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Kinetic Study of Saponin Extraction from Sapindus rarak DC by Ultrasound-Assisted Extraction Methods

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Abstract

Saponins is an important material derived from plant, such as Sapindus rarak DC. Its extraction assisted by ultrasonic wave is find to be an effective methods. Saponin is extensively used in plenty industrial application including detergent, emulsifying agent in cleanser, shampoos and cosmetics. Hence, the study of saponin extraction is highly needed, especially its kinetic study. The kinetic study is needed in coherence with it use in industrial equipment design such as reactor design. In this study, a simple kinetics analysis of saponin extraction was construct following several equation. Pelleg's model was observed to be the most compatible model to describe the extraction process with R^2 value more than 0.9 in all variance. In addition, the influence of solute-solvent ratio and extraction temperature on extracted saponin were studied. It was found that the concentration of extracted saponin increased with the increase of extraction temperature as well as the solute ratio in the solution. The highest concentration of saponin was obtained in the solute-solvent ration of 1:50, with extraction temperature condition of 50^{o}C. A further analysis by FTIR was used to ensure the existence of saponin in the extract solution. The investigation was conduct based on the peak of pure saponin functional group.

Keywords:

Saponin; Sapindus rarak DC; Ultrasound-assisted extraction; Kinetics study

1. Introduction

Saponin has been classified as one of natural surfactants derived from plants belonging to the genus of Sapindaceae. It appears to have a great potential to alternate synthetic surfactant. These plants produce saponaceous substances called saponins, it can form foam or lather in water solution

[1]. This kind of natural surfactant is easily degradable in nature, show a low toxicity, and very similar characteristic to synthetic surfactant. Saponin is extensively used in plenty industrial application including detergent, emulsifying agent in cleanser, shampoos and cosmetics.

The molecular structure of saponin consist of a hydrophobic and hydrophilic structure. The hydrophobic triterpenoidal or steroidal backbone, it also formed by one or two hydrophilic glycoside moieties attached to the backbone. This molecular structure of saponins give soap-like foams in water, which also known as amphiphilic glycoconjugates [2]. The combination of the nonpolar sapogenin and water-soluble side chain of saponin is very similar to the structure of most synthetic surfactants having lipophilic and hydrophilic molecular parts [3].

Saponins belongs to a various group of naturally occurring surface-active compounds. Near 100 families of plant species was also producing saponin from its different tissues. Other part of plant tissue which saponin can be found is including fruits, flowers, roots, seeds and shoots. Although saponin is predominant in angiosperms plants, it also occur in some ferns, such as the species of Polypodium and Cyclamen. Some algae and certain lower marine organisms also found to be one of the source of saponin.

In Asia region the species of sapindaceae is one of the main source of saponin. The Sapindus genus is widely distributed in all tropical and subtropical regions of Asia, four species of sapindus plants are found in South China [4]. The studies on the phytochemical properties of the Sapindus have revealed that the metabolites are particularly important as triterpenoid saponins. Some of the study show the activity of molluscum, anti-inflammatory, cytotoxic, and anti-platelet aggregation of saponin [5-8]. In ancient China, Sapindus mukorossi Gaertn. (Sapindaceae), which is a tall trunked tree, has been used as expectorant and natural surfactant [9]. And in recent years, S. mukorossi has attracted much attention because of its fruit as a cosmetic shampoo and cleanser.

Because of its importance, the extraction of saponin has been widely studied. Ultrasound-assisted extraction (UAE) is one of the non-conventional and green technologies for the extraction of bioactive compounds from plant materials. The previous study has been state that UAE can significantly reduce energy consumption and reduce extraction times, which gives a better extraction efficiency. Because of its characteristics of shorter extraction time and less use of organic solvents, it is found to be an environmental friendly and efficient way to extract natural bioactive compound from plant tissues [10-12]. Both direct and indirect used of ultrasonic waves has been applied on the extraction of saponins from different varieties of ginseng roots [13]. In the specific case of saponin extraction from edible seeds, the UAE is also novel and scarce.

Ultrasound assisted extraction methods has also been conducted to extract saponin from some plant sources such as Panax notogingseng, soybean, chickpea, Allium nigrum L., and T. terrestris

[14]. However, the studies of kinetics for saponin extraction from Sapindus rarak DC was rarely found. Even though, determining kinetic parameters is very important for designing efficient extraction processes to produce compounds from various materials. The knowledge of extraction kinetics parameter is essential to allow their adequate use in industrial scale. The data also improving the procedure precision, minimizing processing errors and increase the product final quality [15-16]. The data from kinetic studies is needed to design the production equipment such as reactor or extractor. For that reason, this study was aimed to investigate the process extraction of saponin from the pericarps of Sapindus rarak DC. Specifically, this research focused on the effect of the solvent and solute ratios as well as the effect of temperature on the yield of saponin extract. The reaction kinetic study of UAE for saponin is also investigated. Four different models are investigate to find the most suitable equation to describe the extraction process.

2. Materials and Methods

2.1. Materials

The raw materials used in this study is raw pericarps of Sapindus rarak DC purchased from Batik Zie, Semarang. Distilled water as the solvent is obtained from Laboratory of MeR-C, UPT, Undip, Semarang. Pure saponin in practical (P.A) grade is procured from Sigma Aldrich as the comparative standard. Before extraction, the pericarps was dried using oven in temperature of 100±2°C, under normal aeration conditions, for an hour. After drying, the fruit pericarps was crushed in a Miyako BL-151 PF-AP dry grinder. The powder was sieved through 100 mesh sieve to obtain homogeneous fine powder of Sapindus rarak. The ready to use dry-grounded powder are stored in the closed vacuum storage by the temperature of 7 °C, silica gel are added to keep the powder dry.

2.2. Extraction Process

Ultrasound-assisted extraction was carried out in an ultrasonic bath (Krisbow Bath Ultrasonic) device with a constant power of 280 W, at the frequency of 40 kHz. The ultrasonic bath device is equipped with digital sonication time and temperature control system. The dry Sapindus rarak powder with various solid-to-solvent ratio (1:25, 1:50, 1:100 (w/v)) is placed in capped glass bottle and mixed with distilled water as the solvent. The bottle with suspension is sonicated for an hour in the ultrasonic device contenting 4 L water at various temperature (30°C, 40 °C, 50 °C). In each batch of extraction, all samples are placed in the center of ultrasonic bath with the depth of fifteen centimeters. To make sure that each samples were sonicated equivalently, the sample was kept at the same position. Then the solution was filtered using filter cloth and centrifuged at 4.500 rpm for 15 minutes to separate out the solid particles. The supernatant solution was used as analysis samples.

2.3. Determination of saponin content

The most common methods applied for saponin quantification is spectrophotometric analysis by using UV light scatter [14, 17]. Pure saponin purchase from Sigma, Aldrich is used as the standard solution reference to make the calibration curves. The maximum absorption wavelength is determined by UV-VIS Spectrophotometer. The preparation of calibration curve is performed by analyzed the absorbance of pure saponin solution in various concentration (5000-100 ppm). Spectrophotometric analysis is performed by measuring the absorbance at the maximum absorption wavelength using reagent blank as reference. The regression equation of the standard curve based on the concentrations (x) versus the absorbance value (y) is made and should have a linear correlation of more than 0,95.

2.4. Statistical Analysis for Kinetic Modeling Extraction s

Under conditions maximized in the section before, extraction of saponin was investigated at 30, 40, and 50 °C for 5, 10, 20, 40, 80, and 120 min. Experimental data (milligrams saponin per gram of dry pericarps powder) as function of time were fitted to kinetic models presented in Table 1. The estimation conducted using the GRG non-linear method with the help of solver engine from Ms. Excel 2013 MSO 32-bit (Microsoft Office Profesional Plus 2013, USA). In the equations, Co and C represent the saponin extracted (mg/g) at time zero and time t (in minutes), respectively, and k (in minutes) is the extraction rate constant at a given temperature. Table 1 shows several mathematical equations that have been used in previous publications.

Table 1. Kinetic models for extraction of compounds from plant matrices

Equation	Model	References
1.	$C = \frac{C_s^2 . k . t}{1 + C_s . k . t}$	[18]
2.	$C = C_s - \frac{C_s}{exp\,(k.t)}$	[19]
3.	$C = \frac{t}{K_1} + K_2 . t$	[20]
4.	$C = A[1 - exp(-B.t)] + C[1 - exp(-D.t)]$	[21]

The evaluation of fitted model was conduct by considering the statistical criteria. Coefficient of determination (r^2), and sum of squared errors (SSE) were the evaluated statistical criteria. Calculation of SSE and r^2 is done by the equation (5) and (6) respectively:

$$SSE = \sum_{i=1}^{n} \left(a_{measured} - a_{predicted}\right)^2 \quad (5)$$

$$r^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \quad (6)$$

Where n is the number of observations. The model with the lowest SSE, and higher r^2 for saponin extraction, is considered as the best choice for modeling the anthocyanin extraction behavior during processing [22].

2.5. Data Analysis

All experiments were conducted in triplicate, in order to diminish experimental errors. Results were expressed as a mean ± standard deviation (SD) for n=3. Graphical plots were performed using Microsoft Excel 2007, representing the average of at least three measurements with a relative standard deviation (RSD) lower than 5%. The statistical analysis of the data was performed using the GRG non-linear method with the help of solver engine from Ms. Excel 2013 MSO 32-bit (Microsoft Office Profesional Plus 2013, USA).

3. Results and Discussion

Ultrasound Assisted Extraction of saponin from the pericarps of Sapindus rarak DC was studied under various condition. In this study the experimental design focused on two main extraction parameters: temperature and solid/liquid ratio (weight/volume). Distilled water is used as a solvent on the extraction. And green method of extraction was conducted with assistance of ultrasonic wave. The extraction using ultrasonic-assisted is widely use to extract various substance from natural sources. Some experiment proved that using ultrasonic wave for extracting natural substance from plants were provided more benefits than other methods, such as anthocyanin from blackberry and sweet cherry cultivar [23], polyphenol from Picea abies bark [18], antioxidant from Jatropha integerrima [24] and saponin from Allium nigrum L. [25]. The comparison of ultrasonic assisted extraction and conventional maceration extraction (ME) was also conduct by Aryanti et al. [26]. for the extraction of anthocyanin from red and purple roselle calyces. The research proved that extraction by assistance of ultrasonic wave giving better result than the conventional extraction by maceration. The extractions using UAE methods were giving anthocyanin concentration about 16 times higher than extraction using ME. The result of the extraction of saponin from Sapindus rarak DC are presented below.

3.1. Influence of solid to solvent ratio on total saponin of the extracts

The evolutions of extracted total saponin versus time, obtained at different operating conditions are present in Figure 1. The first investigation is conducted for various ratio of solid to solvent, and the temperature is set for 30ºC. It is important to optimize the solid to liquid ratio as the excess of solvent doesn't have a significant role on extraction yield. Therefore, resulting in wastage of solvent, decreased throughput and larger vessel size which affect the equipment design.

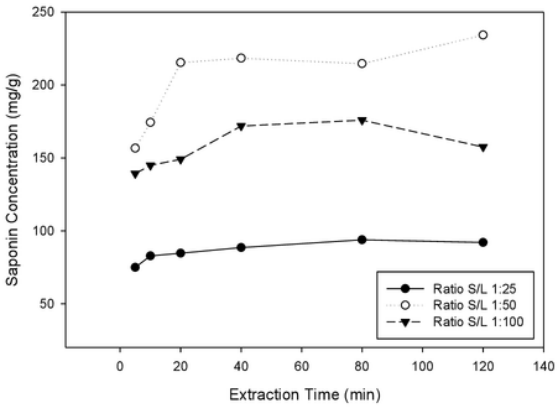

Figure 1. Effect of solid to liquid (S/L) ratio on the extracted saponin on the ultrasound-assisted extraction of Sapindus rarak DC. Experimental conditions: Temperature 30 ºC

From Figure 1, it was observed that ratio of solute to solvent less than 1:25 (w/v) was inadequate to disperse the dry pericarps powder of Sapindus rarak DC properly. This is proved by the lowest of saponin concentration. Thus, the solution requiring higher solid to liquid ratio. However, the increase of solid to solvent ratio into 1:100 (w/v) giving less saponin concentration. Instead, the maximum concentration of saponin has reached at solid to solvent ratio of 1:50 (w/v) with the concentration of 234 mg/g solid.

Higher solid to liquid ratio ensures homogenous mixing and allows penetration of solvent into deep interior parts. This implies greater concentration gradient between the interior plant cells and the solvent and thus enhancing the diffusion of saponin from solid to solvent. As shown in Figure 1, the increase of total saponin concentration with the increase of the solid-to-solvent ratio is consistent with mass transfer principles. The driving force during mass transfer within the solid is considered to be the concentration gradient, which was greater when a higher ratio of solvent was used. The process also resulting in an increase of the diffusion rate. However, the solid-to-solvent ratio did not significantly affect diffusivity. The extractions were slow down and even stopped when equilibrium was reached. Saponin concentration should have also been affected by the extraction conditions, such

as the modifications of solubility and solute–solvent interactions. A solid's solubility is affected by changes in the activity coefficient, which varies with the temperature and composition of the solution [21]. Interactions of the compounds with the solvent could also have modified the activity coefficients and thus the solubility of the compounds. In summary, the main effect of the solvent-to-solid ratio was to modify the solubility and equilibrium constants and thus increase total saponin concentration to a maximum at the highest solvent-to-solid ratio. Thus, the optimum solid to liquid ratio of 1:50 g.ml-1 was finalized for the subsequent studies.

3.2. Influence of temperature condition on total saponin of the extracts

The second investigation is conducted for various temperature condition with solid to solvent ratio is set for 1:50 (g/mL). The result of saponin extraction under 3 level of temperature is showed in Figure 2.

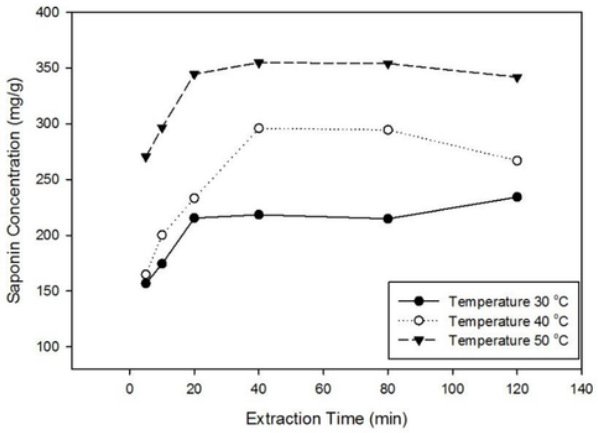

Figure 2. Effect of temperature condition on the extracted saponin on the ultrasound-assisted extraction of Sapindus rarak DC. Experimental conditions: solid to solvent ratio of 1:50 (g/ml)

Figure 2 shows the positive effects of temperature on saponin concentration, with almost 50% increased of saponin concentration at 50ºC compared to 30ºC. In this study, it was observed that extraction yield keep increase with the increase of temperature condition. The highest saponin concentration achieve at the temperature of 50ºC, with the value of 354.92 mg/g of solid. In the process of natural compound extraction, the condition of extraction temperature was highly influenced the result.

Literature data reports that higher temperatures have positive influence on the extraction rates of natural compounds from different biomass. At higher temperatures the solubility and diffusivity of the phenolic compounds is increased because the viscosity of the spruce bark extracts is decreased,

improving in this way the mass transfer and accelerating the extraction process [18].

Theoretically, temperature affects the mass transfer rate and the solubility of some compound in the extraction process [27]. Therefore, the increased of temperature could be used to find the efficient condition of extraction from interior parts. In the extraction process assisted by ultrasonic wave, increase of temperature will affects both of the bubble formation and collapse. It is also decreases the surface tension, making the compound inside the plant tissue can be easily comes out. While at lower temperature, only few bubbles are formed. More formation of bubble helps the cell disruption because of the increased of collapse intensity. Since the extraction with higher temperature produce more bubble, the distribution of energy and collapse of the bubble around the molecule was better. Resulting in more cell disruption and increased of mass transfer [28].

However, previous study has shown that most natural compound cannot stand high temperature. Anthocyanin from Roselle verified to visibly degrade at temperature of 60°C [29]. The extraction of camptothecin from Nothapodytes nimmoniana plant assisted with ultrasonic wave also shows a negative effect beyond 50°C, and only slightly decline from 30°C to 40°C [27]. This phenomena could be happening due to some reason. The most commonly cause is the heat-sensitive molecular structure of natural compound. In the ultrasonic assisted process, the phenomena also affected by the decreased of cavitation arising out, increase of vapour pressure and a decrease of surface tension within micro bubbles, causing the damping of ultrasonic waves [28, 30, 31]. Taking into account about the theoretically negative result by too much addition of heat, 50°C was finalized as the maximum temperature.

3.3. Kinetic study of saponin extraction

In the solid–liquid extraction context, temperature and solid-to-solvent ratio are significant process variables on the extraction yield aiming to industrial applications. Therefore, the investigation of optimal conditions of temperature and solid-to-liquid ratio was evaluated. Mathematical models consist of equations that provide an output based on a set of input data can be consider as a concise way to express the physical behavior. Various phenomena might govern the removal of compounds from plant matrices, including sorption/desorption, washing and swelling of plant material, diffusion, among others.

Choosing the best mathematical model to represent processing curves is fundamental in order to minimize processing errors, maximize final product quality, and facilitate designing and simulation of industrial processes. The maximum condition giving highest yield of saponin known from section before is the ratio solid-to-solvent of 1:50, and extraction temperature of 50°C. The experimental data (milligrams saponin per gram of dry pericarps powder) from maximized condition were fitted to

kinetic models presented in Table 1 as function of time.

Second order kinetics model equation (eq. 1) represent the comparison of extracted compound based on time. Where Cs is the concentration of total polyphenols at saturation in the liquid extraction (mg/L) and k is the second-order extraction rate constant (L/g.min) [18]. Pseudo-first-order model (Eq. 2) considers that the compound concentration in the extract tends to"plateau" [19]. The Equation 3 is based on the sorption/desorption mechanism to remove compounds from the plant material [32]; in the model, K_1 represents the extraction rate constant and K_2, Peleg's capacity constant. In Equation 4, the extraction of compounds is assumed to happen in two distinct periods, related to accessible and inaccessible compounds (outside and inside plant cells, respectively) [21]. B and D parameters are the extraction rates of the two different classes of compounds, A and C are constants.

Extraction kinetics is usually expressed in terms of solute concentration extracted from the solid to the solvent per unit time [33]. Mechanism of extraction of natural compounds from plant matrices is not fully enlightened; therefore, several mathematical equations have been described in literature. Statistical errors for fitting the experimental data of extraction of saponin from Sapindus rarak DC to models used in previous publications are shown in Table 2.

Table 2. Summary of errors performance of selected models to describe extraction of saponin from Sapindus rarak DC

No	Equation	SSE (Sum Square of Errors)	R^2
1	Second order kinetics model (Eq. 1)	557.22	**0.929**
2	Pseudo first-order model (Eq. 2)	1026.12	0.829
3	Modified Pelleg's model (Eq. 3)	288.33	0.887
4	Two-step transfer model (Eq. 4)	1026.12	0.829

Analysis of data presented in Table 2 clearly demonstrates that the best model to describe extraction of anthocyanins from grape marc is the pseudo-first order (Eq.3). The R^2 values were the highest, and low SSE. The second order kinetics model is representing that the saponin extraction in the conditions studied is continuous.

3.4 The practical calculation based on the second-order kinetic model

The experimental data of the UAE extraction were processed and plotted in the specific coordinates of the second-order kinetic model. The dissolution rate of saponin contained in the solid can be described by second-order kinetic model presented in Eq. (7):

$$\frac{dC_t}{dt} = k(C_s - C_t)^2 \quad (7)$$

Where C_t is the concentration of total polyphenols in the liquid extraction (mg/L) at a given extraction time t (min), C_s is the concentration of total polyphenols at saturation in the liquid extraction (mg/L) and k is the second-order extraction rate constant (L/g.min). In order to determine the kinetic parameters, Eq. (7) is integrated under the boundary conditions $C_t = 0$ to Ct and t = 0 to t. The equation was then linearized in the form of Eq. (8), where h was the initial extraction rate (g/L.min) when t and C_t approach 0:

$$\frac{t}{C_t} = \frac{1}{k.C_s^2} + \frac{t}{C_s} = \frac{1}{h} + \frac{t}{C_s} \quad (8)$$

Figure 3 present the experimental data showing a linear line after the plot of equation models.

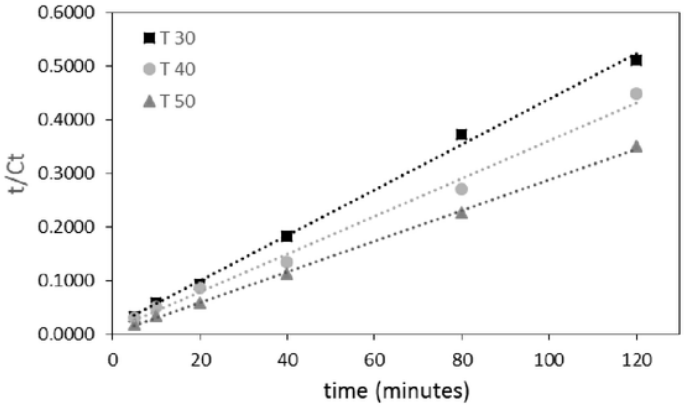

Figure 3. Validation of the second-order kinetics model

The specific kinetic parameters, such as extraction capacity (concentration at saturation – Cs), extraction rate constant (k) and initial extraction rate (h) were determined based on the mathematical regression equations for each temperature. It can be determined experimentally from the slope and intercept of the plotting t/C_t against t. Fitting of the second-order kinetic model for all experimental data is confirmed by the value of mathematical regression coefficient ($R^2 > 0.99$).

Table 3. Kinetic parameters for the second-order kinetic model

T (°C)	h (mg/L.min)	k (L/mg/min)	C_s (mg/L)	R^2
30	74.63	0.00138	232.56	0.9972
40	126.58	0.00155	285.71	0.9925
50	588.24	0.00495	344.83	0.9988

The second-order kinetic model was also used to describe the release kinetics of total phenolics from oak chips into a model wine, under ultrasounds action into a bath system [34]. In the kinetic modeling study of UAE of oil from pomegranate seeds using hexane as solvent, [35] reported that the best fits were given when the second-order law was applied [36]. The second-order model also used to describe the solid–liquid extraction processes of antioxidants from pomegranate marc, by stirring, using water as a solvent. The value of extraction rate constant (k) observed to be increased as the temperature rise. The change of k values and other kinetic parameter as the temperature change could be observed that temperature has a strong influence on it. The plotting of experimental result versus practical calculation using the obtained model also gives good comparison by the average error range of 1.5 until 19% as showed in Figure 4.

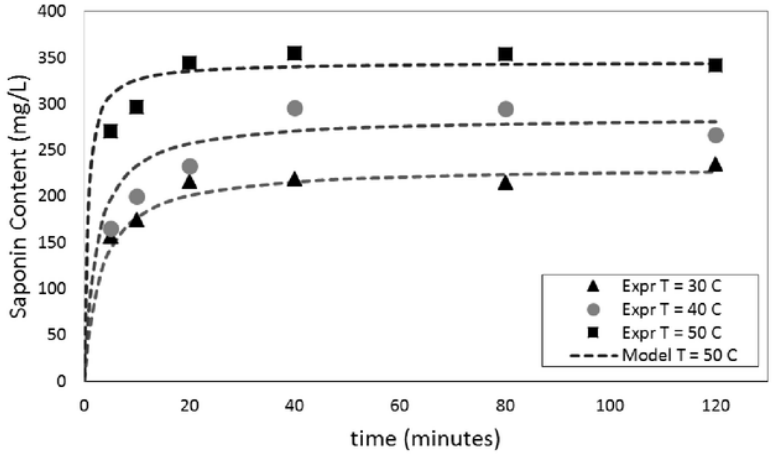

Figure 4. The comparison of experimental data with the practical calculation based on the second-order kinetic model

4. Conclusions

In this study, the investigation of natural saponin extraction and its extraction kinetics was conducted. The study of extraction kinetic of saponin from the pericarps of Sapindus rarak DC assisted by ultrasonic wave was performed to to elucidate the phenomena involved in this kind of process. The results were able to use for further possible applications in industrial projects. The process condition showing a better performance among all of the variable used in this study was the extraction at 50 ºC using water as solvent at a solute to solvent ratio of 1:50 (w/v). With the highest yield achieved in this process was 354.92 mg of saponin per gram of dried-ground pericarps of Sapindus rarak DC. Second order kinetics model was the equation that represent the extraction process better, with value of R^2 of 0.929. The comparison of experimental results and the practical

calculation using the obtained model also gives good comparison by the average error range of 1.5 until 19%. The extraction of saponin from Sapindus rarak DC shows a good results and has possibility as alternative of synthetic surfactant. The knowledge about kinetics extraction of saponin from plant resource was present as the result of this study, to support industrial application such as elucidate the phenomena and better equipment dimensioning.

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