Effect of extraction conditions on the antioxidant activity of *Vernonia amygdalina* Del. (*Asteraceae*)

Dinh Chung Duong*, Ngoc Yen Nguyen Thi, Hung Lam Hoa

***Abstract*—In this study, the effect of extraction conditions on the antioxidant activity of *Vernonia amygdalina* Del. (Asteraceae) was evaluated by Response surface methodology and central composite design (RSM-CCD) to predict the content of phenolic compounds with maximum antioxidant activity. Total phenol and flavonoid contents were determined by spectrophotometry method, especially the flavonoid content was identified by HPLC-DAD system. The antioxidant activity was estimated by the DPPH and the FRAP method. Results showed that extracting time, extracting temperature and solvent-to-material ratio had a significant effect on phenolic content ($p < 0.001$). The interactions between the three factors were also found to be significant at 0.05 level of probability. After re-estimating predicted variables on the experiment, we found that the polyphenol content was 137.15 ± 1.36 mg gallic acid /g dry weight (dw), the flavonoid content was 96.78 ± 1.39 mg quercetin/g dw, the total antioxidant activity was 1.95 ± 0.09 mg ascorbic acid/g dw and iron reduction activity was 5.90 ± 0.12 mg $FeSO_4$/g dw at optimum conditions of 34.82 hours at 53.09 °C with solvent to material ratio is 43.64 (ml/g). The correlation coefficients were greater than 0.995 observed between the predicted and actual values for the response variables, which are evidences that the regression model can represent the experimental data well. HPLC showed that leaves contain at least six flavonoids, two of which are apigenin and luteolin. The flavonoids apigenin and luteolin were identified in the extract from *Vernonia amygdalina* with high levels of apigenin (2.72 mg/g dw), luteolin (3.76 mg/g dw).**

***Keywords*—*Vernonia amygdalina* Del., extraction conditions, polyphenol, antioxidant activity, oxidative stress.**

1 INTRODUCTION

Free radicals play important roles and necessary for life. It was produced continuously in all cells as part of a normal cellular function. Free radicals and oxidants contain both toxic and beneficial compounds. Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses [1] but cannot gradually be destroyed, following their accumulation in the body. This process is partly reposible for the development of diseases such as arthritis, vasculitis, lupus erythematous, adult respiratory diseases syndrome, hypertension, heart diseases, stroke, intestinal is chemianeurological disorder (Alzheimer's disease, Parkinson's disease, muscular dystrophy) [2, 3].

Antioxidants act as a radical scavenger, a hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, a enzyme inhibitor, synergist, and metal chelating agents. Both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS (reactive oxygen species) [4]. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, such as the superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase which are either naturally produced or externally supplied through foods and/or supplements such as vitamin A, C , E [5, 6],

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glutathione [7] and polyphenol antioxidants originated from plants [8-11].

Vernonia amygdalina is a shrub that grows predominantly in Africa and Asia. That is a plant widely used for application in natural medicine. It is commonly known as "bitter leaf" which is due to its bitter taste [12]. It is characterized by a soft-wooded tree of 2 to 5 m with an elliptical leaf from the genus Vernonia [2]. The phytochemical screening of the plants studied showed that the presence of flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, sesquiterpene lactones, triterpenoids [13, 14] was represented by polysaccharides[15], luteolin, luteolin 7-O-β-glucoside luteolin 7-O-glucuronide [12], vernolide, vernolepin, vernodalin, hydroxyvernolide, vernodalol, vernomygdin, vernomenin, 4,15-dihydrovernodalin, 1,2,11,12′,3′ hexahydrovernodalin, 1,2,4,15,11,13,2′,3′ octahydrover nodalin, epivernodalol, and vernonioside [16-19]. The pharmacological properties of *V. amygdalina* have been reported to following antidiabetic [20], antioxidant [12, 21], antimicrobial[22], antifungal[23], antiplasmodial [24], cathartic [25], hepatoprotective [26], and antitumor activity [27, 28].

Vernonia amygdalina Del. is a plant widely used for application in natural medicine. The study of medicinal plants starts with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. Hence, selection of proper extraction method needs meticulous evaluation. Traditional methods such as maceration and soxhlet extraction are commonly used in the laboratory research. However, extensive extraction time, experimental numbers with low extraction productivity and unstable results [29]

Response surface methodology is commonly used to reduce experimental numbers and evaluate the interaction between the design factors for improving materials and methods for further application in many industries. In this study, optimal conditions for extraction were determined by RSM to predict the content of phenolic compounds with maximum antioxidant activity from *V. amydalina* Del. leaves.

2 MATERIALS AND METHODS

2.1 Plant Material

Leaves of *V. amygdalina* were collected at Cu Chi ward, Ho Chi Minh city in November 2017 and were identified by Botanical department of Nguyen Tat Thanh University. The leaves of the plant were air-dried in shade and finely powdered.

2.2 Experimental design

Experimental variables of extraction process were performed based on RSM combined with Box-Behnken design for extraction of polyphenols and antioxidant activity from *V. amygdalina* leaves. The variables were designed of three levels (lower, middle and higher value, being coded as −1, 0 and +1) (Table 1) and a total of 15 runs including 3 at central experiments were carried out to optimize the level of chosen variables, such as extraction temperature (X_1, ^oC), extraction time (X_2, hour) and solvent to sample ratio (X_3, g/ml) (Table 2). The total polyphenol content (Y_1), total flavonoid content (Y_2), radical scavenging activity (DPPH) (Y_3) and ferric ion reducing antioxidant power (Y_4) were expressed individually as a function of the independent variables. The generalized second-order polynomial model used in the response surface analysis as follows:

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

where Y is the predicted response, β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for the intercept, linearity, square, and interaction, respectively, X_i and X_j (i=1–3, j=1–3 and i≠j) are the independent variables.

The analysis of variance (ANOVA) using Design Expert trial version 7.0.0 (State Ease, Inc.) was carried out to determine maximal values of reponses. The significance of all the terms of polynomial equation was analyzed statistically by computing the P-value < 0.05.

Table 1. Independence factors and corresponding levels

Independent variables	Unit	Values of coded levels		
		-1	0	+1
Extraction temperature (X_1)	^oC	45.0	52.5	60.0
Extraction time (X_2)	hour	16.0	32.0	48.0
Solvent-to-material ratio (X_3)	ml/g	20.0	40.0	60.0

2.3 Chemicals and Reagents

Folin-ciocalteu, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl triazine (TPTZ), luteolin, apigenin, aluminium chloride ($AlCl_3$), and sodium carbonate (Na_2CO_3) were purchased from Sigma Aldrich (Singapore). All the chemicals were analytical grades.

Table 2. Box–Behnken design matrix and experimental responses

Runs	Variables			Polyphenol Content (Y_1)	Flavonoid content (Y_2)	Antioxidant activity (Y_3)	Ferous reducing activity (Y_4)
	X_1 (°C)	X_2 (hour)	X_3 (ml/g)				
1	52.5	32.0	40.0	135.18	97.85	1.91	5.87
2	45.0	16.0	40.0	84.59	69.70	1.20	2.05
3	52.5	32.0	40.0	139.07	98.93	1.97	5.96
4	60.0	32.0	60.0	106.96	67.57	1.51	3.52
5	45.0	32.0	20.0	81.55	68.47	1.15	2.08
6	52.5	16.0	20.0	88.21	67.39	1.25	3.41
7	52.5	32.0	40.0	137.14	98.68	1.94	5.94
8	45.0	32.0	60.0	89.30	75.13	1.26	2.67
9	60.0	32.0	20.0	88.77	59.47	1.25	2.65
10	60.0	16.0	40.0	87.22	55.66	1.23	2.55
11	60.0	48.0	40.0	114.75	66.49	1.62	2.96
12	52.5	48.0	20.0	94.83	77.15	1.34	3.19
13	52.5	16.0	60.0	88.21	80.21	1.25	3.66
14	45.0	48.0	40.0	86.37	71.06	1.22	2.24
15	52.5	48.0	60.0	116.08	83.70	1.64	4.46

Y_1 = mg gallic acid/g dw; Y_2 = mg quercetin/g dw; Y_3 = mg ascorbic acid/g dw; Y_4 = mg $FeSO_4$/g dw

2.4 Determination of total phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method [30]. Samples (0.5 ml) were introduced into test tubes, mixed thoroughly with 2.5 ml of Folin–Ciocalteu reagent for 5 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 90 min in the dark at room temperature, and absorbance was measured at 760 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

$$TFC = \frac{CxFxV}{Wx(1-h)}$$

Where C: sample concentration calculated from calibration curve (mg/ml), F: dilution factor; V: total volumn of ethanol extract (ml), W: sample weight (g), h: sample moiture content.

2.5 Determination of total flavonoid content

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method of Thaipong (2006) [31]. In brief, 1 ml of crude extract (1 mg/ml ethanol) were mixed with 4 ml of distilled water and then 0.3 ml of 5% $NaNO_2$ solution; 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 2 min. Then, 2 ml of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 415 nm. The total flavonoid content was calculated from a calibration curve established by quercetine solution 20 – 200 µg/ml, and the result was expressed as mg rutin equivalent per g dry weight.

$$TFC = \frac{CxFxV}{Wx(1-h)}$$

Where C: sample concentration calculated from calibration curve (mg/ml), F: dilution factor; V: total volumn of ethanol extract (ml), W: sample weight (g), h: sample moiture content.

2.6 DPPH method of antioxidant assay

The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay of Yuvaraj (2013) [32] with some modifications. Briefly, 0.5 ml of each extract (was diluted with ethanol to suitable concentration) were mixed with 2,5 ml DPPH solution (0.25 µM) and incubated in the dark at room temperature for 30 min. A blank containing 2.5 ml of DPPH and 0.5 ml methanol was prepared and treated as the test samples. The absorbance of the mixture was then measured at 517 nm. The ability of the sample to scavenge DPPH radical was determined from:

$$\text{DPPH radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/ Abs_{control}]x100$$

Ascorbic acid with concentrations of 3 – 15 µg/ml was used as a positive control to set up

calibration curve and the result was expressed as mg ascorbic acid equivalent per g dry weight.

2.7 Ferric ion Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted according to the method reported by Benzie and Strain (1999) [33]. FRAP reagent was prepared freshly by mixing three solutions, sodium acetate buffer at pH = 3, 6, 10 mM TPTZ solution in 40 mM HCl solution and 20 mM ferric chloride ($FeCl_3$) solution in proportions of 10:1:1 (v/v/v). For the assay, 0.5 ml of plant extracts was mixed with 2.5 ml of FRAP reagent. The samples were vortexed for 1 min and incubated in dark for 30 min at 40°C. The absorbance of reaction mixture was measured at 593 nm. The standard ferrous sulfate solution ($FeSO_4$) of 10 – 100 µg/ml was used for calibration curve. The results of FRAP activity expressed as ferrous equivalent per g dry weight (mg $FeSO_4$/g dw) were then extrapolated from the standard curve.

2.8 High pressure liquid chromatography test condition

The sample (10 mg crude extract) was added 100 ml of methanol: water (1: 1) solution, ultrasonic extraction in 15 minutes (no heat) and after that centrifuge 6000 rpm for 10 minutes, take solution, add 100 ml of 20% acid HCl hydrolyzed in 3 hours at 85°C. Then, the aglycon flavonoids were extracted by 20 ml of ethyl acetate (x3), combine the extract, and rotate the solvent. The residue is dissolved in 3 ml mobile phase. The sample washed with column Bond Elut C18 SPE (Agilent - USA) activated by 3ml water. Wash diluted solution of 5 ml with mobile phase, filter through PTFE membrane 0.45 µm for chromatography injection. Condition chromatography analysis was performed using an Agilent Technologies 1260 infinity I, with a photodiode array detector (PDA - G1315D) and an automatic injector. Stationary phase was used a Zorbax XDB reversed phase (SB-C18 150 x 4.6 mm), 5 µm particle size. The mobile phase composed of acetonitrile and 1 % phosphoric acid aqueous solution (68:32, v/v) at a flow rate of 0.7 ml/min. The injection volume was 50 µL and the temperature was maintained at 40°C during the analysis. Detection was realized at wavelength 384 nm. Two reference standards, luteolin and apigenin [12, 34], were simultaneously used in this experiment as markers.

2.9 Statistical Analysis

Data were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance followed by the Tukey test. was considered significant.

3 RESULTS AND DISCUSSION

3.1 Effect of extraction variables on total polyphenol content (TPC)

The experimental data showing the total phenolic content was 81.55 – 139.07 mg gallic acid equivalents/g dry weight. The ANOVA showed the model F value of 182.21 with probability ($p < 0.0001$) which implied that the model was significant and there was only 0.01% chances that this large F value could occur due to noise. The coefficient of determination R^2 was 0.9970 expressing the strong correlation between input variables and TPC. Indeed, phenolic content of extracts was significantly influenced ($p < 0.05$) by linear (X_1, X_2, X_3), interaction parameters (X_1X_2, X_1X_3, X_2X_3) and quadratic parameters (X_1^2, X_2^2, X_3^2) (Table 3). The curved surface plot (Figure 1a-c) demonstrated the role of three extraction variables effect positively on TPC at medium levels of these factors. The final empirical regression model of their relationship between responses and the three tested variables could be expressed by the following quadratic polynomial equation:

$$Y_1 = 137.13 + 6.99X_1 + 7.98X_2 + 5.90X_3 + 6.44X_1X_2 + 2.61X_1X_3 + 5.31X_2X_3 - 24.54X_1^2 - 19.35X_2^2 - 20.94X_3^2 \quad (2)$$

3.2 Effect of extraction variables on total flavonoid content (TFC)

The experimental data showing the total flavonoid content was 55.66 – 98.93 mg rutin equivalents/g dry weight. The ANOVA showed the model F value of 369.62 with probability ($p < 0.0001$) which implied that the model was significant and there was only 0.01% chances that this large F value could occur due to noise. The coefficient of determination R^2 was 0.9985 expressing the strong correlation between input variables and TPC. Indeed, phenolic content of extracts was significantly influenced ($p < 0.05$) by linear (X_1, X_2, X_3), interaction parameters (X_1X_2, X_1X_3, X_2X_3) and quadratic parameters (X_1^2, X_2^2, X_3^2) (Table 3). The curved surface plot (Figure 1a-c) demonstrated the role of three extraction variables effect positively on TPC at medium levels of these factors. The final empirical regression model of their relationship between responses and the three tested variables could be

expressed by the following quadratic polynomial equation:

$$Y_2 = 98.49 - 4.40X_1 + 3.18X_2 + 4.27X_3 + 2.27X_1X_2 + 0.36X_1X_3 - 1.571X_2X_3 - 21.11X_1^2 - 11.65X_2^2 - 9.72X_3^2 \quad (3)$$

3.3 Effect of extraction variables on antioxidant capacity

The antioxidant capacity of the extract was determined by two methods: DPPH and FRAP assay. The results of ANOVA analysis showed that the antioxidant activity significantly affected by the extraction temperature, extraction time, and solvent-to-material ratio with three linear effects (X_1, X_2, X_3), three quadratic effects (X_1^2, X_2^2, X_3^2), and three interactive effects (X_1X_2, X_1X_3, X_2X_3). The model P value of 0.0001 obtained for the antioxidant capacity implied that the model is hingly significant (Table 3). The regression equation predicted by mathematical models for Y_3, Y_4 were given below:

$$Y_3 = 1.94 + 0.097X_1 + 0.11X_2 + 0.084X_3 + 0.093X_1X_2 + 0.037X_1X_3 + 0.075X_2X_3 - 0.35X_1^2 - 0.27X_2^2 - 0.30X_3^2 \quad (4)$$

$$Y_4 = 5.92 + 0.33X_1 + 0.15X_2 + 0.37X_3 + 0.055X_1X_2 - 0.007X_1X_3 + 0.26X_2X_3 - 2.21X_1^2 - 1.26X_2^2 - 0.98X_3^2 \quad (5)$$

Table 3. ANOVA analysis for model

Source	TPC content (Y_1)		TFC content (Y_2)		Antioxidant activity (DPPH) (Y_3)		Ferrous reducing power (FRAP) (Y_4)	
	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Model	182.21	< 0.0001	369.62	< 0.0001	175.58	< 0.0001	1501.80	< 0.0001
X_1	106.29	0.0001	195.15	< 0.0001	99.41	0.0002	437.06	< 0.0001
X_2	138.51	< 0.0001	102.11	0.0002	129.43	< 0.0001	87.32	0.0002
X_3	75.78	0.0003	183.78	< 0.0001	73.35	0.0004	556.88	< 0.0001
X_1 X_2	45.13	0.0011	28.29	0.0031	44.74	0.0011	6.07	0.0470
X_1 X_3	7.42	0.0416	0.65	0.4553	7.35	0.0422	9.83	0.0258
X_2 X_3	30.73	0.0026	12.41	0.0169	29.41	0.0029	130.48	< 0.0001
X_1^2	605.44	< 0.0001	2075.91	< 0.0001	591.25	< 0.0001	9060.61	< 0.0001
X_2^2	376.55	< 0.0001	632.85	< 0.0001	358.40	< 0.0001	2948.54	< 0.0001
X_3^2	440.85	< 0.0001	440.36	< 0.0001	427.18	< 0.0001	1785.03	< 0.0001
Lack of Fit	0.950	0.549	3.465	0.2320	0.750	0.6148	0.820	0.5901
R^2		0.9970		0.9985		0.9968		0.9990
Adj R^2		0.9915		0.9958		0.9912		0.9960
Pre R^2		0.9686		0.9793		0.9699		0.9964

The effect of the variables and their interaction on the antioxidant capacity of the *V. amygdalina* leaf extracts is shown in three-demensional response surface in Figure 1. A higher antioxidant capacity was obtained in the extraction by increasing extraction temperature, time and solvents. However, the yield of antioxidant compounds tended to reduce at elevated temperature and elongated time because of the rate of decomposition of these compounds. The temperature utilized during extraction influenced the stability of antioxidant compounds due to chemical and enzymatic degradation; these factors have been suggested to be the main mechanisms underlying reduction of the polyphenol content in the extraction. Besides, further increase of the solvent to material ratio may dilute the extraction solution thereby lowering the antioxidant activity.

The three-dimensional surface response in Figure 1 evaluated the relationship between three input variables and the contribution of each parameter on the values of responses.

The RSM model and ANOVA analysis showed that the values of TPC and TFC content and antioxidant activity were affected proportionally by three variables: extraction temperature, extraction time, and solvent-to-material ratio. By increasing these parameters, the results of responses tended to decrease due to the decomposition of phenolic compounds. The maximum level was determined under the following experimental conditions: a temperature of 53.09°C, extraction time of 34.82 hours, and a solvent-to-material ratio of 43.64 (ml/g). In order to validate the suitability of the mathematical model for predicting the optimal response value,

verification experiments were carried out under the optimal conditions. The values of TPC, TFC content, antioxidant power (DPPH and FRAP assay) obtained from experiment were 137.15 ± 1.36 mg gallic/g dw, 96.78 ± 1.39 mg quercetin/g dw. 1.95 ± 0.09 mg ascorbic/g dw và 5.90 ± 0.12 mg $FeSO_4$/g dw, respectively. Based on the results, the experimental values of responses were found to be quite comparable with predicted values at 95% confidence level.

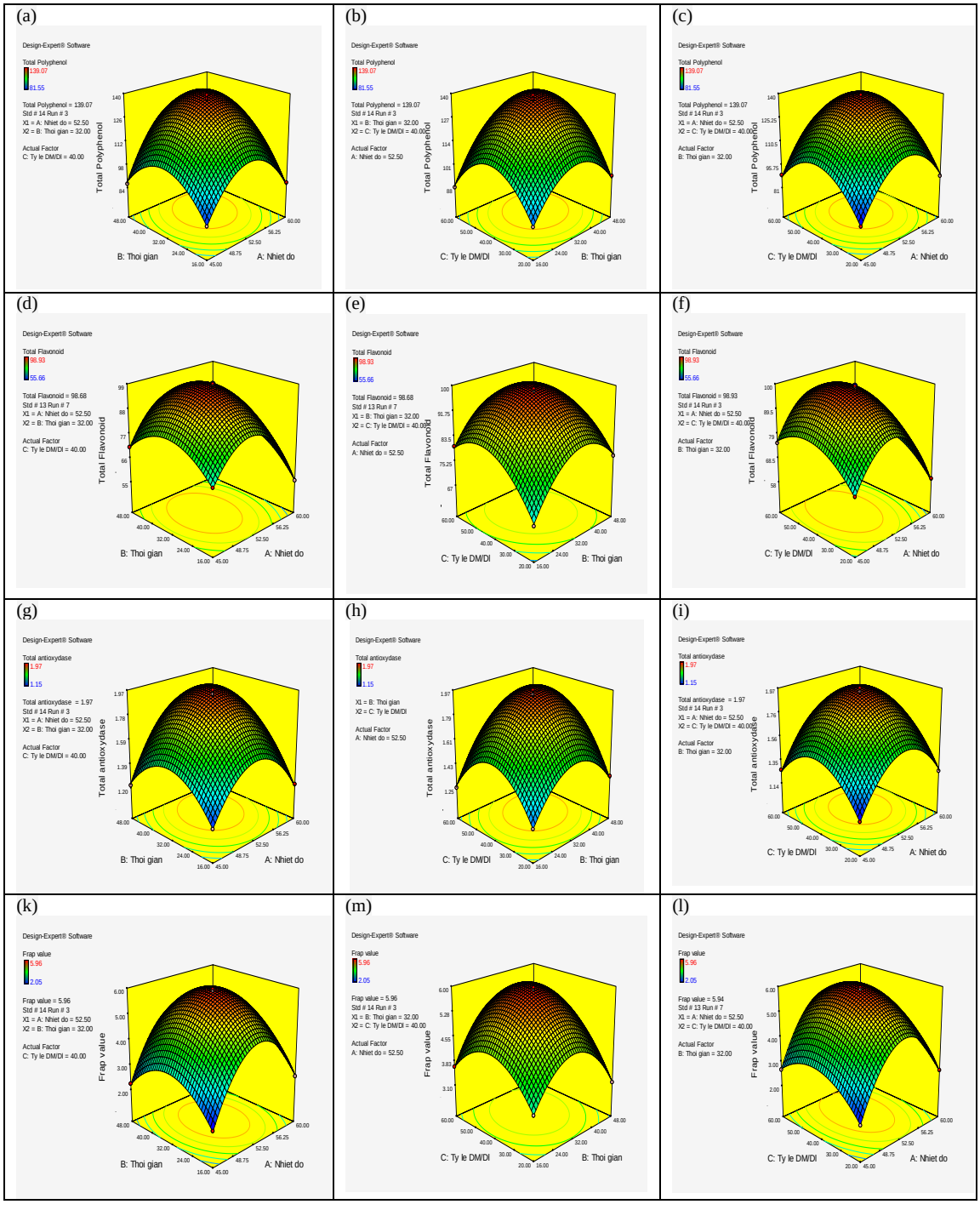

Figure 1. The three-dimensional response surface for TPC (1a-c), TFC (1d-f), antioxidant activity (1g-i) and ferrous reducing antioxidant power (1k-l)

3.4 Analysis of the ethyl acetate fraction by HPLC

The HPLC chromatographic conditions allowed the determination of the flavonoid content in the hydrolyzed extract from *V. amygdalina* leaves. In Figures 2, retention time of luteolin (6.45), apigenin (9.99) and the respective UV spectra are shown in Figures 3. The result identified that the contents of luteolin and apigenin were 3.76 and 2.47 (mg/g dw) respectively.

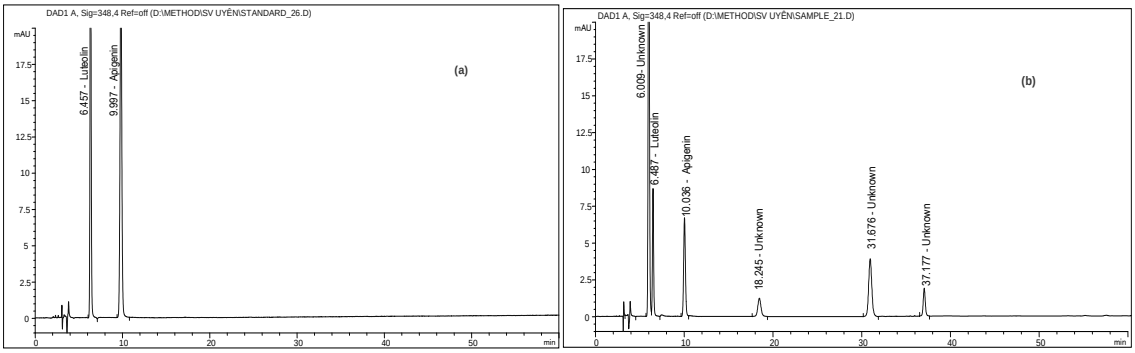

Figure 2. HPLC chromatogram of (a) apigenin and luteolin reference standards and (b) the hydrolyzed sample of *V. amygdalina.*

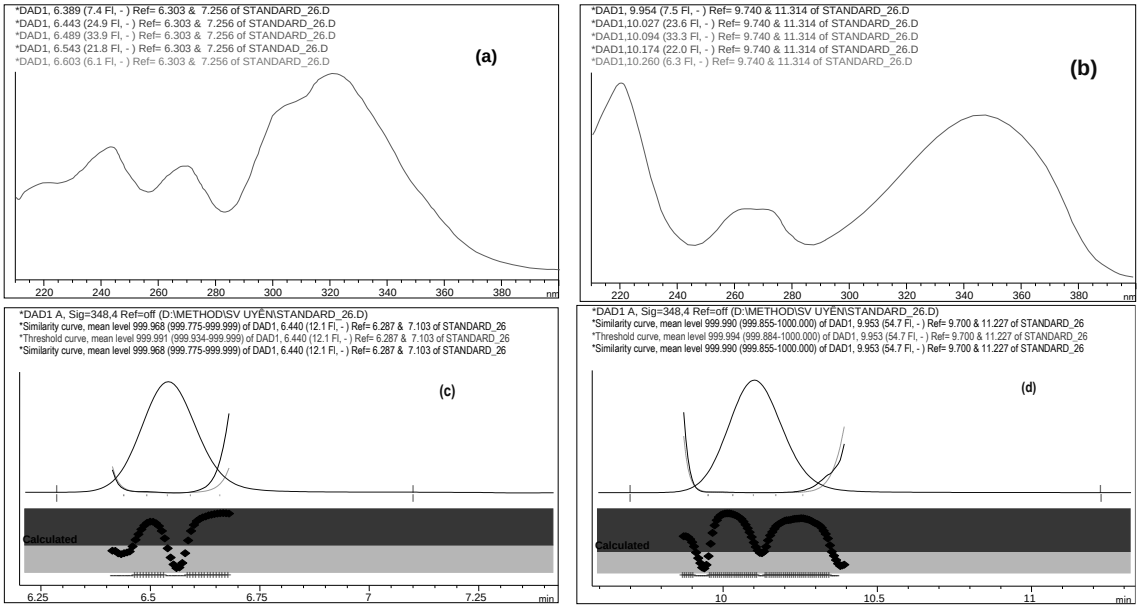

Figure 3. UV spectra of (a) apigenin, (b) luteolin, and the purity of (c) apigenin and (d) luteoli

4 CONCLUSION

Response surface methodology with central composite design (RSM-CCD) on Desige Expert® software is a powerful mathematical technique being widely used in research for optimizing experimental models because of reducing the number of experiments, proceeding time and evaluting the relationship between the responses and input variables as well as finding out the optimal solutions as suggested by the software.

The experimental designs were found to be adequate to predict the extraction process of phenolic compounds with antioxidant activity from *V. amygdalina* Del. leaves. Optimal extraction conditions were found when the following parameters were applied: a temperature of 53.09 °C, extraction time of 34.82 hours, and a solvent-to-material ratio of 43.64 (ml/g).

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Ảnh hưởng của điều kiện chiết xuất đến hoạt tính chống oxy hóa của cây lá đắng (*Vernonia amygdalina* Del.; *Asteraceae*)

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Tóm tắt—Trong nghiên cứu này, sự ảnh hưởng của các điều kiện chiết lên hoạt tính kháng oxy hóa của cây lá đắng *Vernonia amygdalina* Del. (Asteraceae) được đánh giá bởi Phương pháp đáp ứng bề mặt và thiết kế cấu trúc có tâm (RSM-CCD) để dự đoán hàm lượng các hoạt chất phenolic đạt hoạt tính kháng oxy hóa cực đại. Hàm lượng phenol và flavonoid tổng cộng được xác định bằng phương pháp quang phổ, đặc biệt hàm lượng flavonoid được xác định bằng hệ thống HPLC-DAD. Hoạt tính kháng oxy hóa được xác định bằng phương pháp DPPH và FRAP. Kết quả cho thấy thời gian chiết, nhiệt độ chiết và tỉ lệ dung môi/ nguyên liệu ảnh hưởng có ý nghĩa trên hàm lượng phenolic ($p < 0,001$). Tương tác giữa 3 yếu tố trên có ý nghĩa thống kê ($p = 0,05$). Tiến hành đánh giá lại mô hình trên thực nghiệm cho thấy hàm lượng polyphenol đạt 137,15 ± 1,36 mg gallic acid /g, hàm lượng flavonoid đạt 96,78 ± 1,39 mg quercetin/g, hoạt tính kháng oxy hóa đạt 1,95 ± 0,09 mg ascorbic acid/g, hoạt tính khử sắt đạt 5,90 ± 0,12 mg $FeSO_4$/g ở điều kiện tối ưu là thời gian chiết 34,82 giờ ở nhiệt độ 53,09°C với tỉ lệ dung môi/ nguyên liệu 43,64 (ml/g). Hệ số tương quan giữa giá trị dự đoán và giá trị thực cao hơn 0,995 chứng tỏ rằng mô hình hồi quy mang tính đại diện tốt cho dữ liệu trong thực nghiệm. Kết quả HPLC cho thấy lá mật gấu có chứa ít nhất là 6 flavonoid, hai trong số đó là apigenin và luteolin. Flavonoid apigenin và luteolin được tìm thấy với nồng độ cao trong lá khô: apigenin (2,72 mg/g) và luteolin (3,76 mg/g).

Từ khóa—*Vernonia amygdalina* Del., điều kiện chiết, polyphenol, hoạt tính kháng oxy hóa, stress oxy hóa.