JURNAL FARMASIMED (JFM)
E-ISSN: 2655-0814
https://ejournal.medistra.ac.id/index.php/JFM

# Uji Aktivitas Antidiare Kombinasi Ekstrak Daun Jambu Biji *(Psidium guajava L.)* dan Rimpang Kencur *(Kaempferia galanga) pada Tikus Jantan (Mus musculus)*

# Antidiarrheal Activity Test of a Combination of Guava Leaf Extract (*Psidium guajava* L.) and Kencur Rhizome (*Kaempferia galanga*) on Male Mice (*Mus musculus*)

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**Abstract**

***Background*** *Diarrhea is the leading cause of high morbidity and mortality in children in developing countries such as Indonesia. Diarrhea can be caused by bacterial infections. Guava leaves and kencur have astringent properties that can help reduce the frequency of bowel movements and stop diarrhea.* ***Aim****: The study aims to determine the most effective dose concentration of a combination of guava leaf ethanol extract (PGEE) and kencur rhizome ethanol extract (KGEE) as an antidiarrheal medicine.* ***Methods:*** *The study used an experimental method with stages of simplisia preparation, extract preparation by maceration, simplisia characteristics, phytochemical screening, and antidiarrheal efficacy testing using 25 male mice divided into 5 groups, namely F1 = negative control (NaCMC); F2 = positive control (Loperamide); F3, F4, and F5 were combinations (PGEE:KGEE) with 3 different dose concentrations, namely (25%:75%); (50%:50%), and (75%:25%), where the base doses used were (PGEE=400mg/kg/BW) and (KGEE=27.5mg/kgBW).* ***Results:*** *The parameters observed included the onset time of diarrhea, duration, frequency of bowel movements, and stool consistency. Statistical data analysis was performed using ANOVA and Tukey's post hoc test with the help of SPSS software. Statistical analysis with Tukey's post hoc test showed that there was a significant difference ($p < 0.05$) in fecal weight between the treatment group and the negative control group.* ***Conclusion****: The dose concentration (50%:50%) showed the most significant effect in reducing fecal weight comparable to loperamide, so that the combination of PGEE and KGEE at that dose concentration proved to be the most effective in reducing fecal weight and overcoming diarrhea, both visually (graph) and based on statistical analysis results.*

***Keywords***: *Diarrhea; Guava Leaf Extract (Psidium guajava L.); Galanga (Kaempferia galanga); Castor Oil; Combinations*

## 1. INTRODUCTION

Diarrhea is one of the leading causes of morbidity and mortality, especially among children in developing countries such as Indonesia. Diarrhea is defined as three or more episodes of abnormal bowel movements (defecation) with loose, watery, or liquid stools. Excessive fluid loss in diarrhea is associated with an imbalance between the mechanisms of absorption and secretion of water and electrolytes in the intestinal tract and is accompanied by hypermotility [1].

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Doi : 10.35451/m2vf9674
Received : March 18, 2026, Accepted: April 23, 2026 , Published: April 30, 2026

Several factors that can cause diarrhea include bacterial infections such as *Escherichia coli, Shigella spp, Vibrio cholera, Bacillus cereus, Salmonella spp, Campylobacter spp, Yersinia spp, viruses, parasites*, food poisoning, medications, allergies, and psychological factors [1]. Pharmacological treatment of diarrhea often involves synthetic drugs such as loperamide, which effectively reduces the frequency of defecation. This has prompted the search for alternative therapies from medicinal plants that are considered safer, more affordable, and have long been used empirically in traditional medicine.

Guava leaves (*Psidium guajava* L.) contain active compounds such as tannins, flavonoids, alkaloids, and saponins, which are known to have astringent, antibacterial, and antidiarrheal effects. Meanwhile, galangal rhizome (*Kaempferia galanga* L.) is rich in essential oils, flavonoids, and alkaloids with carminative, antimicrobial, and digestive support activities. The combination of these two plants is thought to provide synergistic effects. The anti-inflammatory effects of galangal rhizome and guava leaves can reduce fluid secretion and intestinal infections, thereby helping to treat diarrhea, where guava plays a role in reducing intestinal fluid secretion and frequency of defecation, while galangal adds antimicrobial effects and helps mask the bitter taste of guava extract [2].

Loperamide administration was used as a comparator because it can restore cells in a state of hypersecretion to normal resorption, and can increase small intestine transit time and absorption of water, sodium, and chloride in the body when electrolyte disturbances occur. Loperamide with guava leaves and kencur have the same mechanism of action, namely slowing down bowel movements and reducing fluid secretion [2].

In this study, oleum ricini was used as a diarrhea-inducing agent. Oleum ricini is a laxative that hydrolyzes into ricinoleic acid in the intestine. Ricinoleic acid stimulates fluid and electrolyte secretion, as well as intestinal peristalsis, causing diarrhea. The anti-diarrheal effect test was conducted on a mouse model. Mice were chosen as subjects for experimental research in the field of health because they have physiological and biochemical characteristics that are almost similar to humans [3].

The purpose of combining guava leaf extract and kencur rhizome is to mask the bitter taste of guava leaves with kencur rhizome. However, research on the effectiveness of the combination of guava leaf ethanol extract (PGEE) and kencur rhizome ethanol extract (KGEE) as an antidiarrheal drug is still limited, especially regarding the most effective dose concentration. Therefore, this study aims to evaluate the antidiarrheal activity of the combination of PGEE and KGEE in male mice and determine the optimal dosage concentration compared to negative and positive controls.

## 2. METHODS

### Materials

The materials in this study are divided into two parts, namely plant materials and chemical materials. The plant materials used were guava leaves (*Psidium guajava* L.) and galangal rhizome (*Kaempferia galanga*), and the test animals were mice. The chemical materials used in this study were 96% ethanol, lopramide, ricini oleum, 0.5% Na-CMC, 1% $FeCl_3$, Mg and HCl(p), 2N $H_2SO_4$, Ammonia, Ethyl Acetate, Chloroform, Maeser's Reagent, Wagner's Reagent, Drageendorff's Reagent, and Liebermann-Buschardat's Reagent.

### Tools

The equipment used in this study consisted of laboratory glassware (Iwaki Pyrex), a grinder (Miyako), a camera (Samsung A15), a gas stove (Rinnai), a drying cabinet, an analytical balance (And), a rotary evaporator (Buchi), scales (Kenmaster), a mortar, a stamper, an animal box, parchment, a syringe, an oral sonde, an animal balance, a water bath, and a set of moisture content testing equipment.

### Method

### Plant Identification

Plant identification was carried out at the Medanense Herbarium Laboratory (MEDA) of the Faculty of Mathematics and Natural Sciences, University of North Sumatra. The purpose of identification was to ensure the accuracy of the plants to be used in the study. The samples used were guava leaves and galanga rhizomes

(*Kaempferia galanga*). The parts used were young guava leaves (from the top to the third set of leaves) and fresh rhizomes selected from healthy plants free from physical defects, taken from the village of Serbajadi, Dusun IV Diski, North Sumatra.

**Ethanol Extract Of Guava Leaves (*Psidium guajava* L.) And Kencur Rhizome (*Kaempferia galanga*)**

Weigh 100 grams each of kencur rhizome and guava leaves. Place the ingredients in a 2-liter dark tube and soak them in 1 liter of 96% ethanol. Stir and let stand for 3x24 hours at room temperature. Then, after 3x24 hours, filter the soaked ingredients using a funnel and filter paper until the residue is separated. The maceration product is placed in a porcelain dish and evaporated using a rotary evaporator until a concentrated extract of kencur rhizome and guava leaves is obtained [4].

The ethanol extract yield is determined using the formula:

$$\%\text{ yield} : \frac{extract\ weight\ (g)}{simplisia\ weight\ (g)} \text{ x } 100\%$$

**Characteristics Of Simplisia**

**Macroscopic Examination**

Macroscopic examination was performed by observing the external morphology, namely the length, width, color, smell, taste, and shape of guava leaves (*Psidium guajava* L.) and galangal rhizomes (*Kaempferia galanga*) [5].

**Determination Of Moisture Content**

The determination of ash content is carried out gravimetrically. 2 grams of ethanol extract is placed on a dish and heated in an oven at 105ºC for 30 minutes. The sample is cooled in a desiccator for 15 minutes and then weighed to a constant weight [6].

Calculation of moisture content using the formula:

$$\%\text{moisture content} = \frac{(initial\ weight\ (g) - dry\ weight\ (g))}{\text{initial weight (g)}} \text{ x } 100\%$$

**Determination Of Water-Soluble Extract Content**

5 g of ethanol extract was macerated with 100 ml of chloroform P (2.5 ml of chloroform in 1000 ml of distilled water) for 24 hours in a stoppered flask, occasionally shaken. The sample is quickly filtered using filter paper, 20 ml of the filtrate is evaporated over a water bath until dry, and the residue in the dish is heated at 105ºC and weighed to a constant weight [7].

The water-soluble extract content is determined using the formula:

$$\%\text{water solubility} = \frac{weight\ of\ extract}{weight\ of\ initial\ sample} \text{ x } 100\%$$

**Determination Of Soluble Extract Content In Ethanol**

5 g of crude drug powder is macerated with 100 ml of 95% ethanol for 24 hours in a stoppered flask and shaken occasionally. The sample is quickly filtered using filter paper, 20 ml of the filtrate is evaporated in a dish over a water bath until dry, the residue in the dish is heated at 105ºC and weighed to a constant weight [7].

The water-soluble extract content is determined using the formula:

$$\text{water solubility} = \frac{dry\ weight}{dry\ ethanol\ extract\ weight} \text{ x } 100\%$$

**Phytochemical Screening**

**Alkaloid Examination**

Alkaloid compound analysis is carried out by dissolving the extract in 10 ml of chloroform and ammonia, adding 0.5 ml of $H_2SO_4$, then homogenizing and allowing it to settle until two layers form. The upper layer is then taken and 1 drop each of Meyer, Dragendorf, Bouchardart and Wagner reagents are added. If a white precipitate forms with the Mayer reagent, an orange-red precipitate with the Dragendorf reagent, a brown precipitate with the Bouchardart reagent, and a brown precipitate with the Wagner reagent, then the extract is declared positive for alkaloids [8].

**Flavonoid Testing**

1 ml of sample extract is placed in a test tube, 2 drops of Mg and concentrated HCl reagents are added, causing the solution to change color from greenish yellow in guava leaves to orange in kencur rhizomes [9].

**Tannin Test**

Take 1 ml of sample extract and place it in a test tube. Then add 2-4 drops of 1% $FeCl_3$. A positive result indicating the presence of tannin in the sample is evidenced by a blue-black or greenish-black color change [10].

**Saponin Test**

Place 0.1 grams of sample in a test tube, add hot water, and shake vigorously. The result is positive if foam ±1 cm high forms on guava leaves and stable foam forms on kencur rhizomes; if 1 drop of 2N HCl is added, the foam does not disappear [11].

**Terpenoid And Steroid Test**

Add 1 ml of chloroform phase to the drop plate, then add 5 drops of Liebermann-Burchard reagent to the drop plate. The presence of steroids will form a blue or green ring layer, while terpenoids will produce a deep green color [12].

**Preparation Of Experimental Animals**

The animals used were 25 male mice weighing 20–30 g, divided into 5 groups, each consisting of 5 mice. Before use, the mice were kept for 2 weeks to standardize their diet and living conditions so that they were considered suitable for research [13].

**Preparation Of Test Materials**

Test material preparation includes sodium carboxymethyl cellulose (Na-cmc 0.5%) solution as a carrier, loperamide HCl solution as a positive control, ethanol extract suspension of guava leaves with an initial dose of 400 mg/kg BW and kencur rhizome 27.5 mg/kg BW developed in 3 concentrations of PGEE:KGEE (25%:75%), PGEE:KGEE (50%:50%), and PGEE:KGEE (75%:25%). Thus, each dose for guava leaf suspension was 100 mg/kg BW, 200 mg/kg BW, and 300 mg/kg BW, while the ethanol extract suspension of kencur rhizome was 6.75 mg/kg BW, 13.5 mg/kg BW, and 20.25 mg/kg BW, and ricinus oil was used as an inducer in the experimental animals.

**Preparation of 0.5% CMC Suspension (B/V)**

Sprinkle 500 mg of Na-CMC into a mortar containing hot distilled water. Leave for 15 minutes, then grind until a transparent mass is obtained, grinding until homogeneous. Dilute with distilled water, homogenize, and transfer to a volumetric flask. Make up the volume to 100 ml with distilled water [14].

**Preparation Of Ethanol Extract Suspension From Guava Leaves (*Psidium guajava* L.) and Kencur Rhizome (*Kampferia galanga*)**

**Guava Leaves (*Psidium guajava* L.)**

The PGEE suspension was made in three concentrations: 25%, 50%, and 75%, with an initial dose of 400 mg/kgBW, with the first dose being 100 mg/kgBW (0.2 grams), the second dose being 200 mg/kgBW (0.4 grams), and the third dose being 300 mg/kgBW (0.6 grams). The ethanol extract of guava leaves is weighed, then placed in a mortar and Na-CMC 0.5% suspension is added little by little while grinding until homogeneous. The suspension is then placed in a 50 ml vial.

**Kencur Rhizome (*Kampferia galanga*)**

The PGEE suspension was made with 3 concentration variants: 25%, 50%, and 75%. with an initial dose of 27.5 mg/kgBW with the first dose of 6.8 mg/kgBW amounting to 0.6 grams, the second dose of 13.75 mg/kgBW amounting to 0.017 grams, and the third dose of 20.6 mg/kgBW amounting to 0.04 grams. The guava leaf ethanol extract was weighed, then placed in a mortar and gradually mixed with 0.5% Na-CMC suspension while grinding until homogeneous. The suspension was then placed in a 50 ml vial.

**Combination Of Guava Leaf Ethanol Extract And Kencur Rhizome**

PGEE and KGEE suspensions were made with three concentration ratios: 25%:75%, 50%:50%, and 75%:25%.
Example: for the 25%:75% concentration ratio group.

The body weight of the mouse is 20 grams and the dosage volume is 0.5 ml, so 0.5 ml is taken from the 25% concentration PGEE suspension and 0.5 ml from the 75% concentration KGEE suspension, then homogenized.

**Testing the Antidiarrheal Effects of PGEE Combined with KGEE**

This experiment used 25 male mice that were randomly divided into 5 treatment groups. Before the experiment began, the mice were fasted for 18 hours but were still given water.

The study consisted of five treatment formulations. Formulation 1 (F1) served as the negative control using NaCMC, while Formulation 2 (F2) was the positive control using Loperamide. Formulations 3 to 5 contained combinations of PGEE and KGEE at different ratios, namely F3 (25%:75%), F4 (50%:50%), and F5 (75%:25%). PGEE refers to the ethanol extract of guava leaves, whereas KGEE refers to the ethanol extract of kencur rhizome.

The mice were grouped and treated according to the grouping section and given treatment according to the grouping section and dosage. Thirty minutes after treatment, diarrhea was induced by administering 0.5 ml of castor oil to each mouse. The test animals were then placed in transparent cages (mouse chambers) with filter paper bases that had been weighed beforehand and observed every 30 minutes for 8 hours after castor oil administration. The parameters observed included the onset of diarrhea, the duration of diarrhea, the frequency of mouse feces, and the weight of feces. In this experiment, the observation of the antidiarrheal effect was carried out until the experimental animals recovered [14].

**Data Analysis**

The ratio values were then averaged for each group, and the values of each group were compared with those of the other groups. The observation data were analyzed statistically using the ANOVA (analysis of variance) method. This statistical analysis was performed using the SPSS (Statistical Product and Service Solution) program [13].

## 3. RESULTS

In this study, mice (Mus musculus) were used as test animals to test the antidiarrheal effect. Male mice were chosen to minimize biological variations related to fluctuating hormonal influences that could reduce the accuracy of data analysis, and male mice are known to be more stable than female mice [15]. The diarrhea inducer used in this study was castor oil (oleum ricini). Castor oil is an irritant or stimulant laxative. In the small intestine, castor oil is hydrolyzed by the enzyme lipase into glycerol and ricinoleic acid, a local irritant that increases motility. It works quickly and continues until the compound is excreted through the column. Ricinoleic acid is the active ingredient. Castor oil also has emollient properties. As a laxative, this medicine is no longer widely used because there are many other safer medicines available. Castor oil causes colic and dehydration accompanied by electrolyte disturbances [15].

**Characteristic Test Results**

**Results of Macroscopic Testing of Crude Drugs And Extracts**

Macroscopic examination was conducted by observing the external morphology, namely the length, width, color, smell, taste, and shape of guava leaves (*Psidium guajava* L.) and galangal rhizomes (*Kaempferia galanga*)

Table 1. Results of Macroscopic Observation of Simplisia and Guava Seed Extract

| Sample | Organoleptic | | weight | % yield |
|---|---|---|---|---|
| Guava leaf simplisia | inspection<br>colour<br>smell<br>taste | : fine powder form<br>: dark green<br>: distinctive<br>: bitter | 400 grams | 40 % |
| Guava Leaf Ethanol Extract | inspection<br>colour<br>smell<br>guava<br>taste | : bentuk kental<br>: blackish green<br>: characteristic of<br><br>: bitter | 18 grams | 18 % |

Table 2. Results of Macroscopic Observation of Simplisia and Extracts of Kencur Rhizome

| Sample | Organoleptic | weight | % yield |
|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Simplisia kencur rhizome | inspection<br>colour<br>smell<br>taste | : fine powder form<br>: yellow<br>: distinctive<br>: spicy | 500 grams | 50% |
| Ethanol extract of kencur rhizome | inspection<br>colour<br>smell<br>taste | : thick<br>: blackish yellow<br>: distinctive<br>: spicy | 11 grams | 11% |

**Results Of Moisture Content Measurement**

Moisture content determination aims to ensure the quality of raw materials so that they are not overgrown with mold or other microorganisms. The method used is gravimetry because it is simple and cost-effective, by heating until all the water in the extract separates. The results of the study showed that the moisture content of guava leaf simplisia was 10% and that of kencur rhizome was 9%, both of which met the requirements as they did not exceed thez 10% limit.

- Guajava leaf

% water content $= \frac{(initial\ weight - weight\ after\ drying)}{initial\ weight} 100\ \%$

$= \frac{(2\ gram - 1{,}8\ gram)}{2\ gram}$

$= 10\ \%$

- kencur rhizome

% water content $= \frac{(initial\ weight - weight\ after\ drying)}{initial\ weight} 100\ \%$

$= \frac{(2\ gram - 1{,}820\ gram)}{2\ gram} x\ 100\%$

$= 9\ \%$

**Water-Soluble Extract Content Results**

The determination of water-soluble extract content aims to find out the amount of polar compounds that can be extracted. The results show that guava leaves contain 16% and kencur rhizomes contain 15%, both of which meet the Indonesian Herbal Pharmacopoeia requirements (⩾11.5%), so they can be declared suitable for use.

- Guajava leaf

% water solubility $= \frac{specific\ gravity}{Initial\ weight} x100\%$

$= \frac{0{,}8\ gram}{5\ gram} x\ 100\%$

$= 16\%$

- Kencur rhizome

% water solubility $= \frac{specific\ gravity}{Initial\ weight} x100\%$

$= \frac{0{,}75\ gram}{5\ gram} x100\% = 15\%$

**Ethanol Solubility Levels**

Determining the ethanol extract content aims to find out the amount of polar and non-polar compounds extracted. The results show that guava leaves contain 15% and kencur rhizome contains 16%, both of which meet the Indonesian Herbal Pharmacopoeia requirements (>10–15%). The higher content in kencur rhizome is acceptable because ethanol is more effective at extracting semi-polar compounds.

- Guajava leaf

% solubility in ethanol $= \frac{specific\ gravity}{initial\ weight} x100\%$

$= \frac{0{,}75\ gram}{5\ gram} x100\%$

= 15%

- Kencur rhizome

% solubility in ethanol $= \frac{specific\ gravity}{initial\ weight} x 100\%$

$= \frac{0{,}8\ gram}{5\ gram} x 100\%$

= 16%

**Phytochemical Screening Results**

Phytochemical screening aims to identify active compounds or secondary metabolites found in plants. Results of phytochemical screening tests on guava leaves (*Psigium Guajava* L.) and kencur rhizome (*Kaempferia galanga*).

Table 3. Results of Phytochemical Screening Tests of Guava Leaf Concentrate Extract (*Psidium guajava* L.)

| **No.** | Compound group | Guava leaf crude drug content | **Result** |
|---|---|---|---|
| 1 | Alkaloids | (+) | - Maeyer (white precipitate) |
| | | (+) | - Wagner (yellow precipitate) |
| | | (-) | - Dragendorff (no red precipitate) |
| 2 | Terpenoids/steroids | (+) | - Liberman-buchar (blue/green ring layer) |
| 3 | Flavonoid | (+) | - Mg+HCL (p) (dark greenish-yellow) |
| 4 | Saponins | (+) | - Foam forms to a height of ± 1 cm |
| 5 | Tannins | (+) | - Blackish green |

Table 4. Results of Phytochemical Screening Tests of Concentrated Extracts of Kencur Rhizome (*Kaempferia galanga*).

| **No.** | Compound group | Simplisia rhizome content | **result** |
|---|---|---|---|
| 1 | Alkaloids | (+) | - Maeyer (white precipitate) |
| | | (+) | - Wagner (brown precipitate) |
| | | (-) | - Dragendorff (orange) |
| | | (+) | - Bouchardart (brown) |
| 2 | Terpenoids/steroids | (-) | - Liberman-buchar (no blue ring formed) |
| 3 | Flavonoid | (+) | - Mg+HCL (p) (bubbles appear and turn orange) |
| 4 | Saponins | (+) | - Stable white foam |
| 5 | Tannins | (+) | - Blackish green |

Description:

(+) Contains Compounds

(-) Does not Contain Compounds

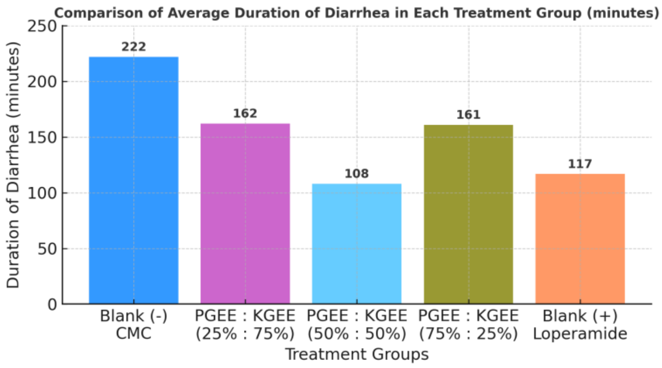
**Results Of Antidiarrheal Effect Testing**

Table 5. Summary of the Results of the Antidiarrheal Effect Test of the Combination of Guava Leaf Extract (*Psidium guajava* L.) and Kencur Rhizome (*Kaempferia galanga*) on Male Mice

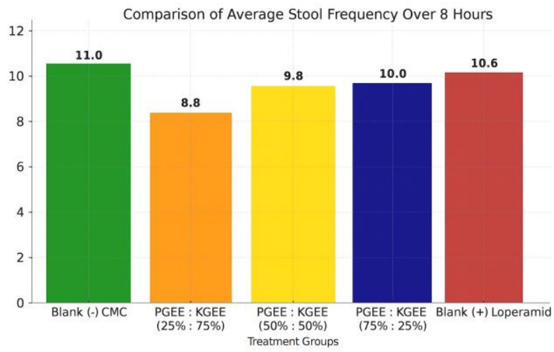
| Parameters | Formulation 1 (F1) | Formulation 2 (F2) | Formulation 3 (F3) | Formulation 4 (F4) | Formulation 5 (F5) |
|---|---|---|---|---|---|
| onset of diarrhea (Menit) | 46.8 | 80.4 | 72.6 | 90.4 | 64.2 |
| duration of diarrhea | 222 | 117 | 162 | 108 | 161 |

| | | | | | |
|---|---|---|---|---|---|
| (Menit) | | | | | |
| stool frequency (Kali/8jam) | 11 | 10.6 | 8.8 | 9.8 | 10 |
| stool weight (Gram) | 1,14 | 0.82 | 1.12 | 0.80 | 0.81 |

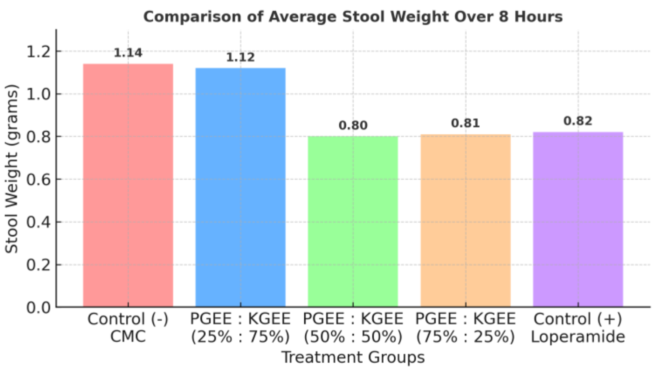
There was a difference in the average time of onset of diarrhea in each treatment, with the negative control (0.5% Na-CMC) showing the fastest response at 46.8 minutes because it did not contain any active antidiarrheal ingredients. The combination of guava leaf extract and kencur rhizome was most effective at a balanced ratio (50%:50%) with a time of 90.4 minutes, presumably due to the tannin and flavonoid content that plays a role in inhibiting diarrhea. As a comparison, the positive control (loperamide) showed a time of 80.4 minutes, so the effectiveness of the balanced combination is close to that of the standard drug, even though the mechanism of action of herbal extracts is not yet fully understood [16].




Graph 2. Comparison Of The Average Duration Of Diarrhea In Each Treatment (Minutes)




Graph 3. Comparison Of The Average Frequency Of Diarrhea In Each Treatment Over 8 Hours (Times)



Graph 4. Comparison of average fecal weight for each treatment over 8 hours (grams)

Table 6. Summary of Tukey's Post Hoc Test Results on the Antidiarrheal Effects of a Combination of Guava Leaf Extract (*Psidium guajava* L.) and Kencur Rhizome (*Kaempferia galanga*) in Male Mice

| Parameters | Kelompok Perlakuan | Average | **Subset homogen** (α=0,05) |
|---|---|---|---|
| Onset of Diare (Menit) | control negative (NaCMC) | 48.6 | a |
| | Combination PGEE:KGEE (25% : 75% ) | 64.2 | b |
| | Combination PGEE:KGEE (50% : 50%) | 90.4 | b |
| | Combination PGEE:KGEE (75% : 25% ) | 71.6 | b |
| | Control positive (loperamide) | 80.4 | b |
| duration of diarrhea (Menit) | control negative (NaCMC) | 222.0 | a |
| | Combination PGEE:KGEE (25% : 75%) | 161.0 | a |
| | Combination PGEE:KGEE (50% : 50%) | 110.0 | b |
| | Combination PGEE:KGEE (75% : 25%) | 162.0 | b |
| | Control positive (loperamide) | 117.0 | c |
| stool frequency (Kali/8jam) | control negative (NaCMC) | 11.0 | a |
| | Combination PGEE:KGEE (25% : 75% ) | 8.8 | ab |
| | Combination PGEE:KGEE (50% : 50%) | 9.8 | ab |
| | Combination PGEE:KGEE (75% : 25%) | 10.0 | b |
| | Control positive (loperamide) | 10.6 | b |
| stool weight (Gram) | control negative (NaCMC) | 1.14 | a |
| | Combination PGEE:KGEE (25% : 75%) | 1.12 | b |
| | Combination PGEE:KGEE (50% : 50% ) | 0.80 | b |
| | Combination PGEE:KGEE (75% : 25%) | 0.81 | b |
| | Control positive (loperamide) | 0.82 | b |

Note: Data are presented as means. Different letters in the Homogeneous Subset column indicate statistically significant differences between treatment groups (Tukey HSD test, $p < 0.05$).

## 4. PEMBAHASAN

### Results of Macroscopic Testing of Crude Drugs And Extracts

Macroscopic observation showed that guava leaf simplisia was a fine dark green powder with a distinctive odor and bitter taste, while its ethanol extract was thick, blackish green, and had an 18% yield. Galangal rhizome simplisia was a fine yellow powder with a distinctive odor and spicy taste, whereas its ethanol extract was thick, dark yellow, and had an 11% yield. Overall, both materials retained their characteristic organoleptic properties after extraction, despite changes in weight and form due to the removal of active compounds by ethanol.

### The phytochemical content

Phytochemical screening of ethanol extracts from guava leaves and kencur rhizomes revealed alkaloids, flavonoids, tannins, saponins, and terpenoids, which contribute to antidiarrheal activity. Alkaloids help suppress intestinal peristalsis and provide antibacterial effects, flavonoids reduce intestinal motility, tannins decrease electrolyte secretion, while saponins and terpenoids act as antimotility, antisecretory, and antimicrobial agents. These combined secondary metabolites support the antidiarrheal potential of both plants. The onset of diarrhea differed among treatments. The negative control (0.5% Na-CMC) showed the fastest onset at 46.8 minutes due to the absence of active compounds. The most effective treatment was the balanced combination of guava leaf extract and kencur rhizome (50%:50%), with an onset time of 90.4 minutes, likely due to the synergistic effects of tannins and flavonoids. This result was close to the positive control, loperamide, which showed an onset time of 80.4 minutes. [16].

The results show that the negative control group (CMC) had the longest duration of diarrhea, namely 222 minutes, indicating that there were no active compounds that could stop diarrhea. In contrast, the combination of PGEE:KGEE (F4) produced the shortest duration, 108 minutes, which was even shorter than the positive control (loperamide, 117 minutes). This confirms the potential of the extract combination to match the effectiveness of standard drugs. The (F4) ratio was found to be the most effective because the two active substances complement each other's antidiarrheal mechanisms, such as antimotility and adsorbent effects that work together. Meanwhile, the (F3) and (F5) combinations showed a duration of around 160 minutes, remaining effective but not as good as the balanced combination [17].

**Antidiarrheal Effect Testing**

The negative control group (0.5% CMC) showed the highest bowel movement frequency, averaging 11 times in 8 hours, due to the absence of active antidiarrheal compounds. In contrast, treatment with the combination of guava leaf extract and kencur rhizome reduced stool frequency as the dose increased, likely because of tannins, flavonoids, and alkaloids [18]. The most effective ratio was PGEE:KGEE (50%:50%), with results approaching the loperamide group, while the 75%:25% ratio was less effective but still better than the negative control. These findings confirm the potential of the extract combination as a natural antidiarrheal agent.

The negative control group (CMC-Na) showed the highest feces weight (1.14 g), indicating severe diarrhea due to the absence of active compounds. The PGEE:KGEE combination (25%:75%) showed a similar result (1.12 g), indicating low antidiarrheal activity. In contrast, the balanced combination (50%:50%) produced the lowest feces weight (0.80 g), comparable to loperamide (0.82 g), and was the most effective treatment [19].

This effectiveness is likely due to the balanced secondary metabolites of both plants. Guava leaves are rich in tannins that reduce intestinal fluid secretion, while kencur rhizomes contain flavonoids that decrease intestinal motility and provide antibacterial effects. Their complementary actions create a synergistic effect, resulting in better antidiarrheal activity than unbalanced formulations [20,21].

**Statistical analysis**

Statistical analysis results show that the negative control group (NaCMC) had significant differences from the treatment group in all parameters. The onset of diarrhea in the negative control group occurred faster than in the extract combination group and the positive control group ($p<0.05$). Meanwhile, the extract combination at various concentration ratios showed no significant difference from the positive control (loperamide), particularly in terms of onset and stool weight. This indicates that the combination of guava leaf and kencur extracts can delay the onset of diarrhea and reduce stool weight with efficacy comparable to loperamide [22].

In terms of diarrhea duration, the positive control group showed a significant difference compared to all combination groups, indicating that loperamide still has a stronger effect in shortening the duration of diarrhea. However, the frequency of bowel movements and stool weight in the extract combination did not differ significantly from the positive control, especially in the 75%:25% ratio, which produced results close to those of loperamide. Overall, statistical tests proved that the extract combination had a significant effect on improving diarrhea symptoms compared to the negative control and had the potential for an antidiarrheal effect close to that of loperamide [23,24].

## 5. CONCLUSION

This study proves that the combination of guava leaf extract (*Psidium guajava* L.) and galangal rhizome (*Kaempferia galanga*) has anti-diarrheal activity in male mice induced by oleum ricini. The combination with a 50%:50% ratio showed the most optimal effect in delaying the onset of diarrhea, shortening its duration, and reducing the frequency and weight of feces, with results comparable to the positive control (loperamide). Thus, the combination of guava leaf extract and galangal rhizome has the potential to be developed as a natural-based antidiarrheal therapy candidate.

## ACKNOWLEDGMENT

The author would like to thank colleagues, supervisors, and examiners who provided input during the writing of this journal.

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