JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Assessment of Roots of *Panax notoginseng* in China: Regional and Seasonal Variations in Its Active Constituents

TINA T. X. DONG,[†] XIU M. CUI,[†,‡] ZONG H. SONG,[†] KUI J. ZHAO,[†,§] ZHAO N. JI,[†] CHUN K. LO,[†] AND KARL W. K. TSIM*,[†]

Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay Road, Hong Kong, China, Department of Pharmacognosy, China Pharmaceutical University, 1 Shennong Road, Nanjing, China, and Department of Pharmacy, Beijing Military Medical College of PLA, Beijing, China

Root of *Panax notoginseng* (Radix Notoginseng, Sanqi) is a well-known traditional Chinese medicine and is mainly cultivated in Wenshan of Yunnan, China. The active constituents include saponin, dencichine, flavonoid, and polysaccharide; however, the levels of these components vary in different geographical regions of growth and also show a seasonal variation. By using high-performance liquid chromatography and spectrophotometry, the contents of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, R_d, dencichine, flavonoid, and polysaccharide were determined and compared with Radix Notoginseng collected from different regions of growth in China, as well as from different seasons of harvest and market grades. Using the contents of these active constituents as markers, the best quality of Radix Notoginseng is found in the southwestern parts of Wenshan, and the best season for the harvest is September to October. In addition, the unseeded plants produced a better quality of Radix Notoginseng. The current results provide useful information for the quality control of Radix Notoginseng and its further development in establishing the good agriculture practice standard of *P. notoginseng* in China.

KEYWORDS: ***Panax notoginseng*; GAP; quality control; polysaccharide; saponin; seasonal variation; Chinese medicine**

INTRODUCTION

Radix Notoginseng, the root of *Panax notoginseng* (Burk.) F. H. Chen, is a well-known traditional Chinese medicine named Sanqi and belongs to the Ginseng genus. A wide variety of therapeutic uses of Radix Notoginseng have been reported including promotion of blood circulation, removal of blood stasis, induction of blood clotting, relief of swelling, and alleviation of pain (*1*−*3*). It has also been used for the treatment of coronary heart disease and cerebral vascular disease with favorable results (*4*−*6*). Recent analyses have provided several lines of evidence that saponin (*7*, *8*), flavonoid (*9*), and polysaccharide (*10*, *11*) are the main active constituents in roots of *P. notoginseng*. Saponins of Radix Notoginseng have been reported to increase the blood flow of the coronary arteries (*12*, *13*) and to decrease the consumption of oxygen by heart muscles (*14*). Currently, over 20 different saponins have been identified in *P. notoginseng* root (*15*, *16*) including ginsenosides, notoginsenosides, and gypenosides. Among these saponins, ginsenoside R_{g1}, R_{b1}, R_d, and notoginsenoside R_1 are considered to be the major components found in Radix Notoginseng (*15*). Thus, the contents of different saponins are important for quality control of Radix Notoginseng. Similarly, flavonoid can increase coronary flow, reduce myocardial oxygen consumption, and lower arterial pressure (*9*). Polysaccharide extracted from *P. notoginseng* root is also considered to be an active constituent with immunostimulating activities in vitro (*10*, *11*, *17*). On the other hand, dencichine, an amino acid isolated from Radix Notoginseng, has a function on hemostasis (*18*).

For over a thousand years, Radix Notoginseng has been grown primarily in Wenshan of Yunnan, where over 85% of the total production of about 2 million kg per year in China is produced. Different regions of Wenshan produce different qualities of Radix Notoginseng; however, which region produces the best quality of herb is still controversial. The harvest season of Radix Notoginseng is normally on 3 year old plants, which is mainly dependent on the total yield of the plant rather than on their chemical composition. Besides, the seasonal variation of active constituents in Radix Notoginseng has not been determined. The aforementioned parameters are, therefore, important to establish the quality control of Radix Notoginseng

* To whom correspondence should be addressed. Tel: (852)2358-7332. Fax: (852)2358-1559. E-mail: botsim@ust.hk.
[†] The Hong Kong University of Science and Technology.
[‡] China Pharmaceutical University.
[§] Beijing Military Medical College of PLA.

10.1021/jf034229k CCC: $25.00 © 2003 American Chemical Society
Published on Web 06/28/2003

and, in particular, to actualize the good agriculture practice (GAP) in China.

In the present study, we compared various active constituents of Radix Notoginseng from different regions of Wenshan, different seasons of harvest, different grades, and manipulations. The levels of ginsenoside R_{g1}, R_{b1}, R_d, notoginsenoside R_1, dencichine, flavonoids, and polysaccharides were determined by reverse phase high-performance liquid chromatography (HPLC) and spectrophotometry. The contents of these active constituents were discussed to give useful information for quality control and GAP farming of Radix Notoginseng in Wenshan.

MATERIALS AND METHODS

Plant Materials. Fresh *P. notoginseng* plants were collected from Wenshan of Yunnan Province, Jingxi of Guangxi Province, and Nanxiong of Guangdong Province. The 3 year old plants were collected from different counties of Wenshan from March to November in the year 2000. The botanical origins of all of the materials in forms of whole plants were identified morphologically during the field collection. Roots of *P. notoginseng* from different geographical properties at the same region were collected and dried under vacuum. Individual samples were prepared from ~500 g of powder that was ground from ~20 plants of the same population. These grinding processes were done during the field collection before they were delivered to the laboratory. The collected powder was stored with silica gel that stabilized the chemical constituents. Unless otherwise stated, the best grade of Radix Notoginseng collected in September to October was selected for analysis. Their corresponding voucher specimens in forms of dry roots were deposited in the Department of Biology, The Hong Kong University of Science and Technology, Hong Kong, China.

Extraction of Chemical Constituents. For saponin and flavonoid, about 5 g of ground powder was Soxhlet extracted in 100 mL of 70% methanol for 2 h, and the extraction was repeated three times. The combined methanol extracts were loaded onto a D101 macroporus resin column. After it was washed by 3 volumes of water, the column was eluted by 70% methanol, and the elution was evaporated to about 2 mL final volume by vacuum. The residues after methanol extraction were further extracted for dencichine in 25 mL of water by ultrasonication (Brason 5200 Danbury, CT) for 1 h and repeated twice. The supernatant was collected by centrifugation at 3500 rpm for 10 min. All samples were filtered through a Millipore filter unit. Ten microliters of the sample was injected to reverse phase HPLC and diluted 20 times with 70% methanol for spectrophotometry. For polysaccharide, about 1 g of powder sample was ultrasonically extracted in 25 mL of water for 1 h, and the extracts were filtered. The filtrate was evaporated to about 2 mL by vacuum, and 95% ethanol was added to the concentrated extract until reaching 85% ethanol in final. The solution was kept still and airtight for 24 h. Then, it was filtered with an air extractor. The mud cake was washed with 70% ethanol and dissolved in water at 60 °C. The solution was centrifuged at 2000 rpm for 5 min to discard the insoluble matter. The supernatant was adjusted to 100 mL in a measuring flask and stored for further analysis.

Quantitative Analysis. Notoginsenoside R_1, R_2, R_3, ginsenoside R_{g1}, R_{g2}, R_{g3}, R_{b1}, R_d, R_{h1}, R_e, and quercetin (a flavonoid standard) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products from Beijing, China. Dencichine was a gift from Prof. Chong Kun Gao of the Institute of Pharmacy in Yunnan Baiyao Gruop. The identities of these chemical markers were confirmed by NMR and mass spectrometers, as determined by the suppliers. Dextran with a molecular mass of 15–20 kDa was from Sigma (St. Louis, MO). HPLC grade reagents were from Fischer and Labscan (Dublin, Ireland).

For the calibration of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, R_d, the standards were weighed and dissolved in 1 mL of methanol to give serial concentrations. Three injections were performed for each dilution. The concentrations of these compounds in the samples were calculated according to the regression parameters derived from the standard curves. The HPLC system consisted of Waters PC 800 Integrator, Waters 486 Tunable Absorbance Detector, and Waters 600 Pump. Chromatographic separations were carried out on a NOVA-PAK C_{18} column (300 mm × 3.9 mm i.d., particle size 4 μm) with a guard column (NOVA-PAK C_{18}, 20 mm × 3.9 mm i.d., particle size 4 μm), with CH_3CN/50 mM KH_2PO_4 (20:80) as an eluent at a flow rate of 1.0 mL/min at room temperature and monitored at 203 nm. The running condition was developed as below: a linear gradient of CH_3CN/50 mM KH_2PO_4 from a ratio of 20:80 to 30:70 from 2 to 6 min; a linear gradient of CH_3CN/50 mM KH_2PO_4 from a ratio of 30:70 to 50:50 from 6 to 14 min; a linear gradient of CH_3CN/50 mM KH_2PO_4 from a ratio of 50:50 to 30:70 from 14 to 16 min; an isostatic CH_3CN/50 mM KH_2PO_4 (30:70) from 16 to 30 min; a linear gradient of CH_3CN/50 mM KH_2PO_4 from a ratio of 30:70 to 20:80 from 30 to 35 min. This running condition was optimized to give the best resolution of all HPLC peaks. The content of total saponins was determined by HPLC using R_{g1} as a reference standard, which was the summation of the above-mentioned major peaks of R_1, R_{g1}, R_{b1}, R_d and minor HPLC peaks of notoginsenosides R_2 and R_3 and ginsenosides R_{g2}, R_{g3}, R_{h1}, and R_e from Radix Notoginseng. Similar HPLC by using NOVA-PAK C_{18} column (150 mm × 3.9 mm i.d., particle size 4 μm) was performed for the determination of dencichine in the extracts, where the mobile phase was CH_3CN:methanol:0.001% acetic acid in a ratio of 18:4:78, and the flow rate was 0.5 mL/min at room temperature and monitored at 213 nm.

For the calibration of polysaccharide, the anthrone–sulfuric acid method was used (*19*). Dextran was weighed and dissolved in 100 mL of water to give serial concentrations. Standard solution (0.6 mL), or prepared sample, was adjusted to 2.0 mL final volume. Then, 4.0 mL of freshly prepared 0.2% anthrone–sulfuric acid was added. Absorbance at 625 nm was measured after 30 min of color reaction. For flavonoid calibration, quercetin was weighed and dissolved in 50 mL of 70% methanol to give serial concentrations. The absorbances of standard solutions and samples were detected at 249 nm by UV spectrophotometry (Beckman DU^R 650). In hierarchical clustering analysis of different samples, SPSS software (version 11.0 from Statistical Product and Service Solutions, Chicago, IL) was used. Data were evaluated for statistical significance on a minimum of six replicates using the unpaired *t* test. Differences were considered to be significant when p values were ≤ 0.05.

RESULTS AND DISCUSSION

Notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, and R_d are the key active constituents in roots of *P. notoginseng*, and they are different according to their carbohydrate side chains (**Figure 1**). Dencichine is an amino acid in root of *P. notoginseng*. When the extract, derived from roots of *P. notoginseng*, was subjected to HPLC analysis under gradient elution, the peaks corresponding to different saponins were well-separated. **Figure 2** shows a typical chromatogram of methanol extract from Radix Notoginseng. The individual peaks for notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, and R_d were distinct. Besides, dencichine could also be determined by HPLC analysis with different wavelength and retention time. The peaks of these saponins, or dencichine, were further identified by two means: (i) by comparing the retention times of the unknown peaks with those of the standards eluted under the same conditions and (ii) by spiking the sample with stock standard solutions of saponins or dencichine. The quantitation was carried out by measuring the peak area according to the regression equation (**Table 1**). By using the established HPLC method, the amounts of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, and R_d were calculated from the calibration curves that were prepared with the standard solution of each compound. These four saponins accounted for ~80% of total saponins in root of *P. notoginseng*. The correlation coefficients of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, and R_d were from 0.9988 to 0.9998. The precision and repeatability of the constituents' tests were excellent with relative standard deviation (RSD) < 3%. The recovery experiment was carried out to evaluate the accuracy of the method. Known

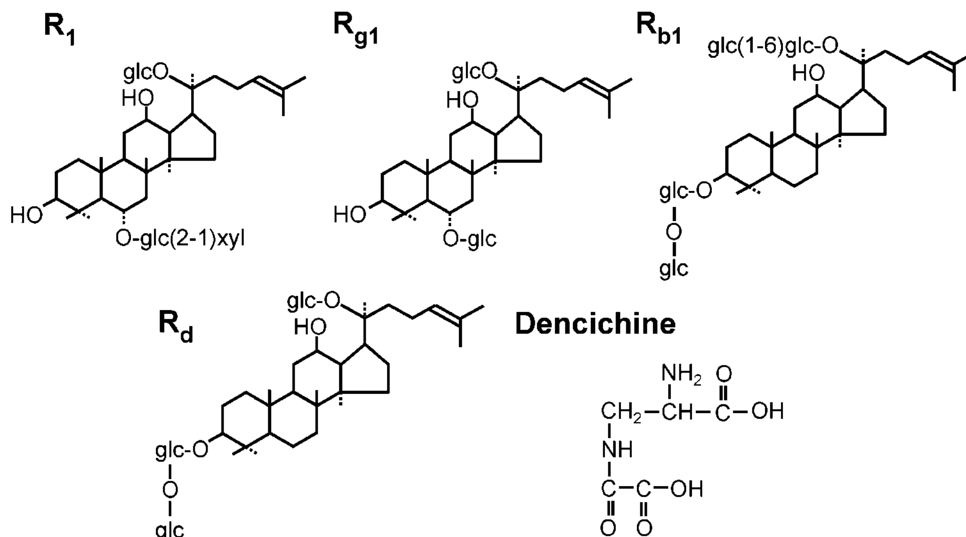

Figure 1. Chemical structures of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, R_d, and dencichine from Radix Notoginseng. Glc, glucose; xyl, xylose.

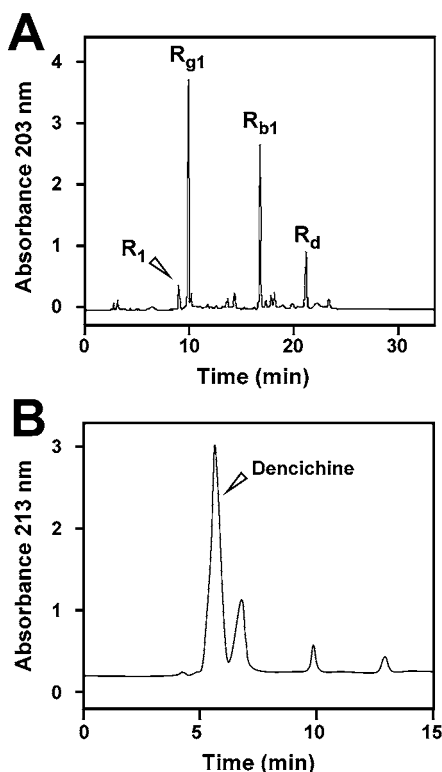

Figure 2. Determinations of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, R_d, and dencichine from Radix Notoginseng. By using a NOVA-PAK C_{18} column in HPLC analyses, different saponins (panel **A** at absorbance 203 nm) and dencichine (panel **B** at absorbance 213 nm) were separated; the running conditions were described in the Materials and Methods. Peaks corresponding to R_1, R_{g1}, R_{b1}, R_d, and dencichine are indicated. Typical chromatograms are shown.

amounts of saponins were added to the sample and extracted accordingly. The recoveries of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, and R_d were from 96 to 101% (**Table 1**). From the same HPLC profile, total saponins were calibrated by adding the major peaks of R_1, R_{g1}, R_{b1}, R_d and the minor peaks of notoginsenosides R_2 and R_3 and ginsenosides R_{g2}, R_{g3}, R_{h1}, and R_e. The identities of these minor peaks of saponins were confirmed by retention times of the corresponding standards. The determinations of dencichine, polysaccharide, and flavonoid were similar to that of saponins, where the calibration curve and the recovery were as good as the analyses of saponins (**Table 1**).

Table 1. Calibrations of Notoginsenoside R_1, Ginsenoside R_{g1}, R_{b1}, R_d, Dencichine, Polysaccharide, and Flavonoid[a]

standard	regression equation	r^2	recovery (%)
notoginsenoside R_1	$y = 547\,904x - 240\,765$	0.9988	96
ginsenoside R_{g1}	$y = 388\,057x - 678\,882$	0.9991	98
ginsenoside R_{b1}	$y = 240\,491x - 175\,137$	0.9998	99
ginsenoside R_d	$y = 127\,725x - 65\,269$	0.9993	101
dencichine	$y = 2.8 \times 10^{-7}x + 0.0072$	0.9996	98
dextran	$y = 0.028x + 0.1745$	0.9982	99
quercetin	$y = 0.07052x + 0.05587$	0.9992	103

[a] HPLC performance and spectrophotometry were described in Materials and Methods. Recovery was determined by adding a known amount of constituents into the plant, where the amount of active constituents was known. These samples were subjected to HPLC analysis. The regression equation was used to calibrate the concentration of various active constituents. The mean values are expressed here, and the SD values of the tested chemicals were less than 5% of the mean. The RSDs were <3%. The calibration was repeated five times ($n = 5$). The regression equation was used to calibrate the concentration of various constituents as listed in **Tables 2** and **3**.

Wenshan of Yunnan is known to produce the best quality of Radix Notoginseng in China; however, the variation within the subregions of Wenshan has not been determined. The contents of various saponins in roots of *P. notoginseng* derived from different regions of Wenshan and from other provinces of China were determined by HPLC. Twenty-eight different populations of *P. notoginseng* from various geographical regions were collected; each chosen population contained 10−15 different batches of samples. Wenshan Prefecture contains eight different counties, and most of the cultivated areas for *P. notoginseng* are found in the southwestern parts, such as Wenshan, Maguan, and Yanshan counties (**Figure 3A**). Despite different subregions, the whole Wenshan Prefecture contained a 20−70% higher amount of R_1, R_{g1}, R_{b1}, R_d, and total saponins in comparison to Radix Notoginseng cultivated in Guangxi and Guangdong (**Table 2**). Different subregions of Wenshan Prefecture, Lianhuatang, Leshicong, Laohuilong, and Pingba of Wenshan county, Zhela and Jiangna of Yanshan county, and Bazai and Dalishu of Maguan county, produced better quality of Radix Notoginseng in term of their contents of R_1, R_{g1}, R_{b1}, R_d, and total saponins (**Table 2**). In addition, the amounts of dencichine,

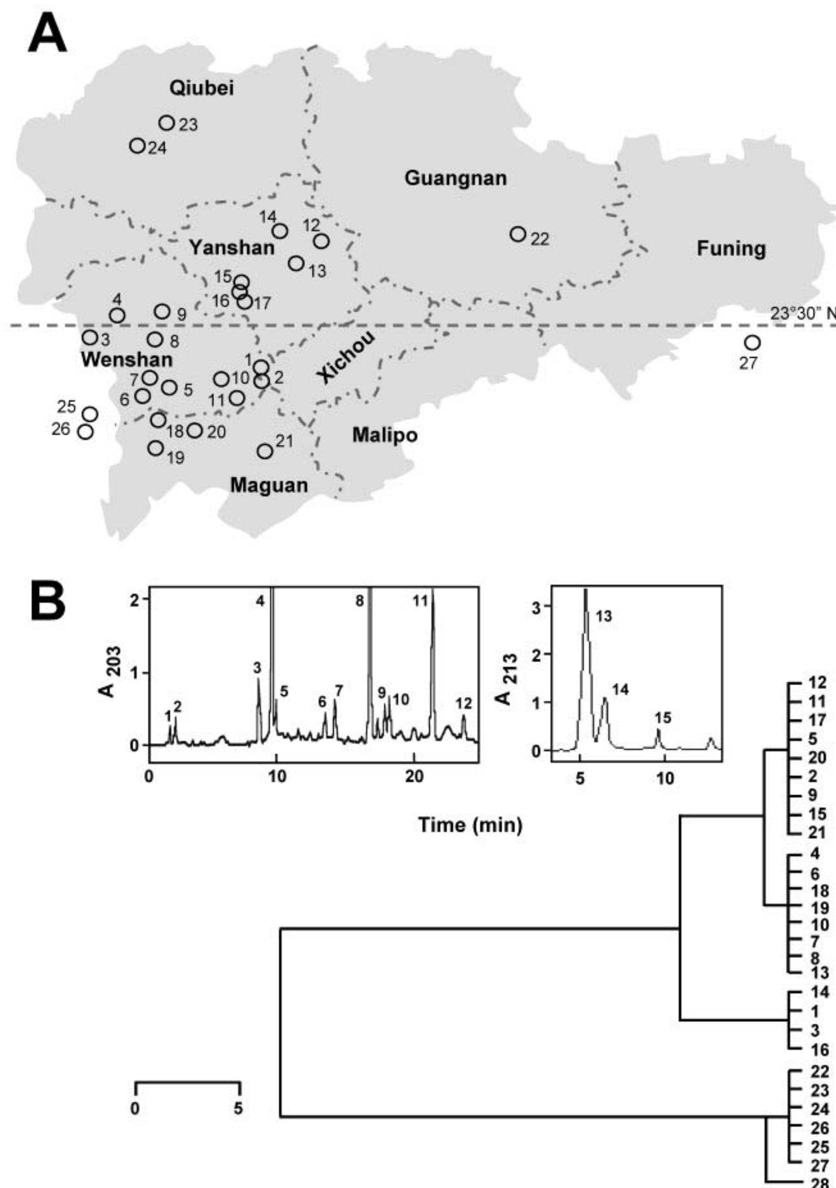

Figure 3. Regional variation of chemical composition of Radix Notoginseng in different counties of Wenshan Prefecture. (**A**) Geographic distribution of *P. notoginseng* in the eight counties of Wenshan, separated by dotted lines. The sources of Radix Notoginseng are indicated by circles and numbers, which are the same denotation as in **Table 2**. The Wenshan Prefecture is shaded. Location numbers 27 and 28 are not shown here. (**B**) The 15 chosen HPLC peaks were derived from absorbance 203 and 213 nm (insert). The numbers from 1 to 15 are indicated. The hierarchical clustering analysis of different *P. notoginseng* roots. The clustering was done by SPSS software from 15 HPLC peaks analyzed from the tested 28 *P. notoginseng* roots; these samples were chosen randomly from the 28 populations, one single sample from each population, as listed in **Table 2**. Bar, cluster distance.

flavonoid, and polysaccharide were also determined, and they were in line with the results from the analysis of individual saponin but the differences were insignificant.

The quality of crude drugs is closely related to their chemical constituents and could be assessed by chemical pattern recognition method. By using the results from HPLC analysis, different samples of Radix Notoginseng were subjected to hierarchical clustering analysis. Within the same HPLC analytic condition, 15 constituents revealed in absorbance 203 and 213 nm including all of the tested saponins and dencichine were eluted, and the chemical data were obtained (**Figure 3B**, insert). These 15 constituents were quantified based on their peak areas by using R_{g1} (peak 4) as a reference standard. A matrix of 28 × 15 was obtained, which gave the content differences of 15 constituents among the tested 28 populations, as listed in **Table 2**. In the hierarchical clustering analysis, a method named as average linkage between groups was applied, and Pearson correlation was selected as a measurement (**Figure 3B**). The tested 28 populations of Radix Notoginseng were divided into two main clusters: samples 1−21 as cluster one and samples 22−28 as cluster two. This clustering agrees very well with the results of the contents of saponins as determined in **Table 2**. Members of cluster one were roots collected from three counties: Wenshan, Yanshan, and Maguan. Indeed, roots of *P. notoginseng* from these counties contained higher amount of saponins than the others. Members of cluster two, containing smaller amount of active constituents, were collected from other counties of Wenshan Prefecture, or those areas in the nearby regions of Wenshan including Guangxi and Guangdong. The hierarchical clustering analysis suggests that *P. notoginseng* root cultivated in the southwestern part of Wenshan is distinct, and these regions are located on, or nearby, the north longitude of 23° 30″ and that could be the cause of its superior quality.

Table 2. Contents of Active Constituents from Different Populations of Radix Notoginseng

no.[a]	source[b]	saponins R_1	saponins R_{g1}	saponins R_{b1}	saponins R_d	saponins total	dencichine	TP[c]	total flavonoids
				Wenshan					
1	Zhuilijie	0.1592	3.1934	2.2800	0.7597	8.4985	0.4780	0.0581	0.1439
2	Lianhuatang	0.4217	3.4752	3.0399	1.0327	9.8105	0.4340	0.1599	0.1483
3	Leshicong	0.5851	3.1784	3.0989	0.9247	9.8110	0.3820	0.0321	0.1259
4	Laohuilong	0.6005	3.8171	3.1512	1.2499	10.9126	0.5570	0.1142	0.1251
5	Pingba	0.4139	3.7247	2.8046	0.9323	11.2930	0.4050	0.1750	0.1468
6	Xinjie	0.4816	3.0436	2.9635	0.7528	8.9985	0.6150	0.1115	0.1266
7	Xiaojie	0.5117	3.2111	2.9488	0.8270	9.2555	0.3900	0.0488	0.1188
8	Baxin	0.5038	3.2474	2.8240	0.7865	9.3138	0.5260	0.0474	0.1266
9	Matang	0.3239	2.9785	2.6487	0.7893	8.3438	0.4170	0.0666	0.1265
10	Gumu	0.5119	2.8900	3.0070	0.9103	9.1204	0.3850	0.1080	0.1287
11	Yangliuling	0.4247	2.7376	2.4873	0.7435	8.0116	0.5990	0.1122	0.1332
				Yanshan					
12	Ameng	0.4335	3.1363	2.7404	1.0296	9.2655	0.4890	0.1098	0.1318
13	Zhela	0.6400	3.3551	2.6681	1.1330	10.2663	0.5710	0.1210	0.1496
14	Ganhe	0.2593	2.8267	2.3961	0.7774	7.8034	0.5670	0.1304	0.1008
15	Jiangna	0.4814	3.2990	2.9313	1.0574	9.8762	0.5250	0.1080	0.1086
16	Jiaozhi	0.2141	3.0708	2.5475	1.0212	8.9554	0.4810	0.0602	0.0953
17	Panlong	0.4447	2.8180	2.5354	0.9685	8.3722	0.4940	0.1072	0.1228
	Maguan								
18	Langqiao	0.4278	2.7745	2.5933	0.9558	8.4044	0.3850	0.1281	0.1303
19	Bazai	0.4986	3.1196	2.5814	1.1640	9.6822	0.3280	0.0131	0.1321
20	Dalishu	0.5347	3.2063	2.8438	1.0739	9.6916	0.3250.	0.1611	0.0990
21	Mabai	0.3882	2.6702	2.5819	1.0678	8.4309	0.5990	0.1762	0.1062
				Guangnan					
22	Majie	0.4614	2.8171	2.6696	0.5439	8.2330	0.3930	0.1250	0.1287
				Qiubei					
23	Xingou	0.3704	2.8260	2.3198	0.8083	7.9442	0.3320	0.1056	0.1287
24	Badashao	0.4224	2.4641	2.2080	0.6415	7.6671	0.4320	0.0171	0.1304
				Menzi					
25	Mingjiu	0.4834	3.1274	3.0170	1.1348	9.7534	0.4650	0.1369	0.1221
26	Laozai	0.5266	3.1720	3.2672	1.2118	9.9173	0.5120	0.0807	0.1142
	Guangxi								
27	Jingxi	0.4534	2.6711	2.6839	0.7556	8.2671	0.4250	0.0117	0.1326
				Guangdong					
28	Nanxiong	0.2954	2.6503	2.6037	0.7184	6.7767	0.4570	0.1348	0.1304

[a] Twenty-eight populations of *P. notoginseng* roots were collected fresh and dried under vacuum as described in Materials and Methods. The geographical locations of these areas are shown in **Figure 3A**. [b] From 10 to 15 individual samples from each population were analyzed. All samples were collected in September to October, and they were all 3 year old plants. [c] Total polysaccharides. Values are in g/100 g dry roots and in means, $n = 10$–15. The SD values, which are less than 10% of the means, are not shown for clarity.

P. notoginseng is a perennial herbage, and normally, its root is harvested after 3 years of growth. The chemical composition of the root, however, could be changed according to different seasons. **Figure 4** shows different levels of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, R_d, total saponins, dencichine, flavonoids, and polysaccharides in the roots of 3 year old *P. notoginseng* that were harvested from March to November. The concentrations of total saponins and gingsenoside R_{g1} and R_{b1} were higher in March to April, then decreased to the lowest level in July, then increased again during August to October, and then slightly declined in November (**Figure 4A**). In parallel, the content of total polysaccharides was similar to that of saponins, which had the highest value during March to April and declined to the lowest level in July (**Figure 4B**). In contrast, the amount of dencichine and flavonoids showed insignificant variation in responding to seasonal change. The great majority of active constituents from traditional Chinese medicine is metabolic byproducts, and thus, the contents of active constituents have a close correlation with the stages of plant growth. Our results show that the content of active constituents is higher in March to April when the plant has higher metabolic rate. In July,

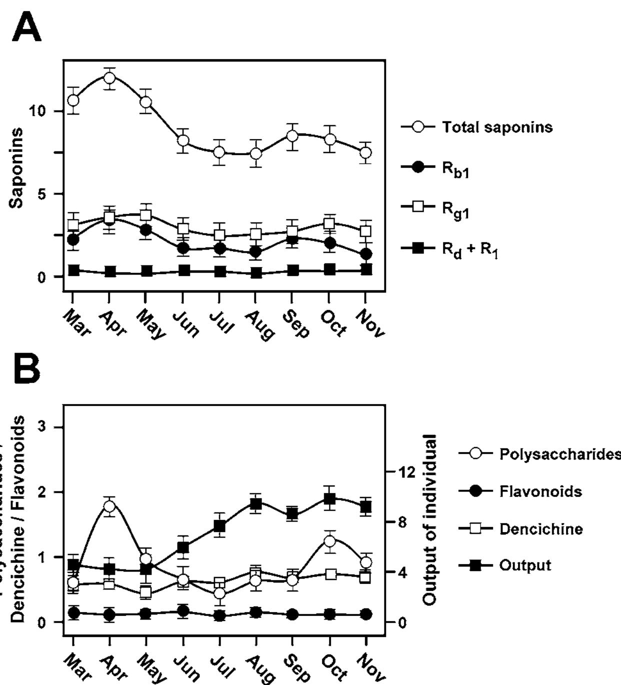

Figure 4. Seasonal change of chemical composition in Radix Notoginseng. Cultivated *P. notoginseng* from Laohuilong of Wenshan county was used for analysis; they were 2–3 years old, depending on the time of harvest. (**A**) The amounts of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, R_d, and total saponins are in g/100 g of dried roots. (**B**) Dencichine, total flavonoids, and polysaccharides are in g/100 g of dried roots. Total output is in grams per individual plant. Values are means ± SD, $n = 10$.

flowers of *P. notoginseng* have anthesis, which subsequently causes the decrease of the active constituents in the roots. The total yield of *P. notoginseng* root showed a gradual increase starting in May and reached a plateau in August to October.

The levels of saponins and total yield of Radix Notoginseng were further determined and compared between the seed-reserved plant (seeded) and the flower-picked plant (unseeded); the latter showed a higher content of saponins as well as higher output yield in the production of *P. notoginseng* roots, in particular from August to November where the difference could reach over 30% (**Figure 5**). These results agree very well with the traditional practice that roots harvested from seed-reserved *P. notoginseng* (named as winter Radix Notoginseng in China) are not as good as those harvested from flower-picked *P. notoginseng* (spring Radix Notoginseng). Another parameter of Radix Notoginseng that could affect its quality, as well as its price, is the size of the roots, namely, Tou (means the number of individual roots in ~500 g), on the market. Commercially, 20 Tou is generally considered as the largest and the best grade of Radix Notoginseng. **Table 3** shows that the 20 Tou (the largest in size) Radix Notoginseng contains higher amounts of saponin, dencichine, polysaccharide, and flavonoid than other grades. The amounts of these tested active constituents decrease in an order of 20 > 30 > 40 > 60 > 120 Tou. The amount of R_{g1} and R_d shows almost 2-fold differences between 20 and 120 Tou of Radix Notoginseng.

Traditionally, Chinese herbalists used only the root of *P. notoginseng* as herbal medicine (*5*). The rhizome of *P. notoginseng* is not used because of its irregular appearance. Although the chemical study on rhizome is very little, saponins have been isolated from rhizomes of *P. notoginseng* (*7*, *20*). A single plant of *P. notoginseng* produces about 8 g of root per harvest, and the total yield of rhizome is about 25% of that. Here, the contents

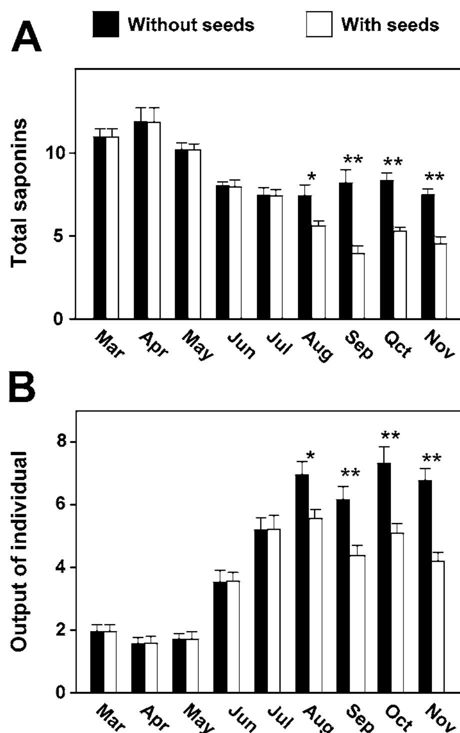

Figure 5. Roots of unseeded *P. notoginseng* contain higher amounts of saponins and total output as compared to the seeded plants. Cultivated *P. notoginseng* from Laohuilong of Wenshan county was used for the analysis, and they were 2–3 years old, depending on the time of harvest. Flowers were picked on those unseeded plants in July of every year. Values for total saponins (g/100 g of dry roots) and total output (g per individual plant) are means ± SD, $n = 10$. Statistical comparison was made by using the unpaired *t* test between with and without seeds, where $^{*}P < 0.05$ and $^{**}P < 0.01$.

Table 3. Contents of Active Constituents from Different Grades of Radix Notoginseng

grade[a]	saponins R1	Rg1	Rb1	Rd	total	dencichine	TP[b]	total flavonoids
20 Tou	0.6005	3.8171	3.1512	1.2499	10.9126	0.5570	0.1142	0.1251
30 Tou	0.9263	3.2058	2.1056	1.2967	10.1843	0.5307	0.0798	0.1296
40 Tou	0.7040	2.8009	2.1601	1.0615	9.0595	0.5447	0.0637	0.1279
60 Tou	0.4276	2.0792	1.8340	1.1345	7.2062	0.5231	0.0781	0.1180
120 Tou	0.4965	2.1269	1.1923	0.5222	6.1613	0.4273	0.0382	0.0817

[a] Roots of *P. notoginseng* were collected fresh and dried under vacuum as described in Materials and Methods; different grades in Tou were collected, where 20 Tou refers to totally ~20 roots in ~500 g of sample. Ten to 15 individual samples from each grade were analyzed. All samples were collected in September to October from Laohuilong of Wenshan, and they were all 3 year old plants. [b] Total polysaccharides. Values are in g/100 g dry roots and in means, $n = 10$–15. The SD values, which are less than 10% of the means, are not shown for clarity.

of saponins, flavonoids, dencichine, and polysaccharides contained in rhizomes of *P. notoginseng* were determined and compared with roots that derived from the same plants. The contents of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, R_d in rhizome were markedly higher than that in the roots (**Figure 6**). Besides, the content of total flavonoids and dencichine in the rhizome showed slightly higher amount than the root. This result suggests that rhizomes of *P. notoginseng* are good materials as herbal medicine and have great economic value, which merits further exploitation.

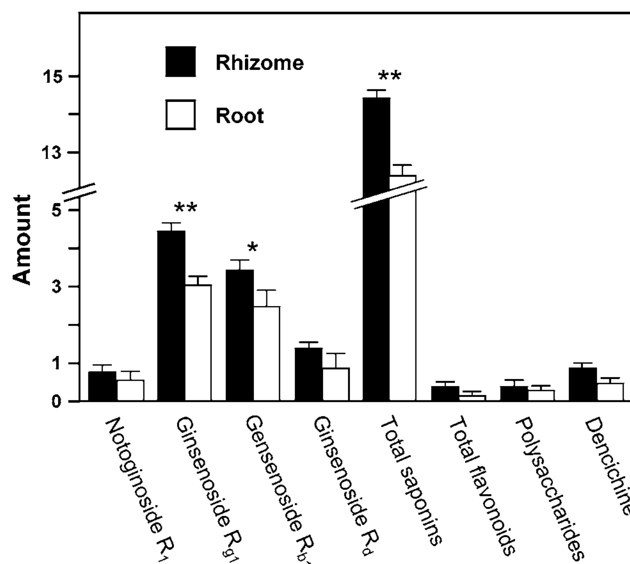

Figure 6. Rhizome contains a higher amount of active constituents than roots in *P. notoginseng*. Cultivated *P. notoginseng* from Laohuilong of Wenshan county was used for the analysis, and they were 3 years old collected in October 2000. Rhizome and root were derived from the same plant for each collection. Amounts of notoginsenoside R_1, ginsenoside R_{g1}, R_{b1}, R_d, total saponins, flavonoids, and polysaccharides were determined as described in Materials and Methods. Values in g/100 g of dry roots are means ± SD, $n = 10$. Statistical comparison was made by using the unpaired *t* test between rhizome and root, where $^{*}P < 0.05$ and $^{**}P < 0.01$.

P. notoginseng belongs to the Ginseng genus, is a member of Araliaceae, and is an archaic plant that originates 25 million years ago in southwest of Yunnan China (*4*). The quality of Radix Notoginseng is dependent on its growth region. Besides the geographical properties, farmers in Wenshan have the experience of cultivating Radix Notoginseng for over a thousand years. Within Wenshan Perfecture, roots of *P. notoginseng* from different counties showed variation in their chemical composition. The counties including Wenshan, Yanshan, and Maguan were shown to have better production of Radix Notoginseng. For over a thousand years, the quality of traditional Chinese medicines has heavily depended on the cultivated regions and the timing of harvest, and in China, they called that "Dao Di" to describe the highest quality of herbs that are collected from the best region and at the best time. Indeed, this ancient concept of quality control is not only applied to Radix Notoginseng here; similar observations have also been revealed in the cultivation of Radix Astragli (*19*) and Radix Angelica (*21*). Nevertheless, these analyses on the quality control of herbs are crucial in the development of the GAP farming of Chinese medicine in China as well as in other parts of Asia.

Roots from *Panax quinquefollius* L. (American ginseng) and *Panax ginseng* C. A. Meyer (Korean ginseng) share a high degree of similarity in their chemical and genetic compositions in comparison to that of *P. notoginseng*. For instance, the spacerdomains of 5S-rRNA were sequenced and compared, which showed over 75% DNA identity among different members of the *Panax* family (*22*). Higher sequence identity was revealed in other regions of the genome (*23*−*25*). Genetically, the distinction of *P. notoginseng* to other members of the *Panax* family could be achieved by random amplification of polymorphic DNA (*22*). Moreover, roots of *P. notoginseng* also show a close chemical resemblance with roots of *P.*

quinquefollius and *P. ginseng*; two other common herbal medicines that contain saponins as their active constituents. However, American and Korean ginsengs, when their ethanol extracts were analyzed on HPLC, contained predominantly R_{b1} as the major ginsenoside (*24*). In contrast, R_{g1} was the major component in our analyses. In addition, the existence of notoginsenoside R_1 and dencichine could also serve as distinct chemical markers of root of *P. notoginseng*.

ACKNOWLEDGMENT

We are grateful to Dr. Shao P. Li from Macau University and Dr. Roy C. Y. Choi from HKUST for their expert technical assistance and fruitful discussion during the study, to Mr. Z. J. Chen and Mr. C. L. Wang at Wenshan of Yunnan for their help in sample collection, and to Prof. C. K. Gao of Yunnan Baiyao Group for providing dencichine.

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Received for review March 10, 2003. Revised manuscript received May 28, 2003. Accepted June 1, 2003. This work was supported by New World Company and Innovation Technology Fund (ITF; UIM/9) and the Area of Excellence Scheme established under the University Grants Committee of Hong Kong SAR, China (AoE/B-15/01) to K.W.K.T. and Yunnan Province Science and Technology Office Application Basic Research Fund to X.M.C.

JF034229K