Southeast-Asian J. of Sciences, Vol. 9, No. 1 (2022) pp. 20-30

OPTIMIZATION OF EXTRACTION CONDITIONS OF TOTAL ALKALOID CONTENT (TAC) in CATHARANTHUS ROSEUS (L.) G. DON APOCYNACEAE by UV-VIS SPECTROPHOTOMETRY

Duong Phuong Quynh, Nguyen Thi Hong Phuc*

Faculty of Pharmacy at HCM city
Nguyen Tat Thanh University, Ho Chi Minh city, Vietnam
e-mail: nthphuc@ntt.edu.vn

Abstract

Catharanthus. roseus is a well-recognized herbal medicine due to its anticancer bisindole alkaloids (vinblastine, vincristine and vindesine). In the Ayurvedic system of medicine, different parts of C. roseus are used in folklore herbal medicine for treatment of many types of cancer, diabetes, stomach disorders, kidney, liver and cardiovascular diseases.

Aim of the study: The main ideas behind this scientific article are to extract the alkaloid total extract; and to optimize the alkaloid extract conditions by using response surface methodology (RSM). The plant part used was the whole plant. The effects of three independent variables (extraction solvent/ material ratio (shorten: solvent/ material ratio):, X1 material soaking time in extraction solvent (shorten: soaking time): X2 and pH of extraction solvent (shorten: pH of solvent) X3) on one response variable (total alkaloid content calculated by vinblastine content, (shorten: TAC): Y) was determined using central composite design (CCD) while phytochemical profiling of the extracts was determined by UV- Vis spectrophotometer. Quadratic models produced a satisfactory fitting of the experimental data with regard to total alkaloid content (r2?=?0.972, p ¡ 0.05). The best extraction conditions observed for total alkaloid content was at solvent/ material ratio of 1?:?5?g/ml; material

Key words: : response surface methodology (RSM), central composite design (CCD); Vinblastine, Catharanthus roseus.

soaking time in extraction solvent; X2 for 18 (hour); pH of extraction solvent, X3: 8 with the optimum value for total alkaloid content was 0, 5841 mg/g. The main phytochemical compound in the optimized C. roseus extract is vinblastine. As conclusion, this study clearly demonstrated the best conditions to obtain higher phytochemical compound which can be further used for the development of cancer, diabetes, stomach disorders agents.

1. Introduction

Catharanthus roseus (L.) G. Don is a well known medicinal plant belonging to family Apocynaceae that have been traditionally used as medicine since ancient times [1]. This plant was found to possess potent antihyperglycemic, and anticancer activities. Interestingly, many phytochemical compounds such as bisindole alkaloids (vinblastine, vincristine and vindesine) have been found in the aerial parts of this plant [2, 3].

Extraction is considered to be one of the important steps in discovering potential bioactive compounds from plant materials, considering their quantity and type of bioactive compounds as they are widely used in pharmaceutical products. The type and concentration of solvent, temperature, time, pH, solid-liquid ratios, pressure, and the particle size of the plant have been identified from the previous studies as important factors affecting the extraction efficiency of the plant bioactive compounds [1-4]. Traditional method, which is known as the one-factor-at-a-time, has been used widely by many researchers in optimizing specific parameters in which only one factor plays a role as a variable at a time while keeping all other factors constant. However, this method is considered to be less reliable as it does not include interactive effects among factors, time-consuming, and expensive. To overcome this problem, response surface methodology (RSM) has been introduced and widely used nowadays for optimizing the extraction conditions. RSM is considered to be a powerful mathematical technique used widely in many industries for technological operations in order to optimize certain experimental conditions. Moreover, it evaluates the effects between the multiple factors and their interactions towards one or more response variables simultaneously, producing lesser number of experimental measurements [5-7].

Visblastine is an bis- indole alkaloid assay used widely in determining the anticancer activity of the plant extracts. Optimization of extraction method to obtain higher total alkaloid from C. roseus is essential for future pharmaceutical product development especially for the anticancer treatment. Therefore, this study aimed at optimizing the solvent/ material ratio; soaking time, and pH of solvent for the extraction of total alkaloid content (TAC) from C. roseus whole

plant using RSM.

2. Materials and Methods

2.1. Chemicals and Reagents

Vinblastine was purchased from Cipla Pharma. Solvent and Reagents were of the high purity (90-99%).

2.2. Plant Materials

The plant parts used were the whole plants of C. roseus. They were collected in May 2018 at traditional pharmaceutical shop on H?i Th??ng Ln ng street, District 5 at Ho Chi Minh city. The samples were cleaned, dried and grounded to obtain a fine powder. The moisture content of powder (5.74%) is carried out according to Appendix 9.6 of Vietnamese Pharmacopoeia V.

2.3. Sample Extraction

Accurately weigh 0.5 g powder of C. roseus , extract this powder with a studied solvent, the extract were vaporized and filted. The filtrate was agited with the second solvent for moving the chlorophyll. The rest solution was vaporized until the sample for using the identification and quantification the alkaloid (calculated to Vinblastine sulfate). Dissolve the sample with nitric acid 1% in a 50 mL volumetric flask, filter and dilute 1 mL of the filtrate solution into a 100 mL volumetric flask with purified water prior to UV-Vis analysis.

2.4. Experimental Design

Using Modde 5.0 software according to the D-Optional model to design 13 randomized experiments out of 27 experiments, then repeat the experiment 3 times at the basic level of factors. Perform 16 designed experiments, enter the obtained data and analyze the results based on the mathematical analysis of Modde 5.0 software.

Box behnken design (BBD) was developed in order to obtain optimized extraction conditions for higher total alkaloid content, of *C. roseus.* BBD was chosen in this study as it provides more design points on each variable. Three selected independent variables in this study were the solvent/ material ratio (X_1: 5:1-15:1 ml/ g), soaking time (hour) (X_2: 18-30), and pH of solvent (X_3: 8-10), while the dependent variable chosen were total alkaloid content (Y). The responses of total alkaloid content was critical to be studied as the presence of it in the plants have been reported to be the reason for hoping of the treating

the anticancer disease of the plants. The complete design was generated by a statistical software package (BBD Design) which consists of 16 combinations (Table 1 and Table 2).

Table 1: Process parameters and Experimental levels

Number	Parameter	Sign	Basic level	High level (+)	Low level (-)	Variation (Δ)
1	solvent/ material ratio	X1	10:1	15:1	5:1	5
2	soaking time	X2	24	30	18	6
3	pH of solvent	X3	9,0	10,0	8,0	1,0

Table 2. Experimental design matrix and the responses for BBD.

Run	X_1 (min)	X_2 (°C)	X_3 (%)
1a	5:1	18	8
2	15:1	18	8
3	5:1	30	8
4	15:1	30	8
5	5:1	18	10
6	15:1	18	10
7	5:1	30	10
8	15:1	30	10
9	5:1	18	9
10	5:1	24	8
11	5:1	24	10
12	10:1	18	10
13	10:1	24	9
14	10:1	24	9
15	10:1	24	9
16	10:1	24	9

2.5. UV- Vis Spectroscopy Analysis

A Shimadzu (Japan) UV 1800 UV-Vis spectrometer was used to perform the measurements of Vinblastine sulfate absorbance. Background correction was

achieved with a deuterium lamp.

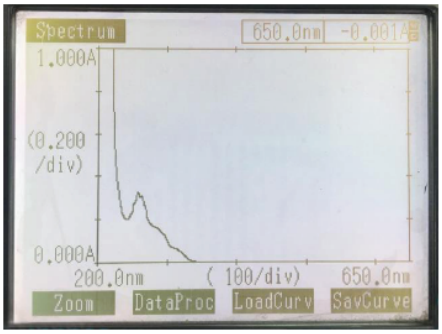

Figure 1: UV-Vis spectrophotogram of vinblastine sulfate / nitric acid 1%

2.6. Total alkaloid Content (TAC) Determination

TAC of the plant extract was determined by UV - Vis spectrophotometry using vinblastine as a standard. The absorbance of the mixture was measured spectrophotometrically at 258?nm, and the results obtained were expressed as milligram vinblastine equivalent per gram (mg/g) based on the standard curve of various concentrations of vinblastine sulfate (0-100μg/ml).

Construction of a standard curve for vinblastine sulfate in nitric acid 1 % Samples preparation: Prepare 6 sample solutions as follows to obtain solutions with different concentrations (g/l).

Table 3. Samples preparation

Vinblastine sulfate Samples	1	2	3	4	5	6
Concentrations (g/l).	0,0020	0,0018	0,0014	0,0010	0,0008	0,0006
Absorbances	0,641	0,576	0,441	0,319	0,259	0,197

2.7. Statistical Analysis

A response surface analysis was employed to determine the regression coefficients and statistical significance of the model terms and to fit the mathematical models of the experimental data. The adequacy of the model was predicted through the regression analysis (r2) and the analysis of variance (ANOVA) ($p < 0.05$).

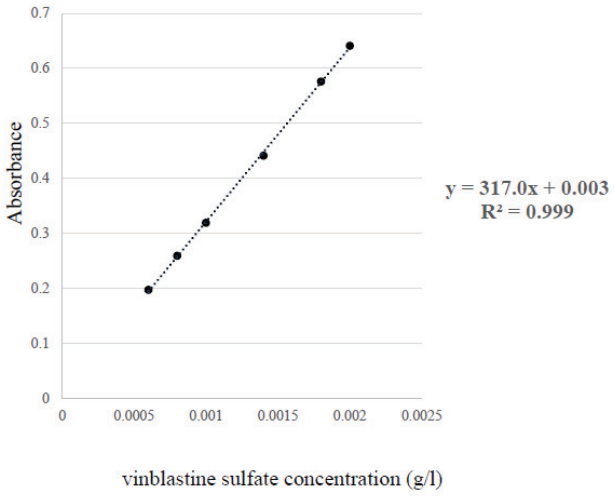

Figure 2: Linearity curve with a correlation coefficient of calibration curve

3. Results and Discussion

3.1. Fitting the Response Surface Models

Fitting the models are crucial in interpreting the accuracy of the RSM mathematical models for prediction of the TAC of C.roseus extract. In this present study, the relationship between the response function (TAC, and the independent variables (solvent/ material ratio, X1 ; soaking time X2 and pH of solvent, X3 was successfully identified by CCD. The results of the responses for the 16 runs following the experimental design are shown in Table 4.

The regression equation is set up as follows:

$$Y = 0,4484 - 0,0558X_1 + 0,0181X_2 - 0,0135X_3 + 0,0429X_1X_2 \qquad (1)$$

- Y: TAC (total alkaloid content calculated to vinblastine content mg/g) where X_1, X_2, and X_3 are the coded variables for Ratio of solvent/ material ratio, soaking time, and pH of solvent, respectively. A negative sign in each equation represents an antagonistic effect of the variables, and a positive sign represents a synergistic effect of the variables Positive coefficient for X_2 was observed in equation (1), indicating that TAC was increased with the increase of soaking time (18-30 hour). In contrast, negative coefficients for X_1 and X_3 were observed in equation, indicating that TAC was decreased with the increase of solvent/ material ratio (5:1-15:1 ml/g), and pH of solvent (8-10).

The RSM model coefficients were validated by analysis of variance (ANOVA) of the response variables for the quadratic polynomial model summarized in

Table 4. Experimental design matrix and the responses for BBD.

Run	X_1 (min)	X_2 (°C)	X_3 (%)	Y (mg/g)
1	5:1	18	8	0,5921
2	15:1	18	8	0,3851
3	5:1	30	8	0,5307
4	15:1	30	8	0,4801
5	5:1	18	10	0,5184
6	15:1	18	10	0,3332
7	5:1	30	10	0,4932
8	15:1	30	10	0,4831
9	5:1	18	9	0,5387
10	5:1	24	8	0,5079
11	5:1	24	10	0,5184
12	10:1	18	10	0,4451
13a	10:1	24	9	0,4580
14a	10:1	24	9	0,4496
15a	10:1	24	9	0,4328
16a	10:1	24	9	0,4515

[a]Center point.

Table 5. ANOVA results for optimization by BBD.

	Coeff. SC	Std. Err.	P	Conf. int (±)
Constant	0.448381	0.0079695	2.11763E-09	0.0195008
X1	-0.0557682	0.00547111	5.1927E-05	0.0133874
X2	0.0181318	0.00547112	0.0161223	0.0133874
X3	-0.0135018	0.00522973	0.0416722	0.0127968
X1*X1	-0.0114026	0.0149299	*0.473972*	0.0365325
X2*X2	0.0195726	0.0119638	*0.152959*	0.0292745
X3*X3	0.0195924	0.0140653	*0.213052*	0.0344168
X1*X2	0.0428904	0.0057156	0.000289698	0.0139857
X1*X3	0.00147962	0.00528752	*0.789002*	0.0129382
X2*X3	0.0124347	0.0057156	*0.0725031*	0.0139857

Supplementary Materials (Table 6).

Table 6. ANOVA results for optimization by BBD.

	DF	SS	MS	F	p
Regression	9	51.6567	5,76235	16.0459	**0.004**
Residual	5	1.79558	0,359117		
Lack of fit	3	0.527269	0,175756	0.277149	**0,157**
Pure Error	2	1.26831	0,634157		
Total Corrected	14	53.6567	3,83262		
R^2 = 0.972; Q^2 = 0.767; R^2 adj = 0.930					

For the response of TAC, the model was highly significant when the computed F-values were greater than the tabulated F-value and the probability values were low ($p < 0.001$), indicating that the individual terms in each response model were significant on the interaction effect. In addition, the coefficient of determination (r_2) of the models was 0.972 indicating that 97.2 % match between the values of the predicted model and the values that were attained in the experimental data. Besides, the r_2 value was comparable to adjusted r_2, demonstrating good statistical model. The p values for the lack of fit were identified as 0.157, highlighting that the lack of fit of the models was significant at $p > 0.05$. Hence, the result indicate that the models could predict the TAC of *C. roseus* extract efficiently when independent variables were within the ranges depicted here.

3.2. Effect of Extraction Parameters on Total Alkaloid Content (TAC)

All independent variables (Solvent/ material ratio, X_1; X_2, soaking time; and X_3, pH of solvent) had a significant effect on TAC of *C. roseus* extract. Among these, X_2 : soaking time was found to be the major effect on TAC in comparison with other variables, which may be due to the high F-value of 16,05.

3.3. Optimization of Extraction Conditions

Based on the optimization software Modde 5.0, the optimal conditions for extracting alkaloids from C. roseus were obtained as follows (tables 7 & 8)

Table 7: Predictive optimization conditions

Stt	parameters	Values	Predicted TAC (g)
1	Solvent/ material ratio	5:1	0,5841
2	soaking time (hour)	18	
3	pH of solvent	8	

Repeat the test 3 times with the predicted optimization condition, evaluating the predicted optimization condition

Table 8: Results of performing 3 times of optimal conditions

	Experimentations	Obtained TAC (g)
1	1st	0,6021
2	2nd	0,5794
3	3rd	0,5869

Experiments give $p = 0.029 < 0.050$ with a confidence interval of 95%, so the results are statistically significant. The yield of the TAC ranged from 0. 5794; 0,5869; 0,6021 mg/g. As suggested by the Design Expert software, a quadratic polynomial model was selected and fitted well for all three independent variables and responses. In terms of coded values, the predicted response for the TAC could be expressed by the second-order polynomial equation via multiple regression analysis.

In this study, the desirability function approach suggested that the optimal extraction conditions for C. roseus extract in obtaining maximum TAC (0,6021; 0,5794; 0,5869 mg/g), were at 18 hour with 15:1 of solvent / material ratio and pH =8. The predicted values of all the responses were close to the experimental values with less than 10% error, which is relatively desirable and all the experimental data were within ±95% prediction intervals. Besides, the accuracy of our model was confirmed as no significant difference was observed

($p > 0.05$) between the experimental values and the predicted values (Table 9). Therefore, it can be concluded that the model from BBD was accurate and reliable of *C. roseus.*

Table 9: Differences between the predicted value and the experimental values.

Parameters	Predicted value	Experimental values	Percentage of error (%)
Total alkaloid content (mg /g)	0,5841 ± 0.00a	0,6021; 0,5794; 0,5869	4.81

Values were presented as mean ±standard deviation. Same superscripts within the row indicate no significant difference ($p > 0.05$) between predicted and experimental values.

4. Conclusion

RSM was successfully employed to optimize the phytochemical compound vinblastine of *C. roseus* where BBD proved to be an efficient tool for the optimization of these parameters. The second-order polynomial models for predicting responses were obtained, and the best combination of X_1 : Ratio of solvent / material ; X_2 : soaking time ; X_3 : pH of solvent. were found to be 5: 1 with 18 hour and 8, respectively. Further investigation on the secondary metabolites present in the optimized extract of *C. roseus* using UV Vis spectrometry analysis revealed the presence of vinblastine in TAC. Hence, *C. roseus* could be a natural source of alkaloid compounds which serve as be used in pharmaceuticals for the treatment of anticancer.

References

[1] C.N .Sternberg, A. Yagoda , H.I. Scher, R.C. Watson, N. Geller, H.W. Herr, M.J. Morse, P.C. Sogani, E.D. Vaughan, N. Bander, *Methotrexate, vinblastine, doxorubicin, and cisplatin for advanced transitional cell carcinoma of the urotheliu,* Cancer, **64**(12)(1989), 2448-2458.

[2] Giuseppe Musumarra, Salvatore Scire (2001), *MODDE, Version 5.0, available from UMETRICS AB,* Journal of Chemometrics, pp. 199-200.

[3] Kratika Kumari, Sharmita Gupta (2011), *Antifungal properties of leaf extract of Catharanthus roseus (G.) Don,* American Journal of Phytomedicine and Clinical Therapeutics.

[4] Kyakulaga A. Hassan, Alinda T. Brenda, Vudriko Patrick, Ogwang E. Patrick , *In vivo antidiarrheal activity of the ethanolic leaf extract of C. roseus Linn. (Apocyanaceae) in Wistar rats,* African Journal of Pharmacy and Pharmacology, **5**(15)(2011), 1797-1800.

[5] Prasad K. N., Kong K. W., Ramanan R. N., Azlan A., Ismail A., *Selection of experimental domain using two-level factorial design to determine extract yield, antioxidant capacity, phenolics, and flavonoids from Mangifera pajang Kosterm,* Separation Science and Technology, **47**(16) (2012), 2417-2423. doi: 10.1080/01496395.2011.606257.

[6] Aybastier Ö, Isik E., Sahin S., Demir C., *Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology,* Industrial Crops and Products, **44**(2013), 558-565.

[7] 7. Silva E., Rogez H., Larondelle Y., *Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology,* Separation and Purification Technology, **55**(3) (2007), 381-387.