

## Formulation Of Physical Stability Test And Antibacterial Activity Test Of Hand Sanitizer Spray Ethanol Extract Of Biddara Leaves (*Ziziphus Mauritiana L.*) Against *Staphylococcus Aureus* Bacteria

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### Abstract

Bidara leaves (*Ziziphus mauritiana L.*) are a plant that has antibacterial activity, one of which is against *staphylococcus aureus* bacteria, so it can be formulated as an active substance in spray hand sanitizer preparations. This research aims to formulate bidara leaf extract and test its antibacterial activity against *staphylococcus aureus* bacteria using the disc diffusion method. In this research, 4 formulas were made with varying concentrations of bidara leaf extract (*Ziziphus mauritiana L.*). Then a physical stability test was carried out with test parameters including organoleptic, pH, homogeneity, freezer and thaw, irritation. The results of research based on the physical stability test showed that organoleptically, pH, homogeneity, irritation, for the 4 formulas, they were stable during storage in the freezer and thaw tests at F0 and F1 remained stable at F2 and F3, physical changes occurred, namely slightly viscous. Furthermore, based on the antibacterial spray hand sanitizer test against *staphylococcus aureus*, the results showed that the concentration of bidara leaf extract had an effect on inhibiting the growth of *staphylococcus aureus* bacteria with an inhibitory power of 14.9 mm for a concentration of 0.7%, 17.3 mm for a concentration of 1.4% and 19.75 mm for a concentration of 2.1%.

Keywords: Bidara Leaf; Hand Sanitizer Spray; *Staphylococcus aureus*.

### 1. Introduction

*Staphylococcus aureus* is a microorganism commonly found on the skin. This bacteria is a gram-positive bacteria that can cause skin infections and serious infections such as mild skin infections (furunculosis and impetigo). Skin diseases caused by bacteria and fungi can be prevented by cleaning the skin with antiseptics. Antiseptics are chemical compounds that can inhibit or kill microorganisms in living tissue, which has the effect of limiting and preventing infections from becoming more severe. One form of pharmaceutical preparation that can be used to clean hands is hand sanitizer spray [1].

Hand sanitizer is antiseptic with an alcohol content of 60-95%. According to the Food and Drug Administration (FDA), hand sanitizers can kill bacteria in less than 30 seconds. The alcohol contained in hand sanitizers has a good bactericidal effect against gram-positive and gram-negative bacteria. Apart from that, hand sanitizers also contain antibacterial ingredients such as triclosan or other antimicrobial ingredients which can prevent the growth of bacteria such as *E-coli* and *Staphylococcus aureus* on the hands [2].

Hands are one of the most important means of spreading germs. Therefore, hand sanitizer is very important. One preparation that is often used as a hand antiseptic is a spray. The spray form created is very practical to use and has a fast-drying profile so it is easy for

users to use [3].

One of the natural ingredients that is often used as traditional medicine is bidara leaves, known by the Latin name *Zizhipus mauritiana* L. Flavonoids, alkaloids, triterpoids, saponins, lipids and proteins are the main components of the bidara plant [4]. According to [5] it can be concluded that the bidara leaf plant has many benefits. The main advantage of the bidara leaf plant is that it has antimicrobial properties. Apart from that, it has many other benefits such as analgesic, antipyretic, anti-inflammatory and anti-cancer and also acts as a protector for body cells such as the kidneys, liver and brain.

## 2. Methodology

This research was conducted in July-August 2023 at the Pharmacy Laboratory, Pharmacy Study Program, Faculty of Health Sciences, Palopo Muhammadiyah University.

### Research Variables

Independent variables: are variables that are not influenced by other factors. In this study, the independent variable tested was hand sanitizer with an active substance concentration of F1 0.7%, F2 1.4%, and F3 2.1%.

The dependent variable: is the variable that will be affected by the independent variable. In this research, the dependent variables include antibacterial hand sanitizer testing, organoleptic testing, pH testing, homogeneity testing, freezer and thaw testing, and irritation testing.

### Tools and materials

The tools that will be used in this research are blenders, test tubes, analytical scales, dropper pipettes, petri dishes, beakers, ovens, magnetic stirres, vortexes, porcelain dishes, L rods, tweezers, oscillating needles, rulers, water baths, hot plates, incubator, and autoclave. The materials that will be used in this research are ethanol extract of bidara leaves, staphylococcus aures bacteria, nutrient agar (NA), glycerin, hydrogen peroxide, methyl paraben, 70% ethanol and distilled water.

### Sample processing (*Ziziphus mauritina* L)

Bidara leaves are picked manually using hands, the leaves that are picked are old leaves that are not eaten by caterpillars, which is done in the morning during photosynthesis. Then the bidara samples that have been collected are wet sorted, then washed using running water to remove dirt and other contaminants that are still attached to the leaves, then weigh the wet weight as much as 2 kg. After washing, the bidara leaves are cut into pieces and then dried, placed on a tray to be drained and aired. Followed by dry sorting so that there are no unwanted particles. Then the sample was ground with a blender and sieved until a fine powder was obtained. Samples of bidara leaf powder were stored in tightly closed containers [6].

### Making bidara leaf simplicia (*Ziziphus mauritina* L)

500 grams of bidara leaf powder was added, 5 liters of 70% ethanol were added, then

extracted using the maceration extraction method, namely soaking with stirring for 48 hours (2 days). Then filtered, the filtrate is obtained and it will be concentrated using a rotary evaporator at a temperature of 50° - 60° C, then the extract is thickened using a water bath at a temperature of 60° C to obtain a thick extract [6].

### **Making Hand Sanitizer Spray with Bidara Leaf Extract**

Prepare all the ingredients, mix the ingredients, namely bidara leaf extract (extract concentration 0.7%, 1.4% and 2.1%), 5 grams of glycerin, 1 ml hydrogen peroxide, methylparaben 0.18 grams, 70% ethanol (10 mL ). mixed until homogeneous, then poured into a measuring cup, added 100ml of distilled water, stirred until homogeneous, then poured into a bottle. This was done repeatedly for each formulation with different extract concentrations.

### **Testing Hand Sanitizer Spray Preparations**

#### **Organoleptic Test**

This is done by describing the shape or composition (e.g. solid, thick, liquid), color (e.g. yellow, brown) and odor (e.g. aromatic, odorless) (Iswandana et al., 2017).

#### **pH test**

Measurements were carried out at room temperature. Pour 10 ml of product into a container, dip the pH meter into the container and record the pH reading value (Iswandana et al., 2017).

#### **Homogeneity test**

The purpose of this examination is to determine whether there are lumps or impurities in the solution (Depkes RI, 1979).

#### **Freeze and thaw stability test**

Stability testing using the Freez and Thaw method was carried out to see the stability of a preparation with the influence of temperature variations during a certain storage time. The preparation was kept at a cold temperature ( $4 \pm 2$  ° C) for 24 hours, then transferred to an oven at  $40 \pm 2$  ° C for 24 hours. This treatment is called 1 cycle (Huynh-Ba, 2008).

#### **Irritation test**

The dermal acute irritation test is a test on animals to detect toxic effects that appear after exposure to a single dose of the test preparation via the dermal route. The degree of irritation is assessed at certain time intervals, namely at 24 hours and 48 hours after exposure to the test preparation. The dermal acute irritation test is designed to determine the presence of an irritating effect on the skin and to assess and evaluate the characteristics of a substance when exposed to the skin (BPOM, 2014). Skin irritation studies are designed to mimic human exposure and are usually carried out on test animals such as rabbits, guinea pigs, rats and mice

#### **Tool sterilization**

Sterilization of tools is carried out on all used equipment by wrapping all tools in paper and sterilizing in an autoclave at a pressure of 121°C and 2 atm for 15 minutes.

### Making Nutrient Agar (NA) Media

In an Erlenmeyer flask, 1.6 grams of Nutrients (NA) are dissolved in 80 ml of distilled water (20 grams per 1000 ml). The solution is then stirred evenly using a magnetic stirrer until it reaches boiling point. After reaching the boiling point, the medium is cooled to a temperature of around  $\pm 45-50^{\circ}\text{C}$ . The basic media that has been prepared will be used as a base layer in making media (Dima et al., 2016)

### Making Bacterial Suspensions

The bacterial suspension solution was made by taking 1 dose of bacteria, placing it in a test tube containing 10 ml of 0.9% physiological NaCl solution, with the pure culture in the test tube and shaking until homogeneous (Misna and Khusnul, 2016).

## 3. Result and Discussion

### 3.1. Result

**Table 1. Maceration Results of Ethanol Extract of Bidara Leaves (*Ziziphus mauritina* L.)**

Plant	Powder (gr)	thick extract (gr)	Rendement (%)
Bidara leaves	500	98,3	19,66

The resulting yield in the table above is 19.66%. The yield is a comparison between the number of metabolites obtained after the extraction process and the weight of the sample used. The higher the yield value, the greater the extract produced, which indicates that more nutritious compounds are extracted [7].

The yield quality is considered good if the value is more than 10%, so the yield of bidara leaf ethanol extract obtained is declared good because it exceeds 10% in accordance with established standards [8].

**Table 2. Drying Loss and Water Content**

No	Simplicia Parameters	Standard	Concentration Examination Results (%)	Condition (%)
1.	Drying shrinkage		7,4 %	$\leq 10$ %
2	Water content		7,1 %	$\leq 10$ %

The drying loss test results in the table above reached 7.4%. Based on this drying loss percentage, simplicia has fulfilled the specified drying loss requirements, namely less than 10%. Water content above 10% can cause simplicia to be easily overgrown by microbes and can reduce the stability of the extract [9]. The water content test results in the table above reached 7.1% for bidara leaf extract so that the water content complies with FHI requirements, namely less than 10%, which can prevent the growth of microorganisms so that simplicia can still be stored for a long period of time [9].

**Table 3. Organoleptic Test Results of Spray Hand Sanitizer Extract of Bidara Leaves**

Formulation		Testing
F0	Form	Liquid
	Color	Colorless
	Smell	Alcohol
F1	Form	Liquid
	Color	Yellowish brown
	Smell	Lemons
F2	Form	Liquid
	Color	Brown
	Smell	Lemons
F3	Form	Liquid
	Color	Blackish brown
	Smell	Lemons

Information :

F0 = No Extract

F1 = 0.7% concentration of ethanol extract of bidara leaves

F2 = Concentration 1.4% Ethanol Extract of Bidara Leaves

F3 = Concentration 2.1% Ethano Extract of Bidara Leaves

Based on the results of the organoleptic tests that have been carried out, each dosage formula stored during the period from week 0 to week 4 shows good stability. There is no color change in each formula. The color of the preparation in F0 remains colorless because it does not contain the concentration of bidara leaf extract. Meanwhile, F1 is yellowish brown, F2 is brown and F3 is blackish brown. The color of these three preparations is related to the presence of bidara leaf extract with different concentrations, namely 0.7%, 1.4%, 2.1%, in the spray hand sanitizer preparation. The physical forms of F0, F1, F2, and F3 remain fluid. This condition is caused by the characteristics of the liquid constituent components in the spray hand sanitizer preparation. The aroma of the preparation did not change in odor as long as the preparation was stored from week 0 to week 4. In F1, F2, F3, the spray hand sanitizer preparation still had the same aroma, namely lemon aroma. So it can be concluded that the three formulas are stable.

**Table 4. pH Test Results**

Formulation	Testing
F0	pH 5
F1	pH 5
F2	pH 5
F3	pH 5

Based on the data above in table 4.4, the results of the pH test in this study, it can be seen that the spray hand sanitizer preparations F0, F1, F2, and F3 still maintained a pH of pH 5 during the storage period from week 0 to week 4. Conclusions from the results of the pH test in the study This is that the spray hand sanitizer preparations obtained at F0, F1, F2, and F3 meet the specified requirements. The pH test carried out showed that the bidara leaf extract hand sanitizer was at a pH that generally met the requirements of SNI No. 06-2588 ranges from 4.5- 6.5. Because if the hand sanitizer product is at an acidic pH, it will

cause irritation to the skin. Meanwhile, if the hand sanitizer is at an alkaline pH, it will cause peeling on the hands.

**Table 5. Homogeneity Test Results**

Formulation	Testing
F0	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous

Based on the data in the table above, the results of the homogeneity test on the spray hand sanitizer preparations F0, F1, F2, and F3 remained homogeneous or clear during the storage period from week 0 to week 4, without any visible dirt or foreign particles. In the homogeneity test of this study, it can be concluded that F0, F1, F2, and F3 produced homogeneous and clear preparations, indicating that the preparations in this study were mixed perfectly. The homogeneity test also meets the criteria set for spray hand sanitizer preparations, namely preparations that are clear and do not contain foreign particles. One guarantee of the quality of a dosage form is the homogeneity of ingredients, which is an important evaluation in pharmaceutical dosage formulations to determine the mixability of ingredients in the formula.

**Table 6. Freeze and thaw test results**

Formulation	Pengujian	
F0	Form	Liquid
	Color	Colorless
	Smell	Alcohol
F1	Form	Liquid
	Color	Yellowish brown
	Smell	Lemons
F2	Form	A bit thick
	Color	Brown
	Smell	Lemons
F3	Form	A bit thick
	Color	Blackish brown
	Smell	Lemons

In the results of the table above, it can be seen that in F0 and F1 there was no physical change, while in F2 and F3 there was a physical change, namely it was a little thick after being put in the freezer at a temperature of  $4 \pm 2^{\circ}\text{C}$  for 24 hours, then transferred to an oven at that temperature.  $40^{\circ}\text{C}$  for 24 hours.

**Table 7. Irritation Test Results**

Time	Occurrence of Erythema				Occurrence of Edema			
	F0	F1	F2	F3	F0	F1	F2	F3
24 Clock	0	0	0	0	0	0	0	0
48 Clock	0	0	0	0	0	0	0	0
72 Clock	0	0	0	0	0	0	0	0

Information ;

(0) = No irritation occurs

Based on table 4.5, the results of the irritation test in this study, the spray hand sanitizer preparation from the ethanol extract of bidara leaves in formula 0, formula 1, formula 2, and formula 3, did not cause irritation to rabbits. In this irritation test it can be concluded that the spray hand sanitizer preparation sprayed on the skin does not show any skin irritation effects such as erythema and edema.

**Table 8. Antibacterial Activity Test Results of Hand Sanitizer Spray Hand Sanitizer Extract of Bidara Leaves (*Ziziphus mauritina* L.)**

Bacteria	Concentration	Replication			Average (mm)	Strength Resistor	Sig
		I	II	III			
<i>S.aureus</i>	F1 0,7%	14,5	17,5	12,75	14,9	Strong	0,00
	F2 1,4%	18,75	18	15,25	17,3	Strong	
	F3 2,1%	20,5	20	18,75	19,75	Strong	
	K +	12	17,75	16,75	15,5	Strong	
	K -	0	0	0	0	-	

The results of observations during incubation for 24 hours showed that the largest average inhibition zone in formula 3 was 19.75 mm in the strong inhibition category, in formula 2 the inhibition zone was 17.3 mm which was included in the strong category, while in formula 1 it was obtained inhibition zone of 14.9 mm which is included in the strong category. This is because the concentration of the extract added to formula 1 is smaller than formulas 2 and 3. The result of the positive control is 15.5 mm which is included in the strong category. The diameter of the antibacterial inhibition zone is grouped into four categories, namely, weak activity (<5mm), medium (>5-10mm), strong (>10-20mm), very strong 20 mm or more [10]. The antibacterial activity contained in bidara leaf extract is caused by the secondary metabolite compounds contained therein, namely alkaloids, flavonoids, tannins and saponins. As an antimicrobial, flavonoids can inhibit cell membrane function and bacterial energy metabolism and form complex compounds with extracellular and dissolved proteins which disrupt the integrity of bacterial cell membranes which can damage cell membranes. The antibacterial mechanism of tannin compounds is by contracting cell walls, this can disrupt cell permeability so that it can cause cell wall damage [11]. Meanwhile, saponin can reduce the surface tension on bacterial cell walls, resulting in damage to cell wall permeability where the cell wall experiences leaks so that intracellular compounds will come out and

result in cell death [12].

To determine whether there is an effect of differences in the concentration of the spray hand sanitizer preparation and the comparison on the diameter of the inhibition zone for *Staphylococcus aureus* bacteria, a statistical test of the One Way Anova parameters was carried out. The results of the normality test for the antibacterial activity of the spray hand sanitizer preparation obtained by the normality test results can be seen in (attachment 12). F1  $p > 0.712$ , F2  $p > 0.391$ , F3  $p > 0.328$ , KP  $p > 0.312$ , which is greater than  $p > 0.05$ . So it can be said that the data is normally distributed. Where the data is normally distributed if the p value is  $> 0.05$ . The results of the homogeneity test obtained a significant value for each data  $> 0.05$ , which means it has a homogeneous value, which can be seen in (attachment 12). It can be said to be significant if  $p > 0.05$  [13]. From these results, further testing can be carried out using the One Way Anova test. The One Way Anova test results obtained a result of 0.00. The value obtained is smaller than  $p < 0.05$ , which is said to be significant if  $p < 0.05$  [13] 5 me. This shows that the data on the diameter of the inhibition zone for the spray hand sanitizer preparation of bidara leaf extract (*Ziziphus mauritiana* L.) has a significant difference.

#### 4. Conclusion

Based on the research that has been carried out, the following conclusions are obtained:

1. In the spray hand sanitizer preparation, the ethanol extract of bidara leaves has a larger inhibition zone at F3 with a concentration of 2.1 with an average inhibition zone of 19.75 mm.
2. In the preparation of hand sanitizer spray, the ethanol extract of bidara leaves has the best physical stability test in F1 with a concentration of 1.4%.

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#### 6. Reference

- [1] Ningrum, W. and Rahmatullah, S, "Uji Aktivitas Antibakteri Ekstrak Etanol Daun Bidara (*Ziziphus Mauritiana* Lamm) Dalam Formulasi Sediaan Sabun Cair Sebagai Antiseptik Terhadap Bakteri *Stapylococcus Aureus* Atcc 25923," *Medical Sains: Jurnal Ilmiah Kefarmasian*, 5(1), 89–98, 2020.
- [2] Rini, E. and Nugraheni E. R, "Uji Daya Hambat Berbagai Merek Handsanitizer Gel Terhadap Pertumbuhan Bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Journal of Pharmaceutical Science and Clinical Research*, 1(10), 18-26, 2018.
- [3] Firmansyah, F, "Formulasi Dan Evaluasi Hand sanitizer Spray Ekstrak Buah Belimbing Wuluh (*Averrhoa Bilimbi* L)," *Prepotif: Jurnal Kesehatan Masyarakat*, 2021.

- [4] Putri, R, “Uji Aktivitas Daun Bidara Arab (*Ziziphus spina-christ L.*) sebagai Antibakteri pada Sel Kanker Kolon (*WiDr*) Melalui Metode MTT dan Identifikasi Senyawa Aktif dengan Metode LC-MS’, *Skripsi*,” Fakultas Sains dan Teknologi, Universitas Islam Negeri Maulana Malik Ibrahim Malang, 2017.
- [5] Siregar, “Berbagai Manfaat Daun Bidara (*Ziziphus mauritina Lam.*) bagi Kesehatan di Indonesia,” *Meta-Analisis*, Vol. 2, No. 1, 2020.
- [6] Ekanursyahfitri. “Mutu Fisik Dan Aktivitas Terhadap Bakteri *Staphylococcus Aureus* Sediaan Masker Gel Peel-Off Ekstrak Daun Bidara (*Ziziphus Mauritiana L.*) Sebagai Antijerawat,” *Akademi Farmasi Putra Indonesia Malang*, 2017.
- [7] Syamsul, E. Anugerah, and Supriningrum, ”Penetapan Rendamen Ekstrak daun Jambu Mawar Determination of Mawar Jambu Leaf Extract (*Syzygium jambos*)’, *Jurnal Riset Kefarmasian*, Vol. 2, No. 3, pp. 147-157, 2020.
- [8] Madjid, A. Rahmawati, and Fasya, “Variasi Komposisi Eluen pada Isolasi Steroid dan Triterpenoid Alga Merah *Eucheuma cottonii* dengan Kromatografi Kolom Basah’, “*Alchemy*, Vol. 8, No. 1, pp. 35-40, 2020.
- [9] Utami, Y. et al, “Standarisasi Simplisia dan Ekstrak Etanol Daun Leilem (*Clerodendrum minahassae L.*)’, *Journal of Pharmaceutical and Medicinal Sciences*, Vol. 2, No. 3, pp. 32-39, 2017.
- [10] Hanizar, E. And Sari, “Aktivitas Antibakteri *Pleurotus Ostreatus* Varietas Grey Oyster Pada *Staphylococcus Aureus* Dan *Pseudomonas Aeruginosa*,” *Pustaka Kesehatan*, 6(3), P. 387, 2018.
- [11] Sapara, T. U. Waworuntu, and Juliatri, ”Efektivitas Antibakteri Ekstrak Daun Pacar Air (*Impatiens balsamina L.*) Terhadap Pertumbuhan *Poryphyromonas gingivalis*. *Pharmacon : Jurnal Ilmiah Farmasi*, 05 (04) : 10-17, 2016.
- [12] Sudarmi, K. Darmayasa, I. and Muksin, “Uji Fitokimia dan Daya Hambat Ekstrak Daun Juwet (*Syzygium cumini*) terhadap Perumbuhan *Escherichia coli* dan *Staphylococcus aureus* ATCC. *Jurnal Simbiosis*, 05 (02) : 47-51, 2017.
- [13] Trisia, A. Philyria, R. And Toemon, “Antibacterial Activity Test Of Ethanol Extract From Kalanduyung Leaf (*Guazuma Ulmifolia Lam.*) On *Staphylococcus Aureus* Growth With Diffussion Method (Kirby-Bauer),” *Anterior Jurnal*, 17(2), Pp. 136–143, 2018.