



Research article

Investigating chemical features of *Panax notoginseng* based on integrating HPLC fingerprinting and determination of multiconstituents by single reference standard



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ARTICLE INFO

Article history:

Received 6 February 2017

Accepted 17 April 2017

Available online 21 April 2017

Keywords:

chemical profile
geographical region
HPLC fingerprinting
multivariate analysis
Panax notoginseng

ABSTRACT

Background: *Panax notoginseng* is a highly valued medicine and functional food, whose quality is considered to be influenced by the size, botanical parts, and growth environments.

Methods: In this study, a HPLC method integrating fingerprinting and determination of multiconstituents by single reference standard was established and adopted to investigate the chemical profiles and active constituent contents of 215 notoginseng samples with different sizes, from different botanical parts and geographical regions.

Results: Chemical differences among main root, branch root, and rotten root were not distinct, while rhizome and fibrous root could be discriminated from other parts. The notoginseng samples from Wenshan Autonomous Prefecture and cities nearby were similar, whereas samples from cities far away were not. The contents of major active constituents in main root did not correlate with the market price.

Conclusion: This study provided comprehensive chemical evidence for the rational usage of different parts, sizes, and growth regions of notoginseng in practice.

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1. Introduction

Panax notoginseng (Burk.) F. H. Chen, commonly called notoginseng, or *Sanqi* or *Sanchi* in Chinese, is primarily produced in southwest of China, which is highly valued for its hemostatic and cardiovascular protective effects [1,2]. In east Asia, especially China, notoginseng has been historically consumed as a medicine and functional food [3,4]. Additionally, various notoginseng products are available in the health food market in the USA as dietary supplements [5,6].

Underground parts of *P. notoginseng* are the main portions for usage, which include rhizome, main root, branch root, and fibrous root (Fig. 1A) [7]. Both root and rhizome (*Notoginseng Radix et Rhizoma*) are officinal in the Pharmacopoeia of the People's Republic of China. Branch root and fibrous root are often used as the raw materials of Chinese tonic soup, or ground to yield powder as health food. Because of the specific growing conditions, notoginseng is vulnerable to root rot during planting [8,9]. The rotten

root is called *Chouqi* in Chinese, and is considered to be of inferior quality and sometimes adulterated into the normal raw materials. The quality of rotten root has rarely been studied, not to mention its comparison with the normal underground parts of notoginseng.

Notoginseng primarily grows in Wenshan Autonomous Prefecture of Yunnan Province, China [10]. In Wenshan Autonomous Prefecture, the southwestern counties, such as Wenshan County, Yanshan County, Maguan County, and Qubei County, are the most important notoginseng-producing areas. As the demand continues to increase, the notoginseng production in Wenshan Autonomous Prefecture is insufficient, and several areas near Wenshan Autonomous Prefecture, such as Honghe Autonomous Prefecture, Kunming City, and Yuxi City, also cultivate this herb. The geographical distribution of *P. notoginseng* is illustrated in Fig. 1B. Notoginseng is not only the product of the specific genome [11], and the chemical composition is strongly influenced by the growing conditions such as terrain, soil and climate [12]. The

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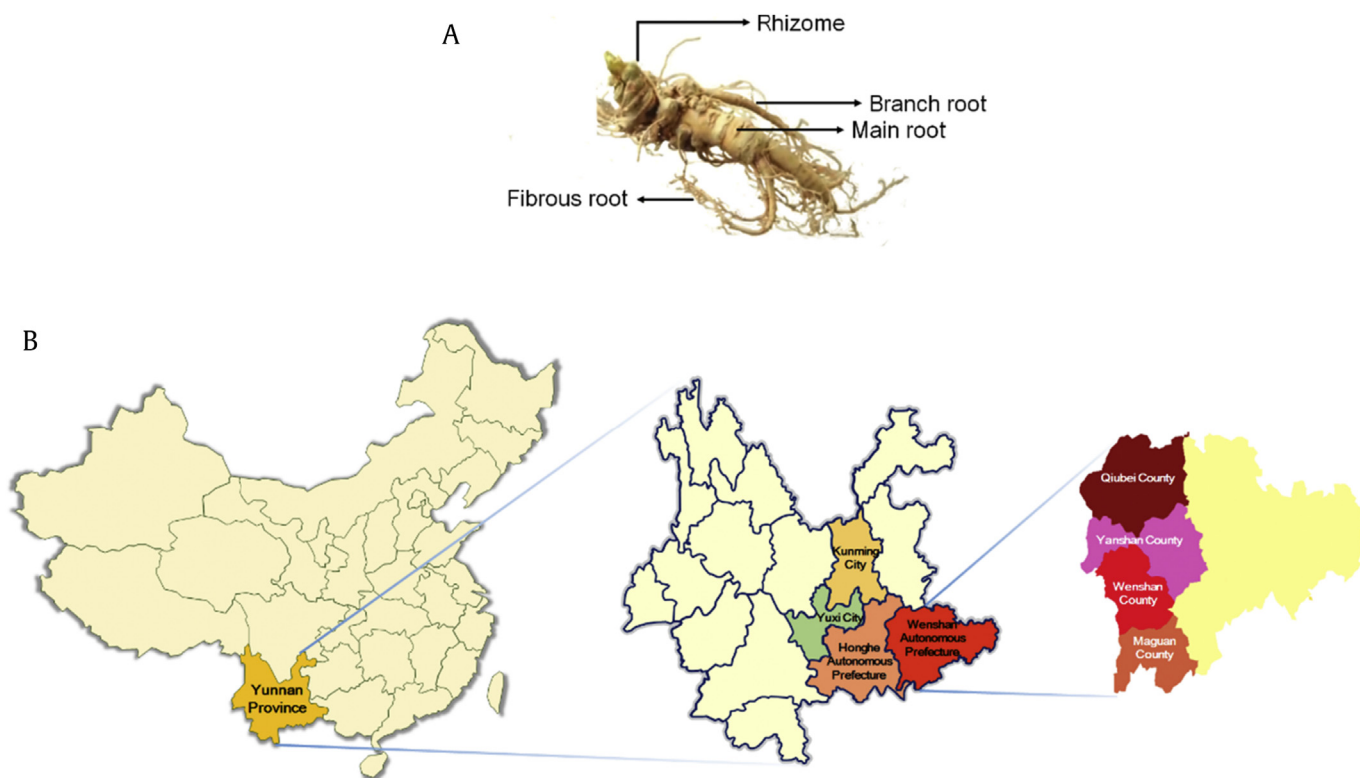


Fig. 1. Underground parts and geographical distribution of *Panax notoginseng*. (A) Underground parts of *P. notoginseng* include rhizome, main root, branch root, and fibrous root. (B) *P. notoginseng* primarily grows in Wenshan Autonomous Prefecture (right) of Yunnan Province (middle) in China (left), and the regions investigated in this study were highlighted.

qualities of notoginseng derived from different geographical origins should be investigated comprehensively.

The size of main root is usually considered as an indication of its quality, which also greatly affects its price at the market. The size of main root is called *Tou*, which means the number of individual dry main roots in 500 g. At the market, the price of 20 *Tou* main root is about 2-fold higher than that of 60 *Tou* on December 12, 2016 (Fig. S1). Whether the main root of large size deserves the high price should be assessed.

The chemical constituents in notoginseng are closely related to its quality, thus it is rational to assess the quality of notoginseng by investigating chemical composition of raw materials. Due to the multicomponent, multitarget synergistic action of notoginseng [13,14], quantification of the bioactive constituents is a key way to assess the quality of raw materials used. In notoginseng, saponins are thought to play the major role in its health function and therapeutic effect [15–17]. Several methods have been established to determine the contents of saponins in *P. notoginseng*. Jia et al. [7] established a method for determination of 11 saponins in 1-, 2- and 3-yr-old main root, rhizome and fibrous root of notoginseng by HPLC–diode array detection. Wan et al. [18] developed a HPLC–evaporative light scattering detection method for determining eight major saponins in root, fiber root, rhizome, stem, leaf, flower and seed of notoginseng. Wang et al. [19] established a method to determine eight saponins in different parts of notoginseng using UPLC–quadrupole–time of flight mass spectrometry. Peng et al. [20] developed an HPLC–charged aerosol detector method for the analysis of saponin contents in notoginseng. Among these saponins determined, notoginsenoside R₁ (NG-R₁), ginsenoside Rg₁ (G-Rg₁), ginsenoside Re (G-Re), ginsenoside Rb₁ (G-Rb₁), and ginsenoside Rd (G-Rd) were regarded as the major active constituents [21]. In these studies, multiple reference standards are needed for quantitative determination of these

constituents with routine methods, i.e., external standard method. However, shortage, cost and instability of chemical reference standards are issues that should be considered [22,23]. With this limitation in mind, determination of multiconstituents by single reference standard (DMS) is an outstanding solution. DMS is a quantitative analysis method for simultaneous determination of a number of constituents using only one reference standard, which is also mentioned as single standard to determine multi-components (SSDMC) or quantitative analysis of multi-component with single marker (QAMS) [24–27]. Wang et al. [28] established the DMS method for quantitative determination of saponins in notoginseng; however, little attention was paid to the minor constituents in notoginseng. To comprehensively reflect the quality of notoginseng, an ideal quality assessment should include not only quantitative information of contents of major active constituents from DMS, but also the information about minor constituents. Fingerprinting could identify as many constituents as possible, thus is a practical method to obtain information of minor constituents. In our previous study, chromatographic and spectroscopic fingerprints of notoginseng were developed [29]. It is urgently needed to integrate these two methods to investigate the quality of notoginseng holistically.

In this study, an integrated method combining HPLC fingerprinting and DMS was developed to determine the contents of bioactive constituents and the chemical profiles, and notoginseng samples with different sizes, from different botanical parts and different geographical regions were analyzed. The contents of major active constituents and HPLC fingerprinting coupled with multivariate analysis were used to investigate the quality of different notoginseng samples in term of chemical constituents. To the best of our knowledge, this is the most comprehensive report on chemical features of underground parts of *P. notoginseng*, and accomplished with the largest sample size. This study provides

comprehensive chemical evidence for the rational usage of different raw materials of notoginseng in practice.

2. Materials and methods

2.1. Chemicals and reagents

Notoginsenoside R₁ (NG-R₁) and ginsenoside Rg₁ (G-Rg₁), ginsenoside Re (G-Re), ginsenoside Rb₁ (G-Rb₁), ginsenoside Rd (G-Rd) (purity >99%) were purchased from Ronghe Pharmaceutical Technology Development Co. Ltd. (Shanghai, China). HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Distilled water was purified by a Milli-Q system (Millipore).

2.2. Sample preparation

A total of 215 *P. notoginseng* samples were collected from different regions of Yunnan Province in China (Fig. 1B). More specifically, these samples included rhizome ($n = 78$), main root ($n = 119$), branch root ($n = 6$), fibrous root ($n = 7$), and rotten root ($n = 5$). The materials were authenticated by Associate Professor Liurong Chen, College of Pharmaceutical Sciences, Zhejiang University.

The sample was ground using a disintegrator and then passed through a sieve (hole diameter, 280 μm). A total of 0.5 g powdered sample was extracted ultrasonically in 40 mL of 70% methanol (v/v) for 60 min. The mixture was then filtered and the filtrate was evaporated to dryness in vacuum. The residue was redissolved, transferred to a 5-mL volumetric flask, and then diluted to this volume with 70% methanol. The solution was filtered through a 0.22- μm filter membrane before HPLC analysis.

2.3. HPLC fingerprinting

The HPLC fingerprinting was performed on an Agilent 1100 HPLC system (Agilent, USA) consisting of a quaternary solvent delivery system, an online degasser, an autosampler, a column temperature controller and an ultraviolet detector. The chromatographic separation was carried out with reference to our previous study [29]. Briefly, the separation was performed on an Agilent Zorbax C18 column (4.6 mm \times 50 mm, 1.8 μm) at a solvent flow rate of 0.8 mL/min at 35°C. Water and acetonitrile were used as mobile phases A and B, respectively. The solvent gradient adopted was as follows: 0–22 min, 17–19% B; 22–30 min, 19–27% B; 30–35 min, 27% B; 35–47 min, 27–46% B; 47–70 min, 46–90% B. The detection wavelength was 203 nm and the injection volume was 3 μL .

All peaks in the chromatograms were integrated and analyzed by the professional software Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A, Chinese Pharmacopoeia Commission, China). The SIMCA-P 12.0 (Umetrics, Sweden) was used to perform the multivariate analysis.

2.4. Determination of multiconstituents by single reference standard

The chromatographic conditions for determination of the five major bioactive saponins in notoginseng by single reference standard were the same as that of HPLC fingerprinting in Section 2.3. G-Rb₁, one of the major saponins in notoginseng, was selected as the reference standard. G-Rb₁ served as the external standard to determine the content of G-Rb₁, and as the internal standard to

Table 1
Linear regression data of the five saponins

Compound	Calibration curve	Linearity range (mg/mL)	Correlation coefficient (r)
NG-R ₁	$y = 851.5137x + 1.8752$	0.0505–4.0392	0.9994
G-Rg ₁	$y = 811.0652x + 21.8680$	0.0982–7.8568	0.9993
G-Re	$y = 836.0123x - 2.0903$	0.0297–2.3780	0.9996
G-Rb ₁	$y = 590.5959x + 52.3356$	0.1031–8.2468	0.9991
G-Rd	$y = 762.3572x + 23.9033$	0.0512–4.0972	0.9992

simultaneously determine the rest four major bioactive saponins in notoginseng according to the conversion factors (F_x).

The F_x of the rest four major bioactive saponins could be obtained with solutions of reference standards and calculated via Eq. (1). With the value of F_x , the concentration of the saponin (c_x) could be obtained via Eq. (2).

$$F_x = \frac{c_x/A_x}{c_r/A_r} \quad (1)$$

$$c_x = \frac{A_x \times c_r \times F_x}{A_r} \quad (2)$$

in which c_x and A_x represent the concentration and peak area of the analyte, c_r and A_r represent that of the reference standard.

Standard method difference (SMD) [30] was applied to evaluate the DMS method and the external standard method, which was calculated via Eq. (3).

$$\text{SMD} = \frac{(c_{ES} - c_{DMS})}{c_{ES}} \times 100\% \quad (3)$$

where c_{ES} and c_{DMS} represent the concentrations of an analyte determined by external standard method and DMS method, respectively.

3. Results and discussions

3.1. Method validation

The linearity of the quantitative analysis method was determined by spiking a series of mixed standard solutions with different concentrations. The calibration curves were constructed by plotting the peak areas (y) vs. the concentrations (x , mg/mL) of the five saponins. The results of calibration are summarized in Table 1. Within relatively wide ranges, all the compounds showed good linearity ($r \geq 0.999$). For intraday precision test, a sample solution was analyzed for six replicates within the same day, and for interday precision test, a sample was analyzed for 6 consecutive days. The intra- and interday precisions were 0.35–0.54% and 0.83–4.94%, respectively, which indicated an acceptable precision of the developed method. Repeatability was evaluated by analyzing six independent replicate preparations of a sample, and the relative standard deviation (RSD) values of the peak area of the five saponins were 1.49–1.72%, demonstrating that the developed method was of satisfactory repeatability. The stability test was performed by analyzing the same sample solution at different time intervals (0 h, 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h), and the RSD values of the peak area of the five saponins were 1.00–1.78%, which indicated that it was feasible to analyze samples within 24 h. The accuracy was validated by recovery rate through the standard addition method. The samples were spiked with a known amount (high, medium, and low levels) of standard solution, and then analyzed with the established HPLC method. The results showed that the mean

recovery rates of the five saponins were in the range of 95.77–104.85% with RSDs < 4.80%, which demonstrated that the developed method had acceptable accuracy.

G-Rb₁ was selected as internal reference standard, because it was easy to obtain, low cost, and stable. The conversion factors were calculated via Eq. (2) in Section 2.4, and the conversion factors of NG-R₁, G-Rg₁, G-Re, and G-Rd were 0.73, 0.78, 0.74, and 0.79, respectively. Taguchi design was used to evaluate the stability of the DMS method. According to the Taguchi's L₉ (3³) design, nine experiments were conducted under various conditions of three factors, namely HPLC instruments systems (Agilent 1100, Agilent 1260, and Waters 2695), analytical columns (SB-C18, Eclipse Plus C18, and Extend-C18) and concentrations of analytes (high, middle, and low), as listed in Table S1. Signal-to-noise (S/N) was introduced to evaluate the influence of these factors to conversion factors.

As shown in Table 2, analysis of variance revealed that all of these three factors, i.e., HPLC instruments systems, columns and concentrations of analytes, did not significantly fluctuate the conversion factors. Although the conversion factors were inevitably influenced under different analytical conditions, the variation was acceptable. Thus, in a certain range, this DMS method was robust enough to be applied in different conditions.

SMD, which represented the difference between the results assayed by external standard method and DMS, were used to evaluate the accuracy of the DMS. SMDs of DMS were below 5% for NG-R₁, G-Rg₁, G-Re, and G-Rd, which implied that the DMS method had a high accuracy in comparison with external standard method and could be used as the substitutive method in the quantitative determination of multiple active constituents.

3.2. Comparison of different parts of notoginseng

Underground parts of *P. notoginseng* are the main portions for usage, and individual parts are used separately. Rhizome and root are both officially approved for the medicinal usage. However, there are still different applications of these two parts, for example, rhizome and main root are the raw materials of Chinese medicine *Xuesaitong* [31] and *Xueshuantong* [32], respectively. Branch root and fibrous root are mainly used for producing health food and food supplement. Due to the special growing environment, notoginseng is easily given rise to various diseases. Among the commonly seen diseases, root rot is a vital one and is thought to harm notoginseng

mostly [33]. Rotten root with poor appearance is available in the market at a low price, and even sometimes adulterated into the normal raw materials. To investigate the variation of the chemical features among different botanical parts of notoginseng, samples of rhizome, main root, branch root, fibrous root, as well as rotten root, were collected.

The representative HPLC chromatograms of rhizome, main root, branch root, fibrous root, and rotten root are displayed in Fig. 2. A total of 19 peaks were selected as the common peaks for further multivariate analysis. Principal component analysis (PCA) was adopted for the exploratory analysis. The first component of samples explained 47.2% of the systematic variation. The PCA score plot is displayed in Fig. 3A. The rhizome samples tended to cluster to the right part, while the other parts of notoginseng scattered on the left. Interestingly, there was no clear separation among main root, branch root, as well as rotten root samples. The fibrous root was mainly located at the higher left quarter, separated from other parts of samples to a certain extent. The result indicated that the chemical profiles of rhizome and fibrous root were different from other underground parts of notoginseng, while it was highly similar among that of main root, branch root and rotten root.

The quantitative results are shown in Fig. 3B. The contents of NG-R₁, G-Rg₁, G-Re, G-Rb₁, and G-Rd in rhizome samples were all higher than that in main root, branch root, fibrous root, and rotten root samples ($p < 0.05$). There were no significant differences in the contents of these five saponins between the main root and branch root samples. To get rid of the variation from diverse origin, growth environment, and harvest time, comparison was also carried out among samples collected from the same field with the same collection time, as shown in Fig. 4, and similar results were observed. As shown in Fig. 3B, the contents of NG-R₁, G-Rb₁, G-Rd, and the sum of the five saponins in main root were higher than that in fibrous root. The contents of G-Rd in rotten root were higher than that in main root, while G-Rg₁ and G-Rb₁ were lower than that in main root. The sum contents of these five saponins in rotten root were not different from that in main root, which agreed with the result from the chemical profile analysis.

Previous studies reported that the contents of saponins in different parts of *P. notoginseng* were in the order of rhizome > main root ≥ branch root > fibrous root [19], which was consistent with the results here. Differentiating main root, branch root, and fibrous root is mainly based on the diameter of the parts.

Table 2
Analysis of variance results of each factor on conversion factors

Compound	Source	DF	Seq SS	Adj SS	Adj MS	F	p
NG-R ₁	HPLC instruments	2	1015.5	1015.5	507.7	4.14	0.195
	Analytical columns	2	205.4	205.4	102.7	0.84	0.544
	Concentrations of analytes	2	319.7	319.7	159.9	1.3	0.434
	Residual error	2	245.2	245.2	122.6		
	Total	8	1785.9				
G-Rg ₁	HPLC instruments	2	1168	1168	584.01	6.98	0.125
	Analytical columns	2	210.1	210.1	105.07	1.25	0.443
	Concentrations of analytes	2	405.5	405.5	202.73	2.42	0.292
	Residual error	2	167.5	167.5	83.73		
	Total	8	1951.1				
G-Re	HPLC instruments	2	1421.41	1421.41	710.7	3.65	0.215
	Analytical columns	2	60.55	60.55	30.28	0.16	0.865
	Concentrations of analytes	2	154.84	154.84	77.42	0.4	0.716
	Residual error	2	389.48	389.48	194.74		
	Total	8	2026.29				
G-Rd	HPLC instruments	2	633.87	633.867	316.933	2.52	0.284
	Analytical columns	2	219.08	219.08	109.54	0.87	0.535
	Concentrations of analytes	2	1.87	1.869	0.934	0.01	0.993
	Residual error	2	251.86	251.863	125.932		
	Total	8	1106.68				

Adj SS, Adjusted sum of squares; Adj MS, Adjusted mean squares; DF, Degrees of freedom; Seq SS, Sequential sum of squares.

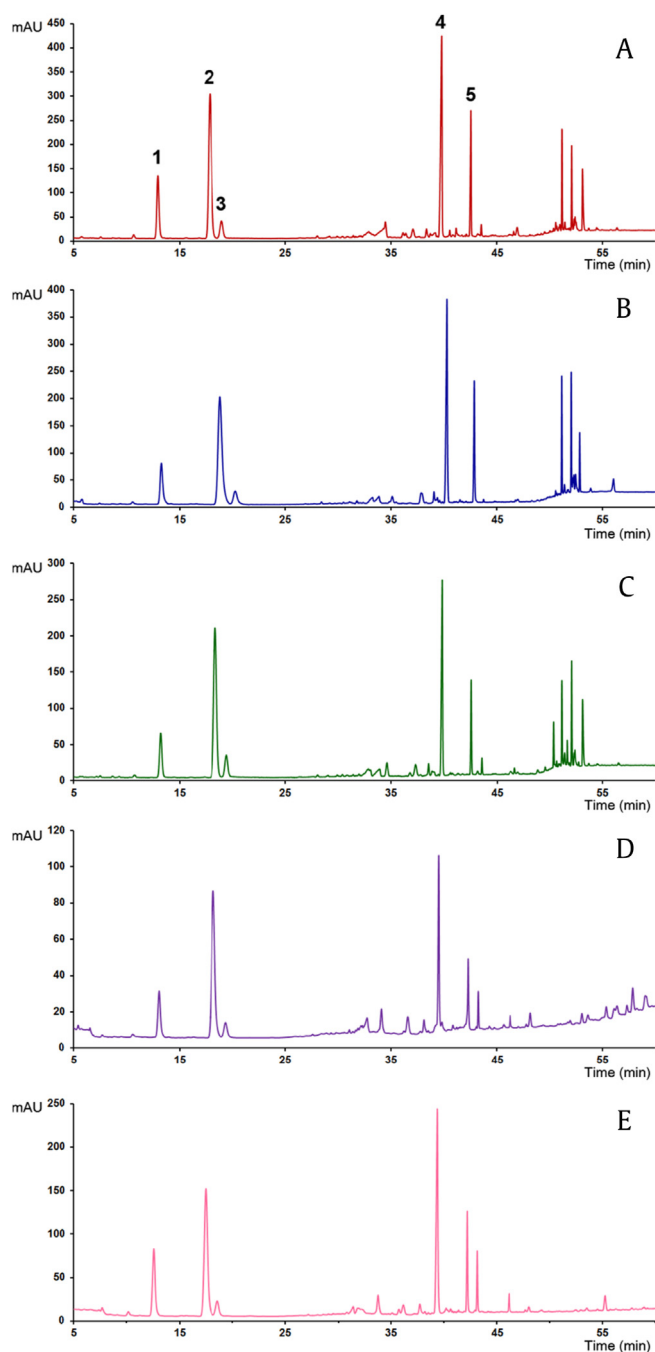


Fig. 2. Representative chromatographic fingerprints of different notoginseng samples: (A) rhizome, (B) main root, (C) branch root, (D) fibrous root, (E) rotten root. Peaks: notoginsenoside R₁ (1), ginsenoside Rg₁ (2), ginsenoside Re (3), ginsenoside Rb₁ (4) and ginsenoside Rd (5).

Within a certain range of the diameter, the chemical profiles of the parts will not vary dramatically. Previous studies about chemical information of rotten root were quite limited. It was interesting to find that the chemical profiles of rotten root could not be differentiated from that of main root, which indicated the high similarity of the chemical profiles between normal and rotten root. And the quantitation results also confirmed this opinion. In an early report, the saponins in rotten root had been contrasted with that in normal root collected from corresponding fields [34]. The contents of NG-R₁, G-Rg₁, and G-Rb₁ in rotten root ranged from 64% to 99% of that in normal root [34]. In view of these findings, rotten root should be

used rationally. For safety reasons, the rotten root might not be a suitable raw material for food or medicine; however, it might be proposed to be collected for manufacturing active constituents.

3.3. Comparison of notoginseng from different geographical regions

Geographical region is usually regarded as one of the most important factors that affects the quality of traditional Chinese medicines [35–37]. The traditional best cultivated region of herbs is called *Daodi* region in China [38]. The *Daodi* region of *P. notoginseng* is Wenshan Autonomous Prefecture of Yunnan Province, China, where over 90% of the worldwide total of this herb is produced. In Wenshan Autonomous Prefecture, the cultivated areas for *P. notoginseng* are mainly located in the southwestern parts [39]. Wenshan County, Yanshan County, Maguan County, and Qiubei County are all traditional cultivated regions of *P. notoginseng* in Wenshan Autonomous Prefecture. Main root and rhizome were collected from these four counties, and the PCA score plot is displayed in Fig. 5A and 5C. The main root from these four counties were superimposed with each other, which indicated that the chemical profiles of the main root from these four counties were not significantly different.

There were no significant differences among the contents of NG-R₁, G-Rg₁, G-Re, and G-Rd in the main root samples collected from these four counties, except the content of G-Rb₁ (the content of this saponin in main root from Wenshan County was lower than that from Maguan County and Qiubei County). The quantitation results agreed well with the results of PCA.

The rhizome samples from these four counties exhibited a similar chemical profile pattern as shown in Fig. 5C, and there was also no significant differences in the contents of these five saponins among the four counties.

Thus, there is no significant difference in the chemical profiles and the major active constituents' contents of rhizome and main root derived from traditional cultivated regions of *P. notoginseng* in Wenshan Autonomous Prefecture, including Wenshan County, Yanshan County, Maguan County and Qiubei County.

The demand for notoginseng, both for medicine and food, continues to increase. However, the cultivation of *P. notoginseng* needs appropriate soil, climate, and geographical conditions, and is also affected by continuous cropping obstacle [40,41]. The production of notoginseng in Wenshan Autonomous Prefecture cannot meet the enormous demands. Thus, several regions nearby, e.g. Honghe Autonomous Prefecture, Kunming City, and Yuxi City, begin to cultivate *P. notoginseng* [42]. A total of 89 batches of main root were collected from these four prefectures/cities. The PCA score plot is displayed in Fig. 5B. Samples of main root from Wenshan Autonomous Prefecture, Honghe Autonomous Prefecture and Kunming City were superimposed with each other; however, samples from Yuxi City were located on the lower left away from other three prefectures/cities.

The quantitation results are shown in Fig. S2. The contents of G-Rb₁ and the sum of these five saponins in Wenshan Autonomous Prefecture were higher than that in Kunming City and Yuxi City. The levels of G-Rg₁ and G-Rd in Wenshan Autonomous Prefecture were higher than in Yuxi City. The contents of NG-R₁ in Honghe Autonomous Prefecture were higher than that in other three prefectures/cities.

As shown in Fig. 5D, the chemical profile pattern of rhizome samples from Wenshan Autonomous Prefecture, Honghe Autonomous Prefecture and Kunming City were similar, while samples from Yuxi City were located left lower of the PCA score plot. The contents of the five saponins in rhizome samples from Wenshan Autonomous Prefecture, Honghe Autonomous Prefecture and

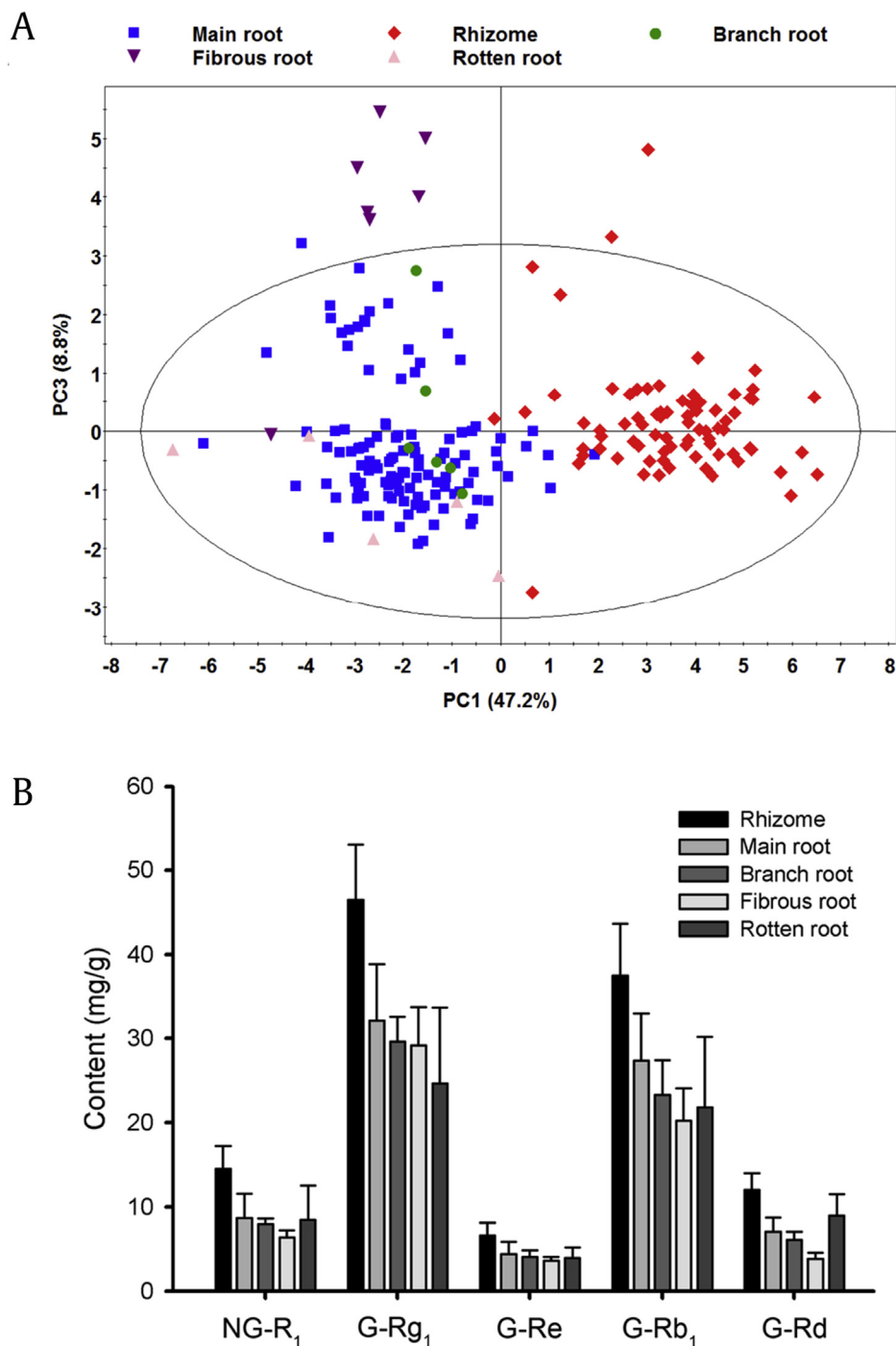


Fig. 3. Principal component analysis scores plot of HPLC fingerprint data (A) and contents of major active constituents (B) of rhizome, main root, branch root, fibrous root, and rotten root of *Panax notoginseng*.

Kunming City were not significantly different from each other, which was in line with the results from PCA.

Thus, within a certain range, there is no significant difference in the chemical profiles and the major active constituents' contents of rhizome and main root among adjacent geographical regions. When beyond the range, the difference could not be ignored.

3.4. Comparison of notoginseng with different sizes

Size is an important parameter for the commercial grades of the main root, which is thought to be associated with its quality [43].

However, the correlation between size and chemical profile has not yet been investigated. The main root samples collected were classified to three parts. Samples larger than 40 *Tou* were treated as large group, smaller than 60 *Tou* as small group, and 40-60 *Tou* as middle group. The PCA score plot is displayed in Fig. 6A. The samples of large, middle, and small groups were superimposed with each other, which indicated that the chemical profiles of main root with different sizes were similar to a certain degree. In the quantitative assays, the contents of NG-R₁ and G-Rd in large main root samples were higher than those in the small group, while G-Rg₁, G-Re, and G-Rb₁ were not significantly different among different sizes (Fig. 6B).

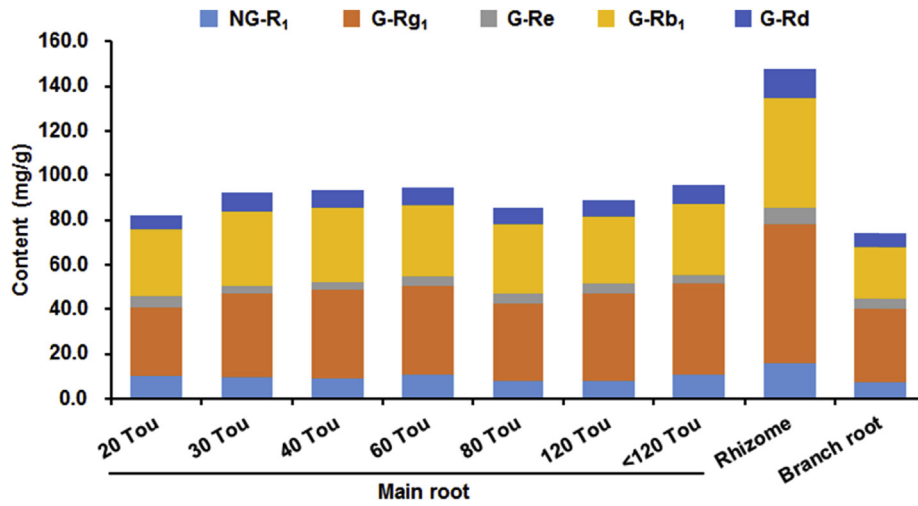


Fig. 4. Contents of major active constituents in raw material samples of *Panax notoginseng* from the same field with the same collection time. The raw material samples included rhizome, branch root, and main root of 20 Tou, 30 Tou, 40 Tou, 60 Tou, 80 Tou, 120 Tou, and <120 Tou.

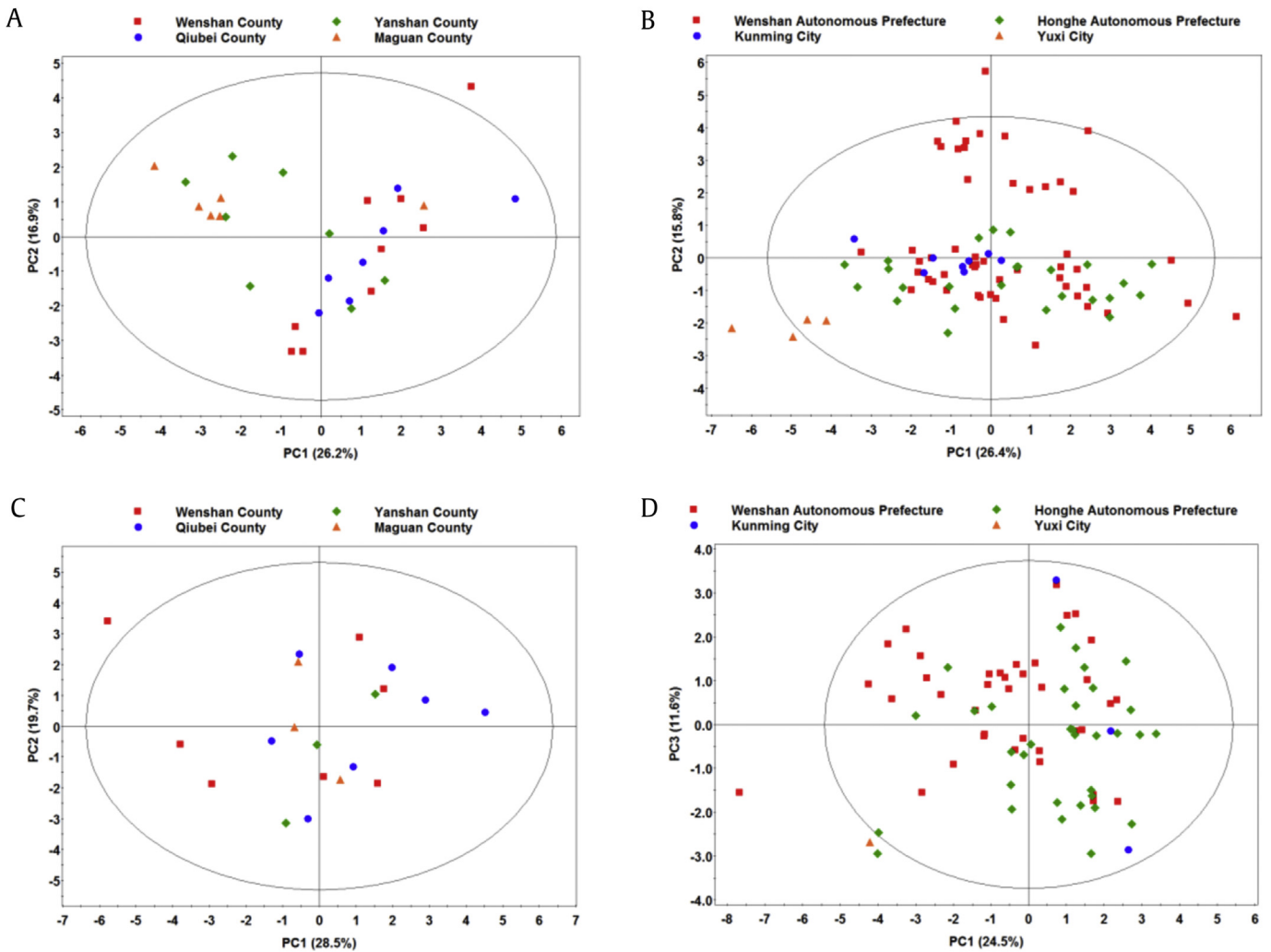


Fig. 5. Principal component analysis scores plot of HPLC fingerprint data. Main root (A) and rhizome (C) from different counties in Wenshan Autonomous Prefecture, and main root (B) and rhizome (D) from different cities in Yunnan Province.

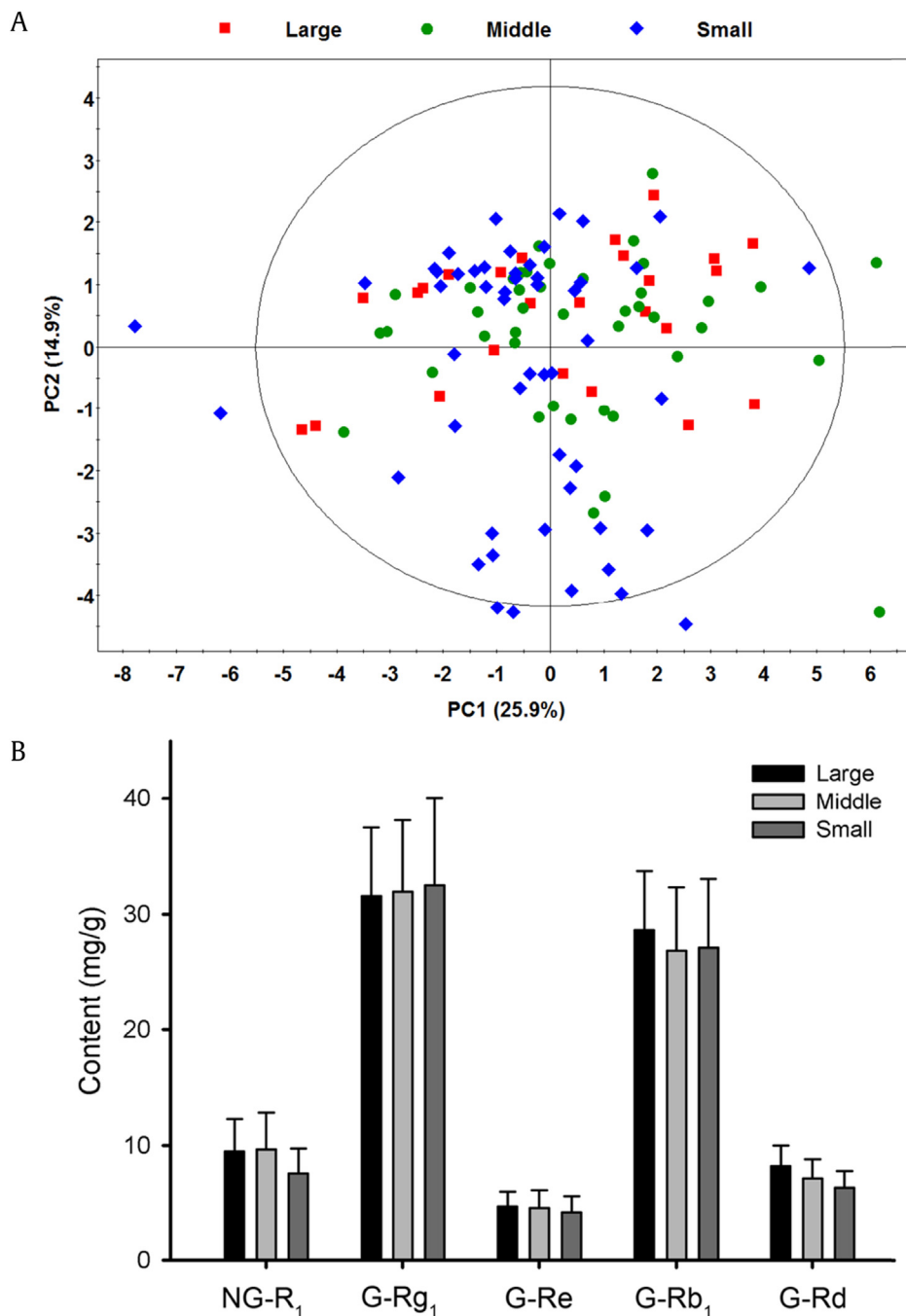


Fig. 6. Principal component analysis scores plot of HPLC fingerprint data (A) and contents of major active constituents (B) of main root with different sizes.

In main root of different *Tou*, collected from the same field at the same harvest time, the contents of these five saponins were compared. Among main root of 20 *Tou*, 30 *Tou*, 40 *Tou*, 60 *Tou*, 80 *Tou*, 120 *Tou*, and <120 *Tou*, as shown in Fig. 4, the variation was insignificant.

Thus, there is a certain degree of variation of the chemical profiles when the size of main root enlarges. This observation was consistent with previous studies [39]. However, it should be mentioned that the chemical profiles and the contents of these major active constituents in main root with different sizes did not show a good correlation with the corresponding market price (Fig. S1).

4. Conclusions

An HPLC fingerprinting and DMS method was established and used to investigate the chemical profiles and active constituent contents of notoginseng samples with different sizes, from different parts and different geographical regions. The chemical feature of rhizome was different from other underground parts of notoginseng. There was no significant difference in the chemical constituents of rhizome/main root from Wenshan Autonomous Prefecture, Honghe Autonomous Prefecture, and Kunming City. Among different sizes of main root, the chemical constituents varied, but did not correlate with the market price. This study

provided comprehensive chemical evidence for the differentiated uses and exploitations of individual notoginseng parts in practice.

Conflicts of interest

The authors declare no competing financial interests.

Acknowledgments

This study was financially supported by National standardization of traditional Chinese medicine (TCM) project (No. ZYBZH-C-HLJ-18).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jgr.2017.04.005>.

References

- [1] Wang LC, Zhang WS, Liu Q, Li J, Aolga RN, Liu K, Liu BL, Li P, Qi LW. A standardized notoginseng extract exerts cardioprotection by attenuating apoptosis under endoplasmic reticulum stress conditions. *J Funct Foods* 2015;16:20–7.
- [2] Wang D, Liao P, Zhu H, Chen K, Xu M, Zhang Y, Yang C. The processing of *Panax notoginseng* and the transformation of its saponin components. *Food Chem* 2012;132:1808–13.
- [3] Yang Y. Scientific substantiation of functional food health claims in China. *J Nutr* 2008;138: 1199S–205S.
- [4] Chen L, Tai WCS, Hsiao WLW. Dietary saponins from four popular herbal tea exert prebiotic-like effects on gut microbiota in C57BL/6 mice. *J Funct Foods* 2015;17:892–902.
- [5] Sun S, Wang C, Tong R, Li X, Fishbein A, Wang Q, He T, Du W, Yuan CS. Effects of steaming the root of *Panax notoginseng* on chemical composition and anticancer activities. *Food Chem* 2010;118:307–14.
- [6] Rhule A, Navarro S, Smith JR, Shepherd DA. *Panax notoginseng* attenuates LPS-induced pro-inflammatory mediators in RAW264.7 cells. *J Ethnopharmacol* 2006;106:121–8.
- [7] Jia X, Wang C, Liu J, Li X, Wang X, Shang M, Cai S, Zhu S, Komatsu K. Comparative studies of saponins in 1–3-year-old main roots, fibrous roots, and rhizomes of *Panax notoginseng*, and identification of different parts and growth-year samples. *J Nat Med* 2013;67:339–49.
- [8] Guan H, Zhang Y, Chen Y, Yang J, Sun S. On the connections between *sanchi* root rot and edaphon dynamics in no-tillage *sanchi*-planted soil. In: International conference on computer distributed control and intelligent environmental monitoring (CDCIEM). IEEE; 2011. p. 1598–602.
- [9] Xia P, Guo H, Zhao H, Jiao J, Deyholos MK, Yan X, Liu Y, Liang Z. Optimal fertilizer application for *Panax notoginseng* and effect of soil water on root rot disease and saponin contents. *J Ginseng Res* 2016;40:38–46.
- [10] Wang D, Li H, Chen K, Zhang Y, Yang C. HPLC comparative analysis of ginsenoside saponins indifferent underground parts of *Panax notoginseng*. *Acta Botanica Yunnanica* 2005;27:685–90.
- [11] Hong DYQ, Lau AJ, Yeo CL, Liu XK, Yang CR, Koh HL, Hong Y. Genetic diversity and variation of saponin contents in *Panax notoginseng* roots from a single farm. *J Agr Food Chem* 2005;53:8460–7.
- [12] Ma J, Mi YH, Li QW, Chen L, Du LJ, He LZ, Lei M. Reduction, methylation, and translocation of arsenic in *Panax notoginseng* grown under field conditions in arsenic-contaminated soils. *Sci Total Environ* 2016;550:893–9.
- [13] Efferth T, Koch E. Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. *Curr Drug Targets* 2011;12: 122–32.
- [14] Yang ZZ, Liu W, Zhang F, Li Z, Cheng YY. Deciphering the therapeutic mechanisms of *Xiao-Ke-An* in treatment of type 2 diabetes in mice by a Fangjiomics approach. *Acta Pharmacol Sinic* 2015;36:699–707.
- [15] Ng TB. Pharmacological activity of *sanchi* ginseng (*Panax notoginseng*). *J Pharm Pharmacol* 2006;58:1007–19.
- [16] Liu JJ, Wang YT, Qiu L, Yu YY, Wang CM. Saponins of *Panax notoginseng*: chemistry, cellular targets and therapeutic opportunities in cardiovascular diseases. *Expert Opin Investig Drugs* 2014;23:523–39.
- [17] Kim DH. Chemical diversity of *Panax ginseng*, *Panax quinquefolium*, and *Panax notoginseng*. *J Ginseng Res* 2012;36:1–15.
- [18] Wan JB, Yang FQ, Li SP, Wang YT, Cui XM. Chemical characteristics for different parts of *Panax notoginseng* using pressurized liquid extraction and HPLC-ELSD. *J Pharmaceut Biomed* 2006;41:1596–601.
- [19] Wang J, Yau L, Gao W, Liu Y, Yick P, Liu L, Jiang Z. Quantitative comparison and metabolite profiling of saponins in different parts of the root of *Panax notoginseng*. *J Agr Food Chem* 2014;62:9024–34.
- [20] Peng M, Zhang T, Ding Y, Yi YX, Yang YJ, Le J. Structure-based prediction of CAD response factors of dammarane-type tetracyclic triterpenoid saponins and its application to the analysis of saponin contents in raw and processed *Panax notoginseng*. *RSC Adv* 2016;6:36987–7005.
- [21] Wang L, Li Z, Shao Q, Li X, Ai N, Zhao X, Fan X. Dissecting active ingredients of Chinese medicine by content-weighted ingredient–target network. *Mol Bio-Syst* 2014;10:1905–11.
- [22] Da J, Cheng CR, Yao S, Long HL, Wang YH, Khan IA, Li YF, Wang QR, Cai LY, Jiang BH, et al. A reproducible analytical system based on the multi-component analysis of triterpene acids in *Ganoderma lucidum*. *Phytochemistry* 2015;114:146–54.
- [23] Pan WD, Yang LX, Feng WH, Lin LM, Li C, Liu WW, Gan GF, Fan JH, Zou JG, Wang ZM, et al. Determination of five sesquiterpenoids in *Xingnaojing* injection by quantitative analysis of multiple components with a single marker. *J Sep Sci* 2015;38:3313–23.
- [24] Song Y, Wang Z, Zhu J, Yan L, Zhang Q, Gong M, Gao H, Feng W, Wang W. Assay of evodiol, evodiamine and rutaecarpine in *Fructus evodiae* by QAMS. *China J Chinese Materia Med* 2009;34:2781–5.
- [25] Hou JJ, Wu WY, Da J, Yao S, Long HL, Yang Z, Cai LY, Yang M, Liu X, Jiang BH, et al. Ruggedness and robustness of conversion factors in method of simultaneous determination of multi-components with single reference standard. *J Chromatogr A* 2011;1218:5618–27.
- [26] Gao XY, Jiang Y, Lu JQ, Tu PF. One single standard substance for the determination of multiple anthraquinone derivatives in rhubarb using high-performance liquid chromatography–diode array detection. *J Chromatogr A* 2009;1216:2118–23.
- [27] Gao W, Wang R, Li D, Liu K, Chen J, Li HJ, Xu X, Li P, Yang H. Comparison of five *Lonicera* flowers by simultaneous determination of multi-components with single reference standard method and principal component analysis. *J Pharmaceut Biomed* 2016;117:345–51.
- [28] Wang CQ, Jia XH, Zhu S, Komatsu K, Wang X, Cai SQ. A systematic study on the influencing parameters and improvement of quantitative analysis of multi-component with single marker method using notoginseng as research subject. *Talanta* 2015;134:587–95.
- [29] Zhu J, Fan X, Cheng Y, Agarwal R, Moore CMV, Chen ST, Tong W. Chemometric analysis for identification of botanical raw materials for pharmaceutical use: a case study using *Panax notoginseng*. *PLoS One* 2014;9:e87462.
- [30] Kuang GJ, Zhou J, Yao MC, Tan QL, Qi LK, Liang JH, Li Y, Zhao ZX, Zhang L. Systematic study on QAMS method for simultaneous determination of triterpenoid saponins in *Ilex pubescens* by HPLC and UPLC. *Anal Method* 2015;7: 6579–87.
- [31] Yao H, Shi PY, Shao Q, Fan XH. Chemical fingerprinting and quantitative analysis of a *Panax notoginseng* preparation using HPLC-UV and HPLC-MS. *Chin Med* 2011;6:8.
- [32] Wang XM, Wang SX, Wang JX, Guo H, Dong ZP, Chai LJ, Hu LM, Zhang Y, Wang H, Chen L. Neuroprotective effect of *Xueshuantong* for injection (lyophilized) in transient and permanent rat cerebral ischemia model. *Evid Based Compl Alt* 2015;2015:1–13.
- [33] Zheng YK, Miao CP, Chen HH, Huang FF, Xia YM, Chen YW, Zhao LX. Endophytic fungi harbored in *Panax notoginseng*: diversity and potential as biological control agents against host plant pathogens of root-rot disease. *J Ginseng Res* 2017;41:353–60.
- [34] Sun Y, Ke J, Ma N, Chen Z, Wang C, Cui X. Effects of root rot on saponin content in *Panax notoginseng*. *J Chinese Med Mater* 2004;27:79–80.
- [35] Bondia-Pons I, Savolainen O, Torronen R, Martinez JA, Poutanen K, Hanhineva K. Metabolic profiling of Goji berry extracts for discrimination of geographical origin by non-targeted liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Food Res Int* 2014;63:132–8.
- [36] Qiu YQ, Lu X, Pang T, Zhu SK, Kong HW, Xu GW. Study of traditional Chinese medicine volatile oils from different geographical origins by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC × GC-TOFMS) in combination with multivariate analysis. *J Pharmaceut Biomed* 2007;43:1721–7.
- [37] Li H, He JX, Li FJ, Zhang ZJ, Li RR, Su JC, Zhang JD, Yang B. Application of NIR and MIR spectroscopy for rapid determination of antioxidant activity of *Radix Scutellariae* from different geographical regions. *Phytochem Analysis* 2016;27: 73–80.
- [38] Zhao ZZ, Guo P, Brand E. The formation of *daodi* medicinal materials. *J Ethnopharmacol* 2012;140:476–81.
- [39] Dong TTX, Cui XM, Song ZH, Zhao KJ, Ji ZN, Lo CK, Tsim KWK. Chemical assessment of roots of *Panax notoginseng* in China: Regional and seasonal variations in its active constituents. *J Agr Food Chem* 2003;51:4617–23.
- [40] Zheng D, Wang L, Ou X, Guo L, Hao Q, Liu D, Xiao Y. Comparison of agronomic traits of *Panax notoginseng* between traditional cultivated fields and new cultivated fields. *China J Chinese Materia Med* 2014;39:558–65.
- [41] Sun XT, Li L, Long GQ, Zhang GH, Meng ZG, Yang SC, Chen JW. The progress and prospect on consecutive monoculture problems of *Panax notoginseng*. *Chinese J Ecol* 2015;34:885–93.
- [42] Cui XM, Huang LQ, Guo LP, Liu DH. Chinese *sanqi* industry status and development countermeasures. *China J Chinese Materia Med* 2014;39:553–7.
- [43] Shan LM, Zhao YL, Hong W, Zhang P, Kong WJ, Liu ZG, Xiao XH. Correlation analysis between hemostatic activity and commercial grades of *Panax notoginseng*. *Chinese Tradit Herbal Drugs* 2011;42:1779–82.