

Supplementary data

Extraction, purification and anticancer activity studies on triterpenes in pomegranate peel

Mengqi Zhang^{a, b, †}, Yutao Zhang^{a, c, †}, Xu Guo^a, Yingying Chen^a, Huimin Li^d, Guihua Zhou^d, Shutao Sun^a,
Qidong Ren^a, Jesus Simal-Gandara^e, Jinyue Sun^{a, *}, Ningyang Li^{c, f, *}, Chao Liu^{a, *}

^a Key Laboratory of Novel Food Resources Processing, Ministry of Agriculture and Rural Affairs/Key Laboratory of Agro-Products Processing Technology of Shandong Province, Institute of Agro-Food Science and Technology, Shandong Academy of Agricultural Sciences, 23788 Gongye North Road, Jinan, 250100, PR China;

^b Shandong Huatai Industrial Technology Institute of Nutrition and Health Co, LTD, 9 Hongji Road, Jinan, 251400, PR China;

^c Key Laboratory of Food Processing Technology and Quality Control in Shandong Province, College of Food Science and Engineering, Shandong Agricultural University, Tai'an, 271017, PR China;

^d Development Center of melon, fruit and vegetable industry in Kashi, Kashi, 844000, PR China;

^e Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, E32004 Ourense, Spain;

^f College of Food Science and Engineering, Ocean University of China, Qingdao, 266003, PR China

[†] These authors contributed equally to this work.

*Corresponding authors:

Tel: +86 0531 66659825; Fax: 86-531-66659825;

E-mail addresses: liuchao555@126.com (C. Liu), ningyangli@126.com (N. Li),
moon_s731@hotmail.com (J. Sun).

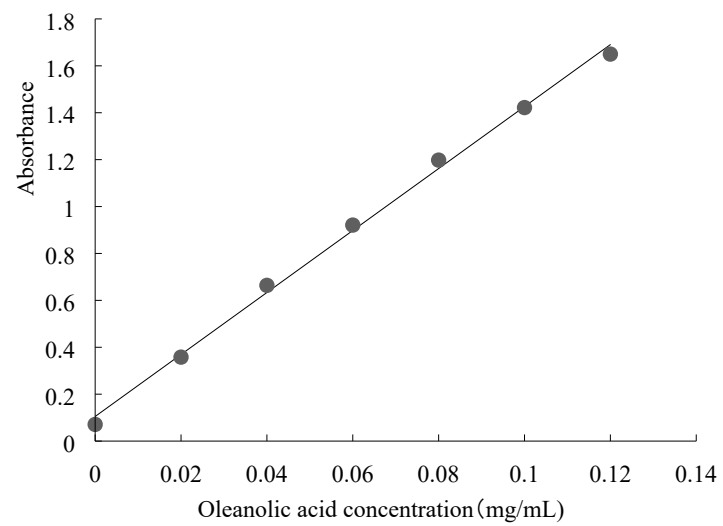


Figure S1. Standard curve of triterpene.

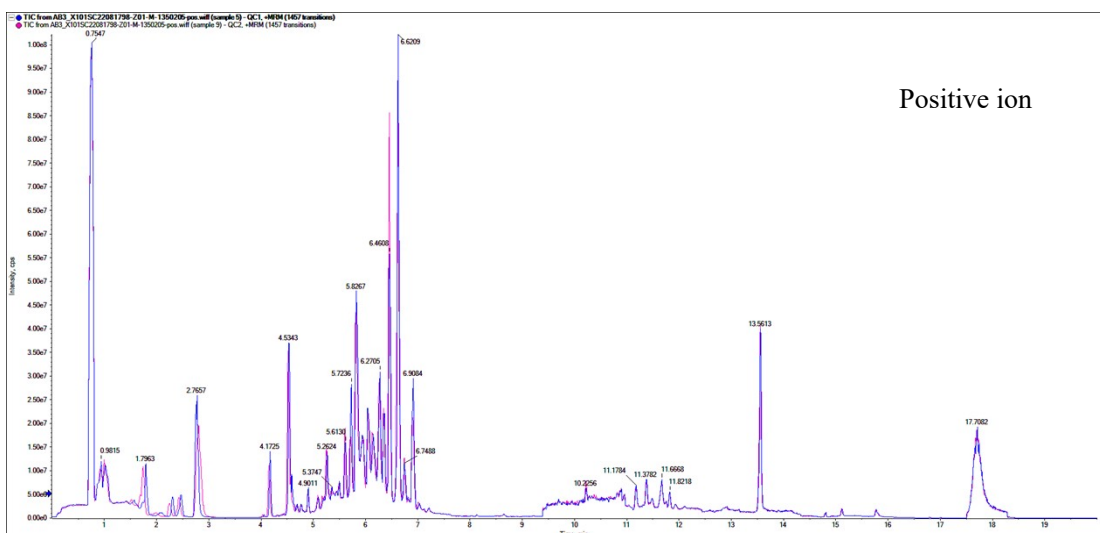
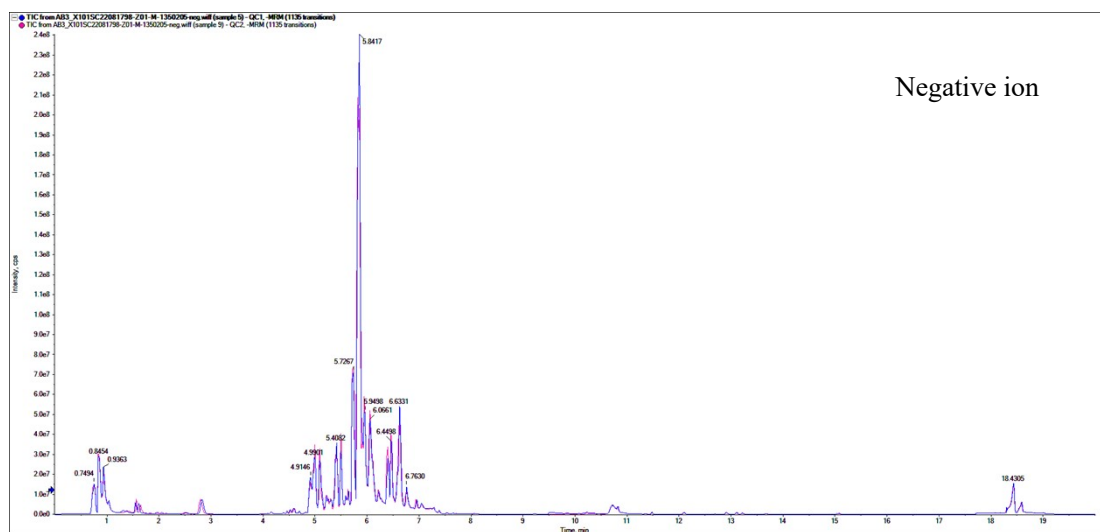
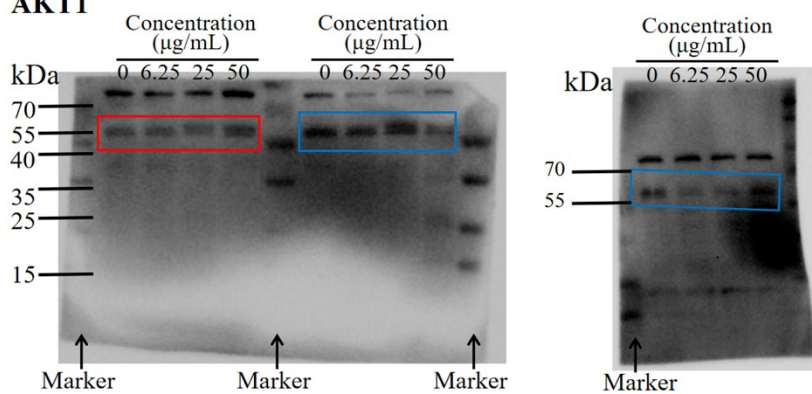
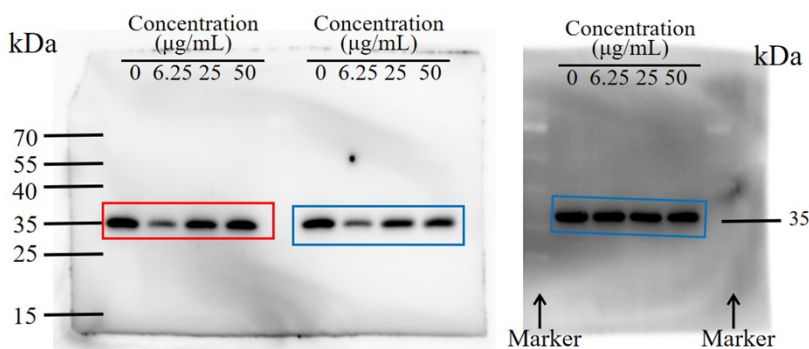


Figure S2. The total ion chromatography of TPP.

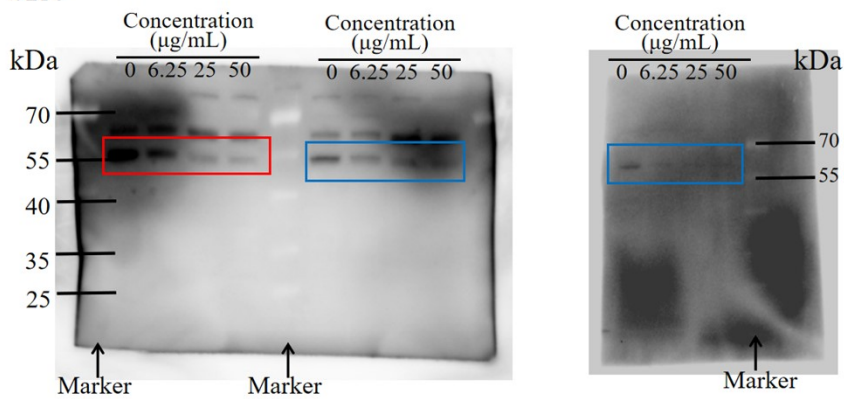
AKT1



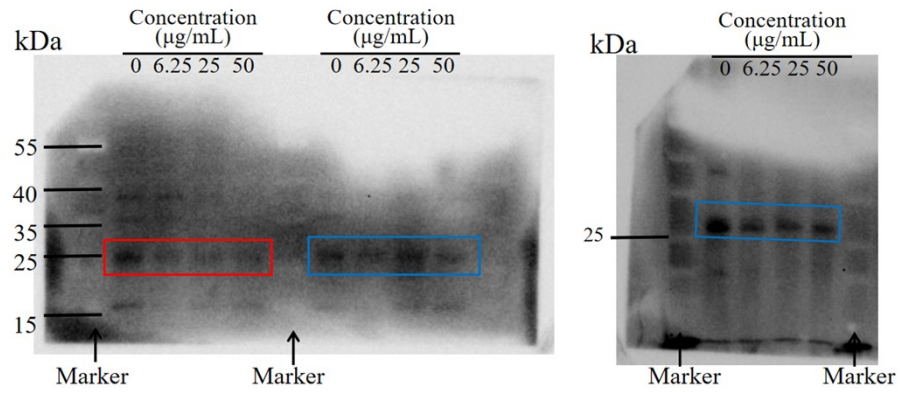
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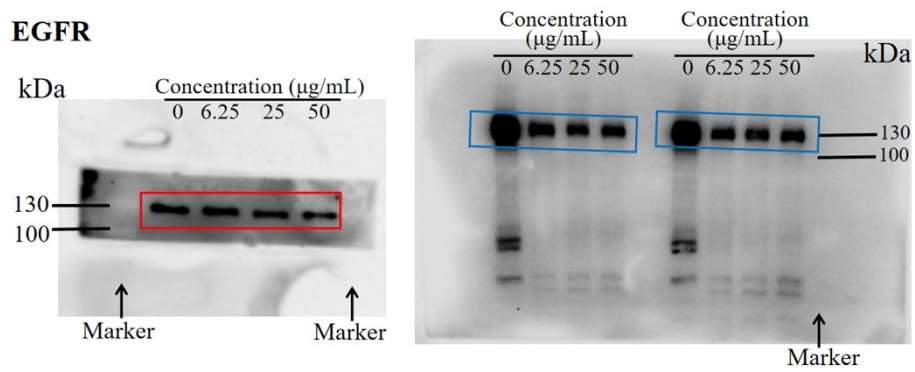
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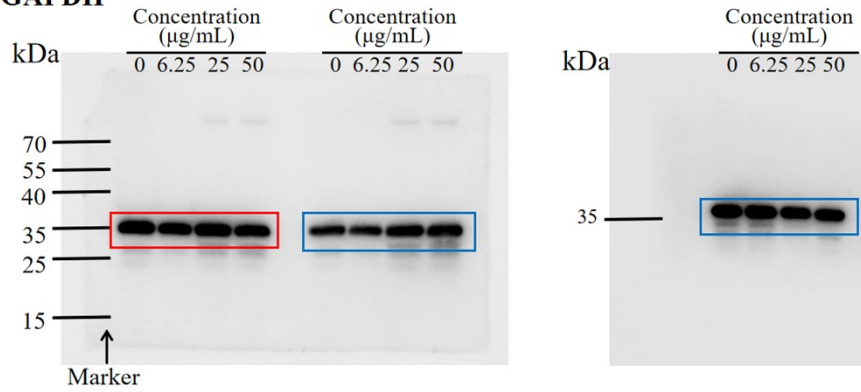
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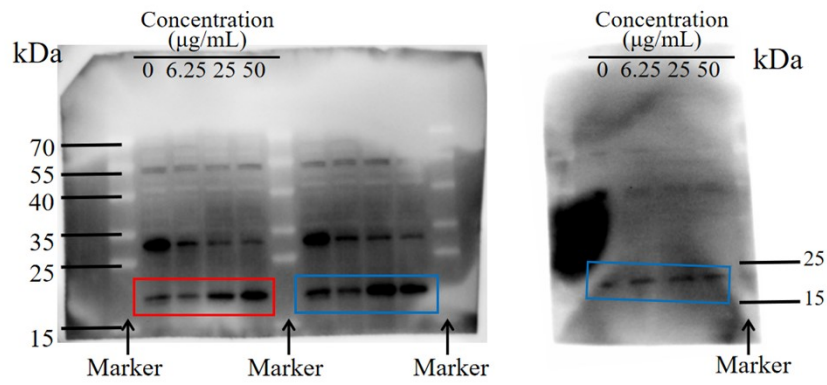
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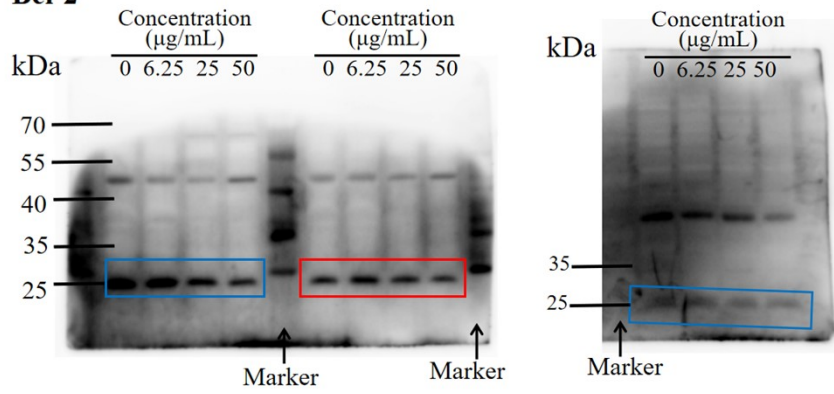
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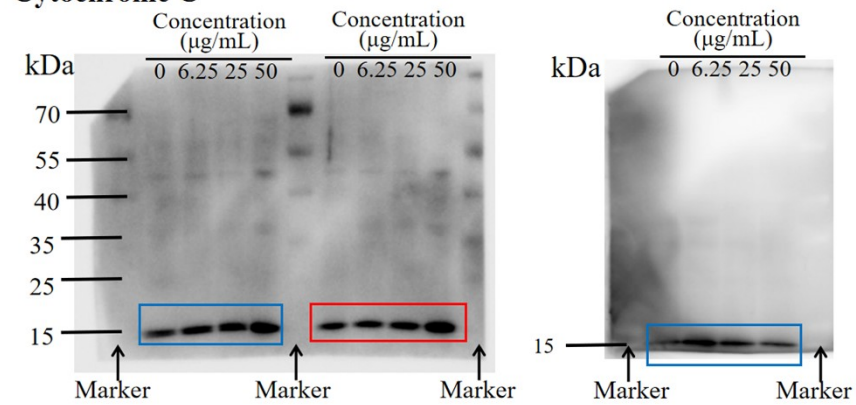
Bax



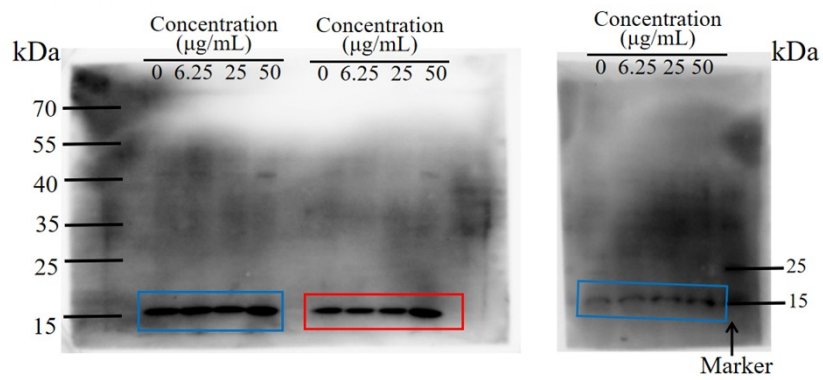
Bcl-2



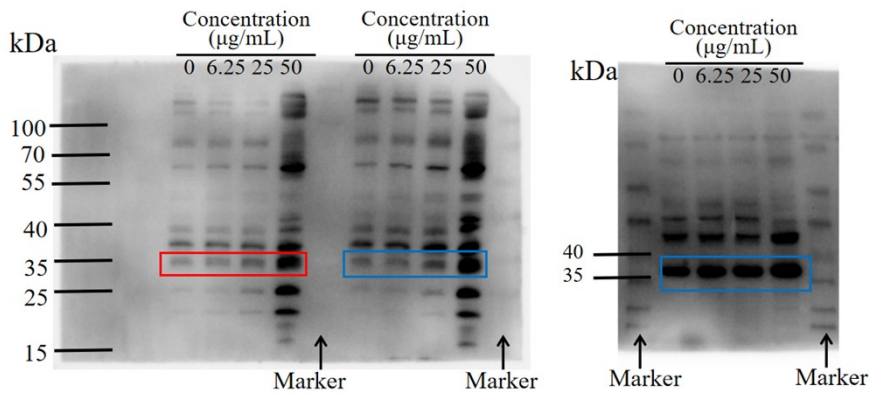
Cytochrome C



Cleaved cas-3



Cleaved cas-9



GAPDH

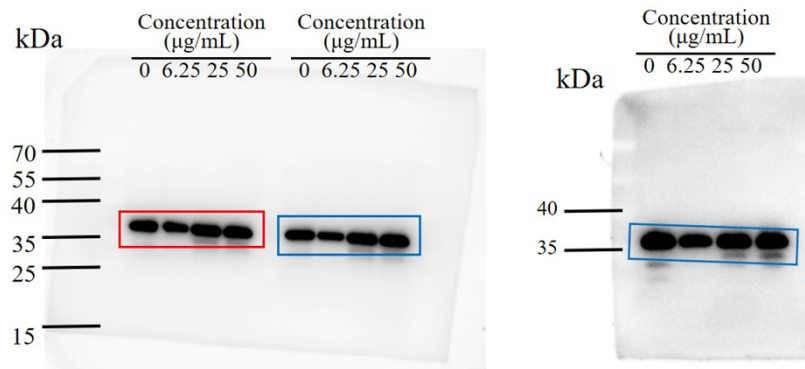


Figure S3. The image of the uncropped and unprocessed Western blot labelled with closest molecular mass markers. Note: The target proteins were labelled with the red box, which were shown in the manuscript. And the bands labelled with blue boxes were the replicate experiments. Some of the raw images contained multiple bands, which may be caused by poor specificity of primary antibody. Therefore, the corresponding bands of the target proteins were determined by the molecular weight compared with the Marker.

Table S1. Level table of Box-Behnken test factors.

Factor	Level		
	-1	0	1
Ethanol Concentration (%)	70	80	90
Solid-liquid Ratio (g/mL)	1:20	1:30	1:40
Ultrasonic Power (W)	100	200	300
Ultrasonic Temperature (°C)	50	60	70

Table S2. The liquid-phase separation gradient conditions of TPP.

Gradient			
1	0 min	98% A	2% B
2	2 min	98% A	2% B
3	15 min	0% A	100% B
4	17 min	0% A	100% B
5	17.1 min	98% A	2% B
6	20 min	98% A	2% B

Table S3. Experimental scheme and results of triterpenes extracted from pomegranate peel.

	Ethanol Concentration (%)	Solid-liquid Ratio (g/mL)	Ultrasonic Power (W)	Ultrasonic temperature (°C)	Triterpene Content (µg/mg)
1	70	1:20	200	60	206.38
2	90	1:20	200	60	224.35
3	70	1:40	200	60	205.21
4	90	1:40	200	60	234.25
5	80	1:30	100	50	220.39
6	80	1:30	300	50	221.3
7	80	1:30	100	70	251.91
8	80	1:30	300	70	229.68
9	70	1:30	200	50	188.94
10	90	1:30	200	50	190.24
11	70	1:30	200	70	169.39
12	90	1:30	200	70	228.6
13	80	1:20	100	60	246.95
14	80	1:40	100	60	237.84
15	80	1:20	300	60	226.19
16	80	1:40	300	60	227.95
17	70	1:30	100	60	174.58
18	90	1:30	100	60	225.94
19	70	1:30	300	60	201.35
20	90	1:30	300	60	207.56
21	80	1:20	200	50	216.5
22	80	1:40	200	50	186.35
23	80	1:20	200	70	248.51
24	80	1:40	200	70	233.21
25	80	1:30	200	60	281.48
26	80	1:30	200	60	280.95
27	80	1:30	200	60	269.73
28	80	1:30	200	60	251.01
29	80	1:30	200	60	275.48

Table S4. Results of variance analysis of quadratic regression equation model of response surface.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	22573.14	14	1612.37	9.43	< 0.0001	significant
A-Ethanol Concentration (%)	2271.23	1	2271.23	13.28	0.0027	
B-Solid-liquid Ratio (g/mL)	161.85	1	161.85	0.95	0.3471	
C-Ultrasonic Power (W)	158.27	1	158.27	0.93	0.3523	
D-Ultrasonic Power (°C)	1577.35	1	1577.35	9.22	0.0089	
AB	30.64	1	30.64	0.18	0.6785	
AC	509.63	1	509.63	2.98	0.1063	
AD	838.39	1	838.39	4.9	0.0439	
BC	29.54	1	29.54	0.17	0.684	
BD	55.13	1	55.13	0.32	0.5792	
CD	133.86	1	133.86	0.78	0.3912	
A ²	13476.9	1	13476.9	78.82	< 0.0001	
B ²	1654.15	1	1654.15	9.67	0.0077	
C ²	2274.43	1	2274.43	13.3	0.0026	
D ²	5665.13	1	5665.13	33.13	< 0.0001	
Residual	2393.87	14	170.99			
Lack of Fit	1766.41	10	176.64	1.13	0.4946	Not significant
Pure Error	627.45	4	156.86			
Cor Total	24967.01	28				

Table S5. The information of identified triterpene compounds.

No.	Name	Formula	Molecular Weight	RT [min]	CAS	Peak area
1	Corosolic acid	C ₃₀ H ₄₈ O ₄	472.7	13.22	4547-24-4	457400
2	Ursolic Acid	C ₃₀ H ₄₈ O ₃	456.7	15.069	77-52-1	8560
3	Betulonic acid	C ₃₀ H ₄₈ O ₃	456.7	14.842	472-15-1	63030
4	Oleanic acid	C ₃₀ H ₄₈ O ₃	456.71	15.03	508-02-1	4820
5	Ginsenoside-Ro	C ₄₈ H ₇₆ O ₁₉	957.119	8.86	34367-04-9	984
6	Maslinic acid	C ₃₀ H ₄₈ O ₄	472.7	13.077	4373-41-5	656500
7	Asiatic Acid	C ₃₀ H ₄₈ O ₅	488.7	10.75	464-92-6	3644000
8	Dipsacoside B	C ₅₃ H ₈₆ O ₂₂	1075.238	6.756	33289-85-9	34780
9	Isomangiferolic acid	C ₃₀ H ₄₈ O ₃	456.36	14.72	13878-92-7	50130
10	Betulonic acid	C ₃₀ H ₄₆ O ₃	454.68	11.061	4481-62-3	67210
11	Cucurbitacin I	C ₃₀ H ₄₂ O ₇	514.293	0.67	2222-07-3	16500
12	Kaji-ichigoside F1	C ₃₆ H ₅₈ O ₁₀	650.85	6.71	95298-47-8	162337