Differences in extraction methods to antidiarrheal activity in vitro and in vivo in unripe Kayu banana fruit (Musa paradisiaca L. Var. Kayu)

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Differences in extraction methods to antidiarrheal activity *in vitro* and *in vivo* in unripe Kayu banana fruit (*Musa paradisiaca L. Var. Kayu*)

Diferencias en los métodos de extracción de la actividad antidiarreica *in vitro* e *in vivo* en frutos de banano inmaduros Kayu (*Musa paradisiaca L. Var. Kayu*)

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SUMMARY

Introduction: *Kayu banana is a fruit found in Lumajang Regency, East Java, with antidiarrheal activity. Phenolic compounds in the unripe Kayu banana fruit have antidiarrheal activity in vivo and in vitro. This study aimed to prove the effectiveness of the extraction method on the phenolic acid content of the unripe Kayu banana fruit (Musa paradisiaca L. var. Kayu) as an antidiarrheal drug and to determine the significant difference between the extraction method on the phenolic acid content and antidiarrheal activity of the unripe Kayu banana fruit (Musa paradisiaca L. var. Kayu).*

Methods: *The research method used is an experimental quantitative method. There are 2 extraction methods used in this study, namely: cold extraction (remaceration and maceration) and hot extraction (reflux and Soxhlet).*

Results: *The results obtained in the extraction method with the remaceration extraction method were 99.31±1.11 mg GAE/g, with the highest antidiarrheal activity at the average marker trajectory. The Soxhlet extraction method has the highest antibacterial activity with an average inhibition zone of 24 0.82 mm.*

Conclusion: *Differences in extraction methods affect the phenolic levels of the extract and the antidiarrheal activity in vitro and in vivo in the raw fruit extract of banana wood. Based on the percentage of inhibition produced by the extract of the unripe fruit of the wood banana (Musa paradisiaca L. Var. Kayu), the extraction method of remacetation was 55.82 %, with the highest phenolic content. The soxhlet method has the highest antidiarrheal activity.*

Keywords: *Extraction methods, antidiarrheal, Musa paradisiaca L. Var. Kayu*

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RESUMEN

Introducción: El banano Kayu es una fruta que se encuentra en Lumajang Regency, East Java, con actividad antidiarreica. Los compuestos fenólicos en la fruta de banano Kayu verde tienen actividad antidiarreica in vivo e in vitro. Este estudio tuvo como objetivo probar la efectividad del método de extracción sobre el contenido de ácido fenólico de la fruta de banano Kayu inmaduro (Musa paradisiaca L. var. kayu) como fármaco antidiarreico y determinar la diferencia significativa entre el método de extracción sobre el contenido de ácido fenólico y actividad antidiarreica de la fruta de banano Kayu inmadura (Musa paradisiaca L. var. kayu).
Métodos: El método de investigación utilizado es un método cuantitativo experimental. Hay 2 métodos de extracción utilizados en este estudio, a saber: extracción en frío (remaceración y maceración) y extracción en caliente (reflujo y Soxhlet).
Resultados: Los resultados obtenidos en el método de extracción con el método de extracción por remaceración fueron de 99,31±1,11 mg GAE/g, con la mayor actividad antidiarreica en la trayectoria promedio del marcador. El método de extracción Soxhlet tiene la actividad antibacteriana más alta con una zona de inhibición promedio de 24 0,82 mm.
Conclusión: Las diferencias en los métodos de extracción afectan los niveles fenólicos del extracto y la actividad antidiarreica in vitro e in vivo en el extracto de fruta cruda de madera de banano. Con base en el porcentaje de inhibición que produce el extracto del fruto inmaduro del banano de madera (Musa paradisiaca L. Var. Kayu), el método de extracción de remacetación fue de 55.82 %, con el mayor contenido fenólico. El método Soxhlet tiene la mayor actividad antidiarreica.

Palabrasclave: *Métodos de extracción, antidiarreico, Musa paradisiaca L. Var. kayu*

INTRODUCTION

The unripe fruit of the Kayu banana (*Musa paradisiaca L. var. Kayu*) is a medicine that is often used empirically by the people of Senduro village, Lumajang, East Java, to treat diarrhea. Empirically, the use of unripe Kayu banana fruit (*Musa paradisiaca L. var. Kayu*) in Senduro is used by burning, steaming, and boiling. In a previous study, bananas also had an antidiarrheal effect in rats induced by oleum ricini showed that the phytochemical test results of the ethanol extract of unripe Kayu banana fruit (*Musa paradisiaca L. var. Kayu*) showed positive tannin content which had antidiarrheal activity induced by Oleum Ricini at a dose of 100 mg/kg, b.w. (1/3) unripe wooden banana has been used empirically as an antidiarrheal by the people in Senduro village, Lumajang, East Java. The study aimed to prove the antidiarrheal effect of ethanol extract of unripe wooden banana (*Musa paradisiaca L.*).

The active compounds contained in unripe Kayu banana fruit are tannins, flavonoids, alkaloids, and saponins (4-7). These compounds have pharmacological activity as antibacterial. To get the active compound it is necessary to do an extraction. The extraction method used can affect the concentration or loss of the therapeutic effect of Simplicia because some Simplicia is relatively stable and can be decomposed depending on the extraction method used (8,9).

Techniques for obtaining phenolic compounds can use several cold and hot extraction methods. The cold extraction method uses remaceration and maceration extraction, while the hot extraction method uses Soxhlet and reflux extraction methods. Several large industries have long applied continuous filtration so that it is time efficient, solvent savings, and the more unripe material is extracted (10). Based on the above background, a study was conducted on the antidiarrheal activity of the unripe fruit extract of the unripe Kayu banana fruit (*Musa paradisiaca L. var. Kayu*) with several extraction methods.

MATERIALS AND METHODS

Materials

The tools used in this research are a hotplate, oven, filter paper, Bunsen, Buchner funnel, macerator, rotary evaporator, Soxhlet, reflux, percolation, Uv-Vis spectrophotometry, analytical balance, surgical table and surgical instruments, Petri dish, incubator, oven, bunsen, autoclave, water bath, paper disk. The materials used in this study were unripe Kayu banana fruit (*Musa paradisiaca L. var. Kayu*), 96 % ethanol, sterile distilled water, Mayer reagent, Dragendoorf reagent, 2 M HCl, magnesium, concentrated HCl,

$FeCl_3$, CH_3COOH, H_2SO_4, acid error, and Folin Ciocalteau reagent, Na_2CO_3, Oleum ricini, CMC-Na, loperamide HCl, Chinese ink, *Escherichia coli* bacteria, MHA (Muller Hinton Agar) media, 70 % alcohol, chloramphenicol, $BaCl_2$ solution, H_2SO_4 solution, NaCl 0.9 %.

Stage of Study

1. Plant Determination

Determination of unripe Kayu bananas was carried out at Purwodadi Pasuruan Botanical Gardens (LIPI) and the Food Security and Agriculture Office of Lumajang Regency. This determination was made to ensure the correctness of the plants used in the study.

2. Making Simplicia Raw Fruit Banana Kayu (*Musa paradisiaca L. var. kayu*)

A total of 12 959.9 g of unripe Kayu banana fruit were washed under running water until clean, drained, and weighed wet. Then cut, dried in a drying rack at a temperature of 50°C, sorted dry, and weighed dry. The dry samples were then blended, sieved, and stored in plastic containers (11).

3. Microscopic Examination

Microscopic examination was carried out by performing some Simplicia powder on a glass object that had been dripped with chloral hydrate solution, covered with a cover slip, and viewed under a microscope.

4. Sample Extraction

a. Remaceration

A total of 500 g of Simplicia powder of unripe Kayu banana fruit (*Musa paradisiaca L. var. Kayu*) was macerated using 96 % ethanol solvent at room temperature and stirred. The powder is soaked for 24 h. Remaceration was carried out 2 times and filtered to separate the residue and filtrate. The resulting macerate was then evaporated with a rotary evaporator at a temperature of 50°C and evaporated until it became a thick extract (12).

b. Maceration

An amount of 500 g of Simplicia powder of unripe Kayu banana fruit was added to as much as 3 750 mL of 96 % ethanol solvent and maceration. After all the powder is soaked, stirred slowly, and soaked for 5 days with stirring. The resulting macerate was then evaporated using a rotary evaporator at a temperature of 50°C (13).

c. Soxhlet

A total of 500 g of Simplicia powder was wrapped in filter paper, tied, and then put into a Soxhlet extractor. 1.5 L of 96 % ethanol solvent was put into a round bottom flask. Then the Soxhlet device was assembled with a condenser. Extraction was carried out at a temperature of 60-80°C until the liquid was colorless. The extract obtained was evaporated using a rotary evaporator at a temperature of 50°C (14).

d. Reflux

A total of 500 g of Simplicia powder was put into a round bottom flask, and then 96 % ethanol solvent was added. Assemble the reflux device, and the sample was extracted at 50°C for 2 hours. The solution was filtered using filter paper and evaporated using a rotary evaporator at a temperature of 50°C (12).

5. Phytochemical Screening

Alkaloid Test: The filtrate was divided into 3 parts, each added with Mayer, Dragendorf, and Wagner reagents. The positive results for alkaloids are that Mayer formed white or yellow on the surface, Wagner on the brown surface, and Dragendorf on the brown surface (15).

Saponin Test: A total of 0.5 g of powder was put into a test tube, added to 10 mL of hot air,

and then shaken for 10 seconds. If it is formed as high as 1 minute to 10 cm, which is stable, it indicates the presence of saponins (16).

Flavonoid Test: 5 mL of the filtrate was added with 0.1 g of Mg powder, 2 mL of amyl alcohol, and 1 mL Dragendoorf reagent of concentrated hydrochloric acid, shaken and allowed to separate. The red, yellow, or orange colors formed on the amyl alcohol layer indicate a positive presence of flavonoids (17).

Tannin Test: 2 mL was taken and added with 1 to 2 drops of 1 % $FeCl_3$. Changes in color to green, blue or blackish indicate a positive result for tannins (18).

Polyphenol Test: The filtrate was divided into 3 parts (A, B, and C). Filtrate A was used as a blank, filtrate B was added with 3 drops of $FeCl_3$, and filtrate C was added with gelatin salt. The color change from green to blue-black indicates the presence of phenolic compounds (19).

Triterpenoid and Steroid Test: As much as 0.1 g of extract was added 3 drops of concentrated HCl and 1 drop of H_2SO_4. If red or purple color is formed, it is positive that it contains terpenoids. If green color is formed, it is positive that it contains steroids (20).

Anthraquinone Test: A total of 0.3 g of the extract was extracted with 10 mL of distilled water, then the filtrate was extracted with 3 mL of toluene and added with ammonia. There is a color change to red which indicates positive anthraquinone.

Glycoside Test: 1 g of thick extract was dissolved in ethanol, evaporated over a water bath, then dissolved in 5 mL of anhydrous acetic acid P. and added 10 drops of sulfuric acid Blue or green color formed indicates the presence of glycosides (21).

6. Determination of the content of phenolic compounds

a. Preparation of concentration test solution

Weighed 10 mg of ethanol extract from raw wood banana fruit, then put it into a 10 mL volumetric flask. The volume was filled with methanol p.a to the limit mark (22).

b. Preparation of gallic acid solution

Gallic acid solution was prepared with a concentration of 1 000 ppm. We weighed as much as 10 mg of gallic acid and then dissolved it in 10 mL of methanol pro analysis (22).

c. Determination of the maximum absorption wavelength

Take 3 mL of solution with a concentration of 30 ppm, add 0.4 mL of Folin-Ciocalteau reagent, shake it and leave it for 4-8 minutes, add 4.0 mL of 7 % Na_2CO_3 solution, and shake until homogeneous. Added bi-distilled water up to 10 mL and allowed to stand for 2 hours at room temperature. The absorbance was measured at a maximum wavelength of 600 – 1 100 nm. A calibration curve was made for the relationship between gallic acid concentration (μg/mL) and absorbance (22).

d. Gallic acid standard curve measurement

For each concentration of 10, 20, 30, 40, and 50 ppm. Take 0.1 mL, add 0.4 mL of Folin-Ciocalteau reagent, shake, and leave for 4-8 minutes. Add 4.0 mL of 7 % Na_2CO_3 solution and shake until homogeneous. Added distilled water up to 10 mL and allowed to stand for 2 hours at room temperature. The absorbance was measured at a maximum wavelength of 759 nm. A calibration curve was made for the relationship between gallic acid concentration (μg/mL) and absorbance (22).

e. Determination of the total phenolic content of raw wood banana extract (*Musa paradisiaca L. Var. Kayu*)

The extract solution was pipetted as much as 1 mL of unripe Kayu banana fruit extract. The sample was added with 0.4 mL of Folin-Ciocalteau reagent, shaken, and left for 4-8 minutes, adding 4.0 mL of 7 % Na_2CO_3 solution until homogeneous. Add bi-distilled water to 10 mL and let stand for 2 hours at room temperature. Measure the absorption at the maximum absorption wavelength of 744.8 nm. Perform

3 repetitions to obtain the phenol content as mg gallic acid equivalent/g extract (22).

7. Antibacterial Activity Test

a. Tool Sterilization and Production of Mueller Hinton Agar (MHA) Media

The tools and materials used are first sterilized in an autoclave at 121°C for 15 minutes at a pressure of 2 Atm. Tools such as ose and tweezers were sterilized by immersing them in 70 % alcohol for 5 minutes, then ignited with a Bunsen flame (23). A total of 3.42 g of MHA media were suspended in 90 mL of sterile distilled water and then heated to boiling. The fully suspended media was sterilized in an autoclave at 121°C for 15 min (24,25).

d. Bacterial Rejuvenation Process

The test bacteria were grown on a slanted agar medium by taking a pure bacterial loop with a needle and scraping it aseptically in a Laminar Air Flow (LAF) cabinet. Then incubated at 37°C for 24 h.

e. Preparation of Standard Turbidity Solution (Mc. Farland's Solution)

Mc. Farland's standard solution was made by taking 0.05 mL of $BaCl_2$ solution and 9.95 mL of H_2SO_4 solution and then shaking them until a cloudy solution was formed. This turbidity was used as a standard for the turbidity of the bacterial test suspension (25).

f. Preparation of *Escherichia coli*. Bacterial Suspension

The *Escherichia coli* test colonies were suspended by taking one ose of colonies from the rejuvenation medium, then suspended into a test tube containing 2 mL of 0.9 % NaCl solution until the turbidity was the same as the turbidity standard of Mc. Farland's solution (24).

g. Negative Control Creation

A total of 5 mL of DMSO solution was diluted with distilled water to 100 mL.

h. Positive Control Creation

Weighed 0.0015 g of chloramphenicol, put the powder into a beaker glass, added sterile distilled water a little while stirring until homogeneous, and then diluted with sterile distilled water to 10 mL.

i. Preparation of Motherboard Test Solution for Banana Wood Fruit Ethanol Extract

The test solution was made by dissolving the ethanolic extract of unripe Kayu banana with 5 % DMSO solution by weighing 10 g of extract, adding 5 % DMSO solution little by little, stirring until homogeneous, and then diluting with 5 % DMSO solution to 10 mL.

j. Determination of Antibacterial Activity of Banana Kayu

Unripe Kayu banana fruit extract prepares sterilized Petri dishes. Then 15 mL of MHA medium was poured into each Petri dish, then 1 mL of bacterial suspension was inoculated on the media and allowed to solidify. The paper disc is immersed in the sample to be tested, and then the paper disc is placed on an agar plate, then incubated at 37°C for 24 h. Then the diameter of the inhibition zone (mm) was measured for each sample (20).

8. Antidiarrheal Activity Test

a. Preparation of CMC-Na 0.5 % w/v and Loperamide HCl Suspension 0.02 % w/v and 0.01 % w/v

A total of 0.5 g of CMC Na was sprinkled into a mortar containing 20 mL of hot distilled

water, covered, and left for 30 minutes until a transparent mass was obtained, crushed, and then diluted with distilled water to 100 mL (15). Lodia tablets contain 2 mg of Loperamide HCl; 10 tablets were taken for Lopramid HCl content of 0.02 % w/v. The tablets were ground until homogeneous. The powder was put into a mortar, and then 0.5 % CMC-Na suspension was added little by little the chili was ground homogeneously and then diluted with 0.5 % CMC-Na suspension to 100 mL (4).

c. Preparation of Unripe Kayu Banana (*Musa Paradisiaca L. var. Kayu*) Ethanol Extract Suspension

Testing the antidiarrheal effect of the unripe Kayu banana fruit extract suspension included the antimotility activity test (inhibiting intestinal movement so that the frequency of diarrhea was reduced), ethanol extract of the unripe Kayu banana fruit with the intestinal transit method (comparing the length of the intestine through the marker to the overall length of the intestine). Mice were randomly grouped into six groups, each consisting of five mice, and then weighed each mouse. 1) Negative control group (Given CMC-Na 0.5 % 0.2 mL/20 g, b.w. orally and given 1 mL Chinese ink). 2) Positive control group (Given HCl suspension 0.02 % w/v at a dose of 2 mg/kg as much as 0.2 mL/20 g, b.w. and given 1 mL Chinese ink). 3) Test Group (Given the suspension of unripe Kayu banana fruit ethanol extract 2 % w/v with a dose of 200 mg/kg, b.w. on remaceration, percolation, reflux, and Soxhlet methods.

Ethanol extract from unripe Kayu banana fruit and loperamide was given at the beginning of the experiment. One hour after treatment, all rats were given 2 mL of oleum ricini. After 1 hour of administration of oleum ricini, 1 mL of Chinese ink was given orally to mice. After an hour of giving the Chinese ink, all the animals were sacrificed by dislocation of the cervical spine. Next, animals are dissected, and their intestines are carefully removed. The length of the intestine traversed by the Chinese ink marker was measured from the pylorus to the ileocecal valve of each animal, and then each animal has calculated the percent of the path traversed by the Chinese ink marker to the total length of the intestine (15).

Data analysis

In this study, for data analysis using SPSS calculations do normality testing before proceeding to the next test. When the results show normal, you can continue the parametric test, but nonparametric testing will be carried out if the data is not normal. For this study, a follow-up test was carried out using homogeneity testing, One way ANOVA, and Tukey test. In addition, an ethical feasibility test has been carried out with the number 170/HRECC.FODM/IV/2022.

RESULTS

Plant Determination

Sampling was carried out in Lumajang Regency, East Java. The selection of fruit taken is a fruit that is still fresh and unripe with a green color without a yellow color, hard, and 3 months old after the flower bunches come out. The unripe Kayu banana fruit that has been obtained is then determined to ensure the correctness of the plants used in the study. Determination of Kayu banana raw fruit was carried out at Purwodadi Pasuruan Botanical Gardens (LIPI), and wooden banana raw fruit was indeed a woody variety by proving the determination of wooden banana raw fruit at the Lumajang Regency Food and Agriculture Security Service.

Sample Making

The unripe Kayu banana fruit, as much as 12 959.9 g, was washed with running water to remove dirt and sap that was still attached to the unripe Kayu banana fruit. Unripe Kayu banana fruit that has been washed is then dried by aerating, then the wooden bananas are cut into thin strips to facilitate a drying process. Drying was carried out using a food dehydrator oven at a temperature of 50° and obtained dry Simplicia results. The dried Simplicia was then mashed using a blender until it became powder, and the resulting fine powder was 4 152.6 g.

Table 1

Results of Simplicia Characteristics of Unripe Kayu Banana Fruit Powder

Simplicia	Temperature	Weight of fresh Simplicia (g)	Weight of dry Simplicia (g)	% Drying shrinkage	Water rate	Organoleptic
Kayu Banan unripe fruit	Temperature 50°C	12 959.9 g	4152.6 g	32.04 %	2.74 %	Aromatic characteristic smell, ivory color, and in white powder form

Microscopic Examination

The results of the research on raw wood banana fruit powder showed that unripe Kayu banana fruit powder with observations under a microscope found fragments or parts contained in the unripe Kayu banana fruit including the trachea, oily glands, starch, and fibers.

Extract Manufacture

Simplicia extraction uses 2 extraction methods, namely cold and hot methods. Cold extraction methods use remaceration and maceration while hot extraction methods use reflux, and Soxhlet uses 96 % ethanol solvent with a temperature of 50°C according to the results of Table 4.1 % the highest yield using the remaceration extraction method with 16.45 % yield.

Table 2

Result RendeMen of Kayu Banana Unripe Fruit Extract

No	Extraction Method	Powder Weight	Extract Weight	Rendemen Extract (%)	Organoleptic Extract
1	Remaserasi	750 g	123.4 g	16.45	Color: brownish green Smell: slightly pungent Taste: slightly bitter and astringent
2	Maserasi	750 g	106.3 g	14.17	Color: brownish green Smell: slightly pungent Taste: slightly astringent and very bitter
3	Reflux	750 g	85.7 g	11.42	Color: dark green Smell: slightly pungent Taste: slightly bitter and slightly astringent
4	Soxhlet	750 g	76.8 g	10.24	Color: dark green Smell: very pungent Taste: bitter

Phytochemical Screening Examination

Phytochemical screening was conducted to determine the content of secondary metabolites in the unripe Kayu banana fruit extract. The phytochemical screening included tannins, alkaloids, saponins, flavonoids, polyphenols, glycosides, anthraquinones, terpenoids, and steroids. Based on the results of the phytochemical screening test with remaceration

and maceration extraction methods, it produced significant secondary metabolite compounds containing alkaloids, saponins, flavonoids, tannins, polyphenols, and anthraquinones. While the reflux and Soxhlet extraction method produces secondary metabolites containing alkaloids, saponins, flavonoids, tannins, polyphenols, anthraquinones, and triterpenoids. In this study, the results of phytochemical screening produced different secondary metabolites because time and temperature greatly affected the number of compounds extracted, so the extraction method by heating where the extraction method would provide an opportunity to obtain maximum secondary metabolites.

Table 3
Results of Phytochemical Screening of Unripe Kayu Banana Fruit

Organoleptic Examination	Extraction Method			
	Remaceration	Maceration	Reflux	Soxhlet
Alkaloid	+	+	+	+
Saponin	+	+	+	+
Flavonoid	+	+	+	+
Tannin	+	+	+	+
Polyphenol	+	+	+	+
Anthraquinone	+	+	+	+
Glycoside	-	-	-	-
Steroid	-	-	-	-
Triterpenoid	-	-	+	+

Antibacterial Activity Test

The method used in the antibacterial activity test is the disc diffusion method. The results of the antibacterial test were based on the measurement of the diameter of the zone of inhibition of bacterial growth formed around the paper disc. The bacteria used were *Escherichia coli* bacteria with Muller Hinton Agar (MHA) media.

Based on the study's results, it was shown that the unripe Kayu banana fruit extract with different extraction methods at a concentration of 100 % had a very strong inhibitory power against the test bacteria. The positive control was categorized as very strong inhibitory, while the negative control did not show any inhibition against the test bacteria. The positive control used was chloramphenicol at a dose of 150 μg. The reason for using chloramphenicol as a positive control was because chloramphenicol is a broad-spectrum antibiotic capable of treating infections caused by gram-positive and gram-negative bacteria. The highest average inhibition zone was the Soxhlet extraction method with an inhibition zone diameter of 24 mm. Next, the remaceration extraction method with an inhibition zone diameter of 23.25 mm, then the reflux extraction method with an average inhibition zone diameter of 23 mm, and the lowest is the maceration method with an average inhibition zone diameter of 20.5 mm.

***In vivo* Antidiarrheal Activity Testing**

The method used in the test of antidiarrheal activity is the intestinal transit method. The results of the antidiarrheal activity test are based on the effect of the ratio of the distance of the intestine traveled by the marker at a certain time to the overall intestinal length in mice or rats.

Based on the results of the study, it can be seen that the different extraction methods can affect the antidiarrheal activity of the unripe Kayu banana fruit extract. The extract using the CMC-Na extraction method (Negative Control) had the highest average cross-marker length in the intestines of mice, which was 78 %. On the other hand, the extract using the Loperamide HCl extraction method (Positive Control) had the lowest average cross-marker length in the intestines of mice, which was 32 %.

Table 4

Results of Inhibitory Zone Diameter for Antibacterial Activity Test

Sample Group	Concentration	Replication	Inhibition Zone	Average	SD	Inhibition Zone ± SD	Interpretation
Chloramphenicol	150 μg	1	27	29.75	2.63	29.75 ± 2,63	Susceptible
		2	28				
		3	32				
		4	32				
DMSO	5 %	1	0	0	0	0 ± 0	No Inhibition
		2	0				
		3	0				
		4	0				
Remaceration	100 %	1	24	23.25	1.5	23.25 ± 1.5	Susceptible
		2	22				
		3	25				
		4	22				
Maceration	100 %	1	22	20.5	1	20.5 ± 1	Susceptible
		2	20				
		3	20				
		4	20				
Soxhlet	100 %	1	25	24	0.82	24 ± 0.82	Susceptible
		2	24				
		3	23				
		4	24				
Reflux	100 %	1	23	23	0.82	23 ± 0,82	Susceptible
		2	23				
		3	22				
		4	24				

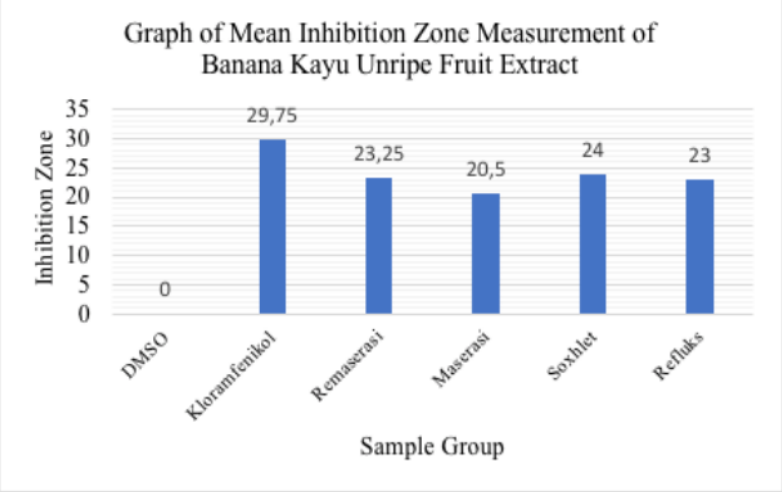

Figure 1. Graph of Average Inhibition Zone Measurement of Raw Fruit Extract.

Table 5
Results of Cross Marker Length Test in Intestines of Mice

Test Material	Replication	Intestine Length (CM)	Ink Distance (CM)	Cross-Ratio Markers	(%) inhibition	Standard Deviation
Loperamide HCL	2	53	20	0.3773	58.38	0.0920
	3	55	19.5	0.3545		
	4	55	21	0.3818		
	5	53	10	0.1886		
		Average		0.3256		
CMC-Na	1	52	39	0.75	0	0.0954
	2	52	45	0.8653		
	3	53	45	0.8490		
	4	50	33	0.66		
		Average		0.7811		
Remaceration	2	55	22	0.4	55.77	0.1100
	3	55	15	0.2727		
	4	55	26	0.4727		
	5	55	13	0.2363		
		Average		0.3454		
Maceration	1	52	24	0.4615	42.86	0.0646
	2	50	26	0.52		
	3	50	22	0.44		
	5	55	20	0.3636		
		Average		0.4462		
Reflux	2	55	30	0.5454	45.06	0.0916
	3	56	24	0.4285		
	4	56	18	0.3214		
	5	57	24	0.4210		
		Average		0.4291		
Soxlhet	2	53	25	0.4716	30.81	0.0527
	3	57	30	0.5263		
	4	55	32	0.5818		
	5	55	32	0.5818		
		Average		0.5404		

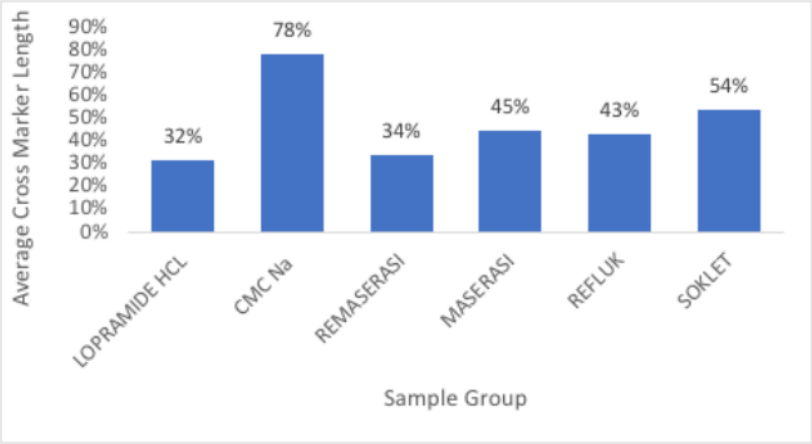

Figure 2. The results of the average length of cross markers in the intestines of mice.

DISCUSSION

In this study, the extract yield from the remaseration extraction method was 16.45 %, and for the results of obtaining phenolic compound, levels produced 99.31±1.11 mg GAE/g, the result of the maceration extract yielded was 14.17 % and for the results of obtaining phenolic compound levels produced by 53.96±0.81 mgGAE/g. In previous research, differences in pomegranate extraction methods show differences in anthocyanin levels (26). The difference in amendments and phenolic levels obtained is suspected to be because the remaceration method is carried out at room temperature and protected from sunlight and heat, the withdrawal of active compounds for 2 days is carried out by soaking the Simplicia powder with the appropriate solvent for 2 days and changing the solvent every day. When it reaches the equilibrium phase, the plant cell will be entered by the solvent by passing through the cell wall. The equilibrium process occurs by exiting secondary metabolite compounds inside the cell because the concentration inside the cell is different from the concentration outside the cell. The equilibrium process occurs because there is a diffusion process caused by a difference in concentration. A higher concentration inside the cell will cause the secondary metabolite compounds to come out and be replaced by solvent liquid outside the cell whose concentration is lower. The event repeatedly occurs until there is a balance of concentration outside and inside the cell. During the remaseration process, a replacement of the igniting fluid is carried out every day for 2 days to maximize the effectiveness of withdrawal. In the maceration extraction method, there is no repetition of the addition of solvents so that the active compounds contained in the raw fruit of wood bananas are not attracted to the maximum. Therefore, the levels of phenolic compounds produced are low.

The result of the reflux method extract yield was 11.42 %, and the resulting phenolic content was 54.65±0.80 mg GAE/g. In the Soxhlet method, an extract yield of 10.24 % was obtained, and the resulting phenolic compound content was 54.47±0.65 mg GAE/g. The reflux method is a method of extraction with the help of heating. The addition of heating greatly affects extraction using reflux, and the solvent used will remain fresh due to the submerged re-evaporation in the material. Therefore, reflux extraction extracts heat-resistant materials with a rough texture (22). The principle of the Soxhlet method is that it is carried out continuously using relatively few solvents. When the extraction is complete, the solvent can be evaporated to obtain an extract. Usually, the solvent used is volatile or has a low boiling point (10). Hmidani et al. 2019 revealed that all extracts obtained from various extraction methods of *Thymus atlanticus* showed different phenolic concentrations, and the extraction time used in the extraction also affected the acquisition of phenolic levels (27).

In the reflux method, the level of phenolic compounds that are high enough should produce high yield levels, but the time made for extraction is quite short, namely for 2 hours, so that the withdrawal process of secondary metabolite compounds is not attracted to the maximum, but the levels of phenolic compounds produced are quite high due to the heating process, causing the cell wall of wood banana fruit powder to open larger, in addition, the heating process also results in the viscosity of the solvent decreasing so that the ability of the solvent to penetrate the cell wall becomes easier and the amount of phenolic compounds extracted becomes high. In the Soxhlet extraction method, the extraction process is quite long, which is 5 hours, so the time in the extraction process greatly affects the optimal extraction time. However, if the extraction time is too long, it will damage the active compounds, and if the extraction time is too short, it will result in low extract yield levels. The results of high phenolic compounds are suspected to be caused by the heating process so that the cell walls of banana powder can break and can secrete active compounds that can withstand heating.

The remaceration method is a modified method of the maceration method where the remaceration method is carried out by repeatedly adding solvent after the first extract has been filtered. In this study, the extract yield from the remaceration extraction method was 16.45 %, and the phenolic compounds yielded 99.31±1.11 mg GAE/g, allegedly producing the highest yield and phenolic compounds because at room temperature and protected from sunlight and heat, the extraction was carried out. The active compound was for 2 days by soaking the

Simplicia powder with the appropriate solvent for 2 days and changing the solvent every day. When it reaches the equilibrium phase, the plant cell will be penetrated by the solvent by passing through the cell wall. The equilibrium process occurs through the release of secondary metabolites in the cell because the concentration inside the cell is different from the concentration outside the cell. This equilibrium process occurs because there is a diffusion process caused by a difference in concentration where a higher concentration inside the cell will cause secondary metabolites to come out and be replaced by a lower concentration of solvent outside the cell. These events occur repeatedly until there is a balance of concentration outside and inside the cell. During the remaceration process, the filtered fluid is replaced every day for 2 days so that the effectiveness of the withdrawal will be maximized.

The maceration method is a cold extraction method and the simplest method in which the liquid filter will penetrate the plant cell wall and will enter the cell cavity containing the active substance so that the active substance, which is the concentrated solution, will be forced out of the cell due to the difference in concentration between the solute solution. Active inside the cell with those outside the cell (28). The yield of the resulting extract was 14.17 %, and the yield of phenolic compounds was 53.96±0.81 mg GAE/g. High yield results due to the long enough time and stirring many times. Therefore, the compounds contained in the raw fruit of the wooden banana are attracted quite a lot. The yield is high enough so that the levels of phenolic compounds should be high, but the principle of the maceration extraction method is not to repeat the addition of solvents so that the active compounds contained in the unripe fruit of Kayu bananas are not maximally attracted. Therefore, the levels of phenolic compounds produced are low.

The reflux method is an extraction method with the help of heating. The addition of heating greatly affects the extraction using reflux, and the solvent used will remain in a fresh state because of the re-evaporation that is submerged in the material. Reflux extraction extracts heat-resistant and coarse-textured materials (22). The extract yield was 11.42 %, and the phenolic content produced was 54.65±0.80 mg GAE/g. High levels of phenolic compounds should produce high yields, but the extraction time is quite short, namely for 2 hours, so the secondary metabolite withdrawal process is not maximally attracted, but the levels of phenolic compounds produced are quite high due to the heating process, causing the cell walls of the wood banana fruit powder. The opening is larger; besides that, the heating process also causes the solvent's viscosity to decrease so that the solvent's ability to penetrate the cell wall becomes easier, and the amount of phenolic compounds extracted is high. Soxhlet extraction time will affect the attraction of chemical compounds. The Soxhlet method takes a long time for extraction, so the Soxhlet method can draw more leverage than the reflux method. The remaceration method has a higher average diameter of the inhibition zone than the reflux method, and it is possible that in this reflux method, several chemical compounds function as an antibacterial that has lower levels than the remaceration method. Hence, the remaceration method has a higher average inhibition zone than the reflux method.

Based on the results of phytochemical screening, the extract using the positive heat extraction method contains triterpenoid compounds while the cold method does not contain triterpenoid compounds, where these triterpenoid compounds have antidiarrheal activity so that the extract using the Soxhlet extraction method provides higher antidiarrheal activity than the maceration method. The reflux method has a lower average cross-marker length in the intestines of mice than the Soxhlet method. The extraction time will affect the attraction of chemical compounds. The Soxhlet method takes a long time for extraction, so the Soxhlet method can draw more leverage than the reflux method. The remaceration method has a higher average cross-marker length in the intestines of mice than the reflux method, and it is possible that in this reflux method, several chemical compounds function as antidiarrheals that have lower levels than the remaceration method, so the remaceration method has an average cross-marker length in mice. Mice intestine is higher than the reflux method.

Based on the results of the one-way ANOVA test on phenolic content testing, it can be concluded that the results of hypothesis testing

have significant performance differences between the extraction methods. This result is indicated by the Fable value of 2.8951073, and the probability value (sig) of 0.000 H0 is rejected, where Fount Fable and the probability (sig) 0.05. The Fount value from the ANOVA Table is 3404,728, and the Fable value is 2.8951073, so it is 3404,728 2.8951073, and the probability value (sig) in the ANOVA Table is 0.0001 while the significance level = 0.05 so 0.0001≤0.05. Therefore, it can be concluded that there is a significant difference in the results of the phenolic compound levels. The analysis of the inhibition zone data and the average cross-marker length was continued using nonparametric analysis with the Kruskal-Wally's test aimed at seeing the difference in the diameter of the inhibition zone between the different extraction methods, positive control, and negative control. Based on the results of the Mann-Whitney Test, it was shown that the difference in the extraction method was statistically significant between the positive and negative controls. The negative control and the different extraction methods had a significant difference in terms of inhibitory activity because the negative control did not produce bacterial inhibitory activity and antidiarrheal activity.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that differences in extraction methods affect the phenolic content of the extract and the antidiarrheal activity in vitro and in vivo in the extract of the unripe Kayu banana fruit. So it can be concluded that the ethanolic extract of the unripe Kayu banana fruit (*Musa paradisiaca L.Var.Kayu)* raw fruit has activity as an antidiarrheal *in vitro* and *in vivo*. Based on the percentage of inhibition produced by the unripe fruit extract of wood banana (*Musa paradisiaca L. Var. Kayu),* the remaceration extraction method was 55.82 %, with the highest phenolic content. The Soxhlet method has the highest antidiarrheal activity.

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