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Study on the Process of Ultrasonic Extraction of Total Saponins from Panax Notoginseng Peduncles

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Abstract

Objective: To investigate the best process for extracting total saponins from Panax notoginseng peduncles. **Methods:** The extraction process of total saponins of Panax notoginseng was optimized by orthogonal test method, and the content of total saponins in Panax notoginseng was measured by ultraviolet-visible spectrophotometer, and four factors were selected for the experiment: ethanol concentration, ultrasonic extraction time, ultrasonic extraction times and material-liquid ratio, and each factor was taken at three levels for experimental exploration. **Results:** The optimal extraction process of Panax notoginseng peduncle saponins A 3 B 2 C_3D_2 was ethanol with a concentration of 70%. The material-liquid ratio of 1:40 was sonicated 3 times in an ultrasonic cleaner with a duration of 50 minutes each time, and the total saponins content of the stalks extracted were the highest. **Conclusion:** In this study, the content of total saponins in the peduncles extracted by the best process was the highest, which provided a certain experimental theoretical basis for the further development and utilization of Panax notoginseng peduncles.

Keywords

Panax notoginseng peduncles, total saponins, orthogonal test, extraction process

1. Introduction

Panax notoginseng (Burk) is also known as "Tianqi", "Jin Bu Change", etc., and is a plant in the family *Panax notoginseng* (Burk.) Dried roots and rhizomes of F. H. Chen, the main production areas are Wenshan Prefecture, Yunnan Province, Wenshan County, Yanshan County, and other places, and also planted in Tianyang, Tiandong, Debao and other places in Guangxi. Yunnan Wenshan Prefecture has a long history of cultivation of Sanqi, where the production is large and the quality is good, people are used to call "Wen Sanqi", "Wenshan Sanqi" and "Kaihua Sanqi" Sanqi is a famous local medicinal herb. Panax notoginseng mainly contains Panax notoginseng saponins (PNS), flavonoids, volatile oils, amino acids, sugars and other active ingredients that are beneficial to the human body, and each of its components has different physiological activities and physiological effects. Modern pharmacological research has found that Panax notoginseng has many beneficial effects on human health, such as protecting brain tissue, protecting cardiomyocytes, stopping bleeding, lowering blood lipids, antithrombotic, enhancing immunity, anti-inflammatory, anti-fibrosis, anti-tumor, removing oxygen free radicals, antioxidant, etc., taking some Panax notoginseng and Panax notoginseng products in people's daily life can effectively reduce the occurrence of some cardiovascular and cerebrovascular diseases, improve the body's immunity, and improve people's lives.

People have also been studying the composition of Panax notoginseng saponins for a certain period of time, as early as the eighties of the twentieth century, Taniyasu et al. reported on the saponins in Panax notoginseng flowers. Through their own investigation, they found that Panax notoginseng flowers contain ginsenosides Rb1, Rb2, Rc, Rd and F2. Through the investigation of literature, it was found that as early as the nineties of the twentieth century, experts and scholars had discovered Panax notoginseng saponin and separated and extracted a variety of active ingredients in Panax

notoginseng saponin through a series of extraction methods, and then a lot of research was carried out. In the literature "Research on Panax notoginseng saponinseng" published by Wei Junxian et al., it is recorded that Wei Junxian et al. discovered Panax notoginseng glycosides A, B, C, D, through the study of Panax notoginseng flowers E and other five crystals, and they also identified the trinotoginseng glycosides A, C, D, E through a series of subsequent experiments, respectively A, ginsenusengdiol, singoscin C and septoginseng D, their discovery expanded the gap of knowledge about notoginseng saponin at that time and enriched the research progress at that time. Zuo Guoying et al. mentioned in the literature "Study on the Saponin Components of Panax notoginseng budsaponins" that they isolated Panax notoginseng saponin Fe, gynostemma saponin IX and ginsenoside saponins other than ginsenoside Rc for the first time in Panax notoginseng through repeated atmospheric pressure column chromatography, low pressure column chromatography and dry column chromatography RB3, which was the first time that other active ingredients other than ginsenoside RC were isolated in Panax notoginseng flowers. At the beginning of the 21st century, Li Xian used ultrasonic extraction, silica gel column chromatography and recrystallization to study the chemical composition of Panax notoginseng saponinseng in detail, and obtained 11 monomeric saponins from Panax notoginseng through experiments, and he also identified the structure of 10 of them Among the 11 monomeric saponins he developed at that time, 20R ginsenoside Rg3 and ginsenoside Re were separated for the first time in Panax notoginsenghua. The above-mentioned experts and scholars on Panax notoginseng total saponin research is mainly a qualitative study of Panax notoginseng total saponins, through the study of new chemical components and new saponin components of Panax notoginseng total saponinseng and new saponin components, but at that time there was no in-depth study of the quantitative study and distribution of Panax notoginseng total saponin components and monomeric saponin content, people at that time did not know the content of saponins in various parts of Panax notoginseng. At that time, the distribution of Panax notoginseng saponin components urgently needed to be supplemented by the research of experts and scholars. Therefore, also at the beginning of the 21st century, Zhang Yuan et al. published the literature "HPLC Determination of Saponin Content in Panax notoginseng flower buds" through investigation and research to establish the HPLC determination method of 8 saponins in Panax notoginseng flowers, which passed A large number of experimental data from HPLC assay indicate high levels of ginsenoside Rc and Rb3 in Panax notoginsenghua [9]. Wei Li et al. [10] measured the content of total saponins and monomeric saponins in Panax notoginseng flower buds of different origins and growth years by colorimetric method and HPLC method It is proved that the content of total saponins and monomeric saponins in Panax notoginseng flowers has a particularly significant difference in different origins, for Panax notoginseng in different growth environments, it will breed Panax notoginseng containing different saponin content, and the different growth years of Panax notoginseng are also very different for its total saponins and monomeric saponin content. In the literature "Analysis of Chemical Composition and Quality Control Methods of Panax notoginseng Traditional Medicinal Parts" published by Li Chunying et al. [11], by using ultraviolet spectrophotometry on Panax notoginseng flower and Panax notoginseng root experimental study, it was found that by comparing the total saponin content of Panax notoginseng flower and Panax notoginseng rose, the results showed that the total saponin content in Panax notoginseng flower was higher than that in Panax notoginseng root. However, there is a significant difference in the content of monomeric saponins in the traditional medicinal parts of Panax notoginseng and Panax notoginseng. Panax notoginseng flower is a high content of total saponins in Panax notoginseng, which can be considered as a substitute for the main root of Panax notoginseng to make rational use of existing medicinal resources. Pedicels are also called peduncles with the stem of a solitary flower or the sprig of each flower in the inflorescence. It supports the flower and places the flower in a certain space. At the same time, the peduncle is the channel where the stem and the flower connect. The length of the peduncle varies depending on the plant species, and there are also flowers without stalks. Pedicels have unbranched and branched peduncles, and branched peduncles are called peduncles. Its structure is similar to the primary structure of the stem, usually green in color, cylindrical in shape, and is the connecting part of the flower to the stem, which can spread the flower in a prominent position on the branch. When the fruit is formed, the peduncle becomes the stalk. Due to the special morphological structure of Panax notoginseng flower, the peduncle of Panax notoginseng flower has a certain length, which has great development value.

Panax notoginseng whole plant has good medicinal and health effects. Due to people's traditional habit of using the root into medicine, and only a small amount of the stems and leaves of Panax notoginseng are used to extract extracts or make tea bags, after digging Panax notoginseng is abandoned in the wilderness in large quantities, and there are few studies on the comprehensive utilization of the resources of Panax notoginseng flowers and Panax notoginseng stems and leaves. At present, most of the higher price of Panax notoginseng flower peduncles on the market have been removed, the price of Panax notoginseng flower peduncles is about one-tenth of the price of Panax notoginseng flowers, but our preliminary research found that the total saponin content in the Sanchi flower peduncle is higher, the market is more Panax notoginseng flower peduncles, with higher resources and development advantages, in 2016 data show that the aboveground part of Panax notoginseng has sufficient resources, and the annual output reaches 10 million kg or so, but today its development and utilization is less than 5% [12, 13]. This series of actions has caused a great waste of resources. In order to avoid excessive waste of resources and better use of the stalks that people have not used before, this paper uses orthogonal design method to investigate the main factors affecting the extraction of total saponins from

Panax notoginseng peduncles, and screens out the best process for extracting Panax notoginseng saponins, which lays a theoretical foundation for the in-depth study and practical production of Panax notoginseng peduncles.

2. Instruments and drug trials

2.1 Instrument

AL204 Electronic Balance (METTLER TOLEDO Instruments Co., Ltd.), DZKW-S-6 Electric Constant Temperature Water Bath (Beijing Yongguang Medical Instrument Co., Ltd.), SB-5200DTDN ultrasonic cleaner (Ningbo Xinzhi Biotechnology Co., Ltd.). UV-1800PC UV/VIS spectrophotometer (Shanghai Meipuda Instrument Co., Ltd.).

2.2 Test the drug

Ginsenoside Re (Shanghai source leaf bioscience and technology are limited Provided by the company , CAS#52286-95-6HPLC ≥98%)), Panax notoginseng peduncle (born from Yunnan blue diamond Provided by Wu Technology Co., Ltd., identified by the company's quality department as Panax notoginseng flower peduncle), methanol (Sinopharm Group Chemical Reagent Co., Ltd.), glacial acetic acid (Tianjin Feng Chuan Chemical Reagent Technology Co., Ltd.), perchloric acid (Tianjin Zhengcheng Chemical Products Co., Ltd.), ethanol (Chongqing Chuandong Chemical Co., Ltd.), macroporous adsorption resin D101 (Shanghai Yuanye Biotechnology Co., Ltd.), neutral alumina (Sinopharm Chemical Reagent Co., Ltd.), Vanillin (Sinopharm Chemical Reagent Co., Ltd.), purified water prepared by Yunnan Blue Diamond Biotechnology Co., Ltd.

3. Determination of total saponins

3.1 Specimen handling

3.1.1 Solid specimens

Weigh a sample of about 1.00g (pre-treated Panax notoginseng flower powder), put it in a 100mL volumetric flask, add a certain amount of water, sonicate for a certain time, a certain number of times, and then set the volume with water to 100mL , shake well, place, and pipette 1.0 mL of supernatant for column chromatography.

3.2 Column chromatography

A 10mL syringe was used as a chromatography tube filled with 3cm Amberlite-XAD-2 macroporous resin and 1cm neutral alumina was added. Wash the column with 25mL of ethanol and discard the eluate, then wash the column with 25mL of water, discard the eluate, and add exactly 1.0mL of the treated sample solution (see 3.1), wash the column with 25mL of water, discard the eluate, elute ginsenosides with 25mL of ethanol of a certain concentration, collect the eluate in an evaporation dish, and place it in a 60°C water bath to drain. Use this for color development.

3.3 Color rendering

Accurately add 0.2mL of 5% vanillin glacial acetic acid solution to the above evaporation dish that has been vaporized, turn the evaporation dish to dissolve the residue, add 0.8mL of perchloric acid, mix well, move into a 5mL graduated centrifuge tube with plug, and heat on a 60°C water bath Take out in 10min, cool in an ice bath, add 5.0mL of glacial acetic acid accurately, shake well, and perform colorimetric determination with the standard tube at 560nm wavelength with an lcm colorimetric cell.

3.4 Standard tube

Pipette 100uL of Ginsenoside Re Standard Solution (1.0mg/mL) into an evaporation dish and drain in a water bath (below 60°C) or dry with hot air (do not overheat), the following operation "From 3.2 column chromatography...", is the same as the specimen. Determine the absorbance value.

4. Methods and results

4.1 Experiments and results

4.1.1 Extraction process design

Taking the total saponin content of Panax notoginseng peduncles as an index, according to the results of the pre-experiment, four factors such as ultrasonic time, ultrasonic frequency, material-liquid ratio, and ethanol concentration were selected for four-factor three-level orthogonal design, and the best process was preferred, and the factor level table was shown in Table 1, and the orthogonal test results of $L_9(3^4)$ were shown in Table 2. Analysis of variance is shown in Table 3.

Table 1. Table of orthogonal experimental factor levels

level	factor			
	A Ultrasound time (**min**).	**B** Feed-to-liquid ratio	**C** Number of ultrasounds (times).	**D** Ethanol concentration (%).
1	**30**	**1:20**	**1**	**60**
2	**40**	**1:40**	**2**	**70**
3	**50**	**1:60**	**3**	**80**

Table 2. Panax notoginseng peduncle total saponin orthogonal test results

Experiment number	factor				Average total saponin content (g/100g)
	A	B	C	D	
1	1	1	1	1	2.403
2	1	2	2	2	3.182
3	1	3	3	3	3.253
4	2	1	2	3	2.716
5	2	2	3	1	3.146
6	2	3	1	2	2.821
7	3	1	3	2	3.568
8	3	2	1	3	2.552
9	3	3	2	1	2.801
K1	2.946	2.896	2.592	2.783	
K2	2.894	2.96	2.9	3.19	
K3	2.974	2.958	3.322	2.84	
R	0.08	0.064	0.73	0.407	

Table 3. Analysis of variance table

Source of variance	Sum of squared deviations from the mean	degree of freedom	variance	F-value	P	Salience
A	0.01	2	0.005	1.25	＞0.05	
B	0.008	2	0.004	1	＞0.05	
C	0.807	2	0.404	100.875	＜0.01	****
D	0.291	2	0.146	36.375	＜0.05	**
Error SS	0.01	2				

note: F 0.05(2,2)=19 F 0.025(2,2)=39.00 $F_{0.01}(2,2)=99$

From the orthogonal test table of the yield of Panax notoginseng saponins and its analysis of variance, it can be seen that in this experiment, the number of ultrasonic sounds (C) has a very significant effect on the extraction of Panax notoginseng saponinseng saponins, and the ethanol concentration has a significant effect on the extraction of Panax notoginseng saponinseng saponinseng, while the other two factors are ultrasonic time (A) and material-liquid ratio (B). The effect on its extraction was not significant within the range selected for this experiment, and the order of influence of the factors was: ultrasonic number (C)> ethanol concentration (D) > ultrasonic time (A) > material-liquid ratio (B). Combined with the visual analysis results, the optimal extraction process of Panax notoginseng peduncle saponins is $A3B2C_{3D2}$, that is, ethanol with a concentration of 70% is used. With a material-liquid ratio of 1:40, the ultrasonic cleaning machine was sonicated 3 times, each time the ultrasonic time was 50 minutes, and the content of total saponins extracted from Panax notoginseng stems was the highest.

4.1.2 Validation experiments for preferred processes

In order to further investigate the accuracy and feasibility of the above preferred process, weighed according to the

above preferred process conditions 1.0g. The saponin powder of Panax notoginseng stalks is selected at a concentration of 70%ethanol, ultrasonic in an ultrasonic cleaner 3 times, the duration of each ultrasound is 50 minutes, proceed 6. The results show that the results obtained by the optimal process are more satisfactory and calculated RSD%=1.459%＜2%. It shows that the optimal process is relatively stable, and the average content of total saponins in Panax notoginseng peduncles is 3.735 (g/100g), and the total saponin content of Panax notoginseng peduncles measured in each experiment of the preferred process in the verification test was higher than that measured in the orthogonal test, which proved that the optimal process was feasible, and the experimental results are shown in the table 4.

Table 4. Repeatability results of preferred processes

numbering	1	2	3	4	5	6	Average content	RSD%
Content(g/100g)	3.725	3.764	3.802	3.659	3.683	3.776	3.735	1.459

5. Methodological examination

5.1 Establishment of standard curves

5.1.1 Preparation of total saponin reference solution for peduncles

Accurately weigh 0.010 g of the Ginsenoside Re standard and volume it to 10.0 mL with methanol, that is, 1.0 mg of Ginsenoside Re1.0 mg per ml.

5.1.2 Drawing of standard curves

Aspirate Ginsenoside Re standard solution (1.0mg/mL) 100μL, 200μL, 300μL, 400μL 500μL were placed in 5 evaporation dishes, placed in a water bath to drain (below 60°C), and 0.2mL of 5% vanillin glacial acetic acid solution was accurately added to the above evaporated evaporation dish, and the evaporation dish was turned to dissolve the residue, and then added 0.8mL perchloric acid, mix well, transfer to a 5mL graduated centrifuge tube with plug, heat on a 60°C water bath for 10min to take out, after the ice bath cools, add glacial acetic acid accurately 5.0 mL, shaken well, colorimetric determination at 560 nm with an LCM colorimetric cell. The standard curve is obtained by returning the absorbance (Y) to the mass (X): Y=3.11X+0.0654 (R2=0.9998). The results showed that the linear relationship between Panax notoginseng saponins at 1mg/mL was good, and the measurement results were shown in Table 5 and the standard curve was shown in Figure 1.

Table 5. Standard curve data table

Control mass (**mg**).	0.1	0.15	0.2	0.25	0.3
Absorbance (A)	0.376	0.532	0.685	0.849	0.995

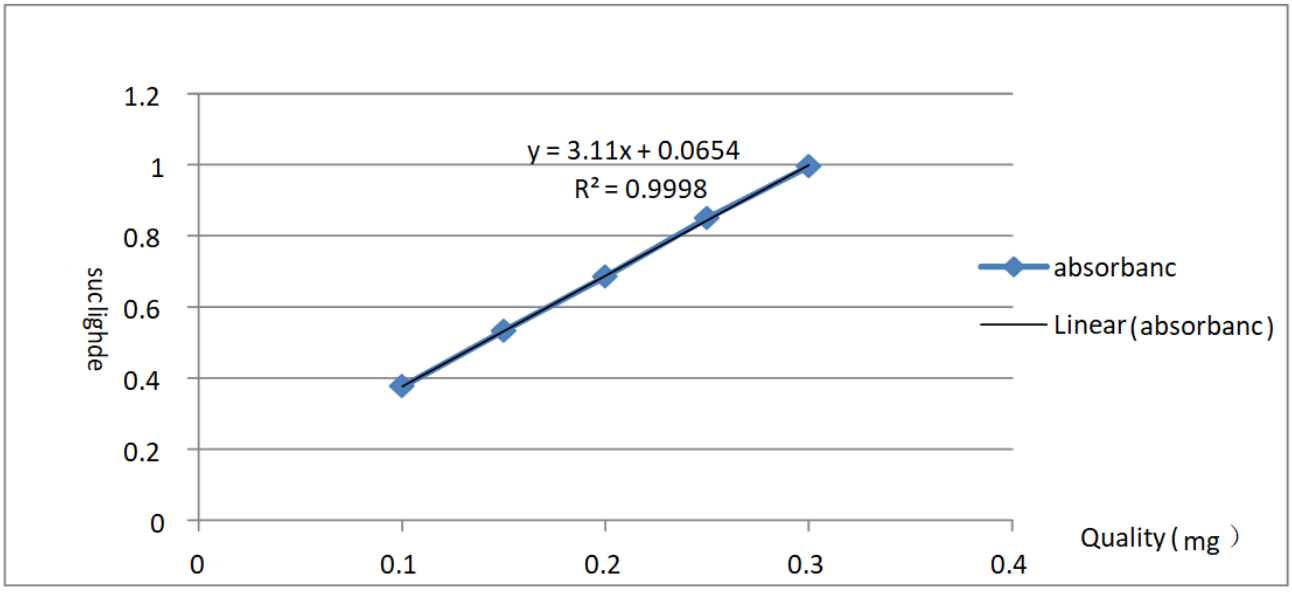

Figure 1. Standard plot of saponin absorbance.

5.2 Instrument precision investigation

The same reference solution was taken for continuous determination 5 times, the measurement structure is RSD% = 0.326 ＜2%. It shows that the precision of the instrument is good, see the table 6.

Table 6. Ultraviolet spectrophotometry precision experimental results

Reference solution	Experiment number					RSD%
	1	2	3	4	5	
Absorbance (A)	0.376	0.375	0.376	0.375	0.378	1.459

5.3 Stability checkout

The total saponin content of sample solution and reference solution was determined at 560nm wavelength at 0, 0.5, 1.0, 2.0 and 4.0h respectively, and calculated RSD. RSDs are 0.041% and 1.010%, both less than 2%. It was shown that both the sample solution and the control solution were stable within 4 hours, and the experimental results are shown in Table 7.

Table 7. Stability test results

Time (**h**).	Sample solution content (**g/100g**).	Reference solution content (m**g**).
0	3.7250	0.0100
0.5	3.7250	0.0098
1.0	3.7260	0.0099
2.0	3.7240	0.0100
4.0	3.7280	0.0098
RSD(%)	0.0410	1.0101

5.4 Spike recovery experiment

5 parts of the known concentration extract (A: containing Panax notoginseng saponins) were taken, a certain amount of Panax notoginseng stalk reference (B) was added precisely, and the content was measured, and the average recovery rate was 99.76% and RSD was 0.23%＜2%, indicating that the recovery rate of spikes is better.

Table 8. Spiked recovery measurements

A(**mg**)	B(**mg**)	Real measurement (**mg**).	Spiked recovery rate (**%**).	Average spiked recovery rate (**%**).	**RSD(%)**
1.326	1.2	2.523	99.77		
1.335	1.2	2.529	99.55		
1.361	1.2	2.556	99.63	99.76	0.23
1.125	1.2	2.322	99.73		
1.452	1.2	2.654	100.14		

6. Summary and discussion

(1) In this experiment, the determination of total saponins in Panax notoginseng peduncles was based on the "Technical Specifications for the Testing and Evaluation of Health Foods (2003 Edition)", the determination method of total saponins in health food and the actual detection, the process research has good reference significance for the development of Panax notoginseng flower peduncles into food and health food.

(2) The average recovery rate of spiked in the test results was 99.76%, indicating that it was feasible to determine the content of total saponins in Panax notoginseng stems by ultraviolet spectrophotometry, the analysis method was feasible and the data analysis was reliable, and the determination of the total saponins in Panax notoginseng stems by ultraviolet spectrophotometry had the advantages of simple operation, easy to use, cheap price, good reproducibility, etc., and the amount of Panax notoginseng peduncles could be better controlled in the actual production process.

(3) In this experiment, ultrasound-assisted extraction was used to refer to the determination method of total saponins in health food in the "Technical Specifications for the Testing and Evaluation of Health Food (2003 Edition)", and the method of extracting total saponins from Panax notoginseng peduncles in this experimental method is also environmentally friendly and pollution-free, and the extracted total saponins of Panax notoginseng peduncles have high purity,

stable quality, and low requirements for resource equipment [14], to provide a certain reference for investing in actual production in the factory in the future.

(4) At present, most of the higher price of Panax notoginseng flower peduncles on the market have been removed, the price of Panax notoginseng flower peduncles is about one-tenth of Panax notoginseng flowers, but our preliminary research found that the total saponin content in the Sanchi flower peduncles is higher, and there are more Panax notoginseng peduncles on the market, so the comparison can find that the Sanchi stalks contain higher Panax notoginseng saponins and its price has a huge advantage over the Panax notoginseng stalks, we may be able to use the less used and less concerned Sanchi flower stalks that people discard, Extracting saponins and beneficial ingredients to make health food and other foods beneficial to the human body, Panax notoginseng flower peduncles have high resource and development advantages. This paper is a simple experiment and introduction to the Sanchi flower stalk, and the conclusions reached have certain reference significance, but the experiment still has many inadequacies and omissions, and the follow-up research still needs relevant professionals to carry out professional scientific research.

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