

Antimicrobial of ethanolic extract from marpuyan stem bark (*Rhodamnia cinerea* Jack)

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DOI: 10.29303/aca.v7i1.193

Article info:

Received 01/02/2024

Revised 12/04/2024

Accepted 15/04/2024

Available online 18/04/2024

Abstract: The Bark of Marpuyan Tree (*Rhodamnia cinerea* Jack) is a plant that has been empirically used by communities in Indonesia for the treatment of infectious diseases caused by bacteria and fungi. The bark of Marpuyan contains flavonoids, phenolics, and saponins. This study aimed to evaluate the antibacterial and antifungal activities of the ethanolic extract of the bark of Marpuyan using the disc diffusion method at various extract concentrations, namely 30%, 25%, 15%, 10%, and 5%. The results of the antibacterial activity test against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*) showed inhibitory zones with respective diameters of 11.67±0.21 mm, 12.29±0.43 mm, and 12.78±0.14 mm. These results indicate weak antibacterial activity. In the antibacterial activity test against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*), inhibitory zones were found with respective diameters of 13.05±0.05 mm, 12.58±1.78 mm, and 10.98±0.21 mm. These results also indicate weak antibacterial activity. However, in the antifungal activity test, no activity was found against *Candida albicans* and *Malassezia furfur*. Nevertheless, the ethanol extract of Marpuyan bark showed moderate antifungal activity against *Trichophyton mentagrophytes*, with an inhibitory zone diameter of 15.44±2.02 mm.

Keywords: antimicrobial, ethanol extract, Marpuyan, Stem bark.

Citation: Nasution, M. R. ., Cahyani, A. R. ., Aqilla, A., Fitri, V. ., & Triani, L. . (2024). Antimicrobial of Ethanolic Extract from Marpuyan Stem Bark (*Rhodamnia cinerea* Jack). *Acta Chimica Asiana*, 7(1), 426–236. <https://doi.org/10.29303/aca.v7i1.193>

INTRODUCTION

Infections are often caused by bacteria, fungi, and viruses, and they commonly occur in developing countries.[1] The usual treatment approach involves the use of antibiotic therapy. However, the frequent use of antibiotics has led to various issues and has become a global threat to health, particularly in terms of bacterial resistance to antibiotics. Therefore, treatment utilizing natural ingredients has gained increasing popularity among the public due to their minimal side effects.[2]

Several bacteria that cause infections include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*, which can lead to skin infections and abscess formation.

Additionally, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi* can cause diarrhea transmitted through food contamination. Infections caused by *Candida albicans* can result in candidiasis, while *Trichophyton mentagrophytes* is a causative agent of dermatophytosis, and *Malassezia furfur* causes a condition known as versicolor or tinea.[3]

Marpuyan (*Rhodamnia cinerea* Jack) is a type of a medicinal plant belonging to the Myrtaceae family.[4] This plant is found in several countries such as Malaysia, China, Thailand, and Indonesia. In Indonesia, Marpuyan is known by various names such as ampuyan, andong, ki beusi, marampuyan, siri-siri, talinga, merapin, and sekala. In the Riau region, Marpuyan leaves have been traditionally used to treat wounds, scabies,

stomachaches, and diarrhea. Furthermore, Marpuyan leaves also have an effect as a remedy for relieving skin itching caused by fungi or insect bites. The Dayak Ngaju community in Central Kalimantan uses Marpuyan tree bark as a remedy for treating diarrhea.[5]

The research conducted by Lakornwong revealed that stem of *Rhodamnia dumetorum* exhibits antibacterial activities.[6] A study by Aminah reported that the acetone extract of Marpuyan leaves can inhibit the growth of various types of bacteria, including *Staphylococcus*, Enteropathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, and *Shigella sp.*[7] Furthermore, Wiart et al (2004) also reported that the ethanol extract of Marpuyan leaves has antibacterial activity against *Staphylococcus aureus*.[8]

Research conducted by Nasution and Sopiarti reported that the ethanol extract of Marpuyan leaves is a traditional medicinal plant that contains flavonoid and phenolic compounds with antioxidant and sunscreen activities.[9,10] The chemical composition of *Rhodamnia cinerea* Jack includes α -pinene, β -pinene, α -eudesmol, β -eudesmol, γ -eudesmol, caryophyllene, globulol, viridiflorol, spathulenol, 1,8-cineole, and sesquiterpenes [11].

The phytochemical content in a plant is influenced by several factors, both internal and external. Internal factors include plant genetics, while external factors include light, temperature, humidity, pH, nutrient content in the soil, and altitude.[12] Secondary metabolites tend to accumulate during various stages of plant growth, including roots, stems, leaves, and flowers. This process is influenced by genetic, ontogenic, morphogenetic, and environmental factors.[13,14]

Based on the aforementioned explanations, it has been reported that Marpuyan leaves exhibit significant antibacterial activity. However, there is still limited research reporting on the antimicrobial activity of Marpuyan tree bark from Riau Province. Therefore, in this study, the researchers were interested in testing the antibacterial and antifungal activities of the ethanol extract of Marpuyan tree bark (*Rhodamnia cinerea* Jack) using the disc diffusion method. This research is expected to provide scientific data on the potential antimicrobial activity of Marpuyan tree bark,

which can serve as a basis for the development of more effective natural drugs in combating bacterial and fungal infections. Additionally, this study contributes to the scientific understanding of the properties of the Marpuyan plant and its potential use in traditional medicine.

METHODS

Equipment

The equipment used in this research included a spirit lamp, Petri dishes, test tubes, stirring rods, tissues, plastic wrap, corn threads, masks, gloves, test tube racks, tweezers, Ose needles, calipers, Erlenmeyer flasks (Pyrex®), beakers (Pyrex®), Laminar Air Flow (LAF), analytical balance (Shimadzu®), incubator (Mettler®), autoclave (GEA®), oven (Mettler®), micropipettes (Nesco®), hot plate (Thermo®), vortex mixer (As One®), UV-VIS spectrophotometer (Spectrum SP-UV 300SRB®), and a refrigerator (Aqua®).

Materials

The materials used in this research included Marpuyan tree bark (*Rhodamnia cinerea* Jack), bacterial cultures, fungal cultures, sterile disc papers, sterile distilled water (aquadest), 96% ethanol, Nutrient Agar (NA) media, Potato Dextrose Agar (PDA) media, Ciprofloxacin 5 μ g discs, Ketoconazole 15 μ g discs, Dimethyl Sulfoxide (DMSO), 0.9% physiological NaCl solution, chloroform, concentrated HCl, Mg metal, FeCl₃, activated charcoal (norit), 0.05 M ammonium chloroform, concentrated sulfuric acid, 2 N sulfuric acid, Mayer's reagent, and Lieberman Bouchard reagent.

Antimicrobial Activity

Test Phytochemical Screening of the Extract

Preliminary screening was conducted to identify the presence of secondary metabolites in the crude extract of Marpuyan tree bark. The parameters examined included alkaloids, flavonoids, saponins, phenolics, steroids, and terpenoids.

Bacterial and Fungal Subculturing

Subculturing was performed by inoculating one or two microbial isolates from each pure stock into fresh media. For bacteria, inoculation was done by streaking them on Nutrient Agar (NA) medium and then incubating at 37°C for 24 hours. Meanwhile, for fungi, inoculation was done by placing them on Potato Dextrose Agar

(PDA) medium and incubating at 25°C for 72 hours.

Preparation of Bacterial and Fungal Suspensions

The revitalized microorganisms were streaked with 3-4 streaks and then transferred into test tubes filled with physiological saline solution (NaCl). Next, the samples were homogenized using a vortex. The turbidity of each suspension was measured using a UV-Vis spectrophotometer to obtain a suspension with 25% transmittance at a wavelength of 580 nm for bacteria and 90% transmittance at a wavelength of 530 nm for fungi.

For the inoculation of bacteria and fungi, 0.3 mL of the test microbial suspension was added to a Petri dish. Then, 15 mL of Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media were poured into the Petri dish. The mixture was then homogenized and allowed to solidify.

Preparation of Test Solutions

0.3 grams of ethanol extract of Marpuyan bark was weighed and dissolved in 1 mL of DMSO solution, resulting in a solution with a concentration of 30% (w/v). From the stock solution, dilutions were made using 1 mL of DMSO to obtain solutions with concentrations of 25%, 15%, 10%, and 5% (w/v).

Determination of Antibacterial and Antifungal Activity with Disc Method

10 µL of ethanol extract of Marpuyan bark was dropped onto each disc using a micropipette, and the discs were then placed on separate Petri dishes until partially dried. The discs that have been treated with the test solution were inoculated onto the solidified culture media. The Petri dishes were then covered with plastic wrap and incubated for 24 hours at 37°C for bacterial growth, while for fungal growth, they were incubated for 120 hours at 25°C in an inverted position. Microbial growth was observed, and the diameter of the inhibition zones formed was measured using a caliper. As a negative control, discs treated with 10 µL of DMSO were used, while a positive control was established using discs containing 5 µg/disk of the antibiotic Ciprofloxacin for bacteria and 15 µg/disk of Ketoconazole for fungi.

Data Analysis

The antibacterial activity data can be observed by measuring the diameter of the inhibition zones around the paper discs at each test

concentration using a caliper, and then calculating the average diameter of inhibition. Next, the data will be analyzed using ANOVA method, and Tukey's test will be conducted to determine significant differences between treatment groups.

RESULTS AND DISCUSSION

The study utilized samples of Marpuyan bark (*Rhodamnia cinerea* Jack) obtained from Kuapan Village, Tambang District, Kampar Regency, Riau Province. Subsequently, the samples were identified at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau (Table 1).

Table 1. Sample Identification Results.



Marpuyan Plant	Simplisia (Marpuyan Stem Bark)
	

Table 2. Sample Identification Results.

Secondary Metabolite	Result	
	Simplisia Stem Bark of Marpuyan	Ethanol Extract of Marpuyan
Alkaloid	-	-
Flavonoid	+	+
Fenolic	+	+
Saponin	+	+
Terpenoid	-	-
Steroid	-	-

The extraction of Marpuyan bark was conducted using the maceration method with 96% ethanol as the solvent. The maceration process was repeated three times with the replacement of 96% ethanol solvent in each repetition, using different volumes for each repetition. The resulting macerate was then concentrated using a rotary evaporator to obtain a thick extract. Prior to conducting the antibacterial and antifungal activity tests, phytochemical screening was performed on the crude bark and ethanol extract of Marpuyan. Phytochemical screening aims to identify the presence of secondary metabolites in the sample. Based on the phytochemical screening, flavonoids, phenolics, saponins,

and terpenoids were found in both the fresh sample and extract (Table 2).

The basic principles of antimicrobial activity testing using the Kirby-Bauer disc diffusion method in this study involve the use of ethanol extract being tested as the disc that is applied onto the disc paper. Next, the discs are placed on a medium that has been inoculated with the test microorganism. Afterward, the samples are incubated until a zone of inhibition is formed around the discs.[15]

The test bacteria used in this study consisted of Gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*. Meanwhile, the Gram-negative bacteria used included *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, representing the Gram-negative bacterial group. Additionally, the test fungi used included *Candida albicans*, *Malassezia furfur*, and *Trichophyton mentagrophytes*.

The test microorganisms to be used in this study need to be revitalized before their usage. Revitalization of the test microorganisms is important because during the logarithmic growth phase, the metabolic activity of microorganisms is at its optimum and most active state. During this phase, the synthesis of cellular materials occurs rapidly and at a constant rate. Therefore, using microorganisms in this logarithmic phase as the inoculum has advantages in research.[16] In this study, the media used for bacterial revitalization and inoculum cultivation is Nutrient Agar (NA), while for fungi, Potato Dextrose Agar (PDA) is used. NA and PDA media contain the necessary nutrients to support microbial growth. The available nutrients in these media provide optimal conditions for microorganisms to grow and develop well.[17]

The test bacteria are then suspended in a physiological NaCl solution (0.9% w/v) to create a bacterial suspension. The use of physiological NaCl solution as the suspension medium is because it provides an isotonic environment for the test bacteria. 18 An isotonic environment refers to a condition where the concentration of the surrounding fluid is equal to the concentration of the fluid inside the bacterial cell. This prevents excessive movement of water into or out of the bacterial cells. The purpose of creating a bacterial suspension is to achieve population

uniformity within the suspension, enabling accurate testing. The turbidity of the bacterial suspension is measured using a UV-Vis spectrophotometer at a wavelength of 580 nm. At this wavelength, the bacterial transmittance should reach 25% to ensure that the cell density in the suspension is at an optimal condition for antibacterial testing .[19]

Bacteria have the ability to cause light scattering. Therefore, when light passes through a cuvette containing a bacterial suspension, some of the light will be scattered by the bacteria, while the remaining portion will be transmitted and detected by the detector. The intensity of light detected by the detector is then measured as a percentage of transmittance. This measurement provides information about the level of turbidity of the bacterial suspension used in the research.

The antibacterial and antifungal activity testing of the ethanol extract from the bark of Marpuyan was conducted using five concentration series: 30%, 25%, 15%, 10%, and 5%. These concentrations were obtained by dissolving the extract in Dimethyl Sulfoxide (DMSO) as the solvent. The use of DMSO as a solvent is commonly employed in biological activity testing to aid in the solubility of compounds that are insoluble in water.[20] In this testing, a negative control is included, which consists of discs containing only DMSO solvent. This is important to ensure that the observed effects are due to the ethanol extract from the bark of Marpuyan tree and not from the DMSO solvent itself.

Furthermore, there are also positive controls used as a reference in the antibacterial and antifungal testing. In the antibacterial testing, the positive control used is Ciprofloxacin with a concentration of 5 µg/disk. Meanwhile, in the antifungal testing, the positive control used is Ketoconazole with a concentration of 15 µg/disk. The use of positive controls is useful for comparing the activity of the extract with tested antimicrobial substances that have known effects. In evaluating the test results, the Clinical Laboratory Standards Institute (CLSI) provides criteria for classifying the strength of antibacterial and antifungal activity based on the diameter of the inhibition zones formed. According to the CLSI categories, an inhibition zone diameter of ≥20 mm is considered strong, 15-19 mm is considered moderate, and ≤14 mm is considered weak.[21]

Based on the results of the antibacterial activity testing, the ethanol extract from the bark of Marpuyan (*Rhodamnia cinerea* Jack) exhibited weak antibacterial activity against Gram-positive bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*, as well as Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. The testing was conducted using five concentration series: 30%, 25%, 15%, 10%, and 5%.

For *Staphylococcus aureus*, the ethanol extract of Marpuyan tree bark showed antibacterial activity with inhibition zone diameters of 11.67 ± 0.21 mm, 8.45 ± 0.18 mm, 7.18 ± 0.29 mm, 6.72 ± 0.17 mm, and 6.12 ± 0.04 mm, respectively, categorizing it as weak activity. Similarly, for *Staphylococcus epidermidis*, the ethanol extract exhibited weak antibacterial activity with inhibition zone diameters of 12.29 ± 0.43 mm, 8.90 ± 0.25 mm, 7.05 ± 0.26 mm, 6.67 ± 0.20 mm, and 6.12 ± 0.03 mm. The antibacterial activity against *Bacillus subtilis* was also weak, with inhibition zone diameters of 12.78 ± 0.14 mm, 8.48 ± 0.19 mm, 7.60 ± 0.80 mm, 6.47 ± 0.20 mm, and 6.17 ± 0.08 mm. Table 3 and Figure 1 present detailed results of the testing. Overall, the antibacterial activity testing indicated that the ethanol extract from Marpuyan bark has weak antibacterial activity against the tested bacteria.

The results indicate that the ethanol extract from the bark of Marpuyan exhibits stronger antibacterial activity against Gram-positive bacteria compared to Gram-negative bacteria. This difference in activity could be attributed to the variations in the cell wall structures of Gram-positive and Gram-negative bacteria, which can influence their susceptibility to the active compounds in the extract. [22] Although the antibacterial activity is categorized as weak, it is important to note that it should not be disregarded, as there is still potential for further development by optimizing the formulation and exploring combinations with other active ingredients.

Based on the results of the antibacterial activity testing of the ethanol extract from the bark of Marpuyan against Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, using concentrations of 30%, 25%, 15%, 10%, and 5%, the findings indicate weak antibacterial activity. In the case of *Escherichia*

coli, the extract displayed antibacterial activity with inhibitory zone diameters of 13.05 ± 0.05 mm, 12.90 ± 0.05 mm, 11.04 ± 0.01 mm, 9.63 ± 0.10 mm, and 7.30 ± 0.08 mm, classified as weak. Similarly, for *Pseudomonas aeruginosa*, the observed antibacterial activity was weak, with inhibitory zone diameters of 12.58 ± 1.78 mm, 12.18 ± 1.85 mm, 9.93 ± 0.68 mm, 8.93 ± 0.16 mm, and 7.37 ± 0.94 mm. The results also showed weak antibacterial activity against *Salmonella typhi*, with inhibitory zone diameters of 10.98 ± 0.21 mm, 9.73 ± 0.68 mm, 8.53 ± 0.25 mm, 7.98 ± 0.45 mm, and 7.02 ± 0.43 mm.

These findings indicate that the ethanol extract from the bark of Marpuyan has weaker antibacterial activity against Gram-negative bacteria compared to Gram-positive bacteria, as previously explained. This difference could be attributed to the variations in the cell wall structures of Gram-positive and Gram-negative bacteria, which can affect their susceptibility to the active compounds in the extract. In this case, the weak antibacterial activity against Gram-negative bacteria may indicate that the active compounds in the ethanol extract from the bark of Marpuyan are not as effective against the tested Gram-negative bacterial strains. It is important to note that although the observed antibacterial activity is weak, it should not be disregarded, as there is still potential for further development by optimizing the formulation and exploring combinations with other active ingredients. Additionally, further research is needed to understand the mechanism of action and the potential applications of the ethanol extract from the bark of Marpuyan in the development of more effective antibacterial agents.

The results indicate that the ethanol extract of marpuyan bark has more effective antibacterial activity against Gram-positive bacteria, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*, compared to Gram-negative bacteria. This can be attributed to the differences in the cell wall structure of Gram-positive and Gram-negative bacteria, which influence their susceptibility to the active compounds in the extract (activity could be attributed to the variations in the cell wall structures of Gram-positive and Gram-negative bacteria, which can influence their susceptibility to the active compounds in the extract.[22] Although the antibacterial activity exhibited is considered weak, the results still demonstrate the potential use of ethanol

extract of marpuyan bark as a source of active compounds for the development of antimicrobial agents. Further research is needed to understand the mechanism of action of the extract and optimize formulations to enhance the effectiveness of antibacterial activity against Gram-negative bacteria. Additionally, combining it with other active ingredients or techniques such as nanoemulsions or nanoparticles could be an interesting approach to enhance the potential use of ethanol extract of marpuyan bark in the field of antimicrobials.[23]

In the test results, the ethanol extract of marpuyan bark exhibited weak antibacterial activity against both Gram-positive and Gram-negative bacteria. One factor that may contribute to this is the difficulty of antibacterial compounds in the extract to penetrate the bacterial cell wall. According Kasim. (2016), for the compounds to effectively kill bacteria, they need to enter the cell through the bacterial cell wall.[21]

In this case, the cell wall structure of Gram-negative bacteria has a thick outer layer of lipopolysaccharides, which can act as a barrier for the active compounds in the ethanol extract of marpuyan bark to reach their target. On the other hand, the cell wall of Gram-positive bacteria is thinner and less complex, making it easier for the active compounds to penetrate.

To enhance the effectiveness of the antibacterial activity of the ethanol extract of marpuyan bark, several strategies can be considered. One approach is to improve the penetration of active compounds into the cell wall of Gram-negative bacteria. Techniques such as combining the extract with membrane-permeabilizing peptides or nanoparticles can enhance the penetration of active compounds into Gram-negative bacterial cells. Furthermore, optimization of extraction methods and purification can increase the concentration of effective active compounds in the extract.

Further research is needed to identify the active compounds in the ethanol extract of marpuyan bark and understand their mechanisms of action against Gram-positive and Gram-negative bacteria. With a deeper understanding, more effective formulation development and combination with other antimicrobial agents can be explored to enhance the potential of marpuyan bark ethanol extract as an antibacterial agent.

The explanation of the differences in the cell wall structure of Gram-positive and Gram-negative bacteria can shed light on their sensitivity to antibacterial compounds.[22] The cell wall of Gram-positive bacteria consists of approximately 40 layers of peptidoglycan, with peptidoglycan accounting for about 70% of the dry weight of the cell wall. This makes the cell wall of Gram-positive bacteria thick and rigid. On the other hand, Gram-negative bacteria have a peptidoglycan layer that accounts for only about 10% of the dry weight of the cell wall, resulting in a thinner cell wall. Gram-negative bacteria also have a higher lipid content and porin proteins that act as entry channels for active compounds into the bacterial cell.

These differences have implications for the sensitivity of bacteria to antibacterial compounds. The thicker cell wall of Gram-positive bacteria hinders the penetration of antibacterial compounds into the cell. The antibacterial compounds must be able to penetrate the peptidoglycan layer to reach their targets within the bacterial cell. On the other hand, the thinner cell wall of Gram-negative bacteria facilitates the penetration of active compounds into the cell. The porin proteins in the outer membrane of Gram-negative bacteria also act as entry channels for active compounds into the bacterial cell.

Once the active compounds enter the bacterial cell, they can interact with various targets, such as enzymes or structural components within the cell. These interactions can disrupt the activity of important enzymes or interfere with structural functions, ultimately leading to damage and death of the bacterial cell.[24]

This explanation highlights that the differences in cell wall structure between Gram-positive and Gram-negative bacteria have an impact on their sensitivity to antibacterial compounds. This understanding is crucial for the development of effective treatment strategies and the discovery of new antibacterial compounds that can selectively target Gram-positive and Gram-negative bacteria.[22]

Furthermore, Gram-negative bacteria have an outer membrane system composed of a phospholipid layer on the inner side and lipopolysaccharide on the outer side. The nonpolar nature of this layer can affect the permeability of active compounds into the bacterial cell. The phospholipid layer in the outer membrane is hydrophobic, which can

inhibit the penetration of polar compounds into the cell. The lipopolysaccharide on the outer side of the outer membrane also has a complex structure that strengthens the membrane barrier and prevents the entry of active compounds into the bacterial cell.[25]

The nonpolar nature of the outer membrane layer in Gram-negative bacteria provides additional protection against polar antibacterial compounds since polar compounds typically have difficulty crossing nonpolar layers. This may explain why Gram-negative bacteria are often more resistant to antibacterial compounds compared to Gram-positive bacteria. By understanding the differences in cell wall structure and the outer membrane system between Gram-positive and Gram-negative bacteria, we can develop more effective strategies in designing antibacterial compounds that can penetrate these barriers and inhibit bacterial growth more efficiently.

In this study, a positive control in the form of the antibiotic ciprofloxacin was used, which showed an average diameter of inhibition zone of 28.73 mm. The use of ciprofloxacin as a positive control is based on its effectiveness against both Gram-negative and Gram-positive bacteria. Ciprofloxacin works by a bactericidal mechanism at the ribosome within the cell. Additionally, ciprofloxacin is also effective against bacteria that are resistant to other antibiotics such as penicillin, aminoglycosides, cephalosporins, and tetracycline.[26]

The use of an appropriate positive control in the testing is crucial to compare the antibacterial activity of the ethanol extract of marpuyan stem bark with a proven effective standard. By using ciprofloxacin as the positive control, we can evaluate the extent to which the ethanol extract of marpuyan stem bark has potential as an antibacterial agent in influencing the growth of Gram-negative and Gram-positive bacteria.

The negative control used in this test is DMSO. DMSO is an organic solvent that does not possess bactericidal properties, thus it does not have a direct effect on bacterial growth. Additionally, DMSO is capable of dissolving both polar and non-polar compounds.

The use of DMSO as a solvent in diluting the extract aims to obtain the desired concentration of the extract. By using DMSO as the negative control, we can ensure that

any observed inhibition zone response is solely due to the active components of the extract and not the solvent used.

The use of a negative control is crucial in antibacterial activity testing as it allows us to differentiate the actual effects caused by the extract from any potential effects caused by the solvent. Consequently, the test results can be more accurate and correctly interpreted when evaluating the effectiveness of the ethanol extract of marpuyan stem bark as an antibacterial agent.

The results of the antibacterial activity test of the ethanol extract of marpuyan stem bark against the negative control DMSO showed no inhibition zones. This result confirms that the use of DMSO as a solvent does not affect the test results, and the observed antibacterial response is attributed to the extract itself. In the antifungal activity test, the ethanol extract of marpuyan stem did not exhibit antifungal activity against *Candida albicans* and *Malassezia furfur*. This can be observed from the absence of inhibition zones at various concentrations of the ethanol extract of marpuyan stem bark, namely 30%, 25%, 15%, 10%, and 5%. Only the positive control showed inhibition zones in the antifungal test against *Candida albicans* and *Malassezia furfur*, with inhibition zone diameters of 37.78 ± 3.97 mm and 38.77 ± 1.97 mm, respectively.

However, in the antifungal test against *Trichophyton mentagrophytes*, the ethanol extract of marpuyan stem bark showed weak to moderate activity. The diameter of the inhibition zones varied depending on the extract concentration, with average diameters of 8.72 ± 0.44 mm, 12.16 ± 1.66 mm, and 15.44 ± 2.02 mm at concentrations of 15%, 25%, and 30%, respectively. However, at concentrations of 15% and 5%, the extract did not show antifungal activity, as indicated by the absence of inhibition zones at those concentrations (Table 5, Figure 3).

The increase in concentration of the ethanol extract of stem bark in this study was associated with an increase in the size of the inhibition zones. This indicates that the higher the extract concentration, the stronger its ability to inhibit bacterial growth. The cause of this phenomenon can be explained by the presence of bioactive compounds in the extract.

Previous research conducted by Debalke has demonstrated that the ability of an extract to inhibit bacterial growth depends on its concentration.[27] The higher the concentration of the extract, the higher the level of bioactive compounds involved in antimicrobial activity. Therefore, increasing the concentration of the ethanol extract of marpuyan stem bark can enhance its antimicrobial activity.

Furthermore, the effectiveness of the ethanol extract of marpuyan stem bark in inhibiting bacterial growth may be attributed to the presence of secondary metabolites such as phenolics, flavonoids, and saponins. Phenolic and flavonoid compounds have been shown to have significant antimicrobial activity against various types of bacteria. Saponins have also been recognized as potential antimicrobial agents. [28] The presence of these compounds in the ethanol extract of marpuyan stem bark may contribute to its antimicrobial activity.

Table 3. Inhibition Diameter Measurement of Ethanol Extract of Marpuyan (*Rhodamnia cinerea* Jack) Stem Bark Against 3 Types of Gram-Positive Bacteria.

Bacteria	% b/v	Inhibition Zone Diameter (mm)			Mean±SD	Inhibition Category
		1	2	3		
<i>Staphylococcus aureus</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	22,43	23,58	23,94	23,31±0,78 ^e	Strong
	30%	11,45	11,68	11,88	11,67±0,21 ^d	Weak
	25%	8,25	8,50	8,60	8,45±0,18 ^c	Weak
	15%	7,51	7,09	6,94	7,18±0,29 ^b	Weak
	10%	6,55	6,89	6,73	6,72±0,17 ^{ab}	Weak
	5%	6,11	6,09	6,18	6,12±0,04 ^{ab}	Weak
<i>Staphylococcus epidermidis</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	23,15	23,85	24,58	23,86±0,71 ^e	Strong
	30%	12,63	12,45	11,80	12,29±0,43 ^d	Weak
	25%	9,15	8,65	8,92	8,90±0,25 ^c	Weak
	15%	7,35	6,92	6,88	7,05±0,26 ^b	Weak
	10%	6,44	6,75	6,82	6,67±0,20 ^b	Weak
	5%	6,12	6,09	6,15	6,12±0,03 ^a	Weak
<i>Bacillus subtilis</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	22,47	22,75	22,63	22,61±0,14 ^f	Strong
	30%	12,76	12,64	12,94	12,78±0,15 ^e	Weak
	25%	8,61	8,58	8,26	8,48±0,19 ^d	Weak
	15%	7,69	7,53	7,59	7,60±0,80 ^c	Weak
	10%	6,24	6,61	6,57	6,47±0,20 ^b	Weak
	5%	6,14	6,11	6,27	6,17±0,08 ^{ab}	Weak

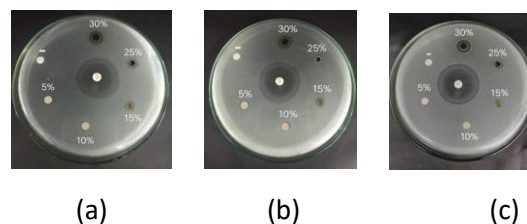


Figure 1. Activity Test of Ethanol Extract of Marpuyan (*Rhodamnia cinerea* Jack) Stem Bark Against (a) *Staphylococcus aureus*, (b) *Staphylococcus epidermidis* and (c) *Bacillus subtilis*.

Table 4. Inhibition Diameter Measurement of Ethanol Extract of Marpuyan (*Rhodamnia cinerea* Jack) Stem Bark Against 3 Types of Gram-Negative Bacteria.

Bacteria	% b/v	Inhibition Zone Diameter (mm)			Mean±SD	Inhibition Category
		1	2	3		
<i>Escherichia coli</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	31,8	31,4	31,5	31,56±0,20 ^f	Strong
	30%	13,05	13,00	13,10	13,05±0,05 ^e	Weak
	25%	12,85	12,90	12,95	12,90±0,05 ^e	Weak
	15%	11,05	11,05	11,03	11,04±0,01 ^d	Weak
	10%	9,55	9,75	9,60	9,63±0,10 ^c	Weak
	5%	7,35	7,35	7,20	7,37±0,08 ^b	Weak
<i>Pseudomonas aureginosa</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	38,50	37,30	37,80	37,87±0,60 ^e	Strong
	30%	14,60	11,25	11,90	12,58±1,78 ^d	Weak
	25%	14,20	11,80	10,55	12,18±1,85 ^d	Weak
	15%	9,95	10,60	9,25	9,93±0,68 ^c	Weak
	10%	9,05	9,00	8,75	8,93±0,16 ^{bc}	Weak
	5%	7,15	6,55	8,40	7,37±0,94 ^b	Weak
<i>Salmonella typhi</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	29,40	28,30	28,50	28,73±0,59 ^f	Strong
	30%	10,75	11,15	11,05	10,98±0,21 ^e	Weak
	25%	9,85	9,00	10,35	9,73±0,68 ^d	Weak
	15%	8,25	8,65	8,70	8,53±0,25 ^c	Weak
	10%	7,50	8,40	8,05	7,98±0,45 ^b	Weak
	5%	6,70	7,50	6,85	7,02±0,43 ^b	Weak

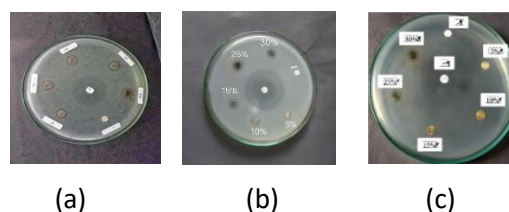
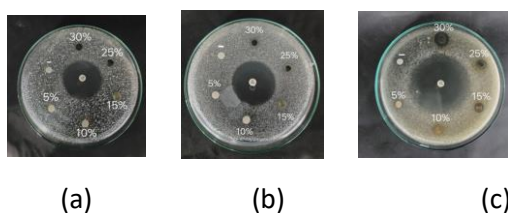


Figure 2. Results of Gram-Negative Antibacterial Activity Test of Ethanol Extract of Marpuyan (*Rhodamnia cinerea* Jack) Stem Bark Against (a) *Escherichia coli*, (b) *Pseudomonas aureginosa*, and (c) *Salmonella typhi*.

Table 5. Measurement of Inhibition Zone Diameter of Ethanol Extract of Marpuyan (*Rhodamnia cinerea* Jack) Stem Bark Against 3 Types of Fungi

Fungi	% b/v	Inhibition Zone Diameter (mm)			Mean±SD	Inhibition Category
		1	2	3		
<i>Candida albicans</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	34,65	36,45	42,26	37,78±3,97 ^b	Strong
	30%	6,00	6,00	6,00	6,00 ^a	None
	25%	6,00	6,00	6,00	6,00 ^a	None
	15%	6,00	6,00	6,00	6,00 ^a	None
	10%	6,00	6,00	6,00	6,00 ^a	None
	5%	6,00	6,00	6,00	6,00 ^a	None
<i>Malassezia furfur.</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	37,72	37,55	41,06	38,77±1,97 ^b	Strong
	30%	6,00	6,00	6,00	6,00 ^a	None
	25%	6,00	6,00	6,00	6,00 ^a	None
	15%	6,00	6,00	6,00	6,00 ^a	None
	10%	6,00	6,00	6,00	6,00 ^a	None
	5%	6,00	6,00	6,00	6,00 ^a	None
<i>Trichophyton mentagrophytes</i>	-	6,00	6,00	6,00	6,00 ^a	None
	+	27,38	36,03	39,29	34,23±6,15 ^e	Strong
	30%	17,78	14,38	14,17	15,44±2,02 ^d	Medium
	25%	12,44	10,37	13,67	12,16±1,66 ^c	Weak
	15%	8,73	8,27	9,16	8,72±0,44 ^b	Weak
	10%	6,00	6,00	6,00	6,00 ^a	None
	5%	6,00	6,00	6,00	6,00 ^a	None

**Figure 3.** Results of Antifungal Activity Test of Ethanol Extract of Marpuyan (*Rhodamnia cinerea* Jack) Stem Bark Against (a) *Candida albicans*, (b) *Malassezia furfur*, and (c) *Trichophyton mentagrophytes*

The mechanism of action of phenolic compounds against antibacterial activity is through disruption of the peptidoglycan in the cell wall of Gram-positive bacteria. Phenolic compounds can inhibit the formation of N-acetylmuramic acid bonds in the mucopeptide structure, which is responsible for maintaining the rigidity of the cell wall. As a result, the synthesis of the bacterial cell wall is disrupted and not formed properly. This causes damage to the cell wall of Gram-positive bacteria and inhibits their growth. Flavonoids, on the other hand, have a different mechanism of action in their antibacterial activity. Flavonoids can exert energy transduction on the bacterial cytoplasmic membrane and inhibit bacterial motility. The hydroxyl groups present in the structure of flavonoids can cause changes in the organic components within the bacteria

and disrupt nutrient transportation, ultimately leading to a toxic effect on bacteria.[29]

Saponins have the ability to act as antibacterial agents with a different mechanism of action as well. The mechanism of action of saponins against bacteria involves disruption of the permeability of the bacterial cell membrane. Saponins can cause damage to the cell membrane, leading to lysis of bacterial cells and release of cell components such as proteins, nucleic acids, and nucleotides. Disruption of the bacterial cell membrane results in bacterial death and cessation of growth.[27]

CONCLUSION

The conclusion of this study is that the ethanol extract of *Rhodamnia cinerea* Jack bark contains secondary metabolites, including phenolics, flavonoids, and saponins. The bark extract exhibits antibacterial activity against Gram-positive bacteria, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*, as well as Gram-negative bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. The antibacterial activity is categorized as weak in the concentration range of 5-30%. However, against fungi, the ethanol extract of *Rhodamnia cinerea* Jack bark at concentrations of 5-10% does not show antifungal activity against *Candida albicans* and *Malassezia furfur*. However, against *Trichophyton mentagrophytes*, the bark extract exhibits moderate antifungal activity at concentrations of 15-30% and weak to moderate activity. It should be noted that the antimicrobial activity of the ethanol extract of *Rhodamnia cinerea* Jack bark is lower compared to the positive controls used in this study.

ACKNOWLEDGEMENTS

We thank the management of Riau College of Pharmaceutical Sciences for the enabling environment for the research for the use of their facilities for laboratory work.

CONFLICT OF INTERESTS

All authors declare to have no conflict of interests

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