Extraction and identification of active components from *Lilium lancifolium* based on NADES-UHPLC-MS/MS technology

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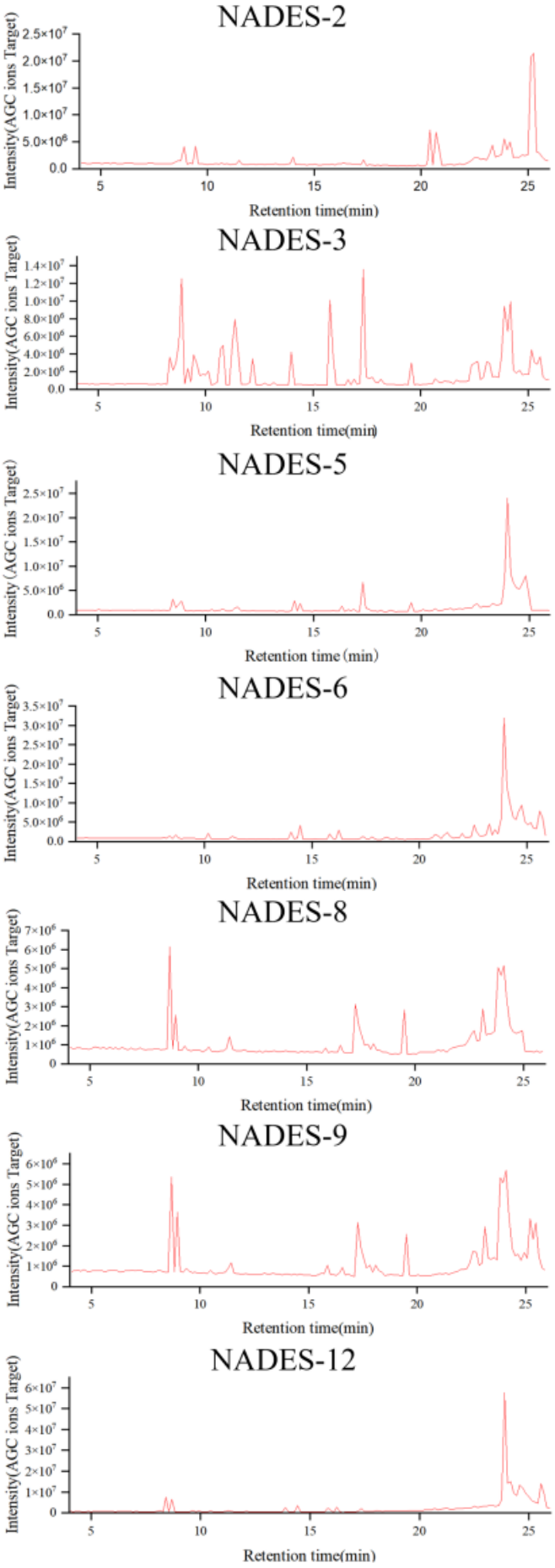
NADES-2
Intensity(AGC ions Target)
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2.0×10^7
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1.0×10^7
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5
10
15
20
25
Retention time(min)
NADES-3
Intensity(AGC ions Target)
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1.2×10^7
1.0×10^7
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4.0×10^6
2.0×10^6
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5
10
15
20
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Retention time(min)
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Intensity(AGC ions Target)
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5
10
15
20
25
Retention time (min)
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Intensity(AGC ions Target)
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5
10
15
20
25
Retention time(min)
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4×10^6
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1×10^6
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5
10
15
20
25
Retention time(min)
NADES-9
Intensity(AGC ions Target)
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1×10^6
0
5
10
15
20
25
Retention time(min)
NADES-12
Intensity(AGC ions Target)
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15
20
25
Retention time(min)

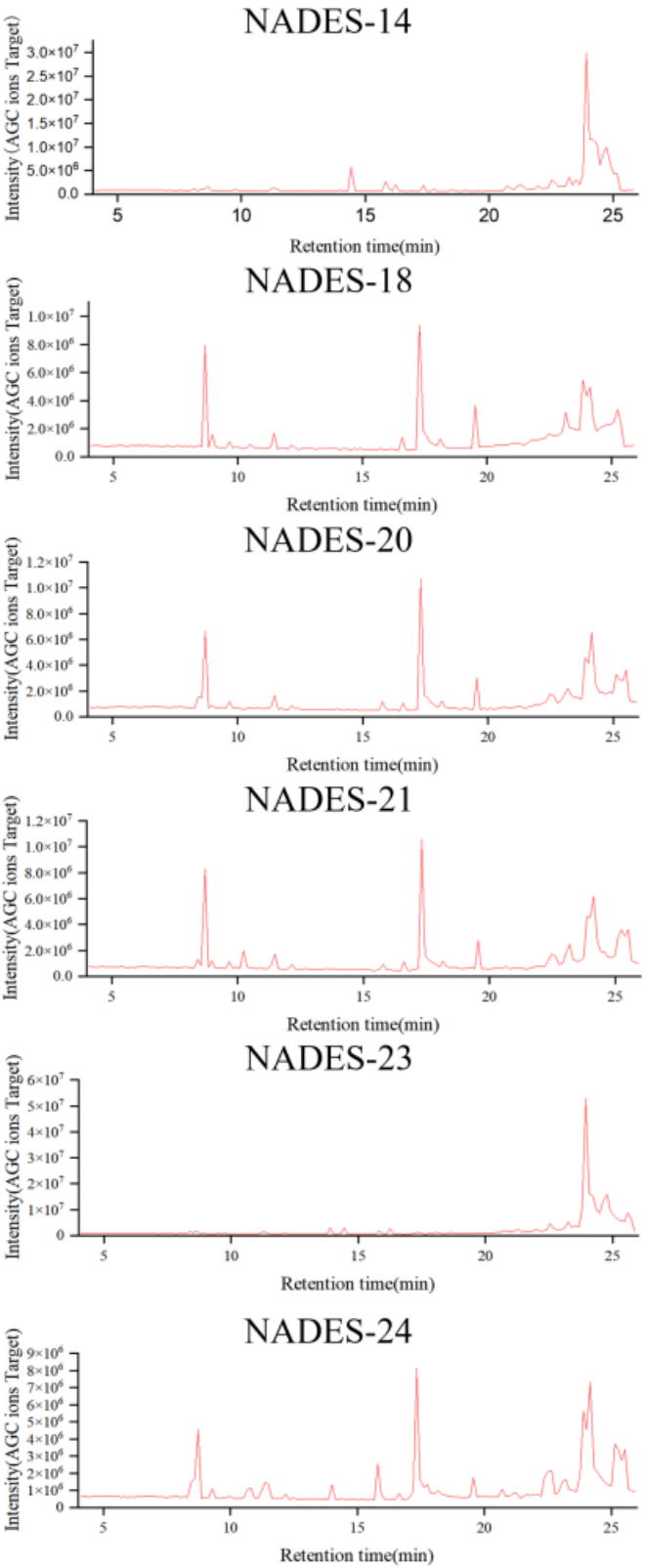


Figure S1 Extraction peak chromatograms (m/z 700-1200) of the remaining 13 stable NADES extracts from Lilium lancifolium bulbs

The molecular ion peak of m/z 904.33578 is lily saponin A, and its predicted molecular formula is $C_{45}H_{74}O_{18}$. After preliminary cracking, three characteristic signal peaks of m/z 742.57691, 580.64551, and 418.22058 were generated, indicating that it contains three hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 400.19514, 274.61436, and 256.47253, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.

The molecular ion peak of m/z 942.46247 is lily saponin B, and its predicted molecular formula is $C_{48}H_{76}O_{18}$. After preliminary cracking, three characteristic signal peaks of m/z 780.62959, 618.27258, and 456.71606 were generated, indicating that it contains three hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 438.36721, 312.36720, and 294.43992, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.

The molecular ion peak of m/z 1046.72542 is lily saponin C, and its predicted molecular formula is $C_{51}H_{80}O_{22}$. After preliminary cracking, four characteristic signal peaks of m/z 884.51417, 722.82482, 561.24067, and 399.33406 were generated, indicating that it contains four hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 381.54712, 255.68624, and 237.31722, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.

The molecular ion peak of m/z 885.37469 is lily saponin D, and its predicted molecular formula is $C_{45}H_{73}NO_{16}$. After preliminary cracking, three characteristic signal peaks of m/z 722.82501, 560.36209, and 397.61846 were generated, indicating that it contains three hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 379.71229, 254.75134, and 236.23394, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.

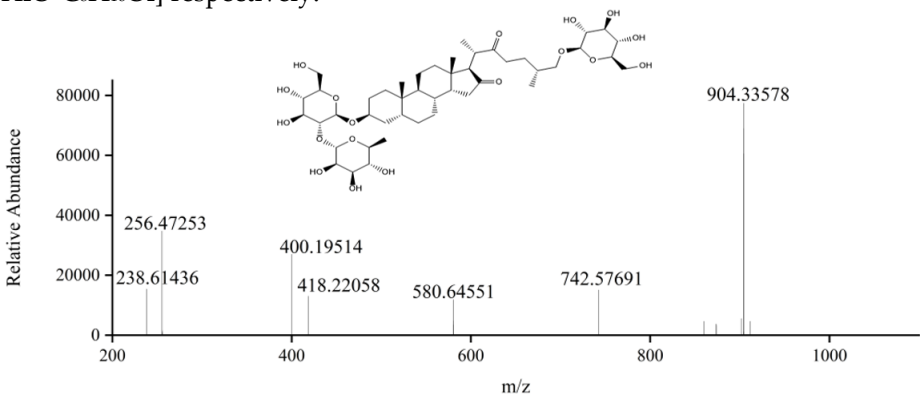
The molecular ion peak of m/z 856.78524 is lily saponin E, and its predicted molecular formula is $C_{44}H_{70}O_{16}$. After preliminary cracking, three characteristic signal peaks of m/z 694.51236, 533.14694, and 370.44392 were generated, indicating that it contains three hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 352.63545, 226.34055, and 208.57821, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.

The molecular ion peak of m/z 902.65168 is lily saponin F, and its predicted molecular formula is $C_{45}H_{82}O_{23}$. After preliminary cracking, three characteristic signal peaks of m/z 740.54061, 578.21436, and 416.31345 were generated, indicating that it contains three hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 398.59172, 272.45695, and 254.31445, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.

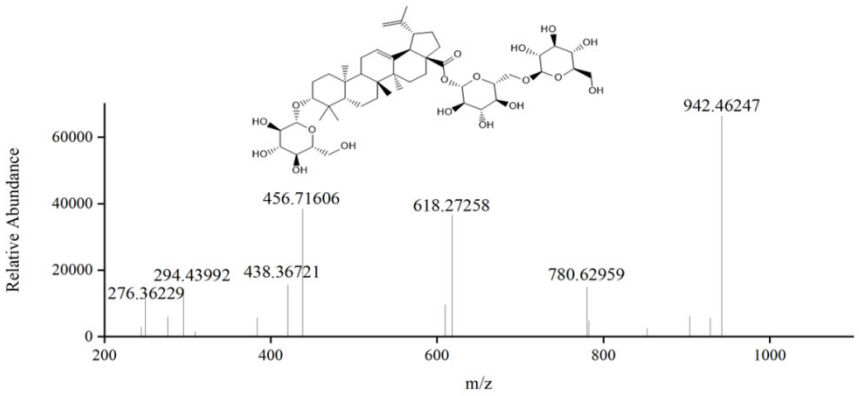
The molecular ion peak of m/z 1064.48567 is lily saponin G, and its predicted molecular formula is $C_{51}H_{82}O_{23}$. After preliminary cracking, four characteristic signal peaks of m/z 903.45601, 739.71998, 577.85162, and 417.20393 were generated, indicating that it contains four hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 398.63406, 272.81262, and 255.23345, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.

The molecular ion peak of m/z 884.68749 is lily saponin H, and its predicted molecular formula is $C_{45}H_{70}O_{17}$. After preliminary cracking, three characteristic signal peaks of m/z 723.74039, 561.33407, and 498.54537 were generated, indicating that it contains three hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 380.29914, 255.65781, and 237.34742, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.

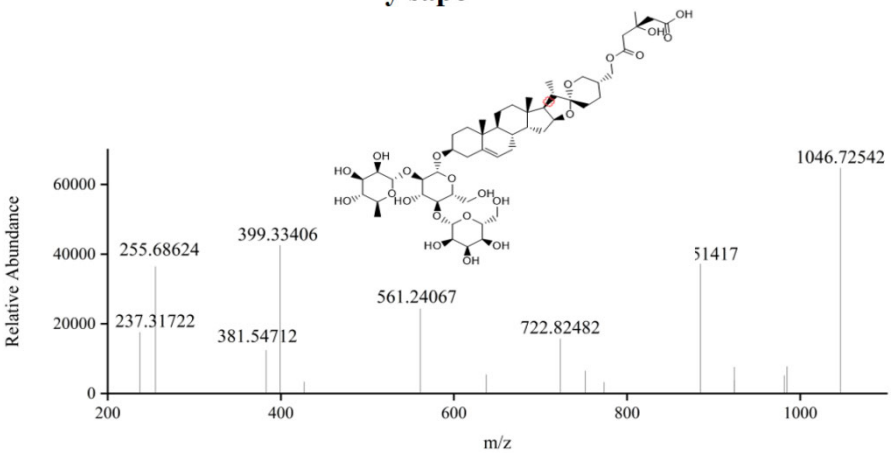
The molecular ion peak of m/z 740.42548 is lily saponin I, and its predicted molecular formula is $C_{39}H_{62}O_{13}$. After preliminary cracking, two characteristic signal peaks of m/z 579.82357 and 417.22031 were generated, indicating that it contains two hexose molecules. After removing the sugar chain, further cracking generated aglycone fragment peaks of m/z 398.26946, 273.42952, and 255.17017, which belong to [Agly.+-H_2O], [Agly.+-$C_8H_{16}O_2$], and [Agly.+-H_2O-$C_8H_{16}O_2$] respectively.



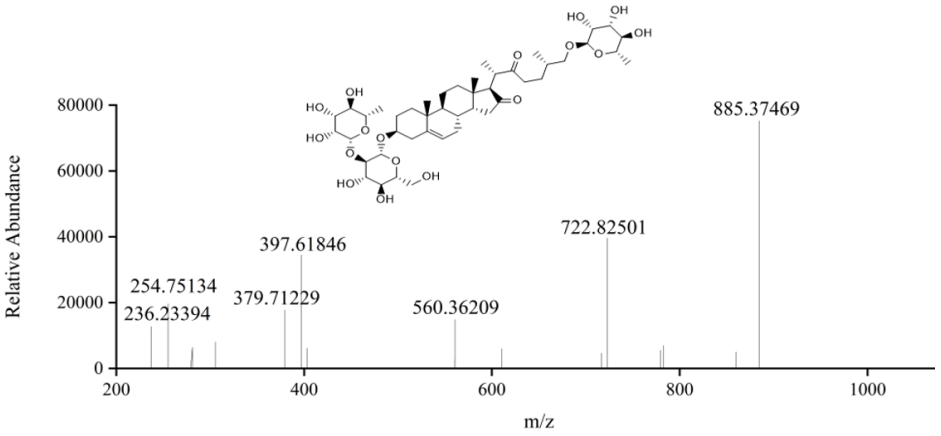
Lily saponin-A



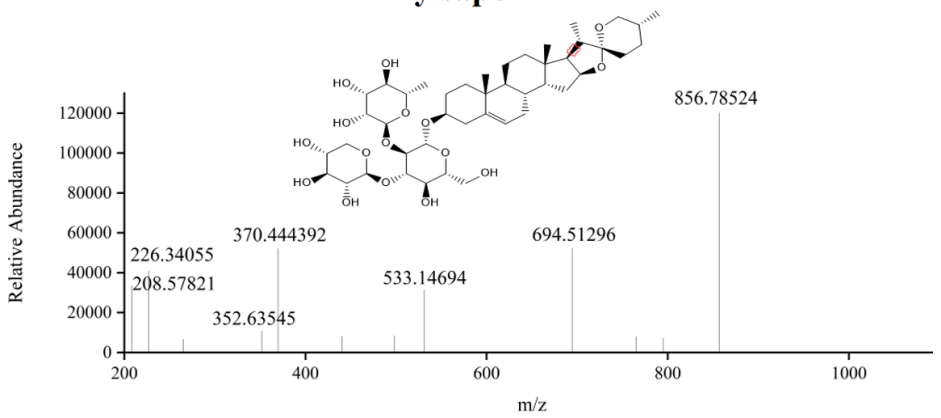
Lily saponin-B



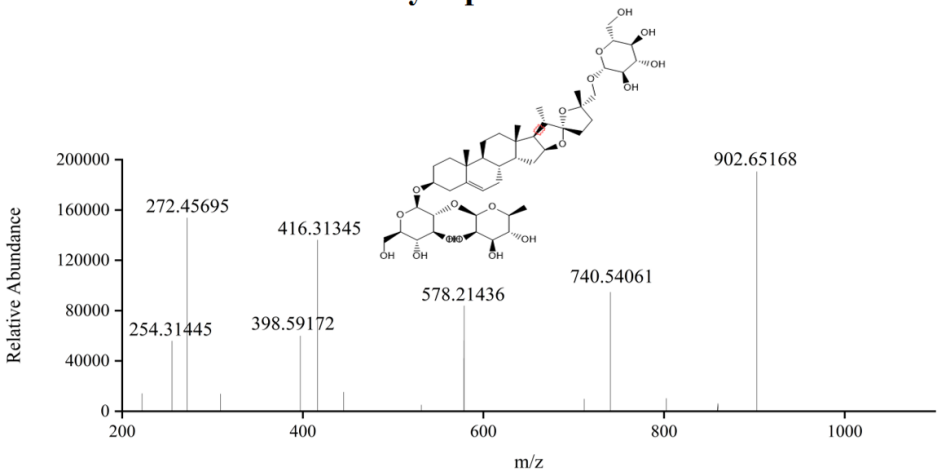
Lily saponin-C



Lily saponin-D



Lily saponin-E



Lily saponin-F

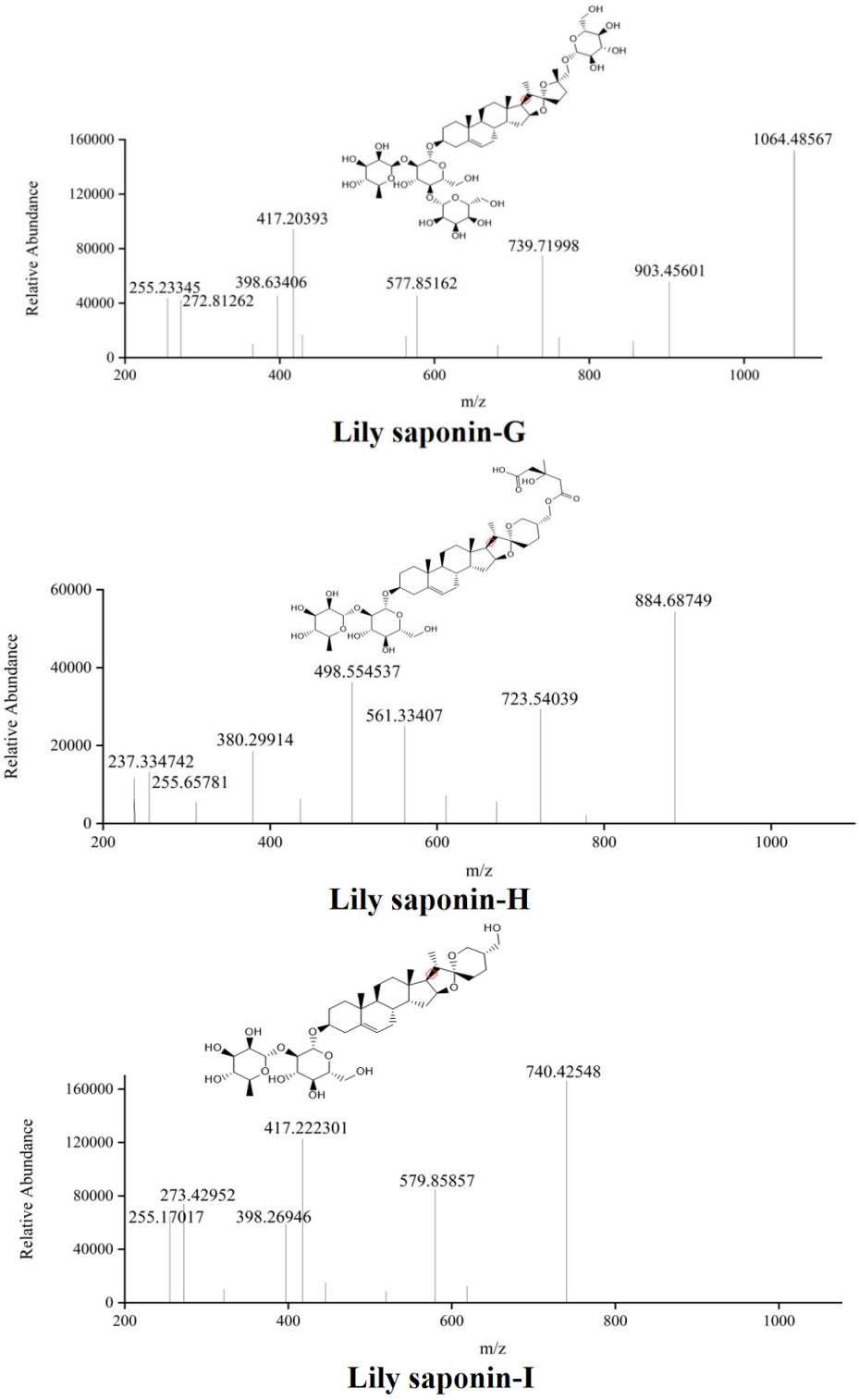


Figure S2 Secondary mass spectrometry of 9 saponins of Lilium lancifolium, The tentative identification of these saponins (Lily saponin A-I) was based on their accurate mass, MS/MS fragmentation patterns, and comparison with data reported for analogous steroidal saponins in Lilium species [38][40-44]. These spectra were used to confirm the presence of these known compounds in the NADES-15 extract.

Table S1 Analysis of Chemical Constituents in Lilium lancifolium NADES-15 Extracts by UHPLC-MS/MS

peak number	Preliminary identification results	Tr/min	m/z	molecular formula	mass error/ppm
1	2-Aminoisonicotinic acid	1.25	139.05013	$C_6H_6N_2O_2$	0.29
2	Isoleucine	1.35	132.10184	$C_6H_{13}NO_2$	0.52
3	Beta-sitosterol	6.71	415.27316	$C_{29}H_{50}O$	1.63
4	Trans-3-indole acrylic acid	7.84	188.07033	$C_{11}H_9NO_2$	1.63
5	Isobornyl methacrylate	8.24	240.19519	$C_{14}H_{22}O_2$	2.51
6	Glycerol caprylate	8.41	388.25332	$C_{16}H_{34}O_9$	2.22
7	Lily saponin A	8.96	904.33578	$C_{45}H_{74}O_{18}$	1.14
8	Lily saponin B	9.43	942.46247	$C_{48}H_{76}O_{18}$	4.63
9	3-Oxocyclopentene-1-carboxylic acid	9.49	177.05434	$C_{10}H_8O_3$	1.61
10	Suberic acid	9.87	173.08107	$C_8H_1O_4$	4.87
11	Citrus flavone	9.91	373.12723	$C_{20}H_{20}O_7$	2.52
12	Azelaic acid	11.15	187.09687	$C_9H_{16}O_4$	3.76
13	Ethyl parahydroxybenzoate	11.32	167.07002	$C_9H_{10}O_3$	1.49
14	Lily saponin C	11.72	1046.72542	$C_{51}H_{80}O_{22}$	2.42
15	Choline	12.03	104.10728	$C_5H_{13}NO$	2.78
16	Lily saponin D	12.63	885.37469	$C_{45}H_{73}NO_{16}$	3.16
17	2,5-Di-tert-butylhydroquinone	13.05	221.15445	$C_{14}H_{22}O_2$	1.14
18	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	13.11	235.16878	$C_{15}H_{22}O_2$	1.42
19	Lily saponin E	13.45	856.78524	$C_{44}H_{70}O_{16}$	1.75
20	Nootkatone	13.97	219.17397	$C_{15}H_{22}O$	1.73
21	Lily saponin F	14.52	902.65168	$C_{45}H_{82}O_{23}$	2.19
22	Lilium regale saponin A	15.84	402.13794	$C_{18}H_{24}O_{10}$	0.804
23	Lily saponin G	16.18	1064.48567	$C_{51}H_{82}O_{23}$	2.36
24	Hexadecanamide	17.75	256.26291	$C_{16}H_{33}NO$	2.28

Table S1 Analysis of Chemical Constituents in *Lilium lancifolium* NADES-15 Extracts by UHPLC-MS/MS(continuous)

peak number	Preliminary identification results	Tr/min	m/z	molecular formula	mass error/ppm
25	Valine	18.15	118.08636	$C_5H_{11}NO_2$	0.11
26	Lily saponin H	18.23	884.68749	$C_{45}H_{70}O_{17}$	1.51
27	D-Sphingosine	18.59	282.27856	$C_{18}H_{37}NO_2$	1.09
28	Lilium regale saponin E	19.47	461.36139	$C_{25}H_{47}O_8$	2.285
29	Lily saponin I	19.58	740.42548	$C_{39}H_{62}O_{13}$	0.21
30	2,4,5-Trimethylaniline	21.95	136.11188	$C_9H_{13}N$	1.44
31	L-Histidine	22.43	154.06114	$C_6H_9N_3O_2$	3.82

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Table S2 Analysis of Chemical Components in Ethanol Extracts of Lilium lancifolium by UHPLC-MS/MS

peak number	Preliminary identification results	Tr/min	m/z	molecular formula	mass error/ppm
1	Beta-sitosterol	6.48	415.39641	$C_{29}H_{50}O$	2.03
2	Trans-3-Indoleacrylic acid	7.36	188.16874	$C_{11}H_9NO_2$	2.31
3	Stigmasterol	8.41	388.25332	$C_{29}H_{48}O$	2.22
4	Lily saponin A	8.86	904.47568	$C_{45}H_{74}O_{18}$	2.31
5	Lily saponin B	9.44	942.46247	$C_{48}H_{76}O_{18}$	3.26
6	3-Oxo-1-indanic acid	9.63	177.28953	$C_{10}H_8O_3$	1.72
7	Beta-terpineol	9.84	155.97268	$C_{10}H_{18}O$	3.54
8	Hesperetin	10.36	373.35652	$C_{20}H_{20}O_7$	2.84
9	Azelaic acid	10.68	187.26546	$C_9H_{16}O_4$	3.68
10	Ethylparaben	11.48	167.58762	$C_9H_{10}O_3$	1.36
11	Geranyl formate	12.23	183.348	$C_{11}H_{18}O_2$	2.64
12	Lily saponin D	12.74	885.45876	$C_{45}H_{73}NO_{16}$	2.61
13	Lilium regale saponin A	15.65	402.13786	$C_{18}H_{24}O_{10}$	1.14
14	Lily saponin G	16.34	1063.65872	$C_{51}H_{82}O_{23}$	3.21
15	Lily saponin H	18.43	884.76324	$C_{45}H_{70}O_{17}$	2.09
16	Lily saponin I	19.42	740.48647	$C_{39}O_{13}H_{62}$	1.48
17	L-Histidine	21.96	154.32568	$C_6H_9N_3O_2$	2.96