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Assessment of flavonoids and volatile compounds in tea infusions of water lily flowers and their antioxidant activities

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ABSTRACT

Water lily, a member of the Nymphaeaceae family, can be made into tea on the basis of outstanding fragrance characteristics and health care functions. In this study, 16 flavonoids were identified and quantified in tea infusions prepared from the petals of 33 water lily cultivars using HPLC–DAD and HPLC–ESI-MS/MS. The infusions were analyzed with HS-SPME coupled with GC–MS; 29 volatile compounds were detected, of which nine were found to be scent components. The cultivars were clustered into three clusters characterized according to scent components. The 'Conqueror' and 'Virginia' cultivars had the highest antioxidant activities. The concentrations of polyphenols and flavonoids showed significant positive correlations with antioxidant activity as measured by DPPH$^{\cdot}$, ABTS$^{\cdot+}$, and FRAP assays. This study is valuable for a fuller understanding of this important tea and can also be used for the development of water lily.

1. Introduction

Water lily (a herb of the genus *Nymphaea*, family Nymphaeaceae), a perennial aquatic flower plant, is divided into two ecological groups based on geographic occurrence, namely the tropical water lily group and the hardy water lily group (Huang, Deng, Li, & Li, 2009). Water lily is widely used as an ornamental plant in landscaping and is also used in water purification applications. Water lily flowers, roots and stems are also used in the medical domain, and have been shown to have anesthetic, roborant, astringent, and diuretic properties, and are used as a therapy for nephritis (Agnihotri et al., 2008; Daboor & Haroon, 2012). Totally, 74 flavonoids and phenolic acid compounds have been isolated from genus *Nymphaea* in previous works (Zhao, Xu, Ji, Gu, & Li, 2014). Due to the polyphenols that it contains, water lily is regarded as a natural source of antioxidant (Kerio, Wachira, Wanyoko, & Rotich, 2013). Scented tea, a unique category of Chinese tea, is made from elegant and aromatic flowers as well as tea. It therefore has both full-bodied fragrance and a deep tea infusion color. Water lily has great developing potential in the functional process of tea beverage industry with both high ornamental value and health care functions.

Flavonoids, which occur widely in plants, are an important class of plant natural products. Many flavonoids have exhibited obvious physiological activities in the human body, such as anti-ulcer, antibacterial, anti-inflammatory, anti-hyperlipidemia, and analgesia activities (Zafra-Stone et al., 2007). Research by Zhao et al. (2014) showed that water lily contains polyphenols such as kaempferol, quercetin, and gallic acid, compounds known to extend the shelf life of food, inhibit lipid oxidation, restrain bacterial growth, resist aging, and prevent tumor growth and cardiovascular disease. Additionally, water lily contains quercetin and chalcone compounds that have been reported to possess antioxidant and antitumor activity (Nafisi, Hashemi, & Rajabi, 2008; Yagura et al., 2008). According to Agnihotri et al. (2008), the EtOAc fraction of *Nymphaea caerulea* flowers can be used as a new natural product for the treatment of oxidative stress-related diseases. Previous studies in this vein have focused primarily on water lily itself, paying little attention to teas prepared from water lilies (Yuan et al., 2014; Zhu, Wang et al., 2012; Zhu, Zheng et al., 2012). Therefore, it is of great significance to identify the flavonoids and evaluate the antioxidant activity in tea infusions of

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water lily in order to lay a scientific foundation for the development and utilization of water lily tea.

Tea fragrance is one of the most important factors impacting the characteristics and quality of tea, contributing from 25% to 40% of organoleptic quality (Wang, 2006). Since water lily contains abundant volatile compounds with desirable fragrances, its contributions to tea infusions merit study (Yuan et al., 2014). Volatile compounds in tea contribute to its fragrance and include alcohols, hydrocarbons, aldehydes, ketones, esters, nitrogenous compounds, and phenolic compounds (Du et al., 2014). It is important to characterize how the scented components differ from volatile compounds in water lily tea. Solid-phase microextraction (SPME) in headspace mode (HS) has an excellent performance record for the extraction and analysis of volatile compounds. It is an ideal sample preparation technique because it is fast, solvent-free, and cost-effective (Yang, Baldermann, & Watanabe, 2013).

In this study, we identified and quantified a number of flavonoids from tea infusions of 33 water lily cultivars using high-performance liquid chromatography (HPLC) with photodiode array detection (DAD) and electrospray ionization mass spectrometry (ESI-MS). Assays of 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot+}$), and ferric reducing antioxidant potential (FRAP) are widely used to detect the antioxidant activity of extracts or single material (Benzie-Iris & Strain, 1996; Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). SPME–GC–MS was used to analyze volatile compounds in the water lily tea infusions. Furthermore, the 33 cultivars were classified into three groups using cluster analysis based on specific scent components and associated fragrances. Additionally, we employed three assay types to evaluate the relationships of total polyphenol content (TPC) and volatile compounds with antioxidant activities. We anticipate that this study will help characterize the edible and medicinal value of water lily and provide a theoretical foundation for the development of water lily scented tea in the future.

2. Materials and methods

2.1. Plant materials

Thirty-three water lily cultivars were used in this study and they were divided into two ecological groups. The tropical water lily group included 21 cultivars: ‘Steven Strawn’ (1), ‘Ray Davies’ (2), ‘Lausanne’ (3), ‘Arethusa’ (4), ‘Perry’s Baby Red’ (5), ‘Aflame’ (6), ‘Alba’ (7), ‘Princosi’ (8), ‘Somptuosa’ (9), ‘Venusta’ (10), ‘Conqueror’ (11), ‘Hollandia’ (12), ‘Madame Wilfron Gonnere’ (13), ‘Sultan’ (14), ‘Rembrandt’ (15), ‘Marliacea Carnea’ (16), ‘Newton’ (17), ‘Celebration’ (18), ‘Rene Gerard’ (19), ‘Ellisina’ (20), and ‘Virginia’ (21). The hardy water lily group included 12 cultivars: ‘9’ (22), ‘Roxburgh’ (23), ‘Ai Ji Bai’ (24), ‘Lan He’ (25), ‘18’ (26), ‘America’ (27), ‘Hu Die Lan’ (28), ‘Subra’ (29), ‘Royal Purple’ (30), ‘Mexicana Zuccarni’ (31), ‘Zanzibar’ (32), and ‘Fo Shou Lian’ (33). Numbers in parentheses indicated the number of water lily cultivars in Fig. 2, Tables 2 and 3. The plants were grown in the lotus germplasm nursery of the Beijing Botanical Garden at the Institute of Botany of the Chinese Academy of Science (IBCAS) in Beijing, China. These cultivars originated from locations all over the China. Each cultivar was planted in the same size cylinder (diameter 40 cm; height 30 cm) and placed in the same sized pool (140 cm × 140 cm × 70 cm). The cultivation conditions included fertilization, irrigation, and pest control measures, and these were the same for each cultivar. Three fully expanded flower petals (the first day after flowering) as three repeats were collected from each cultivar in the morning between five and seven o’clock and immediately processed; sampling took place in May of 2013. In order to facilitate the comparison analyses, petals were freeze-dried for 12 h. 0.5 g of dry petals, chosen at random from each entire flower, was steeped for 10 min in 100 ml of hot water. These and subsequent procedures were repeated three times for each cultivar.

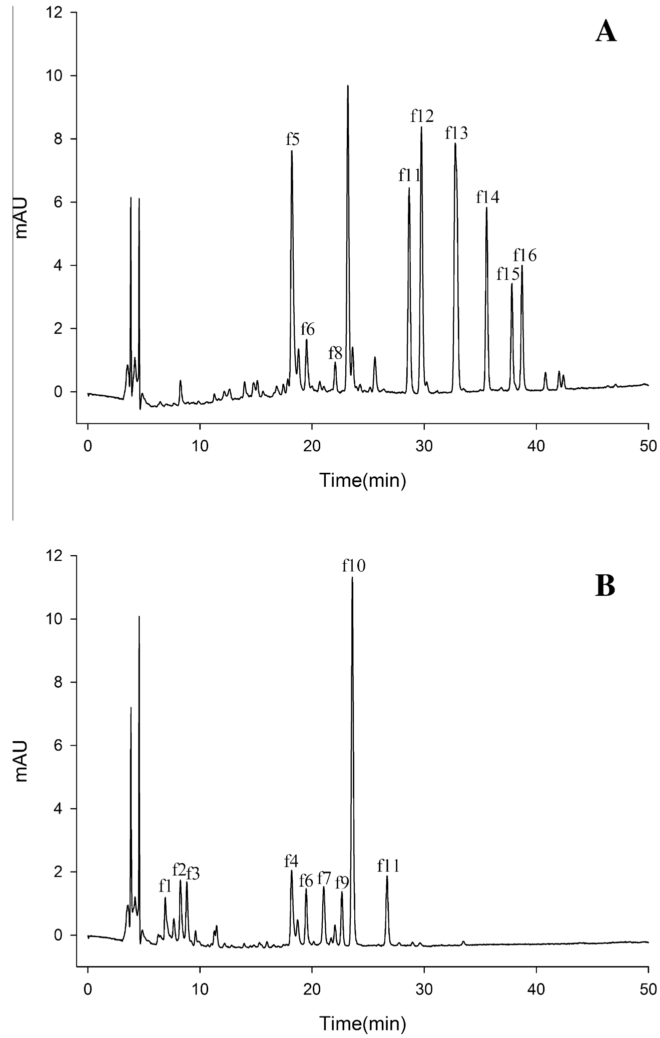

Fig. 1. HPLC chromatograms at 350 nm of water lily tea infusions. The upper figure is the chromatogram of the ‘Lausanne’ cultivar (A). The lower figure is ‘LanHe’ (B). Peak identification information is detailed in Table 1.

2.2. Collection of volatiles

Ten milliliters of the tea infusion soup was aliquoted into a brown glass vial (40 mL) and enclosed with a plastic cap with a polytetrafluoroethylene septum. Volatile compounds were extracted by headspace SPME using a 100 μm polydimethyl-siloxane coated fiber attached to a manual SPME holder (Supelco, Bellefonte, PA, USA). The fiber was exposed to each sample by manually penetrating the septum for 30 min at room temperature (20 °C). The fiber was then introduced into the injection port of the GC, which was set at 250 °C. The fibers were conditioned in the GC injection port for 30 min at 250 °C before volatile collection. The carrier gas flow rate was 0.9 mL min^{-1} without diversion.

2.3. SPME–GC–MS

The analysis was carried out using a gas chromatograph-mass spectrometer (GC6890N/MS5973, Agilent) fitted with a HP-5MS capillary column (5% phenyl methyl siloxane, 30 m × 0.25 mm