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Geraniol quality improvement on citronella oil as raw material for making anti-bacterial perfumes

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Geraniol quality improvement on citronella oil as raw material for making anti-bacterial perfumes

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Abstract. In this study, optimization was carried out to increase the levels of geraniol compounds present in citronella oil using vacuum distillation. Geraniol is a compound that has been legalized by IFRA (Fragrance International Research Association) as a scent compound, Geraniol has been studied in addition to having a pleasant fragrance, geraniol is also very effective in inhibiting bacterial growth. So geraniol is a reference to use it as formulated as a raw material for anti-bacterial products, but the levels of geraniol in citronella oil are still very low. This is what encourages the processing of fragrant citronella oil to be processed, by increasing geraniol levels using a vacuum distillation method, with variations in pressure: 8mmHg, 6mmHg, 4mmHg, 1mmHg during the operating time: 20 minutes; 40 minutes; 60 minutes and until Final boiling point. The samples were analysed using GC-MS (Gas Chromatography of Mass Spectrometry, FTIR (Fourier Transform Infra-Red Spectrophotometer), refractive index, and bacterial testing of research products. The results showed that higher temperature usage had an effect on increasing geraniol levels and showed that This vacuum distillation method is quite effective. Geraniol concentrations before and after isolation increased by 38%. namely from 20.32% to 57.39%. This shows that this method is quite effective in increasing geraniol concentrations in fragrant citronella oil. Analysis of FTIR shows 3365.78 cm-1 according to the hydroxyl polymer group (OH).

Keywords. Anti-bacterial perfume; Citronella oil; geraniol; vacuum distillation.

1. Introduction

Consumer demand for safe and non-hazardous perfume is a challenge for the perfume industry. This has led to an increase in the discovery of perfume content by trying to find natural and renewable contaminants. Dermatologists explain that the most common reaction is the use of cosmetics, one of which is perfume, especially perfume [1]. One way that producers do to avoid these allergies is by controlling the concentration and mixture of aroma in perfume and avoiding the activity of bacteria figure 1 show the bacteria causes of S. Aureus and E. Coli body odor.

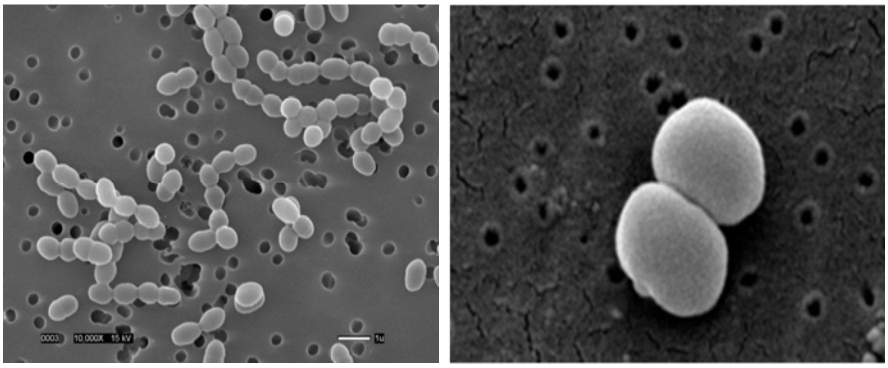


Figure 1. Bacteria causes of S. Aureus and E. Coli body odour.

Perfume is a solution to overcome body odor caused by sweat mixed with bacteria. At present, there are many types of perfumes on the market, but perfumes that have been produced, only prioritize fragrances, where combining these substances can trigger the emergence of allergen compounds. Most perfumes that have been tested, show that the concentration of a perfume is outside the safe limit, so it can potentially cause allergic contact dermatitis [2].

Dermatitis is considered as one of the diseases that cause damage to the skin both permanently and temporarily. Contact that occurs between the skin and contaminants causes an allergic reaction. Dermatologists explain that the most common reactions due to the use of cosmetics, one of which is fragrance [1]. Previous research shows that citronella oil is one component that can prevent the occurrence of allergies, due to the presence of geraniol as an anti-bacterial agent contained in fragrant lemongrass [3]. Geraniol is an alcoholic terpene which has an important role in the perfume industry and is found in various essential oils from several aromatic plants [4]. Antioxidants [5].

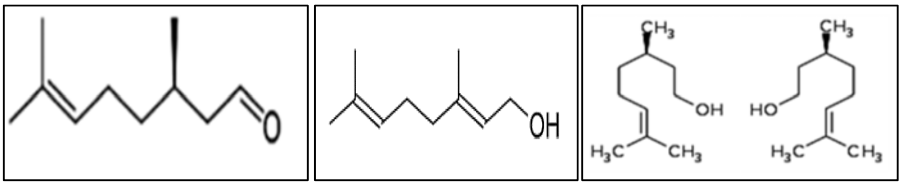
So in this study, synthesis of perfume will be made with alternative ingredients that are safer and more natural, that is by using citronella oil. The essential oils of the fragrant citronella sold in the market will increase the quality of the geraniol substance using the vacuum distillation method, a function of the vacuum distillation itself to prevent damage/removal from geraniols which have a high boiling point. Previous research in increasing geraniol levels has been carried out using the vacuum distillation method by varying temperatures, which at higher temperatures will affect the increase in geraniol [6]. It is known that the boiling point is directly proportional to the pressure, therefore in this study variations in pressure on temperature and short operating times will be carried out [7]. By obtaining high geraniol levels from citronella fragrance (citronella oil) will produce antimicrobial perfumes and antiseptic inhibitors of microorganisms (bacteria

Figure 2. Fragrant lemongrass.

Fragrant lemongrass (as shown in figure 2) is one of the plants that produce essential oils. Essential oil is a basic ingredient of natural fragrance which can be developed into several products such as soap, shampoo, lotion, and perfume. Currently, citronella oil (sample shown in figure 3) is exported abroad to be processed into essential oils and then made into various cosmetics and perfume mixtures, which are then imported back into and abroad to increase foreign exchange. The main components of citronella oil-based on equipment GC / MS analysis of oil extracted using the hydrodistillation method, the results revealed a chromatogram plot obtained about 95% of commercially important compounds namely, citronellal (55.23%), geraniol (26.29%) and citronellol (13.41%), indicating high-quality citronella oil [8].

Figure 3. Citronella essential oil.

There are 3 main components in citronella oil: Citronellal with molecular weight C10H16O and boiling point at 201-207°C, Geraniol with molecular weight C10H18O and boiling point 229-502oC and then Citronellol with molecular weight C10H20O and boiling point at 225°C, 498 K, 437°F. As in figure 4, the following shows the formula for building up of these three compounds [9].



(a) (b) (c)

Figure 4. (a) Citronellal (b) Geraniol and (c) Citronellol.

Geraniol is monoterpenoid & alcohol. Geraniol can also be obtained from rose oil, palmarosa oil, and citronella oil. Geraniol can also be obtained in low amounts in geraniums, lemons, and many other essential oils. Geraniol is also often called rose oil. Geraniol is a pale yellow liquid. It smells pungent and is often used as a perfume. Geraniol content in fragrant citronella oil is 11-15%. Geraniol is also used to attract insects or repel insects because of anti-bacterial & Antiseptic substances.

Researches on geraniol as an anti-bacterial agent have been summarized a collection of research studies from geraniol compounds, it can be concluded that geraniol is one of the most important molecules in the fragrance industry and is used as raw materials in perfume industries [10]. In addition to a pleasant odor, geraniol is known to show insecticide-repelling properties and is used as a natural pest controller that shows low toxicity. Geraniol also plays an important role in the medical world as a compound that contains drugs for anti-tumor, leukemia and hepatoma. Table 1 show the physical properties of Geraniol compound

Table 1. Physical properties of Geraniol compounds.

Properties	Details
Solubility	Not soluble in water
Steam Pressure	0.02mmHg at 20°C
Flash point	> 37°C
Boiling point	229°C–230°C

Another study on geraniol has also been conducted which aims to identify the anti-microbial efficacy of geraniol in interventions in inoculated pathogenic spinach samples stored and cooled at 5°C and then temperature varied at (15°C), (25°C) research time for 10 days. The main purpose of this research is to inhibit or reduce the number of pathogens in inoculants by using geraniol as an anti-bacterial. Based on the observation that geraniol can inhibit the growth of pathogens at a temperature variation (25°C), the application of geraniol can reduce pathogens on the surface of spinach, prevent them from entering the body, and help avoid any health problems [11]. So Geraniol has been suggested to represent a medical solution as a cure for cancer. Benefits of Geraniol in biological activities; anti Insecticide, anthelmintic activities, antimicrobial effects, anti-oxidant effect and anti-dermatitis.

The isolation process here is a separation between citronella oil and geraniol compounds contained in it using a vacuum distillation method, which is a distillation where the pressure is below the atmosphere. The use of vacuum distillation was chosen for strong reasons because of the existence of a physical principle in which liquid will boil below its normal boiling point if the pressure on the surface of the liquid is minimized or vacuumed. Vacuum distillation is usually used when the distilled compound is a mixture that has a high boiling point difference [11]. The geraniol isolation process has been carried out by extracting it using the soxhlet method, but the results obtained are unsatisfactory because there are still citronellal compounds which are included in the geraniol compound due to the very similar similarity between the two compounds. Therefore, in this study, an increase in geraniol (isolation) by using a pressure drop variation which is expected to later reduce the boiling point of the citronellal so that the compound will evaporate first, so only the geraniol compounds left behind in the boiling.

Research on the separation of citronellal and geraniol compounds using vacuum distillation was carried out by varying the pressures covering 5, 10, and 15 bar using reflux ratios aimed at investigating column performance. Vacuum distillation is operated in batch mode to increase the concentration of citrus oil contained in fragrant lemongrass. The results obtained for the concentration of citrus oil produced in citronella at optimal valencene-rich operations were (20.5 % wt) at 10 bar [12]. In this study, the elimination of Limone compounds was carried out using the vacuum method and the results of vacuum performance in the batch mode were said to be satisfactory.

The focus of this study was to improve the quality of geraniol levels in fragrant lemongrass essential oils (Citronella oil) by using a vacuum distillation method to produce geraniol isolates which are effective as anti-bacterial compounds in perfumes.

2. Methods

2.1. Materials

Lemongrass obtained from Aceh Tengah, water, peppermint oil, Potato Dextrose Agar (Merck, Germany), Staphylococcus aureus and Escherichia coli bacteria and 95% alcohol. before the vacuum process.

2.2. Geraniol isolation uses vacuum distillation

Add 200ml of citronella oil into the glass receiver, then enter the boiling stone into the flask (it can be 5-7mm in size), transfer the fragrant oil to the flask, place the flask on the bottom heater (the power cable has not been installed), plug the clamp between the connections and tighten the fastening bolts,

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give a few drops of white oil to the thermometer flask hole, then turn on the bath and set circulator temperature at 30 – 80□, turn on the control system, turn on vacuum pump and wait 1-2mmHg, then check the connections for leaks can be seen from the presence of incoming air bubbles, if there is a tightening connection, after the condenser temperature is reached and the bubbles in the fragrant citronella oil have disappeared there is also no leakage in the connections. After all are sure to safely, turn on the vacuum distillation equipment on the computer, click 'Vacuum Start Run', enter sample data such as sample volume, density, pressure/temperature, then click 'OK'. When it reaches the desired temperature, click 'STOP', so that the compound is protected from cracking process (damage to the compound). Wait until the bottom and distillate are completely at room temperature. The cooling process will be finished after exiting the 'Finish Distillation' button, then take the distillate sample and bottom into the sample bottle. to print the results of the final data analysis [11].

2.3. The process of making perfume
Peppermint oil is used as 15ml of top notes, 25ml of nutmeg oil as 25 notes and geraniol obtained from the isolation of fragrant citronella oil is used as a base note (figure 5), and do not forget that carrier oil, Cusson Baby, functions for the aroma of perfume that will produce later.

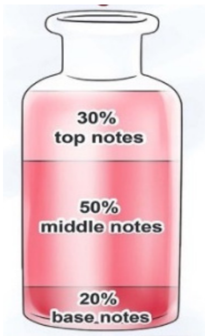

Figure 5. Notes for making perfume.

Making perfume begins by inserting a carrier oil that is 30ml Cusson oil into a 250ml beaker glass, adding 17ml peppermint oil, then adding 25ml of nutmeg oil as a middle note. After that 15ml of selected citronella oil were added which would be high in geraniol compounds and 96% alcohol by 90ml. After all the solutions have been put into the glass beaker, the residence is carried out for 48 hours. The beaker glass is covered in all its surface using aluminum foil. This is intended so that no sunlight will affect the perfume so that it will not cause less perfume from the perfume to be produced [13].

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis
Analysis of citronella samples was carried out by GC-MS gas chromatography (GC-Shimadzu 2010) with auto sampler and ionization detector. GC-MS was connected with a mass spectrometer (Agilent 5975C) using DB-1MS capillary column (30 x 0.25mm ID .0,25µm thick layer.) The injector and detector temperature are set at 250°C. The oven temperature is programmed at 40°C for 8 minutes, raised at 3°C/minute to 240°C and then held for 10 minutes Helium as the carrier gas is set to flow rate of 1.2 ml/minute The volume of sample injected is 1.0ml with the aim of identifying qualitatively the presence of fragrant compounds in citronella oil.

2.5. FTIR analysis (fourier transform infra-red spectrophotometer)
Infrared spectroscopy from fragrant citronella oil was obtained by using KBr pallet (Potassium bromide) using Shimadzu FTIR Spectrophotometer. Fragrant citronella oil obtained from community distillation will be analyzed for IR spectra in the "IR Affinity1" FTIR spectrometer (Shimadzu Corporation, Japan), measured in a range between 4000 – 400cm^{-1} with a resolution of 2 cm^{-1} and 30 scans per spectrum, against the background of melting KBr empty.

2.6. Bacterial vulnerability test on perfume products

Antibacterial perfume activities were investigated using agar media methods on Petri dishes [7]. Bacterial cultures that grow in the mid-logarithmic phase are placed in agar media. Escherichia coli and Staphylococcus aureus are injected into agar media. After compacting the agar, the perfume solution (15 mm diameter) with different concentrations (5% and 10% by weight) is placed on the agar surface. The layers were incubated at 37°C for 24 hours and 28°C for the next 72 hours. Next, the results data. Antibacterial activity testing was obtained by calculating the inhibition diameter formed in the petri dish.

3. Results and discussion

The raw material used in this study is Citronella Oil, which was purchased from distillates in the Sp. Keramat area, Lhokseumawe city. This distillation directly produces citronella oil of its fragrance. Before going through the vacuum distillation process, the raw material must be tested for the concentration of the composition or compounds contained therein, using GC-MS tools.

Perfume was made which consisted of several rules for making a perfume which consisted of several notes, namely top notes; middle notes; and base notes. In this study peppermint oil was chosen as a top note because it has a fresh fragrance, nutmeg oil as a middle note because its aroma tends to have a concept towards a sweet but fresh aroma so that it functions to calm (Aromatherapy), while the base notes used are geraniol obtained from Citronella oil from the results of research that has been done. Before making perfume, it is necessary to determine the size of the bottle used because each note has its composition. For top notes, 30%, middle notes 50% and base note 20%. After leaving it for 48 hours in a room that is not exposed to sunlight, aluminium foil that wraps the beaker glass is opened and then add 30 ml of aquadest and a bacterial killer perfume ready for use

3.1. Gas chromatography-mass spectrometry (GC-MS) analysis

The results of the examination by GC-MS showed that this material contained 63 components, but the dominant and related to this study were 3 main compounds, namely: Citronellal, Citronellol, and Geraniol. Results of GC-MS testing data. According to GC-MS examination, this material contains 59.28% Cironellal, 9.57% Citronellol, and 20.32% Geraniol. According to international market standards, the citronellal content must be higher than 35%, therefore this material meets international market quality standards and besides that, the physical and chemical properties of Citronella oil used as material for this study also meet the quality requirements based on the Indonesian National Standard (SNI). The following are the results of the GC-MS examination on early citronella oil at figure 6:

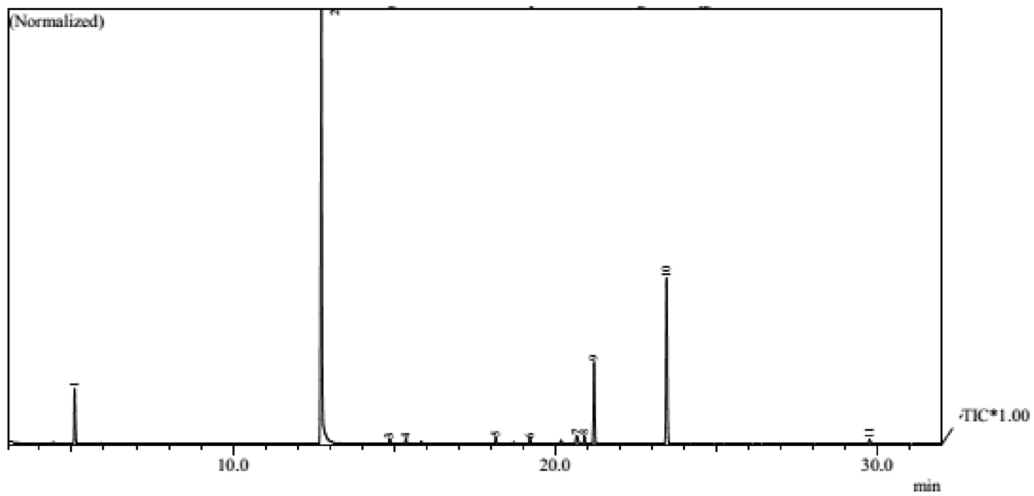

Figure 6. GC-MS testing on citronella oil.

The serial number of the components above shows the order in which the "peak" image or peak of the components concerned is released when analyzed using GC-MS. This can also be used as an indication to determine the magnitude of the boiling point of the component in charge. The later release of the peak results of GC-MS analysis of a component means the higher the boiling point of the component concerned [12]. Thus, if you want high levels of the results of this separation, you must pay attention to the amount of vacuum pressure used, temperature or boiling point of the desired fraction, reflux ratio and process time [13]. In this study, it was focused on the separation of citronellal, citronellol and geraniol compounds, or more precisely the isolation of geraniol compounds. Geraniol isolation was carried out using the ASTM (Koehler) D1160 Vacuum Distillation device by varying the pressure of 8mmHg, 6mmHg, 4mmHg and 1mmHg with operating time variations: 20, 30, 40 minutes and final boiling point minutes.

Geraniol isolation in citronella oil was carried out by evaporating the citronellal component contained in fragrant citronella oil so that the geraniol concentration was expected to be higher. The vacuum distillation method was chosen because of the high citronellal boiling point so that if the distillation process was carried out vacuum it would reduce the citronellal boiling point [14]. This is to keep the components of fragrant citronella oil from being damaged by high heating. In the geraniol isolation process by vacuum distillation method, the most optimum operating pressure is at a pressure of 1mmHg and the boiling temperature is automatically measured at 229□, this produces a distillate of 23 ml. The resulting distillate (citronellal) is clear and has a scent that is not too strong. While the bottom (geraniol) obtained as much as 34 ml brownish color has a strong aroma [15].

Figure 7 shows the results of the optimum geraniol isolation using the vacuum distillation method at a pressure of 1mmHg and on boiling point and the automatic boiling temperature is measured at 280°C. Where the geraniol concentration is as big as 59.8% and is the component that has the highest concentration in this oil. The GC-MS analysis of isolation at a pressure-temperature of 1mmHg is presented in figure 7

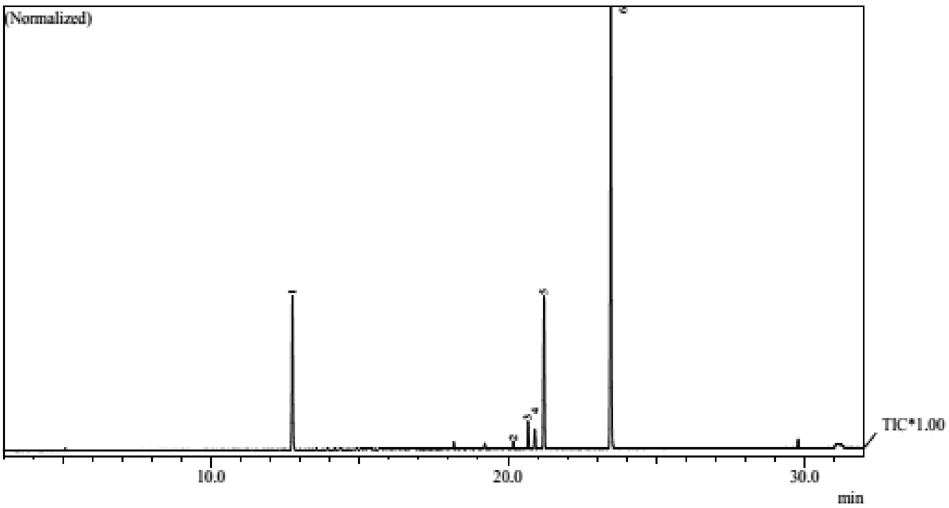

Figure 7. Optimum pressure of 1mmHg at final boiling point.

Geraniol concentrations before and after isolation increased by 38%. namely from 20.32% to 57.39%. This shows that this method is quite effective in increasing geraniol concentrations in fragrant citronella oil. While this isolation process also reduced citronellal levels by 59.28% to 23.5%. The citronellal concentration drops dramatically compared to the citronellal concentration contained in pure citronella oil [17]. This proves and justifies Boyle's law that gas is at a certain volume, pressure & temperature. If the temperature remains constant, the volume of a gas with a certain period is inversely proportional to the pressure. And the smaller the pressure, the greater the volume obtained. The volume pressure of a gas is inversely proportional to the gas mass & constant temperature [18].

3.2. FTIR (fourier transform infra-red spectrophotometer) analysis
Qualitative analysis of different organic compounds can be carried out to ensure that the characteristics of the vibration band appear in the infrared spectrum region at certain frequencies influenced by certain functional groups [19]. The percentage of transmittance corresponding to the wave number is concluded in the total attenuated reflectance IR spectrum as shown in the figure 8. There is an intense broad peak in the range 3600–3200cm^{-1} specifically at 3365.78cm^{-1} according to the hydroxyl polymer group (OH). Other intense and branching peaks in the range of 2935–2915 cm^{-1} are suitable for stretching methyl C and methylene asymmetric, most aliphatic alkyl groups are observed. The medium peak at 2719.63cm^{-1} validates the stretching of terminal C aldehyde carbonyl compounds.

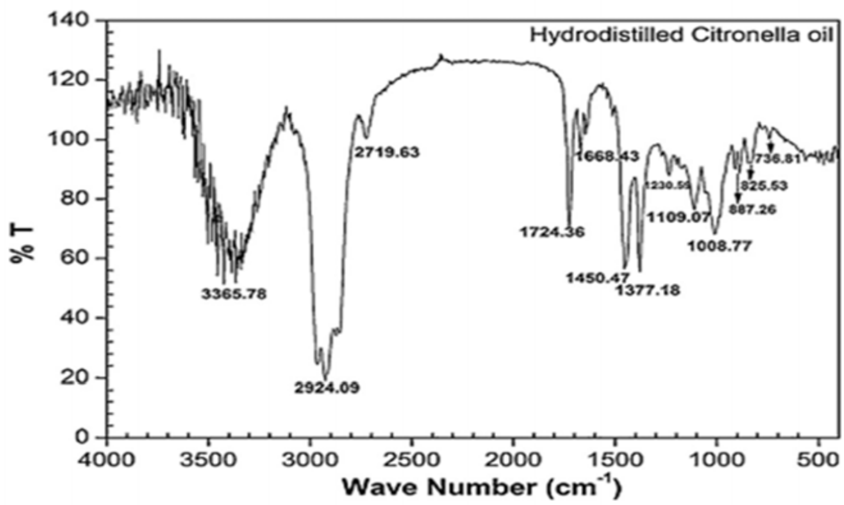

Figure 8. Index bias graph for the value of citronella oil fragrance.

The peaks are different and sharp in the range 1,750-1705cm^{-1} Aldo, keto, Estero and or acido (C O) stretch. The strong and relatively narrow absorption peak at 1668.43cm^{-1} contributed to unsaturated olefinic. Group C-C. Sharp and strong peaks were observed for methylene C H (1485–1445cm^{-1}), symmetrical bend CH methyl (1380–1371cm^{-1}) and stretch of Aryl-O H (1270–1230cm^{-1}). Moreover, the C O bends (1140–1050cm^{-1}), simple OH stretches (1200-1000cm^{-1}) and trans-unsaturated CH=CH (910–860cm^{-1}) functional groups with moderate peaks are also observed. A moderate peak that describes the strain at or tri-substitution of alkene (C H) is detected at 825.53cm^{-1}. Small vibrations in the range 750–660cm^{-1} are associated with the presence of aromatics, vinyl C H groups. This result is very much in line with the previous work carried out [19].

3.3. Bacterial vulnerability test on perfume products
The anti-bacterial activity of geraniol itself was carried out in Lin Yue (2017) study, focusing on geraniol compounds as anti-microbial agents against gram-positive and gram-negative bacteria and very effective in inhibiting the growth of Staphylococcus aureus and Escherichia coli bacteria [10].

On observation and measurement of inhibition zones carried out on the first day (24 hours) the inhibition zone is formed. To find out whether red onion skin extract has bactericidal properties, the inhibitory zone observation was continued for 48 hours. After being observed at 48 hours the bacteria did not grow in the inhibitory area. This shows that Geraniol perfume can kill bacteria (bactericidal). From this explanation, the results of this research are bacteriostatic and bactericidal against the

Staphylococcus aureus bacteria. Figure 9 and figure 10 show the results of inhibition zone of both Escherichia coli and Staphylococcus aureus bacteria.

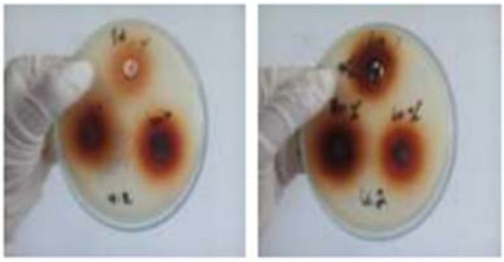

Figure 9. Inhibition zone (inhibition diameter) Escherichia coli , incubation time 24 hours 48 hours.

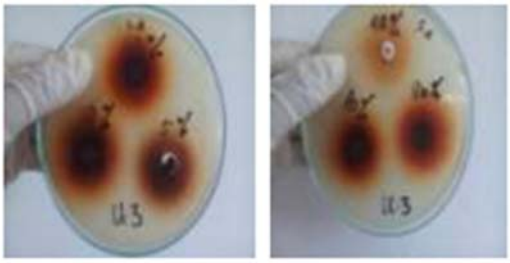

Figure 10. Inhibition zone (inhibition diameter) Staphylococcus aureus, incubation time 24 hours 48 hours.

Based on the result data summarized in table 2, it is shown that the formula which has the strongest antibacterial activity is at the concentration 48 gives the largest diameter barrier 13.23mm against Staphylococcus aureus and at 13.13mm against Escherichia coli almost the same as the diameter of the resulting resistance on comparative perfume. From the data above shows, the obstacles produced by the Escherichia coli bacterial preparations look smaller than those of Staphylococcus aureus, this is because Escherichia coli is a gram-negative bacterium that has thinner and more complex cell walls with high lipid content that is difficult to penetrate [19]. Sadness Staphylococcus aureus is a gram-positive bacterium that has simple and thick cell walls, single layer, low lipid content, cytoplasmic membrane layer composed of peptidoglycan and teichoic acid in the form of water-soluble polymers, so that gram-positive bacteria are more easily penetrated by polar substances comes from preparations [20]. Overall the results of the study of antibacterial perfumes which provide the most powerful antibacterial activity are perfume research results with 24-hour incubation time.

Table 2. Inhibition diameter calculated.

Effectivity of bacteria against perfume	Diameter of inhibition (mm)			
	S.aerus		*E.Coli*	
	24hours	48hours	24hours	48hours
Perfume researched	6.06	13.23	5.06	13.13
Perfume on the market	8.06	16.43	8.03	16.03

4. Conclusions

The conclusion that can be observed from this study is that vacuum distillation technology can be used for the perfume process of fragrant citronella oil. the smaller the control pressure, the greater the volume that will be obtained so that the geraniol concentration will increase, which at a pressure of 1mmHg can increase geraniol levels by 37.2%. Geraniol in citronella has properties as an anti-bacterial agent and it is proven that geraniol formulated in fragrant anti-bacterial perfume has the strongest antibacterial activity at a concentration of 10% with an activity diameter of 13.23mm against Staphylococcus aureus and 13.13mm against Escherichia coli , almost the same as Dettol as a comparative perfume which is 16.43 mm.

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