Antifouling activity of *Rhizophora stylosa* and *Rhizophora mucronata* in Morotai Island, North Maluku

[1]Rinto M. Nur, [1]Iswandi Wahab, [1]Nurafni, [1]Djainudin Alwi, [1]Kismanto Koroy, [2]Irfan H. A. Rahman, [3]Ningsi Saibi, [4]Resmila Dewi

[1] Faculty of Fisheries and Marine Sciences, Pasifik Morotai University, Morotai, Indonesia; [2] Faculty of Engineering, Pasifik Morotai University, Morotai, Indonesia; [3] Faculty of Teacher Training and Education, Khairun University, Ternate, Indonesia; [4] Department of Pharmacy, STIKES Assyifa Aceh, Banda Aceh, Indonesia. Corresponding author: R. M. Nur, rintomnur777@gmail.com

Abstract. Fouling organisms' activity is extremely detrimental, particularly in the fishing and shipping industries. They are avoided through the use of paint. However, because antifouling paints containing heavy metals pollute the water and harm marine life, it is necessary to develop an alternative to antifouling paints, preferably made of natural materials. This study aimed to determine the class of bioactive compounds contained in the leave and bark extracts of *Rhizophora stylosa* and *Rhizophora mucronata*. The extraction was carried out by using the maceration method and methanol. The antifouling capacity of the obtained extract was determined by mixing varnish. The control blocks were (1) treated with varnish, (2) treated with paint, and (3) without painting. Each treatment was applied to a 10×10×2 cm^3 wooden block immersed 50 cm below sea level at low tide. For 11 weeks, weekly observations were made. The percentage of fouling organisms covering the blocks treated with *R. stylosa* leave extract (ELRs) was 15% and in those treated with *R. stylosa* bark extract (EBRs), the percentage was 10%. Meanwhile, the percentage of cover of fouling organisms in *R. mucronata* leave extracts (ELRm) was 100% and in bark extract (EBRm) it was 98%. ELRs contained alkaloids, saponins, and steroids; EBRs contained alkaloids and steroids; ELRm contained saponins and steroids; and EBRm contained alkaloids, flavonoids, saponins, and steroids. The antifouling activity of EBRs is the highest because it contains alkaloids and steroids. Extract fractionation is necessary to realize the potential of *R. stylosa* as a natural antifouling paint.

Key Words: bioactive compound, extraction, fouling organisms' activity, mangrove, paint.

Introduction. Biofouling is the attachment/agglomeration of organisms on the surface of objects or buildings submerged in air, causing damage to the building's structure. This buildup has a detrimental effect on exposed surfaces involving public transportation vessels, recreational vessels, naval fleets, heat exchangers, oceanographic sensors, and aquaculture systems (Callow & Callow 2011; Kirschner & Brennan 2012). The biofouling phenomenon has many consequences, particularly in the fisheries and shipping sectors (Plouguerné et al 2010). Biofouling can be corrosive and increase the weight of the boat or ship, which increases the ship's traction and efficiency. Additionally, Sabdono (2010) explained that biofouling could damage wood structures due to wood borer activity. Bressy et al (2014) reported that biofouling losses totalize approximately $150 million annually. Schultz et al (2011) also report that biofouling can result in annual losses of $180 and $260 million to the US Navy Fleet. In general, this is controlled by painting. The use of paints containing heavy metals as coatings and antifouling is often done to reduce this impact. It is one of the causes of heavy metal pollution in the water, which accumulates over time and endangers biota and the environment. Today's antifouling paints are primarily composed of tributyltin (TBT) and copper sulfate. According to Lamoree et al (2002), these compounds can contaminate the marine environment. TBT can be toxic to organisms that are not intended to be affected (Fernández-Alba et al 2002; Thomas et al 2002). According to Park et al (2012), TBT can result in imposex, decreased gonadal index, and delayed growth in *Gomphina veneriformis* species. According to Bressy et al (2014), copper sulfate can kill certain marine biota and trigger salmon migration. In light of the dangers associated with TBT use, the International Maritime Organization (IMO) has proposed that antifouling paint made from TBT be phased out beginning January 1, 2008. One alternative to using TBT-based paint to control biofouling is to isolate natural antifouling agents from marine organisms. Numerous marine organisms naturally produce secondary metabolites that inhibit fouling organisms from adhering to the substrate's surface. Numerous research findings indicate that certain marine biota possesses antifouling properties (Plouguerné et al 2010; Qian et al 2009; Habsah et al 2011; Acevedo et al 2013; Santi et al 2014; Shao et al 2015; Fitrianingrum et al 2016).

Numerous mangrove species have been reported to possess antifouling properties (Manilal et al 2009; Prabhakaran et al 2012). Nandhini & Revathi (2016) demonstrated antimicrobial activity of *Rhizophora apiculata* bark, root, and leaf extracts. Balasubramanian (2015) also observed antifouling activity of *R. apiculata* leaves against *Vibrio alginolyticus*. *R. apiculata*, *R. stylosa*, and *R. mucronata* are found in Morotai Island Regency. Nur & Rahmawati (2019) demonstrated that a methanol extract of *R. apiculata* leaves from Morotai Island had an antifouling activity with 0% macrofouling organism attachment after eight weeks of immersion.

Antifouling tests on *R. apiculata* have previously been conducted using biofilm (microfouling) and anti-macrofouling bacteria. However, *R. stylosa* and *R. mucronata* have not been tested for their ability to inhibit fouling, particularly macrofouling. This study aimed to determine the class of bioactive compounds contained in methanolic extracts of *R. stylosa* and *R. mucronata* leaves and stems. As a result, natural materials suitable for antifouling paint are obtained.

Material and Method

Material. *R. stylosa* and *R. mucronata* leaves and bark were collected in Daruba Village (N2º3’19,39788”/E128º17’46,068”), Morotai Island, North Maluku, Indonesia. This research was conducted from May to November 2019. The following materials were used in this study: filter paper (Circles 150 mm, Cat No. 1001.150, Whatman), methanol (Merck), aqua-dest, and bioactive compound identification reagents.

Sample preparation and its extraction. The leaf and bark samples of *R. stylosa* and *R. mucronata* obtained were washed and dried. Next, the sample was mashed. Extraction was carried out by a repeated maceration method using methanol (ratio 1:4 w/v) for 48 hours, then the extract was filtered, and the filtrate was evaporated with a water bath. The extract obtained was weighed and stored in a flacon bottle.

Evaluate the antifouling properties of R. stylose and R. mucronata extracts. Antifouling tests on extracts were conducted by mixing each extract with varnish to determine the bioactive compounds' ability to protect the structure from fouling organism attachment. Several 10×10×2 cm^3 wooden blocks were prepared and immersed in a varnish solution mixed with each of the prepared extracts. As a control, varnish and paint were used. Each treatment was repeated three times. Following that, each solution was applied to the available wooden blocks. After approximately three days of drying, the wooden blocks are tied together with rope and installed beneath the bridge. For 11 weeks, the block was immersed 50 cm below the sea level at the lowest tide in Juanga Village (N2º1'0,19596"/E128º16'53,256"), Morotai Island, North Maluku, Indonesia. Each week, observations were made to determine the percentage of fouling organisms.

Bioactive compounds isolated from R. stylosa and R. mucronata extracts. The crude extracts of *R. stylosa* and *R. mucronata* leaves and stems were then subjected to phytochemical analysis to determine the content of a group of bioactive compounds. The bioactive compounds *R. stylosa* and *R. mucronata* were identified qualitatively using a chemical detector and by observing the color change, precipitate formation, and foam formation reactions. Alkaloids, flavonoids, saponins, and steroids/triterpenoids are all examples of bioactive compounds.

Results and Discussion

Extraction. Extraction results indicate that bark extraction yields more than leaf extraction. EBRs produced the highest yield (13.79%) followed by EBRm (13.41%), ELRs (3.67%), and ELRm (2.67%) Table 1. This indicates that *R. stylosa* contains a greater concentration of bioactive compounds than *R. mucronata*, when extracted with methanol. Additionally, the bark contains more bioactive compounds than the leaves.

Table 1

Extract and yield of *Rhizophora stylosa* and *Rhizophora mucronata*

Material	*Extract code*	*Simplicia weight (g)*	*Extract weight (g)*	*The yield (%)*	*Extract colors*
Leave *R. stylosa*	ELRs	150.00	5.50	3.67	Blackish green
Bark *R. stylosa*	EBRs	43.50	6.00	13.79	Blackish green
Leave *R. mucronata*	ELRm	150.00	4.00	2.67	Blackish green
Bark *R. mucronata*	EBRm	41.00	5.50	13.41	Dark green

The methanol extract yield value is used to assess the efficacy of the material containing bioactive metabolites. The results indicated that extraction with methanol (a polar solvent) yielded more extract in bark than in leaves and that the bioactive compounds in the bark are polar. However, Andayani et al (2018) found that the yield of the methanol extract of leaves was higher (24.8%) than that of the bark (17.9%). Nur & Rahmawati (2019) reported an 8.2% yield of *R. apiculata* leaves extracted with methanol. Additionally, the extract obtained was blackish green and dark green, in a paste, with granules resembling crystals. According to Nur & Nugroho (2018), the methanol leaf extract contained clear granules similar to crystals. The obtained extracts were then evaluated for their antifouling activity.

Antifouling activity. The antifouling test results indicate that the leave and bark extracts of *R. stylosa* had a greater antifouling activity than the leave and bark extracts of *R. mucronata*. Fouling organisms began to appear on wood blocks with EBRs treatment at week 9, on those with ELRs treatment at week 6, on those with EBRm and ELRm treatments at week 4, on those with paint and varnish treatment at week 3, and on those without painting at week 2 (Figure 1).

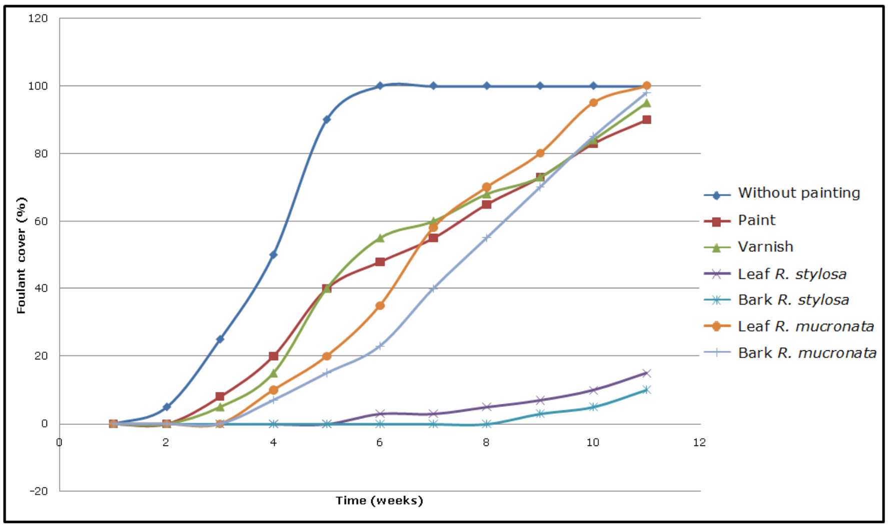

Figure 1. Cover percentage of fouling on wood blocks treated with *Rhizophora stylosa* and *Rhizophora mucronata* bark and leave extracts.

After being immersed for 11 weeks, the percentages of fouling cover on the treated wood blocks were 10, 15, 98, 100, 95 and 90% for the treatment with EBRs, ELRs, EBRm, ELRm, varnish and paint, respectively, while on the woodblock without painting, the percentage of fouling cover reached 100 percent after six weeks of immersion. Four different fouling organisms were discovered, including macroalgae: *Ectocarpus* sp., *Cladophora* sp., *Enteromorpha* sp., and *Padina australis*. Figure 2 depicts the fouling organisms.

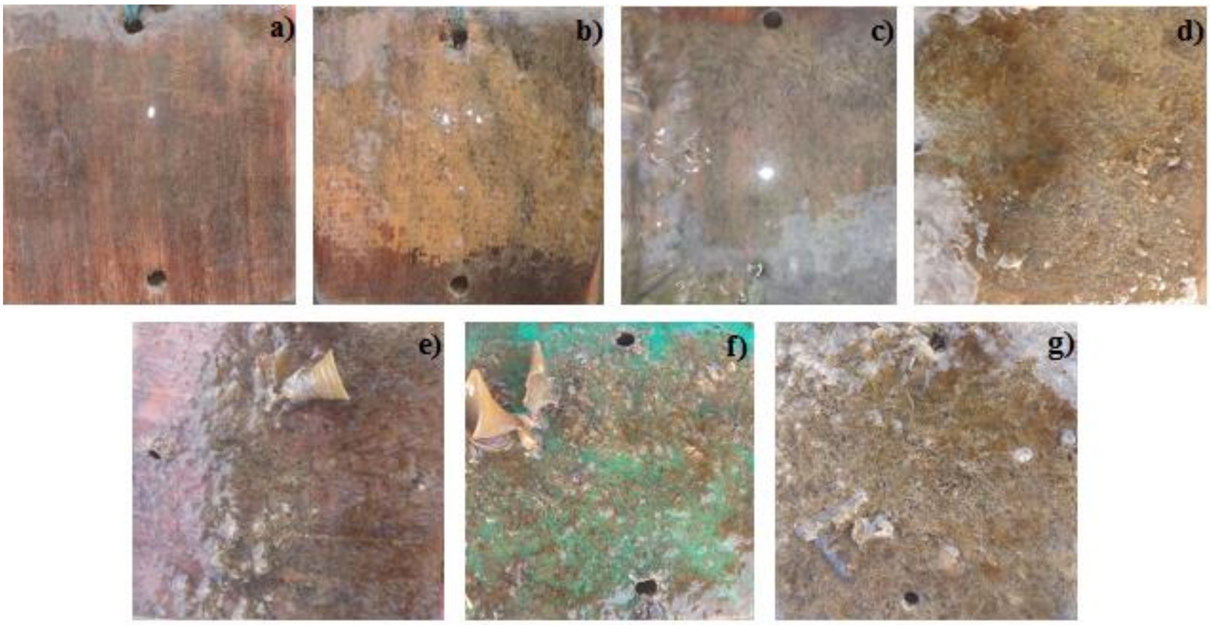

Figure 2. Wooden blocks immersed in the sea for 11 weeks. a) extract of bark of *Rhizophora stylosa* (EBRs); b) extract of leave of *Rhizophora stylosa* (ELRs); c) extract of bark of *Rhizophora mucronata* (EBRm); d) extract of leave of *Rhizophora mucronata* (ELRm); e) varnish; f) paint; g) without painting.

Since 1863, antifouling paint has been applied using tar. However, heavy metals, such as copper (Cu), are still present in this paint (Almeida et al 2007). These compounds have the potential to contaminate marine ecosystems. As a result, it is necessary to develop antifouling paint entirely made of natural materials. The results indicated that mangrove extracts had antifouling activity, with the bark extract of *R. stylosa* having the highest activity. When the antifouling activity was evaluated, fouling organisms and macroalgae were discovered on the test plate. Almeida et al (2007) reported that algae, in addition to invertebrates found on the test plate, are fouling organisms. Rejeki (2010) identified macrofouling as macroalgae (*Demesteria* sp., Enteromorpha clatharata, *Ectocarpus* sp., *Enteromorpha* sp., and *Pterosiphonia* sp.) in floating cage nets. Faÿ et al (2019) also reported the presence of algae on plain painted plates after one month of immersion.

Identification of bioactive compounds. The identification of bioactive compounds in *R. stylosa* and *R. mucronata* leave and bark extracts reveals that ELRs contain alkaloids, saponins, and steroids; EBRs contain alkaloids and steroids; ELRm contains saponins and steroids; and EBRm contains alkaloids, flavonoids, saponin, and steroids (Table 2).

The antifouling activity of *R. mucronata* and *R. stylose* extracts is inextricably linked to their bioactive compound content. Williams (1999) also reported the *R. mucronata* contains steroids, diterpenoids, and triterpenoids, whereas *R. stylosa* contains triterpenoids. According to Tarman et al (2013), tannins, saponins, flavonoids, hydroquinone phenols, triterpenoids, and alkaloids are present in the leaf extract of *R. mucronata*.

Table 2
The identification of bioactive compound in an extract of leave and bark of *Rhizophora stylosa* and *Rhizophora mucronata*

Material	*Extract code*	*Bioactive compounds*			
		Alkaloid	*Flavonoid*	*Saponin*	*Steroid*
Leave *R. stylosa*	ELRs	+	-	+	+
Bark *R. stylosa*	EBRs	+	-	-	+
Leave *R. mucronata*	ELRm	-	-	+	+
Bark *R. mucronata*	EBRm	+	+	+	+

According to Nebula et al (2013), *R. mucronata's* leaves, bark, root bark, and fruit contain steroids, diterpenoids, and triterpenoids, whereas *R. stylosa's* stems, twigs, and leaves contain steroids, triterpenoids, and flavonoids. Andayani et al (2018) also discovered alkaloid compounds in the methanol extract of *R. mucronata* leaves and bark. According to Chitra et al (2019), the methanol extract of *R. mucronata's* bark contains steroids, fatty acids, carbohydrates, tannins, and alkaloids.

Conclusions. The results of extraction indicate that bark yield is greater than leaf yield. In general, the yield of *R. stylosa* extract (17.46%) was greater than that of *R. mucronata* extract (16.08%). When extracted with methanol, *R. stylosa* contains a greater concentration of bioactive compounds than *R. mucronata*. After 11 weeks of immersion, four distinct types of fouling organisms, including macroalgae, were discovered. When the fouling cover was 20%, extracts of the bark of *R. stylosa* (EBRs) and extracts of the leaves of *R. stylosa* (ELRs) exhibited the highest antifouling activity. Alkaloids, saponins, and steroids are found in ELRs, whereas in EBRs only alkaloids and steroids were found.

Conflict of interest. The authors declare no conflict of interest.

References

Acevedo M. S., Puentes C., Carreño K., León J. G., Stupak M., García M., Pérez M., Blustein G., 2013 Antifouling paints based on marine natural products from Colombian Caribbean. International Biodeterioration & Biodegradation 83(2013):97–104.

Almeida E., Diamantino T. C., de Sousa O., 2007 Marine paints: The particular case of antifouling paints. Progress in Organic Coatings 59(1):2–20.

Andayani D. G. S., Anggraeni S. R., Liviawaty E., Chrisentia R. M., Srikandace Y., 2018 Isolation, identification of alkaloid from *Rhizophora mucronata* and the activity of its methanol extract against Barnacles. IOP Conference Series: Earth and Environmental Science 160(1):160.

Balasubramanian V., Rajaram R., Rajesh P., 2015 Antifouling potentials of mangrove *Rhizophora apiculata* against *Vibrio alginolyticus* strain VBM-01. Indian Journal of Geo-Marine Sciences 44(10):1654–1661.

Bressy C., Hellio C., Nguyen M. N., Tanguy B., Maréchal J. P., Margaillan A., 2014 Optimized silyl ester diblock methacrylic copolymers: A new class of binders for chemically active antifouling coatings. Progress in Organic Coatings 77(3):665–673.

Callow J. A., Callow M. E., 2011 Trends in the development of environmentally friendly fouling-resistant marine coatings. Nature Communications 2(1):244–253.

Chitra J., Ali M. S., Anuradha V., 2019 Identification of bioactive compounds in the crude bark extract of *Rhizophora mucronata* by GCMS analysis and HPTLC fingerprinting of the C. Universal Review 8(6):31–33.

Faÿ F., Gouessan M., Linossier I., Réhel K., 2019 Additives for efficient biodegradable antifouling paints. International Journal of Molecular Sciences 20(2):1-12.

Fernández-Alba A. R., Piedra L., Mezcua M., Hernando M. D., 2002 Toxicity of single and mixed contaminants in seawater measured with acute toxicity bioassays. The

Scientific World Journal 2:1115-1120.
Fitrianingrum N., Sabdono A., 2016 [Activity of crude extract of seaweed (*Sargassum echinocarpum*) and ZnO as active pigments on antifouling paints]. Journal of Marine Research 5:11. [In Indonesian].
Habsah M., Kamariah B., Aisha M. R. S., Julius Y. F. S., Desy F. S., Asnulizawati A., Faizah S., 2011 The potential of local *Sargassum granuliferum* crude extract as antibacterial and antifouling properties. Empowering Science, Technology and Innovation Towards a Better Tomorrow 721-726.
Kirschner C., Brennan A., 2012 Bio-inspired antifouling strategies. Annual Review of Materials Research 42:211–229.
Lamoree M. H., Swart C. P., Van Der Horst A., Van Hattum B., 2002 Determination of diuron and the antifouling paint biocide irgarol 1051 in Dutch Marinas and Coastal Waters. Journal of Chromatography A 970(1–2):183–190.
Manilal A., Sujith S., Kiran G. S., Selvin J., Shakir C., Gandhimathi R., Panikkar M. V. N., 2009 Biopotentials of seaweeds collected from Southwest Coast of India. Journal of Marine Science and Technology 17(1):67–73.
Nandhini S., Revathi K., 2016 Antifouling activity of extracts from mangroves against biofouling bacteria isolated from Boats in Royapuram, Chennai, India. International Journal of Current Microbiology and Applied Sciences 5(8):324–335.
Nebula M., Harisankar H. S., Chandramohanakumar N., 2013 Metabolites and bioactivities of Rhizophoraceae mangroves. Natural Products and Bioprospecting 3(5):207–232.
Nur R. M., Nugroho L. H., 2018 Cytotoxic activities of fractions from *Dioscorea bulbifera* L. chloroform and methanol extracts on T47D breast cancer cells. Pharmacognosy Journal 10(1):33–38.
Nur R. M., Rahmawati R., 2019 [Antifouling activity of *Rhizophora apiculata* in Morotai Island Regency]. Jurnal Ilmu-Ilmu Perikanan dan Budidaya Perairan 14(1):7-16. [In Indonesian].
Park K., Kim R., Park J. J., Shin H. C., Lee J. S., Cho H. S., Lee Y. G., Kim J., Kwak I. S., 2012 Ecotoxicological evaluation of tributyltin toxicity to the equilateral venus clam, *Gomphina veneriformis* (Bivalvia: Veneridae). Fish and Shellfish Immunology 32(3):426–433.
Plouguerné E., Hellio C., Cesconetto C., Thabard M., Mason K., Véron B., Pereira R. C., da Gama B. A. P., 2010 Antifouling activity as a function of population variation in *Sargassum vulgare* from the littoral of Rio de Janeiro (Brazil). Journal of Applied Phycology 22(6):717–724.
Prabhakaran S., Rajaram R., Balasubramanian V., Mathivanan K., 2012 Antifouling potentials of extracts from seaweeds, seagrasses and mangroves against primary biofilm forming bacteria. Asian Pacific Journal of Tropical Biomedicine 2(1 SUPPL.):S316–S322.
Qian P. Y., Xu Y., Fusetani N., 2009 Natural products as antifouling compounds: Recent progress and future perspectives. Biofouling 26(2):223–234.
Rejeki S., 2010 [The succession of biofouling on a marine net cage in Hurun Bay, Lampung]. Indonesian Journal of Marine Sciences 14(2):112-116. [In Indonesian].
Sabdono A., 2010 [Effect of antifouling of coral bacteria *Pelagiobacter variabilis* strain USP3.37 extract on barnacle attachment in Teluk Awur Coastal Waters, Jepara]. Indonesian Journal of Marine Sciences 12(1):18–23. [In Indonesian].
Santi I. W., Radjasa O. K., Widowati I., 2014 [The potential of *Sargassum duplicatum* seaweed as a source antifouling compounds]. Journal of Marine Research 3(3):274–284. [In Indonesian].
Schultz M. P., Bendick J. A., Holm E. R., Hertel W. M., 2011 Economic impact of biofouling on a naval surface ship. Biofouling 27(1):87–98.
Shao C. L., Xu R. F., Wang C. Y., Qian P. Y., Wang K. L., Wei M. Y., 2015 Potent antifouling marine Dihydroquinolin-2(1H)-one-containing alkaloids from the Gorgonian coral-derived fungus *Scopulariopsis* sp. Marine Biotechnology 17(4):408–415.
Tarman K., Purwaningsih S., Negara A. A. A. P. P., 2013 [Antibacterial activity of

Rhizophora mucronata against diarrhea causing bacteria]. Jurnal Pengolahan Hasil Perikanan Indonesia 16(3):249-258. [In Indonesian].
Thomas K. V., McHugh M., Waldock M., 2002 Antifouling paint booster biocides in UK Coastal Waters: Inputs, occurrence and environmental fate. Science of the Total Environment 293(1–3):117–127.
Williams L. A. D., 1999 *Rhizophora mangle* (Rhizophoraceae) triterpenoids with insecticidal activity. Naturwissenschaften 86(9):450–452.

Received: 16 October 2022. Accepted: 09 January 2023. Published online: 22 January 2023.
Authors:
Rinto Muhammad Nur, Pasifik Morotai University, Faculty of Fisheries and Marine Sciences, Jl. Siswa Darame, 97771, Morotai, Indonesia, e-mail: rintomnur777@gmail.com
Iswandi Wahab, Pasifik Morotai University, Faculty of Fisheries and Marine Sciences, Jl. Siswa Darame, 97771, Morotai, Indonesia, e-mail: iswandi.fpik@gmail.com
Nurafni, Pasifik Morotai University, Faculty of Fisheries and Marine Sciences, Jl. Siswa Darame, 97771, Morotai, Indonesia, e-mail: nurafni1710@gmail.com
Djainudin Alwi, Pasifik Morotai University, Faculty of Fisheries and Marine Sciences, Jl. Siswa Darame, 97771, Morotai, Indonesia, e-mail: djainudinalwi@gmail.com
Kismanto Koroy, Pasifik Morotai University, Faculty of Fisheries and Marine Sciences, Jl. Siswa Darame, 97771, Morotai, Indonesia, e-mail: kismantokoroy@gmail.com
Irfan Haji Abdul Rahman, Pasifik Morotai University, Faculty of Enginnering, Jl. Siswa Darame, 97771, Morotai, Indonesia, e-mail: irfanabdrahman86@gmail.com
Ningsi Saibi, Khairun University, Faculty of Teacher Training and Education, Jl. Batu Angus, 97728, Ternate, Indonesia, e-mail: ningsi.saibi19@gmail.com
Resmila Dewi, STIKES Assyifa Aceh, Department of Pharmacy, Jl. Mr. Teuku Moh. Hasan No.110, 23245, Banda Aceh, Indonesia, e-mail: resmila_dewi@yahoo.com

How to cite this article:
Nur R. M., Wahab I., Nurafni, Alwi D., Koroy K., Rahman I. H. A., Saibi N., Dewi R., 2023 Antifouling activity of *Rhizophora stylosa* and *Rhizophora mucronata* in Morotai Island, North Maluku. AACL Bioflux 16(1):252-258.