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# Optimization of Extraction Conditions for Phenolic Compounds Extracted from Cassumunar Ginger (*Zingiber montanum*)

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**Abstract:** Phenolic compounds are found in plant materials and regarded as an important human dietary element with a wide range of biological activities and health benefits. Extraction of phenolic compounds can be done by various extraction methods and conditions, but the phenolic compounds yield will be affected by many factors. Therefore, the aim of this study is to evaluate the effect of three parameters (solid-to-solvent ratio, extraction temperature, extraction time) on total phenolic compounds (TPC) yield, and to optimize the extraction conditions for phenolic compounds extracted from cassumunar ginger. In this study, ethanol extraction (1/5 to 1/15 g/ml), extraction temperature (30 to 70 °C) and extraction time (30 to 180 min). While, response surface methodology (RSM) with Box-Behnken design was utilized for the optimization of extraction condition. The results showed the three investigated factors significantly affected TPC yield (p-value < 0.05). TPC yields were increased as the function of solid-to-solvent-ratio and temperature, but the TPC yield tended to be stable at prolong extraction time from 105 to 180 min. However, the optimum extraction condition of phenolic compounds extracted from cassumunar ginger was at solid-to-solvent ratio of 1/15 g/ml, extraction temperature of 70 °C, and extraction time of 143.5 min. These results are useful by providing the optimum condition for phenolic compounds extraction from cassumunar ginger rhizome and other rhizomes, and useful data for developing extraction process.

Keywords: Optimization; Solvent extraction; Phenolic compounds; Cassumunar ginger



# 1. INTRODUCTION

In science, there are various significant methodologies in the mathematic field for dealing with issues on the efficiency of any systems. Among those methodologies, process optimization

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is one of a powerful mathematical technique that can be employed for integration process, and can provide the best solution [1]. Currently, many researches have been used Response Surface Methodology (RSM), which is a common mathematical and statistical method for modeling and analyzing a process that is impacted by various variables [2]. Whereas, Box-Behnken design (BBD), is the main experimental design that has been used frequently in the design of experiments. This is because it can generate high response with experimental runs lesser than other traditional factorial techniques [3].

In extraction processes, there are many factors that can affect the extraction yield including extraction time, temperature, solid-to-solvent ratio, solvent type, and so on. Theoretically, extraction processes can be described by two concepts such as equilibrium and mass transfer rate, as the process will rely on how rapidly the compound dissolves and reaches the equilibrium state in liquid phase [4]. For instance, the extraction rate can be enhanced by increasing the concentration gradient or increasing the diffusion coefficient. Several researches had conducted studies on the effect of solidto-solvent ratio, temperature, and extraction time, and they found that these parameters are the prominent factors that can cause significant changes in extraction yield extracted from plant materials. For example, in the studies of Wong et al. [5] and Anuar et al. [6], solid-to-solvent ratio and temperature significant impacted on the yield of phenolic compounds and anthocyanin extracted from plants. As increasing these two parameters during the extraction could result in high extraction yields. However, this tendency could not always increase at prolong extraction time because the extraction process can reach an equilibrium constant at any period. This means a positive or negative effect of each parameter in extraction processes are not always apparent. Hence, the effect of each extraction parameter should be always evaluated in order to maintain the high extraction yield of bioactive compounds extracted from plants.

Under Zingiberaceae family, the genus of Zingiber consist of around 85 species, and many of them are consumed or used medicinally [7]. Among them, cassumunar ginger (Zingiber *montanum*), is one of aromatic plant that mostly found in tropical and subtropical Asia countries. Extracts from cassumunar ginger obtained from either ethanol [8] or methanol [9] extraction were reported to have high antioxidant capacity and activity. In addition, it is observed that this rhizome contains several phytochemicals with biological activities and pharmacological effects. Polyphenols or phenolic compounds are found to be rich in many rhizomes, consequently many studies have recently emphasized on phenolic compounds extraction from rhizomes. However, the extraction is challenging because the presence of phenolic compounds in plant are typically trapped in insoluble vacuole structures of plant cells and lipoprotein bilayers. Additionally, the compounds can be binded to sugars and proteins, or be esterified with organic acids, acetylated, or polymerized derivatives [10]. Consequently, a suitable extraction technique should be used to enhance the extraction efficiency. Conventional solvent extraction is a simple method for the extraction of phenolic compounds from solid matrices. In general, the solvent's property can facilitate a physical carrier to transfer the target molecules between the solid, liquid, or vapor phases [11].

In this study, the effects of solid-to-solvent ratio, temperature, and extraction time on phenolic compounds extracted from cassumunar ginger rhizome were investigated. Specifically, optimization of the extraction condition by applying Response surface methodology (RSM) with Box-Behnken design is also undertaken in this work. Because conventional solvent extraction technique has low sensitivity, large ranges of solid-to-solvent ratio (1:5 to 1:15 g/ml), temperature (30 to 70 °C), and time (30 to 180 min) are chosen in this study.

# 2. METHODOLOGY

#### 2.1. Chemicals

Chemical reagents used in this study were analytical grade. Those reagents were ethanol (Merck, Germany), gallic acid (Himedia, India), Folin-ciocalteau (Sigma Aldrich, Switzerland), and sodium carbonate (Merck, Denmark).

#### 2.2. Sample collection and preparation

In this study, cassumunar ginger (*Zingiber montanum*) as given in Fig. 1 was collected from local farm at Phnom Kulen  $(13^{\circ}42'01.5"N 104^{\circ}02'22.9"E)$ , Siem Reap province in January 2022. The raw material was initially cleaned to remove soil and then sliced into small pieces prior to be freeze dried at - 55 °C for 48 h [12]. Freeze-dried sample (moisture content of 7.0 %) was then ground into powder, using for the extraction of the phenolic compounds.



Fig. 1. Cassumunar ginger (Zingiber montanum) used in this work

#### 2.3. Experimental design

Response Surface Methodology (RSM) with Box-Behnken design (BBD) was used to optimize the extraction condition. The design of experiment for three parameters and statistical analysis were randomly generated using JMP 16 software. Table 1 illustrates the settings of three independent variables including solid-to-solvent ratio  $(X_1)$ , temperature  $(X_2)$ , and extraction time  $(X_3)$  at three levels. Through this design of experiment, there are total of 30 runs including six center points and duplication.

**Table 1** Condition of independent variables for RSM with Box-Behnken design

Independent variables		Levels			
	-1	0	1		
$X_1$ : Solid-to-solvent ratio (g/ml)	1/5	1/10	1/15		
$X_2$ : Temperature (°C)	30	50	70		
$X_3$ : Time (min)	30	105	180		

With the three parameters and three levels using the RSM with BBD, yield of phenolic compound has correlation with linear, interaction, and quadratic coefficients as given in Eq. 1. The adequacy and significance of model was identified by Analysis of Variance (ANOVA).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$
(Eq. 1)

Where *Y* is response (yield of total phenolic compound),  $\beta_0$  is intercept,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  are linear coefficient,  $\beta_{12}$ ,  $\beta_{13}$ ,  $\beta_{23}$  are interaction coefficient,  $\beta_{11}$ ,  $\beta_{22}$ ,  $\beta_{33}$  are quadratic coefficient, and  $X_n$  is independent parameter.

#### 2.4. Extraction of phenolic compounds

Extraction of phenolic compounds from cassumunar ginger was carried out using solvent extraction method with assistance from water bath shaker (SSB-18, SciLab, Korea). Initially, 1 g of cassumunar ginger powder was mixed with ethanol (5 to 15 ml), then the mixture was shaken in the water bath at different extraction temperatures (30 to 70 °C) and extraction times (30 to 180 min). After that, liquid (extract) was separated from solid residue using centrifugation (Hermle, Germany). The liquid was further removed solid residue using membrane filter before subjected to the analysis of phenolic contents.

#### 2.5. Analysis of total phenolic compounds

Folin–Ciocalteu colorimetric method was applied to analyze total phenolic content (TPC) in the extract. The procedure of the colorimetric method was based on procedure reported by Sepahpour et al. [13]. In this method, UV-vis spectrophotometer was used and the content of TPC in the extract was reported as mg GAE/g DW, where GAE is gallic acid equivalent and DW refers to dried weight sample.

# 2.6. Statistical analysis

Design of experiment and all statistical data were generated and analyzed by JMP 16 software. Data analysis was carried out in duplicated. One-way ANOVA (Analysis of Variance) was expressed as the statistical analysis to determine the significance of differences in the means at the 95 % confident level.

# 3. RESULTS AND DISCUSSION

#### 3.1. Yield of total phenolic compounds

Table 2 shows the result of total phenolic content (TPC) extracted from cassumunar ginger at various levels of solid-tosolvent ratio, extraction temperature, and extraction time. Through the solvent extraction method, it is found that TPC yield extracting from the cassumunar ginger ranged from 3.92 to 7.85 mg GAE/g DW. The highest TPC was found at extraction condition of using solid-to-solvent ratio of 1/15 g/ml, extraction temperature of 70 °C, and extraction time of 105 min, whereas the lowest TPC was obtained at solid-to-solvent ratio of 1/5 g/ml, temperature of 50 °C, and extraction time of 30 min. The results can be influenced by the effect of each extraction condition (solid-to-solvent ratio, time, and temperature) and the interaction effect of those selected extraction parameters.

#### 3.2. Effect of solid-to-solvent ratio

Fig. 2 shows the effect of solid-to-solvent ratio on the extraction yield of total phenolic compounds (TPC) extracting from cassumunar ginger. It is observed that TPC yield increased with increasing solid-to-solvent ratio. This result can be compatible with the mass transfer principle, as the concentration gradient between the solid and the solvent is thought to be the driving force for mass transfer. A high solid-to-solvent ratio could contribute to enhance concentration gradient, then improving the diffusion rate and enabling high extraction rate [14]. Similarly, Zhang et al. [15] reported that high amount of solvent can increase the contact between solid and solvent, then increasing amount of solute releasing from solid material; however, this trend will be constant once an extraction equilibrium is reached. Similar to this work, a previous study also found that increasing ethanol amount in the extraction led to an increase in the extraction yield of TPC from Phyllanthus niruri [5]. In general, the primary impact of solid-to-solvent ratio is related to the changes of solubility and equilibrium constant between solid, solute, and solvent, and it can result in greatest total phenolic yield at the optimum solid-to-solvent ratio of extraction condition.

#### 3.3. Effect of extraction temperature

Fig. 3 illustrates the effect of temperature on TPC yield extracting from cassumuanar ginger using ethanol extraction method. Result showed that TPC yield is increased with increasing extraction temperature from 30 °C to 70 °C. In

general, extraction temperature is an important factor which influences the extraction of phenolic compounds. Increasing temperature could promote the solute solubility and coefficient of diffusion during extraction. Evidently, the ethanolic extraction of TPC from ginger (*Zingiber officinale Roscoe*) was significant increased with the increase of extraction temperature from 40 to 100 °C [16]. This phenomenon could be induced by heating process which provides energy to molecules in the system to vibrate, weakening interactions between compounds, disrupting cell membranes, and lastly spilling out desired compounds from the cell compartment into the solvent [6].

Table 2 Total phenolic content (TPC) in cassumar ginger extracts

Dung	Inde	pendent va	Total phenolic	
Kulls -	$X_1$	$X_2$	$X_3$	— content [mg GAE/g DW]
1	0	0	0	5 74
2	Ő	Ő	Ő	5.25
3	0	-1	1	5.06
4	1	0	-1	5.20
5	1	-1	0	4.63
6	0	0	0	6.03
7	-1	0	1	3.94
8	0	-1	-1	4.49
9	1	1	0	6.21
10	1	0	1	6.52
11	0	1	-1	5.36
12	-1	0	-1	4.33
13	-1	1	0	4.08
14	-1	-1	0	4.71
15	0	1	1	4.96
16	0	0	0	5.98
17	0	0	0	5.69
18	0	-1	1	5.89
19	1	0	-1	5.27
20	1	-1	0	5.33
21	0	0	0	6.04
22	-1	0	1	4.71
23	0	-1	-1	4.46
24	1	1	0	7.85
25	1	0	1	6.83
26	0	1	-1	6.01
27	-1	0	-1	3.92
28	-1	1	0	4.45
29	-1	-1	0	4.25
30	0	1	1	5.97

 $X_1$ : solid-to-solvent ratio (g/ml),  $X_2$ : extraction temperature (°C),  $X_3$ : extraction time (min)

#### 3.4. Effect of extraction time

The result of total phenolic content (TPC) extracted under various extraction times (30, 105, and 180 min) is indicated in

Fig. 4. TPC yield was increased markedly between the extraction time of 30 and 105 min. This tendency may be related to the increase of extraction time allowing more solvent to contact with the plant material, breaking the plant cells and causes the solvent to penetrate more easily into the solid matrix. Then, a huge quantity of solutes was released from the sample cell leaking into the solvents at the longer time of extraction.



**Fig. 2.** Effect of solid-to-solvent ratio on extraction yield of total phenolic compound (TPC)



**Fig. 3.** Effect of extraction temperature on extraction yield of total phenolic compound (TPC)

However, TPC yield extracted from cassumanar ginger was almost constant when the extraction time was increased from 120 to 180 min. The extraction process was observed to reach an equilibrium constant, showing that the extraction time used to extract phenolic compounds from the sample was sufficient. In comparison to previous study which used microwave-assisted extraction method, it has been reported that TPC extracted from ginger rhizome yielded the higher concentration using only 1 min of extraction time [17]. According to their finding, nonconvectional method, microwave-assisted extraction took shorter extraction time and offered better yield of phenolic compounds than conventional methods utilized in this study. However, operation cost can be much different between the two extraction methods. Economical study should be taken into account to apply the extraction methods as practical stage.



Fig. 4. Effect of extraction time on extraction yield of total phenolic compound (TPC)

#### 3.5. Optimization using RSM with Box-Behnken design

#### 3.5.1. Model fitting and statistical analysis

In this work, Response Surface Methodology (RSM) with Box-Behnken Design (BBD) was used to find the optimum extraction conditions for phenolic compounds from cassumunar ginger. The effect of three independent parameters on the response, such as solid-to-solvent ratio, extraction temperature, and extraction time, was examined. It is found that solid-tosolvent ratio, extraction temperature, and extraction time were all significant effect (*p*-value < 0.05) on the TPC yield (see Table 3).

Model fitting has been used to exhibit the RSM mathematical model's accuracy in predicting the optimum variance and relationship between the parameters. Statistical values previewed in Tables 3 and 4 were used to identify the fitted of model, and the regression model of relationship between response and different factors. The mathematical model is represented in following equation:

Y = 5.78 +	$0.84X_{1} +$	$0.37X_2 +$	$0.30X_3 +$	$0.56X_1X_2 +$	$0.31X_{I}X_{3} -$
$0.305X_2X_3$	$-0.39X_1^2$	$-0.20X_2^2$	$-0.30X_3^2$		(Eq. 2)

where Y is yield of TPC,  $X_1$  is solid-to-solvent ratio,  $X_2$  is extraction temperature, and  $X_3$  is extraction time.

It is observed that linear effect of solid-to-solvent ratio, extraction temperature, and extraction time exhibited significant and positive effect (*p*-value < 0.0001 and  $\beta_1 = 0.84$ ; *p*-value = 0.0029 and  $\beta_2 = 0.37$ ; *p*-value = 0.0138 and  $\beta_3 = 0.30$ ). This does not imply that increasing solid-to-solvent ratio, temperature, or extraction time will always enhance TPC yields; nevertheless, it may result in undesirable effects such as denaturation or oxidation of phenolic compounds.

In interaction term, it is noticed that there was a significant positive effect (*p*-value = 0.0019,  $\beta_{12} = 0.56$ ) between solid-to-solvent ratio and temperature variables, meaning that once these two variables are increased, the response (TPC yield) will be increased either. Whereas the interactions effect of temperature and extraction time showed a negative effect ( $\beta_{23} = -0.305$ ) and not statistically significant effect (*p*-value = 0.0686).

For quadratic term, only solid-to-solvent ratio and extraction time are the most significant parameters since the *p*-value of them are smaller than 0.05. As a result, it might be claimed that increasing levels of all three parameters provided a negative influence (decreased TPC yields) on the extraction of phenolic compounds from cassumunar ginger.

Table 4 depicts the analysis of variance (ANOVA), which includes the model value, lack of fit, pure error, cor total, and determination coefficient (R<sup>2</sup>). All values are necessary for the evaluation of the model's efficiency. Principally, a test for regression model's significance and model coefficients with lack of fit was done in order to fit a satisfactory model. A greater Fvalue and lower p-value (prob > F) meant that the associated coefficient was more significant [18]. According to the ANOVA data given in Table 4, the R<sup>2</sup> value was 0.84, indicating that the model explained 84 % of total variation. The model is regarded acceptable (fitted model), when the coefficient of determination  $(\mathbb{R}^2)$  is equal to or greater than 80 % [19]. Hence, it means the model is well-fitting to the actual experimental data. Moreover, F-value and *p*-value of this model were 11.63 and lower than 0.0001, respectively, indicating that the model is considered to be significant. Furthermore, the *p*-value of lack of fit tests was greater than 0.05 (*p*-value = 0.72), showing that this result was not significant. This pointed out that regression models could be used to predict the response (TPC yield). Based on the obtained results, it can be assumed that phenolic compounds were impacted by all extraction parameters, including solid-to-solvent ratio, temperature, and extraction time. Additionally, the model was assumed to be adequate enough for predicting TPC yield extracted from cassumunar ginger rhizome.

#### 3.5.2. Response surface plot

Response surface plots are defined as three-dimensional data diagrams using to display the relationship between response and two independent factors, as well as to determine the optimum combinations of the two parameters. Figs. 5 (A), (B), and (C) showed three response plots, and each of them previewed the effect three independent variables, while one parameter was regulated as constant at zero level. The colors in diagrams represent differently, as the red, green, and blue color demonstrate the maximum, average, and minimum yields of phenolic compounds, correspondingly.

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Table 3	Regression	coefficients	OT	second	order	polvi	iomiai
	regression	ecenterento	· ·	seeona	01001	Porj.	

Variables	Regression coefficient	Standard error	<i>p</i> -value
Intercept	•••••		
β	5.78	0.18	< 0.0001
Linear			
β <sub>1</sub>	0.84	0.11	< 0.0001
β,	0.37	0.11	0.0029
β <sub>3</sub>	0.30	0.11	0.0138
Interaction			
β <sub>12</sub>	0.56	0.15	0.0019
β <sub>13</sub>	0.31	0.15	0.0645
β <sub>23</sub>	-0.305	0.15	0.0686
Quadratic			
β <sub>11</sub>	-0.39	0.16	0.0274
β <sub>22</sub>	-0.20	0.16	0.2232
$\beta_{33}^{22}$	-0.30	0.16	0.0783

**Note:** '1', '2', and '3' represents the solid-to-liquid ratio, temperature, and extraction time, respectively

 Table 4 Analysis of variance (ANOVA) for response surface quadratic model

Source	Sum of	DF	Mean	F-	<i>p</i> -value
	squares		of	value	(Prob >
			square		F)
Model	21.03	9	2.33	11.63	< 0.0001
Lack of	0.29	3	0.09	0.44	0.72
fit					
Pure	3.72	17	0.21		
error					
Cor	25.05	29			
total					
$R^{2} =$					
0.84					

\*DF = Degree of freedom

As can be observed in Fig.5 (A), TPC yield increased by the function of solid-to-solvent ratio and temperature. It is observed that the combination of these two elements had a good effect on TPC yield. Surface plot of the effect of solid-to-solvent ratio and extraction time on TPC yield was depicted in Fig.5 (B).



**Fig. 5.** Response surface plots: (A) solid-to-solvent ratio and temperature, (B) solid-to-solvent ratio and extraction time, and (C) extraction time and temperature

It was noticed that increasing solid-to-solvent ratio from 1/10 to 1/15 g/ml and the extraction time from 105 to 180 min can cause the increase of the yield of TPC. It seems that to obtain the maximum yield of TPC from cassumunar ginger rhizome, a high-level of solid-to-solvent ratio and longer extraction time was crucial factor to be considered. For Fig.4 (C), it showed the effect of extraction time and temperature on TPC yield. The yield of TPC is increased when extraction time increased from 30 to 105 min and extraction temperature increased from 30 to 70 °C. However, when prolonging the extraction time from 105 to 180 min, the TPC yield started to decrease gradually. So, it is appeared that increasing extraction time while increasing temperature could not give a positive effect to the extraction of phenolic compounds from cassumunar ginger.

Based on the depicted plots, it can be observed that the optimum condition for extracting phenolic compounds from cassumunar ginger was predicted to be at the temperature of 70 °C, solid-to-solvent ratio of 1/15 g/ml, and extraction time of 143.5 min. The predicted value at the optimal condition which was optimized by RSM performing the statistical software revealed the maximum value of total phenolic compounds was 7.05 mg GAE/g DW.

# 5. CONCLUSION

In the study, response surface methodology with Box-Behnken design was applied to optimize the extraction conditions for phenolic compounds from cassumunar ginger. At the designed extraction conditions, total phenolic compounds extracted from the cassumunar ginger was in the range of 3.92 to 7.85 mg GAE/g DW. The effect of solid-to-solvent ratio, temperature, and extraction time were also investigated. It showed that these parameters were found to be the most important factor impacting the phenolic compounds extraction. Based on statistical analysis, the model fitted the data well, meaning it is possible to predict extraction yield of phenolic compounds in the designed ranges of extraction condition. After performing optimization, the optimum condition for extraction phenolic compounds were at 1/15 g/ml of solid-to-solvent ratio, 70 °C of temperature, and 143.5 min of extraction time. The findings of this study are beneficial and reliable for future research and upscale the extraction by providing an optimum condition for extracting phenolic compounds from cassumunar ginger rhizomes or other rhizomes to produce a greater yield. However, in terms of economic and time consuming at practical stage, further study should be considered.

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