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Optimizing the extraction of total saponins from *Ornithogalum Caudatum* Ait. by response surface methodology

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ABSTRACT

To optimize the extraction process of total saponins from *Ornithogalum Caudatum* Ait. (OC), ethanol was selected as the extraction solvent and the extraction rate of total saponins was selected as evaluation index. Response surface methodology were used to optimize the extraction process of OC saponins. The result showed that the best extraction process of total saponins from OC was as follows: the temperature was 65 °C, the ethanol concentration was 82% and the extraction time was 4.3 h. Under the optimum condition, the actual extraction rate of total saponins from OC was 0.6743% which was higher than that under the other experimental conditions. From the above, we could draw the conclusion that response surface methodology was a feasible and effective method for optimizing extraction process of the OC total saponins and the results could provide reference for the industrial production of total saponins from OC.

KEYWORDS

Ornithogalum Caudatum Ait.;
Total saponins;
Response surface methodology;
Extraction process.

INTRODUCTION

Ornithogalum Caudatum Ait. (OC) is a plant of Liliaceae perennial herb who is also called pearl grass. It is native to southern parts of Africa and now cultivated all over China[10]. OC is sweet, slightly cold and Channel tropism to liver and spleen. As OC has the efficacy of clearing and detoxifying and dispersing clumping, it is used for the treatment of anti-inflammatory, healing bloated sore and cholecystitis in Chinese folk[10]. Experimental studies have shown that the ethanol extract of OC not only can significantly combat inflammation and ease pain[9], but also can effectively inhibit the proliferation of cancer cells such as gastric cancer[4], liver cancer[3,5,7], breast cancer[1] and so on. Further study shows that saponins and polysaccharides are the main efficacy material basis of anti-tumor effect[6]. This paper is to optimize the extraction process of total saponins from OC by response surface methodology (RSM). The result will lay foundations for further ex-

ploration and utilization of OC[2].

MATERIALS AND METHODS

Ornithogalum Caudatum Ait. (purchased from Jilin Changbai Korean Autonomous County Changbai Mountain Institute of TCM); Yam saponins standard (purity e™ 98%. BBT INC, batch number: S22-111012).

Testing

Determination of the content of total saponins from OC

Preparation of standard solution

After precision weighing 1.5 mg Yam saponins standard into 10 mL volumetric flask, methanol was added to dissolve the saponins standard and diluted to the final volume. The standard solution was shaked and reserved.

Preparation of the sample solution

Dissolve dry paste that had been dried to constant weight with methanol in 100ml volumetric flask and dilute to the final volume. The sample solution was shaked and reserved.

Preparation of standard curve

Put 0, 0.20, 0.40, 0.60, 0.80, 1.00 mL of Yam saponins standard solution into 10 mL tubes with stoppers. Evaporate to dryness, add 0.2 mL of the fresh 5 % vanillin - glacial acetic acid solution, 0.8 mL of perchloric acid and shake up. The samples were cooled immediately with water when they were taken out. 5 mL glacial acetic was added and the standard absorbance was measured at 454 nm. Each concentration was measured 3 times in parallel and the average value was calculated. Then, calculate the regression equation of standard curve using linear regression method, and draw Standard Curve with the amount of reference substance as the abscissa and absorbance as the vertical axis. The regression equation was obtained as $y = 2.6162x + 0.0033$ ($r = 0.9997$). It showed a good linear relationship of Yam saponins among 0.03 - 0.15 mg.

Methodology study

(1) Precision experiment: Draw the standard solution 0.2 mL precisely and placed into tubes with stoppers. After color processing, the absorbance of standard was detected 6 times continuously at 454 nm. The RSD was 0.17 % (n = 6).

(2) Stability test: The same test sample solution was taken and the absorbance was measured at 0, 10, 20, 30, 40, 50, 60, 70, 80 min after color processing. The RSD was 0.23 % (n = 9).

(3) Reproducible experiment: 6 copies of OC medicinal powder (10g) were taken and were extracted 3 h at the temperature of 70 °C with 80 % ethanol. The absorbance was measured at 454 nm after color processing. The RSD was 1.08 % (n = 6).

(4) Recovery experimentÿ6 copies of OC medicinal powder (0.5 g, the total saponin content is 0.63 %) and 3.0 mg dioscin were taken and were extracted 3 h at the temperature of 70 °C with 80 % ethanol. The absorbance was measured at 454 nm after color processing. Recovery is 98.35 %±1.12 % and the RSD was 1.14 % (n = 6).

Determination of sample solution

Draw the total saponins sample solution 0.2 mL precisely and placed into tubes with stoppers. After color processing, the absorbance was detected at 454 nm. Then calculate the content of total saponins of OC by the regression equation.

Response surface methodology

According to the principle of Box - Behnken experiment design and the result of single factor experiments, we fixed material liquid ratio at 1:15, extraction times at 3 times. Extraction temperature, ethanol concentration and extraction time were chosen as 3 factors (A, B, C). 3 different levels were encoded as 1, 0, -1 (showed in TABLE 1). The extraction rate of total saponins was indicated as response value *Y*. The experimental data were analyzed by Minitab 16 software.

TABLE 1 : Factor of factorial experiment design and code value

Level	Temperature (℃)	Ethanol concentration (%)	Extraction time(h)
-1	60	70	3
0	70	82.5	4
1	80	95	5

FULL PAPER

RESULT AND DISCUSSION

Selection of response surface factors and levels

Result of ANOVA analysis

The experimental results were shown in TABLE 2. Through the ANOVA analysis by Minitab 16 Software, the quadratic regression equation of OC total saponins extraction rate to the conditions of temperature (A), ethanol concentration (B) and time (C) was as follows: $Y=0.60717 - 0.09913A - 0.05038B + 0.04350C - 0.09483A^2 - 0.04708B^2 - 0.09633C^2 - 0.119AB - 0.0535AC - 0.15BC$. The analysis of variance and significance tests of model were shown in Table 3. The *F*

TABLE 2 : Box-Behnken design plan and experimental results

StdOrder	RunOrder	Point class area	Block	Temperatu-reT/℃	Ethanol concentration%	Extractio-n time/h	Total saponin extraction yield %
5	1	2	1	60	82.5	3	0.4445
13	2	0	1	70	82.5	4	0.6550
2	3	2	1	80	70.0	4	0.5575
6	4	2	1	80	82.5	3	0.3070
9	5	2	1	70	70.0	3	0.3185
12	6	2	1	70	95.0	5	0.3090
7	7	2	1	60	82.5	5	0.6320
11	8	2	1	70	70.0	5	0.6122
3	9	2	1	60	95.0	4	0.6110
1	10	2	1	60	70.0	4	0.4715
14	11	0	1	70	82.5	4	0.6395
8	12	2	1	80	82.5	5	0.2805
4	13	2	1	80	95.0	4	0.2210
10	14	2	1	70	95.0	3	0.5155
15	15	0	1	70	82.5	4	0.6454

TABLE 3 : Response values estimated and regression coefficients

Term	Freedom	Coefficient	SD	T	P	Significance
Constant		0.60717	0.02294	26.464	0.000	**
A-temperature/℃	1	-0.09913	0.01405	-7.055	0.001	**
B-ethanol concentration%	1	-0.05038	0.01405	-3.585	0.016	*
C-extraction times/h	1	0.04350	0.01405	3.096	0.027	*
A2	1	-0.09483	0.02068	-4.586	0.006	*
B2	1	-0.04708	0.02068	-2.277	0.072	
C2	1	-0.09633	0.02068	-4.658	0.006	*
AB	1	-0.11900	0.01987	-5.989	0.002	*
AC	1	-0.05350	0.01987	-2.693	0.043	*
BC	1	-0.15000	0.01987	-7.549	0.001	**
Residual and error	5	0.007896				
Lack of fit	3	0.004373		0.83	0.588	
Pure error	2	0.003523				
Total	14	0.346859				

Note: * *P* d" 0.05, significant; ** *P* d" 0.01, very significant.

value is 23.85, *P* value is 0.001, the *P* value of loss of quasi is 0.588. The analysis results made known that the model was remarkable and the test method was reliable. The result of $R^2 = 0.9772$ indicated that 97.72 % of the extraction rate of total saponins was from the selected independent variable that also showed the precision of the model was excellent. According to the *P* value, we could know that the influence ranking of factors on the extraction rate of total saponins was as follows: extraction temperature > ethanol concentration > extraction time.

Result of surface plot analysis

The contour plots and response surface graphs after regression optimization were shown in Figure 1-Figure 3. The results in TABLE 3 and the figures showed that the interaction between the temperature and ethanol concentration, the interaction between the temperature and extraction time, the interaction between the ethanol concentration and extraction time were all significant. The *P* values were 0.002, 0.043 and 0.001 respectively.

The best extraction conditions

To obtain the optimum condition of total saponins of OC, the experimental data were further analyzed by response surface optimization. The result showed that the theoretical maximum value of total saponins extrac-

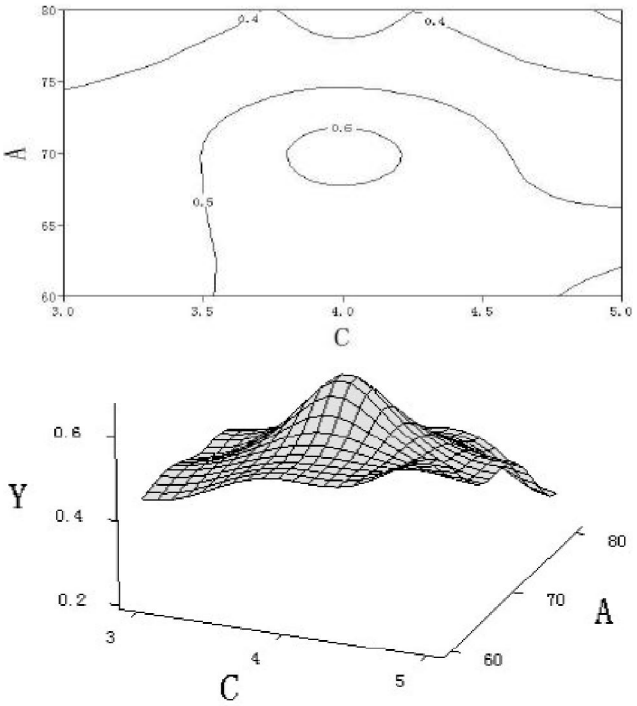

Figure 2 : The contour plot and surface graph of the effect of temperature and extraction time on the extraction rate of total saponins

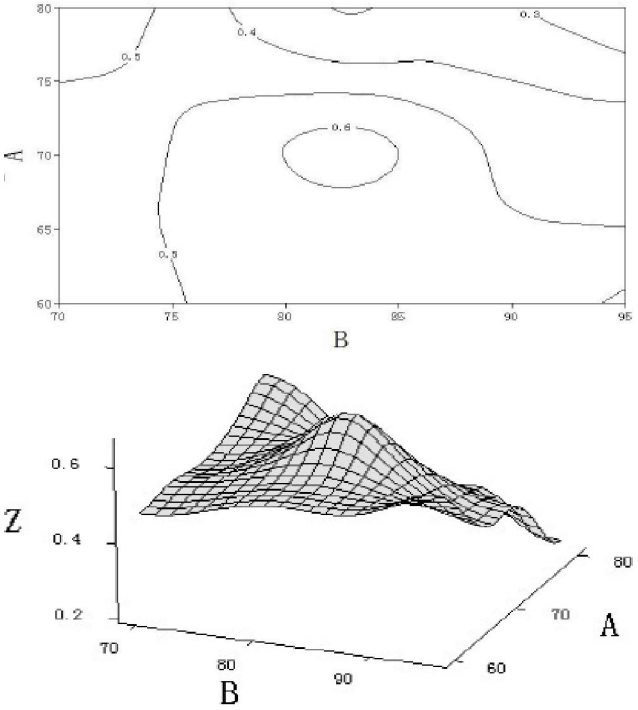

Figure 1 : The contour plot and surface graph of the effect of temperature and ethanol concentration on the extraction rate of total saponins

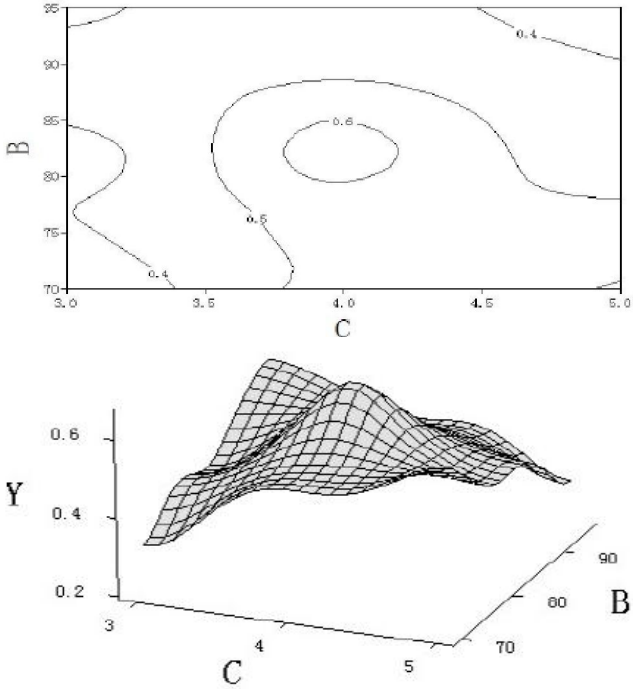

Figure 3 : The contour plot and surface graph of the effect of ethanol concentration and extraction time on the extraction rate of total saponins

FULL PAPER

tion rate was 0.68 %, and the best condition was as follows: temperature (A) was 64.65 °C, the ethanol concentration (B) was 82.12 %, extraction time (C) was 4.25 h.

Result of verification testing:Considering the actual operation, the experiment condition was selected as follows: solid-liquid ratio of 1:20, the temperature of 65 °C, ethanol concentration of 82 %, extraction for 4.3 h. The verification testing was taken 3 times and the average actual extraction rate of OC total saponins was 0.6743 % that was higher than that of the other conditions. The difference between the experimental extraction rate and the theoretical extraction rate was only 0.0032 %.

CONCLUSION

In the paper, we optimize the extraction process of OC total saponins using Minitab 16 software and the Box - Behnken design. The results showed that any two of all the three conditions (temperature, ethanol concentration and extraction time) interaction had significant influence on the extraction rates of OC total saponins. The optimum condition obtained was as follows: temperature was 65 °C, ethanol concentration was 82 %, extraction time was 4.3 h. Under the optimum condition, the actual average extraction rate of OC total saponins was 0.6743%, which was higher than that under the other conditions. The response surface analysis and validation experiment showed that the model was feasible and effective. The experimental result could provide the reference for the industrial production of total saponins from OC.

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