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Antibacterial Activity of Tropical Plants (Morinda citrifolia and Melaleuca leucadendra) against Pneumonia-causing Bacteria

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Abstract

This study aims to determine the antibacterial activity of tropical plants against bacteria that cause pneumonia. The initial stage is to prepare samples of tropical plants that are macerated with methanol and produce a thick extract, namely 38.388 grams of noni fruit with a yield of 13.422%, and cajuput leaves as many as 11.211 grams with a yield of 3.38%. In the next step, each sample was fractionated using VLC and produced ethyl acetate and methanol fractions. The bacterial activity test was carried out by disc diffusion method, and the MIC value was determined by liquid dilution method using UV-Vis spectrophotometry. Based on the results of antibacterial testing, the noni fruit extract and cajuput extract obtained the most active fraction in response to inhibiting bacteria causing pneumonia *(S. pneumonia*, *S. aureus*, and *K. pneumonia)* was the ethyl acetate fraction with a strong average response zone of inhibition up to very strong with MIC value of ethyl acetate fraction from noni fruit extract 6250 ppm each and MIC value of ethyl acetate fraction from cajuput extract *(Melaleuca leucadendra)* 390.625 ppm each; 390.625 ppm; and 195.312 ppm.

Keywords: Noni fruit, cajuput leaves, pneumonia bacterial

Abstrak (Indonesian)

Penelitian ini bertujuan untuk mengetahui aktivitas antibakteri tumbuhan tropis terhadap bakteri penyebab pneumonia. Tahap awal adalah menyiapkan sampel tumbuhan tropis yang dimaserasi dengan metanol dan menghasilkan ekstrak kental yaitu buah mengkudu sebanyak 38,388 gram dengan rendemen 13,422%, dan daun kayu putih sebanyak 11,211 gram dengan rendemen 3,38%. Tahap selanjutnya masing-masing sampel difraksinasi menggunakan KVC dan dihasilkan fraksi etil asetat dan metanol. Uji aktivitas bakteri dilakukan dengan metode difusi cakram, dan nilai KVC ditentukan dengan metode pengenceran cairan menggunakan spektrofotometri UV-Vis. Berdasarkan hasil pengujian antibakteri, ekstrak buah mengkudu dan ekstrak kayu putih diperoleh fraksi paling aktif dalam merespon bakteri penghambat penyebab pneumonia (*S. pneumonia*, *S. aureus*, dan *K. pneumonia)* adalah fraksi etil asetat dengan rata-rata kuat. Fraksi teraktif dihitung nilai KHMnya yaitu fraksi etil asetat dari ekstrak buah mengkudu masing-masing 6250 ppm dan nilai KHM fraksi etil asetat dari ekstrak kayu putih (*Melaleuca leucadendra*) masing-masing 390,625 ppm; 390,625 ppm; dan 195.312 ppm.

Kata Kunci: Buah mengkudu, daun kayu putih, bakteri pneumonia

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INTRODUCTION

Traditional medicine has long employed tropical plants to treat pneumonia and coughing. Tropical plants frequently encounter, such as cajuput (*Melaleuca leucandendra*) and noni (*Morinda citrifolia*), have a history of being utilized as medicinal herbs to treat a range of illnesses. Antibacterial activity in tropical plants has also been investigated in published scientific studies.

Pneumonia is an acute infection or inflammation in the lung tissue caused by various microorganisms, such as bacteria, viruses, parasites, and fungi, exposure to chemicals, or physical damage to the lungs. According to data on pneumonia from the WHO [1],

14% of all deaths in children under five years in the world are due to pneumonia. *Streptococcus pneumoniae* is the main cause of pneumonia in children. Pneumonia occurs in adults, especially those aged > 65 year, the main pathogens are microorganisms such as *Streptococcus pneumonia*, species of *Legionella, Hemophilus influenzae, Moraxella catarrhalis,* and *Staphylococcus aureus* [2].

Noni fruit was previously known to have activity in inhibiting the growth of *Escherichia coli* and *Salmonella typhy* bacteria [3]. Noni fruit is also reported to contain compounds from the flavonoid group which is thought to have antibacterial activity [4]. The research was also carried out on noni fruit extract (*Morinda citrifolia)* which was tested as an antibacterial against *Streptococcus mutants* bacteria [5].

Cajuput leaves are known to contain 1,8-sienol (30%-60%) which has antibacterial benefits [6]. Methanol extract from cajuput leaves also has antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* with inhibition zone diameters of 13.6 mm and 6.3 mm [7]. Research on the antibacterial ability of the Indian plant, *Melaleuca bracteata* which is still in the same genus as cajuput (*Melaleuca leucadendra)* showed that the plant had an antibacterial activity that was tested against pneumonia-causing bacteria such as *Staphylococcus aureus, Klebsiella pneumonia,* and *Streptococcus mutans* [8].

MATERIALS AND METHODS

Materials

Materials that used in this research were noni fruits (*Morinda citrifolia),* cajuput leaves (*Melaleuca leucadendra)*, technical n-hexane, technical ethyl acetate, technical methanol, aluminum foil, TLC plate, silica gel 60 $F_{254,}$ silica gel p.a, silica gel 60 (0.2-0.5) mm, *Blood Agar*, *Nutrient Agar* (NA), *Nutrient Broth* (NB), distilled water, $BaCl_2$, concentrated H_2SO_4, dimethyl sulfoxide (DMSO), ampicillin antibiotics, *Streptococcus pneumonia* bacteria from collection of Atma Jaya Catholic University Faculty of Medicine Laboratory, *Staphylococcus aureus* from collection of Laboratory Microbiology FMIPA Sriwijaya University, and *Klebsiella pneumonia* from Faculty of Medicine, University of Indonesia Laboratory.

Preparation Sample

Samples of noni fruits (*Morinda citrifolia)* and cajuput leaves (*Melaleuca leucadendra)* were collected from KM. 12 Sukarami district, Palembang city, South Sumatra. Fresh samples were then washed until clean from the impurities and dried at room temperature until dry. After drying, the sample was mashed with a grinder until it formed a powder called simplicia. Simplicia from noni fruits (*Morinda citrifolia)* and cajuput leaves (*Melaleuca leucadendra)* was obtained.

Extraction Sample

The samples were added to the bottle maceration as many as 286 g of noni fruits (*Morinda citrifolia)* simplicia and 300 g of cajuput leaves (*Melaleuca leucadendra)* simplicia. Take a sample then soak it in technical methanol. Maceration was conducted for 3 x 24 hours with repetition 3 times and stored in a protected place from sunlight every 24 hours was stirred.

Using filter paper Whatman 42, the sample filter separates the extract from the residue and gets a residue-free extract. The extract from the maceration sample is then concentrated using a rotary evaporator set to the boiling point of the solvent used. After getting the concentrated extract, then dried at room temperature. Each thick extract has determined results in the yield.

Vacuum Liquid Chromatography (VLC)

Vacuum liquid chromatography (VLC) is then used to separate thick methanol extracts of noni fruits and cajuput leaves. The stationary phase used is silica gel pa entered into the column. The sample was prepared by impregnation using silica gel 60 (0.2-0.5) mm with a ratio of 1: 2 to form a powder, then put into a column and eluted using 100% n-hexane followed by ethyl acetate 100% then 100% methanol. Collect eluate in a 500 mL bottle and then concentrated with a rotary evaporator. Concentration results eluate then stored at room temperature.

Bacterial Suspension Culture

Bacteria suspension culture was conducted from bacteria by taken one ose needle then inoculated with a scratch on the surface of the medium so that it is tilted after that incubated for 24 hours at 37 °C. Culture suspension bacteria where test bacteria are taken as much as 1 ose needle from results rejuvenation, then suspended to in 5 mL *of Nutrient Both* (NB) and stirred. Then suspension bacteria were equated the cloudiness with solution standard 0.5 McFarland (1.5×10^8 CFU/mL) using a UV-Vis spectrophotometer with a long 625 nm wave and absorbance range between 0.08-0.1.

Antibacterial Activity Test

Antibacterial activity test used the method of disc diffusion using a 6 mm diameter paper disc with test bacteria of *Streptococcus pneumonia, Staphylococcus*

aureus, and *Klebsiella pneumonia*. Activity test conducted with three repetitions. The bacteria to be tested were rubbed onto *Nutrient Agar* (NA) media using a *cotton swab* and allowed to stand for 15 minutes. Disc paper is dipped in methanol extract and fractionation results in noni and cajuput leaves that have been diluted to a concentration of 5%; 50,000; 25,000; 12,500; 6,250 ppm with the positive control (ampicillin) and negative control (DMSO 10%). A Paper disc that has been dipped earlier is placed using an inoculating loop on the surface of the media with bacteria. Then the media containing bacteria incubate for 24 hours at 37 °C. After incubation, observe the bacteria's growth and check the inhibition zone formed with the ruler [9].

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration determines the fraction that shows the most activity or gives t. MIC is determined by dilution method or graded dilution with a ratio of 1:2 (w/v). Prepared as many as 11 test tubes that have been sterilized. Then each tube was labeled 1-9, then tube 10 was labeled K (+) and was a positive control containing a bacterial suspension that had been adjusted to McFarland's standard. Tube 11 was labeled K (-) or negative control containing 10% DMSO. Tube 1 was filled with 4 mL of selected active fraction from the extract with a concentration of 6250 ppm. Tubes 2-9 were filled with 2 mL of *Nutrient* liquid medium *broth*. Then, 2 mL of the solution was taken from tube 1 and put into tube 2, mixed until homogeneous so that a concentration of 50% was obtained. The same thing was done until tube 9 to get the concentration of the extract with a ratio of 1:2 (w/v). After that, 1 mL of the bacterial suspension which had been equalized with McFarland's standard was added into tubes 1-9.

All tubes were then incubated for 1×24 hours at 37 °C with 3 repetitions of incubation. Each time incubated, the tube observed by the Turbidimetry method or the observation of turbidity. If the turbidity of the tube is still equal to or cloudier than the K (+) tube, it can be said that the bacteria can still thrive, but if the solution in the tube looks clearer than the K (+) tube, it means that growth is starting to be inhibited. Furthermore, absorbance (OD) measurements were carried out using a UV-Vis spectrophotometer at a length of 600 nm wave, measurement absorbance conducted before and after incubation. Testing processes were conducted in 3 repetitions to determine MIC value from difference score absorbance before and after incubation [10].

Data Analysis

Result data testing the antibacterial activity of the extra fraction of most active noni fruits and cajuput leaves was analyzed statistically using the one-way ANOVA with the Statistical program Product Services Solution (SPSS) for comparative data on each sample concentration to the zone of inhibition.

RESULTS AND DISCUSSION

Sample Extraction

Fruit 3 kg of fresh noni fruits and cajuput leaves 1 kg fresh dried at room temperature. The weight of noni fruit was collected after drying as much as 700 g with a water content of 76.67% and b cajuput leaves were obtained after drying as much as 780 g with a water content of 22%. After drying, the samples were mashed using a grinder until producing the simplicia powder of noni fruits and cajuput leaves. Simplicia powder weight noni fruits produced as many as 500 g with a yield of 16.67 % as well as simplicia powder of cajuput leaves as many as 660 g with a 66% yield.

Simplicia powder 286 g of noni fruits and simplicia powder 331 g of cajuput leaves were extracted by maceration using methanol solvent and noni fruits produce black maser brown and cajuput leaves produce macerate colored dark green. Macerate was then concentrated using a rotary evaporator and the results obtained in the form of each thick extract of noni fruits as much as 38.388 g with a yield of 13.442% and cajuput leaves as much as 11.211 g with 3.38% yield.

Fractionation Extract Methanol Sample

Separation of crude extract methanol of noni fruit as many as 25 g and cajuput leaves as many as 8 g with a 1:2 ratio with silica gel 60 (0.2-0.5) mm was carried out using vacuum liquid chromatography (VLC). Separation of crude extract methanol of noni fruit and cajuput leaves using vacuum liquid chromatography (VLC) was eluted using 100% n-hexane eluent 3 times each 400 mL then followed by 100% ethyl acetate 3 times each 400 mL and 100% methanol 3 times each 400 mL and then results in eluate obtained concentrated with rotary evaporator then dried at room temperature. The results obtained in the n-hexane fraction are very small, in the noni fruits obtained an ethyl acetate fraction of 0.5052 g and the fraction of methanol of 0.5605 g, and cajuput leaves obtained an ethyl acetate fraction of 3.256 g and fraction methanol of 3.7547 g.

Antibacterial Activity of Extract Methanol Samples and Fractions to Bacteria Causes of Pneumonia

The disc diffusion method used to evaluate the antibacterial activity of noni fruits (*Morinda citrifolia)* and cajuput leaves (*Melaleuca leucadendra)* as well as the fractions were carried out against bacteria that cause pneumonia, namely gram-positive bacteria *S. pneumonia* and *S. aureus* and gram-negative bacteria *K pneumoniae.* A test for antibacterial activity was performed to determine the effect of the extract methanol noni fruits (*Morinda citrifolia)* and cajuput leaves (*Melaleuca leucadendra)* as well as its fractions in inhibiting the growth of bacteria that cause pneumonia. The results of the antibacterial activity of noni fruits and cajuput leaves, as well as their fractions, on the growth of bacteria (*S. pneumonia, S. aureus,* and *K. pneumonia)* are characterized by the formation of a clear zone of inhibition, as shown in **Table** 1 and **Table** 2.

The data of the inhibition zone measurements in **Table** 1, the average diameter of the inhibition zone by the positive control using ampicillin was 23 mm with a very strong category, and in the negative control using dimethyl sulfoxide (DMSO) there was no inhibition zone diameter. The extract of methanol noni fruits, for crude extract methanol at a concentration of 50.000 ppm, and 25.000 ppm has a strong inhibition zone response in inhibitory activity bacteria, *S. aureus,* and moderate inhibition zone response in inhibitory activity bacteria *S. pneumonia,* and *K. pneumonia.*

Concentrations of 12.500 ppm and 6.250 ppm have moderate inhibition zone response in inhibitory activity bacteria *S. pneumonia, S. aureus,* and *K. pneumonia.* Ethyl acetate fraction of noni fruits at a concentration of 50.000 ppm, and 25.000 ppm have strong inhibition zone responses in inhibitory activity bacteria, *S. aureus,* and *K pneumonia.* Concentrations 12.500 ppm and 6.250 ppm have moderate inhibition zone response in inhibitory activity bacteria *S. pneumonia, S. aureus,* and *K. pneumonia.* Fraction methanol at a concentration of 50.000 ppm has strong inhibition zone response inhibitory activity bacteria, *S. aureus,* and *K pneumonia.* Concentrations of 25.000 ppm, 12.500 ppm, and 6.250 ppm have moderate inhibition zone response in inhibitory activity bacteria *S. pneumonia, S. aureus,* and *K. pneumonia.*

Classification of the zone of inhibition after 24 hours of incubation formed around the *disc* which has a size of 5 mm, then the inhibition zone formed is included in the weak category. If the inhibition zone formed is 6-10 mm in diameter, then the inhibition zone formed is in the medium category, 11-20 mm in diameter is in a strong category, and 20 mm in diameter is in the very strong category [11].

The data of the inhibition zone measurements in Table 2, the average diameter of the inhibition zone by the positive control using ampicillin was 23 mm with a very strong category, and in the negative control using dimethyl sulfoxide (DMSO) there was no inhibition zone diameter. The extract of methanol cajuput leaves, for crude extract methanol at concentrations of 50,000 ppm, 25,000 ppm, 12,500 ppm, and 6,250 ppm had moderate inhibition zone response in inhibitory activity bacteria *S. pneumonia,* and *K. pneumonia,* while at concentrations of 50,000 ppm, 25,000 ppm, 12,500 ppm had strong inhibition zone response in inhibitory activity bacteria *S. aureus* and at a concentration of 6250 ppm had moderate inhibition zone response in inhibitory activity bacteria *S. aureus.*

Ethyl acetate fraction of cajuput leaves at a concentration of 50,000 ppm, and 25,000 ppm has very strong inhibition zone response in inhibitory activity bacteria *S. pneumonia,* and *S. aureus,* and has strong inhibition zone response in inhibitory activity bacteria *K. pneumonia,* at concentration 12,500 ppm has very strong inhibition zone response in inhibitory *S. aureus.* Concentrations of 12,500 ppm and 6,250 ppm have strong inhibition zone responses in inhibitory activity bacteria *S. pneumonia,* and *K. pneumonia.* Fraction methanol at concentrations of 50,000 ppm and 25,000 ppm has a strong inhibition zone response in inhibitory activity bacteria *S. pneumonia,* and *S. aureus* and have a moderate inhibition zone response in inhibitory activity bacteria *K. pneumonia,* at concentrations of 12,500 ppm and 6,250 ppm had strong inhibition zone response in inhibitory activity bacteria *S. pneumonia* and has moderate inhibition zone response in inhibitory activity bacteria *S. aureus* and *K. pneumonia.*

Determination of the effectiveness comparison data of each sample concentration and test compound against the zone of inhibition, statistical analysis was carried out using the test results *one-way ANOVA* with the *Statistical program Product Services Solutions* (SPSS). Test results *of one-way ANOVA* can be against the three bacteria can be figured in **Table** 3-8.

Based on Table 3-8, the results show that the significance value for the one-way *test* on the extract methanol of noni fruits as well as its fractions and the extract methanol cajuput leaves as well as the fractions for all test bacteria (*S. pneumonia, S. aureus* and *K. pneumonia)* showed that the significance value of $p < 0.05$ which means H_0 is rejected and H_1 is accepted, then extract methanol noni fruits as well as its fractions

and extracts methanol cajuput leaves as well as its fractions in inhibiting the growth of *S. pneumonia*, *S. aureus* and *K. pneumonia* have a comparison of the effectiveness of each sample concentration to the zone of inhibition.

Table 1. The diameter inhibition zone of extract methanol noni fruits (*Morinda citrifolia*) and their fractions against pneumonia-causing bacteria (*S. pneumonia, S. aureus*, and *K. pneumonia*).

Extracts	Conc. (ppm)	Inhibition zone diameter (mm) SD Average±		
		S pneumoniae	*S. aureus*	*K. pneumonia*
Positive control (ampicillin)	500	25 ±1	24 ±2.64	20.33 ±1.53
Negative control (DMSO)		6 ±0	6 ±0	6 ±0
Crude extract methanol	50,000	9.33 ±2.31	12 ±1	10.67 ±0.57
	25,000	8 ±1.73	11.33 ±0.57	9.67 ±0.57
	12,500	7.67 ±1.15	9.67 ±0.57	9 ±0
	6,250	7 ±0.86	9 ±1	8.33 ±0.57
Fraction ethyl acetate	50,000	10 ±1	14.33 ±0.57	16.67 ±1.52
	25,000	9.33 ±0.57	12 ±1	14 ±0
	12,500	8.67 ±0.57	10 ±0	13 ±1
	6,250	8 ±1	9.33 ±0.57	11.67 ±2.08
Fraction methanol	50,000	9.67 ±1.53	12.33 ±0.57	14.33 ±0.57
	25,000	8.67 ±1.53	11 ±0	12.67 ±0.57
	12,500	8.33 ±1.15	10.67 ±0.57	11 ±1
	6,250	7.5 ±0.86	9.67 ±0.57	9.67 ±0.57

Remarks: inhibition zone including disc diameter (6 mm).

Table 2. The diameter of the extract inhibition zone methanol extract of cajuput leaves (*Melaleuca leucadendra)* and their fractions against pneumonia-causing bacteria (*S. pneumonia, S. aureus*, and *K. pneumonia)* using the disc diffusion method

Test compound	Conc. (ppm)	Inhibition zone diameter (mm) SD Average±		
		S pneumoniae	*S. aureus*	*K. pneumonia*
Positive control (ampicillin)	500	25 ±1	24 ±2.64	20.33 ±1.53
Negative control (DMSO)		6 ±0	6 ±0	6 ±0
Crude extract methanol	50,000	9 ±1	26.33 ±0.57	11.67 ±1.15
	25,000	8.33 ±0.57	24.67 ±0.57	11 ±1
	12,500	7.67 ±0.57	19 ±2.64	9.67 ±0.57
	6,250	6.83 ±0.28	14 ±1.73	9 ±1
Fraction ethyl acetate	50,000	27.33 ±2.52	30 ±3	18.67 ±1.15
	25,000	20.67 ±1.15	25.67 ±1.15	15.67 ±1.15
	12,500	18.67 ±0.57	23.33 ±1.15	14.33 ±0.57
	6,250	15.67 ±3.21	17 ±1.73	13 ±0
Fraction methanol	50,000	18.33 ±4.04	13 ±1	9 ±1
	25,000	16.67 ±4.93	11.67 ±0.57	8 ±1
	12,500	12 ±1.73	10 ±1	8 ±1
	6,250	11 ±1	9.33 ±0.57	7.33 ±1.52

Remarks: inhibition zone including disc diameter (6 mm)

Table 3. The test results *one-way ANOVA* antibacterial activity of noni fruits extract against the bacteria *S. pneumonia*

ANOVA

DIAMETER (MM)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	781.231	12	65.103	42.672	.000
Within Groups	39.667	26	1.526		
Total	820.897	38			

Table 4. The test results *one-way ANOVA* antibacterial activity of noni fruits extract against *S. aureus bacteria*

ANOVA

DIAMETER (MM)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	575.641	12	47.970	81.341	.000
Within Groups	15.333	26	.590		
Total	590.974	38			

Table 5. The test results *one-way ANOVA* antibacterial activity of noni fruits extract against bacteria *K. pneumonia*

ANOVA

DIAMETER (MM)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	409.231	12	34.103	34.103	.000
Within Groups	26.000	26	1.000		
Total	435.231	38			

Table 6. The test results *one-way ANOVA* antibacterial activity of cajuput leaves extract against bacteria *S pneumoniae*

ANOVA

DIAMETER (MM)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1612.667	12	134.389	26.982	.000
Within Groups	129.500	26	4.981		
Total	1742.167	38			

Table 7. The test results *one-way ANOVA* antibacterial activity of cajuput leaves extract against bacteria *S. aureus*

ANOVA

DIAMETER (MM)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1774.923	12	147.910	63.390	.000
Within Groups	60.667	26	2.333		
Total	1835.590	38			

Table 8. The test results *one-way ANOVA* antibacterial activity of cajuput leaves extract against bacteria *K. pneumonia*

ANOVA

DIAMETER (MM)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	636.308	12	53.026	48.093	.000
Within Groups	28.667	26	1.103		
Total	664.974	38			

Minimum Inhibitory Concentration (MIC) of Ethyl Acetate Fraction from Noni fruits Extract and Cajuput Leaves Extract

The results of measuring the OD (optical density) value in the MIC test using a spectrophotometer (600 nm) showed inhibition of the growth bacteria of *S. pneumoniae*. *S. aureus* and *K. pneumonia* with certainty by looking at the decrease in OD values before and after incubation are presented in **Table** 9 and 10. The MIC value can be determined by measuring the difference between the OD values before and after incubation. A negative OD value indicates a decrease in the absorbance value, which means a decrease in the number of bacteria after 24 hours of incubation, while a positive OD value indicates no decrease but an increase in absorbance value, which means that there is still bacterial growth after 24 hours of incubation [11].

The most active fraction from the samples of noni fruits extract and cajuput leaves extract is ethyl acetate fraction which has a strong average inhibition zone response in inhibitory activity bacteria *S. pneumonia*. *S. aureus* and *K. pneumonia*. OD (optical density) values on *S. pneumonia*, *S. aureus*, and *K. pneumonia* displayed in **Tables** 9-10.

The data of value the difference in optical density (ΔOD) ethyl acetate fraction from noni fruits extract in the Table 9 has the positive and negative control, the OD value is negative. It means that the positive and negative control could inhibit the growth of bacteria.

The value minimum inhibition concentration of ethyl acetate fraction from noni fruits extract on bacteria *S.* pneumonia with a concentration of 6250 ppm has an OD value is negative with an OD value is -0.638 which means a concentration of 6250 ppm could inhibit the growth of bacteria. Concentrations of 3125 ppm to 24.414 ppm have positive OD values. Bacteria *S. aureus* on the test tube with concentrations of 6250 ppm and 3125 ppm have OD values is negative -1.213 and -0.028 which means concentrations up to 3125 ppm could inhibit the growth of bacteria. Concentrations of 1562.5 to 24.414 ppm have positive OD values. Bacteria *K. pneumoniae* in test tubes with concentrations of 6250 ppm and 3125 has OD values

is negative -0.693 and -0.051028 which means concentrations up to 3125 ppm could inhibit the growth of bacteria. Concentrations of 1562.5 ppm to 24.414 ppm have positive OD values.

Table 9. The value of the difference in optical density (ΔOD) ethyl acetate fraction from noni fruits extract to bacteria causes of pneumonia (*S. pneumoniae, S. aureus and K. pneumoniae)*

Conc. fraction (ppm)	ΔOD		
	S pneumoniae	*S. aureus*	*K. pneumonia*
Control (+) (ampicillin)	-0.062	-0.095	0.136
Control (-) (extract)	-0.005	-0.003	-0.001
6250	-0.638	-1.213	-0.693
3125	0.030	-0.028	-0.051
1562.5	0.213	0.042	0.005
781.25	0.111	0.175	0.235
390.625	0.245	0.009	0.417
195.312	0.479	0.215	0.402
97.656	0.769	0.557	0.578
48.828	0.552	0.663	0.364
24.414	0.461	0.431	0.282

Table 10. The value of the difference in optical density (ΔOD) ethyl acetate fraction from cajuput leaves extract to bacteria causes of pneumonia (*S. pneumoniae, S. aureus,* and *K. pneumonia)*

Conc. fraction (ppm)	ΔOD		
	S pneumoniae	*S. aureus*	*K. pneumonia*
Control (+) (ampicillin)	-0.062	-0.095	0.136
Control (-) (extract)	-0.005	-0.003	-0.001
6250	-1.32	-1.2	-2.299
3125	-0.203	-1.154	-2.246
1562.5	-0.141	-0.72	-2.010
781.25	-0.482	-0.566	-1.674
390.625	-0.007	-0.118	-0.648
195.312	0.097	0.289	-0.184
97.656	0.428	0.326	0.327
48.828	0.457	0.229	0.261
24.414	0.526	0.015	0.255

The data of value the difference in optical density (ΔOD) ethyl acetate fraction from cajuput leaves extract **Table** 10, the positive and negative control, the results of OD value are negative, this means that the positive and negative control could inhibit the growth of bacteria. The value minimum inhibition concentration of ethyl acetate fraction from cajuput leaves extract on bacteria *S. pneumonia* with concentrations of 6250 ppm to 390.625 ppm have OD value is negative with each OD value of -1.32; -0.203; -0.141; -0.482 and -0.007 which means concentrations up to 390.525 ppm could inhibit the growth of bacteria. Concentrations of 195.312 ppm to 24.414 ppm have positive OD values which means concentrations more than 195.312 ppm could not inhibit the growth of bacteria after 24 hours of incubation.

Bacteria *S. aureus* on the test tube with concentrations of 6250 ppm to 390.625 ppm have a negative OD value of -1.2; -1.154; -0.72; -0.566 and –0.118 which means concentrations up to 390.625 ppm could inhibit the growth of bacteria. Concentrations of 195.312 ppm to 24.414 ppm have positive OD values which means concentrations more than 195.312 ppm could not inhibit the growth of bacteria after 24 hours of incubation. Bacteria *K. pneumonia* on the test tube with concentrations of 6250 ppm to 195.312 ppm have OD value is negative with each OD value of -2.299; -2.246; -2.01; -1,647; -0.648 and -0.184 which means concentrations up to 195,312 ppm could inhibit the growth of bacteria. Concentrations of 97.656 ppm to 24.414 ppm have positive OD values which means concentrations more than 97.656 ppm could not inhibit the growth of bacteria after 24 hours of incubation.

Based on the results of research antibacterial activity of tropical plants (*Morinda citrifolia)* and *(Melaleuca leucadendra)* against pneumonia-causing bacteria (*S. pneumonia, S. aureus,* and *K. pneumonia*), has antibacterial activity and can inhibit the growth of bacteria. Because it is suspected that there are secondary metabolites that can inhibit the growth of *S. pneumonia, S. aureus,* and *K. pneumonia.* According to Nugraheni [12], noni fruits contained flavonoids and coumarin compounds as antibacterial candidates. Activities of flavonoids, moreover, are related to their ability to form complexes with proteins from the cell wall, which will result in damage to the permeability of the bacterial cell wall. Flavonoids have an antibacterial effect because of their ability to interact with DNA of bacteria. Each flavonoid compound could damage the hydrogen bridge bonding of the strands of the DNA double chain, disrupting the stability of the double chain structure of bacterial DNA later influencing the whole process of bacterial growth and metabolism. Flavonoids can also produce energy transduction that will affect the bacterial cytoplasm and slow the motility of bacteria. It is known based on the presence of hydroxyl ions in flavonoids that can chemically alter organic compounds and nutrient

transport that can cause toxic effects on the bacterial cells.

According to Siddique [13], cajuput leaves extract contains tannin, benzyl alcohol, and eugenol as an antibacterial candidate. Tannins exhibit their antibacterial activity by forming a complex compound with the protein-rich amino acid proline, which causes protein leakage, breakdown of cell walls, and bacterial death. Benzyl alcohol has solvent properties of fat and protein denaturation, which can cause damage to the bacterial cell membrane. The protein denaturation process involves changes in molecular protein stability and causes changes in protein structure and coagulation. Proteins that undergo denaturation will lose their physiological activity and ability to function properly. Changes that occur in the protein in the cell wall will lead to increased cell permeability. Damage and increased permeability of the cell then will damage the bacterial cells. Shapiro [14] reported eugenol can significantly increase the membrane's permeability profile and has disruptive action on the cytoplasmic membrane. Eugenol has been scientifically proven to be pharmacologically active against several bacteria, both Gram-negative and Gram-positive, as well as fastidious and facultative anaerobic oral bacteria.

This research showed high concentrations of *Morinda citrifolia* fruits and *Melaleuca leucadendra* leaves extract to inhibit the growth of *S. pneumonia, S. aureus*, and *K. pneumonia* bacteria. The differences in the antibacterial activity of type Gram-positive and negative bacteria are Gram-positive bacteria have a larger inhibition zone than gram-negative bacteria. This can be caused by the type of bacteria used in the research. *K. pneumonia* bacteria is a Gram-negative bacteria that have high phospholipids on its cell wall, making them more permeable than the Gram-positive bacteria. Gram-negative bacteria have two membranes and a distinct periplasm gap between them that Gram-positive bacteria do not have. There are enzymes in the periplasm that can break down foreign compounds that enter the bacterial cell from the outside. Additionally, Gram-negative bacteria have a lipopolysaccharide-rich hydrophilic coating on their outer membrane that acts as a barrier to the entry of antimicrobial compounds. However, *S. pneumonia* and *S. aureus* Gram-positive bacteria have outer membrane structures and cell walls that differ from Gram-negative bacteria. Gram-positive bacteria only have a single plasma membrane, and the majority of Gram-positive bacteria are more sensitive to antimicrobial or antibacterial materials [15].

CONCLUSION

Tropical plants include noni fruits, and cajuput leaves can inhibit pneumonia-causing bacteria. Ethyl acetate fraction from noni fruits (*Morinda citrifolia*) and ethyl acetate fraction from cajuput leaves (*Melaleuca leucadendra)* have the strongest zone inhibition. They inhibit the growth of pneumonia-causing bacteria *S. pneumonia, S. aureus*, and *K. pneumonia* with a minimum inhibitory concentration value (MIC) each of 6,250 ppm, and minimum inhibitory concentration value (MIC) each of 390.625 ppm; 390.625 ppm; and 195.312 ppm.

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REFERENCES

[1] World Health Organization, Pneumonia Fact Sheets. 2020. [Online]. Available: https://www.who.int/news-room/factsheets/detail/pneumonia

[2] A. Krishnamurthy, and E. Palombo, "The Microbiology of Respiratory System Infections," In *Clinical Microbiology: Diagnosis, Treatments and Prophylaxis of Infections*. Australia, Melbourne: Academic Press, 2016, vol. 1, pp. 263–279.

[3] F. Malinggas, Pangemanan, and N.W. Mariati, "Inhibitory test of noni fruit extract (M. Citrifolia, L) against streptococcus growth mutants in vitro," *Pharmaceutical Scientific Journal UNSRAT*, vol. 4 pp. 2302 – 2493, Apr 2015.

[4] W. Darwis, V. S. Anggun, M. Choirul, "The effectiveness of noni Juice (Morinda citrifolia L.) against Salmonella typhi," Department of Biology FMIPA Bengkulu University, Biological Conservation, vol. 6, pp. 6-12, 2009.

[5] S. Sogandi, P. Nilasari, "Identification of active compounds of noni fruit extract (Morinda citrifolia L.) and its potential as a dental caries inhibitor," *Indones. J. Pharm*., vol. 9, no. 2, pp. 73-81, Nov 2019.

[6] K. K. Noor, and R. Anto, "Potential development of cajuput oil industry", *Department of Forestry Bogor Forestry Research and Development Agency*, vol. 2 pp. 26-39, Mar 2014.

[7] N. M. Al-Abd, Z. M. Nor, M. Mansor, F. Azhar, M. S. Hasan, and M. Kassim, "Phytochemical characterization of Melaleuca cajuputi extract." *BMC Complement Altern. Med.*, vol. 10, pp. 1–13, Oct 2015.

[8] P. Goswami, K. V. Sajendra, C. Amit, K. T. Venkatesha, S. V. Ram, R. S. Ved, P. D. Mahendra, S. C. Chandan, and C. P. Rajendra. "Chemical composition and antibacterial activity of Melaleuca bracteata essential oil from india: a natural source of methyl eugenol," *Nat. Prod. Commun*.,vol. 12, pp. 965-968, Apr 2017.

[9] Y. Andriani, H. Mohamad, B. Kesaven, M. I. Abdullah, H. Amir, "Phytochemicals, antibacterial and antibiofilm activities against pseudomonas aeruginosa of various extracts and fractions of mangrove-associated, Hibiscus tiliaceus," *J. Sustain. Sci. Manag*., vol 12, no. 2, pp. 45-5, Mar 2017.

[10] D. R. Ningsih, Zusfahair and D. Kartika, "Soursop (annona chloroform extract activity Muricata Linn.) as antibacterial against propionibacterium acne," *J. Sci. Res*., vol. 22, pp. 90-97, Nov 2017.

[11] F. Riska, S. Puguh, and Sarwiyono. "Inhibition activity of Moringa oleifera juice to growth of Streptococcus agalactiae and Streptococcus uberis bacteria caused mastitis dairy cows," *Journal of the Faculty of Agriculture Universitas Brawijaya*, vol 1, no. 1, pp. 1-18, Jan 2015.

[12] E.R. Nugraheni, "Antibacterial activity of ethyl acetate the extract of noni fruit (Morinda citrifolia L.) against bacterial spoilage in fish," *Proc. IOP Conference Series*, 2017, vol. 193, pp. 12-19,

[13] S. Siddique, "Chemical composition, antibacterial and antioxidant activities of essential oils from leaves of three Melaleuca species of Pakistani Flora Arabian," *J. King. Saud Univ Sci*., vol. 13, pp. 67-74, Jan 2020.

[14] M. Abers, S. Schroeder, L. Goelz, A. Sulser, T.S. Rose, K. Puchalski, and J. Langland, "Antimicrobial activity of the volatile substances from essential oils," *BMC Complement Med. Ther*., vol. 21, no. 1, pp. 124-138, Apr, 2021.

[15] K. Konate, A. Hilou, J.F. Mavoungou, A.N. Lepengue, A. Souza, N. Barro, J.Y. Datte, B.M. Batchi, and O.G. Nacoulma, "Antimicrobial activity of polyphenol-rich fractions from Sida alba L. (Malvaceae) against cotrimazol-resistant bacteria strains," *Ann. Clin. Microbiol. Antimicrob*., vol. 1, pp. 1-6, 2012.