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# A comparative analysis of chemical compositions in *Camellia sinensis* var. *puanensis* Kurihara, a novel Chinese tea, by HPLC and UFLC-Q-TOF-MS/MS

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ABSTRACT

*Camellia sinensis* var. *puanensis* Kurihara (Puan tea) is a kind of ancient tea plant newly found in Jiangxipo and the surrounding areas of Puan County (Guizhou, China). People there always believe that drinking Puan tea is beneficial to the promotion of health and prevention of diseases. However, detailed information on its compositions has not been reported. Therefore, in this study, the varieties and contents of purine alkaloids and polyphenols in Puan tea were identified and determined by HPLC and UFLC-Q-TOF-MS/MS. Our results showed that theacrine, but not caffeine, was the dominated purine alkaloid detected in Puan tea. Meanwhile, Puan tea contained B-type procyanidin dimer, trimer and dimer monogallate, which were not detected in *Camellia sinensis*, *Camellia ptilophylla* and *Camellia assamica* var. *kucha*. The obtained results could support the local uses of Puan tea in health and nutrition and contribute to the research of tea variety.



## 1. Introduction

Tea is the most popular and widely consumed beverage and has become one of the three major non-alcoholic drinks in the world. Drinking tea is believed to prevent several diseases, such as cardiovascular disease (Zhang et al., 2015), cancer (Wang, Zhang, Liu, Shen, & Li, 2014), depression (Dong et al., 2015), and help to reduce body weight (Chen, Liu, Chiu, & Hsu, 2016). The chemical compositions of tea are diversity, purine alkaloids and polyphenols are the main bioactive compounds in tea which have been widely reported to play important roles in reducing the risk of diseases (Higdon & Frei, 2003; Zhu et al., 2004). Caffeine and theobromine are the most familiar purine alkaloids in tea (Del Rio et al., 2004; Ye, Lin, Zhou, Chen, & Li, 1996). Theacrine, a novel purine alkaloid that is less common in tea beverages, has widely caused concern in recent years (Zheng, Ye, Kato, Crozier, & Ashihara, 2002). Meanwhile, the main polyphenols in tea include (−)-gallocatechin gallate (GCG), (−)-epigallocatechin gallate (EGCG), gallic acid (GA), (+)-catechin (C), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and (−)-epicatechin gallate (ECG) (Del Rio et al., 2004).

Different chemical compositions, which mainly affected by climate, environment and disposal processes, determine the features of different kinds of tea. For example, *Camellia sinensis* (*C. sinensis*) abundantly contains caffeine, but contains less theobromine and theophylline (Peng, Song, Shi, Li, & Ye, 2008). High contents of caffeine in *C. sinensis* make it possess the central excitability (Gramza-Michałowska, 2014). *Camellia ptilophylla* (*C. ptilophylla*) mainly contains theobromine but contain less caffeine, which contributes to its function of reducing blood pressure and less central excitability (Mitchell et al., 2011; Yang et al., 2011). The dominant purine alkaloid in a new camellia species named *Camellia assamica* var. *kucha* (*C. assamica* var. *kucha*) is theacrine. This novel purine alkaloid in *C. assamica* var. *kucha* has been reported to possess multiple psychoactive properties (Feduccia et al., 2012; Li et al., 2015a, 2015b;

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Tsoi et al., 2015; Wang et al., 2010). In addition, the contents and varieties of polyphenols also make contribution to the diversity functions and tastes of different species of tea (Cheynier, 2005; Lesschaeve & Noble, 2005). All that highlighted the great values of discovering and developing of the novel species of tea.

*Camellia sinensis* var. *puanensis* Kurihara (Puan tea) is an ancient tea plant newly found in Jiangxipo and the surrounding areas of Puan County, Guizhou Province, China. It has become an important and popular drinking tea at local with long history. People there always hold a belief that its intake is beneficial to the promotion of health and prevention of diseases. However, because of its narrow and special distribution, there is still less research on its various aspects including chemical synthesis and chemical compositions. Therefore, as a novel Chinese tea, it is necessary to reveal its chemical compositions.

In this study, the major goal is to analyze the chemical compositions of Puan tea. High performance liquid chromatography (HPLC) for quantitative analysis was conducted to determine the varieties and contents of purine alkaloids and polyphenols in Puan tea, which was compared with *C. sinensis*, *C. ptilophylla* and *C. assamica* var. *kucha*. Ultra fast liquid chromatography (UFLC) coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS/MS) was carried out for qualitative analysis.

## 2. Materials and methods

### 2.1. Chemicals and materials

Caffeine, theobromine, GCG, EGCG were purchased from Wako Pure Chemical Co. (Osaka, Japan). GA and C were purchased from Sigma Chemical Co. (St Louis, MO, USA). EC, EGC and GC were purchased from Kurita Co. (Tokyo, Japan). ECG was purchased from Shanghai Jingchun Scientific Co. (Shanghai, China). Theacrine was self-prepared by extraction of *C. assamica* var. *kucha* as we described previously (Xu, Kurihara, Zhao, & Yao, 2007). HPLC-grade formic acid was purchased from Dima Technology Inc. (Beijing, China). HPLC-grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA, USA). HPLC-grade water generated by Milli-Q system (Millipore, Bedford, MA, USA) was used for the preparation of the HPLC mobile phase. All other agents were commercially available.

### 2.2. Preparation of standard solution

Standard solutions of GA, EGC, theacrine, C, caffeine, EC, EGCG, GCG, ECG, GC, theobromine were prepared by dissolving them in HPLC-grade water to generate stock concentrations of 2.10, 0.82, 2.08, 1.01, 2.29, 2.67, 0.73, 0.58, 0.49, 0.56 and 0.37 mg/mL, respectively. The working solution contained 150 μL of GA, EGC, theacrine, C, caffeine, EC, EGCG, GCG; 200 μL of ECG, GC; and 300 μL of theobromine. HPLC-grade water was added to the solution to make up a 2 mL solution. It was diluted to obtain a series of standard solution.

### 2.3. Collection and pretreatment of the tea sample

Puan tea and *C. sinensis* were collected in Puan County, Guizhou Province, China. *C. ptilophylla* was collected from Nankun Mountain in Longmen County, Guangdong Province, China. *C. assamica* var. *kucha* was collected in Jinping County, Yunnan Province, China. Puan tea was authenticated by Prof. Guang-Xiong Zhou (Department of Pharmacognosy, College of Pharmacy, Jinan University, Guangzhou, China). Voucher specimen (KH-201503-2) was maintained in Institute of Traditional Chinese Medicine and Natural Products of Jinan University. All the fresh tea leaves were steamed and then dried for the following experiments.

### 2.4. Preparation of tea sample solution

Dry leaves were ground using a ceramic mortar with pestle and sifted through a 20-mesh sifter immediately before use. Powdered tea leaves (0.1 g) were extracted in 15 mL of 90 °C distilled water, and placed in a hot water bath for 40 min. The precipitate was removed by centrifugation at 12,000*g* for 15 min after cooling. The supernatant of four tea leaves extracts was then filtered by a 0.45 μm nylon filter and analyzed by HPLC.

### 2.5. Chromatographic conditions of HPLC

The chromatographic conditions were conducted following Li et al. (2013) with a slight modification. The HPLC system (Dionex, America) consisted of pumps, auto-sampler, DAD detector and a column heater. The separation was performed on a COSMOSIL 5C18-AR-II column (4.6 × 250 mm, 5 μm, Nacalai tesque. Inc., Tokyo, Japan). The sample was detected by an UV detector at 231 nm, with a flow rate of 1.0 mL/min. The injection volume of sample was 20 μL and column temperature was 35 °C. The mobile phases were composed of acetonitrile (A) and water with 0.1% formic acid (B) using a gradient elution of 4–6% A at 0–20 min, 6–22% A at 20–65 min.

### 2.6. Method evaluation

The standard curves of three purine alkaloids and eight polyphenols were constructed using six different standard concentrations. Detection limit and system adaptability were assessed using the standard compounds. Detection limit was based on a 3-fold signal-to-noise ratio under the selected conditions. Recovery tests were performed by adding known amounts of caffeine and EGCG, which are the representative purine alkaloid and polyphenol in tea extracts, to the six tea samples with known content. Then tea samples were prepared to sample solution according to the above method and analyzed under the selected chromatographic condition. The percentage of recovery was calculated by comparing the measured amount of standards with the spiked amount. In order to evaluate the repeatability of the method, five independent same tea samples were precisely weighed, prepared to sample solution according to the above method and injected twice in parallel under the selected condition. The accuracy of the apparatus was evaluated by analyzing a tea sample solution repeatedly for five times under the selected condition. The stability of method was evaluated by analyzing tea sample solution prepared and stored at 4 °C at 0, 3, 6, 12 and 24 h respectively under the selected condition. Measurements of the accuracy of apparatus, the repeatability of method and the stability of method were calculated and expressed as relative standard deviation (RSD). RSD (%) = (SD/mean) × 100.

### 2.7. UFLC-Q-TOF-MS/MS analysis

Chromatographic analysis was performed on a Shimadzu UFLC XR instrument (Shimadzu Corp., Kyoto, Japan). The column was a Phenomenex Kinetex C18 column (2.1 × 100 mm, 2.6 mm, Phenomenex, CA, USA). Column temperature was 35 °C. The mobile phases were composed of acetonitrile (A) and water with 0.1% formic acid (B) using a gradient elution of 4–6% A at 0–4 min, 6–22% A at 4–15 min, with the flow rate at 0.3 mL/min. The injection volume of sample was 20 μL. Mass spectrometry was performed on the Triple TOFTM5600 (AB SCIEX, Foster City, CA) a hybrid triple