Jurnal Ilmu Kehutanan
https://jurnal.ugm.ac.id/v3/jik/
ISSN: 2477-3751 (online); 0126-4451 (print)

Phytochemical Content of Tingi (*Ceriops tagal*) Bark Extract by Ultrasonication Process

Kandungan Fitokimia pada Ekstrak Kulit Kayu Tingi (Ceriops tagal) dengan Proses Ultrasonikasi

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RESEARCH ARTICLE

DOI: 10.22146/jik.v18i2.6780

MANUSCRIPT:
Submitted : 24 January 2023
Revised : 22 April 2024
Accepted : 29 May 2024

KEYWORD
Ceriops tagal, ultrasonication, extraction, yield, phytochemical

ABSTRACT

Tingi (*Ceriops tagal*) becomes one of the natural dyes used in textile dyeing. *Ultrasonication* is an extraction method that requires less time than conventional methods. Therefore, this study aimed to determine the effect of the ultrasonication duration (20, 40, 60, 80, and 100 minutes) on the total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC). The extraction yield was calculated by drying the solution with a freezer dryer, and phytochemical content was determined using UV-vis spectrophotometry. The results indicated that the yield value of the duration treatment was in the range of 3.74% to 5.53%, which was lower than the conventional method (10.58%). However, TPC, TFC, and TTC had more optimal values than the conventional method, with TPC values ranging from 268.1 to 314.1 mgGAE/g sample, TFC ranging from 68.3 to 87.1 mgQE/g sample, and TTC ranging from 244.8 to 338.1 mgTAE/g sample. The highest compound content was observed at 20 minutes, indicating that the ultrasonication method can be applied to extract Tingi bark as natural dyes.

KATA KUNCI
Ceriops tagal, ultrasonikasi, ekstraksi, rendemen, fitokimia

INTISARI

Tingi (Ceriops tagal) merupakan bahan pewarna alami soga yang dapat digunakan pada proses pewarnaan kain. Salah satu metode ekstraksi pewarna dapat dilakukan dengan ultrasonikasi yang memerlukan waktu lebih singkat dibandingkan metode konvensional. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh durasi ekstraksi (20, 40, 60, 80, dan 100 menit) pada kulit kayu Tingi dengan ultrasonikasi terhadap nilai rendemen, kadar fenolat total (KPT), kadar flavonoid total (KFT), dan kadar tanin total (KTT). Rendemen dihitung dengan pengeringan larutan dengan freezer dryer, sedangkan kadar fitokimia dihitung dengan metode pembacaan pada spektrofotometri UV-vis. Hasil percobaan menunjukkan nilai rendemen perlakuan durasi ada pada rentang 3,74%-5,53% yang lebih rendah dari metode konvensional (10,58%). Namun, KPT, KFT, dan KTT memberikan nilai yang lebih optimal daripada metode konvensional dengan nilai KPT berkisar antara 268,1-314,1 mgGAE/g sampel, KFT pada rentang 68,3-87,1 mgQE/g sampel, dan KTT pada rentang 244,8-338,1 mgTAE/g sampel. Kandungan senyawa fitokimia terbanyak ada pada durasi 20 menit. Hal ini menunjukkan metode sonikasi dapat diaplikasikan untuk mengekstraksi pewarna alami kulit kayu Tingi.

Introduction

Natural dyes are experiencing a good trend due to their more environmentally friendly advantages than synthetic dyes. Natural dyes in textiles use raw materials from bark, fruit peel, roots, wood, leaves, and others. The process of obtaining natural dye ingredients is carried out using extraction technology, including aqueous, acid alkali, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), fermentation, and others (Cuong et al. 2019) (Mansour 2018). Tingi (*Ceriops tagal*) bark is one of the dye materials in textiles that produces reddish-brown color results. This mangrove plant has a 24% tannin content when extracted with water for three hours at 70°C (Handayani and Maulana 2014). Moreover, Pujilestari (2017) analyzed the optimal process in cotton fabric dyes at a temperature of 90°C for 1 hour. The conventional extraction process is time-consuming and needs refinement to reduce the duration and improve the outcome.

The water immersion method in the Tingi bark extraction process is simple and includes immersion and boiling to obtain dye extract. This method is easy but requires a long extraction time, lots of water, high temperature, and low yields because it only extracts water-soluble compounds (Mansour 2018). Meanwhile, the ultrasonication extraction method with ultrasonicators could increase extract yield. Ultrasonication requires no chemical addition to facilitate the extraction process, and the yield has no significant changes in the chemical structure, particles, and compounds of the materials used (Iida et al. 2008). This research aimed to determine the effect of the ultrasonication duration process (five duration levels) in extracting Tingi bark. The parameters observed were yield, total phenolic, flavonoid, and tannin contents.

Materials and Method

Materials

This research used commercial Tingi bark from a natural dye distributor in Yogyakarta. Other materials used were distilled water, distilled water, gallic acid (Merck), quercetin (Merck), tannic acid (Merck), ethanol, Folin Ciocalteau reagent, Folin Denis reagent, saturated Na_2CO_3 solution, 2% $AlCl_3$ solution, and 120 nM potassium acetate solution. Furthermore, the tools were scales, freeze dryer, Scilogex rotary evaporator, and Bel Engineering UV-vis spectrophotometry device UV M51.

Preparation and Yield Calculation

A total of 50 g of Tingi bark was extracted and put into 300 ml of distilled water. The samples were extracted with a ultrasonicator at 20, 40, 60, 80, and 100 minutes. Furthermore, the control treatment was according to the method conducted by Pujilestari (2017), which was heating to 100°C for 120 minutes. Each solution was filtered with filter paper and concentrated using a rotary evaporator. Subsequently, the solution was dried using a freeze dryer for 3x24 hours to obtain the yield. The experiment had five replications, and the calculation of extractive yield used the following formula.

$$\% \text{ extract yield} = \frac{\text{Weight of extract obtained (g)}}{\text{Number of samples used (g)}} \times 100$$

Total Phenolic Content (TPC) Test

TPC was measured by making gallic acid standard solutions with concentrations of 3, 13, 23, 33, 43, and 53 ppm. Each solution was added with 0.4 ml of Folin Ciocalteu reagent, homogenized, and allowed to stand for about 4-8 minutes. Subsequently, 4 ml of Na_2CO_3 was added to the solution, homogenized, and 10 ml of distilled water was added. The solution was allowed to stand for 2 hours, and absorbance measurements were made at a wavelength of 776.2 nm. The absorbance of each wavelength was recorded, and a regression equation was generated using the wavelength values recorded at each concentration (Figure 1). A 10 mg sample of Tingi bark extract was dissolved in 10 ml of ethanol p.a. (to 1000 ppm), and 1 ml of solution was taken and reacted until the wavelength absorption value. In addition, the value was entered into the regression equation, and phenolic content was obtained. The phenolic content was expressed as mg gallic acid equivalent per gram of sample (Tahir et al. 2017).

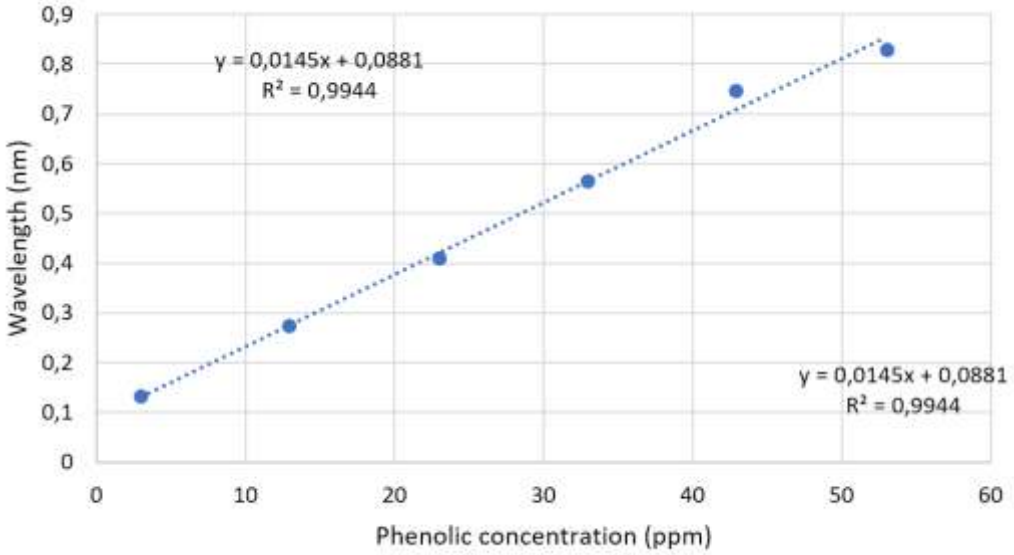

Figure 1. Regression equation of phenolic concentration

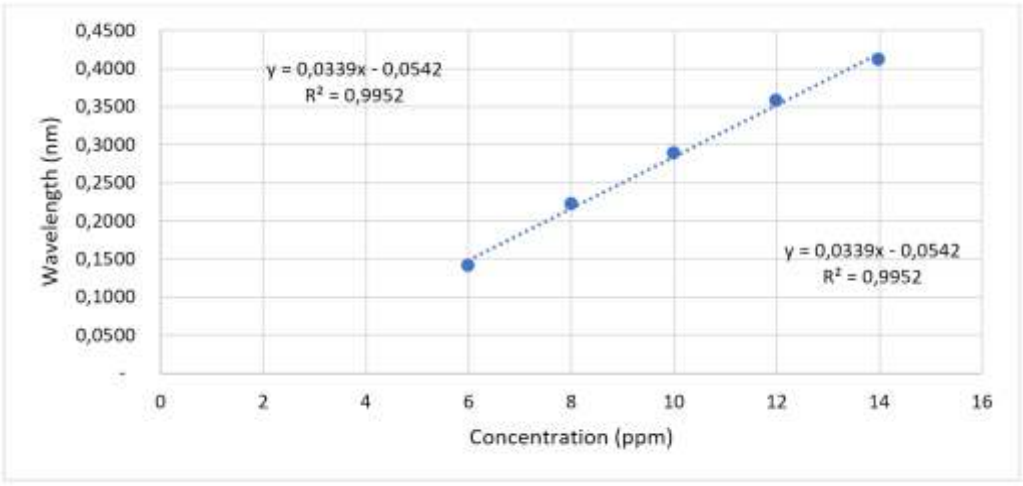

Figure 2. Regression equation of flavonoid content

Total Flavonoid Content (TFC) Test

TFC was measured with the quercetin quotient solutions of 6, 8, 10, 12, and 14 ppm concentrations. Furthermore, 1 ml was collected from each concentration, to which 1 ml of 2% AlCl3 and 1 ml of 120 nM potassium acetate were added. The solution was incubated for 60 minutes at room temperature, and wavelength absorbance was measured at 423 nm to construct a standard curve (Figure 2). A sample of about 10 mg of Tingi bark extract was obtained and dissolved in 10 ml ethanol. A parent solution of 0.5 ml was collected, and 10 ml ethanol was added. The solution was then reacted, and the wavelength absorption was measured. In addition, the wavelength value was entered into the regression equation to obtain TFC. The flavonoid content was expressed as mg quercetin equivalent per gram sample (Aminah et al. 2017; Phuyal et al. 2020).

Total Tannin Content (TTC) Test

Tannic acid was made at 10, 15, 20, 25, 30, and 35 ppm concentrations; 1 ml was collected from each solution, and 1 ml of Folin Dennis reagent was added and incubated for 3 minutes. The solution was then added to 1 ml of saturated Na2CO3 and incubated for 40 minutes under closed conditions. The wavelength absorbance was measured at 782.4 nm. Furthermore, the wavelength value made a standard curve to

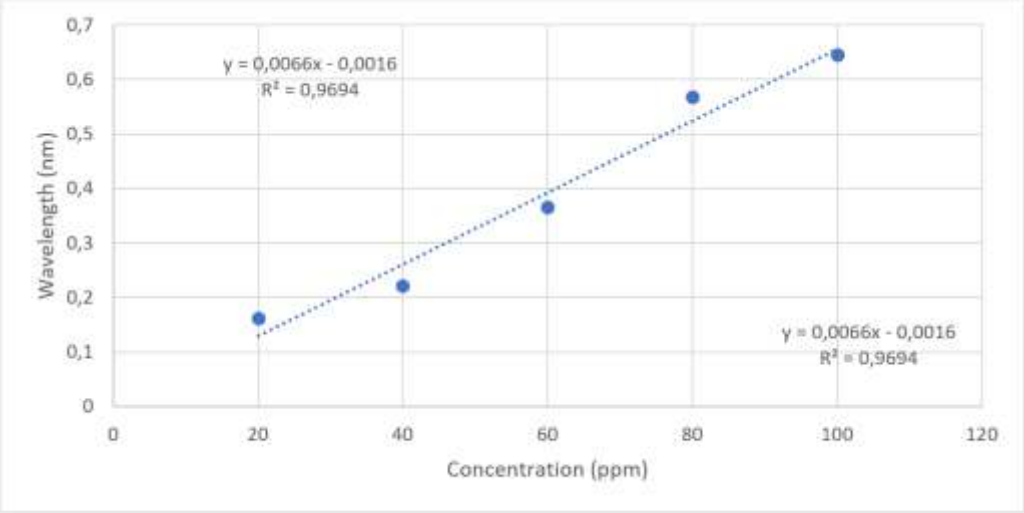

Figure 3. Regression equation of tannin content

determine TTC (Figure 3). A 10 mg of Tingi bark and 0.5 ml of mother liquor were dissolved in 10 ml of distilled water, the solution was reacted, and the wavelength absorbance was calculated. The wavelength value was entered into the regression equation to calculate TTC expressed as mg tannic acid equivalent per gram of sample (Pratama et al. 2019).

Data Analysis

The yield was analyzed with ANOVA (Analysis of Varian) at a 1% significant level. Significant results from the ANOVA test were followed by a further examination using the Duncan Multiple Range Test (DMRT). The TPC, TFC, and TTC extractive data were analyzed using descriptive statistics on Microsoft Excel, with the addition of add-on Data Analysis and SmartstatXL Premium.

Result and Discussion

Extractive Content

The extraction of Tingi bark using ultrasonicators produced a yield that tended to increase with increasing extraction time, with the highest value at 100 minutes (5.53%). This result aligned with the previous research, which suggested that extraction duration became a crucial factor in determining the yield (Silva et al., 2007). In addition to duration, other factors included particle size, solvent type, temperature, and material type. The results in Figure 4 showed that the yield value was 3.74%-5.53%. The 20-minute duration yielded the least and contained only certain solvent compounds. The control (conventional) treatment produced more yield than the ultrasonicator because the control applied higher temperature and longer time (soaking and heating for up to 2 hours). The control generated more yield and contained more sugar than all the ultrasonicator methods.

In addition, ultrasonication affected the induction of polysaccharide chain degradation and reduced phenolic thermal degradation, leading to lower yield with higher phenolic content. This condition was found in experiments on *Choerospondias axillaries* peels extracted with hot water and an ultrasonicator (Wang et al. 2023). The yield value in this experiment was lower than the extraction on *Gnetum gnemon* peels, which reached 15% using methanol solvent (Rujiyanti et al., 2020) due to the difference in solvent types. However, the results for the phenolic, flavonoid, and tannin content did not follow the higher yields due to the contribution of sugar or salt compounds produced during the extraction process.

The results of the ANOVA test analysis showed that the treatment yield differed significantly. Furthermore, the DMRT (Duncan) test showed no significant difference between the extraction durations of 20 and 80 minutes (Table 1). However, there was a significant difference between the extraction durations of 20 and 80 minutes and the 100-minute duration. Therefore, short-duration

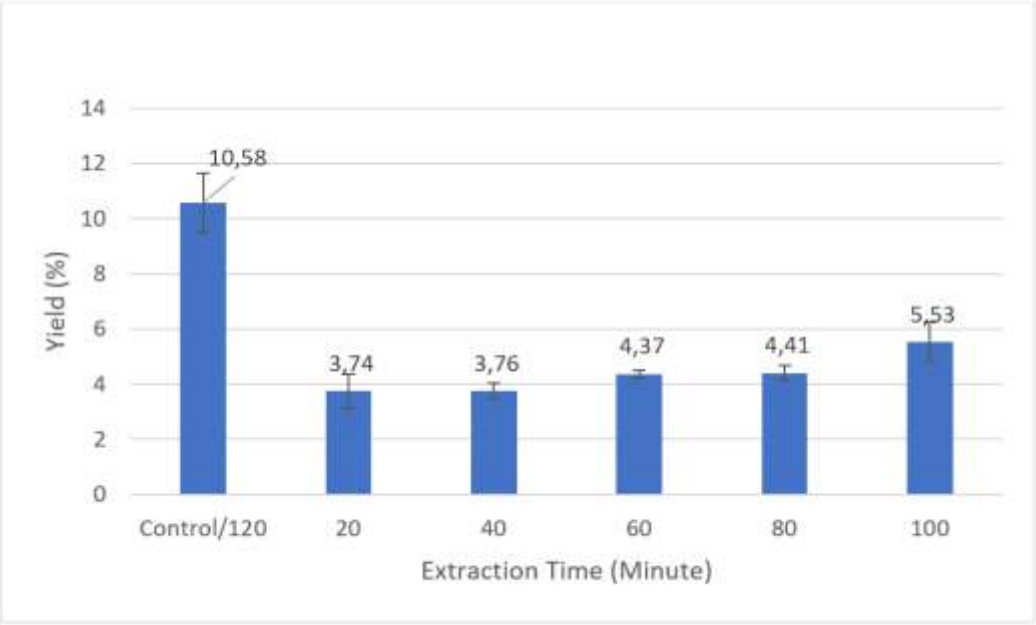

Figure 3. Regression equation of tannin content

Table 1. DMRT of TPC, TFC, and TTC on Tingi Bark Extract

Extraction sample	TPC (mgGAE/g)	TFC (mgQE/g)	TTC (mgTAE/g)
Control (120 minute)	304.3 ± 17.5^{bc}	78.3± 2.6^{b}	279.4 ± 10.5^{bc}
20 minutes	314.1 ± 21.6^{c}	87.1 ± 8,6^{c}	338.1 ± 17.1^{d}
40 minutes	274.4 ± 25.6^{ab}	81.4 ± 3,9^{bc}	291.8 ± 7.6^{c}
60 minutes	272.8 ± 29.1^{ab}	74.8 ± 5,7^{ab}	269.3 ± 13.7^{b}
80 minutes	268.2 ± 21.7^{a}	68.3 ± 4,9^{a}	244.8 ± 16.2^{a}
100 minutes	268.1 ± 28.4^{a}	70.5 ± 2,9^{a}	278.4 ± 8.3^{bc}

Note:
The same letter on the same column is not statistically different at P < 0.05 by DMRT
TPC: Total phenolic content; TFC: Total flavonoid content; TTC: Total tannin content.

extraction was more effective than 100-minute extraction as it required more energy. All ultrasonicator treatments were significantly different from the control.

Phytochemical Analysis

Total Phenolic Content (TPC)

The phenolic content extract decreased with a longer extraction duration; the highest value was in 20 minutes. At 20 minutes, the solvent and solute conditions experienced equilibrium, and the phenolic content decreased. This decrease could be caused by a decrease in the rate and area of diffusion due to the sonification process (Annegowda et al. 2010) or the oxidation of phenolic compounds because phenolic compounds are sensitive to heat and easily oxidized (Khoddami et al. 2013). A similar decrease also occurred in the research on *Gnetum gnemon* peels after 20 minutes(Rujiyanti et al. 2020).

By ANOVA, the duration factor significantly affected TPC (p<0.01), TFC (p<0.01), and TTC (p<0.01). The results of DMRT (Table 1) on TPC showed a significant difference in the treatment duration. This finding has practical implications, as it suggests that the duration of 40 to 80 minutes was not significantly different, therefore it can be concluded that the extraction results produced the same phenolic content. The 20-minute duration had results that were not significantly different from the control, but significantly different from other treatments. It was assumed that using an ultrasonicator could give the same results as conventional methods and even better because it required a shorter time.

Total Flavonoid Content (TFC)

TFC of Tingi bark extract values tended to decrease with increasing extraction time (Table 1). The highest phenolic content was at 20 minutes, and after

20 minutes, the content decreased. The decreased flavonoid content was due to the reduced condition of flavonoids with more extended ultrasonication treatment or thermal degradation (Lu et al. 2013). The phenolic content of the control was less than that of the ultrasonicator treatments. Therefore, using an ultrasonicator produced more flavonoids with a shorter duration than the conventional method.

The DMRT Test (Table 1) revealed a significant difference at the 1% level in each treatment. Notably, the 20-minute treatment was significantly different from the others, including the control (120 min). These results suggest that a short ultrasonication duration can yield higher flavonoid values than the conventional method with a longer duration. Furthermore, the ultrasonication method's advantage of increasing the surface contact of the sample can lead to a positive effect by boosting the extraction yield up to a specific duration, inspiring new approaches to extraction processes (Pan et al. 2012)

Total Tannin Content (TTC)

The highest TTC value was at 20 minutes with a 338.12 mgTAE/g sample. This result was higher than the control method, worth 279.4 mgTAE/g of the sample. Furthermore, the results showed that the value of tannin content decreased by adding the extraction duration up to 80 minutes. However, an anomaly was that the TTC increased from 80 to 100 minutes. The decrease in tannin value caused damage during cavitation in the long extraction process. In addition, there were factors of decreased diffusion area and rate, increased diffusion distance (Annegowda, et al. 2010), and heat effect that damaged tannin (Sukardi, et al. 2007). A similar decrease occurred in research conducted on red *Gnetum gnemon* peels (Rujiyanti et al. 2020) and *Averrhoa bilimbi* leaves (Andriani et al. 2019). Tannin in *Gnetum gnemon* peels increased until 30 minutes and then decreased, similar to the Tingi bark treatment, which decreased by 20 minutes.

The DMRT for TTC (Table 1) resulted in a significant difference between the treatments at the 1% level. The DMRT indicated that the TTC at 20 minutes significantly differed from others, including the control. Moreover, the 40 and 60-minute treatments were not significantly different from the control and 100 minutes, and the treatment at 80 minutes was significantly different from the others. The 20-minute extraction produced high compounds with a short extraction duration. A similar high result of TTC was obtained from 100 minutes and control, but the extraction process required more energy than the shorter extraction duration. Therefore, extraction with an ultrasonicator was more effective in producing tannin compounds (Iida et al. 2008).

Conclusion

In conclusion, the use of an ultrasonicator for extracting natural dyes from Tingi bark, while yielding less than the conventional method (120 minutes boiling), is a significant finding. The fact that a 20-minute ultrasonication resulted in the highest TPC, TFC, and TTC, indicating that the ultrasonication for extracting phenolic compounds of Tingi bark for natural dyes requires a shorter time, is a key finding. However, it is crucial for future research to delve into the analysis of other non-phenolic components that affected yield, as this will provide a more comprehensive understanding of the process.

Acknowledgments

The authors are grateful to the Center of Standardization and Industrial Services for Crafts and Batik Ministry of Industry, Faculty of Forestry UGM, Integrated Research and Development Institute UGM, Center for Food and Nutrition Studies UGM, and Education Fund Management Institution (LPDP).

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