Supporting Information

Rec. Nat. Prod. 14:2 (2020) 116-128

Efficacy of *Gynostemma pentaphyllum* Extract in Anti-obesity Therapy

Yoon Hee Kim¹, So Mi Kim², Jae Kyoung Lee¹, Sung Kwan Jo¹, Hyung Joong Kim¹, Kyu Min Cha¹, Cho Young Lim¹, Joo Myung Moon¹, Tae Young Kim¹, Eun Ji Kim²

¹BTC Corporation, Ansan, Korea
²Center for Efficacy Assessment and Development of Functional Foods and Drugs, Hallym University, Chuncheon, Korea

Table of Contents

| Table of Contents | Page |
|-------------------|------|
| S1: Experimental Details | |
| S1.1: LC/MS Analysis Conditions | 1 |
| S1.2: LC/MS Data Analysis | 1 |
| S1.3: Method Validation | 1 |
| Figure S1: HPLC spectrum and full scan total LC-MS spectra at 10eV (ESI+) revealed two gypenosides and one ginsenoside peak in an extract from GPE | 2 |
| Figure S2: Identification of compound 1(GL), 2(GLI), and 3(Rg3) from GPE | 3 |
| Figure S3: Linearity of GL | 4 |
| Figure S4: Linearity of GLI | 4 |
| Figure S5: Linearity of Rg 3 | 5 |
| Table S1: Precision-repeatability of GL, GLI, and Rg3 | 6 |
| Table S2: Accuracy of GL, GLI, and Rg3 | 6 |
| Table S3: ¹³C NMR spectroscopic data for isolated GL, GLI, and Rg3 in pyr-d5 | 7 |
| Figure S6: ¹H NMR (400 MHz) spectrum of isolated GL | 8 |
| Figure S7: ¹³C NMR (100 MHz) spectrum of isolated GL | 9 |
| Figure S8: ¹H NMR (400 MHz) spectrum of isolated GLI | 10 |
| Figure S9: ¹³C NMR (100 MHz) spectrum of isolated GLI | 11 |
| Figure S10: ¹H NMR (500 MHz) spectrum of isolated Rg3 | 12 |
| Figure S11: ¹³C NMR (125 MHz) spectrum of isolated Rg3 | 13 |
S1: Experimental Details

S1.1: LC/MS Analysis Conditions

GPE and purified compounds were dissolved in methanol and analyzed by a Thermo U3000-LTQ XL ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an electrospray ionization (ESI) mass source. Chromatographic separation of the compounds was achieved using a HSS T3 C18 column (2.1 × 150 mm; 2.5 μm particle size; Waters, Milford, MA, USA) at a flow rate of 0.3 mL/min. Mobile phases A and B were water and acetonitrile, respectively, both containing 0.1% formic acid. Gradient elution was conducted as follows: 5–100% B for 0–15 min with a linear gradient, followed by 5 min of 100% B. The MS/MS system was operated in ESI mode. The typical operating parameters were as follows: spray needle voltage, +5 kV; ion transfer capillary temperature, 275°C; nitrogen sheath gas, 35; and auxiliary gas, 5 (arbitrary units). The ion trap contained helium damping gas, which was introduced in accordance with the manufacturer’s recommendations. Mass spectra were acquired in an m/z range of 50–1000, with 3 microscans and a maximum ion injection time of 200 ms. The data-dependent mass spectrometry experiments were controlled using the menu-driven software provide with the Xcalibur system (version 2.2 SP1.48; Thermo Fisher Scientific).

S1.2: LC/MS Data Analysis

Raw data files were processed using Mass Frontier 7.0 software (Thermo Fisher Scientific). The program modules used were Chromatogram Processor and Database Manager. Mass Frontier software was then employed to interpret MS/MS spectra by assigning structures to the fragment ions automatically.

S1.3: Method Validation

The Method was validated by the linearity, Precision and Accuracy of the results. Correlation coefficient was 0.999 for Gypenoside L, Gypenoside LI and Ginsenoside Rg3 which prove that the method is linear (Figure S3, S4, and S5). Precision was measured by repeatability. Repeatability was demonstrated by repeated measurements of three concentrations the intended range of samples. The method is precise as % RSD of peak area was 0.743-1.433 in case of Gypenoside L, 0.730-1.230 in case of Gypenoside LI and 1.500-1.804 in case of Ginsenoside Rg3 (Table S2). Accuracy was assessed by analyzing a sample with known concentration and comparing the measured value with the true value. In case of Gypenoside L % recovery was 100.04% -103.12% (average 101.73%, % RSD 1.532), in case of Gypenoside LI % recovery was 100.05% -101.29% (average 100.85%, % RSD 0.686) and in case of Ginsenoside Rg3 % recovery was 101.39% -102.69% (average 101.85%, % RSD 0.709) (Table S3).
**Figure S1**: HPLC spectrum and full scan total LC-MS spectra at 10eV (ESI+) revealed two gypenosides and one ginsenoside peak in an extract from GPE. (A) HPLC spectrum of GPE. (B) LC-MS spectra of Peaks 1, 2, and 3.
Figure S2: Identification of compound 1(GL), 2(GLI), and 3(Rg3) from GPE. (a)–(b) HPLC spectra of GL standard and Isolated GL, (c)–(d) HPLC spectra of GLI standard and Isolated GLI, (e)–(f) HPLC spectra of Rg3 standard and Isolated Rg3
Figure S3: Linearity of GL

Figure S4: Linearity of GLI
Figure S5: Linearity of Rg3
Table S1: Precision-repeatability of GL, GLI, and Rg3

| Compound | Mean of 5 samples |  |
|----------|-------------------|---|
|          | Concentration (mg/ml) | Peak area | Conc. (mg/g) | RSD (%) |
| GL       | 8.99              | 552.530    | 18.025       | 0.743   |
|          | 10.07             | 621.510    | 18.040       | 1.433   |
|          | 11.05             | 683.269    | 18.047       | 0.575   |
| GLI      | 9.03              | 391.147    | 14.019       | 1.230   |
|          | 10.01             | 434.550    | 14.028       | 1.167   |
|          | 11.05             | 480.293    | 14.038       | 0.730   |
| Rg3      | 9.01              | 39.698     | 1.408        | 1.804   |
|          | 10.12             | 44.341     | 1.402        | 1.500   |
|          | 11.09             | 48.932     | 1.409        | 1.680   |

Table S2: Accuracy of GL, GLI, and Rg3

| Compound | Mean of 3 samples |  |
|----------|-------------------|---|
|          | Concentration. (mg/mL) | Standard added (µg/mL) | Recovery (%) | Mean recovery (%) | RSD (%) |
| GL       | 7.38              | 94.24         | 103.12   | 101.73            | 1.532   |
|          | 10.32             | 102.04        | 102.04  | 101.73            | 1.532   |
| GLI      | 7.56              | 101.76        | 101.21  | 100.85            | 0.686   |
|          | 10.04             | 100.05        | 101.29  | 100.85            | 0.686   |
| Rg3      | 7.60              | 19.68         | 101.49  | 101.39            | 0.709   |
|          | 10.13             | 101.85        | 102.69  | 101.85            | 0.709   |

© 2019 ACG Publications. All rights reserved.
| Position | GL<sup>a</sup> δ<sup>R</sup> | GL<sup>a</sup> δ<sup>C</sup> | GLP δ<sup>R</sup> | GLP δ<sup>C</sup> | Rg3<sup>b</sup> δ<sup>R</sup> | Rg3<sup>b</sup> δ<sup>C</sup> |
|----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| C1       | 47.8            | 47.79           | 47.9            | 47.80           | 38.4            | 39.15           |
| C2       | 68.0            | 66.70           | 68.1            | 66.70           | 25.8            | 27.11           |
| C3       | 96.5            | 95.61           | 96.6            | 95.62           | 88.0            | 88.92           |
| C4       | 41.8            | 41.01           | 41.8            | 41.03           | 38.2            | 36.93           |
| C5       | 57.1            | 56.22           | 57.2            | 56.23           | 55.4            | 56.38           |
| C6       | 19.3            | 18.51           | 19.3            | 18.54           | 17.7            | 18.45           |
| C7       | 35.7            | 35.07           | 35.8            | 35.10           | 34.3            | 35.18           |
| C8       | 40.9            | 39.98           | 40.9            | 40.01           | 36.2            | 35.9            |
| C9       | 51.2            | 50.39           | 51.2            | 50.69           | 49.3            | 50.40           |
| C10      | 38.8            | 37.85           | 38.8            | 37.86           | 38.6            | 39.72           |
| C11      | 32.2            | 32.27           | 32.2            | 32.41           | 31.0            | 32.07           |
| C12      | 71.9            | 71.29           | 71.8            | 71.31           | 69.5            | 71.03           |
| C13      | 48.8            | 48.52           | 49.5            | 49.18           | 47.8            | 48.60           |
| C14      | 52.5            | 51.73           | 52.6            | 51.80           | 50.1            | 54.83           |
| C15      | 32.0            | 31.32           | 32.0            | 34.41           | 30.4            | 31.35           |
| C16      | 27.3            | 27.07           | 27.2            | 26.68           | 25.5            | 26.86           |
| C17      | 55.0            | 54.82           | 50.8            | 50.40           | 49.4            | 51.73           |
| C18      | 16.2            | 15.81           | 16.2            | 15.83           | 16.4            | 17.02           |
| C19      | 17.9            | 17.70           | 17.9            | 17.73           | 15.8            | 16.63           |
| C20      | 74.3            | 72.90           | 74.5            | 72.92           | 71.4            | 72.96           |
| C21      | 26.6            | 26.87           | 22.4            | 22.65           | 22.0            | 25.83           |
| C22      | 36.3            | 35.92           | 43.3            | 43.27           | 41.9            | 40.00           |
| C23      | 23.3            | 23.02           | 22.8            | 22.82           | 21.4            | 23.02           |
| C24      | 126.2           | 126.37          | 126.0           | 126.11          | 124.9           | 126.33          |
| C25      | 131.9           | 130.77          | 131.9           | 130.78          | 129.5           | 130.77          |
| C26      | 25.9            | 25.84           | 25.9            | 25.86           | 25.4            | 26.75           |
| C27      | 17.8            | 17.65           | 17.8            | 17.65           | 17.5            | 17.70           |
| C28      | 28.7            | 28.32           | 28.7            | 28.32           | 27.4            | 28.14           |
| C29      | 17.8            | 17.54           | 17.7            | 17.57           | 15.7            | 16.38           |
| C30      | 17.2            | 16.98           | 17.5            | 17.29           | 15.4            | 15.84           |
| C1'      | 104.7           | 105.72          | 104.8           | 105.73          | 103.5           | 105.14          |
| C2'      | 80.7            | 82.46           | 80.7            | 82.47           | 81.0            | 83.51           |
| C3'      | 78.1            | 78.43           | 78.2            | 78.44           | 76.4            | 78.29           |
| C4'      | 72.0            | 71.91           | 72.0            | 71.91           | 69.7            | 71.66           |
| C5'      | 77.9            | 78.20           | 77.9            | 78.2            | 76.2            | 78.14           |
| C6'      | 63.2            | 62.95           | 63.2            | 62.95           | 60.9            | 62.86           |
| C1"      | 104.3           | 104.53          | 104.4           | 104.54          | 103.8           | 106.12          |
| C2"      | 76.1            | 76.75           | 76.1            | 76.77           | 75.0            | 77.20           |
| C3"      | 78.5            | 78.58           | 78.6            | 78.58           | 75.7            | 77.99           |
| C4"      | 71.1            | 70.93           | 71.2            | 70.83           | 69.6            | 71.64           |
| C5"      | 77.9            | 78.34           | 78.0            | 78.35           | 76.7            | 78.37           |
| C6"      | 62.3            | 62.37           | 62.4            | 62.39           | 60.7            | 62.71           |

<sup>a</sup>Recorded at 100 MHz for 13C NMR data in pyridine(pyr)-d5.

<sup>b</sup>Recorded at 125 MHz for 13C NMR data in pyridine(pyr)-d5.

<sup>c</sup>Reference chemical shift.
Figure S6: $^1$H NMR (400 MHz) spectrum of isolated GL
Figure S7: $^{13}$C NMR (100 MHz) spectrum of isolated GL.
Figure S8: $^1$H NMR (400 MHz) spectrum of isolated GLI
Figure S9: $^{13}$C NMR (100 MHz) spectrum of isolated GLI
Figure S10: $^1$H NMR (500 MHz) spectrum of isolated Rg3
Figure S11: $^{13}$C NMR (125 MHz) spectrum of isolated Rg3