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Optimization of Plectranthus amboinicus (Lour.) Spreng Extraction Process using Microwave-Assisted Technique

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Plectranthus amboinicus (Lour.) Spreng is a medicinal herb with bioactive compounds and known to have pharmacological properties. To obtain the extract of this herb, efficient extraction technique with high extraction rates is needed. Microwave-assisted extraction (MAE) is a green extraction technique with shorter irradiation time, less solvent used and lower CO_2 emission compared to other conventional extraction methods. The objective of the study was to determine the optimum operating extraction condition of *P. amboinicus* using MAE by varying the irradiation time, solvent concentration and solvent-to-solid ratio to obtain optimum yield extract and conducted using response surface methodology (RSM). The extract obtained at optimum condition was characterized for its antioxidant properties. The RSM results show that the optimum condition of *P. amboinicus* extraction was at 2 min, 30 % ethanol concentration and solvent-to-solid ratio at 30 mL/g with yield extract obtained at 39.81 wt %. The result antioxidants activity of the *P. amboinicus* extract showed IC_{50} value of 4.63 mg/mL while the inhibition of DPPH radical scavenging activity from the trolox standard was 5.48 ± 0.77 mg trolox equivalents (TEQ)/g extract. The study proved MAE is a better alternative compared to conventional extraction method with 16.8 % higher yield and the extract of *P. amboinicus* can be potentially applied in healthcare and pharmaceutical industry.

1. Introduction

Plectranthus amboinicus (Lour.) Spreng. is small flowering herbs which scattered naturally throughout the tropical countries and middle east regions like India, Asia and Australia. It is usually called as Indian borage due to its fleshy and famous succulent as it has oregano properties and refreshing odour (Arumugam et al., 2016). In Malaysia, *P. amboinicus* are known with various names such as *pokok bangun-bangun*, *bebangun, sedingin* or *hati-hati hijau* (Sabrina et al., 2014). Besides*, P. amboinicus* is among the most cited species in the family Lamiaceae mainly for its medicinal properties (Lukhoba et al., 2006) such as for digestive condition, skin condition, respiratory condition, infections and fever (Morton, 1992). This indicates that *P. amboinicus* has a pharmacological properties which is suitable for curing disease like cardiovascular, respiratory, skin, oral, digestive and urinary diseases.

Traditionally, the hydro distillation and soxhlet extraction methods are used to extract natural oils and this includes *P. amboinicus*. Soxhlet extraction requires a long 16 h of extraction time and uses more than 300 mL of solvent (Hadkar et al., 2013) with the yield ranged only between 23 wt% (Megha Rani et al., 2013) to 23.7 wt% (Megha Rani et al., 2016). To overcome this problem, microwave-assisted extraction (MAE) process was a simple and cheap equipment (Atirah et al., 2017) that has been proven to reduce extraction time, lessen solvent usage and improve the extraction yield as it uses microwave energy to heat solvents rapidly and efficiently with an ability to conduct homogeneous heating on natural product and solvent (Jain et al., 2009). MAE requires only 10 to 20 mL of solvent and 30 to 45 s of irradiation time depending on the natural product used for the extraction (Hadkar et al., 2013). Study by Wang and Weller (2006) has reported that MAE is a comparable extraction technique to other technique such as subcritical water extraction, supercritical fluid

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extraction (SFE) and ultrasonic assisted extraction (UAE). MAE has also been reported to extract natural colorants (Jusoh et al., 2018) for food application. From the literature study, there is no previous research on the extraction of *P. amboinicus* essential oil using MAE technique. However, several studies using MAE method to obtain essential oils from various medicinal plants has been conducted. Previous study done on *Mythus Communis* L. leaves (Dahmoune et al., 2015), *Crytotaenia japonica Hassk* (Lu et al., 2013) and *Pistacia lentiscus* leaves (Dahmoune et al., 2014) can act as a basis in determining the operation parameter for this study.

Essential oil of *P. amboinicus* contains high amount of bioactive compounds such as thymol at 79.20 % (Hussein et al., 2017), 11.90 %, (El-Ahmady, 2014) and 21.66 % (Senthilkumar and Venkatesalu, 2010), carvacrol at 37.7 % (Swamy et al., 2017), 90.55 % (Santos et al., 2016) and 14.3 % (Asiimwe et al., 2014), ρ-cymene at 10.83 % (Manjamalai et al., 2012), α-terpineol at 3.28 % (Senthilkumar and Venkatesalu, 2010) and β-caryophyllene at 3.09 % (Santos et al., 2016). Study by Arumugam et al. (2016) stated that the presence of thymol and carvacrol indicates that the *P. amboinicus* extract has an antioxidants properties against stress-created in cell line-induced lung cancer either for (in vitro and in vivo) models. Moreover, work by Manjamalai and Berlin Grace (2012) also reported that the presence of thymol and carvacrol showed a significant inhibition in DPPH free radical formation. This shows that *P. amboinicus* has a good potential to be used in the formulation for healthcare and pharmaceutical industry application.

This study aimed to optimize the extraction process of *P.amboinicus* using MAE technique by varying the irradiation time, solid-to-solvent ratio, and solvent concentration. Optimization experiment was designed using Design Expert® software (year) with Box-Behnken design (2006). Response surface methodology (RSM) was used to study the interaction of significant process variables after screening to predict the optimum process condition for extraction of active compound in this study. The response of oil yield was evaluated for the optimization while antioxidants activity was analyzed based on the optimum extraction condition.

2. Experimental

2.1 Reagents and materials

P. amboinicus (Lour.) Spreng leaves were collected from SMK Ulu Tiram, Johor, Malaysia (Latitude 1˚36'08.5"N, Longitude 103˚48'57.0"E). The plant was identified and authenticated taxonomically at the Forest Research Institute Malaysia (FRIM), Malaysia. The voucher specimen, PID210518-20, was deposited in the department. The chemical used were deionized water and distilled water, while 95 % ethanol, sodium sulphate and sodium carbonate were purchase from VNK Supply & Services whereas 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent, Folin-Ciocalteu reagent and gallic acid were purchased from Merck Millipore, USA.

2.2 Sample preparation

P. amboinicus leaves was cleaned by using a wet cloth to remove the dirt on the surface of the leaves and then, the collected leaves were dried in a Drying Oven (Model FCH-9036A, Constance Lab) at 60 °C (Wang and Weller, 2006) for 24 h and then ground using an electric grinder (Waring Commercial Blender 8011S Model HGB2WTS3, USA) at speed 2 for 20 s. Then, the dried leaves powder was stored in a sealed container under 4 °C for further usage (Asiimwe et al., 2014).

2.3 Microwave-assisted extraction

The extraction procedure was performed via MAE using MAS-II Plus Microwave Synthesis/Extraction Reaction Workstation (SINEO Microwave Chemistry Technology Co. Ltd., China) referring to Dahmoune et al. (2015). The powdered *P. amboinicus* sample was mixed with 40 mL of ethanol and placed in schott bottle to be soaked for 3 h and then poured into 4 neck flasks to be extracted in MAE at different parameter. Table 1 shows the independent variables of Box-Behnken Design (BBD) which includes the three parameters namely irradiation time (A), solvent-to-solid ratio (B) and solvent concentration (C) that influence the MAE which were varied in the optimization process. Total of 17 run of experiment was conducted according to Table 2. The filtrate was concentrated by using a rotary evaporator at 55 °C until all the solvent was evaporated. The screw cap amber test tubes were used to store the extract produced and kept refrigerated at 4 °C before analysis (Asiimwe et al., 2014). Weight of extracted oil per weight of dried herb was used to calculate the yield in percentage as shown in Eq(1):

$$\text{Yield wt (\%)} = \frac{\text{Weight of Extracted Produced (g)}}{\text{Weight of Dried Sample (g)}} \times 100\ \% \qquad (1)$$

Table 1: Independent Variables of P. amboinicus extract

Parameter	Coded Factor	Factor Level		
		-1	0	+1
Irradiation Time (min)	A	2	6	10
Solvent to Solid Ratio (mL/g)	B	10	20	30
Solvent Concentration (%)	C	30	50	70

2.4 Antioxidants activity analysis

The antioxidants activity was determined by using DPPH radical scavenging activity**.** 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of *P. amboinicus* extraction was measured by the method of Swamy et al. (2017) with different extract concentration range. Under the optimized condition, the extract produced was prepared from concentration 100 to 10 000 ppm. The DPPH reagent was prepared by 1.97 mg of DPPH reagent in 50 mL of 95% ethanol. Then, 120 μl of different concentration of extract was prepared. 660 μl of DPPH reagent was mixed gently together and placed in a dark condition at room temperature for 30 min. The absorbance changes of the solution was measured at a wavelength of 517 nm against the blank (95% ethanol) by using EPOCH Microplate Spectrophotometer (BioTek Instrument Inc., USA) and it was compared with that of Trolox, which was used as the standard control. The percentage of DPPH radical scavenging activity was measured by using Eq(2):

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Control Absorbance-Sample Absorbance}}{\text{Control Absorbance}} \text{ x } 100\ \% \quad (2)$$

3. Result and discussion

3.1 Optimization of MAE condition

3.1.1 Models fitting

The result for yield of P. amboinicus from the MAE is shown in Table 2.

Table 2: Box-Behnken (BBD) Matrix and Observed Response

Run	Factor 1	Factor 2	Factor 3	Response
	A: Irradiation Time (min)	B: Solvent-to-Solid Ratio (mL/g)	C: Ethanol Concentration (%)	Yield (%)
1	6	20	50	27.64
2	6	10	70	11.60
3	2	30	50	33.07
4	6	30	30	45.19
5	6	30	70	9.30
6	2	20	70	7.34
7	6	20	50	26.22
8	2	20	30	30.29
9	6	20	50	30.63
10	6	10	30	32.16
11	10	10	50	23.23
12	10	30	50	27.51
13	6	20	50	28.19
14	6	20	50	22.90
15	10	20	30	38.28
16	10	20	70	4.94
17	2	10	250	26.20

The regression quadratic equation in terms of coded factor was obtained as shown in Eq(3). The equation can be used to obtain the optimal values of P. amboinicus extract yield percentage (Y) by taking into considerations the parameters of MAE (A: Irradiation time; B: solvent-to-solid ratio; C: solvent concentration).

$$Y = 27.11 - 0.37A + 2.73B - 14.09C - 0.65AB - 2.60AC - 3.83BC - 1.98A^2 + 2.37B^2 + 4.92C^2 \quad (3)$$

From the analysis of variance (ANOVA) of the second-order polynomial regression model, the P value for the model was 0.0002. This suggests that the model is significant. The coefficient estimated for the optimization processes suggested that linear coefficients (*B* and *C*), interaction coefficient (*BC*) and quadratic coefficient (C^2) were significant models that affected the *P. amboinicus* extract yield. Furthermore, the "Lack of Fit F-value" of 1.03 implies the Lack of Fit is not significant relative to the pure error with a 46.92 % chance that a "Lack of Fit F-value" this large could occur due to noise. Hence, non-significant lack of fit is good as it indicates the model to fit.

Moreover, the values of determination coefficients (R^2), adjusted determination coefficients (R^2_{adj}) and predicted determination coefficients (R^2_{pred}) for *P. amboinicus* extract yield were 0.9704, 0.9324 and 0.7677. Hence, from the predicted determination coefficients (R^2_{pred}) is in a reasonable agreement with the adjusted determination coefficient (R^2_{adj}) as the differences is below 0.30. Thus, it indicates reasonable correlations between the predicted and observed data obtained.

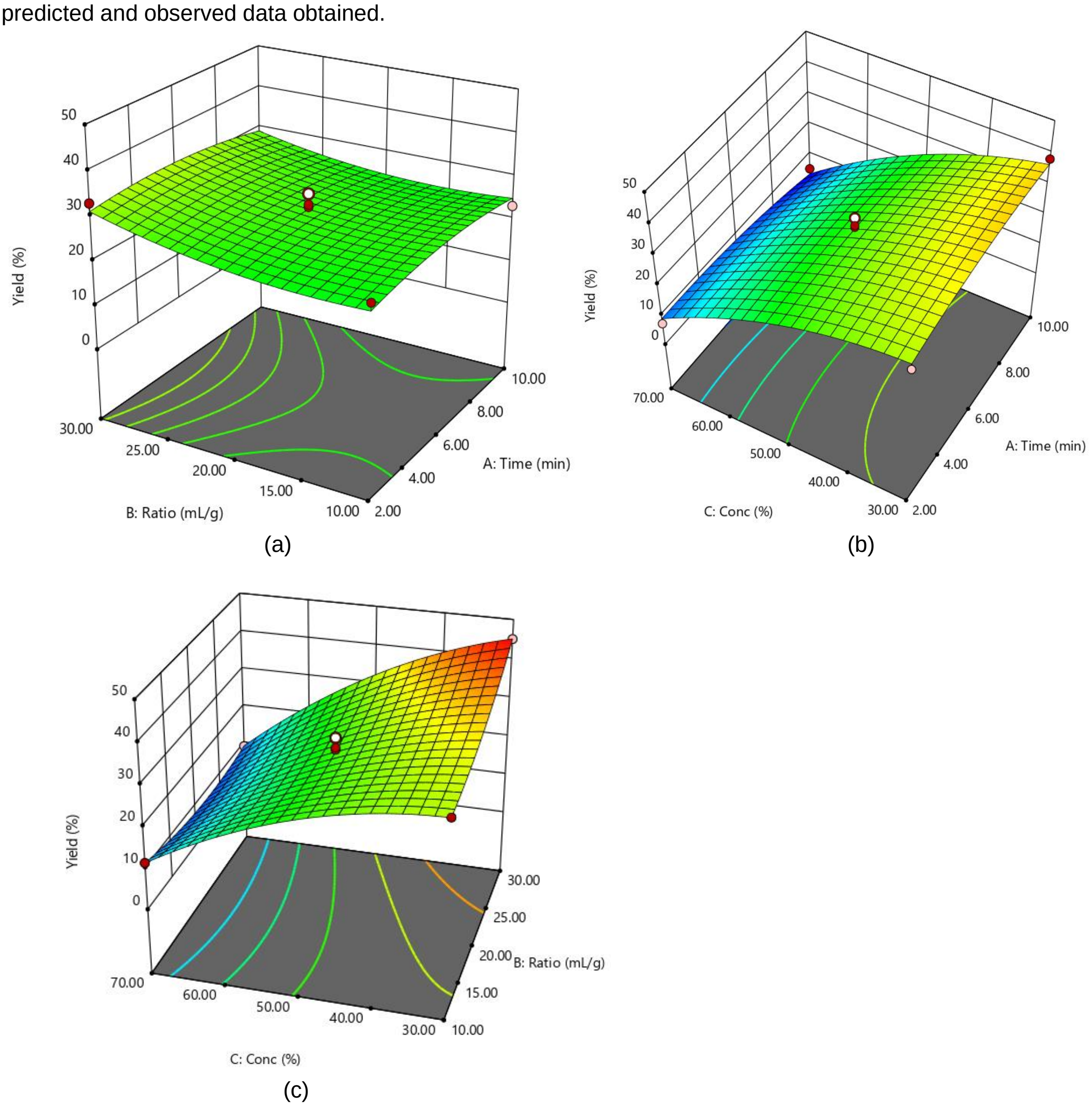

Figure 1: Response surface plot of P.amboinicus yield extract from MAE for effect of (a) irradiation time vs solvent-to-solid ratio, (b) irradiation time vs solvent concentration, (c) solvent-to-solid ratio vs solvent concentration on yield of P. amboinicus extract

3.1.2 Analysis of response surface plots

From Figure 1a, it can be observed that the yield decreases with increase in irradiation time and decrease in solvent-to-solid ratio when the ethanol concentration was kept constant at 50 %. The maximum recovery yield of *P. amboinicus* extract at 33.07 % was attained at an irradiation time of 2 min and solvent to solid ratio of 30 mL/g. Moreover, from Figure 1b, the yield declined with decrease in irradiation time and increases with ethanol concentration as the solvent to solid ratio were kept constant at 20 mL/g. The minimum recovery yield of *P. amboinicus* extract at 4.94 % was achieved at an irradiation time of 10 min and 70 % ethanol concentration. Besides, from Figure 1c, the yield increased with decrease in ethanol concentration as the irradiation time was kept constant at 6 min. The maximum recovery yield of *P. amboinicus* extract at 45.19 % was reached at 30 mL/g of solvent to solid ratio and 30 % ethanol concentration while the minimum recovery yield of *P. amboinicus* extract at 9.30 % was obtained at 30 mL/g of solvent to solid ratio and 70 % ethanol concentration. In addition, from the analysis of variance (ANOVA) of the second-order polynomial regression models, solvent-to-solid ratio and ethanol concentration have a significant model terms that affected the *P. amboinicus* extract yield.

3.1.3 Model validation

The model was verified based on the results obtained from response surface analysis, the optimal extraction for ethanol as a solvent was 2 min irradiation time, 30 % of ethanol concentration and solvent-to-solid ratio 30 mL/g. From the optimal conditions, the predicted yield value was 41.65 wt %. On the other hand, the actual yield value from experimental was 39.81 wt %. However, the optimal condition was reasonable as the percentage error was 4.42 %. This shows that MAE exhibit higher yield compared to studies by Megha Rani et al. (2013) obtain 23 wt % while Megha Rani et al. (2016) obtained 23.7 wt % when extracting P. *amboinicus* using soxhlet extraction method. Hence, MAE method produced a higher yield compared to soxhlet extraction method. Thus, MAE method is highly potential and recommended for *P. amboinicus* extraction.

3.2 Antioxidants activity

The extract of the leaves which has the highest polyphenolic content showed an appreciable DPPH radical scavenging activity as well as total antioxidant capacity. The antioxidants of the *P. amboinicus* extract was calculated from the experimental calibration curve of $y = 5.2993x + 24.812$, $R^2 = 0.9789$ where the concentration of extracts increased as the percentage of inhibition of DPPH radical scavenging activity were increased in its absorbance at 517 nm.

The IC_{50} value for ethanol extract was 4.75 mg/mL. Moreover, the inhibition of DPPH radical scavenging activity was 5.48 ± 0.77 mg Trolox eq/g extract as obtained from the trolox calibration curve of $y = 1573.7x + 2.8741$, $R^2 = 0.9913$. Result reported by Khanum et al. (2011) using column extractor also showed the same pattern of antioxidants activity where the antioxidant activity of extracts increased with increasing concentration. This finding shows the potential of ethanol extract of the leaves of *P. amboinicus* from Indian borage to act as bio-preservatives since it showed high antioxidant and antibacterial activities. Besides, this indicates that, *P. amboinicus* extract has a very good potential to be applied in the healthcare and pharmaceutical industry.

4. Conclusions

The optimum condition for extraction of *P. amboinicus* using MAE was at irradiation time 2 min, ethanol concentration 30 % and solvent-to-solid ratio 30 mL/g with the actual yield value from experimental of 39.81 wt%. Therefore, MAE has a great potential as an alternative towards conventional method given shorter extraction time and less usage of solvent with high yield of essential oil. From the antioxidants analysis of *P. amboinicus* extract the IC_{50} value was 4.63 mg/mL and the inhibition of DPPH radical Scavenging Activity was 5.48 ± 0.77 mg Trolox eq/g extract. Thus, *P. amboinicus* extract has a very good potential to be applied in the healthcare and pharmaceutical industry.

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