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Systematic study on phytochemicals and antioxidant activity of some new and common mulberry cultivars in China

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ABSTRACT

Mulberry is a delicious fruit with high nutritional value. However, there are significant differences in the nutritional values among different mulberry cultivars. Therefore, in this study, the composition of some new and common mulberry cultivars was compared and their potential antioxidant activity evaluated. HPLC and LC–MS analyses revealed that mulberry fruits are rich in polyphenols such as cyanidin-3-O-glucoside (1.25–3.35 g/kg), cyanidin-3-O-rutinoside (0.25–1.50 g/kg), rutin (0.01–0.12 g/kg) and quercetin (0.0068–0.0081 g/kg). Correlation studies explained that the antioxidant activity of mulberry cultivars was closely associated with phenolics, including flavonoids. However, weak correlation was observed between cellular reactive oxygen species (ROS) scavenging ability and bioactive components. Besides, principal component analysis was employed to compare the differences between mulberry cultivars. Concerning high content of nutritional components and antioxidant activity, Hanguo variety was found to be the most beneficial and commercial cultivar among all the tested varieties.

1. Introduction

Mulberry (*Morus* spp.) was domesticated thousands of years ago and is widely distributed across the world. It is adapted to a wide range of environmental conditions such as temperate, subtropical and tropical regions of Asia, Europe, North and South America, and Africa (Jiang & Nie, 2015). In most Asian countries, mulberry leaves were largely used for sericulture purpose. However, in most European countries mulberry trees were cultivated for fruit production rather than foliage (Gerasopoulos & Stavroulakis, 1997).

Despite being high nutritional and tasty, mulberry was also proven to have high medicinal values. The therapeutic importance of mulberry was well documented in various traditional Chinese medicinal records. Traditionally black mulberry fruits were used for the treatment of sore throat, anaemia, iron deficiency, tonsillitis and hypertension (Jiang, Xu, Liu, & Huang, 2011). Due to the use of mulberry fruits in large extent for various purposes, the study of chemical composition of mulberry fruits has been taken into more consideration. Researchers have ascertained that mulberry fruits are rich in phytochemicals such as ascorbic acid, phenolic compounds, anthocyanins and other flavonoid compounds (Ercisli & Orhan, 2008; Wu et al., 2013a).

Given that the presence of a large number of bioactive components, mulberry fruits were determined to have a broad range of biological activities such as antioxidant activity,

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anti-hyperlipidaemia, anti-atherogenic and anti-cancer effect (Chan et al., 2013; Chen et al., 2006; Natic et al., 2015).

Further studies on mulberry varieties suggested that the content of phytochemicals, flavonoids, anthocyanins, proteins, and fatty acids of mulberry is dependent on the type of cultivar. Despite the type of cultivar, the region and conditions applied for cultivation of mulberry varieties are known to have a significant impact on their composition and biological properties. Studies found that, sometimes even with the same genotype, mulberry varieties are likely to have differences among the same variety (Song et al., 2009). To the best of our knowledge, some mulberry cultivars such as "Da 10," "Hongguo," "Tianquan," "Taiwanguosang," and "Zhenzhubai" have been investigated so far (Isabelle, Lee, Ong, Liu, & Huang, 2008; Qi et al., 2014; Song et al., 2009). The anthocyanin contents of Da 10 (15.38–62.93 mg/g dry weight), Taiwanguosang (15.38–62.93 mg/g dry weight), Hongguo (14.73 mg/g dry weight), Tianquan (13.00 mg/g dry weight) and Zhenzhubai (0.09 mg/g dry weight) have been evaluated (Isabelle et al., 2008; Li et al., 2014; Song et al., 2009). However, there was no systematic approach on these cultivars about phytochemicals and antioxidant activity. In addition, some new mulberry cultivars such as "Mengsi," "J33," "Hanguo," "Riben," "Zhongshenyihao," "Guangdong" and "Xiaodianhong" have not been reported so far. Therefore, in the present study, some new and common mulberry cultivars have been systematically analysed concerning their phytochemicals and antioxidant activity.

Reactive oxygen species (ROS) is a series of oxygen free radicals which are generated during cell metabolism process including oxygen radicals such as superoxide ($O_2^{\bullet-}$), hydroxyl ($^{\bullet}OH$), peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), as well as some non-radical species. Overproduction of ROS leads to an imbalance in intracellular redox status, and further it is associated with the oxidative stress (Chen, Xu, Zhang, Li, & Zheng, 2016; Chen, Zhuang, Li, Shen, & Zheng, 2013). Some studies indicated that eruption of ROS could cause cytotoxicity and DNA damage (Chen, Feng, Huang, & Su, 2012; Chen et al., 2009). Hydrogen peroxide induced oxidative stress is a widely used model to study the oxidative stress. It is an oxidizing agent which can produce intracellular ROS, subsequently leading to oxidative damage (Swalwell, Latimer, Haywood, & Birch-Machin, 2012).

Earlier studies were conducted to evaluate the nutritional ingredient, polyphenol and antioxidant capacity in mulberry cultivars grown in different areas. Unfortunately, so far, there is no systematic report on differences in ROS scavenging ability and role of phytochemicals in a wide range of mulberry varieties. Moreover, as we know, there are no comparative studies on the phytochemicals composition, *in vitro* antioxidant and intracellular ROS scavenging capacity of these mulberry cultivars. Therefore, in this study, we aimed to evaluate nutrients and polyphenols composition and determine *in vitro* and intracellular antioxidant activity of various mulberry varieties. Our focus also admits correlation studies and principal component analysis, which may provide a relationship between bioactive compounds and antioxidant properties of mulberry cultivars, as well as guidance for fruit processing and selection for commercial cultivars with the high content of functional profiles as functional foods.

2. Materials and methods

2.1. Chemicals and materials

Sodium hydroxide, monobasic potassium phosphate, ascorbic acid, Folin–Ciocalteu reagent, chromatographic grade methanol and acetonitrile were purchased from Aladdin (Shanghai, China). 2,2 diphenyl-1-picrylhydrazyl radical (DPPH), 2-2′azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridy-S-triazine (TPTZ), 2′,7′-dichlorofluorescin diacetate (DCFH-DA), cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, rutin, quercetin, myricetin and dihydroxycoumarin were obtained from Sigma Chemicals (St. Louis, MO, USA). All other reagents were of analytical grade. Syringe membrane filters (13 mm, PTFE, 0.45 μm) were obtained from Supelco (Bellefonte, PA, USA).

2.2. Mulberry samples

Mulberry fruits of the following 12 cultivars were analysed: black mulberry cultivars (*Morus alba* L.) "Tianquan," "Mengsi," "J33," "Hanguo," "Riben", (*Morus atropurpurea* Roxb.) "Da 10," "Taiwanguosang," "Zhongshenyihao," "Guangdong" (*Morus multicaulis* Perr.), "Hongguo" and white mulberry cultivars (*M. atropurpurea* Roxb.) "Zhenzhubai" and "Xiaodianhong" were collected from farms of Zhejiang province, China in May 2015. Common varieties of mulberry included "Da 10," "Hongguo," "Tianquan," "Taiwanguosang," "Zhenzhubai"; new mulberry cultivars involved "Mengsi," "J33," "Hanguo," "Riben," "Zhongshenyihao," "Guangdong" and "Xiaodianhong" (Fig. S1). For each mulberry cultivar, three groups of samples were selected for uniformity in shape and colour as though they were picked from several different plants of the same cultivar. After harvesting, the fruits were kept on ice for several hours and then stored at −80 °C until analysis.

2.3. Sample preparation

Three replicates of ten grams of mulberry fruits were homogenized, followed by extraction with 100 mL of ethanol/water (70:30, v/v) in the dark at room temperature for 1 h. The extraction procedure was repeated twice, then the extracts were filtered, and samples were collected. Collected samples were further concentrated by rotary evaporation under reduced pressure at 40 °C for further analysis.

2.4. Determination of moisture, ash, pH, the titratable acid, total sugars, reducing sugars and ascorbic acid of mulberry fruits

Followed by AOAC standard procedures, the parameters such as moisture, ash, pH, the titratable acid, total sugars, reducing sugars were determined (AOAC, 1995). The vitamin C content of mulberry fruits was determined according to AOAC (1984) standard procedure.

2.5. Determination of total phenolic, total flavonoid and procyanidin contents in mulberry fruits

Total phenolic content (TPC) of mulberry fruits was determined by Folin–Ciocalteu method (Obanda, Owuor, & Taylor,