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Antibacterial Effect of *Caesalpinia sappan* L. Aqueous Decoction on *Staphylococcus aureus*

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ABSTRACT

Secang (Caesalpinia sappan L.) is a plant frequently utilized in herbal medicine due to its therapeutic properties. Sappan wood contains various bioactive compounds, such as flavonoids, saponins, and tannins, which can inhibit the growth of Staphylococcus aureus. Staphylococcus aureus is a bacterium responsible for conditions such as pressure sores, abscesses, impetigo, cellulitis, and staphylococcal scalded skin syndrome. The aim of this study was to determine the differences in antibacterial activity of sappan wood decoctions at concentrations of 70%, 80%, 90%, and 100% in inhibiting the growth of Staphylococcus aureus. This study employed a quasi-experimental method with a purposive sampling technique. The samples consisted of 70%, 80%, 90%, and 100% concentrations of sappan wood decoction, each repeated six times for a total of 24 samples. Antibacterial testing was conducted using the Kirby-Bauer diffusion method. The results showed that 100% of the tested sappan wood decoction concentrations successfully inhibited the growth of Staphylococcus aureus, with the average inhibition zones being 25 mm at a 70% concentration, 27.16 mm at 80%, 28.58 mm at 90%, and 30.58 mm at 100%. There was a significant relationship between the concentration of sappan wood decoction and its antibacterial activity ($p = 0.000$). Based on these results, even the lowest concentration of 70% is highly effective for inhibiting the growth of Staphylococcus aureus.

Keywords: *Caesalpinia sappan L., Staphylococcus aureus, Antibacterial Activity, Decoction, Inhibition Zone.*

ABSTRAK

Secang (*Caesalpinia sappan* L.) merupakan salah satu tanaman yang banyak dimanfaatkan sebagai obat tradisional karena memiliki berbagai khasiat terapeutik. Kayu secang mengandung senyawa bioaktif seperti flavonoid, saponin, dan tanin yang berpotensi menghambat pertumbuhan bakteri *Staphylococcus aureus*. Bakteri *Staphylococcus aureus* merupakan salah satu penyebab berbagai infeksi, seperti ulkus dekubitus, abses, impetigo, selulitis, dan staphylococcal scalded skin syndrome. Penelitian ini bertujuan untuk mengetahui perbedaan aktivitas antibakteri rebusan kayu secang pada konsentrasi 70%, 80%, 90%, dan 100% dalam menghambat pertumbuhan *Staphylococcus aureus*. Penelitian ini menggunakan metode kuasi-eksperimental dengan teknik purposive sampling. Sampel penelitian terdiri atas rebusan kayu secang dengan konsentrasi 70%, 80%, 90%, dan 100%, masing-masing dilakukan enam kali pengulangan sehingga diperoleh total 24 sampel. Uji aktivitas antibakteri dilakukan menggunakan metode difusi cakram Kirby-Bauer. Hasil penelitian menunjukkan bahwa seluruh konsentrasi rebusan kayu secang mampu menghambat pertumbuhan *Staphylococcus aureus* dengan rata-rata diameter zona hambat sebesar 25 mm pada konsentrasi 70%, 27,16 mm pada konsentrasi 80%, 28,58 mm pada konsentrasi 90%, dan 30,58 mm pada konsentrasi 100%. Analisis statistik menunjukkan adanya hubungan yang signifikan antara konsentrasi rebusan kayu secang dan aktivitas antibakteri terhadap *Staphylococcus aureus* ($p = 0,000$). Berdasarkan hasil tersebut, konsentrasi terendah yaitu 70% telah menunjukkan efektivitas yang sangat kuat dalam menghambat pertumbuhan *Staphylococcus aureus*.

Kata kunci: *Caesalpinia sappan* L., *Staphylococcus aureus*, aktivitas antibakteri, rebusan, zona hambat.

INTRODUCTION

The escalating utilization of herbal medicines is driven by growing public awareness of their safety and therapeutic benefits (1). *Caesalpinia sappan* L. (Secang) is a medicinal plant frequently employed in traditional medicine, particularly for topical applications such as alleviating irritation, treating ocular inflammation, and acting as an antiseptic wash for cutaneous wounds (2,3). The pharmacological efficacy of sappan wood, specifically its antibacterial and anti-inflammatory properties, is attributed to bioactive compounds including resorcinin, brazilin, gallic acid, flavonoids, saponins, terpenoids, and tannins (4,5). Bacterial skin infections typically occur following an epidermal barrier breach caused by factors like maceration, excoriations, or xerosis. These breaches facilitate the entry of opportunistic pathogens, which can cause localized inflammation or, if disseminated, severe systemic conditions (6).

Previous research has established the general antibacterial potential of *C. sappan*. A study by Amallia Putri Megarani demonstrated that sappan wood decoction exhibits antibacterial activity against *Escherichia coli*, noting an inverse relationship between solvent volume and the diameter of the inhibition zone, peaking at 13.56 mm (7). Similarly, an *in vitro* study by Ni Made Irna Prasetiari evaluated an aqueous extract against *Streptococcus mutans*, finding that a 50% concentration yielded the highest efficacy with an inhibition zone of 15.4 mm (8).

However, studies examining the specific antibacterial efficacy of high-concentration aqueous decoctions against *Staphylococcus aureus*—a primary pathogen responsible for severe skin infections like abscesses and cellulitis—are still limited. While previous research has focused on lower extract concentrations, different extraction methods, or Gram-negative and oral bacteria, there is a distinct research gap regarding how standardized traditional decoctions at high concentrations (70% to 100%) perform against this specific cutaneous pathogen. Building upon this need, the present study aimed to investigate the antibacterial activity of high-concentration sappan wood aqueous decoctions against *Staphylococcus aureus* using the disk diffusion method

METHOD

Research Design, Variables, and Sampling

This study utilized a quasi-experimental design, which approximates a true experiment without the strict control or manipulation of all relevant variables. The independent variable was the concentration of *Caesalpinia sappan* L. aqueous decoction (70%, 80%, 90%, and 100%), while the dependent variable was the antibacterial activity, measured by the diameter of the inhibition zone. Samples were selected using purposive sampling, specifically targeting high-concentration ranges to observe their efficacy. Based on the experimental formula $(t - 1)(r - 1) \geq 15$ for a completely randomized design, the number of replications (r) was calculated to be six per concentration, resulting in a total sample size of 24 (9). To ensure validity, inclusion criteria for the samples required the use of a pure *Staphylococcus aureus* culture matching the McFarland turbidity standard and sterile Mueller-Hinton Agar (MHA). Exclusion criteria included any contaminated bacterial suspensions or improperly solidified MHA plates.

1. Examination Method & Principle

The antibacterial assay was conducted using the Kirby-Bauer disk diffusion method. Blank paper discs impregnated with the antimicrobial agent were placed onto MHA plates previously inoculated with the test microorganism. The diameter of the resulting zone of inhibition served as an indicator of the microorganism's susceptibility to the extract (10).

2. Working Procedure

a. Preparation of Sappan Wood Decoction (10)

- 1) Weigh 300 grams of sappan wood, section it into smaller pieces, and thoroughly wash with sterile distilled water.
- 2) Boil the wood in 1000 mL of distilled water at 75°C on a hotplate for approximately 7 hours until the volume reduces to 300 mL.
- 3) Filter the decoction to remove solid residues. The initial filtrate is designated as the 100% concentration.
- 4) Prepare subsequent dilutions (70%, 80%, and 90%) using 10 mL volumetric flasks according to the table below:

Table 1: Preparation of Secang Wood Decoction Concentration

No	Concentration	Secang Wood Decoction	Description
1	70%	7 mL	Add 7 mL of 100% decoction, then fill to 10 mL with sterile distilled water.
2	80%	8 mL	Add 8 mL of 100% decoction, then fill to 10 mL with sterile distilled water.
3	90%	9 mL	Add 9 mL of 100% decoction, then fill to 10 mL with sterile distilled water.
4	100%	10 mL	Use 10 mL of the pure decoction directly.

* Dilution using a 10 mL volumetric flask

b. Preparation of the McFarland Turbidity Standard (11)

- 1) Pipette 0.5 mL of 1.175% BaCl₂ and 99.5 mL of 1% sulfuric acid into a test tube and homogenize.
- 2) Seal the tube tightly to prevent evaporation. This suspension standard shows bacterial turbidity equal to 10⁸ CFU/mL.

- 3) Store in a dark environment at room temperature (valid for up to six months). Agitate prior to use.
- c. Preparation of Bacterial Suspension (11)
 - 1) Take a *Staphylococcus aureus* colony from a pure culture using a round loop.
 - 2) Transfer it to a test tube containing 5 mL of sterile NaCl and homogenize.
 - 3) Compare the turbidity with the McFarland standard.
 - d. Preparation of Antimicrobial Discs (12)
 - 1) Prepare blank discs.
 - 2) Soak blank discs in each concentration (70%, 80%, 90%, and 100%) of boiled sappanwood water using sterile tweezers, one disc at each concentration, for 15 minutes.
 - 3) Then, remove the discs and place them in the center of the plate inoculated with the microbial culture.
 - e. Inoculating the Sample (11)
 - 1) Dip a sterile cotton swab into the suspension in the test tube.
 - 2) Use a pressing and rotating motion to apply pressure to the sterile cotton swab against the wall of the test tube to allow it to drain.
 - 3) Then, spread the swab evenly over the surface of the Mueller-Hinton agar plate.
 - 4) Let it sit for 5-15 minutes to allow the bacterial suspension to penetrate the agar.
 - 5) Place the discs containing the respective concentrations of sappanwood decoction on the surface of the agar plate and gently press them with tweezers to adhere to the agar surface.
 - 6) Incubate at 37°C for 24 hours in the incubator.
 - 7) Check for the formation of a zone of inhibition around the discs.
 - f. Preparation of negative controls (11)
 - 1) Dip a sterile cotton swab into the bacterial suspension in the test tube.
 - 2) Press and rotate the swab against the wall of the test tube.
 - 3) Then, spread the swab evenly over the surface of the MHA medium.
 - 4) Place blank paper discs soaked in distilled water on top of the MHA.
 - 5) Incubate at 37°C for 24 hours in the incubator. Observe growth.
 - g. Reading the diameter of the inhibition zone (11)

Reading or measuring the inhibition zone is done on paper or a dark background, with the naked eye without a loop, measure the diameter of the inhibition zone formed on the MHA cup using a ruler or dial caliper.

Table 2: Inhibition Zone Diameter Categories

Inhibition Zone Diameter (mm)	Description
>20	Very Strong
11 – 20	Strong
5 –10	Moderate
<5	Weak

*Source: Davis and Stout, 1971 dalam Silap, Wewengkang and Rotinsulu, 2020

3. Data Analysis Techniques
 - 1) Univariate Analysis

Univariate analysis aims to explain or describe the characteristics of each variable studied. This type of analysis is tailored to the type of data used. For numerical data, it is typically analyzed using the mean, median, and standard deviation (13).

2) Bivariate Analysis

Bivariate analysis is used to examine the relationship between two variables. These two variables are the primary variables: the influencing (independent) variable and the affected (dependent) variable (Siyoto and Sodik, 2015). Using the One-Way ANOVA test, the data obtained from the research results were analyzed and processed computerized using SPSS.

RESULTS

This study evaluated the antibacterial efficacy of various concentrations of *Caesalpinia sappan* L. aqueous decoction against *Staphylococcus aureus*.

Tabel 3: Descriptive Characteristics of the Inhibition Zone Diameter (mm)

Concentration	N	Minimum	Maximum	Mean	Std. Deviation
70%	6	24,0	26,0	25,0	0,8367
80%	6	26,0	28,0	27,16	0,8165
90%	6	27,5	30,0	28,58	0,9174
100%	6	29,0	32,0	30,58	1,1143

As shown in Table 3, the average inhibition zone diameter increased sequentially with higher concentrations. At a 70% concentration, the average inhibition zone was 25 mm, increasing up to a maximum of 30.58 mm at a 100% concentration. Based on standard categorization, all tested concentrations produced inhibition zones exceeding the 20 mm threshold, classifying them as possessing "very strong" antibacterial activity.

Tabel 4: Analysis of Relationship (One-Way ANOVA Test)

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	99,583	3	33,194	38,486	0,000
Within Groups	17,250	20	0,862		
Total	116,833	23			

Based on Table 4, there was a significant relationship between the concentration of the sappan wood decoction and the resulting diameter of the antibacterial inhibition zone ($p = 0.000$).

DISCUSSION

This finding indicates that the concentration level of the *Caesalpinia sappan* L. aqueous decoction directly influences its antibacterial efficacy against *Staphylococcus aureus*. Higher concentrations of the extract yielded progressively larger zones of inhibition, demonstrating a dose-dependent response where even the lowest tested concentration (70%) was sufficient to produce a very strong inhibitory effect.

This may occur because the sappan wood decoction contains potent bioactive secondary metabolites, specifically flavonoids and tannins. Phytochemical screenings indicate that these compounds function as aggressive antibacterial agents. Flavonoids specifically inhibit bacterial

growth or induce cell death through several distinct mechanisms, including the disruption of bacterial cell membranes, inhibition of cell wall formation, interference with nucleic acid synthesis, and the prevention of toxin release (14). Furthermore, tannin compounds demonstrate significant antibacterial activity by damaging bacterial cell membranes and inducing the contraction of bacterial cell walls. This disrupts cellular permeability, preventing the bacteria from executing essential metabolic activities and effectively leading to cellular death (Sazali et al., 2024).

This result is consistent with previous studies which reported that sappan wood possesses strong, broad-spectrum antimicrobial properties. These findings align with research by Silviani and Handayani, which established that tannins are the primary active compounds present in boiled sappan wood water (11), as well as research by Duniaji and Nocianitri, which corroborated that sappan wood extract contains significant levels of flavonoids (6.02%) and anthocyanins (2.43%) serving as antimicrobials (15). Furthermore, the dose-dependent efficacy observed in this study mirrors an in vitro study conducted by Ni Made Ina Prasetiari on *Streptococcus mutans*. That research also proved that increasing the concentration of sappan wood extract (from 10% up to 50%) resulted in statistically significant increases in the inhibition zone diameter (8).

CONCLUSION

There was a significant relationship between the concentration of *Caesalpinia sappan* L. aqueous decoction and its antibacterial activity against *Staphylococcus aureus* ($p = 0.000$). All tested concentrations demonstrated very strong inhibitory effects, with the 70% concentration already proving highly effective.

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