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Research Article

Optimizing extraction of polyphenols from red *Fallopia multiflora* Thunb. root in raw and processed form by response surface method: A comparison

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ABSTRACT

Fallopia multiflora (Thunb.) Haraldson (abbreviated as *F. multiflora*), belonging to *Polygonaceae* family, has long been used in traditional medicine. Polyphenols, main compounds in *F. multiflora* roots, have high antioxidant, anti-inflammatory, anti-aging, etc. Processing *F. multiflora* roots is necessary to reduce their negative effect on liver. Study on processing red *F. multiflora* roots and optimizing the extraction of polyphenols from processed red *F. multiflora* root product is to decrease the liver toxicity and to find the optimal conditions for extract of polyphenols from processed product. This study deals with the influence of some factors including to the ratio of solvent and medicinal herbs (mL/g), extraction temperature (°C), extraction time (min) on polyphenol content and its activity in red *F. multiflora* roots. Raw material has been processed by the stewing method with black bean water, then by ultrasonic extraction immersed on an ES-600N device (the ultrasonic capacity of 600W, the particle size of powder of 0.5-1.0 mm). Polyphenol content and its activities were evaluated by Folin-Ciocalteu technique and DPPH method. The response surface method combined with the Box-Behnken design was used to optimize polyphenol extraction. The optimization results obtained on the raw sample were the solvent/medicinal herb ratio of 5.7/1, the extraction temperature of 42.4°C, the extraction time of 47.6 min while the optimal extraction conditions for processed sample were the solvent/medicinal herb ratio 11.2/1, extraction temperature of 53°C, extraction time of 52 min. At the optimal extraction conditions of *F. multiflora* roots, the polyphenol content and its activity of raw sample were 60.41±0.14 mgGAE/g and 65.98±0.22%, respectively. They were lower than those of the processed sample, 59.91±0.17 mgGAE/g and 80.18±0.21%, respectively. These results indicated that the processing is necessary for polyphenol extraction from *F. multiflora* roots to obtain the products having high anti-oxidation activity.

Keywords:
Fallopia multiflora (Thunb.) Haraldson, Total polyphenols content, Anti-oxidation activity, Response surface method, Optimal extraction conditions

1. INTRODUCTION

Polyphenol compounds are widely distributed in the plant kingdom, attracting the interest of scientists in recent years thanks to their strong antioxidant ability and prevention of diseases related to oxidative stress, free radical formation, anti-inflammatory and anti-cancer[1].

Red *Fallopia multiflora* (Thunb.) Haraldson

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(abbreviated as *F. multiflora*) or *Polygonum multiflorum* (Thunb.), belonging to *Polygonaceae* family, has been used for a long time in traditional medicine in Asian countries. The *F. multiflora* root contains many phenolic compounds such as flavonoids, anthraquinones, stilbene, tannins, etc. In which the main compounds having anti-oxidant activity are 2,3,5,4'-tetrahydroxystilben-2-O-β-D-glucoside (THSG), physcion, emodin, chrysophanol,[2-3]. However, the problem of liver damage caused using products from *F. multiflora* has also been reported world-wide[4-8]. The components and mechanisms of hepato-toxicity are still unclear, ambiguous, and controversial. The stilbene glucoside and anthraquinone compounds have been confirmed as one of the components that are toxic to hepatocytes[9-11]. Processing can significantly reduce the toxicity of *F. multiflora* raw form, so the processed products from *F. multiflora* are considered relatively safe.

In processing, the content of active compounds is one of important parameters to optimize the efficiency of processing as well as to evaluate the quality of processed products. Among active compounds extracted from medicinal plants in general and *F. multiflora* root in particular, polyphenols have been known as a key compound for optimizing extraction process[12-13]. Poly-phenols could be extracted from medicinal plants by immersion heater (or traditional boiler), Soxhlet, ultra-sonic assisted extraction, microwave extraction, super-critical liquid extraction, and enzyme assisted extraction, and so on[14-15]. The extraction efficiency of these processes varies widely depending on the technological parameters, surfactants and used solvents.

In optimization of extraction process, many modern experimental planning methods have been mentioned, in which response surface method (RSM) with the support of data processing software has become a useful tool to help experts conducting research on multi-factor optimization processes, to save time and costs[12-13,16-17]. Experimental planning according to the Box-Behnken model helps to estimate the parameters of the quadratic model, build sequential designs, detect model mismatches, predictive values in the experimentally verified model, therefore, it has many advantages over the traditional one-by-one approach, which is relatively expensive and time consuming[16,18].

Although polyphenols extraction from *F. multiflora* roots have been reported previously[19-20], the optimiza-tion of polyphenol extraction process from *F. multiflora* roots in raw form or processed form by RSM based on factors such as the ratio of solvent and medicinal herbs, extraction temperature, extraction time has been still limited in research. Therefore, the purpose of this study is to optimize the polyphenols extraction conditions from *F. multiflora* roots in both raw and processed products. From that, a comparison of investigated parameters between *F. multiflora* root raw and processed products could be given. Moreover, the effect of factors on the anti-oxidation activity of polyphenols extraction has been also reported and discussed.

2. MATERIALS AND METHODS

2.1. Raw materials

The roots of *F. multiflora* were collected from Ky Son district, Nghe An province (Vietnam) by Muong Long Herbals Joint Stock Company (Ky Son district, Nghe An province, Vietnam). The company is allowed to plant and harvest the roots of *F. multiflora*. The planting area belonged to Pu Hoat Nature Reserve which was recognized by UNESCO in 2011. The *F. multiflora* roots were washed, sliced to a thickness of 0.5-1 mm, and dried in an oven at 60°C. After that, the raw materials were processed by soaking in rice swill for 24 hours and stewing with black beans (ratio of *F. multiflora* root: black bean is 1:1) in distilled water for 12 hours. The mixture of black bean decoction obtained after stewing is evenly impregnated on medicinal herbs and the stewed *F. multiflora* roots were then dried at a temperature of 40°C. This process was repeated until the black bean decoction is gone. This stage was carried out according to report by Li et al.[11]. Black been decoction was used as an auxiliary material.

The humidity of the processed *F. multiflora* roots was determined on an ADAM AMB 310 automatic meter. The products must meet the requirement of humidity less than 12%. Raw and processed samples were packaged under vacuum and stored at room temperature for further studies.

2.2. Chemicals and reagents

The chemicals and reagents in this study were obtained from Sigma-Aldrich (USA) and Merck (Ger-many) such as gallic acid, Folin-Ciocalteu, 2,2-diphenyl 1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO). Ethanol 95%, water used met the analytical standard.

2.3. Determination of polyphenol content and the antioxidant activity of extracts

The total polyphenol content (TPC) of the *F. multiflora* products was determined by the method reported by Singleton et al.[21-22] with a few minor adjust-ments. The regression equation of gallic acid was defined as $y=14.06x+0.034$ ($R^2=0.9992$). The antioxidant activity of extracts was determined by the DPPH method[23].

2.4. Experimental design to optimize extraction conditions

Extraction of *F. multiflora* roots in 95% ethanol

(EtOH) was carried out by immersion combined with ultrasonic extraction methods. The *F. multiflora* root samples in raw and processed form were soaked in EtOH for 60 minutes at room temperature before ultrasonically extracted. The ratio of solvent and medicinal herbs was changed from 3/1-20/1, extraction temperature was changed from 30-70°C, extraction time was varied from 30-70 minutes. The number of extractions is two times. The extract was filtered and distilled under vacuum to collect dry residue to determine the TPC and the antioxidant activity of the extract.

Based on initial experiments, there are three main factors that greatly affect to the polyphenol extraction process from *F. multiflora* products including to extraction temperature, extraction time and solvent/medicinal herb ratio. Therefore, the influence of these parameters on TPC and its activity was estimated using the response surface method combined with the Box-Behnken design[16,24].

The optimal conditions were selected from 17 experiments, using Design expert 7.0.0 software to obtain the experiment matrix. The objective functions are Y1 (mg GAE/g) - total phenolic content and Y2 (%) - free radical scavenging capacity.

2.5. Data processing

Each experiment was repeated three times. The results are presented in the form of mean±SD. SPSS 20.0 software was used to analyze the obtained data. One-way ANOVA analysis was used to compare means. Duncan test was used. Experimental planning designs were according to Box- Behnken model, using Design expert 7.0.0 software. The free radical scavenging capacity (SC) value was determined using TableCurve AISN software (Jandel Scientific, USA). Drawings are made on Excel 2010 software.

3. RESULTS AND DISCUSSION

3.1. Assessment of factors affecting to the extraction process of *F. multiflora* roots

3.1.1. Effect of solvent/medicinal herb ratio

F. multiflora roots were soaked in EtOH 95% for 60 minutes and then, ultrasonically extracted for 50 minutes at room temperature (two times). The solvent/medicinal herb ratios are 3/1, 5/1, 7/1, 9/1, 11/1 for raw sample and 3/1, 5/1, 10/1, 15/1, 20/1 for processed sample, respectively. The obtained results were shown in Table 1.

From Table 1, the solvent/medicinal herb ratios had a significant influence on the dried mass, TPC and the antioxidant activity of the extracts. The dried mass, TPC and the antioxidant activity of extracts obtained from raw and processed samples have a significant difference (p<0.05). The dried mass of both extracts from raw and processed samples increased as increasing the solvent/

Table 1. The TPC and its activity for raw and processed samples which were extracted at different solvent/medicinal herb ratios.

Sample	Solvent/medicinal herb ratios (ml/g)	Signature	Medicinal herb (g)	Dried mass (%)	TPC (mgGAE/g)	SC (%)
Raw	3/1	RFMT1	2.08	13.54	49.14±0.26	59.21±0.40
	5/1	RFMT2	2.03	14.07	51.02±0.32	59.02±0.90
	7/1	RFMT3	2.09	14.17	47.20±0.27	58.17±0.70
	9/1	RFMT4	2.10	14.32	44.87±0.64	53.72±0.40
	11/1	RFMT5	2.09	14.53	43.72±0.55	52.77±0.40
Processed	3/1	PFMT1	2.01	3.13	47.85±0.46	75.38±0.20
	5/1	PFMT2	2.09	3.93	49.18±0.24	76.04±0.90
	10/1	PFMT3	2.00	5.48	51.82±0.48	78.22±0.50
	15/1	PFMT4	2.01	8.63	46.78±0.67	73.16±0.70
	20/1	PFMT5	2.02	9.05	45.40±0.43	72.01±0.80
				p<0.05	p<0.05	p<0.05

medicinal herb ratio due to the acceleration of mass transfer as well as the increase in the diffusion of components into solvents[25]. In case of raw sample, the TPC gradually increased when increase the solvent/medicinal herb ratio from 3/1 to 5/1 and reached the maximum value at the solvent/medicinal herb ratio of 5/1 (51.02± 0.32 mg GAE/g). This could be explained by a rise in concentration gradient between the solute and the solvent at the surface of the raw material as more solvent was introduced[26]. However, the TPC in the extract from raw sample had a decreased tendency as increasing the solvent/medicinal herb ratio. This may be due to the content of polyphenols in the herb was depleted. Moreover, using high solvent/medicinal herb ratio in extraction process could affect on the purity of the extract because of the coextraction of non-desirable compounds[26]. The same trend was also observed for antioxidant activity of the extract from raw sample. The SC value reached 59.21±0.40% at the solvent/medicinal herb ratio of 5/1. For the processed sample, both TPC and SC of the extract got to the maximum values at the solvent/medicinal herb ratio of 10/1, 51.82±0.48 mg GAE/g and 78.22±0.50 %, respectively.

Although the solvent/medicinal herb ratio depends on the medicinal herbs, solvents, extraction techniques, extracted compounds, etc., the solvent/medicinal herb ratio is the most important factor of extraction process[27]. The aim of experiments was to extract TPC with the highest efficiency and to minimize the cost of the process. Therefore, the low optimal solvent/medicinal herb ratio for extraction polyphenols (higher TPC) from both raw and processed *F. multiflora* roots is an interesting result of this study because the cost for extraction was saved remarkably (the optimum solvent/medicinal herb ratio for the extraction polyphenols from raw material was 5/1 and that from processed product was 10/1). Quoc LPT found the optimal acetone/material ratio of 40/1 for extraction of polyphenols from *F. multiflora* roots (obtained from Cao Bang province, Vietnam). The maximum value of TPC was 33.72±0.44 mgGAE/g dry weight (DW)[20]. In another study, Quoc LPT and Muoi NV reported that the solvent (acetone/water 60/40 v/v)/ material ratio of 30/1 is optimal condition for ultrasound-assisted extraction process of polyphenols from *F. multiflora* roots (obtained from Cao Bang province, Vietnam)[27]. The TPC was 43.28±0.44 mgGAE/g DW[27]. Zhu et al. found the optimal solid-liquid (water) ratio for ultrasonic-assisted extraction of polysaccharides from *F. multiflora* roots was 1/20[28]. Li YX and his co-workers reported that the solvent (ethanol 50%)/herb ratio of 20/1 was suitable for extraction of physcion, chrysophanol, rhein, emodin, acid gallic, catechin, stilbene glycoside, aloe emodin from *F. multiflora* roots by ultrasonic-assisted extraction immersion method[29]. Chen et al. used a solid-liquid ratio of 1/3 and EtOH 95% for extraction *F. multiflora* roots by cold immersion method[2].

Another interesting result of this study was the extracts from processed sample had the antioxidant activity better than that from raw material although the TPC in extracts was not much difference. For examples, at the same solvent/medicinal herb ratio of 5/1, the TPC and SC values of the extract from raw material were 51.02±0.32 mg GAE/g and 59.21±0.40%, respectively while that of the extract from processed sample were 49.18±0.24 mg GAE/g and 76.04±0.90%. This can be due to content of non-desirable compounds in the extract of processed sample was lower than that in the extract of raw sample (lower dried mass). This result recommends that processing is necessary to improve the antioxidant activity of the extract of *F. multiflora* roots.

Table 2. The TPC and its activity for raw and processed samples which were extracted at different extraction temperature.

Sample	Temperature (°C)	Signature	Medicinal herb (g)	Dried mass (%)	TPC (mgGAE/g)	SC (%)
Raw	30	RFMT6	2.02	14.05	51.09±0.18	59.12±0.40
	40	RFMT7	2.00	15.28	52.71±0.74	60.61±0.20
	50	RFMT8	2.02	13.70	36.36±0.43	44.83±0.50
	60	RFMT9	2.10	12.86	33.84±0.62	45.33±0.50
	70	RFMT10	2.06	11.69	32.89±0.28	44.91±0.20
Processed	30	PFMT6	2.02	5.50	51.87±0.97	78.16±0.70
	40	PFMT7	2.04	4.00	57.49±0.76	79.44±0.80
	50	PFMT8	2.01	3.49	59.96±0.32	81.99±0.80
	60	PFMT9	2.08	3.28	44.25±0.70	54.61±0.62
	70	HTOC10	2.03	2.98	40.63±0.65	52.91±0.31
				$p<0.05$	$p<0.05$	$p<0.05$

3.1.2. Effect of extraction temperature

The materials were immersed in EtOH 95% for 60 minutes at room temperature before ultrasonically extracted for 50 minutes (once) with the ratio of solvent/ medicinal herb of 5/1 for raw sample and 10/1 for processed sample. The extraction temperature varied from 30-70°C. The results are presented in Table 2.

When changing the temperature from 30-70°C, the dried mass, the content and activity of polyphenols in *F. multiflora* roots varied significantly and have a significant difference ($p<0.05$). For raw sample, the extraction efficiency increased and peaked at 40°C as increasing the temperature from 30-40°C. The TPC and SC values of the extract obtained from raw sample at 40°C were 52.71±0.74 mgGAE/g and 60.61±0.20%, respectively. If the extraction temperature increased higher than 40°C, the dried mass, the content and antioxidant activity of polyphenols in raw *F. multiflora* root extract were inclined to decrease. The same trend was observed to the processed *F. multiflora* root extract with the TPC and SC values reached maximum value at 50°C, 59.96±0.32 mg GAE/g and 81.99±0.80%, respectively. It can be seen that the solubility and diffusion of active substances were increased at high temperature. However, the too high temperature may cause the lost of solvent, resulting to extracts of undesirable impurities and the degradation of thermolabile components[15]. The most suitable extraction temperature in this study was 40°C for raw sample and 50°C for processed sample, similar to other reports[20,29].

3.1.3. Effect of extraction time

The materials were immersed in EtOH 95% for 60 minutes at room temperature before ultrasonically extracted for 50 minutes at 40°C for raw sample and at 50°C for processed sample. The ratio of solvent/medicinal herb of raw sample and processed sample was 5/1 and 10/1, respectively. The extraction time varied from 30-70 minutes. The obtained results are shown in Table 3. The extraction time had a remarkable effect on extraction efficiency, TPC and antioxidant activity of the extracts. The suitable extraction time for the extraction of polyphenols from raw sample and processed sample was 40 minutes and 50 minutes, respectively. At this condition, the dried mass, TPC and SC values of raw sample extract reached 14.41%, 58.27±0.64 mgGAE/g, 65.39±0.20%, respectively while the dried mass, TPC and SC values of raw sample extract reached 3.50%, 59.98±0.77 mgGAE/g, 82.16±0.80%, respectively. The increase of time affects negligibly to the extraction due to the solute of active compounds outside and inside the solid herb reached equilibrium[15]. Moreover, the long time for extraction also cause to the decomposition of compounds.

Table 3. The TPC and its activity for raw and processed samples which were extracted at different extraction time.

Sample	Time (min)	Signature	Medicinal herb (g)	Dried mass (%)	TPC (mgGAE/g)	SC (%)
Raw	30	RFMT11	2.06	14.25	43.81±0.58	51.31±0.60
	40	RFMT12	2.09	14.41	58.27±0.64	65.39±0.20
	50	RFMT13	2.00	15.29	52.75±0.92	60.54±0.20
	60	RFMT14	2.09	15.47	42.87±0.67	50.39±0.50
	70	RFMT15	2.03	15.61	37.97±0.85	50.13±0.60
Processed	30	PFMT11	2.04	2.16	46.32±0.29	63.62±0.30
	40	PFMT12	2.06	2.19	49.00±0.31	65.62±0.30
	50	PFMT13	2.02	3.50	59.98±0.77	82.16±0.80
	60	PFMT14	2.05	3.93	56.55±0.96	79.27±0.40
	70	PFMT15	2.01	4.05	46.86±0.72	64.12±0.30
				$p<0.05$	$p<0.05$	$p<0.05$

3.2. Optimization of polyphenol extraction process of raw and processed *F. multiflora* samples

3.2.1. Setting up the model and building the experimental design matrix

Owing to experimental data on the influence of univariate technological parameters on the objective functions including to TPC (Y1) (mgGAE/g) and SC (Y2) (%), we choose a quadratic model and design experimental according to the Box-Behnken model. The base (0), low (-1) and high (+1) levels of the factors (k=3) and variation range are shown in Table 4. The number of experiments at the center is 5.

In this work, based on the results in 3.1 subsection, the independent variables which were chosen for optimization by response surface methodology (RSM) were extraction temperature (code variable A), solvent/medicinal herb ratio (code variable B) and extraction time (code variable C). For raw sample, the extraction time was changed from 30 to 50°C, the solvent/medicinal herbal ratio was varied from 3 to 7 mL/g and the extraction time was set from 30 to 50 minutes. For the processed sample, the extraction time, the solvent/medicinal herbal ratio and the extraction time were varied from 40 to 60°C, 5 to 15 mL/g and 40 to 60 minutes, respectively.

The Design expert 7.0.0 software was used to build the experimental design matrix with 17 experiments. Box-Behnken model was set for objective functions: Y1 (mg GAE/g) corresponding to TPC and Y2 (%) corresponding to free radical scavenging capacity, respectively. The experimental results are given in Tables 1S and 2S.

Table 4. Experimental level of technological variation.

Sample	Real variable	Code variable	Variation range (Δ)	Level		
				-1	0	1
Raw	Z1: Extraction temperature (°C)	A	10	30	40	50
	Z2: Solvent/medicinal herb ratio (mL/g)	B	2	3	5	7
	Z3: Extraction time (min)	C	10	30	40	50
Processed	Z1: Extraction temperature (°C)	A	10	40	50	60
	Z2: Solvent/medicinal herb ratio (mL/g)	B	5	5	10	15
	Z3: Extraction time (min)	C	10	40	50	60

3.2.2. Checking model validity

The model validation and the coefficients were tested by analysis of variance (ANOVA) and are listed in Tables 3S and 4S. The analysis results of the model in Tables 3S and 4S showed that this model was completely compatible with the experiment results, which was proven with the F (Fisher) standards of the model valid for the functions Y1 (51.35); Y2 (16.62) and Y1 (85.67); Y2 (21.42) for raw and processed samples, respectively. These variances have a statistical significance with high reliability ($p<0.05$).

The suitability of the experimental model is also verified by the coefficient of determination (R^2). The closer the R^2 value is to 1, the closer the experimental value is to the model's predicted value. The R^2 values according to the data in Table 3S of the two objective functions Y1 and Y2 of raw sample were 0.9851 (98.51%) and 0.9553 (95.53%), respectively. Besides, the additional determination coefficients (Adj-R^2) values of Y1 and Y2 were 0.9659 (96.59%) and 0.8978 (89.78%), respectively and Adeq Precision values of Y1 and Y2 were 21.868 and 14.268, respectively. For processed sample, the data in Table 4S showed R^2 value of 0.9910 for Y1 and R^2 value of 0.9650 for Y2; Adj-R^2 value of 0.9794 for Y1 and Adj-R^2 value of 0.9199 for Y2; Adeq Precision value of 25.575 for Y1, Adeq Precision value of 14.530 for Y2; lack of fit of both Y1 and Y2 has $p>0.05$).

According to Guan and Yao[30] and Zabeti et al.[31], the model has a high compatibility with the experiment data when both R^2 and Adj-R^2 values are greater than 0.8 and the Adeq Precision index is greater than 4. In case combined with "Lack of fit" of both objective functions Y1 and Y2 ($p>0.05$ -the lack of fit is not significant), it can be confirmed that the built model is highly compatible with experiment.

After removing the non-significant variables ($p>0.05$), the objective functions Y1, Y2 of the model was also determined and represented by a quadratic regression equation owing to the relative importance of the main effects and their interactions with $p<0.05$.

For raw sample:

$$\mathbf{Y1} = 59.14 + 8.40A + 5.72B + 2.03C - 2.45AB - 7.59A^2 - 3.93B^2 - 4.29C^2 \quad (1)$$

$$\mathbf{Y2} = 63.96 + 2.54A + 1.61B + 2.62C - 5.16A^2 - 6.15B^2 + 2.06C^2 \quad (2)$$

For processed sample:

$$\mathbf{Y1} = 59.16 + 4.73A + 2.15B + 1.46C - 5.7A^2 - 3.98B^2 - 7.45C^2 \quad (3)$$

$$\mathbf{Y2} = 80.87 + 1.43A + 1.46B + 1.72C - 3.55BC - 3.27A^2 - 4.23B^2 - 1.94C^2 \quad (4)$$

The influence of linear effects (A, B, C) on the objective function values is the largest, followed by the influence of double interaction effects (AB, AC, BC) and the least influence on the objective function values are the squared effects (A^2, B^2, C^2).

From regression equation (1), it can be seen the influence of technological factors on the objective function Y1 (TPC). All three factors (A, B and C) had a great influence and show a positive interaction effect on the function Y1. In which, the influence of technological factors was arranged in the order A>B>C corresponding to their coefficients in the regression equation (1). The squared effects (A^2, B^2, C^2) exhibit a negative interaction influence on the objective function Y1 corresponding to their coefficients in the regression equation (1).

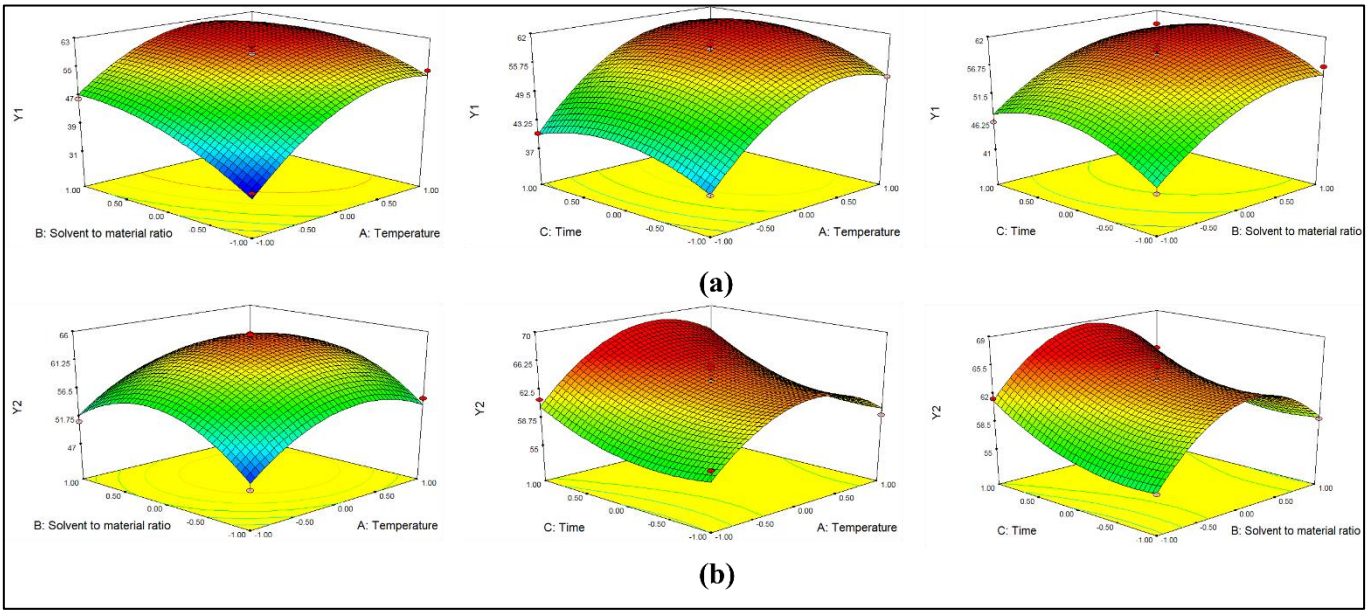

Figure 1. The response surfaces of TPC (a) (raw sample); SC (b) (raw sample) and TPC (c) (processed sample); SC (d) (processed sample). Y1: TPC; Y2: SC.

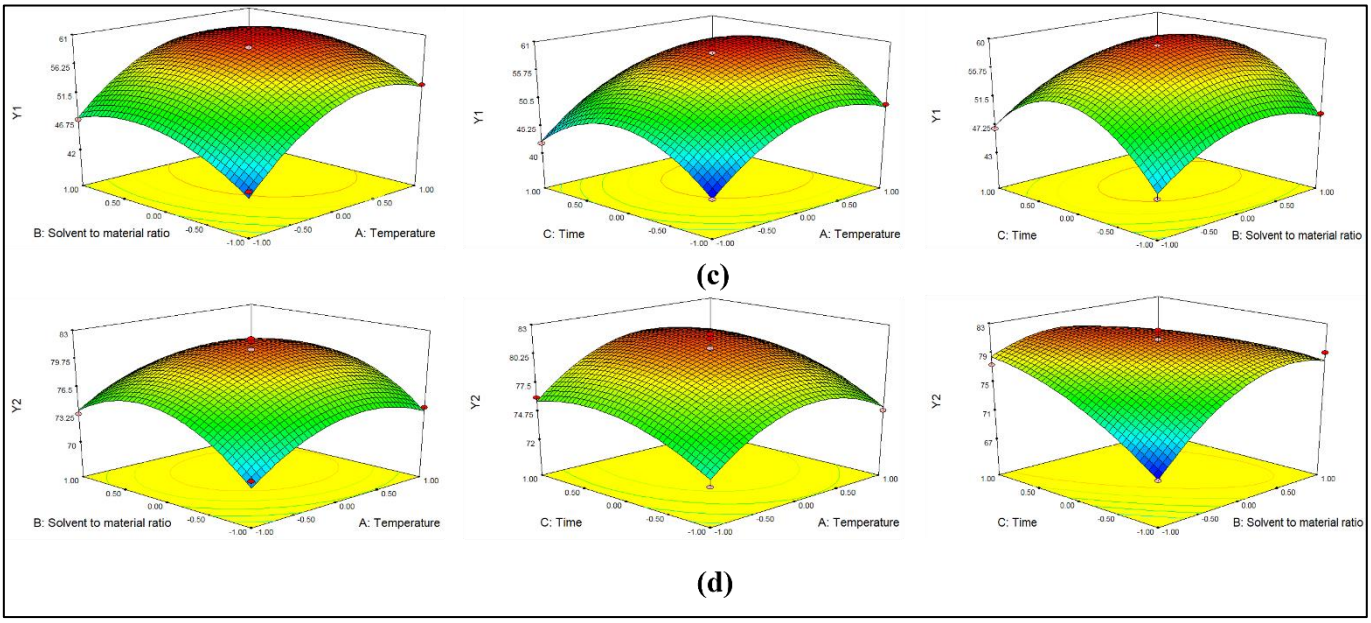

Figure 1. The response surfaces of TPC (a) (raw sample); SC (b) (raw sample) and TPC (c) (processed sample); SC (d) (processed sample). Y1: TPC; Y2: SC.

Similar to the regression equation (1), the regression equation (2) shows the influence of all three technological factors (A, B and C) on the value of the objective function Y2. Linear effects had a positive interaction influence on the function Y2 (C>A>B) while the squared effects (A^2, B^2) had a negative interaction influence and C^2 had a positive interaction influence.

As seen from Equation (3) and Equation (4), A, B, C exhibited positive interaction effects while A^2, B^2, C^2 had negative interaction effects on both objective functions Y1 and Y2.

The influence of the technological factor pairs expressed in the double interaction effect on the target functions was shown through the response surfaces of TPC (Y1) and SC (Y2) in Figures 1. Where, the dark red region is the optimal region on response surfaces. At this region, the objective function values Y1 and Y2 are in the maximum value region. The regions in yellow, green, and blue color represent the objective function values become gradually small.

3.2.3. Optimizing extraction process

The extraction process of raw and processed *F. multiflora* roots needs to be optimized so that both the objective functions Y1 and Y2 have the maximum value. This is solved by resolution of the optimization problem using Design expert 7.0.0 software according to the aspiration method with the priority levels (from 1 to 5). In this problem, with the set objectives, we choose the priority for the objective functions as follows:

+ TPC Y1 (level 3)

+ SC Y2 (level 5)

Optimal results using Design expert 7.0.0 software give one best solution corresponding to one set of optimal technology data. The optimal results are presented in Table 5 and Figure 2. In terms of technological parameters, the predicted values of the objective functions Y1 and Y2 of raw sample are 61.12 (mg GAE/g) and 67.29%, respectively while the predicted values of the objective functions Y1 and Y2 of processed sample are 60.26 mg GAE/g and 81.30%, respectively.

3.2.4. Rechecking the optimization model

The experiment was repeated three times with a set of technological parameters at optimal conditions for raw sample including extraction temperature of 42.4°C, solvent/medicinal herb ratio of 5.7/1 mL/g and extraction time of 47.6 minutes. The experimental results are shown in Table 5. The results of Table 5 show that the experimental results at the optimal conditions are close to the predicted values of the objective function. This once again confirms that the optimal model is compatible with the experiment.

For processed sample, the experiment was set at optimal conditions including extraction temperature of 53.0°C, solvent/medicinal herb ratio of 11.2/1 mL/g and extraction time of 52.0 minutes. The results in Table 5 show that the experimental results at the optimal conditions are close to the predicted values of the objective function.

At the optimal conditions as listed in Table 5, the TPC and SC values of raw sample extract were 60.41± 0.14 mgGAE/g and 65.98±0.22%, respectively, lower than those of processed sample extract (TPC = 59.91± 0.17 mgGAE/g and SC = 80.18±0.21%). The TPC in both raw and processed samples when extracted at the optimal conditions (corresponding to each sample) is the same and close to the theory values calculated from

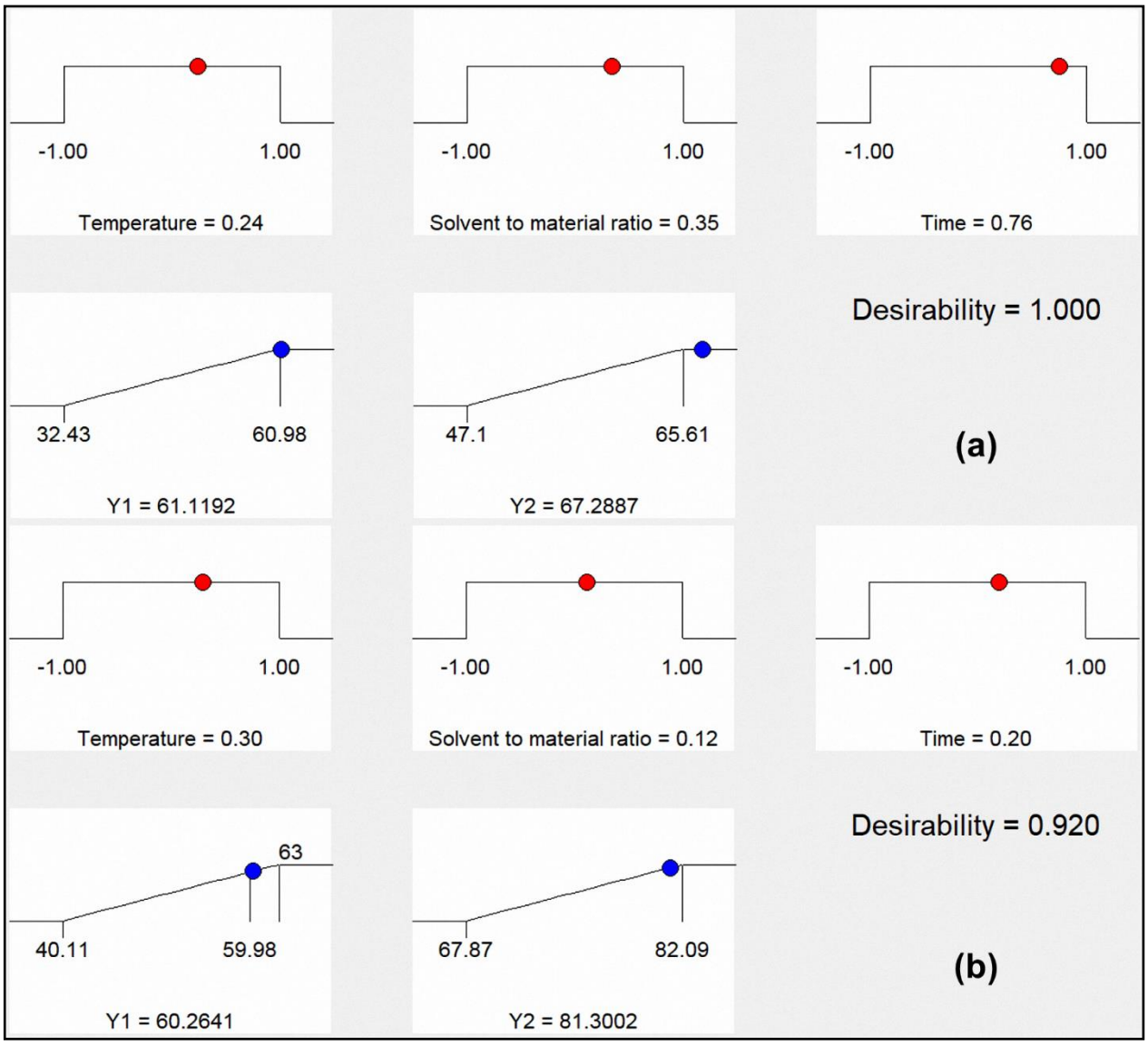

Figure 2. Optimal conditions and values of objective functions (the red dots are the optimal point of investigated variations, and the blue dots are the maximum values of objective functions): (a) raw sample and (b) processed sample. Y1: TPC; Y2: SC.

Table 5. Experiment results of objective functions at the optimal conditions.

Sample	Technological parameters			Objective function	Result	
	Extraction temperature (°C)	Solvent/medicinal herb ratio (mL/g)	Extraction time (min)		Experimental	Theory
Raw	42.4	5.7/1	47.6	Y1	60.41±0.14	61.12
	(A= 0.24)	(B= 0.35)	(C= 0.76)	Y2	65.98±0.22	67.29
Processed	53.0	11.2	52.0	Y1	59.91±0.17	60.26
	(A = 0.3)	(B = 0.12)	(C = 0.2)	Y2	80.18±0.21	81.30

Table 6. Extraction parameters.

No.	Method/size of material/technological parameter	Device/optimal condition	
		Raw FMTH sample	Processed FMTH sample
1	Immersion, ultrasonically assisted extraction	ES-600N device, power of 600W	ES-600N device, power of 600W
2	Size of FMTH root slice (mm)	0.5-1	0.5-1
3	Extraction solvent	Ethanol 95%	Ethanol 95%
4	Extraction temperature (°C)	42.4	53.0
5	The number of extractions (time)	2	2
6	Solvent/medicinal herb ratio (mL/g)	5.7	11.2
7	Extraction time (min)	47.6	52.0

optimization process. This indicated that after processing, the TPC was remained with a high content. The antioxidant activity of the processed sample differs significantly to that of the raw sample; however, these values are close to the theory values that calculated from optimization process. The antioxidant activity of the processed sample is 1.2 time higher than that of the raw sample. This result once again confirmed that the processing is necessary to enhance the antioxidant activity of *F. multiflora* roots. This is complied with the report of Xinyan et al.[32].

Table 6 was summary of parameters and optimal results for extracting polyphenols from *F. multiflora* roots in raw and processed forms. De Brito Maia Miamoto et al. optimized the extraction of polyphenols and antioxidant capacities from two types of *Solanum gilo* Raddi using RSM[33]. The authors found the optimal time of sonication was 30 minutes with the concentration of ethanol was 65-70%. At the optimal conditions, the concentration of sample at which the percent inhibition reaches 50% by DPPH method was 0.1 mg/mL and the TPC in the extract was 6.83 mg GAE/g dry fruit. Nida Anis and Dildar Ahmed also optimized the polyphenol and antioxidant extraction from *Rumex hastatus* by green glycerol-water solvent according to RSM[13]. The independent variables were chosen including glycerol concentration, extraction temperature and extraction time. By RSM, the authors found the predicted optimum conditions were 52.155% glycerol concentration, 50°C extraction temperature and 41.303 minutes extraction time with the predicted values for TPC and anti-radical activity as 21.65 mg GA/g DW and 84.93%. Olfa et al. also selected the extraction temperature, extraction time, solvent composition, and solid to liquid ratio for optimization of polyphenol extraction from *Schinus molle* L. peel using RSM[34]. These reports show a similarity in the selection of independent variables for optimization using RSM in this study. A very good agreement was observed between experimental and predicted values of TPC and DPPH radical scavenging activity. The processed sample has better radical scavenging activity thanks to soaking the *F. multiflora* roots with rice swill and stewing them with black been decoction. The processing contributed to the enrichment of multiple phenolic compounds which enhance the antioxidant activity, and other biological activities in general[11,35]. These results in this work can support the use of the model to quantitatively describe the extraction of polyphenols from red *Fallopia multiflora* Thunb. Haraldson roots.

4. CONCLUSION

In this study, the response surface method (RSM) combined with the Box-Behnken design has been applied to optimize the extraction process of polyphenols from the roots of *Fallopia multiflora* Thunb. Haraldson by immersion combined ultrasonication method with three experimental parameters including ratio solvent/ medicinal herb (mL/g), extraction temperature (°C) and extraction time (min). The *F. multiflora* roots consist of two kinds of sample, the raw and the processed sample (raw *F. multiflora* roots were processed with soaking in rice water and stewing with black beans). At the optimal extraction conditions (extraction time of 47.6 minutes, solvent/medicinal herb ratio of 5.7 mL/g and extraction temperature of 42.4°C), the extract of raw *F. multiflora* roots has the TPC and SC values of 60.41±0.14 mgGAE/g and 65.98±0.22%, respectively. At the extraction time of 52.0 minutes, solvent/medicinal herb ratio of 11.2 mL/g and extraction temperature of 53.0°C, the extract of raw *F. multiflora* roots has the TPC and SC values of 59.91±0.17 mgGAE/g and 80.18± 0.21%, respectively. The processed sample exhibits greater antioxidant activity than raw sample. This study contributes to the development of optimal conditions for extracting polyphenol compounds from the *F. multiflora* roots, getting high efficiency for industrial use, providing basic information for further research on *F. multiflora* roots in the future.

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