

Article

UPLC-QTOF/MS-Based Nontargeted Metabolomic Analysis of Mountain- and Garden-Cultivated Ginseng of Different Ages in Northeast China

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Abstract: Aiming at further systematically comparing the similarities and differences of the chemical components in ginseng of different ages, especially comparing the younger or the older and mountain-cultivated ginseng (MCG), 4, 5, 6-year-old cultivated ginseng (CG) and 12, 20-year-old MCG were chosen as the analytical samples in the present study. The combination of UPLC-QTOF-MS^E, UNIFI platform and multivariate statistical analysis were developed to profile CGs and MCGs. By the screening analysis based on UNIFI, 126 chemical components with various structural types were characterized or tentatively identified from all the CG and MCG samples for the first time. The results showed that all the CG and MCG samples had the similar chemical composition, but there were significant differences in the contents of markers. By the metabolomic analysis based on multivariate statistical analysis, it was shown that CG_{4–6 years}, MCG_{12 years} and MCG_{20 years} samples were obviously divided into three different groups, and a total of 17 potential age-dependent markers enabling differentiation among the three groups of samples were discovered. For differentiation from other two kinds of samples, there were four robust makers such as α -linolenic acid, 9-octadecenoic acid, linoleic acid and panaxydol for CG_{4–6 years}, five robust makers including ginsenoside Re₁, -Re₂, -Rs₁, malonylginsenoside Rb₂ and isomer of malonylginsenoside Rb₁ for MCG_{20 years}, and two robust makers, 24-hydroxyoleanolic acid and palmitoleic acid, for MCG_{12 years} were discovered, respectively. The proposed approach could be applied to directly distinguish MCG root ages, which is an important criterion for evaluating the quality of MCG. The results will provide the data for the further study on the chemical constituents of MCG.

Keywords: mountain-cultivated ginseng; identification; metabolomic analysis; UPLC-QTOF-MS

1. Introduction

Ginseng, the king of herbs in the Orient, has always received a lot of attention, not only as a therapeutic medicinal herb, but also as a health supplement. According to the different growing environments and diverse cultivation methods, there two kinds of ginseng are distinguished in the Chinese Pharmacopoeia: cultivated ginseng (CG) and mountain-cultivated ginseng (MCG). CG is cultivated artificially in gardens, while MCG is grown for at least 10 years [1,2]. MCG, also called “Lin-Xia-Shan-Shen”, can be regarded as a replacement of wild ginseng. MCG is of better quality than CG and offers more production than wild ginseng [3]. Actually, the adulteration or falsification of the cultivation age of MCG has always been a serious problem in the MCG commercial market. As we all know, the chemical components and biological activities of ginseng with different cultivation ages are

distinct [4,5], and more aged ginseng is usually of higher economic value. In an investigation of the characteristic components for distinguishing CG (4–7-year of age) and MCG (with 15-years of growth), 12 compounds, including ginsenoside Ra₃/isomer, gypenoside XVII, quinquenoside R₁, ginsenoside Ra₇, notoginsenoside Fe, ginsenoside Ra₂, ginsenoside Rs₆/Rs₇, malonyl ginsenoside Rc, malonyl ginsenoside Rb₁, malonyl ginsenoside Rb₂, palmitoleic acid, and ethyl linoleate were regarded as the characteristic chemical markers for the discrimination [6]. Recently, a UPLC/QTOF-MS-based metabolomics approach was applied to the global metabolite profiling of MCG leaf samples aged from 6 to 18 years, and the authors claimed that the approach could also be applied to discriminate MCG root ages indirectly [7]. It is undoubted that the developed method can be used as a standard protocol for discriminating and predicting MCG leaf ages directly, but there might be some inaccuracy and uncertainty when discriminating MCG root ages indirectly.

In the past decades, some analytical methods focusing on ginsenosides had been used to distinguish MCG from CG, such as thin layer chromatography (TLC), or high performance liquid chromatography (HPLC) [8,9]. However, these technologies require lots of time and energy, and the results cannot provide a comprehensive or accurate discrimination between them. Currently, untargeted metabolomics, combined with multivariate statistical methods such as principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), are widely used to profile diverse classes of metabolites and to better understand the chemical diversity and the multiple pharmacological effects of ginsenosides or ginseng [10,11]. Given the multi-component property, the combination of LC-MS-based metabolomic profiling with multivariate statistical analysis methods was used as a rapid means of characterization and was increasingly applied for analyzing ginseng from different herbs, cultivation environments/areas, cultivation ages or different parts [12,13]. As an example, for different herbs belonging to the same genus, specific biomarkers including chikusetsusaponin IVa, ginsenoside Rf and ginsenoside Rc were selected and verified for ginseng [14]. In another example of different parts analysis, the metabolic profiles of root, leaf, flower bud, berry and seed of ginseng were investigated [12,15]. In addition, the approach for the discrimination of different red ginseng root parts was reported. As a result, fine roots had the highest protopanaxadiol (PPD)/protopanaxatriol (PPT) ratio, which could clearly distinguish the main roots from the lateral roots and fine roots parts [16]. Such analysis was also applied to make metabolite profiling and age discrimination of 4- and 6-year-old red ginseng [17], or 1–6 years ginseng [18].

In addition, UNIFI, the automated data processing software, is an integrated informatics platform that possesses the ability to incorporate scientific library into a streamlined workflow, aiming at identifying chemical components from complex raw data [19]. The combination of UPLC separation, Q/TOF-MS detection and UNIFI platform has been frequently applied in the characterization of chemical constituents of herbs [20,21].

Normally, CG is harvested after a 4–6 years cultivation period, and MCG is collected at ages of 10–20 years. To develop a more direct and more efficient discrimination method for the cultivation ages and to explore potential age-dependent markers, we chose 4, 5, 6-year-old CG and 12, 20-year-old MCG as the analytical samples in the present study. UPLC-QTOF-MS^E, UNIFI platform and multivariate statistical analysis were then used to profile these two kinds of ginseng. The aims were to systematically screen the chemical components and to perform the non-targeted metabolomic analysis, and in turn will lay the foundation for the establishment of CG and MCG quality criteria in the future. In one hand, this study will reveal the structural diversity of secondary metabolites and the different patterns in CG and MCG. In the other hand, the present study could provide a reference point for a reliable, accurate method for distinguishing among CG and MCG samples of different ages.

2. Materials and Methods

2.1. Materials and Reagents

A total of 40 batches of CG and MCG root products, including 24 batches of CGs and 16 batches of MCGs, were collected from different cultivation areas in Jilin Province, the main source of ginseng in China. A detailed sample list is given in Table 1. All samples were harvested and collected by Professor Li Ping-ya from Jilin University Institute of Frontier Medical Science, according to China Pharmacopoeia (2015 version) [22]. Voucher specimens have been deposited at the Research Center of Nature Drug, School of Pharmaceutical Sciences, Jilin University, Changchun, China.

Table 1. Details of the MCG and CG samples.

Sample No.	Source	Collection Time
CG _{3years} -1, CG _{3years} -2; CG _{4years} -1, CG _{4years} -2; CG _{5years} -1, CG _{5years} -2; MCG _{12years} -1, MCG _{12years} -2; MCG _{20years} -1, MCG _{20years} -2	Ji'an City, Jilin Province, China	2017.09–2017.10
CG _{3years} -3, CG _{3years} -4; CG _{4years} -3, CG _{4years} -4; CG _{5years} -3, CG _{5years} -4; MCG _{12years} -3, MCG _{12years} -4; MCG _{20years} -3, MCG _{20years} -4	Fusong County, Jilin Province, China	2017.09–2017.10
CG _{3years} -5, CG _{3years} -6; CG _{4years} -5, CG _{4years} -6; CG _{5years} -5, CG _{5years} -6; MCG _{12years} -5, MCG _{12years} -6; MCG _{20years} -5, MCG _{20years} -6	Tonghua City, Jilin Province, China	2017.09–2017.10
CG _{3years} -7, CG _{3years} -8; CG _{4years} -7, CG _{4years} -8; CG _{5years} -7, CG _{5years} -8; MCG _{12years} -7, MCG _{12years} -8; MCG _{20years} -7, MCG _{20years} -8	Jingyu Country, Jilin Province, China	2017.09–2017.10

Acetonitrile, methanol were all UPLC-MS pure grade (Fisher Scientific Inc., Geel, Belgium). Formic acid (MS grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Leucine enkephaline was provided by Waters (Waters Technologies, Milford, MA., USA). Distilled water was prepared in-house via a Millipore water purification system (Millipore, Billerica, MA, USA). All other chemicals were analytical grade. For reference substances, ginsenoside F₁ (R20151040), -F₂ (R20151040), notoginsenoside R₁ (R20170210), notoginsenoside R₄ (R20170212) were provided by the Research Center of Natural Drugs, School of Pharmaceutical Sciences, Jilin University, China. Ginsenoside Rb₁, -Rb₂, -Rb₃, -Rc, -Rd, -Re, -Rf, -F₅, -Rg₁, 20(R)-Rg₂, 20(S)-Rh₁, 20(R)-Rh₁, 20(S)-Rg₃, 20(R)-Rg₃, -Ro, gypenoside XVII, ginsenoside Rs₁, -Rs₂ were isolated in our laboratory and identified by spectroscopic data. Adenine (101774299), tryptophane (73-22-2), palmitoleic acid (101491588) were purchased from Sigma-Aldrich. Notoginsenoside Fe (8105-29-5), D-adenosine (110879-200502), histidine (624-200304) were purchased from the National Institutes for Food and Drug Control. Ginsenoside Rg₅ (wkq16051002, Victory Biological Technology Co., Ltd., Sichuan, China), α -linoleic acid (B21469; Yuanye Biological Technology Co., Ltd., Shanghai, China), D-arginin (130701; Nuoye Biological Engineering Co., Ltd., Anhui, China) and phenylpropionic acid (A20160211), quillaic acid (A20171109) were purchased from Beijing Zhongke Quality Inspection Biotechnology Co., Ltd. (Beijing, China) with the Chinese National Standard Sieve No. 3 (R40/3 series).

2.2. Sample Preparation and Extraction

All the CG and MCG samples were air-dried, grinded (Baijie Stainless Steel Grinder, BJ-800A, Deqing Baijie Electric Appliance Co. Ltd., Zhejiang, China) and sieved (Chinese National Standard Sieve No. 3, R40/3 series) to get the homogeneous powder respectively. Then, the powder of 40 samples (200 mg accurately weighed per sample) were refluxed respectively with 85% methanol (2 L) at 80 °C for three times (2 h, 2 h, 1 h each time, respectively). Then, the extracts of each sample were combined, concentrated and evaporated to dryness. Each powder was dissolved in 5.0 mL of 80% methanol. After being filtered, each methanolic solution was injected directly into UPLC system.

Meanwhile, 20 μ L aliquots of each CG and MCG sample were mixed to obtain a quality control (QC) sample, which contained all of the components in the analysis. The QC sample was run randomly to monitor the stability of the system. All of the above solutions were stored at 4 °C prior to LC-MS analysis and the injection volume was 2 μ L.

2.3. UPLC/QTOF-MS^E

The chromatographic separation and mass spectrometry detection were conducted on the Waters Acquity UPLC system coupled with a Xevo G2-S QTOF mass spectrometer equipped with an electrospray ionization source (ESI). Separation was performed on Waters ACQUITY UPLC BEH C₁₈ column (100 mm \times 2.1 mm, 1.7 μ m) at 40 °C. The mobile phase consisted of eluent A (0.1% formic acid aqueous solution) and eluent B (0.1% formic acid in acetonitrile) at flow rate of 0.4 mL/min with the following gradient program: 0~2 min, 10% (B); 2~26 min, 10%~100% (B); 26~28 min, 100% (B); 28~28.1 min, 100%~10% (B); 28.1~30 min, 10% (B). Mixtures of 10/90 and 90/10 water/acetonitrile were the strong wash and the weak wash solvent, respectively. The optimized conditions were employed: source temperature was 120 °C, the desolvation temperature was 300 °C, capillary voltage was 2.6 kV (ESI⁺) or 2.2 kV (ESI⁻), cone voltage was 40 V, desolvation gas flow was 800.0 L/h, cone gas flow was 50 L/h. The energy of low energy function and the collision energy of high energy function were set at 6 V and 20 V~40 V respectively in MS^E mode. The mass spectrometer was calibrated with sodium formate in the range of 200–1500 Da. The lockmass compound used was leucine-enkephaline (external reference to the ion m/z 556.2771 in positive mode and 554.2615 in negative mode). Data were collected with MasslynxTM V4.1 workstation in continuum mode.

2.4. Chemical Information Database for the Components of CG and MCG

In addition to the Waters Traditional Medicine Library in UNIFI software, a systematic investigation of chemical constituents from the target herbs based on the literature was conducted. A self-built database of compounds, such as saponins, flavonoids, volatile oil, amino acids and so on, isolated from CG and MCG was established by searching online databases such as China Journals of Full-Text Database (CNKI), PubMed, Medicine, Web of Science and ChemSpider. The name, molecular formula and structure of components from CG and MCG were obtained in the database.

2.5. The Screening Analysis Based on UNIFI Platform

UNIFI 1.7.0 software (Waters, Manchester, UK) was used to perform the screening analysis on the structural characteristics and MS fragmentation behaviors, especially for characteristic fragments. Main parameters were set as follows: peak intensity of high energy over 200 counts and the peak intensity of low energy over 1000 counts were the selected parameters in peak detection; mass error up to ± 10 ppm for identified compound; retention time tolerance was set in the range of ± 0.1 min; positive adducts containing +H, +Na or negative adducts containing -H, +HCOOH were all selected; the reference compound was leucine-enkephalin (556.2766 for positive ion, 554.2620 for negative ion). The MS raw data were processed using the streamlined workflow of UNIFI software to quickly identify the chemical components that met the match criteria with the in-house Traditional Medicine Library and the self-built database [20,21].

2.6. The Metabolomics Analysis Based on Multivariate Statistical Analysis

To differentiate MCG and CG, MarkerLynx XS V4.1 software (Waters, Milford, DE, USA) was used to process the raw data by deconvolution, alignment, data reduction and to perform the multivariate statistical analysis [20,21]. The following steps were performed: acquiring data, creating a MarkerLynx processing method, processing the acquired data and viewing results Extended Statistics (XS) Viewer. The main parameters in the method set to process the raw data were as follows: retention time range 5–28 min, mass range 200–1400 Da, mass tolerance 5 mDa, intensity threshold 2000 counts, mass

window 0.05 Da, retention time window 0.20 min. In resulting database list, RT- m/z pairs represent an identifier of ion in the order of their elution time. The same value of RT and m/z in different batches of samples were regarded as the same compound. Multivariate statistical analysis was then performed to find the potential biomarkers that significantly contributed to the difference among the groups. During the analysis, principal component analysis (PCA) was firstly used to show the maximum variation and pattern recognition in order to get the overview and classification, and the orthogonal projections to latent structures discriminant analysis (OPLS-DA) was then performed aiming to get the maximum separation between two groups. S-plots was then available to provide visualization of the OPLS-DA predictive component loading to facilitate model interpretation. Variable importance for the projection (VIP) was also used to help screen the different components, and the metabolites with VIP value above 1.0 were considered as potential markers. Additionally, a permutation test was performed to provide reference distributions of the R^2/Q^2 -values that could indicate the statistical significance. Simca 15.0 software (Umetrics, Malmö, Sweden) was used to show the analysis results.

3. Results and Discussion

3.1. Identification of Components from MCG and CG Based on UNIFI Platform

As a result of our analysis, a total of 126 compounds, including triterpenoids (the main ingredients), flavonoids, organic acids and organic acid esters, alcohol phenols, aldehyde ketones and amino acids, etc., were characterized or tentatively identified from the MCG and CG in both ESI⁺ and ESI⁻ modes. 85 compounds were identified in ESI⁺ mode and 41 compounds were identified in ESI⁻ mode. Base peak intensity (BPI) chromatograms are shown in Figure 1, the identification information is listed in Table 2, and the chemical structures are shown in Figure 2.

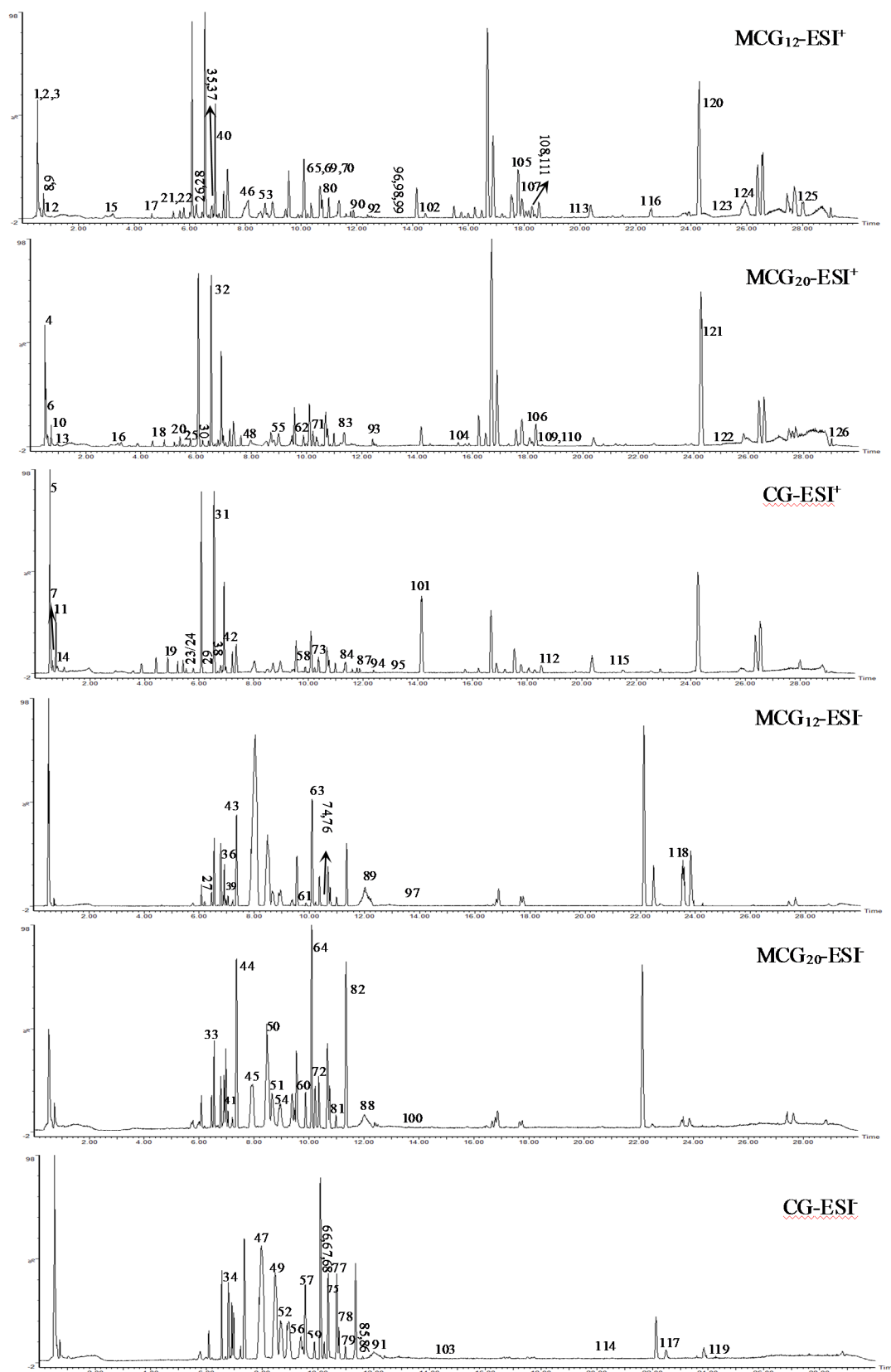


Figure 1. The representative BPI chromatograms of CG and MCG in positive and negative modes.

Table 2. Compounds identified from MCG and CG by UPLC-QTOF-MS^E.

No.	t _R (min)	Formula	Calculated Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)	MS ^E Fragmentation	Identification	Sources	Ref.
1	0.49	C ₅ H ₈ N ₂ O ₅	176.0431	176.0433	−1.5	177.0503[M + H] ⁺ ; 130.0495[M − 2 × OH − NH ₂] ⁺	Dencichine	CG, MCG ₁₂ , MCG ₂₀	[23]
2	0.54	C ₆ H ₁₄ N ₄ O ₂	174.1115	174.1117	−1.1	175.1188[M + H] ⁺ ; 158.0912[M − NH ₂] ⁺ ; 116.0704[M − NH ₂ − CN ₂ H ₂] ⁺ ; 114.1015[M − NH ₂ − CHO ₂] ⁺	Adipodihydrazide	CG, MCG ₁₂ , MCG ₂₀	a
3	0.55	C ₃₀ H ₂₂ O ₁₀	542.1257	542.1213	8.1	543.1330[M + H] ⁺ ; 273.0833[M − C ₁₅ H ₁₀ O ₅] ⁺ ; 242.1025[M − OH − C ₁₅ H ₁₀ O ₆] ⁺ ; 127.0388[M − C ₂₄ H ₁₇ O ₇] ⁺ ; 116.0703[M − 2 × OH − C ₂₁ H ₁₂ O ₈] ⁺ ; 109.0284[M − C ₂₄ H ₁₅ O ₈] ⁺	Chamaejasmine	CG, MCG ₁₂ , MCG ₂₀	a
4	0.59	C ₁₂ H ₂₂ O ₁₁	342.1156	342.1162	−1.6	365.1055[M + Na] ⁺ ; 203.0550[M − OH − C ₄ H ₈ O ₄] ⁺ ; 185.0444[M − 2 × OH − C ₄ H ₈ O ₄] ⁺	α-Maltose	CG, MCG ₁₂ , MCG ₂₀	[24]
5	0.69	C ₆ H ₁₄ N ₄ O ₂	174.1115	174.1117	−0.9	175.1188[M + H] ⁺	D-Arginin	CG, MCG ₁₂ , MCG ₂₀	s
6	0.74	C ₁₉ H ₁₈ O ₁₁	422.0842	422.0849	−1.7	423.0915[M + H] ⁺ ; 268.1040[M + H − C ₆ H ₆ O ₅] ⁺ ; 119.0349[M − C ₁₅ H ₁₀ O ₇] ⁺	Isomangiferin	CG, MCG ₁₂ , MCG ₂₀	[25]
7	0.75	C ₅ H ₅ N ₅	135.0546	135.0545	0.5	136.0618[M + H] ⁺ ; 119.0352[M − NH ₂] ⁺	Adenine	CG, MCG ₁₂ , MCG ₂₀	s
8	0.76	C ₁₀ H ₁₃ N ₅ O ₄	267.0974	267.0968	2.4	268.1050[M + H] ⁺ ; 136.0618[M − C ₅ H ₉ O ₄] ⁺ ; 119.0352[M − C ₅ H ₉ O ₄ − NH ₂] ⁺	D-Adenosine	CG, MCG ₁₂ , MCG ₂₀	s
9	0.80	C ₂₀ H ₁₅ NO ₆	365.0876	365.0899	−6.3	366.0949[M + H] ⁺	Integriamide	CG, MCG ₁₂ , MCG ₂₀	a
10	0.81	C ₂₅ H ₃₀ O ₁₂	538.1677	538.1686	−1.7	561.1569[M + Na] ⁺ ; 393.1138[M − C ₆ H ₈ O ₄] ⁺ ; 381.0788[M − CH ₃ − C ₇ H ₁₀ O ₃] ⁺ ; 366.0930[M − OH − C ₈ H ₁₀ O ₃] ⁺ ; 366.0930[M − C ₁₉ H ₂₁ O ₈] ⁺	Linearoside	CG, MCG ₁₂ , MCG ₂₀	[26]
11	0.82	C ₉ H ₁₁ NO ₂	165.0782	165.0790	−0.5	166.0862[M + H] ⁺ ; 120.0805[M − COOH] ⁺ ; 103.0543[M − COOH − NH ₂] ⁺	Phenylpropionic acid	CG, MCG ₁₂ , MCG ₂₀	s
12	0.91	C ₆ H ₉ N ₃ O ₂	155.0762	155.0695	−3.5	156.0762[M + H] ⁺	Histidine	CG, MCG ₁₂ , MCG ₂₀	s
13	1.05	C ₁₁ H ₁₂ N ₂ O ₂	204.0898	204.0899	−0.5	205.0971[M + H] ⁺ ; 188.0706[M − NH ₂] ⁺ ; 143.0723[M − NH ₂ − COOH] ⁺ ; 118.0649[M − NH ₂ − COOH − C ₂ H ₃] ⁺	Tryptophan	CG, MCG ₁₂ , MCG ₂₀	s
14	1.06	C ₆ H ₁₄ N ₄ O ₂	174.1117	174.1117	0.0	175.1190[M + H] ⁺	Argentine	CG, MCG ₁₂ , MCG ₂₀	[27]
15	3.13	C ₂₅ H ₂₈ O ₄	392.2009	392.1988	5.5	393.2082[M + H] ⁺	Glabrol	CG, MCG ₁₂ , MCG ₂₀	[28]
16	3.27	C ₂₅ H ₂₄ O ₅	404.1646	404.1624	5.1	405.1719[M + H] ⁺	Puerarol	CG, MCG ₁₂ , MCG ₂₀	[29]
17	4.42	C ₂₇ H ₃₈ O ₆	458.2716	458.2668	10.0	481.2609[M + Na] ⁺ ; 436.2642[M − COOH] ⁺	Lucideric acid	CG, MCG ₁₂ , MCG ₂₀	a
18	4.64	C ₃₆ H ₅₈ O ₈	618.4107	618.4132	−4.0	619.4180[M + H] ⁺ ; 421.3446[M − Glc − OH] ⁺	β-D-Glucopyranosyl oleanolate	CG, MCG ₁₂ , MCG ₂₀	[30]
19	4.89	C ₄₈ H ₈₂ O ₁₉	962.5484	962.5450	3.3	985.5312[M + Na] ⁺ ; 765.4795[M − Glc − OH] ⁺ ; 541.2637[M − Glc − OH − C ₁₅ H ₂₈ O] ⁺ ; 421.3463[M − Glc − Glc/Rha − 2 × OH] ⁺	Majoroside F6	CG, MCG ₁₂ , MCG ₂₀	[31]
20	5.21	C ₃₁ H ₄₆ O ₈	546.3248	546.3193	9.7	569.3140[M + Na] ⁺ ; 133.0859[M − C ₂₅ H ₃₃ O ₅] ⁺	Methyl ganoderate G	CG, MCG ₁₂ , MCG ₂₀	a

Table 2. Cont.

No.	t _R (min)	Formula	Calculated Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)	MS ^E Fragmentation	Identification	Sources	Ref.
21	5.53	C ₃₅ H ₄₈ O ₉	612.3344	612.3298	7.4	613.3416[M + H] ⁺ ; 582.3264[M – OCH ₃] ⁺ ; 526.2986[M – C ₄ H ₇ O ₂] ⁺	Cinobufagin 3-hemisuberate methyl ester	CG, MCG ₁₂ , MCG ₂₀	a
22	5.56	C ₄₅ H ₇₄ O ₁₇	886.4877	886.4926	−5.4	909.4769[M + Na] ⁺ ; 745.4383[M – CH ₂ OH – C ₆ H ₁₀ O ₂] ⁺ ; 729.4136[M – OH – C ₈ H ₁₄ O ₂] ⁺ ; 601.2768[M – 2 × OH – C ₁₆ H ₂₆ O ₄] ⁺ ; 431.1870[M – Glc – C ₁₉ H ₂₇ O ₂] ⁺	Shatavarin IV	CG, MCG ₁₂ , MCG ₂₀	[32]
23	5.64	C ₂₅ H ₃₂ O ₁₃	540.1800	540.1843	3.0	541.1873[M + H] ⁺ ; 347.0906[M – CH ₃ O – C ₂ H ₄ – C ₈ H ₉ O ₂] ⁺ ; 195.1008[M – Glc – C ₂ H ₄ O ₂ – C ₆ H ₅ O ₂] ⁺	Oleuropein	CG, MCG ₁₂ , MCG ₂₀	[33]
24	5.65	C ₁₄ H ₁₆ O ₄	248.1025	248.1049	−9.7	271.0917[M + Na] ⁺ ; 195.1008[M – C ₂ H ₄ – COH] ⁺ ; 189.1348[M – OH – COOH] ⁺	Isohistiopterosin A	CG, MCG ₁₂ , MCG ₂₀	a
25	5.79	C ₄₅ H ₇₄ O ₁₇	886.4881	886.4926	−5.0	909.4773[M + Na] ⁺ ; 707.4360[M – Glc] ⁺ ; 689.4262[M – Glc – OH] ⁺ ; 657.3636[M – Glc – OH – 2 × CH ₃] ⁺ ; 609.3646[M – Glc – C ₆ H ₁₀ O ₂] ⁺ ; 523.3626[M – Glc/Glc – C ₃ H ₆] ⁺	Malonylginsenoside Rf	CG, MCG ₁₂ , ## MCG ₂₀	[34]
26	6.21	C ₄₂ H ₇₀ O ₁₄	798.4778	798.4766	1.6	799.4851[M + H] ⁺ ; 439.3563[M – Glc/Rha – 2 × OH] ⁺ ; 421.3441[M – Glc/Rha – 3 × OH – H ₂ O] ⁺	Ginsenoside Rg ₈	CG, MCG ₁₂ , MCG ₂₀	[34]
27	6.23	C ₂₂ H ₃₂ O ₁₃	504.1840	504.1843	−0.6	503.1767[M – H] [−] ; 457.1715[M – OH – CH ₂ OH] [−] ; 293.0878[M – C ₂ H ₄ O – CH ₂ OH – C ₈ H ₉ O ₂] [−]	Cistanoside H	CG, MCG ₁₂ , MCG ₂₀	[35]
28	6.24	C ₂₃ H ₂₈ O ₁₁	480.1594	480.1632	−7.7	481.1667[M + H] ⁺ ; 317.0803[M – C ₁₀ H ₁₂ O ₂] ⁺	Peoniflorin	CG, MCG ₁₂ , MCG ₂₀	a
29	6.28	C ₂₄ H ₃₀ O ₁₂	510.1702	510.1737	−6.9	511.1775[M + H] ⁺ ; 317.0803[M – C ₁₁ H ₁₄ O ₃] ⁺	Mudanpioside D	CG, MCG ₁₂ , MCG ₂₀	[36]
30	6.39	C ₅₄ H ₉₂ O ₂₄	1124.5943	1124.5978	−3.1	1147.5835[M + Na] ⁺ ; 585.2870[M – C ₂₅ H ₄₇ O ₁₂] ⁺ ; 325.1130[M – C ₄₂ H ₇₁ O ₁₄] ⁺	Ginsenoside V	CG, MCG ₁₂ , MCG ₂₀	[31]
31	6.49	C ₄₈ H ₈₂ O ₁₉	962.5484	962.5450	3.3	985.5302[M + Na] ⁺ ; 865.4789[M – C ₆ H ₉ O] ⁺ ; 823.4787[M – C ₈ H ₁₁ O ₂] ⁺ ; 805.4668[M – C ₈ H ₁₃ O ₃] ⁺ ; 555.2763[M – C ₁₂ H ₂₆ O ₅] ⁺ ; 423.3602[M – Glc – Glc/Glc – OH] ⁺ ; 405.3507[M – Glc – Glc/Glc – 2 × OH] ⁺	Ginsenoside Re ₁	CG, MCG ₁₂ , ##,* MCG ₂₀	[37]
32	6.59	C ₂₃ H ₂₈ O ₁₁	480.1587	480.1632	−9.3	481.1660[M + H] ⁺ ; 317.0810[M – C ₇ H ₅ O – C ₃ H ₅ O] ⁺	Mudanpioside I	CG, MCG ₁₂ , MCG ₂₀	[38]
33	6.64	C ₄₁ H ₇₀ O ₁₄	786.4762	786.4766	−0.4	831.4744[M + HCOO] [−] ; 653.4270[M – H – C ₅ H ₈ O ₄] [−] ; 491.3710[M – H – C ₁₁ H ₁₈ O ₉] [−]	Notoginsenoside Rw ₂	CG, MCG ₁₂ , MCG ₂₀	[39]
34	6.67	C ₄₇ H ₈₀ O ₁₈	932.5335	932.5345	−1.0	977.5317[M + HCOO] [−] ; 785.4693[M – Ara – CH ₃] [−] ; 653.4282[M – Glc – 2 × OH – C ₅ H ₉] [−]	Quinquenoside F ₆	CG, MCG ₁₂ , MCG ₂₀	[37]
35	6.77	C ₃₆ H ₆₀ O ₉	636.4217	636.4237	−3.1	637.4290[M + H] ⁺ ; 621.42740[M – OH] ⁺ ; 423.3605[M – Glc – 2 × OH] ⁺	Ginsenoside Rh ₈	CG, MCG ₁₂ , MCG ₂₀	[40]
36	6.84	C ₄₈ H ₈₂ O ₁₉	962.5469	962.5450	1.9	1007.5456[M + HCOO] [−] ; 799.4848[M – Glc] [−] ; 637.4317[M – Glc/Glc] [−] ; 179.0545[Glc – H] [−]	20-β-D-Glucopyranosyl-ginsenoside Rf	CG, MCG ₁₂ , MCG ₂₀	[41]
37	6.80	C ₄₂ H ₇₀ O ₁₃	782.4773	782.4816	−5.4	805.4665[M + Na] ⁺ ; 765.4734[M – OH] ⁺ ; 677.4220[M – 2 × OH – C ₄ H ₇ O] ⁺ ; 661.4265[M – 3 × OH – C ₄ H ₇ O] ⁺ ; 439.3562[M – Glc – Man – OH] ⁺	Ginsenoside Rh ₁₄	CG, MCG ₁₂ , MCG ₂₀	[40]

Table 2. Cont.

No.	t _R (min)	Formula	Calculated Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)	MS ^E Fragmentation	Identification	Sources	Ref.
38	6.82	C ₁₇ H ₂₄ O ₈	356.1460	356.1472	−3.1	379.1352[M + Na] ⁺ ; 145.0495[M − OH − C ₁₁ H ₁₃ O ₃] ⁺	Erigeside II	CG, MCG ₁₂ , MCG ₂₀	[42]
39	6.96	C ₄₇ H ₈₀ O ₁₈	932.5410	932.5345	6.7	977.5392[M + HCOO] [−] ; 799.4825[M − Xyl] [−] ; 769.4724[M − H − Glc] [−] ; 637.4291[M − (Glc/Xyl)] [−] ; 179.0539 [Glc − H] [−]	Notoginsenoside R ₁	CG, MCG ₁₂ , MCG ₂₀	s
40	6.99	C ₂₈ H ₄₄ O ₁₂	572.2810	572.2833	−3.9	573.2883[M + H] ⁺ ; 555.2779[M − OH] ⁺ ; 531.2860[M − C ₂ H ₃ O] ⁺	Picrasinoside G	CG, MCG ₁₂ , MCG ₂₀	a
41	7.05	C ₄₈ H ₈₂ O ₁₉	962.5425	962.5450	−2.6	1007.5415[M + HCOO] [−] ; 799.4822[M − Glc] [−] ; 637.4333[M − (Glc/Glc)] [−]	Notoginsenoside N	CG, MCG ₁₂ , MCG ₂₀	[43]
42	7.20	C ₄₈ H ₈₂ O ₁₉	962.5422	962.5450	−2.9	985.5314[M + Na] ⁺ ; 703.4371[M − Glc − 2 × OH − CH ₂ OH] [−] ; 439.3565[M − Glc − Glc/Glc − OH] [−]	Ginsenoside Re ₂	CG, MCG ₁₂ , ##,***MCG ₂₀	[40]
43	7.34	C ₄₂ H ₇₂ O ₁₄	800.4934	800.4922	1.4	845.4916[M + HCOO] [−] ; 637.4344[M − Glc] [−] ; 475.3798[M − Glc − Glc] [−] ; 179.0553[Glc − H] [−] ;	Ginsenoside Rg ₁	CG, MCG ₁₂ , MCG ₂₀	s
44	7.36	C ₄₈ H ₈₂ O ₁₈	946.5524	946.5501	2.3	991.5506[M + HCOO] [−] ; 783.4912[M − Glc] [−] ; 637.4344[M − (Glc/Rha)] [−] ; 475.3798[M − Glc − (Glc/Rha)] [−]	Ginsenoside Re	CG, MCG ₁₂ , MCG ₂₀	s
45	7.74	C ₄₅ H ₇₄ O ₁₇	886.4925	886.4926	−0.1	885.4853[M − H] [−] ; 781.4740[M − HOCOCH ₂ COOH] [−] ; 619.4197[M − Glc(Mal)] [−] ; 161.0438[Glc − H ₂ O] [−]	Malonylginsenoside Rg ₁	CG, MCG ₁₂ , MCG ₂₀	[39]
46	7.93	C ₄₈ H ₇₆ O ₁₉	956.4960	956.4981	−2.2	979.4852[M + Na] ⁺ ; 799.4161[M − CO ₂ − CH ₂ OH − C ₆ H ₁₂] ⁺ ; 641.4008[M − Glc − C ₄ H ₆ O ₅] ⁺ ; 439.3562[M − Glc − Glc/Glc(mal)] ⁺ ; 145.0493[Glc − OH] ⁺	Isomer of ginsenoside Ro	# CG, MCG ₁₂ , MCG ₂₀	[31]
47	8.04	C ₅₁ H ₈₄ O ₂₁	1032.5532	1032.5505	2.6	1031.5460[M − H] [−] ; 987.5564[M − CO ₂] [−] ; 927.5337[M − HOCOCH ₂ COOH] [−] ; 781.4759[M − Rha(Mal)] [−] ; 619.4222[M − (Rha(Mal)/Glc)] [−]	Malonylginsenoside Re	CG, MCG ₁₂ , MCG ₂₀	[39]
48	8.08	C ₄₈ H ₇₆ O ₁₉	956.4950	956.4981	−3.1	979.4842[M + Na] ⁺ ; 817.4311[M − Glc] ⁺ ; 439.3571[M − Glc/Glc − Glc − OH] ⁺	Isomer of ginsenoside Ro	CG, MCG ₁₂ , MCG ₂₀	[44]
49	8.09	C ₄₄ H ₇₄ O ₁₅	842.5032	842.5028	0.5	841.4959[M − H] [−] ; 799.4861[M − CH ₂ O] ⁺ ; 781.4741[M − CH ₂ O − OH] ⁺ ; 637.4316[M − Xyl(mal)] ⁺ ; 619.4228[M − Xyl(mal) − OH] ⁺ ; 475.3798[M − Xyl(mal) − Glc] ⁺ ; 179.0550[Glc − H] ⁺ ; 161.0439[Glc − OH] ⁺	Yesanchinoside D	CG, MCG ₁₂ , MCG ₂₀	[45]
50	8.10	C ₄₅ H ₇₄ O ₁₇	886.4931	886.4926	0.6	885.4858[M − H] [−] ; 781.4741[M − H − HOCOCH ₂ COOH] [−] ; 619.4228[M − H − Glc(Mal)] [−] ; 161.0439[Glc − H − H ₂ O] [−]	Isomer of malonylginsenoside Rg ₁	CG, MCG ₁₂ , MCG ₂₀	[39]
51	8.49	C ₄₁ H ₇₀ O ₁₃	770.4801	770.4816	−1.5	815.4784[M + HCOO] [−] ; 637.4321[M − Xyl] [−]	Notoginsenoside R ₂	CG, MCG ₁₂ , MCG ₂₀	[39]
52	8.50	C ₅₆ H ₉₄ O ₂₄	1150.6124	1150.6135	−1.1	1149.6051[M − H] [−] ; 1119.5951[M − CH ₂ OH − 2 × OH] [−] ; 807.4861[M − Glc/Glc − OH] [−] ; 605.4423[M − Glc/Glc − Glc(mal)] [−] ; 325.1119[Glc/Glc − OH] [−]	Quinquenoside R ₁	CG, MCG ₁₂ , ## MCG ₂₀	[46]

Table 2. Cont.

No.	t _R (min)	Formula	Calculated Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)	MS ^E Fragmentation	Identification	Sources	Ref.
53	8.60	C ₂₂ H ₃₀ O ₄₇	406.1957	406.1992	−8.5	407.2030[M + H] ⁺ ; 376.1859[M − OCH ₃] ⁺	Nigakilactone K	CG, MCG ₁₂ , MCG ₂₀	[47]
54	8.87	C ₄₈ H ₈₂ O ₁₉	962.5445	962.5450	−0.5	1007.5427[M + HCOO] [−] ; 797.4706[M − Glc] [−] 1187.5821[M + Na] ⁺ ; 1147.5803[M − OH] ⁺ ;	Ginsenoside Re ₃	CG, MCG ₁₂ , MCG ₂₀	[37]
55	8.96	C ₅₆ H ₉₂ O ₂₅	1164.5929	1164.5928	0.1	805.4305[M − Ara/Glc − CH ₂ OH − CH ₃] ⁺ ; 443.3868[M − Ara/Glc − Glc/Glc(mal)] ⁺ 1285.6740[M + HCOO] [−] ; 945.5421[M − (Ara/Xyl)] [−] ;	Malonylginsenoside Rb ₂	CG, MCG ₁₂ , ^{##,*} MCG ₂₀	[44]
56	9.41	C ₅₉ H ₁₀₀ O ₂₇	1240.6488	1240.6452	2.8	913.5184[M − (Glc/Glc)] [−] ; 783.4900[M − (Ara/Xyl) − Glc] [−] 845.4903[M + HCOO] [−] ; 637.4319[M − Glc] [−] ;	Notoginsenoside R ₄	CG, MCG ₁₂ , MCG ₂₀	s
57	9.56	C ₄₂ H ₇₂ O ₁₄	800.4921	800.4922	−0.1	475.3786[M − (Glc/Glc)] [−] ; 1,3A _{2β} 221.0658; 161.0439[Glc − H − H ₂ O] [−] , 2,5A _{1β} 101.0235	Ginsenoside Rf	CG, MCG ₁₂ , MCG ₂₀	s
58	9.79	C ₁₈ H ₃₄ O ₅	330.2398	330.2406	−2.3	353.2290[M + Na] ⁺ ; 213.1459[M + H − COOH − C ₅ H ₁₁] ⁺	12,13,15-Trihydroxy-9-octadecenoic acid	CG, MCG ₁₂ , MCG ₂₀	[48]
59	9.87	C ₄₁ H ₇₀ O ₁₃	770.4809	770.4816	−1.0	815.4791[M + HCOO] [−] ; 475.3783[M − (Glc/Xyl)] [−] ; 161.0437[Glc − H − H ₂ O] [−]	Ginsenoside F ₅	CG, MCG ₁₂ , MCG ₂₀	s
60	9.89	C ₆₀ H ₁₀₂ O ₂₈	1270.6635	1270.6558	5.9	1315.6617[M + HCOO] [−] ; 841.4991[M − Glc/Glc − OH − C ₄ H ₄] [−] ; 769.4777[M − Glc/Glc/Glc − CH ₃] [−] 1255.6340[M + HCOO] [−] ; 1077.5833[M − Xyl] [−] ;	Ginsenoside Ra ₀	CG, MCG ₁₂ , MCG ₂₀	[49]
61	9.94	C ₅₈ H ₉₈ O ₂₆	1210.6358	1210.6346	1.0	1047.5719[M − Glc] [−] ; 955.4871[M − Glc − OH − C ₄ H ₇] [−] ; 783.4892[M − Glc/Xyl/Rha] [−] 469.1084[M + Na] ⁺ ; 429.1154[M − OH] ⁺ ; 385.0884[M − OH − CH ₃ − CH ₂ OH] ⁺ ; 341.0661[M − C ₄ H ₈ O ₃] ⁺ ;	Ginsenoside Ra ₂	CG, MCG ₁₂ , MCG ₂₀	[50]
62	10.00	C ₂₂ H ₂₂ O ₁₀	446.1192	446.1213	−4.5	237.0746[M − C ₁₀ H ₁₃ O ₅] ⁺ ; 193.0483[M − C ₁₂ H ₁₆ O ₆] ⁺ 1285.6444[M + HCOO] [−] ; 1107.5964[M − Xyl] [−] ;	Glycitin	CG, MCG ₁₂ , MCG ₂₀	a
63	10.01	C ₅₉ H ₁₀₀ O ₂₇	1240.6462	1240.6452	0.8	945.5424[M − (Glc/Xyl)] [−] ; 783.4912[M − Xyl − GlcGlc] [−] 1153.6083[M + HCOO] [−] ; 945.5437[M − Glc] [−] ;	Notoginsenoside Fa	CG, MCG ₁₂ , MCG ₂₀	[50]
64	10.05	C ₅₄ H ₉₂ O ₂₃	1108.6101	1108.6029	6.2	783.4888[M − (Glc/Glc)] [−] ; 621.4382[M − (Glc/Glc) − Glc] [−] ; 459.3835[M − (Glc/Glc) − (Glc/Glc)] [−] ; 2,5A _{1β} 101.0235	Ginsenoside Rb ₁	CG, MCG ₁₂ , MCG ₂₀	s
65	10.10	C ₄₂ H ₇₀ O ₁₂	766.4863	766.4867	−0.5	767.4936[M + H] ⁺ ; 443.3866[M − Rha − Glc] ⁺ ; 425.3762[M − Rha − Glc − OH] ⁺	Ginsenoside Rg ₄	CG, MCG ₁₂ , MCG ₂₀	[34]
66	10.20	C ₅₇ H ₉₄ O ₂₆	1194.6087	1194.6033	4.5	1193.6015[M − H] [−] ; 1149.6098[M − CO ₂] [−] ; 783.4908[M − Glc/Glc] [−] ; 179.0545[Glc − H] [−]	Isomer of malonylginsenoside Rb ₁	CG, MCG ₁₂ , ^{##,*} MCG ₂₀	[39]
67	10.22	C ₄₂ H ₇₂ O ₁₃	784.4997	784.4973	2.9	829.4979[M + HCOO] [−] ; 637.4336[M − Rha] [−] ; 475.3809[M − (Glc/Rha)] [−] ; 161.0449 [Rha − H] [−]	20(R)-Ginsenoside Rg ₂	CG, MCG ₁₂ , MCG ₂₀	s
68	10.25	C ₃₆ H ₆₂ O ₉	638.4407	638.4394	2.9	683.4389[M + HCOO] [−] ; 161.0449[Glc − H − H ₂ O] [−]	Ginsenoside Rh ₁	CG, MCG ₁₂ , MCG ₂₀	s

Table 2. Cont.

No.	t _R (min)	Formula	Calculated Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)	MS ^E Fragmentation	Identification	Sources	Ref.
69	10.27	C ₄₁ H ₇₀ O ₁₃	770.4779	770.4816	−4.7	793.4672[M + Na] ⁺ ; 587.4276[M − Ara(p) − 2 × OH] ⁺ ; 423.3589[M − Ara(p)/Glc − 2 × OH] ⁺	Ginsenoside F ₃	CG, ^{ΔΔ} MCG ₁₂ , MCG ₂₀	[34]
70	10.29	C ₃₆ H ₆₀ O ₈	620.4292	620.4288	0.7	621.4365[M + H] ⁺ ; 390.2277[M − C ₁₇ H ₂₆] ⁺ ; 187.1473[M − OH − Glc − C ₁₆ H ₂₄ O] ⁺	Ginsenoside Rh ₄	CG, MCG ₁₂ , MCG ₂₀	[40]
71	10.32	C ₅₃ H ₉₀ O ₂₂	1078.5939	1078.5924	1.3	1101.5805[M + Na] ⁺ ; 939.5312[M − Glc] ⁺ ; 929.5452[M − Ara(f)] ⁺ ; 789.4784[M − Ara(f) − Glc] ⁺	Ginsenoside Rc	CG, MCG ₁₂ , MCG ₂₀	s
72	10.34	C ₅₈ H ₉₈ O ₂₆	1210.6356	1210.6346	0.7	1255.6338[M + HCOO] [−] ; 1077.5851[M − Xly] [−] ; 1047.5702[M − Glc] [−] ; 945.5396[M − (Xly/Ara(p))] [−] ; 621.4323[M − (Xly/Ara(p)/Glc − Glc) [−]	Ginsenoside Ra ₁	CG, MCG ₁₂ , MCG ₂₀	[50]
73	10.38	C ₄₂ H ₇₀ O ₁₂	766.4872	766.4867	0.6	767.4945[M + H] ⁺ ; 605.4423[M − Glc] ⁺ ; 443.3870[M − Glc/Xyl] ⁺ ; 407.3660[M − Glc − 2 × OH] ⁺ ; 163.0591[Glc − OH] ⁺ ; 145.04901[Glc − OH − H ₂ O] ⁺	Ginsenoside Rg ₅	CG, MCG ₁₂ , MCG ₂₀	s
74	10.47	C ₅₆ H ₉₂ O ₂₅	1164.5947	1164.5928	1.6	1163.5874[M − H] [−] ; 1119.5961[M − CO ₂] [−] ; 927.5320[M − Ara(f) − HOCOCH ₂ COOH] [−]	Malonylginsenoside Rc	CG, MCG ₁₂ , MCG ₂₀	[44]
75	10.51	C ₄₈ H ₇₆ O ₁₉	956.5001	956.4981	2.1	955.4928[M − H] [−] ; 793.4399[M − Glc] [−] ; 613.3739[M − Glc − Glc − OH] [−]	Ginsenoside Ro	# CG, MCG ₁₂ , MCG ₂₀	s
76	10.57	C ₅₇ H ₉₄ O ₂₆	1194.6059	1194.6033	2.2	1193.5986[M − H] [−] ; 1149.6062[M − CO ₂] [−] ; 1089.5851[M − HOCOCH ₂ COOH] [−] ; 945.5428[M − Glc(Mal)] [−] ; 783.4926[M − (Glc/Glc)] [−]	Malonylginsenoside Rb ₁	CG, MCG ₁₂ , MCG ₂₀	[39]
77	10.63	C ₅₃ H ₉₀ O ₂₂	1078.5979	1078.5924	4.9	1123.5961[M + HCOO] [−] ; 945.5448[M − Ara(p)] [−] ; 783.4896[M − (Ara/Glc)] [−] ; 149.0443[Ara(p) − H] [−]	Ginsenoside Rb ₂ /Rb ₃	CG, MCG ₁₂ , MCG ₂₀	s
78	10.77	C ₅₆ H ₉₂ O ₂₅	1164.5986	1164.5928	5.0	1163.5913[M − H] [−] ; 1101.5822[M − CO ₂] [−] ; 765.4782[M − H − Glc(Mal) − Ara(p) − OH] [−]	Malonylginsenoside Rb ₂	CG, MCG ₁₂ , MCG ₂₀	[44]
79	11.06	C ₃₆ H ₆₂ O ₉	638.4391	638.4394	−0.4	683.4373[M + HCOO] [−]	20(R)-Ginsenoside Rh ₁	CG, MCG ₁₂ , MCG ₂₀	s
80	11.14	C ₃₆ H ₆₂ O ₉	638.4399	638.4394	0.7	661.4291[M + Na] ⁺ ; 376.2462[M − C ₁₇ H ₂₄ O ₂] ⁺	Ginsenoside F ₁	CG, MCG ₁₂ , MCG ₂₀	s
81	11.15	C ₅₆ H ₉₂ O ₂₅	1164.5971	1164.5928	3.7	1163.5898[M − H] [−] ; 1119.6000[M − CO ₂] [−] ; 1059.5772[M − H − C ₃ H ₄ O ₄] [−]	Malonylginsenoside Rb ₃	CG, MCG ₁₂ , MCG ₂₀	[39]
82	11.27	C ₄₈ H ₈₂ O ₁₈	946.5482	946.5501	−1.9	991.5464[M + HCOO] [−] ; 783.4878[M − Glc] [−] ; 621.4350[M − (Glc/Glc)] [−] ; 161.0435[Glc − H] [−]	Ginsenoside Rd	CG, MCG ₁₂ , MCG ₂₀	s
83	11.31	C ₅₅ H ₉₂ O ₂₃	1120.6049	1120.6029	1.7	1143.5941[M + Na] ⁺ ; 831.4874[M − Glc(mal)] [−]	Ginsenoside Rs ₁	CG, MCG ₁₂ , ^{##,*} MCG ₂₀	s
84	11.36	C ₄₂ H ₇₀ O ₁₂	766.4875	766.4867	1.0	767.4947[M + H] ⁺ ; 605.4423[M − Rha] ⁺ ; 587.4300[M − Rha − OH] ⁺ ; 569.4211[M − Rha − 2 × OH] ⁺ ; 443.3866[M − Rha/Glc] ⁺ ; 425.3769[M − Rha/Glc − OH] ⁺ ; 145.0491[Rha − H − H ₂ O] ⁺	Ginsenoside Rg ₆	CG, MCG ₁₂ , MCG ₂₀	[44]
85	11.42	C ₅₁ H ₈₄ O ₂₁	1032.5515	1032.5505	0.9	1131.5442[M − H] [−] ; 765.4785[M − Glc(mal) − OH] [−] ; 621.4372[M − (Glc/Glc(mal))] [−]	Malonylginsenoside Rd	CG, MCG ₁₂ , MCG ₂₀	[45]
86	11.53	C ₅₅ H ₉₂ O ₂₃	1120.6065	1120.6029	3.0	1165.6047[M + HCOO] [−] ; 1077.5851[M − Ac] [−] ; 1059.5745[M − Ac − OH] [−]	Ginsenoside Rs ₂	CG, MCG ₁₂ , MCG ₂₀	s

Table 2. Cont.

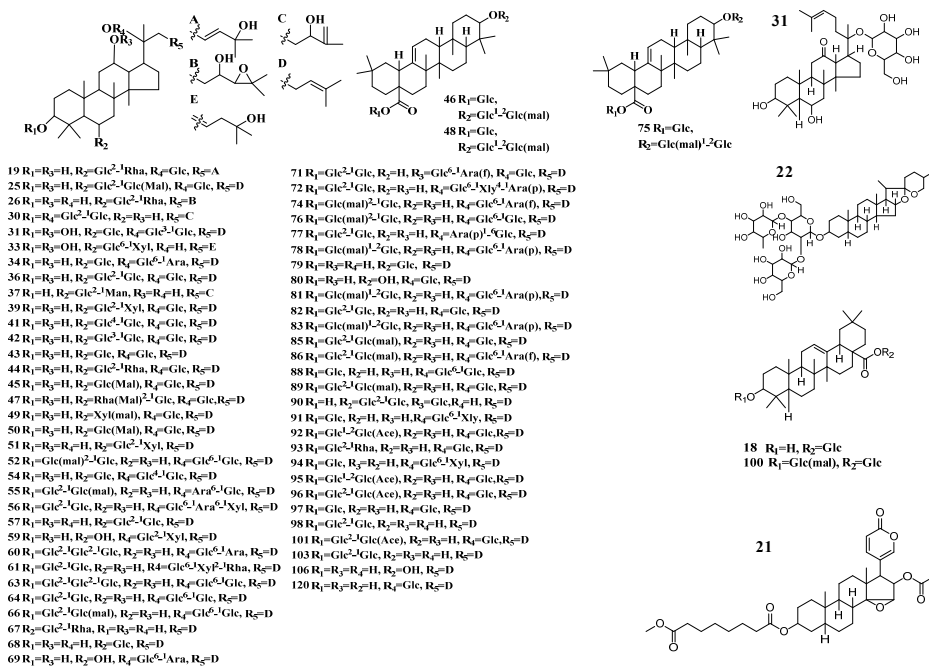
No.	t _R (min)	Formula	Calculated Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)	MS ^E Fragmentation	Identification	Sources	Ref.
87	11.69	C ₄₂ H ₇₀ O ₁₃	782.4738	782.4816	−9.7	805.4631[M + Na] ⁺ ; 621.4354[M − Glc] ⁺ ; 311.0902[Glc/Glc − CH ₂ OH] ⁺	Ginsenoside Rg ₁₀	CG, MCG ₁₂ , MCG ₂₀	a
88	11.79	C ₄₈ H ₈₂ O ₁₈	946.5494	946.5501	−0.7	991.5476[M + HCOO] [−] ; 927.5308[M − OH] [−] ; 783.4926[M − Glc] [−] ; 621.4412[M − (Glc/Glc)] [−]	Gypenoside XVII	CG, MCG ₁₂ , MCG ₂₀	s
89	11.81	C ₅₁ H ₈₄ O ₂₁	1032.5504	1032.5505	−0.1	1031.5431[M − H] [−] ; 987.5535[M − CO ₂] [−] ; 621.4412[M − (Glc/Glc(mal))] [−] ; 179.0546[Glc − H] [−]	Isomer of malonylginsenoside Rd	CG, MCG ₁₂ , MCG ₂₀	[49]
90	11.88	C ₄₈ H ₈₂ O ₁₈	946.5476	946.55021	−2.6	969.5368[M + Na] ⁺ ; 605.4394[M − Glc/Glc] ⁺ ; 587.4312[M − Glc/Glc − OH] ⁺ ; 425.3744[M − Glc/Glc − Glc] ⁺ ; 407.3661[M − Glc/Glc − OH − Glc] ⁺	Chikusetsusaponin FK ₁	CG, MCG ₁₂ , MCG ₂₀	[40]
91	12.18	C ₄₇ H ₈₀ O ₁₇	916.5398	916.5396	0.2	961.5380[M + HCOO] [−] ; 783.4870[M − Xyl] [−] ; 621.4388[M − (Xyl/glc)] [−]	Notoginsenoside Fe	CG, MCG ₁₂ , MCG ₂₀	s
92	12.39	C ₅₀ H ₈₄ O ₁₉	988.5565	988.5607	−4.1	1011.5458[M + Na] ⁺ ; 831.4819[M − Glc] ⁺ ; 425.3763[M − Glc/Glc(ace) − Glc] ⁺	Quinquenoside III	CG, MCG ₁₂ , MCG ₂₀	[51]
93	12.45	C ₄₇ H ₈₀ O ₁₇	916.5376	916.5396	−2.1	939.5268[M + Na] ⁺ ; 789.4754[M − 2 × OH − CH ₆ O ₃] ⁺	Vinaginsenoside R ₁₆	CG, MCG ₁₂ , MCG ₂₀	[40]
94	12.59	C ₄₇ H ₈₀ O ₁₇	916.5361	916.5396	−3.7	939.5253[M + Na] ⁺ ; 407.3672[M − Glc − (Glc/Xyl) − OH] ⁺	Gypenoside IX	CG, MCG ₁₂ , MCG ₂₀	[52]
95	12.91	C ₅₀ H ₈₄ O ₁₉	988.5569	988.5607	−3.8	1011.5461[M + Na] ⁺ ; 789.4784[M − Glc − 2 × OH] ⁺	Quinquenoside III isomer	CG, MCG ₁₂ , MCG ₂₀	[51]
96	13.29	C ₅₂ H ₈₆ O ₁₉	1014.5753	1014.5763	−1.0	1037.5645[M + Na] ⁺ ; 857.5032[M − C ₄ H ₈ O ₄ − 2 × OH] ⁺ ; 393.1376[Glc/Glc(ace) − OH] ⁺	Quinquenoside I	CG, MCG ₁₂ , MCG ₂₀	[53]
97	13.34	C ₄₂ H ₇₂ O ₁₃	784.4984	784.4973	1.4	829.4966[M + HCOO] [−] ; 621.4373[M − Glc] [−] ; 161.0437[Glc − H − H ₂ O] [−]	Ginsenoside F ₂	CG, MCG ₁₂ , MCG ₂₀	s
98	13.55	C ₄₂ H ₇₂ O ₁₃	784.4977	784.4973	0.5	807.4869[M + Na] ⁺ ; 605.4402[M − Glc] ⁺ ; 587.4286[M − Glc − OH] ⁺ ; 425.3765[M − Glc/Glc − OH] ⁺ ; 407.3659[M − Glc/Glc − 2 × OH] ⁺	20(R)-Ginsenoside Rg ₃	CG, MCG ₁₂ , MCG ₂₀	s
99	13.57	C ₁₇ H ₂₄ O ₂	260.1774	260.1776	−0.8	261.1847[M + H] ⁺	Panaxydol	^{ΔΔ,##} CG, MCG ₁₂ , MCG ₂₀	[54]
100	13.77	C ₄₂ H ₆₆ O ₁₄	794.4464	794.4453	1.4	793.4391[M − H] [−] ; 731.4375[M − CO ₂ − OH] [−] ; 613.3746[M − Glc] [−]	Chikusetsusaponin Iva	CG, MCG ₁₂ , MCG ₂₀	[51]
101	14.02	C ₅₂ H ₈₆ O ₁₉	1014.5750	1014.5763	−1.3	1037.5642[M + Na] ⁺ ; 789.4732[M − Glc − C ₂ H ₄ O ₂] ⁺	Isomer of Quinquenoside I	CG, MCG ₁₂ , MCG ₂₀	[51]
102	14.38	C ₁₇ H ₂₆ O ₃	278.1879	278.1882	−1.1	279.1952[M + H] ⁺	Panaxtriol	CG, MCG ₁₂ , MCG ₂₀	[55]
103	14.46	C ₄₂ H ₇₂ O ₁₃	784.4970	784.4973	−0.3	829.4966[M + HCOO] [−] ; 621.4373[M − Glc] [−] ; 407.3672[M − Glc/Glc − 2 × OH] ⁺	20(S)-Ginsenoside Rg ₃	CG, MCG ₁₂ , MCG ₂₀	s
104	15.05	C ₁₈ H ₃₄ O ₄	314.2444	314.2457	−3.8	337.2336[M + Na] ⁺	Dibutyl sebacate	CG, MCG ₁₂ , MCG ₂₀	a
105	17.90	C ₁₆ H ₂₂ O ₄	278.1516	278.1518	−0.7	301.1408[M + Na] ⁺ ; 149.0230[M − C ₄ H ₉ − C ₄ H ₉ O] ⁺	n-Butyl isobutyl phthalate	CG, MCG ₁₂ , MCG ₂₀	a

Table 2. Cont.

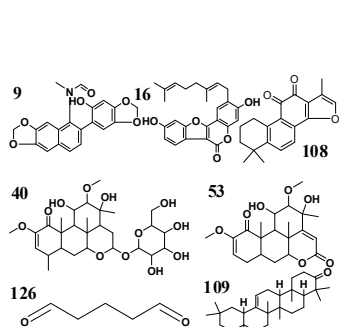
No.	tr (min)	Formula	Calculated Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)	MS ^E Fragmentation	Identification	Sources	Ref.
106	17.93	C ₃₀ H ₅₂ O ₄	476.3856	476.3866	−2.2	499.3747[M + Na] ⁺ ; 441.3728[M − 2 × OH] ⁺ ; 423.3590[M − 3 × OH] ⁺ ; 317.2049[M − 2 × CH ₃ − C ₈ H ₁₅ O] ⁺	20(R)-Protopanaxatriol	CG, MCG ₁₂ , MCG ₂₀	[56]
107	17.95	C ₁₆ H ₃₀ O ₂	254.2246	254.2268	8.2	277.2161[M + Na] ⁺	Palmitoleic acid	CG, ^{Δ, **} MCG ₁₂ , MCG ₂₀	s
108	18.07	C ₁₉ H ₁₈ O ₃	294.1258	294.1256	0.5	317.1150[M + Na] ⁺	Tashinone IIA	CG, MCG ₁₂ , MCG ₂₀	[57]
109	18.08	C ₃₀ H ₄₈ O ₄	472.3546	472.3553	−1.4	495.3438[M + Na] ⁺	β-Amyrone	CG, MCG ₁₂ , MCG ₂₀	[58]
110	18.08	C ₆ H ₆ O ₃	126.0331	126.0317	9.4	149.0223[M + Na] ⁺	Pyrogallol	CG, MCG ₁₂ , MCG ₂₀	a
111	18.09	C ₃₀ H ₄₈ O ₄	472.3546	472.3553	−1.8	495.3438[M + Na] ⁺	24-Hydroxyoleanolic acid	CG, ^{ΔΔ, **} MCG ₁₂ , MCG ₂₀	[59]
112	18.09	C ₂₄ H ₃₈ O ₅	406.2720	406.2719	0.3	429.2613[M + Na] ⁺ ; 319.1950[M − CH ₃ − C ₄ H ₇ O] ⁺ ; 261.2213[M − 2 × C ₂ H ₄ O ₂ − C ₂ H ₃] ⁺	Vitetrifolin	CG, MCG ₁₂ , MCG ₂₀	a
113	20.14	C ₃₁ H ₄₆ O ₂	450.3535	450.3498	8.0	473.3428[M + Na] ⁺ ; 430.2889[M − C ₃ H ₇] ⁺	Vitamin K ₁	CG, MCG ₁₂ , MCG ₂₀	[60]
114	20.97	C ₁₈ H ₃₀ O ₂	278.2224	278.2252	−7.9	277.2151[M − H] [−] ; 232.2172[M − COOH] [−]	α-Linolenic acid	^{ΔΔ, ##} CG, MCG ₁₂ , MCG ₂₀	[61]
115	21.18	C ₂₁ H ₃₈ O ₄	354.2758	354.2770	−3.1	377.2650[M + Na] ⁺	β-Monolinolein	CG, MCG ₁₂ , MCG ₂₀	[62]
116	22.11	C ₁₈ H ₃₂ O	264.2452	264.2453	−0.5	265.2525[M + H] ⁺ ; 149.1320[M − CH ₂ − C ₆ H ₁₂ O] ⁺ ; 135.1166[M − CH ₂ − C ₇ H ₁₃ O] ⁺ ; 121.1008[M − CH ₂ − C ₈ H ₁₅ O] ⁺ ; 109.1010[M − C ₈ H ₁₅ O − C ₂ H ₃] ⁺	(Z)-9,17-Octadecadiene	CG, MCG ₁₂ , MCG ₂₀	[63]
117	22.49	C ₁₈ H ₃₂ O ₂	280.2386	280.2402	−5.9	279.2313[M − H] [−] ; 234.2325[M − COOH] [−]	Linoleic acid	^{ΔΔ, ##} CG, MCG ₁₂ , MCG ₂₀	s
118	23.85	C ₁₄ H ₂₀ O ₂	220.1478	220.1463	5.6	265.1460[M + HCOO] [−]	Thymyl isobutyrate	CG, MCG ₁₂ , MCG ₂₀	[64]
119	24.25	C ₁₈ H ₃₄ O ₂	282.2541	282.2559	−6.3	281.2468[M − H] [−] ; 236.2481[M − COOH] [−]	9-Octadecenoic acid	^{ΔΔ, ##} CG, MCG ₁₂ , MCG ₂₀	a
120	24.40	C ₃₆ H ₆₂ O ₈	622.4454	622.4445	1.6	623.4527[M + H] ⁺ ; 316.2842[M − OH − Glc − C ₈ H ₁₄] ⁺	Compound K	CG, MCG ₁₂ , MCG ₂₀	[40]
121	24.89	C ₄₀ H ₅₆ O ₄	600.4219	600.4179	6.7	601.4292[M + H] ⁺ ; 557.4021[M − C ₂ H ₄] ⁺	Violaxanthin	CG, MCG ₁₂ , MCG ₂₀	[65]
122	25.31	C ₂₀ H ₃₈ O ₂	310.2862	310.2872	−3.2	311.2935[M + H] ⁺ ; 277.1995[M − C ₆ H ₁₃] ⁺	Ethylolate	CG, MCG ₁₂ , MCG ₂₀	a
123	25.35	C ₄₀ H ₅₆ O ₄	600.4212	600.4179	5.6	601.4285[M + H] ⁺ ; 497.3800[M − OH − C ₄ H ₈ O ₂] ⁺	Neoxanthine	CG, MCG ₁₂ , MCG ₂₀	[65]
124	26.38	C ₂₄ H ₃₈ O ₄	390.2758	390.2770	−2.8	413.2653[M + Na] ⁺ ; 301.1406[M − 3 × C ₂ H ₅] ⁺ ; 189.0153[M − C ₂ H ₅ − C ₄ H ₉ − C ₈ H ₁₇] ⁺ ; 167.0327[M − 2 × C ₂ H ₁₇] ⁺	Bis(2-ethylhexyl) phthalate	CG, MCG ₁₂ , MCG ₂₀	a
125	28.01	C ₃₀ H ₄₆ O ₅	486.3334	486.3345	−2.2	509.3226[M + Na] ⁺	Quillaic acid	CG, MCG ₁₂ , MCG ₂₀	s
126	29.04	C ₅ H ₈ O ₂	100.0512	100.0524	−10.0	123.0404[M + Na] ⁺	Pentanedial	CG, MCG ₁₂ , MCG ₂₀	[66]

^s Identified with standard. ^a Compared with spectral data obtained from Wiley Subscription Services, Inc. (USA). ^{Δ, ΔΔ}: Represented the content either in CG_{4–6 years} group or in MCG_{12 years} group was significantly higher than the other one (^Δ $p < 0.05$, ^{ΔΔ} $p < 0.001$) ^{#, ##}: Represented the content either in CG_{4–6 years} group or in MCG_{20 years} group was significantly higher than the other one ([#] $p < 0.05$, ^{##} $p < 0.001$) ^{*, **}: Represented the content either in MCG_{12 years} group or in MCG_{20 years} group was significantly higher than the other one (^{*} $p < 0.05$, ^{**} $p < 0$).

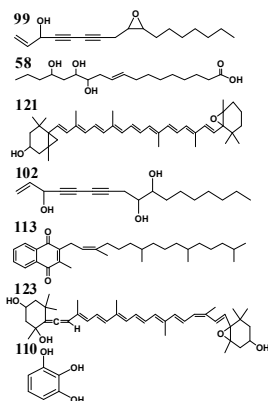
Ginsenosides



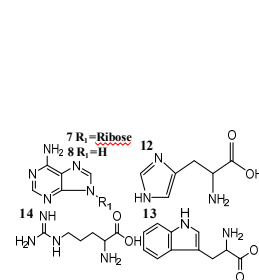
Aldehydes and ketones



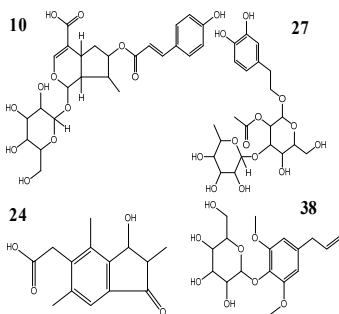
Alcohols and phenols



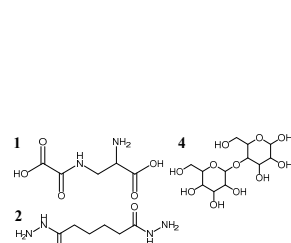
Amino acids



Phenylpropanoids



Others



Flavonoids

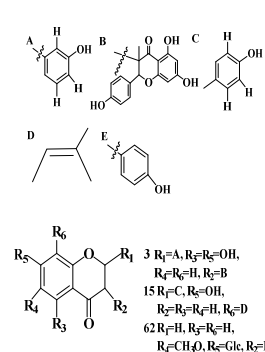


Figure 2. Cont.

Organic acids and organic acid esters

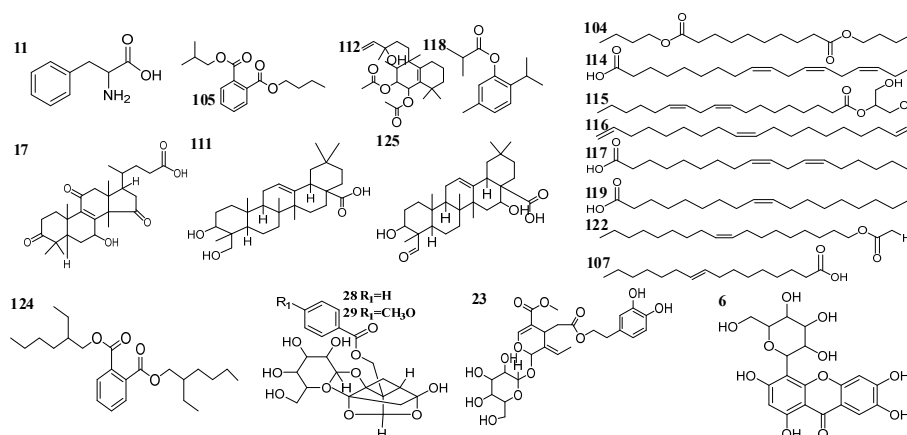


Figure 2. Chemical structures of compounds identified in MCG and CG.

For the isomers, they could be compared with the retention time of the standards or distinguished by the characteristic MS fragmentation patterns reported in literature. Taking compounds **82** and **88** as example, both of them had the same protonated ion $[M + HCOO]^-$ at m/z 991.5464 and 991.5476. In a result, one of them was identified as ginsenoside Rd due to the same retention time, and the other one was tentatively identified as gypenoside XVII because it was matched with the characteristic MS fragmentation pattern of gypenoside XVII reported in the literature [31].

3.2. Biomarker Discovery for Distinguishing MCG and CG

The MS^E data of CG and MCG samples were statistically analyzed via PCA and OPLS-DA. As seen in PCA 2D plots (Figure 3), there was no obvious difference among of 4–6-year-old CG samples, but the $MCG_{20\text{ years}}$, $MCG_{12\text{ years}}$ and $CG_{4-6\text{ years}}$ groups were obviously separated, indicating that these three groups could be differentiated. With the aim of distinguishing MCG from CG, or $MCG_{20\text{ years}}$ from $MCG_{12\text{ years}}$, OPLS-DA plot, permutation test, and S-plot, VIP values were obtained to understand which variables were responsible for the separation (Figures 4–6). The variables showing $VIP > 1$ and $p < 0.05$ (in t -test) were considered as potential biomarkers. The robust known biomarkers enabling the differentiation between CG and MCG were discovered and marked in S-plots. In order to systematically evaluate the biomarkers, heatmaps (Figure 7) were generated from these biomarkers. The hierarchical clustering heatmaps, intuitively visualizing the differential levels of potential biomarkers concentration in different ginseng groups, are shown in Figure 7. The larger contents were represented by red squares and smaller values by green squares.

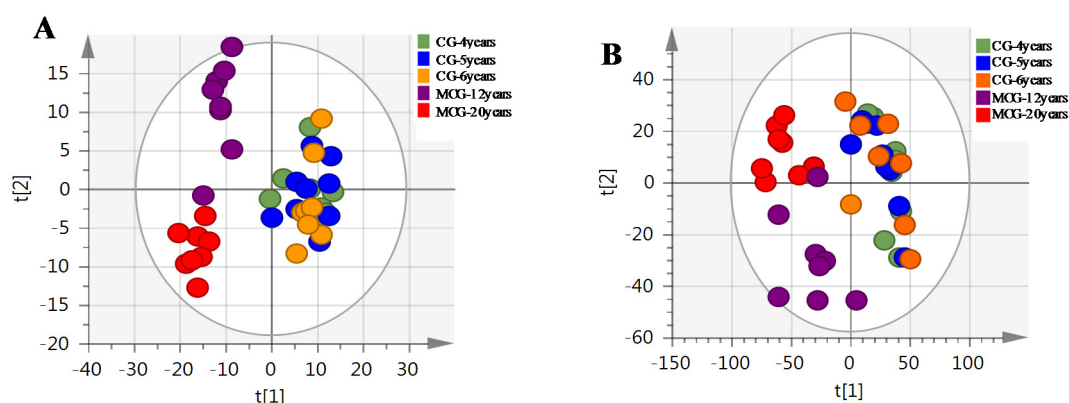


Figure 3. The PCA of CG and MCG in positive mode (A) and negative mode (B).

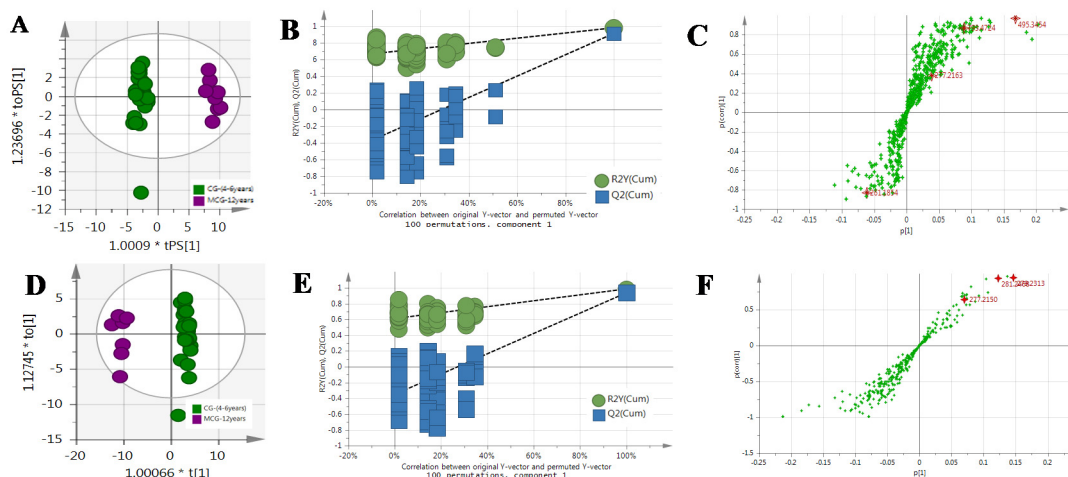


Figure 4. The OPLS-DA/Permutation test/S-Plot of CG₄₋₆ years and MCG₁₂ years in positive mode (A/B/C) and negative mode (D/E/F).

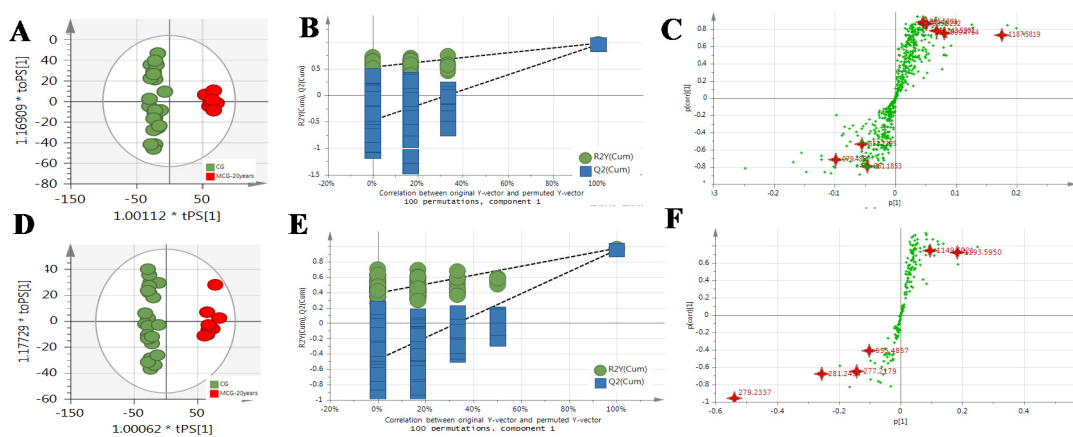


Figure 5. The OPLS-DA/Permutation test/S-Plot of CG₄₋₆ years and MCG₂₀ years in positive mode (A/B/C) and negative mode (D/E/F).

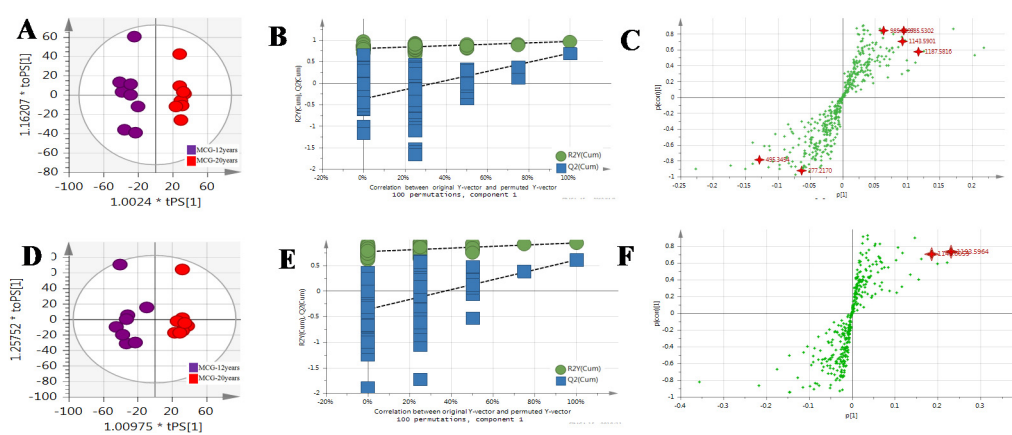


Figure 6. The OPLS-DA/Permutation test/S-Plot of MCG₁₂ years and MCG₂₀ years in positive mode (A/B/C) and negative mode (D/E/F).

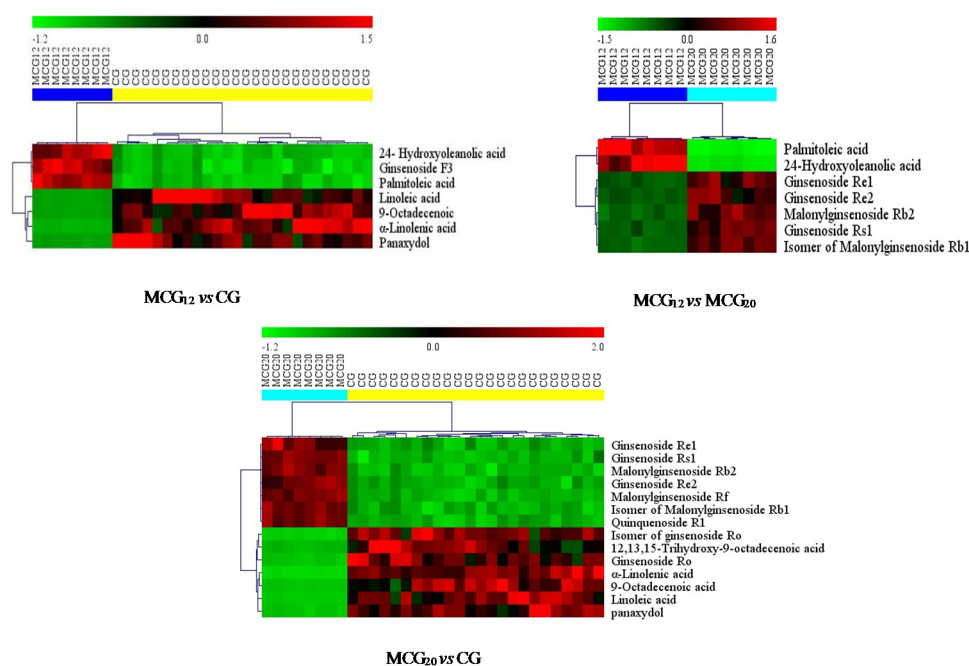


Figure 7. The heatmaps visualizing the intensities of potential biomarkers.

Between the $CG_{4-6\text{ years}}$ and $MCG_{12\text{ years}}$ groups, the contents of 24-hydroxyoleanoic acid, ginsenoside F_3 and palmitoleic acid in MCG_{12} samples were significantly higher. While, the contents of α -linolenic acid, 9-octadecenoic acid, linoleic acid and panaxydol in all the CG samples were significantly higher.

Between the $CG_{4-6\text{ years}}$ and $MCG_{20\text{ years}}$ groups, the contents of ginsenoside Re_1 , $-Re_2$, $-Rs_1$, malonylginsenoside Rb_2 , $-Rf$, isomer of malonylginsenoside Rb_1 and quinquenoside R_1 in the samples of $MCG_{20\text{ years}}$ were higher. On the contrary, the contents of ginsenoside Ro and the isomer of ginsenoside Ro , 12,13,15-trihydroxy-9-octadecenoic acid, linoleic acid, 9-octadecenoic acid, α -linolenic acid, panaxydol were rather higher in CG samples.

Between the $MCG_{12\text{ years}}$ and $MCG_{20\text{ years}}$ groups, the contents of palmitoleic acid and 24-hydroxyoleanoic acid in $MCG_{12\text{ years}}$ samples were significantly high, while the contents of ginsenoside Re_1 , $-Rs_1$, malonylginsenoside Rb_2 , $-Re_2$ and isomer of malonylginsenoside Rb_1 were rather higher in $MCG_{20\text{ years}}$ samples.

Overall, on one hand, the contents of α -linolenic acid, linoleic acid, 9-octadecenoic acid and panaxydol in CG samples were significantly higher than those in all MCG samples. On the other hand, ginsenoside Re_1 , $-Re_2$, $-Rs_1$, malonylginsenoside Rb_2 and isomer of malonylginsenoside Rb_1 in $MCG_{20\text{ years}}$ samples were really higher than those both in $MCG_{12\text{ years}}$ and in all of CG samples, but there is no significant difference between $MCG_{12\text{ years}}$ and $CG_{4-6\text{ years}}$ samples. The summary with variable identity, VIP and p value were shown in Table 3.

Table 3. The summary table with variable identity, VIP and p value.

Groups for Comparison		Marker Name	VIP Value	p Value
CG _{4–6} years vs. MCG ₁₂ years	CG _{4–6} years	α-linolenic acid	1.23	<0.001
		9-octadecenoic acid	2.17	<0.001
		linoleic acid	2.57	<0.001
		panaxydol	1.49	<0.001
	MCG ₁₂ years	24-hydroxyoleanolic acid	4.13	<0.001
		ginsenoside F3	2.15	<0.001
		palmitoleic acid	1.54	0.037
CG _{4–6} years vs. MCG ₂₀ years	MCG ₂₀ years	ginsenoside Re1	1.60	<0.001
		ginsenoside Re2	1.75	<0.001
		ginsenoside Rs1	1.59	<0.001
		malonylginsenoside Rb2	4.10	<0.001
		ginsenoside Rf	1.83	<0.001
		isomer of malonylginsenoside Rb1	2.30	<0.001
		quinquenoside R1	1.21	<0.001
	CG _{4–6} years	ginsenoside Ro	1.39	0.017
		isomer of ginsenoside Ro	2.31	0.022
		12,13,15-trihydroxy-9-octadecenoic acid	1.25	0.003
		linoleic acid	7.08	<0.001
		9-octadecenoic acid	3.45	<0.001
		α-linolenic acid	1.86	<0.001
MCG ₁₂ years vs. MCG ₂₀ years	MCG ₁₂ years	palmitoleic acid	2.07	<0.001
		24-hydroxyoleanolic acid	3.26	<0.001
MCG ₂₀ years	MCG ₂₀ years	ginsenoside Re1	1.16	0.002
		ginsenoside Rs1	1.89	0.024
		malonylginsenoside Rb2	2.76	0.026
		ginsenoside Re2	1.60	<0.001
		isomer of malonylginsenoside-Rb1	3.87	<0.001

4. Discussion

Although MCG and CG both belong to *Panax ginseng*, their chemical ingredients and pharmacological activities are different due to their significantly different growth environment [3,67]. As we all know, MCG has been regarded as a replacement of wild ginseng. Recently, the UPLC-QTOF-MS/MS-based approach has been developed to distinguish MCG (grown for 15 years) and CG (grown for 4–7 years) [6]. As a result, 40 ginsenosides in both MCG and CG were unambiguously identified and tentatively assigned, and the potential chemical markers identifying different ginseng products were characterised [6]. Additionally, the study on 6–18-year-old Mountain Cultivated Ginseng Leaves (MGL) samples showed that the MGL were obviously divided into three main groups according to different age brackets (6~10, 11~13 and 14~18 years) [7]. Although the sample of the study was the leaf of MCG, it could be indirectly speculated that the MCG roots with different cultivation ages are also different. In order to further systematically compare the similarities and differences at the chemical level between different ages of ginseng, especially to compare the younger or the older MCG, 4, 5, 6-year-old CG and 12, 20-year-old MCG were chosen as the analytical samples in the present study.

Firstly, based on UNIFI platform, intelligent and automatic workflows, the screening analysis of metabolites in different cultivation ages of ginseng were rapidly performed. As a result, a total of 126 compounds were characterized from CG_{4–6} years, MCG₁₂ years and MCG₂₀ years samples. Among of them, ginsenosides were the main ingredients. Both CG and MCG had the similar chemical composition, but the components were variously distributed in CG and MCG samples at different contents. That means in CG and MCG, the secondary metabolites had the features of structural diversity and the different content patterns. As far as we know, this is the first time that the comprehensive

screening analysis of MCG_{12 years} and MCG_{20 years} samples by using UPLC-QTOF-MS^E combined with UNIFI platform. It could provide the scientific data for clarifying the chemical composition of MCG.

Secondly, the combination of LC-MS based metabolomic profiling with multivariate statistical analysis method was used to profile the CG, MCG_{12 years} and MCG_{20 years} samples. A total of 17 potential age-dependent markers enabling differentiation among the CG and MCG samples were discovered. (1) There were four robust markers including α -linolenic acid, 9-octadecenoic acid, linoleic acid and panaxydol being the characteristic components for CG samples, that distinguished them from both MCG_{12 years} and MCG_{20 years} samples. The results showed that CG samples contained more non-ginsenosides. Both linoleic acid and α -linolenic acid, the main products of the acetate-malonate pathway, are two essential fatty acids necessary for health. Linoleic acid is used in the biosynthesis of arachidonic acid and thus some prostaglandins, leukotrienes, and thromboxane [68,69]. Panaxydol, one of the C17 polyacetylenic compounds, originates from acetyl-CoA/malonyl-CoA via fatty acids with crepenynate as the intermediate [70]. It is considered a potential antitumor agent due to its significant anticancer activity [71]. (2) In CG samples, there were three other characteristic components such as ginsenoside Ro, the isomer of ginsenoside Ro, and 12,13,15-trihydroxy-9-octadecenoic acid, that could be used to differentiate them from MCG_{20 years} samples. From this, we could draw a conclusion that pentacyclic triterpenoids decreased significantly in older MCG samples. (3) Five robust biomarkers including ginsenoside Re₁, -Re₂, -Rs₁, malonylginsenoside Rb₂ and isomer of malonylginsenoside Rb₁ were found to enable differentiation of MCG_{20 years} from CG and MCG_{12 years} samples. These five compounds might be used for rapid identification of MCG_{20 years} samples. A proposed biosynthetic pathway of ginsenosides is as follows: with the action of squalene epoxidase, squalene was converted to 2,3-oxidosqualene. Dammaranes can be synthesized by dammarenediol synthase, and oleananes by β -amyrin synthase [72]. Ginsenosides were found to have both antimicrobial and antifungal properties and the molecules are naturally bitter-tasting, discouraging insects and other animals from consuming the plant, so ginsenosides likely serve as mechanisms for plant defense [73,74]. (4) In MCG_{20 years} samples, another two markers, ginsenoside Rf and quinquenoside R₁, were discovered that distinguished them from all CG samples. (5) In MCG_{12 years} samples, 24-hydroxyoleanolic acid and palmitoleic acid were the two robust markers for distinguished from both CG and MCG_{20 years} samples. These two compounds might be used for rapid identification of MCG_{12 years} samples. Palmitoleic acid is biosynthesized from palmitic acid by the action of the enzyme stearoyl-CoA desaturase-1, a key enzyme in fatty acid metabolism [75]. (6) Ginsenoside F₃ was another marker for MCG_{12 years} samples that differentiated them from CG samples. However, there are still some unresolved issues. For example, as shown in BPI chromatograms, though 126 compounds were identified, there are still some unidentified components. there are still some unidentified components. Further research should be carried out based on the formula of these unknown compounds.

5. Conclusions

By combining the UPLC-Q/TOF-MS^E and UNIFI platform, 126 chemical components with various structural types, such as triterpenoids, flavonoids, organic acids and organic acid esters, etc., were characterized or tentatively identified from CG_{4-6 years}, MCG_{12 years} and MCG_{20 years} samples for the first time. All the CG and MCG samples had the similar chemical composition, but there were significant differences in the content of each component. Further nontarget metabolomic analysis combined with multivariate statistical analysis showed that CG_{4-6 years}, MCG_{12 years} and MCG_{20 years} samples were obviously divided into three different groups. A total of 17 potential age-dependent markers enabling differentiation among the CG and MCG samples were discovered. Among of these markers, four robust markers, including α -linolenic acid, 9-octadecenoic acid, linoleic acid and panaxydol, could be the characteristic components for differentiation of CG from all other MCG samples. Five robust markers including ginsenoside Re₁, -Re₂, -Rs₁, malonylginsenoside Rb₂ and isomer of malonylginsenoside Rb₁ were found to enable differentiate MCG_{20 years} samples from all other samples, while 24-hydroxyoleanolic acid and palmitoleic acid were the robust markers for

distinguishing MCG_{12 years} samples from all the CG samples and MCG_{20 years} samples. The proposed approach could be applied to directly distinguish MCG root ages, which is an important criterion for evaluating the quality of MCG. The results will provide the data for the deficient study on the chemical constituents of MCG and provide reference for the quantitative determination in the quality control criterion of MCG.

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Sample Availability: Samples of the compounds are available from the authors.



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