

Investigation of Extraction and Purification Technology of Total Saponins from *Sindora glabra* Seeds

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Abstract. Ginsenoside Re is employed as the reference substance for the determination of contents, elution rate of the total saponins from *Sindora glabra* seeds is as the evaluation index, the optimum extraction technology derives from the L₉ (3⁴) orthogonal experiments designed for purpose of the maximum extraction of total saponins. The results showed that the optimum extraction process is: concentration of ethanol 70%(v/v), temperature for extraction 50°C, ratio of crude materials to liquid 1:15, time 2.5h, XAD resin is employed to extract the desired substrates total saponins, flow rate 1.0mL/min, desorption first by 6bv distilled water, followed by ethanol 7BV70%. The adsorption capacity of XAD macroporous resin on the total saponins is proved to be large and easy to be desorption.

Introduction

Sindora glabra Merr. Ex de Wit is widely used in tropics areas with high economical values [1]. It is a large tree mainly distributed in Southeast Asia, Hainan island of China and other tropical and subtropical regions [2]. *Sindora glabra* is rich in oil, similar to the flammability of diesel, and so is called "diesel tree". Currently, research on *S. glabra* is limited to its resin oil [3-5]. Our research group studied the chemical compositions of *S. glabra* seeds. It was found that it is high in total saponins, which is considered to bear anti-inflammatory, analgesic and anti-tumor effects [6,7]. In this paper, orthogonal test, separation and purification with macroporous adsorption resin, were applied to extract and purify total saponins.

Experimental

All chemicals and solvents were of analytical grade and commercially available, and used without any purification. *S. glabra* seeds were collected in a mountain in mid Hainan Island and identified by Dazhou Li, senior engineer, from Hainan Forestry Science Institute. The seeds were kept in Key Laboratory of Ministry of Education for Advanced Materials in Tropical Island Resources.

Analysis of the content in *S. glabra* seed

Preprocess of the seed: *S. glabra* seeds were mill crushed into powder, packed in a dry bag and spared. A curtained amount of powder was taken, which was then introduced into petroleum ether (60~90), ultrasonicated and filted to discard petroleum ether liquid, repeated 3 to 4 times, filtering, remove solvents, and spared after drying as degreased seed powder. Set aside.

Samples preparations: 1.0 g the seed powder was placed in a round bottom flask, and ethanol was then introduced, refluxed, centrifuged, and the supernatant was collected and dried over a water bath, and then distilled water was used to dissolve. After that, the residues were defatted with ether, and water saturated n-butanol was employed to repeatedly extract 3 times. The organic solvents were combined and dried, and methanol was added to dissolve until to a final 10 mL.

Preparation of reference solution: 5.0mg of ginsenoside Re was introduced into 50 mL volumetric flask, then methanol was added to dissolve, diluted, and shaken.

Determination of wavelength: A solution of 0.10 mL control sample was introduced into a 75mL evaporation dish, remove water over a water bath. A fresh vanillin glacial and acetic acid solution of 5%, 0.2 mL, and a solution of perchloric acid 0.8 mL were combined together, heated to 60°C for 15min, and then cooled over an ice-water bath for 5min, an additional 5ml of acetic acid was then added to the final control sample. Which was then canned, and at the wavelength of 550nm it was found a maximum absorption.

Calibration: Sample solutions of 0.00 mg/mL, 0.10 mg/mL, 0.20 mg/mL, 0.40 mg/mL, 0.60 mg/mL, and 0.80 mg/mL were well prepared, and samples of 2mL of the corresponding solutions were taken, the water was removed and the residues were introduced to a combination of 0.2mL of fresh solution of vanillin glacial and acetic acid and 0.8mL of solution of perchlorate, the mixtures were then heated to 60°C for 15min, cooled with ice bath for 5min, and additional 5mL of ice acetic acid were added to the corresponding solution to form final solutions. The calibration equation is: $A=0.8185x+0.0152$, $R^2=0.9968$. It was indicated that the calibration has a good linear correlation.

Verification: The experimental results showed that the average content of total saponins was 4.33% and RSD was 2.12%, and the extraction process has a good repetition.

Results and discussion

Extraction technology of total saponins of *S. glabra* seed [8]

Through pre-experiments, ethanol was chosen as the solvent for extraction and purification, for purpose of an optimized extraction conditions, concentration of ethanol (A), temperatures (B), ratio of solid to liquid (C) and extraction times (D) were as the possible factors for the determination of the content of total saponins, orthogonal test of $L_9(3^4)$ were carried out. The results are listed in table 1.

Table 1 Orthogonal results

Entry	Factor				Absorption
	A[%]	B[°C]	C[m:v]	D[h]	
1	60	50	1:10	1	1.017
2	60	60	1:15	2	0.568
3	60	70	1:20	2.5	0.717
4	70	50	1:15	2.5	2.117
5	70	60	1:20	1	0.553
6	70	70	1:10	2	0.524
7	80	50	1:20	2	2.062
8	80	60	1:10	2.5	1.276
9	80	70	1:15	1	1.039
K1	0.767	1.732	0.939	0.870	
K2	1.065	0.799	1.241	1.051	
K3	1.459	0.760	1.111	1.370	
R	0.692	0.972	0.302	0.500	

Results in Table 1 indicated that, the optimized extraction condition is: concentration of ethanol, 70%, extraction temperature, 50oC, ratio of solid to liquid, 1:15, and soaking time, 2.5h. A total of 4.29% of total saponins could be extracted from *S. glabra* seeds.

Purification of macroporous resin

Saturated adsorption determination. Samples of 10mg/mL of extraction solution from *S. glabra* seeds were dealt with columns filled with 8mL XAD resin, 8 collections were collected and determined the absorption, and the results were listed in Table 2.

Table 2 Determination of saturated adsorption

BV	1	2	3	4	5	6	7	8
Absorption	0.014	0.128	0.348	0.480	0.636	0.659	0.671	0.673

Results in Table 2 indicated that with increased samples, a increase in absorbance could be observed, nevertheless, after collection 5, no obvious absorbance could be detected. Which means that the absorbance comes to saturation after acceptance of 10mL collections. Therefore 10mL collections proved to be the maximum absorption for the employed 8mL XAD resin.

Screening for elution solvent. 3BV of extraction solution of 10mg/mL from *S. glabra* seeds were subjected to go through a column filled with 8mL XAD resin, repeated 3 times. And washed with 6BV distilled water, eluted with 2BV elution solutions ethanol of 30%, 40%, 50%, 70%, 90%, respectively. One sample collection was made every 1BV, there are 16 collections in total, whose absorptions were measured and the results were listed in Table 3.

Table 3 Choice of the elutions

BV	1	2	3	4	5	6	7	8
Absorption	1.439	0.538	0.431	0.201	0.128	0.016	1.078	0.249
BV	9	10	11	12	13	14	15	16
Absorption	1.089	0.249	0.105	0.133	2.438	1.951	1.508	0.864

Results in Table 3 showed that when 6BV elution solutions were carried out, almost no absorption could be detected. The amount of elution was changed with increased elution solution, and when ethanol of 50% was used, the amount of elution became to be the smallest among all elution solutions tested, which proved to be the largest in elution amount when ethanol of 70% was employed.

Volume of employed elution solution. 3BV of extraction solution of 10mg/mL from *S. glabra* seed were subjected to go through a column filled with 8mL XAD resin, washed with 6BV distilled water, and eluted with 8BV ethanol solution of 70%, one sample was collected every 1BV, totally 8 ones were collected, the absorptions were measured and the results were listed in Table 4.

Table 4 Amount of ethanol of 70%

BV	1	2	3	4	5	6	7	8
Absorption	2.276	1.812	1.006	0.476	0.227	0.163	0.102	0.041

Results in Table 4 indicated that after the eighth collection, the absorption decreased sharply, which means that the concentration of total saponins in the collection is very low, most of the total saponins was eluted. Therefore, 7BV of elution solution ethanol of 70% proves to be enough for desorption.

Effect of flow rate on absorption. Four samples of extraction solution 2BV equipped with 4 steles of 8mL XAD resin, washed with 6BV distilled water, eluted with 1BV ethanol solution of 70%, collected and measured the corresponding absorptions, and the results were listed in Table 5.

Table 5 Relation between the absorption and flow rate

Flow rate[mL/min]	1.0	2.0	3.0	4.0
Absorption	0.807	0.675	0.592	0.220

Results in Table 5 indicated that XAD resin has better adsorption capacity with low flow velocity, and the adsorption capacity decreased when flow rate increased. For consideration of easy operation and producing rate, the flow rate of 1mL/min was thus chosen.

Conclusions

In this work, the extraction process of total saponin from *Sindora glabra* seeds was subjected to orthogonal test. The optimized conditions are: ethanol concentration, 70%, extraction temperature 60 °C, soaking time, 2h, and the ratio of solid to liquid, 1:15. The extraction rate of crude saponins can be reached to 4.29%.

Experimental results proved that the XAD macroporous resin performed better for absorption of total saponins, which is proved to have larger adsorption capacity, easy adsorption and desorption. The optimal conditions among all the tested ones are: being eluted with 1.0 mL/min distilled water, and then eluted with 6BV, and then 7BV ethanol of 70% was used to get the total saponins. The pure total saponins is obtained in the yield of 59.65%, and the extraction rate was 2.56%.

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