

Evaluation of formula and antibacterial activity of spray preparation from combination of aloe vera (*Aloe vera* L.) and lime concentrate (*Citrus aurantiifolia*) as hand sanitizer

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Abstract: Aloe vera plant (*Aloe vera* L.) has antioxidant activity and can be used as a moisturizer, especially on the inside of the leaves. Lime plant (*Citrus aurantiifolia*) has antibacterial activity, one of the compounds it contains is limonene. This study aims to evaluate the formula and antibacterial activity of a spray preparation combining aloe vera and lime concentrate as a Hand Sanitizer. The Hand Sanitizer spray formulation consists of four formulas with variations in the concentration of aloe vera and lime concentrate, namely F0 (0), F1 (30:20), F2 (25:25), and F3 (20:30). The evaluation carried out for 28 days includes organoleptic tests, homogeneity, pH, viscosity, drying time, and antibacterial activity. The organoleptic and homogeneity test results showed that F1, F2, and F3 were in homogeneous liquid form, cloudy orange in color, and had a distinctive lime aroma. The pH test obtained a range of 3.75-5.79. The viscosity test obtained a viscosity range of 1.06-1.29 cps. The drying time test obtained a time range of 33-46 seconds. The results of the antibacterial activity test showed that the highest inhibitory value was in formula F3 with values (14.5±0.2nm), followed by Formula F2 (11.5±0.45nm) and F1 (8.2±0.25nm). Based on statistical testing, it was concluded that there was an effect of each formula on antibacterial activity.

Keywords: *Aloe vera*; Anti-bacterial; Hand-sanitizer; Lime; Spray.

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Introduction

Personal hygiene is an action taken to maintain the cleanliness and health of each person. One of the actions taken to improve a person's hygiene is washing their hands (Lestari et al., 2022). In addition to cleaning hands with hand soap, we can also use hand sanitizer as a hand cleaner that does not require running water (Saputri et al., 2021). Several hand sanitizer preparations available on the market can be used very quickly and easily, by pouring it into the palm of the hand and then spreading it evenly over the surface area of the hand (Diana et al., 2013). Most of the hand sanitizer products on the market contain alcohol with a high concentration of between 70-90% and synthetic chemicals which

function as antibacterials. These chemicals can cause skin health problems, such as making the skin dry, irritated, and rashes (Gusman et al., 2022).

The Aloe vera (*Aloe vera* L.) which comes from the Liliaceae family can help maintain skin moisture in the gel part of its leaves. The compounds contained in the aloe vera plant include aloin, emodin, aloe-emodin, salicylic acid, and cinnamic acid which can function as antioxidants, moisturizing agents, and antibacterials (Marhaeni, 2020).

The lime (*Citrus aurantiifolia*), especially in their concentrate, contain citric acid, limonene, hesperidin, alkaloids, flavonoids, and phenolic compounds that have the ability to inhibit bacterial growth (Puspita et al., 2020). The flavonoid compounds most commonly found

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in lime juice have the activity of causing damage to bacterial cells (denaturing) (Mengga et al., 2021). Hand sanitizer spray preparations containing a combination of Aloe vera and lime concentrate have several advantages compared to other dosage forms, namely they are more practical to use, easy to absorb, easy to carry, do not contain alcohol, and have good antibacterial activity.

Materials and Methods

Materials

The tools used in this study include mortar and stamper, analytical balance, homogenizer, magnetic stirrer, beaker glass, watch glass, dropper pipette, pH meter, Brookfield viscometer, petri dish, incubator, loop needle, tweezers, and commonly used laboratory tools.

The materials used in this study included aloe vera gel, lime concentrate, carbopol, propylene glycol, methyl paraben, propyl paraben, triethanolamine, oleum citri, agar nutrient, 70% alcohol, *Staphylococcus aureus* bacteria, and distilled water.

The Plant Determination

Determination of aloe vera and lime plants was carried out at the Herbarium Laboratory of the Bandung Institute of Technology (ITB), West Java.

The Preparation of Simplicia

Aloe vera leaves are taken as much as 2 kg and lime fruit is taken as much as 1 kg. Aloe vera leaves are cleaned and the gel part is taken and then washed with running water. The cleaned lime is then cut into two parts, then squeezed using a squeezer and filtered to get lime concentrate (Martono, 2018).

The Spray Hand Sanitizer Preparation

In this study, formula optimization was carried out with variations in the concentration of active ingredients aloe vera and lime concentrate.

Table 1. Hand sanitizer spray preparation formulation

Ingredients	Concentration (%)			
	F0	F1	F2	F3
Aloe vera	-	30	25	20
Lime Concentrate	-	20	25	30
Carbopol	0,5	0,5	0,5	0,5
Propylenglycol	10	10	10	10
Methyl Paraben	0,18	0,18	0,18	0,18
Propyl Paraben	0,02	0,02	0,02	0,02
Triethanolamine (TEA)	0,4	0,4	0,4	0,4
Oleum citri	0,4	0,4	0,4	0,4
Aquadest	ad	ad	ad	ad
	100	100	100	100

Each ingredient in **Table 1.** according to the formula is weighed using an analytical scale. The volatile materials are weighed using a watch glass or beaker glass. Carbopol material is dissolved with distilled water and TEA is added until a transparent and slightly thick gel mass is formed. After that, propylene glycol material is used to dissolve methyl paraben and propyl paraben (mass 2). The active ingredients of aloe vera, lime concentrate, and mass 2 are put into the gel mass then dripped with oleum citri and homogenized using a homogenizer until homogeneous and then put into a spray bottle container (Agustin et al., 2023).

The Evaluation of Hand Sanitizer Spray Preparations The Organoleptic Test

The organoleptic test was carried out by observing changes in odor, color and aroma of the spray hand sanitizer preparation during the storage time. Each hand sanitizer spray preparation was tested 3 times replication (Agustin et al., 2023).

The pH Test

The pH test was carried out using a pH meter by dipping the pH meter into 10 ml of hand sanitizer spray preparation. Previously, the pH meter was calibrated with pH 4.7, and 9. Each hand sanitizer spray preparation was tested 3 times (Agustin et al., 2023).

The Homogeneity Test

The homogeneity test was carried out by placing 20 ml of hand sanitizer spray on transparent glass. Homogeneity is indicated by the absence of coarse grains. Each hand sanitizer spray preparation was tested 3 times replication (Agustin et al., 2023).

The Viscosity Test

The viscosity test was conducted using a Brookfield viscometer. A sample of 100 ml of hand sanitizer spray preparation was taken, then the preparation was put into a beaker, then the spindle was dipped into the preparation and the viscosity value was recorded. Each hand sanitizer spray preparation was tested 3 times. The viscosity test that shows a good preparation is if the viscosity value is at 800-3000 cPs (Agustin et al., 2023).

The Dry Time Test

The sample of the hand sanitizer spray preparation was taken as much as 2 ml and then applied to one hand and rubbed. The drying time of the preparation was calculated using a stopwatch when the preparation was completely dry and not sticky. Each hand sanitizer spray preparation was tested 3 times replication (Martono et al., 2018).

The Antibacterial Activity Test

Each tool used for testing antibacterial activity was sterilized, including petri dishes, test tubes, droppers, Erlenmeyer flasks, measuring cups, tweezers, and loop needles according to the sterilization method of each tool.

The preparation of Nutrient Agar (NA) Media is done by dissolving 0.43 grams of NA powder into 15 ml of distilled water for one petri dish, then the solution is heated until boiling while stirring, then sterilized.

The antibacterial activity testing was carried out by calculating the inhibition zone of each formula. A sterile ose needle was dipped into the *Staphylococcus aureus* bacterial suspension and rubbed evenly over the NA media. Sterile disc paper was soaked into each sample preparation for 15 minutes, then the disc paper was placed on the NA media containing *Staphylococcus aureus* bacteria, then incubated at 37°C for 24 hours. Sample observation was carried out by calculating the diameter of the inhibition power in each sample which was marked by the presence of a clear area if no bacteria were growing. Testing was carried out on negative control preparations (-), positive control (+) using 70% alcohol, F0, F1, F2, and F3 with 3 replications (Agustin et al., 2023).

The Data Analysis

The data obtained from this study were tested using the One-Way ANOVA statistical test and each test was replicated with 3 samples to obtain the standard deviation.

Result and Discussion

The results of the determination test of aloe vera and lime plants obtained from the Herbarium Laboratory of the Bandung Institute of Technology showed that the aloe vera plant comes from the *Asphodelaceae* family with the species *Aloe vera* L. While the lime plant comes from the *Rutaceae* family with the species *Citrus aurantiifolia*. This plant determination aims to determine the genealogy and species of a plant.

This hand sanitizer spray preparation containing aloe vera and lime concentrate is used as an active antibacterial ingredient. Additional ingredients used to make this hand sanitizer spray preparation include carbopol as a thickener, propylene glycol as a humectant, methyl paraben and propyl paraben as a combination of preparation preservatives to maintain product quality during storage, triethanolamine is used as a pH stabilizer, oleum citri as a flavoring, and aquadest as a preparation solvent. All additional ingredients used use safe concentrations and meet cosmetic safety standard requirements so that the risk of

irritation or allergies can be avoided (Rollando et al., 2023).



Figure 1. Base (1), F1 (2), F2 (3), F4 (4)

The results of formula optimization evaluation in Table 1. for the spray hand sanitizer preparation in organoleptic testing were carried out by testing the color, odor, aroma, and texture to ensure consumer acceptance. The results of organoleptic testing show a comparison of the base and formulas F1, F2, and F3 based on the visual appearance shown in Figure 1. The spray hand sanitizer preparation has a cloudy orange color, especially in the formula containing aloe vera and lime, while the base preparation has a transparent appearance. The aroma of the spray hand sanitizer preparation has a refreshing aroma due to the content of natural lime and the addition of oleum citri. The texture of the hand sanitizer is not sticky and easy to apply and feels moist due to the content of propylene glycol. The results of the organoleptic test showed that there were no significant physical changes in each formula during 28 days of storage.

The homogeneity testing conducted on hand sanitizer spray preparations for formulas F1, F2, and F3 has a mixture of preparations that can be dispersed well, although the preparation looks cloudy. The higher the concentration of lime concentrate, the cloudier the preparation looks because the lime concentrate contains small grains of lime, while the base or F0 has good homogeneity.

In pH testing, the F0 or base formulation gave a pH value ranging from 5.64 ± 0.005 to 5.79 ± 0.011 . Formula F1 had a fluctuating pH but remained stable in the range of 4.56 ± 0.02 – 4.77 ± 0.01 . Formula F2 had a pH range of 4.27 ± 0.01 – 4.35 ± 0.01 . Formula F3 showed a low pH value in the range of 3.75 ± 0.02 – 3.86 ± 0.03 . The pH testing was carried out for 28 days during the shelf life and 3 replications. In formulas F1 and F2, the active ingredients did not cause major changes in the pH value compared to F0, which was still within the ideal pH range for topical preparations of 4–7, in accordance with the natural pH of human skin. This pH range helps prevent irritation and ensures the stability of the ingredients [Ayun et al., 2020]. Meanwhile, F3 shows a pH value below 4, which is assumed to cause irritation to sensitive skin. In the F3 formula, the highest

concentration of lime is 30%, making the pH value tend to be acidic. The statistical test results show that there is no significant difference in the pH value of each formula against the observation time interval (28 days) ($p>0.05$), and there is a significant difference in the pH value in each formula ($p<0.05$).

The viscosity test is carried out to determine the consistency of the spray hand sanitizer preparation, ensure the product can be applied easily and evenly on the skin, and maintain the stability of the preparation. A good viscosity range for this preparation is 800-3000 cPs (Indalifiany et al, 2021). In the viscosity test conducted on each formula, a stable and low viscosity value was obtained, namely in the range of 1.06-1.09 cPs during a 28-day shelf life in formula F0. From the three formulas F1, F2, and F3, the highest viscosity value was in formula F1, which indicated that the use of carbopol and a higher concentration of aloe vera than the other formulas had an effect on increasing viscosity. The viscosity value obtained in F1 was in the range of 1.21-1.29 cPs during a 28-day shelf life. Meanwhile, F2 and F3 have moderate viscosity indicating a balance between the thickener and other ingredients, which is in the range of 1.14-1.21 cPs for F2, and 1.09-1.10 cPs for F3 during a 28-day shelf life. The results of statistical tests show that the formula significantly affects viscosity ($p<0.05$), while there is no effect of storage time on changes in viscosity in each formula ($p>0.05$).

The dry time test on hand sanitizer spray preparations is carried out to determine how long the product dries so that it can increase user comfort. The ideal dry time range is 15-60 seconds. Drying time that is too fast (<15 seconds) is not effective enough to kill microorganisms, while drying time that is too long (>60 seconds) can interfere with user activities and reduce user interest in using it (Asngad et al., 2018). In the test, formula F1 had the longest drying time, which was 46 ± 1 seconds, because it contained the most aloe vera, thus slowing down evaporation and drying. Meanwhile, F3 showed the fastest drying time, which was 37 ± 1.7 seconds when compared to F1 and F2. Formula F2 showed a drying time of 41 ± 1 seconds and F0 at 35 ± 1 seconds. Overall, each formula was still within the ideal drying time range. In statistical testing, each formula affected the length of drying time ($p<0.05$).

The antibacterial activity testing was carried out to evaluate the ability of the hand sanitizer spray formula to inhibit the growth of *Staphylococcus aureus* bacteria which often cause skin infections (Hita et al, 2023). Testing was carried out on each formula F0 as a negative control, F1, F2, F3, and positive control using 70% alcohol.

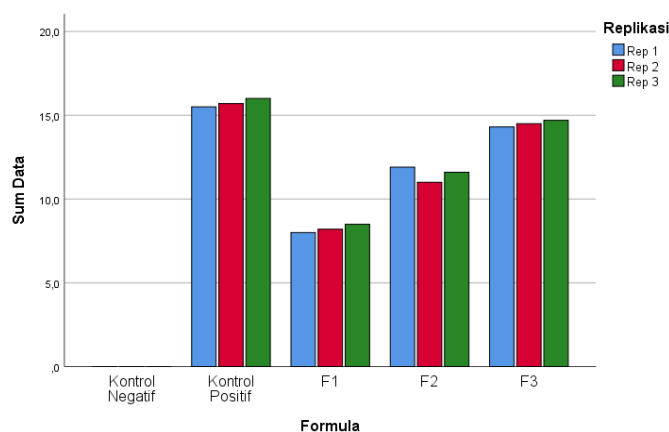


Figure 2. The Chart of inhibition zone results for hand sanitizer spray preparations.

The test results showed that the negative control had no inhibition against *Staphylococcus aureus* bacteria, while the positive control showed an average inhibition zone of 15.7 ± 0.25 mm. Formula F1 showed moderate antibacterial effectiveness with an average inhibition zone of 8.2 ± 0.25 mm, while F2 and F3 showed strong antibacterial effectiveness with an average inhibition zone of 11.5 ± 0.45 mm (F2) and 14.5 ± 0.2 mm (F3) (showed in **Figure 2** an **Figure 3**).

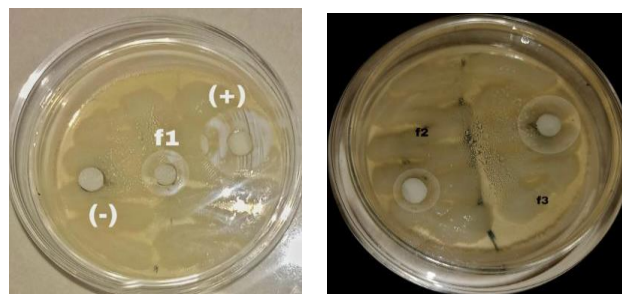


Figure 3. The Inhibition zone results of each formula

The formula F3 showed almost the same antibacterial effect as the positive control. This shows that the higher the concentration of lime concentrate, the greater the antibacterial activity, because it contains citric acid and high flavonoid compounds. The active component of aloe vera that has antibacterial activity is aloe-emodin compound which is an anthraquinone compound that is effective in gram-positive and negative bacteria. The mechanism of action as an antibacterial is by inhibiting bacterial growth by disrupting the process of protein synthesis and DNA replication and the destruction of bacterial cell walls that cause bacterial death (Rahardjo, 2017). While lime concentrate has a high citric acid component which can also disrupt protein synthesis and can stimulate antibody production and phagocyte activity (Sari et al, 2022).

Based on statistical testing, it shows that there is a significant difference in the inhibition zone of each formula ($p < 0.05$). So, it can be assumed that the difference in the composition of active ingredients makes a significant difference in antibacterial activity.

Conclusion

The conclusion of the results of this study is that of the three formulas, formula F2 containing 25% aloe vera and 25% lime concentrate showed the most optimal formula during 28 days of storage, was physically stable, safe for the skin, and effective as an antibacterial in the strong category.

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