

Mint leaf extract (*Mentha x piperita* L.) and Bay Leaves (*Syzygium polyanthum* (Wight) Walp.) As Additional Ingredients for Making Hand Sanitizer

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Abstract

Spray Hand Sanitizer In this study is a product used as an alternative hand cleansing with the addition of mint leaf extract and bay leaves that contain secondary metabolic compounds and can form an antibacterial inhibitory zone. This study aims to determine the effectiveness of the addition of mint leaf extract (*Mentha x piperita* L.) and bay leaves (*Syzygium polyanthum* (Wight.) Walp.) As a spray hand sanitizer formulation. The method used is quantitative with a complete random design research design (RAL) consisting of 4 treatments, namely: P0 (30% Bay leaf extract: Control), P1 (3% Mint Leaf Extract + 30% Salam Leaf Extract), P2 (5 % Mint leaf extract + 30% bay leaf extract) and P3 (7% mint leaf extract and 30% bay leaf extract). Data analysis techniques in the form of ANOVA tests and further DMRT tests. The results showed that the addition of mint leaf extract and bay leaves gave effectiveness to the hand sanitizer formulation with the ability to form an inhibitory zone for the staphylococcus aureus bacteria. The largest inhibitory zone is formed by the P3 formulation with an average diameter of 15.78 mm category of strong inhibition. The spray hand sanitizer formulation that is preferred by panelists is the P3 formulation (7% of mint leaf extract + 30% bay leaf extract). The average value of the P3 formulation in the color parameter of 3.57, the aroma parameter of 3.63, the parameter of the use of 3.4 and the absorption rate of 3.57 with a fairly like category.

Keywords: Bay leaves; hand sanitizer; mint leaves.

INTRODUCTION

Health problems are one of the conversations that never end to be questioned, one of the factors that causes health problems in the body quickly is a dirty hand (Holifah et al., 2020). Therefore, one of the most important limbs to be kept clean is hands, because it is vulnerable to being used as a place for nesting bacteria and viruses. Bacteria are typical and unicellular prokaryotic cells. Bacteria are microorganisms that cannot be seen in plain view by humans (Adinda Fitri Salsabila et al., 2022). Types of bacteria that are often found in the hands are *Staphylococcus aureus* bacteria. *Staphylococcus aureus* is a bacterium that is easily found everywhere and is pathogenic for humans. *Staphylococcus aureus* bacteria are round-shaped gram-positive bacteria with a diameter of 0.7-1.2 μm , arranged in irregular groups such as grapes, anaerobic facultative, not forming spores and immovables (Yanto et al., 2021).

Efforts can be made to maintain a very simple hand health, namely by washing hands. Hand washing is a method used to eliminate bacteria or germs that are on the surface of the hand through the process of rubbing both palms simultaneously using soap or cleaning

substances that are appropriate and then rinsed using running water (Sultan & Zikri, 2021). The habit of washing hands using soap is an action that is still difficult to apply to the community, especially if you are traveling or in a journey and clean water is not available, then the dirty hand will be used to eat and touch other limbs (Huliatunisa et al., 2020).

Hand Sanitizer or hand cleansing spray is a hand-cleaning innovation that can be used without running water. Hand Sanitizer is a health product that can directly kill germs without rinsing with water (less et al., 2020). Hand Sanitizer began to bloom on the market because of its use which tends to be practical and its packaging is easy to carry everywhere (Nakoe et al., 2020). The selection of materials in making hand sanitizers is very important to note, so that the hand sanitizer products used are skin-friendly products and do not irritate the skin. The addition of natural ingredients is an alternative that can be applied to overcome these problems (Aprilia & Yanti, 2019). Examples of types of plants that have various properties besides being used as food sources are mint plants and greeting plants.

Mint plants (*Mentha x piperita* L.) and Salam (*Syzygium polyanthum* (Wight) Walp.) can be processed as a natural ingredient to make hand sanitizers by utilizing the leaves. Mint plants are plants that are included in the Lamiaceae family with active ingredients and distinctive aroma, in addition to being antibacterial and antifungal mint leaves can also be used to increase skin moisture, treat acne, remove dead skin cells, smooth the skin and be able to control excess oil in Skin (Hasibuan Maimunah Aisah, 2022). Mint leaves are widely used in the pharmaceutical industry, cigarettes, and food, among others, for the manufacture of toothpaste, wind oil, balm, confectionery and others. Salam plants are one type of woody plant that is often found in everyday life. This plant can grow wild in the forests and mountains, or commonly planted in the case and the environment around the house. Salam plants also have a diuretic, analgesic and antibacterial effect. The benefits of bay leaves are produced by the content of chemical compounds they have (Fitri et al., 2020).

Both of these plants can be processed into hand sanitizer preparations because they contain antibacterial compounds that can kill germs. Antibacterial compounds found in mint leaves and bay leaves are flavonoids, saponins, tannins and alkaloids (Taba et al., 2019). This research is expected to provide information about the use of mint leaves and bay leaves that contain antibacterial compounds both for students and the community. So, this study aims to make natural spray hand sanitizer preparations using the additives of mint leaf extract (*Mentha x piperita* L.) and bay leaves (*Syzygium polyanthum* (Wight) Walp.) Which contain antibacterial compounds as a solution that can be used to clean the hands practical after doing various activities.

MATERIALS AND METHODS

Materials

This research material was conducted at the UIN Raden Intan Lampung Biology Education Laboratory and Lampung State Polytechnic Agricultural Product Technology Laboratory in February to May 2024. Equipment used in this study was analytic scales, spatel, stirring rods, dropper pipette, erlenmeyer, shaker, blender, vaporizer, rotary evaporator, glass object, watch glass, measuring cup, pH meter, sieve, flannel, spray hand sanitizer bottle. The ingredients used in this study are mint leaves, bay leaves, glycerin, methylparaben, propylparaben and ethanol 70%.

Methods

The required sample is mint leaves and bay leaves. Mint leaves are obtained from Jl. Abdul Kadir, Rajabasa District, Bandar Lampung City. Salam leaves are obtained from Jl. Sukardi Hamdani, Labuhan Ratu District, Bandar Lampung City. In this study, the type of

research used is quantitative research with the experimental method. Research using a Complete Randomized Design (RAL). Provision of concentration treatment from mint leaf extract and bay leaves consists of 4 treatments and 3 repetitions. From this treatment, 12 experiments will be obtained with the following concentration:

- P0 = 0% mint leaf extract and 30% bay leaf extract
- P1 = 3% mint leaf extract and 30% bay leaf extract
- P2 = 5% mint leaf extract and 30% bay leaf extract
- P3 = 7% mint leaf extract and 30% bay leaf extract

Mint leaves are washed thoroughly with running water, and then separated with the leaf stalks. After that it is weighed and dry the mint leaves by aerating \pm 1 week. After drying, the mint leaves are blended and sifted until it becomes a powder preparation. Furthermore, the making of the extract is done by the maceration method. The extraction process is carried out by simplicia mint leaves inserted into a container and then soaked with 96% Ethanol solvent for 1x24 hours. Furthermore, the extract solution is inserted on the shaker and then covered with the lid of the container for 3x24 hours, every day stirred \pm 5 minutes then filtered using a flannel. Furthermore, it is concentrated using a rotary evaporator until 100 ml of thick extract is obtained.

Before entering the process of making hand sanitizer, some of each mint leaf extract and bay leaves are taken first for the process of phytochemical screening and testing antibacterial inhibition. The phytochemical screening test was carried out to find out what secondary metabolites compounds are contained in the plant leaf extract (Sulistyarini et al., 2019). The antibacterial activity test was carried out to determine the potential for inhibition of antibacterial mint leaf extract and bay leaf extract which was added as a preparation in the spray hand sanitizer formulation (Karmilah et al., 2023). This antibacterial activity test was carried out on *Staphylococcus aureus* bacteria through the well method. The steps in testing antibacterial effectiveness are, sterilization of tools, manufacturing nutrient broth (NB) and nutrients agar (Na), bacterial inoculation, making bacterial suspension, manufacturing test media, observation and measurement. Observation and measurement were carried out after the incubation process for 24 hours (Ishimora et al., 2023). Observation and measurement of the diameter of the inhibitory zone (clear zone) is carried out using the calipers. Given positive control treatment using clindamycin and negative control using aquadest.

After all ingredients are weighed and recorded the weight, mint leaf extract and bay leaves are put into a test tube according to the concentration in each treatment, then dissolved with 70% ethanol until dissolved (L1) (Zuhri & Dona, 2021). Propyl paraben as much as 0.04 g and methyl paraben as much as 0.18 g are inserted into a test tube and dissolved with 70% ethanol until dissolved (L2). Glycerin as much as 15 ml is put into a test tube

(L3). Furthermore, L1, L2 and L3 are put into a spray hand sanitizer bottle and added with 70% ethanol as much as 100 ml, stirred until evenly distributed and evaluated the preparation.

Table 1. Hand Sanitizer Formulation.

Component	Uses	Formulation			
		P0	P1	P2	P3
Mint leaf ekstrak	Active substance	0 ml	0,3 ml	0,5 ml	0,7 ml
Bay leaf ekstrak	Active substance	3 ml	3 ml	3 ml	3 ml
Metyl paraben	Preservatives	0,18 g	0,18 g	0,18 g	0,18 g
Propil paraben	Preservatives	0,04 g	0,04 g	0,04 g	0,04 g
Glycerin	Humectant	15 ml	15 ml	15 ml	15 ml
Ethanol 70%	Solvent	100 ml	100 ml	100 ml	100 ml

RESULTS AND DISCUSSION

Results

The results of phytochemical screening test

The results of phytochemical screening testing can be seen in Table 2.

Table 2. The results of phytochemical screening test.

No.	Materials	Compound	Results	Detected
1.	Mint leaf ekstrak	Flavonoid	+	Yellow
		Tanin	+	Dark green
		Saponin	+	Stable foam
		Alkaloid	+	White precipitate
2.	Bay leaf ekstrak	Flavonoid	+	Orange
		Tanin	+	Dark blue black
		Saponin	+	Stable foam
		Alkaloid	+	White precipitate

The results of the antibacterial activity test

The results for testing antibacterial activity can be seen in Table 3.

Table 3. The results of the antibacterial activity test.

Deuteronomy	Test of the Treatment					
	K+	K-	P0	P1	P2	P3
1	32,27	0,00	14,93	15,07	15,47	15,83
2	32,27	0,00	14,73	15,20	15,53	15,73
3	32,37	0,00	14,63	15,23	15,37	15,78
Average (mm)	32,27	0,00	14,76	15,17	15,45	15,78

One way anova test results

One way anova test results can be seen in Table 4.

Table 4. One-way anova test results.

ANOVA					
DH					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1566.614	5	313.323	39165.344	.000
Within Groups	.096	12	.008		
Total	1566.710	17			

Duncan Further Test Results

Duncan's advanced test results can be seen in Table 5.

Table 5. Duncan Further Test Results.

Duncan ^a		Subset for alpha = 0.05					
sample	N	1	2	3	4	5	6
KN	3	.00					
P0	3		14.76				
P1	3			15.14			
P2	3				15.46		
P3	3					15.78	
KP	3						32.27
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Homogeneity Test Results

Homogeneity testing gets the results that hand sanitizer preparations have homogeneous variations.

pH Test Results

pH test results can be seen in Table 6.

Table 6. pH Test Results.

Testing	Observation Results			
	P0	P1	P2	P3
pH	6,0	6,1	6,2	6,3

Organoleptic Test Results

Organoleptic test results can be seen in Table 7.

Table 7. Organoleptic Test Results Average.

Parameter	Treatment			
	P0	P1	P2	P3
Color	3,4	3,4	3,37	3,57
Aroma	3,6	3,4	3,37	3,63
Properties of Use	3,3	3,4	3,4	3,47
Absorption rates	3,27	3,2	3,13	3,57

Discussion

This research discussion was conducted from February to May 2024 by utilizing the UIN Raden Intan Lampung Biology Education Laboratory and Lampung State Polytechnic Agricultural Product Technology Laboratory. The research design used is experimental using a complete random design (RAL). Provision of concentration treatment from mint leaf extract and bay leaves consists of 4 treatments and 3 repetitions. The resulting spray hand sanitizer product has a brownish color characteristic, has an aroma of mint leaves and bay leaves, gives a cold sensation to the skin after being sprayed and absorbs on the skin. The results of spray hand sanitizer products can be seen in Figure 1.



Figure 1. Spray Hand Sanitizer Product.

The results of phytochemical screening tests show that in mint leaf extract contained secondary metabolite compounds in the form of flavonoids characterized by the detection of the yellow color in the extract after mixed with the reagent solution. Tannins marked by the detection of dark green color on the extract after mixed with reagent solution. Saponins were marked by the detection of stable foam after the extract was mixed with a reagent solution, as well as an alkaloid compound characterized by the detection of white deposits in the extract after mixed with the reagent solution. Whereas in bay leaf extract it appears that the metabolite compound contained in the form of flavonoids is characterized by the detection of orange colors in the extract after mixed with reagent solution. Tannin is characterized by the detection of dark blue in the extract after being mixed with a reagent solution. Saponins are marked by the

detection of stable foam after the extract is mixed with reagent solution, as well as alkaloid compounds are characterized by the detection of white deposits in the extract after mixed with reagent solution (Darma & Marpaung, 2020). Phytochemical screening testing is carried out to detect secondary metabolic compounds contained in a plant extract.

The observations of the antibacterial activity test show the diameter of the inhibitory zone which means that the compounds contained in mint leaf extract and bay leaves can inhibitory power to the staphylococcus aureus bacteria. The biggest inhibition is made by extracts with P3 formulations, namely the concentration of bay leaves by 30% and mint leaf concentrations of 7% with an average inhibitory power of 15.78 mm strong category. While the smallest inhibition is made by extracts with P0 formulation, namely the concentration of bay leaves by 30% without the addition of the concentration of mint leaf extract with an average inhibitory power of 14.76 mm strong category. In the P1 formulation the average inhibition produced is 15.17 mm of the strong category, and in the P2 formulation the average inhibitory power produced is 15.45 mm of the strong category. The inhibition produced by the clindamycin as a positive control of 32.27 mm is very strong category and aquadest as a negative control does not indicate the presence of antibacterial activity with the formation of the inhibition zone. clindamycin as a positive control produces a diameter of the inhibitory zone which is classified as very strong because of clindamycin is a type of antibiotic that has antibacterial activity by inhibiting protein synthesis in bacteria (Ardhany et al., 2023). While Aquadest as a negative control does not produce an inhibitory zone because the compounds of aquadest are neutral which will not have an effect on bacterial growth or do not have antibacterial activity (Pangestu & Kusuma, 2023).

The Anova test results show a significant value of 0.000 which means that there is an effectiveness of the addition of mint leaf extract and bay leaves as a hand sanitizer formulation. Then in the Duncan follow-up test the results were obtained that all formulations experienced a significant difference, but the concentration of the extract formulation whose most effective inhibition was found in the P3 formulation with a mint leaf concentration of 7% and the concentration of bay leaves by 30%, this was due to the test Duncan P3 formulation is right after the KP column (positive control).

Homogeneity testing aims to find out and observe the mixing of substance components in the hand sanitizer preparations that are made so that they don't seem to have rough granules. Homogeneity testing of hand sanitizer solutions is done by means of a hand sanitizer solution, dropped on 1 ml of object glass using a measuring pipette to form a flat surface. The results show the absence of rough grains visible. These results indicate that the substances contained in the hand sanitizer

formulation are evenly dissolved so that there are no coarse grains in the observation process. These results indicate that the formulation of each treatment has a homogeneous variation. The pH test was conducted to determine the sensitivity of the hand sanitizer preparation for the skin. The normal pH range of preparations that can be applied to the skin is 4.5-6.5 (Arina et al., 2023). PH testing is measured by a pH meter to determine the degree of acidity or taste of the preparation and to ensure that spray hand sanitizer preparations do not cause irritation or damage to the surface of the skin. PH preparations are too acidic (low pH) can irritate the skin while the pH is too alkaline (high pH) and can make the skin dry (Aprilianti et al., 2020).

The hand sanitizer preparation shows that the higher the addition of the concentration of mint leaf extracts gives the influence the level of the Hand Sanitizer produced, namely the pH produced is increasing. The four hand sanitizer preparations formulations that are made meet the size of the skin pH ranging from 4,56.5. The P0 formulation has the lowest pH of 6.0, the P1 formulation has a pH of 6.1, the P2 formulation has a pH of 6.2 and the P3 formulation has the highest pH of 6.3. The degree of acidity (pH) is one of the important factors in bacterial growth, this affects the high and low density of the bacteria produced. The minimum and maximum pH value for bacterial growth in general is 4-9, but the most optimal pH ranges from 6.57.5. PH is very influential on the growth of bacteria because it is related to the activity of the enzymes needed by bacteria to catalyze reactions associated with bacterial growth. If the pH in a medium or the environment is not optimal, it will interfere with the work of these enzymes and eventually disrupt the growth of the bacteria itself (Razmi et al., 2023). The PH of Spray Hand Sanitizer products in this study is classified as acidic and not in the optimal range of bacterial growth pH.

The study was followed by organoleptic testing using a questionnaire sheet involving 30 panelists in the panelist category not trained, to find out the response of community acceptance of the Hand Sanitizer products produced. This test includes 4 parameters, namely color, aroma, nature of use and absorption rate. The ability of the sensory device to form a response which will later become an assessment of the products tested in accordance with the stimuli received by the senses. Based on the organoleptic test that has been carried out shows that in the color parameter the average organoleptic test results of the P0 formulation are 3.4. The P1 formulation is 3.4, the P2 formulation is 3.37 and the P3 formulation is 3.47. In the aroma parameter of the P0 formulation, the lowest was 3.6, the P1 formulation was a score of 3.4, the P2 formulation received a score of 3.37, and the P3 formulation received a score of 3.63. Then in the parameter the nature of the use of the P0 formulation received a score of 3.3, the P1 formulation was a score of 3.4, the P2 formulation was a score of 3.4 and the P3 formulation was a score of 3.47. Based on the

highest score absorption level parameter obtained by the P3 formulation with an average value of 3.57 while the lowest score is in the P2 formulation with an average value of 3.13, the P0 formulation is a score of 3.27 and the P1 formulation is a score of 3, 1. Overall based on the results of the 4 parameters, each hand sanitizer formulation in this study can be accepted by panelists and is in the category quite liked by panelists.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that there is the effectiveness of the addition of mint leaf extract (*Mentha x piperita* L.) and bay leaf extract (*Syzygium polyanthum* (Wight) Walp.) As a hand sanitizer formulation. Optimal concentration on hand sanitizer products with the addition of mint leaf extract and bay leaves based on antibacterial activity tests and organoleptic tests, namely in the P3 formulation with a concentration of adding mint leaves as much as 7% and the addition of bay leaves as much as 30%. The inhibition zone formed by this concentration has an average of 15.78 mm and the average results of organoleptic testing results of a color parameter of 3.57, aroma parameter of 3.63, parameter of the use of 3.47 and absorption level parameter of 3.57 with whole parameter in the category quite like.

Competing Interests: The authors declare that there are no competing interests.

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