

FORMULATION AND TEST OF ANTIBACTERIAL ACTIVITY OF LIQUID SOAP EDAMAME SEED EXTRACT (*Glychine max. Merr*) AGAINST BACTERIAL GROWTH *Staphylococcus aureus*

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ABSTRACT

Skin infections can be caused by *Staphylococcus aureus* bacteria that attack normal skin. Infections can be prevented by giving antibiotics, but bacterial resistance is reported to be increasing. One of the product development innovations that will be made is liquid soap of edamame seed extract by utilizing natural ingredients. to formulate and test the antibacterial activity of liquid soap of edamame seed extract against the growth of *Staphylococcus aureus* bacteria. This study aims to formulate and test the antibacterial activity of liquid soap of edamame seed extract against the growth of *Staphylococcus aureus* bacteria. Furthermore, a liquid soap preparation of edamame seed extract was formulated with concentrations of F1 (5%), F2 (10%), F3 (15%). Then after that, physical properties such as pH test, organoleptics, foam height, dispersion, homogeneity and antibacterial activity. The data was analyzed using *the One Way anova* test method followed by *the Post hoc* test, namely *LSD*. Liquid soap of edamame seed extract is tested for physical. The average antibacterial test results for the concentration of F1 (5%) in the inhibition zone were 14.66 mm, F2 (10%) in the inhibition zone was 15.29 mm, and F3 (15%) in the inhibition zone was 16.57 mm and the Control (+) inhibition zone was 18.83 mm. The results of *the One Way Anova* analysis on the anti bacterial test with a significant value of 0.199 showed that there was no difference in each concentration on the effect of the inhibitory power of *Staphylococcus aureus* bacteria. It can be concluded that the liquid soap formulation that has been tested for physical quality has met the predetermined requirements. For the antibacterial test carried out on the three formulations made, the best results were produced by F3 (15%) with an inhibitory zone diameter of 16.57 mm.

Key words: Edamame, liquid soap, *Staphylococcus aureus*, *Glychine max. Merr*

INTRODUCTION

The human body is a preferred host for various microorganisms and can cause infectious diseases, especially pathogenic microorganisms. Skin infections can be caused by *Staphylococcus aureus* bacteria that attack normal skin. Diseases caused by bacterial infections can be treated with antibacterial soap. Therefore, it is important to conduct research to obtain new sources of antimicrobial innovation from natural materials.

The use of natural ingredients as traditional medicine in Indonesia has been carried out by ancestors since centuries ago both for health maintenance and for the treatment of certain diseases. Therefore, it takes various efforts to find and find new

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compound materials from natural sources that are proven to be naturally antibacterial. One of the natural ingredients used as traditional medicine is a plant of the type of edamame soybean.

Edamame soybean plants are Jember's flagship products because of their high productivity and nutritional content such as high protein, rich in fiber, calcium, magnesium iron and folic acid. Jember is a city that has good quality edamame agricultural production, as evidenced by the high export of edamame to various countries. In addition, edamame also contains genistein which is one of the main isoflavones in edamame soybeans has antibacterial, antioxidant and anti-inflammatory effects. Soybeans also have bioactive compounds of isoflavones (one of the flavonoid groups that are polyphenolic compounds) which is one of the components of the main chemical compounds that are antibacterial, because phenolic components can interfere with bacterial growth by inhibiting nucleic acid synthesis, enzyme activity, cytoplasmic membrane function, and energy metabolism (Akhita et al., 2020). This natural ingredient can be developed into a new innovative product as the basic ingredient for making bath soap. Bath soap is a topical preparation in solid or liquid form that is used to clean the skin. The basic ingredient of soap is made from the addition of surfactants, foam stabilizers, preservatives, dyes, and fragrances that are allowed to be used without causing side effects during use (Amilia, 2022)

In the background with the characteristics of Jember products, it is necessary to develop the potential of Jember products to be more differentiated. One form of preparation that will be developed is liquid soap preparation. Several studies state that edamame seeds have antibacterial activity against *Eshcerichia coli bacteria* ⁽²⁾. This study aims to make a liquid soap preparation of edamame seed extract and determine the antibacterial effect of liquid soap of edamame seed extract (*Glycine max. Merr*) against the growth of *Staphylococcus aureus* bacteria because no one has yet researched for the formulation of liquid soap edamame seed extract.

Based on the background and previous research that has proven the efficacy of the edamame plant as an antibacterial, a study will be conducted with the title of formulation and test of the antibacterial activity of liquid soap of edamame seed extract (*Glycine max. Merr*) was subjected to the growth of *Staphylococcus aureus* bacteria which aimed to formulate and test the antibacterial activity of liquid soap extract of edamame seed extract (*Glycine max. Merr*) against the growth of *Staphylococcus aureus bacteria*.

MATERIAL AND METHODS

The tools used in this study are *ovens (memmert)*, tools used in this study pH meters, calipers, mortars and stempers, measuring cups, stirring rods, droppipettes, micropipettes (*DragonLab*), yellow tips, borer corks, Erlenmeyer (*Iwaki*), analytical scales, bunsen, *Biological Safety Cabinet (biobase)*, petri dishes (*Anumbra*), ose wire, incubator (*Memmert*), autoclave, blender, UAE (*Biobase*), *UV-Vis Spectrophotometer (Shimadzu 1900i)*, *Waterbath, Rotary Evaporator (Intra)*, picnometer, tweezers, *hand mixer (Cosmos)* and mesh sieve 80.

The materials used in this study are edamame seeds (*Glycine max L.*), *Staphylococcus aureus* bacterial isolate, Aluminum Foil (*Good*), Filter Paper



(Whatman), Brown Paper, olive oil (*Living Tree*), Potassium Hydroxide (KOH), Sodium Carboxy Methyl Cellulose (CMC), Sodium Lauryl Sulfate (SLS), stearic acid, Butyl Hydroxyl Anisol (BHA), BaCl₂ 1%, H₂SO₄ 1%, Vanilla Flavoring (*Sicher Ecosystem*), Ethanol 96%, Nutrient Agar (*Himedia*), MHA Muller Hinton Agar (*Himedia*), *Staphylococcus aureus* bacteria (ATCC 25923), Aquaades.

Making Simplisia

Edamame seeds are collected and then sorted wet, then cleaned from dirt that sticks by washing in running water. After it is clean, it is peeled and separated between the skin and the seeds. After completion, the seeds to be used are then baked at a temperature of 50°C until completely dry, after the sample is dry, then blended until it becomes a fine powder. The powder is then sifted using a mesh sieve no 80 to produce a very fine powder (Trimanto et al., 2018)

Extraction of Edamame Bark and Seeds

The preparation of edamame seed extract uses the UAE (*Ultrasonic Assisted Extraction*) method. Edamame seed powder is weighed as much as 100 grams, put into erlenmeyer, then 96% ethanol solvent is added with a ratio of ingredients: solvents is 1:10 A total of 5 grams of simplicia powder is extracted with 96% ethanol solvent as much as 50 mL. Then it was extracted by the UAE method at a frequency of 40 KHz at a temperature of 50°C for 15 minutes. The filtrate obtained was then filtered with *whatman* filter paper no. 1 and the filtrate was evaporated with a *rotary evaporator* at a temperature of 70°C so that a thick extract of edamame seeds was obtained.

Liquid Soap Preparation Formulation

All materials to be used are weighed in advance according to the formulation made. 15 mL of olive oil is added to a porcelain cup, then 8 mL of 40% potassium hydroxide is added in a small way while continuing to heat at 50°C until a soap paste is obtained. Soap paste is added with 15 mL of aquaades, as much as 0.5 grams of Na-CMC is developed in hot aquaade, then stirred until homogeneous. A total of 0.5 grams of SLS is added and stirred until homogeneous. BHA 0.5 grams is added, then stirred until homogeneous. And add 1 mL of vanilla flavoring, then stir until evenly distributed. Finally, edamame seed extract is added and then stirred until homogeneous. Liquid soap is added with 100 mL ad aquaades, put in a clean prepared container (Umami, 2019)

Test of Physical Properties of Liquid Soap

The pH test is carried out by weighing 1 gram of preparation then put it in a beaker glass, then adding 10 mL of aquaade, stirring until dissolved, then tested using Universal pH. Good pH test results in liquid soap preparations 8.0 – 10.8 (Ayuni et al., 2023)

The foam height test is carried out by weighing 1 gram of liquid soap preparation then put into the test tube and then adding 10 mL of water, then the mouth of the test tube is closed with your thumb then shaken for 20 seconds then measure the height of the foam (Rusli et al., 2016)



The diffusion test was carried out by placing 0.5 grams of liquid soap preparation on a round glass with a diameter of 15cm, then placing another glass on top of it and putting a load of 50 grams on it for 1 minute, then recording the diameter. Then add a load of 100 grams until constant, then record the resulting diameter, then add a load of 150 grams, then record the diameter. Test good dispersion according to the requirements, namely 3-5 cm (Adri et al., 2023)

The organoleptic test was carried out using the five senses which were then observed directly such as color, shape and smell (Dimpudus et al., 2017)

The homogeneity test was carried out by weighing 1 gram of the preparation and then applying it to the preparation glass, then observing the soap preparation whether there were any substances on or clumps on the liquid soap preparation (Pebrianti et al., 2023)

Sterilization of Tools and Media

The sterilization of the tools used in this antibacterial activity research is sterilized first using an oven. The oven is a sterilizer with hot and cold air (Rishliania, 2022) The tools to be used are first washed and dried before being sterilized. Petri cups, ose wire, L rods, stirring rods, droppers, tweezers are wrapped using brown paper. *Erlenmeyer*, test tubes and *beakerglass* were sealed with a cup wrapped in sterile gauze and then wrapped in brown paper and sterilized in an *autoclave* for 15 minutes at a temperature of 121°C. For tweezers, ose wire and L rods if they were to be used were sterilized again by being lit directly close to the Bunsen flame.

Media Creation

In this study, NA (*Nutrient Agar*) media was used for antibacterial rejuvenation, which was made by weighing NA as much as 2.8 grams, then dissolved in 100 mL of aquatics using erlenmeyer. The medium is then homogenized by stirring using (*stirrer*) on a water bath until it boils. The homogeneous media is sterilized in an *autoclave* at 121°C for 15 minutes. Media that has been sterilized, cooled to a temperature that can be used as needed, media that has been sterilized when not in use is stored in a refrigerator (Septiyawati et al., 2020)

MHA (*Muller Hinton Agar*) powder is used for anti-bacterial test media. MHA media is made by weighing 3.8 grams and then inserted into an Erlenmeyer tube. Then add 1000 mL of aquades to the tube, mix and stir evenly then heat until boiling and dissolved. Then the solution is put into the autoclave at 121°C for 20 minutes. Then 20 mL of the solution in the tube is poured into a petri dish by aseptically and then put into an incubator with a temperature of 37°C (Utomo et al., 2018)

Staphylococcus aureus Bacteria

Bacterial rejuvenation is carried out using the scratching method. A pure culture of *Staphylococcus aureus* bacteria was taken from one ose and then inoculated by *Zig-Zag* scratching on NA (*Nutrient Agar*) media aseptically. It is then incubated at 37°C for 24 hours (Pananginan et al., 2020).



McFarland Standard Manufacturing 0.5

McFarland 0.5 solution can be used as a comparison of turbidity of bacterial cultures in liquid medium with a density between 1×10^7 cells/mL and 1×10^8 cells/mL. The working order of making *Mc Farland* 0.5 solution is to mix 0.05 mL of Barium Chloride (BaCl_2) 1% in an aqueous plus 9.95 mL of sulfuric acid (H_2SO_4)1% (Muttaqin et al., 2021)

Staphylococcus aureus Suspension Manufacturing

The inoculated test bacteria were taken with a sterile ose wire and then suspended into a tube containing 2 mL of 0.9% NaCl solution until the same turbidity was obtained as the standard turbidity of *Mc. Farland* 0.5 solution using a Uv-vis spectrophotometer at a wavelength of 625 nm. The same treatment was performed on the test bacteria (Septiyawati et al., 2020)

Well Diffusion Antimicrobial Test

The manufacture of test media uses the agar diffusion method modified by the well method. The suspension of *Staphylococcus aureus* bacteria was inserted into a petri dish containing *Muller Hinton Agar* media by taking 100 μL and leveled using an L rod, then a well hole with a diameter of 6 mm was made using a *cork borer* tool to insert the test material. Then positive control, negative control and liquid soap preparation of edamame seed extract with concentrations of 5%, 10%, and 15% were put into a well of *Muller Hinton agar* media as much as 50 μL using a micropipette with aseptic processing. Next, a petri dish containing bacteria with various concentrations of extracts is placed in an incubator at 37°C for 24 hours to allow the compound to diffuse (Lisa Potti et al., 2022)

Observation and measurement of the inhibition zone

After being incubated for 1x24 years, the observed area is a clear zone around the well on the medium measured using a caliper. After the data was obtained, a data analysis test was carried out using the SPSS *One Way Anova* method to see if there was a difference in the diameter of the growth inhibition of *Staphylococcus aureus* bacteria on the test medium.

RESULT AND DISCUSSION

Plant Determination

The determination of edamame aims to find out the identity of the plant used. Based on the results of the determination, the samples used in this study have Kingdom/Regnum: Plantae, Devisio: Spermatophyta, Sub Devisio: *Magnoliophyta*, Class: *Magnilopsida*, Order: Polypetale, Family: *Leguminoseae*, Genus: *Glychine*, Species: *Glycine max*, Merr.

Edamame Seed Extraction

In the extraction process, 200 g of simplicia powder was used and extracted by the *Ultrasonic Assisted Extraction* (UAE) method with a temperature of 50°C for 15



minutes using a 96% ethanol solvent in a ratio (1:10). The extraction results are then filtered and evaporated with a *Rotary evaporator* at a temperature of 70°C. The thick extract produced has a characteristic blackish-brown color that smells like soy milk, and has a bitter taste. The viscous extract obtained from the ethanol extraction of edamame seeds was 21.251 grams, so that the total yield obtained was 10.625%. The yield can be said to be good if the yield value is more than 10%, therefore the yield of edamame seed extract is said to be good because it is >10% (Anggraini et al., 2021) Based on the results of the resulting yield, it meets the requirements. This is due to several factors that can influence, such as the extraction method used, the solvent used, the extraction temperature and extraction time. In this study, the extraction method used is UAE where this method uses ultrasonic vibrations that can accelerate the extraction time. The solvent used is 96% ethanol where this solvent is widely used because it is polar and has the property of a material that can penetrate the cell wall so that it can attract bioactive wax faster.

In this study, the author used the *Ultrasonic Assisted Extraction* (UAE) extraction method. UAE is an extraction method that can increase the mass transfer rate and break the cell wall with a lot of *microactivity* so that it will shorten the process time and optimize the use of solvents. The advantage of the ultrasonication method is that it can speed up the extraction time and does not require heat in the process, so it will not damage the two chemical components in plants that are easily damaged by heat and can increase the extraction yield with a small volume of solvent (Tua, 2022) The UAE extraction process using extraction time that is too long and exceeds the optimum limit can cause bioactive compounds to undergo changes in chemical structure. The results of the study (Rachma, 2019) showed that high levels of flavonoids and antioxidant activity were obtained in teak leaf extract extracted for 15 minutes using the UAE method with 96% ethanol solvent and ingredient ratio : solvent is 1:5.

Ethanol is a solvent that is often used for the extraction process and there have been many studies that use ethanol solvents. The reason for choosing ethanol as a solvent is because it is easy to obtain, efficient, safe for the environment and has a high extraction rate (Rusli et al., 2016) The extraction process is influenced by several factors, including the type of solvent, the comparison of the solvent with the extraction material, temperature, pressure and extraction time, and the bioactive components of the plant. In research (Yunita & Khodijah, 2020) it was shown that extraction using ethanol with a concentration of 96% provided greater curcumin levels compared to ethanol solvents with a concentration of 50% or 70%.

Edamame Seed Extract Liquid Soap (*Glycine max.* Merr)

Liquid soap preparations made with 3 different formulations and concentrations of extracts, namely 5%, 10% and 15%. In each concentration, 100 mL was made and replication was carried out 3 times.

Physical Properties Test of Liquid Soap Preparation Edamame Seed Extract pH Testing

The pH test is carried out using beaker glass then tested using beaker glass then tested using a pH meter. The test results obtained in the test table above show that the



liquid soap preparation of edamame seed extract that has been made obtained an average pH test of F1 obtained pH 8.78, F2 obtained pH 9.12 and F3 obtained pH 9.38 meeting the vulnerable requirements of pH 8-11 test. The purpose of the pH test on liquid soap preparations is to avoid irritation during use. In general, soap is alkaline because the basic ingredient in making soap is KOH. It can be concluded that the higher the edamame seed extract, the higher the pH obtained.

Foam Height Testing

Foam is produced because there are ingredients that contain surfactants or other ingredients that make up soap. The results of the foam height test were obtained on average, F1 is 9.23 cm, F2 is 9.66 cm and F3 is 9.93 cm. The results of the three formulations meet the requirements of the foam height test, which is around 13-220 mm. The foam height test is used to see if the soap made is stable in producing foam. Where foam is one of the attractions of consumers in using liquid soap products. Foam is produced due to the addition of SLS material which functions as a saponification or foam enhancer in soap.

Spread Power Testing

The spreadability test was carried out using round glass with a diameter of 15 cm, then given a load of 50 grams-150 grams and then recorded the diameter. The results of the spread test of F1 (3 cm, 4 cm and 4.6 cm), F2 (3.5 cm, 4.5 cm and 5 cm) and F3 (4.6 cm, 5 cm, and 5 cm) of the three formulations met the requirements of the spread test. Where the condition for a good dispersion test is 2.7 cm- 5 cm. The diffusion test is used to see if the liquid soap made is able to spread when applied to the skin. Based on these results, it can be seen that the variation in the concentration of edamame seed extract used affects the dispersion of liquid soap. In this case, the more extracts are added to the liquid soap preparation, the dispersion of the preparation will increase.

Organoleptis Testing

Organoleptic tests are carried out with the five human senses, where we observe shapes, smells and colors. The results obtained after observation are shown in table 3. Where in F1 it has a thick liquid form, smells of vanilla and is yellowish-white in color. In F2 it has a thick liquid form, smelling of vanilla and brownish-yellow color. Seangkan F3 has a thick liquid form, smells of vanilla and is light brown in color. It can be concluded that each preparation made has a different color due to the addition of extracts to different preparations.

Homogeneity Testing

The homogeneity test was carried out by weighing 1 gram of the preparation and then applying it to the preparation glass then observing whether there were any particles that clumped together on the preparation.

The results obtained from the homogeneity test of liquid soap of seed extract were homogeneous and no granules or coarse powder were found. The good homogeneity of a preparation indicates that the active substance is evenly distributed in the soap base, so that when applied to the skin there are no solid particles. Where in this homogeneity test can be used as a benchmark whether the soap made has



consistent properties or not.

Antibacterial Activity Test

Based on research that has been carried out in the manufacture of liquid soap preparations of edamame seed extract (*Glycine max.* Merr) then carried out antibacterial activity tests on each formulation made.

The antibacterial activity test was carried out to determine whether the liquid soap preparation of edamame seed extract (*Glycine max.* Merr) which is made has a function as an antibacterial. Where edamame contains several types of isoflavones including genistein, daidzin, genistin, and melanoyl isoflavones which have antibacterial properties (Studi et al., 2023) One of the antibacterial mechanisms by isoflavones is by inhibiting the function of the cytoplasmic membrane, namely by interfering with its permeability which will later cause lysis in bacterial cells (Sandra et al., n.d.) In the tests that have been carried out on the negative control group in the form of liquid soap base, no inhibition zone response was found. The negative control ingredient uses a liquid soap base without the use of edamame seed extract (*Glycine max.* Merr), but in it there are preservatives that do not affect the response to the inhibitory power of the antibacterial activity carried out. The positive control used is green dettol liquid soap where this product is widely known among the public, which in the inhibition test has an influence on the inhibition zone. Testing on each liquid soap formula made was accompanied by the average diameter of the bacterial inhibition zone of 14.36 mm – 16.57 mm. These results can be said to meet the requirements of a strong inhibition zone. Where it can be said that edamame seed liquid soap has antibacterial activity. The categories of inhibition zones can be seen in the table below.

In the liquid soap preparation made, the smallest inhibition zone is produced by formulation 1 with a concentration of edamame seed extract of 5% and the largest inhibition zone is produced by formulation 3 where the concentration of the extract given is 15%, this is because the extract in F3 is the most abundant so that the inhibition power produced is also greater than F1 and F2. So the more extracts are included in the formula, the more antibacterial activity in the liquid soap is made. Antibacterial activity can also be affected by several things such as the concentration of the extract used, the solvent used and the extraction method also affect the antibacterial activity. The difference between the research I conducted and the previous research conducted by (Akhita et al., 2020) where in the previous research I used *Escherichia coli* bacteria while in the research I conducted I used *Staphylococcus aureus* bacteria. The method used during the extraction process is also different, where in the previous research using the maceration method and the research I conducted using the UAE method. In the previous study, only antibacterial activity tests were carried out on edamame seed extract (*Glycine max.* Merr). Meanwhile, in the research I conducted using a new innovation, namely by making a liquid soap formula with edamame seed extract against the growth of *Staphylococcus aureus* bacteria.

In this study, the data obtained by statistical method was carried out using *one way anova*. The criteria for testing *the one-way anova test with* a sig.p value of < 0.05 were concluded to be significantly different. And if the sig.p value > 0.05, it can be concluded that there is no significant difference (Daud et al., 2023) The data generated from the *one-way anova* test < 0.05 of 0.000 can be concluded that there is a difference in antibacterial effectiveness of edamame seed extract liquid soap. Post Hoc follow-up



tests were used to find out if there was a significant difference in the methamphetamine formulation made. The results in this study are marked (*) which means that there is a significant difference. Overall, this study shows that the liquid soap of edamame seed extract has an inhibition zone at each concentration made.

This study concluded that edamame seed ethanol extract (*Glycine max*) can be formulated into liquid soap preparations and the three formulations made meet the requirements of the physical properties of liquid soap such as pH test, foam height, dispersibility, organoleptis and homogeneity. The liquid soap of edamame seed extract meets the requirements of the antibacterial activity test with a diameter of 14.36 mm – 16.57 mm and the diameter of the inhibition zone is said to be strong if it has a diameter of about 11-20 mm. the best formula is F3 (15%) because it has adequate physical stability and *the antibacterial activity of Staphylococcus aureus* is the best compared to F1 (5%) and F2 (15%). For the next researcher, the suggestion that can be done is to develop a formulation of liquid soap preparations using other samples that contain antibacterial. And it is expected to be able to conduct antibacterial activity tests using other bacteria.

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TABLE

Table 1. Formulation of Edamame Seed Extract Liquid Soap Preparation

| Material | Formula % | | | | Uses |
|-----------------------|-----------|--------|--------|--------|----------------------|
| | F0 (%) | F1 (%) | F2 (%) | F3 (%) | |
| Extract Edamame Seeds | 0 | 5 | 10 | 15 | Active ingredients |
| Olive Oil | 15 | 15 | 15 | 15 | Foaming |
| Koh | 8 | 8 | 8 | 8 | Basis (alkali agent) |
| Na-CMC | 0,5 | 0,5 | 0,5 | 0,5 | Emulgator |
| SLS | 0,5 | 0,5 | 0,5 | 0,5 | Foaming |
| Asam Stearat | 0,25 | 0,25 | 0,25 | 0,25 | Emulgator |
| BHA | 0,5 | 0,5 | 0,5 | 0,5 | Preservatives |
| Vanilla Flavoring | 1 | 1 | 1 | 1 | Moneyoma |
| Aquadest | Ad 100 | Ad 100 | Ad 100 | Ad 100 | Solvent |

Table 2. Results of edamame seed extraction

| Plant name | Powder weight | Weight of condensed extract | Rendermen % |
|---------------|---------------|-----------------------------|-------------|
| Edamame Seeds | 200 gram | 21,25 gram | 10,625 % |

Table 3. pH test results

| Replication | F1 | F2 | F3 |
|----------------|--------------------|--------------------|--------------------|
| 1 | 8,99 | 9,03 | 9,44 |
| 2 | 8,97 | 9,49 | 9,52 |
| 3 | 8,38 | 8,84 | 9,02 |
| pH ± SD | 8,78 ± 0,36 | 9,26 ± 0,32 | 9,32 ± 0,26 |



Table 4. Foam Height Test Results

| Replication | F1(cm) | F2 (cm) | F3 (cm) |
|---------------------------------|----------------|----------------|----------------|
| 1 | 8,7 | 9,5 | 10 |
| 2 | 9,0 | 9,0 | 8,8 |
| 3 | 10 | 10,5 | 11 |
| Average Foam Height ± SD | 9,23 cm ± 0,68 | 9,66 cm ± 0,76 | 9,93 cm ± 1.10 |

Table 5. Dispersion Test Results

| Soap Formulation | Spreadibility ± SD |
|------------------|--------------------|
| F1 | 3,8 cm ± 0,80 |
| F2 | 4,3 cm ± 0,76 |
| F3 | 4,8 cm ± 0,23 |

Table 6. Organoleptis Test Results

| Formula | Bentuk | Bau | Warna |
|---------|----------------|--------|-----------------|
| F1 | Viscous Liquid | Vanila | Yellowish white |
| F2 | Viscous Liquid | Vanila | Brownish yellow |
| F3 | Viscous Liquid | Vanila | Light brown |

Table 7. Homogeneity Test Results

| Soap Formulation | Homogenitas |
|------------------|---|
| F1 | Homogeneous, no small particles on the F1 liquid soap preparation |
| F2 | Homogeneous, no small particles in the F2 liquid soap preparation |
| F3 | Homogeneous, no small particles on F3 liquid soap preparations |



Table 8. Antibacterial activity test results

| Inhibition Zone Diameter <i>Staphylococcus aureus</i> (cm) | | | | | |
|---|---|---------------------|---------------------|---|-----------|
| Edamame Seed Extract Liquid Soap | Edamame Seed Extract Liquid Soap | | | Edamame Seed Extract Liquid Soap | |
| | F1 (5%) | F2 (10%) | F3 (15%) | K+ | K- |
| Replication 1 | 14,17 | 14,35 | 15,17 | 19,58 | 0 |
| Replication 2 | 15,28 | 16,18 | 16,06 | 19,89 | 0 |
| Replication 3 | 13,08 | 14,26 | 17,33 | 18,48 | 0 |
| Replication 4 | 14,77 | 15,65 | 16,23 | 17,07 | 0 |
| Replication 5 | 14,54 | 16,01 | 17,62 | 19,03 | 0 |
| Average ± SD | 14,36 ± 0,82 | 15,29 ± 0,91 | 16,57 ± 0,98 | 18,83 ± 1,08 | 0 |

Table 9. Category of Inhibition Zone (Tjiptoningsih, 2021)

| Inhibition Zone Diameter | Obstacle Response |
|-------------------------------------|--------------------------|
| >21 mm | Very Strong |
| 11 – 20 mm | Strong |
| 6 – 10 mm | Keep |
| < 5 mm | weak |

