

Brine shrimp cytotoxicity bioassay of red mangrove (*Rhizophora mucronata*) leaves using different solvents and its potency as antibacterial

ILHAM ZULFAHMI¹, EPA PAUJIAH^{2,*}, ZULTIRA HARINA ROZA³, KAMALIAH HELMI⁴,
BADRATUN NAFIS³, FIRMAN MUHAMMAD NUR⁵, PUTROE NURUL FAZILA³

¹Department of Fisheries Resources Utilization, Faculty of Marine and Fisheries, Universitas Syiah Kuala. Jl. Teuku Nyak Arief Darussalam, Banda Aceh 24415, Aceh, Indonesia

²Department of Biology Education, Faculty of Tarbiyah and Teacher Training, Universitas Islam Negeri Sunan Gunung Djati Bandung. Jl. AH. Nasution, No. 105, Bandung 40292, West Java, Indonesia. Tel.: +62-22-7800525, *email: epapaujiah@uinsgd.ac.id

³Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. Jl. Teuku Nyak Arief Darussalam, Banda Aceh 24415, Aceh, Indonesia

⁴Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Ar-Raniry. Jl. Syekh Abdul Rauf Darussalam Banda Aceh, Banda Aceh 23111, Aceh, Indonesia

⁵Research Center for Biosystematics and Evolution, Badan Riset dan Inovasi Nasional (BRIN). Jl. M.H. Thamrin No. 8, Gedung B.J. Habibie, Jakarta Pusat, Jakarta 10340, Indonesia

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Abstract. Zulfahmi I, Paujiah E, Roza ZH, Helmi K, Nafis B, Nur FM, Fazila PN. 2024. Brine shrimp cytotoxicity bioassay of red mangrove (*Rhizophora mucronata*) leaves using different solvents and its potency as antibacterial. *Biodiversitas* 25: 3227-3236. This study evaluates the cytotoxicity of ethanol, ethyl acetate, and n-hexane extracts of red mangrove (*Rhizophora mucronata*) leaves and their potential against pathogenic bacteria. Red mangrove leaf was evaluated for its cytotoxicity using the Brine Shrimp Lethality Test (BSLT). Each solvent's LC₅₀ - 24-hour value was determined using probit analysis and measured using one-way ANOVA with a 95% confidence interval (P<0.05). The secondary metabolic compounds in each extract were presented and analyzed descriptively using GC-MS analysis. The effect of incubation time on the bacterial inhibition zone was tested using independent t-test analysis against *S. aureus*, *V. parahaemolyticus*, and *A. hydrophyla*. The results showed that the LC₅₀ value of ethanol extract was 402.45 ppm, ethyl acetate extract was 230.97 ppm, and n-hexane extract was 180.31 ppm. The secondary metabolite compounds observed in red mangrove leaves extracted with different solvents (ethanol, ethyl acetate, and n-hexane) include terpenoids, alkaloids, ketones, phenols, turpentine oil, essential oils, unsaturated fats, and *camphor*. The ethyl acetate extract from red mangrove leaves inhibited the growth of *S. aureus* and *V. parahaemolyticus*. *A. hydrophyla* and *V. parahaemolyticus* exhibited the lowest inhibition zone, which varied based on time and concentration.

Keywords: Antibacterial, brine shrimp, BSLT method, cytotoxicity, red mangrove

INTRODUCTION

Indonesia is one of the countries with the world's largest area of mangrove forests. Approximately 22.6% (3.4 million hectares) of the world's total mangrove cover is estimated to be found within Indonesia's borders (Kusmana et al. 2020). This vast expanse of mangrove forests makes Indonesia a global center of mangrove biodiversity, playing a crucial role in maintaining coastal ecosystem balance and mitigating climate change (Jennerjahn et al. 2022). Most of Indonesia's mangrove forests are across Papua, East Kalimantan, South Kalimantan, Riau, and South Sumatra (Arifanti et al. 2022). A total of 202 types of mangroves (47 true mangroves) have been identified in Indonesia. Some common types of mangroves are from the genus *Rhizophora*, *Avicennia* *Sonneratia*, and *Bruguiera* (Irawan et al. 2021; Kathiresan 2021).

The red mangrove (*Rhizophora mucronata*), classified as a true mangrove species, predominates in coastal regions characterized by tropical and subtropical climates, including those found in Indonesia (Kumari et al. 2015). These mangroves have a crucial ecological role (Bento et al.

2024), such as protecting beaches from erosion, providing habitat for various marine animals, and absorbing carbon dioxide, which helps mitigate climate change (Alongi 2022). Besides their ecological benefits, red mangrove has various pharmacological potentials (Awuku-Sowah et al. 2022). Setyawan et al. (2019) reported that red mangrove has the potential to be anti-bacterial, anti-viral, and anti-fungal. Several types of secondary metabolite compounds that have been identified in red mangrove are alkaloids and hydrolyzed tannins, polyphenols, flavonoids, polysaccharides, triterpenes, inositol, saponins, anthocyanidins (Anjaneyulu and Rao 2001). These compounds have various beneficial biological activities, such as antioxidant, anti-inflammatory, and anticancer (Sudhir et al. 2022). In the fisheries sector, red mangrove has been utilized for its potential to combat various diseases caused by viruses and bacteria, including White Spot Syndrome Virus (WSSV) (Zhao et al. 2017), *Vibrio harveyi* (Mulyani et al. 2020) and *Aeromonas salmonicida* (Park et al. 2020). Baskaran and Mohan (2012) also reported that red mangrove is effective as an alternative medicine for vibriosis in lobster larvae (*Nephropidae*).

Brine shrimp (*Artemia salina* L.) cytotoxicity assay (BSLT) is one of the cytotoxic tests commonly used to assess the pharmacological activity and toxicity of a plant extract (Anderson et al. 1988; McLaughlin 1991; Syamsurizal et al. 2023). This method continues to develop and has been applied globally for over 20 years. According to Orno and Rantesalu (2020), this method is relatively easy to apply, effective, fast, and cheap. Various indigenous Indonesian plant extracts have been evaluated for their toxicity levels using this method, including Tiwai onion bulbs (*Eleutherine bulbosa*) (Lestari et al. 2019), mangosteen leaves (*Garcinia mangostana* L.) (Ansori 2020; Said et al. 2023) and red algae (*Eucheuma spinosum*) (Hamrun et al. 2020; Sugrani and Fitriana 2021). The toxicity values of several types of mangrove extracts were also previously reported, including *Avicennia marina*, *Rhizophora mucronata*, *Sonneratia alba*, and *Xylocarpus granatum* (Darmadi et al. 2021).

One factor influencing a plant extract's toxicity value is the solvent used for extraction. Research results by Ningdyah et al. (2015) revealed that Tampoi fruit peel (*Baccaurea macrocarpa*) extracted with a semi-polar solvent (ethyl acetate) tends to have higher toxic properties compared to polar (methanol) and non-polar (n-hexane) solvents. So far, cytotoxic testing of red mangrove leaves using the BSLT method has been restricted to polar solvents, specifically methanol (Musalipah et al. 2021) and ethanol (Saragih et al. 2020). However, the comparative toxicity of red mangrove (*R. mucronata*) leaf extracts using different solvents (polar, non-polar, and semi-polar) remains undisclosed. Therefore, this study aims to evaluate the cytotoxicity of red mangrove (*R. mucronata*) leaves extracted using three different solvents (ethanol, ethyl acetate, and n-hexane) using the BSLT method, as well as to assess its potential against various pathogenic bacteria.

MATERIALS AND METHODS

Collection and preparation of red mangrove leaf samples

A total of 2 kg of fresh red mangrove (*R. mucronata*) leaves were collected from the coastal area of Alue Naga, Banda Aceh City (5.59°98'4"N, 95.35°25'9"E). The leaves were thoroughly cleaned and cut into small pieces. Subsequently, they were air-dried at room temperature for seven days. After drying, the leaves were finely ground using a blender and filtered through a sieve to obtain a fine dry powder (Ghosh et al. 2017).

100 g of red mangrove leaf powder was placed into three separate beakers, each containing different solvents: ethanol p.a, ethyl acetate p.a, and n-hexane p.a (Merck) each of 1 L (comparison 1:10) for 24 hours. After 24 h maceration, the filtrate is filtered. Maceration is performed twice until the filtrate is nearly transparent (Karim et al. 2020). The filtrate obtained from maceration was composited, and the solvent was removed using a vacuum rotary evaporator (Buchi R300; Switzerland) at temperatures close to the boiling points of each solvent (ethanol: 78.37°C, ethyl acetate: 77.1°C and n-hexane: 69°C). The resulting thick extract was further dried over a water bath maintained at

45°C until a dry, concentrated extract was obtained (Lestari et al. 2019).

Preparation of stock solution and hatching of artemia salina larvae

1 g of dry extract from each solvent was placed in separate Erlenmeyer. Each extract was dissolved in 2 mL of sterile seawater, serving as the standard solvent. Homogenized sterile seawater was added to obtain the total volume of 500 mL as a stock solution with a concentration of 2000 ppm (Indriaty et al. 2022).

A. salina eggs were obtained from local ornamental fish traders in Baiturrahman District, Banda Aceh City. Hatching of *A. salina* eggs refers to the protocol by Lestari et al. (2019). Briefly, 50-150 mg of *A. salina* were soaked in distilled water for 1 hour. Next, the eggs are filtered and hatched in a plastic container with a volume of 500 mL of sterile seawater (salinity 30 ppt, pH 7-8, temperature 28-29°C) equipped with aeration and a 40-watt lighting lamp. *A. salina* eggs are left for 36-48 hours until they hatch into nauplii. *A. salina* larvae that are 48 hours old and have active movement will be selected for further testing (Bhuvaneshwari et al. 2017).

Gas Chromatography and Mass Spectrometry (GC-MS) identification

The secondary metabolic compounds in each extract were identified using Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu QP2010 SE system). A Rxi-1ms column was utilized under the following conditions: an oven temperature range of 50°C to 290°C, an interface temperature of 290°C, a split control mode, a pressure of 20.8 psi, a total flow rate of 23.7 mL/min, a split ratio of 200:1, a split flow rate of 199 mL/min, helium as the carrier gas, and a mass selective detector (MSD) (Al-Rubaye et al. 2017).

Brine shrimp cytotoxicity bioassay

A total of 20 *Artemia salina* larvae were placed into vials with a volume of 10 mL added with red mangrove leaf extract of 100, 250, 500, and 1000 ppm. The test vials were placed under a 40-watt lamp for 24 hours at room temperature (28-29°C). Each concentration of treatment was replicated five times. Mortality observations, defined by the absence of movement for several seconds and a tendency for the larvae to remain at the bottom of the container, were conducted every 6 hours over 24 hours (Roy et al. 2023).

Antibacterial activity

The antibacterial activity test was carried out using the disc diffusion method on Mueller Hinton Agar (MHA) media following Igbinsosa et al. (2022) with modification. The concentrated extract of red mangrove leaves from ethyl acetate solvent was diluted using Dimethyl Sulfoxide (DMSO) to a concentration of 20%, 30%, and 40%. The ethyl acetate extract of red mangrove leaf was used to determine the antibacterial activity due to the extract yield and its acute toxicity value against *A. salina* (Table 1, Table 3). The bacteria tested were *Vibrio parahaemolyticus*,

Aeromonas hydrophila, and *Staphylococcus aureus*. The positive control was chloramphenicol, while the negative control was distilled water. Bacterial inoculation on the growth medium was done using a sterile cotton swab (Damtie and Mekonnen 2020).

40 µL of red mangrove leaf extract was applied to a 6 mm diameter paper disc (Oxoid CT0998) and incubated at 37°C for 48 hours (Rahman et al. 2017). The diameter of the clear zone produced by each extract concentration against bacteria was measured using a digital caliper (Sigmat Sketch) with an accuracy of 0.01 mm. Measurements of the inhibition zones were conducted at 24 and 48 hours. Each extract and bacterial concentration treatment was replicated five times.

Data analysis

Data on the percentage of *A. salina* mortality on different solvents and the inhibition zones (clear zones) against each bacterium were presented as mean values with standard deviations. The LC₅₀-24-hour value for each solvent was determined using probit analysis. The significance of *A. salina* mortality between solvents at 24 hours and the significance of the effect of extract concentration on bacterial inhibition zones were measured using one-way ANOVA with a 95% confidence interval (P<0.05). The content of secondary metabolic compounds in each extract was presented and analyzed descriptively. The effect of incubation time on the bacterial inhibition zone was tested using independent t-test analysis. Statistical analyses were performed using SPSS 22 software.

RESULTS AND DISCUSSION

The yield of extract and secondary metabolic content of red mangrove extract

The yield of red mangrove leaves produced from each solvent was 14.7 g (14.7%) for ethanol, 4.4 g (4.4%) for ethyl acetate, and 1.92 g (1.92%) for n-hexane (Table 1). The ethanol and ethyl acetate extracts have a thick texture and a dark green color. Meanwhile, hexane extract of red mangrove leaves extracted using n-hexane solvent have a thick, oily texture and a deep green color.

Twenty-six secondary metabolic compounds were successfully identified in n-hexane red mangrove leaves, 12 compounds from ethanol extract, and 13 compounds from ethyl acetate extract (Table 3). The class of compounds that have been successfully identified includes alkaloids (2-Pentanol, 2-methyl- (CAS) 2-Methyl-2-p, Cyclopentanol, 1-methyl-, 2-Hexen-1-ol,(E)-(CAS)trans-2-Hexen-1-, Tetradecanoic acid, Tetradecanoic acid, ethyl ester (CAS) Ethyl, Hexadecanoic acid, methyl ester (CAS) Me., Hexadecanoic acid (CAS) Palmitic acid, Hexadecanoic acid, ethyl ester (CAS) Ethyl, 1-Octadecene (CAS) alpha-Octadecene, Oleic acid, and Elaidic acid, (E)-9-Octadecenoic acid ethyl, 2-Pentanol, 2-methyl- (CAS) 2-

Methyl-2-p, and Cyclopentanol, 1-methyl-), unsaturated fats (Dodecanoic acid (CAS) Lauric acid, and Dodecanoic acid, ethyl ester (CAS) Ethyl la), terpenoids (1,8-Cineole), essential oils (Borneol L, and 1-Heptadecene), limestone (Camphor), turpentine oil (Alpha.-Pinene), ketones (2-Hexanone n-Butyl methyl ketone), Andphenol (Styrene Benzene, ethenyl-, and Benzene, 1,3,5-trimethyl- (CAS) 1,3,5-Tri) (Table 2).

Alkaloid compounds and unsaturated fats were identified in red mangrove leaves, which were extracted using three solvents. Terpenoids compound was detected both in n-hexane and ethyl acetate extract. Meanwhile, ketones, phenols, camphor, and turpentine oil are only found in n-hexane red mangrove leaf extract. Types of secondary metabolic compounds found in the three solvents were Hexadecanoic acid (CAS), Tetradecanoic acid (CAS), and Dodecanoic acid (CAS) (Table 3). The highest percentage in hexane red mangrove leaves extract is 1,8-Cineole (26.51%), 2-Pentanol, 2-methyl- (CAS) 2-Methyl-2-p (10.33%), and -Hexanonen-Butyl methyl ketone (7.45%). Meanwhile, the ethanol solvent is Octadec-9-Enoic acid (31.10%), Hexadecanoic acid (CAS), Palmitic acid (25.11%), and Dodecanoic acid (CAS) Lauric acid (11.10%). In the ethyl acetate solvent, they are Dodecanoic acid, 1,2,3-propanetriyl ester (29.08%), 1(2H)-Naphthalenone, octahydro-4a,8a-dim (19.18%), and Glycerol Tridodecanoate (10.55%) (Table 2).

Artemia salina mortality rate and LC₅₀-24-hour

The mortality rate of *A. salina* exposed to red mangrove leaf extract of three different solvents (ethanol, n-hexane, and ethyl acetate) at 6 hours of observation and a concentration of 100 ppm was 5%. At a concentration of 250 ppm, the mortality rates were 11.67%±2.89%, 10%±0%, and 6.25%±2.5% for ethanol, n-hexane, and ethyl acetate, respectively. At a concentration of 500 ppm, the mortality rates were 9%±4.18%, 13.75%±4.79%, and 8%±3.46% for ethanol, n-hexane, and ethyl acetate, respectively. At a concentration of 1000 ppm, the mortality rates were 8%±2.74%, 50%±14.58%, and 39%±6.52% for ethanol, n-hexane, and ethyl acetate, respectively.

A similar pattern occurred at 12, 18, and 24 hours for all concentrations between solvents (Figure 1). Statistical analysis showed that there was no significant effect of different solvents on mortality values of *A. salina* at the 24-hour at concentrations of 100 and 250 ppm (p>0.05) (Table 3). However, there was a significant increase in mortality in the treatment of n-hexane extract compared to the other 2 solvents at a concentration of 500 ppm (p<0.05) (Table 3). At a concentration of 1000 ppm, there was a significant increase in mortality value in the treatments of n-hexane and ethyl acetate extract compared to the ethanol solvent (p<0.05) (Table 2). The lowest LC₅₀-24-hour values in red mangrove leaves were n-hexane extract followed by ethyl acetate and ethanol extracts, i.e., 180.31 ppm, 243.55 ppm, and 402.45 ppm, respectively (Table 3).

Table 1. Extract yield of red mangrove leaves in various solvents

Solvent	Powder (g)	Extract (g)	Yield (%)
Ethanol	100	14.7	14.7
Ethyl acetate	100	4.4	4.4
N-hexane	100	1.92	1.92

Table 2. Identified compounds of *Rhizophora mucronata* leaf extracts using GC-MS analysis

Compounds	Ethanol extract		Ethyl acetate extract		Hexane extract	
	Retention Time (minute)	Area (%)	Retention Time (minute)	Area (%)	Retention Time (minute)	Area (%)
Ethane, 1,1-diethoxy- (CAS) 1,1-Diethoxye	3.413	9.60	-	-	-	-
5 Methyl Furfural	6.662	0.60	-	-	-	-
5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl	8.010	1.19	-	-	-	-
Dodecanoic acid (CAS) Lauric acid	15.733	11.10	15.414	10.19	15.427	3.26
(-) Caryophyllene oxide	15.950	0.43	15.597	0.78	-	-
Globulol	16.802	0.56	-	-	-	-
4,8-Dimethyl-Nona-3,8-Dien-2-One	17.370	0.92	-	-	-	-
Tetradecanoic acid (CAS) Myristic acid	17.981	10.82	17.643	5.32	-	-
Hexadecanoic acid (CAS) Palmitic acid	20.065	25.11	-	-	19.734	3.74
Octadec-9-Enoic Acid	21.731	31.10	-	-	-	-
Octadecanoic acid	21.947	7.09	-	-	-	-
9,12-Octadecadienoic acid (Z,Z)-(CAS) Li	22.410	1.47	-	-	-	-
.alpha.-Guaiene	-	-	13.845	1.16	-	-
Seychellene	-	-	13.963	2.24	-	-
5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahyd	-	-	14.154	1.46	-	-
1-Hexadecanol	-	-	15.733	1.48	-	-
1(2H)-Naphthalenone, octahydro-4a,8a-dim	-	-	16.570	19.18	-	-
Hexadecanoic acid, methyl ester	-	-	19.249	1.46	19.270	0.87
n-Hexadecanoic acid	-	-	19.714	7.92	-	-
Dodecanoic acid, 1,2,3-propanetriyl ester	-	-	20.888	29.08	-	-
Heptadecene-(8)-Carbonic Acid	-	-	21.364	9.18	-	-
Glyceryl Tridodecanoate	-	-	22.649	10.55	-	-
2-Pentanol, 2-methyl- (CAS)2Methyl-2-	-	-	-	-	3.303	10.33
-Hexanonen-Butyl methyl ketone	-	-	-	-	3.872	7.45
Cyclopentanol, 1-methyl-	-	-	-	-	4.007	3.72
2-Hexen-1-ol, (E)- (CAS) trans-2-Hexen-1-	-	-	-	-	4.746	3.73
Styrene Benzene, ethenyl-	-	-	-	-	5.451	1.51
Alpha.-Pinene	-	-	-	-	6.296	4.43
Benzene, 1,3,5-trimethyl- (CAS) 1,3,5-Tri	-	-	-	-	7.174	1.78
5-Hexen-2-one (CAS) Allylacetone	-	-	-	-	7.671	5.47
1,8-Cineole	-	-	-	-	7.783	26.51
Camphor	-	-	-	-	9.414	0.98
Borneol L	-	-	-	-	9.936	1.37
Dodecanoic acid (CAS) Lauric acid	-	-	-	-	15.427	3.26
Dodecanoic acid, ethyl ester (CAS) Ethyl la	-	-	-	-	15.618	1.91
Benzene, (2-decyldodecyl)- (CAS) 11-Benz	-	-	-	-	17.028	1.32
Tetradecanoic acid	-	-	-	-	17.662	2.25
Tetradecanoic acid, ethyl ester (CAS) Ethyl	-	-	-	-	17.890	1.64
1-Heptadecene	-	-	-	-	18.019	0.61
Hexadecanoic acid, ethyl ester (CAS) Ethyl	-	-	-	-	19.956	3.73
1-Octadecene (CAS) .alpha.-Octadecene	-	-	-	-	20.076	2.09
Phytol	-	-	-	-	21.151	1.35
Oleic Acid	-	-	-	-	21.389	1.38
Elaidic acid, (E)-9-Octadecenoic acid ethyl	-	-	-	-	21.582	5.26
Octadecanoic acid, ethyl ester	-	-	-	-	21.838	1.32
1-Heneicosanol	-	-	-	-	21.952	1.96

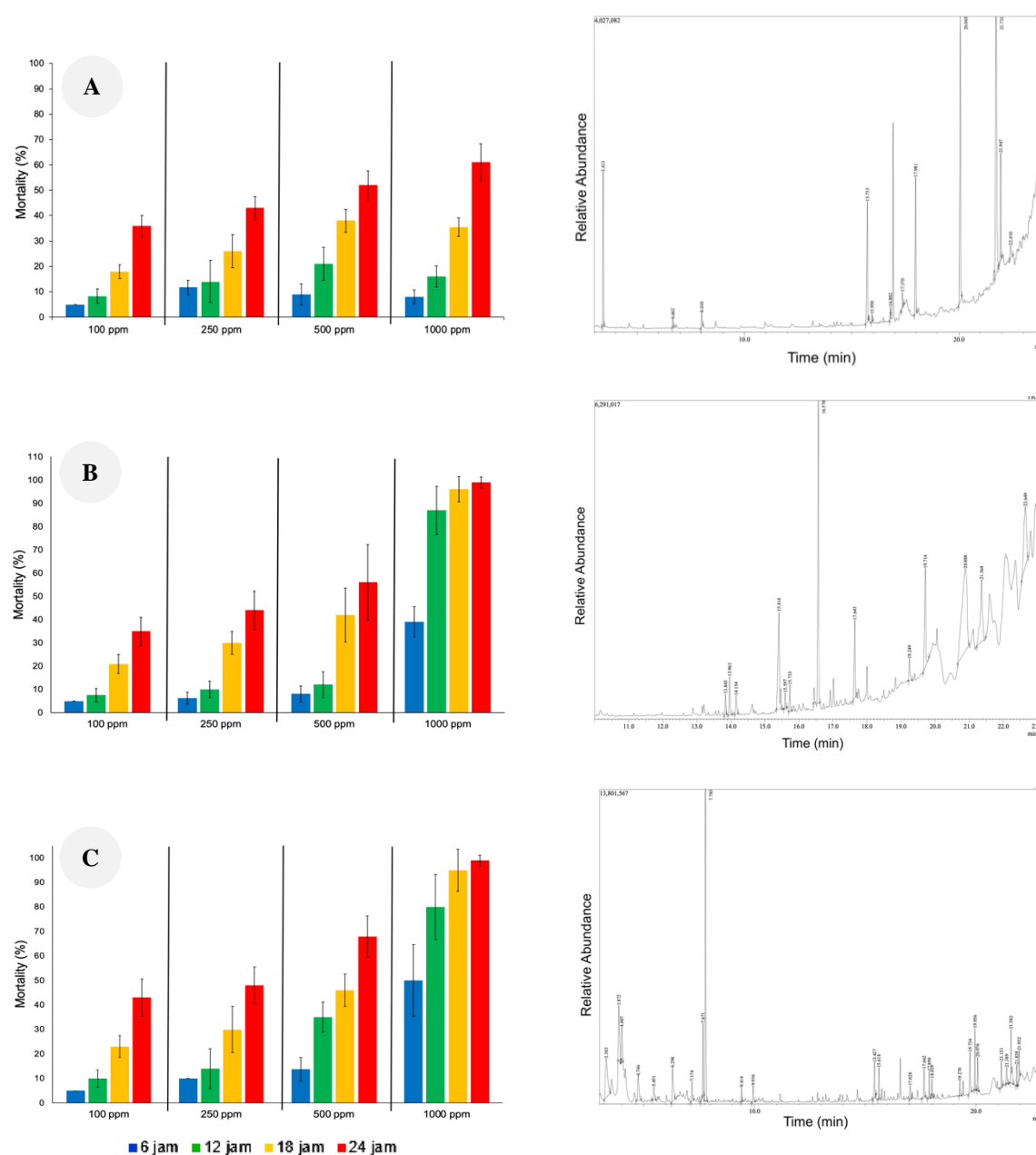


Figure 1. Mortality percentage of *Artemia salina* treated with red mangrove extracts using various solvents and concentrations. A. Ethanol extract; B. Ethyl acetate extract; C. n-hexane extract; D. GC-MS chromatogram of ethanol extract of red mangroves (*Rizophora mucronata*); F. GC-MS chromatogram of ethyl acetate extract of red mangrove (*Rizophora mucronata*); G. GC-MS chromatogram of n-hexane extract of red mangrove (*Rizophora mucronata*)

Table 3. Mortality and LC₅₀ - 24-hour values of *Artemia salina* treated with red mangrove (*Rizophora mucronata*) leaf extract

Solvent	<i>Artemia salina</i> mortality (%)				LC ₅₀ - 24-hour (ppm)
	100 ppm	250 ppm	500 ppm	1000 ppm	
Ethanol	36±4.18 ^a	43±4.47 ^a	52±5.70 ^a	61±7.42 ^a	402.45
Ethyl acetate	35±6.12 ^a	42±8.37 ^a	50±10.80 ^a	99±2.24 ^b	243.55
N-hexane	43±7.58 ^a	48±7.58 ^a	68±8.37 ^b	99±2.24 ^b	180.31

Note: *: Different superscripts in the same column indicate significant differences (p<0.05)

Effect of the concentration of red mangrove leaf extract on the inhibition of bacterial growth

The red mangrove leaf extract at concentrations of 20% and 30% had the same inhibitory zone diameter against *S. aureus* and *A. hydrophila* bacteria up to 48 h ($p > 0.05$). A significant increase in the diameter of the inhibition zone against *S. aureus* and *A. hydrophila* occurred at a concentration of 40% with the inhibitory zone of 15.07 ± 1.13 mm (*S. aureus*) and 11.53 ± 1.96 mm (*A. hydrophila*) (Figure 2.A; 2.B). In 24 hours, the diameter of the inhibition zone in various red mangrove leaf extract concentrations against *V. parahaemolyticus* increases significantly as the extract concentration increases ($p < 0.05$). However, in 48

hours, the diameter of the inhibition zone of 30% and 40% extract concentration showed no significant difference with values of 9.35 ± 1.28 - 9.76 ± 0.62 mm ($p > 0.05$) (Figure 2C).

The research results show that the diameter of the inhibition zone against *S. aureus* in all treatments was higher and significantly different from that of the bacteria *A. hydrophila* (Figure 2D). However, when the inhibition zone is compared to the inhibition zone of *V. parahaemolyticus*, the significant difference is only at concentrations of 20% and 40%. Meanwhile, the diameter of the inhibition zone between *A. hydrophila* and *V. parahaemolyticus* was not significantly different, except for a concentration of 30% at 48 hours (Figure 2.E).

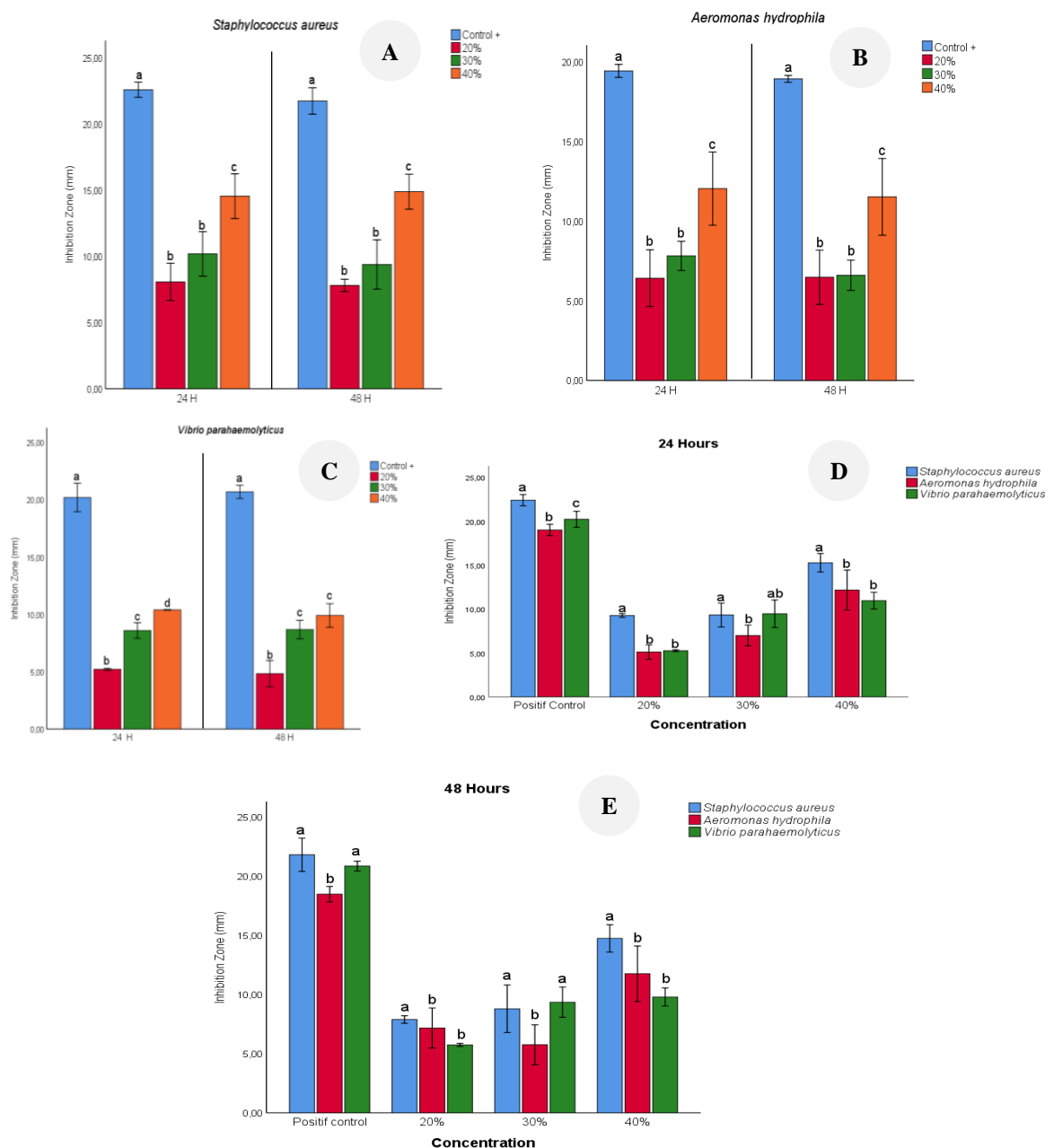


Figure 2. The effect of ethyl acetate extract of red mangrove leaves on the inhibition zone against: A. *Staphylococcus aureus*; B. *Aeromonas hydrophila*; C. *Vibrio parahaemolyticus*. The inhibition zone of ethyl acetal extract of red mangrove leaves against several bacteria was observed at: D. 24 hours; E. 48 hours

Table 4. Effect of time on the inhibition zone of fish pathogenic bacteria

Bacteria	Treatment	Inhibition zone diameter (mm)		Criteria	Sig. t-test
		24 h	48 h		
<i>Staphylococcus aureus</i>	Control -	0	0	-	0
	Control +	22.41±0.63	22.21±1.00	Very strong	0.761
	20%	8.30±1.33	7.57±1.39	Moderate	0.072
	30%	9.91±1.56	9.43±1.75	Moderate	0.148
	40%	14.55±1.69	15.07±1.13	Strong	0.572
<i>Aeromonas hydrophila</i>	Control -	0	0	-	0
	Control +	19.01±0.64	18.49±0.66	Strong	0.065
	20%	5.92±1.76	6.02±1.65	Moderate	0.262
	30%	7.41±1.17	5.89±1.20	Moderate	0.060
	40%	12.04±1.87 ^a	11.53±1.96 ^b	Strong	0.015 [*]
<i>Vibrio parahaemolyticus</i>	Control -	0	0	-	0
	Control +	20.23±0.91	20.86±0.41	Strong	0.213
	20%	5.26±0.10	5.74±0.13	Moderate	0.891
	30%	9.46±1.56	9.35±1.28	Moderate	0.876
	40%	11.24±0.96	9.76±0.62	Strong	0.107

Effect of the length of incubation on the bacterial inhibition zone

The results showed no significant differences in the diameter of the inhibition zones against *S. aureus* and *V. parahaemolyticus* as the incubation time increased in all treatments. A similar pattern was observed in the diameter of the inhibition zones against *A. hydrophila* for the negative control, positive control, and extract concentrations of 20% and 30%. However, at an extract concentration of 40%, the diameter of the inhibition zone significantly decreased after 48 hours (11.53±1.96 mm) compared to 24 hours (12.04±1.87 mm) (Table 4).

Discussion

Two species of mangroves have been tested for their cytotoxicity using polar, semi-polar, and non-polar solvents, i.e., *Rhizophora apiculata* and *Acanthus ilicifolius* (Maulana 2021). The hexane extract of *A. ilicifolius* leaves exhibited the highest toxicity, with a value of 245.25 ppm, and the highest toxicity of *R. apiculata* leaves was obtained in the ethanol extract (40.45 ppm). This research revealed that the n-hexane extract was more toxic than other extracts. However, the yield produced was lower than that of the other extracts. The effectiveness of the extraction process influences the yield. According to Rodríguez De Luna et al. (2020), factors influencing extraction results are time, temperature, stirring, and solvent. Apart from the type of solvent, sample size also influences the extract yield. The smaller the surface area of the sample, the greater the contact and the greater the interaction with the solvent (Płotka-Wasyłka et al. 2017).

The difference in toxicity values among solvents is closely related to the type of bioactive compounds extracted by the solvent. It is caused by its active compounds, such as terpenoids in mangrove leaves, extracted by hexane and ethyl acetate, which are toxic to *Artemia*. The terpenoid compounds in the n-hexane extract of red mangrove (*R. mucronata*) leaves include 1,8-cineole and phytol. Terpenoid compounds were also identified in the ethyl acetate, i.e., 1(2H)-Naphthalenone, octahydro-4a,8a-dim, which causes

toxicity. Phytol and cineole are toxic against bacteria primarily due to their disruptive effects on bacterial cell membranes and essential cellular processes. Phytol, a diterpene alcohol, integrates into the bacterial cell membrane, altering its permeability and leading to the leakage of vital cellular contents, eventually resulting in cell death (Okoh et al. 2015). Phytol interferes with the functions of enzymes and proteins within the cell membrane, further contributing to its antibacterial activity (Sung et al. 2018).

On the other hand, cineole, also known as eucalyptol, is a cyclic ether and monoterpene that exhibits antimicrobial properties by similarly disrupting bacterial cell membranes. Its lipophilic nature allows cineole to penetrate the lipid bilayer of bacterial membranes, causing structural and functional damage (Van et al. 2024). Moreover, cineole may interfere with bacterial respiration and energy production, making survival difficult for the bacteria (Calo et al. 2015). These combined effects of phytol and cineole on bacterial integrity result in potent antibacterial activity and are widely used in natural preservatives and various treatments to control bacterial pathogens (Messaoudi et al. 2023).

The ethyl acetate extract of red mangrove leaves showed various antibacterial activity against each bacteria tested. *S. aureus* was more susceptible than the *A. hydrophila* and *V. parahaemolyticus* bacteria. It might be influenced by the characteristics of the cell wall structure of *S. aureus* as a Gram-positive bacteria. According to Dörr et al. (2019), Gram-negative bacteria (including *A. hydrophila* and *V. parahaemolyticus*) are less susceptible to antibiotics because it has a complex cell wall structure with 3 layers, namely the outer membrane (OM), the peptidoglycan cell wall, and the cytoplasmic or inner membrane (IM). Gram-positive bacteria possess a cell wall structure with more peptidoglycan, fewer lipids, and polysaccharides (teichoic acid) (Siegel et al. 2016). Teichoic acid is a water-soluble polymer that acts as a conduit for transporting positive ions in or out of the cell. This water solubility indicates that the cell walls of Gram-positive bacteria are more polar. Flavonoid and tannin are polar compounds that penetrate the polar peptidoglycan layer more quickly than the non-

polar lipid layer. It causes the inhibitory activity of Gram-positive bacteria to be greater than Gram-negative bacteria's.

A similar result was also reported by Jamili et al. (2014), where *S. aureus* (Gram-positive bacteria) had a broader zone of inhibition compared to *Salmonella typhi* (Gram-negative bacteria). The research results by Danada and Yamindago (2014) showed that *Avicennia marina* mangrove leaf extract was more effective in inhibiting *S. aureus* than *V. parahaemolyticus*. A study by Sungkar et al. (2018) showed that face powder enriched with *R. mucronata* mangrove fruit extract demonstrated a more significant inhibition zone against *S. aureus* than *Escherichia coli*.

Differences in the antibacterial activity of each extract are influenced by the composition of the active compounds. Flavonoids can damage cell walls, cause cell death, and exhibit antibacterial activity by disrupting the metabolic function of microorganisms. They achieve this by damaging cell walls and denaturing the cell proteases of microorganisms. Tannins can also damage cell membranes and the formation of bacterial conidia. The high density of bacterial cells may also influence the effectiveness of the active antibacterial substances in each extract (Stokes et al. 2019).

The concentration of the bioactive compounds influences the effectiveness of antibacterial activity. Increasing the concentration of substances causes an increase of active antibacterial compounds so that their ability to kill bacteria also increases (Díez-Pascual 2018). According to Li et al. (2017), the concentration of antimicrobial substances significantly affects the growth of microorganisms. Higher concentrations lead to a greater number of microbial deaths. Consequently, varying concentrations of antimicrobial substances could result in different inhibitory zones for each microbe.

The study results showed no difference in inhibitory zone in different incubation time ($P < 0.005$) for the *S. aureus* and *V. parahaemolyticus* bacteria. However, the ethyl acetate extract of red mangrove leaves at a concentration of 40% at 48 hours of incubation was decreased compared to 24 hours of incubation. Siegel et al. (2016) stated that different incubation times impacted antibacterial activities. Seagrass extract incubated for 48 hours at a concentration of 15% exhibited higher inhibitory activity than that of 24 hours and 72 hours. Generally, a more extended incubation period results in a broader inhibition zone as the active compounds in the extract increase and have a more extensive inhibitory zone. However, the inhibition rate slows down beyond a certain point due to the decrease in the amount of active compounds in the extract (Pina-Pérez and Pérez 2018). A prolonged incubation period generally allows the active compounds in an extract to accumulate, leading to an increased antimicrobial effect. This accumulation enhances the interaction between the active compounds and microbial cells, resulting in a broader inhibition zone around the extract. The initial stages of incubation possess a rapid expansion of this inhibition zone as the active compounds are at their highest concentration, effectively disrupting microbial growth and leading to a clear area devoid of bacterial or fungal activity. However, as the incubation continues, the inhibition zone's expansion rate begins to slow down due to the active compounds in

the extract diminishing over time, either due to degradation or diffusion away from the application site. As these compounds decrease in concentration, their ability to inhibit microbial growth decreases. Therefore, optimizing incubation times to achieve maximum antimicrobial efficacy is important.

Ethyl acetate extract of red mangrove (*R. mucronata*) leaves had the highest inhibitory zone against *S. aureus* ATCC 25923 compared to the *A. hydrophila* ATCC 7966 and *V. parahaemolyticus* at 24 hours. However, at 48 hours and 30% extract concentration, the inhibition zone against *S. aureus* ATCC 25923 was almost equal to that of *V. parahaemolyticus*. The lowest inhibition zone was in the *A. hydrophila* ATCC 7966 and *V. parahaemolyticus* bacteria, depending on time and concentration.

It can be concluded that based on the Brine Shrimp Lethality Test (BSLT), the LC_{50} value for the ethanol, ethyl acetate, and n-hexane extracts of red mangrove (*R. mucronata*) were 402,450 ppm, 230,977 ppm, and 180,310 ppm, respectively. It indicated that all three extracts were categorized as toxic. The secondary metabolite compounds in the ethanol extract with the highest LC_{50} were terpenoids, alkaloids, ketones, phenols, turpentine oil, essential oils, unsaturated fats, and camphor. The ethyl acetate extract of red mangrove leaves shows increased antibacterial activity against *S. aureus* and *V. parahaemolyticus* by increasing extract concentration. At 48 h of incubation, the largest inhibition zone diameter was obtained against *S. aureus* (15.07 ± 1.13 mm). Inter-time comparison showed no significant difference in inhibition zone diameter against *S. aureus* and *V. parahaemolyticus*. The inhibition zone diameter against *A. hydrophila* was decreased from 12.04 ± 1.87 mm at 24 hours to 11.53 ± 1.96 mm at 48 h at 40% extract concentration.

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REFERENCES

- Al-Rubaye AF, Hameed IH, Kadhim MJ. 2017. A review: Uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. *Intl J Toxicol Pharmacol Res* 9 (1): 81-85. DOI: 10.25258/ijtr.v9i01.9042.
- Alongi DM. 2022. Climate Change and Mangroves. In: Das SC, Pullaiah, Ashton EC (eds). *Mangroves: Biodiversity, Livelihoods and Conservation*. Springer, Singapore. DOI: 10.1007/978-981-19-0519-3_8.
- Anderson JE, Chang CJ, McLaughlin JL. 1988. Bioactive components of *Allamanda schottii*. *J Nat Prod* 51: 307. DOI: 10.1021/np50056a018.
- Anjaneyulu AS, Rao VL. 2001. Rhizophorin A, a novel secolabdane diterpenoid from the Indian mangrove plant *Rhizophora mucronata*. *Nat Prod Lett* 15: 13-9. DOI: 10.1080/10575630108041252.
- Ansori ANM, Fadholly A, Hayaza S, Susilo RJK, Inayatillah B, Winarni D, Husen SA. 2020. A review on medicinal properties of mangosteen

- (*Garcinia mangostana* L.). Res J Pharm Technol 13 (2): 974-982. DOI: 10.5958/0974-360X.2020.00182.1.
- Arifanti VB, Sidik F, Mulyanto B, Susilowati A, Wahyuni T, Subarno, Yulianti, Yuniarti N, Aminah A, Suita E, Karlina E, Suharti S, Pratiwi, Turjaman M, Hidayat A, Henti, Rachmat H, Imanuddin R, Yeny I, Darwiati W, Sari N, Hakim SS, Slamet WY, Novita N. 2022. Challenges and strategies for sustainable mangrove management in Indonesia: A review. Forests 13 (5): 695. DOI: 10.3390/f13050695.
- Awuku-Sowah EM, Graham NA, Watson NM. 2022. Investigating mangrove-human health relationships: A review of recently reported physiological benefits. Dialogues in Health 1: 100059. DOI: 10.1016/j.dialog.2022.100059.
- Baskaran R, Mohan PM. 2012. In vitro antibacterial activity of leaf extract of *Rhizophora mucronata* L. against multi drug resistant *Vibrio* spp. isolated from marine water lobster's larvae hatcheries. Indian J Geom Sci 41 (3): 218-222.
- Bento JPSP, Victório CP, Praça LF, Oliveira Arruda RdoCde. 2024. Salt extrusion, a new function of lenticels in red mangrove leaves. Environ Exp Bot 223: 105798. DOI: 10.1016/j.envexpbot.2024.105798.
- Bhuvaneshwari M, Sagar B, Doshi S, Chandrasekaran N, Mukherjee A. 2017. Comparative study on toxicity of ZnO and TiO₂ nanoparticles on *Artemia salina*: Effect of pre-UV-A and visible light irradiation. Environ Sci Pollut Res 24: 5633-5646. DOI: 10.1007/s11356-016-8328-z.
- Calo JR, Crandall PG, O'Bryan CA, Ricke SC. 2015. Essential oils as antimicrobials in food systems-A review. Food Control 54: 111-119. DOI: 10.1016/j.foodcont.2014.12.040.
- Damtie D, Mekonnen Y. 2020. Antibacterial activity of essential oils from Ethiopian thyme (*Thymus serrulatus* and *Thymus schimperi*) against tooth decay bacteria. PLoS One 15 (10): e0239775. DOI: 10.1371/journal.pone.0239775.
- Danada RH, Yamindago A. 2014. Analisis aktivitas antibakteri ekstrak daun mangrove *Avicennia marina* dari Kabupaten Trenggalek dan Kabupaten Pasuruan terhadap pertumbuhan *Staphylococcus aureus* dan *Vibrio alginolyticus*. Jurnal Kelautan: Indonesian J Mar Sci Technol 7 (1): 12-19. DOI: 10.21107/jk.v7i1.792. [Indonesian]
- Darmadi J, Batubara RR, Himawan S. 2021. Evaluation of Indonesian mangrove *Xylocarpus granatum* leaves ethyl acetate extract as potential anticancer drug. Sci Rep 11: 6080. DOI: 10.1038/s41598-021-85383-3.
- Díez-Pascual AM. 2018. Antibacterial activity of nanomaterials. Nanomaterials 8 (6): 359. DOI: 10.3390/nano8060359.
- Dörr T, Moynihan PJ, Mayer C. 2019. Bacterial cell wall structure and dynamics. Front Microbiol 10: n487209. DOI: 10.3389/fmicb.2019.02051.
- Ghosh D, Mondal S, Ramakrishna K. 2017. Pharmacobotanical, physicochemical and phytochemical characterisation of a rare salt-secreting mangrove *Aegialitis rotundifolia* Roxb., (Plumbaginaceae) leaves - a comprehensive pharmacognostical study. South Afr J Bot 113: 212-229. DOI: 10.1016/j.sajb.2017.08.012.
- Hamrun N, Nabilah T, Hasyim R, Ruslin M, Dammar I, Arianto MAA. 2020. Toxicity test of bioactive red alga extract *Euclima spinosum* on shrimp *Artemia salina* Leach. Syst Rev Pharm 11: 672-676. DOI: 10.31838/srp.2020.5.94.
- Igbinoza E O, Ogofure AG, Beshiru A. 2022. Evaluation of different agar media for the antibiotic susceptibility testing of some selected bacterial pathogens. Univ Lagos J Basic Med Sci 8 (1-2): 34-42.
- Indriaty I, Ginting B, Hasballah K. 2022. Assessment cytotoxic assay of *Rhizophora* plants mangrove using brine shrimp (*Artemia salina* L.) model. IOP Conf Ser: Earth Environ Sci 951: 012070. DOI: 10.1088/1755-1315/951/1/012070.
- Irawan A, Chikmawati T, Sulistijorini S. 2021. Diversity and zonation of mangrove flora in Belitung Island, Indonesia. Biodiversitas 22 (5): 2981-2992. DOI: 10.13057/biodiv/d220563.
- Jamili MA, Hidayat MN, Hifizah A. 2014. Uji daya hambat ramuan herbal terhadap pertumbuhan *Staphylococcus aureus* dan *Salmonella thypi*. Jurnal Ilmu dan Industri Peternakan 1 (3): 227-239. DOI: 10.24252/jiip.v1i3.1547. [Indonesian]
- Jennerjahn TC, Ardlie ER, Boy J, Jill Heyde J, Lukas MC, Nordhaus I, Sastranegara MH, Máñez KS, Yuwono E. 2022. Mangrove ecosystems under threat in Indonesia: The Segara Anakan Lagoon, Java, and other examples. Science for the protection of Indonesian coastal ecosystems (SPICE). Academic Press, Amsterdam. DOI: 10.1016/B978-0-12-815050-4.00004-3.
- Karim MA, Islam MA, Islam MM, Rahman MS, Sultana S, Biswas S, Hosen MJ, Mazumder K, Rahman MM, Hasan MN. 2020. Evaluation of antioxidant, anti-hemolytic, cytotoxic effects and anti-bacterial activity of selected mangrove plants (*Bruguiera gymnorhiza* and *Heritiera littoralis*) in Bangladesh. Clinical Phytosci 6: 8. DOI: 10.1186/s40816-020-0152-9.
- Kathiresan K. 2021. Mangroves: Types and Importance. In: Rastogi RP, Phulwaria M, Gupta DK (eds). Mangroves: Ecology, Biodiversity and Management. Springer, Singapore. DOI: 10.1007/978-981-16-2494-0_1.
- Khan MF, Alqahtani AS, Almarfadi OM, Ullah R, Nasr FA, Noman OM, Siddiqui NA, Shahat AA, Ahamad SR. 2021. The reproductive toxicity associated with *Dodonaea viscosa*, a folk medicinal plant in Saudi Arabia. Evid-Based Complement Alternat Med 2021: 6689110. DOI: 10.1155/2021/6689110.
- Kumari CS, Yasmin N, Hussain MR et al. 2015. In vitro anti-inflammatory and anti-arthritic property of *Rhizophora mucronata* leaves. Intern J Pharma Scie Res 6: 482-485. DOI: 10.22159/ajpcr.2020.v13i8.38017.
- Kusmana C, Dwiyantri FG, Malik Z. 2020. Comparison of several methods of stands inventory prior to logging towards the yield volume of mangrove forest in Bintuni Bay, West Papua Province, Indonesia. Biodiversitas 21 (4): 1438-1447. DOI: 10.13057/biodiv/d210423.
- Lestari D, Kartika R, Marlina E. 2019. Uji Brine Shrimp Lethality Test (BSLT) umbi bawang tiwai (*Eleutherine bulbosa* (Mill.) Urb) dan uji toksisitas akut fraksi aktif. Jurnal Riset Kefarmasian Indonesia 1 (1): 1-10. DOI: 10.33759/jrki.v1i1.43. [Indonesian]
- Li J, Xie S, Ahmed S, Wang F, Gu Y, Zhang C, Chai X, Wu Y, Cai J, Cheng G. 2017. Antimicrobial activity and resistance: Influencing factors. Front Pharmacol 8: 364. DOI: 10.3389/fphar.2017.00364.
- Maulana DM. 2021. The dose effect of mangrove leaf extract (*Rhizophora apiculata*) on anticancer activity in HeLa cells. J Stem Cell Res Tissue Eng 5 (1): 1-15. DOI: 10.20473/jsrcte.v5i1.29380.
- McLaughlin JL, Chang CJ, Smith DL. 1991. Bench-Top, Bioassay for The Discovery of Bioactive Natural Products, An Update, Natural Product Chemistry. Elsevier, Amsterdam.
- Messaoudi M, Bouzidi N, Khaldi A. 2023. Antimicrobial effects of essential oils and their components on bacteria: Mechanisms and applications. J Nat Prod Therapeutics 10 (2): 245-261.
- Mulyani Y, Haetami K, Baeha LK, Arsad S, Prasetya FS. 2020. In vivo test of *Rhizophora mucronata* mangrove extract from Pangandaran coast towards Nile tilapia *Oreochromis niloticus* infected by *Vibrio harveyi*. J Aquaculture Fish Health 9: 131-142. DOI: 10.20473/jafh.v9i2.16211.
- Musdalipah M, Tee SA, Karmilah K, Sahidin S, Fristiohady A, Yodha AWM. 2021. Total phenolic and flavonoid content, antioxidant, and toxicity test with BSLT of *Meistera chinensis* fruit fraction from Southeast Sulawesi. Borneo J Pharm 4 (1): 6-15. DOI: 10.33084/bjop.v4i1.1686.
- Ningdyah AW, Alimuddin AH, Jayuska A. 2015. Uji toksisitas dengan metode BSLT (*Brine Shrimp Lethality Test*) terhadap hasil fraksinasi ekstrak kulit buah tampoi (*Baccaurea macrocarpa*). Jurnal Kimia Khatulistiwa 4 (1): 91-103. [Indonesian]
- Okoh O, Sadimenko A, Afolayan A. 2015. Comparative evaluation of the antibacterial activity of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem 172: 374-379. DOI: 10.1016/j.foodchem.2009.09.084.
- Orno TG, Rantesalu A. 2020. In vitro cytotoxicity assays of seagrass (*Enhalus acoroides*) methanol extract from Soropia Coastal waters Southeast Sulawesi Regency. Indonesia J Med Laboratory Sci Technol 2 (1): 27-33. DOI: 10.33086/ijmlst.v2i1.1463.
- Park SY, Ha JE, Kwon H, Park SC, Kim JH. 2020. Recent insights into *Aeromonas salmonicida* and its bacteriophages in aquaculture: A comprehensive review. J Microbiol Biotechnol 30: 1443. DOI: 10.4014/jmb.2005.05040.
- Pina-Pérez MC, Pérez MF. 2018. Antimicrobial potential of legume extracts against foodborne pathogens: A review. Trends Food Sci Technol 72: 114-124. DOI: 10.1016/j.tifs.2017.12.007.
- Plotka-Wasyłka J, Rutkowska M, Owczarek K, Tobiszewski M, Namieśnik J. 2017. Extraction with environmentally friendly solvents. TrAC Trends Anal Chem 91: 12-25. DOI: 10.1016/j.trac.2017.03.006.
- Rahman FA, Haniastuti T, Utami TW. 2017. Skrining fitokimia dan aktivitas antibakteri ekstrak etanol daun sirsak (*Annona muricata* L.) pada *Streptococcus mutans* ATCC 35668. Majalah Kedokteran Gigi Indonesia 3 (1): 1-7. DOI: 10.22146/majkedgiind.11325. [Indonesian]
- Roy UK, Sarkar C, Jamaddar S, Mondal B, Ramproshad S, Zulfiquar TN, Panthi S, Mondal M, Mukerjee N, Rahman MH, Roy P, Ahmad Z, Khan FS, Sweilam SH. 2023. A detailed assessment of the traditional applications, bioactive content, pharmacology, and toxicity of

- Rhizophora mucronata*. J Herbal Med 41: 100702. DOI: 10.1016/j.hermed.2023.100702.
- Rodríguez De Luna SL, Ramírez-Garza RE, Serna Saldívar SO. 2020. Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. Sci World J 2020: 6792069. DOI: 10.1155/2020/6792069.
- Said F, Rahmawati I, Ningsih NS. 2023. Clinical study and toxicity tests of disclosing agent *Aloe vera* gel. Asian J Pharm Res Develop 11 (2): 15-19. DOI: 10.22270/ajprd.v11i2.1243.
- Saragih G, Tamrin M, Nasution DY. 2020. Phytochemical screening and toxicity of ethanolic extract of mangrove (*Rhizophora mucronata*) leaves from Langsa, Aceh Timur. Rasaya J Chem 13 (1): 476-480. DOI: 10.31788/RJC.2020.1315524.
- Setyawan AD, Ragavan P, Basyuni M, Sarno S. 2019. *Rhizophora mucronata* as source of foods and medicines. Intl J Bonorowo Wetlands 9 (1): 42-55. DOI: 10.13057/bonorowo/w090105.
- Siegel SD, Liu J, Ton-That H. 2016. Biogenesis of the Gram-positive bacterial cell envelope. Curr Opin Microbiol 34: 31-37. DOI: 10.1016/j.mib.2016.07.015.
- Stokes JM, Lopatkin AJ, Lobritz MA, Collins JJ. 2019. Bacterial metabolism and antibiotic efficacy. Cell Metabolism 30 (2): 251-259. DOI: 10.1016/j.cmet.2019.06.009.
- Sudhir S, Arunprasath A, Vel VS. 2022. A critical review on adaptations, and biological activities of the mangroves. J Nat Pesticide Res 1: 100006. DOI: 10.1016/j.napere.2022.100006.
- Sugrani A, Fitriana. 2021. Antibacterial and anticancer activity of protein from red algae (*Euclima cottonii*). Saudi J Med Pharm Sci 7 (12): 594-598. DOI: 10.36348/sjmps.2021.v07i12.001.
- Sung WS, Lee IS, Lee DG. 2018. Damage to the cytoplasmic membrane and cell death caused by the large-scale effects of phytol on staphylococci. Appl Environ Microbiol 84 (11): e00338-18.
- Sungkar OF, Khanza S, Pangestu RA. 2018. Aktivitas antibakteri bedak yang diperkaya dengan konsentrasi ekstrak buah *Rhizophora mucronata*. Jurnal Teknologi Pangan 2 (2): 135-141. DOI: 10.14710/jtp.2018.21170. [Indonesian]
- Syamsurizal S, Utami DT, Sutrisno S, Efrini E, Jumardi MI. 2023. Toxicity test of red palm (*Cyrtostachys renda* Blume.) using the brine shrimp lethality test (BSLT) method. J Pharm Sci 1: 407-413. DOI: 10.36490/journal-jps.com.v6i5-si.429.
- Van YT, Dinh D, Tran DM, Tran TN, Nguyen H, Duong TT, Doan TQ, Nguyen HT, Ogunwande IA. 2024. The antimicrobial activity and essential oil constituents of the leaves and trunks of *Aquilaria banaensis* PH Hô (Thymelaeaceae) from Vietnam. Nat Prod Res 38 (5): 744-752. DOI: 10.1080/14786419.2023.2196624.
- Zhao C, Fu H, Sun S, Qiao H, Zhang W, Jin S, Jiang S, Xiong Y, Gong Y. 2017. Experimental inoculation of oriental river prawn *Macrobrachium nipponense* with white spot syndrome virus (WSSV). Dis Aquat Organism 126 (2): 125-134. DOI: 10.3354/dao03165.