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Antibacterial Activity of Laja Gowah (*Alpinia malaccensis* (Brum.f) Roscoe) Oil in Reducing the Number of *Staphylococcus aureus* Colonies in Hospital Wards

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ABSTRACT

Staphylococcus aureus is one of the pathogenic bacteria that cause nosocomial infections in hospitals. The use of essential oil-based antibacterials for hospital wards was developed to prevent nosocomial infections. Laja gowah oil is an essential oil isolated from the *Alpinia malaccensis* plant, which belongs to the Zingiberaceae plant group. The purpose of this study was to determine the antibacterial activity of laja gowah oil in reducing the number of *S. aureus* colonies in hospital inpatient rooms through the air diffusion method. The compound content in laja gowah oil was analyzed with GC-MS. The study of antibacterial activity using the total plant counts continued with the colony count test, Gram staining, catalase test, and coagulase test. Laja gowah oil contains methyl cinnamate and 1,8-cineole. The installation time of the diffuser containing laja gowah oil affects the number of colonies of *S. aureus* bacteria in hospital wards. The antibacterial activity of laja gowah oil was influenced by the synergistic effect of methyl cinnamate and 1,8-cineole compounds.

Keywords: laja gowah; Staphylococcus aureus; nosocomial infection; hospital wards; diffuser essential oil

INTRODUCTION

The hospital is a community health service center providing comprehensive services, healing disease (curative), and disease prevention (preventive). The treatment room is a space for patients who need continuous care and nursing services and treatment for more than 24 hours (Kemenkes, 2016). The hospital environment has a significant influence on the patient's healing process. Unclean environmental conditions can be an excellent place for breeding disease vectors and can be a factor in the occurrence of nosocomial infections (Hidayati *et al*., 2017).

Nosocomial infection is an infection that develops in a hospital environment (Guo & Li, 2019). Nosocomial infections are among the biggest causes of death in hospital treatment patients, especially in the third class of hospital wards, which have a crowded home environment. The activity of moving patients from one unit to another is mainly done in that place. The percentage of nosocomial infections in the world's hospitals reaches 9% or more than 1.4 million hospitalized patients worldwide. The causes of nosocomial infections include the use of medical equipment, treatment items, aids for defecation and urination, as well as through air circulation (Khan *et al*., 2017). Nosocomial infections can occur in patients, health workers, And hospital visitors. This infection can be transmitted through staff health, sick people, visitors with status career, or hospital conditions.

One of the causes of nosocomial infections in hospital inpatient rooms is *Staphylococcus aureus* (Horváth *et al*., 2016). Some infections caused by the pathogenic bacterium *S. aureus* are associated with mortality rates comparable to those caused by HIV/AIDS, tuberculosis, and viral hepatitis. (Coté *et al*., 2016). Bazargani & Rohloff (2016) reported that the infection caused by *Methicillin-Resistant Staphylococcus aureus* (MRSA) is relatively high; there are about 14 million health cases for the skin.

In most cases of infection due to pathogenic bacteria, the spread is through air media (airborne). Antibacterial materials need to be used to suppress the spread of pathogenic bacteria in the air in hospital inpatient rooms. Handling to suppress the spread of pathogenic bacteria in hospital wards has been carried out by cleaning the floor with carbolic fluid and spraying a disinfectant with an active ingredient of sodium hypochlorite. Most floor cleaners and disinfectants contain chlorine which is corrosive and has a long-term adverse effect on the respiratory tract (Clausen *et al*., 2020).

Therefore, other methods are needed to use antibacterial ingredients, one of which is by using essential oils that can suppress the growth of pathogenic bacteria. One method of distributing essential oil molecules as antibacterial in the air is the air diffusion method by utilizing a humidifier

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equipped with an ultrasonic diffuser. The humidifier is a device that functions as an air humidifier that can increase humidity by spraying water vapor into the air. The water vapor that is sprayed can bind to bacteria and viruses in the air. A humidifier equipped with an ultrasonic diffuser can break down essential oil molecules into small particles due to ultrasonic vibrations, which are then diffused through water vapor (Hugentober, 2018). Nisyak & Hartiningsih (2020) reported that using a diffuser in the distribution of fennel oil can suppress the growth of *S. aureus* up to 68.2% within 48 hours.

The essential oil used in the study was laja gowah (*Alpinia malaccensis* (Brum.f) Roscoe) oil. Laja gowah or forest galangal (*Alpinia malaccensis* (Burm.f.) Roscoe) is a plant from the Zingiberaceae family related to white galangal and red galangal. Laja gowah has been used as a medicinal plant by the people of Indonesia. The rhizome of laja gowah is used as a medicine for ulcers, wound care, and stomachache. Laja gowah oil was isolated from the rhizome of the laja gowah plant through the steam distillation method. Laja gowah oil is one of Indonesia's newly developed essential oil commodities with promising business prospects. The main content of laja gowah essential oil is methyl cinnamate, cineol, and α-pinene (Riyanto *et al*., 2012). Laja gowah oil is reported to have antibacterial activity against Gram-negative and Gram-positive bacteria (Sitorus & Satria, 2016). The antibacterial activity of laja gowah is associated with the content of methyl cinnamate compounds. Methyl cinnamate is an ester group compound of cinnamic acid with the formula $C_{10}H_{10}O_2$, which has a benzene ring and a double bond in its structure. (Ernawati *et al*., 2016). Methyl cinnamate is a derivative of cinnamic acid, was widely used in the cosmetic industry (Padalia *et al*., 2017). In this study, the antibacterial activity of laja gowah oil was tested against the spread of *S. aureus* bacteria in the hospital wards using the air diffusion method.

METHODOLOGY

Materials

The research was conducted in the third class hospital wards at Rumah Sakit Umum Anwar Medika Sidoarjo, Microbiology Laboratory in STIKES Rumah Sakit Anwar Medika and Instrumentation Laboratory of Brawijaya University Malang in May – August 2019. The materials used include laja gowah oil (Galanga Oil pure grade 100%, PT. Nusaroma Essential Indonesia), Mannitol Salt Agar (Merck), distilled water, Lugol's reagent, safranin, and crystal violet. The instruments used include Gas Chromatography-Mass Spectrophotometer (GCMS QP 2010, Shimadzu), autoclave (GEA), incubator (Memmert), and essential oil diffuser.

Methods

Laja Gowah Oil Characterization

Laja gowah oil was analyzed for its chemical compound content using GC-MS to obtain information on the content of chemical compounds and their composition through the chromatogram pattern and mass spectrum of each peak. The column used for essential oil analysis by GC-MS is the Rtx-5MS column (5% diphenyl – 95% dimethyl polysiloxane) which has low polarity with N2 carrier gas.

Identification of *S. aureus* in third class hospital wards

The inpatient room for air sampling is 5 m x 7 m (35 m^2), with five patient beds. The room used uses a fan, has two windows (0.75 m x 1 m), has one bathroom, the room temperature reaches 31 C, and the humidity is 71%. Examination of the total number of bacteria in the room was carried out by the Total Plate Count method using Nutrient Agar (NA) media (Wikansari, 2012). The plates were placed in a placement point with an open condition for 30 minutes and incubated for 24 hours at 37°C. The results of the calculation of bacterial colonies with colony counters were analyzed descriptively and tabulated. Analysis of the number of bacteria follows the following equation (Tselebonis *et al*., 2020).

$$\text{Total bacteria count} = \frac{\text{total of colonies in all Petri dishes}}{\text{total of Petri dishes}} \text{ x CFU/m}^3 \quad (1)$$

Initial identification of *S. aureus* bacteria in third class inpatient room was carried out using the Total Plate Count method following the *S. aureus* test protocol in SNI 2332.9:2011(Imaniar, Apriliana, & Prambudi, 2011). The media used was Mannitol Salt Agar (MSA) which is a selective medium for *S. aureus*. A total of 15 mL of MSA media solidified in a petri dish was placed in a placement point with an open condition for 30 minutes. Figure 1 shows the point of placing the petri dish in the room. The number of Petri dishes placed is 15 plates; at each point of air sampling, there are three plates. After 30 minutes, the Petri dishes were closed and incubated for 24 hours at 37°C.

A quantitative test was carried out in the next step by counting the colonies using the Colony Counter. Colonies were taken for qualitative test using Gram staining method by adding crystal

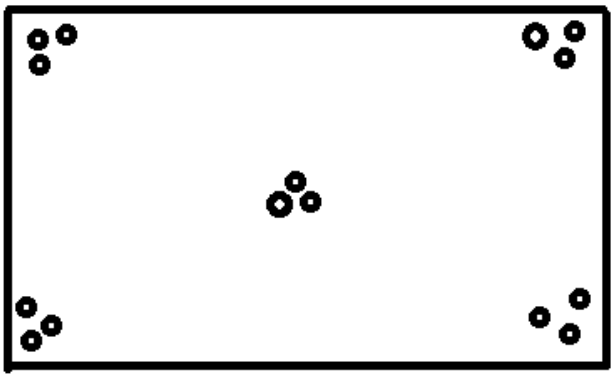

Figure 1. Petri dish placement point

violet and allowed to stand for 2 minutes, then rinsed with running water. The next step is adding Lugol solution, standing for 1 minute, and rinsing with running water. Then the preparation is rinsed with alcohol for 10-20 seconds and rinsed with running water. The last step was the addition of safranin and left for 1 minute, then rinsed with running water. The slide is ready to be identified with a low magnification microscope. The magnification was carried out gradually until a purple cocci form was obtained, which indicated Gram-positive bacteria.

The following identification is the catalase test is carried out by taking a few colonies from MSA media and placing the colonies on a slide dripped with H_2O_2. The presence of air bubbles in the bacterial colony isolates indicates that the bacterial colonies include *Staphylococcus* sp., if no air bubbles appear, it indicates the *Streptococcus* sp. The last identification was a coagulase test with plasma citrate, where the object-glass was given one drop of plasma citrate and sodium citrate and 0.9% sodium citrate, then took the colonies that grew on MSA media with one needle and homogenized. The presence of lumps on the slide shows that the isolate of the bacterial colony is *S. aureus*. The quantitative and qualitative tests were used to ensure that the isolates of bacterial colonies obtained from air caught in the hospital wards were *S. aureus* bacteria. The results of the calculation of *S. aureus* bacterial colonies with colony counters were analyzed descriptively and tabulated. Calculation of the number of *S. aureus* bacteria follows the following equation (Tselebonis *et al*., 2020).

$$S.aureus \text{ bacteria count} = \frac{\text{a total of } S.aureus \text{ colonies in all Petri dishes}}{\text{total of Petri dishes}} \text{ x CFU/m}^3 \quad (2)$$

Laja Gowah Oil Antibacterial Activity Test

A total of 1 mL of laja gowah oil used as a test material was inserted into the essential oil diffuser set, which already contained 400 mL of distilled water and was connected to a power source. Every 6 hours, distilled water was replaced, and essential oil was added. The diffuser that is ready to be placed in the corner of the inpatient room is on. Bacterial sampling was carried out at the 6th, 12th, 24th, 36th, and 48th hours. The method of analyzing the *S. aureus* bacteria present in the room was carried out by the total plate count method using the same procedure as the initial identification of *S. aureus* in the inpatient room. Petri dishes that already contain MSA media are placed in petri dish placement point (Figure 1), and the position of the essential oil diffuser is placed on top of the patient's cupboard, which is located in the corner of the room. The decrease in the number of *S. aureus* colonies was calculated based on the following equation (Nisyak & Hartiningsih, 2020):

$$\text{Decrease in } S.aureus \text{ colonies (\%)} = \frac{\text{Xo}-\text{Xt}}{\text{Xo}} \text{ x } 100\% \quad (3)$$

Where X_o is the initial number of *S. aureus* colonies, and X_t is the number of *S. aureus* colonies at the time of observation.

Data Analysis

The data obtained from the study include GC-MS chromatogram data and identification data of *S. aureus* bacteria. In the GC-MS chromatogram data, it can be seen that the chemical compound content and levels in laja gowah oil by matching the fragmentation pattern, where the fragmentation pattern of the compound with a Similarity Index (SI) value> 90, is suspected to be the chromatogram of the volatile oil content compound. The identification data of *S. aureus*

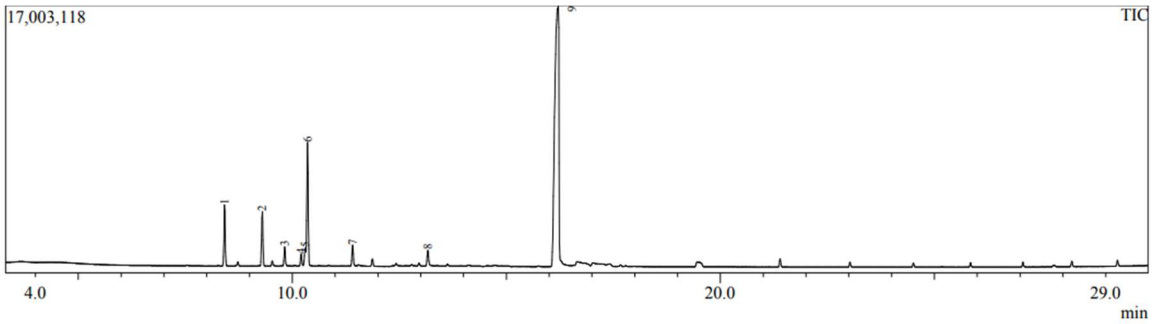

Figure 2. Total Ionic Chromatogram of Laja Gowah Oil

Table I. Chemical Compounds of Laja Gowah oil

No	Compound	yield (%)*	SI
1.	Alpha-pinene	5,14	98
2.	Beta-pinene	4,84	97
3.	phellandrene	1,58	96
4.	o-cymene	1,01	96
5.	limonene	0,60	91
6.	1,8-cineole	9,69	96
7.	fenchone	1,72	96
8.	Linalyl propionate	1,47	95
9.	Methyl cinnamate	73,94	97

* area on the Laja Gowah oil GC chromatogram

bacteria with installing a diffuser containing laja gowah oil on the number of *S. aureus* colonies were analyzed statistically using SPSS 21 software with linear regression test.

RESULT AND DISCUSSION

Chemical Compounds of Laja Gowah Oil

Laja gowah oil was used as an antibacterial agent and analyzed for its chemical compounds using GC-MS. Based on the results of the GC-MS analysis, laja gowah oil contains nine chemical compounds, as shown in the chromatogram (Figure 2). The content of chemical compounds and their compositions is shown in Table I, where information on the types of compounds is identified from the mass spectrum pattern. The mass spectrum pattern of the main compounds found in laja gowah oil is methyl cinnamate as much as 73.94% (peak number 9) and 1.8-cineole as much as 9.69% (peak number 6). In this study, the methyl cinnamate content obtained was lower than that of Riyanto (2012), 80.86%.

Identification of *S. aureus* Bacteria in The Third Class Hospital Wards

The condition of the inpatient room does not meet the standards set by the Decree of the Minister of Health of the Republic of Indonesia No. 1204/MENKES/SK/X/2004, where the temperature reaches 31 ºC (above the range 24 - 26 ºC) and the humidity is 71% (above the range 45 – 60%). Based on the calculation of microbial load in the room, the number shows 1210 CFU/m^3; this value exceeds the standard threshold for air quality hospital wards (above the range 200-500 CFU/m^3). The identification of *S. aureus* bacteria in the third-class hospital wards was made using selective media, Mannitol Salt Agar (MSA). Colonies growing on MSA media were round, smooth, prominent, and shiny. A yellow zone surrounds colonies growing on yellowish white due to fermenting mannitol (Boyd dan Morr, 2014).

Based on Gram staining results, bacterial isolates from the third-class hospital ward that grew in MSA media could include *Staphylococcus aureus* bacteria with Gram-positive coccus results, shown in Figure 3. *Staphylococcus aureus* is a Gram-positive bacterium and cocci-shaped, producing a purple color on Gram stain. The purple color is caused by the bacteria retaining the first color, which is crystal violet.

The function of the catalase test on coccus-shaped bacteria is to distinguish between *Staphylococcus* and *Streptococcus*, where the *Staphylococcus* group is catalase positive. Catalase is an enzyme that catalyzes the breakdown of

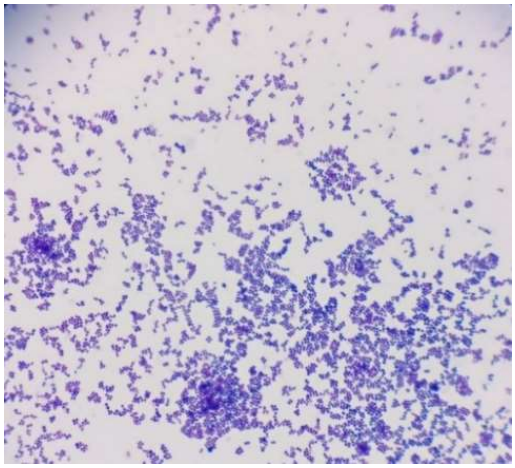

Figure 3. Gram stain of bacterial isolates from the inpatient room (10x100 magnification)

Figure 4. Positive catalase test results for bacterial isolates (the presence of oxygen gas bubbles in both spheres)

Figure 5. Coagulase test results (A) are positive and (B) are negative

hydrogen peroxide into H_2O_2 and O_2. Hydrogen peroxide is toxic to cells because it inactivates enzymes in cells. Hydrogen peroxide is formed during aerobic metabolism, so microorganisms growing in an aerobic environment must decompose the material. The catalase test carried out by bacterial isolates from the inpatient room showed a positive reaction (Figure 4), so it can be concluded that the bacterial isolates were a group of *Staphylococcus* bacteria.

The coagulase test aims to determine the ability of bacteria to produce coagulase enzymes. Coagulase production is the most commonly used criterion for the identification of *Staphylococcus aureus*. A positive coagulase reaction is essential to distinguish *Staphylococcus aureus* from other *Staphylococcus* species. Based on the coagulase test results that have been carried out, the bacterial isolates showed a positive coagulase reaction as shown in Figure 5, which indicates that the bacterial isolates were *Staphylococcus aureus* bacteria.

Antibacterial activity of Laja Gowah Oil

The observation data on the number of colonies of *S. aureus* bacteria after treatment with essential oils are presented in Table II. Based on the data on the number of colonies in Table II, it can be observed that the number of colonies of *S. aureus* bacteria decreased from time to time when the diffuser was installed. The decrease in *S. aureus* colonies by laja gowah oil can be seen

Table II. The effect of installing a diffuser on the microbial load and the number of *S. aureus* colonies

Diffuser Installation Time (h)	Microbial load (CFU/m³)	Number of SA Bacterial Colonies (CFU/m³)	Decrease in the number of SA colonies (%)
control	1210	32 (±0.82)	0
6	985	27 (±0.47)	16
12	780	21 (±0.47)	34
24	634	18 (±0.47)	44
36	585	15 (±0.82)	53
48	390	9 (±0.82)	72

Table III. The results of data analysis on antibacterial activity of Laja gowah oil

Test — **Result**

Normality

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Unstandardized Residual	.228	6	.200*	.949	6	.733

*. This is a lower bound of the true significance; a. Lilliefors Significance Correction

Linear regression

ANVOA

Model	Sum of Squares	df	Mean Square	F	Sig.
1	Regression	335.878	1	335.878	68.712
	Residual	19.553	4	4.888	
	Total	355.431	5		

a. Dependent Variable: number of S. aureus colonies

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.972[a]	.945	.931	2.21093

a. Predictors: (Constant), time of diffuser installation (h)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	29.640	1.441		20.575	.000
	Tim of diffuser installation	-.443	.053	-.972	-8.289	.001

a. Dependent Variable: number of S. aureus colonies

from statistical analysis results using the SPSS 21 program with a simple linear regression test. A linear regression test was used to determine whether or not the installation time of an essential oil diffuser containing Laja Gowah oil on the number of *S. aureus* colonies. Before the linear regression test, the data were tested for normality using the Shapiro-Wilk method because samples were <50 (Suyono, 2015). The normality test results can be presented in Table III, where the significance value obtained is 0.733 (sig > 0.05), so it can be interpreted that the data is normally distributed.

The results of the linear regression test show that the calculated F value is 68.712 while the F table value is 6.61 so that the calculated F value > F table with a significance level of 0.001 (sig < 0.05), which means that the installation time of a diffuser containing laja gowah oil (X) effects on the number of *S. aureus* colonies (Y). The correlation

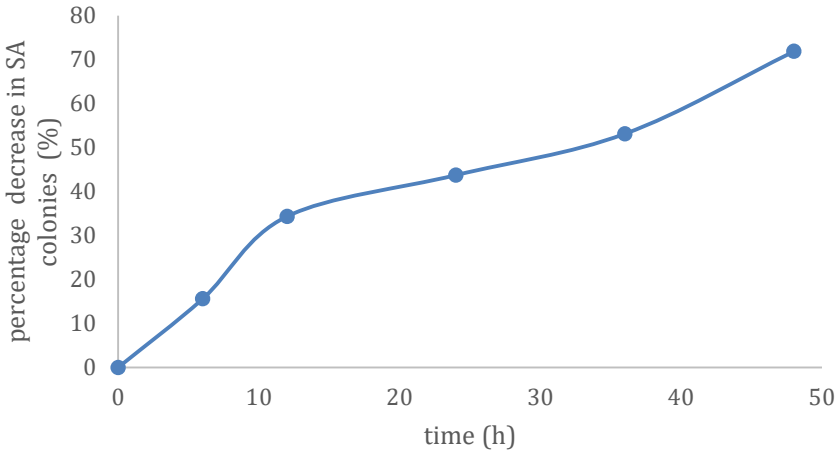

Figure 6. The relationship between the time of installation of the EO diffuser on the decrease in *S. aureus* colonies

value (R) obtained is 0.972, so that the strength of the effect between the length of installation of a diffuser containing laja gowah oil (X) on the number of *S. aureus* colonies (Y) is included in the strong category (0.80-1,000). The value of the coefficient of determination (R2) of 0.945 shows the effect of the installation time of a diffuser containing laja gowah oil (X) on the number of *S. aureus* colonies (Y) of 95.3%.

Based on the linear regression test, the constant (a) value of 29.64 was obtained while the treatment value of the diffuser installation time (b) was -0.443 so that the regression equation could be written as y = 29.64 - 0.443x. The regression coefficient value is negative, indicating that the length of installation of the Laja Gowah oil diffuser harms the number of *S. aureus* colonies, where the more extended the installation time of the diffuser containing Laja Gowah oil (X), the lower the number of *S. aureus* (Y) bacterial colonies. In the use of laja gowah oil (Figure 6), at the 6th hour, there was a 16% decrease in the number of *S. aureus* colonies. The decrease in the number of *S. aureus* colonies increased by increasing the installation time of the essential oil diffuser.

S. aureus is a Gram-positive bacteria that have a thick layer of peptidoglycan. Gram-positive bacteria are more sensitive to essential oils than gram-negative bacteria because of differences in the cell envelope structure (Ebani *et al*., 2017). The cell wall structure of Gram-positive bacteria is more straightforward, single-layered with low lipid content (1-4%), making it easier for antibacterial agents to enter the cell. This interaction is indicated to be the cause of antibacterial ingredients such as essential oils that can easily cause cytoplasmic leakage and coagulation, causing cell death of *S. aureus* bacteria.

The activity of laja gowah oil as an antibacterial agent is determined by the components of its compounds, namely methyl cinnamate, and 1,8-cineole. Methyl cinnamate belongs to the phenylpropanoid group and is an ester of cinnamic acid. Methyl cinnamate is reported to have broad-spectrum antimicrobial activity, working effectively against Gram-negative bacteria, Gram-positive bacteria, and fungi. Methyl cinnamate has been reported to inhibit bacterial cell proliferation (Huang *et al*., 2009). Ernawati *et al*. (2016) reported that pure methyl cinnamate compounds were less effective in inhibiting the growth of *S. aureus* in vitro. The activity shows a synergistic effect between the compounds found in Laja Gowah oil as an antibacterial. Sitorus & Satria (2016) reported that laja gowah oil effectively inhibited the growth of *S. aureus* bacteria.

Another compound that is thought to have antibacterial activity in laja gowah oil is 1,8-cineole. 1,8-cineole is a terpenoid compound (monoterpene) that is also widely found in the Eucalyptus genus. (Li *et al*., 2014) reported that 1,8-cineole could induce cytoplasmic leakage, which caused bacterial cell death so that its antibacterial activity was intense. Hendry *et al*. (2009) reported that pure 1,8-cineole had the vigorous growth-inhibiting activity of *S. aureus*, but the antibacterial activity was more potent when 1,8-cineole was tested with other compounds in essential oils.

Based on the research results, the synergistic effect between the compounds in laja gowah oil, used as an antibacterial, can reduce the

number of *S. aureus* colonies found in the third-class hospital wards. Another factor that affects the decrease in the number of *S. aureus* colonies is the circulation and humidity of the air in the inpatient room. In addition, the parameters that affect the antibacterial activity test results of Laja Gowah oil on the air quality of the inpatient room include access to and from patients and visitors, the number of patients with different diseases, and the cleanliness of the bathroom in the room.

The use of an essential oil diffuser also helps the distribution of essential oil particles throughout the room. The ultrasonic waves in the essential oil diffuser can break down the essential oil molecules into small particles due to ultrasonic vibrations, which are then diffused through water vapor. Water vapor containing essential oils, when sprayed, can bind bacteria and viruses in the air (Hugentober, 2018). The use of laja gowah oil as an antibacterial in the inpatient room needs further research on its side effects on the patient's healing process and safety.

CONCLUSION

Using laja gowah oil as an antibacterial agent vaporized through a diffuser can reduce the number of *S. aureus* bacteria colonies in the third-class hospital wards. The installation time of the diffuser containing laja gowah oil affects the number of colonies of *S. aureus* bacteria, where the more extended the installation time of the diffuser causes a decrease in the number of colonies of *S. aureus* bacteria in the third class of hospital wards. The antibacterial activity of laja gowah oil was influenced by the synergistic effect of methyl cinnamate and 1,8-cineole compounds.

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