Supporting Information

Rec. Nat. Prod. X:X (2019) XX-XX

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

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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).

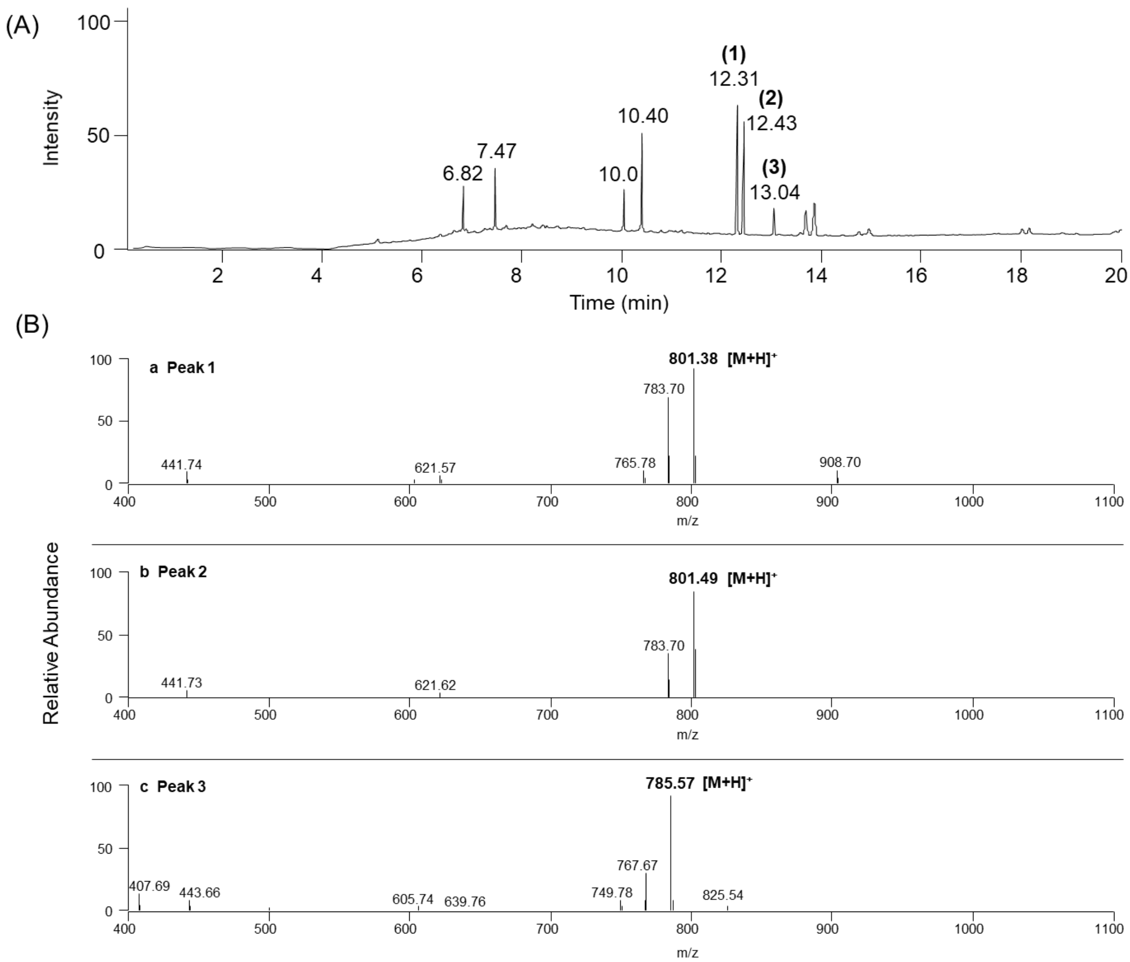

Figure S1: HPLC spectrum and full scan total LC-MS spectra at 10eV (ESI+) revealed two gypenosides and one ginsenoside peak in an extract from GPE. (A) HPLC spectrum of GPE. (B) LC-MS spectra of Peaks 1, 2, and 3.

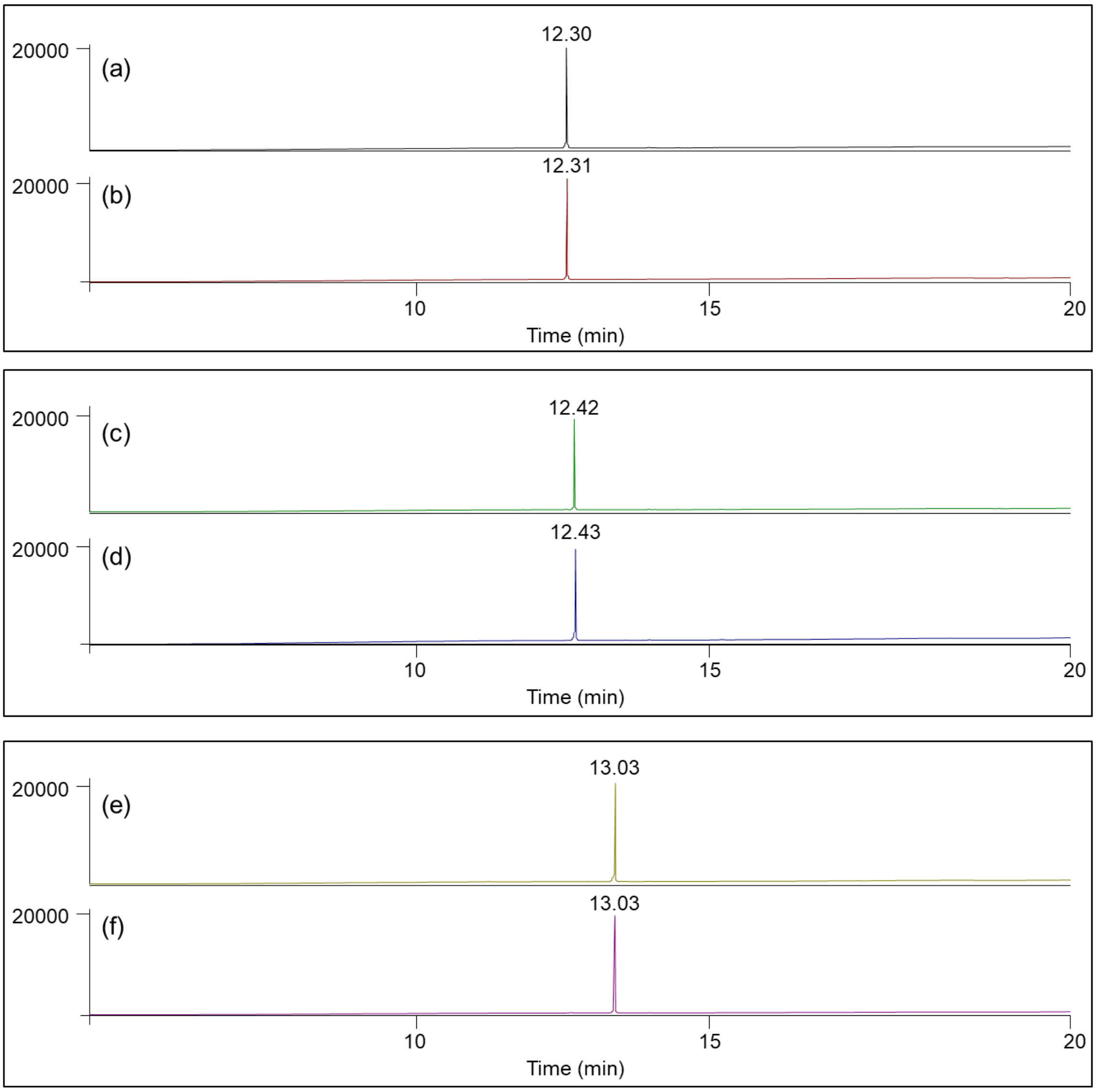

Figure S2: Identification of compound 1(GL), 2(GLI), and 3(Rg3) from GPE. (a)–(b) HPLC spectra of GL standard and Isolated GL, (c)–(d) HPLC spectra of GLI standard and Isolated GLI, (e)–(f) HPLC spectra of Rg3 standard and Isolated Rg3.

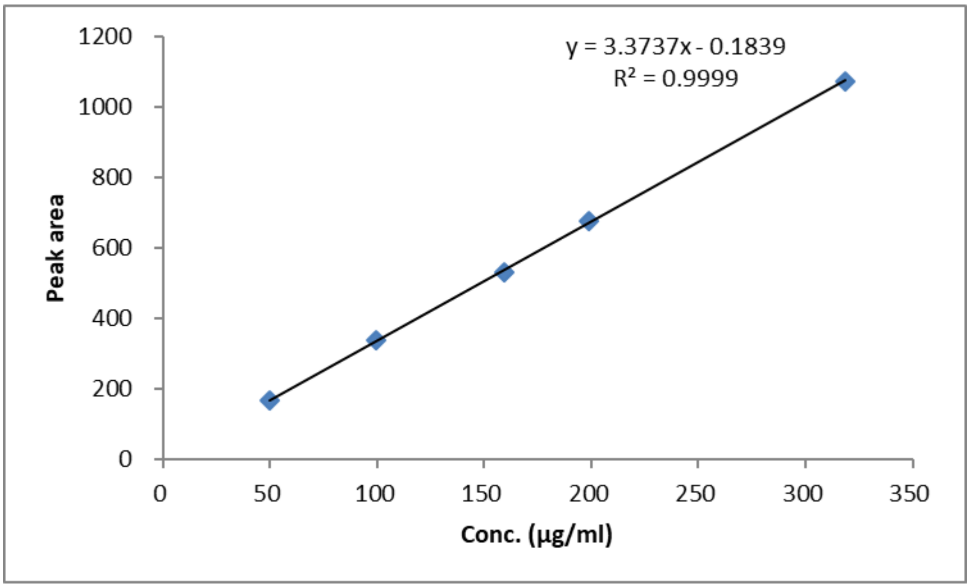

Figure S3: Linearity of GL.

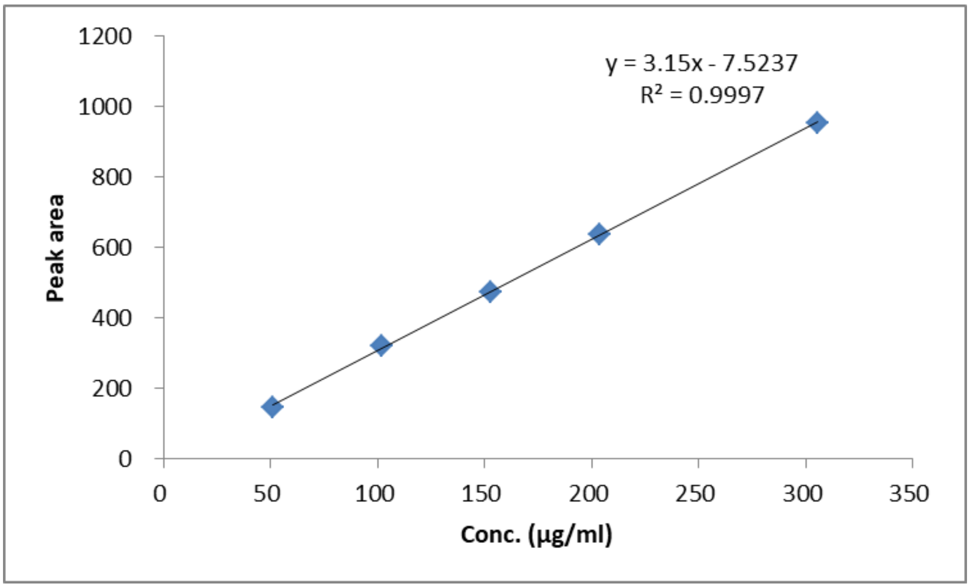

Figure S4: Linearity of GLI.

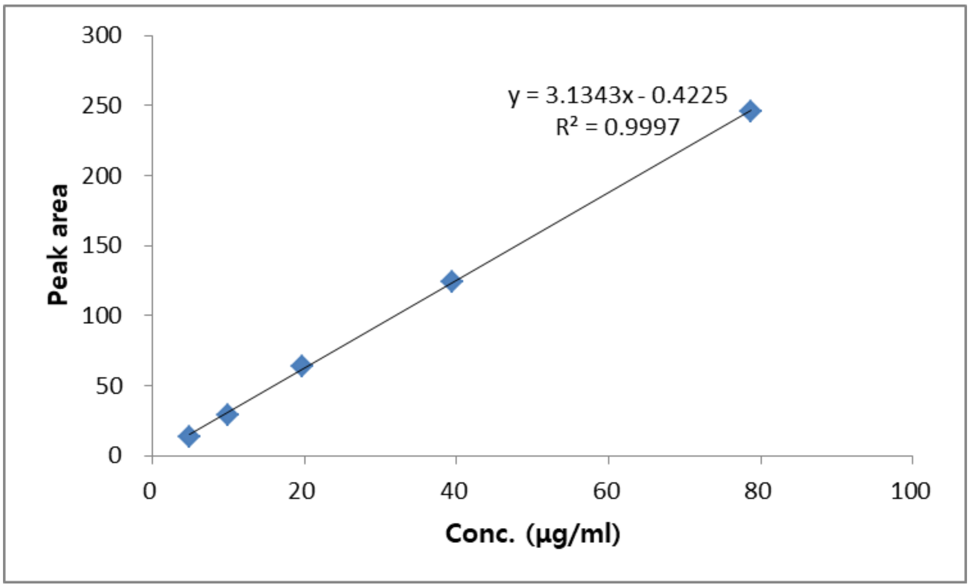

Figure S5: Linearity of Rg3.

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			Mean recovery (%)	RSD (%)
	Concentration. (mg/mL)	Standard added (μg/mL)	Recovery (%)		
GL	7.38	94.24	103.12	101.73	1.532
	10.32		100.04		
	12.42		102.04		
GLI	7.56	101.76	101.21	100.85	0.686
	10.04		101.29		
	12.53		100.05		
Rg3	7.60	19.68	101.49	101.85	0.709
	10.13		101.39		
	12.61		102.69		

Table S3: ^{13}C NMR spectroscopic data for isolated GL, GLI, and Rg3 in pyr-*d*5.

Position	GL[a]		GLI[a]		Rg3[b]	
	δ^R	δ_C	δ^R	δ_C	δ^R	δ_C
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

[a]Recorded at 100 MHz for ^{13}C NMR data in pyridine(pyr)-*d*5.
[b]Recorded at 125 MHz for ^{13}C NMR data in pyridine(pyr)-*d*5.
[R]Reference chemical shift.

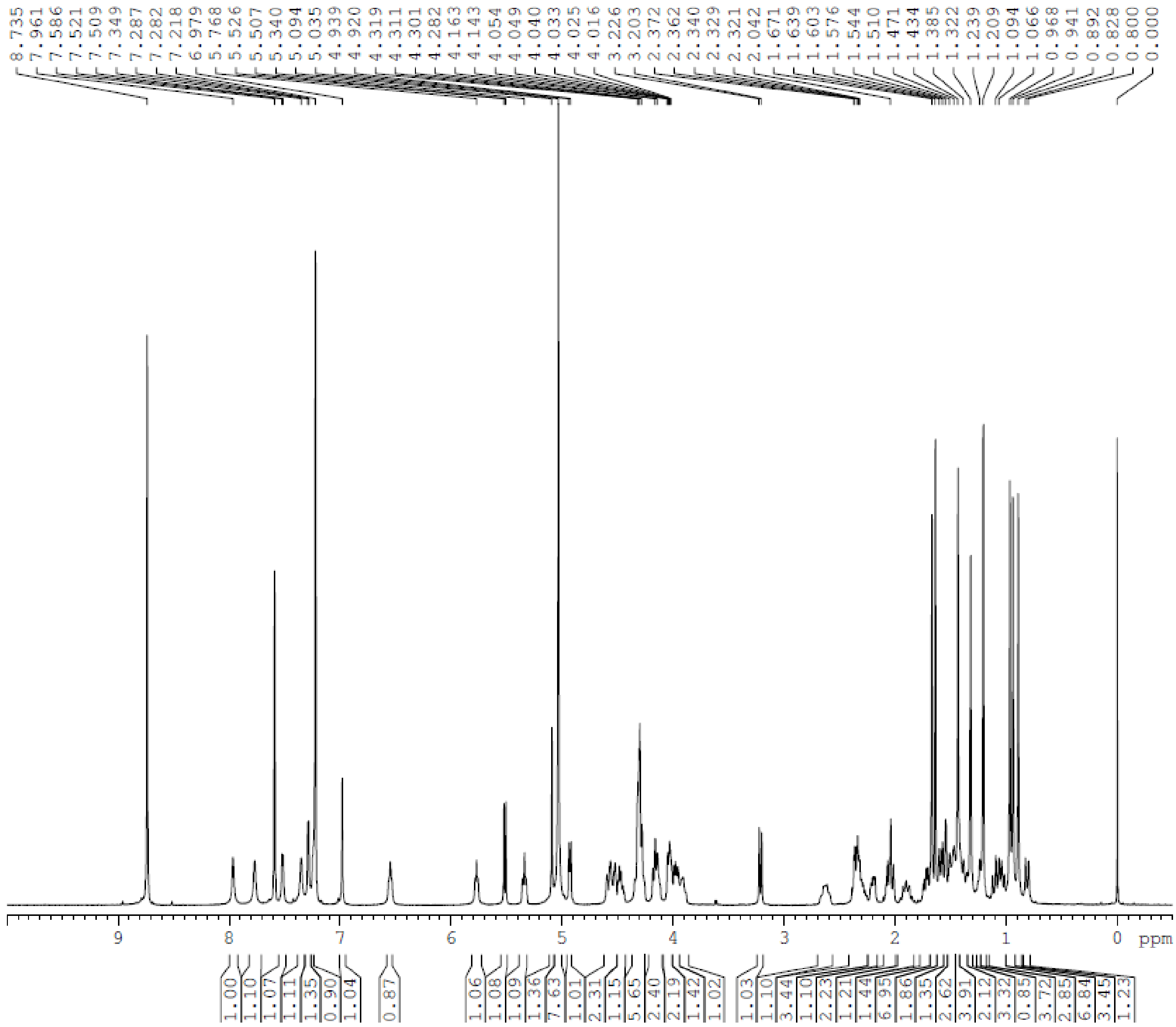

Figure S6: ^{1}H NMR (400 MHz) spectrum of isolated GL.

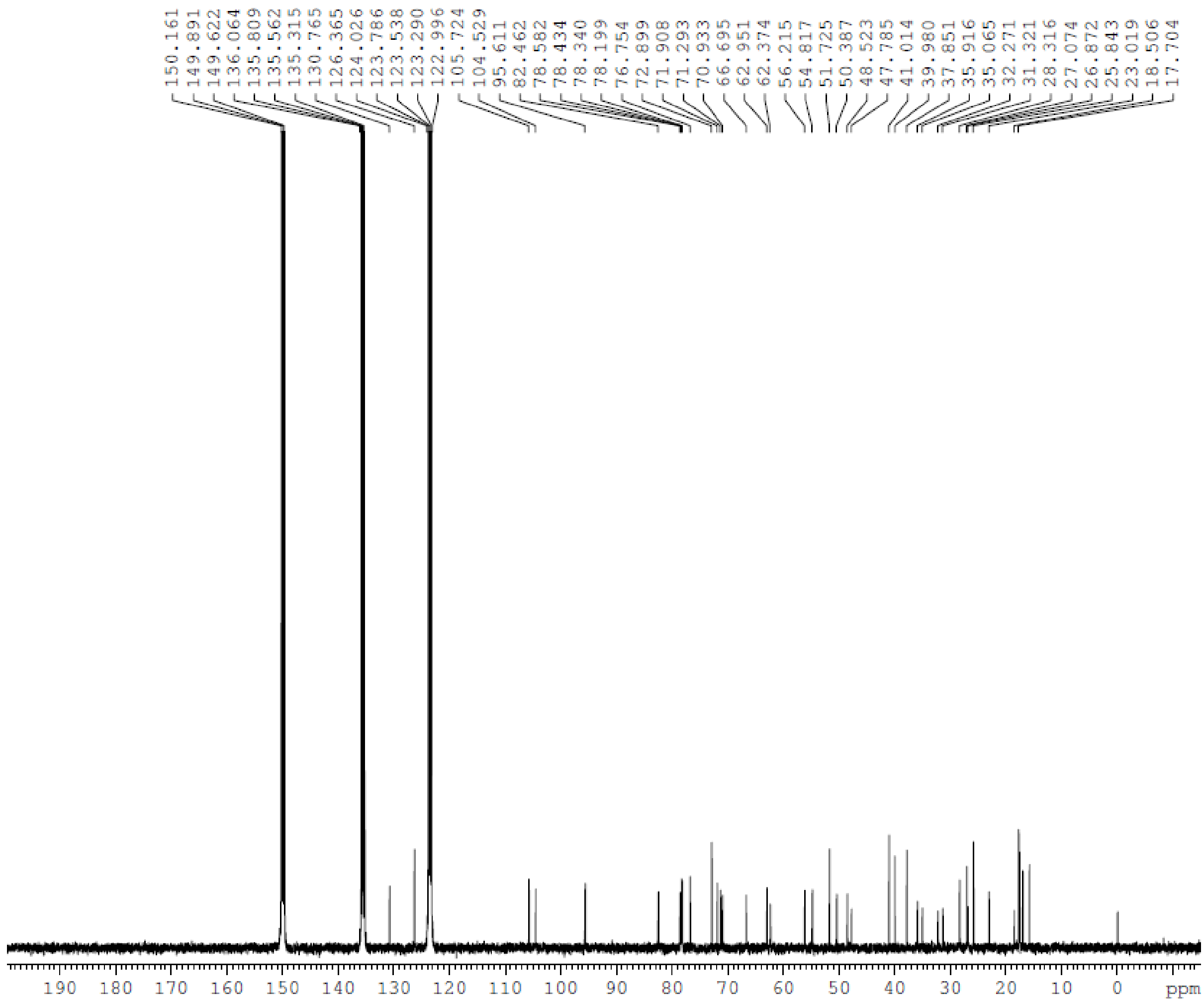

Figure S7: ^{13}C NMR (100 MHz) spectrum of isolated GL.

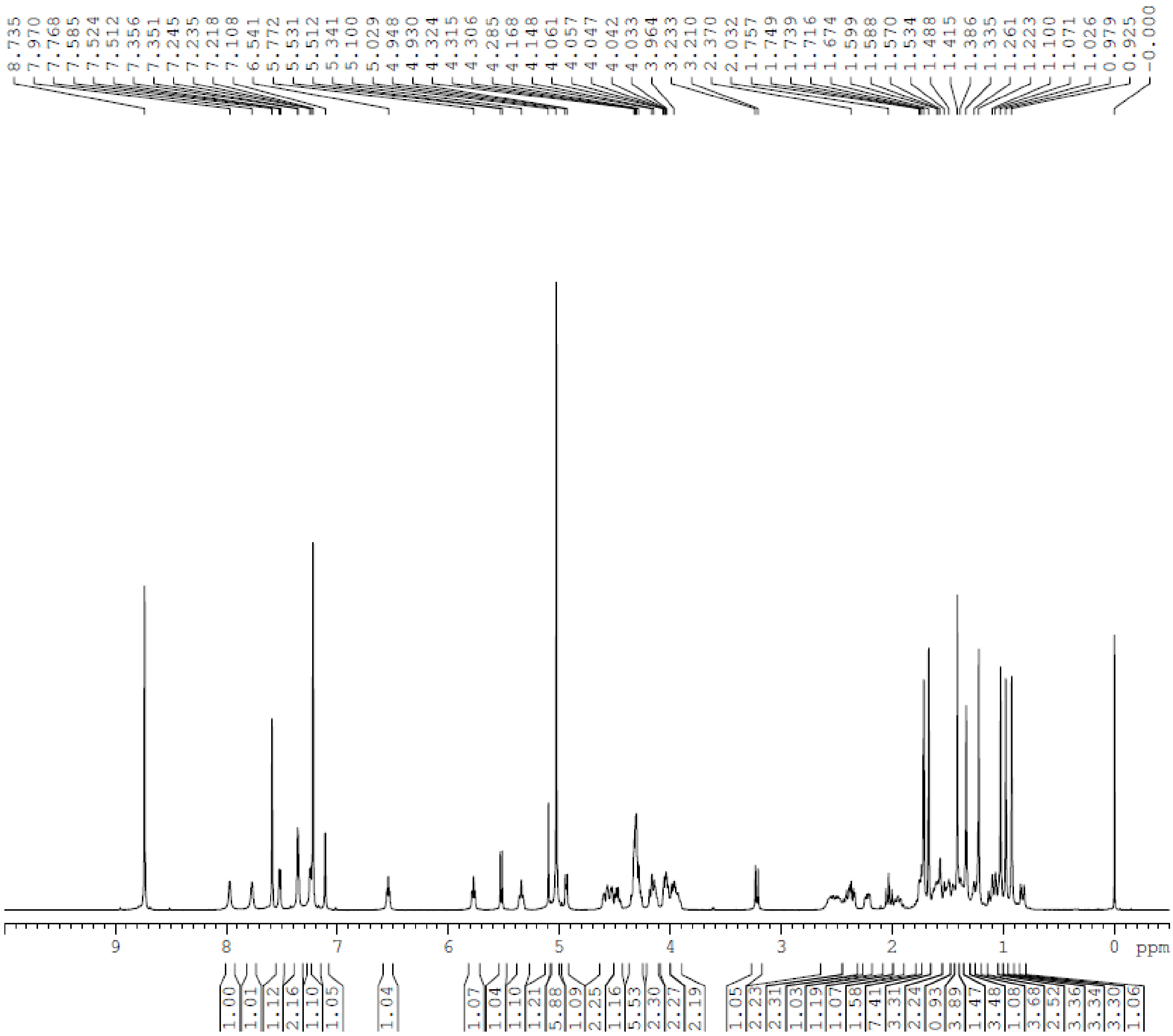

Figure S8: ^{1}H NMR (400 MHz) spectrum of isolated GLI.

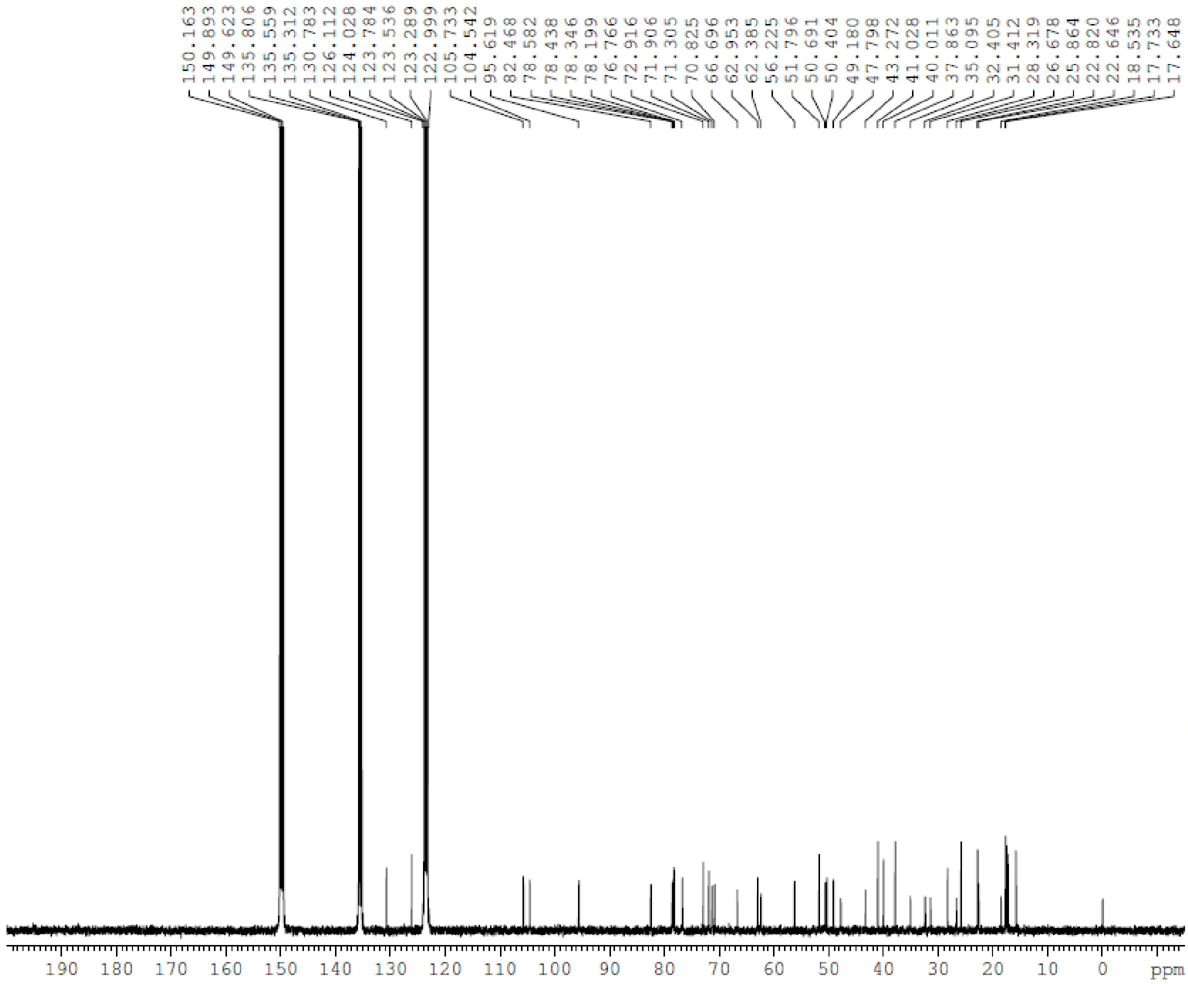

Figure S9: ^{13}C NMR (100 MHz) spectrum of isolated GLI.

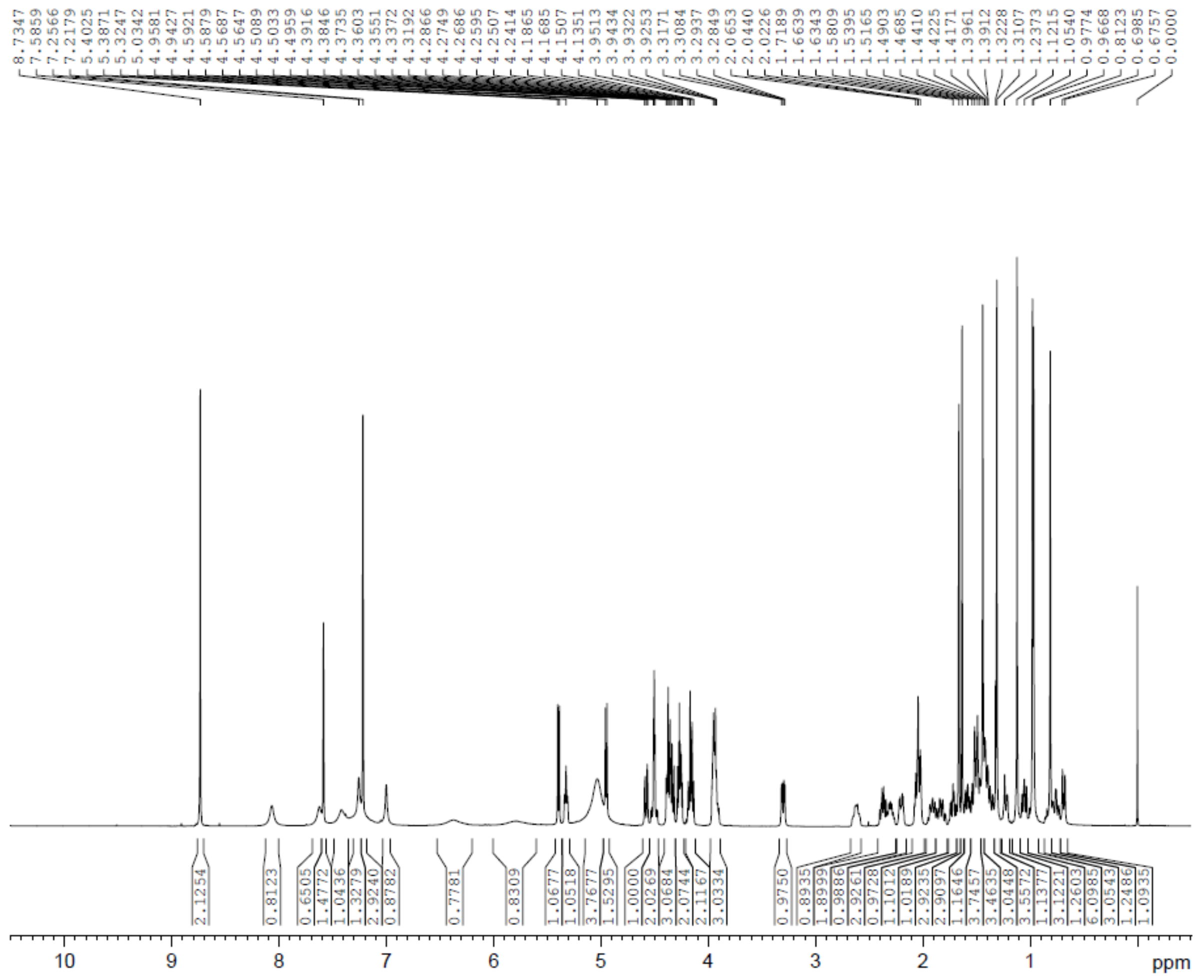

Figure S10: ^{1}H NMR (500 MHz) spectrum of isolated Rg3.

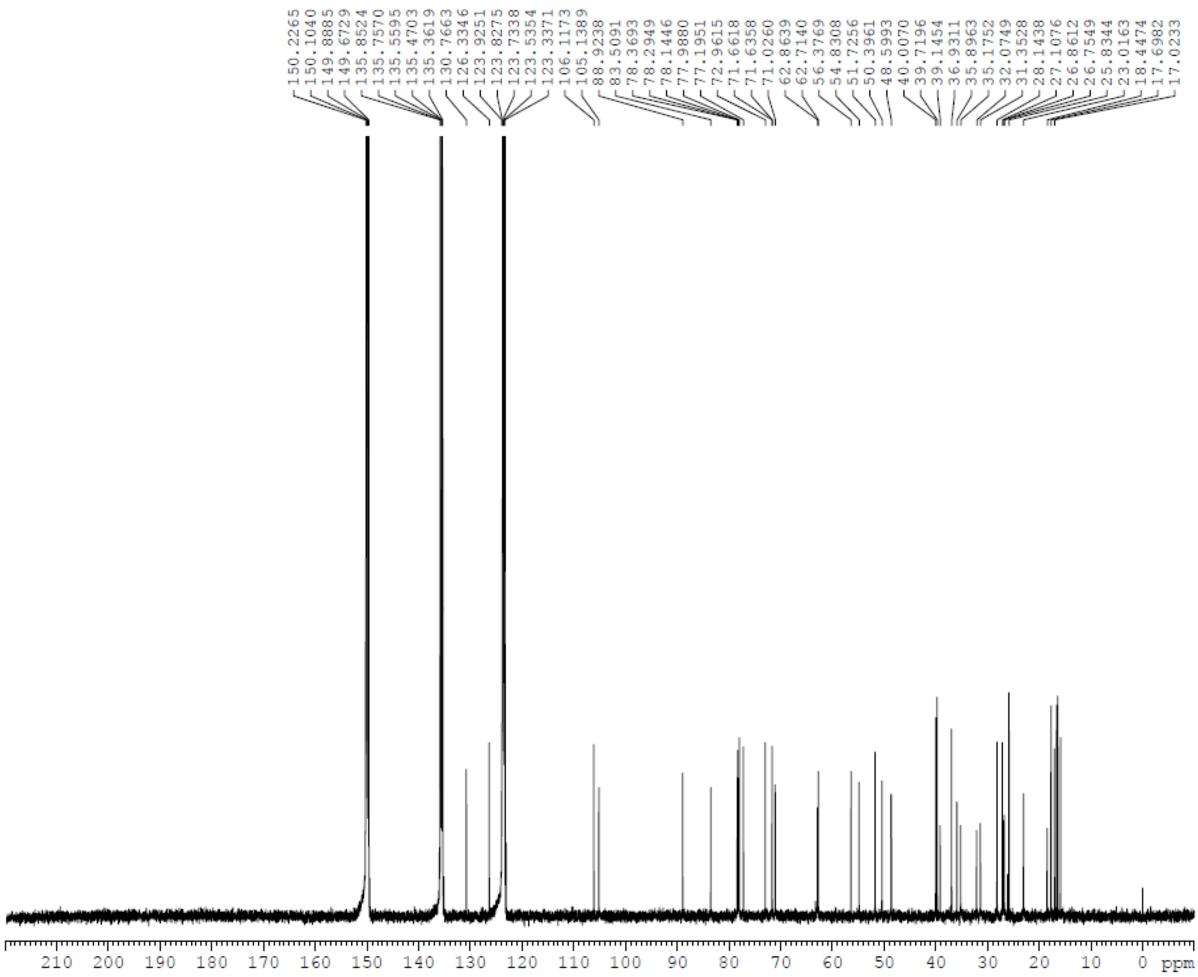

Figure S11: ^{13}C NMR (125 MHz) spectrum of isolated Rg3.