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Two new saponins from tetraploid jiaogulan (*Gynostemma pentaphyllum*), and their anti-inflammatory and α-glucosidase inhibitory activities

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ABSTRACT

Jiaogulan tea has been commercialised globally. This study investigated the chemical components and health properties of a new jiaogulan genotype, tetraploid *Gynostemma pentaphyllum*. Two new saponins, (23*S*)-21β-*O*-methyl-3β,20ξ-dihydroxy-12-oxo-21,23-epoxydammar-24-ene-3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranoside (**4**) and 23β-H-3β,20ξ-dihydroxy-19-oxo-21,23-epoxydammar-24-ene-3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside (**5**), together with one lactone, 3,5-dihydroxyfuran-2(5H)-one (**1**), and two flavonoids, rutin (**2**) and kaempferol 3-*O*-rutinoside (**3**), were characterised in the aerial parts of tetraploid jiaogulan. The chemical structures of the five isolated compounds were elucidated by NMR, HR-MS spectra and chemical degradation. The five compounds were also examined and compared with the methanol extract and *n*-butanol soluble fraction of the jiaogulan for their inhibitory activities on lipopolysaccharide (LPS)-induced IL-1β, IL-6 and COX-2 mRNA expression in RAW 264.7 mouse macrophages, and their *in vitro* α-glucosidase suppressing capacities. The results from this study may be used to promote the potential application of jiaogulan in functional foods.

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1. Introduction

Jiaogulan tea has been commercially available in the United States, China and many other Asia countries including Japan (Xie et al., 2012). Jiaogulan, *Gynostemma pentaphyllum* (Thunb.) Makino, also has been used in functional foods (Gao & Yu, 1993; Zheng & Liu, 2008). It may have anti-inflammatory (Liou, Huang, Kuo, Yang, & Shen, 2010), hypolipemic (Megalli, Aktan, Davies, & Roufogalis, 2005), hypoglycemic (Norberg et al., 2004), and anti-cancer activities (Cheng et al., 2011; Li et al., 2012). Gypenosides, one type of saponins, were considered as the major bioactive constituents in *G. pentaphyllum*. To date, more than 170 gypenosides were obtained from *G. pentaphyllum* (Shi, Song, Pan, & Liu, 2011b) since the first two gypenosides were reported by Nagai et al. in 1976 (Nagai, Izawa, Nagumo, Sakurai, & Inoue, 1981). The previous studies also revealed that the gypenoside compositions differed in *G. pentaphyllum* samples collected from different origins (Qin et al., 2012) and seasons (Liu & Hu, 2005), sweet and bitter taste variants (Lu et al., 2013), and genotypes (Xie et al., 2012).

Multiploid phenomenon is inherent with *Gynostemma* plants, and *G. pentaphyllum* has diploid, tetraploid, hexaploid and octoploid (Gao, Chen, Gu, & Zhao, 1995; Jiang, Qian, Guo, Wang, & Zhao, 2009). Most of the previous studies were performed on the diploid *G. pentaphyllum*. Our recent research suggested that tetraploid *G. pentaphyllum* may be a better source for gypenosides than its diploid counterpart (Xie et al., 2012). To date, little is known for the gypenoside compositions in the tetraploid *G. pentaphyllum*. As a continuation of our research on jiaogulan, this study was performed to characterise saponins in the tetraploid *G. pentaphyllum*. The anti-inflammatory and hypoglycemic activities of the isolated compounds were also examined. These data will be used to promote the application of tetraploid jiaogulan in improving human health.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were measured in CH_3OH on a Jasco P-2000 polarimeter (Tokyo, Japan) with a sodium lamp operating at 589 nm. IR spectra were recorded using a Nicolet 6700 FTIR spec-

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trophotometer (Madison, WI, USA). Semi-preparative HPLC was carried out on a Waters liquid chromatograph system equipped with 1525 pump and 2489 detector, and an Agilent Zorbax Eclipse XDB-C18 column (250 mm × 9.4 mm, i.d., 5 μm). The leucine derivatives of sugars were identified using an Agilent 7890A gas chromatograph system (Santa Clara, CA, USA). Column separations were also performed on a Buchi Sepacore medium-pressure chromatography system (Flawil, Switzerland). Silica gel (200–300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, Shandong, China) and ODS (S-50 and 75 μm, YMC Co., Ltd., Kyoto, Japan) were used for regular and medium-pressure column chromatography fractionations. IScript Advanced cDNA Synthesis kit was purchased from Bio-Rad Laboratories (Hercules, CA, USA), while AB Power SYBR Green PCR Master Mix was purchased from ABI (Applied Biosystems, Carlsbad, CA, USA). The α-glucosidase enzyme inhibition was analysed on a Synergy 2 Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA).

2.2. Plant material and reagents

The aerial parts of tetraploid *G. pentaphyllum* (Thunb.) Makino were collected and identified by the Asian Citrus Holdings Ltd. (Hong Kong) from the Dabashan area of Pingli, Shaanxi province of China in 2010. Botanicals were kept at 4 °C before use. A voucher specimen (No. 2010005) has been deposited in our laboratory, School of Agriculture and Biology, Shanghai Jiao Tong University.

Mouse RAW 264.7 macrophage cell line was bought from Chinese Academy of Sciences (Shanghai, China). Lipopolysaccharide (LPS, from *Escherichia coli* O111:B4) was obtained from Millipore (Billerica, MA, USA). Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS) and 1× PBS (pH 7.4) were purchased from Gibco (Life Technologies, Carlsbad, CA, USA). TRIzol reagent was obtained from Invitrogen (Life Technologies). α-Glucosidase, p-nitrophenyl-α-D-glucopyranoside (pNPG) and acarbose were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solvents for isolation were of analytical grade, and those for HPLC analysis were of chromatographic grade and for UPLC–Q-TOF-MS analysis were of mass spectrometry grade without further purification.

2.3. Extraction and isolation

The dried and powdered sample (3.8 kg) of tetraploid *G. pentaphyllum* was extracted three times (2 h for each time) with methanol at 70 °C. The combined extract was concentrated under reduced pressure to give a dark brown residue (800 g). The residue was suspended in deionised water, then extracted with petroleum ether, ethyl acetate and *n*-butanol sequentially. The *n*-butanol fraction (390 g) was fractionated further on a silica gel (200–300 mesh) column with CH_2Cl_2–MeOH (100:1, 20:1, 10:1, 5:1, 1:1, and 1:2, v/v) to obtain four sub-fractions. Fraction B-1 (10 g) was further chromatographed on a silica gel column with EtOAc–MeOH (40:1, v/v) to remove non-polar compounds, and eluted with MeOH to obtain compound **1** (40 mg). Fraction B-3 (223 g) was separated on a silica gel (200–300 mesh) column with EtOAc–MeOH (10:1, 5:1, 2:1, 1:1, and 1:2, v/v) to obtain five fractions: B-3-1 to B-3-5. Fraction B-3-2 (12 g) was subjected to a ODS medium pressure column chromatography, eluted with MeOH–H_2O (1:4, 1:1, 3:1, and pure methanol, v/v) to yield compounds **2** (110 mg) and **3** (320 mg). Fraction B-3-4 was subjected to a ODS medium pressure column chromatography, washed with MeOH–H_2O (1:19, 1:4, 2:3, 3:2, 4:1 and pure methanol, v/v) to afford ten fractions, and Fraction B-3-4-7 was passed through a silica gel (200–300 mesh) column, washed with $CHCl_3$–MeOH (5:1 and 1:1, v/v) to yield eleven fractions, and the Fraction B-3-4-7-5 was further purified by semi-preparative HPLC (MeOH–H_2O, 65:35, v/v; 2 ml/min, and the detection wavelength was 205 nm) to obtain two new saponins, compounds **4** (48 mg) and **5** (24 mg).

2.4. NMR spectroscopy

The NMR spectra were recorded on a Bruker AVANCE DRX-500 spectrometer (Rheinstetten, Germany) equipped with a 5 mm probe at 300.0 K. Compound **4** or **5** was dissolved in 0.5 ml of pyridine-d_5. All chemical shifts were given on the δ scale and referenced to tetramethylsilane (TMS) at 0.00 ppm for proton and carbon. The NMR experimental conditions were as follows: for the ^{1}H spectrum, spectrometer frequency (SF) of 500.13 MHz, acquisition time (AQ) of 2.18 s, relaxation delay (RD) at 2.0 s, 90° pulse width at 9.7 us, spectral width (SW) of 15,015 Hz, Fourier transform (FT) size at 64 k; for the ^{13}C NMR spectrum, SF was 125.75 MHz, AQ was 0.747 s, RD was 2 s, 90° pulse width was 10.2 us, SW was 43859 Hz, line broadening (LB) was 1 Hz, FT size was 64 k; for the ^{1}H–^{1}H COSY, NOESY and TOCSY spectra, AQ was 0.12 s, RD was 2 s, SW was 4252 Hz, number of points (NP) was 1 k, number of increments (NI) was 512, the mixing time of NOESY was 1 s, the spin-lock time of TOCSY was 600 ms; for the HSQC and HMBC spectra, AQ was 0.12 s, RD was 2 s, SW at 4252 Hz (^{1}H) and 25000 Hz (^{13}C), NP at 1 k, NI at 512, FT size of 1 k × 512.

2.5. UPLC–Q-TOF-MS analysis

The HR-ESIMS data of compounds **4** and **5**, and confirmation of the natural occurrence of compound **4** were obtained on a Waters Xevo G2 Q-TOF mass spectrometer (Milford, MA, USA). The UPLC analysis was performed at 40 °C using an Acquity UPLC BEH C_{18} column (100 mm × 2.1 mm, i.d., 1.7 μm), with a 5 mm × 2.1 mm i.d. guard column of the same material (Waters, Milford, MA, USA). The elution gradient (solution A, water; solution B, acetonitrile) was used as follows: 20% B for 1 min, 20–90% B in 14 min, and 90% B for 2 min. The flow rate was 0.4 ml/min, and the injection volume was 2 μl. Mass data were obtained by an electrospray ionisation in negative ion mode and was calibrated using the lock-mass function with leucine encephalin. MS conditions were: capillary voltages, 2.8 kV; sampling cone voltages, 55.0 V; source temperature, 100 °C; desolvation temperature, 300 °C; desolvation gas flow, 500.0 L/h; cone gas flow, 50.0 L/h; scan range, *m*/*z* 100–1500; scan time, 0.3 s; interscan time, 0.02 s. Data were collected and analysed with Waters MassLynx v4.1 software.

2.6. Structure identification of new compounds

(23*S*)-21β-*O*-Methyl-3β,20ξ-dihydroxy-12-oxo-21,23-epoxydammar-24-ene-3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L-arabinopyranoside, **4**. White amorphous powder; $[\alpha]_D^{19}$+ 3.3° (*c* 0.12, MeOH); IR (KBr): ν_{max} 3433, 2968, 2934, 2878, 1706, 1638, 1451, 1384, 1075, 1038, 981, 611 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR (pyridine-d_5) data are presented in Table 1; HRESIMS: *m*/*z* 941.5114 [M−H]$^-$ (calculated for $C_{48}H_{77}O_{18}$, 941.5110), 779.4590 [M−Glc−H]$^-$, 633.3999 [M−Glc−Rha−H]$^-$, 501.3566 [M−Glc−Rha−Ara−H]$^-$.

23β-H-3β,20ξ-Dihydroxy-19-oxo-21,23-epoxydammar-24-ene-3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside, **5**. White amorphous powder; $[\alpha]_D^{28}$+ 4.7° (*c* 0.19, MeOH); IR (KBr): ν_{max} 3426, 2942, 2876, 1706, 1639, 1450, 1384, 1261, 1134, 1074, 1043, 979, 610 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR (pyridine-d_5) data are provided in Table 1; HRESIMS: *m*/*z* 881.4908 [M−H]$^-$ (calculated for $C_{46}H_{73}O_{16}$, 881.4899), 749.4482 [M−Xyl−H]$^-$, 603.3893 [M−Xyl−Rha−H]$^-$, 471.3460 [M−Xyl−Rha−Ara−H]$^-$.

Compounds **4** and **5** (3 mg each) were hydrolysed with 10% HCl/dioxane (1:1, v/v, 1 ml) at 85 °C for 4 h in water bath, respectively

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