

Optimization of Parameters for the Extraction of Phenolic Antioxidants from Boxberry Tree (*Myrica esculenta*) Bark Using Response Surface Methodology

Do Thi Kieu Trinh, Nguyen Thi Thanh Tinh, Ho Thi Thu Hoa and Nguyen Tien An*

Department of Postharvest Technology, Faculty of Agriculture and Forestry, Dalat University, Da Lat, Vietnam

Pham Ngoc Tuan and Phan Hoang Dai

Department of Agronomy, Faculty of Agriculture and Forestry, Dalat University, Da Lat, Vietnam

* Corresponding author. E-mail: annt@dlu.edu.vn DOI: 10.14416/j.asep.2023.09.001

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Abstract

The boxberry tree (*Myrica esculenta*) bark has been known to have multiple health benefits and is used as a traditional medicine. A critical gap in knowledge exists on a simple but effective method to isolate the bioactive components from the bark. This study aimed to optimize the operating conditions, including temperature, ethanol concentration, and time, for the extraction of phenolic antioxidants from the boxberry bark sample using a response surface methodology. Results showed that the second-order polynomial regression models were statistically significant and sufficient to estimate the responses. Response surface optimization for all responses was successfully carried out to determine the optimum extraction conditions, which were a temperature, an ethanol concentration, and an extraction time of 75.8 °C, 48.3% (v/v), and 117 min, respectively. At these conditions, total phenolic and total flavonoid contents, 3-ethylbenzothiazoline-6-sulphonic acid diammonium salt (ABTS) scavenging capacity, and ferric-reducing antioxidant power were predicted to be 205.9 mg GAE/100 g, 37.8 mg CE/100 g, 271.3 mg AAE/100 g, and 111.4 mg AAE/100 g, respectively. The insignificant difference between the estimated and the experimental values suggested that the predictive models were valid to predict the process outcomes.

Keywords: Antioxidant, Boxberry, *Myrica esculenta*, Phenolic, Response surface methodology

1 Introduction

The boxberry tree (*Myrica esculenta*) is widely distributed in regions that have temperate and subtropical climates, such as India, Nepal, China, and Southeast Asia [1]. The tree has been known to have many health benefits, including anxiolytic [2], anti-helminthic [3], anti-inflammatory and anti-allergic [4], anti-asthmatic [5] and mast cell stabilizing activities [6]. In addition, the fruits, leaves, and bark of the boxberry tree also have free radical scavenging, antioxidant, and antibacterial properties [1]. The boxberry tree bark is commonly used as a traditional medical treatment for conditions including abdominal lump, fever, irregular bowel function, anemia, nausea, cough, and anorexia [1]. Health-promoting properties of the boxberry tree

have been reported to derive from a wide variety of chemical components from different parts of the tree, such as phenolic compounds, steroids, triterpenoids, proanthocyanidins, and some volatile compounds [7]. For example, myricitrin in boxberry tree bark extract has been reported to ameliorate diabetic nephropathy and suppress inflammation [8]. In addition, the ethanolic bark extract of this tree was documented to exhibit antipsychotic activity in rats [9].

Compared with the fruits and leaves, the stem bark of the boxberry tree probably attracted more research on pharmacological and therapeutic effects due to the diversity in phytochemical composition as well as its traditional use as a medicine by the local community. In particular, the boxberry tree stem bark has been reported to possess many health-promoting

and disease-prevention effects [10], which are partly derived from many phenolic components, such as tannins, gallic acid, epigallocatechin 3-*O*-gallate, myricetin and procyanidins [11], [12]. Although medicinal properties, as well as phenolic composition, were intensively studied, a critical gap in knowledge exists on how to effectively isolate antioxidant polyphenols from the raw materials.

Process optimization is highly important for the extraction of bioactive phytochemicals from natural sources because it helps set the right extraction parameters for obtaining optimal output. A one-factor-at-a-time or a full factorial experiment can be employed for process optimization. For example, optimal conditions, including ethanol concentration, extraction time and ethanol to rice ratio, were successfully established for the extraction of antioxidant phenolics and GABA from germinated Sangyod rice by employing the one-factor-at-a-time method [13]. However, this approach typically requires a large number of experimental units, and thus consumes much time and effort [14]. Response surface methodology (RSM) was introduced as a powerful approach to model and optimize a process in which an output is dependent upon several input variables [15]. In addition, the RSM also enables the evaluation of the impact of process-independent variables at both linear and quadratic levels as well as the interaction between the variables [14]. In order to apply an RSM, an appropriate experiment design, which defines the experimental region to be studied, should be carefully selected. It showed that central composite design was used successfully in conjunction with RSM to optimize the extraction conditions of phenolic substances from various plant materials [16]–[19]. In addition, a face-centered central composite design coupled with response surface methodology was employed to investigate and obtain maximum oil yield from *Gliricidia sepium* seeds by supercritical carbon dioxide extraction, as affected by extraction temperature, pressure and CO₂ flow rate [20].

Therefore, this study aimed at evaluating the impacts of extraction conditions, namely extraction time, temperature, and ethanol concentration, on the isolation of phenolic antioxidants from the stem bark of the boxberry tree by using RSM, thus establishing optimal conditions. This research will provide a reference for the pharmaceutical industry to further

develop an efficient method to obtain the maximum yield of phenolic antioxidants from boxberry stem bark.

2 Materials and Methods

2.1 Materials

The stem bark of *Myrica esculenta* growing wildy in Da Lat, Vietnam, was harvested from a ~10-year-old tree at around 0.7 m from the ground. The stem bark pieces were then dried at 40 °C until obtaining ~10 % moisture content (wb) and finely ground into powder passing through a 75-mesh sieve. The samples were kept at –20°C for further experiments.

Folin-ciocalteu's phenol reagent and 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich (St. Louis, MO), L(+)-Ascorbic acid was purchased from Merck (Darmstadt, Germany), and gallic acid was provided by HiMedia (Mumbai, India). All other chemicals met analytical standards.

2.2 Extraction procedure

Around 250 mg of samples were suspended in 50 mL of aqueous ethanol at different concentrations and maintained at experimented temperatures controlled by a waterbath. The mixture was vigorously shaken at 10 min intervals. After some periods of time as described in Table 1, the solutions were centrifuged at ~2000 xg for 10 min and the resulting supernatants were kept at –20 °C for further analyses.

2.3 Total phenolic content

The measurement of total phenolic content (TPC) was carried out following the Folin-Ciocalteu method [21]. In brief, 100 µL of the extract was mixed with 100 µL of Folin-Ciocalteu phenol's reagent, 300 µL of 20% sodium carbonate solution and 4.5 mL of water. The reaction was allowed to occur at ambient temperature for 2 h. The absorbance of the reaction solution was then measured at 760 nm and used to calculate TPC. The result was reported in milligrams of gallic acid equivalent per 100 g of boxberry tree bark (mg GAE/100 g).

2.4 Total flavonoid content

Total flavonoid content (TFC) was measured according to a method reported by Adom and Liu [22]. Briefly, 125 μL of the extract was mixed with 37 μL of 5% sodium nitrite and 1.025 mL of water. Then, 75 μL of 10% aluminum chloride was added to the reaction solution after 4–6 min of incubation at room temperature. The mixture was maintained at room temperature for another 5–7 min before 0.25 mL of 4 M sodium hydroxide was added. Finally, the absorbance of the sample at 510 nm was monitored. Flavonoids were reported in milligrams of catechin equivalent per 100 g of boxberry tree bark (mg CE/100 g).

2.5 Antioxidant activities

2.5.1 ABTS scavenging capacity

The ABTS scavenging capacity was estimated using the method of Re *et al.* [23]. Briefly, 36 mg ABTS and 66 mg potassium persulphate were dissolved into 100 mL of water to produce the free radicals. The mixture was incubated in darkness at room temperature for a period of 14 h. The resulting solution was then diluted with water until it reached an absorbance of 0.700 ± 0.005 at 734 nm. Forty microliters of the boxberry tree bark extract were combined with 4 mL of ABTS' diluted solution. The reaction solution was allowed to occur in darkness for 6 min. The absorbance of the resulting solution at 734 nm was then measured and used to calculate the ABTS scavenging capacity of the sample, which was reported in milligrams of ascorbic acid equivalent per 100 g of boxberry tree bark (mg AAE/100 g).

2.5.2 FRAP

The ferric-reducing antioxidant power (FRAP) of the sample was estimated using a method reported by Ho *et al.* [16]. In brief, 0.5 mL of the extract was combined with 2.5 mL of 1% (w/v) potassium ferricyanide and 2.5 mL of phosphate buffer (0.2 M, pH 6.6). The solutions were incubated for 20 min at 50 $^{\circ}\text{C}$ for the reaction to occur. Next, 2.5 mL of 10% trichloroacetic acid was added to the solution to terminate the reaction. Then, the resulting solution was centrifuged at 2000 xg for 10 min and an aliquot

of 2.5 mL of supernatant was mixed with 0.5 mL of 0.1% ferric chloride and 2.5 mL of distilled water. The absorbance of the solution at 700 nm was then monitored and used to calculate FRAP, which was reported in milligrams of ascorbic acid equivalent per 100 g of boxberry tree bark (mg AAE/100 g).

Table 1: Experimental space of the CCD

Extraction Parameters	Levels				
	- α	-1	0	1	+ α
x_1 : Temperature ($^{\circ}\text{C}$)	43.2	50	60	70	76.8
x_2 : Ethanol concentration (%)	16.4	30	50	70	83.6
x_3 : Time (min)	12.7	40	80	120	147.3

2.6 Design of experiment

RSM was used to optimize the operating parameters for the isolation of polyphenols from the boxberry stem bark. A circumscribe central composite design (CCD) was used for the experiment. Extraction conditions, including temperature, ethanol concentration, and time were selected as operating parameters and varied at 5 corresponding levels (Table 1) with the range being determined by preliminary experiments (data not shown). The TPC, TFC, ABTS, and FRAP were selected as responses. The CCD included eight (23) factorial points, six star points that were distanced ± 1.682 away from the central level, and six replicates of central points (Table 2). The extraction parameters were coded, which had a relation with actual values in the following Equation (1):

$$x = (X_i - X_0) / \Delta X \quad (1)$$

where x was the coded value; X_i was the actual value corresponding to the coded value; X_0 was the value at the middle of the actual range; and ΔX was the actual range between the midpoint and upper or lower points.

The regression equation to estimate the responses corresponding to the model was:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

where Y was the responses, namely TPC, TFC, TTC, ABTS, and FRAP; β_0 was the intercept; β_i , β_{ii} , and β_{ij} were the coefficients of linear, quadratic, and interactive effects, respectively; and ε was the random error.

Table 2: The CCD and observed responses

Std Order ^a	Coded Variables			Observed Responses ^b			
	x ₁	x ₂	x ₃	TPC (mg GAE/100 g)	TFC (mg CE/100 g)	ABTS (mg AAE/100 g)	FRAP (mg AAE/100 g)
1	-1	-1	-1	204.9	37.2	233.4	86.2
2	1	-1	-1	199.3	35.6	248.4	101.2
3	-1	1	-1	200.7	38.4	230.0	101.1
4	1	1	-1	195.5	37.6	253.2	104.9
5	-1	-1	1	210.5	38.6	237.9	97.9
6	1	-1	1	206.1	37.1	262.9	102.1
7	-1	1	1	205.8	39.8	237.2	87.2
8	1	1	1	199.9	37.5	268.5	102.6
9	-1.682	0	0	215.8	38.9	237.4	106.6
10	1.682	0	0	198.2	37.4	263.4	110.9
11	0	-1.682	0	200.7	34.6	232.3	88.1
12	0	1.682	0	180.7	36.6	256.2	95.9
13	0	0	-1.682	190.7	35.1	226.9	100.6
14	0	0	1.682	208.9	39.0	242.7	106.3
15	0	0	0	202.8	37.1	250.6	102.0
16	0	0	0	201.0	38.3	238.9	111.4
17	0	0	0	207.1	38.9	254.4	109.7
18	0	0	0	209.9	39.6	247.5	109.3
19	0	0	0	205.5	38.2	261.1	104.7
20	0	0	0	209.6	38.2	258.1	101.3

Note: ^a The experiment was conducted randomly; ^b Means of triplicate determination

2.7 Statistical analysis

The significance of the model, as well as the linear, quadratic, and interactive terms, was estimated by multiple regression analysis. The fit of the model to the experimental data was checked by the lack-of-fit test. The difference between predicted responses and experimental data was determined by One-way ANOVA. A term was considered significant if the corresponding p -value ≤ 0.05 . The optimization study was performed, and the goal was set to maximize the responses. Minitab V.19 (Minitab Inc., State College, PA) was used to perform all analyses.

3 Results and Discussion

3.1 Fitting the model

The TPC, TFC, and antioxidant activities as measured by ABTS scavenging capacity and FRAP of the extracts at different extraction conditions are shown in Table 2. The ANOVA analysis indicated that the models

estimating TPC, TFC, ABTS scavenging capacity, and FRAP were statistically significant with p -values of 0.009, 0.018, 0.011, and 0.030, respectively. Besides, the non-significant p -values for lack-of-fit tests of the TPC, TFC, ABTS scavenging capacity, and FRAP estimation models (0.205, 0.393, 0.627, and 0.288, respectively) confirmed that the models were of good fit to the data. In addition, the coefficient of estimation for the models showed an adequate explanation of the variances of the data obtained from the experiments (Table 3). All these statistics indicated that the models were well-fitted to the experimental data and could be used to predict the TPC and TFC yields of the extraction as well as the antioxidant activity of the extracts. Table 4 shows the coefficients of the predictive models described in Equation (2) as estimated by multiple regression analyses. As only significant terms shown in Table 3 were used to formulate the predictive models, the fitted quadratic models in coded units to estimate the TPC, TFC, ABTS scavenging capacity, and FRAP of the boxberry stem bark are given in the following Equations (3)–(6):

Table 3: ANOVA results for terms contributing to the predictive models

Source	df	Sum of Square	Mean Square	F-value	p-value
<i>TPC (mg GAE/ 100 g)</i>					
Model	9	946.78	105.198	5.08	0.009
Linear	3	592.2	197.401	9.53	0.003
x ₁	1	188.22	188.216	9.09	0.013
x ₂	1	202.1	202.098	9.76	0.011
x ₃	1	201.89	201.888	9.75	0.011
Square	3	353.35	117.783	5.69	0.016
x ₁ ²	1	21.5	21.497	1.04	0.332
x ₂ ²	1	297.25	297.247	14.35	0.004
x ₃ ²	1	25.27	25.272	1.22	0.295
2-Way Interaction	3	1.23	0.411	0.02	0.996
x ₁ x ₂	1	0.15	0.151	0.01	0.934
x ₁ x ₃	1	0.03	0.031	0	0.97
x ₂ x ₃	1	1.05	1.051	0.05	0.826
Error	10	207.15	20.715		
Lack-of-Fit	5	142.28	28.456	2.19	0.204
Pure Error	5	64.87	12.974		
Total	19	1153.93			
R ²		0.8205			
<i>TFC (mg CE/ 100 g)</i>					
Model	9	29.8932	3.32147	4.18	0.018
Linear	3	18.9272	6.30905	7.93	0.005
x ₁	1	5.5712	5.57122	7.01	0.024
x ₂	1	4.8799	4.8799	6.14	0.033
x ₃	1	8.476	8.47603	10.66	0.009
Square	3	10.4011	3.46702	4.36	0.033
x ₁ ²	1	0.1533	0.15326	0.19	0.67
x ₂ ²	1	9.1872	9.18718	11.55	0.007
x ₃ ²	1	1.177	1.177	1.48	0.252
2-Way Interaction	3	0.565	0.18833	0.24	0.869
x ₁ x ₂	1	0	0	0	1
x ₁ x ₃	1	0.245	0.245	0.31	0.591
x ₂ x ₃	1	0.32	0.32	0.4	0.54
Error	10	7.9523	0.79523		
Lack-of-Fit	5	4.484	0.89679	1.29	0.392
Pure Error	5	3.4683	0.69367		
Total	19	37.8455			
R ²		0.7899			

Table 3: ANOVA results for terms contributing to the predictive models (*Continued*)

Source	df	Sum of Square	Mean Square	F-value	p-value
<i>ABTS (mg AAE/ 100 g)</i>					
Model	9	2393.78	265.98	4.8	0.011
Linear	3	1896.65	632.22	11.4	0.001
x ₁	1	1399.05	1399.05	25.24	0.001
x ₂	1	158.29	158.29	2.86	0.122
x ₃	1	339.31	339.31	6.12	0.033
Square	3	428.37	142.79	2.58	0.112
x ₁ ²	1	1.48	1.48	0.03	0.873
x ₂ ²	1	49.51	49.51	0.89	0.367
x ₃ ²	1	388.87	388.87	7.01	0.024
2-Way Interaction	3	68.76	22.92	0.41	0.747
x ₁ x ₂	1	26.28	26.28	0.47	0.507
x ₁ x ₃	1	40.95	40.95	0.74	0.41
x ₂ x ₃	1	1.53	1.53	0.03	0.871
Error	10	554.39	55.44		
Lack-of-Fit	5	235.12	47.02	0.74	0.627
Pure Error	5	319.27	63.85		
Total	19	2948.17			
R ²		0.8120			
<i>FRAP (mg AAE/100 g)</i>					
Model	9	801.09	89.01	3.57	0.03
Linear	3	189	62.999	2.52	0.117
x ₁	1	152.47	152.469	6.11	0.033
x ₂	1	33.9	33.904	1.36	0.271
x ₃	1	2.62	2.624	0.11	0.752
Square	3	508.33	169.445	6.79	0.009
x ₁ ²	1	0.35	0.352	0.01	0.908
x ₂ ²	1	479.09	479.087	19.2	0.001
x ₃ ²	1	42.51	42.514	1.7	0.221
2-Way Interaction	3	103.76	34.587	1.39	0.303
x ₁ x ₂	1	0	0	0	1
x ₁ x ₃	1	0.08	0.08	0	0.956
x ₂ x ₃	1	103.68	103.68	4.15	0.069
Error	10	249.59	24.959		
Lack-of-Fit	5	157.03	31.406	1.7	0.288
Pure Error	5	92.56	18.512		
Total	19	1050.68			
R ²		0.7625			

$$TPC = 205.85 - 3.71x_1 - 3.85x_2 + 3.84x_3 - 4.54x_2^2 \quad (3)$$

$$TFC = 38.36 - 0.64x_1 + 0.6x_2 + 0.79x_3 - 0.8x_2^2 \quad (4)$$

$$ABTS \text{ scavenging capacity} = 251.64 + 10.12x_1 + 4.98x_3 - 5.19x_3^2 \quad (5)$$

$$FRAP = 106.5 + 3.34x_1 - 5.77x_2^2 \quad (6)$$

Where, x_1 , x_2 , and x_3 are the extraction temperature, ethanol concentration, and time, respectively.

Table 4: Regression coefficients for total phenolic content, total flavonoid content, ABTS scavenging capacity, and ferric-reducing antioxidant power

Term	Coefficient			
	TPC (mg GAE/100 g)	TFC (mg CE/100 g)	ABTS (mg AAE/100 g)	FRAP (mg AAE/100 g)
Intercept	205.85	38.355	251.64	106.5
x_1	-3.71	-0.639	10.12	3.34
x_2	-3.85	0.598	3.4	1.58
x_3	3.84	0.788	4.98	0.44
x_1^2	1.22	0.103	0.32	0.16
x_2^2	-4.54	-0.798	-1.85	-5.77
x_3^2	-1.32	-0.286	-5.19	-1.72
$x_1 x_2$	-0.14	0	1.81	0
$x_1 x_3$	0.06	-0.175	2.26	0.1
$x_2 x_3$	-0.36	-0.2	0.44	-3.6

3.2 Effect of extraction conditions on responses

As shown in Table 3, all extraction parameters and the quadratic term of ethanol concentration had a significant effect on the extraction yield of TPC. The quadratic models as visually illustrated by contour plots and 3-dimensional response surfaces showed that the higher TPC yields (>210 mg GAE/100 g) were obtained at the lower extraction temperatures (<55 °C, Figure 1(a), (c), (d), and (f) and ethanol concentrations (<55%, Figure 1(a), (b), (d), and (e) and longer extraction time (>80 min, Figure 1(b), (c), (e), and (f) in comparison with higher temperature and ethanol concentration and shorter time. Similarly, the yield of flavonoid compounds also varied correspondingly with the changes in extraction temperature, ethanol concentration, extraction duration, and the quadratic term of ethanol concentration

(Table 3). As such, higher TFC yields of over 39 mg CE/100 g were obtained at a temperature range of 50–53°C (Figure 2(a), (c), (d), and (f)) in combination with ethanol concentrations of 50–60% (Figure 2(a), (b), (d), and (e)) and extraction time of 100–120 min (Figure 2(b), (c), (e), and (f)). In addition, Table 3 showed that only the temperature, time, and quadratic term of time had significant effects on ABTS scavenging capacity while the temperature and quadratic term of ethanol concentration significantly contributed to FRAP. In this study, longer extraction time (80–120 min) in combination with higher temperature (63–70 °C) produced higher levels of ABTS scavenging capacity of above 250 mg AAE/100 g (Figure 3). Alternatively, the level of FRAP varied markedly depending on extraction time and ethanol concentration. Specifically, the maximum FRAP value of over 105 mg AAE/100 g could be achieved at a temperature of above 68°C, incubation time of ~80 min, and ethanol concentration of ~53% (v/v) (Figure 4).

Temperature is a critical environmental condition affecting the extraction kinetics of substances from the solid matrix [24]. Various studies showed that incubation temperature greatly affects the extraction yield of phenolic substances from different materials, such as peach [25], olive leaves [26] or mandarin peel [27]. In the current study, the temperature significantly affected the extraction of both TPC and TFC (Table 3). A temperature lower than 55 °C tends to yield higher levels of TPC and TFC from boxberry tree bark (Figures 1 and 2). However, a slightly higher temperature (i.e. > 55 °C) was reported to be more favorable for the extraction of phenolics from artichoke leaves [16]. In contrast, a lower temperature (i.e. 35–36 °C) was documented to improve the extraction of polyphenols from the *Parkia speciosa* pod [28]. This discrepancy in optimal temperature for the extraction of phenolics from various plant matrixes may imply that the diversity in the chemical and structural nature of different phenolic compounds as well as their interaction with the plant matrixes influence their mobility as affected by temperature.

Solvent concentration was reported to be another important factor determining the extraction yield of phenolic compounds as it affects the polarity of the solvents, hence affecting their affinity with polyphenols [16], [29]. On the other hand, extraction time determines the maceration of plant materials and the

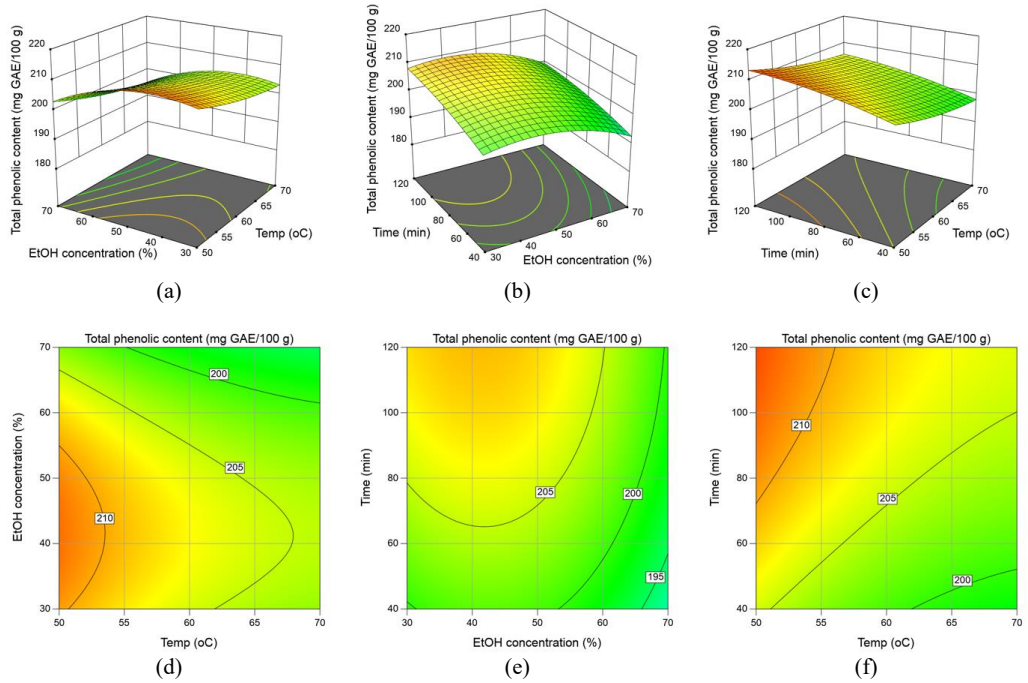


Figure 1: The effect of extraction parameters on total phenolic content as illustrated by (a)–(c) 3D-response surface and (d)–(f) contour plots.

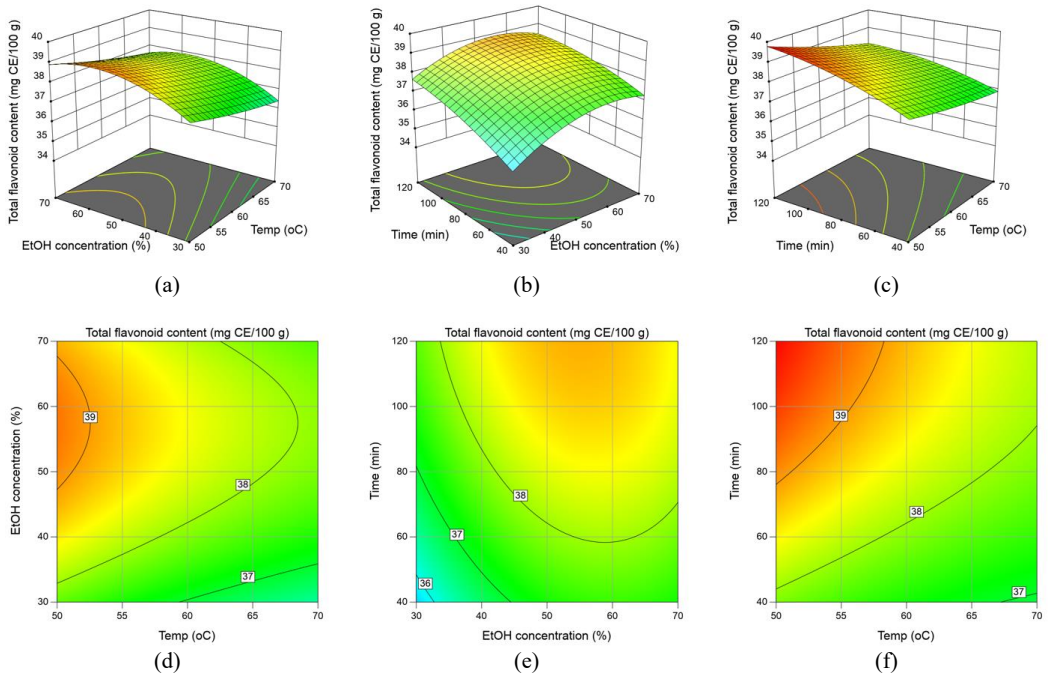


Figure 2: The effect of extraction parameters on total flavonoid content as illustrated by (a)–(c) 3D response surface and (d)–(f) contour plots.

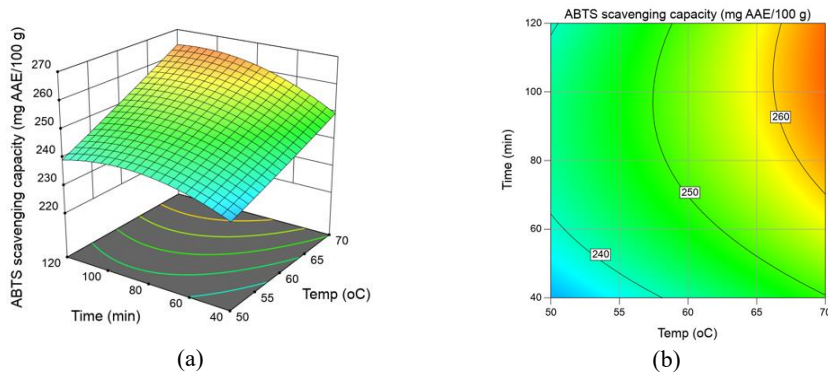


Figure 3: The effect of extraction parameters on ABTS scavenging ability as illustrated by (a) 3D response surface and (b) contour plots.

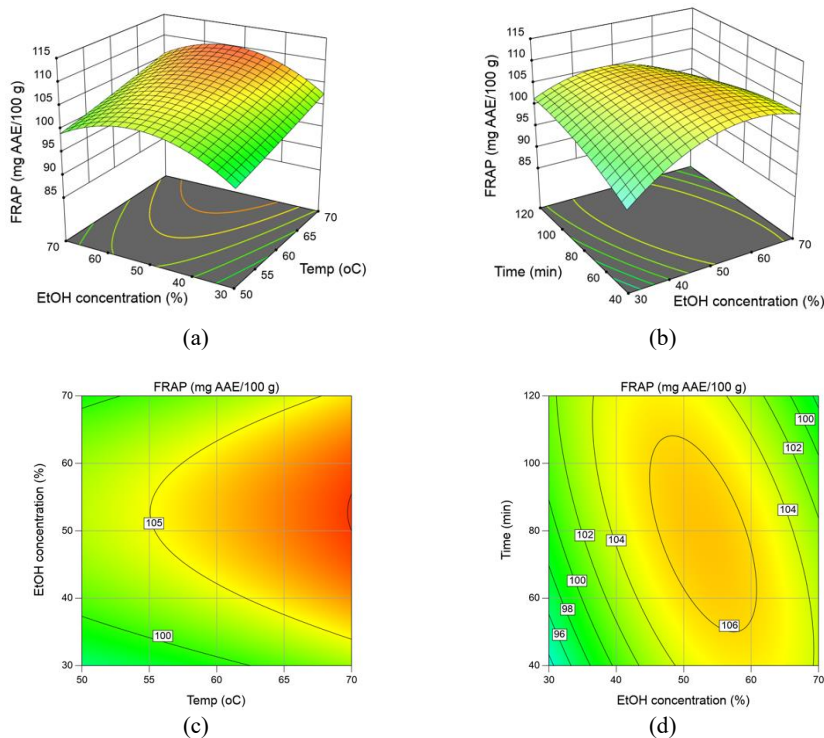


Figure 4: The effect of extraction parameters on FRAP as illustrated by (a)–(b) 3D response surface and (c)–(d) contour plots.

diffusion of substances to the solvent [29]. Gan and Latiff [28] documented that an increase in extraction time from 100–150 min improves the yield of phenolic extraction from *Parkia speciosa* pod. Similarly, results from our current study indicated that a longer extraction time was more favorable for the extraction of polyphenols and flavonoids from

boxberry tree bark (Figures 1 and 2).

3.3 Optimization of extraction conditions

The Response Optimizer tool of Minitab V.20 (Minitab Inc. Stage College, PA) was utilized to optimize the conditions for the extraction of phenolics from

boxberry tree bark. As an optimal extraction condition would yield the highest values of responses, the target of the optimization was set to maximize individual process output as well as a set of multiple outputs. Table 5 shows the predicted and experimental values of TPC, TFC, ABTS scavenging capacity and FRAP. The maximum TPC can be obtained at a temperature of 43.2 °C, an aqueous ethanol concentration of 40.8%, and a duration of 139 min. The highest level of TFC was yielded at a temperature of 43.2 °C, an aqueous ethanol concentration of 53.1%, and a duration of 147 min. The optimal temperature for both ABTS and FRAP was 76.8 °C while the optimal ethanol concentrations for these two responses were 83.6 and 52.4% and the optimal extraction times were 116 and 82 min, respectively. At such optimal conditions, the TPC, TFC, ABTS scavenging capacity, and FRAP were predicted to be 219.1 mg GAE/100 g, 40.8 mg CE/100 g, 279.5 mg AAE/100 g, and 112.7 mg AAE/100 g, respectively. In addition, the optimization study for all responses was also carried out. Results showed that the optimal condition for the set of all responses was a temperature of 75.8 °C, an ethanol concentration of 48.3% (v/v), and an extraction time of 117 min. These optimal conditions were predicted to yield TPC, TFC, ABTS scavenging capacity, and FRAP of 205.9 mg GAE/100 g, 37.8 mg CE/100 g, 271.3 mg AAE/100 g, and 111.4 mg AAE/100 g, respectively. It was noted that the predicted and experimental values of all responses did not differ significantly, suggesting the validity of the estimation model for the prediction of phenolic compounds from boxberry tree bark.

According to Sun *et al.* [30], gallic acid, myricanol, myricanone, epigallocatechin 3-*O*-gallate, epigallocatechin-(4β→8)-epigallocatechin 3-*O*-gallate and 3-*O*-galloyl epigallocatechin-(4β→8)-

epigallocatechin 3-*O*-gallate are the phenolic compounds present in the ethyl acetate extract of boxberry tree bark. In another study, Patel *et al.* [31] reported that the bark contains myricetin at a level of around 2.25 mg/kg. However, the chemical composition of the extract obtained using methanol as a solvent is not available to date, suggesting a further characterization study.

4 Conclusions

In summary, the quadratic model was sufficient to describe the experimental data. It was shown that extraction conditions, including ethanol concentration, time and temperature, as well as a quadratic term of ethanol concentration significantly affect TPC and TFC while antioxidant activity measured by ABTS scavenging capacity was affected by temperature, time, and quadratic term of time and FRAP was impacted by temperature and quadratic term of ethanol concentration. The optimization by RSM showed that the optimal temperature was 75.8 °C, ethanol concentration was 48.3% (v/v), and the extraction time was 117 min. At such conditions, a yield TPC, TFC, ABTS scavenging capacity, and FRAP was predicted to be 205.9 mg GAE/100 g, 37.8 mg CE/100 g, 271.3 mg AAE/100 g, and 111.4 mg AAE/100 g, respectively. The validity of the estimation model was verified by the insignificant difference between the experimental and predicted values of extraction outputs at optimum conditions.

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Table 5: Predicted and experimental values of responses under optimal extraction conditions

Optimal Conditions			Predicted Responses				Experimental Responses			
x ₁ (°C)	x ₂ (%)	x ₃ (%)	TPC (mg GAE/ 100 g)	TFC (mg CE/ 100 g)	ABTS (mg AAE/ 100 g)	FRAP (mg AAE/ 100 g)	TPC (mg GAE/ 100 g)	TFC (mg CE/ 100 g)	ABTS (mg AAE/ 100 g)	FRAP (mg AAE/ 100 g)
43.2	40.8	40.8	219.1 ^{ns}	-	-	-	210.4 ± 9.8 ^{ns}	-	-	-
43.2	53.1	53.1	-	40.8 ^{ns}	-	-	-	39.1 ± 1.8 ^{ns}	-	-
76.8	83.6	83.6	-	-	279.5 ^{ns}	-	-	-	271.3 ± 5.4 ^{ns}	-
76.8	52.4	52.4	-	-	-	112.7 ^{ns}	-	-	-	113.7 ± 7.8 ^{ns}
75.8	48.3	48.3	205.9 ^{ns}	-	271.3 ^{ns}	111.4 ^{ns}	201.2 ± 5.3 ^{ns}	36.8 ± 1.4 ^{ns}	263.7 ± 8.7 ^{ns}	112.5 ± 3.6 ^{ns}

Note: ^{ns} is Non-significant difference between predicted and corresponding experimental values.

Author Contributions

D.T.K.T.: research design, methodology, investigation, writing original draft; N.T.T.T.: investigation, data curation; H.T.T.H.: investigation, data analysis; N.T.A.: conceptualization, supervision, writing original draft and reviewing; P.N.T.: funding acquisition, project administration; P.H.D. writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interests

The authors declare no conflict of interest.

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