Antibacterial Potency of Dayak Onion Extract (*Eleutherine Americana merr*.) Against *Escherichia coli*

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Abstract

This research was conducted to determine the antibacterial effect of Dayak onions on Escherichia coli using the dilution method. Dayak onions (Eleutherine americana Merr.) contain several active substances that act as antibacterial agents, such as flavonoids, tannins, terpenoids, alkaloids, and phenols. Dayak onions were extracted using a maceration method with 96% ethanol as the solvent. Dayak onions were then diluted with sterilized water (aquadest) and 1% CMC Na. Escherichia coli is the bacterium that causes the most diarrhea in both humans and animals. This research used two types of E. coli: E. coli ATCC 25922 and E. coli environmental isolates. The dilution method utilized four treatments of each bacterium and two controls to determine the Minimum Inhibitory Concentration (MIC), followed by streaking on Mueller Hinton Agar (MHA) medium to assess the Minimum Bactericidal Concentration (MBC). The treatments used were 1%, 5%, 10%, and 15%, along with a negative control and a positive control. The treatment group consisted of E. coli and dayak onion extract, the negative control contained only a suspension of E. coli, and the positive control contained only dayak onion extract; each treatment was repeated three times respectively. The MIC value cannot be determined because all the MIC test tubes show similar turbidity, and there is no difference in turbidity between the pre-incubation and post-incubation phases. The MBC test showed different results between E. coli ATCC 25922 and E. coli environmental isolates. The MBC test results indicated that the Dayak onion extract acted as a bactericide against E. coli ATCC 25922 at a 5% concentration, while the MBC test results showed that the Dayak onion extract acted as a bactericide against E. coli environmental isolates at a 1% concentration.

Keywords: Dayak Onion, Antibacteria, *Escherichia coli*.

1. Introduction

Indonesia is known for its rich and diverse flora, many of which have been utilized by its people to create various forms of medicine, commonly referred to as herbal remedies (Zuhud, 2012). One plant that serves as an alternative medicine and has been empirically used within several community groups is the Dayak onion plant [1].

Dayak onions (Eleutherine americana Merr.) are a type of onion found in the interior forests of Kalimantan and are still not widely known to the public [1]. This plant possesses various properties, including antioxidant, sunscreen activity, anti-inflammatory, antidiabetic, antiplatelet aggregation, antibacterial, anticancer, antiamoebic, antifungal, and subacute toxicity [1]. According to this research, the efficacy of Dayak onion bulbs as an antibacterial can serve as an alt'ernative medicine alongside antibiotics.

Antibiotics are typically administered to reduce the incidence of diseases caused by bacteria, including E. coli, which is part of the normal flora but can become pathogenic if its numbers are excessive and the body's immune system is weakened [2]. *Escherichia coli* is also used as an indicator of contamination in livestock and product manufacturing [3]. The use of antibiotics can lead to new health issues, specifically antibiotic resistance. Furthermore, the use of antibiotics incurs costs that the general public may find unaffordable [4].

Previous research conducted by [5] showed that Dayak onions contain many phytochemical compounds, some of which function as antibacterials. These compounds, such as flavonoids, alkaloids, tannins, phenolics and triterpenoids, are known to exhibit antibacterial properties [6].

Based on this, the author seeks to offer an alternative solution by using ethanol extract from Dayak onion (Eleutherine americana Merr.). This research aims to assess the efficacy of ethanol extract from Dayak onion bulbs (Eleutherine americana Merr.) in inhibiting the growth of, and killing, E. coli.

2. Materials and Methods

2.1 Place and Materials

The primary ingredient used is Dayak onion bulbs, which are sourced from the North Samarinda District in Samarinda, East Kalimantan. The materials for the antibacterial test include E. coli ATCC 25922 and E. coli field isolates. Other ingredients consist of 96% ethanol, sterile distilled water, and 0.9% physiological NaCl. The tools utilized are test tubes, micro pipettes, microtips, syringes, vortex mixers, microscopes, incubators, water baths, refrigerators, Petri dishes, test tube racks, Bunsen burners, measuring cups, Erlenmeyer flasks, cover slips, aluminum foil, digital analytical scales, autoclaves, ovens, and evaporators.

2.2 Creating Simplicia.

4 kg Dayak onion bulbs, washed until clean. Then the tubers are sliced thinly and dried without being exposed to direct sunlight. Next, it is ground into powder (simplisia).

2.3 Extract Creation

The Dayak onion simplicia is macerated by soaking it in 96% ethanol solvent until it is fully submerged for 3x24 hours, followed by filtration using filter paper. The resulting filtrate is collected and evaporated at a temperature of 30-40˚C (Harbone, 1996), producing a pure, thick liquid extract. The obtained extract is prepared in various concentrations: 1%, 5%, 10%, and 15%. The Dayak onion extract is then diluted with distilled water and 1% CMC Na.

2.4 Preparation of Bacterial Suspension

To prepare a bacterial suspension, collect several colonies and suspend them in 0.9% physiological NaCl, stirring thoroughly until the turbidity matches that of McFarland standard no. 1, which corresponds to a bacterial count of 3 x 10^8 cells/ml (Clinical and Laboratory Standards Institute, 2018). Then, perform serial dilutions to achieve a bacterial count of 3 x 10^6 cells/ml.

2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC determination was performed using four different concentrations for each bacterium, namely 1%, 5%, 10%, and 15%, along with negative and positive controls, with three

repetitions. One milliliter of bacterial suspension was added to each tube. Then, the extract was added according to the concentration level of one milliliter. The negative control comprised two milliliters of bacterial suspension, while the positive control consisted of two milliliters of Dayak onion extract without bacteria. The samples were then incubated at 37˚C for 24 hours.

The minimum bactericidal concentration (MBC) is determined to identify the lowest concentration that is effective at killing bacteria. To determine the MBC, all tubes used for the minimum inhibitory concentration (MIC) test are inoculated on Mueller-Hinton agar (MHA) media by streaking. The tubes are then incubated at 37˚C for 24 hours.

3. Results

The identification results of the two E. coli bacteria are presented in Table 1, confirming that the two isolates used were indeed Escherichia coli. In this study, the MIC values for all tubes in the MIC test could not be determined since the clarity in each tube was not sufficiently visible for both E. coli ATCC 25922 and E. coli field isolates Figure 1.

The MBC data obtained indicated that in E. coli ATCC 25922 bacteria, the MBC value was at a concentration of 5%, while E. coli field isolates exhibited an MBC value at a concentration of 1%. This study demonstrates that dayak onion extract is effective in killing Escherichia coli bacteria. The MBC data in this study can be seen in Table 2, and a depiction of bacterial growth in MHA is presented in Figure 2 and Figure 3.

Table 1 Results of identification tests of *E. coli* ATCC 25922 bacteria and *E. coli* field isolates

Identification Test		***E. coli ATCC 25922***	***E. coli* field isolates**
Macroscopic	Colony color	Metallic green	Metallic green
	Colony formation	Round	Round
	Colony type	Flat	Flat
Microscopic	Gram staining	Gram -	Gram -
	Cell shape	Rod	Rod
Biochemistry	TSIA	Positive (acid, acid, gas (+), H_2S (−))	Positive (acid, acid, gas (+), H_2S (−))
	Urea agar	Negative	Negative
	SIM	Motile (+), indole (+),H_2S (−)	Motile (+), indole (+),H_2S (−)
	MR	Positive	Positive
	VP	Negative	Negative
	SCA	Negative	Negative
	Glukose	Positive, gas (+)	Positive, gas (+)
	Maltose	Positive, gas (+)	Positive, gas (+)
	Mannitol	Positive, gas (+)	Positive, gas (+)
	Lactose	Positive, gas (+)	Positive, gas (+)
	Sucrose	Positive, gas (+)	Positive, gas (+)

Table 2 MBC test results for E. coli ATCC 25922 and E. coli field isolates

Treatment	Ulangan		
	I	II	III
1% *E.coli* ATCC 25922	+	+	+

5% *E.coli* ATCC 25922	-	-	-
10% *E.coli* ATCC 25922	-	-	-
11% *E.coli* ATCC 25922	-	-	-
1% *E. coli* field isolates	-	-	-
5% *E. coli* field isolates	-	-	-
10% *E. coli* field isolates	-	-	-
15% *E. coli* field isolates	-	-	-
Control -	-	-	-
Control +	+	+	+

Information: Control (-) : Only suspension of extract; control (+) : only suspension of bacteria; (+) : There is bacterial colony growth; (-) : There is no bacterial colony growth.

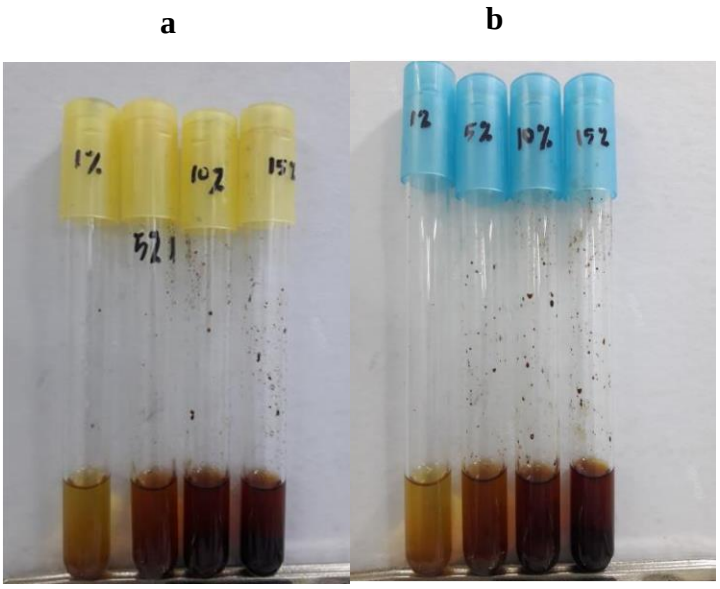

Figure 1 MIC shows the results of the dayak onion extract against E. coli ATCC25922 (a) and E. coli field isolate (b). In the MIC results, it is challenging to discern the level of clarity at all concentrations due to the dense color of the dayak onion extract.

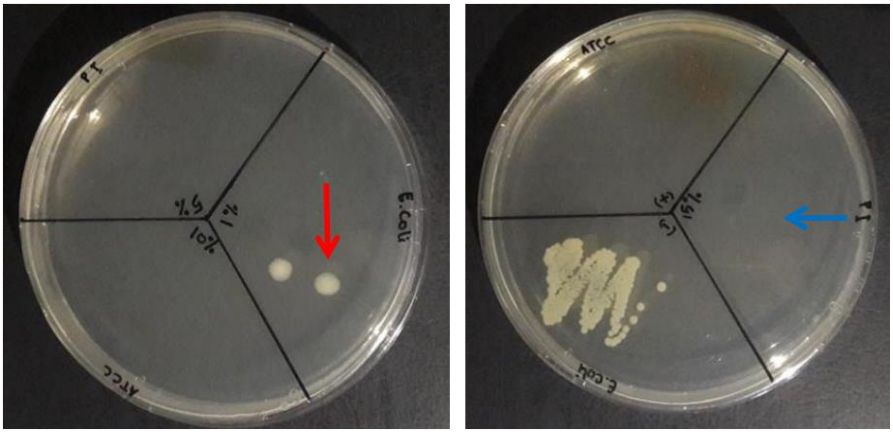

Figure 2 Dayak onion extract against Escherichia coli isolate ATCC 25922 (Red arrow: bacterial growth), (blue arrow: no bacterial growth).

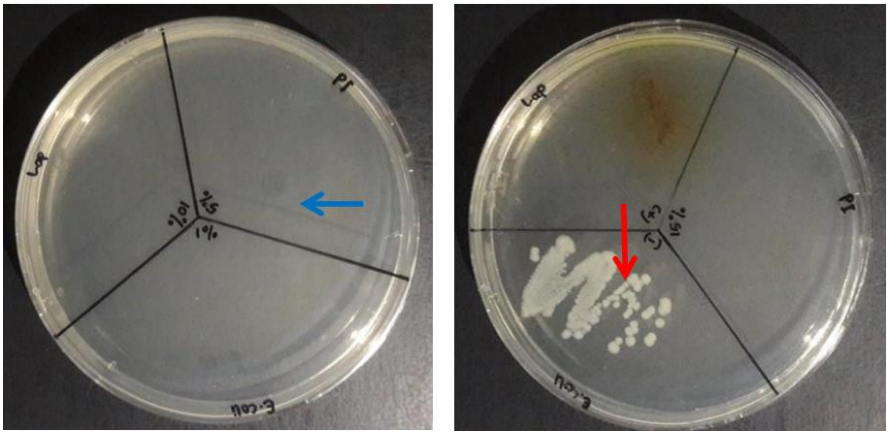

Figure 3 MBC results of Dayak onion extract against E. coli isolates from the field (Red arrow: there is bacterial growth), (blue arrow: there is no bacterial growth).

4. Discussion

The differences in MBC values in this study may arise from variations in bacterial origins, which lead to differences in genes and subsequently differences in resistance mechanisms. The E. coli ATCC 25922 strain is a standard bacterial product established by the Clinical Laboratory Standards Institute (CLSI) for antibacterial testing (ATCC, 2018). In contrast, field isolates of Escherichia coli are derived from environmental sources, resulting in strains that are not yet characterized.

Dayak onion extract (Eleutherine americana Merr.) can kill E. coli bacteria because it contains compounds that damage the components of bacterial structures. Flavonoids possess antibacterial mechanisms, which include directly killing bacteria, reducing pathogenic bacteria, and forming extracellular protein complexes and bacterial membranes. This inhibits the development of bacteria, leading to their eventual death [1; 2]. Flavonoids can disrupt the synthesis of peptidoglycan and ribosomes in Escherichia coli cells. The study by [8] states that the antibacterial activity of flavonoids is associated with three mechanisms: the first is the inhibition of nucleic acid synthesis. The second is the inhibition of cytoplasmic membrane function, and the third is the inhibition of energy metabolism.

Phenol acts as an antibacterial agent by degrading cell proteins and damaging the bacterial plasma membrane[9]. It binds to proteins via hydrogen bonds, leading to structural damage. The effectiveness of phenol compounds can be bacteriostatic or bactericidal, depending on their concentration [10].

Alkoloid memiliki mekanisme penghambat dengan cara mengganggu komponen penyusun sel bakteri yaitu peptidoglikan, sehingga lapisan sel tidak terbentuk secara utuh serta lisis dan menyebabkan kematian sel tersebut [11]. Bakteri *Escherichia coli* termasuk bakteri gram negatif. Dinding sel bakteri gram negatif tersusun atas membran luar, peptidoglikan dan membran dalam. Peptidoglikan yang terkandung dalam bakteri gram negatif memiliki struktur yang lebih kompleks dibandingkan gram positif [12].

Tannins can serve as bacteriostatic agents and combat infections in wounds [13]. Tannin compounds are polyphenols that inhibit bacteria by inactivating enzymes, blocking microbial cell adhesion, and disrupting protein transport within the inner layer of cells [14]. Tannins can shrink the bacterial cell membrane, leading to alterations in the cell's permeability, which disrupts cellular functions and ultimately results in cell death.

Meanwhile, other compounds recognized for their antibacterial activity include terpenoid compounds. These terpenoid compounds are part of essential oils that can disrupt the formation of cell walls or membranes, preventing their perfect development. The antibacterial mechanism of these compounds likely involves an increased disruption of the lipophilic components of bacteria [15]. Plants known to contain these compounds include dayak onions (Eleutherine americana Merr.).

The content of compounds in Dayak onions will be maintained depending on the extraction process. In this study, cold extraction was utilized through maceration to prevent damage to the compounds in the extract. The solvent used was 96% ethanol, which is a polar compound that attracts many polar compounds to the extract [16]. The use of ethanol as a solvent effectively dissolves the alkaloid and polyphenol compounds found in Dayak onions [17]. Based on this study, it can be concluded that flavonoids, terpenoids, phenols, tannins, and alkaloids can dissolve in ethanol solvent.
To dilute the dayak onion extract (Eleutherine americana Merr.) to achieve the desired concentration, this study utilized a 1% CMC-Na solvent. This 1% CMC-Na solvent has been tested and is effective as a solvent. Additionally, CMC-Na does not kill bacteria or is not bactericidal against bacteria.

5. Conclusion

Research results concluded that Dayak onion extract (Eleutherine americana Merr.) possesses antibacterial properties against E. coli. The minimum concentration of Dayak onion extract that can inhibit the growth of E. coli remains unknown. The minimum killing concentration for E. coli ATCC 25922 is at a concentration of 5%, whereas for field isolates of E. coli, it is at a concentration of 1%.

Conflict of Interest
The authors have no conflicts of interest regarding this investigation.

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