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Antibacterial Potential of Kepok Sobo Londo Banana Peel (*Musa paradisiaca* L.) Against Diarrhea Causing Bacteria Enterohemorrhagic *Escherichia coli*

DHIRA SATWIKA*, KEZIA YOVANKA IRAWAN, AND TRI YAHYA BUDIARSO

Department of Biology, Faculty of Biotechnology, Universitas Kristen Duta Wacana, Yogyakarta 55224, Indonesia

The administration of excessive doses of antibiotics and frequency of diarrhea outbreaks caused by Enterohemorrhagic *E. coli* (EHEC) need to be reexamined, and one of it measures is considering the potency of local plants, such as kepok sobo londo banana. This study is conducted to examine the antibacterial potential of kepok sobo londo banana peel extract (*Musa paradisiaca* L.) through its inhibitory activity on the growth of EHEC. The bacterial isolates were molecularly identified using specific primers encoding EHEC virulence genes, namely stx1 and eae, respectively. Banana peel samples were extracted by maceration method and tested for phytochemicals using GC-MS. Antibacterial testing was carried out using the agar well diffusion method, as well as MIC and MBC assay. The data from GC-MS analysis results in the identification of main antibacterial compounds contained in the extract, namely fatty acids, tannin alkaloids, and phenols, respectively. The results of the antibacterial assay using agar well diffusion method for inhibition of EHEC bacteria by kepok sobo londo banana peel extract were found to be greatest at a concentration of 28% which is considered as intermediate inhibitory power. Obtained MIC dan MBC value of extract occurred at concentration of 17% and 22%, respectively against EHEC.

Keywords: Antibacterial, diarrhea, Enterohemorrhagic *E. coli*, Kepok sobo londo banana skin

Infeksi diare oleh bakteri diarrheagenic *E. coli* (DEC), khususnya strain Enterohemorrhagic *E. coli* (EHEC) menjadi salah satu penyebab mortalitas pada balita di negara berkembang. EHEC merupakan bakteri patogen penghasil shiga toxin yang menginvasi usus besar. Pertambahan kasus resistensi karena pemberian dosis antibiotik berlebih dan peningkatan frekuensi kasus kejadian luar biasa (KLB) diare mendorong terjadinya eksplorasi potensi bahan alam lokal sebagai alternatif pengganti antibiotik, seperti tanaman dengan senyawa antibakteri. Pisang kepok sobo londo termasuk komoditas buah lokal yang tersebar di Pulau Jawa. Kulit pisang diketahui memiliki potensi bioaktivitas yang baik. Saat ini, belum banyak penelitian yang mengangkat potensi kulit pisang kepok sobo londo, oleh karena itu penelitian ini akan mengkaji potensi antibakteri ekstrak kulit pisang kepok sobo londo (*Musa paradisiaca* L.) melalui aktivitas daya hambatnya terhadap pertumbuhan bakteri EHEC penyebab diare. Sampel isolat bakteri diidentifikasi secara molekuler menggunakan primer spesifik yang mengkode gen virulensi EHEC, yaitu stx1 dan eae. Sampel kulit pisang diekstraksi dengan metode maserasi dan diuji fitokimia menggunakan GC-MS. Penentuan aktivitas antibakteri dilakukan melalui metode difusi agar sumuran, serta pengukuran nilai MIC dan MBC terhadap EHEC. Analisa hasil GC-MS mengidentifikasi senyawa antibakteri utama yang terkandung pada ekstrak adalah asam lemak, tanin, alkaloid, dan fenol. Hasil uji antibakteri metode difusi agar sumuran terhadap penghambatan bakteri EHEC oleh ekstrak kulit pisang kepok sobo londo paling besar terjadi pada konsentrasi 28% yang dikategorikan ke dalam daya hambat sedang (intermediate). Didapatkan nilai MIC pada konsentrasi ekstrak 17% dan MBC pada konsentrasi ekstrak 22% terhadap EHEC.

Kata kunci: Antibakteri, diare, Enterohemorrhagic *E. coli*, Kulit pisang kepok sobo londo

Diarrhea is a disease with high mortality rate in the world, especially in developing countries. Cases of acute diarrhea in infants are generally dominated by diarrheagenic *E. coli* (DEC), specifically the Enterohemorrhagic *E. coli* pathotype. EHEC is a pathogenic bacterium that causes outbreaks of bloody diarrhea in humans because of its ability to invade and irritate the large intestine. EHEC has a specific virulence factor in the form of "shiga toxin" (stx) which is able to bind to intestinal endothelial cells and allows the spread of the toxin to other organs through the blood circulation system (Lin *et al.* 2012). Although "shiga toxin" (stx) being the major virulence factor of EHEC, other factors

*Corresponding author: Phone: + 62-274-563929 (ext. 459); Fax: + 62-274-513235; email: dhira@staff.ukdw.ac.id

such as the intimin structural protein encoded by the eae gene also contributes to the pathogenesis of EHEC through its mechanism of bacterial colonization in the intestinal mucous membrane (Roussel *et al.* 2016).

Diarrhea treatment therapy is generally carried out by administering conventional antibiotics, but as cases of antibiotic resistance increase, people are starting to explore the potential of natural ingredients. In addition, the high cases of diarrhea outbreaks in developing countries have added to the urgency of finding alternative natural ingredients as therapeutic treatments that are easily accessible and abundantly available. Previous studies have shown the presence of several secondary metabolites in plants that have antibacterial activity. One of the plants that contain antibacterial compounds is the kepok sobo londo banana (*Musa paradisiaca* L.). Kepok sobo londo banana is a local fruit commodity in Java with high production and consumption levels. In general, almost all parts of the fruit can be utilized, but the banana peel is often not used, even though the banana peel is known to have a higher phenolic content than the flesh (Fathemeh *et al.* 2012). At present, there are not many studies that address the potential of the kepok sobo londo banana peel, therefore this study will focus on the antibacterial potential of the kepok sobo londo banana peel extract through its inhibitory activity against EHEC that causes diarrhea.

MATERIAL AND METHODS

Test Bacteria. This study uses *E. coli* ATCC 35218 and *E. coli* ATCC 45894 obtained from the collection of the Microbiology Laboratory, Faculty of Medicine, Universitas Gadjah Mada.

Molecular Identification. Bacterial molecular identification was performed to confirm the type of pathotype based on its virulence, *i.e. eae*, and *stx1* gene. DNA isolation is carried out using Thermo Scientific GeneJET Genomic DNA Purification Kit. The DNA obtained from the isolation process is then amplified using an eae gene primer (F (5'GTT CCT TGA CCG CCT TTC CGA TAC CGT C'3) and R (5'GCC GGT CAG CCA CCC TCT GAG AGT AC3')), and *stx1* gene primer (F (5'GTT CCT TGA CCG CCT TTC CGA TAC CGT C'3) and R (5'GCC GGT CAG CCA CCC TCT GAG AGT AC3')) produced 180 bp and 384 bp amplicon, respectively, with PCR program conditions: pre-denaturation (95°C, 5 minutes), denaturation (95°C, 60 seconds), annealing (56°C, 30 seconds), extension (72°C, 60 seconds, 30 cycles), and "final extension" (72°C, 8 minutes) (Manhique-Coutinho *et al.* 2022). PCR results were sequenced using a sequencing service at PT. Genetika Science Indonesia to determine the nucleotide sequence. Constructed sequence data forms a phylogenetic tree.

Sample Preparation and Extraction. 3 kg of kepok sobo londo banana peel samples with maturity level in phase 1-2 were obtained from Kebun Plasma Nutfah Giwangan, Yogyakarta. Samples were dried, mashed, and sieved using a "mesh" 40 sieve. Simplicia powder was extracted by maceration method using 70% ethanol solvent with a ratio of 1:3.5 in a maceration vessel placed on a "rotary shaker" with a speed of 120 rpm for 5 day (Venkatesh *et al.* 2013). The maserate is filtered and evaporated using a "vacuum rotary evaporator" with a temperature of 40°C and water bath. The extract obtained was diluted using distilled water until it reached a concentration of 28, 22, 17 and 11%.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. Identification of the active compounds contained in the extract was carried out using Shimadzu's GC-MS QP2010 at the UNDIP Chemistry Laboratory. The molecular weight and separation pattern of each active compound is adjusted to the "database" so that the name, formula, and molecular structure of the identified compound could be identified.

Pre-enrichment and Preparation of Bacterial Suspension. Re-culture of *E. coli* ATCC 35218 and *E. coli* ATCC 45894 was carried out by inoculating pure isolates on slanted Brain-Heart Infusion Broth (BHIB) media and incubating at room temperature for 24 hours. Bacterial suspension was prepared by inoculating the bacteria culture in sterile 0.9% NaCl and adjusted to the McFarland turbidity standard of 0.5 (equivalent to 1.5×10^8 CFU/ml).

Antibacterial Test. Antibacterial testing was carried out using the agar well diffusion method. Bacterial suspension of *E. coli* ATCC 35218 and *E. coli* ATCC 45894 was inoculated on MHA medium (Mueller-Hinton Agar) using the "spread plate" technique. On the MHA surface, wells with a diameter of 6 mm were made and 80 µL of the test extract with various concentrations, negative control (aquadest) and positive control (2 mg/ml of Ciprofloxacin) were poured, and incubated for 24 hours at room temperature. This test was made 3 repetitions. The Diameter of Inhibition Area (DDH) in the form of a clear zone around the wells is calculated in mm units and interpreted according to the CLSI standard.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration(MBC) Test. The MIC test was carried out using the multilevel dilution method, the extract was diluted with sterile

distilled water until it reached a concentration of 22; 17; 11; 5.5; 2.75 and 1.375%. As much as 2 ml of each extract, 2 ml of sterile BHIB media and 2 ml of bacterial suspension were put into a test tube and incubated for 18 hours. Such treatment was also carried out for positive and negative controls by replacing the test extract with the antibiotic ciprofloxacin at a dose of 2 mg/ml and sterile distilled water. Absorbance was measured before and after incubation using a UV spectrophotometer at a wavelength of 600 nm. The lowest concentration without turbidity was determined as the MIC value. The MBC test is carried out by streaking a sample of the MIC tube into MHA, then incubating it for 18-24 hours. If there is no growth, then the concentration of the extract was determined as the MBC value.

RESULT

Molecular Identification. Amplification of bacterial isolates using PCR method with eae and stx1 primer produced DNA fragments of 384 bp and 180 bp, respectively (Figure 1). The results of the phylogenetic tree construction of the sample isolates show a very close relationship between the sample isolates and the EHEC group (Figure 2), based on that *E. coli* ATCC 35218 and *E. coli* ATCC 45894 can be identified as a clinical isolate of EHEC bacteria that produce "shiga toxin".

Kepok Sobo Londo Banana Peel Extraction. The maceration of produce a viscous extract in the form of a blackish brown paste with a distinctive smell of banana weighing 28 grams with a total yield of 28%.

Analysis of Phytochemical Content of Sobo Londo Banana Peel Extract Using GC-MS. The results of GC-MS analysis that has been carried out identify 15 chemical compound components with varying peak areas, retention times, and molecular weights (Table 1). The peak area determines the quantity of chemical components contained in the test sample where the wider the area, the more chemical compounds detected. The identified chemical compounds were dominated by fatty acids, tannins, alkaloids, and phenols.

Antibacterial Activity Using Agar Well Diffusion. The antibacterial activity of the ethanol extract of kepok sobo londo banana peel was determined by the formation of an inhibition zone around the wells. The results of the antibacterial test of kepok sobo londo banana peel extract against two strains of EHEC bacteria, namely *E. coli* ATCC 35218 and *E. coli* ATCC 45894 showed the antibacterial activity of kepok sobo londo banana peel extract with concentrations of 28% and 22% (Table 2).

MIC and MBC Test. The inhibitory activity (bacteriostatic) of the extract in the MIC test can be observed visually through the parameters of turbidity and absorbance values. Inhibition of bacterial growth is indicated by a decrease in the absorbance value, while an increase in the absorbance value indicates that there is still growth of bacterial cells. The results of the MIC test analysis showed that the sobo londo banana peel extract was able to inhibit bacterial growth of *E. coli* ATCC 35218 and *E. coli* ATCC 45894 at the smallest concentration of 17% (Table 3). In the MBC test the bactericidal activity of the extract was determined by

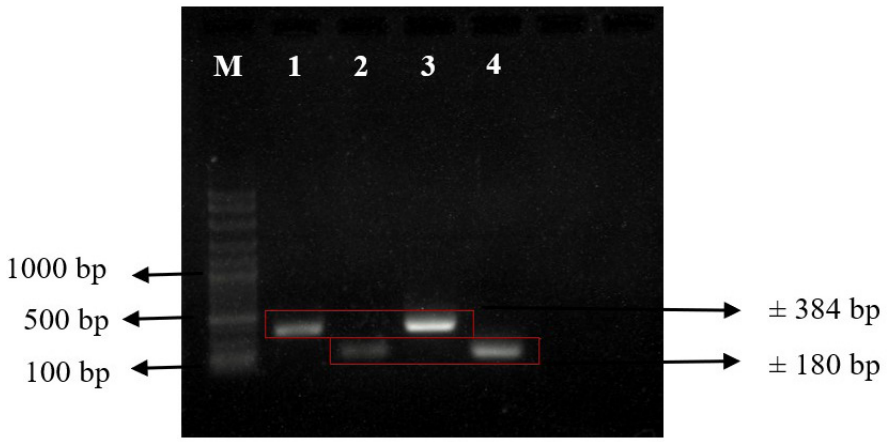

Figure 1. PCR product of *E. coli* using a primer which encoded eae and stx1 gene on agarose 0.9%. Well 1 and 3: Isolate *E. coli* ATCC 35218 with eae primer. Well 2 and 4: Isolate *E. coli* ATCC 45894 with stx1 primer. M: "Marker" 100 bp DNA Ladder (Vivantis)

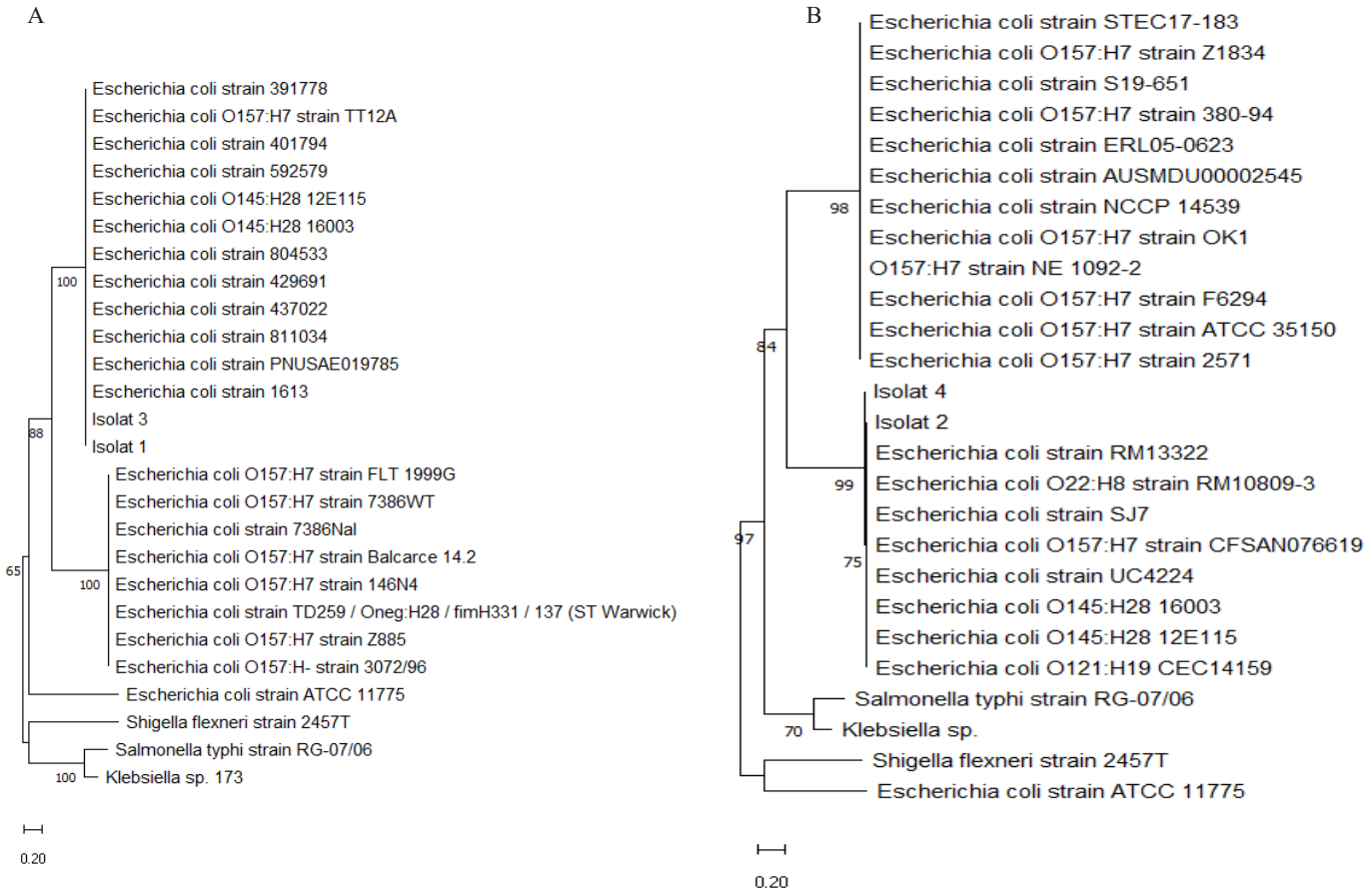

Figure 2. Phylogenetic tree of isolates 1 and 2 (*E. coli* ATCC 35218) and isolates 3 and 4 (*E. coli* ATCC 45894) by eae gene (A) and stx1 gene (B)

Table 1. Main chemical compounds of kepok sobo londo banana peel extract

Chemical compound	Formula	Area (%)	R. Time (min)	MW	SI	Class
Pyrolidine	$C_4H_5D_4N$	5.59	3.391	71	87	Alkaloid
Propanoic acid	$C_5H_{10}O_5$	3.38	3.430	118	97	Fatty acid
Heptadecane	$C_{17}H_{36}$	2.95	18.368	240	97	Alkane
Methyl palmitic	$C_{17}H_{34}O_2$	2.80	28.441	284	95	Fatty acid
Ethyl palmitic	$C_{18}H_{36}O_2$	25.12	29.822	284	95	Fatty acid
Phytol	$C_{20}H_{40}O$	2.71	32.363	296	93	Alkaloid
Stearic acid	$C_{19}H_{34}O_2$	6.67	33.139	294	91	Fatty acid
Ethyl oleate	$C_{20}H_{38}O_2$	13.08	33.243	310	91	Fatty acid
Oleate acid	$C_{20}H_{38}O_2$	5.64	33.385	310	86	Fatty acid
Ethyl stearic	$C_{20}H_{40}O_2$	4.35	33.719	312	90	Fatty acid
Tetratetracontane	$C_{40}H_{82}$	8.64	37.420	618	93	Tannin
Tetracontane	$C_{44}H_{90}$	9.61	37.572	618	93	Tannin
Dotriacontane	$C_{35}H_{72}$	2.67	37.718	450	78	Benzoic acid
Sinamic acid	$C_{14}H_{18}O_5$	4.38	38.703	266	71	Phenol
Monogliceride	$C_{19}H_{38}O_4$	2.40	39.802	330	81	Glycerol

observing the presence or absence of bacterial growth. The MBC test results showed that there was no growth of bacterial colonies at a concentration of 22%, so the MBC value of the sobo londo banana peel extract for *E. coli* ATCC 35218 and *E. coli* ATCC 45894 is present at a concentration of 22% (Figure 3).

DISCUSSION

This study focused on the antibacterial ability of kepok sobo londo banana peel extract against the two isolates of *E. coli* cause of diarrhea. Molecular identification was carried out to confirm the pathotype using specific primers

Table 2. Inhibition zone (mm) of kepok sobo londo banana peel against EHEC

Isolate	Concentration of extract (%)	Diameter (mm) ± SD				Category
		R1	R2	R3	Mean	
E. coli ATCC 35218	28	13	13	14	13.3±0.58	Intermediate
	22	11	11	13	11.7±1.15	Resistant
	17	0	0	0	0.0	-
	11	0	0	0	0.0	-
	Ciprofloxacin (0.2)	40	40	42	40.6±1.15	Susceptible
E. coli ATCC 45894	28	14	13	15	14.0±0.0	Intermediate
	22	12	10	12	11.3±1.15	Resistant
	17	0	0	0	0.0	-
	11	0	0	0	0.0	-
	Ciprofloxacin (0,2)	40	39	38	39.0±0.0	Susceptible

Table 3. Absorbance value of MIC test

Concentration (%)	*E. coli* ATCC 35218			*E. coli* ATCC 45894		
	OD Value (before)	OD Value (after)	Turbidity	OD Value (before)	OD Value (after)	Turbidity
1,375	0.03	0.76	++	0.03	0.76	++
2.75	0.03	0.73	++	0.03	0.72	++
5.5	0.03	0.68	++	0.03	0.67	++
11	0.03	0.67	++	0.03	0.65	++
17	0.03	0.0	-	0.03	0.0	-
22	0.03	0.0	-	0.03	0.0	-
Kontrol (+)	0.03	0.0	-	0.03	0.0	-
Kontrol (-)	0.03	0.98	+++	0.03	0.87	+++

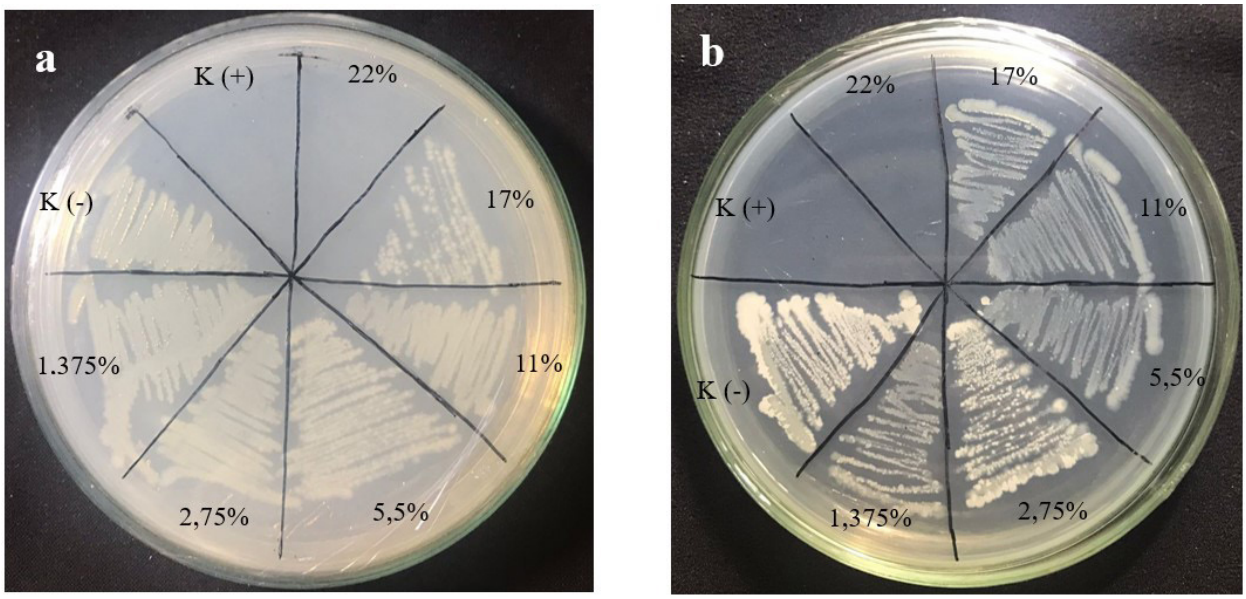

Figure 3. MBC results of kepok sobo londo banana peel extract against bacterial isolates off *E. coli* ATCC 35218 (a) and *E. coli* ATCC 45894 (b). 22 – 1,375%: kepok sobo londo banana peel extract, K(+) = Ciprofloxacin dose of 2 mg/1 ml, and K(-) = aquadest

to detect the target gene belonging to EHEC bacteria, ie stx1 and eae. The *stx1* gene is the most important EHEC virulence factor capable of producing holotoxins which will bind to glycolipid globotriaosylceramide (Gb3) on the surface of intestinal endothelial cells thereby interfering protein synthesis mechanism and systemic absorption of the human digestive system (Gomes *et al.* 2016). On the other hand, the eae gene also plays a role in the pathogenicity of EHEC because of its role in encoding the intimin protein on the bacterial cell surface which will attach to its receptor (Translocated Intimin Receptor) and cause irritation to the human

gastrointestinal tract (Abbasi *et al.* 2014). PCR results of sample isolates showed the formation of PCR products with a size of ± 384 bp for isolates 1 and 3 (*E. coli* ATCC 35218) and size ± 180 bp for isolates 2 and 4 (*E. coli* ATCC 45894). This indicates the success of the isolates in amplifying each targeted gene.

The sequencing of the amplicon product identified its kinship with the nucleotide sequences encoding the eae and stx1 gene belongs to other species found in the "online database" through the construction of a phylogenetic tree. The result of kinship analysis based on evolutionary relationships indicates that the sample isolates were in the same clade as most of the clinical EHEC strains, like *E. coli* O145:H28 strains 12E115 and 16003 isolated from feces of patients with bloody diarrhea in Japan and Belgium (Nakamura *et al.* 2020). This statement showed there is a monophyletic relationship between the sample isolates and the EHEC clinical bacterial group because of their very close kinship. This closeness might occur because they are descended from the same ancestor with similar genetic material and characteristics. This is also supported by the bootstrap value of the clade are 100% and 99%, respectively. On the other hand, other clades were dominated by non-clinical EHEC group isolated from environment, food, or ruminants (zoonotic). This shows the sensitivity and specificity of the primers applied in this study so that they are able to separate and detect EHEC bacterial groups down to the strain level based on their clinical features.

Banana peel sample for antibacterial test was extracted by maceration using 70% ethanol. Maceration is the method of choice because it includes cold extraction without heating, thereby reducing the risk of damage to active compounds that are easily damaged by heat. Referring to the results of the GC-MS chromatogram analysis, several main compounds with bioactivity were identified in the kepok sobo londo banana peel extract, including fatty acids (61.04%), tannins (18.25%), alkaloids (8.3%), and phenol (4.38%). The "similarity index" values of these compounds range from 71-97, this indicates that the compounds are highly resembling standard compounds in the "database library". These compounds are known to have various pharmacological activities, such as antibacterial, antioxidant, antifungal, antidiabetic, and antidiuretic. The phytochemical content of the kepok sobo londo banana peel has a fairly good utilization potential because of the high content of fatty acids and tannins.

The antibacterial test results showed that there was antibacterial activity of kepok sobo londo banana peel extract against two strains of EHEC bacteria at a concentration of 28% and 22% which were categorized into intermediate and resistant for the two test bacteria with the greatest inhibition found in concentration of 28% each of 13.3±0.58 mm (*E. coli* ATCC 35218) and 14 mm (*E. coli* ATCC 45894) as could be seen in Figure 3, 4 and 5 below. Based on the formation of the inhibition zone, it can be seen that there was an increase in the inhibition zone with increasing concentration of the extract. This indicates that there is a directly proportional relationship between the concentration of the extract and its inhibitory activity, presumably the higher the concentration the greater the inhibitory activity. Other methods that can be used in the antibacterial test of an extract are MIC and MBC to determine the concentration needed to initiate antibacterial activity both as bacteriostatic and as bactericidal. The results of the MIC test analysis showed that the kepok sobo londo banana peel extract was able to inhibit bacterial growth of *E. coli* ATCC 35218 and *E. coli* ATCC 45894, indicated by clear media and decrease in absorbance values after incubation, at the smallest concentration of 17% (170 mg/ml) which was considered less sensitive (weak) compared to Ciprofloxacin (2 mg/ml). Inhibition occurs due to contact between the antibacterial compounds contained in the extract and bacterial cells. Based on the MIC test results, it is assumed that the multilevel dilution method in this study is more sensitive because it allows direct contact between the bacteria and the extract so that the inhibitory activity tends to be more optimal. In the MBC test results, there was no growth of bacterial colonies at a concentration of 22%. From this statement, it can be seen that the MBC value of banana peel extract against bacteria *E. coli* ATCC 35218 and *E. coli* ATCC 45894 is present at a concentration of 22%. On the other hand, at a smaller concentration, bacterial growth is still found. This condition in line with Ayini & Dewi (2014) which stated that the inhibition and killing power of an antibacterial substance were stronger if the concentration of the treatment given was high.

The antibacterial activity of kepok sobo londo banana peel extract is thought to be the role of several compounds that have been identified through the GC-MS method, such as fatty acids, tannins, alkaloids, and phenols. Palmitic acid compounds belonging to the fatty acids are known to have inhibitory effect against *E. coli* by interfering with the permeability of the cell membrane which results in leakage of the cell cytoplasm (Zhong-hui *et al.* 2010). In addition to fatty acids, tannin derivative compounds identified as tetracontana in this study are

also known to have antibacterial activity by coagulating the protoplasm of cells so that growth activity is inhibited which results in cell death. The mechanism of inhibition of alkaloid compounds is carried out by inhibiting nucleic acid synthesis, bacterial homeostasis, and depolarizing the cytoplasmic membrane resulting in leakage of cytoplasmic fluid (Cushnie *et al.* 2014). Furthermore, the phenol secondary metabolites present also provide quite good antibacterial activity because of their ability to trigger toxin neutralization reactions and nucleic acid synthesis. The existence of compounds that have various mechanisms of inhibiting bacterial growth further supports the potential utilization of the part of the banana that is often not used, namely the kepok sobo londo banana peel as an antibacterial against two clinical EHEC strains, *Escherichia coli* ATCC 35218 and *Escherichia coli* ATCC 45894 (Kapadia *et al.* 2015). Based on the research results, kepok sobo londo banana peel is able to inhibit bacterial growth, even in small concentrations so that this extract is possible to have greater antibacterial activity when the concentration is increased. In addition, the inhibitory activity of the test extract against EHEC pathogenic bacteria which have virulence properties can support its application against other Diarrheagenic *E. coli* pathotypes to develop kepok sobo londo banana peel extract as an antibacterial product made from natural ingredients that is safe, easily apply, and environmentally friendly.

REFERENCES

Abbasi P, Kargar M, Doosti A, Mardaneh J, Ghorbani-Dalini S, Dehyadegari MA. 2014. Characterization of Shiga-toxin producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) using multiplex real-time PCR assays for *stx1, stx2, eaeA.* Iranian Journal of Microbiology. 6(3):169. https://doi.org/10.17795/acr-22721

Ayini U, Dewi TC. 2014. Efek antibakteri ekstrak daun mimba (*Azadirachta indica* A. Juss) terhadap bakteri *Vibrio algynoliticus* secara *in vitro*. Biosaintifika Journal of Biology & Biology Education. 6(1):67-75.

Cushnie TT, Cushnie B, Lamb AJ. 2014. Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. International Journal of Antimicrobial Agents. 44(5):377-386. https://doi.org/10.1016/j.ijantimicag.2014.06.001

Fatemeh SR, Saifullah R, Abbas FM, Azhar ME. 2012. Total phenolics, flavonoids and antioxidant activity of banana pulp and peel flours: influence of variety and stage of ripeness. International Food Research Journal. 19(3):1041-1046.

Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigue JF, Piazza RM, Martinez MB. 2016. Diarrheagenic *Escherichia coli.* Brazilian Journal of Microbiology. 47:3-30. https://doi.org/10.1016/j.bjm.2016.10.015

Kapadia SP, Pudakalkatti PS, Shivanaikar S. 2015. Detection of antimicrobial activity of banana peel (*Musa paradisiaca* L.) on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*: an *in vitro* study. Contemporary Clinical Dentistry. 6(4):496-499 https://doi.org/10.4103/0976-237X.169864

Lin A, Nguyen L, Clotilde LM, Kase JA, Son I, Lauzon CR. 2012. Isolation of Shiga toxin–producing *Escherichia coli* from fresh produce using STEC heart infusion washed blood agar with mitomycin-C. Journal of Food Protection. 75(11):2028-2030.

Manhique-Coutinho L, Chiani P, Michelacci V, Taviani E, Bauhofer AFL, Chissaque A, Cossa-Moiane I, Sambo J, Chilaúle J, Guimarães EL, Salência J, Cassocera M, Bero DM, Langa JP, de Deus N. 2022. Molecular characterization of diarrheagenic *Escherichia coli* isolates from children with diarrhea: a cross-sectional study in four provinces of mozambique: diarrheagenic *Escherichia coli* in mozambique. International Journal Infection Disease. 121:190-194. https://doi.org/10.1016/j.ijid.2022.04.054

Nakamura K, Murase K, Sato MP, Toyoda A, Itoh T, Mainil JG, Pierard D, Yoshino S, Kimata K, Isobe J, Seto K, Etoh Y, Narimatsu H, Saito S, Yatsuyunagi J, Lee K, Iyoda S, Ohnishi M, Ooka T, Gotoh Y, Ogura Y, Hayashi T. 2020. Differential dynamics and impacts of prophages and plasmids on the pangenome and virulence factor repertoires of Shiga toxin-producing *Escherichia coli* O145:H28. Microbial Genomics. 6(1):1-13. https://doi.org/10.1099/mgen.0.000323

Roussel C, Cordonnier C, Galia W, Goff OL, Thevenot J, Chalancon S, Alric M, Thevenot-Sergentet D, Leriche F, Wiele TV, Livrelli V, Blanquet S. 2016. Increased EHEC survival and virulence gene expression indicate an enhanced pathogenicity upon simulated pediatric gastrointestinal conditions. Pediatric Research. 80:734-743. https://doi.org/10.1038/pr.2016.144

Venkatesh KV, Girish KK, Pradeepa K, Santosh KSR. 2013. Antibacterial activity of ethanol extract of *Musa paradisiaca* cv. Puttabale and *Musa acuminate* cv. grand naine. Asian J. Pharm. Clin. Res. 6(2):167-170.

Zhong-hui P, Yu-qun Z, Zhong-qiong, Y, Jiao X, Ren-yong J, Yang L, Fan Y. 2010. Antibacterial activity of 9-Octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-Diyl ester from neem oil. Agricultural Sciences in China. 9(8): 1236-1240.