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Chemical and DNA authentication of taste variants of *Gynostemma pentaphyllum* herbal tea

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ABSTRACT

Gynostemma pentaphyllum Makino (Gp) was once used as a sweetener in Japan and is now widely consumed as an herbal tea worldwide for lowering cholesterol levels. Two taste variants, bitter and sweet, of Gp exist in the commercial market, but they cannot be differentiated morphologically nor by existing chemical analytical methods. This has been creating a problem in quality control of Gp products. In the present study, using HPLC-DAD and HPLC–ESI-MS analysis, we found that the Gp saponins, not flavonoids, from the sweet and bitter variants have distinctly different profiles. In addition, the two variants share only 69.01% homology in the ribosomal ITS-1 region, suggesting a phylogenic gap between these two variants. The combinations of chemical profiling and phylogenic analysis clearly confirm, for the first time, the distinction between these two taste variants. This information has direct application in the authentication and quality assessment of the various *Gynostemma* tea products.

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1. Introduction

Gynostemma pentaphyllum Makino (Cucurbitaceae) (Gp) is a perennial creeping plant that supplies the herbal tea known as jiaogulan in China. For centuries, the herbal tea made from the aerial part of Gp has been consumed in China as a general tonic. Today, it is increasingly popular in Europe and Northern America for lowering the serum lipid and cholesterol levels (Lin, Huang, & Lin, 2000). Similar to green tea, jiaogulan tea also possesses antioxidant and anti-carcinogenic activities, according to *in vitro* and animal studies (Chen, Chung, & Chen, 1999; Lin et al., 2000; Ma & Yang, 1999; Wang, Chen, Hsieh, Cheng, & Hsu, 2002; Zhou, Wang, Zhou, & Zhang, 1998). This herb was once used as a sweetener in Japan, probably owing to the presence of a wide variety of glycosides in the plant. The major active components of this plant are a series of dammarane-type saponins, which are also known as gypenosides. More than 100 gypenosides have been isolated and identified from Gp (Razmovski-Naumovski et al., 2005). These include eight saponins structurally identical to the known ginsenosides Rd, Rb_1, Rb_3, F_2, Rc, Rg_3 and malonylginsenosides Rb_1 and Rd, which were initially isolated from ginseng (Cui, Eneroth, & Bruhn, 1999; Hu, Chen, & Xie, 1996; Kuwahara, Kawanish, Komiya, & Oshio, 1989; Liu et al., 2004; Yin, Hu, Lou, & Pan, 2004; Yin, Hu, & Pan, 2004).

Two taste variants, sweet and bitter, exist in the market (Zheng, 2004). However, the chemical profile and phylogenetic relationships of these two variants have not been examined and compared. The quality control of Gp mainly depends on the determination of the total quantity of gypenosides by thin layer chromatography or colorimetry. Recently, chromatographic fingerprinting of saponins was employed for this purpose with different samples of jiaogulan from different regions of China (Kao, Huang, Inbaraj, & Chen, 2008; Liu et al., 2008); however, chemical profiling of the taste variants of Gp has not been reported.

In the present study, we performed chemical profiling and ribosomal DNA sequence analysis of sweet and bitter variants of Gp collected from different regions of China. Our results using HPLC-DAD and HPLC–ESI-MS showed that these two variants possess distinct and non-overlapping saponin profiles. Sequencing of the ribosomal DNA revealed a phylogenic distinction between them. These assessment methods and data provide useful information for the accurate authentication and quality control of jiaogulan tea and related products.

2. Materials and methods

2.1. Plant materials and extraction

Gp total saponins were purchased from commercial sources in nine provinces of China, as listed in Table 1. Details provided by these sources regarding the areas of growth, taste of the plants

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from which the Gp saponins were extracted and purity are also listed in Table 1. Pure gypenosides **1**–**17** were previously isolated in our lab as described (Liu et al., 2004, 2005; and unpublished data), and the chemical structures are illustrated in Fig. 1. These compounds are **1**: 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-2α-hydroxy-12β,23(*R*)-12,23-epoxydammar-24-en-20(*S*)-yl-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside; **2**: 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-2α,12β-dihydroxy-24ζ-hydroperoxy-25,26-en-dammaran-20(S)-yl *O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (Liu et al., 2004); **3**: 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-3α,12β-dihydroxy-25-en-24-one-dammar-20(*S*)-yl-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (unpublished data); **4**: 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-2α,12β-dihydroxy-23,25-dien-dammar-20(*S*)-yl-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (unpublished data); **5**: gynoside D; **6**: gynoside C; **7**: gypenoside XLII; **8**: gypenoside LVI; **9**: gypenoside XLIII; **10**: gypenoside XLVI; **11**: gypenoside IV; **12**: gypenoside VIII; **13**: 3-*O*-β-D-glucopyranosyl-2α-hydroxy-24-en-dammaran-20(S)-yl *O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (Liu et al., 2004); **14**: gypenoside LVII; **15**: gypenoside XLV Ed; **16**: gypenoside LXXVII; **17**: gynosaponin TN1. Gp saponins were dissolved in methanol at 10 mg/ml and sonicated for 30 min. Samples were filtered through a 0.4 μm membrane. Compounds **1**–**17** were dissolved in methanol at 2 mg/ml and used as standard compounds. All other chemicals were of the analytical grade.

In addition to the acquired Gp total saponins, methanolic extracts were prepared from three sweet- and three bitter-taste jiaogulan plants collected from six different regions in China and Japan. Dried aerial part of plant material (1 g) was frozen, pulverised and extracted with 15 ml methanol for 30 min by sonication at room temperature. The residue was further extracted with 10 ml methanol for 30 min by sonication at room temperature. The methanol extracts were pooled and stored at 4 °C for later usage.

2.2. High-performance liquid chromatography (HPLC) analysis

The HPLC was conducted with Agilent 1100 series equipped with G1312A binary pump, G1329A automatic sample injector and G1315B Diode Array Detector (DAD) (Santa Clara, CA). The separation was performed on an Eclipse XDA-C8 column (150 × 4.6 mm i.d., 5 μm) (Agilent, Santa Clara, CA). The mobile phase consisted of 0.1% formic acid in water (A), and 0.1% formic acid in acetonitrile (B) was used for separation. The flow rate was kept constant at 1 ml/min at 25 °C for a total run time of 45 min. The system was programed with the following gradients: from 18% to 60% B in 32 min, followed by 60–100% B in 13 min. The detection was performed at 203 nm. The filtrate of Gp saponins was diluted with methanol to 0.5 mg/ml. Compound **1**–**17** were diluted with methanol to 0.1 mg/ml. The volume of sample injection was 20 μl.

2.3. Mass spectrometry

The Gp saponin samples were analysed using the Bruker Daltonics micrOTOF-Q quadrupole time-of-flight mass spectrometer with an electrospray ionisation (ESI) source (Bremen, Germany) coupled to the HPLC system. The filtrate of the Gp saponins was first diluted with methanol to 0.1 mg/ml. Compounds **1**–**17** were diluted with methanol to 0.02 mg/ml. For the filtrate of the Gp total saponin extracts, equal volume of the samples was injected. The injection volume of each sample was 20 μl. The spray voltage was set at 4.5 kV and the capillary offset voltage was at −500 V. All spectra were acquired at a capillary temperature of 180 °C. The calibration of the mass range (400–2000 Da) was performed in negative-ion mode. The raw mass spectra, without smoothing or baseline correction were processed with DataAnalysis 4.0 (Bruker Daltonics, Bremen, Germany).

2.4. Identification of gypenoside compounds

Gypenosides were identified based on their retention times and then compared with the mass spectra of the gypenoside standards (compounds **1**–**17**). In addition, putative gypenosides were identified through the comparison of the mass of $(M+HCOO)^-$ ion obtained from the extracted ion chromatogram (EIC) with the theoretical mass of gypenosides in our previous reports (Liu et al., 2004, 2005; and unpublished data) and the CHMIS-C database (Fang, Shao, Zhang, & Wang, 2005). Each of the EIC was confined to a width of ±0.02 *m*/*z*. To distinguish a gypenoside peak from background noise, a threshold value of signal intensity for a valid peak was set at 500 counts. Meanwhile, the tolerance of the retention time shift of the gypenoside candidate between samples was set at ±0.1 min. The corresponding $(M-H)^-$ ion of the putative gypenoside was detected in the mass spectra to support the identification.

2.5. Cluster analysis of the HPLC and MS chromatograms

Clustering 11 taste variants of Gp is based on the peaks obtained from the chromatogram of the total saponins of the plant. For the HPLC-DAD chromatograms, the peaks were identified and aligned using the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (SESCFTCM) Ver. 2004A (State Pharmacopoeia Committee of China, PR China). Hierarchical clustering analysis was performed on the HPLC-DAD chromatograms using Cluster 3.0 software (University of Tokyo, Human Genome Center) based on the area of the aligned peaks of individual chromatograms. The similarities between different samples were calculated as correlation coefficients (C.E.) using the Pearson correlation. For the HPLC–ESI-MS chromatograms, the peak area of $(M+HCOO)^-$ ion of the corresponding identified gypenosides and the putative gypenosides were extracted from

Table 1
Gp saponins obtained from different regions of cultivation in China.

Code	Plant source[a]	Taste[a]	Purity (%)[a]	Supplier
S-GD	Guangdong Province	Sweet	85	Huaduo Natural Products Company, Guangzhou
S-FJ	Fujian Province	Sweet	85	Hui Zhou Shi Orient Plant Health Care SCL & Tech. CD., Ltd., Huizhou
S-SC	Sichuan Province	Sweet	80	Chengdou Jubang Bio-engineering Co. Ltd., Chengdu
B-FJ	Fujian Province	Bitter	80	Hui Zhou Shi Orient Plant Health Care SCL & Tech. CD., Ltd., Huizhou
B-SC	Sichuan Province	Bitter	98	Chengdou Jubang Bio-engineering Co. Ltd., Chengdu
B-HB	Shen-Nong-Jia, Hubei Province	Bitter	>95	Yichang Night Pearl Natural Botanical Product Co. Ltd., Yichang
B-HN	Hunan Province	Bitter	90	Changsha Organic Herb Inc., Changsha
B-AH	Anhui Province	Bitter	80	Xuancheng Baicao Plants Industry and Trade Co.,Ltd., Xuancheng
B-GX	Da-Yao Mountain, Jinxiu County Guanxi Province	Bitter	85	Guilin Sanleng Biological Products Co. Ltd., Guilin
U-YN	Yunnan Province	Unspecified	>95	Yunnan Yuxi Wangzilong Pharmaceautical Co. Ltd., Yuxi
U-SX	Ankang, Shaanxi Province	Unspecified	98	Shaanxi Undersun Biomed Tech Co. Ltd., Xian

[a] Plant source, taste and purity information were obtained from the suppliers.

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