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Optimization of microwave-assisted extraction of total flavonoids extraction from *Coreopsis tinctoria* by response surface methodology

Xiaofeng Liu[1], Lin Liu[1], Xiaoli Wang[2], Yonggang Wang*[1] and Feifan Leng[1]

[1]School of Life Science and Engingeering, Lanzhou University of Technology, Lanzhou,
[2]Lanzhou Institute of Animal Husbandry and Veterinary Medicine, China Academy of Agricultural Sciences, Lanzhou

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ABSTRACT

Response surface methodology (RSM) was applied to predict optimum conditions for microwave-assisted extraction of total flavonoids extraction from Coreopsis tinctoria . A central composite design was used to monitor the effect of ratio of water to material, extraction time, microwave power on yield of total flavanoid. the optimal extraction conditions were obtained as ration of water to material of 70 mL/g, extraction time of 8 min and microwave power 590 W. under this conditions, the average total flavonoids yield was of 9.95% which matched with the predicted value of 9.99%. The extraction method was applied successfully to extract total flavonoids from Coreopsis tinctoria.

Keywords: *Coreopsis tinctoria*; flavonoid; microwave-assisted extraction; Response surface methodology

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INTRODUCTION

Coreopsis tinctoria is an annual forb which is spread in Canada, Northeast Mexico, much of the United States, especially the Great Plains and Southern states [1,2]. it is often called “calliopsis.”. *Coreopsis tinctoria* plants attain heights of 12 to 40 inches. Leaves are pinnately-divided, glabrous and tending to thin at the top of the plant where numerous 1 to 1.5 inches flowers sit atop slender stems. Flowers are brilliant yellow with maroon or brown centers of various sizes which is rich in flavonoids [3,4].

Microwave assisted extraction utilized microwave to destroy rapidly cell wall to accelerate digestion of active ingredient and to get preferable dissolution for many insoluble matters, thereby to be enhanced the speed and yield of extraction. Compared to conventional method [5,6], Microwave Assisted Extraction has high selectivity, fast and efficient, consumed fewer solvent, high yield of active ingredient, noiseless, suited to extract thermally unstable substance. Be more availably development and utilization Coreopsis tinctoria and more sufficiently extract general flavones from Coreopsis tinctoria, this article used response surface design processed the study of Microwave Assisted Extraction so as to improve traditional extracting method.

EXPERIMENTAL SECTION

1.1 Materials

Coreopsis tinctoria was provided from Guazhouyide biotechnology limited company in March 2012 and accredited Compositae coreopsis by Dr. yanglin, School of Life science and engineering, Lanzhou University of Technology.

1.2 Methods

1.2.1 Sample preparation

Paved Coreopsis tinctoria to a 5mm lamina, dried 1h at 60℃, smashed and overed 50 mesh, finally putted it in dryer.

1.2.2 Draw standard curve
Weighed precisely 20mg rutin standard, dissolved and metered it volume to 100ml with 95%ethanol. Taken above solution 0ml, 0.5ml, 1.0ml, 2.0ml, 3.0ml, 4.0ml to 10ml volumetric flask, added 0.3ml 5% $NaNO_2$, shaken up, injected 0.3ml 10% $Al(NO_3)_3$ after sat for 6 min, shaken well and sat again. Putted in 4 ml 4% NaOH and confirmed volume with 95%ethanol, sat for 12min after shaken, employed colorimetric analysis at 550nm, one drawing is made with absorbance A as ordinate and concentration *C* as abscissas, matched regression equation: y=0. 0819x+0.0005， R^2=0.9987.

1.2.3 Microwave assisted extraction
Weighed precisely 2.00g Coreopsis tinctoria power in 100ml conical flask, mixed ethanol of a certain volume and concentration, under some kind of temperature microwave processed a period of time. Filtered with vacuum suction while hot, the capacity to 100ml. Referred standard curve drawing method to determined the yield of general flavones.

1.2.4 Appropriate level of various factors
The single factor experiment was used for determining a proper value range among a ratio of water to raw material, extraction time and microwave power, in order to selected response surface design factor level.

1.2.4.1 a ratio of water to raw material
Under a ratio of water to raw material at 1:35, 1:45, 1:55, 1:65, 1:75, 1:85 respectively, microwave power 500W, extraction time 10min and extraction times 1 condition, studied the influence that a ratio of water to raw material to the yield.

1.2.4.2 extraction time
Under extraction time 2, 4, 6, 8, 10, 12, 15min respectively, a ratio of water to raw material 1:55, microwave power 500W and extraction times 1 condition, studied the influence that extraction time to the yield.

1.2.4.3 microwave power
Under microwave power 200, 300, 400, 500, 600W respectively, a ratio of water to raw material 1:55, extraction time 10min and extraction times 1 condition, studied the influence that microwave power to the yield.

1.2.5 Response surface analysis
According to Box-Behnken Central composite design principle, we synthesize the results of single factor experiment and chosen extraction times 1 to design 3-level and 4-variable response surface experiment for the three major factors of influence the general flavones yield in Coreopsis tinctoria. Levels and coding schedule were shown in Tab.1.

Tab.1 Factors and levels of RSM

Factor	level		
	-1	0	1
A microwave power（W）	500	550	600
B extraction time（min）	5	7.5	10
C a ratio of water to raw material（ml/g）	50	60	70

RESULTS AND DISCUSSION

2.1 Single factor design
2.1.1 Different microwave powers produced the influence to the general flavones yield in Coreopsis tinctoria
When a ratio of water to raw material at 1:35 and extraction time 10min, we tested different microwave powers (200-600W) produced the influence to the general flavones yield in Coreopsis tinctoria.Fig.1a shown the result. Before 500W, it increase of the yield along with the augment of microwave powers while decrease after 500W. Hence, we selected microwave power at 500W.

2.1.2 Different extraction time produced the influence to the general flavones yield in Coreopsis tinctoria
When a ratio of water to raw material at 1:35 and microwave power 500W, we tested different extraction time (2-15min) produced the influence to the general flavones yield in Coreopsis tinctoria. The results were shown in Fig.2b. The yield is increase as microwave radiation time extended at the beginning and arrive submit at 10min. Later the yield present downtrend. It is possible that microwave radiation time too long lead solvent excess temperature which destroyed effective constituent of Coreopsis tinctoria extractive and also high temperature makes protein coagulation to difficult dissolving out for flavones. Hence, we selected extraction time 10min.

2.1.3 Different a ratio of water to raw material produced the influence to the general flavones yield in Coreopsis tinctoria

When microwave power 500W and extraction time 10min, we tested different a ratio of water to raw material (1:35-1:85) produced the influence to the general flavones yield in Coreopsis tinctoria. The results were shown in Fig.3c. From Fig.3c, it is observed that the yield amplification larger with the aggrandize dosage of extracting solution. The increase yield rate can be divided into two phases: 1:35-1:45 is low yield stage, extraction efficiency is larger; 1:45-1:55 is high yield stage, the yield is get to maximum at 1:55. The yield leveling out and extraction efficiency is very small when a ratio of water to raw material exceed 1:55. Generally speaking, the more a ratio of water to raw material and the more yield. But too much solvent bring about difficult to subsequent dispose process and increase the cost of production. To the extraction effects premise, we should lessen solvent dosage and evaporation concentrated load to the greatest extent. Composited economic results and production management, we select a ratio of water to raw material 1:55.

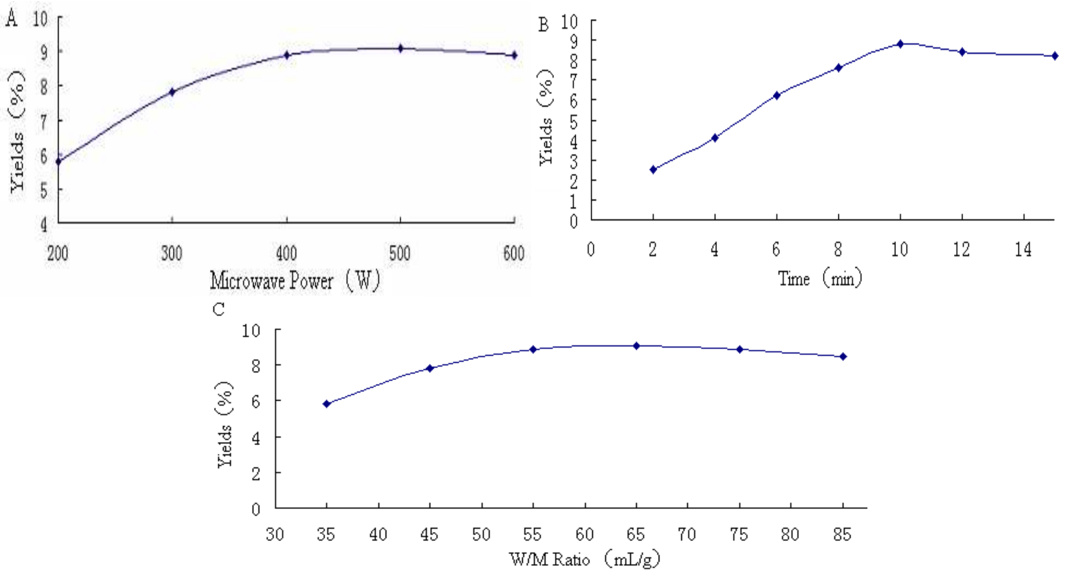

Fig. 1 Effects of different microwave power (A), extraction time (B), and W/M ratio (C) on extraction yields of flavones.

2.2 Optimized extraction conditions by Box-Behnken

2.2.1 The results of response surface design

Employed Design Expert7.0, we will be a ratio of water to raw material, extraction time and microwave power as response variable and be the general flavones yield as response value to deal with data in Fig.2. Number 1-14 are factorial experiment and 15-21 are centre experiment. Twenty test points were divided into factorial points and null points. Thereinto, factorial points are three-dimensional peak which were constituent by independent variable at A, B, C. And null points are regional centre points. The null experiment repeats six times so as to estimate experiment error. We carry out regression analysis for the obtained data by Design Expert7.0. The results were shown in Tab.3. We also carry out quadratic polynomial fitting of nonlinear regression and the following prediction model are obtained: $R= - 102.91064 + 0.22740A + 2.40285B + 0.23775C + 0.00137500AB - 0.000591667AC - 0.000983333BC - 0.00263636A^2 - 0.014945B^2 - 0.000860606C^2$

Tab.2 Design and results of RSM

NO.	A Microwave power （W）	B Extraction time （min）	C W/M （mL/g）	R Yield （%）
1	500.00	5.00	50.00	9.57
2	600.00	5.00	50.00	9.52
3	500.00	10.00	50.00	9.62
4	600.00	10.00	50.00	9.73
5	500.00	5.00	70.00	9.69
6	600.00	5.00	70.00	9.75
7	500.00	10.00	70.00	9.82
8	600.00	10.00	70.00	9.88
9	500.00	7.50	60.00	9.86
10	600.00	7.50	60.00	9.89
11	550.00	5.00	60.00	9.88
12	550.00	10.00	60.00	9.94
13	550.00	7.50	50.00	9.92
14	550.00	7.50	70.00	10.08
15	550.00	7.50	60.00	10.06
16	550.00	7.50	60.00	10.01
17	550.00	7.50	60.00	10.09
18	550.00	7.50	60.00	10.04
19	550.00	7.50	60.00	10.06
20	550.00	7.50	60.00	10.02

Tab.3 Analysis of variance for fitted quadratic polynomial model

Term	Quadratic sum	df	Mean square	F value	Pvalue	significance
model	0.58	9	0.064	66.29	< 0.0001	**
A	0.004410	1	0.004410	4.55	0.0587	
B	0.034	1	0.034	34.72	0.0002	**
C	0.074	1	0.074	76.34	< 0.0001	**
AB	0.0032	1	0.0032	3.30	0.0992	
AC	0.00045	1	0.00045	0.46	0.5110	
BC	0.000	1	0.000	0.000	1.0000	
A^2	0.079	1	0.079	81.59	< 0.0001	**
B^2	0.050	1	0.050	51.38	< 0.0001	**
C^2	0.005457	1	0.005457	5.63	0.0391	
Residual	0.009688	10	0.009688			
Lack of fit	0.005355	5	0.001071	1.24	0.4110	
Net error	0.004333	5	0.0008667			
Total devition	0.59	19				
		R^2=0.9835 R^2_{Adj}=0.9687				

*“**” indicate highly significant（P<0.01）, “*” indicate significant（0.01<P<0.05）*

It is show that this model is regress significant and lack of fit is not significant from Tab.3. R^2=0.9835, R_{Adj}^2=0.9687 illustrate the model is better fitting with practical experiment. Linear relation is significant between response variable and response value. Therefore, the model can be used for prediction of Microwave Assisted extract general flavones in Coreopsis tinctoria.

2.2.2 The influence of the interaction among with factors

As can be from Tab.3, Model first term B, C and quadratic term A^2, B^2 impact highly significant to response value R (P＜0.01). C^2 ,too(P＜0.05). Interaction term impact on the yield was shown in Fig.4; we combine with these data from Tab.3 and conclude that interaction term is not significant to R. It is well known that the more F value and the more significant which the factor influence on the response value. According to F value in Tab.3, we can get know the influence extent of three factors which influence Microwave Assisted extract general flavones in Coreopsis tinctoria : a ratio of water to raw material＞extraction time＞microwave power.

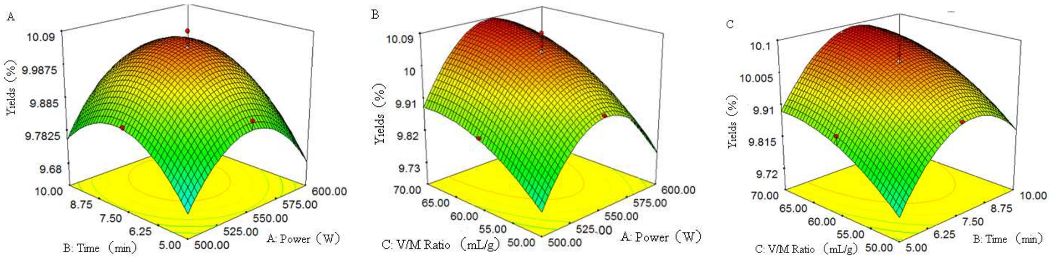

Fig.2 Response surface of factors and interactions on the content of total Flavones

2.2.3 Optimization for process variables and verification

In order to confirm further optimum point and selected model concentration range as start, used Fast-rising to optimize and got preferred plan: a ratio of water to raw material 70 ml/g, extraction time 8.2min, microwave power 592.90W. On this condition, the flavones yield is 9.9974%. we take into consideration actual operation convenience and extraction conditions： a ratio of water to raw material 70ml/g, extraction time 8min, microwave power 590W. With the test result of the above conditions for vivificate, we repeat experiment three times and score the flavones yield respectively 9.87%, 10.01%, 9.97%, average yield 9.95%. Compared with theory predict value, we obtain the relative error about 0.60%and that state the regression equation have certain practical guiding significance after optimized extraction conditions by Box-Behnken.

CONCLUSION

By single factor experiment and response surface analysis, we determine optimum technology parameter of Microwave assisted extract general flavones from *Coreopsis tinctoria*: a ratio of water to raw material 70 ml/g, extraction time 8min, microwave power 590 W and average flavones yield 9.95% .

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