EFFECTS OF MICROWAVE-ASSISTED EXTRACTION ON SAPONIN CONTENT FROM *Schefflera heptaphylla*

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ABSTRACT

This study was conducted to determine the factors affecting the extraction yield of saponins from *Schefflera heptaphylla.* Surveyed factors include type of solvent (methanol, ethanol, water), material/solvent ratios (1/15, 1/20, 1/25, 1/30, and 1/35, w/v), microwave powers (150, 300, 450, 650, and 800 W) and microwave times (30, 60, 90, 120 and 150 sec). The extraction yield was shown by the saponin content obtained by UV-Vis spectroscopy. From there, optimize the saponin extraction conditions such as solvent/material ratio, microwave power, and time by response surface method (RSM). The results showed that at optimal conditions, the ratio of material/solvent ratio was 1/25 (w/v), microwave power 360.06W and 110.29 sec, and the obtained saponin content was 52.67 (mg/g_{db}).

Keywords: Extraction, microwave, optimization, saponins, *Schefflera heptaphylla*.

1. INTRODUCTION

Schefflera heptaphylla, growing wildly in many Vietnamese northern provinces, is considered the largest genus with 56 species. It is a source of natural compounds that own a variety of potential properties. Triterpenoids are the major and biologically active components in it. Thus, it has been used in traditional medicine for a long time to support some disorder treatments of inflammation, rheumatism, fever, bleeding after trauma, etc due to it being various biological activities, such as anti-inflammatory, antibacterial, antitumor, and antiviral. *S. heptaphylla* is combined with other medicinal herbs for the treatment of rheumatoid arthritis, bone pain, cold feet, and swollen feet [1], and its leaves have also been widely used in the treatment of traumatic hemorrhage in China [2]. It can increase the tolerance to hypoxia, anti-aging, increase physical strength and intelligence and anabolic processes, increase metabolism and promote regeneration, and clear sedation. In addition, it has anti-viral, anti-cancer, immunomodulatory effects, etc [3].

Saponin is a secondary metabolite synthesized from many different plant species that distribute throughout the bark, leaves, rhizomes, and flowers of plants. In recent years, saponins have received considerable attention due to their biological properties such as anti-tumor, anti-insect, hepatoprotective, hemolytic, and anti-inflammatory activities, lowering blood cholesterol levels. It has been used to make soaps, detergents, shampoos, beer, and cosmetics. It is said that young *S. heptaphylla* plants own higher saponin content than mature ones despite several impacted factors such as physiological state and environmental elements [4].

Saponin extraction using solvents has some disadvantages such as long time, high amount of solvent, and low extraction efficiency. Microwave-assisted extraction of saponins from

natural materials has been widely applied due to the reduction of extraction time and a significant increase in extraction yield [5]. The extraction process is affected by extraction factors such as solvent type, material/solvent ratio, power, and time microwave. Beside, one of the primary advantages of RSM is that a large amount of information is obtained from a limited number of experiments. Building models and graphical illustrations can study the main effects of variables and their interaction on the response. It determines the factor levels providing the optimum response and optimum conditions resulting from multiple responses. This study was carried out to determine the basic factors of microwave-assisted extraction as well as the optimal conditions for saponin extraction from *S. heptaphylla*, thereby offering the basic foundation for further research to apply this biological compound in a large-scale application.

2. MATERIALS AND METHODS

2.1. Materials

S. heptaphylla was picked in Huong Khe district, Ha Tinh province. The leaves are pale yellow when young, and when they are mature, they turn green. The leaves are oblong, growing in the form of double propellers, for each cluster of leaves, there will be 6-8 leaves of the same type. Evergreen plants grow year round if well cared for even in low light indoor conditions. After being harvested, *S. heptaphylla* would be washed and dried at 60 ºC until under 10% moisture content, then ground with dimension of powder about 0.15 mm and stored in PE bags at room temperature.

Chemicals: Distilled water, Methanol (China), oleanolic acid (USA), perchloric acid (India), vanillin (China), acetic acid (China), ethyl acetate (China). The chemicals in the study were at analysis grade.

Microwave oven Electrolux EMM2318X (800W), Hermale Z206A centrifuge centrifuge (50 mL tube), Spectrophotometer (UV-VIS) Genesys 10S UV-Vis, 6-/1-cell.

2.2. Methods

2.2.1. Effects of microwave on saponin extraction

The single-factor investigation includes solvent type, material/solvent ratio, capacity, and time. Experiments were conducted based on the principle of changing the parameters of one experimental factor and fixing the remaining factors, the results of previous experiments are used as fixed factors for subsequent experiments. After the extraction solution was centrifuged with a Hermale Z206A centrifuge (50 mL tube) at a speed of 5500 rpm for 10 min to remove the residue, the supernatant was determined the saponin content. The experiments were repeated 3 times. The response was the total saponin content in the extract (mg/g_{db}) [6].

Table 1. Experimental layout - to investigate the effects of microwave on the saponins extraction

Experiment	Fixed factor	Survey factor
Experiment 1. Effects of solvent type	Material weight: 1 mg/g_{db} Solvent concentration: 70% Material/solvent ratio: 1/30 w/v Temperature: 50°C Time: 120 min	Solvent type: MeOH, EtOH, H_2O

Experiment 2: Effects of microwave power	Material weight: 1 mg/g_{db} Solvent type: test result 1 Material/solvent ratio: 1/30 w/v Microwave time: 60s	Microwave powers: 150, 300, 450, 650 and 800W
Experiment 3: Effects of microwave time	Material weight: 1 mg/g_{db} Solvent type: test result 1 Microwave power: test result 2 Material/solvent ratio: 1/30 w/v	Times: 30, 60, 90, 120, and 150 sec
Experiment 4: Effects of material/solvent ratio	Material weight: 1 mg/g_{db} Solvent type: test result 1 Microwave power: test result 2 Microwave time: test result 3	Material/solvent ratios: 1/15, 1/20, 1/25, 1/30 and 1/35 w/v

In experiment 1, the convention method method will be used, which is by surveying solvents MeOH, EtOH, H_2O. When the solvent with the highest saponin content is selected, it will be used for the Electrolux Emm2318x microwave-assisted extraction method with the maximum microwave power: 800 W, adjusting the survey powers 150, 300, 450, 650 and 800W and fixed time is 60s, material/solvent ratio: 1/30. Continue to perform the same operation with experiments 3 and 4.

2.2.2. Optimization of microwave-assisted extraction by response surface method

The response surface method (RSM) was used to determine the influence of factors during microwave-assisted extraction on saponin content. The CCD complex center model was applied to optimize the saponin extraction conditions with 3 factors such as the ratio of material/solvent ratio X_1 (w/v), microwave power X_2 (W), microwave time X_3 (sec), and the dependent variable was saponin content (Y, mg/g_{db}) [6]. The experimental levels used are shown in Table 2.

Table 2. Experimental levels used in the RSM model

Variables	Low	Middle	High
Material/solvent ratio (X_1, w/v)	1/20	1/25	1/30
Power (X_2, W)	150	300	450
Time (X_3, sec)	60	90	120

The CCD design included 17 experiments (8 experiments factorial point, 3 center point, and 6 experiments at the axial points). The linear regression equation with quadratic form was determined by JMP 10 software:

$$Y(\%) = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_{12} + b_{22}X_{22} + b_{33}X_{32} + b_{12}(X_1X_2) + b_{13}(X_1X_3) + b_{23}(X_2X_3)$$

In there: b_0, b_1, b_2, b_3, b_{11}, b_{22}, b_{33}, b_{12}, b_{13}, b_{23} were the coefficients of the variables X_1, X_2, X_3, X_{11}, X_{22}, X_{33}, X_1X_2, X_1X_3, X_2X_3, respectively.

2.2.3. Saponin content determination

The oleanolic acid solution was mixed with a concentration of 1000 ppm, then put into test tubes with different volumes, then added vanillin-acetic acid (8%), perchloric acid (concentrated), and ethyl-acetate, to heat at 70°C for 20 min until the solution purple, and measure the absorbance by spectrophotometer (UV-VIS) Genesys 10S UV-Vis, 6-/1-cell at

550 nm [7]. 1 mL extract (exactly), 0.3 mL of vanillin-acetic acid solution (5%), and 1 mL of perchloric acid were mixed and incubated at 70°C, for 20 min. Then, the mixture was cooled with cold water for 2 min before adding ethyl acetate until 10 mL in the total volume. Saponin content was determined by colorimetric spectroscopy at a wavelength of 550 nm [8]. The results were expressed in mg/$g_{db.}$ by the following formula:

$$\text{Saponin content} = \frac{C_x \times V \times N \times 100}{m_{\text{dr.wt}}}$$

In there: C_x: Saponin concentration from the calibration curve (mg/mL), V: Volume of extract (mL), N: Dilution, $m_{dr.wt}$: Mass of dry matter in the raw material sample (g).

2.3. Data analysis

The experiments were repeated three times. The results were presented as mean ± SD. Using IBM SPSS Statistics 20.0 software to analyze experimental data and evaluate the difference between samples. Optimization experiments were processed using JMP 10 software. Charts were drawn with Microsoft Excel 2019 software.

3. RESULTS AND DISCUSSION

3.1. Effects of microwave on saponin extraction from *S. heptaphylla*

3.1.1. Effects of solvent type on saponin extraction

The selectivity, solubility, cost, and safety should be considered when selecting a solvent for biological compound extraction, saponins included. The similar polarity of solvents and the target compounds would result in better extraction performance. In this study, the effects of MeOH, EtOH, and distilled water on saponin extraction from *S. heptaphylla* were investigated (Fig.1).

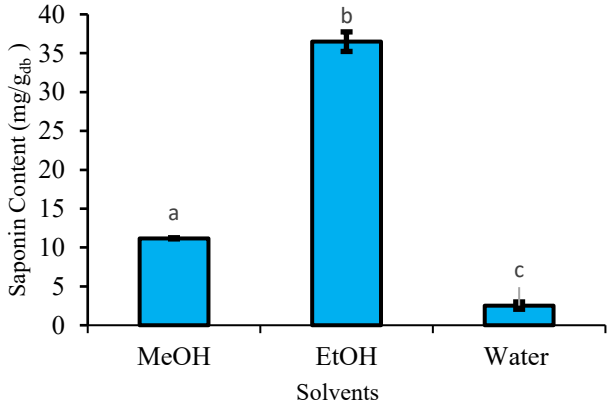

Fig. 1. Effects of solvents on saponin extraction
Note: Different letters in the column represent statistically significant differences at p < 0.05.

Saponin has a strong polarity with lots of -OH groups in its molecular structures, so it would dissolve well in highly polar solvents such as methanol, ethanol, n-butanol, etc. Distilled water was a solvent with higher polarity compared with the other two solvents, but it resulted in the lowest saponin content with 2.53±0.47 mg/g_{db} compared with 36.49 ±1.28 mg/g_{db} (70% EtOH) and 11.18±0.07 mg/g_{db} (70% MeOH). In addition, EtOH and MeOH rapidly denature, destroy cell membranes, and create favorable conditions for penetration and

contact with other active ingredients in raw materials [9]. That explains why the two organic solvents gave a higher saponin content than that of distilled water.

So, EtOH was selected for the next experiments. In line with the finding, Vaishali *et al.* also indicated that EtOH was better than MeOH and chloroform in extracting saponins and alkaloids from *Pogestemon patchouli* with saponin contents was 13.33% w/w (EtOH), 12.6% w/w (MeOH), and 7.16% (chloroform).

3.1.2. Effects of microwave powers on saponins extraction

The effects of five microwave power levels of 150, 300, 450, 650, and 800W were shown in Fig.2.

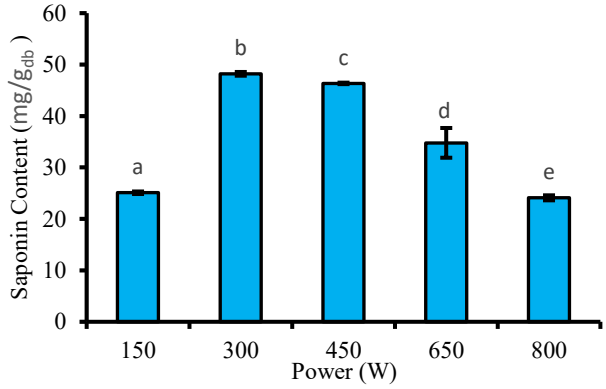

Fig. 2. Effects of microwave powers on saponin content

Note: Different letters in the column represent statistically significant differences at $p < 0.05$.

The saponin content increased quite significantly from 25.08 ± 0.25 mg/g_{db} (150W) to 48.20 ± 0.37 mg/g_{db} (300W). The saponin content was no statistically significant difference at the power range from 450 to 800W (46.34 ± 0.15 mg/g_{db}, 34.78 ± 2.89 mg/g_{db} and 24.09 ± 0.49 mg/g_{db}) (Fig. 2). The higher microwave power would create the greater the pressure that helps solvents penetrating the cells, increasing the mass transfer to the interface the upper cell wall. The surface and interior of the material were broken, and the target compounds escapes more easily. Once a certain equilibrium threshold and power level were reached, the saponin content would no longer increase significantly [11]. The study of Feng-Jie *et al.* also noted the same rule of microwave energy affecting extraction ability [12]. So, 300W power is chosen in this experiment.

3.1.3. Effects of microwave treatment time on saponin extraction

Besides the power factor, time is also a significant factor affecting the extraction of saponins, the microwave time to obtain the most optimal saponin content is shown in Figure 3.

Fig. 3 indicated that the saponin content increased significantly from 37.76 ± 0.39 mg/g_{db} (30 sec) and reached the highest point of 50.68 ± 4.03 mg/g_{db} (90 sec). Then, the longer time would not result in a higher saponin content or even a decrease significantly to 41.64 ± 64 mg/g_{db} at 150 sec. Microwave treatment enhanced the pressure exerted on the cell wall, the better the solvent penetrates cells, and the mass transfer ability. However, longer treatment times also increased other impurities together with saponins [13]. In this study, 90 sec of microwave treatment was selected for follow-up experiments.

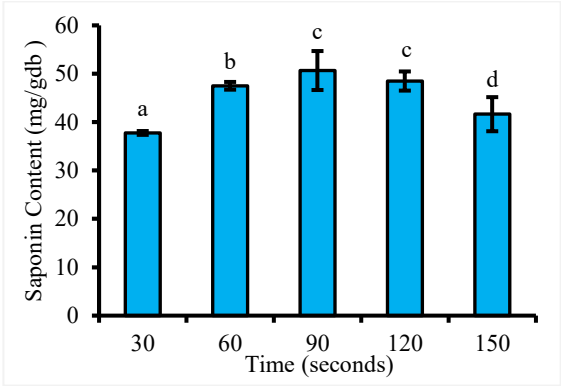

Fig. 3. Effects of microwave treatment time on saponin content
Note: Different letters in the column represent statistically significant differences at $p < 0.05$.

3.1.4. Effects of material : solvent ratios on saponins extraction

The ratio of raw material/solvent has certain interactions with each other and affects the ability to recover the saponin content of the extract of *S. heptaphylla*. The effects of the ratios of material/solvent were shown in Fig. 4.

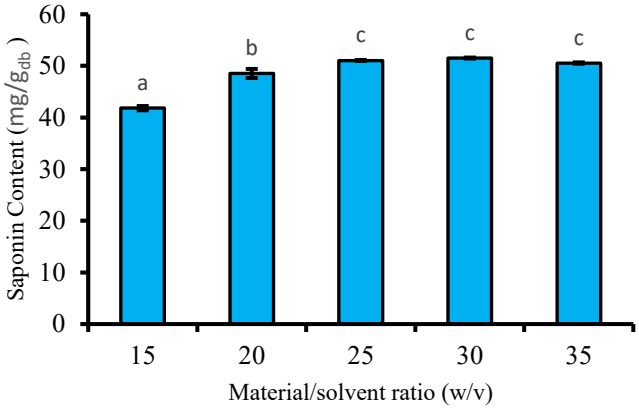

Fig. 4. Effects of material/solvent ratios on saponin content
Note: Different letters in the column represent statistically significant differences at $p < 0.05$.

The results from Fig. 4 showed that the saponin content was material/solvent ratio dependent. It increased significantly from 41.82 ± 0.41 mg/g_{db} (1/20 w/v) to 51.05 ± 0.05 mg/g_{db} (1/25 w/v) and remained at no significant difference at the ratios 1/30 and 1/35 (w/v). The process of dissolving biologically active substances into a solvent was physical. As the amount of solvent increased, bioactive substances were more likely to enter into the solvents, leading to higher permeability and faster and more efficient extraction [15].

3.2. Optimization of saponin extraction by EtOH from *S. heptaphylla*

In this study, the effects of the material/solvent ratio, microwave power, and microwave treatment time were the three selected parameters for optimizing the extraction. The results of obtained saponin content at the experimental levels were presented in Table 2.

Table 3. Experimental planning matrix and results

No.	Encoding variables			Unencoded variables			Saponin content (mg/g_{db})
	X_1	X_2	X_3	Material/solvent ratio (w/v)	Microwave power (W)	Microwave time (sec)	
1	-1	-1	-1	20	150	60	48.63
2	-1	-1	1	20	150	120	46.11
3	-1	1	-1	20	450	60	47.5
4	-1	1	1	20	450	120	48.35
5	1	-1	-1	30	150	60	49.47
6	1	-1	1	30	150	120	50.52
7	1	1	-1	30	450	60	49.65
8	1	1	1	30	450	120	51.79
9	-1.682	0	0	16.59	300	90	47.03
10	1.682	0	0	33.41	300	90	51.12
11	0	-1.682	0	25	47.73	90	48.3
12	0	1.682	0	25	552.27	90	49.56
13	0	0	-1.682	25	300	39.55	47.39
14	0	0	1.682	25	300	140.45	50.78
15	0	0	0	25	300	90	51.83
16	0	0	0	25	300	90	52.07
17	0	0	0	25	300	90	51.62

Table 4. The results of R_{adj}^2 và R^2 and Lack of fit

Summary of Fit				
R_{Square}	0.89			
$R_{Square\ Adj}$	0.77			
Lack Of Fit				
Source	DF	Sum of Squares	Mean Square	F_{Ratio}
Lack Of Fit	5	65.03	13.00	16.60

According to Jon *et al.* (2002), the experimental model was reliable when R^2 was in the range of 0.80 - 0.97; the closer the R^2 value was to 1, the better the regression function described the experimental results. In addition, the goodness of fit of the model was also evaluated by the F value of Lack of fit. The P value was used to test the significance of each regression coefficient. Specifically, factors with $P < 0.05$ were considered to influence on the response [16]. Among the 9 regression coefficients (except b_0), 2 regression coefficients that weren't significant with the confidence $P > 0.05$ as b_2 and b_{12}, this proved the interaction between X_2 and X_1X_2 had no significant effect on the response. For negative values, the regression coefficients b_{11}, b_{22}, and b_{33} were the factors that had a negative impact on the response, reducing the saponin content.

Regression coefficient b_1 had the largest positive value, showing that X_1 (material/solvent ratio) had the highest positive effect on the saponin content.

Table 5. The results of the significance analysis of the coefficients of the regression equation

Regression coefficient	Coeff. SC	Std. Err.	P-value
b_0	51.83	135.02	<0001*
b_1	1.30	7.20	0.0002*
b_2	0.34	1.90	0.0991
b_3	0.53	2.93	0.0219*
b_{12}	0.04	0.18	0.8619
b_{13}	0.60	2.58	0.0365*
b_{23}	0.55	2.37	0.0498*
b_{11}	-0.95	-4.77	0.0020*
b_{22}	-1.00	-5.03	0.0015*
b_{33}	-0.94	-4.75	0.0021*

Table 3 showed the influence of material/solvent ratio (X_1), microwave power (X_2), and microwave time (X_3) on the obtained saponin content. The regression equation was bellowed.

$$Y = 51.83 + 1.30X_1 + 0.53X_3 + 0.60X_{13} + 0.55X_2X_3 - 0.95X_1^2 - 1.00X_2^2 - 0.94X_3^2$$

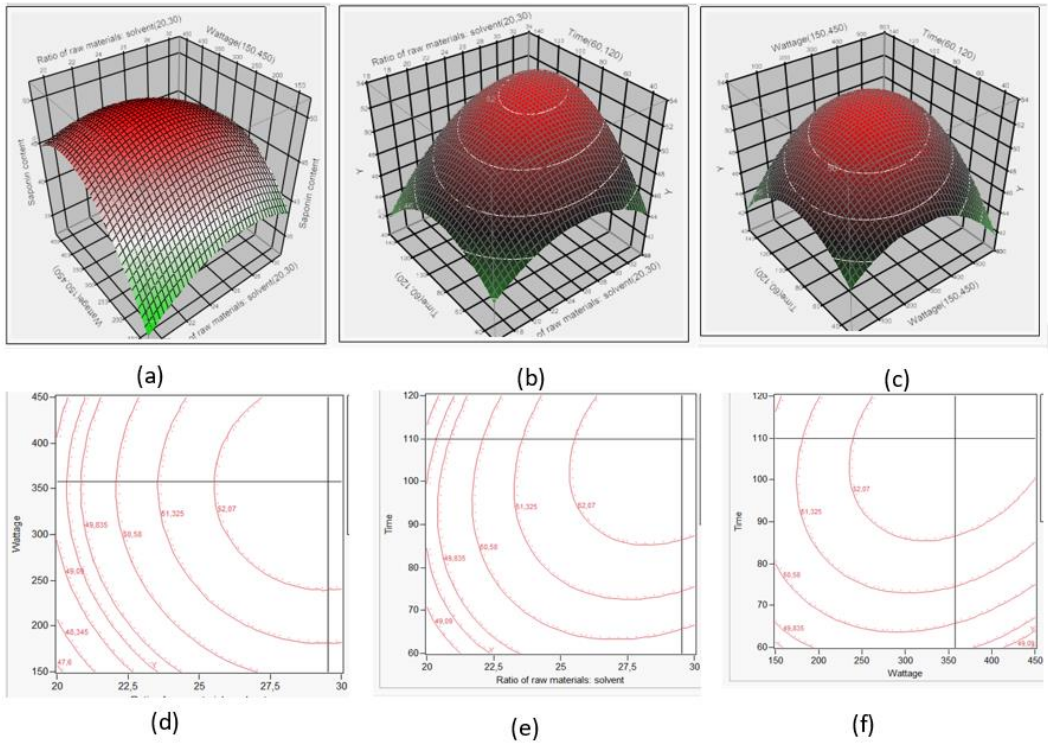

Fig. 5. Response surface models (a, b, c) and contour lines (d, e, f) show the influence of factors (material/solvent ratio, X_1; microwave power, X_2; microwave time, X_3) on saponin content; effect of material/solvent ratio and microwave power (a, d); effect of material/solvent ratio and microwave time (b, e); effect of microwave power and microwave time (c, f).

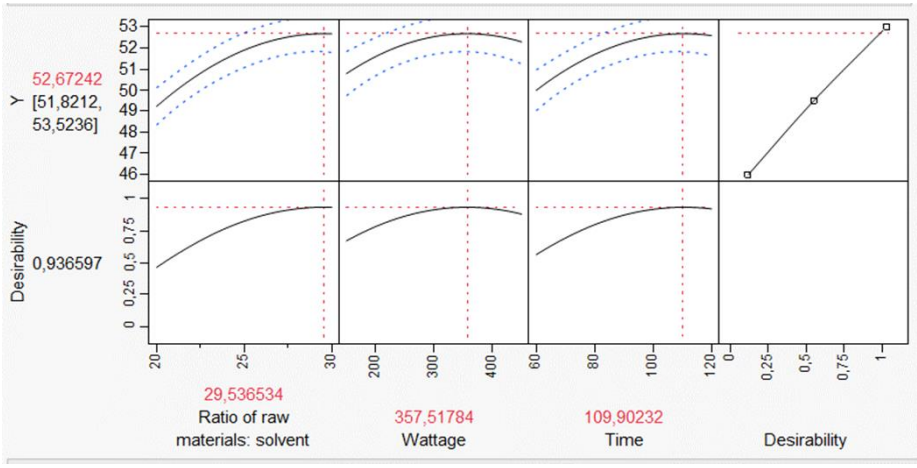

Fig. 6. Predictive model for saponin content

The maximum saponin content was 52.67 (mg/g_{db}) which was obtained at the extraction conditions including material/solvent ratio 1/25 (w/v), microwave power 360.06W in 110.29 sec of microwave treatment time. The response surface model showed the influence of the survey factors on the saponin content and predict the optimal conditions (Fig.5 and Fig.6).

After receiving the optimal extraction conditions, repeat the experimental experiment 3 times at the obtained optimal conditions, comparing the actual results and the predicted results from the optimal model. The experimental results indicated that the saponin content was 52.05 (mg/g_{db}), a difference of 2.99% (<5%) compared to the saponin content predicted from the regression equation. This showed that the experimental saponin content was not significantly different from the prediction from the quadratic regression model. Thus, the quadratic equation used was consistent with reality and practical value.

Similarly, in the study of Kwon *et al.* (2003), the optimal microwave-assisted saponin extraction for ginseng obtained the optimal conditions of 77.3% EtOH in the extraction treatment time of 3295.1 seconds [17]. Akbari *et al.* also optimized conditions for saponins, and phenolics extraction from *Trigonella foenum-graecum* seeds using the response surface method received the optimal conditions of 63.68% EtOH in microwave time 170.4 sec and microwave power 572.50 W. The results in the current study also showed that there was not a significant difference significance between the actual values and the predicted values, and the regression equation was suitable.

4. CONCLUSION

The study proved that ethanol was a suitable solvent to extract saponins from *S. heptaphylla*. The optimal conditions was obtained via the response surface method and the saponin content was determined by UV-Vis spectroscopy (mg/g_{db}). The results showed that the optimal conditions included material/solvent ratio 1/25 (w/v), microwave power 360.06 W, and treatment time 110.29 sec. With these conditions, the highest obtained saponin content was 52.67 (mg/g_{db}). Further studies should be done to evaluate the main biological compound and the biological properties of the extract.

Acknowledgments

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TÓM TẮT

ẢNH HƯỞNG CỦA VI SÓNG ĐẾN TRÍCH LY SAPONIN TỪ *Schefflera heptaphylla*

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Nghiên cứu này được thực hiện để xác định các yếu tố ảnh hưởng đến năng suất chiết xuất saponin từ cây ngũ gia bì chân chim *Schefflera heptaphylla.* Các yếu tố được khảo sát bao gồm loại dung môi (metanol, ethanol, nước), tỷ lệ nguyên liệu/dung môi (1/15, 1/20, 1/25, 1/30 và 1/35, w/v), công suất vi sóng (150, 300, 450, 650 và 800 W) và thời gian vi sóng (30, 60, 90, 120 và 150 giây). Hiệu quả chiết xuất được đánh giá qua hàm lượng saponin thu được đo bằng phương pháp quang phổ UV-Vis. Từ đó, tối ưu hóa các điều kiện chiết saponin như tỷ lệ dung môi/nguyên liệu, công suất vi sóng, thời gian bằng phương pháp bề mặt đáp ứng (RSM). Kết quả cho thấy ở điều kiện tối ưu, tỷ lệ nguyên liệu liệu/dung môi là 1/25 (w/v), công suất vi sóng 360,06W và 110,29 giây, hàm lượng saponin thu được là 52,67 (mg/g_{db}).

Từ khóa: Chiết xuất, vi sóng, tối ưu hóa, saponin, *Schefflera heptaphylla*.