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RESEARCH ARTICLE

Rapid Characterization of Constituents in *Tribulus terrestris* from Different Habitats by UHPLC/Q-TOF MS

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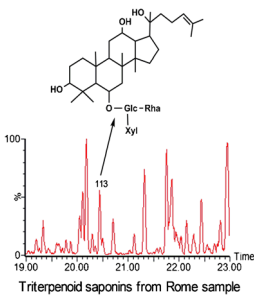

Abstract. A strategy for rapid identification of the chemical constituents from crude extracts of *Tribulus terrestris* was proposed using an informatics platform for the UHPLC/Q-TOF MS^E data analyses. This strategy mainly utilizes neutral losses, characteristic fragments, and in-house library to rapidly identify the structure of the compounds. With this strategy, rapid characterization of the chemical components of *T. terrestris* from Beijing, China was successfully achieved. A total of 82 steroidal saponins and nine flavonoids were identified or tentatively identified from *T. terrestris*. Among them, 15 new components were deduced based on retention times and characteristic MS fragmentation patterns. Furthermore, the chemical components of *T. terrestris*, including the other two samples from Xinjiang Uygur Autonomous region, China, and Rome, Italy, were also identified with this strategy. Altogether, 141 chemical components were identified from these three samples, of which 39 components were identified or tentatively identified as new compounds, including 35 groups of isomers. It demonstrated that this strategy provided an efficient protocol for the rapid identification of chemical constituents in complex samples such as traditional Chinese medicines (TCMs) by UHPLC/Q-TOF MS^E with informatics platform.

Keywords: *Tribulus terrestris*, Rapid characterization, UHPLC/Q-TOF MS, informatics platform of MS data

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Introduction

The efficacy of traditional Chinese medicines (TCMs) has been extensively validated [1]. However, obtaining the overall chemical information of numerous TCM components remains a major challenge. Therefore, establishing a strategy for rapid and comprehensive identification of chemical ingredients in TCMs is critical.

Tribulus terrestris is an annual creeper herb that grows worldwide, especially in the North temperate zones. The fruit of *T. terrestris* is a TCM termed "Ji Li," which possesses various activities, including activities against heart diseases [2], eye inflammation, skin irritation [3], abdominal distention, etc. *T. terrestris* has been extensively used in clinical practice in China many years. However, there are some significant differences in its therapeutic effects between domestic and foreign countries. In Europe and the USA, it is mainly used for increasing muscle strength [4] and improving sexual function [5], whereas it is always used to treat cardiovascular disease [6] in China. The differences in therapeutic functions of *T. terrestris* could be related to their different habitats. *T. terrestris* contains phenolic compounds [7], saponins [8], sterols [9], flavonoids [10, 11], alkaloids [12] etc., and the chemotypes are different in *T. terrestris* from Southeastern Europe, East Asia, and South Asia [13]. Based on previous phytochemical studies on *T. terrestris* from Beijing from our own laboratory [14], we choose three different areas, including Beijing, Rome, and Xinjiang to identify the chemical constituents *T. terrestris* in this study.

Owing to the advantages of high resolution and sensitivity, UHPLC/Q-TOF MS is used for the analyses of complex samples, including individual herbs and their components [15, 16]. However, conventional identification requires analysts to rapidly

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examine each individual peak, which is time-consuming, and a rapid workflow of chemical identification is urgently required.

In this study, we proposed a strategy to utilize the UHPLC/Q-TOF MS with the informatics platform of MS data for identification of multiple components of *T. terrestris*. The compounds were rapidly separated by ultra-high-performance liquid chromatography (UHPLC) and accurately measured by TOF mass spectrometry. Then the data was processed and analyzed by the informatics platform, which has a TCM component in-house database and the ability to automatically identify compounds [17].

A total of 82 steroidal saponins and nine flavonoids were identified or tentatively characterized from *T. terrestris* in Beijing. Of these, the chemical structures of 15 new components were deduced based on their characteristic MS fragmentation patterns and common neutral loss settings. In addition, the chemical ingredients of *T. terrestris*, from Xinjiang and Rome, were also similarly investigated. Hitherto, a total of 141 chemical components were identified from these three samples, including 35 groups of isomers and 39 of these components were identified or tentatively identified as new compounds (Table 1, Figure 1). This study established an efficient approach for the rapid identification of chemical constituents in TCMs by UHPLC/Q-TOF MS^E with the informatics platform, which has the advantages of automation, accuracy, and time-saving.

Experimental

Chemicals and Herb Materials

Acetonitrile (HPLC grade) was purchased from Fisher Scientific Co. (Loughborough, UK). Distilled water was purchased from Watsons (Guangzhou, China). Formic acid (HPLC grade) was purchased from Acros Co. Ltd. (St. Louis, MO, USA). Other reagents were obtained commercially in analytical purity (Beijing, China).

The fruits of *T. terrestris* were collected from Beijing and Xinjiang in China and Rome, Italy in October 2015. The plant was identified by Professor Bao-lin Guo of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Twenty-three steroidal saponin standards, Ginsenoside Rb_1, and Ginsenoside Re (Figure 2) were previously isolated and their structures were confirmed by MS as well as 1D and 2D NMR spectra.

Preparation of Herbal Extract

The dried powder of *T. terrestris* fruits (7 g) was extracted two times with hot 60% aqueous EtOH for 1.5 h per extraction. The combined extracts were filtered and concentrated at reduced pressure. The extract was subjected to a SP825 column, eluted with 10% aqueous acetonitrile and 95% aqueous acetonitrile to yield fractions A and B. Ten mg of fraction B was dissolved in 1.5 mL of 60% ethanol and filtered through a 0.22 μm membrane prior to use, and 2 μL was injected for UHPLC/Q-TOF MS^E analysis.

UHPLC/Q-TOF MS^E Analysis

UHPLC/Q-TOF MS^E analysis was performed on an Acquity UPLC system (Waters Corp., Milford, MA, USA) coupled to a Synapt G2-Si MS system (Waters Corp.). A Waters Acquity UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm) was used with the column temperature at 45 °C. Mobile phases were water with 0.1% formic acid (A), and acetonitrile (B). The gradient used was as follows: (0–0.5) min, 5%→15% B; (0.5–8.0) min, 15%→20% B; (8.0–14.0) min, 20%→25% B; (14.0–15.0) min, 25%→28% B; (15.0–18.0) min, 28%→35% B; (18.0–23.0) min, 35%→45% B; (23.0–27.0) min, 45%→50% B; (27.0–32.0) min, 50%→70% B; (32.0–34.0) min, 70%→80% B; (34.0–34.1) min, 80%→96% B; (34.1–36.1) min, 96% B; (36.1–36.2) min, 96%→5% B; (36.2–37.2) min, 5% B. The flow rate was 600 μL min^{-1}. The injection volume of sample was 2 μL. The data acquisition mode was MS^E. Each extract was injected twice: once for ESI^+ analysis, and once for ESI^- analysis; data were acquired from 50 to 1500 Da. The source temperature was 100 °C, and the desolvation temperature was 450 °C, with desolvation gas flow of 850 L h^{-1}. Leucine-enkephalin was used as lock mass. The capillary voltage was 3 kV. At low CE scan, the cone voltage was 30 V, and the collision energy was 6 eV (trap), and 4 eV (transfer). At high CE scan, the cone voltage was 30 V, and the collision energy was 45–60 eV (trap), and 12 eV (transfer). The instrument was controlled by Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

Data Analysis by Informatics Platform

Data was analyzed using software UNIFI 1.8.1 (Waters Corp., Milford, MA, USA). The maximum allowed number of peaks detected was 1000 for 2D peak detection. The peaks intensity threshold was 80 counts of high energy and 200 counts of low energy in 3D peak detection. Mass error and fragment error were both set at 10 mDa for identification, which would be exactly predicted fragments from the structure. We selected $+H-H_2O$, +H, +Na, –e as positive adducts and $+HCOO^-$, –H as negative adducts. Using leucine-enkephalin as the reference compound to confirm the mass accuracy, $[M-H]^-$ 554.2620 was used in negative ion and $[M+H]^+$ 556.2766 was used in the mode of positive ion.

Results and Discussion

Identification of the Fragmentation Patterns and Retention Times of Reference Standards

Samples were tested in both positive and negative ion modes with the same LC mobile phase in order to obtain more information on the fragmentation and chromatography patterns of steroidal saponins. The reference standards were classified into six types because of different aglycones, containing: Type I (tigogenin), Type II (gitogenin), Type III (hecogenin), Type IV (diosgenin), and their furostane-type and spirostane-type saponins, respectively;

Table 1. Compounds Identified from Three Samples of *T. terrestris* by UPLC/Q-TOF MS

No.	Rt min	Formula	$[M-H]^-$ Experimental	$[M-H]^-$ Theoretical	Error (ppm)	(-)MS and MS^E *m/z*	(+)MS and MS^E *m/z*	Identification	Source		
1	1.19	$C_{33}H_{40}O_{22}$	787.1913	787.1933	-2.5	833.1985, 787.1913, 625.1400, 463.0883, 301.0337	789.2099, 627.1595, 465.1026, 303.0525	Quercetin 3,7-diglucoside+Glc [10]		R	
2	1.28	$C_{33}H_{40}O_{21}$	771.1989	771.1984	0.6	817.2025, 771.1989, 609.1475, 463.0839, 301.0337	773.2139, 611.1631, 465.1026, 303.0525	Quercetin-3-*O*-sophoroside-7-*O*-glucoside [10]		R	
3	1.75	$C_{33}H_{40}O_{22}$	787.1913	787.1933	-2.5	787.1913, 625.1400, 463.0883, 301.0337	811.1981, 789.2094, 627.1546, 465.1026, 303.0525	Isomer of Quercetin 3,7- diglucoside+Glc [10]		R	
*4	1.87	$C_{32}H_{38}O_{21}$	757.1838	757.1827	1.5	757.1838, 301.0337, 300.0256,	781.1886, 759.1923, 627.1569, 465.1034, 303.0560	Quercetin 3,7-diglucoside+Xyl	B	R	
5	2.35	$C_{27}H_{30}O_{17}$	625.1400	625.1405	-0.8	625.1400, 300.0256	627.1569, 465.1058, 303.0560	Quercetin 3-gentiobioside [10]	B	R	X
6	2.49	$C_{32}H_{38}O_{20}$	741.1885	741.1878	0.9	741.1885, 300.0256	765.1886, 743.2082, 303.0560	Quercetin-3-*O*-β-(2(*G*)-O-β-xylopyranosyl-6(*G*)-*O*-α-rhamnopyranosyl)glucopyranoside [18]		R	X
*7	2.60	$C_{33}H_{40}O_{21}$	771.1989	771.1984	0.6	771.1989, 314.0427	795.1930, 773.2131, 641.1705, 479.1196, 317.0666	Isorhamnetin-3,7-diglucoside+Xyl	B	R	X
8	2.86	$C_{26}H_{28}O_{16}$	595.1292	595.1299	-1.2	595.1292, 301.0337, 300.0256,	619.1233, 597.1415, 465.1058, 303.0560	Isomer of Quercetin 3-*O*-arabinosyl galactoside [19]	B	R	
9	3.25	$C_{27}H_{30}O_{16}$	609.1475	609.1456	3.1	609.1475, 285.0410	633.1467, 611.1633, 449,1050, 287.0541	Kaempferol-3-gentiobioside [10]	B	R	X
10	3.55	$C_{33}H_{40}O_{20}$	755.2018	755.2035	-2.3	755.2018, 609.1433, 463.0883, 300.0256	779.2061, 757.2173, 465.1026, 303.0529	Quercetin-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-*D*-glucopyranoside) [20]	B	R	
11	3.61	$C_{21}H_{20}O_{12}$	463.0883	463.0877	1.3	463.0883, 300.0256	487.0845, 465.1070, 303.0525	Isoquercitrin [10]			X
12	3.71	$C_{28}H_{32}O_{17}$	639.1533	639.1561	-4.4	639.1533	641.1717, 479.1151, 317.0657	Astragaloside [10]	B	R	X
*13	4.22	$C_{27}H_{30}O_{16}$	609.1475	609.1456	3.1	609.1475	633.1412, 611.1611, 479.1196, 317.0657	Isorhamnetin 3-glucoside+Xyl	B		X
14	4.60	$C_{27}H_{30}O_{15}$	593.1528	593.1506	3.7	593.1528, 285.0401	617.1496, 595.1688, 449.1077, 287.0571	Kaempferol-glucosyl-(1→2) -rhamnoside [10]		R	
15	4.69	$C_{51}H_{84}O_{26}$	1111.5216	1111.5173	3.9	1111.5216, 949.4618, 787.4097, 625.3550	1135.5157, 1095.5272, 933.4679, 771.4120, 609.3643, 447.3136, 429.3076, 315.2364	Tribufuroside I [21]	B	R	X
*16	4.75	$C_{27}H_{30}O_{16}$	609.1475	609.1456	3.1	609.1475,	633.1412, 611.1631, 479.1196, 317.0693	Isorhamnetin 3-glucoside+Xyl		R	
*17	4.92	$C_{51}H_{84}O_{26}$	1111.5216	1111.5173	3.9	1157.5287, 1111.5216, 949.4681, 787.4155, 625.3602	1135.5178, 1095.5272, 933.4674, 771.4177, 609.3693, 447.3093, 429.3033, 315.2327	Isomer of Tribufuroside I	B	R	X
*18	5.12	$C_{56}H_{92}O_{30}$	1243.5604	1243.5595	0.7	1243.5604, 1111.5148, 1081.5054, 949.4592, 787.4039, 625.3555	1267.5527, 1227.5679, 1095.5272, 933.4742, 771.4348, 609.3643, 447.3093, 429.3033, 315.2400	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-12-one-2α,3β,22α,26-tetrol-3-*O*-β-*D*-xylopyranosyl-(1→3)-[β-*D*-glucopyranosyl-(1→2)]-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside	B		X
19	5.20	$C_{28}H_{32}O_{16}$	623.1603	623.1612	-1.4	623.1603	647.1586, 625.1786, 479.1191, 317.0693	Microcephalin I [10]		R	
*20	5.25	$C_{56}H_{92}O_{30}$	1243.5604	1243.5595	0.7	1289.5702, 1243.5604, 1111.5148, 1081.5054, 949.4618, 787.4097, 625.3550	1267.5564, 1227.5649, 1095.5272, 933.4742, 771.4234, 609.3693, 447.3093, 429.2991, 315.2291	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-2α,3β,22α,26-tetrol-3-*O*-β-*D*-xylopyranosyl-(1→3)-[β-*D*–glucopyranosyl-(1→2)]-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside	B		X
21	5.42	$C_{45}H_{74}O_{21}$	949.4618	949.4644	-2.7	995.4717, 949.4618, 787.4097, 625.3550, 463.2999	973.4644, 933.4679, 771.4120, 609.3593, 447.3093, 429.2906, 315.2327	Tribufuroside D [22]	B	R	X
22	5.59	$C_{22}H_{22}O_{12}$	477.1037	477.1033	0.8	477.1037	501.1029, 479.1196, 317.0693	Isorhamnetin or nepetin hexoside [10]	B		
*23	5.65	$C_{51}H_{86}O_{26}$	1113.5295	1113.5329	-3.4	1159.5360, 1113.5295, 951.4755, 789.4220, 627.3639	1137.5344, 1097.5253, 935.4725, 773.4130, 611.3654, 449.3275, 431.3188, 413.3082, 269.1347	12OH-26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-2α,3β,22α,26-tetrol-3-*O*-β-*D*-galactopyranosyl-(1→2)-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside			X
24	5.69	$C_{45}H_{74}O_{21}$	949.4618	949.4644	-2.7	995.4717, 949.4618, 787.4097, 625.3602,	973.4516, 933.4679, 771.4177, 609.3593, 447.3136, 429.2991, 315.2364, 297.2327	Tribufuroside E [22]	B	R	X
*25	5.97	$C_{51}H_{86}O_{26}$	1113.5295	1113.5329	-3.4	1159.5360, 1113.5295, 951.4755, 789.4220, 627.3690	1137.5313, 1097.5388, 935.4750, 773.4144, 611.3640, 449.3131, 431.3103, 413.2999, 269.1881	12OH-26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-2α,3β,22α,26-tetrol-3-O-β-*D*-galactopyranosyl-(1→2)-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside			X
26	6.89	$C_{51}H_{82}O_{25}$	1093.5045	1093.5067	-2.0	1139.5073, 1093.5045, 931.4526, 769.3974, 607.3447	1117.4969, 1077.5122, 915.4576, 753.4072, 591.3506, 429.2991, 315.2291, 297.2233	26-*O*-β-*D*-glucopyranosyl-(25*R*,*S*)-furostan-4(5)-en-12-one-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→2)-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [23]	B	R	X
27	7.17	$C_{51}H_{84}O_{25}$	1095.5232	1095.5223	0.8	1095.5232, 933.4722, 771.4150, 609.3648, 447.3136	1119.5191, 1079.5273, 917.4700, 755.4185, 593.3668, 431.3145, 317.2015, 299.2067	25*S*-Terrestrosin I [24]	B	R	X
28	7.41	$C_{51}H_{84}O_{25}$	1095.5232	1095.5223	0.8	1095.5232, 933.4696, 771.4150, 609.3597, 447.3136	1119.5217, 1079.5273, 917.4700, 755.4185, 593.3668, 431.3145, 317.1978, 299.1967	25*R*-Terrestrosin I [24]	B	R	X
29	7.69	$C_{56}H_{92}O_{29}$	1227.5644	1227.5646	-0.2	1273.5682, 1227.5644, 1095.5165, 1065.5046, 933.4660, 771.4150	1251.5642, 1211.5760, 1079.5542, 917.4948, 755.4016, 593.3638, 431.3188, 317.2551, 299.2403	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-xylopyranosyl-(1→3)-[β-*D*-galactopyranosyl-(1→2)]-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [25]		R	

Table 1 (continued)

No.	Rt min	Formula	$[M-H]^-$ Experimental	$[M-H]^-$ Theoretical	Error (ppm)	(-)MS and MS^E *m/z*	(+)MS and MS^E *m/z*	Identification	Source
30	7.87	$C_{56}H_{92}O_{29}$	1227.5646	1227.5646	0	1227.5646, 1065.5114, 933.4660, 771.4150, 609.3648	1251.5570, 1211.5690, 1049.5202, 917.4748, 755.4184, 593.3688, 431.3188, 317.2515, 299.2367	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-xylopyranosyl-(1→3)-[β-*D*-galactopyranosyl-(1→2)]-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [25]	B R X
31	8.08	$C_{45}H_{74}O_{20}$	933.4660	933.4695	-3.7	979.4757, 933.4660, 771.4150, 609.3648	957.4613, 917.4700, 755.4185, 593.3638, 431.3145, 317.2515, 299.2367	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [26]	B R X
32	8.14	$C_{56}H_{92}O_{29}$	1227.5676	1227.5646	2.4	1227.5676, 1065.5114, 933.4660, 771.4150, 609.3648	1251.5615, 1211.5690, 1049.5202, 917.4776, 755.4185, 593.3638, 431.3145, 317.2515, 299.2367	Polianthoside D [27]	B R X
33	8.17	$C_{51}H_{84}O_{24}$	1079.5265	1079.5274	-0.8	1125.5328, 1079.5265, 917.4758, 771.4150, 609.3648	1103.5133, 1063.5351, 901.4807, 755.4297, 593.3738, 431.3188, 317.2478, 299.2367	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [26]	B R X
34	8.34	$C_{45}H_{74}O_{20}$	933.4660	933.4695	-3.7	979.4757, 933.4660, 771.4150, 609.3648	957.4677, 917.4700, 755.4185, 593.3638, 431.3103, 317.2515, 299.2367	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [28]	B R X
35	8.39	$C_{51}H_{84}O_{24}$	1079.5265	1079.5274	-0.8	1125.5328, 1079.5265, 917.4734, 771.4150, 609.3622	1103.5168, 1063.5351, 917.4763, 755.4185, 593.3688, 431.3103, 317.2478, 299.2393	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [28]	B X
*36	8.80	$C_{51}H_{84}O_{23}$	1063.5338	1063.5325	1.2	1109.5359, 1063.5338, 917.4758, 771.4150, 753.4062, 591.3538	1087.5396, 1047.5365, 885.4813, 739.4257, 593.3538, 431.3145, 317.2503, 299.2403	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-α-*L*-rhamnopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside	R
*37	9.08	$C_{51}H_{84}O_{23}$	1063.5338	1063.5325	1.2	1109,5359, 1063.5338, 917.4756, 771.4164, 753.4062, 591.3538	1087.5396, 1047.5365, 885.4852, 739.4200, 593.3688, 431.3188, 317.2478, 299.2397	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-α-*L*-rhamnopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside	R
*38	9.35	$C_{56}H_{92}O_{28}$	1211.5714	1211.5697	1.4	1257.5719, 1211.5714, 1079.5265, 917.4696, 753.4022, 591.3538	1235.5615, 1195.5754, 1063.5319, 901.4730, 755.4228, 593.3638, 431.3103, 317.2461, 299.2330	Isomer of Terrestrinin p	B R X
*39	9.40	$C_{45}H_{74}O_{19}$	917.4758	917.4746	1.3	963.4822, 917.4758, 771.4150, 609.3597, 447.3092	941.4716, 901.4814, 755.4227, 593.3688, 431.3188, 317.2515, 299.2383	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-α-*L*-rhamnopyranosyl-(1→4)-β-*D*-galactopyranoside	R
*40	9.57	$C_{56}H_{92}O_{28}$	1211.5714	1211.5697	1.4	1257.5719, 1211.5714, 1079.5265, 917.4758, 753.4022, 591.3538	1235.5643, 1195.5745, 1063.5318, 901.4730, 755.4228, 593.3638, 431.3103, 317.2415, 299.2367	Isomer of Terrestrinin p	B X
*41	9.79	$C_{39}H_{64}O_{15}$	771.4150	771.4167	-2.2	817.4264, 771.4150, 609.3648	795.4107, 755.4225, 593.3688, 431.3130, 413.3029, 287.2062, 269.1917	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-5(6)-en-2α,3β,22α,26-tetrol-3-*O*-β-*D*-galactopyranoside	B R X
42	9.92	$C_{51}H_{86}O_{25}$	1097.5370	1097.5380	-0.9	1097.5370, 935.4815, 773.4296, 611.3782, 449.3253	1121.5348, 1081.5444, 919.4901, 757.4356, 595.3884, 433.3361, 289.2167, 271.2057	Terrestrosin G [24]	B R X
43	10.16	$C_{51}H_{86}O_{25}$	1097.5370	1097.5380	-0.9	1097.5370, 935.4815, 773.4296, 611.3782, 449.3253	1121.5348, 1081.5444, 919.4909, 757.4337, 595.3884, 433.3347, 289.2167, 271.2061	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-2α,3β,22α,26-tetrol-3-*O*-β-*D*-glucopyranosyl-(1→2)-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [24]	B R X
*44	10.29	$C_{44}H_{72}O_{18}$	887.4647	887.4640	0.8	933.4660, 887.4647, 741.4059, 609.3647, 447.3092	911.4702, 871.4650, 725.4107, 593.3688, 431.3082, 413.3082, 395.2953, 269.1917, 251.1801	Isomer of Ceparoside B	R
45	10.42	$C_{56}H_{94}O_{29}$	1229.5824	1229.5803	1.7	1229.5824, 1097.5370, 1067.5242, 935.4835, 773.4296, 611.3782	1253.5767, 1213.5846, 1081.5437, 919.4909, 757.4380, 595.3834, 433.3304, 415.3192, 271.2057	Parvispinoside A [29]	B R X
46	10.52	$C_{56}H_{94}O_{29}$	1229.5813	1229.5803	0.8	1229.5813, 1097.5370, 1067.5242, 935.4835, 773.4296, 611.3782	1253.5765, 1213.5846, 1081.5437, 919.4909, 757.4380, 595.3834, 433.3304, 415.3192, 271.2057	Purpureagitosid [14]	B R X
47	10.73	$C_{51}H_{86}O_{24}$	1081.5441	1081.5431	0.9	1127.5499, 1081.5441, 919.4899, 773.4322, 611.3803, 449.3277	1105.5416, 1065.5477, 903.4948, 757.4354, 595.3884, 433.3330, 415.3208, 271.1990, 253.1908	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-2α,3β,22α,26-tetrol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [24]	B X
48	10.79	$C_{45}H_{72}O_{20}$	931.4508	931.4539	-3.3	977.4577, 931.4508, 769.3974, 607.3467		Terrestrinin I [14]	B R X

Table 1 (continued)

No.	Rt min	Formula	[M-H]⁻ Experimental	[M-H]⁻ Theoretical	Error (ppm)	(-)MS and MS^E *m/z*	(+)MS and MS^E *m/z*	Identification	Source		
							955.4561, 933.4679, 793.4044, 771.4106, 609.3642, 591.3525, 429.3076, 317.2451, 299.2348				
49	10.94	$C_{45}H_{76}O_{20}$	935.4815	935.4852	-4.0	981.4890, 935.4815, 773.4296, 611.3782, 449.3253	959.4852, 919.4908, 757.4349, 595.3844, 433.3304, 415.3192, 271.2057	Isomer of Terrestrosin F [24]	B	R	X
50	11.05	$C_{61}H_{100}O_{32}$	1343.6116	1343.6119	-0.2	1389.6179, 1343.6116, 1211.5704, 1079.5265, 917.4758, 771.4150, 609.3633	1367.6119, 1327.6090, 1195.5591, 1033.5060, 901.4669, 755.4072, 593.3588, 431.3060, 317.2369, 299.2212	Terrestrinin N [30]	B	R	X
51	11.16	$C_{45}H_{76}O_{20}$	935.4815	935.4852	-4.0	981.4890, 935.4815, 773.4296, 611.3782, 449.3253	959.4857, 919.4907, 757.4312, 595.3844, 433.3304, 415.3192, 271.2057	Terrestrosin F [24]	B	R	X
52	11.29	$C_{61}H_{100}O_{32}$	1343.6116	1343.6119	-0.2	1389.6179, 1343.6116, 1211.5704, 1079.5265, 917.4758, 771.4150, 609.3648	1367.6104, 1327.6118, 1195.5561, 1033.5068, 901.4692, 755.4083, 593.3538, 431.3003, 317.2367, 299.2267	Terrestrinin O [30]	B	R	X
*53	11.74	$C_{47}H_{76}O_{20}$	959.4822	959.4852	-3.1	1005.4911, 959.4822, 917.4696, 899.4608, 771.4150, 753.3965, 609.3648, 591.3538, 447.3092, 429.3011	983.4839, 943.4936, 797.4368, 755.4297, 593.3738, 413.3082, 269.1917, 251.1801	Ethanoyl+26-*O*-β-*D*-glucopyranosyl-(25*S*)-furostan-5(6)-en-2α,3β,22α,26-tetrol-3-*O*-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-galactopyranoside		R	
*54	12.06	$C_{47}H_{76}O_{20}$	959.4822	959.4852	-3.1	1005.4911, 959.4822, 917.4634, 771.4150, 609.3597, 591.3538, 447.3092, 429.3011	983.4839, 943.4936, 797.4368, 755.4297, 593.3788, 413.3041, 269.1917, 251.1834	Ethanoyl+26-*O*-β-*D*-glucopyranosyl-(25*R*)-furostan-5(6)-en-2α,3β,22α,26-tetrol -3-*O*-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-galactopyranoside		R	
55	12.12	$C_{56}H_{90}O_{29}$	1225.5453	1225.5490	-3.0	1271.5522, 1225.5453, 1093.5045, 931.4564, 769.3974, 607.3447	1249.5457, 1209.5598, 1077.5148, 915.4591, 753.4093, 591.3532, 429.2991, 315.2318, 297.2230	26-*O*-β-*D*-glucopyranosyl-(25*S*)-furostan-5(6)-en-12-one-3β,22α,26-triol-3-*O*-β-*D*–glucopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*–glucopyranosyl-(1→4)-β-*D*-galactopyranoside [31]			X
*56	12.24	$C_{41}H_{66}O_{16}$	813.4255	813.4273	-2.2	859.4286, 813.4255, 771.4150, 609.3627, 447.3092	837.4285, 797.4310, 635.3769, 593.3688, 431.3188, 413.3029, 269.1917, 251.1801	Ethanoyl+26-*O*-β-*D*-glucopyranosyl-(25*S*)-furostan-5(6)-en-2α,3β,22α,26-tetrol -3-*O*-β-*D*-galactopyranoside		R	
57	12.25	$C_{56}H_{92}O_{28}$	1211.5704	1211.5697	0.6	1257.5719, 1211.5704, 1031.5054, 899.4685, 737.4132, 575.3529	1235.5643, 1230.6113, 1213.5862, 1033.5210, 901.4792, 739.4281, 577.3725, 415.3250, 273.2234, 255.2089	Terrestrinin P [30]	B		
*58	12.58	$C_{41}H_{66}O_{16}$	813.4296	813.4273	2.8	859.4286, 813.4296, 771.4150, 609.3629, 447.3092	837.4285, 797.4310, 635.3763, 593.3638, 431.3145, 413.3082, 269.1917, 251.1801	Ethanoyl+26-*O*-β-*D*-glucopyranosyl-(25*R*)-furostan-5(6)-en-2α,3β,22α,26-tetrol-3-*O*-β-*D*-galactopyranoside		R	
59	13.39	$C_{45}H_{76}O_{19}$	919.4895	919.4903	-0.9	965.4917, 919.4895, 757.4374, 595.3840	943.4876, 903.4969, 741.4472, 579.3866, 417.3349, 273.2238, 255.2104	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [32]		R	X
*60	13.62	$C_{51}H_{82}O_{23}$	1061.5182	1061.5169	1.2	1107.5229, 1061.5182, 915.4588, 769.4031, 607.3497	1085.5136, 1045.5252, 883.4671, 737.4125, 591.3536, 429.2991, 315.2364, 297.2216	26-*O*-β-*D*-glucopyranosyl-(25*R*,*S*)-5α-furostan-12-one-5(6)-en-3β,22α,26-triol-3-*O*-α-*L*-rhamnopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside		R	
61	14.17	$C_{51}H_{84}O_{24}$	1079.5265	1061.5169	-0.8	1125.5328, 1079.5265, 917.4796, 755.4213, 593.3672	1103.5268, 1063.5385, 901.4730, 739.4265, 577.3748, 415.3250, 271.2057, 253.1940	Isomer of Terrestrosin J [33]	B	R	X
62	14.33	$C_{51}H_{84}O_{24}$	1079.5265	1061.5169	-0.8	1125.5328, 1079.5265, 917.4758, 755.4213, 593.3672	1103.5268, 1063.5318, 901.4730, 739.4268, 577.3747, 415.3212, 271.2057, 253.1940	Terrestrosin J [24]	B	R	X
63	14.42	$C_{33}H_{54}O_{10}$	609.3629	609.3639	-1.6	655.3695, 609.3629, 447.3110	633.3611, 593.3670, 431.3145, 317.2485, 299.2367	Terrestrinin F [14]		R	
64a	14.6	$C_{56}H_{92}O_{28}$	1211.5704	1211.5697	0.6	1257.5719, 1211.5704, 1049.5165, 917.4758, 755.4213	1235.5665, 1195.5732, 1033.5210, 871.4681, 739.4257, 577.3752, 415.3257, 273.2238, 255.2194	terrestrinin Q [30]	B	R	X
64b	14.6	$C_{57}H_{96}O_{29}$	1243.5979	1243.5959	1.6	1289.6030, 1243.5979, 1081.5458, 919.4895, 757.4374, 595.3840	1267.5903, 1227.6023, 1065.5406, 903.4931, 741.4416, 579.3905, 417.3373, 273.2204, 255.2104	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→2)-[β-*D*-glucopyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [33]			X
65	14.65	$C_{51}H_{86}O_{24}$	1081.5458	1081.5431	2.5	1127.5483, 1081.5458, 919.4905, 757.4374, 595.3840	1105.5421, 1065.5406, 903.4931, 741.4416, 579.3875, 417.3373, 273.2204, 255.1694	Isomer of Terrestrosin H [33]	B	R	X
66	14.80	$C_{51}H_{86}O_{24}$	1081.5458	1081.5431	2.5	1127.5465, 1081.5458, 919.4905, 757.4374, 595.3840	1105.5426, 1065.5406, 903.4946, 741.4493, 579.3866, 417.3379, 273.2204, 255.1687	Terrestrosin H [24]	B	R	X
67	15.04	$C_{56}H_{94}O_{28}$	1213.5822	1213.5853	-2.6	1259.5913, 1213.5822, 1081.5458, 919.4926, 757.4374, 595.3840	1237.5798, 1197.5909, 1035.5356, 903.4908, 741.4404, 579.3866, 417.3349, 273.2204, 255.2127	Parvispinoside B [29]	B	R	X

Table 1 (continued)

No.	Rt min	Formula	[M-H]⁻ Experimental	[M-H]⁻ Theoretical	Error (ppm)	(-)MS and MS^E *m/z*	(+)MS and MS^E *m/z*	Identification	Source
68	15.05	$C_{56}H_{90}O_{28}$	1209.5558	1209.5540	1.5	1255.5598, 1209.5558, 1047.5008, 915.4589, 753.4059, 591.3536	1233.5524, 1079.5232, 917.4753, 755.4241, 593.3668, 431.3157, 317.2460, 299.2365	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-20(22)-en-3β,26-diol-3-*O*-β-*D*-xylopyranosyl-(1→3)-[β-*D*-galactopyranosyl-(1→2)]-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*- glucopyranoside [25]	X
69	15.17	$C_{33}H_{50}O_{10}$	605.3329	605.3326	0.5	651.3398, 605.3329,	589.3398, 427.2849, 313.2132	Terrestrinin D [14]	B R X
70	15.19	$C_{56}H_{94}O_{28}$	1213.5822	1213.5853	-2.6	1259.5913, 1213.5822, 1081.5439, 919.4902, 757.4321, 595.3842	1237.5838, 1197.5980, 1065.5467, 903.4908, 741.4493, 579.3905, 417.3349, 273.2204, 255.2127	Uttroside B [27]	B R X
71	15.44	$C_{51}H_{82}O_{22}$	1045.5234	1045.5219	1.4	1091.5217, 1045.5234, 899.4657, 737.4147, 575.3531	1047.5378, 901.4730, 739.4252, 577.3750, 415.3215, 271.2071, 253.1954	Pseudoprotogracillin [34]	B R X
72	15.46	$C_{45}H_{76}O_{19}$	919.4905	919.4903	0.2	965.4980, 919.4905, 757.4374, 595.3840	903.4908, 741.4460, 579.3905, 417.3349, 273.2204, 255.2127	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D* -galactopyranoside [34]	R X
73	15.53	$C_{51}H_{86}O_{23}$	1065.5448	1065.5482	-3.2	1111.5557, 1065.5448, 919.4905, 757.4374, 595.3840, 433.3325	1089.5438, 1049.5500, 903.4908, 741.4404, 579.3817, 417.3349, 273.2204, 255.2127	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [21]	B R X
74	15.61	$C_{51}H_{86}O_{23}$	1065.5475	1065.5482	-0.7	1111.5557, 1065.5475, 919.4905, 757.4374, 595.3840, 433.3325	1089.5438, 1049.5500, 903.4908, 741.4404, 579.3815, 417.3308, 273.2204, 255.2127	26-*O*-β-*D*-glucopyranosyl-(25*R*)-furostan-3β,22α,26-triol-3-*O*-β-*D*–glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside [35]	B R X
75	15.66	$C_{51}H_{84}O_{22}$	1047.5349	1047.5376	-2.6	1093.5430, 1047.5349, 901.4777, 739.4275	1071.5320, 1049.5523, 887.5018, 741.4425, 579.3899, 417.3370, 273.2218, 255.2113	Tribulosaponin B [36]	B X
76	15.68	$C_{33}H_{52}O_{10}$	607.3447	607.3482	-5.8	653.3533, 607.3447, 445.2952,	631.3412, 591.3526, 429.3027, 411.2897	Terrestrinin G [14]	B X
77	15.71	$C_{51}H_{84}O_{22}$	1047.5413	1047.5376	3.5	1093.5452, 1047.5313, 885.4872, 739.4224, 593.3672, 431.3187	1071.5314, 1031.5442, 869.4897, 723.4328, 577.3752, 415.3249, 271.2056, 253.1905	Protodioscin [13]	B R X
78	15.80	$C_{51}H_{86}O_{22}$	1049.5520	1049.5532	-1.1	1095.5571, 1049.5503, 903.4938, 757.4374, 595.3840, 433.3325	1033.5554, 887.5049, 741.4460, 579.3866, 417.3391, 273.2204, 255.2127	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-3β,22α,26-triol-3-*O*-α-*L*-rhamnopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [37]	R
79	15.87	$C_{51}H_{86}O_{22}$	1049.5520	1049.5532	-1.1	1095.5577, 1049.5520, 903.4948, 757.4373, 595.3856, 433.3332	1073.5507, 1033.5554, 871.5020, 725.4456, 579.3981, 417.3446, 273.2210, 255.2105	Neoprotodioscin [37]	B R X
80	15.91	$C_{51}H_{84}O_{21}$	1031.5458	1031.5427	3.0	1077.5438, 1031.5458, 885.4811, 739.4268, 577.3758, 415.3218	1055.5460, 1033.5552, 887.5007, 741.4401, 579.3881, 417.3346, 273.2210, 255.2105	Tribulosaponin A [36]	B R X
81	16.04	$C_{51}H_{84}O_{23}$	1063.5319	1063.5325	-0.6	1109.5380, 1063.5319, 901.4796, 755.4219, 593.3653	1087.5302, 1047.5342, 885.4874, 739.4231, 577.3748, 415.3212, 271.2051, 253.1949	Protogracillin [38]	B R X
82	16.05	$C_{45}H_{74}O_{18}$	901.4798	901.4797	0.1	947.4853, 901.4798, 755.4219, 593.3756	925.4726, 885.4849, 739.4250, 577.3747, 415.3232, 271.2051, 253.1949	Trigofoenoside A [39]	B R X
*83	16.16	$C_{61}H_{100}O_{30}$	1311.6201	1311.6221	-1.5	1357.6235, 1311.6201, 1179.5786, 1047.5362, 885.4827, 739.4265, 577.3738	1335.6178, 1313.6343, 1181.5948, 1049.5558, 887.4996, 741.4429, 579.3873, 417.3342, 273.2202, 255.2106	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-20(22)-en--3β,26-diol-3-*O*-β-*D*-xylopyranosyl-(1→2)-[β-*D* -xylopyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside	B R X
84	16.17	$C_{61}H_{100}O_{32}$	1343.6116	1343.6119	-0.2	1389.6179, 1343.6116, 1211.5686, 1049.5174, 917.4786, 771.4169, 609.3634	1367.6095, 1235.5662, 1073.5106, 941.4731, 795.4149, 633.3626, 471.3056, 449.3231, 411.2980, 335.2545, 299.2367, 281.2303	Terrestrinin K [30]	X
85	16.23	$C_{45}H_{74}O_{18}$	901.4762	901.4797	-3.9	901.4762, 739.4280, 593.3672	885.4874, 723.4388, 577.3748, 415.3250, 271.2057, 253.1940	Isomer of Trigofoenoside A [39]	R
86	16.27	$C_{45}H_{76}O_{18}$	903.4938	903.4953	-1.7	903.4938, 757.4374, 595.3890	887.4991, 741.4437, 579.3817, 417.3366, 273.2238, 255.2094	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-3β,22α,26-triol-3-*O*-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-galactopyranoside [39]	R
*87	16.49	$C_{61}H_{100}O_{31}$	1327.6199	1327.6170	2.2	1327.6199, 1195.5740, 1063.5338, 901.4762, 755.4256, 593.3672	1311.6239, 1179.5752, 1017.5239, 885.4852, 739.4200, 577.3796, 415.3233, 271.2023, 253.1905	Isomer of Terrestrinin M or Isomer of Terrestrinin L	B X
88	16.53	$C_{51}H_{82}O_{22}$	1045.5210	1045.5219	-0.9	1091.5282, 1045.5210, 899.4670, 753.4078, 591.3538	1069.5204, 1029.5255, 867.4723, 721.4102, 575.3564, 413.3041, 271.2057, 253.1940	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-5(6),22(23)-diene-3β,20α,26-triol-3-*O*-α-*L*-rhamnopyranosyl -(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [40]	R
89	16.55	$C_{61}H_{100}O_{31}$	1327.6183	1327.6170	1.0	1327.6183, 1195.5769, 1063.5372, 901.4762, 755.4256, 593.3622	1311.6291, 1149.5649, 1017.5239, 577.3796, 415.3292, 273.2070, 255.1927	Terrestrinin J [30]	B R X

Table 1 (continued)

No.	Rt min	Formula	$[M-H]^-$ Experimental	$[M-H]^-$ Theoretical	Error (ppm)	(-)MS and MS^E *m/z*	(+)MS and MS^E *m/z*	Identification	Source		
90a	16.56	$C_{51}H_{84}O_{24}$	1079.5265	1079.5274	-0.8	1125.5328, 1079.5265, 917.4796, 755.4256, 593.3672	1103.5272, 1081.5421, 941.4779, 757.4393, 595.3884, 433.3389, 415.3250, 289.2237, 271.2023, 253.1973	26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-20(22)-en-2α,3β,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→2)-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [41]	B		X
90b	16.56	$C_{61}H_{100}O_{31}$	1327.6199	1327.6170	2.2	1327.6199, 1195.5769, 1063.5372, 901.4762, 755.4256, 593.3682	1311.6239, 1149.5671, 1017.5239, 885.4874, 739.4200, 577.3796, 415.3212, 271.2057, 253.1955	Terrestrinin M [30]	B	R	X
91	16.65	$C_{61}H_{102}O_{31}$	1329.6350	1329.6327	1.7	1329.6350, 1197.5927, 1065.5448, 903.4938, 757.4374, 595.3890	1313.6348, 1181.5908, 1049.5566, 887.5032, 725.4438, 579.3866, 417.3391, 273.2238, 255.2127	Terrestrinin B [42]	B	R	X
92	16.67	$C_{61}H_{102}O_{31}$	1329.6350	1329.6327	1.7	1329.6350, 1197.5927, 1065.5448, 903.4938, 757.4374, 595.3890	1313.6348, 1181.5908, 1049.5566, 887.5032, 725.4438, 579.3866, 417.3391, 273.2204, 255.2127	Tribuluside A [42]	B	R	X
93	16.69	$C_{61}H_{100}O_{30}$	1311.6241	1311.6221	1.5	1357.6210, 1311.6241, 1179.5801, 1047.5388, 901.4803, 739.4273, 577.3754	1313.6396, 1181.5939, 1049.5580, 887.5009, 725.4462, 579.3902, 417.3368, 273.2220, 255.2114	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-20(22)-en-3β,26-diol-3-*O*-β-*D*-xylopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [43]	B	R	X
*94	17.72	$C_{51}H_{82}O_{22}$	1045.5210	1045.5219	-0.9	1091.5282, 1045.5210, 899.4608, 753.4022, 591.3539	1069.5170, 1047.5365, 885.4874, 739.4257, 593.3688, 431.3145, 413.3082, 271.2057, 253.1940	26-*O*-β-*D*-glucopyranosyl-(25*R*,*S*)-5α-furostan-22(23),24(25)-diene-2α,3β,26-triol-3-*O*-α-*L*-rhamnopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside		R	
*95	17.95	$C_{51}H_{84}O_{22}$	1047.5347	1047.5376	-2.8	1093.5433, 1047.5347, 901.4762, 755.4213, 593.3672	1071.5348, 1049.5566, 887.5092, 741.4404, 595.3834, 433.3347, 415.3233, 273.2272, 255.2127	Isomer of 26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-20(22)-en-3β,26-diol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside		R	
96	17.97	$C_{51}H_{84}O_{22}$	1047.5349	1047.5376	-2.6	1093.5430, 1047.5349, 901.4791, 739.4293,	1071.5352, 1049.5531, 887.5080, 741.4411, 579.3822, 417.3370, 273.2212, 255.2110	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-20(22)-en-3β,26-diol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [44]		R	
97	18.40	$C_{45}H_{72}O_{18}$	899.4608	899.4640	-3.6	945.4658, 899.4608, 753.4022, 591.3504,	923.4647, 901.4794, 739.4268, 593.3688, 431.3188, 317.2454, 299.2388	Hecogenin-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [45]		R	
98	18.50	$C_{56}H_{92}O_{27}$	1195.5740	1195.5748	-0.7	1241.5856, 1195.5740, 1063.5372, 901.4724, 739.4224, 577.3708	1219.5718, 1197.5909, 1065.5473, 903.4939, 741.4404, 579.3814, 417.3391, 273.2204, 255.2114	Terrestrinin T [30]	B		X
99	18.72	$C_{45}H_{72}O_{19}$	915.4550	915.4590	-4.4	915.4550, 753.4065, 591.3588, 429.3072	939.4593, 917.4758, 755.4242, 593.3688, 431.3104, 317.2412, 299.2346	26-*O*-β-*D*-glucopyranosyl-5α-furostan-12-one-20(22)-en-3β,26-diol-3-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [46]	B	R	
100	18.93	$C_{45}H_{74}O_{19}$	917.4734	917.4746	-1.3	963.4858, 917.4734, 755.4213, 593.3722,	941.4716, 919.4971, 757.4399, 595.3848, 433.3390, 415.3216, 271.2009, 253.1931	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-20(22)-en-2α,3β,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-glucopyranoside [47]	B	R	X
101	18.97	$C_{45}H_{74}O_{19}$	917.4758	917.4746	1.3	963.4858, 917.4758, 755.4213, 609.3648	941.4728, 779.4202, 415.3233, 273.2072, 255.1959	(23*S*,24*R*,25*S*)-5α-spirostan-3β,23,24-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [41]	B	R	X
102	19.20	$C_{45}H_{74}O_{18}$	901.4762	901.4797	-5.1	947.4817, 901.4762, 739.4280, 593.3622	925.4759, 903.4963, 885.4874, 723.4363, 577.3726, 415.3233, 273.2232, 255.2167	(24*S*,25*S*)-5α-spirostan-3β,24-diol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [24]	B		X
*103	19.31	$C_{63}H_{104}O_{31}$	1355.6475	1355.6483	-0.6	1401,6590, 1355.6475, 1209.5954, 1047.5377, 885.4899, 739.4214, 607.3803, 475.3789,	1379.6432, 441.3322, 423.3223, 405.3181	Ginsenoside Re+Rha+Xyl+Xyl		R	
104	19.47	$C_{61}H_{100}O_{30}$	1311.6201	1311.6221	-1.5	1357.6209, 1311.6201, 1179.5732, 1047.5347, 885.4872, 739.4280, 577.3708	1313.6397, 1181.5938, 1049.5500, 887.4998, 741.4422, 579.3812, 417.3369, 273.2220, 255.2114	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-20(22)-en-3β,26-diol-3-*O*-β-*D*-xylopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside [43]	B		X
*105	19.79	$C_{59}H_{100}O_{26}$	1223.6433	1223.6425	0.7	1269.6472, 1223.6433, 1061.5848, 915.5394, 769.4713, 607.4203, 475.3744	1247.6453, 1225.6508, 441.3722, 423.3613, 405.3516	Ginsenoside Re+Rha+Xyl		R	
106	20.02	$C_{50}H_{80}O_{24}$	1063.4938	1063.4961	-2.2	1109.5018, 1063.4938, 901.4401, 769.4088, 607.3497, 445.2934	1087.4989, 903.4587, 771.4153, 609.3696, 447.3149, 429.3065, 315.2346	25*R*-spirostan-2α,3β-diol-12-one-3-*O*-β-*D*-glucopyranosyl-(1→2)-[β-*D*-xylpyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [48]	B		X

Table 1 (continued)

No.	Rt min	Formula	[M-H]⁻ Experimental	[M-H]⁻ Theoretical	Error (ppm)	(-)MS and MS^E *m/z*	(+)MS and MS^E *m/z*	Identification	Source
*107	20.11	$C_{63}H_{104}O_{31}$	1355.6451	1355.6483	-2.4	1401,6590, 1355.6451, 1209.5965, 1047.5345, 885.4899, 739.4214, 607.3803, 475.3789,	1379.6432, 441.3322, 423.3225, 405.3186	Ginsenoside Re+Rha+Xyl+Xyl	R
108	20.15	$C_{45}H_{72}O_{20}$	931.4526	931.4539	-1.4	977.4581, 931.4526, 815.4078, 653.3581	955.4517, 933.3654, 793.3914, 631.3414, 469.2931, 447.3136, 429.3050, 315.2301	Tribufuroside J [22]	B X
*109	20.17	$C_{58}H_{98}O_{25}$	1193.6348	1193.6319	2.4	1239.6326, 1193.6348, 1047.5745, 885.5219, 739.4669, 607.4203, 475.3789	1217.6225, 1195.6451, 459.3831, 441.3722, 423.3695, 405.3586	Ginsenoside Rg2+Rha+Xyl+Xyl	R
110	20.21	$C_{45}H_{72}O_{19}$	915.4612	915.4590	2.4	961.4661, 915.4550, 753.0425, 591.3538	939.4594, 917.4703, 755.4210, 593.3644, 431.3104, 317.2493, 299.2328	Terrestrosin C [49]	X
111	20.24	$C_{50}H_{82}O_{23}$	1049.5172	1049.5169	0.3	1095.5232, 1049.5172, 917.4758, 755.4213, 593.3672	1073.5109, 1051.5366, 919.4948, 757.4386, 595.3835, 433.3301, 415.3216, 271.2046	Neogitogenin-3-*O*-β-*D*-galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside [50]	B
*112	20.37	$C_{53}H_{90}O_{22}$	1077.5830	1077.5845	-1.4	1123.5896, 1077.5830, 915.5314, 769.4713, 607.4203, 475.3744	1101.5816, 939.5270, 441.3722	Ginsenoside Re+Xyl	R
*113	20.47	$C_{47}H_{80}O_{17}$	915.5314	915.5317	-0.3	961.5383, 915.5314, 769.4769, 607.4203, 475.3744	939.5293, 917.5414, 755.4928, 441.3722,	Ginsenoside Rg2+Xyl	R
*114	20.71	$C_{52}H_{88}O_{21}$	1047.5745	1047.5740	0.5	1093.5790, 1047.5745, 901.5131, 739.4614, 607.4203, 475.3744	1071.5782, 1049.6030, 441.3765	Ginsenoside Rg2+Xyl+Xyl	R
115	21.21	$C_{50}H_{78}O_{23}$	1045.4812	1045.4856	-4.2	1091.4917, 1045.4812, 913.4426, 751.3902, 589.3389	1069.4835, 1047.5047, 885.4426, 753.4025, 591.3583, 429.3023, 315.2301, 297.2299	Neoaspidistrin [51]	B
116	21.30	$C_{50}H_{78}O_{23}$	1045.4812	1045.4856	-4.2	1091.4917, 1045.4812, 913.4463, 751.3959, 589.3339	1069.4802, 1047.5079, 885.4409, 753.4062, 591.3534, 429.3065, 315.2347, 297.2299	Isomer of Neoaspidistrin [52]	B
117	21.56	$C_{50}H_{80}O_{23}$	1047.5016	1047.5012	0.4	1093.5045, 1047.5016, 915.4550, 753.4022, 591.3538	1071.4979, 1049.5136, 917.4714, 755.4297, 593.3638, 431.3188, 317.2442, 299.2367	Terrestrosin D [24]	B R X
118	21.76	$C_{45}H_{72}O_{19}$	915.4550	915.4590	-4.4	961.4621, 915.4550, 753.4025, 591.3538	939.4506, 917.4709, 755.4210, 593.3649, 431.3104, 317.2493, 299.2328	Terreside A [53]	B R X
119	21.87	$C_{45}H_{72}O_{17}$	883.4677	883.4691	-1.6	929.4727, 883.4677, 737.4121, 591.3538	907.4607, 885.4852, 739.4257, 593.3708, 431.3103, 317.2488, 299.2396	Hecogenin-3-*O*-α-*L*-rhamnopyranosyl-(1→2)-[α-*L*-rhamnopyranosyl-(1→4)]-β-*D*-galactopyranoside [54]	R
*120	21.99	$C_{64}H_{108}O_{29}$	1339.6890	1339.6898	-0.6	1385.6957, 1339.6890, 1193.6377, 1031.5778, 869.5248, 723.4681, 591.4235, 459.3812	1363.6874, 1341.7048, 425.3795, 407.3623	Ginsenoside F2+Rha+ Rha + Xyl +Xyl	R
*121	22.15	$C_{51}H_{82}O_{21}$	1029.5270	1029.5270	0	1075.5339, 1029.5270, 883.4694, 737.4182, 575.3543	1053.5232, 885.4847, 739.4232, 577.3778, 415.3224, 271.2075, 253.1971	Yamogenin-3-*O*-α-*L*-rhamnopyranosyl-(1→2)-[α-*L*-rhamnopyranosyl-(1→4)]-glucopyranosyl-(1→4)-β-*D*-galactopyranoside	B R X
*122	22.23	$C_{51}H_{82}O_{21}$	1029.5270	1029.5270	0	1075.5372, 1029.5270, 883.4615, 737.4182, 575.3562	1053.5264, 885.4870, 739.4280, 577.3778, 415.3299, 271.2012, 253.1924	Diosgenin-3-*O*-α-*L*-rhamnopyranosyl-(1→2)-[α-*L*-rhamnopyranosyl-(1→4)]-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside	B X
123	22.32	$C_{45}H_{72}O_{17}$	883.4677	883.4691	-1.6	929.4727, 883.4677, 737.4121, 591.3538	907.4645, 885.4852, 739.4221, 577.3781, 415.3208, 271.2078, 253.1931	Gracillin [55]	B R X
*124	22.81	$C_{39}H_{62}O_{13}$	737.4125	737.4112	1.8	783.4157, 737.4281, 591.3538	761.4076, 721.4156, 575.3515, 413.3059, 269.1985, 251.1836	Spirost-5-ene-3,16-diol+Gal+Rha	B R X
*125	23.02	$C_{39}H_{62}O_{13}$	737.4125	737.4112	1.8	783.4157, 737.4225, 591.3538	761.4076, 721.4156, 575.3521, 413.3059, 269.1985, 251.1836	Isomer of Spirost-5-ene-3,16-diol+Gal+Rha	B
126	23.44	$C_{50}H_{82}O_{23}$	1049.5172	1049.5169	0.3	1095.5232, 1049.5172, 917.4707, 887.4630, 755.4213	1073.5142, 1051.5363, 889.4722, 757.4305, 595.3885, 433.3304, 415.3233, 271.2057	Desglucolanatigonin II [24]	B X
*127	23.45	$C_{58}H_{98}O_{24}$	1177.6371	1177.6370	0.1	1223.6465, 1177.6371, 1031.5778, 869.5248, 723.4681, 591.4285, 459.3856	1201.6332, 1179.6521, 425.3795	Ginsenoside F_2+Rha+Rha+Rha+Xyl-Glc	
128	23.58	$C_{50}H_{82}O_{23}$	1049.5172	1049.5169	0.3	1095.5232, 1049.5172, 917.4707, 887.4630, 755.4213	1073.5142, 1051.5363, 889.4722, 757.4305, 595.3885, 433.3304, 415.3233, 271.2057	F-Gitonin [24]	B X
*129	23.60	$C_{58}H_{98}O_{24}$	1177.6371	1177.6370	0.1	1223.6465, 1177.6371, 1031.5778, 869.5248, 723.4681, 591.4235, 459.3856	1201.6332, 1179.6511, 425.3795	Isomer of Ginsenoside F_2+Rha+Rha+ Rha+Xyl-Glc	R
130	25.25	$C_{39}H_{64}O_{13}$	739.4224	739.4269	-6.1	785.4339, 739.4224, 577.3733,	763.4295, 741.4491, 579.3859, 417.3393, 273.2225, 255.2140	Isomer of Tigogenin-3-*O*-β-*D*-glucopyranosyl-(1→4) -β-*D*-galactopyranoside [36]	B R X
131	25.58	$C_{39}H_{64}O_{13}$	739.4224	739.4269	-6.1	785.4326, 739.4224, 577.3716	763.4213, 741.4493, 579.3866, 417.3349, 273.2219, 255.2159	Tigogenin-3-*O*-β-*D*−glucopyranosyl-(1→4)-β-*D*-galactopyranoside [36]	B R X
132	26.24	$C_{50}H_{82}O_{22}$	1033.5222	1033.5219	0.3	1079.5265, 1033.5222, 901.4762, 739.4213, 577.3716	1057.5168, 1035.5322, 925.4772, 741.4472, 579.3878, 417.3391, 273.2204, 255.2127	Gitonin [24]	B R X
133	26.50	$C_{45}H_{72}O_{16}$	867.4709	867.4742	-3.8	913.4791, 867.4709, 721.4149,	891.4728, 869.4896, 415.3210,	Dioscin [56]	B R X

Table 1 (continued)

No.	Rt min	Formula	$[M-H]^-$ Experimental	$[M-H]^-$ Theoretical	Error (ppm)	(-)MS and MS^E *m/z*	(+)MS and MS^E *m/z*	Identification	Source
134	26.89	$C_{55}H_{90}O_{25}$	1149.5658	1149.5693	-3.0	1195.5749, 1149.5658, 1017.5258, 885.4827, 739.4224, 577.3708	1173.5654, 1151.5818, 1019.5404, 887.5032, 725.4473, 579.3823, 417.3349, 273.2243, 255.2114	Tribulosin [57]	X
135	26.94	$C_{55}H_{90}O_{25}$	1149.5683	1149.5693	-0.9	1195.5749, 1149.5683, 1017.5257, 885.4827, 739.4286, 577.3742	1173.5641, 1151.5809, 1019.5404, 887.5032, 725.4473, 579.3823, 417.3349, 273.2270, 255.2114	25*R*-Tribulosin [24]	X
136	27.12	$C_{45}H_{74}O_{16}$	869.4886	869.4899	-1.5	915.4922, 869.4886, 723.4326, 577.3708	893.4849, 871.5073, 725.4493, 579.3817, 417.3349, 273.2204, 255.2094	Tigogenin3-*O*-α-*L*- rhamnopyranosyl- (1→2)-[α-*L*-rhamnopyranosyl-(1→4)]-β-*D*-galactopyranoside [58]	R
137	27.25	$C_{45}H_{74}O_{17}$	885.4830	885.4848	-2.0	931.4902, 885.4830, 739.4238, 577.3739	909.4802, 887.5008, 741.4437, 579.3817, 417.3391, 273.2238, 255.2159	25*S*- Terrestrosin B [24]	R
138	27.55	$C_{45}H_{74}O_{17}$	885.4830	885.4848	-2.0	931.4901, 885.4830, 739.4238, 577.3739	909.4813, 887.5001, 741.4425, 579.3818, 417.3391, 273.2204, 255.2154	Isoterrestrosin B [36]	R
139	27.72	$C_{39}H_{62}O_{12}$	721.4167	721.4163	0.6	767.4221, 721.4167, 575.3582,	745.4124, 723.4323, 577.3704, 415.3249, 271.2091, 253.1954	Isomer of Tribestin [39]	B R X
140	28.71	$C_{39}H_{62}O_{12}$	721.4152	721.4163	-1.5	767.4235, 721.4152, 575.3584	745.4169, 723.4333, 577.3706, 415.3275, 271.2023, 253.1973	Tribestin [59]	B R X
141	29.29	$C_{39}H_{64}O_{12}$	723.4341	723.4320	2.9	769.4372, 723.4241, 577.3708	747.4298, 725.4473, 579.3823, 417.3391, 273.2238, 255.2112	Tigogenin-3-*O*-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-glucopyranoside [60]	B R X

T. terrestris from Beijing, China (B), *T. terrestris* from Xinjiang, China (X), *T. terrestris* from Rome, Italy (R).
* Tentatively identified as novel compounds.

Type V (Terrestrinin D), and Type VI (Terrestrinin K). To our knowledge, most steroidal saponin glycosides obtained from Zygophyllaceae were 3-O-glycosides, in which the inner sugar was usually galactose and the outer sugars were rhamnose, xylose, and glucose, respectively. The precursor ions and characteristic fragment ions of these reference standards are discussed in detail below.

In ESI^-, Parvispinoside B (a furostane-type saponin of Type I) produced a deprotonated molecular ion at *m/z* 1213.5822 $[M-H]^-$ and an adduct ion at *m/z* 1259.5913 $[M+HCOO]^-$ at low CE (Figure 3a); at high CE, Parvispinoside B produced fragment ions at *m/z* 1213.5822 $[M-H]^-$, 1081.5458 $[M-H-Xyl]^-$, 919.4926 $[M-H-Xyl-Glc]^-$, 757.4374 $[M-H-Xyl-Glc-Gal]^-$, and 595.3840 $[M-H-Xyl-Glc-Gal-Glc]^-$ (Figure 3b).

In ESI^+, Parvispinoside B produced major fragment ions at *m/z* 1237.5798 $[M+Na]^+$, 1197.5909 $[M+H-H_2O]^+$, 1035.5356 $[M+H-H_2O-Glc]^+$, 903.4908 $[M+H-H_2O-Glc-Xyl]^+$, 741.4404 $[M+H-H_2O-Glc-Xyl-Gal]^+$, 579.3866 $[M+H-H_2O-Glc-Xyl-Gal-Glc]^+$, and 417.3349 $[M+H-H_2O-Glc-Xyl-Gal-Glc-Gal]^+$ at low CE (Figure 3c), and at high CE, the characteristic fragment ions at *m/z* 273.2204 and 255.2127 were observed because of consecutive losses of 144 (formula $C_8H_{16}O_2$) and one molecule of water (18 Da) from the fragment ion at *m/z* 417.3349 (Figure 3d). The fragmentation pathways of Parvispinoside B are shown in Figure 4.

In ESI^-, Terrestrinin R (a spirostane-type saponin of Type I) produced a $[M-H]^-$ ion at *m/z* 1327.6170 and a $[M+HCOO]^-$ ion at *m/z* 1373.6226 at low CE, and then the major fragment ions were observed at *m/z* 1327.6170 $[M-H]^-$, 1195.5740 $[M-H-Xyl]^-$, 1063.5338 $[M-H-2Xyl]^-$, 901.4794 $[M-H-2Xyl-Glc]^-$, 755.4213 $[M-H-2Xyl-Glc-Rha]^-$, and 593.3729 $[M-H-2Xyl-2Glc-Rha]^-$ at high CE. In ESI^+, Terrestrinin R produced major ions at *m/z* 1351.6177 $[M+Na]^+$, 1149.5667 $[M+H-H_2O-Glc]^+$, 723.4333 $[M+H-H_2O-2Glc-2Xyl]^+$, 577.3747 $[M+H-H_2O-2Glc-2Xyl-Rha]^+$, 415.3233 $[M+H-H_2O-2Glc-2Xyl-Rha-Gal]^+$, 273.2238 $[415.3233-C_8H_{14}O_2]^+$, and 255.2127 $[273.2238-H_2O]^+$. When the hydroxyl group substituted on the F-ring was lost, one more double bond was added to the F ring of the aglycone. This made the neutral loss 142 Da.

Parvispinoside A (a furostane-type saponin of Type II) produced the major ions at *m/z* 1229.5824 $[M-H]^-$, 1275.5659 $[M+HCOO]^-$, 1067.5242 $[M-H-Glc]^-$, 935.4835 $[M-H-Glc-Xyl]^-$, 773.4296 $[M-H-Glc-Xyl-Gal]^-$, and 611.3782 $[M-H-Glc-Xyl-Gal-Glc]^-$ in ESI^-. In high CE ESI^+, Parvispinoside A produced fragment ions at 1253.5767 $[M+Na]^+$, 1213.5846 $[M+H-H_2O]^+$, 1051.5322 $[M+H-H_2O-Glc]^+$, 919.4909 $[M+H-H_2O-Glc-Xyl]^+$, 757.4380 $[M+H-H_2O-Glc-Xyl-Gal]^+$, 595.3834 $[M+H-H_2O-2Glc-Xyl-Gal]^+$, 433.3304 $[M+H-H_2O-2Glc-Xyl-2Gal]^+$, 415.3192 $[433.3304-H_2O]^+$, 289.2167 $[433.3304-C_8H_{16}O_2]^+$, 271.2057 $[415.3192-C_8H_{16}O_2]^+$, and 253.1940 $[271.2057-H_2O]^+$. Comparison of Type I and Type II produced different characteristic fragmentations at *m/z* 433, 415, 289, 271, and 253, attributable to one hydroxyl group at C-2 position of glycone.

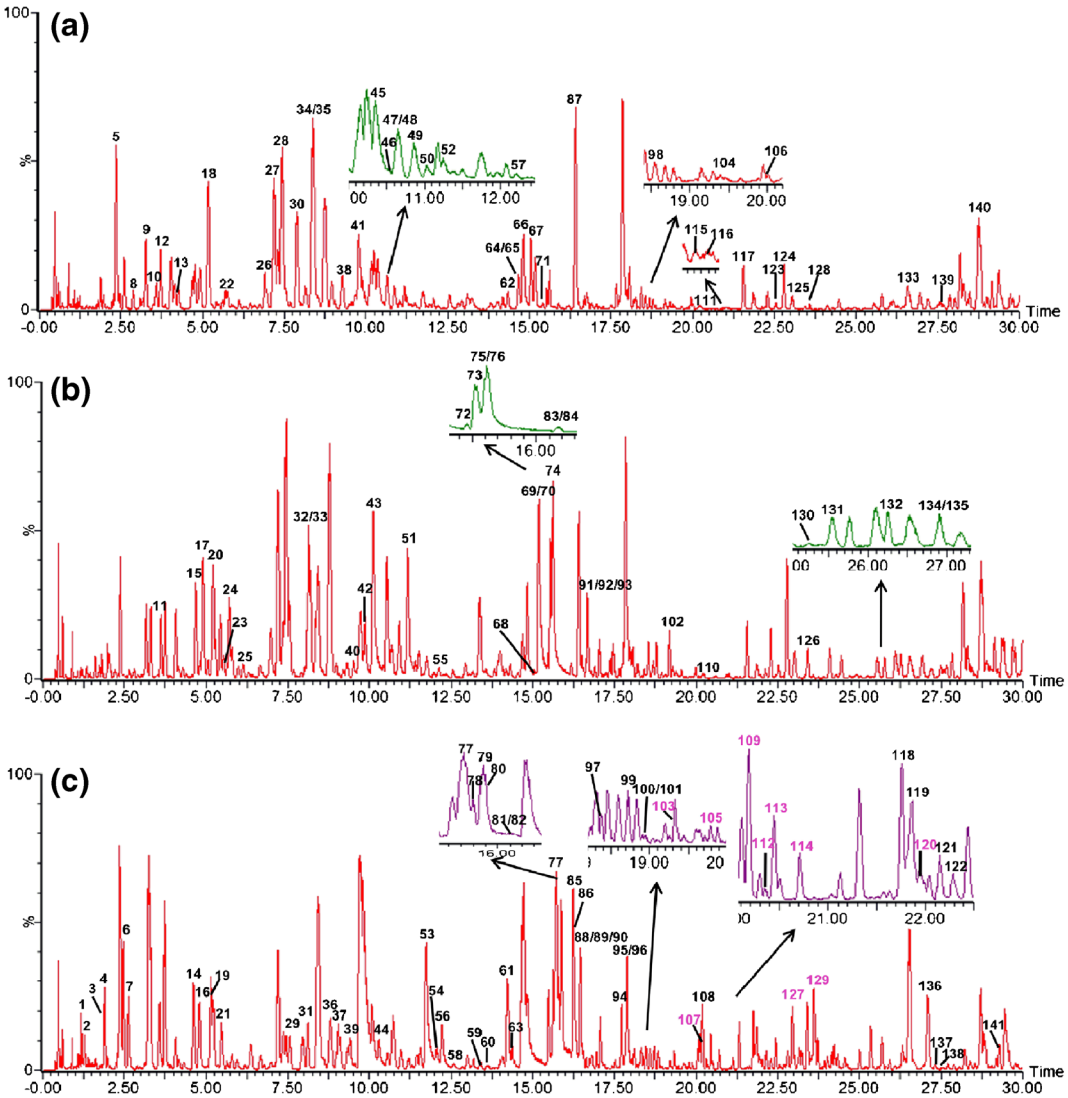


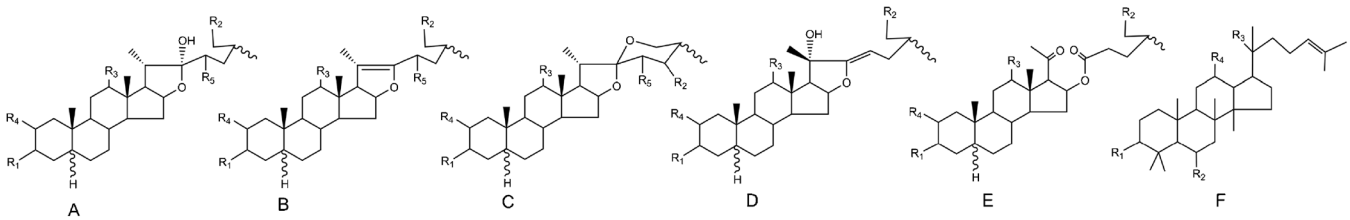
Figure 1. The base peak ion (BPI) chromatograms of the extracts from the negative mode in *T. terrestris* analyzed by UHPLC/Q-TOF MS^E. (**a**) *T. terrestris* from Beijing; (**b**) *T. terrestris* from Xinjiang; (**c**) *T. terrestris* from Rome. Pink numbers, triterpenoid saponins are the major components that differed between the three samples.

25S-Terrestrosin I is a furostan-type saponin of Type III, which produced a $[M-H]^-$ ion at *m/z* 1095.5223 at low CE in ESI$^-$. In high CE ESI$^+$, the fragment ions observed at *m/z* 917.4700, 755.4185, 593.3688, 431.3145, 317.1978, and 299.1967 were observed, attributed to loss of two glucose and two galactose residues, one formula of $C_6H_{10}O_2$ (114 Da), and one molecule of water from $[M+H-H_2O]^+$ (*m/z* 1079.5273) (Figure 4).

Terrestrinin S (pseudosapogenins of Type IV) produced ions at *m/z* 1239.5634 $[M+HCOO]^-$, 1193.5570 $[M-H]^-$, 1061.5182 [M-H-Xyl]$^-$, 899.4608 [M-H-Xyl-Glc]$^-$, 737.4138 [M-H-Xyl-2Glc]$^-$, 575.3582 [M-H-Xyl-3Glc]$^-$ in ESI$^-$. Meanwhile, in high CE ESI$^+$, Terrestrinin S produced fragment ions at *m/z* 1217.5568 $[M+Na]^+$, 1195.5745 $[M+H]^+$, 1063.5385 [1195.5745-Xyl]$^+$, 901.4753 [1063.5385-Glc]$^+$, 739.4262 [901.4753-Glc]$^+$, 577.3747 [739.4262-Glc]$^+$, 415.3233 [577.3747-Gal]$^+$, 271.2091 [415.3233-$C_8H_{16}O_2$]$^+$, and 253.1973 [271.2091-H_2O]$^+$. The ions at *m/z* 415, 271, and 253 in high CE ESI$^+$ can be considered as diagnostic ions for this type of steroidal saponin.

Type V is a type of steroidal saponins with a ketone group at C-3 position, such as Terrestrinin D. In the negative ion mode, Terrestrinin D produced a deprotonated molecular ion at *m/z* 605.3329 $[M-H]^-$, an adduct ion at *m/z* 651.3398 $[M+HCOO]^-$, and fragment ions at *m/z* 443.2798 [M-H-Glc]$^-$. In the positive ion mode, Terrestrinin D produced major fragment ions at *m/z* 629.3333, 589.3398, 427.2849, and 313.2132. The characteristic fragment ions at *m/z* 427.2849 and 313.2132 resulted from loss of one molecule of glucosyl and one formula of $C_6H_{10}O_2$ (114 Da) from the fragment ion at *m/z* 589.3398 $[M+H-H_2O]^+$, respectively.

In ESI$^-$, Terrestrinin K (Type VI) produced major fragment ions at *m/z* 1389.6179 $[M+HCOO]^-$, 1211.5686, 1049.5174, 917.4786, 755.4269, and 609.3634 which were attributed to the sequential loss of one xylose, one glucose, one xylose, one glucose, one rhamnose, and one galactose residues from the



No.	Tpye	R_1	R_2	R_3	R_4	R_5	25-C	H_5	Name
1	A	S_4	S_9	=O	H	H	*S*	α	25S-terrestrosin I
2	A	S_4	S_9	=O	H	H	*R*	α	25R-terrestrosin I
3	A	S_2	S_9	=O	H	H	*R*	α	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-xylopyranosyl-(1→3)-[β-*D*-galactopyranosyl-(1→2)]-*O*-β-*D*-glucopyranosyl-(1→4)-β-D-galactopyranoside
4	A	S_3	S_9	=O	H	H	*R*	α	Polianthoside D
5	A	S_6	S_9	=O	H	H	*R*	α	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside
6	A	S_2	S_9	H	OH	H	*R*	α	Parvispinoside A
7	A	S_3	S_9	H	OH	H	*R*	α	Purpureagitosid
8	C	S_6	S_9	=O	H	H	*R/S*	α	Terrestrinin I
9	A	S_1	S_9	=O	H	H	*R*	α	Terrestrinin O
10	D	S_2	S_9	H	H	H	*R*	α	Terrestrinin P
11	A	S_2	S_9	H	H	H	*R*	α	Parvispinoside B
12	A	=O	S_9	=O	H	H	*R*	$\Delta^{4(5)}$	Terrestrinin D
13	E	S_1	S_9	H	H	H	*R*	α	Terrestrinin K
14	A	S_1	S_9	H	H	H	*R*	α	Tribuluside A
15	C	S_1	S_9	H	H	H	*R*	α	Terrestrinin R
16	B	S_3	S_9	H	H	H	*R*	$\Delta^{5(6)}$	Terrestrinin S
17	B	S_3	S_9	H	H	H	*R*	α	Terrestrinin T
18	B	S_1	S_9	H	H	H	*R*	α	26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-20(22)-en-3β,26-diol-3-*O*-β-*D*-xylopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside
19	C	S_3	H	=O	H	H	*R*	α	Terrestrosin D
20	C	S_1	H	=O	H	H	*R*	α	(25*R*)-5α-spirostan-12-one-3β-ol-3-*O*-β-*D*-xylopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside
21	C	S_2	H	H	OH	H	*R*	α	Desglucolanatigonin II
22	C	S_3	H	H	OH	H	*R*	α	F-gitonin
23	C	S_1	H	H	H	H	*R*	α	25*R*-tribulosin
24	F	S_5	H	S_7	OH	-	-	α	Ginsenoside Rb1
25	F	OH	S_8	S_9	OH	-	-	α	Ginsenoside Re

S1= *O*-β-*D*-Gal-(1→4)-[α-*L*-Rha-(1→2)]-β-*D*-Glc-(1→2)-[β-*D*-Xyl-(1→3)]-β-*D*-Xyl, S2= *O*-β-*D*-Gal-(1→4)-β-*D*-Glc-(1→2)-[β-*D*-Xyl-(1→3)]-β-*D*-Gal, S3= *O*-β-*D*-Gal-(1→4)-β-*D*-Glc-(1→2)-[β-*D*-Xyl-(1→3)]-β-*D*-Glc , S4= *O*-β-*D*-Gal-(1→4)-β-*D*-Glc-(1→2) -β-*D*-Gal, S5= *O*-β-*D*-Glc-(1→2)-β-*D*-Glc, S6= *O*-β-*D*-Gal-(1→4)-β-*D*-Glc, S7= *O*-β-*D*-Glc-(1→6)-β-*D*-Glc, S8= *O*-β-*D*-Glc-(1→2)-β-*D*-Rha, S9= *O*-β-*D*-Glc
Glc: glucosyl; Gal: galactosyl; Rha: rhamnosyl; Xyl: xylosyl

Figure 2. The structures of 25 standard compounds

$[M-H]^-$ ion (*m/z* 1343.6116), respectively. In high CE ESI^+, Terrestrinin K produced fragment ions at *m/z* 1367.6095 $[M+Na]^+$, 449.3231 $[M+H-2Xy-2Glc-Rha-Gal]^+$, 335.2545 $[449.3231-C_6H_{10}O_2]^+$, and 299.2367 $[335.2545-2H_2O]^+$.

Therefore, it can be concluded that the precursor ion can be identified from the low CE ESI^-, and the exact mass of the saponin could determine its molecular formula. The type of aglycone in the ESI^+ can also be deduced. For example, the saponin must be a furostanol saponin with the $[M+Na]^+$ and $[M+H-H_2O]^+$ ions, and without the $[M+H]^+$ ion. If diagnostic ions were detected at *m/z* 417, 273, and 255, the saponin should be sarsasaponin with no substituent. On the base of the sarsasaponin, hydroxyl groups and double bonds with the different characteristic ions should be identified. The characteristic neutral loss of 144 ($C_8H_{16}O_2$) and 114 ($C_6H_{10}O_2$) Da could identify whether a carbonyl is located at C-12, Moreover, 142 ($C_8H_{14}O_2$) and 112 ($C_6H_8O_2$) Da can determine whether a double bond is present in the F ring. Furthermore, at high CE ESI^- and the ESI^+, the analysis of its characteristic fragment ions can reveal the number, type, and carbohydrate sequence of sugar moieties. Based on the retention times of reference compounds, it can be deduced that the number and position of hydroxyl group substituent at the aglycone will affect the retention times of saponins, and also affect by the number of carbohydrate units. On a C18 column, the retention times of steroidal saponins with the 25S configuration were shorter than those with the 25R configuration [19, 20]; the retention times of steroidal saponins in which the terminal sugar was galactose were shorter than those with glucose. The mass fragmentation patterns of reference standards were summarized and proposed, respectively. With these typical fragment ions, we utilized informatics platform for automatic identification.

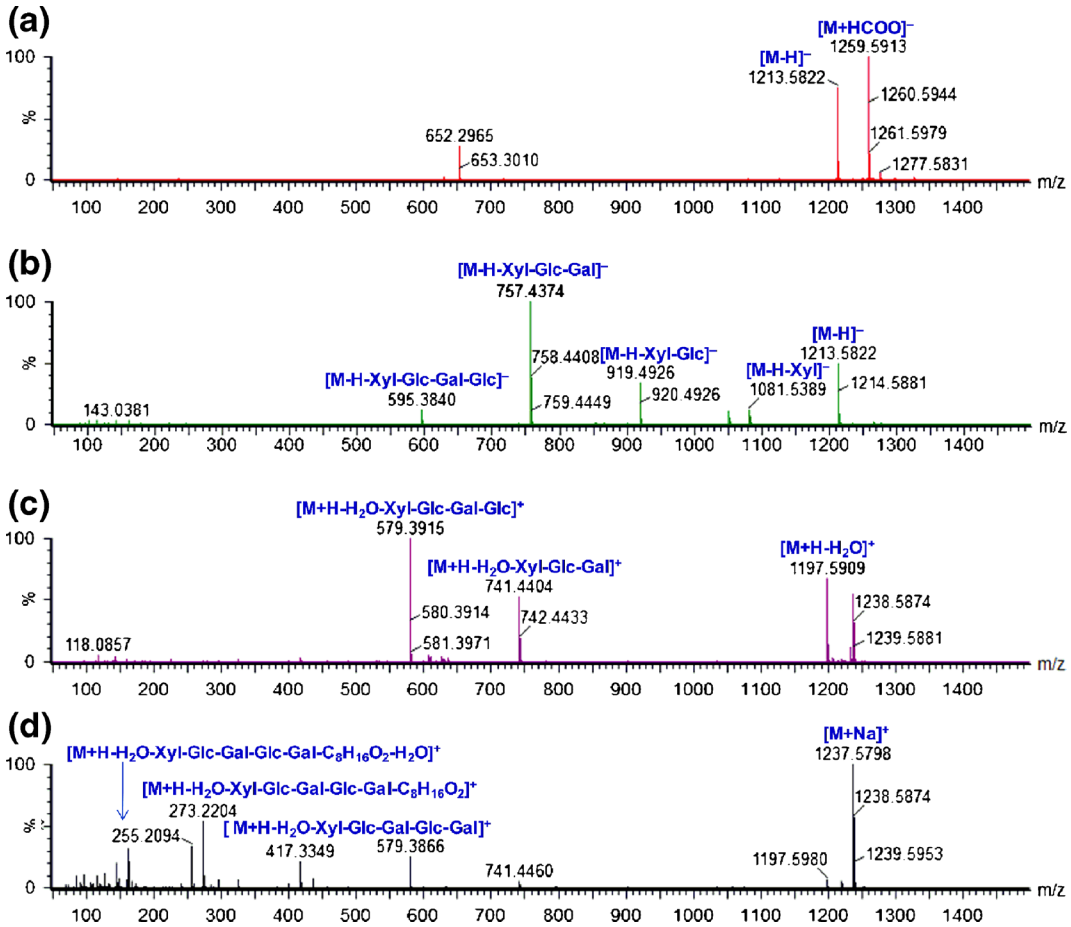


Figure 3. MS and MS^E spectra of Parvispinoside B (peak **67**). (**a**) (–) low CE ESI-MS spectrum; (**b**) (–) high CE ESI-MS spectrum; (**c**) (+) low CE ESI-MS spectrum; (**d**) (+) high CE ESI-MS spectrum

The Workflow for Rapid Characterization from TCMs by Informatics Platform

First, it was to supplement an in-house library on the TCM component database, including the information on component names and structures from *T. terrestris* based published reports. Then, after importing the acquisition data into the informatics platform, it could automatically detect and filter the data. The third step was to summarize and propose individual fragmentation patterns from reference compounds and set up characteristic fragment ions and neutral loss to screen out the components with similar MS fragment behaviors. For instance, the characteristic fragments at *m/z* 449, 431, 417, and 415 were set in the Common Fragment Settings. Meanwhile, the characteristic neutral loss at *m/z* 144, 142, 114, 112, 162, 146, and 132 were set in the Common Neutral Loss Settings. The characteristic fragments at *m/z* 431 and 417 are the main types of nuclear fragments in steroidal saponins of *T. terrestris*. The structures of E and F rings are always marked by the characteristic neutral loss at *m/z* 144, 142, 114, and 112. The sugar moieties are deduced by the characteristic neutral loss at *m/z* 162, 146, and 132. Finally, the chemical structures of the potential novel components from *T. terrestris* were identified or characterized (Figure 5). The advantage of this strategy mainly involves utilizing neutral losses and characteristic fragments to rapidly identify the structure of the compounds.

Characterization of Compounds in T. terrestris from Beijing by the Informatics Platform

After processing the data in the informatics platform, all components were further verified based on the fragment ions, retention times, and structures described in published reports. As a result, 91 compounds were tentatively identified or identified from the extract of *T. terrestris* from Beijing, including 82 saponins and nine flavonoids.

Saponins

Saponins were the major compositions identified in *T. terrestris*. This study mainly focused on saponins to propose a strategy to rapidly characterize components (Figure 6). In the results interface, a total of 76 saponins were automatically matched. Among them, peaks **33**, **35**, **61**, **62**, and **90a** were isomers with the same deprotonated molecular ion at *m/z*

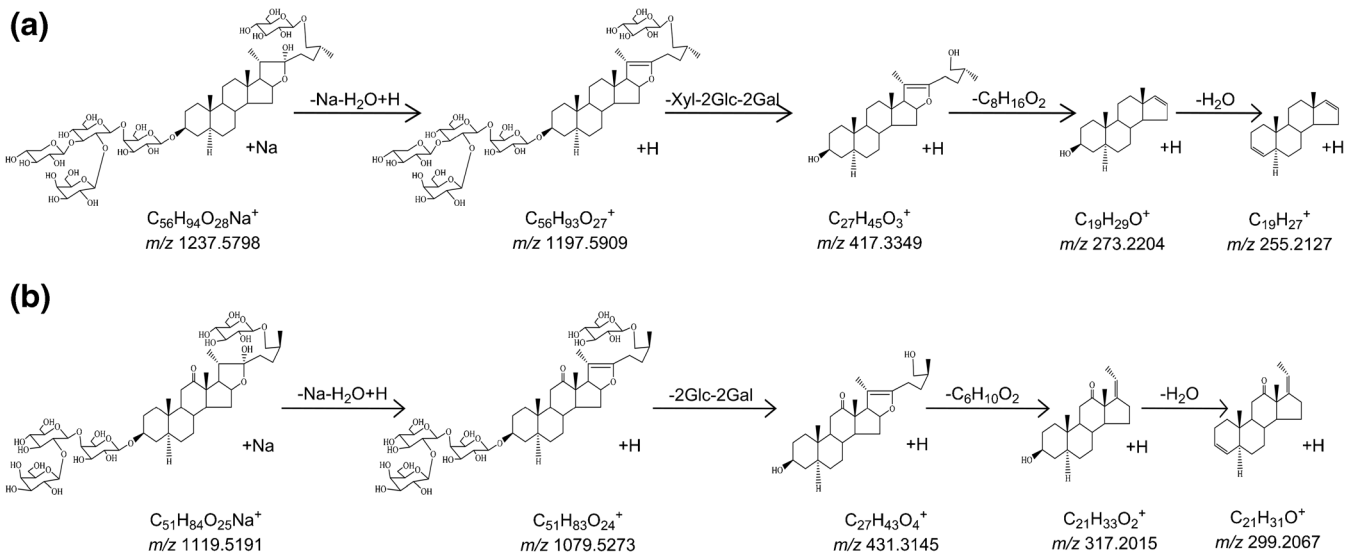


Figure 4. Proposed fragmentation pathways and characteristic ions of Parvispinoside B and 25S-terrestrosin I

1079.5265 $[M-H]^-$ at low CE in ESI^-. As an exception, in ESI^+, only peak **90a** had a protonated ion at *m/z* 1081.5421 $[M+H]^+$ rather than $[M+H-H_2O]^+$ ion at *m/z* 1063.5385. Both peaks **33** and **35** showed the same diagnostic ions at *m/z* 431, 317, and 299 in the ESI^+. Based on their similar fragmentation behaviors and the chromatography behaviors on a C18 column [23, 39, 61], **35** was tentatively identified as the 25*R* stereoisomer of **33** or its isomer. The diagnostic ions at *m/z* 415, 271, and 253 in the ESI^+ of peaks **61** and **62** indicated that their aglycone was proto-diosgenin. With the diagnostic ions at *m/z* 433, 415, and 271 of **90a** in the ESI^+, the aglycone can be deduced as gitogenin. Therefore, based on their retention times, fragment ions and comparison with known compounds from previous reports, peaks **33**, **35**, **61**, **62**, and **90a** were tentatively identified as 26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside, 26-*O*-β-*D*-glucopyranosyl-(25*R*)-5α-furostan-12-one-3β,22α,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-galactopyranoside, 25*S*-Terrestrosin J, Terrestrosin J, and 26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-20(22)-en-2α,3β,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→2)-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside, respectively.

At low CE ESI^-, peak **90** showed two pairs of ions at *m/z* 1079.5265 $[M_1-H]^-$ and 1125.5328 $[M_1+HCOO]^-$, and 1327.6199 $[M_2-H]^-$ and 1373.6176 $[M_2+HCOO]^-$, which indicated that there were two compounds in peak **90**. At high CE ESI^+, the major fragment ions were observed at *m/z* 1103.5272, 941.4779, 757.4393, 595.3884, 433.3389, 415.3250, and 271.2023; and at 1311.6239, 1149.5671, 1017.5239, 885.4874, 739.4200, 577.3796, 415.3212, 271.2057, and 253.1955. Based on their retention times, fragment ions, and comparison with known compounds in the literature, they were tentatively identified as 26-*O*-β-*D*-glucopyranosyl-(25*S*)-5α-furostan-20(22)-en-2α,3β,26-triol-3-*O*-β-*D*-glucopyranosyl-(1→2)-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside and Terrestrinin M, respectively.

A class of new compounds with characteristic fragmentation at *m/z* 431 without 317 and 299 were found in the characteristic fragments by the informatics platform (Figure 7). It could be deduced that the aglycones of these compounds were not hecogenin. The characteristic fragments of peak **41** were at

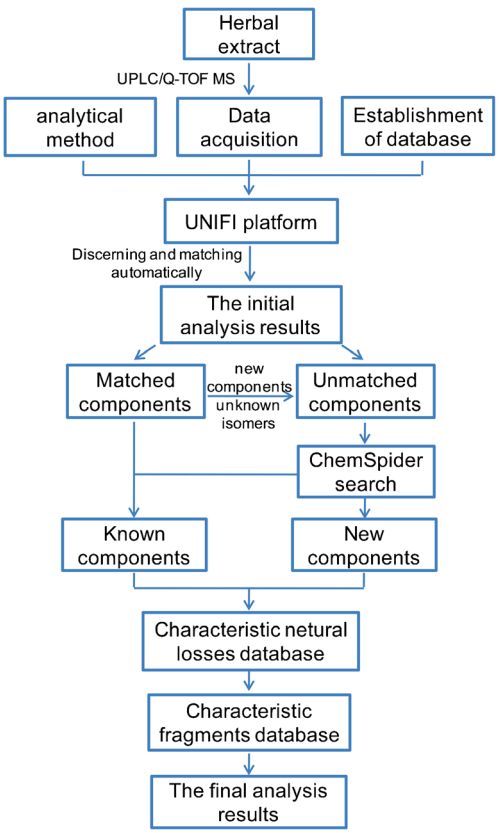


Figure 5. Workflow for rapid characterization of TCMs by the informatics platform

m/z 431.3130, 413.3029, 287.2062, and 269.1917. In contrast to the characteristic fragments of gitogenin, the aglycone of peak **41** had one more degree of unsaturation. So, peak **41** was tentatively identified as 26-*O*-β-*D*-glucopyranosyl-(25*R*)-furostan-5(6)-en-2α,3β,22α,26-tetrol-3-*O*-β-*D*-galactopyranoside.

Flavonoids

A total of nine flavonoids were automatically matched from *T. terrestris* from Beijing. Based on the literatures, the major aglycones were Quercetin, Isorhamnetin, and Kaempferol in *T. terrestris*. According to the automatic matching function of the informatics platform, in the ESI^+ at high CE, Quercetin revealed a characteristic fragment at *m/z* 303; with specific ions at *m/z* 317, it could be deduced that the aglycone was Isorhamnetin; and the characteristic fragment in 287 was caused by Kaempferol. Based on the match of sugar residues, the number and types of glycosyl groups could be deduced.

Characterization of Compounds in T. terrestris from Rome by the Informatics Platform

According to the existing method, 98 compounds were screened out in both positive and negative mode of ESI, including 84 saponins, 14 flavonoids, 35 groups of isomers, and 20 new components. There was a type of new components, which could not be matched with the characteristic fragments for aglycones. According to its characteristic fragments and related literature, these compounds were tentatively identified as a type of triterpenoid saponins, which might be explained by different therapeutic functions from different habitats. Moreover, we selected two reference samples to verify its fragmentation patterns.

In ESI^-, Ginsenoside Rb_1, a type of protopanaxadiol saponin, produced the major ions at *m/z* 1153.6084 $[M+HCOO]^-$, 945.5457, 783.4906, 621.4366, and 459.3817 caused by consecutive losses of four glucosyls from the fragment ion at *m/z* 1107.5948 $[M-H]^-$. In ESI^+, the major ions were 1131.6000 $[M+Na]^+$, and 1109.6113 $[M+H]^+$. The characteristic fragment ions at *m/z* 443.3863, 425.3778, 407.3669 were attributed to successive loss of one molecule of water.

Ginsenoside Re, a type of protopanaxatriol saponin, produced $[M+HCOO]^-$ (*m/z* 991.5457), $[M-H]^-$ (*m/z* 945.5482), $[M-H-Glc]^-$ (*m/z* 783.4901), $[M-H-Rha]^-$ (*m/z* 799.4845), $[M-H-Rha-Glc]^-$ (*m/z* 637.4354), and $[M-H-Rha-Glc-Glc]^-$ (*m/z* 475.3799) in ESI^-. In high CE ESI^+, in comparison with Ginsenoside Rb_1, Ginsenoside Re produced different characteristic ions at *m/z* 441.3727, 423.3627, and 405.3528 for the sequential loss of two molecules of water. After addition of 475 and 459 into the common fragment settings in the negative mode, as well as 441 and 425 in the positive mode, 10

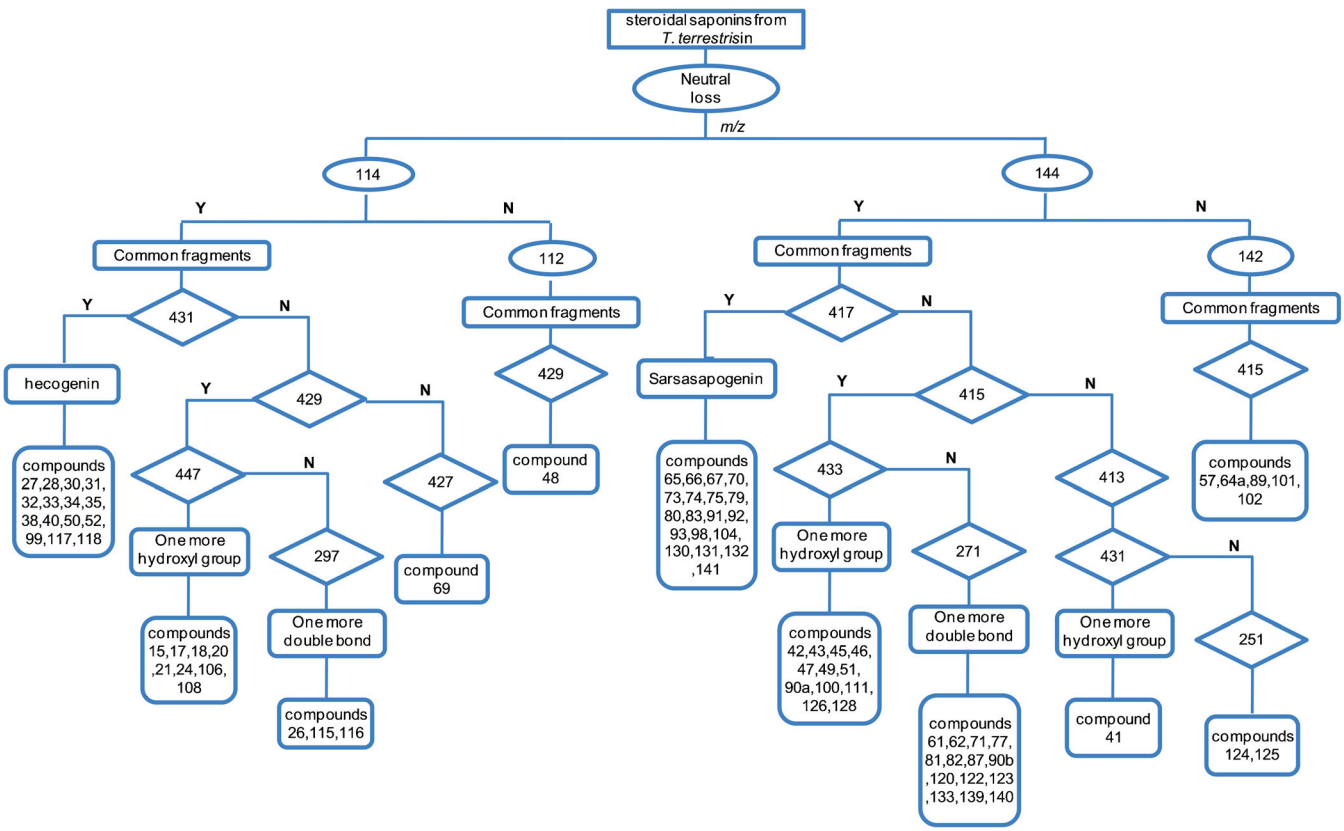


Figure 6. Strategy for rapid identification of steroidal saponins from *T. terrestris* from Beijing by the informatics platform

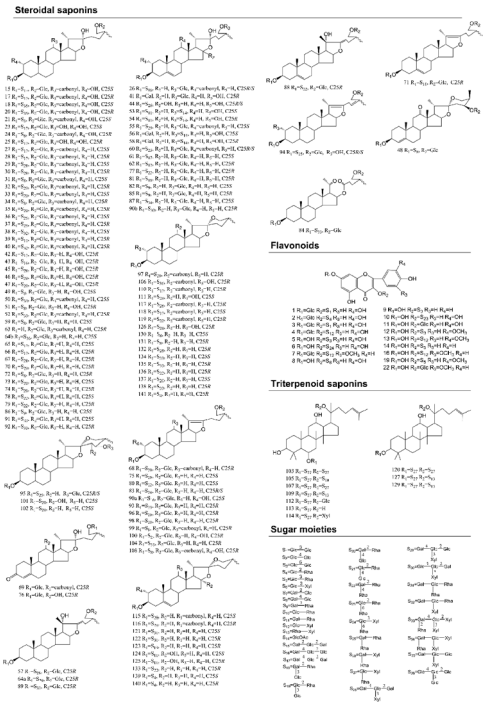


Figure 7. The identified chemical structures of compounds in *T. terrestris* by UPLC/Q-TOF MS

compounds were matched by automatic matching function of the informatics platform, including three protopanaxadiols and seven protopanaxatriols.

As a result, a total of 108 chemical components were identified from *T. Terrestris* from Rome, including 84 steroidal saponins, 14 flavonoids, and 10 triterpenoid saponins, 35 groups of isomers, and 30 new components.

Characterization of Compounds in T. terrestris from Xinjiang by the Informatics Platform

By comparing with *T. terrestris* from Beijing, the sample from Xinjiang had similar chemical composition. Based on the established detection methods, the chemical composition of *T. terrestris* from Xinjiang could be quickly identified or characterized. After artificially processing the data of the informatics platform with the confirmation, 94 compounds were identified or tentatively identified, including 87 saponins, seven flavonoids, 32 groups of isomers, and 15 new components.

Conclusions

In this study, an analytical approach for rapid characterization of chemical ingredients in TCMs was established using UHPLC/Q-TOF MS coupled with the informatics platform. Specifically, a valid strategy for identification of components of *T. terrestris* from different geographical regions was proposed based on the summarized fragmentation patterns. Based on the exact mass, fragmentation behaviors, retention times of different types of compounds, and known structures in literature, a total of 141 chemical components (Figure 7) were identified from these three samples of one species, including 35 groups of isomers, and 39 of these components were identified or tentatively identified as new compounds. The study of the total components of *T. terrestris* from these three areas revealed that although they belonged to the same species, their compositions were very different. The main components of *T. terrestris* from Beijing and Xinjiang were similar, 81 common compounds, mainly including flavonoids and steroidal saponins. In contrast, the *T. terrestris* from Rome contained some ginsenosides, which explained the different therapeutic effects of *T. terrestris* from different regions. This method could rapidly, effectively, and comprehensively screen and identify multiple components from TCMs.

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