RiCE: Research in Chemical Engineering
Volume 4. No 1, 2025
Journal homepage: https://ejournal.ump.ac.id/index.php/rice

Potential of Biosurfactant Extracted from *Averrhoa bilimbi* Leaves Using the Maceration Method

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Article Info

Submitted:
20 April 2025

Revised:
6 May 2025

Accepted:
15 May 2025

ABSTRACT

The utilization of natural resources as raw materials for biosurfactants is increasingly urgent to reduce dependence on synthetic surfactants that have the potential to pollute the environment. One potential natural source is Averrhoa bilimbi leaves, which contain chemical compounds such as saponins. This compound acts as a natural biosurfactant that is not only effective but also environmentally friendly, so it can be a solution in overcoming the negative impact of synthetic surfactant use. This study aims to determine the optimal level of saponin as a biosurfactant from starfruit wuluh (*Averrhoa bilimbi*) by varying the ratio of starfruit leaf extraction and solvent, as well as examining the height and stability of foam produced by saponin from starfruit wuluh leaves. In addition, this study evaluated the effect of pH on the performance of saponin from star fruit as a biosurfactant. The method used was maceration extraction using ethanol solvent. Saponin testing was carried out by Thin Layer Chromatography (TLC) test to determine the saponin content of each sample. The test results showed that the highest saponin content was found in the starfruit leaf extraction: solvent ratio variation of 1:12, namely 6.12%, while the best foam height and foam stability test results were found in the starfruit leaf extraction: solvent ratio variation of 1:10, where no foam disappeared for 15 minutes. The pH test showed that pH 7 is considered safe for the environment. Thus, saponin extract from belimbing wuluh leaves has the potential to be an effective and sustainable biosurfactant, which can be a more environmentally friendly alternative to synthetic chemical-based surfactants.

Keywords: saponins, biosurfactants, natural surfactants, maceration extraction

1. INTRODUCTION

In recent decades, the use of synthetic surfactants has increased significantly in various industries, such as the textile industry, food industry, and oil industry. However, the use of these synthetic surfactants has several disadvantages, such as high toxicity, cannot be decomposed naturally, and can cause environmental pollution [1]. Synthetic surfactants are often difficult to degrade naturally and can have a negative impact on water and soil ecosystems, such as in a study conducted by Larasati 2021 [2] who evaluated the impact of detergents containing surfactants and phosphate compounds in the waters of the Tapak River Estuary, Semarang. The surfactant content resulted in a decrease in water quality in the estuary and caused a significant contribution to pollution in the estuary. Efforts to reduce the content of synthetic surfactant compounds require environmentally friendly technologies such as biosurfactants derived from saponin compounds. Saponin compounds can be found in various plants, one of which is *Averrhoa belimbi* [3]. The plant *Averrhoa beli*mbi contains saponins found in the fruit, leaves, and stalks. Attach the data about the amount of saponin in the bilimbii/star fruit have a higher saponin content compared to the stalk. Therefore, star fruit leaves are highly recommended to be used as biosurfactant [4].

Star fruit leaves have been used traditionally as medicines and have complex chemical content. One of the bioactive compounds contained in star fruit leaves is saponin. Saponins are glycoside compounds that have the ability to form foam when shaken with water. This ability makes saponins a potential candidate for use as a biosurfactant.

Biosurfactants are compounds capable of lowering the surface tension between two immiscible liquids, such as water and oil. which are amphiphilic compounds with the ability to lower the surface tension between two immiscible phases [5]. Biosurfactants can be obtained from various sources, including microorganisms such as bacteria and yeast, which produce rhamnolipids and sophorolipids, plants that contain saponins such as star fruit leaves, and mammals that produce lung surfactants to aid breathing [5].

Several researchers have conducted research on biosurfactants. One of them is a research by Nurhasanah in 2023 [6] which optimized the production of biosurfactant from local isolate bacteria from the sediment of Panjang Port waters, Lampung. This study showed that local isolate bacteria can produce biosurfactants under certain optimum conditions. However, this study has shortcomings related to the sustainability of bacterial isolate sources, especially if produced on a large scale. Furthermore, in a study conducted by [7], this study aimed to evaluate the potential and effectiveness of saponins extracted from bidara leaves and papaya skin as biosurfactants. This study found that saponins from bidara leaves and papaya skin have a good ability to form biosurfactants. However, this study has the disadvantage of not calculating the foam stability of saponins extracted from bidara leaves and papaya skin.

In Sari Andira Tahir's research [8] using maceration extraction with various sample: solvent ratios that will be carried out in this study. The selection of ethanol solvent was revealed by Ali Rakhman Hakim [9] who revealed that ethanol solvent is very good for obtaining flavonoid and phenolic compounds. Furthermore, evaporation was carried out using a rotary evaporator which in Roffiatul Qorriaina's research [10] revealed that at a temperature of 50 ° C the extract results were obtained more optimally. This research was conducted by following the operating conditions set by previous researchers to achieve the best optimization. By following methods and parameters that have proven effective before, this research can ensure that the results obtained will be more accurate.

This research aims to explore the potential of belimbing wuluh (*Averrhoa bilimbi*) leaf waste as a source of saponins that can be utilized as biosurfactants in the formulation of environmentally friendly liquid detergents. In an effort to achieve these objectives, this research will involve a series of tests, including a saponin content test to determine optimal levels, a foam height and stability test to assess foam formation ability and resilience. The results of this research are expected to provide a more environmentally friendly alternative to synthetic surfactants in the cleaning industry.

2. MATERIALS AND METHODS

2.1 Materials

This study used fresh star fruit leaves (*Averrhoa bilimbi*) as the main ingredient. The leaves were washed, dried, then pulverized into powder (simplicia). The solvent used was 96% ethanol. Additional materials such as distilled water and chemical reagents are used in laboratory analysis. The tools used include analytical scales, measuring cups, Erlenmeyer flasks, rotary evaporators, pH meters, and Thin Layer Chromatography (TLC) analysis was conducted at the Standardized Testing Center for Medicinal and Aromatic Spice Plants Instrument (BPSI TROA).

2.2 Experimental procedure

2.2.1Saponin Extraction

The extraction process was carried out using the maceration method. A total of 20 grams of simplicia was macerated with 96%

ethanol solvent at a variation of the ratio of simplicia to solvent, namely 1:4, 1:6, 1:8, 1:10, and 1:12 (**Table 1**). Starfruit leaf simplicia was extracted for 3 days in an erlenmeyer covered with aluminum foil and shaken twice a day (**Figure 1**). After filtering, the pulp was re-extracted for 2 days with 100 mL new solvent, then filtered. The first and second filtrates were mixed, then evaporated using a rotary vacuum evaporator until a thick extract was obtained.

Table 1. Sample Ratio Variation

Sample	Averrhoa belimbi leaves powder (mL)	Solvent (mL)
1:4	20	80
1:6	20	120
1:8	20	160
1:10	20	200
1:12	20	240

Figure 1. Maceration Process

2.2.2 Identification of Saponins by Thin Layer Chromatography (TLC)

Saponin testing was conducted at the BPSI TROA Laboratory in Bogor using the Thin Layer Chromatography (TLC) method. A total of ±0,25 g of sample was put into a 25 mL volumetric flask, then aquadest was added as much as ±¼ volume of the flask and shaken using a shaker for 2 hours. After the shaking process was complete, the volume of the solution was adjusted to the mark and kept for 24 hours. Next, the solution was filtered and the filtrate obtained was photographed as much as 5 µL on a silica gel 60 GF 254 aluminum sheet plate, along with the saponin comparison material that had been dissolved in distilled water with a concentration of 100 ppm as much as 5 µL. Elution was carried out using a mixture of $CHCl_3$:Ethanol:Ethyl Acetate eluent until the eluent limit was about ± 15 cm. After the elution process was complete, the TLC plate was allowed to dry and the results were analyzed using a Camag 3 TLC scanner at a wavelength of 301 nm.

2.2.3 Foam Height and Stability Tests

A total of 5 mL of extract was dissolved in 100 mL of distilled water, then shaken for 10 seconds in a measuring tube. The foam height was immediately measured, and its stability was observed for 15 minutes with recording every 5 minutes. This test aims to assess the effectiveness of foam formation and durability of the biosurfactant producer.

2.3.4 pH Test

Each extract was measured for its pH value using a calibrated digital pH meter. Measurements were taken at room temperature to determine the level of acidity and its potential.

2.3 Methods of analysis

2.3.1 Test for saponin content with Thin Layer Chromatography (TLC) scanner

In this test, the concentrated extract of star fruit leaves with a variety of starfruit leaf extraction:solvent ratios, namely 1:4, 1:6, 1:8, 1:10, 1:12 as much as 20 mL each separated to be examined for saponin content in the Laboratory of the Research Center for Spice and Medicinal Plants (BPSI TROA) Bogor, West Java. The results of the data obtained are the percentage of active ingredient content of the 5 samples sent for testing.

2.3.2 Foam height and stability test

The foam stability test was conducted by dissolving 2 mL of Averrhoa belimbi extract in 5 mL of water and shaking for 1 minute. The resulting foam height was then measured after 0 and 15 minutes. Calculation of foam stability using the **equation 1**:

$$\%foam\ stability = \frac{H}{H_0}\ x\ 100\% \qquad (1)$$

With: H: foam height after 15 min, cm

H0: initial foam height, cm

The extract foam height test was conducted by dissolving 1 mL of extract in 5 mL of water and shaking for 1 minute. The resulting foam height was then measured.

2.3.3 pH Test of Viscous Extract

Data analysis was carried out by comparing the pH value of each viscous extract sample with a ratio of 1:4, 1:6, 1:8, 1:10, and 1:12 obtained from the measurement results using a pH meter. The pH data obtained were analyzed descriptively to determine the trend of pH changes based on variations in the ratio of extract to solvent. The results of this analysis were used to evaluate the pH stability of the star fruit saponin extract and assess its suitability as an environmentally friendly biosurfactant.

3. RESULTS AND DISCUSSION

The extraction process was carried out for five days. On the first three days, the first filtrate was taken from the mixture of ethanol and star fruit leaf powder. After the first extraction, a second filtrate was carried out for the next two days to obtain additional filtrates as obtained in **Table 2**.

Table 2. Maceration Extraction Results

Sample	Extraction Result 1	Extraction Result 2
1:4	23 ml	38 ml
1:6	54 ml	79 ml
1:8	90 ml	112 ml
1:10	134 ml	163 ml
1:12	179 ml	198 ml

The extraction results in the second maceration using ethanol solvent showed a higher amount compared to the first maceration. Ethanol as a semi-polar solvent has the ability to dissolve active compounds such as saponins more effectively [11]. In the second maceration, the remaining active compounds that have not been extracted in the first stage have a greater chance of dissolving, resulting in more extracts. This proves the efficiency of ethanol in dissolving active compounds gradually during the extraction process.

The extraction that has been done is then evaporated using a *Rotary Evaporator* with a temperature of 50 ° C and a rotation of 25 rpm. This process is done to evaporate the solvent and produce a thick extract containing active compounds from star fruit leaves. Evaporation using Rotary Evaporator obtained results as in **Table 3**. The decrease in extraction yield at a 1:10 ratio could be due to several factors, such as solvent saturation, limited diffusion efficiency, or compound degradation due to prolonged exposure to ethanol during the extraction process. These factors have been discussed in previous literature, as described by Elboughdiri [12], where increased extraction time can lead to degradation of saponins and decreased extraction yield. In addition, research by Zhang et al. [13]showed that high ethanol concentrations can lead to solvent saturation, which inhibits saponin extraction efficiency. This suggests that an optimal solvent ratio is essential to maximize saponin extraction yield.

Table 3 Rotary Evaporator Results

Sample Variation	Extraction Results (g)
1:4	5.3
1:6	5.21
1:8	7.14
1:10	6.96
1:12	7.13

3.1 Thin Layer Chromatography Scanner Test

Table 4 is the results of Thin Layer Chromatography (TLC) tests conducted with the same extract variables as before, namely the ratio of 1:4, 1:6, 1:8, 1:10, and 1:12, using ethanol solvent. The test was conducted in the laboratory of the Standardized Testing Center for Medicinal and Aromatic Spice Plants Instrument (BPSI TROA) in Bogor.

Table 4. Thin Layer Test Results

Sample Variation	Test Result (%)
1:4	2.15
1:6	2.70
1:8	3.14
1:10	4.45
1:12	6.02

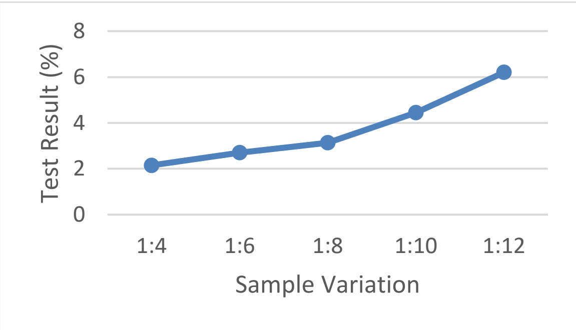

Figure 2. TCL Test Results

Figure 2 is the result of the TCL test, which explains that the greater the amount of solvent used, the more effective the solvent is in dissolving saponins, as the solvent's ability to capture active compounds increases before reaching saturation conditions, as described by [14] which emphasizes the importance of sufficient solvent volume to ensure optimal solubility of target compounds.

Based on the results of laboratory tests, the use of ethanol solvents with a larger ratio was shown to increase the saponin content extracted from star fruit leaves. At 1:4, the saponin content only reached 2.15%, but increased gradually as the amount of solvent increased to 4.45% at 1:10. This shows that the extraction efficiency increases with an increase in solvent volume. Scientifically, this can be explained through the principle of diffusion equilibrium, where higher solvent concentration accelerates the dissolution of active compounds from the plant matrix into the solvent, thereby increasing the extraction yield.

The peak saponin content was reached at 1:12, which amounted to 6.02%, making it the most effective ratio in the extraction process. This high saponin content is important because saponins are known to have amphipathic properties, namely containing hydrophilic and hydrophobic groups, so they are very potential as biosurfactants. Thus, it can be concluded that the greater the solvent ratio, the higher the saponin extraction yield obtained.

3.2 Foam Height and Foam Stability Tests

This study also involved testing the foam height and stability of the resulting viscous extraction. This test was conducted to evaluate the ability of the extract to produce foam as well as its stability under certain conditions. The results of this test are presented in **Table 5**, which shows the initial and final foam height measurements of the extract samples at different solvent ratios, along with the calculated foam stability percentage after 15 minutes.

Table 5. Foam Height and Stability Test

Sample	Initial Height (0 minutes)	Final Height (15 minutes)	Foam Stability Calculation Results
1:4	0.6 cm	0.4 cm	66,66%
1:6	1 cm	0.8 cm	80%
1:8	1 cm	0.9 cm	90%
1:10	1 cm	1 cm	100%
1:12	1.6 cm	1.6 cm	100%

Foam height testing is carried out to obtain foam from the extraction results that have been carried out, The foam that appears during this test occurs due to the nature of glycosides that can produce foam when mixed with water. In addition, glycosides also undergo a hydrolysis process that produces glucose and other compounds [15].

Foam stability testing shows that foam stability tends to decrease over time due to the reduced ability of foam bubbles to maintain their shape [14] which significantly affects surfactant performance, surfactants that are able to produce stable foam demonstrate higher effectiveness in maintaining foam structure for a longer period, and several studies indicate that saponins can be considered effective biosurfactant candidates when they exhibit high foaming ability and foam stability, as demonstrated in a study where a 0.6% saponin solution achieves 80% foam stability [16]

Based on the foam height and foam stability test calculations, the sample with a ratio of 1:12 showed the best foam stability with a result of 100%, characterized by a foam height that remained at 1,6 cm for 15 minutes without decreasing. This indicates the ability of the sample to maintain the foam structure, which is important in biosurfactant applications. Thus, the ratio of 1:12 was considered the most optimal to produce stable foam.

3.3 PH Test of Viscous Extract

Table 6. PH Test Results

Sample Variation	pH Test Results
1:4	6.89
1:6	7.74
1:8	7.74
1:10	7.74
1:11	7.89

The test results showed that the pH of the saponin extract from *Averrhoa bilimbi* leaves varied depending on the solvent ratio used, with values ranging from 6.89 to 7.89 (**Table 6**). The 1:4 ratio had the lowest pH, while the 1:11 ratio showed the highest pH. A pH value close to neutral, especially at pH 7, is very important because it indicates the stability and safety of biosurfactants for various applications. Based on SNI 4075-1.2017, the pH value of liquid detergent should be in the range of 5-10 to ensure environmental and health safety, and the results of this study show that all samples are within this range.

4. CONCLUSION

This study aims to determine the optimal saponin content that can be extracted from the leaves of *Averrhoa bilimbi*, which has the potential to be used as a natural biosurfactant by varying the ratio of sample and solvent. The results showed that ethanol extraction with a ratio of 1:12 produced the highest saponin concentration of 6.02%, which identified it as the most efficient ratio to maximize extraction yield. In addition, foam stability testing showed that the 1:12 ratio exhibited the most stable foam, maintaining the initial height over a 15-minute period an important characteristic for biosurfactant function in detergent applications. The pH values of the resulting extracts ranged from 6.89 to 7.89, all within the acceptable safety range specified by SNI 4075-1:2017, thus confirming the suitability of the extracts for safe and environmentally responsible use. Collectively, these results suggest that saponins from Averrhoa bilimbi have strong potential as a sustainable alternative to synthetic surfactants, especially for application in the formulation of environmentally friendly liquid detergents.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Cindy Dwi Sekar Arum: Conceptualization, methodology, investigation, data curation, writing-original draft.

Cintiya Septa Hasannah: Supervision, writing-review & editing, validation

Alfieta Rohmaful Aeni: Supervision, writing-review & editing

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