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The Effect of Bay Leaf (*Syzygium Polyanthum*) Extract Hand Washing Liquid Soap Preparation on The Growth of Escherichia Coli Bacteria in Vitro

Wahdaniah[1], Asep Sabolakna[1], Bella Ramadhanti[1], Bagus Muhammad Ihsan[1*], Yeremiah Rubin Tjamin[2]

[1] Department of Medical Laboratroy Technology, Poltekkes Kemenkes Pontianak, Pontianak, Indonesia.
[2] Faculty of Biology, National University Jakarta, Jakarta, Indonesia.

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Corresponding Author:
Bagus Muhammad Ihsan
ihsanfillah24@gmail.com

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Abstract: Bay leaf (*Syzygium polyanthum*) is known as a complement to cooking spices, also used by the community for treatment. The content of antibacterial compounds from bay leaves such as alkaloids, flavonoids, saponins and tannins which can inhibit bacterial growth. This study aims to determine the effect of hand washing liquid soap with bay leaf extract on the growth of *Escherichia coli* bacteria. This study uses a Quasi Experiment research design. The population in this study is located on Jalan Atot Ahmad, West Pontianak District. The sample used was liquid hand washing soap with bay leaf extract at a concentration of 3, 6, and 9%, 9 repetitions with a total of 27 samples, using the disc diffusion method. The results of the antibacterial study showed that the average diameter of the inhibition zone at 3% concentration was 8.27 mm, 6% concentration was 10.55 mm and 9% concentration was 11.16. The results of the Linear Regression test using the Anova test obtained $p_{value} = 0.000$, which is $p_{value} < \alpha$, with $\alpha = 0.05$, so that it was stated that H1 was accepted so that there was an effect on the preparation of liquid hand washing soap (*Syzygium polyanthum*) extract on the growth of Escherichia coli bacteria in vitro.

Keywords: Bacteria; Escherichia coli; Extract; Hand washing liquid soap; *Syzygium polyanthum*

Introduction

Bay leaf (*Syzygium polyanthum*) is a medicinal plant that can be used in everyday life. Its use is only based on experience passed down from generation to generation. Empirically, the boiled water of bay leaves is used by the community for the treatment of hypertension, high cholesterol, diabetes, gastritis, and diarrhea (Utami, 2020). The active compounds contained in bay leaves include alkaloids, flavonoids, saponins, tannins, and steroids which act as antibacterial (Evendi, 2017; Falade et al., 2022; Mursyida et al., 2021). Escherichia coli is a gram-negative rod-shaped bacterium that does not form spores and is a normal intestinal flora. Nonetheless, several types of Escherichia coli can be pathogenic. Various studies have shown that some strains or strains of Escherichia coli bacteria can cause outbreaks of diarrhea or vomiting, especially in children (Werner et al., 2024).

The active compound of bay leaf extract has an inhibition zone against the growth of Salmonella typhi and Escherichia coli bacteria. The response to inhibition of the growth of Salmonella typhi and Escherichia coli bacteria by bay leaf extract (*Syzygium polyanthum*) is included in the strong category (Irshad et al., 2024). A study conducted by Kilis et al. (2020) stated that the formulation of bay leaf extract (*Syzygium polyanthum*) had antibacterial activity against Staphylococcus aureus

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bacteria with a diameter of inhibition zone produced in an ointment concentration of 10%, namely 2.4 mm, 20%. ie, 3.9 mm and 40% ie, 7.4 mm. The largest diameter of the inhibition zone is at a concentration of 40%.

One of the intermediaries for the spread of disease is the hands; therefore, washing hands with soap is one way to prevent the emergence of a chain of transmission of various infectious diseases such as diarrhea (Ningsih et al., 2024). Medicinal plants can be used as an alternative in dealing with diarrhea. The presence of compounds that act as antibacterial is possible to inhibit the growth of Escherichia coli bacteria that cause diarrhea (Septhimoranie et al., 2022; Plaatjie et al., 2024). Based on the description above, the researchers are interested in formulating and testing whether Salam leaf extract (*Syzygium polyanthum*) made in the form of liquid hand-washing soap can inhibit the growth of Escherichia coli bacteria.

Novel aspects worth highlighting, the study investigates the potential antibacterial properties of bay leaf extract, which is a novel approach to exploring natural sources for antibacterial agents. In an era where antibiotic resistance is a growing concern, exploring alternative sources like plant extracts can provide sustainable solutions. The study utilizes a quasi-experimental research design to evaluate the antibacterial effect of hand washing liquid soap containing bay leaf extract. While experimental designs are common in scientific research, quasi-experimental designs are particularly useful when true randomization is not feasible. This approach enhances the applicability of the findings to real-world settings. Overall, the combination of exploring natural antibacterial agents, employing a quasi-experimental design, conducting community-based sampling, assessing concentration-dependent activity, and performing in vitro evaluation contributes to the novelty and significance of the journal article. These aspects collectively advance our understanding of natural antibacterial agents and their potential applications in promoting hygiene and preventing bacterial infections.

Method

The research design used was quasi-experimental (Quasi Experiment), using the disc diffusion method to measure the inhibition zone of hand washing liquid soap with bay leaf extract (*Syzygium polyanthum*) concentration of 3, 6, and 9% on the growth of Escherichia coli bacteria. The principle of the test is that a paper disc that has been soaked in the test solution is placed on a solid medium that has been inoculated with the test bacteria. Bacterial growth was observed to see the clear zone around the disc.

The tools used in the study were blender, analytical balance, Erlenmeyer, beaker glass, hotplate, stir bar, autoclave, incubator, oven, petri dish, tweezers, spirit lamp, blank disk, cotton, needle loops, universal pH, ruler, test tube and spirit lamps. The materials used in the study were bay leaf extract, Escherichia coli bacteria, Virgin Coconut Oil (VCO), Potassium hydroxide (KOH), glycerin, Propylene glycol, cocamide-DEA, aquadest, Mueller Hinton Agar (MHA) medium, Eosin Methylene Blue Medium Agar (EMBA), barium chloride ($BaCl_2$) and sulfuric acid ($H2SO_4$). This research was conducted in June 2023 at the Pontianak Ministry of Health's Integrated Polytechnic Laboratory.

Preparation of Bay Leaf Extract

4.3 kg of sorted bay leaves were dried using an oven at 40^{o}C; after drying, they became 1.4 kg and then mashed using a blender to produce 1.3 kg of dried simplicia. Then the extraction process was carried out using the maceration method using 96% ethanol solvent; after that, evaporation was carried out using a rotary evaporator to separate the solvent from the resulting extract so that a thick extract was obtained. From this process, 116 gr of bay leaf extract was obtained.

Manufacture of Washing Liquid Soap

VCO is weighed as much as 3 grams for each concentration, then put into a beaker glass and heated on a hotplate until it reaches a temperature of 75^{o}C, then removed from the hotplate. Add 1 gram of KOH, and stir until homogeneous. Then add the other supporting ingredients, 0.45 grams of glycerin, 0.85 grams of propylene glycol, 0.2 grams of cocamide-DEA, and a little distilled water, and stir until homogeneous. The finished soap base is added to the bay leaf extract according to the calculation of each concentration contained in Table 1.

Table 1. How to Make Liquid Hand Soap Preparations of Bay Leaf Extract

Concentration	Base	Concentration 3%	Concentration 6%	Concentration 9%	Function
Bay leaf extract	0 gr	0.3 gr	0.6 gr	0.9 gr	Active substance
VCO	3 gr	3 gr	3 gr	3 gr	Base soap
KOH	1 gr	1 gr	1 gr	1 gr	Emulsifier
Glycerin	0.45 gr	0.45 gr	0.45 gr	0.45 gr	Humectant
Propylene glycol	0.85 gr	0.85 gr	0.85 gr	0.85 gr	Humectant
Cocamide-DEA	0.2 gr	0.2 gr	0.2 gr	0.2 gr	Foaming surfactant
Aquadest	Add 10 ml	Add 10 ml	Add 10 ml	Add 10 ml	Solvent

Evaluation of Liquid Hand Washing Soap Preparations

The organoleptic test is a physical test of liquid soap, including color, smell, and shape. The pH test was carried out using Universal pH by dissolving 1 ml of liquid soap sample in 9 ml of distilled water and homogenizing it. Then dip the universal pH. Results are read on the pH scale and recorded (Maksumah et al., 2021). The foam height test was carried out by dissolving 1 ml of liquid soap sample with 9 ml of distilled water and homogenizing it. Then shake vigorously for 2 minutes, observe the foam's height, and measure the foam's height using a ruler (Kurniawati et al., 2022).

Inhibitory Power Test of Liquid Hand Washing Soap Preparations of Bay Leaf Extract by Disc Diffusion Method

Suspension of Escherichia coli bacteria in the swab evenly on Mueller Hinton Agar (MHA) media. Paper discs with a diameter of 6 mm were soaked in the liquid hand-washing soap with bay leaf extract that had been made. Then using tweezers, the disc paper was placed on the surface of the MHA media and pressed slightly, and then incubated at 37^{o}C for 24 hours. The inhibition zone formed was measured using a ruler (Savitri et al., 2018).

Data Analysis

The data obtained from measuring the diameter of the inhibition zone was then analyzed using the Linear Regression Test using a computer application program.

Result and Discussion

Table 2. Results of Phytochemical Screening of Bay Leaf Extract (*Syzygium polyanthum*)

Phytochemical Test	Test results
Alkaloid	Positive
Flavonoid	Positive
Saponin	Positive
Tanin	Positive

Table 3. Organoleptic Test Results for Hand Washing Liquid Soap with Bay Leaf Extract

Concentration	Color	Odor	Shape
3%	Brownish	Typical of bay leave	A little thick
6%	Greenish brown	Typical of bay leave	A little thick
9%	Greenish brown	Typical of bay leave	A little thick
Soap base control	White	VCO	A little thick

Table 4. Test Results for the Degree of Acidity (pH) for Hand Washing Liquid Soap with Bay Leaf Extract

Concentration	Degree of Acidity (pH)
3%	9
6%	9
9%	9
Soap base control	9

Table 5. High Foam Test Results for Hand Washing Liquid Soap with Bay Leaf Extract

Concentration	Foam Height(mm)
3%	20
6%	20
9%	20
Soap base control	60

Table 6. Inhibitory Test Results for Hand Washing Liquid Soap with Bay Leaf Extract

	Obstacles zone			
Sample code	3%	6%	9%	Base Control
R1	9	10.5	10.5	0
R2	8.5	11	11	0
R3	7.5	10	11	0
R4	8.5	10	10.5	0
R5	8	11	12	0
R6	9	10.5	11.5	0
R7	7	11	11.5	0
R8	8.5	10.5	11	0
R9	8.5	10.5	11.5	0
Average	8.27	10.55	11.16	0
Inhibition zone category	Medium	Medium	Strong	There is no inhibition zone

Based on Table 6, the results of the inhibition test of liquid hand washing soap with bay leaf extract on the

growth of Escherichia coli bacteria with a concentration of 3% obtained an average inhibition zone diameter of 8.27 mm, a concentration of 6% obtained an average inhibition zone of 10, 55 mm and a concentration of 9% obtained an average inhibition zone diameter of 11.16 mm. The results of the phytochemical screening showed that bay leaf extract contains antibacterial compounds, namely alkaloids, flavonoids, saponins, and tannins. The formation of a brick-red precipitate indicates a positive alkaloid result. The formation of yellow color indicates a positive result for flavonoids. The foam formation indicated saponin-positive results and tannin-positive results were indicated by the formation of a greenish-black color.

The results of the organoleptic examination of liquid hand-washing soap with bay leaf extract included color, smell, and shape. Bay leaf extract hand washing liquid soap is brownish to greenish brown, has a distinctive bay leaf odor, and is slightly thick in shape. The results of the pH examination showed that the pH of the liquid hand washing soap with bay leaf extract concentrations of 3, 6, and 9% was 9. This indicated that the liquid hand washing soap was safe because the pH of the liquid hand washing soap had a pH according to the standard, where the pH for liquid hand sanitizer is in the range of 4-10. Check the pH using universal pH.

The results of the foam height examination showed that the foam height in the sample for liquid hand-washing soap with bay leaf extract was 20 mm. For the control soap base, it was 60 mm indicating that the height of the soap in the control soap base was higher than the sample for liquid hand-washing soap with leaf extract. Regards. This aligns with research conducted by Pangestika et al. (2021), which states that adding extracts to making soap reduces the foam height. The height standard for soap foam set by the Indonesian National Standard (SNI) is 13-220 (Hutauruk et al., 2020; Pangestika et al., 2021).

The results of the antibacterial inhibition test from the liquid hand washing soap sample obtained the average diameter of the inhibition zone at each concentration; namely a 3% concentration of 8.27 mm, 6% concentration of 10.55 mm, and 9% concentration of 11.16 mm and the soap base control has no inhibition zone. This is in line with research conducted by Utami (2020), which states that the greater the concentration in each formulation, the greater the inhibition of the bacteria (Maramis & Asri, 2022; Mulyani & Hendick, 2022; Mudjahid et al., 2022).

The inhibition zone formed is due to the antibacterial compounds in the bay leaf extract, namely alkaloids, flavonoids, saponins, and tannins, which act as antibacterial. This is in line with the research of Kilis et al. (2020) and Damayanti et al. (2021), which states that bay leaf extract contains secondary metabolite compounds, including alkaloids, flavonoids, saponins, and tannins which have antibacterial activity. The mechanism of action of alkaloids as an antibacterial is by interfering with the constituent components of peptidoglycan in bacterial cells. The peptidoglycan layer is used for the survival of bacteria, so if the layer is damaged, stiffness occurs in the bacterial cell wall, which can cause cell death (Nau'e et al., 2020; Pananginan et al., 2020; Apriliana et al., 2018).

The mechanism of action of flavonoids is antibacterial because they can interfere with the function of the bacterial cell wall through the formation of complexes with extracellular proteins and inhibit bacterial motility. Damage to the bacterial cell wall will react with the alcohol group of the flavonoid compound, causing the absorption of these compounds into the nucleus of the bacterial cell. Then the DNA contained in the nucleus of the bacterial cell will react with the flavonoid compound through the difference in polarity between the alcohol groups and the lipids that make up the DNA, causing the nucleus of the bacterial cell to lysis (Nurhayati et al., 2020; Mudjahid et al., 2022; Utami, 2020).

The action of saponins as antibacterial is to reduce cell surface tension and damage membrane permeability because saponins contain hydrophilic and lipophilic molecules. Disruption of the surface tension of the cell wall causes the antibacterial content to easily enter the cell, which will eventually cause cell death. In contrast, damaged cell membranes can cause bacterial cells to experience protein and enzyme deficiencies (Fatonah et al., 2022; Sadiah et al., 2022; Mudjahid et al., 2022). The mechanism of action of tannins as an antibacterial is by inhibiting bacterial extracellular enzymes and taking over the substrates needed for bacterial growth, besides that tannins can also attack cell wall polypeptides which can eventually cause damage to the bacterial cell wall (Sukmawati et al., 2019; Adrar et al., 2019).

Conclusion

The diameter of the inhibition zone at each concentration was obtained: a 3% concentration of 8.27 mm, a 6% concentration of 10.55 mm, and a 9% concentration of 11.16 mm. Based on the results of a simple linear regression test analysis using ANOVA, it was found that $p_{value} = 0.00$, which means $p_{value} < \alpha$ ($\alpha = 0.05$), it can be concluded that H1 is accepted so that it is stated that there is an effect of liquid hand washing soap with bay leaf extract (*Syzygium polyanthum*) on the growth of Escherichia coli bacteria in vitro.

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The authors declare that they have no conflict of interest.

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