

## Metabolit Profiling and Antibacterial Activity of Ethanol Extract of the Unripe Fruit Peel of Kayu Banana (*Musa paradisiaca* L. Var. Kayu)

Arista Wahyu Ningsih<sup>1,2</sup>, Achmad Syahrani<sup>1</sup>,  
Abdi Wira Septama<sup>3</sup> and Sukardiman<sup>1,\*</sup>

<sup>1</sup>Faculty of Pharmacy, Airlangga University Surabaya, Surabaya, Indonesia

<sup>2</sup>Faculty of Health Sciences, Pharmacy Study Program, Anwar Medika University, Jawa Timur, Indonesia

<sup>3</sup>Research Centre of Pharmaceutical Ingredients and Traditional Medicine, Badan Riset dan Inovasi Nasional, Jakarta, Indonesia

(\*Corresponding author's e-mail: [sukardiman@ff.unair.ac.id](mailto:sukardiman@ff.unair.ac.id))

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### Abstract

According to Basic Health Research data in Indonesia in 2020 shows that the diarrhea morbidity rate in Indonesia is still high. In Indonesia, the unripe fruit of the Kayu banana (*Musa paradisiaca* L. var. Kayu) is a drug that is often used empirically by the people of Senduro village, Lumajang, East Java, Indonesia, to treat diarrhea. This study was conducted to investigate the antibacterial activity of unripe fruit peel, unripe fruit flesh and unripe fruit. A disc diffusion test was selected to assess the sensitivity of each sample to *Escherichia coli*. The disc dilution was used to determine the value of MIC and MBC in *Escherichia coli*. Total phenolic content to determine phenolic levels in samples that are suspected of providing antibacterial activity. Analysis of secondary metabolite profiles using the UPLC-Q-TOF/MS instrument. Unripe fruit peel extract shows more optimal antibacterial activity compared to other extracts. The diameter value of the inhibitory zone of the unripe fruit peel extract is 17 mm and the yield of a large  $\Delta$ OD is  $-1.3620$  at a concentration of 10 % w/v. Based on the calculation results from the regression equation, TPC fruit extract was obtained by 8.6 %, TPC flesh extracts by 6.1 % and TPC from bark extract by 10.8 %. Based on the data from the interpretation of compound content analysis using UPLC-QToFMS, it can be seen that there are 25 mayor compounds. These results suggest that the unripe fruit peel extract of banana Kayu has the potential to be antibacterial against *Escherichia coli*. The application of this research is as a reference for the development of antidiarrheal standardized herbal medicines and the implication of this research is to support the independence of national medicinal raw materials in Indonesia.

**Keywords:** Antibacterial, *Musa paradisiaca*, *Escherichia coli*, Banana Kayu

### Introduction

Diarrhea disease is an endemic disease that has the potential to cause outbreaks and contribute to the mortality rate in Indonesia, especially in toddlers [1]. Based on 2019 World Health Organization (WHO) data, diarrhea is one of the diseases with the highest incidence and mortality rates in the world. It is reported that there are about 1.7 trillion cases annually. According to Basic Health Research data in 2020, it shows that the diarrhea morbidity rate 2020 for all ages was 270/1,000 residents while for toddlers it was 843/1,000 population. In 2021, the service coverage for people with diarrhea at all ages is 33.6 % and for toddlers is 23.8 % of the set target. In 2021, diarrhea is still the leading cause of death in the postnatal period, at 14 % and in the group of children under 5 (12 - 59 months) at 10.3 % of deaths due to diarrhea [2]. Treatment of diarrhea is done by taking chemical drugs such as Loperamid. Such treatment can cause side effects such as nausea, vomiting, abdominal pain and rashes on the skin. The side effects cause people to prefer medicinal plants as an alternative treatment. Some previous studies explain that some medicinal plants are effective in treating diarrhea because of the content of tannins, phenols, saponins, essential oils, alkaloids and flavonoids of unripe banana fruit. This study aims to raise the potential of unripe fruit of wood banana as an antidiarrheal herbal medicine [3]. Cases of diarrhea patients served at all ages were 44.4 % and in toddlers at 28.9 % [4]. *Escherichia coli* is one of the bacteria that cause diarrhea in humans. *Escherichia coli* causes diarrhea because the intestines produce toxins that irritate the intestinal membranes. Diarrhea can result in the loss of minerals and fluids in the body it can trigger dehydration, especially in infants and children. Dehydration or lack of fluids in the body can lead to death [5].

In Indonesia, people in Senduro village, Lumajang, East Java, frequently utilize the unripe fruit of the Kayu banana (*Musa paradisiaca* L. var. Kayu) as a medicine to treat diarrhea. Empirically, the Senduro people steam, boil and burn the unripe fruit of the Kayu banana (*Musa paradisiaca* L. var. Kayu). The use of medicinal plants for the treatment of diarrhea in traditional medicine is a routine practice in Indonesia. However, in the majority of cases, this practice is passed down from generation to generation empirically without knowing the plausible mechanisms, safety and efficacy of herbal remedies. Despite this traditional claim and previous research, it was not fully investigated scientifically to validate its therapeutic effects. In previous studies, the unripe fruit of the Kayu banana also has an antidiarrheal effect on Oleum Ricini-induced mice and phytochemical test results of ethanol extract of unripe fruit of banana Kayu (*Musa paradisiaca* L. var. Kayu) showed positive tannin content that had Oleum Ricini-induced antidiarrheal activity at a dose of 200 mg/kg BB [6] and provide inhibitory and killing power to the growth of *Escherichia coli* bacterial media with concentrations of 25 and 50 %.

Phenolic compounds are one of the secondary metabolites of plants and phenolic compounds are contained in several groups of secondary metabolites such as flavonoids, tannins and polyphenols [7]. The mechanism of action of the phenolic compound group that works as an antidiarrheal work to stop diarrhea by inhibiting intestinal motility to reduce the secretion of fluids and electrolytes while tannins are one of the compounds that have a mechanism of action as gastrointestinal astringencies by shrinking the pores of the intestinal wall and intestinal mucous membranes and binding proteins so that they form and damage the cell walls of bacteria that cause diarrhea [8]. Meanwhile, phenolic compounds that have an antibacterial function, namely flavonoids, have a mechanism of action by damaging the permeability of bacterial cell walls and inhibiting bacterial motility and tannins which have a mechanism of action by inactivating bacterial cell adhesions, inactivating enzymes, and also attacking cell wall polypeptides so as to cause cell wall damage to bacteria [9]. Phenolic compounds are able to damage cell membranes, inactivate enzymes and denature proteins so that cell walls are damaged due to decreased permeability. Changes in the permeability of the cytoplasmic membrane allow disruption of the transport of important organic ions into the cell, resulting in inhibition of growth even to cell death. In high concentrations, phenolic content penetrates and disrupts the bacterial cell wall and precipitates proteins in bacterial cells. In lower concentrations, phenols inactivate important enzyme systems in bacterial cells. The unripe fruit of Kayu bananas contains phenolic compounds including flavonoid compounds that have a mechanism of action by inhibiting intestinal motility movements so as to reduce fluid and electrolyte secretion and prolong intestinal transit time.

Phenolics are a group of compounds consisting of aromatic rings containing one or more hydroxyl groups [10]. All plants contain phenolic compounds in the form of glycosides and bind to proteins and then form a complex bond through hydrogen bonds [11]. Phenolic compounds in the unripe fruit of Kayu bananas have pharmacological activity as antidiarrheals *in vitro* and *in vivo*.

Based on the above background, phenolic levels and antidiarrheal activity *in vitro* will be determined on fruit peel, fruit flesh and Kayu banana unripe fruit. It is hoped that this study will provide an overview of the effectiveness of antidiarrheals on fruit peels, fruit flesh and unripe fruits of Kayu bananas and phenolic compounds that have optimal *in vitro* antidiarrheal activity to provide benefits for the treatment of diarrhea caused by bacteria.

## Materials and methods

### **Kayu banana unripe fruit, fruit peel and fruit flesh simplisia (*Musa paradisiaca* L. var. Kayu)**

A total of 13 kg of unripe Kayu banana fruits and unripe fruit banana Kayu is separated between the peel the fruit and the flesh of the fruit aged for approximately 3 months are cleaned, drained and weighed after being washed under running water by wet weight. Then plowing is carried out, dried at a temperature in a drying rack of 50 °C using a food dehydrator, dry distorted and weighed dry weight. The dried sample is then blended and then sifted and stored in a tightly closed container [12].

### **Extraction process**

A total of 500 g of crude drug powder was macerated at normal temperature with a 96 % ethanol solvent and stirring was carried out. The powder takes for 24 h. Remaceration occurs 7 to 8 times until the solvent becomes clear again and colorless, and filtration is carried out to separate residues and filtrates. The resulting master is then 50 °C in a rotary evaporator, where it was evaporated until it became a thick extract [13]. The extraction method carried out in this study is stratified remaceration by adding solvent repeatedly and until clear with a solvent ratio of 1:4 with the amount of solvent needed as 1,600 mL so that the compound attracted by the solvent is high. The duration of maceration is 24 h with 2 stirring, after 24 h a

change of solvent and soaking 24 h again. The soaking process is repeated until the color of the solvent is clear.

#### **Phytochemical screening**

Examined phytochemical screening is alkaloid test, saponin test, flavonoid test, tannin test, polyphenol test, triterpenoid steroid test, antraquinon test and glikosida test. According to Elza *et al.* [14], the alkaloid test was carried out by weighing 50 mg of viscous extract, adding 1 mL of 2N hydrochloric acid (HCl) and 9 mL of water, then heated on a water bath for 2 min, put into a test tube, then added each test tube 2 drops of Meyer, Bouchardat and Dragendroff reagent solution. If alkaloids are present, a precipitate is formed. The flavonoid test was carried out by weighing 50 mg of thick extract, weighed 20 mL of hot water, boiled for 10 min and filtered hot, added 0.1 g of magnesium powder and 1 mL of concentrated hydrochloric acid and 2 mL of amyl alcohol to 5 mL of filtrate, and left to separate, positive containing flavonoids if red, yellow, orange colors occur in the amyl alcohol layer. The tannin test was carried out by weighing 50 mg of viscous extract with 10 mL aquades for 15 min and added 2 drops of 10 % FeCl<sub>3</sub> solution. A change to blue or green indicates the presence of tannins.

Polyphenol test can be done by means of a thick extract as much as 50 mg boiled for 3 min in 10 mL distilled water after which it is cooled and then filtered then added 1 - 2 drops of FeCl<sub>3</sub> reagent. The positive results of polyphenols are characterized by the formation of green to black blue color. Anthraquinone test was carried out 50 mg of extract plus 10 mL of water then heated for 5 min and filtered then added a few drops of NaOH 1 N solution if positive then formed a red solution and tube 2 as a control. Triterpenoid or steroid tests are carried out by weighing 50 mg of viscous extract, added ether or n-hexane, then allowed to stand for 2 h, filtered, added acetic acid anhydride, then dripped with sulfuric acid (Liebermann Burchart reagent). The onset of purple and red colors or changing to a blue green color determines the presence of triterpenes or steroids. The glycoside test was carried out by means of a viscous extract dissolved in 96 % ethanol solvent, evaporated on a water tank, dissolved the rest in 5 mL of anhydrous acetic acid P, and added 10 drops of sulfuric acid P. The blue or green color formed indicates the presence of glycosides. (Endarini, 2016) (Rahmawati, 2018)[15-18].

#### **Determination of total phenol content**

One mL of the Kayu banana unripe fruit extract solution was pipetted into the extract solution, then the sample was added with 0.4 mL of Folin Ciocalteu reagent shaken and left for 4 - 8 min, adding 4.0 mL of 7 % Na<sub>2</sub>CO<sub>3</sub> solution to beat until homogeneous. Add aquabidestillata to 10 mL and let stand for 2 h at room temperature. Measure absorption at a maximum absorption wavelength of 745 nm. Do 3 replication so that the phenol levels obtained are obtained as mg equivalent to gallic acid/g extract [17]. Method TPC (Total Phenolic Content): Making test samples by weighing unripe fruit extract of Kayu bananas as much as 10 mg with added as much as 10 mL methanol pro analysis using a 10 mL measuring flask then taken a parent sample of 1 mL plus Folin Ciocalteu reagent as much as 0.4 mL then shake vigorously let stand for 4 - 8 min, then add 7 % Na<sub>2</sub>CO<sub>3</sub> solution as much as 4 mL then add 10 mL aquadest and let stand for almost 2 h. Five replications are used. The standard used is gallic acid. Gallic acid solution is made at a 1,000 ppm concentration. Ten mg of gallic acid was weighed out and then diluted in 10 mL of methanol for analysis.

#### **Determination of the inhibitory zone**

MHA medium is poured as much as 15 mL into each petri dish then 1 mL of bacterial suspension is inoculated on the medium and allowed to stand until it solidifies. The paper disc is immersed in the sample to be tested, then the paper disc is placed on the agar plate, then incubated at 37 °C for 24 h. The disc paper size used is 6 mm. Positive control using chloramphenicol and negative control used is DMSO (Dimetyl Sulfoksida). This inhibitory zone measurement is done by taking a horizontal line on the clear zone around the paper disc and using caliper tools. Then measured the diameter of the inhibitory zone (mm) of each foreign sample.

#### **Determination of minimal inhibitory concentration (MIC)**

MIC determination was carried out using 1 mL of NB (*Nutrient Broth*) media which was inserted into the test tube, then added with 1 mL of *Escherichia coli* bacterial suspension and added 1 mL of test solution, positive control and negative control respectively. After that, all samples were incubated at 37 °C for 24 h until bacterial growth occurs. The positive control used was 0.0015 g of chloramphenicol and dissolved in 10 mL of sterile aquadest. The negative control preparation was 5 mL of concentrated DMSO and dissolved in 100 mL of sterile aquades. One g of extract test solution is dissolved in 1 mL of solvent. The test method used is the OD dilution method with  $\lambda$  max 625 nm. The number of bacterial cells can be measured by

knowing the turbidity (turbidity) of the culture. Then turbidity is observed in the solution and the absorbance value is measured before and after incubation [18].

#### Determination of minimal bactericidal concentration (MBC)

Determination of MBC is carried out with EMB (Eosin Methylene Blue) media. A total of 15 mL of EMB media was put into a petri dish and then 1 mL of the incubated test solution was inoculated on the media and homogenized. Then the medium was left until it solidified and incubated at a temperature of 37 °C for 24 h, then observed. The parameter used is the presence or absence of bacterial growth on the agar media which is characterized by the presence or absence of white areas or spots on the agar media [19].

#### Metabolite profiling using UPLC/Q-TOF MS

Thoroughly weighed 10.00 mg of the extract, which was then dissolved in 10 mL of methanol. A microsyringe containing 5 µL of the extract in methanol is used to take the extract and inject it into the sample site before adding it to the UPLC column. Four replications are completed. The liquid sample will be transformed into droplets by passing past the needle that has received a positive ESI charge (+). A Q-ToF analyzer will next separate the ions that the detector has produced. The gradient elution method shown in **Table 2** was used to elute a mixture of 1) Water: Formic acid (99.9:0.1) and 2) Acetonitrile: Formic acid (99.9:0.1) at a flow rate of 0.2 mL/min. A polar compound will show up first on a chromatogram, followed by a compound with a lower polarity. A chromatogram peak is produced after the QToF-MS detector reads the separation findings.

In this study the stationary phase used was column C18 (2.1, 100 mm, particle size 1.7 µm) or octadecyl silica. The potential of octadecyl silica separating compounds with low, medium and high polarities is one of its advantages as a stationary phase. With a gradient elution system and an eluent flow rate of 0.2 mL/min, the eluent is a mixture of 1) Water: Formic acid (99.9:0.1) and 2) Acetonitrile: Formic acid (99.9:0.1). Chromatograms with polar compounds will appear first followed by compounds with lower polarity. The UPLC-MS system employed in this investigation has a Q-ToF MS analyser and an MS detector with an ESI ion source (+). The instrument has a number of benefits, including being selective, sensitive and rapid so that analysis times are reduced. Ten mg of the extract were weighed, and they were then dissolved in 10 mL of methanol. A microsyringe containing 5 µL of the extract in methanol was used to inject it into the sample site and add it to the UPLC column. Four replications are completed. Through a needle with a positive ESI charge (+), the liquid sample will be transformed into droplet grains. Q-ToF analyzer will next separate the ions that the detector has created. The chromatogram peaks are produced once the QToF-MS detector reads the separation findings. The Masslynx application is then used to interpret the chromatogram peaks. Peak chromatograms are then interpreted using the Masslynx application [22].

#### Statistical analysis

The data are presented as average values and standard deviations. The data analysis used is a descriptive method. The data obtained will then be tested for normality using SPSS 21. Data were analyzed using one way ANOVA for comparison between groups, followed by Tukey's post hoc HSD test which showed statistically significant differences. *p*-value < 0.05 is considered statistically significant.

#### Results and discussion

The percent yield of simplicia yields obtained was as much as *Musa paradisiaca* Fructus 32.04 %, *Musa paradisiaca* Fructus Cortex 12.11 %, and *Musa paradisiaca* Fructus Pulp 26.01 %.

**Table 1** Simplicia results.

No	Group	Fresh weight (g)	Dry weight (g)	% Rendemen	% Moisture content	Oragnoleptis
1	<i>Musa paradisiaca</i> Fructus	12.960	4.153	32.04	2.74	Characteristic aromatic smell, ivory white color and powder form
2	<i>Musa paradisiaca</i> Fructus Cortex	4.759	577	12.11	2.50	Characteristic aromatic smell, brown color, and powder shape

No	Group	Fresh weight (g)	Dry weight (g)	% Rendemen	% Moisture content	Oragnoleptis
3	Musa paradisiaca Fructus Pulp	3.844	1.000	26.01	2.60	Characteristic aromatic smell, light white color, and powder form.

The results obtained showed that the largest percentage of extract amendments in Kayu banana unripe fruit extract was 18.7 % and close to Kayu banana unripe fruit peel extract by 18.52 %.

**Table 2** Extract result.

No	Group	Dry weight (g)	Extract weight (g)	% Rendemen	% Moisture content
1	Musa paradisiaca Fructus	705	94.5	13.4	The smell has aromatic, orange-white color, viscous extract form
2	Musa paradisiaca Fructus Cortex	705	130.6	18.52	Characteristic aromatic smell, blackish-green color, liquid, and viscous extract form.
3	Musa paradisiaca Fructus Pulp	705	131.9	18.7	Aromatic characteristic smell, intense blackish-green color, liquid and viscous extract form.

The difference in the percent yield obtained is influenced by the amount of organic solvent, the characteristics of the content of secondary metabolite compounds and the extraction method used [20].

**Table 3** Phytochemical screening results of unripe fruit peel extract, unripe fruit flesh extract and unripe fruit extract of banana Kayu (*Musa paradisiaca* L. var. Kayu).

No	Phytochemical test	Reagents	Result	UFPE	UFFE	UFE
1	Alkaloid	Dragendorf	There are sludge	+	+	+
		Wagner	There are sludge	+	+	+
		Mayer	There are sludge	+	+	+
2	Flavonoid	Wilstater	orange	+	-	+
3	Glikosida	Molisch	Brown color	+	+	+
4	Saponin	Busa	Forming froth	+	+	+
5	Tanin	FeCl <sub>3</sub>	Blackish green	+	+	+
6	Triterpenoid	Lieberman-Burchard	red or purple color	+	+	+
7	Steroid	Lieberman-Burchard	green color	+	+	+
8	Polyphenol		green to blue-black color	+	+	+
9	Antrakuinon	KOH	Red	+	+	+

Note: UFPE is unripe fruit peel extract, UFFE is unripe fruit flesh extract and UFE is unripe fruit extract.

Based on phytochemical screening carried out, the compounds contained in unripe fruit extracts and unripe fruit peel extracts of banana Kayu are alkaloids, flavonoids, saponins, tannins, triterpenoids, polyphenols and anthraquinones. The unripe fruit flesh extract of the Kayu banana contains alkaloid compounds, saponins, tannins, triterpenoids and polyphenols. Secondary metabolite compounds that are not present in the unripe fruit extract of Kayu banana but are present in unripe fruit extract and peel extract of unripe fruit of banana Kayu are flavonoids and anthraquinones.

On the identification of tannin compounds using reagents of iron (III) chloride. The formation of a blackish-green color occurs after the addition of  $\text{FeCl}_3$  10 % due to the formation of complex compounds produced by the reaction of the hydroxyl group with  $\text{Fe}^{3+}$  ions. flavonoids can be identified, then glycoside bonds with flavonoids in plants must be broken by reducing these bonds where the results obtained are positive because they are formed orange color and the addition of HCl can hydrolyze flavonoids into aglikon.

Identification of alkaloid compounds using Mayer, Wagner and Dragendorff reagents as alkaloid compound detectors. The purpose of adding HCl 2 N is to pull alkaloids from the extract, with the addition of HCl salts will form because alkaloids are alkaline, then heated to break the salt that is not in the form of alkaloid bonds. A positive result of alkaloids on the Mayer test is characterized by the formation of a white precipitate. It is thought that such deposits are potassium-alkaloid complexes. In the manufacture of Mayer's reagent, a solution of mercury (II) chloride plus potassium iodide will react to form a red precipitate of mercury (II) iodide. If excess potassium iodide is added, potassium tetraiodomercurate (II) will form. Alkaloids contain nitrogen atoms that have lone pairs of electrons so that they can be used to form coordinate covalent bonds with metal ions. In the alkaloid test with Mayer's reagent, it is estimated that the nitrogen in the alkaloid will react with  $\text{K}^+$  metal ions of potassium tetraiodomercurate (II) to form a potassium-alkaloid complex that precipitates.

Positive alkaloid results on the Wagner test are characterized by the formation of light brown to yellow deposits. It is thought that such deposits are potassium-alkaloids. In the manufacture of the Wagner reagent, iodine reacts with the  $\text{I}^-$  ions of potassium iodide to give  $\text{I}_3^-$  ions which are brown in color. In the Wagner test,  $\text{K}^+$  metal ions will form coordinate covalent bonds with nitrogen in alkaloids to form potassium-alkaloid complexes that precipitate. Positive alkaloid results on the Dragendorff test are also characterized by the formation of light brown to yellow deposits. Such deposits are potassiumalkaloids. In the manufacture of Dragendorff reagents, bismuth nitrate is dissolved in HCl so that hydrolysis reactions do not occur because bismuth salts are easily hydrolyzed to form bismutil ions ( $\text{BiO}^+$ )

In the saponin compound test, the result indicates positive because it contains saponins that form foam 1 cm high in no less than 10 min and the froth does not disappear when added 1 drop of HCl 2 N. Through its ability to form saponin foam is a surface active compound that is easily detectable. The glycoside bond component. The weight of the extract used to be able to form foam is 3 g of extract. Shaking is done for 30 s and for observation it is done 30 s also if within 30 s the foam does not disappear then it is declared a positive extract.

**Table 4** Results of measuring the diameter of the inhibitory zone at a concentration of 10 % w/v.

No	Treatment	Replication	Diameter of the inhibitory (mm)	Average $\pm$ SD	Category (CLSI, 2013)
1	Unripe fruit flesh extract	1	10	$11 \pm 1^a$	Resistant
		2	12.5		
		3	9.5		
2	Unripe fruit extract	1	11.5	$15 \pm 3^{ab}$	Intermediate
		2	14.5		
		3	18.5		
3	Unripe fruit Peel extract	1	19	$17 \pm 1^b$	Intermediate
		2	16		
		3	17		

No	Treatment	Replication	Diameter of the inhibitory (mm)	Average $\pm$ SD	Category (CLSI, 2013)
4	K+	1	32.5	$33 \pm 1^c$	Susceptible
		2	34		
		3	33.5		
5	K-	1	0	$0 \pm 0^d$	Resistant
		2	0		
		3	0		

Note: The significance level of  $p$ -value  $< 0.05$  was compared with the control group and in the follow-up test with the Tukey HSD test.

The antibacterial activity test has the purpose of measuring the antibacterial power activity of unripe fruit extract, unripe fruit flesh extract and unripe fruit peel extract of Kayu banana which is suspected to have the ability to inhibit growth.

**Table 5** Results of optical density (OD) Measurement at minimum inhibitory concentration with dilution method at a concentration of 10 % w/v.

No	Treatment	Average value OD MIC			Category
		Before Incubation	After Incubation	$\Delta OD \pm SD$	
1	Unripe fruit extract	0.8513	0.504	$-0.3473 \pm 0.127$	down
2	Unripe fruit flesh extract	0.1025	0.0755	$-0.027 \pm 0.007$	down
3	Unripe fruit Peel extract	1.5313	0.1783	$-1.3620 \pm 0.193$	down
4	K+	2.2850	1.0678	$-1.2173 \pm 0.112$	down
5	K-	0.0383	0.2970	$+0.2588 \pm 0.172$	up

Note: "Up" indicates the absorbance value after incubation  $>$  the absorbance value before incubation, which means that there is bacterial growth; while "Down" indicates the absorbance value after incubation  $\leq$  the absorbance value before incubation, which means that bacterial growth is inhibited. OD is Optical Density.

The test method used is the dilution method OD  $\lambda$  max 625 nm with incubation time. The bacteria used in *Escherichia coli*. This antibacterial test aims to determine the ability to inhibit bacterial growth within a certain incubation time. The number of bacterial cells can be measured by knowing the turbidity (turbidity) of the culture [21]. The more turbid a culture is the greater the number of cells. The light emitted on the spectrophotometer will hit the cell so that part of the light will be absorbed and part of it will be passed on. The magnitude of light in a spectrophotometer absorbed by cells in a cuvette is calculated as the absorbance value [25,26].

The number of bacterial cells can be measured by knowing the turbidity of the culture. The cloudier a culture, the greater the number of cells. The light emitted on the spectrophotometer will hit the cell so that some of the light will be absorbed and some of it is transmitted. The amount of light in the spectrophotometer absorbed by the cell in the cuvette is calculated as the absorbance value [22]. Minimum Inhibitor Concentration (MIC) is the lowest concentration of an antibacterial substance in inhibiting bacterial growth within a certain incubation time. The MIC value is obtained by looking at the growth of bacteria in the test tube using a UV-Vis spectrophotometer. Bacteria that grow and die can be observed by looking at the difference in absorbance from before incubation to after incubation called the change in Optical Density ( $\Delta OD$ ) [23].

Based on **Table 5**, it is seen that unripe fruit extracts, unripe fruit flesh extracts and unripe fruit peel extracts show  $\Delta OD$  negative value. This shows the ability of unripe fruit peel extract in inhibiting the growth of *Escherichia coli* bacteria more optimally compared to unripe fruit extract and unripe fruit flesh extract [24]. Extracts of fruit peel, pulp and unripe fruit of Kayu banana have antibacterial activity, but the mechanisms and molecular pathways have not been studied [25].

**Table 6** Observation results of *Escherichia coli* bacterial colonies on Eosin Methylene Blue Agar (EMBA) media at a concentration of 10 % w/v.

No	Treatment	Replication 1	Replication 2	Replication 3
1	Unripe fruit extract	–	–	–
2	Unripe fruit flesh extract	–	–	–
3	Unripe fruit Peel extract	–	–	–
4	K+	–	–	–
5	K–	+	+	+

Note: + means grow colonies of *Escherichia coli* bacteria. “–” means do not grow colonies of *Escherichia coli* bacteria.

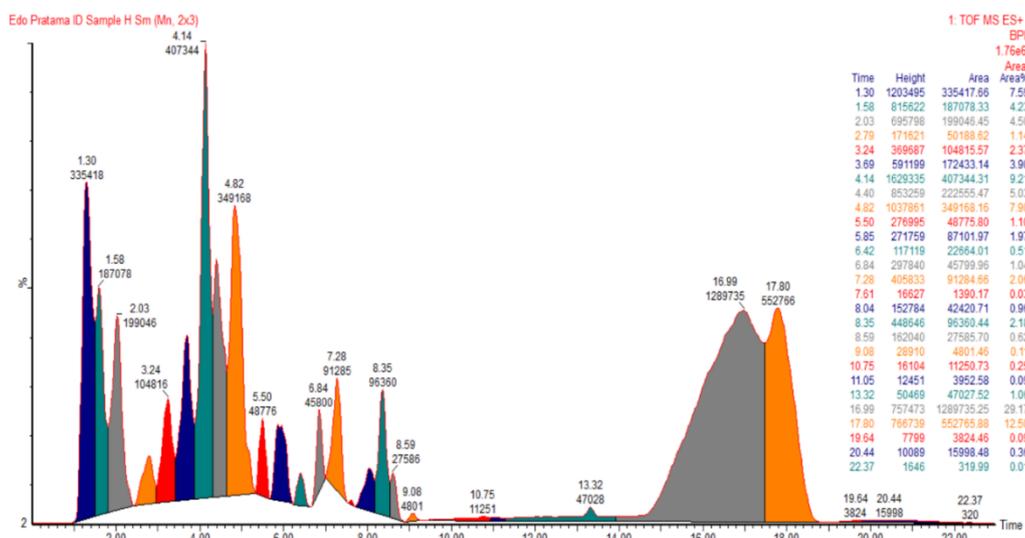
**Table 7** Results of measuring the absorbance of total phenolic levels.

No	Sample	Average absorbance	Total phenolic content (mgGAE/g ekstrak) ± SD	% Total phenolic
1	Unripe fruit extract	0.9072	86.42 ± 0.50 <sup>a</sup>	8.6
2	Unripe fruit flesh extract	0.1184	60.75 ± 0.65 <sup>b</sup>	6.1
3	Unripe fruit Peel extract	0.2164	107.80 ± 0.50 <sup>c</sup>	10.8

The significance level of  $p$ -value < 0.05 was compared with the control group and in the follow-up test with the Tukey HSD test.

TPC analysis on samples by the Folin-Ciocalteu (FC) method using a UV-Vis spectrophotometer at a maximum wavelength ( $\lambda$  max) of 745 nm. Phenolic ions are formed through the dissociation of protons of phenolic compounds, but this reaction runs slowly, therefore it is necessary to have an alkaline atmosphere-carrying solution so that the reaction takes place quickly, so sodium carbonate is used as a carrier of alkaline atmosphere [23]. The total phenol content is influenced by the type of solvent. Phenols are compounds that are polar so their solubility is highest in polar solvents. Polar solvents can dissolve phenols better so that their levels in the extract become high [26]. The mechanism may also be related to the permeability of the surface of the bacterial cell wall with the extract [27]. According to research by Septama and Panichayupakaranant [31], the content of the phenolic compound artocarpanone provides antibacterial activity against *Escherichia coli* by disrupting membrane permeability. The antibacterial mechanism is still unclear and it is suspected that the bacterial cell wall membrane is one of the places of action by interacting on the lipophilic side of the cell wall membrane, causing damage to the cell wall membrane. The absorption of phenolic compounds by the bacterial cell membrane and the interaction of phenolic compounds with active enzymes on the bacterial cell wall greatly affect its antibacterial activity. In unripe fruit peel extract banana Kayu has optimal activity with the largest total phenol content [17].

Colorimetric oxidation and reduction reactions to measure all phenolic compounds in the test sample. Ciocalteu folin reagent is a complex solution of polymeric ions formed from phosphomolybdic acid and heteropolyphosphotungstic acid. The formation of a blue complex compound that can be measured at a wavelength of 765 nm. This reagent oxidizes phenolics (alkali salts) or phenolichydroxyl groups reducing heteropoly acid (phosphomolybdate-phosphotungstate) present in the FC reagent to a molybdenumtungsten complex. Phenolic compounds react with FC reagents in an alkaline atmosphere in order to dissociate protons in phenolic compounds into phenolic ions, and to create alkaline conditions 7 % Na<sub>2</sub>CO<sub>3</sub> is used. The hydroxyl group in the phenolic compound reacts with the FC reagent to form a blue molybdenumtungsten complex that can be detected with a spectrophotometer. The greater the concentration of phenolic compounds, the more phenolic ions will reduce heteropoly acid (phosphomolybdate-phosphotungstate) into a molybdenumtungsten complex so that the blue color produced is more concentrated.



**Figure 1** Chromatogram UPLC-MS unripe fruit peel extract.

In comparison to HPLC, the analytical method using UPLC can reduce the phase of motion consumption by up to 80 % in a relatively quicker period of time of roughly 1.5 min. The UPLC-MS system used in this investigation included a Q-ToF MS analyzer and an MS detector with an ESI ion source (+). Such instruments provide a number of benefits, including selective, sensitive, high-speed resolution performance that reduces analysis time. After injecting the sample, it enters the column where a process of metabolite component separation takes place as part of the profile analysis of the metabolite of unripe fruit peel extract. Column C18, or octimethyl silica, was employed as the solitary phase in this research. Chromatogram peak and quantitative data are obtained from the percentage content of each compound based on the area of the chromatogram peak. One of the LC-MS technique's later-stage analytical methods that can be used to examine a sample's metabolite profile is UPLC-MS. This analytical method offers a number of benefits, including accurate mass measurement, high-resolution, robust and consistent chromatogram results and structural information, and allows extensive detection of metabolites from plant samples. Faster mobile phase and analysis time contribute to increased efficiency and cost-effectiveness.

**Table 8** Results of interpretation of metabolite data profiling unripe fruit peel extract.

No.	R. Time (min)	% area	m/z	Formula	Predictions compound name
1	1.30	7.59	137.0600	C8H8O2	Methyl benzoate
2	1.58	4.23	137.0604	C8H8O2	unknown
3	2.03	4.50	178.0865	C10H11NO2	unknown
4	2.79	1.14	230.0930	C17H11N	Benz(a)acridine
5	3.24	2.37	208.1339	C12H17NO2	unknown
6	3.69	3.90	272.0925	C15H13NO4	unknown
7	4.14	9.21	222.1501	C13H19NO2	unknown
8	4.40	5.03	256.1334	C16H17NO2	unknown
9	4.82	7.90	434.2038	C19H31NO10	unknown
10	5.50	1.10	482.2610	C21H39NO11	unknown
11	5.85	1.97	197.1177	C11H16O3	unknown
12	6.42	0.51	280.1913	C16H25NO3	unknown
13	6.84	1.04	496.2765	C22H41NO11	unknown
14	7.28	2.06	466.2713	C22H35N5O6	unknown

No.	R. Time (min)	% area	m/z	Formula	Predictions compound name
15	7.61	0.03	211.1702	C13H22O2	Altretamine
16	8.04	0.96	428.2816	C26H37NO4	unknown
17	8.35	2.18	538.3225	C25H47NO11	unknown
18	8.59	0.62	538.3224	C25H47NO11	unknown
19	9.08	0.11	181.1229	C11H15O2	unknown
20	10.75	0.25	214.2526	C14H31N	unknown
21	11.05	0.09	327.2048	C16H22N8	unknown
22	13.317	1.06	279.1596	C16H21O4	unknown
23	16.99	29.17	960.8902	C58H109N11	unknown
24	17.80	12.50	960.9007	C61H117NO6	unknown
25	20.44	0.36	327.2073	C20H26N2O2	unknown

Based on the data from the interpretation of compound content analysis using UPLC-QToFMS, it can be seen that there are 25 compounds. The major compounds are methyl benzoate, benza(a)acridine and altretamine.

The purpose of drying is to obtain simplicia that is not easily damaged so that it can be stored for a longer time. Reducing the moisture content and stopping the enzymatic reaction, will prevent deterioration or destruction of simplicia. How to dry the sample by drying using a food dehydrator. It is chosen because it is to avoid damage to compounds or substances contained in the plant if drying with sunrays is carried out [28]. Storage of simplicia that has become a powder is carried out by putting the fine simplisia into a tightly closed container and tightly closed to avoid exposure to sunlight or insect ingress and giving silica gel to absorb moisture [29]. The difference in the percent yield obtained is influenced by the amount of organic solvent, the characteristics of the content of secondary metabolite compounds and the extraction method used [20]. The unripe fruit flesh extract of the Kayu banana contains alkaloid compounds, saponins, tannins, triterpenoids and polyphenols [34,35].

On the identification of tannin compounds using reagents of iron (III) chloride. A positive result indicates the formation of a blackish-green color. The formation of a blackish-green color occurs after the addition of  $\text{FeCl}_3$  10 % due to the formation of complex compounds produced by the reaction of the hydroxyl group with  $\text{Fe}^{3+}$  ions [30]. In addition, tannin compounds are compounds that are polar due to the presence of OH. According to Makatamba *et al.* [38], if the results show a blue-black color, it indicates that the tannin is hydrolyzed, while the condensed tannin when adding  $\text{FeCl}_3$  will show a blackish-green color [37].

In the identification of flavonoid compounds using Mg powder, concentrated HCl and amyl alcohol as detectors. The purpose of adding concentrated magnesium and HCl powders is to reduce glycoside bonds with flavonoids [31]. So that flavonoids can be identified, then glycoside bonds with flavonoids in plants must be broken by reducing these bonds where the results obtained are positive because they are formed orange color and the addition of HCl can hydrolyze flavonoids into aglikonnya, thus it can be easily dunripen by amyl alcohol so that color will appear on the amyl alcohol layer [32].

In the identification of alkaloid compounds using Mayer, Wagner and Dragendorff reagents as alkaloid compound detectors. The purpose of adding HCl 2 N is to pull alkaloids from the extract, with the addition of HCl salts will form because alkaloids are alkaline, then heated to break the salt that is not in the form of alkaloid bonds [33]. The results obtained from the formation of white deposits on Mayer reagents, reddish brown on Wagner reagents, and orange deposits on Dragendrof reagents, it can be concluded that the extract contains alkaloid compounds [34]. The formation of steroid compounds and triterpenoids is marked by forming a blue or green color for steroids, and red or purple for triterpenoids [35].

In the saponin compound test, the extract that has been added to heated water and the extract is then put into a test tube after which it is shaken with foam. The result indicates positive because it contains saponins that form foam 1 cm high in no less than 10 min and the froth does not disappear when added 1 drop of HCl 2 N [36]. The addition of hydrochloric acid to saponin testing aims to increase the polarity of saponin compounds so that there is a change in the location of their constituent groups [37].

The antibacterial activity test has the purpose of measuring the antibacterial power activity of unripe fruit extract, unripe fruit flesh extract and unripe fruit peel extract of Kayu banana which is suspected to have the ability to inhibit growth or even kill bacteria based on the principle, the antibacterial activity test can be carried out by 2 methods, namely the diffusion method and the dilution method [38]. In the diffusion method, the growth of the test organism can be inhibited from spreading along antibacterial diffusion (a clear zone is formed around the reserve) so it can be said that the bacteria are bacteria that are sensitive to antibacterial compounds [39]. The inhibition zone is one of the parameters for determining MIC, as has been reported in this study. In the dilution method, MIC can be seen either visually or in semiautomatic and automatic tools. The dilution method can be applied to qualitative and quantitative determinations together [40].

The test method used is the dilution method OD  $\lambda$  max 625 nm with incubation time. The bacteria used in *Escherichia coli*. This antibacterial test aims to determine the ability to inhibit bacterial growth within a certain incubation time. The more turbid a culture is, the greater the number of cells [41]. The light emitted on the spectrophotometer will hit the cell so that part of the light will be absorbed and part of it will be passed on. The magnitude of light in a spectrophotometer absorbed by cells in a cuvette is calculated as the absorbance value [22]. MIC is the lowest concentration of an antibacterial substance in inhibiting bacterial growth within a certain incubation time. The MIC value is obtained by looking at the growth of bacteria in the test tube using a UV-Vis spectrophotometer. Bacteria that grow and die can be observed by looking at the difference in absorbance from before incubation to after incubation called  $\Delta$ OD changes. [23].

Based on **Table 5**, it is seen that unripe fruit extracts, unripe fruit flesh extracts and unripe fruit peel extracts show  $\Delta$ OD negative value. This suggests that bacterial growth is inhibited, which is characterized by a decrease in  $\Delta$ OD after the incubation period. The same is also shown in the positive control  $\Delta$ OD is negative. This indicates stunted bacterial growth. Unripe fruit extracts, unripe fruit flesh extracts and fruit extracts are thought to have penetrated the cell walls of *Escherichia coli* bacteria and can suppress their growth characterized by negative  $\Delta$ OD [42]. Unripe fruit peel extract showed a large  $\Delta$ OD yield of  $-1.3620$  compared to unripe fruit extract and unripe fruit flesh extract. This shows the ability of unripe fruit peel extract in inhibiting the growth of *Escherichia coli* bacteria more optimally compared to unripe fruit extract and unripe fruit flesh extract [24]. Extracts of fruit peel, pulp and unripe fruit of Kayu banana have antibacterial activity, but the mechanisms and molecular pathways have not been studied [25].

TPC analysis on samples by the FC method using a UV-Vis spectrophotometer at a maximum wavelength ( $\lambda$  max) of 745 nm [43]. Phenolic ions are formed through the dissociation of protons of phenolic compounds, but this reaction runs slowly, therefore it is necessary to have an alkaline atmosphere-carrying solution so that the reaction takes place quickly, so sodium carbonate is used as a carrier of alkaline atmosphere. Phenolic ions when reacting with the FC reagent form a blue molybdenum-tungsten complex that can be detected with a spectrophotometer. Based on the calculation results from the regression equation, TPC fruit extract was obtained by 8.6 %, TPC flesh extracts by 6.1 %, and TPC from peel extract by 10.8 % [26,48]. The total phenol content is influenced by the type of solvent. Phenols are compounds that are polar so their solubility is highest in polar solvents. Polar solvents can dissolve phenols better so that their levels in the extract become high [29,52].

The activity of antibacterial barriers is due to the presence of multiple compounds in the extract of unripe fruit peels that contribute to the antibacterial effect [44]. The presence of antibacterial activity in the extract is due to the presence of secondary metabolites of the phenolic group such as tannins, flavonoids, phenolics and polyphenols in the extract [45]. The hydroxyl group is thought to be related to its toxicity activity against microorganisms, where the increase in hydroxylation results in an increase in toxicity [23]. The mechanism of extracts in the inhibition of *Escherichia coli* may be related to obstacles in electron transport, protein translocation, phosphorylation and other reactions involving enzymes, which lead to an increase in plasma membrane permeability and eventually an ion imbalance in the cell wall. In addition, the mechanism may also be related to the permeability of the surface of the bacterial cell wall with the extract [27]. According to Septama and Panichayupakaranant [31], the content of the phenolic compound artocarpanone provides antibacterial activity against *Escherichia coli* by disrupting membrane permeability [46]. The antibacterial mechanism is still unclear and it is suspected that the bacterial cell wall membrane is one of the places of action by interacting on the lipophilic side of the cell wall membrane, causing damage to the cell wall membrane [47]. Rita *et al.* [54] stated that Pecah Seribu bananas have flavonoid and phenolic content of 2033.53 mg QE/100 g and 250.25 mg GAE/100 g respectively can inhibit the growth of *Staphylococcus aureus* bacteria by 12.27 mm at a concentration of 20 % and 13.60 mm at a concentration of 50 %, while *Escherichia coli* bacteria by 11.87 mm at a concentration of 20 % and 12.73 mm at a concentration of 50 %. The difference in sensitivity between fruit extract, flesh extract and unripe fruit peel

extract of banana Kayu is due to the difference in the content of secondary metabolites contained. The absorption of phenolic compounds by the bacterial cell membrane and the interaction of phenolic compounds with active enzymes on the bacterial cell wall greatly affect its antibacterial activity [48]. In unripe fruit peel extract banana Kayu has optimal activity with the largest total phenol content [17].

## Conclusions

Unripe fruit peel extract of Kayu banana (*Musa paradisiaca* L. var. Kayu) has the potential to be antibacterial against *Escherichia coli*. The results of this study showed that the peel extract of the unripe fruit of banana Kayu has an inhibitory zone diameter of 17 mm and there is a decrease in absorbance with a large  $\Delta OD$ , which is  $-1.3620$  with a total phenol content of 107.8 mg of gallic acid equivalent per gram of unripe fruit peel extract. More research is needed to explain the mechanism of unripe fruit peel extract of banana Kayu against pathogenic bacteria of diarrhea. Further research is needed in validating the antibacterial activity and safety of the extract so that comprehensive perspective results are obtained such as conducting animal or clinical studies.

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