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Characterization of phenolics, betacyanins and antioxidant activities of the seed, leaf, sprout, flower and stalk extracts of three *Amaranthus* species

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ABSTRACT

Hydrophilic extracts from different parts including leaves, stalks, seeds, flowers and sprouts of 3 *Amaranthus* species (*Amaranthus hypochondriacus*, *Amaranthus caudatus* and *Amaranthus cruentus*) were characterized for their phytochemical profiles including the phenolics and betacyanins by UHPLC and LC–ESI–MS, and their antioxidant activities by FRAP and ORAC assays. The main betacyanins in *Amaranthus* samples were identified to be amaranthine and isoamaranthine. Eleven phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, gentistic acid, 2,4-dihydroxybenzoic acid, ferulic acid, salicylic acid, rutin, ellagic acid, kaempferol-3-rutinoside and quercetin) were identified in the extracts of different parts of *Amaranthus*. The total phenolic content (TPC) ranged from 1.04 to 14.94 mg GAE/g DW; the total flavonoid content (TFC) ranged from 0.27 to 11.40 mg CAE/g DW; while the total betalain content (TBC) ranged from 0.07 to 20.93 mg/100 g DW. FRAP values ranged from 0.63 to 62.21 μmol AAE/g DW and ORAC ranged from 30.67 to 451.37 μmol TE/g DW. The leaves of *Amaranthus* showed the highest TPC, TFC, TBC, FRAP and ORAC values; while the seeds and stalks the lowest. There was a strong correlation between TPC, TBC, TFC and the antioxidant activity. The result suggests that all parts of the *Amaranthus* plant can be a good source of antioxidants.

1. Introduction

The genus *Amaranthus* (family Amaranthaceae), including quinoa and amaranth species, is a valuable food source of nutrients with high quality proteins, vitamins, minerals and bioactive compounds such as phenolics (Silva-Sánchez et al., 2008). In addition to being consumed as a staple food, both the grain and vegetable *Amaranthus* have also been commercially exploited for natural dyes, pharmaceuticals and skin lubricants (Science, 1984). The grains of quinoa and amaranth in particular have become one of the most favored new foods in recent years in North America, largely because they are gluten-free, and contain a balanced essential amino acid profile. Apart from the macronutrients, phytochemicals in both the grain and vegetable portion of *Amaranthus* plants have also been shown to possess many beneficial health effects. The aqueous extracts of *Amaranthus gangeticus* leaves showed anticancer potential by inhibiting the proliferation of liver (HepG2), breast (MCF-7) and colon (Caco-2) cancer cell lines (Sani et al., 2004). *Amaranthus tricolor* leaves have been reported to be potent inhibitor of cyclooxygenase and human tumor cells including colon HCT-116, breast MCF-7, lung NCl-H460, stomach AGS and central nervous system SF-268 (Jayaprakasam et al., 2004).

Abbreviations: AAE, ascorbic acid equivalent; AAPH, 2,2′-azobis-(2-methylpropionamidine) dihydrochloride; ACa, *Amaranthus caudatus*; ACr, *Amaranthus cruentus*; AH, *Amaranthus hypochondriacus*; CAE, catechin equivalent; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; LC–ESI–MS, liquid chromatography–electrospray ionization–mass spectrometry; ORAC, oxygen radical absorption capacity; TBC, total betalain content; TE, trolox equivalent; TFC, total flavonoid content; TPC, total phenolic content; TPI, total phenolic index; TPTZ, 1,3,5-tri(2-pyridyl)-2,4,6-triazine; UHPLC, ultra high performance liquid chromatography.

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The color of *Amaranthus* leaf and stalk varies from red to green, and that of the seed ranges from black to white (Science, 1984). Highly pigmented vegetables or fruits such as anthocyanin-rich vegetables usually have higher nutritional value compared to other species of the same plant as found by Li et al. (2012). The visual appearance of most *Amaranthus* plants is bright red-violet, and betalains are generally considered to be responsible for the maroon pigment. However, the similarity in color may lead to misidentification of the pigments in *Amaranthus* as anthocyanins. While the overwhelming majority of the literature suggests betalains to be the pigments in *Amaranthus* plants, there are reports that estimated the pigments spectrometrically as anthocyanins (Paśko et al., 2009). It is well established, however, that phytochemically, betalains (betacyanins and betaxanthins) and anthocyanins in plants are mutually exclusive, i.e. plants producing anthocyanins will not synthesize betalains and vice versa (Stafford, 1994). Betalains exhibit broader pH stability than anthocyanins, thus they can be used in low-acid foods as a food colorant (Stintzing and Carle, 2007).

Betacyanins are natural colorants and have been widely used in the food industry. Betacyanins in *Amaranthus* are mainly amaranthine and isoamaranthine (a C15 epimer of amaranthine), which share the same aglycone betanidin with the betanins, but different glycosidic moiety; they are betanidin 5-*O*-β-glucuronosylglucoside. Cai et al. (2003) showed that the amaranthines in *Amaranthus* possessed very strong antioxidant activities and could be used as a substitute source for the well-known betanins from red beets in the food colorants and natural antioxidants (Cai et al., 2003; Klimczak et al., 2002). Total phenolic content and antioxidant activity of amaranth leaf and sprout have been studied; however, the composition has not been elucidated, and the pigments were analyzed as total anthocyanins instead of betacyanins (Paśko et al., 2009). There is a need to systematically study the composition of the phenolics and betalains in different *Amaranthus* species and the different parts of *Amaranthus* plants, in order to find out which of these phytochemicals contribute to the potential health benefits.

While quinoa and amaranth were originally cultivated in South and Latin America, adaptation trials and commercial production of these two plants are currently being conducted in Ontario, Canada to meet the high consumer demand. Data reported herein are part of the initial evaluation of the quality of Ontario-produced amaranth. Three *Amaranthus* species were evaluated under the growing conditions in Ontario. *Amaranthus hypochondriacus* and *Amaranthus cruentus* are native to Mexico and Guatemala, and *Amaranthus caudatus* is native to Andean highlands of Argentina, Peru, and Bolivia. These three species produce large seed heads that are loaded with edible seeds. The main objective of this study was to identify and quantify the phenolic compounds and betalains in different parts (leaves, stalks, seeds, flowers and sprouts) of three amaranth species grown in Ontario, and to discover how these phytochemicals contribute to the antioxidant activities.

2. Materials and methods

2.1. Plant materials

All three *Amaranthus species*, *A. hypochondriacus* (AH), *A. caudatus* (ACa), and *A. cruentus* (ACr) were produced on farm in Port Burwell, Ontario, Canada, 2011. The amaranth field site was planted on June 2, 2011 at a high seeding rate to achieve a stand of approximately 300,000 seeds/ha. Seed was mixed with cornmeal in order to obtain the appropriate seeding density. Each variety consisted of 8 rows per plot, 20 m long with 76 cm row spacing. The field was hand weeded fortnightly throughout the growing season. The leaves, stalks, seeds and flowers of all three species were harvested by removing (cutting) the whole plant at the stem, in November 2011 for AH and ACa, and in December 2011 for ACr stalks and seeds. ACa samples were collected from two different plots. Ten random plants for each species were selected by pacing 2 m in a diagonal line along the test plot starting 1 m from the plot edge. Plants were collected, air-dried at room temperature in dim light for 5 days and separated into stalks, leaves, flowers, and seeds. The flowers and seeds were hand threshed from the head. Moisture levels of the stalks and leaves ranged from 57 to 78%. Stalks were cut up into 4–5 cm sections. All parts were pooled separately for analysis, i.e. five independent samples. ACa seeds and sprouts were planted in October 2011 and harvested 10 days post-seeding from one area. To reduce the number for sample analysis, only ACa was examined for all parts (including the sprouts), and ACr was tested for seeds and stalks to explore its potential for food and non-food uses. Sprouts were immediately sent to the laboratory for drying. All plant samples were vacuum-dried in the laboratory at room temperature for >36 h till consistent weight and then ground into fine powder. The ground materials were stored in polyethylene tubes at −80 °C prior to analysis.

2.2. Chemicals and standards

Gallic acid (purity: 97%), protocatechuic acid (97%), chlorogenic acid (95%), gentistic acid (98%), 2,4-dihydroxybenzoic acid (97%), ferulic acid (99%), salicylic acid (99%), rutin (95%), ellagic acid (96%), kaempferol-3-rutinoside (95%), quercetin (95%), betanin (95%), 1,3,5-tri(2-pyridyl)-2,4,6-triazine (TPTZ), L-ascorbic acid (98%), Folin–Ciocalteu's phenol reagent, fluorescein, Trolox (98%) and 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Sigma (St. Louis, MO, USA). Sodium acetate, ferric chloride hexahydrate, sodium phosphate monobasic, sodium phosphate dibasic and HPLC-grade solvents, including methanol and formic acid were purchased from Caledon Laboratories (Georgetown, ON, Canada).

2.3. Colorimetric study

Color characteristics are important for studying pigments in food. Instrumental measurements of color were conducted in a Minolta Chroma Meter (CR-200; Minolta Camera Co. Ltd., Osaka, Japan) by placing the plant powder in a 15 mm thick and transparent plastic cell without cover and by using a black plate as the background to standardize the measurements. The chromameter consisted of an 8 mm diameter measuring area and diffuse illumination/viewing was utilized. CIE 1976 uniform color space was taken into account for the colorimetric analysis. The color characteristics of CIE L^*, a^*, b^* were calibrated against a standard white plate. Data were the average of triplicate measures on equidistant points of the plant powder (Li et al., 2011). Plant powder instead of actual plant parts were used in this experiment for better reproducibility and uniformity. The color characteristics are L^* (lightness, ranging from 0, black, to 100, white), a^* (positive values for the direction of redness and negative values for the direction of the complement green) and b^* (positive for yellowness and negative for blueness) (Arslan and Musa Özcan, 2010). The values a^* and b^* were used to calculate the hue angle (H = arctan (b^*/a^*)) and metric chroma value ($C = (a^{*2} + b^{*2})^{1/2}$). All the procedures were conducted at room temperature in the laboratory.

2.4. Sample extraction

Amaranthus powder samples (1.5 g) were accurately weighed and transferred into separate 50 mL tubes containing 30 mL of 80% methanol (Ali et al., 2009; Cai et al., 2001; Li et al., 2011). The extraction was carried out on a rotary shaker (Scientific Industries Inc., USA) overnight (ca. 15 h; 400 rpm) at room