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Comparative study on phenolics and antioxidant property of some new and common bayberry cultivars in China

Wei Chen *, Jingcheng Zhao, Tao Bao, Jiahong Xie, Wenkang Liang, Vemana Gowd

Department of Food Science and Nutrition, Zhejiang Key Laboratory for Agro-Food Processing, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

Fruits tend to possess different nutritional values among different cultivars of same family. Therefore, in this study, the composition and antioxidant activity of some new and common bayberry cultivars were evaluated. Significant amounts of phenolics (0.47 to 2.31 g gallic acid equivalents/kg), flavonoids (0.34 to 2.08 g rutin equivalents/kg) and procyanidins (0.24 to 1.99 g catechin equivalents/kg) were found in all tested bayberry varieties. Anthocyanin cyanidin-3-glucoside and flavonol myricetin was detected in almost all cultivars, however, cyanidin-3-glucoside was not detected in new cultivar Xishanbai. Antioxidant activity of bayberry was highly correlated with contents of phenolics, including flavonoids. However, no positive correlation was observed between cellular reactive oxygen species scavenging ability and bioactive compounds. The new cultivar Yingsi was found to have higher levels of nutritional components and potent antioxidant activity among all tested cultivars.

1. Introduction

Endogenous sources such as mitochondria, peroxisomes, and inflammatory cell activations and exogenous sources including environmental agents, pharmaceuticals, and industrial chemicals are known to produce cellular reactive oxygen species (ROS), thereby producing oxidative stress (Klaunig, Kamendulis, & Hocevar, 2010). Oxidative stress aroused by ROS leads to DNA, protein and lipid damage (Chen, Zhou, & Zheng, 2015; Sekiya, Hiraishi, Touyama, & Sakamoto, 2008) which further leads to the development of chronic disorders and age-related diseases (Chen, Feng, Huang, & Su, 2012; Khansari, Shakiba, & Mahmoudi, 2009; Klaunig et al., 2010; Rahman, Hosen, Islam, & Shekhar, 2012). There are multiple antioxidant systems in humans which can protect the cells from oxidative stress. However, oxidative stress-induced cellular dysfunctions occurred when antioxidant defense system and DNA repair systems are overwhelmed (Rahman et al., 2012). Therefore, oxidative stress has become serious issue in human beings. The

* *Corresponding author.* Department of Food Science and Nutrition, Zhejiang University, 866 Yuhangtang Road, Xihu District, Hangzhou 310058, China. Fax: +86 571 88982191

E-mail address: zjuchenwei@zju.edu.cn (W. Chen).

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antioxidant defense system could be protected by supplementation of antioxidant molecules in form of diet and medication. So far, several drugs are available to enhance the antioxidant defense in humans. However, these drugs are in limited use due to their negative impact on human system (Hirose et al., 1998; Sun & Fukuhara, 1997). Therefore, there is a growing interest to combat the side effects of the drugs available for reducing oxidative stress leading to the development of green medicines as potent antioxidant molecules (Zhou et al., 2009), and they are considered as safeguard of human system. Plethora of studies state that consumption of polyphenol rich diet can be proposed as a healthy diet for the treatment of oxidative stress (Liu, Qi, Cao, & Li, 2014). According to World Health Organization (WHO) reports, 80% of the traditional medicines are found to be bioactive compounds which were extracted from plant sources (Krishnaiah, Sarbatly, & Nithyanandam, 2011).

Chinese bayberry *(Myrica rubra Sieb. et Zucc.)* is native to China with high health promoting nature, and largely cultivated in southern China since 2000 years (Chen et al., 2015). All parts of the Chinese bayberry plant were used in Chinese traditional medicine for various medicinal purposes (Sun, Huang, Xu, Li, & Chen, 2013). Bayberries are important sources of phytochemicals such as phenolic acids, anthocyanins, and flavonol glycosides. Most of the berry fruits were found to be rich in anthocyanins especially cyanidin 3-*O*-glucoside (C3G) and tend to be red or dark in color (Zhang et al., 2011). Fruit derived phytochemicals have received more attention due to their antioxidant property (Chen, Su, Xu, Bao, & Zheng, 2016a; Chen, Xu, Zhang, Li, & Zheng, 2016b; Fang et al., 2009) and their potent anti-inflammatory (Farrell et al., 2015), anti-obesity (Meireles et al., 2016), anti-hyperlipidemia (Jurgonski, Juskiewicz, & Zdunczyk, 2008; Valcheva-Kuzmanova et al., 2007), anti-atherosclerosis (Alarcon et al., 2015; Chan et al., 2014; Zhang, Zhang, Geng, & Geng, 2014), anti-diabetes (Sun et al., 2012; Zhang et al., 2016), and anti-cancer activities (Sun et al., 2012). Studies on bayberries and its associated processed foods such as juice and jam proved their potential antioxidant capacity (Fang et al., 2009; Sun et al., 2013; Szajdek & Borowska, 2008). Cyanidin 3-*O*-glucoside from bayberry had a significant antitumor activity in a dose dependent manner in gastric adenocarcinoma (Sun et al., 2012). Further studies on flavonoid extract of Chinese bayberry showed that it has a protective role against ethanol induced oxidative damage in mice liver (Liu et al., 2014). Recent findings from our laboratory revealed the protective effect of bayberry fruit extract on peroxynitrile-induced oxidative DNA damage and cytotoxicity (Chen et al., 2015). Researchers also pinpointed that, supplementation of red bayberry extract to mice fed with high fat diet showed protective role in prevention of high fat induced metabolic disorders (Yu, Cai, Zhang, Feng, & Huang, 2015). So far, there are considerable studies on phytochemicals and antioxidant properties of different cultivars of Chinese bayberry. However, there were no systematic and comparative studies on phytochemicals, *in vitro* antioxidant and intracellular ROS scavenging capacities of different cultivars of bayberry. Therefore, in this study, we aimed to systematically evaluate the phytochemicals and antioxidant properties of some common and new bayberry cultivars. Further, our study provides a correlation between bioactive compounds and antioxidant property of bayberry cultivars using Pearson's correlation coefficient studies and principal component analysis.

2. Materials and methods

2.1. *Chemicals and materials*

Ascorbic acid, 2,4-Dinitrophenylhydrazine, Folin–Ciocalteu reagent, vanillin, chromatographic grade methanol and acetonitrile were purchased from Aladdin (Shanghai, China). 2-2'azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2 diphenyl-1-picrylhydrazyl radical (DPPH), 2,4,6- tripyridy-S-triazine (TPTZ), 2',7'-dichlorofluorescin diacetate (DCFH-DA), gallic acid, rutin, catechin, cyanidin-3-O-glucoside and myricetin were obtained from Sigma Chemicals (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. *Bayberry samples*

Following 4 common Chinese bayberry *(M. rubra Sieb. et Zucc.)* cultivars such as "Biqi", "Dongkui", "Fenhong", "Xianju" and 4 new bayberry cultivars such as "Yingsi", "Xishanbai", "Heitan", "Ruansi" were used in the present study (Supplementary Fig. S1). Some of the bayberry cultivars namely "Biqi", "Dongkui", "Fenhong", "Xianju", "Xishanbai" and "Heitan" were collected from farms of Zhejiang province, China in June 2015. Remaining cultivars such as "Yingsi" and "Ruansi" were collected from farms of Fujian province, China in June 2015. All bayberries were harvested randomly according to shape and uniform color at their commercial maturity stage. After harvesting, the fruits were kept on ice for several hours and then stored at –80 °C until analysis.

2.3. *Preparation of samples*

Samples were prepared in triplicates. Fifteen grams of bayberry fruits were homogenized, followed by extraction with 150 mL of ethanol/water (70:30, v/v) at room temperature for 1 h in dark. The extraction procedure was repeated twice, then the extracts were filtered, and the solution was collected. Collected solution was further concentrated at 40 °C for 30 min by rotary evaporation under reduced pressure (0.1 MPa) for further analysis.

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

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

2.5. *Determination of total phenolic, total flavonoid and procyanidins contents in bayberry fruits*

Total phenolic content (TPC) of bayberry fruits was determined by Folin–Ciocalteu method (Chen, Xu, Zhang, Li, & Zheng,