Optimization of Ultrasound-Assisted Extraction of Total Phenol from *Piper Betle* Linn. Using Response Surface Methodology

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Received: 13 May 2021; Accepted: 19 June 2021

Abstract
Piper betle Linn. leaves extract contains phytochemicals with various therapeutic effects. While these phytochemicals are susceptible to degradation by extreme extraction conditions, novel extraction techniques enable the preservation of the phytochemicals and thus enabling recovery of higher quality phytochemical extract. This research aimed to study the effect of parameters of ultrasound-assisted extraction (UAE) on the quality of phytochemicals, measured by total phenol content and antioxidant activity of *Piper betle* leaves extract. The parameters studied are sonication power (*i.e*., 50W, 70W, 90W), extraction time (*i.e*., 20, 25, 30 minutes), and temperature (*i.e*., 45ºC, 50ºC, 55ºC). Ethanol of 96% concentration was used as solvent and the ultrasound bath was operated at 45 kHz. Response surface methodology is used to analyze the result of experiment. Sample testing were done by completely randomized design (CRD) and the result was statistically analyzed by using central composite design (CCD) to find the most optimal parameter combination towards total phenol content and antioxidant activity. The optimum parameters for UAE of *Piper betle* leave that gave the maximum amount of total phenolic content and antioxidant activity are as follows: temperature of 55ºC, extraction time of 27.55 minutes, and sonication power of 73.04 Watt.

Keywords: *Piper Betle*, Ultrasound-Assisted Extraction, Yield, Total Phenolic Content, Antioxidant Activity

Introduction

Nowadays, scientific practices demand more environmentally friendly methods to achieve sustainability. To realize it, Green Chemistry and Green Analytical Chemistry (GAC) have become the key focus on conducting scientific experiments, even outside the field of environmental science. This applies to food analysis as well, where waste produced by the experiments could pose threats to the environment. The 12 major principles of green chemistry were established in 1998 by Anastas & Warner (Anastas & Warner, 1998).

Out of all usages of green chemistry, extraction of essential oils from plants using novel technology is considered important to minimize damages to the phytochemicals in the plants. Pretreatments of plant samples prior to analysis are the most challenging steps to be made environmentally friendly as they involve some reagents which may be toxic to the environment (Armenta *et al.*, 2015). Microwave-assisted extraction (MAE), enzyme-assisted extraction (Li *et al.*, 2006), ultrasound-

assisted extraction (UAE) are among green extraction techniques which can help preserving phytochemicals and at the same time enable commercialization due to cost-lowering process which can be applied in industrial scale (Chavan & Singhal, 2013).

Ultrasound is a sound wave which operates at frequency of 16 kHz or higher. Ultrasound-assisted food extraction can be done within minutes as compared to conventional solid-liquid extraction methods. This is due to ultrasound effects, which disrupt cell wall and assist mass transfer. As far as green extraction theory goes, UAE also comes with other benefits such as reduced amount and non-toxic solvent being used thus it is safer than other methods. Ultrasound's output source is none other than a vibrating body. This happen when the surrounding medium vibrates to the ultrasound wave and transmits energy to the neighboring particles. The major parameters, which play essential roles in ultrasound extraction process, are ultrasound power, frequency, and amplitude. There are 3 ways of expressing the energy level at which an ultrasound propagates through a medium, namely ultrasound power (Watt), intensity (Watt/cm^2), and acoustic energy density (Watt/cm^3) (Esclapez *et al.*, 2011). Ultrasound wave can cause cavitation, mixing, crushing and vibration in the medium it is propagating on. For extraction process, the effective frequency used ranges from 20kHz to 50 kHz (Wen *et al.*, 2018).

It is generally believed that in UAE, cavitation, thermal and mechanical effects play important roles. These three effects lead to destruction of cell wall, reduction in particle size, and an increase in mass transfer rate without changing the structure and functions of the solutes (Ashokkumar, 2015). As ultrasound wave propagates through medium, a series of alternating pressure (negative and positive) compress and expand the liquid medium. This compression and rarefaction cycle creates a phenomenon called 'stable cavitation' where the cavitation bubbles vary with the frequency of the wave. As the extraction process proceeds, the bubbles grow until it reaches a critical value with a high temperature (5000 K) and high pressure (100 MPa) generated around cavitation zone. Due to the high temperature and pressure caused at the moment of the collapse, shear forces and turbulence are produced; this cavitation process is called 'transient cavitation'. The heat and volume generated, though large in values, are not sufficient to affect macroscopic system due to the small size of the parameters, yet they are still able to affect cell structure and facilitate in mass transfer (Brennen, 1995).

Piper betle Linn. (Betel vine) is an important remedial plant in Southeast Asia. This plant belongs to *Piperaceae* family and is cultivated widely in India, Nepal, Bangladesh, Burma, and Sri Lanka. Measuring 10-18 cm long and 5-10 cm wide, the leaves of *Piper betle* Linn. has numerous therapeutic effects. Due to that reason, betel leaves have been widely investigated for its contents, mainly the phytochemicals in the leaves, for potential medicinal properties (Madhumita *et al.*, 2020). The spicy odor from the leaves is due to the phenol and terpenes content in the leaves. Polyphenolic compounds belong to one of the six groups of important bioactive groups in phytochemicals. Polyphenolic compounds are characterized by the benzene ring with one or more hydroxyl groups attached to it. Production of polyphenolic compounds starts with phenylalanine as a precursor progressing through 3 biosynthetic routes: 1) succinyl benzoate pathway, 2) acetate/malonate pathway, 3) acetate/melavonate pathway (Bhattacharya *et al.*, 2010). Polyphenolic compounds can be found in the

form of flavonoids, tannins, phenolic acids, lignans, stilbenes, and coumarins (Holland *et al.*, 2017).

Phenolic compounds in betel leaves contribute to its antimicrobial activity. According to Ali *et al*., (2018), in a fundamental study of *Piper betle* antimicrobial activity, a toothpaste which is enriched with *Piper betle* extract exhibit larger inhibition zone as compared to toothpaste with triclosan and fluoride alone. Extraction of polyphenol must be done at a lower temperature as polyphenol in plants degrade at high temperature. Depending on the type of the plants, thermal treatment on samples may degrade polyphenol or may increase the content of polyphenol (Sikora & Borczak, 2014). The objective of this study was to find the optimal extraction parameters of betel leaves ultrasound-assisted extraction, encompassing extraction time, ultrasound bath power, and extraction temperature, on total phenolic compounds and antioxidant activity in the leaf extract.

Methodology

Plant Materials

Fresh betel leaves were obtained from a single vendor at Peterongan Wet Market, Semarang, Indonesia. Betel leaves were picked based on the size uniformity and maturity.

Extraction of Piper betle

Fresh betel leaves were dried in vacuum dryer with temperature of 50^{o}C and air pressure of 0.5 atm (Wahida *et al.*, 2012) for 6 hours. After the leaves were dried, the leaves were shredded using a blender (Phillips) and then sifted through mesh 30. The dried leaves were then stored in freezer at -20^{o}C. To prepare the leaf flakes for extraction, 3 g of flakes was added with 90 g of 96% ethanol technical grade (1:30 solid to solvent ratio). The dried leaves in the solvent was then subjected to various extraction conditions as follows: extraction time (X_1): 20 minutes, 25 minutes, and 30 minutes ultrasound bath power (X_3) : 50W, 70W, 90W (Chavan & Singhal, 2013), and extraction temperature (X_2): 45^{o}C, 50^{o}C, and 55^{o}C. Feed to solvent ratio is kept constant at 1:30. The responses studied in this experiment were extract yield and total phenol. The ultrasound bath (Biobase 45/80 kHz Small Size Double Frequency) was operated at 45 kHz and the solvent used in this experiment was 96% ethanol. The experiment was conducted by using completely randomized design (CRD). After ultrasound-assisted extraction was carried out, the extract was concentrated by using an oven (Venticell 111, MM Medcenter GmbH) operated at 40^{o}C for 36-48 hours or until the solvent all evaporate, leaving the crude extract.

Total phenol was measured by using Folin-Ciocalteau Assay, which is regularly used to measure phenolic antioxidants that present in a sample. To prepare a 0.025% sample solution, 0.025 gram of crude extract was diluted to 100 ml mark in a 100 ml volumetric flask. This 0.025% sample solution was used for both total phenol assay and antioxidant activity assay.

Gallic Acid Standard

Stock solution was prepared by adding 2 mg of gallic acid (Sigma-Aldrich, Co., China) into 10 ml of ethanol. Gallic acid with concentrations of 100, 50, 25, and 12,5 µg/ml were prepared from the stock solution. 200 microliters of each concentration were then placed in a test tube and added with 1.5 ml of 10% Folin-Ciocalteau Reagent (FCR) solution (MERCK KGaA, Darmstadt, Germany). The solution was then incubated for 5 minutes and then 1.5 ml of 6% Na_2CO_3 (MERCK KGaA, Darmstadt, Germany) solution was added to the sample solution. The mixtures were incubated for 2 hours while the test tubes were covered with aluminium foil. The absorbance of the solutions were then measured using a spectrophotometer (UV-1280, Shimadzu) at 760 nm. (Said *et al.*, 2018). The absorbance of each gallic acid solution was then plotted on a graph to obtain the standard curve for Folin-Ciocalteau Assay. The equation obtained from the curve was as follows:

$$y = 0.0024x + 0.0221$$

Where y is sample's absorbance and x is total phenol concentration measured in ppm.

Total Phenol

1.5 ml of 10% FCR reagent was added into 200 µl of betel leaves extract. The solution was incubated for 5 minutes. 1.5 ml of 6% Na_2CO_3 solution was added and then the mixture was incubated for 2 hours while the test tubes were covered with aluminium foil. The absorbance of the solution was measured using spectrophotometer at 760 nm. The total phenol concentration was measured by using the equation in section 2.2.1

Antioxidant Activity

Antioxidant activity of sample was evaluated by using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. As much as 100 µl of 0.025% sample solution was added with 3.9 ml of 250mM DPPH (Sigma-Aldrich, Co., Saint Louis, USA) solution in a dark room (DPPH solution was prepared fresh in a dark room). The test tubes containing the mixture were shaken well before being incubated for 30 minutes in a dark room at room temperature. A blank (negative control) was prepared similarly by substituting the 0.025% sample solution with 96% ethanol as the sample solvent. At the end of incubation time, the absorbance of the mixture was analyzed using UV-vis spectrophotometer at wavelength of 517 nm. Each sample was analyzed in triplicates. The antioxidant activity was presented as % Inhibition and the formula used to calculate % Inhibition was as follows:

$$\%Inhibition = {(A_{sample} - A_{blank})}/{A_{blank}} \times 100$$

Where A_{sample} is sample's absorbance and A_{blank} is blank's absorbance.

Response Surface Methodology – Central Composite Design

Response Surface Methodology (RSM) was used to study the effect of multiple factors on an experimental response. Central Composite Design (CCD) was used for the experiment design. The statistical analysis of response surface model was conducted using the application JMP Pro version 13.

Results and Discussion

In Central Composite Design analysis, p-value indicates the significance of each coefficient in the polynomial regression model constructed; where the lower the p-value is, more significant the coefficient towards the overall regression model is (Zhong & Wang, 2010). The full design of experiment and the result of yield, total phenolic content, and antioxidant activity are shown in Table 1. The symbols on 'Pattern' column is explained as follows: '+' and '-'are factorial points where '+' indicates the highest value of each corresponding factor's factorial point, while '-' indicates the lowest value of each corresponding factor's factorial point. The symbol 'A' and 'a' represent axial points where 'A' indicates the highest value of each corresponding factor's axial point while 'a' indicates the lowest value of each corresponding factor's axial point. The number '0' is used to indicate the middle value of each corresponding factor *(i.e.,* time= 25 minutes, temperature= 50^{o}C, and power= 70 Watt).

Table 1: Effect of extraction variable of UAE on total phenolic content, and antioxidant activity (% inhibition) in *Piper betel* extract

		Independent Variables			Dependent Variables	
No.	Pattern	Time (minutes)	Temperature (^{o}C)	Power (Watt)	Total Phenol Content (ppm)	Antioxidant Activity (% Inhibition)
1	− − −	20	45	50	27.65 ± 1.50	18.28 ± 0.42
2	− − +	20	45	90	31.44 ± 1.18	17.38 ± 2.63
3	− + −	20	55	50	48.69 ± 1.18	26.91 ± 1.59
4	− + +	20	55	90	48.79 ± 1.50	25.97 ± 0.68
5	+ − −	30	45	50	36.48 ± 0.99	20.16 ± 2.03
6	+ − +	30	45	90	37.38 ± 2.45	22.66 ± 1.32
7	+ + −	30	55	50	50.48 ± 2.46	29.66 ± 1.76
8	+ + +	30	55	90	53.10 ± 1.94	31.32 ± 0.96
9	a00	20	50	70	38.54 ± 1.58	21.30 ± 0.65
10	A00	30	50	70	38.19 ± 2.59	23.10 ± 1.94
11	0a0	25	45	70	35.45 ± 1.19	19.59 ± 0.35
12	0A0	25	55	70	53.85 ± 1.37	32.76 ± 1.87
13	00a	25	50	50	40.30 ± 0.61	22.27 ± 0.28
14	00A	25	50	90	38.47 ± 1.84	22.26 ± 0.26
15	000	25	50	70	40.92 ± 1.03	23.49 ± 1.15
16	000	25	50	70	42.38 ± 1.15	23.09 ± 0.93
17	000	25	50	70	43.73 ± 0.60	23.70 ± 1.13
18	000	25	50	70	42.88 ± 0.67	22.16 ± 1.66
19	000	25	50	70	42.51 ± 1.28	23.42 ± 1.78
20	000	25	50	70	41.77 ± 2.06	22.61 ± 2.29

*Numbers show the average ± standard deviation (n=3)

The data from Table 1 shows the full design of response surface experiment with dependent variables: extraction time, extraction temperature, ultrasound power; and

dependent variables: total phenolic content, and antioxidant activity. Lowest yield was obtained from sample 15 with response yield of 17.05% while the highest response yield was shown by sample 8 with yield value of 19.50%. The lowest total phenolic content (TPC) was obtained from sample 1 with TPC value of 27.65 ± 1.50 ppm gallic acid equivalent while the highest TPC value was obtained from sample 12 with TPC value of 53.85 ± 1.37 ppm gallic acid equivalent. Correspondingly, sample with highest antioxidant activity, expressed by % inhibition was sample 12 with % inhibition value of 32.76 ± 1.87, while the sample with the lowest antioxidant activity was sample 2 with % inhibition value of 17.38 ± 2.63.

In addition to the full design and result of experiment, the effect summary of the experimental result is displayed in Table 2. The effect summary shows the p-value of each coefficient. A p-value below 0.05 indicates that the corresponding coefficient is significant in the constructed regression model at 95% confidence level. A p-value under 0.01 means, that the corresponding coefficient is significant in the regression model at 99% confidence level.

Table 2: Effect Summary of Response Surface Analysis on experimental results

Source	LogWorth	*p*-Value
Temperature (45,55)	7.404	0.00000
Time (20,30)	3.296	0.00051
Temperature*Temperature	2.971	0.00107
Time*Time	1.326	0.04719
Time*Temperature	0.894	0.12767
Power*Power	0.789	0.16242
Time*Power	0.707	0.19655
Power (50,90)	0.596	0.25344
Temperature*Power	0.402	0.39636

Table 2 shows the effect summary of experimental analysis. It shows 9 effects in which 3 are single effects (time, temperature, and power) and the rest of the 6 effects are quadratic effects (temperature^2, time^2, power^2, time*temperature, time*power, and temperature*power). The most significant variable towards the model was temperature with p-value of 0.00000, followed by time with p-value of 0.00051 and then temperature*temperature with p-value of 0.00107, and the last significant coefficient is time*time with p-value of 0.04719. The rest of the coefficients are insignificant in the regression model. The interaction between temperature and power showed the lowest significance with p-value of 0.39636.

Prediction Profiler

The prediction profiler can be used to find the most desired value of the dependent variables (total phenolic content and antioxidant activity) based on the objective of the experiment. Figure 1 shows the prediction profiler when it is set to maximizing response.

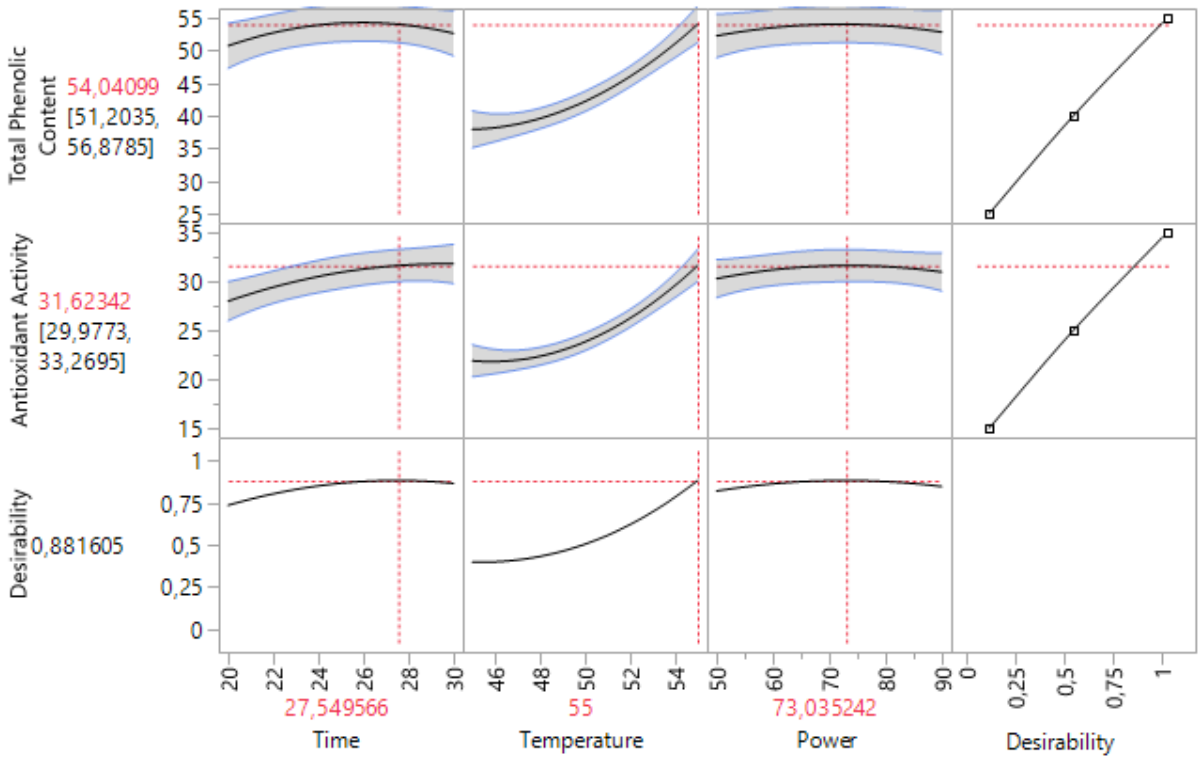

Figure 1: Prediction profiler of three extraction factors on total phenolic content and antioxidant activity

Figure 1 shows the predicted response value when it was set to maximize the value of responses. It can be seen that to achieve maximum desirability of total phenolic content and antioxidant activity, the extraction duration needs to be set at 27.55 minutes, the sonication power to be set at 73.04 Watt, and the temperature needs to be set at 55 ºC. This set of condition has a desirability value of 0.881605 as shown by the bottom row figure. Since the value is close to 1, this set of conditions fits the purpose of this study, which is to maximize the responses: total phenolic content and antioxidant activity.

Total Phenolic Content

The statistical result for total phenolic content comprising summary of fit, ANOVA and surface plot are displayed below in Table 3 and Figure 2.

Predicted Model and Statistical Analysis

Table 3: Analysis of Variance of total phenolic content response surface model

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	849,30190	94,3669	27,6721
Error	10	34,10185	3,4102	**Prob > F**
C. Total	19	883,40374		<,0001*

Table 3 indicates that the probability of the regression model is lower than 0.0001 which means that the quadratic model is significant and can be used to optimize

extraction factors. Since the model is significant, the regression formula below can be used to predict the value of total phenolic content based on the value of independent variables

$$Y = 41.77 + 2.05(X_1) + 8.65(X_2) + 0.56(X_3) - 1.08(X_1X_2) - 0.48(X_1X_3) - 0.25(X_2X_3) - 2.52(X_1^2) + 3.77(X_2^2) - 1.49(X_3^2)$$

Legend:
Y= Total phenolic content
X_1= Time
X_2= Temperature
X_3= Power

Model Plots
The total phenolic content from 0.025% of sample solution at ultrasound power 70 Watt can be seen in Figure 2.

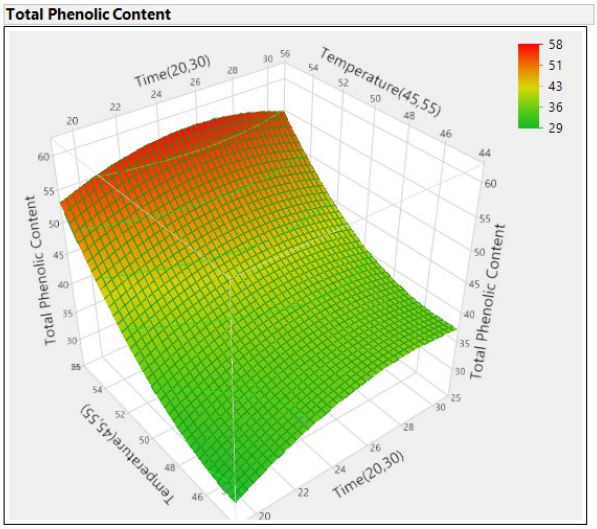

Figure 2: Surface plot of total phenolic content against extraction time and temperature at ultrasound power of 70 Watt

The surface plot above shows the predicted value of total phenolic content over a surface area. The total phenolic content was plotted against time at range 19-31 minutes and temperature at range 44ºC-56ºC. Figure 2 shows the surface plot at power level 70 Watt and the lower bound of predicted total phenolic content was shown to be 29 ppm galic acid equivalent while the upper bound was shown to be 58 ppm galic acid equivalent.

Antioxidant Activity
The statistical result for antioxidant activity comprising ANOVA and surface plot are displayed below in Table 4 and Figure 3.

Predicted Model and Statistical Analysis

Table 4: Analysis of variance of antioxidant activity response surface model

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	293,55120	32,6168	28,4208
Error	10	11,47640	1,1476	**Prob > F**
C. Total	19	305,02760		<,0001*

Table 4 indicates shows that the probability of the regression model is lower than 0.0001 which means that the quadratic model is significant and can be used to optimize extraction factors. Since the model is significant, the regression formula below can be used to predict the value of antioxidant activity based on the value of independent variables

$$Y = 23.14 + 1.71(X_1) + 4.85(X_2) + 0.41(X_3) + 0.12(X_1X_2) + 0.52(X_1X_3) - 0.34(X_2X_3) - 1.04(X_1^2) + 2.94(X_2^2) - 0.97(X_3^2)$$

Legend:
Y= Antioxidant activity
X_1= Time
X_2= Temperature
X_3= Power

Model Plots

Antioxidant activity of 0.025% sample solution at ultrasound power 70 Watt can be seen in Figure 3.

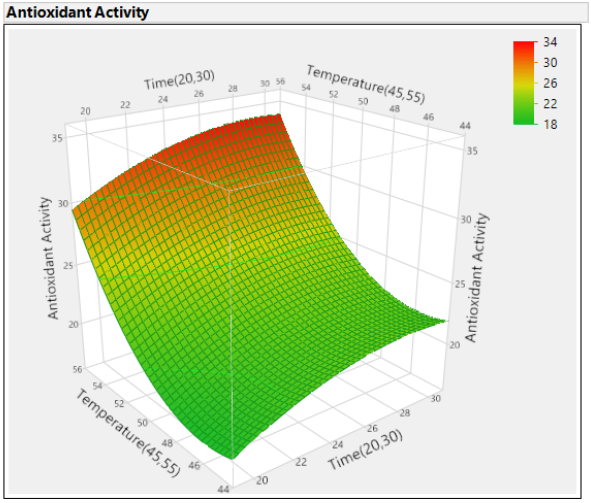

Figure 3: Surface plot of % Inhibition against extraction time and temperature at ultrasound power of 70 Watt

The surface plot above shows the predicted value of antioxidant activity over a surface area. The antioxidant activity (expressed as % Inhibition) was plotted against time at range 19-31 minutes and temperature at range 44°C-56°C. Figure 3 shows the surface

plot at power level 70 Watt and the lower bound of predicted antioxidant activity was shown to be 18% while the upper bound was shown to be 34%.

Statistical Analysis of Response Surface Method on Ultrasound-Assisted Extraction

Table 2 shows that the linear coefficient that is temperature and time, also quadratic coefficients temperature^2 and time^2 have values below 0.05 which means that they are significant. The rest of the coefficients have p-value of higher than 0.05 which means that these are insignificant coefficients in the model (Wang *et al.*, 2014). Table 3 and Table 4 show the ANOVA of the 3 extraction factors on total phenolic content and antioxidant activity respectively. Since both ANOVA for total phenol content and antioxidant activity showed probability of <0.0001 ($p<0.05$), the response surface quadratic models for both responses (dependent variables) were significant and can be used to optimize the extraction factors. (Wang *et al.*, 2014).

Effect of Ultrasound-assisted Extraction on Total Phenolic Content and Antioxidant Activity

In this study, the efficacy of UAE on betel leaves is indicated by the amount of total phenolic content and antioxidant activity present in the crude extract of betel leaves. Figure 2 show the effect of different combination of UAE factors on total phenolic content while Figure 3 show the effect of different combination of UAE factors on antioxidant activities. The pattern of both sets of figures are similar and this could be due to the fact that antioxidant activity from crude extract comes from the phenolic content of the extract (Pin *et al.*, 2010; Murata *et al.*, 2009).

Effect of Temperature on Total Phenolic Content and Antioxidant Activity of Crude Extract

In studying the phenolic compound stability during extraction, temperature becomes the main factor to be taken into consideration (Setyaningsih *et al.*, 2019). Based on Table 2, the p-value of temperature as a coefficient and as quadratic coefficient (temperature*temperature) were below 0.05 which means there is a significant correlation between temperature and total phenolic content and antioxidant activity. This correlation was depicted in Figure 2. From the surface plot, there is a positive trend where higher temperature showed an increase in the value of total phenolic content. The same trend can be seen from Figure 3 where an increase in the temperature is followed by an increase in the antioxidant activity.

Both surface plots are in accordance with several studies on UAE with temperature as one of the factors studied. Vuong *et al.*, (2015) reported similar effect of temperature where an increase in temperature results in an increase in efficiency of terpenoid extraction, such as euphol. Similarly, Muruganandam *et al.,* (2017) who studied about optimization of betel leaves' phytochemicals extraction also reported that an increase in the extraction temperature would lead to an increase in yield of phytochemical components. However, despite the overall positive trend of temperature, from prediction plot in Figure 2 there was a slight downward trend beyond 51^{o}C at higher extraction time value. Some studies have concluded that at higher temperature for a prolonged period of time, phenolic compounds can be damaged (Bahadur & Hathan, 2017). Thermal degradation of phenolic compounds mainly occurs when the phenolic compounds are subjected to high temperature for a prolonged period of extraction time (Wahida *et al.*, 2012).

Increasing extraction temperature has several effects on the efficacy of extraction process in general, not only in UAE. Higher heat value subjected to solvent used during extraction can break cell wall easier which assist in the mass transfer of phenolic compounds out of the cell (Wang *et al.*, 2007). Furthermore, an increase in the extraction temperature was found to be able to promote extraction of active compounds, especially phenolic compounds by increasing the diffusion coefficient and solid to solvent solubility which means now per volume unit of solvent used, there are more solutes extracted (Al-Farsi & Lee, 2008).

Effect of Time on Total Phenolic Content and Antioxidant Activity of Crude Extract
In this study, time has a similar effect as temperature and is one of the main factors to be considered in doing ultrasound-assisted extraction (Vuong *et al*., 2015). In Table 2, the significance of time linear coefficient and time*time quadratic coefficient were lower than 0.05 which means that these two coefficients are significant in the regression model. Figure 2 and 3 depict the effect of time and time*time coefficient on response total phenolic content and antioxidant activity respectively. In this experiment, the range of extraction duration used was 20-30 minutes.

In a study about extraction of bioactive components from areca nut (*Areca catechu*) using ultrasound-assisted extraction, sonication time of up to 35 minutes increases the amount of total phenolic content. Above 35 minutes of extraction at 30^{o}C, there is a decrease in the total phenolic content in the extract (Chavan & Singhal, 2013). As seen Figure 2 and Figure 3 there is an upward trend of total phenolic content and antioxidant activity when extraction time is increased while keeping the other two variables constant. At higher time and temperature combination *(i.e.,* Time = 30 minutes, temperature = 55^{o}C), there is a slight drop in both TPC and antioxidant as mentioned above. At a fixed temperature, as extraction proceeds, the TPC and antioxidant activity increase but after a certain extraction duration, the value of TPC and antioxidant activity decreases. This is due to the decomposition of some phytochemical contained in the sample (Chavan & Singhal, 2013; Wang *et al*., 2008).

Loss of polyphenolic compounds can happen due to thermal degradation. In natural plant extract, the rate of degradation is slower as compared to pure phenolic compound. This observed effect could be due to higher concentration of phenolic compounds in natural samples which can resist thermal degradation better than pure single phenolic compound (Fischer *et al.*, 2013). To find the characteristic of phenolic compound degradation in different sample, a study exclusively conducted for each sample is needed, as the complexity of phenolic compounds in each sample is different, thus affecting the overall stability of polyphenolic compounds.

Effect of Power on Total Phenolic Content and Antioxidant Activity of Crude Extract
The effect of sonication power was proven to be the least significant factor out of all three factors. This can be seen from Table 2 where p-value of 0.23544 ($p>0.05$) which mean that power is not a significant coefficient. This same conclusion can be seen from quadratic coefficients involving power too, such as power*power, power*time and power*temperature whereby none of these quadratic coefficients were significant. This observation is backed up by some studies about optimization of ultrasound-assisted extraction using RSM, sonication power often has insignificant effect on the extraction process (Vuong *et al.,* 2015; Chavan & Singhal, 2013). Therefore, setting sonication power at 70 Watt would be the best alternative as shown by Figure 1 where sonication

power of 73.03 Watt gives out the maximum values for both responses. This value is further ascertained by Wang *et al.*, (2014) where they studied the effect of sonication power on extraction of polysaccharides from *Trametes robiniophila*. When sonication power gets too high, some active components are subjected to hydrolysis.

Optimization of Extraction Factors and Suggestions to Improve the Result

Based on Figure 1, the most optimum combinations of factors to achieve the maximum values of TPC and antioxidant activity are extraction temperature of 55ºC, extraction time of 27.55 minutes and sonication power of 73.04 Watt. This result was in accordance with the experimental result obtained in Table 1 where the highest and second highest values of total phenolic content and antioxidant activity were recorded at extraction time of 25 minutes, extraction temperature of 55ºC and sonication power of 70 Watt, and extraction time of 30 minutes, extraction temperature of 55ºC, and sonication power of 90 Watt respectively. As shown in Figure 1, the optimum extraction condition was in between the extraction conditions, which gave the highest and second highest total phenolic content, and antioxidant activity. However, as seen in the prediction profiler in Figure 1, extraction temperature hits a maximum of 55ºC without reaching a peak or plateau, which means that the value of both responses could still be increasing with increasing extraction temperature.

Conclusion

In conclusion, from this study the temperature of 55ºC with sonication power of 73.04 Watt and extraction time of 27.55 minutes are the most optimal ultrasound-assisted extraction of betel leaves, maximizing the total phenolic content and antioxidant activity of the leaf extract. The findings from this study could be used for the substantial production of the bioactive compound of interest in *Piper betle* Linn.

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