**Indonesian Journal of Chemical Research**

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 10(1), 19-26, 2022

# Chemical Composition Oil and Ethanol Extract of Nutmeg Leaf and Antibacterial Test Against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Received: January 2022
Received in revised: March 2022
Accepted: May 2022
Available online: May 2022

## Abstract

This study aims to determine the yield and composition of the essential oil and ethanol extract of nutmeg leaves and determine its antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. Nutmeg leaf oil was obtained by isolation using steam-water distillation and extraction methods using maceration with ethanol as solvent. The moisture content of nutmeg leaves is 11.11%. From the distillation method, 0.26% nutmeg oil was obtained, while the yield of nutmeg oil was 29.01% from the extraction method. Gas Chromatography-Mass Spectrometer analysis showed that distilled nutmeg oil contains 20 components with the main composition, namely myristicin (15.92%), β-phellandrene (14.35%), limonene (11.20%), β-pinene (10.81%), and α-pinene (8.59%). The ethanol extract of nutmeg leaf contains 37 components with the main composition being myristicin (7.64%), 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethylheptasiloxane (7.14%), 2,2-dimethyl-1-decanol (7.12%), bis(2-ethylhexyl) phthalate (5.55%), and 9-dodecane-1-al (4.63%). The antibacterial activity test of nutmeg oil was carried out using the good diffusion method. The inhibitory power of nutmeg oil and ethanol extract of nutmeg leaves against *S. aureus* bacteria were 20.31 mm and 23.56 mm, while against *P. aeruginosa* bacteria were 11.79 mm and 8.86 mm, respectively.

*Keywords: Antibacterial, Essential oil, Ethanol, GC-MS, Nutmeg leaf.*

## INTRODUCTION

The nutmeg plant is one of the native plants in the eastern part of Indonesia, especially Maluku, and has many widely known benefits (Kamelia & Silalahi, 2018). The economic value of the nutmeg plant can improve the community's economy so that it is primarily managed in the form of plantations in several areas of Maluku (Fauziyah, Kuswantoro, & Sanudin, 2015). The nutmeg plant consists of several parts that generally correspond to trees: roots, stems, leaves, flowers, and nutmeg. According to Rijal (2017), nutmeg consists of fruit flesh (77.8%), mace (4%), shell (5.1%), and seeds (13.1%). Nutmeg flesh is used in sweets, pickles, jams, syrups, and nutmeg lunkhead. Nutmeg seeds and mace are processed into nutmeg oil through a refining process. According to Kapelle & Laratmese (2014), nutmeg oil is a raw material for medicine, especially for antibacterial and anti-inflammatory, the beverage industry, and cosmetics. Traditionally, nutmeg leaves are used as a spa herb for relaxation. However, if underutilized, nutmeg leaves are often left to dry on the ground to become waste which is then burned.

The benefits of nutmeg oil can not be separated from the chemical composition. Nutmeg seed oil from Soya Ambon Village contains eight main components (Souhoka, Sohilait, & Fransina, 2018). Lekatompessy (2020) stated that mace nutmeg oil from Air Lafa Hamlet, Central Maluku Regency contained three major components and 14 minor components. According to Rastuti et al. (2013), nutmeg leaf oil from Banyumas contains 33 components, similar to the results of Puspa, Syahbanu, & Wibowo (2017), who obtained 33 components in nutmeg leaf oil from West Kalimantan.

Generally, people treat wound infections caused by bacteria (such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*) using antibiotics. Antibiotics often used are ampicillin, tetracycline, and other antibiotics obtained from a doctor's prescription. The use of antibiotics that are not as recommended can cause resistance. Resistance leads to the failure of the treatment of infectious diseases. For this reason, alternative antibiotics from natural ingredients are

needed that can inhibit *S. aureus* and *P. aeruginosa* bacteria (Sulistyarsi & Pribadi, 2018). *S. aureus* and *P. aeruginosa* bacteria are pathogenic bacteria or normal flora on humans' skin, respiratory tract, and digestive tract. Both bacteria often form colonies in external wounds, including cuts and burns. The bacteria *P. aeruginosa* and *S. aureus* are the primary contaminants in burns and the fungi *Candida spp*., *Aspergillus*, and *Fusarium* (Al-Akayleh, 1999).

According to Wibowo et al. (2018), Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MBC) of nutmeg seed oil against *S. aureus* bacteria, namely at concentrations of 0.625% and 10%. However, the nutmeg oil could not inhibit the growth of *P. aeruginosa* bacteria. These results are following a study from Tully & Wibowo (2019), in which a mixture of fresh and dried nutmeg leaf essential oils had an antibacterial activity of *S. aureus* with an inhibition zone diameter of 3.96–5.71 mm, due to the terpenoid compounds contained in it. Ibrahim, Naem, & Abd-Sahib (2018) reported that the ethanolic extract of nutmeg seeds also had antibacterial activity due to the formation of a zone of inhibition against *S. aureus*, but this did not occur in *P. aeruginosa*. Research conducted by Ifriana & Kumala (2018) stated that ethanol extract of nutmeg seeds with a concentration of 20–80% could inhibit the growth of *P. aeruginosa* bacteria by 1.5–4.83 mm. The antibacterial activity of *S. aureus* also increased as the concentration of the ethanol extract of nutmeg leaves increased and was inhibited by 6.43–16.63 mm (Rizal, 2018).

In this study, young nutmeg leaves were taken near the shoots because, according to Permata & Asben (2017), young leaves contain many polyphenols compared to old leaves. After that, nutmeg leaf oil was distilled using the steam-water distillation method, and nutmeg leaf extraction using ethanol using the maceration method. The choice of ethanol solvent for extraction is based on dissolving polar, semi-polar, and non-polar compounds (Susanti et al., 2012). Several phenolic group compounds are included in the semi-polar compound and the terpenoid group, including non-polar compounds. Therefore, the ethanol solvent is selective in dissolving these compounds. The oil and ethanol extract of nutmeg leaves were analyzed for their components by Gas Chromatography-Mass Spectrometer (GC-MS), and antibacterial tests were carried out against *S. aureus* and *P. aeruginosa*.

## METHODOLOGY

### Materials and Instrumentals

The materials used were nutmeg leaves (*Myristica fragrans* Houtt), 96% ethanol, anhydrous sodium sulfate (p.a. Merck), filter paper, *S. aureus* bacteria, *P. aeruginosa* bacteria, Nutrient Agar (NA) (Pa Merck), distilled water, amoxicillin, dimethyl sulfoxide (DMSO) (p.a. Merck).

The tools used are a set of glassware (Pyrex), analytical balance (Denver Instrument XP-3000), oven (Memmert), desiccator, scissors, a set of steam-water distillation apparatus, electric heater (Cimarec 20D+), separating funnel (Pyrex), glass vial, Rotary evaporator (Rotavapor R-215 Buchii), spatula, GC-MS spectrometer (QP-2010 Plus, Shimadzu), loop needle, autoclave, petri dish, sterile perforator, micropipette, incubator, and vernier caliper.

### Methods

#### Sample Collection and Preparation

Nutmeg leaf samples were taken from Manipa District, West Seram Regency, Tumalehu Barat Village. Fresh nutmeg leaves are sorted or separated from small twigs and cleaned of adhering dirt. A total of 8 kg of nutmeg leaves were air-dried for five days. The moisture content of nutmeg leaves was determined by weighing 2–3 g of dried Simplicia, then dried in an oven at 105 °C for two hours, then put in a desiccator for 30 minutes and weighed. The treatment was repeated until a constant weight of Simplicia was achieved, and the water content was calculated using equation (1) (Departemen Kesehatan RI, 1989). After that, the dried Simplicia is cut into 14-16 parts.

#### Nutmeg Leaf Distillation

The Simplicia pieces were weighed as much as 6 kg and put in a sieve in the kettle. After that, the distillation boiler was closed, and distillation was carried out for 6–8 hours (Kapelle & Laratmese, 2014). The resulting distillate will form two layers, the top layer is nutmeg leaf oil, and the bottom layer is water. Next, the oil is separated using a separatory funnel, and then anhydrous sodium sulfate is added (Souhoka, Sohilait, & Fransina, 2018). The obtained oil is filtered and weighed, then the oil yield is calculated.

#### Nutmeg Leaf Extract

A total of 200 g of Simplicia pieces were put into a 2.5 L glass bottle, then 800 mL of 96% ethanol (1:4) was added (Morsy, 2016). The mixture was allowed to stand for 3x24 hours at room temperature and protected from direct sunlight while occasionally stirring (Fawwaz, Nurdiansyah, & Baits, 2017). Then the macerate was filtered and evaporated using a rotary evaporator at a temperature of 45 °C to obtain a thick extract of nutmeg leaves (Atmaja, Mudatsir, & Samingan, 2017). Ethanol from the evaporation is put

back into the maceration bottle, and the maceration process is carried out for 5x24 hours, then evaporated. The viscous extract was then combined, and anhydrous sodium sulfate was added. The extract obtained was filtered and weighed, then the yield was calculated.

**Chemical Composition Analysis**

The results of distillation and maceration in nutmeg leaf oil and ethanol extract of nutmeg leaf were analyzed using GC-MS.

**Antibacterial Activity Test of *Staphylococcus aureus* and *Pseudomonas aeruginosa***

The tools used in the antibacterial activity test were sterilized first. Glassware was sterilized in the oven at 145 °C for 1 hour. Ose needles and tweezers are sterilized by burning with a Bunsen flame. Antibacterial activity test using pathogenic bacteria *S. aureus* and *P. aeruginosa* has been purified and rejuvenated. The bacterial suspension was made and adjusted with standard Mc. Farland 0.5. Weighed 5 g of NA stock media, added 250 mL of distilled water, and dissolved on an electric heater. Media stock that is ready to be sterilized using an autoclave at a temperature of 121 °C for 15 minutes.

Furthermore, the suspension of the two test bacteria and the stock of NA media were simultaneously put into eight Petri dishes. Wait for the mixture to solidify, then three holes were made in each petri dish using sterile perforations with a diameter of 6 mm. The sample solution, the positive control solution, namely amoxicillin, and the negative control solution, namely DMSO with a concentration of 30% each as much as 20 L, were added to each well in each petri dish. Then the agar media was allowed to stand for 1 hour, then incubated at 37 °C in an incubator for 24 hours (Yunita, Permatasari, & Lestari, 2020). The clear zone formed in each petri dish was measured using a vernier caliper. The results of the inhibition zone measurement from 3 holes in each petri dish were taken as the average value.

**Data analysis**

The water content of sampels were calculated using Equation 1.

$$\text{Moisture content} = \frac{(a-b)}{a} \times 100\% \quad (1)$$

Description:
a = Fresh sample weight (g)
b = Weight of dry sample (g)

## RESULTS AND DISCUSSION

**Sample Collection and Preparation**

Nutmeg leaf samples were air-dried for five days to reduce the water content in the sample (Feriyanto, Sipahutar, & Hakim, 2013). The leaves are chopped into several pieces to open the oil glands optimally, so volatile oil evaporation from the sample is faster during the distillation process. In addition, to increase the contact surface area between the sample and the solvent when the extraction process occurs (Suardhika, 2018). Moisture content in nutmeg leaf samples was analyzed using the drying method (thermogravimetry). The moisture content of nutmeg leaves is 11.11%, according to the Departemen Kesehatan RI (1989), which states that the water content in Simplicia ranges from 10%.

**Nutmeg Leaf Distillation**

Isolation of nutmeg leaf oil was carried out by the steam-water distillation method, in which a condensation process took place in the cooler through a pipe so that a layer of oil and water was formed again. Layers that do not coalesce are accommodated and separated by a separating funnel (Kapelle & Laratmese, 2014). The addition of anhydrous sodium sulfate binds the remaining water in the oil. The nutmeg leaf oil is clear, light yellow (Figure 1), and has a characteristic nutmeg odor.

Figure 1. Nutmeg leaf oil distilled

The yield of nutmeg leaf oil is 0.26%. The yield is slightly lower than the research of Rastuti et al. (2013), Puspa, Syahbanu, & Wibowo (2017), and Tully & Wibowo (2019). Differences in yield results may be due to differences in sampling locations. It is suspected that the drying process using the wind-dry method has not been optimal, so it has not been effective in opening the oil glands and removing water in the sample. This result is by Nurdjannah (2007) opinion, which states that several factors affect the quality and yield of nutmeg oil, namely pre-harvest and post-harvest. Pre-harvest factors consist of varieties or types of plants,

harvest locations, cultivation methods, and harvest methods and times. Post-harvest factors consist of material handling, refining, packaging, and transportation.

**Nutmeg Leaf Extract**

The nutmeg leaf extraction process was carried out using 96% ethanol as solvent using the maceration method. The maceration method is a simple extraction method because it is only done by immersing the sample for a particular time while stirring occasionally. The repeated maceration process by re-entering the remaining evaporation solvent into the maceration bottle aims to optimize the time and ability of the solvent to extract the bioactive compounds contained in the sample. The evaporation process carried out serves to evaporate the solvent that is still contained in the extract so that the resulting nutmeg leaf extract is free of ethanol solvent. The ethanol extract of nutmeg leaves produced is brownish red (Figure 2) and has a characteristic nutmeg odor.

Figure 2. Ethanol extract of macerated nutmeg leaves

The yield of nutmeg leaf ethanol extract was 29.01%. This yield is more than Anggriani, Rahim, & Syamsuddin (2018) research, which uses 96% ethanol solvent with a yield of 11.74%, and Moningka et al. (2020), which uses 70% ethanol solvent with a yield of 7.52%. According to Chairunnisa, Wartini, & Suhendra (2019), several factors influencing the extraction include temperature, time, type of solvent, the ratio of material and solvent, and particle size. The longer the maceration process, the longer the contact between the sample and the solvent will increase the number of broken cells and dissolved active ingredients (Wahyuni & Widjanarko, 2015).

**Chemical Composition Analysis**

**Chemical Composition of Nutmeg Leaf Oil**

The results of the GC-MS analysis of nutmeg leaf oil obtained 20 peaks (Figure 3), indicating the presence of 20 components. The five main components of nutmeg leaf oil are myristicin (15.92%), β-phellandrene (14.35%), limonene (11.20%), β-pinene (10.81%), and α-pinene (8.59%). The main components of nutmeg leaf oil are presented in Table 1.

Table 1. Main Components of Nutmeg Leaf Oil

| No. | Retention Time (Minutes) | Concentration[a] (%) | Molecular Formula | Compound Name |
|---|---|---|---|---|
| 1. | 6.325 | 8.59 | $C_{10}H_{16}$ | α-pinene |
| 2. | 7.242 | 14.35 | $C_{10}H_{16}$ | β-phellandrene |
| 3. | 7.308 | 10.81 | $C_{10}H_{16}$ | β-pinene |
| 4. | 8.319 | 11.20 | $C_{10}H_{16}$ | Limonene |
| 5. | 14.350 | 15.92 | $C_{11}H_{12}O_3$ | Myristicin |

a = concentration *Flame Ionisasi Detector* GC

The main component of nutmeg leaf oil, according to the research of Puspa, Syahbanu, & Wibowo (2017), namely limonene compounds (25.73%) and research from (Rastuti et al., 2013)with the same three compounds, namely α-Pinene, β-Pinene, and β-Felandrene. However, the myristicin component was not the main component of the two studies. This could be due to differences in sampling locations. The myristicin content in nutmeg leaf oil is greater than that of nutmeg seed oil, namely 6.56%

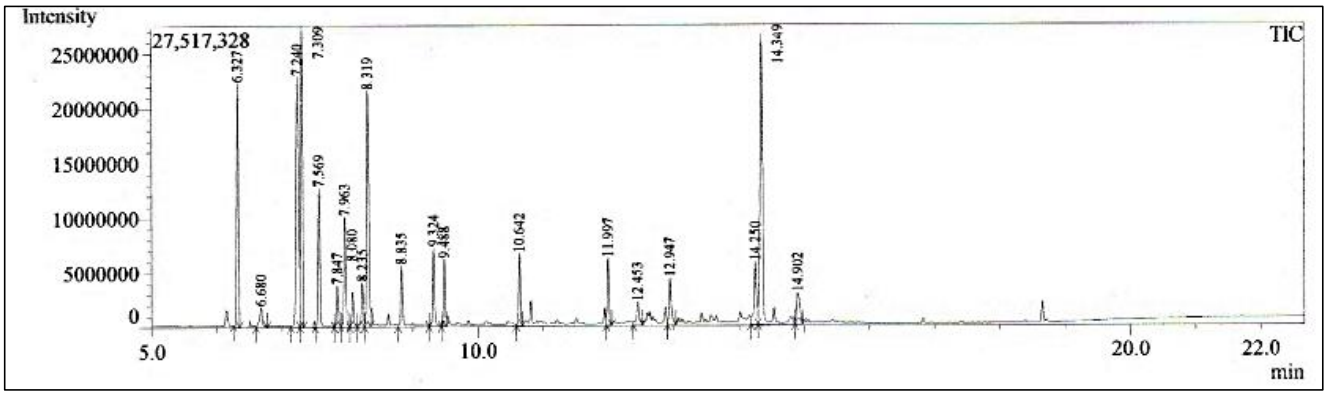


Figure 3. The results of the GC-MS analysis of nutmeg leaf oil

(Souhoka, Sohilait, & Fransina, 2018), but relatively small compared to nutmeg mace oil 20.45% (Sahbandar, 2019). Liunokas & Karwur (2020), stated that myristicin compounds are allylphenol derivatives of the phenylpropanoid group, a group of phenolic compounds that are antimicrobial bioactive compounds.

**Chemical Composition of Nutmeg Leaf Ethanol Extract**

The results of the GC-MS analysis of nutmeg leaf ethanol extract obtained 37 peaks (Figure 4), indicating the presence of 37 components. The five main components of nutmeg leaf ethanol extract are presented in Table 2.

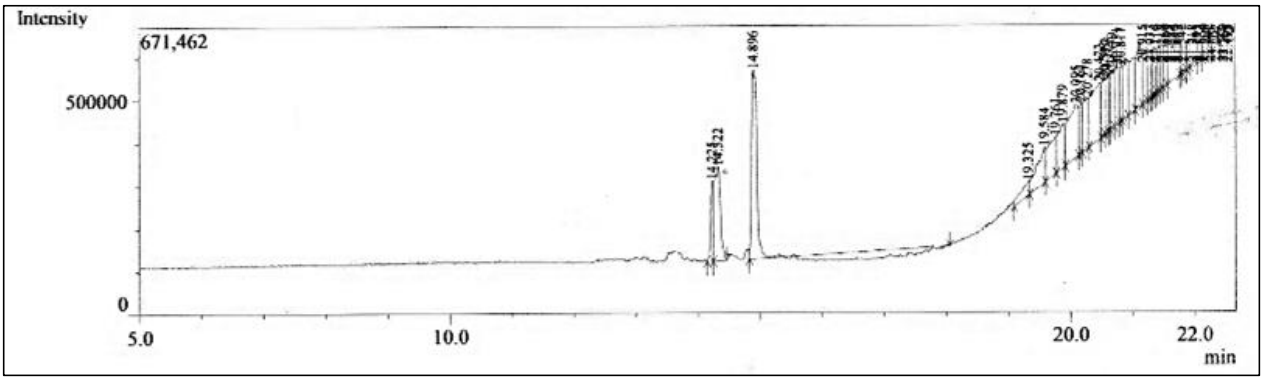


Figure 4. The results of the GC-MS analysis of nutmeg leaf ethanol extract

Table 2. Main Components of Nutmeg Leaf Ethanol Extract

| Retention Time (Minutes) | Concentration [a] (%) | Molecular Formula | Compound Name |
|---|---|---|---|
| 14.325 | 7.64 | $C_{11}H_{12}O_3$ | Myristicin |
| 20.092 | 7.12 | $C_{12}H_{26}O$ | 2,2-dimethyl-1-decanol |
| 20.475 | 7.14 | $C_{14}H_{44}O_6Si_7$ | 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradeca methyl heptasiloxane |
| 21.117 | 4.63 | $C_{12}H_{22}O$ | 9-dodecane-1-al |
| 21.717 | 5.55 | $C_{24}H_{38}O_4$ | Bis(2-ethylhexyl) ftalat |

a = concentration *Flame Ionisasi Detector* GC

The five main components of the ethanolic extract of nutmeg leaves were myristicin (7.64%), 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethylhepta siloxane (7.14%), 2,2-dimethyl-1-decanol (7.12%), bis(2-ethylhexyl) phthalate (5.55%), and 9-dodecane-1-al (4.63%). Not only nutmeg leaf oil contains myristicin, but extracts from nutmeg leaves also contain myristicin (Fawwaz, Nurdiansyah, & Baits, 2017). According to Sirwutubun, Ludong, & Rawung (2016), ethanol solvent is a semipolar solvent that can dissolve polar and non-polar compounds. Myristicin, a non-polar compound, is dissolved in the ethanol solvent used.

**Antibacterial Activity Test of *Staphylococcus aureus* and *Pseudomonas aeruginosa***

Antibacterial testing was carried out by the good diffusion method. The agar medium used was NA medium which *S. aureus* and *P. aeruginosa* bacteria had inoculated. Oil samples, ethanol extract, and positive control (Amoxicillin) were dissolved in DMSO solvent and made up 30% each. DMSO solvent was used as a negative control. The clear zone indicates the presence of inhibitory activity against bacteria. The diameter of the resistance of each petri dish was measured using a vernier caliper. The results of testing the antibacterial activity of *S. aureus* and *P. aeruginosa* are presented in Table 3.

Table 3. Testing the Antibacterial Activity of Samples Against *S. aureus* and *P. aeruginosa*

| Sample | Average Inhibitory Zone Diameter (mm) | |
|---|---|---|
| | *S. aureus* | *P. aeruginosa* |
| Nutmeg leaf oil | 20.31 | 11.79 |
| Nutmeg Leaf Ethanol Extract | 23.56 | 8.86 |
| Positive control (+) Amoxicillin | 45.31 | 39.64 |
| Negative control (-) DMSO | 0 | 0 |

Table 3 shows that samples of nutmeg leaf oil, ethanol extract of nutmeg leaves, and positive control (Amoxicillin) inhibited the growth of *S. aureus* and *P. aeruginosa* bacteria, while the negative control (DMSO) did not show an inhibition zone. DMSO is a

suitable extract solvent without affecting the inhibition of the test bacteria (Lestari, Ardiningsih, & Nurlina, 2016), so the inhibition of bacterial growth only comes from the test sample used.

Antibacterial activity is divided into four categories: weak category if the diameter of the inhibition zone is <5 mm, moderate 5-10 mm, strong 10-20 mm, and powerful >20 mm (Riski et al., 2020). These criteria classified nutmeg leaf oil's antibacterial activity and nutmeg leaf's ethanol extract against *S. aureus* bacteria as very strong. Antibacterial activity against *P. aeruginosa* bacteria in nutmeg leaf oil was strong, while the ethanol extract was moderate. The antibacterial activity of positive control against the two test bacteria was potent because amoxicillin is a generic antibiotic and is classified as a penicillin drug. Infectious diseases caused by gram-positive and gram-negative bacteria are generally treated using these drugs (Atmaja, Mudatsir, & Samingan, 2017).

Nutmeg leaf oil and ethanol extract significantly differed in the zone of inhibition between gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*P. aeruginosa*). This is because gram-negative bacteria have a relatively more complex cell wall structure consisting of three layers: the outer layer in the form of lipoprotein (lipid), the middle layer in the form of lipopolysaccharide (lipid), and the inner layer in the form of peptidoglycan. Gram-positive bacteria have relatively few or simple cell wall structures, namely only peptidoglycan and teichoic acid, making it easier for antibacterial compounds to enter cells and find targets to inhibit the growth of these bacteria (Lingga, Pato, & Rossi, 2015).

The ethanol extract of nutmeg leaf had an average diameter of the inhibition zone against *S. aureus* greater than that of nutmeg leaf oil. For *P. aeruginosa* bacteria, nutmeg leaf oil had a larger average inhibition diameter than nutmeg leaf extract. According to (Lestari, Ardiningsih, & Nurlina, 2016), four factors affect the antibacterial activity: the concentration of the extract, the content of bioactive compounds, the diffusion power of the extract, and the type of bacteria inhibited. The difference in the number of components and the percentage of myristicin content of the two samples affected the average diameter of the resulting inhibition zone.

The flavonoid compounds can inhibit bacterial growth by remodeling the cell membrane structure. Flavonoid compounds can be bound to the surface of the bacterial cell membrane, disrupting the function of the bacterial cell membrane and causing death in bacterial cells (Mere, Bintang, & Safithri, 2021). The phenolic compounds in the oil and ethanol extract of nutmeg leaves can break peptidoglycan cross-links while breaking down cell walls. After the cell wall is broken down, phenolic compounds will cause cell nutrient leakage by destroying the hydrophobic bonds of cell membrane components (such as proteins and phospholipids) and dissolving hydrophilic and hydrophobic components that bind. This process results in impaired cell membrane permeability. The formation of damage to the cell membrane inhibits the activity and biosynthesis of specific enzymes needed in metabolic reactions and bacterial growth (Yuk & Marshall, 2005).

## CONCLUSION

The yield of nutmeg leaf oil was 0.26%, while the ethanol extract of nutmeg leaf was 29.01%. The chemical composition of nutmeg leaf oil consists of 20 components, with five main compounds, namely myristicin (15.92%), β-phellandrene (14.35%), limonene (11.20%), β-pinene (10.81%), and α-pinene (8.59%). The chemical composition of nutmeg leaf ethanol extract consists of 37 components with five main compounds, namely myristicin (7.64%), 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethylhepta siloxane (7.14%), 2,2-dimethyl-1-decanol (7.12%), Bis(2-ethylhexyl) phthalate (5.55%), and 9-dodecane-1-al (4.63%). The antibacterial activity of oil and ethanol extract of nutmeg leaf against *S. aureus* was classified as very strong with inhibition zone diameters of 20.31 mm and 23.56 mm, respectively. Antibacterial activity of oil and ethanol extract of nutmeg leaf against *P. aeruginosa* was classified as strong and moderate, with inhibition zone diameters of 11.79 mm and 8.86 mm, respectively.

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