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Investigation of ginsenosides in different parts and ages of *Panax ginseng*

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Abstract

Panax ginseng (*P. ginseng*) has been used as a traditional medicine for thousands of years. It includes *P. ginseng* root, leaf, root-hair, rhizome and stem. *P. ginseng* root is usually considered to be the main part for medicine, and other parts of *P. ginseng* are neglected. In this paper the content of ginsenosides in different parts and ages of *P. ginseng* was determined. Separation and determination of seven major ginsenosides, including Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3 and Rd, has been achieved by high performance liquid chromatography (HPLC) with UV detector at 203 nm. The extraction of ginsenosides from *P. ginseng* material was performed by microwave-assisted extraction (MAE). The results indicate that the content of ginsenosides is higher in the leaf and root-hair, and lower in stem than that in other parts of *P. ginseng*. The content of ginsenosides in root and root-hair increases with increase in age of *P. ginseng* from one to five years. However, the total content of ginsenosides in *P. ginseng* leaf decreases with the increase in age.

Keywords: High performance liquid chromatography; *Panax ginseng*; Ginsenosides; Microwave-assisted extraction

1. Introduction

Panax ginseng (*P. ginseng*) is a valuable agricultural commodity used in many traditional medicinal therapies. Nowadays *P. ginseng* is used mainly to increase resistance to physical, chemical and biological stress and boost general vitality, and it is frequently featured in traditional medicine used by cancer patients (Chang, Seo, Gyllenhaal, & Block, 2003; Kiefer & Pantuso, 2003). Ginsenosides are the main active constituents in *P. ginseng*, and the main ginsenosides are derived from the every part of *P. ginseng*. Up to now more than 30 ginsenosides have been reported from *P. ginseng* and most of them exhibit four types of aglycone moieties: protopanaxadiol, protopanaxatriol, ocotillol and oleanolic acid types (Fuzzati, 2004; Fuzzati, Gabetta, Jagaker, Pace, & Peterlongo, 1999). *P. ginseng* root is considered to be the main part used for medicinal purposes, and most studies on the ginsenosides have focused on the *P. ginseng* root, although there exist publications on ginsenoside contents of the other parts of *P. ginseng* (Attele et al., 2002; Popovich & Kitts, 2004; Wang, Chen, & Chang, 2001; Xie et al., 2004; Yip, Lau, But, & Kong, 1985). Samukawa, Yamashita, Matsuda, and Kubo (1995) examined contents of ginsenosides in Japanese ginseng according to the differences in the year of growth and the part of the plant, including lateral-root, root, root-hair, rhizome and periderm. The change of ginsenosides with age is related to the growing area of *Panax* sp., for example, Wang, Wang, Wu, Osinski, and Yuan (2005) determined and compared major ginsenosides in American ginseng root of different age grown in different areas. Mizuno et al. (1994) indicated that the total content of gisenosides in wild *P. ginseng* and cultivated *P. ginseng* were 30.17 and 32.26 mg/g dry weight, respectively. Ginsenoside Re content was highest and occupied about 45% and 37% of the total ginsenosides in wild *P. ginseng* and cultivated *P. ginseng*, respectively. However, these reports had no studies about change of content of ginsenosides in *P. ginseng* leaf and root-hair in relation to the age of the plant.

HPLC has been used extensively to determine the ginsenosides in *P. ginseng*. The methods reported in the literatures used mainly C18 columns. Water and acetonitrile

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mixtures were used as mobile phase in gradient elution mode and different detection techniques were applied (Chen, Hu, Yi, & Wang, 2000; Court, Hendel, & Glmi, 1996; Fuzzati, 2004; Lau, Woo, & Koh, 2003; Kwon et al., 2001). Park, Park, Han, and Shin (1996) studied the ginsenosides in *P. ginseng* root on amino column. The UV detector and reversed-phase column were used in this study and this method was developed for simultaneous determination of the main ginsenosides. Microwave-assisted extraction (MAE) has been shown to enhance the extraction efficiency of interested components and was used as a sample preparation technique, and this method has been employed for the extraction of Chinese herbal medicine (Ding, Li, Li, Liu, & Zhang, 2003; Li et al., 2004; Lucchesi, Chemat, & Smadja, 2004). Microwave-assisted procedures have been also used for the extraction of ginsenosides from *P. ginseng* root and the degradation of ginsenosides in aqueous solution (Kwon, Belanger, Pare, & Yaylayan, 2003; Ren & Chen, 1999; Shu, Ko, & Shiun, 2003).

In this paper some ginsenosides in *P. ginseng* were extracted by microwave-assisted extraction and determined by reverse phase HPLC. The contents of ginsenosides in five parts of *P. ginseng* and the changes of contents of ginsenosides in *P. ginseng* root, leaf and root-hair in plants from one year to five years of age were studied.

2. Experimental

2.1. Materials

The acetonitrile used in this study was of HPLC grade. Water was treated with a Milli-Q water purification system (Millipore, France). The standards of ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3 and Rd were purchased from Chinese Medical and Biological Products Institute (Beijing, China). Other reagents used in this study were of analytical grade.

The fresh *P. ginseng* material was harvested in fall and obtained in Jingyu county of Jilin province in China. Five parts of *P. ginseng* were detached, rinsed with water, and dried at 50 °C in a hot drier. The dried *P. ginseng* was crushed and passed through a 40 mesh sieve.

2.2. Preparation of sample

2.2.1. MAE

The ginsenosides in the *P. ginseng* material were extracted with a WR-C special microwave sample preparation system with a pressure, temperature and time control system (Meicheng, China). 0.5 g of *P. ginseng* material powder was accurately weighed and transferred into the liner vessel and 50 ml of 70% (v/v) ethanol solution was added into it. The pressure was set at 500 kPa, time was set at 15 min and the oven was turned on. The irradiation time was counted from the moment the pressure reached 500 kPa. The extraction was carried out continuously at the preset pressure for 15 min. When the extraction was completed, the sample was allowed to cool to room temperature. Finally the extract in the vessel was filtered and transferred into a 100 ml flask, the solvent was evaporated using a rotary evaporator at 60 °C, and the residue was then dissolved in 30 ml water. The aqueous solution was first extracted two times with 25 ml ether, the aqueous layer was then extracted three times with 20 ml of water–saturated *n*-butanol. The *n*-butanol fraction was evaporated and the residue was dissolved in 10 ml of methanol. The sample solution was filtered through a 0.45 μm filter prior to analysis.

2.2.2. Soxhlet extraction

P. ginseng material (0.500 g) was placed in soxhlet extractor and 100 ml of 70% (v/v) ethanol solution was added into it. The extract was evaporated to dryness using a rotary evaporator at 60 °C, and the residue was then dissolved in 30 ml pure water. The aqueous solution was first extracted two times with 25 ml of ether each time, the aqueous layer was then extracted three times with 20 ml of water–saturated *n*-butanol each time. The *n*-butanol fraction was evaporated and the residue was dissolved in 10 ml of methanol. The sample solution was filtered before analysis.

2.3. Standard solution

Ginsenoside Rg_1 (0.5155 mg/ml), Re (0.5075 mg/ml), Rb_1 (0.5188 mg/ml), Rc (0.5071 mg/ml), Rb_2 (0.5070 mg/ml), Rb_3 (0.5000 mg/ml) and Rd (0.5036 mg/ml) mixed standard stock solution was prepared in methanol. A series of standard operating solutions of different concentrations were obtained by diluting the mixed standard stock solution.

2.4. Condition of HPLC

An Agilent 1100 liquid chromatograph (Agilent Technologies, USA) equipped with quaternary gradient pump and a UV detector was used. A HPLC method was developed using a reversed-phase C18 column (Pinnacle, 250 mm × 4.6 mm I.D, 5 μm, Restek). Sample injection quantity was 20 μl and temperature of column was controlled at 25 °C. The binary gradient elution solvent consisted of acetonitrile (A) and water (B). A gradient elution procedure was used: 0–30 min, 19% A, 81% B; 30–31 min, 19%–30% A, 81%–70% B; 31–65 min, 30% A, 70% B. The flow rate was kept at 1.5 ml/min, and the absorbance was measured at a wavelength of 203 nm for the detection of ginsenosides.

3. Results and discussion

3.1. Analytical performance of HPLC

Ginsenoside Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3 and Rd in extract from samples were identified by comparison of the retention times with authentic ginsenoside standards

obtained from the chromatogram of mixed standards. The relationships between the analyte concentration and measured signal for seven ginsenosides are listed in Table 1. The linearity of the calibration curves was verified by the correlation coefficients and the results are also given in Table 1. The injection precision was obtained by analysing the peak area variations of six injections of a mixture of seven standard ginsenosides. The intra-day and inter-day (six days) precisions are 0.6%–2.06% ($n = 6$) and 2.51%–6.0% ($n = 6$), respectively. The recoveries of the ginsenosides were determined with spiked samples. Ginsenosides Rg_1 (0.82 mg), Re (0.81 mg), Rb_1 (0.83 mg), Rc (0.81 mg), Rb_2 (0.81 mg), Rb_3 (0.80 mg), and Rd (0.80 mg) were added into 1 g of two-year-old *P. ginseng* root and extracted by the microwave-assisted extraction method. The recoveries of all seven ginsenosides were within the range of 92.29%–98.50% ($n = 3$). In this paper, each value is the average of three replicated samples.

3.2. Comparison of MAE and Soxhlet extraction

The results obtained by MAE and Soxhlet extraction were compared and presented in Table 2. As shown in Table 2, compared with the Soxhlet extraction, when the MAE was applied the extraction yield is higher and the extraction time is shorter, so microwave-assisted extraction method was used in this study.

3.3. Comparison of different parts of P. ginseng

The different parts of five-year-old *P. ginseng* were studied, and the contents of seven ginsenosides from five parts are different. From Table 3, we have come to the conclusion that the total content of ginsenosides in root-hair is the highest, however that in stem is the lowest. It should be pointed out that the content of ginsenosides in one-year-old *P. ginseng* leaf is remarkably higher than that in five-year-old *P. ginseng* root-hair and leaf. The content of ginsenoside Re in *P. ginseng* leaf and root-hair is about five times higher than that of Rg_1, but the content of Re is lower than that of Rg_1 in the root. In a previous pharmacological study, the results shown that ginsenoside Re possesses anti-diabetic activity (Attele et al., 2002). Based on the results obtained in the present studies, *P. ginseng* leaf may be more beneficial for diabetic patients than the other parts. Rg1, Re and Rb1 are the three main ginsenosides in extracts of *P. ginseng* root. In the previous study, it was found that Re and Rb1 are the main components in American ginseng root, and the quantity of Rg1 was lower (Wang et al., 2005). The content of ginsenoside Rb_1, Rc, Rb_2 and Rd in root-hair is the highest, moreover Rc, Rb_3 almost were not detected in *P. ginseng* stem. Samukawa et al. (1995) reported the distribution of ginsenosides in the parts of *P. ginseng* cultivated in Nagano, Japan. They found that the contents of ginsenosides are at the highest level in the lateral root, followed by the rhizome, the root hair and the main root.

Table 1
Calibration curve and concentration range of seven ginsenosides

Ginsenosides	Calibration curve[a]	r^2	Concentration range (mg/ml)
Rg_1	$Y = 3776.1\ X - 20.169$	0.9983	0.030–0.50
Re	$Y = 2186.2\ X + 6.5537$	0.9991	0.040–0.50
Rb_1	$Y = 3386.5\ X - 12.677$	0.9967	0.030–0.50
Rc	$Y = 2974.6\ X + 1.7754$	0.9987	0.030–0.50
Rb_2	$Y = 3424.2\ X - 3.7611$	0.9991	0.020–0.50
Rb_3	$Y = 3520.9\ X - 0.5758$	0.9969	0.020–0.50
Rd	$Y = 3892.4\ X - 4.8759$	0.9976	0.020–0.50

[a] Where Y and X are the peak area and concentration of the analytes, respectively.

3.4. Comparison of different ages of P. ginseng

It can be seen from Fig. 1 that the content of seven ginsenosides in *P. ginseng* root increases with age and the increase rate of the content of ginsenoside is different. Content of Re in one-year-old *P. ginseng* root is higher compared with other ginsenosides and the content increases slightly with the increase of age. The content of Rc, Rb_2 and Rb_3 increases with age. The content of Rg_1, Rb_1 and Rd increases from one to four-year-old *P. ginseng* and then has a decrease. Changes of ginsenoside content with age may be related to the growing area of *Panax* sp., for example, Wang et al. (2005) investigated ginsenosides extracted from American ginseng cultivated in both Wisconsin and Illinois. The amounts of Re in 5- and 7-year Illinois-cultivated samples were greater than those found in ginseng cultivated for 3 or 4 years in Wisconsin, whereas the level of Rb1 was higher in the younger Wisconsin samples. The total content of seven ginsenosides changed slowly in one and two-year-old *P. ginseng*, while rapidly in two to four-year-old and tardily in four and five-year-old. However, ginsenoside content in Japanese ginseng root increased annually for three years, and it decreased at the fourth year and increased again at the fifth and the sixth years (Samukawa et al., 1995). Results obtained in our study are different

Table 2
Comparison of the results obtained by MAE and Soxhlet extraction

Extraction method	Content of ginsenosides (mg/g[a])							Extraction time (min)	Extraction pressure (kPa)
	Rg_1	Re	Rb_1	Rc	Rb_2	Rb_3	Rd		
MAE	8.70 ± 0.17	6.67 ± 0.18	6.69 ± 0.21	3.77 ± 0.11	3.65 ± 0.09	2.13 ± 0.13	1.80 ± 0.23	15	500
Soxhlet	5.94 ± 0.22	3.94 ± 0.14	5.80 ± 0.16	2.87 ± 0.13	2.69 ± 0.11	0.47 ± 0.08	0.48 ± 0.11	300	101

[a] Mean value ± standard deviation ($n = 3$).

Table 3
Content of ginsenosides in different parts of *P. ginseng*

Part of *P. ginseng*	Content of ginsenosides (mg/g[a])							Total content (mg/g[a])
	Rg_1	Re	Rb_1	Rc	Rb_2	Rb_3	Rd	
Root	8.70 ± 0.17	6.67 ± 0.19	6.69 ± 0.21	3.77 ± 0.12	6.65 ± 0.09	2.13 ± 0.11	1.80 ± 0.23	36.41 ± 0.95
Leaf	8.80 ± 0.57	42.02 ± 0.47	5.04 ± 0.09	2.40 ± 0.11	2.64 ± 0.14	2.19 ± 0.27	6.76 ± 0.23	69.85 ± 1.99
Root-hair	4.37 ± 0.48	25.95 ± 0.92	16.23 ± 0.91	13.70 ± 0.54	12.40 ± 0.78	1.85 ± 0.27	11.40 ± 0.67	85.90 ± 4.57
Rhizome	5.36 ± 0.24	8.16 ± 0.35	9.08 ± 0.22	5.92 ± 0.19	5.46 ± 0.62	1.02 ± 0.25	3.33 ± 0.41	38.33 ± 2.28
Stem	2.70 ± 0.11	5.02 ± 0.23	0.52 ± 0.07		0.54 ± 0.13		1.01 ± 0.18	9.79 ± 0.72

[a] Mean value ± standard deviation ($n = 3$).

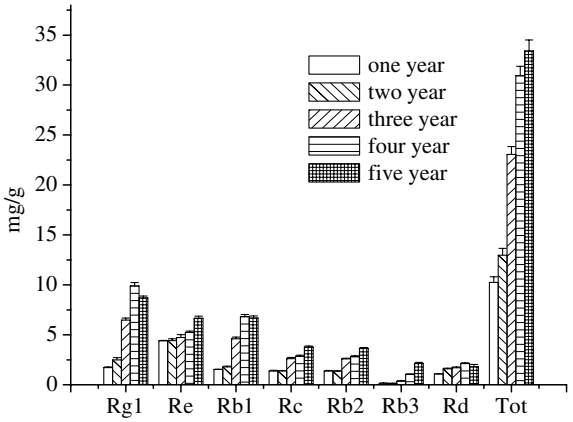

Fig. 1. The changes of ginsenoside contents (mg/g dry plant material) in *P. ginseng* foot extracts in relation to the age, tot represents the total content of ginsenosides.

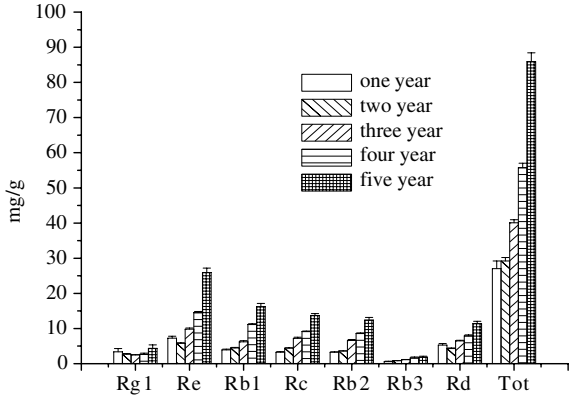

Fig. 3. The changes of ginsenoside contents (mg/g dry plant material) in *P. ginseng* foot-hair extracts in relation to the age, tot represents the total content of ginsenosides.

from the previous study, which may be due to the difference of genetic, cultivation conditions as well as the geographic location of *P. ginseng*.

As shown in Fig. 2, the changes of the content of seven ginsenosides in *P. ginseng* leaf are not obviously dependent on age. Interestingly, the content of ginsenoside Re in *P. ginseng* leaf is highest, and the change of the content of ginsenoside Re is not obviously dependent on age. The content of ginsenoside Re is the highest in four-year-old *P. ginseng* leaf, but the content in five-year-old is the lowest.

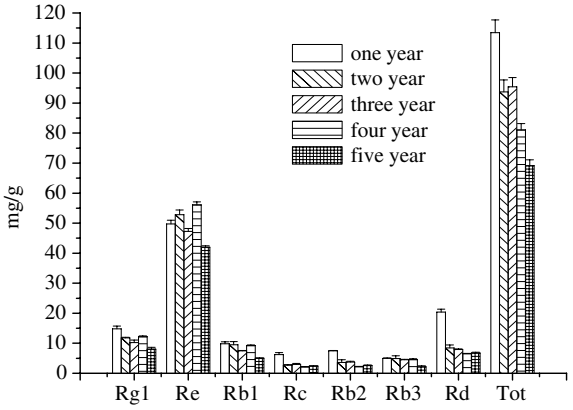

Fig. 2. The changes of ginsenoside contents (mg/g dry plant material) in *P. ginseng* leaf extracts in relation to the age, tot represents the total content of ginsenosides.

The total content of ginsenosides in *P. ginseng* leaf decreases with the increase of age except three-year-old leaf. It is likely that the leaves are newly grown annually, therefore the content is conform in each year.

Fig. 3 shows the variation trend of the content of ginsenoside in *P. ginseng* root-hair of different ages. It can be concluded that the content of Re is the highest and the total content of seven ginsenoside increases with age. The change of the total content of ginsenoside is slowly from one to two-year-old and that is more significant from three to five-year-old.

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