OPTIMIZATION OF EXTRACTION OF POLYPHENOLS FROM VIETNAMESE *ZINGIBER OFFICINALE* RHIZOME

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ABSTRACT

Zingiber officinale is a popular herb in Vietnam, used as a spice and a traditional medicinal plant, especially its rhizome. The main chemical components are polyphenols. This study was conducted to optimize the extraction of polyphenols from Vietnamese *Zingiber officinale* rhizome using the magnetic-stirring assisted extraction method and response surface methodology. The solvent used in this study was ethanol 96%. The highest polyphenol content of (12.36±0.04) mg GAE/g dried material was obtained after triplicate repetition of extraction at the optimal conditions of the extraction process as follows: the solvent/dried material ratio (mL/g) of 35/1, the extraction time of 19 minutes, the stirring speed of 400 rpm at the room temperature. In addition, the study also determined the total flavonoid content in the extract by colorimetric method with aluminum chloride reagent and the antioxidant capacity through the DPPH (2,2-diphenyl-1-picryl hydrazyl radical) free radical scavenging method. The results showed that the total flavonoid content was (0.80±0.06) mg QE/g dried material, and 1 mL of the obtained extract inhibited (96.84 ± 0.26)% of DPPH free radical.

Keywords: Zingiber officinale, polyphenol, optimization, RSM, CCD.

1. INTRODUCTION

Zingiber officinale (*Z. officinale*), or ginger, is one of the herbs belonging to the Ginger family that has been widely used in food and traditional medicine. *Z. officinale* rhizome has been known as a natural source containing many polyphenols that are very helpful to health due to their biological activities [1–4].

There are many scientific studies related to the investigation of polyphenol extract methods and the determination of biological activities from *Z. officinale* in the world. For example, Sharif et al. studied the effects of maceration and reflux extraction methods in different solvents on the polyphenol content from *Z. officinale* [5]. Their results showed that between the two extraction methods, the maceration method gave the better polyphenol content; among the solvents, ethanol was the best solvent. Ezez D et al. investigated the effects of solvents on total phenolic content and antioxidant activity of ginger extracts [3]. The magnetic-stirring assisted extraction method was used at 900 rpm for 24 h at room temperature. Their data showed that among four different solvents (ethanol, methanol, acetone, and ethyl acetate), methanol gave the maximum phenolic content (10.22 - 11.84 mg GAE/g) and the highest DPPH radical scavenging activity (82.88% - 84.87%) for ginger rhizomes collected at two local markets in Ethiopia. Mukherjee S et al. optimized the extraction of polyphenolic antioxidants from *Z. officinale* using the continuous stirring extraction method [6]. The results demonstrated that the optimum conditions for the highest polyphenol content (16.20 mg

GAE/g) from 1 gram of ginger root were 75% ethanol, a temperature of 40 °C, and an extraction time of 60 min. Optimization of the ultrasound-assisted extraction method of the total phenolic compounds from *Z. officinale* was studied by Anuar A. et al. [7]. The results showed that the maximum phenolic content (22.33±0.25 mg GAE/g) from ginger rhizome was obtained at the optimum parameters as follows: a temperature of 80 °C, 468 mg ginger powder/20 mL solvent, the solvent concentration of 70%, and the extraction time had an insignificant effect on the extraction process. In a similar study, Murphy A et al. indicated that the optimum parameters for the obtained maximum total phenolic content (1039.64 mg GAE/g) from ginger powder purchased from the local supermarket in Ireland were 1.2 g of spice/20 mL of 86% ethanol, sonication time of 11 minutes at 65 °C [2].

In Vietnam, Trang Đ et al. reported that the extracts of some rhizomes of plants belonging to the Zingiberaceae family in An Giang province possessed antioxidant and antifungal ability [8]. Thy et al. evaluated the polyphenol content, antioxidant activity, and tyrosinase inhibitory activity of plant rhizomes belonging to the Zingiberaceae family in An Giang province [1]. The results showed that among the four rhizome extracts, *Z. officinale* extract had the great polyphenol content, the best DPPH free radical scavenging ability, and inhibited tyrosinase. However, optimization of the extraction conditions to obtain maximum polyphenols from Vietnamese *Z. officinale* has been limited. Most studies limited themselves to screening or evaluating the total polyphenol content in Vietnamese *Z. officinale* rhizome. Therefore, this study conducts experiments to achieve the optimal extract parameters to obtain the maximum polyphenol content from Vietnamese *Z. officinale* rhizome. The magnetic-stirring assisted extraction method was applied because it is easy to perform and cheap. The response surface methodology (RSM) was used to design the extract experiments because it is an optimization tool that can help recognize the interrelationship between variables in the extraction method from herbs [9,10]. The results will contribute to the polyphenol extraction theory of *Z. officinale* in Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

Fresh *Z. officinale* rhizomes were purchased from March to May 2024 at An Giang Province, Vietnam. After washing with water and removing the damaged parts, the *Z. officinale* rhizomes were washed with 96% ethanol and dried naturally. Then, these rhizomes were sliced thinly (Figure 1) and dried at 50 °C until their moisture content was less than 10%. All dried samples were ground into powder, classified using sieves with different diameters (500 µm, 300 µm, and 212 µm), and stored in plastic zipper bags at 4 °C for the next experiments.

Figure 1. Image of the (a) fresh and (b) sliced Vietnamese *Z. officinale* in this study

The chemicals used in this study were ethanol (96%, Vietnam), sodium carbonate (99.8%, Vietnam), Folin-Ciocalteu (Merck), gallic acid (≥ 98%, Merck), sodium hydroxide (96%, Vietnam), sodium nitrite (99%, China), aluminum chloride hexahydrate (97%, China),

quercetin dihydrate (98%, India), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Merck), ascorbic acid (P ≥ 99%, Merck) and distilled water that was supplied by the laboratory of Faculty of Chemical Engineering, Ho Chi Minh City University of Industry and Trade.

2.2. Methods

2.2.1. Magnetic-stirring assisted extraction method

The magnetic-stirring assisted extraction method was used in this study to extract the polyphenols from Vietnamese *Z. officinale* rhizomes. This extraction process was carried out as follows: First, weigh 1 gram of dried *Zingiber officinale* rhizome powder, ensuring it is of suitable size, and place it into a 100 mL Erlenmeyer flask. Next, add a specific volume (V mL) of 96% ethanol to the flask and cover it with an alumina membrane. The mixture was then placed on a magnetic stirrer and stirred at 400 rpm for a designated time (t minutes) at room temperature. After stirring, filter the mixture to collect the extract. The remaining residue was then treated with an additional volume (V mL) of solvent, and the extraction process was repeated two more times, making a total of three extractions. All extracts were combined, brought up to a final volume of 100 mL, and shaken thoroughly. Finally, the total polyphenol content of the extract was determined.

2.2.2. Optimization of extraction conditions and validation of the model

Analysis of variance and RSM on the experimental data was performed using Design Expert Version 13 Software. A Central Composite Design (CCD) was employed to find the optimal magnetic-stirring assisted extracting conditions of polyphenols from Vietnamese *Z. officinale* rhizomes (Table 1). The ratio of solvent/dried material (mL/g) and extraction time, named coded variables X_1 and X_2, respectively, were chosen for this study based on the preliminary study (data not shown). The only dependent variable was total polyphenol content, which was selected as a response. Each variable had two levels, -1 and +1. The matrix consisted of 13 experimental runs.

Table 1. CCD experimental design matrix

Experimental runs	Coded variables		Real variables	
	X_1	X_2	The ratio of solvent/dried material (mL/g)	Extraction time (min)
1	-1	-1	10	5
2	1	-1	40	5
3	-1	1	10	25
4	1	1	40	25
5	-1.41421	0	3.78680	15
6	1.41421	0	46.21320	15
7	0	-1.41421	25	0.85786
8	0	1.41421	25	29.14214
9	0	0	25	15
10	0	0	25	15

Experimental runs	Coded variables		Real variables	
	X_1	X_2	The ratio of solvent/dried material (mL/g)	Extraction time (min)
11	0	0	25	15
12	0	0	25	15
13	0	0	25	15

The mathematical models were evaluated using a multiple regression method. The response function applied was a second-order polynomial model as equation (1):

$$Y = K + AX_1 + BX_2 + ABX_1X_2 + A^2X_1^2 + B^2X_2^2 \quad (1)$$

in which Y is the dependent variable (total polyphenol content in this study), K is the intercept, A& B are the linear coefficients, AB is the interaction coefficient, and A^2& B^2 are the quadratic coefficients.

2.2.3. Determination of total polyphenol content

Total polyphenol content (TPC) was determined using the Folin-Ciocalteu method [1]. The standard of gallic acid was used in this study, and the calibration curve was plotted in the range of 0.25 – 7.50 µg/mL. The calibration curve equation was determined, y = 0.086x + 0.0076, with a correlation coefficient of $R^2 = 0.999$, in which y is the absorbance of the gallic acid solution and x is the concentration of gallic acid (µg/mL). TPC of the sample was calculated using the following equation (2). This value is expressed as milligram gallic acid equivalents per gram of sample (mg GAE/g).

$$\text{TPC (mg GAE/g)} = \frac{C \times V \times f}{m_m \times 10^3} \quad (2)$$

in which C is TPC in test sample calculated from the equation of gallic acid and the absorbance of the sample (µg/mL), V is volume of test sample (mL), f is diluted factor, and m_m is the mass of the initial sample (g).

2.2.4. Determination of total flavonoid content

Total flavonoid content (TFC) was determined using the aluminum chloride assay in the presence of sodium nitrite [11]. The experiment was conducted with a slight modification: Pipette accurately V mL of extract into a test tube containing 2.5 mL of distilled water. Add 0.15 mL of 5% sodium nitrite solution, shake, and rest at room temperature for 6 minutes. Then, add 0.3 mL of 10% aluminum chloride solution and rest for 5 minutes before adding 1 mL of 1 M sodium hydroxide. The mixture was made up to 5 mL with distilled water, shaken, and immediately measured its absorbance at 510 nm.

The standard of quercetin was used in this study, and the calibration curve was plotted in the range of 0.25 – 10.00 µg/mL. The calibration curve equation was determined, y = 0.1022x + 0.0605, with a correlation coefficient of $R^2 = 0.9985$, in which y is the absorbance of the quercetin solution and x is the concentration of quercetin (µg/mL). TFC of the sample was calculated using the following equation (3). This value is expressed as milligram quercetin equivalents per gram of sample (mg QE/g).

$$\text{TFC (mg QE/g)} = \frac{C \times V \times f}{m_m \times 10^3} \quad (3)$$

in which C is TFC in the test sample calculated from the equation of quercetin and the absorbance of the sample (µg/mL), V is the volume of the test sample (mL), f is the dilution factor, and m_m is the mass of the initial sample (g).

2.2.5. Determination of antioxidant ability

The antioxidant ability of the extract was determined due to its ability to scavenge DPPH radicals [12]. The experiment was conducted with a slight modification as follows: Pipette accurately 1 mL of extract. Then, add 2 mL of DPPH solution to the test tube. Finally, add 1 mL of ethanol. After being incubated for 30 minutes in the dark at ambient temperature, the mixture was measured for its absorbance at 517 nm. Ascorbic acid (3 µg/mL) was used as a positive control in this study. The DPPH free radical scavenging ability was evaluated using inhibitory percentage (I%). It was calculated using the following equation (4).

$$I\% = \left(1 - \frac{A_i}{A_0}\right) \times 100 \qquad (4)$$

in which A_i and A_o were absorbances of the tested sample and control sample in the same condition of the experiment.

2.2.6. Data analysis

All experiments were conducted in triplicate, and the data were analyzed using Microsoft Excel. Results were shown as mean ± SD (standard deviation).

3. RESULTS AND DISCUSSION

3.1. Optimization of extraction conditions and validation of the model

Many scientific studies have investigated the effect of a single factor on the polyphenol extraction process, such as the particle size of the material, the kind of solvent, the solvent composition, the ratio of solvent/material, the extraction time, the extraction temperature, … [2,3,5,10,13]. However, it takes a lot of time to investigate each factor one by one, and the obtained result is the best value among the tested values. When using an optimization method, such as RSM, we can evaluate the effect of multiple factors on the extraction; thus, the obtained extract conditions based on a predictive model have been more optimal to achieve the best response value [14–17]. The TPC results of 13 extraction conditions according to the CCD experimental design matrix are displayed in Table 2. The TPC values obtained from the actual experiment and the prediction model of 13 experimental runs were not significant. The highest and lowest TPC values were in experiments 13 and 5, respectively.

The ANOVA is shown in Table 3. Evaluation of the fitness of the model was determined by the coefficient of R-squared (R^2) and the *F*-test for lack of fit [9]. As shown in Table 3, the R^2 value of 0.9589 indicated that 4.11% of the variability of TPC could not be explained by the predictive model. Usually, with $R^2 \geq 0.8$, the predictive model and experimental data are evaluated as a relatively high correlation [2]. The R^2 value in this study was higher than 0.8, which showed a high fit between the two models. Thus, this model could be used to assess the effect of two factors (the ratio of solvent/material and extraction time) on the TPC from Vietnamese *Z. officinale* rhizomes. In addition, the predicted R^2 value of 0.8162 is consistent with the adjusted R^2 value, the difference is less than 0.2. The lack of fit value of the model was 0.5871, higher than 0.5, which indicated that the predictive model is very consistent with the experiment. This proves that there is a good correlation between the experimental data and

the predicted data by the model (Table 2), thereby showing the reliability of the built model. The model had a high *F*-value (*F*-value = 32.70) and a low *p*-value ($p < 0.05$), which demonstrated that the predictive model is highly reliable.

Table 2. Results of 13 extraction conditions according to the CCD experimental design matrix

Experimental runs	Real variables		TPC (mg GAE/g)	
	The ratio of solvent/dried material (mL/g)	Extraction time (min)	Experimental	Predicted
1	10	5	8.28	8.33
2	40	5	11.38	11.24
3	10	25	10.60	10.29
4	40	25	12.01	11.90
5	3.78680	15	7.91	7.86
6	46.21320	15	11.90	11.92
7	25	0.85786	9.13	9.44
8	25	29.14214	11.54	11.68
9	25	15	11.32	11.85
10	25	15	11.71	11.88
11	25	15	12.09	11.93
12	25	15	12.10	11.94
13	25	15	12.19	11.99

Table 3. ANOVA for fitting a quadratic model of polyphenol extraction from Vietnamese *Z. officinale* rhizomes

Source	Sum of squares	df	Coefficients	Mean square	*F*-value	*p*-value
Model	26.12	5		5.22	32.70	0.0001
Intercept		1	11.88			
A	12.88	1	1.27	12.88	80.66	< 0.0001
B	5.05	1	0.7948	5.05	31.63	0.0008
AB	0.7140	1	–0.4225	0.7140	4.47	0.0723
A^2	5.35	1	–0.8766	5.35	33.46	0.0007
B^2	3.05	1	–0.6616	3.05	19.06	0.0033
Residual	1.12	7		0.1597		
Lack of fit	0.5871	3		0.1957	1.47	0.3485
Pure error	0.5311	4		0.1328		
Correlation total	27.24	12				

Source	Sum of squares	df	Coefficients	Mean square	*F*-value	*p*-value
Standard deviation	0.3997	R^2		0.9589	Adeq Precision	15.2018
Mean	10.94	Adjusted R^2		0.9296		
C.V %	3.65	Predicted R^2		0.8162		

The *p*- and *F*-values of each regression coefficient are used to test the significance of each coefficient, thereby indicating the interaction between variables in the model. The smaller the *p*-value, the more significant the coefficient, and it can be concluded that the corresponding variables affect the TPC. The coefficients A, B, A^2, and B^2 were significant with a *p*-value < 0.05. However, the coefficient AB was not significant with a *p*-value > 0.05. Therefore, in the range of evaluation, TPC was affected by the first-order and second-order of both factors (X_1 and X_2), but was not significantly affected by the correlation of the factor pair X_1X_2. The final equation in terms of coded factors was:

$$Y = 11.88 + 1.27X_1 + 0.7948X_2 - 0.8766X_1^2 - 0.6616X_2^2$$

The final equation in terms of real factors was: TPC (mg GAE/g) = 11.88 + 1.27 *(The ratio of solvent/dried material (mL/g)) + 0.7948*(Extraction time (min)) – 0.8766*(The ratio of solvent/dried material^2 (mL/g)) – 0.6616*(Extraction time^2 (min))

The 3D surface plot for TPC of Vietnamese *Z. officinale* rhizomes is shown in Figure 2. The colors blue, green, yellow, and red represent the increasing order of TPC values.

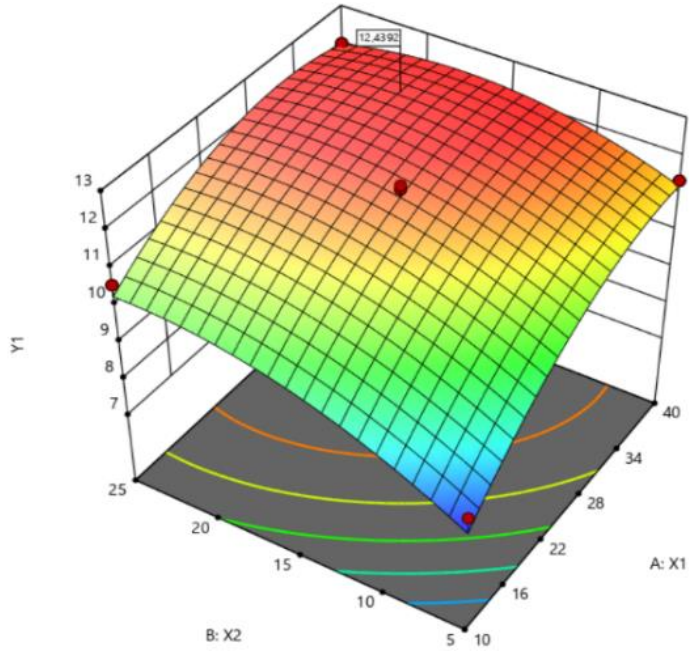

Figure 2. 3D surface plot showing the effects of solvent-to-material ratio (mL/g, X-axis) and extraction time (minutes, Y-axis) on total polyphenol content (TPC, mg GAE/g, Z-axis) of Vietnamese *Zingiber officinale* rhizomes. The color scale indicates increasing polyphenol content from blue (low) to red (high).

In addition, it can also be seen that an independent variable affects TPC when another variable is fixed. For example, when the ratio of solvent/material (mL/g) was increased, TPC increased and reached a maximum at the ratio (mL/g) of 34.588/1. This is because at lower ratios, the amount of the solvent is not high enough to extract all the polyphenols in the sample; however, at higher ratios, the ability to dissolve polyphenols from the sample into the solvent is not increased, thus it is not necessary to use a much larger amount. When the extraction time was increased, TPC also increased and reached a maximum at 18.863 minutes. It is explained that at shorter extraction times, the polyphenols in the sample do not have enough time to interact and dissolve into the solvent, resulting in a low TPC. On the contrary, when the extraction time is extended, the interaction and dissolution of polyphenols into the solvent will occur more thoroughly, thereby increasing TPC. However, at extraction times longer than 18.863 minutes, TPC does not increase significantly because the amount of polyphenol in the solvent has reached saturation.

The highest TPC (12.439 mg GAE/g) of Vietnamese *Z. officinale* rhizomes from the model was obtained at the optimal conditions as a ratio of solvent/material (mL/g) of 34.588/1 and extraction time of 18.863 minutes. This study tested the optimal condition from the model by conducting experimental triplicate with the ratio of ethanol/material (mL/g) of 35/1, extraction time of 19 minutes at a stirring speed of 400 rpm. The result showed that the obtained TPC was 12.36 ± 0.04 mg GAE/g. This result is equivalent to the prediction from the model; the error is less than 5% compared to the model prediction. Therefore, the model in this study is completely suitable and meaningful for polyphenol extraction from Vietnamese *Z. officinale* rhizomes.

3.2. Total flavonoid content

The TFC of the obtained extract in this study was determined as 0.80 ± 0.06 mg QE/g. According to Manuhara Y et al. [18], the TFC of Indonesian *Z. officinale* was 0.52 ± 0.05 mg QE/g. This value was much lower than that in this study. Therefore, it allows conclude that Vietnamese *Z. officinale* has great potential for application due to its high content of natural active compounds.

3.3. Antioxidant ability

The extract from Vietnamese *Z. officinale* under optimal conditions in the study was evaluated for its antioxidant ability through its ability to scavenge DPPH free radicals. The results are presented in Table 4.

Table 4. Inhibitory percentage (I%) of extract and ascorbic acid

Sample	$I_{average}$ ± SD (%)
Z. officinale extract (1 mL)	96.84 ± 0.26
Ascorbic acid (3 µg/mL)	94.92 ± 0.17

Data from Table 4 showed that Vietnamese *Z. officinale* extract possessed a strong ability to scavenge DPPH free radicals; 1 mL of this extract could inhibit 96.84% of free radicals. This ability was equivalent to the DPPH free radical scavenging ability of 1 mL of ascorbic acid (3 µg/mL). Therefore, Vietnamese *Z. officinale* extract has strong biological activity, can be applied in pharmaceuticals, cosmetics, and functional foods.

4. CONCLUSION

This study applied the magnetic-stirring assisted extraction method and RSM to optimize the polyphenol extract conditions from Vietnamese *Zingiber officinale* rhizome. The predictive model was tested on fitness and reliability based on ANOVA. The TPC value at the optimal conditions was verified by experiment, the result showed that the error was less than 5%, thereby the model is completely suitable and meaningful for polyphenol extraction. The obtained extract was also evaluated the TFC and antioxidant ability. Compared to the previous study and the positive control, respectively, the extract in this study indicated that the Vietnamese *Zingiber officinale* rhizome has great potential for application in food, cosmetics, and medicine.

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TÓM TẮT

TỐI ƯU HÓA QUÁ TRÌNH THU DỊCH CHIẾT GIÀU POLYPHENOL TỪ THÂN RỄ GỪNG VIỆT NAM

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Gừng *(Zingiber officinale)* là một loại thảo dược phổ biến ở Việt Nam, được sử dụng làm gia vị và làm thuốc theo y học cổ truyền, đặc biệt là thân rễ. Thành phần hóa học chính là các polyphenol. Nghiên cứu này được tiến hành nhằm tối ưu hóa quá trình thu dịch chiết giàu polyphenol từ thân rễ gừng Việt Nam bằng phương pháp chiết xuất hỗ trợ khuấy từ và phương pháp bề mặt đáp ứng. Dung môi được sử dụng trong nghiên cứu này là ethanol 96%. Hàm lượng polyphenol cao nhất (12,36 ± 0,04) mg GAE/g nguyên liệu khô thu được sau khi lặp lại quá trình chiết ba lần ở các điều kiện tối ưu của quy trình chiết xuất như sau: tỷ lệ dung môi/nguyên liệu khô (mL/g) là 35/1, thời gian chiết 19 phút, tốc độ khuấy 400 vòng/phút ở nhiệt độ phòng. Ngoài ra, nghiên cứu cũng xác định tổng hàm lượng flavonoid trong dịch chiết bằng phương pháp so màu với thuốc thử nhôm clorua và khả năng kháng oxy hóa thông qua phương pháp loại bỏ gốc tự do DPPH (2,2-diphenyl-1-picryl hydrazyl). Kết quả cho thấy, tổng hàm lượng flavonoid là (0,80 ± 0,06) mg QE/g nguyên liệu khô và 1 mL dịch chiết thu được có khả năng ức chế (96,84 ± 0,26)% gốc tự do DPPH.

Từ khóa: Zingiber officinale, polyphenol, tối ưu, RSM, CCD.