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Optimization of Microwave-Assisted Extraction of Flavonoid from *Radix Astragali* using Response Surface Methodology

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Abstract: Response surface methodology (RSM) was applied to predict optimum conditions for microwave-assisted extraction (MAE) of flavonoid from *Radix Astragali.* A central composite design was used to monitor the effect of temperature, extraction time, solvent-to-material ratio, and the ethanol concentration on yield of total flavanoid (TFA). Optimum extraction conditions were predicted as 108.2°C, 26.7 min, 23.1 ml/g solvent-to-material ratio and 86.2% ethanol. The maximum yield 1.234 ± 0.031 mg/g was close to the yield of Soxhlet and higher than that of ultrasound assisted extraction and heat reflux extraction. MAE was an effective alternative to conventional extraction methods.

Keywords: Microwave assisted extraction, response surface methodology, optimization, flavonoid, *Radix Astragali*

INTRODUCTION

Radix Astragali (root of Astragalus; Huangqi), a common traditional Chinese medical herb, has been proved to be an immunostimulant, tonic (adaptogenic), hepatoprotective, diuretic, antidiabetic, analgesic, expectorant, and sedative

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drug (1). The main bioactive components of *Radix Astragali* are flavonoids including calycosin, formononetin, calycosin-7-O-β-D-glucoside, and ononion (2–4). Several studies revealed that the four flavonoids in *Radix Astragli* had strong antioxidant activity (5–7). The pharmacological properties of *Radix Astragli* flavonoid has also been researched by some workers. Fan observed the effect of calycosin on impairment of barrier function induced by hypoxia (8). Calycosin-7-O-β-D-glucoside was reported to have anti-coxsackie virus activity and alleviate osteoarthritis (9, 10).

There is increasing interest in the extraction of flavonoid from *Radix Astragli*. Many methods such as heat reflux extraction, ultrasound assisted extraction and Soxhlet extraction had been employed in the extraction of *Radix Astragli* flavonoid (2, 3, 11, 12). However, the main drawback of these traditional methods is time-consuming, laborious, and requiring bulk amount of solvents. Microwave-assisted extraction (MAE), as a newly developed process (13), has many advantages over the traditional extraction methods, such as shortened extraction time and lower consumption of solvents with satisfying recovery (14–16).

The general objective of the present work was to apply MAE in the extraction of flavonoid from *Radix Astragli* and develop an effective extraction process. Response surface methodology (RSM) was used to optimize the extraction conditions (temperature, extraction time, solvent-to-material ratio, and the ethanol concentration). The extraction efficiency was validated by comparing MAE with conventional methods.

EXPERIMENTAL

Plant Material and Chemicals

Radix Astragali was bought from Beijing Tong Ren Tang (Beijing Tong Ren Tang Technology Development Co., Ltd. Pharmaceutical Factory) in June 2006. 500 g of *Radix Astragali* was ground with a blade-mill (FW135 medicine mill, P. R. China) to obtain a relative homogenous drug powder and then sieved through a 10-mesh screen. The powder was dried at 60°C until constant weight.

All analytical grade solvents were from Beijing Chemical Plant (Beijing, P. R. China) and HPLC grade chemicals were from Fisher (Fisher Scientific, Fair Lawn, New Jersey). Standard of calycosin(>90%) was obtained from, Shanghai R & D Center for Standardization of Chinese Medicine, calycosin-7-O-β-D-glucoside (>98%) from the national natural product standard lab, formononetin and ononion (>98%) from Chromadex (Santa Ana, CA, USA).

Extraction

Microwave-assisted extraction was performed on Microwave apparatus using a closed-vessel system with pressure (ETHOS® T Microwave

digestion/extraction system, Milestone Co. Italy). After setting the microwave power at 1000 W, the powdered *Radix Astragali* was placed into the extraction vessel in addition with solvent up to volume of 50 ml and subjected to set temperatures for predefined irradiation time for two cycles. Two samples were extracted simultaneously. After the extraction time had elapsed, the vessels were allowed to cool to lower than solvent boiling point before opening.

Soxhlet extraction was performed in a Soxhlet apparatus. Exhaustive extraction with methanol (85°C) was performed on 4.0 g drug powder, placed in an extraction bag filter, and impregnated with methanol. Extraction was performed for about 4 h with 100 ml methanol.

HRE was conducted in a water bath at 75°C. 10.0 g *Radix Astragali* powder was placed into a glass flask with 250 ml 90% (v/v) aqueous ethanol and extracted for two cycles of 2 h.

Ultrasound-assisted extraction was conducted in an ultrasonic bath (Shumei®KQ-800TDV ultrasonic instrument, Kunshan, P.R.China). 5.0 g *Radix Astragali* powder was placed into a 250 ml volumetric flask with 100 ml methanol and sonicated in a water bath at 60°C for two cycles of 30 min.

All of the obtained solvent was then evaporated to dry, dissolved in methanol for HPLC analysis.

Experimental Design

The RSM used a four-factor and rotatable central composite design (CCD) consisting of 31 experimental runs with 7 replicates at the center point was applied to determine the working conditions of microwave equipment for the extraction of flavonoid from *Radix Astragali*. The effect of independent variables temperature (70–110°C; X_1), extraction time (10–30 min; X_3), solvent-to-material ratio (15–35 ml/g; X_3), and the ethanol concentration (EtOH: H_2O, 80–100%, v:v; X_4) at five variation levels (in Table 1) in the extraction process, is shown in Table 2.

Table 1. Independent variables and their levels in the response surface design

	Symbol		Factor level		
Independent variables	Uncoded	Coded	−1	0	+1
Temperature °C	X_1	x_1	80	90	100
Time min	X_2	x_2	15	20	25
Solvent/material ratio ml/g	X_3	x_3	20	25	30
Ethanol concenration %	X_4	x_4	85	90	95

Table 2. Central composite design with the observed responses for TFA

Run	Coded variable levels				Y(mg/g)
	x_1	x_2	x_3	x_4	
1	−1	−1	−1	−1	0.743
2	−1	−1	−1	1	0.697
3	−1	−1	1	−1	0.806
4	−1	−1	1	1	0.758
5	−1	1	−1	−1	0.839
6	−1	1	−1	1	0.785
7	−1	1	1	−1	0.881
8	−1	1	1	1	0.833
9	1	−1	−1	−1	1.007
10	1	−1	−1	1	0.895
11	1	−1	1	−1	1.014
12	1	−1	1	1	0.894
13	1	1	−1	−1	1.178
14	1	1	−1	1	1.022
15	1	1	1	−1	1.148
16	1	1	1	1	0.969
17	−2	0	0	0	0.741
18	2	0	0	0	1.160
19	0	−2	0	0	0.896
20	0	2	0	0	1.095
21	0	0	−2	0	0.910
22	0	0	2	0	1.082
23	0	0	0	−2	0.999
24	0	0	0	2	0.639
25	0	0	0	0	1.160
26	0	0	0	0	1.086
27	0	0	0	0	1.094
28	0	0	0	0	1.065
29	0	0	0	0	1.068
30	0	0	0	0	1.082
31	0	0	0	0	1.074

HPLC-Analysis

Quantitative analysis of *Radix Astragali* flavonoid (calycosin, formononetin, calycosin-7-O-β-D-glucoside, and ononion) was carried out by the HPLC method described by Wu, Annie, Gu, Wang, Liu and Cheng et al. (2) with minor modification. A Hitachi system (Japan) consisting of two MODEL L-7100 pumps, a MODEL L-7200 auto-sampler and a MODEL L-7420 UV detector were used. Experimental data were acquired and processed by Weimalong Software (Nanning, P.R.China).

Chromatographic separations were carried out using a Supelcosil^{TML}C18 column (250 mm × 4.6 mm, 5 μm, Supelco USA) with a Pelliguard modular guard column LC-18 (250 mm × 4.6 mm, 5 μm, Supelco, USA). A gradient elution regime was employed using water (eluent A) and MeCN (eluent B). The composition of the eluent was varied from 0% to 28% B in 15 min, while simultaneously the flow rate was changed from 1.2 ml/min to 1.0 ml/min; 28% to 38% B from 15 min to 30 min, 38% B from 30 min to 45 min and 38% to 100% B from 45 min to 60 min, the flow rate was kept at 1.0 ml/min. Column temperature was kept constant at 40°C. UV detection: 230 nm.

The four standards were mixed and four six points calibration curves were obtained. Quantitative determination of the target compounds in the extracts was performed using external standards by means of calibration curves. The yield of calycosin, formononetin, calycosin-7-O-β-D-glucoside, and ononion was summed as the yield of total flavonoids per gram of material which was used to evaluate the extraction yield.

Statistical Analysis

SAS (SAS version 9.00, SAS Institute Inc., USA) was used to design central composite rotatable design (CCRD) and analyze the experimental data. All the experiments were carried out in duplicate. Experimental data were fitted to a second-order polynomial model and regression coefficients obtained. The generalized second-order polynomial model used in the response surface analysis was as follows:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i<j=1}^{4} \beta_{ij} x_i x_j \quad (1)$$

where β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms respectively and x_i, and x_j are the coded independent variables. Y is the response value of TFA. SAS was used to generate response surfaces while holding a variable constant in the second-order polynomial model.

RESULTS AND DISCUSSION

Multiple Regression Results and Analysis of the Adequacy of the Fitted Model

Multiple regressions using the second-order polynomial model (Eq. 1) were performed on the results of Table 2 and the predicted model can be

described by the following equation Eq. (2):

$$Y = 0.9555 + 0.1093x_1 + 0.05163x_2 + 0.0200x_3 - 0.0618x_4 - 0.0232x_1x_4 - 0.0408x_1^2 - 0.0296x_2^2 - 0.0295x_3^2 - 0.0737x_4^2$$

Good fits were achieved and most of the responses' variability was explained by the model, the coefficients of multiple determination (R^2) being 0.94.

The lack of fit test was used to verify the adequacy of the fit. ANOVA for the lack of fit test did not show inadequacy of the model ($p > 0.05$), indicating that the model could adequately fit the experimental data (Table 3).

Analyses of the Regression Coefficients and the Response Surface

The regression coefficients of the model obtained by the multiple regressions are reported in Table 4. Variables in their coded form (Table 1) permitted a direct interpretability of the effects (linear, quadratic and interaction) of the independent variables, and the surface plots (Fig. 1) facilitated the visualization of the statistically significant factors (denoted by the superscript letters on the regression coefficients of Table 4) derived from the statistical analysis.

Regarding the temperature, the linear effects were verified to be statistically significant, as indicated by the *p* value in Table 4. A negative quadratic effect of x_1 was obtained, indicating that there is a maximum TFA extraction at a certain temperature. TFA increased sharply when the temperature is below 100°C and decreased slightly before 110°C (Fig. 1 (A)). That might be due to the loss of thermo-sensitive flavonoids under high temperature as extensive heat treatment had been known to cause degradation of flavonoids (17, 18).

For the time of extraction, all the response variables exhibited significant linear and negative quadratic effects. The negative quadratic effects for x_2 confirm the deceleration in the extraction yield, as Fick's second law of diffusion predicts a final equilibrium between the solute concentrations in the solid. Fig. 1(B) shows the effect of the MAE time on the extraction of TFA. The yield of TFA was increased with the increase of MAE time first and reached a high point in 25 min. The extraction of TFA was decreased

Table 3. ANOVA for the lack of fit testing for total flavanoids

Source	DF[a]	SS[b]	MS[c]	*F* ratio
Lack of fit	15	0.0033	0.0022	2.0652
Pure error	6	0.0064	0.0011	0.1900
Total error	21	0.0392	0.0019	

[a]Degree of freedom.
[b]Sum of squares.
[c]Mean square.

Table 4. Regression coefficients of the predicted second-order model for the response variables

Variables	DF	SS	MS	F	p
x_1	1	0.2867	0.2867	151.4883	0.0001^a
x_2	1	0.0640	0.0640	33.8006	0.0001^a
x_3	1	0.0096	0.0096	5.0942	0.0383^c
x_4	1	0.0916	0.0916	48.4245	0.0001^b
x_1^2	1	0.0477	0.0477	25.1901	0.0001^a
x_2^2	1	0.0250	0.0250	13.2208	0.0022^b
x_3^2	1	0.0248	0.0248	13.1093	0.0023^b
x_4^2	1	0.1553	0.1553	82.0872	0.0001^a
$x_1\ x_2$	1	0.0019	0.0019	0.9885	0.3349^{NS}
$x_1\ x_3$	1	0.0053	0.0053	2.7968	0.1139^{NS}
$x_1\ x_4$	1	0.0086	0.0086	4.5459	0.0488^c
$x_2\ x_3$	1	0.0009	0.0009	0.4997	0.4898^{NS}
$x_2\ x_4$	1	0.0008	0.0008	0.4069	0.5326^{NS}
$x_3\ x_4$	1	0.0000	0.0000	0.0241	0.8786^{NS}

Statistically significant at $^a p < 0.001$, $^b p < 0.01$, $^c p < 0.05$ and ^{NS}non-significant

with the increase of time after 25 min. The results also indicate that overexposure under microwave caused the loss of flavonoids and that was also observed in the microwave assisted extraction of polyphenols and caffeine (19)

For the solvent-to-material ratio, linear effects were verified to be statistically significant, as indicated by the p value in Table 4. A negative quadratic effect of x_3 was obtained, indicating that there was a maximum TFA yield at a certain solvent-to-material ratio. Fig. 1(C) showed that TFA increased with the increase of solvent volume until solvent-to-material ratio arrived at 20–25 ml/g and decreased as the ratio was over 25 ml/g. Excessive solvent may cause the dissolution of other contents like polysacharide which is the adsorbent for the target compounds. That was similar with the result on soy isoflavones with MAE (20).

Regarding the ethanol concentration, a negative linear effect was verified to be statistically significant, as indicated by the p value in Table 4. A negative quadratic effect of x_4 was obtained, indicating that there is a maximum in the TFA extraction at a certain EtOH concentration. The yield of TFA started to decrease above this concentration. Figure 1(C) showed that the extraction of TFA was greatly influenced by the ethanol concentration in water. When the ethanol volume percentage in the solvent was lower than 85% (v/v), the extraction yield increased obviously with the rise of ethanol concentration. When the ethanol volume percentage in the solvent was higher than 85% (v/v), the extraction yield decreased markedly. From these results, it was clear that the addition of some amount of water improved the extraction yield. One possible reason for the increased efficiency with a presence of some water might be the increase in swelling of plant material by water,

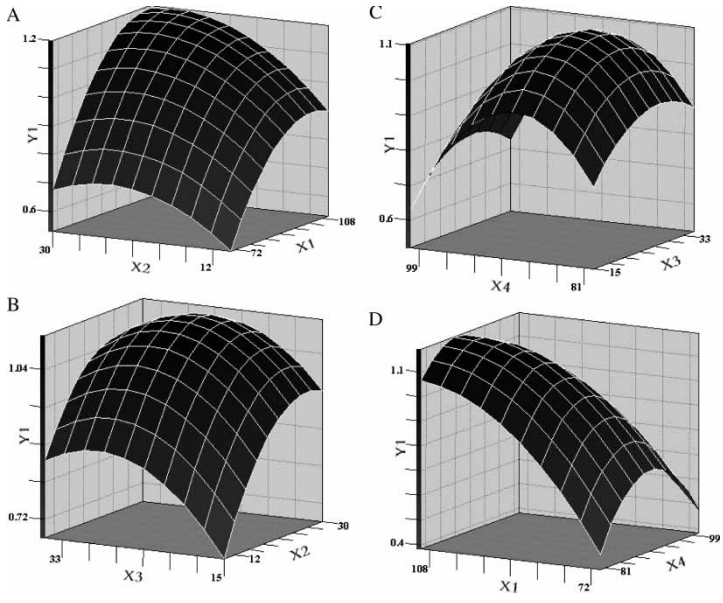

Figure 1. The surface plots of yield of TFA from *Radix astragali* as affected by temperature, extraction time, solvent/material ratio and ethanol concentration. Where (A) is actual temperature and extraction time (fixed solvent/material ratio 25 ml/g and ethanol concentration 90%); (B) is actual extraction time and solvent/material ratio (fixed temperature 90°C and ethanol concentration 90%); (C) is actual solvent/material ratio and ethanol concentration (fixed temperature 90°C, extraction time 20 min); (D) is actual ethanol concentration and temperature (fixed extraction time 20 min and solvent/material ratio 25 ml/g).

which increased the contact surface area between the plant matrix and the solvent (21). So 80–90% (v/v) aqueous ethanol concentration was chosen for the extraction of *Radix Aatragali* flavonoids.

Interestingly, a negative interaction (cross-effect) between the temperature and the ethanol concentration was obtained. The yield of TFA increased rapidly in the high temperature and low ethanol concentration area (Fig. 1 (D)). It could be imputed to the microwaves heat the polar solvent or solvent mixture directly, and the direct interaction of microwaves with the free water results in the subsequent rupture of the plant tissue and the release of the active compounds into the organic solvent (13)

Determination and Experimental Validation of the Optimal Conditions

In order to verify the predictive capacity of the model, an optimum condition was determined using the simplex method and the maximum desirability for

Table 5. Comparison between the predicted value and observed value

Optimized condition		Predicted value (mg/g)	Observed value (mg/g)
Temperature/°C	108.2	1.244	1.234 ± 0.031
Time/min	26.7		
Liquid/solid ratio/(mL/g)	23.1		
Ethanol concenration/%	86.2		

TFA, and it was used for an extraction test (Table 5). The measured values lay within a 95% mean confidence interval of the predicted value for TFA. These results confirm the predictability of the model for the extraction of flavonoid from *Radix Astragali* in the experimental condition used.

Comparison of MAE with Soxhlet Extraction, Heat Reflux Extraction, and Ultrasound-Assisted Extraction

The most common method used for the analysis of the *Radix Astragali* flavonoids was Soxhlet method (2–4). UAE was the newly developed method for the extraction of *Radix Astragali* flavonoids (12) and HRE was the widely used method for the production on a large scale (11). It can be seen in Table 6 that the flavonoid yield of MAE was significantly higher than the heat reflux extraction (HRE) and the ultrasound-assisted extraction (UAE) and sligthtly lower than the soxhlet method while MAE takes less than 1/4 time of the Soxhlet and the extraction solvent (90% ethanol) and is much safer than the methanol used in the Soxhlet extraction. Therefore, MAE can save a lot of time as compared to Soxhlet and the heat reflux method and brings a higher yield of flavonoid than heat reflux and UAE. MAE is a good alternative to the soxhlet method for sample preparation and to heat reflux extraction in the practical production of *Radix Astragali* flavonoids.

Table 6. Comparison of MAE with other extraction methods

Number	Extraction methods	Extraction time	Solvent	Solvent consumption	Yield of flavonoids (mg/g)
1	Soxhlet	4 h	Methanol	25 ml/g	1.292 ± 0.033
2	UAE	30 min × 2	Methanol	20 ml/g	0.736 ± 0.038
3	HRE	2 h × 2	90%Ethanol	25 ml/g	0.934 ± 0.021
4	MAE	Optimal conditions			1.234 ± 0.031

CONCLUSIONS

The response surface methodology was successfully employed to optimize the flavonoid extraction from *Radix Astragali*. The second-order polynomial model gave a satisfactory description of the experimental data. An optimized condition for the extraction of TFA was determined. Temperature and ethanol concentration were the most important factors affecting extraction, and the influence of extraction time and solvent-to-material ratio was limited. The optimal predicted flavonoid yield of 1.244 mg/g was obtained when the extraction conditions were at a temperature of 108.2°C, extraction time was 26.7 min, and the solvent-to-material ratio 23.1 ml/g and the ethanol concentration 86.2% The experimental values under the optimal conditions agreed with the predicted value within a 95% confidence interval, thus indicating the suitability of RSM in optimizing the extraction of flavonoid from *Radix Astragali*. Compared with the traditional methods MAE resulted in good yield with high efficiency which indicated it was an effective alternative to sample preparation of *Radix Astragali* and promising development for industrial extraction processes.

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