

Full Length Research Paper

The ginsenoside profile of ginseng cultivated under mountainous forest

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Ginseng cultivated under forest was formally called “*Lin-Xia-Shan-Shen*” (LXSS) according to Chinese Pharmacopoeia (2010 edition) meaning that the seeds of ginseng were disseminated in mountainous forest and the plants grew naturally without pesticide and fertilizer. The accumulation of ginsenosides in LXSS and profile comparison between garden ginseng and LXSS were studied, thus a chromatographic method to distinguish LXSS from garden ginseng was established. In the study, external standard method and multi-components quantification with one marker based on the peak area recorded on HPLC were applied to determine the contents of components in LXSS. Correlation analysis was used to find the correlations between ginsenoside contents and growing years, between appearances and growing years of LXSS. It was found that most components exhibited a trend of first increasing, and then stabilizing before finally declining, with the ratio of protopanaxatriol and protopanaxadiol-type ginsenosides decreasing in LXSS. The total weight and length of LXSS were positively correlated to the growing years. The results provided the scientific bases for the optimal collection time of LXSS. Fingerprints of LXSS and garden ginseng were also set up with ginsenoside Rb₁ as reference peak, and 19 and 13 characteristic peaks were identified, respectively. Analysis of components between ginsenosides Re and Rf showed that ginseng samples from different sources were characterized with peak numbers and ratios in this area. Those of LXSS showed several peaks eluted just before ginsenoside Rf, while those of garden ginseng embodied a relative high peak with retention time at 24.3 min.

Key words: Lin-Xia-Shan-Shen (LXSS), ginsenosides, accumulation trend, HPLC fingerprint.

INTRODUCTION

Garden ginseng now is still the main resources of ginseng materials but its forest–ruined cultivation mode severely destroyed the ecological balance. In recent two decades, the cultivation of ginseng under mountain forest has been spread at large scale so as to preserve forest and imitate the growing conditions of wild ginseng. Ginseng cultivated under this condition was formally called “*Lin-Xia-Shan-Shen*” (LXSS) in Chinese Pharmacopoeia and named customarily “*Zi-Hai*”, meaning that the LXSS was cultivated directly by seeds and disseminated in forest and grew naturally. The

cultivation of LXSS makes good use of forest to change the forest–ruined cultivation mode of garden ginseng. The natural growth of LXSS without pesticide and fertilizer reduces environment contamination and provides green medicines. The similar appearance of LXSS to wild ginseng formed by longer growing time is also one of its attracting characteristics in the culture of traditional Chinese medicine. All these destined the planting of LXSS to become a prospecting cultivation mode of ginseng. Now LXSS over 10 years of growth is available at great amount, the elucidation of accumulation of ginsenosides in LXSS with age increasing and profile difference of ginsenosides in LXSS and garden ginseng is feasible so as to explore the effect of natural environment on ginsenosides in ginseng and to establish the quality standard for LXSS.

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Previously the bioactivities and hemolytic interaction of ginsenosides were studied (Cheng et al., 2007; Dou et al., 2001a; Qiu et al., 2009) and the SOP for cultivation and fingerprint of LXSS were proposed (Jiang HP et al., 2007; Jiang HP et al., 2008a). More recently, the monthly accumulating profile of ginsenosides in LXSS was studied and the optimal harvest month of LXSS was discussed (Zhou et al., 2010). And this paper deals with the accumulation and profiles of ginsenosides in LXSS and the ginsenoside comparison between LXSS and garden ginseng.

MATERIALS AND METHODS

Materials

Samples of LXSS was collected in the city of Benxi, Liaoning province were identified as *Panax (P.) ginseng* C. A. Meyer by Feng-Yun Liu and Bing Wang. The growing years of LXSS were determined by the number of rhizome nodes together with the different collection areas, where the seeds of ginseng were disseminated in different years. The voucher specimens were deposited in college of pharmacy, Liaoning University of Traditional Chinese Medicine. Authentic samples of ginsenosides Rg₁, Re, Rf, Rb₁, Rc, Rb₂, Rd were prepared from ginseng extract with the purity of over 98%, which was qualified for content determination. Acetonitrile and methanol were chromatographical grade and purchased from Tedia Tele Chem International Inc. and Yu-Wang reagent company. Deionized water was supplied by Xinchengyuan Company, while analytical grade phosphoric acid was purchased from Ruiquan reagent company.

Instruments

Agilent 1100 HPLC instrument (Agilent Technologies, Inc.), AT-130 column oven (Dalian Zhonghuida Scientific instrument Co., Ltd), Sartorius CP225 analytical balance (Sartorius Co., Ltd), and FW80 high-speed grinder (Tianjin Taisite instrument Co., Ltd) were employed during the process of chemical analysis.

HPLC analysis

The chemical analysis was achieved on an Agilent 1100 HPLC instrument with Agilent C18 column (150×4.6 mm, 5 μm), column temperature at 30°C, and flow rate at 1.0 ml · min⁻¹. The ginsenosides were separated in 70 min by mobile phase consisted of acetonitrile (A) and 0.1% phosphoric acid (W) with the gradients as follows: 0~30 min, A : W from 19 : 81 to 29 : 71; 30~50 min, A : W from 29 : 71 to 32 : 68; 50~70 min, A : W from 32 : 68 to 51 : 49. While the determinations of ginsenosides Rg₁ and Re were achieved by isocratic elution with A : W (20 : 80) within 30 min. Components with retention time between Re and Rf in the first condition were separated by gradient elution as follows: 0~30 min, A : W 20 : 80; 30~38 min, A : W from 20 : 80 to 21 : 79; 38~41 min, A : W from 21 : 79 to 21.5 : 78.5; 41~57 min, A : B from 21.5 : 78.5 to 28 : 72.

Contents of ginsenosides with authentic samples such as Rg₁, Re, Rf, Rb₁, Rc, Rb₂, Rd were determined by external standard method. While compounds without authentic samples were determined by method of multi-components quantification with one marker. Since the relative low level of these compounds and the relative correction factors (RCF) of usual ginsenosides to Rb₁ were

around 1 (Zhu et al., 2008), so the RCFs of compounds without authentic samples to Rb₁ were determined approximately as 1.

Sample preparation

The powder of ginseng was weighed accurately (0.5 g) and refluxed by methanol 2 times, 1 h for each time. The solvent was evaporated and the residue was dissolved by methanol to a constant volume, then filtered to give the sample solution. Authentic samples were also dissolved in methanol.

Statistics

All values are expressed as mean ± SEM. The correlation analysis of data was achieved by SPSS.

RESULTS AND DISCUSSION

Method validation

The method was validated by linearity, precision, repeatability, stability and recovery, and all were up to the demands for HPLC determination.

Accumulation trend of ginsenosides in LXSS

The ginsenosides were determined by the procedure mentioned above. The following regularities could be concluded from the data:

- 1) The contents of total and protopanaxadiol (PPD) ginsenosides accumulated at the initial growing period (from the beginning to the eighth year) of LXSS, and reached a peak at the seventh year of growth, then fell off a little and then kept constant in the following years of growth. While the content of protopanaxatriol (PPT) ginsenosides increased, then kept constant, and then fell off at the twelfth year of growth at first glance (as shown in Table 1 and Figure 1). But negative correlation between content of PPT ginsenosides and growing years was figured out after SPSS analysis with the correlation coefficient (R value) of -0.733 (P<0.01). The content ratio of PPT and PPD ginsenosides was also negatively correlated to the growing years with R value of -0.659 (P<0.05).
- 2) Ginsenosides Re, Rf, Rb₁, Rc, Rb₂ and Rd showed the same accumulating trend with total ginsenoside (as shown in Table 2 and Figure 2), while Rg₁ was negatively correlated to the growing years with R value of -0.723 (P<0.01), which might indicate the transformation from Rg₁ to other ginsenosides as the whole plant grew.
- 3) The relative contents of components with retention times at 43.0, 52.5, 54.2, 57.1 and 66.8 min also increased at the initial growing period, then kept stable or decreased a little, and that of component with retention time at 46.5 min increased through the growing years

Table 1. Contents of total, protoanaxatriol and protoanaxadiol ginsenosides (mg/g) in LXSS with different growing years together with those of garden ginseng (n=5)

LXSS		Total ginsenosides	PPT	PPD	PPT/PPD
Growing years	1	20.61±0.09	9.02±0.00	11.59±0.00	0.78±0.01
	2	21.35±0.02	9.15±0.03	12.20±0.03	0.75±0.03
	4	26.94±6.39	9.50±0.40	17.44±6.79	0.59±0.25
	7	27.23±3.02	8.10±1.49	19.13±2.57	0.43±0.10
	9	24.98±3.29	8.05±0.18	16.93±3.46	0.49±0.11
	12	24.94±7.35	6.67±1.33	18.27±6.69	0.40±0.17
Garden ginseng		16.23±3.28	5.37±0.86	10.87±2.67	0.51±0.11

Figures in the brackets indicates the percentage of samples containing the component at the corresponding time.

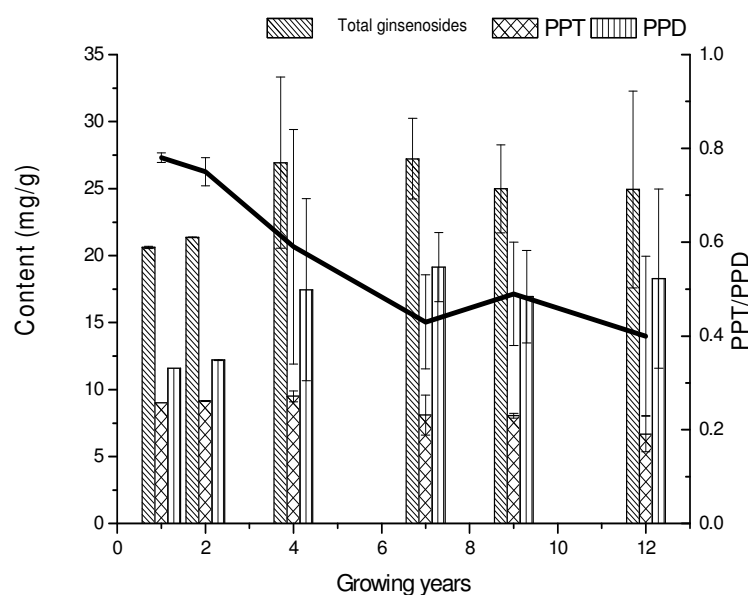


Figure 1. The accumulation trend of ginsenosides of LXSS. The columns stand for the contents of total, PPT and PPD ginsenosides, respectively, and the solid line standing for the ratio of PPT and PPD. As shown in the figure above, the contents of total and PPD ginsenosides accumulated at the initial growing period, and reached a peak at the seventh year of growth, then fell off a little and kept constant. And content protoanaxatriol (PPT) ginsenosides increased, then kept constant, fell off at the twelfth year of growth. The ratio of PPT/PPD fell during the growing period.

Table 2. Contents of ginsenosides (mg/g) in LXSS with different growing years together with those of garden ginseng (n = 5).

LXSS		Rg ₁	Re	Rf	Rb ₁	Rc	Rb ₂	Rd	Rg ₁ /Re
Growing years	1	4.10±0.00	4.02±0.00	0.90±0.00	4.20±0.00	3.65±0.03	2.83±0.03	0.91±0.04	1.02±0.00
	2	4.15±0.01	4.08±0.01	0.92±0.01	4.60±0.01	3.60±0.03	2.96±0.03	1.04±0.03	1.02±0.01
	4	3.79±0.57	4.48±0.59	1.23±0.42	6.70±2.83	5.40±2.45	4.19±2.45	1.16±0.26	0.86±0.24
	7	3.69±0.89	3.40±0.62	1.01±0.25	6.49±1.16	6.13±0.86	4.88±0.86	1.63±0.60	1.09±0.27
	9	3.44±0.79	3.35±0.56	1.26±0.06	5.17±0.04	5.19±1.09	4.36±1.09	2.22±0.93	1.07±0.42
	12	2.35±0.36	3.52±1.14	0.79±0.12	6.24±0.43	5.46±2.98	5.01±2.98	1.56±0.67	0.71±0.22
Garden ginseng		1.94±0.33	2.67±0.45	0.76±0.19	3.35±0.95	3.03±0.75	2.96±0.74	1.53±0.39	0.73±0.08

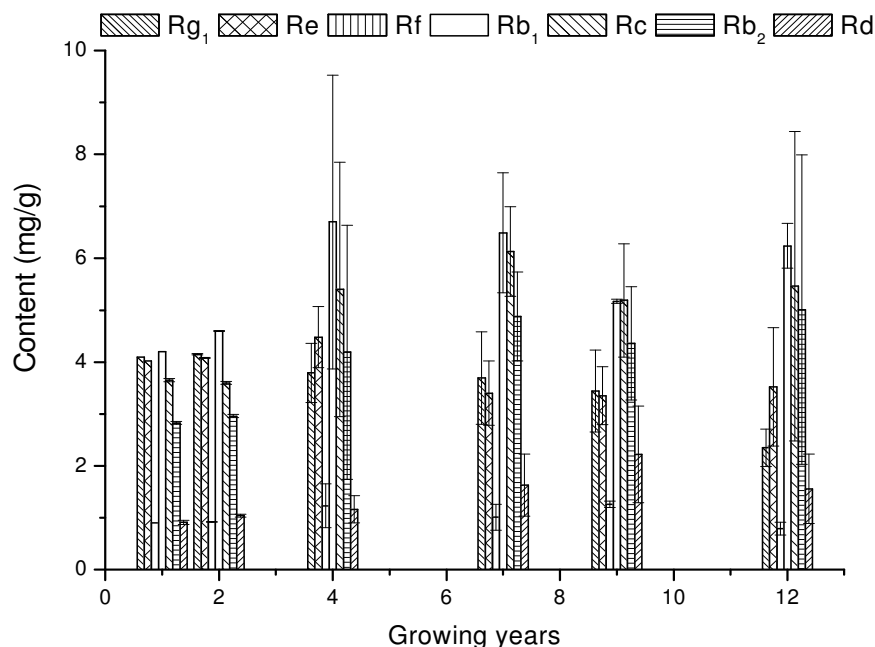


Figure 2. Accumulating trend of ginsenosides in LXSS. The columns stand for the contents of ginsenosides Rg₁, Re, Rf, Rb₁, Rc, Rb₂ and Rd, respectively. As shown in the figure above, ginsenosides Re, Rf, Rb₁, Rc, Rb₂ and Rd all increased at the initial growing period, and fell a little, then kept stable. But ginsenoside Rg₁ decreased during the growing period.

detected (from the very beginning to the twelfth year), which indicated that those components might have a higher accumulating rate than Rb₁ at the initial growing period of LXSS. The relative contents of components at 15.6 and 21.7 min were positively correlated to the growing years with the correlation coefficients of 0.614 ($P < 0.05$) and 0.583 ($P < 0.05$), which indicated that the increasing rate of these compounds were higher than those of Rb₁. While those at 12.8, 13.8 and 56.3 min were negatively correlated to the growing years with R values of -0.875 ($P < 0.01$), -0.603 ($P < 0.05$) and -0.619 ($P < 0.05$), respectively, which indicated that the decreasing rate of these compounds were higher than those of Rb₁ (Table 3 and Figure 3).

4) The total weight and length were positively correlated to the growing years with R values of 0.847 ($P < 0.01$) and 0.644 ($P < 0.01$). The length ratio of the rhizome and main root increased as the growth period extended, which suggested that the growth rate of the rhizome was higher than that of the main root (as shown in Table 4 and Figure 4).

From the results above, the correlations of the inner quality and the outer standard of LXSS, which meant the contents of ginsenosides and the length of the rhizome, respectively, with the growing years of LXSS were established.

Total content of ginsenosides reached the maximum at the seventh year, then decreased a little, and kept stable in the following years. But LXSS with this growing span

was not qualified for sales from commercial considerations with the weight of 3.76 g and rhizome length of 1.82 cm. While after twelve years of growing, the appearance of LXSS approximated that of wild ginseng, and the contents of ginsenosides were stable for quality control.

The contents of ginsenosides and appearances of garden ginseng were compared with those of LXSS on sales, with twelve years of growth. As could be seen in Tables 1, 2 and 4, the contents of ginsenosides were higher in LXSS with lower total weight, and the length and weight percentage of rhizome were also higher in LXSS.

Profiles of LXSS and garden ginseng

The HPLC fingerprints were established and 13 and 19 characteristic peaks were confirmed from the profiles of garden ginseng and LXSS. The similarities were determined by Cosine, which were among 0.95 and 1.00. The RSD of relative retention time and relative peak area to Rb₁ of characteristic peaks in the fingerprints were shown in Table 5. The reference fingerprints were shown in Figures 5 and 6).

The diversity of secondary metabolites in LXSS might be due to its mountainous forest growing condition without pesticide and fertilizer and LXSS might become a source of novel natural products for various pharmacological uses. So components with retention time

Table 3. Relative contents (mg/g) of components without authentic standard to Rb₁ in LXSS with different growing years (n = 5).

Growing years	11.1	12.0	12.8	13.8	15.6	20.0
1	0.43±0.09	0.92±0.00	0.53±0.01	1.33±0.01	0.03±0.00	0.12±0.00
2	0.42±0.02	0.96±0.03	0.58±0.03	1.32±0.03	0.04±0.01	0.10±0.01
4	0.31±0.23	0.95±0.01	0.55±0.02	0.69±0.98	0.02±0.01	0.19±0.10
7	0.37±0.06	0.17±0.11	0.37±0.07	0.18±0.17	0.10±0.03	0.09±0.06
9	0.33±0.04	0.40±0.41	0.31±0.03	0.17±0.12	0.08±0.03	0.11±0.03
12	0.30±0.13	1.10±1.59	0.37±0.06	0.37±0.06	0.08±0.03	0.10±0.04
Growing years	21.1	21.7	S-Rg ₂	R-Rg ₂	Rb ₃	42.0
1	0.92±0.00	0.00±0.05	0.26±0.06	0.20±0.09	0.10±0.00	0.21±0.03
2	0.94±0.01	0.00±0.03	0.23±0.03	0.25±0.03	0.19±0.01	0.29±0.04
4	0.69±0.42	0.00±0.00	0.46±0.28	0.11±0.15	0.32±0.25	0.38±0.16
7	0.50±0.21	0.06±0.11	0.49±0.15	0.33±0.43	0.36±0.11	0.34±0.11
9	0.63±0.21	0.03±0.04	0.40±0.12	0.22±0.21	0.29±0.16	0.29±0.11
12	0.73±0.47	0.29±0.26	0.42±0.20	0.64±0.63	0.17±0.18	0.41±0.05
Growing years	43.0	46.5	47.0	50.5	51.5	52.5
1	0.40±0.00	0.20±0.00	0.07±0.04	0.10±0.04	0.20±0.00	0.47±0.05
2	0.80±0.01	0.25±0.01	0.08±0.04	0.08±0.04	0.25±0.01	0.52±0.04
4	1.29±0.69	0.36±0.21	0.14±0.06	0.06±0.08	0.29±0.07	0.41±0.11
7	1.67±0.25	0.37±0.06	0.18±0.02	0.08±0.03	0.22±0.06	0.70±0.22
9	1.51±0.61	0.31±0.07	0.15±0.06	0.09±0.02	0.24±0.04	0.88±0.29
12	1.69±1.21	0.42±0.10	0.18±0.15	0.09±0.03	0.22±0.16	0.79±0.42
Growing years	54.2	56.3	57.1	57.7	66.8	Rg ₃
1	0.20±0.04	0.11±0.00	0.02±0.05	0.06±0.00	0.14±0.00	0.00±0.05
2	0.26±0.04	0.12±0.01	0.04±0.04	0.05±0.01	0.16±0.01	0.00±0.04
4	0.23±0.01	0.13±0.01	0.06±0.01	0.06±0.00	0.20±0.04	0.02±0.02
7	0.24±0.06	0.10±0.03	0.09±0.03	0.08±0.03	0.20±0.10	0.00±0.00
9	0.40±0.15	0.10±0.01	0.14±0.04	0.10±0.04	0.32±0.01	0.00±0.00
12	0.27±0.12	0.07±0.04	0.08±0.08	0.09±0.06	0.08±0.08	0.09±0.06

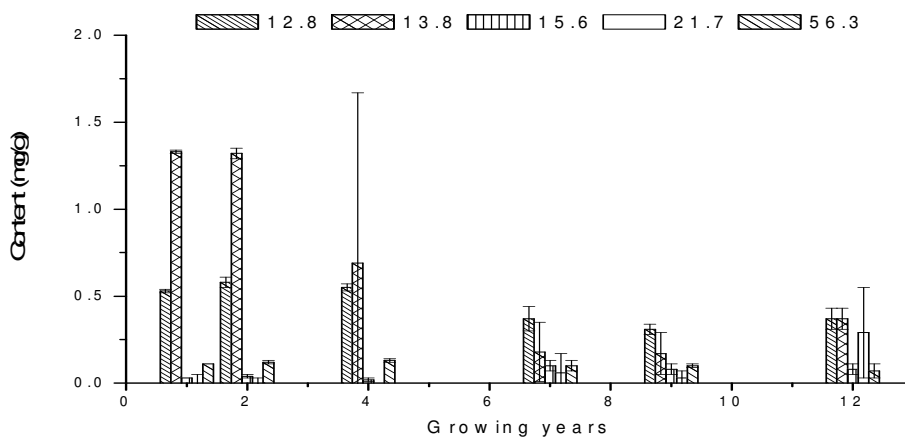
**Figure 3.** Components without authentic standard correlated to the growing years in LXSS. The columns stand for the relative contents of components without authentic standards with retention times at 12.8, 13.8, 15.6, 21.7 and 56.3 min, respectively. As shown in the figure above, the relative contents of components with retention times at 15.6 and 21.7 min to Rb₁ increased, while those with retention times at 12.8, 13.8 and 56.3 min decreased.

Table 4. Appearances of LXSS with different growing years together with those of garden ginseng (n = 5).

LXSS		Total weight (g)	Weight of main root (%) ^a	Weight of rhizome (%) ^a	Weight of fibrous root (%) ^a	Weight of adventitious root (%) ^a	Total length (cm)	Length of main root (%) ^b	Length of rhizome (%) ^b	Length of rhizome/length main root
Growin g years	1	0.25±0.00	78.70±0.00	4.62±0.00	16.67±0.00	0.00±0.03	1.70±0.04	88.24±0.05	11.76±0.00	0.13±0.00
	2	0.55±0.01	81.82±0.01	5.74±0.01	12.45±0.01	0.00±0.03	2.60±0.03	84.62±0.03	15.38±0.01	0.18±0.01
	4	0.85±0.21	77.51±1.51	7.79±3.50	14.71±1.99	0.00±0.00	5.10±0.99	65.40±7.15	34.61±7.15	0.54±0.19
	7	3.76±2.26	79.33±1.14	5.68±1.59	14.53±0.55	0.46±0.80	6.17±3.12	70.50±9.89	29.50±9.89	0.44±0.21
	9	4.41±0.07	80.34±0.18	6.31±1.65	12.41±2.13	0.93±1.31	8.45±1.91	64.78±7.96	35.22±7.95	0.56±0.19
	12	6.98±2.87	73.92±6.35	9.39±4.59	15.25±3.88	1.45±2.51	8.40±4.54	62.05±4.21	37.95±4.21	0.65±0.14
Garden ginseng		17.48±3.09	87.44±5.85	3.07±1.90	9.52±4.06	0.00±0.00	9.80±1.30	91.85±4.62	5.48±5.63	0.09±0.05

^a the percentage of total weight of LXSS, ^b the percentage of total length of LX.

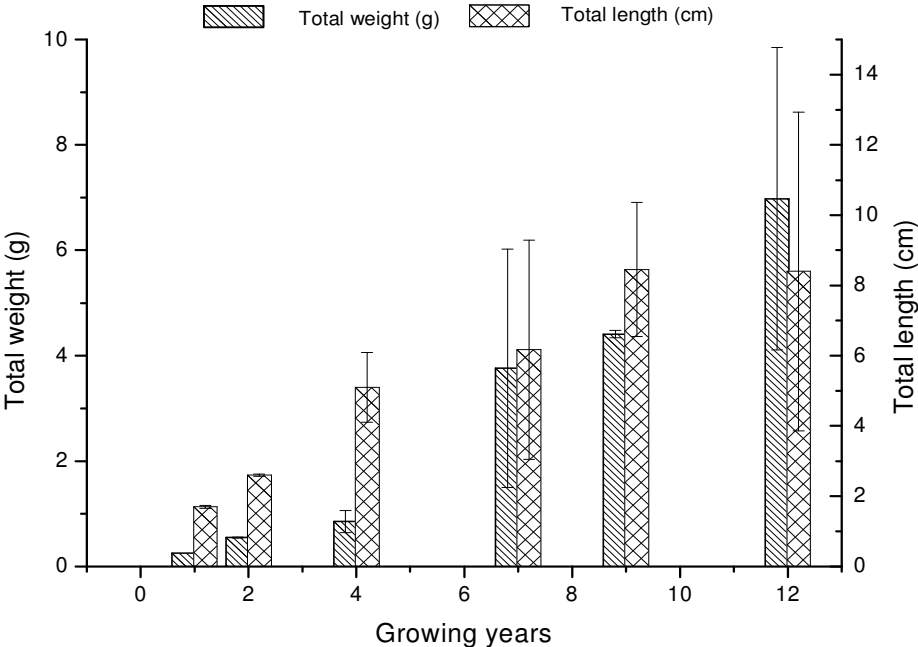
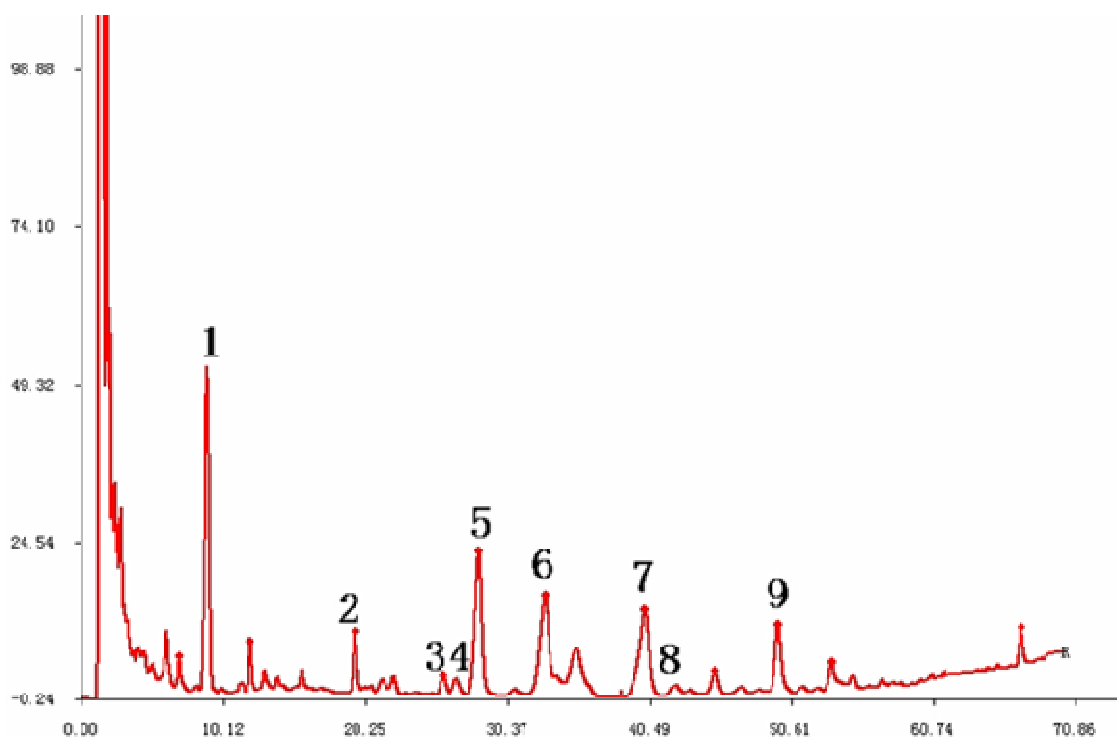


Figure 4. Weight and length of LXSS with different growing years. The columns stand for the total weight and length of LXSS, respectively, which increased as the growing of the plant.

Table 5. Characteristic peaks of garden ginseng and LXSS (components without authentic samples were expressed by the retention times) (n = 10).

Characteristic peaks of garden ginseng	12.0	13.0	Rf	25.2	Rb₁	Rc	Rb₂	42.0	45.8	48.6
RSD of retention time (%)	1.31	1.16	0.81	1.08	1.65	1.86	1.55	1.35	1.12	0.96
RSD of peak area (%)	55.43	44.11	25.66	25.23	30.59	25.65	17.17	60.00	32.86	26.99
Characteristic peaks of garden ginseng	52.5	54.3	57.7							
RSD of retention time (%)	0.67	0.60	0.56							
RSD of peak area (%)	64.07	32.95	48.07							
Characteristic peaks of LXSS	11.2	13.0	Rf	20.5	S-Rg₂	Rb₁	Rc	Rb₂	42.0	44.0
RSD of retention time (%)	1.15	1.05	0.99	1.56	1.28	1.57	1.54	1.70	1.53	1.51
RSD of peak area (%)	27.90	35.53	26.85	133.95	31.66	14.76	18.49	19.47	42.51	34.62
Characteristic peaks of LXSS	45.8	47.3	Rd	51.4	52.5	54.3	57.2	57.7	66.8	
RSD of retention time (%)	1.47	1.40	1.34	1.14	1.15	1.07	0.83	0.83	0.24	
RSD of peak area (%)	16.41	43.32	25.04	42.84	25.95	32.88	47.53	24.0	69.0	

**Figure 5.** The reference fingerprint of garden ginseng.

between those of Re and Rf were further analyzed. Since most samples tested contained peak at 27.1 min with satisfying resolution, this peak was selected as reference, and the ratios of other peaks detected to it were calculated. As shown in Table 6, the ratios of 20.0 and 31.5 to 27.1 min of LXSS were similar to those of

garden ginseng with similar detected percentages, and the ratio of 41.0 min was a little higher than that of garden ginseng with higher detected percentage, while the ratio of 23.5 min was lower in LXSS with lower detected percentage. It is the most important that 50% of LXSS detected showed several peaks below the limit of

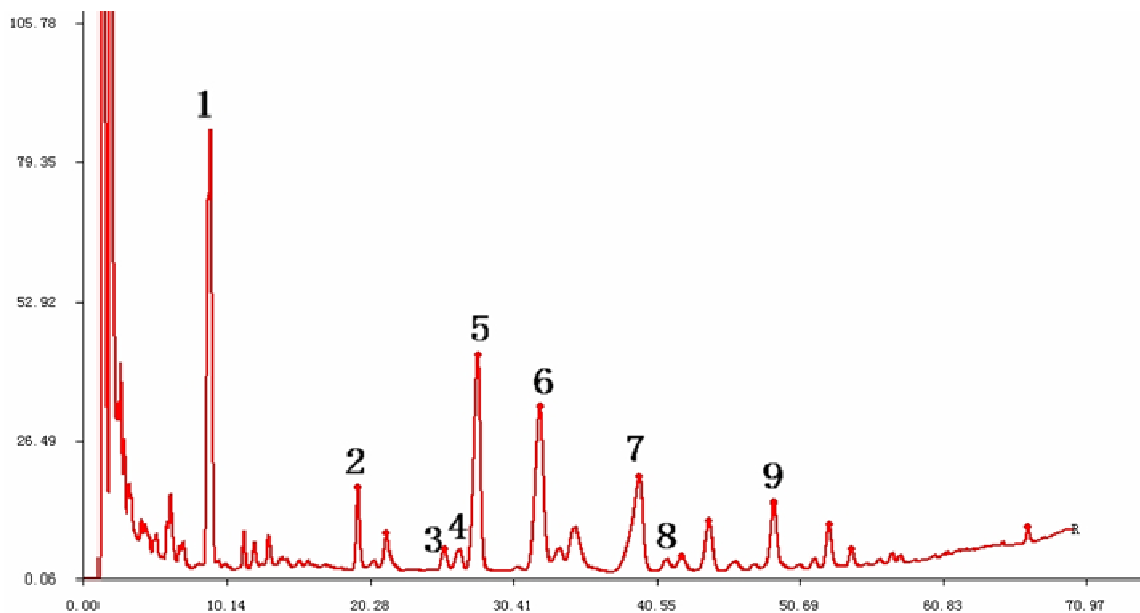


Figure 6. The reference fingerprint of LXSS. **1** Rg₁ and Re, **2** Rf, **3** S-Rg₂, **4** R-Rg₂, **5** Rb₁, **6** Rc, **7** Rb₂, **8** Rb₃, **9** Rd.

Table 6. Ratios of components with polarity between Re and Rf to that with retention time at 27.1 min (n = 10).

Sample	Source	Retention time (min)					
		20.5 (%)	24.0 (%)	27.1 (%)	31.8 (%)	41.4 (%)	50.0~54.0 (%)
LXSS	Benxi	4.00 (100)	0.20 (30)	1.00 (100)	0.70 (30)	0.31 (40)	(50)
garden ginseng		5.32 (83)	3.18 (83)	1.00 (58)	0.68 (33)	0.19 (8)	(0)

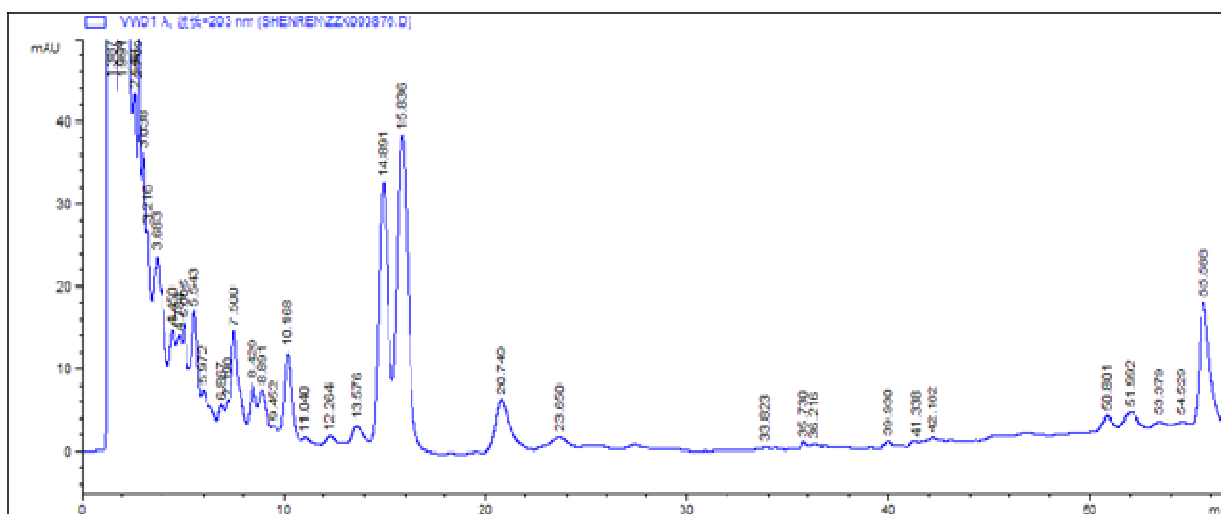


Figure 7. Chromatographic analysis of components with retention time between those of Re and Rf of LXSS.

quantification (LOQ) between 50.0 and 54.0 min under this chromatographic condition, while none of garden ginseng showed these peaks. So LXSS and garden ginseng could be distinguished from the area ratios of

other peaks to 27.1 min as well as their profiles (Figures 7, 8 and 9).

Peak identifications of Figures 7 and 8 were still under investigation. Based on HPLC analysis of authentic

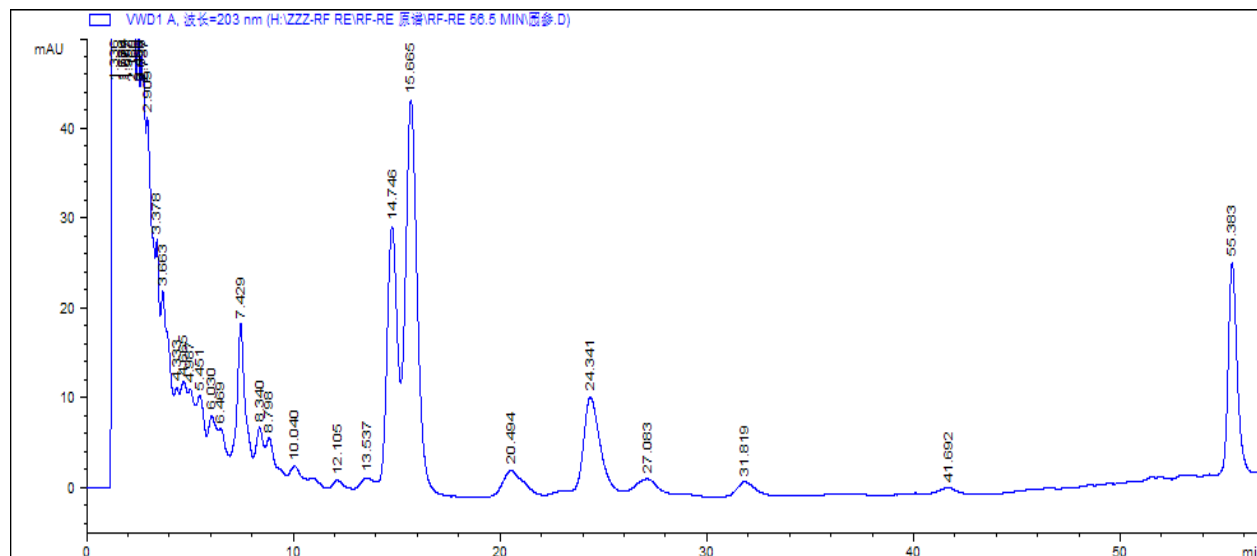


Figure 8. Chromatographic analysis of components with retention time between those of Re and Rf of garden ginseng.

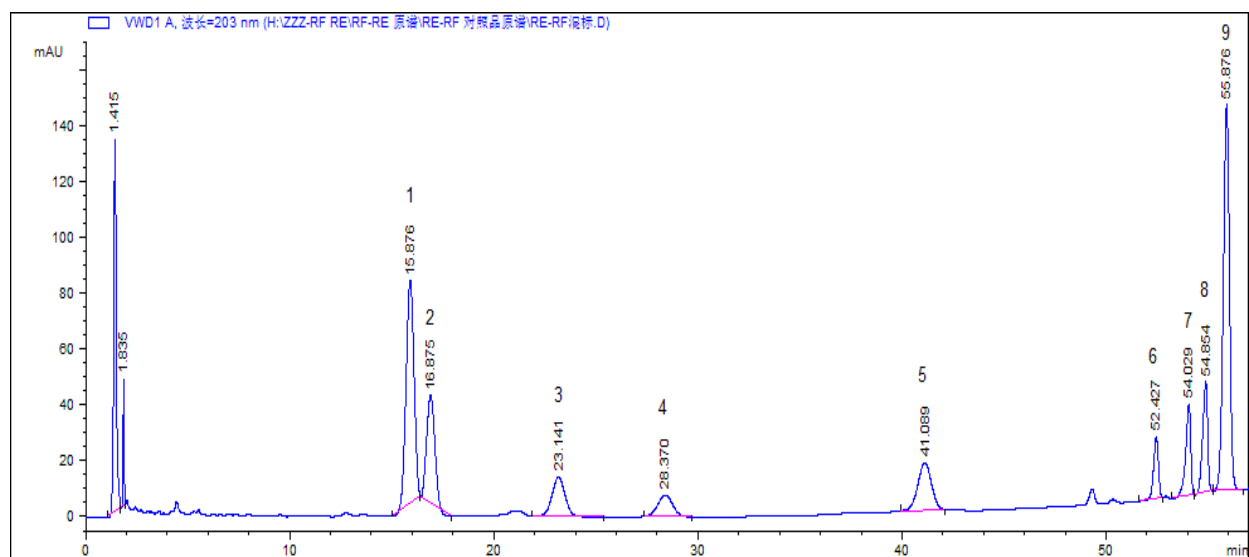


Figure 9. Chromatographic analysis of authentic samples available under condition for isolation of compounds between Re and Rf. **1:** Ginsenoside Rg₁; **2:** Ginsenoside Re; **3:** Quinquefoloside Lb; **4:** Ginsenoside Ra₃ and Ginsenoside Le; **5:** Ginsenoside Rh₉; **6:** Vinaginsenoside R₈; **7:** 3 β , 12 β , 20(S), 24(S)-tetrahydrodammar-25-ene 3-O-[β -D-glucopyranosyl (1 \rightarrow 2) - β -D-glucopyranoside]-20-O-[α -L-arabinopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside]; **8:** 3 β , 12 β , 20(S), 24(S)-tetrahydrodammar-25-ene 3-O-[β -D-glucopyranosyl (1 \rightarrow 2) - β -D-glucopyranoside]-20-O-[β -D-xylopyranosyl (1 \rightarrow 6) - β -D-glucopyranoside]; **9:** Ginsenoside Ia and Rf.

samples at hand, most triterpenoid saponins appeared in this area had changes in the side chain of dammarane skeleton, such as cyclization (Quinquefoloside Lb (Jiang et al., 2008b), Ginsenoside Rh₉ (Dou et al., 2001b), Ginsenoside Le (Zhou et al (accepted) Two new dammarane-type tetraglycosides from leaves of *Panax Quinquefolium*. Journal of natural medicine.), hydroxylation (Vinaginsenoside R₈ (Nguyen et al., 1994),

and double bond migration {3 β , 12 β , 20(S), 24(S)-tetrahydrodammar-25-ene 3-O-[β -D-glucopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside]-20-O-[α -L-arabinopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside] (Kaikura et al., 1991), 3 β , 12 β , 20(S), 24(S)-tetrahydrodammar-25-ene 3-O-[β -D-glucopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside]-20-O-[β -D-xylopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside (Yoshikawa et al., 1987)]. This indicated that the different growing

circumstance of LXSS and garden ginseng might effect the biosynthesis of dammarane triterpenoids with changes in side chain.

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REFERENCES

- Cheng DR, Fu R, Dou DQ, Kang TG (2007). Hemolytic and its Protective Action of Ginsenosides. *Modern Chinese Med.*, 9(9): 7-8.
- Dou DQ, Chen YJ, Liang LH, Pang FG, Shimizu N, Takeda T (2001b). Six New Dammarane-type Triterpene Saponins from the Leaves of *Panax ginseng*. *Chem. Pharm. Bull.*, 49(4): 442-446.
- Dou DQ, Zhang YW, Zhang L, Chen YJ, Yao XS (2001a). The inhibitory effects of ginsenosides on protein tyrosine activation induced by hypoxia/reoxygenation in cultured human umbilical vein endothelial cells. *Planta Medica.*, 67(1): 19-24.
- Jiang HP, Dou DQ, Jing SQ, Liu FY (2008a). Study on ginsenoside and fingerprint of Lin-Xia-Shan-Shen by HPLC. *Modern Chinese Med.*, 10(4): 12-15.
- Jiang HP, Liu FY, Dou DQ, Li JH (2007). The SOP for cultivation of Lin-Xia-Shan-Shen. *Modern Chinese Med.*, 10(9): 34-38.
- Jiang HP, Qiu YK, Chen DR, Kang TG, Dou DQ (2008b). Structure elucidation and complete NMR spectral assignments of two new dammarane-type tetraglycosides from *Panax quinquefolium*. *Magnetic Resonance in Chemistry.*, 46: 786-790.
- Karikura M, Miyase T, Tanizawa H, Taniyama T, Takino Y (1991). Studies on absorption, distribution, excretion and metabolism of ginseng saponins. VI. The decomposition products of ginsenoside Rb₂ in the stomach of rats. *Chem. Pharm. Bull.*, 39(2): 400-404.
- Nguyen MD, Kasai R, Ohtani K, Ito A, Nguyen TN, Yamasaki K, Tanaka O (1994). Saponins from Vietnamese ginseng, *Panax vietnamensis* Ha et Grushv. Collected in central Vietnam. II. *Chem. Pharm. Bull.*, 42(1): 115-122.
- Qiu YK, Dou DQ, Cai LP, Jiang HP, Kang TG, Yang BY, Kuang HX, Li Michael ZC (2009). Dammarane-type saponins from *Panax quinquefolium* and their inhibition activity on human breast cancer MCF-7 cells. *Fitoterapia.*, 80: 219-222.
- Yoshikawa K, Takemoto T, Arihara S (1987). Studies on the constituents of Cucurbitaceae plants. XVI. On the saponin constituents of *Gynostemma pentaphyllum* Makino. *Yakugaku Zasshi.*, 107(4): 262-267.
- Zhou ZX, Dou DQ, Zhao HX (2010). The dynamic accumulation of ginsenosides in *Panax ginseng* "Linxia Shanshen" in different seasons. *Ginseng Res.*, 88(2):12-18.
- Zhu JJ, Wang ZM, Kuang YH, Zhang QW, Gao QP, Ma N (2008). A quantitative method using one marker for simultaneous assay of ginsenosides in *Panax ginseng* and *P. notoginseng*. *Acta Pharmaceutica Sinica.*, 43(12): 1211-1216.