Electronic Supplementary Information

Extraction and isolation of polyhydroxy triterpenoids from *Rosa laevigata* Michx. fruit with anti-acetylcholinesterase and neuroprotection properties

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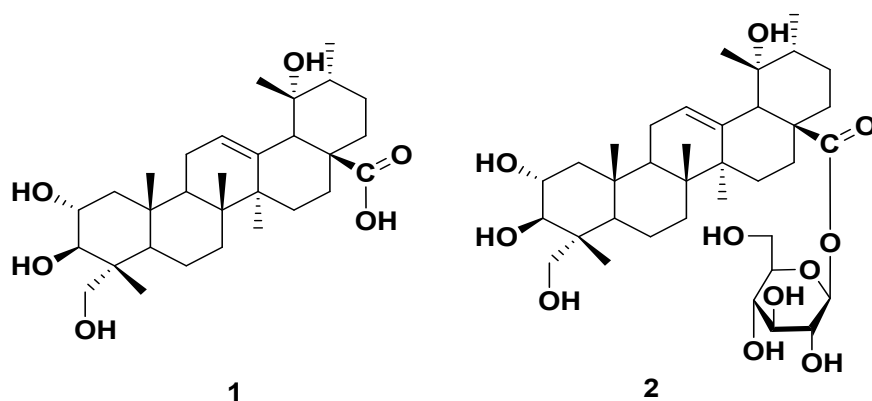**Table   S4   Recoveries   of   compounds   1   and   2.**

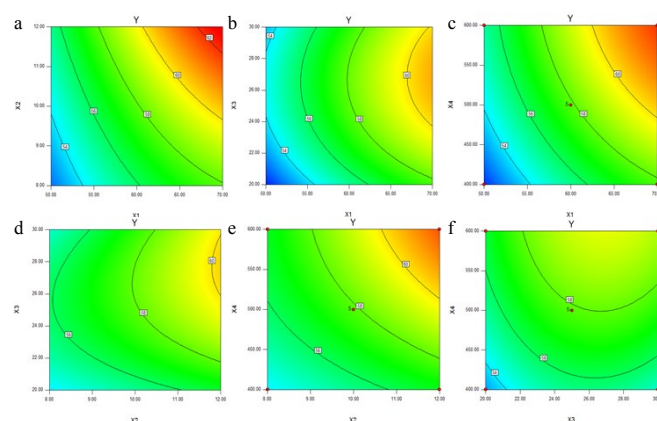**Figure S1.1** Structures of compounds **1** and **2**.

Figure. S2.1 Contour plots displaying the interaction effects of X_1 and X_2 (a), X_1 and X_3 (b), X_1 and X_4 (c), X_2 and X_3 (d), X_2 and X_4 (e), X_3 and X_4 (f) on the saponin content yield of *Rosa laevigata* Michx. fruits. X_1: ethanol concentration; X_2: extraction time; X_3: ratio of liquid to raw material; X_4: microwave power

S. 3.1 Acid Hydrolysis of compound 2 and PMP-HPLC analysis

Compound **2** (50.0 mg) was individually hydrolyzed by 2N HCl (50.0 mL) under reflux for 3 h. The reaction mixtures were extracted with EtOAc, and the organic layer evaporated under reduced pressure to yield **1**. The dried aqueous layer and D-glucose (each 5 mg) were dissolved in 0.5 mL water and treated with 0.5 mL of PMP methanol solution (0.5 mol/L) and 1 mL of NaOH solution (0.6 mol/L) at 70 °C for 100 min. Cooled to room temperature, added 2 mL of HCl solution (0.3 mol/L) for neutralization of residual alkali. The reaction mixture was added distilled water (30 mL) and then extracted with CH_2Cl_2 (30 mL) 3 times. The PMP derivative of **2** was determined by HPLC equipped with a Promosil C_{18} Column (5 μm, 250 mm × 4.6 mm) at 30 °C and the flow rate was 0.8 mL/min. The mobile phase was methanol (**A**): phosphate buffer solution (0.1 mol/mL) (**B**) (A:B = 18:82, v/v). The operating conditions were as follows: detective wavelength, 245 nm; injection volume, 20 μL.

S. 3.1 Characteristic data of compounds
2α, 3β, 19α, 23-tetrahyd roxyurs-12-en-28-oic acid (1):

an amorphous white powder (C$_5$D$_5$N), mp 280~282 °C. ESI-MS (m/z): 1031.67 [2M+Na]$^+$, 527.32 [M+Na]$^+$. ^{1}H-NMR (500 MHz, C$_5$D$_5$N): δ$_H$ 5.55 (1H, br s), 4.23 (1H, dt, J = 9.6, 4.2 Hz), 4.17 (1H, d, J = 10.8 Hz), 4.15 (1H, d, J = 10.8 Hz), 3.69 (1H, d, J = 9.6 Hz)，3.06 (1H, dt, J = 8.4, 2.4 Hz), 3.01 (1H, s), 1.62 (3H, s), 1.48 (3H, s), 1.10 (3H, s), 1.09 (3H, d, J = 6.4 Hz), 1.07 (3H, s), 1.04 (3H, s). ^{13}C-NMR (125 MHz, C$_5$D$_5$N): δ$_C$ 47.8 (C-1), 68.8 (C-2), 78.3 (C-3), 42.1 (C-4), 47.9 (C-5), 18.6 (C-6), 33.1 (C-7), 40.4 (C-8), 47.8 (C-9), 38.3 (C-10), 24.1 (C-11), 127.9 (C-12), 139.9 (C-13), 42.1 (C-14), 29.2 (C-15), 26.3 (C-16), 48.3 (C-17), 54.5 (C-18), 72.6 (C-19), 42.3 (C-20), 26.9 (C-21), 38.3 (C-22), 66.5 (C-23), 14.3 (C-24), 16.7 (C-25), 17.2 (C-26), 24.6 (C-27), 180.6 (C-28), 27.0 (C-29), 17.3 (C-30).

2α, 3β, 19α, 23-tetrahydroxyurs-12-en-28-oic acid-28-*O*-β-D-glucopyrannoside (2)：

colorless needles (MeOD), mp 228～230 °C. ESI-MS (m/z): 1355.79 [2M+Na] $^+$, 689.38 [M+Na]$^+$. ^{1}H-NMR (500 MHz, MeOD): δ$_H$ 5.31 (2H, m), 3.79 (1H, dd, J = 12.0 Hz, J = 2.4 Hz), 3.67 (2H, m), 3.49 (1H, d, J = 10.8 Hz), 3.33-3.34 (5H, m), 3.26 (1H, d, J = 10.8 Hz), 1.33 (3H, s), 1.20 (3H, s), 1.02 (3H, s), 0.92 (3H, d, J = 6.8 Hz), 0.77 (3H, s), 0.69 (3H, s). ^{13}C-NMR (125 MHz, MeOD): δ$_C$ 47.9 (C-1), 69.7 (C-2), 78.3 (C-3), 42.9 (C-4), 48.8 (C-5), 19.2 (C-6), 33.5 (C-7), 41.2 (C-8), 48.2 (C-9), 39.0 (C-10), 24.8 (C-11), 129.5 (C-12), 139.7 (C-13), 41.2 (C-14), 29.6 (C-15), 26.5 (C-16), 48.5 (C-17), 54.9 (C-18), 73.6 (C-19), 42.8 (C-20), 27.2 (C-21), 38.3 (C-22), 66.4 (C-23), 13.8 (C-24), 16.6 (C-25), 17.6 (C-26), 24.7 (C-27), 178.5 (C-28), 27.0 (C-29), 17.6 (C-30), 95.7 (C-1′), 73.8 (C-2′), 78.3 (C-3′), 71.1 (C-4′), 78.5 (C-5′), 62.4 (C-6′).
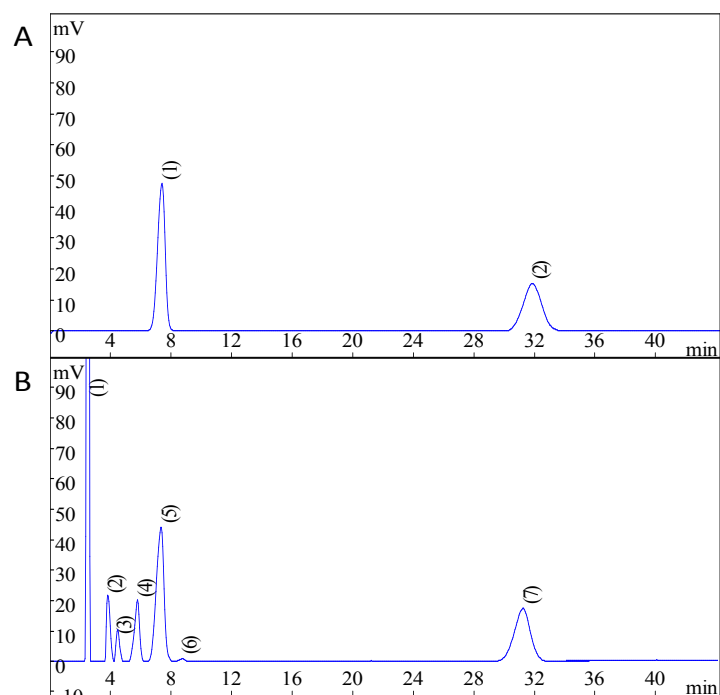

Figure S3.1 High-performance liquid chromatography of standard solution and samples solution. A: standard solutions, (1): saponin , (2): sapogenin; B: sample solutions, (5): saponin, (7): sapogenin.
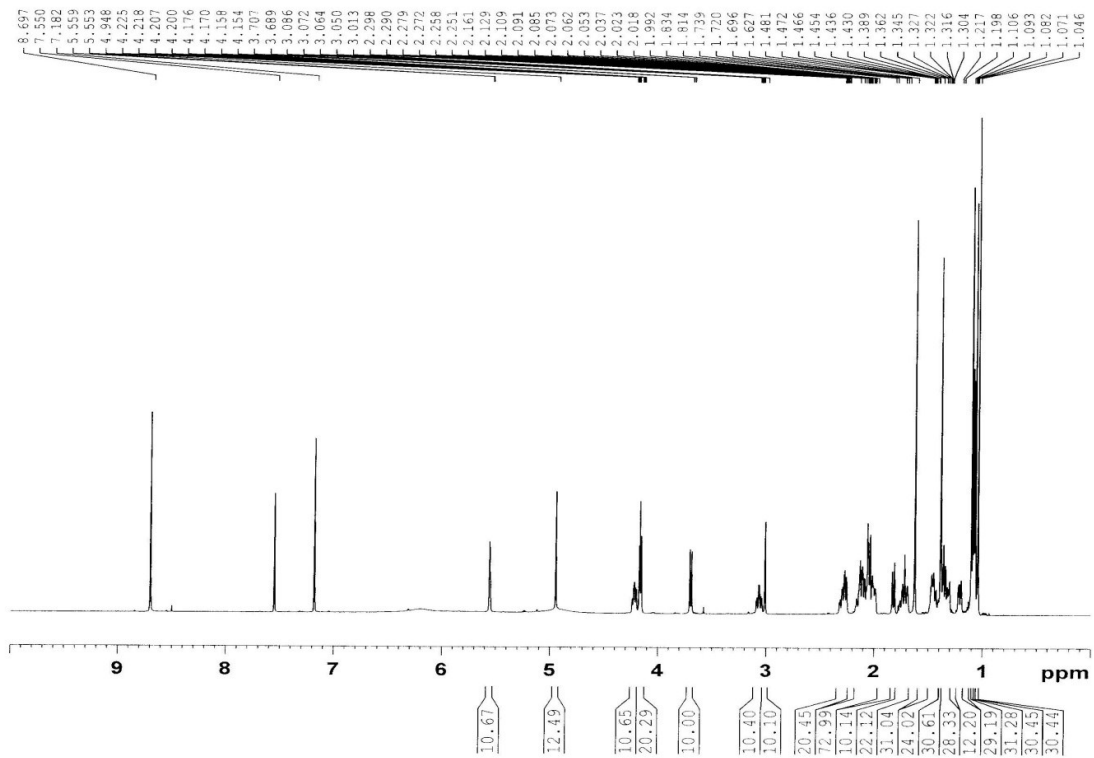
Figure S4.1 ^{1}H NMR spectrum (500 MHz, C$_5$D$_5$N) of compound **1**

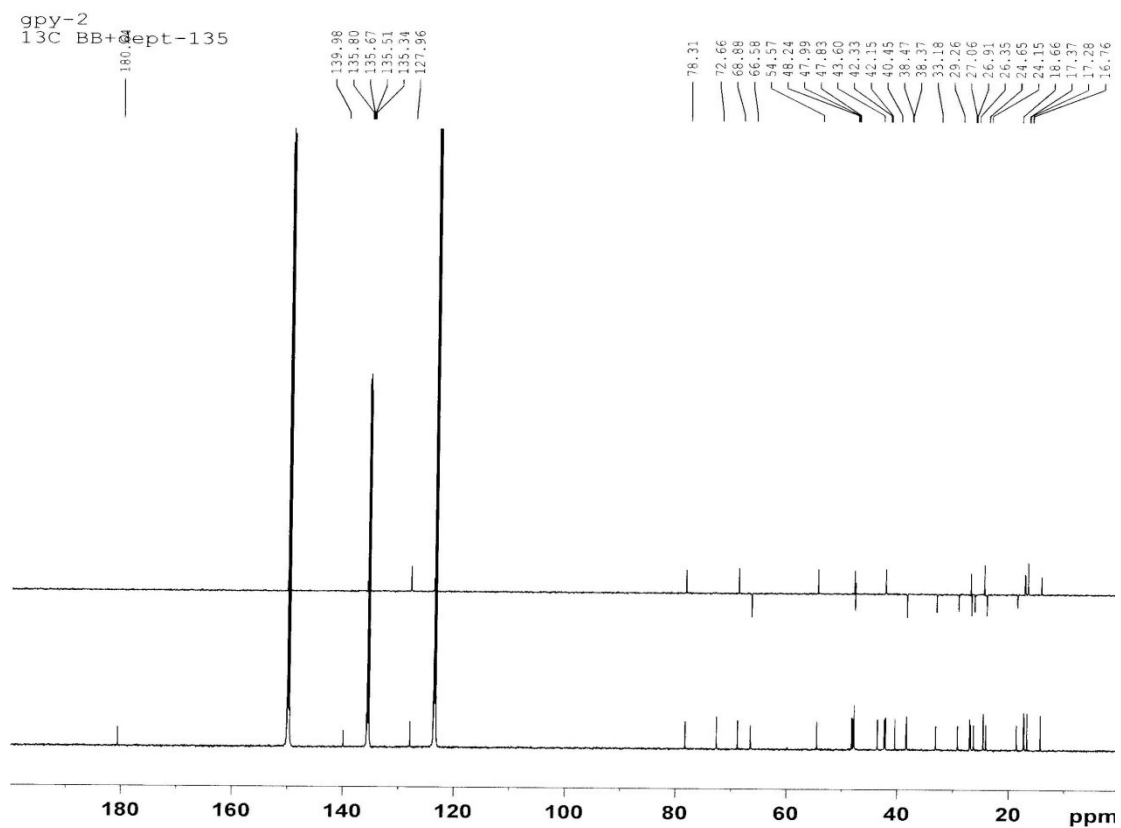**Figure S4.2** ^{13}C NMR spectrum (150 MHz, C$_5$D$_5$N) of compound **1**
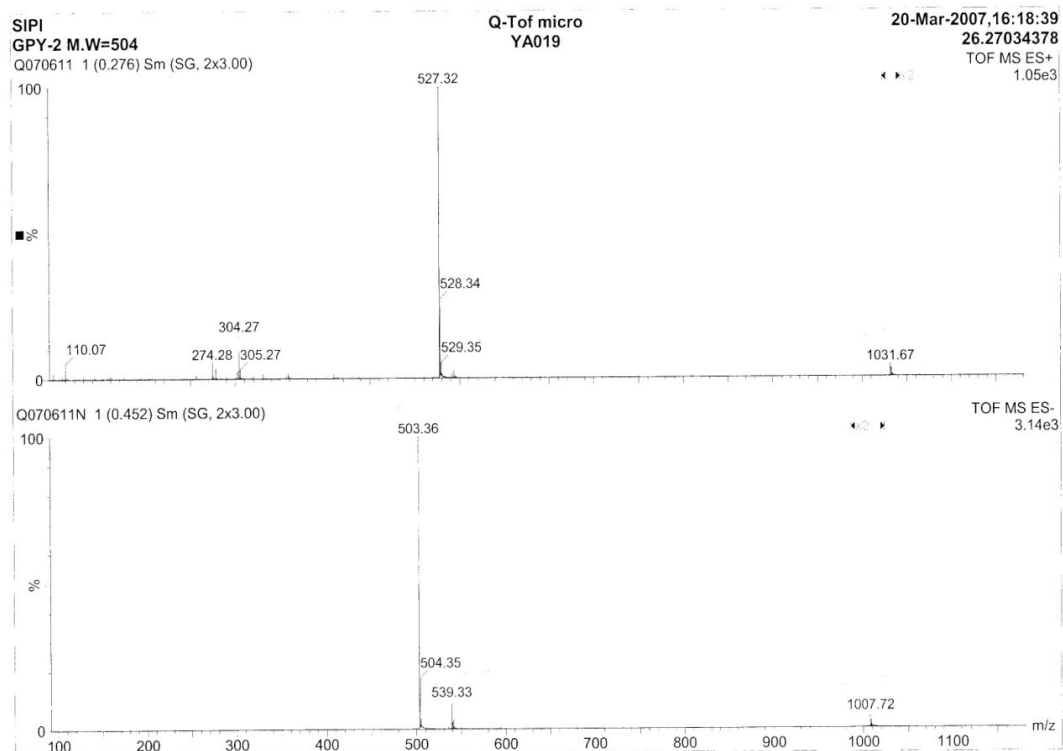
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YA019

20-Mar-2007,16:18:39
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100

%

527.32

528.34

304.27

529.35

110.07
274.28 305.27

1031.67

0

Q070611N 1 (0.452) Sm (SG, 2x3.00)

TOF MS ES-
3.14e3

100

503.36

%

504.35

539.33

1007.72

0
100   200   300   400   500   600   700   800   900   1000   1100   m/z

Figure S4.3 ESI-MS spectrum of compound **1**
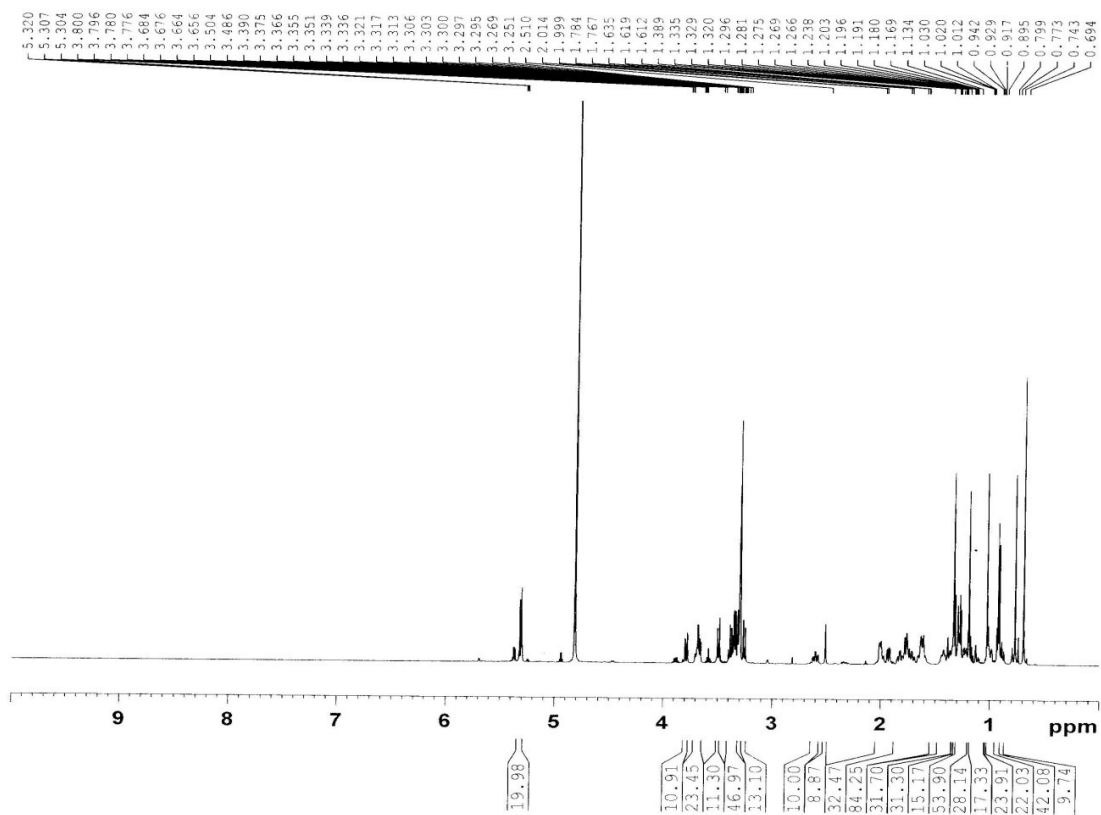
Figure S4.4 ^{1}H NMR spectrum (500 MHz, MeOD) of compound **2**
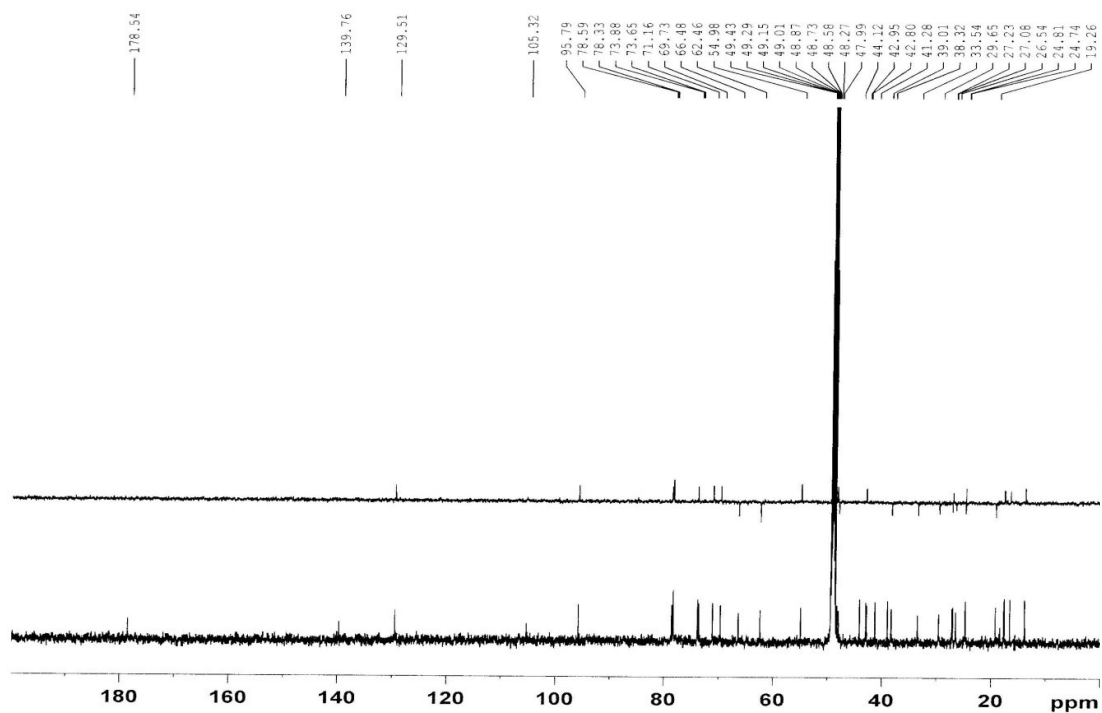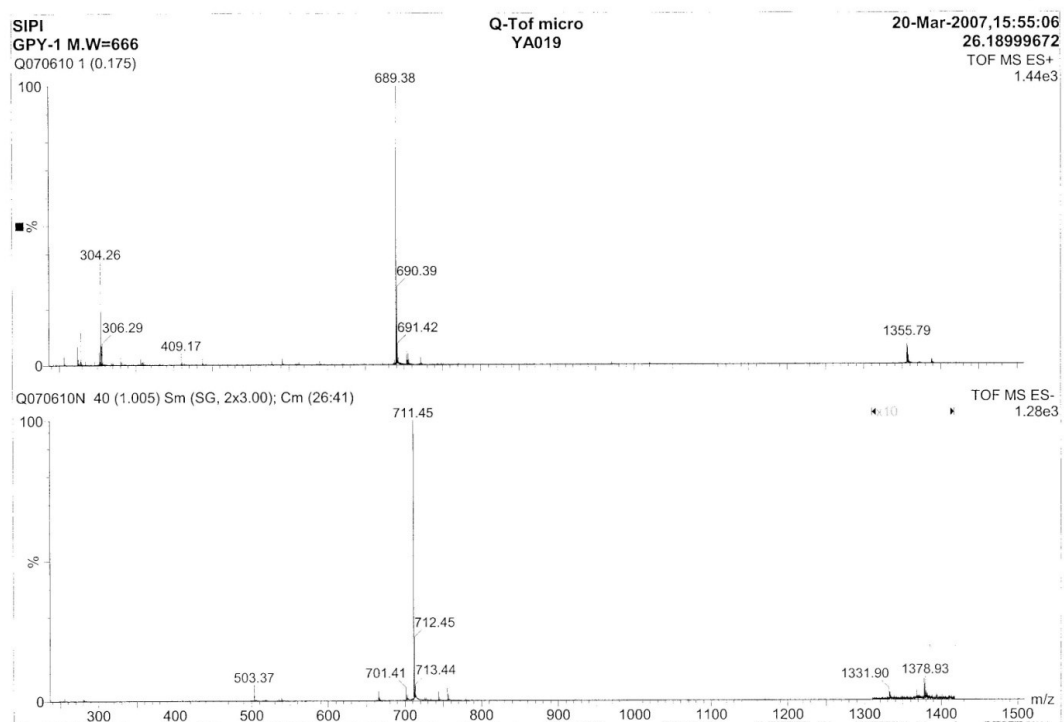
Figure S4.5 ^{13}C NMR spectrum (150 MHz, MeOD) of compound **2**

Figure S4.6 ESI-MS of compound **2**

Table S1 ^{13}C NMR (125MHz) data for compounds **1** and **2** (in C_5D_5N and MeOD).

No.	1	2
	δc	δc
1	47.8	47.9
2	68.8	69.7
3	78.3	78.3
4	42.1	42.9
5	47.9	48.8
6	18.6	19.2
7	33.1	33.5
8	40.4	41.2
9	47.8	48.2
10	38.3	39.0
11	24.1	24.8
12	127.9	129.5
13	139.9	139.7
14	42.1	41.2
15	29.2	29.6
16	26.3	26.5
17	48.3	48.5
18	54.5	54.9
19	72.6	73.6
20	42.3	42.8
21	26.9	27.2
22	38.3	38.3
23	66.5	66.4
24	14.3	13.8
25	16.7	16.6
26	17.2	17.6
27	24.6	24.7
28	180.6	178.5
29	27.0	27.0
30	17.3	17.6
1′		95.7
2′		73.8
3′		78.3
4′		71.1
5′		78.5
6′		62.4

Table S2 Levels and code of variable used for Box–Behnken design (BBD).

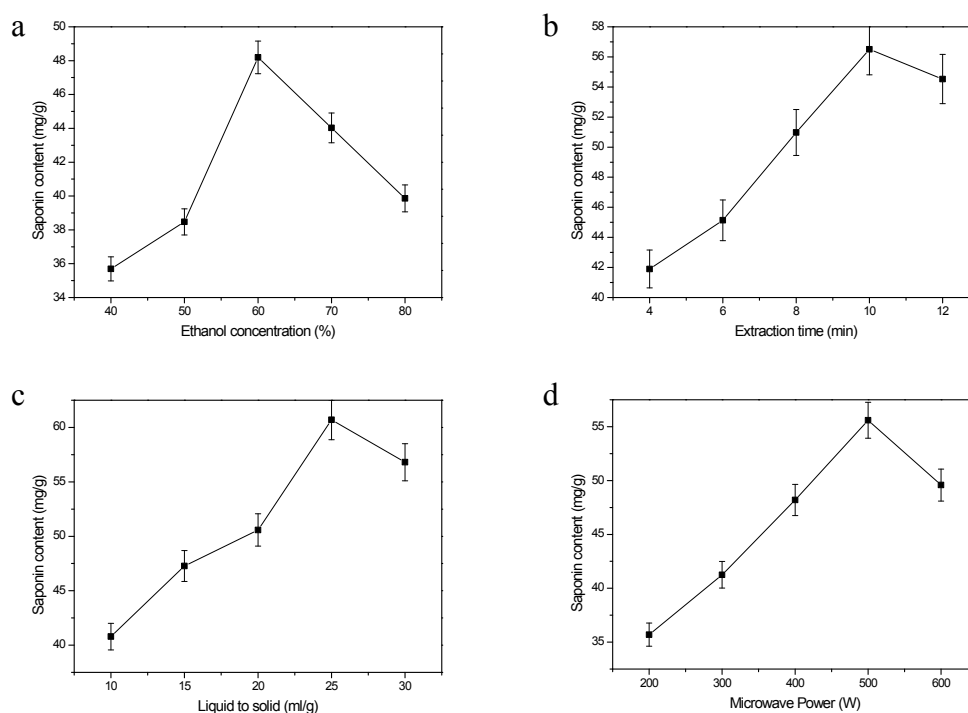| Independent variables	Symbol	Range and level		
		-1	0	1
ethanol concentration (%)	X_1	50	60	70
extraction time(min)	X_2	8	10	12
ratio of liquid to raw material(mL/g)	X_3	20	25	30
microwave power(W)	X_4	400	500	600

Figure S6.1 Effects of the extraction time (X_1), extraction time (X_2), liquid to raw material (X_3) and microwave power (X_4) on the saponin content of *Rosa laevigata* Michx. fruits.

Table S3 Predicted and experimental values of the responses at optimal conditions.

Optimum condition				content of TTSs (Y; mg/g)	
Ethanol concentration (X_1;%)	extraction time (X_2; min)	ratio of liquid to raw material (X_3; mL/g)	microwave power (X_4; W)	Experimental	Predicted
69	12	26:1	528	62.48±0.25	62.69

S. 8.1 Preparation of standard solutions and sample solutions

S. 8.1.1 The preparation of standard solutions

Dried compounds **1** (5 mg) and **2** (2.8 mg) were accurately weighed, and dissolved in a 10 mL volumetric flask with 10 mL methanol.

S. 8.1.2 The preparation of sample solutions

Dried RMLF were powdered and sieved (40 mesh), and accurately weighed 5 g, respectively. They were dissolved in a 50 mL volumetric flask with 50 mL of CH_2Cl_2 and MeOH (5:1), then performed using ultrasonic wave for 30 min and dried under reduced pressure. The dried samples were diluted to 50 mL in a volumetric flask with 50 mL methanol, and then also conducted using ultrasonic wave for 30 min.

S. 9.1 Validation of the predictive model

S. 9.1.1 Linearity studies.

According to HPLC analysis conditions, the standard solutions of 5, 10, 15, 20, 25, 30, 40 μL were filtered using a 0.22 μm filter, and injected into high performance liquid phase instrument. The calibration curve was acquired by the linear regression analysis. The standard curves of compounds **1** and **2** were showed in **Figure S9.1**. The linearity equations of compounds **1** and **2** measured at 210 nm were y = 5868.69x + 6.6558 and y = 9945.21x - 0.90256, and their regression coefficients were 0.9970 and 0.9969, respectively. Good linearity was confirmed with above description.

S. 9.1.2 The precision studies

The standard solution of 10 μL was filtered using a 0.22 μm filter, and determined for five times using HPLC analysis conditions. Then peak areas were recorded. RSDs of compounds **1** and **2** were 0.95% and 0.62%, which displayed good precision.

S. 9.1.3 Reproducibility of this method

RLMF powder of three equal parts was precisely weighed. The sample solution of 25 μL was determined using HPLC analysis conditions, and filtered using a 0.22 μm filter each time, then recorded peak areas. The reproducibility research was performed by calculating peak areas. RSDs

confirmed of compounds **1** and **2** were 2.5% and 1.4%, which showed satisfactory reproducibility.

S. 9.1.4 Recovery studies

The recovery research was carried out via standard addition method. Compounds **1 (**3.2 mg, 3.19 mg, 3.2 mg) and **2** (3.4 mg, 3.39 mg, 3.38 mg) were separately added to RLMF powders (5 g), and analyzed at each addition. From Table 3-1, it indicated that the recoveries of compounds **1** and **2** were 101.56% (RSD: 1.58%) and 102.94% (RSD: 0.97%), respectively. Good recovery was confirmed with above result.

S. 9.1.5 Stability test of the solutions

The sample solution of 25μL was filtered using a 0.22 μm filter, and injected into high performance liquid phase instrument every 3 h for 5 times. Peak shapes of 5 were stable, which implied good stability of the sample solution. Thus, this method offered fit accurateness for determination of the sample solution in RLMF.
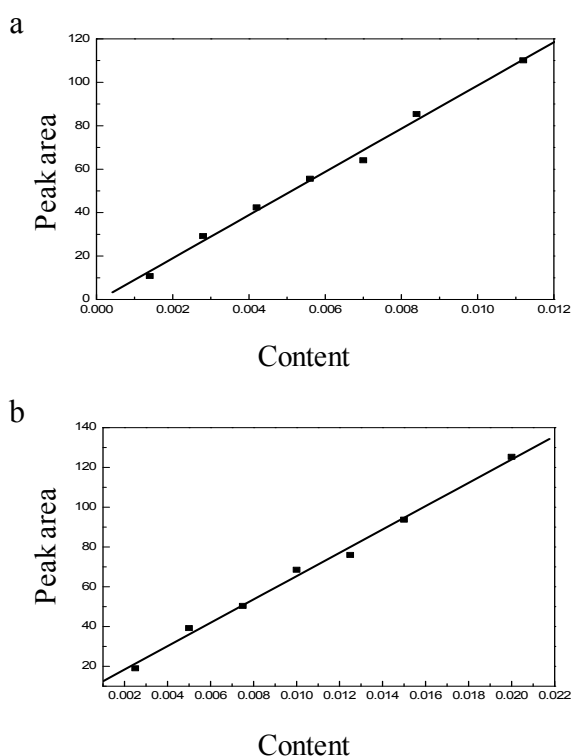

Figure S9.1 The standard curves of compounds **1** and **2** (a and b) for Linearity studies.

Table S4 Recoveries of compounds **1** and **2**

Triterpenoids	Added (mg/g, n=3)	Test values (mg/g, n=3)	Recovery (%, n=3)	RSD (%, n=3)

1	0.64	0.65	101.56	1.58
2	0.68	0.70	102.94	0.97