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Simultaneous determination of ten ginsenosides in Panacis Quinquefolii Radix by ultra performance liquid chromatography and quality evaluation based on chemometric methods

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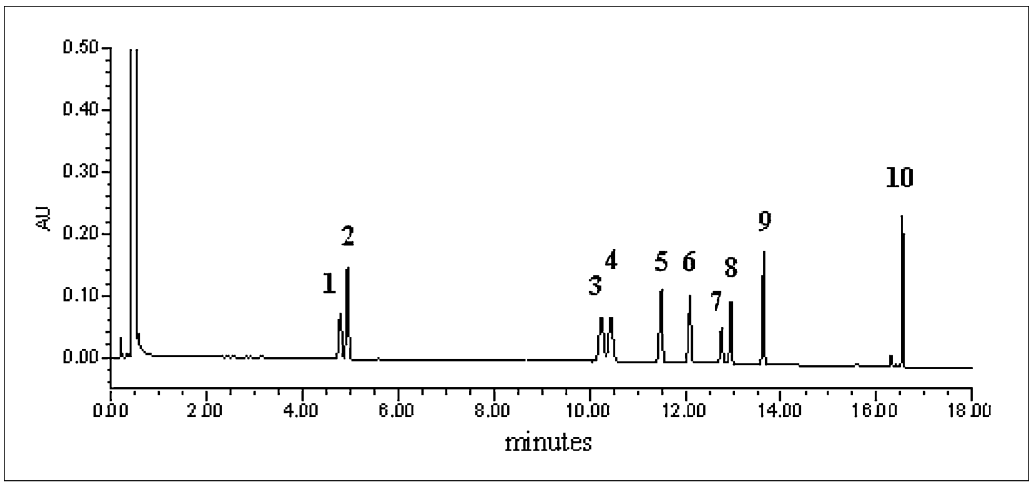
A rapid, sensitive and reliable method based on ultra performance liquid chromatography coupled with a photodiode array detector (UPLC-PAD) was developed for both the quantitative analysis of ten bioactive ginsenosides and a chemical-fingerprint analysis. The chromatography was performed on an ACQUITY UPLC™ BEH C_{18} column using a gradient elution with acetonitrile/water as the mobile phase. To compare the UPLC fingerprints and to evaluate their quality, chemometric methods including similarity analysis (SA) and hierarchical-clustering analysis (HCA) were implemented when classifying the Panacis Quinquefolii Radix samples. The Panacis Quinquefolii Radix samples were successfully grouped in accordance with their geographic origins.

1. Introduction

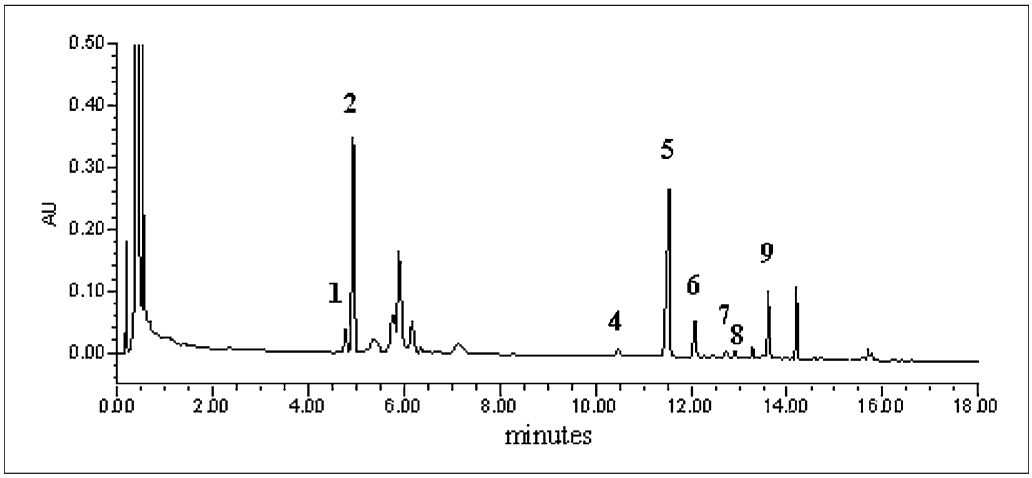
American ginseng, or Panacis Quinquefolii Radix (Huaqishen or Xiyangshen in Chinese), is derived from *Panax quinquefolius* L. (Family Araliaceae), a perennial plant indigenous to the central and eastern regions of Canada and the United States (Jia and Zhao 2009). American ginseng possesses various pharmacological actions such as modulating neurotransmission in learning, memory improvement and neuroprotection, strengthening the immune system to fight cancer, and promoting longevity equally as well as Asian ginseng (Attele et al. 1999; Jia et al. 2009). American ginseng has been recorded in *the Supplement to the "Compendium of Materia Medica"* for medicinal purposes in China since the Qing dynasty and is widely used in traditional medicines and health foods. Since the late 1800 s, shaded cultivation has been encouraged to aid in its conservation and to accommodate the high demand for wild American ginseng (Jia and Zhao 2009). In recent years, American ginseng was abundant in supply for medicinal and tonic consumption in China. It has been extensively and successfully cultivated in Northeastern and Eastern China, including the provinces of Liaoning, Jilin, Heilongjiang, Beijing and Shandong (Xiao 2002). Previously, Yuan et al. compared the pharmacological effects of American-cultivated and Chinese-cultivated Panacis Quinquefolii Radix and examined differences between Wisconsin-cultivated and Illinois-cultivated Panacis Quinquefolii Radix. The results indicated that the potency of extracts from the Panacis Quinquefolii Radix cultivated in different countries or regions varied significantly due to their differing ginsenoside profiles (Yuan et al. 1998; Yuan et al. 2001). Therefore, quality control is very important in guaranteeing the desired pharmacological effects.

Ginsenosides, a unique group of triterpenoid saponins, are responsible for the various pharmacological activities such as antihyperglycemic, aphrodisiac, anticancer or immunotherapeutic effects (Jia and Zhao 2009). Of the more than 150 naturally occurring ginsenosides found in the *Panax* genus, only ginsenosides Rb_1, Re and Rg_1 have been designated as quality-control markers for Panacis Quinquefolii Radix in the Chinese pharmacopoeia (State Pharmacopoeia Committee of the People's Republic of China 2010). A number of the bioactive ginsenosides are currently not thoroughly quantified due to the lack of corresponding ginsenoside standards; however, the high quantities of ginsenosides Rg_1, Re, Rb_1, Rg_2, Rb_2, Rc, Rg_3 and Rd have been recognized as the main active ingredients in American ginseng (Wang et al. 2005; Court et al. 1996; Chen et al. 2007; Zhang et al. 2008). Quality control through quantitative analysis of one or more bioactive constituents or marker compounds remains of prime importance, but consistent quality cannot be guaranteed based on the contents of only a few of the ginsenosides. It has become increasingly clear that a synergism among the multiple active analytes plays an important role in Ginseng bioactivity and the quality assessment based on multiple constituents as much as possible are comprehensive. The development of "chromatographic fingerprints" conforms to the concept of a comprehensive quality-control program for herbal medicines.

Today, these chromatographic fingerprints have become the most powerful approaches in the quality control of herbal medicines, along with quantitative analysis (Zeng et al. 2009; Jin et al. 2008; Tian et al. 2009; Wei et al. 2010). A chromatographic fingerprint is a chromatographic profile representing multiple components with characteristic peaks, known or unknown, for the herbal medicine under investigation. Fingerprint chromatograms are complex multivariate data sets because the herbal medicines tested are complex mixtures of components. The differences among chromatographic fin-



(A)



(B)

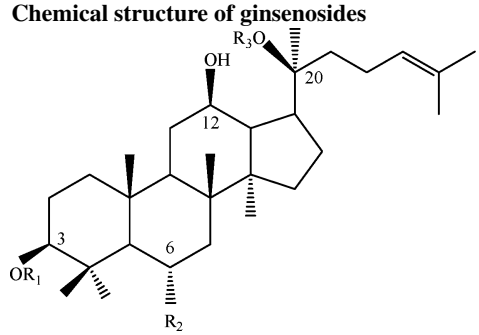
Fig. 1: The UPLC chromatograms of mixed standard compounds (A) and Panacis Quinquefolii Radix sample (B). The chromatogram of mixted standard compounds (A): (1) G-Rg_1; (2) G-Re; (3) G-Rh_1; (4) G-Rg_2; (5) G-Rb_1; (6) G-Rc; (7) G-Rb_2; (8) G-Rb_3; (9) G-Rd; (10) G-Rg_3.

gerprints from various sources are difficult to discriminate visually or to analyze quantitatively. Thus, chemometric methods, such as similarity analysis (SA) and hierarchical clustering analysis (HCA), should be considered to facilitate the classification of an herbal medicine (Liu et al. 2010; Kong et al. 2009).

High-performance liquid chromatography (HPLC) coupled with various detectors is the most commonly employed analytical method for the detection of ginsenosides (Chen et al. 2007; Shangguan et al. 2001; Li et al. 2005). Unfortunately, to acquire a chemical fingerprint and a quantitative analysis for the ginsenosides of Asian or American ginseng by traditional HPLC generally requires one or more hours per sample run (Wang et al. 2005; Chen et al. 2007; Zhang et al. 2008). In comparison, ultra performance liquid chromatography (UPLC) not only has a lower flow rate, a shorter analysis time and a lower solvent consumption, it also performs satisfactory separations, has good resolution and sensitivity and is a powerful tool for chromatographic fingerprints and quantitative analyses of complex systems such as herbal medicines (Kong et al. 2009; Chen et al. 2008).

In the present study, a rapid, high-throughput UPLC–PDA method was developed for the simultaneous determination of ten ginsenosides in Panacis Quinquefolii Radix (Table 1). Additionally, the chemical fingerprints of Panacis Quinquefolii Radix from various sources were investigated, and SA and HCA were performed.

Table 1: Chemical structure of ginsenosides



No.	Ginsenosides	R_1	R_2	R_3
1	G-Rg_1	H	-O-Glc	-Glc
2	G-Re	H	-O-Glc^2-Rha	-Glc
3	G-Rh_1	H	-O-Glc	H
4	G-Rg_2	H	-O-Glc^2-Rha	H
5	G-Rb_1	-Glc^2-Glc	H	-Glc^6-Glc
6	G-Rc	-Glc^2-Glc	H	-Glc^6-Ara(f)
7	G-Rb_2	-Glc^2-Glc	H	-Glc^6-Ara(p)
8	G-Rb_3	-Glc^2-Glc	H	-Glc^6-Xyl
9	G-Rd	-Glc^2-Glc	H	-Glc
10	G-Rg_3	-Glc^2-Glc	H	H

G: Ginsenoside; Glc: β-D-glucose; Rha: α-L-rhamnose; Ara(p): α-L-arabinose (pyranose); Ara(f): α-L-arabinose (furanose); Xyl: β-D-xylose

Table 2: Calibration curve, limit of detection, limit of quantification of the analytes

Compounds	Regression equation	Linear range (mg/ml)	R^2	LOD (ng)	LOQ (ng)
G-Rg_1	Y = 2293897X + 5572	2.51−502	1.0000	1.7	5.8
G-Re	Y = 1886809x + 1809	51.7−1014	0.9998	1.7	6.0
G-Rh_1	Y = 3568176X + 7082	2.55−510	1.0000	1.7	6.0
G-Rg_2	Y = 3367695X + 9565	2.56−512	0.9999	1.7	3.0
G-Rb_1	Y = 1792394X + 7041	51.3−1026	1.0000	1.7	6.0
G-Rc	Y = 1829870X + 6204	41.5−830	1.0000	1.4	4.8
G-Rb_2	Y = 1855268X + 3433	2.00−401	1.0000	1.3	4.7
G-Rb_3	Y = 2262722X + 5570	2.52−504	0.9999	1.7	5.9
G-Rd	Y = 2136235X + 8066	40.3−806	1.0000	1.3	4.7
G-Rg_3	Y = 3402357X + 8797	2.57−128.7	0.9991	1.7	6.0

Y, peak area; X, the concentration of each reference compound (mg/ml); *R*, correlation coefficient of regression equations; LOD, limit of detection (*S/N* = 3); LOQ, limit of quantification (*S/N* = 10).

2. Investigations, results and discussion

2.1. Selection of extraction procedure

The efficiency of the extraction procedure was evaluated using different solvents, i.e., different concentrations of methanol in water (100% methanol, 70% methanol and 50% methanol), ethanol or *n*-butanol saturated with water. The results demonstrated that methanol extraction yielded the highest contents of the target ginsenosides compared to the other solvents. Various extraction techniques such as sonication, refluxing, Soxhlet extraction, and marinating at room temperature were also investigated. The sonication extractions gave concentrations of ginsenosides higher than those for the marinating extractions and equivalent to those for the reflux and Soxhlet extractions, but took less time. Thus, sonication was subsequently chosen as the extraction method for the test samples. The duration of the extraction was also investigated; however, the results demonstrated that the established method was adequate and appropriate for the analysis.

2.2. Selection of chromatographic conditions

The chromatographic conditions were optimized using both the standards for the ten ginsenosides and the Panacis Quinquefolii Radix samples based on conditions described in previous reports (State Pharmacopoeia Committee of the People's Republic of China 2010; Zhang et al. 2008; Guan et al. 2007). First, variations through gradient elution in the ratio of water to acetonitrile in the mobile phase were investigated to obtain the baseline separation of the target ginsenosides, especially ginsenosides Rg_1 and Re, Rb_2 and Rb_3. Optimized chromatographic conditions were achieved to provide the best resolution and a good separation of the ten ginsenosides by considering the effects of flow rate and column temperature. Here, the flow rate was set at 0.3 mL·min^{-1} when comparing the various column temperatures (20, 25, 30 and 40 °C), and the column temperature was kept at 30 °C when varying the flow rate (0.3, 0.4 and 0.5 mL·min^{-1}). System backpressure and running time were also taken into consideration during these trials. Under the optimal conditions, all ten ginsenosides and the other components in the extracts of the Panacis Quinquefolii Radix samples were well separated, as shown in the representative chromatogram in Fig. 1. The use of the UPLC-PAD method produced a significant increase in peak capacity, which resulted in a better chromatographic resolution and increased signal-to-noise ratio.

2.3. Method validation

The newly established method was validated for linearity, limits of detection and limits of quantification (LODs and LOQs), precision (inter-day and intra-day precision), reproducibility, stability and accuracy.

2.3.1. Linearity, limits of detection (LOD) and limits of quantification (LOQ)

Linearity was evaluated using calibration curves constructed in triplicate by plotting the peak area of the analyte for each ginsenoside against its standards at six different concentrations; the equations and correlation coefficients corresponding to the linear-regression curves are listed in Table 2. Here, the limit of detection (LOD) was defined as the concentration giving a signal three times higher than the noise level. The limit of quantification (LOQ) was defined as the concentration giving a signal 10 times greater than the noise (*S/N* = 10) (Table 2).

2.3.2. Accuracy and precision

To estimate sample-matrix effects in this newly optimized UPLC method, ginsenoside recoveries from samples spiked with the ten standards were evaluated. The spiked samples (Sample 5) were extracted, processed and quantified in accordance with the sample preparation and chromatographic-analysis methods described above, and the analyte recoveries were determined for three separate concentrations (low, medium and high). The average recoveries for the three extracted aliquots of the spiked samples were found to be within the range of 95.3% to 104.6%, with relative standard deviations (*RSD*) between 0.19% and 5.98% (Table 3).

The intra-day and inter-day accuracies were evaluated under the optimal conditions by performing six replicate analyses on standard solutions prepared and analyzed every four hours for intra-day analysis and twice a day for three consecutive days for the inter-day study. The intra-day and inter-day accuracies showed good coefficients of variance, with *RSD*s ranging from 2.07% to 3.20% (Table 3), which was acceptable.

2.4. Repeatability and stability

To further evaluate the repeatability and stability of the developed assay, Panacis Quinquefolii Radix sample was analyzed in six replicate analyses as described above. The contents were calculated and the *RSD*s were taken as measures of the repeatability and stability of the method. Stability was tested and analyzed at 0 h, 2 h, 4 h, 8 h and 12 h, respectively. The *RSD*s for the repeatability and stability were within 3.83% and 2.45%, respectively.

Table 3: Precision and recovery for ten ginsenosides in Panacis Quinquefolii Radix

Analytes	Accuracy ($n = 6$)			Recovery ($n = 3$)		
	Concentration (μg/mL)	Intra-Day *RSD* (%)	Inter-Day *RSD* (%)	Spiked (mg)	Mean (%)	*RSD* (%)
G-Rg_1	0.1337	2.17	3.20	0.80	98.8	3.10
				1.36	99.8	0.62
				1.80	100.7	0.28
G-Re	0.2585	2.07	3.10	1.20	96.9	0.78
				2.16	104.3	0.93
				3.24	98.5	0.19
G-Rh_1	0.1394	2.18	2.96	0.20	95.8	1.45
				0.32	98.8	1.34
				0.42	101.1	1.11
G-Rg_2	0.1367	2.37	3.29	0.25	99.0	1.86
				0.40	95.8	1.48
				0.50	101.8	1.15
G-Rb_1	0.2565	2.13	3.08	2.40	96.9	1.00
				3.60	100.2	1.15
				4.80	96.7	0.69
G-Rc	0.2075	2.20	3.12	0.08	103.3	4.53
				0.40	99.8	2.04
				0.64	96.6	0.94
G-Rb_2	0.1069	2.45	3.15	0.15	97.5	3.87
				0.27	100.8	1.05
				0.36	98.9	3.96
G-Rb_3	0.1345	2.41	3.12	0.16	104.6	5.98
				0.32	95.3	1.38
				0.44	96.3	4.01
G-Rd	0.2015	2.60	3.11	0.42	98.6	5.68
				0.63	104.6	3.04
				0.84	103.3	4.95
G-Rg_3	0.1373	2.08	2.92	0.20	103.5	0.68
				0.36	95.6	0.45
				0.48	95.7	0.26

2.5. Sample analysis

The established method was applied to determine the contents of ten ginsenosides in 16 batches of Panacis Quinquefolii Radix from locations of varying origin. The results are shown in Table 4 as the mean values of the three replicate injections. Among the ten target ginsenosides, ginsenosides Rh_1 and Rg_3 were not detected; ginsenoside Rg_2 was not detected in half of the samples, and only very small amounts were detected and quantified in the remaining half. It is clear from the results that the contents of the ginsenosides detected in the Panacis Quinquefolii Radix samples varied remarkably. The highest contents of two ginsenosides, Re and Rb_1, exceeded 0.73% and 0.94%, respectively. Comparatively, the contents of three other ginsenosides, Rg_1, Rc and Rd, which are bioactive components known to be present in higher amounts, ranged from 0.054% to 3.94% and were higher than ginsenosides Rb_2 and Rb_3. In Panacis Quinquefolii Radix samples from China and elsewhere, ginsenosides Re, Rb_1 and Rg_1 were found to be the main bioactive components, together with ginsenosides Rc and Rd. Regrettably, quality control in the Chinese pharmacopoeia utilizing quantitative analysis remains infeasible for detecting ginsenosides Re, Rb_1, and Rg_1 (State Pharmacopoeia Committee of the People's Republic of China 2010). However, trace ginsenosides and unknown ginsenosides are known to possess therapeutic effects; synergic effects among bioactive ginsenosides play important roles. To evaluate the comprehensive quality of Panacis Quinquefolii Radix, the chromatographic fingerprints established on the rapid UPLC technique combined with HCA were performed.

2.6. Establishment of chromatographic fingerprint for Panacis Quinquefolii radix and its similarity evaluation

These experiments showed that the proposed UPLC method could also be satisfactorily employed in the fingerprinting of Panacis Quinquefolii Radix while simultaneously performing a quantitative analysis of the ten ginsenosides. The information gathered from the fingerprints was more comprehensive than that provided from the typical approach, which only focuses on the quantitation of individual markers or active constituents.

The Similarity Evaluation System for Chromatographic Fingerprinting of Traditional Chinese Medicines, a computer-aided-similarity-evaluation software developed for the statistical evaluation of chromatographic patterns, was employed for the synchronization and the quantitative comparison of the different samples. The correlation coefficients of all the chromatographic profiles for the samples were calculated, and simulated mean chromatograms were calculated and generated by this program. The different chromatographic patterns for each investigated sample were compared for their similarities with the mean chromatogram.

The chromatograms of the sixteen Panacis Quinquefolii Radix samples, containing 11 distinct common peaks within an elution time of 18 min, are shown in Fig. 2. All the chromatograms were evaluated in terms of their similarity to the mean chromatogram through the calculation of their angle cosine value from the original data. In other words, the similarity analyses were conducted based on standard fingerprints, and the results are shown in Table 5. Here, cosine values closer to 1 indicate a high similarity of a given chromatogram to the mean chro-

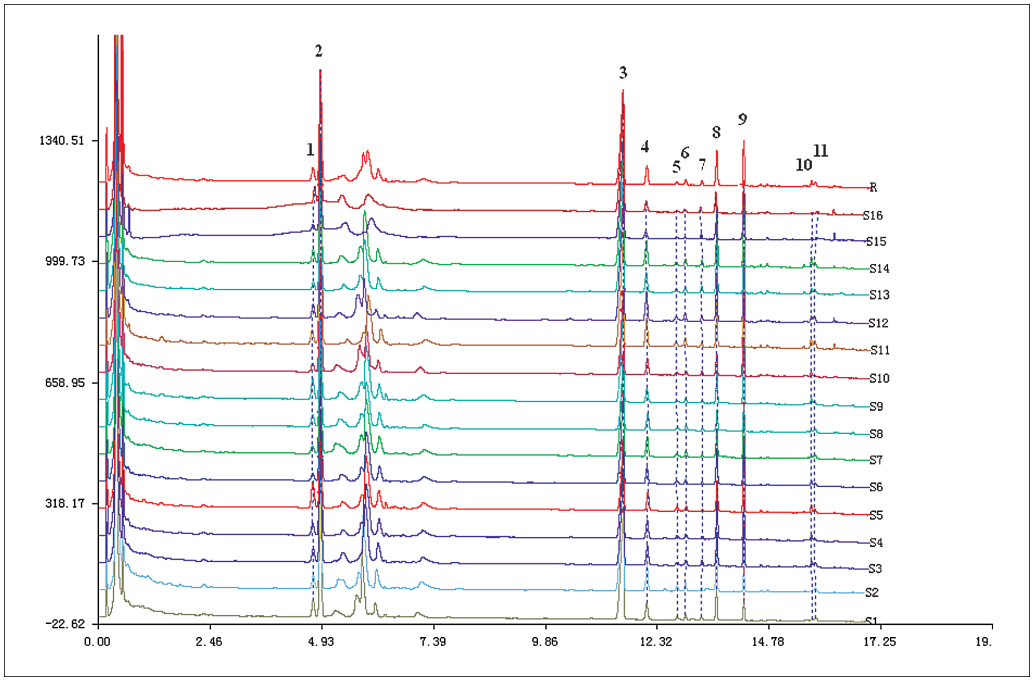


Fig. 2: The UPLC chromatograms of 16 batches of Panacis Quinquefolii Radix samples. Numbers 1–10 stand for the common peaks: (1)G-Rg_1; (2) G-Re; (3) G-Rb_1; (4) G-Rc; (5) G-Rb_2; (6) G-Rb_3; (8) G-Rd; (7), (9), (10) and (11) were unknown; Numbers S1–S16 stand for the different samples.

matogram. The similarity values of all 16 samples were more than 0.90, except Sample 3.

2.7. HCA based on the chromatographic fingerprint for quality assessment and classification

Relative peak area of each characteristic peak related to the weight was calculated for the quantitative expression of the chemical properties in the chromatographic pattern. This information allowed us to assess the quality and use HCA to classify the Panacis Quinquefolii Radix samples.

HCA is a statistical method for finding relatively homogeneous clusters in cases based on measured characteristics. In this analysis, the ratio between the areas of the 11 common peaks and the weights determined for the 16 Panacis Quinquefolii Radix samples formed an 11 × 16 matrix. Similarities among the 16 samples were calculated using the SPSS software, and a dendrogram was generated, which revealed the relationships among the samples. The results of the HCA are shown in Fig. 3, and they reveal that the samples could be divided into two levels and three clusters: Samples 3–10 and 15 were in Cluster One, belonging to Level I, while Samples 11–14, 1–2 and 16 fell in Clusters Two (A) and Three (B), both belonging to Level II. Cluster One consists of Sample 15 collected from Canada and Samples 3–10 collected from the Jilin and Heilongjiang provinces. Cluster Two consists of two samples, 11 and 12, collected from Shandong. Cluster Three consists of five samples collected from the Beijing

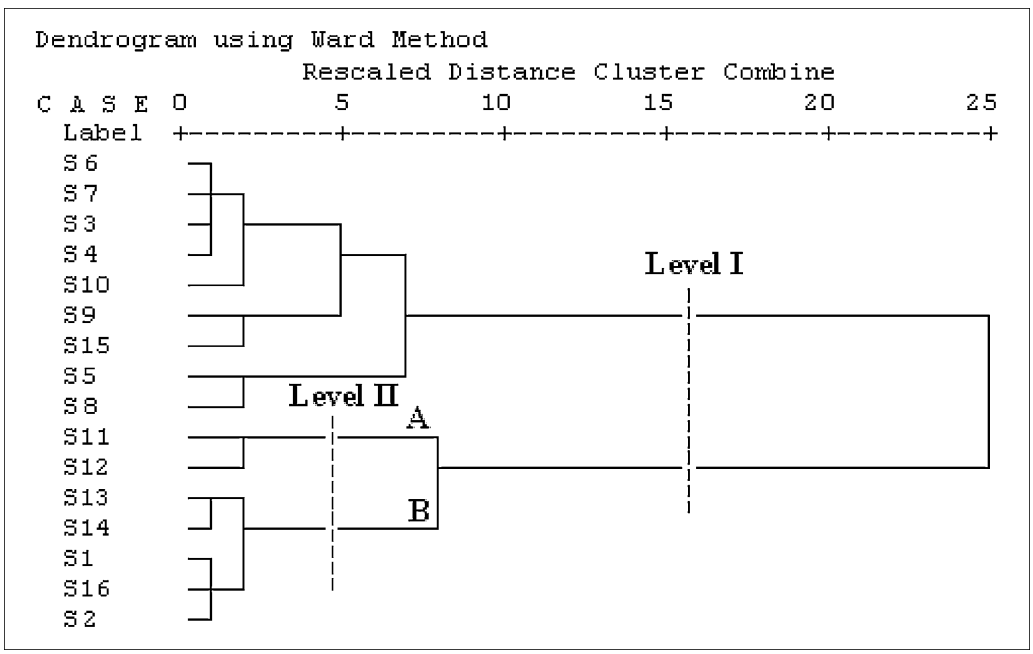


Fig. 3: Dendrogram of clustering of Panacis Quinquefolii Radix samples. This dendrogram was performed using SPSS16.0 software (Chicago, USA). The Ward's method as the amalgamation rule and the Squared Euclidean distance as metric were used to establish clusters: Numbers S1–S16 stand for the different samples.

Table 4: Contents of ten ginsenosides in Panacis Quinquefolii Radix ($n = 3$, mg·g^{-1})

Sample No.	Sources	G-Rg_1	G-Re	G-Rg_2	G-Rb_1	G-Rc	G-Rb_2	G-Rb_3	G-Rd
S1	Qingyuan, Liaoning	1.5834 ± 0.0398	12.2038 ± 0.1535	n.d.	17.4665 ± 0.1316	1.6371 ± 0.0055	0.2787 ± 0.0053	0.3013 ± 0.0128	1.8590 ± 0.0452
S2	Qingyuan, Liaoning	1.2710 ± 0.0060	12.9160 ± 0.0372	n.d.	18.5732 ± 0.0526	2.6595 ± 0.0155	0.5715 ± 0.0057	0.4927 ± .0085	2.9461 ± 0.0089
S3	Tonghua, Jilin	1.3696 ± 0.0351	12.7009 ± 0.0681	0.0015 ± 0.0025	14.0092 ± 0.0446	2.1434 ± 0.0098	0.3808 ± 0.0038	0.3987 ± 0.0188	2.3481 ±0.0211
S4	Ji'an, Jilin	1.2594 ± 0.0170	13.6195 ± 0.1173	n.d.	13.4775 ± 0.1404	1.8672 ± 0.0176	0.2753 ± 0.0046	0.3072 ± 0.0032	2.4369 ± 0.0267
S5	Fusong, Jilin	2.7560 ± 0.0086	7.3664 ± 0.0083	n.d.	9.4496 ± 0.0072	1.9374 ± 0.0024	0.4175 ± 0.0065	0.3297 ± 0.0043	1.9612 ± 0.0093
S6	Fusong, Jilin	0.7532 ± 0.0028	11.9148 ± 0.0427	n.d.	13.3744 ± 0.0385	1.6670 ± 0.0051	0.2603 ± 0.0036	0.2632 ± 0.0034	2.2955 ± 0.0098
S7	Jingyu, Jilin	0.7991 ± 0.0038	12.8802 ± 0.0169	0.1081 ± 0.0053	14.3186 ± 0.0706	1.8947 ± 0.0018	0.3174 ± 0.0010	0.4415 ± 0.0018	0.8831 ± 0.0092
S8	Jingyu, Jilin	1.2451 ± 0.0050	10.0103 ± 0.0159	n.d.	9.8180 ± 0.0391	1.7271 ± 0.0097	0.2648 ± 0.0045	0.2830 ± 0.0028	1.2829 ± 0.0020
S9	Anning, Heilongjiang	2.0528 ± 0.0044	8.9944 ± 0.0459	n.d.	13.8959 ± 0.0775	1.3939 ± 0.0052	0.2118 ± 0.0040	0.2473 ± 0.0080	2.0198 ± 0.0077
S10	Muling, Heilongjiang	0.8350 ± 0.0240	12.3826 ± 0.0137	0.0241 ± 0.0042	11.6521 ± 0.0051	1.6685 ± 0.0065	0.3076 ± 0.0038	0.3136 ± 0.0115	1.4985 ± 0.0013
S11	Wendeng, Shandong	1.4713 ± 0.0164	16.7431 ± 0.0598	0.0347 ± 0.0026	18.1929 ± 0.0368	3.1073 ± 0.0059	0.6381 ± 0.0227	0.7920 ± 0.0307	3.0964 ± 0.0163
S12	Wendeng, Shandong	1.3846 ± 0.0239	17.0314 ± 0.0190	0.1362 ± 0.0078	21.3261 ± 0.1457	3.1563 ± 0.0117	0.7362 ± 0.0234	0.6837 ± 0.0206	3.1552 ± 0.1200
S13	Huairou, Beijing	0.7226 ± 0.0010	10.6368 ± 0.0093	0.0977 ± 0.0124	19.9807 ± 0.0275	2.0780 ± 0.0058	0.3383 ± 0.0042	0.3999 ± 0.0048	3.2671 ± 0.0092
S14	Huairou, Beijing	1.3716 ± 0.0058	11.7095 ± 0.0311	0.0699 ± 0.0103	19.6912 ± 0.0169	2.6624 ± 0.0049	0.4157 ± 0.0262	0.4789 ± 0.0191	3.9489 ± 0.0126
S15	Canada	0.5484 ± 0.0030	8.8464 ± 0.0854	0.0497 ± 0.0055	17.6850 ± 0.2354	0.6875 ± 0.0045	0.1498 ± 0.0041	0.1155 ± 0.0099	0.9890 ± 0.0562
S16	Canada	1.1627 ± 0.0057	11.9101 ± 0.0409	n.d.	18.8115 ± 0.0460	1.0976 ± 0.0218	0.1904 ± 0.0027	0.1651 ± 0.0009	1.2566 ± 0.0072

Both ginsenosides Rh_1 and Rg_3 were not detected in the Panacis Quinquefolii Radix samples; N.D. = not detected.

Table 5: Similarity values of 16 batches of Panacis Quinquefolii Radix samples

Sample No.	Similarity	Sample No.	Similarity
S1	0.915	S9	0.921
S2	0.912	S10	0.910
S3	0.871	S11	0.975
S4	0.958	S12	0.945
S5	0.912	S13	0.929
S6	0.952	S14	0.933
S7	0.925	S15	0.926
S8	0.941	S16	0.944

and Liaoning provinces and from Canada. It can be seen that the rescaled distance between Clusters Three and One (or Clusters Two and One) is 25 (arbitrary units), whereas the rescaled distance between Clusters Two and Three is 7.6. These distances indicate that the qualities of Clusters Two and Three are more similar to each other than with those of Cluster One. From the plot, all the samples from Jilin and Heilongjiang were clustered in Level I, which tells us that the qualities of these samples were homogenous and stable. The samples from Liaoning and Beijing were clustered in B, which indicates that their cultivation climates and other factors vary only a little. The UPLC profiles for the Canadian-grown samples were similar to those of the Chinese samples, ensuring that Chinese-cultivated Panacis Quinquefolii Radix should provide the same pharmacological effects as the Canadian form.

3. Experimental

3.1. Reagents and standards

Ginsenosides Rg_1, Re, Rg_2, Rg_3, Rb_1, Rb_2, Rb_3, Rc and Rd were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and ginsenoside Rh_1 was obtained from Shanghai Tongtian Biotechnology Company (Shanghai, China). Their structures were shown in Table 1.

HPLC-grade acetonitrile was purchased from Merck Company (Darmstadt, Germany). Water was prepared from Millipore water purification system (Millipore, MA, USA). Solvents were filtered through 0.22 μm membrane filters (Waters Co., MA, USA) before use. Other solvents were of analytical grade (Peking Chemical Factory, Beijing, China).

3.2. Plant materials

Fourteen raw material samples were collected from the provinces of Liaoning, Jilin, Heilongjiang, Beijing and Shandong in China and were identified as Panacis Quinquefolii Radix by Professor Yulin Lin (Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College). Two commercial sliced samples were purchased from herbal stores in Beijing, marked as "American ginseng root, made in Canada." Voucher specimens were deposited in the Herbarium of the Institute of Medicinal Plant Development at the Chinese Academy of Medical Sciences & Peking Union Medical College.

3.3. Sample preparation

The powdered samples (1.0 g, sieved to a size of 250 μm) were accurately weighed and macerated in 50 mL methanol in a measuring flask for 10 min. These samples were extracted in an ultrasonic bath for 30 min (Power 500 W). Of the methanol extracts remaining after removing the solvent *in vacuo*, 25 mL residue was dissolved in 10 mL methanol. The sample solution was filtered through a membrane filter (0.22 μm pore size) prior to injection into the UPLC.

3.4. Apparatus and chromatographic conditions

The experiments were performed on an ACQUITY UPLC™ system (Waters Co., MA, USA) consisting of a binary solvent delivery system, a sample manager, a column oven, and a photodiode array detector (PDA). Data were analyzed with the Waters Empower 2 Chemstation software.

Chromatographic separation was performed on an ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm particle size, Waters Co., MA, USA). The column was maintained at 30 °C, and analysis was monitored at 203 nm. The mobile phase consisted of acetonitrile as solvent A and water as solvent B. Separation was performed by gradient elution: 0–3 min 19% A, 4 min 21% A, 5 min 26% A, 9 min 27% A, 12 min 32% A, 15 min 43% A, 17 min 60% A and 20 min 100% A, with a flow rate of 0.3 mL·min^{-1}. The injection volumes of the test sample solutions and the standard solution were each 2 μL. Each wash cycle consisted of 200 μL of a strong solvent (acetonitrile/water, 8:2, v/v) and 600 μL of a weak wash solvent (acetonitrile/water, 2:8, v/v).

3.5. Data analysis

The similarity analysis (SA) of the fingerprints is one of the most important aims in the quality control of traditional Chinese medicines. Data analysis was performed using the commercial software program "Similarity Evaluation System for Chromatographic Fingerprinting of Traditional Chinese Medicines" (Version 2004), which was recommended by the State Food and Drug Administration (SFDA) of China. A simulated mean chromatogram was calculated and generated by this software, which represented the standard fingerprint chromatograms for the Panacis Quinquefolii Radix samples from different sources. The different chromatographic patterns for all the tested samples were compared for their similarities with the standard chromatograms.

Hierarchical-clustering analysis (HCA) is a multivariate analysis method used for finding relatively homogeneous clusters in cases based on measured characteristics; its aim is to classify samples into groups. In this study, the HCA of samples was performed using SPSS 16.0 software (SPSS, Chicago, USA). Ward's method was selected as the amalgamation rule, and the squared Euclidean metric distance was used to establish clusters. The results of this hierarchical clustering process were represented in a dendrogram.

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