Antioxidation capacity of fruit and flower extracts from American elderberry (*Sambucus canadensis*)

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Antioxidants are free radical scavengers that are commonly found in fruit and other plant organs. This study investigates the antioxidation potential of *Sambucus canadensis* (elderberry) flowers (n=6) and fruits (n=6) in order to determine their human health benefits. We used the oxygen radical absorbance capacity (ORAC) assay, which is commonly used as an index of total antioxidant capacity. Both flowers and fruits showed high ORAC values relative to other natural sources; the fruit values ($\bar{x}$= 213.1 ± 61.30) were higher than those found for flowers ($\bar{x}$= 83.0 ± 27.80). These findings highlight the importance of both fruit and flower parts as valuable antioxidant sources for humans.

INTRODUCTION

Antioxidants and Human Health

Over the past few decades, concerns about nutrition and aging have stimulated many people to seek out ways to incorporate health-promoting foods into their diets. These foods provide both basic nutrition and additional physiological benefits such as prevention of chronic diseases (Kaur & Kapoor, 2001). One group of foods that may provide such benefits do so through the capture of the free radicals that contribute to many human health problems including cardiovascular disease, atherosclerosis, chronic inflammation, cataracts, neurodegenerative diseases, and even cancer (Zhang, Lin Han, & Zhang, 2009; Rafat, Philip, & Muniandy, 2010). Our bodies release free radicals during oxidative stress linked with diet and lifestyle factors, including exposure to solar radiation, pesticides, air pollution, and pharmaceuticals (Limón-Pacheco & Gonsebatt, 2009).

Fruit and Flower Antioxidants

Antioxidants synthesized by many plants help delay or inhibit oxidation by free radicals in humans, which supports their use for disease prevention (Zhang, Lin Han, & Zhang, 2009). Overall antioxidant activity in plants is correlated with high production of flavonoids and other related polyphenols (Einbond et al., 2004; Kaur & Kapoor, 2001).

Anthocyanins, a subclass of flavonoids, are important flower and fruit pigments that function to attract pollinators and protect plant tissues from oxidation resulting from photosynthesis (Einbond et al., 2004). Environmental stress such as UV-B sunlight exposure increases the production of reactive oxygen species (Agati & Tattini, 2010). In response to these stress-related compounds, plants produce flavonoids and other phenolics that have the potential to reduce oxidative damage (Agati & Tattini, 2010). Plants can increase the content and activity of oxidant scavengers that behave as hydrogen-donating antioxidants using their hydroxyl groups (Grace & Logan, 2000).

These natural antioxidants are distributed in many plant organs including fruits, flowers, stems, leaves, and roots (De Pascual-Teresa & Sanchez-Ballesta, 2007), but plant organs with dark pigments such as purple, blue, or red typically contain the highest anthocyanin concentrations and therefore the highest antioxidation capacities (Einbond et al., 2004; Wu et al., 2004). Dark colors indicate that plant organs absorb UV radiation, which is where antioxidants will be concentrated to combat harmful oxidants produced by photo-oxidation (Cooper-Driver & Bhattacharya, 1998). For this reason, the focus of consumers should be on the dark-colored fruits with the highest antioxidation potential.

S.canadensis Antioxidation Potentials

Elderberry (*Sambucus canadensis L.*) plants are rich in polyphenols, even in comparison to common sources of antioxidants in the human diet such as cranberry and blueberry (Cejpek et al., 2009). Elderberry fruits are known to be one of the richest sources of phenolics among small fruits (Özgen et al., 2010). Elderberry flowers are also recognized as a valuable source of bioactive polyphenols in the human diet (Cejpek et al., 2009) and are known to have at least moderate antioxidant activity (Harokopakis et. al., 2006). Both fruits and flowers of elderberry are therefore used as sources of natural antioxidants that can reduce oxidative damage (Bagchi et al., 2004).

Uses in Traditional Medicine

The well-documented and widespread historical uses of elderberry fruits and flowers support their health-providing benefits. Practitioners of traditional medicine have long recognized elderberry as the “country medicine chest” (Austin, 2004). For example, many indigenous groups in

North America utilized the elderberry stems, leaves, flowers, fruits, and bark as medicine, food, and to fashion toys for children (Moerman, 2002; Lee & Finn, 2007). Fruits were used as a febrifuge, purgative, and to help with rheumatism (Moerman, 2002). Flowers were used as a febrifuge, purgative, diaphoretic, topical anti-inflammatory emulsion, and to control colic in babies (Moerman, 2002; Harokopakis et al, 2006). Although nearly all parts of the elderberry plant were reportedly used for medicinal purpose, fruits and flowers were the most commonly used, which suggest that they confer the highest health benefits (Cejpek et al., 2009).

Motivation for Comparison of S.canadensis *Flower and Fruit ORAC Values*

Despite the many medicinal uses of elderberry flowers, its fruits have received the most attention from researchers interested in antioxidation capacity. Given the dark color of elderberry fruits, this emphasis is justified but we nevertheless wanted to compare their antioxidant capacity with those of flowers from the same species. We used the Oxygen Radical Absorbance Capacity (ORAC) assay to reflect total antioxidant activity (Ou et.al., 2002) of *S.canadensis* flowers and fruits.

METHODS

Plant Material

Elderberry shrubs produce clusters of white flowers that develop into dark purple berries. It grows on the margins of ditches and streams in many parts of the United States but is very common in the Southeast. Berries of this shrub are prized by both frugivorous birds and humans. Flower and fruit samples from wild elderberry shrubs were collected from different populations within 50 km of Gainesville, Florida. Samples were refrigerated immediately after collection and then frozen at 0°C until sample extraction. Berries and flowers were separated from their stalks immediately before extraction. Only fully ripe fruits (based on their dark color) were used.

Chemicals

AAPH (2,2’-azobis(2-amidinopropane)) was a product of Wako Chemicals Inc. (Bellwood, RI). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), HPLC grade methanol, acetic acid, fluorescein, sodium phosphate dibasic heptahydrate, and sodium phosphate monobasic dihydrate were purchased from Fischer Scientific Co. (Pittsburg, PA).

Extraction and sample preparation

Elderberry flower and fruit extractions were carried out similar to the method by Sandhu et al. (2010). Thawed berries were weighed and then pulverized in a mortar until very small amounts of solid material remained. The samples were extracted (0.81-1.0 g) with 15 mL of methanol/water/acetic acid (85:15:0.5; v/v/v) in plastic tubes, vortexted for 30 s, sonicated for 5 min, and then kept in the dark at room temperature for 10 min. The tubes were then centrifuged at 1320 g-force (g) at 20°C for 5 min and the supernatant was removed. The samples were extracted again with 10 mL of methanol/water/acetic acid using the same procedure, but centrifuged for only 8 min. The supernatants from the two extractions were combined and transferred into 25-mL volumetric flasks. The methanol/water/acetic acid solvent was added to reach the final volume to 25 mL and stored at 0°C until analysis.

Oxygen Radical Absorbance Capacity (ORAC)assay

ORAC assays were run on a Spectra XMS Gemini Plate Reader (Molecular Devices, Sunnyvale, CA). Briefly, 50 µL of standard and samples were added to the designated wells of a 96-well black plate to which 100 µL of fluorescein (20nM) was added. The mixture was mixed for three minutes and incubated at 37°C for 10 min before the addition of 50µL of AAPH. Fluorescence was monitored using 485 nm excitation and 530 nm emissions at 1 min intervals for 40 min. Trolox was used to generate a standard curve. The antioxidant capacities of extracts were expressed as micromoles of trolox equivalents (TE) per gram of fruit or flower fresh weight (µmol of TE/g; Sandhu et al., 2010). Flower and fruit assays were run in duplicate and triplicate, respectively.

Results were expressed as mean ± standard deviation. A Student’s t-test was performed for the comparison of the means between flowers and fruits.

RESULTS & DISCUSSION

ORAC values for *S. canadensis* fruits ($\bar{x}$= 213.07 ± 61.3) were higher than for flowers ($\bar{x}$= 83.02 ± 27.8; Fig. 1; $P<0.01$). All fruit samples showed a higher ORAC values than their associated flower samples (Table 1). The data confirmed that elderberry flowers and fruits both have high antioxidant potentials but the ORAC values for fruits were much higher (Figure 1). It should be noted that the free-radical scavenging activity of elderberry flowers were similar to those of many other fruits well-known for their antioxidants. For example, the ORAC values for elderberry flowers were similar to published values for blackberries (95.8-115.2 µmol TE/g), strawberries (136.0 µmol TE/g) , and black raspberries (122.7 µmol TE/g; Wang & Lin, 2000). A popular source of antioxidants, the lowbush blueberry, did show higher ORAC (92.60 µmol TE/g) than elderberry flowers but lower than elderberry fruits (Wu et al., 2004).

Since both fruits and flowers showed high antioxidant potential relative to other berry species, they should both

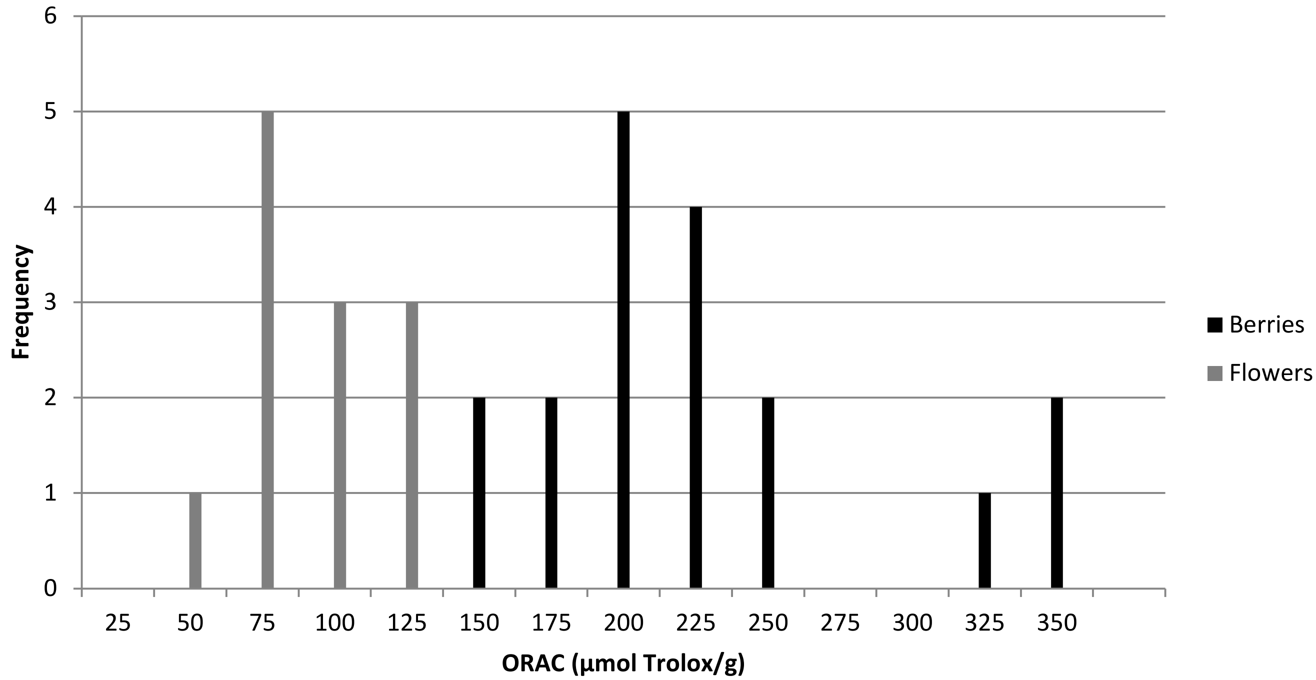

Figure 1. Histogram of ORAC values comparing fruit and flower values for all samples.

Table 1. Range of ORAC Values for *S.canadensis* Fruit and Flower Samples

Sample	Range ORAC (TE, μmol/g) Fruit	Range ORAC (TE, mol/g) Flower
1	300.41-346.00	44.08-53.02
2	210.46-218.11	58.42-60.12
3	147.08-180.97	122.95-123.80
4	149.82-179.77	71.80-94.59
5	223.94-233.51	69.53-88.80
6	178.77-192.36	87.17-121.97

be considered for their human health benefits. Plant parts with naturally high concentrations of phenolics, which correlate with high radical absorbency, can be used to protect against free radicals (Rafat, Philip, & Muniandy, 2010). Our bodies constantly produce reactive oxygen species in our cells, especially in the mitochondria via release of electrons through the electron transport chain which reduces oxygen to superoxides (Salganik 2001). Oxidative stress can be increased by external factors such as solar radiation, tobacco smoke, and industrial pollutants (Salganik 2001). Therefore plant antioxidants should be consumed to help combat these damaging free radicals.

By scavenging for radicals, antioxidants can be protective against cancer (Salganik 2001). Reactive oxygen species can prompt oxidative carcinogenic damage in DNA, and antioxidants can help prevent this damage from happening. (Stoner 2009). Human trials have shown that antioxidants in berries can prevent the conversion of premalignant cells to malignancy at doses that cause minimal to no cytotoxicicty; therefore, berries are good candidates for chemopreventitive uses (Stoner, 2009). The general public is intrigued with food-based approaches to cancer and disease prevention, so information about the easy accessibility and cost-effective ways to incorporate antioxidants into a diet in the form of eating elderberry flowers and fruits is a potential strategy for disease prevention.

We have shown that *S.canadensis* flowers and fruits have high radical scavenging activity. People seeking to enhance their diet with antioxidants could grow or harvest their own flowers and fruits of this widespread and easily cultivated species. The diversity of habitats in which this species naturally occurs also makes it an easily available source of natural antioxidants. It is a type of preventative medicine that can grow in backyards or nearby forest margins. Natural antioxidants as opposed to synthetics are highly valued by food industries in part because some of the latter are toxic or have been found to have carcinogenic side effects (Zhang, Lin Han, & Zhang, 2009).

Antioxidant capacity is dependent on many factors. Environmental factors such as location, light, temperature, agronomic practices, and environmental stress can contribute to differences in anthocyanin concentrations (Ou et al., 2002; Kalt et al., 1999). Different cultivars of species and harvest times can also influence in antioxidant capacity (Wang & Lin, 2000). For example, Wang found that blackberry, raspberry, and strawberry fruits harvested during their green (unripe) stage yielded the highest ORAC values (Wang & Lin, 2000). In this study we only used ripe fruit but nevertheless observed high antioxidant activity, which could suggest different harvest times are suitable for optimal antioxidant capacity for different fruits.

The ORAC assay fails as a measure of total antioxidant activity assay because it only measures activity against peroxyl radicals (Ou et al., 2002). To achieve a full summary of antioxidant activity against various radicals, comprehensive assays are required (Ou et al., 2002). Despite this limitation, ORAC assays are widely accepted in many industries as a standard tool in measuring antioxidant activity (Huang et al., 2002). This study demonstrates the value of studying more than just the fruit of antioxidant-containing plants. Perhaps in the future more flower species will be tested for their antioxidation potentials. This information can be utilized by agricultural researchers striving to enhance antioxidant levels and consumers looking for local sources of antioxidant-rich plants.

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