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Optimization Model of Total Phenolic Compounds in Zingiber Officinale via Ultrasound-Assisted Extraction Technique

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Abstract: Ginger (Zingiber Officinale) as a medicinal herb is frequently neglected for other possible applications and usually only be consumed as spices. It shows unpredictable potentialities in both food and pharmaceutical industries. This study aims to provide the comprehensive view on the optimization model of extraction of polyphenols from ginger as well as the antioxidant and antimicrobial capacity of ginger extracts. Extraction parameters screening of various parameters assessed the influence of these parameters against the efficiency of recovering polyphenols from ginger. This includes the extraction temperature (60-80 °C), quantity of spice which represent the concentration of ginger (200-600 mg/20 ml solvent) and the solvent concentration (60-80 %) for the extraction of polyphenols from ginger. The optimum parameters were found to be at 80 °C, 468 mg/20 ml solvent and the solvent concentration at 70 % and the extraction time was found to have minimal influence on the extraction process. The total yield of phenolic content under optimum condition were found to be at 22.333 ± 0.2462 mg GAE/g of dry weight of ginger extract.

Key words: *Zingiber Officinale, extraction,polyphenol,optimization*

INTRODUCTION

With the outbreaks of infectious disease rising all around the world and direct responsibility of infectious disease which contributes for nearly 25 % of mortality rate globally [1]. Viruses and bacteria are known as most common inducer of the infectious disease. In the same way, microbial threatened not only global health but as well as the food security today. Henceforth, there are more and more studies and research on the use of natural herbs in lieu of commercially available antibiotics and synthetic food preservatives. In fact, the usage of natural herbs and spices to fight spectrum of microbial threats can be traced back even before antibiotics and chemical derived food preservatives was discovered and applied. Ginger is defined as an ayurvedic which is one of those natural herbs which possess the necessary bioactive compound that have effect in inhibiting the microbial growth. The ginger is a member of the *Zingiberaceae* family and Zingiber genus of plant, and its scientific name is *Zingiber officinale* (*Z. officinale*). The ginger is a perpetual plant with thick tuberous rhizomes. A major part of ginger which is consumed is known as the rhizome. It is a commonly consumed dietary condiments, spices and the rhizome part is also used extensively as herbal medicine for a long time by Chinese and Indians [2]. The Chinese and Indians are known to utilize ginger for over 5000 years to cure abundant of ailments [3]. The ginger has been discovered to possess numerous biological activities such as anti-inflammatory, antioxidant [4], anticancer [5] and antimicrobial activities [6]. The bioactivities of the ginger make it capable to avert and control several diseases, for instance neurodegenerative diseases [7] cardiovascular diseases [8], obesity, obesity mellitus [9] chemotherapy-induced nausea and emesis [10], and respiratory disorders [11]. Due to the unbranched

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alkyl chain, gingerols are identified as analogous phenolic ketones and subsist as 6, 8, and 10-gingerols among all the bioactive compounds found in ginger. These polyphenols possess bioactive compounds which have significant effect in treating various diseases. The methodology chosen to complete this review is the Ultrasound-Assisted Extraction (UAE). UAE is the first choice chosen for this extraction process as the UAE method possess several interesting advantages over the conventional method such as the time required for extraction is faster using UAE. Next, the UAE is generally safer as it does not require high pressure. Moreover, the whole procedure is simpler and less prone to contamination. However, there are some parameters that need to be extra cautious when adopting UAE method which is the particle size and the ultrasonic probe surface ageing problem. The optimal extraction condition of ginger is study in this review to achieve the polyphenols maximum yield per gram of ginger. DPPH assay is used to determine the antioxidant activity of the extracted polyphenols using optimizes parameters. This is to maximize polyphenols extraction from ginger with greater activity of antioxidant and find out the most profitable analytical model. Factors that enhancing the diffusivity and solubility will accelerate the extraction process. The extraction efficiency will be influenced by the choice of extraction solvent, the solvent-to-solid ration (mg/20 ml solvent), particle size of ginger powder, the extraction temperature (°C), solvent concentration (%) and the extraction duration (minutes). Generally, all the factors should be considered to get highest yield of the extraction. The extractive capability of phenolic components from ginger is considerably rely on the type of solvent. The solubility level of phenolic compounds in different solvent is different. To extract the phenolic compounds, a ratio of solid-to-solvent which is high is found to be favorable for the extraction. The concentration gradient amongst the solid and the solvent is the driving force for mass transfer and the results are accordant with mass transfer principle.In UAE, there is a significant repercussion of temperature on the yield of extraction. In overall, increases in extraction yields of the phenolic compounds caused by an increase in temperature. There are various factors that correlates with extraction temperature such as the ruptures of matrix bonds being initiated, increase of the solvent diffusion rate, solubility of compound, decrease in tension and viscosity of the solvent and mass transfer [12]. To evaluate the effects of three independent variables, the extraction temperature (X1: 40–80 °C), extraction solvents concentration (X2: 50 %–90 %), quantity of spice (X3: 200–1000 mg/20 ml solvent) and time (X4: 0.5–1.5h) on the extract properties. The UV-vis spectrophotometer is used to measure the TPC, TFC and DPPH radical scavenging activity of the ginger extract. In this review, the action mechanisms of ginger are summarized. The total flavonoid and phenolic contents of ginger is investigated and the optimum conditions for extraction of ginger is analyzed.

EXPERIMENT

Ultrasound-Assisted Extraction (UAE)

The UAE was used for the extraction method with liquid solvents used on specimens in solid matrices. The UAE was carried out by following the quantity of spices and the amount of solvents that were set earlier. Extraction was conducted by mixing the powdered ginger with 20 ml of extract solvent in a 100 ml conical flask with different extract parameters. The contents ~~in~~ of the conical flask were subjected to ultrasonication at various pre-set extract parameters. The samples were filtered by cotton for the first time, followed by the transferring of the supernatant to a centrifuge tube and centrifugation at 8 °C, 3000g for 30 minutes. The resultant supernatant was collected and went through TPC.

Screening of Extraction Solvent Type

Screening of solvent types was carried out by using three types of solvent, which were distilled water, 50% methanol and 50% ethanol. Solvent screening was carried out in an ultrasound water bath with constant power. 600 mg of powdered ginger was extracted in 20 ml of different solvents under two different temperatures, which were 30 °C and 50 °C. The extraction was carried out in triplicate, and the TPC were evaluated using the Folin-method to determine the type of solvent for further study in optimization of the extract parameters.

Screening of Extraction parameters

In the screening of parameters, the extraction parameters, which were quantity of spice, extraction solvent concentration, and extraction temperature, were evaluated. The screening of extraction parameters was carried out by the one factor at a time (OFAT) method, which uses one factor variable with a holding constant of the other two parameters. The extraction parameters were tested between 200 mg/20 ml solvent and 1000 mg/20 ml solvent for spice quantity, 50–90 % ethanol

concentration, and 40 °C–80 °C for extraction temperature.

RESULT AND DISCUSSION

Screening of Extraction Parameters

The yield of polyphenols can be affected by the solvent type, as different solvent has different polarity. Ethanol is commonly used as a solvent when polyphenol extraction is carried out, as it is safe for human consumption too. Methanol, on the other hand, is more efficient when polyphenols with a lower molecular weight are used [13]. However, there was no record of any previous research on the screening of different types of extraction solvents for ultrasound assisted extraction of ginger rhizome. The solvent screening in this study was carried out under the same conditions of 600 mg/20 ml of solvents for 30 minutes at 50 °C. The variable parameter was the type of solvents, distilled water, 50 % methanol, and 50 % ethanol were used to determine the best solvents to produce the highest recovery of polyphenols in ginger. The quantitative analysis of TPC by various solvents recorded decreased in the following order: 50 % aqueous ethanol, 50 % aqueous methanol, distilled water, respectively.

Table 1. Quantitative analysis of ginger extract with different type of solvents.

Solvent Type	Total Phenolic Content (TPC) (mg GAE/g)
Distilled Water	10.67 ± 4.4746
Methanol	14.00 ± 2.5511
Ethanol	15.33 ± 0.8184

The extraction time was screened under the conditions of 600 mg of ginger to 20 ml of solvent, 50 °C, and 50 % ethanol; the results are tabulated in Table 2. The screening of extraction time shows no obvious order from 10 minutes to 60 minutes. As elucidated by [14], extraction time was found to have a lower effect on phenolic compound recovery using ultrasound assisted extraction. This screening result can also be supported by the fact that the response surface plot results show a relatively insignificant effect of extraction time as compared to other extracting parameters [15]. Therefore, the extraction time was held constant at 30 minutes.

Table 2. Quantitative analysis of screening of extraction time.

Extraction Time (min)	Total Phenolic Content (TPC) (mg GAE/g)
5	15.33 ± 1.0472
10	16.67 ± 1.6700
20	14.67 ± 2.0873
30	18.67 ± 0.5978
40	18.33 ± 1.1811
50	16.67 ± 1.0000
60	18.00 ± 0.3300

The solid to solvent ratio will influence the ultrasound assisted extraction due to its surface area and mass transfer during the diffusion process [16]. Theoretically, a higher solid to solvent ratio will increase the extraction of polyphenols in common consent [17]. The screening of the quantity of spice was carried out under conditions of 50 °C, 50 % ethanol, and 30 minutes. The yield of TPC increases significantly from 200 mg/20 ml solvent to 400 mg/20 ml solvent and the trend of the graphs gradually declines from 400 mg/20 ml solvent to 800 mg/20 ml solvent and there is a slight rise in 1000 mg/20 ml solvent. An equilibrium constant trend was observed from 600 mg/20 ml solvent to 1000 mg/20 ml solvent.

Table 3. Quantitative analysis of screening of quantity of spice.

Quantity of Spice (mg/20 ml solvent)	Total Phenolic Content (TPC) (mg GAE/g)
200	15.00 ± 0.9909
400	17.50 ± 0.7238
600	15.33 ± 2.7372
800	12.25 ± 1.3987
1000	16.20 ± 0.4784

The hydroalcoholic mixtures reportedly enhanced the extraction efficiency. Water not only increases the contact surface, but it also plays a role as a swelling agent in the plant matrix, and ethanol can incite the bond breaking between solutes and matrix [17]. The screening of ethanol concentration was carried out with the holding constant of the quantity of spice at 600 mg/20 ml of solvent, time at 30 minutes and temperature at 50 °C. The trend of the TPC graph shows a gradual increase with the variation of ethanol concentration from 50 % to 70 %, 70 % ethanol has the

highest TPC of 18.20 ± 0.9923 mg GAE/g and decreases gradually for the subsequent concentrations.

Table 4. Quantitative analysis of Solvent Concentration.

Solvent Concentration (%)	Total Phenolic Content (TPC) (mg GAE/g)
50	9.67 ± 0.4950
60	15.33 ± 1.3654
70	18.20 ± 0.9923
80	17.90 ± 1.8520
90	13.00 ± 1.7583

The extraction temperature has a similar theoretical assumption as the quantity of spice. A higher temperature accelerates the breaking of matrix bonds, increasing the mass transfer rate and diffusion rate of solvent as well as the solubility of bioactive compounds. The viscosity and tension of the solvent are also reduced with the increase of temperature during extraction [14]. The denaturation of polyphenols at high temperatures should be considered, thus too high of a temperature is not recommended. The trend in screening of extraction temperatures shows a steady rise of yield in polyphenols from 40 °C to 70 °C and then slightly dropped at 80 °C for TPC. The extraction temperature was tested under the conditions of 600 mg/20 ml of solvent, 30 minutes, and 50 % ethanol. The extraction temperature range was set from 60 °C to 80 °C in future optimization extractions.

Table 5. Quantitative analysis of extraction temperature.

Temperature (°C)	Total Phenolic Content (TPC) (mg GAE/g)
40	17.67 ± 1.6817
50	19.44 ± 0.9160
60	20.33 ± 2.3400
70	21.33 ± 1.4465
80	16.67 ± 0.6600

The range of extraction parameters which were adopted for further optimization were set from the optimum range of graphs obtained from screening of extraction parameters. To further optimize ginger extract, raw material of 200 mg/20 ml solvent, 400 mg/20 ml solvent, and 600 mg/20 ml solvent were used, as well as extraction solvent concentrations of 60 %, 70 %, and 80 %, and extraction temperatures of 60 °C, 70 °C, and 80 °C. The optimization process was carried out using the response surface method.

Optimization of Ultrasonication for extraction of polyphenols

For RSM, a three-level numerical factor with ~~3~~ three center points were executed. This study included three numerical factors, and the experimental data was evaluated to fit a quadratic statistical model. Conditions that were used were as follows: concentration of extraction solvent (20-80 %), extraction time (10-30 minutes), temperature (30-70 °C) and quantity of spice (400-1200 mg/20 ml solvent) [18]. These ranges were used as references in this study and screening of extraction parameters was carried out, so there were some modifications in the range of extraction parameters used for the optimization step in this study.

Table 6. Analysis of variance (ANOVA) table for TPC.

Source	Sum of squares	df	Mean square	F-value	P-value
Model	143.56	9	15.95	236.44	< 0.0001
A-Temperature	10.67	1	10.67	158.18	< 0.0001
B-Quantityof Spice	38.01	1	38.01	563.36	< 0.0001
C-Solvent Concentration	23	1	23	340.9	< 0.0001
AB	0.2244	1	0.2244	3.33	0.1109
AC	5.54	1	5.54	82.19	< 0.0001
BC	22.24	1	22.24	329.73	< 0.0001
Lack of Fit	0.3056	5	0.0611	0.7334	0.6632
Pure Error	0.1667	2	0.0833		

From Table 6, the quadratic interaction model of three parameters for TPC was evaluated. The quadratic parameters AC, BC, A^2, B^2 and C^2 were significant as $p < 0.05$. However, the parameter AB was not significant as the p-value > 0.10. All three of the extraction parameters were significant at $p < 0.05$. The contribution of the quadratic model was significant, fitted the quadratic model for TPC in coded variables.

The variable with the largest effect on TPC decreases in the order of the linear terms of quantity of spice (B) and solvent concentration (C), followed by the interaction term of BC. The lack of fit test measured the failure of the model, which denoted the data in the experimental domain at those points which were not included in the regression. The result showed-a lack of fit p-value of 0.6632 ($p > 0.05$) which indicates the experimental data fitted well to the model and is adequate to predict the TPC. The value of the determination coefficient R^2 from the result obtained was 0.9967 and R_{adj}^2 was 0.9925. The R^2 value indicates that the model is fitted at 99.67 % of the variability of TPC, and the R_{adj}^2 of 99.25 % indicates that the data is adequately fit. This result shows that the experimental value and predicted values have a high correlation. In addition, the coefficient of variation (CV) is a standard deviation in percentage of the mean. It is expected that a lower CV will have fewer residuals when compared to the predicted value. The result obtained was 1.60, which suggests high reliability and preciseness of the experiment performed.

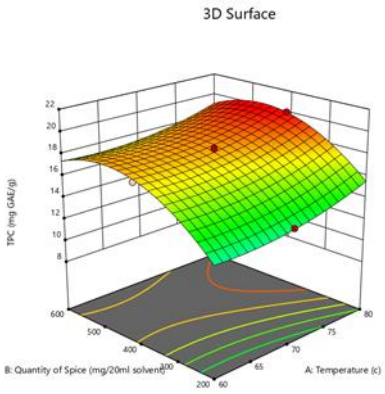
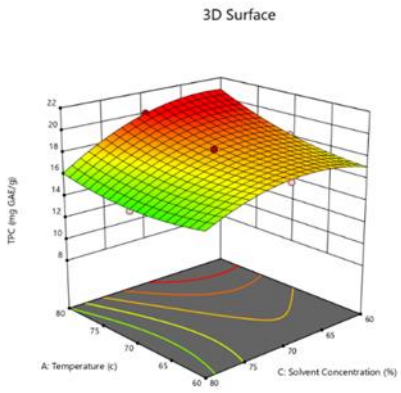


Figure 1 3D surface plot of extraction temperature and solvent concentration

Figure 2 3D surface plot of extraction temperature and quantity of spice



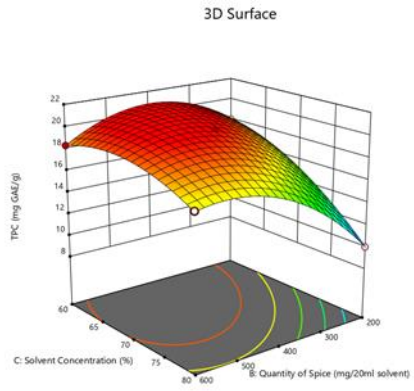


Figure 3 3D surface plot of quantity of spice and solvent concentration

CONCLUSION

In conclusion, this present study successfully adopted the one factor at a time (OFAT) method, the response surface method, and disc diffusion method to comprehensively assess the optimization model, antioxidant, and antibacterial study of ginger rhizome. The screening of extraction parameters concluded that the solvent type of ethanol results in the highest recovery of polyphenols in the extraction of ginger rhizomes, quantity of spice, solvent concentration, and extraction temperature were applied in the optimization model. The optimal extraction condition of quantity of spice, solvent concentration and extraction temperature were 469 mg/20 ml solvent, 70.77 % and 80 °C with corresponding recovery of TPC at 22.333±0.2462 mg/20 ml solvent. From the results, it can be concluded that these extraction parameters showed a significant effect on the recovery efficiency of TPC with a p-value less than 0.05. These results indicated the Central Composite Design (CCD) of Response Surface Methodology (RSM) was efficient in analysing the optimization of polyphenols in ginger rhizomes.

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