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# Conjugated linolenic acids and nutraceutical components in Jiaogulan (*Gynostemma pentaphyllum*) seeds

Chen Zou [a, 1], Haiming Shi [a, 1], Xin Liu [a], Yiqing Sheng [a], Tingting Ding [a], Jun Yan [a], Boyan Gao [a, b], Jie Liu [a], Weiying Lu [a], Liangli (Lucy) Yu [b, *]

[a] Institute of Food and Nutraceutical Science, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China
[b] Department of Nutrition and Food Science, University of Maryland, College Park, MD 20742, United States

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ABSTRACT

Diploid and tetraploid Jiaogulan (*Gynostemma pentaphyllum*) seeds were examined for their conjugated linolenic acids and nutraceutical components. Both di- and tetraploid Jiaogulan seeds were rich in conjugated linolenic acids possibly including α-eleostearic, catalpic and β-eleostearic acids for about 80% of total fatty acids. α-Eleostearic acid was the primary conjugated isomer at a level of 98.8 and 187.7 mg/g dried seeds in diploid and tetraploid seeds, respectively. The seeds also contained significant levels of essential amino acids (616.3 and 541.5 μmol/g dry seeds), phytosterols (1292.0 and 2316.0 μmol/kg dry seeds), tocopherols, phenolics and flavonoids. In addition, the seeds extract showed no difference in their anti-inflammatory effects in LPS-stimulated RAW 264.7 mouse macrophage cells, whereas the diploid seed extract had stronger radical scavenging activities and a ferric reducing capability than those of the tetraploid seed extract on a per seed weight basis. The results may be used to promote the value-added utilization of Jiaogulan seeds.



## 1. Introduction

Edible seeds are generally rich in protein, oil, dietary fiber and other health promoting components, and are considered as a potential source for functional food ingredients. Many edible seeds or their components, including nuts and pulses, have showed potential in reducing the risk of cardiovascular disease, cancer, chronic inflammation and Alzheimer's disease (Bagchi et al., 2000; Ros & Hu, 2013).

Jiaogulan leaf, *Gynostemma pentaphyllum* (Thunb.) Makino, has been used in food and beverage products for thousands of years for its potential in reducing the risk of hyperlipidemia, cancer, diabetes and chronic inflammation (Razmovski-Naumovski et al., 2005), but Jiaogulan seeds are not well utilized. A preliminary study detected three conjugated linolenic acids (CLNAs) including α-eleostearic (*c*9, *t*11, *t*13-18:3), catalpic (*t*9, *t*11, *c*13-18:3) and β-eleostearic (*t*9, *t*11, *t*13-18:3) acids, together with palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids, in a diploid Jiaogulan seed oil (Jiang, Ma, & Xiao, 2013) according to the GC-MS data and retention indices. Conjugated fatty acids including conjugated linoleic and linolenic acids, have been shown to have many health benefits including anti-carcinogenic and anti-inflammatory activities (Yuan, Chen, & Li, 2014). Together, these data suggested a potential utilization of Jiaogulan seeds in developing nutraceutical and functional food ingredients, and warranted additional research of other nutraceutical components and health properties of Jiaogulan seeds.

Jiaogulan have several polyploid species, which differed in their chemical components and health properties (Xie et al., 2012; Xie et al., 2011b). Tetraploid Jiaogulan seeds have never been evaluated for its nutraceutical components and health beneficial properties. As a continuation of our research on Jiaogulan, this study aimed to examine the nutraceutical properties for the seeds of diploid and tetraploid Jiaogulan, and to evaluate their anti-inflammatory, radical scavenging and ferric reducing activities. The results from this study may be used to develop value-added utilization of Jiaogulan seeds for improving human health.

* Corresponding author.
*E-mail address:* lyu5@umd.edu (L. Yu).
[1] Equal contribution.

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## 2. Materials and methods

### 2.1. Materials and chemicals

The seeds of diploid and tetraploid *G. pentaphyllum* (Thunb.) Makino were purchased from the Baicaotang Biotechnology Co. Ltd., Pingli, Shaanxi province of China in 2013, and kept at 4 °C until analysis.

Gallic acid, rutin, α-, β- and γ-tocopherols, 2, 4, 6-tripyridyl-S-triazine (TPTZ), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), Fluorescein, Folin-Ciocalteu reagent, and 2, 2-diphenyl-1-picryhydrazyl radical (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stigmasterol (purity, 95%), β-sitosterol (purity, 75%), and β-cholestanol (purity, 96%) were obtained from Acros Organics (Geel, Belgium). All solvents used for HPLC analysis were of chromatographic grade, and for UPLC-Q/TOF-MS analysis were of mass spectrometry grade. All other chemicals and solvents were of the highest commercial grade.

Mouse RAW 264.7 macrophage cell line was purchased from the 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), fetal 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). IScript Advanced cDNA Synthesis kit was purchased from Bio-Rad Laboratories (Hercules, CA, USA), while AB Power SYBR Green PCR Master Mix was purchase from ABI (Applied Biosystems, Carlsbad, CA, USA).

### 2.2. Proximate analysis

Moisture content was determined according to the AOAC method 925.10 (AOAC, 1990). Ash content was determined according to the AOAC method 923.03 (AOAC, 1990). Total protein content was determined based on AOAC method 984.13 (AOAC, 1990).

### 2.3. Dietary fiber analysis

The soluble and insoluble dietary fiber contents were determined with a commercial kit purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland) according to a previously reported procedure (Xie et al., 2012).

### 2.4. Oil extraction

The seeds were ground in an IKA A11 basic analytical mill (Königswinter, Germany) to a particle size that would pass through a 100-mesh screen. Approximately 5 g ground seeds were extracted with 75 mL hexane for 6 h using a Soxhlet apparatus. The hexane was evaporated using a rotary evaporator under a reduced pressure, and the residual solvent was removed under nitrogen. The remaining oils were weighed and the yield of oils was calculated. The oils were stored at 4 °C until further testing.

### 2.5. Anti-inflammatory and antioxidant extraction

The defatted seed flour was dried in fume hood overnight at ambient temperature. Approximately 1 g of defatted seed flour was extracted with 10 mL pure ethanol by sonication for 2 h at ambient temperature. The extracts were centrifuged at 3082 *g* for 10 min. After centrifuging, the supernatants were collected. The extracts were kept in the dark at 4 °C until further analysis.

### 2.6. Fatty acid profile

Fatty acid methyl esters (FAME) were prepared according to a laboratory method, and subjected to gas chromatography-mass spectrum (GC-MS) analysis (Xie et al., 2011a). The FAMEs were analyzed on a Perkin-Elmer AutoSystem XL GC (Waltham, MA, USA) equipped with a Turbomass MS (Waltham, MA, USA), with a DB-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA). Helium was the carrier gas at a flow rate of 1.0 mL/min. Injection volume was 1 μL at a split ratio of 5:1 (v/v). Oven temperature was initially 70 °C where it was held for 5 min, increased 10 °C/min until 200 °C, and increased by 5 °C/min to a final temperature of 280 °C. Electron impact ionization mass spectra were obtained at an ionization energy of 70 eV with a scan range of *m/z* 29–700. The ion source temperature was 200 °C. Individual fatty acid was identified by comparing their mass spectra with that of the standard compounds and NIST 2005 mass spectral reference library. The quantification was based on the area under each fatty acid peak as compared to the total area of all fatty acid peaks.

### 2.7. Triacylglycerol profile

Approximate 5 mg of each seed oils were dissolved in 5 mL methanol/isopropanol (1:1, v/v) to a final concentration of 1 mg/mL. The sample solution was analyzed by a Waters UPLC system coupled to a Waters Xevo G2 Q-TOF mass spectrometer (Milford, MA, USA). The separation was performed at 40 °C using an ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm i.d.; 1.7 μm). The elution gradient (eluent A, acetonitrile/isopropanol, 90:10; eluent B, acetonitrile/isopropanol, 45:55, v/v) was: started at 10% B; increased via linear gradient to 30% B at 20 min, 90% B at 22 min, and 90% B for 6 min. The flow rate was 0.3 mL/min and the injection volume was 2.0 μL. MS conditions were: capillary voltages for positive ion mode at 2.5 kV; sampling cone voltage of 35.0 V; source temperature 120 °C; desolvation temperature 250 °C; desolvation gas flow rate of 800.0 L/h; cone gas flow at 50.0 L/h; and a scan range of *m/z* 100–1500. $MS^E$ technology was used in two separate scan functions which were programmed for the MS acquisition method. The first scan function was set at low collision energy (6 eV) which provided parent ions, and the second scan function was set a high collision energy (ramped from 30 to 40 eV) which provided fragment ions. Data were collected and analyzed with Waters MassLynx v4.1 software (Milford, MA, USA).

### 2.8. Phytosterol content analysis

The phytosterol content of the seed oil was determined by GC-FID. Briefly, 200 mg oil was ultrasonically mixed with 2 mg internal standard (β-cholestanol), to which 5 mL of 2.0 mol/L KOH-ethanol was added. After reacting 5 min in ultrasonic bath, the mixture was kept at 60 °C for 1 h. To stop the reaction, 6 mL *n*-hexane and 4 mL water were added, and the mixture was centrifuged for 3 min at 4000 *g*. The upper organic layer was collected, and another 6 mL of *n*-hexane was used to extract the aqueous layer. After the solvent was removed, the residue was derivatized with 100 μL of BSTFA:TMCS (99:1, v/v) at 105 °C for 15 min. Finally, 1 μL of the derivatized solution was analyzed on an Agilent 6890 GC system (Agilent, Wilmington, DE, USA) equipped with an FID detector and an HP-5 capillary column (30 m × 0.32 mm, 0.25 μm film thickness, Agilent). Helium was the carrier gas at a flow rate of 1 mL/min. Injection volume was 1 μL at a split ratio of 50:1 with an injector temperature of 300 °C. Column temperature was initially 200 °C and increased by 2 °C/min until a final temperature 300 °C. The temperature of FID detector was kept at 360 °C. The