SUPPLEMENTARY MATERIAL

A new triterpenoid saponin from *Clinopodium chinense* (Benth.) O.Kuntze

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A new triterpenoid saponin from *Clinopodium chinense* (Benth.) O.Kuntze

A new triterpene saponin, 3*β*, 16*β*, 23*α*, 28*β*, 30*β* - pentahydroxyl - olean -11, 13(18) - dien - 3*β* - yl - [*β*- D - glucopyranosyl- (1→2)] - [*β* - D - glucopyranosyl - (1 → 3)] - *β* - D - fucopyranoside, was named Clinoposaponin D (**1**), together with six known triterpene saponins, buddlejasaponin IVb (**2**), buddlejasaponin IVa (**3**), buddlejasaponin IV (**4**), clinopodisides D (**5**), 11*α*, 16*β*, 23, 28-Tetrahydroxyolean - 12-en - 3*β* - yl - [*β* - D - glucopyranosyl - (1→2)] - [*β* - D - glucopyranosyl - (1→3)] - *β* - D - fucopyranoside (**6**), prosaikogenin A (**7**), and two known triterpenes, saikogenin A (**8**), saikogenin F (**9**) was isolated from *Clinopodium chinense* (Benth.) O. Kuntze. Their structures were elucidated on the basis of 1D, 2D NMR and MS analysis. Meanwhile, the effects of all compounds on rabbit platelet aggregation and thrombin time (TT) were investigated in vitro. Compounds **4** and **7** had significant promoting effects on platelet aggregation with EC_{50} value at 53.4 μM and 12.2 μM, respectively. In addition, the highest concentration (200 μM) of compounds **2** and **9** shortened TT by 20.6% and 25.1%, respectively.

Key words: *Clinopodium chinense* (Benth.) O.Kuntze; triterpene saponin; platelet aggregation; thrombin time

NMR Spectra of Compound 1

Herein, we provide the original 1D and 2D NMR spectra and NMR chemical shifts of Compound **1** .

The ^{1}H-NMR spectrum see Figure S1-S3, ^{13}C-NMR spectrum see Figure S4-S6, ^{1}H-^{1}H COSY spectrum see Figure S7, HSQC spectrum see Figure S8, HMBC spectrum see Figure S9, and ROESY spectrum see FigureS10; NMR chemical shifts see Table S1.

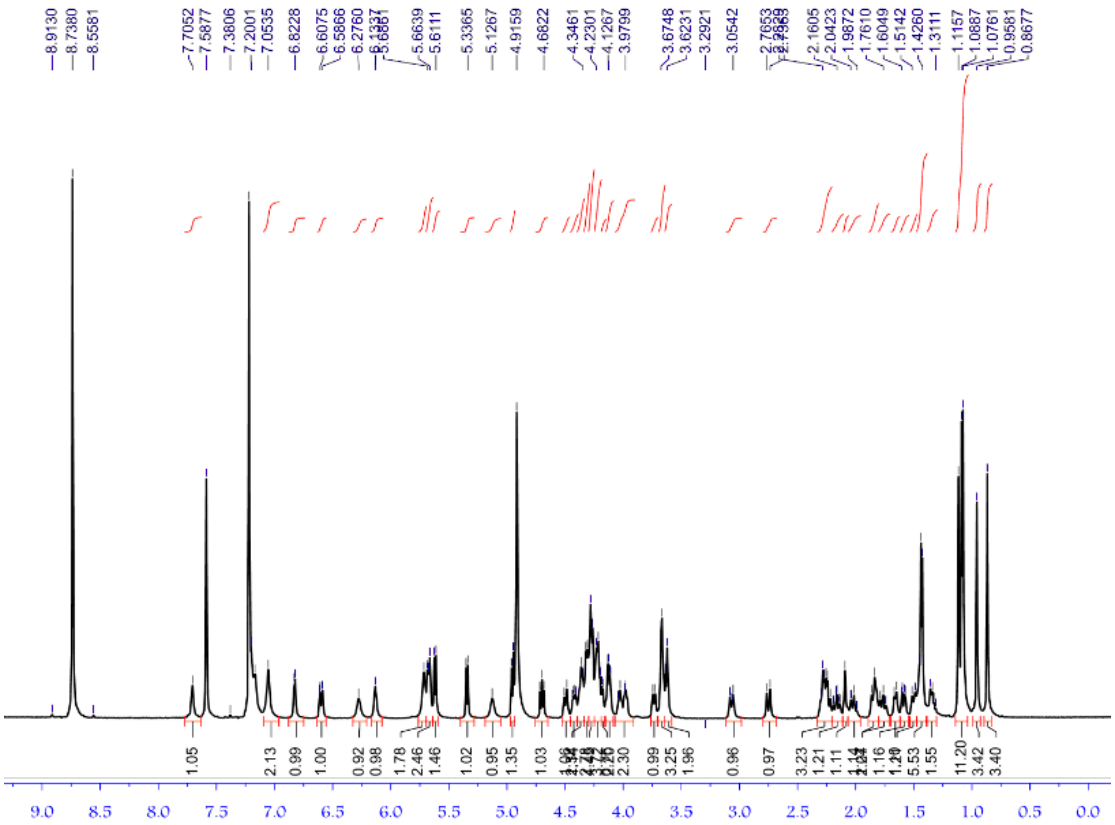

Figure S1. ^{1}H-NMR spectrum of Compound **1** (pyridine-d_5, 500 MHz)

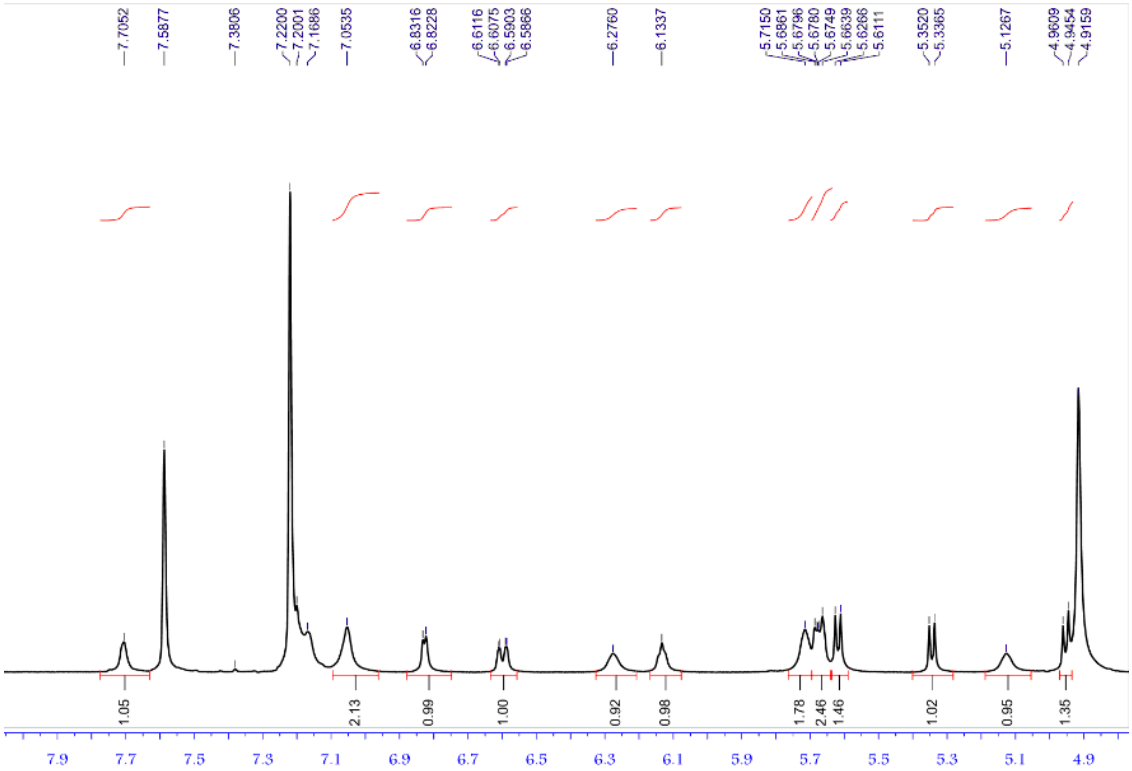

Figure S2. ^{1}H-NMR spectrum of Compound **1** (pyridine-d_5, 500 MHz)

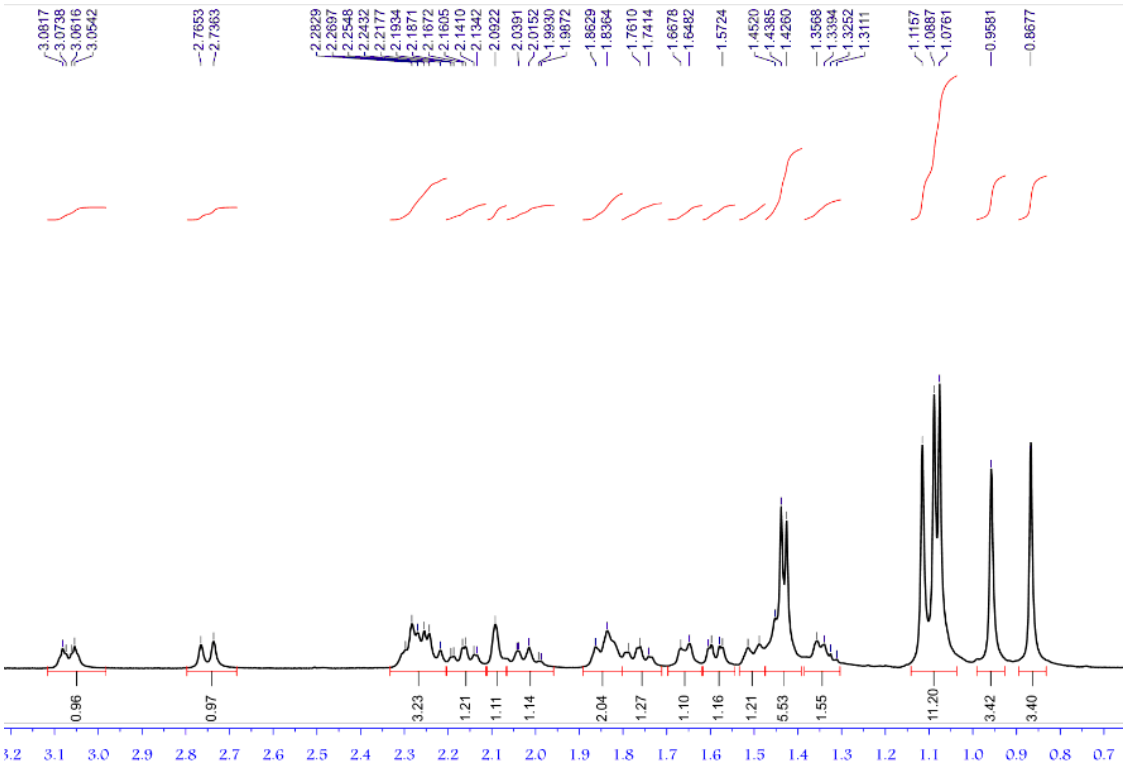

Figure S3. ^{1}H-NMR spectrum of Compound **1** (pyridine-d_5, 500 MHz)

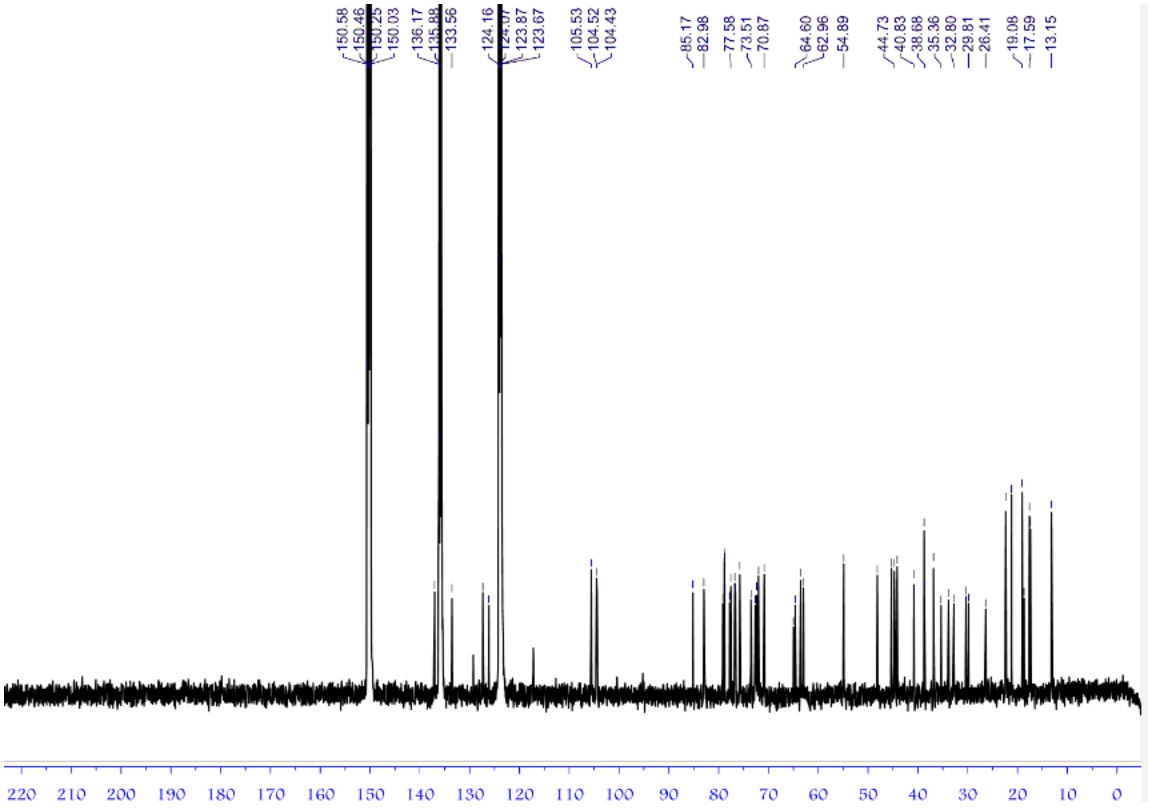

Figure S4. ^{13}C-NMR spectrum of Compound **1** (pyridine-d_5, 125 MHz)

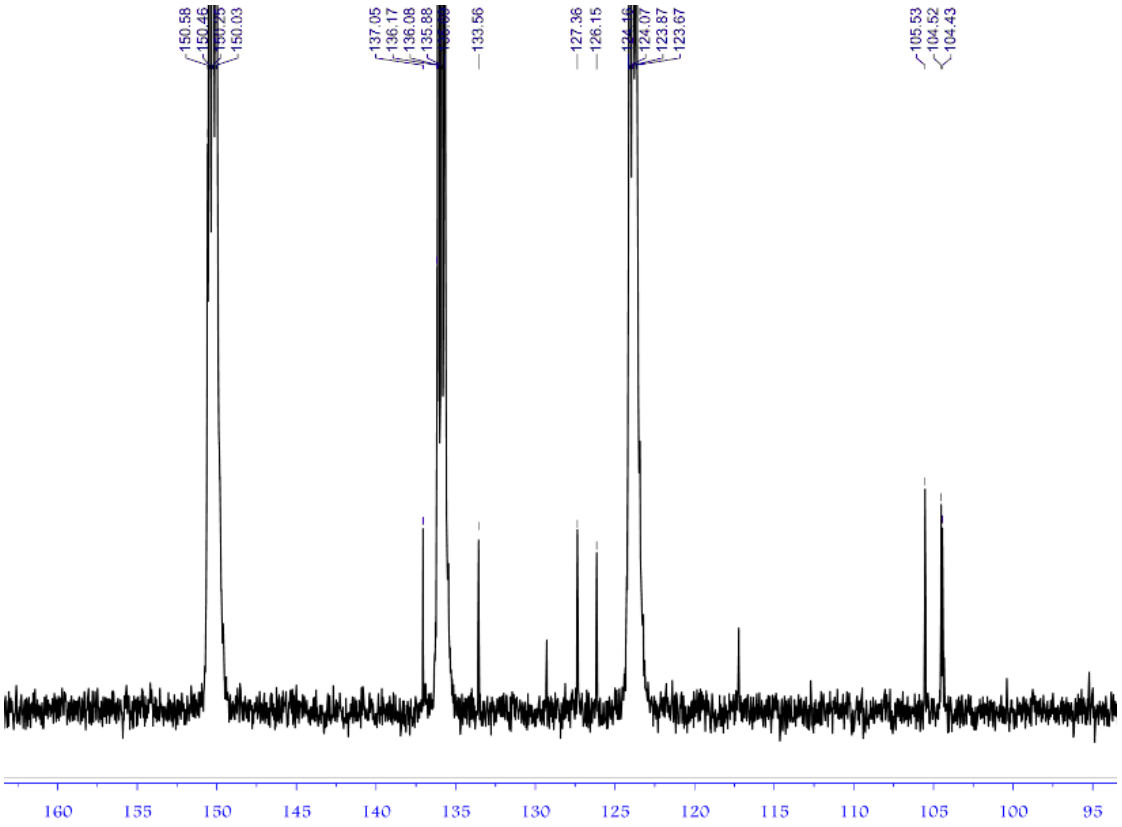

Figure S5. ^{13}C-NMR spectrum of Compound **1** (pyridine-d_5, 125 MHz)

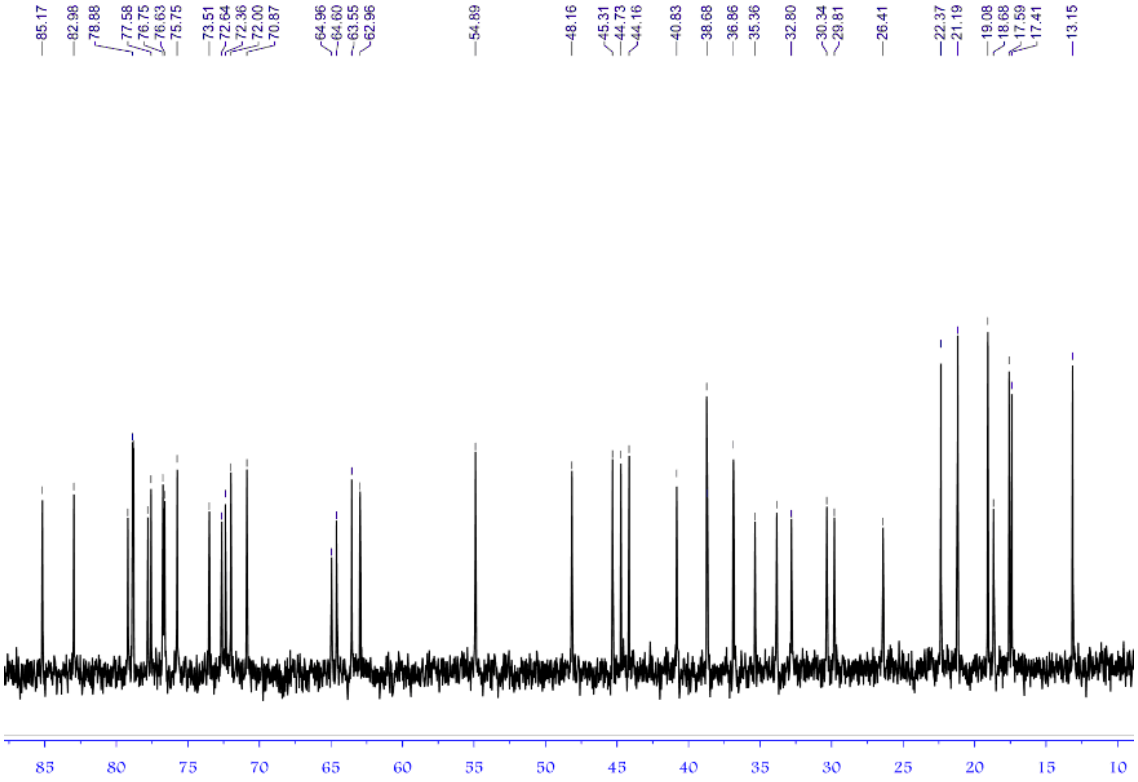

Figure S6. ^{13}C-NMR spectrum of Compound **1** (pyridine-d_5, 125 MHz)

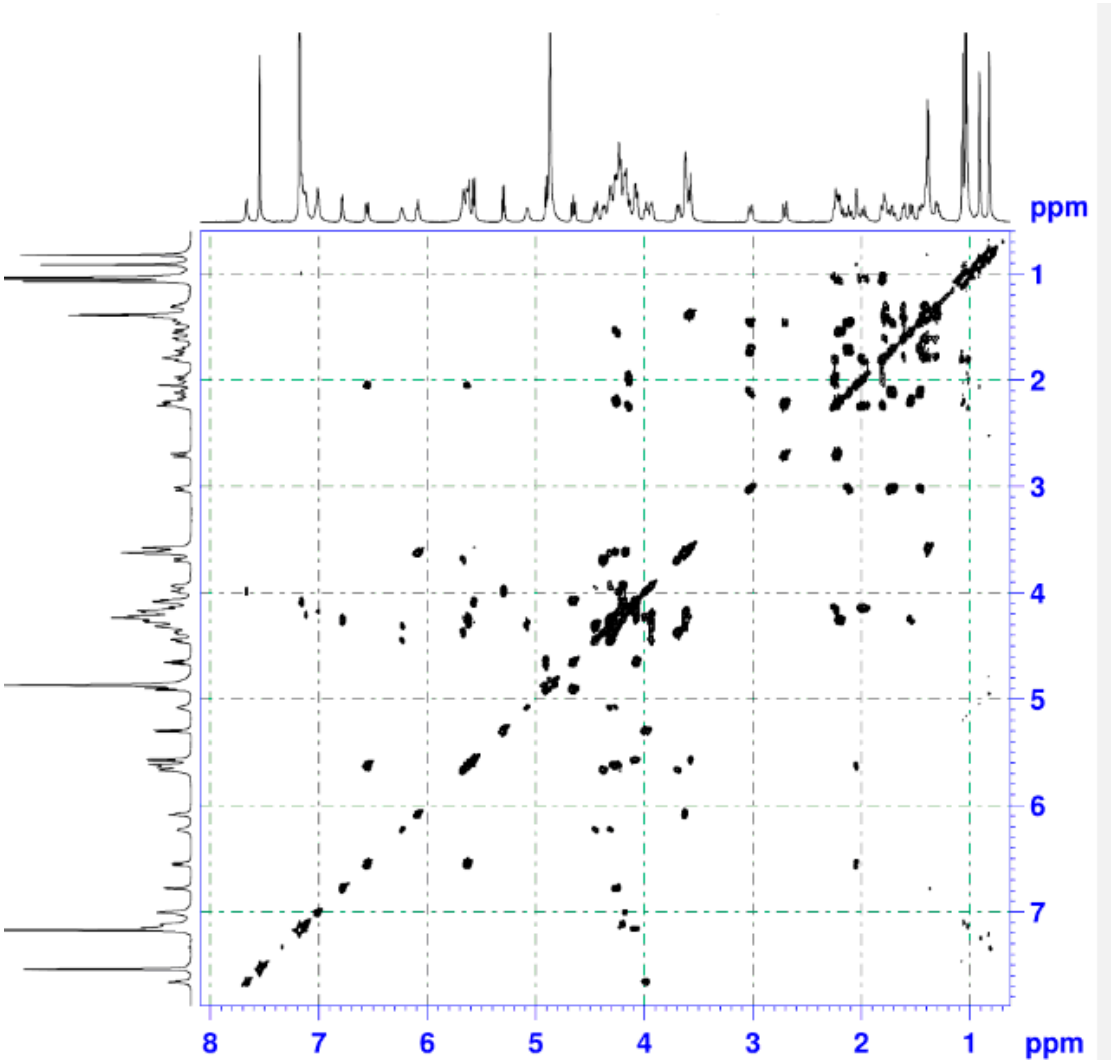

Figure S7. ^{1}H-^{1}H COSY spectrum of Compound **1** (pyridine-d_5, 500 MHz)

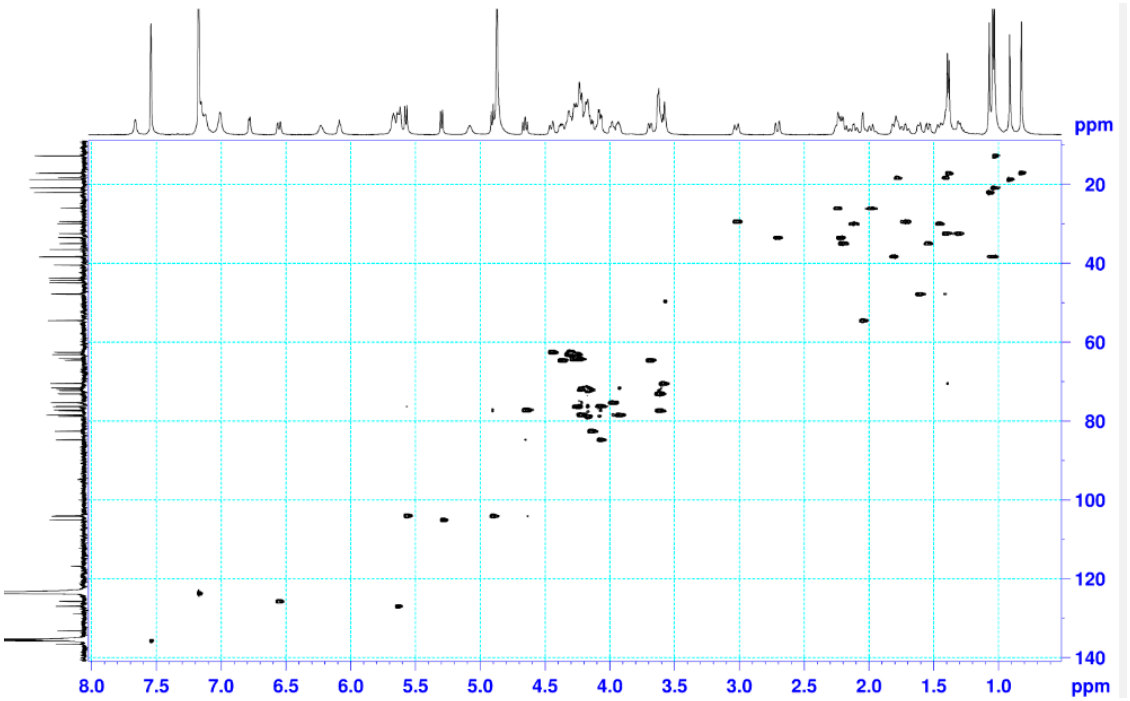

Figure S8. HSQC spectrum of Compound **1** (pyridine-d_5, 500 MHz)

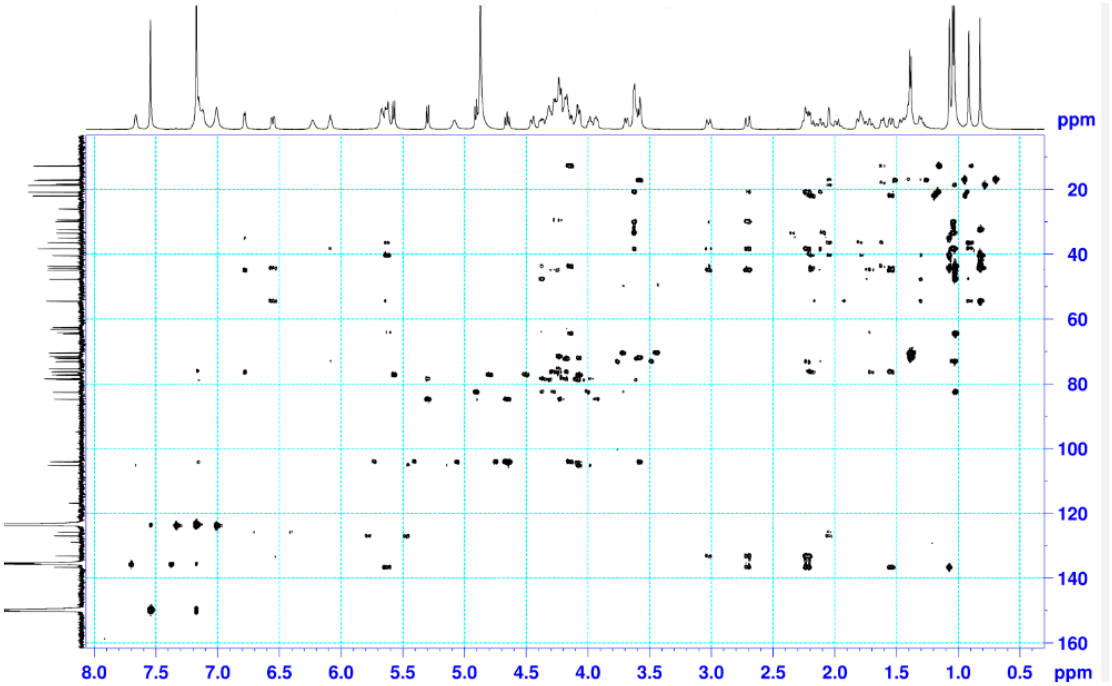

Figure S9. HMBC spectrum of Compound **1** (pyridine-d_5, 500 MHz)

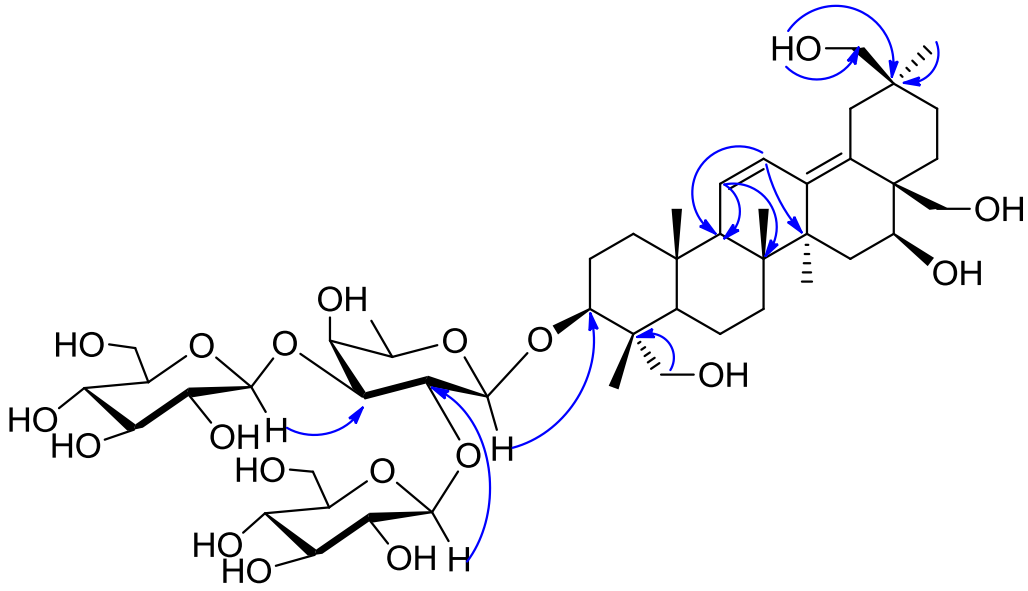

Figure S10. Specific HMBC correlations of compound **1** (arrows point from H to C)

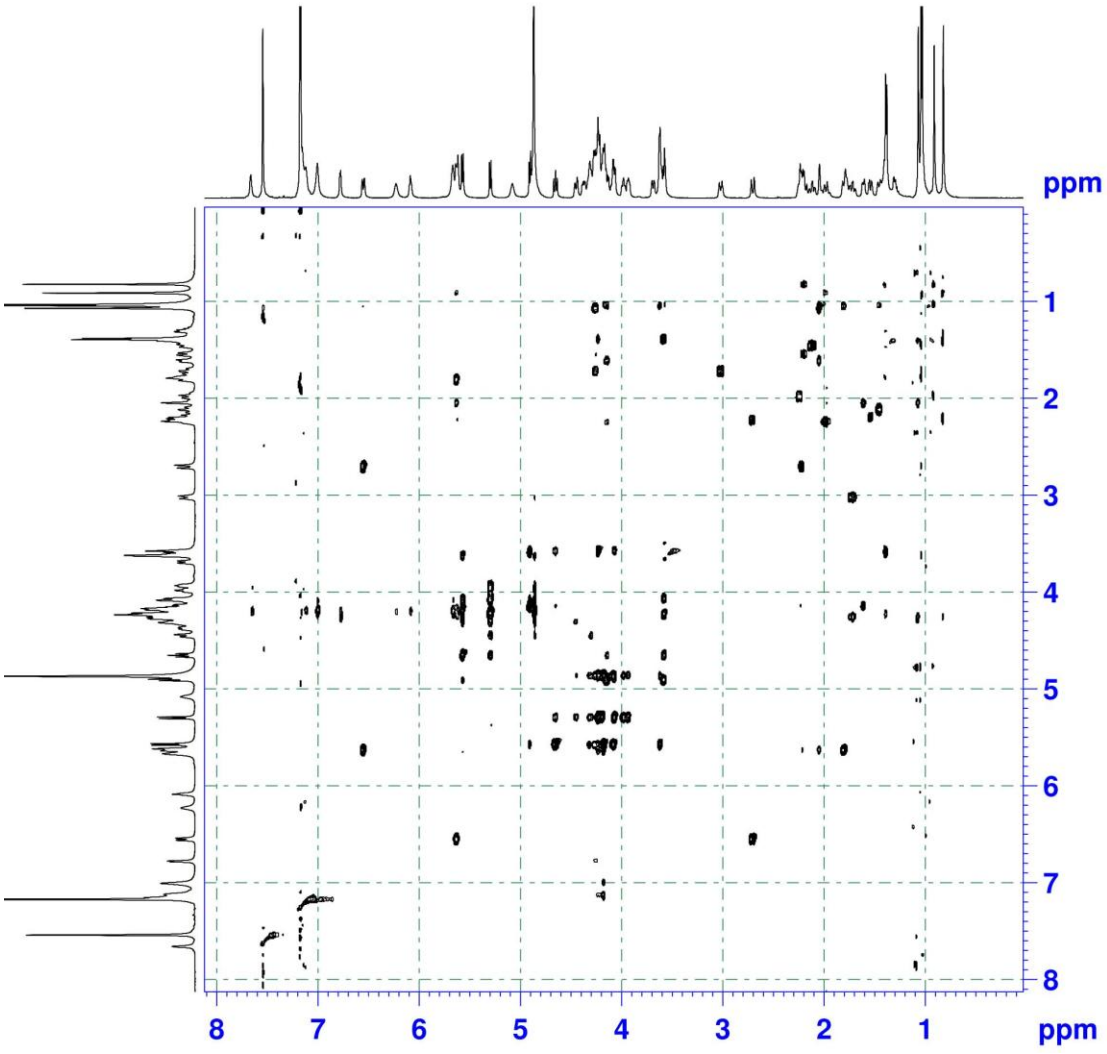

Figure S11. ROESY spectrum of Compound **1** (pyridine-d_5, 500 MHz)

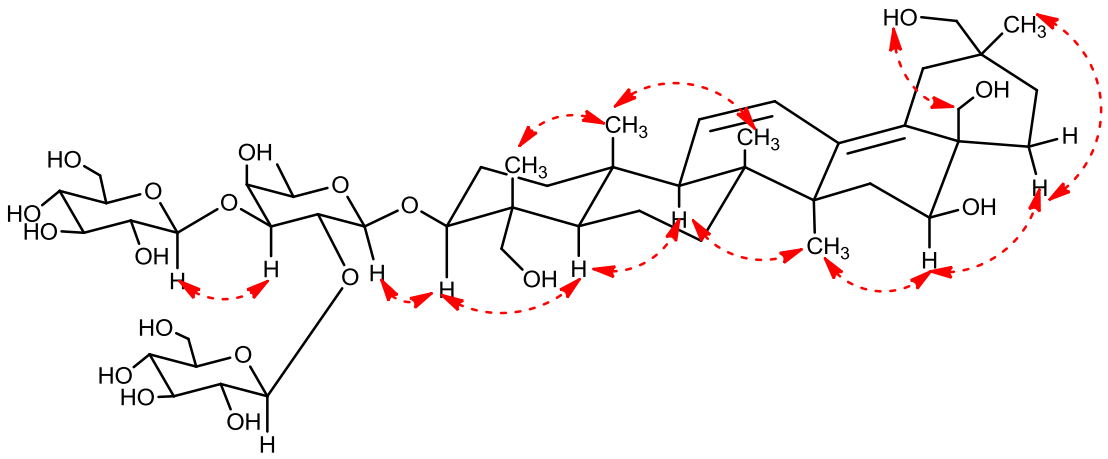

Figure S12. Specific ROESY correlations of compound **1**

Table S1 NMR chemical shifts of compound **1** and **2** in pyridine-d_5 (500/125 MHz)

NO.	**2**	**1**	
	δ_C	δ_C	δ_H
1	38.7	38.7	1.84(1H, m)/1.08(1H, m)
2	26.3	26.4	2.28(1H, m)/2.03(1H, m)
3	82.9	83.0	4.19(1H, dd, *J* = 12.1, 4.6 Hz)
4	44.1	44.2	
5	48.0	48.2	1.66(1H, d, 9.8 Hz)
6	18.6	18.7	1.84(1H, m)/1.45(1H, m)[a]
7	32.7	32.8	1.45(1H, m)[a]/1.35(1H, d, 8.7 Hz)
8	40.8	40.8	
9	54.8	54.9	2.09(1H, s)
10	36.8	36.9	
11	127.4	127.4	5.68(1H, d, *J* = 10.7 Hz)
12	125.9	126.2	6.60(1H, dd, *J* = 10.7, 2.1 Hz)
13	136.7	137.1	
14	44.6	44.7	
15	35.1	35.4	1.59(1H, dd, *J* = 3.7, 12.7 Hz)/2.23(1H, d, *J* = 12.7 Hz)
16	76.9	76.8	4.31(1H, m)[a]
17	44.7	45.3	
18	133.6	133.6	
19	38.6	33.8	2.75(1H, d, *J* = 14.5 Hz)/2.27(1H, d, *J* = 14.1 Hz)
20	32.9	38.7	
21	35.4	30.3	2.16(1H, td, *J* = 13.3, 3.8 Hz)/1.50 (1H, m)[a]
22	30.2	29.8	3.07(1H, dt, *J* =14.7, 3.8 Hz)/1.76(1H, td, *J* = 13.8, 3.6 Hz)
23	64.3	65.0	3.73(1H, d, *J* = 10 Hz)/4.42(1H, dd, *J* = 10.5, 4.75 Hz)
24	13.1	13.2	1.08(3H, s)
25	19.0	19.1	0.96(3H, s)
26	17.5	17.4	0.87(3H, s)

27	22.2	22.4	1.12(3H, s)
28	64.7	64.6	4.28(2H, m)[a]
29	25.1	21.2	1.09(3H, s)
30	32.5	73.5	3.67(2H, s)
Fuc			
1′	104.2	104.4	4.95(1H, d, *J* = 7.8 Hz)
2′	77.3	77.6	4.70(1H, dd, *J* = 9.2, 8.1 Hz)
3′	85.3	85.2	4.12(1H, dd, *J* = 9.7, 3.1 Hz)
4′	71.9	72.0	4.27(1H, m)[a]
5′	70.8	70.9	3.63(1H, m)
6′	17.3	17.6	1.44(3H, d, *J* = 6.3 Hz)
Glu			
1″	104.5	104.5	5.62(1H, d, *J* = 7.8 Hz)
2″	76.5	76.6	4.12(1H, m)[a]
3″	79.1	79.2	4.21(1H, m)[a]
4″	72.6	72.6	4.21(1H, m)[a]
5″	77.9	77.8	3.65(1H, m)
6″	63.5	63.4	4.35(1H, m)[a]/4.31(1H, m)[a]
Glu			
1‴	105.5	105.5	5.34(1H, d, *J* = 7.8 Hz)
2‴	75.7	75.8	4.03(1H, t, *J* = 8.3 Hz)
3‴	78.7	78.8	4.28(1H, m)[a]
4‴	71.9	71.6	4.27(1H, m)[a]
5‴	78.7	78.9	3.98(1H, m)
6‴	62.8	63.0	4.50(1H, dd, *J* =11.8, 2.5 Hz)/ 4.35(1H, m) [a]

[a] Proton signals were overlapped with other signals.

Pharmacological activity data

Table S2 Effects of compounds on blood platelet aggregation

Compounds	EC_{50} value (μM)	Maximum platelet aggregation (%)
Control	—	2.2±0.43
1	>100	27.2±1.7**
2	>100	8.6±1.2*
3	>100	5.0±0.7
4	53.4±1.6	69.9±1.8**
5	>100	2.0±0.5
6	>100	11.4±1.2*
7	12.2±2.5	74.1±2.6**
8	>100	5.0±0.7
9	>100	9.0±1.1*
ADP (10 μM)		69.88±1.65**
polyphyllins II (100 μM)		44.5±5.5**

PRP was incubated with different concentrations (3, 10, 30, 50, 100 μM) of individual compounds in turbidimetric cup for 5 min at 37 ℃. The changes in the light transmittance of the reaction mixture were continuously recorded for 5 min and the maximal aggregation was recorded by Agg-RAM-Aggregometer. Polyphyllins II (100 μM) and ADP (10 μM) was used as positive control. Data are expressed as mean ± S.D., n = 6. Significant differences compared with control group. * $p < 0.05$. ** $p < 0.01$

Table S3 Effects of compounds from *C. chinense* on plasma TT

Compounds	TT(s)	
	100 μM	200 μM
control	24.88±2.76	
1	25.16±3.79	23.16±3.89
2	21.06±3.29	19.76±2.8**
3	23.74±2.66	23.34±5.72
4	22.66±3.40	22.53±3.56
5	26.16±2.47	24.14±3.49
6	25.78±3.78	25.34±3.78
7	22.3±3.84	23.50±3.56
8	25.24±4.20	24.88±2.40
9	21.78±2.38	18.64±2.76**
Thrombin (0.006 U)	19.41±0.75**	

Data are expressed as mean ± S.D., n = 6. Significant differences compared with control group.

* $p < 0.05$, ** $p < 0.01$.