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RESEARCH ARTICLE

Optimization of the water extraction process on the total phenolic content from *Labisia pumila*

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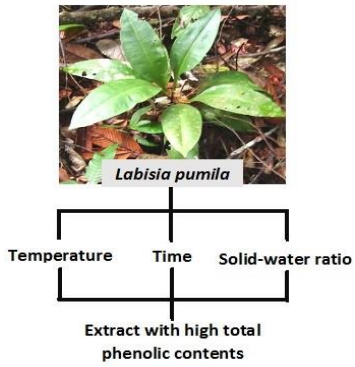
Graphical abstract



Abstract

Labisia pumila is one of the most widely used medicinal herbs among women in Southeast Asia.There is an increasing demand force for this herb in pharmaceutical and food industries. Most of these products are registered without knowing the level of bioactivity in the extracts and not in standardised form. Non-standardized extract is perceived as a low-quality herbal product, hence lowering its market value. Therefore, this study aimed to standardize the optimal water extraction conditions for maximum total phenolic content (TPC) of *L. pumila.* In this study, Response Surface Methodology (RSM) was used to optimize the extraction process of TPC from *L. pumila.* Dried whole plant of *L. pumila* was extracted in water as solvent at different temperatures, times, and solid to water ratios that have been identified to be significantly affecting the recovery of TPC. A Box-Behnken design was used to investigate the effects of three independent variables that were coded at three levels consisted of 30 experimental points using decoction method. A second-order polynomial model was used for predicting the response. Regression analysis showed that more than 91.99 % of the variation was explained by the models. Results identified temperature as the most significant ($p<0.05$) factor affecting the TPC. The optimal conditions obtained from RSM were 60°C for the temperature, 2.67 hours for the extraction time and 1:10 for the solid to water ratio. Under these optimal conditions, the response value of the experimental values agreed with the predicted value of TPC. In conclusion, the present study has succesfully standardized optimal temperature, time and solid-water ratio of *L. pumila* water extraction process for high TPC.

Keywords: Total phenolic content, *Labicia pumila*, water extract, optimization, standardization

INTRODUCTION

L. pumila or Kacip Fatimah is a well-known wild forest herb belongs to Myrsinaceae family. It is widely inhabited throughout lowland and hill forest of Peninsular Malaysia at an altitude between 300 and 700 m. This economical and medicinal important herb consists of three varieties including var. *alata*, var. *pumila* and var. *lanceolata* [1]. The plant decoction has been used since ancient by women of Malay Archipelago to ease child birth delivery, improve post-partum health and promote the health of female reproductive system [2,3]. The usage of this herb in traditional medicine is supported, at least a part, by accumulating scientific evidence. *L. pumila* extract exhibits wide range bioactivities including anti-inflammatory [4], anti-proliferation [5], anti-oxidant [6,7] and anti-photoaging [8] . Clinical data reported that *L. pumila* can improve cardiovascular risk factor (total cholesterol and low-density lipoprotein cholesterol) in pre- and post-menopausal women which may help in maintaining cardiovascular health [9]. The presence of phenolics and flavonoids are believed to be responsible for such wide-spectrum health-benefits properties of herbs [10]. Therefore, it is important to maximize the recovery of phenolics and flavonoids during extraction process. Traditionally, decoction of *L. pumila* has been prepared using water [11]. Based on the previous studies by Choi *et al.* [12], Fazliana *et al.*[13], Pihie *et al.*[14] and Mukrish *et al.* [15], only three main factors that have been reported to affect the extraction process when water is used as solvent, which are temperature of extration, process duration of extraction (time) and solid to solvent ratio. The ratio of plant quantity to solvent is one-part plant to six parts of solvent, one-part plant to eight parts of solvent and one-part plant to ten parts of solvent [12,13]. These critical components should be considered when a high solid to solvent ratio increases the concentration gradient and hence, increases the rate of diffusion of bioactive compounds to the solvent [16]. The temperature and time of extraction are important in minimizing energy and cost of the extraction process in order to extract most of the desired bioactive compounds. The standardization of extraction temperature and time is crucial as insufficient time means incomplete extraction or overheated causes deteriaration of bioactive compounds [17,18]. Extraction process was carried out for whole plant (leaves, stem and roots) of *L. pumila* plant material at three different temperatures of 60°C, 80°C and 100°C with continuous stirring for 2, 3 and 4 hours [13,14,15]. Therefore, the present study was designed to optimize temperature, time and solid-solvent ratio for obtaining the highest total phenolic

content in water extract of *L. pumila* using statistical experimental design.

EXPERIMENTAL

Materials

Dried whole plant (raw material) of *L. pumila* was obtained from Institute Bioproduct Develempont, UTM. The solvent used for the *Labisia pumila* extraction process was distilled water by Nano Ultra-pure water system (Barnstead, USA). Folin–Ciocalteu reagent, sodium carbonate and gallic acid standard were purchased from Sigma Chemical Co (St. Louis, MO, USA).

Optimization of extraction process by Response Surface Methodology (RSM)

The RSM was employed for optimization on three relevant reaction factors which were temperature, time and solid to water ratio (Table 1). The experiments were carried out in randomized run order to determine characteristic response which was the total phenolic content. The independent factors and results were taken as design as in Table 2. The experimental design and analysis of data were done using a statistical approach by using the MINITAB 15 software. The extraction processes were done through the decoction of dried whole plant in the water. The beaker was fully covered with aluminium foil to minimize evaporation of water to occur in order to maintain the water-to-sample ratio. The resultant extract was then filtered to remove suspended solids through filter paper (Whatman No.1) and then it was concentrated by using rotary evaporator. The concentrated extracts were dried via vacuum drying oven at 30^{o}C for 16 hours in the vacuum of 10-15 Mb to form the final extract in dry powder form.

Table 1 Experimental range and levels of factors influencing total phenolic contents in a Box-Behnken design.

Factors (g L^{-1})	Low (-1)	Centre (0)	High (+)
Temperature (^{o}C)	60	80	100
Time (hour)	2	3	4
Solid to water ratio (g/mL)	1:6	1:8	1:10

The TPC of *L. pumila* extracts was taken as response or dependent variables (Y_1). The experimental value of the response was recorded in the experimental set up as presented in Table 2. A second order polynomial (Eq. 1) was fitted and explained each of the response. Where Y, predicted response; intercept; β_0, linear coefficients: β_1, β_2, β_3, β_4, squared coefficients: β_{11}, β_{22}, β_{23}, β_{33}, β_{44}, interaction coefficient: β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{33}. From the Box-Behnken design, contour plots that delineated and predicted responses over a certain range in the design surface could be plotted. The contour plots between the 3 factors were analyzed and the numerical optimization was chosen to generate optimal conditions by setting a goal as 'maximum' for TPC that was analyzed by the Box-Behnken design.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} AA + \beta_{22} BB + \beta_{33} CC + \beta_{12} AB + \beta_{23} BC + \beta_{13} AC \quad (1)$$

Table 2 Actual levels for the experimental design for total phenolic content in *L. pumila* extracts.

Exp.	Temperature (^{o}C)	Time (hour)	Sample ratio (g/mL)	TPC (mg GAE)/g
1	80	4	6	23.04
2	60	3	6	60.68
3	60	4	8	47.00
4	60	2	8	57.99
5	80	4	10	36.13
6	100	3	6	13.66
7	80	4	10	30.19
8	80	4	6	28.00
9	80	3	8	39.60
10	100	3	10	10.20
11	60	4	8	61.79
12	60	3	10	71.96
13	60	3	6	38.68
14	80	2	10	39.86
15	100	3	10	11.16
16	60	3	10	80.94
17	80	3	8	38.79
18	80	2	10	38.55
19	80	2	6	32.49
20	80	3	8	46.09
21	60	2	8	63.23
22	100	2	8	14.43
23	100	2	8	13.03
24	80	3	8	46.60
25	100	4	8	13.78
26	80	2	6	52.95
27	100	4	8	9.01
28	80	3	8	40.85
29	100	3	6	15.88
30	80	3	8	39.09

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the *L. pumila* extract was determined using Folin–Ciocalteu reagent as described by Singleton and Rossi [20]. An aliquot of 1 mL extract (12.5 mg/mL) was mixed with Folin–Ciocalteu reagent (50 µl) and 2% of sodium carbonate (2 mL). The contents were mixed thoroughly and made up to 10 mL with distilled water before being incubated for 2 hours. The absorbance of the solution was measured at 765 nm using a UV–Vis spectrophotometer (Agilent Technologies Cary 60). Gallic acid was used as a reference standard and the results were expressed as milligram gallic acid equivalent (mg GAE)/g extracts.

RESULTS AND DISCUSSION

Optimization of water extraction condition using experimental design and statistical analysis (ANOVA) for total phenolic content of *L. pumila*

Analysis of Experimental Data of Box-Behnken Design as shown in Table 2 indicated that treatment runs 16 and 12 had the highest TPC value (ranging from 71.96 - 80.94 mg GAE/g). The present study showed that highest TPC value was obtained when temperature was at 60°C, time at 3 hours and sample ratio at 1:10. On the other hand, the lowest TPC value was obtained when the temperature was at 100°C, time at 4 hours and sample ratio at 1:8. These findings suggested that temperature at 60°C can help to promote the extraction of solutes [20]. ANOVA was also used to investigate the main effects and interactions among temperature, time and sample ratio on phenolic compound extraction (Table 3). For TPC as the response, the probability values for terms lower than 0.05. $P<0.05$ indicated that the model was considered statistically significant. The results showed that the linear term of extraction temperature was the major contributing factor in

phenolic extraction of *L. pumila* (F value = 203.17, P value = 0.000). This was followed by the linear term of extraction time (F value = 5.66, P value = 0.027) which also significantly affected the extraction of phenolic compounds from *Labisia pumila*. Besides, the interactions term between temperature and sample ratio (F value = 10.66, p value = 0.004) also played significant roles in *Labisia pumila* phenolic extractions. In this experiment, the response gave r^2 above 90 %, indicating that this study signified a good correlation between the experimental data and the predicted values which overall only 10 % was not explained by the model. The acceptance of the model was supported by lack of fit of 0.299 =p >0.05 which was insignificant, indicating that the model was significant [21, 22].

Fig.1(a) illustrates the normal probability plot of residuals which showed that the residuals were fallen on the straits line, implying that the errors were distributed normally and also reflected the accuracy and applicability of the independent variables to the responses. Fig. 1(b-c) depict residual versus fitted value plot and TPC which showed no unusual pattern and the data was scattered randomly. The following equations are the second order polynomial equation (2) in term of uncoded variables, which was adequate for predicting the response.

Total phenolic content = -48.7656+1.019A+9.64297B-0.0192891AC *(2)*

The contour plots provided information about the interaction between two variables and interpreted the optimum value for the desired response. The interactions between the variables were determined through the shape of the contour plots. An elliptical contour plot indicates that the interaction between the variables is significant, while a circular contour plot means negligible interaction [23].

As shown in Fig. 2(a), the converging parameters for sample ratio and time were found to be at 1:8 and 3 hours of extraction at a constant hold value of temperature at 80°C. The maximum response obtained was 42 mg GAE/g while the minimum response obtained was 30 mg GAE/g. Hence, it was clear that variation of sample ratio and extraction time above and below 1:8 and 3 hours, respectively, decreased total phenolic content. The converging concentrations for sample ratio and extraction temperature were found to be 1:8 and 80°C, respectively, as shown from Fig. 2(b) at a constant hold value of extraction time of 3 hours. It showed different trend to Fig.2(b) when sample ratio and extraction time were varied from the lower to upper limit. There was no significant difference when temperature of extraction was varied above 60°C as high temperature could cause deteriaration of bioactive compounds [17,18]. However, sample ratio showed significant effect when varied the sample ratio due to increase of concentration gradient that would increase the rate of diffusion of bioactive compounds to the solvent [16]. The maximum response obtained from the contour was 60 mg GAE/g while the minimum response obtained from this contour was 20 mg GAE/g.

Table 3 Analysis of variance (ANOVA) of main effects and interactions among temperature, time and sample ratio on total phenolic content of *Labisia pumila* water extraction.

Source	DF	Seq SS	Adj SS	Adj MS	F
Regression	9	10260.4	10260.4	1140.04	25.51
Linear	3	9510.4	9510.4	3170.12	70.95
Temperature (A)	1	9077.9	9077.9	9077.92	203.17
Time (B)	1	252.7	252.7	252.71	5.66
Sample ratio (C)	1	179.7	179.7	179.73	4.02
Square	3	204.1	204.1	68.02	1.52
Temperature* Temperature (A^2)	1	18	30.5	30.46	0.68
Time*Time (B^2)	1	159.1	168.4	168.36	3.77
Sample ratio*Sample ratio (C^2)	1	27	27	26.96	0.6
Interaction	3	545.9	545.9	181.98	4.07
Temperature*Time (AB)	1	7.5	7.5	7.53	0.17
Temperature*Sample ratio (AC)	1	476.2	476.2	476.25	10.66
Time*Sample ratio (BC)	1	62.2	62.2	62.16	1.39
Residual Error	20	893.6	893.6	44.68	
Lack-of-Fit	3	169.3	169.3	56.45	1.32
Pure Error	17	724.3	724.3	42.61	
Total	29	11154			

R-Sq = 91.99 % R-Sq(pred) = 80.13% R-Sq(adj) = 88.38%

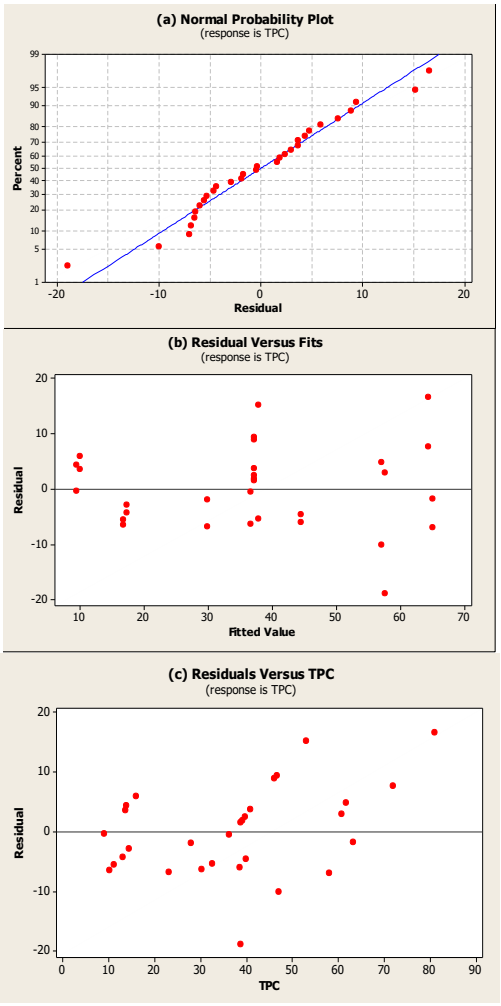


Fig.1 Normal probability plot (a), residual versus fitted value (b), and residual versus total phenolic content of the variables to the total phenolic content.

Fig. 2(c) demonstrates similar results when extraction time and temperature value were varied from the lower to upper limit at constant sample ratio of 8. The converging values for extraction time and temperature were found to be 3 hours and 80°C, respectively. The maximum response obtained was 60 mg GAE/g while the minimum response obtained was about 20 mg GAE/g. Hence, it was clear that variation of extraction time and temperature resulted in the decrease of total phenolic content. Furthermore, Fig. 2(c) indicates that temperature played an important role as the lower temperature was required for optimal phenolic compound extraction. This was in line with those reported by Idris & Sulaiman [17] and Md Salehan *et al.* [24], which showed that the extraction temperature was an important parameter which influenced the yield of gallic acid at a certain temperature. Further increase of the extraction temperature will begin to decline the extraction yield due to decomposition of the compound.

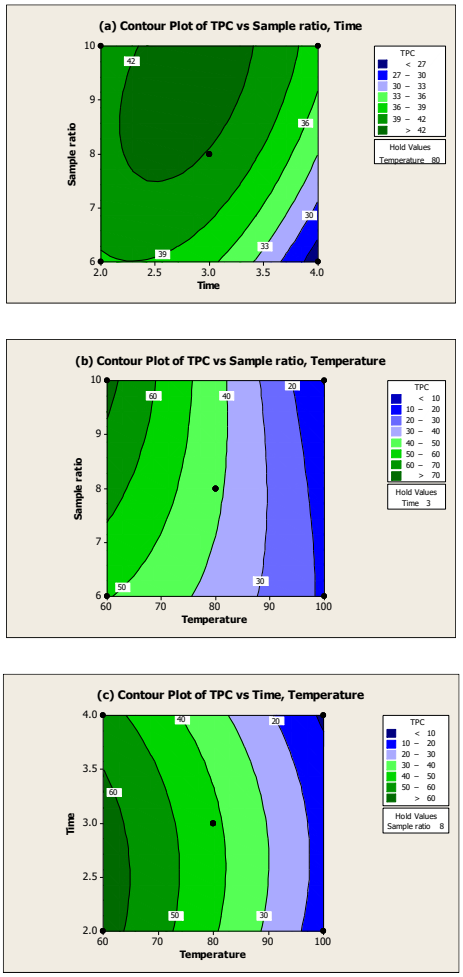


Fig. 2 Contour plot interactions between independent variables (temperature, time and sample ratio) and the total phenolic content value.

Validation of optimization study

The conditions producing the maximum extraction of the TPC in *L. pumila* extracts were determined based on a polynomial equation. The optimal condition of temperature, time of extraction and ratio of water to herbal formula was 60°C, 2.67 hours and 1:10, respectively. The optimized total phenolic content was predicted to be 73.02 mg/GAE, which was very close to the actual value of 70.91 (mg/GAE), as shown in Table 4. These results showed that the model for the total phenolic content from water extraction of *L. pumila* was able to predict the experimental conditions.

Table 4 Optimum conditions and the predicted and experimental values of the response at the optimum conditions.

Parameters	Temperature (A)	Time (B)	Sample ratio (C)	TPC (mg GAE)/g)
Predicted	60 ^{O}C	2.67 hrs	100	73.02±7.87
Actual	60 ^{O}C	2.67 hrs	100	70.91±5.99

CONCLUSION

This study indicated that the optimal conditions for water extraction were 60^{0}C for the temperature, 2.67 hours for the extraction time and 1:10 for the solid to water ratio. These optimized conditions could yield the highest TPC in *L. pumila* which would benefit consumers and industries.

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