

Antibacterial Activity of Bay Leaf (*Syzygium polyanthum*) Ethanol Extract on *Escherichia coli* Growth

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Abstract

Acute diarrhea is liquid stool with a frequency of more than 3 times a day and lasts less than 14 days. One of the most common causes of acute diarrhea is *Escherichia coli*. Diarrhea caused by bacteria can be treated with the use of antibiotics, but the relatively high intensity of antibiotic use causes various health problems, especially bacterial resistance. Therefore, the search for antibacterial agents derived from natural ingredients was carried out as an alternative treatment. Bay leaf (*Syzygium polyanthum*) is one of the natural ingredients that has the potential as an antibacterial because it contains active compounds such as essential oils, flavonoids (quercetin), saponins, tannins, and alkaloids. This study aimed to analyze the antibacterial activity of the ethanolic extract of bay leaves on the growth of *Escherichia coli*. This research uses the dilution method which aims to determine the Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). The results showed that the MIC value at a concentration of 200mg/ml and the MBC value at a concentration of 500mg/ml.

Key words: bay leaf extract, *Escherichia coli*, Minimum bactericidal concentration, Minimum inhibitory concentration

Abstrak

Diare akut merupakan feses yang berbentuk cair dengan frekuensi lebih dari 3 kali dalam sehari dan berlangsung kurang dari 14 hari. Salah satu penyebab tersering terjadinya diare akut adalah *Escherichia coli*. Diare yang disebabkan oleh bakteri dapat diobati dengan penggunaan antibiotik, namun intensitas penggunaan antibiotik yang relatif tinggi menimbulkan berbagai permasalahan bagi kesehatan terutama resistensi bakteri. Oleh karena itu, dilakukan pencarian agen antibakteri yang berasal dari bahan alam sebagai alternatif pengobatan. Daun salam (*Syzygium polyanthum*) merupakan salah satu bahan alam yang berpotensi sebagai antibakteri karena mengandung senyawa aktif seperti minyak atsiri, flavonoid (kuersetin), saponin, tanin, dan alkaloid. Penelitian ini bertujuan menganalisis aktivitas antibakteri ekstrak etanol daun salam terhadap pertumbuhan *Escherichia coli*. Metode penelitian ini menggunakan metode dilusi yang bertujuan untuk mengetahui Kadar Hambat Minimum (KHM) dan Kadar Bunuh Minimum (KBM). Hasil penelitian didapatkan nilai KHM pada konsentrasi 200mg/ml dan nilai KBM pada konsentrasi 500mg/ml.

Kata kunci : ekstrak daun salam, *Escherichia coli*, Kadar Bunuh Minimum, Kadar Hambat Minimum.

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1. Introduction

Acute diarrhea is liquid stool with a frequency of more than 3 times a day or more often than normal and lasts less than 14 days. Globally, it is estimated that 99,000,000 adults experience acute diarrhea each year. The incidence of acute diarrhea in developing countries including Indonesia is 2-3 times higher than developed countries (Setiati *et al.*, 2014). Acute diarrhea is caused by various microbes such as bacteria, viruses, and parasites. The bacteria that most often causes diarrhea is *Escherichia coli* (World Health Organization, 2017).

Escherichia coli is a normal flora found in the large intestine of humans and is opportunistic which can cause primary infection in the intestine. *Escherichia coli* is a Gram-negative rod-shaped bacteria (cocobacil) with a size of 0.4-0.7x1.4µm, most of which are motile, and some strains have capsules. *Escherichia coli* can produce enterotoxins, namely LT toxin (Thermolabile) and ST toxin (Thermostabile). LT toxin works by stimulating the enzyme adenyl cyclase contained in the epithelial cells of the small intestine mucosa, causing an increase in the activity of these enzymes and an increase in the permeability of intestinal epithelial cells, resulting in fluid accumulation in the intestine and causing diarrhea. ST toxin works by activating the enzyme guanylate cyclase to produce cyclic guanosine monophosphate, causing impaired absorption of chloride (Cl⁻) and sodium (Na⁺), in addition ST toxin also causes a decrease in small intestinal motility (Syahrurachman *et al.*, 2019).

Diarrhea caused by a bacterial infection can be treated with antibiotics. However, the relatively high intensity of use of antibiotics creates various problems and is a global threat to health, especially bacterial resistance to antibiotics. Initially resistance occurred at the hospital level, but gradually developed in the community, especially *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*. Antimicrobial Resistant in Indonesia (AMRIN-Study) has conducted research and proved that out of 2494 individuals in the community, 43% of *Escherichia coli* were resistant to ampicillin (34%), cotrimoxazole (29%), and chloramphenicol (25%). In addition, the results of a study of 781 hospitalized patients found that 81% of *Escherichia coli* were resistant to ampicillin (73%), cotrimoxazole (56%), chloramphenicol (43%), ciprofloxacin (22%), and gentamicin (18%) (Depkes RI, 2011).

According to Murhadi *et al* (2007) as cited by Hakim *et al.*, (2016), due to cases of antibiotic resistance, currently the search for new and more effective antibacterial agents, especially those from natural ingredients, can be used as an alternative for treatment. One of the plants that has the potential as medicine is bay leaf. Bay leaves are known to have antibacterial effects due to the active compounds contained in them such as saponins, triterpenoids, flavonoids (quercetin), polyphenols, alkaloids, tannins, and essential oils (simple phenols, phenolic acids such as gallic acid, sesquiterpenoids and lactones). The mechanisms of

these compounds as antibacterial include disrupting the permeability of cell membranes (saponins), damaging cell membranes by lipophilic compounds (triterpenoids), disrupting the integrity of bacterial cell membranes (flavonoids), and damaging bacterial cytoplasmic membranes (polyphenols) and influencing osmotic pressure. between bacteria and their environment (alkaloids), tannins by inhibiting replication and damaging bacterial cell walls, then essential oils by denaturing proteins and damaging bacterial cell membranes (Hakim *et al.*, 2016).

According to research Evendi (2017), bay leaf methanol extract has antibacterial activity against the growth of *Salmonella typhi* at concentrations of 25µg/well, 50µg/well, 100µg/well, 200µg/well, and 400µg/well with a diameter of 11.11mm, 11.78mm, 12.56mm, 12.78mm, and 14.67mm as well as for *Escherichia coli* with an average diameter of 11.33mm, 10.44mm, 11.33mm, 12.11mm, and 12.00mm. Another study, Dewanti and Wahyudi (2013) found that bay leaf infusion at concentrations of 10%, 20%, 30%, 40%, 80%, and 100% was unable to inhibit the growth of *Escherichia coli* by diffusion method.

Based on the above background, due to the presence of antibiotic resistance against *Escherichia coli*, a study was conducted to test the antibacterial activity of bay leaf extract (*Syzygium polyanthum*) using 96% ethanol against *Escherichia coli* which later could provide information to the public about the benefits of bay leaf extract as an antibacterial especially for diarrhea.

2. The Methods

Time and Location

This research was conducted in June 2021 at the Abdurrah Vocational School Laboratory, Microbiology and Parasitology Laboratory, Universitas Abdurrah.

Tools and Materials

The instrument used in this study was a spectrophotometer. The materials used in this study were bay leaf, *Escherichia coli* ATCC 25922 bacteria.

Bay Leaf Extract

2 kg of bay leaves washed, then dried in the sun to dry. Next, the dried bay leaves are blended to obtain the simplicia powder. A total of 500g of simplicia powder was extracted by maceration using 96% ethanol solvent in a 1:2 ratio for 24 hours and then filtered to produce a filtrate. Then the filtrate was evaporated with a vacuum rotary evaporator at a temperature of 60°C to obtain a thick extract.

Making Concentration of Bay Leaf Extract

Stock solution of 1000mg/ml bay leaf extract was made by adding 25g of extract plus 25ml of distilled water. Then, the initial stock of the extract was diluted into various concentrations, including 200mg/ml, 300mg/ml, 400mg/ml, and 500mg/ml.

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Phytochemical Screening

Phytochemical screening of bay leaves was carried out on tannins, flavonoids, saponins, alkaloids, and essential oils. The tannin test was carried out by taking 1ml of bay leaf extract, then adding 5 drops of FeCl_3 solution. If a green to blue-black color is formed, it indicates the presence of tannin compounds. The flavonoid test was carried out by taking 1ml of bay leaf extract, then evaporated to dryness, then added 1-2ml of ethanol, a little magnesium powder, and 2ml of 5M HCl. If a red to purple or red orange color is formed, it indicates the presence of flavonol compounds (quercetin), flavonones, flavonolol, and dihydroflavonol.

Saponin test, 1 ml of bay leaf extract was shaken with 2ml of water. If the foam is formed for ten minutes, does not disappear, as high as 1-10cm, the addition of 1 drop of 2N HCl indicates the presence of saponin compounds. Mayer's Alkaloid Test was carried out by adding 1ml of bay leaf extract with 2 drops of Mayer's reagent along the side of the test tube. The formation of a creamy white precipitate indicates the presence of alkaloid compounds. Wagner's test, 1ml of bay leaf extract was added to 1ml of Wagner's reagent along the side of the test tube. If a reddish brown precipitate is formed, it indicates the presence of alkaloid compounds. Essential Oil Test, (1). A total of 2ml of plant extracts were added with 5 drops of KMnO_4 solution, there will be a change in the color of KMnO_4 to pale or lost, (2) A total of 2ml of plant extracts were added with 1 ml of $\text{C}_4\text{H}_6\text{O}_3$ solution, then 1 ml of concentrated H_2SO_4 solution was added so that a green blue color would appear.

Bay Leaf Extract Antibacterial Activity Test against *Escherichia coli*

The initial step was carried out by mixing the isolates of *Escherichia coli* bacteria into a test tube containing 9ml of 0.9% NaCl which was in accordance with the 0.5% McFarland standard. Next, the bacterial suspension obtained was diluted to 10^7CFU/ml by taking 1ml of the bacterial suspension from 10^8CFU/ml which was inoculated into 9ml of 0.9% NaCl. Then it was diluted again to 10^6CFU/ml by taking 1ml of bacterial suspension from 10^7CFU/ml which was inoculated into 9ml NB. Then the bacterial suspension is ready to be tested for MIC and MBC.

The MIC test was carried out by taking 1ml of bay leaf extract with various concentrations (200mg/ml, 300mg/ml, 400mg/ml, and 500mg/ml) which was inoculated into a test tube containing 1ml of cultured *Escherichia coli* bacteria. In the control treatment the bacteria contained 2ml of suspension of *Escherichia coli* bacteria, while in the control treatment the solvent contained 1ml of ethanol extract 1000mg/ml of bay leaf with 1ml of distilled water added. All treatments were homogenized using a vortex and incubated at 37°C for 24 hours. After incubation time, 2ml of treatment was taken and the value of Optical Density (bacterial density seen as turbidity in the medium) was measured using a UV-Vis spectrophotometer

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(λ 630nm). The MIC value was obtained from the lowest extract concentration which was indicated by the clarity of the solution in the test tube.

Furthermore, to determine the MBC value, it was carried out using the spread plate method on NA medium, each of which came from the MIC tube (concentration 200mg/ml, 300mg/ml, 400mg/ml, and 500mg/ml) as much as 0.1ml using a micropipette. Then, it was incubated at 37°C for 24 hours. After incubation, the MBC value was obtained from the absence of bacterial colony growth at the lowest concentration.

Data analysis

The data obtained were analyzed descriptively and presented in the form of tables and figures.

3. Result and Discussion

Phytochemical test results showed that bay leaf extract contains active compounds such as tannins, flavonoids, saponins, alkaloids, and essential oils (Tabel 1). This is in accordance with research conducted by Wilapangga and Syaputra (2018) and Algabri *et al.*, (2018) which showed that bay leaves contain active compounds such as alkaloids, flavonols (kaempferol, myricetin, and quercetin), flavones (apigenin and luteolin), glycosylated flavonoids, sesquiterpene, lactones, monoterpene, germacrane alcohols, and essential oil (1.8-cineol (44.72%), α -terpinyl acetate (12.95%), and sabinene (12.82%)) as an antimicrobial.

Table 1. Phytochemical screening results

Compound	Results
Tannins	+
Flavonoids	+
Alkaloids	
- Mayer's	+
- Wagner's	+
Essential oils	
- KMnO ₄	+
- C ₄ H ₆ O ₃ and H ₂ SO ₄	+

The results of the MIC test showed that the 200mg/ml concentration of bay leaf extract could inhibit the growth of *Escherichia coli* which was indicated by an average absorbance value of 0.95. This can be seen from the average absorbance value for solvent control which is 0.17 and the average absorbance for bacterial control is 1.23 (Table 2). According to Nasution (2014), the higher the concentration, the level of turbidity in the test tube will decrease or the test tube will appear clearer. This can be proven by the absorbance value where the higher the concentration of the extract, the closer the absorbance value to the control solvent will be.

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Table 2. Average Absorbance Value of Bay Leaf Extract

Treatment	Mean \pm SD
Solvent control	0.17 \pm 0.044
Bacterial control	1.23 \pm 0.029
Concentration 200mg/ml	0.95 \pm 0.056
Concentration 300mg/ml	0.88 \pm 0.024
Concentration 400mg/ml	0.77 \pm 0.013
Concentration 500mg/ml	0.59 \pm 0.088

In the MBC test, the MBC value of bay leaf extract was found at a concentration of 500mg/ml which was indicated by the absence of growth of *Escherichia coli* bacteria on NA medium (Figure 1). According to research by Utami (2020) it is known that 96% ethanol extract of bay leaves can inhibit the growth of *Escherichia coli* at concentrations of 25%, 50%, and 75% with an average inhibition zone diameter of 14mm, 16mm, 20mm. This is different from the research conducted by Dewanti and Wahyudi (2013) who found that bay leaf infusion was not able to inhibit the growth of *Escherichia coli*. This may be influenced by several factors such as the type and origin of the bay leaf, thus affecting the percentage of active compounds contained in the bay leaf, as well as ineffective drying and extraction methods causing the active compounds in the leaves to not reach their maximum levels.

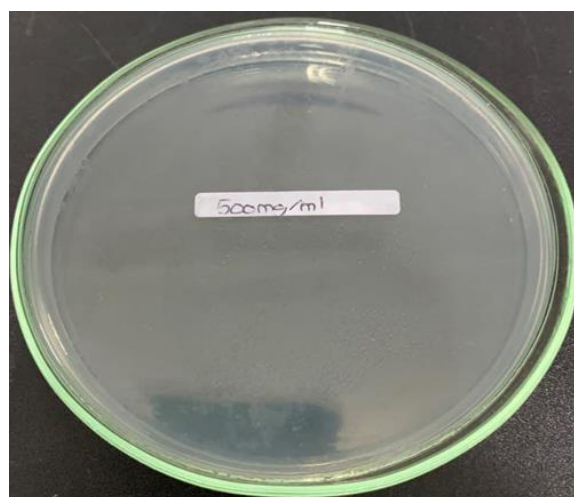


Figure 1. Results of MBC Bay Leaf Extract on the Growth of *Escherichia coli* at a concentration of 500mg/ml in NA medium

4. Conclusion

Bay leaf extract has active compounds such as tannins, flavonoids, saponins, alkaloids, and essential oils. The MIC value of bay leaf extract was found at a concentration of 200mg/ml and the MBC value at a concentration of 500mg/ml.

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