Study on Extraction Technology for Polysaccharide from Blood-Supplementing Angelica Sinensis Decoction

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Abstract: Purpose:Optimize water-alcohol technology for extracting polysaccharide from blood-supplementing angelica sinensis decoction to improve the extraction rate. Method: Draw the standard curve of glucose reference substance and build the regression equation to calculate the polysaccharide content. Investigate the effect of water addition times, extraction duration, extraction times, ethanol concentration for ethanol precipitation and times of ethanol precipitation on the extraction rate of polysaccharide. Result Add 8 times the medicinal material quality of water, and perform 2 extractions for 120min each time; it shows that conducting 2 ethanol precipitations with 80% ethanol concentration results in the maximum polysaccharide content, indicating the best extraction condition. Conclusion: The experiment establishes an easy and convenient water-alcohol method for extracting polysaccharide from blood-supplementing angelica sinensis decoction, and lays a research foundation on pharmacodynamics of polysaccharide in blood-supplementing angelica sinensis decoction.

Keywords: Blood-Supplementing angelica sinensis decoction; Angelica sinensis; Milkvetch; Polysaccharide; Extraction Technology

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1. Introduction

Blood-supplementing angelica sinensis decoction is created over 800 years ago by Li Dongyuan, one of the four eminent physicians of the Jin-yuan Dynasty. As recorded in Theory of Syndrome Differentiation on Internal and External Injuries, it consists of angelica sinensis and milkvetch with the ratio of 1:5 to achieve the effect of replenishing "qi" and nourishing blood[1]. It is mainly created to treat overstrains and internal injuries, week qi and blood deficiency, floating Yang qi, while it also can be used for menstrual or postpartum blood deficiency, fever and headache, or for long-lasting illnesses and symptoms such as hemorrhoids and ulcerations[2]. The Decoction is also widely used in modern treatment with the critical effect of its polysaccharide macromolecules. Milkvetch in the decoction contains milkvetch macromolecules which can balance the immune performance, improve the metabolism function and hematopoiesis of human body, regulate collagen synthesis and its metabolism, and protect the activity of damaged cells, as well as achieve the effect of anti-oxidation and anti-tumor; angelica sinensis polysaccharide can improve immune and hematopoiesis function of human body and has the function of anti-virus, anti-tumor, anti-radiation injury and anti-oxidation[1]. For further research and development, this test studies its extraction technology by means of water-alcohol method to optimize the extraction process, so as to provide reference for extraction of polysaccharide from blood-supplementing angelica sinensis decoction and lay a foundation for studies on its pharmacology.

2. Experimental Instruments and Materials

2.1 Experimental Instruments

Ultraviolet spectrometry photometer (Beijing Persee General Instrument Co., Ltd.); Electronic analytical balance (Shanghai Sunny Hengping Scientific Instrument Co., Ltd.); Rotary evaporator (Gongyi Kerui Instrument Co., Ltd.); Vacuum pump (Henan Yuhua Instrument Co., Ltd.); Thermostat water bath (Beijing Zhongxing Weiye Instrument Co., Ltd.); Centrifuge (Jinan Boxin Biotechnology Co., Ltd.).

2.2 Experimental Medicinal Materials and Reagents

Angelica sinensis, Milkvetch (National Institute for the Control of Pharmaceutical and Biological Products) Anhydrous glucose (National Institute for the Control

of Pharmaceutical and Biological Products), anhydrous ethanol (AR, Chengdu Chron Chemicals), concentrated sulfuric acid (AR, Chengdu Chron Chemicals), trichloromethane (AR, Chengdu Chron Chemicals), n-butanol (AR, Chengdu Chron Chemicals), phenol (AR, Chengdu Chron Chemicals), distilled water.

3. Testing Method

3.1 Determination Principle of Polysaccharide Content

The study uses phenol-sulfuric acid method to determine the content of polysaccharide. Principle: Polysaccharide reacts with concentrated sulfuric acid to produce monosaccharide, which then dehydrates to produce furfural derivatives, and the furfural derivatives combine with phenol to produce orange compound, thus the polysaccharide content can be calculated by determining the absorbance.

3.2 Preparation of 5%Phenol Solution

Put 100 g phenol, 0.1 g aluminum flake and 0.05 g sodium bicarbonate into round-bottom flask, heat to boiling and take the distillate at 182℃ (the boiling point of phenol), then put 5 g of the distillate into 100ml volumetric flask with distilled water added to 100 mLto get 5% phenol solution.

3.3 Drawing Glucose Standard Curve

Put glucose in the dryer at 105℃ to constant weight, accurately take 40.00 mg and add distilled water to 1000 mL to get 40 μg/mL standard glucose solution, respectively take 0.4 mL, 0.8 mL, 1.2 mL, 1.6 mL, 2.0 mL solution into dry test tubes and add distilled water in each tube to 2.0 mL, then each add 1.0 mL 5% phenol solution before 10 min standing, and eventually test the absorbance value at 490 nm after evenly shaking up the tubes and placing them at room temperature for 20 min. Take 2.0 mL distilled water as blank control. Take absorbance value as ordinate and polysaccharide microgramme as abscissa to draw the standard curve.[2]

3.4 Remove Protein From Crude Polysaccharide

Measure the crude polysaccharide product, add two times of distilled water and stir for dissolvement before adding 1/5 its quality of chloroform solution and 1/30 its quality of butanol solution, after 25 min strong shaking to mix it evenly. put it into centrifugal machine to centrifuge at 2,500 r/min for 5 min, separate centrifuged supernatant and precipitation, then put the separated water layer into centrifugal machine again with 5 repetitions for 5 min each, then put the final gained water layer solution into water bath at 70-80℃ to heat and concentrate to appropria te amount, and eventually put the product in the dryer for vacuum drying at 80℃ before weighing to obtain non-protein polysaccharides.

3.5 Determination of Polysaccharide Content

Take 1.0 mL polysaccharide extracting solution, dilute 15 times, stir evenly, accurately measure 1.0 mL, operate according to procedures stipulated in Item 2.3, measure the absorbance value and then calculate the sugar content in the sample referring to the standard curve.

3.6 Extracting Technology Research on Blood-Supplementing Angelica Sinensis Decoction

3.6.1 Decide the Water Addition

Accurately take 18 g medicinal materials of the decoction and put them into the flask for pretreatment. Respectively add 6 times, 8 times, 10 times its quantity of water to decoct 2 times for 2 h each time; after thoroughly decocting, mix the two filtered solutions together and put the mixture into rotary evaporator to rotate and evaporate to optimal concentration; then, add 80% ethanol solution to precipitate for 24 h before putting it into the centrifugal machine for 5min at 400 r/min, then put the precipitation into the dryer at 70-80℃ for vacuum drying, protein of dried crude polysaccharide can thus be removed, dry the product to obtain protein-removed crude polysaccharide.

Prepare gained crude polysaccharide as per preparation method of sample solution. Accurately take 2.0 mL prepared polysaccharide solution by transfer pipette, place it at 488 nm to measure its absorbance as per phenol-sulfuric acid method (after adding 1.0 mL 5.0% phenol solution, rapidly add 15.0 mL sulfuric acid and then shake evenly), the maximum absorbance indicates the highest polysaccharide content, that means, the corresponding water addition is the optimal water extracting condition.

3.6.2 Decide the Decoction Duration

Measure 3 portions of blood-supplementing angelica sinensis decoction material with 18 g each for pretreatment, add 8 times the decoction's quantity of distilled water to decoct respectively for 60, 120 and 150 min; collect extracted solution and put it into rotary evaporator for evaporation and concentration; as per method of the above procedure (add 80% ethanol solution for 24 h precipitation before putting it into the centrifugal machine at 400 r/min for 5 min, then put the collected precipitation in the dryer at 70-80℃ for vacuum drying; protein of dried crude polysaccharide can thus be removed, dry the product to obtain protein-removed crude polysaccharide), prepare the gained crude polysaccharide as per preparation method of sample solution. Accurately take 2.0 ml prepared polysaccharide solution by transfer pipette, place it at 488 nm to measure its absorbance as per phenol-sulfuric acid method (after adding 1.0 mL 5.0% phenol solution, rapidly add

15.0 mL sulfuric acid and then shake evenly), the maximum absorbance indicates the highest polysaccharide content, that means, the corresponding decoction duration is the optimal water extracting condition.

3.6.3 Decide Decoction Times

Measure 3 portions of blood-supplementing angelica sinensis decoction material with 18 g each for pretreatment, add 8 times the decoction's quantity of distilled water to respectively decoct once, twice and 3 times for 120 min each time, collect the decocted solutions, respectively put them into the rotary evaporator for evaporation and concentration. As per method of the above procedure (add 80% ethanol solution for 24 h precipitation before putting it into the centrifugal machine at 400 r/min for 5 min, then put the collected precipitation in the dryer at 70-80℃ for vacuum drying; protein of dried crude polysaccharide can thus be removed, dry the product to obtain protein-removed crude polysaccharide), prepare the gained crude polysaccharide as per preparation method of sample solution. Accurately take 2.0 mL prepared polysaccharide solution by transfer pipette, place it at 488 nm to measure its absorbance as per phenol-sulfuric acid method (after adding 1.0 mL 5.0% phenol solution, rapidly add 15.0 mL sulfuric acid and then shake evenly), the maximum absorbance indicates the highest polysaccharide content, that means, the corresponding decoction times are the optimal water extracting condition.

3.7 Decide the Ethanol Precipitation Technology of Blood-Supplementing Angelica Sinensis Decoction

3.7.1 Decide the Ethanol Concentration

Accurately take 3 portions of blood-supplementing angelica sinensis decoction concentration solution with 3 mL each for control test, and then respectively take ethanol solution with concentration of 75%, 80%, and 85% to conduct ethanol precipitation to learn and decide which concentration could produce the highest polysaccharide extraction ratio.

After 24 h ethanol precipitation is finished, separate the precipitation and supernatant, collect the precipitation and place it in the dryer at 70-80℃ for vacuum drying before weighing. Remove protein from gained crude polysaccharide as per the protein removing method, and protein-removed crude polysaccharid can be produced after vacuum drying. Accurately take 0.10 mg of the crude polysaccharide product, add distilled water to prepare 0.10 mg/mL sample solution, accurately take 2.0mL by using transfer pipette, place it at 488 nm to measure the absorbances of three solutions as per phenol-sulfuric acid method, and then calculate concentrations and percentage contents to select the optimal ethanol concentration.

3.7.2 Decide the Ethanol Precipitation Times

Take 3.0 g concentration solution of blood-supplementing angelica sinensis decoction, add distilled water to appropriate density, use 80% ethanol solution to prepare ethanol precipitation respectively for once, twice and 3 times, after 24 h, collect the precipitation and place it in the dryer at 70-80℃ for vacuum drying before weighing, protein of dried crude polysaccharide can thus be removed and vacuum dry the product to obtain protein-removed crude polysaccharide; accurately take 0.10 mg of the finally-gained crude polysaccharide, add distilled water to prepare 0.10 mg/mL sample solution, accurately take 2.0 mL by using transfer pipette, place it at 480 nm to measure absorbance A value as per phenol-sulfuric acid method, then calculate the crude polysaccharide percentage content.

4. Experimental Results

4.1 Glucose Standard Curve

After glucose with different concentrations are colored by phenol-sulfuric acid, use spectrophotometer to measure the absorbance at 490 nm. The results are shown in the following table. According to the data, calculate the regression equation: Y=0.017X-0.047, R2=0.993.

Table 1. Absorbance values of glucose standard solution with different concentrations

Sugar quality (μg)	16	32	48	64	80
Absorbance A	0.079	0.20	0.33	0.49	0.64

4.2 Research Result of Water Extracting Technology

4.2.1 Influence of Water Addition on Polysaccharide Contents

As shown in Table 2, adding 8 times quantity of water produces the highest absorbance value, indicating the highest polysaccharide content that means, 8 times of water shall be taken as optimal.

Table 2. Influence of water addition on polysaccharide content measurements

Water addition times	6	8	10
Absorbance A	0.180	0.215	0.209

4.2.2 Influence of Decoction Duration on Polysaccharide Contents

As shown in Table 3, 120 min decoction produces the highest absorbance value, indicating that 120 min is the optimal duration for extracting.[3]

Table 3. Influence of decoction duration on extraction ratios of polysaccharide

Decoction time (min)	60	120	150
Absorbance A	0.190	0.210	0.201

4.2.3 Influence of Extracting Times on Polysaccharide Content Measurements

As shown in Table 4, 2 times of extracting produces the highest polysaccharide content, thus, 2 times extracting are optimal.

Table 4. Influence of extracting times on polysaccharide content measurements

Extracting times	1	2	3
Absorbance A	0.179	0.211	0.200

4.3 Research Result of Ethanol Precipitation Technology

4.3.1 Influence of Ethanol Concentration on Polysaccharide Contents

Among the 75%, 80% and 85% ethanol solution used in ethanol precipitation, the highest ethanol absorbance value is produced by the 80% concentration, that indicates the highest polysaccharide content, thus, 80% concentration is optimal.

Table 5. Influence of ethanol concentration on polysaccharide percentages

Ethanol concentration(%)	75	80	85
Absorbance A	0.166	0.212	0.199

4.3.2 Influence of Ethanol Precipitation Times on Polysaccharide Contents

As shown in Table 6, 2 times of ethanol precipitation produces the highest absorbance value, namely the highest polysaccharide content, thus, 2 times of ethanol precipitation is optimal.

Table 6. Influence of ethanol precipitation times on polysaccharide content

Ethanol precipitation times	1	2	3
Absorbance A	0.177	0.221	0.201

5. Discussion

Use the water-alcohol method to extract polysaccharide from blood-supplementing angelica sinensis decoction, during which experiment the water extracting indicators are optimized to obtain the optimal experiment condition.[4] Decide the optimal method of extracting polysaccharide component from blood-supplementing angelica sinensis decoction by measuring absorbance values. By univariate experimental selection, the optimal water extracting process is obtained as: Add 8 times of water and conduct reflux extraction for 2 times with 120 min for each time.

Use the water-alcohol method to analyze polysaccharide of blood-supplementing angelica sinensis decoction, during which experiment the ethanol precipitation indicators are optimized to obtain the optimal condition. Add blood-supplementing angelica sinensis decoction concentrated solution into ethanol solutions with different concentrations by the phenol-sulfuric acid method (after adding 1.0 mL 5.0 % phenol solution, rapidly add 15.0 mL sulfuric acid and stir evenly), for example: Respectively add 70%, 80% and 90% concentration ethanol solutions with once, twice and 3 times of precipitate, and finally measure the absorbance values, the higher the absorbance value, the higher polysaccharide content is.[5] The result shows that 80% ethanol and 2 times of ethanol precipitation produces the highest polysaccharide absorbance, that is, the highest content, thus it is the optimal condition.

6. Conclusion

From the experiment, it can be concluded that the optimal water extracting ethanol precipitation technology of blood-supplementing angelica sinensis decoction is: add 8 times of water with 2 times of decoction for 120 min each time; water extracting solution shall be precipitated twice by 80% ethanol.

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