OPTIMIZATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID EXTRACTION CONDITIONS FROM LEAVES OF LAUNAEA SARMENTOSA USING THE RESPONSE SURFACE METHODOLOGY

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The Central composite design (CCD) of response surface methodology (RSM) was used to investigate the effects of three factor as extraction temperature (°C), extraction time (min) and ethanol concentration (%) of Launaea sarmentosa leaves on the responses: total phenolic content (TPC) and total flavonoid content (TFC). The optimal conditions obtained from response RSM were 90% v/v for the solvent ratio, 54°C for extraction temperature and 110min for extraction time. The experimental values of TPC and TFC were 318.85±0.32 mgGAE/g, 8.21±0.14 mgCE/g.
Keywords: *Launaea sarmentosa, total phenolic content (TPC), total flavonoid content (TFC), response surface methodology (RSM), extraction.*

1. INTRODUCTION

Launaea sarmentosa (Willd) Schultz-Bip.ex Kuntze, belongs to family of Asteraceae, in Vietnam, It is grown at sandy coasts of Thai Binh, Nghe An, Ha Tinh, Ben Tre, Quang Tri [1], it is a creeping herb, native to tropical Indian coastlines. All parts of the *Launaea sarmentosa* (Willd.) plant especially leaves of contain high amounts of phenolic and flavonoid compounds with potential antioxidant properties. This Plant also synthesize huge amount of aromatic compound among which phenols or their oxygen substituted derivatives are predominant. These compounds provide protection against microbes for the plant [2].

Launaea has great importance due to its ethnobotanics, phytochemistry and biological activity, and various secondary metabolites including sequiterpenoids, terpenoids and flavonoids [3]. It's root contains the following chemical components: calcium oxalate crystals, tannin content, contains alkaloids, aminoacids, carbohydrates, glycosides, tannin, and steroids [4].

The role of flavonoids is to be the "biochemical repairman of nature", helping to correct errors for metabolic reactions, the biosynthesis processes of living ingredients, supporting endocrine regulation. Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups [5].

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 [6,7]. 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 leaves of *Launaea sarmentosa* because these are two compounds found very much in genus *launaea* [8].

2. MATERIAL AND METHODS

2.1. Material

Leaves of *Launaea sarmentosa* were collected in Nghi Xuan District of Ha Tinh Province, Vietnam in September 2019 and identified by Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen was deposited at the herbarium of the School of Chemistry, Biology and Environment, Vinh University. The material is dried, crushed and stored at 4^{o}C for further experiments.

2.2. Methods

2.2.1. Total Phenolic Content (TPC)

The TPC of the *Launaea sarmentosa* leaves 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. 1ml of sample was mixed with 5ml of Folin-Ciocalteu's solution. After 3 min, 4ml of 7.5% sodium carbonate solution was added to a mixture and adjusted to 10ml with deionized water. The mixture was kept at room temperature in a dark environment for 60min. The color change was determined by scanning the wavelength at 765nm (Agilent 8453 UV – Visible Spectrophotometer) since maximum absorbance was obtained. TPC of the *Launaea sarmentosa* leaves extract was determined as mg gallic acid equivalent using the standard curve prepared at different concentrations of gallic acid and reported as mgGAE/g dry weight (DW).

2.2.2. Total Flavonoid Content (TFC)

The TFC of the *Launaea sarmentosa* leaves extract was estimated according to the procedures described by D. Marinova et al.[10] with slight modification. An aliquot (1ml) of extracts or standard solution of catechin (0.01 ÷ 0.07mg/ml) was added to 10 volumetric flask containing 4 ml of dd H_2O. To the flask was added 0.3ml 5%$NaNO_2$. After 5 min, 0.3ml 10% $AlCl_3$ was added. At 6th min, 2ml 1M NaOH was added and the total volume was made up to 10ml with ddH_2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm (Agilent 8453 UV-Visible Spectrophotometer). Total flavonoid content of *Launaea sarmentosa* leaves extract was expressed as mg Catechin equivalents mgCE/g DW.

2.2.3. 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) and ethanol concentration (%) as extraction medium on two responses TPC and TFC in the *Launaea sarmentosa* leaves extracts. These three factors, namely extraction temperature (X_1), extraction time (X_2) and ethanol concentration (X_3) were coded into three levels (-1, 0, +1). Ranges of extraction temperature, extraction time 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 response surface models

The responses consisting of total phenolic content and total flavonoid content for *Launaea sarmentosa* leaves extract were optimized based on the central composite design (CCD), the CCD was used to identify the relationship between the response functions and process variables as well as to find out the

conditions that optimized the extraction process. The experimental design and corresponding three response variables are presented in Table 1. This design consisted of 20 experimental points with six replicates at the central point. In the present study, according to the sequential model sum of squares, the highest order polynomials were utilized to select the models where the additional coefcients estimates were signifcant and the models are not aliased. Hence, for all three independent variables and responses, a quadratic polynomial model was selected and fitted well as suggested by the software.

Table 1: The experimental data obtained for the three responses based on the CCD matrix

RUN	X_1 (°C)	X_2 (min)	X_3 (%)	TPC Y_1 (mgGAE/g)	TFC Y_2 (mgCE/g)
1	50	120	90	318.15	8.32
2	60	100	80	318.05	8.18
3	60	100	80	318.48	8.17
4	70	120	70	318.76	8.01
5	60	100	80	318.16	8.17
6	76.82	100	80	331.61	7.95
7	43.18	100	80	311.98	8.03
8	60	100	96.82	315.01	8.29
9	60	66.36	80	311.16	8.25
10	60	133.64	80	318.11	8.21
11	50	80	70	294.65	8.02
12	70	80	70	317.65	8.15
13	60	100	80	318.08	8.15
14	50	120	70	312.62	7.93
15	70	120	90	318.27	8.16
16	70	80	90	327.83	8.12
17	60	100	80	318.39	8.16
18	60	100	63.18	300.93	7.99
19	60	100	80	318.09	8.17
20	50	80	90	311.67	8.27

The values of the two evaluation indices for each extracting condition were listed in Table 1. At extracting condition: 76.82°C, 80% ethanol concentration in 100min, the maximal TPC was 331.61 mgGAE/g and the maximal TFC was 8.32 mgCE/g at 50°C, 90% ethanol concentration in 120 min.

The final empirical regression model of their relationship between responses and the three tested variables for phenolic and favonoid contents could be expressed by the following quadratic polynomial equation [Eqs. (1–2)]:

$$Y_1 = 318.20 + 5.74X_1 + 2.03X_2 + 4.09X_3 – 4.11X_1X_2 – 1.61X_1X_3 – 2.27X_2X_3 + 1.34X_1^2 – 1.19X_2^2 – 3.54X_3^2 \quad (1)$$

$$Y_2 = 8.17 – 0.017X_1 – 0.015X_2 + 0.093X_3 – 0.065X_1X_3 + 0.04X_2X_3 – 0.061X_1^2 + 0.024X_2^2 – 0.008X_3^2 \quad (2)$$

Where Y_1 is total phenolic content, Y_2 is the total flavonoid content, X_1 is the temperature, X_2 is the time and X_3 is the solvent ratio (ethanol concentration ratio).

Table 2: Analysis of variance (ANOVA) for the model

Source	Y_1 – Total phenolic content			Y_2 – Total flavonoid content		
	Mean Square	F- value	p- value	Mean Square	F- value	p-value
Model	132.24	1586.23	< 0.0001***	0.027	181.35	< 0.0001***
X_1 (temperature)	450.45	5403.24	< 0.0001***	0.004	27.55	0.0004***
X_2 (time)	56.136	673.36	0.0001***	0.003	21.52	0.0009***
X_3 (solvent ratio)	228.96	2746.50	0.0001***	0.117	800.83	< 0.0001***
X_1X_2	135.30	1622.94	< 0.0001***	4.5E-004	3.08	0.1099^{NS}
X_1X_3	20.672	247.97	< 0.0001***	0.034	231.18	< 0.0001***
X_2X_3	61.38	736.29	0.0001***	0.013	87.55	< 0.0001***
X_1^2	26.00	311.95	0.0001***	0.054	370.82	< 0.0001***
X_2^2	20.34	244.00	0.0001***	0.008	54.51	< 0.0001***
X_3^2	181.06	2171.79	< 0.0001***	9.94E-004	6.80	0.0262*
Lack of Fit	0.13	4.06	0.0751^{NS}	1.857E-004	1.74	0.2787^{NS}
R^2	0.9993			0.9939		
C.V%	0.091			0.15		

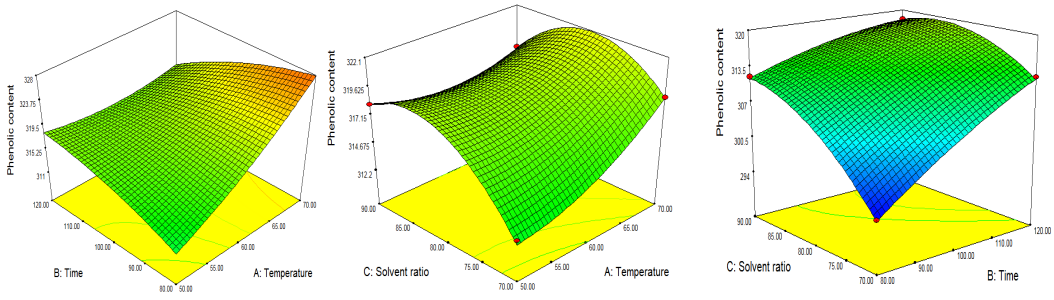
*p< 0.05; **p< 0.01; ***p< 0.001; NS: non-significant.

The RSM model coefcients were validated by analysis of variance (ANOVA) of the response variables for the quadratic polynomial model summarized in Table 2. The ANOVA analysis results for multiple regression and response surface quadratic model of Y_1 and Y_2 were evaluated using the corresponding p and R^2 values. F values of Y_1 and Y_2 were calculated to be 1586.23 and 181.35, both leading to a p value <0.05, suggesting both the models were statistically significant. The models' coefficient of determination (R^2) were 0.9993 and 0.9939, indicating that more than 99.93%; and 99.39% 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 Y_1 and Y_2 were 4.06 and 1.74, 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

Three factor that temperature, time and ethanol concentration effects the extraction condition of the maximum total phenolics and total favonoids content. This section discusses how these conditions work on natural antioxidants extraction. Three-dimensional model graphs were plotted as shown in the respective figures. The response surface plots of the model were done by varying two variables, within experimental range under investigation and holding the other variables at its central level.

3.2.1. Response surface analysis of total phenolic content



a) b) c)

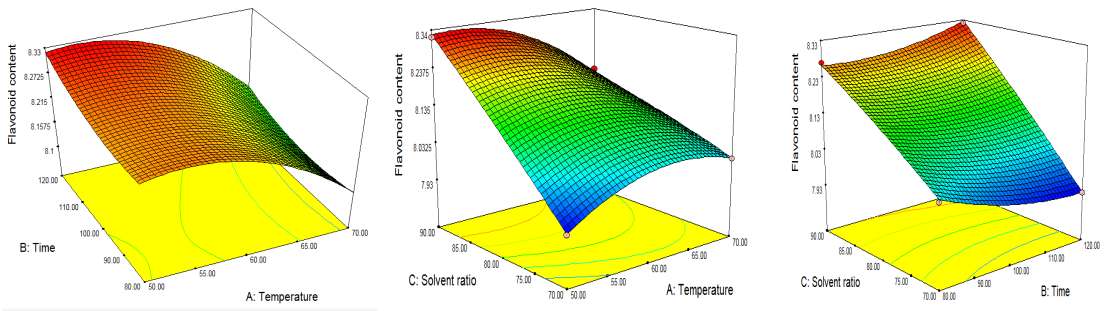
Figure 1: The response surface plot of TPC

The response surface plots for total phenolic extraction of *Launaea sarmentosa* leaves 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 2, all of three factors (extraction temperature, extraction time and ethanol concentration ratio) have showed negative quadratic effects ($p<0.0001$). In fig. 1a, The surface plot demonstrates the function of extraction temperature versus time effect on TPC at fixed ethanol concentration (80%). We can be observed that the yields of total phenolic content increased with the increase of extraction temperature from 50°C to 70°C and the maximum amount of phenolics can be achieved at the highest temperature of 65÷70°C at the shortest extraction time at 120 min. Higher solubility and diffusion coefficient of polyphenols were observed with increased temperature, allowing more extraction rate [11]. However, an upper limit of temperature must be respected in order to prevent decomposition of thermo sensitive phenolics during extraction [12]. These results are similar to a study reported by of Rajha et al. [13] which showed the total phenolics from grape by products increased with the increment of temperature and reduction of time.

The surface plot in Fig. 1b show the function of temperature versus solvent ratio effect on TPC at extraction time (120min). The yields of TPC increased with the increase of ethanol concentration from 70%v/v to 90%v/v and the maximum phenolic content in *Launaea sarmentosa* leaves can be achieved at highest ethanol concentration (90%). The higher phenolic content could be explained by the natural polarity of the solvents used [14]. Ethanol and water were used in this study because they are safer to handle as compared to other organic solvents and more importantly, they are acceptable for human consumption. Samuagam et al. [15] stated that a suitable solvent ratio is able to improve the effciency of extraction. The maximum total phenolic content in *Launaea sarmentosa* leaves can be obtained with optimum ethanol concentration and an extraction temperature of approximately 80÷90 v/v% and 65÷70°C respectively.

In Fig. 1c. The surface plots revealed that the higher TPC in *Launaea sarmentosa* leaves can be obtained when conducted at increasing ethanol concentration at fixed extraction time. Based on the result at constant extraction time of 120 min, 90% of ethanol concentrations yielded the most TPC as compared with 70% ethanol concentrations. These overall results of phenolic content indicate a similar trend as observed in the phenolic content in other study [16], [17], where the TP contents increased with increasing the independent variables ethanol concentration and processing time until a maximum amount of phenolic was reached

3.2.2. Response surface analysis of total flavonoid content



a) b) c)

Figure 2: The response surface plot of TFC

The 3D in Fig.2a shows the response surface plot of temperature (X_1) and time (X_2) at fixed extraction solvent ratio (80%). Response surface plot showed that extraction temperature exhibited a weaker efect whereas extraction time represented a relatively signifcant effect on the favonoids yield. An increase in the yield of favonoid could be signifcantly achieved with the increase of extraction time, at any level of extraction temperature. Therefore, the optimum amount of favonoid was achieved in this study at 50÷55°C and 110÷120 min of extraction time.

The 3D surface plots in Fig. 2b shows the interaction between extraction temperature (X_1) and solvent ratio (X_3) at the fixed 100 min. According to Bazykina et al. [18] favonoids and their glycosides are thought to be effciently extracted from plant materials by ethanol solvent. It was observed that the value of TFC in *Launaea sarmentosa* leaves increased when ethanol concentration was increased from 70 to 90v/v% at fixed 60°C extraction temperature. In contrast, increasing the extraction temperature at highest ethanol concentrations resulted to decreased, TFC values.

Fig. 2c shows the interaction between extraction time (X_2) and ethanol concentration (X_3) at the fixed extraction temperature at 60°C. An increase in ethanol concentration promoted the breakdown of the cell membrane that enhanced the permeability of the solvent into a solid matrix. In this study, highest favonoids content can be achieved when conducted at highest ethanol to water ratio 90% as compared with 30% with increasing extraction time. A great increase in the yield also resulted when extraction time was increased in the range of 80÷120min.

3.3. Optimization and Model Verification

The final result for the simultaneous optimization using the desirability function approach suggested that the optimal ethanolic extraction conditions for *Launaea sarmentosa* leaves extract were at 54°C with 110 min and 90% of ethanol concentration to achieve the best combination for highest total phenolic and favonoids content. Table 3 shows the predicted and experimental values for the extraction of target compounds from *Launaea sarmentosa* leaves. The actual values obtained from the experimental gave the extraction yields of total phenolic and total flavonoid as 318.85±0.32mgGAE/g and 8.21±0.14mgCE/g. These experimental values were close to the predicted values (TPC = 320.53mgGAE/g, TFC = 8.23mgCE/g) derived from the respective regression models with the CV ranging from 0.24% to 0.52%.

Table 3: Comparison between the predicted and experimental values for antioxidants from extracts of Launaea sarmentosa leaves

Condition	Response values	
	Total phenolic content mgGAE/g	Total flavonoid content mgCE/g
Predicted	320.53	8.23
Experimental	318.85±0.32	8.21±0.14
% Difference (CV)	0.52%	0.24%

4. CONCLUSION

Use response surface methodology (RSM) with central composite design (CCD) were successfully developed to determine the optimum process parameters and the second order polynomial models for predicting responses were obtained. The best combination of extraction temperature, time and ethanol concentrations were found to be 54°C with 110 min and ethanol concenration ratio 90% which rendered a mean phenolic content of 318.85±0.32 mgGAE/g and 6.12 ± 0.23 mgCE/g of total favonoid content from experimental run and thus indicated good antioxidant activities from the leaves of *Launaea sarmentosa*.

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