

Optimisation of Essential Oil Yield and Zerumbone Content in Zingiber Zerumbet Extract Using Hydrodistillation Process

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The optimisation of zerumbone content in essential oil of Zingiber zerumbet was investigated using hydrodistillation extraction method. The extractions were carried out to identify the optimum condition of the processing parameters (solvent-to-solid loading ratio, particle size and time) on the essential oil and zerumbone recovery. The extraction variables (solvent-to-solid ratio, particle size and time), on the other hand, were optimised by central composite design. The optimum conditions of the Z. zerumbet essential oil extraction were at the solvent-to-solid loading ratio, 187 mL/10 g; particle size, 2,000 μm ; and extraction time, 74.28 min, whereas the concentration of Z. zerumbet essential oil and zerumbone as high as 3.73 % and 1.58 % were obtained. Hence, the central composite design model can be used to predict the zerumbone content in essential oil extraction from Z. zerumbet in a hydrodistillation extraction system.

1. Introduction

Zingiber zerumbet (Z. zerumbet) is an herbal species from ginger family and widely cultivated in South East Asia countries (Rashid and Pihie, 2005). It has been used as a traditional medicine to treat swelling, sores and loss of appetite. Z. zerumbet, on the other hand, has been used to treat enterobiasis (Somchit et al., 2003). The essential oils of Z. zerumbet have been studied by many researchers, whereas zerumbone has been reported as major constituents contained in the essential oils (Bhuiyan et al., 2009).

The identification of zerumbone in the essential oil was known to have certain medicinal properties such as anti-inflammatory activity and anti-tumor activity (Ghasemzadeh et al., 2017). Based on the previous studies, zerumbone was demonstrated to inhibit colonic tumor marker formation in rats and induces apoptosis in colon cancer cell lines (Murakami et al., 2004). Zerumbone has been reported as major components present in the essential oil compared to other components such as α -caryophyllene and camphene (Bhuiyan et al., 2009).

Many extraction methods have been used in the recovery of bioactive compounds from plant materials, such as hydrodistillation, steam distillation, soxhlet and simultaneous distillation extraction (Lucchesi et al., 2004). The hydrodistillation method is the most commonly used since it is economically viable and safe. This method showed that the amount of bioactive compounds of the plant materials was only influenced by specific processing parameters (Mohamed et al., 2004). Further studies on the influence processing parameters to extract the target bioactive compounds in the optimum yield, need to be carried out.

It is important to observe the processing parameters of extraction such as solvent-to-solid ratio, particle size and extraction time, in order to obtain the optimal yield of zerumbone in essential oil extraction from Z. zerumbet. In the present study, response surface methodology (RSM) was used to optimise the extraction conditions of zerumbone from the essential oil. In this methodology, mathematical and statistical approaches are combined for designing experiments, building models, correlating the effects of processing parameters and identifying the optimum condition of processing parameters for desirable yield (Liu et al., 2015).

The work on optimisation of zerumbone extraction from the essential oil of Z. zerumbet is still very limited from literature. This study was conducted to investigate the effects of processing parameters (extraction time, solvent-to-solid ratio and particle size) on the yield of essential oil extract and the yield of zerumbone by using RSM.

2. Materials and methods

2.1 Chemicals

A standard chemical of zerumbone with 98 % purity was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade), ethanol (95 %), ethyl acetate, methanol (HPLC grade), n-hexane and potassium dihydrogen phosphate were obtained from Qrec (New Zealand).

2.2 Plant material

Z. zerumbet, a traditional medicinal plant material, was purchased from a local farm in Batu Pahat, Malaysia. The plant materials were identified by the Herbarium, Universiti Putra Malaysia (UPM), Bangi, Malaysia. The fresh rhizomes of *Z. zerumbet* were thoroughly washed which were then cut into 1 cm lengths. The cut-rhizomes were oven-dried for three days at 50 °C. The dried rhizomes were grounded into powder by using commercial grade grinder (Model EBM-9182, ELBA, Malaysia). The powder was passed through a sieve filter of 250 µm, 500 µm, 710 µm, 1,000 µm and 2,000 µm, and was stored in a sealed container prior to analysis.

2.3 Extraction of *Z. zerumbet* Essential Oil

The extraction of *Z. zerumbet* essential oil was conducted using a hydrodistillation extraction method. The dried plant samples were immersed in 100 mL of an extraction solvent composed of water. The crude extracts were then heated to 100 °C and evaporated using a rotary evaporator to retrieve the essential oil. The essential oil extracts were then stored in vials at 4 °C prior to analysis.

2.4 Experimental Design

2.4.1 Processing Parameters

The extractions were conducted by manipulating three parameters including time (minutes, x_1), solvent-to-solid ratio (mL/10 g, x_2) and particle size (µm, x_3). The range for the time, solvent-to-solid ratio and particle size was set according to Central Composite Design (CCD) with three factors at five levels ($-\alpha$, -1, 0, +1 and $+\alpha$) as presented in Table 1. The performance of extraction process was evaluated in terms of *Z. zerumbet* essential oil (Y_1) and zerumbone content (Y_2). There were 63 runs of experiment in total with 5 central points in this design. Experimental data were fitted to the quadratic model Eq(1):

$$Y = B_0 + \sum_{i=1}^k B_i x_i + \sum_{i=1}^k B_{ii} x_i^2 + \sum_{i=1}^k \sum_{j=i+1}^k B_{ij} x_i x_j + e_i \quad (1)$$

where Y is the response, x_i and x_j are variables (i and j range from 1 to k), B_0 is the intercept coefficient, B_i is the linear coefficient; B_{ii} is the quadratic coefficient and B_{ij} is the second-order terms coefficient, k is the number of independent variables and e_i is the error.

Table 1: Processing parameters of the extraction of *Z. zerumbet* 's essential oil

Parameter	Level				
	$-\alpha$	-1	0	1	$+\alpha$
Extraction time (min)	51.66	60	90	120	128.34
Solvent-to-solid ratio (mL/10g)	136.1	150	200	250	263.9
Particle Size (µm)	2,000 – 2,800 (A), 710 – 1,000 (B), 250 - 500 (C)				

2.5 Statistical analysis

All the experimental results were analysed using the statistical analyses such as multiple regressions analysis and analysis of variance (ANOVA). These analyses were performed using Design Expert software version (Version 6.0.6, Stat-Ease Inc., USA). Multiple regressions analysis was used to analyse the relationship between several independent parameters and a dependent parameter. F-test was used to evaluate the significance of regression coefficients (R^2). The design modelling with quadratic model was developed. The adequacy of model is determined by R^2 , adjusted R^2 and prediction error sum of squares (PRESS) by using ANOVA. Response surface plots and contour plots were developed from the regression model.

In this study, the optimisation was performed to optimise the yield of *Z. zerumbet* essential oil and zerumbone content. The optimisation for the extraction process was evaluated based on the desirability value (D). By setting the target of processing parameters of the optimisation, the function can find out the optimum value for each variable by satisfying the requirement of all responses. By setting the target of parameters of the optimisation, the function can find out the optimum value for each variable by satisfying the requirement of all responses. The

validity of the model was conducted by using the predicted optimised values. The experimental value was then compared with the predicted value for confirmation.

2.6 Determination of zerumbone

The concentration of zerumbone was determined using a gas chromatography system (Perkin Elmer, Autosystem XL, USA), equipped with a flame ionisation detector, split injector and a capillary column of fused silica (HP-5, 30 m × 0.25 mm × 0.25 µm, J&W Scientific, USA). The column temperature was heated to 50 °C for 5 min and programmed at 5 °C/min to 280 °C. The separation was performed by using nitrogen as carrier gas at the flow of 1.7 mL/min while the injector and detector temperature were maintained at 240 °C and 280 °C. The standard solution was prepared by dissolving 1 mg of zerumbone in 1 mL of methanol. The stock standard solution was diluted with different dilution factors to prepare a desired concentration of standard solutions (15, 20, 25, 30, and 35 µg/mL). Solutions were filtered by using nylon membrane filters with 0.45 µm pore size before GC-FID injection.

3. Results and discussion

3.1 Statistical Analysis and Model Fitting

Table 2 shows ANOVA results and regression equation coefficients. Quadratic model is significant for the extraction process as the p-value is less than 0.05. The F-value of the yield Z. zerumbet essential oil (31.25) and the zerumbone content (39.11) from the ANOVA analysis has further proven the significance of the model. In this study, the R^2 of the yields of Z. zerumbet essential oil and zerumbone are 0.93 and 0.94, which indicate that the goodness of the fit for the quadratic model is satisfactory. The probabilities of regression (p-value) for both yields of Z. zerumbet essential oil and zerumbone are less than 0.05, which indicates the multiple regression relationship between the independent variables and responses are statistically significant. The lack of fit, which investigates the fitness of the model, shows p-value more than 0.05, indicating the model is sufficiently accurate for the prediction of the responses. The significance of the factors can be observed by the p-value, where the smaller the p-value is, the more significant the factor is. Extraction time is the most significant factor that affects the yield of Z. zerumbet essential oil; whereas time, solvent-to-solid ratio and particle size are the most significant factors that affect the yield of zerumbone.

Table 2: Analysis of variance (ANOVA) results for quadratic model

Source	Sum of Square	Degree of freedom	Mean Square	F-value	Probability > F
Yield of Z. zerumbet essential oil ($R^2 = 0.9305$)					
Model	9.55	9	1.06	31.25	<0.0001
Residual	0.71	21	0.034		
Lack of Fit	0.25	5	0.051	1.77	0.1751
Pure Error	0.46	16	0.029		
Cor Total	10.26	30			
Yield of zerumbone ($R^2 = 0.9437$)					
Model	12.72	9	1.41	39.11	<0.0001
Residual	0.76	21	0.036		
Lack of Fit	0.31	5	0.062	2.21	0.1042
Pure Error	0.45	16	0.028		
Cor Total	13.48	30			

The relationships between the responses of the extraction process (yield of Z. zerumbet essential oil and zerumbone content) with the processing parameters (time, solvent-to-solid ratio and particle size) are described by second order equations in terms of coded factors as presented in Eq(2) and (3). These equations show the polynomial equations for the yield of Z. zerumbet essential oil (Y_1) and yield of zerumbone content (Y_2). The processing parameters, time, solvent-to-solid ratio and particle size, are denoted as x_1 , x_2 and x_3

$$Y_1 = 3.49 + 0.35 x_1 + 0.16 x_2 - 0.024 x_3 - 0.59 x_1^2 - 0.61 x_2^2 + 0.71 x_3^2 + 0.090 x_1 x_2 + 0.17 x_1 x_3 - 0.23 x_2 x_3 \quad (2)$$

$$Y_2 = 1.26 - 0.78 x_1 - 0.24 x_2 + 0.36 x_3 + 0.18 x_1^2 + 0.027 x_2^2 - 0.66 x_3^2 + 0.27 x_1 x_2 - 0.21 x_1 x_3 - 0.20 x_2 x_3 \quad (3)$$

3.2 Effect of Extraction Parameter on yield of *Z. zerumbet* essential oil

The effect of the extraction variables on the yield of *Z. zerumbet* essential oil (%) and zerumbone (%) can be explained by response surface plot. The response surface plots show the relative effects of any two variables in three-dimensional plot, while the rest of the parameter is kept constant. Figure 1 shows the effect of each parameter on the yield of *Z. zerumbet* essential oil. Figure 1a shows the yield of *Z. zerumbet* essential oil increases when the solvent-to-solid ratio is increased from 150 to 200 mL/10 g. When a larger ratio is used, the capacity of solvent to diffuse into the plant cells is higher, which will accelerate desorption of phytochemicals from the cells (Lau et al., 2014). This process is consistent with mass transfer principles where the concentration gradient between solid and solvent is the driving force for the mass transfer during extraction (Bulduk et al., 2015). The yield of *Z. zerumbet* essential oil decreased when the solvent-to-solid ratio was further increased from 200 to 250 mL/10 g. The similar result was reported in the extraction of *Ingaedulis*; where increasing of ratio solvent-to-solid above 200 mL/10g could limit the extraction yield (Silva et al., 2007). This implies that the extraction yield is limited by the total available solute when excessive solvent is used.

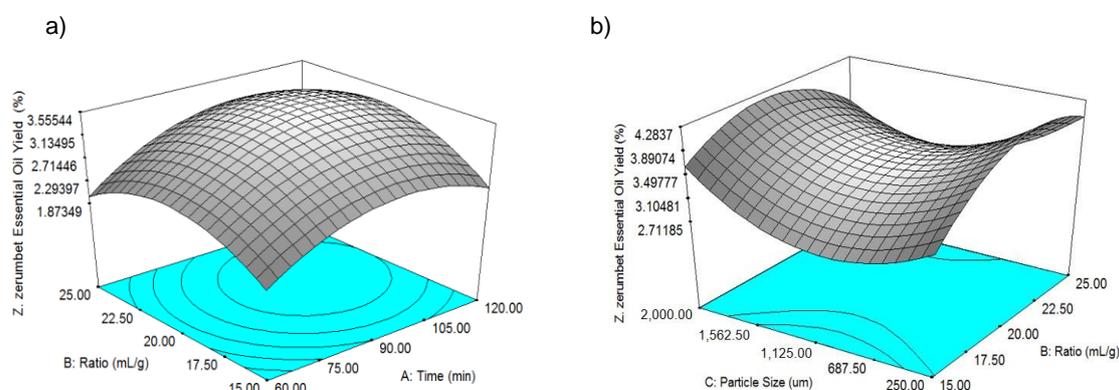


Figure 1: Response surface plots of yield of zerumbone (%) versus (a) solvent-to-solid ratio (mL/g) and time (min) (b) solvent-to-solid ratio (mL/g) and particle size (μm)

Figure 1b shows the interaction between solvent-to-solid ratio and particle size was significantly exist where at solvent ratio 200 mL to 10 g and using particle size 2,000 μm , *Z. zerumbet* essential oil are highly obtained. This is because the rate of diffusion between water and plant particles increases when the solvent-to-solid ratio and particle size are increased, which will disrupt the cell walls of plant particles and aid the release of essential oil into the solvent. The yield of *Z. zerumbet* essential oil decreased when the solvent-to-solid ratio was further increased from 200 to 250 mL/10 g. This may be due to the oil cells that are not rebuilt due to fully rupture of parenchyma cell wall (Buang et al., 2013).

The yield *Z. zerumbet* essential oil increases when the extraction time is increased from 60 to 90 min and it was found to be decreased as the extraction time was further increased from 90 to 120 min. This condition can be described by the Fick's second law of diffusion, indicating that a final equilibrium between the solute concentrations in the plant matrix and in the solvent might be reached after a certain time, leading to reduction in the extraction *Z. zerumbet* essential oil yield. Furthermore, prolonged extraction time accelerates the decomposition and oxidation of essential oil components due to the long exposure to unfavourable environmental factors such as temperature, light and oxygen (Tan et al., 2013).

3.3 Effect of extraction parameter on yield of zerumbone contain in *Z. zerumbet*

Figure 2a shows a reduction in the yield of zerumbone when the solvent-to-solid loading ratio is increased. This might be due to the amount of zerumbone decreases as the solvent-to-solid loading ratio is further increased. Similar results were reported in many studies where the total bioactive compounds of plant material had no significant change in higher solvent-to-solid loading ratio (Lau et al., 2014).

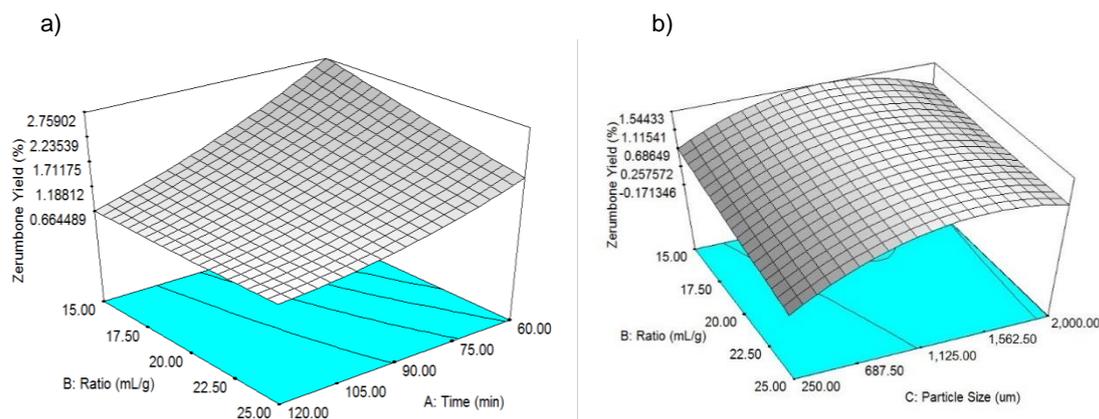


Figure 2: Response surface plots of yield of zerumbone (%) versus (a) solvent-to-solid ratio (mL/g) and time (min) (b) solvent-to-solid ratio (mL/g) and particle size (μm)

Figure 2b illustrates the yield of zerumbone increases when the plant particle size is increased. This is because when the particle size ranges from 250 to 2,000 μm is used, the ability of solvent to diffuse into the plant particle cell is higher, which will elevate the removal of zerumbone from the plant particles cells. Besides that, smaller particle size could also enhance the dissolution rates of zerumbone from the plant particle cell into the bulk fluid. This might be due to the larger surface area allows more plant particle exposed to the surrounding bulk fluid (Naveena et al., 2005).

The yield of zerumbone was observed to be decreased as the extraction time was further increased to 120 minutes. This is because prolonged extraction contributes to deterioration in the zerumbone due to the degradation of zerumbone compounds (Wang and Wei, 2015). The increased extraction time is not economic and time consuming from the industrialisation perspective, also potentially increasing the loss of solvent via vaporisation which directly affects the loss of solvent-to-solid ratio of extraction (Tan et al., 2013).

4. Conclusion

In this study, the optimum conditions of zerumbone extraction from the essential oil of *Z. zerumbet* were obtained through the hydrodistillation extraction method, whereas the centre composite design was employed to optimize these conditions. The optimal conditions suggested by design expert to obtain the highest yield of essential oil extract, as well as the highest yield of zerumbone were at the extraction time, 74.30 min; 187 mL solvent/10 mg solid ratio; and particle size, 2,000 μm . The quadratic model is sufficient to describe the extraction process under the optimal conditions of the extraction variables. Previous studies show that zerumbone possess strong anti-inflammatory and antinociceptive effect. Therefore, in the present study we suggest that higher zerumbone content in *Z. zerumbet* extract will present stronger pharmacological activities.

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