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Analytical Methods

Study on ginsenosides in different parts and ages of *Panax quinquefolius* L.

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ABSTRACT

The contents of 12 ginsenosides (Rg_1, Re, F_{11}, Rf, Rg_2, Rh_1, Rb_1, Rc, Rb_2, Rb_3, Rd, Rh_2) in different parts and ages of *Panax quinquefolius* L. (American ginseng) were quantified by high pressure microwave-assisted extraction (HPMAE) high-performance liquid chromatography coupled with evaporative light scattering detection (HPLC-ELSD). The analytical method was established and analytical performances were evaluated. The chemical marker of American ginseng F_{11} was detected, and Rf which is the chemical marker of Asian ginseng was not found. Rare ginsenoside Rh_1, Rg_2 and Rh_2 were also studied in this experiment. The total contents of these 12 ginsenosides in the different parts of 5-year-old American ginseng follow this order: leaf > root-hair > rhizome > root > stem. Therefore, compared with the other parts of American ginseng, the leaf is a better available source of ginsenosides. The contents of ginsenosides in root and leaf also change with age.

1. Introduction

Ginsenosides are the primary bioactive components of Asian ginseng (*Panax ginseng* C.A. Meyer), American ginseng (*Panax quinquefolius* L.) and San qi (*Panax notoginseng*). All these kinds of ginsengs are well known for their use in healthy food and traditional medicine (Zhang, Chen, Wu, & Wang, 2006). Asian ginseng is the most familiar herbal medicine which has been used as a tonic, sedative, anti-fatigue, or anti-gastric ulcer drug, and also has antidiabetic and antitumor activities (Lee, Lee, Kim, Park, & Lee, 1997; Shin, Bae, & Kim, 2006). American ginseng, which is cultivated in United States and Canada, is used to reduce stress, lower high blood sugar and adjust immunity (Vuksan et al., 2001). Modern pharmacological studies have shown that San Qi has anticarcinogenic and hepatoprotective activities, as well as protective effects on cardiovascular and cerebrovascular systems (Konoshima, Takasaki, & Tokuda, 1999). Ginsengs have been not only used as therapeutic agents but also marketed as dietary supplements and raw materials of health food (Shen, Ren, & Chen, 2003; Wang et al., 2008). For example, the root of Asia ginseng has been used as additives to drinks for hundreds of years.

More than 40 ginsenosides have been identified, isolated and characterised till now (Teng et al., 2003). Based on their aglycone moieties, ginsenosides can be mainly divided into two categories: 20(S)-protopapaxdiol (ginsenoside Rb_1, Rb_2, Rb_3, Rc, Rd and Rh_2) and 20(S)-protopapaxtriol (ginsenoside Re, Rf, Rg_1, Rg_2 and Rh_1) (Fig. 1a) (Kim, Ha et al., 2007). Other ginsenosides which do not belong to the two categories are also identified, such as oleanolic acids (Ro, Rhs, R_1, F_4) and pseudoginsenoside F_{11} (Fig. 1b) (Leung, Chan, Bensoussan, & Munroe, 2007; Wang, Wang, Wu, Osinski, & Yuan, 2005). Each kind of ginseng has its own characteristic ginsenoside which does not exist in other kinds of ginsengs. For example, ginsenoside F_{11} is the characteristic component of American ginseng, while ginsenoside Rf is the chemical marker of Asian ginseng. Reverse C18 column was usually employed to separate ginsenosides (Chen, Xie, Fu, Lee, & Wang, 2007; Liu, Han, Duan, Huang, & Wang, 2007; Shangguan et al., 2001; Wan, Lai et al., 2006; Wan, Yang, Li, Wang, & Cui, 2006; Wood, Bernards, Wan, & Charpentier, 2006; Zhang et al., 2006). However, polyvinyl alcohol-bonded stationary phase was also used to separate ginsenosides (Quiming, Denola, Soliev, Saito, & Jinno, 2007).

Only few articles reported the changing trends of contents of ginsenosides in ginsengs along with the age and parts. Wang et al. (2005) investigated the amounts of Rg_1, Re, Rf, Rb_1, Rc, Rb_2 and Rd in the root of 5- and 7-year Illinois-cultivated American ginseng and those cultivated for 3 or 4 years in Wisconsin. Li, Mazza, Cottrell, and Gao, (1996) studied ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2, Rd in the root and leaf of American ginseng cultivated at different sites. While Lim, Mudge, and Vermeylen, (2005) also studied the genotype effects. In a previous study in our laboratory (Shi, Wang, Li, Zhang, & Ding, 2007), seven major ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3 and Rd in different parts and ages of Asian ginseng were studied by HPLC coupled with UV detection.

The conventional methods for extracting Chinese herbal medicine include reflux extraction (RE), Soxhlet extraction (SE), ultrasonic extraction (UE) and supercritical fluid extraction (SFE) etc. These extraction methods confront with some problems, such as

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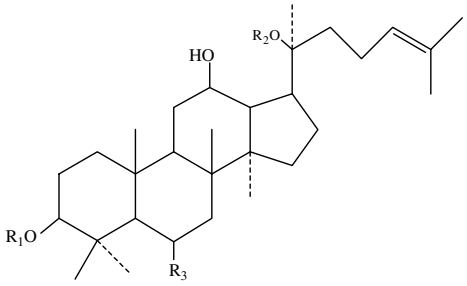
a



Ginsenoside	R_1	R_2	R_3
Rg_1	-H	-glc	-O-glc
Re	-H	-glc	-O-glc(2-1)glc
Rf	-H	-H	-O-glc(2-1)glc
Rg_2	-H	-H	-O -glc(2-1)rha
Rh_1	-H	-H	-O-glc
Rb_1	-glc(2-1)glc	-glc(6-1)glc	-H
Rc	-glc(2-1)glc	-glc(6-1)ara (f)	-H
Rb2	-glc(2-1)glc	-glc(6-1)ara (p)	-H
Rb3	-glc(2-1)glc	-glc(2-1)xyl	-H
Rd	-glc(2-1)glc	-glc	-H
Rh_2	-glc	-H	-H

Glc = glucose, ara (p) = alpha-L arabinopyranose, ara (f) = alpha-L arabinofuranose, rha = rhamnose

b

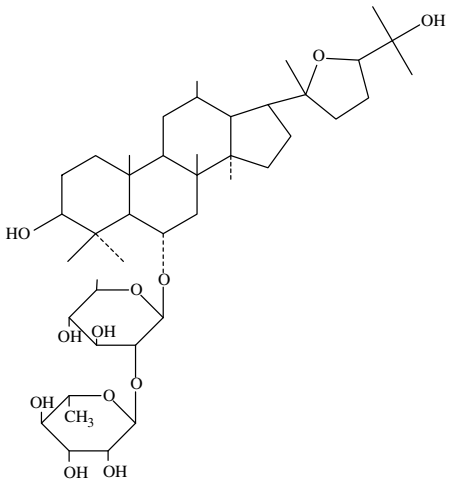


Fig. 1. Structure of Rg_1, Re, Rf, Rg_2, Rh_1, Rb_1, Rc, Rb_2, Rb_3, Rd and Rh_2 (a) and F_{11} (b).

long time and low efficiency. Recently other extraction methods were also employed, e.g. Li's group used pressurised liquid extraction to extract ginsenosides (Wan, Lai et al., 2006; Wan, Yang et al., 2006). And microwave-assisted extraction (MAE) in which the extraction time is dramatically reduced is popular in these years (Kim, Murthy, Hahn, Lee, & Paek, 2007; Kwon, Belanger, Jocelyn Pare, & Yaylayan, 2003; Shi et al., 2007; Shu, Ko, & Chang, 2003; Zhang et al., 2006). It includes atmospheric pressure microwave-assisted extraction (APMAE) and high pressure microwave-assisted extraction (HPMAE).

HPLC-UV method, which uses acetonitrile and water as elution solvents and measures the absorbance at wavelength of 203 nm, is a general method to determine ginsensosides. Acetonitrile used in the gradient elution also has absorption at 203 nm and makes baseline drift. Besides UV detector, evaporative light scattering detector (ELSD) was also used to detect ginsenosides (Kim et al., 2007; Kim et al., 2000; Kwon et al., 2001). Kim's group (Kim et al., 2007) simultaneously quantified 14 ginsenosides in Korean red ginseng by HPLC-ELSD. The working principle of ELSD is to evaporate the elution solvent and remain the non-volatile objects to be detected, so ELSD can solve the baseline drift problem well. Another reason for the use of ELSD in our study was that the chemical marker ginsenoside F_{11} does not have UV absorption at 203 nm. Recently Kwon, Jeong, Lee, and Hong, (2008) used pulsed amperometric detection to determine glycosides which include ginsenoside Rg_1, Re, Rf, Rb_1, Rc, Rb_2 and Rd.

Many scientists usually focused on the root of different kinds of ginsengs. In the present study, besides the root, we also studied the

leaf, root-hair, rhizome and stem of American ginseng. This would provide more data and information in the fields of food and medicine.

2. Experimental

2.1. Materials and instruments

The standards of ginsenoside Rg_1, Re, F_{11}, Rf, Rg_2, Rh_1, Rb_1, Rc, Rb_2, Rb_3, Rd and Rh_2 were purchased from Chinese Medical and Biological Products Institute (Beijing, China). Ginsenoside Rg_1 (0.5570 mg mL^{-1}), Re (0.6475 mg mL^{-1}), F_{11} (0.5700 mg mL^{-1}), Rf (0.5160 mg mL^{-1}), Rg_2 (0.4800 mg mL^{-1}), Rh_1 (0.4380 mg mL^{-1}), Rb_1 (0.5390 mg mL^{-1}), Rc (0.4600 mg mL^{-1}), Rb_2 (0.5310 mg mL^{-1}), Rb_3 (0.5170 mg mL^{-1}), Rd (0.4620 mg mL^{-1}) and Rh_2 (0.4340 mg mL^{-1}) mixed stock standard solution was prepared in methanol and diluted to a series of different concentrations to establish the calibration curves. HPLC grade acetonitrile and methanol were obtained from Fisher Corporation, USA. Water used in all experiments was purified by Milli-Q system (Millipore Corporation, USA).

A WR-C microwave preparation system (Michem Technology Co. Ltd., Beijing, China) with a pressure and temperature control system was used to perform HPMAE. Agilent 1100 liquid chromatograph (Agilent Technologies, USA) equipped with quaternary gradient pump was used to separate the ginsenosides in American ginseng. SEDEX75 evaporative light scattering detector (Digma Co. Ltd., France) coupled with a Chromatograph Workstation (Jiangshen Co. Ltd., Dalian, China) was used for detecting ginsenosides.

The fresh American ginseng materials were harvested in fall and obtained from Jingyu County in Jilin Province, China. The root, root-hair, rhizome, stem and leaf of American ginseng were detached, rinsed with water and dried at 38 °C in ventilated drier to constant weight. The dried ginseng was crushed and then passed through a 40 mesh sieve with 0.425 mm aperture. The samples were put into Soxhlet extractor and enough quantity of chloroform was added. Then the samples were refluxed for 3 h to remove fats and chlorophyll (Zhang et al., 2006). The degreased sample was placed at a ventilating place to make the chloroform volatilise completely and then the analytical sample was obtained.

2.2. Procedures

2.2.1. High pressure microwave-assisted extraction (HPMAE)

Five hundred milligrams of analytical sample was accurately weighed and put into the extraction vessel, followed by adding fifty millilitres of 70% (v/v) ethanol solution. Then the vessel was closed tightly and put into a WR-C special microwave sample preparation system. The pressure, temperature and time were set at 450 KPa, 125 °C and 10 min, respectively. After the extraction was finished, the samples were cooled down to room temperature and filtered and the solvent was evaporated by a rotary evaporator at 50 °C. Then the residue was dissolved in 25 ml methanol and the sample solution was filtered through 0.45 μm filter. 20 μl of sample solution was injected into HPLC system for analysing.

2.2.2. Soxhlet extraction (SE)

Five hundred milligrams of 5-year-old leaf of American ginseng was accurately weighed and placed into a 250 ml flask of a Soxhlet extractor. One hundred millilitres of 70% (v/v) ethanol solution was added into it. The mixture was refluxed in a water-bath (80 °C) for 5 h to extract the ginsenosides. Then the extract was evaporated, and the residue was dissolved in a fixed volume in methanol, and then filtered for analysis.

2.2.3. The conditions of evaporative light scattering detection

The evaporative light scattering detector makes the elution solvent evaporate and the non-volatile objects remain to be detected. The response values are closely related to the pressure of carrier gas and the temperature of drift tube. The pressure of carrier gas and temperature of drift tube in our experiment were set at 2.0 bar and 40 °C, respectively.

2.2.4. Determination of ginsenosides by HPLC-ELSD

A reversed-phase C18 column Prevail (5 μm, 250 mm × 4.6 mm ID) was used to separate ginsenosides in a binary solvent consisted of acetonitrile (A) and water (B) with a gradient elution: 0–31 min, 21% A, 79% B; 31–32 min, 21–30% A, 79–70% B; 32–53 min, 30% A, 70% B; 53–54 min, 30–32% A, 70–68% B; 54–64 min, 32% A, 68% B; 64–65 min, 32–39% A, 68–61% B; 65–72 min, 39% A, 61% B; 72–74 min, 39–75% A, 61–25% B; 74–81 min, 75% A, 25% B. The column was then washed with 100% A for 10 min at a flow rate of 1.5 ml min^{-1}. After then, the elution solvent was kept at 21% A and 79% B for 20 min at a flow rate of 1.5 ml min^{-1} to analyse next sample.

3. Results and discussion

3.1. Evaluation of the methods

3.1.1. Calibration curve, limit of detection (LOD) and limit of quantification (LOQ)

The calibration curves and the LOD for the ginsenosides are shown in Table 1. The correlation coefficients are all better than 0.9875, which show good linearity. The LODs, which are in the range from 7.49 to 11.55 μg ml^{-1} for the 12 ginsenosides, were determined as the lowest concentrations injected that yielded a signal-to-noise (S/N) ratio of 3. The LOQs were determined as the concentrations that yielded a S/N ratio of 10 and are in the range from 25.0 to 38.5 μg ml^{-1}.

3.1.2. Precision

The precision of this HPLC method for determining ginsenosides was evaluated by intra-day and inter-day variations. Five hundred milligrams of 5-year-old root sample was treated with the method described in Section 2.2.1, then analysed by the method mentioned in Section 2.2.4 to study the intra-day precision. The experiment was finished in one day and three parallel experiments were carried out. The inter-day precision was studied by performing the same process in three different days. This HPMAE-HPLC-ELSD method shows good reproducibility for the quantification of the 12 ginsenosides. The intra-day precisions of 12 ginsenosides which were worked out with the relative standard deviations (RSD) are between 0.59% and 3.00%, and inter-day precisions of these ginsenosides are between 1.15% and 6.41%.

3.1.3. Analysis of spiked samples

The accuracy of this method was evaluated by recovery experiment. The recoveries were calculated by the following formula: recovery (%) = (found amount–original amount)/added amount × 100%. Five hundred milligrams of the mixture of 4-year-old and 5-year-old rhizome was treated as described in Sections 2.2.1 and 2.2.4 to get the original amounts of these ginsenosides. Then fixed quantities of 12 ginsenosides were added into 500 mg of the mixture of 4-year-old and 5-year-old rhizome and analysed in the same way. All the data were the average values obtained by three parallel experiments. The recoveries of the 12 ginsenosides were listed in Table 2. The recoveries are between 92.06% and 107.46%. These results demonstrate that the method

Table 1
Calibration curve, LOD and LOQ for 12 ginsenosides.

Ginsenoside	Calibration curve	r	Concentration range (μg ml^{-1})	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
Rg_1	$Y = 1.9494X + 20.9660$	0.9993	46.50–557.00	7.49	25.0
Re	$Y = 1.8944X + 20.6405$	0.9996	27.00–647.00	7.70	25.7
F_{11}	$Y = 1.7277X + 21.7345$	0.9999	23.75–570.00	8.45	28.2
Rf	$Y = 1.7313X + 21.6913$	0.9949	21.50–516.00	8.43	28.1
Rg_2	$Y = 1.6405X + 21.0454$	0.9980	20.00–480.00	8.89	29.6
Rh_1	$Y = 1.7406X + 21.1217$	0.9989	18.25–438.00	8.38	27.9
Rb_1	$Y = 1.5540X + 21.0021$	0.9875	22.50–539.00	9.39	31.3
Rc	$Y = 1.6788X + 21.0109$	0.9986	19.13–460.00	8.69	29.0
Rb_2	$Y = 1.6686X + 21.0512$	0.9977	22.13–531.00	8.74	29.1
Rb_3	$Y = 1.7285X + 21.0278$	0.9984	21.50–517.00	8.44	28.1
Rd	$Y = 1.47328X + 21.8415$	0.9906	38.50–462.00	9.90	33.0
Rh_2	$Y = 1.2638X + 19.8391$	0.9978	36.20–434.00	11.55	38.5

Y is the peak area and X is the concentration of analyte.

Table 2
Recovery of ginsenosides determined by standard addition method.

Ginsenoside	Original (mg)	Added (mg)	found (mg)	Recovery (%)	RSD (%)
Rg_1	1.69	3.40	4.82	92.06	0.83
Re	6.63	9.65	16.77	105.08	5.85
F_{11}	1.23	2.28	3.68	107.46	0.28
Rf	ND	0.46	0.48	104.35	8.08
Rg_2	ND	1.97	1.84	93.40	2.21
Rh_1	ND	1.13	1.19	105.31	3.53
Rb_1	3.22	4.20	7.50	101.90	4.81
Rc	0.59	1.59	2.14	97.48	6.61
Rb_2	0.22	1.04	1.24	98.08	8.26
Rb_3	0.71	1.68	2.34	97.02	9.87
Rd	2.15	5.27	7.72	105.69	2.30
Rh_2	ND	6.23	5.93	95.18	3.73

ND, not detectable.

proposed in this paper is accurate for the quantitative determination of 12 ginsenosides in ginseng samples.

3.1.4. Selection of extraction method

The 5-year-old leaf was studied by HPMAE and Soxhlet extraction. The results are shown in Table 3. It shows that almost all the contents of 12 ginsenosides obtained by HPMAE are higher than that obtained by Soxhlet except the contents of ginsenoside Rg_2, Rb_1 and Rc, which obtained by HPMAE are almost the same as those obtained by Soxhlet. The ginsenoside Rf, Rh_1, Rh_2 are undetectable by both methods. What is more, the HPMAE extraction only took 10 min, while Soxhlet extraction costs 5 h. Therefore the HPMAE was applied in this study.

3.2. Ginsenoside contents in different parts of American ginseng

The ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3 and Rd in different parts and ages of Asian ginseng were studied previously (Shi et al., 2007). It was found that the total content of ginsenoside in root-hair is the highest and that in stem is the lowest. The root of American ginseng was studied by some scientists. But other parts of American ginseng were not studied as widely as the root.

In this work, the different parts of 5-year-old American ginseng were studied. The ginsenoside Rg_1, Re, F_{11}, Rf, Rg_2, Rh_1, Rb_1, Rc, Rb_2, Rb_3, Rd and Rh_2 in the root, root-hair, rhizome, stem and leaf of 5-year-old American ginseng were determined. The results are shown in Table 4. The marker of American ginseng F_{11} is detectable in all parts of American ginseng and the content of F_{11} in the leaf is much higher than that in other parts. The contents of ginsenoside Rg_1, Re, Rg_2, Rb_2, Rb_3 and Rd in the leaf are higher than that in other parts, too. But the content of ginsenoside Rb_1 in leaf is relatively low. Rf, which is the chemical marker of Asian ginseng, is proved not existing in American ginseng. Rare ginsenoside Rh_1, Rg_2 and Rh_2 were also studied in this experiment. Ginsenoside Rh_1 and Rh_2 were undetectable. The contents of gisenoside Rg_2 were found to be quite low in the root, root-hair and rhizome. The total contents of ginsenosides in these five parts follow this order: leaf > root-hair > rhizome > root > stem. As medicine or tonic, the main-root, root-hair and rhizome are used as a whole, and the leaf and stem are not used. Due to the high content of ginsenosides in leaf of American ginseng, the leaf should be fully used as a new ginsenoside supplier. Different ginsenosides have different pharmacological effects, so we can do the extraction, separation and purification based on the content differences in different parts.

3.3. Ginsenoside contents in different ages of American ginseng root

Fig. 2 shows that the main ginsenosides in the root of American ginseng are ginsenoside Re and Rb_1, which is consistent with the results obtained by Wang et al. (2005) and Li et al. (1996). However, besides ginsenoside Re and Rb_1, Rg_1 is also a main ginsenoside in the root of Asian ginseng (Shi et al., 2007). The contents of ginsenoside Re and Rb_1 in root increase gradually from 1-year-old to 5-year-old. The same rule is observed for the total contents of 12 ginsenosides in root. But the increase rates for the contents of ginsenoside Re and Rb_1 are different. The content of ginsenoside Re in root increases gradually with the increase of age, but the change of content of ginsenoside Rb_1 in root is slight from 1 to 2-year-old and that is significant from 2 to 5-year-old.

3.4. Ginsenoside contents in different ages of American ginseng leaf

Mazza's group studied the 1-month-old and 4-month-old leaf of American ginseng (Li et al., 1996). 1-month-old leaf contains total

Table 3
Comparison of the results obtained by HPMAE and Soxhlet extraction.

Extraction method	Content of ginsenosides in 5-year-old leaf (mean ± SD) (mg g^{-1})												Extraction time (min)	Extraction pressure (kPa)
	Rg_1	Re	F_{11}	Rf	Rg_2	Rh_1	Rb_1	Rc	Rb_2	Rb_3	Rd	Rh_2		
MAE	9.58 ± 0.12	34.19 ± 1.93.	19.41 ± 0.11	ND	1.06 ± 0.16	ND	2.77 ± 0.42	5.60 ± 0.51	18.22 ± 1.35	46.36 ± 0.28	28.06	ND	10	450
Soxhlet	7.23 ± 0.17	33.49 ± 0.06	13.35 ± 0.15	ND	1.37 ± 0.17	ND	2.78 ± 0.34	5.66 ± 0.24	17.67 ± 0.30	32.03 ± 0.63	18.10 ± 0.06	ND	300	101

ND, not detectable.

Table 4
Contents of ginsenosides in different parts of 5-year-old American ginseng (mean ± SD) (mg g^{-1}).

Part of plant	Rg_1	Re	F_{11}	Rf	Rg_2	Rh_1	Rb_1	Rc	Rb_2	Rb_3	Rd	Rh_2	Total content
Rentention time (min)	27.6	30.1	40.0	41.5	49.8	51.3	56.4	60.1	63.4	65.3	67.6	74.1	
Main-root	2.45 ± 0.88	20.54 ± 0.50	2.03 ± 0.18	ND[a]	ND	ND	18.46 ± 0.79	2.88 ± 0.10	0.44 ± 0.07	0.38 ± 0.10	2.19 ± 0.17	ND	49.37 ± 2.79
Root-hair	8.32 ± 0.60	19.07 ± 0.40	3.95 ± 0.08	ND	ND	ND	11.63 ± 0.11	10.68 ± 0.46	2.34 ± 0.04	3.62 ± 0.08	9.48 ± 0.42	ND	69.09 ± 2.19
Rhizome	8.81 ± 0.39	17.80 ± 0.57	3.25 ± 0.13	ND	ND	ND	10.61 ± 0.04	2.49 ± 0.23	0.91 ± 0.06	2.54 ± 0.19	4.61 ± 0.45	ND	51.02 ± 2.06
Leaf	9.58 ± 0.12	34.19 ± 1.93.	19.41 ± 0.11	ND	1.06 ± 0.16	ND	2.77 ± 0.42	5.60 ± 0.51	18.22 ± 1.35	46.36 ± 0.28	28.06	ND	165.25 ± 5.18
Stem	0.45 ± 0.06	5.52 ± 0.18	3.15 ± 0.03	ND	0.22 ± 0.03	ND	1.35 ± 0.02	0.28 ± 0.03	1.06 ± 0.06	3.75 ± 0.03	4.45 ± 0.43	ND	20.23 ± 0.87

[a] ND, not detectable.

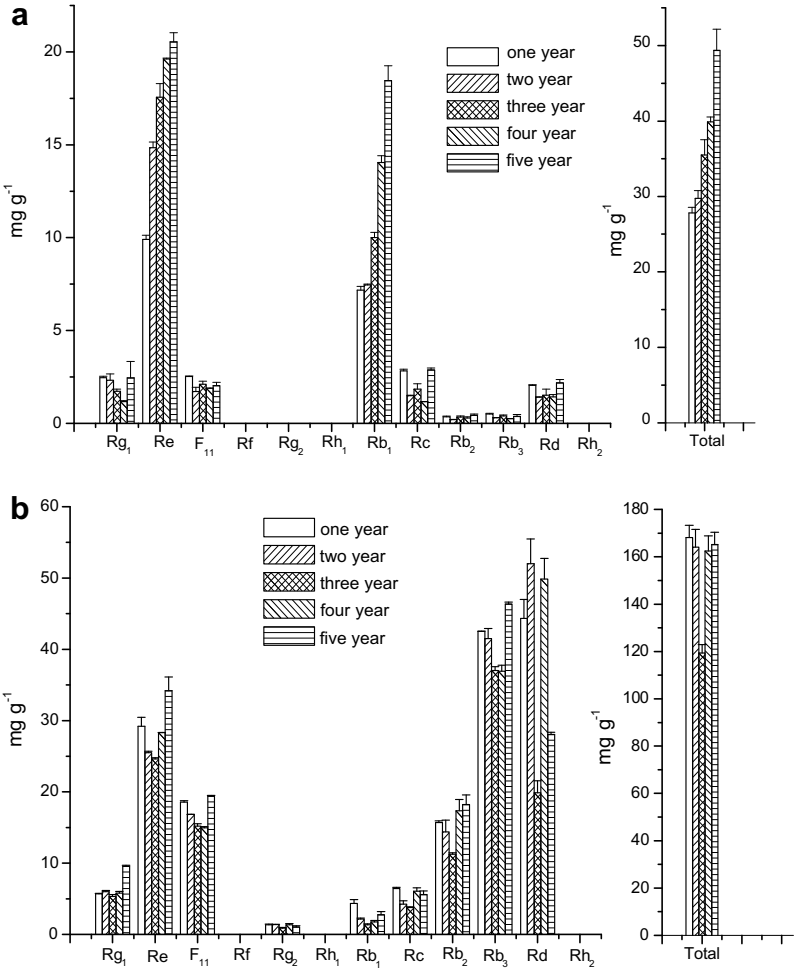


Fig. 2. The changes of ginsenoside contents (mg g^{-1}) in the root (a) and leaf (b) of American ginseng with age.

ginsenoside of 13.3–26.4 mg g^{-1} dry weight, and the ginsenoside Re accounts for >50% of the total ginsenoside. In mature, 4-month-old leaf, the total ginsenoside content ranges from 41.4 to 55.8 mg g^{-1} dry weight, and the ginsenoside Re and Rd each accounts for ~40% of the total ginsenosides.

1-year-old to 5-year-old leaf of American ginseng was investigated in this work. As shown in Fig. 2, ginsenoside Re, F_{11}, Rb_2, Rb_3 and Rd are the main constituents in the leaf of American ginseng, and the contents of Rb_3 and Rd are high. In the leaf of Asian ginseng, the content of ginsenoside Re is the highest among the seven ginsenosides, Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3 and Rd (Shi et al., 2007). The total content of the 12 ginsenosides is not obviously dependent on age. However, the total content of ginsenosides in 3-year-old leaf of American ginseng is lower than that in other ages. It is likely that the leaf is newly grown annually. Therefore, the content is relative to climatic conditions in each year.

4. Conclusion

In this paper, 12 ginsenosides, Rg_1, Re, F_{11}, Rf, Rg_2, Rh_1, Rb_1, Rc, Rb_2, Rb_3, Rd and Rh_2, in different parts and ages of American ginseng were determined by HPMAE HPLC-ELSD. F_{11} was detectable in all parts of American ginseng, and Rf was validated not existing in American ginseng. The total content of these 12 ginsenosides in leaf is much higher than that in the root, root-hair, rhizome and stem. Rare ginsenoside Rh_1, Rg_2 and Rh_2 were also studied in this experiment. Ginsenoside Rh_1 and Rh_2 were not detectable in American ginseng. The content of ginsenoside Rg_2 was found to be quite low. The contents of main ginsenoside Re and Rb_1 in root increase with age. The same rule goes for the total content of 12 ginsenosides. The total content of 12 ginsenosides in leaf is not obviously dependent on age.

In present work, the result reveals that there are more ginsenosides in leaf than those in root. Therefore, the leaf of American ginseng may be considered to be a new source of ginsenosides. The contents of ginsenosides in different parts and ages of American ginseng provide more information of American ginseng. This paper also makes a contribution to quality control of American ginseng.

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