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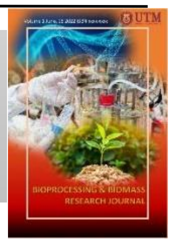

Research Article

Optimization of Ultrasonic-Assisted Extraction Condition for Total Phenolic Compounds from *Citrus hystrix* Peel

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ABSTRACT

This study aims to optimize the extraction conditions of Ultrasonic-Assisted Extraction (UAE) on the extraction of phenolic compound and of *Citrus hystrix* (*C. hystrix*) peel. The experimental design was carried out using Face Centered Central Composite Design. A total of 20 sets of different combination experiment designs were generated from 3 parameters; temperature (30-50 °C), time (20-40 min), and raw material to the solvent ratio (1:30-1:70). The highest yield of total phenolic compounds was 4.36 mg/g with the optimize extraction condition of 50 ºC, 30 min extraction time and 1:50 raw material to the solvent ratio. The result of this study indicates that the phenolic compounds extracted from *C. hystrix* peel could have the potential to be used for pharmaceutical and food applications.

INTRODUCTION

Citrus hystrix (C. hystrix) or known as Kaffir Lime is native to Asia countries, including Thailand, Indonesia, and Malaysia. High concentrations of beneficial organic compounds made *C. hystrix* highly respected as herbal medicine. The strong flavor was due to the high concentration of organic compounds such as alkaloids, citronellol, etc. (Staughton, 2021). Generally, the juice and fruit of *C. hystrix* are used in food condiments, but the peel is discarded. The peel of *C. hystrix* had been discovered to contain a wide range of phenolic compounds, which are secondary metabolites, primarily flavone, and flavanol. These compounds had the potential to be used as bio-preservative of food due to their biological characteristics such as antioxidant, antimicrobial, etc.

Conventional extraction of the beneficial compounds from plants included liquid-liquid extraction (LLE), solid-liquid extraction (SLE), and reflux extraction. On the contrary, non-conventional extraction methods included ultrasonic-assisted extraction (UAE), microwave-assisted extraction, pressurized liquid extraction, etc. The non-conventional method has been proven to be more effective, as the extraction time was shorter with fewer toxic chemicals being used. UAE is one of the alternatives to the conventional extraction method, where the basic principle is to generate rapid movement on solvent, which results in high mass transfer speed and acceleration of extraction. UAE has been proven to be simple, fast, energy-efficient, and high-potential method to obtain desired products (Ofori-Boateng & Lee, 2013).

The low operating temperature of UAE makes this process capable of preventing the damage of heat-sensitive compounds, such as bioactive compounds, during the extraction process. Toma et al. (2001) demonstrated that desired product yield of the sonicated sample was higher compared to the non-sonicated sample (Toma et al., 2001).

The principle of UAE was based on acoustic cavitation ultrasound, which involves a series of compression and rarefaction waves within the molecule. The compression and rarefaction waves will break the plant cell wall to release the bioactive compound. During the sonication process, cavitation bubbles will form and break the cell wall. In short, the ultrasound could break the cell wall rapidly with a highly desired product yield (Syahir et al., 2020). There are two

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types of sonication devices: ultrasonic bath and ultrasonic probe. The difference between these two is that ultrasonic bath won't have indirect contact with the sample, while ultrasonic probes will directly contact the sample (Thilakarathna et al., 2022).

The interaction time of UAE could be classified into two phases. The first phase is within the first 10 to 20 min, known as the washing or rapid extraction phase. In this phase, the soluble compounds on the sample surface are dissolved. Almost 90% of the extraction could be completed within this phase. The second phase (slow extraction phase) is a diffusion process, in which the matrix is transferred to solvent, and this phase occurs within 60 to 100 min (Medina-Torres et al., 2017). A longer extraction time will allow the ultrasound wave more time to break the cell wall, resulting in a higher yield. However, the extraction yield will remain constant after a specific time, which indicates the system is in an equilibrium state. Prolonged time will cause the sample's decomposition, resulting in a lower yield (Syahir et al., 2020).

A few researchers suggested that the optimum extraction temperature of the UAE should be within 30-50 °C to obtain a high yield of desired products. Higher extraction temperature could enhance the diffusion coefficient of the desired compound and improve the compound's solubility in extraction solvent (Zhou et al., 2017a). The high temperature will increase the speed of the bubble, which causes more collapses to occur in the solvent. This promotes the penetration of solvent and speeds up the release of the desired product into the extraction solvent. A temperature of 50 ^oC should be the maximum temperature in UAE, as excessively high temperatures may lead to the damage of bioactive compounds, providing negative results for antioxidants. The cavitation effect had been reported to be less effective at high temperatures, resulting in lower yield (Syahir et al., 2020).

The degree of interaction between raw material and solvent mainly depends on the ratio. At a specific ratios, the efficiency of the extraction process could be improved due to the tremendous concentration difference (Zhou et al., 2017b). The mass transfer driving force is also dependent on the ratio. At low raw material to solvent ratio, the driving force will be greater due to significant concentration differences between the interior and exterior cell, allowing the phenolic compound to dissolve in extraction solvent faster. However, suppose the ratio is beyond a lower range. In that case, the yield may remain unchanged due to prolonged diffusion distance toward the interior cell, resulting in a low diffusion rate (Fuad & Don, 2016).

This paper aims to determine the optimum condition for extraction of phenolic compounds from *C. hystrix* peel using UAE. The effect of extraction temperature, extraction time, and raw material to solvent ratio will be investigated in this study.

MATERIALS AND METHOD

Materials and Apparatus

As much as 2 kg of *C. hystrix* fruit were purchased from Morning Market, Taman Universiti Skudai, Johor Malaysia. The fruit and the peel were separated, and the peel was washed to avoid contamination. The peel was cut into small pieces and freeze-dried using a freeze dryer (Martin Christ Alpha 1-2 LSC Basic) for 24 hours. The peel was then grounded into powder and stored in the refrigerator for up to 12 weeks for further use.

Extraction of *Citrus hystrix* using Ultrasonic-Assisted Extraction

The experimental design was done by using Stat-Ease Design Expert Software version 13, based on the independent variable in **Table 1Error! Reference source not found.**. The 20 sets of generated experiments are shown in **Error! Reference source not found.** by Response Surface Method (RSM) three-factors of face-centred central composite design (FCCD). The actual and predicted response was analyzed through analysis of variance (ANOVA) by Design Expert Software. About 5 g of sample was mixed with pure ethanol (Sigma Aldrich) based on the raw material to solvent ratio. After the sample was sonicated in a solicitor, the extract was passed through a rotary evaporator until the powder form of the extract was obtained. The extract was then collected and stored in a chiller for further analysis.

Table 1 Independent variables and their corresponding levels

Variables	-1	0	+1
Temperature (°C)	30	40	50
Time (min)	20	30	40
Material-to-solvent ratio	1:30	1:50	1:70

Table 2 Design matrix experiment from Design Expert

Run	Temperature (°C)	Time (min)	Material to Solvent ratio	TPC Response (mg/g)	
				Actual	Predicted
1	50	20	1:30	1.65	2.00
2	40	30	1:70	3.77	3.90
3	40	30	1:30	3.41	3.01
4	40	30	1:50	3.27	3.37
5	40	30	1:50	3.27	3.37
6	40	30	1:50	3.27	3.37
7	30	40	1:70	3.18	2.90
8	30	30	1:50	2.87	3.22
9	40	30	1:50	3.27	3.37
10	50	40	1:30	3.56	3.66
11	40	40	1:50	3.61	3.27
12	30	20	1:30	2.93	2.68
13	50	30	1:50	4.36	3.74
14	50	20	1:70	3.72	3.58
15	30	40	1:30	2.49	2.70
16	40	30	1:50	3.27	3.37
17	30	20	1:70	3.54	3.51
18	50	40	1:70	4.30	4.61
19	40	20	1:50	2.68	2.74
20	40	30	1:50	3.27	3.37

Table 3 Analysis of variance of regression model for extraction of phenolic compound concentration and antioxidant response

Source	TPC Response		
	Coefficient	F-value	P-value
Intercept	3.37	4.74	0.0116
X_1 Temperature	0.2585	5.14	0.0468
X_2 Time	0.2625	5.30	0.0441
X_3 Ratio	0.4464	15.34	0.0029
X_1X_2	0.4097	10.33	0.0093
X_1X_3	0.1871	2.16	0.1728
X_2X_3	-0.1558	1.49	0.2496
X_1^2	0.1112	0.2616	0.6201
X_2^2	-0.3603	2.75	0.1284
X_3^2	0.0881	0.1644	0.6937
R^2	0.8101		
Adj R^2	0.6392		
Lack of fit	1.30		

Determination of Total Phenolic Compound Concentration

The concentration of TPC was analyzed through Folin-Ciocalteu reagent based on the method modified from Rodsamran et al. (2019). 0.1 g of extract was mixed with 0.5ml of 10% (v/v) Folin-Ciocalteu reagent. The mixture was then vortex and left for 5 min before 0.4 ml of 7.5% (w/w) sodium carbonate was added into the mixture. Concentration of TPC in the extract was analyzed through UV-Vis Spectrophotometer at a wavelength of 765 nm.

RESULTS AND DISCUSSION

Fitting Response Surface Method

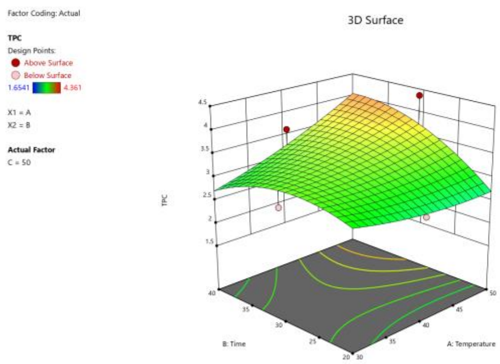
Table 2 shows the actual and predicted response for total phenolic compound (TPC) for 20 sets of experiment design, while **Table 3** shows the analysis of variance (ANOVA) for TPC responses. The highest yield of TPC (4.36 mg/g) in **Table 2** was observed at 50 °C temperature, 30 min extraction time, and 1:50 material-to-solvent ratio. The equations for TPC generated by the Design Expert software is as follow:

$$Y_{TPC} = 3.37 + 0.2585X_1 + 0.2625X_2 + 0.4464X_3 + 0.4097X_1X_2$$

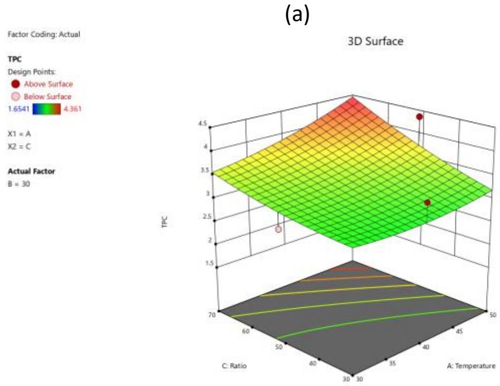
The ANOVA result in **Table 3** indicates that the TPC models is significant, with P-values of 0.0116, respectively. The TPC concentration model has R^2 value of 0.8101, and adjusted R^2 value of 0.6392. If the R^2 value is closer to 1; the model could consider having a strong correlation between the dependent and independent variables. Based on **Table 3**, temperature, time, and material-to-solvent ratio are significant parameters affecting TPC production, as all the P-values are less than 0.05.

Analysis of Response Surface Plot

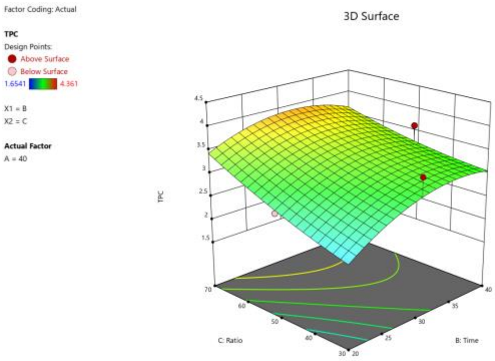
Figure *1*(a), **1(b)**, and **1(c)** show the relationship between two parameters investigated in this study with one parameter fixed. The trend in **Figure 1(a)** shows that with increased temperature and time, the TPC concentration increases, suggesting that future research needs to be conducted in detail as both parameters play an important role in extracting the phenolic compound from *C. Hystrix* peel. Similar 3-D plot was obtained by (Wang et al., 2008) in extracting wheat bran. Wang et al. (2008) explained that the trend was due to the enhanced solubility of the phenolic compound at a higher temperature which caused the viscosity to decrease and accelerate the whole extraction (Wang et al., 2008). **Figure 1(b)** presents the relationship between the raw material to solvent ratio and temperature toward the TPC concentration. The 3-D plot shows that the TPC concentration is higher at a high solvent ratio and high temperature. The results indicate that the volume of ethanol used greatly affected the production of the phenolic compounds. A reasonable explanation for this is that ethanol is an essential solvent in the extraction process. An appropriate ethanol volume should be used to extract the phenolic compounds as low ethanol volume might cause the mixture to become viscous, which then affects the extraction process compared to the high ethanol volume. The relationship between raw material to solvent ratio and time is shown in **Figure 1(c)**. The highest TPC could be observed produced around 30 min extraction time and 1:70 material to solvent ratio.



(a)



(b)



(c)

Figure 1 3D Surface Plot for TPC Model (a) Temperature and Time (b) Raw material to solvent ratio and Temperature (c) Time and Raw Material to Solvent ratio

The highest concentration of TPC obtained in this optimization study was 4.36 mg/g. **Table 4** summarized the concentrations of TPC extracted through other extraction methods. The concentration of TPC extracted through solvent extraction by Chan et al. (2009) was the highest, however, the solvent and retention time used was higher when compared to this study. The result in **Table 4** indicate that UAE may not be able to extract a high concentration of TPC, however, the operating condition was found to be the most less complex with friendly operating conditions such as easier equipment setup, lower retention time, not energy intensive, etc. Maceration process by Ayucitra et al. (2016) had shown higher concentrations but the retention time for the extraction process was 24 hours which is time-consuming when compared to UAE. The yield of TPC was much lower using the pressurized hot water (Khuwijitjaru et al., 2008). Besides, the system setup was complex when compared to the UAE, as the system required an external gas supply to generate a pressurized environment for the extraction process.

Table 4 TPC concentration through other extraction methods

Extraction Method	Concentration of TPC (mg/g)	Reference
Solvent Extraction	12.91	(Chan et al., 2009)
Maceration	55.71	(Ayucitra et al., 2016)
Pressurized Hot Water Extraction	2.37	(Khuwijitjaru et al., 2008)
Ultrasonic Assisted Extraction	4.36	This study

Furthermore, the choice of solvent required a critical consideration, some solvents such as hexane pose good extraction conditions. However, hexane is a toxic chemical that may cause damage to human health. Exposure to hexane would cause damage to the nervous system, etc. Thus, a green and clear solvent such as ethanol, acetone, etc should be considered when designing the extraction process.

In addition to that, the value of the extract obtained should not only be determined by the concentration of TPC obtained. Other evaluations such as bioactivity like antioxidant, antimicrobial, etc should be tested by the extract obtained. Those biological characteristics were heat sensitive which depends on and is easily affected by the extraction process. Thus, the extraction process developed plays an important role in preserving those characteristics.

CONCLUSION

From this paper, the optimum operating condition for extraction of phenolic compound from *C. hystrix* peel has been found at the extraction temperature of $50\,^{o}C$, extraction time of 30 min, raw material to solvent ratio of 1:50, ultrasonic power of 100% and 100% ethanol with the obtained concentration of TPC at 4.36 mg/g.

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