Study on Optimization of polyphenols extraction from Cuscuta chinensis by response Surface Methodology

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Keywords: Cuscuta; polyphenol; extraction; single factor; response surface; optimum process

Abstract: In order to extract polyphenols from Cuscuta chinensis and obtain the best technology for extracting polyphenols from Cuscuta chinensis, the effects of single factor liquid ratio, ethanol concentration, extraction temperature and extraction time on the yield of polyphenols from Cuscuta chinensis were investigated. The reliability of the test was investigated by repeatability test and stability test, and the optimum process of extracting polyphenols from Cuscuta chinensis by alcohol extraction was obtained by the method of screening the best process by response surface software "Design-Expert.V8.0.6.1". The results show that the yield of polyphenols varies greatly under the same factor and different level, the model of response surface is significant, the mismatch is not significant, and the model is effective, the ratio of liquid to material is 25, When the concentration of ethanol is 30%, the extraction temperature is 40 ℃ and the extraction time is 2 h. The results showed that the four single factors had effects on the yield of polyphenols, and there was interaction. The optimum process of response surface screening was effective, and the theoretical extraction rate of polyphenols from Cuscuta chinensis was the highest (0.984%).

1. Introduction

There are mainly two kinds of Cuscuta chinensis and Cuscuta chinensis in the past dynasties in China. They are all Cuscuta species of Cuscuta family, the small ones are Cuscuta chinensis, the large grains are Cuscuta chinensis, all of them have the effects of tonifying the kidney and tonifying essence, nourishing the liver and nourishing the eye, tranquilizing the fetus, etc., and they are commonly used drugs for tonifying the kidney and strengthening yang[1]. The wild Cuscuta chinensis in Changbai Mountain area mainly comes from the dry mature seeds of the genus Cuscuta of the family Cuscutae. Plant polyphenols are a kind of substances with scavenging free radicals and antioxidant capacity[2-3], which play an important role in reducing blood glucose, lowering blood pressure and preventing cardiovascular disease[4-6]. At present, according to the characteristics of plant polyphenols, the main extraction methods are leaching. Waterlogging method, percolation method, decoction method, organic solvent method and so on, these methods often have poor extraction effect, low content of target components, many impurities, affecting the efficacy and so on. Compared with the traditional extraction methods, these new extraction methods, such as ultrasonic assisted extraction[7, 8], microwave assisted extraction[9], enzyme-assisted extraction and supercritical fluid extraction, have the advantages of high purity of target components, high yield and energy saving, but the high cost is limited to laboratory research. The study of polyphenols with high yield and low cost has become the focus of many researchers. The response surface method[10-12] optimizes the design essence. High degree, low cost, suiTable for research.

2. Materials and instruments

2.1 Main drugs

Cuscuta chinensis, Gallic acid standard, fosinophenol, anhydrous sodium carbonate, anhydrous ethanol.

  DOI: 10.25236/isafb.2019.004

2.2 Main tools

100ml beaker, 500ml beaker, 10ml capacity bottle, 25ml capacity bottle, 100ml capacity bottle, glass rod, liquid transfer gun (1ml and 10ml), colorimetric dish, fresh-keeping film, vacuum water circulation pump, water bath pot, ultraviolet spectrophotometer, etc.

3. Method of operation

3.1 Pretreatment

The dried dodder was crushed by an ultra-fine grinder, and the powder was sieved through 100 mesh to obtain fine powder, which was dried and preserved as a sample.

3.2 Pest procedure

sample→ethanol water bath extraction→vacuum filtration→constant volume →dilution→chromogenic reaction→measurement of light absorption→yield.

3.3 Calculation of polyphenol yield of samples

Polyphenol yield(%)=(C×Va×Vb ×N)/(M×1000)×100%

In the formula, C is the concentration measured by polyphenols in the extract (mg/mL); Va is the volume bottle range (mL); Vb is the dilution multiple of the total volume (ml); N of the extract; the mass of M sample is (g); w (%) is the moisture content; v (ml) is the diluent.

3.4 Preparation of standard curve of gallic acid

The standard solution of 0.01g Gallic acid was accurately called 0.01g Gallic acid standard, dissolved in distilled water and fixed in 100mL volume flask. The standard solution of Gallic acid with concentration of 0.1mg/mL was obtained and ready for use. Gallic acid standard solution 0, 0.2, 0.4, 0.8, 0.8, 1.0 mL in 25ml capacity bottle, add distilled water to 5 mL in turn, then add Flynn reagent 0.5 mL in turn, shake well for 1 min, then add 20% Na_2CO_3 solution 1.5 mL in turn, stabilize the volume with distilled water, shake well and avoid light. Using solvent as blank and distilled water as reference, the absorbance was measured by 752nm (scanning its solution with maximum absorption peak under 752nm). Taking Gallic acid concentration as transverse coordinate and absorbance value as longitudinal coordinate, the standard curve was drawn as shown in the figure.

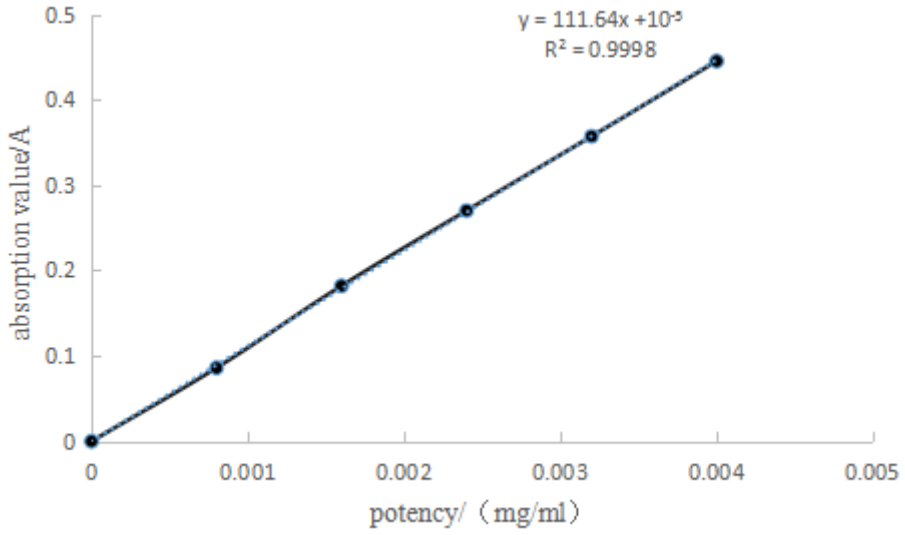

Figure 1 Standard curve of gallic acid

The determination coefficient R^2 is 0.9998, and the standard curve is reasonable.

3.5 Precision test

The standard product of Gallic acid was 0.01g, fixed volume to 25ml capacity bottle, repeated the preparation of standard curve, the concentration of each group was repeated 5 times, and the absorbance value was made into standard curve. The determination coefficient R^2 was above 0.9995, which showed that the precision of the instrument was good.

3.6 Repeatability test

Six samples (fine powder of Cuscuta chinensis) prepared by 1g were accurately extracted and dissolved in 30% ethanol, filtrated, 1ml of the culture medium, added to the 25ml volume bottle respectively, then distilled water was added to 5 mL in turn, then Flynophenol reagent 0.5 mL, was added to shake for 1 min, and finally 20% Na_2CO_3 solution 1.5 mL was added in turn, the volume was fixed with distilled water, shook evenly and placed in the light for 2 hours. Using solvent as blank and distilled water as reference, the absorbance was measured at 752 nm. According to the data of six groups, the absorbance difference R=0.005 was obtained, and the reproducibility was good.

3.7 Breakdown test

The absorbance value of the same sample was observed at 0, 15, 30, 45, 60, 75, 90, 105, 120 min, and the variation of absorbance value A was less than 0.005, which proved that it was sTable within 2 hours.

3.8 Single factor test

3.8.1 Effect of liquid-material ratio on the yield of polyphenols

Using 30% ethanol as extractant, 55 ℃ constant temperature water bath for 2.5 h, the effect of liquid-material ratio on polyphenols extraction was investigated under the conditions of liquid-material ratio of 15, 20, 25, 30 and 35mL/g, respectively. The results are as follows.

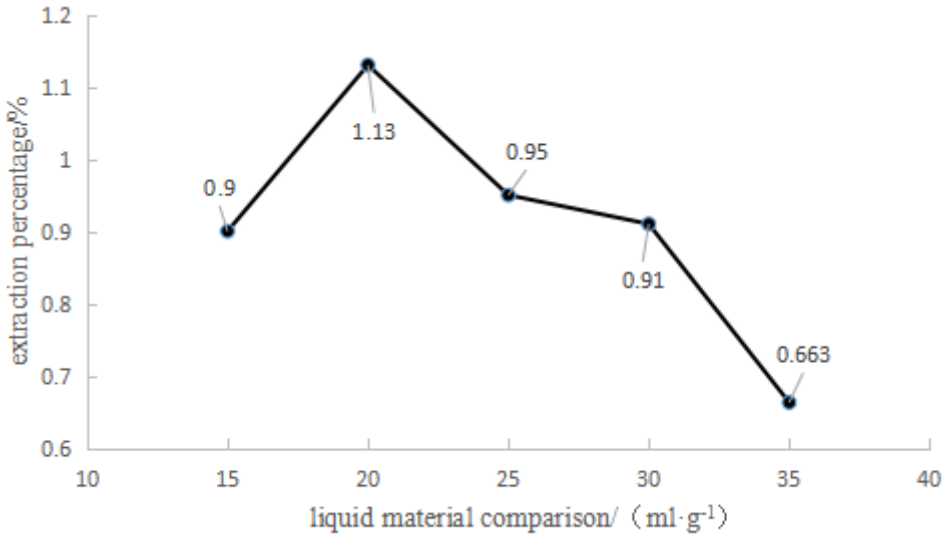

Figure 2 Effect of different ratio of liquid to material on extraction of polyphenols

It can be seen from the diagram that the extreme value R=0.467 shows that the ratio of liquid to material has an effect on the yield of polyphenols, and the yield of polyphenols is the highest when the ratio of liquid to material is 20.

3.8.2 Effect of ethanol concentration on polyphenol yield

The effect of ethanol concentration on the extraction of polyphenols was investigated under the conditions of liquid to material ratio 20, 55 ℃ constant temperature water bath, extraction time 2.5 h, ethanol volume fraction 0, 10%, 30%, 50%, 70%, respectively.

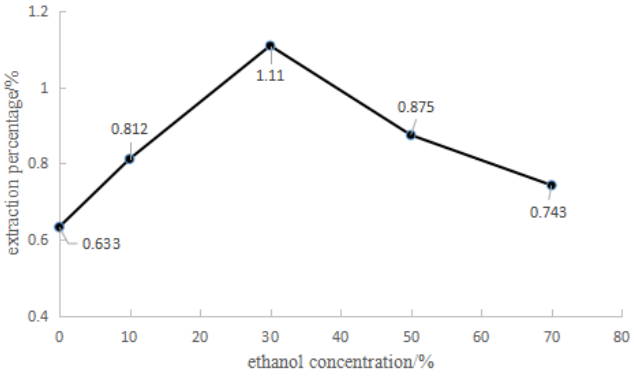

Figure 3 Effect of different ethanol concentrations on the yield of polyphenols

The results show that the extreme value R=0.477 shows that the concentration of ethanol has an effect on the yield of polyphenols, and when the concentration of ethanol is 30%, the yield of polyphenols is the highest.

3.8.3 Effect of extraction temperature on the yield of polyphenols

When 30% ethanol was used as extraction solvent, the ratio of liquid to material was 30 mL/g, the extraction time was 2.5 h, and the water bath temperature was 30, 40, 50 and 60 ℃. The effect of temperature on the extraction of polyphenols was investigated at 70 ℃. The results are as follows.

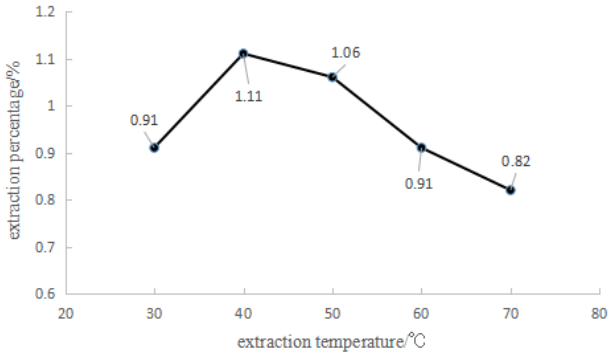

Figure 4 Effect of different extraction temperature on the yield of polyphenols

It can be seen from the diagram that the extreme value R=0.29 shows that temperature has an effect on the yield of polyphenols, and the yield of polyphenols is the highest when the extraction temperature is 40 ℃.

3.8.4 Effect of extraction time on the yield of polyphenols

Using distilled water as extractant, the ratio of liquid to material was 30 mL/g, 70 ℃ constant temperature water bath, hot extraction, extraction for 1.0 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h, 6 h, the effect of time on the extraction of polyphenols was as follows.

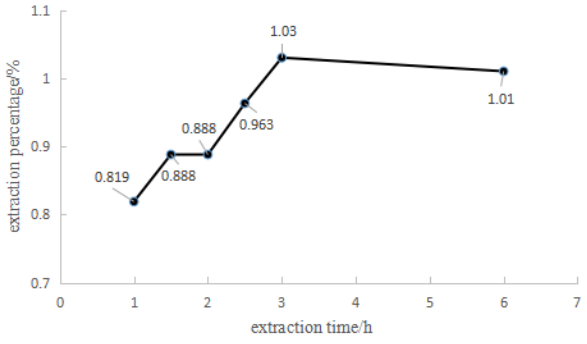

Figure 5 Effect of different extraction time on the yield of polyphenols

It can be seen from the diagram that the extreme value R=0.211 shows that the extraction time has an effect on the extraction rate, and the extraction rate does not change obviously after the extraction time is 3 h, so the extraction time for 3 h is the best.

3.9 Selection of the Optimum Technology for response Surface

The larger influence range of each factor is shown in the Table below.

Table 1 Effective range of influencing factors

Influencing factor	Liquid material comparison/(ml/g)	Ethanol concentration/%	Extraction time/h	Extraction time/℃
The wide range of levels	15-25	20-40	2-4	30-50

Based on the results of the single factor investigation, the “Design-Expert.V8.0.6.1” software, the design test and the results are shown in the following Table.

Table 2 Experimental Design and results of response Surface

Run	Liquid material comparison(A) ml/g	Ethanol concentration (B)%	Extraction time (C) h	Extraction temperature (D)℃	Extraction percentage %
1	25	40	4	30	0.414
2	15	20	2	50	0.488
3	15	40	4	50	0.504
4	20	30	3	20	0.732
5	25	20	2	30	0.754
6	25	40	2	50	0.806
7	25	40	2	30	0.649
8	20	30	3	40	1.12
9	25	40	4	50	0.437
10	20	30	3	60	0.537
11	15	20	2	30	0.389
12	25	20	4	50	0.466
13	20	50	3	40	0.437
14	20	30	3	40	0.943
15	25	20	2	50	0.711
16	20	30	1	40	0.786
17	15	20	4	30	0.449
18	20	30	3	40	0.851
19	15	40	2	30	0.448
20	15	20	4	50	0.554
21	10	30	3	40	0.743
22	20	30	3	40	0.943
23	20	30	3	40	0.904
24	15	40	4	30	0.414
25	25	20	4	30	0.449
26	20	30	5	40	0.899
27	15	40	2	50	0.543
28	30	30	3	40	0.84
29	20	10	3	40	0.673
30	20	30	3	40	0.958

There are 30 groups of tests, of which 8, 14, 18, 22, 23, 30 are central tests, other groups are non-central tests, the three-dimensional vertex is the value of A, B, C, D, and the zero point is the center point. The model obtained by variance analysis is significant, and mis-fitting is not significant, so the construction is effective.

The effect of interaction between four factors on the extraction rate of polyphenols is shown in the figure.

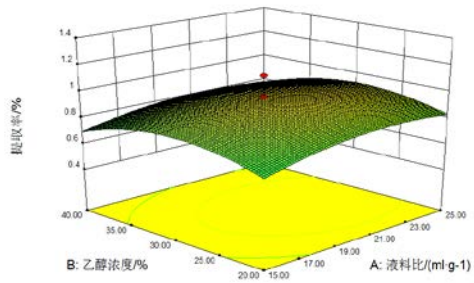

Figure 6 Effect of A / B interaction on extraction rate of polyphenols

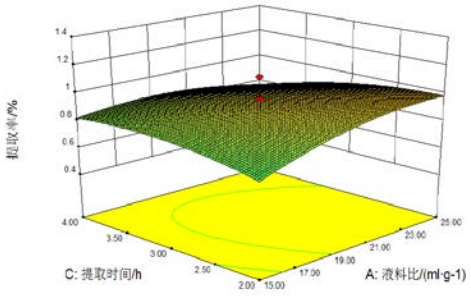

Figure 7 Effect of A / C interaction on extraction rate of polyphenols

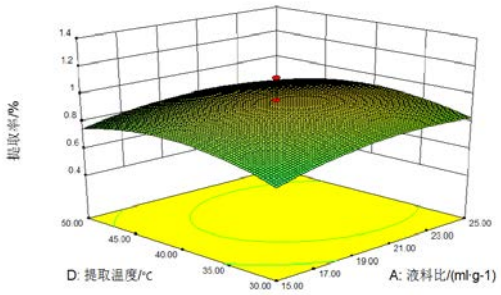

Figure 8 Effect of A / D interaction on extraction rate of polyphenols

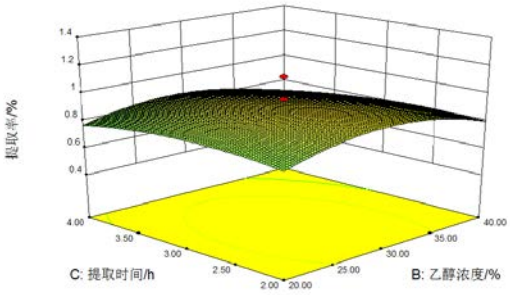

Figure 9 Effect of B/ C interaction on extraction rate of polyphenols

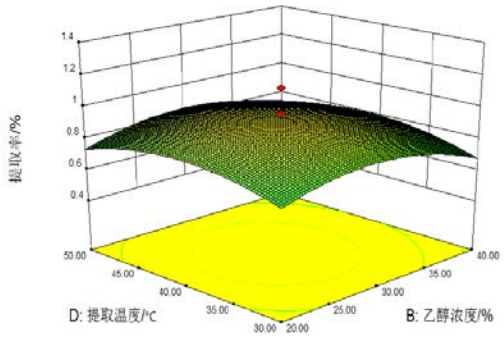

Figure 10 Effect of B / D interaction on extraction rate of polyphenols

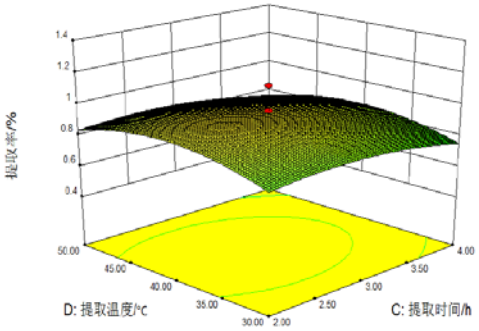

Figure 11 Effect of C / D interaction on extraction rate of polyphenols

4. Results and analysis

The center point is the origin, and when the ratio of liquid to material and the concentration of ethanol is large, the extraction rate decreases, the ethanol concentration increases, and the repulsive force of polyphenols also increases. When the ratio of liquid to material increases, the extraction rate increases, which may be due to the increase of the contact surface area between the extraction solvent and the polyphenols, and when the extraction time increases, the extraction rate decreases, which may be due to the oxidation of polyphenols. From A-D, it can be seen that when the ratio of liquid to material is higher, the extraction rate decreases, which may be due to the destruction of the stability of polyphenols at high temperature. From B-C, it can be seen that when the extraction time and ethanol concentration are large, the extraction rate decreases and the original extraction rate decreases. Because the repulsive force of high concentration ethanol to polyphenols may be increased, it can be seen from B-D that with the increase of ethanol concentration and temperature, the extraction rate will decrease, which may be due to the effect of high concentration ethanol on the dissolution of polyphenols or the destruction of the stability of polyphenols at high temperature. C-D shows that with the increase of extraction temperature and extraction time, the extraction rate

decreases, which may be due to the destruction of the stability of polyphenols by high temperature. The optimum conditions were as follows: the ratio of liquid to material was 25ml/g, the concentration of ethanol was 29.52%, the extraction time was 2.03h, the extraction temperature was 39.79 ℃, and the theoretical extraction rate was 0.984%. Combined with the actual situation, this method is a dodder. The optimum extraction conditions were as follows: the ratio of liquid to material was 25ml/g, the concentration of ethanol was 30%, the extraction time was 2h, the extraction temperature was 40 ℃, and the extraction rate was 0.903%, 0.899%, 0.932%, 1.24%, 0.887%, the average value was 0.972%, which was close to the theoretical value.

5. Discussion

The results show that the square term of the four factors has a great influence on the extraction rate, and the influence degree is as follows: ethanol concentration > temperature > material-liquid ratio > extraction time. The interaction proves that the effect on the extraction rate is not only linear. The experimental results show that the design of the response surface method is reasonable. Orthogonal test is the most commonly used in optimization test, but the accuracy is not enough and the prediction is poor, so the response surface method is used in this method, and the optimization results are more accurate and predictable. The solvent extraction method is low cost, environmental protection, suitable for production research, and provides a reference for the development and utilization of Cuscuta chinensis.

Acknowledgement

Fund Projects: Jilin College of Agricultural Science and Technology Youth Foundation Funding Project, Contract No.: Jilin College of Agriculture Synopsis[2018] No. 3002.

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