Difference inhibitory power between extract of Guava Leaves and Bay Leaves against Escherichia coli Bacterial growth

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Abstract. Escherichia coli is a Gram-negative bacteria that has a natural habitat in the human digestive tract. This bacteria is a normal flora but have a potency to be pathogenic. Escherichia coli is the most common cause of diarrhea. Guava leaves and bay leaves contain essential oils, tannins, flavonoids, and saponins which have antimicrobial effects. The purpose of this study was to determine the difference in the effectiveness of inhibition between ethanol extract of guava leaves and bay leaves on the growth of Escherichia coli bacteria. This research was an experimental laboratory study. The inhibitory test was carried out by measuring the clear zone around the paper disc using calipers. The results of data analysis using one way ANOVA showed that there were significant differences between groups of extracts with $F$ output = 49.83 and $p = 0.00$ ($p \leq 0.05$). Guava leaf extract concentration of 10%, 30% was significantly different from bay leaf extract concentration of 10%, 20%, 30%, with a $p$-value $\leq 0.05$. The conclusion of this study is Ethanol extract of guava leaves has a higher inhibitory ability than the ethanol extract of bay leaves.

1. Introduction
Escherichia coli is a Gram-negative bacteria that has a natural habitat in the human digestive tract [1]. This bacteria is a normal flora but also have a potency to be pathogenic. Escherichia coli is the most common cause of diarrhea. Diarrhea is a condition when the stool consistency is liquid and occur more than three times a day and lasts for 14 days [2]. According to data from Ministry of Health of Indonesia, the incidence of diarrhea morbidity from 2000 to 2010 tend to increase from 301 to 411 per 1000 population. According the age group, the highest prevalence occurs in children and toddlers [3].

Some therapies for treating diarrhea include pharmacological and non-pharmacological therapy. Pharmacological therapy using chemicals can cause various side effects and antibiotic resistance. The use of traditional plants can be an option as a substitute for chemical drugs, in addition to its cheap price, it is also easy to find in the surrounding environment. Medicinal plants that are often used by the community to treat diarrhea are guava leaves.

Guava leaf (Psidium guajava L.) is a type of medicinal plant that has several benefits such as anti-inflammatory, antimicrobial and analgesic. There are several compounds in guava leaves, namely essential oils, polyphenols, carotenes, flavonoids, saponins, and tannins. Based on the research of Yuniarti in Plantus, decoction of guava leaves can inhibit the growth of Escherichia coli bacteria at a concentration of 10%, whereas the study of Ekananda et al., guava leaves extract (Psidium Guajava L.) in hand sanitizer gel preparations can inhibit the growth of Escherichia bacteria coli at a concentration of 10%. According to the study by Nolia et al., guava leaf extract has an inhibitory effect on the growth of
Escherichia coli at concentrations of 20%, 40%, and 60%, with the category of moderate to strong inhibition zone diameters [4-6].

Plants that have antimicrobial effects in addition to guava leaves are bay leaves (Syzygium polyanthum). Plants that are often used as a spice in cooking can also act as traditional medicine with the same content as guava leaves, namely essential oils, tannins, flavonoids, and saponins [7]. Based on the research of Sari, ethanol extract of bay leaves (Syzygium polyanthum) can inhibit the growth of Escherichia coli bacteria at a concentration of 20% [8]. According to Yuliati's study, a test of the ethanolic extract of bay leaves in KLT Bioautography had an effect on Escherichia coli [9].

The purpose of this study was to measure the inhibition of guava leaves and bay leaves ethanol extract with concentrations of 10%, 20%, and 30%, respectively, to the growth of Escherichia coli bacteria, and to analyze the effectiveness of the inhibitory power on the growth of Escherichia coli bacteria.

2. Methods

2.1. Tools and materials
The tools used in this study consisted of a tool for making ethanol extract of guava leaves and bay leaves, which were Erlenmeyer vacuum, evaporator rotary vacuum, autoclave, Wattman filter paper No.2. The tools used in the inhibitory test were petri dishes, spirits lamps, calipers, paper discs, drop pipettes, ose, test tubes, and incubators.

The materials used in this study were guava leaves, bay leaves, Escherichia coli, Tetracycline antibiotics disk 30 µl, 96% ethanol, and sterile aquadest. Mueller-Hinton Nutrien agar was used as a medium for bacterial growth in the inhibitory power test, whereas MacConkey agar was used as a medium for bacterial identification tests.

2.2. Inhibitory test
The first step was carried out by applying culture of Escherichia coli bacteria in the form of suspension on Mueller-Hinton agar plate. Then put randomly the paper disc that has been soaked for ± 60 minutes with guava leaves extract concentrations of 10%, 20%, and 30%, bay leaves extract concentrations of 10%, 20%, and 30%, 96% ethanol solution (negative control ) on the agar media which has been rubbed with bacteria. Put the 30 µl Tetracycline disk (positive control) on the agar media which has been smeared by bacteria. Repeat four times.

The agar media containing culture of Escherichia coli was incubated at 37°C for 24 hours. Measurement of inhibition zones formed around the paper disc using a calipers with a thickness of 0.05 mm. It can be measured by determining the longest distance from the circle of inhibitory zone formed, then each side is marked with a marker and a straight line was drawn to form a diameter. The measurement results were recorded in the recording form.

3. Results
The results of the study showed that each test material has an inhibitory power. This was indicated by the descriptive results presented in Table 1 below.

| No | Test Material     | Mean  | Min.  | Max.  | SD   |
|----|-------------------|-------|-------|-------|------|
| 1  | Positive control  | 23.25 | 22.50 | 24.50 | 0.957|
| 2  | Negative control  | 6.00  | 0.00  | 8.50  | 4.020|
| 3  | Guava leaves 10%  | 25.50 | 24.00 | 26.00 | 0.957|
| 4  | Guava leaves 20%  | 25.00 | 23.00 | 27.00 | 1.632|
| 5  | Guava leaves 30%  | 26.25 | 25.00 | 27.00 | 0.957|
| 6  | Bay leaves 10%    | 22.25 | 21.00 | 23.00 | 0.957|
| 7  | Bay leaves 20%    | 22.25 | 20.00 | 24.00 | 1.707|
| 8  | Bay leaves 30%    | 22.50 | 21.00 | 24.00 | 1.290|
Of the 4 repetitions, the greatest value and the smallest value of the inhibition zone will be measured for each test material. From the mean inhibition zone of each test material against the growth of Escherichia coli bacteria, 30% guava leaves has the largest mean of 26.25, while the smallest mean was negative control that was equal to 6.00.

The normality test of the data using the Kolmogorov Smirnov test shows that the data were normally distributed because the p value was ≥ 0.05. The homogeneity test of the data with Levene Test showed that the data was homogeneous with a p value of ≥ 0.05. Because of the data was normally distributed and homogeneous, the different tests between groups of data were carried out by one way ANOVA test followed by Least Significant Difference (LSD). One way Anova test results can be seen in table 2.

Table 2. One Way ANOVA Test Results between Ethanol Extract of Guava Leaves and Bay Leaves.

|                      | Sum of Squares | DF  | Mean square | F        | P     |
|----------------------|----------------|-----|-------------|----------|-------|
| Between group        | 1184.50        | 7   | 169.214     | 49.83    | 0.00  |
| Within groups        | 81.50          | 24  | 3.396       |          |       |
| Total                | 1266.00        | 31  |             |          |       |

The results of the ANOVA test show that the value of F output = 49.83 with the value of p = 0.00 or p ≤0.05. These results indicate that at least one group has a different diameter of the inhibition zone. To find out which groups of test material were different, Post Hoc Analysis was carried out. Post Hoc analysis between negative controls and extract test materials showed significant differences with p values ≤0.05. Guava leaves and bay leaves with a concentration of 10%, 20%, and 30% has the effect of inhibition because it was greater than negative control. Post Hoc test results can be seen in table 3.

Table 3. Post Hoc LSD test result between Ethanol Extract of Guava leaves and Bay leaves

| Comparative Test Material | Compared Test Material | Mean differences | P value |
|---------------------------|------------------------|------------------|---------|
| Guava leaves 10%          | Guava leaves 20%       | 0.50             | 0.705   |
| Guava leaves 10%          | Guava leaves 30%       | -0.75            | 0.570   |
| Guava leaves 10%          | Bay leaves 10%         | 3.25*            | 0.020   |
| Guava leaves 10%          | Bay leaves 20%         | 3.25*            | 0.020   |
| Guava leaves 10%          | Bay leaves 30%         | 3.00*            | 0.030   |
| Guava leaves 20%          | Guava leaves 30%       | -1.25            | 0.347   |
| Guava leaves 20%          | Bay leaves 10%         | 2.75*            | 0.045   |
| Guava leaves 20%          | Bay leaves 20%         | 2.75*            | 0.045   |
| Guava leaves 20%          | Bay leaves 30%         | 2.50             | 0.067   |
| Guava leaves 30%          | Bay leaves 10%         | 4.00*            | 0.005   |
| Guava leaves 30%          | Bay leaves 20%         | 4.00*            | 0.005   |
| Guava leaves 30%          | Bay leaves 30%         | 3.75*            | 0.008   |
| Bay leaves 10%            | Bay leaves 20%         | 0.00             | 1.000   |
| Bay leaves 10%            | Bay leaves 30%         | -0.25            | 0.849   |
| Bay leaves 20%            | Bay leaves 30%         | -0.25            | 0.849   |

4. Discussion
The results from this study showed that the inhibitory test of the ethanol extract of guava leaves and bay leaves with concentrations of 10%, 20%, and 30%, respectively, has shown the presence of an inhibitory zone with a large diameter based on the classification of the inhibition zone 13, which means that Guava leaves extract and bay leaves have a very strong inhibitory ability. The high diameter of the inhibition
zone produced in both extracts can be caused by several factors, one of which was the high moisture content of guava leaves and bay leaves. According to Melliawati if E. coli was placed in a hypertonic solution then the contents of bacterial cells will experience plasmolysis, in addition, guava leaves and bay leaves also contain anti-bacterial compounds [7]. These compounds are essential oils, polyphenols, carotene, flavonoids, saponins, and tannins. Quite a lot of compounds in guava leaves are tannins and flavonoids, with a tannin content of around 9%. Bay leaves contain essential oils as much as 0.05%.

Tannin has a pyrogalol group which was a phenol group which can inhibit bacterial growth or kill it by reacting to bacterial protein cells resulting in protein denaturation in the bacterial cell wall. Denaturation causes metabolic disorders that result in bacterial lysis [8,9].

Flavonoids are the largest group of phenol compounds. This compound denatures proteins that cause damage to the bacterial cell wall. Essential oils have antibacterial ability by interfering with enzymes that useful in forming energy so that cell growth is inhibited16 In negative controls (ethanol 96%) an inhibitor zone was formed with a moderate diameter, this was because ethanol 96% has a function as antiseptic [10]. According to Trifani in Arifianti et al ethanol is a polar compound, where the compound is a water-soluble compound. E. coli bacteria have a small hole called porin, if the molecule of the active substance is small and polar, it can penetrate the outer wall, pass through the porin and then enter the bacterial cell causing bacterial lysis [11,12].

Ethanol extract of guava leaves and bay leaves has different inhibitory power to the growth of E. coli bacteria. In this study it showed that the ethanol extract of guava leaves had a higher inhibitory ability than the ethanol extract of bay leaves. The ethanol extract of guava leaves which has the most effective ability to inhibit bacterial growth was a concentration of 30%. The difference in the ability of the inhibition between the ethanol extract from guava leaves and bay leaves was probably caused by differences in the content of the anti-bacterial compounds from the two types of extract, therefore further research is needed to measure the levels of anti-bacterial compounds from each extract.

5. Conclusion
The conclusion of this study is Ethanol extract of guava leaves has a higher inhibitory ability than the ethanol extract of bay leaves. The guava leaves ethanol extract which has the most effective ability to inhibit bacterial growth was 30% concentration.

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