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Polyphenol profile and antioxidant activity of the fruit and leaf of *Vaccinium glaucoalbum* from the Tibetan Himalayas

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ABSTRACT

Vaccinium glaucoalbum, a perennial evergreen shrub, is naturally distributed in high-altitude areas. In this study, the composition and content of polyphenolic compounds in the fruit and leaf of *V. glaucoalbum* were characterized. In total, 24 chemical compounds were detected and identified by HPLC-DAD and HPLC-ESI-MS^2. Among all the compounds determined, 15 were anthocyanins and detected in fruit, 5 were flavonols and monitored in leaf, and 4 were chlorogenic acids and found in both fruit and leaf. The total anthocyanin content (TAC) of fruit (682 mg/100 g FW) was the highest among wild *Vaccinium* berries in China which have been investigated for now, and the total flavonol content of leaf was 2764 mg/100 g FW. The antioxidant activity of both fruit and leaf was assessed by DPPH and FRAP assays. Given its high TAC and strong antioxidant activity, the fruit of *V. glaucoalbum* has great potential in functional food.

1. Introduction

Recently, the health-promoting effects of berries, such as blueberry, blue honeysuckle, cranberry, bilberry, and currant, have drawn extensive attention among researchers worldwide. The health benefits of berries are mainly ascribed to their high antioxidant activities, which are related to phytonutrients, especially flavonoids (Prior et al., 1998). Flavonoids are ubiquitous secondary metabolites in the plant kingdom with important physiological functions as colorants, attractants, UV protectants, and detoxicants, among others (Kidd, Llugany, Poschenrieder, Gunsé, & Barceló, 2001; Les & Sheridan, 1990; Winkel-Shirley, 2002). These compounds also have various biological properties, such as antioxidant, antibacterial, anti-inflammatory, astringent, antiproliferative, and anticarcinogenic activities (Antunes-Ricardo, Gutiérrez-Uribe, & Serna-Saldívar, 2015; Landete, 2012).

Vaccinium berries are considered to be richer sources of flavonoids with higher antioxidant activities as compared with other fruits and vegetables (Zoratti, Jaakola, Häggman, & Giongo, 2015). Moreover, *Vaccinium* berries have been exploited into functional food for eyesight protection, memory improvement, and cardiovascular protection, among others (Krikorian et al., 2010; Liu et al., 2011; Zafra-Stone et al., 2007). The genus *Vaccinium* (Ericaceae family) contains approximately 450 species worldwide, with a wide geographical distribution in the temperate and subtropical zones of the Northern Hemisphere, as well as the tropical mountains of America and Asia. More than half of all the species (235 species) are concentrated in Malaysia, whereas 91 species are found in China (Gao, Xu, Wang, & Hou, 2015). Numerous publications regarding *Vaccinium* berries are currently available. Most of the literature focused on three *Vaccinium* berries (i.e., blueberries, cranberries, and lingonberries), which have been commercially utilized (Song & Hancock, 2011). Investigations on wild *Vaccinium* berries are limited as compared with the total number of species.

Vaccinium glaucoalbum J. D. Hooker ex C. B. Clarke (Fig. 1) is a perennial evergreen shrub that grows in high-altitude areas from 2900 m to 3300 m above the sea level and is distributed in Southwest China, Bhutan, Northeast India, Myanmar, Nepal, and Sikkim. The plant blooms in June, and the fruiting period can last from August to December (http://foc.eflora.cn/content.aspx?TaxonId=242444176). The higher the altitude is, the more difficult the plant survival is. The poor living conditions of *V. glaucoalbum* suggest the presence of some special traits, such as cold and wind tolerance, to

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Fig. 1. Leaf and fruit of *Vaccinium glaucoalbum* investigated in this study.

resist environmental stress. Therefore, this species can be used as an exceptional germplasm resource for breeding new varieties with strong adaptability. A recent study noted the increasing accumulation of anthocyanins in *Vaccinium myrtillus* along an altitudinal gradient (Zoratti et al., 2015). The relatively high-altitude habitat for *V. glaucoalbum* may indicate high level of anthocyanins and antioxidant activity. Therefore, this berry has great potential to meet the public demand for higher qualities of natural functional food. In addition, with late fruit maturity period (from August to December), *V. glaucoalbum* can be domesticated into late-maturing varieties, which would be of great economic value. Unfortunately, data regarding this species are limited to date. Given the abovementioned advantages, we estimated the quality of this wild berry. In the present study, the chemical composition and antioxidant activities of the fruit and leaf were analyzed. This paper is the first to report on the polyphenol constituent and antioxidant capacity of *V. glaucoalbum*.

2. Materials and methods

2.1. Plant materials

The fruit and leaf of *V. glaucoalbum* at the fruit full maturation stage were collected from a valley (latitude 27°52′N, longitude 91°47′E, and altitude 2672 m) of Cona county in Tibet Province (China), on December 4, 2014. The botanical authentication of this species was completed by Jian-Fei Ye (an Engineer of Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences). Three bushes with similar morphological characteristics were randomly selected for sampling. Within each bush, approximately 200 g fresh fruits and 20 g fresh leaves were hand-picked and placed into an airtight preserving box with ice bags. Subsequently, all sampled fruits and leaves were brought back to the laboratory and stored at −20 °C for future use.

2.2. Chemicals and reagents

The anthocyanin standard, cyanidin 3-glucoside (Cy3G), was purchased from Extrasynthese (Genay, France). The flavonol standard, quercetin 3-rutinoside (rutin); the chlorogenic acid standard, *trans*-5-caffeoylquinic acid; and the antioxidant activity standard, gallic acid (GA), were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The methanol, formic acid, and acetonitrile for HPLC analysis were of chromatographic quality and obtained from Alltech Scientific (Beijing, China). The Folin–Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH•), and 2,4,6-tripyridyl-*S*-triazine (TPTZ) were employed to assess the antioxidant capacity and were purchased from Sigma–Aldrich (St. Louis, USA). All other analytical chemicals (sodium carbonate, sodium acetate, ferric chloride, methanol, hydrochloric acid, and acetic acid) used in the determination of antioxidant capacity were obtained from Beijing Chemical Works (Beijing, China). Double distilled water was prepared by using a Milli-Q System (Millipore, Billerica, MA, USA).

2.3. Sample extraction

Sample extraction was performed as previously described (Feng et al., 2016; Wang et al., 2015) with some modifications. Approximately 0.5 g frozen fresh fruits and 0.1 g frozen fresh leaves were separately ground into fine powder in liquid nitrogen with mortars and pestles and transferred into 10 mL centrifuge tubes with 3 and 2 mL methanol–formic acid (49:1, v/v), respectively. Subsequently, the mixtures were shaken with a QL-861 vortexer (Kylinbell Lab Instruments, China) for 30 s. The samples were sonicated in a KQ-500DE ultrasonic cleaner (Ultrasonic instruments, China) at 20 °C for 20 min and centrifuged in a SIGMA 3K30 centrifuge (SIGMA centrifuge, Germany) at 12,000*g* for 10 min. The supernatants were collected in sterile 10 mL centrifuge tubes. The abovementioned steps were repeated until the extracts were colorless. Subsequently, 8 and 5 mL extracts were obtained for fruit and leaf samples, respectively. The extracts were passed through 0.22 μm millipore membrane filters (Shanghai ANPEL, China) prior to HPLC-DAD and HPLC-MS^2 analysis. Three biological replicates were performed for both samples; all the concentrations involved in this study were calculated from fresh weight (FW).

2.4. HPLC-DAD and HPLC-ESI-MS^2 analysis

The apparatus and conditions used for HPLC-DAD and HPLC-ESI-MS^2 analyses were identical to those described in a previous study (Wang et al., 2014). HPLC-DAD analysis was carried out using a Dionex system (Sunnyvale, USA) equipped with a P680 HPLC pump, an UltiMate3000 autosampler, a TCC-100 thermostated column compartment, and a Dionex PDA100 photodiode array detector. The column was an ODS-80Ts QA C18 column (150 mm × 4.6 mm, 5 μm i.d., Tosoh, Tokyo, Japan) protected with a C18 guard cartridge (ANPEL, Shanghai, China). Eluent A was 5% formic acid in double distilled water (v/v), and eluent B was absolute acetonitrile. The following gradient elution protocol was used: 5% B at 0 min, 12% B at 30 min, 25% B at 50 min, and 5% B at 60 min. For HPLC-DAD analysis, a 10 μL aliquot of each sample was injected; the flow rate was controlled at 0.8 mL/min, and the column temperature was maintained at 35 °C. Chromatograms were obtained at 525 and 350 nm. The photodiode array spectra were recorded from 200 nm to 800 nm.

HPLC-ESI-MS^2 analysis was performed on an Agilent-1100 HPLC system equipped with an UV detector and a LC-MSD Trap VL ion-trap mass spectrometer (Agilent Technologies, USA). ESI source was used for ionization. The HPLC separation conditions were the same as those mentioned above for the HPLC-DAD analysis. For MS analysis, anthocyanins were analyzed in the positive ion (PI) mode; other phenolic compounds were analyzed in the PI and negative ion (NI) modes. The following mass spectrum detection conditions were employed: capillary voltage, 4.0 kV; nebulization pressure, 241.3 kPa; gas (N_2) temperature, 350 °C; gas flow rate, 8.0 L/min; capillary offset voltage, 77.2 V; capillary exit voltage, 127.3 V; scan range, 100–1000 (m/z).