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Inhibition Effect of Klutuk Banana Fruits Ethanol Extract Against *Shigella dysenteriae* ATCC 13313 In Vivo

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Abstract

The present study was carried out for evaluating the inhibition effect of klutuk banana fruits extract against *S. dysentery* ATCC 13313 in vivo. The extraction of dried Klutuk banana fruits was prepared using a maceration method. The secondary metabolites of the extract were analyzed using standard method. The antibacterial activity was done using the bacterial induction method in male *Mus musculus* mice, followed by treatment with klutuk banana fruits ethanol extract. The results of the phytochemical analysis revealed varying constituents of these extracts, as follows: flavonoids, polyphenols, tannins, monoterpenoids, sesquiterpenoids, quinones and saponins. Our results demonstrated that ethanol extracts of Klutuk banana fruits have active antibacterial activity by decreasing *S. dysenteriae* ATCC 13313 colony number.

Keywords: *Shigella dysenteriae*, colony, Klutuk, banana fruits, in vivo

Introduction

Dysentery is a type of acute diarrheal disease caused by *Shigella dysenteriae*. This infection is characterized by fluid and slimy stools [1]. *Shigella dysenteriae* are relatively resistant to gastric acid, that is why the bacteria can enter the small intestine. Invasion of *Shigella dysenteriae* in the intestinal mucosal cells mediated by phagocytosis, multiplying and spreading to cytoplasm of epithelial cells, then spread to adjacent cells. Further microbes occur in the wall of the colon that can cause damage to mucous membranes ulceration and bleeding[2]. *Shigella dysenteriae* also produce the exotocin that can affect the gastrointestinal and central nervous system [3].

Until now, antibiotics is still the first choice for dysentery treatment caused by *Shigella dysenteriae*. Long term and repeated use of antibiotics can caused bacterial resistance [4]. *Shigella dysenteriae* are known to be resistant to ampicillin and tetracyclines [3].
The usage of botanical thing can be the inovasion to create the new herbal treatment for bacillary dysentery. The banana klutuk ethanol extract are known contains (*Musa balbisisana* Colla) tanin and flavonoid. Flavonoid can affecting the syntesis of extracelular protein and bacterial cell wall [5]. Tanin has the antibacterial activity by causing damage to cell membrane, cell wall and all protein components [6]. All those thing can cause the bacterial cell death.

Other in vitro research show that banana klutuk fruits (*Musa balbisisana* Colla) ethanol extract has antibacterial activity againts *Shigella dysenteriae*. The in vivo analysis is required to prove the antibacillary dysentery activity of banana klutuk ethanol extract againts *Shigella dysenteriae*. Therefore, banana klutuk can be developed into a standardized herbal preparation or fitofarmaka, especially for treating dysenteriae.

Materials and Methods

Material

The chemical used are distilled water, chloride acid solution (Merck®), feric chloride reagents, carbol fuchsin, carbol gentian violet, CMC, etanol 96% (Merck®), pottasium hydroxide, chloroform (Bratachem®), gelatin solution 1%, methanol (Bratachem®), lugol, ciprofloxacin, Mueller-Hinton Broth (MHB-Oxoid), Mueller-Hinton Agar (MHA-Oxoid), sulfuric acid solution, *Dragendorff* reagent, *Liebermann-Burchard* reagen, *Mayer* reagent, vanillin 10% reagent in sulfuric acid solution and magnesium powder.

The animal used in this research was male *Mus musculus* mice and the bacteria used was *Shigella dysenteriae* ATCC 13313.

Plant Material

The samples that used in this study are *Musa balbisiana* Colla fruits from Manoko Garden, Lembang, West Java, Indonesia. Plant sample was identified in Plant Taxonomy Laboratory of Biology Major, Faculty of Mathematics and Natural Science Padjadjaran University.

Methods

Preparation of Fruits Extracts

Dried simplisia of banana klutuk fruits were extracted using maceration method. Dried simplisia extracted by maceration during 3x24 hours using ethanol 70% as the solvent. The extracts were evaporated using a rotary evaporator at 40-50^{O}C, then continued to evaporate on a water bath until dried extract with a constant weight was obtained [7].

Phytochemical Screening of Secondary Metabolites

Phytochemical screening of secondary metabolites was using a standard method to determine the contains alkaloids, flavonoids, tannins, quinones, saponins, steroids, and triterpenoids, in both simplisia and ethanol extracts of banana klutuk fruits.

Preparation of The Bacterial Suspension

Shigella dysenteriae ATCC 13313 in 5 mL Shigella-Salmonella agar in the test tube was incubated for 18-24 h at 37 °C. *Shigella dysenteriae* bacteria that have been cultured then suspended into a sterile physiological NaCl until the turbidity is equal to Mc Farland 4 [8].

Antibacterial Activity Test in Vivo

After all healthy mice were determined, the mice were divided into 5 groups: normal, negative control, positive control, group 1, and group 2. All groups of mice except normal group were induced to be dysentery by administration of 1 mL of *Shigella dysenteriae* bacterial suspension with turbidity equivalent to McFarland 4 intraperitonically. After diarrhea, Group 1 and group 2 were given banana klutuk fruits extract suspention with 125 mg / 20 g BW and 150 mg / 20 g BW peroralally. Positive control were given 20 mg / kg BW orally of antibiotics ciprofloxacin. The negative control group was given orally of 1% CMC

suspension. Normal control groups are fed without dysentery induction [9].

On the 7th day, the calculation of Shigella dysenteriae colony from fresh stools samples was performed. Fresh stools samples from each mice were collected as much as 0.1 g and suspended in 0.9 mL NaCl sterile physiology. The stools suspension in NaCl Physiologically diluted to 10^{-1}, 10^{-2}, 10^{-3} and then 10 µL from each dilution were inoculated on Shigella-Salmonella agar medium using the spread plate method. Then the medium was incubated at 37 ° C for 18 h. Calculation of bacterial colony is done by using colony counter and then Colony Forming Unit (CFU) was calculated using formula: CFU = number of colonies x dilution factor

Results and Discussion

Extraction Result

Extraction is performed to draw the chemical components in the simplicia. The method of extraction used in this research is maceration. The principle of maceration is to draw the active substance by soaking the simplicia in a suitable solvent for three days with the change of solvent every day at room temperature and protected from light [10]. The solvent will enter the cell through the cell wall. The content of the cell will dissolve because there is difference concentration between the solution inside the cell and outside the cell. A high concentration solution will be pushed out and replaced by a low concentration liquid (diffusion process). It repeats until there is a balance concentration between the solution outside and inside the cell [11].

The maceration method is a cold way extraction where the chemical subtance is carried out at room temperature without heating. The maceration method is the easiest method to perform, the tools required are also simple. Because of the chemical subtances is carried out without heating, the risk of destruction of the thermolable active ingredient component can be avoided.

The solvent used is 70% ethanol. The used of 70% ethanol solvent is because the material used is banana klutuk fruits dried simplicia so it takes ethanol with enough water content for wetting process [11]. The presence of water content in the solvent can facilitate the diffusion of secondary metabolites out of cells.

The extract of banana klutuk fruits obtained brownish liquid. The extract were concentrated with rotary evaporator at 50°C until a viscous extract was obtained. This is necessary because ethanol is disinfectant agent, which is able to inhibit the growth of bacteria and also can kill the bacteria, so it will interfere with the results of antibacillary dysentery activity of banana klutuk fruits ethanol extract [12]. In addition, ethanol content can also affect the metabolism of test animals and will affect the test results.

Phytochemical Screening Result

The results of the phytochemical analysis revealed varying constituents of these extracts, as follows: flavonoids, polyphenols, tannins, monoterpenoids, sesquiterpenoids, quinones and saponins**.** The result of phytochemical screening can be seen in Table 1.

Table 1: Phytochemical screening

Secondary metabolites	Results	
	Simplisia	Extract
Alkaloids	-	-
Quinones	+	+
Polyphenols	+	+
Monoterpenoids	+	+
Sesquiterpenoids	+	+

Tannins	+	+
Flavonoids	+	+
Saponins	+	+

Note: (+) presence; (-) absence

From that table, the banana klutuk fruits ethanol extract contains flavonoid, polifenol, tanin, monoterpenoid, seskuiterpenoid, kuinon, and saponin. Various literature study result suggest that the metabolites subtance like polifenol, flavonoid, tanin and saponin have the antibacterial activity with different mechanism. Suji leaves and bay leaves ethanol extract also contained flavonoid, tannin and polyphenol compounds and proven its active antibacterial activity against S. dysenteriae ATCC 13313 [13]. Flavonoids act as antibacterial by forming complex compounds against extracellular proteins that affecting the integrity of bacterial cell membranes [14]. Flavonoids can also cause damage to bacterial cell wall permeability, microsomes, and lysosomes as a result of the interaction between flavonoids and bacterial DNA. Polyphenols have antibacterial activity by denaturing proteins and interfering with cell membrane function, thereby becoming lysis [2]. Tanin has antibacterial activity by damaging components of cell membranes, cell walls, enzymes, genetic material, and other protein components [6]. Tannins can also protect the intestinal mucosa and decreasing bowel peristalsic [15]. The lipophilic terpenoid has antibacterial activity by destroying the bacterial cell membrane, it will react with the active side of the membrane, dissolve the lipid constituent and increase its permeability [16]. Saponin can increase the permeability of bacterial cell membranes, its causing membrane protein denaturation , the cell membranes will be damaged and lysis [14].

Antibacterial Activity Result

All mice used in this experiment were adapted for 7 days before testing. This is done to avoid depression in mice that can cause changes in body condition that may affect the results of the study.

On the 7th day, fresh stool samples were collected from each mice for microbiological number of Shigella dysenteriae colonies. Fresh stools were weighed as 0.1 g and suspended in 0.9 ml of NaCl sterile physiology. The stools suspension diluted into 10^{-1}, 10^{-2}, 10^{-3} concentration ,10 µl of each dilution was grown on Shigella-Salmonella agar and then incubated at 37°C for 18 hours. All is done aseptically to reduce the risk of contamination. Dilution of stools suspension was done to facilitate the observation and calculation of *Shigella dysenteriae* colonies. The results of the number of *Shigella dysenteriae* colonies can be observed in Table 2.

Table 2: Antibacterial activity results

Group	Colonies (cfu/ml)
Normal Control	0
Negatif Control	38998,002
Positif Control	4
Group 1(125 mg/ 20 g BB)	4750
Group 2(150 mg/ 20 g BB)	385,66

From the results, it can be seen that the extract has antibacterial activity against *Shigella dysenteriae*. In the normal control group there is no *Shigella dysenteriae* colony because *Shigella dysenteriae* is not a normal flora in the feces. The positive control had a much smaller number of

colonies than the negative control because the antibiotics of ciprofloxacin were effective to eridicate Gram Negative bacterial infections such as *Shigella dysenteriae.* The administration of the extract also showed differences in the number of *Shigella dysenteriae* colonies compared with the negative control. Banana klutuk ethanol extract showed the presence of antibacillary dysentery activity in the test animals. In group 2, the number of *Shigella dysenteriae* colony was less than group 1. It showed that banana klutuk fruits ethanol extract with dose 150mg / 20g BW gave better antibacillary dysentery activity than the 125mg / 20g BW.

Conclusion

Our results demonstrated that ethanol extracts of Klutuk banana fruits have active antibacterial activity against *S. dysenteriae* ATCC 13313.

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