



Phenolic contents and cellular antioxidant activity of Chinese hawthorn “*Crataegus pinnatifida*”



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ABSTRACT

It is evident from various epidemiological studies that consumption of fruits and vegetables is essential to maintain health and in the disease prevention. Present study was designed to examine phenolic contents and antioxidant properties of three varieties of *Crataegus pinnatifida* (Chinese hawthorn). Shanlihong variety exhibited elevated levels of total phenolics and flavonoid contents, including free and bound phenolics. Procyanidin B₂ was most abundant phenolic compound in all samples, followed by epicatechin, chlorogenic acid, hyperoside, and isoquercitrin.

The free ORAC values, and free hydro-PSC values were 398.3–555.8 μmol TE/g DW, and 299.1–370.9 μmol VCE/g DW, respectively. Moreover, the free cellular antioxidant activity (CAA) values were 678–1200 μmol of QE/100 g DW in the no PBS wash protocol, and 345.9–532.9 μmol of QE/100 g DW in the PBS wash protocol. *C. pinnatifida* fruit could be valuable to promote consumer health.

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1. Introduction

Various epidemiological studies have demonstrated that increased consumption of plant-based whole foods, fruits and vegetables are important for good health and disease prevention, especially in the prevention of chronic diseases, which include: cancer, diabetes, cardiovascular disorders, Alzheimer disease, and age-related functional decline (Eberhardt, Lee, & Liu, 2000; Liu, 2013). Fruits and vegetables are potential sources of phytochemicals particularly phenolic compounds, which are major bioactive compounds and natural antioxidants (Sun, Chu, Wu, & Liu, 2002). In the USDA (2010) dietary intake advise people are recommended to eat at least 9 servings of fruits and vegetables per day for 2000 kcal diet. However, in China, people rarely realized the consequence of fruits and vegetables intake on daily basis.

Crataegus pinnatifida (hawthorn), is a member of family Rosaceae. This species is widely distributed in Asia, Europe and North America (Kwok et al., 2013). Over 1000 species of genus *Crataegus* have been identified worldwide. However, *C. pinnatifida*

and *C. pinnatifida* Bge. var. major N.E.Br. are common in China (Yang & Liu, 2012). Traditionally *C. pinnatifida* is used as Chinese medicinal herb. This species showed strong antioxidant properties (Cui, Nakamura, Tian, Kayahara, & Tian, 2006). To date, over 150 compounds, especially phenolic compounds have been identified in *C. pinnatifida* (Wu, Peng, Qin, & Zhou, 2014). Among these phenolic compounds, procyanidins (procyanidin B₂, procyanidin B₅, and procyanidin C₁), flavonoids (epicatechin, hyperoside, quercetin, rutin, and isoquercitrin), and triterpenoids acid (ursolic acid, corosolic acid, oleanolic acid, and maslinic acid) are the key bioactive components of hawthorn (Chai et al., 2014; Liu, Kallio, Lu, Zhou, & Yang, 2011; Wu et al., 2014; Yang & Liu, 2012). And procyanidins and triterpenoids acid dominate in the fruits, while flavonoids are most abundant in the leaves (Wu et al., 2014; Yang & Liu, 2012). Moreover, chemicals extracted from the species or consumption of fruits from the species were reported to exhibit a variety of pharmacological effects on digestive, cardiovascular, and endocrine systems (Guo, Liu, Gao, & Shi, 2014; Li, Zhu, Guo, et al., 2013; Zhu et al., 2013), and neuro-protective (Chang et al., 2013), and have antiviral, anti-inflammatory, anticancer, and antimicrobial activities (Li, Zhu, Dong, et al., 2013; Tadic et al., 2008). Additionally, fruits of *C. pinnatifida* are consumed fresh or processed into canned fruits, jams, jellies, and soft drinks in food and beverages industries (Yang & Liu, 2012). Currently, different researchers have paid their attention on *C. pinnatifida*, because of

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its health benefits and abundant bioactive compounds (Liu, Yuan, & Zhang, 2010). However, very limited information is known about cellular antioxidant activity (CAA) of different varieties of *C. pinnatifida* used particularly in China.

The cellular antioxidant activity (CAA) assay is a newly developed approach that quantifies the antioxidant capacity of bioactive compounds in cell cultures. In this method, a probe, 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA), is taken up by HepG₂ cells and fluoresces when oxidized to fluorescent DCF by reactive oxygen and reactive nitrogen species. HepG₂ cells treated with pure phytochemical compounds and (or) extracts show the ability of quenching peroxy radical and inhibiting the generation of DCF (Wolfe & Liu, 2007). Comparing to other methods, the HepG₂ CAA assay via probe DCFH-DA to DCF provides a better understanding on how antioxidants will be taken up, distributed and metabolized under physiological conditions? Though the extracellular antioxidant activity of *C. pinnatifida* extract has been studied with chemical methods, there is little information on its antioxidant properties within cell that takes bioavailability into account. Thereby, present investigation was aimed to examine the phytochemical profiles, including both free and bound phenolics and flavonoids, and antioxidant properties of three varieties of *C. pinnatifida* using oxygen radical absorption capacity (ORAC), hydrophilic peroxy radical scavenging capacity (hydro-PSC), and cellular antioxidant activity (CAA) assays.

2. Materials and methods

2.1. Chemicals and reagents

Ascorbic acid, gallic acid, Folin–Ciocalteu reagent, sodium borohydride (NaBH₄), aluminum chloride, chloranil, catechin hydrate, vanillin, epicatechin, hyperoside, isoquercitrin, chlorogenic acid, procyanidine B₂, and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Potassium hydroxide (KOH), sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH₂PO₄), dipotassium hydrogen phosphate (K₂HPO₄) and sodium bicarbonate (NaHCO₃) were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Methanol, absolute ethyl alcohol, acetone, acetic acid, tetrahydrofuran (THF), hydrochloric acid (HCl) were obtained from Guangzhou Reagent Co. (Guangzhou, China). 2,2'-Azobis-amidinopropane (ABAP) was purchased from Aldrich (MO, USA). All reagents used were of analytical grade. Additionally, acetonitrile and methanol used for HPLC analysis was obtained from CNW Technologies GmbH (Dusseldorf, Germany).

2.2. General method

The laboratory analysis work was conducted in department B8, South campus of South China University of Technology, Guangzhou Higher Education Mega Center. All analysis was conducted in triplicate unless specifically described different.

2.3. Sample preparation

Fresh fruit of three varieties of *C. pinnatifida* (hawthorn): Shanlihong (*C. pinnatifida* Bge. var. major N.E.Br.), Shanzha (*C. pinnatifida* Bge), and Dajinxing (*C. pinnatifida* Bge var. major) were harvested from commercial orchard in Shandong, China. The fruits were cleaned with distilled water, and then the pulp and seed were manually separated before use.

2.4. Extraction of soluble free and bound phenolic compounds

Fruit pulp was cut into small pieces, and free phytochemical contents were extracted, following the method Guo, Li, Tang, and Liu (2012) with some modifications. Briefly, 25 g of sample was blended with 150 mL of chilled 80% acetone (1:6, w/v) using a Waring blender (DS-1, Shanghai Specimen and Model Factory, Shanghai, China) for 5 min and further homogenized with a homogenizer (T25, IKA Co., Staufen, Germany) for another 4 min. The mixture was then centrifuged at 3500 rpm for 10 min, then filtered through Whatman No. 2 filter paper under vacuum, and the remaining residue was extracted twice as mentioned above. The supernatants were collected and concentrated to less than 5 mL with a rotary evaporator (Hei-VAP, Heidolph, Germany) under reduced pressure at 45 °C. The extracts were reconstituted in distilled water to a final volume of 25 mL and stored at –40 °C until use. All extractions were performed in triplicate for each sample.

Bound phytochemicals of fresh hawthorn pulp were extracted following the method Sun et al. (2002) with some modifications. Briefly, the residues obtained after extraction of free phytochemical contents were digested with 25 mL of 4 M NaOH at room temperature for 1 h while shaking under nitrogen. The mixture was acidified to pH 2.0 with concentrated hydrochloric acid and extracted five times with ethyl acetate. The ethyl acetate fractions were pooled and evaporated at 45 °C to dryness before reconstituting in 25 mL of water and then stored at –40 °C until use.

2.5. Determination of total phenolics

The total phenolic contents were measured by colorimetric Folin–Ciocalteu method with some modifications (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, an aliquot (0.1 mL) of diluted fruit extracts was mixed with 0.4 mL of distilled water and 0.1 mL of Folin–Ciocalteu reagent. After incubating for 6 min, 7% Na₂CO₃ (1.0 mL) and 0.8 mL distilled water were added and the mixture was allowed to stand for 90 min at room temperature. The absorbance was measured at 760 nm using a Nucleic acid/Protein analyzer (Du730, Beckman Coulter, USA). The total phenolic contents were determined as milligram gallic acid equivalents per 100 g on dry weight basis (mg GAE/100 g DW).

2.6. Determination of total flavonoids

The total flavonoid contents were determined using the sodium borohydride/chloranil protocol (SBC) (Malta, Tessaro, Eberlin, Pastore, & Liu, 2013). After drying under nitrogen gas, phytochemical extracts were reconstituted in 1 mL of tetrahydrofuran/ethanol (THF/EtOH, 1:1, v/v). Catechin hydrate standard (0.3–10.0 mM) was prepared fresh in 1 mL of THF/EtOH. Each test tube (15 × 150 mm) with sample or standard solution was mixed with 0.5 mL of 50 mM NaBH₄ solution and 0.5 mL of 74.6 mM AlCl₃ solution, and was shaken in an orbital shaker at 180 rpm for 30 min at room temperature, following by shaking for another 30 min after adding an additional 0.5 mL of NaBH₄ solution. Chilled acetic acid solution (2.0 mL of 0.8 M, 4 °C) was added into each test tube and kept in the dark for 15 min after thorough mixing. Then 1 mL of 20.0 mM chloranil was added into the mixture and heated at 99 °C with shaking for 60 min. The reaction solutions were cooled with tap water immediately, and volume was brought to 4 mL with methanol. After mixing with 1 mL of 16% (w/v) vanillin, the reaction solution was mixed with 2 mL of 12 M HCl and kept in the dark for 15 min. The reaction mixture were centrifuged at 2500 rpm for 10 min before measuring the absorbance at 490 nm. The standard curve of different catechin hydrate concentration was used to calculate the total flavonoid content of

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