

Correlation analysis between climatic factors and chemical components of *Panax notoginseng* grown in various regions in China

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(Received in revised form: November 13, 2021)

ABSTRACT

Major continuous cropping problems exist in *P. notoginseng* growing fields which lowers its quality than wild types. It is known that climate change significantly influences the plant chemical components. Therefore, analysis of relationship between climatic factors and the chemical components of *P. notoginseng* would be beneficial to find the climatic conditions required to increase the contents of desired chemical components. This can help in searching the new ginseng producing areas, to avoid continuous cropping problem and improve the quality of cultivated *P. notoginseng*. Precipitation and climate changes are the key climatic factors that affects the chemical components of *P. notoginseng*. Different chemical components have distinct or even opposite responses to climate factors. What's more, the effects of climate on different chemical components in *P. notoginseng* could be categorized. In this study, partial least-squares regression was introduced for the first time to analyze the effects of ecological environment on the chemical components. We analyzed the synthetic mechanism of chemical constituents of *P. notoginseng*.

Key words: Chemical components, climatic factors, continuous cropping problems, correlation, ginseng growing areas, ginseng quality, *Panax notoginseng*

INTRODUCTION

'Sanchi' is the dried root and rhizome of *Pana. notoginseng* (Burk.) F.H. Chen (Fam. Araliaceae). Its effects on human body includes hemostasis, blood circulation and blood supplementation. Besides, its active compounds saponins, are very helpful to improve the microcirculation, increase blood flow, dilate blood vessels, inhibit anti-blood cell condensation (21), anti-inflammatory (27), anti-tumor (19) and immune enhancement (4). According to Pharmacological Research, other ingredients in Notoginseng Radix et Rhizoma also have bioactivity. Flavonoids are antiviral, which prominently increases the survival rate of infected mice and also increases the interferon level in mouse spleen (20). Polysaccharides are immunomodulatory, which are used for treating type 2 diabetes and its complication(s) (10). Dencichine is useful to stop bleeding, increasing platelets and releases the blood-clotting substances (23).

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Generally, climatic factors consists of temperature, [including average annual temperature, effective accumulated temperature, mean monthly maximum temperature], precipitation, evapotranspiration and humidity (7,22). Temperature affects the metabolic rate, plants cells nutrients absorption and phytochemicals for adaptive changes (1). Precipitation affects the physical features and the growth of plants (15). It affects the photosynthetic capacity, leaf dark respiration, leaf hydraulic conductivity, leaf nitrogen and phosphorus content. In arid areas, leaf nitrogen, phosphorus content, leaf respiration in dark are high, but photosynthesis efficiency is low (26). The main influence of climatic factors is on the accumulation of secondary metabolites in medicinal plants (3,28).

This study mainly focusses on the chemical components of *Notoginseng Radix et Rhizoma*. Few studies have been done on correlations between the main components of *P. notoginseng* and climatic factors, however these needs further investigation (2). This study aimed to determine the characteristics of climatic factors in different *P. notoginseng* producing regions and to find the correlation between the main chemical components and climatic factors by principal component analysis (PCA), correlation coefficients, multiple stepwise regression analysis (SMRA), partial least-squares regression (PLS) and other analytical methods. The results indicated that climatic factors greatly affected the *P. notoginseng* chemical components. The Ginsenosides (R1, Rg1, Re, Rb1 and Rd) saponin components, were negatively correlated with mean annual temperature, but were positively correlated with precipitation. Likewise, the polysaccharides, total flavonoids and dencichine were positively correlated with precipitation but negatively correlated with mean daily temperature. In this study, we collected the *P. notoginseng* from different producing regions in China and analysed the relationship between chemical components and climatic factors to find the mechanism of production of chemical components (ginsenoside, saponins, flavonoids, polysaccharide and dencichine) which influences the quality of *P. notoginseng*.

MATERIALS AND METHODS

I. Extract preparation

Sixty roots of *Chunqi* (*P. notoginseng*) were collected from October to November 2018 from 16 producing regions in China (Table 1). The samples were washed and dried, then ground to powder and sieved through 100-mesh net screen to make powders. From the above powder, the saponins, flavonoids, polysaccharides, dencichine and other chemical components were extracted.

The specific sampling locations latitude and longitude of growing regions, were obtained through the official website National Catalogue Service For Geographic Information. Afterwards, Arc Gis 2.0 was used to collect the climatic factors in these areas (24).

Table 1. Details of *P. notoginseng* collection sites

Growing region*	Details of collection sites	Geographical Ordinates			Climatic Factors	
		Longitude	Latitude	Altitude (masl)	Temperature (°C)	Precipitation (mm)
Yunnan Province						
Bs-1	Xiyi Old Street, Longyang District, Baoshan City, Yunnan Province	106.43	23.09	1388	19.9	1489.0
Js-1	Dafeilong, Limin Township, Jianshui County, Yunnan Province	102.99	23.95	1797	16.2	1056.0
Km-1	Changhu Town, Shilin County, Kunming City, Yunnan Province	103.24	24.42	1905	15.2	1030.0
Lx-1	Xiaoshama Village, Xiangyang Township, Luxi County, Honghe Prefecture, Yunnan Province	103.88	24.33	2078	17.6	945.0
Lx-2	Taoyuan Village Committee, Baishui Town, Luxi County, Honghe Prefecture, Yunnan Province	103.49	24.40	1815	17.6	937.0
MI-1	Pingdi Village, Wushan Township, Hongxi Town, Mile County, Honghe Prefecture, Yunnan Province	103.57	23.97	1353	17.8	935.0
Mlp-1	Malipo Town, Malipo County, Wenshan Prefecture, Yunnan Province	104.42	23.12	1049	17.4	1322.0
Mz-1	Laozhai Township, Mengzi City, Honghe Prefecture, Yunnan Province	103.47	24.23	1901	18.8	913.0
Qj-1	Panjiadong Village, Baishui Town, Zhanyi County, Qujing City, Yunnan Province	103.98	25.60	2107	14.3	1014.0
Qj-2	Dagenze Villager Group, Wulong Village, Caiyun Town, Shizong County, Qujing City, Yunnan Province	103.56	24.45	1850	15.8	988.0
Ws-2	Baishapo Village, Kaihua Town, Wenshan City, Wenshan Prefecture, Yunnan Province	104.13	23.41	1778	17.4	1086.0
Xsbn-1	Xinghuo, Xiding Township, Menghai County, Xishuangbanna Prefecture, Yunnan Province	100.10	21.56	1805	19.4	1459.0
Ys-1	Zhicheng, Suburb, Jiangna Town, Yanshan City, Yunnan Province	104.20	23.35	1540	16.9	1163.0
Guangxi Province						
Gx-1	One Group, Liangbiao Village, Xinjing Town, Jingxi City, Guangxi Province	106.43	23.09	720	19.9	1489.0
Guizhou Province						
Gz-1	Tiechang Village, Danxia Town, Panzhou City, Guizhou Province	104.55	25.64	1783	14.8	1261.0
Sichuan Province						
Sc-1	Group 10, 6th Brigade, Minzhu Township, Longde Town, Daying County, Sichuan Province	104.52	30.46	305	16.3	939.0

* *P. notoginseng* growing regions Abbreviated names

II. Determination of chemical components

(i). Ginsenoside R1, Rg1, Re, Rb1 and Rd contents

The determination was done as per the Pharmacopoeia of the People's Republic of China (2020 edition). The reference substance of Notoginsenoside R1 and Ginsenoside Rg, Re1, Rb1 and Rd were weighed 5 mg each and dissolved in 50 ml methanol separately to produce a mixture containing 0.5 mg/ml as reference solution (17). The root tubers of *P. notoginseng* were cleaned, dried in shade and powdered. The powdered 600 mg material from different producing areas were separately weighed, dissolved in 50 mL methanol and kept overnight. It was boiled on the water bath at 80 °C for 2 h. After cooling, it was weighed again and made up the lost weight with methanol. Thereafter, it was shaken until homogenised and filtered with a 0.45-micron filter (17). Their contents were determined by a Waters XBridge™ BEH130 C₁₈ column (Waters Corp., Milford, MA, USA; 250 mm × 4.6 mm and 5 µm). The mobile phase was composed of (A) water and (B) acetonitrile and a gradient elution programme was used with 0-20 min at 80 % A, 20-45 min at 80-55 % A, 45-55 min at 55-45 % A and 55-60 min at 45-45 % A. The flow rate was 0.4 mL/min, detection wavelength at 203 nm, column temperature at 25 °C and injection volume at 20 µL. Methodological investigation results confirmed excellent precision, repeatability and stability. The mixed reference substance stock solution was diluted with methanol to 0.3333, 0.2000, 0.1000, 0.0500, 0.0333 mg/mL concentration. According to the chromatographic conditions, 20 µL sample was injected for determination, the linear relationship of each component was judged and the results were illustrated (8).

(ii). Saponins

Sample extracts were prepared by soaking and twice ultrasonically extracting 500 mg of powder in 6 mL of *n*-butanol saturated with water. The extract saponin contents were determined using vanillin-glacial acetic acid-perchloric acid colorimetry. Next, *P. notoginseng* saponins Chemical Reference Substance (CRS) was applied to plot the standard curve and measure the absorbance at the maximum wavelength (18).

(iii). Flavonoids

Flavonoids were measured by soaking 0.5g of powder into 70 % (by vol) ethanol, 1 h of ultrasonication, absorbing 6 mL of extract, addition of 0.1 M AlCl₃ ethanol solution to a final 8 mL volume. The following steps were used to measure the absorbance at maximal wavelengths, produce a standard curve with rutin CRS and measure the absorbance at the maximal absorption wavelength (12).

(iv). Polysaccharide content

Here, 500 mg of powder was extracted with 50 ml ultra-pure water in an ultrasonic water bath at 60 °C for 2 h and filtered while hot. Next, 20 ml of filtrate was reduced by heating at 65 °C to 10 mL. Absolute ethanol was then added to a final 50 mL volume and centrifuged at 9000 RPM for 10 min. The sediment was twice washed with 70 % ethanol and dried. Water was added to make final volume of 40 mL and heated on a water bath for 30 min. After cooling to room temperature, 1 mL of solution was taken and combined with 1 mL distilled water. After that, 1 mL of 5 % benzoquinone solution

was added and then 4 mL of concentrated H₂SO₄ was added. The mixture was heated on a boiling water bath for 30 min. The spectrometer detection wavelength was 490 nm and a standard curve was prepared using anhydrous glucose standards (9).

(v). Dencichine

It was determined as per the method of Li Yun (13). Five hundred mg of dencichine reference substance was weighed, dissolved in water ultrasonically and shaken to make a 0.7 mg/mL standard mother liquid. A Waters X Select HSS T3 column (4.6 × 100 mm, 2.5 μm) was employed with a mobile phase composed of (A) water and (B) acetonitrile. Gradient elution programme was 0-3 min at 91-86 % A, 3-4 min at 86 % A and 4-5 min at 86-91 % A. The flow rate was 0.5 mL/min, column temperature was 25 °C and detection wavelength was 213 nm (9).

One mg/mL *Panax notoginseng* standard was prepared and serially diluted 5-times according to a 40 % gradient. Six standard solutions with different concentration gradients were obtained. The content of dencichine was determined according to the chromatographic conditions under 4.1.2.8. For the content, the peak area was recorded; taking the peak area as the abscissa and the concentration as the ordinate, the linear regression equation was obtained. Then linear index was calculated, which indicated that the linear relationship was good.

III. Statistical analysis

For climatic factors and *P. notoginseng* chemical composition, the common factor score chart of PCA in SPSS 21 software was used to compare the relationships between the producing regions and samples, with factor loading used to evaluate the correlation between the factors. Correlation analysis and SMRA (SPSS 21 software) were used to analyze their relationships as well as the dominant ecological factors that affected chemical composition. A multiple regression model was established. At the same time, PLS of Minitab 19 was used to obtain the relationship between each chemical composition and climatic factor, thus combining with the aforementioned methods for mutual verification and reference.

RESULTS AND DISCUSSION

I. Climatic factors in producing areas

For the *P. notoginseng* sampling sites, we collected following climatic factors :
(i). Temperature (annual average temperature, the average temperature of the hottest month, coldest month, driest season, and wettest season, seasonal changes in temperature, isothermality, mean annual temperature range, mean daily range of temperature) and
(ii). Precipitation (annual precipitation, seasonal changes in precipitation, and precipitation of driest season, wettest season, coldest season and hottest season). Correlation analysis was done among the climatic factors, there was significant correlation between most variables and it was suitable to use PCA to transform highly correlated variables into mutually independent variables.

Although most of these environmental variables were highly correlated, it was impossible to consider the 21 climatic factors in the actual application process at the same time. Therefore, it was necessary to select the principal components from climatic characteristics and find the aggregate variables affecting and reflecting the effects of multiple factors to make sure that the analysis and evaluation was simpler and more accurate. The cumulative contribution of principal component 1, principal component 2 and principal component 3 reached 72.41 % (Table 2). The first three principal components contained most of the characteristic information of the original climatic factors, with high credibility and reflecting the influence of climatic factors. Subsequently, the scores of principal component analyses were used to reflect correlation between the factors (Fig. 1). PCA results of the climatic factors from *P. notoginseng* producing areas were also found to be closely related, except for few outliers. Consequently, the results demonstrated that *P. notoginseng* was greatly affected by the environment of production regions and had higher requirements for climate and environment in some producing regions. These results agree with growth habit of *Panax notoginseng*, grown in controlled environment.

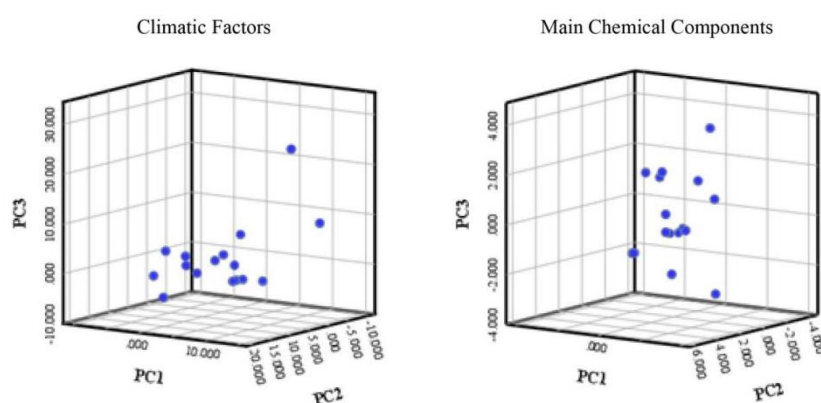


Figure 1. PCA of climatic factors and main components

Table 2. Effects of climatic factors and producing regions on Principal components

Principal component (PC)	Initial Eigen values			Adjusted Sums of Squared Loadings		
	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)
Climatic factors						
PC1	7.65	36.43	36.43	5.853	27.873	27.873
PC2	5.579	26.569	62.999	5.044	24.019	51.891
PC3	4.099	19.519	82.518	4.309	20.519	72.41
PC4	1.573	7.488	90.006	3.075	14.645	87.055
Producing regions						
PC1	3.794	37.945	37.945	3.371	33.713	33.713
PC2	1.735	17.348	55.293	2.107	21.07	54.783
PC3	1.653	16.527	71.819	1.704	17.036	71.819
PC4	0.943	9.425	81.244	-	-	-
PC5	0.639	6.391	87.635	-	-	-

PC : Principal component

II. Chemical components and analysis of samples

Samples collected from different ginseng producing areas were analysed for R1, Rg1, Re, Rb1, Rd, polysaccharides and flavonoids (Table 3). The contents of these chemical components of sample collected from site *qj-1* were low. The range of each chemical composition was relatively large, indicating that the external environment had great influence on the plant composition. Among these components, the coefficient of variation of flavonoids and R1 content was highest, indicating that both were most affected by the external environment.

Table 3. Main chemical components contents (%) in *P. notoginseng* from various growing areas

Ginseng Producing area	Ginsenosides contents (%)					Total saponins	Polysaccharides	Flavonoids	Dencichines
	R1	Rg1	Re	Rb1	Rd				
Bs-1	0.527	5.263	0.621	3.352	0.824	11.212	14.265	0.943	4.700
Js-1	1.181	5.178	0.517	3.177	1.165	9.614	11.979	0.454	3.680
Km-1	0.790	5.008	0.513	3.395	1.098	9.226	13.433	0.874	3.757
Lx-1	1.348	4.124	0.564	2.973	0.925	13.190	16.371	0.504	3.857
Lx-2	0.666	4.155	0.558	2.576	1.044	9.402	16.005	0.576	3.373
Ml-1	0.424	4.493	0.653	4.155	0.926	8.494	12.074	0.161	3.693
Mlp-1	1.318	4.166	0.737	2.851	0.869	9.277	22.527	0.741	3.803
Mz-1	0.838	3.920	0.549	2.451	0.749	11.243	9.057	0.582	3.110
Qj-1	0.625	3.964	0.516	2.812	0.823	6.375	13.319	0.517	3.677
Qj-2	0.679	2.826	0.233	2.569	0.654	8.267	13.844	0.907	5.257
Ws-2	0.622	5.342	0.667	2.920	1.068	8.124	21.605	1.077	3.680
Xsbn-1	0.509	2.887	0.364	2.447	0.637	6.740	18.451	0.439	3.283
Ys-1	0.537	6.231	0.634	3.656	1.550	9.277	22.527	0.741	4.190
Gx-1	0.474	3.684	0.671	3.059	0.948	7.607	17.564	1.181	5.860
Gz-1	0.687	3.433	0.527	2.562	0.840	7.087	19.032	0.279	4.393
Sc-1	0.227	3.142	0.377	1.161	0.226	6.663	10.063	0.468	5.400
CV	0.028	0.014	0.015	0.014	0.020	0.013	0.017	0.027	0.012
Range	1.122	3.406	0.505	2.993	1.324	6.815	13.470	1.020	2.750

PCA done on the chemical components of *P. notoginseng* from 16-producing regions showed that the cumulative contribution of Principal component 1, Principal component 2 and Principal component 3, in producing areas, was 71.82 % (Table 2), indicating that the results are highly reliable. Therefore, these 3- Principal components were the axis, reflects the fundamental characteristics and informations about the sample chemical composition (Fig. 1). The qualities of sample chemical components from different producing regions were fairly scattered, which was inconsistent with the PCA results of climatic factors. This might have been caused by the conditions influencing the content of chemical components and their inability to reflect the climatic factors (9).

Table 4. Correlation coefficients between the chemical components of *P. notoginseng* and climatic factors

Climate factor	Ginsenosides contents (%)					Total saponins	Polysaccharides	Flavonoids	Dencichines
	R1	Rg1	Re	Rb1	Rd				
	Temperature								
Mean temperature	-	-	-	-	-	-	-	-	-
Mean temperature in the driest season	-	-	-	-	-	-	-	-	-.371
Mean temperature of wettest season	-	-	-	-	-	-	-	-	-
Mean annual range of temperature	-.413	-.638	-.647	-.685	-.774	-	-.611	-.360	-
Lowest temperature of coldest month	-	-	.382	-	-	-	.358	-	-
Highest temperature of hottest month	-	-.449	-	-.397	-.357	-	-	-	-
Seasonal changes in temperature	-	-	-	-.543	-.437	-	-	-	.622
Isothermality	-	-	-	.483	-	.367	-	-	-.638
Mean daily range of temperature	-	-	-	-	-	-	-	-	-.520
Lowest temperature of coldest season	-	-	-	-	-	-	-	-	-.364
Lowest temperature of hottest season	-	-	-	-.367	-	-	-	-	-
	Precipitation (Rainfall + Snow etc)								
Precipitation of coldest season	-	-	-	-	-	-	.352	.546	.403
Precipitation of hottest season	-	-	-	-	-	-	.560	.381	.416
Precipitation of driest season	-	-	.371	.382	-	-	-	.585	.454
Precipitation of wettest season	-	-	-	-	-	-.421	.551	-	-
Seasonal changes in precipitation	-	-.449	-	-.607	-.480	-.541	-	-	-
Precipitation of driest month	-	-	-	-	-	-	-	.536	.521
Precipitation of wettest month	-	-	-	-	-	-.452	.566	-	-
Average Annual Precipitation	-	-	-	-	-	-.360	.620	-	-
	Longitude								
Longitude	-	-	-	-	-	-	-	-	-
	Latitude								
Latitude	-	-	-	-.573	-.562	-	-.513	-	.421

III. Correlation analysis between climatic factors and chemical components

Correlation Coefficients

With correlation analysis between the climatic factors and the sum of samples chemical components, the result showed that the 5-saponins and polysaccharides contents were negatively correlated with the annual temperature range. Saponins were negatively correlated with the precipitation, while polysaccharides, flavonoids and dencichine were positively correlated with precipitation (Table 4). The changes in temperature and precipitation were the main factors affecting the accumulation of saponins (active ingredient in *P. notoginseng*). The geographical location significantly impacted the climate and the accumulation of different chemical components in *P. notoginseng*.

Climate

The correlation between specific chemical components and climatic factors was further explored by studying the chemical components of samples from different producing areas as dependent variable *Y* and 21-climatic factors as independent variables. Besides, the content of climatic factors and the chemical composition of *P. notoginseng* were analyzed by SMRA and a stepwise regression equation was developed for each component. Taking the content of R1, Re, polysaccharide and dencichine of *Panax notoginseng* in different production areas as the dependent variable *Y*, and climate factors as the dependent variables, a step-wise multiple regression analysis was done for the climate factors and the chemical composition content of *Panax notoginseng*.

Regression Equations

- (i). **Rg1 and Climate:** The regression equation of Rg1 and climate factors was: $Y=6.840-0.856x_1+0.555x_2+0.074x_3$, x_1 : mean annual range of temperature (°C), x_2 = latitude, x_3 = isothermality (°C), $R=0.842$, $F=8.471$, $P=0.003$.
- (ii). **Rd and Climate:** The regression equation of Rd and climate factors was: $Y=4.866-0.158x_1-0.002x_2$, x_1 : mean annual range of temperature (°C), x_2 =precipitation of wettest month (mm), $R=0.808$, $F=12.264$, $P=0.001$.
- (iii). **Polysaccharide:** The regression equation of polysaccharide and climate factors was: $Y=34.231+0.011x_1-1.352x_2$, x_1 : average annual precipitation (mm), x_2 : mean annual range of temperature (°C), $R=0.768$, $F=9.328$, $P=0.003$.
- (iv). **Dencichine and Climate:** The regression equation of dencichine and climate factors was: $Y=34.231+0.011x_1-1.352x_2$, x_1 : Isothermality (°C), x_2 : precipitation of coldest season (mm), $R=0.828$, $F=14.169$, $P=0.001$.

In the actual process, the *R* was too small in many models (the step-wise regression analysis of R1 content, Re content, Rb1 content, total saponins content and flavonoid content) and the correlation coefficient was low. This indicated that the experimental result was unsatisfactory and needed further optimization.

Model

The accuracy of the model was improved by further analyzing the correlation between the chemical components and climatic factors and the non-linear iterative partial least squares (NIPALS) algorithm developed by Herman Wold, to solve problems associated

with ill-conditioned data (25). Observations with standardized residuals $> +/-2$ were outside the horizontal reference lines on the plot, which meant they were outliers and the leverage value was $> 2m/n$ (m and n , the number of components and observations), respectively. The right side of the vertical dashed line represented a leverage point with extreme values. Because of the existence of outliers and leverage, the R -square value of the step-wise multiple regression analysis was poor, but a new step-wise regression equation was possible after removing the outliers. Considering the content of R1, Re, Rb1, total saponins and flavonoids of *P. notoginseng* in different production areas as the dependent variable Y , and 11-climate factors as the dependent variables, a step-wise multiple regression analysis was performed on the climate factors and the chemical components content of *P. notoginseng* and we obtained following Regression equations.

Regression Equations

- (i). **R1 and Climate:** The regression equation of R1 and climate factors was: $Y=7.625+0.088x_1-0.116x_2-0.092x_3$, x_1 : seasonal changes in precipitation (mm), x_2 =longitude, x_3 = mean annual range of temperature ($^{\circ}\text{C}$), $R= 0.774$, $F= 4.481$, $P= 0.035$.
- (ii). **Re and Climate:** The regression equation of Re and climate factors was: $Y= 0.932-0.040x_1+0.011x_2-0.037x_3+0.011x_4$, x_1 : Mean annual range of temperature ($^{\circ}\text{C}$), x_2 =precipitation of coldest season (mm), x_3 = precipitation of driest month (mm), x_4 = seasonal changes in temperature ($^{\circ}\text{C}$), $R= 0.967$, $F= 28.754$, $P= 0.000$.
- (iii). **Rb1 and Climate:** The regression equation of Rb1 and climate factors was: $Y= 10.462- 0.337x_1+0.161x_2-0.004x_3$, x_1 : Mean annual range of temperature ($^{\circ}\text{C}$), x_2 : average annual precipitation (mm), x_3 = precipitation of wettest season (mm), $R=0.804$, $F= 5.48$, $P= 0.020$.
- (iv). **Total saponins and Climate:** The regression equation of total saponins and climate factors was: $Y= 6.742+0.155x_1-0.005x_2$, x_1 : precipitation of driest season (mm), x_2 = average annual precipitation (mm), $R= 0.793$, $F= 7.642$, $P= 0.011$.
- (v). **Flavonoids and Climate:** The regression equation of flavonoids and climate factors was: $Y= 0.194+0.093x_1-0.069x_2$, x_1 : precipitation of driest month (mm), x_2 = mean daily range of temperature ($^{\circ}\text{C}$), $R= 0.712$, $F= 4.638$, $P= 0.041$.

The above step-wise regression equations showed that the mean annual range of temperature and daily temperature range reduced the most chemical components, while, these were increased by precipitation in driest and wettest seasons, annual precipitation, seasonal changes in precipitation and latitude. Multiple step-wise regression analysis (MRS) was employed to confirm that the variables were independent of each other and did not have multicollinearity. Therefore, the Variance Inflation Factor (VIF) value in the result test was < 10 , which met the assumptions of MSRA. However, there was mutual relationship among the variables (climatic factors), which suggests unreasonable explanations to estimate the regression coefficients. Hence to compensate the shortcomings in this method, it was necessary to evaluate relationships from more perspectives and use PLS to find the relationship between each factor and main component (Fig. 2).

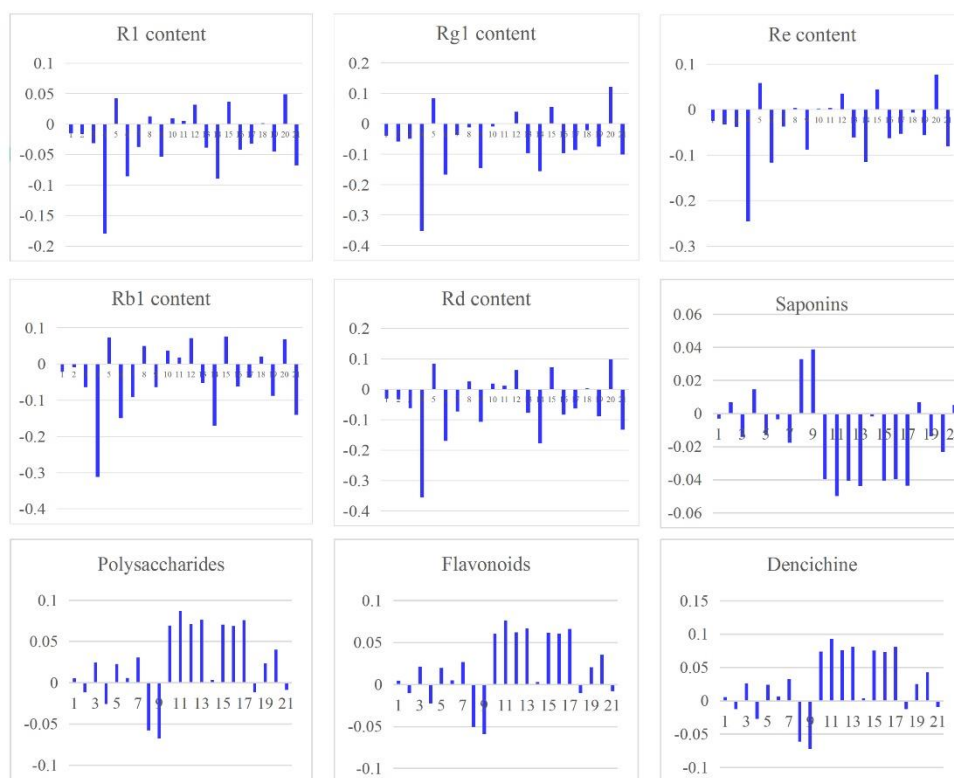


Figure 2. PLS Std Coefficient plot of main components (1-21 on the abscissa correspond to 21 climate factors, respectively).

In the graph of the normalization coefficient of PLS, the element with the longest bar had the largest normalization coefficient and the greatest impact on the response value. The elements above the centre line had positive correlation with the response value and vice versa. Similarly the components affected by climate were congregated into one group. While, the 5-saponins were grouped, these were negatively correlated with the mean annual range of temperature, highest temperature of the hottest month, seasonal range of precipitation and latitude, while, positively correlated with the longitude and lowest temperature of the coldest month. The Flavonoids, polysaccharides and *P. notoginseng* were grouped in one category, because their influence on these components was opposite to saponins. The saponins contents were positively correlated with isothermality (mean daily range of temperature). While, flavonoid, polysaccharide, and dencichine contents were correlated with the precipitation (of coldest, hottest, driest, and wettest quarters, driest and wettest months, annual precipitation), mean temperature of hottest quarter and longitude.

Temperature : There were significant differences in the influence of climate of the producing areas on various chemical components of *P. notoginseng*. The lowest temperature

of coldest month was positively correlated with the content of the five saponins, because the average monthly temperature in winter in Yunnan-Guizhou region (11°C) could support the growth of living stems and leaves of *P. notoginseng* and the higher temperature helped the root in nutrients accumulation (14). The reason why temperature change (mean annual and daily temperature range) was related to chemical components was that the producing area was prone to short-term local frost and low-temperature in winter and spring, late summer and early autumn. These plants are greatly affected by such weather disasters, which decreases the content of 5-saponins, polysaccharides and flavonoids.

Precipitation : The precipitation was negatively correlated with saponins contents, which indicated that moderate drought stress was conducive to saponin accumulation (2). Among the 5-types of saponins, the accumulation of Rg1, Re, Rb1 and Rd of the protopanaxatriol-type ginsenosides were less affected by precipitation than ginsenoside Rb1 and Rd (protopanaxadiol-type ginsenosides). Higher precipitation in the wettest month and quarter could reduce saponins accumulation, which was consistent with our results from previous studies (29).

The temperature and precipitation affects the soil conditions and microbial environment, which affects the growth and the chemical composition of plants. For instance, the soil organic carbon is the most significant factor that determines the upper limit of plant yield. Temperature and precipitation greatly influence the soil organic carbon content. Temperature and precipitation influences the soil microbial composition (7,30) and the soil microbial composition affects the content of secondary metabolites in medicinal plant species (6). The temperature and precipitation influences the chemical components of *Panax notoginseng*, these may be mediated by the microbial community (5,23).

In PCA comprehensive evaluation model, there was a very low correlation between the producing regions with high climatic factor scores and high chemical component scores, which differs from previous literature (13). This might be due to the main component comprehensive score only evaluated the producing areas with high values of climatic factors. However, the high temperature and precipitation had no clear linear correlation with chemical components contents.

CONCLUSIONS

This study analyzed the correlation between climatic factors and the main chemical components (ginsenosides R1, Rg1, Re, Rb1, and Rd, polysaccharides, flavonoids and dencichine) in *P. notoginseng*. MSRA and PLS models were constructed and the results revealed that precipitation and temperature changes were the two most critical factors affecting the chemical content of the samples, with different chemical components having variable or even opposite sensitivities to climatic factors. This indicated that the content of a certain component could be increased by choosing the appropriate ginseng producing region.

The limitations of a single analysis method were avoided by integrating correlation coefficient, PCA, MSRA, and PLS analytical methods, with the integration were found useful to obtain more comprehensive and reliable conclusions. PLS overcome the defect that

the results of correlation coefficients were scattered. There were insufficient independent variables in MSRA for independent variables with correlation, which showed that chemical compositions in *P. notoginseng* was affected by climatic factors. It provided new ideas for correlations between climatic factors and chemical compositions. Nevertheless, the saponins, flavonoids and other chemical components of medicinal plant are very complex. The types of active ingredients in *P. notoginseng* investigated in this study were limited. Further studies will explore the relationship between climatic factors and the clinical effects of *P. notoginseng* in promoting blood circulation and other effects to cultivate *P. notoginseng* with better medicinal qualities.

ACKNOWLEDGEMENTS

This study was financially supported by the Fundamental Research Funds for the Central Universities of China, the “Comprehensive evaluation of the quality of *Panax notoginseng* medicinal materials from different origins based on the concept of precision medicinal materials” project (2020-JYB-ZDGG-049), Open Research Fund of Zhejiang Tiantong Forest Ecosystem National Observation and Research Station (TTK201905), Hangzhou West Lake Scenic Area Science and Technology Development Project (2019-008).

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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