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Antibacterial Activity of 70% Ethanol Extract of Red Hanjuang (*Cordyline fruticosa* L.) Leaves Against *Staphylococcus aureus*

Aktivitas Antibakteri Ekstrak Etanol 70% Daun Hanjuang Merah (*Cordyline fruticosa* L.) terhadap *Staphylococcus aureus*

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ABSTRACT

Productive cough is a common symptom of bacterial respiratory infections. Red hanjuang (*Cordyline fruticosa* L.) leaves have been traditionally used to relieve such conditions. This study aimed to standardize red hanjuang leaf plant material and evaluate the antibacterial activity of its 70% ethanol extract against *Staphylococcus aureus*. Phytochemical screening revealed the presence of flavonoids, saponins, tannins, polyphenols, quinones, steroids, monoterpenoids, and sesquiterpenoids. Antibacterial activity was tested using the well diffusion method at concentrations of 10%, 25%, 35%, and 50%, with minimum inhibitory concentration (MIC) tests ranging from 10% to 0.8%. Specific and non-specific standardization showed water-soluble extract at 34.593%, ethanol-soluble extract at 31.663%, moisture content at 4.67%, drying loss at 9.57%, total ash content at 7.70%, and acid-insoluble ash at 0.89%. The extract demonstrated strong antibacterial activity at all tested concentrations. The lowest active concentration was 10%, with an MIC value at 8%, forming an inhibition zone of 2.43 mm. These findings support the potential of red hanjuang leaves as an herbal antibacterial agent.

Kata kunci:
Antibakteri
Cordyline fruticosa L.
Ekstrak etanol
Obat herbal
Staphyloccous aureus

ABSTRAK

Batuk berdahak merupakan gejala umum dari infeksi bakteri pada saluran pernapasan. Daun hanjuang merah (*Cordyline fruticosa* L.) secara tradisional digunakan untuk meredakan kondisi tersebut. Penelitian ini bertujuan untuk menstandarisasi simplisia daun hanjuang merah serta mengevaluasi aktivitas antibakteri ekstrak etanol 70%-nya terhadap *Staphylococcus aureus*. Skrining fitokimia menunjukkan adanya flavonoid, saponin, tanin, polifenol, kuinon, steroid, monoterpenoid, dan seskuiterpenoid. Uji aktivitas antibakteri dilakukan dengan metode difusi sumur pada konsentrasi 10%, 25%, 35%, dan 50%, sedangkan penentuan Konsentrasi Hambat Minimum (KHM) dilakukan pada konsentrasi 10% hingga 0,8%. Standarisasi spesifik dan nonspesifik menunjukkan kadar ekstrak larut air sebesar 34,59%, larut etanol 31,66%, kadar air 4,67%, susut pengeringan 9,57%, kadar abu total 7,70%, dan kadar abu tak larut asam 0,878%. Ekstrak menunjukkan aktivitas antibakteri yang kuat pada semua konsentrasi yang diuji. Konsentrasi terendah yang masih aktif adalah 10%, dengan nilai KHM sebesar 8% dan diameter zona hambat 2,43 mm. Hasil ini mendukung potensi daun hanjuang merah sebagai agen antibakteri herbal.

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1. INTRODUCTION

Coughing is a reflex response triggered by irritation in the lungs or respiratory tract, often caused by infections from microorganisms such as viruses, bacteria, or fungi (Sari et al., 2022; Hidayatuloh & Suharsono, 2023; Lazamidarmi et al., 2021). In studies involving patients with productive sputum, particularly in individuals with HIV or cystic fibrosis, several bacterial species have been identified, including *Staphylococcus aureus*-, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Enterobacter* spp. (Kamara et al., 2024; Naqvi et al., 2024).

Staphylococcus aureus is a Gram-positive bacterium commonly found in the environment and as part of the human microbiota, particularly on the skin, in the upper respiratory tract, axillae, and intestines, with colonization rates ranging from 20% to 30% (Indiyen et al., 2020; Howden et al., 2023). As an opportunistic pathogen, *S. aureus* can cause severe infections and significantly contributes to global morbidity and mortality. The increasing resistance of *S. aureus* to antibiotics poses a major challenge to effective treatment and highlights the urgency of finding alternative therapies (Rahman et al., 2023).

Herbal medicines are gaining renewed interest due to their accessibility, affordability, and relatively low side-effect profiles. In Indonesia, traditional herbal remedies are widely used across generations (Grenvilco et al., 2023). One such plant is red hanjuang (*Cordyline fruticosa* L.), a member of the Asparagaceae family, which has shown various pharmacological properties. Traditionally, red hanjuang leaves have been used to treat hemoptysis in tuberculosis patients (Dalimartha, 2007) and have demonstrated antioxidant, antidiarrheal, wound-healing, cholesterol-lowering, and anti-obesity effects (Sahara et al., 2021).

The leaves of *Cordyline fruticosa* L. contain secondary metabolites such as tannins, phenols, flavonoids, alkaloids, and saponins, which have potential antibacterial properties (Bogoriani et al., 2021). Prior studies have indicated significant antibacterial effects of ethanol and methanol extracts of red hanjuang leaves against *S. aureus* (Indiyen et al., 2020; Bogoriani et al., 2021). However, studies investigating the antibacterial activity of the 70% ethanol extract of red hanjuang leaves using the well diffusion method are limited.

This study aims to examine the antibacterial effect of the 70% ethanol extract of red hanjuang leaves against *Staphylococcus aureus* using the well diffusion method. The outcomes are expected to provide a scientific foundation for the potential use of red hanjuang as an herbal antibacterial agent for respiratory tract infections.

2. METHODS

2.1. Materials and Equipment

The study utilized red hanjuang (*Cordyline fruticosa* L.) leaves from Tasikmalaya, West Java, identified by the Center for Research and Development of Medicinal Plants and Traditional Medicine (ID: TL.02.04/D.XI.6/2917.117/2025). Other materials included *Staphylococcus aureus* (ATCC-6538 PK/5), 70% ethanol, amoxicillin, dimethyl sulfoxide (DMSO), 0.9% NaCl, and chloral hydrate. Equipment used included a laminar airflow cabinet, analytical balance, rotary evaporator, autoclave, water bath, incubator, microscope, oven, blender, micropipette, and standard glassware.

2.2. Plant Collection and Extract Preparation

The leaves were sorted, washed, chopped, and sun-dried under a black cloth. The dried leaves were ground into powder and extracted by maceration using 70% ethanol for 72 hours, with daily solvent replacement (Utami, 2021). The extract was filtered, concentrated using a rotary evaporator, and thickened using a water bath at 40°C.

2.3. Standardization and Phytochemical Analysis

Macroscopic and microscopic analyses, along with water- and ethanol-soluble extract tests, were conducted as specific parameters. Non-specific parameters included moisture content, drying loss, total ash, and acid-insoluble ash. Phytochemical screening identified flavonoids, alkaloids, polyphenols, tannins, saponins, steroids, triterpenoids, sesquiterpenes, and monoterpenes. Procedures followed the Indonesian Herbal Pharmacopoeia (2nd ed.) and Materia Medica Indonesia (5th ed.).

2.4. Antibacterial Assay

Preparation and Inoculation. All equipment and media were sterilized at 121°C for 15 minutes. Positive and negative controls were prepared using amoxicillin (2.0178 mg/mL in 10% DMSO) and 10% DMSO, respectively. Extract concentrations of 10%, 25%, 35%, and 50% were prepared in 10% DMSO. Nutrient agar was prepared and poured into Petri dishes. *S. aureus* was inoculated onto slant agar and incubated at 37°C for 24 hours. A suspension was prepared using 0.9% NaCl and adjusted to McFarland standard 0.5.

Diffusion Method and MIC Testing. Agar wells were filled with 50 µL of each extract concentration and control. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured with a vernier caliper (Alouw et al., 2022). MIC testing used extract concentrations from 10% to 0.6%, with the MIC defined as the lowest concentration showing inhibition.

2.5. Data Analysis

Statistical analysis was conducted using SPSS. Normality and homogeneity tests preceded one-way ANOVA ($\alpha = 0.05$), and Tukey's HSD test was used to assess significant differences between treatments.

3. RESULTS AND DISCUSSION

3.1. Preparation and Yield of Red Hanjuang Leaf Extract

A total of 3.50 kg of fresh red hanjuang leaves were collected and processed to remove impurities. The leaves were dried and ground into 847.00 g of powder. Maceration using 70% ethanol (1:5 ratio) yielded 134.07 g of extract, corresponding to a 26.81% yield, which is substantially higher than the 16.26% reported by Wahyuningsih et al. (2023), indicating more efficient extraction and potentially higher levels of active compounds (Maesaroh et al., 2021).

3.2. Standardization and Phytochemical Characteristics

Macroscopically, the leaves were lanceolate (~30 cm), purplish red with pinnate veins and wavy edges. Microscopic analysis revealed key identifiers such as collenchyma tissue, actinocytic stomata, needle-shaped calcium oxalate crystals, parenchyma, star-shaped trichomes, and upper epidermis structures (**Figure 1**), confirming the botanical identity.

The specific standardization revealed that the water-soluble extract content was 34.59% while ethanol-soluble content was 31.66%, indicating a predominance of polar compounds (**Table 1**). For non-specific parameters, moisture content was 5.67%, loss on drying was 9.57%, total ash content was 7.70%, and acid-insoluble ash was 0.88% (**Table 2**). All these values complied with acceptable pharmacopoeial standards—e.g., moisture content and drying loss below 10%, total ash below 16.60%, and acid-insoluble ash under 2% (BPOM RI, 2019; Kemenkes RI, 2017; MMI, 1989).

Phytochemical screening indicated the presence of several secondary metabolites (**Table 3**): flavonoids, saponins, tannins, polyphenols, quinones, steroids, and sesquiterpenes/monoterpenes. Alkaloids and triterpenoids were not detected. The presence of these compounds supports the extract's bioactivity. Flavonoids and polyphenols function by disrupting the bacterial membrane and cytoplasmic structures (Guli et al., 2024; Monica et al., 2024), while saponins enhance membrane permeability (Pratiwi et al., 2024), and tannins and quinones interfere with enzymatic activity and structural proteins (Purba et al., 2020; Novitarini et al., 2024).

3.3. Antibacterial Activity and MIC Evaluation

The antibacterial activity against *S. aureus* was tested using the well diffusion method. The extract at concentrations of 10%, 25%, 35%, and 50% produced inhibition zones of 13.09 mm, 14.12 mm, 14.95 mm, and 16.53 mm, respectively (**Table 4**). The positive control, 1% amoxicillin, showed a zone of 12.87 mm, while the negative control (10% DMSO) showed no activity (**Figure 2**).

One-way ANOVA analysis indicated statistically significant differences among treatments ($p = 0.000$). Post-hoc analysis using Tukey's HSD test revealed that the 10% extract concentration was statistically similar to the positive control ($p > 0.05$), suggesting comparable antibacterial effects. In contrast, the 25%, 35%, and 50% concentrations were significantly different from both the control and each other.

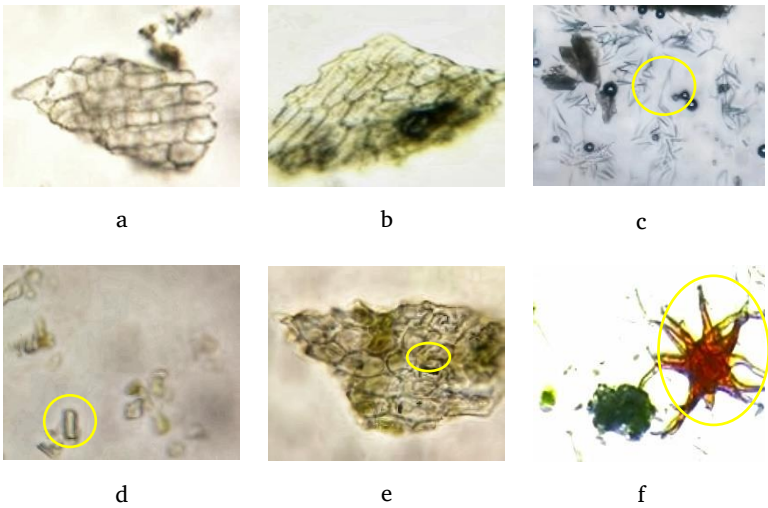

Figure 1. Microscopic observation of red hanjuang leaf powder at 100x magnification. a. Parenchyma; b. Upper epidermis; c. Needle crystal; d. Calcium oxalate crystal; e. Collenchyma with actinocytic stomata; f. Star-shaped trichomes.

Table 1. Results of specific standardization of red hanjuang leaves powder.

Parameter	I	II	III	Mean ± SD
Water-soluble extract (%)	33.16	33.59	37.03	34.59 ± 2.12
Ethanol-soluble extract (%)	29.93	31.68	32.38	31.33 ± 1.26

Table 2. Results of non-specific standardization of red hanjuang leaves powder.

Parameter	I	II	III	Mean ± SD	Reference
Moisture content (%)	5.00	6.00	6.00	5.67 ± 0.58	≤ 10% (BPOM RI, 2019)
Drying loss (%)	9.54	9.54	9.64	9.57 ± 0.06	< 10% (Kemenkes RI, 2017)
Total ash content (%)	7.55	7.76	7.79	7.70 ± 0.13	≤ 16.60% (Kemenkes RI, 2017)
Acid-insoluble ash content (%)	0.82	0.83	0.99	0.88 ± 0.10	< 2% (MMI, 1989)

Table 3. Phytochemical screening of red hanjuang leaves powder and extract.

Compound	Powder	Extract
Flavonoid	+	+
Alkaloid	–	–
Saponin	+	+
Tannin	+	+
Polyphenol	+	+
Quinone	+	+
Steroid	+	+
Triterpenoid	–	–
Sesquiterpene/Monoterpene	+	+

Note: + = detected, - = not detected

Table 4. Antibacterial activity of red hanjuang leaf extract.

Concentration (%)	Inhibition Zone (mm)
Positive control	12.87 ± 0.17
Negative control	0.00
10	13.09 ± 0.35
25	14.12 ± 0.19
35	14.95 ± 0.13
50	16.53 ± 0.23

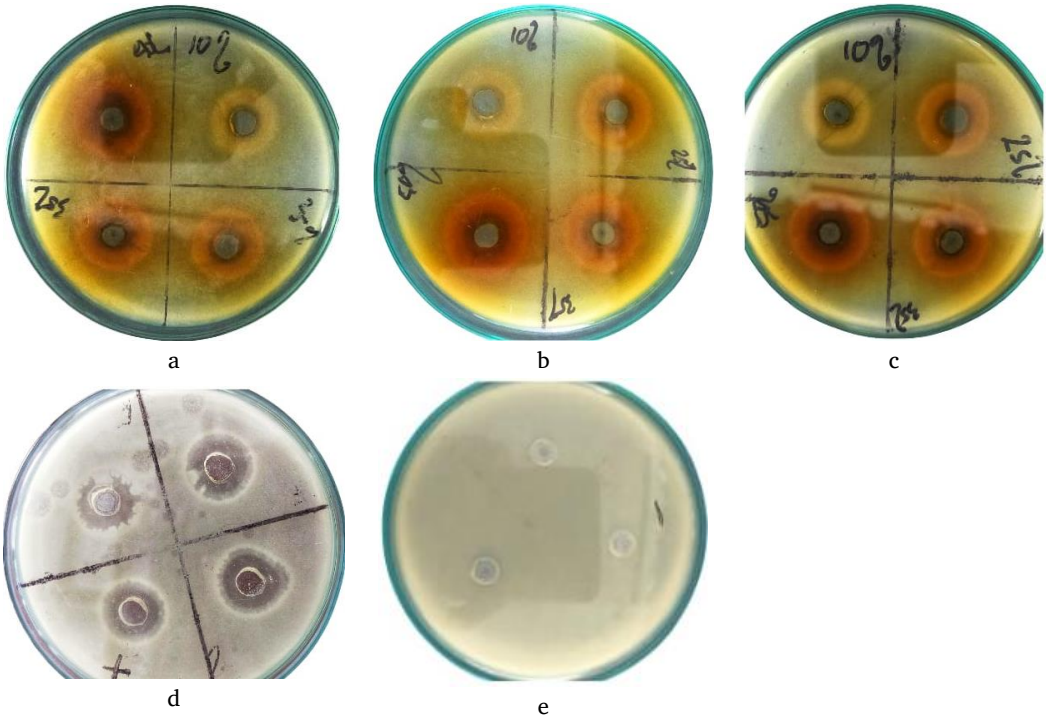

Figure 2. Antibacterial activity test results using the well diffusion method. Petri dishes show clear inhibition zones: (a-c) 10% to 50% extract; (d) positive control (amoxicillin); (f) negative control (DMSO).

The MIC test demonstrated that antibacterial activity of the red hanjuang leaf extract persisted down to 0.80%, producing an inhibition zone of 2.43 mm (**Table 5**). At 1% concentration, the inhibition zone measured 5.00 mm, which was categorized as weak, while at 2% the inhibition zone increased to 6.45 mm, considered moderate activity. From 3% to 9%, the inhibition zones gradually expanded (6.73–11.72 mm), and at 10% the extract produced a 13.09 mm zone, indicating strong activity. Below 0.80% (0.70% and 0.60%), no inhibition was observed (**Figure 3**). Based on Davis and Stout's (1971) classification, these results confirm a clear dose-dependent relationship, with higher concentrations producing progressively stronger inhibition. The MIC value of 0.80% highlights the relatively high potency of the extract compared to other plant-derived antibacterials reported in previous studies. Importantly, although inhibition zones at lower concentrations were smaller than the positive control, the 10% concentration was statistically equivalent to 1% amoxicillin, demonstrating the therapeutic potential of the extract at practical concentrations.

Table 5. MIC determination of red hanjuang leaf extract.

Concentration (%)	Inhibition Zone (mm)
10	13.09 ± 0.35
9	11.72 ± 0.41
8	11.40 ± 0.21
7	10.36 ± 0.65
6	9.43 ± 0.16
5	9.20 ± 0.38
4	7.89 ± 0.61
3	6.73 ± 0.14
2	6.45 ± 0.08
1	5.00 ± 0.16
0.9	3.97 ± 0.15
0.8	2.43 ± 0.34
0.7	0.00
0.6	0.00

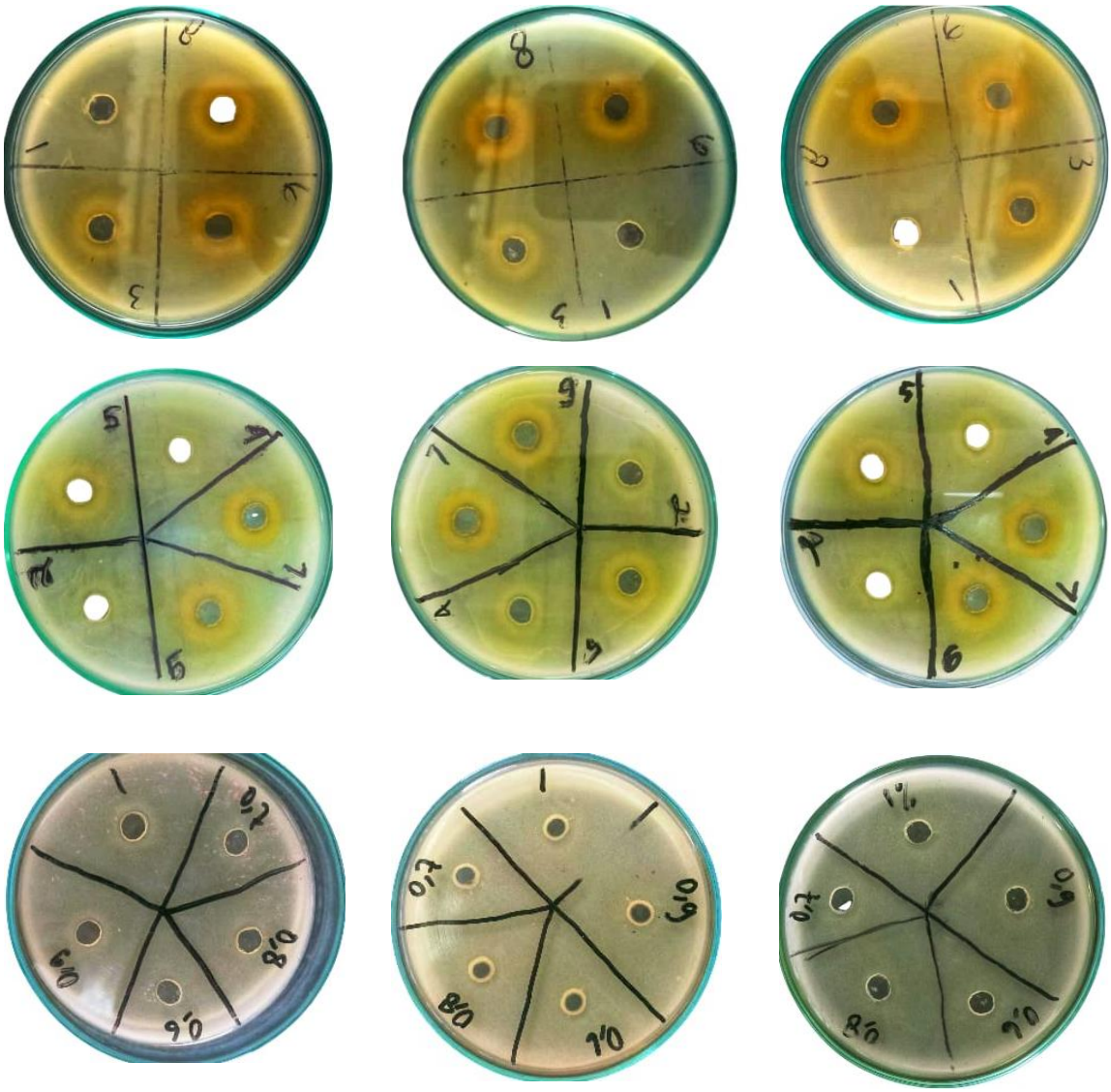

Figure 3. Minimum inhibitory concentration (MIC) test result of the ethanolic extract.

4. CONCLUSION

The 70% ethanol extract of red hanjuang (*C. fruticosa*) leaves met pharmacognostic quality standards and contained secondary metabolites with antibacterial potential. The extract demonstrated strong antibacterial activity against *S. aureus* at all tested concentrations, with an inhibition zone at 10% comparable to amoxicillin. The Minimum Inhibitory Concentration (MIC) was established at 0.80%. These findings confirm the potential of red

hanjuang leaf extract as a natural antibacterial candidate against *S. aureus*.

AUTHOR CONTRIBUTIONS

Conceptualization, VN; methodology, IF & VN; software, IF; validation, IF & DSZ; formal analysis, IF & VN; investigation, IF, VN & DSZ; resources, VN & IF; data curation, IF & VN; writing—original draft, IF; writing—review and editing, IF, VN & DSZ; visualization, IF; supervision, VN & DSZ; project administration, VN & IF; funding acquisition, VN & IF. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study did not involve human participants or animal subjects. All experiments were conducted *in vitro* using bacterial cultures (*Staphylococcus aureus* ATCC-6538).

INFORMED CONSENT STATEMENT

Not applicable. This study did not involve human participants.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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