Antibacterial Activity of Ethanol Extract and Fraction from Mundar (Garcinia forbesii) Pericarp

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Abstract: *Garcinia forbesii is a typical fruit of South Kalimantan, the same genus as the mangosteen, used by the community as medicine and cosmetics. The aimed of the study was to determine the antibacterial activity of ethanolic extract and fraction of G. forbesii pericarp against Staphylococcus aureus and Propionibacterium acne based on the diameter of the inhibition zone and to determine the minimum inhibitory concentration (MIC) and the minimum killing concentration (MBC). G. forbesii pericarp was extracted using maceration method with ethanol 70%, and fractionation was carried out using liquid-liquid fractionation with n-hexane and ethyl acetate as solvents. Antibacterial activity test on the diameter of the inhibition zone was carried out using a paper disc. The test was continued on the MIC and KBM tests at concentrations of 1.5%, 1.25%, 1%, 0.75%, and 0.5%. The MIC test was carried out using the microdilution method, while the MIC was using the streak plate method. The results showed that the diameter of the inhibition zone of the ethanolic extract of G. forbesii pericarp against S. aureus and P. acne was 2.16±0.983 mm and 2.75±1.405 mm, for the n-hexane fraction 2.08±0.664 mm and 4.08±0.664 mm, the ethyl acetate fraction was 5.08±1.020 mm and 14.33±3.326 mm, respectively. The MIC value of ethanol extract against S. aureus and P. acne bacteria was above 1.5%, the n-hexane fraction and ethyl acetate fraction had the same results, namely against S. aureus bacteria above 1.5% and P. acne at 1.5 %. The MBC value was only obtained for the n-hexane and ethyl fractions of P. acne at a concentration of 1.5%. It was concluded that the ethyl acetate fraction of G. forbesii pericarp had strong antibacterial activity against P. acne bacteria based on the value of inhibition zone diameter, MIC, and MBC.*

Keywords: *antibacterial, G. forbesii, P. acne, S. aureus*

Abstrak: *Garcinia forbesii* merupakan buah khas Kalimantan Selatan yang masih satu kerabat dengan manggis, secara tradisional digunakan oleh masyarakat sebagai pengobatan dan kosmetik. Tujuan penelitian ini yaitu menentukan aktivitas antibakteri ekstrak etanol dan fraksi kulit buah *G. forbesii* terhadap bakteri *Staphylococcus aureus* dan *Propionibacterium acne* berdasarkan nilai diameter zona hambat dan menentukan konsentrasi hambatan minimum (KHM) serta konsentrasi bunuh minimum (KBM). Ektraksi kulit buah *G. forbesii* menggunakan metode maserasi dengan pelarut etanol 70%, dan fraksinasi dilakukan menggunakan fraksinasi cair-cair dengan pelarut *n*-heksan dan etil asetat. Uji aktivitas antibakteri pada diameter zona hambat dilakukan dengan menggunakan *paper disc.* Pengujian dilanjutkan pada uji KHM dan KBM pada konsentrasi 1,5%, 1,25%, 1%, 0,75%, dan 0,5%. Pengujian KHM dilakukan dengan metode mikrodilusi, sedangkan KBM menggunakan metode *streak plate*. Hasil penelitian diperoleh diameter zona hambat ekstrak etanol kulit buah *G. forbesii* terhadap bakteri *S. aureus* dan *P. acne* sebesar 2,16±0,983 mm dan 2,75±1,405 mm, pada

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fraksi *n*-heksan sebesar 2,08±0,664 mm dan 4,08±0,664 mm, pada fraksi etil asetat sebesar 5,08±1,020 mm dan 14,33±3,326 mm. Nilai KHM ekstrak etanol terhadap bakteri *S. aureus* dan *P. acne* diatas 1,5%, fraksi n-heksan dan fraksi etil asetat memiliki hasil yang sama yaitu terhadap bakteri *S. aureus* diatas 1,5% dan *P. acne* sebesar 1,5%. Nilai KBM hanya didapat pada fraksi n-heksan dan fraksi etil terhadap *P. acne* pada konsentrasi 1,5%. Disimpulkan bahwa fraksi etil asetat kulit buah *G. forbesii* memiliki aktivitas antibakteri yang kuat terhadap bakteri *P. acne* berdasarkan nilai diameter zona hambat, KHM, dan KBM.

Kata kunci: Antibakteri, G. forbesii, P. acne, S. aureus

Introduction

Bacteria are a group of single-celled living organisms and are microscopic [1]. *Staphylococcus aureus* and *Propionibacterium acne* are gram-positive bacteria [1,2,3]. *Staphylococcus aureus* causes pneumonia, lung abscess, meningitis, laryngeal infections, and skin lesions [4,5]. *Propionibacterium acne* causes pimples and plays a role in the inflammatory chemotactic process in the ducts of the sebaceous glands [6].

Antibacterials are compounds that can inhibit the growth of certain bacteria [4], while antibiotics are used to treat bacterial infections [5]. Antibiotic resistance cause problems that can counteract antibiotic treatment [7]. The World Health Organization states that more than 200,000 newborns die yearly from infections that do not respond to the given drugs; most of these deaths occur in developing countries [8]. Control of antibiotics resistance can be done by using plants that have antibacterial properties. One of the plants with the potential as an antibacterial agent is the peel of mundar fruit (*Garcinia forbesii*).

The *G. forbesii* plant is a typical plant of South Kalimantan, traditionally used by the community as medicine and cosmetics [9,10]. The Garcinia genus contains secondary metabolite compounds such as xanthones, alkaloids, flavonoids, tannins, and saponins [11,12]. Alpha-mangostin is a compound belonging to the xanthone group found in the rind of the mangosteen fruit (*Garcinia mangostana* L.), which has antibacterial and antimicrobial activity and helps prevent various diseases [13,14,15]. This study aimed to determine the antibacterial activity of ethanol extract and fraction from *G. forbesii* pericarp against *Staphylococcus aureus* and *Propionibacterium acne* based on the diameter of the inhibition zone and determine the minimum inhibitory concentration (MIC) and minimum killing concentration (MKC).

Materials and Methods

Materials

The tools used were glassware (Pyrex® Iwaki Glass), measuring instruments (Pyrex® Iwaki Glass), autoclave (TOMY SX-500), stir bar, maceration vessel, blender (Miyako), bunsen, porcelain, separatory funnel, hot plate (MaxBlend), incubator (Memmert), *laminar air flow*, refrigerator, micropipette, analytical balance (Ohauss).

The ingredients used were *G. forbesii* pericarp, distilled water, aluminum foil, bacteriological agar no. 1 (OXOID), barium chloride, ferric chloride, blank paperdisc, cotton swab, Chloramphenicol Supplement (OXOID), liquid spirits, 70% ethanol (technical), ethyl acetate (technical), gelatin, Whatman No.1 filter paper, bacterial culture *S. aureus* ACC 25923, *P. acne* ATCC 6919.

Methods

Plant Determination

Plant determination was carried out at the Basic Biology Laboratory, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University. Plant determination aims to match the morphological characteristics of the plants to be studied by looking at the literature, so there is no mistake in taking plants for research.

Sample Preparation

Samples of *G. forbesii* pericarp were wet sorted, washed, and the pericarp was separated from the fruit flesh. Then cut into thin slices and dried at 60ºC for three days. Dried samples were powdered using a mixer, then weighed and stored in a container. As much as 55 grams of dry powder of mundar pericarp was macerated with 70% ethanol at a ratio of 1:10 for 3 x 24 hours. The maceration results were filtered, the solvent was evaporated with a rotary evaporator, and concentrated with a waterbath [16].

Fractionation using *n*-hexane and ethyl acetate using the liquid-liquid extraction. The extract was dissolved in aquades, added with n-hexane, and leave it until two layers were formed. The *n*-hexane fraction was taken and concentrated using a waterbath. The aquades fraction was added with ethyl acetate solvent, shaken, and left until two layers were separated. The ethyl acetate fraction was separated and then concentrated using a waterbath [17].

Test Sample Preparation

The samples used at concentrations of 1.5%, 1.25%, 1%, 0.75%, and 0.5% (b/v) from ethanol extracts, n-hexane fractions, and ethyl acetate fractions. of G. forbesii pericarp. The positive control used 1% chloramphenicol, while the negative control used DMSO because it was a diluent [18].

Bacterial Rejuvenation

The test bacteria *S. aureus* ACC 25923 and *P. acne* ATCC 6919 came from the Banjarbaru Industrial Research and Standardization Center Laboratory. Each bacteria was taken 1 mL and then inoculated into the natrient broth (NB) medium in a closed test tube and incubated at 37°C for 24 hours [19]. The bacteria incubated were then visually compared for turbidity with a 0.5 McFarland standard (10^8 colonies/mL). Other comparisons were made by plating bacteria on NA media using the pour plate method. The bacteria were diluted, then plated, and put into a petri dish, and sufficient NA media was added and then incubated for 24 hours. The incubation results were observed, and counted manually how many bacteria grew [20].

Inhibition Zone Diameter Test

Inhibition Zone Diameter Test by the diffusion method make us of a paper disc or the so-called Kirby Bauer method. *Nutrient agar* medium is prepared by mixing 15 g of agar with 13 *g* of *nutrient broth* and adding distilled water to a volume of 1000 mL. *Nutrient Agar* sterile medium is poured into the petri dish until evenly distributed and allowed to solidify. The petri dish containing the solidified media was divided into six areas, then the bacterial suspension was smeared on the *Nutrien Agar* medium using a sterile *cotton swab*. A *blank paper disc* is dipped into each extract and fraction according to the concentration, then affixed to the media that has been bacteria smeared, and incubated for 24 hours at 37°C. The diameter of the clear zone formed was measured with a ruler and recorded [21]. The formula can calculate the diameter of the inhibition zone: $\frac{d1+d2}{2} - x$.

Description:

d1 = vertical diameter of the clear zone in the media.

d2 = horizontal diameter of the clear zone in the media.

X = disc paper diameter (6 mm).

Minimum Inhibitory Concentration Test (MIC)

The MIC test was carried out using the microdilution method. The bacteria to be used are equated with the McFarland standard 0.5 (10^5 colonies/mL), which is prepared by taking 1 mL of bacteria from the 10^8 colonies/mL previously made and put into a test tube containing 9 mL of *nutrient broth*, then diluted again by taking 0.5 mL of bacteria and putting it into 9.5 mL of *nutrient broth*, so that 10^5 colonies/mL of bacteria are obtained. 0.1 µL bacterial suspension was put into a *polypropylene PP centrifuge tube* containing 0.7 µL NB, then added with 0.2 µL sample (extract or fraction). This treatment was carried out on all *tubes*, the only difference being the concentration used [20], and then incubated for 24 hours at 37°C. After incubating, the *tube* containing the bacteria and the ethanol extract sample, the *n*-hexane fraction and the ethyl acetate fraction were smeared on the surface of the NA media in a petri dish divided into five parts, then incubated for 24 hours at 37°C. The minimum inhibitory concentration results are taken from the lowest concentration where there is no bacterial growth [22].

Minimum Kill Concentration Test (MKC)

The MKC test was carried out using the *Streak plate* (scratch method). MKC determinations were carried out at concentrations considered as MIC values. The NA media was put into a petri dish and allowed to solidify. Samples with MIC values were taken to be scratched on the surface of the NA media using a *cotton swab*, then incubated for 24 hours at 37°C. Results that show no more bacterial growth are considered MKC. A clear area on the petri dish indicates no bacterial growth. In contrast, an area that looks like streaks indicates bacterial growth, so at this concentration, it can only inhibit bacterial growth [21,22].

Data Analysis

The effects of the inhibition zone diameter on bacterial growth inhibition of ethanol extract samples, n-hexane fractions, and ethyl acetate

fractions were analyzed using SPSS [23]. The data in the second stage were in the form of minimum inhibitory concentration (MIC) and minimum killing concentration (MKC). Each data is presented in tabular form and interpreted according to the results obtained.

Result and Discussion

Plant Determination Test Results

The results of the determination test showed that the sample used in this study was Mundar fruit (*Garcinia forbesii*) based on the test results certificate Number 040a/LB.LABDASAR/I/2019. The results are that the tree has a height of 15-17 m, leaves are oblong with a width of 6.63 cm, and stem height is 3-4 m. The fruit is spherical or round with a diameter of 2-3 cm, weighing less than 90 g; the color of the fruit is red when ripe, and the color of the fruit coating is a white cream with a soft and watery texture. The seeds are light brown with an oblong shape, 1.28 cm long, 0.65 cm wide, and 0.25 cm thick.

Sample Preparation Results

The dry powder result obtained was 43.39 g from the 500 g wet sample. These results indicate that there is shrinkage of the sample after drying. This is due to the large amount of water in the peel of *G. forbesii* fruit. Previous research stated that the water content contained in *G. forbesii* fruit skin was 11% [24].

Mundar fruit dry powder was extracted and then calculated the extract obtained was. The yield calculation results are provided in table 1.

Table 1. Extraction Results of *G. forbesii* pericarp

Sample	Powder Weight	Extract Weigt	Percent (%) yield
G. forbesii ethanol extract	55.00 g	31.573 g	57.40

Based on the table above, the percent yield obtained in this study is not much different from previous studies. The percent yield of *G. forbesii* pericarp obtained in previous research extracted using 70% ethanol had a yield of 57.83% [25,26]. Calculation of percent yield was carried out to determine the percentage of metabolites extracted from the extraction process [27].

The extract obtained was then carried out by liquid-liquid fractionation. The results of *G. forbesii* pericarp fractionation can be seen in Table 2.

Table 2. *G. forbesii* fruit peel fractionation results

Extract weight	*n*-hexane fraction weight	ethyl acetate fraction weight	*n*-heksan Percent (%) yield fraction	e Per
20.00 g	0.201 g	14.01 g	1.005	

Based on the results of yield calculations, the ethyl acetate fraction has a more significant yield percentage than the *n*-hexane fraction. The yield calculation results are related to the number of metabolite compounds extracted in a sample. The sample's high content of metabolite compounds is indicated by the resulting high yield value [28]. The percentage yield obtained from the *n*-hexane fraction of *G. forbesii* pericarp was not much different when compared to the percent yield of the *n*-hexane fraction of mangosteen pericarp (*Garcinia mangostana)* which was 1.34% [29], while the percent yield of the ethyl acetate fraction more significant than that of previous research, namely 17.08% in the ethyl acetate fraction of mangosteen pericarp (*Garcinia mangostana*) [30]. The results obtained differed from the percent yield of the ethyl acetate fraction of *G. forbesii* pericarp in previous studies, which was 28.5% [26].

Inhibitory Power Test Results

The results of the diameter of the inhibition zone of the samples of *G. forbesii* pericarp can be seen in Table 3.

Table 3. Diameter of Zone Inhibition in Bacterial Growth from samples of G. forbesii pericarp

Sample	x ± SD diameter of zone inhibition (mm)	
	S.aureus	*P.acne*
Extract ethanol	2.16±0.983	2.75±1.405
Fraction *n*-hexane	2.08±0.664	4.08±0.664
Fraction ethyl acetate	5.08±1.020	14.33±3.326
Control positive	6.24±0.592	19.5±1.449
Control negative	0±0	0±0

The results showed that the diameter of the inhibition zone formed in the ethyl acetate fraction was more significant than in the ethanol extract and *n*-hexane fraction; this was probably due to the compounds contained in the ethyl acetate fraction of *G. forbesii* pericarp being more active than those in the ethanol extract and the *n*-hexane fractions, and there were specific compounds act as antibacterial in the ethyl acetate fraction [31,32]. The samples of *G. forbesii* pericarp had different inhibition zone diameters against *S. aureus* and *P. acne* bacteria.

Antibacterial activity can be influenced by several factors, including the concentration of the sample (extract or fraction), the content of antibacterial compounds, the diffusing power of the extract or fraction, the type of bacteria that is inhibited, the thickness of the agar plate, and the sensitivity of the bacteria to samples of *G. forbesii* pericarp [33]. Based on the diameter of the inhibition zone, the positive control of 1% chloramphenicol proved that the antibiotic compound could affect positively to the growth of bacteria, while the negative control DMSO did not affect the growth of bacteria by not forming an inhibition zone.

The results showed that the activity as antibacterial of samples against *S. aureus* bacteria was classified as weak. Based on the results of the Shapiro-Wilk test, the value of p> 0.05, or the data is normally distributed, the test is continued with the homogeneity test, the result is p <0.05, or the data is not homogeneous. The analysis continued with a non-parametric test, namely Kruskal-Wallis and the Mann-Whitney test. The results of the Mann-Whitney test of the samples on the growth of *S. aureus* bacteria can be seen in Table 4.

Table 4. Mann-Whitney test results for the samples of *G. forbesii* pericarp based on the diameter of the inhibition zone

Treatment group	Aquades	Ethanol extract	*n*-hexane fraction	Ethyl acetate fraction	Chloramphenicol 1%
Aquades	-	0.002*	0.002*	0.002*	0.002*
Ethanol extract	0.002*	-	0.738	0.004*	0.004*
n-hexane fraction	0.002*	0.738	-	0.004*	0.004*
Ethyl acetate fraction	0.002*	0.004*	0.004*	-	0.053
Chloramphenicol 1%	0.002*	0.004*	0.004*	0.053	-

Description: *p <0.05 there is a significant difference

SPSS analysis showed that the antibacterial activity of the ethanol extract and the *n*-hexane fraction was not much different, while the ethanol extract and ethyl acetate fraction had quite different antibacterial activity. The antibacterial activity of the ethyl acetate fraction and the positive control was not much different; this meant that the ethyl acetate fraction had good antibacterial activity against *S. aureus* bacteria.

The results indicated that the activity as antibacterial of the ethanol extract against *p. acne* belongs to the weak category, the *n*-hexane fraction belongs to the medium category, and the ethyl acetate fraction belongs to the strong category. Based on the results of the Shapiro-Wilk test, the value of p>0.05, or the data is normally distributed, the test is continued with the homogeneity test, the result is p<0.05, or the data is not homogeneous. The analysis was continued with a non-parametric test, namely Kruskal-Wallis, and continued with the Mann-Whitney test. The results of the Mann-Whitney test of the samples of *G. forbesii* pericarp on the growth of *P. acne* bacteria can be seen in Table 5.

Table 5. Mann-Whitney test results for the samples of *G. forbesii* pericarp based on the diameter of the inhibition zone

Treatment group	Aquades	Ethanol extract	*n*-hexane fraction	Ethyl acetate fraction	Chloramphenicol 1%
Aquades	-	0.002*	0.002*	0.002*	0.002*
Ethanol extract	0.002*	-	0.055	0.004*	0.004*
n-hexane fraction	0.002*	0.055	-	0.004*	0.004*
Ethyl acetate fraction	0.002*	0.004*	0.004*	-	0.013*
Chloramphenicol 1%	0.002*	0.004*	0.004*	0.013*	-

Description: * p <0.05 there is a significant difference

SPSS results showed that the activity as antibacterial of the ethanol extract and *n*-hexane fraction was not significantly different, while the antibacterial activity of the ethanol extract, *n*-hexane fraction, and ethyl acetate fraction to the positive control had quite a difference, so it can be interpreted that the resulting antibacterial activity of the ethanol extract, the *n*-hexane fraction and the ethyl acetate fraction were not as substantial as the antibacterial activity of the positive control which had been shown to inhibit bacterial growth because the positive control contained antibiotics.

Minimum Inhibitory Concentration Test Results (MIC)

MIC test results for the samples of *G. forbesii* pericarp can be seen in Table 6.

Table 6. Minimum inhibitory concentration (MIC) of the samples of *G. forbesii* pericarp

Sample	Consentration (%)	*S. aureus*	*P. acne*

Ethanol extract	1.5	+	+
	1.25	+	+
	1	+	+
	0.75	+	+
	0.5	+	+
n-hexane fraction	1.5	+	-*
	1.25	+	+
	1	+	+
	0.75	+	+
	0.5	+	+
Ethyl acetate fraction	1.5	+	-
	1.25	+	-
	1	+	-*
	0.75	+	+
	0.5	+	+
Positive control	1	-	-
Negative control		+	+

Information :

Sign (+) : there is bacterial growth

Sign (-) : no bacterial growth

Sign (-*): MIC value

The results showed that the ethanol extract did not have MIC values for all bacteria, whereas for the *n*-hexane fraction, the MIC values were obtained for *P. acne* at a concentration of 1.5%, and for *S. aureus* bacteria, no MIC values were obtained, as well as for the ethyl acetate fraction obtained MIC values for *P. acne* bacteria at a concentration of 1%, and MIC values were not obtained for *S. aureus* bacteria. Based on the results acquired shows that the ethyl acetate fraction has the smallest MIC value compared to other samples; these results follow the outcomes of previous tests where the ethyl acetate fraction has the largest diameter of the inhibition zone compared to other samples.

Minimum Kill Concentration Test Results (MKC)

The results of the MKC test for the ethanol extract, *n*-hexane fraction, and ethyl acetate fraction of *G. forbesii* pericarp can be seen in Table 7.

Table 7. Minimum killing concentration (MKC) of ethanol extract, *n*-hexane fraction, and ethyl acetate fraction of *G. forbesii* fruit peel

Sample	Consentration (%)	*S. aureus*	*P. acne*
n-hexane fraction	1.5	Not tested	-*
	1.25	Not tested	Not tested
	1	Not tested	Not tested
ethyl acetate fraction	1.5	Not tested	-*
	1.25	Not tested	+
	1	Not tested	+

Information :

Sign (+) : there is bacterial growth

Sign (-) : no bacterial growth (MKC value)

Sign (-*): has no MIC value

The results showed that the *n*-hexane fraction had a MKC value against *P. acne* at a concentration of 1.5%, or the antibacterial activity of the *n*-hexane fraction against *P. acne* was bactericidal. The ethyl acetate fraction obtained the MKC value for *P. acne* at a concentration of 1.5% and was bacteriocidal, while for *P. acne* at a concentration of 1%, it was bacteriostatic because, at that concentration, there was bacterial growth after incubation. This is because the higher the concentration, the more antibacterial compounds are contained in the sample, so the capability to inhibit bacterial growth is higher [34].

Conclusion

The results of this study demonstrated that the ethyl acetate fraction of G. forbesii pericarp was more effective than the ethanol extract and n-hexane fraction against P. *acne* on the basis of zone of inhibition diameter, MIC and MKC. It has been shown to have strong antibacterial activity.

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