

Optimization of Extraction of Triterpense from *Geum japonicum* using Response Surface Methodology

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ABSTRACT

This study aims to determine the best extraction conditions and find out the effects of solvent type (ethanol, acetone, and ionic liquid (1-butyl-3-methylimidazolium bromide)[BMIM]Br), extraction time (20–60min), and microwave power (200–600w) on extraction rate and triterpenes (TTP) of *Geum japonicum*. The three parameters, type solvents, extraction time and microwave power, were optimized using the Box-Behnken design (BBD) with a quadratic regression model built by using response surface methodology (RSM). The experiments were carried out according to 17 runs with 3 variables and 3 levels for the optimization. The extracts were analyzed by spectrophotometric methods for the (TTP). The optimal extraction conditions were determined as follows: (i) [BMIM]Br solvents, microwave power 599.99 w, extraction time 60 min for the best extraction rate 34.1 % and TTP 3.9 mg/g. (ii) ethanol solvents, microwave power 235.77 w, extraction time 37.73 min for the extraction rate 19.8 % and best TTP 33.25 mg/g. This results showed that [BMIM][Br] solvent are more efficient in the extraction of extraction rate compare with ethanol and acetone solvents. While ethanol solvent are more efficient in the extraction of TTP compare with [BMIM][Br] and acetone solvents.

Keywords: [BMIM][Br], *Geum japonicum*, microwave, response surface methodology, triterpenes

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INTRODUCTION

Traditional Chinese medicine has been used by Chinese people from ancient times. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of the more than 12 000 items used by traditional healers, about 500 are in common use. Botanical products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine. In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy. Traditional Chinese medicine is still in common use in China. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China, they account for

approximately one fifth of the entire Chinese pharmaceutical market (1).

The genus *Geum* (Rosaceae family) comprises ca. 70 species of plants, four of which are found in China. *Geum japonicum*, which is abundant in China and commonly known as “Lanbuzheng”, has been used as a herbal medicine in diuretics and astringents in traditional Chinese medicine (2). It is also used for the treatment of dizziness and headache in some regions of China (3). The previous phytochemical studies on the constituents of *Geum japonicum* THUNB. (Rosaceae) led to the discovery of many compounds, including triterpenoids and tannins (4).

Response Surface Methodology (RSM) is widely employed for constructing and exploring estimated functional relationship between a response variable and design

variables (5). Meanwhile successfully been used to model and optimize biochemical and biotechnological process related to food systems (6).

RSM is a one of mathematical and statistical techniques used to identify for optimum conditions of factors for desirable responses; it evaluates the relative significance of several treatment factors even in the presence of complex interactions. The design leads to the generation of contour plots by linear or quadratic effects of the key variables, and a model equation is derived that fits the experimental data to calculate the optimal response of the system (7).

The main objective of this study was to employ response surface methodology to study the effects of microwave power, extraction time, and solvents to determine the best extraction conditions for *Geum japonicum*, in order to maximize

simultaneously the yield of extraction rate and TTP by using response surface methodology.

MATERIAL AND METHODS

Chemicals and Machine

Gallic acid, quercetin reagent, vanillin-glacial and perchloric acid were from Sigma (USA) and D-101 macroporous resin was from Tianjin Dajun Co., Ltd. While ethanol, was from Sinopharm chemical reagent co.ltd. Spectrophotometer (Shanghai-Techcomp, UV 2300), balance (Shanghai-Mettler Toledo, AB 204-N), rotary evaporator (Shanghai-Biochemical Equipment), water bath (Shanghai-Hengzi) and Microwave (Beijing- Xianghu Science & Technology.XH-200A).

Preparation of (1-butyl-3-methylimidazolium bromide) [BMIM] [Br].

Prepared the 1.5 M [BMIM][Br] according to the following equation under the Condition 400 W, 120 °C and 20 min by microwave.

$^1\text{H-NMR}$: 8.73 (1H, s), 7.50 (1H, d, $J = 2.4$ Hz), 7.46 (1H, d, $J = 2.4$ Hz), 4.22 (2H, t, $J = 9.6$ Hz), 3.91 (3H, s), 1.87 (2H, m), 1.34 (2H, m), 0.95 (3H, s, $J = 10.0$ Hz).

Plant materials

The whole parts of *G. japonicum* were bought from Beijing Tong Ren Tang, P.R. China, in July 2012 was deposited at the School of Food Science and Technology, Jiangnan University, Wuxi, P. R. China.

Preparation of extracts using microwave-assisted extraction (MAE)

The *G. japonicum* was air-dried in shade, 5 grams from plant was extracted with 150 ml of 1.5 M [BMIM][Br], 95% ethanol and 50 % aceton in microwave at 80°C, extraction power from 200 to 600w and extraction time from 20 to 60 min under different MAE conditions. MAE was performed on microwave apparatus using vessel system. After extraction, the vessel was allowed to cool at room temperature. The ethanol and aceton extracts were filtered and the solvents were removed using a rotary evaporator. While [BMIM][Br]extract was filtered and then they were loaded onto an D-101macroporous resin column (1.6 × 60 cm) (**Figure 1**). The desorption conditions

were as follows: wash by 1000 ml of water, and eluted by 300 ml of 90% ethanol, 1 ml/min of flow rate then disposed of ethanol by rotary evaporator. Dried extracts were kept refrigerated until use.

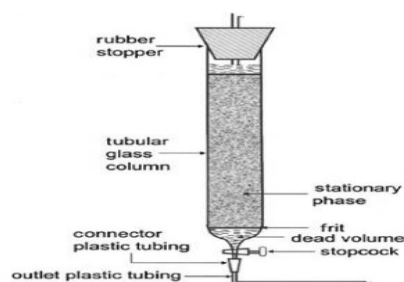


Figure 1: Removing the [BMIM][Br] by D-101 macroporous resin column Triterpenes (TTP)

The TTP determined by the method (8) was taken and dried in a boiling water bath. The dried residue was dissolved in 0.2 mL of 5% vanillin-glacial acetic acid solution and 1 mL of perchloric acid, and the mixture was heated in a water bath at a temperature of 60 °C. After 5 min, the mixture was cooled

immediately in ice water and then added to 2 mL of ethyl acetate. The absorbance was measured at 550 nm after keeping for 5 min. The content of total triterpenes was expressed as gallic acid (mg/g of dry extracted) from (Fig. 2).

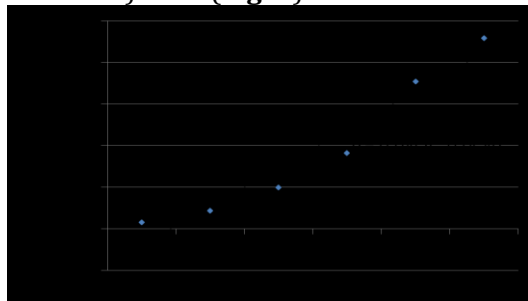


Figure 2: Calibration curve for gallic acid (mg/g of dry extracted)

Single factor experiments

- 1.Extraction of antioxidant using different solvents of [BMIM][Br], ethanol and acetone, By fixing extraction time (40 min) and extraction temperature (80 °C) and 400 w. The best solvent type was selected based on the highest value of extraction rate % and TTP (express as mg gallic acid equivalents/g dry weight).
- 2.The impact of extraction time on the extraction rate and TTP were varied from 20, 40, and 60 minutes. *G. japonicum* extract was extracted using the solvent type and the extraction power chosen in single factor experiments. The extraction procedures were repeated by applying

the solvent [BMIM][Br], ethanol and acetone, and extraction power (400 w) at 80 °C. The best extraction time was chosen according to the highest values of extraction rate and TTP.

- 3.Lastly, the extraction was executed by using the extraction time and solvent type selected in single factor experiments sections (1) and (2). *G. japonicum* were extracted at various extraction powers which ranged from 200 to 600 w at the optimum time determined. Based on the results of single factor experiment, the ranges of three factors (solvent type, extraction time and extraction power) were determined for RSM.

Experiment design of RSM

A three level (-1, 0,+1) and three-factor (X_1 , X_2 and X_3) in Table 1, those factors rotatable central desing was utilized to examine the optimum combination of extraction variables based on the extraction rate and TTP of *G. japonicum*.

The complete BBD design comprised of seventeen experiments with performed five central points at the centre of the design (Table 2) to allow a good estimation of pure error (9). The design variables were the solvent type, X_1 , the extraction time, X_2 , and the extraction power, X_3 (Table 1). All the experiments were performed in a random order.

Table 1: Variables and their levels for central composite design

Independent variable	Units	Symbol	Code levels		
			-1	0	+1
Solvents	Type	X_1	50 % acetone	[BMIM][Br] (1.5 M)	95% ethanol
Time	Min	X_2	20	40	60
power	W	X_3	200	400	600

Statistical analysis

The experimental results in single factor experiments were analyzed using calculated means and standard deviations of three simultaneous assays carried out. Statistical analysis (SPSS, 16) was applied to the data to determine differences ($P < 0.05$) performed by ANOVA.

Multiple linear regression analysis was performed by the software Design-Expert (Version 6.0.10, Stat-Ease Inc., Minneapolis). Experimental data were fitted to the

following second order polynomial model and regression coefficients were obtained. The generalized second-order polynomial model proposed for the response surface analysis was given as shown in equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j$$

Where β_0 was the value of the fitted response at the center point of the design, which was point (0, 0, 0). β_0 , β_i , β_{ii} , and β_{ij} were the constant, linear, quadratic and cross-product regression terms,

respectively. The quality of fit of the polynomial model was expressed by the coefficient of determination R^2 , and the statistical.

RESULTS AND DISCUSSION

Effect of extraction time on the extraction rate and TTP

In this study, the range of extraction time was designed based on the practical and economical aspects. Extraction time was another main parameter in the extraction procedure (10).

An increase in extraction time result in an increase in the extraction rate in all solvents, while the [BMIM][Br] best solvent in extraction rate where the value of the extraction rate in the [BMIM][Br] was $30.42\% \pm 0.18$ at 20 min and rose to $33.31\% \pm$

0.23 . at the 60 min. Figure 3 (a). In general, the maximum concentration of TTP was achieved at extraction time of 40 min at three solvents. After this point, the TTP was decrease. As shown in Figure 3(b) the highest value of TTP for extraction time was at 40 minutes accompanied by a decrease at 60 minutes using ethanol as a solvent. While the ethanol best solvent in TTP, also the maximum concentration of TTP was achieved at extraction time of 40 min at three solvents. After this point in the extraction ethanol the TTP was decreased to 29.1 ± 0.12 at 60 min Figure 3(b). This phenomenon could be explained that final equilibrium will be attained between the solution concentrations and solvent after a particular duration (11).

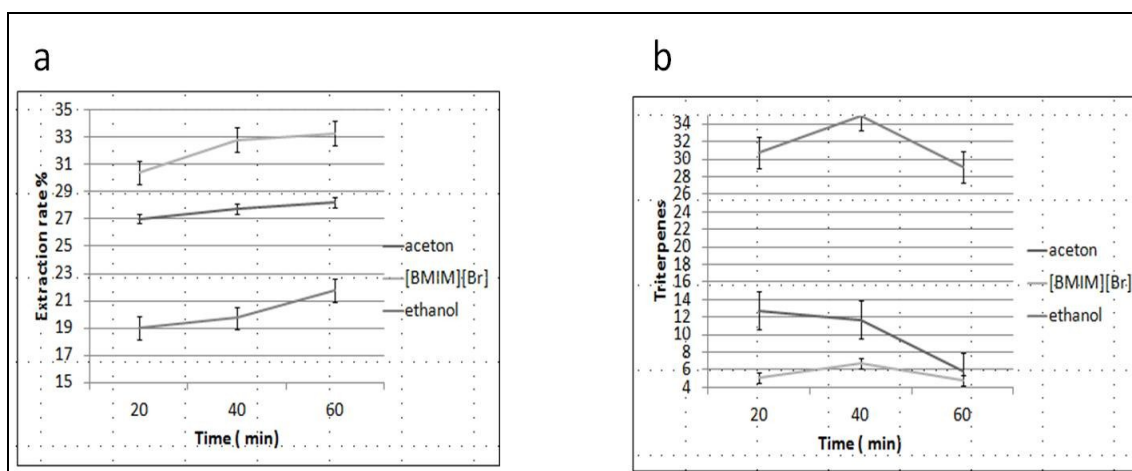


Figure 3: Effect of the extraction time on the (a) extraction rate and (b) Triterpenes from *G. japonicum* at power 400 w and temperature 80 °C

It was found out that prolonged extraction time lead to more exposure to oxygen and thus increase the chances for occurrence of oxidation (12,13). Reduction of TTP in extract with longer extraction time could also be caused by the endogenous enzymes in plant tissues (14). Hence, an excessive extraction time was not useful to extract more TTP (15). Furthermore, prolonged extraction process might lead to oxidation due to light or oxygen exposure. However, there was no difference in extraction of free radical scavenging TTP when compared to shorter time. Time does have a significant effect on extraction of TTP as shown in Figure 3(b). It was obvious that a shorter time will extract the same amount of TTP extracts as longer time while saving cost

Effect of solvents type on the extraction rate and TTP.

Extraction operation are different depending on the type of solvent and solvent polarity. Figure 4 (a) [BMIM][Br] showed the highest extraction rate when compared with ethanol and acetone at all extraction time, where the best extraction rate at 60 minutes. As the value of the extraction rate at use [BMIM][Br] 33.94% , while was 21.78 and 28.24% when the use of ethanol and acetone, respectively, at the same time. Figure 4 (b) showed the highest in TTP was ethanol, followed by acetone than [BMIM][Br] (35.01 ± 0.44 , 12.72 ± 0.12 and 6.75 ± 0.62) mg/g, respectively at the 40 minutes.

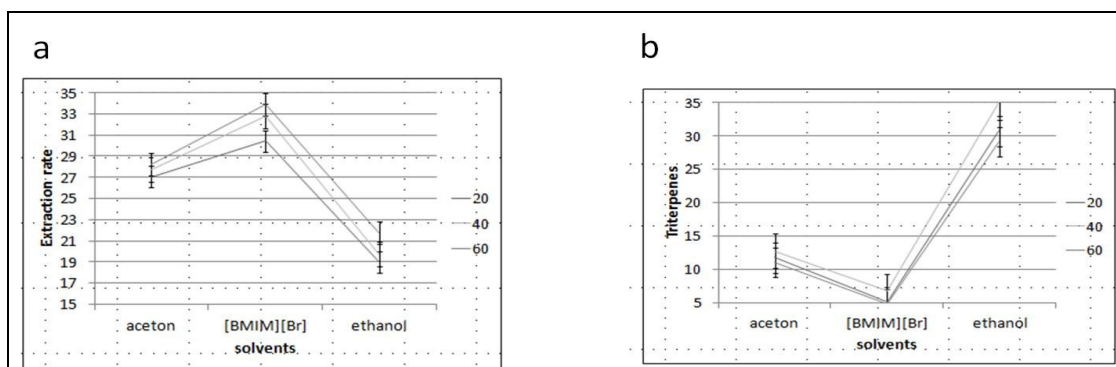


Figure 4: Effect of the solvent type on the (a) extraction rate and (b) Triterpenes from *G. japonicum* at power 400 w and temperature 80 °C

Effect of power on the extraction rate and TTP

Increased in power resulted enhanced compounds solubility, faster diffusion rate, and increased mass transfer. However, it was noted that increasing the power beyond certain values may promote possible concurrent decomposition of compounds which were already mobilized at lower power or even the break down of some compounds that were still in the plant matrix. Additionally, high power may encourage solvent loss through vaporization and increase the cost for extraction process from the industrialization point of view. Therefore, moderate extraction power of 200, 400 and 600w were chosen as the lower, middle and

upper levels, respectively, to be applied in RSM optimization.

There was an increase in the value of the rate of extraction with high power even 600 w. Similarly, there were also high the values of antioxidant and TTP even 400w below which there was a decrease in values after this power in the three solvents under study. Although the best value retention of extraction rate was in [BMIM][Br], where value increased of the extraction rate of $32.34 \pm 0.18 \%$ at power 200 w to $33.94 \pm 0.26 \%$ at power 600w (Fig 5 a). Also increased of the TTP of $33.25 \pm 0.14 \%$ at power 200 w to $35.0 \pm 0.09 \%$ at power 400 w , than after this power in the extration ethanol the TTP was decreased to 30.5 ± 0.23 at 600 w (Fig.5b).

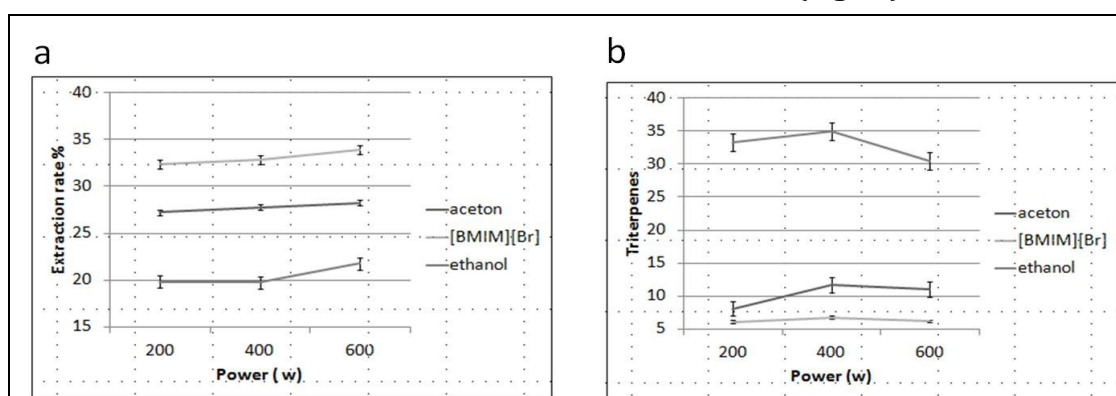


Figure 5: Effect of the power on the (a) extraction rate and (b) Triterpenes from *G. japonicum* at time 40 min and temperature 80 °C

Response surface methodology (RSM) experiments

Fitting the models

Response surface methodology (RSM) is an effective tool for optimizing the process. The basic principle behind response surface methodology (RSM) analysis is to relate the

observed value (dependent variables) to process parameters (independent variables) using statistical methods, yielding a multivariate regression equation, often of second-order. RSM uses an experimental design such as the box-Behnken design (BBD) to fit a model by least squares technique. As revealed by the diagnostic checking provided by an analysis

of variance (ANOVA) and residual plots, contour plots can be usefully employed to study the response surface and locate the optimum (16).

Therefore, the results showed that the experimental model was adequate due to no significant lack of fit and satisfactory levels of R^2 . The R^2 value of the dependent variables was more of 0.80, indicating that a high proportion of variability was explained by the data (17).

Effect of process variables on extraction rate

The extraction rate of experiments under 13 different conditions with 5 central points based on BBD are presented in Table 2. The regression equation in coded levels without insignificant terms were used to calculate the content variation through the response surface analysis as follows:

$$Y = 32.70 - 3.41 X_1 + 1.32 X_2 + 0.68 X_3 + 0.39 X_1 \times X_2 + 0.51 X_1 \times X_3 - 0.28 X_2 \times X_3 - 8.27 X_1^2 - 0.41 X_2^2 - 0.11 X_3^2$$

X_1 , X_2 and X_3 in this equation express solvent type, extraction time and microwave power, respectively.

Table 2: Operating parameters as well as the experimental and predicted values of extraction rate, and Triterpenes of *G. japonicum* for different setups of experimental design

Treatment	Independent variables Coded variables			Uncoded variables			Extract rate %	TTP mg/g extract
	X_1	X_2	X_3	Solvents	Time	Power		
1	0	-1	-1	[BMIM][Br]	20	200	29.7	4.9
2	0	0	0	[BMIM][Br]	40	400	32.82	6.75
3	1	-1	0	ethanol	20	400	19.02	30.75
4	0	-1	1	[BMIM][Br]	20	600	31.38	4
5	-1	1	0	acetone	60	400	28.24	5.83
6	0	1	-1	[BMIM][Br]	60	200	33.54	4.75
7	1	1	0	ethanol	60	400	21.78	29.1
8	0	0	0	[BMIM][Br]	40	400	32.75	6.7
9	0	0	0	[BMIM][Br]	40	400	32.8	6.88
10	0	1	1	[BMIM][Br]	60	600	34.1	3.5
11	-1	-1	0	acetone	20	400	27.04	12.75
12	0	0	0	[BMIM][Br]	40	400	32.42	6.78
13	-1	0	-1	acetone	40	200	27.22	8.08
14	-1	0	1	acetone	40	600	27.82	13.03
15	1	0	-1	ethanol	40	200	19.8	33.25
16	1	0	1	ethanol	40	600	22.44	30.5
17	0	0	0	[BMIM][Br]	40	400	32.70	6.65

The [BMIM][Br] extraction was much higher than those of acetone and ethanol extraction. The extraction rate of [BMIM][Br] extraction ranged from 29.7 to 34.1 % (Table 2). The results of multiple regression analysis showed that the extraction rate contents were significantly ($P < 0.0001$) affected by the linear term of solvents type, extraction time and microwave power with R^2 -value of 0.9965 and adjusted- R^2 value of 0.9920 (Table 3).

There response surface plots of the relationship between extraction rate and solvents type, extraction time and microwave power Figures 6 (a, b and c). Also (Figure 6 a) show the extraction rate different with the different solvent and in the following order ([BMIM][Br], acetone and ethanol). And extraction rate increased with increasing extraction time and microwave power (Figures 6 b, c).

Table 3: Analysis of variance for the fitted model for the extraction rate

Source	Sum of squares	df	Mean square	F- value	P-value Prob>F
Model	404.96	9	45	220.56	0.0001
X1	93.02	1	93.02	455.98	0.0001
X2	13.83	1	13.83	67.81	0.0001
X3	3.75	1	3.75	18.40	0.0036
X1X2	0.61	1	0.61	2.98	0.1278
X1X3	1.04	1	1.04	5.10	0.0585
X2X3	0.31	1	0.31	1.54	0.2550
X ₁ ²	287.90	1	287.90	1411.20	0.0001
X ₂ ²	0.70	1	0.70	3.45	0.1055
X ₃ ²	0.050	1	0.050	0.25	0.6356
Residual	1.43	7	0.20	-	-
Lack of fit	1.32	4	0.44	16.75	0.0099
Pure error	0.11	4	0.026	-	-
Cor total	406.39	16	-	-	-
<u>R²-value</u>			0.9965		
<u>R² value - adjusted</u>			0.9920		

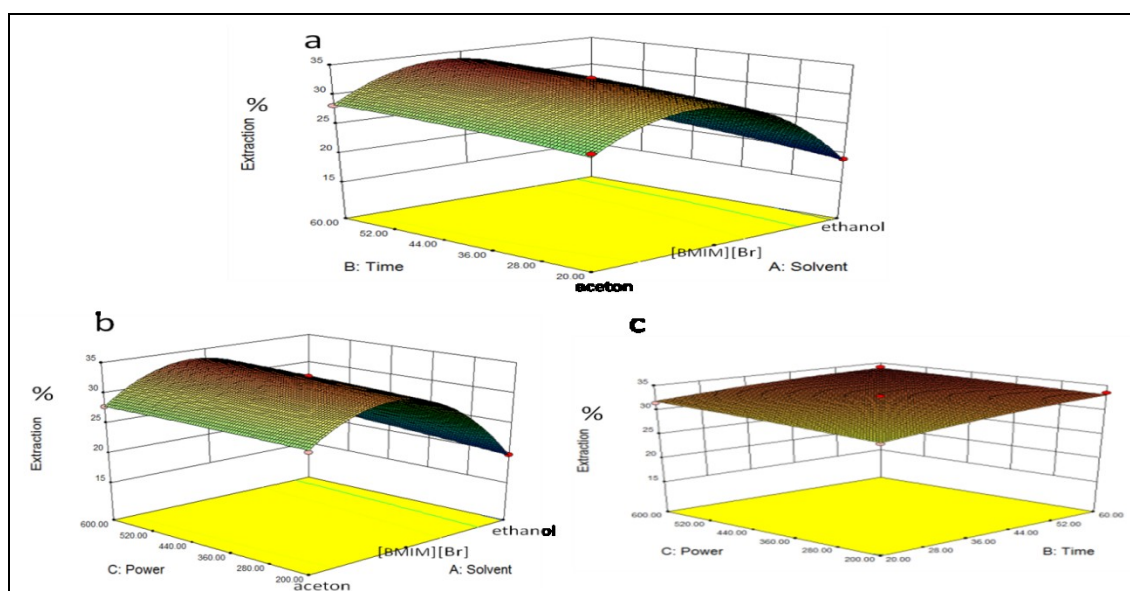


Figure 6: Response surface plot corresponding to (extraction rate) of *G. japonicum* of (a) solvent type and extraction time; (b) solvent type and power; and (c) extraction time and power. The value of the missing independent variable in each plot was kept at the middle level

Effect of process variables on Triterpenes TTP

The triterpenes of *G. japonicum* ranged from 33.25 to 3.5 mg/g. The TTP were much higher in ethanol extraction than acetone after that [BMIM][Br] extraction (Table 2). The results of multiple regression analysis Table 4 showed that the TTP were significantly ($p < 0.0001$) R^2 -value of 0.9944 and adjusted- R^2 value of 0.9872 affected by

Table 4: ANOVA for the fitted model for TTP

the linear term of solvent type . The predicted model obtained for Y is given below:

$$Y = 6.71 + 10.49 X_1 - 1.15 X_2 + 5.000 X_3 + 1.32 X_1 \times X_2 - 1.92 X_1 \times X_3 - 0.087 X_2 \times X_3 + 14.91 X_1^2 - 2.01 X_2^2 - 0.14 X_3^2$$

X_1 , X_2 and X_3 in this equation express solvent type, extraction time and microwave power, respectively.

Source	Sum of squares	df	Mean square	F- value	P-value Prob>F
Model	1855.56	9	206.17	138.18	0.0001
X1	880.32	1	880.32	590.01	0.0001
X2	10.63	1	10.63	7.12	0.0321
X3	2.000	1	2.000	1.340	0.9911
X1X2	6.94	1	6.94	4.65	0.0679
X1X3	14.78	1	14.78	9.91	0.0162
X2X3	0.031	1	0.031	0.021	0.8901
X ₁ ²	936.07	1	936.07	627.37	0.0001
X ₂ ²	17.09	1	17.09	11.45	0.0117
X ₃ ²	0.71	1	0.71	0.47	0.5134
Residual	10.44	7	1.49	-	-
Lack of fit	10.43	3	3.49	1255.51	0.0001
Pure error	0.011	4	2.770	-	-
Cor total	1866.00	16	-	-	-
<u>R²-value</u>			0.9944		
<u>R² value - adjusted</u>			0.9872		

The TTP extraction by the ethanol solvent contributes to higher content of TTC 33.25 mg/g. The same was observed with acetone as extraction solvent, but acetone extracts contained lower content of TTP compare to ethanol extracts. 13.03 mg/g and contained

higher content of TTP compare to [BMIM][Br] extracts 6.88 mg/g (Table 2). Also notes there is a significant effect on the type of solvent content TTP (Figure 7 a and b). While the influence of time and power are no significant (Figure 7 c).

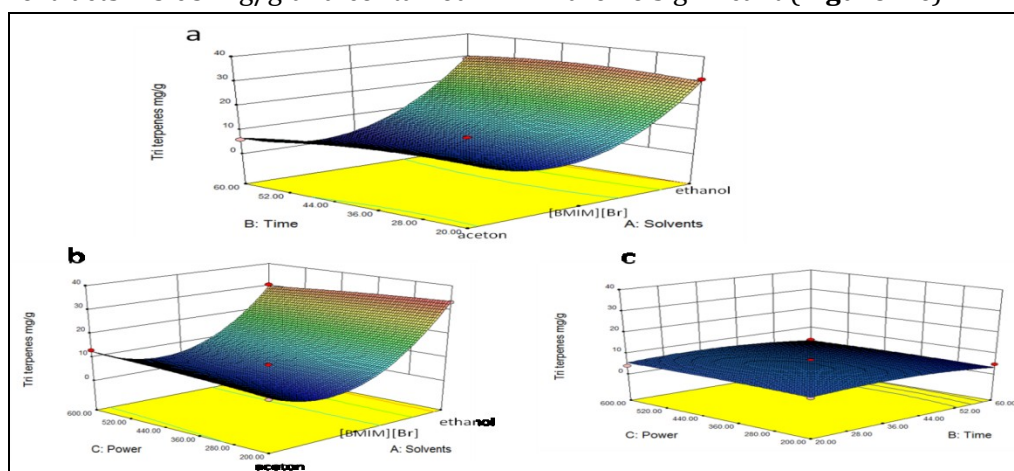


Figure 7: Response surface plot corresponding to (TTP) of *G. japonicum* of (a) solvent type and extraction time; (b) solvent type and power; and (c) extraction time and power. The value of the missing independent variable in each plot was kept at the middle level.

The optimum condition of extraction rate and TTP

According to the desired goals, and in order to verify the predictive capability of the model in the Table 5, optimum conditions were established by RSM and comparisons between the predicted results and the practical values were done by experimental

rechecking using those presumed optimal conditions. Table 5 presented the optimum conditions for (extraction rate and TTP) and its predicted and experimental value.

The optimum conditions for extraction for extraction rate was as follows: solvents type, [BMIM]Br; extraction time, 60 min and microwave power, 599.99 w, the model

predicted a maximum response of 34.1 % for extraction rate.

While TTP was as follows: solvents type ethanol, extraction time, 37.73 min and

microwave power, 235.77 w, the model predicted a maximum response of 33.25mg GAE/g for TTP.

Table 5: The optimum condition of extraction rate and TTP

	Solvents	Extraction time (min)	Microwave power (W)	Predicted
Extraction rate %	[BMIM][Br]	60	599.99	34.1
Triterpenes mg/g	Ethanol	37.73	235.77	33.25

CONCLUSION

This study demonstrates that it is essential to optimize systematically the extraction solvent composition, extraction time and power for accurate of *G. japonicum*. Also the difference in the time and power you do not have an effect is high compared with solvents. Our results showed that [BMIM][Br] solvent are more efficient in the extraction of extraction rate compare with ethanol and acetone solvents. This study confirmed that the [BMIM][Br] (1.5), extraction time 60 min and extraction power 599.99 w were the most efficient for extraction of extraction rate and lowest efficient for extraction of TTP. While ethanol (95%) were the most efficient for TTP extraction of at extraction time 37.73 min and extraction power 235.77 w.

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REFERENCES

1. L. Li. Opportunity and challenge of traditional Chinese medicine in face of the entrance to WTO (World Trade Organization). *Chin. Inform. trad. Chin. Med.* 7 (2000) 7–8.
2. C. Xiangrong, Q. Jiangjiang, Z. Qi, Z. Shoude, Z. Fei, Y. Shikai, J. Huizi, Z. Weidong. Taraxasterane-Type Triterpene and Neolignans from *Geum japonicum* Thunb. var. *chinense* F. Bolle. *Planta Med.* 77 2011 2061–2065.
3. G.N. Li. Dictionary of Chinese Herb Medicines in Yunnan, Yunnan Scientific and Technologic Press, Yunnan. 1990; pp. 153.
4. X. Hong-Xi, S.M. Dong, D. Hui, P.H. Paul. A New Anti-HIV Triterpene from *Geum japonicum*. *Chem. Pharm. Bull.* 48(9) 2000 1367-1369.
5. P. Sharma, L. Singh, N. Dilbaghi. Optimization of process variables for decolorization of disperse yellow 211 by *Bacillus subtilis* using box-behnken design. *J. Hazardous Mater.* 164(2-3) 2009 1024-1029.
6. P.C. Liyana, F. Shahidi. Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chem.* 93(1) 2005 47-56.
7. K.N. Prasad, F.A. Hassan, B. Yang, K.B. Kong, R.N. Raman, A. Azlan, A. Ismail. Response surface optimization for the extraction of phenolic compounds and antioxidant capacities from *Mangifera pajang* Kosterm. peels. *Food Chem.* 128 2011 1121 - 1127.
8. L. Xia, Y.Z. Jun, Wen, Y.G. W. Ying, Y.W. Hai, G.C. Jing, Q.H. Lu. Chemical Composition and Anti-inflammatory and Antioxidant Activities of Eight Pear Cultivars. *J. Agric. Food Chem.* 60 2012 8738–8744.
9. H.N. Sin, S. Yusof, N.S.A. Hamid, et al. Optimization of hot water extraction for sapodilla juice using response surface methodology. *J. Food Eng.* 74 2006 352–358.
10. B. Lapornik, M. Prosek, A.G. Wondra. Comparison of extracts prepared from plant by-products using different solvents and extraction time. *J. Food Eng.* 71 2005 214–222.
11. M. Pinelo, J. Sineiro, M.J. Núñez. Mass transfer during continuous solid–liquid extraction of antioxidants from grape byproducts. *Journal of Food Engineering.* 77(1) 2006 57-63.
12. M. Naczek, F. Shahidi. Extraction and analysis of phenolics in food. *J. Chromatogr. A.* 1054 (1-2) 2004 95–111.
13. R. Chirinos, H. Rogez, D. Campos, R. Pedreschi, Y. Larondelle. Optimization of

- extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruiz and Pavón) tubers. *Separation and Purification Technology*. 55(2) 2007 217-225.
- 14.T. Kuljarachanan, S. Devahastin, N. Chiewchan. Evolution of antioxidant compounds in lime residues during drying. *Food Chemistry*. 113(4) 2009 944-949.
- 15.E.M. Silva, H. Rogez, Y. Larondelle. Optimisation of extraction of phenolics from *Inga edulis* leaves using response surface methodology. *Journal of Separation and Purification Technology*. 55 2007 381-387.
- 16.I.Y.S. Rustom, M.H. López-leiva, B.M. Nair. Optimization of extraction of peanut proteins with water by response surface methodology. *J. Food Sci.* 56(6) 1991 160-163.
- 17.A.I. Varnalis, J.G. Brennan, D.B. Macdougall, S.G. Gilmour. Optimization of high temperature puffing of potato cubes using response surface methodology. *J Food Eng.* 61 2004 153-163.