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The Effect of Extraction Time by Ultrasound Assisted Extraction (UAE) Method on Tannin Levels of Kayu Bulan Leaf (*Pisonia alba* Span.)

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ABSTRACT

Background: Kayu bulan (*Pisonia alba* Span.) is one of the ornamental plant that have been used as an alternative treatment for health because of the tannin compound content in them. Tannin compounds can be obtained through the ultrasound-assisted extraction (UAE) method, which is an extraction method using ultrasonic waves so that the extraction process can run quickly, minimize the use of solvents, and produce better extract quality. Extraction time is one of the factors that affects the tannin content in the extract, so it is necessary to find the optimum extraction time to obtain optimal tannin levels.

Objective: This study aims to determine the effect of extraction time using the Ultrasound Assisted Extraction (UAE) method on the tannin content of Bulan Wood (Pisonia alba Span.) leaves.

Research methods: Tannin levels were measured using the Folin-Ciocalteu method, with UV-Vis spectrophotometry at 760 nm determining tannic acid equivalent (TAE).

Results: The results of the study obtained tannin levels at extraction time variations of 15, 30, 45, 60, 75, and 90 minutes, namely 15.4474; 14.9067; 19.2815; 14.4919; 15.6919; and 14.8326 mg TAE/gram. The study identifies the optimal extraction time for maximizing tannin yield, which is essential for the efficient use of Pisonia alba in pharmaceuticals and antioxidant-rich formulations. The highest tannin levels were obtained at extraction time variations of 45 minutes of 19.2815 mg TAE/gram.

Conclusion: The conclusion of this research is the extraction time affects the levels of tannin compounds in the extract of kayu bulan (*Pisonia alba* Span.).

Keywords: *Pisonia alba* Span; Ultrasound Assisted Extraction; Tannins; Time Optimization

INTRODUCTION

Indonesia has been known as a tropical country with a diversity of plants, especially ornamental plants that have been used as indoor decorations or yards to pamper the eyes for those who see. In addition, some ornamental plants are also used as alternative treatments in health (Maharani, 2017). Ornamental plants that are known to have benefits as alternative treatments for health include kolbana leaf, often called kayu bulan leaf (*Pisonia alba* Span.). The properties that have been known in kayu bulan leaf according to their research Tamizhazhagan & Pugazhendy (2017) are that they can be used as anti-inflammatory, analgesic, antidiabetic, diuretic, and antioxidant. Sarvananda & Premarathna (2022) stated that the antioxidant activity of kayu bulan leaf comes from the bioactive component of secondary metabolites, namely tannins. Because of its activity as an antioxidant, tannin compounds are known to be able to inhibit the proliferation of cancer cells (Marwati et al., 2021). Tannin compounds can be obtained through various extraction methods, one of which is ultrasound-assisted extraction (UAE). UAE is a cutting-edge method that leverages ultrasonic waves to enhance extraction efficiency by promoting cavitation. Compared to traditional methods like maceration, it minimizes solvent use, reduces processing time, and improves extract quality, making it an environmentally friendly and cost-effective alternative.

Extraction time is a critical parameter affecting yield and compound stability, not only for tannins but also for a wide range of bioactive phytochemicals. Understanding this relationship is pivotal for optimizing industrial-scale processes and ensuring reproducibility in plant-based extractions. Research by Ishak *et al*. (2020) reported that extraction using the UAE produces higher tannin levels compared to conventional methods, namely maceration. This can happen due to ultrasonic wave cavitation, which makes the extracted sample molecules enlarge and shrink smoothly and slowly, thus making the tannin molecules optimally attracted to the solvent. Solvents that can be used in the extraction of tannin compounds are ethanol, methanol, or ethyl acetate because tannin compounds are polar to semi-polar so that the extraction principle in the form of like dissolve like polar compounds, will be carried away by solvents that are also polar (Matheos *et al*., 2014; Zhang *et al*., 2018). The benefits of using the UAE extraction method are that the extraction process becomes faster, minimizes the use of solvents, and produces pure extracts with better results *(*Malau *et al*., 2021).

During the ultrasonic extraction process, the extraction results are influenced by several factors including the extraction time. According to Purnama *et al*. (2019), the time and

temperature factors during the extraction process have a clear influence on the tannin content in the extract. Supported by the research of Sukaryo (2017), the extraction time has an effect on tannin levels; namely, the longer the extraction time lasts, the longer the contact of the sample with the solvent, resulting in an increase in tannin volume and content. Based on this background, there has been no research related to kayu bulan leaf extraction for the identification of tannin content, which is focused on the extraction time using the Ultrasound Assisted Extraction (UAE) method, so this research is important to be carried out in order to obtain the optimal time of kayu bulan leaf extraction that produces optimal tannin levels.

RESEARCH METHODS

Tools and Materials

The tools used in this study were a 40-mesh sieve, a 500-mL durian bottle, a flacon, a beaker glass, a measuring cup, a grinder, a watch glass, a measuring flask, a micropipette (Eppendorf), a water bath, a droppipette, a test tube rack, a rotary evaporator, a sonicator (Cole Parmer Waterbath Sonicator), a Genesys UV-Vis Spectrophotometer, a test tube, and an analytical scale (Ohaus). The ingredients used are kayu bulan leaf, aquades, tannic acid, bluetip, sodium carbonate, and foline reagent ciocalteu.

Research Procedure

1. Preparation and Extraction of Kayu Bulan Leaf Samples

   The kayu bulan leaves chosen are fresh yellowish-green leaves. After picking, the leaves are cleaned with running water, then dried, aired, and then cut into small pieces to speed up drying. Small pieces of kayu bulan leaves are dried using a 40°C oven to avoid damage to tannin compounds. Next, the dried leaves of kayu bulan are smoothed with a blender and sifted using a 40-mesh sieve. The fine powder obtained was extracted by the ultrasound-assisted extraction (UAE) method by means of 25 grams of sample dissolved in 250 mL of 70% ethanol and put in a duran bottle. There are 6 duran vials for 6 dissolved sample powders prepared for variation in extraction time. Inserted into a 40 kHz speed sonicator at 40°C with variations in extraction times of 15, 30, 45, 60, 75, and 90 minutes. The extraction results are filtered and evaporated with a rotary evaporator at a temperature of 50 °C until a thick extract is obtained. Shortly, Kayu bulan leaf powder (25 g) was dissolved in 250 mL of 70% ethanol and subjected to ultrasound-assisted extraction using a 40 kHz sonicator at 40°C, with extraction times ranging from 15 to 90 minutes.

2. Determination of Tannin Levels of Kayu Bulan Leaf Extract

   The UV-Vis spectrophotometric analysis followed the standard protocol as outlined by Kresnadipayana & Lestari (2017), and tannin quantification was conducted using the Folin-Ciocalteu method based on the guidelines provided by Kusumaningsih *et al*. (2015). In the analysis of tannin levels, the first step is to determine the maximum wavelength in the range of 600-800 nm and the operating time (OT) of the reaction. Furthermore, the standard curve of tannic acid with concentrations of 10, 15, 20, 25, 30, and 35 ppm was made. As much as 9 mL was taken from each raw concentration and dissolved with aquades up to 10 mL. Then 1000 µL of Folin Ciocalteu reagent and 1M sodium carbonate solution were added and incubated during OT at room temperature. Then the absorption is read using a UV-Vis spectrophotometer with a maximum wavelength. The level was determined at each variation of the extract sample time with a concentration of 1000 ppm using the same procedure as the raw tannic acid (Pratama *et al*., 2019). The tannin compound content is calculated in units of mg TAE/gram using the following equation:

$$\text{Total tannin content} = \frac{\text{calculated content } \left(\frac{\text{mg}}{\text{mL}}\right) \text{ x total volume (mL)}}{\text{sample weight (g)}}$$

Data Analysis

The analysis of this research data was carried out to determine the best time to extract tannins. Using SPSS software, statistical analysis was carried out on the tannin level data to determine whether the data was homogeneous and normally distributed. The Levene homogeneity test was used to test tannin levels, and the normality test was used using Shapiro-Wilk. These tests can be assessed as significant if $p < 0.05$. Continue with non-parametric tests if the data obtained is not homogeneous or normally distributed. One of the non-parametric tests can use the Kruskal-Wallis test.

RESULT AND DISCUSSION

1. Extraction of Kayu Bulan Leaf Samples

   In this study, the viscous extract of kayu bulan leaves was weighed, and the percentage of yield was calculated at each time interval shown in Table I. The yield of the extract obtained was calculated as a percentage of the ratio of the weight of the resulting extract to the weight of the kayu bulan leaf powder used during extraction. Based on these results, it shows that the condensed extract of kayu bulan leaves meets the requirements, which is not less than 10% according to the Indonesian Herbal Pharmacopoeia

(Badriyah & Farihah, 2023). The highest extract yield was obtained at a time variation of 75 minutes, which was 22.334%. This is influenced by the temperature and extraction time. At a temperature and extraction time that is less than the optimum limit, the bioactive components are not extracted completely because the diffusion process does not run optimally so that there are still many bioactive components left in the material. Therefore, the increase in temperature and time during the extraction process should be considered, as it exerts a significant influence. The right temperature and time for extraction can produce extracts with high yields (Ibrahim *et al*., 2015).

TABLE I. % Yield of Kayu Bulan Leaf Extract

Extraction Time (Minutes)	Yield (%)
15	16,058
30	17,762
45	18,724
60	19,746
75	22,334
90	21,790

2. Determination of Tannin Levels in Kayu Bulan Leaf Samples

The maximum wavelength is sought before determining the compound rate. The absorption value of the test sample must be at the maximum wavelength (Kresnadipayana & Lestari, 2017). The maximum wavelength is measured with a UV-Vis spectrophotometer at a wavelength of 600-800 nm. The measurement results showed that the maximum wavelength was 760 nm, which was the same as the research (Sampepana *et al*., 2020). It is followed by determining the operating time (OT), which aims to determine the optimal time for the absorption of the test solution to remain stable (Suharyanto & Prima, 2020). Obtained stable OT for 34 minutes. The determination of the standard curve on the tannin acid standard is to obtain a linear regression equation that will be used to measure the absorbance value obtained on the UV-Vis spectrophotometer. The linear regression equation is obtained by calculating the relationship between the concentration of tannic acid (ppm) and its absorption. The regression equation of the tannic acid raw curve can then be used to determine the tannin content contained in the sample of extraction time variation. Tannin levels were determined with a UV-Vis spectrophotometer instrument, which aimed to obtain the

tannin levels contained in the time variation samples. The treatment to determine tannin levels with the principle of the foline ciocalteu method, which is to reduce phosphotungstomolybdic acid from tannins, and then in an alkaline atmosphere due to the addition of sodium carbonate will form a blue color in the solution so that it can be measured by a visible spectrophotometer (Alfian & Susanti, 2012; Kusumaningsih *et al*., 2015). It was then incubated for 34 minutes and then measured for absorption at a wavelength of 760 nm with a UV-Vis spectrophotometer. The results of determining the tannin levels contained in the time variation sample can be seen in Table II.

TABLE II. Tannin Levels of Kayu Bulan Leaf Extract

Extraction Time (Minutes)	Tannin content (mg TAE/gram)
15	15,4474 ± 0,0486
30	14,9067 ± 0,0257
45	19,2815 ± 0,0245
60	14,4919 ± 0,0148
75	15,6919 ± 0,0323
90	14,8326 ± 0,0074

Based on the results obtained, the highest level of tannins is shown in extraction with a time of 45 minutes, which is 19.2815 ± 0.0245 mg TAE/gram, which means that the longer the extraction time is up to 45 minutes, the extracted tannin compounds will increase so that the tannin content is high. However, in the next minute, the tannin level decreases because, at the time that has reached the optimal point of more than 45 minutes, tannin compounds are damaged by hydrolysis during the extraction process. The observed peak in tannin content at 45 minutes aligns with the principle that prolonged sonication beyond optimal conditions leads to hydrolysis and degradation of tannin molecules. This is consistent with findings from a previous study, who reported similar trends in polyphenol extraction (Andriani *et al*., 2019). There is a difference in the results of tannin levels in each variation of the extraction time produced so that the extraction time greatly affects the content of a compound (Ummat *et al*., 2020). The tannin levels obtained at each variation of extraction time fluctuated so that the results were not significant because at 60 to 90 minutes the tannin levels tended to decrease. This is related to the length of the extraction process where sonication can cause a decrease in diffusion area, diffusion rate, and an increase in diffusion distance, which

can result in a decrease in the yield of a compound (Annegowda *et al*., 2010). Additionally, the results are comparable to studies on other plant materials, such as Musa acuminata, which also demonstrated the superior efficiency of UAE in extracting high levels of tannins and other bioactive compounds within optimized timeframes (Ishak *et al*., 2020).

CONCLUSION

The highest tannin levels were obtained at a 45-minute extraction time of 19.2815 ± 0.0245 mg TAE/gram. Theses study findings demonstrate the potential for optimizing UAE parameters for other medicinal plants, providing a model for maximizing bioactive compound extraction while ensuring compound stability. Of the findings, future studies could explore the effects of varying solvent concentrations, sonication frequencies, and alternative plant materials to further refine UAE protocols for diverse applications.

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