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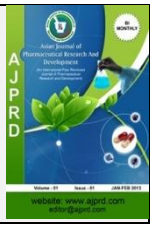

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Research Article

Antibacterial Activity Test of Ethanol Extract From Unripe Klutuk Banana Fruit (Musa Balbisiana Colla Bb) Towards Bacterial of Staphylococcus Aureus and Escherichia Coli

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A B S T R A C T

Object : This study aims to look at antibacterial activity Ethanol extracts from the flesh and peel of unripe klutuk banana fruit (*Musa balbisiana* Colla BB) against *Staphylococcus aureus* and *Escherichia coli.*

Methods : Study included phytochemical screening, simplicial and extract characterization, antibacterial testing using a well diffusion method ethanol extracts from the flesh and peel of unripe klutuk banana fruit (*Musa balbisiana* Colla BB) against *Staphylococcus aureus* and *Escherichia coli.*

Results : The phytochemical test showed that both extracts contained alkaloid compounds, flavonoids, tannins, triterpenoids, and saponins. The fruit simplicial characterization of the flesh and peel contains 5% and 7.17% of water level; 14.55% and 11.61% of total ash level; 10.67% and 9.67% of water-soluble level; 9.67% and 9.67% of ethanol-soluble level. The ethanol extracts of the flesh and peel of unripe klutuk banana contains water level of 28.26% and 15.5%;total ash level of 10.16% and 14.09%; water-soluble level of 33.33% and 71.67%, and ethanol-soluble level of 69.16% and 78.33%. The highest activity of the peel ethanol extract towards *S. aureus* was obtained at 10% concentration with an inhibiting zone diameter of 9.3 mm and the highest activity of the flesh ethanol extract towards *E. coli* was obtained at 20% concentration with an inhibiting zone diameter of 8.63 mm.

Conclusion : Antibacterial activity against *S. aureus* by the ethanol extract from the peel was higher than ethanol extract from the flesh of the fruit. While antibacterial activity towards bacterial *E. coli* by the flesh of fruit showed better activity.

Keywords : Unripe Klutuk banana fruit, *Musa balbisiana* Colla BB, ethanol extract, antibacterial.

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INTRODUCTION

Diarrhea is a digestive system disorder characterized by increased intestinal peristalsis, fluid secretion, volume, and frequency of bowel movements with soft and liquid consistency of stools [1]. The high incidence of diarrhea in developing countries, such as Indonesia, makes diarrhea an endemic and outbreak disease which is often accompanied by death. Diarrhea causing death shows a percentage in infants (31.4%) and toddlers (25.2%), while in all age groups (13.2%)[2]. Several factors that influence the occurrence of diarrhea include nutrition, environmental ,socio-economic conditions, and community behavior. The

causes of diarrheal diseases include viruses, namely *Rotavirus* (40-60%), the parasite *Entamoeba hystolitica* (<1%), and the bacteria *Shigella sp.* (1-2%) and *E. coli* (20-30%) [3]. Diarrhea can be caused by infection, such as Gram-positive bacteria: *Staphylococcus aureus*, and Gram-negative bacteria: *Escherichia coli* [4].

Empirically, people have used medicinal plants to treat various diseases. *Klutuk* banana (*Musa balbisiana BB*) is one of the common plants used traditionally to treat typhus, peptic ulcer, tonsillitis, intestinal bleeding, dysentery, and diarrhea [5]. The plant is typically grown in yards or found in forests. Acehnese people use unripe klutuk bananas as a mixture in the *Rujak Aceh*, believed toprevent stomach aches. In a ripe condition, it has many seeds making it unpalatable and eventually underutilized. The fruit contains secondary metabolites, namely tannins, steroids, terpenoids, saponins, glycosides, and flavonoids[6], along with nutrients including carbohydrates, proteins, fats, and minerals[7].

Several studies regarding the antibacterial activity of *M. balbisiana* banana fruit extract, including the ethyl acetate fraction from the flesh of the *klutuk* banana (*M. balbisiana* Colla), reported the greatest antibacterial activity of *S. aureus* at a concentration of 500 mg/mL [8]. The ethanol extract of unripe klutuk banana (*M. balbisiana* Colla) at the concentration of 40% w/v reported an inhibition zone of 14.03 mm against *Shigella dysenteriae* ATCC 13313[9]. Saraswati [10] states that 96% ethanol extract of ripe banana peel waste (*M. balbisiana*) at the concentration of 100,000 mg/L produces a 12.4 mm diameter of the inhibition zone against *S. aureus*. While the results of Mawarni's research[11] states that the ethanol extract of unripe klutuk banana (*M. balbisiana BB*) against mice (*Mus musculus*) induced by castor oil (*Oleum ricini*) was 50% greater than loperamide HCl as an anti-diarrhea. *M. balbisiana* banana extract has antibacterial activity towards *S. aureus*, but there has not been any research on the activity of *M. balbisiana* banana extract towards *E. coli*. Likewise, there are no studies on the antibacterial activity of the ethanol extract of unripe *M. balbisiana* BB banana towards *S. aureus* and *E. coli*. Based on the description above, the researchers are interested in testing the antibacterial activity of the unripe klutuk banana (*M. balbisiana* BB) fruit extract by obtaining the peel and flesh through separation.

MATERIAL AND METHODS

Sampling technique

The sampling technique was purposive sampling, the sample was taken from a banana *(Musa balbisiana* Colla BB*)* which had green flesh no seeds. The banana samples used came from Indrapuri, Aceh Besar District. Indonesia. The sample had been identified at Herbarium Bogoriense Botanical Department, The Indonesian Institute of Sciences (LIPI) Bogor.

PHYTOCHEMICAL SCREENING

Phytochemical screening extract by qualitatively analyzed,including the presence of alkaloid, flavonoid, tannin, saponin, steroid, and terpenoid compounds[12,13].

CHARACTERIZATION SIMPLICIA AND EXTRACT

Characterization **ethanol-soluble level** simplicia and extract from the flesh and peel including **water level, water- soluble level and total ash level** [12,14].

Preparation of Flesh and Peel of Unripe *Klutuk* Banana Fruit Simplicia

25 kg of flesh and peel of unripe *klutuk* banana were cleaned by running water aimed to remove the impurities attached to the sample results, so it doesnot interfere with the test results. After that, chopped into small parts achieve a faster drying process. Furthermore, the very tender parts of the flesh and peel were weighed separately and dried at room temperature protected from direct sunlight. The simplicial were weighed and crushed using a blender to become coarse powder, then reweighed[15].

Preparation of Flesh and Peel of Unripe *Klutuk* Banana Fruit Extract

The extraction of flesh and peel of unripe klutuk banana was carried out by maceration using distilled ethanol with a simplicial : solvent ratio of 1:10. 1100 g flesh simplicia and 1400 g peel simplicia was put in a maceration container then ethanol was added as much as 7.5 parts of 10 parts of solvent which was 3.75 L, then stirred. Maceration lasted for 5 days while occasionally stirred, and then filtered. The filtrate was concentrated using a rotary evaporator at 50ºC to obtain a viscous extract[16]. The viscous extract acquired was used for phytochemical screening and antibacterial testing.

Preparation And Bacterial Activity Testing

Bacteria from stock culture were taken each using an Ose needle, then suspended into a test tube containing 5 mL of 0.9% NaCl solution, then homogenized with vortex for 15 seconds. Then the bacterial suspension solution was put into a 2-3 mL cuvette and the absorbance was measured using a spectrophotometer at a wavelength of 600 nm (OD 600) = 0.5 (0.5 x 108 CFU)[17]. Antibacterial activity test was carried out using well diffusion method, by making a well or hole in the MHA media containing test bacteria. A bacterial inoculation test on MHA media was carried out by the pouring method. A total of 500 μL of *E. coli* or *S. aureus* bacterial suspension was put into each Erlenmeyer flask containing 20 mL of MHA media that had been sterilized and cooled to temperatures up to 50ºC, then

shaken until homogenous and allow to settle. Then 6 wells or holes were made, each with a 4 mm diameter. Every 20 µL drop of flesh and peel ethanol extract and with concentrations of 5, 10, 20, 30, 40%, distilled ethanol, 2% amoxicillin, and 4% gentamicin were made in separate wells, then the media was incubated at 37ºC for 24 hours[18]. The treatment was repeated three times.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical test results of ethanol extract from flesh and peel of unripe klutuk banana fruit can be seen in Table 1.The result shows that both samples have the same secondary metabolite compounds. This can happen because the sample used was still young and was the same part of the fruit, even though it is separated between flesh and peel. Some researchers have also reported the same content as this study. Salau *et al*[19] mentioned that bananas contain flavonoid compounds, saponins, tannins, alkaloids, anthraquinone, and quinone. And the peel of banana contains alkaloids compounds, flavonoids, saponins, and tannin that can inhibit bacterial growth.

Characterization Simplicia And Extract

Characterization of simplicia and extract from flesh and peel of unripe klutuk banana fruit can be seen in Table 2 and Table 3.

Table: 1. Phytochemical Screening of Extract of *Musa balbisiana* Colla BB

Plant constituent	Flesh	Peel
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Steroid	-	-
Triterpenoids	+	+

Table: 2. Characterization of simplicia from flesh and peel of unripe klutuk banana fruit

Test	Mean Percentage of Yield (%) ± SD	
	Flesh	Peel
Water level	5,00 ± 1,00	7,17 ± 0,58
Total ash level	14,55 ± 0,27	11,61 ± 0,16
Water-soluble level	10,67 ± 0,57	9,67 ± 2,08
Ethanol-soluble level	9,67 ± 2,31	9,67 ± 1,53

Table: 3. Characterization of extract from flesh and peel of unripe klutuk banana fruit

Test	Mean Percentage of Yield (%) ± SD	
	Flesh	Peel
Water level	28,26 ± 0,36	15,5 ± 1,32
Total ash level	10,16 ± 0,52	14,09 ± 3,82
Water-soluble level	33,33 ± 1,44	71,67 ± 1,44
Ethanol-soluble level	69,16 ± 1,44	78,33 ± 2,88

Antibacterial Activity Test Outcome

Antibacterial activity tests were carried out with variations in the concentration of 5, 10, 20, 30, and 40% using a well method with a diameter of 4 mm. Antibacterial activity results were identified by the formation of a clear zone around the wells containing the test substance. The measurement results of the inhibition zone diameter on the antibacterial activity of ethanol extract from flesh and peel of unripe klutuk banana fruit can be seen in Table 4.

Based on Table 4 it can be seen that the ethanol extract from flesh and peel of unripe *klutuk* banana fruit can inhibit the growth of *S. aureus* and *E. coli*. Based on the results of antibacterial activity of the ethanol extract from the flesh of unripe *klutuk* banana fruit on *S.aureus* and *E.coli* obtained the largest inhibition zone diameter of 9.2 mm and 8.63 mm at 20% concentration. Whereas the ethanol extract from the peel of unripe klutuk banana fruit on *S.aureus* was 9,3 mm at 10% concentration and on *E. coli* obtained by 7.97 mm at 40% concentration.

The antibacterial activity shown by the ethanol extract from peel towards *S. aureus* and *E. coli* shows a difference. At a concentration of 10% was able to produce the largest inhibition zone in S. *aureus*, while *E. coli* has the largest inhibition zone at 40%concentration. This is presumed by differences in the cell wall structure of the two bacteria related to the ability of secondary metabolites to inhibit these bacteria. The cell wall

structure of Gram-positive bacteria has a thick peptidoglycan layer and the presence of teichoic acid while Gram-negative bacteria have a lipopolysaccharide layer with thin peptidoglycan. The largest inhibition zone against *S. aureus*, and *E. Coli* by ethanol extract from the flesh was at 20% concentration, while the ethanol extract from the peel at 10% concentration against *S.aureus* showed a decrease in the inhibition zone diameter. This can occur due to differences in diffusion rates of antibacterial compounds on agar media. The different types and concentrations of antibacterial compounds provide different inhibition zone diameters for a certain length of time[20]. As for the results by ethanol extract from the peel, the largest inhibition zone was obtained at a concentration of 40%, confirming the higher concentration of the extract used, the larger inhibition zone. Because high concentration was able to increase the penetration of antibacterial compounds into bacterial cells so that it will interfere with cell metabolism and cause cell lysis[21].

Table 4. Antibacterial activity test results from flesh and peel of unripe *klutuk* banana fruit ethanol extract on *S. aureus* and *E. coli*

Extract concentration (%)	Average inhibitory zone diameter (mm) ± SD			
	Flesh Extract		Peel Extract	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
5	6.70±0.36	6.26±1.00	5.60±0.36	5.10±1.00
10	7.50±0.26	7.10±0.30	9.30±3.40	5.53±1.21
20	9.20±0.40	8.63±0.75	8.51±1.83	6.00±1.45
30	8.63±1.56	7.93±0.73	8.03±0.73	6.67±0.80
40	8.60±1.11	8.06±0.74	8.96±2.85	7.97±0.65
Positive Control	28.50±1.12	35.00±0.92	29.93±1,41	35.63±2.83
Negative Control	0±0	0±0	0±0	0±0

Description : Positive Control :*S. aureus* :amoxicillin *E. coli* : gentamicin Negative Control : Ethanol Solvent

The inhibition zone diameter of the ethanol extract from flesh and peel on *S. aureus* was smaller than the diameter of the amoxicillin inhibition zone which was used as the positive control, the flesh extract tested 28.5 mm, and the peel extract tested 29.93mm. Based on this result, it shows that amoxicillin iscategorized as sensitive, namely ≥29 mm[22]. The diameter of the inhibition zone of flesh and peel extracts in *E. coli* was also smaller than the diameter of the gentamicin inhibition zone which was used as a positive control, the flesh extract tested 35 mm and thepeel extract tested 35.63 mm. It shows that gentamicin is categorized as sensitive, namely > 15 mm, and can inhibit the growth of *E. coli*[22] .

The research results on ethanol extract from unripe *klutuk* banana fruit showed antibacterial activity test towards *S.aureus* and *E. coli* with the largest inhibition zone at 20% concentration, which was different from the study of Sitorus *et al*[8] that reported banana fruit extracts from *M. balbisiana*, including ethyl acetate fraction from the flesh of klutuk banana fruit (*M. balbisiana* Colla), have the largest antibacterial activity towards *S. aureus* at a concentration of 500 mg/mL (50%). If compared with the results of this study, the differences can be caused by the use of different secondary metabolites which are used inthe flesh of unripe klutuk banana, namely 96% ethanol while the study of Sitorus *et al*[8] used ethyl acetate fraction. The type of solvent affects active compounds that are attracted from the extraction process and can produce different phytochemical results. Meanwhile, no research results have been reported for theflesh of unripe klutuk banana using 96% ethanol as a solvent. The ethanol extract from the peel of unripe klutuk banana fruit in this study had the largest activity with the inhibition zone by 9.30 mm at 10% concentration against *S. aureus*. This result is different from the research conducted by Saraswati[10] which reported that 96% of the ethanol extract from ripebanana peelwaste (*M. balbisiana*) at a concentration of 100,000 mg/L (10%) made an inhibition zone diameter of 12.4 mm towards *S. aureus*. The phytochemical results in Saraswati's[10] study obtained the same results as the research conducted on unripe klutuk banana fruit samples, namely the presence of alkaloid compounds, flavonoids, saponins, tannins. The terpenoid and steroid compound tests were not carried out in that study. Although the results of the same phytochemical test were obtained, the differences in the results of this study could be due to different levels of secondary metabolites found in unripe klutuk banana that was used in the extract. This may due to differences in age of the fruit used when making the extract, the environment in which it is grown, the age at which it is harvested, the solvent that is used, and it can also be due to differences in plant parts used in the study. Harvest time is closely related to the formation time of secondary metabolite content so that harvesting is better done when

the secondary metabolite content is at maximum levels[23].

The content of secondary metabolites, namely alkaloids, flavonoids, saponins, triterpenoids,and tannins in the ethanol extract of the flesh and peel provides the antibacterial activity. The two ethanol extracts contain the same secondary metabolite compounds but different inhibition zones can occur because the amount of secondary metabolite content contained in the extract is not known with certainty. This is because the analysis carried out is the qualitative analysis of secondary metabolites, so it is not known the composition of active metabolites that have antibacterial activity. Besides, the compounds contained in the extract still consist of many types, so compound activity may occur whichinhibits the work of other compounds.

The test for the antibacterial activity of flesh and peel extracts showed that the inhibition zone of the flesh and peelwas larger in *S. aureus* than in *E. coli*. This is possible because that the structure of the Gram-positive bacteria cell wall is simpler than the Gram-negative, which has a thick peptidoglycan layer and teichoic acid that creates a rigid cell structure. Therefore, it is suspected that the antibacterial compounds contained in the extract of the flesh and peel of unripe klutuk banana can easily damage the cell walls of *S. aureus*. The teichoic acid, which is also present in the cell walls of Gram-positive bacteria, is known to be polar causing easierpenetration ofthe bacterial cell walls. Meanwhile, Gram-negative bacteria have a more complex cell wall structure so that secondary metabolites are difficult to penetrate, thatact as antibacterial. The cell wall of Gram-negative bacteria consists of lipoproteins, thin peptidoglycan, and the outer membrane consisting of phospholipids and lipopolysaccharides, which are complex lipids. Large antibacterial molecules are relatively slow when penetrating the outer membrane, causing Gram-negative bacteria to be more resistant to antibacterials[24].

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