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Comparison of *in-vivo* and *in-vitro* blood activating effects of *Panax notoginseng*

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ABSTRACT

Through *in-vitro* experiments, we screened the *P. notoginseng* from 10- producing areas for the greater anti-platelet aggregation effects. Anti-mouse tail thrombosis, anticoagulation and anti-hemostasis experiment were done to compare the *in-vivo* blood-activating effects of *P. notoginseng* from 10-different areas. The results showed that the impact of *P. notoginseng* from the Baoshan, Yanshan and Mengzi was better. This study provided a basis for selecting improving blood circulation. It will be helpful to ensure the effectiveness of the clinical application of *P. notoginseng.*

Keywords: Anticoagulation, anti-hemostasis, anti-platelet aggregation, anti-thrombosis, origin difference, *Panax notoginseng*

INTRODUCTION

The *Panax notoginseng* (Burk.) F. H. Chen (family: Araliaceae), dried root and rhizome are called Sanchi in China. It has sweet and slightly bitter taste and warm in nature. It has powerful effects to improve blood circulation, remove blood stasis, reduce swelling, relieve pain, and treat various bleeding diseases and cardiovascular system diseases. It stops bleeding, without blood stasis and injury. The Notoginseng Radix et Rhizoma is effective in anti-atherosclerosis (15), vasodilation (7), anti-thrombosis (5,11), regulates vascular function and tissue remodelling (16), to treat hypertension, coronary heart disease and other cardiovascular diseases (9). In recent years, with the increasing demands of *Panax notoginseng,* its production area has been expanded. To ensure the efficacy of *P. notoginseng* use, it is necessary to study and compare its effects on improving blood circulation.

Guangxi Province was the earliest main producing area of *P. notoginseng* (4,12) in China. However, due to serious continuous cropping obstacles, cultural and social factors, the main producing area of *P. notoginseng* has been moved to Wenshan, Yunnan (12), which is currently recognized as the authentic producing area of *P. notoginseng*. With the increasing demand for *P. notoginseng* and the restriction of continuous cropping obstacles, the production area of *P. notoginseng* has been gradually expanded to Honghe, Kunming,

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Chuxiong, Dali, Qujing, Yuxi, Lincang and Baise City of Guangxi (10). The quality of *P. notoginseng* is greatly affected by internal and external factors, such as germplasm, soil, climate, cultivation, processing, etc. (21). Therefore, *P. notoginseng* produced from different producing areas, may differ in quality and efficacy due to variable soil and climatic conditions.

In the *Pharmacopoeia of the People's Republic of China* (2020 Edition) on Chinese medicine's, strict quality standards have been established for all sources of Chinese medicine. It is necessary to ensure the clinical use of *P. notoginseng* for better human health. This study aimed to determine the blood-activating effects of *P. notoginseng* from 22 different areas, through *in-vivo* and *in-vitro* experiments.

MATERIALS AND METHODS

(i). **Sample collection:** The *P. notoginseng* samples were collected from 22 different producing areas, (19 sites in Yunnan Province and one each in Guangxi, Guizhou and Sichuan provinces) (Table 1). The collection site serial number, longitude, latitude, altitude and annual precipitation are also given.

(ii). **Reagents:** Adenosine diphosphate disodium salt, Carrageen glue, Sodium carboxymethyl cellulose 3.8 % sodium citrate anticoagulant. All chemical reagents were purchased from Shanghai yuanye Bio-Technology Co., Ltd. China.

Experimental animals

Experimental animals: Male SD rats (SPF grade, about 200 g) and male ICR mice (SPF grade, 20-22 g) were purchased from Beijing Huafukang Biotechnology Co. Ltd.

Experimental instrument

Spark 20 m full-wavelength enzyme labeling instrument, Swiss TECAN company; 3K15 centrifuge, German Sigma company.

II. Experimental design

Preparation of *P. notoginseng* powder

The root tubers of *P. notoginseng* were cleaned and dried naturally in shade. Then they were baked at 45 ℃ for 8 h, 55 ℃ for 40 h in the oven. After drying, the root tubers were powdered and screened through 100 mesh. The powder was stored in Refrigerator for later use.

Extraction of *P. notoginseng* saponins

The *P. notoginseng* saponins were extracted as per Li Qing (6) method. We mixed 2.0 g *P. notoginseng* powder with 10 mL methanol for 20 s and extracted by ultrasonic for 2 h at room temperature. The supernatant was transferred to 25 mL volumetric flask. Methanol solution was added to volumetric flask scale and the resulting solution was filtered through 0.22 μm filter. Twenty mL filtrate was dried in a rotary steamer at 65 ℃ and dissolved in 3 mL deionized water. It was dried by freeze-drying with a vacuum freeze-dryer to get the freeze-dried powder of *P. notoginseng* saponins. The freeze-dried powder of *P. notoginseng* saponins was dissolved in distilled water and the concentration was adjusted to 50 mg/mL, which was used to experiment with anti-platelet aggregation *in-vitro*.

Table 1. Sample collection sites of *P. notoginseng* and their Geographical locations

Serial number	Sample collection site	longitude	latitude	Altitude (m)	Annual precipitation (mm)
Yunnan Province					
Ws-1	Xiaojie Village, Wenshan City, Wenshan Prefecture, Yunnan Province	104.0275	23.2580	1778	1128
Ws-2	Baishapo Village, Kaihua Town, Wenshan City, Wenshan Prefecture, Yunnan Province	104.1622	23.4192	1390	2600
Ys-1	Jiaozhi City, Jiangna Town, Yanshan City, Yunnan Province	104.3453	23.6102	1540	990
Ys-2	Asanlong Village, Pingyuan Town, Yanshan County, Wenshan Prefecture, Yunnan Province	103.6714	23.7819	1459	937
Ys-3	Huilong Base, Huilong Community, Pingyuan Town, Yanshan County, Wenshan Prefecture, Yunnan Province	103.7746	23.6841	1476	937
Xc-1	Wangjiatang Village, Jijie Town, Xichou County, Wenshan Prefecture, Yunnan Province	104.8196	23.5225	1073	1294
Mlp-1	Malipo Town, Malipo County, Wenshan Prefecture, Yunnan Province	104.7032	23.1261	1049	1010
Qb-1	Shikazi Village,Nijiaoyizu Township, Qiubei County,Wenshan Prefecture, Yunnan Province	103.7652	23.8763	1494	1200
Ml-1	Pingdi Village, Wushan Township, Hongxi Town, Mile County, Honghe Prefecture, Yunnan Province	103.5868	23.9794	1353	950
Js-1	Dafeilong, Limin Township, Jianshui County, Yunnan Province	103.0085	23.9512	1797	642
Lx-1	Xiaoshama Village, Xiangyang Township, Luxi County, Honghe Prefecture, Yunnan Province	103.8898	24.5233	2078	979
Lx-2	Taoyuan Village Committee, Baishui Town, Luxi County, Honghe Prefecture, Yunnan Province	103.8178	24.6556	1815	900
Mz-1	Laozhai Township, Mengzi City, Honghe Prefecture, Yunnan Province	103.7868	23.3970	1901	1300
Qj-2	Dayize Village, Wulong Village, Caiyun Town, Shizong County, Qujing City, Yunnan Province	103.9251	24.7539	1850	1100
Qj-3	Dawulong Village, Wulong Village, Caiyun Town, Shizong County, Qujing City, Yunnan Province	103.9218	24.7744	1868	1100
Qj-1	Panjiadong Village, Baishui Town, Zhanyi County, Qujing City, Yunnan Province	103.9981	25.6085	2107	988
Bs-1	Xiyi Old Street, Longyang District, Baoshan City, Yunnan Province	99.0254	24.9961	1388	1000
Km-1	Changhu Town, Shilin County, Kunming City, Yunnan Province	103.4055	24.7045	1905	1069
Xsbn-1	Xinghuo Hill, Xiding Township, Menghai County, Xishuangbanna Prefecture, Yunnan Province	100.1590	21.9360	1805	1740
Guanxi Province					
Gx-1	One Group, Liangbiao Village, Xinjing Town, Jingxi City, Guangxi Province	106.4515	23.0963	720	1630
Guizhou Province					
Gz-1	Tiechang Village, Danxia Town, Panzhou City, Guizhou Province	104.5504	25.6404	1783	1424
Sichun Province					
Sc-1	Group 10, 6th Brigade, Minzhu Township, Longde Town, Daying County, Sichuan Province	105.4135	30.6183	305	925

Dose screening and animal administration

The oral dose of *P. notoginseng* was usually 3 g/(d ·50 kg) (3,14), and the equivalent dose of *P. notoginseng* powder in mice was 0.70 g/kg (2), according to body surface area. Zhang (20) and Du (2) *et al*. found that the medium and high dose of *P. notoginseng* powder could significantly inhibit thrombosis. Therefore, to obtain the best administration dose, the human equivalent dose of 1-10 times was set in this experiment to screen the administration

dose. Ten mice in the administration dose group and one normal saline group were assessed. The normal saline group was taken as the blank control group and the mice were given oral administration with the administration volume of 0.2 mL/10g. The control group was given the same volume of normal saline. The animals were randomly divided into 11 groups, with 3 mice in each group and 33 mice in total. Then from the blood of mice in different dose groups and isolated platelet-rich plasma were taken respectively. We adjusted its concentration to a suitable value and added the appropriate amount of ADP solution. The peak of platelet aggregation and peak time were seen, and then the platelet maximum aggregation (%) and aggregation inhibition rate were calculated. The determination of indexes was the same as the anti-platelet aggregation test.

Comparison of anti-platelet aggregation ability *in vitro*

According to Yang (17) method, fresh rat blood containing 3.8 % sodium citrate anticoagulant was centrifuged at 22 ºC for 9 min at 1100 r/min, and the upper liquid was slowly transferred to another centrifuge tube to obtain platelet-rich plasma (PRP). The remaining part was centrifuged at 3000 r/min for 10 min, and the supernatant layer was taken to obtain platelet-poor plasma (PPP).

Twenty-two *P. notoginseng* administration groups from different producing areas, 5 mg/mL aspirin positive control group, and the blank group were set up in a total of 24 groups. Specific grouping and drug administration were shown in Table 2. According to the method of Yu (18), a 96-well plate was laid, and 100 μL platelet/hole was added first, each group was set up in five-holes. Then medication was given according to the grouped situation. The optimal dosage of medicine was obtained by screening in the administration group. The blank and positive control groups were given the same amount of water or aspirin aqueous solution.

Table 2. The specific groups and drug administration

Serial number	Group	Drug administered
1	Blank group	100 μL platelet + 50 μL water + 50 μL ADP
2	Positive control group	100 μL platelet + 50 μL aspirin solution + 50 μL ADP
3-24	Drug administration groups from different places of origin	100 μL platelet + *P. notoginseng* powder solution of different origin +50 μL ADP

50 μL ADP was added to induce blood coagulation 30 min later, mixed for 30 seconds. FLASH continuous spectrum fluorescence enzyme labeling instrument (405 nm wavelength, the 50 s/times, 15 min) was used to detect platelet aggregation dynamically. With PPP as blank control, the maximum aggregation rate (MAR) of platelets was obtained, when the transmittance of PRP changed to maximum. Then we calculated the maximum aggregation (%) and aggregation inhibition (%) to find the anti-platelet aggregation ability.

Comparison of the anti-thrombotic ability

According to the *in vitro* anti-platelet aggregation experiment results, *P. notoginseng* from 10 producing areas with better anti-platelet aggregation effects were selected as the experimental research samples of anti-thrombotic ability, anticoagulation ability and anti-hemostasis ability. Mice were divided into 13 groups, including blank group, model group, aspirin positive control group, and 10 administration groups, with 7 healthy ICR mice male mice in each group. All mice were first fed in an environment of about 15 ℃ with humidity of 30 % - 50 % for 7 days.

We constructed the mice's tail thrombosis model after the adaptive feeding. The mice were given the finest powder of *P. notoginseng* orally. According to previous dose screening experimental results, ICR mice in the administration group, the positive control group, the model group, and the blank group were administered. The administration method was as under : The administration group was intragastrically administered with suspension made of 1 % sodium hydroxymethyl cellulose (CMC-Na) and *P. notoginseng* powder; the positive control group was intragastrically administered with suspension made of 1 % CMC-Na and the appropriate amount of aspirin powder (13); the blank group and model group were given the same volume of CMC-Na at the same time; Once a day for consecutive 7 days, the mice were fasting the night before modelling. One hour after the last administration, the administration group, positive control group, and model group were intraperitoneally injected with carrageenan solution prepared with 0.2 % saline (0.2 mL/10g) to induce thrombosis. In contrast, the blank group was given the same volume of normal saline. Then the tail length and thrombosis length of mice were recorded at 24 h and 48 h after modelling.

Comparison of anticoagulant and anti-hemostatic abilities

Mice were randomly divided into 12 groups, a blank group, an aspirin positive control group, 10 administration groups, with 7 healthy ICR mice in each group. In the same way as in the "Comparison of anti-thrombotic ability," all mice were adaptively reared. After adaptive feeding, mice in each group were given intragastric administration. Each group was given a corresponding solution at a dose of 0.3 g/kg for consecutive 7 days. The blank group was given the same amount of normal saline solution for consecutive 7 days. Then we recorded the clotting time and hemostatic time.

III. Index determination

Determination of the indexes of anti-platelet aggregation *in vitro*

PRP and PPP were prepared respectively from 11 groups of mice in a different dose and normal saline groups. PRP in different groups was adjusted to an appropriate concentration, and then added a proper amount of ADP solution to induce blood clotting. Simultaneously, the same amount of ADP solution of PPP was given as blank control, and platelet aggregation was dynamically detected by FLASH continuous spectrum fluorescence enzyme labeling (405 nm wavelength, the 50 s/times, 15 min). The peak value and time of platelet aggregation in each group were observed. The following indicators are calculated respectively (17):

The maximum percentage of platelet aggregation=$(OD_0-OD_M) / (OD_0-OD_{PPP})$

Where, OD_0 : Absorbance value of initial enzyme marker, OD_M : absorbance value of platelet aggregation peak, and $OD_{PPP:}$ Absorbance value of PPP as blank control.

Aggregation inhibition (%) = (PRP aggregation (%) in saline group- RP aggregation (%) in administration group) / PRP aggregation (%) in saline group x 100 %

Index of anti-thrombotic ability

After the mice modelling, we respectively measured the tail length and tail thrombosis length of all model mice with a ruler at 24 h and 48 h, and then calculated the relative length of tail thrombosis, thrombosis inhibition rate, and thrombosis rate of mice, respectively (18).

Mousetail thrombosis relative length= (A_1/A_2) x 100 %

Thrombotic inhibition rate= ($1-B_1/B_2$) x 100 %

Thrombosis rate= (The number of mice with dark red thrombosis in their tails/Total number of mice samples in this group) x 100 %

A_1, A_2, B_1, B_2 respectively represent the length of blacktail (cm), the length of mice's tail (cm), the mean length of tail thrombosis (cm) of mice in the administration group or the positive control group, and the mean length of the tail thrombosis of mice in the model group (cm).

Indices of anticoagulation and anti-hemostasis

Determination of coagulation time (CT): One h after the last intragastric administration, we collected the mice's orbital blood with a capillary glass tube and dropped it on the slide. Then we measured the time immediately until the needle picked up the fibrin filaments and recorded the time as the coagulation time.

Determination of hemostatic time (HT) (8): One h after the last intragastric administration, we fixed the mice and used sharp scissors to cut 0.5 cm away from the mice's tail tip. When the blood flowed out by itself, the timing began. The blood drops from the mice's tail were absorbed with a filter paper every 30 s until the bleeding stopped naturally, that was when no blood was absorbed with a filter paper, and the hemostasis time was recorded.

IV. Data analysis

In this experiment, we used SPASS22.0 to conduct principal component analysis on the effect of *in vivo* and *in vitro* blood promotion and used software such as Origin2018 and Excel2020 to conduct data analysis and drawing. The percentage of Platelet aggregation inhibition was expressed as an average. Other metrics like BT, HT, and black tail rate were expressed as the mean ± SD ($\bar{x} \pm s$), variable mean was compared by one-way analysis of variance. $P < 0.05$ means a significant difference, and $P < 0.01$ means an extremely significant difference.

RESULTS AND DISCUSSION

I. Dose screening

As shown in Table 3 and Figure 1, the inhibition rate of platelet aggregation at the equivalent human dose of 5-10 times was higher, among which the anti-platelet aggregation rate at the equivalent human dose of 7-times was the highest, up to 54.99 %, followed by the inhibition rate of platelet aggregation at the equivalent human dose of 6-times, up to 47.55 %. It can be concluded that the medium and high dose of *P. notoginseng* powder has an anti-thrombotic effect. Due to the limited total amount of *P. notoginseng* collected, from the perspective of economy, this study selected 6 times equivalent human dose to do the subsequent anti-platelet aggregation experiment and comparative study on the effects of promoting blood circulation *in-vivo*, so as to screen out the producing areas of *P. notoginseng* with better blood circulation effect.

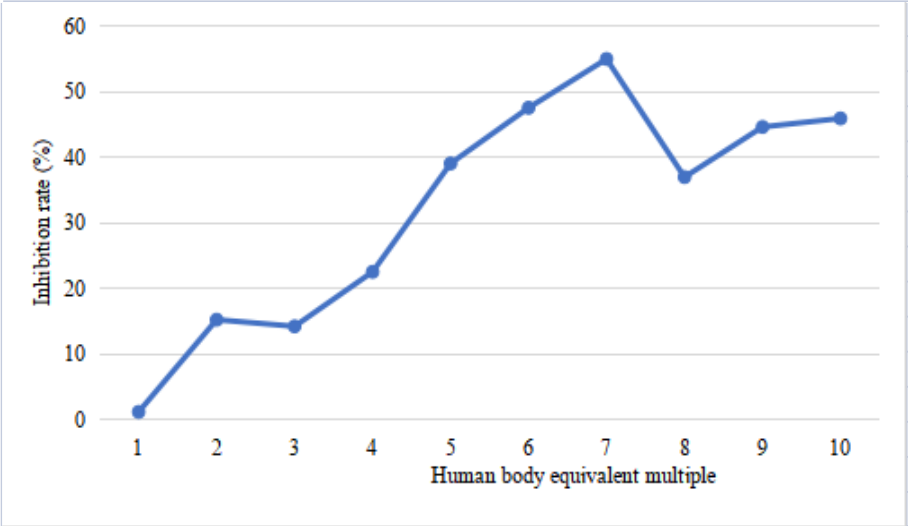

Figure 1. The relationship between different administration doses and inhibition rate

Table 3. Results of experimental data of dose screening

Equivalent multiple of human administration	10	9	8	7	6	5	4	3	2	1	KB
Inhibition rate (%)	45.9289	44.6261	36.9723	54.9971	47.5477	39.051	22.511	14.1931	15.2085	1.1367	——

II. Comparison of anti-platelet aggregation ability of *P. notoginseng* powder from different origins *in-vitro*

After comparing the anti-platelet aggregation ability with the equivalent human body volume of 6 times, the results were shown in Figure 2. The platelet aggregation rate of *P. notoginseng* powder from different origins ranged from 79.32 % to 32.69 %, among which *P. notoginseng* from Baoshan had the best anti-platelet aggregation effect with the platelet inhibition rate of nearly 80 %, the inhibition rate of *P. notoginseng* powder from Mengzi, Mile, Yanshan, Guangxi, and other places also exceeded > 60 %. Due to the limited experimental conditions, *P. notoginseng* from Malipo, Kunming, Mile, Mengzi, Guangxi, Baoshan, Yanshan, Sichuan, Xishuangbanna, and Qujing, were selected to study the anti-thrombotic ability, anticoagulant ability and anti-hemostatic ability of mouse tail.

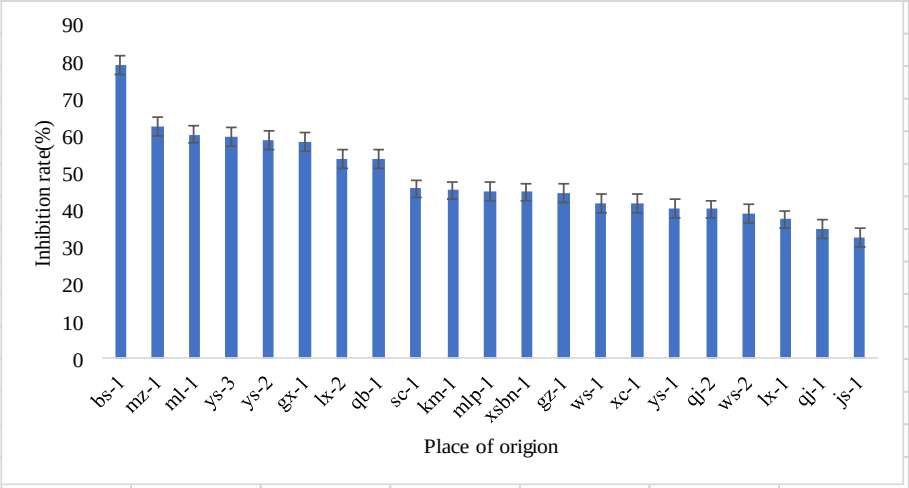

Figure 2. The inhibitory rates of anti-platelet aggregation of 6-times equivalent amount of *P. notoginseng* powder

III. Comparison of anti-mouse tail thrombosis of *P. notoginseng* from different origins

Because after 48 h of modelling, except for the positive control group, Kunming group, and Malipo group, all the other groups suffered tail amputation, resulting in inaccurate experimental data. Therefore, the results only list the black tail length, the blacktail rate in mice, thrombosis inhibition rate, and modelling success rate after 24 h of modelling. The specific data were shown in Table 4.

Table 4. The length and formation rate of thrombosis in mice at 24 h ($\bar{x} \pm s$, n=7)

Group	Blacktail length (cm)	Blacktail rate (%)	Thrombotic inhibition rate (%)	The success rate of modelling (%)
Model group	3.58 ± 0.47	38.95 ± 0.12	——	100%
Positive control group	2.22 ± 0.27	24.36 ± 0.08	37.99	100%
KM-1	3.02 ± 0.74^{aB}	32.44 ± 0.97^{AB}	15.64	100%
XSBN-1	2.40 ± 0.24^{A}	26.967 ± 0.15^{A}	32.96	86%
SC-1	3.05 ± 0.14^{aB}	33.15 ± 0.19^{aB}	14.8	86%
ML-1	3.10 ± 0.14^{aB}	34.07 ± 0.15^{aB}	13.41	100%
YS-1	2.45 ± 0.60^{A}	27.53 ± 0.67^{A}	31.56	100%
MZ-1	2.98 ± 0.44^{Ab}	32.43 ± 0.73^{AB}	16.76	100%
QJ-1	2.40 ± 0.18^{A}	27.59 ± 0.06^{Ab}	32.96	100%
MLP-1	2.58 ± 0.33^{A}	28.39 ± 0.36^{Ab}	27.93	100%
GX-1	2.42 ± 0.23^{A}	26.56 ± 0.25^{A}	32.4	100%
BS-1	3.18 ± 0.12^{aB}	32.82 ± 0.12^{AB}	11.17	100%

Note : Compared with the blank group, the obtained significant difference was represented by a and A. ($^{a}P < 0.05$; $^{A}P < 0.01$). Compared with the positive control group, the obtained significant difference is represented by b and B. ($^{b}P < 0.05$; $^{B}P < 0.01$).

Based on the experimental data, the tail thrombosis model in mice was successfully constructed in this study. Except for one death in the Xishuangbanna group and Sichuan group, the others' success rate was 100 %. By comparing the inhibition rate of thrombosis in each group, we can see that the positive control group, Xishuangbanna group, Qujing group, Guangxi group, and Yanshan group had better inhibition effect, all above 30 %. Therefore, the anti-thrombosis effect of *P. notoginseng* from Xishuangbanna, Qujing, Guangxi, and Yanshan was more substantial.

IV. Comparison of anticoagulant and anti-hemostatic effects of *P. notoginseng* from different origins

The specific CT and HT time data were shown in Table 5. Compared with the blank group, CT and HT of the positive control group and *P. notoginseng* group from different origins were prolonged, which confirmed the effect of aspirin and *P. notoginseng* on promoting blood circulation. Compared with the results of CT examination in each group, the anticoagulant effect of Yanshan Group, Sichuan group, Malipo group, Mile group, and Xishuangbanna group was the best, and the CT time was more than 40 s, which had a significant difference compared with the blank group and positive control group.

Table 5. CT and HT of *P. notoginseng* powder from different areas ($\bar{x} \pm s$, n=7) on7-animals

Group	Dose (g/kg)	CT (sec)	HT (min)
The blank group	0	30.13 ± 0.07	13.98 ± 0.31
Positive control group	0.3	37.40 ± 0.28	22.69 ± 0.29
SC-1	0.3	47.05 ± 0.16^{AB}	18.12 ± 0.91^{aB}
XSBN-1	0.3	44.73 ± 0.91^{AB}	25.24 ± 0.06^{A}
QJ-1	0.3	37.567 ± 0.52^{A}	22.51 ± 0.42^{A}
MLP-1	0.3	45.07 ± 0.76^{AB}	25.82 ± 0.22^{A}
GX-1	0.3	37.31 ± 0.03^{A}	25.28 ± 0.68^{A}
ML-1	0.3	44.95 ± 0.31^{AB}	21.42 ± 0.09
YS-1	0.3	62.51 ± 0.68^{AB}	30.48 ± 0.31^{Ab}
MZ-1	0.3	30.11 ± 0.98^{B}	24.65 ± 9.09^{A}
BS-1	0.3	33.08 ± 0.63^{Ab}	26.83 ± 0.46^{Ab}
KM-1	0.3	31.49 ± 0.34^{B}	26.83 ± 0.66^{Ab}

Note : Compared with the blank group, the obtained significant difference was represented by a and A. ($^{a}P < 0.05$; $^{A}P < 0.01$). Compared with the positive control group, the obtained significant difference is represented by b and B. ($^{b}P < 0.05$; $^{B}P < 0.01$).

By comparing the HT of each group, it was found that the better hemostasis effect was in the Yanshan group, the HT was more than 30 min, and the HT is significantly different from that of the blank group and positive control group. Based on CT and HT analysis, *P. notoginseng* from Yanshan has a better effect of promoting blood circulation. According to the principal component analysis of the data of three *in-vivo* experiments (including anti-thrombotic experiment, anticoagulant experiment, and anti-hemostasis experiment), the results showed that Baoshan, Yanshan, and Mengzi's *P. notoginseng* had a better effect of promoting blood circulation *in-vivo*.

V. Principal component analysis based on various indexes of blood activity

We conducted a comprehensive analysis of the above indexes of blood activity *in-vivo* and *in-vitro*, and the analysis data and sequence were shown in Table 6. According

to the results of principal component analysis, the variance contribution rates of the two principal components with eigenvalues > 1 were 43.23 % and 31.86 % respectively, and the cumulative variance contribution rates were 75.09 % > 60 %, which indicated that the original data was more suitable for principal component analysis.

Table 6. The comprehensive score and ranking of the blood activating effect of *P. notoginseng*

Place of origin	F1	F2	F	Ranking
BS-1	3.107	0.315	1.443	1
YS-1	-0.460	2.051	0.455	2
MZ-1	1.069	-0.617	0.265	3
KM-1	0.472	0.090	0.233	4
ML-1	0.890	-0.963	0.078	5
GX-1	-0.338	-0.590	0.042	6
XSBN-1	-0.991	0.936	-0.130	7
MLP-1	-1.169	-0.533	-0.675	8
QJ-1	-1.955	0.206	-0.779	9
SC-1	-0.625	-0.2074	-0.931	10

Note: F1 and F2 are two principal component scores respectively; F is the comprehensive score obtained from the analysis.

According to the final score, the comprehensive effects of improving the circulation of *P. notoginseng* from high to low in turn is Baoshan > Yanshan > Mengzi > Kunming > Guangxi > Xishuangbanna > Wenshan > Malipo > Qujing > Sichuan. The three producing areas with a better comprehensive effect of promoting blood circulation were Baoshan, Yanshan and Mengzi.

However, due to the limitation of experimental conditions, the reasons for the difference in blood activity of *P. notoginseng* from different origins have not been further explored. At present, it was mainly believed that the blood-activating effects of *P. notoginseng* were closely related to the type and content of saponins (22,24). It was speculated that it might be related to external factors such as germplasm resources, soil, climate, cultivation techniques (1), and subsequent studies on the difference of *P. notoginseng* in promoting blood circulation can also be carried out from these aspects.

CONCLUSIONS

In this study, by comparing the anti-platelet aggregation ability of *P. notoginseng* from 22 producing areas, we selected *P. notoginseng* with better anti-platelet aggregation ability from 10 producing areas The anti-thrombotic experiment, anticoagulant experiment, and anti-hemostasis experiment *in-vivo* were further carried out to find the difference *in-vivo* and *in-vitro* blood-activating effects of *P. notoginseng* from different growing areas.

The results showed that *P. notoginseng* from Baoshan, Mengzi, Mile, and Yanshan had a better anti-platelet aggregation effect *in-vitro*. Based on three *in-vivo* experiments, we found that *P. notoginseng* from Yanshan, Baoshan, Xishuangbanna, and Guangxi promoted the blood circulation. Based on the principal component analysis of both *in-vivo* and *in-vitro* experiments. We found obvious differences in the effects of promoting the blood circulation *in-vitro* and *in-vivo* of *P. notoginseng* from different growing areas. The comprehensive effects of *P. notoginseng* from Baoshan, Yanshan, and Mengzi were the best. This study

provides ideas for future research and for screening the *P. notoginseng* with beneficial effects to promote blood circulation.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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PUBLISHER NOTE