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Optimization of Hand Sanitizer Formulation with the Addition of Lemongrass (*Cymbopogon citratus* (DC.) Stapf) Essential Oil and a Combination of Essential Oils of Local Indonesian *Empon-empon*

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ABSTRACT: This research aimed to optimize the formulation of hand sanitizer by incorporating lemongrass (*Cymbopogon citratus* (DC.) Stapf) essential oil in combination with essential oils of local Indonesian *empon-empon*: *temu giring* (*Curcuma heyneana* Val. & van Zipj.), *temu kunci* (*Kaempferia pandurata* Roxb.), and *temu mangga* (*Curcuma amada* Roxb.). The optimization of hand sanitizer formulation was done based on several tests. The first was organoleptic test by observing the aroma, color, and form; the second was pH, homogeneity, and spreadability testing, and; the third was testing the antibacterial activities of each hand sanitizer formula against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25922. The research stages encompassed isolation of essential oils, formulation and evaluation of hand sanitizers, antibacterial activities test, optimization of hand sanitizer formulation, and identification of the chemical compounds in the hand sanitizer with the optimum formulation using the GC-MS method. The study resulted in the hand sanitizer with the optimum formulation containing the essential oils of lemongrass (3%) and *temu giring* (3%), demonstrating very strong antibacterial bioactivities against both *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, with inhibition zones measuring 29.70 mm and 60.08 mm, respectively. This hand sanitizer had a distinctive aroma, combining the scents of lemongrass and *temu giring*. It was in liquid form, clear, and homogeneous, with pH and spreadibility values of 5.4 and 6.4 cm, respectively. The quality complied with SNI quality standards No. 06-2588. The primary active ingredients were E-citral (22.10%), Z-citral (17.88%), and 1,8-cineol (17.63%).

Keywords: Essential oil, hand sanitizer, lemongrass, *temu giring, temu kunci, temu mangga.*

INTRODUCTION

To safeguard against severe illnesses caused by bacterial infections, people often use antiseptics, including soap and hand sanitizer. Antiseptic soap typically requires water for optimal effectiveness in protecting against a variety of harmful bacteria, while hand sanitizer offers the advantage of being water-free. Hand sanitizer is a convenient method for safeguarding hands against various types of bacteria (Susilaningrum et al., 2021).

Hand sanitizers, typically containing ethanol, effectively reduce bacterial counts on hands rapidly. Nevertheless, ethanol may not eliminate all types of bacteria (Holifah et al., 2020). The World Health Organization (WHO) recommends that the hand sanitizer contains 60-95% ethanol and 3-6% hydrogen peroxide for their efficacy (Hayati et al., 2022). To enhance the effectiveness of hand sanitizers, innovation like the addition of natural compounds with antibacterial properties is needed. Essential oils represent a group of plant-based oils that are liquid at room temperature but easily evaporate, imparting a distinct aroma (Triesty & Mahfud, 2017). These essential oils contain bioactive components that serve as natural antibacterial agents, offering an alternative to chemical sanitizers (Pizzo et al., 2023).

Cymbopogon nardus (citronella) and *Cymbopogon citratus* (DC.) Stapf. (lemongrass) have been known as sources of essential oils with antibacterial properties. Yet, lemongrass is less popular in Indonesia compared to citronella, despite being easier to cultivate and having a higher essential oil content (Zaituni et al., 2016). Tadtong et al. (2014) stated that the chemical components contained in lemongrass essential oil are β-myrcene (3.9%), E-citral (48.7%), selina-6-en-4-ol (1.8%), cis-ocimene (0.5%), and Z-citral (31.7%). The primary compound found in lemongrass oil is citral, known for its distinctive lemon aroma (Sufyan et al., 2018). Several studies demonstrated that lemongrass essential oil could inhibit *S. aureus* bacteria and *E. coli* bacteria (Nisyak & Hartiningsih, 2020; Sufyan et al., 2018).

In Indonesia, *empon-empon* are well-known as plants that hold significant cultural and traditional importance for their vital component of traditional medicine. In this study, essential oils were chosen from three local types of *empon-empon*, namely *Curcuma heyneana* Val & V. Zijp. (*temu giring*), *Kaempferia pandurata* Roxb. (*temu kunci*), and temu mangga (*Curcuma amada* Roxb.), which are widespread in Indonesia and belong to the family *Zingiberaceae* (Putri et al., 2019).

Curcuma heyneana Val & V. Zijp. (*temu giring*) exhibits potential applications in medicine and cosmetics and is categorized as an indigenous species native to Indonesia (Putra et al., 2015). The chemical components present in the essential oil of *temu giring* include acetophenone (18,93%), camphor (17,89%), camphene (8,39%), epicurzereno (8,16%), β-elemene (6,75%), germacrone (6,38%), 1,8-cineole (6,09%), α-guaiene (4,10%), and curzerene (0,55%), (Nuraeni and Yunilawati, 2012). *Temu giring* essential oil also exhibits strong antibacterial bioactivity against *E. coli* bacteria, with MIC value of 3.9 μg/mL (Septama et al., 2023).

Kaempferia pandurata Roxb. (*temu kunci*), which also belongs to the family *Zingiberaceae*, is a native plant in Indonesia, particularly found on the islands of Sumatra and Java, and it can still be found growing wild in the forests of Jawa Tengah and Jawa Timur. Analysis using the GC-MS method has revealed that the essential oil of *temu kunci* comprises 31 chemical components, with the primary constituents including 1,8-cineol (14.83%), transβ-osimen (8.22%), methyl cinnamate (8.28%), camphor (21.20%), and nerol (10.48%) (Simbolon, 2014). Mahmudah and Atun (2017) reported that the ethanol extract of *temu kunci* exhibited inhibitory activity against the growth of *Streptococcus mutans*. Contact bioautography testing results also indicate that compounds from the monoterpene group found in *temu kunci* essential oil possess antibacterial activity against *S. aureus* (Christiana & Soegianto, 2020).

Temu mangga (*Curcuma amada* Roxb.) is a plant native to the Indo-Malesian region, and it is referred to as temu *mangga* due to its distinctive aroma, reminiscent of mango. The main components of *temu mangga* essential oil consist of monoterpenes, including α-pinene, 1,8-cineol, β-pinene, sabinene, camphor, β-myrsene, limonene, trans β-osimene, cis-osimene, perillene, and terpinen-4-ol (Novianti, 2012). Research conducted by Susanti and Mahmudah (2017) showed that *temu mangga* essential oil possesses antibacterial properties against *S. aureus*. Moreover, *temu mangga* essential oil exhibits strong antibacterial bioactivity against *S. aureus* ATCC 25923 bacteria, with diameter of inhibition zone was 11.13 mm (Ariani et al., 2022).

To enhance the antibacterial efficacy of hand sanitizers by incorporating natural compounds with antibacterial properties and to elevate the economic value of locally abundant plants like lemongrass, *temu giring*, *temu kunci*, and *temu mangga* through optimizing the diversification of their essential oils, this research plays a role. A literature review reveals a gap in research; no prior study of the addition of hand sanitizers with a combination of the essential oil of lemongrass and those of *empon-empon* has been found. This research would test the addition of lemongrass essential oil to hand sanitizer, in combination with the essential oils of *temu giring*, *temu kunci*, and *temu mangga*. The anticipated outcome was to develop a hand sanitizer formulation with enhanced antibacterial activities compared to the basic hand sanitizer formula, while still meeting the required standards in terms of physical and chemical properties.

EXPERIMENTAL SECTION

Plant Materials

The lemongrass plants and rhizomes of *temu giring*, *temu kunci*, and *temu mangga* used as this research materials were sourced from the Surakarta region in Jawa Tengah, Indonesia, situated at coordinates 110 °E – 7 °S. Plant determination carried out at the Biology Study Program, FMIPA, UNS Surakarta, proved that the samples to be used in the research were indeed lemongrass [*Cymbopogon citratus* (DC.) Stapf. (043/UN27.9.6.4/Lab/2023)], *temu giring* [*C. heyneana* Val. & van Zipj. (011/UN27.9.6.4/Lab/2023)], *temu kunci* [*Kaempferia pandurata* Roxb. (036/UN27.9.6.4/ Lab/2023)], and *temu mangga* [*Curcuma amada* Roxb. (010/UN27.9.6.4/Lab/2023)].

Isolation of Essential Oils

This research employed all components of lemongrass, comprising leaves and stems, and only the rhizomes of *temu giring*, *temu kunci*, and *temu mangga*. Each material was sorted, cleaned, washed, chopped, and then subjected to a four-day drying process in indirect sunlight. Following the drying process, the dried samples were stored in sealed containers and were ready for essential oil isolation (Ariani et al., 2021; Ariani et al., 2022; Souhoka et al., 2020).

In this research, the isolation of sample essential oils was conducted by Steam-Water Distillation method. Dry lemongrass plants and the rhizomes of *temu giring*, *temu kunci*, and *temu mangga*, each weighing 2.50 kg dry weight (DW), were respectively placed into different distillation apparatus and distilled for a duration of 6 hours. The resulting distillate formed two layers. Subsequently, anhydrous Na_2SO_4 was added to each essential oil to remove any remaining moisture, and the mixture was then filtered. The isolated essential oil was then measured in terms of volume, and its color and aroma were observed (Ariani et al., 2021; Ariani et al., 2022; Souhoka et al., 2020).

Formulation and Evaluation of Hand Sanitizers

The complete hand sanitizers formulation can be found in **Table 1** below. Evaluation of hand sanitizers included organoleptic test by observing the aroma,

Table 1. The formulation of hand sanitizers added with lemongrass essential oil and variations of *temu giring*, *temu kunci,* and *temu mangga* essential oils

No.	Material	Composition (%)				
		HS_1	HS_2	HS_3	HS_4	HS_5
1.	Basic hand sanitizer	94	94	94	97	100
2.	Lemongrass essential oil	3	3	3	3	-
3.	*Temu giring* essential oil	3	-	-	-	-
4.	*Temu kunci* essential oil	-	3	-	-	-
5.	Temu mangga essential oil	-	-	3	-	-
	Total	100	100	100	100	100

Notes: Basic hand sanitizer: ethanol (833.3 mL), 98% glycerin (14.5 mL), 3% hydrogen peroxide (41.7 mL), and distilled water (110.5 mL).

color, and form of the hand sanitizers (Ariani et al., 2023) and physical tests including pH, homogeneity, and spreadability. Each test was performed three times. The pH test was carried out using a digital pH meter (Pirgal et al., 2022). The homogeneity test involved dripping the hand sanitizer sample onto a glass surface to check for any coarse grains or uneven mixing (Putri et al., 2019). The spreadability test was conducted by spraying the hand sanitizer sample onto mica paper from a distance of 5 cm from the funnel's mouth, and the resulting spray's diameter was measured (Arifan et al., 2022).

Antibacterial Activities Test

The antibacterial activities test was conducted on all hand sanitizer samples that had been enriched with lemongrass essential oil and combined with the essential oils of *temu giring*, *temu kunci*, and *temu mangga*. The antibacterial activity test utilized the well diffusion method and was performed against *E. coli* ATCC 25922 and *S. aureus* ATCC 25922. In this test, the positive control involved vancomycin when testing against *E. coli* ATCC 25922, and chloramphenicol when testing against *S. aureus* ATCC 25923. As for the negative control, distilled water was used (Ariani et al., 2022; Nassar et al., 2019).

Optimization of Hand Sanitizer Formulation

The optimization of hand sanitizer formulation was done based on several tests. The first was organoleptic test by observing the aroma, color, and form; the second was pH, homogeneity, and spreadability testing, and; the third was testing the antibacterial activities of each hand sanitizer formula against *E. coli* ATCC 25922 and *S. aureus* ATCC 25922.

Identification of The Chemical Components in The Hand Sanitizer with The Optimum Formulation Using the GC-MS Method

Identification of the chemical compounds in the hand sanitizer sample with the optimum formulation using GC-MS QP-2010 SE Shimadzu with the following specifications: Rtx-5MS capillary column, 0.25 mm column diameter, 30 m column length, 0.25 μm column thickness, helium (He) as the carrier gas with a flow rate of 0.75 mL/minute, starting at an initial temperature of 60°C and gradually increasing at a rate of 10°C per minute until it reached a temperature of 200°C and injector and detector temperature set at 200°C (Ariani et al., 2022; Guimaraes et al., 2020; Habibi, 2020; Hubschmann, 2015).

RESULTS AND DISCUSSION

Isolation of Essential Oils

The characteristics data of the essential oils from this research are presented in **Table 2**.

Formulation and Evaluation of Hand Sanitizers

The basic hand sanitizer was supplemented with lemongrass essential oil, resulting in the hand sanitizer formulation containing lemongrass oil. This formulation was then further enriched with various essential oils from *temu giring*, *temu kunci*, and *temu mangga*. Hand sanitizer evaluation data includes organoleptic tests (aroma, color, and form), pH, homogeneity and spreadability testing can be described below.

Organoleptic test

Based on the organoleptic test results, it was evident that each hand sanitizer preparation had a distinct aroma corresponding to the essential oil added to it. All preparations were in liquid form. The preparation containing lemongrass and *temu giring* essential oils stood out as the best preparation from the organoleptic perspective. This preparation produced the clearest color compared to the others and shared the same color as the basic hand sanitizer. These clarity and similarity to the basic hand sanitizer make it a favorable choice in terms of organoleptic properties.

pH test

The degree of acidity or pH was a critical parameter for assessing the acidity of a hand sanitizer preparation. Preparations with very low pH can potentially cause skin irritation, while those with very high pH may result in dry or scaly skin (Ariani et al., 2023). The pH values of hand sanitizers are presented in **Table 3**.

Table 2. The characteristics data of the essential oils

Essential Oil	Color	Aroma	Content (%)
Lemongrass	Clear	Typical	0.16
Temu giring	Clear	Typical	0.60
Temu kunci	Clear yellowish	Typical	0.68
Temu mangga	Clear yellowish	Typical	0.80

Table 3. The pH values of hand sanitizers

Sample	Average of pH
HS_1	$5.4^a \pm 0.2$
HS_2	$5.3^a \pm 0.2$
HS_3	$5.6^a \pm 0.2$
HS_4	$6.2^b \pm 0.1$
HS_5	$6.3^b \pm 0.2$

Notes:
HS_1 : Hand sanitizer + lemongrass essential oil + *temu giring* essential oil
HS_2 : Hand sanitizer + lemongrass essential oil + *temu kunci* essential oil
HS_3 : Hand sanitizer + lemongrass essential oil + *temu mangga* essential oil
HS_4 : Hand sanitizer + lemongrass essential oil
HS_5 : Basic hand sanitizer
The different superscripts in the same column show a significant difference ($P<0.05$)

Based on the pH value data in **Table 3**, the hand sanitizer preparations containing lemongrass essential oil in combination with the essential oils of *temu giring*, *temu kunci*, and *temu mangga* had average pH values of 5.4, 5.3, and 5.6, respectively. On the other hand, the hand sanitizer formulation containing merely lemongrass oil and the basic hand sanitizer had pH values of 6.2 and 6.3, respectively. Research conducted by Ariani et al. (2023) showed that hand sanitizers containing *lempuyang emprit* essential oil had a pH range of 4.5-6.0. Additionally, Wijana et al. (2020) found that hand sanitizer containing baby orange and sweet orange peel essential oils (0.75:0.25) had a pH of 4.5. Another study by Patricia et al. (2019) revealed that hand sanitizer containing celery (*Apium graveolens*) essential oil had a pH of 6.0.

The pH values of all the hand sanitizers from this study fall within the safe pH range for skin according to SNI No.06-2588 quality standards, in the range of pH scale were 4.5-6.5. Therefore, all of the hand sanitizers were safe for consumer use.

Homogeneity test

The homogeneity test was carried out by putting hand sanitizer into an object glass, then observing. Hand sanitizer is said to have good homogeneity if there are no lumps or coarse grains in the preparation (Zarwinda et al., 2022). The homogeneity data showed that all hand sanitizers were homogenous. Therefore, all the hand sanitizer preparations resulting from this research met the requirements of SNI 06-2588 in terms of homogeneity. Based on the research conducted by Safitri and Fariztamarin (2023), the homogeneity test on hand sanitizer preparations with essential oil from cinnamon bark did not find any granules, and all the preparations were homogenous. Research by Bahri et al. (2021) stated that hand sanitizer preparations with nutmeg essential oil were homogeneously structured, indicated by the thorough mixing of all components. Another study by Haque et al. (2021) found that hand sanitizers containing kalamansi orange essential oil had good homogeneity, as there were no coarse particles present.

Spreadability test

The spreadability test was conducted to assess how effectively a hand sanitizer can spread across the surface of human skin. An effective hand sanitizer should easily and evenly cover the skin when applied. According to SNI No. 06-2588, a good spreadability test should fall within the range of 5.0-7.0 cm. The spreadability data of hand sanitizers are presented in **Table 4**.

The data in the Table indicate that the average spreadability test results for hand sanitizer containing lemongrass essential oil combined with the essential oils of *temu giring*, *temu kunci*, *temu mangga,* hand sanitizer containing lemongrass essential oil and basic hand sanitizer were 6.4, 5.6, 6.4, 6.6, and 6.4 cm, respectively. These results were in accordance with the quality standards of SNI No. 06-2588, within the range of 5.0-7.0 cm, demonstrating that the hand sanitizer preparations met the required spreadability standards.

For comparison, research by Sari et al. (2022), showed that hand sanitizer prepared from *Curcuma aeruginosa* Roxb. rhizome extract had a spreadability of 4.9-6.2, thus considered acceptable based on the range set by SNI. Another study by Legowo et al. (2020) reported that hand sanitizer preparations containing basil essential oil had a spreadability in the range of 6.2-7.0, meeting the SNI requirements for spreadability. Additionally, research by Manus et al. (2016) indicated that hand sanitizer containing lemongrass leaf essential oil with concentrations of 5.0, 10.0, and 15.0% had a spreadability in the range of 5.1-5.4, meeting the spreadability requirements according to SNI.

Antibacterial Activities Test

Based on the diameter of inhibition zone, the antibacterial activities are categorized into four groups: weak (≤5.0 mm), moderate (6.0-10.0 mm), strong (11.0-20.0 mm), and very strong (≥ 21.0 mm) (Davis & Stout, 1971). The antibacterial activities test of *E. coli* as gram negative bacteria and *S. aureus* as gram positive bacteria are presented in **Figure 1**.

Table 4. Spreadability test results

Sample	Average of Spreadability (cm)
HS_1	$6.4^b \pm 0.2$
HS_2	$5.6^a \pm 0.2$
HS_3	$6.4^b \pm 0.2$
HS_4	$6.4^b \pm 0.1$
HS_5	$6.4^b \pm 0.2$

Notes:
HS_1 : Hand sanitizer + lemongrass essential oil + *temu giring* essential oil
HS_2 : Hand sanitizer + lemongrass essential oil + *temu kunci* essential oil
HS_3 : Hand sanitizer + lemongrass essential oil + *temu mangga* essential oil
HS_4 : Hand sanitizer + lemongrass essential oil
HS_5 : Basic hand sanitizer
The different superscripts in the same column show a significant difference ($P<0.05$)

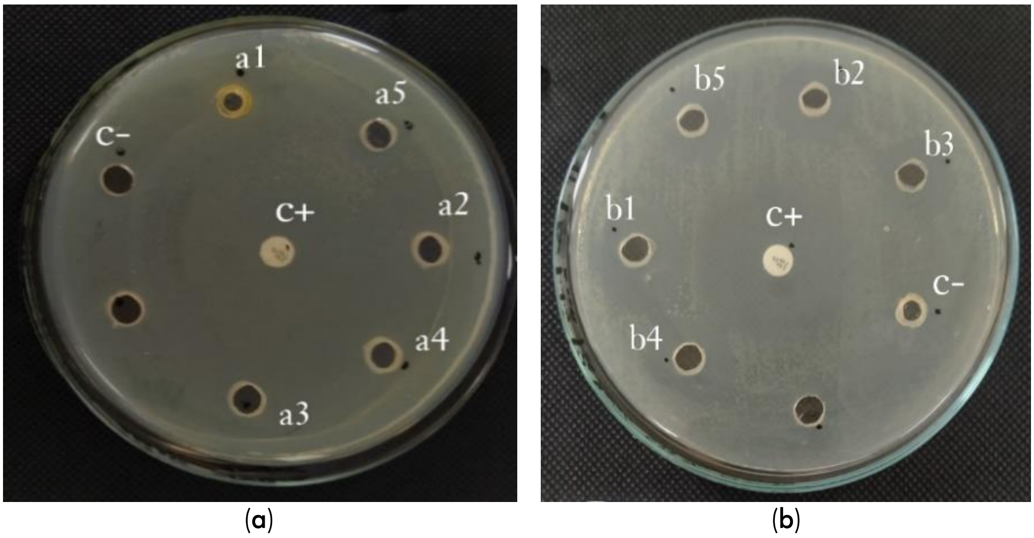

Figure 1. The antibacterial activities test of (**a**) *E coli* ATCC 25922, (**b**) *S. aureus* 25923
Notes:
a1, b1 : Hand sanitizer + lemongrass essential oil + *temu giring* essential oil
a2, b2 : Hand sanitizer + lemongrass essential oil + *temu kunci* essential oil
a3, b3 : Hand sanitizer + lemongrass essential oil + temu mangga essential oil
a4, b4 : Hand sanitizer + lemongrass essential oil
a5, b5 : Basic hand sanitizer
c+ : Control +
c- : Control -

The following **Table 5** presents the inhibitory power values for the hand sanitizer preparations from the research. In the **Table 5**, the results of the antibacterial activities test of hand sanitizer against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 are presented. The hand sanitizers produced exhibited antibacterial activity against *E. coli* ATCC 25922 with diameter inhibition zone ranging from 26.01 to 29.70 mm (very strong antibacterial activity). In contrast, the inhibitory zone diameters for the antibacterial activity against *S. aureus* ATCC 25923 were notably larger, ranging from 50.21 to 60.08 mm (very strong antibacterial activity). The type of bacteria can indeed influence the antibacterial activity of volatile oils. For instance, gram negative bacteria like *E. coli* tend to be more resistant to volatile oils compared to gram positive bacteria like *S. aureus* (Pizzo et al., 2023). Such discrepancy is due to differences in the cell wall structures of these bacteria. *E. coli* possesses a more complex three-layered cell wall structure consisting of an outer lipoprotein layer, a middle lipopolysaccharide layer, and an inner peptidoglycan layer (Gumbart et al., 2014). In contrast, *S. aureus*, as a gram-positive bacterium, has a simpler cell wall structure with a thicker peptidoglycan layer, resulting in a more rigid structure (Pasquina-Lemonche et al., 2020). The relatively simple cell wall of *S. aureus* makes it more susceptible to antibacterial compounds, as these compounds can more easily penetrate the cell and target their sites of action (Kumayas et al., 2015; Muharni et al., 2017). As explained by Vaou et al. (2021), antibacterial agents ca n act through various mechanisms, such as inhibiting protein synthesis, damaging cell walls, interfering with nucleic acid synthesis, disrupting plasma membranes, and hindering the production of essential bacterial metabolites.

Table 5. The results of antibacterial activities of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923

No.	Sample	Bacteria	Inhibitory Power (mm)
1.	MA_1	*E. coli* ATCC 25922	11.74±0.02
		S. aureus ATCC 25923	18.59±0.03
2.	MA_2	*E. coli* ATCC 25922	03.41±0.03
		S. aureus ATCC 25923	10.97±0.03
3.	MA_3	*E. coli* ATCC 25922	00.00±0.00
		S. aureus ATCC 25923	05.16±0.02
4.	MA_4	*E. coli* ATCC 25922	23.18±0.02
		S. aureus ATCC 25923	37.30±0.03
5.	HS_1	*E. coli* ATCC 25922	29.70±0.03
		S. aureus ATCC 25923	60.08±0.02
6.	HS_2	*E. coli* ATCC 25922	28.58 ±0.02
		S. aureus ATCC 25923	57.31±0.02
7.	HS_3	*E. coli* ATCC 25922	28.31±0.04
		S. aureus ATCC 25923	52.40±0.03
8.	HS_4	*E. coli* ATCC 25922	29.52 ±0.03
		S. aureus ATCC 25923	58.50 ±0.03
9.	HS_5	*E. coli* ATCC 25922	26.01 ±0.04
		S. aureus ATCC 25923	50.21±0.03
10.	C+	*E. coli* ATCC 25922	24.57±0.02
		S. aureus ATCC 25923	28.51±0.01
11.	C–	*E. coli* ATCC 25922	00.00±0.04
		S. aureus ATCC 25923	00.00±0.00

Notes:
MA_1 : *Temu giring* essential oil
MA_2 : *Temu kunci* essential oil
MA_3 : *Temu mangga* essential oil
MA_4 : Lemongrass essential oil
HS_1 : Hand sanitizer + lemongrass essential oil + *temu giring* essential oil
HS_2 : Hand sanitizer + lemongrass essential oil + *temu kunci* essential oil
HS_3 : Hand sanitizer + lemongrass essential oil + *temu mangga* essential oil
HS_4 : Hand sanitizer + lemongrass essential oil
HS_5 : Basic hand sanitizer
C+ : Control +
C- : Control –

The research results indicate that hand sanitizer containing the essential oils of lemongrass (3%) and *temu giring* (3%) possessed the strongest antibacterial activity against *E. coli* ATCC 25922 compared to the other formulations. The inhibition zones of the essential oils of lemongrass and *temu giring* against *E. coli* ATCC 25922 are 23.18 mm (strong) and 11.74 mm (strong) in diameter, respectively. Upon the addition of lemongrass essential oil into the basic hand sanitizer, the inhibitory power increased from 26.01 mm (the basic hand sanitizer) to 29.52 mm. Following the addition of *temu giring* essential oil into the hand sanitizer containing lemongrass oil, another increase in the inhibitory power, namely from 29.52 mm (the hand sanitizer containing lemongrass oil) to 29.70 mm, occurred.

The hand sanitizer containing the essential oils of lemongrass (3%) and *temu giring* (3%) had the strongest antibacterial activity against *S. aureus* ATCC 25923 compared to the other formulations. The diameter inhibition zones of the essential oils of lemongrass and *temu giring* against *S. aureus* ATCC 25923 were respectively 37.30 mm (strong) and 18.59 mm (strong) in diameter. After lemongrass essential oil was added to the basic hand sanitizer, the inhibitory power increased from 50.21 mm (the basic hand sanitizer) to 58.50 mm. Furthermore, after *temu giring* essential oil was added to the hand sanitizer containing lemongrass oil, the inhibitory power increased from 58.50 mm (the hand sanitizer containing lemongrass oil) to 60.08 mm. This phenomenon showed that the chemical compounds contained in lemongrass oil could increase the antibacterial activity of the basic hand sanitizer and, furthermore, *temu giring* oil could increase the antibacterial activity of the hand sanitizer added with lemongrass oil, against both *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Such increased antibacterial l activity in the hand sanitizer was possibly due to the synergistic effect between the compounds in lemongrass and *temu giring* essential oils. A synergistic effect is an effect that arises due to the presence of two or more chemical ingredients that have the same properties and are mutually beneficial.

Optimization of Hand Sanitizer Formulation

In terms of organoleptic characteristics, the hand sanitizer formulation containing the essential oils of lemongrass (3%) and *temu giring* (3%) was considered the best preparation, as it exhibited the same clear color as the basic hand sanitizer. The pH value of this preparation was 5.4, which fell within the acceptable pH range of 4.5–6.5 according to SNI quality standards No. 06-2588. It also demonstrated spreadability of 6.4 cm, indicating that it met the SNI requirement No. 06-2588 for spreadability, which should be in the range of 5.0–7.0 cm. Moreover, it met the SNI 06-2588 requirement for homogeneity, as it was homogenous with no granules or coarse grains. Based on these findings, it can be concluded that the best hand sanitizer formulation is the one containing the essential oils of lemongrass and *temu giring*.

The results of this research indicated that hand sanitizer containing essential oils of lemongrass and *temu giring* was the most effective against *E. coli* ATCC 25922, with an inhibitory power value of 29.70 mm. It also demonstrated the strongest antibacterial activity against *S. aureus* ATCC 25923, with an inhibitory value of 60.08 mm.

Identification of the Chemical Compounds in the Hand Sanitizer with the Optimized Formulation Using the GC-MS Method

The GC chromatogram of the hand sanitizer preparation containing lemongrass and *temu giring* essential oils can be seen in Figure 2.

From the GC chromatogram results above, it can be seen that the hand sanitizer preparation containing lemongrass and *temu giring* essential oils contains 17 compounds. The results of identifying chemical components using the GC-MS method of hand sanitizer containing lemongrass (3%) and *temu giring* (3%) essential oils can be seen in Table 6.

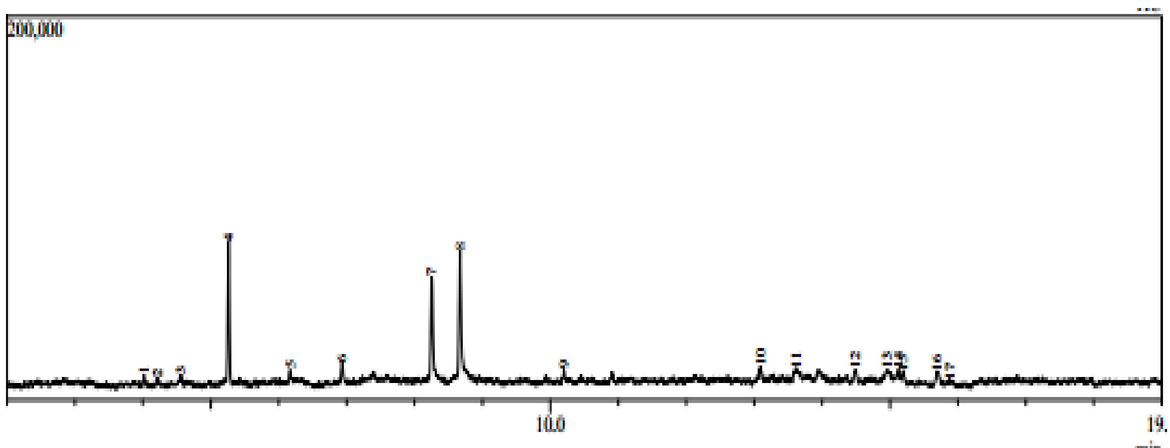

Figure 2. GC chromatogram of hand sanitizer preparation containing lemongrass and *temu giring* essential oils

Table 6. The data of identifying chemical compounds using GC-MS Method of hand sanitizer containing lemongrass (3%) and *temu giring* (3%) essential oils

Peak	Retention Time (Min)	Retention Index Lit (RI_{lit})	%Area	Molecular Weight	Data MS : m/z (Relative Intensity)									Compound Name
1	4.018	935	1.10	136	27(15)	39(20)	53(10)	65(5)	77(30)	93(100)	105(10)	119(10)	136(10)	α-Pinene
2	4.216	946	1.18	136	41(40)	65(10)	67(35)	77(30)	79(40)	93(100)	107(30)	121(60)	136(10)	Camphene
3	4.564	976	2.98	136	27(30)	41(75)	53(15)	65(15)	69(45)	77(20)	93(100)	119(10)	136(5)	β-Pinene
4	5.260	1034	17.63	154	27(15)	41(30)	43(100)	55(30)	69(30)	81(40)	93(20)	108(30)	139(20)	1,8-Cineole
5	6.173	1102	2.50	154	27(20)	39(20)	43(90)	55(70)	67(25)	71(100)	80(35)	93(80)	121(25)	Linalool
6	6.935	1144	3.86	152	27(35)	29(20)	41(80)	55(40)	69(40)	81(70)	95(100)	108(40)	152(30)	Camphor
7	8.254	1212	17.88	152	41(100)	43(10)	53(15)	59(15)	69(85)	83(20)	94(20)	109(20)	119(10)	Z-Citral
8	8.679	1270	22.10	152	27(20)	41(100)	51(5)	65(5)	69(90)	79(5)	84(30)	94(20)	107(10)	E-Citral
9	10.201	1383	1.92	196	43(70)	44(5)	53(10)	69(100)	77(10)	92(5)	93(20)	119(10)	136(20)	Geranyl acetate
10	13.095	1578	3.22	220	41(100)	55(40)	65(10)	69(50)	79(80)	93(0)	105(40)	121(20)	135(10)	Caryophyllene oxide
11	13.620	1597	3.26	206	29(40)	39(30)	41(100)	55(60)	67(45)	79(50)	91(50)	107(35)	121(20)	Patchulene
12	14.491	1713	2.37	218	41(55)	53(30)	67(70)	82(20)	91(30)	107(100)	121(25)	135(80)	147(10)	Germacrone
13	14.955	1745	5.54	220	41(80)	55(65)	67(25)	79(65)	91(100)	105(95)	119(60)	133(40)	147(55)	Valerenol
14	15.130	1757	4.45	220	29(20)	41(80)	55(50)	67(55)	81(40)	95(60)	109(100)	121(30)	135(30)	1,4-Methanoazulen29(50)-7(1H)-one, oct27(40)ahydro-4,8,8,9-28(5)tetramethyl-, (+)-(CAS) Z. alpha.H-longifolan-3-one
15	15.197	1762	3.13	276	29(50)	41(100)	55(75)	67(100)	81(90)	95(70)	109(45)	123(30)	151(20)	Cyclohexene, 1-pentyl-4-(4-propylcyclohexyl)
16	15,691	1796	4.08	152	27(40)	41(80)	53(30)	67(100)	81(50)	93(80)	109(90)	123(70)	137(20)	Isopulegone
17	15.881	1809	2.81	208	28(5)	51(10)	68(15)	75(10)	85(5)	102(20)	137(20)	173(100)	193(10)	Benzene, trichloro-1,3-dimethyl- (CAS) AR, AR, AR-trichloro-o-xylene
Main components of compounds														
Monoterpene hydrocarbons (%) (No. 1-3)														5.26
Oxygenated monoterpens (%) (No. 4-9, 16)														69.97
Oxygenated sesquiterpene (%) (No. 10, 12-14)														15.58
Sesquiterpenes hydrocarbons (%) (No. 11,15)														6.39
Aromatic compounds (%) (No. 17)														2.81
Total identified														100.00

The antibacterial bioactivities of hand sanitizer containing essential oils of lemongrass (3%) and *temu giring* (3%) was closely related to the content of chemical components, such as aldehydes [Z-sitral (17.88%), E-sitral (22.10%)]; ketones [camphor (3.86%), isopulegon (4.08%), 1,4-methanoazulene-7(1H)-one,octahydro-4,8,8,9-tetramethyl-,(+)-(CAS)7.alpha.H-longifolan-3-one (4.45%), germacrone (2.37%)]; alcohol [linalool (2.50%), valerenol (5.54%)]; ethers[1,8-cineole (17.63%), caryophyllene oxide (3.22%)], and esters [geranyl acetate (1.92%)].

The hand sanitizer containing essential oils of lemongrass (3%) and *temu giring* (3%) contained major chemical components in the form of: E-citral (22.10%); Z-citral (17.88%); and 1,8-cineole (17.63%). The results of the research by Tadtong et al. (2014) stated that the chemical components contained in lemongrass oil are Z-citral (31.7%); E-citral (48.7%); cis-ocimene (0.5%); selina-6-en-4-ol (1.8%); and β-myrcene (3.9%). In this research, E-citral (22.10%) and Z-citral (17.88%) are contributed by the essential oil of lemongrass. Citral (3,7- dymethyl-2,6-octadienal) serves as the main component in lemongrass oil, found in 2 isomers, namely Z-citral (neral) and E-citral (geranial) (Phunpee et al., 2017; Majewska et al., 2019). It belongs to the group of oxygenated monoterpenes hydrocarbons with an aldehyde group and has a lemon aroma. Furthermore, it has been observed that E-Citral and Z-Citral have antibacterial properties against gram negative bacteria (like *S. aureus*) and gram positive bacteria (like *E. coli*) bacteria (Shi et al., 2016).

The results of research from Nuraeni and Yunilawati (2012) stated that the components contained in *temu giring* essential oil are acetophenone (18.93%); camphor (17.89%); camphene (8.39%); epicurzereno (8.16%); β-elemene (6.75%); germacrone (6.38%); 1,8-cineole (6.09%); α-guaiene (4.10%); and curzerene (0.55%). 1,8-cineole (17.63%) is contributed by the essential oil of *temu giring.* Research by Li et al. (2014) revealed that 1,8-cineole has strong antibacterial activity due to its hydrophobicity which causes cell wall and cell membrane rupture. 1,8-cineol is a cyclic ether compound belonging to the group of oxygenated monoterpenes hydrocarbons, with the characteristics of being fresh and having a camphor aroma, spicy taste, and antibacterial properties, so it is widely used as a mixture of medicinal ingredients such as nasal sprays and disinfectants (Sikawin et al., 2018). Based on the literature, it can be concluded that E-citral, Z-citral, and 1,8-cineole contained in hand sanitizer containing essential oils of lemongrass and *temu giring* are antibacterial. The antibacterial mechanism of the compounds E-citral, Z-citral and 1,8-cineole in hand sanitizers works through the inhibition of bacterial cell membrane function which causes damage to the cell membrane and, ultimately, death of the bacteria (Nurhedian et al., 2023).

CONCLUSIONS

The study resulted in the hand sanitizer with the optimum formulation containing the essential oils of lemongrass (3%) and *temu giring* (3%), demonstrating very strong antibacterial bioactivities against both *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, with inhibition zones measuring 29.70 mm and 60.08 mm, respectively. This hand sanitizer had a distinctive aroma, combining the scents of lemongrass and *temu giring*. It was in liquid form, clear, and homogeneous, with pH and spreadibility values of 5.4 and 6.4 cm, respectively. The quality complied with SNI quality standards No. 06-2588. The primary active ingredients were E-citral (22.10%), Z-citral (17.88%), and 1,8-cineol (17.63%).

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