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Bioactivity of Red Leaf Extract of Pucuk Merah Plant (*Syzygium myrtifolium* Walp.)

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Abstract

Syzygium myrtifolium Walp. is an ornamental plant traditionally used as an herbal medicine. Ethanol extract of *Syzygium myrtifolium* Walp. has been reported to have activities as an antioxidant, anticancer, and antimicrobial. In this study, Syzygium myrtifolium Walp is extracted. Leaves were carried out using a multistage maceration method using methanol, ethyl acetate, and hexane solvents. Antioxidant, antimicrobial, and toxicity tests were performed, and secondary metabolite compounds, total flavonoid content, and total phenolic content were identified on red leaf extract. Total phenolic content was tested using *the Folin-Ciocalteau reagent,* total flavonoids with the $AlCl_3$ reagent, antioxidants with the DPPH method, antimicrobial using the disc diffusion method, and toxicity tested using the BSLT method. The result showed that *Syzygium myrtifolium* Walp. Red leaf extract contained a secondary metabolite of triterpenoids, steroids, phenolics, flavonoids, alkaloids, and coumarins. The ethyl acetate and methanol extract's total phenolic and flavonoid content were 311.0520 and 420.360 mg GAE/g extract and 211.788 and 105.360 mg QE/g extract. Antioxidant activity of ethyl acetate and methanol showed that the extracts were highly antioxidant with IC_{50} of 19.606 and 12.0656 mg/L. The antimicrobial activity showed that the hexane extract was resistant to the bacteria Staphylococcus aureus and Propionibacterium acnes and the fungus *Candida albicans*. However, no inhibition zones were produced for Pseudomonas aerigunosa and Escherichia coli bacteria. The toxicity test showed that the hexane and ethyl acetate extract had strong toxic properties with LC_{50} of 56.65 and 16.79 mg/L, and the methanol extract was toxic with LC_{50} of 224.2846 mg/L.

Keywords: *Syzygium myrtifolium; total phenolic content; total flavonoids content; antimicrobial activity; toxicity*

Introduction

The community utilizes the diversity of plants in Indonesia as a source of traditional medicinal ingredients to cure various diseases because they contain natural bioactive compounds, including the pucuk merah plant (*Syzygium myrtifolium* Walp.) *Syzygium myrtifolium* Walp. belongs to the genus Syzygium and the family Myrtaceae[1]. The pucuk merah plant is characterized by its young red and older green leaves, often used as an ornamental plant.

Several previous studies have reported compounds isolated from the pucuk merah plant, namely flavonoids and terpenoids, as well as the bioactivity of its extract, which includes anti-inflammatory, anti-angiogenesis, antibacterial, toxicity, antifungal, antiviral, and antioxidant properties[2][3]. The methanol extract

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of red leaves from the pucuk merah plant has been reported to have antifungal, antioxidant, antibacterial, and antiviral activities[3]. The essential oil of pucuk merah leaves has been reported to have antimutagenic and anticancer activities[2][4]. The pucuk merah plant contains phenolic and flavonoid compounds that act as anti-angiogenic and antioxidant agents[5]. However, specific bioactivities of the young red leaves of Syzygium myrtifolium Walp. have yet to be previously reported. This article reports several bioactivities of the red leaf extracts of the plant, including antioxidant, antimicrobial, and toxicity activities. Additionally, it presents the secondary metabolite content, total flavonoid, and total phenolic content of the Syzygium myrtifolium Walp's red leaf extracts in methanol, ethyl acetate, and hexane.

Experimental

Equipment

The instruments used in this research include an analytical balance (Mettler 200), distillation apparatus, grinder, rotary evaporator, TLC plates, oven, UV lamp (254 – 356 nm), autoclave, Petri dishes, laminar air flow, calipers, shrimp larvae breeding container, aerator, and UV-Vis spectrophotometer.

Material

The materials used are red leaves from the pucuk merah plant, hexane (C_6H_{14}), ethyl acetate ($C_4H_8O_2$), distilled water (H_2O) , chloroform ($CHCl_3$), cyanidin test reagent ($C_{15}H_{11}O_6^+$), Liebermann-Burchard reagent, 0.05 M chloroform-ammonia solution ($CHCl_3$ + NH_3), Mayer's reagent ($K_2[HgI_4]$), and 1% sodium hydroxide (NaOH) for secondary metabolite tests. Gallic acid ($C_7H_6O_5$), methanol (CH_3OH), Folin-Ciocalteu reagent, and sodium carbonate (Na_2CO_3) for total phenolic tests. Quercetin ($C_{15}H_{10}O_7$), Sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), and Aluminium chloride ($AlCl_3$) for total flavonoid tests. 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid ($C_6H_8O_6$) for antioxidant tests. DMSO (Dimethyl Sulfoxide, $(CH_3)_2SO$), *Artemia salina* Leach shrimp larvae, and seawater for toxicity tests. Candida albicans fungus, Sabouraud Dextrose Agar (SDA), and 0.5% ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) for antifungal tests. *Propionibacterium acnes* ATCC 11827, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, Mueller-Hinton Agar (MHA), 1.2% clindamycin ($C_{18}H_{33}ClN_2O_5S$), 0.5% ciprofloxacin ($C_{17}H_{18}FN_3O_3$), and 0.1% chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) for antibacterial tests.

Methods

Identification and Sample Preparation

Red leaves from the pucuk merah plant were collected, totaling 20 kg. The plant was identified at the Herbarium Laboratory of Andalas University (ANDA) in the Department of Biology, FMIPA, Andalas University. A total of 20 kg of pucuk merah leaves were air-dried without direct exposure to sunlight, resulting in 5.2 kg of dried leaves, which were then ground using a grinder.

Secondary Metabolite Content Test

Phenolic, flavonoid, coumarin, terpenoid, steroid, alkaloid, and saponin contents were tested using appropriate reagents in hexane, ethyl acetate, and methanol extracts. Phenolic content was tested with $FeCl_3$, flavonoid content with the cyanidin test, coumarin content with NaOH, terpenoid and steroid content with the Liebermann-Burchard reagent, alkaloid content with Mayer's reagent, and saponin content with HCl.

Extraction of Pucuk Merah Leaves

The red leaf powder from the pucuk merah plant was extracted using the maceration method with hexane, ethyl acetate, and methanol solvents. Each filtrate obtained was evaporated using a rotary evaporator, yielding hexane, ethyl acetate, and methanol extracts. Each obtained extract was weighed.

Determination of Total Phenolic Content

Gallic acid (0.0100 g) was weighed and dissolved in methanol to produce a 1000 mg/L standard gallic acid solution concentration. Then, varying concentrations of gallic acid were ready at 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L. A 10 mL volumetric flask was pipetted with 0.5 mL of each standard solution, and then 1 mL of Folin-Ciocalteu reagent was added. The mixture was homogenized and incubated for 10 minutes. After that, 2 mL of 7% Na_2CO_3 was added to each volumetric flask, and the volume was adjusted to the mark. The standard solutions were homogenized and incubated for 60 minutes, and then the absorbance was measured at a wavelength of 765 nm. The total phenolic content was determined in the methanol and ethyl acetate extracts using the same methods as the standard. The regression equation of the standard solution was used to compute the results. The entire phenolic content was measured in milligrams of Gallic Acid Equivalent (GAE)/gram of extract, and the test was run in triplicate [6].

Determination of Total Flavonoid Content

A standard quercetin solution was prepared by weighing 0.0100 g of quercetin and dissolving it in methanol to a volume of 10 mL to obtain a concentration of 1000 mg/L. Then, varying concentrations were prepared at 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L. 1 mL of each standard solution was pipetted into a 10 mL volumetric flask, and 4 mL of distilled water was added. A 0.3 mL 5% $NaNO_2$ solution was added, and the mixture was incubated for five minutes. Subsequently, 2 mL of 1M NaOH was added, and the mixture was incubated for 5 minutes before 0.3 mL of 10% $AlCl_3$ solution was added. Absorbance was measured at 510 nm in wavelength after the volume was adjusted to the proper level with distilled water. The total flavonoid content in the methanol and ethyl acetate extracts of pucuk merah leaves was determined using the same method as the standard quercetin solution test was carried out in triplicate, and the total flavonoid concentration was represented in mg Quercetin Equivalent (QE)/g extract[7].

Antioxidant Activity Test

Antioxidant activity was ascertained using the DPPH (1,1-diphenyl-2-picrylhydrazyl) technique. A standard ascorbic acid solution was prepared by weighing 0.0025 g and dissolving it in methanol to a volume of 50 mL to obtain a concentration of 50 mg/L. Varying concentrations were then prepared at 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, and 25 mg/L. Each concentration (2 mL) was pipetted and mixed with 3 mL of DPPH solution in a vial and then homogenized. Thirty minutes were spent in the dark, incubating the combination. The same procedure was applied to the methanol and ethyl acetate extracts. The blank used in the DPPH method consisted of 2 mL methanol and 3 mL DPPH. Determining antioxidant activity in the methanol and ethyl acetate extracts was performed the same way as the standard solution; the test was conducted in triplicate, and the absorbance was measured at a wavelength of 517 nm[8]. Antioxidant activity is expressed as the IC50 value. The percentage inhibition can be calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{Sample}}{A_{control}} \text{ X } 100\%$$

Antibacterial Activity Test

Disk diffusion was the method used to test for antibacterial activity. Suspensions of the bacteria *Propionibacterium acnes* ATCC 11827, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922 were prepared by taking 2 inoculating loop needles of each rejuvenated bacterium and placing them into test tubes containing 5 mL of physiological NaCl solution, which were then homogenized using a vortex. The bacterial suspension's turbidity was changed to conform to the McFarland 0.5 standard. Using a cotton bud, the bacterial suspensions were then streaked onto MHA media in Petri dishes. Test solutions with concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.5625% w/v were applied in 10 µL

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drops onto paper disks, which were then placed on the surface of the MHA media. The positive controls used were 1.2% clindamycin, 0.5% ciprofloxacin, and 0.1% chloramphenicol, while the negative control used was DMSO. After that, the petri dishes were kept in an incubator for a full day. After 24 hours, the clear zones formed around the disks were measured using calipers[9].

Antifungal Activity Test

Antifungal activity was tested using the disk diffusion method with three repetitions. A suspension of Candida albicans was prepared by taking 2 inoculating loop needles of rejuvenated fungi and placing them into test tubes containing 5 mL of physiological NaCl solution, which were then homogenized using a vortex. The turbidity of the fungal suspension was adjusted to match the McFarland 0.5 standard. Using a cotton bud, the Candida albicans suspension was then streaked onto the surface of Petri dishes containing solid Sabouraud Dextrose Agar media. Test solutions with concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.562% w/v were applied in 10 µL drops onto paper disks, which were then placed on the surface of the media. The petri dishes were covered and wrapped with plastic wrap. The positive control was 0.5% ketoconazole, and the negative control was DMSO. The petri dishes were incubated for 3 x 24 hours at 37℃ in a fungal incubator. The clear zones formed around the disks were measured using calipers[9].

Toxicity Test

The Brine Shrimp Lethality Test (BSLT) protocol was employed for the toxicity test. Artemia salina Leach shrimp larvae were hatched using activated seawater and bred in a two-sided breeding container (dark side and light side) for 48 hours. After 48 hours, the larvae were ready to be used as test organisms. To prepare the solution, 0.0500 g of the extract (hexane, ethyl acetate, and methanol) was weighed and dissolved with the appropriate solvent in a 50 mL flask to obtain a stock solution concentration of 1000 mg/L. The stock solution of each extract was then diluted to various concentrations: 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, 100 mg/L, 150 mg/L, and 200 mg/L. Each stock solution concentration (5 mL) was pipetted into a vial, and the solvent was evaporated. The dried samples were then dissolved in 50 µL of 1% DMSO, and 20 Artemia salina Leach larvae were added. The control included mixing 50 µL of DMSO with 5 mL of seawater and adding 20 Artemia salina Leach larvae. Observations were made over 24 hours. The toxicity activity was determined based on the LC_{50} value obtained through probit analysis and the regression equation.[10]. The test was conducted with four repetitions.

Results and Discussion

Identification and Sample Preparation

The identification results showed that the sample used was the species Syzygium myrtifolium Walp. Twenty kilograms of fresh red leaves were prepared, and 5.2 kilograms of dry powder was obtained.

Results of Pucuk Merah Plant Red Leaf Extraction

Maceration of 5.2 kg of dried red leaf powder from the pucuk merah plant with hexane, ethyl acetate, and methanol produced extracts with varying yields (Table 1).

Table 1 Results of Pucuk Merah Plant Red Leaf Extraction

Extract	Mass of Extract (g)	Extract Yield (%)
Hexane	106.1154	2.0407
Ethyl acetate	312.0033	6.00
Methanol	391.9013	7.5365

Table 2. Secondary Metabolite Content of Pucuk Merah Plant Red Leaf Extracts

Secondary Metabolite	Observation			
	Colors	Hexane	Ethyl acetate	Methanol
Flavonoids	Red-orange	No color (-)	Red-orange (+)	Red-orange (+)
Phenolic	Dark Blue	Orange (-)	Dark Blue (+)	Dark Blue (+)
Saponins	Foam	No foam (-)	No foam (-)	No foam (-)
Triterpenoids	Red ring	Red ring (+)	Green (-)	Red ring (+)
Steroids	Green Ring	Green ring (+)	Larutan hijau (+)	Green ring (+)
Alkaloids	White precipitate	White precipitate (+)	Red orange precipitate (-)	Red orange precipitate (-)
Coumarin	Blue fluorescene	Blue fluorescene (+)	Blue fluorescene (+)	Blue fluorescene (+)

Description: (+) Contains secondary metabolites
(-) It does not contain secondary metabolites

Methanol produced the highest extract yield (7.5365%) with the largest extract mass (391.9013 g), followed by ethyl acetate (6.00%, 312.0033 g), and hexane with the lowest yield (2.0407%, 106.1154 g). This indicates that methanol is the most effective solvent among the three in extracting compounds from *pucuk merah* leaves, likely due to its polarity, which enables it to dissolve a broader range of phytochemicals compared to the less polar solvents[11].

Secondary Metabolite Content

Table 2 displays the outcomes of the secondary metabolite assays for the hexane, ethyl acetate, and methanol extracts of the red leaves of the Pucuk Merah plant.

The methanol extract yielded the most diverse array of secondary metabolites compared to the other two solvents. Flavonoids, phenolics, triterpenoids, steroids, and coumarins were all identified in the methanol extract. The ethyl acetate extract also contained several secondary metabolites, including flavonoids, phenolics, steroids, and coumarins; however, it did not show the presence of triterpenoids or alkaloids. In contrast, the hexane extract exhibited only the presence of steroids, alkaloids, triterpenoids, and coumarins, with no detection of flavonoids, phenolics, or saponins. It is important to note that saponins were not detected in any of the extracts. These findings suggest that solvent polarity plays a crucial role in the extraction efficiency of specific phytochemical classes, with methanol as a highly polar solvent proving to be the most effective for extracting secondary metabolites from the red leaves of *Pucuk Merah*[11].

Total Phenolic Content

The total phenolic content in methanol and ethyl acetate extracts of pucuk merah leaves was determined using the Folin-Ciocalteu reagent as a complexing agent. This reagent produces a blue color when phenolic compounds are reduced in a primary environment by adding Na_2CO_3. Because gallic acid is a stable derivative of hydroxybenzoic acid, it was selected as the reference. Figure 1's regression curve was utilized to calculate the total phenolic content.

The total phenolic content for the methanol and ethyl acetate extracts was 311.0520 and 420.306 mg GAE/gram extract, respectively. A higher concentration of phenolic compounds results in a more significant number of phenolate ions capable of reducing heteropoly acid (phosphomolybdic-phosphotungstic) to a molybdenum-tungsten complex, leading to a more intense blue color produced by the phenolic compounds.

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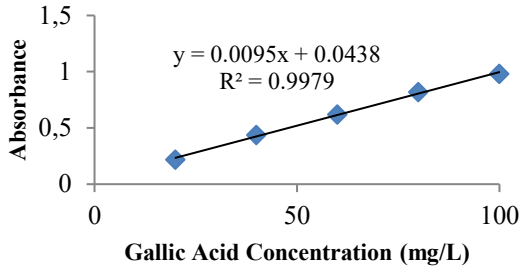


Figure 1. The curve between the concentration of the standard gallic acid solution and absorbance

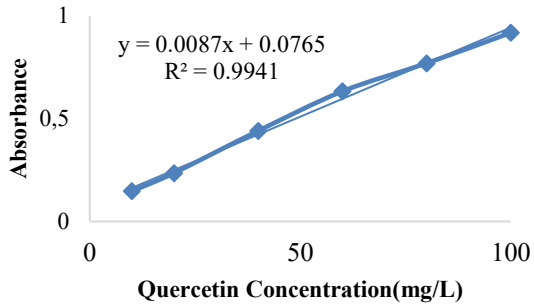
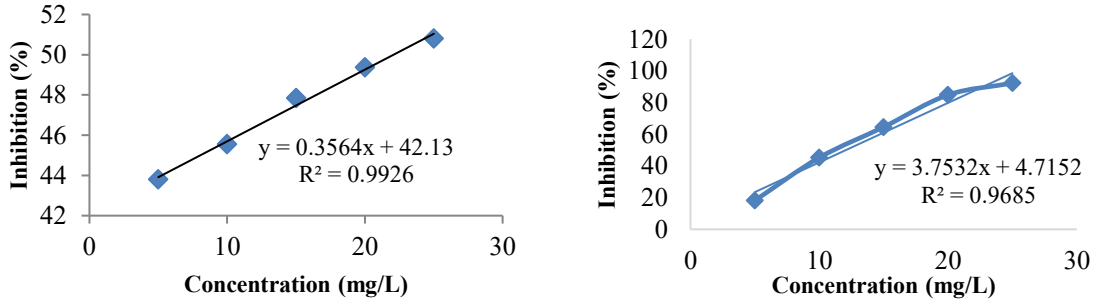


Figure 2. The relationship curve between the concentration of ethyl acetate extract



(a) (b)

Figure 3. The relationship curve between the concentration of ethyl acetate extract (a) and methanol extract (b) with percentage inhibition

Total Flavonoid Content

The total flavonoid content was ascertained using an $AlCl_3$ reagent. The regression curve depicted in Figure 2 was utilized to determine the overall flavonoid content.

Figure 2 shows that as the concentration increases, the absorbance value also increases, indicating a higher flavonoid content in the methanol and ethyl acetate extracts. The total flavonoid content for the ethyl acetate extract was 1058.936 mg QE/g extract, while for the methanol extract, it was 105.7472 mg QE/g extract.

Antioxidant Activity

Antioxidant activity was determined to assess the ability of antioxidant compounds in the ethyl acetate extract of pucuk merah leaves to inhibit free radicals. This testing was conducted using the DPPH method. The results of the antioxidant activity for the ethyl acetate and methanol extracts of pucuk merah leaves can be seen in Figure 3.

Figure 3 shows that as the concentration of an extract increases, the absorbance decreases, and the percentage inhibition increases. This indicates that the antioxidant compounds become more effective at inhibiting DPPH radicals[12]. Antioxidant activity is assessed through the IC_{50} value. The IC_{50} values for the methanol and ethyl acetate extract are 19.606 mg/L and 12.0656 mg/L, respectively. This indicates that the methanol and ethyl acetate extracts have very strong activity inhibiting DPPH radicals.

Antibacterial Activity

Disk diffusion was used to test for antibacterial activity, characterized by clear zones around the disks. The antibacterial activity was tested at varying concentrations of the test solutions: 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.562%. DMSO was used as the negative control. Chloramphenicol was chosen as the positive control for testing against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. At the same time, clindamycin was used for *Propionibacterium acnes* ATCC 11827, as it is an effective antibiotic for treating diseases caused by Gram-positive anaerobic bacteria[13]. Ciprofloxacin is a broad-spectrum antibiotic effective against Gram-negative bacteria and was chosen as the positive control for testing antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853 [14].

Based on the data in **Table 3**, the hexane extract does not exhibit antibacterial activity against the Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. However, for Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Propionibacterium acnes* ATCC 11827), the hexane extract shows antibacterial activity characterized as *resistant*.

Table 3. Results of Antibacterial Activity Testing of Hexane Extract

Concentration (%)	Gram-positive bacteria			
	***P.acnes* ATCC 11827**		***S.aereus* ATCC 29213**	
	Inhibition zone (mm)	**Category**	**Inhibition zone (mm)**	**Category**
1.562	1.00	*Resistant*	3.37	*Resistant*
3.125	1.38	*Resistant*	3.71	*Resistant*
6.25	2.20	*Resistant*	4.90	*Resistant*
12.5	3.74	*Resistant*	7.77	*Resistant*
25	4.35	*Resistant*	8.79	*Resistant*
50	4.66	*Resistant*	9.59	*Resistant*
Control (+)	45.65	*Susceptible*	18.06	*Susceptible*
Control (-)	0		0	
Concentration (%)	**Gram-negative bacteria**			
	***P.aerigunosa* ATCC 27853**		***E.coli* ATCC 25922**	
	Inhibition zone (mm)	**Category**	**Inhibition zone (mm)**	**Category**
1.562	0	-	0	-
3.125	0	-	0	-
6.25	0	-	0	-
12.5	0	-	0	-
25	0	-	0	-
50	0	-	0	-
Control (+)	43.77	*Susceptible*	15.45	*Intermediate*
Control (-)	0		0	

Note: The testing was conducted in triplicate.

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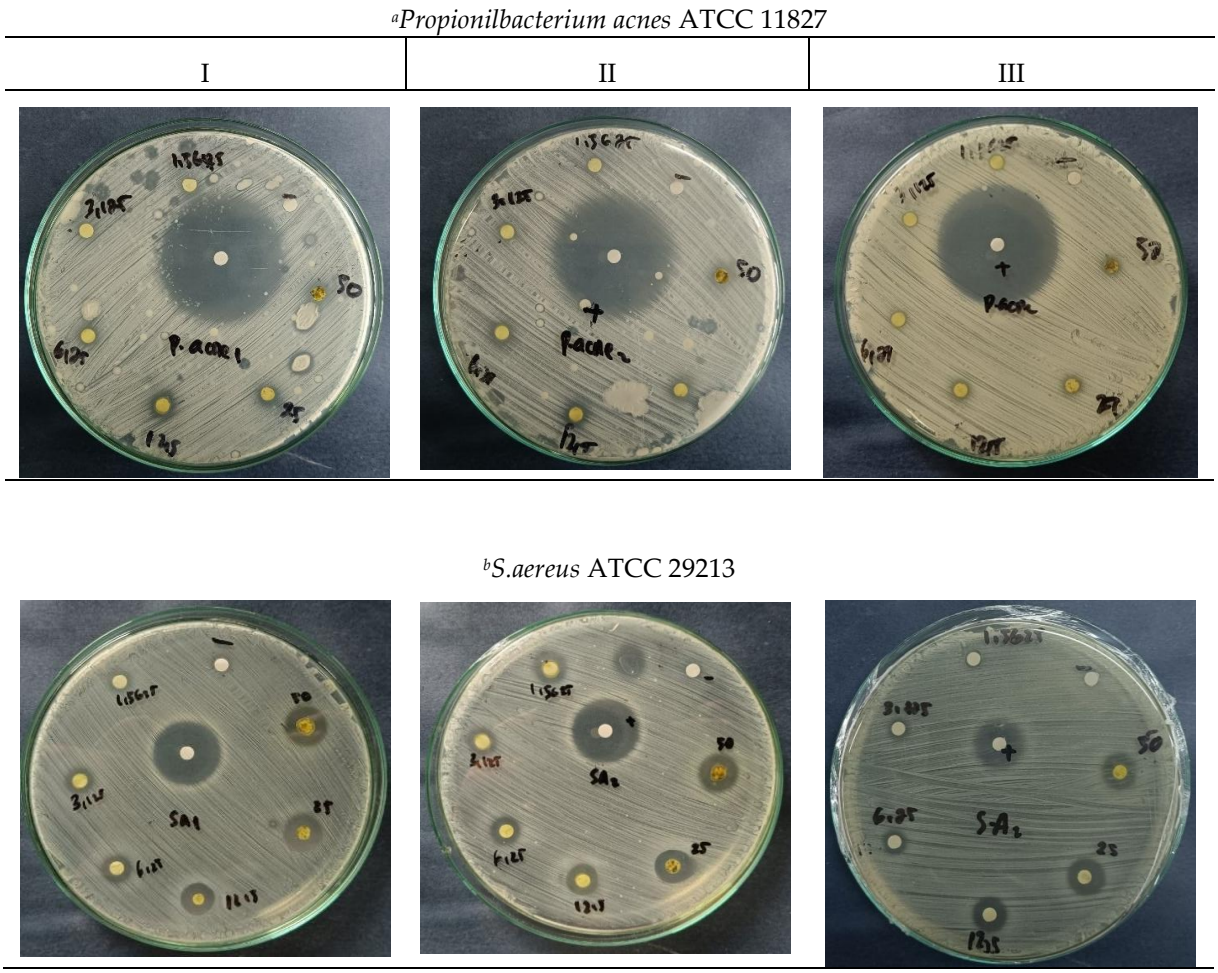

Figure 4. Observation of Antibacterial Activity Test Results of Hexane Extract from Red Leaves of Pucuk Merah Plant

The inhibition zones for both Gram-positive bacteria increased along with the rise in extract concentration. This indicates a positive correlation between extract concentration and inhibition zone diameter, where higher concentrations lead to larger inhibition zones. The increase in inhibition zone occurs because higher extract concentrations contain more antibacterial active compounds. This enhances the ability of antibacterial compounds to penetrate microbial cells, damage their metabolic systems, and ultimately lead to cell death. Bacterial growth tends to decrease significantly with increased antibacterial concentration[15].

The lack of antibacterial activity against Gram-negative bacteria (*Staphylococcus aureus* ATCC 29213 and *Propionibacterium acnes* ATCC 11827) is due to their double-layered cell wall structure, which includes an outer layer of lipopolysaccharides and an inner layer of peptidoglycan. This structure protects against external environmental influences, making it difficult for antibacterial compounds to penetrate the cells of Gram-negative bacteria, thus resulting in no antibacterial activity. In contrast, Gram-positive bacteria such as *Propionibacterium acnes* ATCC 11827 and *Staphylococcus aureus* ATCC 29213 show antibacterial activity because their cell walls contain a higher amount of peptidoglycan, which facilitates the penetration of antibacterial compounds. The growth of Gram-positive bacteria can be inhibited by damaging the cell wall, altering protein molecule structures, modifying cell permeability, and precipitating protoplasm. Damage to the cell wall impedes

bacterial growth and ultimately leads to bacterial cell death.[16].

Secondary metabolites, bioactive substances with antibacterial potential, are responsible for the pucuk merah leaves' antibacterial activity in the hexane extract. Alkaloids and steroids can interfere with the formation of peptidoglycan components, leading to bacterial cell death due to incomplete cell wall formation. Additionally, alkaloids can alter membrane permeability, disrupt metabolism, and interfere with nucleic acid and protein synthesis, thus combating bacterial resistance[17]. Triterpenoids work by interacting with the porins in the outer membrane of bacterial cells, forming solid polymeric bonds that subsequently reduce the permeability of the bacterial cell wall[18].

Antifungal Activity

Antifungal activity was tested using the disk diffusion method, characterized by an inhibition zone (clear area) around the paper disks. The antifungal activity was evaluated using six different concentrations of the test solution: 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.562%. DMSO was used as the negative control, and ketoconazole was used as the positive control.

Table 4. Results of Antifungal Activity Testing of Hexane Extract

Concentration (%)	*Candida albicans*	
	Inhibition zone (mm)	Category
1.562	0.50	*Resistant*
3.125	0.89	*Resistant*
6.25	1.28	*Resistant*
12.5	1.97	*Resistant*
25	3.14	*Resistant*
50	5.31	*Resistant*
Control (+) Ketoconazole 0.5%	20.61	*Resistant*
Control (-) DMSO	0	

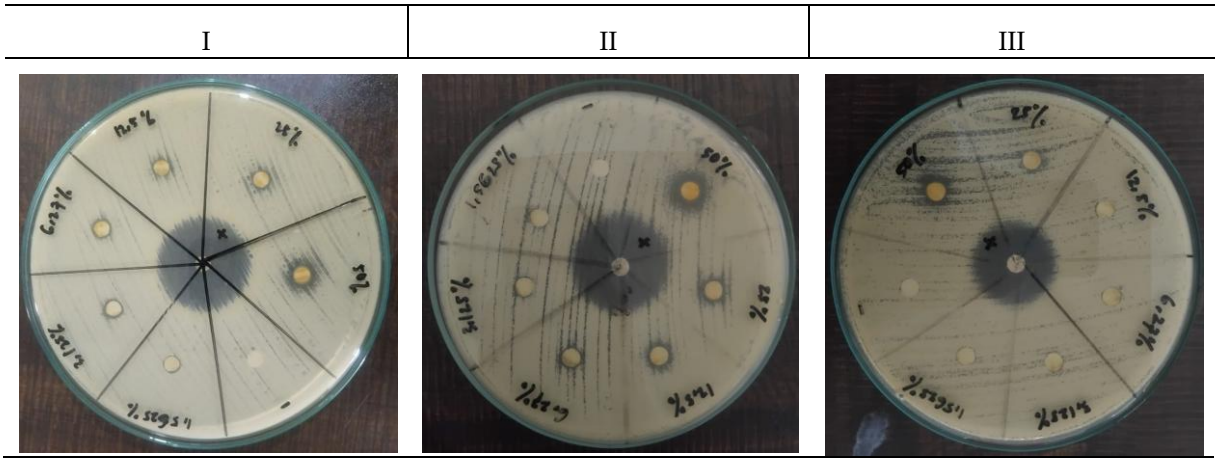

Figure 5. Observation of Antibacterial Activity Test Results of Hexane Extract from Red Leaves of Pucuk Merah Plant

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The data show that the inhibition zones produced by the hexane extract are ≤20 mm for all concentrations, indicating that the hexane extract is resistant to *Candida albicans*. The antifungal effects observed are due to the secondary metabolites in pucuk merah leaves. Steroids can inhibit the reproduction of mycelium and spore germination. Triterpenoids prevent enzyme activity within the cells, damaging cellular organelles and disrupting fungal growth. Coumarins change the structure and function of the plasma membrane by creating pores in the cell wall. As a result, the cells undergo shrinkage and damage[19].

Toxicity Activity

The determination of toxicity activity for hexane, ethyl acetate, and methanol extracts of pucuk merah leaves in this study was conducted using the Brine Shrimp Lethality Test (BSLT). This method serves as an initial step to evaluate the potential anticancer activity of a plant extract by measuring the LC_{50} value, which is based on the number of deaths of Artemia salina Leach larvae after a 24-hour incubation period.

Table 5. Results of Toxicity Testing for Hexane Extract

Concentration (mg/L)	Average Mortality	% Mortality	Probit Value
20	3.5	23.00	4.26
40	5.50	36.00	4.64
60	6.75	45.00	4.87
80	8.75	58.00	5.20
100	9.25	61.00	5.28
150	11.00	73.00	5.64
200	13	86.00	6.08
Control	0	0	0

Table 6 Results of Toxicity Testing for Ethyl Acetate Extract

Concentration (mg/L)	**Average Mortality**	**% Mortality**	**Probit Value**
20	7.30	36.50	4.64
40	8.50	42.50	4.80
60	10.50	52.50	5.05
80	11.50	57.50	5.18
100	12.50	62.50	5.31
150	13.50	67.50	5.44
200	14.80	74.00	5.64
Control	0	0	0

Table 7 Results of Toxicity Testing for Methanol Extract

Concentration (mg/L)	Average Mortality	% Mortality	Probit Value
20	4.00	20.00	4.16
40	5.25	26.25	4.36
60	6.25	31.25	4.50
80	7.00	35.00	4.61
100	7.50	37.50	4.69
150	8.75	43.75	4.85
200	10.00	50.00	5.00
Control	0	0	0

The test results showed toxicity activity in the methanol, ethyl acetate, and hexane extracts, with LC_{50} values of 61.60 mg/L (strongly toxic), 51.75 mg/L (strongly toxic), and 224.28 mg/L (moderately toxic), respectively[20]. The active compounds in the extract enter the larvae's body through the mouth, disrupting the digestive system of *Artemia salina* shrimp larvae. This disruption can impair the larvae's ability to recognize their food, ultimately leading to death.[21].

Correlation Between Flavonoid and Phenolic Compounds and Bioactivity

The flavonoid and phenolic content in red shoot (*Syzygium myrtifolium*) leaves significantly contributes to their antioxidant activity. The IC_{50} values of the methanol and ethyl acetate extracts were 19.606 and 12.065 mg/L, respectively, in line with their high phenolic content (311.052 and 420.306 mg GAE/g) and flavonoid content (211.788 and 105.75 mg QE/g). In comparison, *Peronema canescens* (Sungkai) leaves contain 273.33 mg GAE/g of phenolics with an IC_{50} of 29.72 mg/L[16], while *Scurrula ferruginea* (cacao mistletoe) contains 83.55 mg QE/g of flavonoids with an IC_{50} of 36.75 mg/L[8]. These findings indicate that red shoot leaves have higher levels of bioactive compounds and stronger antioxidant activity compared to other medicinal plants.

Although flavonoids and phenolics are widely known to possess antimicrobial activity[3][9], this study found that the hexane extract of red leaves from the pucuk merah plant (*Syzygium myrtifolium*), which did not contain flavonoids or phenolics based on phytochemical tests, still exhibited inhibitory effects against *Staphylococcus aureus* and *Propionibacterium acnes*. This suggests that the antimicrobial activity of the hexane extract is likely attributed to other secondary metabolites detected in the extract, such as triterpenoids, steroids, and alkaloids. Therefore, even without the direct contribution of flavonoids and phenolics, the red leaf extract of *Syzygium myrtifolium* still demonstrates antimicrobial potential due to the presence of other bioactive constituents.

The red leaf extract of the pucuk merah plant (*Syzygium myrtifolium*) exhibited toxicity against *Artemia salina* larvae, with the ethyl acetate and hexane extracts classified as highly toxic (LC_{50} of 16.79 and 56.65 mg/L, respectively), and the methanol extract classified as moderately toxic (LC_{50} of 224.28 mg/L). Although flavonoids and phenolics can induce oxidative stress, the toxicity of the hexane extract is likely due to other compounds such as triterpenoids and steroids, as identified in the phytochemical screening. As a comparison, the methanol extract of *Lactuca sativa* (red lettuce) has an LC_{50} value of 250.11 mg/L[21], indicating that the red leaf extract of the pucuk merah plant possesses

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stronger toxicity and potential as a natural anticancer [3],[21].

Conclusions

The red leaves of the pucuk merah plant (*Syzygium myrtifolium*) contain secondary metabolites including flavonoids, phenolics, triterpenoids, steroids, alkaloids, and coumarins. The highest flavonoid and phenolic contents were found in the methanol and ethyl acetate extracts, which also exhibited very strong antioxidant activity with IC_{50} values of 19.606 and 12.065 mg/L, respectively. These values reflect a high free radical scavenging ability compared to several other medicinal plants. In the antimicrobial test, the hexane extract despite not containing flavonoids and phenolics demonstrated inhibition zones against *Staphylococcus aureus*, *Propionibacterium acnes*, and *Candida albicans*, suggesting the involvement of other active compounds such as triterpenoids and steroids. Toxicity testing using the BSLT method showed that the ethyl acetate and hexane extracts were highly toxic (LC_{50} values of 16.79 and 56.65 mg/L), while the methanol extract was moderately toxic (LC_{50} of 224.28 mg/L). Based on IC_{50}, LC_{50}, and inhibition zone data, and in comparison to other plants such as *Lactuca sativa*, *Peronema canescens*, and *Scurrula ferruginea*, it can be concluded that red leaves of *Syzygium myrtifolium* possess stronger bioactivity and hold great potential for development as a natural herbal source for antioxidant, antimicrobial, and anticancer agents.

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Declaration of Generative AI and AI-Assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Author Contributions

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Declaration of Conflicting Interests

The authors declare no conflict of interest.

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