Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 11(3), 181-189, 2024

Antibacterial Activity of *Malaleuca Leucadendron* Linn Essential Oil from North Central Timor Against *Escherichia coli* and *Staphylococus Aureus*

Sefrinus M.D. Kolo, Janrigo K. Mere*, Emirensiana H. Klau, Noviana M. Obenu

Departement of Chemistry, Faculty of Agriculture, Science & Health, Timor University, Jl. KM 09, Sasi Sub-discret, Kefamenanu, 85614, Indonesia.

Corresponding author: merejanrigo@gmail.com

Received: June 2023
Received in revised: August 2023
Accepted: October 2023
Available online: January 2024

Abstract

The eucalyptus plant (*Melaleuca leucadendron* Linn) is one of the essential oil production plants in the world. The plant has been widely used as an antiseptic and also for relieving colds, sore throat, and infections. This study aims to identify the whole essential oil compounds of this plant using GC-MS and test the antibacterial activity of the essential oil using the disc diffusion method. Based on the results of the GC-MS analysis of the essential oil content obtained by steam distillation and antibacterial test using discs diffusion method to *Escherichia coli* and *Staphylococcus aureus*, yielded 81 compound peaks with antibacterial activity analysis results classified as relatively very strong at 5 different concentrations. The results of the best antibacterial activity test were indicated by the diameter of the inhibition zone 26.42 ± 0.97 mm for *Escherichia coli* while *Staphylococcus aureus* 23.43 ± 2.09 mm at a concentration of 100%. The study of antibacterial compounds based on the 81-peak GC-MS analysis found that 8 compounds have been reported as antibacterial. These compounds belong to the terpenes group with total percentages of eucalyptol 20.97 %, terpineol 11.74 %, β-pinene 6.24%, 3-carene 1.95%, caryophyllene 4.96 %, (z)-geraniol 0.10%, farnesol 0.10%, and eugenol 0.18% respectively.

Keywords: Eucaliptus oil, Antibacterial, Escherichia coli, Staphylococcus aureus.

INTRODUCTION

Indonesia is a country with an abudance of biodiversity. The diversity of flora and fauna that is spread in almost part of Indonesia is proff that Indonesia has the potential for natural resources that can be utilized in various sectors, especially in the food and medicine industry (Nugroho, 2017). The use of biological resources in the food and medical industry is strongly linked to the diversity of flora. In the drug or pharmaceutical industry, plant species have to be researched before they can be used as ingridients of drugs, either empirically or trough laboratory research (Idrus et al., 2020). This is done so that the ingredients used do not have a negative impact on health like nusea, vomiting, or local hypersensitivity of the skin. One Type of flora that has been researched and empirically proven safe to use as medicine is eucalyptus oil from eucalyptus plant (Koswandy & Ramadhania, 2017).

There are various types of eucalyptus plants that grow in Indonesia. Some of these species are *Asteromyrtus symphyocarpa*, *Melaleuca angustifolia* Gaertn and *Melaleuca leucadendron* latifolia L. Var Latifolia L. F (Yarman & Damayanti, 2012).These species have widely been studied and have various potentials to be used as drug candidates. They can be used as antibacterials, antifungals, antitumors, and anticancer (Hanif et al., 2021). The use of eucalyptus oil as a drug candidate cannot be separated from the secondary metabolite content generated by eucalyptus plants. The location of growth and climatic conditions, play an important role in the production of secondary metabolites produced by a plant. Areas with low precipitation are the places for producing secondary plant metabolites of high quality. This is because low precipitation will prompt plants to produce more secondary metabolites to survive (Ramakrishna & Ravishankar, 2011).

North Central Timor Regency is one of the regions of the East Nusa Tenggara Province Indonesia with relatively low rainfall. This area is also a producer of eucalyptus oil. The type of eucalyptus oil produced from this area is the *Melaleuca leucadendron* species, which until now has not been widely known and studied specifically regarding its chemical composition. Most local people, use eucalyptus oil is still limited as rubbing oil. The research of eucalyptus oil from this area is important to do to provide information to the general public or

researchers about its chemical composition, which make it possible to use it as a drug candidate, especially to overcome the problem of antibiotic resistance by bacteria. This study will examine the whole chemical composition of eucalyptus oil from the North Central Timor and its ability to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria that cause infectious diseases.

METHODOLOGY

Materials and Instrumentals

This section must display the equipment (brand, specifications, the research materials used were old eucalyptus leaves (*Melaleuca Leucadendron* Linn.) from Humusu Village, Insana District, North Central Timor Regency East Nusa Tenggara Province, and two isolated bacterial namely *Escherichia coli* and *Staphylococcus aureus*. Other ingredients, namely anhydrous sodium sulfate (E merk), aquades, DMSO (dimethylsulfoxide), nutrient agar (NA), 70% alcohol (E merk), label paper (E merk), paper discs (Oxoid antibacterial susceptibility test discs), chloramphenicol antibiotic, $BaCl_2.2H_2O$ (E merk), H_2SO_4 (E merk), physiological NaCl 0.9% (E merk). Distillation apparatus, hotplate, autoclave, glassware, (Pyrex), ose needle (no brand), petri dish (Pyrex), stirring rod, measuring pipette, incubator, analytical balance (Fujitsu FSR-A), Bunsen burner (no brand), micropipette (Hirschmann-9475406 Labopette), oven (Memmert UNB 400), caliper, and Gas Chromatography-Mass Spectroscopy (GC-MS) analyzer.

Preparation and Determination of Water Content

Eucalyptus leaves samples were taken from Humusu C Village, Insana District, North Central Timor regency, cleaned, and dried at room temprature for 3 days. The dried leaves as much as 1 gram were put into the porcelain dish with constant weight known, then heated in the oven for 1 hour at 105 ℃. Measurement of water content is determined by the Equation 1.

$$\text{Water content} = \frac{a-b}{a} \text{X } 100\ \% \qquad (1)$$

Where value (a) is related to weight of sample before heated, b is Weight of samples after heated.

Eucalyptus Oil Distilation

Eucalyptus oil distillation is carried out by the steam distillation method. 23 kg of eucalyptus leaves were put into a distillation kettle. Distillation was carried out for 4 hours at a temperature of 90-100 ℃. Water vapor flows into a distillation kettle containing eucalyptus leaves. The resulting distillate is accommodated in a holding kettle. The resulting distillate was added with anhydrous sodium sulfate to absorb water for 15 minutes and then filtered. The results of the distillation of eucalyptus oil were then calculated and analyzed for its chemical content using GC-MS. Eucalyptus oil yield is calculated using the Equation 2.

$$\text{Yield} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \text{X } 100\ \% \qquad (2)$$

Antibacterial Activity Test

The antibacterial activity test was carried out using the disc diffusion method. *Escherichia coli* and *Staphylococcus aureus* bacteria suspension of as much as 5 µL has been adjusted to Mc. Farland 0.5 was spread in Nutrient agar media in each petri dish. Discs paper that was prepared and then soaked in Eucalyptus oil distillate was made in concentrations of 20%, 40%, 60%, 80%, and 100 %. The test medium was then incubated for 24 hours at 37 ℃. Antibacterial activity was measured by looking at the clear zone formed around the discs.

Data Analysis

Antibacterial activity test was analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level ($p<0.05$) with application of Minitab 18.0 to determine the difference in the effect of Eucalyptus oil on *Escherichia coli* and *Staphylococcus aureus* bacteria.

RESULTS AND DISCUSSION

Preparation and Water Content Determination

This Eucalyptus leaves that have been taken from the location are cleaned from the stalks and then dried for 3 days at room temperature. The purpose of drying is to reduce the water content in the material. High water content in the material can trigger enzymatic reactions that cause the material to become easily damaged or decomposed (Najib et al., 2017). The water content in the material was determined gravimetrically by evaporating free water molecules from the material at a temperature of 105 ℃. According to (BPOM, 2014) a good quality Simplicia is simplicia with a water content of less than 10%. Based on the results of measurements of the water content of eucalyptus oil leaves, obtained Simplicia water content of 41%. The water content of this research material is still quite high and needs to be dried again to get good quality simplicia. However, according to (Syarifuddin et al., 2020) drying that is too long from the sample will cause some of the

important components of the essential oil to evaporate with the water which can affect the yield and quality of the essential oil produced.

Eucalyptus Oil Distillation

Eucalyptus oil leaves that have been dried for 4 days are then put into a distillation kettle that has been prepared for distillation. Essential oil distillation in this study was carried out using the steam distillation method. The steam distillation method is the most commonly used in essential oil distillation because it is fast, easy, and simple (Ma'sum & Proborini, 2016). In distillation of essential oils using steam distillation method can produce better yields than other methods (Mbaru et al., 2018). The result of volatile oil used steam distillation in this study was oil with a paleyellow color with a characteristic eucalyptus oil aroma with a yield of 34%. The low yield of essential oils from this because the water content in the sample during distillation was still very high, thus affecting the yield measurements.

Antibacterial Test Of Eucalyptus Oil

The antibacterial activity of eucalyptus oil in this study was carried out using the disc diffusion method. This method is most often used for antibacterial assay because it is easy and simple. Antibacterial activity can be determined by measuring the clear zone that forms around the disc (Mere et al., 2021) Antibacterial activity tests were carried out on two types of bacteria that cause infectious diseases, namely *Escherichia coli* and *Staphylococcus Aureus*. Based on the results of the antibacterial activity test of eucalyptus oil against both types of bacteria with 5 variations in concentration, data were obtained as shown in Table 1.

Table 1. Inhibition Zone of Antibacterial Activity

No	Antibacterial Consentration (%)	Inhibition zone (mm) ± SD		Categorize of Inhibition zone
		E. Coli	*S. Aureus*	*E. coli* & *S. aureus*
1	20	12.83 ± 1.74^{d}	12.12 ± 1.56 ^{e}	Strong
2	40	15.47 ± 1.41^{d}	13.82 ± 1.87 ^{d,e}	Strong
3	60	18.97± 0.15^{c}	16.51± 0.67 ^{c,d}	Strong
4	80	22.92± 1.18^{b}	19.78± 2.25 ^{b,c}	Very strong & Strong
5	100	26.42± 0.97^{a}	23.43± 2.09 ^{a,b}	Very strong
6	DMSO (-)	28.52± 1.15 ^{a}	25.44± 0.82 ^{a}	Very strong
7	Cloramphenicol (+)	0.00 ± 0.00^{e}	0.00 ± 0.00 ^{f}	No inhibition

Note : Anttibacterial acttivity data were interpreted by mean ± standard deviation of 3 repetitions. Different superscripts in the inhibition zone of each concentration were significa.ntly different (p<0.05) in Tukey's test

According to (Riski et al., 2020) the ability of an antibacterial in inhibiting bacterial growth can be categorized into 4 groups, namely very strong, strong, moderate, and weak. Antibacterials can be categorized as very strong if the diameter of the inhibition zone obtained is 20 mm, while the strong, moderate, and weak categories are (10-20 mm), (5-10 mm), and (< 5 mm) respectively. The results of Tukey's test showed that there was a significant difference in the zone of inhibition resulting from increasing the antibacterial concentration at ($p<0.05$). This is because the higher concentration of antibacterial in the medium test of the bacteria will increase the effectiveness of chemical compounds in antibacterial to inhibit bacteria growth sign by clear zone around the disc in the medium test (Lingga et al., 2016). In addition to the effect of increasing the antibacterial concentration, the diameter of the inhibition zone is also strongly influenced by the type of test bacteria and the chemical properties of the antibacterial.

Eucalyptus oil as an antibacterial in this study is a type of antibacterial belonging to the terpenes group (Yong et al., 2019). The antibacterial mechanism of the terpene group generally depends on the type of test bacteria and derivates of terpenes. Based on the results of the antibacterial test in Table 1, it can be seen that the inhibition zone of *Escherichia coli* exposed by anti-bacteria is larger than *Staphylococcus aureus* at the same concentration. This happens because the surface of the *E. coli* bacterial cell wall which is composed of lipopolysaccharide tends to react more easily with terpene groups from eucalyptus oil than *S. aureus* bacteria whose cell surface is mostly composed of peptidoglycan. The terpene group derived from eucalyptus oil has non-polar properties. The mechanism of inhibition of the antibacterial compound of eucalyptus oil against *E. coli* bacteria is thought to originate from non-polar interactions which allow disruption of the surface of bacterial cells much faster than *S. aureus* which only has a

small amount of lipopolysaccharide in their cell walls. This is supported by (Sieniawska et al., 2017) who state that the lipophilic nature of terpenes plays an important role as antibacterial and is responsible for damage to bacterial cell membranes as well as the chemical properties of important functional groups in terpene compounds. The interaction between terpene compounds with the bacterial cell membrane causes alterations in the chemical properties of the bacterial cell membrane that caused the osmotic imbalance. As a result of these interactions, the bacteria lose their function in absorbing nutrients and also doing cell division and finally rapid bacterial death (Khameneh et al., 2019).

GC-MS Analysis Of Eucalyptus Oil

The analysis of eucalyptus oil used as an antibacterial in this study was carried out using Gas Chromatography-Mass Spectroscopy (GC-MS). The aim of analyzing using GC-MS is to know the chemical composition of compounds in eucalyptus oil from Timor Island, North Central Timor Regency, Indonesia. Based on the results of the GC-MS analysis of eucalyptus oil, 81 peaks of the compound were obtained as shown in Figure 1.

Based on the chromatogram of the results of the GC-MS analysis in Figure 1, it can be seen that the pattern of separation of compounds from the peaks looks close to each other.

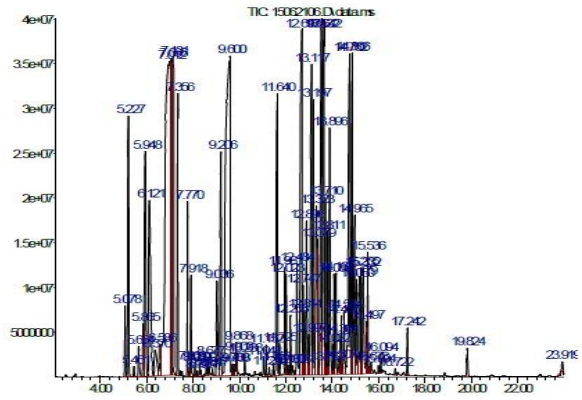


Figure 1. Chromatogram of Eucalyptus oil (*Melaleuca Leucadendron* Linn)

This is caused by the retention time of each compound which is not much different and the chemical composition of eucalyptus oil which is quite a lot. Besides that, there are also a number of the same compounds from different peaks and retention times. This is thought to originate from the separation pattern or the in complete ionization process of the compounds when the sample passes trough the column so that compound with the same separation pattern still appear following the existing compound standards when entering a different retention time. The following is the overall data on eucalyptus oil compounds shown in Table 2.

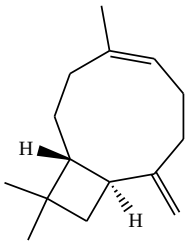
Table 2. Chemical compounds of eucalyptus oil from North Central Timor

Peak	Retention Time	Area (%)	The Name of Compounds
1	5.078	0.44	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-
2	5.227	2.38	1R-.alpha.-Pinene
3	5.461	0.08	Camphene
4	5.664	0.30	Benzaldehyde
5	5.865	0.41	beta.-Phellandrene
6	5.948	2.87	beta.-Pinene
7	6.121	3.14	beta.-Pinene
8	6.370	0.52	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
9	6.586	0.34	(+)-4-Carene
10	7.012	12.77	Eucalyptol
11	7.066	3.37	Eucalyptol
12	7.131	4.83	Eucalyptol
13	7.356	1.95	3-Carene
14	7.770	0.83	Cyclohexene, 1-methyl-4-(1-methylethylidene)-
15	7.869	0.08	Benzoic acid, methyl ester
16	7.918	0.55	1,6-Octadien-3-ol, 3,7-dimethyl-
17	8.089	0.07	2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-
18	8.195	0.03	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-
19	8.300	0.07	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-
20	8.351	0.03	2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-

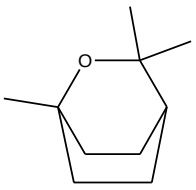
Peak	Retention Time	Area (%)	The Name of Compounds
21	8.581	0.06	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-
22	8.677	0.14	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-
23	8.847	0.04	Isopulegol
24	9.036	1.04	Cyclohexene,1-methyl-4-(2-propanol-2-yl)-
25	9.206	2.03	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-
26	9.600	11.74	p-menth-1-en-8-ol
27	9.700	0.06	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-
28	9.768	0.08	2-Norbornene, 2,3-dimethyl-
29	9.868	0.18	6-Octen-1-ol, 3,7-dimethyl-, (R)-
30	9.900	0.10	(Z)-Geraniol
31	10.238	0.09	2,6-Octadien-1-ol, 3,7-dimethyl-
32	11.048	0.12	(Z)-3,7-Dimethyl-6-oxo-2-octenal
33	11.152	0.17	trans-(-)-Bicyclo[3.1.0]hexane
34	11.290	0.06	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-
35	11.453	0.07	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-
36	11.640	3.22	3-Cyclohexene-1-methanol
37	11.725	0.18	Eugenol
38	11.961	0.58	Copaene
39	12.023	0.56	a-Cubebene
40	12.100	0.06	1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene
41	12.208	0.36	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-
42	12.306	0.08	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a
43	12.484	0.87	1H-Cycloprop[e]azulene
44	12.697	4.96	Caryophyllene
45	12.747	0.50	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)
46	12.814	0.45	1H-Cyclopropa[a]naphthalene
47	12.896	1.16	1H-Cycloprop[e]azulene
48	12.997	0.30	Isoledene
49	13.117	3.52	a-Caryophyllene
50	13.197	1.68	1H-Cycloprop[e]azulene
51	13.323	1.92	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-
52	13.379	0.87	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-
53	13.532	3.64	1H-Cyclopropa[a]naphthalene
54	13.642	3.78	1,3-Dimethyl-5-(propen-1-yl)adamantane
55	13.710	0.90	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-
56	13.762	0.05	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-
57	13.811	0.73	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-
58	13.896	1.83	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (1S-cis)-
59	14.022	0.14	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
60	14.085	0.52	1H-Cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-
61	14.167	0.58	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1methylethylidene)-
62	14.307	0.10	VULGAROL B

Peak	Retention Time	Area (%)	The Name of Compounds
63	14.364	0.23	(1S,2S)-1-Methyl-2-(prop-1-en-2-yl)-4-(propan-2-ylidene)-1-vinylcyclohexane
64	14.407	0.34	(-)-Caryophyllene-(I1)
65	14.514	0.50	4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-
66	14.762	4.67	Globulol
67	14.866	2.99	Ledol
68	14.965	1.06	Ledol
69	15.050	0.86	1H-Cycloprop[e]azulene
70	15.179	1.12	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-
71	15.255	0.60	BICYCLO[4.4.0]DEC-1-EN, 2-ISOPROPYL-5-METHYL-9-METHYLENE-
72	15.332	1.12	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-
73	15.497	0.36	a-Cadinol
74	15.536	0.68	Neointermedeol
75	15.678	0.13	1H-Cycloprop[e]azulene
76	15.994	0.04	Naphthalene, 2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-
77	16.094	0.10	Farnesol
78	16.722	0.05	Benzyl Benzoate
79	17.242	0.29	Cyclononasiloxane, octadecamethyl
80	19.824	0.25	Cyclodecasiloxane, eicosamethyl-
81	23.919	0.05	1,3,5,7-Tetraethyl-1-ethylbutoxysiLoxycyclotetrasiloxane

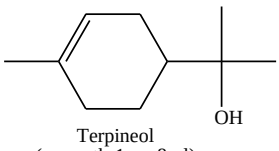
Based on the data in Table 2, it can be seen that most of compounds contained in eucalyptus oil are mostly derivatives of terpenes groups. The study of antibacterial compounds from 81 peaks from GC-MS analysis showed that several compounds have been reported to have antibacterial activity. These compounds with complete chemical structures are shown in Figure 2.



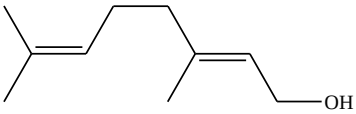
Caryophyllene
(1R,4E,9S)-4,11,11-Trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene)



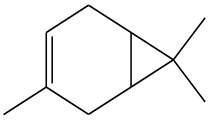
Eucaliptol
(1,3,3-Trimethyl-2-oxabicyclo(2.2.2)octane)



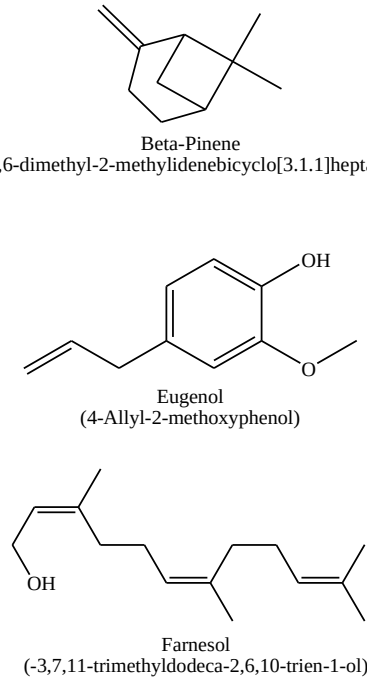
Terpineol
(p-menth-1-en-8-ol)



(Z)-Geraniol
(2E)-3,7-dimethylocta-2,6-dien-1-ol)



3-Carene
(3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene)



Beta-Pinene
(6,6-dimethyl-2-methylidenebicyclo[3.1.1]heptane)

Eugenol
(4-Allyl-2-methoxyphenol)

Farnesol
(-3,7,11-trimethyldodeca-2,6,10-trien-1-ol)

Figure 2. Several Antibacterial Compounds of Eucalyptus Oil

Eucalyptol and terpineol are compounds that have been reported as antibacterial agents. The mechanism of action of these compounds according to (Safitri et al., 2022) is that these two compounds can kill bacteria by damaging the membranes and cell walls of bacteria. This is supported by the data transmission electron microscope of *Escherichia coli* after exposure to terpineol reported by (Li et al., 2014) showing that there was a change in the size of the *E. coli* cell shape to become more irregular. These changes are caused by the loss of cytoplasmic fluid due to the destruction of the bacterial cell wall which results in the death of the bacteria.

Beta-pinene compounds are also reported to have antibacterial activity. The results of the study of enantiomer (+)-β-pinene and (-)-α-pinene by (Da Silva et al., 2012) on several microbes, showed that these two enantiomers were able to kill the test microbes including *C. albicans, C. neoformans, R. oryzae* including *Methicillin-resistant Staphylococcus Aureus* (*MRSA*). Besides pinene, 3-carene is also known to have antibacterial properties against gram-positive and gram-negative bacteria. The results of the study of antibacterial activity and the mechanism of inhibition of microbial growth of the compound 3-carene by (Shu et al., 2019) showed that 3-carene had strong antimicrobial activity against Gram-positive and Gram-negative bacteria from the types of bacteria *Bronchothrix thermospacta* and *Pseudomonas fluorescens*. The results of the search for the mechanism of inhibition of this compound through several biochemical analyzes showed that this compound was able to kill bacteria through three pathways, namely damaging the bacterial cell membrane, interfering metabolism of bacterial cells, and finally disrupting the DNA structure of the genome of bacteria. Although there is no specific study on the mechanism of inhibition of this compound against *E. coli* and *S. aureus*, the mechanism of inhibition by this compound on the growth of the two test bacteria in this study is also suspected to follow the mechanism of inhibition of *B. thermospacta* and *P. fluorescens* because they are still in the same group when viewed from the perspective of the composition of the cell walls.

Caryophyllene as an antibacterial compound also has been reported by (Dahham et al., 2015). The results of the antibacterial activity test of this compound showed an inhibition of the growth of *E. coli* and *S. aureus* bacteria. The activity of bioactive compounds of Geraniol and Eugenol compounds has also been widely reported. Geraniol by (Tahya et al., 2022) reported that this compound has pharmaceutical activity, one of which is antibacterial. The antibacterial mechanism of geraniol that may occur is through interaction with lipid membranes and destabilizing the membrane structure making it more permeable and easily destroyed (Lira et al., 2020). Similar to geraniol, the mechanism of action of eugenol is also known to cause death in bacterial cells by modifying the structure of fatty acids in bacterial cell membranes. In addition, it inhibits the work of enzymes in bacteria such as ATPase, protease, and amylase which play an important role in the metabolic process of bacterial cells. Meanwhile, farnesol is also known to act as an antibacterial agent. Although the mechanism of antibacterial activity is not fully understood, several studies have reported that the results of exposure to farnesol compounds against bacteria indicate inhibition of bacterial growth (Unlu et al., 2018).

CONCLUSIONS

Identification of eucalyptus oil compounds from North Central Timor showed that there were 81 compounds with most of the constituent components derived from terpenes. The results of the antibacterial activity test against *Escherichia coli* and *Staphylococcus aureus* bacteria, eucalyptus oil were effective in inhibiting the growth of both types of bacteria. Tracing the antibacterial properties of 81 compounds that have been identified, it is known that

8 compounds are responsible for inhibiting bacterial growth. The results of the literature study of these 8 compounds indicate that most of the inhibition mechanisms of these compounds on bacterial growth are through the path of destroying the lipid membrane in the bacterial cell wall which disrupts cell structure and bacterial cell metabolism processes resulting in bacterial cell death.

REFERENCES

BPOM. (2014). *Badan Pengawas Obat dan Makanan republik indonesia*.

Da Silva, A. C. R., Lopes, P. M., de Azevedo, M. M. B., Costa, D. C. M., Alviano, C. S., & Alviano, D. S. (2012). Biological Activities of a-Pinene aand b-Pinene Enantiomers. *Molecules*, *17*(6), 6305–6316. https://doi.org/10.3390/molecules17066305

Dahham, S. S., Tabana, Y. M., Iqbal, M. A., Ahamed, M. B. K., Ezzat, M. O., Majid, A. S. A., & Majid, A. M. S. A. (2015). The Anticancer, Antioxidant and Antimicrobial Properties of The Sesquiterpene β-caryophyllene from The Essential Oil of *Aquilaria crassna*. *Molecules*, *20*(7), 11808–11829.

Hanif, H., Elikaei, A., Vazini, H., & Mohammadi, A. (2021). Anticancer and Antibacterial Effect of Eucalyptus *camaldulensis*, in Vitro. *Medical Laboratory Journal*, *15*(1), 26–32.

Idrus, I., Kurniawan, F., Mustapa, F., & Wiboowo, D. (2020). Concentration Effect of Leaf Extract from Kekara Laut (*Canavalia maritima* Thou.) in Inhibiting of *Staphylococcus* epidermidis Bacteria with a Statistical Science Approach. *Indonesian Journal of Chemical Research*, *8*(2), 151–158.

Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on Plant Antimicrobials: A Mechanistic Viewpoint. *Antimicrobial Resistance and Infection Control*, *8*(1).

Koswandy, L. F., & Ramadhania, Z. M. (2017). Kandungan Senyawa Kimia dan Bioaktivitas dari *Eucalyptus globulus* Labill. *Farmaka*, *24*(2), 63–78.

Li, L., Shi, C., Yin, Z., Jia, R., Peng, L., Kang, S., & Li, Z. (2014). Antibacterial Activity of α-Terpineol may Induce Morphostructural Alterations in *Escherichia coli*. *Brazilian Journal of Microbiology*, *45*(4), 1409–1413.

Lingga, A. R., Pato, U., & Rossi, E. (2016). Uji Antibakteri Ekstrak Batang Kecombrang (*Nicolaia speciosa* Horan) terhadap *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal Online Mahasiswa*, *18*(2), 33–37.

Lira, M. H. P. de, Andrade Júnior, F. P. de, Moraes, G. F. Q., Macena, G. da S., Pereira, F. de O., & Lima, I. O. (2020). Antimicrobial Activity of Geraniol: An Integrative Review. *Journal of Essential Oil Research*, *32*(3), 187–197.

Ma'sum, Z., & Proborini, W. D. (2016). Optimasi Proses Destilasi Uap Essential Oil. *Jurnal Reka Buana*, *1*(2), 105–109.

Mbaru, M. E., Victor, M., Proborini, W. D., & K.F, A. C. (2018). Perbandingan Metode Distilasi Minyak Atsiri Daun Kayu Putih Menggunakan Hydrodistillation dan Steam Distillation. *EUREKA: Jurnal Penelitian Teknik Sipil Dan Teknik Kimia*, *2*(2), 215–221.

Mere, J. K., Bintang, M., & Safithri, M. (2021). Antibacterial Effectiveness of *Syzygium cumini* (L.) Skeels Leaves to *Escherichia coli* pBR322. *Indonesian Journal of Chemical Research*, *9*(1), 8–14.

Najib, A., Malik, A., Ahmad, A. R., Handayani, V., Syarif, R. A., & Waris, R. (2017). Standarisasi Ekstrak Air Daun Jati Belanda Dan Teh Hijau. *Jurnal Fitofarmaka Indonesia*, *4*(2), 241–245.

Nugroho, A. W. (2017). Review: Konservasi Keanekaragaman Hayati Melalui Tanaman Obat dalam Hutan di Indonesia dengan Teknologi Farmasi: Potensi dan Tantangan. *Jurnal Sains dan Kesehatan*, *1*(7), 377–383.

Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of Abiotic Stress Signals on Secondary Metabolites in Plants. *Plant Signaling and Behavior*, *6*(11), 1720–1731.

Riski, D. G., Maulana, R. G. R., Permana, E., Lestari, I., & Tarigan, I. L. (2020). Profile Analysis of Fatty Acids of Tengkawang (*Shorea Sumatrana*) Oil Using GC-MS and Antibacterial Activity. *Indonesian Journal of Chemical Research*, *8*(2), 114–119.

Safitri, Y. D., Amalia, A., Fatimah, & Muadifah, A. (2022). The Identification of Antibacterial Compounds in Clove Stem. *Jurnal Biosains (The Journal of Bioscences)*, *8*(1), 24–29.

Shu, H., Chen, H., Wang, X., Hu, Y., Yun, Y., Zhong, Q., … Chen, W. (2019). Antimicrobial Activity and Proposed Action Mechanism of 3-Carene Against *Brochothrix thermosphacta* and *Pseudomonas fluorescens*. *Molecules*, *24*(18), 1–18.

Sieniawska, E., Swatko-Ossor, M., Sawicki, R., Skalicka-Woźniak, K., & Ginalska, G. (2017). Natural Terpenes Influence The Activity of

Antibiotics Against Isolated *Mycobacterium tuberculosis*. *Medical Principles and Practice*, *26*(2), 108–112.

Syarifuddin, Ellysa, Busthan, M., & Thalib, A. (2020). Pengaruh Waktu Penjemuran Terhadap Rendemen Minyak Atsiri Daun *Eucalyptus grandis* dengan Metode Kohobasi. *Majalah Biam*, *16*(02), 64–71.

Tahya, C. Y., Kolo, S. M. D., & Karnelasatri. (2022). Antimicrobial and Antioxidant Properties of *Cymbopogon nardus* L (Citronella Grass) Oil from Kefamenamu, Timor Tengah Utara Regency, Indonesia. *AIP Conference Proceedings*, *2391*.

Unlu, A., Sar, T., Seker, G., Erman, A. G., Kalpar, E., & Akbas, M. Y. (2018). Biofilm Formation by *Staphylococcus aureus* Strains And Their Control by Selected Phytochemicals. *International Journal of Dairy Technology*, *71*(3), 637–646. https://doi.org/10.1111/1471-0307.12520

Yarman, & Damayanti, E. K. (2012). Pemanfaatan dan Upaya Konservasi Kayu Putih (*Asteromyrtus symphyocarpa*) di Taman Nasional Wasur. *Media Konservasi*, *17*(2), 85–93.

Yong, W. T. L., Ades, P. K., Goodger, J. Q. D., Bossinger, G., Runa, F. A., Sandhu, K. S., & Tibbits, J. F. G. (2019). Using Essential Oil Composition to Discriminate between Myrtle Rust Phenotypes In *Eucalyptus globulus* and *Eucalyptus obliqua*. *Industrial Crops and Products*, *140*(July), 111595.