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Original research article

Variation of select flavonols and chlorogenic acid content of elderberry collected throughout the Eastern United States

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ABSTRACT

American elderberries are commonly collected from wild plants for use as food and medicinal products. The degree of phytochemical variation among wild populations has not been established and might affect the overall quality of elderberry dietary supplements. The three major flavonols identified in elderberries are rutin, quercetin and isoquercetin. Variation in the flavonols and chlorogenic acid was determined for 107 collections of elderberries from throughout the eastern United States using an optimized high performance liquid chromatography with ultraviolet detection method. The mean content was 71.9 mg per 100 g fresh weight with variation ranging from 7.0 to 209.7 mg per 100 g fresh weight within the collected population. Elderberries collected from southeastern regions had significantly higher contents in comparison with those in more northern regions. The variability of the individual flavonol and chlorogenic acid profiles of the berries was complex and likely influenced by multiple factors. Several outliers were identified based on unique phytochemical profiles in comparison with average populations. This is the first study to determine the inherent variability of American elderberries from wild collections and can be used to identify potential new cultivars that may produce fruits of unique or high-quality phytochemical content for the food and dietary supplement industries.
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1. Introduction

American elderberry, *Sambucus nigra* subsp. *canadensis* (L.) Bolli (Adoxaceae), is native to eastern and central North America and Central America. Elderberry shrubs are multi-stemmed with small, weak branches that can easily bend under the weight of mature fruit clusters (Martin and Mott, 1997). These clusters can contain as many as 2000 elderberries with diameters ranging from 5 to 9 mm for the individual berries (Charlebois, 2007). Ripe berries have deep purple/black colors that act as attractants for birds and mammals that consume the fruits and disperse seeds. The shrubs are commonly found growing along forest edges, roadsides and in open disturbed land, allowing accessibility for those relying on wild collections.

Traditionally, American elderberries and elderflowers are used in herbal remedies primarily for colds and flu and for anti-inflammatory, antioxidant and tonic activities (Moerman, 1998; Drapeau and Charlebois, 2012; Foster and Duke, 2014). Elderberries are used to manufacture foods such as jams, pies, wines and juices. The majority of the products created with American elderberries come from wild collections, while most European elderberry products are manufactured with berries from established cultivars grown at commercial production sites (Thomas et al., 2013). There are several established American elderberry cultivars that were developed decades ago at agricultural research stations, some of which have high quality berry production (Charlebois, 2007). Elderberries are more frequently promoted for their medicinal benefit, which has spiked demand for their products. This increased demand for berries and flowers has enabled the growth of small and large scale production sites throughout the United States and Canada. These sites are using established cultivars and/or wild cuttings for their elderberry production (Thomas et al., 2009). Although studies of American elderberries have focused on established cultivars, it is important to establish determinants of quality for wild elderberries used in food and medicinal products (Drapeau and Charlebois, 2012; Lee and Finn, 2007; Thomas et al., 2008).

Although small in size, elderberries are packed with non-nutritive compounds responsible for their high antioxidant properties (Ozgen et al., 2010; Mikulic-Petkovsek et al., 2012).

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As the majority of research relating to these phytochemicals in elderberries focuses on established cultivars, little is known about the natural phytochemical distribution of wild American elderberries. Ozgen et al. (2010) determined the total phenolic content of wild American elderberries, after transplantation to a single agricultural site, where they reported similar phenolic contents in comparison with previous reports of American elderberry cultivars (Lee and Finn, 2007). This report gave little information on the wild collections, and transplanting the shrubs minimized environmentally mediated variation, which is unlike the conditions of commercial wild harvest for food production. It is known that phenolic compounds can provide protective effects from pests, predators and environmental stressors such as altitude and UV radiation (Ozgen et al., 2010; Reiger et al., 2008), suggesting that environmental factors may affect the phytochemical composition of wild elderberries.

In order to establish the phytochemical variability of wild American elderberries, collection of wild elderberry fruits was undertaken over a two-year period throughout the eastern United States. Profiles of select flavonols and chlorogenic acid of the berries were determined and those from different geographic regions compared using high performance liquid chromatography with ultraviolet (HPLC-UV) detection. The goal of this project was to determine whether phytochemical diversity within this species is great enough to create a potential for selection of source material for new cultivars based on increased phytochemical content.

2. Materials and methods

2.1. Plant collections

Wild collections of Eastern American Elderberry (*Sambucus nigra* subsp. *canadensis*) took place in 2010 and 2011 throughout the eastern United States. A total of 107 samples were collected. The following states and number of collections per state are summarized: Alabama (7), Arkansas (3), Connecticut (5), Georgia (4), Indiana (1), Kentucky (9), Maine (6), Missouri (2), Mississippi (7), North Carolina (9), New Hampshire (5), New York (6), Ohio (2), Pennsylvania (11), South Carolina (14), Virginia (3), Vermont (1) and West Virginia (12). Fig. 1 shows the distribution of wild elderberry collections. The date and exact coordinates were recorded for all collections, and voucher specimens were preserved in the Missouri Botanical Garden herbarium. Berries were frozen after collection and stored at −20 °C until use for chemical analysis.

2.2. Reagents and reference materials

HPLC grade methanol, acetonitrile, tetrahydrofuran and isopropanol were purchased from VWR International (Mississauga, ON, Canada). HPLC grade phosphoric acid and acetic acid were purchased from VWR International. The primary grade reference standards chlorogenic acid (purity: 93.9%), rutin (purity: 89.3%), quercetin (purity: 93.4%) and isoquercetin (purity: 93.2%) were purchased from Chromadex (Irvine, CA, USA). Water was deionized using a Barnstead water purification system (Fisher Scientific, Ottawa, ON, Canada). Stock solutions of the reference standards were prepared at 1000 μg/mL. Each day mixed calibration solutions ranging from 0.5 to 200 μg/mL were prepared.

2.3. Sample preparation

Several elderberry clusters were collected from individual trees and pooled together as a single collection. Elderberries were separated from the stems and freeze dried. Dried samples were ground to less than 500 μm particle size to ensure a homogeneous sample from each collection location. 150 mg of dried berries were extracted with 15 mL of extraction solvent composed of water: methanol:acetic acid (66:30:4 v/v). The samples were extracted using a wrist action shaker for 1 h and then centrifuged at

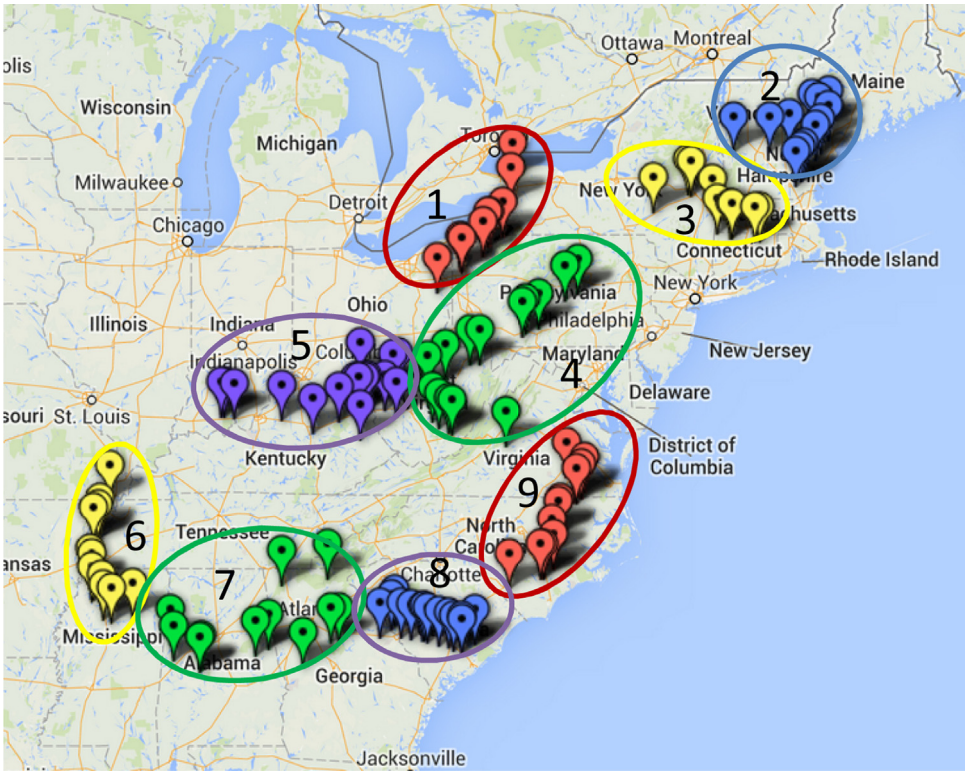

Fig. 1. Geographical distribution of American elderberry (*Sambucus nigra* subsp. *canadensis*) collections in the Eastern United States.

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