Optimization of microwave-assisted extraction of polysaccharides in the flower of Platycodon grandiflorum by response surface methodology

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Abstract: *Platycodon grandiflorum* polysaccharides (PGPs) are naturally occurring compounds derived from the flowers of *platycodon grandiflorum*. the microwave assisted extraction (MAE) of PGPs was studied. A three-level and three-factor Box–Behnken design was used to optimize MAE conditions, and the effects of operating conditions including microwave power (180−540 W), extraction time (20−60 s) and liquid to solid ratio (20−30 v/w) on the extraction yield of PGPs were studied through response surface methodology (RSM). The results showed that the highest yield of PGPs reached 8.35 g/(100 g) at 360 W microwave power, 40 s extraction time and 25.2 v/w liquid to solid ratio. The extraction yield of PGPs was significantly affected by liquid to solid ratio ($P<0.01$), microwave power and extraction time ($P<0.05$). Compared with the conventional solvent extraction, MAE is more efficient and rapid due to the disruption of the flower tissue microstructure as a result of microwave acoustic cavitation as observed with the scanning electron microscopy (SEM). A macroporous resin, HPD400, was found to be effective for purifying the PGPs extract.

Keywords: polysaccharides, extraction, microwave-assisted extraction (MAE), *platycodon grandiflorum*, process optimization, macroporous resin

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1 Introduction

In China, *platycodon grandiflorum* have long been used as traditional foods and medicines[1]. The edible part of *platycodon grandiflorum* contains an abundance of compounds that possess a multitude of biological activities, such as polysaccharides and saponins. They produce various classes of secondary metabolites with interesting biological activities and, thus, have already been used as valuable sources for drug development[2]. Biological properties of *platycodon grandiflorum* polysaccharides (PGPs), as immunological[3,4], anti-complementary[5], and antitussive[6] have been reported. However there are no reports on the effect of microwave-assisted extraction on extraction yield of the PGPs derived from the flower of *platycodon grandiflorum*.

Response surface methodology (RSM) is effective for evaluating responses that are influenced by many factors and their interactions. This methodology was originally described by Yang and Cheng[7]. Many studies indicated that it is useful for developing, improving and optimizing processes. In this study the microwave assisted extraction(MAE) parameters[8] such as ethanol

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content in solution, ultrasonic power and ultrasonic temperature were optimized by RSM, in order to obtain the optimal condition for extraction of PGPs from the flowers of *platycodon grandiflorum*.

Recently, there has been a growing interest in employing macroporous resins to separate bioactive components from crude extracts of herbal raw materials. This study aimed to investigate the conditions of microwave extraction and develop an optimal resin to purify the extracts.

2 Methodology

2.1 Materials

The flowers of *platycodon grandiflorum* harvested from fields in July 2007 were provided by Shan Zhenyuan Company (Zibo Shandong). All flowers were stored at -18℃ until further analysis. Ninety-five percent ethanol and hydrochloric acid were of reagent grade, and all the other chemicals were of analytical grade.

2.2 Conventional extraction of polysaccharides

About (5±0.5) g of frozen samples were smashed into pieces (3–4 mm), and mixed with 70 mL of 1% HCl and 95 mL/(100 mL) ethanol solvents in a 500 mL round bottom flask fitted with a cooling system. Microwave power was set at (360±1) W, which was considered as the optimal power conditions based on single-factors experiments. Extractions time was 40 s. The PGPs extracts were cooled to room temperature and filtered through Whatman No.1 paper under vacuum and collected in a volumetric flask. The residue was taken back and extracted again in the same conditions. The PGPs extracts of both extractions were pooled together and tested for total PGPs content.

2.3 Quantitative analysis

The phenol-sulphuric acid method was used to detect PGPs, which was referred by Wang Liming and Zhang Gui[9,10] with some modifications. A regression equation between milligram (mg) value of oleanolic acid and OD value were obtained as A=0.8465x−0.007, R^2=0.9994, A is OD value, x is oleanolic acid in mg/mL.

According to the standard curve, the concentration of oleanolic acid from the regression equation was obtained, and the corresponding concentration of PGPs in exacts was calculated with the formula of x= (A+0.007)/ 0.8465, where A is OD value of sample solution, x is oleanolic acid in mg/mL. The yield (%) of PGPs was calculated with the formula of c(%)= m/w. Where w is the weight of the flower sample, m is the weight of PGPs.

2.4 Experimental design

The aim of this study was to evaluate the effect of the variables (factors) involved in the MAE and to find the optimum values of those factors that give a maximum in the analytical response. It is usual that many of the operational variables can affect the extraction process, only a few of them are truly important or critical. An orthogonal array experimental design is usually used to identify the most probable active factors and possible interactions. Five different factors, namely microwave power, extraction time, ratio of liquid–solid, pre-extraction time which means the time soaking in the same solvent but at room temperature, and content of ethanol, were investigated with several levels. For each factor, the experimental range and the central point were based on the results of preliminary trials. Thus, in the first stage of the microwave extraction process optimization, the single factor experiments were taken as the preliminary trials to limit the total experimental work to reasonable levels. These single factor experimental designs do not allow one to explore exhaustively a wide range of the experimental space, but they do indicate with minimum experimental effort the possible direction which the future experiments should take.

RSM was used to determine the optimum condition for PGPs extraction. The experimental design and statistical analysis were performed using SAS software. A three-level three-factor Box–Behnken design was chosen to evaluate the combined effect of three independent variables: microwave power, extraction time and ratio of liquid to solid, coded as X_1, X_2 and X_3, respectively. The minimum and maximum values for extraction microwave powers were set at 180 and 540 W, extraction time was between 20 min and 60 min and ratios of liquid to solid were 20(V/W) and 30(V/W) (Table 1). The response values were PGPs yield. The complete design consisted of 15 combinations including three replicates of the center point (Table 2).

Table 1 Coded values of the experimental variables

Code	-1	0	1
X_1 Microwave power /W	180	360	540
X_2 Extraction time /min	20	40	60
X_3 Ratio of liquid to solid /V/W	20	25	30

Table 2 Response surface central composite design and experimental PGPs yield

Run	X_1 Microwave Power/W	X_2 Extraction time /min	X_3 Ratio of liquid to solid /V/W	PGPs yield /g • (100 g)$^{-1}$
1	-1	-1	0	3.66
2	-1	0	-1	5.96
3	-1	0	1	4.02
4	-1	1	0	6.64
5	0	-1	-1	4.42
6	0	-1	1	5.66
7	0	1	-1	7.35
8	0	1	1	6.52
9	1	-1	0	5.69
10	1	0	-1	4.1
11	1	0	1	4.22
12	1	1	0	5.63
13	0	0	0	8.12
14	0	0	0	8.43
15	0	0	0	8.51

The responses function (Y) was partitioned into linear, quadratic and interactive:

$$Y = \beta_0 + \sum_{i=1}^{k} B_i X_1 + \sum_{i=1}^{k} B_{ii} X^2 + \sum_{i>j}^{k} B_{ij} X_i X_j \qquad (1)$$

where β_0 is defined as the constant; B_i is the linear coefficient; B_{ii} is the quadratic coefficient and B_{ij} is the cross-product coefficient; X_i and X_j are the levels of the independent variables. While k equals to the number of the tested factors (here k=3).

The analysis of variance (ANOVA) tables were generated and the responses function was determined. The significances of all terms in the polynomial were judged statistically by computing the F-value at a probability (P) of 0.001, 0.01 or 0.05 levels. The regression coefficients were then used to make statistical calculations to generate contour maps and response contours from the regression models.

Five grams flowers were weighed and then put into a double-neck flask with a cooling system. After the flask was filled with the proper volume of extraction liquids according to the solvent to sample ratio defined in experiment design, it was immersed in a cool water bath with dimension of 250 mm×240 mm×150 mm in the microwave chamber of microwave extraction device (WG900FDL23-K6) with total nominal power of 900W; The flask was taken out and cooled to room temperature by cooling water. The PGPs extracts were filtered through filter-paper and abscise ethanol under the vacuum, and the solution was collected and metered volume in a 100 mL volumetric flask. OD value of the solution was examined. The residue was taken back and extracted again in the same conditions. The PGPs extracts of both extractions were mixed and used for the determination of the total PGPs content.

2.5 Statistics

SAS 8.0 software (version 8.0, SAS institute Inc., USA) was used to design three-level three-factor Box–Behnken design and analyze the experiment result. Trends were considered as significant when mean values of compared sets are different at $P<0.05$ or 0.01, and all the data were reported as standard deviation.

2.6 Tissue preparation and observation by scanning electron microscopy (SEM)

The flower residues after conventional extraction and MAE respectively were fixed in 2.5% glutaral-dehyde and 1% osmic acid for 12 h, and then dried in a critical-point dryer, and mounted on stubs, finally observed on the FEI sinon 200 scanning electron microscope.

2.7 Purification of the PGPs by macro--porous resins

The selectivity of resins was based on the capacities of adsorption samples. Seventeen kinds of macroporous resins were chosen to purify PGPs (Table 3).

The static adsorption and desorption tests of PGPs on macroporous resins were performed as follows: 3 g samples of hydrated test resins together with 10 mL of extraction solutions were added into a flask, shaken (130 r/mim) for 24 h at 25℃. The respective concentrations of madecassoside and asiaticoside in the sample solution after adsorption of a certain time were monitored at equal time intervals till equilibration to produce adsorption kinetic curves.

Table 3 Adsorption rate of different macroporous resin on PGPs

Macroporous resins	Dry resins mass /g	Particle size (mm≥95%)	Apparent density /g • mL^{-1}	Average pore size /nm	Special surface area /m^2 • g^{-1}	Adsorption rate /%
ADS-7	0.301	0.3-1.25	0.28-0.36	25-30	≥100	10.01
ADS-17	0.3	0.3-1.25	0.25-0.32	25-30	90-150	40.68
D101	0.3015	0.3-1.25	0.27-0.34	100-110	≥400	55.77
DM130	0.2995	0.3-1.25	0.27-0.34	90-100	500-550	51.46
AB-8	0.301	0.3-1.25	0.25-0.32	130-140	480-520	44.36
HPD100	0.3003	0.3-1.2	0.28-0.34	85-90	650-700	43.35
HPD300	0.3005	0.3-1.2	0.28-0.34	50-55	800-870	37.26
HPD400	0.3	0.3-1.2	0.28-0.34	75-80	500-550	59.32
HPD700	0.3007	0.3-1.2	0.28-0.34	85-90	650-700	33.21
D312	0.3015	0.32-1.25			800	49.30
XF-800	0.3007				≥400	44.11
FLC-10	0.3004	0.25-0.45				51.84
HPD100A	0.3006	0.3-1.2	0.28-0.34	95-100	650-700	34.09
HPD722	0.3013	0.3-1.25	0.25-0.34	130-140	485-530	34.47
HPD750	0.299	0.3-1.2	0.28-0.34	85-90	650-700	36.12
HPD100C	0.2999	0.3-1.25	0.27-0.34	80-90	720-760	46.39
HPD200A	0.2982	0.3-1.25	0.27-0.34	85-90	700-750	26.62

3 Results and discussion

3.1 Effect of different microwave power levels on extraction yield of PGPs

The effect of different microwave power input levels on extraction yield of PGPs is shown in Figure 1. Extraction was carried out at different microwave power levels (180, 360, 540, 720, 900 W) when other extraction conditions were as follows: extraction time was 40 s; the ratio of liquid–solid was 25(V/W); pre-extraction time was 15 min; content of ethanol was 20%; and the number of extraction was one at room temperature. The extraction yields of the PGPs significantly increased from 5.02% to 7.30% as microwave power increased from 180 W to 360 W shown in Figure 1, which can be attributed to the enhanced mass transfer of the PGPs due to an increase in the driving force[12]. However, when the microwave power continued to increase, the extraction yields decreased with PGPs degradation by microwave power increased .

3.2 Effect of different extraction time on extraction yield of PGPs

Extraction time is another factor that would influence the extraction efficiency. A longer extraction time generally improved the yield of PGPs (Figure 2). When extraction time varied from 0 s to 300 s, the variance of extraction yield was relatively rapid from Figure 2, and PGPs yield reached a maximum at 40 s, and then decreased with further increase in time. Thus, extraction for 20−60 s can be considered sufficient for cost-effective extraction of PGPs from the flower samples.

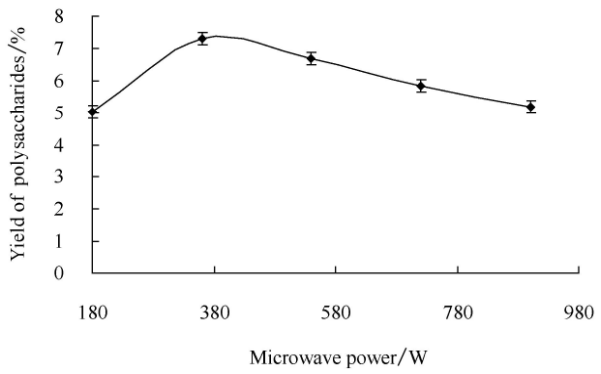

Figure 1 Effect of different microwave power on extraction yield of PGPs

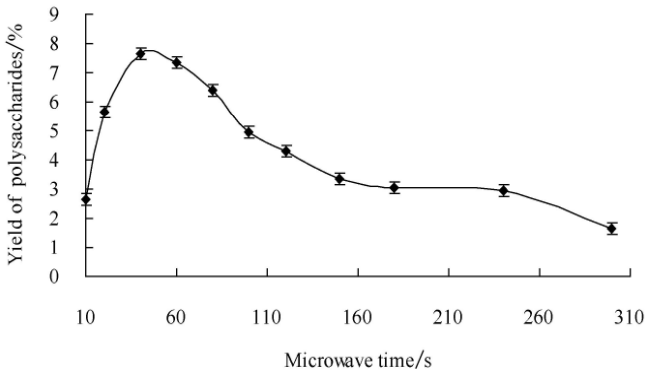

Figure 2 Effect of different extraction time on extraction yield of PGPs

3.3 Effect of different ratios of liquid to solid on extraction yield of PGPs

To study the effect of different ratios of liquid to solid on extraction yield of PGPs, extraction process was carried out at the different ratios of liquid to solid: 10, 15, 20, 25, 30 and 50 (V/W), while keeping other variables constant. The extraction yield of PGPs increased with ratio of liquid to solid increased from 10 to 25 (V/W), especially 20 to 25 (V/W). As shown in Figure 3, the maximum yield of PGPs was observed when ratio of liquid to solid was 25(V/W). As the ratio of liquid to solid increased above 25(V/W), the yield of PGPs decreased. Therefore, ratio of liquid to solid range of 20−30 (V/W) was considered to be optimal in the present experiment. The increase in the PGPs diffusion coefficient and the enhanced solubility of the PGPs in the extracting solvent at higher ratio of liquid to solid is known to cause an increase in the PGPs mass leaching out from the sample into the solution[13], resulting in higher extraction coefficients.

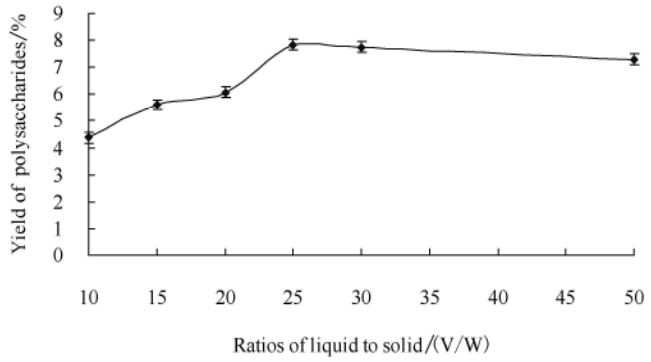

Figure 3 Effect of different ratios of liquid to solid on extraction yield of PGPs

3.4 Effect of different content of ethanol on extraction yield of PGPs

Ethanol was chosen as the extraction solvent of PGPs based on preliminary experimental results. Extraction yields increased with increasing ratio of ethanol to water in the solvent below 20% (V/V), and then decreased with further increase in ethanol concentration (Figure 4). Similarly, the antioxidant activity in the borage meal increased with ethanol concentration up to about 20% and then started to decline with further increase in ethanol concentration[14]. Therefore, 20% (V/V) ethanol was used in the extraction solvent for further experiments.

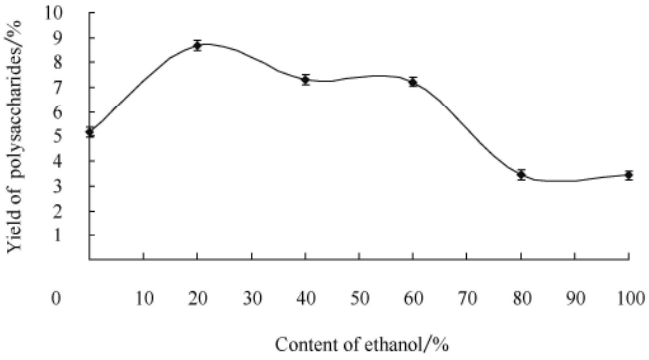

Figure 4 Effect of different content of ethanol on extraction yield of PGPs

3.5 Effect of different pre-extraction time on extraction yield of PGPs

Pre-extraction time was also an important parameter in extraction procedure. To evaluate the effect of pre-extraction time, samples were pre-extracted for 0, 5, 10, 15, 20, 25 and 30 min before they were subjected to normal extraction under these conditions: extraction time 40s, the ratio of liquid to solid of 25(V/W), and 20% ethanol in the solvent at room temperature. It was found that the extraction yield of PGPs increased with the increase in pre-extraction time to a maximum and then leveled off (Figure 5). Based the results, 15 min was selected for the latter experiments.

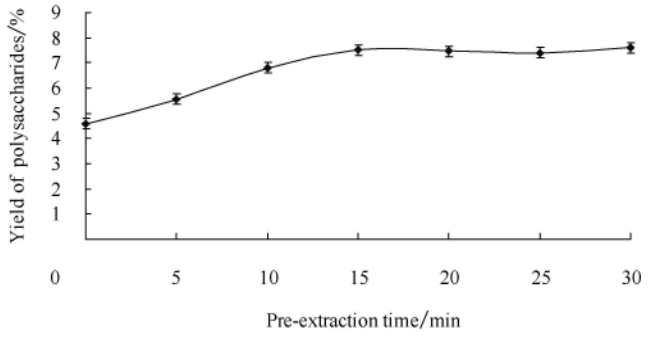

Figure 5 Effect of different pre-extraction time on extraction yield of PGPs

3.6 Optimization of extraction conditions of PGPs

The values of responses (extraction yield of PGPs) to different experimental combinations for coded variables are given in Table 4. The percentage yield ranged from 3.66% to 8.51%.

Table 4 Test of significance for regression coefficients

Variable	DF	Parameter estimate	Standard error	t value	Pr > |t|
Intercept	1	-47.08917	8.92427	-5.28	0.0033
x_1	1	0.04215	0.01108	3.80	0.0126
x_2	1	0.40090	0.09973	4.02	0.0101
x_3	1	3.16158	0.63006	5.02	0.0040
x_{11}	1	-0.00006730	0.00000936	-7.19	0.0008
x_{22}	1	-0.00192	0.00075838	-2.53	0.0524
x_{33}	1	-0.06392	0.01213	-5.27	0.0033
x_{12}	1	-0.00021111	0.00008096	-2.61	0.0478
x_{13}	1	0.00057222	0.00032384	1.77	0.1375
x_{23}	1	-0.00517	0.00291	-1.78	0.1360

The application of RSM offers, based on parameter estimates, an empirical relationship between the response variable (extraction yield of PGPs) and the test variables under consideration. By applying multiple regression analysis on the experimental data, the response variable and the test variables are related by the following second-order polynomial equation:

$$Y=-47.08917+0.04215X_1+0.40090X_2+3.16158X_3-0.00006730X_1^2-0.00192X_2^2-0.00021111X_1X_2+0.00057222X_1X_3-0.00517X_2X_3 \quad (2)$$

The variance analysis indicated that the proposed model was adequate, possessing no significant lack of fit and with very satisfactory values of the R^2 for all the responses. On the other hand, different the value of probability (P) stand for the relevance between different variables in Table 5. The probability (P) values of all regression models were less than 0.05(Table 5), with the level of significant.

Table 5 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	R-Square
Model	9	36.44768	4.04974	11.92	0.0070	0.9555
Error	5	1.69889	0.33978			
Corrected Total	14	38.14657				

According to this model, linear terms of ratio of liquid to solid (X_3, $a<0.01$), microwave power and extraction time (X_1, X_2, $a<0.05$), quadratic terms of microwave power and ratio of liquid to solid (X_1^2; X_3^2; $a<0.01$) are significant, indicating that the change in microwave power, extraction time, and ratio of liquid to solid all significantly affected the PGPs concentration in the extracts. The interaction between microwave power and extraction time was also found significant (X_1X_2, $a<0.05$). On the other hand, the interactions between microwave power and ratio of liquid to solid (X_1X_3), and extraction time and ratio of liquid to solid (X_2X_3), and quadratic term of ultrasonic power (X_2^2) were not significant.

Meanwhile, the whole model including linear level ($a<0.01$) and quadratic level ($a<0.01$) all reached significant ($a<0.01$), which indicated an excellent agreement between the experimental and predicted values. The optimal MAE conditions were obtained from response surface analysis as follows: microwave power was 360 W; extraction time was 40 s, and ratio of liquid to solid was 25.2(V/W). Under these conditions, the experimental total platyconins (8.35 g/(100 g)) was close to the values (8.51 g/(100 g)) calculated from the polynomial response surface model equation. Therefore, the three-level three-factor Box–Behnken desig was adequate to predict the extraction efficiency of PGPs by MAE.

The response surface were used to illustrate microwave power（X_1）, extraction time (X_2) and ratio of liquid to solid (X_3) on the responses. Response surfaces for PGPs yield is shown in Figures 1–5, and the contour map is shown in Figures 6–8.

The effects of microwave power (X_1) and extraction time (X_2) on the total PGPs (Y) of extracts were reflected in Figure 1 and Figure 4, when X_1 was fixed, e.g., at 300 W, with an increase in X_2, the Y increased gradually, and reached the highest value, and then decreased rapidly. When X_2 was kept constant, e.g., at 35 s, an increase in X_1, gradually increased Y to a maximum before it caused Y to decline rapidly. When microwave power was 360 W and extraction time was 40 s, the Y reached the highest value.

Therefore, it could be concluded that the microwave

power (X_1) and extraction time (X_2) played prominent roles in the extraction efficiency of PGPs during MAE, due to the acceleration of disruption of tissues and leaching of solutes from tissues under acoustic cavitation effect, which would be observed by SEM in this work..

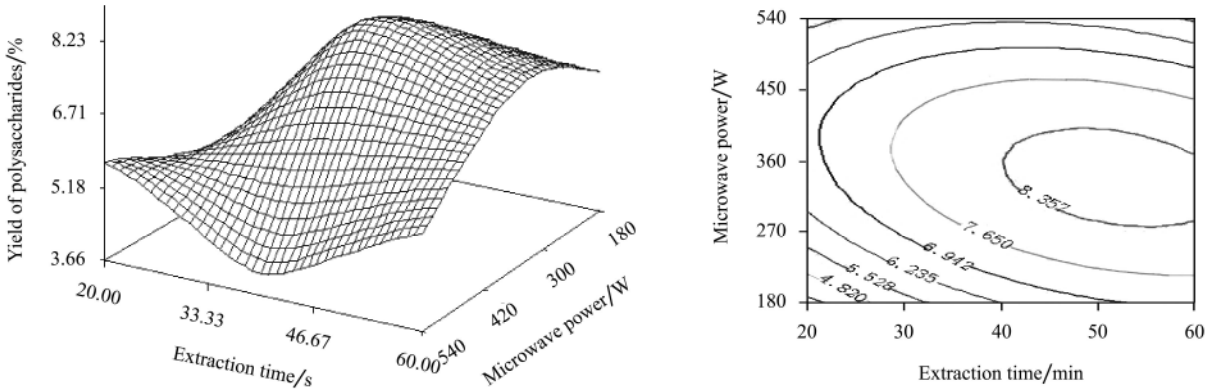

Figure 6 Figure 1 and Figure 4 Interaction of microwave power and extraction time on PGPs yield

The effects of microwave power (X_1) and ratio of liquid to solid (X_3) on the total PGPs (Y) of extracts at the extraction time of 40 s were reflected in Figure 2 and Figure 5. It can be seen that, when X_1 was fixed, with increase in X_3, Y increased gradually, and reached the peak and then decreased. Similarly, when X_3 was fixed, with increase in X_1, Y increased rapidly, and reached the highest value, and then decreased rapidly too. However, the contour gradient in X_1 coordinate direction was less than that in X_3 coordinate direction, indicating that X_3 was not as significant as X_1 in response for Y. When microwave power was 360 W and ratio of liquid to solid was 5.2(V/W), the Y reached the highest value.

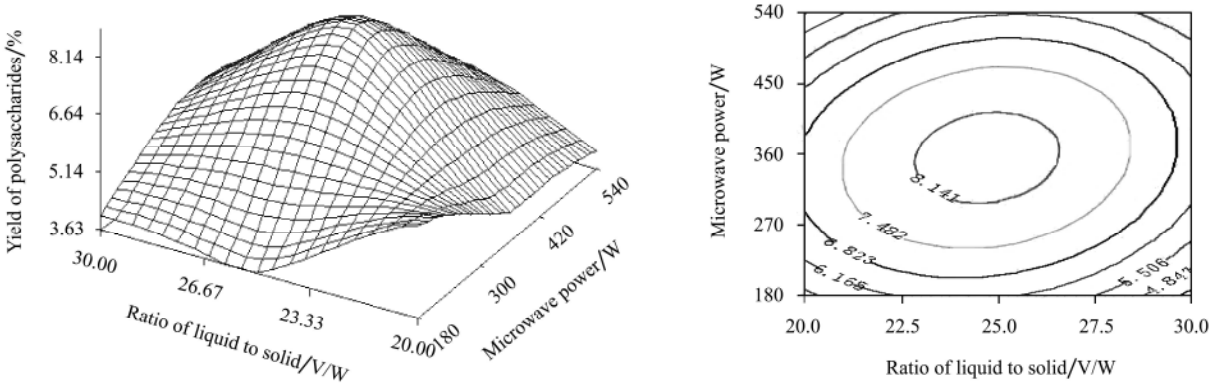

Figure 7 Figure 2 and Figure 5 Interaction of microwave power and ratio of liquid to solid on PGPs yield

The relationship between Y and extraction time (X_2) as well as ratio of liquid to solid (X_3) was illustrated in Figure 3 and Figure 6. The contour plot indicated that increases in X_2 and X_3 benefit the extraction of PGPs. However, the changes in X_2 have more significant effects on Y than the X_3. As X_2 was less than 40 s, the increase in X_3 could significantly affect Y. Y went up with increasing X_2, and reached the highest level at about 40 s, and then decreased after the peak. Similarly, when the ratio of liquid to solid was kept constant, e.g., at 24(V/W), the yield of PGPs increased in the beginning period with an increase in ratio of liquid to solid, and then decreased after it reached its peak. It is well known that the powerful microwave energy may induce chemical reactions, and the degree of the chemical reactions could be examined with some further methods, whether the decomposition of PGPs resulted from the high microwave power or a long treatment time, we need do further research..

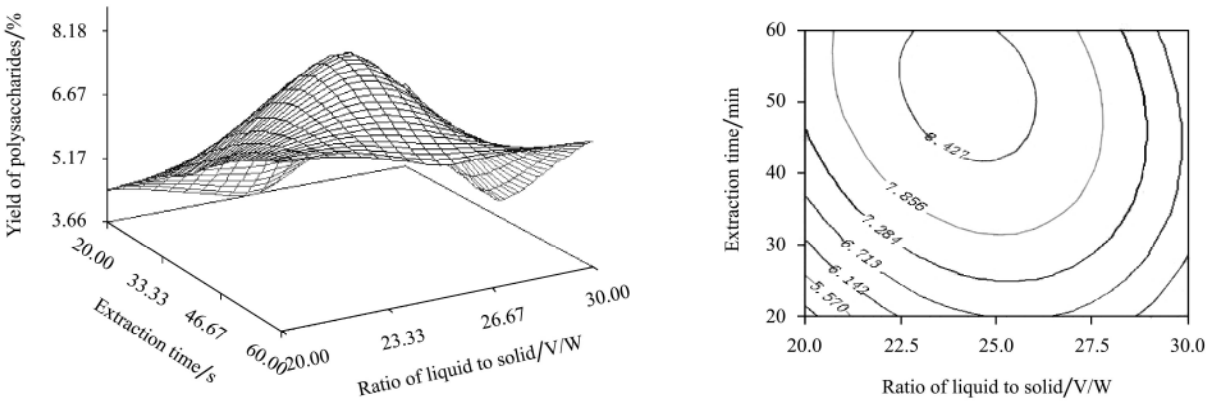

Figure 8 Figure 3 and Figure 6 Interaction of extraction time and ratio of liquid to solid on PGPs yield

3.2 Comparison of MAE with conventional extraction of PGPs

Platycodon grandiflorum samples, which were the same plant materials and pretreated in the same conditions as aforementioned, were extracted with MAE and conventional extraction processes, respectively, in order to evaluate effects of MAE on the extraction efficiency and compositions of PGPs. The microstructure of the flower samples after extraction was studied with SEM. The significant differences were revealed in Figure 9. The structure of MAE treated samples was looser than that of conventionally extracted ones. It seems that microwave treatment results in an explosive disruption of the physical structure of the flower, leading to a direct migration of PGPs into the surrounding solvent, probably because the acoustic cavitation, heating and mechanical action in flower occur during microwave irradiation. Similar results were reported on the effect of microwave vibration on the physical structure of ginger particles[15] and soybean tissue[16]. In conventional extraction process, a heated solvent slowly diffuses through the material, dissolving and carrying away target compounds. The structural disruption caused by microwave irradiation makes MAE a much more efficient and rapid process than conventional extraction methods.

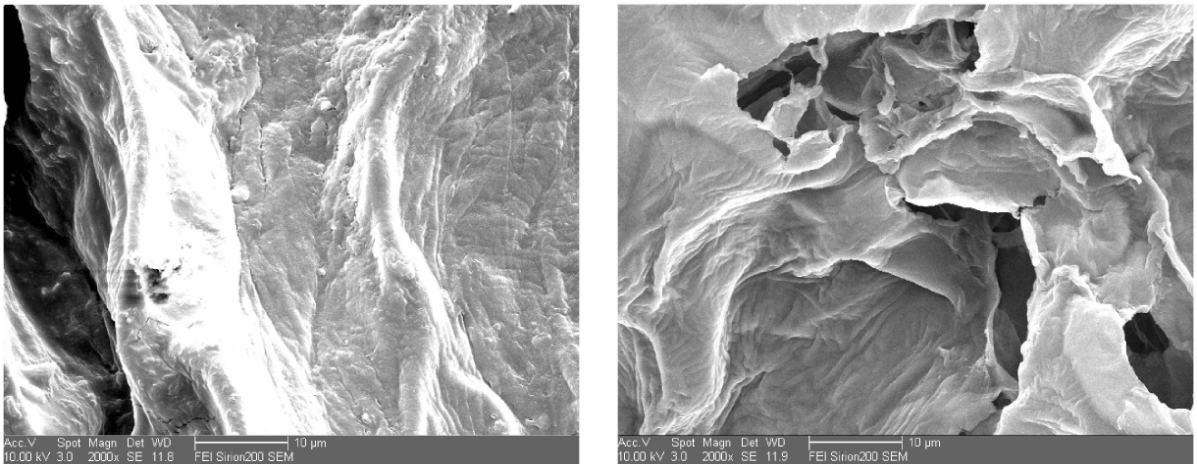

Figure 9 SEM micrographs of conventionally extracted and MAE treated samples. The left one is conventionally extracted sample tissues and the right one is the structure of MAE treated samples

To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. A mean value of (8.35±0.3)g/(100 g), obtained from confirmatory test with the conditions: microwave was 360 W power, extraction time was 40 s and liquid to solid ratio was 25.2 v/w, demonstrated the validation of the RSM model with the P value between the yield of theory

value and experimental value was significantly less than 0.05 obtained by SAS software. The good correlation between these results confirmed that the response model was adequate for arriving at process optimization.

3.3 Adsorption capacities and kinetics on resins

The following equations were used to quantify the capacities of adsorption and desorption as well as the desorption ratio. Adsorption evaluation[17]:

$$qe=(C_0-C_e)\times V_i(1-M)W \quad (3)$$

where qe is the adsorption capacity at adsorption equilibrium, mg/g dry resin; C_0 and C_e are the initial and equilibrium concentrations of PGPs in the solution, respectively, mg/mL; V_i is the volumeof the initial sample solution, mL; M is the ratio of water content; W is the weight of resin, g. As shown in Table 5, all the resins tested in this study showed high adsorption capacity for asiaticoside.

The HPD400 resin had higher adsorption capacity than other resins. The adsorption kinetics curves for PGPs on five different resins were obtained. As what can be seen from Figure 10, HPD400, the adsorption capacity of PGPs increased with the adsorption time, reaching equilibrium at about 60 min, which suggested that HPD400 was effective in absorbing PGPs.

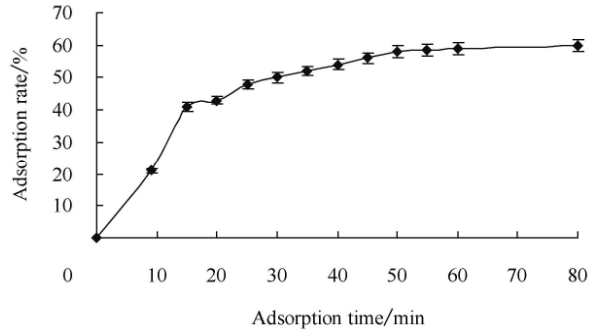

Figure 10. Adsorption curve of HPD400 on PGPs

4 Conclusions

The extraction of PGPs from the flower of *platycodon grandiflorum* was studied with a statistical method based on the response surface methodology to identify and quantify the variables in order to maximize the yield of PGPs. The three variables chosen, namely microwave power, extraction time, and ratio of liquid to solid all have a positive influence on the yield of PGPs using microwave extraction method. The optimal conditions obtained by RSM for production of PGPs are: microwave power of 360 W, extraction time of 40 s, and ratio of liquid to solid of 25.2 (V/W). The PGPs yield obtained using optimized conditions was 8.35%, which confirmed the validity of the models by the analysis of variance obtained from SAS software.

In this study, the adsorption ablities of different macroporous resins were investigated by adsorption capacities and kinetics of PGPs, and the separation process of these from flower of *platycodon grandiflorum* extracts with the selected resin was tested. HPD400 resin was selected because it had the highest adsorption capacities, and the highest adsorption rate among all tested resins.

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