BIODIVERSITAS
Volume 24, Number 3, March 2023
Pages: 1346-1355

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240303

Potential of prospective medicinal plants of Rhizophoraceae from North Kalimantan, Indonesia

SAAT EGRA[1,2,3], HARLINDA KUSPRADINI[4], IRAWAN WIJAYA KUSUMA[4], IRMANIDA BATUBARA[5], IMRA[6], NURJANNAH[2], ETTY WAHYUNI[2], KOSEI YAMAUCHI[3,♥], TOHRU MITSUNAGA[3,♥♥]

[1]United Graduate School of Agriculture Sciences, Gifu University. Yanagido 501-1193 Gifu, Japan
[2]Faculty of Agriculture, Universitas Borneo Tarakan. Jl. Amal Lama No. 1, Tarakan 77123, North Kalimantan, Indonesia
[3]Faculty of Applied Biological Sciences, Gifu University. 1-1 Yanagido, 501-1193 Gifu, Japan. Tel.: +81-58-293-2835, ♥email: kosei_y@gifu-u.ac.jp, ♥♥email: mitunaga@gifu-u.ac.jp, saat.egra.shaumi@gmail.com
[4]Faculty of Forestry, Universitas Mulawarman. Jl. Penajam, Kampus Gunung Kelua, Samarinda 75119, East Kalimantan, Indonesia
[5]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Tropical Biopharmaca Research Center, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia
[6]Faculty of Fishery, Universitas Borneo Tarakan. Jl. Amal Lama No. 1, Tarakan 77123, North Kalimantan, Indonesia

Manuscript received: 19 January 2023. Revision accepted: 1 February 2023.

Abstract. *Egra S, Kuspradini H, Kusuma IW, Batubara I, Imra, Nurjannah, Wahyuni E, Yamauchi K, Mitsunaga T. 2023. Potential of prospective medicinal plants of Rhizophoraceae from North Kalimantan, Indonesia. Biodiversitas 24: 1346-1355.* The abundance of mangrove forests in the equatorial region necessitates proper management, including the sustainable use of plant parts for functional products such as food, medicine, etc. This study aimed to assess the medicinal potential of five mangrove plants through phytochemical analysis, antibacterial assays against *Streptococcus sobrinus*, DPPH free radicals, and toxicity assay using *Artemia salina* L., *Bruguiera parviflora* (Roxb.) Wight and Arn. ex Griff, *Bruguiera cylindrica* (L.) Blume, *Ceriops tagal* (Perr.). The plants used were *Ceriops tagal* (Perr.) C.B. Rob, *Rhizophora mucronata* Poir, and *Rhizophora apiculata* Blume. Plant samples were extracted with n-hexane, ethyl acetate, and methanol in that order, and then the obtained plant extracts were subjected to various assays. The results showed that *B. cylindrica* wood and *C. tagal* leaf extract had the highest antibacterial activity, with more than 50% relative inhibition. The *C. tagal* leaf methanolic extract had the highest antioxidant activity, by 91% relative inhibition. Followed by *R. mucronata* wood ethyl acetate extract and *B. parviflora* leaf methanolic extract, with 87% and 86%, respectively. The highest value in the cytotoxicity assay was discovered in the *B. cylindrica* in the very strong category with an LC_{50} value of 22.9 µg/mL. The present study revealed the potential of mangrove plant extracts to have strong antibacterial, cytotoxic, and antioxidant properties.

Keywords: Antibacterial, antioxidant, mangrove, phytochemical, toxicity

INTRODUCTION

Tarakan City is a small island with an area of 657.33 km^2. The island is located in the northern region of Kalimantan, Indonesia which has mangrove forest areas of 1,244.9 ha. The mangrove forest of Tarakan City is part of the coastal ecosystem. That provides a productive natural resource for fish (fish, shrimps, crabs, gastropods, shells), animals, snakes, monkeys, proboscis's monkeys, birds, and mangrove vegetation, namely mangrove plants from *Rhizophora*, *Avicennia*, *Combretocarpus*, *Nypa*, etc. Specifically, the most mangrove vegetation found on the side of coastal mangrove forest land in Tarakan City is *Rhizophora* plants (Sawitri et al. 2013).

Rhizophora plants, commonly called bakau in Indonesia, are mangrove vegetation that mostly grows on the side of tropical coastal land. Five types of mangrove plants can commonly be found in the Indonesian mangrove forest, i.e., *Rhizophora apiculata* Blume, *Rhizophora mucronata* Lam., *Rhizophora stylosa* Griffith, *Bruguiera gymnorrhiza* (L.) Lam., and *Ceriops tagal* (Perr.) C.B.Rob. This vegetation can also be found mostly in the mangrove conservation area of Tarakan City, North Kalimantan, Indonesia. The Indonesian coastal peoples have long utilized mangroves as materials for paper, coal, and firewood. Mangroves contain a lot of tannins, especially in the stems. Tannins in plants play an important role in protecting plants from predation by herbivores and pests and regulating growth. In addition, tannins are a group of polyphenols with antibacterial activity (Nguyen et al. 2023). Tannins in mangroves can be used as a coating material. People living near the Indonesian coast also utilize mangroves as traditional medicinal for generations. However, the coastal peoples of Tarakan City have not optimally utilized the mangrove plants (Sawitri et al. 2013).

Sough, *Ambay* and *Mandori* tribes from Papua near the coastal area have used mangroves as traditional medicinal plants for generations (Mahmud and Wahyudi 2014). The *Sough* tribe in Papua utilized *Rhizophora* bark for toothache and malaria, while *Rhizophora* root was utilized to cure diarrhea. The *Ambay* tribe utilized *Rhizophora* stem and twig coal for strengthening the toddler's bones. The *Mandori* tribe utilized *Rhizophora* root as a diarrhea curing following the *Sough* tribe. These tribes believed that using mangrove plants as traditional medicines had no side effects. The coastal peoples of Mamuya Village in East

Halmahera District, North Maluku, Indonesia have a long history of using mangrove plants as traditional medicines for rashes, wounds, liver disease, muscle aches, and toothache (Abubakar et al. 2019). Mangroves have long been utilized as traditional medicinal plants by the coastal peoples are considered due to the active compounds in the mangroves that have the potential as natural medicinal materials to cure various diseases. On the other hand, *B. parviflora* is traditionally used as an antitumor. This plant contains carbohydrates, carotenoids, lipids, minerals, phenolic compounds, procyanidins, proteins, and tannins. *R. mucronata* is also used traditionally as a treatment for elephantiasis, hematoma, hepatitis, ulcers, and as a febrifuge. This plant contains alkaloids, anthocyanidins, carotenoids, tannins, gibberellins, flavonoids, inositols, polysaccharides, polyphenols, procyanidins, proteins, saponins, steroids, and triterpenes (Bandaranayake 2002).

The chemical composition and bioactivity of Rhizophoraceae mangrove plants have been widely studied. The Rhizophoraceae family contains the most common natural mangrove plant species, which are found across the tropics and parts of the subtropics. There are 24 species in this family, grouped into four genera. Half of the 24 species have not had their phytochemical components investigated. There have been reports of 268 metabolites from 16 different species. Diterpenoids and triterpenoids have been identified as key phytochemical constituents in the family (Nebula et al. 2013).

R. apiculata leaf originated from the Indian coastal area and had a potential antioxidant activity that could prevent free radicals at 84% (Ramalingam et al. 2018). *R. mucronata* extract displayed antioxidant and hepatoprotective potential with low toxicity levels after *in vivo* tests on the mouse in repetitive doses for 28 days (Widadi et al. 2014). The antibacterial activity of *R. apiculata* extract could inhibit Gram-positive bacteria growth, such as *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* (Sormin et al. 2021). The saponins, tannins, flavonoids, and steroids had antibacterial activity potential in *R. apiculata* extract. The *Rhizophora* mangrove types are known to have antioxidant and potential antibacterial development (Syawal et al. 2020). The leaves and bark of *Soneratia alba* have inhibitory activity with *Streptococcus sobrinus* of 48.51% at a concentration of 20 mg/mL (Egra et al. 2020). This study provides information on the inhibition of *S. sobrinus* in other mangrove species with relatively lower concentrations. This research aims to provide information regarding Rhizophora's potential as a natural medicinal, antioxidant, and antibacterial agent, as its distribution around the mangrove region of Tarakan City, North Kalimantan, Indonesia is very extensive and widespread.

MATERIALS AND METHODS

Plants and chemical materials

Five mangrove plants were obtained from the Mangrove and Proboscis Monkey Conservation Area, Tarakan City, North Kalimantan, Indonesia (3.304693°N, 117.577164°E) (Figure 1). The chemical materials, namely glucose and nutrient broth, were obtained from Merck (Darmstadt, Germany). DPPH was purchased from Wako Pure Chemical Industries, Ltd. (Japan). DMSO (dimethyl-sulfoxide) was purchased from Merck (Darmstadt, Germany). Other chemical materials were retrieved commercially along with HPLC solvent with high quality and purity levels. The plant herbs used were preserved in the Laboratory of Dendrology, Faculty of Forestry, Universitas Mulawarman.

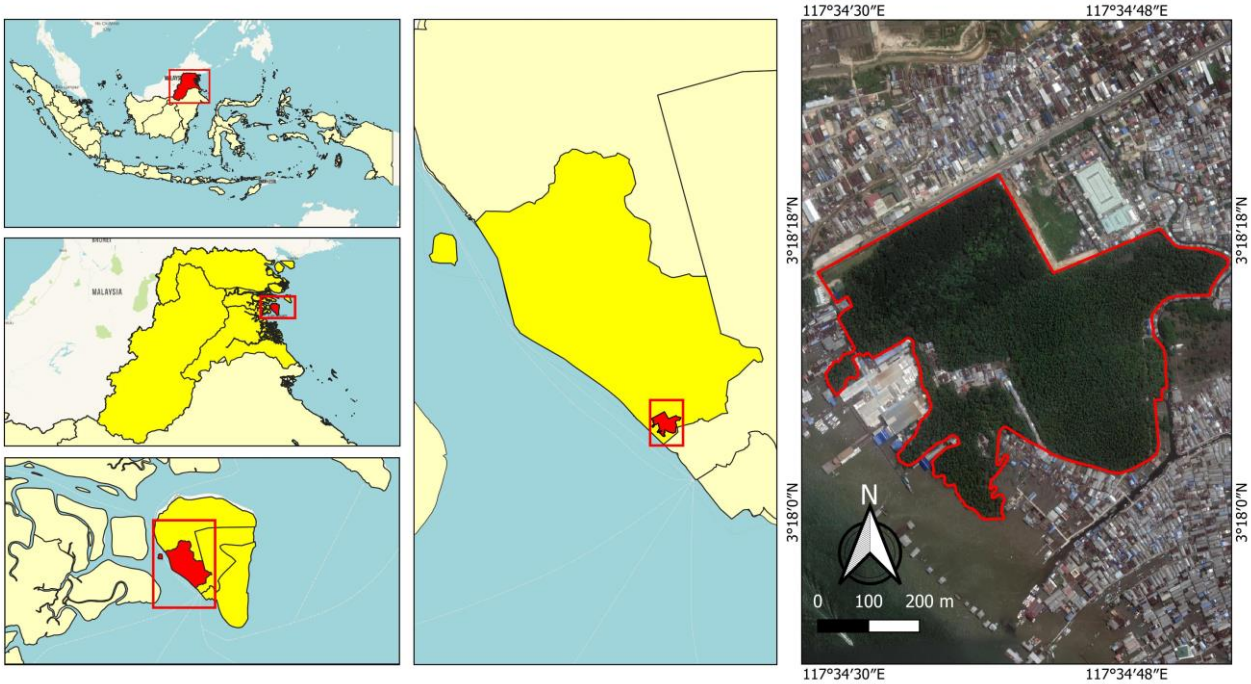

Figure 1. Research sites in the Mangrove and Proboscis Monkey Conservation Area, Tarakan City, North Kalimantan, Indonesia

Plant extraction

The leaves, barks, and woods of Rhizophora species were collected from the field; about 250-500 g was collected for each sample over three days. First, the samples were dried in two ways: one week at room temperature and three days in an oven, after which they were ground to pieces with a Waring (USA) grinder. Next, the samples underwent serial maceration using n-hexane, ethyl acetate, methanol, and shaking (IKA-KS 260-C shaker, Switzerland). Next, the samples were filtered using a Whatman No. 2 paper (Maidstone, UK). Next, the solvent was separated from the extracts using a rotary evaporator controlled at 35-40°C. Finally, the extracts obtained were dried in a Memmert 100-80 vacuum oven.

Phytochemical test

Phytochemical tests were carried out with the principle of color testing referred to by Harborne (1998) and Kokate (2002). Each phytochemical test used 0.01 g of dried samples. The alkaloid test used was Dragendorff's solution. Positive samples contain alkaloids identified with Wagner, Mayer, and Dragendorff reagents. The flavonoid test used NaOH, H_2SO4, and Mg-HCl powder. The presence of flavonoids was indicated by the formation of a red, yellow, or orange color. The saponin test was done by dissolving the extract in hot water and shaking it. The appearance of foam that has not disappeared after standing for 15 minutes indicates the presence of saponins. The phenolic test was carried out by dissolving a few milligrams of the extract into a solvent to which 5% $FeCl_3$ solution was added, then shaken. The formation of a green or bluish-green color indicates the presence of phenolic.

Antioxidant assay

The antioxidant activity was determined using the DPPH radical scavenging method (Shimizu et al. 2001). The test was performed using a room temperature (25°C) spectrophotometer with 514 nm wavelength and DPPH (1,1-diphenyl-2-picrylhydrazyl) solution. First, the 3 mg of mangrove plant extract was dissolved in 1000 µL DMSO. Then, the 33 µL solution sample was moved to the cuvette containing 467 µL ethanol added with 500 µL DPPH at 60 µm (dissolved in ethanol). The test was performed in one extract concentration, 100 µg/mL. Ascorbic acid was used as a positive control with a 50 µg/mL concentration. The mixture was terminated as the sample volume reached 1000 µL (1 mL) and incubated at dark room temperature for 20 minutes. The antioxidant activity was determined from the decolorization of DPPH observed by a spectrophotometer at 514 nm wavelength.

Antibacterial assay

The antibacterial assay was performed using the diffusion method following Ashraf et al. (2015) with modification. The bacteria used in this study were *S. sobrinus* grown in Nutrient agar (NA) from Merck (Darmstadt, Germany) as the media. The media consisted of 20 g of NA with supplemented 1% sucrose, diluted in 1 L distilled water, which was autoclaved for 15 minutes at 121°C. Media were added 20 mL to the petri dish. Then, the medium surface was spread with the bacteria at 20 µL until evenly distributed in parts of the surface perforated at 7 mm. The bacterial concentration in the inoculum was standardized at 0.5 McFarland turbidity scale, equivalent to 10^8 CFU mL^{-1}. Chloramphenicol was used as a positive control at 100 µg/mL concentration. Samples were used in 2000, 1000, and 500 µg/mL concentrations. The test was repeated in triplicate for each sample

Cytotoxicity assay

Artemia larvae were used in this assay (McLaughlin 1991). The first step was to prepare a solution with concentrations of 1000 µL, 100 µL, and 10 µL. The eggs of *A. salina* were then prepared using seawater and placed in a tube with adequate light. The *A. salina* eggs hatch into *A. salina* puppies are called nauplii after 24 to 48 hours. A toxicity assay was performed by placing 3 mL of seawater in a vial and homogenizing it with a sample based on concentration. Then, ten *A. salina* larvae were placed in the vial using a pipette and kept in a well-lit location. The number of dead shrimp larvae for each concentration was counted after 24 hours. The test is repeated with different concentrations of extracts that have a percentage mortality value of 50% at a concentration of 1000 µg/mL.

RESULTS AND DISCUSSION

The extraction process results on each sample were performed through the maceration method and are presented in Table 1. The yield from *C. tagal* showed that the highest amount of extracted leaves, bark, and wood was obtained using methanol solvent at 6.5%, 13.09%, and 0.28%, respectively. Similarly, the yield obtained from *R. mucronata* had the highest amount of extracted leaves, bark, and wood after using methanol solvent at 10.89%, 2.56%, and 0.46%, respectively. Meanwhile, the amount of *R. apiculata* extract yield from leaves, bark, and wood obtained the highest value using methanol solvent at 5.08%, 3.59%, and 1.10%, respectively. Finally, the yield obtained from *B. parviflora* showed that the highest amount of extracted leaves, bark, and wood was obtained using methanol solvent at 3.16%, 1.62%, and 1.74%, respectively. Similarly, the yield obtained from *B. cylindrica* had the highest amount of extracted leaves, bark, and wood after using methanol solvent at 5.80%, 1.48%, and 0.65%, respectively. These conditions presumably indicate that the higher yield value was obtained.

Based on the phytochemical test results, *C. tagal* leaf extract had high steroid contents, while alkaloids, saponins, tannins, phenols, and flavonoids tended to be low. In addition, the bark and wood extract tended to have low alkaloids, steroids, saponins, tannins, phenols, and flavonoids. The *R. mucronata* leaf extract had high steroid contents, while alkaloids, saponins, tannins, phenols, and flavonoids tended to be low. In addition, the bark and wood extract tended to have low alkaloids, steroids, saponins, tannins, phenols, and flavonoids. *R. apiculata* leaf, bark, and wood extracts had high steroids, while alkaloids,

saponins, tannins, phenols, and flavonoids tended to be low.

The antioxidant assay was performed using a spectrophotometer at room temperature (25°C) at 100 µg/mL concentration on 517 nm wavelength. The results of the antioxidant assay are shown in Figure 2. The results show the highest antioxidant level in *C. tagal* leaf extract was obtained from the methanolic extract with inhibition of 91.02%. The highest antioxidant level in *C. tagal* bark extract was obtained from ethyl acetate extract at 82.42%. In addition, the highest antioxidant level in *C. tagal* wood extract was obtained from ethyl acetate extract at 85.66%. The antioxidant test results showed that the highest antioxidant level in *R. mucronata* leaf extract was obtained from methanolic extract with an inhibition of 72.69%, while the highest antioxidant level in *R. mucronata* bark extract was obtained from ethyl acetate extract at 87.28%.

Furthermore, the *R. mucronata* wood methanolic extract obtained the highest inhibition level at 85.91%. Meanwhile, the antioxidant test result on *R. apiculata* extract obtained the highest inhibition level in 100 µg/mL leaf ethyl extract at 47.01%. In bark extract, the highest inhibition level was obtained from ethyl acetate extract at 87.16%, while the highest inhibition level in the wood extract was obtained from methanolic extract at 78.18%. The highest inhibition level of *B. parviflora* leaf extract was obtained using methanol at 86.66%. The highest antioxidant activity in bark and wood extracts was obtained using ethyl acetate at 75.69% and 73.57% inhibition, respectively.

The antioxidant activity of *B. cylindrica* leaf extract was obtained using methanol with an inhibition of 73.94%, while its bark extract was obtained using ethyl acetate solvent with an inhibition of 72.07%. In addition, the antioxidant inhibition level in *B. parviflora* wood extract was obtained using methanol solvent at 78.30%. In comparison, the positive control obtained an inhibition level of 94.51% with a 50 µg/mL concentration.

The results of the antibacterial activity against *S. sobrinus* are shown in Table 2. The inhibition zone revealed that *B. parviflora* leaf extract with n-hexane solvent inhibited *S. sobrinus* bacteria better than the other two wood and bark parts, with the highest inhibition at a concentration of 2000 and an inhibition value of 6.3 mm. *B. cylindrica* wood extract with methanol solvent has better inhibition than other plant parts on *S. sobrinus* bacteria, with the highest inhibition at concentrations of 2000, 1000, and 500 ppm with inhibition zone values of 13.7, 11.7, and 10.5 mm, respectively. The inhibition zone results showed that the *C. tagal* leave ethyl acetate extract showed an antibacterial effect against *S. sobrinus* at 9, 7, and 6 mm in 2000 µg/mL, 1000 µg/mL, and 500 µg/mL concentrations, while hexane and methanolic extracts had no inhibition activity. The bark hexane extracts inhibited *S. sobrinus* growth at 7 and 6 mm in 2000 µg/mL and 1000 µg/mL, respectively. The bark extract using ethyl acetate solvent obtained the inhibition level against *S. sobrinus* at 2000 µg/mL and 1000 µg/mL at 7 and 6 mm. The bark methanolic extract obtained an inhibition level against *S. sobrinus* in 2000 µg/mL concentration at 7 mm inhibition. Based on the inhibition zone results, the *R. mucronata* leaf extract had no inhibition level against *S. sobrinus*. The bark ethyl acetic extract had the inhibition level against *S. sobrinus* at 8, 6, and 6 mm at 2000 µg/mL, 1000 µg/mL, and 500 µg/mL, respectively. The methanolic wood extract had the inhibition level against *S. sobrinus* at 8 mm in 2000 µg/mL. Moreover, the inhibition zone results showed that the *R. apiculata* leaf and bark extracts had no inhibition activity against *S. sobrinus*. In contrast, the wood ethyl acetic extract could inhibit *S. sobrinus* in 2000 µg/mL concentration at 8 mm.

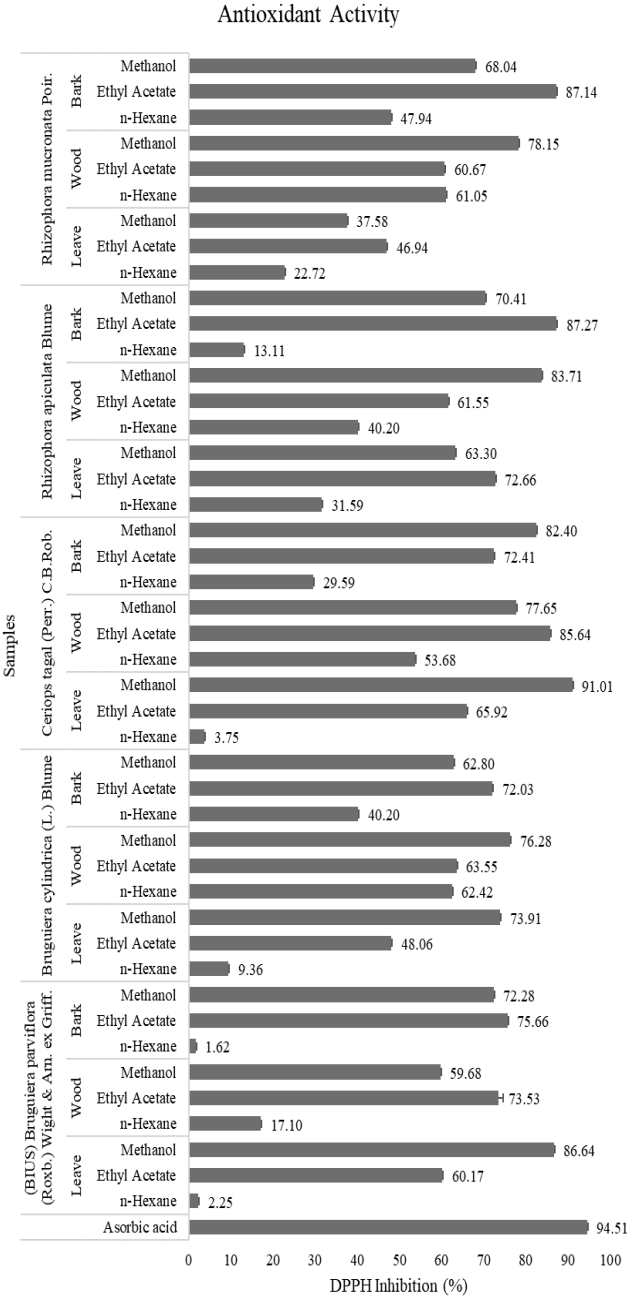

Figure 2. Antioxidant inhibition on five mangrove plants with a concentration of 100 µg/mL

Table 1. Phytochemical screening and yield value of five mangrove samples from the Rhizophoraceae family

Sample	Local name	Part	Solvent	Alkaloids			Steroids	Saponins	Tannins	Phenols	Flavonoids	Yield (%)	Extract color
				Wagner	Mayer	Dragendorff							
B. parviflora	*Bius*		*n*-Hexane	+	-	-	+	-	+	-	-	0.85	Dark green
		Leaves	E. Acetate	+	-	-	+	-	+	+	-	0.54	Dark green
			Methanol	+	-	+	-	+	+	+	-	3.16	Dark green
			n-Hexane	+	+	-	-	-	-	-	-	0.08	Yellow
		Wood	E. Acetate	+	+	-	-	-	-	-	+	0.07	Pale Chocolate
			Methanol	+	+	+	-	+	-	-	-	1.75	Chocolate
			n-Hexane	+	+	-	-	-	-	-	-	0.24	Green
		Bark	E. Acetate	+	-	-	-	-	-	+	-	0.12	Dark green
			Methanol	+	-	+	-	-	+	+	+	1.62	Dark chocolate
B. cylindrica	*Bakau mutut*		*n*-Hexane	+	-	-	+	-	+	+	-	1.37	Dark green
		Leaves	E. Acetate	-	-	-	+	+	-	+	-	1.10	Dark green
			Methanol	+	-	-	+	+	+	+	-	5.80	Dark green
			n-Hexane	+	+	-	-	-	-	-	-	0.30	Yellow
		Wood	E. Acetate	+	-	-	-	-	-	-	-	0.11	Pale chocolate
			Methanol	+	-	-	+	-	+	+	+	0.65	Dark chocolate
			n-Hexane	+	+	+	+	-	-	-	-	0.17	Dark green
		Bark	E. Acetate	+	-	-	+	+	+	-	-	0.22	Dark green
			Methanol	-	-	+	+	+	+	+	+	1.48	Dark green
C. tagal	*Kayu merah*		*n*-Hexane	+	-	-	+	-	-	-	-	0.74	Dark green
		Leaves	E. Acetate	+	-	-	+	-	-	-	-	1.12	Dark green
			Methanol	+	-	-	+	+	+	+	-	6.50	Dark chocolate
			n-Hexane	+	+	-	+	-	-	-	-	0.02	Pale green
		Wood	E. Acetate	+	-	-	-	-	-	-	-	0.10	Dark chocolate
			Methanol	+	-	-	-	-	+	+	+	0.28	Dark red
			n-Hexane	+	-	-	-	-	-	-	-	0.28	Pale green
		Bark	E. Acetate	-	-	-	-	-	-	-	-	0.31	Dark chocolate
			Methanol	+	-	+	-	+	+	-	+	13.0	Dark chocolate
R. mucronata	*Bakau minyak*		*n*-Hexane	+	-	-	+	-	-	-	-	0.19	Dark green
		Leaves	E. Acetate	+	-	-	+	+	-	-	-	1.87	Dark green
			Methanol	+	-	+	+	+	+	+	-	5.08	Dark red
			n-Hexane	-	-	-	+	-	-	-	-	0.46	Pale yellow
		Wood	E. Acetate	+	-	-	+	-	-	-	-	0.07	Chocolate
			Methanol	+	-	-	-	-	+	+	+	1.10	Pale chocolate
			n-Hexane	+	+	-	-	-	-	-	-	0.12	Dark green
		Bark	E. Acetate	+	-	-	-	+	+	+	-	0.16	Dark green
			Methanol	+	-	-	-	-	+	+	+	3.59	Dark chocolate
R. apiculata	*Bakau panggang*		*n*-Hexane	-	-	-	+	-	-	-	-	0.87	Dark green
		Leaves	E. Acetate	-	-	-	+	-	-	-	-	8.99	Dark green
			Methanol	+	+	-	+	+	-	+	-	10.8	Dark chocolate
			n-Hexane	+	+	-	-	+	-	-	-	0.07	Pale chocolate
		Wood	E. Acetate	+	-	-	-	-	-	-	-	0.08	Pale chocolate
			Methanol	+	-	-	+	-	-	+	-	0.46	Dark red
			n-Hexane	-	+	-	+	-	-	-	-	0.14	Dark green
		Bark	E. Acetate	+	-	-	+	+	-	+	-	0.37	Dark green
			Methanol	+	-	+	+	+	+	+	+	2.56	Dark chocolate

Note: (-) negative; (+) exi

Table 2. Antibacterial inhibition against *S. sobrinus*

Plant sample	Part	Extracts	Positive control	Inhibition zone (mm)/extract (µg/well)		
				500	1000	2000
Bruguiera parviflora (Roxb.) Wight & Arn. ex Griff.	Leaves	*n*-Hexane	17.0±0.1	5.7±0.1	6.3±0.1	6.3±0.1
		Ethyl Acetate	16.0±0.1	2.3±0.4	2.7±0.5	6.0±0.1
		Methanol	16.3±0.3	-	-	-
	Wood	*n*-Hexane	16.0±0.0	-	-	6.0±0.0
		Ethyl Acetate	26.0±0.0	-	-	7.7±0.2
		Methanol	15.3±0.4	-	-	-
	Bark	*n*-Hexane	14.6±0.2	-	7.0±0.3	8.7±0.5
		Ethyl Acetate	15.3±0.1	-	5.7±0.1	6.0±0.1
		Methanol	15.3±0.4	2.0±0.3	5.3±0.1	6.0±0.0
Bruguiera cylindrica (L.) Blume	Leaves	*n*-Hexane	19.0±0.0	-	-	-
		Ethyl Acetate	19.0±0.0	-	-	-
		Methanol	19.0±0.0	-	-	6.7±0.1
	Wood	*n*-Hexane	16.0±0.1	-	-	6.0±0.0
		Ethyl Acetate	19.0±0.0	4.0±0.3	5.3±0.5	9.0±0.0
		Methanol	15.6±0.3	10.3±0.1	11.7±0.1	13.7±0.1
	Bark	*n*-Hexane	15.0±0.0	-	-	-
		Ethyl Acetate	15.0±0.0	-	-	3.0±0.5
		Methanol	12.0±0.0	-	5.7±0.1	7.0±0.1
Ceriops tagal (Perr.) C.B.Rob.	Leaves	*n*-Hexane	19.0±0.0	-	-	-
		Ethyl Acetate	19.0±0.0	6.0±0.0	7.0±0.0	9.0±0.0
		Methanol	15.0±0.0	-	-	-
	Wood	*n*-Hexane	20.0±0.0	-	-	-
		Ethyl Acetate	18.6±0.1	-	-	-
		Methanol	20.0±0.0	-	-	-
	Bark	*n*-Hexane	19.0±0.0	-	6.0±0.0	7.0±0.0
		Ethyl Acetate	19.0±0.0	-	6.0±0.0	7.0±0.0
		Methanol	16.0±0.0	-	-	7.0±0.0
Rhizophora apiculata Blume	Leaves	*n*-Hexane	16.0±0.0	-	-	-
		Ethyl Acetate	16.0±0.0	-	-	-
		Methanol	26.0±0.0	-	-	-
	Wood	*n*-Hexane	25.0±0.0	-	-	-
		Ethyl Acetate	25.0±0.0	-	-	8.0±0.0
		Methanol	26.0±0.0	-	-	-
	Bark	*n*-Hexane	27.0±0.0	-	-	-
		Ethyl Acetate	15.0±0.0	-	-	-
		Methanol	25.0±0.0	-	-	-
Rhizophora mucronata Poir.	Leaves	*n*-Hexane	25.0±0.0	-	-	-
		Ethyl Acetate	27.0±0.0	-	-	-
		Methanol	27.0±0.0	-	-	-
	Wood	*n*-Hexane	16.0±0.0	-	-	-
		Ethyl Acetate	27.0±0.0	-	-	-
		Methanol	19.0±0.0	-	-	8.0±0.0
	Bark	*n*-Hexane	27.0±0.0	-	-	-
		Ethyl Acetate	19.0±0.0	6.0±0.0	6.0±0.0	8.0±0.0
		Methanol	25.0±0.0	-	-	-

Note: (-) negative, (+) chloramphenicol 100 µg/mL, (±) Stdev

Table 3 shows the mortality rate of *A. salina* larvae ranging from 0% to 100%, with a high percentage of mortality at a concentration of 1000 µg/mL. The high concentration had a mortality percentage of up to 100% in the bark of the *B. cylindrica* in the n-hexane. Furthermore, *B. cylindrica* bark extract at a 100 µg/mL concentration showed the highest mortality percentage in the samples. At a 100 µg/mL concentration, the mortality percentage was 67% in ethyl acetate. The highest mortality percentage in the samples at 10 µg/mL was 40% in ethyl acetate in the bark of *B. cylindrica*. As a result, the LC_{50} value was 22.9 µg/mL in the very strong category. In this study, nine samples with very strong categories were obtained, namely the ethyl acetate and methanol extract of leaves, and ethyl acetate extract of bark of *B. parviflora*, the hexane and ethyl acetate extract of leaves, and the hexane and ethyl acetate extract of bark of *B. cylindrica*, and the leaves and wood of *R. apiculata*. Each plant's LC_{50} value was less than 250 µg/mL.

Table 3. Toxicity assay using *A. salina*

Samples	Part	Extract	Mortality (%) with concentration (µg/mL)			LC_{50} (µg/mL)	Category
			1000	100	10		
Bruguiera parviflora (Roxb.) Wight & Arn. ex Griff.	Leave	*n*-Hexane	7	-	-	>1000	Weak
		Ethyl acetate	60	43	37	199.1	**Very strong**
		Methanol	80	47	10	145.7	**Very strong**
	Wood	*n*-Hexane	77	20	17	259.5	Strong
		Ethyl acetate	83	17	3	277.3	Strong
		Methanol	53	20	-	570.0	Medium
	Bark	*n*-Hexane	50	20	13	1000	Weak
		Ethyl acetate	93	7	3	236.1	**Very strong**
		Methanol	23	-	-	>1000	Weak
Bruguiera cylindrica (L.) Blume	Leave	*n*-Hexane	80	30	3	241.2	**Very strong**
		Ethyl acetate	93	23	7	146.8	**Very strong**
		Methanol	77	17	10	310.3	Strong
	Wood	*n*-Hexane	57	-	-	>1000	Weak
		Ethyl acetate	53	33	27	865.2	Weak
		Methanol	93	-	-	752.6	Weak
	Bark	*n*-Hexane	100	53	33	27.0	**Very strong**
		Ethyl acetate	90	67	40	22.9	**Very strong**
		Methanol	43	10	-	782.5	Weak
Ceriops tagal (Perr.) C.B.Rob.	Leave	*n*-Hexane	30	20	-	884.0	Weak
		Ethyl acetate	-	-	-	>1000	Weak
		Methanol	3	3	-	>1000	Weak
	Wood	*n*-Hexane	77	7	-	464.1	Strong
		Ethyl acetate	10	-	-	>1000	Weak
		Methanol	43	-	-	>1000	Weak
	Bark	*n*-Hexane	27	-	-	>1000	Weak
		Ethyl acetate	60	3	-	694.9	Medium
		Methanol	7	-	-	>1000	Weak
Rhizophora apiculata Blume	Leave	*n*-Hexane	43	40	10	>1000	Weak
		Ethyl acetate	23	17	-	>1000	Weak
		Methanol	93	23	10	135.0	**Very strong**
	Wood	*n*-Hexane	27	7	-	>1000	Weak
		Ethyl acetate	57	30	20	590.4	Medium
		Methanol	80	23	20	196.6	**Very strong**
	Bark	*n*-Hexane	7	-	-	>1000	Weak
		Ethyl acetate	-	-	-	>1000	Weak
		Methanol	10	-	-	>1000	Weak
Rhizophora mucronata Poir.	Leave	*n*-Hexane	-	-	-	>1000	Weak
		Ethyl acetate	-	-	-	>1000	Weak
		Methanol	40	-	-	>1000	Weak
	Wood	*n*-Hexane	50	13	-	656.6	Strong
		Ethyl acetate	67	37	-	397.4	Strong
		Methanol	47	-	-	>1000	Weak
	Bark	*n*-Hexane	27	-	-	>1000	Weak
		Ethyl acetate	23	17	-	>1000	Weak
		Methanol	43	30	-	614.3	Medium

Discussion

Yield percentage calculation aims to determine the number of samples required for extraction to obtain a desirable amount of extract. Yield is also determined to identify the secondary metabolites from the solvent (Zhang et al. 2018). The extraction method becomes one of the factors that will affect the extract yield (Patel et al. 2019). The extraction process with the solvent containing cold method (maceration and percolation) and hot method (reflux, soxhletation, infuse, decoy, and digestion). The extraction method in this study was a maceration method, which was performed by soaking the samples with solvent through stirring at room temperature. The maceration method was selected as the extraction process because one of the cold extraction methods prevents the heat metabolites from degradation. Extraction was performed using three solvents, such as hexane, ethyl-acetate, and methanol. n-Hexane is a non-polar solvent that can dissolve the non-polar compounds (Sepahpour et al. 2018). Moreover, ethyl acetate is a semi-polar solvent that can

dissolve the semi-polar compounds in the cell wall, while methanol is a polar solvent that could dissolve polar compounds such as phenols.

This study calculated the yield to identify the simplicial amount required to gain the desired amount of extract. Solvent selection is an important part of the extraction process. The yield results showed that methanol had the highest yield value due to its polar characteristics, which can easily dissolve the samples compared to other solvents. In addition to solvent selection, extract yield could also be affected by several factors, including the extraction method (Alara et al. 2021). Leaf, bark, and wood extractions from *B. parviflora* and *B. cylindrica* mangrove plants were performed using the serial maceration method through sample soaking in three solvent types: hexane, ethyl acetate, and methanol. During the maceration, the cell wall and cell membrane will break because pressure difference between the outside and inside cell. The secondary metabolites were released in the cytoplasm and dissolved in the solvent. The maceration method has advantages, namely the active substances contained in the extract are undamaged as the maceration process is carried out without heating or with the cold method (Li et al. 2019)

The phytochemical test in this study aimed to identify the secondary metabolites produced by plants that function to defend the plants from unfavorable environmental conditions, such as temperature, climate, pests, and diseases (Sanchez and Demain 2011). *Bruguiera gymnorrhiza* is one of mangrove plants similar to *B. parviflora* and *B. cylindrica*, *B. gymnorrhiza* contains high secondary metabolite compounds, such as steroids, saponins, flavonoids, triterpenoids, tannins, and alkaloids (Sivaperumal et al. 2010). Based on the phytochemical results, the *B. parviflora* and *B. cylindrica* mangrove plants also had various secondary metabolites: alkaloids, steroids, saponins, tannins, phenols, and flavonoids which are beneficial for antioxidant and antibacterial compounds.

An antioxidant assay using DPPH radical material on mangrove plant extract caused a color change from purple to yellow and clear, which was measured using a spectrophotometer at 517 nm wavelength. Free radicals are unstable and extremely reactive, even tending to react with other molecules. Radicals with high reactivity can start a chained reaction in one formation, which causes abnormal compounds and starts a chained reaction that can damage the important cells in the body (Badarinath et al. 2010). The antioxidant test results in mangrove plant extract samples showed a relatively good inhibition level in C. tagal methanolic extract with inhibition of 91.02%, close to vitamin C as a positive control at 94.51%. Based on this phytochemical, the *C. tagal* leaf methanolic extract contained polyphenols such as tannins. Tannins are secondary active metabolite compounds with several properties, such as astringent, anti-diarrhea, antibacterial, and antioxidant (Hagerman et al. 1998). Tannins commonly obtained from the plant parts, such as wood, bark, and fruit, have complex biological roles, from protein deposition to metal binding. Therefore, these substances can also function as biological antioxidants (Das et al. 2020).

The antioxidant assay was performed using DPPH (1,1-diphenyl-2 picrylhydrazil) at 517 nm wavelength. In this study, vitamin C has used a positive control, and comparative material as vitamin C is a natural antioxidant that relatively causes no toxicity level (Kedare et al. 2011). The *B. parviflora* and *B. cylindrica* mangrove plants had antioxidant activity based on their activity. Furthermore, based on the performed phytochemical test, the *B. parviflora* and *B. cylindrica* mangrove plants contained secondary metabolite compounds, such as tannins, flavonoids, and saponins. Tannins have several characteristics, such as astringent, anti-diarrhea, antibacterial, and antioxidant. Flavonoids have anti-inflammation, antibacterial, and antioxidant properties.

Moreover, saponins show antioxidant and antibacterial effects (Kurniawan et al. 2015). In a previous study, a similar mangrove plant, namely *R. mucronata*, contained alkaloids, flavonoids, and tannins that functioned as an antioxidant that could prevent free radicals (Ridlo et al. 2017). Tannins as antioxidants could act as an H atom donor to muffle the DPPH radical, which occurs as a tannin radical stabilizer due to resonance. Flavonoids directly capture a reactive oxygen species (ROS) and prevent ROS regeneration while indirectly increasing the enzymatic and cellular antioxidants. Preventing ROS formation by flavonoids is performed in several ways, such as inhibiting xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) enzymes and preventing a redox reaction that can produce free radicals. Saponins can muffle superoxide through hydroperoxide intermediate, which prevents biomolecular damage due to free radicals (Liu et al. 2018)

For the antibacterial activity of five mangrove extracts, 40% ethanol was used as negative control and chloramphenicol as a positive control. That was because it is known for its broad-spectrum bacteriostatic characteristics, which remain active on Gram-negative and Gram-positive bacteria, besides inhibiting the attachment of amino acids to bacteria. This study used *S. sobrinus* as a test bacterium to identify plant species inhibiting oral bacteria. The *S. sobrinus* bacteria are gram-positive bacteria with a simpler cell wall, causing the compounds in the extract can easily damage the bacterial cell wall (Badarinth et al. 2010; Conrads et al. 2014). The gram-positive bacteria cell wall comprises one layer, namely the relatively thick peptidoglycan layer (Briaud and Carrol 2020).

This study showed several samples could inhibit bacteria, namely *C. tagal*, *B. parviflora*, and *B. cylindrica* wood extracts. The highest inhibition sample was *C. tagal* leaf ethyl acetic extract at 47% with a concentration of 2000 µg/mL, followed by *C. tagal* leaf methanolic extract at 43%. Based on the phytochemical extract content identification in *C. tagal* leaf, ethyl acetic extract has alkaloids and steroids. Alkaloids are organic compounds commonly found in nature and mostly in plants and contribute to many biological activities and some alkaloids also turn into active metabolites. Furthermore, alkaloids have health benefits, including nervous system improvement, blood pressure maintenance, and attacking

microbial infection (Debnath et al. 2018). Steroids are one of the important compounds in the health sector and are widely used in medicines, namely as antibacterial, anti-inflammatory, and pain-relieving drugs (Cole et al. 2019). The mechanisms of the antibacterial compound in the extract using ethyl acetate were synergistic or supportive, which resulted in an additional effect as there were two or more active compounds (Li et al. 2019). This condition demonstrated the *C. tagal* leaf ethyl acetic extract condition that produced the highest inhibition level against *S. sobrinus*. The *C. tagal* bark methanolic extract could inhibit the *S. sobrinus* bacteria. However, the inhibitory activity was smaller than the inhibitory level produced by the *C. tagal* leaf ethyl acetate extract. This example with great cell reinforcement esteem should be explored further to confine the primary compound and for its potential as a characteristic elective medication because of its solid antimicrobial movement. It is accepted that this is the primary report on the compound substance and bioactivity of five mangrove species.

Plant exploration with high mortality of *A. salina* larvae remains important in the search for natural ingredients for drugs, specifically anticancer drugs. Plant toxicity research is closely related to the concentration of ingredients. Alkaloids, terpenoids, polyphenols, flavonoids, and resins are some chemical plant compounds with anticancer properties (Khan et al. 2022). Toxic compounds in plants are typically used as a self-defense mechanism against the antimicrobial activity. According to Table 3, the higher the concentration value of the extract is directly proportional to the mortality in *A. salina*, implying that it may contain high-toxicity compounds. The extract is also directly proportional to its toxic properties. *Artemia* had died in the test tube that had been given the extract since the beginning of the observation. That demonstrates *A. salina* died due to the toxic properties of the extract induced in the test tube. Another study found that an extract had toxic activity while tested to observe whether it could cause 50% death at a concentration of less than 1000 µg/mL.

The death of *Artemia* in a solution of medicinal plant extracts dissolved in methanol and ethyl acetate indicated the existence of polar and non-polar secondary metabolism. Polar secondary metabolites are flavonoids and alkaloids, while non-polar compounds are terpenoids and steroids. The presence of flavonoids in the cell environment causes the OH groups in flavonoids to bind to fixed proteins in the cell membrane. That prevents the active transport of Na+ to K+. Active transport, which no longer causes uncontrolled penetration of Na+ ions into cells, causes cell membrane damage, leading to cell death (Green 2022).

In conclusion, the leaf, bark, and wood extracts of several plants from the Rhizophoraceae family found in the mangrove and probosci's monkey conservation area of North Kalimantan, Indonesia have strong antimicrobial, toxicity, and antioxidant properties. These study findings will serve as the scientific foundation for further developing several plants with the potential to become medicinal plants. However, additional research is required to isolate potential plant compounds and test effective compounds using cells related to the studied organism.

ACKNOWLEDGEMENTS

This study was supported by SIMLITABMAS funding from the Ministry of Research and Technology/National Research and Innovation Agency (contract no: 002/UN51.9/SP2H-LT/2021) and Research Collaboration between Universitas Borneo Tarakan and the Government office of Forestry in North Kalimantan. The authors would like to thank all young researchers in the laboratory of natural chemical resources, Universitas Borneo Tarakan, for their assistance in finishing this project and the head of the laboratory of forest processing products and renewal resources from the University of Mulawarman. We would also thank Mr. Raymoon Silaban for providing the Map of Tarakan city and Mr. Samsul and teams as a Mangrove and Proboscis Monkey Conservation Area staff in Tarakan City, North Kalimantan, Indonesia.

REFERENCES

Abubakar S, Kadir MA, Wibowo ES, Akbar N. 2019. Manfaat mangrove bagi peruntukan sediaan farmasitika di Desa Mamuya Kecamatan Galela Timur Kabupaten Halmahera Timur (Tinjauan Etnofarmakologis). Jurnal Enggano 4 (1): 12-25. DOI: 10.31186/jenggano.4.1.12-25. [Indonesian]

Alara OR, Abdurahman NH, Ukaegbu CI. 2021. Extraction of phenolic compounds: A review. Food Sci 4: 200-214. DOI: 10.1016/j.crfs.2021.03.011.

Badarinath AV, Rao KM, Madhu C, Chetty S, Ramkanth S, Rajan TVS, Gnanaprakash K. 2010. A review on In-vitro antioxidant methods: Comparisions, correlations and considerations. Intl J Pharm Tech Res 2 (2): 1276-1285.

Bandaranayake WM. 2020. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl Ecol Manag 10 (6): 421-452. DOI: 10.1023/A:1021397624349.

Briaud P, Carroll RK. 2020. Extracellular vesicle biogenesis and functions in gram-positive bacteria. Infect Immun 88 (12): e00433-20. DOI: 10.1128/IAI.00433-20.

Cole TJ, Short KL, Hooper SB. 2019. The science of steroids. Semin Fetal Neonatal Med 24 (3): 170-175. DOI: 10.1016/j.siny.2019.05.005.

Conrads G, de Soet JJ, Song L, Henne K, Sztajer H, Wagner-Döbler I, Zeng AP. 2014. Comparing the cariogenic species *Streptococcus sobrinus* and *S. mutans* on whole genome level. J Oral Microbiol 6 (1): 26189. DOI: 10.3402/jom.v6.26189.

Das AK, Islam MN, Faruk MO, Ashaduzzaman M, Dungani R. 2020. Review on tannins: Extraction processes, applications and possibilities. S Afr J Bot 135: 58-70. DOI: 10.1016/j.sajb.2020.08.008.

Debnath B, Singh WS, Das M, Goswami S, Singh MK, Maiti D, Manna K. 2018. Role of plant alkaloids on human health: A review of biological activities. Materials Today Chem 9: 56-72. DOI: 10.1016/j.mtchem.2018.05.001.

Egra S, Mardhiana M, Rahayu NI, Nurjannah N, Sirait S, Santoso D, Pradana AP, Kuspradini H, Mitsunaga T. Aktivitas senyawa antibakteri ekstrak parepat (*Sonneratia alba*) terhadap pertumbuhan *Ralstonia solanacearum* dan *Streptococcus sobrinus*. J-PEN Borneo Jurnal Ilmu Pertanian 3 (2): 35-43. DOI: 10.35334/jpen.v3i2.1624. [Indonesian]

Green DR. 2022. Nonapoptotic cell death pathways. Cold Spring Harbor Laboratory Press, New York. DOI: 10.1101/cshperspect.a041079.

Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 46 (5): 1887-1892. DOI: 10.1021/jf970975b.

Kedare SB, Singh RP. 2011. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 48: 412-422. DOI: 10.1007/s13197-011-0251-1.

Khan MI, Bouyahya A, Hachlafi NEL, Menyiy N El, Akram M, Sultana S, Zengin G, Ponomareva L, Shariati MA, Ojo OA, Dall'Acqua S, Elebiyo TC. 2022. Anticancer properties of medicinal plants and their bioactive compounds against breast cancer: A review on recent investigations. Environ Sci Pollut Res 29 (17): 24411-24444. DOI: 10.1007/s11356-021-17795-7.

Kurniawan B, Aryana WF. 2015. Binahong (*Cassia alata* L.) as inhibitor of *Escherichia coli* growth. Jurnal Majority 4 (4): 1-5. [Indonesian].

Li H-F, Li L-J, Ji L-M, Peng X-W, Li W. 2019. The extraction ability and mechanism in extraction lithium by several organic extractants. Chem Phys Lett 733: 136668. DOI: 10.1016/j.cplett.2019.136668.

Liu Z, Ren Z, Zhang J, Chuang CC, Kandaswamy E, Zhou T, Zuo L. Role of ROS and nutritional antioxidants in human diseases. Front Physiol 9: 477. DOI: 10.3389/fphys.2018.00477.

Mahmud, Wahyudi. 2014. Pemanfaatan vegetasi mangrove sebagai obat-obatan tradisional pada lima Suku di Papua. Biota 19 (1): 1-8. DOI: 10.24002/biota.v19i1.448. [Indonesian]

Nebula M, Harisankar HS, Chandramohanakumar N. 2013. Metabolites and bioactivities of Rhizophoraceae mangroves. Nat Products Bioprospect 3 (5): 207-232. DOI: 10.1007/s13659-013-0012-0.

Nguyen CNM, Nirmal NP, Sultanbawa Y, Ziora ZM. 2023. Antioxidant and antibacterial activity of four tannins isolated from different sources and their effect on the shelf-life extension of vacuum-packed minced meat. Foods 12 (2): 354.-370. DOI: 10.3390/foods12020354.

Patel K, Panchal N, Ingle P. 2019. Techniques adopted for extraction of natural products extraction methods: Maceration, percolation, soxhlet extraction, turbo distillation, supercritical fluid extraction. Intl J Adv Res Chem Sci 6 (4): 1-12. DOI: 10.20431/2349-0403.0604001.

Ramalingam V, Rajaram R. 2018. Enhanced antimicrobial, antioxidant and anticancer activity of *Rhizophora apiculata*: An experimental report. 3 Biotech 8 (4): 200. DOI: 10.1007/s13205-018-1222-2.

Ridlo A, Pramesti R, Koesoemadji K, Supriyantini E, Soenardjo N. 2017. Aktivitas antioksidan ekstrak daun mangrove *Rhizopora mucronata*. Buletin Oseanografi Marina 6 (2): 110-116. [Indonesian]

Sanchez S, Demain AL. 2011. Secondary metabolites. In: Comprehensive Biotechnology 3rd Edition. Elsevier, Amsterdam. DOI: 10.1016/B978-0-08-088504-9.00018-0.

Sawitri R, Bismark M, Karlina E. 2013. Ekosistem mangrove sebagai obyek wisata alam di kawasan konservasi mangrove dan bekantan di kota tarakan. Jurnal Penelit Hutan dan Konservasi Alam 10 (3): 297-314. DOI: 10.20886/jphka.2013.10.3.297-314. [Indonesian]

Sepahpour S, Selamat J, Manap MYA, Khatib A, Razis AFA. 2018. Comparative analysis of chemical composition, antioxidant activity and quantitative characterization of some phenolic compounds in selected herbs and spices in different solvent extraction systems. Molecules 23 (2): 402-419. DOI: 10.3390/molecules23020402.

Shimizu K, Kondo R, Sakai K, Takeda N, Nagahata T, Oniki T. 2001. Novel vitamin E derivative with 4-substituted resorcinol moiety has both antioxidant and tyrosinase inhibitory properties. Lipids 36 (12): 1321-1326. DOI: 10.1007/s11745-001-0847-9.

Sivaperumal P, Ramasamy P, Inbaneson S. 2010. Screening of antibacterial activity of mangrove leaf bioactive compounds against antibiotic resistant clinical isolates. World J Fish Mar Sci 2 (5): 348-53.

Sormin RBD, Nendissa DM, Mailoa MN, Rieuwpassa F, Wenno MR. 2021. Antibacterial activity of *Rhizophora apiculata* extract originated from Inner Ambon Bay against selected pathogen bacteria. IOP Conf Ser Earth Environ Sci 797 (1): 012017. DOI: 10.1088/1755-1315/797/1/012017.

Syawal H, Hakim L, Effendi I. 2020. Phytochemical analysis of *Rhizophora apiculata* leaf extract and its inhibitory action against *Staphylococcus aureus*, *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. AACL Bioflux 13 (4): 2242-2249.

Widadi, Rahayu I, Purwaningsih S, Wientarsih I. 2014. Toxicological evaluation of syrup from *Rhizophora mucronata* hypocotyls ethanolic extract in sprague-dawley rats. Glob J Biol Agric Heal Sci 1 (2): 2085-9449. DOI: 10.18502/kls.v1i0.98.

Zhang QW, Lin LG, Ye WC. 2018. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Med 13: 20. DOI: 10.1186/s13020-018-0177-x.