ANTIBACTERIAL ACTIVITY TEST OF N-HEXANE AND ETHANOL EXTRACT OF MELINJO PEEL (*GNETUM GNEMON L.*) AGAINST *SHIGELLA DYSENTERIAE* AND *BACILLUS CEREUS*

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ABSTRACT

Melinjo peel (*Gnetum gnemon L.*) contains secondary metabolites with antibacterial characteristics such as alkaloids, flavonoids, saponins, tannins, and steroids. This study aims to determine the antibacterial activity of 96% ethanol and n-hexane extract of melinjo peel resulting from the multilevel maceration against *Shigella dysenteriae* and *Bacillus cereus* bacteria. This research is an experimental study to test the antibacterial activity of melinjo peel extract as measured by the Diameter of Inhibition Zone (DIZ) and Minimum Inhibitory Concentration (MIC) at concentrations of 12.5%, 25%, and 50%. The results found that the extract resulted from maceration with 96% ethanol solvent as much as 70.95 g with a yield of 14.19% and n-hexane solvent as much as 7.07 g with a yield of 1.41%. The 96% ethanol extract of melinjo peel gave the highest DIZ at a concentration of 50% against *Bacillus cereus* compared to *Shigella dysenteriae*. In contrast, the DIZ of n-hexane extract of melinjo peel had no inhibitory power against both of bacteria in all concentrations. MIC of 96% ethanol extract of melinjo peel extract against *Shigella dysenteriae* and *Bacillus cereus* at concentrations of 50% and 25%, respectively, whereas n-hexane extract melinjo peel had no MIC value against both bacteria.

Keywords: Antibacterial activity; *Bacillus cereus*; Extract melinjo peel; *Shigella dysenteriae,*

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INTRODUCTION

Infectious diseases are still a serious health problem with high morbidity and mortality rates in Indonesia. Infectious diseases are caused by microorganisms such as those that infect the body in various ways, such as through the digestive tract which can lead to diarrheal infections. (Rahmawati et al., 2014). *Shigella dysenteriae* bacteria is one of the bacteria that can cause diarrheal infectious diseases, one of which is shigellosis (Prabhurajeshwar and Chandrakanth, 2018).

The pathogenic potential bacteria that causes diarrhea is *Bacillus cereus* which which can cause two different types of gastrointestinal (GI) diseases: the emetic and the diarrheal syndrome (Messelhäußer and Monika, 2018). Selection of other alternative treatments is currently needed to minimize the effects of antibiotic resistance, one of which is by utilizing the content of a natural substance that has benefits as an antibacterial agent. Several studies have shown that some local plants in Indonesia have the ability as antibacterial substances, such as the melinjo plant. Melinjo (*Gnetum gnemon L.*) is one of the plant species of the Gnetaceae family which grows a lot and is spread in the tropics.

Research conducted by Thressia (2019) showed that 96% ethanol extract of melinjo peel extract had antimicrobial activity against *Escherichia coli* bacteria. The results showed that the minimum inhibitory concentration at 50% concentration had the highest antimicrobial activity against *Escherichia coli* bacteria. In another study conducted by Hati

et al. (2018) n-hexane and Ethyl acetate extract of melinjo seeds 15% w/v produced a weak category growth inhibitory response against *Salmonella typhi* bacteria and a medium category against *Streptococcus mutans*. Ethanol extract of 70% melinjo seeds 15% w/v produced a strong growth inhibitory response against *Salmonella thypi* and *Streptococcus mutans* bacteria.

Based on the background description above, the researchers wanted to conduct further research regarding the antibacterial activity of the ethanol extract and n-hexane extract of melinjo peel against *Shigella dysenteriae* and *Bacillus cereus*.

MATERIALS AND METHODS

a. Reagents and Chemicals

Nutrient Agar (Oxoid), Sodium Chloride (Merck), Blank disc (Oxoid), Antibiotic Amoxicillin disc (Oxoid), DMSO (Merck), Crystal violet solution (Merck), Iodine solution (Oxoid), Safranin solution (Merck), Hydrochloric acid (Merck), Chloroform (Merck), Sodium Nitrate (Merck), Ammonia (Merck), Acetic anhydrous acid (Merck), Dragendroff reagent, Bouchardat reagent, n-Hexane, Ethanol 96 % and Aquadest.

b. Peel Samples

The ripe melinjo peel (red color) used in this study were collected from Balai Penelitian Tanaman Obat dan Aromatik, Bogor. The plant was determined by National Research and Innovation Agency (BRIN).

c. Bacteria Strains

Gram-positive bacteria: *Bacillus cereus* and Gram-negative bacteria: *Shigella dysenteriae* were obtained from the Laboratorium of Microbiology Faculty of Medicine Universitas Pembangunan Nasional "Veteran" Jakarta and Laboratorium Microbiology Faculty of Medicine UIN Syarif Hidayatullah Jakarta.

c. Preparation of Extract

Melinjo peel (*Gnetum gnemon L.*) was peeled washed using water and drained. The process of wet sorting and chopping was carried out. Melinjo peel (*Gnetum gnemon L.*) was dried in the oven at 40-50˚C for 3x24 hours. Dried plants are sorted and continued with the grinding process.

500 g of melinjo peel powder was macerated with 5000 mL of n-hexane for 3 days and filtrated. Each filtrate was dried using a water bath at 40˚C to obtain n-Hexane extract. n-Hexane residue was macerated again with 5000 mL of 96% ethanol for 3 days. Filtered and separated from the dregs to obtain a 96% ethanol extract of melinjo peel, then concentrated to obtain a thick 96% ethanol extract.

d. Screening Extract

Identification of Saponins

0.5 g of extract was added with 10 ml of hot water and shaken for 10 seconds. A positive result was indicated by foam formation and stable with 1 drop of 2 N hydrochloric acid (Depkes RI, 1989).

Identification of Steroids/ Triterpenoids

2 g of extract was added with 20 ml of ether and filtered. Filtrate was evaporated, 2 drops of acetic anhydride and 2 ml of chloroform were added to the residue then transferred to a test tube, and 1 ml of sulfuric acid. It was observed that the boundary of the two layers formed a brownish-red or purple ring, while the solution at the top turned green or purple indicating the presence of steroids or triterpenoids (Rijayanti, 2014).

Identification of Flavanoids

0.5 g of extract was added to 10 ml aquadest, boiled for 10 minutes, and filtered. 5 ml of the filtrate and 0.1 g of magnesium powder were added, 1 ml of hydrochloric acid, and 2 ml of amyl alcohol. Flavonoids are positive if red, yellow, and orange colors occur in the amyl alcohol layer (Depkes RI, 1989).

Identification of Tannins

1 g of extract was added to 100 ml of hot water and filtered. 5 ml of filtrate is added to 1% iron (III) chloride solution until a dark blue or blackish green color is formed, indicating the presence of tannin group compounds (Rijayanti, 2014).

Identification of Alkaloids

0.5 g of extract was added with 1 ml of 2 N hydrochloric acid and 9 ml of distilled water then heated over a water bath for 2 minutes, cooled, and filtered. The filtrate was put into 3 different test tubes. Tube I was added with 3 drops of Mayer's reagent to produce a white residue, Tube II was added with 3 drops of Bouchardat to produce a brown precipitate, and Tube III was added with 3 drops of Dragendorff's reagent to produce a red beta precipitate. A positive alkaloid result is indicated by the formation of at least 2 or 3 precipitates from the test (Marjoni, 2019).

e. The Diameter of Inhibition Zone (DIZ)

Antibacterial activity was tested using the disc diffusion method with the spread method for each series of extract concentrations of 50%, 25%, and 12.5%. The suspension of the test bacteria was spread over the surface of the NA media. The antibiotic discs and discs containing 20 µl of melinjo peel extract solution were placed in the inoculum. Incubated at 37° C for 24 hours, the test was carried out triplo.

f. The Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by the solid dilution method. 1 ml of 10^7 CFU/ml bacterial suspension, 1 ml of melinjo peel extract, and NA media were added to a petri dish. Incubated for 24 hours at 37°C. Media that looks clear or does not grow bacteria is designated as MIC.

RESULT

The results found that the extract resulted from maceration with 96% ethanol solvent as much as 70.95 g with a yield of 14.19% and n-hexane solvent as much as 7.07 g with a yield of 1.41%. The calculation of the yield value can be seen in Table 1.

Solvent	Extract (g)	Yield (%)
Ethanol 96%	70,95	14,19
n-Hexane	7,07	1,41

Table 1. Yield of Melinjo Peel Powder (*Gnetum gnemon L.*)

According to the yield percentage of this study, polar components were dominant in melinjo peel and reflected higher yields by using polar solvents.

The results of the phytochemical screening of the 96% ethanol extract of melinjo peel (*Gnetum gnemon L.*) contained alkaloids, flavonoids, saponins, and tannins. In contrast, the n-hexane extract of melinjo peel (*Gnetum gnemon L.*) contained steroid compounds. The results of the phytochemical screening test can be seen in Table 3.

Metabolite Secondary	Extract	
	96% Ethanol Extract	n-Hexane Extract
Alkaloid	+	-
Flavonoid	+	-
Saponin	+	-
Steroid	-	+
Tannin	+	-

Table 2. The Results of the Phytochemical Screening

The results of the Diameter of Inhibition Zone (DIZ) test showed that *Shigella dysenteriae* and *Bacillus cereus* could not be inhibited by n-hexane extract of melinjo peel since even the highest concentration, the growth of this bacteria could not be inhibited. In addition, the 96% ethanol extract inhibition towards *Bacillus cereus* was positively higher than *Shigella dysenteriae* at the concentration of 50%. The results of the Diameter of Inhibition Zone (DIZ) test can be seen in Table 3.

The 96% ethanol extract had the highest MIC value against *Bacillus cereus* at a concentration of 25% while against *Shigella dysenteriae* bacteria at a concentration of 50%. There was not MIC against *Shigella dysenteriae* or *Bacillus cereus* bacteria in n-hexane extract of melinjo peel at all concentrations. The results of the MIC can be seen in Table 4.

Concentrations	*Shigella dysentriae*		*Bacillus cereus*	
(%)	DIZe	DIZn	DIZe	DIZn
12,5	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00
25	10,83 ± 0,12	0 ± 0,00	9,87 ± 0,46	0 ± 0,00
50	12,68 ± 0,00	0 ± 0,00	13,49 ± 2,23	0 ± 0,00
PCo	58,33 ± 0,46	51,4 ± 0,35	13,13 ± 0,36	13,61 ± 0,05
NCo	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00

Table 3. The results of the Diameter of Inhibition Zone (DIZ)

DIZe : Diameter of Inhibition Zone of 96% Ethanol
DIZn : Diameter of Inhibition Zone of n-Hexane
PCo : Positive Control (Amoxicillin)
NCo : Negative Control (DMSO)

Concentrations	*Shigella dysentriae*		*Bacillus cereus*	
(%)	MICe	MICn	MICe	MICn
12,5	++	++	+	++
25	+	++	-	++
50	-	+	-	+
PCo	-	-	-	-
NCo	+	+	+	+

Table 4. The results of the Minimal Inhibition Concentrations (MIC)

MICe : Minimum Inhibitory Concentrations of 96% Ethanol
MICn : Minimum Inhibitory Concentrations of n-Hexane
++ : High Bacterial Growth
+ : Moderate Bacterial Growth
- : No Bacterial Growth

DISCUSSION

Based on this study, phytochemical screening test results were obtained in the form of alkaloids, flavonoids, saponins, and tannins. In several more studies, the results of which are consistent with those in this study, several secondary metabolite compounds have been shown to be involved in antimicrobial activity. The use of 96% ethanol as a solvent in the extraction of bioactive compounds is widely used because ethanol is good for extracting the antibacterial compounds tannins, phenols, and flavonoids because ethanol is easier to penetrate cell membranes to extract intracellular materials from plant materials.

Several factors including content of metabolite compounds, concentration of extract, diffusion capacity and bacterial type have an influence on the antibacterial activity of extracts. The structure of bacterial cell walls influences the activity, penetration, and binding of antibacterial compounds.

As opposed to Gram-negative bacteria, this extract has more inhibiting effect against Gram-positive bacteria since they have several complex layers on the cell walls. Gram-positive bacteria have a thicker peptidoglycan structure, fewer lipids, and contain polysaccharides in the form of teichoic acids. Teichoic acid is a water-soluble polymer that acts as a transport agent

for positive ions in or out. In the case of Gram-positive bacteria, water soluble nature implies that their cell walls are more polarized. (Septiani, 2017).

Flavonoid and tannin compounds are polar compounds so they penetrate the polar peptidoglycan layer more easily than the non-polar lipid layer. This causes the inhibitory activity of Gram-positive bacteria to be greater than Gram-negative bacteria.

According to Rijayanti (2014), Saponin can cause leakage of proteins and enzymes from inside cells. Saponin may be antibacterial because it has a surface-active substance that is similar to detergent, which reduces the surface tension of bacterial cell walls and damages membrane permeability resulting in hemolysis in bacterial cells.

Tannins have antibacterial activity by promoting proteins through their cell membrane interactions, inactivating enzymes and interfering with the function of genetic material, resulting in an inhibitor of reverse transcriptase and DNA topoisomerase enzymes to prevent bacterial cells from forming. Tannins are involved in the ability to inactivate the adhesion of microbial cells. Inactivating enzymes, which interrupt protein transport to the cell's inner layers Rijayanti (2014).

The antibacterial action is also influenced by denaturation of bacterial cell walls. The cell wall that denatures more easily is the cell wall which are composed of polysaccharides compared to cell walls which are composed of phospholipids, where the cell walls of gram-positive (*Bacillus cereus*) contain peptidoglycan while *Shigella dysenteriae* (gram-negative bacteria) have cell walls composed of a small amount of peptidoglycan between the outer membrane and contain phospholipids and proteins.

CONCLUSION

The 96% ethanol extract of melinjo peel (*Gnetum gnemon L*.) has the highest DIZ against *Shigella dysenteriae* and *Bacillus cereus* bacteria at a concentration of 50%, while the n-hexane extract of melinjo peel (*Gnetum gnemon L*.) did not have DIZ against *Shigella dysenteriae* and *Bacillus cereus*.

The 96% extract of melinjo peel (*Gnetum gnemon L*.) has MIC against *Shigella dysenteriae* and *Bacillus cereus* bacteria at concentrations of 25% and 50%. The n-hexane extract of melinjo peel (*Gnetum gnemon L*.) did not have a MIC against *Shigella dysenteriae* and *Bacillus cereus* bacteria at all concentrations.

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