

SUPPLEMENTARY MATERIAL

Dammarane-type saponins from *Gynostemma pentaphyllum* and their cytotoxicities

Wei-Ye Cui^a, Yulian Jin^b, Hui Liu^a, Ma-Li Zu^a, Xin-Fang Zhai^a, Ce Yang^a, Yu-Long Gu^a, Yong Cheng^a and Xiang-Lan Piao^{a,*}

^a Center on Translational Neuroscience and School of Pharmacy, Minzu University of China, Beijing 100081, China.

^b School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China.

*Correspondence

Prof. Xiang-Lan Piao

School of Pharmacy, Minzu University of China

E-mail: xlpio@muc.edu.cn; xlpio@163.com. Tel/fax: +86-10-68939905

ABSTRACT

Heat-processed *Gynostemma pentaphyllum* has shown strong activity against human lung carcinoma A549 cells. In this study, two new dammarane-type saponins together with two known compounds were isolated from the ethanol extract of the heat-processed leaves of *G. pentaphyllum*. They were identified as

2 α ,3 β ,12 β -trihydroxydammar-20(22),24-diene-3-O- β -D-glucopyranoside (**1**, namely damulin E), 2 α ,3 β ,12 β -trihydroxydammar-20(21),24-diene-3-O- β -D-glucopyranoside (**2**, namely damulin F), damulin A (**3**) and damulin B (**4**), respectively, using IR, NMR and mass spectra. Damulin E and damulin F showed strong anti-cancer activity against A549, H1299, T24, SH-SY5Y and K562 cell lines in vitro using CCK-8 assay.

Keywords: *Gynostemma pentaphyllum*, saponin, damulin E, damulin F, anti-cancer activity

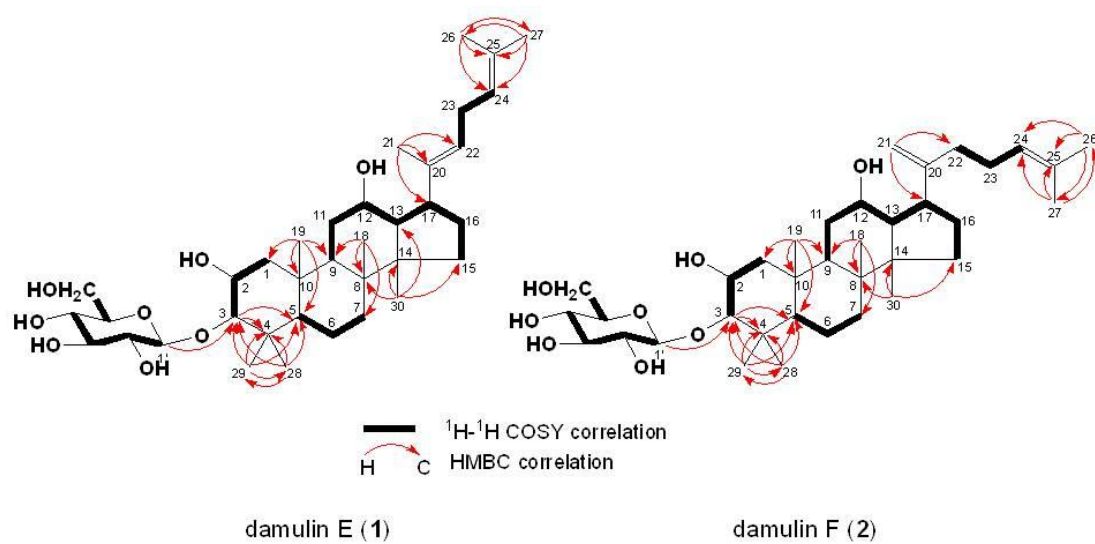


Figure S1. Key COSY and HMBC correlations of compounds **1** and **2**.

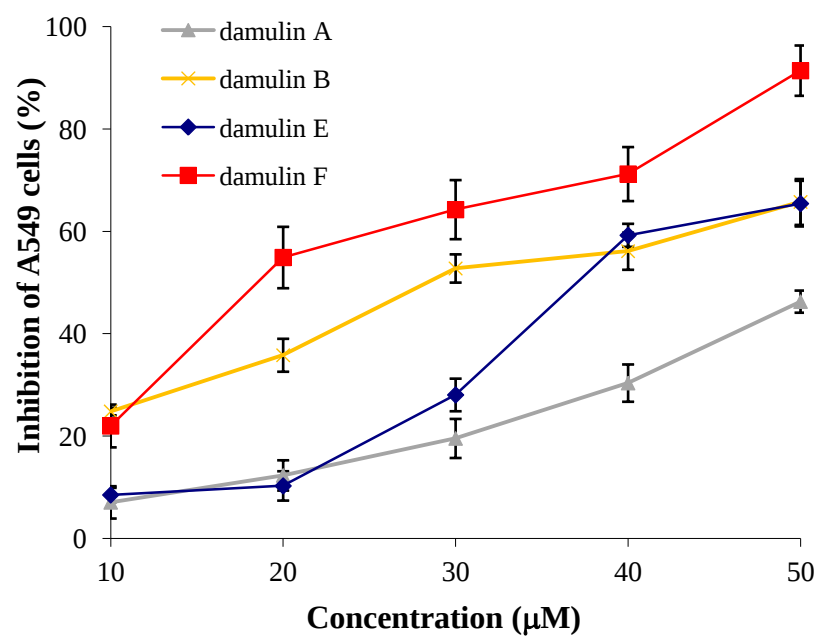


Figure S2. Inhibitory activity of isolated saponins against A549 cells.

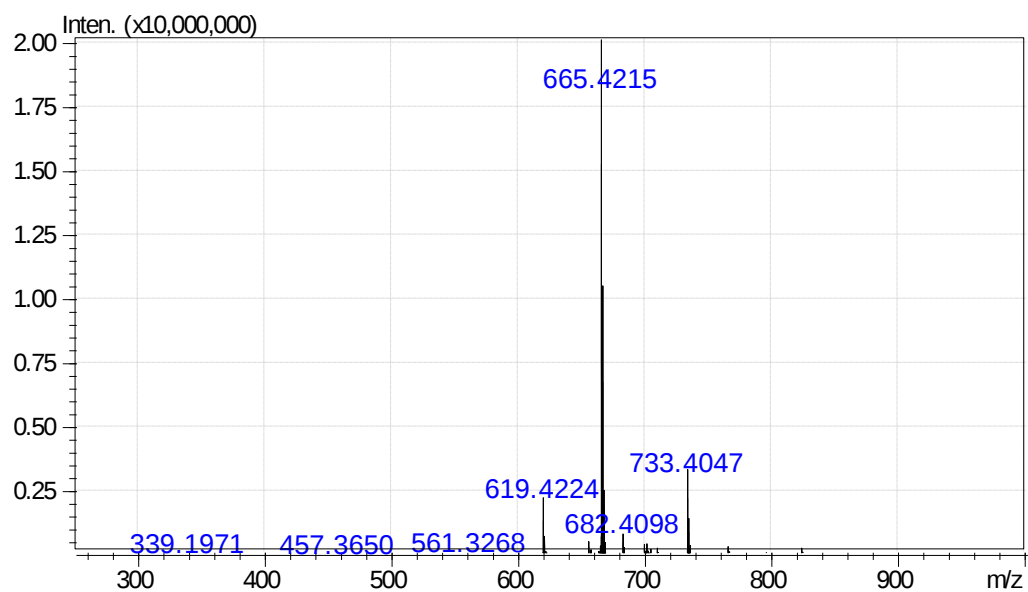
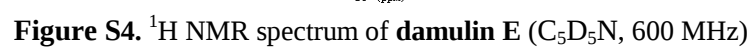


Figure S3. HR-ESI-MS of **damulin E**



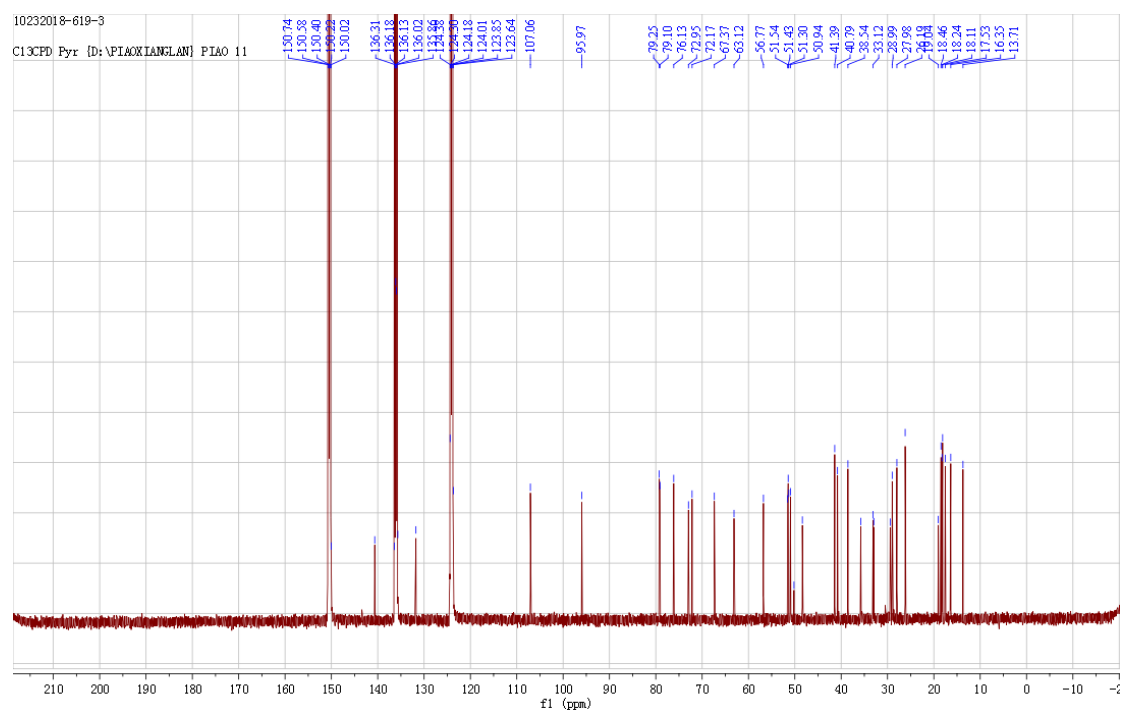


Figure S5. ^{13}C NMR spectrum of **damulin E** ($\text{C}_5\text{D}_5\text{N}$, 150 MHz)

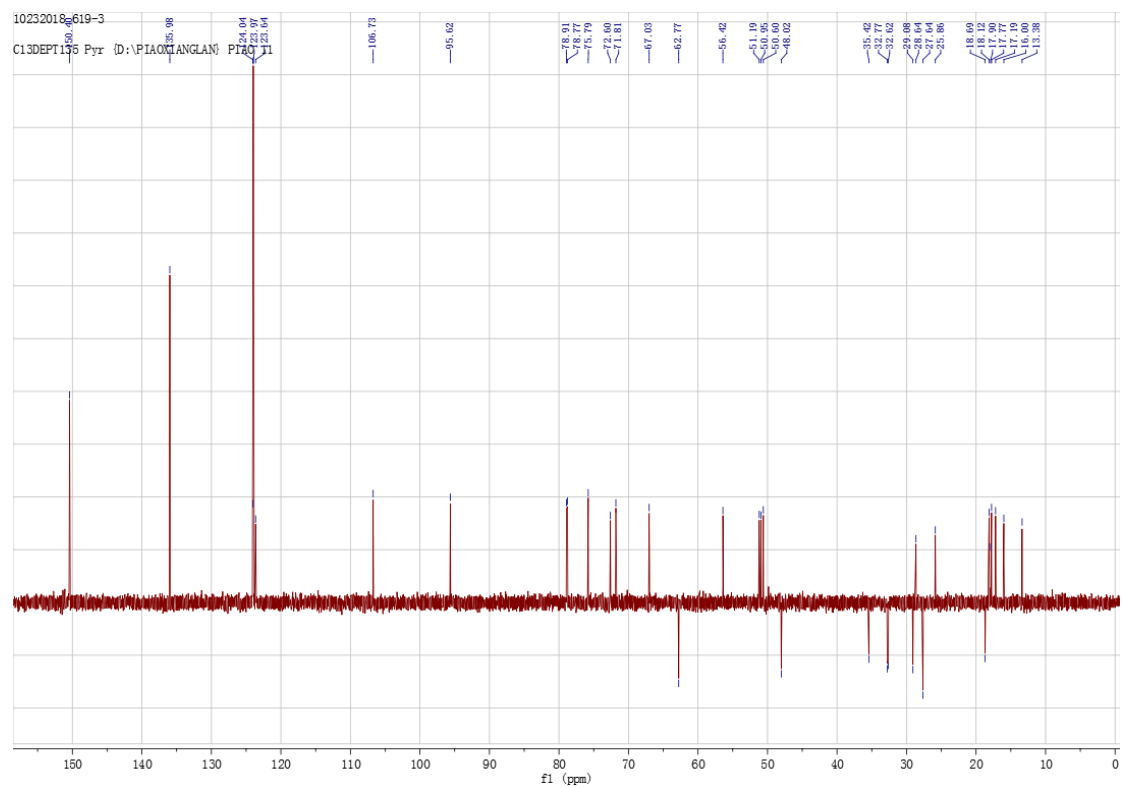


Figure S6. DEPT 135 of **damulin E** (C_5D_5N , 150 MHz)

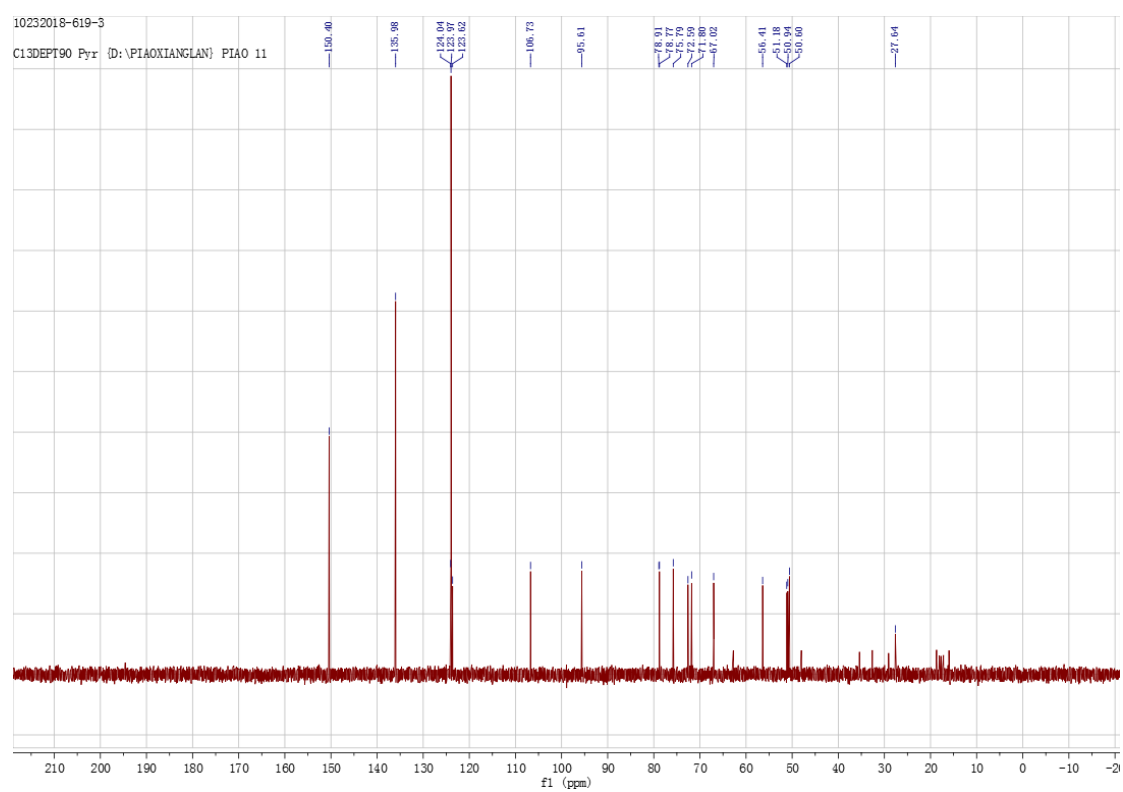


Figure S7. DEPT 90 of **damulin E** (C_5D_5N , 150 MHz)

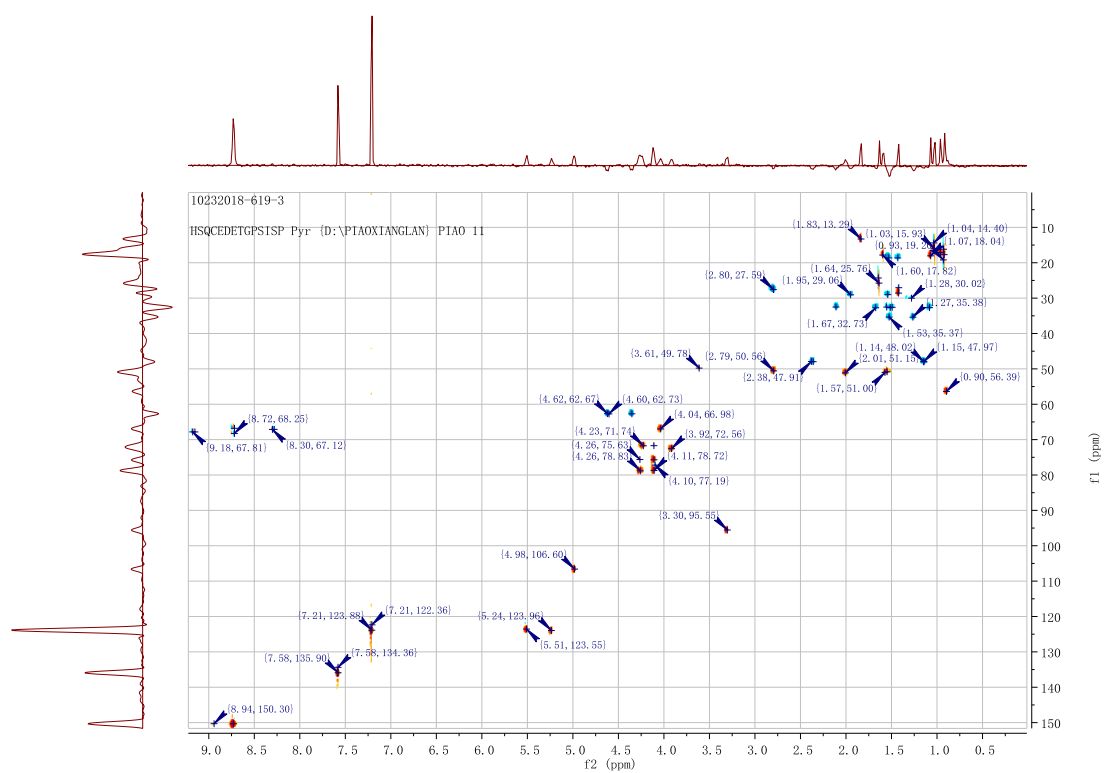


Figure S8. HMQC spectrum of **damulin E** (C_5D_5N , 600 MHz)

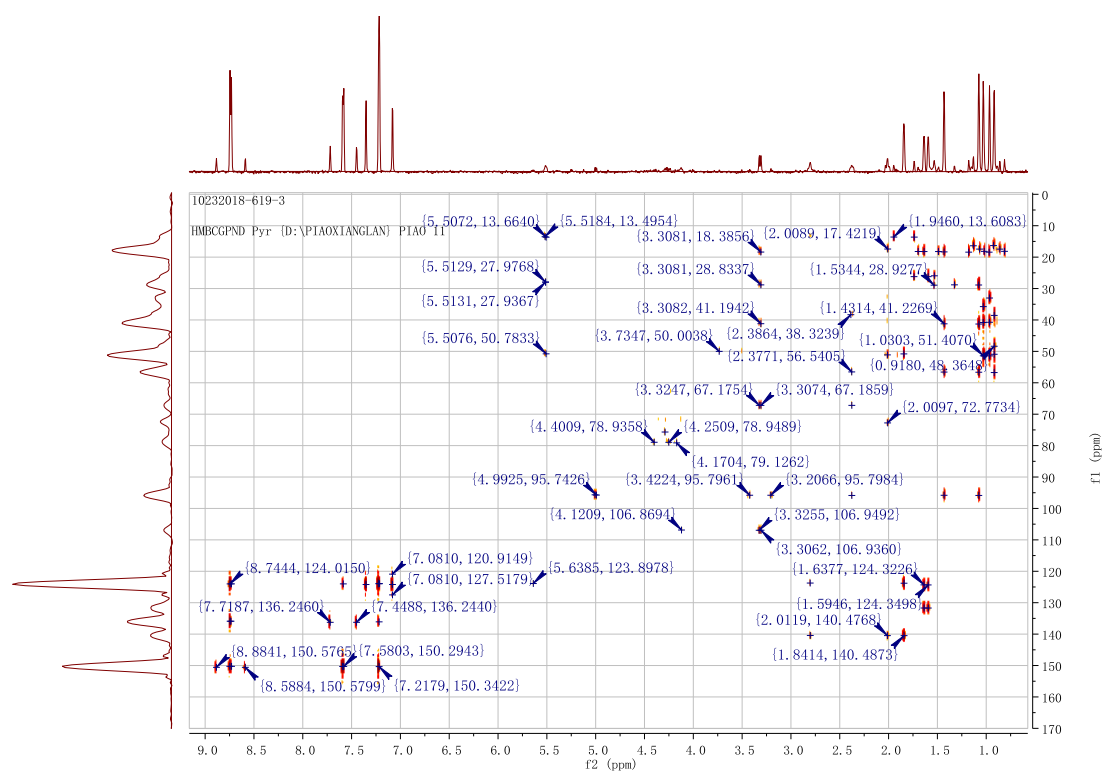


Figure S9. HMBC spectrum of damulin E

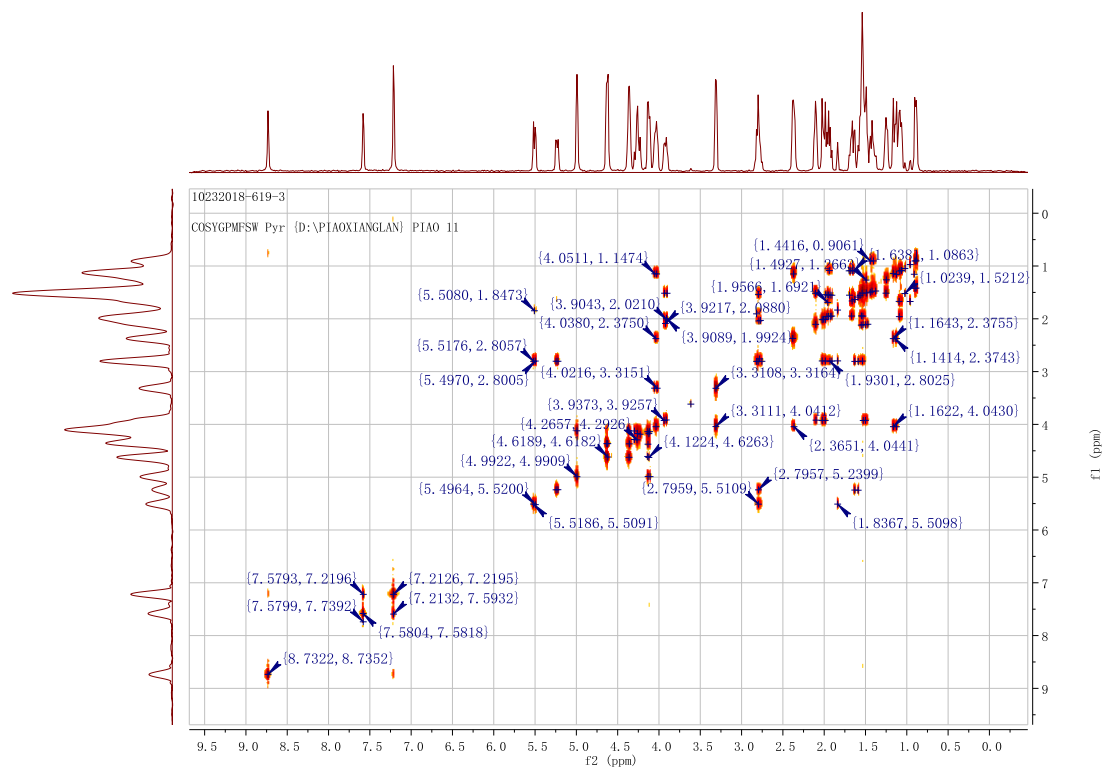


Figure S10. ^1H - ^1H COSY spectrum of **damulin E**

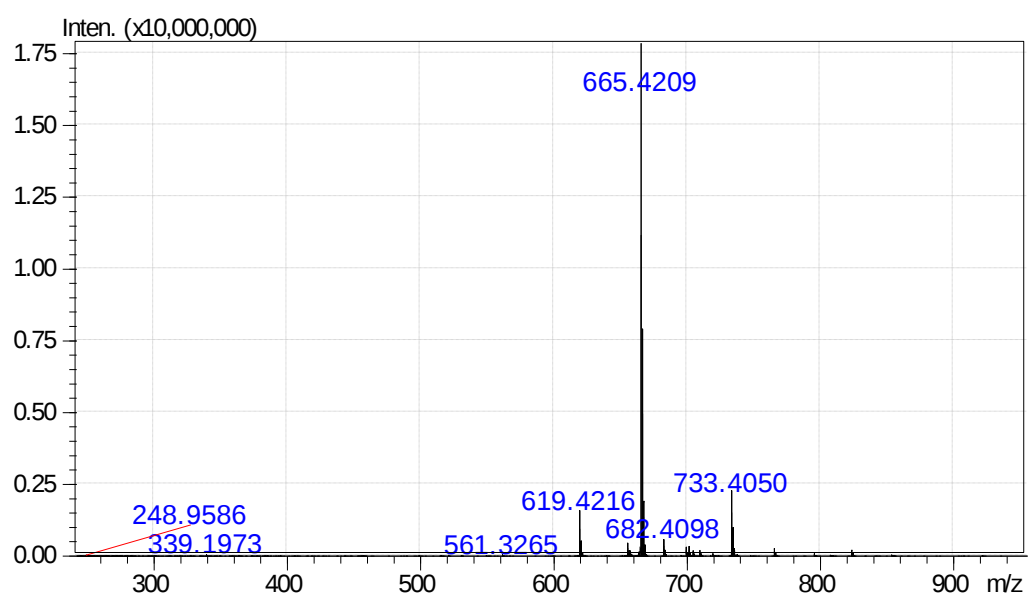
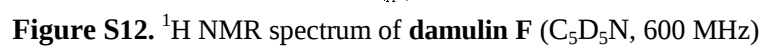


Figure S11. HR-ESI-MS of **damulin F**



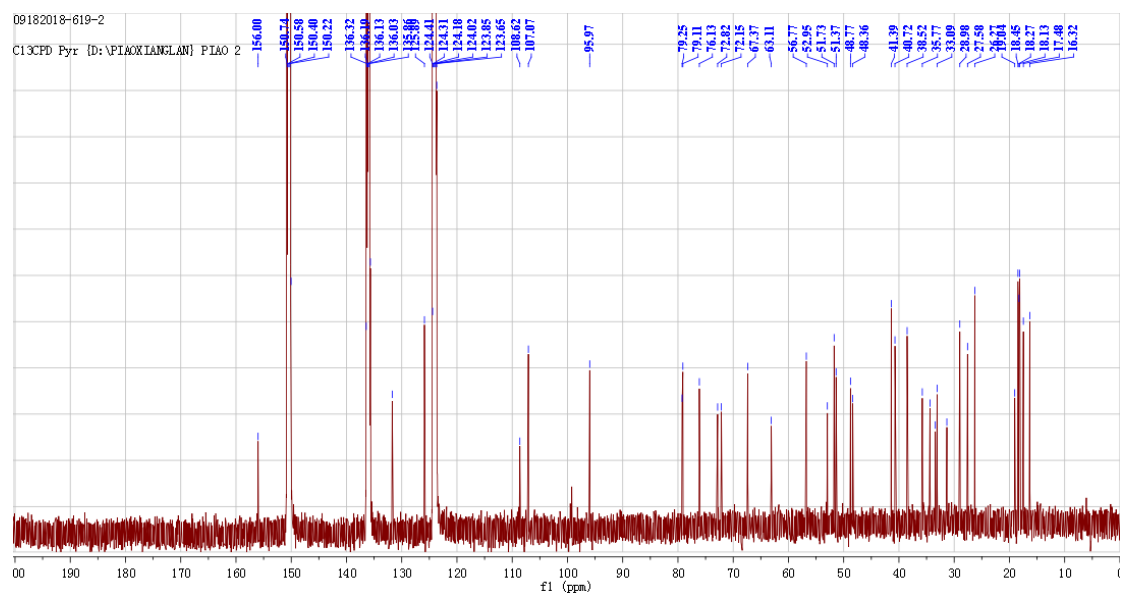


Figure S13. ^{13}C NMR spectrum of **damulin F** ($\text{C}_5\text{D}_5\text{N}$, 150 MHz)

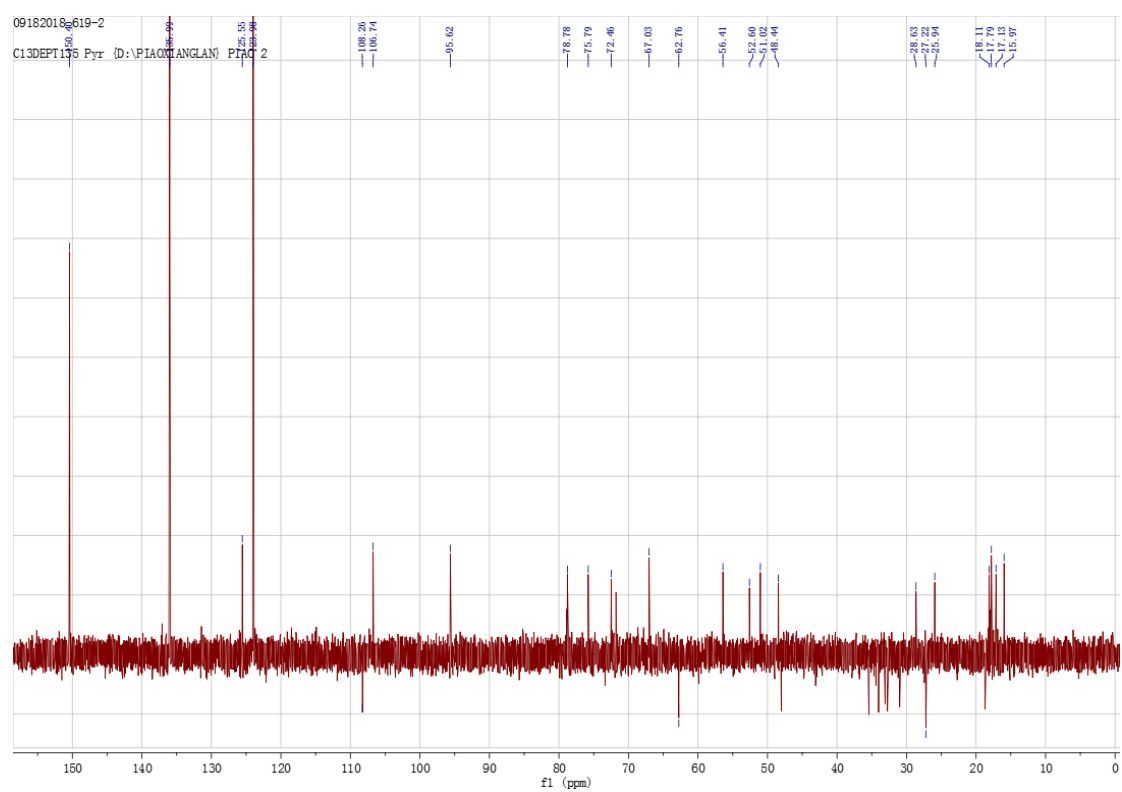


Figure S14. DEPT 135 of **damulin F** (C_5D_5N , 150 MHz)

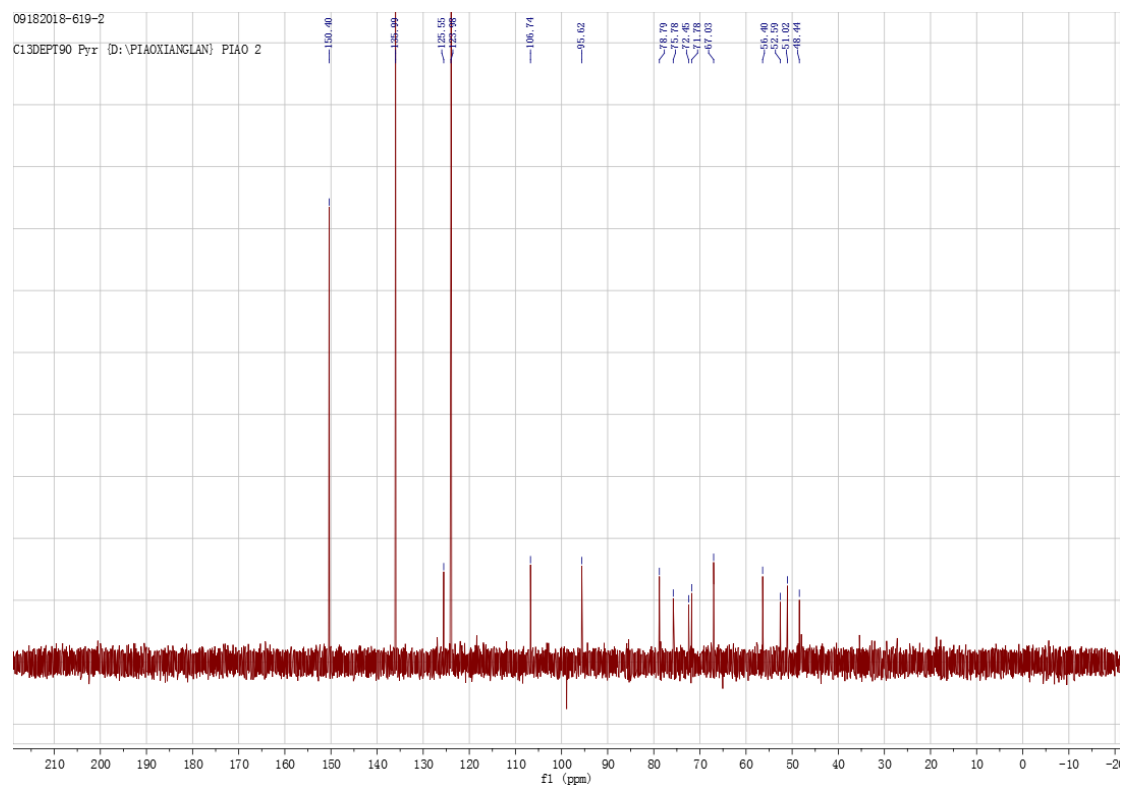


Figure S15. DEPT 90 of **damulin F** (C_5D_5N , 150 MHz)

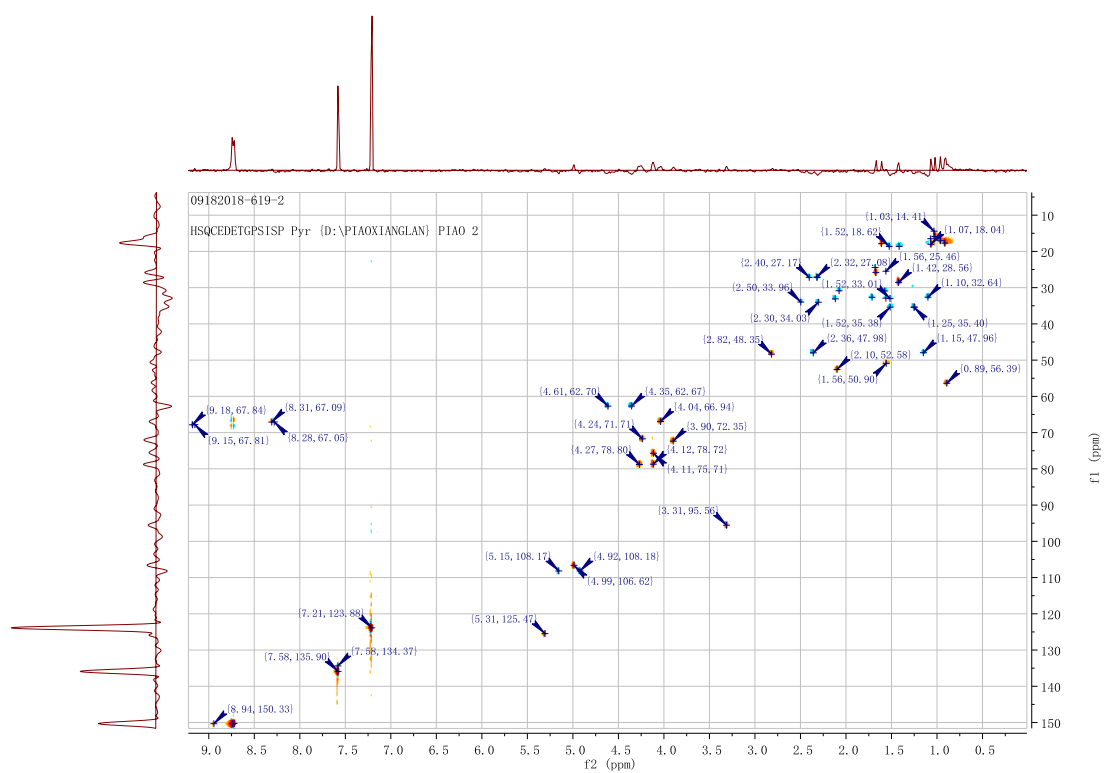


Figure S16. HMQC spectrum of **damulin F** ($\text{C}_5\text{D}_5\text{N}$, 600 MHz)

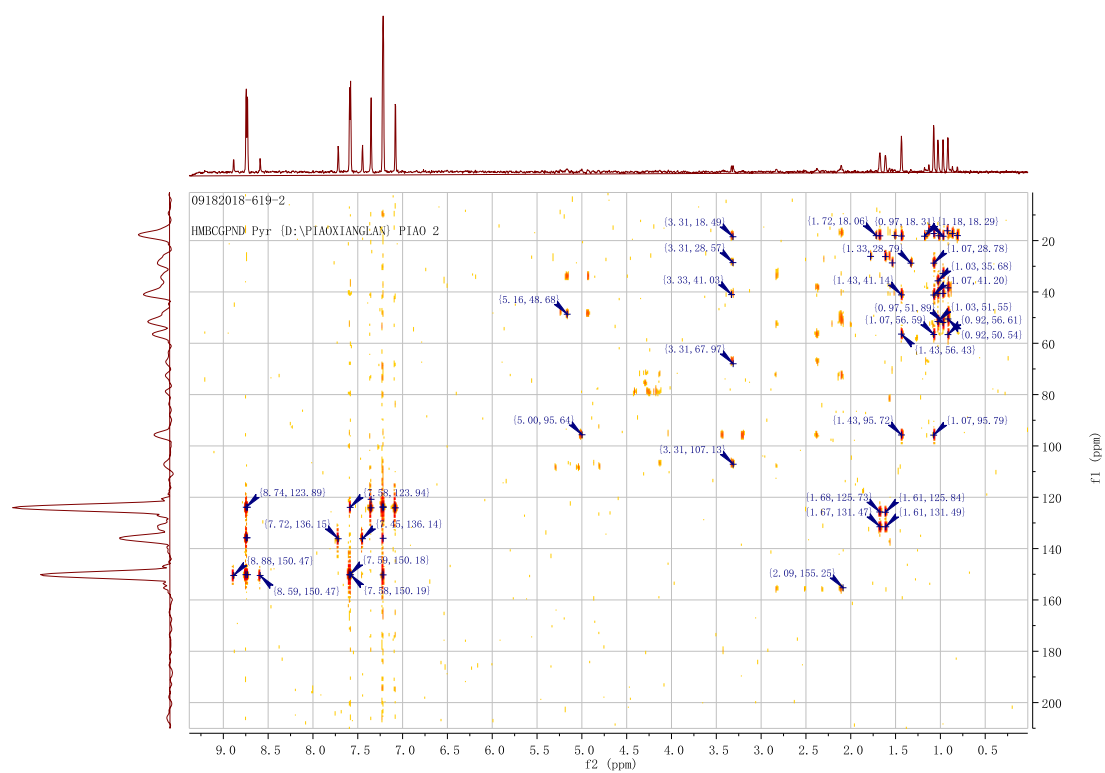


Figure S17. HMBC spectrum of damulin F

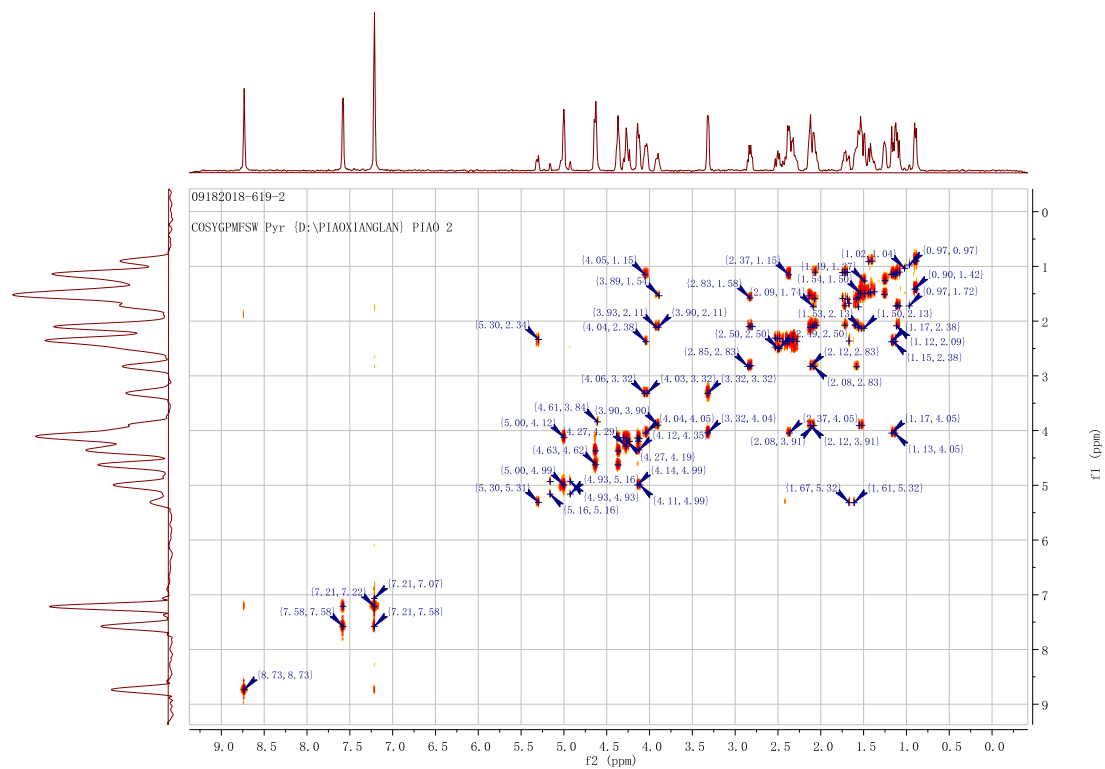


Figure S18. ^1H - ^1H COSY spectrum of **damulin F**

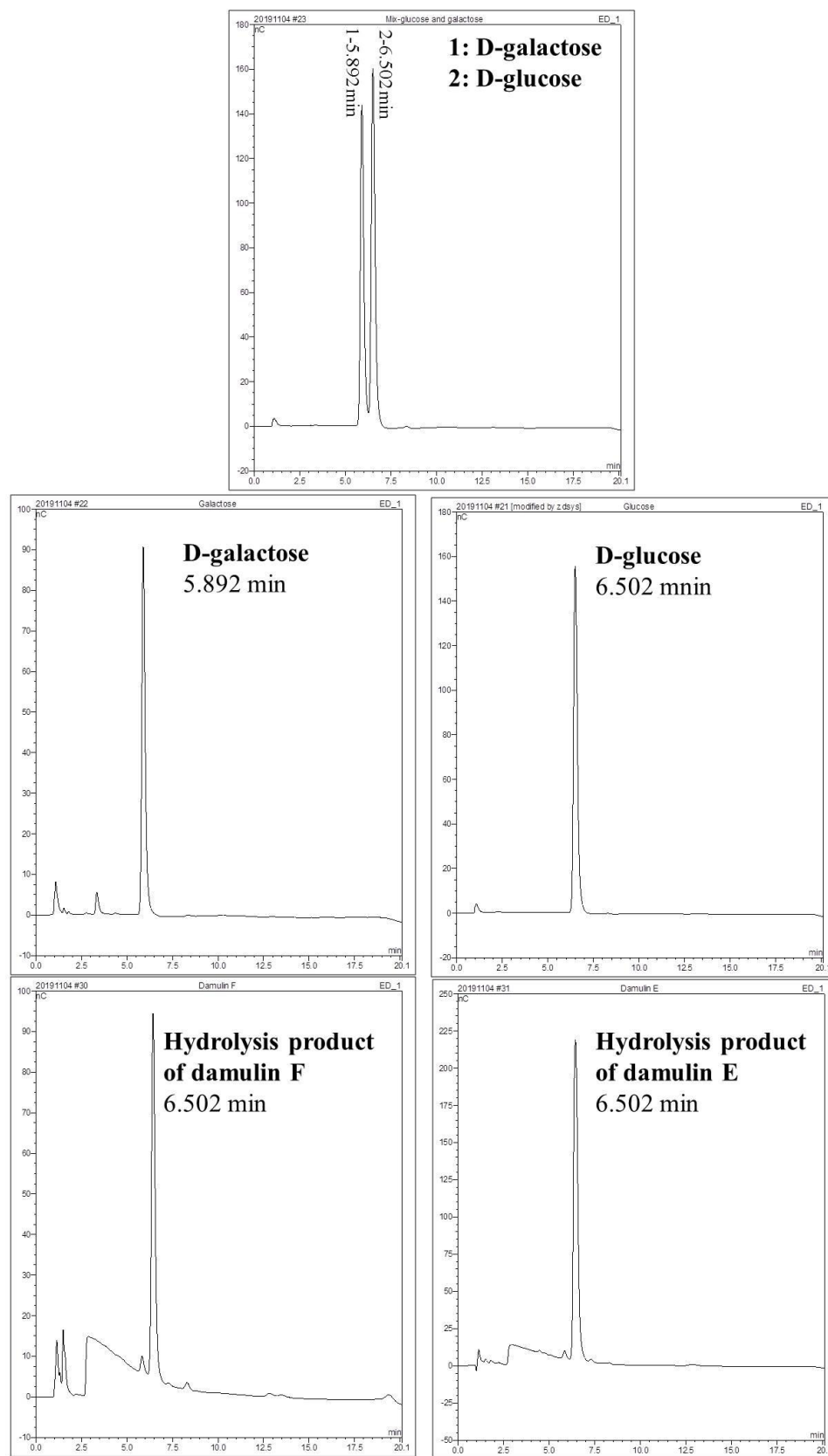


Figure S19. Ion Chromatogram of hydrolysis products of **damulin E** and **damulin F**

Table S1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR chemical shift values of compound **1** and **2** isolated from heat-processed *G. pentaphyllum* in $\text{C}_5\text{D}_5\text{N}$.

No.	Damulin E (1)		Damulin F (2)	
	^1H	^{13}C	^1H	^{13}C
1	2.38 (1H, m), 1.15 (1H, m)	48.4	2.34 (1H, m), 1.18 (1H, m)	48.4
2	4.04 (1H, m)	67.4	4.05 (1H, m)	67.4
3	3.33 (1H, d, $J = 9.2$ Hz)	96.0	3.33 (1H, d, $J = 9.3$ Hz)	96.0
4		41.4		41.4
5	0.89 (1H, m)	56.8	0.89 (1H, m)	56.8
6	1.52 (1H, m), 1.41 (1H, m)	19.0	1.57 (1H, m), 1.41 (1H, m)	19.0
7	1.50 (1H, m), 1.26 (1H, m)	35.8	1.52 (1H, m), 1.28 (1H, m)	35.8
8		40.8		40.7
9	1.54 (1H, m)	51.3	1.59 (1H, m)	51.4
10		38.5		38.5
11	2.11 (1H, m), 1.51 (1H, m)	33.0	2.13 (1H, m), 1.54 (1H, m)	33.4
12	3.93 (1H, m)	73.0	3.92 (1H, m)	72.8
13	2.01 (1H, m)	51.5	2.11 (1H, m)	53.0
14		51.4		51.7
15	1.68 (1H, m), 1.13 (1H, m)	33.1	1.73 (1H, m), 1.16 (1H, m)	33.1
16	1.95 (1H, m), 1.55 (1H, m)	29.4	2.11 (1H, m), 1.59 (1H, m)	31.3
17	2.82 (1H, m)	50.9	2.84 (1H, m)	48.8
18	1.03 (3H, s)	16.4	1.03 (3H, s)	16.3
19	0.92 (3H, s)	18.1	0.92 (3H, s)	18.1
20		140.7		156.0
21	1.84 (3H, s)	13.7	5.17 (1H, bs), 4.93 (1H, bs)	108.6
22	5.51 (1H, m)	124.0	2.50 (1H, m)	34.4
23	2.81 (1H, m), 2.42 (1H, m)	28.0	2.39 (1H, m), 2.33 (1H, m)	27.6
24	5.24 (1H, m)	124.3	5.31 (1H, m)	125.9
25		131.8		131.7
26	1.64 (3H, s)	26.2	1.68 (3H, s)	26.3
27	1.59 (3H, s)	18.2	1.61 (3H, s)	18.3
28	1.43 (3H, s)	29.0	1.44 (3H, s)	29.0
29	1.08 (3H, s)	18.5	1.08 (3H, s)	18.5
30	0.97 (3H, s)	17.5	0.97 (3H, s)	17.5
1'	5.00 (1H, d, $J = 7.9$ Hz)	107.1	5.02 (1H, d, $J = 7.9$ Hz)	107.1
2'	4.14 (1H, m)	76.1	4.14 (1H, m)	76.1
3'	4.29 (1H, m)	79.3	4.30 (1H, m)	79.3
4'	4.25 (1H, m)	72.2	4.28 (1H, m)	72.2
5'	4.13 (1H, m)	79.1	4.15 (1H, m)	79.1
6'	4.65 (1H, m), 4.38 (1H, m)	63.1	4.65 (1H, m), 4.37 (1H, m)	63.1

Table S2. Cytotoxicities of isolated saponins from *G. pentaphyllum*.

Compound	IC ₅₀ (μM)				
	A549	H1299	K562	SH-SY5Y	T24
Damulin E	38.9 ± 0.6	33.4 ± 1.5	57.7 ± 1.8	63.1 ± 1.7	64.7 ± 1.8
Damulin F	19.8 ± 0.4	18.5 ± 1.3	20.6 ± 1.3	31.8 ± 1.5	18.5 ± 1.3
Damulin A	59.2 ± 1.2				
Damulin B	29.6 ± 0.8				
20(S)-Rg3	> 100				
20(R)-Rg3	> 100				