The Exploration of Moringa Leaves' Antibacterial in Biodegradable Detergent Production Through Application of Eco-enzyme Synthesis

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Abstract. Nowadays, organic waste from plants cannot be processed optimally. One of the solutions to protect the environment is to thoroughly process organic waste into an alternative eco-enzyme that is useful in making biodegradable detergents. Alternative sources of biosurfactant production can be substituted with alternative materials that are more environmentally friendly, such as Moringa leaves (*Moringa oleifera*). The purpose of this study was to: (1) produce biodegradable detergents processed through the synthesis of eco-enzymes and Moringa leaves surfactants, (2) find out the value of pH test, organoleptic test, and antibacterial test, and (3) explore the Moringa leaves' antibacterial in biodegradable detergent production. The method used in this study included maceration, filtration, and evaporation using a rotary evaporator to get pure Moringa surfactants. Detergent is made by adding eco-enzymes which have been synthesized through a fermentation from organic matter. The quality control of products including organoleptic tests, pH, and antibacterial tests which are then compared with commercial detergents. Optimum biosurfactant results were obtained at an extract concentration of 45%. Liquid detergent showed physicochemical properties produced in accordance with SNI standards with a pH of 11. The results of the biosurfactant antibacterial test were 15.9 mm, 14.6 mm, and 15.2 mm. This study shows that the quality of the detergent is good, which is categorized as strong antibacterial strength.

1 Background

The waste problem is currently increasing with the increase in population and the rapid pace of industrial growth. This has become a common problem found in big cities around the world, one of which is in Indonesia, namely in big cities such as Jakarta, Bandung, Surabaya. Around 80% of the waste is generated by organic waste which is only seen as residual waste and has no economic value [1]. Organic waste such as food scraps, fruits, and vegetables that are produced by households are simply thrown away without being sorted and without considering the impact on the environment. If organic waste is not managed properly, it can cause serious environmental problems such as the spread of skin diseases in humans and environmental pollution [2].

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To minimize environmental pollution, one way that can be done is convert organic waste into eco-enzyme. Eco-enzyme is a liquid obtained from vegetable and fruit waste with a mixture of sugar and water, using a certain ratio through a fermentation process to produce products that are environmentally friendly and beneficial to humans [3]. By utilizing fruit and vegetable waste, it can create a solution to reduce organic waste. Eco-enzyme is environmentally friendly because it is produced from natural ingredients so that it can be used as an effective substitute substance that can replace chemicals commonly used in the detergent manufacturing process. This can act as an antibacterial agent in fabrics, reduce greenhouse gas emissions, and minimize waste. Eco-enzyme contain a number of bacteria that break down organic waste such as lactic acid bacteria, bacillus, and lactobacillus [4].

The benefits of eco-enzyme can be used as a household cleaner, one of which is as a detergent. Eco-enzyme contain lipase and amylase enzymes. Those enzymes have biocatalyst properties that can be used to reduce the concentration of wastewater contaminants [5]. Previous studies on reducing pollutant concentrations using eco enzymes were able to reduce Chemical Oxygen Demand (COD) concentrations in domestic waste [3]. The increase in detergent consumption in Indonesia is directly proportional to the increase in the volume of detergent waste, which has the potential to pollute the aquatic ecosystem [6]. Ecosystem pollution is caused by harmful *Acrylonitrile Butadiene Styrene* (ABS) and *Linear Alkylbenzene Sulfonates* (LAS) compounds contained in commercial detergents, so these two materials are non-biodegradable [7]. Therefore, the use of natural detergents must be developed, namely detergents from eco-enzyme and Moringa leaves to remove stains and as an environmentally friendly and biodegradable antibacterial.

Biodegradable detergents require alternative sources of surfactants to replace chemicals that are safe for the environment and humans. Alternative sources of biosurfactant production can use plants such as Moringa leaves. Moringa leaves are plants that contain surfactants in the form of active saponins which function as antibacterial and antioxidants [8]. In addition, moringa also has many benefits because it contains secondary metabolite compounds consisting of flavonoids, phenols, ascorbate, and carotenoids [8]. These compounds are able to provide defense against pathogens. The manufacture of liquid detergents uses a rotary evaporator as an extraction tool with the principle of separating the extract from the filter liquid by heating which is accelerated by the rotation of the flask [6]. The purpose of this study was to produce biodegradable detergents which were processed through the synthesis of eco-enzymes and extraction of moringa leaf surfactants, as well as to determine the antibacterial content found in Moringa leaves.

2 Methods

The ingredients used are Biosurfactant (moringa leaf saponin) 20%, Eco-enzyme 20%, MES (*Methyl Ester Sulfonate*) 15%, Aquades 35%, Tetranyl 5%, Ester Compound 5%, natural dye 3%, and methanol 70%. The tools used are (1) Erlenmeyer tube with the Pyrex brand measuring 250ml. This Erlenmeyer tube is used in a sterile and clean manner as a container for biosurfactants when carrying out titration and filtration processes, (2) Pyrex brand beaker used for storing and mixing detergent ingredients. This beaker measures 500 ml and is sterile and resistant to room temperature, (3) pH meter, used to measure the level of acidity or alkalinity of detergent biosurfactants. The brand we use is Hanna because we believe that the accuracy, precision, calibration of the tool is valid. (4) Digital balance with the Eutech brand which has specifications that suit researchers' needs for measuring, calibration and durability processes. (5) IKA brand hotplate used to heat a mixture of detergent ingredients such as MES, tetranyl and biosurfactant with a heating time of 1 hour and a temperature of 60 degrees.

Moringa leaf powder was extracted using 70% methanol solvent with a ratio of 1:10 [7] by maceration method. Maceration is the process of extracting active ingredients from plants using a liquid solvent, such as methanol. Maceration of Moringa leaves aims to obtain antioxidant content, vitamins, minerals and other compounds that are beneficial for health. The steps for macerating Moringa leaves include the following: (1) Select Moringa leaves that are still fresh, young and free from pests or diseases. Wash the Moringa leaves with running water, then dry them with a clean cloth or tissue, (2) cut the Moringa leaves into small pieces then grind them using a blender until they become powder, (3) pour the liquid solvent, namely methanol, into a container containing 100 grams of Moringa leaf powder with a solvent ratio of 7:3 (70% methanol solvent and 30% distilled water), (4) stir the mixed solution then cover with aluminum foil and let sit for 3 days.
Concentrate maserate that has been incubated and filtered is then diluted with the addition of distilled water and activated by adding HCl solution, after standing it produces biosurfactant. In the process of making liquid detergent, it is done by melting MES and then mixing it with biosurfactant until it is homogeneous, which is called solution 1. At the same time, glucose and Na_2SO_4 solutions are made until homogeneous, which is then called solution 2. Solution 1 and solution 2 are shown by Figure 2.

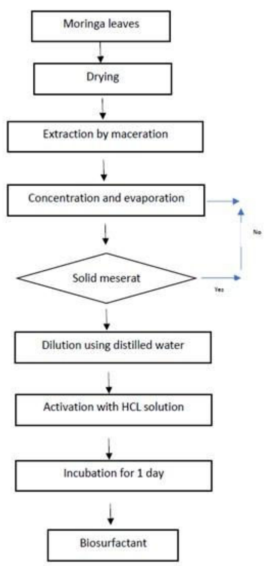

Fig. 1. Flow chart for the manufacture of biosurfactants [7].

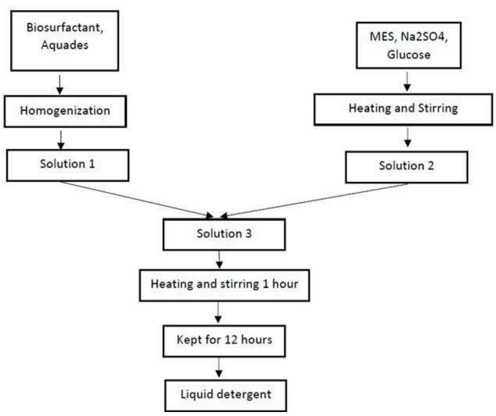

Fig. 2. Flow chart for the manufacture of liquid detergent.

The stage of making eco-enzymes must have been carried out long ago, because eco-enzymes require a fairly long fermentation process. The process of making eco-enzymes from fruit and vegetable waste that are no longer used. The steps for making eco-enzyme are explained as follows: (1) Pouring 500 ml of clean water into a jar, (2) Adding 150 grams of orange peel and 50 grams of palm sugar, (3) Stirring the mixture so that the sugar dissolves with water until it is homogeneous, (4) After all the ingredients are mixed well, the jar is tightly closed so that outside air does not enter because it can interfere with the fermentation process, (5) Store eco-enzymes in a place that is not accessible to sunlight, (6) Perfect fermentation takes up to 3 month (the jar is opened a maximum of 2 times for a few seconds to get rid of the gas that is formed each month, after 3 months of storage the dregs are filtered and the liquid is taken to produce eco-enzyme).

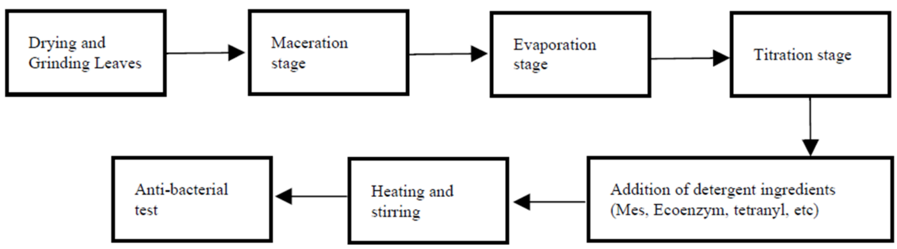

Fig. 3. Flow chart overall stage.

The overall stages are summarized in the flow chart in Figure 3, which starts from the process of making saponin biosurfactant, namely at the stage of drying and grinding Moringa leaves until they are mixed with detergent and ends with the anti-bacterial test stage. After the detergent product is finished, we carry out a physical inspection through an organoleptic test to determine the condition of the color, shape and odor of the detergent that has been made.

3 Results And Discussion

3.1 Process in Making Biodegradable Detergent Production from Moringa Leaves Through the Application of Eco-enzyme Synthesis

The manufacture of biodegradable detergent from Moringa leaves with the addition of eco-enzyme consists of 3 major stages, namely the manufacture of moringa surfactant, the manufacture of eco-enzyme, and the manufacture of biodegradable detergent. The first stage is the manufacture of biosurfactants which are the main components in the manufacture of detergents. Biosurfactants are biodegradable surfactants produced from microorganisms or natural resources [9]. In the process of making this detergent, the biosurfactant is made from moringa leaves, where the manufacturing process begins with the drying process of the moringa leaves.

In addition, the process for making eco-enzymes is produced through the process of collecting waste from fruits and vegetables, such as peels from apples, bananas, oranges, watermelons and vegetables that are no longer used. The production of moringa biosurfactant begins with collecting moringa leaves and then drying the moringa leaves which will be used as powder. The finished moringa powder is then subjected to the maceration process (soaking). Moringa powder is dissolved in water and left for 1-2 days. After going through the soaking process, a filtration process is carried out so that the moringa powder filtrate does not mix together so that a ready-to-evaporate moringa biosurfactant liquid is obtained.

Fig. 4. Drying Process of Moringa Leaves into Powder (left), Filtration Process of Macerated Moringa Biosurfactant Results (right).

Fig. 5. Evaporation Process of Biosurfactant Moringa Result of Filtration (left), Addition of Eco-enzyme in Liquid Detergent Production (right).

Process to make biodegradable detergents is with the addition of biosurfactant moringa and eco-enzymes that have been prepared previously. The steps taken are as follows: (1) Mixing *Methyl Ester Sulfonate* (MES) and salt which has been weighed according to the dosage, (2) Dissolving the mixture of MES and salt into hot water, (3) Stirring the mixture until it thickens and the temperature decreases, (4) Adding eco-enzyme, evaporated moringa extract, and perfume, and (5) Adding additional water to stir the mixture. The end result of the detergent is a liquid detergent that is ready to be tested. Laboratory tests were carried out on organoleptic tests, pH tests, and anti-bacterial tests. After that, a liquid detergent test was also carried out on soiled fabrics. This trial process was also carried out by comparing it with commercial detergents commonly used. MES is an important aspect in detergent manufacturing. *Methyl Ester Sulfonate* (MES) is one type of anionic surfactant derived from vegetable oils, such as coconut oil. MES used in the manufacture of environmentally friendly detergents has several roles such as MES is easily degraded by nature and is non-toxic to humans and the environment. MES also has good detergency, especially in hard water containing calcium and magnesium ions. In addition, MES can replace petroleum-based synthetic surfactants, such as *Linear Alkyl Benzene Sulfonate* (LAS) or *Sodium Lauryl Sulfate* (SLS).

In Figure 6, it can be seen that the ratio of foam produced from the two detergents. The results of the stability of the Moringa leaf saponin extract liquid detergent foam produced were 70%. This is supported by the results according to SNI 06-4075-1996, that the stability of liquid detergent foam must be at least 60%. Biosurfactants are compounds that can lower the surface tension between two phases, such as water and oil, and form stable emulsions. Biosurfactants have advantages over synthetic surfactants, because they are more environmentally friendly, biodegradable, and can be produced from cheap and abundant natural materials [18]. One of the natural materials that can be used as a source of biosurfactant is moringa leaves, which is a fast-growing plant and widely grown in Indonesia. Moringa leaves contain saponin compounds, which are natural biosurfactants that can form foam, anti-inflammatory, antivirus, and antibacterial. Several studies have been conducted to apply biosurfactants from moringa leaves as an environmentally detergent.

Fig. 6. Comparison Test Results of Moringa Detergent with Commercial Detergents.

This detergent can be made by extracting moringa leaves with an acidic solvent, such as HCl, and adding other ingredients such as Na_2CO_3, Na_2SO_4, fragrance, and colorant. Na_2SO_4 is very soluble in water and functions as a detergent filler, which is to increase the volume of material and increase the stability of the detergent. Sodium sulfate can also function as a detergent support material, which is to support the work of surface tension-reducing ingredients, increase washing power, also bind calcium and magnesium ions that cause water hardness. This detergent has physicochemical properties that comply with national standards, such as pH, density, and detergency. In addition, this detergent also has a low pollution level, so it does not damage the environment. Furthermore, eco-enzyme have many benefits, one of which is as a natural detergent ingredient. Natural detergent from eco-enzyme can be used to clean various surfaces of the house, clothes, fruits, and vegetables. This detergent is environmentally friendly, biodegradable, and harmless to human skin. It can also remove stains, grease, and dirt, as well as repel pests and odors.

3.2 The Results of pH Tests, Organoleptic Tests, and Antibacterial Tests

As the final step to test biosurfactant preparations in liquid detergents, several tests were carried out, namely organoleptic tests, pH tests, and liquid detergent antibacterial tests by comparing them with commercial detergents. Based on SNI, the pH value for liquid laundry detergent is in the range of 10-12 (SNI 06-4075-1996). The results obtained were at pH 10 which is in the range of quality standards. The resulting pH level is alkaline so it does not cause disturbance to the ecosystem in the waters. Organoleptic tests can simply be referred to as sensory tests [10]. This test is carried out by directly observing the physical form which includes the shape, color, and smell of liquid detergent with the following results.

We divided the organoleptic test results into 4 samples with codes B1, B2, B3, B4 where each code is different in terms of the color produced. sample B1 is light brown, B2 is

Yellowish-brown, B3 is Dark yellowish-brown, and B4 is dark brown. After the organoleptic test, an antibacterial test was also carried out to determine the antibacterial activity of biosurfactants against Escherichia coli bacteria in liquid detergent preparations. The results of the antibacterial test that has been carried out are as follows.

Table 1. Organoleptic test results.

Sample	Form	Color	Smell
B1	Homogene liquid	Light brown	Typical
B2	Homogene liquid	Yellowish-brown	Typical
B3	Homogene liquid	Dark yellowish-brown	Typical
B4	Homogene liquid	Dark brown	Typical

Table 2. Antibacterial test results

Sample	Obstacles Zone (mm)				
	U1	U2	U3	Average	Antibacterial Category
B1	7,5	7,0	7,5	7,34	Currently
B2	7,7	7,2	7,7	7,53	Currently
B3	8,2	7,7	8,2	8,03	Currently
B4	8,4	8,2	8,4	8,33	Currently

U1, U2, U3: Inhibition zone code

According to Davis and Stout [11] there are four categories of antibacterial power based on the resulting inhibition zones, namely the very strong category with an inhibition zone of >20 mm, the strong category with an inhibition zone ranging from 10-20 mm, the medium category with an inhibition zone ranging from 5-10 mm and the weak category with an inhibition zone <5 mm. The antibacterial power of the four liquid detergent preparations from Moringa leaves and eco-enzyme against Escherichia coli bacteria was categorized in the medium category (5-10 mm). Judging from the resulting inhibition zone ranged from 7 mm to 8.5 mm. Furthermore, Ericsson et al. [12] also explained that the zone of inhibition of bacterial growth will be greater when the higher concentration is added. The difference in the inhibition zone at each concentration is due to the large difference in the active ingredients contained in that concentration. The greater the concentration, the greater the active ingredients contained so that the resulting inhibition zone is greater.

Table 3. Antibacterial test results.

Sample	Obtacles Zone (mm)				
	U1	U2	U3	Average	Antibacterial Category
Positive Control	12,7	12,5	11,5	12,23	Strong
Negative Control	0	0	0	0	Blank
Biosurfactant	15,9	14,6	15,2	15,23	Strong

U1, U2, U3: Inhibition zone code

Based on the results of antibacterial testing on the positive control (KP), negative control (KN), and biosurfactants, it yielded some data that the positive control, which is a commercial detergent, obtained inhibition zones of 12.6 mm, 12.4 mm, 11.4 mm, and 12.2 mm which is categorized as strong antibacterial power. In the negative control in the form of basic detergent preparations without biosurfactants and eco-enzymes, no inhibition zones were produced. This means that there is no antibacterial activity in the liquid detergent preparation because there is no active ingredient that functions as an antibacterial agent. The results of the biosurfactant antibacterial test itself were 15.9 mm, 14.6 mm, and 15.2 mm which were categorized as strong antibacterial strength. This happens because the mechanism of action of saponins as antibacterial is by denaturing proteins, because the surface active substances of saponins are similar to detergents so they can be used as antibacterials where the pressure of the bacterial cell wall will be lowered and the permeability of the bacterial membrane is damaged [13]. Judging from the biosurfactant from Moringa leaves, it functions as a substitute for dangerous surfactants, which functions as a foam producer, the eco-enzyme material functions as an antibacterial agent and is able to remove stains on clothes.

3.3 The Exploration of Moringa Leaves' Antibacterial Activity

In this study, it has been proven that Moringa leaves have strong antibacterial activity against Escherichia coli bacteria both in the form of biosurfactants and in the form of detergent preparations. Based on SNI, the pH value for liquid detergent is in the range of 10-12 (SNI 06-40751996). The results obtained in the liquid detergent that has been produced are in the pH range of 10-11 which is in accordance with the quality standards. The pH level produced by liquid detergent is alkaline so that it does not cause disturbances to the ecosystem in the waters. Moringa leaves contain secondary metabolites and are efficacious as antibacterials. Antibacterial activity is the ability of a substance to inhibit the growth or kill bacteria.

Moringa leaves have antibacterial activity because they contain compounds such as flavonoids, saponins, terpenoids and tannins that can disrupt cell walls, cell membranes, or bacterial enzymes [14]. The antibacterial activity of sengon leaves can be tested by measuring the diameter of the inhibition zone containing sengon leaf extract on media overgrown with bacteria [15]. The antibacterial function is as a herbal ingredient that can be used to treat various diseases, respiratory tract infections, digestive tract infections, skin infections, and others [16]. Sengon leaves have antibacterial mechanisms that vary depending on the type of bacteria inhibited or killed. One of them is to inhibit bacterial protein synthesis, so that bacteria cannot perform their vital functions [15]. An example of bacteria that are sensitive to this mechanism is Escherichia coli.

Furthermore, the antibacterial properties of Moringa leaf surfactant can help clean stains and dirt on clothes caused by bacteria, such as blood, pus, or sweat [17]. The surfactant of sengon leaves can prevent the growth of bacteria on washed clothes, so that the clothes remain hygienic and odorless. The surfactant of sengon leaves can reduce the negative impact of detergents on the environment, because sengon leaves are natural ingredients that are easily decomposed by microorganisms and do not contain harmful substances.

4 Conclusions

The process of processing organic waste into alternative eco-enzymes is used in the manufacture of biodegradable detergents. Alternative sources of biosurfactant production can be substituted with alternative materials that are more environmentally friendly, such as Moringa leaves. Product quality control includes organoleptic tests, pH, and antibacterial tests which are then compared with commercial detergents. Optimal biosurfactant results were obtained at an extract concentration of 45%. Liquid detergent showed a pH value of 11.

The results of the biosurfactant antibacterial test were 15.9 mm, 14.6 mm, and 15.2 mm which were categorized as strong antibacterial strength. Based on the results of research that has been done, it was found that biodegradable detergents processed through the synthesis of eco enzymes and moringa leaf surfactants work effectively and have strong antibacterial properties in removing stains on clothes.

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