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Optimization of ultrasonic-assisted extraction of total flavones from *Coreopsis tinctoria* Nutt.

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Abstract

The conventional extraction methods for flavonoid were time-consuming, laborious and energy-consuming. In this article, ultrasonic-assisted extraction (UAE) was utilized to extract flavonoid from Coreopsis tinctoria Nutt. The factors influencing the extraction efficiency were studied, including the effects of ultrasonic power, extraction time, a ration of materials mass and extraction temperature. By single factor design and response surface methodology (RSM) experiments, the optimum experimental parameters were determined: extraction temperature 70°C, extraction time 62min, a ratio of water to raw material 55mL/g and ultrasonic power 460W,under the most suitable conditions, a maximum yield of total flavonoids 10.90% can be achieved. UAE was an effective way to extract flavonoid from Coreopsis tinctoria Nutt. This technology required short time, less laborious and energy, resulting in low cost and friendly environment.

Keywords: Coreopsis tinctoria Nutt., flavonoid, ultrasonic-assisted extraction, response surface methodology

1. Introduction

Flavonoid also known as bioflavonoid, is a class of compounds with chromone ring and benzene ring as the basic structure, flavonoids widely distributed and various, and has multiple biological activities: in addition to antibacterial, anti-inflammatory, resist mutation, antihypertensive, clearing heat and removing toxicity, calm, diuresis, the significant functions also include antioxidant, antitumor, anti-cancer, inhibition of lipase release, which is a kind of important natural organic antioxidant that has a development prospect[1-3].

The conventional extraction methods include fluid extraction, Soxhlet extraction, supercritical and subcritical extraction and pressurized liquid extraction, but they are require more time and energy. Concerning flavonoids extraction from *bamboo leaves*, *ginkgo biloba*, *hawthorn leaf*, *mulberry leaf, pomegranate leaf* in many of studies have been reported, but about ultrasonic extraction technology of total flavonoids from *C. tinctoria* in research is less. This technology required short time, less laborious and energy, resulting in low cost and friendly environment[4,5].This article optimize the ultrasonic extraction process, aims to find

the best extraction technological parameters of total flavonoids from *C. tinctoria*, to provide theoretical basis for improving the extraction yield of total flavonoids from *C. tinctoria*[6,7,8].

2. Materials and methods

Materials

Coreopsis tinctoria Nutt. (Asteraceae) was provided from Guazhouyide Biotechnology Limited Company in 2012 March and identified as Coreopsis species of *Compositae* by Dr. Yang Lin in College of life science and engineering Lanzhou University of Technology.

Methods

Sample preparation

Paved *C. tinctoria* with a thin layer of 5mm,dried at 60°C for 1h,ground and sieved with 50 meshes. Material sample was kept in dryer.

Draw standard curve

Weighed accurately rutin (>98%) 20mg with 95% ethanol solution at a volumetric flask and constant volume 100ml as standard. The dissolved material 0, 0.5, 1.0, 2.0, 3.0, 4.0 ml were respectively placed in 10ml volumetric flask with 0.3ml 5% $NaNO_2$ solution and mixed. After 6min was added 0.3mL 10% $Al(NO_3)_3$ solution and shook up. Stood 6min after had reacted. Resulting samples were then mixed 4ml 4% NaOH and added 95% ethanol to 10ml, stewed after shook up.

Ultrasonic assisted hydrothermal extraction and determination of extraction yield

The crushed *C. tinctoria* were accurately weighed 2.00 mg and dissolved in 100 ml conical flask, then added a certain amount of distilled water. The experimental process were conducted with predetermined ultrasonic power and time, and immediately filtered with vacuum to 100ml. The content of flavonoid extracted from *Coreopsis tinctoria* was measured according to the method[9]. The optical density of reaction solution was measured at 510 nm. The content of flavonoid in this sample was calculated according to equation of linear regression (Y=0.0819x+0.0005, R^2=0.9987) based on the standard curve whose horizontal coordinate and vertical coordinate denoted the concentration of Rutin (mg/mL) and OD_{510}, respectively. The flavonoid yield could be calculated as follows:

$$\text{yield}\ (\%) = \frac{\text{C} \times \text{N} \times \text{V}}{\text{W} \times 1000} \times 100\%$$

where C is the concentration of flavonoid calculated by the calibrated regression equation (mg/mL); N is the dilution factor; V is the total volume of extraction solution (mL); and W is the weight of raw material (g).

Single factor test

Carried out single factor on ultrasonic power, extraction time, a ration of materials mass and extraction temperature respectively to determine value level of factors, so that selection of BBD factor level.

(1) Extraction temperature

At a ratio of liquid to solid 50 ml/g, ultrasonic power 480 W, extraction times 2 to extract sample at 50, 60, 70, 80°C for 60min. The influence of extraction temperature on flavonoids extraction yield was studied.

(2) Extraction time

At a ratio of liquid to solid 50 ml/g, ultrasonic power 480 W, extraction temperature 70°C and extraction times 2 to extract sample for 30, 40, 50, 60, 70, 80min. The influence of extraction time on flavonoids extraction yield was studied.

(3) A ratio of liquid to solid

At ultrasonic power 480 W, extraction temperature 70°C and extraction times 2 to extract sample at a ratio of liquid to solid 35, 45, 55, 65, 75 ml/g. The influence of a ratio of liquid to solid on flavonoids extraction yield was studied.

(4) Ultrasonic power

At a ratio of liquid to solid 50 ml/g, extraction temperature 80°C, extraction times 2 to extract sample at ultrasonic power 120, 240, 320, 480 W. The influence of ultrasonic power on flavonoids extraction yield was studied.

3. Response surface analysis

According to Box-Behnken Central composite design principle, we synthesized single factor test results, selected 60% alcohol, designed 3-level and 4-variable response surface experiment to study four elements (extraction temperature, extraction time, a ratio of liquid to solid, ultrasonic power) that effect markedly on yield of *C. tinctoria*[8,9], such as the table 1.

Tabel 1. Factors and levels of RSM

Factor	**Level**		
	-1	0	1
A extraction temperature(°C)	65	70	75
B extraction time(min)	50	60	70
C a ratio of liquid to solid(ml/g)	45	55	65
D ultrasonic power (W)	360	420	480

4. Results and discussion

Single factor design

Effects on total flavonoids in C. tinctoria for different extraction temperatures

The condition of ultrasonic power 480 W, a ratio of liquid to solid 55ml/g, extraction time 60min and extraction times 2 were carried out. With the increase of temperature, we can obviously observed that the extraction ratio of flavonoids were increased in 50-70°C and decreased in 70-80°C gradually, which is due to molecular diffusion rate increased slowly as higher temperature and the extraction ratio of flavonoids were increased, but it's easily that oxidative degradation of flavonoids and activity lost with too high temperature (Fig. 1). It's also lead to accelerate the solvent evaporation, energy consumption added, therefore 70°C is a better extraction temperature.

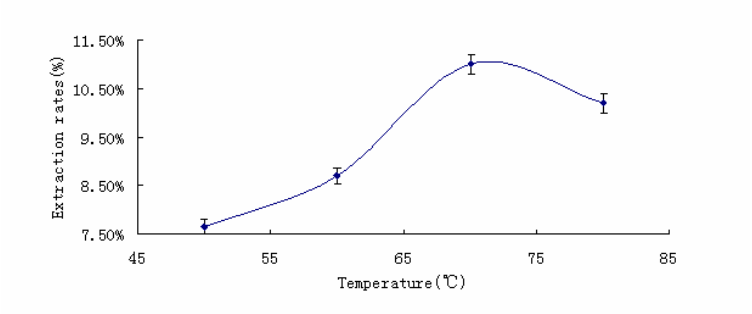

Figure 1. Effect of temperature on flavonoids extraction rates

Effects on total flavonoids in C. tinctoria for different extraction times

The condition of ultrasonic power 480 W, a ratio of liquid to solid 55 ml/g, extraction temperature 70°C and extraction times 2 were carried out. With the extended response time, we can obviously observed that the extraction ratio of flavonoids were increased and achieved to a summit at extraction time 60min (Fig. 2).

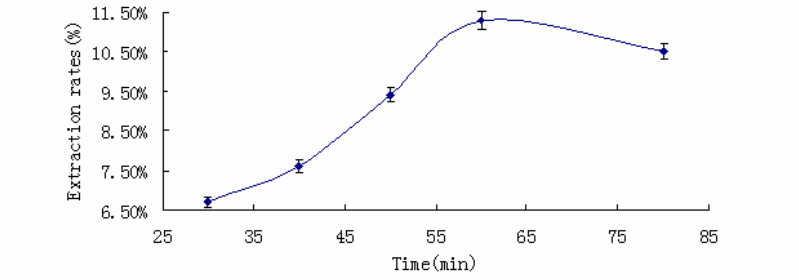

Figure 2. Effect of time on flavonoids extraction rates

Effects on total flavonoids in C. tinctoria at different ratios of materials mass

The condition of ultrasonic power 480W, extraction time 60min,extraction temperature 70°C and extraction times 2 were carried out. With the ratio of liquid to solid augment, we can obviously observed that the extraction ratio of flavonoids were increased among from 35 to 65ml/g. While the ratio was a narrow range behind ratio of liquid to solid 65 ml/g. It is as grow in number of ultrasonic medium, intracellular flavonoids substances began to spread out. As a result, we can achieve to a maximum at a ratio of liquid to solid 65ml/g (Fig. 3).

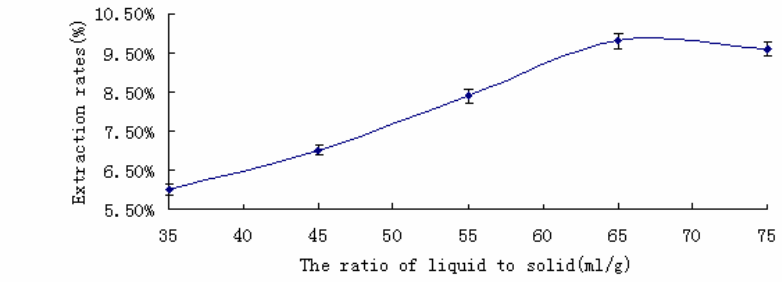

Figure 3. Effect of the ratio of liquid to solid on flavonoids extraction rates

Effects on total flavonoids in C. tinctoria at different ultrasonic powers

The condition of extraction temperature 70°C, a ratio of liquid to solid 55ml/g and extraction times 2 were carried out. With the ultrasonic power augment, we can obviously observed that the extraction ratio of flavonoids were increased and achieve to a summit at 480W. When the ultrasonic power continues to increase, on the country, total flavonoids is a slight decline (Fig. 4). This may be the large ultrasonic power cause flavonoids structure changed. Thus, 480W is better.

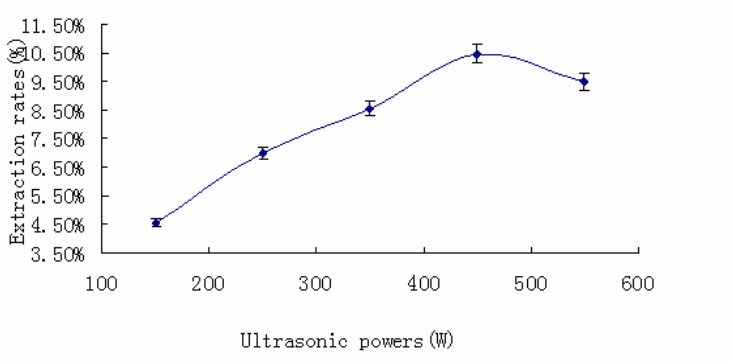

Figure 4. Effect of ultrasonic powers on flavonoids extraction rates

Optimization of extraction conditions by BBD

Box-Behnken design (BBD)

Employ Design Expert 7.0 software, Play with ultrasonic power, extraction time, a ration of materials mass and extraction temperature as response variable and the yield of total flavones as response value processing Table 2. 1-16 experiments are factorial experiment and 17-21 experiments are central experiment in Table 2. The 21 test points divided into factorial point and null point. The factorial points were the independents in the three dimensional vertices which was constituted by A, B, C, D points. Zero point is the center of the area which test repeat 5 times used to estimate test error. Taken Design Expert 7.0 software conducted analysis of regression towards the obtained data. This result was shown in Table 3. Utilized software to conduct nonlinear regression quadratic polynomial fitting and obtained prediction model followed:

$$R= - 78.87157 + 1.92891A+0.065918B + 0.50954C + 0.024480D + 0.00367305AB - 0.00215AC + 0.000389191AD + 0.00025BC + 0.000120608BD - 0.00000833333CD - 0.015654A^2 - 0.00320635B^2 - 0.00334777C^2 - 0.00006451292D^2$$

Tabel 2. Design and results of RSM

NO.	A extraction temperature(°C)	B extraction time(min)	C a ratio of liquid to solid(ml/g)	D ultrasonic power (W)	R Yield (%)
1	75.00	70.00	65.00	360.00	9.41
2	75.00	70.00	45.00	360.00	9.55
3	75.00	50.00	65.00	480.00	9.68
4	65.00	70.00	45.00	480.00	9.61
5	75.00	50.00	45.00	480.00	9.94
6	65.00	50.00	65.00	360.00	9.87
7	65.00	70.00	65.00	480.00	9.88
8	65.00	50.00	45.00	360.00	9.68
9	61.59	60.00	55.00	420.00	9.71
10	78.41	60.00	55.00	420.00	9.76
11	70.00	43.18	55.00	420.00	9.89
12	70.00	76.82	55.00	420.00	9.98
13	70.00	60.00	38.18	420.00	9.82
14	70.00	60.00	71.82	420.00	9.97
15	70.00	60.00	55.00	319.09	9.75
16	70.00	60.00	55.00	420.00	10.62
17	70.00	60.00	55.00	420.00	10.98
18	70.00	60.00	55.00	420.00	10.94
19	70.00	60.00	55.00	420.00	10.97
20	70.00	60.00	55.00	420.00	10.94
21	70.00	60.00	55.00	420.00	10.98

Tabel 3. Analysis of variance for fitted quadratic polynomial model

Term	Sum of squares	df	Mean square	F value	P value	Sig.
model	5.93	14	0.42	27.10	0.0003	**
A	0.001250	1	0.001250	0.080	0.7869	
B	0.00405	1	0.00405	0.26	0.6290	
C	0.00714	1	0.00714	0.46	0.5244	
D	0.38	1	0.38	24.20	0.0027	**
AB	0.11	1	0.11	7.15	0.0369	*
AC	0.092	1	0.092	5.91	0.0511	
AD	0.045	1	0.045	2.89	0.1401	
BC	0.005	1	0.005	0.32	0.5923	
BD	0.017	1	0.017	1.11	0.3328	
CD	0.0002	1	0.0002	0.013	0.9137	
A^2	2.29	1	2.29	146.33	< 0.0001	**
B^2	1.54	1	1.54	98.23	< 0.0001	**
C^2	1.67	1	1.67	107.08	< 0.0001	**
D^2	0.81	1	0.81	51.54	0.0004	**
Residual	0.094	6	0.016			
Lack of fit	0.092	2	0.046	109.72	0.0003	**
Net error	0.00168	4	0.00042			
Total devition	6.03	20				
		R^2=0.9844	R^2_{Adj}=0.9481			

"**"indicate highly significant (P<0.01), "*" indicate significant (0.01<P<0.05)

By regression analysis of variance Table3 significance test showed that the model regression significance ($p<0.0001$) and lack of fit significance. The model $R^2=0.9968$, $R^2_{Adj}=0.9894$ accounted for the model is better fitting with actual experiment and the linear relation significantly between dependent variable and the response value. It can be used to optimize for ultrasonic assisted extraction of *C. tinctoria* flavonoids.

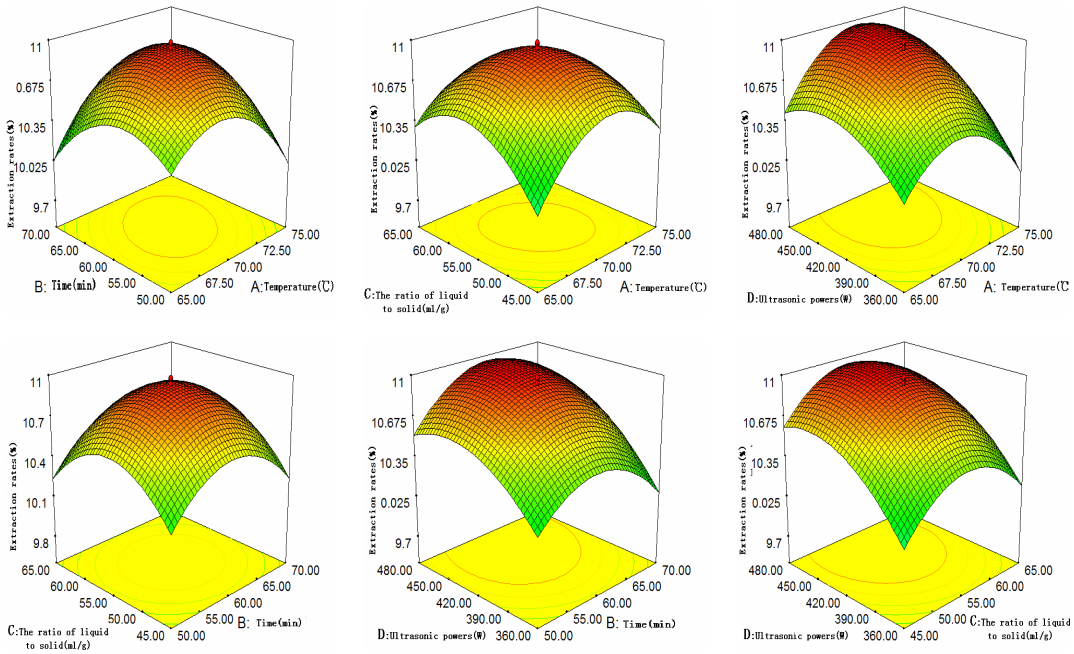

Figure 5. The influence of the interaction among with factors

The influence of the interaction among with factors

As can be seen from Table 3, the first degree D of model indicated extremely significant to response value R ($P<0.01$). Quadratic term A^2, B^2, C^2, D^2 indicated highly significant ($P<0.01$) to R. The interactions have great impact on the yield as shown in Fig 5. Integrated date in Table3, it is observed that AB showed very significant effects on R. While the rest of response not have. The above described interaction of temperature and time have highly significant. We can see by Table 3 that gives us the following list of significant level: ultrasonic power>a ratio of liquid to solid>extraction time>extraction temperature.

The optimum experimental process and verification

The objective of optimization was to select the extraction conditions from model concentration range and use rapidly rising method optimized, the results are extraction temperature 70.27°C, extraction time 62.89min, a ratio of liquid to solid 54.8ml/g, Ultrasonic power 459.31W. In this situation, the yield of flavonoid in *C. tinctoria* is 10.9942%. In order to convenient operate, we modified technological parameters are extraction temperature 70°C, extraction time 62min, a ratio of liquid to solid 55ml/g, Ultrasonic power 460W. With the test result of the above for verification, repeated 3 times determined the yields severally are 10.85%, 10.91%, 10.95%. Average flavonoids extraction yield is 10.90%. Compared with

theoretical value, the relative error is about 0.83%. These explain the regression quadratic has certain practical guiding significance which by optimization of extraction conditions by BBD.

5. Conclusions

Single factor design and BBD was used for optimizing extraction parameters in this work. Results shown that extraction temperature 70°C, extraction time 62min, a ratio of liquid to solid 55 mL/g and ultrasonic power 460 W were the best conditions to produce flavonoids. Under the most suitable conditions, a maximum yield of total flavonoids 10.90% can be achieved.

6. Acknowledgements

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