiment was a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan multiple range test at $P<0.05$ significant level.

RESULTS AND DISCUSSION

Berry weight, protein, pH, total acidity, soluble solid content and reducing sugar in elderberry fruits

Table 1 shows average berry weight, protein, pH, total acidity, soluble solid and reducing sugars in elderberry genotypes. As indicated in Table 1, there were statistical differences in terms of these parameters, except berry weight and pH, among elderberry genotypes ($p<0.05$).

Average individual berry weight of elderberry genotypes were found between 0.14 (AR3) and 0.20 g (AR4). Previously average berry weight of European elderberries was found between 0.13 and 0.21 g (24) which supports our findings. SSC content within elderberry genotypes varied from 11.74% (AR3) to 12.62% (AR4). Lee and Finn (17) reported SSC content between 11.2–15.4% among 10 elderberry cultivars in USA. Our SSC results are in general were lower than this study. As well known a lot of factors including genotype, harvest time, environment strongly affects SSC content in fruits (24).

Total acidity, pH and reducing sugar of elderberry genotypes were between 1.07 to 1.24%; 3.94 to 4.12 and 6.17 to 7.01% respectively (Table 1). Lee and Finn (17) reported total acidity and pH values in elderberry cultivars were 0.53 to 1.43 and 3.80 to 4.50. Mratinic and Fotiric (25) reported reducing sugar in elderberry fruits between 5.60-6.20% which in accordance with our results.

Vitamin C, total antioxidant activity, total phenolic content and total anthocyanins in elderberry fruits

Vitamin C, total antioxidant activity, total phenolic and total anthocyanin content of elderberry genotypes are given in Table 2. Elderberry genotypes revealed statistical differences in terms of these parameters ($p<0.05$).

Vitamin C content of elderberry genotypes were between 28 (AR1) to 34 mg/100 ml (Table 2). Mratinic and Fotiric (25) reported vitamin C in elderberry fruit samples between 20-35 mg/100 ml. Vitamin C is a very important biological parameter. Kaack and Austed (26) found that vitamin C content in 13 different elderberry cultivars were between 6–25 mg/100 ml.

The content of total anthocyanins were ranging from 242 (AR4) to 283 (AR2) mg cy-3-glu/100 g fw within our *Sambucus nigra* genotypes (Table 2). The genotype seemed to influence the extent of total anthocyanin content accumulation in the berries. According to earlier reports, total anthocyanin content in 26 elderberry cultivars and genotypes ranged from 200 to 1000 mg cy-3-glu/100 g fw (27) and 140 to 280 mg cy-3-glu/100 g fw (17).

The results for total phenolics clearly showed that fruits of European elderberry (*Sambucus nigra*) (371-432 mg GAE/100 g fresh matter) had relatively high total phenolic content. Earlier, total phenolic content in elderberry fruits was reported which ranged from 327-582 mg GAE/g fresh matter (17). The production of a number of minor crops (*Vaccinium* L., *Ribes* L., and *Lonicera* L.) is expanding rapidly around the world and numerous papers have reported the berries of these to contain 14–593 mg/100 g total anthocyanin and 191–1790 mg/100g total phenolics in berries (28,29,30). Elderberry samples analyzed in this study, using identical methods, fall within those reported ranges.

The difference of the elderberry genotypes in terms of phenolics is supposed to its genetic derivation as well because all plants found same agroclimatic conditions. The genotypic effect within same fruit species on total phenolic content are well documented by several researches on mulberry (8) and cornelian cherry (9).

Table 1. Berry weight, protein, pH, total acidity, soluble solid content and reducing sugar content of elderberry genotypes

Genotypes	Berry weight (g)	Protein (%)	pH	Total acidity (%)	SSC (%)	Reducing sugar (%)
AR1	0.17^{NS}	2.73ab	3.94^{NS}	1.05c	12.27ab	7.01a
AR2	0.15	2.91a	4.12	1.07c	12.04ab	6.17b
AR3	0.14	2.86ab	4.07	1.24a	11.74b	6.85ab
AR4	0.20	2.68b	4.00	1.19b	12.62a	6.49ab

*Values in the same column with different lower-case letters are significantly different at $P<0.05$.

Table 2. Vitamin C, total anthocyanin, total phenolic and antioxidant activity of elderberry genotypes

Genotypes	Vitamin C (mg/100ml)	Total anthocyanin (mg cy-3-glu/100 g fw)	Total phenolic (mg GAE/100 g fw)	Antioxidant capacity (FRAP) (mmol/100 g fw)
AR1	28ab	271ab	408b	6.09b
AR2	34a	283a	432a	6.37a
AR3	31ab	256ab	384c	5.31c
AR4	29ab	242b	371d	5.04d

*Values in the same column with different lower-case letters are significantly different at $P<0.05$.

The antioxidant activity (FRAP assay) in elderberry fruits was between 5.04 to 6.37 mmol/100 g fw (Table 2). The order of antioxidant capacity expressed as mmol/100 g fw in FRAP assay within elderberry genotypes were AR2 (6.37)> AR1 (6.09)>AR3 (5.31)>AR4 (5.04), respectively (Table 1). Halvorsen et al. (31) found that FRAP value in elderberry genotype grown in Norway was 5.24 mmol/100 g. Small fruits and berries contain very high antioxidant capacity determined by FRAP assay. These included crowberry, wild blueberry, black currant, wild blackberry, wild strawberry, cultivated blackberry and cowberry/cranberry, which all contained between 5.03 and 9.17 mmol/100 g FRAP value. The cultivated varieties of blueberry and strawberry also contained high antioxidant concentrations, i.e., 3.64 and 2.17 mmol/100 g, respectively (31).

CONCLUSION

Folk medicine has been around for millennia exploiting first wild then cultivated plants to prevent or cure a myriad of illnesses. Among plant species, elderberries, particularly black one have been used for centuries throughout world to keep the evil spirits away, to prevent or cure numerous ailments and health problems. Elderberry medicinal potential comes from its antioxidant potential, a property shared by numerous phytochemicals. Human body uses antioxidants from plant origins to neutralize harmful free radicals and elderberry total antioxidant capacity is one of the highest of all the small fruits. This study is also confirmed that consumption of elderberry fruits is very important for human health.

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