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Quality Assessment and Ecotype Distinction for *Panax quinquefolius* L. from China and Canada by ^{1}H NMR and Chemometrics

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ABSTRACT

Panax quinquefolius L. is one of the most widely consumed and cultivated herbal medicines around the world. China and Canada are the two major producing countries. However, research on the ecotype distinction of *P. quinquefolius* from Canada has never been reported, and the quality evaluation of *P. quinquefolius* between China and Canada using nuclear magnetic resonance (^{1}H-NMR) is limited. Here, we investigated the ^{1}H NMR key signals and ecological factors of *P. quinquefolius* samples, and the data were further analyzed by principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). The key signals were identified as sugar and methyl, which distinguished all the samples into two different ecotypes. This is the first report of ecotype division for *P. quinquefolium* worldwide. The results showed quality variation of *P. quinquefolium* from different geographic areas, implying the ecological adaptation and biodiversity. Our findings also demonstrate the critical need for improving quality and quality standardization, appropriate ecological regionalization and promoting industrialized development of *P. quinquefolium*.

INTRODUCTION

Panax quinquefolius L. is one of the most important herbs in the world, and is native to North America in USA and Canada; it is now widely cultured in many parts all over the world, including China (Christensen *et al*., 2006). The history of *P. quinquefolius*, in China, was first recorded in Ben Cao Gang Mu in the Ming Dynasty (Li, 2004). Presently, the cultivation of the herb has been extended on a large scale in Jilin and Shandong provinces. Canada, China and USA are the three major producing areas. China has gradually become the major production, consumption and export country instead of traditional import country.

It was reported that the contents and composition of perceived pharmacological properties varied significantly among populations (Li *et al*., 1996; Tang *et al*., 2016; Qi *et al*., 2016). The pharmacological effects of ginseng roots has been attributed primarily to ginsenosides, a triterpenoid saponin glycoside.

Ginsenosides were recorded as indicator compound in the Chinese Pharmacopoeia and the United States Pharmacopeia. The grading and pricing of *P. quinquefolius* are primarily determined by the ginsenosides of harvested roots (Lim *et al*., 2005). The ecotype is a population concept put forward by Turesson in 1921, and Odum improved it (Liu *et al*., 2004; Odum, 1996). The ecotype of *P. quinquefolius* from China has been investigated in our lab, which showed that two chemoecotypes of *P. quinquefolius* in China, ginsenosides Rb1-Re from outside Great Wall and Rg2-Rd from inside Great Wall with distinct climatic characteristics (Wang and Huang, 2015; Wang *et al*., 2015; Huang *et al*., 2013). To date, some studies have been reported for quality assessment of *P. quinquefolius* (Ludwiczuk *et al*., 2005; Xu *et al*., 2011; Chan *et al*., 2000; Sun *et al*., 2012; Zhao *et al*., 2015; Yang *et al*., 2012), but the ecotype of *P. quinquefolius* from major productive country Canada has never been conducted. The current article investigates the differences in quality and ecotype of *P. quinquefolius* from Canada and China by ^{1}H-NMR spectroscopy coupled with chemometrics, which includes PCA and PLS-DA. This paper aims to establish an effective and rapid metabolomics method for geographical origin traceability of *P. quinquefolius* in the world.

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MATERIALS AND METHODS

Sample collection

The samples of *P. quinquefolius* were collected from 6 locations, which include China and Canada populations (Table 1). Three independent plants were collected from each location. Professor Huang Linfang identified the botanical specimens, and the voucher specimens were deposited in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China. The typical root and aerial part of*P. quinquefolius* is shown in Fig.1.

Fig.1: The root and aerial part of *P. quinquefolius*.

Chemicals and reagents

For ^{1}H NMR analysis, methanol-d4 (CD_3OD, 99.8%), dimethyl sulfoxide-d6 (DMSO-d6, 99.9%), and deuterated pyridine (C_5D_5N, 99.5%) were purchased from Cambridge Isotope Laboratories, Inc. (Miami, FL, USA). The *P. quinquefolius* samples, labeled as CSDa1-3, CSDb1-3, CJLa1-3, CJLb1-3, were collected from China;samplesCANa1-3 and CANb1-3, with origins in Canada, were purchased from Hong Kong Lin Shi Ginseng Antler Co., Ltd. The detailed information of the plant materials in this study is shown in Table 1.

Preparation of samples

CD_3OD, DMSO-d6 and C_5D_5N were tested to dissolve the lyophilized samples; CD_3OD was found the best solvent.30 mg of each samplewas extracted with 1 ml of CD_3OD. The extractions were vortexed vigorously for 30s, and then were sonicated for 40 min at room temperature. NMR samples were prepared by shaking manually, sonicated the insoluble material at 2000 g for a further 5 min, and adjusted to pH 7.0 ± 0.003 using deuterated base and/or acid. Finally, it wastransferring the supernatant of the extracts into 5 mm NMR tubes for NMR analysis (Shin *et al*., 2007).

^{1}H NMR analysis

One-dimensional ^{1}H NMR spectra were measured at a temperature of 300Kon a 600.13MHz BrukerAvance spectrometer (BrukerAnalytische GmbH, Rheinstetten, Germany) equipped with a broad-band-observe (BBO) probe. A zgcppr pulse sequence wasapplied to suppress the residual water signal. A total of 128transients were collected in 32 K data points with relaxation delay of 2s. Aspectral width of 9615.4 Hz and an acquisition time per scan of 1.70s were used.

Prior to Fourier transformation, an exponential line broadening function of 0.30 Hz was applied to the free induction decay. The chemical shifts for all samples were referenced to TMS (tetramethylsilane) at 0.00 ppm.

Table 1: *P. quinquefolius* samples collected from different locations

Number	Lat/Lon	Location	Date	Voucher numbers	State	Years-grow
CSDa1	N37.13° E122.13°	Kouzili, Shandong, China	2015.10	Implad2015ZYH-CSDa1	Fresh	4
CSDa2	N37.13° E122.13°	Kouzili, Shandong, China	2015.10	Implad2015ZYH- CSDa2	Fresh	4
CSDa3	N37.13° E122.13°	Kouzili, Shandong, China	2015.10	Implad2015ZYH- CSDa3	Fresh	4
CSDb1	N37.4° E121.95°	Dashuipo, Shandong, China	2015.10	Implad2015ZYH- CSDb1	Fresh	4
CSDb2	N37.4° E121.95°	Dashuipo, Shandong, China	2015.10	Implad2015ZYH- CSDb2	Fresh	4
CSDb3	N37.4° E121.95°	Dashuipo, Shandong, China	2015.10	Implad2015ZYH- CSDb3	Fresh	4
CJLa1	N42.33° E127.3°	Fusong, Jilin, China	2015.10	Implad2015ZYH- CJLa1	Fresh	4
CJLa2	N42.33° E127.3°	Fusong, Jilin, China	2015.10	Implad2015ZYH- CJLa2	Fresh	4
CJLa3	N42.33° E127.3°	Fusong, Jilin, China	2015.10	Implad2015ZYH- CJLa3	Fresh	4
CJLb1	N41.44° E125.97°	Tonghua, Jilin, China	2015.10	Implad2015ZYH- CJLb1	Fresh	4
CJLb2	N41.44° E125.97°	Tonghua, Jilin, China	2015.10	Implad2015ZYH- CJLb2	Fresh	4
CJLb3	N41.44° E125.97°	Tonghua, Jilin, China	2015.10	Implad2015ZYH-CJLb3	Fresh	4
CANa1	N43.98° W79.19°	Toronto Canada	2015.10	Implad2015ZYH- CANa1	Dry	4
CANa2	N43.98° W79.19°	Toronto Canada	2015.10	Implad2015ZYH- CANa2	Dry	4
CANa3	N43.98° W79.19°	Toronto Canada	2015.10	Implad2015ZYH- CANa3	Dry	4
CANb1	N49.279° W123.05°	Vancouver Canada	2015.10	Implad2015ZYH- CANb1	Dry	4
CANb2	N49.279° W123.05°	Vancouver Canada	2015.10	Implad2015ZYH- CANb2	Dry	4
CANb3	N49.279° W123.05°	Vancouver Canada	2015.10	Implad2015ZYH- CANb3	Dry	4

Table 2: The ecological factors of sampling locations.

Location	Major ecological factors							
	Annual air temperature	Relative humidity	Annual precipitation	Annual solar insulation	Average air temperature in January	Minimum air temperature in January	Average air temperature in July	Maximum air temperature in July
CSD1	14	70.2	723.6	1582.2	6.37	6.33	22.8	22.8
CSD2	13.1	70.2	678.9	1573.76	2.54	1.09	23.7	25.9
CSD3	13.9	71.3	708.1	1543.95	3.69	2.22	24	26.2
CJL1	6.03	64.8	831.6	1431.9	-14.3	-21	21.3	27.3
CJL2	3.17	68.7	759.6	1426.5	-18.8	-26.4	20	26.5
CJL3	4.34	68.9	861.4	1435,36	-16.7	-24.1	20.3	26.5
CAN1	6.27	71.2	1237.35	1240.8	-2.25	-5.9	16.6	26.3
CAN2	7.35	71	964.8	1291.5	-7.66	-12.3	20.8	29
CAN3	5.21	71.3	1047.55	1258.03	-4.05	-8	16.3	26.2

CSD1-3 and CJL1 are Chinese samples data; CAN1-3 are the Canadian samples data.

Ecological factors

The data of ecological factors were collected from the NASA Atmospheric Science Data Center, which covered the whole growth process of *P. quinquefolius* from the year 2011 to 2015. The average values of ecological factors listed in Table 2, including annual air temperature, relative humidity, annual precipitation, annual average solar isolation, average air temperature in January, and minimum air temperature in January, average air temperature in July, and maximum air temperature in July, at 6 locations.

Data analysis

The NMR spectra of all the *P. quinquefolius* samples were bucketed in 0.04 ppm. Bucketing of spectra was performed using MestReNova (version 6.0.4.SantiagodeCompostela, Spain: Mestrelab Research) software and Chenomx NMR suite software (version5.1. Alberta, Canada: Chenomx Inc.). The resulting data were converted to Microsoft Office Excel (version 2007. WA, USA: Microsoft) format, and imported into SIMCA P+ software (version 12.0.Umea, Sweden, Umetrics)(Park *et al*., 2013). The method of mean-centered and scaling with Pareto (Par) scaling, were used in PLS-DA.

RESULTS AND DISCUSSION

^{1}H-NMR spectra profiling

To obtain the best separation for all the integrated signals in ^{1}H NMR spectra, NMR solvents were optimized in this study. Based on the dissolvability of the main pharmacological components, three solvent systems involving CD_3OD, DMSO-d6 and C_5D_5N were investigated. CD_3OD showed better separation for the signals of the analyzed ginsenosides, and it was the preferred ^{1}H NMR solvent finally. In Fig. 2I, examples of NMR spectra of *P. quinquefolius* are shown. Differences can be observed from the spectra, PCA and PLS-DA was utilized to further analyze the differences in the spectra of the samples.

Chemometric analysis

PCA was performed on the pretreated NMR spectra of all the studied *P. quinquefolius* samples. Fig. 3A presents the differences between samples from China and Canada. The samples from Jilin in China and the samples from Canada clustered into one group, while samples from Shandong in China were in a distinct group, which is similar result with our previous research(Huang *et al*., 2013). In order to further analyze the spectra of different samples, 16 main peaks were selected based on their intensity data.

These data were then imported into Excel to generate a curve graph (Fig. 3B). The figure shows the differences in content of the main compounds in various samples. It is interesting to note that the peaks in the range of 3.0 ppm to 5.5 ppm (sugar region) and those in the range of 0.5 ppm to 2.0 ppm (aliphatic region) show an opposite trend (Shin *et al*., 2007; Lee *et al*., 2009; Jocham *et al*., 2007). The peaks in the range of 0.5 ppm to 2.0 ppm and 3.0 ppm to 5.5 ppm are pointing to opposite directions, meaning that if higher 0.5 ppm to 2.0 ppm peaks are present, then lower 3.0 ppm to 5.5 ppm peaks are present, and vice versa.

This result shows that location may influence the quality of *P. quinquefolius* significantly. In United States Pharmacopeia USP35, the content of ginsenosides acceptance criteria is no less than 4.0% of total ginsenosides (Rg_1, Re, Rb_1, Rc, Rb_2 and Rd) on the dried basis, while in Chinese Pharmacopoeia 2015, the acceptance criteria is no less than 2.0% of total ginsenosides (Rg_1, Re and Rb_1) on the dried basis. The content requirement of ginsenosides is different in the two Pharmacopeias, which illustrate the importance of quality control.

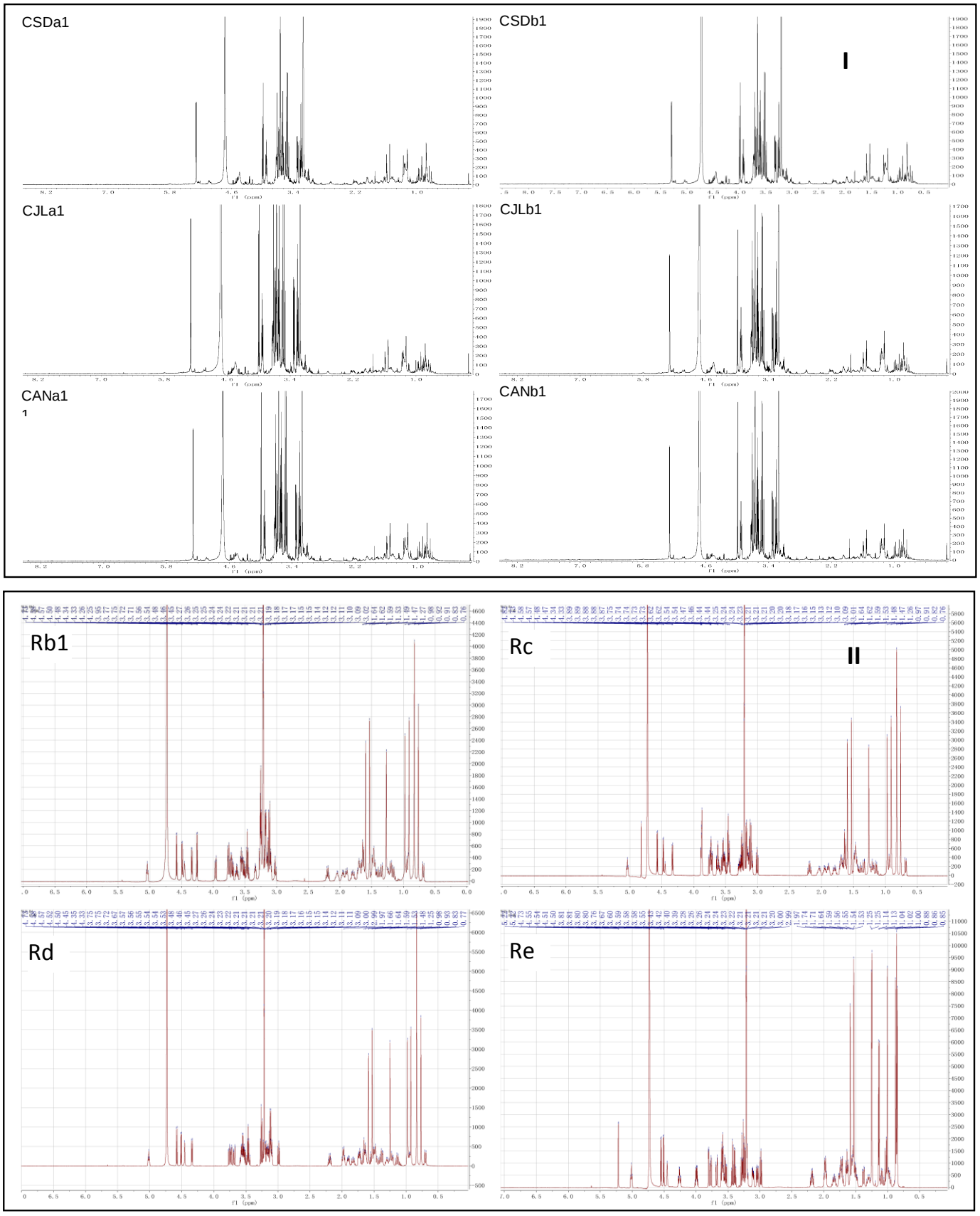

Fig. 2: I Representative ^{1}H NMR spectra of *P. quinquefolius*from China and Canada. II NMR spectra of ginsenoside Rb1, Rc, Rd and Re.

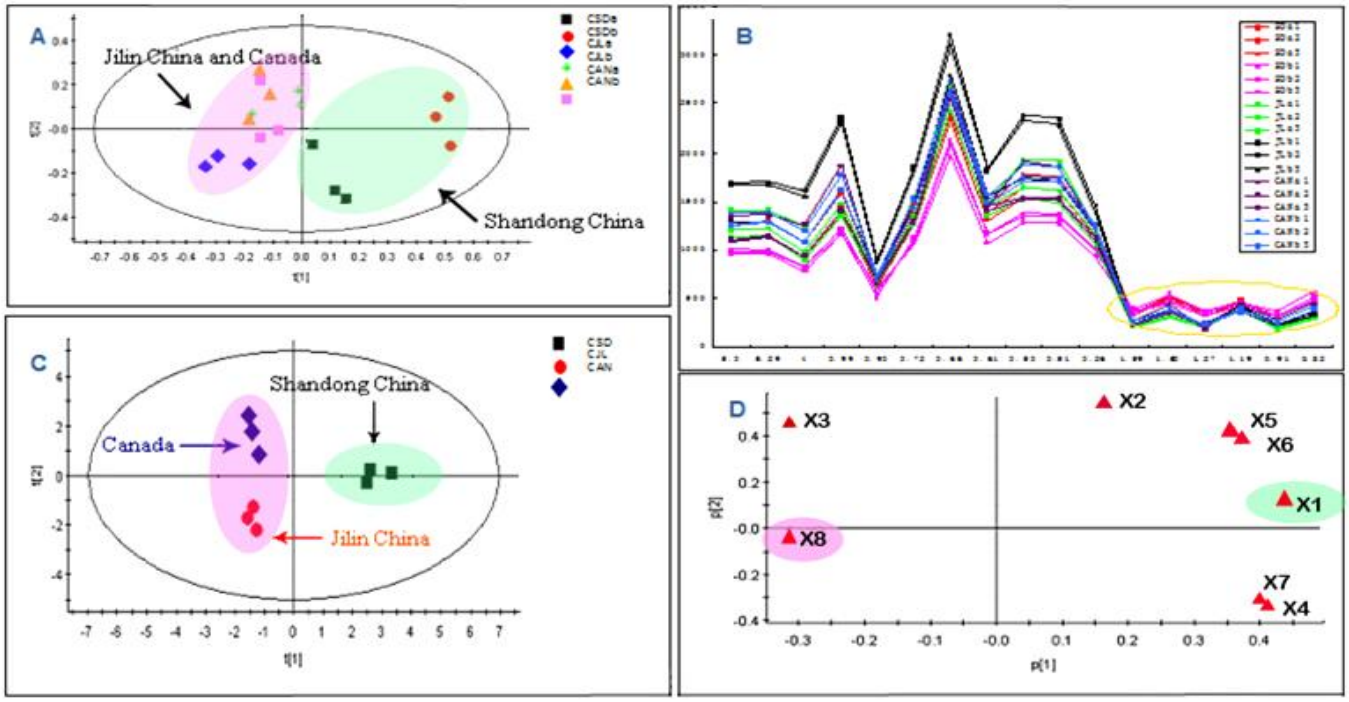

Fig. 3: A The PLS-DA analysis of different *P. quinquefolius* samples (R^2X [1]=0.400836 R^2X [2]=0.173504); B Intensities of selected peaks from 1H NMR profiles of Various *P. quinquefolius*; C The PLS-DA score plots of ecological factors of sampling location (R^2X [1]=0.570915 R^2X [2]=0.299042); D The PLS-DA loading plot of ecological factors of sampling location. X1 Annual air temperature; X2 Relative humidity; X3 Annual precipitation; X4 Annual average solar insolation; X5 Average air temperature in January; X6 Minimum air temperature in January; X7Average air temperature in July; X8 Maximum air temperature in July.

Ecological factors analysis and its effect on phytochemical composition

Principal component analysis was used to analyze the differences in climate in China (Shandong and Jilin) and Canada. The PLA-DA scores plot shows that ecological factors of Jilin in China and those in Canada clustered into one group, and those in Shandong, China clustered into another group (Fig. 3C). The PCA loadings plot indicated that the annual air temperature and maximum air temperature in July made a great influence on the discrimination of the 2 types of climate (Fig. 3D). Shandong has a relatively higher annual air temperature and relatively lower maximum air temperature in July,which of these above all verified our previous results.

The result was also validated by our previous experiments. In 2008, we reported that the northeast of China including Jilin is the most suitable area for the growth of *P. quinquefolius*, because the climate is very similar to North America (Chen *et al*., 2008). And in 2011, our data further showed the temperature was the most important environmental factors affecting the content of ginsenosides of *P. quinquefolius* roots (Dong, 2011). Jochum. GM, *et al*, demonstrated that plants grown, at high temperatures, had less root biomass and greater concentrations of storage root ginsenosides (49%), than plants grown at low temperatures (Jocham *et al*., 2007). In the present study, Shandong samples (grown at high environmental temperature) contain higher signal levels in the range of 0.5 ppm to 2.0 ppm, and the selected peaks, 1.59, 1.53, 1.27, 1.19, 0.91 0.82 ppm, were mainly correlated to the methyl group of ginsenosides that is referred to in the supplementary information (Fig. 2II). The samples from Jilin and Canada (grown at lower environmental temperature) contain relatively higher levels of signals in the sugar region. We performed discriminatory NMR-based chemical profile studies on *P. quinquefolius* roots from China and Canada. Samples from Shandong, China contain higher content of ginsenosides and relatively lower saccharides than those from Jilin in China and those from Canada. And chemical discrimination was in accordance with their ecological discrimination, and temperature factors were concluded as the most influential ecological variable. All the above results are consistent with our previous work. Consequently, we assume that the ecotype of Canadian *P. quinquefolius* was outside Great Wall due to the similar latitude to the Northeast of China.

CONCLUSION

The *P. quinquefolius* are observed with big differences in quality and ecotype between China and Canada, which indicate that the *P. quinquefolius* from Canada is the ecotype of outside Great Wall. This is the first time studied the ecotype distinction of *P. quinquefolius* around the world, and the present approach is

important and reliable to control the quality and distinguish ecotype.

AUTHORS'CONTRIBUTIONS

Linfang Huang initiate and all authors designed the study. The sample extraction was conduct by Zenghui Wang and Labin Wu, The method developments were conducted by Caimei Gu who drafted the manuscript. All authors contributed to the data analyses and to finalizing the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

ChristensenLP, JensenM, KidmoseU.Simultaneous determination of ginsenosides and polyacetylenes in *Panax quinquefolius* L. root (*Panax quinquefolium* L.) by high-performance liquid chromatography. Journal of agricultural and food chemistry. 2006;54(24):8995-9003.

LiSZ. Compendium of Materia Medica (Ben cao Gang mu), Beijing: Foreign Languages Press; 2004.

LiTS,MazzaG, CottrellAC.Ginsenosides in roots and leaves of *Panax quinquefolius* L.Journal of agricultural and food chemistry. 1996; 44(3):717–720.

Tang X, Gan X T, Rajapurohitam V, *et al*. North American ginseng (*Panax quinquefolius*) suppresses β-adrenergic-dependent signalling, hypertrophy, and cardiac dysfunction. Canadian Journal of Physiology and Pharmacology, 2016, 94(12): 1325-1335.

Qi B, Wang S, Wang Q, *et al*. Characterization and immunostimulating effects on murine peritoneal macrophages of a novel protein isolated from *Panax quinquefolius* L. Journal of Ethnopharmacology, 2016, 193: 700-705.

Lim W, MudgeKW, Vermeylen F.Effects of population, age, and cultivation methods on ginsenoside content of wild *Panax quinquefolius* L.Journal of agricultural and food chemistry. 2005; 53(22): 8498–8505.

LiuZY, ChenBG, Xie ZS.Advances in plant ecotype classification. Ecologic Science. 2004; 23(4): 365-369.

Odum EP. Fundamentals of Ecology Saunders: Philadelphia; 1997.

WangZH, HuangLF.*Panax quinquefolius*: An overview of the contaminants. Phytochemistry Letters. 2015; 11: 89-94.

WangYP*et al*. Chemical analysis of *Panax quinquefolius* (North American ginseng): A review. Journal of Chromatography A. 2015; 1426: 1-15.

Huang LF, *et al*.Quality variation and ecotype division of *Panax quinquefolium* in China.Acta Pharmaceutica Sinica. 2013; 48(4): 580-589.

Ludwiczuk A, Nyiredy S, Wolski T.Separation of the ginsenosides fraction obtained from the roots of *Panax quinquefolius* L. cultivated in Poland. Journal of Planar Chromatography-Modern TLC. 2005; 18(102): 104-107.

XuY,LiuY,JieS.Quality Assessment of Saponins from Aerial Parts of *Panax quinquefolius* L. by LC-ELSD Fingerprints and LC Quantitative Analysis. Planta Medica. 2011;77: 94.

ChanTWD *et al*.Differentiation and authentication of Panax ginseng, *Panax quinquefolius*. and ginseng products by using HPLC/MS. Analytical chemistry. 2000;72(6): 1281-1287.

Sun BS, Xu MY, Li Z.UPLC-Q-TOF-MS/MS analysis for steaming times-dependent profiling of steamed *Panax quinquefolius* L. and its ginsenosides transformations induced by repetitious steaming.Journal of ginseng research. 2012;36(3): 277-290.

Zhao HY *et al*. Metabolomic quality control of commercial Asian ginseng, and cultivated and wild *Panax quinquefolius* L. using ^{1}H NMR and multi-step PCA. Journal of Pharmaceutical and Biomedical Analysis. 2015;114: 113-120.

YangSO*et al*.NMR-based metabolic profiling and differentiation of ginseng roots according to cultivation ages. Journal of Pharmaceutical and Biomedical Analysis. 2012; 58: 19-26.

ShinYS, BangKH,InDS.Fingerprinting analysis of fresh ginseng roots of different ages using1H-NMR spectroscopy and principal components analysis.Archives of pharmacal research. 2007;30(12):1625-1628.

ParkSJ, Hyun SH,SuhHW.Biochemical characterization of cultivated Cordycepsbassiana mycelia and fruiting bodies by ^{1}H nuclear magnetic resonance spectroscopy.Metabolomics. 2013;9(1):236-246.

Kang J, Lee S, Kang S.NMR-based metabolomics approach for the differentiation of ginseng (Panax ginseng) roots from different origins.Archives of pharmacal research. 2008;31(3): 330-336.

Lee EJ, Shaykhutdinov R, Weljie AM.Quality assessment of ginseng by ^{1}H NMR metabolite fingerprinting and profiling analysis.Journal of agricultural and food chemistry. 2009;57(16): 7513-7522.

Chen SL, Zhou YQ, Xie CX. Suitability evaluation of *Panax quinquefolius* L.'s producing area based on TCMGIS-1. China Journal of Chinese Materia Medica. 2008; 33(7): 741-745.

Dong L. Study on the correlations between the quality characteristics of Panax ginseng, *Panax quinquefolius* L. and ecological factors. Zhengzhou: Henan University of Traditional Chinese Medicine; 2011.

Jochum GM, Mudge KW, Thomas RB.Elevated temperatures increase leaf senescence and root secondary metabolite concentrations in the understory herb *Panax quinquefolius* L. (Araliaceae). American journal of botany. 2007;94(5): 819-826.

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