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DEVELOPMENT AND CHARACTERIZATION OF HAND SANITIZER CONTAINING LEMPUYANG EMPRIT *(Zingiber amaricans* BL.*)* ESSENTIAL OIL WITH ZERUMBONE AS THE MAIN CHEMICAL COMPONENT

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ABSTRACT

Recently, research on the chemistry of natural products as medicinal has been increasingly developed; this is because natural products have many chemical components, have the potential biological activities without side effects, and are easy to obtain. One of these natural products is Lempuyang Emprit *(Zingiber amaricans* BL.) essential oil. The researchers guided UKM Suti Sehati to develop a new product: a hand sanitizer containing Lempuyang Emprit essential oil (LEEO). This study aims to make ten hand sanitizer formulas by varying LEEO concentrations and determine the best formula based on the physical properties and antibacterial activities. The best formulation obtained was then optimized with variations in fragrance concentration. The best product was selected by a hedonic test using 30 untrained panelists based on the parameters of color, scent, texture, pH, homogeneity, speed of drying, causing no red skin, causing no pain, and causing no dry skin. The antibacterial activity tests were performed against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 using the disk method. The LEEO isolation was carried out using steam and water distillation methods, and the chemical components of the best product was identified using the GC-MS method. Based on the hedonic test, the best hand sanitizer product contains 1.5% LEEO and 3% fragrance. This product has a strong antibacterial activity value against *S. aureus* ATCC 25923, with an inhibition zone of 15.95 mm, and a moderate value against *E. coli* ATCC 25922, with an inhibition zone of 9.42 mm. The hand sanitizer product contains 21.35% of Zerumbone and exhibits antibacterial and antioxidant capabilities.

Keywords: Lempuyang Emprit essential oil, hand sanitizer, zerumbone

INTRODUCTION

Indonesia has mega-biodiversity and is the second-largest biodiversity in the world. Therefore, it has excellent potential to develop in the herbal medicine industry due to the availability of diverse and abundant raw materials. Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and can cause severe respiratory infections in humans [1]. This virus can spread through contact with

exposed people, droplets, air, and surface contamination exposed to droplets of people exposed to COVID-19 [2]. Hand sanitizer is the best choice to prevent transmission of COVID-19 after washing hands with soap. In addition, using hand sanitizers that contain alcohol can kill viruses that stick to your hands [3]. When the Covid-19 pandemic comes, the demand for hand sanitizers increases rapidly, including that for hand sanitizers containing natural products, since people believe that hand sanitizers with natural products have additional benefits. Natural ingredients have many chemical components with various benefits, have the potential to reduce side effects, and are easy to obtain [4].

According to the *World Health Organization* (WHO), hand sanitizer is an alcohol-based hand sanitizer preparation to inactivate microorganisms or temporarily suppress their growth [5]. It is effective to use by those who find it difficult to get water to wash their hands with soap and those who are travelling [6]. It is an antiseptic substance containing 60-95% alcohol and has good bacterial activity against gram-positive and gram-negative bacteria. According to the *Food and Drug Administration* (FDA), hand sanitizer can kill germs in less than 30 seconds [7]. The compounds contained in hand sanitizers kill germs by denaturing and coagulating their cell proteins [8].

One of the plants in Indonesia that is commonly used as traditional medicine is lempuyang. Indonesian people have identified three types of lempuyang: Lempuyang Emprit *(Zingiber amaricans* BL.*)*, Lempuyang Gajah *(Zingiber zerumbet* (L.) Smith*)*, and Lempuyang wangi (*Zingiber aromaticum* Val.*)*. Lempuyang Emprit has small rhizomes, Lempuyang gajah has the largest rhizome size, while Lempuyang wangi has small rhizomes and a fragrant aroma [9].

The part of the Lempuyang plant used as a herb is the rhizome containing essential oils, saponins, flavonoids, and tannins [9]. Essential oils are liquid and volatile compounds from plant parts, such as leaves, stems, rhizomes, roots, flowers, and seeds, through distillation [10]. Essential oils are also known as etheric or volatile oils because they are volatile at room temperature and have an aroma according to their plant origin [11]. Essential oils can be obtained through distillation, making them free from organic solvents, thus requiring no separation process before use [12]. Biologically, essential oils are used by plants for self-defence from predators and pests because they produce a scent that insects and pests do not like [13].

The primary content of Lempuyang essential oil is the sesquiterpenoid zerumbone, an oxygenated sesquiterpene compound with antioxidant [14] and anti-inflammatory [15] activities. In addition, it can potentially be developed as an anticancer agent to treat cervical and ovarian cancers because it can inhibit the growth of cancer cells [16].

Lempuyang Emprit is an annual shrub with pseudo-stems as the extension of the leaf midribs. Its leaves are green, in single alternate arrangements, shaped long ovoid with flat edges and pointed ends [9]. The leaves are alternately arranged along the pseudo-stem and can reach 1-2 m in length

[17]. The flowers are pinecone-shaped, green when young and red when old in color, and emerge from the rhizome. Essential oils from Lempuyang Emprit *(Zingiber amaricans* BL.*)*, Lempuyanggajah *(Zingiber zerumbet* (L.) Smith*)*, and Lempuyang wangi (*Zingiber aromaticum* Val.*)* can inhibit three gram-negative bacteria, namely *E. coli, Salmonella thypii*, and *Pseudomonas aeruginosa* [18].

The main chemical component in Lempuyang Emprit essential oil is zerumbone, reaching 65.06% [19], with a retention time of 21.465, a relative mass of 218, and the structural formula $C_{15}H_{22}O$. Zerumbone is efficacious as an antioxidant, anti-cancer, anti-skin cancer [20], antibacterial, and a drug to treat tinea versicolor and ringworm [21]. Emprit Lempuyangshow that the major component of Lempuyang Emprit from Yogyakarta is zerumbone, and the minor component is a mixture of phytosterols [22].

This study aimed to optimize the best formulation, determine the antibacterial activities (*S. aureus* ATCC 25923 and *E. coli* ATCC 25922), the physical properties, product quality, and stability; and determine the chemical components of hand sanitizer containing Lempuyang Emprit essential oil. The novelty of this research was the addition of essential oils to hand sanitizers to increase their antibacterial activity. However, Lempuyang Emprit is known for its bitter taste, and its use is still limited to traditional medicine, so this research was expected to provide knowledge about the chemical components of hand sanitizer containing Lempuyang Emprit essential oil and its benefits.

METHOD

Sample Determination

Determination of Lempuyang Emprit (*Zingiber amaricans* BL.) has been carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. The sample in the form of Lempuyang Emprit rhizomes was obtained from the Nguter area, Sukoharjo, Jawa Tengah, Indonesia (7º42'44.39" S,110º51'2.62" E with an altitude of 95 masl).

Isolation of Lempuyang Emprit Essential Oil

The essential oil isolation was carried out using steam and water distillation methods refer to Giofana and Putra [23]. The distillation process was carried out until the resulting liquid no longer dripped in the distillate flask [24]. Finally, the essential oil content was measured using the following calculation formula:

$$\text{Content} = \frac{\textit{Volume of Essential oil (mL)}}{\textit{Mass of Sample (kg)}} \times 100\%$$

Optimization of the Hand Sanitizer product

There are two optimization process to get the best product of the hand sanitizer. he first optimization is to find the best formula from ten hand sanitizer formulas based on physical properties observation and antibacterial tests. The second optimization is carried out to get the best hand sanitizer product containing Lempuyang Emprit essential oil (LEEO) and fragrance. The best formula from the first optimization was then optimized with variations in fragrance concentration. Then, the best hand sanitizer product was selected by a hedonic test using

30 untrained panelists based on the parameters of homogeneity, color, texture, scent, speed of drying, causing no red skin, causing no pain, and causing no dry skin. Furthermore, the optimizations were carried out with the following steps:

Making Hand Sanitizer Formulas in various concentrations of Lempuyang Emprit Essential Oil

The hand sanitizers were made following the WHO's prescription [25]. The 100 mL hand sanitizer preparation was made according to the following formulation in Table 1.

Table 1. Formula of Hand Sanitizer Containing Lempuyang Emprit Essential Oil (LEEO)

Composition	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
96% Ethanol (mL)	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33
3% Hydrogen Peroxide (mL)	4.17	4.17	4.17	4.17	4.17	4.17	4.17	4.17	4.17	4.17	4.17
98% Glycerin (mL)	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Distilled Water (mL)	Ad 100.00	Ad 100.00	Ad 100.00	Ad 100.00	Ad 100.00	Ad 100.00	Ad 100.00	Ad 100.00	Ad 100.00	Ad 100.00	Ad 100.00
LEEO (%)	0.00	0.50	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00

Note:

F0	:	Hand Sanitizer (HS)	F4	:	HS + 2.0% LEEO	F8	:	HS + 4.0% LEEO
F1	:	HS + 0.5% LEEO	F5	:	HS + 2.5% LEEO	F9	:	HS + 4.5% LEEO
F2	:	HS + 1.0% LEEO	F6	:	HS + 3.0% LEEO	F10	:	HS + 5.0% LEEO
F3	:	HS + 1.5% LEEO	F7	:	HS + 3.5% LEEO			

Physical Properties assessment of Various Hand Sanitizer Formulas

The physical properties of hand sanitizer formulas containing LEEO and without LEEO (F0) were observed to determine which hand sanitizer formula has the best physical properties. The assessment of physical properties was performed to color, scent, and form.

Antibacterial Activity Test of Various Hand Sanitizer Formulas

The antibacterial activity of various hand sanitizer formulations that have been made were test against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 using the Disc Diffusion Method. Preparation of MHA media and bacterial suspensions refers to Nurhayati, Yahdiyani, and Hidayatullah [26]. Vancomycin and Chloramphenicol were used as positive controls for *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, respectively, while distilled water was used as a negative control.

Optimation of the Hand Sanitizer Formulas Containing Lempuyang Emprit Essential Oil and Fragrance

This optimation aims to get the best product from the best hand sanitizer formula containing LEEO with additional variations of fragrance concentration. The best hand sanitizer formula is added fragrance with

concentration variations of 0 %, 3 % (FA), 6 % (FB) and 9% (FC). The selection of products with the best LEEO and fragrance content was conducted by a hedonic test using 30 untrained panelists. Assessment with this hedonic test referring to the SNI 01-2346-2006 procedures includes eight categories: homogeneity, texture, scent, color, speed of drying, causing no red skin, causing no pain, and causing no dry skin. Characterization of the best hand sanitizer formula with the best fragrance concentration was then determined based on the physical properties, pH, dispersibility, homogeneity, and antibacterial activity.

Characterization of The Best Hand Sanitizer Product

The physical properties and anti-bacterial activity of the best hand sanitizer formula with the best fragrance concentration produced were determined by procedure described above (4b and 4c). Its characterization of pH, dispersibility, homogeneity, and stability were conducted based on SNI No. 06-2588 procedures.

Identification of Chemical Components of the Best Hand Sanitizer Formula containing LEEO and fragrance

Chemical components of the best hand sanitizer formula containing LEEO and fragrance was analyzed using GC-MS Shimadzu QP 2010. Therefore, GC-MS chromatogram and mass spectra were analyzed and compared with the data in the *Wiley7 Library* to determine the chemical components contained in the analyzed sample.

RESULTS AND DISCUSSION

Lempuyang Emprit Supply and Preparation

The sample used in this study was the rhizomes of Lempuyang Emprit *(Zingiber amaricans* BL*.)*. The results of sample preparation can be seen in Figure 1.

(A) (B) (C)

Figure 1. Lempuyang Emprit (A), Lempuyang Emprit Slices (B), and Lempuyang Emprit Essential Oil (C)

Lempuyang Emprit Essential Oil Isolation

The essential oil isolation from 4.9 kg sample was carried out using the steam and water distillation method. The essential oil produced from this isolation (Table 2) is a clear pale yellow, Lempuyang Emprit essential oil is pale yellow [19].

Table 2. Results of the Lempuyang Emprit Essential Oil Characteristics Test

Results	Essential Oil
Initial mass (kg)	4.9
Volume (mL)	14
Color	Clear pale yellow
Form	Liquid
Aroma	Typical of Lempuyang
Content (% v/w)	0.286%

Optimation of the Formula of Hand Sanitizer containing LEEO (F1 – F10)

The color of hand sanitizer containing LEEO became cloudier if the concentration of the LEEO were increased. The hand sanitizer F0 had the distinctive scent of ethanol, in contrast, other formulas containing LEEO have a distinctive scent of Lempuyang tuber and the scent became stronger if the LEEO concentration increased.

Antibacterial Activity Test of Hand Sanitizer Formulation against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 using the Disc Diffusion Method

There are four categories of antibacterial activity strength based on the diameter of the inhibition zone, according to Susanto, Sudrajat, and Ruga (2012), that are weak (≤ 5 mm), moderate (6-10 mm), strong (11-20 mm), and very strong (≥ 21 mm) [27]. The inhibitory values of the hand sanitizers against *S. aureus* ATCC 25923 as gram-positive bacteria and *E. coli* ATCC 25922 as gram-negative bacteria are presented in Table 3.

Table 3. Inhibitory Values of Lempuyang Emprit Essential Oil and Hand Sanitizers against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922

Type of Bacteria	Sample Name	Inhibition Zone (mm)	Category
Staphylococcus aureus ATCC 25923	F0	32.09 ± 0.03	Very Strong
	F1	9.33 ± 0.02	Medium
	F2	8.72 ± 0.02	Medium
	F3	10.13 ± 0.03	Medium
	F4	13.12 ± 0.04	Strong
	F5	13.14 ± 0.02	Strong
	F6	16.16 ± 0.02	Strong
	F7	16.80 ± 0.04	Strong
	F8	17.23 ± 0.02	Strong
	F9	18.62 ± 0.03	Strong
	F10	18.20 ± 0.03	Strong
	F11	16.29 ± 0.03	Strong
	C+ *(Vancomycin)*	23.72 ± 0.03	Very strong
	C- (Aquades)	0 ± 0.00	Weak
Escherichia coli ATCC 25922	F0	8.65 ± 0.03	Medium
	F1	9.92 ± 0.03	Medium
	F2	9.97 ± 0.03	Medium
	F3	11.90 ± 0.02	Strong
	F4	11.60 ± 0.02	Strong
	F5	11.31 ± 0.03	Strong
	F6	9.85 ± 0.03	Medium
	F7	14.15 ± 0.03	Strong
	F8	10.57 ± 0.02	Medium
	F9	8.62 ± 0.02	Medium
	F10	9.55 ± 0.02	Medium
	F11	9.85 ± 0.03	Medium
	C+ (*Chloramphenichol)*	30.20 ± 0.02	Very strong
	C- (Aquades)	0 ± 0.00	Weak

Note:

F0 : Hand Sanitizer (HS) + 0% LEEO
F1 : HS + 0.5% LEEO
F2 : HS + 1.0% LEEO
F3 : HS + 1.5% LEEO
F4 : HS + 2.0% LEEO
F5 : HS + 2.5% LEEO
F6 : HS + 3.0% LEEO
F7 : HS + 3.5% LEEO
F8 : HS + 4.0% LEEO
F9 : HS + 4.5% LEEO
F10 : HS + 5.0% LEEO

The data from the antibacterial activity test results were then carried out by the Shapiro Wilks normality test to determine the distribution of data in each group. The normality test results are presented in Table 4.

The results of the normality test show that there is significant value ($p < 0.05$) so that it can be concluded that the data is not normally distributed. The data was then analyzed using a non parametric test, namely the Kruskal Wallis test which is presented in Table 5.

Table 4. Normality Test Results of Shapiro Wilks

Type of Bacteria	Sample Name	Sig	p value (significan)	Test Results
Staphylococcus aureus ATCC 25923	F0	0,363	p > 0,05	Normally distributed data
	F1	1,000	p > 0,05	Normally distributed data
	F2	0,000	p < 0,05	Not normally distributed data
	F3	0,363	p > 0,05	Normally distributed data
	F4	0,537	p > 0,05	Normally distributed data
	F5	1,000	p > 0,05	Normally distributed data
	F6	1,000	p > 0,05	Normally distributed data
	F7	0,537	p > 0,05	Normally distributed data
	F8	1,000	p > 0,05	Normally distributed data
	F9	0,363	p > 0,05	Normally distributed data
	F10	0,363	p > 0,05	Normally distributed data
	F11	0,363	p > 0,05	Normally distributed data
Escherichia coli ATCC 25922	F0	1,000	p > 0,05	Normally distributed data
	F1	0,363	p > 0,05	Normally distributed data
	F2	1,000	p > 0,05	Normally distributed data
	F3	0,000	p < 0,05	Not normally distributed data
	F4	0,000	p < 0,05	Not normally distributed data
	F5	0,363	p > 0,05	Normally distributed data
	F6	0,363	p > 0,05	Normally distributed data
	F7	0,363	p > 0,05	Normally distributed data
	F8	1,000	p > 0,05	Normally distributed data
	F9	0,000	p < 0,05	Not normally distributed data
	F10	0,000	p < 0,05	Not normally distributed data
	F11	0,363	p > 0,05	Normally distributed data

Table 5. Kruskal Wallis Test Results

Type of Bacteria	df	Asymp. Sig.	p value (significan)	Test Results
Staphylococcus aureus ATCC 25923	11	0,000	p < 0,05	Significantly different
Escherichia coli ATCC 25922	11	0,000	p < 0,05	Significantly different

Table 6. Significantly Different Data Groups

Type of Bacteria	Sample Pair	Asymp. Sig.	p value (significan)	Test Results
Staphylococcus aureus ATCC 25923	F2 dan F9	0,032	p < 0,05	Significantly different
	F2 dan F0	0,008	p < 0,05	Significantly different
	F1 dan F0	0,032	p < 0,05	Significantly different
Escherichia coli ATCC 25922	F0 dan F7	0,024	p < 0,05	Significantly different
	F9 dan F3	0,043	p < 0,05	Significantly different
	F9 dan F7	0,011	p < 0,05	Significantly different

Based on the results of the Kruskal Wallis test in the Table 5, it can be concluded that there is a significant difference in the tests were carried out to find out which groups were significantly different. Further test results showed that only a few pairs of samples were significantly different. diameter of the bacterial inhibition zone *S. aureus* and *E. coli* on the addition of lempuyang emprit essential oil. Then further Significantly different data groups are presented in Table 6.

The antibacterial test results showed that LEEO had very strong antibacterial activity against *S. aureus*. Hand sanitizer

without LEEO addition (F0) had moderate antibacterial activity. Table 3 indicates that the antibacterial activity against *S. aureus* bacteria tends to increase with increasing of LEEO in the hand sanitizer formulas. The hand sanitizer formula with antibacterial activity in the strong category starts from F3, which contains 1.5% of LEEO.

The increase in antibacterial activity in the hand sanitizer after adding LEEO occurred because the essential oil contains hydroxyl and carbonyl groups [28]. The hydroxyl and carbonyl functional groups are antibacterial because they can interact with the bacterial cell wall, which causes protein denaturation, causing lysis of the bacterial cell membrane [29].

The antibacterial test results showed that LEEO and hand sanitizer without LEEO (F0) had moderate antibacterial activity against *E. coli*. However, adding 1.0 %, 2.0%, and 3.0% of LEEO into the pure hand sanitizer (F0) can increase the antibacterial activity from moderate to strong.

The antibacterial test results of the hand sanitizer formula with the LEEO addition at a concentration of 1.5% (F3) showed strong antibacterial activity against *S. aureus* with an inhibition zone of 13.12 mm and *E. coli* with an inhibition zone of 11.60 mm. Adding 1.5 % LEEO to the hand sanitizer formula can increase antibacterial activity against *S. aureus* and *E. coli* bacteria from moderate to strong. The LEEO contain hydroxyl (-OH) and carbonyl groups, which are antibacterial. Based on observations of the inhibition zones of *S. aureus* (gram-positive) and *E. coli* (gram-negative) bacteria, the inhibition zones of gram-positive bacteria are more extensive because gram-positive bacteria have a more straightforward cell wall structure so that antibacterial compounds enter more easily. In contrast, gram-negative bacteria have a more complex cell wall structure consisting of three layers, so antibacterial compounds are challenging to enter [28].

Based on the physical properties test, the hand sanitizer F3 containing 1.5% of LEEO had a clear color with the typical aroma of Lempuyang tuber, while Formula containing LEEO more than 1.5 % seemed cloudy and pale yellow with an increasingly more pungent aroma. So, the researchers decided that the hand sanitizer formula F3 was the most optimum in terms of physical properties and antibacterial activity test against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. However, hand sanitizer F3 still had the typical aroma of Lempuyang tuber, thus requiring fragrance. The optimal fragrance addition was determined through a hedonic test using 30 panellists.

Optimization of the Formulation of Hand Sanitizer Containing Lempuyang Emprit Essential Oil and Fragrance

The hedonic test is a test in organoleptic sensory analysis to determine the magnitude of the difference in quality between several similar products by giving a score to certain characteristics of a product and to determine the level of preference for the product [30]. Panelists try to use hand sanitizer samples and then give a value according to their level of preference. The results of the hedonic test were obtained from tabulated assessments to determine the liking value for each hand sanitizer

preparation by finding the average value of each panelist with a 95% confidence level. The results of the hedonic test involving 30 panelists on the various hand sanitizer are shown in Table 7.

Table 7. Hand Sanitizer Hedonic Test Results on 30 Panelists

Category	Sample of Hand Sanitizer				
	F0	F3	FA	FB	FC
Color	8	7	7	6	6
Scent	6	4	6	6	6
Texture	7	7	6	6	7
Homogeneity	7	7	7	7	7
Speed of Drying	6	7	7	6	7
Causing no Red Skin	7	7	8	7	7
Causing no Pain	7	7	7	7	7
Causing no Dry Skin	7	7	8	7	7
Total	55.00	53.00	56.00	52.00	54.00
Average	6.88	6.63	7.00	6.50	6.75
Standard Deviation (s)	0.60	0.99	0.71	0.50	0.43
Interval (P) (-)	6.66	6.27	6.75	6.32	6.60
Interval (P) (+)	7.09	6.98	7.25	6.68	6.91
P	6.66 ≤ μ ≤ 7.09	6.27≤ μ ≤ 6.98	6.75≤ μ ≤ 7.25	6.32≤ μ ≤ 6.68	6.60≤ μ ≤ 6.91

Note:

F0 : HS FA : HS + 1.5% LEEO + 3% F FC : HS + 1.5% LEEO + 9% F
F3 : HS + 1.5% LEEO + 0% F FB : HS + 1.5% LEEO + 6% F F : Fragrance
1 = extremely dislike, 2 = strongly dislike, 3 = dislike, 4 = quite dislike, 5 = neutral, 6 = quite like, 7 = like, 8 = strongly like, 9 = extremely like

The results of the hedonic test in Table 7 show that FA got the highest average (7.00), thus categorized "like." F0 got an average value of 6.88, F3 got an average value of 6.63, FB got an average value of 6.50, and FC got an average value of 6.75. Therefore, all of them were categorized as "like." Therefore, it can be concluded that hand sanitizer formula FA is the most preferred hand sanitizer by panelists based on the hedonic test.

Observation of the Physical Properties of Hand Sanitizer

The results of observing the physical properties of hand sanitizers can be seen in Table 8.

Table 8. Results of Observation of Physical Properties of Hand Sanitizers

Formulation	**Week-**	**Identification**		
		Aroma	**Color**	**Form**
HS (F0)	0-12	Typical of Ethanol	Clear	Liquid
HS + 1.5% LEEO (F3)	0-12	Typical of Lempuyang	Clear	Liquid
HS + 1.5% LEEO + 3% Fragrance (FA)	0-12	Lemon Scent	Clear Yellowish	Liquid

Hand Sanitizers' Quality and Stability Tests

pH Test

The pH values of the hand sanitizers were in the range of 4-6. According to SNI No. 06-2588, the pH of hand sanitizers should be in the range of 4.5-6.5 [31]. So, all the hand

sanitizers tested met the standards.

Dispersibility Test

The dispersibility test was carried out by spraying each preparation on the mica plastic at a distance of 5 cm and measuring the diameter of the liquid spread. Dispersibility is categorized as good, based on SNI No. 06-2588, if the diameter is 5-7 cm [31]. Based on the results obtained, the diameters of all the samples ranged from 6-7, thus having met SNI.

Homogeneity Test

According to SNI No. 06-2588, each preparation must be free from coarse grains and agglomerates [31]. The results of the observations showed that all hand sanitizers were homogeneous and had no coarse grains, thus having met SNI.

Antibacterial Activity Test against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922

Furthermore, the antibacterial activity of the hand sanitizers was tested against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. The inhibition values of the hand sanitizers against *S. aureus* ATCC 25923 as gram-positive bacteria and *E. coli* ATCC 25922 as gram-negative bacteria are presented in Table 9.

Table 9. Hand Sanitizer Inhibition Value against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922

Type of Bacteria	Sample Name	Inhibition Zone (mm)	Category
Staphylococcus aureus ATCC 25923	HS (F0)	15.54 ± 0.03	Strong
	HS + 1.5% LEEO (F3)	17.82 ± 0.01	Strong
	HS + 1.5% LEEO + 3% F (FA)	15.95 ± 0.02	Strong
	C+ *(Vancomycin)*	21.26 ± 0.01	Very strong
	C- (Aquades)	0 ± 0.00	Weak
Escherichia coli ATCC 25922	HS (F0)	7.31 ± 0.02	Moderate
	HS + 1.5% LEEO (F3)	8.42 ± 0.01	Moderate
	HS + 1.5% LEEO + 3% F (FA)	9.42 ± 0.01	Moderate
	C+ (*Chloramphenichol)*	26.51 ± 0.02	Very strong
	C- (Aquades)	7.46 ± 0.01	Moderate

The data from the antibacterial activity test results were then carried out by the Shapiro Wilks normality test to determine the distribution of data in each group. The normality test results are presented in Table 10.

Table 10. Normality Test Results of Shapiro Wilks

Type of Bacteria	Sample Name	Sig	p value (significan)	Test Results
Staphylococcus aureus ATCC 25923	F0	0,363	p > 0,05	Normally distributed data
	F3	1,000	p > 0,05	Normally distributed data
	FA	0,000	p < 0,05	Not normally distributed data
Escherichia coli ATCC 25922	F0	0,000	p < 0,05	Not normally distributed data
	F3	1,000	p > 0,05	Normally distributed data
	FA	1,000	p > 0,05	Normally distributed data

The results of the normality test show that there is significant value ($p < 0.05$) so that it can be concluded that the data is not normally distributed. The data was then analyzed using a non parametric test, namely the Kruskal Wallis test which is presented in Table 11.

Table 11. Kruskal Wallis Test Results

Type of Bacteria	df	Asymp. Sig.	p value (significan)	Test Results
Staphylococcus aureus ATCC 25923	2	0,027	p < 0,05	Significantly different
Escherichia coli ATCC 25922	2	0,027	p < 0,05	Significantly different

Based on the results of the Kruskal Wallis test in the Table 11, it can be concluded that there is a significant difference in the diameter of the bacterial inhibition zone *S. aureus* and *E. coli* on various hand sanitizer formulas. Then further tests were carried out to find out which groups were significantly different. Further test results showed that only a few pairs of samples were significantly different. Significantly different data groups are presented in Table 12.

Table 12. Significantly Different Data Groups

Type of Bacteria	Sample Pair	Asymp. Sig.	p value (significan)	Test Results
Staphylococcus aureus ATCC 25923	F0 dan F3	0,021	p < 0,05	Significantly different
	F0 dan FA	0,534	p > 0,05	Not significantly different
	F3 dan FA	0,534	p > 0,05	Not significantly different
Escherichia coli ATCC 25922	F0 dan F3	0,534	p > 0,05	Not significantly different
	F0 dan FA	0,021	p < 0,05	Significantly different
	F3 dan FA	0,534	p > 0,05	Not significantly different

Based on the data above, among the three hand sanitizer samples, the hand sanitizer formula F3 had the greatest antibacterial activity against *S. aureus* bacteria and hand sanitizer formula FA had the greatest antibacterial activity against *E. coli* bacteria. The hand sanitizer formula F3 and FA has greater antibacterial activity compared to hand sanitizer formula F0 due to the addition of Lempuyang Emprit essential oil. The results of the test showed that the hand sanitizer formula FA had a strong antibacterial activity value against *S. aureus* ATCC 25923, with an inhibition zone of 15.95 mm, and a moderate value against *E. coli* ATCC 25922, with an inhibition zone of 9.42 mm.

Identification of the Chemical Components Hand Sanitizer Containing Lempuyang Emprit Essential Oil and Fragrance using the GC-MS Method

The GC spectra of hand sanitizer containing Lempuyang Emprit essential oil and fragrance can be seen in Figure 2.

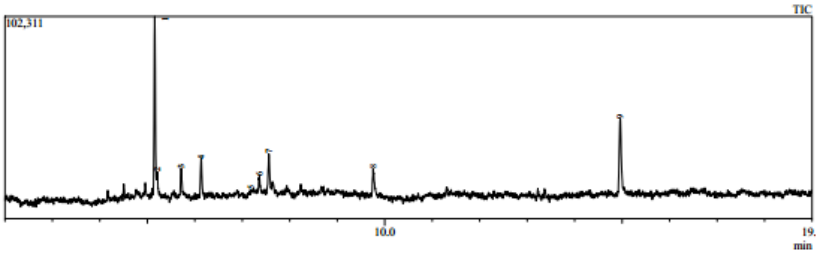

Figure 2. GC Spectra of Hand Sanitizer Containing 1.5% Lempuyang Emprit Essential Oil and 3% fragrance

The results of the identification of the chemical components of the hand sanitizer containing 1.5% Lempuyang Emprit essential oil and 3% fragrance can be seen in Table 13.

Table 13. Results of Identification of the Chemical Components of the Hand Sanitizer Containing 1.5% Lempuyang Emprit Essential Oil and 3% Fragrance

Peak No.	Compound Name	RT (Min)	RI	Molecular Weight	%Area
1	1-Limonene	5.156	1028	136	32.16
2	1,8-Cineole	5.209	1030	154	3.86
3	Dihydromyrcenol	5.713	1058	156	5.42
4	L-Linalool	6.133	1102	154	9.54
5	Busan	7.180	-	309	4.61
6	4-Terpineol	7.361	1177	154	4.65
7	α-Terpineol	7.560	1191	154	11.08
8	Terpinyl Acetate	9.755	1367	196	7.33
9	Zerumbone	14.961	1742	218	21.35
Main Groups of Compounds:					
Monoterpene hydrocarbon (%) (No. 1)					32.16
Oxygenated monoterpenes (%) (No. 2-4, 6-8)					41.88
Oxygenated sesquiterpenes (%) (No. 9)					21.35
Unknown (%) (No. 5)					4.61
Total identified compounds (%)					100.00

Note:
RT: Retention Time, RI: Retention Index in Literature

The results of the identification of the chemical components above show that the largest components contained in the hand sanitizer with the addition of Lempuyang Emprit essential oil and fragrance are 1-limonene (32.16%) and zerumbone (21.35%). Ariani *et al*. (2021) revealed that the chemical components of Lempuyang Emprit essential oil isolated from Jumapolo, Karanganyar, Jawa Tengah, Indonesia were zerumbone (65.06%), humulene oxide (9.66%), α-humulene (9.41%), β-selinene (4.39%), and L-Linalool (3.96%), the first of which had the largest portion. Zerumbone exhibits antibacterial and antioxidant capabilities [19]. Zerumbone has the potential as an antibacterial compound because it contains a carbonyl group, in which the hydroxyl and carbonyl groups are antibacterial.

The mass spectra of zerumbone contained in hand sanitizer with the addition of Lempuyang Emprit essential oil and fragrance can be seen in Figure 3.

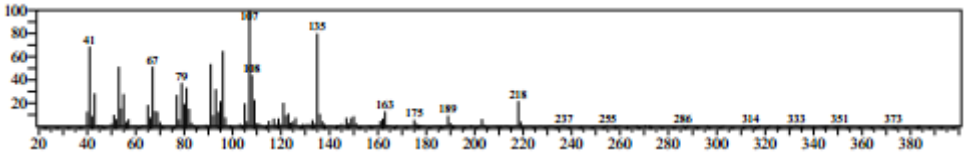

Figure 3. Mass Spectra of Zerumbone Contained in Hand Sanitizer with the Addition of Lempuyang Emprit Essential Oil and Fragrance

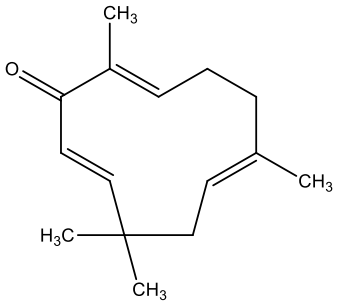

Figure 4. The Structure of Zerumbone

CONCLUSION

Based on the antibacterial activity test against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, physical properties test, and hedonic test, the best formulation of hand sanitizer contained 1.5% Lempuyang Emprit essential oil and 3% fragrance. This hand sanitizer had a strong antibacterial activity value against *S. aureus* ATCC 25923, with an inhibition zone of 15.95 mm, and a moderate value against *E. coli* ATCC 25922, with an inhibition zone of 9.42 mm. Hand sanitizers containing Lempuyang Emprit essential oil contained 21.35% of zerumbone.

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