Enhancement of The Sappanwood Extract Yield by Aqueous Ultrasound-Assisted Extraction Using Water Solvent

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Abstract—Sappanwood (Caesalpinia sappan L) is a member of Leguminosae plant, which is popular for its function as a natural source of red dye and has been traditionally used to prepare food and beverage in Southeast Asia. From the pharmacological point of view, the heartwood extract of this plant exhibits various biological activities, such as antibacterial, anti-photoaging, anti-allergic, anti-inflammatory, antioxidant, etc. Brazilin as the main antioxidant compound can be efficiently extracted by the ultrasound-assisted extraction (UAE) method. This study aimed to evaluate the effect of temperature (30 to 60°C), time (5 to 25 minutes), and solid-liquid ratio (1:5 to 1:8 g/mL) on the batch ultrasound-assisted extraction of antioxidant compounds from sappanwood heartwood. The results showed that the feasible extraction process was at 30°C using the solid-liquid ratio of 1:5 g/mL for 15 minutes to obtain an extract yield of 3.0%. The yield was 1.50 times compared to the conventional extraction without ultrasound. The increase in temperature and solvent resulted in higher yields. However, considering the energy for extraction and product purification, extraction at ambient temperature with minimum solvent volume was favorable. Meanwhile, the pseudo-second-order mass transfer model exhibited good statistical parameters, and \( R^2 \) was higher than 0.99 with lower RSMD values. This model can be used to describe the kinetics of UAE of antioxidant compounds from sappanwood. The model was meaningful in determining the efficient extraction time with reasonable antioxidant performance.

Keywords—Brazilin; mass transfer; operating parameters; sappanwood; ultrasound-assisted extraction.

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I. INTRODUCTION

The use of local plant biodiversity for many purposes, specifically in food, beverage, and traditional medicine, has been steadily improving in today’s society. Sappanwood (Caesalpinia Sappan L) or Brazilwood in English can be easily found in the scrub jungle and limestone hills in Southeast Asia [1]. In Indonesia, this plant is called Kayu Secang and has played a significant role in popular agricultural commodities for centuries. It is used to prepare traditional drinks (Wedang Secang) by the folk of Central Java to boost human health by boiling or steeping the dried sappanwood pieces, ginger, cinnamon, cloves, and lemongrass in hot water [2].

In addition, sappanwood, belonging to the Caesalpiniaceae family, is well-known as a natural source of the coloring agent in the food industry. This water-soluble natural dye is mainly in brazilin and is generally obtained through water extraction of sappanwood heartwood. Brazilin has been reported to exhibit various pharmacological properties, such as antioxidant activity, antibacterial activity, antiacne activity, anti-inflammatory activity, anticancer activity, and hypoglycemic activity [3]–[6].

Depending on the pH, brazilin appears to be amber to red in color, where the red color can be obtained under basic conditions (pH >7) [7]. Exposure to light, pH, and air may induce the oxidation of brazilin to form brazilein due to the alteration of a hydroxyl group to the carbonyl group, which is responsible for the intense red color of the sappanwood extract [7], [8]. Based on his study on the storage of sappanwood water extract at 4°C and 25°C under neutral and basic conditions (pH 7.0, 8.0, and 9.0), Sinsawasdi confirmed that the exposure to light at a given pH gave a more pronounced brazilin degradation to Brazilian that the exposure to higher temperature [9].

Brazilin can be obtained via aqueous extraction of sappanwood heartwood, by which other water-soluble
materials will also be coextracted. Currently, the brazilin extraction was well done by maceration in which can be used for antibacterial, lipase inhibitory, and antioxidant [10]. However, this method requires a long extraction time. Another common sappanwood extraction method is by using distilled water extraction in the soxhlet apparatus [11]. Using the soxhlet apparatus, the extraction time can be shortened. Occasionally, the conventional extraction by the soxhlet apparatus was conducted at a higher temperature that may degrade the ingredients, such as bioactive compounds.

Ultrasonic-assisted extraction method can be a promising green technology to replace the aforementioned conventional extraction methods. Indeed, it offers some superiorities over the formers by minimizing solvent and energy consumption, enhancing the extraction rate, and achieving a high extraction yield in a short extraction time. This is because the ultrasonic wave can generate a cavitation effect that can distract the raw material’s cell walls, leading to a remarkable increase of mass transfer of the target compounds into the solvent [12]–[14].

While temperature, time, solid to liquid ratio, and solvent selection are reported to be the main influential parameters that can affect the UAE performance, polar solvents have been reported to be the suitable solvents for the extraction of phenolic compounds like brazilin. Based on their investigations through comparison of several solvents to extract brazilin from sappanwood heartwood, namely water, methanol, ethanol, acetonitrile, and acetone, Xia et al. reported that methanol and water are the most suitable solvents [15]. Unfortunately, methanol is more toxic, expensive, and may cause serious environmental issues. Therefore, using water as a solvent to extract brazilin from sappanwood heartwood is preferable because it is easy to obtain, cheap, reusable, and environmentally benign.

The previous research about brazilin extraction from sappanwood studied the brazilin yield [1], [15] and its pharmacological properties [1], [7]. The process transfer during the extraction involving diffusion of brazilin to solvent and extraction rate were scared. The aspects are important to estimate process extraction parameters in which are useful to find effective extraction time, the favorable sappanwood to solvent ratio, and reasonable brazilin yield. The parameter estimation in the extraction process was successfully developed in the Vernonia cinerea leaf extract using different extraction methods and time [16].

The present study aimed to investigate the effect of temperature, time, and sappanwood heartwood-water ratio on the yield of antioxidant compounds from sappanwood heartwood extraction by ultrasound-assisted extraction (UAE). In doing so, the pseudo-first order and pseudo-second-order mass transfer models were compared to find the most suitable kinetic model for UAE of sappanwood heartwood.

II. MATERIALS AND METHODS

A. Materials

The sappanwood (Caesalpinia sappan L.) heartwood powders were obtained from a local Herbal Market in Yogyakarta, Indonesia, with a moisture content of about 2.14% (Figure 1). The powders were passed through 80 and 100 mesh sieves to obtain heartwood particles with an average diameter of 0.1635 mm for further use in ultrasound-assisted extraction (UAE) experiments with distilled water as solvent. The distilled water was selected since it is an edible solvent that can dissolve sappanwood extract and is environmentally benign.

![Fig. 1 Sappanwood (a) sheet, (b) powder before sieving](image)

B. Methods

This study was conducted through a series of experimental steps, including sappanwood heartwood powder preparation, ultrasound-assisted extraction (UAE) of antioxidant compounds (BUC 65L, B-One Ultrasonic Cleaner, China), filtration, oven-drying, and UAE mass transfer kinetics model analysis. The kinetics model was then used for determining the optimum extraction time at various temperatures and solid-liquid ratios.

C. Sappanwood Antioxidant Compounds Extraction

The extraction of total antioxidant compounds from the heartwood of sappanwood powder was carried out using 250 mL distilled water as a solvent in an ultrasonic extraction system (Figure 2), in which ultrasonic wave irradiation was used as the agitation mode (40 kHz). The solid to liquid ratios were varied at 1:5, 1:6, 1:7, and 1:8 (g/mL), whereas the studied temperatures were 30°C, 40°C, 50°C, and 60°C, respectively. The extraction was performed at 5 min, 10 min, 15 min, 20 min, and 25 min to enable the extraction kinetics evaluation. At the end of sonication, the suspension was equilibrated to room temperature and further filtered through a vacuum filtration process employing a Buchner funnel connected on a filtering flask with a side tube connected to a vacuum pump. Upon completing the filtration process, the filtrates were dried in an electric oven at 105°C to the attainment of constant weight. The total yield of the antioxidant extract from sappanwood was calculated using equation 1.

\[
\text{Yield} \% = \frac{w_2}{w_1} \times 100\%
\]  

![Fig. 2 Schematic representation of ultrasound-assisted extraction of antioxidant compounds from sappanwood](image)
D. Extraction Kinetic Model

The mass transfer model is important to describe the physical phenomena of the aqueous ultrasound-assisted extraction (UAE) of brazilin from sappanwood heartwood powders. This model was previously used to represent the kinetics of solid-liquid extraction of Tilia sapwood [17]. Here, pseudo-first-order and pseudo-second-order mass transfer models were compared in order to find the proper model. The pseudo-first-order model was derived as expressed in equations 2 and 3. While the pseudo-second-order model was also developed, as seen in equation 4 and 5.

$$\frac{dc}{dt} = k_1(C - C_E) \quad (2)$$

$$C = C_E (1 - e^{-k_1 t}) \quad (3)$$

$$\frac{dc}{dt} = k_2(C - C_E)^2 \quad (4)$$

$$\frac{1}{c} = \frac{1}{k_2 C_E^2} + \frac{t}{C_E} \quad (5)$$

E. Statistical Analysis

The kinetic parameters were evaluated by linear regression. The validation of the mass transfer kinetics model used in this study was performed by evaluating the value of $R^2$ and Root Mean Square Deviation (RMSD). The $R^2$ value close to 1 and the lowest RMSD value were considered indicators for the model's suitability to represent kinetic of ultrasound-assisted extraction of antioxidant compounds from sappanwood heartwood.

F. DPPH Analysis

An antioxidant activity based on the DPPH (1,1-diphenyl-2-picrylhydrazyl) analysis was carried out to observe the antioxidant capacity of the sappanwood extract according to the method developed by Anggraini et al. [18] with some modification. The DPPH analysis was performed by dilution of 0.25 mL of sappanwood extract in 4.75 mL of methanol. A carefully measured 0.2 mL of the dilute solution was taken and was further added into 6 mL of DPPH solution. The mixture was then incubated for 30 minutes at ambient temperature. The analysis of this solution was performed using UV vis spectrophotometer. The color absorbance values of each sample were analyzed to obtain the percentage (%) discoloration as an indication of the antioxidant activity of sappanwood extract.

III. RESULTS AND DISCUSSION

A. The Effect of Extraction Temperature on Yield of Sappan Extract

This study was conducted to investigate the influential operating parameters of ultrasound-assisted extraction of sappanwood total antioxidant extract, including the extraction temperature and sappanwood powders' mass to solvent ratio. The effect of extraction temperature versus solid-liquid ratio is presented in Figure 3. That confirmed it all parameters affected the concentration of total antioxidant extract. The total antioxidant extract concentration increased following the increase of extraction temperature at all solid-liquid ratios. Figure 3 showed that the extraction rate was slower at lower temperatures (30°C to 40°C). As expected, the extraction rate accelerated significantly at temperatures beyond 40°C and achieved the highest antioxidant extract concentration at 60°C. Several aspects influenced the yield of extract compounds from plant materials, such as the extraction method, particle size, storage conditions, and the presence of interfering substances [19], [20]. Therefore, extracts of plant materials still contained the mixture of various phenolics compounds group and the others that were soluble in the solvent. The heartwood contained flavonoids compounds that are water-soluble, namely, brazilin, protosappanin, and hematoxylin [1]. The brazilin was sensitively oxidized by oxygen from the air to form brazilein. When the heartwood sappanwood was quickly extracted by water, the extract was enriched by brazilin. On the other hand, when the extraction process was delayed, the part of brazilein was oxidized. Thus, brazilein content increases appreciably [7].

Figure 3 also demonstrates that the effect of extraction temperature was significant for each solid-liquid ratio. Theoretically, the yield of total antioxidant extract continued to increase as the extraction temperature raised because a higher temperature induced a higher driving force of the dispersed extracting material in the solvent due to an increase in the number of collisions between the extracting materials and solvent. This case accelerated the rates of mass transfer of the solute [20]–[23]. However, bioactive compounds like brazilin may undergo degradation at a temperature higher than 60°C. Therefore, it was plausible that a temperature of 60°C was previously reported as the optimum aqueous extraction temperature of brazilin [15].

Meanwhile, in this research, the highest extraction yield was obtained at a solid-liquid ratio of 1: 5 (g/mL) and the extraction temperature of 60°C. However, the ultrasound-assisted extraction was still good at room temperature rounding 30°C with the extract yield around 3.0%. This achievement was higher than that of a previous study using conventional extraction without ultrasound with an extract yield of around 2% [2].

B. The Effect of Extraction Time on Yield of Sappan Extract

Figure 4 shows the effect of extraction time on the yield of the sappanwood extract. For all cases, prolonging extraction time increased the yield of the extract. However, after 15 minutes, the effect was limited. The sappanwood extract was
bounded in the tissue of the heartwood matrix. It took time to release the extract to the solvent since the bounded extract needed more energy to be broken [19], [24]. The first time, the energy destroyed the linked extract in the solid matrix. After that, the extract diffused to the solvent via the pores of sappanwood layers. However, using ultrasound, more vibration energy can be provided [24], [25]. As a result, more sappanwood extract engaged in the solid matrix can be cracked and diffused, passing the particle layers to water as solvent. For comparison, the conventional sappanwood extraction without ultrasound yielded 2.0% extract for 15 minutes [2]. Meanwhile, at the same extraction time, the yield can be enhanced up to 3.5% while assisting by ultrasound. Thus, the improvement results closed to double.

Prolonging extraction time (longer than 15 minutes), the increase of sappanwood extract yield was not significant anymore. This is because the remained extract in the wood was still bounded in the matrix of wood particles. The bounding can be broken using the other organic solvent such as ethyl acetate, or diaion resin, and the extract's yield was higher [26]. However, the residue of the solvents in the extract can trigger impacts on human health, requiring extra purification processes. Ethyl acetate exposure at above threshold limit can cause irritation (nose, eyes, and throat) and even unconsciousness [27]. In comparison, the use of diaion resin requires a higher purification cost [26].

![Fig. 4](image_url)

Fig. 4 The influence of extraction time on the yield of sappanwood extract, at operational temperature 30°C

C. Fitting Model to Experiment

The kinetics of the overall ultrasound-assisted extraction (UAE) process was developed according to the pseudo-first-order and pseudo-second-order mass transfer (Figures 5 and 6). The models were validated with the experimental data at solid to solvent ratio 1:5 and various extraction temperatures. In Figure 5 part (a), the models were fitted by experiment data at operational 30°C. While the extraction temperatures of 40, 50, and 60°C are presented in Figure 5 part (b), (c), and (d), respectively. For all cases, the pseudo-second-order sounds the better accuracy in describing the process extraction. The data resulted from the model close to experimental data at every extraction time. In comparison, the pseudo-first-order model was precise in the first 5 minutes only.

![Fig. 5](image_url)

Fig. 5 Comparison of experimental data with pseudo-first-order model results for ultrasound-assisted extraction of total sappanwood extract kinetics at temperatures: (a) 30°C, (b) 40°C, (c) 50°C, (d) 60°C solid to solvent ratio 1:5
Figure 6 demonstrates the models fitting in different solid to solvent ratios under extraction temperature 30°C. For all cases, it was clear that after 15 minutes, extending extraction time did not increase the concentration of sappanwood extract significantly. With less solvent, the sappanwood extract can be more concentrated. In contrast, with the excess solvent, the sappanwood concentration becomes lower in which caused the problem in the extract purification. Based on the experiment and models, it can be recommended that the favorable extraction time was about 15 minutes, with a solid to solvent ratio 1:5.

Figure 6, part (a) represents the concentration of sappanwood extract diluted in solvent every extraction time obtained by pseudo-first-order, pseudo-second-order, and experiment under solid to solvent ratio 1:5. Results showed that the extract concentration obtained by pseudo-second-order model closed to experiment at every extraction time. Meanwhile, the pseudo-first-order was accurate in the first five minutes only. The same results were also obtained at various solid to solvent ratios as depicted in Figure 6 part (b), (c), and (d) for solid to solvent ratio 1:6, 1:7, 1:8, respectively.

D. Parameter Estimation of Kinetic Models

Table 1 presents the pseudo-first-order kinetic parameters and goodness of fit at the temperature of 30°C to 60°C and the solid-liquid ratio of 1:5 to 1:8 g/mL. The value of $R^2$ and RMSD for the pseudo-first-order mass transfer model exhibited good conformity between experimental data and calculated values. Table 1 also shows that the k values increased significantly ($p < 0.05$) as the temperature rises, indicating the increase of extraction rate. Based on the $R^2$, the pseudo-first-order was good enough to describe the process transport in sappanwood extraction, especially in the first 5 minutes. After that, the model accuracy was lower.

Meanwhile, the pseudo-second-order kinetic model shows the more consistent result, as depicted in Table 2. For all conditions and extraction time, the value of $R^2$ was higher than 0.99 with lower RSMD values. Considering those values and yield profiles illustrated in Figures 5 and 6, the pseudo-second-order was more favorable to describe the sappanwood extraction assisted with ultrasound. The phenomena can be described in two aspects. Firstly, the ultrasound force vibrated the linkage of brazillin and solid extract components in the surface wood particle-matrix intensively during the extraction time. The vibration of the ultrasound wave broke the extract bounded at the surface. As a result, the brazillin and solid extract are released and diffused to the solvent/water quickly. The introduction of ultrasound was very meaningful since, unlike the other organic solvents, the distilled water as a solvent had limited capability to break the matrix and dissolve the wood extract [26]. More sappanwood extract or brazillin can be diluted by ultrasound vibration compared to conventional without ultrasound [2].

Secondly, the vibration attacked the sappanwood extract at the inside part of the solid particle. Here, the linkage was stronger involving the intermolecular components. With this intensive force, the matrix has been cracked and broken for a longer time. Hence, the brazillin and solid extract components can release to the water. However, the diffusion of the extract was slow since it passed the multi-components layer in the tissue of solid particles. After 15 minutes, the amount of sappanwood extract dissolved into solvent did not increase significantly. The remaining extract is located in the deeper layer or close to the core of particles, requiring more ultrasound power with a shorter wavelength for breaking. Perhaps, the smaller wood particles' size can shallow the layer in which was able to speed up the extract diffusion.

Based on the above phenomena and comparing with the other extraction process such as Tilia sapwood, the pseudo-
second-order was a better option to represent the phenomena in antioxidant extraction from sappanwood [17]. The pseudo-first-order was suitable for quick extraction only. It was good in the first of 5 minutes where the surface extraction process of brazilein and solid wood extract occurred.

### Table I

| Sappanwood to Water Ratio | T(°C) | k₁ (1/minute) | R² | RSMD (10⁻⁴) |
|--------------------------|------|--------------|----|-------------|
| (1 : 5)                  | 30   | 0.408        | 0.990 | 0.003       |
|                          | 40   | 0.412        | 0.990 | 0.004       |
|                          | 50   | 0.417        | 0.992 | 0.002       |
|                          | 60   | 0.319        | 0.977 | 0.006       |
| (1 : 6)                  | 30   | 0.384        | 0.989 | 0.003       |
|                          | 40   | 0.407        | 0.991 | 0.003       |
|                          | 50   | 0.384        | 0.987 | 0.003       |
|                          | 60   | 0.382        | 0.989 | 0.003       |
| (1 : 7)                  | 30   | 0.394        | 0.993 | 0.002       |
|                          | 40   | 0.318        | 0.976 | 0.007       |
|                          | 50   | 0.332        | 0.983 | 0.005       |
|                          | 60   | 0.373        | 0.989 | 0.003       |
| (1 : 8)                  | 30   | 0.368        | 0.981 | 0.006       |
|                          | 40   | 0.394        | 0.991 | 0.003       |
|                          | 50   | 0.361        | 0.986 | 0.004       |
|                          | 60   | 0.366        | 0.993 | 0.002       |

### Table II

| Sappanwood to Water Ratio | T(°C) | k₂ × 10⁻⁴ | R² | RSMD (10⁻⁴) |
|--------------------------|------|-----------|----|-------------|
| (1 : 5)                  | 30   | 2.055     | 0.997 | 4.605       |
|                          | 40   | 1.988     | 0.996 | 6.315       |
|                          | 50   | 2.183     | 0.998 | 3.060       |
|                          | 60   | 1.229     | 0.992 | 1.199       |
| (1 : 6)                  | 30   | 2.160     | 0.997 | 4.294       |
|                          | 40   | 2.340     | 0.998 | 3.469       |
|                          | 50   | 1.859     | 0.996 | 6.280       |
|                          | 60   | 1.725     | 0.997 | 4.530       |
| (1 : 7)                  | 30   | 2.632     | 0.999 | 1.995       |
|                          | 40   | 1.513     | 0.993 | 1.180       |
|                          | 50   | 1.634     | 0.995 | 7.164       |
|                          | 60   | 1.943     | 0.997 | 3.977       |
| (1 : 8)                  | 30   | 2.106     | 0.993 | 12.090      |
|                          | 40   | 2.382     | 0.997 | 4.320       |
|                          | 50   | 1.916     | 0.996 | 7.228       |
|                          | 60   | 2.235     | 0.999 | 1.759       |

E. Effect of Extraction Temperature and Solid-Liquid Ratio on The Antioxidant Activity of Total Sappanwood Extract

The total antioxidant activity of the sappanwood extract at various solid to solvent ratios and extraction temperature was observed as tabulated in Table 3. The results showed that increasing extraction temperature from 30°C to 40°C led to an increase in the total antioxidant activity of the sappanwood extracts. This was due to a higher extraction of freely accessible brazilein in the heartwood of sappanwood as the oxidative product of brazilein. Further increase in extraction temperature to 50°C caused a reduction in the antioxidant activity of the sappanwood extract since less residual brazilein should exist in the inner part of the heartwood of sappanwood and possible thermal degradation brazilein as a phenolic compound [15]. However, as the extraction temperature was increased to 60°C, a higher total antioxidant activity. This was likely due to the extraction of brazilein as a stronger antioxidant compound located in the inner part of sappanwood heartwood. Brazilin has been reported to exhibit the highest DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and ferrie reduction activities than standard vitamin E, braziline sappanahalcone, protosappanin B and C [28]. In this study (with ultrasound-assisted extraction), lowering temperature and amount of solvent are suggested to find a better quality of extract.

### Table III

| Solid-Liquid Ratio | DPPH (%) |
|-------------------|----------|
| 30°C  | 40°C | 50°C | 60°C |
| 1 : 5  | 86.914 ± 0.071 | 87.071 ± 0.080 | 75.591 ± 0.178 | 86.237 ± 0.093 |
| 1 : 6  | 82.747 ± 0.000 | 86.750 ± 0.050 | 77.382 ± 0.057 | 85.987 ± 0.043 |
| 1 : 7  | 86.279 ± 0.064 | 86.964 ± 0.078 | 76.504 ± 0.007 | 77.967 ± 0.029 |
| 1 : 8  | 67.322 ± 0.100 | 68.377 ± 0.000 | 57.125 ± 0.050 | 74.778 ± 0.093 |

IV. Conclusion

An ultrasound assisted extraction (UAE) of antioxidant compounds from sappanwood heartwood powder has been successfully performed. A lower value of solid-solvent ratio and a higher temperature resulted in the highest concentration of total antioxidant compounds extract from sappanwood. Considering the extract yield, antioxidant activity, and energy introduced for heating, the reasonable extraction process condition with UAE was reached at 30°C and the solid-solvent ratio of 1 : 5 g/mL for 15 minutes. Higher extraction temperature increases the extract yield, but the higher temperature also implies more energy cost required for heating. Meanwhile, the excessive solvent can result in more extract. However, it will be costly in the solvent separation since much water must be evaporated to find dry sappanwood extract.

The pseudo-second-order model was a suitable approach to represent batch solid-liquid extraction of brazilein from heartwood powder of sappanwood. The model was meaningful for determining the effective extraction time at various operational temperatures. In brief, shorter extraction time was adequate to obtain a high extract yield in ultrasound-assisted extraction of brazilein from the heartwood of sappanwood. Implementation of this finding on a commercial scale will increase the antioxidant extraction efficiency, reduce the processing cost and subsequently increase the economic value of sappanwood as an agricultural commodity.

### NOMENCLATURE

- C: concentration of total extract at given extraction time mg/L
- k: kinetic constant /minutes
- T: temperature of extraction °C
- t: extraction time minutes
- w₁: the dry weight of the sappanwood powders kg
- w₂: the weight of total dried extract of sappanwood powders kg

### Subscripts

- E: equilibrium
- 1: pseudo-first-order
- 2: pseudo-second-order

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