Ginsenoside Content in The Leaves and Roots of *Panax ginseng* at Different Ages

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Abstract: *Panax ginseng* is one of the most important medicinal plants in Asia, particularly Korea. Triterpene saponins (ginsenosides) are the main bioactive compounds in *P. ginseng*. The present study investigates the growth characteristics and variation of ginsenoside content in the leaves and roots of Korean ginseng (*Panax ginseng*) at different growth stages (from 1 to 6 years). Variation in ginsenoside content of both the leaves and roots was higher at the beginning of growth (1-3 years) and then increased with decreasing rates. Root fresh weight increased by 3.3, 5.0, 2.2, 3.0, and 1.7 times for successive year intervals. In comparison, leaf fresh weight increased by 4.6, 2.6, 3.7, 2.4, and 1.2 times for successive year intervals. Analysis of Korean ginseng leaves and roots indicated the presence of 10 ginsenosides (Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, and Rh_1) with the leaves containing higher levels of ginsenoside than the roots. All ginsenosides were maximally accumulated in the leaves during the early growth stages(1^{st} and 2^{nd} years), whereas maximum accumulation was recorded in the roots during the later growth stages (~5 years) Levels of ginsenoside Re, Rd, Rg_1 and Rc were higher in the leaves than other ginsenosides at all growth stages. Rb_1, Rc, and Rb_2 ginsenosides levels exhibited wide variation in the leaves across each year interval compared to the other 7 ginsenosides. Rb_1 accumulated more in the roots than in the leaves of Korean ginseng. Of the 10 ginsenosides examined, Rf ginsenoside content in the roots exhibited wide annual variation, with a difference of 3.3 times between the highest and lowest content. Overall, ginsenosides levels were much higher in the leaves compared to the roots, with plant age also contributing to variation in the levels of ginsenoside compounds.
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1. Introduction

Asian ginseng, which is commonly known as Korean ginseng (*Panax ginseng* C. A. Meyer, Aralaceae family), has a long history worldwide as a medicinal herb (Kee, 1999). Korean ginseng is a perennial herb that produces flowers and fruits in its third year of growth. Ginseng root is normally harvested between the fourth and sixth year of growth. For at least 2000 years, Korean ginseng has been valued as a medicinal herb in traditional Asian medicine. By the 1900s, demand for ginseng was greater than the available supply of wild plants, hence, Korea began commercial cultivation (Kee, 1999; Ang Lee et al., 2001). Since the beginning of the 20^{th} century, the constituents of ginseng root have been investigated, and several classes of compounds have been isolated. Examples include triterpene saponins, essential oil-containing polyacetylenes and sesquiterpenes, polysaccharides, peptidoglycans, nitrogen-containing compounds, and various ubiquitous compounds, such as fatty acids, carbohydrates, and phenolic compounds (Tang et al., 1992).

Ginsenoside is a major compound of ginseng which has numerous physiological and pharmacological effects (Sticher, 1998). More than 150 naturally occurring ginsenosides have been isolated from different parts of ginseng plants (Christensen and Steve, 2008) with approximately 40 ginsenoside compounds identified in *P. ginseng* alone. While ginsenosides are distributed throughout parts of the ginseng plant, different parts of the plant exhibit distinct ginsenoside profiles, such that these parts may have different pharmacological activities (Attele et al., 1999). The key physiological effect of ginsenosides is that it can influence central nervous system activity and functions as anti-cancer drugs (Shinkai et al., 1996, Iishi et al., 1997, Kubo et al., 1992, Attele et al., 2002,

Dey et al., 2002, Yun, 1996). In addition, ginsenosides can have other activities including its anticarcinogenic, immunomodulatory, anti-inflammatory, antiallergic, antiatherosclerotic, antihypertensive, and antidiabetic effects as well as antistress activity and effects on the central nervous system (Lu et al., 2009).

Ginsenosides are specific types of triterpene saponins, and are thus members of a large group of plant glycoside compounds. In the 1960s, Shibata and others isolated 13 different saponins from Korean ginseng, and classified them according to RF values using thin-layer chromatography (Shibita et al., 1966). Subsequently, more than 40 putative ginsenosides have been isolated from ginseng roots. These ginsenosides are classified into two main groups: (1) the glycosides of 20(S)-protopanaxadiol (20[S]-dammar-24-ene-3b, 12b, 20- triol) (Rb_1, Rb_2, Rc, Rd, Rg_3 and Rh_2, and (2) and the glycosides of 20(S)-protopanaxatriol (6a-hydroxy-20[S]-protopanaxadiol) (Re, Rf, Rg_1, Rg_2, Rh_1 and R_1) (Attele et al., 1999; Awang, 2000; Popovich and Kitts 2004). Ginsenosides are distributed in many parts of the ginseng plant, including the root, leaf, and berry. Different parts of the plant contain distinct ginsenoside profiles (Attele et al, 1999) which may exhibit different pharmacological activity. Several studies have isolation and quantified ginsenosides in different parts of ginseng; however, there have been no studies that have investigated differences in leaf ginseng content at different growth stages. In this study, we quantified the amount of ginsenoside in Korean ginseng leaves and roots of different ages (from 1 to 6 years).

2. Material and Methods

2.1. Plant Material

Leaves and roots of different ages of Korean ginseng were collected at September of 2011 in field of Chungnam National University, Daejeon, Korea. Collected samples were freeze dried for 72h in freeze dryer and dried samples were ground into a fine powder (40-mesh) by mill.

2.2. Extraction of ginsenoside

Samples (approximately 5g) were extracted with 400 ml of 70% EtOH at 37℃ for three days in shaking incubator and repeat three times in same method. The extract was filtrated through filter paper (Whatman No. 42) and evaporated (Heidoph VV2011, 40℃). The evaporated extract was resuspended with 5ml of distilled water and freeze-dried the samples. It stored in room temperature and 1g sample resuspended in 20ml distilled water for HPLC analysis.

2.3. HPLC analysis

The 10 ginsenosides were analyzed using HPLC system of a model NS-4000 (Futecs Co., Daejeon, Korea) equipped with Softa Evaporative Light Scattering Detector (ELSD) 300s (SofTA, Thornton, Co, USA). The separation of ginsenoside was performed on a PRONTOSIL NC (250× 4.6mm) fractionation column, with a flow rate of 0.8 ml min^{-1}. The sample was injected (20 μℓ) and applied gradient elution was as follows our previous work (Kim et al., 2009). Identification and quantification of ginsensides were carried out by comparing the retention times and the peak areas respectively with those of ginsenoside standard or by direct addition of ginsenoside standard into the sample (spike test). Sample aliquots were filtered through a 0.45 μm poly(tetrafluoroethylene) filter prior to injection. All samples were run in triplicate. The standard chemical (ginsenoside Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rh_1) was purchased from canfo chemical, china.

2.4. Statistical analysis

The statistical significance was evaluated by ANOVA using the SAS 9.2 Software (SAS, 2010); SAS Institute Inc., Cary, NC, USA)., followed by individual comparison using Duncan's multiple-range test at $p < 0.05$.

3. Results

3.1. Growth characteristics of the leaves and roots of Korean ginseng at different ages

The growth patterns of the leaves and roots with respect to length, width, and fresh weight at different ages are presented in Table 1, while their morphological development is shown in Fig. 1. There was significant variation in all evaluated parameters for each year. Both leaf and root growth increased with increasing age. There was higher variation in leaf and root growth beginning during early development (1-3 years). Root fresh weight increased by 3.3, 5.0, 2.2, 3.0, and 1.7 times for successive year intervals. Root fresh weight was 5 times higher in the third year of growth compared to the second year of growth. A similar pattern was observed for growth in the length and diameter of roots. In comparison, leaf fresh weight increased by 4.6, 2.6, 3.7, 2.4, and 1.2 times for successive year intervals. The second year yielded 4.6 times higher leaf weight than the first year of growth. A similar pattern was observed for growth in the length and diameter of leaves.

3.2. Ginsenoside contents in the leaves of Korean ginseng at different ages

Analysis of Korean ginseng leaves and roots showed that the leaves contained higher levels of ginsenoside than the roots (Table 2). Ten ginsenosides (Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, and Rh_1) were identified from the analysis of Korean ginseng

leaves (Table 2). Ginsenoside content varied significantly with ginseng age. Early ginseng stages showed maximum accumulation of all ginsenosides. The highest accumulation of ginsenosides was in the first year of growth, and this then generally decreased with increasing plant age. Of the 10 ginsenosides, only Rg_1 had the highest accumulation in year 6. The levels of ginsenosides Re, Rd, Rg_1, and Rc were much higher for all ages compared to the other 7 ginsenosides. Re, Rd, Rg_1, and Rc levels were 56, 41, 32, and 28 times higher than the lowest levels of ginsenoside (Rf) in the first year. Rb_1, Rc, and Rb_2 ginsenoside levels varied widely across each year interval compared to the other 7 ginsenosides. This difference, in Rb_1, Rc, and Rb_2 content was 7.2, 2.8, and 2.7 times, respectively. Total leaf ginsenoside content in Korean ginseng varied significantly at different plant ages (Table 2). The range in total leaf ginsenoside was from 130.09 to 83.47 mg/g D.W. The highest amount of ginsenoside accumulated during the first year, then decreased until the third year, and subsequently slightly increased from the fourth to sixth year.

Table 1: Growth of leaves and root of Korean ginseng at different ages

Age	Leaf			Root		
	Length (cm)	Width (cm)	F.W. (g)	Length (cm)	Diameter (mm)	F.W. (g)
1	3.90 f	2.08 f	0.14 e	13.24 f	4.75 f	0.74 e
2	6.72 e	3.40 e	0.64 e	16.20 e	8.32 e	2.44 e
3	8.52 d	3.94 d	1.66 d	22.54 d	12.14 d	12.24 d
4	12.70 c	5.50 c	6.08 c	27.34 c	17.98 c	26.59 c
5	17.10 b	7.70 b	14.79 b	29.30 b	25.80 b	79.33 b
6	19.40 a	8.52 a	17.85 a	37.50 a	33.59 a	134.43a

3.2. Ginsenoside contents in the leaves of Korean ginseng at different ages

Analysis of Korean ginseng leaves and roots showed that the leaves contained higher levels of ginsenoside than the roots (Table 2). Ten ginsenosides (Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, and Rh_1) were identified from the analysis of Korean ginseng leaves (Table 2). Ginsenoside content varied significantly with ginseng age. Early ginseng stages showed maximum accumulation of all ginsenosides. The highest accumulation of ginsenosides was in the first year of growth, and this then generally decreased with increasing plant age. Of the 10 ginsenosides, only Rg_1 had the highest accumulation in year 6. The levels of ginsenosides Re, Rd, Rg_1, and Rc were much higher for all ages compared to the other 7 ginsenosides. Re, Rd, Rg_1, and Rc levels were 56, 41, 32, and 28 times higher than the lowest levels of ginsenoside (Rf) in the first year. Rb_1, Rc, and Rb_2 ginsenoside levels varied widely across each year interval compared to the other 7 ginsenosides. This difference, in Rb_1, Rc, and Rb_2 content was 7.2, 2.8, and 2.7 times, respectively. Total leaf ginsenoside content in Korean ginseng varied significantly at different plant ages (Table 2). The range in total leaf ginsenoside was from 130.09 to 83.47 mg/g D.W. The highest amount of ginsenoside accumulated during the first year, then decreased until the third year, and subsequently slightly increased from the fourth to sixth year.

3.3. Ginsenoside contents in the roots of Korean ginseng at different ages

Ginsenoside content in Korean ginseng roots varied significantly (Table 3). It is noticeable that the roots of Korean ginseng contained lower levels of ginsenosides compounds compared to ginsenosides content in the leaves except for Rb_1 (Table 3). In the later growth stages (~5 years) the roots maximally accumulated all ginsenosides compared to the leaves, except for a few specimens. There was a greater accumulation of the ginsenoside Rb_1 in the root compared to the leaf. The highest amount of Rb_1 (15.92 mg/g D.W.) accumulated in the fifth year of growth, and was 1.35 times higher compared to the highest leaf content of Rb_1. In comparison, the lowest amount of Rb_1(8.60 mg/g D.W.) accumulated in the root during the first year of growth, and was 5.2 times higher compared to that accumulated in the leaf. Rf ginsenoside levels exhibited wide annual variation compared to the other 9 ginsenosides. There was a 3.3 times difference between the highest and lowest Rf content. There was significant annual variation in the total ginsenoside content in the root of Korean ginseng at different ages (Table 3). Ginsenoside content was much lower in Korean ginseng root compared to total leaf ginsenoside content. Total ginsenoside content in the leaf ranged from 130.09 to 83.47 mg/g D.W., whereas it ranged from 46.0 to 25.77 mg/g D.W. in the root. Ginsenoside accumulation was greater in the root during the later growth stages, which was the opposite of that recorded for the leaf.

4. Discussions

To the best of our knowledge, information about the ginsenosides content in the leaves and roots of ginseng at different plant ages has not been published. Ginsenosides are generally distributed throughout all the parts of the ginseng plant. We found that the highest total ginsenoside content accumulated during the first year of growth, then decreased until the third year, and subsequently slightly increased from the fourth to sixth year in ginseng leaves; however, the highest total ginsenoside content accumulated in the roots during the later stages of ginseng growth, which was the opposite of that recorded for leaf. Liu (1988) reported that total ginsenosides content increases with age in Asian ginseng roots, from 1.15% at 1-year-old to

4.85% at 6-year-old, which is supported by our results for total ginsenoside content in the roots. Court et al. (1996) investigated the influence of root age on the ginsenoside content of American ginseng, and found that ginseng harvested after just 3 years of cultivation contained lower amounts of ginsenosides than ginseng harvested after 4 years

Table 2: Ginsenoside contents in leaves of Korean ginseng at different ages

Age	Ginsenoside content (mg/g D.W.)										
(Year)	Rb_1	Rb_2	Rb_3	Rc	Rd	Re	Rf	Rg_1	Rg_2	Rh_1	Total ginsenosides
1	11.81 a	9.61 a	1.51 a	18.41 a	27.35 b	36.73 a	0.66 c	21.26 ab	2.03 bc	0.74 c	130.09 a
2	3.95 bc	8.51 a	1.32 b	15.26 b	33.80 a	34.05 b	0.68 bc	21.33 ab	1.97 c	0.79 c	121.65 a
3	1.69 d	4.16 cd	0.82 d	6.69 d	22.06 cd	28.21 c	0.78 a	13.23 c	4.54 a	1.30 b	83.47 d
4	1.64 d	3.60 d	0.85 d	6.94 d	24.79 bc	29.84 c	0.72 b	20.11 b	4.31 a	1.66 a	94.45 cd
5	2.92 c	5.16 bc	1.04 c	9.86 c	19.39 d	33.59 b	0.71 b	20.14 b	2.27 bc	0.81 c	95.88 c
6	4.31 b	5.75 b	1.12 c	11.50 c	22.60 c	35.08 ab	0.72 b	22.92 a	2.49 b	0.86 c	107.36 b

Table 3: Ginsenoside contents in roots of Korean ginseng at different ages

Age	Ginsenoside content (mg/g D.W.)										
	Rb_1	Rb_2	Rb_3	Rc	Rd	Re	Rf	Rg_1	Rg_2	Rh_1	Total ginsenosides
1	8.60 d	1.35 f	0.28 f	3.49 d	1.78 c	6.66 c	0.84 f	1.85 f	0.43 c	0.49 e	25.77 e
2	11.39 c	1.81 d	0.35 e	5.23 c	3.25 a	7.55 a	1.26 d	3.33 e	0.78 a	1.03 b	35.98 d
3	12.24 b	2.75 c	0.47 c	7.10 b	2.75 b	6.91 b	1.46 c	4.67 d	0.63 b	0.88 c	39.86 c
4	12.72 b	3.08 b	0.51 b	8.09 a	2.84 b	6.46 d	1.58 b	5.39 c	0.59 b	0.70 d	41.96 b
5	15.92 a	3.77 a	0.57 a	8.35 a	1.81 c	6.23 e	2.19 a	6.47 a	0.41 c	0.28 f	46.00 a
6	15.71 a	1.53 e	0.42 d	5.45 c	0.99 d	6.39 d	1.09 e	5.87 b	0.32 d	1.25 a	39.02 c

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