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Identification of triterpenoid saponins in flowers of four *Camellia Sinensis* cultivars from Zhejiang province: Differences between cultivars, developmental stages, and tissues

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ABSTRACT

Triterpenoid saponins are important bioactive compounds in tea flowers. Using ultra-high performance chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry, a total of 21 triterpenoid saponins were identified and characterized from tea flower extracts of selected cultivars from Zhejiang province. A comparison of the seven main saponins was performed in the four main Zhejiang tea flower samples at different developmental stages. High accumulation of saponins at the early stages was observed in all tea flower cultivars. This may be explained, at least partly, by the high levels of saponins in petals at the green bud stage, when petals constitute over 50% of the whole flower weight. Floratheasaponin A and floratheasaponin D were the most abundant saponins in the flowers of Longjing NO.43, Baiye NO.1, and Jiaming NO.1, which is similar to results with tea flowers from Anhui province. Considerable amounts of chakasaponins I and II were found in Yingshuang flowers, as in the parent cultivar, Fudingdabai, and tea flowers from Fujian province. The quantified saponins profiles were analyzed by principal component analysis (PCA) and hierarchical clustering analysis (HCA) and successfully discriminated between all tea flower samples.

1. Introduction

Saponins are a group of naturally occurring glycosides in higher plants which have strong foam-forming properties in aqueous solution. These are important for providing defense against pathogens and herbivores and anti-fungal, anti-microbial, insecticidal and molluscicidal activities have been observed in numerous saponins studies (Augustin et al., 2011). Triterpenoid saponins are one of the biggest subclasses in the plant kingdom and are widely distributed in *Leguminosae*, *Araliaceae*, *Scrophulariaceae*, *Campanulaceae*, *Caryophyllaceae* and *Theaceae* (Man et al., 2010). In spite of the great variation in chemical structures, triterpenoid saponins have been shown to exhibit excellent anti- tumorigenic effects in numerous studies (Dinda et al., 2010).

Research on *Theaceae* saponins started in 1931, when the saponins in the seeds of *Camellia sinensis* were first separated and named "Theasaponin". In recent years, over 60 kinds of triterpenoid saponins, defined as oleanane-type saponins, have been isolated and characterized in the leaves, seeds, roots, and flowers of *Camellia sinensis* (Xia et al., 2011). Because of the special interest in this plant, the numbers of saponins is updated each year. Saponins are present at high concentrations in seeds and flowers of *Camellia sinensis* (Xia et al., 2011), and tea flowers were used as a food garnish to form foam in Japanese-style dishes in ancient Japan, and have been incorporated into a range of drinks in China, Japan, and India. In addition to being an abundant agricultural by-product, tea flowers are used to produce a well-accepted drink, and have attracted the attention of researchers for the content of functional compounds and medicinal effects in recent years. Triterpenoid saponins are ubiquitous and abundant in tea flowers from China, Japan, and India; these have been isolated and characterized as a vast heterogeneous group of compounds which have been studied extensively for the bioactive properties. Saponins in tea flowers exhibit a wide variety of pharmacological activities, including gastro-protection (Yoshikawa et al., 2008a, 2009), hypoglycemic effects (Yoshikawa et al., 2008a), anti-hyperlipidemia (Yoshikawa et al., 2005, 2008a), anti-allergia (Yoshikawa et al., 2007), and pancreatic lipase inhibition (Yoshikawa et al., 2009).

The structure of tea flowers saponins is characterized by an oleanane-type sapogenin, linked with tigloyl, angeloyl, and acetyl moieties in the C21 and C22 positions. The 21, 22-acyl groups in floratheasaponins A–C are described as being essential for the

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Table 1
Nomenclature and scientific names of five cultivars analyzed.

Sample name	Abbreviation	Genus/Origin	Type	Note
Jiaming No.1	JM-1	*Camellia sinensis*, Zhejiang	Cultivated	Longjing tea material with earliest leaves picking
Yingshuang	YS	*Camellia sinensis*, Zhejiang	Cultivated	Jingshan tea material
Longjing No.43	LJ-43	*Camellia sinensis*, Zhejiang	Cultivated	Longjing tea material
Baiye No.1	BY-1	*Camellia sinensis*, Zhejiang	Cultivated	Albino tea cultivar of Anji white tea material
Fudingdabai	FDDB	*Camellia sinensis*, Fujian	Cultivated	An excellent tea cultivar as breeding standard

effects on serum TG elevation in the olive oil-treated mice model (Yoshikawa et al., 2005). Floratheasaponins extracted from Chinese tea flowers (Anhui Province) also exhibited potently inhibitory effects on gastric mucosal lesions in rats and serum glucose elevation in sucrose-loaded rats (Yoshikawa et al., 2008a). Chakasaponins I–III from Chinese tea flower (Fujian Province) showed an accelerating effect on gastrointestinal transit in mice and a moderately inhibitory effect on pancreatic lipase (Yoshikawa et al., 2009). These findings confirm that tea flowers are a possible resource of nutraceutical products.

Yoshikawa et al. analyzed the floratheasaponins in Indian, Chinese (Anhui), and Japanese flowers, and found that there is similarity in the composition of floratheasaponins from Indian tea flowers and Chinese tea flowers (Yoshikawa et al., 2008a). Further evaluation of 13 kinds of extracts of *Camellia sinensis* collected from China, Taiwan, Japan, and India were conducted and high contents of floratheasaponins were observed in tea flower samples from Anhui, China and Shiga, Japan (Morikawa et al., 2012). In addition, high levels of chakasaponins I and II were found in all Fujian tea flowers, supporting a potential use in chemotaxonomy.

Zhejiang is an important province for tea plantation and production, in addition to Fujian and Anhui provinces, and there are many cultivars for processing Longjing tea, Jingshan tea, and Anji white tea, with an abundant supply of tea flowers every year. The literature on saponin composition of tea flowers in Zhejiang province is sparse, however. With the aim of better utilization of these resources, the saponin monomers of tea flowers (Zhejiang) were separated and identified through ultra-high performance chromatography (UPLC) coupled with electrospray ionization quadrupole time-of-flight mass spectrometry. Efficient measurement of saponin levels was by qualitative analysis performed by UPLC. Saponins distribution in different cultivars, developmental stages, and tissues was also analyzed and the value of this information in identification is discussed in detail.

2. Materials and methods

2.1. Plant materials and chemicals

The tea (*Camellia sinensis*) flowers of five cultivars (Jiaming NO.1, Yingshuang, Longjing NO.43, Baiye NO.1, Fudingdabai) were harvested from the tea plantation of Hangzhou Academy of Agricultural Sciences in Hangzhou City, Zhejiang Province, China and the cultivar information is listed in Table 1. The experimental tea flowers were collected at four different stages: stage 1, green bud (GB), stage 2, white bud (WB), stage 3, half-open flower (HOF), stage 4, full bloom (FB). The tea flowers of the Yingshuang cultivar were divided into three parts at different development stages: Part 1 consisted of petals, Part 2 consisted of stamens, pistils and ovaries, and Part 3 consisted of calyces and receptacles. The samples were frozen in liquid nitrogen, dried by vacuum freeze-drying for 48 h and then stored at −80 °C until analysis.

Purified Ginsenoside Rd ((3β,12β)-20-(β-D-glucopyranosyloxy)-12-hydroxydammar-24-en-3-yl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside) was purchased from Chengdu Must Bio-technology Company (Chengdu, China). UPLC-grade acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ). UPLC-grade formic acid was purchased from Tedia Company (Fairfield, USA). Analytical-grade methanol from TJSHIELD Company (Tianjin, China)was used for sample preparation. The Milli-Q water was prepared by an EASY Pure II UV Ultra-Pure Water System (Barnstead International, Dubuque, IA, USA).

2.2. Sample extraction

Due to high product yields, ultrasound assisted extraction (UAE) has been widely applied in the extraction of bioactive components from food by-products (Rahmaniana et al., 2015). The enhanced mass transfer rates of UAE increases extraction rate and reduces processing time and energy consumption, and therefore was adopted for sample preparation in the present work. The sample preparation method used for *Polygala tenuifolia* (Zhang et al., 2014) was preformed, with some modifications, as follows. Prior to extraction, samples were finely powdered and an accurately weighed 0.5000 g of each tea flower sample was extracted three times with 10 mL methanol/water (80:20 v/v) and allowed to stand for 30 min, followed by sonication for 30 min. After centrifugation of the extracts at 4000 r/min for 10 min, the solution was filtered through a syringe filter (0.22 μm), and an aliquot of 10 μL was subjected to UPLC analysis.

2.3. Chromatographic conditions

The chromatographic separation of extracts was performed on an Agilent UPLC-1290 (CA, USA) and a Welch Ultimate LP-C18 (250 mm × 4.6 mm, i.d., 3 μm). The mobile phase consisted of 0.1% aqueous formic acid (A) and acetonitrile (B) at a flow rate of 1 mL/min. A gradient elution was applied for chromatographic separation: 0 min: 15%B; 15 min: 38%B; 60 min: 43%B; 63 min: 70%B; 64 min: 15%B; 72 min: 15%B. The column temperature was set at 25 °C, the injection volume was 10 μL and the detection wavelength was 210 nm.

2.4. Mass spectrometry conditions

Mass Spectrometry: an AB Triple TOF 5600^{plus} System (AB SCIEX, Framingham, USA) was used. The optimal MS conditions: scan range *m*/*z* 100–2000. Positive ion mode: source voltage was +5.5 kV, and the source temperature was 600 °C. Negative ion mode: source voltage was −4.5 kV and the source temperature was 550 °C. The pressure of Gas 1 (Air) and Gas 2 (Air) were set to 50 psi. The pressure of Curtain Gas (N_2) was set to 35 psi. Maximum allowed error was set to ±5 mDa. Declustering potential (DP) was 100 V and collision energy (CE) was 10 V. For MS/MS acquisition mode, the parameters were almost the same except that the collision energy (CE) was set at 70 ± 20 V, ion release delay (IRD) at 67, ion release width (IRW) at 25. The exact mass calibration was performed automatically before each analysis employing the Automated Calibration Delivery System.