Full paper

Andrographolide from *Andrographis paniculata* Extract: Optimization of Accelerated Solvent Extraction Technique.

Rabe'ah Adam, Mariani Abdul Hamid*

Instititute Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

*Corresponding author: mariani@ibd.utm.my

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Graphical abstract

Abstract

Andrographolide is main diterpenoid lactone isolated from *Andrographis paniculata* present mostly in the leaves and all part of the plant. It is widely use as pharmaceutical and nutraceutical formulation. In this study, andrographolide from *Andrographis paniculata* were extracted applying technique of accelerated solvent extraction (ASE). The extracts obtained were characterized by HPLC. The quantification method showed good linearity (r^2= 0.9107) and precision (RSD < 5%) with low values of detection (LOD) and quantification (LOQ) limits. A Box–Behnken design was used to correlate three independent variables (temperature, extraction time and number of cycles) with the amount of andrographolide extracted. The response surface methodology was applied to optimize the extraction of andrographolide by ASE. The optimal conditions were 80°C, 5 minutes and 2 cycle. The shorter time of extraction and the lower heat energy used justify the ASE technique choice to characterize andrographolide from *Andrographis paniculata*.

Keyword**:** *Andrographis paniculata;* andrograholide; ASE; Box–Behnken design; optimization

Abstrak

Andrographolide adalah lactone utama diterpenoid diasingkan daripada *Andrographis paniculata* membentangkan kebanyakannya dalam daun dan bahagian tumbuhan. Ia secara meluas digunakan sebagai formulasi farmaseutikal dan nutraseutikal. Dalam kajian ini, Andrographolide dari *Andrographis paniculata* telah diekstrak menggunakan teknik yang dipercepatkan oleh ekstrak pelarut(ASE). Ekstrak yang diperolehi dicirikan oleh HPLC. Kaedah kuantifikasi menunjukkan kelinearan baik (r^2= 0.9107) dan ketepatan (RSD <5%) dengan nilai rendah pengesanan (LOD) dan kuantifikasi (LOQ) had. Reka bentuk Box-Behnken telah digunakan untuk mengaitkan tiga pembolehubah bebas (suhu, masa pengekstrakan dan beberapa kitaran) dengan jumlah Andrographolide diekstrak. kaedah gerak balas permukaan telah digunakan untuk mengoptimumkan pengekstrakan Andrographolide oleh ASE. Keadaan optimum ialah 80° C, 5 minit dan 2 kitaran. Masa yang lebih pendek daripada pengekstrakan dan penggunaan tenaga haba yang rendah telah menjadikan teknik ASE sebagai teknik yang wajar digunakan untuk mencirikan Andrographolide dari *Andrographis paniculata.*

Kata kunci**:** *Andrographis paniculata;* andrograholide; ASE; reka bentuk Box–Behnken; pengoptimuman

■1.0 INTRODUCTION

Andrographis paniculata which is known as King of Bitter is mainly grown in South East Asia, China and India. In Malaysia, this plant is locally called Hempedu Bumi. *Andrographis paniculata* leaf and stem have an extremely bitter taste.This therapeutic herb is traditionally used for treating liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection [1]. It was also claimed that the traditional application of this plant can treat allergy and inflammatory diseases[2].

In recent studies, Andrographolide known as major constituents is well incorporated with ethanol, methanol, acetone, acetic acid and pyridine and moderately incorporated with water and ether posses posses potential anti-inflammatory properties [3].

To extract and characterize the andrographolide from *Andrographis paniculata*, Accelerated solvent extraction was used in order to distribute medicinal properties from solid liquid extraction. ASE technique is basically an alternative extraction method because it uses high pressure and slightly higher temperatures that accelerates the extraction kinetics which provides higher extraction yields, less extraction time and lower solvent consumption [4].

The optimization of an extraction technique can be accomplished by using a Response surface methodology (RSM) to optimize the effects of processing parameters of extraction on the yield of *Andrographis paniculata* and a Box-Behnken design (BBD) for the optimization of analytical methods [5,6]. It showed a comparison between this design and composite central, three-level full factorial and Doehlert designs. These models were developed to get a good correlation between the input variables responsible for extraction and the output parameter (andrographolide content) of extraction from *Andrographis paniculata.*

In this study we focused on the optimization accelerated solvent extraction (ASE) techniques to improve the andrographolide characterization of *Andrographis paniculata.* It is done to evaluate Andrographolide content present in the leaves itself on whether it could be useful to improve isolation of andrographolide (colourless, bitter and crystalline diterpene lactone) by this newly developed extraction technique [7].

■2.0 EXPERIMENTAL

2.1 Plant materials

The ground dried leaves of *Andrographis paniculata* were obtained from Herba Bagus Sdn Bhd, Johor. The ground *Andrographis paniculata* was stored in a cool and dry environment to prevent fungus growth.

2.2 Reagents

Purified water was prepared using Barnsted E-pure apparatus. Andrographolide standard was purchased from Sigma-Aldrich, 95% ethanol was purchased from QRëC Reagent Chemical.

2.3 Accelerated Solvent Extraction

The three gram ground dried leaves of *Andrographis paniculata* were extracted in absolute 95% ethanol as solvent using the ASE 100 Dionex System (Dionex Pty Ltd, Lanecove, 1595, NSW). Operating procedure was based on the built set up method 1 by Dionex with the following specifications and extraction conditions: Dionex Part No. 056780 Filter grade: D28, Size: 30mm; Temperature: 100ºC; Pressure: 1,500psi (10MPa); Heating time: 5 min; Static time: 5 min; Flush time: 60%; Purge time: 100s; Static cycle: 3; Total extraction: 30 min per sample [8]. The extracts were then filtered through a Whatman filter paper No. 1. The ethanol was evaporated using a rotary evaporator under vacuum (Biichi Rotavapor, Brinkman Company, Westbury, NY, USA). The concentrated aqueous extracts were dried in laboratory oven. The extract was dissolved in methanol (2 mL) and was filtered through a 0.45 µm filter prior to injection into the HPLC system.

2.4 Experimental design and Statistical analysis

Temperature, cycle number and time affect constituents extraction by ASE techniques. were studied using three factors, three level Box-Behnken response surface design. Process parameters, which affect the efficiency of ASE such as extraction temperature (60-100°C), Cycle number (1-3) and time (3-7 min) were investigated. Experiments (17 runs) were carried out in a single base block, of which three were replicates at the center point measuring experimental error. The level of independent variables studied and definition of dimensionless coded of the independent variables are given in Table 2. For statistical calculations, the independent variables were coded as A (coded temperature), B (coded cycle number) and C(coded time). The correspondence between coded and uncoded variables was fitted according to the linear equations showed in Table 1, which were deduced from their respective variations limits. The dependent variables were Y (andrographolide content, % w/w) .

Table 1
Process variables and their range

Independent variables	Unit	Level		
		-1	0	1
Temperature (A)	ºC	60	80	100
Cycle No (B)		1	2	3
Time (C)	min	3	5	7

The experimental data were evaluated by response surface methodology using Design Expert 9. Effect of each independent variable to the response was fitted by polynomial quadratic equation, Eq. (1), which includes linear, interaction and quadratic terms:

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 \quad (1)$$

where y is predicted response, b_0 is the model constant, A, B, C are independent variables (coded), b_1, b_2 and b_3 are linear coefficients, b_{12}, b_{13} and b_{23} are cross product coefficients and b_{11}, b_{22} and b_{33} are the quadratic coefficients. Dependent variables were optimized using Box Behken Design (BBD).

■3.0 RESULTS AND DISCUSSION

3.1 Mathematical modeling

In the present study, extraction yield of andrographolide was investigated according to BBD (17 batch experiments) and the results are shown in Table 2. Then, BBD experimental data are investigated using multi regression analysis namely the sequential model sum of squares (Table 3). From the results, second order polynomial equation was selected to represent the ASE extraction process, due to higher F-value with low p-value. The equation with coded factors was given below

$$Y = 24.95 - 0.74A + 0.74B + 0.68C - 4.19AB + 4.37AC + 2.03B^2 - 3.65C^2 \quad (2)$$

where Y is extraction yield of andrographolide (% w/w); A, B and C are temperature, cycle number and extraction time respectively.

ANOVA results of the quadratic model in Table 4 indicated that the model equation derived by RSM could adequately be used to describe the extraction process under a wide range of operating conditions. As can be seen in Table 4, F-value of 13.11 implied the model was significant There is only a 0.05% chance that an F-value this large could occur due to noise. Moreover, each term in the model was also tested for significance.

Table 2
BBD experimental design with results

S. No	A	B	C	Y
1	80	3	3	22.60
2	80	2	5	25.08
3	100	1	5	31.44
4	60	3	5	32.16
5	100	2	7	25.33
6	80	2	5	25.08
7	80	2	5	22.21
8	100	2	3	15.13
9	60	2	3	27.27
10	80	1	3	21.57
11	80	2	5	23.93
12	100	3	5	24.21
13	60	2	7	19.99
14	80	2	5	25.95
15	60	1	5	22.64
16	80	1	7	22.04
17	80	3	7	24.61

Table 3
Sequential model sum of squares for extraction of yield of andrographolide

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	Remarks
Mean	9947.01	1	9947.01			
Linear	12.43	3	4.14	0.22	0.8775	
2FI	147.22	3	49.07	5.32	0.0190	
Quadratic	76.41	3	25.47	11.20	0.0046	Suggested
Cubic	7.56	3	2.52	1.21	0.4147	Aliased
Residual	8.36	4	2.09			
Total	10198.97	17	599.94			

Table 4
ANOVA result for extraction yield of andrographolide

Source	Sum of Squares	df	MeanSquare	F Value	Prob > F	Remarks
Model	229.46	7	32.78	13.11	0.0005	significant
A	4.43	1	4.43	1.77	0.2158	
B	4.34	1	4.34	1.73	0.2205	
C	3.66	1	3.66	1.46	0.2572	
AB	70.22	1	70.22	28.08	0.0005	
AC	76.41	1	76.41	30.55	0.0004	
B^2	17.41	1	17.41	6.96	0.0270	
C^2	56.26	1	56.26	22.50	0.0011	
Residual	22.51	9	2.50			
Lack of Fit	14.15	5	2.83	1.35	0.3956	not significant
Pure Error	8.36	4	2.09			
Cor Total	251.97	16				

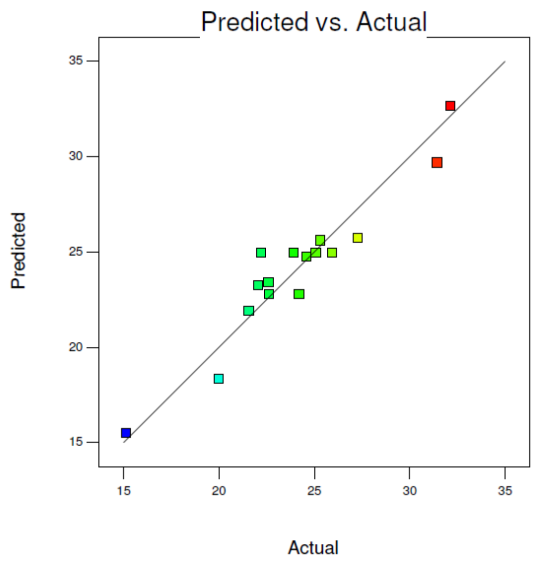

Fig 1. Effect of extraction conditions on the andrographolide yield

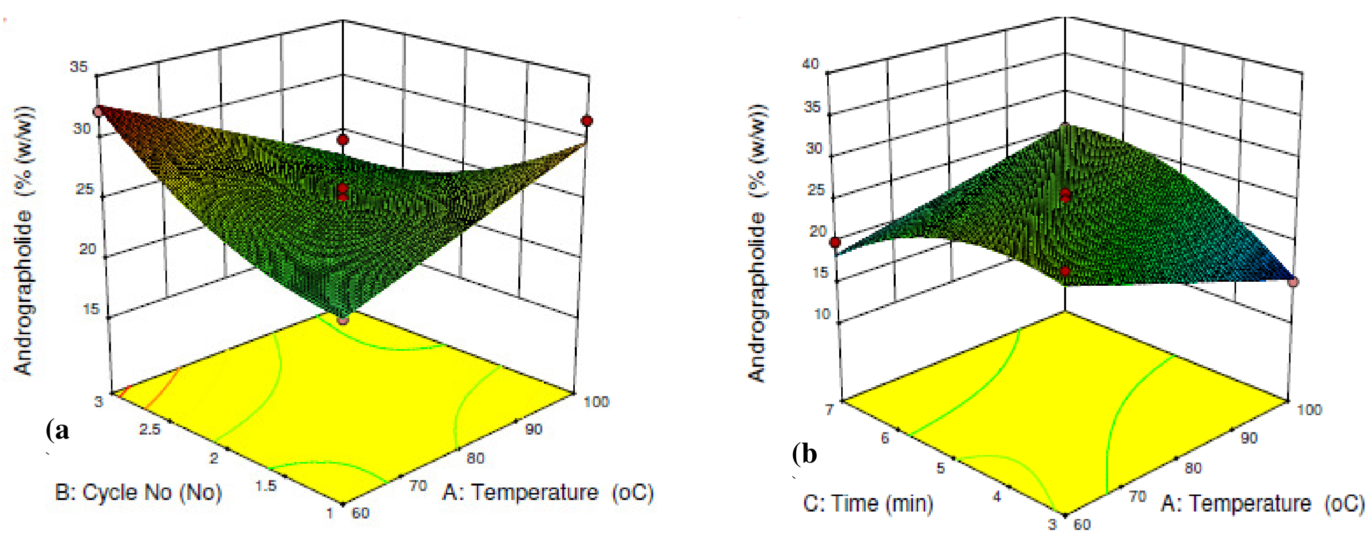

Fig 2. Response surface plots representing the effect of process variables on extraction yield

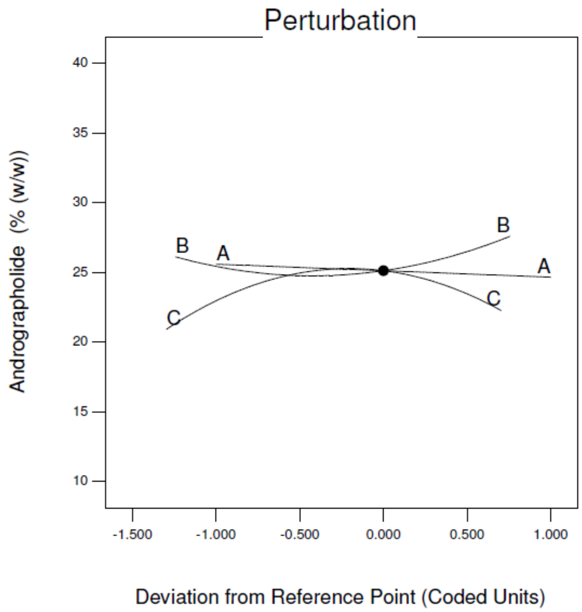

Fig 3. Perturbation plot for extraction yield of andrographolide

A p-value less than 0.05 implies that the corresponding model term is significant. Moreover, individual and interactive model terms also shows the significance of the developed model.The "Lack of Fit F-value" of 1.35 implies the Lack of Fit is not significant relative to the pure error. There is a 39.56% chance that a large number of "Lack of Fit F-value" could occur due to noise. Non-significant lack of fit is good as we want the model to fit. The r^2= 0.9107 shows the reproducible nature. In the present study, adequate precision value was obtained as >4, demonstrate a significant and intense correlation between the observed and predicted values. The actual and predicted pectin yields are plotted in Fig. 1.Actual values are data for each specific run from Table 3, and predicted values are produced by the model, Eq. (3). The data points on this plot lie reasonably close to the straight line and indicate that an adequate agreement between real data and the data obtained from the model [9].

3.2 Effect of extraction conditions on the andrographolide yield

In order to study the interactive effect of the process variables on the response, response surface contour plots are plotted from the developed mathematical model (Eq. (2)) and it is shown in Fig. 2. Temperature is one of the crucial parameters which influence the Fig extraction of andrographolide from Andrographis paniculata leaves. In order to investigate the effect of temperature on andrographolide yield, experiments were carried out in various power and the results are shown in Fig. 2(a). From the results, it is observed that, the andrographolide yield is increased linearly with increasing temperature to 100^{o}C due to the increasing linearity of cycle number from 1 to 3. Faster diffusion rates occur as a result of increasing the temperature of the extraction [10]. Diffusion rates have been shown to increase roughly upon increasing the temperature from 60°C to 100 °C.

Time is one of the important process variables for extraction of andrographolide and it is associated with the mass transfer effect. In order to examine its effect on extraction process, experiments were carried out in various extraction time and the results are shown in Fig. 2(b) and from the results, it is found that, the andrographolide yield is increased rapidly with increasing the time up to 5 min. However, lower andrographolide yield was showed after 5 min as the temperature decreasing linearly from 60^{o}C to 100^{o}C and it was probably due to mass transfer effect. The use of longer time increases the capacity of solvents to solubilize analytes and improved mass transfer and, hence, increased extraction rates would result [11]. Perturbation plot for extraction yield of pectin is shown in Fig. 3 and it showed the effect of process variables on the extraction process deviation from reference point.

3.3 Multi response optimization

According to BBD results, optimal extraction conditions to obtain the maximum extraction yield of andrographolide from *Andrographis paniculata* leaves is determined by Derringer's desired function methodology as follows: temperature of 80◦C, cycle number of 2 and extraction time of 5 min Under this conditions, 25% of andrographolide was extracted with a desirability value of 0.9107. The suitability of the optimized conditions for predicting the andrographolide value was tested experimentally using the selected optimal conditions. Triplicate experiments were performed under the optimized conditions and the mean values (25.47%) obtained from real experiments, demonstrated the validation of the optimized conditions.

4.0 CONCLUSION

In the present study, extraction of andrographolide from *Andrographis paniculata* leaves was investigated under different extraction conditions such as temperature, cycle number and extraction time. Three factors at two levels BBD coupled with RSM were used to develop the second order polynomial model. Maximum extraction yield of andrographolide from *Andrographis paniculata* leaves was found to be as follows: temperature of 80◦C, cycle number of 2 and extraction time of 5 min. Under these optimal conditions, 25% of andrographolide was extracted. HPLC confirms the presence of andrographolide in *Andrographis paniculata* leaves crude extract. Results exhibited that ASE was found to be a suitable technique to extract the andrographolide from *Andrographis paniculata* leaves.

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