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Research Article

Antibacterial Activity of Gaharu (*Aquilaria malaccensis* Lamk) Against the Bacteria *Streptococcus pyogenes*

Aktivitas Antibakteri Daun Gaharu (Acuilaria malaccensis Lamk*) Terhadap Bakteri Streptococcus pyogenes*

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Abstract: *Streptococcus pyogenes is a bacterium that causes throat and skin infections. Diseases caused by Streptococcus pyogenes bacteria can typically be treated with antibiotics. This study aims to determine the antibacterial activity of the ethanol extract of gaharu leaves (Aquilaria malaccensis Lamk) against the development of Streptococcus pyogenes bacteria using the disc diffusion method. The positive control used the antibiotic clindamycin, and the negative control used 1% DMSO. Observations were made by measuring the inhibition zone formed around the paper disc. The average diameter of the inhibition zone formed included a 10% concentration of 9.8 mm, a 15% concentration of 10.08 mm, a 20% concentration of 11.54 mm, and for the positive control, antibiotic clindamycin, it was 29.92 mm in the very strong category. In contrast, the negative control, 1% DMSO, did not form an inhibition zone. The One Way Anova data analysis test using the SPSS IBM version 26 program obtained results (p>0.05), meaning that there were significant antibacterial and differences in the diameter of the inhibition zone of the ethanol extract of gaharu leaves in each treatment.*

Keywords: Antibacterial, Aquilaria malaccensis Lamk, Disc diffusion, Streptococcus pyogenes

Abstrak: *Streptococcus pyogenes* merupakan bakteri menyebabkan infeksi pada tenggorokan dan kulit. Penyakit yang disebabkan oleh bakteri *Streptococcus pyogenes* dapat diatasi dengan menggunakan antibiotik. Penelitian ini bertujuan mengetahui aktivitas antibakteri ekstrak etanol daun gaharu (*Aquilaria malaccensis* lamk) terhadap pertumbuhan bakteri *Strepcoccus pyogenes* dengan metode difusi cakram. Kontrol positif menggunakan antibiotik klindamisin dan kontrol negatif menggunakan DMSO 1%. Pengamatan dilakukan dengan mengukur zona hambat yang terbentuk disekitar kertas cakram. Rata-rata diameter zona hambat yang terbentuk diantaranya konsentrasi 10% sebesar 9,8 mm, konsentrasi 15% sebesar 10,08 mm, konsentrasi 20% sebesar 11,54 mm, serta untuk kontrol positif antibiotik klindamisin sebesar 29,92 mm dengan kategori sangat kuat, sedangkan kontrol negatif DMSO 1% tidak terbentuk zona hambat. Hasil uji analisis data *One Way Anova* program SPSS IBM versi 26 diperoleh hasil *(p>0,05),* artinya terdapat antibakteri dan perbedaan diameter zona hambat ekstrak etanol daun gaharu yang signifikan pada setiap perlakuan.

Kata kunci: Antibakteri, *Aquilaria malaccensis* Lamk, Difusi Cakram, *Streptococcus pyogenes*

INTRODUCTION

Infectious diseases are a leading cause of mortality worldwide, particularly in tropical countries such as Indonesia, due to dusty air conditions, warm temperatures, and high humidity. Microorganisms can cause infections, including fungi, viruses, parasites, and bacteria (Asfi, 2023).

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According to data from the Directorate of Disease Prevention and Control of the Ministry of Health from January to September 2023, cases of acute respiratory infections (ARI) are among the most frequently reported diseases in Indonesia, with a national prevalence of 1.5 to 1.8 million cases (Dong et al., 2018).

One bacterium that can lead to human infections is *Streptococcus pyogenes*. This bacterium belongs to Group A *Streptococcus* and is characterized by its spherical or oval shape, arranged in chains. It causes infections in the throat and skin (Asfi, 2023). Prevention of *Streptococcus pyogenes* infections can be achieved through the use of antibiotics. Effective antibiotics include penicillin G, penicillin V, amoxicillin, erythromycin, azithromycin, cefadroxil, cephalexin, and clindamycin (Febriyanti, 2020). Antibiotics without a doctor's prescription for bacterial infections can increase resistance. *Streptococcus pyogenes* has shown resistance to several antibiotics, including macrolides and tetracyclines. Due to this antibiotic resistance, there is a need to discover medicinal plants with antibacterial effects that can inhibit *Streptococcus pyogenes* and are safe for consumption (Dong et al., 2018).

One plant that shows potential as an antibacterial agent is gaharu leaves. Extracts from gaharu leaves exhibit antibacterial activity against Gram-positive and Gram-negative bacteria (Hasanah, 2020). Previous studies have indicated that gaharu leaves possess antibacterial activity against *Streptococcus mutans* (Febriyanti, 2020). The extract of gaharu (*Aquilaria malaccensis Lamk*) contains active compounds such as flavonoids, triterpenoids, tannins, and glycosides. These compounds are suspected to have antibacterial activity (Hidayatullah, 2023).

However no researchers have studied the effectiveness of gaharu leaves in inhibiting the growth of *Streptococcus pyogenes*. Researchers aim to investigate further the antibacterial activity of ethanol extracts from gaharu leaves (*Aquilaria malaccensis Lamk*) against *Streptococcus pyogenes* at concentrations of 10%, 15%, and 20%. The selection of these three concentrations is based on prior research conducted by Venesia (2019), which demonstrated that the ethanol extract of gaharu leaves could inhibit *Staphylococcus epidermidis* with weak efficacy at a concentration of 5% and moderate efficacy at concentrations ranging from 10% to 15%.

MATERIALS AND METHODS

The materials used in this study were obtained from North Kalimantan, Bulungan Regency, Peso Hilir District, Long Lembu Village. The bacterium *Streptococcus pyogenes* ATCC 19615 was sourced from the Thermo Scientific laboratory. Other materials included anhydrous acetic acid, concentrated hydrochloric acid ethanol, sulfuric acid, sterile aquadest, chloroform, 70% ethanol, Nutrient Agar (NA) (Hinmedia®), Muller Hinton Agar (MHA) (Oxoid®), Meyer reagent, Dragendorf reagent, Wagner reagent, 2N HCl, $FeCl_3$, H_2SO_4, 1% $BaCl_2$, magnesium (Mg), filter paper, brown paper, cotton, clindamycin antibiotics (300 mg), and dimethyl sulfoxide (DMSO).

The equipment utilized in this research included stir bars, Pyrek® beakers, Pyrek® graduated cylinders, desiccators, furnaces (Carbolite Gero 30-3000˚C®), ovens (Memmert®), Buchner funnels (Pyrek®), aluminum foil, Erlenmeyer flasks (Iwaki®), test tubes (Pyrek®), autoclaves (Gea medical®), incubators (LabTech®), clamps, vortex mixers (Pyrek®), analytical balances (Fujitsu®), dropper pipettes (Pyrek®), glass jars, hot plates (DLAB®), inoculating

needles, magnetic stirrers (Pyrek®), calipers, water baths, spirit lamps, Laminar Air Flow (LAF) systems (Qoalca®), macerators, micropipettes, scissors, tripods, and Petri dishes (Pyrek®).

Research Procedure

Preparation of Gaharu Leaf Extract

The powdered simplicia obtained was weighed at 200 grams and placed into a maceration container. Subsequently, 2 liters of 96% ethanol were added until fully submerged. The maceration container was sealed and left in a cool place protected from sunlight for 3×24 hours at room temperature with occasional stirring. The extract solution was then filtered using filter paper. The remaining residue was re-extracted with fresh 96% ethanol in the same amount and concentrated using a water bath at 60°C to obtain a 100% extract. This step prepared extracts at concentrations of 10%, 15%, and 20% use dimethyl sulfoxide (DMSO) solvent.

Phytochemical Screening

Phytochemical testing included tests for flavonoids, alkaloids, saponins, tannins, steroids, and phenols.

Flavonoid

A sample of 0.05 g was placed into a test tube and heated for approximately 5 minutes. Then, 0.05 g of magnesium powder and three drops of concentrated HCl were added. A positive test is indicated by the formation of red, yellow, or orange coloration (Khasanah, 2020).

Alkaloid

A sample of 0.05 g was dissolved in 10 mL of HCl and then filtered. A positive test is indicated by forming a yellowish-white precipitate upon adding Meyer's reagent, a brown precipitate with Wagner's reagent, and an orange precipitate with Dragendorf's reagent (Kartikasari, 2022).

Saponin

A sample of 0.05 g was mixed with 10 mL of aquadest and vigorously shaken for 5 minutes. A positive test is indicated by the formation of stable foam lasting no less than 10 minutes (Qomaliyah, 2023).

Tannin

A sample of 0.05 g was mixed with 10 mL of aquadest and filtered; then several drops of 1% $FeCl_3$ were added until a color change occurred. A positive result is indicated by the formation of a brownish-green or blue-black color (Kimia, 2022).

Steroid

A sample of 0.05 g was dissolved in 1 mL of 70% ethanol and then treated with anhydrous acetic acid and 12 mL of concentrated H_2SO_4. A positive result shows the formation of a greenish-black precipitate (Qomaliyah, 2023).

Phenol

A sample of 0.05 g was treated with two drops of $FeCl_3$ reagent. A positive result is indicated by the formation of green or blue-green coloration (Qomaliyah, 2023).

Rejuvenation of Test Bacteria

One inoculating needle from a pure culture of *Streptococcus pyogenes* was taken using a sterilized wire loop after flaming it and then inoculated onto slant agar by streaking in a zig-zag pattern before being incubated for 24 hours at a temperature of 37°C (Janshen, 2017).

Preparation of Bacterial Suspension

The McFarland solution was prepared by mixing 9.5 mL of 1% H_2SO_4 with 0.5 mL of $BaCl_2$ in a test tube and shaking until a turbid solution formed. This turbidity served as the standard for bacterial suspension turbidity (Wardaniati, 2021).

Testing Antibacterial Activity

Antibacterial activity was tested using the disk diffusion method with 5 replication. A sterilized cotton bud was dipped into the *Streptococcus pyogenes* bacterial suspension and then rubbed on the walls of the test tube before streaking it in a zig-zag pattern on agar media. Disks containing extracts at concentrations of 10%, 15%, and 20%, along with MHA as a positive control for clindamycin and DMSO as a negative control, were placed on the agar surface. The plates were incubated at a temperature of 37°C for 24 hours. Antibacterial activity was observed based on the clear zones surrounding the disks, measured in millimeters using calipers. The inhibition zone was measured from the disk's outer edge to the longest extent of the inhibition zone formed.

Data Analysis

Data analysis in this study utilized SPSS IBM version 26.0 software. The analyzed data comprised measurements of inhibition zones from antibacterial activity tests. The types of data analysis performed included the Shapiro-Wilk test for normality assessment, One-Way ANOVA, and the LSD test to determine significant differences in activity among inhibition zones.

RESULTS AND DISCUSSION

Phytochemical screening tests were conducted as a preliminary analysis on the concentrated extract of gaharu leaves to qualitatively determine the secondary metabolite content by observing color changes in the samples. Based on the phytochemical screening results (Table 1), the ethanol extract of gaharu leaves contains secondary metabolite compounds including alkaloids, flavonoids, tannins, steroids, and phenols.

These findings align with Venesia's (2019) research, which reported that phytochemical screening of gaharu leaf extract revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, and phenols. However, the ethanol extract of gaharu leaves showed negative results in some phytochemical screening tests. Variations in secondary metabolite content in plants can be attributed to factors such as environmental conditions, extraction methods, genetic variation, plant age, plant parts used, and the sensitivity of the testing methods to the quantity of natural chemical constituents being analyzed.

Table 1. Phytochemical screening results of Gaharu leaves

Phytochemical Test	Observation	Results
Alkaloid Test	Formation of yellowish-white precipitate	+
	Formation of brown precipitate	+
	Presence of orange precipitate	+
Flavonoid Test	Presence of orange-red precipitate	+
Saponin Test	No foam formation	-
Tannin Test	Formation of brownish-green color	+
Steroid Test	Formation of dark green precipitate	+
Phenol Test	Formation of blue-green color	+

Notes::
(+) Positive: Presence of secondary metabolites
(-) Negative : Absence of secondary metabolites

Table 2. Average Inhibition Zone Diameter of Ethanolic Extract of Gaharu Leaves Against *Streptococcus pyogenes*

Inhibitory diameter (mm)							
Treatment	**P1**	**P2**	**P3**	**P4**	**P5**	**Mean ± SD**	Inhibitory Strength Category
Negative Control	0	0	0	0	0	0	No inhibition
10%	10,9	8,65	10,35	11,1	8	9,8±1,39	Moderate
15%	13,4	8,45	10,15	10,2	8,2	10,08±2,08	Strong
20%	10,85	9,05	12,8	13,8	11,2	11,54±1,84	Strong
Positive Control	29,2	30,15	31,8	28,15	30,3	29,92±1,36	Very Strong

Based on Table 2, ethanol extract concentrations of Gaharu leaves at 10%, 15%, and 20% could inhibit the growth of *Streptococcus pyogenes*. At a concentration of 10%, the inhibition zone diameters were 10.9 mm, 8.65 mm, 10.35 mm, 11.1 mm, and 8 mm, with an average of 9.8 mm, categorized as moderate inhibition (5–10 mm). At a concentration of 15%, the inhibition zone diameters were 13.4 mm, 8.45 mm, 10.15 mm, 10.2 mm, and 8.2 mm, with an average of 10.8 mm, categorized as strong inhibition (10–20 mm). At a concentration of 20%, the inhibition zone diameters were 10.85 mm, 9.05 mm, 12.8 mm, 13.8 mm, and 11.2 mm, with an average of 11.54 mm, also categorized as strong inhibition (10–20 mm). The inhibition zones formed by these three concentrations did not exceed the positive control's ability to inhibit *Streptococcus pyogenes*, which had an average diameter of 29.92 mm and was categorized as very strong inhibition (>20).
Increasing the concentration of ethanol extract corresponded to stronger antibacterial activity, with the highest inhibition observed at the 20% concentration compared to the 10% and 15% concentrations. The differences in inhibition zones are influenced by several factors, including organism sensitivity, pH levels, microbial type, antimicrobial agent used, culture medium conditions, incubation settings, and agar diffusion rate (Asfi, 2023).

There were notable differences in the diameters of inhibition zones across various concentrations of Gaharu leaf ethanol extract in inhibiting bacterial growth. The size of the inhibition zones increased with higher extract concentrations, resulting in stronger antibacterial

activity due to the higher content of active antibacterial compounds in the extract. Venesia's (2019) research on the antibacterial activity of Gaharu leaf ethanol extract against *Staphylococcus epidermidis* showed no inhibition at a concentration of 5%, while concentrations of 10% and 15% produced inhibition zones of 6.3 mm and 7.14 mm, respectively, categorized as moderate inhibition (Venesia, 2019). Additionally, Sari's (2017) study supported these findings by demonstrating that Gaharu leaf ethanol extract exhibited antibacterial activity against *Staphylococcus aureus* and *Proteus mirabilis* at concentrations of 300 mg/mL, 400 mg/mL, and 500 mg/mL with successive inhibition zone diameters of 10.17 mm, 11.62 mm, and 13.41 mm for both bacteria.

The antibacterial activity of Gaharu leaf ethanol extract is attributed to its secondary metabolite content—alkaloids, flavonoids, tannins, steroids, and phenols—which exhibit diverse mechanisms of action: **Alkaloids** disrupt peptidoglycan synthesis in bacterial cell walls. **Flavonoids** inhibit cell membrane function and damage bacterial energy metabolism (Pranidya, 2021). **Tannins** inhibit reverse transcriptase and DNA topoisomerase enzymes, leading to bacterial cell lysis (Dong et al., 2018). **Steroids** interact with membrane phospholipids to reduce membrane integrity and induce morphological changes that cause cell lysis (Hidayatullah, 2023). **Phenols** denature cellular proteins by forming hydrogen bonds that disrupt protein structure (Zulita, 2018).

The results showed significant differences between the inhibition zones at a concentration of 10% when compared with those at concentrations of 15% and 20%. However, no significant difference was observed between the inhibition zones at concentrations of 15% and 20% (p-value >0.05).

CONCLUSIONS

Based on the research findings, the ethanol extract of Gaharu leaves (*Aquilaria malaccensis* Lamk) exhibits antibacterial activity against the growth of *Streptococcus pyogenes* at concentrations of 10%, 15%, and 20%. The average inhibition zone diameters were 9.8 mm for the 10% concentration, categorized as moderate inhibition; 10.8 mm for the 15% concentration, categorized as strong inhibition; and 11.54 mm for the 20% concentration, also categorized as strong inhibition. In contrast, the positive control demonstrated superior inhibition against *Streptococcus pyogenes* compared to the Gaharu leaf ethanol extract, with an average inhibition zone diameter of 29.92 mm, categorized as very strong inhibition. For further studies recommended to isolate and identify the specific bioactive compounds for this activity.

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