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Effective Purification of Ginsenosides from Cultured Wild Ginseng Roots, Red Ginseng, and White Ginseng with Macroporous Resins

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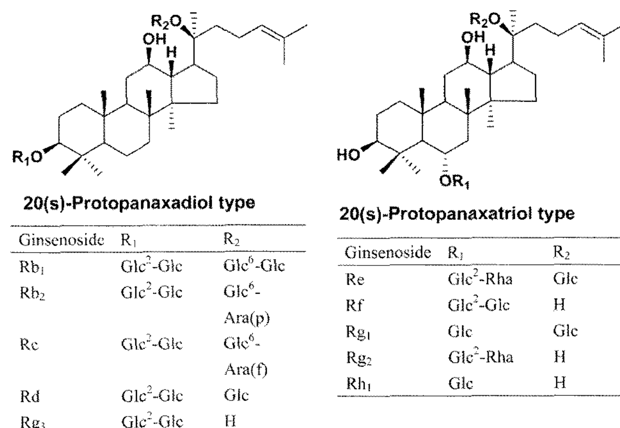
This study was aimed (i) to develop an effective method for the purification of ginsenosides for industrial use and (ii) to compare the distribution of ginsenosides in cultured wild ginseng roots (adventitious root culture of *Panax ginseng*) with those of red ginseng (steamed ginseng) and white ginseng (air-dried ginseng). The crude extracts of cultured wild ginseng roots, red ginseng, and white ginseng were obtained by using a 75% ethanol extraction combined with ultrasonication. This was followed sequentially by AB-8 macroporous adsorption chromatography, Amberlite IRA 900 Cl anion-exchange chromatography, and Amberlite XAD16 adsorption chromatography for further purification. The contents of total ginsenosides were increased from 4.1%, 12.1%, and 11.3% in the crude extracts of cultured wild ginseng roots, red ginseng, and white ginseng to 79.4%, 71.7%, and 72.5% in the final products, respectively. HPLC analysis demonstrated that ginsenosides in cultured wild ginseng roots were distributed in a different ratio compared with red ginseng and white ginseng.

Keywords: Ginsenoside, purification, macroporous resin, HPLC, cultured wild ginseng roots

Ginseng (*Panax ginseng*, C.A. Meyer) has been used as a tonic, antifatigue, sedative, and antigastric ulcer drug for thousands of years. Recently, many studies have suggested that its pharmacological effects are mainly due to ginseng saponins [5, 14]. However, it takes several years to cultivate ginseng in fields and also needs very sophisticated care because its growth conditions (*i.e.*, soil, climate, and pathogenesis) are very difficult to control. For these reasons, low yields and high costs hamper efforts to meet the demand of increasing markets. In general, cultured wild ginseng roots (adventitious root culture of *Panax ginseng*) are easily obtained by using a plant cell culture technique for the production of ginseng and its active ingredient, ginsenosides, rather than natural cultivation. With the cell culture technique, fastidious and complicated conditions for the production of ginseng and ginsenosides can be overcome and optimized. Although many attempts have been made to isolate ginseng saponins [8, 9], these were good only for separation of each single ginsenoside from the total ginsenosides products. Prior to the process, large amounts of total pure ginsenosides should be obtained for its latter separating process and bioassay. Generally, separation of the ginsenosides was usually performed by using organic solvents [12], which are not suitable for use in food or medicine. There is an alternative purification method to use adsorbents such as silica [6] and Diaion HP 20 [7, 11], but this method also needs to use toxic organic solvents for the extraction of crude ginsenosides. In addition, the absorbents have poor specificity to select ginsenosides with low efficiency. Thus, a selective and high-yield purification method for ginsenosides needs to be developed. In this study, three different macroporous resins (AB-8, IRA 900 Cl, and XAD 16) were used to separate total ginsenosides from cultured wild ginseng roots (CWG, adventitious root culture of *Panax ginseng*), red ginseng (RG, steamed ginseng)



Ginsenoside	R_1	R_2
Rb_1	Glc^2-Glc	Glc^6-Glc
Rb_2	Glc^2-Glc	Glc^6-Ara(p)
Rc	Glc^2-Glc	Glc^6-Ara(f)
Rd	Glc^2-Glc	Glc
Rg_3	Glc^2-Glc	H

Ginsenoside	R_1	R_2
Re	Glc^2-Rha	Glc
Rf	Glc^2-Glc	H
Rg_1	Glc	Glc
Rg_2	Glc^2-Rha	H
Rh_1	Glc	H

Fig. 1. Structures of ginsenosides from *Panax ginseng*.
Glc: glucose; Rha: rhamnose; Ara(f): arabinose in furanose form; Ara(p): arabinose in pyranose form.

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and white ginseng (WG, air-dried ginseng). In addition, ten ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rh_1, Rg_1, Rg_2, and Rg_3) (Fig. 1) were quantitatively compared among three kinds of ginseng products by HPLC analysis.

Ten g of each ground sample (CWG, RG, and WG) was extracted with 200 ml of 75% ethanol in an ultrasonic bath for 120 min at 39°C. Then, the concentrated extracts (40 ml) were passed through an AB-8 polar column (bed volume, 80 ml) to eliminate water, soluble impurities at the flow rate of 1 BV (bed volume)/h and the adsorbed ginsenosides were eluted with 3 BV of 70% ethanol (v/v) at 2 BV/h. The eluent was applied onto the Amberlite IRA 900 Cl strong base anion-exchange column at the flow rate of 1 BV/h to remove the pigments and loaded onto the Amberlite XAD 16 column to get rid of nonpolar substances. The final resulting eluent was collected and evaporated to yield dried powders. The purity of the ginsenosides was estimated by using ginsenoside Re as a calibration standard [1]. A good linear relationship was obtained in the range of 0.005 to 0.03 mg/ml, and the regression equation was: $y=30.376x-0.0258$ ($R^2=0.9983$, n=5), where y represents the absorbance at 544 nm, x the concentration of total ginsenosides (mg/ml). Analysis of single ginsenoside content was performed on an HPLC system with a reversed-phase column (Zorbax Bonus-RP 4.6 mm×150 mm, 3.5 μm).

It has been known that macroporous resins are widely used in medicine manufacturing and in extraction of active ingredients in natural plants such as vitexin [2], vanillin [15], arabinogalactan [4], scutellarin [3], flavone compounds [10], *etc.* As the macroporous resin has a lot of advantages of nontoxicity, good specificity, easy operation, low cost, and easy regeneration of resin, it can be a powerful method for industrial use instead of toxic organic solvents. Hence, here we also tried to develop the purification method to obtain high levels of yield and purity of ginsenosides from cultured wild ginseng roots, red ginseng, and white ginseng. As is shown in Table 1, 3.12±0.14 g, 3.72±0.37 g, and 3.50±0.22 g of crude ethanol extracts were obtained from each 10 g of CWG, RG, and WG, respectively. However, the total ginsenosides content of each crude extract was only 4.0%, 12.1%, and 11.3%. After purification with the three macroporous resins, the purity of the ginsenosides increased to 79.4%, 71.7%, and 72.5% for CWG, RG, and WG, respectively, indicating that the purification steps used in this study significantly improved the purity of the ginsenosides in each product.

Table 1. Yield and purity of total ginsenosides from 10 g of ground ginseng (CWG: cultured wild ginseng roots; RG: red ginseng; WG: white ginseng).

		Yield (g)	Purity (%)
Crude ethanol extracts	CWG	3.12±0.14	4.0±0.8
	RG	3.72±0.37	12.1±1.2
	WG	3.50±0.22	11.3±0.9
Purified ginsenosides products	CWG	0.14±0.05	79.4±1.3
	RG	0.46±0.04	71.7±0.9
	WG	0.39±0.02	72.5±1.5

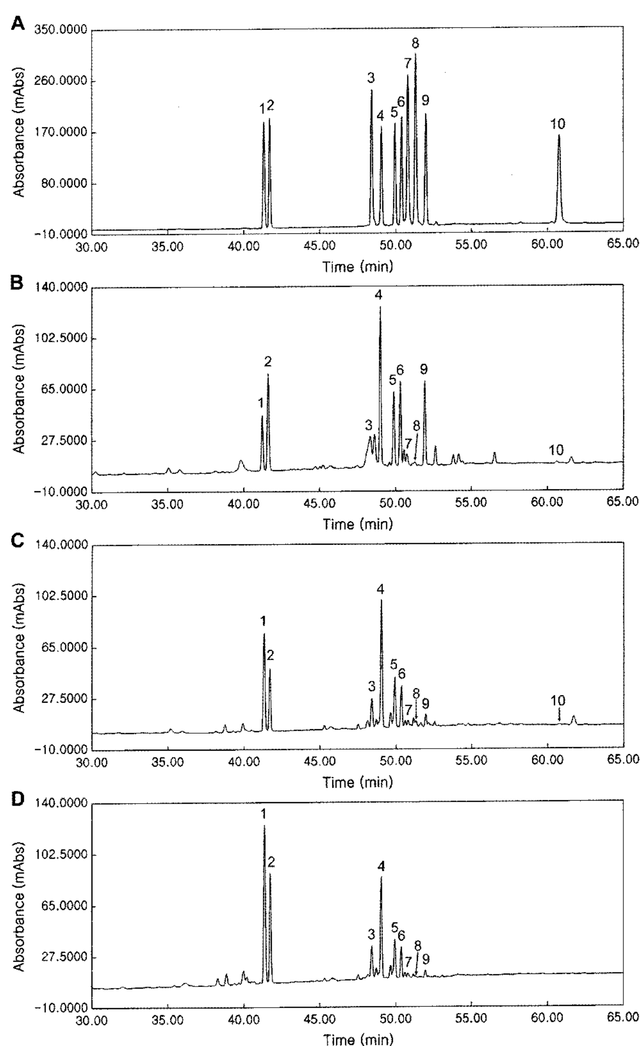

Fig. 2. HPLC chromatograms of (**A**) mixed standards (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rh_1, Rg_1, Rg_2, and Rg_3); purified ginsenosides products of (**B**) CWG, (**C**) WG, and (**D**) RG. HPLC was performed on a Zorbax Bonus-RP column (4.6 mm×150 mm, 3.5 μm).
The binary gradient elution system consisted of water (A) and acetonitrile (B) and separation was achieved using the following gradient: 0–30 min, 20% B; 30–48 min, 20–40% B; 48–60 min, 40–45% B; 60–72 min, 45–55% B. The column temperature was kept constant at 35°C. The flow-rate was 1 ml/min and injection volume was 10 μl for the standard solution (0.5 mg/ml) and 20 μl for the samples (1 mg/ml). The UV detection wavelength was set at 203 nm. 1: Rg_1; 2: Re; 3: Rf; 4: Rb_1; 5: Rc; 6: Rb_2; 7: Rg_2; 8: Rh_1; 9: Rd; 10: Rg_3.

Under the chromatographic conditions used in this study, all ten calibration curves exhibited good linear regressions (data not shown). Standard and representative chromatograms of purified ginsenosides products for CWG, RG, and WG were compared to be shown in Figs. 2A–2D. CWG contained similar ginsenoside types with RG and WG, but they were distributed in different ratios compared with the other two ginseng products (Figs. 2B–2D). Some unknown small

Table 2. Contents[a] of ten investigated ginsenosides in three purified ginsenosides products.

Origins	Protopanaxadiol (μg/mg)					Protopanaxatriol (μg/mg)					Rb_1/Rg_1
	Rb_1	Rb_2	Rc	Rd	Rg_3	Re	Rf	Rg_1	Rg_2	Rh_1	
CWG	157.7±0.7	76.7±2.3	78.5±0.5	74.1±0.9	–[b]	95.1±0.4	54.6±7.8	54.3±0.4	8.0±1.7	–[b]	2.90
RG	129.4±1.5	40.0±0.7	58.1±0.2	10.6±0.8	–[b]	59.0±0.9	22.6±5.7	97.4±0.6	4.1±1.2	4.3±1.1	1.33
WG	102.7±1.3	28.3±0.6	50.0±0.5	6.1±0.5	–[c]	106.1±1.7	26.2±2.9	153.1±0.8	2.8±0.9	–[b]	0.67

[a]Contents=mean±SD (n=3).
[b]Too low to be measured.
[c]Not detected.

peaks observed in Figs. 2B–2D indicated that there are still some uninvestigated minor ginsenosides containing in each ginsenosides product. As shown in Table 2, the content of protopanaxadiol-type saponins (Rb_1, Rb_2, Rc, Rd, Rg_3) was higher than that of protopanaxatriol-type ones (Re, Rf, Rg_1, Rg_2, Rh_1) in CWG and RG, whereas the contents were opposite in the case of WG. This result was inconsistent with the previous data reported by Wan *et al.* [13] that WG contained more protopanaxadiol-type saponins. One of the possible reasons for such a different result is that ginsengs cultivated in different areas might accumulate different ratios of the ginsenosides. The ratio of Rb_1/Rg_1, two major ginsenosides representing protopanaxadiol and protopanaxatriol, for CWG, RG, and WG were 2.90, 1.33, and 0.67, respectively. The Rb_1 content was higher than Rg_1 both in CWG and RG; however, the difference was much more significant in CWG. The Rg_1/Re ratio of CWG, which contained more Re (95.1±0.4 μg/mg) than Rg_1 (54.3±0.4 μg/mg), was contrary with those of the other two ginseng products. The Rd content in CWG (74.1±0.9 μg/mg) was about seven times and twelve times more than that in RG (10.6±0.8 μg/mg) and WG (6.1±0.5 μg/mg), respectively. In addition, Rg_3, a kind of minor ginsenoside, was found both in CWG and RG, but not in WG.

In conclusion, the purification of ginsenosides using three macroporous resins (AB-8, IRA 900 Cl, and XAD 16) significantly increased the purity of the total ginsenosides. In addition, the contents of each investigated ginsenoside in cultured wild ginseng roots, red ginseng, and white ginseng were quite different. These results assist the development of a new ginsenosides purification method and provide a possibility of high-quality but low-price ginsenosides products. Further study on how to improve the efficiency of purification of ginsenosides, and on the relations between ginsenosides distribution and their biological activities, should be undertaken.

REFERENCES

1. Cai, X., Z. Q. Liu, P. X. Wang, and L. Liu. 2001. Study on purification process of ginsenosides with macroreticular resin. *Chinese Trad. Patent Med.* **23:** 631–634.
2. Fu, Y., Y. Zu, W. Liu, C. Hou, L. Chen, S. Li, X. Shi, and M. Tong. 2007. Preparative separation of vitexin and isovitexin from pigeonpea extracts with macroporous resins. *J. Chromatogr. A* **1139:** 206–213.
3. Gao, M., W. Huang, and C. Z. Liu. 2007. Separation of scutellarin from crude extracts of *Erigeron breviscapus* (vant.) Hand. Mazz. by macroporous resins. *J. Chromatogr. B* **858:** 22–26.
4. Huang, Z., G. Fang, and B. Zhang. 2007. Separation and purification of arabinogalactan obtained from *Larix gmelinii* by macroporous resin adsorption. *J. Forest. Res.* **18:** 81–83.
5. Kim, H. A., S. Kim, S. H. Chang, H. J. Hwang, and Y. Choi. 2007. Anti-arthritic effect of ginsenoside Rb1 on collagen induced arthritis in mice. *Int. Immunopharmacol.* **7:** 1286–1291.
6. Kimura, Y., M. Sumiyoshi, K. Kawahira, and M. Sakanaka. 2006. Effects of ginseng saponins isolated from red ginseng roots on burn wound healing in mice. *Brit. J. Pharmacol.* **148:** 860–870.
7. Kwak, Y. S., M. S. Hwang, S. C. Kim, C. S. Kim, J. H. Do, and C. K. Park. 2006. A growth inhibition effect of saponin from red ginseng on some pathogenic microorganisms. *J. Ginseng Res.* **30:** 128–131.
8. Lau, A. J., B. H. Seo, S. O. Woo, and H. L. Koh. 2004. High-performance liquid chromatographic method with quantitative comparisons of whole chromatograms of raw and steamed *Panax notoginseng*. *J. Chromatogr. A* **1057:** 141–149.
9. Li, L., J. L. Zhang, Y. X. Sheng, G. Ye, H. Z. Guo, and D. A. Guo. 2004. Simultaneous quantification of six major active saponins of *Panax notoginseng* by high-performance liquid chromatography-UV method. *J. Pharmaceut. Biomed.* **38:** 45–51.
10. Sun, A., Q. Sun, and R. Liu. 2007. Preparative isolation and purification of flavones compounds from *Sophora japonica* L. by highspeed counter-current chromatography combined with macroporous resin column separation. *J. Sep. Sci.* **30:** 1013–1018.
11. Tran, Q. L., M. M. Than, Y. Tezuka, A. H. Banskota, K. Kouda, H. Watanabe, *et al.* 2003. Wild ginseng grows in Myanmar. *Chem. Pharm. Bull.* **51:** 679–682.
12. Vanhaelen-Fastre, R. J., M. L. Faes, and M. H. Vanhaelen. 2000. High-performance thin-layer chromatographic determination of six major ginsenosides in *Panax ginseng*. *J. Chromatogr. A* **868:** 269–276.
13. Wan, J. B., S. Li, J. M. Chen, and Y. T. Wang. 2007. Chemical characteristics of three medicinal plants of the *Panax* genus determined by HPLC-ELSD. *J. Sep. Sci.* **30:** 825–832.
14. Yun, T. K. 2003. Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds. *Mutat. Res.* **523–524:** 63–74.
15. Zhang, Q. F., Z. T. Jiang, H. J. Gao, and R. Li. 2008. Recovery of vanillin from aqueous solutions using macroporous adsorption resins. *Eur. Food Res. Technol.* **226:** 377–383.