Eksakta : Berkala Ilmiah Bidang MIPA
VOLUME 26 NO 02 2025, pp 163-174
ISSN : Print 1411-3724 — Online 2549-7464
DOI : https://doi.org/10.24036/eksakta/vol26-iss02/584

Eksakta
Berkala Ilmiah Bidang MIPA
http://www.eksakta.ppj.unp.ac.id/index.php/eksakta

Article

Chemical Components and Antibacterial Activity of *Cinnamomum culilaban* Extract from Southeast Maluku

Article Info

Article history :
Received March 15, 2025
Revised April 14, 2025
Accepted April 21, 2025
Published June 30, 2025
In Press

Keywords :
Cinnamomum culilaban (L.) Presl, Lawang bark extract, antibacterial, GCMS analysis

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Abstract. The bark of *Cinnamomum culilaban*, endemic to Southeast Maluku, has the potential as an antibacterial. This study aimed to determine the antibacterial activity of *C. culilaban* bark extract isolated using distillation and maceration methods and to determine the chemical components contained therein. Qualitative tests using GCMS and antibacterial activity testing against *E. coli* and *S. aureus* bacteria. The extraction results using the distillation method obtained a sedimentation of 3.28% and the maceration method of 28.03%. The chemical components of the distillation extract showed four main components: Eucalyptol 4.02%, (+)-2-Bornanon 2.32%, terpineol 1.49% and Safrole 86.78%. The chemical components of the maceration extract have five main components, namely (+)-2-Bornanon 1.35%, Terpineol 1.43%, Safrole 89.06%, Spathulenol 1.26%, and Methoxyeugenol 1.73%. The antibacterial activity of the distillation extract against *E. coli* bacteria is classified as strong, with the highest inhibition zone size at a concentration of 60% (13.47 ± 1.14 mm). In contrast, for *S. aureus* bacteria, there is no antibacterial activity. The antibacterial activity of the maceration extract against *S. aureus* bacteria is classified as strong with the inhibition zone size at a concentration of 100% (11.87 ± 1.15 mm). In contrast, for *E. coli* bacteria, there is no antibacterial activity.

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1. Introduction

Bacterial resistance to conventional antimicrobial agents has become a major health problem worldwide, associated with increased morbidity, mortality, and economic losses [1]. Antimicrobial resistance (AMR) has now emerged as a chronic global public health problem, with an estimated 10 million deaths per year globally by 2050 [2]. One of the causes of infectious diseases is bacteria caused by pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli* [3]. The severity of *S. aureus* infection varies, from mild to life-threatening, depending on the type of infection and the health of the person [4]. *E. coli* are gram-negative bacillus bacteria that cause diarrhea, uncomplicated cystitis, and other extraintestinal diseases: pneumonia, bacteremia, and abdominal infections [5]. Antimicrobials are compounds that can control the growth of certain harmful microbes. Controlling the growth of these microbes generally aims to prevent the spread of disease and infection and prevent spoilage in food. Microbes have developed acquired AMR against many drugs due to high selection pressure due to the increasing use and misuse of antibiotics over the years [6]. Essential oils (EOs) have emerged as alternative antimicrobial products due to their strong and broad-spectrum activity against microorganisms and being environmentally friendly and safe for humans [7-9].

These antimicrobial compounds can be obtained from plant extracts where the antimicrobial activity comes from the bioactive components contained therein [10]. Bioactive components found in plants can inhibit bacterial growth [11-12]. Cinnamon (C. verum) has been shown to have antibacterial effects against clinical strains of several XDR bacteria, including MRSA, vancomycin-resistant (VR) *E. faecium, A. baumannii, P. aeruginosa, E. coli* and *S. aureus* [13-14].

The type of each plant has different secondary metabolite compound components and can affect its activity. Secondary metabolites are organic compounds that help plants adapt to their environment [15-16]. The techniques and solvents used to isolate bioactive compounds from plants can affect the quality and quantity of the extracted compounds as well as the antioxidant activity of the extracted compounds [17-19]. One source of essential oils found in Eastern Indonesia is *C.culilaban*, a member of the Lauraceae family in the cinnamon group. *C. culilaban* plant extract from Seram Island, Maluku, has been shown to have inhibitory activity against Streptococcus mutans and Enterococcus faecalis. Phytochemical testing of the extract contains alkaloids, flavonoids, tannins, saponins, phenolics, and steroids/terpenoids [20]. *C. culilaban* plants from Tamngil Nuhuten Village, Southeast Maluku Regency are used as alternative medicine. The purpose of this study was to determine the antibacterial activity of the bark extract of the lawang tree isolated using the distillation method and the maceration method and to determine the chemical components contained therein.

2. Experimental Section

2.1 Plant Material and Essential Oil Extraction

The *C. culilaban* plant samples were obtained from Tamngil Nuhuten Village, Southeast Maluku Regency, Maluku Province, Indonesia. The bark of the *C. culilaban* plant tree was collected on freshly growing trees, and the bark of the tree was taken. The bark of the C. culilaban plant tree was dried at room temperature for 12 days.

2.2 Steam Distillation Method

The dried pieces of the *C. culilaban* plant weighed as much as 2.5 kg, and a steam distillation apparatus was prepared. The bark of the tree cut into 1x1 cm sizes was placed on a sieve in a distillation kettle. Distillation was carried out using the steam distillation method. Distillation was stopped when no more essential oil was obtained. The distillate obtained was then separated between oil and water. The oil obtained was added with anhydrous Na_2SO_4 to bind the water still contained in the oil. The essential oil obtained was stored in a dark bottle and tightly closed before use.

2.3 Maceration Method

The dried *C.Culilaban* plant bark weighed as much as 0.5 kg, and an Erlenmeyer reactor was prepared for extraction. The maceration extraction method used 96% ethanol solvent, which was carried out for 7 x 24 hours. After the extraction process, it was filtered, and the filtrate was obtained. The filtrate obtained was then evaporated using a rotary evaporator between 40–50 ºC until *C.Culilaban* plant extract was obtained.

2.4 Analysis of the Chemical Composition of Essential Oil Extracts

Determination of essential oil extract content using gas chromatography-mass spectrometry (GC-MS Simadzu QP-5050 A series II, Class-5000 Ver 2.2) equipped with a DBMS detector with a DB10 capillary column 30 meters long and 0.25 mm in diameter, using hydrogen as a carrier gas (1.6 ml/min). Column temperature 60 ºC, injector temperature 280 ºC, detector temperature 300 ºC, interface temperature 320 ºC, column pressure 100 kPa. Equipment conditions are set to identify essential oil groups. The program time lasts for 39 minutes. The percentage of essential oil obtained is the percentage of oil injected (relative percentage). The molecular profile in essential oils is obtained by comparing the chromatogram that appears through the GC-MS digital detector with the molecular chromatogram contained in the WILLEY 229, NIST62 LIB, and PESTICID.LIB library sources. The percentage of essential oil molecule content obtained is relative.

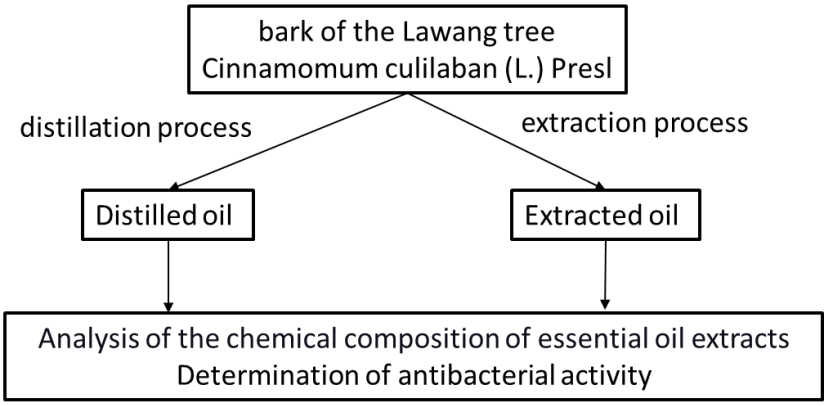

Figure 1. Research method flow chart

2.5 Determination of Antibacterial Activity

2.5.1 Preparation of Test Bacterial Suspension

The suspension of *E. coli* and *S. aureus* test colonies was made by taking one loop of colonies from solid NA media into a test tube containing 5 mL of physiological NaCl. The turbidity of the test colony suspension was standardized with a 0.5 McFarland standard (approximately 1.5x108 CFU/mL). The suspension should be used as an inoculum within 15 minutes.

2.5.2 Well-Diffusion Antibacterial Activity Testing

Antibacterial activity testing in this study used *E. coli*, representing Gram-negative bacteria, and *S. aureus*, representing Gram-positive bacteria that are pathogenic. Antibacterial testing using the well method was carried out for two samples, namely, the extract sample from the distillation of the bark of the *C.Culilaban* plant and the extract from the maceration method of the bark of the *C.Culilaban* plant. Two-layer nutrient agar media were used in this testing process. The first layer of the medium is a nutritious base layer, while the second layer is a mixture of media and pathogenic bacterial suspension with a cell density of 10^6 CFU/mL. The top of the media (second layer) was given a well hole with a 6-7 mm diameter using a sterile punch. Each petri dish was made with three well holes

and two holes in 1 separate dish as a control. The oil sample was dissolved in a DMSO solvent, and the extracted sample was dissolved in a water solvent, each made in five concentration variations, namely 20%, 40%, 60%, 80%, and 100%. Amoxicillin was used as a positive control, and DMSO solvent was used as a negative control. The agar media was incubated at 37 ºC for 24 hours. The diameter of the inhibition zone formed after inoculation was measured. The clear zone indicates the presence of bacterial inhibition activity; the inhibition diameter of each petri dish was measured using a caliper.

3. Results and Discussion

The *C. culilaban* plant came from Southeast Maluku Regency, Maluku Province, Indonesia (Figure 2). The purpose of cutting and drying was to minimize the sample size, making it more effective during the extraction process and reducing the water content. The water content of the sample obtained was 3.9419%.

Figure 2. *C. culilaban* plant bark sample.

The results of the extraction of *C.Culilaban* tree bark using the distillation method obtained a clear yellow liquid with a soaking of 3.2814%. Physical and chemical factors can cause a decrease in oil soaking. Chemical factors are caused by several compound components in essential oils sensitive to oxygen, such as alcohol, aldehydes, and oxides, which can cause compound decomposition. The results of the extraction of *C.Culilaban* tree bark using the maceration method using ethanol solvent obtained a thick brownish liquid with a soaking of 28.0349%. Factors that affect the extraction results are temperature and maceration time. The higher the temperature and maceration time, the more bioactive components can be increased. The higher the temperature and time of a maceration process, the more compounds attracted to the solvent will increase [21]. Separation in the maceration process is carried out using a rotary evaporator; where the selection of this separation method aims for the active substances extracted not to be damaged because they can evaporate the solvent below the boiling point so that the substances contained in the oil are not damaged by high temperatures [22]. The results of GCMS analysis of essential oil from the bark *C. Culilaban* obtained 21 peaks (Figure 3).

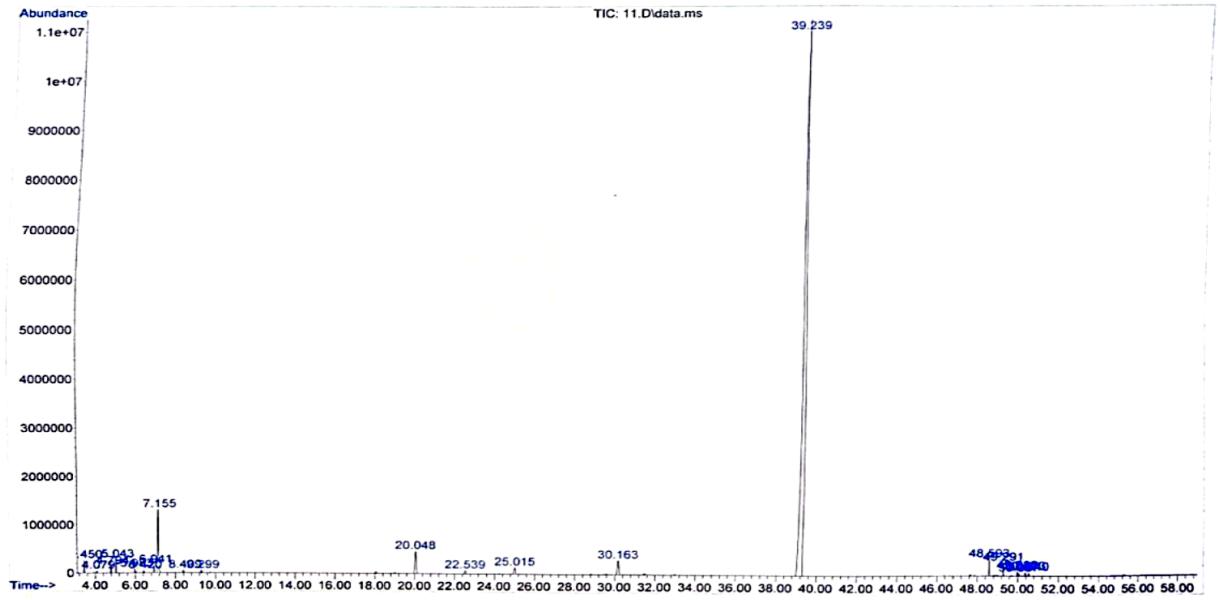

Figure 3. GC chromatogram of distillation oil of the bark of the *C. culilaban*

The main components of the *C. Culilaban* bark essential oil can be seen in Table 1. However, the safrole component is not the most abundant in the study. There are differences in the species of Cinnamomum plants taken and the most dominant components. Safrole is the most dominant component in the bark of the tree of the *C.culilaban* type. The fragment pattern with a molecular ion peak at m / z = 162 indicates the molecular weight of safrole. The mass spectrum of safrole provides fragments of m / z, namely 51, 63, 77, 91, 104, 119, 131, and 162 [$C_{10}H_{18}O_2^+$].

Table 1. Main Components of Oil from Distillation of *C. culilaban* Bark

No	Retention Time	Concentration (%)	Compound Name
1	7.155	4.02	Eucalyptol
2	20.048	2.32	(+)-2-Bornanone
3	30.161	1.49	Terpineol
4	39.236	86.78	Safrole

There were four main components as in the MS data (Figure 4), namely eucalyptol, (+)-2-bornanone, terpineol, and safrole.

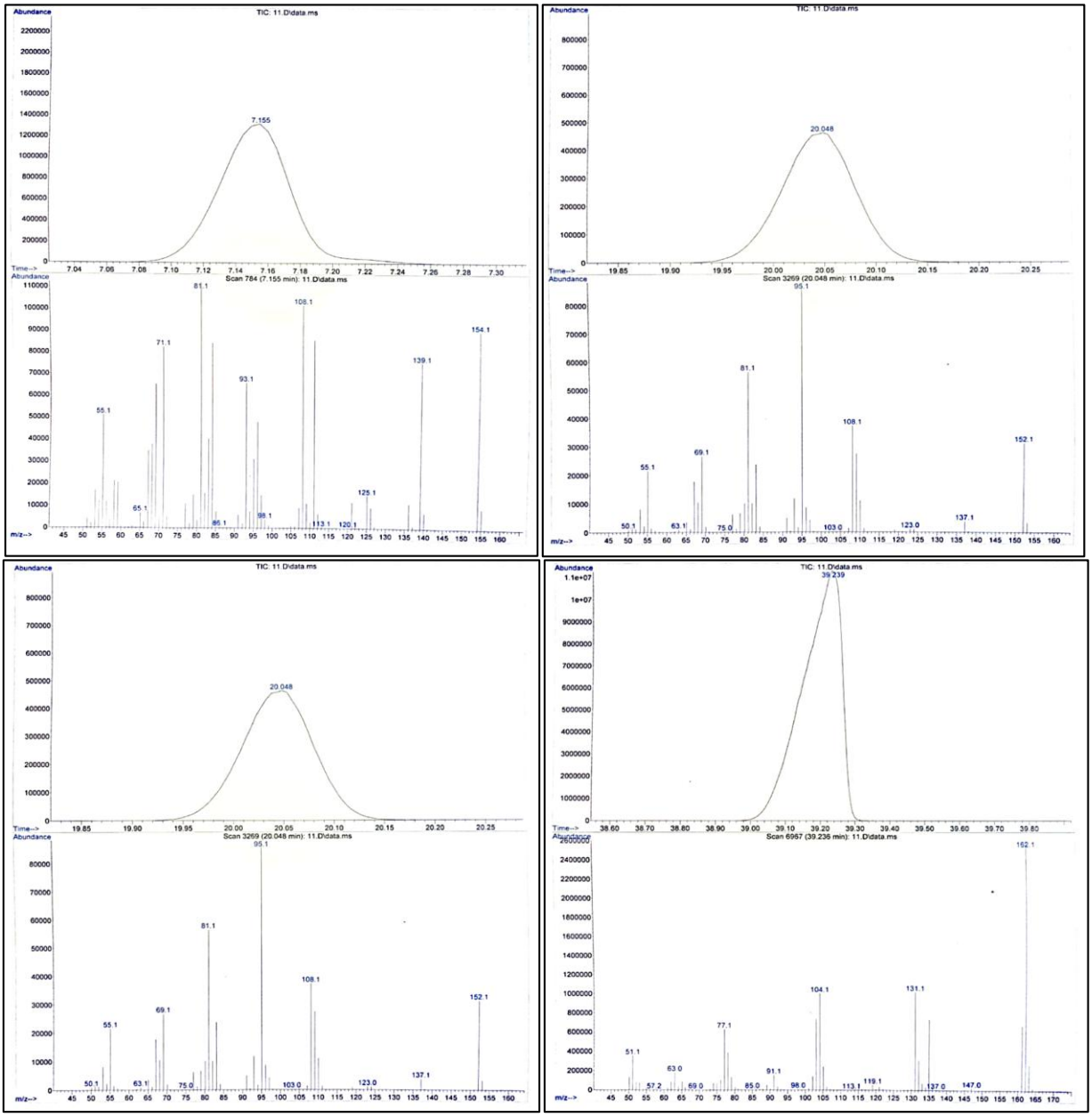

Figure 4. MS data of distillation oil of the bark of the *C. culilaban*

The results of the GCMS analysis of ethanol extract from the bark of *C. culilaban* obtained 25 peaks (Figure 5).

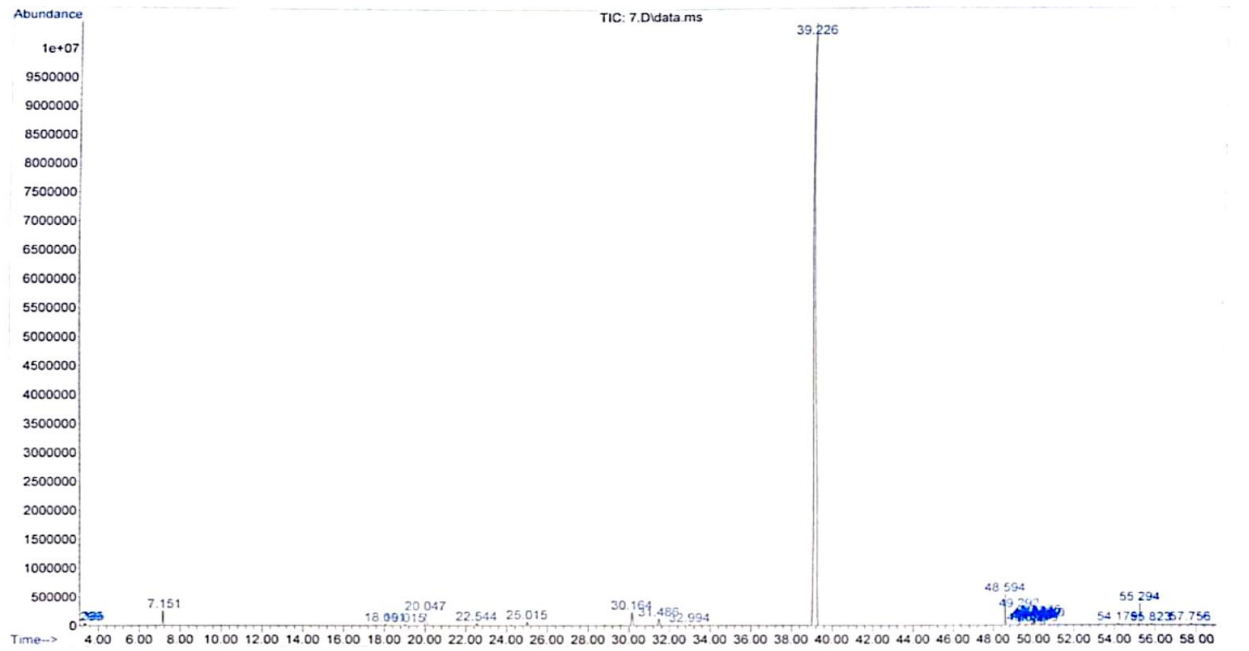

Figure 5. GC chromatogram of oil extracted from the bark of the *C. culilaban*

The main components of the ethanol extract of *C.Culilaban* bark can be seen in Table 2. From the samples analyzed by GCMS, it can be seen that there are differences in the levels of safrole, which is the most dominant component. Safrole produced from the distillation method is 86.78%, while in the maceration method it is 89.06%.

Table 2. The main components of oil extracted from the bark of *C. culilaban* Bark

No	Retention Time	Concentration (%)	Compound Name
1	20.048	1.35	(+)-2-Bornanon
2	30.116	1.43	Terpineol
3	39.226	89.06	Safrole
4	48.596	1.26	Spathulenol
5	55.295	1.73	Methoxyeugenol

Five main components of the extract are visible in the MS data (Figure 6), namely (+)-2-Bornanon, Terpineol, Safrole, Spathulenol, and Methoxyeugenol.

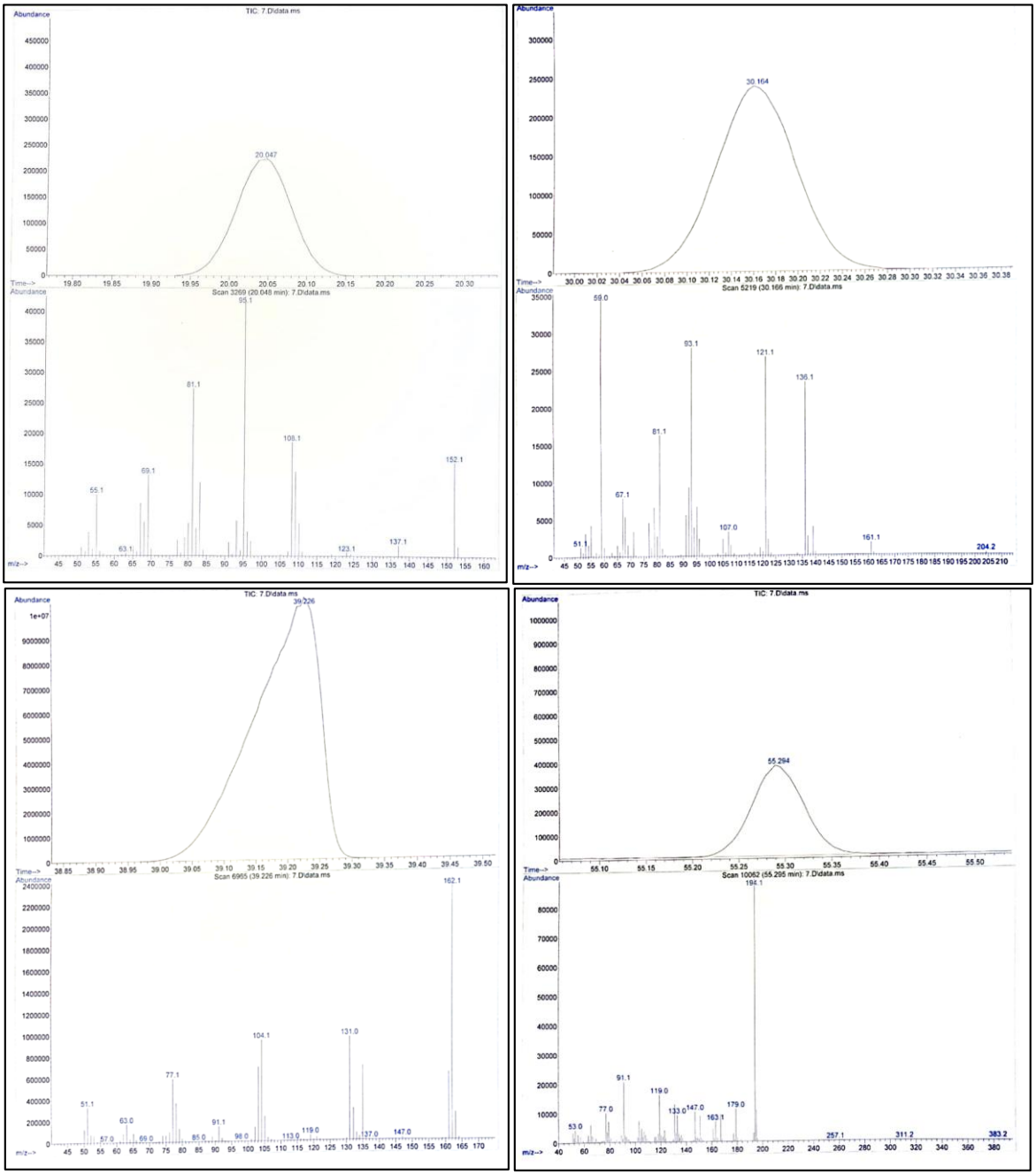

Figure 6. MS data of oil extracted from the bark of the *C. culilaban*

The antibacterial test results of the good diffusion method with the agar medium used are NA media that has been inoculated with S. aureus and E. coli bacteria. The sample was dissolved in DMSO solvent and made in five concentration variations, namely 20%, 40%, 60%, 80%, and 100%. Amoxicillin was used as a positive control, and DMSO solvent was used as a negative control. The clear zone indicates the presence of bacterial inhibitory activity. Antibacterial test value data for both samples are shown in Table 3.

Table 3. Antibacterial activity test values of *C. culilaban* bark distillate and extract samples against *S. aureus* and *E. Coli* bacteria.

Bacteria	Sample concentration	Inhibition zone diameter (mm)			
		Distillation oil sample	Maseration oil sample	Positive control	Negative control
S.aureus	20	NA	10.37 ± 0.21	49.10 ± 0.10	NA
	40	NA	10.67 ± 0.58		
	60	NA	13.47 ± 1.14		
	80	NA	12.84 ± 0.14		
	100	NA	11.70 ± 0.72		
E. coli	20	11.13 ± 0.06	NA	44.00 ± 0.10	NA
	40	11.13 ± 0.10	NA		
	60	11.40 ± 0.10	NA		
	80	11.43 ± 0.06	NA		
	100	11.87 ± 1.15	NA		

Antibacterial is a compound used to inhibit bacteria, and antibacterial is usually found in an organism as a secondary metabolite. Based on these criteria, the antibacterial activity against *E. coli* bacteria in distilled oil is classified as strong. In contrast, the antibacterial activity against *S. aureus* bacteria has no inhibition zone. The antibacterial activity of distilled oil against *E. coli* and *S. aureus* bacteria has a different inhibition zone. Testing the antibacterial activity of each bacteria has its own bacterial inhibition effectiveness time; the effectiveness of bacterial inhibition does not always increase over time due to the bacteriostatic nature [23-24].

The difference in the bacteriostatic properties of *C. culilaban* essential oil against both bacteria is that the inhibition time of bacterial growth in *E. coli* is slower than in *S. aureus*, so when measuring the inhibition zone, there is no inhibition zone in S. Aureus bacteria. The minimum inhibitory concentration for Star anise essential oil (SAEO) against E. coli is lower than against *S. Aureus* [25]. Some SAEO components work on the lipid layer of the cell membrane, while others affect the cell cycle or inhibit DNA replication [26].

Antibacterial activity against *S. aureus* bacteria in the ethanol extract of *C. culilaban* is relatively strong, while antibacterial activity against E. coli bacteria does not have an inhibition zone. The antibacterial activity test of the ethanol extract of star anise skin against E. coli and S. aureus bacteria has different inhibition zones. This is because, in the ethanol extract of star anise skin, E. coli bacteria have better resistance so that no inhibition zone is formed. In contrast, S. aureus bacteria do not resist the *C. culilaban* extract.

Gram-positive bacteria have cell walls mostly made of peptidoglycan, while Gram-negative bacteria have more complex cell walls. This lipopolysaccharide layer strengthens the rigidity of the cell wall of Gram-negative bacteria through intermolecular cationic cross-linking [27]. This causes Gram-negative bacteria to become stronger, making it difficult for antibacterial compounds to penetrate. The thick peptidoglycan layer of Gram-positive bacteria acts as a barrier that makes it difficult for antibacterial compounds to enter the cell [28].

Antibacterial compounds can damage bacterial cells by damaging the cell membrane, DNA, and protein synthesis. Some antibacterial compounds include flavonoids, phenols, and alkaloids [29,30]. Phenolic compounds in essential oils and plant extracts can break peptidoglycan cross-links and break down cell walls. Once the cell wall is damaged, phenolic compounds will cause leakage of cell nutrients by damaging the hydrophobic bonds of cell membrane components (such as proteins and phospholipids) and dissolving the hydrophilic and hydrophobic components that bind. This process results in disruption of cell membrane permeability [9].

In the antibacterial test of ethanol extract of star anise skin for *S. aureus* bacteria, there was no increase in the inhibition zone that increased in proportion to the increase in sample concentration. The factors that influence this are thought to be due to the different diffusion capabilities of each concentration variation, which proves that the diameter of the inhibition zone does not always increase in proportion to the increase in antibacterial concentration; this is likely due to differences in the diffusion rate of antibacterial compounds in agar media, and different types and concentrations of antibacterial compounds (viscosity factors) also provide different diameters of the inhibition zone at certain times [31].

Based on the results of both samples, it can be stated that there are differences in the results of antibacterial activity tests of oil from distillation and maceration of star anise skin against *S. aureus* and E. *Coli* bacteria. This is because there are differences in the production of several main components. These different main components play a role in inhibiting bacterial growth. There was no inhibition zone in the oil antibacterial test using the distillation method against S. aureus bacteria. In contrast, there was an inhibition zone against *S. aureus* bacteria for the ethanol extract maceration method. In the ethanol extract of star anise skin, there are terpineol, spathulenol, and methoxy eugenol compounds, which have an affinity for bacteria with low lipid content (Gram-positive bacteria). Other factors that can affect the results of antibacterial activity tests include Media and incubation temperature, Agar depth, Exposure time, Surface cleanliness, and Surface aging [32].

Testing of distilled oil against *E. coli* bacteria showed an inhibition zone, while in the maceration method, there was no inhibition zone against *E. coli* bacteria. This is because the distilled oil contains eucalyptol and terpineol compounds, which have an affinity for bacteria and have a high lipid content (Gram-negative bacteria). The mechanism of antibacterial compounds, in general, is by damaging cell walls, changing membrane permeability, disrupting protein synthesis, and inhibiting enzyme activity. Compounds that play a role in damaging cell walls include phenols, flavonoids, and alkaloids [33].

As a component of bacterial cell defense, the cell wall is damaged, causing secondary metabolite compounds to enter deeper and disrupt other organelles. The cell membrane located right on the inside of the cell wall can be damaged by phenol, flavonoid, and saponin compounds. Some of these compounds can break down phospholipids into glycerol, carboxylic acid, and phosphoric acid, so the membrane cannot maintain its shape. As a result, the membrane leaks, and substances can enter and exit the cell uncontrollably so that metabolism is disrupted and bacteria are lysed. The overall activity of medicinal plant extracts results from the combined action of several compounds with synergistic, additive, or antagonistic activity [10].

4. Conclusion

The antibacterial activity of distillation oil from *C. culilaban* bark against *E. coli* bacteria is classified as strong with the highest inhibition zone size at a concentration of 60% with a size of 13.47 ± 1.14 mm, while for *S. aureus* bacteria, there is no antibacterial activity. The antibacterial activity of macerated oil from *C. culilaban* bark against *S. aureus* bacteria is classified as strong, with the highest inhibition zone size at a concentration of 100% with a size of 11.87 ± 1.15 mm. At the same time, there is no antibacterial activity against *E. coli* bacteria. The composition of the distillation oil from *C. culilaban* bark shows the presence of 21 components with four main components, namely Eucalyptol 4.02%, (+) -2-Bornanon 2.32%, terpineol 1.49%, and Safrole. The chemical composition of the ethanol extract of *C. culilaban* bark consists of 37 components with five main components, namely (+)-2-Bornanon 1.35%, Terpineol 1.43%, Safrole 89.06%, Spathulenol 1.26%, and Methoxyeugenol 1.73%

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