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Analysis of Ginsenoside Composition of Woods-grown Ginseng Roots

Sung Tai Han, Cha Gyun Shin, Byung Wook Yang, Young Tae Hahm, Uy Dong Sohn[1], Byung Ok Im[2], Soon Hyun Cho[2], Boo-Yong Lee[3]*, and Sung Kwon Ko[4]

Department of Biotechnology, Chung-Ang University, Anseong, Gyeonggi 456-756, Korea
[1]College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea
[2]Korea Ginseng Institute, Chung-Ang University, Anseong, Gyeonggi 456-756, Korea
[3]Graduate School of Complementary Alternative Medicine, Pochon CHA University, Seongnam, Gyeonggi 463-836, Korea
[4]Department of Oriental Medical Food and Nutrition, Semyung University, Jecheon, Chungbuk 390-711, Korea

Abstract The objective of this research is to provide basic information necessary to differentiate between ginseng (*Panax ginseng*) grown in woods environments and cultivated ginseng. The ginseng saponin (ginsenoside) contents of Korean woods-grown, 4 year-old cultivated, and 6 year-old cultivated ginsengs were determined via HPLC analysis. The total saponins in the woods-grown ginseng (0.648%) were approximately twice that of the 4 year-old cultivated (0.270%) and the 6 year-old cultivated ginsengs (0.280%). The protopanaxadiols (PD)/ protopanaxatriols (PT) ratio of the woods-grown ginseng (3.258%) was higher than that of the 4 year-old cultivated (2.456%) and the 6 year-old cultivated ginsengs (2.183%). The Rb_1/Rg_1 ratio of the woods-grown ginseng (10.225%) was also higher than those of the 4 year-old cultivated (3.514%) and the 6 year-old cultivated ginsengs (4.865%).

Keywords: ginsenoside composition, woods-grown ginseng, cultivated ginseng, *Panax ginseng*

Introduction

The roots of ginseng (*Panax ginseng* C. A. Meyer) are routinely used for medical purposes, and ginseng is normally divided into categories based on its cultivation. The normal classifications include: wild ginseng (when naturally grown in mountain settings), ginseng (cultivated ginseng), and woods-grown ginseng (*changnwaesam* or *sanyangsam*, when the ginseng is cultivated in habitats such as mountainous settings). The physiologically active substances in ginseng include the ginsenosides, polyacetylenes, ginseng proteins, polysaccharides, and phenolic compounds, as well as many others (1-4).

Ginsenoside has previously been identified as one of the more effective biochemical and medical constituents of ginseng. Beginnings in the late 1960's, a variety of studies have been conducted concerning this constituent. Beginning with the works of Shibata (5, 6), research groups have examined and defined the chemical structure of ginsenoside. Currently, the ginsenosides are subdivided into several chemical structural categories; protopanaxadiols (PD, 22 types), protopanaxatriols (PT, 13 types), and oleanane saponin (1 type).

The medical usage of ginsenoside involves its anti-cancer (7), anti-diabetic (8), and central nervous system (CNS) sedative effects (9), its arteriosclerosis and hypertension preventive abilities (10, 11), and several other activities, including anti-fatigue, anti-stress (12, 13), anti-oxidation (14), anti-inflammation (15), strength of immunity (16), and other effects.

As mentioned above, the efficacy of ginsenoside has been thoroughly evaluated in both biochemical and medical studies. Researchers are still eagerly attempting to elucidate the efficacy of ginsenoside components.

However, the ginsenoside contents of ginsengs have also been the subject of a number of studies by many researchers in the Orient. For example, Jang *et al.* (17) previously reported on the ginsenoside content of Korean white ginseng correlated with years of cultivation, and Namba *et al.* (18) evaluated the relationship existing between ginsenoside contents and cultivation years of white ginseng (Nagano, Japan). Also, Lee *et al.* (19) evaluated the ginsenoside contents of cultivated ginseng (Korea) at different periods of cultivation. It has been reported that the ginsenoside composition of woods-grown ginseng generally differs from that of cultivated ginseng. However, precious few studies have been conducted to determine systematically and precisely the ginsenoside components of woods-grown ginseng. Therefore, the present research is designed to analyze and compare the components of woods-grown ginseng with that of cultivated ginseng (4 and 6 year-old) in South Korea.

Materials and Methods

Materials Samples of woods-grown ginseng were collected from Sobaeksan(Mt.), Suhsan Palbongsan(Mt.), and Gangwon-do. The cultivated ginseng samples were collected from more than 10 representative ginseng cultivation farms, including Geumsan, Punggi, Ganghwa, Hongcheon, Umsung, Jinan, Yeongwol, Wanju, Gochang, and Gimje, and categorized by the age of the roots (4 and 6 year-old). All of the ginseng samples were frozen and dried prior to analyses of the ginseng saponin contents. These specimens were stored at the Korea Ginseng Institute of Chung-Ang University.

Preparation of ginsenoside extract An exact amount (5

*Corresponding author: Tel: 82-31-725-8371; Fax: 82-31-725-8350
E-mail: bylee@cha.ac.kr
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g) of fine ginseng root powder was mixed with 20 parts of 50% ethyl alcohol. The mixture was refluxed for 2 hr at 90°C in a water bath and then filtered. The filtrate was concentrated with a vacuum evaporator.

Analysis of ginsenoside The ginsenoside composition of the extract was analyzed via HPLC in accordance with the techniques established by Ko *et al.* (20). The quantity of ginsenoside in each sample was compared and analyzed 3 times via HPLC. The ginsenoside standards utilized in this experiment were pure ginseng saponins constructed at the Chung-Ang University Ginseng Research Laboratory, and ginsenosides with purity of more than 99% that were purchased from ChromaDex (Laguna Hills, CA, USA) for this experiment. The ginsenoside standards employed in this study were as follows: Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, and Rh_2.

The HPLC device utilized in this study was an Alltech Binary Gradient HPLC system model 627 (Alltech Associates, Inc., Dearfield, IL, USA), and the column used was a Prevail Carbohydrate ES column (Alltech Asso., 4.6 ×250 mm). A gradient elution system consisting of A (acetonitrile:water:isopropylalcohol = 80:5:15) and B (acetonitrile:water:isopropylalcohol = 67:21:12) was used [10% B (0 min); 85% B (28 min); 80% B (35 min); 75% B (45 min); 90% B (50 min); 100% B (51 min); 25% B (57 min); 10% B (58 min)]. The system was operated at room temperature, and the running fluid speed was set at 0.8 mL/min. The chromatogram was generated using an ELSD detector (Alltech Asso.).

Results and Discussion

The objectives of this study were to provide basic regarding ginsenoside contects via the analysis and comparison of woods-grown ginseng with cultivated ginseng (4 and 6 year-old).

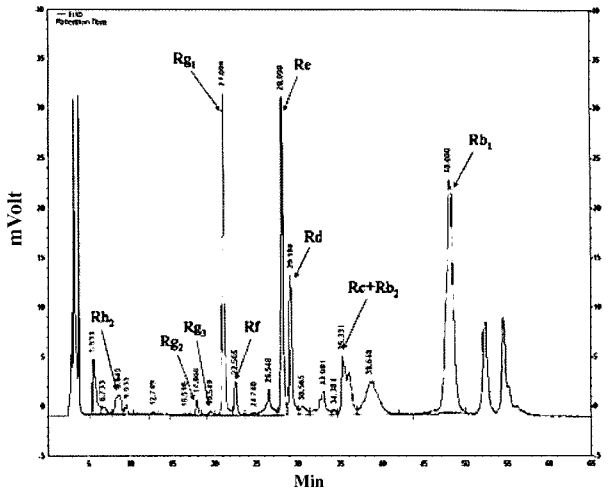

Fig. 1. HPLC chromatogram of ginsenosides detected from the woods-grown ginseng (Sobaeksan(Mt.) 15 year-old).

The ginseng saponins of ginsenoside Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, and Rh_2 were analyzed. The data acquired via HPLC analysis were analyzed using descriptive statistics and analysis of variance. Table 2 shows the results of the total saponin contents of the woods-grown, 4 year-old cultivated and 6 year-old ginsengs. The woods-grown ginseng evidenced a significantly higher saponin content (0.648%) than was observed in both the 4 year-old cultivated (0.270%) and 6 year-old cultivated (0.280%) ginsengs after 2 hr of extraction at 90°C.

However, the study conducted by Chang *et al.* (17) demonstrated the ginsenoside contents of Korean white ginseng at various cultivation years, and the work of

Table 1. The ginsenoside content of the woods-grown ginseng collected in the different areas of Korea[1)] (%, w/w)

Ginsenoside	Sobaeksan(Mt.) (8 year-old)	Sobaeksan(Mt.) (11 year-old)	Sobaeksan(Mt.) (12 year-old)	Sobaeksan(Mt.) (15 year-old)	Gangwon-do (15 year-old)	Palbongsan(Mt.)I (15 year-old)	Palbongsan(Mt.)II (15 year-old)
Rh_2	0.020±0.001	0.016±0.000	0.014±0.001	0.013±0.000	0.029±0.001	0.027±0.002	0.036±0.002
Rh_1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Rg_2	0.003±0.001	0.003±0.000	0.002±0.000	0.002±0.000	0.003±0.000	0.003±0.000	0.000
Rg_3	0.000	0.000	0.003±0.000	0.001±0.000	0.000	0.006±0.000	0.000
Rg_1	0.012±0.000	0.026±0.000	0.024±0.000	0.030±0.000	0.018±0.000	0.018±0.000	0.019±0.000
Rf	0.031±0.002	0.022±0.000	0.010±0.000	0.008±0.000	0.000	0.037±0.001	0.000
Re	0.118±0.001	0.174±0.008	0.082±0.001	0.071±0.002	0.129±0.002	0.134±0.001	0.109±0.005
Rd	0.116±0.003	0.097±0.008	0.064±0.001	0.036±0.001	0.158±0.001	0.478±0.003	0.562±0.010
$Rc+Rb_2$	0.021±0.001	0.069±0.001	0.013±0.000	0.015±0.001	0.020±0.001	0.051±0.010	0.030±0.003
Rb_1	0.106±0.003	0.394±0.009	0.175±0.002	0.351±0.008	0.163±0.003	0.181±0.006	0.180±0.003
Total[2)] ginsenoside	0.429±0.008	0.800±0.010	0.386±0.004	0.526±0.005	0.520±0.006	0.936±0.018	0.936±0.014
PD/PT[3)]	1.598±0.024	2.565±0.138	2.279±0.014	3.752±0.111	2.475±0.029	3.871±0.062	6.268±0.187
Rb_1/Rg_1	8.689±0.183	15.273±0.432	7.273±0.036	11.590±0.233	9.243±0.233	10.185±0.376	9.324±0.285

[1)]Values represent the mean±SE (n=3).
[2)]Sum of individual ginsenosides content.
[3)]Ginsenoside $Rb_1+Rb_2+Rc+Rd+Rg_3+Rh_2$ / $Re+Rf+Rg_1+Rg_2+Rh_1$.

Table 2. The content of total saponin and each ginsenoside of ginsengs (4 year-old cultivated, 6 year-old cultivated, and woods-grown ginsengs) and statistical analysis[1)]

Ginsenoside content	Average (%, w/w)			Variance			Statistic significance	
	4 Year-old cultivated	6 Year-old cultivated	Woods-grown ginseng	4 Year-old cultivated	6 Year-old cultivated	Woods-grown ginseng	F-value[4)]	*p*-value
TS[2)]	0.270±0.024	0.280±0.027	0.648±0.090	0.005	0.005	0.051	56.322	0.000
PD/PT[3)]	2.456±0.286	2.183±0.119	3.258±0.587	0.707	0.112	2.221	7.326	0.001
Rb_1/Rg_1	3.514±0.239	4.865±0.786	10.225±0.978	0.644	5.079	6.285	75.834	0.000
Rb_1	0.060±0.007	0.087±0.013	0.222±0.040	0.000	0.001	0.010	46.981	0.000
Rb_2+Rc	0.015±0.002	0.017±0.002	0.031±0.008	0.000	0.000	0.000	10.212	0.000
Rd	0.070±0.012	0.048±0.008	0.216±0.080	0.001	0.000	0.041	14.809	0.000
Re	0.051±0.007	0.054±0.005	0.117±0.013	0.000	0.000	0.001	58.661	0.000
Rf	0.013±0.002	0.014±0.002	0.015±0.006	0.000	0.000	0.000	0.432	0.651
Rg_1	0.017±0.002	0.020±0.004	0.021±0.002	0.000	0.000	0.000	1.127	0.330
Rg_2	0.001±0.001	0.000	0.002±0.000	0.000	0.000	0.000	12.625	0.000
Rg_3	0.000	0.000	0.001±0.001	0.000	0.000	0.000	10.257	0.000
Rh_1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Rh_2	0.043±0.004	0.040±0.002	0.022±0.003	0.000	0.000	0.000	34.926	0.000

[1)]Values represent the mean±SE.
[2)]Sum of individual ginsenosides content.
[3)]Ginsenoside Rb_1+Rb_2+Rc+Rd+Rg_3+Rh_2 / Re+Rf+Rg_1+Rg_2+Rh_1.
[4)]F-value of the reject is 3.130.

Namba *et al.* (18) reported that total ginsenoside compositions and contents varied according to cultivation period. Also, Lee *et al.* (19) previously conducted a study of the ginsenoside contents of cultivated ginseng (Korea) involving 4 hr of extraction at 90°C, using cultivated ginseng samples of 4 (0.96%), 5 (0.78%), and 6 (0.77%) years.

As can be observed in Table 1 and 2, the PD (protopanaxadiol)/PT (protopanaxatriol) ratios of the woods-grown ginseng (3.258%) were higher than those of both the 4 year-old (2.456%) and 6 year-old cultivated (2.183%) ginsengs. The Rb_1/Rg_1 ratio is a critical element for the discrimination of white ginseng from red ginseng. As compared to the 4 year-old cultivated (3.514%) and 6 year-old cultivated (4.865%) ginsengs, the Rb_1/Rg_1 ratios of the woods-grown ginseng (10.225%) were far higher. Rb_1 is renowned for its physiological activation effects, including its sedative effects on the central nervous system (CNS) (9) and against hypertension (10, 11). In particular, the content of ginsenoside Rb_1 of the woods-grown ginseng (0.222%) was significantly higher than those of the 4 year-old cultivated (0.070%) and 6 year-old cultivated (0.048%) ginseng samples.

The ginsenoside Rd content of the woods-grown ginseng, and the 4 year-old cultivated and 6 year-old cultivated ginseng samples were 0.216, 0.070, and 0.048%, respectively. In addition, the ginsenoside Re content of the woods-grown ginseng (0.117%) was significantly higher than those of the 4 year-old cultivated (0.051%) and 6 year-old cultivated (0.054%) ginsengs.

In conclusion, the total saponin, Rb_1/Rg_1 ratio, as well as the ginsenoside Rd and ginsenoside Re contents of the woods-grown ginseng were generally higher than those of the cultivated ginsengs. The results of this study may prove beneficial to those seeking basic information regarding ginsenoside composition and contents in woods-grown ginseng, especially those interested in its use as a ginseng-herb medicine.

Acknowledgments

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