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Kinetic Study of Total Phenolic Content from *Piper betle* Linn. Leaves Extract Using Subcritical Water

(Kajian Kinetik Jumlah Kandungan Fenolik daripada Ekstrak Daun *Piper betle* Linn. Menggunakan Air Subkritikal)

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ABSTRACT

The green plant-based extraction of phenolic compounds is still challenging and attractive due to their benefit. The mechanism controlling of desorption rate of phenolic compounds, measured as total phenolic content (TPC), from *Piper betle* Linn. (PBL) leaves using subcritical water, and a one-site kinetic desorption model (first order) was studied. One-site kinetic desorption model has well explained the extraction mechanism of phenolic compounds from PBL leaves using subcritical water through desorption and diffusion mechanism. This model fits with the experimental data and presents a good description of the extraction mechanism with R-squared of 0.94. The recovery of TPC from PBL leaves using subcritical water was influenced by intraparticle diffusion, temperature, and extraction time. The desorption rate constant in the one-site kinetic desorption model increased from 100 to 200 °C (0.3975±0.02 to 3.3045±0.00 min^{-1}) and then decreased to 250 °C (3.2093±0.00 min^{-1}). The highest TPC was recovered quickly for 5 min at 200 °C. In addition, a high yield of TPC was also obtained at a slow desorption process for 30 min at a lower temperature of 175 °C. The low activation energy for the diffusion of phenolic compounds from PBL leaves of this study was 8.964 kJ/mol. This result showed that the one-site kinetic desorption model of subcritical water extraction has an excellent opportunity to be applicable in phenolic compounds recovery from PBL leaves. The one-site kinetic desorption rate constant and mathematical kinetic model equation achieved in this study might control the quality of phenolic compounds extracted from PBL leaves through subcritical water.

Keywords: Activation energy; betel leaves; desorption rate; kinetic; subcritical water

ABSTRAK

Pengekstrakan berasaskan tumbuhan hijau sebatian fenolik masih mencabar dan menarik kerana manfaatnya. Mekanisme yang mengawal kadar desorpsi sebatian fenolik, diukur sebagai jumlah kandungan fenolik (TPC), daripada daun *Piper betle* Linn. (PBL) menggunakan air subkritikal dan model penyahserapan kinetik satu tapak (turutan pertama) telah dikaji. Model penyahserapan kinetik satu tapak telah menjelaskan dengan baik mekanisme pengekstrakan sebatian fenolik daripada daun PBL menggunakan air subkritikal melalui mekanisme penyahserapan dan penyebaran. Model ini sesuai dengan data uji kaji dan menunjukkan deskripsi yang baik tentang mekanisme ekstraksi dengan koefisien determinasi (R^2) sebesar 0.94. Pemulihan TPC dalam daun PBL menggunakan air subkritikal dipengaruhi oleh penyebaran antara zarah, suhu dan masa pengekstrakan. Pemalar kadar desorpsi dalam model desorpsi kinetik satu tapak meningkat daripada 100 kepada 200 °C (0.3975±0.02 kepada 3.3045±0.00 min^{-1}) kemudian menurun kepada 250 °C (3.2093±0.00 min^{-1}). TPC tertinggi telah pulih dengan cepat selama 5 minit pada 200 °C. Di samping itu, hasil TPC yang tinggi juga diperoleh pada proses desorpsi perlahan selama 30 minit pada suhu yang lebih rendah 175 °C. Tenaga pengaktifan rendah (Ea) untuk penyebaran sebatian fenolik daripada daun PBL kajian ini adalah 8.964 kJ/mol. Hasil ini mendedahkan bahawa model penyahserapan kinetik satu tapak pengekstrakan air subkritikal mempunyai peluang yang sangat baik untuk digunakan dalam pemulihan sebatian fenolik daripada daun PBL. Kadar penyahserapan kinetik satu tapak pemalar dan persamaan model kinetik matematik yang dicapai dalam kajian ini mungkin mengawal kualiti sebatian fenolik yang diekstrak daripada daun PBL melalui air subkritikal.

Kata kunci: Air subkritikal; daun sirih; kadar penyahserapan; kinetik; tenaga pengaktifan

Introduction

Piper betle Linn. (PBL) leaves are essential plants in Asian countries such as India, Bangladesh, Sri Lanka, Malaysia, Indonesia, Philippines, and East African countries (Haider et al. 2013; Umar et al. 2018). PBL leaves contain phenolic compounds useful in nutraceuticals, cosmetics, or the food and beverage industry. Phenolic compounds are useful for antioxidant, antidiabetic, antimicrobial, anti-rheumatic, alfa amylase inhibitor, and many other biological activities (Abrahim, Kanthimathi & Abdul-Aziz 2012; Madhumita, Guha & Nag 2019; Murugesan et al. 2020; Taukoorah, Lall & Mahomoodally 2016; Yogeswari et al. 2020).

The main problem in obtaining phenolic compounds from PBL leaves was caused by the conventional extraction process that used organic solvent and was time-consuming (Abrahim, Kanthimathi & Abdul-Aziz 2012; Arambewela, Arawwawala & Rajapaksa 2006; Arawwawala et al. 2011; Murugesan et al. 2020; Taukoorah, Lall & Mahomoodally 2016; Yogeswari et al. 2020;). Moreover, another process using acid or alkaline hydrolysis caused the side effect of acid or base residue (Kumar & Goel 2019). Therefore, the promising technology for getting phenolic compounds is using subcritical water extraction (SWE) (Kim & Lim 2020; Nkurunziza, Pendleton & Chun 2019). In addition, water is an environmentally friendly solvent and can modify the dielectric constant by managing the operation time and temperature in subcritical conditions.

The subcritical condition was achieved when water was pressurized at around 0.101-22.064 MPa (1-221 bar) and heated at a high temperature between 100-374 °C. In this condition, water is still in liquid form, and water's polarity is reduced like ethanol or methanol and lower the dielectric constant (Chemat & Strube 2015; Zhang et al. 2020). As pressurized water becomes close to the polarity of organic solvent, it can potentially improve the extraction process of phenolic compounds due to the low polarity. The gallic acid equivalent per g dry weight (mg GAE/g dry weight) can be used to measure the content of total phenols in the whole extract (Essien, Young & Baroutian 2020; Nastić et al. 2018). The concentration of phenolic compounds from the extract is determined by quantifying the total phenolic content (TPC) after the SWE process to indicate antioxidant presence.

TPC could be different in each matrix plant (Mufari et al. 2021). The understanding of the ability of SW to increase the yield of TPC is still interesting to explore. Phenolic compounds as a secondary metabolite of *P. betle* are stored inside the secretory cells in the mesophyll region of the leaf with diameter of 31-33 µm (Raman, Galal & Khan 2012). Rahmah et al. (2022) proved that there were 30 opened pores in a 250 µm particle size of PBL powder with an oval diameter of roughly 30 × 15 µm in PBL residue after SWE. This result proved that there is a strong possibility happened the diffusion phenomenon. SWE is used to extract bioactive compounds from many plant matrices or by-products, but there is little research on the basic mechanism of extracting phenolic compounds for PBL leaves. Studying these mechanisms through one-site desorption kinetic model could enhance our understanding. This research wants to present useful information about the mechanisms of PBL leaves extraction through approaches to the kinetic theory of desorption and diffusion that may be used to increase the scale for future commercial production.

Other authors have studied a one-site kinetic desorption model to describe the extraction of okara isoflavones (Nkurunziza, Pendleton & Chun 2019) and citrus unshiu peel flavonoids (Kim & Lim 2020) using a subcritical water. However, to date, research has yet to be explored on the SWE one-site desorption kinetic of phenolic compounds from PBL leaves and activation energy calculation obtained from the diffusion coefficient. In this study, a one-site kinetic desorption model for PBL leaves extraction using laboratory scale of a batch-type oil and salt bath subcritical water extractor was studied to get the desorption rate constant. Moreover, this study also evaluated the diffusivity and desorption activation energy from the SWE process in recovering phenolic compounds from PBL leaves. These results significantly benefit the industry by reducing trial and error in phenolic compounds extraction, reducing the organic solvent usage, controlling and predicting phenolic compounds quality, and for process optimization.

Materials and Methods

Chemicals and Reagents

All chemicals used in this research were analytical grade. Gallic acid (3,4,5-Trihydroxybenzoic acid monohydrate, 99%) was purchased from Alfa Aesar, Thermo Fisher Scientific (Heysham, Lancashire, United Kingdom), Folin & Ciocalteu's Phenol reagent, and sodium carbonate anhydrous 99.5% analytical reagent were obtained from R&M Chemicals, ethanol 95% (v/v) was purchased from SYSTERM® (ChemPur®),

and distilled water was distilled by laboratory water purification system (model of DW 200, series number of 1309039, Hitech Instrument Co., Ltd).

RAW MATERIAL PREPARATION

Fresh PBL (family: *Piperaceae*) leaves were purchased from the traditional market 'Pasar Borong Selangor' harvested from Banting, Selayang, and Rawang (Selangor, Malaysia). The plant age of PBL leaves was over one year, with the commercial harvest of this leaves being two weeks. These leaves were processed 24 h after they were harvested. The fresh PBL leaves were washed to remove impurities and dried at 70 °C for 4 h using the oven. The dried leaves were pulverized using a blender and sieved at 250 µm to obtain a uniform size with a moisture content of 10.8%. PBL leaves powder was stored in a glass bottle covered using aluminium foil and put in a desiccator until used for extraction.

SUBCRITICAL WATER EXTRACTION EQUIPMENT AND PROCEDURE

This experiment was conducted using two units of equipment: (i) a laboratory scale of a batch-type oil and salt bath subcritical extractor purchased from Thomas Kogaku Co Ltd., Japan and (ii) a stainless-steel reactor cell with a length of 150 mm, 7.5 mm inner diameter and volume capacity 10 mL, purchased from Swagelok Company, USA. The photograph of the oil and salt bath of the subcritical water extractor and reactor cell is shown in Figure 1(a), 1(b), and 1(c), respectively. The kinetic study of SWE from PBL leaves powder utilized batch-type of oil and salt bath subcritical extractor at temperature (100-250 °C) and time (5-30 min). The oil bath was used for extraction at temperatures of 100-175 °C, whereas the salt bath was at 200-250 °C. The temperature controller in the SWE equipment monitored the temperature during extraction. The pressure ranges from 0.102-5.947 MPa.

The extraction was conducted in the stainless-steel reactor cell. PBL leaves powder (0.75 g) was inserted into the reactor followed by 4.25 mL of distilled water and purged with argon gas to release trapped air in the reactor. The reactor was then inserted into the oil or salt bath according to the temperature and time setting. Cooled down in cold water, opened the reactor, transferred the extract to the 15 mL conical centrifuge tube, and centrifugated at 4,000 rpm. Filtered the supernatant using a 0.22 µm nylon filter and moved it into a vial bottle. Keep the PBL leaves extract at -15 °C for further analysis. All the experiments were conducted in two replications.

TOTAL PHENOLIC CONTENT (TPC) ANALYSIS

The total phenolic compounds of PBL leaves extract were measured by total phenolic content (TPC) using a modified Folin-Ciocalteu method (Cliffe et al. 1994; Rahmah et al. 2022; Zakaria et al. 2017). TPC was calculated from the gallic acid standard curve in milligrams of gallic acid equivalent per gram of PBL leaves (mg GAE/g). The gallic acid standard solution 20, 40, 60, 80, 100, 120, and 140 mg/L) was prepared followed by Rahmah et al. (2022). Each 200 µL standard solution was added with 2,800 µL distilled water, and 0.5 mL Folin-Ciocalteu reagent. The mixture was homogenized with a vortex for 20 s then incubated for 3 min in the dark. This solution was added by 20% (w/v) sodium carbonate (2 mL) and mixed using a vortex for 20 s. The measurement of absorbance was conducted using a UV-Vis spectrophotometer at 765 nm after incubation for 60 min in the dark. A blank solution was prepared, similar to the standard without the addition of gallic acid solution.

TPC of PBL extract was similar preparation with standard solution, by changing the standard with the extract. Approximately 20 mg extract was diluted up to 10 mL with distilled water. Each 200 µL extract solution was added with 2,800 µL distilled water, and 0.5 mL Folin–Ciocalteu reagent. The mixture was homogenized using a vortex for 20 s, then incubated for 3 min in the dark. This solution was added with 20% (w/v) sodium carbonate (2 mL) and mixed using vortex for 20 s. The absorbance of the mixture solution was measured using a UV-Vis spectrophotometer at 765 nm after incubation for 60 min in the dark.

KINETIC PARAMETER OF ONE-SITE KINETIC DESORPTION MODEL (FIRST ORDER) CALCULATION

One-site kinetic desorption model of SWE described that extraction is controlled by intra-particle diffusion. Diffusion occurs when a solute in the plant matrix moves from a higher concentration to a lower concentration before migrating to the solvent. Mass transfer of solute happens when the solvent desorbs the solute into the solvent phase. In the first order, the one-site kinetic desorption model considers that the solute was initially uniformly distributed within the matrix, and intra-particle diffusion controlled the extraction. This model was analogous to the hot ball transfer heat model. The

compound may remain in the spherical matrix (Cr) during extraction (t). Therefore, the calculation of the ratio between the compound that remains in the matrix and the initial concentration extractable (Co) is shown in Equation (1) (Islam et al. 2013):

$$\frac{Cr}{Co} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(\frac{-D_e n^2 \pi^2 t}{r^2}\right) \quad (1)$$

where n is an integer and De is the effective diffusion coefficient. Generally, after t > 0.5tc (characteristic time quantity), the curve for Equation (1) tends to become linear at a prolonged time. The logarithm natural of the ratio between Cr and C0 is written by Equation (2):

$$ln\left(\frac{Cr}{Co}\right) = -0.4977 - \frac{t}{t_0} \quad (2)$$

where t0 is the initial time, *tc* (min), defined in Equation (3):

$$t_c = \frac{r^2}{\pi^2 D_e} \quad (3)$$

At the beginning of extraction, no solute migrated to the solvent. Hence, no concentration or zero concentration in the solution. The mathematical model equation for calculating the total mass or concentration of extracted solute (ct) is given in Equation (4) (Anekpankul et al. 2007; Asl & Khajenoori 2013; Islam et al. 2013; Mufari et al. 2021):

$$\frac{ct}{c0} = 1 - exp\,(-kt) \quad (4)$$

where c_0 is the initial mass or concentration (mg/g) of solute in the plant matrix, *k* is the first-order rate constant; *ct* is the mass of the solute removed by the extraction process after time *t* (Asl & Khajenoori 2013; Mufari et al. 2021). Using Microsoft Excel Solver®, the kinetic parameters were obtained by minimizing the errors between the experimental data and the model.

DIFFUSIVITY AND ACTIVATION ENERGY

Mass transfer characteristics could be described by an empirical model approach from a one-site kinetic desorption model through a diffusion model to obtain an effective diffusion coefficient (De, m^2/s). The effective diffusion coefficient, as shown in Equation (5) obtained by entering the value of some variables based on a one-site kinetic model (Islam et al. 2013; Kim & Lim 2020.

$$1 - \frac{Ct}{C0} = \frac{6}{\pi^2} \exp\left(-\frac{D_e \pi^2 t}{r^2}\right) \quad (5)$$

De was calculated by calculating the normal logarithm (ln) from each side of Equation (5), where Ct and C0 were obtained from the mass of phenolic compounds (mg GAE/g) at t time and initial time (0 min), and *r* is the radius of the spherical particle. De in different temperatures, was determined from the slope of time versus ln [1-(Ct/C0)].

STATISTICAL ANALYSIS

The kinetic parameters data (k) were expressed in mean ± deviation standard of replication. Data were subjected to statistical analysis using IBM SPSS Statistics 20. Analysis of variance (ANOVA) was used to know the differences in temperatures toward kinetic parameters with 95% significance (α = 0.05). Duncan's multiple range test was applied for multiple comparisons if those temperatures were significantly different. In addition, the coefficient determination (R^2) using Microsoft Excel Worksheet was used to know the fitness of the model by minimizing the error.

Results and Discussion

ONE-SITE KINETIC DESORPTION MODEL (FIRST ORDER) KINETIC MODEL OF TPC

The one-site kinetic desorption model for the TPC of PBL leaves obtained by subcritical water treatment in this study was easily described by the illustration mechanism, which is shown in Figure 2. This kinetic model was controlled by intra-particle diffusion that was started by the desorption process of the phenolic compounds located in the plant matrix in the cell. Specifically, the phenolic compounds as secondary metabolite was stored in the secretory cell (Raman, Galal & Khan 2012), and this cell is also guarded with the cell wall. The rigid structure of cell wall consists of major cell wall components, i.e., cellulose, hemicellulose, pectin, and lignin (Bar-Peled & O'Neill 2011; Temple et al. 2016). Cellulose is the main polymer in most plant cell walls, and it consists of unbranched β-(1,4)-linked glucan chains (Ding et al. 2012; Zhang et al. 2019). However, the plant cell wall is the least understood cellular structure in plants (Zhang et al. 2021). By considering this mechanism, the relationship between the cell wall, matrix plant, phenolic compounds, cell pores, desorption and diffusion process for the phenolic compounds extraction using subcritical water is more comprehensively understood. Hence, this study describes the extraction mechanism of total phenolic compounds in PBL leaves, especially from the secretory cell.

Figure 2 shows the extraction of total phenolic compounds was started by rapid solvent entry from the solvent phase by damaging the β-(1,4)-linked glucan chains or glycosidic bonds (Das & Arora 2021). In this condition, phenolic compounds in plant matrix are usually linked to polysaccharides (Gong et al. 2015). The desorption occurred when they received enough energy to make the linkage brake. The desorption was influenced by temperature and time. When the phenolic compounds are released from the active site of the plant matrix, they are concentrated in the middle or surface inside the cell. The high concentration of phenolic compounds was controlled by intraparticle diffusion (Mufari et al. 2021). The diffusion depends on the other compound concentration surrounding the surface of the secretory cell because of the desorption process and then undergo the diffusion through pores.

To assess the applicability of a one-site kinetic model in TPC recovery from PBL leaves using SWE, the first-order desorption rate constant (k) was determined by fitting the model to the experimental data. The k value was preferably in high value because it describes the desorption of phenolic compounds from the matrix plant. The k value of TPC of SWE from PBL leaves is presented in Table 1. Based on ANOVA, the k value in this study increased significantly ($p<0.05$) by temperature with an average determination coefficient (R^2) of 0.94. Similar result was also reported by Kim and Lim (2020) that k increased with the temperature when the flow rate was constant. The first-order desorption rate constant value was raised by temperature up to 200 °C, then decreased and constant until 250 °C. It is caused by the lower surface tension, viscosity and dielectric constant of water with the increasing temperature of subcritical water (Islam et al. 2013; Jamaludin et al. 2021).

Based on Table 1, the k value from 100 to 175 is quite low. It might be the low energy exposed to the matrix cell, so it needs more time to desorb the phenolic compounds from the matrix plant. Statistically, the desorption rate increased to 0.4147±0.02 min^{-1} from 100 to 150 °C, then constant to 175 °C (0.4059±0.02 min^{-1}). It might be due to the higher energy by the temperature of subcritical water can desorb the phenolic compounds quickly. When the higher energy from increasing temperature was exposed to the matrix plant, the phenolic compounds bonding was broken, and it released the phenolic compounds in the cell. The diffusion occurred due to the different concentrations between the inside close to the surface of the cell to the outside by migration of the phenolic compounds from inside to outside through cell pores.

From 175 to 200 °C, the increase in desorption rate was very sharp, about 8-fold. This result agreed with this finding where the highest TPC was achieved at 200 °C (7.89±0.26 mg GAE/g) for 5 min and supported by Mufari et al. (2021) that the highest TPC result from malted-quinoa resulted from the optimal temperature of 200 °C. The TPC in this study was higher than TPC from PBL leaves (variety: Banarasisafeda) obtained by maceration for 2 h (using a shaking incubator) with various solvents of 80% methanol (2.62±0.036 mg GAE/g), 80% ethanol (2.04±0.87 mg GAE/g), 80% acetone (2.73±0.30 mg GAE/g), 80% ethyl acetate (1.94±0.27 mg GAE/g), and water (0.29±0.02 mg GAE/g) (Jaiswal et al. 2014). High TPC is related with the increasing of desorption rate of TPC using SWE until 8-fold. The significant rise in the desorption rate was due to the changes in water properties, for instance, lower dielectric constant, viscosity, and surface tension (Jamaludin et al. 2021). This condition can decrease the polarity of water and make it possible to extract moderate polar compounds (Zhang et al. 2020). Therefore, the subcritical water can desorb fast the phenolic compounds up to 3.3045±0.00 min^{-1}.

Raised temperature from 200 to 250 °C significantly decreased the desorption rate value and agreed with the decreased concentration of TPC from 200 to 250 °C. Increasing temperature to 250 °C resulted in the decreasing polarity of water and dielectric constant, and the properties of subcritical water became like methanol and could extract low polar compounds. However, the desorption rate of phenolic compounds becomes slower due to the possibility of secondary metabolites such as terpenes and alkaloid compounds also easily desorb (Jamwal, Bhattacharya & Puri 2018; Kanjwani et al. 2008; Sugumaran et al. 2011). The desorption competition of these compounds can disturb the desorption rate of phenolic compounds. Hence, the crowded product inside the cell might be affect the movement of phenolic compounds to be diffused. In addition, once the diffusion occurs, particular phenolic compounds might damage due to the higher temperature. Zakaria et al. (2017) mentioned that increasing the temperature of subcritical water to 250 °C was not suggested because it might damage the phenolic compounds that have been extracted.

These phenomena are well explained in that one-site kinetic desorption model described intra-particle diffusion with constant flow rate by setting the speed controller in a similar value (50 speed). Therefore, the intra-particle diffusion was influenced by temperature, which was clearly defined in this model because the flow rate of subcritical water was assumed to be relatively low and constant (Asl & Khajenoori 2013).

To understand the desorption rate value with the experimental data and model, Figure 2 was created to fit the one-site kinetic desorption model in first order to the experimental data for the SWE of the phenolic compounds from PBL leaves. The marker shows experimental data, whereas kinds of dash lines show one-site kinetic model equations in different temperatures. Generally, after t > 0.5tc (characteristic time quantity), the curve for the one-site kinetic desorption model tends to become linear at a prolonged time (Islam et al. 2013; Mufari et al. 2021). The relationship between k value and TPC, either in experimental data or model, clearly shows that from 100 to 175 °C, the k value was quite the same. However, if we observed the fit curve of the one-site desorption model and experimental data in Figure 3, TPC well extracted in prolonged time for up to 30 min at temperature 175 °C (7.61±0.08 mg GAE/g). In this operation condition, the TPC reaches equilibrium which has a similar result with condition extraction at 200 °C for 5 min. It showed that at 175 °C, the low value of k can contribute to high yield by prolonged extraction time. This result was similar to Kim and Lim (2020) that a prolonged time to 30 min in subcritical conditions could reach the equilibrium of the flavonoids extraction from citrus unshiu peel in the thermodynamic partitioning model. It is similar to the one-site kinetic model, but they only differ on 'k' and 'keap/kD'.

According to Figure 3, TPC increased from 100 to 200 °C, then decreased to 250 °C. This result was supported by the values of k and De from 100-175 °C is lower than at 200-250 °C. It means that the desorption rate below 200 °C is very low due to the low energy needed to undergo the desorption in the cell matrix. In contrast, starting from 200 °C above, k and De values were high because they had enough energy to desorb the TPC from the plant matrix. The amount of TPC was highly recovered in 5 min (short period) then slightly decreased and became constant at a prolonged time. This result was supported by Bodoira et al. (2017), that the optimum extraction time of phenolics compound of defatted peanut skin using SWE was below 10 min at 220 °C.

DIFFUSIVITY AND ACTIVATION ENERGY

Diffusion occurs when there is a difference in concentration of phenolic compounds between inside and outside the cell. The diffusion migrated the phenolic compounds from inside to the solvent phase through pores that were formed by the breakdown of cell wall components (Temple et al. 2016; Zhang et al. 2019). Increasing temperature at 100-200 °C affected the increase of De (2.390×10^{-4} - 6.726×10^{-4} m^2/s), followed by decreasing in De at elevated temperatures of 250 °C (5.198×10^{-4} m^2/s) as shown in Table 2. De values at 100-175 °C were quite low compared to a higher temperature (200-250 °C). This condition might be affected by the decreasing particle size when extraction was conducted at the higher temperature so that migration or diffusion of phenolic compounds is wider than in lower temperatures. This result was suitable in approving Fick's second law for spherical particles (Crank 1975; Cussler 1984).

The measurement of the activation energy (Ea) of TPC was used to know the reaction dependency on the temperature and was suggested by other authors (Jamaludin et al. 2021; Kim & Lim 2020). The activation energy calculated by the effective diffusion coefficient (De) can be seen as the energy barrier that molecules need to overcome to be able to diffuse. According to Arrhenius's law (van Boekel 2009), Ea was calculated by multiplying Equation (6) by ln of each other. The slope from the linear regression of the curve plot between 1/T (K) as the x-axis and ln De as the y-axis could be used to measure the Ea by multiplying it with the molar gas constant.

$$D_e = D_0 \exp(-\frac{E_a}{RT}) \quad (6)$$

The Ea for the diffusion of phenolic compounds from PBL leaves of this study was 8.964 kJ/mol. The lower the activation energy, the higher the reaction rate. The lower the barrier, the higher molecules would have enough energy to make it over at any given moment (Islam et al. 2013). The Ea of this result was lower than hesperidin (37.2 - 43.8 kJ/mol) (Kim & Lim 2020) and alizarin (62.7 - 64.5 kJ/mol) (Jamaludin et al. 2021) extracted using subcritical water. It means that this result might be useful for phenolic compounds extraction as an antioxidant and proved that the extraction of phenolic compounds from PBL leaves successfully extracted the antioxidant in low Ea and depending on the time extraction. In other words, the flow rate might not significantly affect the TPC extraction. Therefore, the extraction mechanism using subcritical water treatment can be modelled by one-site kinetic model.

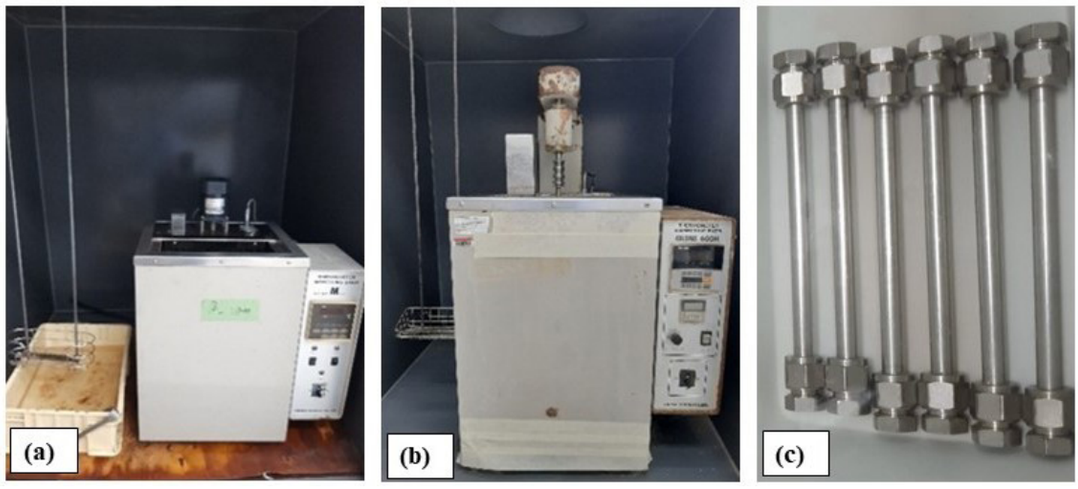

FIGURE 1. Photograph of (a) oil bath subcritical extractor (b) salt bath subcritical extractor, and (c) reactor cell

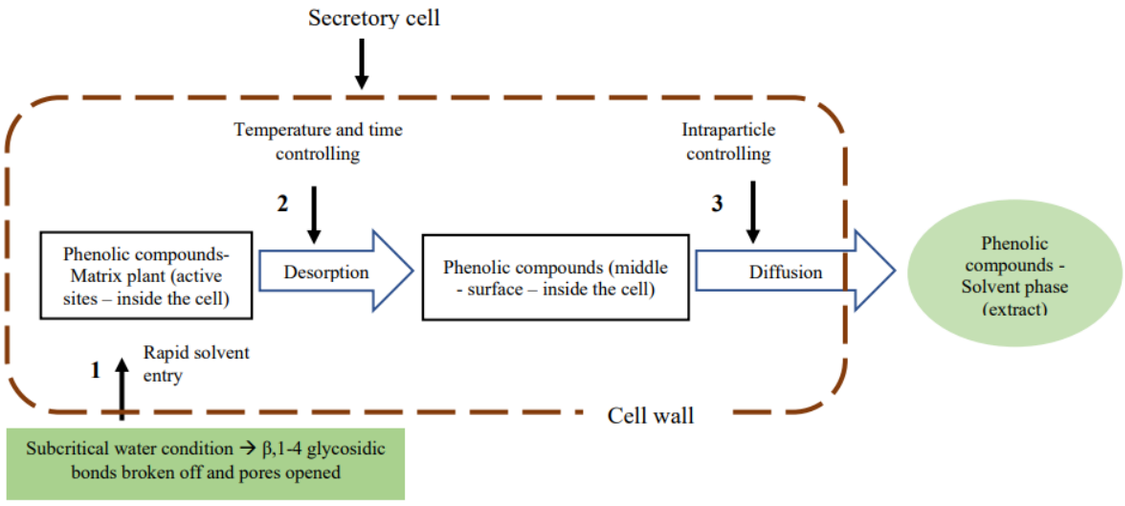

FIGURE 2. The one-site kinetic desorption model illustration

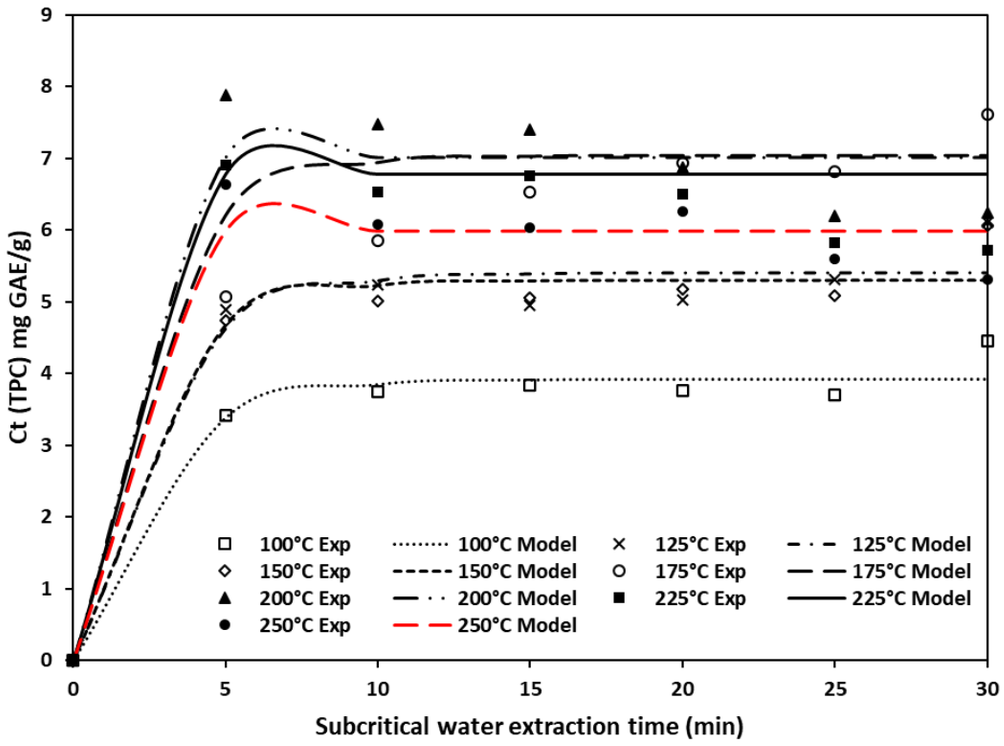

FIGURE 3. The fit curves of the one-site kinetic first-order model of the total phenolic content recovery from PBL leave at various times in various temperatures extracted by SW

TABLE 1. The desorption rate constant of the one-site kinetic desorption model

Temperature (°C)	k (min^{-1})	R^2
100	0.3975±0.02[a]	0.93
125	0.3819 ±0.01[ab]	0.94
150	0.4147±0.02[b]	0.97
175	0.4059±0.02[ab]	0.94
200	3.3045±0.00[d]	0.93
225	3.2277±0.00[c]	0.94
250	3.2093±0.00[c]	0.94
	Average	0.94

TABLE 2. Effective diffusion coefficient, activation energy and determination coefficient of one-site kinetic desorption model in various temperature

Temperature (°C)	One-site kinetic desorption model	
	De (m^2/s) × 10^{-4}	Ea (kJ/mol)
100	2.390	8.964
125	2.891	
150	3.125	
175	2.713	
200	6.726	
225	4.228	
250	5.198	

CONCLUSIONS

The one-site kinetic desorption model for the extraction of phenolic compounds of *Piper betle* Linn. leaves using subcritical water had a good fit to the experimental data. It was evaluated from the high average of R^2 (0.94) from each temperature. The extraction of total phenolic compounds was started by rapid solvent entry by subcritical water by damaging the glycosidic bond in high energy, then continued by desorption and diffusion of total phenolic compounds to the solvent phase as an extract. The desorption rate (k) and effective diffusion coefficient (De) value increased by temperature from 100 to 200 °C then decreased to 250 °C. This result was similar to the highest total phenolic content, which was recovered in a fast process for 5 min at 200 °C. Interestingly, the prolonged time of the slow desorption process to 30 min at 175 °C could achieve an almost similar concentration of phenolic content with a fast process. This result showed that although the desorption rate at 175 °C was slower 8-folds, with the addition of extraction time, the equilibrium was almost achieved. The activation energy for phenolic compound calculated from the effective diffusion coefficient was 8.964 kJ/mol. Hence, the one-site kinetic could give information on the controlling mechanism of phenolic compounds extraction by subcritical water treatment. In further research, the authors recommend investigating the kinetic study of phenolic acids from PBL leaves and their relation with antioxidant compounds.

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