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Rapid Analysis of the Chemical Compositions in *Semiliquidambar cathayensis* Roots by Ultra High-Performance Liquid Chromatography and Quadrupole Time-of-Flight Tandem Mass Spectrometry

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Abstract: *Semiliquidambar cathayensis* Chang was a traditional medicinal plant and used to treat rheumatism arthritis and rheumatic arthritis for centuries in China with no scientific validation, while only 15 components were reported. Thus, a rapid, efficient, and precise method based on ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UHPLC-Q-TOF-MS/MS) was applied in both positive- and negative-ion modes to rapidly analysis the main chemical compositions in *S. cathayensis* for the first time. Finally, a total of 85 chemical compositions, including 35 alkaloids, 12 flavonoids, 7 terpenoids, 5 phenylpropanoids, 9 fatty acids, 7 cyclic peptides, and 10 others were identified or tentatively characterized in the roots of *S. cathayensis* based on the accurate mass within 5 ppm error. Moreover, alkaloid, flavonoid, phenylpropanoid, and cyclic peptide were reported from *S. cathayensis* for the first time. This rapid and sensitive method was highly useful to comprehend the chemical compositions and will provide scientific basis for further study on the material basis, mechanism and clinical application of *S. cathayensis* roots.

Keywords: *Semiliquidambar cathayensis* roots; UHPLC-Q-TOF-MS/MS; chemical profiling; rapid identification; chemical compositions

1. Introduction

Semiliquidambar cathayensis Chang, is an epiphyllum tree belonging to the Hamamelidaceae family, native only to China, and grows in Jiangxi, Guangxi, Guangdong, Hainan and Guizhou [1]. Chinese people call the roots of *S. cathayensis* as *Ban feng he* (Chinese name 半枫荷), which have long been used in traditional Chinese medicine (TCM) for the treatment of rheumatism arthritis and rheumatic arthritis [2]. Modern pharmacological experiments have demonstrated that the crude extracts and/or fractions obtained from the roots of *S. cathayensis* have the effects of analgesia, anti-inflammatory, anti-hepatitis B virus, promoting blood circulation, and removing blood stasis [3–6]. Unfortunately, only 15 chemical compositions—including 7 terpenoids, 3 steroids, 3 tannins, and 2 fatty acids—were reported from the roots of *S. cathayensis* [7,8], which was a significant barrier for further pharmacological, metabolic and pharmacokinetic studies of this medicinal plant. Moreover, due to the indeterminate relationship between pharmacological activities, chemical components, the clinical application and

quality control of *S. cathayensis* roots still faced big challenges. Therefore, a rapid and sensitive method to figure out the chemical components in the roots of *S. cathayensis* was urgently needed.

Conventional separation and identification processes were time and plant material consuming [9–13], whereas the use of a rapid, efficient, and precise method focused on identification chemical components was very important for TCMs. Over the past decade, UHPLC coupled with high-resolution mass spectrometry (HRMS) has become the prime tool for investigating the chemical profiling of TCMs, because of its advantages on the peak capacity, resolution, separation time, and detection sensitivity, all of which are suitable for addressing the complicated characteristics of the constituents in TCMs [14–17]. Furthermore, quadrupole time-of-flight Q-TOF-MS/MS with powerful structural characterization can provide more specific and accurate mass measurements for both precursor and fragment ions. These features can greatly facilitate prediction of elemental compositions and fragmentation pathways [18–20].

In this study, a rapid, sensitive, and reliable approach based on UHPLC-Q-TOF-MS/MS method was established to determine the main chemical components in the roots of *S. cathayensis* for the first time, which will provide a basis for further study in vivo of *S. cathayensis* roots and the information of potential new drug structure for treating rheumatism arthritis and rheumatic arthritis.

2. Results and Discussion

2.1. Optimization of Chromatographic Separation

A series of parameters, including stationary and mobile phases, flow rate, and column temperature were investigated in order to obtain optimal chromatographic separation and analytical sensitivity for multiple constituents in the roots of *S. cathayensis*. A comparative study based on chromatographic selectivity and detection sensitivity revealed that the best performance was achieved with the BEH C₁₈ column as the stationary phase and acetonitrile as the organic part of the mobile phase. Since alkaloid compounds generally exhibits better mass spectrometric responses in positive ionization mode, the addition of 0.1% formic acid into the aqueous part of the mobile phase was found to be beneficial to the subsequent positive electrospray ionization (ESI⁺) analysis. In addition to the optimization of the stationary and mobile phases, control of column temperature and flow rate were also optimized to improve selectivity and resolution. Finally, column temperature of 35 °C and the flow rate of 0.3 mL/min were suitable for the separation. The total ion chromatogram (TIC) of *S. cathayensis* roots extract in positive- and negative-ion modes were shown in Figure 1. Moreover, the tandem mass spectra of the main components were available in Supplementary Materials.

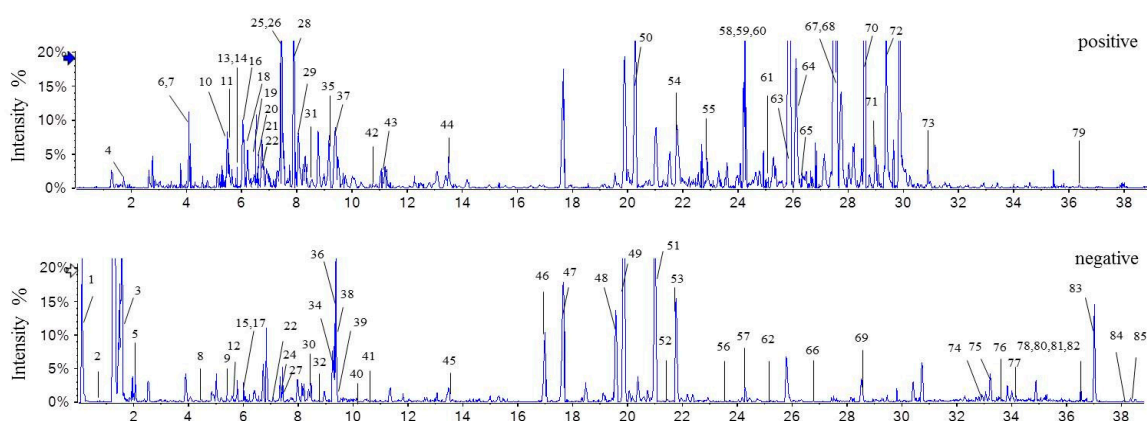


Figure 1. The total ion chromatograms of the *S. cathayensis* roots extract by UHPLC-Q-TOF-MS/MS in positive- and negative-ion modes.

2.2. Identification of Main Constituents in *S. Cathayensis* Extract

A total of 85 chemical compositions, including 35 alkaloids, 12 flavonoids, 7 terpenoids, 5 phenylpropanoids, 9 fatty acids, 7 cyclic peptides, and 10 others were identified. The molecular

formula was accurately assigned within mass error of 5 ppm. Then the fragment ions were used to further confirm the chemical structure. Information including compound name, retention time, formula, precursor ion, and fragment ions of the rest of these compositions can be found in Table 1; Table 2. The chemical structures of the main components in *S. cathayensis* roots extract are showed in Figures 2–9. All the components were identified based on the existing literatures, which includes the database of Chinese medicine ingredients and the free chemical structure database, including ChemSpider, Massbank, and mzCloud. Furthermore, the fragmentation pathways of some compounds were proposed in order to facilitate structural identification.

Table 1. Compounds identified from the roots of *S. cathayensis* by UHPLC–Q-TOF-MS/MS in positive ion mode.

No.	t_R (min)	Compounds	Molecular Formula	Molecular Weight	Measured Mass [M + H]	Error (ppm)	MS ²	Ref.
Alkaloids								
4	1.74	stachydrine	C ₇ H ₁₃ NO ₂	143.0946	144.1020	0.8	144.1004 ^a , 128.0647, 102.0585	[21]
6	4.06	gentianin	C ₁₀ H ₉ NO ₂	175.0633	176.0706	−0.2	176.0714 ^a , 148.0763, 133.0524, 130.0660, 103.0560, 120.0820, 117.0350	[22]
7	4.10	kalacolidine	C ₂₂ H ₃₅ NO ₅	393.2515	394.2593	1.4	394.2588, 376.2485 ^a	[23]
10	5.49	mesaconine	C ₂₄ H ₃₉ NO ₉	485.2625	486.2702	1.0	486.2697 ^a , 436.2332, 404.2070	[23]
11	5.54	16-β-hydroxycar diopetaline	C ₂₁ H ₃₃ NO ₄	363.2410	364.2486	1.0	364.2488 ^a , 346.2382, 328.2275	[24]
13	5.84	senbusine A	C ₂₃ H ₃₇ NO ₆	423.2621	424.2699	1.3	424.2701 ^a , 406.2591, 388.2487	[23]
16	6.05	carmichaeline	C ₂₂ H ₃₅ NO ₄	377.2566	378.2645	1.6	378.2640, 360.2532 ^a , 328.2271	[23]
18	6.10	isotalatizidine	C ₂₃ H ₃₇ NO ₅	407.2672	408.2749	1.2	390.2642 ^a , 358.2378	[23]
19	6.46	aconine	C ₂₅ H ₄₁ NO ₉	499.2781	500.2861	1.3	500.2863 ^a , 450.2495	[15]
20	6.56	songorine	C ₂₂ H ₃₁ NO ₃	357.2304	358.2381	1.2	358.2375, 340.2270 ^a	[23]
21	6.6	napelline	C ₂₂ H ₃₃ NO ₃	359.2460	360.2536	0.7	360.2536, 342.2430 ^a	[15]
22	6.74	hetisine	C ₂₀ H ₂₇ NO ₃	329.1991	330.2069	1.6	330.2062 ^a , 312.1954	[23]
25	7.38	hypaconine	C ₂₄ H ₃₉ NO ₈	469.2676	470.2751	0.6	470.2764 ^a , 438.2498, 406.2216	[24]
26	7.44	senbusine C	C ₂₄ H ₃₉ NO ₇	453.2727	454.2805	1.3	454.2786 ^a , 404.2427	[23]
28	7.91	neoline	C ₂₄ H ₃₉ NO ₆	437.2777	438.2854	0.8	438.2841 ^a , 420.2738, 388.2480, 356.2222, 154.1223	[23]
29	8.08	14-acetyl-karakoline	C ₂₄ H ₃₇ NO ₅	419.2672	420.2749	1.0	420.2757 ^a , 402.2653	[24]
35	9.18	talatisamine	C ₂₄ H ₃₉ NO ₅	421.2828	422.2906	1.2	390.2642 ^a , 358.2379	[24]
37	9.41	denudatine	C ₂₂ H ₃₃ NO ₂	343.2511	344.2589	1.4	344.2587 ^a , 326.2480	[23]
42	10.76	bullatine C	C ₂₆ H ₄₁ NO ₇	479.2883	480.2962	1.2	480.2980 ^a , 462.2858, 430.2587, 398.2295	[23]
43	11.22	chasmanine	C ₂₅ H ₄₁ NO ₆	451.2934	452.3012	1.2	452.3016 ^a , 420.2755, 388.2490	[23]
44	13.41	14-acetyl-talatisamine	C ₂₆ H ₄₁ NO ₆	463.2934	464.3011	1.0	464.3011 ^a , 432.2746	[23]
50	20.28	benzoylmesaconine	C ₃₁ H ₄₃ NO ₁₀	589.2887	590.2966	1.1	590.2941 ^a , 540.2579, 508.2323, 105.0341	[15]
54	21.85	benzoylaconine	C ₃₂ H ₄₅ NO ₁₀	603.3044	604.3122	0.9	604.3110 ^a , 554.2748	[15]
55	22.91	benzoylhypaconine	C ₃₁ H ₄₃ NO ₉	573.2938	574.3015	0.8	574.3017 ^a , 542.2755, 510.2495	[15]

Table 1. Cont.

No.	t_R (min)	Compounds	Molecular Formula	Molecular Weight	Measured Mass [M + H]	Error (ppm)	MS ²	Ref.
58	24.20	benzoyl-3,13-deoxymesaconine	C ₃₁ H ₄₃ NO ₈	557.2989	558.3068	1.1	558.3055 ^a , 540.2955, 508.2697	[15]
59	24.25	10-hydroxy-mesaconitine	C ₃₃ H ₄₅ NO ₁₂	647.2942	648.3024	1.4	648.3002 ^a , 588.2791, 556.2540, 370.1653	[23]
60	24.27	benzoyldeoxyaconine	C ₃₂ H ₄₅ NO ₉	587.3094	588.3172	0.8	588.3174 ^a , 556.2918	[15]
61	25.21	pyrohyapaconitine	C ₃₁ H ₄₁ NO ₈	555.2832	556.2910	0.9	556.2915 ^a , 524.2647, 492.2414, 452.2072, 402.2285, 238.1807, 192.1383	[15]
63	25.87	mesaconitine	C ₃₃ H ₄₅ NO ₁₁	631.2993	632.3070	0.7	632.3032 ^a , 572.2826, 540.2566, 512.2622, 508.2315, 354.1685, 105.0342	[15]
64	26.12	10-hydroxy-aconitine	C ₃₄ H ₄₇ NO ₁₂	661.3098	662.3180	1.4	662.3168 ^a , 602.2957, 570.2702, 384.1809	[15]
67	27.49	hyapaconitine	C ₃₃ H ₄₅ NO ₁₀	615.3044	616.3125	1.5	616.3089 ^a , 556.2876, 524.2618, 496.2638, 342.2055, 338.1739, 105.0340	[15]
68	27.58	aconitine	C ₃₄ H ₄₇ NO ₁₁	645.3149	646.3232	1.5	646.3199 ^a , 586.2985, 554.2735, 526.2783, 522.2487, 368.1850, 105.0342	[23]
70	28.60	deoxyaconitine	C ₃₄ H ₄₇ NO ₁₀	629.3200	630.3281	1.3	630.3256 ^a , 570.3047, 538.2787, 510.2830, 478.2575, 356.2219, 352.1905	[15]
71	28.98	yunaconitine	C ₃₅ H ₄₉ NO ₁₁	659.3306	660.3386	1.2	660.3396 ^a , 572.2866, 540.2591, 354.1735	[24]
72	29.38	3,13-dideoxyaconitine	C ₃₄ H ₄₇ NO ₉	613.3251	614.3326	0.4	614.3302 ^a , 554.3091, 522.2835, 494.2880, 462.2620, 105.0345	[23]
Terpenoids								
14	5.94	oxypaeoniflorin	C ₂₃ H ₂₈ O ₁₂	496.1581	497.1657	0.7	497.2676, 349.1575, 197.0831, 133.0687, 121.0297 ^a	[25]
31	8.56	paeoniflorin	C ₂₃ H ₂₈ O ₁₁	480.1632	481.1711	1.4	319.1245, 197.0808, 179.0691, 151.0750, 133.0650, 105.0342 ^a	[26]
65	26.21	benzoyl paeoniflorin	C ₃₀ H ₃₂ O ₁₂	584.1894	585.1970	0.5	585.3271, 319.1195, 267.0885, 249.0785, 197.0807, 179.0705, 121.0666, 105.0349 ^a	[26]
73	30.98	atractylenolide-1	C ₁₅ H ₁₈ O ₂	230.1307	231.1380	0.0	213.1257, 163.0778, 155.0848, 143.0931, 128.0610, 115.0541, 105.0712 ^a	[27]
79	36.18	3-oxo-olean-12-en-28-oic acid	C ₃₀ H ₄₆ O ₃	454.3447	455.3516	-0.8	455.3539, 437.3426, 247.1668, 233.1531, 229.1584, 197.1332, 189.1615 ^a	[28,29]

^a base peak.

Table 2. Compounds identified from the roots of *S. cathayensis* by UHPLC–Q-TOF-MS/MS in negative ion mode

No.	t_R (min)	Compounds	Molecular Formula	Molecular Weight	Measured Mass [M – H] [–]	Error (ppm)	MS ²	Ref.
Flavonoids								
24	7.33	puerarin	C ₂₁ H ₂₀ O ₉	416.1107	415.1015	–4.6	415.0970, 295.0614, 277.0490, 267.0653 ^a	[30]
33	8.85	scoparin	C ₂₂ H ₂₂ O ₁₁	462.1162	461.1069	–4.4	415.0991 ^a , 252.0361	[30]
34	9.17	isorhamnetin-3-O -neohesperidoside	C ₂₈ H ₃₂ O ₁₆	624.1690	623.1602	–2.5	623.1609, 461.1022 ^a , 417.1203, 315.0710, 153.0226, 145.0338	[31]
36	9.38	kaempferol-3-O -glucorhamnoside	C ₂₇ H ₃₀ O ₁₅	594.1585	593.1485	–4.6	593.1010, 547.1243, 430.0954, 275.0347, 112.9889 ^a	[32]
39	9.44	methyl hesperidin	C ₂₉ H ₃₆ O ₁₅	624.2054	623.1963	–2.9	623.0455, 577.0923, 534.0988, 461.1451, 410.0366, 315.1067, 145.0319 ^a	[33]
17	6.06	catechin	C ₁₅ H ₁₄ O ₆	290.0790	289.0715	–1.1	221.0899, 205.0532, 203.0700 ^a , 187.0372, 159.0452, 125.0280, 123.0486	[34]
46	17.01	naringin	C ₂₇ H ₃₂ O ₁₄	580.1792	579.1716	–0.6	579.1721, 459.1144, 313.0736, 271.0617 ^a , 177.0209, 151.0048	[33]
48	19.58	hesperidin	C ₂₈ H ₃₄ O ₁₅	610.1898	609.1815	–1.6	609.1826, 301.0706 ^a , 286.0481, 242.0583	[35]
56	23.57	5,8-dihydroxy-6,7 -dimethoxyflavone	C ₁₇ H ₁₄ O ₆	314.0790	313.0709	–2.9	297.0313, 283.0226, 266.0197, 255.0309 ^a , 227.0318, 211.0393, 185.0235, 183.0456	[33]
57	24.10	juglanin	C ₂₀ H ₁₈ O ₁₀	418.0900	417.0814	–3.1	161.0578, 135.0527, 129.0226 ^a	[20]
62	25.32	naringenin	C ₁₅ H ₁₂ O ₅	272.0685	271.0609	–1.1	151.0030, 119.0509 ^a , 117.0421	[33]
66	26.53	hesperetin	C ₁₆ H ₁₄ O ₆	302.0790	301.0713	–1.7	258.0578 ^a , 134.0383	[33]
Terpenoids								
76	33.74	2 α ,3 β -dihydrox yolean-12-en-28- oic-acid	C ₃₀ H ₄₈ O ₄	472.3553	471.3466	–3.0	471.3494 ^a , 451.0162, 411.0302, 389.2158, 330.9965, 264.9917	[28,29]
81	36.45	oleanic acid	C ₃₀ H ₄₈ O ₃	456.3604	455.3512	–4.2	455.3495 ^a , 409.2443, 391.2341, 373.2227, 355.2079	[28,29]
Phenylpropanoids								
15	6.01	ferulic acid	C ₁₀ H ₁₀ O ₄	194.0579	193.0510	2.0	134.0379 ^a	[36]
22	7.10	fraxin	C ₁₆ H ₁₈ O ₁₀	370.0900	369.0814	–3.5	223.0462 ^a , 205.0350, 129.0210, 125.0241	[32]
32	8.76	3-(3,4-dihydroxy phenyl)-2-hydroxy -propanoic acid	C ₉ H ₁₀ O ₅	198.0528	197.0456	0.2	162.8375 ^a , 160.8401, 138.0358, 123.0085	[37]
38	9.44	acteoside	C ₂₉ H ₃₆ O ₁₅	624.2054	623.1963	–2.9	623.0455, 577.0923, 461.1451, 315.1067, 145.0319 ^a	[30]
52	21.42	bergaptol	C ₁₁ H ₆ O ₄	202.0266	201.0192	–0.8	228.9172 ^a , 166.8855, 147.8874, 117.0436	[38]
Fatty Acids								
5	2.05	citric acid	C ₆ H ₈ O ₇	192.0270	191.0199	0.7	146.9074, 111.0110 ^a	[36]
69	28.24	trihydroxy- octadecaenoic acid	C ₁₈ H ₃₄ O ₅	330.2406	329.2326	–1.5	329.2354, 229.1443, 211.1346, 183.1371, 171.1026 ^a , 139.1137	[27]

Table 2. Cont.

No.	t_R (min)	Compounds	Molecular Formula	Molecular Weight	Measured Mass [M – H] [–]	Error (ppm)	MS ²	Ref.
74	32.9	dihydroxy- octadecatrienoic acid	C ₁₈ H ₃₀ O ₄	310.2144	309.2069	–0.9	291.1995 ^a , 199.8548, 179.1442, 110.0373	[27]
77	34.13	hydroxy- octadecatrienoic acid	C ₁₈ H ₃₀ O ₃	294.2195	293.2121	–0.4	293.2080 ^a , 199.8526, 149.0939, 125.1018	[28]
78	36.16	linolenic acid	C ₁₈ H ₃₀ O ₂	278.2246	277.2171	–0.7	134.8951 ^a	[28]
80	36.22	stearic acid	C ₁₈ H ₃₆ O ₂	284.2715	283.2640	–0.9	283.2633, 199.8512 ^a	[28]
83	37.05	linoleic acid	C ₁₈ H ₃₂ O ₂	280.2402	279.2329	–0.2	279.2319 ^a , 261.2194	[28]
84	38.09	palmitic acid	C ₁₆ H ₃₂ O ₂	256.2402	255.2332	0.9	256.2333, 255.2327 ^a , 114.9333	[28]
85	38.20	oleic acid	C ₁₈ H ₃₄ O ₂	282.2559	281.2484	–0.9	281.2489 ^a	[28]
Cyclic Peptides								
27	7.59	cyclo trileucyl (or isoleucyl)	C ₁₈ H ₃₃ N ₃ O ₃	339.2522	384.2488 ^b	–1.3	135.0456 ^a	[28]
40	10.07	cyclo tetra-leucyl (or isoleucyl)	C ₂₄ H ₄₄ N ₄ O ₄	452.3363	497.3328 ^b	–1.1	497.1555, 451.3294 ^a , 433.3159, 337.2669, 224.1758, 137.0247	[28]
45	13.48	cyclo pentaleucyl (or isoleucyl)	C ₃₀ H ₅₅ N ₅ O ₅	565.4203	610.4168 ^b	–1.0	564.4112 ^a , 546.4021, 225.1592	[28]
47	17.66	cyclo hexaleucyl (or isoleucyl)	C ₃₆ H ₆₆ N ₆ O ₆	678.5044	723.5020 ^b	0.7	677.4961 ^a	[28]
49	19.86	cyclo heptaleucyl (or isoleucyl)	C ₄₂ H ₇₇ N ₇ O ₇	791.5885	836.5862 ^b	0.8	790.5791 ^a	[28]
51	21.0	cyclo octaleucyl (or isoleucyl)	C ₄₈ H ₈₈ N ₈ O ₈	904.6725	949.6704 ^b	0.9	946.6691, 903.6636 ^a	[28]
53	21.78	cyclo nonaleucyl (or isoleucyl)	C ₅₄ H ₉₉ N ₉ O ₉	1017.7566	1062.7546 ^b	0.9	1062.7547, 1016.7472 ^a	[28]
Others								
1	0.35	glucogallin	C ₁₃ H ₁₆ O ₁₀	332.0744	331.0662	–2.7	169.0124, 125.0257 ^a	[39]
2	0.69	gallic acid	C ₇ H ₆ O ₅	170.0215	169.0149	3.6	124.0178 ^a	[40]
3	1.46	sucrose	C ₁₂ H ₂₂ O ₁₁	342.1162	341.1078	–3.5	221.0641, 179.0592, 161.0419, 119.0379, 113.0253 ^a	[41]
8	4.29	piscidic acid	C ₁₁ H ₁₂ O ₇	256.0583	255.0508	–0.7	218.8641, 180.9830, 165.0550 ^a , 118.9815	[42]
9	5.37	vanillin	C ₈ H ₈ O ₃	152.0473	151.0410	4.1	105.0368 ^a	[43]
12	5.59	protocatechuic acid pentoside	C ₁₂ H ₁₄ O ₈	286.0689	285.0611	–1.6	152.0117, 108.0243 ^a	[44]
30	8.37	vanillic acid	C ₈ H ₈ O ₄	168.0423	167.0354	2.2	108.0206 ^a	[36]
41	10.65	paeonol	C ₉ H ₁₀ O ₃	166.0630	165.0564	3.8	147.0476, 119.0505, 117.0379 ^a , 103.0575, 101.0392	[31]
75	33.10	dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278.1518	277.1444	–0.3	147.0072 ^a	[45]
82	36.52	dimethisterone	C ₂₃ H ₃₂ O ₂	340.2402	339.2326	–1.1	339.2317, 163.1140 ^a	[45]

^a base peak, ^b measured mass [M + HCOO][–].

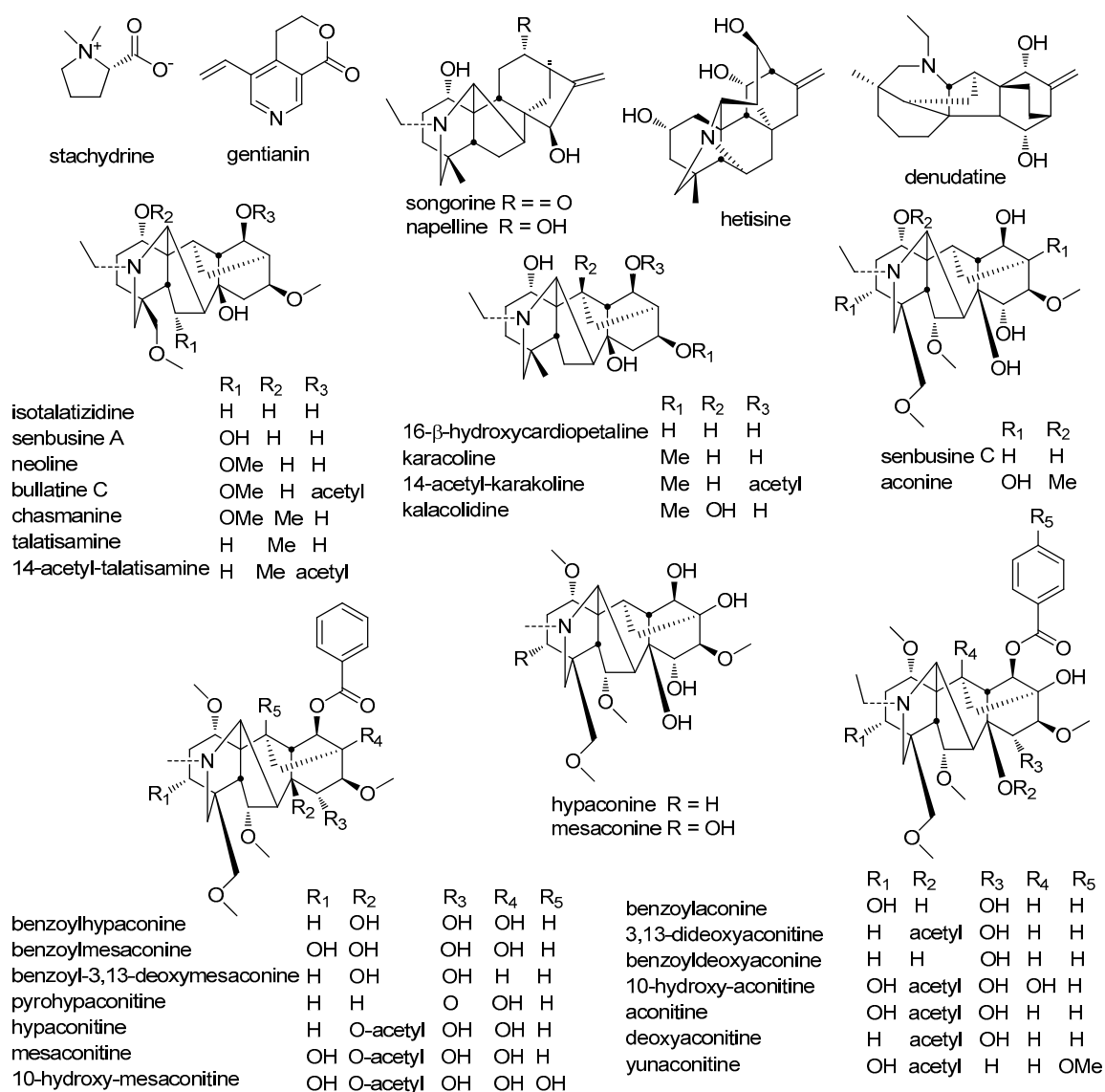


Figure 2. Chemical structures of alkaloids from *S. cathayensis* roots.

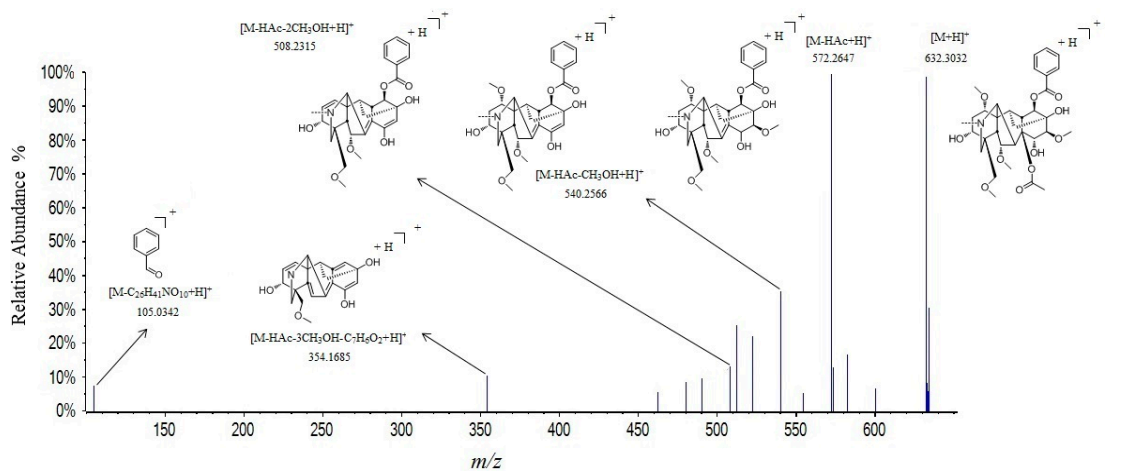


Figure 3. Tandem mass spectra and its fragmentation of mesaconitine in positive ion mode.

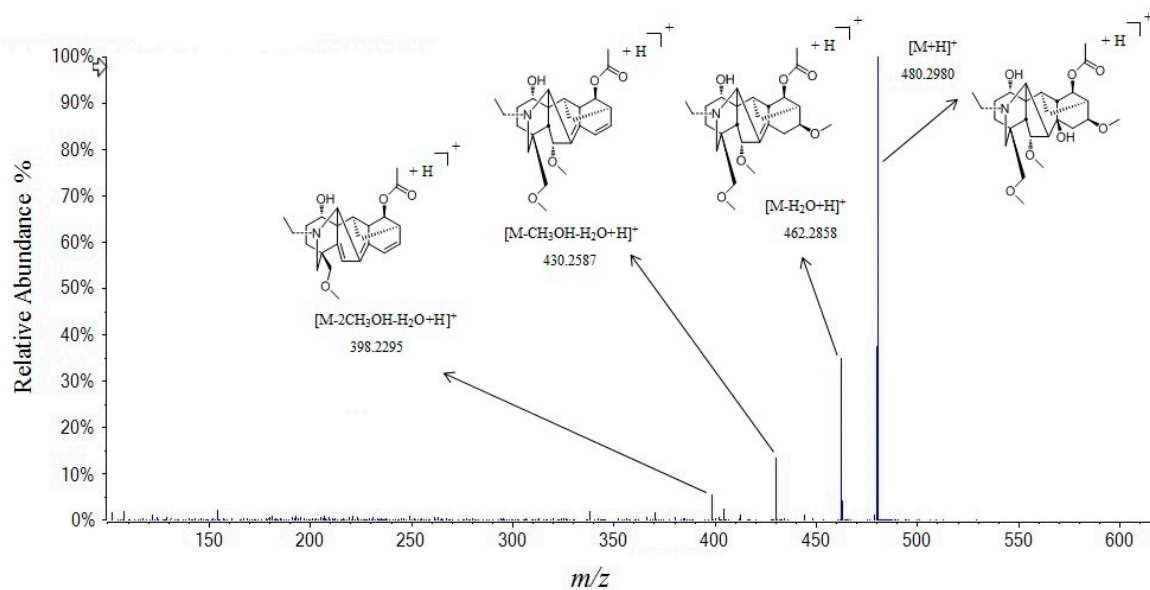


Figure 4. Tandem mass spectra and its fragmentation of bullatine C in positive ion mode.

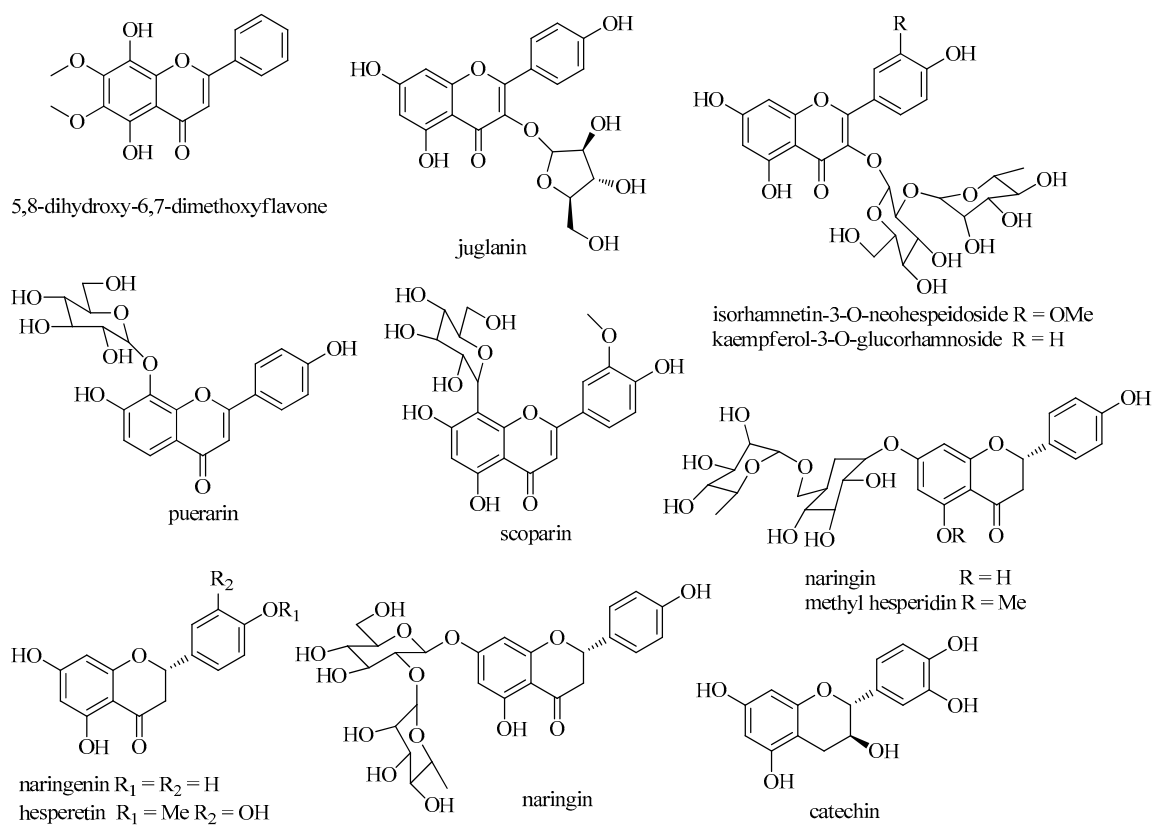


Figure 5. Chemical structures of flavonoids from *S. cathayensis* roots.

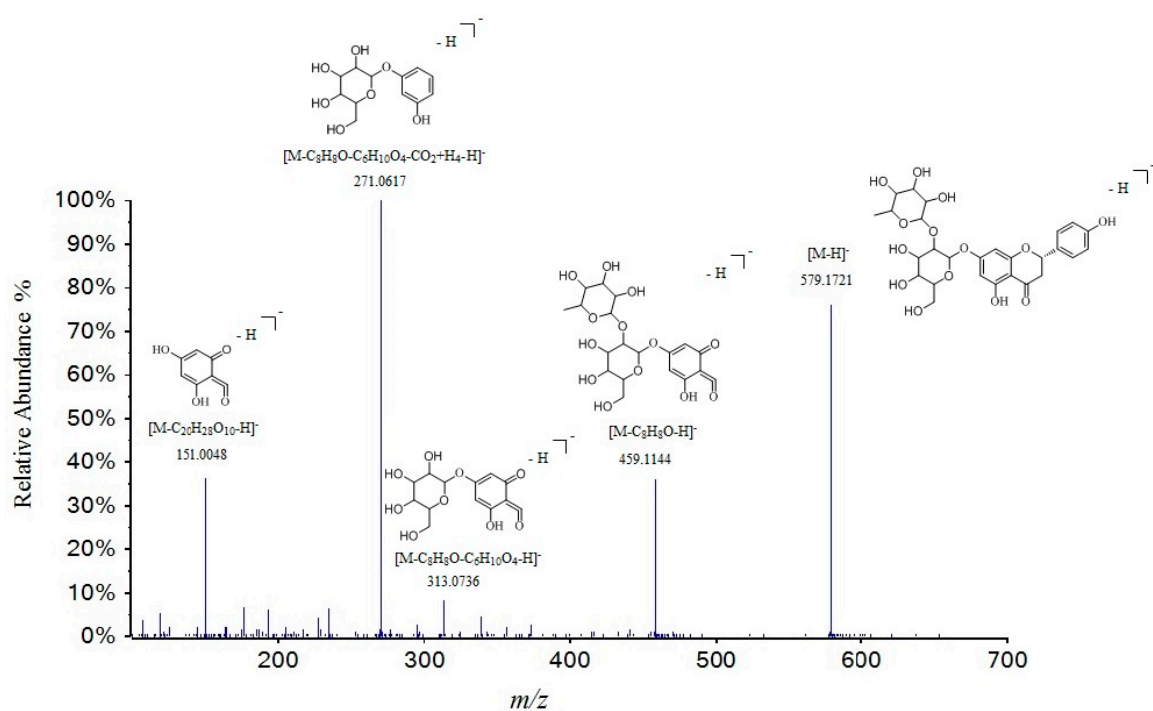


Figure 6. Tandem mass spectra and its fragmentation of naringin in negative ion mode.

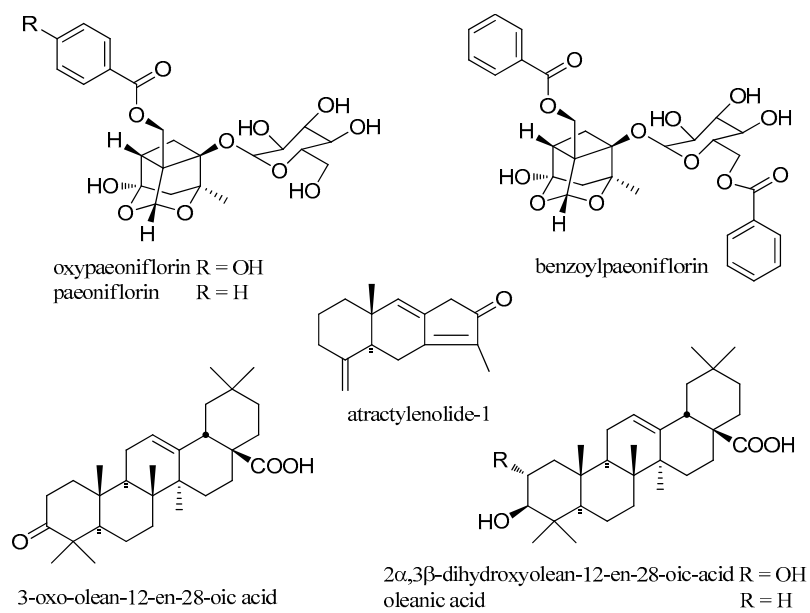


Figure 7. Chemical structures of terpenoids from *S. cathayensis* roots.

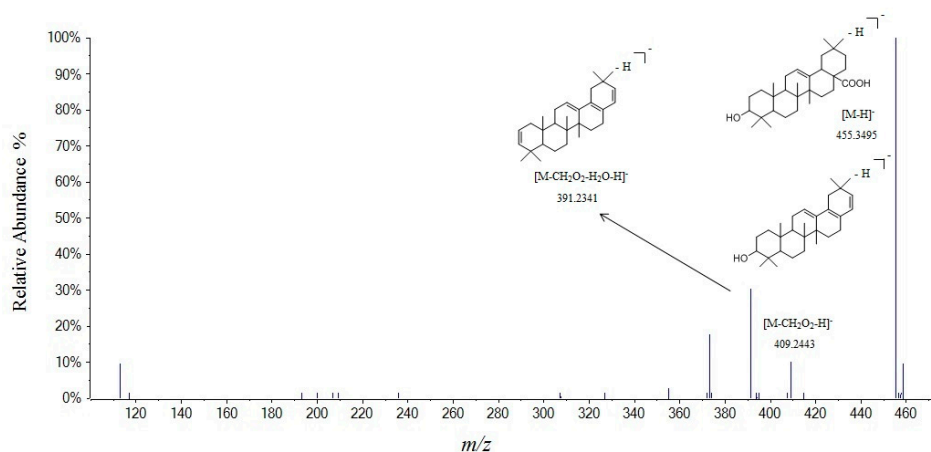


Figure 8. Tandem mass spectra and its fragmentation of oleanolic acid in negative ion mode.

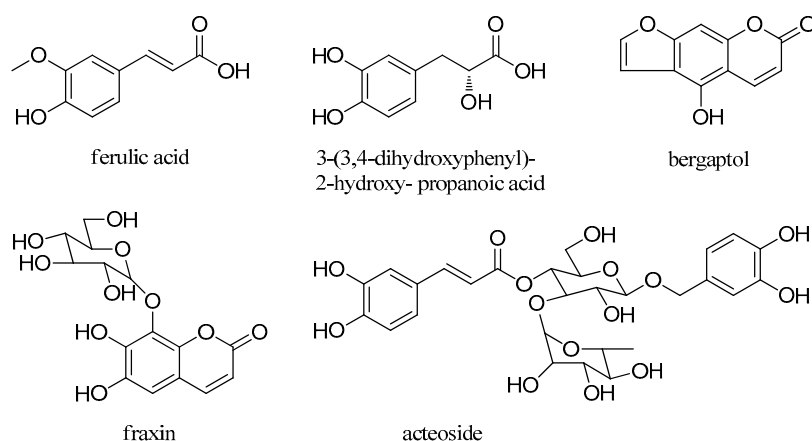


Figure 9. Chemical structures of phenylpropanoids from *S. cathayensis* roots.

2.2.1. Alkaloids

A total of 35 alkaloids (Figure 2) in the roots of *S. cathayensis* extract were identified in positive ion mode, all ones except compounds 4 and 6 were diterpenoid alkaloids, which can be classified into C₁₈-, C₁₉-, and C₂₀- diterpenoid alkaloids according to skeleton carbons [46]. In this study, compounds 7, 20, 21, 22, and 37 were C₂₀-diterpenoid alkaloids, while others were C₁₉-diterpenoid alkaloids, which were belonging to aconitum alkaloids. Moreover, aconitum alkaloids include diester-diterpenoid, monoester-diterpenoid, amine-diterpenoid, and other alkaloids [25]. In tandem mass spectrum of aconitum alkaloids commonly observe the neutral losses of H₂O, MeOH, AcOH, or BzOH. In diester-diterpenoid alkaloids, the hydroxyl groups of C8 and C14 in these compounds are combined with acetic acid and benzoic acid to form esters, respectively. The neutral loss of 28 Da corresponding to eliminate one molecule of CO or C₂H₄ was the feature loss. Meanwhile, the order of eliminations of benzyl, acetyl, carboxyl, ethyl, or methyl and methoxy was also investigated. In monoester-diterpenoid alkaloids, only hydroxyl group of C14 binds with benzoic acid to form esters. Then, hydroxyl at C1 position was the most active site. Unlike them, hydroxyl at C15 position could not be eliminated even at a high fragment or voltage in amine diterpenoid alkaloids [15].

Compounds 59, 63, 64, 67, 68, 70, 71, and 72 were diester-diterpenoid alkaloids. Among them, 63, 67, and 68 showed [M + H]⁺ ion at *m/z* 632.3032, 616.3089 and 646.3199. They have similar fragmentation pathways, including [M + H - HAc]⁺, [M + H - HAc - CH₃OH]⁺, [M + H - HAc - CH₃OH - CO]⁺, [M + H - HAc - 2CH₃OH - CO]⁺, [M + H - HAc - 2CH₃OH]⁺, and [M + H - HAc - 3CH₃OH - C₆H₅COOH]⁺, which were identified as mesaconitine, hypaconitine, and aconitine, respectively. The possible fragmentation mechanism of mesaconitine is depicted in Figure 3.

Moreover, compounds **59** and **64** gave $[M + H]^+$ ion at m/z 648.3002 and 662.3168, 16 Da greater than that of mesaconitine and aconitine, respectively. As C10 position was commonly substituted by hydroxyl in aconitum alkaloids, they were presumed as 10-OH mesaconitine and 10-OH aconitine, respectively. However, compounds **70** and **72** gave $[M + H]^+$ ion at m/z 630.3256 and 614.3302, 16 Da and 32 Da less than aconitine, respectively. Their tandem mass spectra were similar to aconitine, and some fragment ions of compounds **70** and **72** were 16 Da and 32 Da less than the fragment ions of aconitine, respectively. They were reported as deoxyaconitine and 3,13-dideoxyaconitine, respectively. Compound **71** showed $[M + H]^+$ ion at m/z 660.3396, 14 Da greater than that of aconitine. Moreover, as C14 position was commonly substituted by methoxybenzoyl in aconitum alkaloids, **71** was presumed as yunaconitine [24,47].

Compounds **29**, **42**, **44**, **50**, **54**, **55**, **58**, **60**, and **61** are monoester-diterpenoid alkaloids. Compound **29** gave fragment ions at m/z 420.2757 and 402.2653 were corresponding to $[M + H]^+$ and $[M + H - H_2O]^+$, respectively, and it was identified as 14-acetyl-karakoline by comparing with the literature [25]. Compound **42** gave fragment ions at m/z 480.2980, 462.2858, 430.2587, and 398.2295 in the positive mode were corresponding to $[M + H]^+$, $[M + H - H_2O]^+$, $[M + H - H_2O - CH_3OH]^+$, and $[M + H - H_2O - 2CH_3OH]^+$, respectively, and it was tentatively identified as bullatine C (Figure 4). The $[M + H]^+$ ion at m/z 464.3011 of compound **44** also gave the fragment ion at m/z 432.2746 $[M + H - CH_3OH]^+$, and identified as 14-acetyl-talatisamine. Compounds **50**, **54**, **55**, and **60** were 42 Da less than mesaconitine, aconitine, hypaconitine, and deoxyaconitine, respectively. As C8 position was substituted by hydroxyl group instead of acetyl group. Therefore, they could be considered as benzoylmesaconine, benzoylaconine, benzoylhypaconine, and benzoyldeoxyaconine, respectively. Moreover, compound **58** was 32 Da less than that of benzoylmesaconine, and was identified as benzoyl-3,13-deoxymesaconine. Meanwhile, compound **61** was 60 Da less than that of hypaconitine, and was presumed as pyrohypaconitine, attributing to one molecule of acetic acid eliminated from hypaconitine.

Compounds **10**, **11**, **13**, **16**, **18**, **19**, **25**, **26**, **28**, **35**, and **43** are amine diterpenoid alkaloids. Among them, **10**, **16**, **19**, **25**, and **35** were 104 Da less than benzoylmesaconine, 14-acetyl-karakoline, benzoylaconine, benzoylhypaconine, and 14-acetyl-talatisamine; and considered as mesaconine, karakoline, aconine, hypaconine, and talatisamine, respectively, because C14 position was substituted by hydroxyl group instead of benzoyl group. Moreover, **11**, **13**, **18**, **26**, **28**, and **43** could be considered as 16- β -hydroxycardiopetaline, senbusine A, isotalatizidine, senbusine C, neoline, and chasmanine, respectively, based on their molecular weight and tandem fragment patterns.

Compounds **7**, **20**, **21**, **22** and **37** were C_{20} -diterpenoid alkaloids, which gave $[M + H]^+$ ions at m/z 394.2588, 358.2375, 360.2536, 330.2062 and 344.2587, respectively. Thus, they were respectively identified as kalacolidine, songorine, napelline, hetisine, and denudatine by comparing with the literatures [15,48].

The $[M + H]^+$ ion of compound **4** was shown at m/z 144.1004. Its MS^2 fragment ions at m/z 128.0647 and 102.0585 exhibited the loss of CH_4 and continuous loss of two CH_4 , and it was presumed as stachydrine [22]. For compound **6**, the positive mode MS spectrum showed the parent ion at m/z 176.0714 $[M + H]^+$, and MS^2 spectrum showed the fragment ions at m/z 148.0763 $[M + H - C_2H_4]^+$, 130.0660 $[M + H - COOH]^+$, and 120.0820 $[M + H - C_2H_4 - CO]^+$. Compared with literature data, compound **6** was identified as gentianin [23].

2.2.2. Flavonoids

Flavonoids were a kind of basic 2-phenyl chromogenic ketones, which exist widely in nature and were important natural organic compounds. Two flavonoids (**17** and **56**) and 10 flavonoid glycosides in the roots of *S. cathayensis* extract were identified in negative ion mode (Figure 5). For flavonoids, small molecules and radicals like CH_3 (15 Da), H_2O (18 Da) and CO (28 Da) were feature loss. The main MS/MS behavior of aglycones described previously was retro Diels-Alder (RDA) fragmentation pathway. RDA fragments of m/z 135 and 119 were the feature fragments in negative ion mode [20,49].

Taking compound **17** as an example, it had $[M - H]^-$ ion at m/z 289.0715. It yielded fragments at m/z 205.0532, 203.0700, 187.0372, and 125.0280 by loss of $2(C_2H_2O)$, $2(C_2H_3O)$, $2(C_2H_2O)-H_2O$, and $C_9H_8O_3$ moieties. It was consistent with literature and identified as catechin [35].

Moreover, the loss of a glucuronic acid (176 Da) and hexose residue (glucose 162 Da, rhamnose 146 Da) were often seen in flavonoid glycosides. Compounds **24**, **33**, **34**, **36**, **39**, **46**, **48**, **56**, **57**, **62**, and **66** were considered as puerarin, scoparin, isorhamnetin-3-*O*-neohespeidoside, kaempferol-3-*O*-glucorhamnoside, methyl hesperidin, naringin, hesperidin, 5,8-dihydroxy-6,7-dimethoxyflavone, juglanin, naringenin, and hesperetin, respectively [31–34,36]. Take compound **46** as an example (Figure 6), it had $[M - H]^-$ ion at m/z 579.17156 in the spectrum. Four main fragment ions at m/z 459.1144, 271.0617, 177.0209, and 151.0048 were obviously observed. Among them, the most abundant fragment ion m/z 271.0617 was suggested by the loss of rutinose residue $[M - H - 146 - 162]^-$. Fragment ions at m/z 459.1144 and 151.0048 were glycoside and aglycone by RDA. The fragment information at m/z 177.0209 was detected as aglycone without C ring. Compared to the MS spectra data and references [34,36] compound **46** was tentatively identified as naringin.

2.2.3. Terpenoids

Terpenoids were a class of structures derived from methylglutaric acid (MVA) and have two or more isoprene units (C5) on the basic carbon shelf. Seven terpenoids were identified in this study, including three monoterpenes (**14**, **31**, and **65**), one sesquiterpene (**73**) and three triterpenes (**76**, **79**, and **81**) (Figure 7). Monoterpenes usually detected the neutral losses of a benzoic acid at m/z 121 or glucosyl group at m/z 165, and aglycone ions at m/z 195 or 197, or their fragmentations of losing H_2O and CO [15]. In that case, fragmentation behaviors showed that compound **14**, **31**, and **65** were oxypaeoniflorin, paeoniflorin, and benzoylpaeoniflorin, respectively [26,27].

Compound **73** had $[M + H]^+$ ion at m/z 231.13795, and its fragments were at m/z 163.0778 $[M + H - C_5H_8]^+$, 155.0848 $[M + H - HCOOH - C_2H_6]^+$, 143.0931 $[M + H - HCOOH - C_3H_6]^+$, and 105.0712 $[M + H - HCOOH - C_3H_6 - C_4H_2]^+$. Its fragmentation process was the same as the literature [28] and identified as atractylenolide-1.

It was reported that triterpenes (**76**, **79**, and **81**) had similar tandem fragment patterns [29,30]. Taking compound **81** as an example, it had $[M - H]^-$ ion at m/z 455.3495, and its fragments were at m/z 409.2443 $[M - H - CH_2O_2]^-$ and 391.2341 $[M - H - CH_2O_2 - H_2O]^-$. Its fragmentation process was the same as the literature [29,30], and identified as oleanolic acid (Figure 8).

2.2.4. Phenylpropanoids

Phenylpropanoids were structures containing one or more C_6-C_3 units, which were widely distributed in medicinal plants. A total of five phenylpropanoids in the roots of *S. cathayensis* extract were identified in negative ion mode (Figure 9). Compounds **15**, **22**, **32**, **38**, and **52** were considered as ferulic acid, fraxin, 3-(3,4-dihydroxyphenyl)-2-hydroxy-propanoic acid, acteoside, and bergaptol, respectively [31,33,37–39].

Taking compound **38** as an example, it had $[M - H]^-$ ion at m/z 623.0455, and its fragments were at m/z 461.1451 $[M - H - C_9H_6O_3]^-$ and 315.1067 $[M - H - C_9H_6O_3 - C_6H_{10}O_4]^-$. Its fragmentation process was the same as the literature [31] and identified as acteoside.

2.2.5. Fatty Acids

Fatty acids found in medicinal plants vary in length chains from 12 to 22 carbon atoms, of which 16–20 carbon atoms are the most common fatty acids in nature. It was reported that these compositions have a wide range of biological activities, including stabilizing cell membranes, maintaining the balance of cytokines and lipoproteins, and fighting cardiovascular diseases. In this study, nine fatty acids with long aliphatic hydrocarbon chains and a carboxyl group at one end (compounds **5**, **69**, **74**, **77**, **78**, **80**, **83**, **84**, and **85**) were identified based on the existing literatures [37,45,46,50], and in the relevant databases, such as ChemSpider, Massbank, and mzCloud.

2.2.6. Cyclic Peptides

Compounds **27**, **40**, **45**, **47**, **49**, **51**, and **53** had similar fragmentation behaviors, and showed $[M + HCOO]^-$ ions at m/z 384.2488, 497.3328, 610.4168, 723.5020, 836.5862, 949.6704, and 1062.7547, respectively. According to reference mass spectra and fragmentation spectra reported in the literatures [46,51,52], a total of seven cyclic peptides were identified with 3–9 leucyl (or isoleucyl) groups in the roots of *S. cathayensis*.

2.2.7. Others

Other 10 compounds **1–3**, **8**, **9**, **12**, **30**, **41**, **75**, and **82** were considered as glucogallin, gallic acid, sucrose, piscidic acid, vanillin, protocatechuic acid pentoside, vanillic acid, paeonol, dibutyl phthalate, and dimethisterone, respectively [32,37,40–46].

3. Experimental Section

3.1. Chemicals and Reagents

Acetonitrile and formic acid (LC-MS grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Deionized water was purified by a Milli-Q ultrapure water system (Merck Millipore, Milford, MA, USA). All other reagents used of at least analytical grade.

3.2. Plant Material and Extraction

The roots of *S. cathayensis* were collected in the town of Longsheng, Guilin City, Guangxi, China, in October 2016. A botanical voucher specimen of this plant (no. SC20161022) was deposited at authors' laboratory and was identified by one of the authors Ronghua Liu. 1.0 g aliquots of the roots of *S. cathayensis* powders were weighed and transferred into a 100-mL conical flask. 50 mL of 50% aqueous ethanol solution was added, and then extracted with a reflux twice for 60 min. Then, the fluid was filtrated and concentrated under reduced pressure in a rotary evaporator. Subsequently, the concentrated extract was lyophilized and dissolved in ACN. The solution was filtered through a 0.22- μ m PTFE membrane before submitting for instrumental analysis.

3.3. UHPLC-Q-TOF-MS/MS

The UHPLC analysis were carried out on a Shimadzu System (Kyoto, Japan), equipped with a LC-3AD solvent delivery system, a SIL-30ACXR auto-sampler, a CTO-30AC column oven, a DGU-20A3 degasser and a CBM-20A controller. Chromatographic separation was conducted on a ACQUITY UPLC[®] BEH C₁₈ (100 \times 2.1 mm, 1.7 μ m) keeping at 35 °C. 0.1% aqueous formic acid (v/v, A) and acetonitrile (B) were used as the mobile phase. The gradient elution with the flow rate of 0.3 mL/min was performed as follows: 0–5 min 5–15% B; 5–15 min 15–18% B; 15–25 min 18–35% B; 25–35 min 35–95% B; 35–37 min 95–95% B; 37–37.1 min 95–5% B; 37.1–40.0 min 5–5% B. The sample inject volume was 3 μ L.

UHPLC-Q-TOF-MS/MS detection was conducted on a Triple TOF[™] 5600+ system with a Duo Spray source in both positive and negative electrospray ion mode (AB SCIEX, Foster City, CA, USA). The MS analysis was carried out by the ESI source in both positive- and negative-ion modes. The parameters were set as follows: ion spray voltage, –5500 V; ion source temperature, 500 °C; curtain gas, 40 psi; nebulizer gas (GS1), 50 psi; heater gas (GS2), 50 psi; and decluster potential (DP), –100 V. Mass ranges were set at 100–1500 Da for the TOF-MS scan and 100–1500 Da for the TOF MS/MS experiments. In the IDA-MS/MS experiment, the collision energy (CE) was set at 45 eV, and the collision energy spread (CES) was (\pm) 15 eV for the UHPLC-Q-TOF-MS/MS detection. The most intensive five ions from each TOF-MS scan were selected as MS/MS fragmentation. LC-MS/MS data were analyzed using PeakView[®] 1.2 software (AB SCIEX, Foster City, CA, USA).

4. Conclusions

In this study, a rapid, efficient, and precise UHPLC-Q-TOF-MS/MS approach was developed for the separation and identification of the main compositions in the roots of *S. cathayensis* for the first time. By the virtue of high resolution and high separation speed of UHPLC, and accurate MS data of Q-TOF-MS, a total of 85 components, including 35 alkaloids, 12 flavonoids, 7 terpenoids, 5 phenylpropanoids, 9 fatty acids, 7 cyclic peptides, and 10 others were identified by comparisons of their retention times, accurate masses, fragment ions, related literatures. Moreover, alkaloid, flavonoid, phenylpropanoid, and cyclic peptide were reported from *S. cathayensis* for the first time. This rapid and sensitive method was highly useful to comprehend the chemical compositions and will provide a scientific basis for further study on the material basis, mechanism and clinical application of *S. cathayensis* roots.

Supplementary Materials: The following are available online, Figure S1: The tandem mass spectra of compound 25 (hypaconine) in positive ion mode, Figure S2: The tandem mass spectra of compound 26 (senbusine C) in positive ion mode, Figure S3: The tandem mass spectra of compound 28 (neoline) in positive ion mode, Figure S4: The tandem mass spectra of compound 50 (benzoylmesaconine) in positive ion mode, Figure S5: The tandem mass spectra of compound 58 (benzoyl-3,13-deoxymesaconine) in positive ion mode, Figure S6: The tandem mass spectra of compound 59 (10-hydroxy-mesaconitine) in positive ion mode, Figure S7: The tandem mass spectra of compound 60 (benzoyldeoxyaconine) in positive ion mode, Figure S8: The tandem mass spectra of compound 63 (mesaconitine) in positive ion mode, Figure S9: The tandem mass spectra of compound 64 (10-hydroxy-aconitine) in positive ion mode, Figure S10: The tandem mass spectra of compound 67 (hypaconitine) in positive ion mode, Figure S11: The tandem mass spectra of compound 68 (aconitine) in positive ion mode, Figure S12: The tandem mass spectra of compound 70 (deoxyaconitine) in positive ion mode, Figure S13: The tandem mass spectra of compound 72 (3,13-dideoxyaconitine) in positive ion mode, Figure S14: The tandem mass spectra of compound 36 (kaempferol-3-O-glucorhamnoside) in negative ion mode, Figure S15: The tandem mass spectra of compound 46 (naringin) in negative ion mode, Figure S16: The tandem mass spectra of compound 48 (hesperidin) in negative ion mode, Figure S17: The tandem mass spectra of compound 38 (acteoside) in negative ion mode, Figure S18: The tandem mass spectra of compound 83 (linoleic acid) in negative ion mode.

Author Contributions: The list authors contributed to this work as follows: L.Y. and J.-W.H. conceived and designed the experiments; L.Y., R.-H.L. and J.-W.H. performed the experiments and analyzed the data; L.Y. and J.-W.H. wrote the paper; L.Y. and J.-W.H. acquired funding for the research. All authors read and approved the final manuscript.

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