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FORMULATION AND ANTIBACTERIAL EFFECTIVENESS OF FERMENTED SOYBEAN HUSK EXTRACTS FROM INDUSTRIAL WASTE OF TEMPE AS ECO-FRIENDLY HAND SANITIZER GEL

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Abstract: This study aims to determine the formulation and antibacterial effectiveness of fermented soybean seed husk extract from tempe industrial waste as an environmentally friendly hand sanitizer gel. This type of research is an experimental study with variations in fermented soybean husk extract concentration, namely 0%, 5%, 15%, 20%, 50%, and 100%. Observational data were tested by analysis of variance ANOVA at a 5% level using Co-Stat software. Suppose there is a significant difference, a further test of Honest Significant Difference (BNJ) (organoleptic test), a further test of Orthogonal Polynomial Method (MOP) (spreadability, pH, viscosity), descriptive analysis test (flavonoid test, homogeneity test, antibacterial activity test is carried out) and test the total number of germs). The results showed that the fermented extract of soybean seed husk was positive for flavonoids, characterized by a bright yellow color change when added with 10% NaOH. The concentration of fermented soybean husk extract significantly affected the hand sanitizer gel's organoleptic properties, dispersion, total pH, and viscosity. Based on the organoleptic test, the panelists preferred the clear gel color in the F1 treatment (0%) and the thick, slightly liquid gel form with a slightly scented soybean husk in the F3 treatment (10%). The concentration treatment of soybean husk fermented extract (50%) was the best treatment based on good homogeneity, total pH 4.35, viscosity 2405 cPs, following SNI No.06-2588, and spreadability of 4.47 cm which was close to hand gel. For commercial sanitizers, the diameter of the inhibition zone against Escherichia coli bacteria ATCC 2922 and Staphylococcus aureus ATCC 2923 is 14 mm and 12 mm.

Keywords: *Antibacterial, Soy Been Husk, Gel Hand Sanitizer*

INTRODUCTION

Hand sanitizer is a practical and efficient hand-washing medium that has become increasingly widespread in the community since the COVID-19 pandemic. *Hand sanitizers* contain antibacterial compounds or antimicrobial agents that can coagulate germ cell proteins [1]. However, using hand sanitizer too regularly will irritate because it contains the main ingredient, alcohol. So further research is needed regarding the antibacterial activity of hand sanitizer gels with the addition of extracts of natural and environmentally friendly ingredients that can improve the quality of hand sanitizers. One of them is by utilizing soybean-based agro-industrial waste extracts.

Soybean-based agro-industry, especially in the temple business, produces solid waste in the form of soybean husks. A Tempe business unit produces 6.19 kg/day of waste on average. While the number of tempeh production units in Mataram City reached 310 units, the total solid waste produced was 1.919 kg [2]. The high volume of soybean husk waste is different from the utilization, which is generally used as animal feed, and the rest is disposed of, so it will harm the environment and the health of the local community. The soybean seed husk contains 17.98% crude protein, 5.5% fat, 24.84% crude fiber, and 28.29 kcal/kg metabolic energy. This content can grow lactic acid bacteria that produce antimicrobial compounds such as hydrogen peroxide (H2O2) diacetyl and bacteriocins that inhibit or prevent resistance [3].

Lactic acid bacteria from soybean husk could inhibit the growth of Escherichia coli 0157 with an inhibition zone of 8.313 mm and Staphylococcus aureus ATCC 25923 with an inhibition zone of 6.960 mm [4]. The soybean seed husk contains flavonoid compounds, secondary metabolic compounds that function as antimicrobial, antifungal, and antiviral compounds [3]. Flavonoid compounds in soybean seeds can be increased by fermentation using Rhizopus sp [5]. The levels of flavonoids in fermented soybean products such as tempeh are much higher than that of soybeans so they can affect the decrease in the enterotoxin adhesion of Escherichia coli to epithelial cells and humans [6]. Based on these problems and potentials, it is necessary to research related to the formulation and antibacterial effectiveness of fermented soybean husk extract from

the industrial waste of tempe, an aco-friendly hand sanitizer gel.

RESEARCH METHODS

Material and Tool

The materials used in this study were fermented soybean husk extract from industrial waste tempeh, RAPRIMA brand tempe yeast, ethanol 96%, NaCl 0.9%, antibiotic ciprofloxacin, buffer post (PGBP) steril, Carbopol 940, Gliserin, Triethanolamine (TEA), aquadest, Poli Etilen (PE) plastic, NaCl 0.9%, NaoH 10%, 0.5 Mc. Farland solution, bakteri Escherchia coli (ATCC 2922) dan Staphylococcus aureus (ATCC 2923), *media Plate Count Agar* (PCA), media Muller Hinton Agar (MHA).

The tools used in this study were a measuring pipette, dropper, blender, hand blender, maceration jar, 100 ml bottle, gloves, basin, analytical scale (Kern, United States), mask, beaker glass (Iwaki pyrex), measuring cup (Iwaki pyrex), test tube, Erlenmeyer flask, oven, aluminum foil, autoclave, sieve, filter paper, stirring rod, rotary evaporator, millimeter block, extract jar, cotton swab**,** pH meter universal, viscometer, autoclave, bunsen, petri dish, sterile loop wire, incubator, micropipettes, test tubes, tube racks, blue tips, and laminar airflow.

Manufacture of Fermentation of Soybean Arid Skin

A total of 2 kg of soybean seed husk and washed thoroughly, then drained and squeezed. After that, it was steamed for 30 minutes and mixed with 2% RAPRIMA yeast. Wrapped in plastic, perforated and fermented for 24 hours [7].

Manufacture of Fermented Soybean Seeds Aris Peel Extract

The extraction process was carried out by the maceration method. The fermented soybean husks were dried in the sun covered with black cloth for 12 hours or using a drying oven at a temperature of 60-70OC for 5 hours, then mashed and macerated using 96% ethanol solvent in a ratio of 1:5. Stored at room temperature in a dark place for five days and stirred every day. The filtration and evaporation process is followed by a Rotary Evaporator (T=55-60°C, speed 100-120 rpm) [8]. The formula for calculating yield:

$$\text{Yield (\%)} = \frac{\text{Thick extract (gram)}}{\text{Powder (gram)}} \times 100\%$$

Identification of Flavonoid Compounds

Flavonoid compounds were identified by color reaction using 2-4 drops of 10% NaOH solvent into 2 drops of the sample until it changed color from yellow to brownish-yellow [9].

Formulation Gel Hand Sanitizer

Formulation Gel hand sanitizer Fermented Extract Concentration of Soybean husk dengan with concentrations of 0%, 5%, 10%, 15%, 20%, 50%, and 100% can be seen in Table 1.

Table 1. Formulation Gel Hand Sanitizer Fermented Extract Concentration of Soybean Husk

Name Ingredients	Unit	Material Wealing						
		F1	F2	F3	F4	F5	F6	F7
Extract	ml	-	5	10	15	20	50	100
Carbopol 940	g	0.5	0.5	0.5	0.5	0.5	0.5	0.5
TEA	ml	1	1	1	1	1	1	1
Gliserin	ml	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Natrium metabisulfit	g	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Aquadest	ml	100	100	100	100	100	100	100

Formulation Making Gel Hand Sanitizer

The making hand sanitizer gel begins by entering carbopol 940 into 100 mL distilled water that has been heated (T=70˚C), and homogenized using a hand blender until it expands. Add TEA, glycerin, Sodium metabisulfite, and fermented soybean husk extract (5%, 10%, 15%, 20%, 50%, 100%) mixed until homogeneous and a gel mass is formed.

Organoleptic Test

Conducted by 20 respondents with an assessment of the shape, color, and aroma of the gel hand sanitizer fermented extract of soybean husk.

Homogeneity Test

Homogeneity testing is carried out by applying 500 mg of gel on top of the prepared glass. The gel must show a homogeneous arrangement and no coarse grains.[10]

Scatter Power Test

Scatter power testing is done by dripping 500 mg of gel in the middle of a petri dish, covering it with another petri dish, and giving 150 grams for 1 minute. SNI No. 06-2588 shows good gel spread power is -7 cm [10].

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pH Value Test

pH testing is carried out using a pH meter with a ratio of 1:9 (gel: aquades sterile). According to SNI No. 06-2588, the good pH value of the gel is 4-6.5 [10].

Uji Viskositas

Viscosity testing is performed using the Brookfield LV viscometer tool at a speed of 30 rpm. The viscosity value of a good gel is in the range of 2000-4000 cPs [11].

Antibacterial Activity Test

Antibacterial activity tests are carried out using the good diffusion method. The test was conducted by taking staphylococcus aureus ATCC 2923 and Escherichia coli ATCC 2922 bacterial cultures from medium Nutrient Broth (NB) into 2 ml of NaCl 0.9 solution until turbidity of 0.5 Mc. Farland. The culture solution is swabbed into the Muller Hinton Agar (MHA) medium. The hollowed-out media is then filled with fermented extracts of soybean husk (0%, 5%, 10%, 15%, 20%, 50%, 100%) and positive control in the form of ciprofloxacin antibiotics. 3 replications were performed and incubated at 36oC for 24 hours. The inhibition zone is measured using a ruler.

RESULTS AND DISCUSSIONS

Making Extract

The yield of fermented extract concentration of soybean husk can be seen in table 2.

Table 2. Fermented Extract Concentration Of Soybean Husk

Husk Fermented	Extract	Yield (%)
478 gram	65 gram	13.59

Table 2 shows that the extract obtained from the evaporation process was 65 grams with a dull yellow color and yielding 13.59%. The solvent used for the extraction process is 96% ethanol solvent. Ethanol solvent has a level of polarity that resembles the polarity of flavonoid compounds, so it is more effective in dissolving flavonoid compounds in simplest [12].

Identification of Flavonoid Compounds

The results of the identification test for flavonoid compounds can be seen in Table 3.

Table 3. Results of Flavonoid Identification With Color Reactions

Identification of Flavonoid Compounds	Result	Pustaka (Kusnadi, 2017
fermented extract concentration of soybean husk + NaOH 10%	Color change to bright yellow (+)	Yellow color change

Table 3 shows that the fermented soybean husk extract contains positive flavonoids because the color changes to yellow after adding 10% NaOH. Bases decompose cristine compounds derived from flavone compounds into molecules such as acetophenone, which are yellow in color due to breaking bonds in the isoprene structure when NaOH% is added [9]. It proves that the fermented extract of soybean seed husk contains flavonoid compounds.

Organoleptic Test

Adding fermented extract concentration of soybean husk with a concentration level gave a significantly different effect on the scoring test and not significantly different on the hedonic test in the form of hand sanitizer gel. The relationship between the effect of the concentration of fermented soybean husk extract on the shape parameters can be seen in Image 1

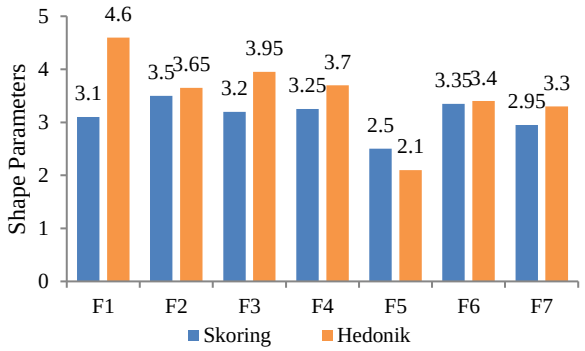

Figure 1. The Effect of Fermented Extract Concentration of Soybean Husk on The Shape of Hand Sanitizer Gel by Scoring and Hedonic

Figure 1 shows that the concentration treatment of fermented soybean husk extract has a significantly different effect on the *hand sanitizer gel* scoring test with a value ranging from 2.5 to 3.5 (thick, slightly liquid to thick). The average panelist gave hedonic test scores ranging from 2.1 to 4.6 (dislike to very like). Panelists preferred the *gel* in the F1 (0%) and F3 (10%) treatments which had a thick, slightly liquid form. The relationship between the effect of the concentration of fermented soybean husk extract on color parameters can be seen in Image 2

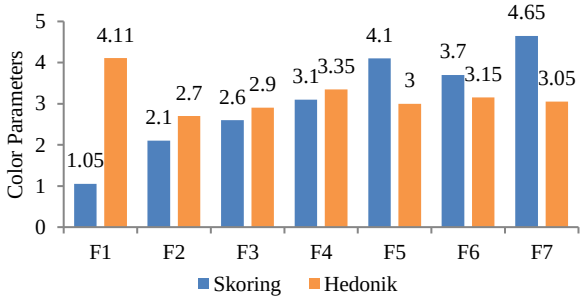

Figure 2. The Effect of Fermented Extract Concentration of Soybean Husk on The Color of Hand Sanitizer Gel by Scoring and Hedonic

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Figure 2 shows that the concentration treatment of fermented soybean husk extract has a significantly different effect on the color scoring test of the *hand sanitizer gel* produced, with a value ranging from 1.05 to 4.65 (clear to very thick yellow). The average panelist gave hedonic test scores ranging from 2.7 to 4.11 (slightly like to like). Panelists preferred *gel* F1 (0%) and F4 (15%) with clear and light yellow gel colors. The higher the concentration of the addition of fermented soybean husk extract, the more concentrated the *hand sanitizer gel*. It is due to the extract, which is dark yellow. The relationship between the effect of the concentration of fermented soybean husk extract on aroma parameters can be seen in Image 3

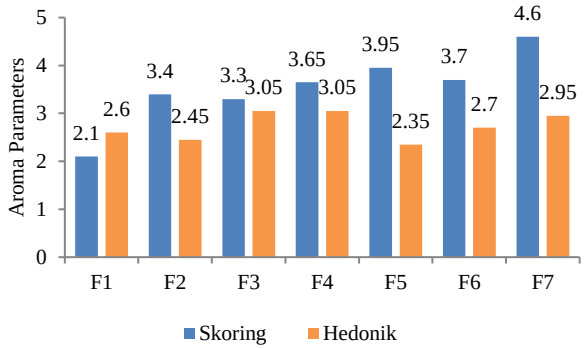

Figure 3. The Effect of Fermented Extract Concentration of Soybean Husk on The Aroma of Hand Sanitizer Gel by Scoring and Hedonic

Figure 3 shows that the concentration treatment of fermented soybean husk extract has a significantly different effect on the hand sanitizer gel, with a value ranging from 2.1 to 4.6 (no scent of soy epidermis to the flavor of soybean epidermis). The average panelist gave hedonic test scores ranging from 2.35 to 3.05 (dislike to somewhat like). The panelists preferred the aroma gels. Soybean seed husks had an unpleasant aroma, but by fermentation treatment with Rhizopus oligosporus, the unpleasant smell of soybean husks could be minimized [13].

Homogeneity Test

The Homogeneity Test aims to determine the degree of homogeneity of fermented soybean husk gel hand sanitizer extract by the qualitative method through direct observation. The homogeneity test results showed no difference in the homogeneity of all gel hand sanitizer formulations. The results of the hand sanitizer gel homogeneity test with a variation in extract concentrations of 0%, 5%, 10%, 15%, 20%, 50%, and 100% have a homogeneous arrangement, namely the absence of coarse granules and parts that are not evenly mixed. Thus it can be known that the difference in the concentration of the extract as a gelling agent does not affect its homogeneity. All formulations of hand sanitizer gel show results that meet the requirements of SNI No.06-2588 [14], that is, if the gel is applied to a piece of glass, indicating the absence of coarse granules or lumps in the gel.

Scatter Power Test

The relation of the concentrating effect of fermented soybean husk extract to the spread power of hand sanitizer gel can be seen in Image 4.

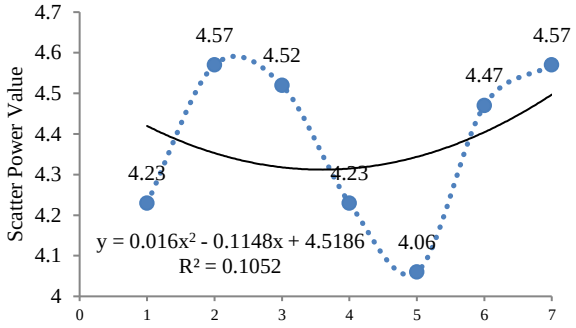

Figure 4. The Effect of Fermented Extract Concentration of Soybean Husk on The Spread Power of Hand Sanitizer Gel

Figure 4 shows the direction of linear regression. The upward regression direction indicates that the higher the concentration of the added extract, the more the value of the hand sanitizer gel spread will increase. The higher the extract concentration will cause the gel structure to become less rigid because the extract still contains water. The increase in the value of the scattering power is affected by the viscosity value. If the viscosity value is low, the spread power value will increase, and vice versa [14]. The treatment of F1 (0%) up to F7 (100%) produces a spread power of 4.06-4.57 cm that does not meet the requirements of SNI No.06-2588 [14], where semisolid preparations that are comfortable to use have a wide power of 5-7 cm. However, all formulations can be said to have good stability and are close to the spread value of commercial hand sanitizer gel (Dettol), which is 4.8 cm.

pH test

The relation of the concentrating effect of fermented extract of soybean husk on the pH of hand sanitizer gel can be seen in Image 5.

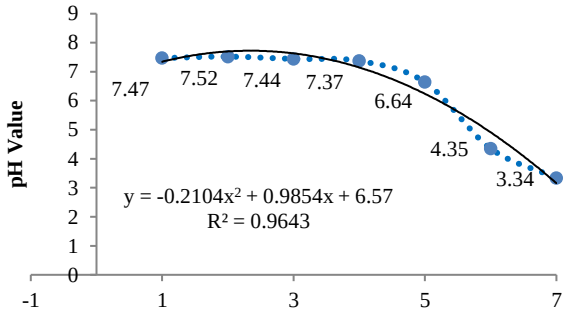

Figure 5. The Effect of Fermented Extract Concentration of Soybean Husk on pH Value of Hand Sanitizer Gel.

Figure 5 shows the direction of linear regression down. The downward regression direction indicates that the higher the concentration of the added extract, the lower the pH of the hand sanitizer gel. The pH value of hand sanitizer gel decreases due to the presence of flavonoid compounds in acidic extracts [16]. In addition, there is a fermentation treatment on the skin of Ari, which produces lactic acid bacteria that produce organic acids so that the pH value decreases [17]. Treatment F5 (20%) and F6 (100%) produce consecutive pH values of 6.64 and pH4.35 that meet the requirements of SNI No.06-2588 [14], namely, the pH gel of hand-washing products ranging from 4-6.5.

Viscosity Test

The relation of the concentrating effect of fermented soybean husk extract to the viscosity of hand sanitizer gel can be seen in Figure 6.

Figure 6 shows the direction of linear regression down. The downward regression direction indicates that the higher the concentration of the extract added, the more viscosity of the hand sanitizer gel will decrease. Decreased viscosity value may be affected by pH Decrease in viscosity value can be affected by the pH and water content of extracts as well as the amount of triethanolamine used [18]. In addition, the influence of temperature and storage time that is getting longer causes syneresis, which is the fluid discharge process entangled in the gel [19]. The treatment of F5 (20%) and F6 (50%) produces viscosities of 3602.67 cPs and 2405 cPs that meet normal viscosity standards ranging from 2000-4000 cPs [2].

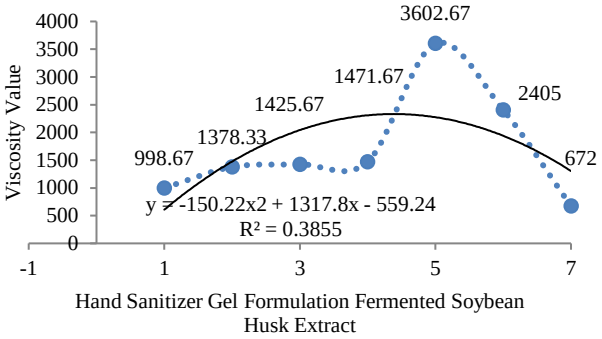

Figure 6. The Effect of Fermented Extract Concentration of Soybean Husk on Vicosity Value of Hand Sanitizer Gel

Antibacterial Activity Test

The effect of fermented soybean husk extract concentration on antibacterial activity can be seen in table 4.

Table 4. Average Observation Results Effect of Fermented Extract Concentration of Soybean Husk on Antibacterial Activity

Formulation (extract concentration)	Resistance Zone Diameter (mm)							
	Staphylococcus aureus				Escherichia coli			
	I	II	III	Average	I	II	III	Average
F1 (0%)	0	-	-	0	0	-	-	0
F2 (5%)	0	0	12	12	0	12	12	12
F3 (10%)	11	0	0	11	0	12	11	11.5
F4 (15%)	11	11	12	11.13	10	11	11	10.6
F5 (20%)	11	11	11	11	13	13	14	13.3
F6 (50%)	10	14	12	12	0	14	14	14
F7 (100%)	11	12	13	12	12	12	11	11.6
Kontrol Positif	30	-	-	30	35	-	-	35

Description: Positive Control: ciprofloxacin
F1 (0%) : Sterile Aquadest
I = First Repetition
II = Second Repetition
III = Third Repetition

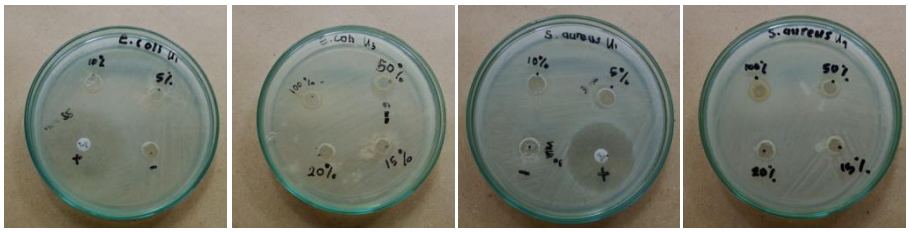

Figure 7. Test Result Antibacterial Activity Test Results

Table 4 shows that the F1 (0%) treatment did not form an inhibitory zone which proves that the inhibition zone formed was not caused by the excipients used but was caused by antimicrobial compounds in the soybean seed coat. The average results of the measurement of the inhibition zone of soybean husk fermented extract against Staphylococcus aureus ATCC 2923 in treatment F2 (5%), F3 (10%), F4 (15%), F5 (20%), F6 (50%), (F7 (100%) 12 mm, 11 mm, 11.13 mm, 11 mm, 12 mm, 12 mm respectively. Meanwhile, the average results of the measurement of the inhibition zone of soybean husk fermented extract against Escherichia coli bacteria in treatment F2 (5%), F3 (10%), F4 (15%), F5 (20%), F6 (50%), (F7 (100%) 12 mm, 11.5 mm, 10, respectively. 6 mm, 13.3 mm, 14 mm, 11.6 mm.

The inhibition zone formed was classified as an active inhibition zone, which ranged from 11-20 mm [20]. The higher the concentration of the fermented extract, the larger the diameter of the inhibition zone formed. Treatment F6 (50%) resulted in the largest diameter of the inhibition zone against Escherichia coli ATCC 2922, which was 14 mm, while the F6 (50%) and F7 (100%) treatments produced the largest diameter of the inhibition zone against Staphylococcus aureus, which was 12 mm. Fermented soybean husk extract produced the best inhibition zone for Escherichia coli ATCC 2922. Treatment F4 (15%) to F7 (100%) was a stable concentration. As evidenced by each replication test, the diameter of the inhibition zone was formed.

The difference in the inhibitory properties of the extract against the two test bacteria was caused by the different sensitivity of each bacterium to antimicrobial substances because it has a structure of antimicrobial compounds in the soybean seed coat. Average inhibition zone measurement results soybean husk fermented extract against Staphylococcus aureus ATCC 2923 in treatment F2 (5%), F3 (10%), F4 (15%), F5 (20%), F6 (50%), (F7 (100%) successively 12 mm, 11 mm, 11.13 mm, 11 mm, 12 mm, 12 mm. Meanwhile, the average results of the measurement of the inhibition zone of soybean husk fermented extract against Escherichia coli bacteria in treatment F2 (5%), F3 (10%), F4 (15%), F5 (20%), F6 (50%), (F7 (100%) 12 mm, 11.5 mm, 10.6 mm, 13.3 mm respectively, 14 mm, 11.6 mm.

The inhibition zone formed belongs to the active inhibition zone, which ranges from 11 to 20 mm [20]. The higher the concentration of the fermented extract, the larger the diameter of the inhibition zone formed. Treatment F6 (50%) produced the largest diameter of the inhibition zone against Escherichia coli ATCC 2922, which was 14 mm, while the F6 (50%) and F7 (100%) treatments produced the largest diameter of the inhibition zone against Staphylococcus aureus, which was 12 mm. ai produced the best inhibition zone for Escherichia coli bacteria ATCC 2922. Treatment F4 (15%) to F7 (100%) was a stable concentration. As evidenced by each replication test, the diameter of the inhibition zone was formed.

The difference in the inhibitory properties of the extract against the two test bacteria was caused by the different sensitivity of each bacterium to antimicrobial substances because they have different cell structures and compositions. Staphylococcus aureus ATCC 2923 is a gram-positive bacterium with a relatively simple cell wall structure, making it easier for antibacterial compounds to enter cells and find targets for work. Escherichia coli is a gram-negative bacterium with a relatively more complex and three-layered cell wall structure. The outer layer is lipoprotein, the middle layer is lipopolysaccharide, and the inner layer is peptidoglycan [20]. Besides being influenced by the test bacteria, the difference in inhibitory activity was influenced by the composition of the active compound. Soybean seed husk fermented extract contains chemical compounds, namely flavonoids. Flavonoid compounds can denature proteases and damage cell walls so that they interfere with metabolic ability; the greater the damage to cell walls. It will cause cell death [21].

CONCLUSION

From the results of the research that has been carried out, it can be concluded the optimum formulation of hand sanitizer gel is in the treatment of adding 50% fermented soybean husk extract based on a homogeneity test, total pH test, and viscosity test following SNI No.06-2588. Meanwhile, based on the organoleptic test, the best treatment based on color was found in the addition of 0% extract, and based on shape and aroma was found in the addition of 10% extract. The fermented extract of soybean husk has antibacterial activity against Escherichia coli and Staphylococcus aureus and has a stable inhibition zone at an extract concentration of 15%-100%.

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