Kinetic Extraction of Antioxidant and Total Phenolic Content of Clinachanthus nutans

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Abstract. Clinacanthus nutans or Belalai Gajah is one of the herbs that contain natural antioxidant. This natural antioxidant can be used commercially in food as well as pharmaceutical industries. This research explored antioxidant of C. nutans and it was extracted by using solid-liquid extraction. Antioxidant of C. nutans extract was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and Total Phenolic Content (TPC) were determined using Folic-Ciocalteau reagent. Screening of the suitable particle size to be used in the extraction of C. nutans shows that mixture and leaves of C. nutans with particle size <63 μm was the best to obtain the highest DPPH scavenging activity. However, only mixture of C. nutans sample was used for the thermal extraction kinetic due to the availability of the samples. Thermal extraction kinetic of DPPH was fitted to the exponential growth model and show a good fit with R² = 0.9921. When transformed, the data gave a linear Arrhenius plot, R²=0.717 with an activation energy of 17.35 ± 0.108 kJ/mol. Thermal extraction kinetic of TPC was fitted to the exponential growth model and show a good fit with R² = 0.9892. When transformed, the data gave a linear Arrhenius plot, R²=0.897 with an activation energy of 5.38³ ± 0.451 kJ/mol. The model is adequate to predict DPPH scavenging activity and TPC of the C. nutans extracts with no significant different of the prediction and validation conducted at temperature of 70 °C for 30 min.

1. Introduction

The enthusiasm in the analysis of active components, especially antioxidants from natural sources has greatly risen up in recent years. Synthetic antioxidants are being used widely compared to natural antioxidants but recently, it has been barred due to the possible negative effects on human health [1]. Natural antioxidants can be obtained from C. nutans which is locally known as Belalai Gajah. It has become an attractive herb due to the high content of flavonoid and other phytochemicals which associates with antioxidant activities. C. nutans is commonly used in traditional Malaysian medicine and being used as an anti-herpes agent and anti-hepatitis due to its nourishing and antioxidant properties. C. nutans is a good and affordable source of high-quality polyphenolic compounds which
can be used in different therapeutic procedures with the purpose of free radical neutralization in biological systems [2].

Many authors investigated solid-liquid extraction of natural antioxidants and their properties from other plant materials as well as methods for their identification [3]. However, literature data about optimization, modelling, and simulation of solid-liquid extraction process are limited. Thus, there is a need for mathematical modelling, as a beneficial engineering tool, which considerably simulation, design, facilities optimization, and control of process and contributes to utilization of time, energy, and solvent. Mathematical models are often used for the description of extraction processes of food materials and one of them is Arrhenius model [4].

In this study, the antioxidant will be determined using DPPH scavenging assay and total phenolic content (TPC). Arrhenius model will be used to examine the solid-liquid extraction. Thus, the goal of this research is to study the extraction kinetics of antioxidant and total phenolic content from C. nutans. The aim of this work is also to determine the best particle size and the best temperature for solid-liquid extraction of antioxidant under experimental conditions.

2. Materials and Methods

2.1. Chemicals
2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) and Folin-Ciocalteu reagent were purchased from Merck. Ethanol and sodium carbonate were purchased from HmBG.

2.2. Plant materials
C. nutans plants were obtained from Kampung Wang Tepus, Jitra, Kedah. The sample was dried using an oven at 50 °C overnight before being shredded into small pieces. C. nutans (leaves (L), stems (S), and both leaves & stems (M)) were ground in a grinder with possibility of particle size regulations and separated by sieving into four particle classes which are <63 μm, 63-125 μm, 125-250 μm, and 250-500 μm.

2.3. Extraction process
Extractions of the samples were carried out by using 80% ethanol as solvent. In a universal bottle, the sample (0.5 g) with different particle sizes (<63 μm, 63-125 μm, 125-250 μm, and 250-500 μm) were mixed with solvent (10 mL) to achieve various solid-liquid ratios of 20 mL/g respectively. The universal bottles were incubated in the water bath (Fisher Scientific, Malaysia) at different temperatures (25, 40, 60, and 80 °C) for 120 min. At every 15 min intervals, the samples were withdrawn and immediately cool in ice bath to stop the reaction. The extracts obtained were centrifuged for 5 min to obtain the supernatant and stored in the fridge. The supernatant will be used for the determination of total antioxidants in C. nutans. Each extraction was performed in triplicate [5].

2.4. Determination of antioxidant activity by DPPH radical scavenging assay
Free radical scavenging activity of the extracts were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH solution (0.1 mM) was prepared in 80% ethanol. Then, 1 mL of the solution was added to 3 mL of extracts and the mixtures were shake gently and incubated at room temperature for 30 min. Immediately, after 30 min incubation, the absorbance of the sample was measured at 517 nm against 80% ethanol as the blank using UV-VIS Spectrophotometer (Helios-Zeta Thermo Scientific, United State) [6]. The experiment was done in triplicate [12]. The percentage of DPPH radical scavenging was calculated using the Equation 1 as given below:

\[
\text{DPPH scavenging activity, (\%)} = \left(\frac{\text{Absorbance after reaction}}{1.85}\right) \times 100
\] (1)
2.5. Determination of Total Phenolic Content (TPC)
The total phenolic content of the C. nutans extracts was determined using the Folin-Ciocalteu reagent [7]. Accurately 0.1 mL of the extracts were added with 0.2 mL Folin-Ciocalteu reagent and 8 mL of distilled water. Then, 1 mL of sodium carbonate (20% Na₂CO₃) was added in the solution and then it was incubated at room temperature for 1 hour. The blue complex mixtures were measured at 765 nm using UV-VIS spectrophotometer (Helios-Zeta Thermo Scientific, United State). Gallic acid was used as standard and the results were expressed as mg GAE/g C. nutans

2.6. Kinetic analysis
Kinetic analysis is performed by using the graphical method as proposed by [8] shown in Equation 2.

\[
\frac{dC}{dt} = k(C_o - C)
\] (2)

where \( C \) is the weight of C. nutans, \( t \) is the extraction time in min, \( C_o \) the initial C. nutans present, and \( k \) is the effective diffusion coefficient. Integrating Equation 2 between the initial moment and a given point at time \( t \) gives rise to Equation 3.

\[
C = C_o(1 - e^{-kt})
\] (3)

The prediction and graph fitting was done using SigmaPlot® Version 12 software.

If the data shows temperature dependence, the secondary model will be conducted by using Arrhenius Equation. Equation 4 was used to determine the rate constant (\( k \)) on temperature, which is described as follows:

\[
k = k_o \exp \left( \frac{E_a}{RT} \right)
\] (4)

where \( E_a \) is the activation energy of the reaction (kJ/mol), \( R \) is universal gas constant (8.3145 J/mol K), \( T \) is absolute temperature (K), and \( k_o \) is frequency factor (min⁻¹). If equation for \( k \) values applies to a reaction in consideration, a plot of the rate constant on semi logarithmic scale as a function of reciprocal absolute temperature (T⁻¹) should yield a straight line, and the activation energy can be determined as the slope of the line multiplied by the gas constant \( R \). the \( R^2 \) values were used to select the best fit equation.

The half-life (\( t_{1/2} \)) (h) of the compound was expressed by the following Equation 5:

\[
t_{1/2} = \frac{\ln 2}{k}
\] (5)

3. Results and Discussion

3.1. Effect of particle sizes DPPH activities of C. nutans extract
Figure 1 shows the effect of different particle sizes on antioxidant activities from C. nutans stems (S), leaves (L), and mixture (M). Strong antioxidant activities for S, L, and M of C. nutans extracts were shown for particle size <63 \( \mu \)m (22.1%, 66.4%, 66.2%) respectively during extraction at 40 °C for 30 min. Among the samples, L and M have the higher antioxidant potential than S. These results are in agreement with study conducted by [9] which found that leaves of C. nutans extract contains higher amount of antioxidant activity compared to the stem.

As expected, the results revealed that smaller particle size has achieved the highest yield and content of antioxidants. It was because smaller particle size means a shorter mass transfer distance and larger resolve surface area, which ultimately increase the extraction efficiency. Similarly, [10] also
reported that the total phenolic contents significantly increased with a reduction in particle size during the extraction of antioxidants from black currant juice press residues.

In general, smaller particle size was preferred for processors to shorten the extraction time because smaller particle size dramatically increased the antioxidant yield and content without much effect on the antioxidant activity. Thus, the mixture of *C. nutans* with the smallest particle size of < 63 μm was used to study the extraction kinetic of DPPH and TPC.

![Figure 1. Effect of different particle sizes on antioxidant activities from *C. nutans* at 40°C, 30 min. Error bars indicates standard error measurement (n=3)](image-url)

3.2. Thermal extraction kinetics of DPPH in *C. nutans* extract

Figure 2 shows the temperature influence on kinetic extraction of DPPH for mixture with particle size of <63 μm. The percentages of scavenging activity were calculated according to Equation 1. The results show that, DPPH scavenging activity of all the extracts tend to reach maximum after 30 min of extraction with 24.1, 68.9, 79.3, and 80.6% scavenging activity for 25, 40, 60, and 80°C respectively.

The data were fitted with exponential growth model (Equation (3)) and show a good fit with $R^2 = 0.9921$. This was in agreement with study conducted by [11] to evaluate the kinetics of essential oil extraction of *Zingiber Cassumunar*. The model shows that extraction kinetics of DPPH scavenging activity has two curves, the rapid extraction in the beginning and started to reach equilibrium over time. The DPPH scavenging activity of the extracts studied were rapidly increased over time and reached equilibrium after 30 min of extraction. DPPH scavenging activity of the samples was temperature dependent. When transformed, the data gave a linear Arrhenius plot (Figure 3) ($R^2 = 0.717$) with an activation energy of 17.35 ± 0.108 kJ/mol. The kinetic extraction parameters of the mixture of *C. nutans* was calculated and tabulated in Table 1.
Figure 2. The temperature influence on kinetic extraction of DPPH with particle size of <63 μm at 25, 40, 60 and 80 °C. Error bars indicate standard error measurement (n=3). Lines interpolating the exponential data point show the fit of exponential growth model.

Figure 3. Arrhenius plot for kinetic extraction of DPPH.

Table 1. Kinetic parameters of *C. nutans* mixture with particle size of <63 μm at 25, 40, 60, and 80 °C for DPPH scavenging activity.

| Temperature (°C) | a (min⁻¹)     | t₁/₂ (h)      | R²        |
|-----------------|--------------|--------------|-----------|
| 25              | 23.7 ± 0.122 | 0.064 ± 0.005 | 0.9699    |
| 40              | 64.3 ± 0.677 | 0.081 ± 0.043 | 0.9751    |
| 60              | 73.2 ± 0.729 | 0.083 ± 0.042 | 0.9672    |
| 80              | 78.7 ± 0.690 | 0.084 ± 0.041 | 0.9786    |

3.3. *Thermal extraction kinetics of TPC in C. nutans extract*

Figure 4 shows the temperature influence on kinetic extraction of TPC for mixture with particle size of <63μm. The percentages of total phenolic content were calculated according to Equation 6. The results show that, TPC of all the extracts tend to reach maximum after 30 min of extraction with 29.4, 29.8, 37.9, and 42.4% TPC for 25, 40, 60, and 80 °C respectively.

$$ y = 1.478x \quad (6) $$

The data were fitted with exponential growth model (Equation 3) and show a good fit with $R^2 = 0.9892$. The model shows that the extraction temperature had effects on the total phenolic yields and contents. The total phenolic yields rapidly increased within the first 30 min, and then start to reach...
equilibrium over time. The total phenolic yields and contents were significantly increased with the increased extraction temperature. This might be due to increased solubility and diffusion coefficient of antioxidants at high temperature [12]. Total phenolic content of the sample was a temperature dependent which the total phenolic content will increase when the temperature increase. When transformed, the data gave a linear Arrhenius plot (Figure 5) ($R^2 = 0.897$) with an activation energy of $5.3832 \pm 0.451$ kJ/mol. The kinetic extraction parameters of the mixture of C. nutans was calculated and tabulated in Table 2.

Figure 4. The temperature influence on kinetic extraction of TPC with particle size of <63 \( \mu \)m at 25, 40, 60, and 80 \( ^\circ \)C. Error bars indicates standard error measurement (n=3). Lines interpolating the experimental data points show the fit of exponential growth model

Figure 5. Arrhenius plot for kinetic extraction of TPC

Table 2. Kinetic parameters of C. nutans mixture with particle size of <63 \( \mu \)m at 25, 40, 60, and 80 \( ^\circ \)C for TPC

| Temperature (\(^\circ \)C) | \( a \) (min\(^{-1}\)) | \( t_{1/2} \) (h) | \( R^2 \) |
|---------------------------|-----------------|----------------|-------|
| 25                        | 28.42 ± 0.271   | 0.067 ± 0.086  | 0.9865|
| 40                        | 29.32 ± 0.240   | 0.068 ± 0.036  | 0.9895|
| 60                        | 37.21 ± 0.258   | 0.071 ± 0.078  | 0.9871|
| 80                        | 38.23 ± 1.114   | 0.072 ± 0.023  | 0.8933|

3.4. Prediction and Validation of DPPH scavenging activity and TPC

Prediction and validation of the model (Table 3) shows that there is no significant different between the prediction and validation for both DPPH scavenging activity and TPC for extraction at temperature of
70 °C for 30 min. The model is adequate to predict DPPH scavenging activity and TPC of the *C. nutans* extracts. However, this model is limited to the extraction of *C. nutans* with the particle size of <63 μm. Further study needs to be conducted to develop more robust model for the extraction of the *C. nutans* and perhaps can be applied to other plant samples as well.

**Table 3.** Prediction and validation values of DPPH scavenging activity and TPC for extraction at 70 °C for 30 min

|                  | DPPH Scavenging Activity (%) | TPC (%) |
|------------------|-------------------------------|---------|
| **Prediction**   | 82.4 ± 6.70^a                 | 35.6 ± 2.88^a |
| **Validation**   | 88.6 ± 1.11^a                 | 32.8 ± 0.25^a |

Data are means ± standard error (n=3). Values that are followed by different letters within each column are significantly different (P<0.05) using Turkey’s Honest Significant Difference test.

4. Conclusion

Screening of the suitable particle size to be used in the extraction of *C. nutans* shows that mixture and leaves of *C. nutans* with particle size <63 μm was the best to obtain the highest DPPH scavenging activity. It also shows that particle size of the samples was inversely proportion to the DPPH scavenging activity. However, only mixture of *C. nutans* sample was used for the thermal extraction kinetic due the availability of the samples. Thermal extraction kinetic of DPPH was fitted to the exponential growth model and show a good fit with $R^2 = 0.9921$. The model shows that the extracts studied were rapidly increased over time and reached equilibrium after 30 min of extraction. When transformed, the data gave a linear Arrhenius plot (Figure 3) ($R^2 = 0.717$) with an activation energy of $17.35 \pm 0.108 \text{ kJ/mol}$. Thermal extraction kinetic of TPC was fitted to the exponential growth model and show a good fit with $R^2 = 0.9892$. The model shows that the extracts studied were increasing over time and reached equilibrium after 30 min of extraction. When transformed, the data gave a linear Arrhenius plot (Figure 5) ($R^2 = 0.897$) with an activation energy of $5.3832 \pm 0.451 \text{ kJ/mol}$. The model is adequate to predict DPPH scavenging activity and TPC of the *C. nutans* extracts with no significant different of the prediction and validation conducted at temperature of 70 °C for 30 min.

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