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ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF RED ANDONG LEAVES (*Cordyline fruticosa* (L.) A.Chev.) AGAINST *Bacillus cereus FNCC-0059*

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ABSTRACT

Introduction: *Cordyline fruticosa* (L.) A.Chev. (red andong) has long been utilised in traditional ethnopharmacological practices by several indigenous communities, including the Dayak and Tolaki Mekongga tribes. The leaves are reported to contain various bioactive secondary metabolites, including flavonoids, tannins, saponins, and triterpenoids, which are associated with antibacterial activity. **Objective:** This study aims to identify the phytochemical content and antibacterial activity of the ethanol extract of red andong leaves (*Cordyline fruticosa* (L.) A. Chev.) against *Bacillus cereus*. **Methods:** This laboratory experimental study involved plant identification, sample collection, ethanol maceration extraction, phytochemical screening, and evaluation of antibacterial activity. Antibacterial activity against *B. cereus* was determined using the agar well diffusion method. The ethanolic leaf extract was tested at concentrations of 30%, 60%, and 90%, with azithromycin 1% serving as the positive control and water for injection as the negative control. **Results:** Phytochemical screening of red andong leaf extract showed positive results for flavonoids, saponins, tannins, phenols, and triterpenoids. Red andong leaf extract had antibacterial activity against *Bacillus cereus* at concentrations of 30%, 60%, and 90%. *Mann-Whitney* analysis showed that all concentrations were significantly different from the negative control ($p \leq 0.05$), but there was no significant difference between concentrations. **Conclusion:** The ethanol extract of red andong leaves positively contained flavonoids, saponins, tannins, phenols, and triterpenoids. *Cordyline fruticosa* (L.) A.Chev. Leaf extract has antibacterial activity against Bacillus cereus FNCC-0059, with moderate to strong inhibition.

Keywords: *Cordyline fruticosa* (L.) A.Chev.; *Bacillus cereus*; Antibacterial activity; Agar well diffusion method; Phytochemical screening.

INTRODUCTION

Cordyline fruticosa (L.) A.Chev. (red andong), A member of the family *Asparagaceae*, it is widely distributed throughout tropical and subtropical regions, including Indonesia. The species has long been utilised in traditional medicine by various indigenous communities, particularly the Dayak and Tolaki-Mekongga tribes, where it is employed to treat a range of ailments. Such ethnopharmacological practices highlight the therapeutic potential of *C. fruticosa* and support its continued investigation as a source of bioactive compounds[1-2].

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Medicinal plants remain an important component of primary healthcare systems, especially in developing countries, where they are widely used because of their accessibility, affordability, and cultural acceptance. Furthermore, natural products derived from medicinal plants have made substantial contributions to the discovery and development of novel therapeutic agents, underscoring the importance of phytochemical and pharmacological investigations[3].

Previous studies have demonstrated a broad spectrum of pharmacological activities associated with *C. fruticosa*. A review by Tematio et al. (2023) reported that species in the *genus Cordyline*, particularly *C. fruticosa*, possess antidiarrheal, anti-inflammatory, antipyretic, antimicrobial, and wound-healing properties, supporting their traditional use for managing skin infections, rheumatism, and other inflammatory conditions[4]. In addition, Andani et al. (2023) reported that ethanol and ethyl acetate extracts of *C. fruticosa* leaves exhibited antibacterial activity against *Propionibacterium acnes* and *Streptococcus mutans*, producing inhibition zones of 12 mm and 9 mm, respectively, at a concentration of 400 µg. These findings indicate that *C. fruticosa* leaves possess promising antibacterial potential.

The pharmacological activities of *C. fruticosa* have been largely attributed to its diverse secondary metabolites. Phytochemical studies have identified flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids, compounds known to exhibit antimicrobial, antioxidant, and anti-inflammatory activities. These bioactive constituents are believed to contribute to the plant's therapeutic properties and support its potential as a natural source of antimicrobial agents[5].

Bacillus cereus is a Gram-positive, endospore-forming bacterium that is a significant cause of foodborne illness through the production of emetic toxin (cereulide) and enterotoxin, which cause emetic and diarrheal syndromes[6]. In addition to its role in food poisoning, B. cereus can also cause opportunistic infections that, under certain conditions, require antimicrobial therapy. However, increasing antimicrobial resistance in *Bacillus* species has encouraged the exploration of new antibacterial sources from natural sources as alternatives or complements to conventional therapies[7].

Although antibacterial activity testing of *C. fruticosa* has been conducted on several pathogenic bacteria, evidence regarding its activity against *B. cereus*, particularly using the reference strain FNCC-0059, is still limited. *B. cereus* FNCC-0059 was selected because it is a well-characterized reference strain commonly used for antimicrobial susceptibility testing. Therefore, this study aims to identify secondary metabolites and the antibacterial activity of the ethanol extract of red andong leaves against *B. cereus FNCC-0059* as a preliminary study to support the development of natural-based antibacterial agents. The results of this study are expected to enrich scientific evidence regarding the antibacterial potential of *C. fruticosa* against bacteria that cause foodborne diseases and provide initial data to support the development of natural materials as a source of antibacterial agents[8].

METHODS

1. Materials and Methods

The materials used in this study were *Cordyline fruticosa* (L.) powder, 70% ethanol, distilled water, *Bacillus cereus FNCC-0059* was obtained from AGAVI, nutrient agar, concentrated acetone, concentrated boric acid, concentrated oxalic acid, concentrated ether, *Dragendorff's*

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reagent, *Mayer's* reagent, chloroform, acetic anhydride, H_2SO_4, $K_2Cr_2O_7$, $FeCl_3$, hydrochloric acid (2N HCl), gelatin, NaCl, water for injection, and azithromycin 1%.

The equipment used in this study included a maceration vessel (glass jar), beakers (AGC IWAKI), a stirring rod, a 70-mesh sieve, filter cloth, a graduated cylinder (Herma), an analytical balance (ACIS), a vernier caliper (KENMASTER), sterile gauze, sterile cotton swabs, an autoclave (HIRAYAMA), a Laminar Air Flow cabinet (INNOTECT), an oven (B ONE), a desiccator, a rotary evaporator (IKA), an incubator (MEMMERT), a colony counter (FUNKE GERBER), a vortex mixer (OHAUS), Petri dishes, an inoculating loop, a Bunsen burner, test tubes (IWAKI PYREX), Erlenmeyer flasks (IWAKI), aluminum foil, parchment paper, a micropipette, a dropping pipette, a volumetric pipette, microscope slides, a microscope, and tissues.

2. Simplicia and Extract Preparation

Identification of the *Cordyline fruticosa* plant was conducted at the Microbiology and Botany Laboratory of BRIN in Cibinong, Bogor, West Java. Red andong leaves were harvested in the Mengwi area, fresh, ±1 year old. The herbal raw material was prepared at the Center for Postharvest Processing of Medicinal Plants (P4TO), Tabanan, Bali, Indonesia.

Red *Cordyline fruticosa* leaves were dried using a greenhouse dryer for 1 day, followed by drying in a blower oven at 50°C for 3 days until the desired moisture content was achieved. The dried plant material was then sorted, ground into powder, and sieved through a 70 mesh sieve to obtain a uniform particle size. The plant extraction process utilised the maceration method with 70% ethanol as the solvent. A total of 500 grams of *Cordyline fruticosa* leaf powder (simplicia) was mixed with 5,000 mL of 70% ethanol at a 1:10 ratio. The mixture was allowed to stand for 3 × 24 hours, with stirring performed every 12 hours. Subsequently, a flannel cloth was used to separate the extract from the solid residue. The collected filtrate was then concentrated using a rotary evaporator at 50°C, yielding a viscous extract[9, 10]. The characterization of the simplicial powder included organoleptic evaluation and moisture content determination, while the extract characterization comprised organoleptic evaluation and phytochemical screening.

3. Phytochemical Screening of the Extract

a. Alkaloids

1 g sample was placed in a test tube, then 5 mL of 2 N HCl was added, heated for a while, and cooled to room temperature. The resulting solution was then divided into three test tubes, each containing 1 mL. The first tube was used as a blank. *Mayer's* reagent was added to the second tube, while *Dragendorff's* reagent was added to the third tube. The test results were declared positive for alkaloids if a white precipitate formed in the tube containing *Mayer's reagent* and/or an orange precipitate in the tube containing *Dragendorff's reagent*[11].

b. Flavonoids

A 0.5 g sample of the extract was mixed with 10 mL of hot distilled water and boiled for 5 minutes, then filtered. 5 mL of the filtrate was transferred to a test tube, followed by the addition of 0.1 g of magnesium powder, 1 mL of concentrated hydrochloric acid (HCl), and 2 mL of amyl alcohol. The mixture was shaken vigorously and allowed to stand until phase separation

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occurred. The appearance of a red, orange, or yellow coloration in the amyl alcohol layer indicated the presence of flavonoids[12].

c. Triterpenoid and steroid

2 mL of the test solution is evaporated. The residue is dissolved in 0.5 mL of chloroform, then 0.5 mL of acetic anhydride is added. Then, 2 mL of concentrated sulfuric acid is added dropwise. The appearance of a brownish ring indicates a positive result for triterpenoids[12].

d. Phenols

1 mL of test solution is reacted with 5% $FeCl_3$ solution; a dark blue or greenish-black colour indicates the presence of Phenols[13].

e. Tannins

1mL of extract was taken and placed in a test tube. Then, a 1% gelatin solution containing sodium chloride was added and shaken. Appearance of a white precipitate indicates the presence of tannins[13].

f. Saponin

1 gram of extract is added to 10 mL of hot water in a test tube. The mixture is shaken for approximately 10 seconds, and foam forms to a height of 1-10 cm. The foam persists after adding one drop of 2 N HCl[12].

4. Antibacterial Activity Assay Against *Bacillus cereus*

a. Sterilization of Equipment and Materials

Sterilization methods were tailored to the specific equipment used. Non-volumetric glassware was sterilized by heating at 160°C for 1 hour. Metal needles and forceps were sterilized by heating them over a Bunsen burner. Subsequently, instruments were sterilized in an autoclave at 121°C and 1 atm pressure for 15 minutes[13].

b. Preparation of Nutrient Agar Medium

A total of 6.72 g of nutrient agar was dissolved in 240 mL of distilled water and sterilised by autoclaving at 121°C for 15 min. Approximately 20 mL of the sterilised medium was poured into each sterile Petri dish and allowed to solidify under aseptic conditions[14].

c. Bacterial Culture

A loopful of *Bacillus cereus* from a pure culture was streaked onto the culture medium using the streak plate method and incubated at 37°C for 24 h[15].

d. Preparation of Bacterial Suspension

A bacterial suspension was prepared by transferring *Bacillus cereus* colonies into 10 mL of sterile 0.9% NaCl solution. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard[16].

e. Preparation of the 0.5 McFarland Standard

The 0.5 McFarland standard was prepared by mixing 0.05 mL of 1% (w/v) $BaCl_2$ with 9.95 mL of 1% (v/v) H_2SO_4. The mixture was vortexed until homogeneous[17].

f. Preparation of Positive Control, Negative Control, and Concentration Variants

Water for injection was used as the negative control. Azithromycin 1% was used as the positive control.

Extracts were prepared at concentrations of 30%, 60%, and 90% by dissolving weighed amounts of the extract in water for injection.

g. Antibacterial Activity Test

Antibacterial activity was tested using the agar well diffusion method. Sterilized Nutrient Agar (NA) medium was inoculated with a *Bacillus cereus* bacterial suspension. Subsequently, wells with a diameter of 6 mm were created using a sterile cork borer and labelled according to the treatment. Test samples at various concentrations, along

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with positive and negative controls, were introduced into the respective wells. The Petri dishes were then incubated at 37°C for 24 hours. Following incubation, antibacterial activity was assessed by measuring the diameters of inhibition zones formed, using a Vernier calliper.

5. Data Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using *SPSS* software. Data normality was assessed using the *Shapiro-Wilk* test for sample sizes ≤50, while variance homogeneity was evaluated using *Levene's test*. Since the data were normally distributed but showed unequal variances, non-parametric analysis was performed using the *Kruskal-Wallis test*, followed by the *Mann-Whitney* test to determine significant differences among treatment groups, positive control, and negative control.

RESULTS

1. Extraction Yield of Simplicia

The extraction yield of the simplicia was determined to evaluate the efficiency of the extraction process. The percentage yield obtained is presented in Table 1.

Table 1. Extraction Yield of Simplicia

Measured Parameters	
Weight of Simplicia Powder (g)	500
Solvent (mL)	5.000
Weight of Extract (g)	303
Extract yield (% w/w)	60,6

2. Extract Characterization

Organoleptic evaluation of the dried plant material and its extract is a specific quality parameter used to assess and identify the material's physical characteristics through sensory observations, including shape, colour, odour, and taste. This evaluation is performed to verify the identity of the plant material, ensure batch-to-batch quality consistency, and facilitate early detection of deterioration, quality changes, or contamination that may affect the material's safety and efficacy. The results of the organoleptic evaluation are presented in Table 2.

Table 2. Organoleptic Characteristics of Simplicia and Extract

Parameter	Results
Simplicia	
Shape	Powder
Odor	Characteristic aromatic odor
Taste	Bitter
Color	Brownish green
Extract	
Consistency	Thick
Odor	Characteristic aromatic odor
Taste	Bitter
Color	Brownish green

3. Moisture Content Determination

The moisture content of the dried plant material was determined using the thermogravimetric method. This method is based on the evaporation of water present in the plant material through heating, followed by repeated weighing until a constant weight is achieved. The mass difference before and after drying was used to calculate the moisture content. Moisture content determination was performed on powdered red *Cordyline fruticosa* leaves in triplicate, and the results are presented in Table 3.

Table 3. Moisture Content Test Results of Simplicia

Rep.	Result(%)	Average(%)	Information
1	9.00	9.01	Condition <10% (Eligible)
2	9.00		
3	9.04		

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4. Phytochemical Screening of the Extract

Phytochemical screening is a qualitative analytical method used to detect the presence of functional groups or classes of secondary metabolites in plant samples. This analysis is based on the ability of these compounds to react with specific chemical reagents, resulting in characteristic colour changes or the formation of precipitates. *Cordyline fruticosa* has been reported to possess considerable potential as a medicinal plant owing to its diverse bioactive secondary metabolites. The results of the phytochemical screening are presented in Table 4.

Table 4. Phytochemical Screening Results of the Extract

Compound group	Extract
Flavonoids	+
Alkaloids	-
Saponins	+
Tannin	+
Triterpenoid	+
Steroid	-
Phenol	+

Description:+:positive for compound; -:no compound

5. Antibacterial Activity Assay

The antibacterial activity of the extract was evaluated using the agar well diffusion method. Antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the wells, which indicates the ability of the extract to inhibit bacterial growth. The results of the antibacterial activity assay are presented in Table 5.

DISCUSSION

Plant identification of *Cordyline fruticosa* (L.) A.Chev. was performed at the Botany-Microbiology Laboratory, National Research and Innovation Agency (BRIN), Cibinong, Bogor, West Java, Indonesia, and the specimen was authenticated on November 22, 2024.

Table 5. Antibacterial Activity Test Results of Red Andong Leaf Extract

Treatment	Rep.	Inhibition Zone Diameter (mm)	Average (mm)±SD	Category
Extract 30%	1	7,68	8,31 ± 1,64^{b}	Medium
	2	7,60		
	3	10,18		
Extract 60%	1	8,19	9,25 ± 0,96^{b}	Medium
	2	9,50		
	3	10,08		
Extract 90%	1	10,54	10,15 ± 0,91^{b}	Strong
	2	9,11		
	3	10,8		
Positive Control	1	23,61	23,16 ± 1,41^{a}	Very Strong
	2	24,20		
	3	21,58		
Negative Control	1	0	0 ± 0^{c}	None
	2	0		
	3	0		

Extract Leaf Andong Category: Weak < 5 mm; Medium 5-10 mm; Strong 10-20 mm; Very Strong >20 mm. ^{abc}Different superscript letters indicate significant differences ($p \leq 0.05$), while identical letters indicate no significant difference based on the *Mann-Whitney test* ($p<0.05$)

The identification confirmed that the plant material used in this study was *C. fruticosa* (L.) A.Chev. (Family: Asparagaceae). The leaf extract of *C. fruticosa* was prepared by maceration, a cold extraction technique performed at room temperature without heat. As shown in Table 1, the extraction yielded 60.6%. This extraction yield exceeded the minimum value generally considered acceptable for plant extracts (>10%)[19].

Specific standardisation based on organoleptic evaluation (Table 2) showed that the simplicial powder of *Cordyline fruticosa* (red *andong*) leaves exhibited a brownish-green colour, a powdery appearance, a characteristic aromatic odour, and a bitter taste. The viscous extract was

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brownish-green in colour, with a semi-solid consistency and a characteristic odour of red *andong* leaves.

Moisture content determination, as one of the non-specific standardisation parameters, was performed to assess the crude drug's water content. Moisture content is an important quality parameter because excessive water content may promote microbial growth and accelerate degradation, thereby affecting the stability and quality of herbal materials[20]. The results presented in Table 3 showed that the moisture content values complied with the requirements specified in the Indonesian Herbal Pharmacopoeia, with values not exceeding 10%[19].

Phytochemical screening of red andong leaf extract showed positive results for flavonoids, saponins, tannins, phenols, and triterpenoids. Table 4 presents the results of the phytochemical screening of *red andong leaf* extract. Phytochemical analysis of the ethanolic extract revealed the presence of flavonoids, as indicated by the formation of a red colouration in the amyl alcohol layer. Concentrated HCl facilitates the hydrolysis of flavonoid glycosides into their corresponding aglycones by cleaving O-glycosidic bonds, allowing the glycosyl group to be replaced by a hydrogen ion (H^+) from the acid. The addition of magnesium metal promotes the reduction of flavonoid compounds, while subsequent acidification with HCl results in the formation of a red-colored flavylium salt[21].

Screening results on the ethanol extract of red andong leaves indicated the presence of terpenoids, based on the formation of a brownish ring. Steroids were not detected due to the lack of a bluish-green color change. This indicates that the secondary metabolites identified in the extract belong to the triterpenoid group[22].

Saponin compounds produce foam due to their amphiphilic nature, possessing both hydrophilic (water-soluble) and hydrophobic (nonpolar) groups, which facilitate foam formation in an aqueous medium[23].

The alkaloid test on the ethanol extract of red andong leaves was negative based on the Mayer and Dragendorff reagent tests, as indicated by the absence of characteristic alkaloid precipitate formation. These results are in line with the research of Usman and Jariah (2023), who reported that the ethanol extract of red andong leaves did not show the presence of alkaloids through phytochemical screening using these specific reagents[24].

Tannin identification using gelatin solution and 10% NaCl showed a positive reaction, as evidenced by the formation of a white precipitate resulting from the interaction between tannin compounds and gelatin, which produced an insoluble complex[25]. The phenol test confirmed the presence of phenolic compounds, as indicated by the formation of a greenish-black colouration[26].

In this study, antibacterial activity was evaluated against *Bacillus cereus*. The antibacterial activity of the extract was assessed using the agar well diffusion method. This method involves forming wells in agar medium that has been previously inoculated with the test microorganism[14]. Using *Bacillus cereus* FNCC-0059 as a test bacterium aims to obtain valid and reproducible test results. The standard strain has a verified identity and quality control, making it widely used in microbiological research and antibacterial activity testing. Nutrient agar was used as the growth medium because it provides essential nutrients to support the optimal growth of *Bacillus cereus*. Its stable gel structure also facilitates uniform diffusion of antibacterial compounds, allowing the formation of clear and measurable inhibition zones as an indicator of antibacterial activity[27,28].

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The ethanolic extract of red *andong* leaves was used as the test sample at three different concentrations (30%, 60%, and 90%). Azithromycin (1%) was used as the positive control. Azithromycin was used as the positive control because it is a macrolide antibiotic with broad-spectrum activity against Gram-positive bacteria, including *Bacillus cereus*. Its antibacterial activity results from binding to the 23S rRNA of the 50S ribosomal subunit, thereby inhibiting protein synthesis and suppressing bacterial growth. Using azithromycin as a positive control provides a reference for comparing the antibacterial efficacy of the extract against a clinically established antimicrobial agent. Furthermore, *B. cereus* has been reported to be susceptible to macrolide antibiotics, making azithromycin an appropriate comparator for evaluating the antibacterial potential of plant extracts [29, 30].

The results showed that all concentrations of red andong leaf ethanol extract exhibited inhibitory activity against the growth of *Bacillus cereus*. The diameter of the inhibition zone was measured using a caliper and expressed in millimeters (mm). As shown in Table 5, the red andong leaf extract exhibited antibacterial activity, as indicated by a clear inhibition zone around the wells, with a diameter varying with the extract concentration.

The 90% extract concentration produced the largest inhibition zone of all extract concentration variations, with an average diameter of 10.15 ± 0.91 mm, indicating strong antibacterial activity. Conversely, the 30% extract concentration showed the smallest inhibition zone, with an average diameter of 8.31 ± 1.64 mm, categorized as moderate antibacterial activity. The positive control, 1% azithromycin, demonstrated the strongest antibacterial activity, with an average inhibition zone diameter of 23.16 ± 1.41 mm, categorized as very strong. Meanwhile, the negative control (*water for injection*) showed no antibacterial activity, as no clear inhibition zone was observed around the wells.

Secondary metabolites present in red *andong* leaves, including flavonoids, saponins, and tannins, have been reported to exhibit antimicrobial activity. Flavonoids exert antibacterial effects through multiple mechanisms, including disruption of bacterial cell membranes and interaction with extracellular proteins, which may alter membrane permeability and impair cellular functions. Furthermore, hydroxyl groups in flavonoid structures can interact with bacterial DNA via intercalation, a process influenced by the compounds' physicochemical properties, thereby interfering with DNA replication and other cellular processes[31].

Saponins exhibit antibacterial activity primarily through protein denaturation and disruption of bacterial cell membranes. These compounds interact with membrane components, increasing membrane permeability, promoting leakage of intracellular constituents, and ultimately leading to bacterial cell death[32]. Tannins are polyphenolic compounds that can bind to and precipitate proteins, thereby inhibiting bacterial protein synthesis. In addition, tannins may inhibit key bacterial enzymes, including reverse transcriptase and DNA topoisomerase, which are involved in nucleic acid synthesis, replication, and bacterial growth[33].

The antibacterial activity test results showed that the ethanol extract of Cordyline fruticosa leaves inhibited the growth of Bacillus cereus FNCC-0059 at all concentrations tested (30%, 60%, and 90%). Based on the *Mann-Whitney analysis*, all extract groups showed significant differences compared to the negative control ($p \leq 0.05$), which indicated the antibacterial activity of the extract. The significant

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difference between the positive control and the extract group indicated that the extract activity was still lower than the standard antibiotic.

However, there was no significant difference between the extract concentration variations ($p>0.05$), indicating that increasing the extract concentration did not provide a significant increase in inhibitory activity against *B. cereus*. This may be due to the content of bioactive compounds in the extract not reaching an optimal concentration to produce a statistically different antibacterial effect.

CONCLUSION

The ethanol extract of red andong leaves positively contained flavonoids, saponins, tannins, phenols, and triterpenoids. Variations in extract concentrations showed antibacterial activity against *Bacillus cereus* at concentrations of 30%, 60%, and 90%, but increasing concentrations did not provide significant differences in inhibitory activity.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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