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Composition of phenolic compounds and antioxidant activity in the leaves of blueberry cultivars

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ABSTRACT

Phenolic compounds in the leaves of 104 blueberry cultivars were investigated using HPLC–DAD and HPLC–ESI–MS^2 analysis. This approach identified 28 constituents, among which eight constituents were identified certainly by authentic standards, while others were tentative. Three anthocyanins, four flavonols and four chlorogenic acids were reported for the first time in blueberry leaves. Furthermore, the antioxidant activity of blueberry leaves was estimated by using $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and FRAP assays. It was found that most rabbiteye blueberry cultivars had higher antioxidant activity than most northern and southern highbush ones. The rabbiteye cultivars 'Vernon', 'Britewell' and 'T-172, Festival' appear to be good sources of antioxidants. Hierarchical cluster analysis classified 104 cultivars into three clusters, and they were distinct from each other. This study contributes to current knowledge on the composition of phenolic compounds in blueberry leaves, and identifies specific cultivars which may be potential resources for tea making and food additive.

1. Introduction

Blueberry, a perennial shrub of the genus *Vaccinium*, family Ericaceae, is popular around the world due to its flavorful fruit and abundant anthocyanins. Numerous studies have been published on the chemical composition and the healthcare applications of blueberry fruit (Barnes, Nguyen, Shen, & Schug, 2009; Lau, Joseph, McDonald, & Kalt, 2009; Norberto, Silva, Meireles, Faria, Pintado, & Calhau, 2013). This has led to a steady increase in the propagation of blueberry around the world. However, there are large amounts of blueberry leaves being discarded following pruning in many countries. Exploring the nutrient contents and application for these

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waste leaves would be strongly beneficial to the related agricultural industry.

Blueberry leaves have been studied since 1927, at which time they were in use as a medicinal tea for diabetic sufferers among alpine peasants (Allen, 1927). It has also been reported that oriental blueberry leaves (*V. bracteatum* Thunb.) have been widely used as a traditional medicinal herb in China for centuries to prevent cataract, premature aging and anemia (Fang & Qin, 2003). Comparing the antioxidant activity of leaves and fruits of highbush blueberry, significantly higher antioxidant activity was found in leaves than in fruits (Ehlenfeldt & Prior, 2001). The biological activity of blueberry leaves is closely related to their chemical composition. A recent study suggested that the leaf extract of lowbush blueberry (*V. angustifolium*) is rich in chlorogenic acid and quercetin glycosides (Harris et al., 2007). In the leaves of rabbiteye blueberry (*V. ashei*), flavan-3-ols and proanthocyanidins were found to be the major phenolic components besides chlorogenic acid and flavonol glycosides (Matsuo et al., 2010). Proanthocyanidins with a polymerization degree of 8 to 9 from rabbiteye blueberry leaves was recently shown to have high potential for suppressing the expression of subgenomic hepatitis C virus RNA (Takeshita et al., 2009). These results suggest that blueberry leaves have great value for practical uses.

Although there have been several studies concerned with blueberry leaves, no more varieties have been systematically studied. The chemical fingerprint chromatographies of leaves from other blueberry types such as southern highbush and northern highbush blueberry have not yet been investigated. Further exploration is needed to confirm whether there are differences in composition between different cultivars. In this study the leaves of 104 blueberry cultivars were assessed by high-performance liquid chromatography with a diode array detector (HPLC–DAD) and HPLC–electrospray ionization–mass spectrometry (HPLC–ESI–MS^2). Multiple radical scavenging assays were used to systematically evaluate the antioxidant activity. This approach allows specific cultivars to be identified as potential valuable resources for tea product or food additive.

2. Materials and methods

2.1. Plant materials

The leaves of 104 blueberry cultivars consisting of 38 rabbiteye blueberry (R), 37 northern highbush blueberry (N) and 29 southern highbush blueberry (S) were collected from the blueberry base of Dalian University in October 16, 2011 which time was before the dormant pruning. At this time, the senescence period of the leaves has begun. The leaves of most rabbiteye cultivars were evergreen, while there was red color presented in the leaves of some northern and southern highbush cultivars. The cultivars had been originally introduced from various locations around the world, but grown under the same cultivation conditions for more than 3 years. The information of the sampled cultivars was shown in the Table S1. For every cultivar, three trees were selected randomly as three replicate sample sources, and approximately 30 leaves were hand-picked across the whole plants at random. They were put into the perforated plastic bag and brought back to the lab on the next day, and then air dried and pulverized. The powder was collected in envelopes and then stored in tawny-glass desiccators until later analysis.

2.2. Chemicals

Cyanidin 3-*O*-glucoside (Cy3G) was purchased from Extrasynthese (Genay, France). Quercetin 3-*O*-rutinoside (rutin), quercetin 3-*O*-galactoside (hyperin), *trans*-5-caffeoylquinic acid, (+)-epicatechin and gallic acid (GA) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 3,5-Dicaffeoylqunic acid and 4,5-dicaffeoylqunic acid were obtained from Beijing Hongyue Innovation technology company (Beijing, China). Standards of quercetin 3-*O*-glucoside and kaempferol 3-*O*-glucoside were generously provided by Dr. Xiao Wang (Shandong Academy of Sciences, China). 1,1-Diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)diammonium salt ($ABTS^{\bullet+}$) and 2,4,6-tripyridyl-*S*-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, USA). HPLC grade acetonitrile and methanol were obtained from Alltech Scientific (Beijing, China). Trifluoroacetic acid (TFA; ≥99%) was purchased from Merck (Darmstadt, Germany). All other regents were of analytical grade and were obtained from Beijing Chemical Works (Beijing, China).

2.3. Sample extraction

The extraction method was modified from the method used by Yang et al. (2009). Approximately 0.1 g of powdered sample was combined with 2 mL of methanol containing 2% formic acid in a 10 mL centrifuge tube and then shaken for even mixing using a QL-861 vortex (Kylinbell Lab Instruments, China). The samples were successively sonicated using a KQ-500DE ultrasonic cleaner (Ultrasonic Instruments, China) at 20 °C for 20 min and centrifuged in a Sigma 3K30 (Sigma, Germany) at 10,000 *g* for 10 min, following which the supernatant was collected. The residue was further extracted with 2 mL and 1 mL of methanol containing 2% formic acid in the same way, respectively. When all of the extract was pooled, 5 mL of petroleum ether was added to remove chlorophyll and other aliphatic soluble impurities. After a period of 2 hours, the supernatant petroleum ether was removed, and the same operation was repeated several times until the added petroleum ether was colorless. The final extract was filtrated through 0.22 μm reinforced nylon membrane filters (Shanghai ANPEL, China) prior to HPLC–DAD and HPLC–MS^2 analysis.

2.4. HPLC–DAD analysis

HPLC analysis for phenolic compounds was performed using a Dionex system (Sunnyvale, USA) with a P680 HPLC pump, an UltiMate 3000 autosampler, a TCC-100 thermostated column compartment and a Dionex PDA100 photodiode array detector. The analytical column was an ODS-80Ts QA C18 column (150 × 4.6 mm, 5 μm i.d.; Tosoh, Japan) protected with a C18 guard cartridge (Shanghai ANPEL, China). Eluent A was double-distilled water containing 0.1% TFA; eluent B was 15% methanol in acetonitrile. The following elution gradient was used: 13% B