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Optimization of Microwave-Assisted Extraction of Antioxidant Compounds from Roots of *Polygonum multiflorum* Thunb. at Vietnam using Response Surface Methodology

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Response surface methodology (RSM) was used to optimize the extraction conditions for the determination of total phenolic content (TPC), total flavonoid content (TFC) and Antioxidant Activity (DPPH) in the roots of *Polygonum multiflorum* Thunb. using Microwave-Assisted Extraction (MAE). The process variables were extraction temperature (40 - 60 ^{o}C), extraction time (30 - 60 min), ethanol concentration (30 - 70 %) and microwave power (300 - 500 W). The results showed that temperature, time, ethanol concentration and microwave power had significant impacts on TPC, TFC and DPPH. The optimum extraction conditions obtained with RSM were: extraction temperature of 53 ^{o}C, extraction time of 49 min, ethanol concentration of 48 % and microwave power level of 420 W. The experimental values obtained for TPC, TFC and DPPH radical scavenging activity were 34.15 ± 0.2 mg GAE/g, 6.52 ± 0.15 mg QE/g and 93.82 ± 0.3 %, respectively.

Keywords: *Polygonum multiflorum*; total phenolic content; total flavonoid content; radical scavenging activity, Response Surface Methodology

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Polygonum multiflorum, a herbaceous perennial plant, was originally called Caulis Polygoni Multiflori in the book "He Shou Wu Zhuan" written during the Tang dynasty [1]. Its roots are tuberous, hypertrophic, oblong and dark brown in colour. *Polygonum multiflorum* is one of the most popular traditional Chinese and Vietnamese medicines. It is also known as Heshouwu (in Chinese) or Ha Thu O Do (HTOD) (in Vietnamese). It has been widely used to treat various diseases. Some studies have proved that it can exhibit antioxidative activity [2] due to its flavonoid and phenolic acid constituents. A previous study [3] investigated the biological composition and antioxidant activity in the leaves, stems and roots of this tree. Results also showed that the polyphenol content and the antioxidant activity were highest in the roots. This created a new foundation for further research on *Polygonum multiflorum* Thunb. roots. Stilbene in *Polygonum multiflorum* has been reported to have anti-aging effects [4, 5], Anthraquinones, another main component of this plant, also have many biological activities, such as effects against cancer [6, 7], developmental anomalies [8] and tonic tension [9].

In this respect, a procedure that could obtain most of the effective constituents in the shortest processing time with low production costs and using the minimum amount of organic solvent would be an ideal technology. Microwave-assisted extraction (MAE) has recently proved to be a promising tool for the extraction of phytochemicals from botanicals [10, 11]. The use of MAE has been shown to have significant advantages over conventional extraction methods, such as shorter extraction times, lesser solvent consumption, higher extraction rates, and better products at a lower cost.

Response surface methodology (RSM) is an effective multivariate statistical method for optimizing complex experimental processes. It generates a second-degree polynomial model by regression fitting of response surface analysis to evaluate the polytomy variables and their interactions, and then determines the best level. The most important advantage of RSM is the reduced number of trials for process optimization. The Box-Behnken design (BBD), a type of RSM, has been effectively applied to optimize parameters in the extraction of bioactive compounds [12]. The objective of this study was to use RSM to optimize the microwave-assisted extraction of the roots of *Polygonum multiflorum* to determine its total phenolic content, total flavonoid content and antioxidant activity.

MATERIALS AND METHODS

Material

The roots of *Polygonum multiflorum* were collected at the Quy Hop District of Nghean Province, Vietnam in September 2020 and identified by the Institute of

Ecology and Biological Resources, Vietnam Academy of Science and Technology. The dried roots were milled to >250 µm, packed in a polyethylene (PE) pail (moisture content about 9 - 10 %), and stored at room temperature (15 ± 3 °C).

Methods

Total Phenolic Content (TPC)

The TPC of the *Polygonum multiflorum* Thunb. root extract was measured using the method reported by Singleton et al. [13] with some modifications. This method measures the colour change caused by phenolates in the presence of sodium carbonate. A 1 ml sample was mixed with 5 ml of Folin-Ciocalteu's solution. After 3 min, 4 ml of 7.5 % sodium carbonate solution was added to the mixture and diluted to 10 ml with deionized water. The mixture was allowed to stand at room temperature in the dark for 60 min. The colour change was determined by scanning at 765 nm (Agilent 8453 UV – Visible Spectrophotometer) to obtain the maximum absorbance. The TPC of the *Polygonum multiflorum* Thunb. root extract was determined as mg gallic acid equivalents using the standard curve prepared with different concentrations of gallic acid and reported as mg GAE/g dry weight (DW).

Total Flavonoid Content (TFC)

The TFC of the *Polygonum multiflorum* Thunb. root extract was determined using the method by Chang et al. [14] with some modifications. The *Polygonum multiflorum* Thunb. root extract (0.5 mL) was mixed with 1.5 mL of 75 % ethanol, 0.1 mL of 10 % aluminium chloride, 0.1 mL of 0.1 M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was kept at room temperature for 30 min and then its absorbance was measured at 415 nm (Agilent 8453 UV-Visible Spectrophotometer). Quercetin was used for the standard calibration curve and the total flavonoid content was expressed in mg of quercetin equivalents/g dry weight (mg QE/g DW).

DPPH Radical Scavenging Activity

The radical scavenging activity of the *Polygonum multiflorum* Thunb. root extract was evaluated using DPPH radicals based on the method by Xu and Chang [15] with slight modifications. The DPPH solution was prepared by dissolving 5.9 mg of DPPH in ethanol (100 ml). Exactly 3.8 ml of DPPH ethanolic solution was added to 0.2 ml of the *Polygonum multiflorum* Thunb. root extract. The mixture was shaken vigorously for 1 min and left to stand in the dark at room temperature for 30 min. Absorbance was measured against the blank reagent at 517 nm (Agilent 8453 UV-Visible Spectrophotometer). All experiments were performed in replicate, with triplicate analyses. The radical scavenging activity was calculated according to Eq. (1) below:

$$\text{Radical scavenging activity (\%)} = [1 - (Abs_{sample}/Abs_{control})] \times 100 \quad (1)$$

Microwave Extraction

The extraction system was comprised of a modified domestic microwave oven extractor equipped with a magnetron of 2450 MHz with a nominal maximum power of 700 W. For MAE, 1 g of the homogenous powder was accurately weighed and mixed with 30 mL solvent. After allowing a preleaching time of 5 min, the suspension was irradiated with microwaves under different experimental conditions to optimize the extraction parameters. The resulting suspensions were centrifuged and filtered with a 0.45 µm syringe filter before further analysis.

Box-Behnken Design

A four-variable, three-level Box-Behnken design (BBD) consisting of 27 experimental runs was used in this optimization study based on the results of preliminary experiments. The independent variables were extraction temperature (X_1), extraction time (X_2), ethanol concentration (X_3) and power level (X_4) while dependent variables were total phenolic content (Y_1), total flavonoid content (Y_2) and DPPH radical scavenging activity (Y_3). Experiments were performed in replicate and the average values were used as the response. Statistical analysis on the means of triplicate experiments was carried out using the ANOVA procedure with Design-Expert® software, version 7.0. All analyses were performed in triplicate and all experimental results were expressed as mean ± SD. P values < 0.05 were significant and P values < 0.01 were very significant.

RESULTS AND DISCUSSION

Fitting the Models

The TPC, TFC and DPPH results for the microwave extraction of *Polygonum multiflorum* Thunb. roots were optimized using the Box-Behnken design. The Box-Behnken used four independent variables: extraction temperature, extraction time, ethanol concentration and microwave power level. The input range of the selected variables was determined by preliminary experiments (Table 1). Three dependent variables including total phenolic content, total flavonoid content and DPPH radical scavenging activity were determined following extraction under optimal conditions.

Table 1. Coded level of independent variables used in the RSM design.

Independent variables	Units	Coded symbols	Coded variable levels		
			-1	**0**	**+1**
Extraction temperature	°C	X_1	40	50	60
Extraction time	min	X_2	30	45	60
Ethanol concentration	%	X_3	30	50	70
Microwave power level	W	X_4	300	400	500

The Design-Expert® program was used to evaluate the effects of the extraction process. The response variable was fitted to a second-order polynomial model as follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j \quad (2)$$

where Y is the predicted response; β_o is the intercept coefficient; β_i is the linear coefficient; β_{ii} is the squared coefficient; β_{ij} is the interaction coefficient; X_i and X_j are the coded independent variables; X_iX_j and X_i^2 are the interaction and quadratic terms respectively.

Table 2. Experimental Design and Response Values.

Run No.	Coded and Processed Variable level				Response		
	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3
	Extraction temperature (°C)	Extraction time (min)	Ethanol concentration (%)	Power level (W)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	DPPH radical scavenging activity (%)
1	0	0	+1	-1	26.14	7.09	88.79
2	+1	-1	0	0	28.97	6.07	91.92
3	0	+1	-1	0	34.51	4.84	92.47
4	-1	0	-1	0	28.10	4.35	90.13
5	-1	0	0	+1	27.61	5.17	91.66
6	0	0	+1	+1	31.35	5.05	92.65
7	0	+1	0	-1	31.47	6.38	92.27
8	0	0	0	0	33.94	6.78	93.67
9	+1	0	+1	0	27.24	6.11	91.25
10	-1	+1	0	0	29.44	5.86	92.07
11	0	+1	0	+1	33.43	5.97	93.61
12	0	-1	0	+1	32.05	4.66	92.66
13	0	0	0	0	35.37	6.89	94.16
14	+1	0	0	+1	34.36	5.49	94.37
15	+1	0	-1	0	33.40	5.42	93.21
16	0	-1	+1	0	26.28	5.38	90.12
17	0	0	0	0	32.76	6.95	94.89
18	+1	0	0	-1	26.08	6.76	91.14
19	-1	0	+1	0	26.28	5.89	88.85
20	-1	-1	0	0	24.36	4.97	90.81
21	-1	0	0	-1	27.84	5.68	91.45
22	0	0	-1	-1	33.04	5.21	94.08
23	+1	+1	0	0	30.73	6.32	92.92
24	0	-1	0	-1	26.41	6.06	88.94
25	0	0	-1	+1	34.16	4.72	93.41
26	0	-1	-1	0	28.78	5.09	91.16
27	0	+1	+1	0	28.30	6.76	89.88

Table 3. Empiric second-order polynomial models for TPC, TFC and DPPH.

Response	Model equations	R^2	p-value
Y_1 – TPC	$Y_1 = 34.02 + 1.43X_1 + 1.75X_2 – 2.20X_3 + 1.83X_4 – 0.83X_1X_2 – 1.09X_1X_3 + 2.13X_1X_4 – 0.93X_2X_3 – 0.92X_2X_4 + 1.02X_3X_4 – 3.56X_1^2 – 2.27X_2^2 – 1.91X_3^2 – 1.12X_4^2$	0.9773	<0.0001
Y_2 – TFC	$Y_2 = 6.87 + 0.35X_1 + 0.33X_2 + 0.55X_3 – 0.51X_4 – 0.16X_1X_2 – 0.21X_1X_3 – 0.19X_1X_4 + 0.41X_2X_3 + 0.25X_2X_4 - 0.39X_3X_4 – 0.56X_1^2 – 0.53X_2^2 – 0.84X_3^2 – 0.54X_4^2$	0.9896	<0.0001
Y_3 – DPPH	$Y_3 = 94.24 + 0.82X_1 + 0.63X_2 – 1.08X_3 + 0.97X_4 - 0.065X_1X_2 – 0.17X_1X_3 + 0.76X_1X_4 – 0.39X_2X_3 – 0.59X_2X_4 + 1.13X_3X_4 – 1.31X_1^2 – 1.43X_2^2 – 1.78X_3^2 – 0.65X_4^2$	0.9005	0.0005

The values of the three evaluation indices for each extraction condition are listed in Table 2. The maximal TPC was 34.51 mg GAE/g, the maximal TFC was 7.09 mg QE/g and the maximal DPPH was 94.89 %. From the multiple linear regression analysis of the 27 data entries, empirical second-order polynomial models for TPC, TFC and DPPH radical scavenging activity were derived (Table 3).

ANOVA results for multiple regression analysis and response surface quadratic models for Y_1, Y_2 and Y_3 were evaluated using the corresponding p and R^2 values (Table 3). F values for Y_1, Y_2 and Y_3 were calculated to be 36.96, 81.78 and 7.75, leading to p values <0.05, suggesting that all the models were statistically extremely significant. The models' coefficients of determination (R^2) were 0.9773, 0.9896 and 0.9005, indicating that more than 97.73 %, 98.96 % and 90.05 % of the response variability were explained, thus supporting the accuracy and ability of the established models within the range limits used. The F-values of Lack of Fit for Y_1, Y_2 and Y_3 were 0.17, 2.07 and 1.88, respectively, implying that the Lack of Fit was not significant relative to the pure error. This indicated that the accuracy of the polynomial models was adequate.

Table 4. Regression coefficients of the predicted second-order polynomial models for total phenolic content, total flavonoid content and DPPH.

Source	Y_1 – TPC		Y_2 – TFC		Y_3 – DPPH	
	F- value	p- value	F- value	p-value	F- value	p- value
Model	36.96	$< 0.0001^S$	81.78	$< 0.0001^S$	7.75	0.0005^S
X_1	46.39	$< 0.0001^S$	107.05	$< 0.0001^S$	12.36	0.0043^S
X_2	69.76	$< 0.0001^S$	90.15	$< 0.0001^S$	7.39	0.0186^S
X_3	109.94	$< 0.0001^S$	262.10	$< 0.0001^S$	21.31	0.0006^S
X_4	76.21	$< 0.0001^S$	221.98	$< 0.0001^S$	17.45	0.0013S
X_1X_2	5.22	0.0414^S	7.28	0.0194^S	0.026	0.8748^{NS}
X_1X_3	8.91	0.0114^S	12.85	0.0038^S	0.18	0.6813^{NS}
X_1X_4	34.27	$< 0.0001^S$	10.27	0.0076^S	3.49	0.0862^{NS}
X_2X_3	6.51	0.0254^S	47.24	$< 0.0001^S$	0.92	0.3563^{NS}
X_2X_4	6.41	0.0263^S	17.43	0.0013^S	2.17	0.1665^{NS}
X_3X_4	7.92	0.0156^S	42.72	$< 0.0001^S$	7.86	0.0159^S
X_1^2	127.76	$< 0.0001^S$	120.20	$< 0.0001^S$	13.95	0.0028^S
X_2^2	51.91	$< 0.0001^S$	106.21	$< 0.0001^S$	16.60	0.0015^S
X_3^2	36.88	$< 0.0001^S$	264.73	$< 0.0001^S$	25.87	0.0003^S
X_4^2	12.58	0.0040^S	112.32	$< 0.0001^S$	3.46	0.0877^{NS}
Lack of Fit	0.17	0.9792^{NS}	2.07	0.3695^{NS}	1.88	0.3971^{NS}
R^2	0.9773		0.9896		0.9005	

S: significant ($p < 0.05$); NS: non-significant.

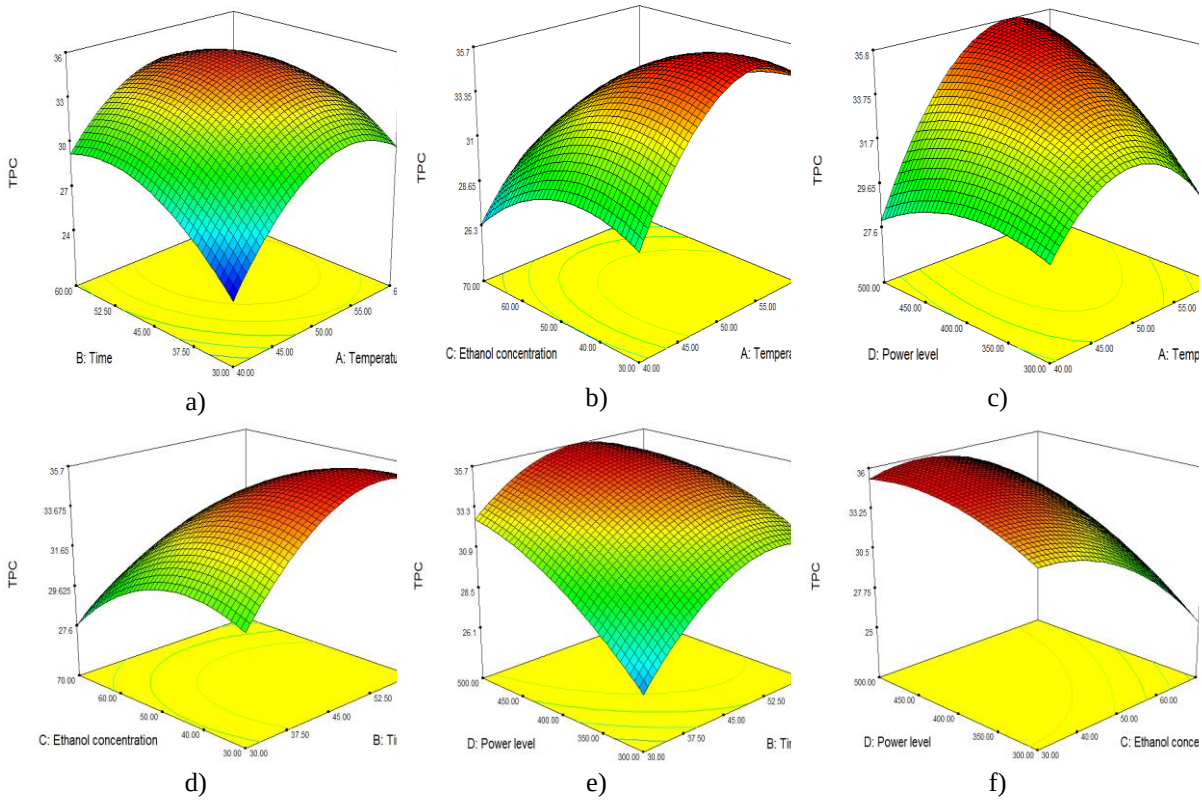


Figure 1. The response surface plot for TPC.

Response Surface Analysis

The effects of extraction temperature, extraction time, ethanol concentration and power level on the total phenolic content, total flavonoid content and DPPH were investigated. Three-dimensional graphs of the models were plotted as shown in Figs. 1, 2 and 3. The response surface plots of the models were created by varying two variables within the experimental range under investigation while holding the other variables constant.

Response Surface Analysis for Total Phenolic Content (TPC)

Fig. 1 investigates the effect of extraction temperature, extraction time, ethanol concentration and power level on the TPC. When extraction temperature was increased from 40 to 55 ºC, the TPC also increased but when extraction temperature continued to increase from 55 to 60 ºC, the TPC tended to decrease, with a constant ethanol concentration of 50 % and microwave power level of 400 W. It has been reported that these compounds degrade at higher temperatures [16]. A higher buffer to solids ratio also showed a positive effect on the recovery of TPC, consistent with mass transfer principles. Normally, phenolic compound yields increase with a higher buffer to solids ratio [17]. It can be observed that the TPC increased when extraction time increased from 30 to 55 min and then decreased, while the TPC also increased when the microwave power level was increased from 300 to 500 W (Fig. 1(e)) In Fig. 1(b), at a constant time of 45 min and microwave power level of 400 W, the TPC increased when the ethanol concentration was increased from 30 %v/v to 50 %v/v, but thereafter decreased. In addition, the ethanol to water ratio (~40–50%) responded well to the TPC posed by the interaction of any buffer to solids ratio. This result was similar to that reported by Gong et al. [18].

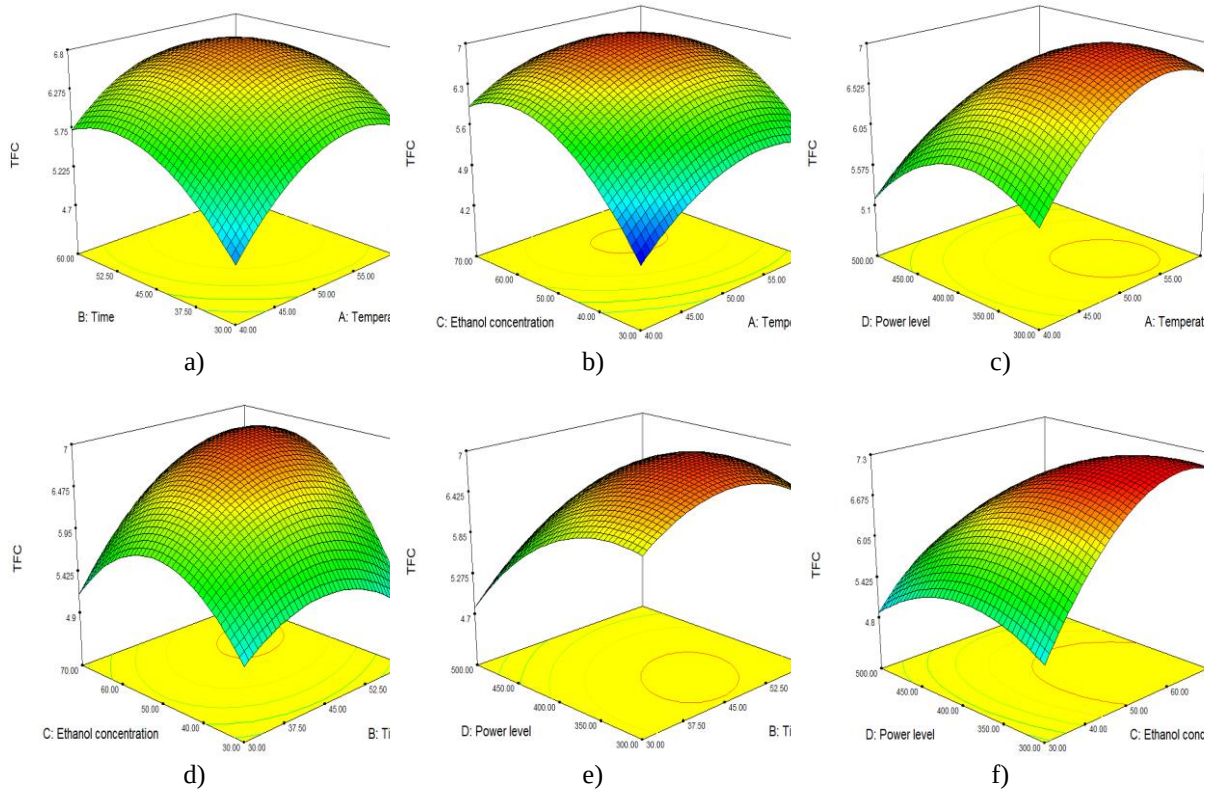
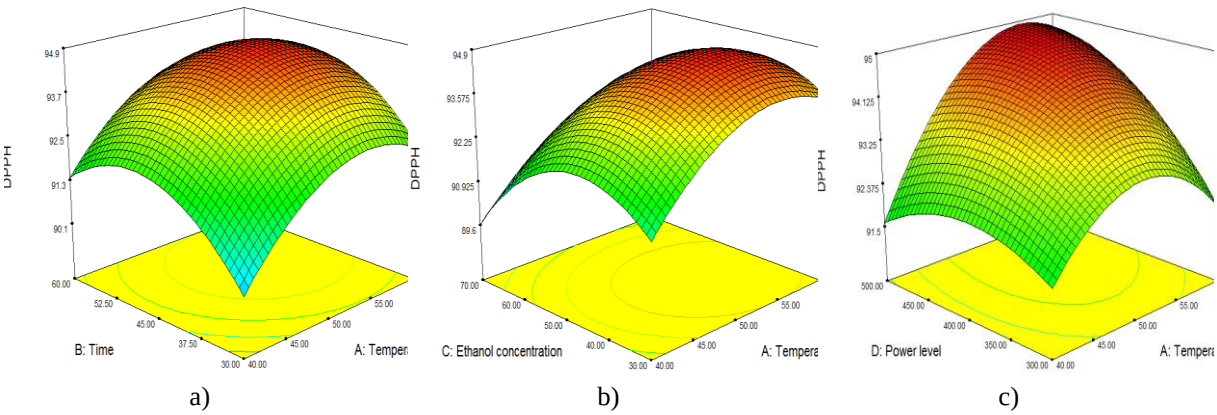
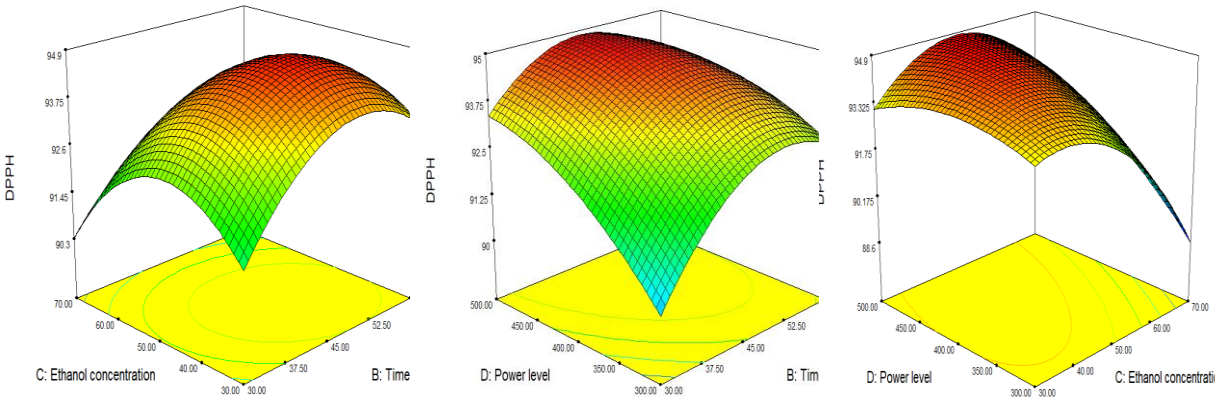


Figure 2. The response surface plot for TFC.

Response Surface Analysis for Total Flavonoid Content (TFC)

Figure 2 presents the combined effects of four variables on the TFC. The plot in Fig. 2(a) combined the effects of temperature and extraction time on the TFC while the other parameters were maintained at constant levels. The TFC increased with increasing extraction temperature regardless of extraction time. When extraction temperature increased from 40 to 55 ^{o}C, the TFC also increased, but then decreased when the extraction temperature continued to rise from 55 to 60 ^{o}C. This was attributed to the positive effect of temperature on the extraction [19]. TFC increased with increasing extraction time from 30 to 52 min, However when the extraction time continued to increase from 52 to 60 min, the TFC decreased. Fig. 2(f) shows the interaction between microwave power level and ethanol concentration at a fixed extraction time (45 min) and temperature (50 ^{o}C). The plot showed that the microwave power level and ethanol concentration had a significant effect on the TFC. It was observed that the TFC increased when the microwave power was increased from 300 to 400 W, but then decreased when the microwave power continued to increase from 400 to 500 W. The TFC also increased when the ethanol concentration was increased from 30 to 60 %, then decreased with higher ethanol concentrations. It was found that the degree of cell membrane rupture of the raw materials and the solubility of phenolic substances were improved when ethanol in water was used. Wang et al. [20] also reported similar results.





d) e) f)

Figure 3. Response surface plot for DPPH.

The plots in Fig. 3 show the effects of extraction temperature, extraction time, ethanol concentration and microwave power level on the DPPH radical scavenging activity. The linear effects of these four factors on the DPPH value were significant. The plot in Fig. 3(a) shows the effects of extraction temperature and extraction time on the DPPH with the remaining factors kept constant. It can be observed that the DPPH increased with the increase in extraction temperature from 40 ^{o}C to 55 ^{o}C, but then decreased when extraction temperature rose to 60 ^{o}C. The DPPH increased with extraction time from 30 min to 50 min, then decreased when the extraction time was increased further. The plot in Fig. 3(f) shows the effects of ethanol concentration and microwave power level on the DPPH when extraction temperature and extraction time were constant. It can be observed that DPPH increased with the increase in ethanol concentration from 30 to 40 % and then decreased at higher ethanol concentrations. The DPPH increased with the increase in the microwave power level from 300 W to 400 W, then decreased when the microwave power level increased from 400 W to 500 W.

Optimization and Model Verification.

According to the second-order polynomial equation, the optimum conditions for total phenolic content, total flavonoid content and antioxidant activity for the roots of *Polygonum multiflorum* were as follows: extraction temperature of 52.86 ^{o}C, extraction time of 48.83 min, ethanol concentration of 47.64 % and microwave power level of 418.31 W. The predicted maximum TPC, TFC and DPPH values were 35.02 mg GAE/g, 6.78 mg QE/g and 94.69 %, respectively. For practical reasons, we modified the optimum conditions slightly to the following: extraction temperature of 53 ^{o}C, extraction time of 49 min, ethanol concentration of 48 % and microwave power level of 420 W. Under these conditions, the TPC, TFC and DPPH values obtained were 34.15 ± 0.2 mgGAE/g, 6.52 ± 0.15 mgQE/g and 93.82 ± 0.3 % (n = 3), respectively. These results imply that the experimental values were consistent with the predicted values.

Table 5. Predicted and experimental values of responses under optimal conditions.

Responses	Optimum extraction conditions				Maximum value		% difference (CV)
	X_1	X_2	X_3	X_4	Experimental[a]	Predicted	
TPC (mgGAE/g DW)	53 ^{o}C	49 min	48 %	420 W	34.15 ± 0.2	35.02	2.48
TFC (mgQE/g DW)					6.52 ± 0.15	6.78	3.83
DPPH radical scavenging activity (%)					93.82 ± 0.3	94.69	0.92

X_1: extraction temperature (^{o}C); X_2: extraction time (min); X_3: ethanol concentration (%); X_4: ultrasonic power level (%); Y_1: TPC (mg GAE/g); Y_2: TFC (mg QE/g); Y_3: DPPH (%).

[a]Responses are the means ± SD (n = 3).

CONCLUSION

In this study, we investigated the optimum conditions for the microwave-assisted extraction (MAE) of the roots of *Polygonum multiflorum* to determine its antioxidant activity. The optimal conditions of MAE were achieved based on response surface methodology using the Box-Behnken design. We concluded that the optimal extraction conditions were: extraction temperature of 53 ^{o}C, extraction time of 49 min, ethanol concentration of 48 % v/v and microwave power level of 420 W. The experimental values obtained for TPC, TFC and DPPH were 34.15 ± 0.2 mg GAE/g, 6.52 ± 0.15 mg QE/g and 93.82 ± 0.3 %, respectively.

REFERENCES

1. Li, Z., Hang, Y. Y. and Zhou, Y. F. (2003) Germplasm appraisements of Plolygonum Multiflorum from main producing areas in China by content of tetrahydroxystilbene. *Chemistry and Industry of Forest Products*, **23**, 37–41.

2. Wang, W. J., Zhang, W. M., Dong, X. L., Zhao, R. H. and Rao, G. X. (2005) Studies on chemical constituents of Polygonum multiflorun from Yunnan. *Journal of Yunnan College of Traditional Chinese Medicine*, **28**, 10–12.

3. Lin, H. T., Nah, S. L., Huang, Y. Y. and Wu, S. C. (2010) Potential antioxidant components and characteristics of fresh *Polygonum multiflorum*. *J. Food Drug Anal*., **18**, 120–127.

4. Long, C. O. and Dougherty, J. (2003) What's new in Alzheimer's disease? *Home Health Nurse*, **21**, 8–14.

5. Lin, H. Q., Ho, M.T., Lau, L. S., Wong, K. K., Shaw, P. C. and Wan, D. C. (2008) Antiacetylcholinesterase activities of traditional Chinese medicine for treating Alzheimer's disease. *Chemico-Biological Interactions*, **175**, 352–354.

6. Ma, Y. S., Weng, S. W., Lin, M. W., Lu, C. C., Chiang, J. H., Yang, J. S., Lai, K. C., Lin, J. P., Tang, N. Y., Lin, J. G. and Chung, J.G. (2012) Antitumor effects of emodin on LS1034 human colon cancer cellsin vitro and in vivo: roles of apoptotic cell death and LS1034 tumor xenografts model. *Food and Chemical Toxicology*, **50**, 1271–1278.

7. Wang, W., Sun, Y., Li, X., Li, H., Chen, Y., Tian, Y., Yi, J. and Wang, J. (2011) Emodin potentiates the anticancer effect of cisplatin on gallbladder cancer cells through the generation of reactive oxygen species and the inhibition of survivin expression. *Oncology Reports*, **26**, 1143–1148.

8. Yon, J. M., Lin, C., Oh, K. W., Baek, H. S., Lee, B. J., Yun, Y. W. and Nam, S. Y. (2013) Emodin prevents ethanol-induced developmental anomalies in cultured mouse fetus through multiple activities. *Birth Defects Research B: Developmental and Reproductive Toxicology*, **98**, 268–275.

9. Lim, K. M., Kwon, J. H., Kim, K., Noh, J. Y., Kang, S., Park, J. M., Lee, M. Y., Bae, O. N. and Chung, J. H. (2014) Emodin inhibits tonic tension through suppressing PKCδ- mediated inhibition of myosin phosphatase in isolated rat thoracic aorta. *British Journal of Pharmacology*, **171**, 4300–4310.

10. Smith, R. M. (2003) Before the injection-modern methods of sample preparation for separation techniques. *J. Chromatogr. A*., **1000**, 3.

11. Mandal, V., Mohan, Y. and Hemalatha, S. (2007) Microwave Assisted Extraction – An Innovative and Promising Extraction Tool for Medicinal Plant Research. *Phcog. Rev*., **1**, 7–18.

12. Zhang, Q., Zhou, M. M., Chen, P. L., Cao, Y. Y. and Tan, X. L. (2001) Optimization of ultrasonic-assisted enzymatic hydrolysis for the extraction of luteolin and apigenin from celery. *J Food Sci*., **76**, 680–685.

13. Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol*., **299**, 152–178.

14. Chang, C. C., Yang, M. H., Wen, H. M. and Chern, J. C. (2022) Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *J Food Drug Anal*, **10**, 178–182.

15. Xu, B. J. and Chang, S. K. C. (2007) A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J Food Sci*., **72**, 159–166.

16. Bimakr, M., Rahman, R. A., Taip, F. S., Adzahan, N. M., Sarker, M. Z. I. and Ganjloo, A. (2012) Optimization of ultrasound-assisted extraction of crude oil from winter melon (*Benincasa hispida*) seed using response surface methodology and evaluation of its antioxidant activity, total phenolic content and fatty acid composition. *Molecules*, **17**, 11748–11762.

17. Ilaiyaraja, N., Likhith, K., Babu, G. S. and Khanum, F. (2015) Optimisation of extraction of bioactive compounds from *Feronia limonia* (wood apple) fruit using response surface methodology (RSM). *Food Chem*, **173**, 348–354.

18. Gong, Y., Hou, Z., Gao, Y., Xue, Y., Liu, X. and Liu, G. (2012) Optimization of extraction para-

meters of bioactive components from defatted marigold (*Tagetes erecta* L.) residue using response surface methodology. *Food Bioprod Process*, **90**, 9–16.

19. Yusof, M., Huzaimi, A., Abd Gani, S., Zaidan, U. H., Halmi, M. I. E. and Zainudin, B. H. (2019) Optimization of an ultrasound-assisted extraction condition for flavonoid compounds from cocoa shells (*theobroma cacao)* using response surface methodology. *Molecules*, **24**, 711–716.

20. Wang, B., Qu, J. and Luo, S. (2018) Optimization of ultrasound-assisted extraction of flavonoids from olive (*olea europaea*) leaves, and evaluation of their antioxidant and anticancer activities. *Molecules*, **23(10)**, 2513–2518.