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RESEARCH ARTICLE

Antiseptic White Honey Liquid Soap Formulation and its Effectiveness against Nosocomial Infections

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* Multiresisten (PaMR) are the main bacteria that cause nosocomial infections. Prevention of MRSA and PaMR transmission can be done by maintaining a clean body through the use of antiseptic bath soap that is sensitive to this bacterium. This study aimed to carry out a white honey antiseptic liquid soap formula that could be useful for killing MRSA and PaMR bacteria. This research was based on laboratory research on white honey as an antiseptic derived from natural ingredients. Organoleptic examination, pH, viscosity, consistency, antibacterial effectiveness for 56 days of storage, then from the evaluation results of the formula was best done a hedonic test. Statistical calculations with fixed random block complete design models were applied for a conclusion of evaluation tests. The results of the test activity of the liquid shower soap showed that all three formulations had activity against the MRSA and PaMR bacteria. Growth Inhibitory Concentration Minimum preparation of antiseptic liquid bath soap was 12.5% w/v. Antiseptic liquid soap with a concentration of 12.5% had a phenol coefficient of 0.4167 for MRSA bacteria and 0.9 for PaMR bacteria. The comparative test results showed that the best formula (F2) activity was 0.859: 1 against MRSA and 0.7167: 1 against PaMR compared to liquid bath soap in the market. The test results showed that the best liquid bath soap formula based on stability and the lowest GICM value was white honey antiseptic liquid soap with a concentration of 12.5% w/v.

Keywords: *Antiseptic bath soap, Nosocomial infections, White Honey, Methicillin-Resistant Staphylococcus aureus (MRSA), Multiresistent Pseudomonas aeruginosa (PaMR).*

Introduction

Nosocomial infections are infections that have been caught in a hospital and are potentially caused by organisms that are resistant to antibiotics. Nosocomial infections account for 7% in developed and 10% in developing countries [1]. The risk of hospital-acquired infection is dependent on the patient's immune status, infection control practices, and the prevalence of the various pathogens in the local community.

The most frequently isolated pathogens were Staphylococcus aureus (20.9%), Klebsiella pneumonia (16.4%) and Pseudomonas aeruginosa (10.7%). Nosocomial infections can be defined as those occurring within 48 hours of hospital admission, 3 days of discharge or 30 days of operation [2-4].The oldest known cosmetics to humans are soap, a skin cleansing agent that is used in addition to cleaning also for skin fragrances. Therefore, consumers must be smart in choosing soap.

Besides being used as a skin cleanser and fragrance, soap must also contain a substance or material that can serve to maintain skin health [5-6]. Besides bathing can also avoid the body from infectious diseases, including nosocomial infections that are often in hospitals. Honey has properties to eradicate and kill bacteria.

Also, the ingredients contained in honey can restore tissues in skin wounds and prevent the death of cells [7]. Honey is reported to have antibacterial activity against Pseudomonas aeruginosa, *E.coli, S. aureus, S. Pyogenus* [7-8]. Honey is produced by honey bees which is a natural liquid from flower essence (floral nectar) or other parts of plants (extrafloral nectar) [9]. White honey and amber honey are examples of types of honey-based on their color.

In previous studies, white honey had better antibacterial activity against MRSA and PaMR when compared to amber honey [10]. This study reports antiseptic white honey liquid soap formulation and its effectiveness against nosocomial infections. Based on the shape of the soap is divided into two types, namely solid form soap, and liquid form. This study chose liquid bath soap. After all, it has advantages when compared with other forms of soap, because it is easy to use, carry and store, is not easily damaged and dirty, and has exclusive packaging [11-12].

S. aureus is around positive Gram bacteria usually arranged in an irregular sequence such as grapes [13]. It is a bacterium that can produce toxins and includes aerobic bacteria. Usually grows above the mucosal layer of the skin and mucous membranes in humans [13]. MRSA is one of the *S. aureus* bacteria that has been resistant to methicillin antibiotics [14]. *P. aeruginosa* is a Gram-negative, aerobic, and moving bacterium using a flagellum. *P. aeruginosa* is a major pathogen for humans and sometimes colonizes humans and causes infection if the host's defense function is abnormal [15-16].

Multiresisten is known for its ability to survive against several types of antibiotics, namely because it has an outer membrane that limits the entry of antibiotics into the cytoplasmic and antibiotic membrane must first diffuse through the pores found in the outer membrane [17].Therefor e *P. aeruginosa* is a dangerous and deadly pathogen.

In this study, an antiseptic liquid soap formulation from white honey will be conducted and its effectiveness against MRSA and PaMR bacteria.

Materials and Methods

Tools

Analytical scales (Mettler Toledo Dragon 204), Magnetic Stirrer (Yellow MAG HS 7, 230V, 50/60 Hz), pH meter (pH-meter 744 Methrohm), Viscotester Rion (VT-04 F), digital cameras (Panasonic), vaporizer cups, Petri dishes, autoclaves (Hirayama HV50), incubators (Hach portable incubator), heaters (Toyomi, HP115FI), micropipets, calipers, Ose wires, spreaders, and glassware that are commonly used in the Pharmacy Laboratory, and the Microbiology Laboratory.

Test Material

The test material used was white honey from the island of Sumbawa, Indonesia, which was produced by C.V. Syan Bimpar Utama, Jakarta and distributed by C.V. **Bacteria Test:** The test bacteria used in this study were *Staphylococcus aureus* Resistant to Methicillin (MRSA) and *Pseudomonas aeruginosa* Multiresisten (PaMR) obtained from Hasan Sadikin Hospital in Bandung, Indonesia.

Bacterial Growth Media

Hatchery media used were Nutrient Agar (oxoid) and Nutrient Broth.

Methods

The research method used in this research was experimental, which consisted of the following stages of work:

Collection of materials: raw materials for research in making soap were obtained from the chemical industry.

Antiseptic liquid bath soap formulation: Four formulations were designed as shown in the table 1.

Testing the physical stability of the preparation: This tests included

Organoleptic: was done by observing the shape, color, and smell of liquid bath soap.

Observation of changes in shape, color, and the odor was done on days 1, 3, 7 and then every week for 56 days of storage.

PH: The pH meter is calibrated with a pH buffer solution, carried out at any time when taking measurements. Measurements were taken on days 1, 3, 7, and then every week for 56 days of storage. The sample to be examined at 25°C.

Consistency and Homogeneity: Done by observing changes in consistency and homogeneity of the soap preparations made, whether there is a separation between the soap-forming material with water. Measurement of Viscosity: Viscosity of the preparation was measured using the Rion VT- 04F Viscotester Stand in the following manner: The spindle was inserted into the container to the boundary markers. The safety valve is released and the rotor is turned on. Then leave it for 5 minutes until the scale shows a stable number. Measurements were made on days 1, 3, 7, and then every week for 56 days of storage.

Measurement of Foam Height: Antiseptic soap preparations containing various concentrations of white honey made a 1% solution in water. Then put into a measuring cup with a lid, and shaken for 20 seconds by turning the measuring cup regularly. Then the height of the foam formed is measured. Measurements were taken every week for 56 days of storage. Testing the effectiveness of antibacterial preparations against MRSA and PaMR bacteria:

Antibacterial Activity Test for Antiseptic Liquid Soap: The agar diffusion method was used, using a cylindrical technique in this technique, as much as 20 ml of nutrient agar (40 - 450C) was poured into a sterile petri dish, then homogenized and allowed to stand for several minutes until it freezes. Each 20 µL test bacterial suspension was put into a petri dish, then spread evenly to all nutrient agar. After that, a sterile cylinder was inserted into the petri dish at a predetermined position, then pressed. The glass cylinder was placed on the surface so that the solid has been inoculated with test bacteria using a spreader. Antibacterial activity was seen as an inhibitory or clear zone around the cylinder.

Determination of Minimum Growth Inhibitory Concentration (MGIC Antiseptic Liquid Soap: was done from various variations of the concentration of antiseptic soap obtained from the results of the activity test.

Each concentration of antiseptic soap (11.5%, 12.5%, and 13.5%) was taken as much as 0.5 g, put into a petri dish, then added 4.5 ml of nutrient agar. After being homogenized and allowed to freeze, an ose of test bacteria was etched on the surface of the agar nutrient. All Petri dishes were incubated in an incubator at 37^0C for 18-24 hours.

MGIC was in the petri dish with the smallest concentration of test material without colony growth.

Determination of Antibacterial Phenol Coefficients of Antiseptic Liquid Soap Soap for Test Bacteria: The purpose of this test was to determine the effectiveness of antiseptics in white honey after a liquid bath soap formulation was made after being stored for eight weeks. The microorganism test was carried out by the phenol coefficient method by observing turbidity.

Comparison Test: A total of 1 g of test material (antiseptic soap) and comparison soap (soap circulating on the market) with various concentrations of concentration were inserted into the cylindrical holes. All Petri dishes were incubated in an incubator at 37^0C for 18-24 hours. The diameter of the inhibition formed around the hole was measured using a caliper.

Safety Testing of Soap Preparations: The safety test of the preparation was carried out using the open patch method (Patch Test Methods) of 10 volunteers based on the Indonesian National Cosmetics Formulary.[18] The preparations tested were liquid antiseptic bath soap from white honey on the back of the hand, allowed to stand for one hour and observed the possibility of irritation to the skin. The test is carried out three times in 1 day for 2-3 consecutive days.

If there is no irritation, it is marked -

If it is hot, a + is given

If erythema develops, it is given a ++ sign

If itching develops, it's marked +++

If you feel sore, marked ++++

Hedonic Test

The hedonic test of this preparation was carried out using hand antiseptic gel preparations which had the best results

during the storage time carried out on 20 volunteers.

Statistical Data Analysis

Data analysis was performed statistically from the results of the evaluation of pH, viscosity, and height of foam preparations, namely the results of measurements of pH, viscosity, and height of foam 1, 3, 7, and then every week for 56 days of storage.

Results and Discussion

Antiseptic Liquid Bath Soap Formulation

Four formulations were designed as shown in table 1. Formulations were made referred to the existing textbook [18-23]. The main raw materials for liquid bath soap were Oils/fats/Esters. In the process of making soap, the type of oil or fat used is vegetable oil or animal fat. The difference between oil and fat is the appearance of both in a state of space. The oil will be liquid at room temperature (± 28 ° C), while fat will be solid [24].

This study used Cocoamidopropyl betaine which was a synthetic surfactant derived from coconut oil and dimethylaminopropylamine. In the form of viscous pale yellow transparent liquid. Its molecular formula C19H38N2O3 and Molecular weight: 342.52 g/mol. Function: surfactants in bath products such as soaps and shampoos, in cosmetics as emulsifying and thickener agents [25, 26]. Palm kernel oil diethanolamine was obtained from palm oil seeds. Palm kernel oil contains fatty acids that were similar to coconut oil so that it could be used as a substitute for coconut oil.

Palm kernel oil has a higher content of unsaturated fatty acids and lower short-chain fatty acids than coconut oil [27]. Sodium lauryl sulfate (SLS) which had synonyms Sodium dodecyl sulfate, dodecyl sodium sulfate, sodium monododecyl sulfate, sodium monolauryl sulfate, texapon K12P. Molecular formula: $NaC12H25SO4$, molecular weight: 288.38 g mol^{-1}. Function as an anionic surfactant, detergent, emulsifying agent, lubricant tablets and capsules, wetting agent. SLS was used with low concentrations in the manufacture of toothpaste, shampoo and shaving foam. The use of SLS that did not irritate the skin is at concentrations below 20% [28].

Sodium Cocoyl Sarcosinate, like sodium lauryl sulfate, was a cleansing and foaming agent, but that was where the similarities end. Derived from sarcosine, an amino acid that occurred naturally in the body, sodium lauroyl sarcosinate was frequently heralded for being a thorough cleanser but also for being gentle. It worked by attracting excess oil and dirt, then carefully removing the grime from the hair by emulsifying it so it rinses easily away with water [29]. Concerning MRSA, *S. aureus* first became an important hospital pathogen in the 1940s. Treatment of this infection using penicillin G (benzylpenicillin) was an antimicrobial of the ß-lactam class. A decade later, penicillin-resistant strains emerged.

This strain inactivates antimicrobials that have the ß-lactam enzyme ring. This enzyme hydrolyzes the cyclic amide bonds that bind to the ß-lactam ring causing a loss of the antimicrobial anti bactericidal activity, therefore efforts were developed to obtain drugs that are resistant to ß-lactamase [30]. Methicillin was modified penicillin which was introduced in the 1960s. This antibiotic was used to treat infections caused by *S. aureus* which are resistant to most penicillins.

In 1961 methicillin-resistant *S. aureus* strain was discovered [31]. *P. aeruginosa* was a bacterium known for its ability to withstand several types of antibiotics. Therefore *P. aeruginosa* was seen as a dangerous and deadly pathogen. These bacteria were naturally resistant to various types of antibiotics because they have an outer membrane that limits the entry of antibiotics into the cytoplasmic membrane. After all, antibiotics must diffuse first through the pores contained in the outer membrane [32].

Antibiotic resistance was genetically coded by genes located on chromosomes or plasmids (R/resistant plasmids). In general, antibiotic-resistant bacteria were caused by the presence of a resistance gene located on plasmid R. In antibiotic resistance encoded by genes in chromosomes, resistance occurred through modification of antibiotic targets. Gene encoded resistance in plasmid R was caused by enzymes that inactivate drugs or enzymes that actively pump drugs out of cells.

Only a few effective antibiotics can fight against P. aeruginosa multiresistant including fluoroquinolones, gentamicin,

cephalosporin, and imipenem [33]. Based on the color, honey can be divided into several types, namely clear (water white), black amber (dark amber), white (white) [34]. The reaction which was catalyzed by the glucose oxidase enzyme was the main factor that determined the antibacterial activity in honey. The antibacterial activity was related to the characteristics and chemical content of honey [35]. Honey was also shown to have activity against several bacteria, including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Streptococcus pyogenus, and Salmonella typhimurium* [36]. It had been reported that white honey was potentially to kill both MRSA) and PaMR [10].

Table 1: Design of antiseptic liquid soap formulations

Composition	Formula			
	F_0	**F_1**	**F_2**	**F_3**
Sodium Lauryl Sulfate (%)	13	13	13	13
Sodium Cocoyl Sarcosinate (%)	6	6	6	6
Palm Kernel Oil Diethanolamide (%)	5	5	5	5
Cocoamidopropyl Betaine (%)	5	5	5	5
White honey	-	11,5	12,5	13,5
Aqua destillata ad	100	100	100	100

Notes:
F0: Formula without white honey
F1: Formula with white honey 11.5%
F2: Formula with white honey 12.5%
F3: Formula with white honey 13.5%

Results of the Physical Stability Evaluation

The results of the physical stability of antiseptic liquid soap soaps can be seen in Table 2. The data in Table 2, it is shown that from the three preparations of Formula F1, F2, F3 it had fairly good organoleptic stability. This was evidenced by the absence of color and odor changes during storage 56 days of storage time. Soap made in the form of thick solution and the addition of white honey caused clear yellow soap preparations.

The addition of honey also causes soap preparations to become thicker when compared to F0 which was not given white honey. Likewise with the homogeneity of each formula was still the same as the initial state. This stable homogeneity was due to the soap formulas that contain surfactants that function as cleaning agents and were also useful as emulsifiers to stabilize the soap dosage form.[37]

PH and Viscosity Measurement Results

The results of pH measurements and viscosity of antiseptic liquid bath soaps with various concentrations of white honey can be seen in Fig.1 and Fig. 2 respectively. From Fig. 1 it could be seen that the pH of antiseptic liquid soap was affected by the addition of various concentrations of white honey.

When the three pHs of liquid bath soap containing various concentrations of white honey were measured, the lowest pH lied in bath soaps containing the highest concentration of white honey. This is because the pH of white honey which tends to be acidic has a pH between 3. 2- 4.5. The more white honey was added, the soap will be more acidic, which causes the pH of the bath soap also decreases [38].

During storage, the pH of the liquid bath soap decreases (more acidic). This was probably due to the presence of CO2 bound in the preparation of liquid bath soap when the liquid bath soap had contact with the air during the storage time. In statistical calculations with a complete random block design, the model found that the null hypothesis (H0) was rejected because the value of F count (10.446) of the three formulas was greater than the Ftable (2.25) with a significant level $\alpha = 5\%$ and $p = .05$ [40,41].

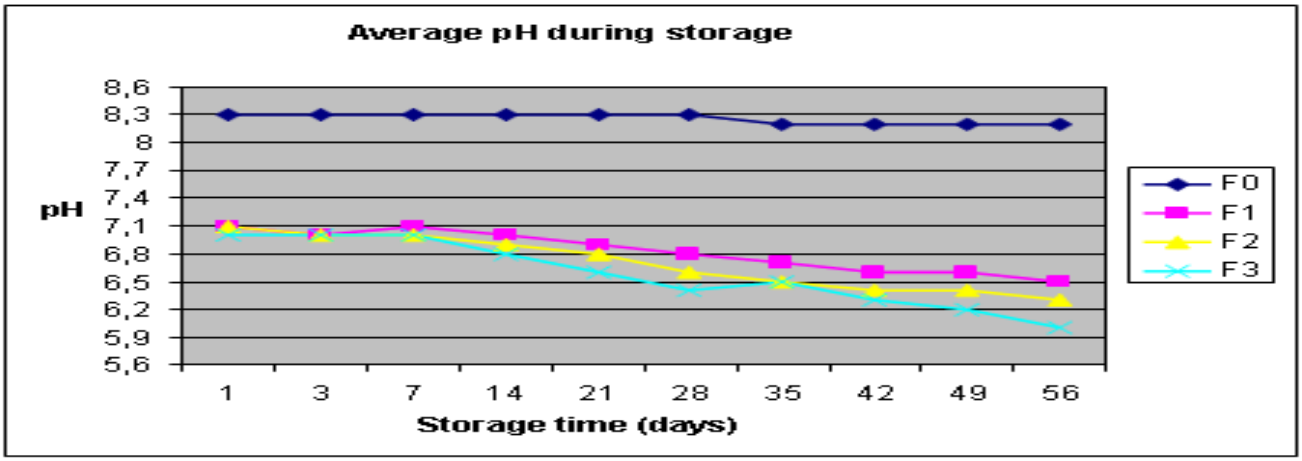
Because Ho was rejected, then the Newman Keuls test was carried out [42] Ho Although there was a decrease in pH in the liquid soap that was made, but the decrease in pH was not significant which meant that the pH of the soap was relatively stable that was relatively safe to use as bath soap with a pH close to the pH of human skin between 5-8 as

stated by Standard of the Ministry of Trade of the Republic of Indonesia [39].

Table 2: Results of Physical Stability Antiseptic Liquid Soap

Observation	Formula	Storage Time Day-to-Day									
		1	3	7	14	21	28	35	42	49	56
Color	**F_0**	b	b	b	b	b	b	b	b	b	b
	F_1	kb	kb	kb	kb	kb	kb	kb	kb	kb	kb
	F_2	kb	kb	kb	kb	kb	kb	kb	kb	kb	kb
	F_3	kb	kb	kb	kb	kb	kb	kb	kb	kb	kb
Odor	**F_0**	kh	kh	kh	kh	kh	kh	kh	kh	kh	kh
	F_1	kh	kh	kh	kh	kh	kh	kh	kh	kh	kh
	F_2	kh	kh	kh	kh	kh	kh	kh	kh	kh	kh
	F_3	kh	kh	kh	kh	kh	kh	kh	kh	kh	kh
Consistency	**F_0**	++	++	++	++	++	++	++	++	++	++
	F_1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	F_2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	F_3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Homogeneity	**F_0**	h	h	h	h	h	h	h	h	h	h
	F_1	h	h	h	h	h	h	h	h	h	h
	F_2	h	h	h	h	h	h	h	h	h	h
	F_3	h	h	h	h	h	h	h	h	h	h

Notes:
F0: Formula without white honey
F1: Formula with white honey 11.5%
F2: Formula with white honey 12.5%
F3: Formula with white honey 13.5%
b: Clear Color
kb: Clear Yellow
kh: Distinctive smell
+/-: Thickness quantity
h: Homogeneous



Notes:
F0: Formulawithout white honey
F1: Formula with white honey 11,5%
F2: Formula with white honey 12,5%
F3: Formula with white honey 13,5%

Fig. 1: Graph of measurement of pH during storage

The results of viscosity measurements of antiseptic liquid bath soaps with various concentrations of white honey can be seen in Fig. 2. It found the viscosity values of the

three liquid bath soap formulas for 56 days experienced significant changes. The addition of white honey causes higher viscosity values. White honey itself was thick and has a viscosity of 18 poise.

The viscosity value of F3 is greater than the value of F2 and F1. Likewise with liquid shower soap without the addition of white honey F0. By using similar statistical calculations with a complete random block design model followed with the Newman Keuls test as above [40-42], it found that the null hypothesis (H0) was rejected because the value of Faccount (12.83) of the three formulas was greater than Ftable (2.25) with a significant level α = 5 % and p = 0.05. This meant that there was a significant difference in the value of viscosity during a storage time of 56 days.

All test preparations had an average viscosity between 31-41 poise. Although there was no limit to the viscosity range in bath soap, the thickness of the soap was important, so that the soap can be poured properly [43].

Foam Height Measurement

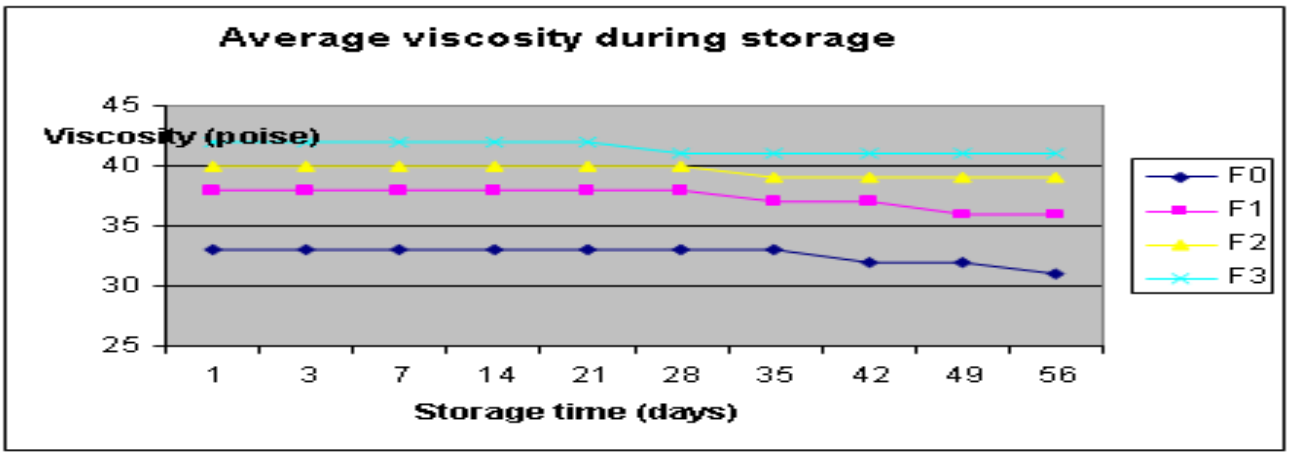
In statistical calculations with the complete random block design of fixed models as in determining the determination of pH and viscosity, it was found that there was a significant difference in the high value of foam during 56 days of storage. All test preparations had an average foam height of between 6.4-6.5 cm. Although there was no limit to the range of high foam in bath soap, the foam produced by bath soap can be one of the satisfactions of consumers when using soap

Antibacterial Activity Test Results for Antiseptic Liquid Soap

Testing the activity of antiseptic liquid bath soap preparations using the agar cylinder method [44].Inhibitory diameters of white honey antiseptic liquid soap against MRSA and PaMR bacteria can be seen in Table 3. From these results, it found F0 which was a formula without white honey had no inhibitory zone diameter.

This implied that the base used in the formulation did not have antiseptic power. From the results of the determination of MGIC in Table 4, it showed that the lowest concentration that did not show the growth of MRSA was 11.5% w/v, while at that concentration PaMR bacteria were still growing. The concentration of white honey that had been found in the formulation did not increase MGIC, this was indicated by the growth of PaMR bacteria at a concentration of 11.5% w/v.

This was consistent with previous studies, that MRSA did not show growth at a concentration of 6.5% w / v whereas PaMR did not show growth at a concentration of 12.5% w/v [10]. The result of the formulation that could kill both MRSA and PaMR bacteria was liquid shower soap formula with the white honey concentration of 12.5% and 13.5%. The result of positive control with 1 bacterial ose showing the growth of bacteria meaning that positive control as a solvent did not have antiseptic power.



Notes:
F0: Formulawithout white honey
F1: Formula with white honey 11,5%
F2: Formula with white honey 12,5%
F3: Formula with white honey 13,5%

Fig. 2: Graph of measurement of viscosity during storage

Table 3: Antibacterial activity test results of Honey white antiseptic liquid soap against MRSA and PaMR bacteria

Formula	Average inhibition zone diameter (cm)	
	MRSA	***PaMR***
F_0	0	0
F_1	1.47633	1.185667
F_2	1.901667	1.42330
F_3	2.3280	1.708330

Notes:
F0: Formulawithout white honey
F1: Formula with white honey 11,5%
F2: Formula with white honey 12,5%
F3: Formula with white honey 13,5%

Table 4: Results of Determination of Minimum Growth Inhibition Concentration (MGIC) Antiseptic Liquid Soap

Formula concentration (% b/v)	Results	
	MRSA	***PaMR***
11.5	-	+
12.5	-	-
13.5	-	-
Positive control	+	+

Notes:
Positive control: Positive control consisted of 1 ml NB (oxoid) and 1 ml of bacterial ose
(+): there is bacterial growth
(-): no bacterial growth

Phenol Coefficient for Antiseptic Liquid Soap: The formula used in the determination of the phenol coefficient was a formula with the white honey concentration of 12.5% w/v. That was because the formula with the white honey concentration of 12.5% had an MGIC against both bacteria namely MRSA and PaMR . From the data in the table above, the killing power of phenols against MRSA at the fastest and longest time is 2.5 'and 15'. Phenol has a killing power at dilution concentrations of 1/2 for the fastest and 1/8 for the oldest (see Table 5). The killing power of formula to the MRSA at the fastest and longest time was 2.5 'and 15'.

The formulation was made to have the killing power at the dilution concentration of 1/4 for the fastest and 1/16 for the longest (Table 6). From the data in Table 5 and Table 6, the calculation results of the phenol coefficient for MRSA bacteria were 0.4167 while the phenol coefficient value of PaMR bacteria was 0.9. The phenol coefficient value obtained had a phenol coefficient value below one. From these results, it appeared that the resulting liquid bath soap had relatively good antibacterial activity. This might be because white honey contains the enzyme glucose oxidase which could function as an antiseptic agent.

Table 5: Average kills of Phenol against MRSA and PaMR

Fenol	1 MRSA/ PaMR	1/2 MRSA/ PaMR	¼ MRSA/ PaMR	1/8 MRSA/ PaMR	1/16 MRSA/ PaMR	1/32 MRSA/ PaMR
2,5 (min)	-/-	-/+	+/+	+/+	+/+	+/+
5 (min)	-/-	-/-	-/+	+/+	+/+	+/+
7,5 (min)	-/-	-/-	-/+	+/+	+/+	+/+
10 (min)	-/-	-/-	-/-	-/+	+/+	+/+
12,5 (min)	-/-	-/-	-/-	-/+	+/+	+/+
15 (min)	-/-	-/-	-/-	-/+	+/+	+/+

Notes:
(+): there was bacterial growth
(-): no bacterial growth

Table 6: Average Kills of Formula (12.5% against MRSA And PaMR

Fenol	1 MRSA/ PaMR	1/2 MRSA/ PaMR	¼ MRSA/ PaMR	1/8 MRSA/ PaMR	1/16 MRSA/ PaMR	1/32 MRSA/ PaMR
2,5 (min)	-/-	-/+	-/+	+/+	+/+	+/+
5 (min)	-/-	-/-	-/+	+/+	+/+	+/+
7,5 (min)	-/-	-/-	-/+	+/+	+/+	+/+
10 (min)	-/-	-/-	-/-	+/+	+/+	+/+
12,5 (min)	-/-	-/-	-/-	+/+	+/+	+/+
15 (min)	-/-	-/-	-/-	-/-	-/+	+/+

Table 7: Average Difference between Treatment Table for α = 5%

Treatment (Day storage)	Mean	Difference 2 Average										RST
		6.4	6.4	6.4	6.45	6.5	6.5	6.5	6.5	6.5	6.5	0.05
56	6.4	-	-	-	0.05*	0.1*	0.1*	0.1*	0.1*	0.1*	0.1*	0.0267
49	6.4	-	-	-	0.05	0.1*	0.1*	0.1*	0.1*	0.1*	0.1*	0.0505
42	6.4	-	-	-	0.05*	0.1*	0.1*	0.1*	0.1*	0.1*	0.1*	0.0356
35	6.45	-	-	-	-	0.05*	0.05*	0.05*	0.05*	0.05*	0.05*	0.0381
28	6.5	-	-	-	-	-	-	-	-	-	-	0.0399
21	6.5	-	-	-	-	-	-	-	-	-	-	0.0415
14	6.5	-	-	-	-	-	-	-	-	-	-	0.427
7	6.5	-	-	-	-	-	-	-	-	-	-	0.439
3	6.5	-	-	-	-	-		-	-	-	-	0.0449
1	6.5	-	-	-	-	-	-	-	-	-	-	0.457

Comparative Test

Comparative results of white honey antiseptic liquid bath soap and standard antiseptic liquid bath soap on the Market using the Cylinder Agar Method. Comparative tests were carried out by comparing the inhibitory diameters of the best antiseptic liquid bath soap from white honey with those on the market.

The best liquid bath soap was bath soap from white honey with a concentration of 12.5% compared to the X stock in the market. Comparative tests were carried out by comparing the inhibitory diameters of the best antiseptic liquid bath soap from white honey with those on the market. The best liquid bath soap was bath soap from white honey with a concentration of 12.5% compared to the X stock in the market. Obtained the ratio of inhibitory diameter for MRSA was 1: 0.859. As for the PaMR bacteria, the diameter inhibition ratio was 1: 0.7167.

Hedonic Test

The antiseptic liquid soap of white honey that was tested favorably was the soap that had the lowest MGIC that be able to kill both bacteria with good physical stability, stable pH value, good viscosity value, liquid bath soap with the white honey concentration of 12.5%.

Safety Test Results: The antiseptic liquid soap of white honey that was tested favorably was the soap that had the lowest MGIC that could and kill both bacteria with good physical stability, stable pH value, good viscosity value, liquid bath soap with the white honey concentration of 12.5%. The results of the safety test of the preparation found that there was no irritation in the volunteer, not found a burning sensation, erythema, itching, and burning so it could be concluded that the formula was safe to use

Statistical Analysis

To get accurate results, statistical tests were performed on pH, viscosity, foam height measurement. The results of this analysis test had been mentioned above. In principle, the statistical analysis by making ANOVA tables from the data obtained. If Ho was rejected if F count> F table.

This meant that there were differences from each data obtained due to the influence of the duration of storage. Then the Newman Keuls test was performed at a significance level of 0.05 so it would get the effect of the length of time of storage of the data obtained. Table 7 was an example of a table obtained in the Newman Keuls calculation, Table of Difference Between Treatment for α = 5%.

From this table, it could be concluded that at the 0.05 significance level it was found that during the storage time of the 35th day, 42nd day, 49th day and 56th day had a different effect on foam height on each each soap. However, the length of storage time is the most influential on the change in foam height of each soap in the storage time is 56 days.

Conclusion

The results of the test activity of the liquid shower soap showed that all three formulations had activity against the MRSA and PaMR bacteria. Growth Inhibitory Concentration Minimum preparation of antiseptic liquid bath soap was 12.5% w/v. Antiseptic liquid soap with a concentration of 12.5% had a phenol coefficient of 0.4167 for MRSA bacteria and 0.9 for PaMR bacteria.

The comparative test results showed that the best formula (F2) activity was 0.859: 1 against MRSA and 0.7167: 1 against PaMR compared to liquid bath soap in the market. The test results showed that the best liquid bath soap formula based on stability and the lowest GICM value was white honey antiseptic liquid soap with a concentration of 12.5% w/v.

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