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Phytochemical Screening of Acetone Extract of Rambai Leaves (*Baccaurea motleyana*) and Its Bioactivity as an Antibacterial Against *Escherichia coli* and *Staphylococcus aureus*

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Abstract: Bacteria are one of the organisms that may cause disease in the human body. Some bacteria which often infect the human body are *Escherichia coli* and *Staphylococcus aureus*. Both bacteria are pathogenic and possibly lead to various diseases such as diarrhea, urinary tract infections, and lung infections that have the potential to result in death. Treatment of infections caused by these bacteria can be done by giving antibiotics as therapy or a quick cure against infection. The use of antibiotics has side effects that result in resistance. Therefore, it is necessary to screen bioactive compounds with bioactivity as better antibacterial agents so that they can be developed into drugs that can cure diseases caused by bacteria. It is urgent to utilize natural antibacterials from plants to treat bacterial infections. This research aims to obtain the compound content, the total phenolic, and antibacterial activity of acetone extract of rambai leaves, so this research focused on rambai leaf acetone extract phytochemical content, total phenolic content, and antibacterial activity examination. Then, Phytochemical testing of rambai leaf acetone extract (*Baccaurea motleyana*) shows rambai leaves contain alkaloid compounds, tannins, flavonoids, saponins, steroids, and terpenoids that have the potential to be antibacterial. The examination of phenolic content was accomplished by using the Folin-Ciocalteau method. The total phenolic test results obtained were 68.63 mg EAG/g. Antibacterial testing was performed by implementing the disk diffusion method. Antibacterial testing of rambai leaf acetone extract Concentration of 20 %, 40%,60%, and 80% showed consecutive results in inhibition zones of 3.86 mm, 6.48 mm, 6.55 mm, and 6.63 mm with weak to medium activity for *E. coli* bacteria. However, *S. aureus* bacteria showed consecutive results in inhibition zones of 6.02 mm, 10.15 mm, 17.10 mm, and 19.46 mm with medium to intense activity. There has not been much research on rambai from Bangka Belitung, so there is an indispensable for further research regarding the total flavonoid and total alkaloid content of rambai leaves. Research may also carried out using a variety of solvents.

Keywords: Aceton; Antibacterial; Phenolic; Phytochemical; Rambai.

Introduction

Pathogenic bacteria can cause various diseases, such as urinary tract infections, diarrhea, and lung infections, and can even result in death. *Escherichia coli* bacteria are generally transmitted through vegetables that are not washed thoroughly, well water, and interactions with animals [1]. Meanwhile, *Staphylococcus aureus* bacteria are transmitted through dirty objects that come into contact with humans so that the bacteria enter the skin, causing skin diseases such as allergies and eczema [2].

Treatment for infections is done by giving antibiotics as a quick cure for infections. The use of antibiotics has side effects resulting in resistance. Resistance occurs because. Changes in bacteria cause a decrease or loss of the effectiveness of antibiotic drugs [3]. Therefore, It is necessary to screen bioactive compounds with bioactivity as better antibacterials so that they can be developed into viable drugs to cure diseases caused by bacteria [4].

The rambai plant is a type of plant belonging to the genus *Baccaurea*. This plant can be found in Kalimantan, Java, Sumatra, and the Bangka Belitung Islands [5]. Rambai Leaves are generally used as a traditional medicine to treat constipation, swelling of the eyes, arthritis, abscesses, and stomach aches, as well as improve menstruation and urination. Extract *motleyana* leaf methanol from East Kalimantan contains flavonoids, phenolics, steroids, and triterpenoids. N-hexane extract from *B. motleyana* leaves from East Kalimantan contains steroids and flavonoids. Meanwhile, the ethyl acetate extract of B. motleyana from East Kalimantan contains phenolics and flavonoids. This secondary metabolite content can act as an antibacterial [6]. The rind of *B. motleyana* fruit from South Kalimantan contains alkaloids, phenolics, saponins, triterpenoids, steroids, and flavonoids, which can be used as antibacterials [7]. This research was carried out to determine the bioactive compounds and bioactivity of the acetone extract of *B. motleyana* leaves. From Bangka Belitung as an antibacterial to obtain antibacterial alternatives derived from plants. Acetone is used as a solvent because the semi-polar nature of acetone can extract both polar and non-polar compounds [8]. This research aims to obtain the compound content, the total phenolic, and antibacterial activity of acetone extract of rambai leaves, so this research focused on rambai leaf

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acetone extract phytochemical content, total phenolic content, and antibacterial activity examination.

Research Methods

Tools

Tools used include blenders, digital calipers, jars, measuring cups, racks and test tubes, rotary evaporators, vials, dropper pipettes, stir sticks, beakers, hotplates, petri dishes, laminar airflow, measuring flasks, paper discs 6 mm, test tube, A4 paper, micropipette, analytical balance, aluminum foil, Bunsen, tripod, erlenmeyer, ose needle, magnetic stirrer, incubator, autoclave, 250 ml Duran bottle, vortex, plastic wrap, filter paper, and UV-Vis.

Materials

The materials used include leaves *Baccaurea motleyana*, aquades, acetone, reagen Wagner, Nutrient Broth, swallow jelly, DMSO, amoxicillin, *Escherichia coli*, dan *Staphylococcus aureus*, $FeCl_3$, ethanol, HCl, Mg powder, NaCl, reagen Follin Ciocalteau, gallic acid, methanol, chloroform, NaHCO3, and H_2SO_4.

Rambai Leaf Extraction

The research samples were dried in an open space, then blended and sieved to produce a fine powder. Then, 300 grams were weighed and macerated for 3x24 hours using acetone with a ratio of 1:10 for sample: solvent. The filtrate obtained was evaporated using a rotary evaporator at 60 ºC.

Phytochemical Screening

Phytochemical screening is performed qualitatively, including testing for alkaloids, tannins, flavonoids, saponins, and steroids.

For the alkaloid test, 0.5 g acetone extract is added with 2 drops of 2% HCl then a drop of Wagner's reagent is added. Wait for 30 minutes. A positive result is indicated by forming a brown precipitate [9].

Tannin test, 0.5 g acetone extract was added with 3 drops of 5% $FeCl_3$ solution. Positive results are indicated by forming a green or greenish-blue color [9].

Flavonoid test, 0.5 g acetone extract was added with 0.2 g Mg powder and 10 drops of HCl. Positive results are indicated by the formation of a reddish-black, yellow, or orange color [10].

Saponin test, 0.5 g of acetone extract was added to 10 mL of hot distilled water and then shaken vigorously for ± 1 minute until foamy. Positive results are indicated by stable foam for 10 minutes [10].

Steroid and Terpenoid test, 0.5 g of extract was added with 3 drops of H_2SO_4, 2 mL of chloroform, and 10 drops of glacial acetic acid. Shake and let sit for approximately 5 minutes. The formation of a blue or green color indicates positive results for steroids. In the Terpenoid Test, 0.5 g of extract was added with 3 drops of sulfuric acid. Positive results for terpenoids are marked with a brownish-red color [11].

Total Phenolic Test

30 mg of extract was added to 10 ml of methanol. Then, pipet 0.5 ml, add 0.5 ml of Folin–Ciocalteau reagent, and vortex for 30 seconds. Next, add 2.5 ml of 7% Na_2CO_3 solution to the mixture and vortex. Leave the solution for 30 minutes at room temperature. Measure absorbance using a UV-Vis spectrophotometer at a wavelength of 760 nm [12].

Antibacterial Test

Media Preparation, 3.2 g of NB was added to 6 g of swallow agar, dissolved in 400 mL of distilled water, and then heated on a hotplate until clear and boiling. Wait 1 minute and sterilize using an autoclave at 121ºC for 15 minutes [13].

Bacterial inoculation: Bacteria are multiplied by taking sufficient pure bacteria using a loop needle heated with Bunsen and scratched on cawan petri filled with media. Then, incubated at 37ºC for 24 hours [14].

Antibacterial test: The positive controls used were amoxicillin, and distilled water was used as a negative control. Antibacterial tests were carried out in duplicate. Sterile 6 mm paper discs impregnated with acetone extract with concentrations of 20%, 40%, 60%, and 80%, which have been dissolved in 1 mL of DMSO, are attached to Petri dishes containing media and bacterial suspensions that have been dissolved in 0.85% solution. NaCl with a turbidity level equivalent to Mc Farland 0.5. After that, vortex for ± 5 minutes to make it homogeneous. They then incubated at 37º C for 24 hours. Antibacterial activity can be seen based on the diameter of the inhibition zone, which can be measured using a calliper [15].

The inhibition zone of the antibacterial test can be calculated using the formula[16]. Zone of inhibition = Clear zone diameter - Disc zone diameter. The strength of antibacterial inhibition is determined based on the provisions of the zone value in the range ≤ 5 mm (weak category), 6-10 mm (medium category), 11-20 (strong category), and >20 mm (very strong category).

Results and Discussion

Phytochemical screening was carried out qualitatively on the acetone extract of rambai leaves (*Baccaurea motleyana*). This test tested alkaloids, tannins, flavonoids, saponins, steroids, and terpenoids. The data, based on the results of the tests, are presented in Table 1.

Table 1. Phytochemical Test Results of Acetone Extract of *B. motleyana* Leaves.

Test	Observation	Results
Alkaloid	A brown precipitate forms	+
Tannin	The solution is green.	+
Flavonoid	The solution is blue.	+
Saponin	Foam forms	+
Steroid	The solution is blue-green.	+
Terpenoid	The solution is red-brown.	+

Notes :
+ : the sample reacts positively to the test reagent
- : the sample reacts negatively to the test reagent

Based on the results of the phytochemical test in Table 1, the acetone extract of rambai leaves showed positive results, containing alkaloids, tannins, flavonoids, saponins, steroids, and terpenoids. This content is similar to the secondary metabolites contained in *Baccaure motleyana* fruit from South Kalimantan [7].

Total Phenolic Test Results

Total phenolic testing was carried out using the Folin-Ciocalteau method to determine the levels of phenolic compounds from a plant extract. Phenolic compounds are a class of compounds most commonly found in all parts of plants that can act as natural antioxidants and antibacterials. The Folin-Ciocalteau method is the most commonly used for total phenolic testing because it is simpler, more sensitive, and more accurate. The working principle of this method is the oxidation reaction of the phenolic group (R-OH) with a mixture of phosphotungsic acid and molybdic acid in the reagent to form a quinoid (R=O) [17].

The data, based on the results of the tests, are presented in Table 2.

Table 2. Total Phenolic Test Results of *B. motleyana* Leaves

Linear Equations	Abs.	Results (mg EAG/g)	Average (mg EAG/g)	Rind (mg EAG/g
y= 0.0325x – 0.5228	1.710	68.70	68.63	109.96
	1.706	68.57		
R^2 = 0.9867				

Based on the results of the phytochemical test in Table 2, a duplo test was carried out on the acetone extract of rambai leaves, resulting in total phenolic levels of 68, 70 mg EAG/g, and 68.57 mg EAG/g, respectively. The average total phenolic content obtained was 68.63 mg EAG/g. Based on a research literature review, the total phenolic content in the ethanol extract of *B. motleyana* fruit peel from Kalimantan was 109.96 mg EAG/g [7]. Rambai leaf acetone extract from Bangka Belitung has a smaller total phenolic content than previous research on rambai fruit skin from South Kalimantan. Differences in the total phenolic value in solvents used in the *B. motleyana* extraction process are also caused by differences in plant parts used as test samples. Differences in plant parts and the maturity of the plant parts tested can influence the biological activity of the plant [18]. Differences in plant organ function can lead to differences in the biosynthetic pathways of phytochemical content in plant parts, including the content of phenolic compounds [19].

Antibacterial Test Result

Antibacterial testing was conducted to determine Zambia leaf extract's antibacterial power against *Staphylococcus aureus* and *Escherichia coli* bacteria. The method used in antibacterial testing is the disc diffusion method. The disc diffusion method is a method that uses a paper disc that has been impregnated with the extract for ± 10 minutes, then placed in a petri dish containing agar media containing new bacteria resulting from inoculation.

Based on the results of the tests carried out, the data is presented in Table 3.

Based on table 3. There are differences in the inhibition zone values for each concentration. Rambai leaf acetone extract at a concentration of 20% to 80% has weak to medium antibacterial strength against *E. coli* bacteria. The data shows that as the sample concentration increases, the antibacterial inhibitory power formed will also increase. The increase in the antibacterial inhibition zone was influenced by the amount of rambai leaf acetone extract used as the test sample. The results of the antibacterial test obtained are relatively smaller when compared to previous research on methanol extracts of *B. motleyana* leaves from Kalimantan against *E. coli* bacteria obtained antibacterial medium activity antibacterial activity at concentrations of 8% and 16% with zone values of 6.5 mm and 8, 75 mm [20].

Based on the results of the tests carried out, the data is presented in Table 4.

Table 3. Antibacterial Test Results of Acetone Extract of *B. motleyana* Leaves Against *E. coli.*

Sample	Zone of Inhibition (mm)		Average Zone of Inhibition (mm)	Category
	D1	D2		
Extract 20 %	3.65	4.07	3.86	Weak
Extract 40 %	6.42	6.55	6.48	Medium
Extract 60 %	6.47	6.63	6.55	Medium
Extract 80 %	6.53	6.72	6.62	Medium
Control +	29.41	29.32	29.36	Very Strong
Control -		0	0	None

Table 4. Antibacterial Test Results of Acetone Extract of *B. motleyana* Leaves Against *S. aureus.*

Sample	Zone of Inhibition (mm)		Average Zone of Inhibition (mm)	Category
	D1	D2		
Extract 20 %	6.22	5.83	6.02	Medium
Extract 40 %	10.86	9.45	10.15	Strong
Extract 60 %	16.70	17.5	17.10	Strong
Extract 80 %	16.65	22.27	19.46	Strong
Control +	36.90	35.92	36.41	Very Strong
Control -		0	0	None

Based on table 4. There are differences in the inhibition zone values for each concentration. Rambai leaf acetone extract at a concentration of 20% to 80% has medium to strong antibacterial power against *S. aureus* bacteria. The data shows that as the sample concentration increases, the antibacterial inhibitory power formed will also increase. The increase in the antibacterial inhibition zone was influenced by the amount of rambai leaf acetone extract

used as the test sample. This B. motleyana antibacterial test results are smaller at concentrations of 20% and 40%. At concentrations of 60% and 80%, the antibacterial activity obtained was greater when compared to previous research on an ethanol extract of *B. dulkis*, with concentrations of 25 %, 50%,75%, and 100 % showed consecutive results in inhibition zones of 12,3 mm, 14,3 mm, 16,3 mm, and 19,0 mm with strong activity for *S. aureus* bacteria [21].

Based on Table 3 and Table 4 regarding the antibacterial test results of the acetone extract of *B. motleyana* rambai leaves, it can be seen that the inhibition zone for *S.aureus* bacteria is much larger than the inhibition zone for *E.coli*. This is caused by differences in the types of test bacteria, namely *S. aureus*, a gram-positive bacteria, and E. coli, a gram-negative bacteria. Gram-positive bacteria have a simpler structure than gram-negative bacteria, so gram-positive bacteria are easier to damage than gram-negative bacteria. Apart from differences in concentration and type of bacteria, the strength of antibacterial inhibition is influenced by the presence of bioactive compounds in the extract. As qualitative phytochemical tests prove, the acetone extract of *B. motleyana* leaves from Bangka Belitung contains alkaloids, tannins, flavonoids, saponins, steroids, and terpenoids. This bioactive compound has the potential to act as an antibacterial [7]. Alkaloids damage the bacterial cell membrane by inhibiting the preparation of peptidoglycan [22], flavonoids cause bacterial cell death by disrupting the components that make the peptidoglycan in cells, and tannin causes bacterial cells to be less perfect because it damages cell wall polypeptides [23], saponins have a detergent-like surface that can damage the permeability of cell membranes [24].

Conclusion

Phytochemical testing of rambai leaf extract (*Baccaurea motleyana*) shows that rambai leaves contain alkaloids, tannins, flavonoids, saponins, steroids, and terpenoids. Testing the total phenolics of the acetone extract of rambai leaves (*Baccaurea motleyana*) resulted in 68.63 mg EAG/g. The antibacterial activity of the acetone extract of rambai leaves (*Baccaurea motleyana*) shows the best activity at a concentration of 80% with a strong category against S. aureus bacteria and a medium category against E. coli at an extract concentration of 80%.

References

[1] Karmali M.A. (2004). Infection by Shiga toxin-producing *Escherichia coli*. *JMol Biotechnol*, *26*, 117-122.

[2] Azzahra, N., Hardiyanti, T., Purnama, Zulfiawan. (2022). Antibacterial Activity Test of Ethanol Extract of Rambai Leaves (*Baccaurea dulkis* Muell.Arg) Against Bacteria (*Staphylococcus aureus*). *Journal of Applied Health*, *9*(2), 54-61.

[3] Utami, E.R. (2011). Antibiotics, resistance, and the rationale of therapy. *El-Hayah, 1*(4), 191-198.

[4] Roanisca, O. dan Mahardika, R. B. (2021). Phytochemical and Antibacterial Screening of Binjai Leaf Ethanol Extract (*Mangiferacaesia*) Against Bacteria *Escherichia coli*. *Pharmascience Journal*, *8*(2), 9-16.

[5] Nurmayani, S., Widodo, W. D., Matra, D. D. (2021). Characterization of Rambai (*Baccaurea motleyana*) genes thought to be involved in sugar metabolism. *IOP Conference Series : Earth and Environmental Sciences*, *694*.

[6] Rachman, F. A., Saleh, C., Marliana, E. (2020). Antibacterial Test of Rambai Leaves (*Baccaurea motleyana* Mull Arg). *Atomic Journal*, *5*(1), 11-17.

[7] Hutasuhut, D. A., Aspriyanto, D., Firdaus, I. W. A. K. (2022). Qualitative and Quantitative Test of Rambai Fruit Skin Extract (*Baccaurea motleyana*) Concentration 100 %. *Journal of Dentistry*, *6*(2), 97-102.

[8] Roanisca, O. dan Mahardika, R. G. (2017). Phytochemical Screening and Antioxidant Activity of Acetone Extract of Iding-iding Shoots (*Stenochlaena palustrisis*). Proceedings of the Research & Community Service Seminar: Bangka Belitung University: Bangka.

[9] Roanisca, O. (2018). Phytochemical Screening and Antibacterial Potential of Ethanol Extract of Iding-Iding Shoots (*Stenochlaena palustris*) Against Antibacterial *Staphylococcus aureus*, *Escherichia coli*, dan *Bacillus subtilis*. *Mulawarman Chemistry Journal*, *15*(2), 99-105.

[10] Harborne, J. B. (1987). *Fitokimia Method*. 2nd Edition Padmawinata K, Soediro I, translation. Bandung: Bandung Institute of Technology. Translation from: Phytochemical Methods.

[11] Hidayah, N., Nurbani, S. Z., Kusuma, J., Siregar, A. N. (2021). Identification of Phytochemical Compounds Extracts of Sea Waru (*Thespesia populnea*) from the Semarus Coast, Natuna Regency. *Bluefin Fisheries Journal*, 2(2), 8-19.

[12] Khadijah, Jayali, A. M., Umar, S., Sasmita, I. (2017). Determination of Total Phenolics and Antioxidant Activity of Samama Leaf Ethanolic Extract (*Anthocephaalus macrophylus*) Originally from Ternate, North Maluku. *Mulawarman Chemical Journal*, 15(1),11-18.

[13] Ramadheni, P., Mukhtar, H., & Prahmono, P. (2017). Test of the Antibacterial Activity of Ethanol Extract of Katuk Leaves (*Sauropus androgynus* (L.) Merr) Against *Staphylococcus aureus* and *Escherichia coli* Using the Agar Diffusion Method. *Indonesian Natural Research Pharmaceutical Journal*,*2*(2), 34-45.

[14] Dhuha, S. (2016). Antibacterial Activity of Ethanol Extract of Seagrass Leaves (*Syiringodium isoetifolium*) Against Bacteria *Pseudomonas aeruginosa*. *UNSRAT Pharmaceutical Scientific Journal*, *5*(1), 2302-2493.

[15] Ariyani, H., Nazemi, M., Hamidah, Kurniati, M. (2018). Antibacterial Test of Lime Peel Extract (*Cytrus hystrix* DC) Against Several Bacteria. *Journal of Current Pharmacheutical Science*, *2*(1), 2590-2095.

[16] Rastina, R., Sudarwanto, M., Wientarsih, I. (2015). Aktivitas Antibakteri Ekstrak Etanol Daun Kari (*Murraya koenigii*) Terhadap *Staphylococcus aureus*, E*scherichia coli*, dan *Pseudomonas Sp*. *Jurnal Kedokteran Hewan Indonesia*, 9(2)

[17] Mardiah, Z., Oktaviani, R., Kusbiantoro, B., & Handoko, D. (2016). Effect of Heating Process on Phenolic Compounds in Colored Rice. P*roceedings of*

the Center for Rice Research. Bogor Agricultural Institute: Bogor.
[18] Duma, Y., Dadomo, M., Di Lucca G., and Grolier, P. (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *Journal of the Science of Food and Agriculture*, *83*(5), 369-382.
[19] Heldt HW, Piechulla B. (2011). Plant Biochemistry 4th Edition. London (UK): *Academic Press*.
[20] Rachman, F. A., Saleh, C., Marliana, E. (2020). Uji Antibakteri Daun Rambai (*Baccaurea motleyana* Mull Arg). *Jurnal Atomik*, 5(1), 11-17.
[21] Azzahra, N., Hardiyanti, T., Purnama, Zulfiawan. (2022). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Rambai (*Baccaurea dulkis* Muell.Arg) Terhadap Bakteri (*Staphylococcus aureus*). *Jurnal Kesehatan Terapan*, 9(2), 54-61.
[22] Liling, V. V., Lengkey, Y. K., Sambou, C.N., & Palandi, R. R. (2020). Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Buah Pepaya *Carica papaya* L.Terhadap Bakteri Penyebab Jerawat *Propionibacterium acnes*. *Jurnal Biofarmasetikal Tropis*, 3(1), 112-121.
[23] Kurama, G. M., Maarisit, W., Karundeng, E., & Potalangi, N. O. (2020). UjiAktivitas Antibakteri Ekstrak Etanol Daun Benalu Langsat (*Dendroptheo sp*)Terhadap Bakteri *Klebsiella peumoniae*. *Jurnal Biofarmasetikal Tropis*, 3(2), 27-33.
[24] Harborne, J.B. (2006). *Metode Fitokimia*, Edisi ke-2. Bandung: ITB.