Studied on the extraction, response surface analysis and antioxidant activity of flavonoids in the *Momordia cochinchinensis* seeds

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Abstract. This text was mainly studied the extraction flavonoids technology from *Momordia cochinchinensis* seeds. The optimum extraction condition was as follows: when the ratio of liquid to solid was 20:1, the ethanol concentration was 90% and the extraction time was 2h. Under this condition, the actual average yield of flavonoids could reach 0.363%.

1 INTRODUCTION

More and more attentions have been paid to the natural products, especially the products derived from medicinal plants. Some plant natural products have many advantages, which could be used in many fields, such as drug discovery, anticancer, immunity, etc. Many diseases of human beings are closely related to the free radicals in the human body^[1]. There are some certain relationships between free radicals and antioxidation. Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, and many other sources^[2]. Compared with synthetic antioxidants, natural plant antioxidants have many advantages, especially safety^[3].

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by initiation or propagation of oxidizing chain reactions^[4]. Natural products contain many functional compounds, and the exertion of these natural products depends to a large extent on these functional compounds^[5]. Flavonoids are parts of these functional compounds and play a preventive role in the development of cancer and heart disease^[6-7].

Momordia cochinchinensis is a kind of medicine plant whose seeds, roots and leaves have been used as food. *Momordia cochinchinensis* contains many functional compounds, such as the flavonoids compounds^[8].

Ultrasound-assisted extraction (USAE) is an important method to obtain high valuable compounds and could contribute to the increase in the value of some food^[9]. In our study, USAE method was used to extract the *Momordia cochinchinensis* seed. The procedure condition was optimized by using response surface. The infrared spectrum and high performance liquid chromatography (HPLC) were also used to confirm the existence of flavonoids.

2 MATERIALS AND METHODS

2.1 Materials

Momordia cochinchinensis seeds were purchased from the local supermarket. Anhydrous ethanol, sodium nitrite, aluminum sulfate, sodium hydroxide were obtained from Beijing North Chemical Fine Chemicals Co., Ltd. Rutin, quercetin, EDTA (disodium ethylenediamine tetraacetic acid), saffron red, o-phenol, disodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium dihydrogen phosphate were purchased from National drug group chemical reagents Co., Ltd. All the reagents were analytically pure.

2.2 Instruments

Dfy-400 high-speed universal mill (Wenling Linda machinery manufacturing co., LTD); 00000792-1 electronic balance (Zhuji chaoze equalizer equipment co., LTD); Kq-250b, ultrasonic cleaner (Kunshan ultrasonic instrument co., LTD); ReactIR 15 infrared analyzer (German mettler company); 1100 chromatographic analyzer (Agilent company).

2.3 Experimental

2.3.1 Extraction procedure of flavonoids

Momordia cochinchinensis seeds were ground to powder and weighed to 1.00 g, the powder and a certain volume ethanol solution were added into the triangular flask. The flask was placed into the ultrasonic cleaner, temperature was set as 50 °C, power was set as 400 W. After extraction, the solution was filtered by filter paper.

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2.3.2 Standard curve of rutin

Figure 1. Standard curve of rutin.

Momordia cochinchinensis seeds were dried at 105 °C. After the powder was smashed and weighed, 95% ethanol was added, the final volume of the solution was 10 ml.

2.3.3 Calculation of flavonoids extraction rate

The flavonoids extraction rate C% was calculated according to the formula (1).

$$C(\%) = \frac{V \times X}{100 \times A \times W} \times 100\% \quad (1)$$

X: flavonoids extraction rate (mg/ml)

V: extraction volume (ml)

A: sample volume (ml)

W: sample weight (g)

2.3.4 Antioxidant analysis

(1) Scavenging of superoxide anion free radical

In this experiment, the phthalic three phenol self oxidation method was used. The absorbance value was determined at 325 nm. The self oxidation rate of o-phenylene three phenol was calculated by using the Tris-HCl buffer solution (pH=8.2) as blank. Inhibition rate was calculated according to the formula (2).

$$\text{Inhibition rate}(\%) = (A_0 - A_1) / A_0 \times 100 \quad (2)$$

A₀: absorbance of blank sample

A₁: absorbance of flavonoids solution

(2) Scavenging effect of hydroxyl radical

The first test tube was added by 0.2 mol/l phosphoric acid buffer (pH=7.4), and 0.2 ml 520 mg/l reddish red solution was added; the other 8 test tubes were added by 1.0 ml 0.2 mol/l phosphate buffer (pH=7.4), 0.2 ml 520 mg/l reddish red solution and 1.0 ml EDTANa²-Fe²⁺. Then the distilled water (7, 6, 5, 4, 3, 3, 3, 0 ml) was added, respectively. Finally 0.8 ml 6% H₂O₂ solution was added, with heated 30 min in water bath pot, the absorbance value at 520 nm was measured. Scavenging rate (S) was calculated according to the formula (3).

$$S(\%) = (A_{\text{Sample}} - A_{\text{Blank}}) / (A_{\text{Contrast}} - A_{\text{Blank}}) \times 100\% \quad (3)$$

3 RESULTS AND DISSCUSSION

3.1 Single factor experiment

3.1.1 Effect of ratio of liquid to solid on flavonoids extraction

Figure 2. Effect of ratio of liquid to solid on flavonoids extraction.

As shown in Figure 2, the increase in the amount of solvent was conducive to the precipitation of flavonoids. However, under a certain amount, the flavonoids extraction rate was increased slowly, when the ratio reached 30:1, the extraction rate began to decline.

3.1.2 Effect of ethanol concentration on flavonoids extraction

As shown in Figure 3, at first, the flavonoids extraction rate was increased and then decreased with the increase of ethanol concentration. The reason might be that with the increase of concentration, the solvent polarity was decreased and the dissolution rate of flavonoids was decreased.

Figure 3. Effect of ethanol concentration on the flavonoids extraction rate.

3.1.3 Effect of ultrasonic time on the extraction effect of flavonoids

Figure 4. Effect of ultrasonic time on flavonoids extraction.

50 °C was set as the extraction temperature, ultrasonic power was set as 400 W, 70% was set as the ethanol concentration. Figure 4 showed that before 3 hours with the extension of ultrasonic time, the flavonoids extraction rate was increased, and after 3 hours, the extraction rate showed a downward trend, the reason might be that with the increase of the extraction time, the solvent was evaporated.

3.2 Experimental results of response surface

The Box Behnken model by software Design Expert 8.0.6 was used to design the response surface experiment. The design parameter contained the ratio of liquid to solid, ultrasonic time and ethanol concentration^[10].

Table 1. Response surface factor and level table.

Code	Ratio of liquid to solid (A)	Ethanol concentration (B)	Time (C)
-1	10:1	50	1
0	20:1	70	2
1	30:1	90	3

Table 2. Design and the results of the response surface.

No	Ratio of liquid to solid (A)	Ethanol concentration (B)	Time (C)	Extraction rate
1	-1	-1	0	0.06
2	1	-1	0	0.14
3	-1	1	0	0.19
4	1	1	0	0.31
5	-1	0	-1	0.07
6	1	0	-1	0.21
7	-1	0	1	0.08
8	1	0	1	0.24
9	0	-1	-1	0.10
10	0	1	-1	0.29
11	0	-1	1	0.10
12	0	1	1	0.27
13	0	0	0	0.12
14	0	0	0	0.12
15	0	0	0	0.13
16	0	0	0	0.14
17	0	0	0	0.13

The multiple regression equation model was obtained:
 $Y=0.13+0.063A+0.083B+ 2.500\times10^{-3} C + 1.000\times10^{-2}AB + 5.000\times10^{-3}AC-5.000\times10^{-3}BC+3.500\times10^{-3}A^2 + 0.043B^2 + 0.018C^2$.

Figure 5. Interaction between ultrasonic time and ethanol concentration on flavonoids extraction rate.

According to the results of response surface, the optimized process condition was that: when ultrasonic time was 1.95 h, the ratio of liquid to solid was 20:1 (ml/g), and the ethanol concentration was 83.26%, the predicted extraction rate of flavonoids was 0.363%. Considering the convenience of the actual operation, the condition in the optimized process was modified to: the ultrasonic time 2 h, the ratio of liquid to solid 20:1 (ml/g) and the ethanol concentration 90%. Three parallel repeated validation experiments were conducted, and the average flavonoids extraction rate under the modified conditions was 0.378%. The experimental value was only 0.015% different from the predicted value of the model equation, indicating that the optimized extraction condition by response surface method was accurate.

Table 3. Variance analysis of the response surface results.

Source of variance	Sum of squares	mean square	F value	P value
Model	0.095	0.011	30.84	<0.0001
A	0.031	0.031	90.02	<0.0001
C	5.000×10^{-5}	5.000×10^{-3}	0.14	0.7156
AB	4.000×10^{-4}	4.000×10^{-4}	1.15	0.3187
AC	1.000×10^{-4}	1.000×10^{-4}	0.29	0.6081
BC	1.000×10^{-4}	1.000×10^{-4}	0.29	0.6081
A ²	5.158×10^{-5}	5.158×10^{-5}	0.15	0.7113
B ²	7.967×10^{-3}	7.967×10^{-3}	22.95	0.0020
C ²	1.441×10^{-3}	1.441×10^{-3}	4.15	0.0310
Residual	2.430×10^{-3}	3.471×10^{-4}		
Unintended term	2.150×10^{-3}	7.167×10^{-4}	10.24	0.0239
Pure error	2.800×10^{-4}	7.000×10^{-5}		
Total deviation	0.099

Figure 6. Interaction of ratio of liquid to solid and ethanol concentration on flavonoids extraction rate.

Figure 7. Interaction between ultrasonic time and ratio of liquid to solid on flavonoids extraction rate.

It could be seen in Figure 5, 6 and 7 that ethanol concentration had the greatest influence on the extraction rate, followed by ratio of liquid to solid and ultrasonic time.

3.3 Infrared spectral characteristics and analysis

Figure 8. Spectrogram of flavonoids in the *Momordica cochinchinensis*.

It could be seen in Figure 8, there was a strong absorption peak at 3359 cm^{-1} , indicating that there was a large amount of phenolic or hydroxyl groups. The -CH stretching vibration was found at 2974 cm^{-1} . $\text{C}=\text{C}$ double bond was found at 1647 cm^{-1} . -CH_3 was appeared at 1382 cm^{-1} . The peak 1269 cm^{-1} was C-C skeleton vibration absorption peak. The absorption peak at 1043 cm^{-1} was the stretching vibration peak of C-O . The peak 878 cm^{-1} might be hexagon double oxygen ring.

3.4 Analysis of HPLC results

Figure 9. HPLC figure of standard rutin.

Figure 10. HPLC figure of standard quercetin.

Figure 11. HPLC figure of the extraction solution.

As shown in the figures above, the peak of rutin was appeared at 1.732 min, the peak of quercetin was appeared at 2.073 min, the peak of the extraction solution was appeared at 1.631 min, followed by 1.691 min, 1.769 min, and 2.103 min, which indicating that the compound might contain substances similar to rutin and quercetin.

3.5 Antioxidant analysis

3.5.1 Scavenging of superoxide anion free radical

Figure 12. Scavenging ability of flavonoids in the kernel of *chiton sinensis* to superoxide anion free radicals.

As shown in Figure 12, the flavonoids solution had a good scavenging ability to superoxide anion free radicals, and the scavenging rate was increased with the increase of flavonoids concentration^[11].

3.5.2 Scavenging hydroxyl radical

Figure 13. Ability of scavenging hydroxyl radical.

Figure 13 showed that flavonoids solution had the higher scavenging ability for hydroxyl radicals. Moreover, its antioxidant capacity was increased with the increase of flavonoids in the extraction solution. There was a good linear relation in the range of 0-700 ug/ml.

Conclusions

According to the optimization of response surface, under the condition of 400 W ultrasonic power, the optimal extraction condition of *Momordia cochinchinensis* seeds flavonoids was: the ratio of liquid to solid was 20:1, the ethanol concentration was 90% and the extraction time was 2 h. Under this condition, the actual average yield of flavonoids could reach 0.363%, which was close to the predicted value of 0.378%.

It is a good research direction to extract functional compounds from natural products and use them as natural antioxidants. The purpose of this study is to provide references for the extraction of natural products by studying the extraction conditions, we hope more and more natural functional compounds could be developed to new pharmaceutical preparations and benefited human health.

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