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PHENOL COMPOUND CONTENT AND ANTIBACTERIAL ACTIVITY OF GAHARU LEAF EXTRACT PRODUCTS (*Aquilaria malaccensis*)

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Abstract
Gaharu leaf extract produces yield extraction, phenol compound, and antibacterial activity in diverse quantities. The purpose of this research was to investigate the influence of the extraction method and type of solvent on the extractability of the polyphenol component and the antibacterial activity of gaharu leaves. Extraction was done through maceration and Soxhlet methods by using solvents of hexane, ethyl acetate, and ethanol. The extraction result showed that the highest yield value of 18.4% was found on the treatment of a combination of ethanol solvent and Soxhlet method. The total content of phenol and tannin of gaharu leaf extract was in the range of 11.2 to 18.62mg. mL^{-1} and 12.82 to 13.41%, respectively. Antibacterial activity of gaharu leaf extract on the Gram-positive test of *Staphylococcus aureus* was higher than that of the Gram-negative test of *Escherichia coli* having a value of zone of inhibition in the range of 5.33 to 6.33 mm and 4.00 to 5.00 mm, respectively.

Keywords: Ethanol. Maceration. Phenol. Solvent. Soxhlet.

1. Introduction

Parwata et al. (2016) reported that gaharu leaf extract contains phenolic compounds (bioflavonoids) and these compounds can prevent the lipid oxidation process by capturing and neutralizing free radicals. Surjanto et al. (2019) added that gaharu leaf extract has a strong category of antioxidant activity with an IC_{50} value of 56.985µg. Kamonwannasit et al. (2013) stated that gaharu leaf also contains tannin, saponin, and *cardiac glycosides* compounds, but has no alkaloid. Tannin is a stringent compound having a bitter taste due to its polyphenol cluster that capable of binding and of precipitating protein. Tannin is a water-soluble phenol compound and has a molecular weight in the range of 500 to 300 Da.

Polyphenol compound contains a hydroxyl cluster that is easily soluble within the polar solvent. Different number and position of hydroxyl clusters make this compound has a wide spectrum in term of solubility characteristics within the solvent having different polarity levels. Therefore, extraction by using different solvents will also produce different polyphenol compounds and antioxidant activity measured (Kratchanova et al. 2010).

The extraction process of a substance is not only affected by type and concentration of the solvent, but also by substance size, extraction period, and temperature as well as an extraction method. Extraction methods of Soxhlet and maceration have respective advantages and disadvantages in producing extracted substances. Therefore, the research related to the extraction of the substance using these two methods is important to be implemented.

The purpose of this research was to investigate the influence of the extraction method and type of solvent on the extractability of the polyphenol component and the antibacterial activity of gaharu leaves.

2. Material and Methods

Materials

Leaf of gaharu plant (*Aquileia malaccensis*) obtained from Bangka Tengah District, Bangka Belitung Province, folin ciocalteu 50%, alcohol, gallic acid, aquadest, gelatin, indigo carmine, kaolin powder, nutrient broth, plate count agar, and extraction solvents of hexane, ethyl acetate, ethanol, *Escherichia coli,* and *Staphylococcus aureus.* and tested bacteria were obtained from the Laboratory of Agricultural Chemistry at Sriwijaya University, Indonesia.

Equipments

Soxhlet extractor, Erlenmeyer flask of 1 L, shaker water bath, rotavapor, spectrophotometer, autoclave, incubator, petri dish, Eppendorf micropipette, OSE needle, and mixer vortex.

Experimental design

The experimental design used in this study was randomized block design consisting of two treatment factors of extraction methods (A_1 = Soxhlet and A_2 = maceration) and solvent types (B_1 = hexane, B_2 = ethyl acetate, and B_3 = ethanol). Data will be processed by using an analysis of variance (ANOVA) and treatment having significant effect will be further analyzed by using the honestly significant different (HSD) test at a 5% level. The observed parameters were yield extraction (Lubis and Nova 2013), total phenol (Alkaltham et al. 2020), percent of tannin (Galvao et al. 2018), and antibacterial activity (Misna and Diana 2016).

Soxhlet extraction

Soxhlet extraction was carried out using the protocol of Ismail et al. (2020). Sample of gaharu leaf powder was weighed with a magnitude of 40 g with a water content of 6% and a size of 40-60 mesh, wrapped with sieving paper, and then put into a Soxhlet tube. Soxhlet flask was filled with a solvent having a volume of 250 mL. Soxhlet unit was set up and equipped with a reverse cooler, heated at boiling point temperature of solvent (hexane, 69°C; ethyl acetate, 77.1°C; ethanol, 78.4°C) let the circulation proceeded for 5-6 hours. The produced solution was subsequently evaporated by using a vacuum rotary evaporator with pressure and temperature according to the solvent until obtained dry extract.

Maceration extraction

Maceration extraction was carried out using the protocol of Vongsak et al. (2011). Sample of gaharu leaf powder was weighed with a magnitude of 40 g with the water content of 6% and size of 40-60 mesh, put into the vessel (macerator), and added with solvent according to treatments having a volume of 250mL. The solution was subsequently macerated for 3 x 24 hours at room temperature and filtrated using sieve paper. The produced filtrate was evaporated by using a vacuum rotary evaporator with pressure and temperature according to the solvent until viscous extract is obtained. This viscous extract was subsequently dried within the oven for 48 hours until dry extract is produced.

3. Results and Discussion

Yield extraction

Results of yield extraction from two extraction methods showed that the higher the solvent polarity level, the higher the yield magnitude. The highest yield value of gaharu leaf extract was 18.4% (A_1B_3 treatment), and the lowest yield value was 0.73% (A_2B_1 treatment) such as shown in Figure 1.

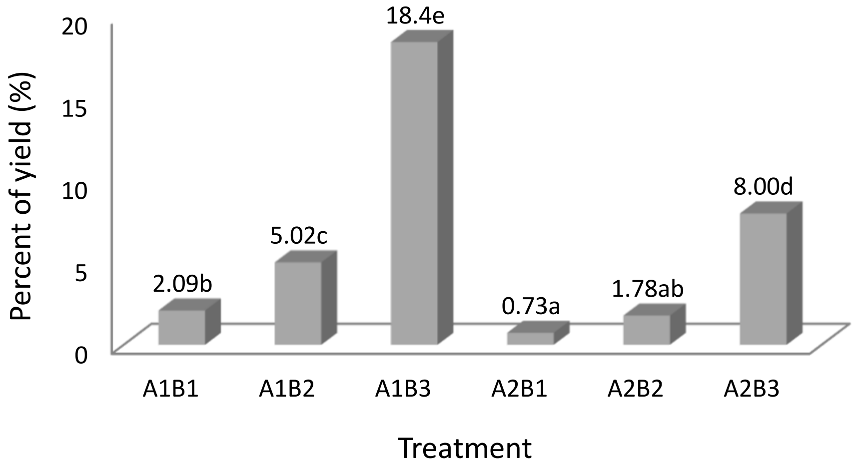

Figure 1. Extraction yield of gaharu leaf using extraction method of Soxhlet (A1) and maceration (A2) with solvents having different polarity levels (B1= hexane, B2 = ethyl acetate, and B3 = ethanol).

Analysis of variance results showed that the extraction method, solvent type, and their interactions had a significant effect on yield extraction. The results of the HSD test were shown in Table 1.

Results of the HSD test (Table 1) showed that the Soxhlet extraction method produced a higher yield value than that of the maceration extraction method. This was due to the fact that the Soxhlet method used heating treatment that had affected the increasing kinetic energy of the solvent. The higher the kinetic energy, the easier the solvent diffusion into cell tissues of gaharu leaf, resulting in a higher quantity of extract. Coelho et al. (2020) stated that the Soxhlet extraction method had produced a higher yield value of guava leaf than that of the maceration method with magnitudes of 3.59% and 0.98%, respectively.

Different threshold values of gaharu leaf extract using different solvents were due to differences in the polarity level of respective solvents. The higher the solvent polarity value, the higher the extraction threshold produced such as shown by HSD test results (Table 1). High solubility is related to the polarity of the solvent and polarity of the extracted substance (like dissolves like). The polarity of the solvent is indicated by dipole moment, dielectric constant, and solubility in water. Ethanol has a higher polarity index than that of ethyl acetate and hexane so that it had produced a higher threshold than that of ethyl acetate and hexane (Taghizadeh et al. 2018).

Table 1. HSD test results related to the effect of the extraction method and solvent type on the yield of gaharu leaf extract.

Extraction method	Yield (%)
A2 (Maceration)	3.50^{a}
A1 (Soxhlet)	8.50^{b}
Solvent type	**Yield (%)**
B1(hexane)	1.41^{a}
B2 (ethyl acetate)	3.40^{b}
B3 (ethanol)	13.20^{c}

Numbers followed by the same letter in the same column are not significantly different (at α =5%).

Results of the HSD test showed that treatment interaction of the Soxhlet extraction method and ethanol solvent had produced the highest yield (Figure 1). Soxhlet extraction method uses a higher temperature that causes lower viscosity of solvent and higher extract solubility which in turn affect the produced threshold. Ethanol has the highest polarity index than the other solvents, the higher the solvent polarity, the higher the produced extract. Hatam et al. (2013) had described that pineapple skin extraction by using the Soxhlet method and ethanol solvent produced higher total phenol than that of the maceration method using the same solvent.

Total phenol

The highest total phenol with a magnitude of 18.62mg. mL^{-1} was found on the treatment of the Soxhlet extraction method using ethyl acetate solvent; by the other hand, whereas the lowest one with a magnitude of 9.62mg. mL^{-1} was found on the treatment of maceration extraction method using hexane solvent. Average values of total phenol from gaharu leaf extract were shown in Figure 2.

Results of variance analysis showed that solvent types had a significant effect on the produced total phenol, whereas the interaction of extraction method and solvent types had no significant effect on the produced total phenol. Results of the HSD test of solvent types on average values of total phenol was presented in Table 2.

Ethyl acetate solvent produced the highest total phenol than that of other solvents. This is due to the fact that the phenol compound has semipolar characteristics so that it will be more soluble within the semipolar solvent. Semipolar solvents such as ethyl acetate can extract phenol, terpenoid, alkaloid, aglycone, and glycoside compounds (Jadid et al. 2017). This is in accordance with the findings of Pambayun et al. (2007), which showed that the highest total phenolate was obtained from the gambier extraction process with the Soxhlet method by using ethyl acetate.

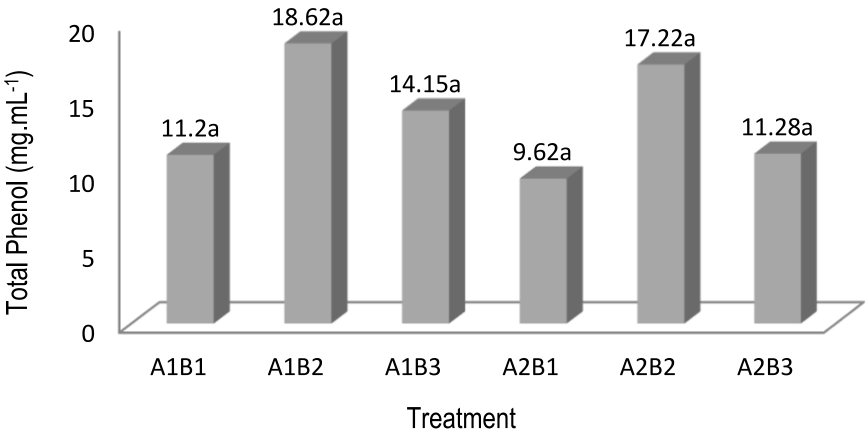

Figure 2. Total phenol (mg. mL^{-1}) of gaharu leaf produced by using extraction with solvents having different polarity levels.

Table 2. Results of HSD test for the effect of solvent types on gaharu leaf yield.

Solvent type	Yield (%)
B_1 (hexane)	10.41^a
B_3 (ethanol)	12.72^a
B_2 (ethyl acetate)	17.92^b

Numbers followed by the same letters in the same column are not significantly different (at α =5%).

Tannin

Analysis of variance results showed that the extraction method, solvent type, and their interactions had no significant effect on tannin. The highest tannin concentration of gaharu leaf extract with a magnitude of 13.41% was found on the treatment of the Soxhlet method and ethyl acetate solvent. In contrast, the lowest tannin concentration with a magnitude of 12.82% was found on the treatment of the maceration method and n-hexane solvent. These results were than produce by Surjanto et al. (2019), which stated that gaharu leaf tea had a spicy taste and contain a tannin concentration of 3.2%. Batubara et al. (2020) had added that gaharu leaf extract contains terpenoid, tannin, and flavonoid. The average value of tannin concentration was shown in Figure 3.

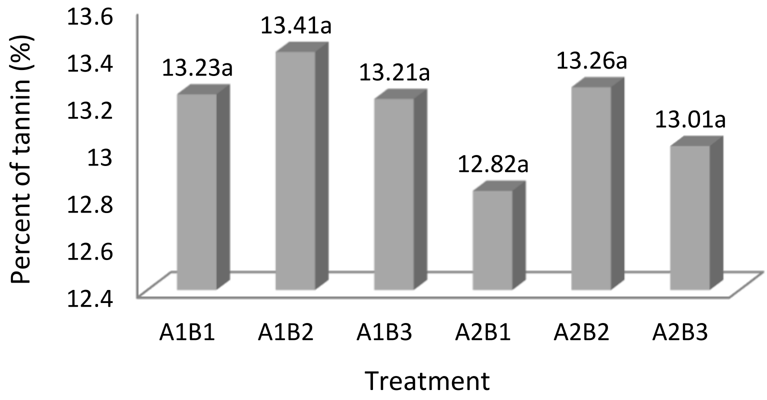

Figure 3. The Percent tannin content of gaharu leaf produced by using extraction methods with solvents having different polarity levels.

Antibacterial activity

Antibacterial activity of gaharu leaf extract obtained by using Soxhlet and maceration methods as well as different solvents was explained by the zone of inhibition toward the tested bacteria. Analysis of variance results showed that the extraction method, solvent type, and their interactions had no significant effect on antibacterial activity. The average value of the zone of inhibition toward *Escherichia coli* and *Staphylococcus aureus* bacterium was shown in Figure 4 and Figure 5.

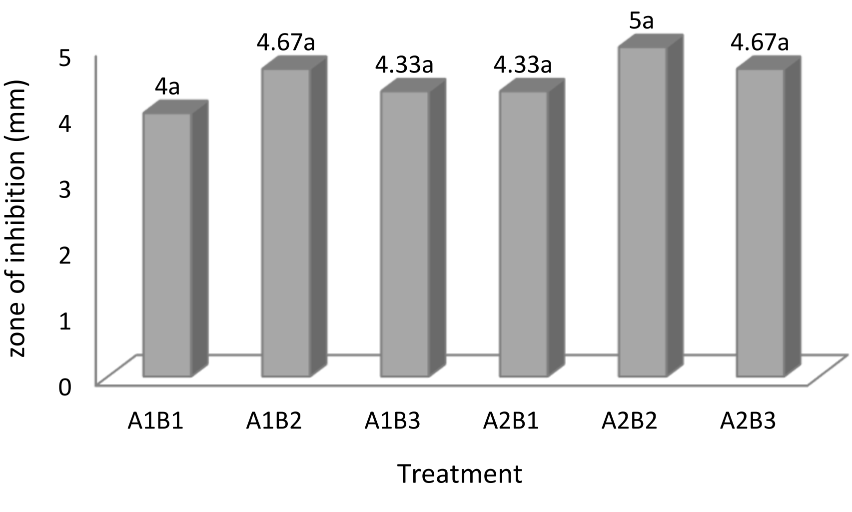

Figure 4. The zone of inhibition (mm) value of the bacterial test of *Escherichia coli* on gaharu leaf extract.

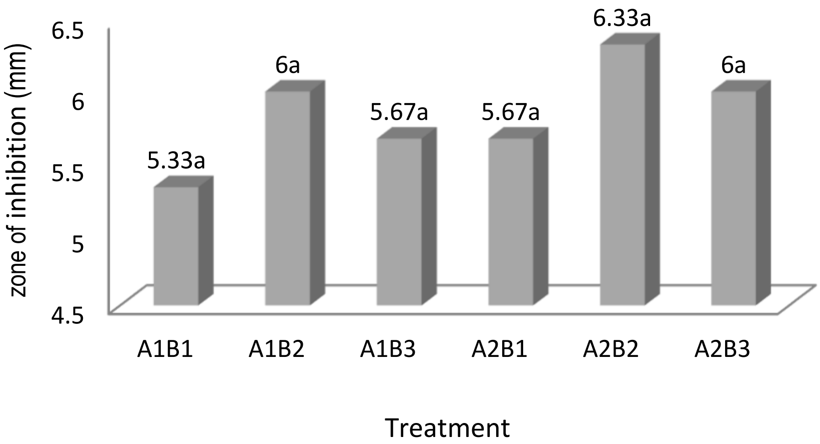

Figure 5. The zone of inhibition (mm) value of the bacterial test of *Staphylococcus aureus* on gaharu leaf extract.

The use of ethyl acetate solvent in the Soxhlet and maceration extraction methods resulted in a higher zone of inhibition value of both *Escherichia coli* (Figure 4) and *Staphylococcus aureus* (Figure 5). This fact was following with the research of Pambayun et al. (2007) stated that the highest antibacterial properties of gambier extract occurred in extracts obtained from extraction using ethyl acetate as a solvent.

The zone of inhibition of gaharu leaf extract toward a bacterial test of *Escherichia coli* (Gram-negative) with a magnitude of 4.67mm was lower than that of the bacterial test of *Staphylococcus aureus* (Gram-positive) with a magnitude of 6.33mm. The capability of gaharu leaf extract as a bacterial agent for Gram-positive and Gram-negative bacteria is due to phenol and tannin compounds within gaharu leaf. However, the zone of inhibition value of gaharu leaf extract toward Gram-positive bacterium was higher than that of Gram-negative bacterium. These results were strengthened by Kamonwannasit et al. (2013) finding which stated that gaharu leaf extract was capable impeding *Staphylococcus epidermidis* bacterium with the zone of inhibition value of 12 mm.

There are two mechanisms related to bacteria growth obstruction by hydroxide (OH^-) ions, i.e. OH^- ions bind the bacterium cell wall that contains a high concentration of peptidoglycan such as found in Gram-positive bacterium. Peptidoglycan found in Gram-positive bacterium is relatively thicker with a magnitude of about 40 nm and located at the surface. In contrast, whereas Gram-negative bacterium has thinner peptidoglycan and located inside. Based on the position of peptidoglycan, peptidoglycan position of Gram-positive bacterium is more accessible by OH^- ions than that of Gram-negative bacterium.

4. Conclusions

Soxhlet extraction method had produced a higher threshold of gaharu leaf extract than that of the maceration method.

The highest total phenol concentration was found on the extraction method using ethyl acetate solvent.

Antibacterial characteristics of gaharu leaf extract were higher toward Gram-positive bacterium than that of Gram-negative bacterium.

Authors' Contributions: SANTOSO, B.: conception and design, drafting the article; ANGGRAINI, N.: analysis and interpretation of data; YULIATI, K.: analysis and interpretation of data, critical review of important intellectual content; PANGAWIKAN, A.D.: analysis and interpretation of data, critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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