Journal of Pharmaceutical and Biomedical Analysis 219 (2022) 114863

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis

Differentiation of three *Asparagus* species by UHPLC-MS/MS based molecular networking identification and chemical profile analysis

Xiaoxia Xue [a,b,1], Runa Jin [a,1], Qishu Jiao [a], Xiwen Li [a], Pengyue Li [a], Gang Shen [c], Shougang Shi [d], Zhengjun Huang [d], Yuntao Dai [a,b,*], Shuosheng Zhang [b,**]

[a] *Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[b] *Shanxi University of Chinese Medicine, Jinzhong 030619, China*
[c] *Chengdu Institute of Chinese Herbal Medicine, Chengdu 610000, China*
[d] *Sunflower Pharmaceutical Group (Xiangyang) Longzhong Co.Ltd, Xiangyang 441003, China*

ARTICLE INFO

Keywords:
Asparagus
Quality assessment
UHPLC-LTQ-Orbitrap-MS
Molecular networking
Fingerprint analysis
HPLC-ELSD

ABSTRACT

Asparagi Radix (AR), a traditional Chinese medicine, is the dried roots of *Asparagus cochinchinensis* (Lour.) Merr. Modern pharmacological studies have shown that AR has various excellent bioactivities, such as antioxidative, antitumor, antibacterial, anti-inflammatory, and hypoglycemic effects. However, the quality control method of AR is incomplete and there are various AR adulterants in markets due to their similar morphological characters. Here, holistic and practical quality evaluation methods were developed to chemically distinguish three common *Asparagus* species in markets, including *Asparagus cochinchinensis* (Lour.) Merr., *Asparagus officinalis* L., and *Asparagus lycopodineus* (Baker) F.T.Wang & Tang. The chemical constituents of three species were rapidly tentatively annotated using a combination of ultra-high pressure liquid chromatography-linear ion trap-orbitrap high resolution mass spectrometry (UHPLC-LTQ-Orbitrap-MS) and molecular networking (MN). Fifty-six steroidal saponins were annotated, including common and characteristic chemical constituents of the three *Asparagus* species. Besides, to establish holistic and practical methods to differentiate three *Asparagus* species, an HPLC-ELSD (evaporative light scattering detector) was applied for fingerprint analysis and content determination of the sum of protoneodioscin and protodioscin of twenty samples. Each *Asparagus* species showed characteristic chemical profile and AR showed much higher level of the sum of protoneodioscin and protodioscin than that in the others. The above analyses showed that the three *Asparagus* species mainly contain steroidal saponins and the developed HPLC-ELSD profile of saponin can be used to differentiate them. In conclusion, this study reveals the different chemical constituents of three *Asparagus* species and provides relatively feasible quality evaluation methods for them which are essential for the rational utilization of these *Asparagus* species.

1. Introduction

The genus *Asparagus* (Liliaceae) is widely distributed from temperate to tropical regions all over the world. Based on the different morphology, the genus *Asparagus* is largely divided into three subgenera, namely *Asparagus*, *Protasparagus*, and *Myrsiphyllum*. Nowadays, many *Asparagus* species are used as food, medicine, and ornamental plants within and outside their native regions [1,2]. There are 31 *Asparagus* species found in China, 12 of them have medical value (*A. cochinchinensis*, *A. officinalis*, *A. lycopodineus*, *A. filicinus*, *A. densiflorus*, *A. dauricus*, *A. oligoclonos*, *A. setaceus*, *A. schoberioides*, *A. subscandens*, *A. gobicus*, and *A. meioclados*) [1,2]. However, only the root of *A. cochinchinensis* is described in the China Pharmacopoeia (2020 edition) as the sole source of Asparagi Radix (Tiandong, or Tianmendong in Chinese, AR). It is widely distributed in China, South Korea, Japan, Philippines, Vietnam, and Laos [1]. Of these regions, Guizhou province, Sichuan province, Yunnan province of China are the main producing areas with high quality.

Asparagi Radix has been used in China for more 2000 years for indications such as dry cough, whooping cough, pain in lower back and

* Correspondence to: Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Nanxiaojie 16, Dongzhimennei Ave, 100700 Beijing, China.
** Corresponding author.
E-mail addresses: ytdai@icmm.ac.cn (Y. Dai), zhangshuosheng@aliyun.com (S. Zhang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jpba.2022.114863
Received 4 January 2022; Received in revised form 21 May 2022; Accepted 27 May 2022
Available online 30 May 2022

Table 1
The information of twenty root samples.

Sample code	Batch number	Growth location	Species	Content of the sum of protodioscin and protoneodioscin (%) (n = 3)
A1	180911	Guiyang City, Guizhou Province	*Asparagus cochinchinensis* (Lour.) Merr.	0.69
A2	180912			0.99
A3	180914			0.76
A4	180915			0.93
A5	20190701	Wanyuan City, Sichuan Province		0.47
A6	20190703			0.52
A7	20190704			0.50
A8	20190705			0.49
A9	18081301	Zunyi City, Guizhou Province		0.60
A10	19030301			0.54
B11	180906	Yulin City, Guangxi Province	*Asparagus officinalis* L.	0.18
B12	180907			0.19
B13	180908			0.20
B14	20190507–2	Zhenba County, Guangyuan City, Sichuan Province		0.12
B15	20190507–5			0.11
C16	180701	Neijiang City, Sichuan Province	*Asparagus lycopodineus* (Baker) F.T. Wang & Tang	0.14
C17	180702			0.13
C18	180703			0.12
C19	180704			0.15
C20	180705			0.11

knees, constipation caused by intestinal dryness, dry throat, and thirst [3]. Modern pharmacological studies have demonstrated that AR has various excellent bioactivities, such as antioxidative, antitumor, antibacterial, anti-inflammatory, and hypoglycemic effects [4,5]. The major chemical constituents of AR are steroidal saponins and polysaccharides, with the steroidal saponins of furostanol as the predominant active ingredients [3]. Besides, it is also a common edible plant that can be used as an ingredient to make tea, wine, porridge, snow pear soup, and sweetmeat in China [4].

Nowadays, adulterated AR is frequently found in market because the morphological characters of different *Asparagus* species are similar. According to our multiple investigations on AR collected from 2017 to 2021 from the major markets in China, we found that two major adulterants of AR were *A. officinalis* and *A. lycopodineus*. The quality control methods of AR in Chinese pharmacopoeia are incomplete without fingerprint and content determination methods, which is correlated to limited researches on AR. At present, existing chemical analyses on different *Asparagus* species were mainly focused on identification or quantification of chemical components in single species, lacking of comparative study among related species. Therefore, methods for distinction of *Asparagus* species are essential for the reasonable and effective use of *Asparagus* species.

Nowadays, LC-MS/MS is widely used for compound annotation in plant materials. Nevertheless, it is time-consuming and difficult to analyze the MS data of herb medicine due to its complex components. Notably, a combination of LC-MS/MS and molecular networking (MN) greatly promotes the annotation of natural products. MN, a method for disposing of complicated MS data, gathers the molecules with similar structures into clusters based on the similarity of their MS/MS fragments, which facilitates compounds annotation [6–8].

In this study, holistic and practical quality evaluation methods were developed to chemically distinguish three common *Asparagus* species. UHPLC-MS/MS-based MN for rapid annotation of chemical constituents of three species and HPLC-ELSD for both fingerprint analysis and quantification analysis were performed. This study provides feasible methods to differentiate and quality evaluate three common *Asparagus* species as well as chemical basis for further study of their activity for their rational use.

2. Material and methods

2.1. Chemicals, reagents, and materials

Twenty cultivated root samples after processing of different *Asparagus* species were purchased from different provinces, including the main producing areas by Sunfower Pharmaceutical Group (Xiangyang) Longzhong Co., Ltd (Hubei, China) (Table 1). The voucher specimens were deposited in institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. Depending on DNA barcoding [9] as well as their morphological characters, all root samples were authenticated as three species, including *Asparagus cochinchinensis* (Lour.) Merr., *Asparagus officinalis* L., and *Asparagus lycopodineus* (Baker) F.T.Wang & Tang. Reference compound, protodioscin (actual a mixture of protodioscin and protoneodioscin) (purity is 94.9 %) was purchased from National Institutes for Food and Drug Control (Beijing, China). Pseudoprotodioscin (purity $\geq$ 98%) was purchased from Chengdu Chroma-Biotechnology Co., Ltd (Sichuan, China). HPLC-grade acetonitrile (Fisher Scientific, United States), optima liquid chromatography-mass spectrometry (LC-MS)-grade formic acid (Fisher Scientific, Czech Republic), and pure water (Wahaha, China) were used as mobile phases.

2.2. Sample preparation

2.2.1. Sample preparation for LC-MS analysis and fingerprint analysis

The reference compounds, protodioscin and pseudoprotodioscin, were accurately weighed and dissolved in methanol at 22 and 24 μg/mL, respectively. The pieces of three *Asparagus* species were ground into fine powder, which passed through a 65 mesh screen. The accurately weighed powder (1 g) was extracted by sonicating for 40 min with 10 mL of 65 % methanol, and the liquid supernatant centrifuged at 8000 rpm for 5 min and then filtered through a 0.22 μm membrane prior to an injection into the UHPLC and HPLC systems.

2.2.2. Sample preparation for content determination

The reference standard of protodioscin was accurately weighed and dissolved in methanol at 1.80 mg/mL. The standard was then diluted to appropriate concentration ranges for the establishment of calibration curves. The accurately weighed fine powder (1 g) of the pieces of root samples was extracted twice by refluxing for 30 min with 10 mL of water, and the liquid supernatant was centrifuged at 8000 rpm for 10 min and filtered through a 0.45 μm membrane prior to injection into the HPLC system.

2.3. Mass spectrometric and chromatographic analysis conditions

2.3.1. UHPLC-LTQ-Orbitrap-MS analysis

UltiMate 3000 UHPLC system (Dionex, USA) coupled with a high-resolution LTQ-Orbitrap XL mass spectrometry (Thermo Scientific, USA) was used to qualitatively compare the main chemical compositions of different species. The mass spectrometry equipped with an electrospray ionization (ESI) and an Xcalibur 2.1 workstation. The chromatographic separation was carried out on an ACQUITY UPLC HSS T3 column (100 mm $\times$ 2.1 mm, 1.8 μm; Waters), maintained at 30 °C. The mobile phase was acetonitrile (A) and water with 0.1 % formic acid (B) run with a gradient program as follows: 0 − 6 min, 24 % A; 6 − 8 min, 24 − 27 % A; 8 − 12 min, 27 − 28 % A; 12 − 14 min, 28 − 32 % A; 14 − 20 min, 32 − 42 % A; 20 − 23 min, 42 − 80 % A; 23 − 25 min, 80 % A at a flow rate of 0.3 mL/min. The injection volume was 2 μL. The conditions of MS analysis were as follows: sheath gas flow rate, 40 (arbitrary units); auxiliary gas, 20 (arbitrary units); spray voltage, 4 kV; capillary voltage of 25 V; probe heater temperature, 350 °C; capillary temperature, 350 °C; scan mode: positive and negative; scan range, *m/z* 100–1400.

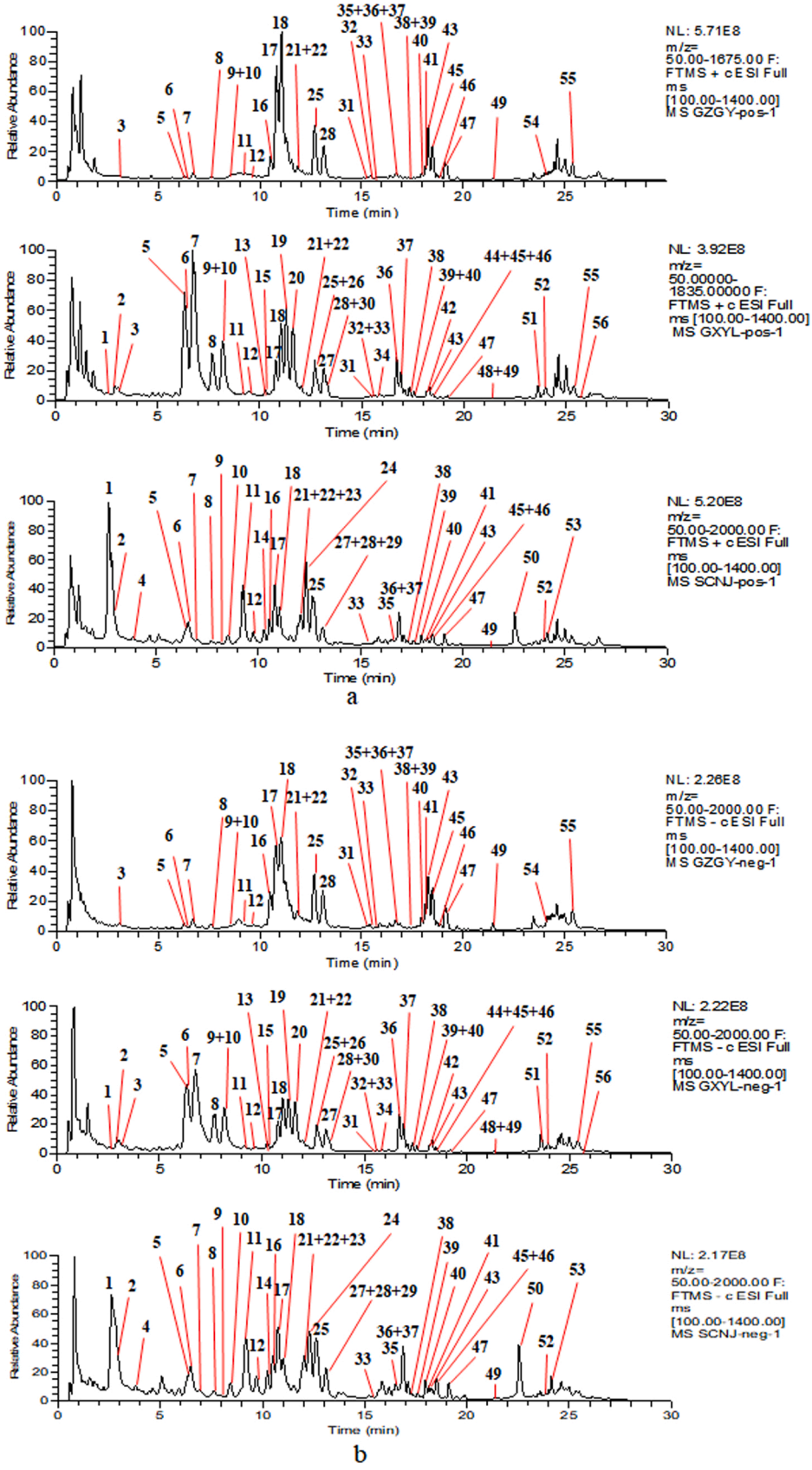

Fig. 1. The UHPLC-LTQ-Orbitrap-MS Total ion chromatograms of *A. cochinchinensis*, *A. officinalis*, and *A. lycopodineus* in positive ion mode (a) and negative ion mode (b). GZGY represents *A. cochinchinensis*, GXYL represents *A. officinalis*, and SCNJ represents *A. lycopodineus*.

Table 2
Annotated steroidal saponins in three *Asparagus* species by UHPLC- MS.

No.	Rt (min)	Formula	Adduct ion	Experiental value (*m/z*)	Theoretical value (*m/z*)	Error (ppm)	Fragment ions	Identification	Source[a]		
									A	B	C
1	2.69	$C_{56}H_{94}O_{28}$	$[M+H-H_2O]^+$	1197.5870	1197.5899	-2.37	1065.5465, 1035.5355, 903.4938, 873.4828, 741.4403, 579.3874, 417.3351	asparagoside H	–	√	√
2	2.87	$C_{51}H_{86}O_{24}$	$[M+H-H_2O]^+$	1065.5463	1065.5476	-1.27	903.4943, 741.4406, 579.3876, 417.3354	asparagoside G	–	√	√
3	3.06	$C_{45}H_{74}O_{19}$	$[M+H-H_2O]^+$	901.4787	901.4791	0.25	739.4266, 593.4447, 431.4406, 413.5232	aspacochinoside M	√	√	–
4	3.85	$C_{55}H_{92}O_{27}$	$[M+H-H_2O]^+$	1167.5776	1167.5793	-1.43	1035.5361, 1005.5256, 903.4938, 873.4831, 741.4403, 579.3876, 417.3352	3*β*,5*β*,22*α*-furostane-3,22,26-triol-3-*O*-*β*-D-xylopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)]-*β*-D-glucopyranosyl 26-*O*-*β*-D-glucopyranoside	–	–	√
5	6.30	$C_{50}H_{84}O_{23}$	$[M+H-H_2O]^+$	1035.5371	1035.5370	-0.83	903.4929, 873.4819, 741.4396, 579.3868, 417.3346	officinalisnin-II	√	√	√
6	6.37	$C_{51}H_{86}O_{23}$	$[M+H-H_2O]^+$	1049.5515	1049.5527	-1.13	903.5400, 887.3966, 741.3399, 725.2604, 887.3966, 579.4051, 417.3601	(25 *S*)− 26-*O*-*β*-D-glucopyranosyl-5*β*-furostane-3*β*,22*α*,26-triol-3-*O*-[*β*-D-glucopyranosyl-(1→4)]-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside	√	√	√
7	6.74	$C_{50}H_{84}O_{23}$	$[M+H-H_2O]^+$	1035.5372	1035.5371	-0.72	903.4929, 873.4822, 741.4399, 579.3869, 417.3347	25-*epi*-officinalisnin II	√	√	√
8	7.71	$C_{45}H_{76}O_{19}$	$[M+H-H_2O]^+$	903.4930	903.4948	-1.94	741.4194, 579.3982, 417.3866, 399.3480	3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl-(25 *S*)− 5*β*-furostane-3*β*,22*α*,26-triol	√	√	√
9	8.23	$C_{45}H_{76}O_{19}$	$[M+H-H_2O]^+$	903.4934	903.4948	-1.53	741.4232, 579.3705, 417.2766, 399.3325	3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl-(25 *R*)− 5*β*-furostane-3*β*,22*α*,26-triol	√	√	√
10	8.43	$C_{50}H_{84}O_{23}$	$[M+H-H_2O]^+$	1035.5360	1035.5371	-1.01	903.4943, 873.4839, 741.4412, 579.3881, 417.3357	aspacochinoside L	√	√	√
11	9.21	$C_{51}H_{86}O_{23}$	$[M+H-H_2O]^+$	1049.5494	1049.5527	-3.10	903.4933, 887.4984, 741.4399, 579.3872, 417.3349	asparoside B	√	√	√
12	9.73	$C_{51}H_{86}O_{23}$	$[M+H-H_2O]^+$	1049.5513	1049.5527	-1.36	903.4946, 887.4995, 741.4409, 579.3880, 417.3356	isomer of asparoside B	√	√	√
13	10.21	$C_{49}H_{82}O_{22}$	$[M+H-H_2O]^+$	1005.5266	1005.5265	0.06	873.4840, 741.4411, 579.3882, 417.3355	aspafilioside C	–	√	–
14	10.22	$C_{50}H_{82}O_{23}$	$[M+H-H_2O]^+$	1033.5198	1033.5214	-1.57	901.0113, 871.0348, 739.4549, 577.5414, 417.4962	26-*O*-*β*-D-glucopyranosyl-furostane-5-ene-3*β*,22*α*,26-triol-3-*O*-[*β*-D-glucopyranosyl-(1→2)]-[*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucopyranoside	–	–	√
15	10.28	$C_{51}H_{86}O_{22}$	$[M+H-H_2O]^+$	1033.5559	1033.5578	-1.81	871.5875, 725.3460, 579.3921, 417.4265	3-O-[*α*-L-rhamnopyranosyl-(1→4)]-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl-5*β*-furostane-3*β*,22*α*,26-triol	–	√	–
16	10.49	$C_{57}H_{94}O_{27}$	$[M+H-H_2O]^+$	1193.5935	1193.5949	-1.21	1047.5373, 1031.5414, 885.4830, 739.4252, 577.3723, 415.3198	26-*O*-*β*-D-glucopyranosyl-furostane-5-ene-3*β*,22*α*,26-triol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside	√	–	√
17#	10.81	$C_{51}H_{84}O_{22}$	$[M+H-H_2O]^+$	1031.5388	1031.5421	-3.21	885.4833, 739.4253, 577.3724, 415.3198	protoneodioscin	√	√	√
18#	11.05	$C_{51}H_{84}O_{22}$	$[M+H-H_2O]^+$	1031.5398	1031.5421	-2.27	885.4831, 739.4249, 577.3721, 415.3196	protodioscin	√	√	√
19	11.31	$C_{51}H_{86}O_{22}$	$[M+H-H_2O]^+$	1033.5541	1033.5578	-3.58	871.5264, 725.4952, 579.5279, 417.3681	isomer of 3-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl-5*β*-furostane-3*β*,22*α*,26-triol	–	√	–
20	11.61	$C_{50}H_{84}O_{22}$	$[M+H-H_2O]^+$	1019.5397	1019.5421	-2.36	887.3126, 725.3671, 579.5277, 417.3175	(25 *R*)− 26-*O*-*β*-D-glucopyranosyl-furostane-3*β*,22*α*,26-triol-3-*O*-[*β*-D-xylopyranosyl-(1→2)]-[*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside	–	√	–
21	11.80	$C_{45}H_{74}O_{18}$	$[M+H-H_2O]^+$	885.4825	885.4842	-1.90	723.4307, 577.3810, 415.3171	aspacochioside D	√	√	√
22	11.93	$C_{51}H_{86}O_{23}$	$[M+H-H_2O]^+$	1049.5499	1049.5527	-2.64	887.4977, 741.4401, 579.3873, 417.3350	sarsaparilloside B	√	√	√
23	12.09	$C_{56}H_{92}O_{27}$	$[M+H]^+$	1197.5897	1197.5898	-0.13	903.4940, 741.4407, 579.3876, 417.3353	filicinin B	–	–	√
24	12.29	$C_{56}H_{92}O_{27}$	$[M+H]^+$	1197.5891	1197.5898	-0.63	903.4935, 741.4405, 579.3873, 417.3351	isomer of filicinin B	–	–	√

(*continued on next page*)

Table 2 (*continued*)

No.	Rt (min)	Formula	Adduct ion	Experiental value (*m/z*)	Theoretical value (*m/z*)	Error (ppm)	Fragment ions	Identification	Source[a]		
									A	B	C
25	12.68	$C_{45}H_{76}O_{18}$	$[M+H-H_2O]^+$	887.4982	887.4998	-1.86	741.4411, 579.3878, 417.3355	aspacochiosidc A	√	√	√
26	12.87	$C_{44}H_{74}O_{18}$	$[M+H-H_2O]^+$	873.4826	873.4842	-1.86	741.4492, 711.4842, 579.4492, 417.4044	isomer of Asp Ⅳ'	–	√	–
27	13.06	$C_{51}H_{84}O_{23}$	$[M+H]^+$	1065.5461	1065.5476	-1.38	903.4931, 741.4401, 579.3872, 417.3350, 399.3247	(25 *R*)− 5*β*-spirostane-3*β*-ol-3-*O*-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→4)]-*β*-D-glucopyranoside	–	√	√
28	13.10	$C_{45}H_{76}O_{18}$	$[M+H-H_2O]^+$	887.4979	887.4998	-2.20	741.4401, 579.3871, 417.3350	isomer of aspacochiosidc A	√	√	√
29	13.21	$C_{57}H_{94}O_{27}$	$[M+H]^+$	1211.6029	1211.6055	-2.16	1065.5450, 1049.5503, 903.4928, 741.4396, 579.3868, 417.3347, 399.3244	$\Delta^{20(22)}$-sarsaparilloside	–	–	√
30	13.32	$C_{44}H_{74}O_{18}$	$[M+H-H_2O]^+$	873.4829	873.4842	-1.50	741.4873, 711.4904, 579.4163, 417.3171	Asp Ⅳ'	–	√	–
31	15.40	$C_{52}H_{86}O_{22}$	$[M+H-H_2O]^+$	1045.5582	1045.5578	0.42	899.5008, 753.4422, 591.4491, 429.3359	methyl protodioscin or methyl protoneodioscin	√	√	–
32	15.61	$C_{51}H_{84}O_{22}$	$[M-H]^-$	1047.5404	1047.5370	3.20	915.5119, 901.5113, 769.4298, 607.4762, 445.8357	coreajaponin B	√	√	–
33	15.68	$C_{52}H_{88}O_{22}$	$[M+H-H_2O]^+$	1047.5721	1047.5734	-1.24	885.4663, 739.4153, 593.4561, 431.4845	methyl 3-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl-(25 *S*)− 5*β*-furostane-3*β*,22*α*,26-triol or its isomer	√	√	√
34	15.86	$C_{51}H_{86}O_{22}$	$[M-H]^-$	1049.5560	1049.5527	3.17	917.6105, 903.4260, 771.5253, 609.6029, 447.5266	Asp Ⅵ	–	√	–
35	16.64	$C_{51}H_{84}O_{21}$	$[M+H-H_2O]^+$	1015.5465	1015.5472	-0.70	853.4374, 707.3708, 561.4357, 415.4202, 397.2885	26-*O*-*β*-D-glucopyranosyl-furostane-3*β*,22*α*,26-triol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-rhamopyranoside	√	–	√
36	16.66	$C_{50}H_{82}O_{22}$	$[M+H]^+$	1035.5362	1035.5370	-1.30	903.4944, 873.4832, 741.4412, 579.3877, 417.3354	asparagoside F	√	√	√
37	16.90	$C_{50}H_{82}O_{22}$	$[M+H]^+$	1035.5359	1035.5371	-1.12	903.4942, 873.4832, 741.4409, 579.3877, 417.3354	isomer of asparagoside F	√	√	√
38	17.33	$C_{45}H_{74}O_{18}$	$[M+H]^+$	903.4946	903.4948	-0.31	741.4415, 579.3883, 417.3358, 399.3245	(25 *R*)− 26-*O*-*β*-D-glucopyranosyl-5*β*-furostane-20 (22)-ene-3*β*,26-diol-3-*O*-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranoside	√	√	√
39	17.53	$C_{45}H_{74}O_{18}$	$[M+H]^+$	903.4941	903.4948	-0.72	741.4409, 579.3881, 417.3356, 399.3252	isomer of (25 *R*)− 26-*O*-*β*-D-glucopyranosyl-5*β*-furostane-20 (22)-ene-3*β*,26-diol-3-*O*-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranoside	√	√	√
40	17.98	$C_{57}H_{92}O_{26}$	$[M+H]^+$	1193.5947	1193.5949	-0.65	1031.5414, 885.4833, 739.4249, 577.3721, 415.3197	(25 *R*)− 26-*O*-*β*-D-glucopyranosyl-furostane-5,20-diene-3*β*,26-diol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside	√	√	√
41	18.12	$C_{57}H_{92}O_{26}$	$[M+H]^+$	1193.5944	1193.5949	-0.50	1031.5415, 885.4833, 739.4248, 577.3720, 415.3196	isomer of (25 *R*)− 26-*O*-*β*-D-glucopyranosyl-furostane-5,20-diene-3*β*,26-diol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside	√	–	√
42	18.16	$C_{50}H_{82}O_{21}$	$[M+H]^+$	1019.5414	1019.5421	-0.68	887.4994, 741.4412, 579.3880, 417.3356	asparanin D	–	√	–
43#	18.27	$C_{51}H_{82}O_{21}$	$[M+H]^+$	1031.5403	1031.5421	-1.80	885.4827, 739.4248, 577.3719, 415.3195, 397.309	pseudoprotodioscin	√	√	√
44	18.37	$C_{50}H_{80}O_{21}$	$[M+H]^+$	1017.5264	1017.5264	-0.11	885.4838, 739.4254, 577.3724, 415.3198	pallidifloside A	–	√	–
45	18.47	$C_{51}H_{82}O_{21}$	$[M+H]^+$	1031.5408	1031.5421	-1.32	885.4830, 739.4252, 577.3721, 415.3197, 397.3094	pseudoprotoneodioscin	√	√	√
46	18.86	$C_{45}H_{72}O_{17}$	$[M+H]^+$	885.4828	885.4842	-1.62	723.5893, 577.3438, 415.3809	3-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl]− 26-*O*-*β*-D-glucopyranosyl-(25 *R*)-furostane-5,20-diene-3*β*,26-diol	√	√	√
47	19.11	$C_{45}H_{74}O_{17}$	$[M+H]^+$	887.4976	887.4999	-2.54	741.4403, 725.4456, 579.3873, 417.3351	aspacochioside C	√	√	√
48	21.34	$C_{39}H_{62}O_{14}$	$[M+H]^+$	755.4205	755.4212	-1.01	593.3673, 431.3147, 413.3044	(25 *R*)− 5*β*-spirostane-12-keto-3*β*-ol-3-*O*-*β*-D-glucopyranosyl-(1→4)]-*β*-D-glucopyranoside	–	√	–
49	21.44	$C_{45}H_{72}O_{17}$	$[M+H-H_2O]^+$	867.4730	867.4736	-0.81	721.4145, 575.3564, 413.3041, 395.2937	yamogenin II	√	√	√

(*continued on next page*)

Table 2 (*continued*)

No.	Rt (min)	Formula	Adduct ion	Experiental value (*m/z*)	Theoretical value (*m/z*)	Error (ppm)	Fragment ions	Identification	Source[a]		
									A	B	C
50	22.53	$C_{50}H_{82}O_{22}$	$[M-H]^-$	1033.5241	1033.5214	2.68	901.5288, 871.5758, 739.4620, 577.5083, 415.4554	filicinin A	–	–	√
51	23.63	$C_{44}H_{72}O_{17}$	$[M+H]^+$	873.4835	873.4842	-0.87	711.4304, 579.3878, 417.3353	(25 *S*)− 5*β*-spirostane-3*β*-ol-3-*O*-*α*-L-arabinopyranosy-(1→4)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranoside	–	√	–
52	23.95	$C_{39}H_{64}O_{13}$	$[M+H]^+$	741.4418	741.4419	-0.18	579.3881, 417.3357	(25 *S*)− 5*β*-spirostane-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside	–	√	√
53	24.13	$C_{45}H_{74}O_{17}$	$[M+H]^+$	887.4986	887.4999	-1.44	741.4407, 725.4457, 579.3874, 417.3349, 399.3248	shatavarin-IV	–	–	√
54	24.14	$C_{45}H_{72}O_{16}$	$[M+H]^+$	869.4882	869.4893	-1.26	723.4302, 577.3721, 415.3196	dioscin	√	–	–
55	25.38	$C_{39}H_{64}O_{12}$	$[M+H]^+$	725.4457	725.4471	-1.89	579.4138, 417.2867	(25 *S*)− 5*β*-spirostane-3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside	√	√	–
56	25.43	$C_{38}H_{62}O_{12}$	$[M+H]^+$	711.4296	711.4314	-2.49	579.3873, 417.3351	(25 *S*)− 5*β*-spirostane-3-*O*-*α*-L-arabinopyranosy-(1→4)-*β*-D-glucopyranoside	–	√	–

#Reference compounds.

[a] A: A. cochinchinensis, B: A. officinalis, C: A. lycopodineus.

2.3.2. HPLC-ELSD conditions for fingerprint analysis

HPLC-ELSD analyses were performed using a 1200 Series HPLC (Agilent)-ELSD system. The chromatographic separation was carried out on a Waters XSelect Hss T3 column (250 mm × 4.6 mm, 5 μm), maintained at 30 °C. The mobile phase was acetonitrile (A) and water (B) run with a gradient program as follows: 0 − 15 min, 24% A; 15 − 20 min, 24 − 27 % A; 20 − 30 min, 27 − 28 % A; 30 − 35 min, 28 − 32 % A; 35 − 50 min, 32 − 42 % A; 50 − 55 min, 42 − 90 % A at a flow rate of 0.8 mL/min. The injection volume was 10 μL. ELSD was performed with nitrogen as the carrier gas at a flow rate of 2.4 L/min, and the tube temperature was set to 104 °C [10].

2.3.3. HPLC-ELSD conditions for content determination of the sum of protodioscin and protoneodioscin

HPLC-ELSD analyses were performed using a 1200 Series HPLC (Agilent)-ELSD system (Alltech 2000 ES). The chromatographic separation was carried out on a Waters XSelect Hss T3 column (250 mm × 4.6 mm, 5 μm), maintained at 30 °C. The mobile phase was acetonitrile (A) and water (B) run with a gradient program as follows: 0 − 20 min, 24 % A; 20 − 25 min, 24–27 % A; 25 − 33 min, 27–28 % A; 33 − 36 min, 28 − 90 % A; 36 − 41 min, 90 − 24 % A at a flow rate of 0.8 mL/min. The injection volume was 10 μL. ELSD was performed with nitrogen as the carrier gas at a flow rate of 2.8 L/min, and the tube temperature was set to 104 °C [10].

2.4. Data analysis

MS/MS spectra were generated using Full MS/MS^2 mode. MS/MS spectra were converted to the mzXML format using the ProteoWizard 3.0.20014. Then, the mzXML file was uploaded by the WinSCP software (https://winscp.net/eng/download.php) to the Global Natural Products Social Molecular Networking (GNPS) platform (https://gnps.ucsd.edu). The following settings were used for generating the network: minimum pairs cos 0.7; parent mass tolerance, 2 Da; MS/MS fragment ion tolerance, 0.5 Da; network top, 10; minimum matched peaks, 6. The molecular networking jobs on GNPS, the platform is on-limits, can be accessed at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=ec9dd1e32bb4422880670187ec778a18 (in the positive mode) and https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task= 3ba7b971a9e34177b41c70f43bbb89c0 (in the negative mode). Visualization of the output from the molecular networking was performed in Cytoscape 3.7.2.

The software "Similarity Evaluation System for Chromatographic Fingerprint of TCM" published by GPC (Version 2012) was employed to generate a reference chromatogram as a representative fingerprint chromatogram for each *Asparagus* species. Main peaks existing in all chromatograms of the samples were assigned as "common peaks". The relative retention time (RRT) and relative peak area (RPA) of each common peak in the reference chromatogram related to a reference peak were calculated, to semi-quantitatively compare the different chemical composition of these samples [11].

3. Results and discussions

3.1. Characterization of constituents in three Asparagus species using MS/MS-based MN

The LC-MS analyses were performed to characterize the overall chemical constituents of three *Asparagus* species. Total ion chromatograms (TIC) of three *Asparagus* species in the positive and negative ions mode with numbered peaks and detailed mass information are displayed in Fig. 1 and Table 2, respectively. The TIC showed that there were great differences in chemical profile of three species, with strong intensity peaks from 10.5 min to 11.5 min in samples of *A. cochinchinensis*, from 6 min to 7 min in *A. officinalis*, and from 2 min to 3 min in *A. lycopodineus*, respectively, both in the negative and positive ions mode.

As shown in Fig. 2a, molecular network (MN) was applied to depict the structural relationship of compounds in the three *Asparagus* species. Compounds of similar structure can be joined together and gathered into clusters based on the similarity of the MS/MS fragments [6–8]. In Fig. 2a, the cluster includes a known saponin, protodioscin, with a precursor ion $[M+H-H_2O]^+$ at *m/z* 1031.5398, and other compounds with similar structures in the cluster can therefore be annotated rapidly. The pie charts for nodes filled with three different colors stand for different species. The proportion of color in each node represents the intensity of the ion peak of each compound in different *Asparagus* species [12]. The result showed that the distribution of compounds in the three *Asparagus* species was greatly different, as shown by the different color compositions of nodes in Fig. 2a.

Researches in recent years have shown that the major chemical constituents of AR are steroidal saponins with different kinds of glycosyls, commonly including Glc (glucose), Rha (rhamnose), Xyl (xylose), and Ara (arabinose). Protodioscin, as one of the main active compounds in *A. cochinchinensis* [3,4,13,14], was selected to investigate the fragmentation pathway of steroidal saponins and deduce the structures of other saponins in the cluster (Fig. 2a, Fig. 2b). In the positive ion mode at 11.05 min, protodioscin ($C_{51}H_{84}O_{22}$) displayed a precursor ion $[M+H-H_2O]^+$ at *m/z* 1031.5398 and it produced main fragment ions at *m/z* 885.4831 $[M+H-H_2O\text{-Rha}]^+$, *m/z* 739.4249 $[M+H-H_2O\text{-}2Rha]^+$,

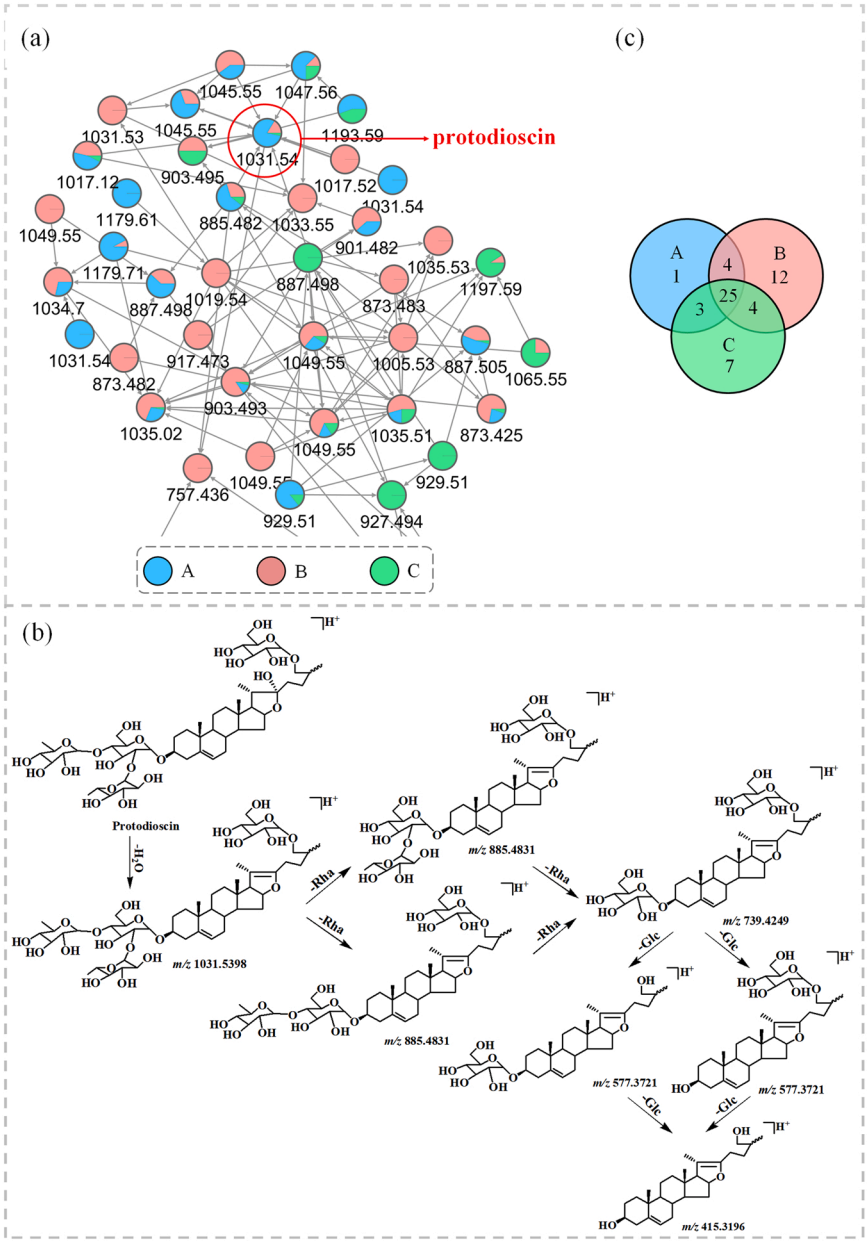

Fig. 2. The molecular network of detected steroidal saponins of three *Asparagus* species (a). A: *A. cochinchinensis*, B: *A. officinalis*, C: *A. lycopodineus*. The proposed fragmentation pathways of protodioscin (b). A diagram showing the amounts of annotated compounds of unique and common to the three *Asparagus* species (c). The pie charts for nodes filled with different colors, blue (*A. cochinchinensis*), red (*A. officinalis*) and green (*A. lycopodineus*), the proportion of color in each node represents the intensity of the ion peak of each compound in different *Asparagus* species. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

m/z 577.3721 $[M+H-H_2O-2Rha-Glc]^+$, and *m/z* 415.3196 $[M+H-H_2O-2Rha-2Glc]^+$ (Fig. 2b, Table 2). Among them, the fragment ion at *m/z* 415.3196 corresponds to the dehydroxyl aglycone of protodioscin [15]. Peak 17 at 10.81 min displayed a precursor ion $[M+H-H_2O]^+$ at *m/z* 1031.5388 ($C_{51}H_{84}O_{22}$), which indicated that it is an isomer of protodioscin. Considering the same fragmentation pathways as protodioscin, as well as different retention time, peak 17 was tentatively annotated to be protoneodioscin by comparing with the previous literature (Table 2) [13]. Peak 31 at 15.40 min ($C_{52}H_{86}O_{22}$) displayed a precursor ion $[M+H-H_2O]^+$ at *m/z* 1045.5582, indicating that there might be one more methyl group than protodioscin or protoneodioscin in its structure. This precursor gave rise to product ions at *m/z* 899.5008 $[M+H-H_2O-Rha]^+$, *m/z* 753.4422 $[M+H-H_2O-2Rha]^+$, *m/z* 591.4491 $[M+H-H_2O-2Rha-Glc]^+$, and *m/z* 429.3359 $[M+H-H_2O-2Rha-2Glc]^+$. Of these fragment ions, *m/z* 429.3359 corresponds to the dehydroxyl aglycone of the compound, which indicated that the additional methyl group should be connected to the aglycone of protodioscin or protoneodioscin. Finally, peak 31 was tentatively annotated to be methyl protodioscin or methyl protoneodioscin by comparing to the previous literature [15]. Peaks 15 and 19 (10.28 and 11.31 min, respectively) are isomers, which have identical precursor ion $[M+H-H_2O]^+$ at *m/z* 1033.5578 and similar fragmentation pathways as protodioscin. Their precursors produced fragment ions at *m/z* 871.5875 $[M+H-H_2O-Glc]^+$, *m/z* 725.3460 $[M+H-H_2O-Glc-Rha]^+$, *m/z* 579.3921 $[M+H-H_2O-Glc-2Rha]^+$, and *m/z* 417.4265 $[M+H-H_2O-2Glc-2Rha]^+$, with *m/z* 417.4265 as the dehydroxyl aglycone of the two compounds. The formula $C_{51}H_{86}O_{22}$ of peaks 15 and 19 indicated one less double bond than protodioscin or protoneodioscin in the structure. Considering different retention time, peaks 15 and 19 were tentatively annotated to be 3-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl-5*β*-furostane-3*β*,22*α*, 26-triol and its isomer by fragment ions comparisons [14]. Peak 33 at 15.68 min displayed a precursor ion $[M+H-H_2O]^+$ of *m/z* 1047.5721 ($C_{52}H_{88}O_{22}$), which indicated that there might be one more methyl group than peaks 15 or 19 in its structure. This precursor produced fragment ions at *m/z* 885.4663 $[M+H-H_2O-Glc]^+$, *m/z* 739.4153 $[M+H-H_2O-Glc-Rha]^+$, *m/z* 593.4561 $[M+H-H_2O-Glc-2Rha]^+$, and *m/z* 431.4845 $[M+H-H_2O-2Glc-2Rha]^+$. Among them, the fragment ion of

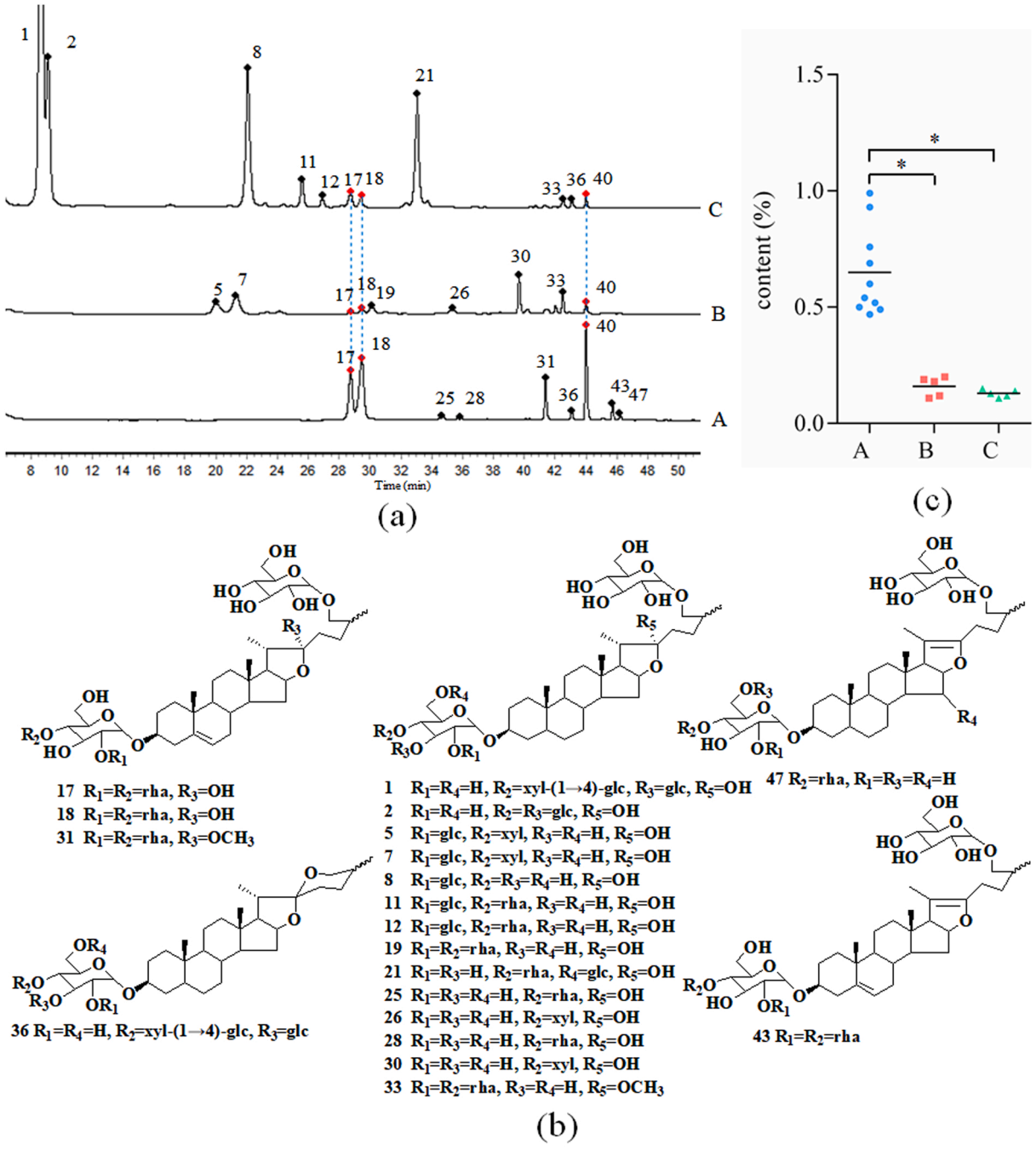

Fig. 3. The representative fingerprint chromatograms of three *Asparagus* species (a). A: *A. cochinchinensis*, B: *A. officinalis*, C: *A. lycopodineus*. Chemical structures of all the annotated steroidal saponins from three *Asparagus* species in the representative fingerprint chromatograms (b). The content of the sum of protodioscin and protoneodioscin (c) in samples of 3 *Asparagus* species from 6 different growth locations. The colored dots represent the content of the index components in 20 samples, and black lines represent the mean content of index components in 3 *Asparagus* species, respectively. *P < 0.05, compared with group A. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

m/z 431.4845 corresponds to the dehydroxyl aglycone of the compound, indicating that the compound has one more methyl on the aglycone than peaks 15 or 19. Finally, peak 33 was tentatively annotated to be methyl 3-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl-(25 *S*)− 5*β*-furostane-3*β*, 22*α*,26-triol or its isomer.

Based on the clusters in the MN, a total of 56 compounds were rapidly annotated from the three *Asparagus* species, which were all steroidal saponins, including 13 pairs of isomers (Table 2) [3,14,15]. Notably, MN is only an auxiliary tool for rapidly annotating compounds, the finally annotation of those compounds were completed by comparing the MS/MS spectrum and fragmentation pathways with those of reference standards or previous literatures. Comparing all the identified compounds in the three *Asparagus* species, 33 compounds were annotated in *A. cochinchinensis* (group A), with one characteristic compound (peak 54) and sharing 29 and 28 compounds with *A. officinalis* (group B) and *A. lycopodineus* (group C), respectively, as shown in Fig. 2c. Forty-five compounds were annotated in *A. officinalis* (group B), with 12 characteristic compounds (peaks 13, 15, 19, 20, 26, 30, 34, 42, 44, 48, 51, and 56) and sharing 29 compounds with

A. lycopodineus (group C). Thirty-nine compounds were annotated from *A. lycopodineus* (group C), with 7 characteristic compounds (peaks 4, 14, 23, 24, 29, 50, and 53). In all, 25 compounds are common to the three *Asparagus* species and each species have their own characteristic compounds (Fig. 2c).

3.2. Chemical profile analysis of three Asparagus species

The HPLC fingerprints of 10 root samples of *A. cochinchinensis*, 5 root samples of *A. officinalis*, and 5 root samples of *A. lycopodineus*, were analyzed with the established fingerprint analysis methods [10]. The obtained overlayed fingerprints of three *Asparagus* species are showed in Fig. S1, respectively. Three mean chromatograms as representative fingerprint chromatograms of three *Asparagus* species were generated with similarity evaluation software and results showed that the three representative fingerprint chromatograms had different common peaks (Fig. 3a). Then the common peaks (Fig. S1, Fig. 3a) were annotated with UHPLC-MS/MS by comparisons with reference standards and previous literatures [3,14,15]. A total of 9 common peaks were tentatively annotated from the representative fingerprint chromatograms of *A. cochinchinensis*, 9 peaks from *A. officinalis*, and 11 peaks from *A. lycopodineus* (Fig. 3a, Fig. 3b, Table 2), which were all steroidal saponins detected in Fig. 1. There are three common compounds among them including protoneodioscin (peak 17), protodioscin (peak 18), and (25 *R*)− 26-*O*-*β*-D-glucopyranosyl-furostane-5,20-diene-3*β*,26-diol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (peak 40). The RRT and RPA of three common peaks were calculated with peak 18 as reference in each chromatogram (Table S1). In addition, asparagoside F (peak 36) was detected in both *A. cochinchinensis* and *A. lycopodineus*, and methyl 3-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl-(25 *S*)− 5*β*-furostane-3*β*,22*α*,26-triol or its isomer (peak 33) was detected in both *A. officinalis* and *A. lycopodineus*. The other peaks in the representative fingerprint chromatograms of three *Asparagus* species are characteristic to each species, which indicates that the three *Asparagus* species have very different chemical profiles of saponins. Compared with the number of characteristic compounds in TIC identified by LC-MS in each species, there are more characteristic peaks in the representative fingerprint chromatograms, indicating that some compounds are common to different *Asparagus* species, but there might be big difference in their content among them. In all, each species has unique chemical fingerprint profile with characteristic peaks, and the fingerprint method established can effectively distinguish the three *Asparagus* species.

3.3. Content determination of common active compounds in three Asparagus species

The common compounds of three *Asparagus* species included protoneodioscin, protodioscin and (25 *R*)− 26-*O*-*β*-D-glucopyranosyl-furostane-5,20-diene-3*β*,26-diol-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside. Among them, protodioscin has hypoglycemic effect in vivo [16] and it is also one of the pharmacodynamic substances of AR [3]. The standard reference of protodioscin provided by National Institutes for Food and Drug Control (Beijing, China) is a mixture of protodioscin and protoneodioscin, which is attributed to that they are isomers with difference in the configuration of C25, protodioscin with *R* configuration and protoneodioscin with *S* configuration [13]. It therefore that protodioscin and protoneodioscin are difficult to be separated by reverse phase chromatography column. Protodioscin and protoneodioscin have no response under ultraviolet detector and HPLC-ELSD is commonly used as in Chinese pharmacopoeia for quantitative analysis of compounds without UV absorption. Based on the above consideration, the contents of the sum of protoneodioscin and protodioscin in the three species were determined using the HPLC-ELSD methods established in our group [10]. The data of method validation for content determination of the sum of protodioscin and protoneodioscin was summarized in Table S2. It was found that the mean content of the sum of protoneodioscin and protodioscin was the highest in *A. cochinchinensis*, which was about 5 times higher than that in *A. officinalis* and *A. lycopodineus* (Table 1, Fig. 3c). The results indicated that although the protoneodioscin and protodioscin are common compounds in the three species, their levels are greatly different among them. It implied that the three species might have very different activities and the healthy function of *A. cochinchinensis* might be reduced if adulterated AR were mixed to it.

In the study, MS/MS-based molecular network was used for rapid identification of chemical constituents from three species, and the result showed that there are common and unique constituents among three species. However, the disadvantage of MS/MS-based molecular network is that it is cost and cannot be widely applied to conventional plant species identification. Subsequently, this problem was solved with the fingerprint analysis and content determination. Each species has unique chemical fingerprint profile and the fingerprint method established can effectively distinguish three *Asparagus* species.

4. Conclusions

In conclusion, relatively basic and practical quality evaluation methods were developed to chemically distinguish three common *Asparagus* species, including *Asparagus cochinchinensis* (Lour.) Merr., *Asparagus officinalis* L., and *Asparagus lycopodineus* (Baker) F.T.Wang & Tang. A total of fifty-six steroidal saponins, including 13 pairs of isomers, were rapidly annotated from the three *Asparagus* species based on a combination of UHPLC-LTQ-Orbitrap-MS and MN. There are common constituents to the three *Asparagus* species and each species also have their own characteristic steroidal saponins, which provides chemical basis for further study of their healthy effect. Besides, the fingerprint analysis and content determination of the sum of protodioscin and protoneodioscin by HPLC-ELSD showed that each species has characteristic fingerprint of saponins and different level of the sum of protoneodioscin and protodioscin, which provides feasible approaches to distinguish different *Asparagus* species. In all, this study reveals the different chemical constituents of three *Asparagus* species and provides holistic and practical quality evaluation methods which is vital important for the rational utilization of these *Asparagus* species.

CRediT authorship contribution statement

Yuntao Dai: Conceptualization, Resources, Supervision, Writing – review & editing. **Shuosheng Zhang:** Conceptualization. **Xiaoxia Xue:** Writing – original draft preparation, Data curation, Software, Visualization. **Runa Jin:** Investigation, Data curation. **Qishu Jiao:** Software. **Xiwen Li:** Project administration. **Pengyue Li:** Investigation. **Gang Shen:** Investigation. **Shougang Shi:** Project administration. **Zhengjun Huang**: Project administration.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors thank the Sunfower Pharmaceutical Group (Xiangyang) Longzhong Co. Ltd., for purchasing root samples of different *Asparagus* species from different provinces.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at doi:10.1016/j.jpba.2022.114863.

References

[1] S. Kubota, I. Konno, A. Kanno, Molecular phylogeny of the genus Asparagus (Asparagaceae) explains interspecific crossability between the garden asparagus (A. officinalis) and other Asparagus species, Theor. Appl. Genet. 124 (2012) 345–354.

[2] N. Štajner, B. Bohanec, B. Javornik, Genetic variability of economically important Asparagus species as revealed by genome size analysis and rDNA ITS polymorphisms, Plant Sci. 162 (2002) 931–937.

[3] Y. Jaiswal, Z. Liang, A. Ho, H. Chen, Z. Zhao, A comparative tissue-specific metabolite analysis and determination of protodioscin content in Asparagus species used in traditional Chinese medicine and Ayurveda by use of laser microdissection, UHPLC-QTOF/MS and LC-MS/MS, Phytochem. Anal. 25 (2014) 514–528.

[4] F. Zhang, Y. Lu, W. Qian, Z. Pei, *Asparagus cochinchinensis* (Lour.) Merr. 天冬 (Tiandong, Chinese Asparagus), in: Y. Liu, Z. Wang, J. Zhang (Eds.), Dietary Chinese Herbs, Springer, Heidelberg, 2015, pp. 83–88.

[5] J.E. Sung, H.A. Lee, J.E. Kim, J. Go, E.J. Seo, W.B. Yun, D.S. Kim, H.J. Son, C. Y. Lee, H.S. Lee, D.Y. Hwang, Therapeutic effect of ethyl acetate extract from Asparagus cochinchinensis on phthalic anhydride-induced skin inflammation, Lab. Anim. Res. 32 (2016) 34–45.

[6] C. Hu, Y. Tang, H.D. Snooks, S. Sang, Novel steroidal saponins in oat identified by molecular networking analysis and their levels in commercial oat products, J. Agric. Food Chem. 69 (2021) 7084–7092.

[7] Z. Wang, J. Li, A. Chambers, J. Crane, Y. Wang, Rapid structure-based annotation and profiling of dihydrochalcones in star fruit (averrhoa carambola) using UHPLC/Q-orbitrap-MS and molecular networking, J. Agric. Food Chem. 69 (2021) 555–567.

[8] H.Y. Wu, J.P. Ke, W. Wang, Y.S. Kong, P. Zhang, T.J. Ling, G.H. Bao, Discovery of neolignan glycosides with acetylcolinesterase inhibitory activity from huangjinya green tea guided by ultra performance liquid chromatography-tandem mass spectrometry data and global natural product social molecular networking, J. Agric. Food Chem. 67 (2019) 11986–11993.

[9] X. Jiang, G. Zeng, L. Ou, C. She, D. Xu, Application of ITS sequences in DNA barcoding of Asparagus L, J. Cent. China Norm. Univ. Nat. Sci. Ed. 45 (2011) 472–476, https://doi.org/10.19603/j.cnki.1000-1190.2011.03.026.

[10] R. Jin, L. Hao, T. Wang, H. Wu, Y. Dai, S. Shi, Z. Huang, Analysis on quality evaluation methods of Asparagi Radix decoction pieces and its standard decoction, Chin, J. Exp. Tradit. Med. Form. 26 (2020) 111–118, https://doi.org/10.13422/j.cnki.syfjx.20201064.

[11] Y. Dai, D. Wang, M. Zhao, L. Yan, C. Zhu, P. Li, X. Qin, R. Verpoorte, S. Chen, Quality markers for astragali radix and its products based on process analysis, Front. Pharmacol. 11 (2020), 554777.

[12] J. Lee, R.R. da Silva, H.S. Jang, H.W. Kim, Y.S. Kwon, J.H. Kim, H. Yang, In silico annotation of discriminative markers of three Zanthoxylum species using molecular network derived annotation propagation, Food Chem. 295 (2019) 368–376.

[13] K. Hu, A. Dong, X. Yao, H. Kobayashi, S. Iwasaki, Antineoplastic agents. II. Four furostanol glycosides from rhizomes of Dioscorea collettii var, hypoglauca, Planta Med 63 (1997) 161–165.

[14] Y. Ling, X. He, R. Jiang, Q. Zhang, S. Yuan, Y. Liang, C. Li, Y. Zhao, Q. Zhang, K. Liu, Rapid detection and characterization of steroidal saponins in the root of asparagus cochinchinensis by high-performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry, J. Chromatogr. Sci. 58 (2020) 454–463.

[15] H. Qi, F. Feng, J. Zhai, F. Chen, T. Liu, F. Zhang, F. Zhang, Development of an analytical method for twelve dioscorea saponins using liquid chromatography coupled to Q-Exactive high resolution mass spectrometry, Talanta 191 (2019) 11–20.

[16] C. Guo, Y. Dong, H. Zhu, Y. Liu, G. Xie, Ameliorative effects of protodioscin on experimental diabetic nephropathy, Phytomedicine 51 (2018) 77–83.