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Evaluation of Antibacterial Activity for Semi-Polar and Polar Fractions of Red Palm Fruit (*Cyrtostachys renda* Blume) against the *Staphylococcus aureus* and *Escherichia coli*

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Abstract

The red palm tree (*Cyrtostachys renda* B.) has potential as an antibacterial agent. This study aims to identify the most promising polar and semi-polar fractions of red palm fruit for development as antibacterial agents. Differences in solvent polarity were used to separate the active compounds. The methods used included the separation of secondary metabolites through fractionation using Vacuum Liquid Chromatography (VLC) and Column Chromatography (CC), followed by antibacterial activity testing against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria using the well method. The results showed that the semi-polar fraction had strong activity against both types of bacteria, reaching a maximum inhibition zone of 12.51 mm in *Staphylococcus aureus* and 14.38 mm in *Escherichia coli* at a concentration of 92%. Meanwhile, the polar fraction only showed antibacterial activity against *Escherichia coli* with a maximum inhibition zone of 12.28 mm at a concentration of 92% and was inactive against *Staphylococcus aureus*. Overall, the semi-polar fraction of red palm fruit is the most promising fraction because it exhibits antibacterial activity against two types of bacteria with a very strong inhibition zone observed at the highest concentration (92%).

[Keywords: *Antibacterial, Cyrtostachys renda Blume, fractionation, Phenolics, Saponins, Tannins*]

Introduction

An unfavorable climate may contribute to the spread of infections. Infectious diseases can be caused by bacteria, fungi, viruses, or parasites. Consequently, there is a pressing need for innovative efforts to develop materials or molecules that can suppress or inhibit bacterial

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growth and effectively kill bacteria (Utami *et al*., 2022). These substances are commonly referred to as antibacterial components (Irmawati, 2018).

Indonesians have a long history of using plants to heal various ailments, drawing on local wisdom that is deeply embedded in their communities. This knowledge is adaptable to environmental conditions and the availability of local plants (Amin *et al*., 2024). Communities believe that utilizing these plants offers several benefits, including safety, accessibility, and a lower risk of side effects (Sadiah *et al*., 2022).

Plants produce two main types of metabolites: primary metabolites and secondary metabolites. Primary metabolites are essential for growth, development, and reproduction, while secondary metabolites play various roles in these processes, including how plants interact with their environment (Yulisma and Fathiya, 2023). Many secondary metabolites exhibit a broad range of pharmacological properties, particularly antibacterial activity. Examples of secondary metabolites with antibacterial properties include phenolics, alkaloids, saponins, and terpenoids (Abdallah *et al*., 2023).

Red palm plants belong to the *Arecaceae* family, also known as the palm family, and are native to the islands of Sumatra and Kalimantan. Plants of the *Arecaceae* family have pharmacological activities, one of which is antibacterial. The antibacterial activity of *Arecaceae* plants varies depending on the type of solvent used. One example of a plant from the *Arecaceae* family that has antibacterial activity is the nipah plant (*Nypa fruticans*) at a concentration of 1000 ppm, which shows antibacterial activity of n-hexane and ethyl acetate fractions on gram-negative bacteria of 9.012 mm and 9.387 mm, respectively. Then, for antibacterial activity on gram-positive bacteria, the n-hexane and ethyl acetate fractions were 11.250 mm and 9.512 mm, respectively (Lestari *et al*., 2016). Organic compounds from plant parts have different affinities to the polarity of the solvent used. Therefore, to extract phenolic compounds contained in plant tissues, it is best to use solvents with different levels of polarity (Sembiring *et al*., 2016). Red palm fruit contains various potential antibacterial compounds, as evidenced by phytochemical screening of ethyl acetate extracts from the fruit, which identified several chemicals with antibacterial properties. These components include alkaloids, phenolics, flavonoids, tannins, steroids, and terpenoids (Syamsurizal *et al*., 2023).

Based on this background, it appears that red palm has potential as an antibacterial agent. The antibacterial activity of red palm can be maximized using solvents with different polarity levels. Differences in the polarity of the solvents used can identify the most promising fractions

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for development as antibacterial agents. Therefore, further research is needed to investigate the antibacterial characteristics of the polar and semi-polar fractions of red palm fruit.

Material and Methods

Tools and Materials

The following laboratory equipment and instruments are essential for various experimental procedures: rotary evaporator, separatory funnel, grinder, oven, TLC chamber, analytical balance, TLC plate, distillation apparatus, vacuum liquid chromatography apparatus, 366 nm UV light detector, sonicator, micropipette, measuring flask, test tubes, test tube rack, pipettes, erlenmeyer, measuring glassware, petri dish, Laminar Air Flow (LAF), autoclave, bunsen, ose needle, incubator, spiritus, vortex. This research examined red palm fruit from the Buluran region in Jambi Province as the main material, solvents: methanol, ethyl acetate, *n*-hexane, and dichloromethane. Bacteria cultures of *Staphylococcus aureus* and *Escherichia coli*, Nutrient Agar (NA), Mueller Hinton agar (MHA), Chloramphenicol and sterile distilled water.

Determination and Simplicia Preparation

The samples of red palm fruit were analyzed at the laboratory of Padjadjaran University. The fruit was collected in Buluran Village, Telanaipura District, Jambi City. After being sorted through wet and dry methods, the red palm fruit was dried using a combination of air drying for 72 hours and oven drying for 24 hours at 60°C. Subsequently, it was ground using a grinder.

Sample Extraction

The maceration method was used to extract 500 grams of red palm fruit simplicia over three days using *n*-hexane as the solvent. A concentrated extract was obtained by filtering the resulting macerate and then evaporating the solvent with a rotary evaporator. Next, the macerate residue was re-macerated with methanol, and the extract was once again concentrated and filtered using a rotary evaporator. Following this, the maceration residue was extracted using the reflux method three times over two hours, with methanol as the solvent to optimize the extraction process. Finally, the extract was filtered and concentrated (Syamsurizal *et al*., 2023).

Fractionation by Liquid Extraction

Using a separating funnel and solvents of varying polarities of *n*-hexane, ethyl acetate, and dichloromethane in order to separate of 165 grams of methanol extract were subjected to extraction until saturation was achieved. This process continued until the desired outcome was

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evident (Efendi, 2019). A rotary evaporator was then employed to concentrate each fraction and produce a thick extract.

Advanced Fractionation by Vacuum Liquid Chromatography

Vacuum liquid chromatography (VLC) was employed to fractionate the previously obtained ethyl acetate fraction. First, the sample was weighed, and silica gel 60 (0.063–0.200 mm) was used for impregnation. A total of 460 grams of silica gel 60 (0.040–0.063 mm) was poured into a vacuum column that measured 10 cm in diameter and 13.5 cm in height, filling the column halfway. Based on the Thin Layer Chromatography (TLC) profile, an eluent with an increasing polarity gradient was utilized during the elution process.

Different ratios of *n*-hexane, *n*-hexane:ethyl acetate, ethyl acetate, and methanol were used as eluents. The obtained fractions were then evaporated using a rotary evaporator to concentrate them. After concentration, the fractions were evaluated for their chromatogram profiles (Haeria *et al*., 2019). To simplify the analysis procedure, fractions exhibiting the same spot colors and chromatogram profiles were combined into one.

Purification by Column Chromatography

Seven fractions were obtained through VLC. Fraction (F-6) was then subjected to the next column by using combination eluen *n*-hexane and ethyl acetate with gradient polarity (1:1; 1:5), ethyl acetate, and methanol were obtained major component which contained small impurities. Fraction ethyl acetate and methanol has a single spot but still has impurities.

The eluate from the CC stage was collected in several test tubes and separated fractions were analyzed by TLC. According to Aisyah *et al*. (2018), spots with identical *Rf* values are merged. Subsequently, the major fractions were determined its antibacterial activities.

Identification of Secondary Metabolites

Screening phytochemicals were conducted through TLC plates which staining utilized with different reagents *i.e.* Dragendorff for alkaloids, $FeCl_3$ 10% for phenolics and tannins, citroborate for flavonoids, Liebermann-Burchard for saponin and steroid/terpenoid. Before staining spotted samples were eluted with the mobile phase. A stain-revealing reagent was then sprayed onto the spots. The plates were inspected under UV light at wavelengths of 366 nm and 254 nm, both before and after applying the spot-revealing reagent (Wilujeng and Anggraini, 2021).

a. Alkaloids

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Dragendorff's reagent was sprayed onto the plate to identify the alkaloid components. Methanol was used as the mobile phase. If the result showed an orange-yellow color, it was considered positive. Caffeine was used as a positive control (Lestari *et al*., 2021).

b. Phenolics

A 10% $FeCl_3$ reagent was sprayed onto the TLC plate to detect phenolic components. Methanol was used as the mobile phase. If blue-black dots appeared when the plate was exposed to UV light at 254 nm and 366 nm, the result was considered positive. Gallic acid was used as a positive control (Fajriaty *et al*., 2018).

c. Flavonoids

Citrate reagent was sprayed onto the plate to detect flavonoid components. Methanol was used as the mobile phase. Dark yellow marks indicate positive results. Quarsetin was used as a positive control (Fitrya *et al*., 2019).

d. Saponins

The TLC plate was sprayed with Lieberman-Burchard reagent to identify saponin components. Methanol was used as the mobile phase. A bluish-green spot indicates the presence of steroid saponins, while pink and purple spots indicate the presence of triterpenoid saponins; both are considered positive results. Saponins was used as a positive control (Trisharyanti and Febriani, 2017).

e. Tannins

To detect tannin components, a 10% $FeCl_3$ reagent was sprayed onto the plate. The mobile phase used was a methanol:water mixture in a 6:4 ratio. If a grayish-black smear appeared under visible light, the result was considered positive. Tannic acid was used as a positive control (Widyastuti, 2017).

f. Steroids/Terpenoids

The Lieberman-Burchard reagent was sprayed onto the plate to identify flavonoid components. Methanol served as the mobile phase. A blue-green spot indicated a positive result for steroids, while a pink and purple spot suggested a positive result for terpenoids. The visible spots were then used to calculate the Rf value. β-sitosterol was used as a positive control (Fajriaty *et al*., 2018).

Preparation of materials and samples for Antibacterial Activity Test

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The autoclave was used to sanitize the glassware for 15 minutes at a temperature of 121°C. The microbiology culture lab at Universitas Jambi provided the *Staphylococcus aureus* and *Escherichia coli* microorganisms that used in this experiment. Both bacteria were cultured in an oxygen incubator for 18 to 24 hours after being inoculated into NA media. Additionally, a tube containing 3,8 grams of MHA was autoclaved for 15 minutes at 121 °C to ensure sterilization. Subsequently, 15 milliliters of the sterilized MHA were added to Petri dishes to serve as the antibacterial test medium (Hudaya *et al*., 2014).

To prepare the McFarland standard solution, 9,5 mL of a 1% sulfuric acid (H_2SO_4) solution is combined with 0,5 mL of a 1,75% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) solution in an erlenmeyer. The mixture is shaken to create a turbid solution, which serves as the standard turbidity for the bacterial suspension test. According to Kosasi *et al*. (2019), a bacterial cell suspension with a concentration of $1,5 \times 10^8$ CFU/mL corresponds to a McFarland standard solution of 0,5. To achieve a turbidity level matching the McFarland turbidity standard, the test bacteria are suspended in a tube containing 3 mL of 0,9% sodium chloride (NaCl) solution after inoculation with a sterile inoculation needle. This identical procedure is followed for each type of test bacteria (Kosasi *et al*., 2019).

A chloramphenicol solution with a concentration of 250 µg in 50 µL was used as the positive control. This solution was prepared by disolving 25 mg of chloramphenicol powder from 250 mg capsules in 5 mL of 10% DMSO. For the negative control, 10 mL of DMSO was mixed with 100 mL of sterile distilled water to create a negative control, which is a 10% DMSO solution (Yani *et al*., 2024).

Antibacterial Testing Using Well Method

The well method was utilized for antibacterial testing. MHA was poured into petri dishes, and sterile cotton buds were employed to spread suspensions of *Staphylococcus aureus* and *Escherichia coli* bacteria, adjusted to the standard McFarland solution, evenly across the surface of the medium. A sterile 6-mm-diameter bluetip was then used to create wells in the agar. Each Petri dish was prepared with six wells. Next, 50 µl of each fraction at varying concentrations, along with positive controls and negative controls, were added to the wells. After incubating the Petri dishes for 24 hours at 37 °C, the clear zones around the wells were observed (Emelda *et al*., 2021).

Data Interpretation

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A The one-way ANOVA test method was performed to determine whether there were significant differences in each test group of polar and semi-polar fractions from red palm fruit against S. aureus and E. coli bacteria, followed by Duncan's test to pick the optimum treatment. Significant one-way ANOVA test results were defined as $p < 0.05$ with a 95% confidence level ($\alpha = 0.05$). (Dahlan, 2013).

Results and Discussion

Preparation of Simplicia and Extraction

Red palm fruit, which weighs up to 4,000 grams, was processed into dry simplicia powder, resulting in a yield of 1,500 grams, which represents 37.5% of the original weight. The simplicia was extracted using *n*-hexane and methanol solvents. The extraction process begins with defatting the sample using *n*-hexane to remove oil, fat, and other semi-polar components. This is followed by the use of methanol to dissolve polar components and secondary metabolites. The *n*-hexane thick extract from red palm fruit yielded 3.48%, while the methanol thick extract produced a yield of 11.08%. The yield of a sample indicates the amount of extract collected during the extraction process. A yield is considered good if it exceeds 10%; generally, a higher yield suggests a greater presence of secondary metabolite components in the extract (Novianti and Nursaidah, 2023).

Fractionation by Liquid Extraction

The methanol extract of red palm fruit underwent liquid extraction using the solvents *n*-Hexane, Ethyl Acetate, Dichloromethane, and Water. The results are summarized in Table 1:

Table 1. Yield of Liquid Extract

Liquid Partition Extract	Result		
	Total Volume (mL)	Extract Weight (gr)	Yield (%)
n-Hexane	73	166,216	25,64%
Ethyl Acetate	82	166,216	44,84%
Diclorometha ne	21	166,216	11,98%
Water	25	166,216	15,38%

On the table above, shows that the ethyl acetate extract had the highest yield at 44.84%. This data indicates that the secondary metabolites in the methanol extract are primarily soluble in ethyl acetate, which has semi-polar characteristics. These indicate that the ethyl acetate extract has antibacterial potential. Therefore, the VLC method will be employed to fractionate the active secondary metabolites based on their polarity.

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Advanced Fractionation by Vacuum Liquid Chromatography

Vacuum Liquid Chromatography method fractionation was conducted using a column filled with silica gel G60 *Merck* (particle size: 0.040-0.063 mm) as the stationary phase and gradient eluents as the mobile phase. The eluents used included 100% *n*-hexane, mixture of *n*-hexane and ethyl acetate with gradient polarity (15:1), (10:1), (5:1) and (1:1), 100% ethyl acetate, and 100% methanol. A total of seven fractions were collected from the VLC method's fractionation, which were then categorized based on their node patterns using TLC under UV light at 254 nm and 366 nm. One of the selected fractionates of 100% methanol eluent with a single spot stain based on the TLC observation results was labeled as F-6. Fractionate F-6 was obtained as much as 23 mL with a specific gravity of 0.878 g/mL so that it has a total weight of 20.21 g. However, based on TLC identification, fractionate F-6 still shows *tailing* or impurities, possibly due to the presence of contaminants in the sample. Therefore, CC was used to purify the sample from impurities.

Purification by Column Chromatography

Each fractionation was conducted using silica gel G60 *Merck* (0.063 - 0.200 mm) as the stationary phase. The mobile phases used were mixture of *n*-hexane and ethyl acetate with gradient polarity (1:1) and (1:5), 100% ethyl acetate, and 100% methanol. The results of the fractionation indicated that only the eluents of 100% ethyl acetate and 100% methanol produced a concentrated color. These two eluents were subsequently identified using the TLC method, as illustrated below.

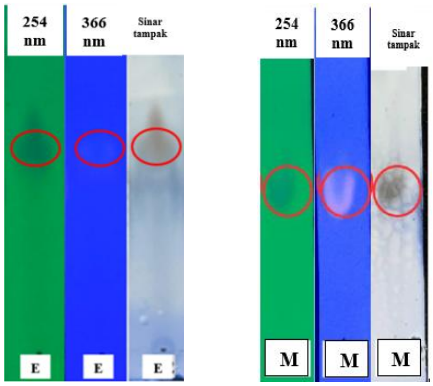

Figure 1. Chromatogram Profiles of Semi-polars (Left) and Polar (Right) Fractionate

Description: E = Ethyl Acetate (Semi-polar) ; M = Methanol (Polar)

Based on the chromatogram profile, the *Rf* value of the ethyl acetate fraction is 0,67, while the *Rf* value of the methanol fraction is 0,584. Components with a higher *Rf* value are

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less polar, whereas those with a lower *Rf* value are more polar due to the polar stationary phase used in chromatography. Therefore, the ethyl acetate fraction contains semi-polar molecules, while the methanol fraction contains polar components (Saputra *et al*., 2023).

Secondary Metabolite Identification

Table 2. Secondary Metabolites Identification

Secondary Metabolite Components	Samples	Observation After Being Given Reagents	Description
Alkaloids	Ethyl Acetate Fraction	No color	-
	Methanol Fraction	No color	-
	Caffeine	Orange	+
Phenolics	Ethyl Acetate Fraction	Black	+
	Methanol Fraction	Black	+
	Gallic acid	Blackish-blue	+
Flavonoids	Ethyl Acetate Fraction	No color	-
	Methanol Fraction	No color	-
	Quarsetin	Yellowish-green	+
Tannins	Ethyl Acetate Fraction	Black	+
	Methanol Fraction	Black	+
	Tannic acid	Blackish-blue	+
Saponins	Ethyl Acetate Fraction	Purplish-brown	+
	Methanol Fraction	Brown	+
	Saponins	Purplish-brown	+
Terpenoids/Steroids	Ethyl Acetate Fraction	No color	-/-
	Methanol Fraction	No color	-/-
	Beta Sitosterols	Purplish-red	+

Description: (+) = Detected secondary metabolite components
(-) = Undetected secondary metabolite components

According to the table above, both the Semi-polar and Polar Fractionates contain the same secondary metabolites: Phenolic, Tannins, and Saponins. Phenolic compounds exhibit antibacterial activity by disrupting the peptidoglycan layer of the bacterial cell wall, which is typically rigid. This disruption leads to irregular development of the cell wall, resulting in the bacteria losing their protective barrier. Consequently, the cell membrane becomes exposed to damage and leakage. Tannins, which possess a *hydroxyl* (OH) group arrangement similar to that of polyphenolic substances, can inhibit the action of key enzymes necessary for bacterial survival. Additionally, tannins can interact with proteins, causing protein denaturation and

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negatively impacting cellular metabolism. (Pangisian *et al*., 2022). Saponins exhibit antibacterial activity primarily by reducing the surface tension of bacterial cell walls. This reduction leads to the leakage of cellular contents, ultimately resulting in cell death. Additionally, saponins increase the permeability of cell membranes, which can cause bacterial cells to undergo hemolysis. Saponins are composed of polar glycosyl groups along with nonpolar steroid or triterpenoid groups(Sujana *et al*., 2024).

Antibacterial Activity Test

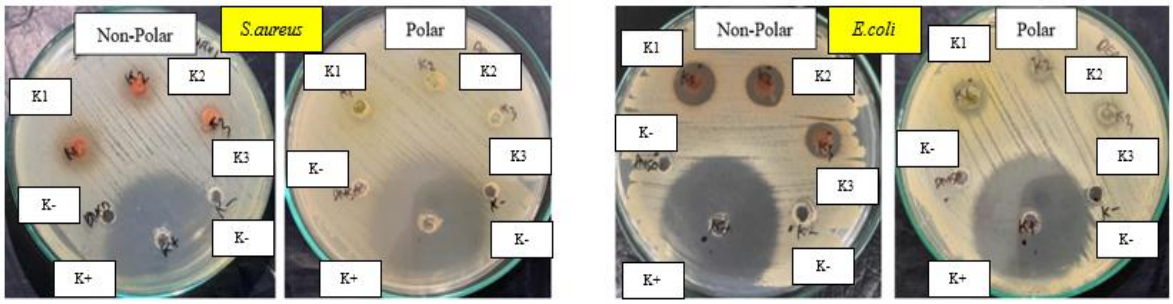

Figure 2. Antibacterial Test Results of Semi-polar and Polar Fractions on *Staphylococcus aureus* and *Escherichia coli*

Description: K1 = 92%
K2 = 46%
K3 = 23%
K- = Ethyl acetate (semi-polar), Methanol (Polar), DMSO 10%
K+ = Chloramphenicol

The following table shows the data collected from the antibacterial activity test results of Polar and Semi-polar Fractions of Red Palm Fruit against *Staphylococcus aureus* and *Escherichia coli* bacteria:

Table 3. Antibacterial Test results on Staphylococcus aureus

Samples	Labels	Average (mm) ± SD	Interpretation
	K1	0[a]	No result
Polar Fraction	K2	0[a]	No result
	K3	0[a]	No result
Chloramphenicol	K+	24,57±4,737[b]	Very strong
	K1	12,51±0,328[c]	Strong
Semi-polar Fraction	K2	11,14±0,696[c]	Strong
	K3	7,34±0,490[b]	Medium
Chloramphenicol	K+	35,83±2,363[d]	Very strong
Methanol **Ethyl Acetate** **DMSO 10%**	K-	0[a]	No result

Based on *One Way Anova* statistical analysis in table 3, semi-polar patterns were identified with a p-value of less than 0.05, indicating significant differences in the frequency

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of bacterial infections with S. *aureus* and E. *coli*. The purpose of the analysis is to determine which activities have the same or different effects, as well as the effects that range from little to major. Duncan's test on the diameter of the inhibition zone of S. *aureus* bacteria was only observed in the semi-polar fraction, with K3 (7.34 mm) being significantly different from the concentrations in the other fractions. This suggests that the concentrations have distinct inhibitory effects on S. *aureus* bacteria. Meanwhile, there was no significant difference between K2 (11.14 mm) and K1 (12.51 mm), indicating that both quantities have the same inhibitory impact on S. *aureus* bacteria.

The positive control chloramphenicol has an inhibition of 35.83 mm including a very strong category and in the negative control of methanol or DMSO 10% has no inhibition. In this study, the absence of antibacterial activity of the polar fraction against *Staphylococcus aureus* bacteria is likely due to the sensitivity factor of the test bacteria to antibacterial compounds in plant samples that affect the diameter of the inhibition zone. Seen in the polar fraction petri disk, the growth of *Staphylococcus aureus* bacteria is very dense which can be caused by the concentration of bacteria during the inoculation process too much. If the concentration of test bacteria in the media is high, it requires antibacterial compounds with high concentrations as well. If the concentration between the two is not comparable, it will be seen that the diameter of the inhibition zone tends to be narrow or even absent (Saudale, 2018).

The antibacterial activity of semi-polar fractions that create inhibitory zones exhibits a bacteriostatic effect against Staphylococcus aureus. The presence of an irradical zone indicates that the test substance is bacteriostatic, meaning it inhibits bacterial growth without completely eradicating the visible germs in the well (Wardoyo *et al*., 2020). The regulation of this process is believed to be influenced by the structure of the cell wall in gram-positive bacteria. These bacteria possess cell walls that are approximately 90% thicker due to a peptidoglycan layer, which measures between 20 to 80 nm. This layer contains polar teichoic acid and teichuronic acid, creating a barrier that makes it challenging for semi-polar antibacterial chemicals such as those found in the semi-polar components of red palm fruit to penetrate and disrupt the bacterial cell wall. Consequently, the bacteria are able to thrive in their environment (Ishmora *et al*., 2023).

Table 4. Antibacterial Test results on *Escherichia coli*

Samples	Labels	Average (mm) ± SD	Interpretation
Polar Fraction	K1	12,28±0,319^{c}	Strong

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	K2	9,98±0,556[b]	Medium
	K3	9,67±0,338[b]	Medium
Chloramphenicol	K+	39,97±0,422[d]	Very strong
	K1	14,38±0,221[d]	Strong
Semi-polar Fraction	K2	12,98±0,548[c]	Strong
	K3	10,81±0,337[b]	Strong
Chloramphenicol	K+	37,75±0,901[e]	Very strong
Methanol Ethyl Acetate DMSO 10%	K	0[a]	No result

Duncan's test on the tabel 4 shows that diameter of the E. *coli* bacterial inhibition zone in the polar fraction showed that K3 (9.67 mm) and K2 (9.98 mm) did not show any significant difference, which means that these concentrations had the same effect in inhibiting E. *coli* bacteria. Meanwhile, K1 (12.28 mm) showed a significant difference. This means that these concentrations had different effects on inhibiting E. *coli* bacteria compared to other concentrations. In the semi-polar fraction, each K1 (14.38 mm), K2 (12.98 mm), and K3 (10.81 mm) showed a significant difference from each other. This means that these concentrations had different effects in inhibiting E. *coli* bacteria.

In contrast, the positive control, chloramphenicol, showed very strong inhibition. However, the samples of methanol, ethyl acetate, or 10% DMSO exhibited no antibacterial activity. The findings indicate that the antibacterial activity of polar and semi-polar fractions against *Escherichia coli* bacteria is similar. This similarity may be attributed to the presence of the same secondary metabolites specifically phenolics, tannins, and saponins in both fractions. These compounds work together to exert their effects. The concentration of saponins in the semi-polar fraction has the ability to lyse the phospholipid cell wall, which leaves a thin peptidoglycan layer that can be further broken down by polar chemicals such as phenolics and tannins.

Both polar and semi-polar fractions exhibited bactericidal activity against Escherichia coli. The appearance of a clear zone around the well indicates the absence of bacterial growth, confirming that the test sample is effective in killing bacteria (Wardoyo *et al*., 2020). This effect is believed to be influenced by the cell wall structure of gram-negative bacteria. Gram-negative bacteria possess a thinner peptidoglycan layer, which ranges from 5 to 10 nanometers in thickness, making them more susceptible to secondary metabolites that can inhibit cell wall production and ultimately kill the bacteria. Additionally, these bacteria are surrounded by an

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outer membrane composed of two layers of phospholipids, proteins, and lipopolysaccharides. Lipopolysaccharides play a crucial role in the structure of the outer membrane of gram-negative bacteria (Astriani *et al*., 2021).

Antibacterial testing is performed using the well diffusion method, which offers the advantage of achieving higher concentrations compared to the disc method. This allows for faster, maximum, and even diffusion in the media (Rahmawati and Rahardhian, 2025). Variations in the size of the inhibition zone at different doses may result from several factors, including the rate of diffusion of antimicrobial components into the medium, the sensitivity of bacterial growth, the incubation temperature and time, and the metabolic activity of the microorganisms (Salni, *et al*., 2011).

The negative control that used, is the solvents of each extract which is ethyl acetate and methanol to demonstrate that these solvents do not influence the antibacterial activity of the test solution. This indicates that the observed activity is due to the substances present in the extract, rather than the solvents used. The positive control used in this experiment is chloramphenicol, a broad-spectrum antibiotic that inhibits bacterial protein synthesis by targeting the 50S subunit of the ribosome. The purpose of including a positive control in this test is to compare the effectiveness of standard antibacterial medications with the test solutions (Mengko, *et al*., 2022). Chloramphenicol was dissolved in 10% DMSO, a solvent that can effectively dissolve both polar and nonpolar molecules. Additionally, since DMSO does not inhibit bacterial growth, it does not affect the results of antibacterial activity testing. Therefore, 10% DMSO was used in this test as a control for chloramphenicol, demonstrating that the solvent had no impact on the antibacterial efficacy of the positive control. It is important to note that because DMSO can permeate cell membranes, the concentration of DMSO used as a solvent should not exceed 10%, as higher concentrations could disrupt the integrity of the cell membrane (Manalu, 2017).

Conclusions

This study shows that the semi-polar and polar fractions of red palm fruit contain the same secondary metabolites, namely phenolics, tannins, and saponins. However, with different levels of polarity, the two fractions also exhibit different antibacterial activities. With the highest concentration in both fractions, the semi-polar fraction (ethyl acetate) exhibited antibacterial activity against both type of bacteria, reaching a maximum inhibition zone of 12.51 mm in *Staphylococcus aureus* and 14.38 mm in *Escherichia coli*. In contrast, the polar

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fraction (methanol) only has antibacterial activity against *Escherichia coli* bacteria with a maximum inhibition zone of 12.28 mm. Overall, the semi-polar fraction of red palm fruit is the most promising fraction to become the antibacterial compounds.

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