

Evaluation of Antibacterial Activity Test For Active Extract Of Red Palm Fruit (*Cyrtostachys renda* Blume) against *Staphylococcus aureus* and *Escherichia coli*

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Abstrak

**Article
Information**
Received:30/08/25
Accepted: 30/10/25

The two bacterial species most commonly associated with human illnesses are *Staphylococcus aureus* and *Escherichia coli*. The red palm (*Cyrtostachys renda* Blume) shows promise as a natural source of antibacterial agents due to its high concentration of metabolite compounds, which include phenolics, flavonoids, saponins, tannins, and terpenoids. Antibacterial activity was evaluated using the pitting diffusion method at concentrations of 92%, 46%, and 23%. The results indicated that the ethyl acetate extract exhibited the strongest antibacterial activity, with an inhibition zone measuring 26.93 mm against *Escherichia coli* and 20.50 mm against *Staphylococcus aureus* at the highest dose. Additionally, the aqueous extract demonstrated significant inhibition, while the *n*-hexane and dichloromethane extracts showed no antibacterial activity. The validity of the method was confirmed by using chloramphenicol as the positive control and 10% DMSO as the negative control. These findings suggest that red palm fruit has potential as a natural antibacterial agent derived from local Indonesian plants.

Keywords: [*Cyrtostachys renda* Blume, *Escherichia coli*, *Ethyl Acetate Extract*, *Staphylococcus aureus*, *Water Extract*]

Introduction

Infectious diseases are among the leading causes of death worldwide, and this is also true in Indonesia. According to the World Health Organization (WHO), infectious diseases were the primary cause of mortality among Indonesian children under the age of five in 2005. These diseases are caused by microorganisms, which include bacteria and fungi (Nursidika et al., 2014). The most common bacterial species responsible for human illnesses are *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* can lead to various conditions, including local abscesses, endocarditis, pharyngitis, pneumonia, meningitis, and empyema. *Escherichia coli*, which is naturally found in the human digestive tract, can become harmful when its numbers increase or when it exits the colon and produces enterotoxins, leading to diarrhea (Nurviana et al., 2018).

The rising prevalence of infectious diseases and the growing concern over antibiotic resistance are driving efforts to develop medications based on natural ingredients. Many people prefer pharmaceuticals derived from natural sources because they are believed to have milder side effects compared to chemical compounds, are generally more affordable, and are more readily available (Listyani et al., 2022). Red palm fruit (*Cyrtostachy renda* Blume) is one of the natural resources that can be used to make antibacterial products.

The Red Palm plant, also known as the red areca nut or lipstick palm, belongs to the Arecaceae family, commonly referred to as the palm tribe. This plant is native to the islands of Sumatra and Borneo and is characterized by the striking red color of its leaf stalks, mature fruits, and young roots (Heatubun, 2009). Additionally, the Red Palm is recognized for its potential as a natural antibacterial source, attributed to the presence of various antibacterial compounds. Preliminary research, including phytochemical screenings, reveals that red palm fruits contain significant amounts of phenolic compounds, flavonoids, saponins, terpenoids, steroids, alkaloids, and tannins (Syamsurizal et al., 2023).

Based on this information, it seems that red palm fruit may contain chemicals that act as antibacterial agents. However, there is currently limited research on the antibacterial properties of the components found in red palm fruit. Therefore, further studies are needed to fully understand the antibacterial effects of red palm fruit extract.

Materials and Methods

Tools and Materials

In this study, the red palm fruit was collected from the Buluran, Telanaipura area of Jambi City. The additional ingredients included methanol, distilled water, 0.9% sodium chloride (NaCl), dimethyl sulfoxide (DMSO), spiritus liquid, 70% antiseptic alcohol, and chloramphenicol (250 mg). The tools utilized in the experiment consisted of scissors, a tray, a cutting board, a knife, an oven, a hammer mill, dark glass bottles, an analytical balance, a rotary evaporator, glass funnels, Erlenmeyer flasks, filter paper, a measuring cup, beakers, aluminum foil, a vacuum device, a reflux apparatus, stirring rods, spatulas, a laminar airflow cabinet, an autoclave, petri dishes, volumetric flasks, a refrigerator, an incubator, a vernier caliper, an ose needle, a hot plate, a micropipette, a glass dropper, test tubes, vial bottles, tweezers, a vortex mixer, and a Bunsen burner.

Plant Sampling and Determination

The samples utilized in this investigation were red palm plants with fruits as the plant sections. Sampling was done in the vicinity of Buluran, Telanaipura area of Jambi City, Jambi Province. Plants were determined at Padjajaran University's Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences.

Simplicia Preparation

After harvesting 4.5 kg of red palm fruit, the sample was cleaned and air dried for 5 days before being dried in an oven at 60° C for 72 hours. The simplicia is then ground with a grinder until it is finely powdered and ready for extraction.

Red Palm Fruit Extraction

The maceration process was employed to extract 500 g of finely powdered simplicia, which was placed in a dark glass bottle and mixed with 5000 mL of *n*-hexane distillate solvent for 24 hours. The maceration continued for a total of 72 hours, with regular shaking. Following this, an additional 5000 mL of methanol distillate solvent was used for further maceration. The resulting macerate was then concentrated using a rotary evaporator to yield a thick extract of crimson palm fruit (Dewi, 2018). For additional extraction, a reflux process was implemented. A sample of 250 mg of palm fruit was placed in a round-bottom reflux flask, and methanol solvent was added until the entire sample was submerged. Reflux was conducted for 2.5 hours, with a maximum of three repetitions. The filtrate from the reflux extraction was then concentrated with a rotary evaporator to produce a thick reflux extract of red palm fruit (Hastuti & Mulangsri, 2021).

Liquid Extraction

The thick methanol extract of red palm fruit was placed in a separating funnel and mixed with distilled water in a 1:1 ratio. This mixture was then partitioned using various solvents based on a polarity gradient, starting with *n*-hexane, followed by ethyl acetate, dichloromethane, and water. The top layer, which was clear, was collected using a pipette. The same procedure was repeated for the ethyl acetate, dichloromethane, and water solvents. Finally, the extracted materials were evaporated using a rotary evaporator until a thick extract was obtained (Rollando, 2019).

Phytochemical Screening

Phytochemical screening involves analyzing various compounds, including alkaloids, phenolics, flavonoids, terpenoids/steroids, saponins, and tannins. The reagents used in the tests for these compounds are as follow. The following reagents are employed in the alkaloid test: Wagner, Mayer, and Dragendorff reagents; flavonoid test with ethanol, concentrated HCl, and Mg powder; saponin test with hot water reagent; tannin test with 1% FeCl₃ reagent; and steroid and terpenoid test with acetic acid reagent and sulfuric acid (Mangalu *et al.*, 2022) and phenolic test using FeCl₃ reagent (Wilujeng dan Anggraini, 2021).

Preparation of Antibacterial Test Tools and Materials

The first step in testing antibacterial activity is to sterilize the tools. The tools were rinsed and allowed to air dry before being wrapped in paper for Petri dishes, gauze for test tubes, and aluminum foil for Erlenmeyer flasks. Sterilization involves placing the tools in an autoclave at 121°C for 20 to 30

minutes (Listyani et al., 2022). For this test, *Staphylococcus aureus* and *Escherichia coli* cultures were obtained from the microbiology lab at Universitas Jambi.

To prepare the McFarland solution, combine 9.95 mL of a 1% H₂SO₄ solution with 0.5 mL of a 1.175% BaCl₂·2H₂O solution in an Erlenmeyer flask. Shake the mixture until turbidity forms. This turbidity will be used to standardize the bacterial suspension for testing. For the bacterial inoculation, use a sterile inoculating needle to transfer the bacteria into a tube containing 3 mL of 0.9% NaCl solution. Mix until the turbidity level of the bacterial suspension matches that of the McFarland turbidity standard. Each type of test microorganism should undergo the same treatment (Kosasi et al., 2019).

To sanitize Mueller-Hinton agar (MHA), 3.8 grams were placed in a tube and autoclaved at 121°C for 15 minutes. After autoclaving, the agar was cooled to 45°C (Hudaya et al., 2014). Petri dishes were then prepared, and 20 mL of the MHA medium was poured into each dish, allowing it to harden. *Staphylococcus aureus* and *Escherichia coli* bacteria were added to the medium and cultured in an oxygen incubator for 18 to 24 hours to facilitate their absorption into the agar (Listyani et al., 2022).

Preparation of Test Solution, Control (+) and (-) Concentrations

The concentration of the test solution were determined by diluting the extract to three concentrations: 92%, 46%, and 23%. To prepare the sample solution, 0.025 g, 0.05 g, and 0.1 g of red palm thick extract were weighed accordingly. The solvent for each extract was then used to dilute the concentrations until the total volume reached 1 mL in a volumetric flask. A positive control solution was created using chloramphenicol antibiotic by dissolving 25 mg of chloramphenicol capsule powder in 5 mL of 10% DMSO, resulting in a concentration of 5000 ppm. The solvent used for the extract served as the negative control.

Wells Method Antibacterial Activity Test

To assess antibacterial activity using the pitting method, first prepare *Mueller-Hinton* Agar (MHA) medium. Suspend the bacteria to be tested by applying the bacterial suspension to the surface of the media using a sterile cotton swab in a horizontal zigzag motion. Next, use a sterile blue tip to puncture the test media, creating up to six holes, each measuring 6 mm in diameter. Add extracts with concentrations of 23%, 46%, and 92%, as well as positive and negative controls, to each well, with a maximum volume of 50 µL per well. After incubating the Petri dish at 37°C for 24 hours, measure the diameter of the clear zones using a caliper.

Data Analysis

The data collected from the test results were evaluated descriptively. The study's findings were interpreted based on the presence or absence of clear zones developed around the wells, along with the documentation obtained. The measurement data were then categorized into four types of inhibitory zones.

Table 1. Zone of Inhibition Classification

Inhibition Zone Diameter (mm)	Inhibition Strength
≤ 5	Weak
6-10	Moderate
11-20	Strong
≥ 21	Extreme strong

Results and Discussion

Plant Sampling and Determination

The samples used were obtained from red palm plants, specifically from the fruits. Sampling were conducted in and around Buluran, located in the Telanaipura area of Jambi City, Jambi Province. The plant taxonomy was identified at the Plant Taxonomy Laboratory within the Department of Biology at the Faculty of Mathematics and Natural Sciences, Padjajaran University, with the reference number 93/HB/10/2024.

Simplicia Preparation

The process of making simplicia begins by weighing the red palm fruit sample, aiming for a total of up to 4,000 grams. First, the fruit is sorted by detaching it from the bunch. Next, the fruit is further sorted to separate those that are still in good condition from the rotten ones, along with any impurities other than ripe red palm fruit. After this sorting, the fruit is washed with running water. The drying process occurs in two stages: it is first air-dried for 5 days, followed by oven drying at 60°C for 8 hours, until a predetermined dry weight is achieved. Subsequently, any contaminants not related to red palm fruit are removed through dry sorting, along with any samples that were damaged or burned during the drying process. The dried sample is then ground into fine powder simplicia using a hammer mill. The final weight of the fine powder simplicia is approximately 1,500 grams. The yield value is calculated to be 37.50%, which meets the minimum yield requirement of 10% (Kalalo et al., 2024).

Red Palm Fruit Extraction

The initial extraction procedure selected is maceration, which does not involve heating in its treatment and is also known as cold extraction. Maceration was chosen as the extraction method in this study because it is feasible, does not require any specific tools, and can be performed independently. Maceration also seeks to keep molecules that cannot survive heat from degrading, as heating can destroy some of the chemical components found in simplicia (Suhaenah et al., 2023).

In this investigation, reflux were used as an extraction method that involves heat. The reflux extraction method uses a solvent that has been heated to its boiling point. Reflux extraction normally takes 2-4 hours. The solvent used is also determined by how many samples are placed in the round bottom flask until they are completely submerged. The red palm fruit extract was refluxed in a methanol distillate solvent at 64°C for 2.5 hours before filtering. The procedure was then done two times with the same solvent and approach. The reflux methanol extract of red palm fruit was evaporated using a rotary

evaporator at 64°C until a thick methanol reflux extract was produced (Syamsul et al., 2020). After that, the thick extracts from the maceration and reflux procedures were mixed and re-extracted in the rotary evaporator. The methanol thick extract of red palm fruit was then weighed to get the extract yield value indicated in **Table 2**.

Table 2. Red Palm Fruit Extract Yield

Sample	Initial Weight of Simplicia	Extract Weight	Yield
Methanol extract	165,419 gr	1500 gr	11,08%

Liquid Extraction

The thick methanol extract of red palm fruit was then treated to liquid extraction employing *n*-hexane, ethyl acetate, and dichloromethane solvents in sequential order of polarity and instruments in the form of a separating funnel. The premise of liquid extraction studies is based on the law of like dissolves like, which states that polar compounds dissolve in polar compounds and non-polar compounds dissolve in non-polar solvents, resulting in the solute being split into two solvents that do not mix. The partition method for isolating secondary metabolite chemicals seeks to classify compounds based on their polarities. A partition process is deemed to be successful if it generates two distinct phases and is ended if after shaking the solvent layer does not change color or clear after standing for a few minutes. After getting the liquid partition findings, the specific gravity value of each extract is calculated by weighing it with a 1 mL pycnometer using solvents with varying degrees of polarity. Table 3 shows the findings of the specific gravity value and the calculation of extract yield for each extract of red palm fruit liquid partition.

Table 3. Yield and Specific gravity values of liquid extracts

Extract	Results			
	Total Volume (mL)	Specific gravity (g/mL)	Sample Weight (Gram)	% Yield
<i>n</i> -hexane	73	0,584	42,632	25,64%
Ethyl Acetate	82	0,909	74,538	44,84%
Dichloromethane	21	0,949	19,929	11,98%
Water	25	1,023	25,57	15,38%

Phytochemical Screening

Phytochemical screening can reveal the group of chemicals found in each liquid extract of red palm fruit. The phytochemical screening findings are shown in Table 4.

Table 4. Phytochemical Screening Results of Liquid Extract

Compound Identification	Reagents	Screening Result				
		Extract <i>n</i> -hexane	Extract Ethyl Acetate	Extract DCM	Extract Water	Positive Control
Alkaloids	Dragendorff	+	+	-	-	Caffeine

Compound Identification	Reagents	Screening Result				
		Extract <i>n</i> -hexane	Extract Ethyl Acetate	Extract DCM	Extract Water	Positive Control
Phenolic	Wagner	-	+	+	-	Gallic Acid
	Mayer	+	+	+	-	
	FeCl ₃ 10%	-	+	+	+	
Flavonoids	Concentrated HCL + Mg powder	-	+	-	+	Quarsetin
	H ₂ SO ₄ 2N	-	+	+	+	
Tannins	NaOH 10%	-	+	+	+	Tannic Acid
	1% gelatin solution + NaCl	-	+	+	+	
Saponins	FeCl ₃	-	+	+	+	Saponins Beta
Steroids	H ₂ O, HCL 2N	-	-	-	+	
	Chloroform, CH ₃ COOH, concentrated	+	-	+	-	Sitosterol
Triterpenoids	H ₂ SO ₄	-	+	-	+	
	Concentrated H ₂ SO ₄ , glacial CH ₃ COOH	-	+	-	+	

Description : (+) = Indicates a positive response (detected compounds)

(-) = Indicates a negative response (undetected contain compounds)

The phytochemical screening results revealed that the ethyl acetate and the water extract of red palm fruit contained the same secondary metabolite components, *i.e.* alkaloid, phenolic, flavonoid, tannin, and terpenoid. These chemicals are thought to contribute to the extracts' antibacterial activity.

Antibacterial Test Results of Red Palm Fruit Extract

Table 5. Activities Agents *Escherichia coli*

Extract	Dose (%)	Inhibition Zone Diameter (mm)		Average	Category
		1	2		
<i>n</i> -hexane	23	0	0	0	-
	46	0	0	0	-
	92	0	0	0	-
	K+	37,99	36,33	37,16	Extreme strong
	K-	0	0	0	-
	DMSO 10%	0	0	0	-
Ethyl acetate	23	14,53	14,67	26,93	Strong
	46	19,37	21,24	20,04	Strong
	92	26,45	27,80	14,66	Extreme Strong
	K+	38,17	35,20	36,31	Extreme strong
	K-	0	0	0	-
	DMSO 10%	0	0	0	-
Dicloromethane	23	0	0	0	-
	46	0	0	0	-
	92	0	0	0	-
	K+	32,83	35,96	34,39	Extreme stong
	K-	0	0	0	-
	DMSO 10%	0	0	0	-
Water	23	15,50	12,82	10,45	Strong

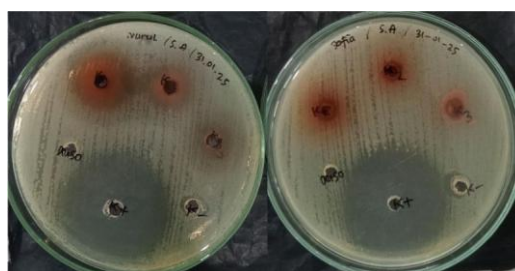
	46	15,14	14,33	15,36	Strong
	92	17,30	16,65	20,50	Extreme Strong
	K+	36,82	34,76	38,90	Extreme strong
	K-	0	0	0	-
	DMSO 10%	0	0	0	-



Picture 1. Test Results of Ethyl Acetate and Water Extracts against *Escherichia coli*

Table 6.

Extract	Dose (%)	Inhibition Zone Diameter (mm)		Average	Category
		1	2		
<i>n</i> - Hexane	23	0	0	0	-
	46	0	0	0	-
	92	0	0	0	-
	K+	30,65	32,65	31,65	Extreme strong
	K-	0	0	0	-
	DMSO 10%	0	0	0	-
Ethyl acetate	23	9,68	10,68	10,45	strong
	46	14,93	15,97	15,36	Strong
	92	19,25	21,30	20,50	Extreme Strong
	K+	40,25	37,05	38,90	Extreme strong
	K-	0	0	0	-
	DMSO 10%	0	0	0	-
Dicloromethane	23	0	0	0	-
	46	0	0	0	-
	92	0	0	0	-
	K+	31,61	32,29	31,95	Extreme strong
	K-	0	0	0	-
	DMSO 10%	0	0	0	-
Water	23	6,07	7,83	6,05	Strong
	46	6,82	6,78	6,80	Strong
	92	7,56	6,04	7,69	Extreme Strong
	K+	36,86	40,32	38,59	Extreme strong
	K-	0	0	0	-
	DMSO 10%	0	0	0	-



Picture 2. Test Results of Ethyl Acetate and Water Extracts against *Staphylococcus aureus*

The test results in **Tables 4** and **5** reveal that *n*-hexane and dichloromethane extracts had no antibacterial action against the two microorganisms tested in this investigation. Meanwhile, ethyl acetate and water extracts of red palm fruit have antibacterial action against both gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*). In this investigation, ethyl acetate extract and water extract were employed at three doses diluted from the maximum. As a negative control, the solvents of each extract, namely ethyl acetate and water, were used to demonstrate the absence of solvent influence on the antibacterial activity of the test solution, allowing it to be determined that the activity is influenced by the substances contained in the extract rather than the solvent used.

Extracts of *n*-hexane and dichloromethane solvents had no antibacterial action against either type of bacterium. This means that the active chemicals in red palm fruit are not soluble in nonpolar or semipolar *non*-ionic solvents. Saponins, flavonoids, and phenolic compounds are more likely to be soluble in polar and semi-polar solvents, which explains why only ethyl acetate and water extracts shown antibacterial action. According to Rollando (2019), the choice of solvent has a significant impact on the extract's efficacy in separating bioactive components. As a result, the liquid partitioning method must take the solvent's solubility into account, as well as the target compound's properties.

The positive control employed is chloramphenicol, which is a broad-spectrum antibiotic that inhibits bacterial protein synthesis by increasing the ribosome's 50S subunit. The use of a positive control in this test serves to compare the activity of typical antibacterial medicines to test solutions (Utomo et al., 2018). Chloramphenicol was dissolved in 10% DMSO, a solvent capable of dissolving both polar and nonpolar molecules. Furthermore, because DMSO does not prevent bacterial growth, the results of antibacterial activity testing are unaffected. As a result, 10% DMSO was utilized in the test as a correction control for chloramphenicol to demonstrate that the solvent had no effect on the positive control's antibacterial efficacy. Because DMSO can penetrate cell membranes, the concentration of DMSO used as a solvent should not exceed 10% or the cell membrane would break (Rizki et al., 2024).

Based on the findings of this investigation, the ethyl acetate extract has better antibacterial activity than the water extract, however the difference in inhibition against *Escherichia coli* bacteria is not significant. These findings could be attributed to the high concentration of secondary metabolite chemicals in ethyl acetate extract, which attracts both *non*-polar and polar molecules. The phytochemical screening of the ethyl acetate extract of red palm fruit revealed alkaloids, tannins, flavonoids, terpenoids, and phenolics, whereas the water extract of red palm fruit contained flavonoids, terpenoids, phenolics, saponins, and tannins. Variations in the size of the inhibition zone at different concentrations can be attributed to a variety of factors, including the velocity of diffusion of antimicrobial chemicals into the medium, bacterial growth sensitivity, incubation temperature and time, and microorganism metabolic activity (Salni et al., 2011).

The greatest inhibition was detected at the maximum concentration (K1), while the least inhibition was observed at the second dilution concentration. This component demonstrates that

differences in the diameter of the inhibition zone created are impacted by variations in the concentration of the test solution used; the higher the concentration, the larger the inhibition zone. The concentration of antibacterial compounds has a significant impact on their ability to prevent the development of germs; the higher the concentration of an extract, the more secondary metabolite chemicals are present, and the larger the inhibition zone. According to CLSI 2020 guidelines, there are four categories of inhibition zones: very strong (more than 20 mm wide), strong (10-20 mm), moderate (10-5 mm), and weak (less than 5 mm) (Alouw et al., 2022).

The ethyl acetate extract and water extract have been tested for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, respectively. They exhibit different modes of action: the ethyl acetate extract has a radical (bactericidal) effect, while the water extract possesses an irradical (bacteriostatic) effect. The radical zone appears as a clear area surrounding the wells where there is no bacterial growth, indicating that the test material is bactericidal. In contrast, the irradical zone shows that the test substance is bacteriostatic, as it only suppresses bacterial growth, leaving visible colonies of germs around the well.

Gram-positive bacteria have a cell wall structure characterized by a significantly thicker peptidoglycan layer, which comprises 90% of their cell wall and ranges from 20 to 80 nm in thickness. This layer contains polar teichoic acid and teichuronic acid, making it challenging for *non*-polar antibacterial substances found in red palm fruit extract to penetrate and disrupt the bacterial cell wall. As a result, these bacteria continue to grow around the wells. In contrast, gram-negative bacteria possess a thinner peptidoglycan layer, measuring between 5 to 10 nm, which makes them more vulnerable to secondary metabolites that can interfere with cell wall formation or even kill the bacteria. Additionally, gram-negative bacteria are surrounded by an outer membrane that includes a thin layer of peptidoglycan, as well as a double layer of phospholipids, proteins, and lipopolysaccharides. The presence of lipopolysaccharides in their outer layer distinguishes gram-negative bacteria. Consequently, the *non*-polar composition of red palm fruit extract enhances its bactericidal effectiveness against gram-negative bacteria (Putri et al., 2019).

In conclusion, this investigation shows that red palm extract has potential as an environmentally friendly and sustainable antibacterial phytopharmaceutical. To validate these findings, further testing is essential, including compound isolation, toxicity studies, and *in vivo* experiments. Additionally, more detailed follow-up research is necessary to fully realize the potential of red palm extract in the development of locally and natively sourced health and pharmaceutical products.

Conclusion

Based on the results of phytochemical tests and antibacterial activity tests, it concluded that the antibacterial activity of ethyl acetate and water extracts using the well method against *Escherichia coli* and *Staphylococcus aureus* bacteria is the largest, with a very strong inhibition zone category occurring

at the first concentration (K1), where the average inhibition zone formed is 20.50 mm in ethyl acetate extracts and 7.69 mm in water extracts and the results of

Acknowledgements

The authors would like to thank PNPB Universitas Jambi for giving chances and financial support to ensure that this research is conducted effectively.

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