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Non-Targeted Metabolomic Analysis of Methanolic Extracts of Wild-Simulated and Field-Grown American Ginseng

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Abstract: Aiming at revealing the structural diversity of secondary metabolites and the different patterns in wild-simulated American ginseng (WsAG) and field-grown American ginseng (FgAG), a comprehensive and unique phytochemical profile study was carried out. In the screening analysis, a total of 121 shared compounds were characterized in FgAG and WsAG, respectively. The results showed that both of these two kinds of American ginseng were rich in natural components, and were similar in terms of the kinds of compound they contained. Furthermore, in non-targeted metabolomic analysis, when taking the contents of the constituents into account, it was found that there indeed existed quite a difference between FgAG and WsAG, and 22 robust known biomarkers enabling the differentiation were discovered. For WsAG, there were 12 potential biomarkers including two ocotillol-type saponins, two steroids, six damarane-type saponins, one oleanane-type saponins and one other compound. On the other hand, for FgAG, there were 10 potential biomarkers including two organic acids, six damarane-type saponins, one oleanane-type saponin, and one ursane. In a word, this study illustrated the similarities and differences between FgAG and WsAG, and provides a basis for explaining the effect of different growth environments on secondary metabolites.

Keywords: wild-simulated American ginseng; field-grown American ginseng; screening analysis; metabolomic analysis

1. Introduction

American ginseng (*Panax quinquefolius* L.) is grouped into four categories: wild, wild-simulated, woods-grown, and field-grown [1,2]. The herb growing in its native habitat is called wild American ginseng. Wild-simulated American ginseng (WsAG) refers to a method of growing ginseng in a hardwood forest environment under natural conditions without any other human intervention [2–4]. As such, WsAG roots are indeed indistinguishable from the wild roots due to the similar characteristics. When the seeds are planted in hardwood forests, and are grown in prepared rows or beds, or with removed ground vegetation or fertilizer and pesticides being available [5–7], it is called as wood-grown ginseng. This variety of American ginseng requires 6 to 9 years to mature before harvesting [8]. Different from wild-simulated category, the quality of wood-grown ginseng is between that of the wild and field-grown categories. That means, wood-grown American ginseng cannot be considered a substitute of the wild one. Field-grown American ginseng (FgAG), also called cultivated American ginseng, is intensely cultivated under artificial shade structures using fertilizers and pesticides [4,9].

Generally speaking, FgAG is harvested after 3–4 years, while WsAG is collected at least after 10–20 years or longer [10].

Modern pharmacological studies have shown that American ginseng has immunomodulatory [11], anti-tumor [12], anti-fatigue [13,14], anti-diabetic [15–17], anti-oxidant effects [18] and the functions of improving impaired memory and learning functions [14,19], etc. Furthermore, it is the traditional belief that roots from the wild are more medicinally efficacious, more potent and more valuable than those from cultivated sources, and wild roots thus command much higher prices on the Chinese medicine market [20–22]. But, since the late 18th century, natural wild American ginseng resources suffered a sharp decline due to predatory exploitation in North America under the influence of economic interests, and are nearing extinction now [23]. Meanwhile WsAG, with high quality and four to ten times the retail value of field-grown roots, is similar to the wild one [4]. Actually, planting wild-simulated ginseng is encouraged with the aim of reducing harvest pressure on wild populations [24]. Wild and wild-simulated roots could share the same export and trade regulations due to the similar morphology phenotype and market value [8,25]. Recently, because the so-called wild-simulated American ginseng could capitalize on the premium paid for wild-appearing roots, and the species appeared well suited to the practice of forest farming, American ginseng has been recommended as an agroforestry crop candidate [26]. With the continuous expansion of the folk and clinical applications of American ginseng, it is necessary to conduct an in-depth study on the chemical constituents of American ginseng aiming to clarify the material basis of efficacy. So far, there are a few comparative analysis on FgAG and wild American ginseng [27,28]. These results showed that ginsenosides are different between them [26], especially, the ratios of Rg_1/Rd, Rg_1/Rb_1 and (Rg_1 + Re)/Rd are characteristic markers for differentiating these two groups [28]. However, a comparative study on the chemical composition between FgAG and WsAG does not exist.

Untargeted metabolomics, being able to profile diverse classes of metabolites, has been successfully applied to compare and identify the overall small-molecule components of different groups of samples [29]. Ultra-high performance liquid chromatography (UPLC) combined with quadrupole time-of-flight tandem mass spectrometry (QTOF-MS) and multivariate statistical analyses are often applied to profile the different groups. For multivariate statistical analyses, principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) are the most common statistical methods. Meanwhile, UPLC-QTOF-MS combined with the automated data processing software UNIFI was often applied recently in the characterization of chemical components of herbal medicines [30–35]. When the coeluting constituents possessed different m/z values, HR-MS can provide a specific and accurate mass. While, UNIFI, a highly comprehensive, high throughput, efficient and simple platform, offers a method for integrating data acquisition, data mining, library searching and reporting generation.

Aiming to find out the similarities and differences between FgAG and WsAG, and to provide a reference for quality control and material basis of efficacy, the screening and the comparative analysis of chemical constituents in FgAG and WsAG is conducted for the first time in this paper. The shared constituents would be evaluated with the UPLC-QTOF-MS method combined with UNIFI. The characteristic components were to be found using the untargeted metabolomics method. The results will also be helpful in explaining the different pharmacological activities and controlling the quality of FgAG and WsAG.

2. Results

2.1. Identification of Components from FgAG and WsAG Based on the UNIFI Platform

As a result of our screening analysis, a total of 121 compounds were identified or tentatively characterized in both positive and negative mode from FgAG and WsAG (Table 1), the base peak intensity (BPI) chromatograms are shown in Figure 1, and their chemical structures are shown in Figure 2.

Table 1. Compounds identified from FgAG and WsAG by UPLC-QTOF-MS^E.

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
1	0.57	$C_7H_{12}O_6$	192.0636	192.0634	1.0	191.0563[M − H]$^-$, 173.0454[M − H-H_2O]$^-$, 127.0407[M − H-H_2O-HCOOH]$^-$, 109.0452[M − H-2H_2O-HCOOH]$^-$, 91.0352[M − H-3H_2O-HCOOH]$^-$	Quinic acid	WsAG, FgAG	s
2	0.64	$C_{12}H_{22}O_{11}$	342.1162	342.1165	0.8	341.1092[M − H]$^-$, 179.0562[M − H-Glu]$^-$	*α*-Maltose	WsAG, FgAG	s
3	0.77	$C_{10}H_{13}N_5O_4$	267.0959	267.0968	−3.3	268.1031[M+H]$^+$, 237.0874[M + H-CH_2OH]$^+$, 226.0898[M + H-CN_2H_2]$^+$, 136.0612[M + H-Rib]$^+$, 130.0495[M + H-CH_2OH-$C_4N_4H_3$]$^+$	Adenosine	WsAG, FgAG	s
4	0.93	$C_{12}H_{22}O_{11}$	342.1168	342.1162	1.7	341.1095[M − H]$^-$, 287.1097[M − H-3H_2O]$^-$, 179.0563[M − H-Glu]$^-$	Sucrose	WsAG, FgAG	s
5	0.95	$C_9H_{11}NO_2$	165.0777	165.0782	−3.0	166.0850[M + H]$^+$, 150.0589[M + H-NH_2]$^+$, 132.0486[M + H-H_2O-NH_2]$^+$, 120.0807[M + H-HCOOH]$^+$, 91.0559[M + H-CH-NH_2-HCOOH]$^+$	L-Phenylalanine	WsAG, FgAG	s
6 *	1.02	$C_{14}H_{18}O_{10}$	346.0903	346.0900	1.0	345.0830[M − H]$^-$, 327.0598[M − H-H_2O]$^-$, 309.0728[M − H-2H_2O]$^-$, 165.0195[M − H-Glu]$^-$, 150.0115[M − H-Glu-CH_3]$^-$	Methyl gallate 3-*O*-*β*-D-glucoside	WsAG > FgAG VIP: 14.18 $p < 0.001$	s
7	1.51	$C_{11}H_{12}N_2O_2$	204.0899	204.0899	−0.1	203.0826[M − H]$^-$, 141.0660[M − H-HCOOH-NH_2]$^-$, 129.0506[M − H-$C_3H_6O_2$]$^-$	L-Tryptophane	WsAG, FgAG	s
8	4.71	$C_{17}H_{20}O_9$	368.1106	368.1107	−0.4	367.1033[M − H]$^-$, 191.0754[M − H-$C_{10}H_9O_3$]$^-$, 193.0466[M − H-GluA]$^-$, 177.0758[M − H-GluA-CH_3]$^-$, 127.0350[M − H-GluA-H_2O-OCH_3]$^-$	3-*O*-trans-Feruloylquinic Acid	WsAG, FgAG	[36]
9	4.92	$C_{48}H_{82}O_{19}$	962.5440	962.5450	−1.0	1007.5432[M + HCOO]$^-$, 763.2898[M − H-2H_2O-Glu]$^-$, 815.4784[M − H-Rha]$^-$, 781.3155[M − H-Glu]$^-$, 635.2307[M − H-Glu-Rha-H_2O]$^-$, 437.1863[M − H-2Glu-Rha-3H_2O]$^-$	Majoroside F_6	WsAG, FgAG	[37]
10	5.18	$C_{36}H_{58}O_8$	618.4130	618.4132	−0.3	619.4203[M + H]$^+$, 439.3712[M + H-Glu]$^+$, 422.3451[M + H-Glu-OH]$^+$, 383.2823[M + H-Glu-C_4H_8]$^+$, 297.2336[M + H-Glu-$C_9H_{16}O$]$^+$	Oleanolic acid -28-*O*-*β*-D-glucopyranoside	WsAG, FgAG	s
11	5.32	$C_{48}H_{82}O_{20}$	978.5399	978.5397	−0.3	1023.5379[M + HCOO]$^-$, 997.5331[M − H]$^-$,815.4972[M − H-Glu]$^-$, 797.4718[M − H-Glu-H_2O]$^-$, 653.3389[M − H-2Glu]$^-$, 491.2724[M − H-3Glu]$^-$	Yesanchinoside B	WsAG, FgAG	[38]
12 *	5.54	$C_{47}H_{80}O_{19}$	948.5305	948.5294	1.2	993.5270[M + HCOO]$^-$, 815.4921[M − H-Ara]$^-$, 653.3392[M − H-2Glu-Ara]$^-$, 473.3030[M − H-2Glu-Ara]$^-$, 455.3922[M − H-2Glu-Ara-H_2O]$^-$, 391.2582[M − H-2Glu-Ara-$C_6H_{12}O$]$^-$	Yesanchinoside C	WsAG > FgAG VIP: 6.18 $p < 0.001$	[38]
13 #	5.78	$C_{48}H_{82}O_{19}$	962.5443	962.5450	−1.0	1007.5436[M + HCOO]$^-$, 961.5388[M − H]$^-$, 799.4784[M − H-Glu]$^-$, 637.2307[M − H-2Glu]$^-$, 475.5863[M − H-3Glu]$^-$	Notoginsenoside N	WsAG < FgAG VIP: 11.83 $p < 0.001$	[39]

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
14 #	5.94	$C_{42}H_{74}O_{15}$	818.5031	818.5028	0.4	863.5006[M + HCOO]$^-$, 667.4323[M − H-Rha]$^-$, 533.2329[M − H-$C_6H_{13}O_2$-$C_{11}H_{19}O$]$^-$, 506.3845[M − H-Glu-Rha]$^-$, 477.2169[M − H-$C_{20}H_{36}O_4$]$^-$	Quinquenoside L_9	WsAG < FgAG VIP: 7.20 p = 0.0002	s
15	5.94	$C_{42}H_{72}O_{14}$	800.4915	800.4922	−0.9	801.4988[M + H]$^+$, 621.4983[M + H-Glu-H_2O]$^+$, 459.3659[M + H-2Glu]$^+$, 423.3450[M + H-2Glu-3H_2O]$^+$	Majoroside F_2	WsAG, FgAG	[40]
16	6.25	$C_{26}H_{34}O_{11}$	522.2097	522.2101	−0.7	567.2079[M + HCOO]$^-$, 521.2204[M − H]$^-$, 458.2935[M − H-H_2O-C_2H_5O]$^-$, 341.1396[M − H-Glu-H_2O]$^-$, 178.0559[M − H-$C_{20}H_{23}O_5$]$^-$	Urolignoside	WsAG, FgAG	[41]
17	6.48	$C_{54}H_{92}O_{23}$	1108.6034	1108.6029	0.4	1153.6016[M + HCOO]$^-$, 961.5452[M − H-Rha]$^-$, 799.4902[M − H-Glu-Rha]$^-$, 637.2950[M − H-2Glu-Rha]$^-$, 475.2681[M − H-3Glu-Rha]$^-$	Yesanchinoside E	WsAG, FgAG	[42]
18	6.77	$C_{47}H_{80}O_{18}$	932.5348	932.5345	0.3	977.5318[M + HCOO]$^-$, 931.5271[M − H]$^-$, 799.4697[M − H-Ara]$^-$, 769.4734[M − H-Glu]$^-$, 637.3146[M − H-Glu-Ara]$^-$	Quinquenoside F_6	WsAG, FgAG	[37]
19	6.88	$C_{48}H_{82}O_{19}$	962.5450	962.5450	−0.1	1007.5432[M + HCOO]$^-$, 859.4881[M − H-$C_5H_{10}O_2$]$^-$, 799.4204[M − H-Glu]$^-$, 696.4328[M − H-Glu-C_5H_9O]$^-$, 637.3158[M − H-2Glu]$^-$, 601.2316[M − H-2Glu-2H_2O]$^-$	Quinquenoside L_2	WsAG, FgAG	[43]
20	7.01	$C_{42}H_{72}O_{14}$	800.4914	800.4922	−1.0	845.4896[M + HCOO]$^-$, 799.4836[M − H]$^-$, 653.4319[M − H-Rha]$^-$, 491.2475[M − H-Glu-Rha]$^-$	(24*S*)-Pseudoginsenoside F_{11}	WsAG, FgAG	s
21 *	7.05	$C_{47}H_{80}O_{18}$	932.5349	932.5345	0.4	977.5346[M + HCOO]$^-$, 840.4930[M − H-2H_2O-C_4H_7]$^-$, 799.4859[M − H-Xyl]$^-$, 769.4735[M − H-Glu]$^-$, 637.4321[M − H-Glu-Xyl]$^-$	Notoginsenoside R_1	WsAG > FgAG VIP: 24.59 p < 0.001	s
22 *	7.10	$C_{28}H_{48}O$	400.3723	400.3705	4.3	423.3620[M+Na]$^+$, 382.2862[M + H-CH_3]$^+$, 339.2934[M + H-H_2O-C_3H_7]$^+$, 255. 2948[M + H-H_2O-C_9H_{19}]$^+$	Methylcholesta-7-en-3β-ol	WsAG > FgAG VIP: 4.69 p < 0.001	[44]
23	7.12	$C_{48}H_{82}O_{19}$	962.5435	962.5450	−1.5	1007.54171[M + HCOO]$^-$, 961.5329[M − H]$^-$, 799.3722[M − H-Glu]$^-$, 637.4321[M − H-2Glu]$^-$, 475.3722[M − H-3Glu]$^-$, 391.4833[M − H-3Glu-C_6H_{12}]$^-$	Notoginsenoside R_6	WsAG, FgAG	[42]
24	7.25	$C_{47}H_{80}O_{18}$	932.5335	932.5345	−1.0	977.5317[M + HCOO]$^-$, 931.5282[M − H]$^-$, 799.4701[M − H-Xyl]$^-$, 673.3294[M − H-Xyl-Glu]$^-$, 475.2148[M − H-Xyl-2Glu]$^-$	Notoginsenoside ST5	WsAG, FgAG	[45]
25	7.29	$C_{54}H_{90}O_{24}$	1122.5818	1122.5822	0.4	1167.5812[M + HCOO]$^-$, 1121.5747[M − H]$^-$, 959.5120[M − H-Glu]$^-$, 797.4669[M − H-2Glu]$^-$, 473.4334[M − H-4Glu]$^-$	Quinquenoside IV	WsAG, FgAG	[42]
26	7.40	$C_{42}H_{72}O_{14}$	800.4918	800.4922	−0.5	845.4900[M + HCOO]$^-$, 784.4683[M − H-CH_3]$^-$, 637.4340[M − H-Glu]$^-$, 471.3787[M − H-2Glu]$^-$	Ginsenoside Rg_1	WsAG, FgAG	s

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
27	7.47	$C_{48}H_{82}O_{18}$	946.5491	946.5501	−1.0	991.5473[M + HCOO]$^-$, 945.5413[M − H]$^-$, 783.5142[M − H-Glu]$^-$, 637.4125[M − H-Glu-Rha]$^-$, 475.5147[M − H-2Glu-Rha]$^-$	Ginsenoside Re	WsAG, FgAG	s
28	7.47	$C_{28}H_{48}O$	400.3715	400.3705	2.4	423.3617[M+Na]$^+$, 401.3540[M + H]$^+$, 383.2861[M + H-H_2O]$^+$, 325.2982[M + H-H_2O-CH_3-C_3H_7]$^+$, 284.1420[M + H-H_2O-C_7H_{15}]$^+$, 175.1221[M + H-H_2O-$C_{15}H_{28}$]$^+$	Campesterol	WsAG, FgAG	a
29	7.69	$C_{45}H_{74}O_{17}$	886.4926	886.4925	−0.1	885.4853[M − H]$^-$, 799.4748[M − H-Mal]$^-$, 637.4303[M − H-Mal-Glu]$^-$, 475.3751[M − H-Mal-2Glu]$^-$	Malonyl-ginsenoside Rg_1	WsAG, FgAG	[46]
30	7.85	$C_{42}H_{72}O_{14}$	800.4929	800.4922	0.8	845.4911[M + HCOO]$^-$, 799.4837[M − H]$^-$, 653.3687[M − H-Rha]$^-$, 491.2354[M − H-Rha-Glu]$^-$	Quinquenoside L_{11}	WsAG, FgAG	s
31 #	7.84	$C_{51}H_{84}O_{21}$	1032.5505	1032.5532	2.6	1031.5414[M − H]$^-$, 945.5212[M − H-Mal]$^-$, 783.4173[M − H-Mal-Glu]$^-$, 637.4385[M − H-Mal-Rha-Glu-Ac]$^-$, 475.3932[M − H-Mal-2Glu-Rha]$^-$	Malonyl-ginsenoside Re	WsAG < FgAG VIP: 5.65 $p = 0.0060$	[39]
32	7.94	$C_{47}H_{80}O_{19}$	948.5282	948.5294	−1.2	947.5209[M − H]$^-$, 815.4786[M − H-Xyl]$^-$, 653.2758[M − H-Glu-Xyl]$^-$, 491.1787[M − H-2Glu-Xyl]$^-$	Vinaginsenoside R_6	WsAG, FgAG	[39]
33	8.15	$C_{47}H_{80}O_{17}$	916.5409	916.5396	1.4	961.5377[M + HCOO]$^-$, 915.5306[M − H]$^-$, 783.4819[M − H-Ara]$^-$, 753.4732[M − H-Glu]$^-$, 621.4290[M − H-Ara-Glu]$^-$, 459.4687[M − H-2Glu-Ara]$^-$	Quinquenoside L_{14}	WsAG, FgAG	[47]
34	8.23	$C_{44}H_{74}O_{15}$	842.4996	842.5028	−3.6	887.4978[M + HCOO]$^-$, 841.4939[M − H]$^-$, 799.4833[M − H-Ac]$^-$, 695.4459[M − H-Glu]$^-$, 653.4321[M − H-Ac-Rha]$^-$, 684.3932[M − H-CH_3-$C_8H_{14}O_2$]$^-$, 491.4219[M − H-Rha-Glu-Ac]$^-$	Vinaginsenoside R_1	WsAG, FgAG	[48]
35 *	8.26	$C_{54}H_{94}O_{24}$	1126.6162	1126.6135	2.4	1171.6110[M + HCOO]$^-$, 1125.6094[M − H]$^-$, 975.5349 [M − H-Xyl-H_2O]$^-$, 963.5502[M − H-Glu]$^-$, 801.3547[M − H-2Glu]$^-$, 831.3214[M − H-Glu-Xyl]$^-$, 507.3214[M − H-3Glu-Xyl]$^-$	Quinquenoside F_3	WsAG > FgAG VIP: 3.35 $p < 0.001$	s
36	8.57	$C_{44}H_{74}O_{15}$	842.5028	842.5012	−1.7	887.4995[M + HCOO]$^-$, 841.4939[M − H]$^-$, 637.4321[M − H-Glu-Ac]$^-$, 475.3030[M − H-2Glu-Ac]$^-$, 391.4158[M − H-2Glu-Ac-C_6H_{11}]$^-$	Acetyl-Ginsenoside Rg_1	WsAG, FgAG	[39]
37 *	8.59	$C_{41}H_{70}O_{13}$	770.4808	770.4816	−1.0	815.4798[M + HCOO]$^-$, 769.4722[M − H]$^-$, 637.4321[M − H-Ara]$^-$, 475.2678[M − H-Ara-Glu]$^-$, 391.1748[M − H-Ara-Glu-C_6H_{11}]$^-$	Notoginsenoside R_2	WsAG > FgAG VIP: 4.83 $p < 0.001$	[42]
38	8.63	$C_{48}H_{82}O_{19}$	962.5423	962.5450	−2.7	1007.5405[M + HCOO]$^-$, 961.5371[M − H]$^-$, 815.4317[M − H-Rha]$^-$, 799.4622[M − H-Glu]$^-$, 653.4385[M − H-Glu-Rha]$^-$, 617.4316[M − H-Glu-Rha-H_2O]$^-$, 491.2912[M − H-2Glu-Rha]$^-$	Majoroside F_5	WsAG, FgAG	[37]

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
39	8.64	$C_{30}H_{48}O_2$	440.3646	440.3654	−1.9	441.3719[M + H]$^+$, 423.3606[M + H-H_2O]$^+$, 339.2908[M + H-HCOOH-C_4H_8]$^+$, 248.2948[M + H-$C_{14}H_{24}$]$^+$, 203.1849[M + H-HCOOH-$C_{14}H_{24}$]$^+$	Deoxyoleanolic acid	WsAG, FgAG	[46]
40	8.78	$C_{48}H_{80}O_{18}$	944.5338	944.5345	−0.6	989.5320[M + HCOO]$^-$, 943.5250[M − H]$^-$, 781.4541[M − H-Glu]$^-$, 619.4143[M − H-2Glu]$^-$, 457.5876[M − H-3Glu]$^-$	Quinquenoside L_1	WsAG, FgAG	[49]
41	8.83	$C_{53}H_{88}O_{23}$	1092.5718	1092.5716	0.2	1137.5696[M + HCOO]$^-$, 1091.5641[M − H]$^-$, 959.5571[M − H-Xyl]$^-$, 929.4601[M − H-Glu]$^-$, 797.4852[M − H-Glu-Xyl]$^-$	Yesanchinoside G	WsAG, FgAG	[50]
42 *	8.89	$C_{47}H_{78}O_{17}$	914.5225	914.5239	−1.5	959.5225[M + HCOO]$^-$, 913.5147[M − H]$^-$, 733.2547[M − H-H_2O-Glu]$^-$, 619.4527[M − H-Glu-Xyl]$^-$, 457.3254[M − H-2Glu-Xyl]$^-$	Quinquenoside L_8	WsAG > FgAG VIP: 3.97 $p = 0.0004$	s
43	8.97	$C_{48}H_{82}O_{19}$	962.5438	962.5450	−1.2	1007.5420[M + HCOO]$^-$, 946.5212[M − H-CH_3]$^-$, 781.4533[M − H-Glu-H_2O]$^-$, 637.4321[M − H-2Glu]$^-$, 475.3932[M − H-3Glu]$^-$	Majoroside F_1	WsAG, FgAG	[40]
44 *	9.14	$C_{42}H_{70}O_{13}$	782.4325	782.4816	1.2	781.4747[M − H]$^-$, 619.4181[M − H-Glu]$^-$, 457.4798[M − H-2Glu]$^-$, 376.4797[M − H-2Glu-C_6H_9]$^-$	Quinquenoside F_1	WsAG > FgAG VIP: 4.10 $p = 0.0004$	[51]
45	9.27	$C_{15}H_{10}O_6$	286.0480	286.0477	0.8	285.0407[M − H]$^-$, 227.0521[M − H-$C_2H_2O_2$]$^-$, 151.0037[M − H-$C_8H_6O_2$]$^-$, 106.0148[M − H-$C_9H_7O_4$]$^-$, 112.0351[M − H-$C_9H_5O_5$]$^-$	Kaempferol	WsAG, FgAG	s
46 #	9.32	$C_{53}H_{86}O_{24}$	1118.5514	1118.5509	0.5	1117.5436[M − H]$^-$, 1040.5481[M − H-CH_3OH]$^-$, 955.3219[M − H-Glu]$^-$, 793.2905[M − H-2Glu]$^-$, 453.1095[M − H-3Glu-GluA]$^-$	Ginsenoside R_{OA}	WsAG < FgAG VIP: 12.60 $p < 0.001$	[52]
47 *	9.54	$C_{41}H_{70}O_{14}$	786.4775	786.4766	1.1	831.4775[M + HCOO]$^-$, 767.4297[M − H-H_2O]$^-$, 653.4318[M − H-Xyl]$^-$, 491.2015[M − H-Glu-Xyl]$^-$	Majonoside R_2	WsAG > FgAG VIP: 25.80 $p < 0.001$	[39]
48	9.61	$C_{48}H_{80}O_{19}$	960.5285	960.5294	−0.9	1005.5267[M + HCOO]$^-$, 941.5316[M − H-H_2O]$^-$, 797.4287[M − H-Glu]$^-$, 635.3221[M − H-2Glu]$^-$, 473.2684[M − H-3Glu]$^-$	Notoginsenoside G	WsAG, FgAG	[42]
49	9.68	$C_{42}H_{72}O_{14}$	800.4922	800.4922	0.0	845.4904[M + HCOO]$^-$, 799.4844[M − H]$^-$, 783.2451[M − H-Rha]$^-$, 621.3547[M − H-Glu-Rha]$^-$	Pseudo-ginsenoside F_{11}	WsAG, FgAG	s
50	9.73	$C_{36}H_{62}O_{10}$	654.4340	654.4343	−0.4	699.4322[M + HCOO]$^-$, 653.4262[M − H]$^-$, 635.4312[M − H-H_2O]$^-$, 491.3254[M − H-Glu]$^-$	Pseudo-ginsenoside RT_5	WsAG, FgAG	s
51	9.78	$C_{36}H_{62}O_{10}$	654.4337	654.4343	−0.9	655.4410[M + H]$^+$, 599.4418[M − H-3H_2O]$^+$, 493.3437[M − H-Glu]$^+$, 457.2651[M − H-Glu-2H_2O]$^+$	Pseudo-ginsenoside RT_4	WsAG, FgAG	[39]

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
52	9.82	$C_{59}H_{100}O_{27}$	1240.6458	1240.6452	0.5	1239.6380[M − H]$^-$, 1107.6376[M − H-Xyl]$^-$, 954.6930[M − H-Xyl-Glu]$^-$, 783.4833[M − H-Xyl-2Glu]$^-$, 621.4431[M − H-Xyl-3Glu]$^-$, 459.4943[M − H-Xyl-4Glu]$^-$	Ginsenoside Ra_3	WsAG, FgAG	[53]
53	9.96	$C_{41}H_{70}O_{13}$	770.4810	770.4816	−0.8	815.4804[M + HCOO]$^-$, 751.4804[M − H-H_2O]$^-$, 637.4904[M − H-Ara]$^-$, 475.3804[M − H-Glu-Ara]$^-$	Ginsenoside F_5	WsAG, FgAG	[39]
54	9.98	$C_{58}H_{98}O_{26}$	1210.6350	1210.6346	0.3	1209.6272[M − H]$^-$, 1077.5814[M − H-Xyl]$^-$, 945.4706[M − H-Xyl-Ara]$^-$, 783.4803[M − H-Xyl-Ara-Glu]$^-$, 459.4799[M − H-Xyl-Ara-3Glu]$^-$	Ginsenoside Ra_2	WsAG, FgAG	[53]
55	10.02	$C_{41}H_{66}O_{11}$	734.4590	734.4605	−2.1	735.4663[M + H]$^+$, 589.3646[M + H-Rha]$^+$, 457.3705[M + H-Rha-Ara]$^+$, 441.5712[M + H-Rha-Ara-HCOOH]$^+$	Eleutheroside K	WsAG, FgAG	[54]
56	10.04	$C_{48}H_{80}O_{18}$	944.5320	944.5345	−2.6	989.5302[M + HCOO]$^-$, 943.5263[M − H]$^-$, 781.4839[M − H-Glu]$^-$, 619.4206[M − H-2Glu]$^-$, 457.5701[M − H-3Glu]$^-$	Quinquenoside L_6	WsAG, FgAG	-
57	10.07	$C_{30}H_{48}O_2$	440.3638	440.3654	−3.6	441.3717[M + H]$^+$, 394.3508[M + H-H_2O-CHO]$^+$, 328.3504[M + H-CHO-C_6H_{12}]$^+$, 219.1792[M + H-$C_{15}H_{26}O$]$^+$, 205.1619[M + H-H_2O-$C_{15}H_{22}O$]$^+$	3β-Hydroxyolean-12-en-28-al	WsAG, FgAG	a
58	10.14	$C_{59}H_{100}O_{27}$	1240.6452	1240.6462	0.8	1285.6444[M + HCOO]$^-$, 1107.5976[M − H-Xyl]$^-$, 945.4900[M − H-Xyl-Glu]$^-$, 783.4835[M − H-Xyl-2Glu]$^-$, 459.4929[M − H-Xyl-4Glu]$^-$	Notoginsenoside Fa	WsAG, FgAG	[53]
59	10.24	$C_{54}H_{92}O_{23}$	1108.6039	1108.6029	0.8	1153.6021[M + HCOO]$^-$, 1107.5961[M − H]$^-$, 943.5414[M − H-Glu]$^-$, 763.4784[M − H-2Glu]$^-$, 615.4417[M − H-3Glu]$^-$	Ginsenoside Rb_1	WsAG, FgAG	s
60	10.24	$C_{30}H_{48}O$	424.3692	424.3705	−3.1	425.3765[M + H]$^+$, 409.3102[M + H-H_2O]$^+$, 371.3759[M + H-CH_3-C_3H_5]$^+$, 189.1614[M + H-$C_{16}H_{26}O$]$^+$, 205.1775[M + H-$C_{15}H_{26}O$]$^+$	Olean-18-en-3-one	WsAG, FgAG	[55]
61	10.31	$C_{57}H_{94}O_{26}$	1194.6054	1194.6033	1.7	1193.5981[M − H]$^-$, 1077.5402[M − H-mal]$^-$, 945.5097[M − H-mal-Glu]$^-$, 783.4906[M − H-mal-2Glu]$^-$, 621.4906[M − H-mal-3Glu]$^-$	Malonyl-ginsenoside Rb_1	WsAG, FgAG	[53]
62	10.33	$C_{42}H_{72}O_{13}$	784.4975	784.4973	0.3	829.4957[M + HCOO]$^-$, 768.4744[M − H-CH_3]$^-$, 635.4330[M − H-Rha]$^-$, 471.3782[M − H-Glu-Rha]$^-$	20(*R*)-Ginsenoside Rg_2	WsAG, FgAG	s
63	10.35	$C_{36}H_{62}O_9$	638.4391	638.4394	−0.4	683.4373[M + HCOO]$^-$, 637.4313[M − H]$^-$, 475.2658[M − H-Glu]$^-$, 457.2235[M − H-Glu-H_2O]$^-$	20(*S*)-Ginsenoside Rh_1	WsAG, FgAG	s
64	10.36	$C_{41}H_{70}O_{13}$	770.4817	770.4816	0.0	815.4799[M + HCOO]$^-$, 678.4450[M − H-2H_2O-C_4H_7]$^-$, 637.4321[M − H-Ara]$^-$, 590.2706[M − H-C_4H_7-C_9H_{16}]$^-$, 475.2622[M − H-Glu-Ara]$^-$	Ginsenoside F_3	WsAG, FgAG	[39]

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
65	10.39	$C_{53}H_{90}O_{22}$	1078.5931	1078.5924	0.6	1123.5913[M + HCOO]$^-$, 943.5423[M − H-Araf]$^-$, 854.4890[M − H-H_2O-Araf-C_4H_7]$^-$, 763.4850[M − H-Glu-Araf]$^-$	Ginsenoside Rc	WsAG, FgAG	s
66	10.42	$C_{36}H_{60}O_8$	620.4276	620.4288	−1.9	621.4349[M + H]$^+$, 603.4238[M + H-H_2O]$^+$, 441.3714[M + H-Glu]$^+$, 423.3612[M + H-Glu-H_2O]$^+$, 350.2971[M + H-Glu-2H_2O-C_4H_7]$^+$, 341.1160[M + H-Glu-$C_6H_{12}O$]$^+$	Ginsenoside Rh_4	WsAG, FgAG	[56]
67	10.46	$C_{58}H_{98}O_{26}$	1210.6353	1210.6346	0.6	1209.6275[M − H]$^-$, 1077.5914[M − H-Xyl]$^-$, 945.4807[M − H-Xyl-Ara]$^-$, 783.4687[M − H-Xyl-Ara-Glu]$^-$, 459.4329[M − H-Xyl-Ara-3Glu]$^-$	Ginsenoside Ra_1	WsAG, FgAG	[53]
68	10.58	$C_{56}H_{92}O_{25}$	1164.5932	1164.5928	0.3	1163.5859[M − H]$^-$, 1119.5976[M − H-CO_2]$^-$, 1077.6021[M − H-Mal]$^-$, 1031.5694[M − H-Araf]$^-$, 945.4900[M − H-Araf-Mal]$^-$, 783.4835[M − H-Glu-Araf-Mal]$^-$	Malonyl-ginsenoside Rc	WsAG, FgAG	[53]
69	10.62	$C_{48}H_{76}O_{19}$	956.4976	956.4981	−0.5	955.4903[M − H]$^-$, 783.4214[M − H-GluA]$^-$, 631.4157[M − H-2Glu]$^-$, 459.4174[M − H-2Glu-GluA]$^-$	Ginsenoside Ro	WsAG, FgAG	s
70 #	10.69	$C_{30}H_{46}O_2$	438.3486	438.3498	−2.7	439.3563[M + H]$^+$, 424.3600[M + H-CH_3]$^+$, 411.1114[M + H-CO]$^+$, 233.1676[M + H-$C_{15}H_{24}$]$^+$, 205.1928[M + H-$C_{15}H_{22}O_2$]$^+$, 190.1778[M + H-$C_{16}H_{23}O_2$]$^+$	3,11-dioxo-β-amyrene	WsAG < FgAG VIP: 6.49 $p < 0.001$	[57]
71	10.70	$C_{53}H_{84}O_{23}$	1088.5402	1088.5403	−0.1	1087.5326[M − H]$^-$, 955.5235[M − H-Ara]$^-$, 925.4610[M − H-Glu]$^-$, 793.2350[M − H-Ara-Glu]$^-$, 455.4611[M − H-Ara-2Glu-GluA]$^-$	Stipuleanoside R2	WsAG, FgAG	[39]
72	10.78	$C_{53}H_{90}O_{22}$	1078.5924	1078.5924	0.0	1123.5906[M + HCOO]$^-$, 913.5403[M − H-Glu]$^-$, 779.4886[M − H-Glu-Ara]$^-$, 615.4431[M − H-2Glu-Ara]$^-$	Ginsenoside Rb_2	WsAG, FgAG	s
73	10.79	$C_{53}H_{90}O_{22}$	1078.5924	1078.5924	0.0	1123.5320[M + HCOO]$^-$, 913.4581[M − H-Glu]$^-$, 779.3696[M − H-Glu-Xyl]$^-$, 615.4912[M − H-2Glu-Xyl]$^-$, 451.3672[M − H-3Glu-Xyl]$^-$	Ginsenoside Rb_3	WsAG, FgAG	s
74	10.89	$C_{55}H_{92}O_{23}$	1120.6009	1120.6029	−1.8	1165.5991[M + HCOO]$^-$, 1077.3151[M − H-Xyl]$^-$, 945.5076[M − H-Ara-Xyl]$^-$, 783.3942[M − H-Ara-Xyl-Glu]$^-$, 621.4742[M − H-Ara-Xyl-2Glu]$^-$	Notoginsenoside Fc	WsAG, FgAG	[53]
75	10.94	$C_{56}H_{92}O_{25}$	1164.5937	1164.5928	0.8	1163.5864[M − H]$^-$, 1077.5570[M − H-Mal]$^-$, 945.5302[M − H-Ara-Mal]$^-$, 783.4540[M − H-Glu-Ara-Mal]$^-$, 621.4570[M − H-2Glu-Ara-Mal]$^-$	Malonyl-ginsenoside Rb_2	WsAG, FgAG	[53]
76	11.01	$C_{56}H_{94}O_{24}$	1150.6138	1150.6135	0.3	1195.6120[M + HCOO]$^-$, 1149.6060[M − H]$^-$, 1107.4997[M − H-Ac]$^-$, 987.4976[M − H-Glu]$^-$, 945.6047[M − H-Glu-Ac]$^-$, 783.4864[M − H-2Glu-Ac]$^-$	Quinquenoside R_1	WsAG, FgAG	[53]
77	11.02	$C_{47}H_{74}O_{18}$	926.4864	926.4875	−1.2	925.4791[M − H]$^-$, 793.4272[M − H-Ara]$^-$, 612.3784[M − H-GluA-Ara]$^-$, 540.3784[M − H-Glu-$C_{14}H_{21}O$]$^-$, 455.2841[M − H-Glu-Ara-GluA]$^-$	Chikusetsu saponin IV	WsAG, FgAG	[39]

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
78	11.14	$C_{56}H_{92}O_{25}$	1164.4967	1164.4958	0.8	1163.4925[M − H]$^-$, 1077.5760[M − H-Mal]$^-$, 945.5503[M − H-Mal-Xyl]$^-$, 783.4735[M − H-Mal-Xyl-Glu]$^-$, 621.4269[M − H-Mal-Xyl-2Glu]$^-$	Malonyl-ginsenoside Rb_3	WsAG, FgAG	[53]
79	11.16	$C_{36}H_{62}O_9$	638.4395	638.4394	0.2	683.4366[M + HCOO]$^-$, 637.4317[M − H]$^-$, 475.2574[M − H-Glu]$^-$, 457.2147[M − H-Glu-H_2O]$^-$	20(*R*)-ginsenoside Rh_1	WsAG, FgAG	s
80	11.18	$C_{43}H_{72}O_{15}$	828.4864	828.4871	−0.8	873.4846[M + HCOO]$^-$, 784.4798[M − H-$COCH_3$]$^-$, 695.2912[M − H-Xyl]$^-$, 491.4938[M − H-Xyl-Glu-Ac]$^-$, 455.2535[M − H-Xyl-Glu-Ac-2H_2O]$^-$	Vinaginsenoside R_2	WsAG, FgAG	[39]
81 #	11.20	$C_{48}H_{82}O_{17}$	930.5546	930.5552	−0.7	929.5474[M − H]$^-$, 767.4642[M − H-Glu]$^-$, 605.4365[M − H-2Glu]$^-$, 443.1196[M − H-3Glu]$^-$	Vinaginsenosides R_3	WsAG < FgAG VIP: 7.60 $p < 0.001$	[58,59]
82	11.34	$C_{42}H_{66}O_{14}$	794.4447	794.4453	−0.8	793.4368[M − H]$^-$, 631.3279[M − H-Glu]$^-$, 613.4222[M − H-Glu-H_2O]$^-$, 569.2927[M − H-Glu-HCOOH]$^-$, 455.1562[M − H-Glu-GluA]$^-$	Chikusetsu saponin II	WsAG, FgAG	[39]
83	11.36	$C_{48}H_{82}O_{18}$	946.5508	946.5501	0.7	991.5490[M + HCOO]$^-$, 945.5430[M − H]$^-$, 783.5147[M − H-Glu]$^-$, 459.3241[M − H-3Glu]$^-$	Ginsenoside Rd	WsAG, FgAG	s
84	11.39	$C_{55}H_{92}O_{23}$	1120.6029	1120.6049	1.7	1119.5941[M − H]$^-$, 1077.5699[M − H-Ac]$^-$, 943.4874[M − H-Ac-Ara]$^-$, 779.1224[M − H-Ac-Ara-Glu]$^-$, 451.2649[M − H-Ac-Ara-3Glu]$^-$	Ginsenoside Rs_1	WsAG, FgAG	s
85	11.52	$C_{51}H_{84}O_{21}$	1032.5503	1032.5505	−0.2	1031.5425[M − H]$^-$, 987.5520[M − H-CO_2]$^-$, 945.5192[M − H-mal]$^-$, 783.3540[M − H-mal-Glu]$^-$, 621.2570[M − H-mal-2Glu]$^-$, 459.3458[M − H-mal-3Glu]$^-$	Malonyl-ginsenoside Rd	WsAG, FgAG	[53]
86	11.70	$C_{55}H_{92}O_{23}$	1120.6039	1120.6029	0.8	1165.6021[M + HCOO]$^-$, 1119.5961[M − H]$^-$, 987.3684[M − H-Ara]$^-$, 914.4587[M − H-Glu-Ac]$^-$, 458.5471[M − H-3Glu-Ara-Ac]$^-$	Ginsenoside Rs_2	WsAG, FgAG	s
87	11.88	$C_{48}H_{82}O_{18}$	946.5501	946.5500	−0.1	991.5482[M + HCOO]$^-$, 783.4871[M − H-Glu]$^-$, 603.4416[M − H-2Glu]$^-$	Gypenoside XVII	WsAG, FgAG	s
88	12.20	$C_{19}H_{36}O_5$	344.2565	344.2563	0.6	343.2486[M − H]$^-$, 329.0232[M − H-CH_3]$^-$, 311.2112[M − H-H_2O-CH_3]$^-$, 294.1609[M − H-H_2O-OCH_3]$^-$, 255.1494[M − H-OCH_3-C_4H_9]$^-$, 242.1610[M − H-OCH_3-C_5H_{11}]$^-$, 228.2523[M − H-OCH_3-C_6H_{12}]$^-$	Methyl-9,10,11-trihydroxy-12-octadecenoate	WsAG, FgAG	[60]
89	12.27	$C_{47}H_{80}O_{17}$	916.5398	916.5396	0.2	961.5380[M + HCOO]$^-$, 866.4901[M − H-H_2O-CH_2OH]$^-$, 783.4749[M − H-Ara]$^-$, 753.4664[M − H-Glu]$^-$, 621.4616[M − H-Glu-Ara]$^-$, 459.4008[M − H-2Glu-Ara]$^-$	Notoginsenoside Fe	WsAG, FgAG	[39]
90	12.38	$C_{50}H_{84}O_{19}$	988.5594	988.5607	−1.3	1033.5576[M + HCOO]$^-$, 987.5539[M − H]$^-$, 945.5428[M − H-$COCH_3$]$^-$, 809.4326[M − H-2H_2O-$C_8H_{14}O_2$]$^-$, 797.4813[M − H-Glu-C_2H_4]$^-$	Quinquenoside III	WsAG, FgAG	[61]

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
91 #	12.48	$C_{47}H_{80}O_{17}$	916.5385	916.5396	−1.1	961.5385[M + HCOO]$^-$, 900.5146[M − H-CH_3]$^-$, 783.49.6[M − H-Ara]$^-$, 630.4290[M − H-Glu-2H_2O-C_5H_9]$^-$, 621.3500[M − H-Glu-Ara]$^-$, 459.2328[M − H-2Glu-Ara]$^-$	Chikusetsu saponin III	WsAG < FgAG VIP: 12.78 $p < 0.001$	[39]
92	12.57	$C_{30}H_{46}O_2$	766.4855	766.4867	−1.6	767.4928[M + H]$^+$, 749.3674[M + H-H_2O]$^+$, 621.2398[M + H-Rha]$^+$, 459.2280[M + H-Glu-Rha]$^+$, 207.1780[M + H-Glu-Rha-$C_{16}H_{26}O$]$^+$	(20*E*)-Ginsenoside F_4	WsAG, FgAG	[62]
93	12.68	$C_{47}H_{80}O_{17}$	916.5399	916.5396	0.3	961.5381[M + HCOO]$^-$, 814.4616[M − H-H_2O-C_6H_{11}]$^-$, 783.4923[M − H-Xyl]$^-$, 621.4390[M − H-Glu-Xyl]$^-$	Gypenoside IX	WsAG, FgAG	[53]
94	13.14	$C_{42}H_{70}O_{14}$	798.4748	798.4765	−2.1	797.4676[M − H]$^-$, 651.4246[M − H-Rha]$^-$, 489.4158[M − H-Glu-Rha]$^-$	Ginsenoside Rg_8	WsAG, FgAG	[63]
95	13.18	$C_{52}H_{86}O_{19}$	1014.5756	1014.5763	−0.6	1059.5738[M + HCOO]$^-$, 1013.5685[M − H]$^-$, 945.5933[M − H-C_4H_5O]$^-$, 851.4510[M − H-Glu]$^-$, 833.4903[M − H-Glu-H_2O]$^-$, 620.2875[M − H-2Glu-C_4H_5O]$^-$, 458.2663[M − H-3Glu-C_4H_5O]$^-$	Quinquenoside I	WsAG, FgAG	[61]
96	13.31	$C_{48}H_{82}O_{17}$	930.5548	930.5552	−0.4	929.5470[M − H]$^-$, 783.4642[M − H-Rha]$^-$, 767.4695[M − H-Glu]$^-$, 621.4365[M − H-Glu-Rha]$^-$, 459.3956[M − H-2Glu-Rha]$^-$	Gypenoside X	WsAG, FgAG	-
97	13.43	$C_{42}H_{70}O_{12}$	766.4861	766.4867	−0.8	765.4783[M + HCOO]$^-$, 610.2361[M − H-CH_2OH-C_8H_{13}-CH_3]$^-$, 603.2375[M − H-Glu]$^-$, 441.1811[M − H-2Glu]$^-$, 340.2323[M − H-$C_{30}H_{49}O$]$^-$	Ginsenoside Rk_1	WsAG, FgAG	[39]
98	13.50	$C_{47}H_{74}O_{18}$	926.4862	926.4875	−1.4	925.4789[M − H]$^-$, 793.4879[M − H-Ara]$^-$, 731.4457 [M − H-Ara-HCOOH]$^-$, 727.4338[M − H-Glu-H_2O]$^-$, 659.4254[M − H-Glu-C_3H_4-HCOOH]$^-$, 569.4945 [M − H-Ara-Glu-HCOOH]$^-$, 455. 4979 [M − H-Ara-Glu-GluA]$^-$	Chikusetsu saponin Ib	WsAG, FgAG	[39]
99 #	13.52	$C_{42}H_{72}O_{13}$	784.4974	784.4973	−0.1	783.4896[M − H]$^-$, 737.4755[M − H-CH_2OH-CH_3]$^-$, 660.4330[M − H-3H_2O-C_5H_9]$^-$, 621.4361[M − H-Glu]$^-$, 459.3782[M − H-2Glu]$^-$	Ginsenoside F_2	WsAG < FgAG VIP: 17.01 $p < 0.001$	[39]
100	13.57	$C_{18}H_{30}O_4$	310.2141	310.2144	−1.0	309.2068[M − H]$^-$, 291.1960[M − H-H_2O]$^-$, 185.1181[M − H-COOH-C_6H_9]$^-$, 171.1024[M − H-CH_2COOH-C_6H_9]$^-$	13S-hydroperoxy-9Z,11E,15Z-octadecatrienoic acid	WsAG, FgAG	[64]
101	13.62	$C_{36}H_{60}O_7$	604.4334	604.4339	−0.8	605.4407[M + H]$^+$, 586.4285[M + H-H_2O]$^+$, 443.3860[M + H-Glu]$^+$, 405.3657[M + H-Glu-H_2O]$^+$, 333.0939[M + H-2H_2O-$C_{16}H_{26}O$]$^+$, 296.1006[M + H-Glu-H_2O-C_8H_{13}]$^+$	Isoginsenoside Rh_3	WsAG, FgAG	[65]
102 *	13.93	$C_{42}H_{66}O_{14}$	794.4449	794.4453	−0.5	793.4387[M − H]$^-$, 613.3751[M − H-Glu]$^-$, 569.3830[M − H-Glu-H_2O-CO_2]$^-$	Chikusetsusaponin Iva	WsAG > FgAG VIP:16.17 $p < 0.001$	[53]

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
103	14.51	$C_{42}H_{72}O_{13}$	784.4967	784.4973	−0.7	829.4951[M + HCOO]$^-$, 783.4892[M − H]$^-$, 621.4442[M − H-Glu]$^-$, 459.3684[M − H-2Glu]$^-$	20(*R*)-Ginsenoside Rg_3	WsAG, FgAG	s
104	14.64	$C_{17}H_{30}O_2$	266.2246	266.2246	−0.1	311.2228[M + HCOO]$^-$, 168.1023[M − H-C_7H_{13}]$^-$, 154.1074[M − H-C_8H_{15}]$^-$, 137.2250[M − H-C_7H_{13}-OCH_3]$^-$, 115.0352[M − H-C_4H_7-C_6H_9O]$^-$, 96.0352[M − H-$C_{11}H_{21}O$]$^-$	5-Hexenoic acid, 10-undecenyl ester	WsAG, FgAG	a
105 #	14.74	$C_{18}H_{28}O_2$	276.2081	276.2089	−2.9	277.2156[M + H]$^+$, 150.1312[M + H-$C_7H_{11}O_2$]$^+$, 137.0951[M + H-H_2O-C_9H_{14}]$^+$, 110.1017[M + H-$C_{10}H_{15}O_2$]$^+$	Palmitoleic acid	WsAG < FgAG VIP: 8.57 $p < 0.001$	s
106	14.75	$C_{42}H_{72}O_{13}$	784.4940	784.4973	−4.1	829.4951[M + HCOO]$^-$, 783.4865[M − H]$^-$, 621.4942[M − H-Glu]$^-$, 459.4578[M − H-2Glu]$^-$, 441.5214[M − H-2Glu-H_2O]$^-$	20(*S*)-Ginsenoside Rg_3	WsAG, FgAG	s
107	14.90	$C_{41}H_{70}O_{12}$	754.4874	754.4867	0.8	799.4856[M + HCOO]$^-$, 621.3141[M − H-Xyl]$^-$, 459.3887[M − H-Glu-Xyl]$^-$, 351.2556[M − H-Xyl-H_2O-$C_{16}H_{28}O_2$]$^-$, 275.1442[M − H-Glu-Xyl-2C_6H_{11}]$^-$	Gypenoside XIII	WsAG, FgAG	[66]
108	14.98	$C_{41}H_{64}O_{13}$	764.4345	764.4347	−0.3	763.4272[M − H]$^-$, 613.3766[M − H-Xyl]$^-$, 569.3856[M − H-Xyl-HCOOH]$^-$	Pseudo-ginsenoside Rp_1	WsAG, FgAG	[39]
109	15.05	$C_{17}H_{30}O_2$	266.2244	266.2246	−0.7	311.2226[M + HCOO]$^-$, 222.1128[M − H-C_3H_7]$^-$, 139.0826[M − H-C_9H_{19}]$^-$, 127.1127[M − H-$C_8H_{11}O_2$]$^-$	(2E,4E)-Hydroprene	WsAG, FgAG	a
110	15.75	$C_{43}H_{68}O_{14}$	808.4610	808.4609	0.1	807.4532[M − H]$^-$, 609.3820[M − H-Glu-H_2O]$^-$, 455.3519[M − H-Glu-Glu acid methyl ester]$^-$, 319.1792[M − H-Glu acid methyl ester-$C_{21}H_{32}$]$^-$	Chikusetsusaponin IVa methyl ester	WsAG, FgAG	[39]
111	15.98	$C_{18}H_{30}O_3$	294.2194	294.2195	−0.3	293.2122[M − H]$^-$, 275.2013[M − H-H_2O]$^-$, 171.1024[M − H-C_9H_{15}]$^-$, 121.1020[M − H-$C_9H_{15}O_3$]$^-$	(*E*,*E*)-9-Oxooctadeca-10,12-dienoic acid	WsAG, FgAG	[34]
112 *	16.90	$C_{36}H_{62}O_8$	622.4431	622.4445	−2.1	667.4442[M + HCOO]$^-$, 621.4360[M − H]$^-$, 459.2656[M − H-Glu]$^-$, 441.4772[M − H-Glu-H_2O]$^-$	Ginsenoside Rh_2	WsAG > FgAG VIP: 4.68 $p = 0.0032$	s
113	17.34	$C_{18}H_{32}O_3$	296.2347	296.2351	1.4	295.2274[M − H]$^-$, 278.2172[M − H-H_2O]$^-$, 233.2273[M − H-HCOOH-O]$^-$, 184.1182[M − H-C_8H_{15}]$^-$, 171.1023[M − H-C_9H_{16}]$^-$, 148.1125[M − H-$C_8H_{15}O_2$]$^-$, 125.1174[M − H-H_2O-$C_{10}H_{17}O$]$^-$	9-Hydroxyoctadeca-10,12-dienoic acid	WsAG, FgAG	[67]
114	17.37	$C_{42}H_{70}O_{12}$	766.4860	766.4867	−0.9	811.4933[M + HCOO]$^-$, 747.4834[M − H-H_2O]$^-$, 603.4833[M − H-Glu]$^-$, 585.4309[M − H-Glu]$^-$, 459.0768[M − H-Glu-Rha]$^-$, 421.4457[M − H-Glu-Rha]$^-$	Ginsenoside Rg_5	WsAG, FgAG	[53]
115 #	17.45	$C_{18}H_{30}O_2$	278.2245	278.2246	−0.1	279.2321[M + H]$^+$, 218.1936[M + H-HCOOH-CH_3]$^+$, 184.1479[M + H-C_7H_{11}]$^+$	α-Linolenic Acid	WsAG < FgAG VIP: 5.24 $p < 0.001$	s

Table 1. *Cont.*

No.	t_R (min)	Formula	Calculated Mass (Da)	TheoreticalMass (Da)	Mass Error (ppm)	MS^E Fragmentation	Identification	Sources	Ref.
116	18.24	$C_{32}H_{50}O_4$	498.3722	498.3709	2.4	521.3614$[M+Na]^+$, 484.3365$[M + H\text{-}CH_3]^+$, 439.3322$[M + H\text{-}C_2H_3O_2]^+$, 303.3080$[M\text{-}C_{12}H_{20}O_2]^+$, 263.2783$[M\text{-}C_{15}H_{24}O_2]^+$, 248.2610$[M + H\text{-}C_{16}H_{26}O_2]^+$, 203.0991$[M + H\text{-}C_2H_3O_2\text{-}C_{15}H_{22}O_2]^+$	3-*O*-Acetyloleanolic acid	WsAG, FgAG	a
117 *	18.50	$C_{19}H_{24}O_2$	284.1773	284.1776	−1.1	285.1843$[M + H]^+$, 259.2243$[M + H\text{-}C_2H_2]^+$, 243.1701$[M + H\text{-}C_2H_2O]^+$, 159.1308$[M + H\text{-}C_8H_{13}O]^+$, 122.1168$[M + H\text{-}C_{11}H_{11}O]^+$	Androsta-1,4-diene-3,17-dione	WsAG > FgAG VIP: 6.16 $p < 0.001$	a
118	19.97	$C_{16}H_{28}O_3$	268.2039	268.2038	0.4	291.1950$[M+Na]^+$, 223.1536$[M + H\text{-}HCOOH]^+$, 123.0441$[M + H\text{-}H_2O\text{-}C_7H_{13}O_2]^+$, 95.0141$[M + H\text{-}C_4H_9O\text{-}C_5H_9O_2]$	13-Hydroxy-9,11-hexadecanedioic acid	WsAG, FgAG	[34]
119	20.15	$C_{17}H_{24}O_2$	260.1766	260.1776	−3.8	261.1839$[M + H]^+$, 243.1708$[M + H\text{-}H_2O]^+$, 221.1479$[M + H\text{-}CH_3\text{-}C_2H_3]^+$, 159.0791$[M + H\text{-}H_2O\text{-}C_6H_{13}]^+$	Panaxydol	WsAG, FgAG	[68]
120	20.49	$C_{17}H_{26}O_3$	280.3138	280.3130	3.3	303.3030$[M+Na]^+$, 252.2401$[M + H\text{-}C_2H_5]^+$, 149.1310$[M + H\text{-}C_{10}H_{21}]^+$,140.1322$[M + H\text{-}C_{10}H_{21}]^+$, 97.1025$[M + H\text{-}C_{13}H_{27}]^+$	1-Eicosene	WsAG, FgAG	a
121	22.88	$C_{19}H_{38}O_4$	330.2766	330.2770	−1.2	353.2658$[M+Na]^+$, 313.2725$[M + H\text{-}H_2O]^+$, 280.2603$[M + H\text{-}2H_2O\text{-}CH_3]^+$, 239.2352$[M + H\text{-}C_3H_7O_3]^+$, 99.0871$[M + H\text{-}C_4H_7O_4\text{-}C_8H_{17}]^+$	Monopalmitin	WsAG, FgAG	[30]

* Characteristic component in WsAG. # Characteristic component in FgAG. s Identified with a standard, a Compared with spectral data obtained from Wiley Subscription Services, Inc. (New York, NY, USA).

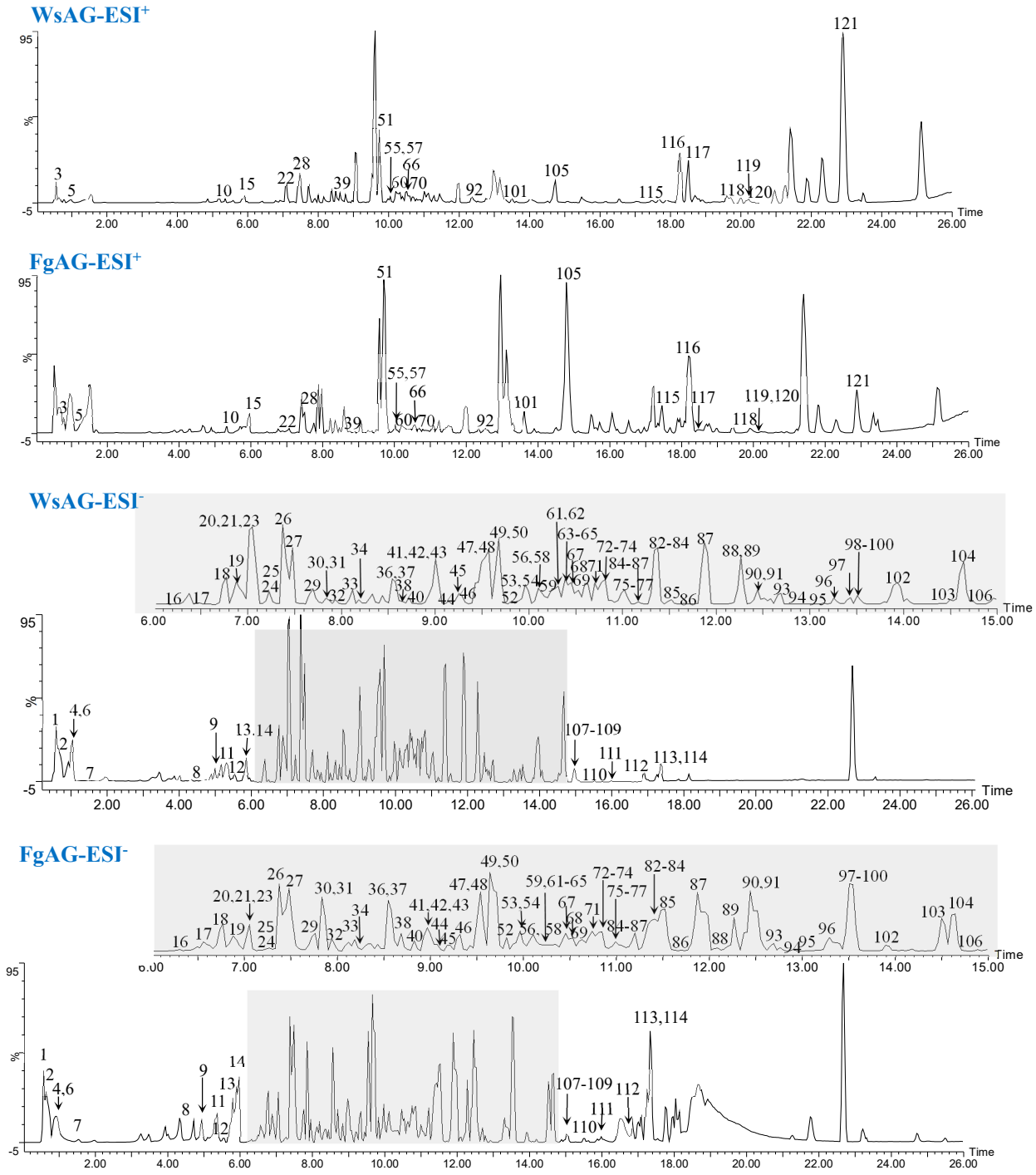
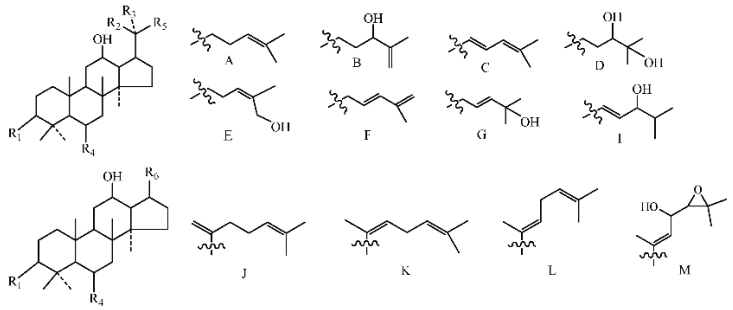


Figure 1. The representative base peak intensity (BPI) chromatograms of FgAG and WsAG in negative and positive modes.

Triterpenoids

Dammarane ginsenosides

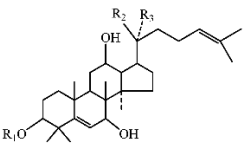


Protopanaxtriol-type saponins

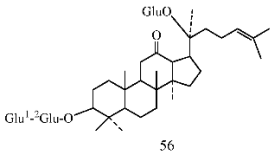
13. R_1–OH, R_2–OGlu, R_3–CH_3, R_4–OGlu^4-^{1}Glu, R_5–A
17. R_1=OH, R_2=OGlu^6-^{1}Glu, R_3=CH_3, R_4=OGlu^2-^{1}Rha, R_5=A
18. R_1–OH, R_2–OGlu^6-^{1}Araf, R_3–CH_3, R_4–OGlu, R_5–A
21. R_1–OH, R_2–OGlu, R_3–CH_3, R_4–OGlu^2-^{1}Xyl, R_5=A
23. R_1=OH, R_2=OGlu^6-^{1}Glu, R_3=CH_3, R_4=OGlu, R_5=A
26 R_1=OH, R_2=OGlu, R_3=CH_3, R_4=OGlu, R_5=A
27 R_1–OH, R_2–OGlu, R_3–CH_3, R_4–OGlu^2-^{1}Rha, R_5–A
29 R_1=OH, R_2=OGlu, R_3=CH_3, R_4=OGlu^6-Mal, R_5=A
31. R_1–OH, R_2–OGlu, R_3–CH_3, R_4–OMal-^{6}Glu^2-^{1}Rha, R_5–A
36. R_1–OH, R_2–OGlu^6-Ac,, R_3–CH_3, R_4–OGlu R_5–A
37. R_1=OH, R_2=OH, R_3=CH_3, R_4=OGlu^2-^{1}Arap, R_5=A
53. R_1=OH, R_2=OGlu^2-^{1}Araf, R_3=CH_3, R_4=OH, R_5=A
61. R_1–OH, R_2–CH_3, R_3–OH, R_4–OGlu^2-^{1}Rha, R_5=A
63. R_1=OH, R_2–OH, R_3=CH_3, R_4–OGlu, R_5=A
64. R_1–OH, R_2–OGlu^2-^{1}Arap, R_3=CH_3, R_4–OH, R_5=A
79. R_1=OH, R_2=CH3, R_3=OH, R_4=OGlu, R_5=A
9. R_1–OH, R_2–OGlu, R_3–CH_3, R_4–OGlu^2-^{1}Rha, R_5–G
14. R_1=OH, R_2=OH, R_3=CH_3, R_4=OGlu^2-^{1}Rha, R_5=D
30. R_1–OH, R_2–OH, R_3=CH_3, R_4–OGlu^2-^{1}Rha, R_5=E
38. R_1–OH, R_2–OGlu, R_3–CH_3, R_4–OGlu^2-^{1}Rha, R_5–I
66. R_1=OH, R_4=OGlu, R_6=K
92. R_1–OH, R_4–OGlu^2-^{1}Rha, R_6–L
94. R_1–OH, R_4–OGlu^2-^{1}Rha, R_6–M

Protopanaxdiol-type saponins

33. R_1=OGlu^2-^{1}Glu, R_2=O-Ara, R_3=CH_3, R_4=H, R_5=A
52. R_1–OGlu^2-^{1}Glu, R_2–OGlu^6-^{1}Glu^3-^{1}Xyl, R_3–CH_3, R_4=H, R_5=A
54. R_1–OGlu^2-^{1}Glu, R_2–OGlu^6-^{1}Araf^2-^{1}Xyl, R_3–CH_3, R_4–H, R_5–A
58. R_1=OGlu^2-^{1}Glu^2-^{1}Xyl, R_2=OGlu^6-^{1}Glu, R_3=CH_3, R_4=H, R_5=A
59. R_1=OGlu^2-^{1}Glu, R_2=OGlu^6-^{1}Glu, R_3=CH_3, R_4=H, R_5=A
62. R_1–OGlu^2-^{1}Glu^6-Mal, R_2–OGlu^6-^{1}Glu, R_3–CH_3, R_4–H, R_5–A
65. R_1=OGlu^2-^{1}Glu, R_2=OGlu^6-^{1}Araf, R_3=CH_3, R_4=H, R_5=A
67. R_1–OGlu^2-^{1}Glu, R_2–OGlu^6-^{1}Arap^4-^{1}Xyl, R_3–CH_3, R_4–H, R_5–A
68. R_1–OGlu^2-^{1}Glu^6-Mal, R_2–OGlu^6-^{1}Ara, R_3–CH_3, R_4–H, R_5–A
72.R_1=OGlu^2-^{1}Glu, R_2=OGlu^6-^{1}Arap, R_3=CH_3, R_4=H, R_5=A
73. R_1–OGlu^2-^{1}Glu, R_2–OGlu^6-^{1}Xyl, R_3–CH_3, R_4–H, R_5–A
74. R_1=OGlu^2-^{1}Glu^6-Ac, R_2=OGlu^6-^{1}Arap, R_3=CH_3,R_4=H, R_5=A
75. R_1–OGlu^2-^{1}Glu^6-Mal, R_2–OGlu^6-^{1}Arap, R_3–CH_3, R_4–H, R_5–A
76. R_1=OGlu^2-^{1}Glu^6-Ac, R_2=OGlu^6-^{1}Glu, R_3=CH_3, R_4=H, R_5=A
78. R_1–OGlu^2-^{1}Glu^6-Mal, R_2–OGlu^6-^{1}Xyl, R_3–CH_3, R_4–H, R_5–A
81. R_1–OGlu^6-^{1}Glu, R_2–OGlu, R_3–CH_3, R_4–H, R_5–A
83. R_1=OGlu^2-^{1}Glu, R_2=OGlu, R_3=CH_3, R_4=H, R_5=A
84. R_1–OGlu^2-^{1}Glu^6-Ac, R_2–OGlu^6-^{1}Arap, R_3–CH_3, R_4–H, R_5–A
85.R_1=OGlu^2-^{1}Glu-Mal, R_2=OGlu, R_3=CH_3, R_4=H, R_5=A
86. R_1=OGlu^2-^{1}Glu^6-Ac, R_2=OGlu^6-^{1}Araf, R_3=CH_3, R_4=H, R_5=A
87. R_1–OGlu, R_2–OGlu^6-^{1}Glu, R_3–CH_3, R_4–H, R_5–A
89. R_1=OGlu, R_2=OGlu^6-^{1}Araf, R_3=CH_3, R_4=H, R_5=A
90. R_1=OGlu^2-^{1}Glu^6-Acetyl, R_2=OGlu, R_3=CH_3, R_4=H, R_5=A
91.R_1=OArap^3-^{6}Glu^2-^{1}Glu, R_2=OH, R_3=CH_3, R_4=H, R_5=A
93. R_1=OGlu, R_2=OGlu^6-^{1}Xyl, R_3=CH_3, R_4=H, R_5=A
95. R_1=OGlu^2-^{1}Glu^6-1-oxo-2-butenyl, R_2=OGlu, R_3=CH_3, R_4=H, R_5=A
96. R_1=OGlu, R_2=OGlu^6-^{1}Rha, R_3=CH_3, R_4=H, R_5=A
99. R_1–OGlu, R_2–OGlu, R_3–CH_3, R_4–H, R_5–A
103. R_1=OGlu^2-^{1}Glu, R_2=CH_3, R_3=OH, R_4=H, R_5=A
106. R_1=OGlu^2-^{1}Glu, R_2=OH, R_3=CH_3, R_4=H, R_5=A
107. R_1=OH, R_2=OGlu^2-^{1}Xyl, R_3=CH_3, R_4=H, R_5=A
112. R_1–OGlu^2, R_2–OH, R_3–CH_3, R_4–H, R_5–A
15. R_1=Glu, R_2=-OGlu, R_3=CH_3, R_4=H, R_5=B
43. R_1=OGlu^2-^{1}Glu, R_2=-OGlu, R_3=CH_3, R_4=H, R_5=B
44. R_1=OGlu^2-^{1}Glu, R_2=OH, R_3=CH_3, R_4=H, R_5=C
35. R_1=OGlu^2-^{1}Glu, R_2=OGlu^6-^{1}Xyl, R_3=CH_3, R_4–H, R_5=D
19. R_1=OGlu^2-^{1}Glu, R_2=OGlu, R_3=CH_3, R_4=H, R_5=E
40. R_1=OGlu^2-^{1}Glu, R_2–OGlu, R_3–CH_3, R_4–H, R_5=F
42. R_1–OGlu, R_2–OGlu^2-^{1}Xyl, R_3–CH_3,, R_4–H, R_5–F
24. R_1–OGlu^2-^{1}Glu^2-^{1}Xyl, R_2–OH, R_3–CH_3 R_4–H, R_5–G
97. R_1–OGlu^2-^{1}Glu, R_4–H, R_6–J
101. R_1=OGlu, R_4=H, R_6=K 114. R_1–OGlu^2-^{1}Glu, R_4=H, R_6–K



25. R_1–Glu^6-^{1}Glu, R_2–Glu^6-^{1}Glu, R_3–CH_3
41. R_1–Glu^2-^{1}Glu, R_2–Glu^6-^{1}Xyl, R_3–CH_3
48. R_1=Glu^2-^{1}Glu, R_2=Glu, R_3=CH_3



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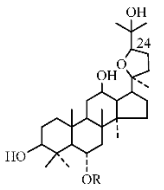
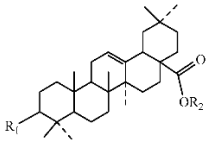
Ocotillol-type saponins

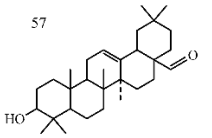


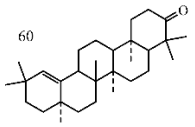
11. R=OGlu^1-^{6}Glu^2-^{1}Glu 24*R*
12. R=OGlu^2-^{1}Glu^2-^{1}Ara 24*R*
20. R=OGlu^2-^{1}Rha 24*S*
32. R=Glu^1-^{6}OGlu^2-^{1}Xyl 24*S*
34. R=Ac-^{6}OGlu^2-^{1}Rha 24*S*
47. R=OGlu^2-^{1}Xyl 24*S*
49. R=OGlu^2-^{1}Rha 24*R*
50. R=OGlu 24*R*
51. R=OGlu 24*S*
80. R=Ac-^{6}OGlu^2-^{1}Xyl 24*S*

Oleanane-type saponins

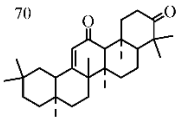


10. R_1=OH, R_2=Glu
39. R_1=H, R_2=H
46 R_1=OGluA^4-^{1}Glu, R_2=Glu^6-^{1}Glu
55. R_1=OArap^2-^{1}Rha, R_2=H
69. R_1–OGluA^2-^{1}Glu, R_2–Glu
71. R_1=OGlu^1-^{3}OGluA^4-^{1}Araf, R_2=Glu
77. R_1–OGluA^4-^{1}Araf, R_2–Glu
82. R_1–OGluA^6-^{1}Glu, R_2–H
98. R_1–OAraf^4-^{4}GluA^6-^{1}Glu, R_2=H
102. R_1=OGluA, R_2=Glu
108. R_1=OGluA^2-^{1}Xyl, R_2=H
110. R_1–OGluA-methyl ester, R_2–Glu
116. R_1=OCOCH$_3$ R_2=H





Ursane



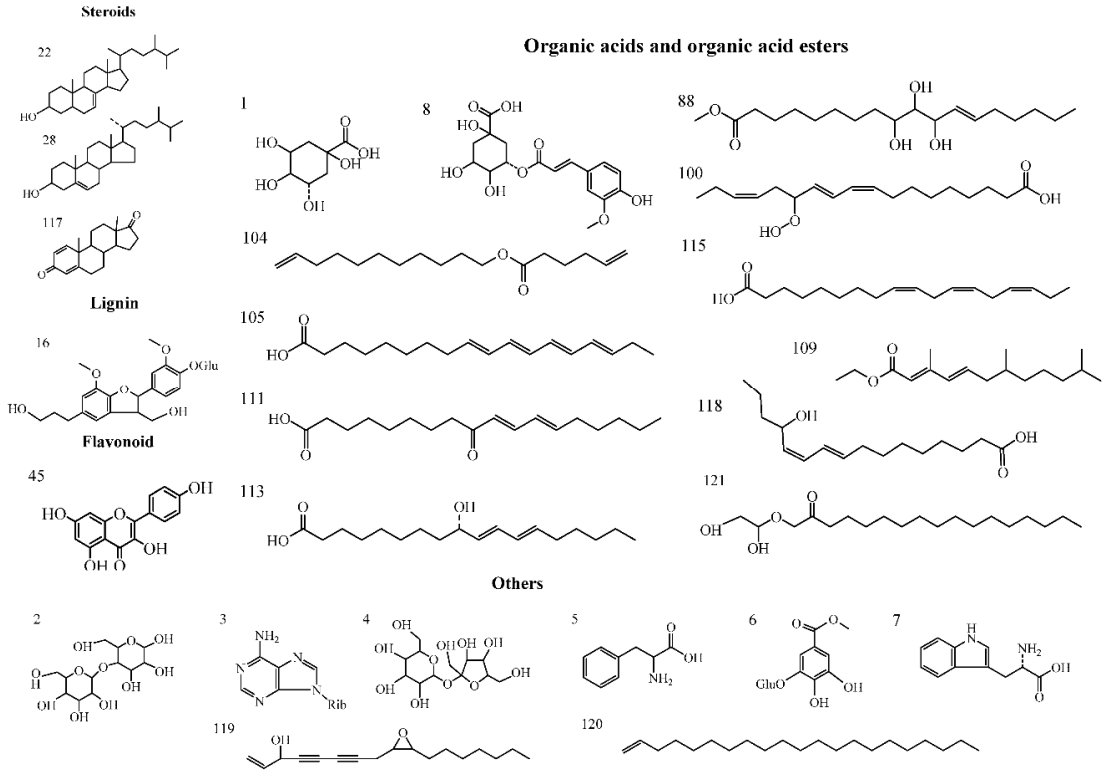


Figure 2. Chemical structures of compounds identified in FgAG and WsAG.

In FgAG and WsAG, these compounds were all shared constituents, including 47 protopanaxdiol-type saponins, 23 protopanaxtriol-type saponins, 15 oleanane- type saponins, 10 ocotillol-type saponins, one ursane, one flavonoid, one lignin, 12 organic acids and organic acid esters, three steroids, and eight other compounds.

2.2. Biomarker Discovery for FgAG and WsAG

The QC injections were clustered tightly in PCA indicating a satisfactory stability of the system. The PCA 2D plots of the samples from FgAG and WsAG groups were classified into two clusters according to their common spectral characteristics (Figure 3), with the FgAG samples of different years clustered into one group, while the WsAG samples were clustered into another group. The FgAG and WsAG samples were clearly separated, indicating that these two herb species could be easily differentiated.

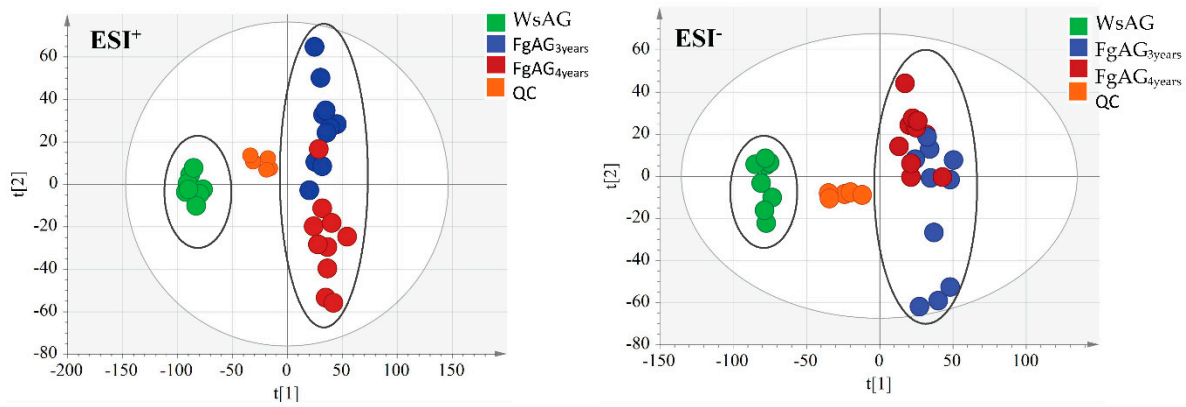


Figure 3. The PCA of FgAG and WsAG in positive mode and negative mode.

After OPLS-DA plots (Figures 4A and 5A) in both negative and positive modes were generated, the maximum separation between MsAG and FgAG groups was available. In the sufficient permutation test, the lines of grouping samples were significantly located underneath the random sampling lines (Figures 4B and 5B), which indicated a fine validity for the following characteristic metabolites biomarkers identification [42]. S-plots were then created to explore the potential chemical markers that contributed to the differences. Based on p values ($p < 0.05$) and VIP values (VIP > 3) [30,61] from univariate statistical analysis, 22 robust known biomarkers enabling the differentiation between FgAG and WsAG, were marked and listed (Figures 4C and 5C and Table 2). Additionally, a heatmap was generated from these biomarkers in order to systematically evaluate the biomarkers (Figure 6), which visually showed the intensities of potential biomarkers between two species.

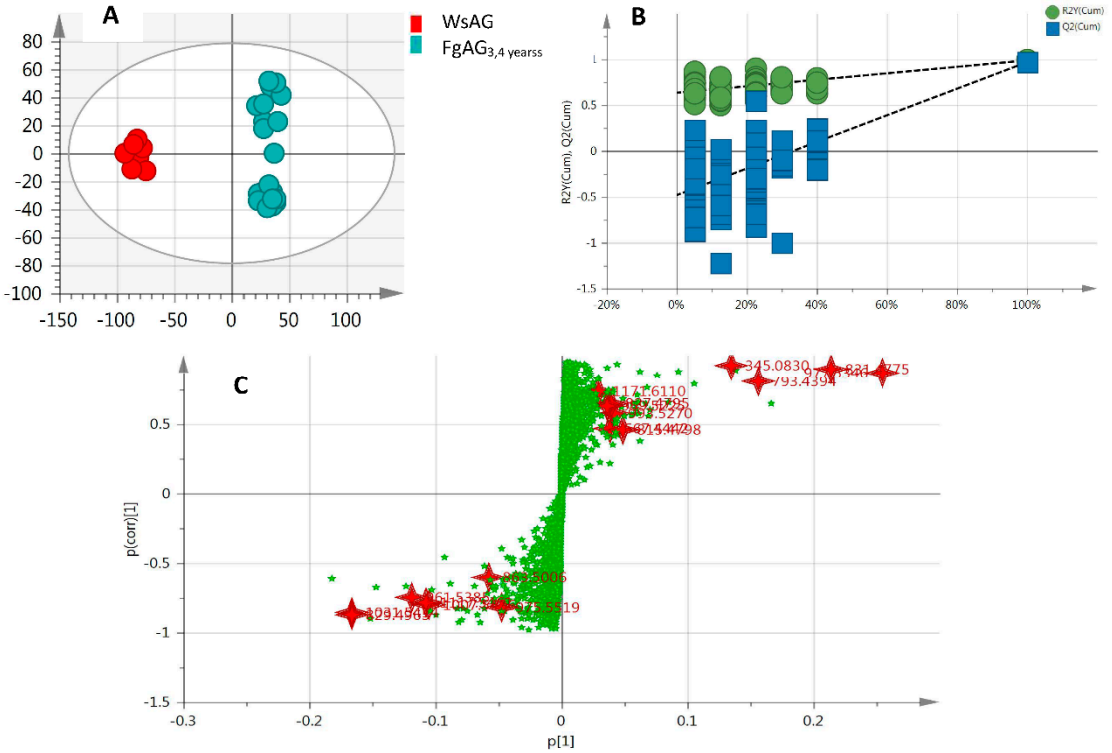


Figure 4. The OPLS-DA (**A**); permutation tests (**B**) and S-plot (**C**) in negative mode.

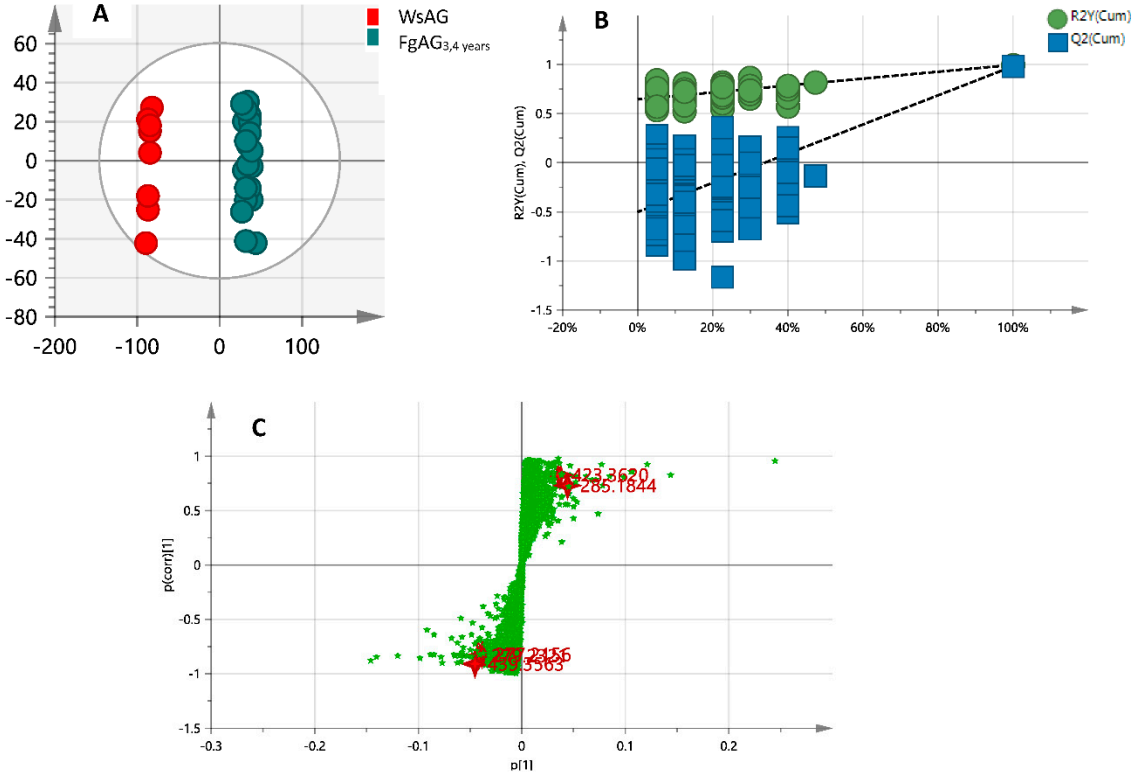


Figure 5. The OPLS-DA (**A**); permutation tests (**B**) and S-plot (**C**) in positive mode.

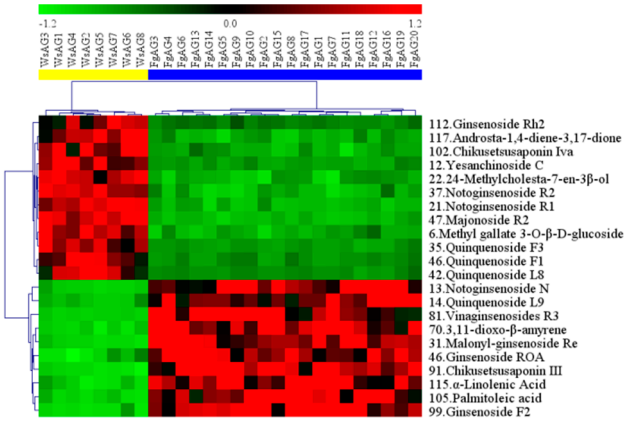


Figure 6. The heatmap visualizing the intensities of potential biomarkers.

3. Discussion

In the screening analysis, 121 compounds were characterized in FgAG and WsAG, respectively. The results showed that both of these kinds of American ginseng were rich in natural components. These 121 compounds were all shared constituents in FgAG and WsAG, which means that they were similar in terms of the kinds of compound they contained. It has been reported that there are high ginsenoside contents in American ginseng. In this study, ginsenosides were also the main chemical components. Besides the most common dammarane-type and oleanane-type saponins, the ocotillol-type saponins are also occupying a notable proportion. The ocotillol-type is the characteristic type of saponin enabling American ginseng to be differentiated from Asian ginseng. So far, the studies on the mechanism of biosynthesis were focused on dammarane-type and oleanane-type ginsenosides. For example, dammaranediol was obtained by DS (dammarenediol synthase), and then modified by CYP450 to obtain dammarane-type saponins. Another example, oleanane-type

ginsenosides were obtained by modifing β-amyrin with CYP450 and UGT (UDP-glycosyltransferase). Actually, there were little literature about the mechanism of ocotillol-type ginsenoside biosynthesis. The phytochemicals in WsAG and FgAG might provide a material basis for mechanistic studies. In short, this comprehensive and unique phytochemical profile study revealed the structural diversity of secondary metabolites and the similar patterns in FgAG and WsAG.

Furthermore, in non-targeted metabolomic analysis, when taking the contents of the constituents into account, it was found that there indeed existed quite a few differences between FgAG and WsAG, and 22 robust known biomarkers enabling the differentiation were discovered. This study illustrated the differences between FgAG and WsAG, and provided a basis for explaining the effect of different growth environments on secondary metabolites. For WsAG, there are 12 potential biomarkers, including two ocotillol-type saponins (**12**, **47**), two steroids (**22**, **117**), six damarane-type saponins (**21**, **35**, **37**, **42**, **44**, **112**), one oleanane-type saponin (**102**) and one other compound (**6**). The contents of these 12 components in WsAG were much greater than in FgAG. On the other hand, for FgAG, there are 10 potential biomarkers including two organic acids (**105, 115**), six damarane-type saponins (**13, 14, 31, 46, 81, 91, 99**), one oleanane-type saponin (**46**), and one ursane (**70**), which contents in FgAG were much greater than in WsAG. It has been reported that wild American ginseng has better biological activity than the FgAG. As is known, biological activity is caused by the high contents of phytochemicals. Correlation studies between potential markers and biological activities could be performed in the future.

Even so, there are still several unresolved issues. For example, as shown in BPI chromatograms, though 121 compounds were identified, there are still some unidentified components. Further research should be carried on based on the formula of these unknown compounds.

4. Materials and Methods

4.1. Materials and Reagents

Twenty eight batches of commercially available FgAGs and WsAGs root products were collected or purchased from different cultivation areas in China and American, including 20 batches of FgAGs and eight batches of WsAGs. A detailed sample list is provided in Table 2.

For FgAGs, six roots of each sample were selected for analysis, while for WsAGs, 2–3 roots of each sample were analyzed. All the herbs were authenticated by the authors and the corresponding voucher specimens have been deposited in the Research Center of Natural Drug, School of Pharmaceutical Sciences, Jilin University, China.

A total of 25 saponins were isolated in our laboratory and identified by spectroscopic data. Among of these saponins, ginsenoside Ro [69], 15 ginsenosides [70,71] (Rb_1, Rb_2, Rb_3, Rc, Rd, Re, 20(*S*)-Rg_3, 20(*R*)-Rg_3, 20(*S*)-Rh_2, Rg_1, 20(*R*)-Rg_2, 20(*S*)-Rh_1, 20(*R*)-Rh_1, pseudo-ginsenoside F_{11}, pseudo-ginsenoside RT_5) and another six saponins [71] (quinquenoside L_8, L_9, L_{11}, F_3, 24(*S*)-pseudo-ginsenoside F_{11}, gypenoside XVII) were isolated and identified by our group.

Oleanolic acid-28-*O*-β-D-glucopyranoside, ginsenoside Rs_1, -Rs_2 and methyl gallate-3-*O*-β-D-glucoside were also isolated in our laboratory and identified by NMR spectroscopy. Adenosine, α-maltose, L-tryptophan, notoginsenoside R_1, kaempferol, L-phenylalanine, sucrose, palmitoleic acid, quinic acid and α-linolenic acid were purchased from Beijing Zhongke Quality Inspection Biotechnology Co., Ltd. (Beijing, China).

Acetonitrile and methanol suitable for UPLC-MS were purchased from Fisher Chemical Company (Geel, Belgium). Formic acid was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Deionized water was purified using a Millipore water purification system (Millipore, Billerica, MA, USA). All other chemicals were of analytical grade.

Table 2. Details of FgAG and WsAG samples.

Species and the Morphological Features	Source	Growth Year	Collection Time	Batch No.
FgAGs *Main roots 9~15 cm (length) × 1.5~3.0 cm (diameter); 2~3 branch roots with diameters of 2~3.5 cm; fibrous roots with diameters of 0.1~0.2 cm; 3~4 stem scars in rhizomes; no adventitious roots.*	Ji'an City, Jilin Province, China	3, 4	2017.09–2017.10	FgAG1, 11
	Fusong County, Jilin Province, China	3, 4	2017.09–2017.10	FgAG2, 12
	Tonghua City, Jilin Province, China	3, 4	2017.09–2017.10	FgAG3,13
	Jingyu Country, Jilin Province, China	3, 4	2017.09–2017.10	FgAG4, 14
	Antu Country, Jilin Province, China	3, 4	2017.09–2017.10	FgAG5, 15
	Hunchun City, Jilin Province, China	3, 4	2017.09–2017.10	FgAG6, 16
	Helong City, Jilin Province, China	3, 4	2017.09–2017.10	FgAG7, 17
	Huadian City, Jilin Province, China	3, 4	2017.09–2017.10	FgAG8, 18
	Huairou District, Beijing Province, China	3, 4	2017.10–2017.11	FgAG9, 19
	Wendeng Area, Shandong Province, China	3, 4	2017.10–2017.11	FgAG10, 20
WsAGs *Main roots 5.0~6.0 cm (length) × 1.5~2.0 cm (diameter); 2~3 branch roots with diameters of 0.5~0.9 cm; fibrous roots with diameters of 0.1~0.2 cm; 15~25 stem scars in rhizomes; adventitious roots with diameters of 0.5~0.8 cm*	Lawton Coumtry, Michigan State, American	>15	2017.09–2017.11	WsAG1, 3, 6
	Schoharie County, Catskill region, American	>15	2017.09–2017.10	WsAG2, 4, 8
	Monongalia County, West Virginia, American	>15	2017.10–2017.11	WsAG5, 7

4.2. Sample Preparation and Extraction

All samples were respectively air-dried, grinded and sieved (Chinese National Standard Sieve No. 3, R40/3 series) to get a homogeneous powder. Then each fine powder was accurately weighed (0.2 g) and soaked with 10 mL of methanol overnight. On the second day, each powder was extracted in an ultrasonic bath (power of 250 W, frequency of 40 kHz) for half an hour. After cooling to room temperature, the lost weight was replenished with methanol. After filtering through a syringe filter (0.22 μm), the extracts were injected directly into the UPLC system. In addition, to ensure the stability and suitability consistency of MS analysis, a quality control (QC) sample was prepared by pooling the same volume (50 μL) from every sample and five QC injections were performed randomly through the whole worklist. The volumes injected for samples and QC were all 5 μL for each run.

4.3. UPLC-QTOF-MS

UPLC-QTOF-MS^E analysis was performed on a Waters Xevo G2-XS QTOF mass spectrometer (Waters Co., Milford, MA, USA) equipped with a UPLC system through an electrospray ionization (ESI) interface. Chromatographic separation was performed on an ACQUITY UPLC BEH C_{18} (100 mm × 2.1 mm, 1.7 μm) from Waters Corporation (Milford, MA, USA). The mobile phases were composed of eluent A (0.1% formic acid in water, v/v) and eluent B (0.1% formic acid in acetonitrile, v/v) with flow rate of 0.4 mL/min. The elution conditions applied were: 0→2 min, 10% B; 2→26 min, 10~100% B; 26→29 min, 100% B; 29→29.1 min, 100~10% B; 29.1→32 min, 10% B. The temperature of the autosampler and the UPLC column manager were set at 15 °C and 30 °C respectively. Mixtures of 90/10 and 10/90 water/acetonitrile were used as the weak wash solvent and the strong wash solvent respectively. The mass spectrum was acquired from 100 to 1500 Da in MS^E mode. The positive mode conditions were: capillary voltage, 2.6 kV; cone voltage, 40 V; source temperature, 150 °C; desolvation temperature, 400 °C; cone gas flow, 50 L/h; desolvation gas flow, 800 L/h. Negative mode conditions were identical to the positive mode conditions except for capillary voltage (2.2 kV). In MS^E mode, data acquisition was performed via the mass spectrometer by rapidly switching from a low-collision energy (CE) scan to a high-CE scan during a single LC run. The low energy function was set to 6 V, while ramp collision energy of high energy function was set to 20~40 V. Leucine enkephalin (m/z 556.2771 in positive mode and 554.2615 in negative mode) was used as external reference of Lock Spray™ infused at a constant flow of 10 μL/min. During acquisition, data were collected in continumn mode for the screening analysis, and in centroid mode for the metabolomics analysis.

4.4. Chemical Information Database for the Components of FgAG and WsAG

Besides the in-house Traditional Medicine Library in the UNIFI software, a systematic investigation of chemical components was conducted [34]. A self-built database of compounds that were isolated from FgAG and WsAG was established by searching online databases or internet search engines such as PubMed, Full-Text Database (CNKI), ChemSpider, Web of Science and Medline. Chemical information including the component name, molecular formula and structure of the components from the herbs were obtained from the database [56].

4.5. The Screening Analysis by the UNIFI Platform

To quickly identify the chemical compounds, the MS raw data, compressed with Waters Compression and Archival Tool v1.10, was automatically screened and identified by using the streamlined workflow of UNIFI 1.7.0 software (Waters, Manchester, UK) [30]. The parameters were as follows: the minimum peak area of 200 was set for 2D peak detection; the peak intensity of low energy over 1000 counts and the peak intensity of high energy over 200 counts were selected for 3D peak detection. Mass error up to ±5 ppm for identified compounds, retention time in the range of ±0.1 min was allowed to match the reference substance. The matching compounds would be generated predicted fragments from structure. The negative adducts containing +COOH and -H

and positive adducts containing +H and +Na were selected in the analysis. Leucine-enkephalin was selected as the reference compound, and $[M - H]^-$ 554.2620 was used for negative ion and $[M + H]^+$ 556.2766 was used for positive ion [72].

4.6. The Metabolomics Analysis

The raw data were processed by MarkerLynx XS V4.1 software (Waters, Milford, CT, USA) for alignment, deconvolution, data reduction, etc. [73]. A MarkerLynx processing method was firstly created, and the main parameters were as follows: retention time range 0–26 min, mass range 100–1500 Da, mass tolerance 0.10, minimum intensity 5%, mass window 0.10, retention time window 0.20, marker intensity threshold 2000 counts and noise elimination level 6. After processing the data, the results could be showed in the Extended Statistics (XS) Viewer. m/z-RT pairs with corresponding intensities for all the detected peaks from each data file were listed. The same value of RT and m/z in different batched of samples were regarded as the same component. Then, multivariate statistical analysis was performed. Firstly, principal component analysis (PCA) was used to show the pattern recognition and maximum variation aiming to obtain the overview and classification. Secondly, orthogonal projections to latent structures discriminant analysis (OPLS-DA) in both positive and negative modes was performed in order to get the maximum separation between MsAG and FgAG group and to explore the potential chemical markers that contributed to the differences. Then, S-plots was created to provide visualization of the OPLS-DA predictive component loading to facilitate model interpretation. Meawhile, variable importance for the projection (VIP) was helpful to screen the different components, and the metabolites with VIP value (>3.0) were considered as potential markers [29]. In addition, the permutation test was performed to provide reference distributions of the R^2/Q^2-values that could indicate the statistical significance [30–33]. Simca 15.0 software (Umetrics, Malmö, Sweden) was used to show the analysis results [56,74].

5. Conclusions

In a comprehensive and unique phytochemical profile study, a total of 121 chemical compounds with different structural types were identified from WsAG and FgAG. The structural patterns included protopanaxdiol-type saponins, protopanaxtriol-type saponins, ocotillol-type saponins, oleanane-type saponins and other glyosides, organic acid and organic acid esters, steroids, etc. The results showed that WsAG and FgAG were rich in natural components. Furthermore, these 121 compounds were all shared constituents in them, meaning that they were similar in the kinds of compounds they contain. In metabolomic analysis, it was found that there indeed existed several differences in the contents of the constituents between FgAG and WsAG, and 22 robust known biomarkers enabling the differentiation were discovered. In a word, the results will fill the data gap in the study on the chemical constituents of WsAG and provide a reference for quantitative determinations in its quality control.

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Conflicts of Interest: The authors declare that they have no conflicts of interest concerning this article.

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