미네랄수를 이용한 한국산양산삼으로부터 추출성분 평가

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Components evaluation through extraction process from Korean cultivated wild ginseng using mineral water

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Introduction

Ginseng is cultivated in China, Korea, Japan and Russia, as well as in the United States and Canada. Ginseng is one of the most well-known herbal medicines widely used in East Asia as a tonic, restorative and anti-aging agent in traditional Korea medicine [1]. Ginseng is a slow-growing, deciduous, perennial plant of the *Araliaceae* family which includes *Panax ginseng* (*Renshen*, Chinese or Korean ginseng), *Panax japonicus* (Japanese ginseng) and *Panax quinquefolius* (*Xiyangshen*, American ginseng) [2]. Ginseng is used as a dietary supplement in the United States [3]. In Korean medicine practice, ginseng root is the most commonly used part of the plant. It contains ginsenosides as the major bioactive components known to have complex and multiple pharmacological effects [4].

While ginseng leaf-stem was less studied, a recent report indicates that American ginseng leaf contains similar pharmacologically active ingredients more abundantly than ginseng root [5]. *Panax ginseng* leaf-stem is rich in containing several ginsenosides. Therefore, this article reviews the constituents and pharmacological profile of ginseng leaf-stem, including its chemical components, biological activities, pharmacological properties and adverse effects. Preparations from cultivated Korean ginseng have been used traditionally to support the immune system, strengthen the nervous system, and to help prevent certain chronic conditions. Research on

Korean ginseng has focused on blood sugar control for type 2 diabetes and quality of life in cancer patients. A patented, chemically defined (polysaccharide-based) extract made from cultivated Korean ginseng roots has shown efficacy in helping to prevent and treat upper respiratory tract symptoms related to colds and flu.

The objectives of this study were to 1) extract ginsenosides within cultivated and wild-type Jinseng using mineral water and 2) evaluate ginsenosides from extracted solutions.

Experimental

Plant materials

Samples of cultivated wild jinseng was collected in the Kangwodo identified as *Panax (P.) ginseng* from Jangsu Wild Jinseng Agricultural Association Co.. 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. Spring water was supplied by Yongkoong Sea Hot Spring Company and mineral waters removed salts from sea hot spring in Yongkoong Hot Spring area in Kanghwado was done by LaNube-Yonsei University joint research team.

Charaterisation

The chemical analysis was achieved on an Agilent 1100 HPLC instrument with Agilent C18 column (150x4.6 mm, 5 μm), column temperature at 30^{o}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. Since the relative low level of these compounds and the relative correction factors (RCF) of usual ginsenosides to Rb1 were around 1, so the RCFs of compounds without authentic samples to Rb1 were determined approximately as 1.

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

Results and Discussions

Ginsenosides Re, Rf, Rb1, Rc, Rb2 and Rd showed the same accumulating trend with total ginsenoside (as shown in Table 1 and Figure 1), 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.

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 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. 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 2, Figure 2).

From the results above, the comparison of extraction process for different solvents such as methyl alcohol, butyl alcohol and deionized water with mineral water, mineral water is superior to others at a rate of about 1.5 times.

Conclusion

The analysis of ginsenosides for growing years showed distinctly differences and amount of total ginseonides using mineral waters were also observed to be extracted much specific Rg components than existing solvents.

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Table 1. Contents of ginsenosides (mg/g) with different growing years of cultivated wild ginseng (n = 5).

		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

Table 2. Relative contents (mg/g) of components without authentic standard to Rb1 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
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
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
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

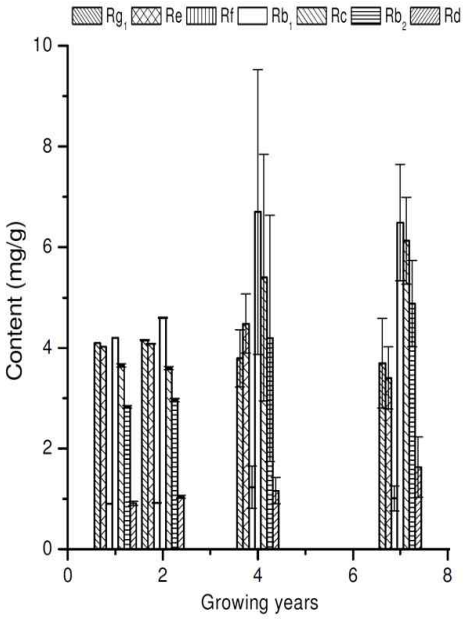

Figure 1. Accumulating trend of ginsenosides. The columns stand for the contents of ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2 and Rd, respectively. As shown in the figure above, ginsenosides Re, Rf, Rb1, Rc, Rb2 and Rd all increased at the initial growing period, and fell a little, then kept stable. But ginsenoside Rg1 decreased during the growing period.

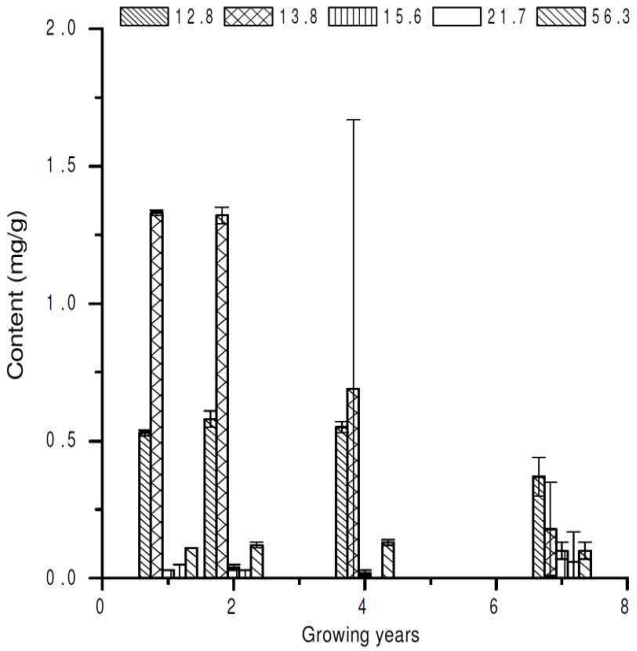

Figure 2. Components without authentic standard correlated to the growing years. The columns stand for the relative contents of components without authentic standards with retention times at 12.8, 13.8, 15.6, 21.7 and 56.3 min, respectively. As shown in the figure above, the relative contents of components 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.