OPTIMIZATION OF EXTRACTION OF TOTAL PHENOLIC AND TOTAL FLAVONOID FROM SEEDS OF *Alpinia blepharocalyx* K. SCHUM. USING THE RESPONSE SURFACE METHODOLOGY

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Received: 24 September 2019; Accepted for publication: 2 November 2019

Abstract. Seeds of *Alpinia blepharocalyx* contain high amounts of phenolic and flavonoid compounds with potential antioxidant properties. In this study, the effects of the extraction method on the recovery of phenolic and flavonoid compounds from *Alpinia blepharocalyx* seeds were investigated. Response surface methodology (RSM) has been used to optimize the extraction conditions of total phenolic and total flavonoid from seeds of *Alpinia blepharocalyx*. A Box-Behnken design was used to investigate the effects of four independent variables, namely extraction temperature (°C), extraction time (min), solvent/material ratio (ml/g) and ethanol concentration (%) on the responses: total phenolic content (TPC) and yield. The optimal conditions obtained from response RSM were 52.66 % v/v for the solvent composition, 62.34 °C for extraction temperature, 34.48/1 ml/g for solvent/material ratio and 125.42 min for extraction time. The experimental values of TPC, TFC and yield were 39.31 ± 0.05 mg GAE/g, 12.75 ± 0.07 mg CE/g and 6.97 ± 0.05 %, respectively.

Keywords: *Alpinia blepharocalyx*, total phenolic content (TPC), total flavonoid content (TFC), response surface methodology (RSM), extraction.

Classification numbers: 1.1.3, 1.3.1, 1.4.4

1. INTRODUCTION

Phenolic and flavonoid compounds derived from vegetables and plants have gained attention due to their capability to prevent age-related diseases [1]. These compounds reduce the oxidative stress by the scavenging of free radicals. The genus *Alpinia* is an important member of the Zingiberaceae family. It includes ca. 230 species [2]. Most of them are distributed in tropical and subtropical Asia, including India, Vietnam, Malaysia, China, and Japan. To date, *Alpinia* plants are also broadly used as traditional medicines in India, China, and Japan to treat many diseases such as indigestion, gastralgia, vomiting, enterozoa, etc.[3, 4]. *A. blepharocalyx* K.
Schum. is a natural dye [5]. The genus *Alpinia* contains many groups of substances with biological activity such as: terpenoids, diarylheptanoids, phenylpropanoids, flavanones, phenolics, steroids, alkaloids, etc.[6].

Response surface methodology (RSM) is an effective statistical method for optimizing experimental conditions and investigation of critical processes as well as reducing the number of experimental trials. RSM helps to define effects of the independent variables, whether it is alone or combination in the process [7, 8]. One of the most important points in the implementation of this method is that the predicted values in the model should be verified experimentally. Thus, RSM is a useful tool for optimizing the technology process over the conventional one-factor-at-a-time approach, which is relatively expensive and time-consuming. In this study, we have optimized the extraction conditions of total phenolic and total flavonoid from seeds of *Alpinia blepharocalyx* because these are two compounds found very much in genus *Alpinia* (flavonoid account for 13%, phenolic account for 12% of total compounds in genus *Alpinia*).

2. MATERIAL AND METHODS

2.1. Material

Seeds of *Alpinia blepharocalyx* were collected in Ky Son District of Nghean Province, Vietnam in September 2018 and identified by Prof. Dr. Ngo Anh from the Department of Biology, Hue University. A voucher specimen was deposited at the herbarium of the School of Chemistry, Biology and Environment, Vinh University. The material was dried, crushed and stored at -20°C for further experiments.

2.2. Methods

2.2.1. Total Phenolic Content (TPC)

The TPC of the *Alpinia blepharocalyx* seeds extracts was measured according to the method reported by Singleton *et al.* [9] with a little modification. This method is based on measuring color change caused by reagent by phenolates in the presence of sodium carbonate. 1 ml of 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 a mixture and adjusted to 10 ml with deionized water. The mixture was kept at room temperature in a dark environment for 60 min. The color change was determined by scanning the wavelength at 765 nm (Agilent 8453 UV – Visible Spectrophotometer) since maximum absorbance was obtained. TPC of the *Alpinia blepharocalyx* seeds extract was determined as mg gallic acid equivalent using the standard curve prepared at different concentrations of gallic acid and reported as mg GAE/g dry weight (DW).

2.2.2. Total Flavonoid Content (TFC)

The TFC of the *Alpinia blepharocalyx* seed extracts was estimated according to the procedures described by D. Marinova *et al.* [10] with slight modification. An aliquot (1 ml) of extracts or standard solution of catechin (0.01 ÷ 0.07 mg/ml) was added to 10 volumetric flask containing 4 ml of H2O. To the flask was added 0.3 ml 5% NaNO2. After 5 min, 0.3 ml 10% AlCl3 was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with H2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm (Agilent 8453 UV-Visible Spectrophotometer). Total
flavonoid content of *Alpinia blepharocalyx* seeds extract was expressed as mg catechin equivalents mg CE/g DW.

2.2.3. Determination of extraction yield

The method was described by Tian *et al.* [11] in which the yield of the *Alpinia blepharocalyx* seeds extract can be calculated as the following equation:

\[
\text{% yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of powder (g)}} \times 100.
\]

2.2.4. Experimental design

Before the development of the study by RSM, determination of experimental ranges for independent variables namely extraction time, extraction temperature, solvent/material ratio and ethanol concentration were carried out using total phenolic content as a determinant factor. Then, RSM was used to determine the optimum levels of extraction time (min), temperature (°C), solvent/material ratio (ml/g) and using other ethanol concentration (%) as extraction medium on three responses namely, TPC, TFC and Yield in the *Alpinia blepharocalyx* seeds extracts. These four factors, namely extraction temperature (X₁), extraction time (X₂), solvent/material ratio (X₃) and ethanol concentration (X₄) were coded into three levels (-1, 0, +1). The coded independent variables used in the RSM design are shown in Table 1. Ranges of extraction temperature, extraction time, solvent/material ratio and ethanol concentration and the central point were selected based on preliminary experimental results. Statistical analysis on the means of triplicate experiments was carried out using the ANOVA procedure of the design expert software, version 7.0.

3. RESULTS AND DISCUSSION

3.1. Fitting the models

*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          | X₁            | 50  | 60 | 70  |
| Extraction time       | min         | X₂            | 100 | 120| 140 |
| Solvent/material ratio| ml/g        | X₃            | 30/1| 35/1| 40/1|
| Ethanol concentration  | %           | X₄            | 30  | 50 | 70  |

The responses consisting of TPC, TFC and yield for *Alpinia blepharocalyx* seeds extract by using ultrasonic extraction were optimized based on the Box-Behnken. The Box-Behnken with four independent variables was used as follows: extraction temperature, extraction time, solvent/material ratio and ethanol concentration. This design consisted of 27 experimental points with three replicates at the central point. The input range of the selected variables was determined by the preliminary experiments (Table 1). Three dependent variables including total phenolic compounds, total flavonoid compounds and yield were determined following extraction under optimal conditions. These experimental values were compared with those of the predicted values to check the validity of the model.
The effects of the extraction parameters were evaluated using the program Design-Expert®, version 7.0.0. The response variable was fitted to be a second-order polynomial model as follows:

\[ y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j \]

where \( Y \) is the predicted response; \( \beta_0 \) is the intercept coefficient; \( \beta_i \) is the linear coefficient; \( \beta_{ii} \) is the squared coefficient; \( \beta_{ij} \) is the interaction coefficient; \( X_i \) and \( X_j \) are the coded independent variables; term of \( X_i X_j \) and \( X_i^2 \) are the interaction and quadratic terms, respectively.

| Table 2. Experimental design and response values. |
|-----------------------------------------------|
| **RUN** | **\( X_1 \) (°C)** | **\( X_2 \) (min)** | **\( X_3 \) (ml/g)** | **\( X_4 \) (%)** | **TPC \( Y_1 \) (mgGAE/g)** | **TFC \( Y_2 \) (mgCE/g)** | **Yield \( Y_3 \) (%)** |
|--------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1      | 60(0)           | 120(0)          | 40/1(+1)        | 30(-1)          | 29.12           | 6.12            | 7.09            |
| 2      | 70(+1)          | 100(-1)         | 35/1(0)         | 50(0)           | 32.71           | 8.35            | 6.07            |
| 3      | 60(0)           | 140(+1)         | 30/1(-1)        | 50(0)           | 38.52           | 9.48            | 4.84            |
| 4      | 50(-1)          | 120(0)          | 30/1(-1)        | 50(0)           | 31.33           | 6.81            | 4.35            |
| 5      | 50(-1)          | 120(0)          | 35/1(0)         | 70(+1)          | 31.18           | 7.87            | 5.17            |
| 6      | 60(0)           | 120(0)          | 40/1(+1)        | 70(+1)          | 34.98           | 9.84            | 5.05            |
| 7      | 60(0)           | 140(+1)         | 35/1(0)         | 30(-1)          | 35.11           | 9.09            | 6.38            |
| 8      | 60(0)           | 120(0)          | 35/1(0)         | 50(0)           | 38.28           | 11.89           | 6.78            |
| 9      | 70(+1)          | 120(0)          | 40/1(+1)        | 50(0)           | 30.37           | 7.05            | 6.11            |
| 10     | 50(-1)          | 140(+1)         | 35/1(0)         | 50(0)           | 32.84           | 8.69            | 5.86            |
| 11     | 60(0)           | 140(+1)         | 35/1(0)         | 70(+1)          | 37.31           | 11.76           | 5.97            |
| 12     | 60(0)           | 100(-1)         | 35/1(0)         | 70(+1)          | 35.76           | 9.87            | 4.66            |
| 13     | 60(0)           | 120(0)          | 35/1(0)         | 50(0)           | 39.89           | 12.87           | 6.89            |
| 14     | 70(+1)          | 120(0)          | 35/1(0)         | 70(+1)          | 38.75           | 11.29           | 5.49            |
| 15     | 70(+1)          | 120(0)          | 30/1(-1)        | 50(0)           | 37.66           | 10.96           | 5.42            |
| 16     | 60(0)           | 100(-1)         | 40/1(+1)        | 50(0)           | 29.79           | 6.74            | 5.38            |
| 17     | 60(0)           | 120(0)          | 35/1(0)         | 50(0)           | 37.16           | 12.33           | 7.16            |
| 18     | 70(+1)          | 120(0)          | 35/1(0)         | 30(-1)          | 29.07           | 6.83            | 6.76            |
| 19     | 50(-1)          | 120(0)          | 40/1(+1)        | 50(0)           | 29.29           | 6.25            | 5.89            |
| 20     | 50(-1)          | 100(-1)         | 35/1(0)         | 50(0)           | 27.74           | 6.16            | 4.97            |
| 21     | 50(-1)          | 120(0)          | 35/1(0)         | 30(-1)          | 30.84           | 7.45            | 5.68            |
| 22     | 60(0)           | 120(0)          | 30/1(-1)        | 30(-1)          | 36.87           | 8.69            | 5.21            |
| 23     | 70(+1)          | 140(+1)         | 35/1(0)         | 50(0)           | 34.08           | 10.34           | 6.32            |
| 24     | 60(0)           | 100(-1)         | 35/1(0)         | 30(-1)          | 29.44           | 6.42            | 6.06            |
| 25     | 60(0)           | 120(0)          | 30/1(-1)        | 70(+1)          | 38.13           | 11.35           | 4.72            |
| 26     | 60(0)           | 100(-1)         | 30/1(-1)        | 50(0)           | 32.09           | 6.86            | 5.09            |
| 27     | 60(0)           | 140(+1)         | 40/1(+1)        | 50(0)           | 31.56           | 6.31            | 6.76            |
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The values of the three evaluation indices for each extracting condition were listed in Table 2. At extracting condition: 60 °C, 50 % ethanol and solvent/material ratio is 35/1 ml/g in 120 min, the maximal TPC was 39.89 mg GAE/g and the maximal TFC was 12.87 mg CE/g. Also at these parameters, the maximal Yield was 7.16 %. From the regression analysis of the 27 data entries, empirical second-order polynomial models of TPC, TFC and Yield scavenging capacity were derived (Table 3).

Table 3. Empiric second-order polynomial model of TPC, TFC and Yield.

| Response | Model equations | R² | p-value |
|----------|----------------|----|---------|
| Y₁ – TPC | Y₁ = 38.44 + 1.62X₁ + 1.82X₂ – 2.46X₁ + 2.14X₄ - 0.93X₁X₂ - 1.31X₁X₃ + 2.34X₁X₄ - 1.17X₂X₃ - 1.03X₁X₄ + 1.15X₂X₄ – 4.09X₁⁻² - 2.71X₂⁻² - 2.36X₃⁻² - 1.51X₄⁻² | 0.9784 | <0.0001 |
| Y₂ – TFC | Y₂ = 12.36 + 0.97X₁ + 0.94X₂ – 0.99X₃ + 1.45X₄ - 0.84X₁X₃ + 1.01X₂X₄ - 0.76X₂X₂ + 2.28X₃⁻² - 2.03X₃⁻² - 2.48X₄⁻² - 1.22X₄⁻² | 0.9546 | <0.0001 |
| Y₃ – Yield | Y₃ = 6.94 + 0.35X₁ + 0.32X₂ + 0.55X₃ – 0.51X₄ - 0.16X₁X₂ – 0.21X₁X₃ – 0.19X₁X₄ – 0.41X₂X₃ + 0.25X₂X₄ – 0.39X₃X₄ – 0.60X₁² – 0.56X₂² – 0.87X₃² – 0.58X₄² | 0.9863 | <0.0001 |

ANOVA analysis results for multiple regression and response surface quadratic model of Y₁, Y₂ and Y₃ were evaluated using the corresponding p and R² values (Table 3). F values of Y₁, Y₂ and Y₃ were calculated to be 38.83; 18.02; 61.67, both leading to a p value < 0.05, suggesting both the models were statistically significant. The models’ coefficient of determination (R²) were 0.9784; 0.9546 and 0.9863, indicating that more than 97.84 %; 95.46 % and 98.63 % of the response variability were explained, and supporting a good accuracy and ability of the established model within the range limits used. The F-values of Lack of Fit of Y₁, Y₂ and Y₃ were 0.21; 2.14; 0.40, respectively, implying that the Lack of Fit was not significant relative to the pure error. This indicated that the accuracy of the polynomial model was adequate.

3.2. Response surface analysis

To visualize the interactions of two operational parameters on extraction efficiency, the responses were generated as response surfaces (Figure 1, Figure 2 and Figure 3). Two variables unshown in the Figures were kept constant at their respective central experimental values and the other two variables presented on the two horizontal axis varied within their experimental ranges in order to understand their main and interactive effects on the dependent variables.

The X- and Y- axes of the three-dimensional response surfaces represented two factors, for extraction temperature and extraction time (ethanol concentration 50 %, solvent/material ratio 35/1 ml/g), extraction temperature and solvent/material ratio (extraction time 120 min, ethanol concentration 60 %), extraction temperature and ethanol concentration (extraction time 120 min, solvent/material ratio 35/1 ml/g), extraction time and solvent/material ratio (extraction temperature 60 °C, ethanol concentration 60 %), extraction time and ethanol concentration (extraction temperature 60 °C, solvent/material ratio 35/1 ml/g), ethanol concentration and solvent/material ratio (extraction temperature 60 °C, extraction time 120 min). The Z-axes represented one of the four evaluation indices (TPC, TFC or Yield). Three dimensional response surfaces were constructed as depicted in Fig. 1, Fig. 2 and Fig. 3.
3.2.1. Response surface analysis of total phenolic content

The response surface plots for total phenolic extraction of *Alpinia blepharocalyx* seeds extract are shown in Fig. 1 demonstrating the effect and interaction of independent variables on the yields of total phenolics. As shown in Fig. 1 and Table 4, all of four factors (extraction temperature, extraction time, ethanol concentration and solvent/material ratio) have showed negative quadratic effects ($p < 0.0001$). It can be observed that the yields of total phenolic content increased with the increase of extraction temperature from $30 \, ^\circ C$ to $62.33 \, ^\circ C$, followed by a decline thereafter. TPC increases linearly with the increases of extraction time from 100 to 125.44 min and then the TPC level falls slightly when extending extraction time from 125.44 to 140 min. Higher solubility and diffusion coefficient of polyphenols were observed with increased temperature, allowing more extraction rate [12]. However, an upper limit of temperature must be respected in order to prevent decomposition of thermo sensitive phenolics during extraction [8]. The yields of TPC increased with the increase of ethanol concentration from 30 % v/v to 52 % v/v, and thereafter decreased. This indicated that low to medium concentrations of ethanol were favorable for extracting phenolic compounds from such plants. This was due to the addition of ethanol in water which improved the breakage degree of cell membranes of plant raw material and improved the solubility of phenolic substances [13, 14]. In the present study, TPC increases linearly with increasing of solvent/material ratio from 40/1 to 30/1 (ml/g). The results of this research are in line with that of Şahin and Şamli [15].
Table 4. Regression coefficients of the predicted second-order polynomial models for the total phenolic content, total flavonoid content, and yield.

| Source | $Y_1$ – TPC | $Y_2$ – TFC | $Y_3$ – Yield |
|--------|-------------|-------------|---------------|
|        | F-value     | p-value     | F-value       | p-value       | F-value       | p-value       |
| Model  | 38.83       | <0.0001***  | 18.02         | <0.0001***    | 61.67         | <0.0001***    |
| $X_1$  | 48.87       | <0.0001***  | 23.82         | 0.0004***     | 78.42         | <0.0001***    |
| $X_2$  | 62.09       | <0.0001***  | 22.53         | 0.0005***     | 66.04         | <0.0001***    |
| $X_3$  | 112.69      | <0.0001***  | 24.86         | 0.0003***     | 192.00        | <0.0001***    |
| $X_4$  | 85.32       | <0.0001***  | 53.57         | <0.0001***    | 162.62        | <0.0001***    |
| $X_1X_2$ | 5.41      | 0.0384*     | 0.16          | 0.7006NS      | 5.34          | 0.0395*      |
| $X_1X_3$ | 10.71     | 0.0067**    | 5.97          | 0.0310*       | 9.41          | 0.0098**     |
| $X_1X_4$ | 33.91     | <0.0001***  | 8.68          | 0.0122*       | 7.52          | 0.0178**     |
| $X_2X_3$ | 4.34      | 0.0132*     | 4.95          | 0.0460*       | 34.61         | <0.0001***   |
| $X_2X_4$ | 6.60      | 0.0246*     | 0.32          | 0.5799NS      | 12.77         | 0.0038**     |
| $X_3X_4$ | 8.23      | 0.0141*     | 0.60          | 0.4544NS      | 31.29         | 0.0001***    |
| $X_1^2$ | 139.07     | <0.0001***  | 59.16         | <0.0001***    | 99.34         | <0.0001***   |
| $X_2^2$ | 60.85      | <0.0001***  | 46.80         | <0.0001***    | 88.44         | <0.0001***   |
| $X_3^2$ | 46.34      | <0.0001***  | 69.91         | <0.0001***    | 210.52        | <0.0001***   |
| $X_4^2$ | 18.85      | 0.0010**    | 16.80         | 0.0015**      | 93.21         | <0.0001***   |
| Lack of Fit | 0.21  | 0.9648NS | 2.14          | 0.3603NS      | 0.40          | 0.8670NS     |

R$^2$ 0.9784 0.9546 0.9863

*p < 0.05; **p < 0.01; ***p < 0.001; NS: non-significant.

3.2.2. Response surface analysis of total flavonoid content

The mean experimental data has showed the total flavonoid content from *Alpinia blehmarocalyx* seeds at various extraction conditions in the total range of 6.12±12.87 mg CE/g of extract. The highest content of total flavonoid was observed at experimental run number 13 meanwhile the lowest yield of total flavonoid was seen in experimental run number 1. The ANOVA analysis showed the model $F$ value of 18.02 with probability (p < 0.0001) which implies that the model is significant and there are only 0.01% chances that this large $F$ value could occur due to noise. Total flavonoid content was significantly influenced (p < 0.05) by all four linear ($X_1, X_2, X_3, X_4$), interaction parameters ($X_1X_3, X_1X_4, X_2X_3, X_1^2, X_2^2, X_3^2, X_4^2$) (Table 4). The effect of their variables on the responses can be seen in Fig. 2.

The 3D in Fig. 2a shows the response surface plot of temperature ($X_1$) and time ($X_2$) at fixed extraction ethanol concentration (50%) and solvent/material ratio (35/1 ml/g). The 3D showed extraction temperature observed a weaker effect while extraction time showed a relatively significant effect on the total flavonoids content. At any level of extraction temperature, an increase in the yield of flavonoid could be significantly achieved with the
increase of extraction time. Thus, the optimum amount of total flavonoid was achieved in this study at 55±65 °C and 120±130 min of extraction time. The results of the present research for time and temperature were similar compared with Choi et al. [16] studies.

Figure 2c shows the interaction between extraction temperature (X1) and ethanol concentration (X4) at the fixed extraction ethanol concentration (120 min) and solvent/material ratio (35/1 ml/g). The 3D plots indicated that extraction temperature and ethanol concentration exerted significant effect on the total flavonoid content. Statistical analysis reveals that the most significant factor with p < 0.0001 in total flavonoid was ethanol concentration. Flavonoids and their glycosides are thought to be efficiently extracted from plant materials by ethanol solvent [17]. It was observed that the value of total flavonoid content increased when ethanol concentration was increased from 30 to 70% at fixed 30 °C extraction temperature. Similarly, increasing the extraction temperature and ethanol concentrations resulted in increased TFC values.

The 3D Fig. 2b, 2d, 2e showed the effect of solvent/material ratio to the total flavonoid content. The yields of total flavonoid content increased when the solvent/material ratio decreased from 40/1 to 30/1 (ml/g). The results of this research are in line with that of Yingngam et al. [18].

![Figure 2c](image)

**Figure 2.** The response surface plot of TFC.

### 3.2.2. Response surface analysis of yield

Figure 3a–f showed three-dimension surface plots describing the relationship between independent and dependent variables for yield from *Alpinia blepharocalyx* seeds extract. Ethanol concentration, extraction temperature, time and solvent/material ratio showed negative quadratic
effects on the yield from *Alpinia blepharocalyx* seeds extract (Table 4, p < 0.05). The presence of an appropriate water content in the extraction solvent also enhanced swelling of plant material, followed by increased extraction yield. Thus, factor of ethanol concentration contributes significant effects on extraction yield. The solvent/material ratio exhibited a weaker effect on the extraction yield. In this study, when increasing the solvent/material ratio, extraction yield was improved. However, excessive addition of solvent could result in larger cost due to increased energy consumption for solvent removal.

![Figure 3. Response surface plot of yield.](image)

### 3.3. Optimization and model verification

The optimal values of the independent variables were obtained by solving second-order regression equations using a numerical optimization method. Experimental data suggested that the highest total phenolic content, total flavonoid content and yield occurred with solvent/material ratio 34.48 ml/g, 52.66 % v/v ethanol for 125.42 min at 62.34 °C. Table 5 shows the predicted and experimental values for the extraction of target compounds from *Alpinia blepharocalyx* seeds. The actual values obtained from the experimental gave the extraction yields of total phenolic, total flavonoid and yield as 39.31 ± 0.05 mgGAE/g, 12.75 ± 0.07 mgCE/g and 6.79 ± 0.05 %. These experimental values were close to the predicted values (TPC = 39.4 mgGAE/g, TFC = 12.87 mgCE/g and Yield = 6.8875 %) derived from the respective regression models with the CV ranging from 0.23 % to 1.13 %.
Table 5. Optimum conditions, predicted and experimental values of responses on extraction of *Alpinia blepharocalyx* seeds extract.

| Independent variables | Dependent variables (Response) | Experimentalb | Predicted | % Difference (CV) |
|-----------------------|---------------------------------|---------------|-----------|------------------|
| X₁       | X₂       | X₃       | X₄       | Y₁       | 39.31 ± 0.05 | 39.4042 | 0.23 |
| 62.34    | 125.42   | 34.48/1  | 52.66    | Y₂       | 12.75 ± 0.07 | 12.8701 | 0.93 |
|          |          |          |          | Y₃       | 6.79 ± 0.05  | 6.8875  | 1.13 |

*X₁*, extraction temperature (°C); *X₂*, extraction time (min); *X₃*, solvent/material ratio (ml/g); *X₄*, ethanol concentration (%); *Y₁*, TPC (mg GAE/g); *Y₂*, TFC (mg CE/g); *Y₃*, Yield (%). bMean ± standard deviation (SD) of four determinations (n= 4) from two crude extract replications.

4. CONCLUSION

Response surface methodology was successfully used to optimized the process of extracting *Alpinia blepharocalyx* seeds extract with the optimal parameters: solvent/material ratio 34.48 ml/g, ethanol concentration 52.66 % v/v, extraction temperature 62.34 °C and extraction time 125.42 min. Under the optimum conditions, the experimental values of TPC, TFC and yield were 39.31 ± 0.05 mg GAE/g, 12.75 ± 0.07 mg CE/g and 6.79 ± 0.05 %. This study can be useful in the development of industrial extraction process.

Acknowledgments. The authors gratefully acknowledge grant from the Ministry of Education and Training (MOET), Vietnam (No. B2018-TDV-09).

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