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Comparison of Unripe Banana Peel of Kepok (*Musa paradisiaca* L.) and Klutuk (*Musa balbisiana* Colla): Phytochemical and Anti- dysenteriae Activity

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Abstract

Aim: The objective of this study is to compare the phytochemical and antidysentriae activities of unripe banana peel ethanolic extract of kepok (*Musa paradisiaca* L.) and klutuk (*Musa balbisiana* Colla) variants.

Methods: The peel of the unripe banana fruits was dried and macerated using ethanol 70%. The extracts were screened for its phytochemical contents using standard methods and confirmed with thin layer chromatography. The antibacterial activity of the extracts against *Shigella dysenteriae* ATCC 13313 was assessed using the agar diffusion method. The values of minimum inhibitory concentration and minimum bactericidal concentration were determined using microdilution method followed by subculturing the result onto the surface of agar medium.

Results: The phytochemical screening results showed that both of banana peel extracts contain the same secondary metabolites, such as flavonoids, tannins, monoterpenoids and sesquiterpenoids. Saponin was found only in the extract of Klutuk banana peel. This result was linear with the antidisentrial activity test which reported that Klutuk banana peel ethanol extract had produced inhibition diameter greater than that of kepok. The MIC values of both extracts were in the range 5-10% w/v while the MBC values were 10-20% w/v.

Conclusion: It can be concluded that peel extract derived from unripe Klutuk banana provides a potential drug for dysentery disease.

Keywords: S*higella dysenteriae*, extract, peel, *Musa paradisiaca* L., *Musa balbisiana* Colla

INTRODUCTION

Dysentery is still one of the infectious disease problems that existed in Indonesia. Dysentery can be caused by many different things, but the cause of dysentery was reported most often is the bacteria *Shigella flexneri* and particularly *Shigella dysenteriae* [1]. From data on Indonesia, mentioned that 29% of deaths caused by dysentery occurred at age 1-4 y, caused by Shigella [2]. In some cases of dysentery deaths occur because of delays in handling the dehydration [3]. Potassium is one of the elements that play a role in maintaining the balance of electrolytes and acid-base regulation in the body [4]. Normal levels of potassium in serum is 3.5-5.0 mEq/l when the potassium in plasma levels less than 3.5 mEq/l, dehydration will occur [5].

Trimetoprim-sulfametoksazol were recommended drug by the WHO for treating basilar dysentery. But resistance cases of *S. dysenteriae* against trimetoprim-sulfametoksazol has been found [6]. In addition, *S. dysentriae* has been resistant to ampicillin, kotrimoksazol, chloramphenicol and tetracycline [7]. The raising of resistance cases against several potential antibiotics made the healing infection *S. dysentriae,* were more complicated.

In recent years, due to the increasing rate of diseases with multidrug resistant microorganisms, increasing interest in finding new anti-dysentery drugs [8,9]. This shows that the investigation of antimicrobial activity of medicinal plants, are very important. Plants produce highly secondary metabolites as bioactive compounds that capable in inhibiting microbial growth. The discovery of antidysentriae basilar candidate can be assessed through the utilization of Indonesian native herbs. The banana is one of the most popular fruits distributed all over the world. In Indonesia, the production of banana fruit is very high. Traditionally, unripe banana fruits are used in dysentery and diarrhea disease [10,11]. The part of banana fruit which not used like banana peels had been reported contain flavonoids and tannins [12]. Another study also reported that the ethanolic extract of banana fruits contains flavonoid and tannin compounds that had been proved as the antibacterial agent against *S. dysentriae* [13]. Flavonoids have the ability to form complexes with protein extracellular solute dissolved or not, and can form a complex with the cell wall. The more lipophilic a flavonoid, its ability to damage the cell wall of the bacteria will be more powerful. While tannins could play a role in damaging components of cell membranes, cell walls, enzymes, genetic material, as well as other protein components [14]. Despite antibacterial metabolites, the banana peels have also reported as a source of natural potassium and donated 78 mg potassium in 100 g banana peel [15]. It can overcome dehydration caused by increased diarrhea frequency because dysentery disease is an important cause of morbidity and mortality associated with diarrhea. Oral Rehydration Therapy (ORT) has been identified as a key factor in the decline of child mortality rate due to diarrhea, although it does not reduce the volume or duration of diarrhea [16]. Diarrheal episodes that begin with dysentery are more likely to become persistent than those that start with watery stools. This is one of the common causes of hypokalemia. Hypokalemia is a common electrolyte disorder caused by changes in potassium intake, altered excretion, or transcellular shifts [17]. Thus in addition as antidysentrial drug, the ethanol extract of banana peel can supply potassium and prevent dehydration caused by hypokalemia.

MATERIALS AND METHODS

Materials

The fruits of Klutuk and Kepok banana (3 months) were collected from Cimincrang Village, District Gedebage, Bandung, West Java, Indonesia and identified in Plant Taxonomy Laboratory of Biology Major, Faculty of Mathematics and Natural Science Padjadjaran University.

Preparation of Peel Extract

The peels of banana fruit were manually separated from the whole fruits, dried in hot air woven, and pulverized into coarse powder using a mechanical grinder. About 300 g of the dried powder materials was maserated in 70% ethanol for 3 d with filtrate taken every 24 h. The total filtrate was concentrated using a rotary evaporator then continued by evaporation on a water bath at a temperature of 40-50 °C to render the thick ethanolic extract (20,52 g).

Phytochemical Screening

The ethanol extracts were screened for the presence of alkaloids, saponins, tannins, flavonoids, steroids, triterpenoids, polyphenols, quinones, and terpenes using standard methods as described by Harborne (1973) [18].

Thin Layer Chromatography Assay

TLC assay was conducted using silica gel 60 F_{254} as the stationary phase and the upper layer of chloroform: acetone: formic acid

(75:16.5:8.5) that had been saturated for 24 h as the moving phase. The Rf values were obtained from the spots detected by 254 and 366 nm UV rays [19].

Preparation of 0.5 Mc Farland Standard Solution

McFarland standards are made to standardize microbial testing by adjusting the turbidity of bacterial suspensions. Standard solution Mc. Farland consists of two components, namely 1% $BaCl_2$ and 1% H_2SO_4. A total of 0.05 mL of 1% $BaCl_2$ solution was mixed with 9.95 mL of 1% H_2SO_4 solution and shaken homogeneously. The solution turbidity was measured at a wavelength of 620 nm with using distilled water as its blank. The optical density value of the standard solution should be in the range of 0.08 to 0.13. Standard solution Mc. Farland 0.5 is equivalent to a bacterial cell suspension with a concentration of 1.5 x 10^8 CFU/mL.

Preparation of Bacterial Suspension

A loopful of *S. dysenteriae* colony from a slant agar was suspending into a sterile physiological NaCl solution (0.9%) aseptically. The turbidity of cell bacterial was adjusted to 0.5 Mc Farland solution [20].

Antibacterial Activity

The antibacterial activity of the extracts against *S. dysenteriae* was conducted using the agar diffusion method with perforator technique. The 20 µL bacterial suspensions with 0.5 McFarland in turbidity were inoculated into a sterile petri dish containing the volume of 20 ml MHA medium. The mixture of bacterial suspension and agar was homogenized, then allowed to solidify. The medium was then perforated to make holes for storing a volume of 50 µl extract and incubated at 37 ° C for 24 h. Each of these extracts were dissolved and diluted using dimethyl sulfoxide to achieve concentrations of 20, 30, 40 and 50 %w/v. The diameter of inhibitory zones was observed using caliper.

Statistical Analysis

The data of antibacterial activity were exported to SPSS for further analysis. The diameter for each extract was analyzed using one-way analysis of variance (ANOVA). P value < 0.05 was considered as significant. SPSS 21.0 was employed for statistical analysis [21]. If the data obtained were significantly different at the 5% test level ($\alpha \leq 0.05$), then followed by further tests (Tukey test) to analyze the difference of each concentration of the extract in inhibiting bacterial growth [22]. Furthermore, the students-T test was conducted to determine the significant difference effect of extract concentration in inhibiting the growth of *S. dysenteriae* ATCC 13313.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

The determination of MIC value of each extract was done using a microdilution method. This test was performed at ten concentrations of each extract (40%, 20%, 10%, 5%, 2.5%, 1.25%, 0.625%, 0.3125%, 0.15625% and 0.078125% w/v) employing doubling dilutions of extract in Mueller Hinton Broth (MHB) up to the tenth dilution. A volume of 100 µL MHB was put in each of 96 wells-microtitre plate. The first well was used as a negative control that contain only sterile MHB. The extract concentration of 80 %w/v was made and piped in the same volume (100 µL) into the second well. The serial dilution was done for the next well with the until eleventh well with the last 100 µL being discarded. The twelfth well was used as positive control that contain inoculated MHB. Then onto the second well until the twelfth well was added 100 µL of bacterial cell suspension that of equivalent to 5x10^6 CFU/mL. The microtiter plate then incubated overnight at 37 °C, after which 0.01 ml incubation result, a loop full from each well was sub cultured and spread on the surface of Mueller Hinton Agar to determine the Minimum Bactericidal Concentration (MBC).

RESULT AND DISCUSSION

Yield of the Extract

To evaluate the quality of the extract, then several extract examinations such as organoleptic examination, calculation of extract rendement and determination of extract water content. Organoleptically, both banana peel of ethanol extract was brown, has a distinctive aroma of an extract, a thick texture, and has a bitter taste. The extraction of 300 g Klutuk banana peel yielded 20.52 g extract and the rendement was 9.28%. Meanwhile the Kepok banana peel yielded 27.86 g and the rendement was 6.84%. The water content of both extracts was 5%. The results are in accordance with the requirement that the water content in the extract should not exceed 10% [23].

Phytochemical Screening Result

The preliminary phytochemical screening of the banana peel extracts gave positive tests for the presence of flavonoids, tannins, monoterpenoids and sesquiterpenoids. Other than that metabolites, the klutuk banana peel contains saponins. Both extract exhibited different kinds of secondary metabolites. This probably contributed to their antibacterial activity. The result can be seen in table 1.

Table 1: Phytochemical Screening Result

Metabolites	Banana Peel Extract	
	Kepok	Klutuk
Alkaloids	-	-
Flavonoids	+	+
Tannins	+	+
Saponins	-	+
Triterpenoids	-	-
Steroids	-	-
Polyphenols	-	-
Quinones	-	-
Monoterpenoids & Sesquiterpens	+	+

Note: (+) presence; (-) absence

The results were similar to the literature which mentioned that the phytochemical content in banana peels in the genus of Musa contain flavonoids, tannins, flotannins, alkaloids, glycosides, terpenoids [24]. Based on the literature, flavonoids, tannins, and saponins have antibacterial activity that contribute to bacterial growth inhibition [25].

TLC Result

Determination of Thin Layer Chromatography profile was purposed to find out the profile of the compound contained in the extract. The TLC result was performed in table 2-3.

Table 2: TLC Result of Klutuk Banana Peel Etanolic Extract

Spot No.	Rf	Visible	Ultraviolet (nm)	
			254	366
1	0.9375	brown	Light green	red
2	0.8	brown	-	yellow
3	0.6875	brown	blue	yellow

Table 3: TLC result of Kepok Banana Peel Etanolic Extract

Spot No.	Rf	Visible	Ultraviolet (nm)	
			254	366
1	0.7375	-	blue	yellow
2	0.6625	-	-	yellow

The yellowish fluorescent was visible at UV 366 nm, showed the existence of flavonoid. While, red fluorescent is chlorophyll. Based on these results, both of banana peel ethanol extract were

confirmed to contain flavonoid compound class of flavonols [19, 26].

Antibacterial Activity Results

The mean inhibitory zone of both ethanol extracts against *S. dysenteriae* was summarized in table 4.

Table 4: Diameter of Antibacterial Activity

Extract concentration (%w/v)	Inhibitory diameter zones (mm)	
	Kepok	Klutuk
20	11.5	11.9
30	12.0	12.6
40	12.3	13.2
50	12.5	13.6
Positive control	+	+
Negative control	-	-

Table 5: Anova Test Result of Kepok Peel Antidysentrial Activity

Source	Type III Sum Of Squares	Df	Mean Square	F	Sig.
Corrected Model	1.471[a]	3	0.472	37.778	0.000
Intercept	1761.763	1	1761.763	140941.067	0.000
Concentration	1.417	3	0.472	37.778	0.000
Error	0.100	8	0.013		
Total	1763.280	12			
Corrected Total	1.517	11			

Table 6: Anova Test Result of Klutuk Peel Antidysentrial Activity

Source	Type III Sum Of Squares	Df	Mean Square	F	Sig.
Corrected Model	5.940[a]	3	1.980	1.980	0.000
Intercept	1966.080	1	1966.080	46260.706	0.000
Concentration	5.940	3	1.980	46.588	0.000
Error	0.340	8	0.043		
Total	1972.360	12			
Corrected Total	6.280	11			

Based on the results of Anova test on an ethanol extract of kepok and klutuk banana peels, obtained significant value 0.000 less than the test level α = 0,05, so Ho was rejected. This means that the increased concentration of ethanol extract of banana kepok and klutuk peels effected the diameter inhibition zones. Furthermore, the Tukey test was done to analyze the difference of each extract concentration in inhibiting bacterial growth. The Tukey test results of banana peel ethanol extract was performed in table 7-8.

Table 7: Tukey Test Result of Kepok Peel Antidysentrial Activity

Concentration (% w/v)	N	Subset		
		1	2	3
20	3	11.5667		
30	3		12.1333	
40	3		12.2667	12.2667
50	3			12.5000
Sig.		1.000	0.501	0.124

Table 8: Tukey Test Result of Klutuk Peel Antidysentrial Activity

Concentration (% w/v)	N	Subset		
		1	2	3
20	3	11.8000		
30	3		12.5000	
40	3			13.3000
50	3			13.6000
Sig.		1.000	1.000	0.1347

From Tukey test results, it can be seen there were a significant difference in the range of concentration of 20-50 %w/v. But spesifically, at range of concentration between 30% - 40% w /v and 40-50 %w/v concentrations, there was no significant difference in effect. This means that those concentrations had the same tendency effect in inhibiting the growth of *S. dysenteriae*. Furthermore, T-test analysis was conducted to compare the potency of both extracts in inhibiting growth of *S. dysenteriae*. The T-test results of the extract peels can be seen in table 9.

Table 9: T- Test Result of Klutuk and Kepok Peel Antidysentrial Activity

	T-test Means		
	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Equal variances assumed	0.001	-0.68333	0.24303

From the result of T-test above, significant value 0,01 was smaller than the test level α = 0.05, so Ho was rejected. This showed that between both of banana peels extract had significant differences in inhibiting the growth of *S. dysenteriae*. But, based on the diameter of inhibition zone graphs in figure 1, *S. dysenteriae* was comparatively more inhibited by Klutuk banana peel extracts. This result might be correlated with the antibacterial secondary metabolite content were more abundant in Klutuk extract than Kepok. The antimicrobial properties of this extract had been attributed to the presence of alkaloids and flavonoids. Flavonoids and tannins have been known to show medicinal activity as well as exhibiting physiological activity [27]. Tannins in plants have been shown to confer antidiarrheal properties of plants [28]. This result indicates a possibility of the use of the Klutuk peel extract to treat infections due to *S. dysentriae*.

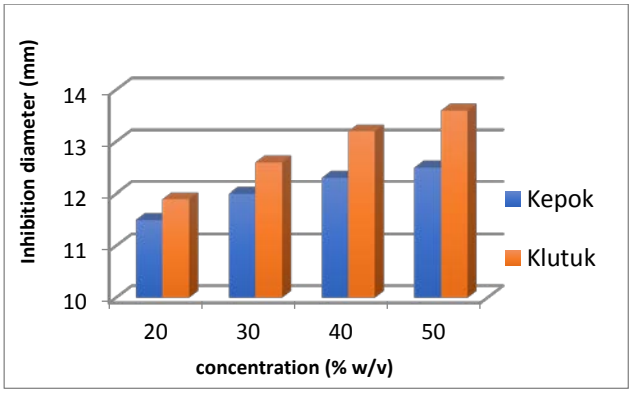

Figure 1. Comparison of Antibacterial Activity

MIC and MBC Results

The lowest concentration of the banana peels extract required for inhibiting the growth of *S. dysentriae* was considered as MIC. MBC is the lowest concentration of a drug that results in killing

99.9% of the bacteria being tested [29]. The MIC and MBC results obtained showed the minimum concentration of both extract inhibited the growth of *S. dysentriae* in the same range concentration, as presented in table 10-11.

Table 10: MIC Value of Banana Peel Extract

Concentration (%w/v)	Cell Bacterial Growth	
	Kepok extract	Klutuk extract
40	-	-
20	-	-
10	-	-
5	+	+
2.5	+	+
1.25	+	+
0.625	+	+
0.3125	+	+
0.15625	+	+
0.078125	+	+
Positive control	+	+
Negative control	-	-

Note: (+) cell growth presence, (-) cell growth absence

Table 11: MBC Value of Banana Peel Extract

Concentration	**Bacterial Colonies Growth**	
(%w/v)	**Kepok extract**	**Klutuk extract**
40	-	-
20	-	-
10	+	+
5	+	+

Note: (+) colony presence; (-) colony absence

The MIC values of both extracts against *S. dysenteriae*, ranged from 5-10 %w/v and MBC values were two times higher than MIC value, ranged from 10-20 %w/v. The MBC values less than four times the MIC value demonstrated that the extract was bactericidal agent.

CONCLUSION

The results of this study showed the ethanolic extract of Klutuk banana peels more potent as antidysentrial medicinal plant than Kepok peel.

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