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Research Article

Chemical and bioactive comparison of *Panax notoginseng* root and rhizome in raw and steamed forms

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ABSTRACT

Background: The root and rhizome are historically and officially utilized medicinal parts of *Panax notoginseng* (PN) (Burk.) F. H. Chen, which in raw and steamed forms are used differently in practice.
Methods: To investigate the differences in chemical composition and bioactivities of PN root and rhizome between raw and steamed forms, high-performance liquid chromatography analyses and pharmacologic effects evaluated by tests of anticoagulation, antioxidation, hemostasis, antiinflammation, and hematopoiesis were combined.
Results: With the duration of steaming time, the contents of ginsenosides Rg_1, Re, Rb_1, Rd, and notoginsenoside R_1 in PN were decreased, while those of ginsenosides Rh_1, 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rh_4, and Rk_3 were increased gradually. Raw PN samples steamed for 6 h at 120°C with stable levels of most constituents were used for the subsequent study of bioeffects. Raw PN showed better hemostasis, anticoagulation, and antiinflammation effects, while steamed PN exhibited stronger antioxidation and hematopoiesis activities. For different parts of PN, contents of saponins in PN rhizome were generally higher than those in the root, which could be related to the stronger bioactivities of rhizome compared with the same form of PN root.
Conclusion: This study provides basic information about the chemical and bioactive comparison of PN root and rhizome in both raw and steamed forms, indicating that the change of saponins may have a key role in different properties of raw and steamed PN.

1. Introduction

The root and rhizome are historically and officially utilized medicinal parts of *Panax notoginseng* (PN) (Burk.) F. H. Chen, which is one of the major herbal plants in genus *Panax* (Araliaceae) [1]. PN has been widely used for treating blood disorder diseases for centuries, which is now included as a herbal medicine in pharmacopeias of China, USA, Britain, Europe and so on and also included as a dietary supplement by the US Dietary Supplement Health and Education Act in 1994 [2–6]. During hundreds of years of medication, there was a description for PN properties that "the raw materials eliminate and the steamed ones tonify". The so-called "eliminate" means raw PN can stop bleeding, eliminate blood stasis, promote blood circulation, diminish swelling, and ease pain. The "tonify" means that steamed PN can nourish the blood and improve the health [7,8]. Pharmacologic studies have shown that the effects of PN changed when steamed. Lau et al [9] compared the bleeding time (BT) in rats orally treated with raw and steamed PN roots and found that the treatment with raw PN extract resulted in shorter BT compared with rats treated with the steamed PN extract. Besides, raw PN extract displayed a much better lipid-lowering effect than steamed PN by investigating the levels of total

Abbreviations: PN, *Panax notoginseng*; HPLC, high-performance liquid chromatography; BT, bleeding time; TCM, traditional Chinese medicine; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; MeOH, methyl alcohol; MeCN, acetonitrile; PPP, platelet-poor plasma; PT, prothrombin time; DPPH, 1,1-diphenyl-2-picrylhydrazyl; CT, coagulation time; FEJ, *Fufang E'jiao Jiang*.

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cholesterol and triglyceride in steatotic L02 cells [10]. Such difference might be attributed to the change in the contents, relative proportion, and structures of chemical constituents in PN. Wang et al [11] used high-performance liquid chromatography (HPLC) to analyze saponins of PN root during the steaming process and found that the contents of five main saponins (ginsenosides Rg_1, Rb_1, Rd, Re, and notoginsenoside R_1) in raw PN root were decreased gradually and other new saponins were formed. Some of those newly produced constituents were responsible for the effect of steamed PN. Sun et al [12] reported that the steaming process influenced significantly the transformation of Rg_3, an anticancer compound, of which the content was 5.23-fold higher in root steamed for 2 h at 120°C and 3.22-fold higher when steamed for 4 h than for 1 h at 120°C. Nevertheless, most pharmacologic studies on raw and steamed PN were focused on the root, whereas little attention was paid to the effect difference between raw and steamed form of rhizome, another officially medicinal part of PN.

According to recent researches, the chemical composition of different parts of PN was reported to be various. Wang et al [13] employed ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry to compare quantitatively eight dammarane-type saponins in different parts of raw PN, finding that the content of each saponin was always the highest in rhizome, followed by main root and branch root, and then fibrous root, which was consistent with previous researches [14,15]. Other components such as polysaccharides, flavonoids, and heavy metals were also found to be distributed differently in different parts of PN [16–18]. Among those components, dammarane-type saponins (including ginsenosides and notoginsenosides) are considered to be major active ones of PN, which might contribute to its pharmacologic and therapeutic effects [19]. However, the bioeffects related to the traditional uses of PN root and rhizome have not been compared comprehensively.

Modern pharmacologic researches attributed the blood-nourishing and body-tonifying functions of herbal medicines to their antioxidant and immunomodulatory activities [20]. And since the blood deficiency in the theory of traditional Chinese medicine (TCM) is considered to be similar to the blood-loss anemia, blood routine indicators such as the levels of white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), and platelet are often used to evaluate the efficacy of blood nourishing of medicines [21,22]. In this work, saponins in PN root and rhizome during the steaming process were investigated to determine the suitable steaming condition for steamed PN. And we carried out a comprehensive comparison of pharmacologic effects of PN root and rhizome in raw and steamed forms firstly, which were evaluated by anticoagulation, antioxidation, hemostasis, and antiinflammation tests, as well as the model of hydracetin-induced anemia mice.

2. Materials and methods

2.1. Chemicals

The reference standards of ginsenosides Rg_1, Re, Rb_1, Rd, Rh_1, 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rh_4, Rk_3, and notoginsenoside R_1 were purchased from the National Institutes for the Control of Pharmaceutical and Biological Products (Beijing, China). Methyl alcohol (MeOH) and acetonitrile (MeCN) (HPLC grade) were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Ultrapure water was generated with an UPT-I-20T ultrapure water system (Chengdu Ultrapure Technology Inc. Sichuan, China). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). 1,10-phenanthroline and L-ascorbic acid were purchased from the Xilong Chemical Co. (Guangdong, China). All other chemicals used were of analytical grade.

2.2. Sample preparation

The roots and rhizomes of PN were obtained from a single batch of samples in Yunnan, China. Steamed PN samples were prepared by steaming the crushed raw PN root and rhizome in an autoclave (Shanghai, China) for 2, 4, 6, 8, and 10 h at 105°C, 110°C, and 120°C, respectively. The steamed powder was then dried in a heating-air drying oven at about 45°C to constant weight, then powdered and sieved through a 40-mesh sieve.

2.3. Animals

Kunming (KM) mice, male and female, weighing 18–22 g, were purchased from Tianqin Biotechnology Co. Ltd., Changsha, Hunan [SCXK (Xiang) 2014-0011]. Before the experiments, the mice were given 1-week acclimation period in a laboratory at room temperature (20–25°C) and constant humidity (40–70%) and fed with standard rodent chow and tap water freely. Animal experimental procedures in the study were strictly conformed to the Guide for the Care and use of Laboratory Animals and related ethics regulations of Kunming University of Science and Technology.

2.4. HPLC analyses

The sample solutions were prepared according to the method described in Chinese Pharmacopoeia [2]. A mixed standard solution, containing (in mg/mL) 0.40 notoginsenoside R_1, 0.55 ginsenosides Rg_1, 0.50 Re, 0.60 Rb_1, 0.50 Rd, 0.60 Rh_1, 1.00 Rk_3, 1.00 Rh_4, 0.45 20(*S*)-Rg_3, and 0.55 20(*R*)-Rg_3, was prepared by adding each standard into a volumetric flask and dissolving with methanol. A series of standard solutions of seven concentrations were prepared by diluting the mixed standard solution with methanol for the determination of the standard curves.

HPLC analyses were done on a 1260 series system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1311B Pump, a G4212B diode-array detector, and a G1329B autosampler. A Vision HT C_{18} column (250 mm × 4.6 mm, 5 μm) was adopted for the analyses. The mobile phase consisted of A (ultrapure water) and B (MeCN). The gradient mode was as follows: 0–20 min, 80% A; 20–45 min, 54% A; 45–55 min, 45% A; 55–60 min, 45% A; 60–65 min, 100% B; 65–70 min, 80% A; 70–90 min, 80% A. The flow rate was set at 1.0 mL/min. The detection wavelength was set at 203 nm. The column temperature was set at 30°C and sample volume was set at 10 μL.

2.5. Anticoagulation test in vitro

Blood was collected via the posterior orbital venous plexus of mice anesthetized with ether and was directly transferred into citrated tubes (0.109 M citrate, 9:1). The supernatant platelet-poor plasma (PPP) was obtained by centrifuging the blood samples above at 3000 rpm for 10–15 min. The mixture of PPP and thrombokinase of various concentrations at the proportion of 2:1 (v/v) of total 50 μL was added into the test cup and incubated for 3 min at 37°C in a blood coagulation instrument (XN06 series, Diagnostic Technology Ltd of Wuhan, Jingchuan, China). 10 U/mL thrombokinase of 100 μL dissolved in 0.1 mol/L Tris–HCl buffer solution (pH 7.4) was subsequently added and incubated at the same condition. The prothrombin time (PT) was determined in accordance with the manufacturer's recommended protocols. The prolongation rate of PT was calculated according to the following equation:

$$\text{Prolongation rate of } PT(\%) = (PT - PT_0)/PT_0 \times 100\% \quad (1)$$

where, PT_0 was the prothrombin time of control (blank, the normal saline replaced of thrombokinase) and PT was the prothrombin time in the presence of thrombokinase.

The standard curve was drawn with the concentration of thrombokinase (U_i) as the X axis and the lg [prolongation rate of PT (%)] as the Y axis. PN samples of 5 g, in the powdered form, were extracted with pure water (50.0 mL) by refluxing twice for 2 h at 80°C. The combined solution was filtered and concentrated under reduced pressure to the extract containing 0.1 g/mL of PN. The extract was then diluted with the normal saline to different concentrations. The PT of the mixed plasma sample containing PPP and PN extract (PT') of different concentrations was determined. The prolongation rate of PT' was calculated according to the following equation:

$$\text{Prolongation rate of } PT'(\%) = (PT' - PT_0')/PT_0' \times 100\% \quad (2)$$

where, PT_0' was the prothrombin time of control (blank, the normal saline replaced of extracts) and PT' was the prothrombin time in the presence of extracts.

The corresponding concentration of thrombokinase (U_i) was determined according to the standard curve and the thromboplastin inhibition rate (%) was calculated according to the following equation:

$$\text{Thromboplastin inhibition rate}(\%) = (U_i - 10)/10 \times 100\% \quad (3)$$

where, U_i was the concentration of thromboplastin determined by the standard curve.

2.6. Antioxidation test in vitro

The extracts prepared in "Section 2.5" were diluted with normal saline to 0.5, 1, 1.5, 2, 2.5, 3, and 3.5 mg/mL, respectively. The scavenging activities for DPPH free radicals and hydroxyl radicals were measured according to the procedure described by Zhao et al [23].

2.7. Hemostasis test in vivo

Sixty KM mice, half male and half female, were randomly divided into six groups, namely the control group, Vitamin K group, low-dose raw PN group, low-dose steamed PN group, moderate-dose steamed PN group, and high-dose steamed PN group. The mice in control group were administered with 0.9% normal saline, whereas other groups were administered with Vitamin K (0.4 g/kg), raw PN powder (0.4 g/kg), and steamed PN powder (0.4 g/kg, 0.8 g/kg, and 1.6 g/kg, respectively), respectively, by gavage for 7 days.

The tail bleeding model was based on previous methods with minor modifications [24,25]. After 1 h of the last administration following anesthetization, the mice tail was precisely transected at 5 mm from the tip. The time between the start of transaction to bleeding cessation was recorded as the BT. Bleeding cessation was considered to be the time when the flow of blood stopped for at least 30 s.

After 1 h of the last administration following anesthetization, the blood was collected via the posterior orbital venous plexus with a glass capillary. The glass capillary filled with blood was broken off

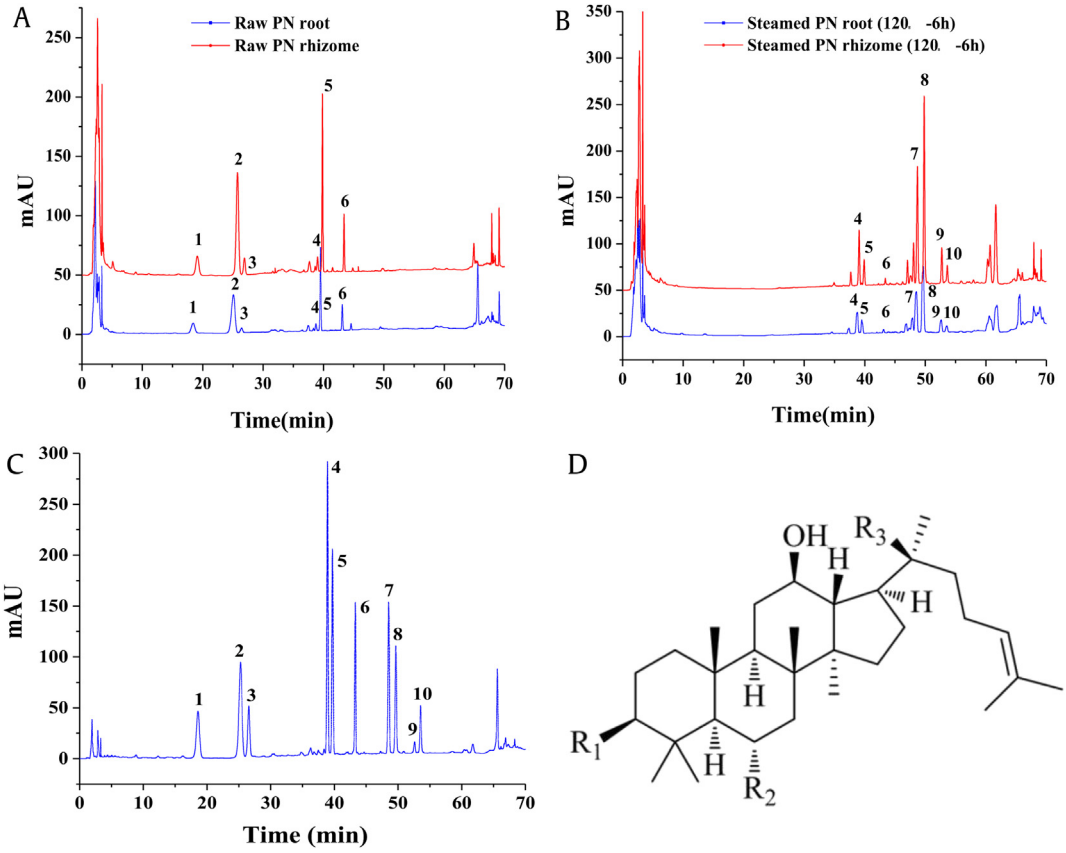

Fig. 1. (A) HPLC chromatogram of raw PN root and rhizome. (B) Chromatogram of PN root and rhizome steamed for 6 h at 120°C. (C) Chromatogram of reference substances. (D) Chemical structures of saponins present in raw and steamed PN. HPLC separation was done on a Vision HT C_{18} column (250 mm × 4.6 mm, 5 μm) at 30°C. The mobile phase consisting of A (ultrapure water) and B (MeCN) was used at a flow rate of 1.0 mL/min as the following gradient mode: 0–20 min, 80% A; 20–45 min, 54% A; 45–55 min, 45% A; 55–60 min, 45% A; 60–65 min, 100% B; 65–70 min, 80% A; 70–90 min, 80% A. The detection wavelength was set at 203 nm and the injection column was set at 10 μL. Peak 1, notoginsenoside R_1; 2-10, ginsenosides Rg_1, Re, Rh_1, Rb_1, Rd, Rk_3, Rh_4, 20(*S*)-Rg_3, and 20(*R*)-Rg_3. HPLC, high-performance liquid chromatography; PN, *Panax notoginseng*.

alternately from both ends in every 30 s, in order to investigate the presence of blood coagulation. Once the blood coagulation was presented in either end, the other end was broken off to verify. The time between the BT to the presence of blood coagulation was recorded as the coagulation time (CT).

2.8. Anti-inflammation test in vivo

Forty KM mice, half male and half female, were randomly divided into six groups, namely the control group, aspirin group, raw PN group, and steamed PN group. The mice in control group were administered with 0.9% normal saline, whereas other groups were administered with aspirin (0.45 g/kg), raw PN powder (1.8 g/kg), and steamed PN powder (1.8 g/kg), respectively, by gavage for 5 days.

Xylene-induced ear edema model was applied to evaluate the antiinflammatory activity based on previous methods with minor modifications [26,27]. After 30 min of the last administration, edema was induced in each mouse by applying 50 μL of xylene to the inner surface of the right ear. Mice were sacrificed under ether anesthesia after 45 min. Both ears were cut off, sized, and weighed to calculate the swelling degree and the inhibition rate of swelling.

2.9. Measurements of blood parameters

Seventy KM mice, half male and half female, were randomly divided into seven groups, namely the control group, model group, *Fufang E'jiao Jiang* (FEJ) group, high-dose raw PN group, low-dose steamed PN group, moderate-dose steamed PN group, and high-dose steamed PN group, 10 mice in each group. The hydracetin and cyclophosphamide-induced anemia model was applied to evaluate the "blood tonifying" function of PN based on previous methods [28]. The anemia model was established by intraperitoneal injection of cyclophosphamide of 70 mg/kg for the first 3 days and hypodermic injection of hydracetin of 0.02 mg/kg at the fourth day. Mice in the control group were administered with 0.9% normal saline, whereas other groups were administered with FEJ (8 mL/kg), raw PN powder (1.8 g/kg), and steamed PN powder (0.45 g/kg, 0.90 g/kg, and 1.8 g/kg, respectively), respectively, by gavage for 12 days. Then the blood was collected for the routing blood analysis, including levels of WBC, RBC, Hb, and platelet after 30 min of the last administration.

2.10. Statistical analyses

Results were obtained in the mean ± standard deviation. SPSS v21.0 (Statistical Program for Social Sciences, Chicago, IN, USA) was applied to carry out the *t* test and principal component analysis (PCA). A p value < 0.05 was considered significant. A p value < 0.01 was considered highly significant. The median effective concentration (EC_{50}) value was fitted by probit regression with Origin 7.5 for windows (OriginLab, Northampton, MA, USA). Heml 1.0 (CUCKOO Workgroup, Wuhan, Hubei, China) was used for the clustering analysis and drawing heatmap.

3. Results and discussion

3.1. HPLC analyses

The results of HPLC analyses indicated a distinct difference in saponin composition between the raw and steamed PN. During the

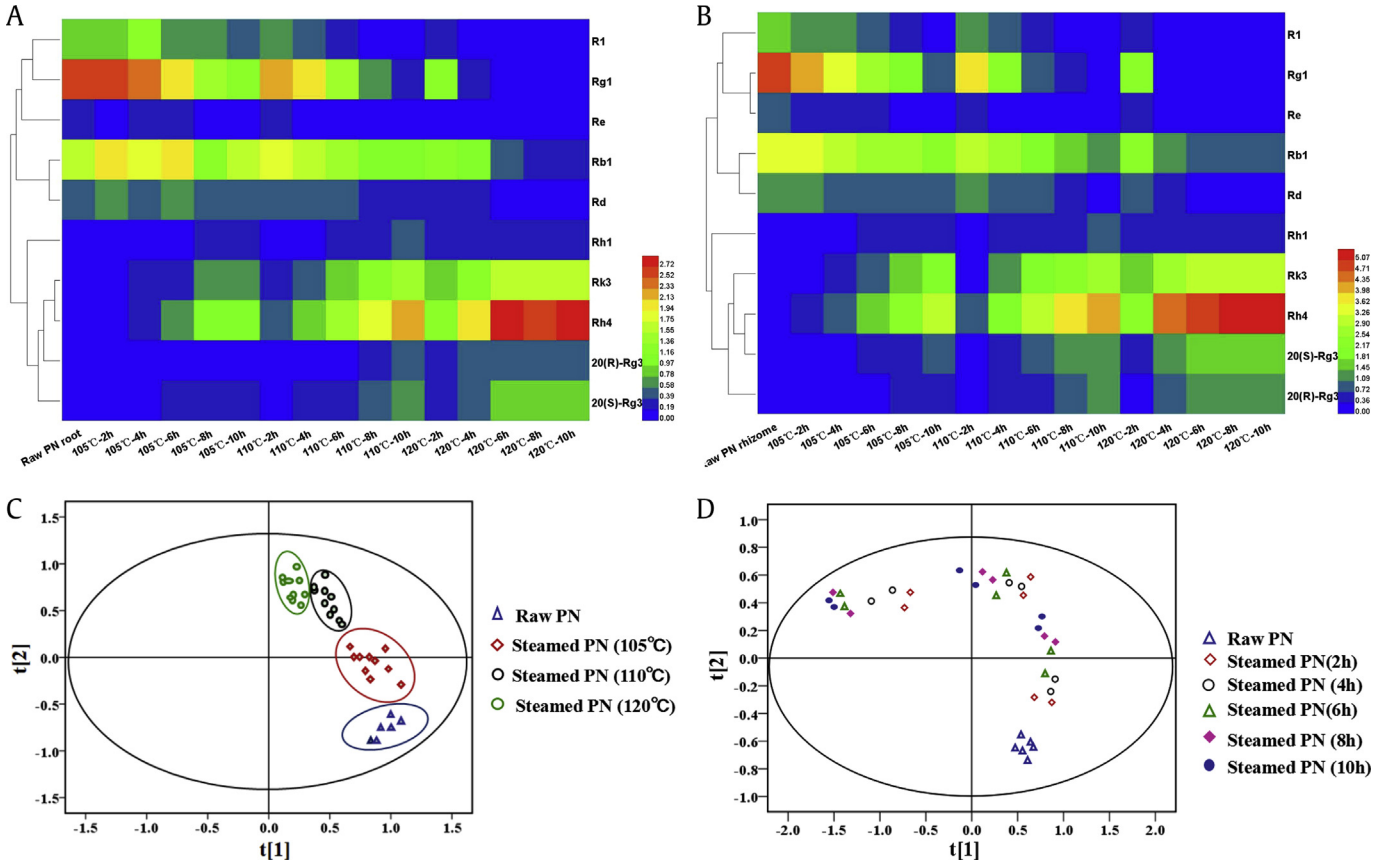

Fig. 2. (A) Heatmap of saponins contents in PN roots. (B) Heatmap of saponins contents in PN rhizomes. (C) PCA of raw and steamed PN samples discriminated by the steaming temperature. (D) PCA of raw and steamed PN samples discriminated by the steaming time. PCA, principal component analysis; PN, *Panax notoginseng*.

steaming process, the contents of five major saponins in the raw PN, including ginsenosides Rg_1, Re, Rb_1, Rd, and notoginsenoside R_1, were decreased gradually, whereas other new saponins were formed (Figs. 1A, 1B). By comparing the chromatograms of PN samples to that of the mixed standard solution, five new converted saponins were identified as ginsenosides Rh_1, 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rh_4, and Rk_3 (Figs. 1C, 1D). The result was coincident with the report by Wang et al [11]. That transformation might be due to the cleavage of glycosidic bond induced by high temperature. The hydrolyzation or dehydration at C-20 could form new saponins during the steaming process. Therefore, high temperature was helpful for these reactions.

By comparing the steaming conditions, it was easy to find that the content change of saponins was in a time-dependent manner at lower steaming temperatures of 105°C and 110°C. While for the same steaming time, the formation as well as the degradation of saponins was in a temperature-dependent way. Ten saponins were clustered into two groups, namely the content-decreased group including ginsenosides Rg_1, Re, Rb_1, Rd, and notoginsenoside R_1, and content-increased group including ginsenosides Rh_1, 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rh_4, and Rk_3 (Figs. 2A, 2B). After 6 h of steaming at 120°C, the content change of most saponins in PN root and rhizome became steady. The overall levels of saponins in PN rhizome were higher than those in the root. According to the PCA result in Fig. 2C, PN samples could be well classified into two groups according to the steaming temperature, with the total variance of 98.634%. PCA1 and PCA2 accounted for 54.997% and 43.636%, respectively. It indicated that the levels of saponins were significantly different in raw PN and samples steamed at 105°C, 110°C and 120°C. However, samples of different steaming time could not be distinguished (Fig. 2D). Based on the above analysis, PN root and rhizome steamed for 6 h at 120°C were determined as steamed samples for the subsequent pharmacologic tests.

3.2. Anticoagulation test in vitro

PT is used to evaluate the overall efficiency of extrinsic clotting pathway. A prolonged PT indicates a deficiency in coagulation factors V, VII, and X [29]. In the study, the EC_{50} determined by the logarithm of PT prolongation rate was applied to evaluate the anticoagulation effect of PN. The standard curve between the concentration of thrombokinase and logarithm of PT prolongation rate was shown in Fig. 3A, exhibiting a good linearity ($R = -0.9991$). According to the comparison of effect in Fig. 3B, raw PN showed a significantly stronger anticoagulation than steamed PN. The result provides evidence for the traditional use of PN, that raw PN is better in the efficacy of activating blood flow and removing blood stasis than steamed PN, of which the beneficial effect could be related to the coagulation system. Meanwhile, for different parts of PN under the same process, the EC_{50} values of PN rhizome were lower than PN root, indicating that the anticoagulation effect of rhizome was better than the root, which could be related to the higher levels of saponins in the PN rhizome.

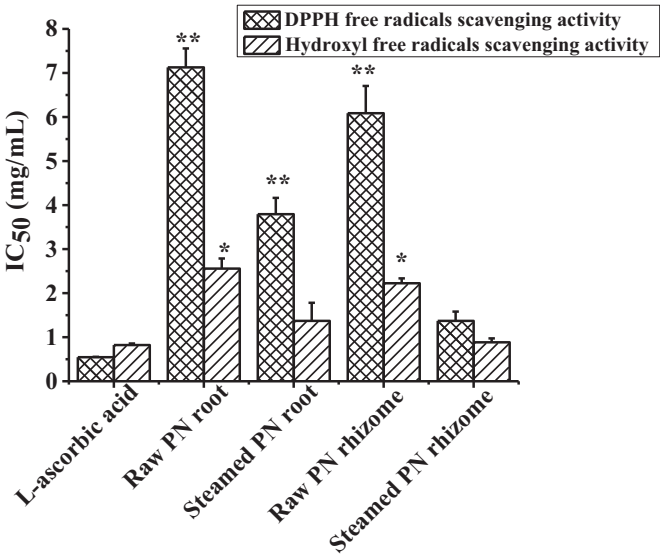

Fig. 4. DPPH and hydroxyl free radicals scavenging activities of PN root and rhizome in raw and steamed forms. Each value represents the mean ± SD ($n = 3$); $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with the L-ascorbic acid group; $\triangle p < 0.05$ and $\triangle\triangle p < 0.01$, compared with the corresponding part of raw PN. DPPH, 1,1-diphenyl-2-picrylhydrazyl; PN, *Panax notoginseng*; SD, standard deviation.

3.3. Antioxidation test in vitro

PN root and rhizome are mostly consumed as popular food tonic in the soup form by people in the southern region of China. Various studies have suggested that the tonifying functions of Chinese herbal medicines could be due to, at least partially, the protective effects against oxidation [20]. DPPH molecule that contains a stable

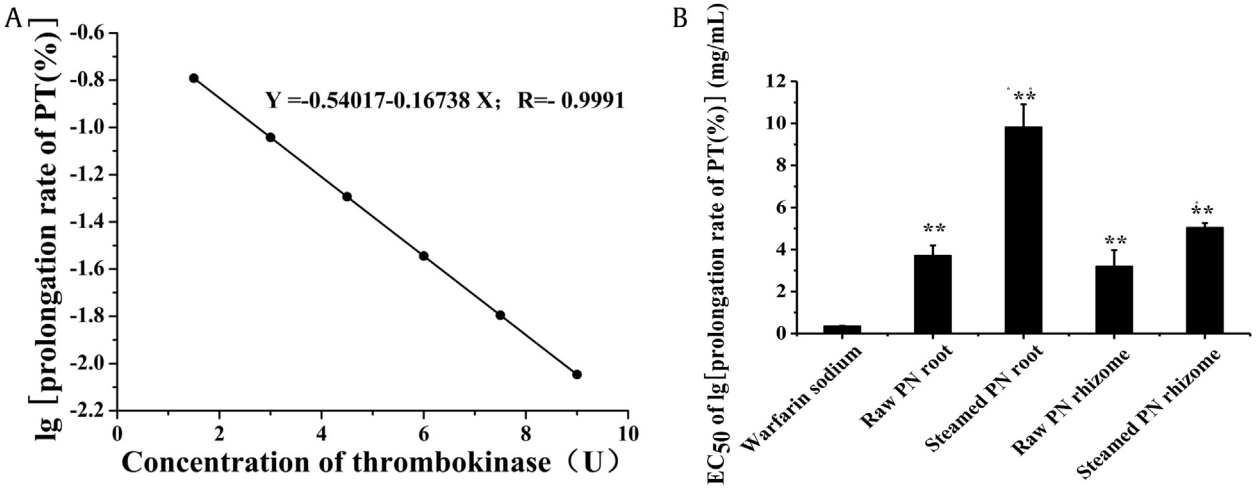

Fig. 3. (A) The standard curve of thrombokinase. (B) Anticoagulation effects of raw and steamed PN samples ($n = 3$). $^{**}p < 0.01$, compared with the warfarin sodium group; $\triangle p < 0.05$ and $\triangle\triangle p < 0.01$, compared with the corresponding part of raw PN. PN, *Panax notoginseng*; PT, prothrombin time.

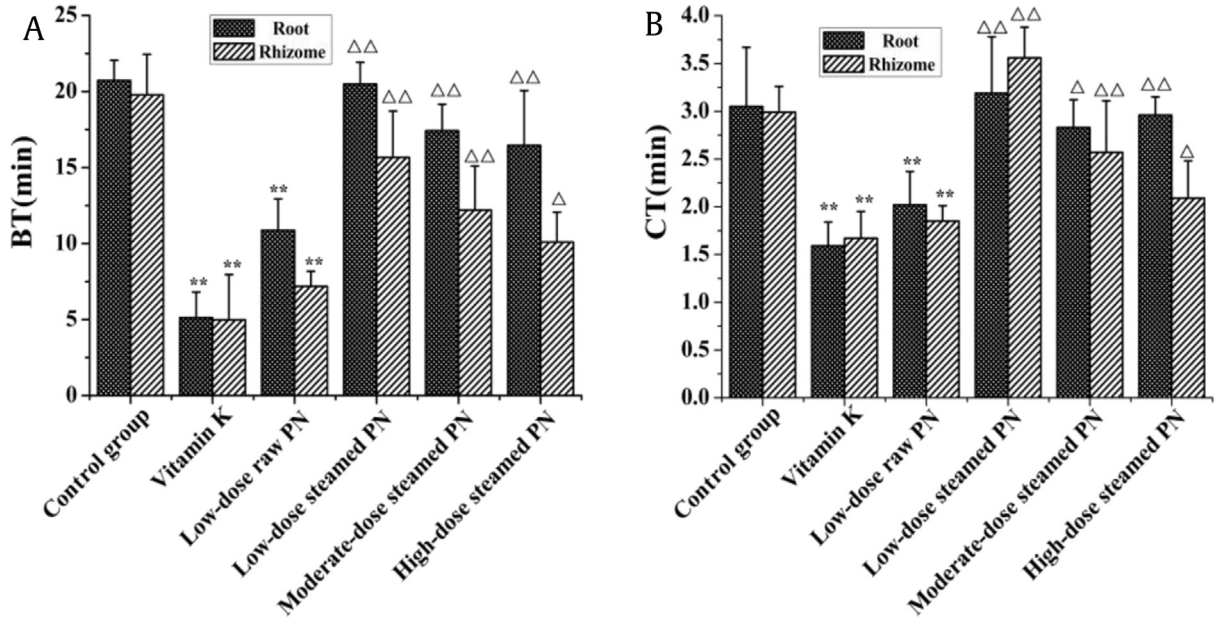

Fig. 5. (A) BT of mice treated with PN root and rhizome in raw and steamed forms. (B) CT of mice treated with PN root and rhizome in raw and steamed forms. Each value represents the mean ± SD ($n = 10$); $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with the control group; $\triangle p < 0.05$ and $\triangle\triangle p < 0.01$, compared with the corresponding part of raw PN. BT, bleeding time; CT, coagulation time; PN, *Panax notoginseng*; SD, standard deviation.

free radical has been widely used to evaluate the radical scavenging ability of antioxidants, and hydroxyl radical is very reactive which can be generated in biological cells through the Fenton reaction. DPPH and hydroxyl radical scavenging assays are commonly used for the determination of antioxidant activities of plant extracts. Therefore, the two methods were applied to investigate the antioxidation effect of raw and steamed PN root and rhizome, with results shown in Fig. 4, where the median inhibitory concentration (IC_{50}) was the concentration of PN scavenging 50% free radicals.

L-ascorbic acid was served as the positive control, exhibiting a stronger activity of scavenging DPPH free radicals than scavenging the hydroxyl radicals. Conversely, PN samples showed higher IC_{50} values of scavenging DPPH radicals than those of scavenging hydroxyl radicals, suggesting that PN could eliminate hydroxyl radicals at lower concentration. Although both raw and steamed PN reacted directly with DPPH and hydroxyl radicals, steamed PN root and rhizome showed stronger scavenging effects on radicals generally than the corresponding raw part. Since the antioxidation activity is partially correlated with the tonifying function of herbal medicines [20], the result gives evidence for the efficacy difference of raw and steamed PN that steamed PN is better in the tonifying effect than the raw form. For different parts of PN under the same

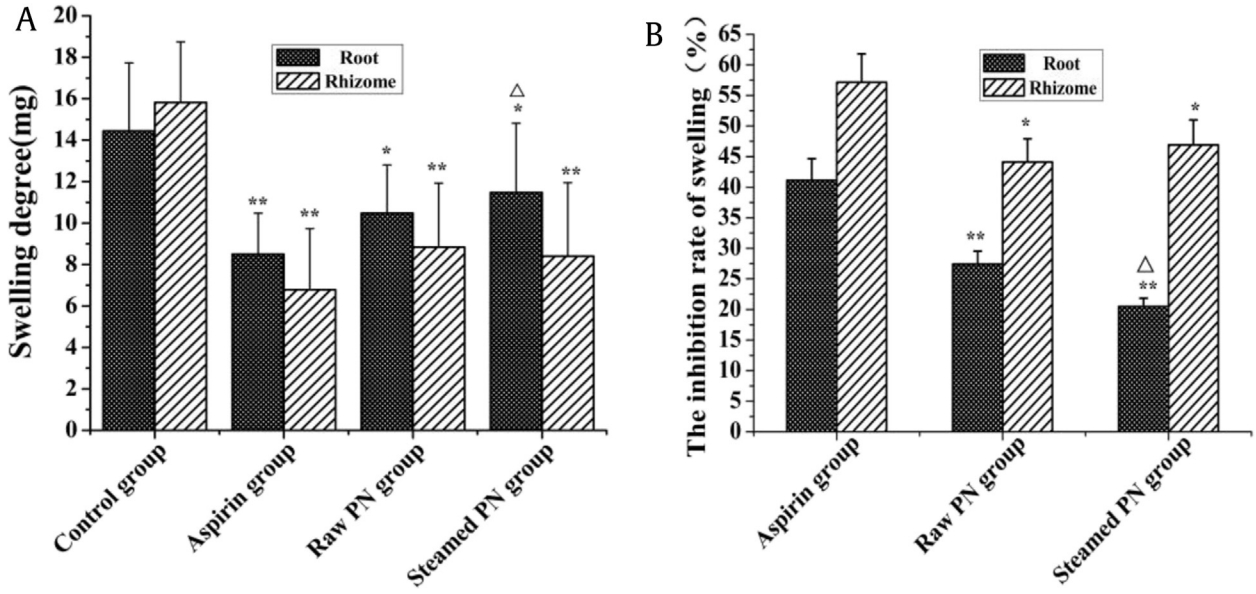

Fig. 6. (A) The swelling degree of ear of mice treated with PN root and rhizome in raw and steamed forms. (B) The inhibition rate of swelling of mice treated with PN root and rhizome in raw and steamed forms. Each value represents the mean ± SD ($n = 10$); $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with the control group; $\triangle p < 0.05$ and $\triangle\triangle p < 0.01$, compared with the corresponding part of raw PN in Fig. A. $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with the aspirin group; $\triangle p < 0.05$ and $\triangle\triangle p < 0.01$, compared with the corresponding part of raw PN in Fig. B. PN, *Panax notoginseng*; SD, standard deviation.

process, PN rhizome also showed significantly stronger activity of scavenging both DPPH and hydroxyl free radicals than the root in the same steaming condition ($p < 0.05$).

3.4. Hemostasis test in vivo

BT and CT of mice were affected by the interplay of factors involved in platelet aggregation and plasma coagulation. To gain insights into the overall hemostatic effect *in vivo*, we investigated the effect of raw and steamed PN root and rhizome on BT and CT, using an established mice's tail bleeding model. Vitamin K, which was served as the positive control, shortened BT and CT significantly compared with the blank control group. As shown in Fig. 5, oral treatment with steamed extract resulted in longer BT and CT compared with mice treated with raw PN. Dencichine, a nonprotein amino acid showing beneficial effects on hemostasis, presents a higher content in the raw PN [30] and is often denatured under high temperature, which provides a plausible explanation for the hemostatic difference between raw and steamed PN. For different parts of PN, BT of mice treated with PN rhizome was significantly shorter than those treated with the same dose of root ($p < 0.05$), and mice treated with high-dose (1.6 g/kg) steamed PN rhizome showed significantly shorter CT than mice treated with the same dose of steamed root ($p < 0.05$).

3.5. Antiinflammation test in vivo

The ear edema model permits the evaluation of antiinflammatory steroids and is less sensitive to nonsteroidal antiinflammatory agents [27]. As shown in Fig. 6, compared with the control group, oral administration of aspirin at 0.45 g/kg inhibited the development of ear edema induced by the topical application of

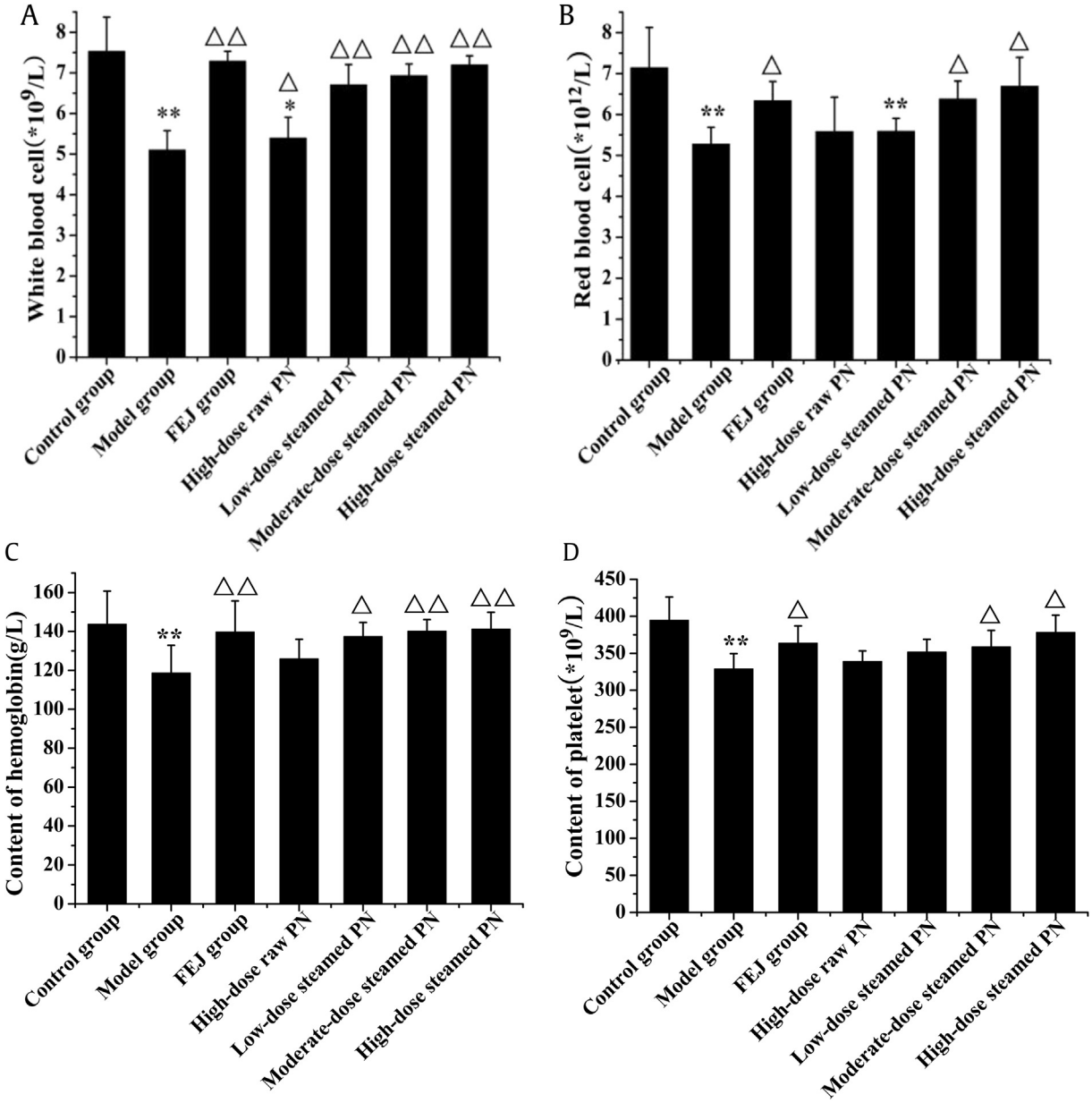

Fig. 7. The blood parameters of mice after treating raw and steamed PN root. (A) The content of WBC. (B) The content of RBC. (C) The content of Hb. (D) The content of platelet. Each value represents the mean ± SD ($n = 10$); *$p < 0.05$ and **$p < 0.01$, compared with the control group; △$p < 0.05$ and △△$p < 0.01$, compared with the model group. Hb, hemoglobin; PN, *Panax notoginseng*; RBC, red blood cell; SD, standard deviation; WBC, white blood cell.

xylene significantly. The swelling degrees after treating PN root and rhizome were inhibited at the dose of 1.8 g/kg significantly ($p < 0.05$ for PN root and $p < 0.01$ for PN rhizome) compared with the control group. The inhibition by 1.8 g/kg of PN rhizome was greater than the same dose of root significantly ($p < 0.01$), suggesting that PN rhizome had better antiinflammatory effect. For the same part of PN under different process, there was no significant difference between raw and steamed PN, except for the inhibition rates between the treatment of raw and steamed PN root ($p < 0.05$).

3.6. Blood parameters after treating raw and steamed PN root

Anemia is a very common and difficult-to-treat syndrome which is regarded as the decrease of hemoglobin in modern medicine, which is also considered to be similar to the blood deficiency in the theory of TCM [21]. The treatment of blood deficiency often includes the use of medicinal formula with blood-enriching and body-tonifying functions. FEJ, a famous TCM formula made up of Asini Corii Colla (donkey-hide gelatin prepared by stewing and concentrating from the hide of *Equus asinus* Linnaeus.), Codonopsis Pilosulae Radix [the root of *Codonopsis pilosula* (Franch.) Nannf.], Ginseng Rubra Radix (the steamed and dried root of *Panax ginseng* Meyer), Crataegi Fructus (the fruit of *Crataegus pinnatifida* Bunge), and Rehmanniae Preparata Radix [the steamed and sun dried tuber of *Rehmannia glutinosa* (Gaertn.) Libosch. ex Fisch. & C.A. Mey.], is used widely in clinic to replenish *qi* (vital energy) and nourish blood; it is approved by China Food and Drug Administration [31]. In the research, FEJ was served as the positive control to investigate the blood-tonifying function of PN. Cyclophosphamide, as a cytotoxic agent used commonly to induce aplastic anemia, is applied to establish the anemia model.

After the administration for 15 days, the quantities of WBC, RBC, Hb, and platelet from the peripheral blood of mice were shown in Fig. 7. Compared with the control group, the levels of WBC, RBC, Hb, and platelet in the model group were decreased significantly ($p < 0.01$), indicating the anemia model was established successfully. Compared with the model group, WBC and Hb levels in mice treated with FEJ and three doses of steamed PN were increased significantly ($p < 0.01$), and the RBC and platelet levels of the FEJ group, moderate-dose and high-dose steamed PN groups were increased significantly ($p < 0.05$). Besides, there were no significant differences in the levels of the above four parameters between the control group and steamed PN groups (except the RBC level induced by low-dose steamed PN), suggesting that steamed PN could reverse the decrease of the quantities of WBC, RBC, Hb, and platelet in a dose-dependent manner significantly. Whereas for raw PN, it elevated the level of WBC significantly, but made no significant difference to levels of RBC, Hb, and platelet compared with the model group, indicating that the blood-enriching effect of raw PN was generally weaker than steamed PN. According the results, steamed PN could enhance the hematopoietic effect of mice with chemotherapy-induced anemia, which was consistent with the traditional use of steamed PN [8].

4. Conclusions

PN root and rhizome have been used traditionally in both the raw and steamed forms. However, the chemical composition and bioactive effects related to the clinic use of raw and steamed PN medicinal parts have not been compared comprehensively, lacking evidence for the differentiated use of PN root and rhizome under different processing conditions. In the research, we confirmed clearly the better anticoagulation, hemostasis and antiinflammatory effects of raw PN, as well as the stronger antioxidation and hematopoiesis effects of steamed PN, which was consistent with the properties description of PN that “the raw materials eliminate and the steamed ones tonify”. These differences in effects are probably attributed to the chemical composition of PN in different forms. For example, the higher levels of ginsenosides Rg_1, Re, Rb_1, Rd, and notoginsenoside R_1 were shown in raw PN, whereas the increased levels of Rh_1, 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rh_4, and Rk_3 were investigated in steamed PN. For different parts of PN, levels of those saponins in PN rhizome were found to be generally higher than those in the root, which could be related to the stronger activities of rhizome including anticoagulation, antioxidation, hemostasis, and antiinflammation effects compared with the same form of PN root. The present study provided an experimental basis for applying raw and steamed PN in clinic differentially, that raw PN is preferable in treating hemorrhages, blood stasis, and swelling, while steamed PN is more beneficial for nourishing the blood and tonifying the body. Furthermore, active constituents correlated to different pharmacologic effects of PN and mechanisms involved will be investigated in the subsequent research.

Conflicts of interest

The authors have declared no conflicts of interest.

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References

[1] Li F, Li Q, Wang J, Lv C, Song D, Liu P, zhang D, Lu J. Chemical and bioactive comparison of flowers of *Panax ginseng* Meyer, *Panax quinquefolius* L., and *Panax notoginseng* Burk. [J]. J Ginseng Res 2017;41:487–95.
[2] Chinese pharmacopoeia. *Notoginseng Radix et Rhizoma*, vol. 1; 2015.
[3] U.S.A Herbal medicines Compendium 1.0. *Notoginseng Radix et Rhizoma*.
[4] British pharmacopoeia. *Notoginseng root*, vol. IV; 2014.
[5] European pharmacopoeia 8.0. *Notoginseng root*.
[6] 103rd Congress. Dietary supplement health and education act of 1994. Pub Law 1994; 103-417. 108 Stat 4325..
[7] Ge F, Huang ZJ, Yu H, Liu DQ. Transformation of *Panax notoginseng* saponins by steaming and *Trichoderma longibrachiatum* [J]. Biotechnol Biotechnol Equip 2016. https://doi.org/10.1080/13102818.2015.1102611.
[8] Gu C, Lv J, Zhang X, Qiao Y, Yan H, Li Y, Wang D, Zhu H, Luo H, Yang C, et al. Triterpenoids with promoting effects on the differentiation cells from the steamed roots of *Panax notoginseng* [J]. J Nat Prod 2015;78:1829–40.
[9] Lau AJ, Toh DF, Chua TK, Pang YK, Woo SO, K HL. Antiplatelet and anticoagulant effects of *Panax notoginseng*: comparison of raw and steamed *Panax notoginseng* with *Panax ginseng* and *Panax quinquefolium*. J Ethnopharmacol 2009;125:380–6.
[10] Chen Z, Li CM, Yang CX, Yu J. Lipid regulation effects of raw and processed Notoginseng Radix et Rhizome on steatotic hepatocyte L02 cell. Biomed Res Int 2016:1–9.
[11] Wang D, Liao P, Zhu H, Chen K, Min X, Zhang Y, Yang C. The processing of *Panax notoginseng* and the transformation of its saponin components [J]. Food Chem 2012;132(4):1808–13.
[12] Sun S, Wang CZ, Tong R, Li XL, Fishbein A, Wang Q, He TC, Du W, Yuan CS. Effects of steaming the root of *Panax notoginseng* on chemical composition and anticancer activities [J]. Food Chem 2010;118:307–14.
[13] Wang JR, Yau LF, Gao WN, Liu Y, Yick PW, Liu L, Jiang ZH. Quantitative comparison and metabolite profiling of saponins in different parts of the root of *Panax notoginseng*. J Agric Food Chem 2014;62(36):9024.
[14] Dan M, Xie G, Gao X, Long X, Su M, Zhao A, Zhao T, Zhou M, Qiu Y, Jia W. A rapid ultra-performance liquid chromatography -electrospray ionization mass spectrometric method for the analysis of saponins in the adventitious roots of *Panax notoginseng*. Phytochem Anal 2009;20:68–76.
[15] Jia XH, Wang CQ, Lui JH, Li XW, Wang X, Shang MY, Cai SQ, Zhu S, Komatsu K. Comparative studies of saponins in 1-3-year-old main roots, fibrous roots, and rhizomes of *Panax notoginseng*, and identification of different parts and growth-year samples. J Nat Med 2013;67:339–49.
[16] Lai MX, Liu HG, Liang QY. Determination of heavy metals and trace elements of *Panax notoginseng*. J Guanxi Acad Sci 2006;13(1):46–7 (in Chinese).
[17] Liu Y, Fan K, Li LJ, Huang S, Liu FH, Wei MT. Studies on content determination the polysaccharides in *Panax notoginseng* and change of polysaccharides in

Panax notoginseng collected at different parts. Chin J Exp Trad Med Formulae 2012;18(19):118–20 (in Chinese).
[18] Liu Y, Qu Y, Wang CX, Yang Y, Cui XM. Determination of the flavonoids in the different parts of *Panax notoginseng* from different areas. J Anhui Agric Sci 2015;43(15):54–5 (in Chinese).
[19] Wu JH, Leung GPH, Kwan YW, Sham TT, Tang JY, Wang YH, Wan JB, Lee SMY, Chan SW. Suppression of diet-induced hypercholesterolaemia by saponins from *Panax notoginseng* in rats. J Funct Foods 2013;5(3):1159–69.
[20] Yim TK, Ko KM. Antioxidant and immunomodulatory activities of Chinese tonifying herbs. Pharm Biol 2008;40(5):329–35.
[21] Shi XQ, Tang YP, Zhu HX, Li WX, Li ZH, Li W, Duan JA. Comparative tissue distribution profiles of five major bio-active components in normal and blood deficiency rats after oral administration of Danggui Buxue Decoction by UPLC-TQ/MS. J Pharm Biomed Anal 2014;88:207–15.
[22] Zhou XH, Long LL, Li CM, Zhao RH, Zhang RP, Mao XJ. Raw versus steamed *Panax notoginseng* in pharmacological effects. Global Chin Med 2014;7(6):420–6 (in Chinese).
[23] Zhao GR, Xiang ZJ, Ye TX, Yuan YJ, Guo ZX. Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem 2006;99:767–74.
[24] Beviglia L, Poggi A, Rossi C, McLane MA, Calabrese R, Scanziani E, Cook JJ, Niewiarowski S. Mouse antithrombotic assay. Inhibition of platelet thromboembolism by disintegrins. Thromb Res 1993;71:301–15.
[25] White CM, Fan C, Song J, Tsikouris JP, Chow M. An evaluation of the hemostatic effects of bydrophilic, alcohol, and lipophilic extracts of *notoginseng*. Pharmacother 2001;21:773–7.
[26] Hosseinzadeh Hossein, Younesi Hani M. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacol 2002;2:7.
[27] Akindele AJ, Adeyemi OO. Antiinflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus*. Fitoterapia 2007;78(1):25–8.
[28] Shi XQ, Shang EX, Tang YP, Zhu HX, Guo JM, Huang MY, Li WX, Duan JA. Interaction of nourishing and tonifying blood effects of the combination of *Angelicae sinensis* Radix and Astragali Radix studied by response surface method. Acta Pharm Sin 2012;47(10):1375–83.
[29] Chan KC, Yin MC, Chao WJ. Effect of diallyl trisulfide-rich garlic oil on blood coagulation and plasma activity of anticoagulation factors in rats. Food Chem Toxicol 2007;45:502–7.
[30] Koh HL, Lau AJ, Chan ECY. Hydrophilic interaction liquid chromatography with tandem mass spectrometry for the determination of underivatized dencichine (β-N-oxalyl-L-α-diaminopropionic acid) in *Panax* medicinal plant species. Rapid Commun Mass Spectrom 2005;19:1237–44.
[31] Liu MX, Tan H, Zhang XK, Wang FS. Hematopoietic effects and mechanisms of *Fufang E'jiao Jiang* on radiotherapy and chemotherapy-induced myelosuppressed mice. J Ethnopharmacol 2014;152:575–84.