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Original Article

ANTIDIARRHEAL ACTIVITY OF EXTRACT ETHANOL MELINJO LEAVES (*GNETUM GNEMON* L. (LINN.)) IN WISTAR MALE WHITE RATS INDUCED BY *ESCHERICHIA COLI* AND EXTRACT STANDARDIZATION

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ABSTRACT

Objective: Determination of the antidiarrheal activity of extract ethanol melinjo leaves (*Gnetum gnemon* L. (Linn.)) in Wistar male white rats induced by *Escherichia coli* and extract standardization has been performed.

Methods: This research was conducted by *in vivo* method using white male rats of Wistar strain induced by *Escherichia coli*. The treatment group was divided into 6 groups: normal, negative control, positive control, and groups with doses 150, 300, and 600 mg/kg BW. Negative control was given Na-CMC and positive control Gentamicin. The initial time diarrhea occurred 24-30 h after administration of *Escherichia coli* suspension.

Results: The results of the phytochemical screening of the ethanolic extract of melinjo contain secondary metabolites of flavonoids, steroids, tannins, and saponins. Standardization of the extract ethanol melinjo leaves (*Gnetum gnemon* L.) meet the predetermined standards, while the acid insoluble ash content parameters did not meet the predetermined standards. The parameters feces weight, feces consistency, frequency diarrhea, body weight, number of *Escherichia coli* colonies feces dose 600 mg/kg BW had an effect that was almost close to positive control. The results showed that anti-diarrheal effect dose 150 mg/kg BW 35.75%, dose 300 mg/kg BW 43.02%, dose 600 mg/kg BW 50.14%. This shows that ethanol extract melinjo leaves dose 600 mg/kg BW wasn't significantly different from the positive control ($p<0.05$). Effective Dose (ED_{50}) Ethanol Extract Melinjo Leave as antidiarrheal was 578.2468 mg/kg BW.

Conclusion: Extract ethanol melinjo leaves (*Gnetum gnemon* L. (Linn)) dose 600 mg/kg BW has the potential to be used as antidiarrheal and its extract meet the predetermined standards, except the acid insoluble ash content parameters did not meet the predetermined standards.

Keywords: Melinjo leaves, Antidiarrheal, *Escherichia coli*, Effective dose 50

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INTRODUCTION

Diarrhea is an increase in frequency and decreased consistency of feces when compared to normal individuals, in other words, diarrhea is loose or liquid bowel movements that occur three or more times within 24 h [1]. In Indonesia, diarrhea is the second leading cause of death among children under five and number five for all ages. One of the side effects of diarrhea is the body experiencing dehydration (loss of fluids and electrolytes) so that if it is not addressed it can immediately cause death [2]. One of the causes of diarrhea is a bacterial infection. *Escherichia coli* bacteria are the bacteria that most often cause diarrhea with a ratio of 19% [3]. *Escherichia coli* bacteria become pathogenic when the number of these bacteria in the digestive tract increases. Modern medicine in the form of antibiotics to fight *Escherichia coli* infection has developed rapidly.

The irrational use of antibiotics in various fields of medical science is one of the causes of resistance that can be obtained. The use of natural ingredients as traditional medicine can be an alternative to the use of antibiotics [4]. One of the plants that have antibacterial properties is the leaves of Melinjo (*Gnetum gnemon* L.). Melinjo leaves have many benefits in the health sector, such as preventing cancer, lowering blood sugar, as an antioxidant, high nutritional food, inhibiting the aging process, antibacterial [5]. Melinjo contains active compounds such as alkaloids, flavonoids, saponins, and tannins [6]. Flavonoid compounds as antibacterial can damage bacterial cell membranes, followed by the release of bacterial intracellular compounds [7]. Alkaloid compounds as antibacterials work by disrupting the constituent components of the cell wall layer so that they are not formed completely [8]. Tannin compounds have antibacterial power by inhibiting the reverse transcriptase and DNA topoisomerase enzymes so that bacterial cells cannot be formed.

Based on the results of observations, a concentration of 60% was able to inhibit *Escherichia coli* bacteria in a maximum inhibition zone of 11 mm with the category of strong inhibition [6]. In this study, the researchers wanted of antidiarrheal activity of extract ethanol melinjo leaves (*Gnetum gnemon* L. (Linn.)) in Wistar male white rats induced by *Escherichia coli* and extract standardization.

MATERIALS AND METHODS

Plant material

The materials used in this research consisted of melinjo leaves (*Gnetum gnemon* L.). The sample used was the leaves of the melinjo plant (*Gnetum gnemon* L.) with wet weight of 3.2 kg obtained from the Inderalaya region, South Sumatra, Indonesia. The plant determination of the melinjo leaves (*Gnetum gnemon* L.) is carried out with the aim of macroscopically identifying the truth of the plant to be used. Determination was carried out at the Laboratory of the Plant Conversion Center of the Purwodadi Botanical Gardens, Purwodadi, Pasuruan, East Java, Indonesia (No. 0044/IPH.06/HM/I/2019).

Chemical and reagent

Male white rat Wistar strain (Abduh Tikus Palembang), ethanol 70% (Dira Sonita®), concentrated hydrochloric acid (Brataco®), sodium acetate (Brataco®), filter paper (Whatman®), Mayer reagent (Paison®), Wagner reagent (Paison®), Liebermann-Bourchard reagent (Paison®), Dragendrof reagent (Paison®), metal magnesium (Brataco®), aluminum chloride (Brataco®), iron (III) chloride (Merck®), aluminum foil, standard rat food, and Gentamicin injection.

Preparation of extract ethanol melinjo leaves

Extract Ethanol Melinjo leaves were made using the maceration method. A total of 1 kg of Melinjo leaf powder was extracted by the maceration method for 3 x 24 h using 5 L of 70% ethanol solvent. Remaceration was carried out after 72 h by replacing the solvent of 2.5 l 70% ethanol for 3 x 24 h. The next remaceration is carried out in the same way as the first remaseration. Stirring is done 2 times a

day to achieve a saturated state. The resulting macerate was then filtered with Whatman filter paper. The resulting filtrate was then concentrated with a rotary evaporator at a temperature of 60 °C until a thick extract is obtained [9].

Phytochemical screening

Preliminary examinations of secondary metabolite compounds on 70% ethanol extracts of melinjo leaves included flavonoid, alkaloid, steroid, triterpenoid, tannin, and saponin [10].

Determination of levels tannins totals

Catechin standard mother liquor is prepared at a concentration of 100 ppm by weighing 1 mg of standard catechin in ethyl acetate pa up to 10 ml. Dilution was carried out with various concentrations of 10, 20, 30, 40, and 50 ppm. The absorbance was measured with a UV-Vis spectrophotometer at its maximum wavelength, then a calibration curve and a regression equation were made. Determination of total sample tannin content was carried out by weighing 1 mg of the sample, then dissolving it in ethyl acetate pa up to 10 ml. The absorbance was measured with a UV Spectrophotometer at the maximum wavelength of catechins (279 nm) [11].

Extract characterization

Determination extract characterization of extract ethanol of melinjo leaves includes specific parameters: organoleptic (form, color, smell, and taste), water-soluble content, and ethanol soluble content. The nonparameters: water content, shrinkage on drying, total ash content, acid insoluble ash content, microbial contamination test, and metal contamination test [12].

Determination of the density of the number of bacteria *Escherichia coli*

McFarland 0.5 standard solution was made to be compared with the *Escherichia coli* bacterial suspension that has been prepared. The standard solution of 0.5 McFarland standard is made by pipetting a 1% $BaCl_2$ solution of 0.05 ml, then inserting it into a test tube. Then pipette also 1% H_2SO_4 solution of 9.95 ml, then mix it into a test tube that already contains 1% $BaCl_2$ solution. The solution is vortexed until it is completely mixed or homogeneous. Measurement of the absorbance value of the test run and the absorbance value of the McFarland 0.5 standard solution was tested by UV-Vis spectrophotometry at a wavelength of 625 nm. The result of the absorbance value of the test must be close to the absorbance value of the McFarland solution of 0.5 so that it is at the bacterial density of 1.5 x 10^8 CFU/ml [13].

Preparation and treatment of test animals

The research had approval for the methodology and concerning ethical issues by Ethical Approval No 022011028 Komite Etik Penelitian Univ Ahmad Dahlan (KEP UAD) 30 December 2020. The tested animals were weighed and grouped randomly into 6 groups, each group consisting of 5 Wistar rats. The test animals were acclimatized for 1 w at room temperature and then given enough food and drink. The rats have fasted for 18 h before the study was carried out. Rats in the negative control, positive control, group 1, group 2, and group 3 were induced by a single dose of *Escherichia coli* suspension 5 ml/kg BW orally and waited until a diarrhea response occurred which was marked by the discharge of stool with a liquid consistency at least three times after giving [14].

Furthermore, 30 rats were divided into 6 groups of treatment as follows:

a. Normal control: without treatment.

b. Negative control: only given 0.5% NaCMC suspension.

c. Positive control: given Gentamicin 8 mg/kg BW intraperitoneally.

d. Group I: Melinjo leaf ethanol extract suspension 150 mg/kg BW orally

e. Group II: Melinjo leaf ethanol extract suspension 300 mg/kg BW orally

f. Group III: Melinjo leaf ethanol extract suspension 150 mg/kg BW orally.

Antidiarrheal testing parameters

Determination antidiarrheal testing parameters included: onset diarrhea occurs [15], feces weight [15], feces consistency [16, 17], diarrhea frequency [15, 17], and change in rat body weight [14].

Antidiarrheal effects

The weight of the liquid or irregular stool can be used to calculate the %antidiarrheal effect. This antidiarrheal effect parameter can be done by calculating the percentage of the anti-diarrheal effect with the formula [18].

The number of *Escherichia coli* bacterial colonies in rat feces

The rat feces test was carried out on EMBA (Eosin *Methylene Blue Agar*) media carried out on the last day of acclimatization as a preliminary test to determine that the mice were in good health, when the rats had diarrhea, and when the rats recovered from diarrhea. 1 g of feces is taken and then diluted with 10 ml of sterile distilled water. 10 ml of fecal solution, then diluted from 10^{-1} to 10^{-6} into 9 ml of distilled water. Taken 1 ml from 10^{-5} and 10^{-6} dilutions then tested on EMBA (a Methylene Blue Agar) medium and incubated for 24 h at 37 °C. This test is carried out on all tested animals [19]. Colonies of growing *E. coli* are characterized by a metallic green colony with a mucoid shape and a dark center. The number of Coliform and E. coli colonies was calculated on the number of colonies [20].

Effective dosage determination (ED_{50})

The effective dose (ED_{50}) is the dose of a drug that can provide a therapeutic response in 50% of the population to be tested. The effective dose value (ED_{50}) can be calculated using a linear regression equation based on the relationship between the extract concentrations analyzed using the formula: y = a+bx

RESULTS AND DISCUSSION

Phytochemical extract screening test

Phytochemical screening on the melinjo leaves ethanolic extract has the aim to determine the presence of secondary metabolite compounds contained in the extract. The results of the phytochemical screening of the extract ethanol melinjo leaves are shown in table 1. Based on the results of the phytochemical screening of the melinjo leaves ethanolic extract in the table above, it shows that the positive extract contains flavonoids, steroids, tannins, and saponins, and negative alkaloids and terpenoids. Melinjo contains active compounds such as alkaloids, flavonoids, saponins, and tannins [6].

Table 1: Results of phytochemical screening for ethyl acetate fraction of melinjo leaves

Compound group	Result
Flavonoids	+
Alkaloids	
-Mayer	-
-Wagner	-
-Dragendorf	+
Steroids	+
Triterpenoids	-
Tannins	+
Saponins	+

Determination of levels tannins total

Determination of total tannin content in extract ethanol melinjo leaves was carried out because the active compound that is effective in dealing with diarrhea is tannin compounds which act as astringents. Determination of total tannin content was carried out to determine the percentage of the content of tannin compounds contained in extract ethanol melinjo leaves. Scanning the catechin wavelength was obtained at 279 nm. Testing of tannin content in melinjo leaves carried out at a concentration of 100 ppm obtained a total tannin of 67.1538 mg/gCE or 6.7% [11].

Extract characterization

The results of characterization of the extract ethanol melinjo leaves with materia medika requirements were obtained as in table 2.

Table 2: Characterization of melinjo leaves ethanolic extract

Parameter	Result (mean±SD)	Requirements for the ministry of health of the republic of Indonesia (2008)
Specific parameters:		
-Organoleptic	Form: Thick Color: Blackish green Smell: Extract Taste: Bitter	-
-Water-soluble content (%)	53.33±10.4083	>31%
-Ethanol soluble content (%)	78.33±2.8868	>70.5%
Non-specific parameters:		
-Water content (%)	8±1.4142	≤10%
-Shrinkage of drying (%)	9.11±1.4905	<11%
-Total ash content (%)	4±1	≤16.6%
-Acid insoluble ash content (%)	2.675±0.9768	≤ 0.7%
-Microbial contamination test	65 CFU/g	1 × 104 CFU/g
-Metal contamination test (mg/kg)	Pb metal: 0	≤ 10 mg/kg

Note: Data was given in mean+SD, n = 3

Organoleptic extract ethanol melinjo leaves show that extract has a thick extract form, blackish green color, the distinctive odor of the extract, and a chelish taste. Ethanol extract of melinjo leaves obtained has a slightly pungent odor and a bitter taste. The blackish-brown color is obtained due to changes in plant color when dried and soaked with a liquid finder [7].

The drying shrinkage test is carried out to maintain the quality of the sample from possible mold or fungal growth in the sample. The result of the drying shrinkage test of the sample shows the percent solvent content, water, and essential oils in the sample which evaporated after heating at 105 °C. Drying shrinkage of extract ethanol melinjo leaves was obtained that percent depreciation of drying amounted to 9.11% was qualify. Examination of water content is done to find out the percentage of the water content contained in the sample so that it can guarantee quality from the sample the. The water content of melinjo leaves ethanolic extract is 8% qualify. It is known that the drying shrinkage value of ethanol extract of melinjo leaves is 26.39±1.91%. Based on the literature, this result does not meet the requirements ie; the extraction loss of the extract does not exceed 12%. This can be due to the high content of essential oils in the extract [7].

Total ash content and acid insoluble ash content on The extract ethanol melinjo leaves was obtained; a percentage ash content of total amounting to 4% qualify and ash content insoluble acid amounting to 2.6725% was not qualify. This is because the processing of melinjo leaves in the wet washing and sorting stages is not clean enough, resulting in a large number of silicates originating from sand or soil. In addition, there may be elements of silver metal (Ag) or mercury (Hg) contained in the extract. The results of the determination of total ash content in the ethanol extract of melinjo leaves were 12.43±0.05% and acid insoluble ash content was 2.277±0.01%. High levels of total ash and acid insoluble ash are caused by the environment in which plants grow in places prone to smoke so there is a lot of copper, lead, and other substances [7].

There is a water-soluble compound obtained equal to 53.33% and the content of soluble compounds in ethanol is equal to 78.33%. This shows that the semi-polar compounds contained in melinjo leaves are more than the polar compounds. Ethanol extract of melinjo leaves obtained water-soluble extract content was 85.00±11.13%, while ethanol-soluble extract content was 81.12±15.50%. This value indicates that the content of a water-soluble extract is greater than the concentration of soluble ethanol. This shows that more compounds are found which are dissolved in water [7].

Based on the test results, microbial contamination shows that the extract ethanol melinjo leaves were not existed microbial contamination that stated by value 65 cfu/g. This shows that melinjo leaves ethanolic extract did not contain pathogenic and non-pathogenic microbes, so that safe extract can be used as raw material for traditional medicine. Based on the test results obtained Pb metal contamination of 0.00 mg/kg. This shows that the melinjo leaves ethanolic extract is safe and non-toxic so that it can be used as a raw material for medicine.

Determination of the density of *Escherichia coli bacteria*

Determination of the density of *Escherichia coli* bacteria was carried out by comparing the turbidity in the suspension solution obtained with the McFarland standard solution of 0.5 OD. The McFarland standard solution is used as a reference to adjust the bacterial turbidity of the suspension so that the number of bacteria is within the range given to standardizing the test microbes [21]. The 0.5 OD McFarland solution has a density of 1.5 x 10^8 cfu/g. The obtained bacterial suspension solution has the same turbidity as the McFarland solution of 0.5 OD, so the value of the density of the *Escherichia coli* bacterial suspension is 1.5 x 10^8 cfu/g. The density value or the number of bacterial cells obtained is close to the density value or the number of bacterial cells of the Escherichia coli bacterial suspension, namely 2 x 10^8 cfu/g, which can cause diarrhea in rats [14].

Antidiarrheal activity test

Early time diarrhea occurs

Diarrhea is characterized by defecating where the frequency increases from normal and the consistency of the stool becomes softer and more fluid because it contains a lot of water. The initial time of occurrence of diarrhea in the mice studied occurred at 24 h to 30 h after administration of the *Escherichia coli* bacterial suspension.

Feces weight

The weight of the feces in rats at the beginning of the observation was higher because the feces contained more water than normal, which is a sign of diarrhea. The effect of healing diarrhea is one of which is marked by a decrease in fecal weight [17].

In the graph above, it is found that in the normal control group, the weight of the rats did not increase and decrease significantly. This is because the normal group is only given NaCMC, which expands in the presence of water so that the feces that are excreted do not contain a lot of water and do not experience diarrhea. Meanwhile, in the positive control group and the test group, there was a significant increase and decrease in fecal weight. In the negative control group there was a very high increase in fecal weight at the 8th hour and only experienced a decrease in fecal weight after several hours or days. This is because the negative group was not given anti-diarrheal drugs, but only NaCMC was given after the induction of *Escherichia coli* bacteria. The positive control group experienced a faster decrease in fecal weight than the test group. However, in the test group with the highest dose, 600 mg/kg BW experienced a decrease in fecal weight almost closer to the positive control group. This shows that the test group with a dose of 600 mg/kg BW has almost the same diarrhea cure effect as the positive control group. The content of tannin compounds in a dose of 600 mg/kg BW is more than the doses of 150 and 300 mg/kg BW. Tannins act as astringents where these substances will cause the tightness and shrinkage of the cell layer, thereby inhibiting tissue secretion [22]. Based on the results of in the study, the fecal weight parameters obtained were comparable to the results study, where the larger the dose used, the faster the stool weight decreased and at the largest dose, the stool weight decreased, which was almost close to the positive control group [16].

Feces consistency

Feces consistency is determined by scoring the feces; namely, a score of 1 for normal feces, score 2 for slightly soft feces, score 3 for very soft feces, and score 4 for soft liquid feces. The smaller the feces consistency value, the greater the anti-diarrhea effect produced [23].

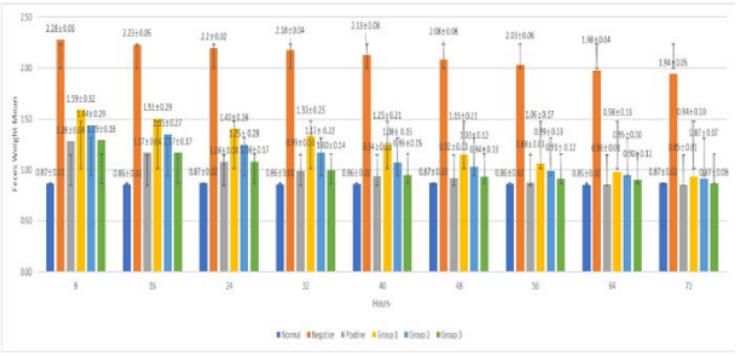

Fig. 1: Graph of the average weight of the test animal feces (n = 5)

Table 3: Average feces consistency of test animal

Hours-	Mean and±SD value of feces consistency					
	Normal	Negative	Positive	Group 1	Group 2	Group 3
8	1.00±0.00	2.80±0.84	2.80±0.84	3.20±0.84	3.20±0.84	2.80±0.84
16	1.00±0.00	2.80±0.45	2.60±0.55	2.80±0.45	2.60±0.55	2.80±0.45
24	1.00±0.00	2.60±0.55	2.00±0.71	2.80±0.45	2.40±0.55	2.00±0.00
32	1.00±0.00	2.40±0.55	1.60±0.89	2.20±0.84	2.20±0.84	1.60±0.89
40	1.00±0.00	2.00±0.71	1.80±0.84	1.80±0.45	1.80±0.45	1.60±0.55
48	1.00±0.00	1.80±0.45	1.20±0.45	1.80±0.45	1.60±0.55	1.20±0.45
56	1.00±0.00	1.60±0.55	1.20±0.45	1.60±0.55	1.40±0.55	1.20±0.45
64	1.00±0.00	1.20±0.45	1.00±0.00	1.20±0.45	1.20±0.45	1.00±0.00
72	1.00±0.00	1.20±0.45	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00

Note: data was given in mean+SD, n =5

The positive control group showed that the consistency of the stool changed back to the normal stool at 64 h. The extract ethanol melinjo leaves also had an effect on changing the consistency of feces to normal or healing diarrhea in rats. The group of extract ethanol melinjo leaves at a dose of 150 mg/kg BW and 300 mg/kg BW experienced a change in fecal consistency at 72 h. Meanwhile, the group of extract ethanol melinjo leaves at a dose of 600 mg/kg BW experienced a change in the consistency of feces at the 64th h. This shows that the extract ethanol melinjo leaves at a dose of 600 mg/kg BW has almost the same effect as the positive control group.

Changes in stool consistency in the test group at a dose of 600 mg/kg BW returned to normal stool faster than the test group at a dose of 150 and 300 mg/kg BW. This is because the tannin content in the 600 mg/kg BW dose may be higher than the 150 and 300 mg/kg BW doses. Tannin compounds have an antidiarrheal effect because they are astringents whose effects can precipitate proteins on the intestinal surface [24]. This situation can form a layer formation (barrier) on the surface of the gastrointestinal tract so that it makes the intestinal surface more resistant [25]. The layer (barrier) also causes the outermost cell to close, thereby inhibiting the secretion of fluids and electrolytes that are excreted into the intestine [26]. Based on the results of this study, the stool consistency paramters obtained are comparable to the results of the study, where the larger the dose used, the faster the stool consistency changes to normal and at the largest dose, the results of stool consistency are almost close to the positive control group [16].

Diarrhea frequency

The frequency of diarrhea was determined by observing how many times the rats had diarrhea for 3 d. The higher the frequency of diarrhea, the weaker the antidiarrheal effect produced [27].

Table 4: Average diarrhae frequency of test animal

Hours-	Mean and±SD value of diarrhae frequency					
	Normal	Negative	Positive	Group 1	Group 2	Group 3
8	0.00±0.00	1.80±0.84	2.00±0.71	2.00±0.71	1.80±0.84	2.00±0.71
16	0.00±0.00	2.00±0.71	0.80±0.84	1.60±0.55	1.20±0.45	1.40±0.55
24	0.00±0.00	1.60±0.55	0.40±0.55	1.00±0.00	1.20±0.45	0.80±0.84
32	0.00±0.00	1.80±0.45	0.20±0.45	1.00±0.00	0.60±0.55	0.60±0.55
40	0.00±0.00	1.00±0.00	0.20±0.45	0.60±0.55	0.80±0.45	0.20±0.45
48	0.00±0.00	1.00±0.00	0.00±0.00	0.40±0.55	0.40±0.55	0.20±0.45
56	0.00±0.00	1.00±0.00	0.00±0.00	0.20±0.45	0.20±0.45	0.00±0.00
64	0.00±0.00	1.00±0.00	0.00±0.00	0.20±0.45	0.00±0.00	0.00±0.00
72	0.00±0.00	0.60±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Note: Data was given in mean+SD, n = 5

The negative control experienced a decrease in the frequency of diarrhea over time, but it took longer or more of 72 h. This is because the negative control group was not given diarrhea inhibitor drugs. However, the mice had their own immune system, which caused the mice to begin to recover in a longer time than the group treated with the drug or extract. Group kpositive control has decreased in frequency or stop diarrhea at the 48th h. The 600 mg/kgBW dose test group experienced a decrease in the frequency of diarrhea or stopped diarrhea at 56th h. Whereas at dose test group 300 mg/kgBW have stopped diarrhea at the 64th h, while the dose test group 150 mg/kgBW stopped diarrhea at 72 h. The test group with the highest dose, namely 600 mg/kgBW, experienced a decrease in diarrhea frequency almost close to the positive control group.

The frequency of diarrhea in the 600 mg/kgBW dose group stopped faster than the 150 and 300 mg/kgBW doses. This is because the content of tannin compounds in a dose of 600 mg/kg BW is more than other doses. Tannins act as an astringent which is able to shrink the intestinal mucous membrane and harden the intestinal wall so that it may be expected to block the absorption of bacteria and toxins while reducing excessive fluid expenditure [26]. Based on the results of this study, the diarrhea frequency parameters obtained are comparable to the results of the study, where the larger the dose used, the faster the frequency of diarrhea stops and at the largest dose the rats experience diarrhea stopping, which is almost close to the positive control group [16].

Changes in rat body weight

Diarrhea is defined as an increase in fluid volume and frequency of defecate, increased secretion and decreased absorption of fluids that cause a lot of loss of fluids and electrolytes from the body, which are typical signs of intestinal disease [28]. The group of rats that had been induced by the *Escherichia coli* bacteria experienced a decrease in body weight. This is because it fits clinical manifestations from sufferers of specific diarrhea, which resulted in a lot loss of fluids in the body. In the negative control group, the mice lost weight up to day 3 and will return to normal again over time with the immune system of the mice itself. This is because they are not given diarrhea-inhibiting drugs.

Table 6: Average % changes in body weight of an animal

Days-	Mean and±SD Value of % changes in body weight					
	Normal	Negative	Positive	Group 1	Group 2	Group 3
1	2.54±1.02	-2.31±1.15	-1.9±1.08	-1.97±0.71	-2.12±0.83	-1.85±0.50
2	4.13±1.19	-3.25±1.76	-0.81±1.86	-2.23±1.84	-1.66±0.85	-1.43±1.63
3	5.00±1.73	-4.05±1.73	-1.89±0.93	-0.59±1.42	-1.54±1.09	-1.52±1.12

Note: Data was given in mean+SD, n = 5

The positive control group experienced the highest weight gain on the first day. The 600 mg/kgBW group of extracts had a higher body weight gain on the first day compared to the other two doses. Increase in weight in the group extract dosage 600 mg/kgBW almost close with a positive control group.

This is presumably because the secondary metabolite compounds contained in tannins are more, where the tannin compounds can precipitate proteins so that the mucous membranes become dry and form tight junctions that are resistant to inflammation and act as astringents which can cause the cell layer to become denser and shrink thereby inhibiting secretion network [22].

Based on the output data obtained, the six treatment groups indicated that the significance value was<0.05. This shows that there is a significant difference between the percent changes in body weight on the first day to the third day in the six treatment groups. The results obtained were then followed by a further test of the Duncan method.

Table 7: Duncan advanced test results percent change in body weight

Treatment group (mg/kg BW)	Mean and±SD value of percent change in body weight on day-		
	1	2	3
Normal	2.54^{b}±1.01	4.14^{b}±1.18	4.80^{c}±3.38
Negative	2.31^{a}±1.15	3.64^{a}±2.33	4.73^{a}±2.53
Positive	1.91^{a}±0.97	0.70^{a}±2.35	2.01^{bc}±1.56
Dosage 1	1.97^{a}±0.71	1.66^{a}±2.45	0.50^{b}±2.40
Dosage 2	2.12^{a}±0.83	1.32^{a}±1.42	1.74^{bc}±1.86
Dosage 3	1.85^{a}±0.50	1.26^{a}±2.23	1.69^{bc}±1.82

Note: The numbers followed by a different lowercase letter are significantly different in Duncan's continued test at P = 5%, Data was given in mean+SD, n = 5

The negative control group experienced a large weight loss because they were not given drugs to inhibit diarrhea but were only given NaCMC. The positive control group experienced the highest weight gain. The results of Duncan's continued test showed that to group test with doses of 300 and 600 mg/kgBW obtained, the results of the analysis were not significantly different from the positive control group on the last day of observation, namely the 3rd day. These results prove that the extract ethanol melinjo leaves at doses of 300 and 600 mg/kgBW has almost the same activity to cure diarrhea as the positive control group. The results of the study are in line with the research study that there was a decrease in body weight of rats when infected with pathogenic bacteria [13].

Percent of antidiarrheal effects

Based on the output data obtained, the four treatment groups indicated that the significance value was<0.05. This shows that there is a significant difference between the percent antidiarrheal effects in the four treatment groups. The percentage of antidiarrheal effect obtained is the magnitude of the healing effect of diarrhea in rats. The results obtained were then followed by a further test of the Duncan method. The results of Duncan's advanced test and the percentage of anti-diarrheal effects can be seen in table 8 below.

Table 8: Percent of anti-diarrheal effect and Duncan's continued test results

Dosage (mg/kgBW)	% Antidiarrheal effect
Positive	50.4357^{b}
150	35.7538^{a}
300	43.0172^{a}
600	50.1444^{b}

Note: fig. followed by different lowercase letters are significantly different in Duncan's advanced test P = 5%, n=5

Based on the output data obtained, it can be seen that extracting ethanol melinjo leaves has an anti-diarrheal effect which over time increases up to 50%. Result Duncan follow-up test analysis, the test group with doses of 300 and 600 mg/kgBW there was a significant difference between the positive and control group test with dose 600 mg/kgBW have percent antidiarrheal effect was not significantly different from the positive control group.

This shows that the extract dose 600 mg/kgBW more effective at inhibiting diarrhea than extracts dose 150 and 300 mg/kgBW and the effect was almost the same as the positive control. The 600 mg/kgBW dose extract had a P value<0.05 with the positive control, which means that the 600 mg/kgBB dose extract was not significantly different from the Gentamicin drug as a positive control. Based on the results of this research, the percentage of antidiarrheal effect parameters obtained is comparable to the results of Anas's (2016) research, where the greater the dose used, the greater the antidiarrheal effect produced and at the greatest dose the antidiarrheal effect obtained is almost close to the positive control group [22].

Table 9: The results of duncan's continued test of the number of Escherichia coli bacteria colonies in rat feces

Treatment Group (mg/kg BW)	Mean value and±SD number of bacterial colonies x 105 (cfu/g) in rat faeces		
	Before induction	After induction day 1	After induction day 3
Normal	76.4^{a}±9.711	75.6^{a}±.85	75^{a}±5.61
Negative	75.2^{a}±11.10	128^{b}±13.47	124.6^{c}±16.99
Positive	71.4^{a}±9.07	133.6^{b}±7.02	102^{b}±5.29
Dosage 1	71.2^{a}±9.04	134.6^{b}±8.02	111.6^{bc}±10.78
Dosage 2	70^{a}±9.85	131.8^{b}±8.47	108.6^{b}±10.36
Dosage 3	73.8^{a}±9.99	132.4^{b}±10.60	104.6^{b}±11.01

Note: The numbers followed by different lowercase letters are significantly different in Duncan's continued test at P = 5%; Data was given in mean+SD, n = 5

Based on the results of Duncan's follow-up test, the test group with doses of 300 and 600 mg/kgBW had no significant difference with the positive control group. This shows that the extract at doses of 300 and 600 mg/kgBW has almost the same diarrhea-healing effect as the positive control group.

In addition, the test group with doses of 300 and 600 mg/kgBW contained more tannin compounds than the 150 mg/kgBW dose. The mechanism of tannin compounds in the extract in killing Escherichia coli bacteria by forming polysaccharide complexes that can damage bacterial cell walls so that bacterial metabolism is disrupted and causes bacterial death [29].

The highest decrease in the number of bacterial colonies in feces occurred in the positive control group. Gentamicin is able to kill Escherichia coli bacteria by penetrating the bacterial wall and binding to the 30S and 50S subunits of the ribosome (where protein synthesis occurs). The translation process (RNA and DNA) is disrupted so that the protein causing holes in the outer membrane, resulting in leakage and the release of bacterial intracellular content [30].

Effective dosage (ED50)

The effective dose is a dose that is pharmacologically effective or effective in 50% of the population. The percentage of antidiarrheal effect of each extract dose was searched for linear regression to obtain the effective dose of the anti-diarrhea effect of the extract ethanol melinjo leaves. The table below shows the percent antidiarrheal effect of each test group at doses of 150, 300, and 600 mg/kgBW. The dose of 150 mg/kg body weight resulted in an anti-diarrheal effect of 35.75%. The dosage of 300 mg/kg body weight resulted in an anti-diarrheal effect of 43.02%. The dose of 600 mg/kg body weight produced an anti-diarrheal effect of 50.14%.

The result of the calculation of the ED_{50} value from antidiarrheal effect of the extract ethanol melinjo leaves was 578.25 mg/kgBW, while the dose of Gentamicin as a positive control was 8 mg/kgBW. ED_{50} obtained falls into the predetermined dose range, but the value ED_{50} was much greater than the Gentamicin dose which was used as a positive control. This is because the Gentamicin used is in the form of pure Gentamicin and the extract dosage ranges used are large, namely the doses of 150, 300, and 600 mg/kgBW.

The content of secondary metabolite compounds contained in this dose is more than the other doses. One of them is the compound tannin has an antidiarrheal effect because it is adstringent whose effect can deposit proteins on the surface of the intestine [27]. This condition can form a barrier formation on the surface of the gastrointestinal tract, making the surface of the intestine more resistant [25]. This layer (barrier) also causes the tightening of the outer cells, thereby inhibiting the secretion of fluids and electrolytes that are released into the intestine [1].

The use of extract ethanol melinjo leaves can cure diarrhea in rat because it contains active antibacterial and anti-diarrheal compounds that work synergistically that can improve intestinal mucosa that is inflamed and ulcers, resulting in improved or normal absorption of electrolytes and food juices. The synthetic drug used as a comparison is the antibiotic Gentamicin, the aminoglycoside group, which is sensitive to the *Escherichia coli* bacteria, which is the cause of diarrhea. The extract ethanol melinjo leaves has an anti-diarrheal effect that is almost close to the antibiotic Gentamicin, so the extract ethanol melinjo leaves can be used as a safer alternative for the treatment of specific diarrhea caused by *E. coli* bacteria.

CONCLUSION

Extract ethanol melinjo leaves (*Gnetum gnemon* L. (Linn)) dose 600 mg/kg BW has the potential to be used as antidiarrheal and its extract meet the predetermined standards, except the acid insoluble ash content parameters did not meet the predetermined standards

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AUTHORS CONTRIBUTIONS

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CONFLICT OF INTERESTS

Declared none

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