ORIGINAL ARTICLE

Antibacterial Activity of Defatted Ethanolic Extract of *Cordyline fruticosa* (L.) A. Chev. Leaves Against Respiratory Pathogen

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ABSTRACT

Red hanjuang (*Cordyline fruticosa* L.) leaves are traditionally used to treat phlegmy coughs, that a symptom of respiratory tract infections, but scientific evidence is still very limited. Therefore, this study examined the antibacterial activity of *Staphylococcus aureus*, one of the causes of respiratory tract infections, which is part of the scientific evidence for the development of traditional medicine. Previous studies have reported that this bacterium can be inhibited by several polar compounds, such as saponins, tannins, polyphenols, and flavonoids, so a defatting process was carried out to maximize the extraction of these compounds. The standardized crude drug was defatted with n-hexane and then macerated using 70% ethanol. Phytochemical tests were performed on the crude drug and its extracts. The antibacterial activity of the extracts (5%, 20%, 35%, and 50%) was evaluated using the agar well diffusion method, while the minimum inhibitory concentration was determined in the range of 0.5% to 5%. The phytochemical analysis revealed the presence of flavonoids, polyphenols, tannins, saponins, quinones, steroids, monoterpenoids, and sesquiterpenoids. All tested concentrations exhibited concentration-dependent antibacterial activity. The lowest inhibitory concentration observed in the diffusion assay was 0.7%, with an inhibition zone of 1.828 mm. The conclusion is defatted 70% ethanolic extract of *C. fruticosa* leaves demonstrated significant antibacterial activity against *S. aureus* in an *in vitro* diffusion system, supporting its potential as a promising plant-derived antibacterial preparation for further pharmacological development.

Keywords: *Cordyline fruticosa*; defatted ethanolic extract; antibacterial activity; *S. aureus*; agar well diffusion

Introduction

Acute respiratory tract infections (ARTIs) remain a global health burden, with *S. aureus* being one of the main pathogens causing pneumonia and bronchitis, especially in vulnerable populations such as immunocompromised patients [1]. The prevalence of *S. aureus* in respiratory infections can reach 20–35% of sputum isolates, with an increasing proportion of methicillin-resistant isolates (MRSA) complicating conventional antibiotic therapy [2,3]. Global studies show that *S. aureus* is an important cause of severe lower respiratory tract infections and is often associated with multidrug-resistant isolates, requiring the development of new therapeutic alternatives [4].

In Indonesia, traditional herbal medicine is still widely used, especially to treat coughs with phlegm and respiratory tract infections. *C. fruticosa*, a traditional medicinal plant in West Java, is empirically used by the people of Tasikmalaya to treat coughs with phlegm caused by respiratory infections. *C. fruticosa* leaves contain many secondary metabolites, including flavonoids, polyphenols, tannins, saponins, and quinones, which are pharmacologically known to act as antibacterial agents by damaging cell membrane integrity and inhibiting essential bacterial enzymes [5,6,7]. Preliminary studies indicate that crude ethanol extracts of *C. fruticosa* leaves can inhibit the growth of *S. aureus* with an

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inhibition zone of 8–16 mm at concentrations of 10–50% [8,9]. However, to date, there has been no systematic study evaluating the antibacterial activity of purified ethanol extracts of *C. fruticosa* leaves against *S. aureus* as a respiratory tract pathogen. Purification processes, such as solvent-based fractionation, are known to reduce non-polar or interfering compounds and may enhance the relative concentration of bioactive secondary metabolites, thereby improving extract selectivity and potentially optimizing antibacterial evaluation [10,11,12].

In addition, the morphological characteristics of red hanjuang leaves, which exhibit a thick and glossy surface, suggest the presence of cuticular wax and other lipophilic constituents. Such waxy components may limit solvent penetration and reduce the extraction efficiency of polar and semi-polar bioactive compounds. Accordingly, a defatting step using n-hexane was introduced to remove non-polar surface constituents and enhance extraction selectivity toward antibacterial metabolites. Therefore, this study aims to evaluate whether defatting-assisted ethanol extraction can maintain antibacterial activity while improving extract selectivity.

METHODS

Materials

The materials used in this study included *C. fruticose* (red hanjuang) leaves, *S. aureus* (ATCC 6538 PK/5®), nutrient agar (Merck®, Germany), n-hexane (Bratachem®, Indonesia), 70% ethanol (OneMed®, Indonesia), amoxicillin (Hexpharm®), 10% dimethyl sulfoxide (DMSO) (Merck®, Germany), 0.9% NaCl (Otsuka®, Japan), chloroform hydrate (Merck®, Germany), and standard reagents for phytochemical testing.

Equipment

The equipment used included an analytical balance (OHAUS Pioneer PX, China), water bath (Memmert, Schwabach, Germany), rotary evaporator (IKA®, Germany), autoclave (Biobase, China), incubator (B-ONE, Indonesia), blender (Philips®, China), oven (Memmert®, Germany), laminar airflow (B-ONE, China), binocular microscope (Shinar XSZ-107, Indonesia), micropipette (Thermo Scientific, United States), and standard glassware (Pyrex®, United States).

Sample Preparation

C. fruticosa leaves were collected from Tasikmalaya Regency and identified in the certified herbarium of the Ministry of Health's research and product supply support facility, Sardjito Hospital, Yogyakarta, with specimen number TL.02.04/D.XI.6/2917.115/2025. The dried leaves were ground into a powder. A defatting process was then carried out using n-hexane at a 1:5 ratio. After defatting, the residue was dried and extracted by maceration with 70% ethanol at the same ratio (1:5). The maceration was repeated three times to obtain optimal results. The filtrate was concentrated by rotary evaporation, yielding a concentrated extract.

Phytochemical Analysis

The presence of phytochemical compounds such as alkaloids, polyphenols, flavonoids, tannins, saponins, quinones, steroids, triterpenoids, monoterpenoids, and sesquiterpenoids was assessed through qualitative screening. Detection was carried out using standard reagents, with color changes serving as indicators of compound presence [13].

Antibacterial Activity Assay

A diffusion-based minimum inhibitory concentration screening was performed using the well agar diffusion method. Defatted ethanolic extracts were prepared at serial concentrations ranging from 0.5% to 5% (w/v) using 10% DMSO as the solvent. A standardized bacterial suspension of *S. aureus* was adjusted to a 0.5 McFarland standard and spread evenly onto sterile Nutrient Agar plates. Wells were filled with 50 µL of each extract concentration and incubated at 37 °C for 24 hours.

A stepwise concentration reduction approach was applied in the agar well diffusion assay to determine the lowest concentration that produced a visible inhibition zone. Extract concentrations were gradually reduced to below 1% until no inhibition zone was observed. The lowest concentration producing a clear inhibition zone was recorded as the minimum inhibitory concentration. in the diffusion system. All tests were performed in triplicate [14].

Data Analysis

Data were expressed as mean ± standard deviation (SD). Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Differences among groups were analyzed

using one-way ANOVA. Statistical significance was set at $p < 0.05$.

RESULT AND DISCUSSION

Extraction Results

The extraction of *C. fruticosa* leaves was carried out by defatting with n-hexane, followed by maceration using 70% ethanol. The defatting process is carried out to remove non-polar constituents such as lipids, chlorophyll, and waxes that can interfere with the extraction of targeted bioactive compounds [13]. The removal of these compounds increases the selectivity and efficiency of subsequent extraction processes, particularly for polar and semi-polar secondary metabolites. The resulting extract was concentrated by evaporation, resulting in an extract yield of 26.93%. These findings indicate that the extraction and purification methods employed were effective in producing bioactive compounds.

Phytochemical Analysis

Phytochemical screening of ethanol extracts and crude drugs was positive for polyphenols, flavonoids, tannins, saponins, quinones, steroids, monoterpenoids, and sesquiterpenoids **(Table 1)**. These compounds specifically contributed to the observed inhibition of *S. aureus*. Flavonoids and tannins bind to *S. aureus* cell wall proteins, causing membrane damage and ion leakage, with a typical MIC of 50 - 200 µg/mL against MRSA. Quercetin and condensed tannins from medicinal plant leaves have been reported to increase *S. aureus* membrane permeability 3–5-fold compared to crude extracts [15]. Saponins damage the lipid bilayer of the *S. aureus* cytoplasmic membrane, forming steroid-saponin complexes that cause cell lysis at concentrations of 100–500 µg/mL. This activity is synergistic with flavonoids in the extract [16,17,18]. Quinones inhibit enzymes. Topoisomerase II and DNA gyrase in *S. aureus*, thereby impairing bacterial DNA replication. 1,4-naphthoquinone from medicinal plants exhibits bacteriostatic activity at 25–100 µg/mL against clinical strains [19,20,21]. Steroids disrupt the synthesis of *S. aureus* membrane lipids and increase membrane fragility to saponins [22,23].

Table 1. Phytochemical Screening Simplicia and extract of Hanjuang leaves

Chemical compounds	Simplicia	Extract
Alkaloid	-	-
Polyphenol	+	+
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Quinon	+	+
Steroid	+	+
Triterpenoid	-	-
Monoterpenoid	+	+
Sesquiterpenoid	+	+

Note: (+) detected; (-) not detected

Antibacterial Activity Test

The agar well diffusion assay demonstrated that the defatted ethanolic extract exhibited antibacterial activity against *S. aureus* at all tested concentrations (5–50%). As shown in **Table 2**, the inhibition zone diameter increased consistently with increasing concentration, indicating a clear dose–response relationship. At 20%, the inhibition zone (12.366 ± 0.265 mm) was comparable to that produced by 1% amoxicillin (12.826 ± 0.17 mm), while at 35% and 50%, the inhibition zones exceeded that of the positive control. Statistical analysis revealed significant differences among treatment groups ($p < 0.05$), and the consistent concentration-dependent pattern supports the biological relevance of these findings within the diffusion assay system. Comparable

Table 2. Antibacterial Activity of Cordyline fruticosa Leaf Defatted Extract Against Staphylococcus aureus

Treatment	Inhibition Zone (mm) Mean ± SD
Extract 50%	16.450 ± 0.025^{a}
Extract 35%	15.383 ± 0.095^{b}
Positive control (C+)	12.826 ± 0.170^{c}
Extract 20%	12.366 ± 0.265^{c}
Extract 5%	10.293 ± 0.074^{d}

Notes: Values are expressed as mean ± SD (n = 3). Different superscript letters indicate significant differences ($p < 0.05$) according to LSD post hoc test after one-way ANOVA.

Table 3. Inhibition zone diameter of defatted ethanolic extract at minimum concentrations (Agar Diffusion Assay)

Extract Concentration (%)	Mean ± SD (mm)	Category
5	10.250 ± 0.13	Strong
4	8.470 ± 0.15	Moderat
3	7.690 ± 0.09	Moderat
2	4.278 ± 0.24	Weak
1	2.550 ± 0.03	Weak
0.9	2.270 ± 0.09	Weak
0.8	2.128 ± 0.09	Weak
0.7	1.828 ± 0.07	Weak
0.6	-	No activity
0.5	-	No Activity

inhibition zones have been reported for ethanolic extracts of flavonoid-rich tropical plants, such as *Psidium guajava* and *Syzygium polyanthum*, where diffusion assays yielded inhibition diameters between 10–15 mm against *S. aureus* at higher extract concentrations [24,25]. This similarity suggests that the antibacterial potency observed in the present study falls within the range commonly reported for semi-purified plant fractions.

Nevertheless, direct comparison with amoxicillin should be interpreted with caution. Amoxicillin is a pure compound with a defined molecular structure, meaning that the entire 1% concentration represents an active antibacterial substance. In contrast, the ethanolic extract is a multi-component mixture composed of various secondary metabolites, of which only a proportion contributes to antibacterial activity. Although both samples are expressed in percentage units (% w/v), their chemical composition and proportion of active constituents are not pharmacologically equivalent. Furthermore, the diffusion assay is influenced by physicochemical factors such as molecular weight, compound solubility, and diffusion capacity within the agar matrix [14,26]. Therefore, the present results are more appropriately interpreted as evidence of significant and concentration-dependent antibacterial activity of the ethanolic extract against *S. aureus* in *an in vitro* diffusion system, with inhibition zone diameters at certain concentrations comparable to or greater than the positive control.

The lowest inhibitory concentration observed in the diffusion assay was 0.7%, indicating that the fraction could inhibit *S. aureus* growth at this concentration. The proportional increase in inhibition zone diameter with increasing concentration further supports a consistent biological response. In the context of plant-derived fractions, this finding reflects measurable antibacterial activity; however, precise pharmacological comparison would require expression in mass-based units (µg/mL). These results suggest that the ethanolic extract contains bioactive components capable of suppressing bacterial growth at defined concentrations, while its potency cannot be directly equated with that of pure antibiotics.

Compared with our previous study [8] using non-defatted ethanolic extract, the current defatted ethanolic

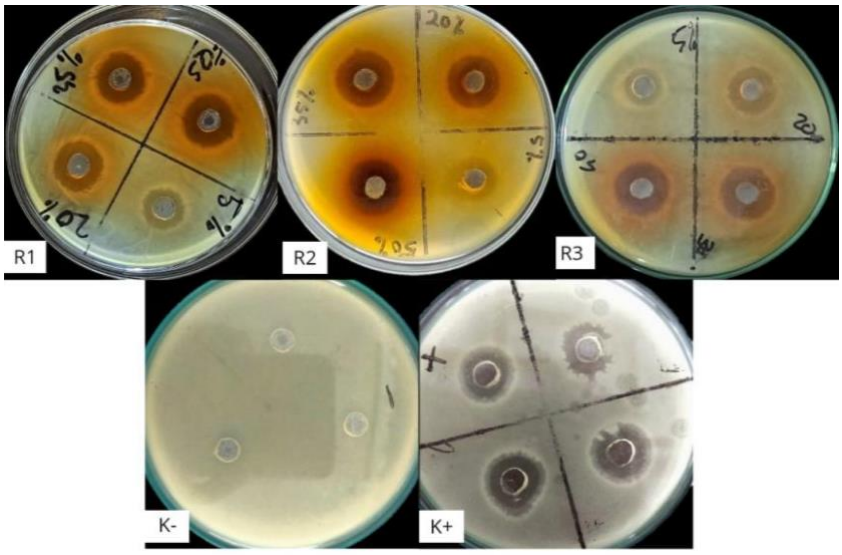

Figure 1. Inhibition zone diameter of defatted ethanolic extract at serial concentration (Agar Diffusion Assay)

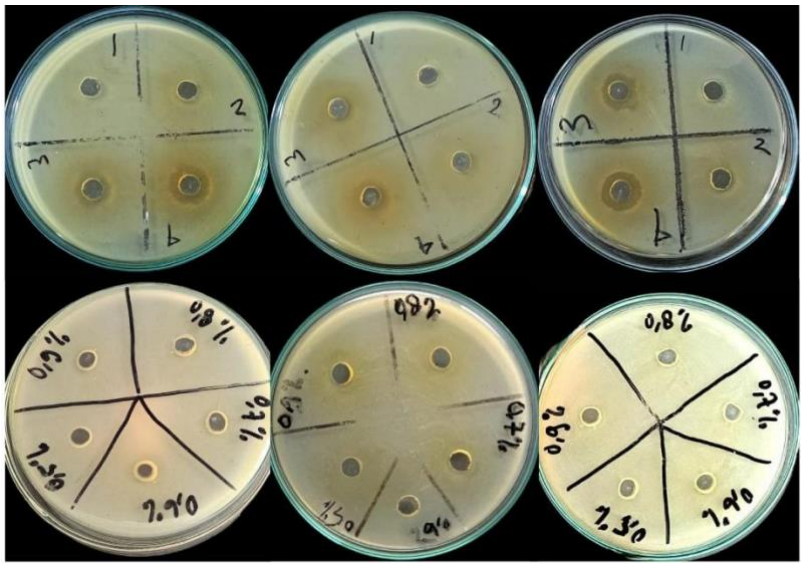

Figure 2. Inhibition zone diameter of defatted ethanolic extract at minimum concentrations (Agar Diffusion Assay)

extract still exhibited consistent antibacterial activity against *S. aureus* in diffusion assays, suggesting that the key antibacterial constituents are likely enriched in the polar–semi-polar fraction extracted by 70% ethanol. However, confirmation of enrichment requires quantitative phytochemical profiling and mass-based potency evaluation.

The antibacterial effect is likely associated with the presence of flavonoids, phenolics, saponins, and alkaloids. Flavonoids have been reported to disrupt bacterial membrane integrity through interactions with phospholipid bilayers and to inhibit essential enzymes such as DNA gyrase and topoisomerase IV [27,28]. Phenolic compounds induce protein denaturation and increased membrane permeability, leading to leakage of intracellular contents [29]. Alkaloids have been shown to interfere with peptidoglycan synthesis and bacterial cell division [30]. In Gram-positive bacteria such as *S. aureus*, the thick peptidoglycan layer without an outer lipopolysaccharide membrane facilitates interaction of hydrophilic and semi-polar compounds with cellular targets compared to Gram-negative bacteria [31]. Additionally, the multi-component nature of plant fractions may promote synergistic multi-target inhibition, thereby enhancing antibacterial activity even when individual compounds are present at relatively low concentrations [32]. Overall, the defatted 70% ethanolic extract of *C. fruticosa* leaves demonstrates significant concentration-dependent antibacterial activity supported by plausible biological mechanisms.

CONCLUSION

The defatted 70% ethanolic extract of *C. fruticosa* leaves demonstrated significant antibacterial activity against *S. aureus* in an agar diffusion assay, with inhibition zone diameters increasing proportionally to concentration. The lowest concentration capable of producing a visible inhibition zone was 0.7%, indicating measurable diffusion-based inhibitory activity. Compared with previous studies using non-defatted extracts, the present findings indicate that defatting maintained comparable antibacterial potency while potentially enhancing the selective enrichment of bioactive secondary metabolites. These results support the potential of the defatted ethanolic extract as a bioactive antibacterial preparation, although further quantitative phytochemical characterization and standardized dilution-based assays are required to confirm enrichment and pharmacological equivalence.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest related to this study.

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