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Extraction of anti-cancer damnacanthal from roots of *Morinda citrifolia* by subcritical water

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Abstract

Roots of *Morinda citrifolia* (Noni or Yor in Thai) are the source of important compounds, anthraquinones, which have been proven to have anti-viral, anti-bacterial, anti-cancer activities. The most medicinally valuable anthraquinones in the roots of this plant is damnacanthal, which has been used for treatment of chronic diseases such as cancer and heart disease. In this study, subcritical water extraction was investigated as a benign alternative for solvent extraction of damnacanthal from the dried root of *Morinda citrifolia*. The experiments were conducted in a continuous flow system at a pressure of 4 MPa at different temperature between 150 and 220 °C and water flow rates of 1.6, 2.4, 3.2 and 4 ml/min. The quantitative analysis of damnacanthal was performed using RP-HPLC with UV detection at 250 nm. The results of the study revealed that the highest amount of damnacanthal extracted with subcritical water was obtained at 170 °C. In addition to the effect of temperature, extractions were conducted at various flow rates and the data were fitted with mathematic models to determine the extraction mechanism. The results suggested that the overall extraction mechanism was influenced by solute partitioning equilibrium with external mass transfer through liquid film. Nevertheless, the desorption model could describe the extraction behavior of *Morinda citrifolia* reasonably at high flow rates.

Keywords: Subcritical water; Extraction; *Morinda citrifolia*; Anthraquinones; Damnacanthal; Modeling

1. Introduction

For centuries, scientists and medical professionals have been investigating chemical constituents in all parts of *Morinda critifolia* (Noni or Yor), including leaf, fruit, bark, and root. The plants contain several medicinally active components that exhibit various therapeutic effects. These include anti-bacterial [1,2], anti-viral [3,4], and anti-cancer [5,6] activities as well as analgesic effects. Critical reviews of the therapeutic properties of the plants are given by Chan-Blanco et al. [8] and Wang et al. [7]. A group of compound in *Morinda citrifolia* that were shown to be responsible for the plant's therapeutic properties is anthraquinones and among the different anthraquinones, damnacanthal which is present mainly in the root is of particular interest, due to its important activity in fighting against cancers [6].

Nowadays, the desire to reduce the use of the organic solvent in food and medicine processing has led to new extraction methods including supercritical fluid extraction (SFE) and subcritical water extraction (SWE). For extraction of slightly polar compounds such as anthraquinones, subcritical water is more preferred over supercritical carbon dioxide. Water at subcritical condition refers to liquid water whose temperature lies between boiling (100 °C) and critical temperature (374 °C). At such condition, the intermolecular hydrogen bonds of water break down, causing water polarity to decrease. As a result, water becomes a more effective solvent for several organic compounds. The review on extraction of medical botanicals with subcritical has recently been available [9].

In our previous study, we have shown the feasibility of extracting anthraquinones from noni roots with subcritical water [10]. The subsequent study on antioxidant activity of the root extracts showed that subcritical water extraction yields the extract with high antioxidant activity compared with that obtained by conventional solvent extraction techniques [11]. In the previous study, spectrophotometric analysis was used for

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the quick determination of the total amount of anthraquinones according to the method described by Zenk et al. [12]. In this work however, the target anti-cancer compound, damnacanthal, in the extracts was quantified using reversed-phase high performance liquid chromatography (RP-HPLC). The effects of various factors such as temperature and flow rate on extraction efficiency of this compound were determined. In addition, the experimental data were fitted with four simple models: thermodynamic partition model, one-site and two-site kinetic desorption models, and thermodynamic partitioning with external mass transfer resistance model, in order to determine which of these models could best describe the behavior of subcritical water extraction of damnacanthal. This will provide useful information for the initial sizing and the economic evaluation of the system in a commercial scale.

2. Experimental

2.1. Plant material and chemicals

Morinda citrifolia used in this study were grown locally in Thailand. The roots of these plants were harvested, washed, and then oven dried at 50 °C for 2 days. The dried sample was then ground to small size using mortar and pestle with liquid nitrogen. The ground samples were oven dried at 50 °C for 1 day, and then stored in a dry place until use.

Standard damnacanthal (99% purity) was purchased from Merck, Germany. Ethyl alcohol was purchased from Fisher Scientific, UK and dimethyl sulfoxide (DMSO) was purchased from Merck, Germany.

2.2. Subcritical water extraction

Subcritical water extraction was performed using an apparatus shown in Fig. 1. The extraction system consisted of two HPLC pumps (PU 980, JASCO, Japan) used for delivering water and solvent, a degassing instrument (ERC 3215, CE, Japan), an oven (D63450, HARAEUS, Germany), in which the extraction vessel (10 ml, Thar Design, USA) was mounted, a pressure gauge, and a back-pressure regulator (AKICO, Japan). All connections are made with stainless steel capillaries (1/16 in. diameter).

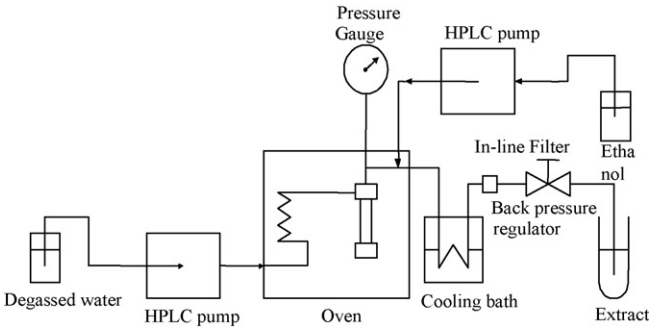

Fig. 1. Diagram of experimental setup of subcritical water extraction.

Distilled water was passed through a degassing equipment to remove dissolved oxygen. The degassed water was then delivered, at a constant flow rate with the first HPLC pump, to a 3-m preheating section installed in the oven, and then passed through the extraction vessel, preloaded with 1 g of ground noni roots. The pressure of the system was adjusted to the desired condition (4 MPa) by using the back-pressure regulator at the outlet coil to ensure that water was in liquid state at the temperatures tested. The oven was then turned on, and when the temperature reached the set point, the extraction started. Ethanol was delivered by the second pump at a constant flow rate to wash off any residual product in the outlet line behind the extractor. The extract was passed through a cooling coil immersed in a water bath to prevent possible product degradation, and was then collected in fractions. In this study, the extraction temperatures studied were 150, 170, 200, and 220 °C, and the water flow rates employed were 1.6, 2.4, 3.2 and 4 ml/min. To determine the percent extracted, after each extraction, the sample residue was extracted in three 10 ml volumes of ethanol, or until the extract was clear. The sum of the amount of damnacanthal extracted and that remained after each extraction would account for the total damnacanthal in the root samples (100%). At the temperatures of 170 °C and below, this value is comparable to the initial amount of the compound in the sample determined by Soxhlet extraction in ethanol for 4 h. All SWE experiments in this study were performed at least in duplicate and all samples were analyzed by RP-HPLC.

2.3. Sample preparation for RP-HPLC

Subcritical water extracts were evaporated under vacuum to dryness, and redissolved in DMSO. The solution was then filtered through a membrane filter (0.45 μm, Millipore, USA) before being subjected to HPLC analysis.

2.4. Analysis RP-HPLC procedure

The HPLC apparatus consisted of pump (Prostar 240, Varian, USA), equipped with photodiode array detector (Prostar 335, Varian, USA). The analysis was carried out at room temperature on a phenomenex Luna C18, 100 Å pore size, 5 μm particle size, 250 mm × 4.60 mm i.d. column. The mobile phase used was modified from that described by Dabiri et al. [13], which consisted of a mixture of (70:30) acidic methanol (50 mM TFA)–buffer (50 mM KH_2PO_4, pH 3). The flow rate of the mobile phase was 1 ml/min and an injection volume of 50 μl was used. The UV detection wavelength was 250 nm. Each analysis was carried out at ambient temperature. A standard calibration curve was made from a plot of peak areas versus concentrations for a series of standard solutions in DMSO.

3. Extraction mechanism and mathematical models

3.1. Extraction mechanism

In general, extraction of any compound from a single plant particle involves the following steps: (a) transport of the com-

pound through the matrix or down its pores (intra-particle diffusion), (b) diffusion through stagnant liquid film around the solid plant particles (external diffusion), and (c) elution or removal of the compound from a solid matrix by thermodynamic partitioning into the flowing solvents.

The plots of the amount of compound extracted versus solvent flow rates and versus solvent volume can determine the relative importance of these steps. For example, if the rate of extraction is controlled by intra-particle diffusion or kinetic desorption, the increase in bulk fluid flow rate would have little effect on extraction rate. On the other hand, if the extraction is controlled by external film transfer diffusion, extraction rates increase with solvent flow rate. In the case where the extraction rate is controlled by thermodynamic partitioning, doubling the bulk fluid flow rate would double the extraction rate, while the curves of extraction efficiency versus the volume of water passed for all flow rates would overlap. In this study, four models will be considered and used to fit with the experimental data. These include (1) partitioning coefficient model, (2) one-site and (3) two-site desorption models and (4) external mass transfer resistance model.

3.2. Mathematical models

3.2.1. Partitioning coefficient (K_D) model

Partitioning coefficient model, adopted from Kubátová et al. [14], describes the extraction process that is controlled by partitioning of solute between matrix and solvent similar to elution of solute from a partition chromatography column. For extraction, this type of behavior occurs when the initial solute concentration in the plant matrix is small. This model assumes that the initial desorption step and the subsequent fluid-matrix partitioning is rapid. Here the thermodynamics partitioning coefficient, K_D, is defined as:

$$K_D = \frac{\text{Concentration of solute in the matrix}}{\text{Concentration of solute in the extraction fluid}}; \quad \text{at equilibrium} \tag{1}$$

If the size of the solid particles is uniform, the mass of the solute in each unit mass of extraction fluid and that remains in the matrix for a particular period can be calculated based on the K_D value of the solute. Thus,

$$\frac{S_b}{S_0} = \frac{(1-(S_a/S_0))}{(K_D m/(V_b - V_a)\rho) + 1)} + \frac{S_a}{S_0} \tag{2}$$

in which S_a is the cumulative mass of the analyte extracted after volume V_a (mg/g), S_b the cumulative mass of the analyte extracted after volume V_b (mg/g), S_0 the initial total mass of analyte in the matrix (mg/g), ρ the density of extraction fluid at given conditions (mg/ml), and m is the mass of the extracted sample (mg).

It should be noted from the model that the cumulative amount of solute extracted is described as a function of water volume passed the extractor, and does not depend on the extraction time. If the flow rate is doubled, the volume of water passed would be doubled for the same unit time, leading to the doubling of the amount of solute extracted per unit time. Therefore, if the cumulative amount of solute extracted was plotted against extraction time, the curve for the higher flow rate would lie above that for the lower flow rate. On the other hand, if the same data were plotted in terms of volume, the theoretical curves from all flow rates would overlap completely.

3.2.2. One-site kinetic desorption model

One-site kinetic desorption model describes the extractions that are controlled by intra-particle diffusion. This occurs when the flow of fluid is fast enough for the concentration of a particular solute to be well below its thermodynamically controlled limit. The one-site kinetic model was derived based on the mass transfer model that is analogous to the hot ball heat transfer model [15,16]. The assumptions are that the compound is initially uniformly distributed within the matrix and that, as soon as extraction begins, the concentration of compound at the matrix surfaces is zero (corresponding to no solubility limitation). For a spherical matrix of uniform size, the solution for the ratio of the mass, S_r, of the compound that remains in the matrix sphere after extraction time, t, to that of the initial mass of extractable compound, S_0 is given as.

$$\frac{S_r}{S_0} = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(\frac{-D_e n^2 \pi^2 t}{r^2}\right) \tag{3}$$

in which n is an integer and D_e is the effective diffusion coefficient of the compound in the material of the sphere (m^2/s).

The curve for the above solution tends to become linear at longer times (generally after $t > 0.5t_c$), and $\ln(S_r/S_0)$ is given approximately by

$$\ln\left(\frac{S_r}{S_0}\right) = -0.4977 - \frac{t}{t_c} \tag{4}$$

where t_c (min) is a characteristic time quantity, defined as:

$$t_c = \frac{r^2}{\pi^2 D_e} \tag{5}$$

The exact model gives the y-intercept for the linear plot at 0.4977, but in practice, the value of the y-intercept depends on the particle shape and size distribution and also on the distribution of solute within the matrix particles (i.e. whether the solute is primarily located near the surface or in the interior of the particle) [17].

An alternative form of Eq. (4), or so called a one-site kinetic desorption model, can be written for the ratio of mass of analyte removed after time t to the initial mass, S_0, as given by:

$$\frac{S_t}{S_0} = 1 - e^{-kt} \tag{6}$$

in which S_t is the mass of the analyte removed by the extraction fluid after time t (mg/g), S_0 the total initial mass of analyte in the matrix (mg/g), S_t/S_0 the fraction of the solute extracted after time t, and k is a first order rate constant describing the extraction.

3.2.3. Two-site kinetic desorption model

Two-site kinetic model is a simple modification of the one-site kinetic desorption model that describes extraction which occurs from the "fast" and "slow" part [14]. In such case, a certain fraction (F) of the analyte desorbs at a fast rate defined by k_1, and the remaining fraction $(1 - F)$ desorbs at a slower rate defined by k_2. The model has the following form:

$$\frac{S_t}{S_0} = 1 - [F\mathrm{e}^{-k_1 t}] - [(1 - F)\mathrm{e}^{-k_2 t}] \quad (7)$$

in which k_1 is the first-order rate constant describing the quickly released fraction (min^{-1}) and k_2 is the first-order rate constant describing the slowly released fraction (min^{-1}).

3.2.4. Thermodynamic partition with external mass transfer resistance model

This model describes extraction which is controlled by external mass transfer whose rate is described by resistance type model of the following form:

$$\frac{\partial c_\mathrm{s}}{\partial t} = -k_\mathrm{e} a_\mathrm{p} \left[\left(\frac{c_\mathrm{s}}{K_\mathrm{D}} \right) - c \right] \quad (8)$$

in which c is the fluid phase concentration ($\mathrm{mol/m^3}$), c_s the solid phase concentration ($\mathrm{mol/m^3}$), k_e the external mass transfer coefficient (m/min) and a_p is specific surface area of particles ($\mathrm{m^2/m^3}$). If the concentration of the solute in the bulk fluid is assumed small and the solute concentration in the liquid at the surface of solid matrix is described by partitioning equilibrium, K_D, the solution of Eq. (8) for the solute concentration in the solid matrix, c_s, becomes:

$$c_\mathrm{s} = c_0 \exp\left(-\frac{k_\mathrm{e} a_\mathrm{p} t}{K_\mathrm{D}} \right) \quad (9)$$

Eq. (9) can be rewritten as the ratio of the mass of diffusing solute leaving the sample to the initial mass of solute in the sample, S_t/S_0, as given by the following equation.

$$S_t = 1 - S_0 \exp\left(-\frac{k_\mathrm{e} a_\mathrm{p} t}{K_\mathrm{D}} \right) \quad (10)$$

Because a_p is difficult to be measured accurately, a_p and k_e are usually determined together as $k_\mathrm{e} a_\mathrm{p}$, which is called overall volumetric mass transfer coefficient. The factors that influence the value of $k_\mathrm{e} a_\mathrm{p}$ include the water flow rate through the extractor and the size and shape of plant sample.

4. Results and discussion

4.1. RP-HPLC chromatograms of extract

In this study RP-HPLC was chosen as a means to quantitatively determine the amount of damnacanthal target compound. It was necessary to concentrate the water extract and this could be achieved by first evaporating off water under vacuum to dryness. The dried extract was redissolved in dimethyl sulfoxide (DMSO) and analyzed. The chromatograms of damnacanthal standard and the extract are shown in Fig. 2. The retention time of the target compound was approximately 12 min.

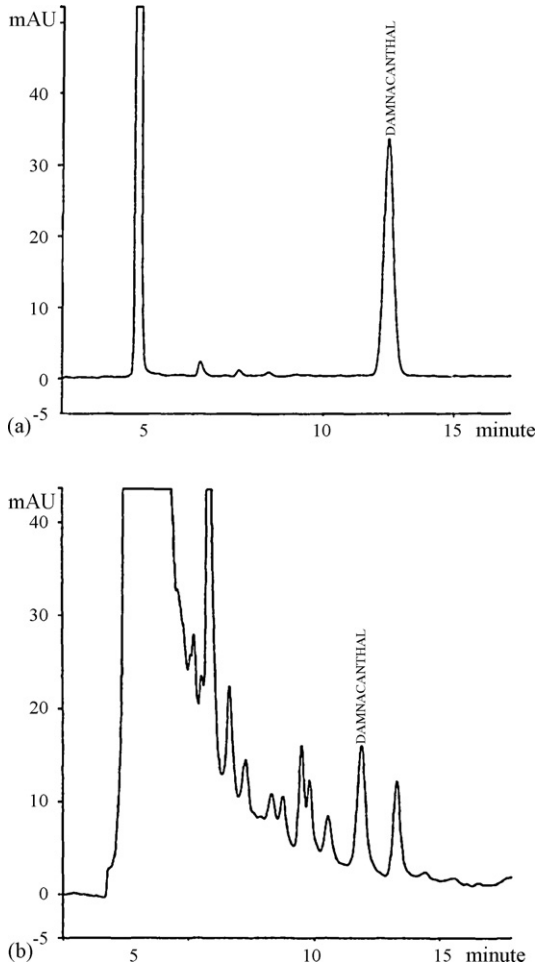

Fig. 2. HPLC chromatogram of damnacanthal (retention time, t_R = 12.44 min) (a) standard, (b) dried extract redissolved into DMSO.

4.2. Effect of subcritical water temperature

The effect of subcritical water temperature on the extraction yield was determined for the temperatures range between 150 and 220 °C and at the water flow rate of 4 ml/min. At 150 °C, 0.659 mg of damnacanthal per g of dried roots was extracted and the amount extracted increased to 0.722 mg/g of dried roots when the temperature increased to 170 °C (Fig. 3). At higher temperature water polarity decreases as a result of reduced polar forces and hydrogen bonding between water molecules, making it more suitable for extraction of organic compounds. Moreover, at elevated temperature, the water density and viscosity decrease, resulting in increased mass transfer of the solvent into the matrix of the plant sample. However, at 200 and 220 °C, the yields were only 0.227 and 0.197 mg/g of dried roots, which were 69 and 73% lower than the yield at 170 °C. Analysis of the sample residue showed that negligible amount of damnacanthal remained, which confirmed that degradation of the product occurred. This finding differed from that obtained from the previous study which showed the highest amount of

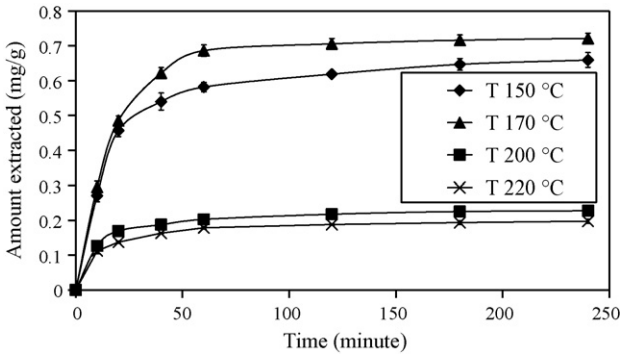

Fig. 3. Effect of water temperature on yield of damnacanthal (flow rate 4 ml/min at pressure of 4 MPa).

total anthraquinones was extracted at the highest temperature of 220 °C [10]. This demonstrated that the temperature have different effects on degradation of different though related compounds.

4.3. Effect of subcritical water flow rate

In this study, the experimental extraction curves were obtained for the volumetric flow rate of 1.6, 2.4, 3.6, and 4 ml/min and for the temperatures of 170 °C at the fixed pressure of 4 MPa. The results were plotted in Fig. 4a, which shows that the rate of damnacanthal extracted increases when the volumetric flow rate increased from 1.6 to 2.4 ml/min and the difference in the rate of extraction was more apparent up to 200 min. After 200 min, the effect was small possibly due to the depletion of the solute from the plant matrix. When the flow rate increased from 2.4 to 3.2 and 4 ml/min, there was little effect of flow rate on the extraction efficiency per unit time, which seems to suggest that extraction is controlled by intra-particle diffusion. However when the extraction yield was plotted against volume of water (Fig. 4b), the data for all flow rates lied almost on the same curve, suggesting that extraction behavior is controlled by partitioning equilibrium. These results therefore demonstrate that extraction could be controlled by a combination of different processes, and that the mechanism controlling extraction behavior may change depending on the extraction conditions. A more quantitative account was given in the following section, in which the data were fitted with the partition model, kinetic desorption models, and external mass transfer resistance model, to determine the mechanism of extraction behavior.

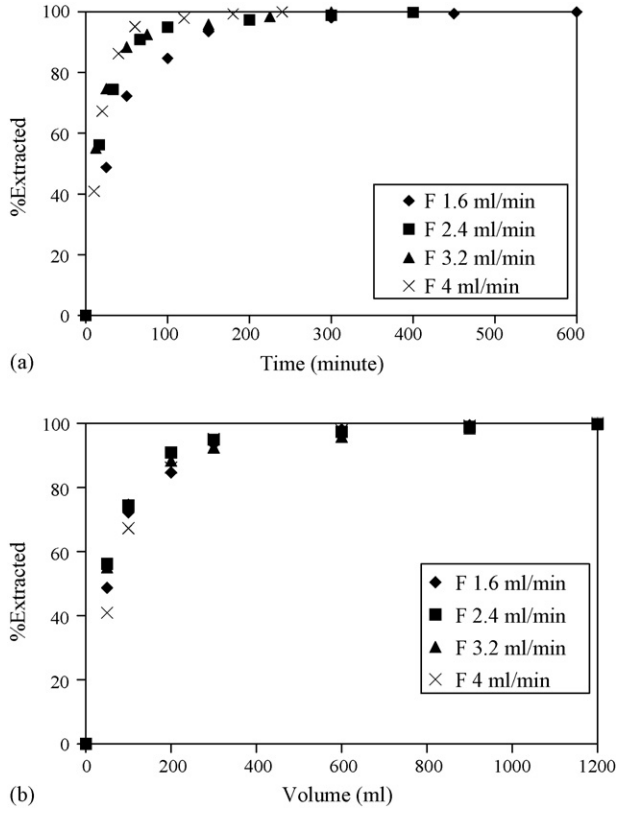

Fig. 4. (a) Percent damnacanthal extracted vs. extraction time, (b) percent damanacanthal extracted vs. volume of water (temperature 170 °C and pressure 4 MPa).

Table 1
K_D values of partitioning coefficient model for different volumetric flow rates

Parameter	Volumetric flow rate (ml/min)			
	1.6	2.4	3.2	4
K_D	55.60	43.27	45.68	66.46

4.4. Modeling of extraction behavior

4.4.1. Partitioning coefficient (K_D) model

The model Eq. (2) and the experimental data from all volumetric flow rate plots were used to determine the K_D value by minimizing the errors between the measured data and the K_D model using Microsoft EXCEL solver. The K_D values determined for different flow rates are summarized in Table 1. The calculated K_D values determined at 1.6 ml/min ($K_D = 55.60$), was used to calculate the model curves for all the other flow rates, which as shown in Fig. 5, the K_D model agreed reasonably with the experimental data. Nevertheless, if the extraction is strictly controlled by partitioning equilibrium, K_D values for all flow rates must be equal. The slight deviation found was

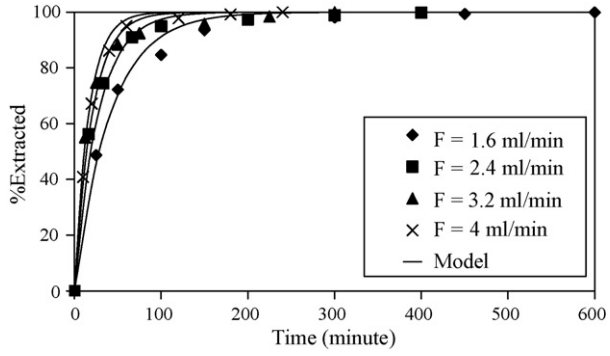

Fig. 5. Comparison of the K_D model fit with experimental data for subcritical water extraction of damnacanthal at various flow rates (symbols represent experimental data and lines were calculated based on the $K_D = 55.60$).

Table 2
k-values for one-site kinetic desorption model for different volumetric flow rates

Parameter	Volumetric flow rate (ml/min)			
	1.6	2.4	3.2	4
k (min^{-1})	0.0243	0.0442	0.0565	0.0530

possibly due to the existence of external film transfer resistance whose model would later be discussed.

4.4.2. One-site kinetic desorption model

Microsoft EXCEL solver was used to determine the desorption rate constant, k, from the data for all flow rates. The values are show in Table 2 and the plot of the calculated and experimental percentage of damnacanthal extracted, $S_t/S_0 \times 100$ (%) versus time are shown in Fig. 6. As mentioned, the kinetic desorption model does not include a factor describing extraction flow rate, k should be the same value for all flow rate if the model is said to fit the experimental data. However, this was not the case (Table 2). The kinetic desorption rate increased for the volumetric flow rate of 1.6–3.2 ml/min. Nevertheless, the rate constant for higher volumetric flow rates of 3.2 and 4 are comparable. This indicated that the kinetic desorption model better describe the data at high flow rate rather than at low flow rates.

4.4.3. Two-site kinetic desorption model

For the two-site kinetic desorption model, the values of k_1 and k_2 were determined by fitting experimental data with the two-site kinetic desorption models by minimizing the errors between the data and the model results, using the value of 0.76 for F. Note that the value of F of 0.76 used was the average of the F values determined from the data obtained at different flow rates found using EXCEL solver. In the one-site model, the extraction rate should not be dependent on the flow rate. The k_1 and k_2 values shown in Table 3 demonstrated that the extraction rates were not completely independent of flow rate particularly at lower flow rates and minimal improvement was found over the one-site kinetic model.

Table 3
k_1 and k_2 values for two-site kinetic desorption model for different volumetric flow rates ($F = 0.76$)

Parameter	Volumetric flow rate (ml/min)			
	1.6	2.4	3.2	4
k_1 (min^{-1})	0.0357	0.065	0.090	0.064
k_2 (min^{-1})	0.0078	0.0144	0.0143	0.029

Table 4
Parameters K_D and $k_e a_p$ for external mass transfer model

Volumetric flow rate (ml/min)	Mass flow rate (mg/min)	Parameter	
		K_D	$k_e a_p$ (min^{-1})
1.6	1485.04	55.70	1.35
2.4	2227.56	55.83	2.47
3.6	2970.08	55.61	3.14
4	3712.60	55.65	2.95

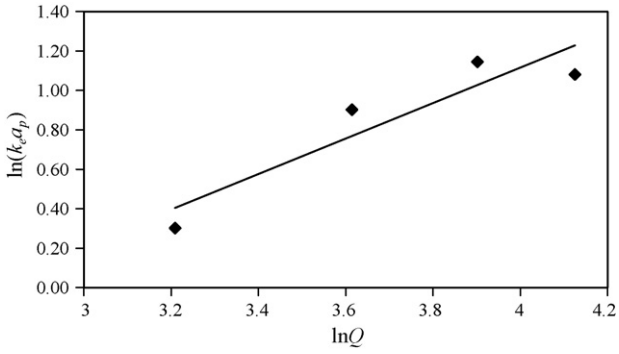

Fig. 7. $\ln(k_e a_p)$ vs. $\ln Q$.

4.4.4. Thermodynamic partition with external mass transfer model

The values for the model parameters, K_D and $k_e a_p$ in Eq. (10) determined by Microsoft EXCEL solver from experimental data obtained at 170 °C are summarized in Table 4 for different mass flow rate (Q, mg/min). Linear regression of the plot between $\ln(k_e a_p)$ and $\ln Q$ (Fig. 7) gives the following correlation for

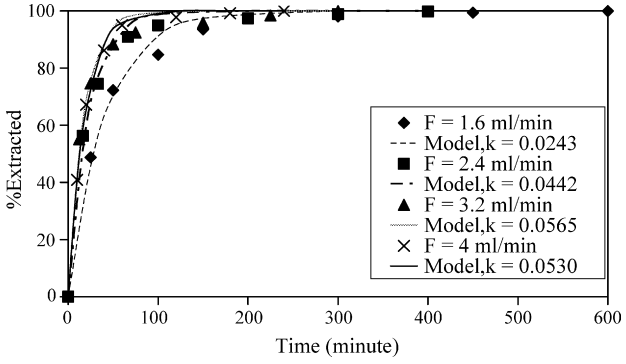

Fig. 6. One-site kinetic desorption model fit of subcritical water extraction data (symbols represent experimental data and lines are based on curve fitting of experimental data).

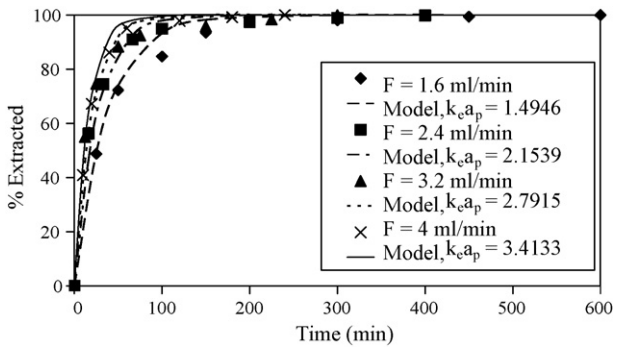

Fig. 8. External mass transfer model fit of subciritcal water extraction data (symbols represent experimental data and lines are based on curve fitting experimental data).

Table 5
Mean percent absolute errors between experimental data and extraction model results

Model	Parameter model	%Mean absolute errors at difference flow rate (ml/min)			
		1.6	2.4	3.2	4
Partitioning coefficient	K_D	2.27	2.69	3.44	2.26
One-site kinetic desorption	k	8.66	3.11	3.07	0.95
Two-site kinetic desorption	k_1, k_2	8.57	3.12	2.49	0.83
External mass transfer	K_D, $k_e a_p$	2.27	2.35	2.97	0.95

$k_e a_p$ and Q:

$$k_e a_p = 0.084 Q^{0.9} \quad (11)$$

Fig. 8 shows the experimental extraction efficiency of damnacanthal versus time, compared with the model prediction which suggested that predicted value agree reasonably with experimental data.

4.5. Comparison of extraction models

To quantitatively compare the extraction models, the mean percentage errors between the experimental data and the models were considered. For the K_D model, the value of K_D calculated from fitting the data at the flow rate of 1.6 ml/min was used to calculate model curves for the other flow rates. For the kinetic desorption model on the other hand, since the model more accurately describe the data at higher flow rates, the value of ks determined from the data for the flow rate of 4 ml/min were used to represent the kinetic desorption models.

Based on the result in Table 5, the K_D model was generally suitable for the description of extraction over all the volumetric flow rates tested. On the other hand, one-site and two-site kinetic desorption models describe the extraction data reasonably at higher volumetric flow rates. Of all the models considered however, the external film transfer model could best describe the experimental data.

5. Conclusions

In summary, subcritical water provides a promising alternative for extraction of the anti-cancer damnacanthal from roots of *Morinda citrifolia*. The most suitable condition for subcritical water extraction of damnacanthal was at the temperature of 170 °C and the water flow rate of 2.4–4 ml/min. At higher temperature than 170 °C, the decomposition of damnacanthal occurred. Overall, a mathematical model base on the combination of partition coefficient (K_D) and external mass transfer gave a good description of subcritical water extraction of damnacanthal, while the kinetic model reasonably described the extraction behavior at higher flow rates.

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