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EFFECT OF LIQUID SMOKE FROM PYROLYSIS OF DURIAN SKIN AND ETANOL EXTRACT OF ORANGE PEEL AS BIO HAND SANITIZER

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Abstract

Liquid smoke resulting from pyrolysis of durian skin has antibacterial potential which is used as a basic ingredient for making bio hand sanitizer. Durian skin is difficult to degrade because it contains high levels of lignin, cellulose, and hemicellulose compounds, so with the use of durian skin, it is expert that it will be able to reduce durian skin waste. Bio hand sanitizer is formulated with essential oils to reduce the pungent aroma of smoke. This study aims to determine the antibacterial activity and characteristics of the durian skin liquid smoke bio hand sanitizer. This study used the Completely Randomized Design (CRD) method, with a comparison between durian peel liquid smoke and orange peel extract and the addition of 10 mL NaOH (1:3, 1:1,57, 1:1, and 3:1). The results of research based on characteristic tests obtained pH values of 4.81-7.36, viscosity 1392-3664 cps. Formula E (3:1) emerged as the best sample through organoleptic tests on each bio hand sanitizer preparation formula. It exhibited a yellow color, a runny texture, and a smoky aroma with the mixture. The antibacterial test demonstrated that the bio hand sanitizer preparation possessed antibacterial activity, and the resistance diameter for the test bacteria S. Aureus and E. Coli ranged from 21.51 to 31.14 mm.

INTRODUCTION

According to WHO data and through research hands contain between 39,000 to 460,000 CFU / cm^2 bacteria [1]. In daily life, hands are often contaminated by very small microbes and viruses, so they can be a medium for bacteria and viruses to enter the body. One of the efforts to prevent infection through hands is the use of *hand sanitizers*. The use of *hand sanitizers* is considered more effective, because it does not require water and soap, with antibacterial abilities in inhibiting or killing microbes. Generally, *hand sanitizers* are made of materials that contain alcohol. However, alcohol used in excess can result in skin irritation. Therefore, other safer materials are needed.

The need for alternative raw materials is possibly used as an antiseptic, especially hand sanitizer other than alcohol. One of the finished ingredients considered is liquid smoke [2].

In general, liquid smoke is a result of distillation/separation or condensation (condensation) of combustion vapors biomass through the pyrolysis process. This burning is carried out either indirectly or directly from materials that contain a lot of carbon and other compounds. Liquid smoke is a product produced through the condensation process from the combustion of raw materials containing lignin, cellulose, and hemicellulose. One of the ingredients that can be processed into liquid smoke is durian skin because it contains high lignocellulose components. The use of durian skin as a raw material in making *hand sanitizers* is expected to reduce the amount of durian waste.

Pyrolysis is a thermal process at high temperatures without the presence of oxygen by decomposing organic materials [3]. The liquid used to heat this product is obtained by condensing the steam resulting from pyrolysis in a protective container. The liquid resulting from pyrolysis is known as bio-oil, tar, bio-crude, liquid smoke, wood oil, or wood distillate. The characteristic of this liquid smoke is that it is a blackish-brown liquid [4]

Several studies related to liquid smoke. The pyrolysis results of palm shell liquid smoke obtained a yield of 30%, acetic acid content of 4.8%, phenol of 18.4%, and pH ranging from 2.2-2.4 [5]. At a liquid smoke concentration of 25%, it can inhibit the growth of *Bacillus substillis* bacteria with an inhibitory diameter of 6.5 mm. Bacterial *S.*

aureus with an inhibitory diameter of 5.6 mm. Bacteria *E.coli* with an inhibitory diameter of 5.5 mm.

Acetic acid is the main component of liquid smoke biomass from eucalyptus (45.35%) and teak wood biomass (25.35%) [6]. In addition to acetic acid, phenol is also contained in both liquid smokes. Liquid smoke from eucalyptus contains 6.53% phenol, and liquid smoke from teak wood biomass contains 11.19% phenol. The high content of phenol and acetic acid in teak wood liquid smoke is thought to affect its ability to inhibit bacterial growth.

Liquid smoke contains phenol, carbonyl, and carboxylic acid compounds that have antibacterial properties, so it is very effective to use as raw material in the manufacture of *hand sanitizers* [7]. However, liquid smoke has a strong/sharp aroma and a charred and sour smell. So other additional ingredients are needed to reduce the aroma.

The use of orange peel essential oil is expected to reduce the pungent aroma of liquid smoke because orange peel has a distinctive fragrant aroma. In addition, orange peels contain phenolic compounds (flavonoids and tannins) that have pharmacological activity as antibacterial [8].

Based on the explanation of the above problems, it is necessary to conduct a study on the effect of bio hand sanitizer on characteristics or the preparation and antibacterial activity of *bio hand sanitizer* from durian skin liquid smoke through pH, viscosity, organoleptic, GC-MS, and diameter resistance (DDH) tests.

EXPERIMENT

This research was conducted at the Waste Processing Technology Laboratory, Chemical Engineering Study Program, Faculty of Industrial Technology, for 5 months.

Material

The materials used in this study were waste from durian monthong peel, mandarin orange peel, 98% ethanol, NaOH, *aquadest*, LPG gas, *paper disc*, Nutrient Agar (NA), and S bacteria isolate. *Aureus* and E., filter paper, *tissue, market hand sanitizer* (control), and *Aluminum foil.*

Instrumentation

The main equipment used in this study is a pyrolysis reactor, distillation, and goblet glass as a container for the maceration process (**Figure 1**).

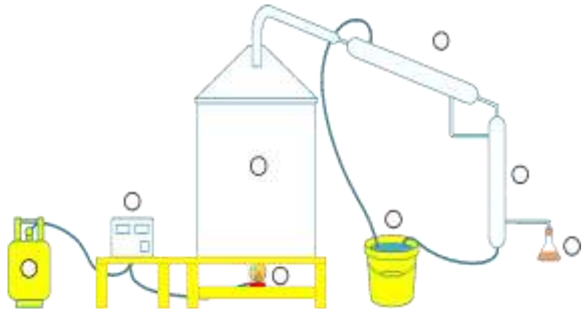

Figure 1. Pyrolysis tools services (reactor, LPG gas cylinder, thermocouple reader, refrigeration, first condenser, second condenser, and erlenmeyer, stove).

Procedure

Liquid Smoke Making

Durian skin is cut into pieces until it reaches a size of ±1-5 cm and then dried by drying in the sun for a day. Durian skin is placed into the pyrolysis tool as much as 2 kg, after the tool is tightly closed, pyrolysis is carried out for 8 hours with a temperature of 350°C. Pyrolysis products in the form of liquid smoke are placed in a container and then allowed to settle tar for 24 hours. The solid black liquid smoke is distilled at a temperature of 105ºC. The resulting product is then re-distilled to produce *grade* 1 liquid smoke [9].

Essential oil manufacturing

Orange peel is reduced in size to a size of ± 0.5 cm, then dried in the oven for 15 hours at a temperature of 55oC. Subsequently, it is crushed using a blender. The resulting powdered orange peel is weighed, and approximately 100 g is placed into a closed container, shielded from light. 98% ethanol solvent is added with a ratio of 1: 10 (w / v). Maceration is carried out for 3 cycles of 24 hours each at room temperature. The solution is later filtered using filter paper. The obtained filtrate is then distilled at a temperature of 70ºC until the entire solvent evaporates [10].

Making Bio hand sanitizer

Making a *bio hand sanitizer* formulation with a ratio between durian peel liquid smoke and orange peel extract, Formulation A (1: 3) v/v, Formulation B (1: 1,57), Formulation C (1: 1), Formulation D (1.57: 1) v/v and Formulation E (3: 1) v/v and adding NaOH 10% 10 mL as a *pH adjuster*. Then, the chemical components contained using GC-MS QP.2010S SHIMADZU were analyzed. Bio *hand sanitizer* testing namely pH, viscosity, organoleptic, GC-MS, and antibacterial activity.

pH

To find out the pH value of liquid smoke from durian skin waste, use a pH meter.

Viscosity

Viscosity measurement to determine the viscosity level of a liquid. The tool used is a viscosity meter

Organoleptic

Organoleptic tests are carried out visually on bio hand sanitizer preparations, including color, aroma and texture.

GC-MS

The liquid smoke produced from the process of making activated charcoal is then analyzed to determine the contents contained in it. Liquid smoke analysis was carried out using GC-MS

RESULT AND DISCUSSION

The general characteristic of liquid smoke is that it is a brownish liquid after the pyrolysis process, after the distillation process it will experience a color change in the form of a yellow liquid, which has a characteristic smell of burning smoke.

Acidity Level (pH)

Based on **Figure 2**, the pH of *biohand sanitizer* in formulas A, B, C, D, and E was obtained at 7.36; 6,41; 6,04; 5,58; and 4.81. This shows that *bio hand sanitizers* made from liquid smoke and *essential oils* tend to be acidic (pH < 7).

From these data, it can be seen that the higher the concentration of liquid smoke, the pH of the *bio hand sanitizer* decreases. In making antiseptic gel from liquid smoke from orange peel, there are differences in pH values due to differences in the composition and concentration of the active substances in the antiseptic preparation. [11]. The pH value of *bio hand sanitizer* ranges between 4.54-7.61.

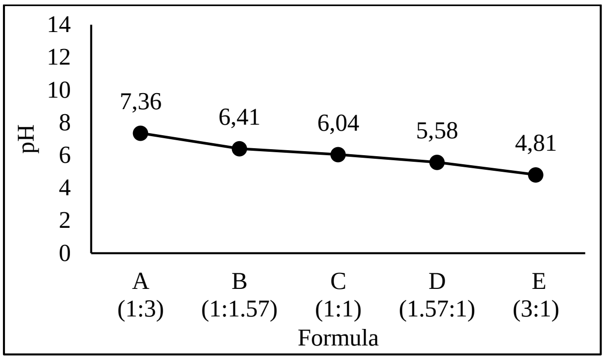

Figure 2. The relationship between formula to pH.

This is influenced by the concentration of active compound components in liquid smoke which is getting higher along with the increasing concentration of liquid smoke. The part of the durian fruit that is more commonly consumed is the flesh. The weight percentage of this part is low, namely only 20% –35%. This means skin (60% – 75%) and seeds (5% –15%). One way to handle durian waste so that it can be utilized is by reprocessing it to make durian skin into a raw material for making activated charcoal and liquid smoke. As biomass, durian skin has a composition of cellulose, hemicellulose, and lignin so it has the potential to be used as raw material for making activated charcoal and liquid smoke by pyrolysis. The main constituent components of durian skin are hemicellulose and cellulose which, when decomposed, produce acidic compounds such as acetic acid.

The addition of essential oils can reduce the acidity level of liquid smoke because it has a pH range of 6-10, so the higher the concentration of *essential oils*, the pH of *bio hand sanitizer* preparations increases. The pH of *hand sanitizers* that are safe for the skin is in the range of 4-10 [12]. This is because hand sanitizers with an excess acidic pH can cause skin irritation, while if the *hand sanitizer* has an alkaline pH it will exfoliate the skin on the hands [13]. The pH of the *bio hand sanitizer* in this study was in the range of 4.81-7.36, which means that it has met the standards that have been set.

Viscosity

Viscosity measurements are carried out on *bio-hand sanitizer* preparations to determine the consistency of these preparations. The results of *bio hand sanitizer* viscosity measurement can be seen in **Figure 3**.

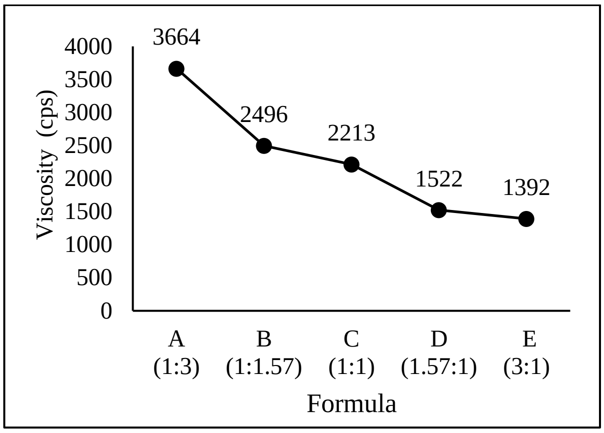

Figure 3. The relationship between formula and viscosity.

The results of bag viscosity measurement can be seen in Figure 3, where viscosity for formulas A, B, C, D, and E is 3664; 2496; 2213; 1522; and 1392 CPS. From these data, it can be seen that the higher the concentration of liquid smoke and the lower the concentration of *essential oil*, the viscosity of *bio hand sanitizer* decreases.

The decrease in viscosity is influenced by the pH of acidic preparations. pH parameters are very influential on physical preparations where the pH of the preparation increases, the viscosity of the preparation will also increase and vice versa [14].

In addition, the decrease in viscosity is also influenced by the presence of a carboxyl group that reacts when adding a neutralizing base (NaOH), where the carboxyl group will bind ions to Na^+ thereby increasing the solubility of *bio hand sanitizer* preparations.

If the pH of the bio hand sanitizer preparation is increased by the addition of a base compound [15], then progressively the carboxyl group will be ionized with the base to increase the solubility of the *bio hand sanitizer* preparation, which causes *viscosity* to decrease. Based on the US Patent, the viscosity standard for *spray gel* preparations is between 500-5000 cps. This shows that the viscosity of *bio hand sanitizer* for each formula in this study has met the standards [16]

Organoleptic

Organoleptic testing was carried out on 30 panelists and given a questionnaire containing an assessment of the interest in organoleptic tests carried out visually on *biohand sanitizer* preparations, including color, aroma, and texture. The assessment results are given a scale of 1-5 as follows: 1 (dislike), 2 (dislike), 3 (neutral), 4 (quite like), and 5 (like). The results of organoleptic tests can be seen in **Figure 4**.

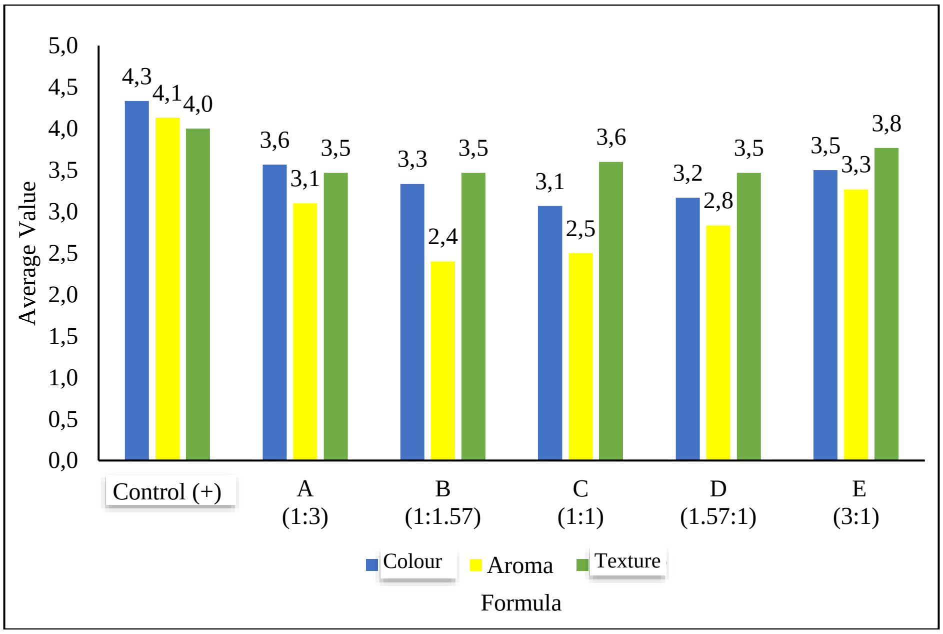

Figure 4. Result organoleptic test of color, aroma, and texture parameters.

The concentration ratio between liquid smoke and *essential oils* influences on panelists' level of preference for the color, aroma, and texture of *bio hand sanitizer*. The higher the concentration of liquid smoke and the lower the concentration of *essential oils*, the color of the *bio hand sanitizer* preparation becomes yellow, and panelists tend to like yellow. The higher the concentration of *essential oils*, the darker the color obtained. The essential oil content in the formulation mixture affects the color characteristics of the formulations, the higher the concentration of the *essential oil*, the darker and thicker the resulting color.

The texture of the *bio hand sanitizer* preparation gets thinner as the concentration of liquid smoke increases and the concentration of *essential oils* decreases and panelists tend to like preparations that have a thin texture. The watery texture makes the bio hand sanitizer preparation more comfortable to use on the skin.

The aroma produced tends to smell of smoke as the concentration of liquid smoke increases, while as the concentration of *essential oils* increases, the aroma of smoke from *bio hand sanitizer* decreases. The distinctive aroma of *limonene* contained in orange peel reduces the smoke aroma from *bio hand sanitizer* preparations. The higher the concentration of *essential oil*, the more concentrated the aroma of *bio hand sanitizer*. The aroma produced is due to the influence of *essential oil* content which makes the aroma distinctive [17]. *Essential oils* are mixtures of various volatile organic compounds that are easily soluble in organic solvents and have a unique aroma depending on the type of plant [18] Based on the assessment given, panelists tend to like *bio hand sanitizer* preparations that produce a weak smoke aroma and moderate *essential oil* aroma.

In **Figure 5**. It can be seen that the *bio hand sanitizer* preparation formula that is most in demand by panelists is formula E (3:1) which ranks 1st. Then rank 2 is formula A (1:3), rank 3 is formula D (1.57:1), rank 4 is formula C (1:1), and rank 5 is formula B (1:1.57).

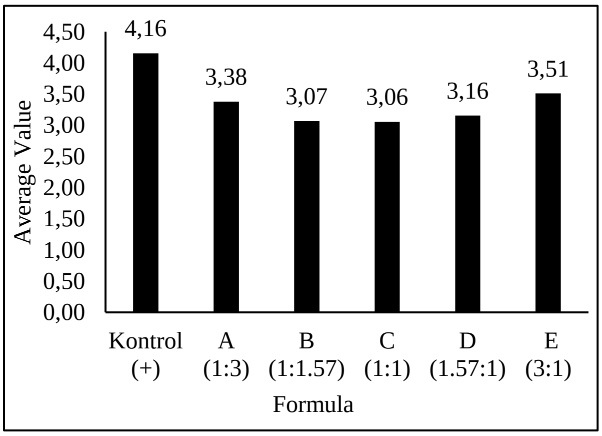

Figure 5. Average value of each *bio hand sanitizer formula.*

The formulation of the best bio hand sanitizer preparation (Formula E) is then compared with hand sanitizer preparations that have been distributed in the market (K+) which have a texture and aroma that resembles the *bio hand sanitizer* formulation made. This assessment was carried out using *Paired Sample T-Test* analysis to determine the level of difference between hand sanitizers made with *hand sanitizers* on the market and obtained the following data (**Table 1**).

Table 1. Paired sample T-test analysis data.

Value	Panelists' Favorability Percentage		t count	t table	P
	K+	E (3:1)			
5	53,3%	23,3%			
4	53,3%	45,3%			
3	35,6%	24,0%	1,2487	2,7764	0,1399
2	0,7%	9,3%			
1	0,0%	0,3%			

Bio hand sanitizer can be compared to *hand sanitizer* preparations on the market with a Paired T-sample Test analysis. In this analysis, there are two hypotheses, namely:

a. $\mu_0 = \mu_1 \leq \mu_2$ (assessment of the level of consumer interest in *bio hand sanitizers* produced from this study and those on the market that do not have a significant difference).
b. $\mu 1 = \mu_1 > \mu_2$ (assessment of the level of consumer interest in bio hand sanitizers resulting from this study and *hand sanitizers* on the market have significant differences) [19].

Based on the test result data above, it can be seen the value of |t calculate| < t *table* or P value > 0.05 so that the hypothesis of μ_0 can be accepted with the statement that the assessment of the level of consumer interest in *bio hand sanitizers* resulting from this study and those on the market does not have a significant difference. This shows that the formulation of *the bio hand sanitizer* preparation has the opportunity to be distributed to the community.

Gas Chromatography Analysis–Mass Spectrometry (GC-MS)

GC-MS analysis was carried out to identify the compounds contained in *bio hand sanitizer* preparations. Based on the results of antibacterial activity testing, formula A (1:3) was obtained as the formula that has the largest Inhibitory Diameter (DDH), so the formula was chosen to be characterized by GC-MS (**Figure 6**).

The compound components contained in *the preparation of bio hand sanitizer*, liquid smoke, durian peel, and *orange peel essential oil* as in **Table 2**.

Table 2. Bio hand *sanitizer* constituent components.

No.	Component	Area (%)
1.	*l-Limonene*	17.10
2.	*2-Pentanon, 3-Methyl- (CAS) 3-Methyl-2-Pentanone*	1.31
3.	*Acetic Acid (CAS) Ethylic Acid*	2.54
4.	*Linalool*	1.29
5.	*Tetracosamethylcyclododecasiloxane*	7.11
6.	*Silana*	4.15
7.	*Octadecamethylcyclononasiloxane*	3.17
8.	*Octadecanoic acid, 2,3-Dihydroxypropyl ester (CAS) 1-Monostearin*	2.19

As shown in **Table 2**, the largest compound found in *bio hand sanitizer* preparations is *Limonene* by 17.10%. *Limonene* is a hydrocarbon in the terpene cycle, which is a liquid that has a distinctive aroma from oranges, therefore it is named *limonene* because it is mostly found in orange peels.

In addition to *limonene*, there is a 2.54% acetic acid compound that has antibacterial properties. Based on research conducted [20], the antibacterial potential of *limonene* was studied using microscopic methods, namely, Scanning Electron Microscopy, Transmission Electron Microscopy, and *Fluorescent Microscopy* which showed membrane disruption, cellular leakage, and E.coli cell death when interacting with *limonene* compounds. Further, the interaction of *limonene* with DNA can lead to the release of plasmids, which can ultimately inhibit DNA transcription and translation.

Other compounds contained in the components of Bio Hand Sanitizier are 31% trans-caryophyllene and 4.03% phenol.

Acetic acid has antibacterial activity through the mechanism of influencing the environment around the growth of these bacteria into acid [21]. The mechanism of antimicrobial inhibition in acetic acid is caused by membrane damage, inhibition of essential metabolic reactions, hemostasis of internal cell pH [22], accumulation of acid residual anions in the cytoplasm that are toxic, disrupt the protein synthesis or genetic system (DNA / RNA synthesis), and microbial death due to ATP depletion.

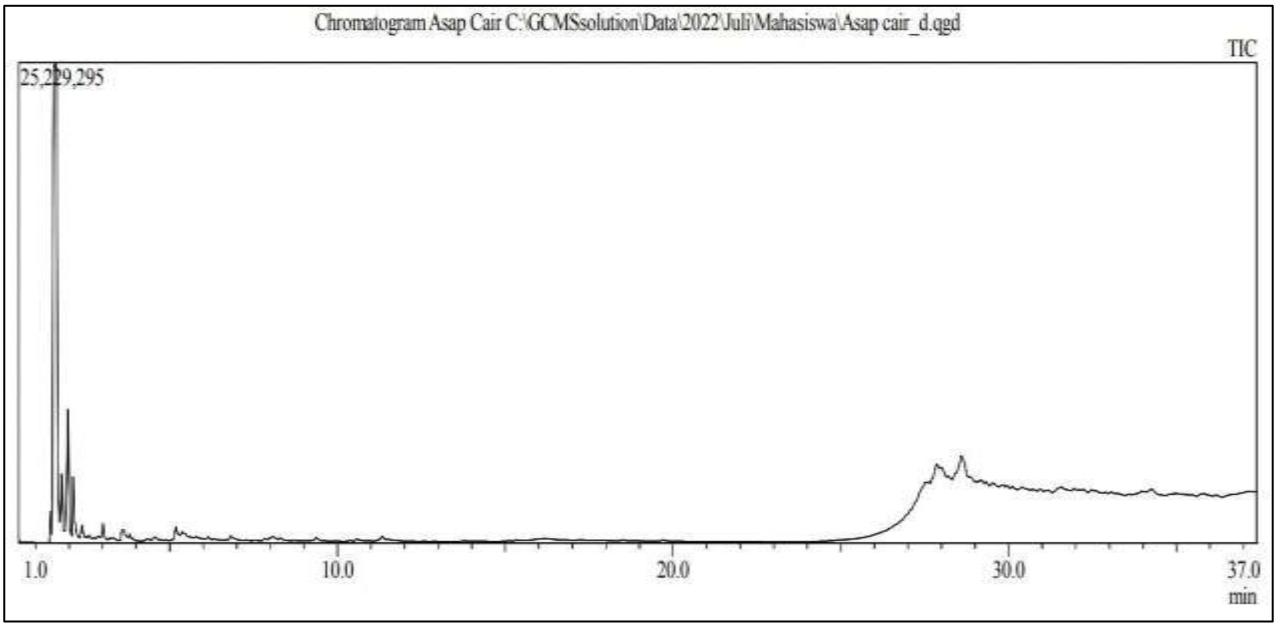

Figure 6. Chromatogram GC-MS *Bio hand sanitizer* preparation.

Antibacterial Activity

Testing the antibacterial activity of *bio hand sanitizer* from durian peel liquid smoke and orange peel *essential oil* against *S. Aureus* and *E. coli* bacteria. Performed using disc diffusion method in petri dish that has contained nutrient agar medium (NA) with 3 times replication. Based on **Table 3**, it can be seen that *bio hand sanitizer* from durian peel liquid smoke and orange peel *essential oil* has antibacterial activity characterized by the formation of clear zones around the disc paper. The size of the diameter of the inhibitory power formed varies, due to the difference in the amount of concentration between liquid smoke and *essential oils*.

Table 3. Average diameter of inhibitory power in S. *Aureus* and *E.Coli*.

Formula (Liquid smoke: Orange Peel Extract)	Ureus *S.Aureus* Inhibitory Zone (mm)	Oil *E.Coli* Inhibitory Zone (mm)
Active Compound (+)	15,20	19,16
A (1:3)	31,14	26,51
B (1:1.57)	28,36	26,39
C (1:1)	27,72	26,33
D (1.57:1)	26,09	25,30
E (3:1)	21,51	24,85

Based on the Resistance Diameter (DDH) formed, essential oils have a greater influence than liquid smoke, this is because there are more antibacterial compounds in essential oils. So the higher the concentration of *essential oil*, the diameter of the inhibitory power formed is greater. Variations in the concentration of Kalamansi orange peel *essential oil* affect the inhibitory zone formed where the higher the concentration, the larger the inhibitory zone formed with significant differences [23]. The increasing amount of *orange peel essential oil* added causes an increase in the content of active compounds that have an antibacterial role so that antibacterial activity will be greater [24]

There is antibacterial activity in liquid smoke bio hand sanitizer preparations and *essential oils* because there are phytochemical compounds in the *bio hand sanitizer* preparations. Liquid smoke contains phenol and acetic acid, while *essential oils* contain terpenoid compounds (*limonene*). The *limonene* compound is more dominant in terms of antibacterial activity. These secondary metabolite compounds will interfere with the constituent components of the cell membrane or cell membrane permeability so that there is a shrinking of the components in the outgoing cell, it can even inhibit the formation of cell membranes and cause inhibition of bacterial growth.

Measurement of bacterial DDH based on both types of bacteria (**Figure 7**) shows that the quality of *bio hand sanitizer* has greater DDH in gram-positive bacteria (*S. Aureus*) than gram-negative bacteria (*E.coli*). This is due to differences in cell walls in both bacteria. Gram-positive bacteria (*S. aureus*) have a simpler cell wall structure consisting of thick peptidoglycan. The thick peptidoglycan layer causes gram-positive bacteria to be more sensitive to antibacterial administration, while gram-negative bacteria (*E.coli*) have a more complex arrangement of bacterial cell walls. The existence of an outer membrane structure in gram-negative bacteria can limit the access of antibacterial compounds into the cell membrane so that bacteria are more resistant to antibacterial. Bacterial cell wall *E.coli* has several polymer layers, namely the outer layer of lipoproteins, the middle layer of lipopolysaccharides, the inner layer of peptidoglycan, and the outer membrane in the form of a *lipid bilayer* that acts as a selective barrier [25].

The diameter of the resistance is determined by measuring the yield of the resistance zone minus the diameter of the paper disc. The diameter of the resulting inhibitory power can be caused by several factors, namely the concentration of bacterial material, the number of bacteria in agar media, and the condition of bacteria [26].

The criteria for Diameter of Inhibitory Power (DDH) are divided into 4 groups, namely very strong if the inhibitory zone > 20 mm, strong 10-20 mm, medium 5-10 mm, and weak < 5 mm [27]. The diameter of the inhibitory power obtained in the *bio hand sanitizer* formula is 21.51-31.14 mm for *S.Aureus* and *E.Coli* test bacteria, DDH is included in the criteria of being very strong because it is greater than 20 mm. Inhibition zone diameter the weak category has a diameter of ≤ 5 m, the medium category has a diameter zone of inhibition approximately between 6-10 mm, and the diameter of the strong inhibition zone approx between 11-20 mm [28].

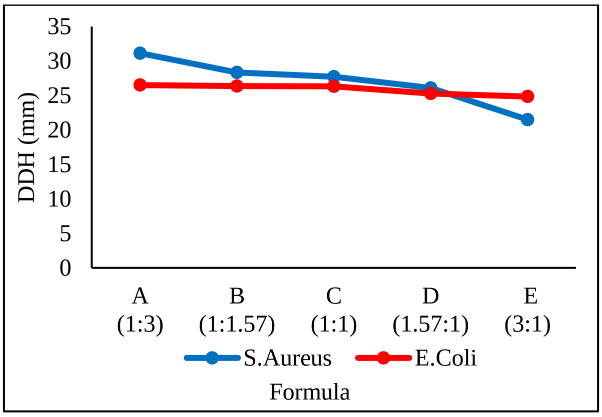

Figure 7. Diameter of inhibitory power in *S.Aureus* Bacteria and *E.Coli*.

CONCLUSION

Based on the dosage characteristics test obtained pH 4.81-7.36, viscosity 1392-3664 cps, organoleptic tests on each *bio hand sanitizer* preparation formula yellow, watery texture and smoke aroma obtained formula E (3: 1) as the best sample. Antibacterial tests show that bio-hand sanitizer preparations have antibacterial activity and obtained an inhibitory diameter in the *bio-hand sanitizer* formula, which is a range of 21.51-31.14 mm for *S.Aureu*s and *E. coli* test bacteria.

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