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TOXICITY AND ANTIBACTERIAL ACTIVITY OF *Rhizophora apiculata* MANGROVE USING DIFFERENT SOLVENT EXTRACTION

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Abstract

Rhizophora apiculata is a type of mangrove widely used as a traditional medicine. This potential is produced by the content of secondary metabolites that environmental conditions, age, and other factors can influence. This study aims to determine the best solvent for *R. apiculata* extract based on toxicity and antibacterial parameters. *R. apiculata* used in this study came from the Kuala Langsa area and Telaga Tujuh Island (East Aceh), with different tree diameters (2.4, 4.8, 5, 13, 23.3, & 26 cm). The samples were macerated three times for 24 hours using 99.8% methanol (MeOH) and 99.9% ethanol (EtOH) solvents. The samples were boiled for 15 minutes at a temperature of 85–90°C using aquades as the solvent. Extraction using a ratio of 10:1 (v/w) with 2 g of simplicia powder and 20 mL of solvent. The toxicity test was conducted using the brine shrimp lethality assay method and the antibacterial test using the disk diffusion assay method. Toxicity tests showed that the ethanol extract had the highest toxicity value with an LC_{50} value of 26.879 µg/mL. Aquadest extract produced the highest inhibition zone with a value of 11.37±0.85 mm in *Escherichia coli* and 17.67±1.19 mm in *Staphylococcus aureus*. The results of two-way ANOVA showed that the solvent had a significant effect on the bacterial inhibition zone ($p<0.05$), while tree diameter had no significant effect ($p<0.05$). Ethanol solvent extract produced the highest toxicity value, while aquadest solvent extract produced the largest inhibition zone.

Keywords: *Artemia salina*, East Aceh, environmental condition, maceration, pathogen

Toksisitas dan Aktivitas Antibakteri Mangrove *Rhizophora apiculata* dengan Pelarut Ekstraksi yang Berbeda

Abstract

Rhizophora apiculata merupakan jenis mangrove yang banyak digunakan sebagai obat tradisional. Potensi ini dihasilkan oleh kandungan metabolit sekunder yang dapat dipengaruhi oleh kondisi lingkungan,

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usia, dan beragam faktor lainnya. Penelitian ini bertujuan untuk menentukan pelarut terbaik ekstrak *R. apiculata* berdasarkan parameter toksisitas dan antibakteri. *R. apiculata* yang digunakan dalam penelitian ini berasal dari daerah Kuala Langsa dan Pulau Telaga Tujuh (Aceh Timur), dengan ukuran diameter pohon yang berbeda (2,4; 4,8; 5; 13; 23,3; & 26 cm). Ekstraksi dengan pelarut metanol 99,8% (MeOH) dan etanol 99,9% (EtOH) dilakukan dengan maserasi (3x24 jam), ekstraksi dengan pelarut akuades dilakukan dengan perebusan (15 menit pada suhu 85-90°C). Ekstraksi menggunakan perbandingan 10:1 (v/w) dengan 2 g serbuk simplisia dan 20 mL pelarut. Uji toksisitas dilakukan dengan metode *brine shrimp lethality assay* dan uji antibakteri menggunakan metode *disk diffusion assay*. Uji toksisitas menunjukkan ekstrak etanol memiliki nilai toksisitas tertinggi dengan nilai LC_{50} sebesar 26,879 µg/mL. Ekstrak akuades menghasilkan zona hambat tertinggi dengan nilai 11,37±0,85 mm pada bakteri *Escherichia coli* dan 17,67±1,19 mm pada bakteri *Staphylococcus aureus*. Hasil ANOVA dua arah menunjukkan pelarut berpengaruh nyata terhadap zona hambat bakteri (p<0,05), sementara diameter pohon tidak berpengaruh nyata (p<0,05). Ekstrak pelarut etanol menghasilkan nilai toksisitas tertinggi, sedangkan ekstrak pelarut akuades menghasilkan zona hambat terbesar.

Kata kunci: Aceh Timur, *Artemia salina*, kondisi lingkungan, maserasi, patogen

INTRODUCTION

Rhizophora apiculata species is widely found in Indonesia and is commonly used as medicine and food. *Rhizophora apiculata* can treat diarrhea, dysentery, used as anti-vomiting drug, and cure other stomach aches (Wardina *et al*., 2023). *Rhizophora apiculata* is known to have pharmacological activities such as antifungal, antibacterial, antiseptic, anti-inflammatory, anti-ulcer, and has properties in wound healing (Sormin *et al.*, 2021). The use of *R. apiculata* in traditional medicine leads to the development and research of its secondary metabolites in biopharmaceuticals or natural products. Studies about the various potentials of secondary metabolites of *R. apiculata* have been carried out previously. Research by Ramalingam & Rajaram (2018) found the potential of *R. apiculata* as an antioxidant and anticancer agent. Research by Syawal *et al*. (2019) showed ethyl acetate extract of *R. apiculata* can inhibit the growth of *Aeromonas hydrophila* and is more sensitive than n-hexane, ethanol, and hot water solvents. Research by Dewanto *et al*. (2021) also showed that *R. apiculata* extract could inhibit *Listeria monocytogenesada* bacteria. Research by Yuniarti *et al*. (2020) also showed that *R. apiculata* extract could inhibit melanosis in whiteleg shrimp (*Litopenaeus vannamei*).

Various factors, including the environment and the plant's physiological adaptation, greatly influence organisms' ability to produce secondary metabolites. In addition to that, a study by Yang *et al*. (2018) reveals for most plants, environmental factors such as light, temperature, soil water, soil fertility, and salinity can have a significant impact on several processes related to plant growth and development, including the ability of plants to synthesize secondary metabolites. This can ultimately change the overall phytochemical profile of the plant, which is very important in producing bioactive compounds. Plant age can also affect secondary metabolites, which are related to environmental conditions. A Study by Li *et al.* (2020) shows environmental cues can influence the initiation and subsequent differentiation of particular cellular structures involved in the biosynthesis and storage of SMs at different developmental stages. Previous studies on *R. apiculata* extract were conducted using various solvents with different polarities, such as water, methanol, ethanol, ethyl acetate, acetone, and n-Hexane (Kurniawan *et al*., 2023; Syawal *et al*., 2019). The results of previous studies stated that *R. apiculata* produced the highest extract using polar solvents (Maulana, 2021). Based on the chemical properties of secondary metabolites, phytochemicals need to be extracted in solvents with different polarities because no single solvent can be relied on to extract all phytochemical compounds and antioxidants present in plants (Nawaz *et al*., 2020). The results of *R. apiculata* extract using polar solvents are known to produce compounds in the polar fraction, such as alkaloids, flavonoids, phenolics, saponins, tannins, and triterpenoids (Maulana, 2021; Mutik *et al*., 2022)

This study was also conducted considering that the increasing resistance of microorganisms to antibiotics is one of the serious problems in the health sector in the world with the increase in infectious diseases that cause many deaths (Abeysinghe, 2010). In this study, antibacterial testing of *R. apiculata* extract was carried out against *Escherichia coli* and *Staphylococcus aureus* bacteria. These two bacteria were chosen because they potentially develop multidrug-resistant properties (Ibrahim *et al.*, 2012; Sonola *et al.*, 2021). The use of these two types of bacteria is also based on the fact that *E. coli* is a gram-negative bacteria and *S. aureus* is a gram-positive bacteria (Usman, 2018). Foreseeing the necessity for new drugs to fight multidrug-resistant microorganisms, research has been carried out to find new types of medications with natural ingredients such as plant extracts. Following the result of Li *et al.* (2020) and Maulana (2021) extraction in this study was carried out with three polar solvents, namely methanol, ethanol, and sterile aquades (distilled water) combined with *R. apiculata* in different diameters, to find the combination that produced the best extract. This study aims to determine the best solvent for *R. apiculata* extract based on toxicity and antibacterial parameters.

MATERIALS AND METHODS

Research Location and Sample Collection

Mangrove sampling and environmental data were conducted at two locations in Aceh, namely Kuala Langsa (98°0' 37.800" E and 4°31' 17.820" N) (Figure 1) and Telaga Tujuh Island (98°3' 27.366" E and 4°33' 27.809" N) (Figure 1). Sampling was conducted by a joint team from Institut Pertanian Bogor (IPB University), Badan Riset and Inovasi Nasional (BRIN), and Aceh Wetland Foundation (AWF) in 2022.

Sampling of the leaves was performed using the random sampling method. Sampling at each location was conducted with nine plots in three transect lines with a transect size of 10 m x 10 m. Data collection of *R. apiculata* diameter was also carried out. Data collection of tree diameters was done using the diameter at breast height (DBH) measurement method. Diameter at breast height is a standard method for expressing the diameter of a tree trunk or stem and is a vital measurement factor in forest resource survey management. Tree diameters were measured at a height of 1.3 m (Figure 2) above the ground using a DBH tape or caliper (Wu *et al.*, 2019). Samples were taken to the Laboratory of Marine Microbiology in the Department of Marine Science and Technology, Institut Pertanian Bogor, for further testing. Research in the laboratory was conducted from November 2023 until May 2024.

Extraction of *R. apiculata*

The primary materials in this study were *R. apiculata* leaves from six different trees. The samples were taken from Kuala Langsa and Telaga Tujuh Island, which have different environmental conditions, such as rehabilitated ecosystems (Kuala Langsa) and pristine and natural ecosystems (Telaga Tujuh Island). Environmental parameter measurement includes pH, salinity, organic matter, and substrate type. *R. apiculata* leaves were transformed into powder and were used for further analysis. The method of making *R. apiculata* powder was modified from Gazali *et al.* (2020). The *R. apiculata* sample was washed and cleaned of dirt. The sample was then dried in an oven at 60°C and ground into simplicial powder. Each *R. apiculata* simplicial was extracted with three different solvents. Extraction was carried out using methanol and ethanol (Merck, Germany) and sterile aquades (distilled water), with a ratio of 10:1 (v/w) from 2 g of simplicial powder. Extraction of *R. apiculata* powder is a modification of previous research. Extraction with methanol and ethanol solvents was carried out by maceration for 3×24 hours (Karim *et al.*, 2020; Nurany, 2019; Subayu *et al.*, 2021). Meanwhile, extraction with aquades solvent was carried out by boiling for 15 minutes at 85-90°C with 20 mL of aquades (Puspitasari, 2019). The extract was separated from the solvent by centrifugation at a speed of 5,000 rpm for 10 minutes, with a modification from (Malik *et al.*, 2017). The solvent was evaporated using a hot plate in a laminary hood until the solvent

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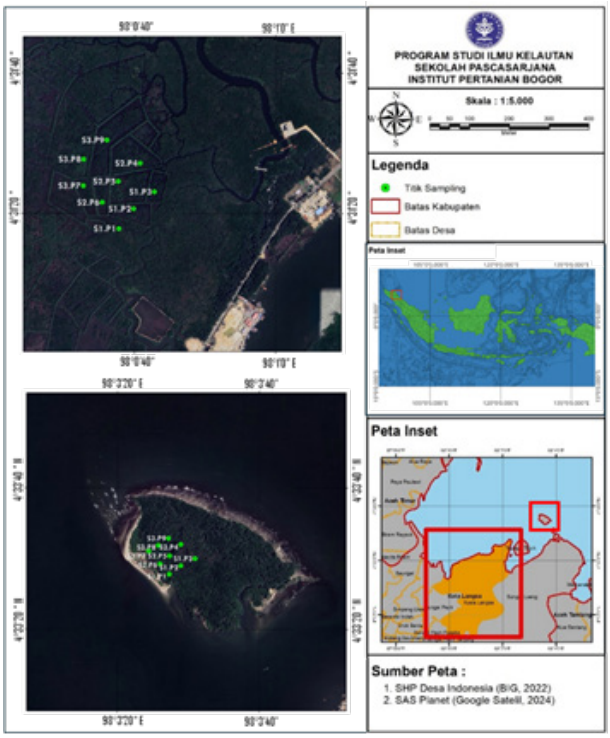

Figure 1 Study location in Kuala Langsa (98°0' 37.800" E and 4°31' 17.820" N) and Telaga Tujuh Island (98°3' 27.366" E and 4°33' 27.809" N

Gambar 1 Lokasi penelitian di Kuala Langsa (98°0' 37.800" BT dan 4°31' 17.820" LU) dan Pulau Telaga Tujuh (98°3' 27.366" BT dan 4°33' 27.809" LU)

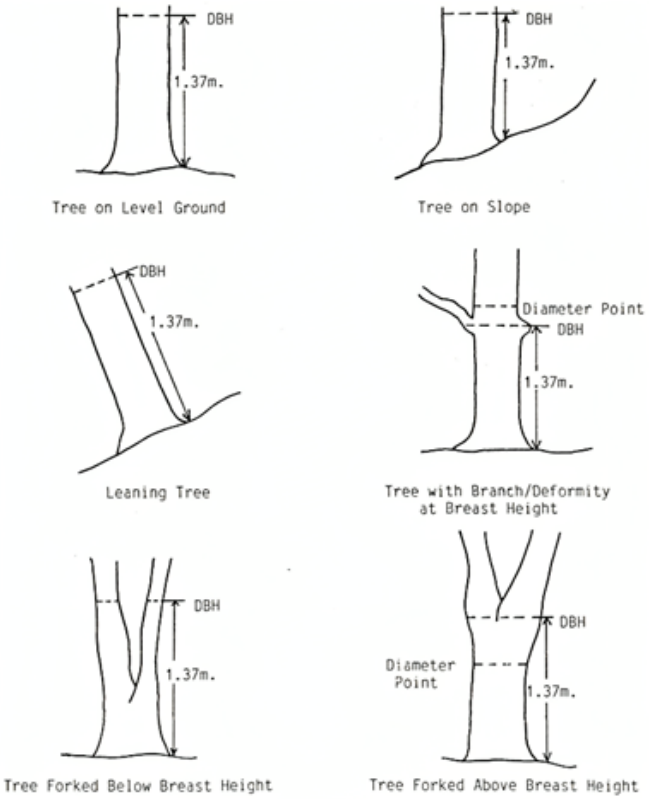

Figure 2 *R. apiculata* diameter data collection method (Miller *et al.*, 1996)

Gambar 2 Metode pengumpulan data diameter *R. apiculata* (Miller *et al.*, 1996)

entirely evaporated (Odey *et al*., 2012). The calculation of the extract yield refers to the calculation (Abbas *et al*., 2021):

$$\text{Yield (\%)} = \frac{\text{Weights of solvent free extract (g)}}{\text{Dried raw material weight (g)}} \times 100$$

Toxicity Testing

Toxicity testing was carried out using the LC_{50} test. LC_{50} testing was performed using extract concentrations of 125, 250, 500 µg/mL, and a control solution. Toxicity testing refers to the brine shrimp lethality assay (BSLA) (Meyer *et al*., 1982). *Artemia salina* used in the toxicity test was obtained from the Laladon Ornamental Fish Exchange, Bogor. About 500 mg of *Artemia salina* was hatched in a Duran bottle or Erlenmeyer flask filled with 200 mL of seawater for 48 hours. The toxicity test was carried out on a 24-well plate. Around 10 nauplii of *Artemia salina* (age 48 hours) were transferred into each well plate. The prepared *R. apiculata* extract was also transferred to each well plate. The volume of the test solution tested was 1 mL (Banti & Hadjikakou, 2021). The toxicity is then determined in terms of the mortality rate of brine shrimp larvae. Brine shrimp assay is a low cost, safe, no required feeding during the assay, while it requiring only a small amount of the tested agent. The test was carried out for 24 hours; the dead *Artemia* was counted and recorded. The test was carried out in three replicates. Data analysis refers to the calculation (Rasyid *et al*., 2022).

$$\%\text{Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of test larvae}} \times 100$$

Cumulative mortality data of *A. salina* in the LC_{50} test were analyzed using the AAT Bioquest (2024) LC_{50} calculator application (https://www.aatbio.com/tools/lc50-calculator) to determine the 24-hour LC_{50} value. LC_{50} calculation was done using AAT Bioquest (2024) LC_{50} calculator, which utilizes LC_{50} Regression.

Antibacterial Activity Testing

Antibacterial activity was tested using the Disk Diffusion Assay (DDA) method (Hudzicki, 2012). *Escherichia coli* and *Staphylococcus aureus* bacteria were cultured for 24 hours in the Oxoid Tryptic Soy Broth (TSB) medium before conducting the antibacterial testing with the Tryptic Soy Agar (TSA) medium. The concentration of the simplicial extract was 10 mg/mL dissolved in dimethyl sulfoxide (DMSO) solution to aid the process of dissolving the extract due to its strong polarity. Dimethyl sulfoxide was also used because DMSO can stabilize plant extract (Engeloch *et al*., 2008). The extract was dropped onto sterile 6 mm Oxoid™ Blank Antimicrobial Susceptibility discs (20 µL) and placed on a petri dish with targeted bacteria that had been incubated for 24 hours. Once all disks are in place, incubate for a full 24 hours. Positive control was done using Tetracycline. The inhibition zone was measured in millimeters (Nurdiani *et al*., 2012). The test was carried out in three replications.

Data Analysis

The study used an experimental design to test the effect of diameter on the toxicity and antibacterial activity of *R. apiculata* extract in different solvents (methanol, ethanol, and aquades). The relationship between tree diameter and toxicity was tested using Correspondence analysis in the XLSTAT. Correspondence Analysis (CA) is a multivariate graphical technique designed to explore relationships among categorical variables. The goal of CA is to illustrate the most important relationships among the variables' response categories using a graphical representation (Sourial *et al*., 2010). The effect of tree diameter and solvent type on the bacterial inhibition zone was tested using two-way ANOVA using the IBM SPSS Statistics 27 to observe the differences between treatments.

RESULTS AND DISCUSSION

Trunk Diameter of *R. apiculata*

R. apiculata mangrove in Telaga Tujuh Island has a larger average tree diameter (ATD) compared to *R. apiculata* in Kuala Langsa (Table 1). The TT-ST 2-4-RA sample from Telaga Tujuh Island has the largest ATD with a value of 23.3 cm, while the KL-ST 3-7-

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Table 1 Size classes of *R. apiculata*
Tabel 1 Kelas ukuran *R. apiculata*

Location	Code	Number of individuals/plot	Average tree diameters (cm)
Kuala Langsa	KL-ST 2-5-RA	1	5±0.0
Kuala Langsa	KL-ST 3-7-RA	2	2.4±0.0
Telaga Tujuh Island	TT-ST 1-1-RA	1	13±0.0
Telaga Tujuh Island	TT-ST 1-2-RA	1	23.3±0.0
Telaga Tujuh Island	TT-ST 2-4-RA	1	4.8±0.0
Telaga Tujuh Island	TT-ST 3-9-RA	4	26±4.2

(A) (B)

Figure 3 *R. apiculata* tree from (A) Kuala Langsa, (B) Telaga Tujuh Island
Gambar 3 Pohon *R. apiculata* dari (A) Kuala Langsa, (B) Pulau Telaga Tujuh

RA sample from Kuala Langsa has the smallest ATD with a size of 5 cm. The actual age of the *R. apiculata* samples in this study are unknown; however, looking at their phenotypes, some of the trees taken from this location are probably more than 100 years old (Figure 3).

It is assumed that the larger the tree diameter, the older the tree. This assumption is supported by research by Kurt *et al.* (2021) and Lukaszkiewicz & Kosmala (2008) which shows that the size of the diameter at breast height in trees increases with increasing tree age. In a comparison of the vegetation history, this study refers to (Hanafi *et al.*, 2021; Maysaroh *et al.*, 2023), that stated the Telaga Tujuh Island mangrove forest is a pure and intact mangrove ecosystem which is estimated to have existed for more than two centuries, while the mangrove ecosystem in Kuala Langsa has undergone rehabilitation efforts since 2006.

Toxicity Test of *R. apiculata* Extract

The extraction results showed that aqueous extract had the highest yield value in both Kuala Langsa from *R. apiculata* with diameter of 2,4 cm (11,25%) and Telaga Tujuh Island from *R. apiculata* with diameter of 26 cm (11,65%); these results indicate that the compounds extracted from the *R. apiculata* mangrove leaf simplicia are in polar fraction. A toxicity test of *R. apiculata* extracts was conducted as a preliminary result of bioassay-guided stages for examining the bioactivity of plant extract. Extracts known to have toxic

Table 2 LC_{50} toxicity test of *R. apiculata*
Tabel 2 Uji toksisitas LC_{50} *R. apiculata*

Location	Tree diameters (cm)	Solvent	Yield extract (%)	LC_{50} (µg/mL)	Toxicity
Kuala Langsa	5	Methanol	3.65	489.4660	Toxic
		Ethanol	2.5	296.2215	Toxic
		Aquades	11.15	ND**	Non Toxic
Kuala Langsa	2.4	Methanol	3.75	149.5659	Toxic
		Ethanol	3.35	230.5517	Toxic
		Aquades	11.25	400.3442	Toxic
Telaga Tujuh Island	13	Methanol	5.9	400.3442	Toxic
		Ethanol	4.65	26.879	Toxic
		Aquades	5.7	655.5693	Toxic
Telaga Tujuh Island	23.3	Methanol	8	230.0557	Toxic
		Ethanol	4.4	467.9903	Toxic
		Aquades	1.75	3,921.66	Non Toxic
Telaga Tujuh Island	4.8	Methanol	4.7	131.2878	Toxic
		Ethanol	4.25	397.3331	Toxic
		Aquades	0.55	ND**	Non Toxic
Telaga Tujuh Island	26	Methanol	3.25	230.0557	Toxic
		Ethanol	3.55	502.4651	Toxic
		Aquades	11.65	ND**	Non Toxic
-	DMSO	DMSO	-	ND*	Non Toxic

*Controls showed no mortalities; **ND: No Data.

properties can be used for further testing. Toxic plant extracts indicate that the sample has potential as an anticancer, antibacterial, and others (Osman & Omar, 2019). AAT Bioquest (2024) analysis of the Brine Shrimp Lethality (BSLA) test of *R. apiculata* extract is shown in (Table 2).

This result shows the preliminary assessment of the toxicity of mangrove plant extract at different diameters, and all of the extracts from ethanol and methanol show toxic properties. *R. apiculata* with a diameter of 13 cm from Telaga Tujuh Island (TT-1-1-RA) extracted with ethanol showed the highest toxicity values (LC_{50}) compared to other extracts with a concentration of 26.879 µg/mL. This result shows a higher toxicity value than the result of Maulana (2021), in which *R. apiculata* ethanolic leaf extract has an LC_{50} value of 49.45 µg/mL. The results of the BSLA test on the aqueous extract did not show any death at diameters of 4,8 cm; 5 cm; and 13 cm samples, which could not be included in the LC_{50} calculation. The results of this study indicate that the process of boiling *R. apiculata* leaves, which the people of Aceh usually do, shows that the boiling results have toxic values. *R. apiculata* leaf extract from Kuala Langsa (KL-ST 3-7-RA) shows a safe concentration of mangrove leaf decoction of 400 µg/mL, while *R. apiculata* leaf extract from Telaga Tujuh Island (TT-ST 1-1-RA) shows a safe concentration of mangrove leaf decoction of 655 µg/mL.

Antibacterial Activity of *R. apiculata* Extract

The aqueous extract showed the highest inhibition zone in both bacteria. The highest inhibition zone in *E. coli* was produced by

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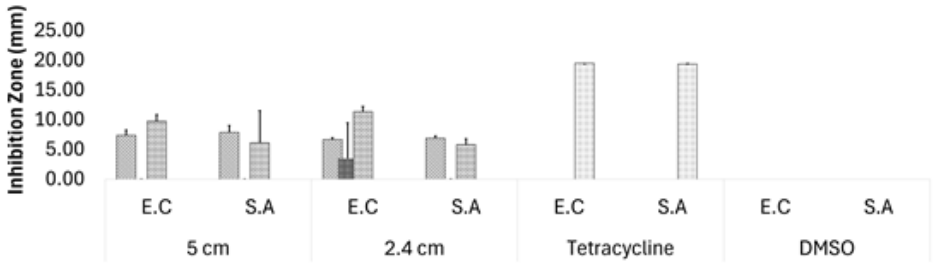

Figure 4 Antibacterial activity of *R. apiculata* extract (▨) methanol, (■) ethanol, (▩) sterile aquades, and (▩) tetracycline from Kuala Langsa

Gambar 4 Aktivitas antibakteri ekstrak *R. apiculata* (▨) metanol, (■) etanol, (▩) akuades steril, dan (▩) tetrasiklin dari Kuala Langsa

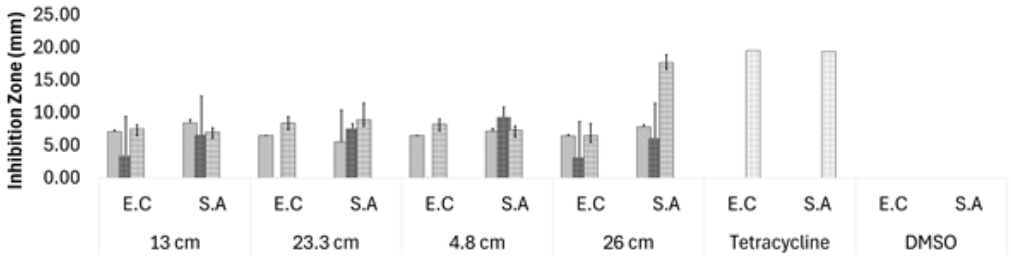

Figure 5 Antibacterial activity of *R. apiculata* extract (▨) methanol, (■) ethanol, (▩) sterile aquades, and (▩) tetracycline from Telaga Tujuh Island

Gambar 5 Aktivitas antibakteri ekstrak *R. apiculata* (▨) metanol, (■) etanol, (▩) akuades steril, dan (▩) tetrasiklin dari Pulau Telaga Tujuh

aqueous extract of *R. apiculata* with a diameter of 2.4 cm from Kuala Langsa (KL-ST 3-7-RA) with inhibition zone of 11.37 mm (Figure 4), while in *S. aureus* it was produced by aqueous extract *of R. apiculata* with a diameter of 26 cm from Telaga Tujuh Island (TT-ST 3-9-RA) with inhibition zone of 17.67 mm (Figure 5). The inhibition activity was determined following the Davis & Stout (1971) inhibition zone classification, where an inhibition zone <5 mm is categorized into weak activity, an inhibition zone of 5-10 mm into moderate, and an inhibition zone of 10-20 mm is categorized into strong activity. Based on this categorization, it is shown that *R. apiculata* with a diameter of 2.4 cm from Kuala Langsa (KL-ST 3-7-RA) and *R. apiculata* with a diameter of 26 cm from Telaga Tujuh Island (TT-ST 3-9-RA) have strong inhibition power.

The results of the aqueous extract in this study showed more minor results (11.37 mm) in *R. apiculata* with a diameter of 2.4 cm from Kuala Langsa (KL-ST 3-7-RA) against *E. coli* compared to the study of Jhurani & Jadhav (2010), which showed an inhibition zone of 14 mm. In contrast to *E. coli*, the aqueous extract of *R. apiculata* with a diameter of 26 cm from Telaga Tujuh Island (TT-ST 3-9-RA) in this study showed a higher inhibition zone against *S. aureus* (17.67 mm) compared to the study of Seepana *et al.* (2016) with 14 mm of inhibition zone. These findings suggest that the boiling method commonly employed in traditional practices of *R. apiculata* enhances the antibacterial properties of the water extract. Water is a widely used solvent due to its low cost, availability, stability, non-toxicity, and non-volatility, making it one of the most common choices for extracting plant-soluble active ingredients (Senduk *et al.*, 2020). DMSO was used as a solvent to aid the process of dissolving the extract, and no interference was observed in the inhibition zone. However, DMSO may affect some ethanol extracts high standard deviation values. Ethanol solvent is a volatile solvent that evaporates quickly.

Some ethanol extracts can have a higher concentration due to evaporation, resulting in a relatively high standard deviation. These results indicate that DMSO is not good at stabilizing ethanol extracts. Other stabilizers, such as tween, can be tested for their ability to stabilize ethanol extracts in subsequent studies.

Moreover, this study also showed differences in *R. apiculata* extracts against targeted bacteria due to sample location. *R. apiculata* extract from Kuala Langsa was more effective against *E. coli*, whilst *R. apiculata* from Telaga Tujuh Island was more effective against *S. aureus*. These results indicate that environmental factors could play a role in secondary metabolites activity produced by *R. apiculata*. Variations in the living environment of *R. apiculata* can lead to differences in the composition of secondary metabolites, resulting in varying effectiveness in inhibiting bacterial growth.

The sampling location has different environmental conditions where Telaga Tujuh Island has a higher sand fraction than Kuala Langsa; in addition, the redox potential value on Telaga Tujuh Island is higher than Kuala Langsa. The difference in environmental conditions is also supported by the opinion of Maysaroh *et al.* (2023), who stated that Telaga Tujuh Island has a higher organic matter value than Kuala Langsa. A study by Elsharkawy *et al.* (2021) stated that the variations in environmental conditions across different ecosystems can influence plant metabolism, significantly impacting both primary and secondary metabolites and overall plant biological activity. Additionally, this study demonstrated that the aqueous extract of *R. apiculata* exhibits activity against targeted pathogenic bacteria in *R. apiculata* with diameter of 5 cm from Kuala Langsa (KL-ST 2-5-RA), *R. apiculata* with diameter of 26 cm from Telaga Tujuh Island (TT-ST 3-9-RA), and *R. apiculata* with diameter of 4.8 cm from Telaga Tujuh Island (TT-ST 2-4-RA) while showing little to no toxicity towards *Artemia salina* nauplii. As stated by (Meyer *et al.*, 1982) extracts with toxic properties have the potential to be antibacterial, while in this study, extracts without toxic properties against *Artemia salina* can inhibit bacterial growth. This phenomenon can be attributed to the properties of selective toxicity. Antimicrobial agents are designed to exhibit selective toxicity, meaning they effectively target and harm microorganisms while ideally leaving eukaryotic cells unaffected (Shivanand *et al.*, 2018)

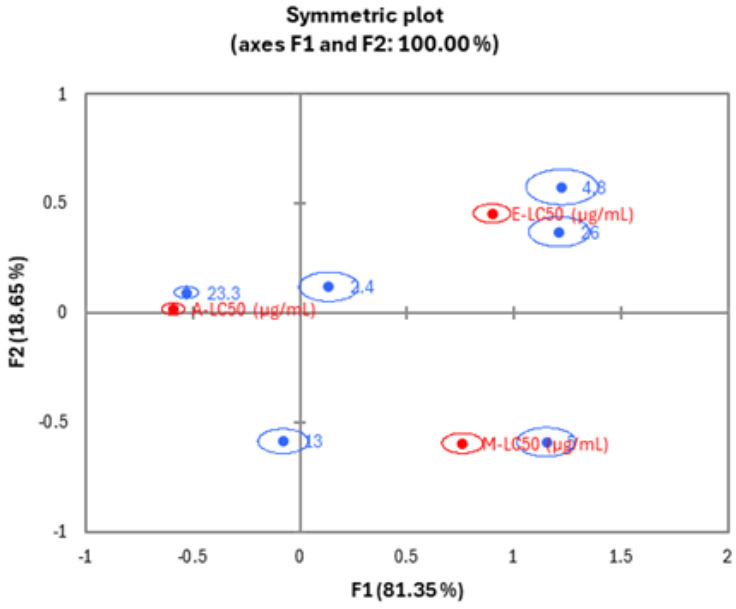

Figure 6 Effect of tree diameter on toxicity value
Gambar 6 Pengaruh diameter pohon terhadap nilai toksisitas

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Correlation between Tree Diameter and Bioactivity of *R. apiculata* Extracts

Many factors, such as environmental conditions and the age of the plant influence secondary metabolite activity. Research by Farias *et al*. (2023) shows the toxicity value decreases with the increasing age of the plant. Farias stated that the age of the plant significantly affects the chemical composition of essential oils in plants, causing significant changes in the viability of its cells. This study conducted a correspondence analysis test (Figure 6) to see the relationship between the tree diameter and toxicity. The result of the Correspondence Analysis (CA) showed that 81.35% of the total variation was accounted for by the first axis (F1) and 18,65% was accounted for by the second axis (F2), the two axes together accounting for 100% of the total variation. The results of the CA analysis showed that *R. apiculata* with diameters (23.3; 4.8; and 26 cm) contributed to F1, while *R. apiculata* with diameters (5; 2.4 and 13 cm) contributed to F2. The CA results (Figure 6) show that *R. apiculata* with 4.8 and 26 cm diameters was directly proportional to to the LC_{50} value in ethanol solvent (E-LC_{50}). The results of this test indicate that tree diameter does not contribute to the dependency between variables.

Furthermore, the results of the two-way ANOVA analysis showed that tree diameter did not significantly affect on the strength of the bacterial inhibition zone with a significance of 0.736 (p<0.05). In contrast, the type of solvent showed a significant impact on the bacterial inhibition zone with a significance of 0.002 (p<0.05). The combination of tree diameter and solvent also did not show a difference in the inhibition zone with a significance of 0.879 (p<0.05), which means that the type of solvent had the highest effect on the strength of the inhibition zone in this study.

Even though Debalke *et al*. (2018) stated that the age of the plant used for extraction is an essential parameter that can affect the ethnopharmacological activity of the extract, this result shows that diameter has an insignificant influence on the bioactivity of mangrove extract. Plant age is an important parameter for the ethnopharmacological activity of the extract because many secondary metabolites frequently arise at a particular stage of plant growth because of the apparent differences in the life cycles of various plants (Li *et al*., 2020). In general, the results of this study support the hypothesis of the medicinal use of *R. apiculata*, especially as local wisdom or traditional medicine. The results of this study can also be used as data for the development of drugs from *R. apiculata*, mainly due to the prevailing antibiotic resistance of many pathogenic bacteria. The variation in tree diameter observed in this study may not significantly impact secondary metabolite production, as factors beyond environmental influences play a role in this process. One such factor is the diversity of rhizosphere bacteria within an ecosystem, which is thought to influence the bioactivity of secondary metabolites. This assumption aligns with findings by Maysaroh *et al*. (2024) who reported that rhizosphere bacteria in Kuala Langsa and Telaga Tujuh Island exhibit different levels of diversity despite experiencing similar environmental conditions. Specific plant species growing in distinct ecosystems can affect the type and content of secondary metabolites they produce, a phenomenon closely tied to the native microbes present in their environment (Köberl *et al*., 2013). Microbes that adapt to particular ecosystems and associate with specific plants can create unique effects on their host, including alterations in secondary metabolite production (Huang *et al*., 2018).

CONCLUSION

This study demonstrated that the choice of extraction solvent had a more significant impact than the age of the plant. Both ethanol and methanol solvents yielded toxic extracts, while aquades solvent proved the most effective, significantly enhancing the bacterial inhibition zone value. For future research, minimum inhibitory concentration testing can be conducted to see the lowest concentration of *R. apiculata* extract from Kuala Langsa dan Telaga Tujuh Island, which can inhibit bacterial growth.

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