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Comparative analyses of flavonoids compositions and antioxidant activities of Hawk tea from six botanical origins

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ABSTRACT

The aim of this study was to compare the flavonoids compositions, content and antioxidant activities of Hawk tea from six botanical origins for screening the most suitable botanical origins. The main flavonoids of Hawk teas from *Litsea coreana* Levl. var. lanuginose were identified, and six high contents of individual flavonoids (hyperin, isoquercitrin, quercitrin, astragalin, quercetin and kaempferol) were quantitative and comparative analysis. Moreover the potential antioxidant capacity of Hawk tea was measured by using in vitro antioxidant assays. The above experimental data were all used for the principal component analysis. The statistical results showed that the extracts from *Machilus chuanchienensis* S. Lee and *L. coreana* Levl. var. lanuginose appeared closely positioned in the positive regions, indicated the higher flavonoids and antioxidant activity compared with other species. Thus, they were temporarily considered as good sources of Hawk tea.

1. Introduction

Tea is one of the most popular soft drink in the world. Since twenty-first Century, the annual consumption of tea in the world has been stable at around 2.5 million tons, and it is an important cash crop (Gupta et al., 2014). Tea is recognized as one of the healthy food, and the healthy benefits have been confirmed, including antioxidant, anti-inflammation, anti-carcinogenesis, cardiovascular health, oral health and antimicrobial (Gomes et al., 1995; Jigisha et al., 2012). As the tea birthplace, China has the rich tea resources. While black, green and Oolong tea dominates in china, where tea drinking is a lifestyle and a ritual. In many minority areas, it is fashionable to drink certain kinds of herbal teas or fruit teas such as bitter buckwheat tea, medlar leaves tea and Kuding tea et al. (Zeng et al., 2013). Herbal teas are hot water infusions of fresh or dried plant parts: roots, leaves, fruits, or grains of plants. Previous studies also proved that some herbal teas had excellent antioxidant capacities compared with black tea (Dalar and Konczak, 2013; Deetae et al., 2012). Thus, it is a kind of important resource worth of exploitation and utilization.

Hawk tea, an edible and medicinal plant, is a traditional herbal infusions in southwestern China for thousands of years (Yinhai and Xincheng, 1998). The infusion of Hawk tea smells a slight camphor-aromatic and it also has effects on the reduction in blood lipid,

Abbreviations: DPPH, 1,1-dipheny2-*b*-picrylhydrazyl; ABTS, 2,2-azobis-3-ethylbenzthiazoline-6-sulphonicacid; EDTA, ethylenediaminetetraacetic acid; TCA, trichloroacetic acid; TBA, 4,6-dihydroxy-2-mercaptopyrimidine; BHT, Butylatedhydroxytoluene; VC, ascorbic acid; HPLC–DAD, high-performance liquid chromatography with diode array detector; LC–ESI–QTOF–MS–MS, HPLC-electrospray ionisation hyphenated with tandem mass spectrometry.

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blood sugar, detumescence, eyesight protection and digest promotion. Furthermore, chemical research shows that Hawk tea contains flavonoids, saponins, organic acid, coumarin and tannin, of which flavonoids have the high content and are proved to be the main active ingredient of the hawk tea (Jia et al., 2013; Wang et al., 2012). Hawk tea is mainly made from buds of *Litsea coreana* Levl. var. lanuginose leaves. However, many other material plants, for example, *Machilus chuanchienensis* S. Lee, *Actinodaphne cupularis* (Hemsl) Gamble, *Machilus rehderi* Allen, *Litsea pungens* Hemsl and *Lindera communis* Hemsl were are also used as the plant source of Hawk tea in Guizhou and Sichuan regions. The use of different cultivated varieties led to a confusion of the botanical origin of Hawk tea, blocking the establishment of uniform quality standards and further product development for Hawk tea. So far, no in-depth comparative investigation has been carried out on the chemical composition and biological activity among different botanical origins of Hawk tea.

In this paper the composition of the main flavonoids of Hawk tea from *L. coreana* Levl. var. lanuginose were identified, and the high contents of individual flavonoids were quantitative and comparative analysis. Additionally, the antioxidant activities of the six botanical origins of Hawk tea extracts were evaluated by multiple antioxidant assays including ABTS, DPPH and Hydroxyl radical scavenging capacities, ferric reducing power and inhibition properties against linoleic acid peroxidation. Besides, the principal component analysis (PCA) was conducted due to large number of variable parameters in studies. The aim of our research is to compare and analyze the flavonoids composition, content and the antioxidant capacity of Hawk tea extracts with different botanical origins. This study may provide important references to choose the most suitable botanical origin of Hawk tea and to standardize the Hawk tea resource, which can lay the foundation for the development of the hawk tea resources.

2. Materials and methods

2.1. Chemicals materials

Rutin, quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, quercetin-3-rhamnoside, quercetin, kaempferol and astragaline (purity 99.0% each) were purchased from the Natl. Inst. (Beijing, China). DPPH, VC, 2-deoxy-D-ribose, β-carotene, Tween 40, BHT, ABTS and linoleic acid were purchased from Aladdin Co. (California, USA). TCA, ferric chloride, ammonium thiocyanate, EDTA, potassium ferricyanide, TBA were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methanol and acetonitrile for HPLC–DAD and LC–ESI–QTOF–MS–MS analysis were of chromatographic grade and purchased from Alltech Scientific (Beijing, China). The remaining reagents were of analytical grade and purchased from Chongqing Chemical Works Co., Ltd. (Chongqing, China).

2.2. Plant material and extraction

All of the six young leaf samples were picked from Youyang County, Chongqing City, China and authenticated by Professor Dan Zhang (Chongqing Medical University). The leaves were air dried in shade and powdered using household food processing machine. After that, 5 g of the sample powder was fully mixed with 50 mL of 70% ethanol aqueous for 2 h at room temperature, followed by the reflux for 2 h. After cooled to room temperature, the mixture was filtered and the residue was re-extracted twice. The pooled extract was concentrated by a rotary evaporator under vacuum at 60 °C. The volume of concentrate was adjusted to 50 mL and stored in dark at 4 °C for further analysis. All samples were extracted in triplicate.

2.3. Identification of flavonoid compounds by LC–ESI–QTOF–MS–MS

According to previous studies, flavonoid compounds were the main active substance of Hawk tea (Yu and Gu, 2001), so the flavonoids compounds of the main variety (*L. coreana* Levl. var. lanuginose) were identified by a Triple TOF 5600 mass spectrometer (AB Sciex, Framingham, MA, USA), which was equipped with anelectro spray ionization (ESI) source scanning in the positive ion mode with spectra acquired over a mass range from *m*/*z* 200–800. The mobile phase used for flavonoid analysis was acetonitrile (A) and 0.1% aqueous formic acid solution (B). Gradient program was: 0–25 min, 11–13% A; 25–40 min, 13–16% A; 40–50 min, 16–18% A; 50–60 min, 18–32% A; 60–70 min, 32–40% A; 70–73 min, 40–11% A; 73–75 min, 11% A. The MS condition was as followed: capillary voltage was set to 5.5 kV (ESI+) or −5.5 kV (ESI−); gas 1 nitrogen (55 psi); gas 2 nitrogen (55 psi); ion-spray voltage (IS) 5500 V; ion-source temperature 500 °C; curtain gas: nitrogen (10 psi); collision energy (CE) was optimized to 10 and −10 V; declustering potential (DP) voltage was optimized to 80 and −80 V. Data were obtained across the *m*/*z* 50–1000 mass range with an accumulation time of 0.01 s. The mass spectrometer was controlled by Analyst® TF 1.6 software (AB Sciex) and the accurate mass data for the molecular ions were processed by PeakView® 1.2.0.3 software (AB Sciex).

2.4. Comparative and quantitative analysis of flavonoids compositions

Based on the identification results by LC–ESI–QTOF–MS–MS, the high contents of individual flavonoids (quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, quercetin-3-rhamnoside, quercetin, kaempferol and astragaline) were used for quantitative analysis. The samples were analyzed by LC2010A HT HPLC with a DAD photodiode array detector (Shimadzu, Kyoto, Japan). An ODS-AP column (150 × 4.6 mm, 5 μm i.d., SinoChrom, Dalian, China) was used at the flow rate of 1 mL/min with a 10 μL of injection volume. The column oven temperature was set at 30 °C. UV absorption spectra were conserved for flavonoid at 350 nm during HPLC analysis. The chromatographic separation condition was the same with the identification of flavonoid compounds with some modification.

2.5. Determination of total flavonoids content

Total flavonoids content in the Hawk tea extract was determined according to the method (Karadeniz et al., 2005). Rutin solutions (10–200 μg/mL in methanol) were used for the standard calibration curve. To start the reaction, a mixture (8 mL) of 50% ethanol as well as 1 mL of 5% sodium nitrite was added with 1 mL 10% aluminum nitrate. After 6 min incubation at room temperature, 10 mL of sodium hydroxide and 3 mL of double distilled water were added to the mixture. The solution was mixed thoroughly and the absorbance was read at 510 nm with a Shimadzu UV-1750 spectrophotometer. All the samples were analyzed in triplication.

2.6. Antioxidant activity

2.6.1. Scavenging effect on DPPH radical

The scavenging activity against DPPH radical by Lauraceae extract was evaluated according to a known protocol (Brand-Williams et al., 1995) with some modifications. Preparation of the sample extracts (200 μL) was mixed with DPPH (800 μL) solution in methanol. Ultrapure water was used as control instead of extract. Each mixture was shaken vigorously and incubated in dark at room