Technical paper

Optimization of Microwave-assisted Ethanol Reflux Extraction Process of Flavonoids and Saponins Simultaneously from Radix Astragali Using Response Surface Methodology

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The simultaneous microwave-assisted ethanol reflux extraction of flavonoids and saponins from *Astragalus* was studied. Response surface methodology (RSM) was used to develop predictive models for simulation and optimization of the extraction process. Based on the single factor test, the multivariate regression equation was obtained by the RSM of five-factors and five-levels, which designed from the Central Composite Circumscribed Design program. The response surface and contour lines were determined by using the yields of *Astragalus* flavonoids and saponins as response values. The optimum conditions of the microwave-assisted ethanol reflux extraction were obtained as follows: liquid to solid ratio of 22 mL/g, extraction temperature of 62°C, ethanol concentration of 64% (v/v), extraction time of 22 min, and microwave power of 590 W. The yields of *Astragalus* flavonoids and saponins reached as high as 25.63% and 0.98%, respectively. Multiple regression equation is extremely significant for the prediction of practical production.

Keywords: response surface methodology, Radix astragali, flavonoids, saponins, microwave-assisted ethanol reflux extraction

Introduction

Radix Astragali (root of *Astragalus membranaceus*; Huangqi) is one of the best-known Chinese medical herbs Ginseng and Notoginseng. This amazing plant has been used for thousands of years in the traditional Chinese medicine and is well known for its beneficial effects on the human body. *Astragalus* is a strong adaptogenic herb and it has an impressive ability to balance and increase the function of body immune system. The effects of *Astragalus* on the reduction of proteinuria (Ma *et al.*, 2002) and hyperlipidaemia (Wang *et al.*, 1999), immune modulations (Pullen *et al.*, 1998; Shen *et al.*, 2007) and neuroprotection (Li *et al.*, 2010).

The main active constituents of Radix Astragali include

isoflavonoids, triterpene saponins, polysaccharides, aminoacids and other trace elements. Recent studies reveal that the isoflavonoids of Radix Astragali show strong antioxidant activity (Zheng *et al.*, 2012; Zhao *et al.*, 2011), and pharmacological properties such as effect on impairment of barrier function induced by hypoxia (Zheng *et al.*, 2011). *Astragalus mongolicus* is the commonly used species and the main flavonoid contents are calycosin, formononetin, calycosin-7-O- β -D-glucoside and ononin (Chang *et al.*, 2012). *Astragalus* saponins have shown interesting pharmacological properties, including immunostimulation (Bedir *et al.*, 2000; Nalbantsoy *et al.*, 2012), anti-protozoal (Yesilada *et al.*, 2005); antiviral (Lee *et al.*, 2012), cytotoxic (Radwan *et al.*, 2004; Choudhary *et al.*, 2008) and cardiotoxic activities (Navarrete

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Fig. 1. The structure of Astragaloside A and formononetin

et al., 2005). Moreover, Astragaloside A, a widely encountered cycloartane-type saponin found in *Astragalus* species, has been proven to be a neuroprotective agent (Wang *et al.*, 2009), and proposed as a potential agent in the treatment of Parkinson's disease (Fong *et al.*, 2013).

Several techniques are available for the extraction of flavonoids and saponins from *Radix Astragali* including traditional heat reflux extraction (HRE) and Soxhlet extraction (Qi *et al.*, 2006; Qi *et al.*, 2008), ultrasound-assisted extraction (UAE)(Cai *et al.*, 2012). Methanol was used as extraction solvent in the Soxhlet and ultrasound-assisted extraction methods, which was hazardous both to the operators and to the environment. HRE with ethanol is now in general practice applied for the large-scale industrial production in spite of it being time-consuming and labour intensive. Many reports have been published on the application of microwave-assisted extraction (MAE)(Li *et al.*, 2012; He and Xia, 2011; Chan *et al.*, 2011) of activity from plants. The main advantages of MAE are the considerable reduction in time and solvent as compared to conventional techniques.

Response surface methodology is a statistical method that uses quantitative data from an appropriate experimental design to determine or simultaneously solve multivariate equation. (Xiong *et al.*, 2011; Sinha *et al.*, 2013; Sahin and Samli, 2013) Besides, this experimental methodology can generate a mathematical model and optimize the process levels (Sinha *et al.*, 2013). So far, available publications on the microwave-assisted simultaneous extraction of isoflavonoids and saponins from *Radix Astragali* with response surface methodology are very limited. The objective of this work was to investigate the effects of above five variables, including ethanol concentration, ratio of solid/liquid, irradiation time, extraction temperature, microwave irradiation power, on the yield of isoflavonoids and saponins extracted from *Radix Astragali* by response surface methodology. Optimization of the extraction was also performed.

Materials and Methods

Plant material, standards and reagents The roots of *Astragalus membranaceus* were purchased from Sanjiu Chemist's Shop, Wuhan, Hubei, which was identified as *Astragalus mongholicus* Bunge. its production place is in Inner Mongolia,

China. The cut pieces were ground with a blade-mill (FW135 medicine mill, PR China) to obtain a relatively homogenous drug power and them sieved through 20-mesh screen. The powder was dried at 60°C until constant weight and was well blended before use.

Formononetin (HPLC>99%, No.0080-9705) was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Astragaloside A (HPLC>95.0%, No.TCM067-101228) were obtained from the Nanjing Tcm Institute of Chinese Materia Medica (Nanjing, China).

Vanillic aldehyde (>98.0%) was added to the standard stock solution as a colour-producing agent for total spanions determination. All the chemicals and reagents used for analysis were of AR grade.

Colorimetric method for quantification analysis The standard curve of isoflavones and saponins in *Astragalus membranaceus*

To determine the content of isoflavones in *Astragalus membranaceus*, formononetin is used as standard samples which was weighed and dissolved in 50 mL of 95% methanol to give serial concentrations from 8 to 80 mg/L. The absorbances of samples were detected at 259 nm by a Shimadzu UV/Vis 2450 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The concentration of total flavonoids was calculated using equation:

$$Y_1 = 30.3571X_1 + 0.0136 \quad R = 0.9999$$

Where Y_1 is the absorbance value (259 nm) of the sample with 95% ethanol-water solution in a 1 cm width cuvette, and X_1 is the mass concentration of gross flavonoids (g/L).

The determination of the total content of triterpenoid saponins was performed as described. (Chen *et al.*, 2007) The standard curve which was used as the benchmark for the yield determination was obtained as follows. A stock solution consisting of Astragaloside A (0.45 g/L) was prepared. The different volumes of the stock solution with 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL were transferred into a 10 mL test tube, respectively. After the solvent was heated to evaporation in a water-bath, 0.2 mL new mixed 5% (w/v) vanillin–acetic acid solution and 0.8 mL perchloric acid were added, mixed and incubated at 70°C for 20 min. The tubes were taken out and cooled in running water for 2 min. Then ethyl acetate was added in

Table 1. Experimental factors level table for the RSM Study

Levels	S X ₁ /mL/g	T X ₂ /°C	C X ₃ /%	t X ₄ /min	P X ₅ /W
2	30	80	75	28	800
1	25	70	70	24	700
0	20	60	65	20	600
-1	15	50	60	16	500
-2	10	40	55	12	400
Distance	5	10	5	4	100

order to make the total volume being 5 mL. After being cooled to room temperature, with a blank solution as reference, the absorbance was scanned using a UV/Vis spectrophotometer in the range of 200 – 800 nm. Scanning results showed that the maximum adsorption was at 456 nm, so the absorbance A (Y₂) at Vis-456 nm was determined with a glass cell of 1 cm. The concentration of total saponins was calculated using equation and X₂ is the mass concentration of gross saponins (g/L):

$$Y_2 = 3.0378X_2 - 0.0599 \quad R = 0.9993$$

Determination of total isoflavonoids and total triterpenoid saponins

The material was passed through 60 mesh sieve to get uniform particle size sample. A sample of 5.0 g *Radix Astragali* crude extract powder was transferred into a 100 mL volumetric flask and dissolved with ethanol-water solution and the final volume was adjusted to 100 mL. The concentrations of total isoflavonoids in the samples were calculated according to the regression parameters derived from the standard curve. The 0.2 mL extract solutions were added to a tube. The absorbance of the total triterpenoid saponins was determined by the colorimetric method as described above. The contents of triterpenoid saponins were determined by reading the values from the standard curve.

Simultaneous microwave-assisted ethanol reflux extraction of Astragalus flavonoids and saponins The material was passed through 100 mesh sieve to get uniform particle size sample. Radix Astragali powder (5.0 g) and 100 mL ethanol-water solution were put in the microwave-assisted extractor for extraction in a designed solid-liquid ratio, extraction time, ethanol concentration, microwave temperature and microwave power. After the extraction, the mixture was centrifuged at 4000 r/min for 10 min. The concentration of the total isoflavonoids and total triterpenoid saponins in the supernatant was measured.

Experimental Design for the RSM Study Response surface methodology was applied to investigate the effects of “ethanol concentration”, “microwave temperature”, “reaction time”, “ratio of liquid to solid”, “microwave power” on extraction and to model the extraction formula. Central Composite Circumscribed Design (CCD) (Wang *et al.*, 2007; Firatligil-Durmus and Evranuz, 2010) with 5 factors and 5 levels (−2, −1, 0, 1, 2) was used to generate factor combinations by using Design-Expert v.8.0.5b (2021 East Hennepin Ave, Stat-Ease, Inc.). The levels for the chosen factors

Table 2. Response surface central composite design (encoded) and results for extraction yield of TSA and TFA, respectively

Run	X ₁	X ₂	X ₃	X ₄	X ₅	Y _{TSA} (%)	Y _{TFA} (%)
1	−1	−1	1	1	1	20.94	0.66
2	−1	−1	−1	1	−1	22.13	0.79
3	−1	−1	1	−1	1	21.05	0.8
4	1	1	−1	−1	1	21.98	0.84
5	−1	−1	−1	−1	−1	22.87	0.76
6	1	1	1	−1	1	24.92	0.67
7	−1	1	−1	1	1	21.31	0.92
8	1	−1	−1	1	−1	23.71	0.78
9	1	1	1	1	−1	24.03	0.81
10	1	−1	1	−1	−1	22.62	0.81
11	1	−1	1	1	1	24.89	0.65
12	0	0	0	−2	0	22.87	0.66
13	2	0	0	0	0	24.71	0.73
14	1	−1	1	1	−1	22.5	0.62
15	0	0	0	0	2	24.34	1.05
16	1	−1	1	−1	1	22.9	0.68
17	−2	0	0	0	0	20.01	0.71
18	0	−2	0	0	0	25.96	0.55
19	−1	1	1	1	−1	21.67	0.71
20	0	0	0	0	−2	24.98	0.78
21	−1	−1	−1	1	1	23.41	0.7
22	1	−1	−1	−1	1	23.51	0.78
23	−1	1	1	−1	1	20.58	0.67
24	0	2	0	0	0	23.92	0.63
25	−1	−1	−1	−1	1	22.71	0.78
26	1	1	1	1	1	26.37	0.81
27	1	1	−1	1	−1	22.88	0.88
28	1	−1	−1	1	1	23.84	0.9
29	1	−1	−1	−1	−1	21.94	0.69
30	1	1	−1	−1	−1	22.5	0.84
31	−1	1	1	1	1	22.52	0.88
32	0	0	0	2	0	22.84	0.7
33	−1	−1	1	−1	−1	23.07	0.83
34	0	0	−2	0	0	21.81	0.92
35	−1	1	1	−1	−1	22.92	0.94
36	−1	1	−1	−1	−1	21.04	0.8
37	−1	−1	1	1	−1	23.42	0.77
38	1	1	1	−1	−1	23.03	0.88
39	1	1	−1	1	1	23.79	0.89
40	−1	1	−1	1	−1	20.85	0.87
41	0	0	2	0	0	21.84	0.89
42	−1	1	−1	−1	1	20.47	0.82
43	0	0	0	0	0	24.95	1.08
44	0	0	0	0	0	25.35	0.97
45	0	0	0	0	0	25.99	1.05
46	0	0	0	0	0	26.25	0.98
47	0	0	0	0	0	25.85	0.99
48	0	0	0	0	0	25.95	1.02
49	0	0	0	0	0	26.05	0.9
50	0	0	0	0	0	25.75	1.05

were: ratio of liquid to solid (S, x/1), (10/1 – 30/1); microwave temperature (T, °C), (40 – 80°C); ethanol concentration (C, %), (55 – 75%); reaction time (t, min), (12 – 28 min) and microwave power (P, W), (300 – 800 W). The independent variables and experimental design are shown in Table 1. A total of 50 experimental runs with 8 center points (0, 0, 0, 0, 0) were generated.

Results and Discussion

Single factor experiment Effect of ratio of liquid to solid on the yields of Astragalus flavonoids and saponins

As shown in Fig. 2a, the yields of Astragalus flavonoids and saponins increased with the increase of ratio of liquid to material at first. Results demonstrated that yield of Astragalus flavonoids reached highest at the special ratio point of 20:1, and yield of Astragalus saponins reached highest at the ratio point of 25:1. After this point, yields reduced slowly with ratio increasing. Higher ratio of liquid to material may need to be coordinated with more cost. Therefore, according to Central Composite Circumscribed design principle, the range of ratio of liquid to material was chosen between 10:1 and 30:1 (mL:g).

Effect of extraction temperature on the yields of Astragalus flavonoids and saponins

As shown in Fig.2b, the yields of Astragalus flavonoids and saponins increased with extraction temperature from 50°C to 60°C. Results demonstrated that yield of Astragalus flavonoids and saponins both reached the highest at the special temperature point of 60°C. After this point, it decreased with temperature rising. The reason may be that as temperature rising effective components was gradually decomposition. 50°C to 82°C was chosen as the extraction temperature in the Central Composite Circumscribed design process.

Effect of ethanol concentration on the yields of Astragalus flavonoids and saponins

In this study, ethanol was selected to be the experimental solvent. Fig.2c showed that the yields of Astragalus flavonoids and saponins increased with the ethanol concentration between 50 and 65%, and decreased in the range of 65 – 75%. When the ethanol concentration was 60%, the yield of Astragalus saponins reached highest. And it remained relatively stable with concentration increasing. But the yield of Astragalus flavonoids reached the highest value at ethanol concentration of 65%. With ethanol concentration increased, the liquid solubility of flavonoids increased. Too high ethanol concentration leads to lower the solubility of Astragalus flavonoids and saponins, resulting in the decrease of the yield value. Therefore, in the Central Composite Circumscribed design process, 50 – 75% ethanol water solution was chosen as the test solvent.

Effect of extraction time on the yields of Astragalus flavonoids and saponins

As shown in Fig.2d, the yields of Astragalus flavonoids and

saponins increased with extraction time from 0 to 20 min. Results demonstrated that yield of Astragalus flavonoids and saponins both reached the highest approximately at the special time point of 20 min. After this point, it decreased with time. The reason may be that as effect components gradually decompose as time goes. 14 – 22 min was chosen as the extraction time in the Central Composite Circumscribed design process.

Effect of microwave power on the yields of Astragalus flavonoids and saponins

As shown in Fig.2e, the yields of Astragalus flavonoids and saponins rapidly increased with the power from 400 W to 500 W. Results proved that yields of Astragalus flavonoids and saponins both reached the highest at the special power point of 500 W. After this point, yields reduced slowly with the power increasing. Higher power may need to be coordinated with more cost. Thus, according to Central Composite Circumscribed design principle, the range of microwave power was chosen between 400 W and 800 W.

Statistical Analysis Regression analysis and statistical significance and response surface applications were performed by using Design-Expert v.8.0.5b Central Composite Circumscribed Design (CCD) program. Compliance of the model was evaluated by results of variance analysis (ANOVA). The quadratic response surface model was fitted to the following equation:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \dots \text{Eq. 1}$$

Where Y is the response (response: incorporation of SDA), β_0 is the intercept, β_i linear (first order model), β_{ii} quadratic, β_{ij} interaction regression coefficients and X_i and X_j are the independent variables. The variables were coded according to the equation:

$$x_i = (X_i - X_0) / \Delta X \dots \text{Eq. 2}$$

Where x_i is the dimensionless coded value of the variable X_i , X_0 is the value of X_i at the centre point, and ΔX is the step change.

Model Fitting For this purpose five-factor, five-level CCD was employed for the reactions assisted by microwave extraction, and the respective design points together with the observed response (yield (%)) of TFA and TSA are given in Table 2. Multiple regression analysis was applied to obtain the best fitting model. Regression coefficients (b) and significance (p) values are given in Table 3 and Table 4.

As can be seen from Table 3, all first order parameters was statistically significant ($P < 0.05$). Among first order parameters, temperature had negative effects on yield of TFA. The most important effect on TFA yield was found as “microwave temperature”. All second order parameters and interaction terms except “substrate molar ratio or time” were found to be statistically significant ($P > 0.05$). The model equation for the response1 (yield of TFA; %) and the response 2 (yield of TSA; %) can be written as respectively:

Fig. 2. The effect ratio of liquid to solid, extraction temperature, ethanol concentration, extraction time, and microwave power on the yields of Astragalus flavonoids and saponins

$$Y(TFA) = 0.99 - 5.594E-003 * X_1 + 0.037 * X_2 - 0.011 * X_3 - 7.531E-003 * X_4 + 5.156E-003 * X_5 + 5.938E-003 * X_1 * X_2 - 0.015 * X_1 * X_3 + 7.813E-003 * X_1 * X_4 + 2.344E-003 * X_1 * X_5 - 4.063E-003 * X_2 * X_3 + 0.018 * X_2 * X_4 - 2.031E-003 * X_2 * X_5 - 0.025 * X_3 * X_4 - 0.012 * X_3 * X_5 + 0.011 * X_4 * X_5 - 0.054 * X_1^2 - 0.086 * X_2^2 - 7.563E-003 * X_3^2 - 0.064 * X_4^2 - 1.266E-003 * X_5^2 \quad \cdots \cdots \text{Eq. 3}$$

$$Y(TSA) = 25.77 + 0.85 * X_1 - 0.22 * X_2 + 0.21 * X_3 + 0.25 * X_4 + 0.068 * X_5 + 0.37 * X_1 * X_2 + 0.18 * X_1 * X_3 + 0.22 * X_1 * X_4 + 0.44 * X_1 * X_5 + 0.44 * X_2 * X_3 + 0.057 * X_2 * X_4 + 0.063 * X_2 * X_5 + 0.011 * X_3 * X_4 - 0.068 * X_3 * X_5 + 0.24 * X_4 * X_5 - 0.86 * X_1^2 - 0.2 * X_2^2 - 0.99 * X_3^2 - 0.73 * X_4^2 - 0.28 * X_5^2 \quad \cdots \cdots \text{Eq. 4}$$

Table 3. Regression analysis and statistical significance of TFA model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.61	20	0.031	4.66	< 0.0001	***
X_1	4.23E-04	1	4.23E-04	0.064	0.8014	
X_2	0.048	1	0.048	7.37	0.0111	*
X_3	0.021	1	0.021	3.16	0.086	
X_4	4.23E-04	1	4.23E-04	0.064	0.8014	
X_5	1.10E-03	1	1.10E-03	0.17	0.6847	
X_1X_2	1.13E-03	1	1.13E-03	0.17	0.6813	
X_1X_3	7.50E-03	1	7.50E-03	1.14	0.2935	
X_1X_4	1.95E-03	1	1.95E-03	0.3	0.5893	
X_1X_5	7.03E-04	1	7.03E-04	0.11	0.7456	
X_2X_3	5.28E-04	1	5.28E-04	0.081	0.7785	
X_2X_4	0.01	1	0.01	1.55	0.2232	
X_2X_5	5.28E-04	1	5.28E-04	0.081	0.7785	
X_3X_4	0.02	1	0.02	2.98	0.0952	
X_3X_5	0.019	1	0.019	2.83	0.1034	
X_4X_5	0.015	1	0.015	2.27	0.1427	
X_1^2	0.093	1	0.093	14.14	0.0008	***
X_2^2	0.24	1	0.24	36.37	< 0.0001	***
X_3^2	1.83E-03	1	1.83E-03	0.28	0.6012	
X_4^2	0.13	1	0.13	19.88	0.0001	***
X_5^2	8.20E-04	1	8.20E-04	0.13	0.7261	
Residual	0.19	29	6.56E-03			
Lack of Fit	0.17	22	7.60E-03	2.31	0.1289	not significant
Pure Error	0.023	7	3.29E-03			
Cor Total	0.8	49				

* $p < 0.05$ significant, ** $p < 0.01$ highly significant, df, degree of Freedom; $R^2=0.7625$, $R^2_{Adj}=0.5988$

As shown in Table 3, the experimental data fitted well to the TFA quadratic model by ANOVA. The second-order polynomial regression model was in good agreement with the experimental results, with R^2 of 0.7625. At the same time, the lack-of-fit statistics, which was used to test the adequacy of the model, indicated that the p-value (0.1289) for yield value was not significant. No abnormality was obtained from the diagnoses of residuals. Thus, it can be concluded that the model was statistically sound. The TFA model F-value of 4.66 ($p < 0.0001$), which implied that the quadratic response surface model was highly significant. The P-value denoting the significance of the coefficients was important to understand the pattern of the mutual interactions between the variables, whose value below 0.05 indicated that the test parameter was significant at the 5% level of significance. It can be seen that the variable with significant effect was the linear term of microwave temperature ($p = 0.0111$). The quadratic terms of microwave temperature ($p = 0.0008$), ratio of liquid to material ($p < 0.0001$) and extraction time had highly effects on the yield of Astragalus flavonoids.

The TSA second-order polynomial regression equation ($R^2 = 0.9006$) was fit to the experimental data and yielded the estimated regression coefficients shown in Table 4. The lack-of-fit

statistics, the p-value (0.0504) for it, was not significant. Therefore, this TSA model can be used to navigate the design space. The TSA model F-value of 13.14 ($p < 0.0001$), which implied that the quadratic response surface model was highly significant. It can be proved that the variable with highly significant effect was the linear term of ratio of liquid to material ($p < 0.0001$), significant as well as the linear term of extraction time ($p = 0.0324$). The two-level interaction between ratio of liquid to material and microwave temperature, ratio of liquid to material and microwave power, as well as microwave temperature and ethanol concentration had highly effects on the yield of Astragalus saponins. The quadratic terms of ratio of liquid to material ($p < 0.0001$), ethanol concentration ($p < 0.0001$) and extraction time ($p < 0.0001$) had higher effects on the yield of Astragalus saponins, followed by the quadratic term of microwave power ($p = 0.0331$).

To determine optimal levels of the test variables for the yields of Astragalus flavonoids and saponins, the 3D response surface described by the regression model is presented in Fig.3a, b, c, d. To depict the interactive effects of operational variables on responses, one variable was kept constant while the other four variables varied in defined ranges. The shapes of response surfaces and contour plots indicate the nature and extent of the interaction between

Table 4. Regression analysis and statistical significance of TSA model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
<i>Model</i>	132.36	20	6.62	13.14	< 0.0001	***
X_1	28.65	1	28.65	56.87	< 0.0001	***
X_2	1.91	1	1.91	3.78	0.0615	
X_3	1.83	1	1.83	3.63	0.0668	
X_4	2.55	1	2.55	5.05	0.0324	*
X_5	0.19	1	0.19	0.37	0.5478	
X_1X_2	4.37	1	4.37	8.68	0.0063	**
X_1X_3	1.03	1	1.03	2.04	0.1642	
X_1X_4	1.56	1	1.56	3.1	0.0888	
X_1X_5	6.1	1	6.1	12.11	0.0016	**
X_2X_3	6.08	1	6.08	12.07	0.0016	**
X_2X_4	0.1	1	0.1	0.2	0.6555	
X_2X_5	0.13	1	0.13	0.26	0.6169	
X_3X_4	3.83E-03	1	3.83E-03	7.60E-03	0.9311	
X_3X_5	0.15	1	0.15	0.3	0.5896	
X_4X_5	1.88	1	1.88	3.73	0.0634	
X_1^2	23.43	1	23.43	46.52	< 0.0001	***
X_2^2	1.42	1	1.42	2.82	0.1038	
X_3^2	31.33	1	31.33	62.19	< 0.0001	***
X_4^2	17.14	1	17.14	34.03	< 0.0001	***
X_5^2	2.52	1	2.52	5.01	0.0331	*
<i>Residual</i>	14.61	29	0.5			
<i>Lack of Fit</i>	13.36	22	0.61	3.41	0.0504	not significant
<i>Pure Error</i>	1.25	7	0.18			
<i>Cor Total</i>	146.96	49				

* $p < 0.05$ significant, ** $p < 0.01$ highly significant, df, degree of Freedom; $R^2 = 0.9006$, $R^2_{Adj} = 0.8321$

different variables.

Figure 3a showed the effect of ratio of liquid to solid and extraction temperature on the yields of Astragalus flavonoids and saponins. When extraction temperature and ratio of liquid to solid increased, the yield value rose to the climax then fell down. Figure 3b showed the effect of ratio of liquid to solid and microwave power on the yields of Astragalus flavonoids and saponins. Figure 3c shows the contour map of the effect of extraction temperature and ethanol concentration on the yields. The convex response surface suggested well-defined optimum variables (extraction temperature and ethanol concentration) and indicates that the sum of the peak area of flavonoids and saponins increased with the increase of temperature and concentration up to maximum values of 62°C and 64%, respectively. The effect of extraction time and ethanol concentration on the yields of Astragalus flavonoids and saponins was shown in Fig.3d. Higher yields of Astragalus flavonoids and saponins were generally obtained with increasing extraction time. There were rather sharp dropoffs in the yield value as the ethanol concentration approached either 60% or 65%. Therefore, the yield of flavonoids mainly depends on the extraction temperature, ratio of liquid to material and extraction time were the factors that less influenced. Similarly, the yield of saponins mainly

depends on the ratio of liquid to material and extraction time, and the ethanol concentration and extraction time were the factors that less affected.

In order to corroborate the optimal value, Eq.(3) and (4) were gained as follows: three experiments were performed to show that when the optimum extraction parameters were ratio of liquid to material 22.3:1 (mL:g), extraction temperature 61.72°C, ethanol concentration 63.87%, extraction time 21.32 min, microwave power 591.0 W. For the limit of the instruments, the following process parameters were used: ratio of liquid to material 22:1 (mL:g), extraction temperature 62°C, ethanol concentration 64%, extraction time 22 min, microwave power 590 W. Triplicate experiments were performed under the determined conditions and the yields of Astragalus flavonoids (0.97%) and saponins (25.63%) in agreement with the predicted values of flavonoids (0.99%) and saponins (25.77%) were obtained. This indicated that all the model statistics and diagnostic plots are OK, the TFA and TSA model was adequate for this extraction process.

Conclusion

In this paper, simultaneous microwave-assisted ethanol reflux extraction technology was used to extract Astragalus flavonoids

(a)

(b)

(c)

(d)

Fig. 3. 3D response surface and contour plots of the effect for the yields of Astragalus flavonoids and saponins

and saponins from Astragalus. The optimal processing parameters determined by response surface methodology were as follows: ratio of liquid to material 22.3:1 (mL:g), extraction temperature 61.72°C, ethanol concentration 63.87%, extraction time 21.32 min, microwave power 591.0 W. Under these conditions, the experimental yield values were flavonoids (0.97%) and saponins (25.63%), agreed well with the predicted yield values of flavonoids (0.99%) and saponins (25.77%). Moreover, compared to the original ethanol reflux extraction, simultaneous microwave-assisted ethanol reflux extraction technology has advantages on saving time, extracting flavonoids and saponins simultaneously and getting more of flavonoids and saponins. It is proved to be an efficient method for extraction of flavonoids and saponins from Astragalus.

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