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Detection of Adulteration of Notoginseng Root Extract with Other *Panax* Species by Quantitative HPLC Coupled with PCA

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Reverse phase high-performance liquid chromatography (HPLC) coupled with a principal component analysis (PCA) method was used to distinguish the extract of notoginseng root from that of other species in the genus *Panax*. The content of 12 saponins in notoginseng root extracts from different sources was evaluated. Herbal extracts from different plant parts of notoginseng, Asian ginseng, and American ginseng were also evaluated. With an HPLC assay, however, it is difficult to determine whether notoginseng root extract has been adulterated with other plant parts or other *Panax* species before extraction. Therefore, PCA was introduced to identify adulteration in notoginseng root extract. PCA was performed on the data set obtained from the HPLC chromatogram. The HPLC-PCA assay distinguished notoginseng root extract not only from the extract of other plant parts of notoginseng but also from the extract of Asian or American ginseng plant parts.

KEYWORDS: *Panax notoginseng*; extract authentication; adulteration; genus *Panax*; dammarane saponins; HPLC; PCA

INTRODUCTION

The root of *Panax notoginseng* (Burk.) F. H. Chen (Araliaceae) is a remedy that has a long history of use in China, Japan, and other Asian countries (*1, 2*). Notoginseng is cultivated commercially in the southwestern regions of China, especially in Yunnan province. The main bioactive compounds in notoginseng are dammarane saponins, commonly referred to as notoginseng saponins (**Figure 1**) (*2, 3*). Notoginseng root extract exerts various effects on the cardiovascular system, the central nervous system, the endocrine system, and the body's inflammatory response (*1, 4*). Although the saponins from notoginseng are similar to those in other ginsengs such as *Panax ginseng* (Asian ginseng) and *Panax quinquefolius* (American ginseng), these herbs differ in their effects on symptoms (*2, 5*). To reduce the expense of ginseng products in the marketplace, however, some notoginseng leaf and flower extracts or extracts of other Asian ginseng are designated and sold as notoginseng extract in China (*6*). Because different ginseng saponins may possess opposing pharmacological activities (*7*), knowing the real content of saponins and their proportions is vital for the safe use of notoginseng extract. If notoginseng extract is adulterated, its therapeutic effects are not achieved. Moreover, adulteration may cause an adverse or opposite effect (*8, 9*). New regulations by the U.S. FDA for alternative complementary supplements require botanical extracts to be standardized (*10*). Therefore, accurate identification of notoginseng root extract is important.

Currently, the most popular herbal products are in extract forms (*10, 11*). The identification of the correct species used to make the extract is an essential step in the production of an herbal product. Several methods have been used to identify the source of an extract, including chemical, spectroscopic, and chromatographic techniques (*12, 13*). Among these methods, high-performance liquid chromatography (HPLC) has been preferred (*14*). HPLC studies of notoginseng root have been performed by different groups (*15*−*19*). In those papers, two HPLC methods to identify the roots of three *Panax* species were developed. However, only the constituents in the roots were determined; aerial plant parts were not assayed (*18, 19*). Because notoginsenoside R1 is found in notoginseng, R1 was used as the marker compound for the identification of notoginseng (*18, 19*). In the process of preparation and before extraction, notoginseng root can be adulterated with its other aerial parts or with other *Panax* species. Previous studies have not considered adulteration (*18, 19*) because notoginsenoside R1 can be detected from the adulterated extracts.

In this study, we determined the saponin content of extracts from different *Panax* species and different plant parts. We established a quantitative method with HPLC for the simulta-

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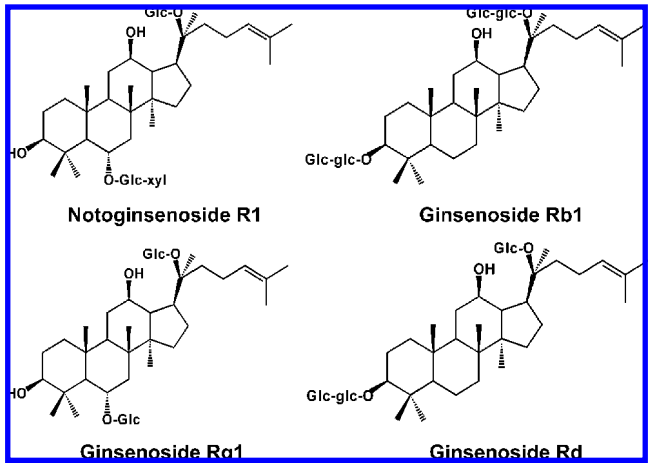

Figure 1. Chemical structures of four major saponins in notoginseng roots. Abbreviations for carbohydrates are as follows: Glc, β-D-glucose; Xyl, β-D-xylose.

neous determination of 12 saponins in notoginseng. If notoginseng root is adulterated with other ginsengs before extraction, the chromatograms of adulterated product may be similar to those of notoginseng root extract. We introduced principal component analysis (PCA), therefore, to find patterns in data and express their statistical similarities and differences (*20, 21*). Data obtained from HPLC were subjected to PCA to identify adulteration of notoginseng root extract.

MATERIALS AND METHODS

Chemicals. Standards for ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 were obtained from Indofine Chemical Co. (Somerville, NJ); ginsenosides Rb3, Rg2, 20*R*-Rg2, Rg3, Rh1, and notoginsenoside R1 were obtained from the Delta Information Center for Natural Organic Compounds (Xuancheng, Anhui, China). All standards were of biochemical reagent grade and at least 95% pure as confirmed by HPLC.

Herbal Materials. Different plant parts of *P. notoginseng*, the root, rootlet, corm, leaf, flower, and berry, were obtained from Wenshan, Yunnan, China. Fourteen lots of notoginseng root were obtained from various pharmacies in Beijing and Nanjing, China. The root, rootlet, leaf, and berry of Asian ginseng (*P. ginseng* C. A. Meyer) were obtained from Fusong, Jilin, China. Standard Asian ginseng root and leaf extracts were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. The roots of notoginseng and Asian ginseng were identified according to the Chinese Pharmacopoeia (2005 edition). American ginseng (*P. quinquefolius* L.) root, rootlet, leaf, and berry were obtained from Roland Ginseng, LLC (Wausau, WI), and American ginseng roots were identified according to the United States Pharmacopoeia NF 21. Voucher samples were deposited at the Tang Center for Herbal Medicine Research at the University of Chicago (**Table 1**).

-Sample Preparation. Dried herbal samples from different plant parts from notoginseng, Asian ginseng, and American ginseng were pulverized into powder and passed through a 40-mesh screen. The sample powder was extracted with 70% ethanol. The extract solution was condensed under vacuum and extracted with water-saturated *n*-butanol. The saponins were extracted into the *n*-butanol phase. The *n*-butanol phase was evaporated under vacuum and then lyophilized. The herbal extract was dissolved in methanol (1 and 10 mg/mL) and filtered through a Millex 0.2-μm nylon membrane syringe filter (Millipore Co., Bedford, MA) before use.

HPLC Analysis. The HPLC system was a Waters 2960 instrument (Milford, MA) with a quaternary pump, an automatic injector, a model 996 photodiode array detector, and Waters Millennium 32 software for peak identification and integration. The separation was carried out on an 250 mm × 3.2 mm i.d., 5 μm, Alltech Ultrasphere C18 column (Deerfield, IL) with a 7.5 mm × 3.2 mm i.d. guard column. For HPLC analysis, a 20-μL sample was injected into the column and eluted at room temperature with a constant flow rate of 1.0 mL/min. For the mobile phase, acetonitrile (solvent A) and water (solvent B) were used. Gradient elution started with 17.5% solvent A and 82.5% solvent B, followed by a 20-min linear gradient from 17.5 to 21% A, a 3-min linear gradient from 21 to 26% A, a 19-min isocratic elution with 26% A, a 13-min linear gradient from 26 to 36% A, a 9-min linear gradient from 36 to 50% A, a 2-min linear gradient from 50 to 95% A, a 3-min isocratic elution with 95% A, a 3-min linear gradient from 95 to 17.5% A, and an 8-min isocratic elution with 17.5% A. The detection wavelength was set to 202 nm.

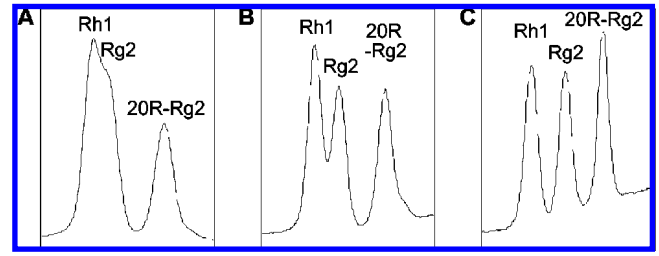

Figure 2. Representative HPLC chromatograms of the separation of ginsenosides Rh1, Rg2, and 20*R*-Rg2. After 23 min, eluting solvents were held for 19 min (**A**) in 28% acetonitrile and 72% water, (**B**) in 27% acetonitrile and 73% water, or (**C**) in 26% acetonitrile and 74% water.

Table 1. *Panax* Extracts from Different Plant Sources

group	classification	part	no. of samples
1	*P. notoginseng* underground	root	16
		rootlet	3
		corm	2
2	*P. notoginseng* aerial	leaf	2
		flower	1
		berry	1
3	*P. ginseng* underground	root	3
		rootlet	1
4	*P. ginseng* aerial	leaf	2
		berry	1
5	*P. quinquefolius* underground	root	4
		rootlet	1
6	*P. quinquefolius* aerial	leaf	3
		berry	4
7	*P. notoginseng*	root, leaf	1
8	*P. notoginseng*	root	1
	P. ginseng	root	
9	*P. notoginseng*	root	1
	P. ginseng	leaf	

Principal Component Analysis (PCA). A similar evaluation system for the chromatographic fingerprint of the different plant parts of notoginseng, Asian ginseng, and American ginseng was used to process a two-dimensional fingerprint. The HPLC fingerprint data were processed on a Pentium IV computer. PCA and other involved programs were coded in MATLAB (Mathworks Inc.).

RESULTS AND DISCUSSION

Optimal Separation of Notoginseng Saponins. The separation of 12 notoginseng saponins was evaluated by changing the gradient eluting programs. In the chromatogram, 12 saponins were separated into three groups: (1) notoginsenoside R1 and ginsenosides Rg1 and Re; (2) ginsenosides Rh1, Rg2, and 20*R*-Rg2; and (3) ginsenosides Rb1, Rc, Rb2, Rb3, Rd, and Rg3. Saponins in groups 1 and 3 were relatively easy to separate. Separation of the three ginsenosides in group 2 was difficult using the gradient eluting system of acetonitrile and water. The modification of HPLC

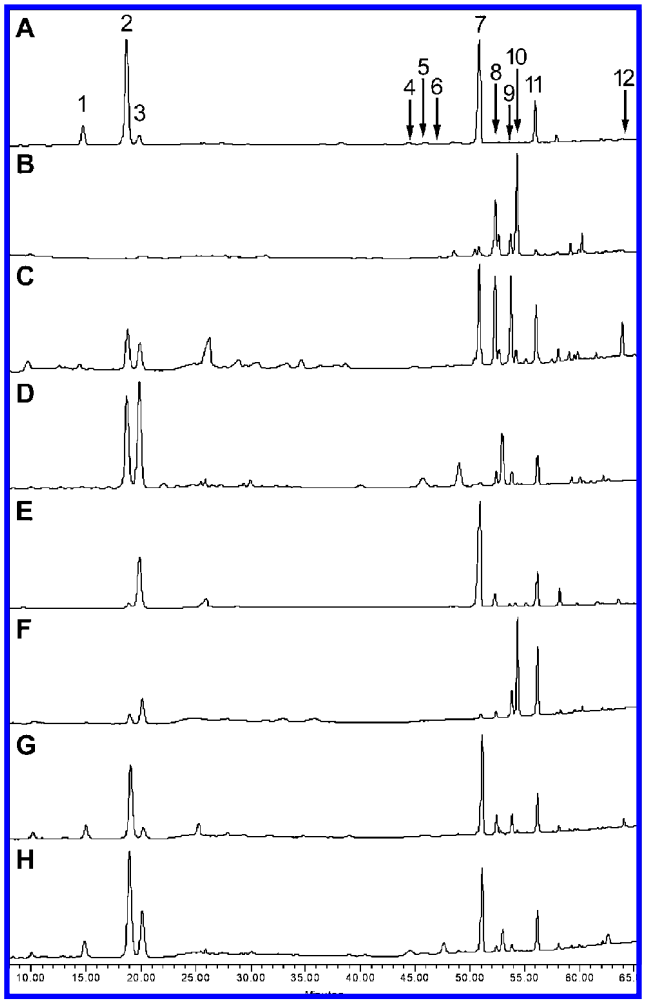

Figure 3. Typical chromatograms of extracts from (**A**) the root and (**B**) leaf of *Panax notoginseng*; (**C**) the root and (**D**) leaf of *Panax ginseng*; (**E**) the root and (**F**) leaf of *Panax quinquefolius*; and a mixture of *Panax notoginseng* root with (**G**) *Panax ginseng* root and (**H**) leaf. Saponin peaks: notoginsenoside R1 (1) and ginsenosides Rg1 (2), Re (3), Rh1 (4), Rg2 (5), 20*R*-Rg2 (6), Rb1 (7), Rc (8), Rb2 (9), Rb3 (10), Rd (11), and Rg3 (12).

conditions in this study was focused on the separation of ginsenosides Rh1, Rg2, and 20*R*-Rg2.

The first 20 min of elution, beginning with 17.5% acetonitrile and 82.5% water and then changing to 21% acetonitrile, was for the separation of notoginsenoside R1 and ginsenosides Re and Rg1. The percentage of acetonitrile was then increased to separate ginsenosides Rh1, Rg2, and 20*R*-Rg2. As shown in **Figure 2**, when the composition of the eluting solvents was kept at 28% acetonitrile and 72% water beginning at 23 min, ginsenosides Rh1 and Rg2 could not be separated. At a composition of 27% acetonitrile and 73% water, ginsenosides Rh1 and Rg2 began to separate, but with low separation efficiency. When the composition of acetonitrile was changed to 26%, baseline separation for Rh1 and Rg2 was achieved. Small changes in the composition of eluting solvents influenced the separation of ginsenosides Rh1 and Rg2 noticeably.

Validation of Analytical Method. The calibration curves for all 12 saponins showed good linearity ($R^2 > 0.9990$) in the concentration ranges of 1−200 μg/mL for ginsenosides Rb2, Rc, Rd, Rg2, 20*R*-Rg2, Rh1, and Rg3 and notoginsenoside R1 and 2−400 μg/mL for ginsenosides Rb1, Rb3, Re, and Rg1.

The precision of the HPLC method was determined for intra- and interday variations. A notoginseng root extract was weighed and dissolved in methanol. The intraday variability was

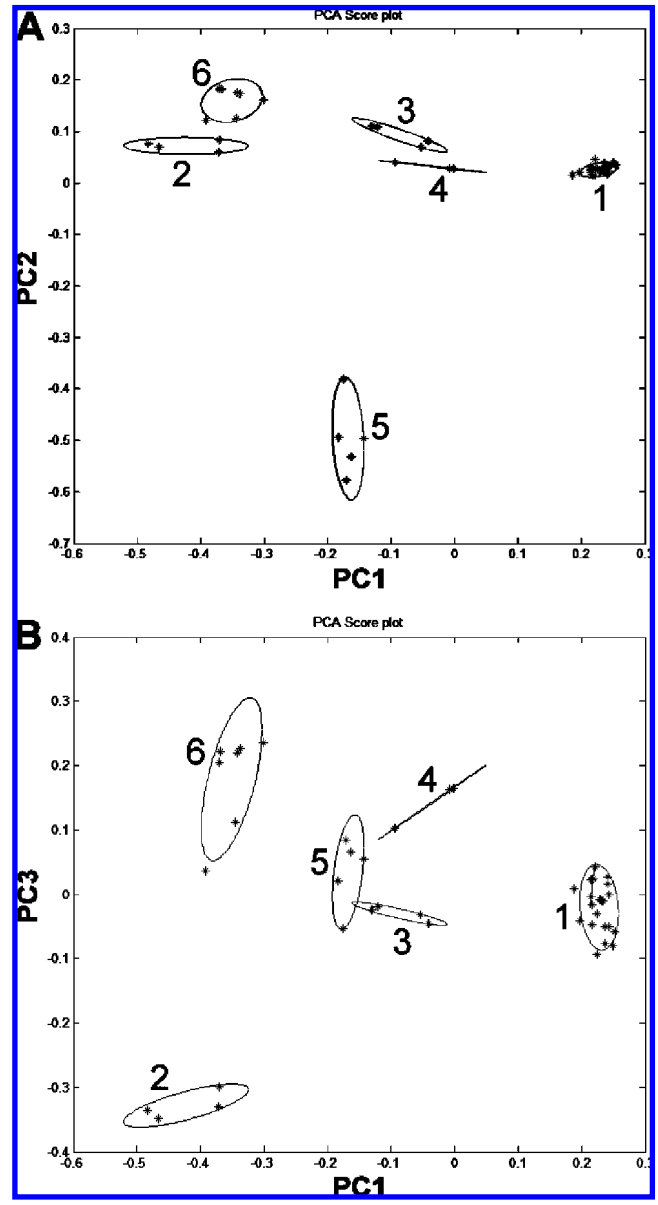

Figure 4. Principal component analysis (PCA) plots with (**A**) principal components 1 and 2 and (**B**) principal components 1 and 3, using contents of 12 saponins as input data. The 12 saponins are notoginsenoside R1, ginsenosides Rg1, Re, Rh1, Rg2, 20*R*-Rg2, Rb1, Rc, Rb2, Rb3, Rd and Rg3. The group numbers of *Panax* extracts are listed in **Table 1**.

performed five times on the same extract prepared on a single day. The interday reproducibility was determined by analyzing the samples on three separate days. The validation studies showed overall intra- and interday variations (RSD) of less than 9.2 and 11.0%, respectively.

The percentage difference between amounts determined and spiked was considered to be a measure of accuracy. Various amounts of 12 saponins (2 and 1 mg) were added to 10 mg of notoginseng extract and then prepared as a test solution. The determination was performed in triplicate, and the average recoveries and relative standard deviation (RSD) were calculated. An accuracy of >87.1% was obtained for each of the analytes tested, and the RSD was <11.5%.

Constituent Analysis of Samples. The HPLC method was subsequently applied to determine the 12 saponins in the botanical extracts simultaneously (**Figure 3**). **Table 2** shows typical analytical results of extracts from root, rootlet, corm,

Table 2. Typical Saponin Content (Milligrams per Gram) in *Panax* Extracts

extract		R1	Rg1	Re	Rh1	Rg2	20*R*-Rg2	Rb1	Rc	Rb2	Rb3	Rd	Rg3
P. notoginseng	root	60.2[a]	299.8	33.9	6.7	9.2	*b*	269.9	2.7	1.3	0.6	70.3	0.5
	rootlet	65.7	250.1	30.8	9.0	8.3		175.2	1.6	0.4	0.7	34.3	0.6
	corm	71.4	270.7	33.6	3.9	6.7		243.5	0.8	7.5	0.7	66.8	0.6
	leaf	0.4	0.5	10.4		1.1		15.6	119.8	35.8	182.1	7.6	0.4
	flower	0.7	0.5	6.7		1.6		54.5	112.9	46.4	152.4	18.0	5.2
	berry		0.1	1.9		0.0		8.7	69.6	27.8	111.5	5.1	0.2
P. ginseng	root		33.6	27.8		3.8		64.5	62.0	52.4	7.9	30.1	0.5
	leaf		146.0	204.2	2.0	46.4		3.2	16.8	14.6	1.7	26.0	
	berry		16.7	212.6	4.1	76.6	0.4	5.2	32.9	42.2	8.0	100.5	0.6
P. quinquefolius	root		14.0	197.9				341.8	34.2	4.6	6.8	65.0	0.6
	leaf		32.8	97.5	0.4	3.1	0.6	8.3	16.2	54.5	204.8	101.6	0.7
	berry		1.4	68.7	0.3	2.2		5.5	30.5	70.0	245.7	47.6	1.5

[a] The data are the average of duplicates from one extract of each plant part. [b] Blank cell, trace content (<0.1 mg/g) or not detected.

leaf, flower, and berry of notoginseng and root, leaf, and berry of Asian ginseng and American ginseng. Although the HPLC method has been used to identify ginseng saponins (*18*, *19*, *22*), the results of the comparison studies on the different plant parts of the *Panax* species are not sufficient. Our HPLC data supplied comprehensive information. The quantitative HPLC assay, however, could not identify notoginseng root extract from that adulterated with its aerial parts or other *Panax* plant parts (**Figure 3G**,**H**). Therefore, it was necessary to introduce an additional method to identify notoginseng root extract.

PCA Assay. To evaluate the similarity/diversity of notoginseng root extract and adulterated extract, we used a PCA assay. PCA assay was performed on the data set obtained from the HPLC chromatogram. Twelve characteristic saponins, notoginsenoside R1 and ginsenosides Rg1, Re, Rh1, Rg2, 20*R*-Rg2, Rb1, Rc, Rb2, Rb3, Rd, and Rg3, were chosen to build a 12-dimensional data set, which represents 12 vectors; the contents are the values of the vectors. The data were normalized to reduce the effect of signal strength, which corresponded to a different concentration in each sample. As shown in **Figure 4**, all samples were classified into one of six groups: (1) notoginseng underground parts; (2) notoginseng aerial parts; (3) Asian ginseng underground parts; (4) Asian ginseng aerial parts; (5) American ginseng underground parts; and (6) American ginseng aerial parts. The points of the notoginseng rootlet and corm extracts were in the area of notoginseng root extracts, suggesting that because of high similarity of chemical constituents, either the rootlet or corm of notoginseng can be used as root.

To simplify data management, array optimizations were performed. In this evaluation, some characteristic saponins were "removed" from the array to make a smaller one. Fewer vectors simplified data collection requirements for model building. Because the content of ginsenosides Rh1, Rg2, 20*R*-Rg2, and Rg3 in the different extracts were relatively low, they may be removed from the array. A subset of eight saponins, notoginsenoside R1 and ginsenosides Rg1, Re, Rb1, Rc, Rb2, Rb3, and Rd, was chosen to build the PCA model. This array performed separation as good as that with the 12-peak array and simplified the mode (**Figure 5**).

We also selected as few as four saponins, with a subset of notoginsenoside R1 and ginsenosides Rg1, Re, and Rc, for PCA assay, which improved separation among different groups. This set separated the groups with more scatter of the notoginseng underground group. The first two components (PC1 + PC2) kept variance information at 95%. The third component contributed little to the separation. Data obtained from this study suggest that the set of eight saponins described above is the better choice for the simplified mode.

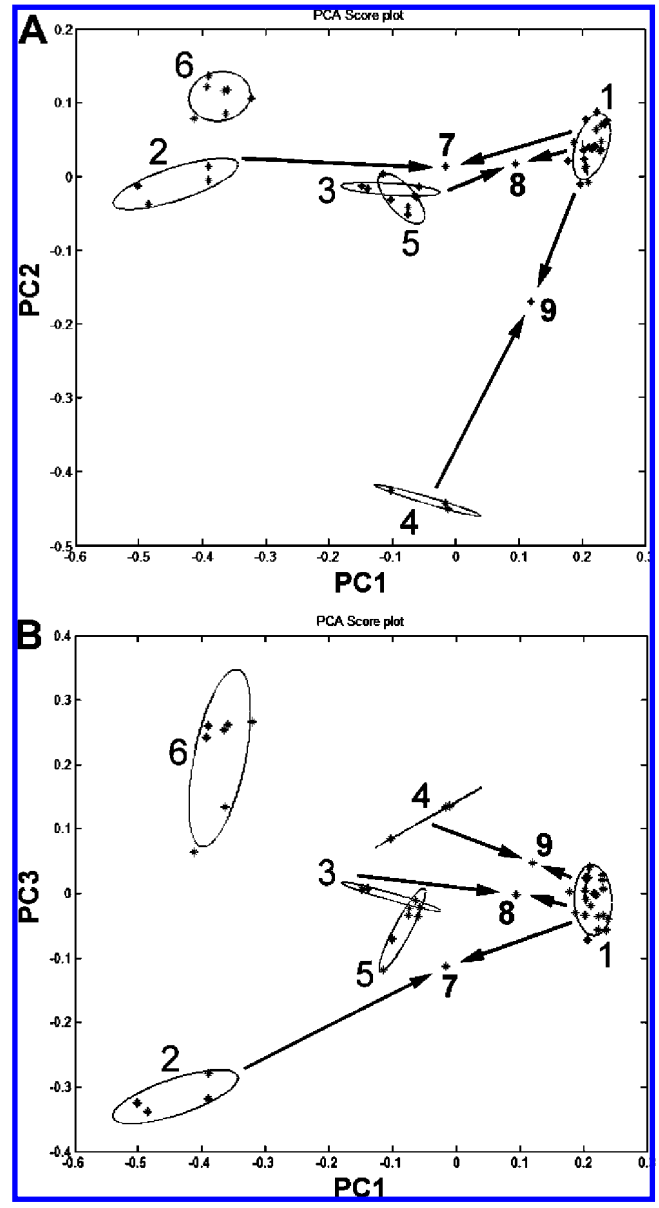

Figure 5. Principal component analysis (PCA) plots with (**A**) principal components 1 and 2 and (**B**) principal components 1 and 3, using contents of 8 saponins as input data. The 8 saponins are notoginsenoside R1 and ginsenosides Rg1, Re, Rb1, Rc, Rb2, Rb3, and Rd. The group numbers of *Panax* extracts are listed in **Table 1**. Arrows indicate groups 7−9 were extracted from mixed plant parts.

Byproducts of notoginseng, such as leaf and flower, and other species in the genus *Panax*, such as Asian ginseng root and leaf, are cheaper than notoginseng root; hence, commercially designated "notoginseng extract" may have been extracted from these other plant resources. Moreover, the pesticide concentration in the leaf extract could be high. Former identification methods were unsatisfactory because of the similarities of chemical constituents between notoginseng extracts and its adulterants. This HPLC-PCA method may identify adulterations in notoginseng such as notoginseng leaf or other ginsengs mixed with notoginseng root before extraction. Using the PCA method, we tried to identify the adulterations. As shown in **Figure 5**, when notoginseng root is mixed with notoginseng leaf (1:1), it can be separated from notoginseng root extract (point 7 in **Figure 5**). Separation was also good when notoginseng root was mixed with the root or leaf of Asian ginseng (1:1) (points 8 and 9 in **Figure 5**). Separation was similar when notoginseng root was mixed with American ginseng root or leaf before extraction, although the possibility of adulteration with American ginseng is low, given that American ginseng is a product of North America. Data obtained from this study suggest that use of our HPLC-PCA method can accurately identify adulterants added to notoginseng root such as notoginseng leaf, Asian ginseng root or leaf, or American ginseng root or leaf. This method ensures the safe use of notoginseng root extract.

The HPLC fingerprint is a powerful method for the identification of constituents in botanical extracts and has been used with many herbal medicines (*23*), avoiding the time-consuming isolation of compounds to be identified (*14*, *24*). Because both Asian and American ginseng are commonly used herbs that are taxonomically related to notoginseng, the differentiation of extracts of notoginseng from Asian or American ginseng is difficult. Our study supplies a new method for distinguishing notoginseng root extract from its adulterants.

In summary, the present study proposes a quantitative HPLC method for the analysis of 12 saponins in extracts from the root and other plant parts of *P. notoginseng*, *P. ginseng*, and *P. quinquefolius*. Our data showed comprehensive results for various plant parts of the commonly used species in the genus *Panax*. PCA was applied to obtain information about the different extracts in a data set of chromatographic fingerprints. A subset of eight saponins, notoginsenoside R1 and ginsenosides Rg1, Re, Rb1, Rc, Rb2, Rb3, and Rd, was distinguished. Notoginseng root extract can be separated from its aerial plant parts and from Asian or American ginseng. With this method, adulteration of the extract from notoginseng root by other ginseng plant parts or species can be revealed. The HPLC-PCA method supplies both a quantitative assay and qualitative identification. It could be a critical reference for the future evaluation of notoginseng products.

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