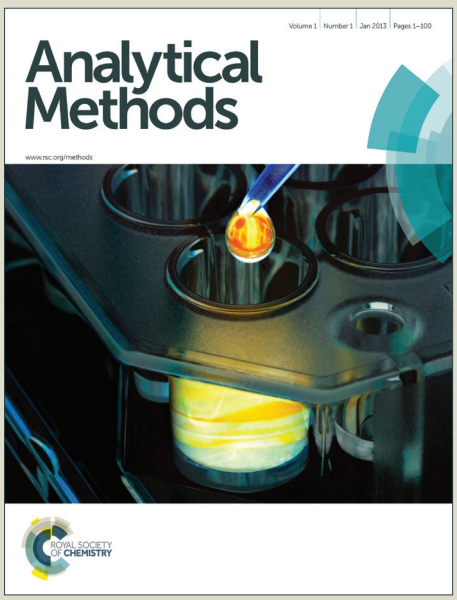
Analytical Methods

Accepted Manuscript



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A correlation model of UPLC fingerprint and anticoagulant activity for quality assessment of *Panax notoginseng* by hierarchical clustering analysis and multiple linear regression analysis

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Running title: UPLC fingerprint and anticoagulant activity of *Panax notoginseng*

Abstract

Panax notoginseng (Burk.) F.H. Chen (commonly known as Tianqi or Sanqi) is a famous traditional Chinese medicine and has been widely used for treatment in cardio- and cerebro-vascular diseases. However, the quality assessment of Sanqi is quite difficult because of ingredients complexity. In this work, the chemical fingerprints of 15 batches of *P. notoginseng* from different sources were investigated by ultra-performance liquid chromatography (UPLC). Fourteen common peaks in these samples were detected including 20(S)-protopanaxatriol saponins (PTS) and 20(S)-protopanaxadiol saponins (PDS). These samples were divided into three clusters by hierarchical clustering analysis (HCA). Cluster I and III possessed stronger anti-coagulation effects than cluster II. Multiple linear regression analysis (MLRA) showed that notoginsenoside R1, ginsenoside Rb1, ginsenoside Rd and an unknown compound might be the major effective compoounds for the quality control of Sanqi. PDS (Rb1, Rd, and the unknown compound) possesses a stronger anticoagulant activity than PTS (R1 only). This conjecture was confirmed by the dose–effect relationship evaluation of PDS and PTS. This study provides a general correlation model of fingerprint and efficacy relationship for the quality control of Sanqi and other TCMs.

Keywords: *Panax Notoginseng*; fingerprint–efficacy; quality control; ultra-performance liquid chromatography; anticoagulant activity; saponins

1. Introduction

Traditional medicine (TM) plays an important role in public health-care. Almost 80% of the population in Africa, Asia, and Latin America rely on TMs to meet their primary health-care needs[1]. Traditional Chinese medicine (TCM) is the part and parcel of TM. Although the TCM industry has developed rapidly in recent years, several issues on quality have not been addressed. Quality control of TCM evaluates only a few ingredients, provides vague descriptions of complex TCM compositions, and ignores synergic actions among different ingredients[2]. The chromatographic fingerprint can reflect the total complex compositions of herbal medicines, and it has been accepted as a strategy for quality assessment of TCM[3]. Chromatographic fingerprinting technique was also introduced by the World Health Organization for assessment of natural products[4]. However, chromatographic fingerprinting only contains the information of chemical substances, but hardly provide the efficacy of TCM[5]. The fingerprint–efficacy relationship provides us a more powerful way for TCM quality assessment. The relationship between fingerprint and efficacy has been established to determine the main active components by chemo-metric methods[6]. These years, the fingerprint–efficacy has been applied to discover the principal components of TCM for quality evaluation and control [7, 8].

Panax notoginseng (Burk) F.H. Chen, commonly known as Tianqi or Sanqi, is a highly valued and important Chinese medicinal herb produced mainly in Yunnan Province. *P. notoginseng* has been widely used in China to stop internal and external bleeding, reduce swelling and pain, disperse blood clots, eliminate blood stasis, and

promote blood circulation because of its hemostatic and cardiovascular effects [9]. Modern pharmacological studies have demonstrated that *P. Notoginseng* possess anti-carcinogenic[10] and hepatoprotective properties[11], as well as protective effects on the cardiovascular and cerebrovascular systems[12]. The main bioactive ingredients of this herb have been thought to be the dammarane-type *P. notoginseng* saponins, including protopanaxadiol (PDS), protopanaxatriol (PTS), and other low-abundance groups[13]. In previous studies, saponins in *P. notoginseng* have been identified as the main components responsible for the anticoagulation activity[14]. However, it is still unclear that which ingredient plays a leading role. Therefore, it is important to elucidate the relationship between fingerprint and efficacy, determine the main active ingredients in *P. notoginseng* fingerprints and establish a reliable method for quality assessment.

In this work, an appropriate UPLC was applied to establish fingerprints of *P. notoginseng* from various sources. The anticoagulant activity of these samples was analyzed by PT assay. A correlation model of UPLC fingerprint and anticoagulant activity was proposed by multiple linear regression analysis (MLRA) for quality assessment of *P. notoginseng.*

2. Materials and methods

2.1. Materials and reagents

A total of 16 batches of *P. notoginseng* samples from various sources in Yunnan Province were authenticated by Professor Yuejin Zhang (College of Life Science,

Northwest A&F University). The *P. notoginseng* samples are numbered and listed in Table 1.

The standards were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R. China). The standards include notoginsenoside R1, ginsenoside Rg1, Rb1, and Rd. Methanol and acetonitrile for ultra-performance liquid chromatography (UPLC) were purchased from Merck (Darmstadt, Germany). Deionized (ultra-pure) water was prepared by using a Millipore Milli Q-Plus system (Millipore, Bedford, MA, USA).

Warfarin sodium was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dade Ci-Trol Coagulation Control level 1 was purchased from Dade Behring Marburg Gmbh (Marburg, Germany). The prothrombin time (PT) assay kit was purchased from Steellex Science Instrument Corporation (Beijing, P.R. China).

All other chemicals in this study were of analytical grade and were obtained from standard sources.

2.2. UPLC fingerprints

2.2.1. Preparation of sample and standard solution

After pulverizing and sifting through a sieve (pore diameter: 0.3 mm), the 15 samples (20 g) from various sources were extracted with 70% methanol (200 mL) by ultrasonication for 20 min. After filtration, each residue was re-extracted with 70% methanol. The combined filtrates from each sample were then evaporated to dryness. The residue was then reconstituted with 70% methanol (100 mL) for the UPLC analysis.

The mixed standard solutions of the four saponins (R1: 0.124 mg/mL; Rg1: 0.6162 mg/mL; Rb1: 0.589 mg/mL; Rd: 0.1468 mg/mL) were prepared with methanol.

2.2.2. UPLC conditions

All samples were analyzed by using the Waters Acquity UPLC system (Waters, MA, USA), which comprises a binary solvent manager, sampler manager, column compartment, and photodiode-array detector connected to the Waters Empower 3 software. An Acquity UPLC BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm; Waters, MA, USA) was also used. The standards and samples were separated by using a gradient mobile phase consisting of water (A) and acetonitrile (B). The gradient conditions[15] were as follows: 0 to 0.2 min, 2% B; 0.2 to 2.0 min, 25% B; 2.0 to 2.5 min, **25**% B; 2.5 to 4.0 min, 35% B; 4 to 5.7 min, 60% B; 5.7 to 7.5 min, 80% B; 7.5 to 9.3 min, 90% B; 9.3 to 10.5 min, 98% B; 10.5 to 11.0 min, 2% B; 11.0 to 13.0 min, 2% B.

The temperatures of the column and sample injector were maintained at 45 and 15 °C, respectively. The flow rate was set at 0.4 mL/min, and the injection volume was 2 μL. The detection wavelength was set at 203 nm.

All standard and sample solutions were filtered through a 0.22 μm Millipore membrane prior to use. After filtration, the solutions were injected directly into the LC system for analysis.

2.2.3. Method validation

Method validation was performed in conformity to the ICH Harmonised Tripartite

Guideline (2006) on precision, repeatability, and stability.

2.2.4. Similarity analysis

The UPLC chromatogram profiles of congeneric samples from different origins were similar. The similarity degrees of these samples should first be evaluated by similarity analysis, which has been compulsorily performed by the SFDA of China[16]. Thus, the fingerprints of 15 batches of *P. notoginseng* were established and matched automatically by the Similarity Evaluation System for Chromatographic Fingerprint of TCM (Version 2004 A Chinese Pharmacopoeia Committee, Beijing, China). The simulative mean chromatogram, as a representative standard chromatogram for the 15 fingerprints, was calculated and generated automatically by this software by using the median method. Similarities between the entire chromatographic profiles of the 15 *P. notoginseng* samples and the reference chromatogram were also calculated by using the same software.

2.2.5. Hierarchical clustering analysis (HCA)

Clustering is the art of grouping pattern vectors that belong together because of similar characteristics. Clustering provides a visual representation of complex data and a method for measuring similarity between experiments. The similarity and dissimilarity between samples (objects) are usually represented in a dendrogram for ease of interpretation. An object is similar to other objects within its group but is different from those in other groups with respect to a predetermined selection criterion[17]. The HCA of 15 *P. notoginseng* samples was performed by SPSS statistics software (SPSS 17.0 for Windows, SPSS Inc., USA) based on the

between-groups linkage method and squared Euclidean distance.

2.3. In vitro PT assay

PT assay experiments [18] were performed by using an LG-PABER-I platelet-aggregation factor analyzer (Steellex Science Instrument Corporation, Beijing, China).

Approximately 100 mL of 70% methanol solutions from each sample were evaporated to dryness. The residue of each sample was then reconstituted and diluted with phosphate buffered saline (PBS; pH 7.4; 0.01 M) to a concentration of 20 mg/mL for the in vitro experiments.

The lyophilized pooled human plasma (Ci-Trol level 1) was freshly reconstituted with water. Plasma mixtures were prepared by mixing 200 μL of plasma with 100 μL of each test sample, as well as a positive control (5 mg/mL, warfarin sodium) and a negative control (PBS only), before performing the PT assay.

Each plasma mixture (50 μL) was incubated at 37 °C for 180 s. The reaction was initiated by adding 100 μL of thromboplastin. The test was then started immediately. The reactions were performed in four concurrent repetitions, which were recorded and averaged.

The data from the in vitro PT assay were analyzed to detect statistical significant differences at the 0.05 probability level by using one-way ANOVA via SPSS statistics software (SPSS 17.0 for Windows, SPSS Inc., USA).

2.4. Multiple linear regression analysis (MLRA)

MLRA attempts to model the relationship between two or more variables and a response by fitting a linear equation to the observed data. The general purpose of performing MLRA is to learn about the relationship between several independent variables and a dependent variable. MLRA can be generally represented by the following form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \ldots + b_nX_n \quad (1)$$

where Y is the estimated value and represents the dependent variable; X_1, X_2, X_3, . . . , X_n are measures of uncorrelated variables that may help in estimating Y. For example, X_1 is the known score of the first independent variable, and X_2 is the known score of the second independent variable. The coefficient b_0 is the estimated constant, and b_1, b_2, b_3 . . . , b_n are the regression coefficients[19].

In this section, MLRA was applied to establish the fingerprint–efficacy relationship between the values of the peak area in UPLC fingerprints and the PT time of 15 *P. notoginseng* samples by using SPSS statistics software (SPSS 17.0 for Windows, SPSS Inc., USA) for finding the possible anticoagulation components.

2.5. Testing the "dose–response" relationships of PTS and PDS

The S14 sample (40 g) was extracted by using the same extraction procedure. The residue was then reconstituted and diluted with 200 mL of water. The solution was separated into two types of saponins, namely, 20(S)-PDS and 20(S)-PTS, by using macroporous resins after centrifugation[20]. The separation conditions were as follows: a glass column (50 cm × 2.2 cm i.d.) wet-packed with 15 g (dry weight) of selected D-101 resin. After adsorption equilibrium, the adsorbents were eluted by

gradient flushing with water followed by 20%, 30%, 40%, and 50% aqueous ethanol at a flow rate of 1 mL/min. According to the fingerprint obtained from UPLC, the PTS and PDS were in the 30% (E-30) and 50% (E-50) aqueous ethanol eluents, respectively. The PTS and the PDS were evaporated to dryness, and then reconstituted and diluted with PBS for the in vitro experiments.

Samples of the 30% aqueous ethanol eluent were evaporated to dryness, and then reconstituted and diluted with PBS to obtain residue concentrations of 5, 10, 15, 20, 25, 30, and 40 mg/mL. Samples of the 50% aqueous ethanol eluent and S14 extract were also prepared by using this procedure. Subsequently, the 21 samples, along with the positive and blank controls, were subjected to PT tests.

3. Results

3.1. UPLC fingerprint of P. notoginseng

3.1.1. UPLC fingerprint and similarity analysis

The results of the methodology validation showed that the relative standard deviation (RSD) was less than 1.73% for precision, 0.76% to 1.35% for repeatability, and 0.79% to 1.46% for stability. All results indicated that the developed methodology was applicable for establishing the UPLC fingerprint of *P. notoginseng* from various sources. The UPLC fingerprints of *P. notoginseng* methanol extracts from various sources were obtained under optimized conditions (Fig. 1a). The generated reference standard fingerprint is shown in Fig. 1b. Peaks existing in more than 10 chromatograms of *P. notoginseng* samples from different sources and with

good segregation from consecutive peaks were regarded as "common peaks." A total of 14 common peaks were found from the reference chromatogram by ultraviolet spectra and UPLC retention time comparisons (Fig. 1b).

However, differences still existed among the chromatograms of the samples from different sources. The discrepant normalized peak areas calculated by the Similarity Evaluation System for Chromatographic Fingerprint of TCM (Version 2004 A Chinese Pharmacopoeia Committee, Beijing, China) are shown in the supplementary table 1. The similarities of the 15 batches of *P. notoginseng* samples between the entire chromatographic profiles and reference fingerprints were evaluated. The correlation coefficients of the 15 samples were as follows: 0.994, 0.971, 0.977, 0.995, 0.995, 0.994, 0.995, 0.998, 0.998, 0.997, 0.993, 0.990, 0.992, 0.995, 0.993, and 0.995. The data showed that the differences of the correlation coefficients came from the different growing years and types of the samples. The data also showed the diversity of the fingerprints. However, the fingerprint differences among samples were difficult to explain clearly and concretely based only on the similarity data.

3.1.2. HCA

The HCA procedure can find the natural cluster of samples according to their fingerprint data; the calculated method is called the "linkage method." We conducted the average linkage and calculated the squared euclidean distance. The HCA result is shown in Fig. 2. The samples were divided into three clusters according to the distance. Cluster I comprised samples S1 (three years, spring), S15 (three years, spring), S14 (three years, spring), S5 (three years, spring), and S13 (three years, spring). Cluster II

was formed by samples S6 (two years, spring), S7 (four years, spring), S11 (three years, winter), S12 (two years, spring), S8 (four years, spring), S9 (four years, spring), S10 (three years, winter), and S4 (four years, spring). Cluster III consisted of samples S2 (three years, spring) and S3 (three years, spring). The results showed that S2 and S3 were firstly separated from the other samples. S3 was from Wensan area, where the best quality Sanqi was traditionally produced. S2 was from Pingyuan in Yanshan area, which is very close to Wenshan. Then, all the spring Sanqi within three years were separated from the others. The samples in Cluster I (S1, S15, S14, S5, S13) and Cluster III (S2 and S3) were all "three years, spring," whereas Cluster II (S6, S7, S11, S12, S8 S9, S10, and S4) were not "three years, spring," but "two years, spring," "three years, winter," and "four years, spring." It was indicated that producing area and harvest time were the principal influencing factor for Sanqi quality.

3.2. In vitro PT assay of P. notoginseng samples

The above results validated the similarity analysis between the entire chromatograms of *P. notoginseng* samples and the reference fingerprint. Thus, the differences of the constituents in different samples of *P. notoginseng* would lead to different efficacies. The anti-coagulation activity is one of the main effects of *P. notoginseng*. The PT assay was introduced in this section to evaluate the anti-coagulation activity of the *P. notoginseng* samples.

Our preliminary experiments showed that the anticoagulation effect was significantly different from the blank control group and warfarin positive control group when the concentration of *P. notoginseng* samples was more than 15 mg/mL.

Thus, the test concentration was set at 20 mg/mL to obtain a significant effect.

The effects of *P. notoginseng* samples on anticoagulation were evaluated by PT assay. Fig. 3 shows that effects of the different samples obtained from various sources were all significantly different compared with those of the control group ($P < 0.05$).The PTs of the samples collected in Cluster II, which was from 16.150 s to 17.975 s, were much shorter than that of the samples in the other two clusters (Fig. 3). The PTs of the samples in Clusters I and III were almost the same, and the samples in Cluster III showed slightly longer PTs than that in Cluster I. These differences were evaluated by one-way ANOVA, which showed that the mean difference between Cluster II and the other two clusters were 1.026 (I) and 1.341 (III), respectively. This result indicated that the samples in Cluster II possessed a significant difference from Clusters I and III, which shared almost no difference.

3.3. MLRA

The relationship between the 14 independent variables X_1, X_2, X_3, …, X_{14} (normalized common peak areas) and a dependent variable Y (PT values of samples) was evaluated by using the MLRA model. A value of r^2 is 0.829 and RMSE is 0.342. The *p* values of peak X_1 - X_{14} coefficients are: 0.017, 0.027, 0.003, 0.005, 0.010, 0.004, 0.011, 0.003, 0.008, 0.000, 0.007, 0.016, 0.019, 0.015.

The following equation was established according to the SPSS output:

$$Y = 0.362\,X_1 + 1.346\,X_2 + 0.013\,X_3 + 0.315\,X_4 - 0.102\,X_5 + 1.183\,X_6 - 0.186\,X_7 + 1.460\,X_8 + 0.464\,X_9 - 1.342\,X_{10} + 1.082\,X_{11} - 0.649\,X_{12} - 0.184\,X_{13} - 1.644\,X_{14}$$

This equation showed that the anticoagulation effect of *P. notoginseng* samples on prolonging PT had a close correlation with the 14 common peaks in the UPLC fingerprints, particularly with X_2, X_6, X_8, and X_{11}. The standardized coefficients of X_2, X_6, X_8, and X_{11} were 1.346, 1.183, 1.460, and 1.082, respectively, thus indicating that these peaks had significant influence on the PT value. Peak 2 (notoginsenoside R1), peak 6 (ginsenoside Rb1), peak 8 (ginsenoside Rd), and peak 11 (unknown compound) had a vital role in prolonging PT and might be the anticoagulation components of *P. notoginseng*. The areas (content) of peak 11 were small but had a large influence on anticoagulation effect. Conversely, peak 3 (ginsenoside Rg1), which had the largest peak area, contributed little to the anticoagulation effect of *P. notoginseng*. These results suggested that compounds with high contents might not be the main effective components of CMM. In addition, the chemical structure of compound 11 needs to be determined by other analytical methods.

3.4. The "dose–response" relationship of 20(S)-PTS and 20(S)-PDS

According to the retention time of these 14 common peaks and the structures of the defined components, we found that peaks 1 to 3 belong to PTS, whereas peaks 4 to 9 belong to PDS. The coefficients of these peaks showed that PDS played a more important role than PTS in the anticoagulation effect. This finding was inconformity with previous reports[14]. Thus, the evaluation of the anticoagulation effect of PTS and PDS from *P. notoginseng* was conducted in this section.

3.4.1. UPLC profiles of PDS and PTS

Fig. 4a shows the mixed standard UPLC profile under the optimized UPLC condition, in which the resolutions between notoginsenoside R1 (1) and ginsenoside Rg1 (2) and between ginsenoside Rb1 (3) and ginsenoside Rd (4) are 2.55 and 3.02, respectively. The main contents in the 30% and 50% aqueous ethanol eluents are shown in Fig. 4b. Compared with the retention times of the peaks in Fig. 4a, the peaks in Fig. 4b confirmed that PTS and PDS were in the 30% aqueous ethanol eluent (E-30) and 50% aqueous ethanol eluent (E-50), respectively.

3.4.2. PT assay of S14, PTS, and PDS

Each sample was tested for PT in four replications. The data were averaged to generate the "dose–response" curve shown in Fig. 5.

The PTs of each sample in this test increased with increasing dose concentration (Fig. 5). This result indicated that saponins in *P. notoginseng* had an obvious "dose–response" relationship in prolonging PT. Moreover, the PT of E-50 was always higher than that of E-30 from the lowest dose to the highest one, as well for the PT of S14 under a dose less than 25 mg/mL. This result showed that PDS had a more significant role than PTS in the anticoagulation properties of *P. notoginseng*, specifically in lower concentrations.

4. Discussion

The chromatographic fingerprinting technology of TCM has gradually been accepted in the world during the past years. Chromatographic fingerprinting is strongly recommended for the quality control of TCM because it appropriately

represents “chemical integrities.” Although HPLC is an effective method for the quality control of *P. notoginseng* and its products[21], UPLC offers higher peak capacity, greater resolution, better sensitivity, and higher analytic speed for saponins in *P. notoginseng*[22]. Therefore, the developed UPLC method was used in this study to achieve a stable and reproducible chemical fingerprint of *P. notoginseng*. According to the results, UPLC has better resolution, and only took 15 min to separate saponins in *P. notoginseng*. This analysis duration is 1/5 of the general analytic time of HPLC.

The correlation between the chemical constituents and pharmacologic actions of TCM is still unclear. Previous studies hardly show the efficacy of a TCM from its fingerprint. Therefore, the fingerprint–efficacy study has been proposed [23, 24]. The fingerprint–efficacy study combines the study of chemical components and pharmacologic actions through chemometrics to illustrate the curative effects of components and their correlations. Aside from the information of active components, the study also provides information on inactive or relatively toxic (opposite effect) components; these information are essential for the total quality control of TCM. TCM needs the quantitative determination of effective components and a limit test of the toxic components of a medicinal material [25]. Kong et al. [7] elucidated the relationship between the chemical fingerprint and anti-bacterial efficacy of artificial *Calculus bovis* by using chemometric methods. Their results showed that cholic acid, taurocholate sodium, hyodeoxycholic acid, and one unknown compound might be the corresponding anti-bacterial components. Moreover, the spectrum–effect

relationship between UPLC fingerprints and the anti-bacterial activities of *Rhizoma coptidis* were investigated by using canonical correlation analysis. The results showed that berberine, jateorrhizine, and palmatine might be the main anti-bacterial components [8]. In the current study, MLRA was conducted to combine components of the fingerprint and anticoagulation effect of *P. notoginseng* for the first time. The results showed a good linear relationship among the 14 common peaks and PT values of *P. notoginseng* samples. According to the coefficients of the common peaks, components with positive coefficients prolong the PT, whereas components with negative ones shorten the PT. This finding suggested that active (positive components), inactive, or even relatively toxic components (negative components) exist simultaneously. Therefore, MLRA reflected the quality of *P. notoginseng* more completely and accurately than other methods.

According to the MLRA results, notoginsenoside R1, ginsenoside Rb1, ginsenoside Rd, and an unknown compound might be the principal components responsible for the anticoagulation effect of *P. notoginseng*. Rb1 and Rd are the typical constituents of PDS, and 1 belongs to PTS. It indicated that anticoagulation effects of PDS was stronger than PTS. This result was also demonstrated by the results of the “dose–response” relationship of PTS and PDS, specifically under lower concentrations. This suggested that PDS is a potential novel anticoagulation drug. The effect should be further identified by in vivo test. However, previous experiments that were performed on animals (in vivo) such as rats and rabbits demonstrated that PTS played an important role in blood activation instead of

PDS[21]. However, the effects of PDS on in vivo anticoagulation have been seldom reported prior to this study. Previous studies have demonstrated that despite the hard absorption of PDS in the gastrointestinal tract, the pharmacokinetics of PPD, the metabolite of PDS, and the actual effective motif of several PPD-type ginsenosides after oral administration shows low oral bioavailability[26]. Similar to PDS, the metabolite of PTS (PPT) is the in vivo main active substance. However, the bioavailability of PPT is higher than that of PPD[27, 28]. This finding may be the reason that PTS has a more important role in blood activation than PDS. Therefore, if the lower bioavailability of PDS can be improved, its clinical use is possible. Jin et al.[29] developed cubic nanoparticles to deliver PPD, which could enhance the dissolution and permeation to improve oral bioavailability. Moreover, taking Rb1 orally with P-gp inhibitor is an effective way to improve the bioavailability of Rb1 [25]. Thus, these methods provide solutions for using PDS as a novel anticoagulation drug.

5. Conclusions

The chemical fingerprints of 15 batches of *P. notoginseng* from different sources were investigated by ultra-Performance liquid chromatography (UPLC). Fourteen common peaks in these samples were detected including 20(S)-protopanaxatriol saponins (PTS) and 20(S)-protopanaxadiol saponins (PDS). These samples were divided into three clusters by hierarchical clustering analysis (HCA). The “three years, spring type” possessed better anticoagulation effect. Multiple linear regression analysis (MLRA) showed that notoginsenoside R1, ginsenoside Rb1, ginsenoside Rd

and an unknown compound might be the major effective components for the quality control of Sanqi. PDS (Rb1, Rd, and the unknown compound) possessed a stronger anticoagulant activity than PTS (R1 only). This conjecture was confirmed by the dose–effect relationship evaluation of PDS and PTS. This study provided a general correlation model of fingerprint and efficacy for the quality control of Sanqi and other TCMs.

Acknowledgement

This material is based upon work funded by the Major Industrial Cluster Project in Shaanxi Province (2012KTCL02-07), National Natural Science Foundation of China under (No.81373908 and No.81403033), Science Foundation of Zhejiang Sci-Tech University (No.1204806-Y), Zhejiang Provincial Natural Science Foundation of China (No.LQ13C020003), Public Project of Technological Application in Zhejiang Province (No.2014C32108), and China Postdoctoral Science Foundation Funded Project (No.2014M551771). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors gratefully acknowledge Prof. David Warrington for providing help with the language.

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Fig. 1. (a) UPLC fingerprints of the 15 batches of *P. notoginseng* samples and (b) the reference chromatogram. The reference chromatogram was generated from the fingerprints of the 15 batches of artificial *P. notoginseng* samples by using the Similarity Evaluation System for Chromatographic Fingerprint of TCM with the median method.

Fig. 2. Dendrogram showing the HCA results for the chemical fingerprints of the 15 batches of *P. notoginseng*. This result was obtained by the SPSS statistics software (SPSS 17.0 for Windows, SPSS Inc., USA) by using the between-groups linkage method as the amalgamation rule and squared Euclidean distance as the metric.

Fig. 3. Effect of various *P. notoginseng* extracts on PT in vitro. The sample concentrations were 20 mg/mL in PBS solution. The positive control was warfarin sodium with a concentration of 10 mg/mL. The blank control was PBS. PT was determined by using a plasma coagulation analyzer. The data were expressed as mean ± standard deviation for two determinations: significance at ## $p < 0.05$ compared with the blank control and ** $p < 0.05$ compared with the positive control.

Fig. 4. (a) UPLC chromatogram of mixed standards (1, Notoginsenoside R1; 2 to 4, ginsenoside Rg1, Rb1, and Rd). (b) UPLC chromatogram of 30% (E-30) and 50% (E-50) aqueous ethanol eluents from macroporous resins (1, Notoginsenoside R1; 2, ginsenoside Rg1 in E-30; 1, ginsenoside Rb1; 2, ginsenoside Rd in E-50).

Fig. 5. "Dose–response" relationships of PTS and PDS for extracts at various concentrations: 30, 30% aqueous ethanol eluent (E-30, PTS); 50, 50% aqueous ethanol eluents (E-50, PDS); S14, *P. notoginseng* extract of S14.

478 **Table 1** Raw herbs used in this study

Sample number	Sources	Growing years	Types	Description
S1	Mengzi, Honghe	3	Spring	Dried
S2	Pingyuan, Yanshan	3	Spring	Dried
S3	Gumu, Wenshan	3	Spring	Dried
S4	Dongshan, Mile	4	Spring	Dried
S5	Dongshan, Mile	3	Spring	Dried
S6	Dongshan, Mile	2	Spring	Dried
S7	Weimo, Yanshan	4	Spring	Dried
S8	Zhela, Yanshan	4	Spring	Dried
S9	Pingyuan, Yanshan	4	Spring	Dried
S10	Ganhe, Yanshan	3	Winter	Dried
S11	Panlong, Yanshan	3	Winter	Dried
S12	Dabukan, Mile	2	Spring	Dried
S13	Dabukan, Mile	3	Spring	Dried
S14	Changhu, Shilin	3	Spring	Dried
S15	Xiyi, Mile	3	Spring	Dried