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Mangrove plants species from Delta Mahakam, Indonesia with antimicrobial potency

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Abstract. *Saptiani G, Asikin AN, Ardhani F, Hardi EH. 2018. Mangrove plants species from Delta Mahakam, Indonesia with antimicrobial potency. Biodiversitas 19: 516-521.* This research was aimed to study biodiversity of mangrove that have potential as an antimicrobial material. The leaves of 4 types of mangroves, namely *Avicennia marina*, *Sonneratia alba*, *Rhizophora stylosa*, and *Acanthus ilicifolius* were chopped, dried and extracted with 3 types of solvents 80% ethanol, water and seawater. The Antimicrobial used Agar Disc Diffusion (ADD) method, and Minimal Inhibitory Concentration (MIC). Microbes that used for the test were *Staphylococcus aureus*, *Escherichia coli*, *Vibrio harveyi, Aeromonas hydrophila* and *Saprolegnia* sp. The ADD test showed ethanol extract of *A. marina* and *A. ilicifolius* can inhibit *S. aureus* with inhibition zone 13.33±0.58 mm. All the extract can inhibit *A. hydrophila* 13.00±1.00 mm and *E. coli* 12.67±0.58 mm. Ethanol extract of *S. alba* can inhibit *V. harveyi* 12.67±0.58 mm and *A. ilicifolius* 12.33±0.58 mm against *Saprolegnia* sp. The best of MIC is ethanol extract of *A. ilicifolius*, following *A. marina*, *S. alba* and *R. stylosa*.

Keywords: Antimicrobial, Delta Mahakam, extract, mangrove

INTRODUCTION

Various types of mangrove plants are commonly found in the Mahakam Delta of East Kalimantan, Indonesia. There are mangroves that grow as trees and shrubs. According to Suyatna et al. (2010) the area of the Mahakam Delta is interpreted about 107,221.9 ha, which is mangrove forest area of about 49,309.4 ha. The types of mangroves present in the Mahakam Delta include *Sonneratia alba, Sonneratia caseolaris, Avicennia* sp., *Rhizophora* sp., *Bruguiera* sp., *Xylocarpus granatum, Nypa fruticans* (Sidik 2010). Mahakam Delta is mainly covered by nipa palms (*Nypa fruticans*) and various types of mangroves (*Rhizophora* spp.), other species of vegetation are *Sonneratia alba* and *Oncosperma* sp. (Persoon and Simarmata 2014). The mangrove ecosystem in the Mahakam Delta has physical and biological functions. The physical function of the mangrove forest is an abrasion barrier, and biological function is the reduction of pollution and sources of natural food for fish and other biotas (Setiawan et al. 2015). Mangrove has ability to prevent erosion, coastal abrasion, filters and sources of life for animals; mangroves are also used for various purposes, such as home utensils, firewood, food, and medicines.

For thousands of years, natural products have been used in traditional medicine worldwide, even long before we know antibiotics and other modern medicine (Khan et al. 2009). The coastal communities use fruit of mangroves as food, and food coloring. The leaves are used as medicine for wounds, skin diseases, fever, diarrhea, gastrointestinal disorders and tonic. A number of investigations indicated that the plant possesses significant antioxidant, antinociceptive, antihyperglycemic, antimicrobial, and anticancer activities (Mahmud et al. 2014). *Rhizophora, Sonneratia, Nypa, Avicennia*, and *Acanthus* are known to contain that can be used as antibacterial and immuno-stimulant materials, for fish and shrimp (Chakraborty et al. 2007; Saptiani et al. 2012a; Ramesh et al. 2014). Mangrove has antibacterial properties against *Vibrio* such as *Avicennia*, *Bruguiera*, and *Acanthus* (Manilal et al. 2009; Saptiani et al. 2012a). Groups of alkaloids and carbohydrates such as glucoside, in mangrove extracts, have a good response to pharmacological activity, as antimicrobial and anti-inflammatory materials (Kokoli et al. 2008). The mangrove plant tissues contained highly polar bioactive compounds (alkaloids, saponins, tannins flavonoids and reducing sugar). The presence of these metabolites is an indication of their potentials as medicinal plants (Edu et al. 2015).

In East Kalimantan, the surrounding shrimp ponds still retain mangrove plants which are rarely affected by disease and rarely experience excessive mortality. This indicates that mangrove leaves that fell into the pond have the potential as antimicrobials. The hatcheries still use antibiotics and chemicals for prevention and protection of infectious pathogens. However, these used uncontrolled and had become resistant and potential toxic effects on fish. (Saptiani et al. 2016b). Antimicrobial activity of plant and plant material products can be tested in vitro before being tested to organisms (Saptiani et al. 2015). The aim is to study types of mangroves, such as *Avicennia marina, Sonneratia alba, Rhizophora stylosa,* and *Acanthus ilicifolius*, which have potential as antimicrobial agent against bacteria and fungi.

MATERIALS AND METHODS

Leaf mangrove extract

Mangrove species *Avicennia marina*, *Sonneratia alba*, *Rhizophora stylosa*, and *Acanthus ilicifolius* from the Mahakam Delta Region of Muara Badak Subdistrict, Kutai Kartanegara District, East Kalimantan, Indonesia. Each of the mangrove leaves is cleaned, washed and drained, after dried chopped and dried in a room without direct expose to sunlight, for about 21-28 days until dry. The leaves are macerated with three different solvents: 80% ethanol, water and seawater 20 ‰ for 24 hours, with leaf and solvent ratio of 1:8. The result of maceration is extracted by evaporation method with rotary evaporator. When extracts began to concentrate, salt extraction is done in the plant with liquid method, until the salt is exhausted. The extraction results are evaporated over the water bath until the solvent runs out, while in the water extract and seawater the process is stopped when the liquid remains 10% out of the original (Saptiani et al. 2013; Saptiani et al. 2015).

The microbes

The microbes used for the test are bacteria namely; *Staphylococcus aureus*, *Escherichia coli*, *Vibrio harveyi*, *Aeromonas hydrophila* and also fungi such as; *Saprolegnia* sp, derived from the Laboratory of Aquatic Microbiology Faculty of Fisheries and Marine Sciences Mulawarman University, Samarinda, Indonesia. Prior to use for the test, each microbe was cultured on the fertilizer medium. *V. harveyi* were cultured on Tryptone Soya Agar (TSA) medium plus 3% NaCl, *S. aureus* and *A. hydrophila* were cultured on Tryptone Soya Agar (TSA), all of which were incubated for 24 hours at 33°C, *E. coli* was cultured on Brilliant Green Bile Lactose Broth (BGBL) media. Fungi, *Saprolegnia* sp., cultured on Potato Dextrose Agar (PDA) medium incubated at 36ºC for 48 hours (Saptiani et al. 2016c).

Antimicrobial tests

Antimicrobial tests were performed by inhibitory method for disc diffusion (ADD) and Minimum Inhibitory Concentration (MIC) tests. ADD test of mangrove leaf extract to *V. harveyi*, was done by bacteria cultured on TSB medium plus NaCl 3%, and then incubated for 24 hours with temperature 30 ºC. After incubation, the bacteria were diluted to 10^3 CfumL^{-1} and cultured on TSA media plus 3% NaCl in Petri dishes. ADD test of mangrove leaf extract on *S. aureus*, *E. coli* and *A. hydrophila* was done in the same way that each bacterium was cultured on TSB medium and incubated for 24 hours with temperature 33ºC. After incubation, the bacteria were diluted to 10^5 CfumL^{-1} and cultured on TSA media. ADD test of mangrove leaf extract to *Saprolegnia* sp. done by fungi cultured on Potato Dextrose Broth medium (PDB), then incubated at temperature 36 ºC, for 24 hours. After incubation, then diluted to 10^5 CfumL^{-1} and cultured on PDA media in a petridish. Furthermore, each microbe that has been cultured in TSA and PDA media were treated with extract. Treatment of concentration of each extract was 200, 400, 600, 800, and 1,000 ppm and negative control using 0.85% Phosphate Buffer Saline (PBS) solution and positive control using 5 mgxmL^{-1} oxytetracycline solution. Each treatment performed three replications. The treatment was administered by dripping the extract solution on Whatman filter paper disc which was 6 mm in diameter, then planted and arranged in such a way as to bacterial culture and fungus culture. After incubation, observations were performed at 24, 36, 48, and 60 hours after incubation. Observations and measurements were made on the diameter of the clear zone formed around the filter paper (Khajure and Rathod 2010; Saptiani et al. 2013; Saptiani et al. 2016c).

The MIC test was performed on a sterile microplate having 96 holes. All holes are filled with 0.5 mL TSB plus 3% NaCl. In the first row hole, each treated ethanol extract, water and seawater from each mangrove leaf, and negative control of PBS and positive control of antibiotics as much as 0.5 mL (2.5mgxmL^{-1}), with three replications. Next was dilution, until the 8th hole. Then all the holes were filled with *V. harveyi* 0.1 mL with concentration 10^5 Cfux mL^{-1} and microplate incubated for 24 hours with temperature 33 ºC. Similarly, in MIC tests of *Staphylococcus aureus*, *Escherichia coli* and *Aeromonas hydrophila* were performed in the same manner on TSB media without NaCl added. While *Saprolegnia* sp., was diluted using PDA media, and incubated at 36ºC. Observation of MIC test results is based on occurrence of turbidity in each hole after 24-48 hours incubation. The cloudy medium indicates the growth of bacteria or fungi while the clear sign shows the extract is able to inhibit bacteria or fungi. To corroborate the MIC test results, in dilute holes which results are clear but dubious, isolation and culture are performed on TSA or PDA media by scattering method. MIC test results were analyzed by observing bacterial growth in each treatment (Bussmann et al. 2011).

Analysis

This study used a complete randomized design with three replications. The observation data of inhibitory zone diameter in ADD test was analyzed using ANOVA test, if there were significant differences followed by Duncan test. The MIC test observations were analyzed descriptively based on the highest dilution results or the lowest extract concentrations that were still able to inhibit bacteria.

RESULTS AND DISCUSSION

Mangrove

Various types of mangrove plants are found in the area of the Mahakam Delta, especially in the Subdistrict of Muara Badak, Kutai Kartanegara District, East Kalimantan Province, Indonesia. Mangrove is widely used by the community as food, traditional medicine i.e. the class of *Avicennia* sp., *Sonneratia* sp., *Rhizophora* sp., *Bruguiera* sp., *Acanthus ilicifolius*, and many other types of mangroves. Vegetation growing in the Mahakam Delta is a mangrove forest distributed in different zones of the pedada zone (*Sonneratia alba* and *Avicennia* sp.), Rhizophora zone, transition zone, nipah zone (*Nypa fruticans*) and

nibung zones (Sidik 2010). In Delta Mahakam, there are eight dominant mangrove families, namely Rhizophoraceae, Avicenniaceae, Sonneratiaceae, Combretaceae, Meliaceae, Myrsinaceae, Euphorbiaceae, and Palmae (Zairin et al. 2014).

Avicennia marina is a large and branched plant, with long and dense roots called pneumatophores or air roots. Leaves are single leathery, opposite, ovate, dark glossy green on upper surface, and dull grayish on the lower surface; underside of the leaf has special glands for secreting excess salt. According to Noor et al. (2012), *A. marina* tree that grows spread with a height of 25 m, has a root breath and form a horizontal root system in the shape of a finger. Smooth leaf surface, shiny green, pale bottom, shaped: lanceolate, sometimes ellipse with pointed tip. *S. alba* plants with roots of trees underground and appear on the surface as pneumatophore, its oval-shaped leaves are rounded like an upside-down, e.g., *S. alba* grows spread with altitude up to 15 m. The roots are wired underground, and surfaces appear as a dull cone-shaped root, and the height is 25 cm. The leaves are skinned, round-shaped like an upside-down egg with a rounded end (Noor et al. 2012).

Rhizophora stylosa, has stilt roots, and pneumatophore roots, the leaves are elliptical with pointed ends. According to Noor et al. (2012), *R. stylosa* plants with one or more stems, up to 10 m tall, have long roots of up to 3 m, and pneumatophore roots growing from the lower branch. The leaves are skinned, regularly spotted in the lower layer, ellipse-shaped widen with tapered ends (Noor et al. 2012). *A. ilicifolius* is mangrove with shrub habitus that has leaves with large serrated edges and sharp spikes. This mangrove grows like a shrub that includes mangrove associates. Smooth leaf surface, large zigzag/jagged leaf edge with wide-pointed and sharp-pointed edges (Noor et al. 2012).

The extracts produced from the four mangrove leaves above have different aromas. In general, the aroma is fragrant and is not bitter in taste. In extracts that use water the aroma and the flavor (if used as a drink), almost the same as tea. People around the Mahakam Delta often use mangroves as herbal medicine.

Agar disc diffusion method

The clear zone formed in the ADD method showed that the filter paper that had been deposited in mangrove extracts microbial growth did not occur, which means that mangrove extract is able to inhibit microbes. The inhibitory zone begins to appear vaguely after 12 hours incubation, increasingly apparent after 24 hours incubation and subsequently enlarging to 48-50 hours of incubation. Furthermore, the inhibitory zone is degraded after 60 hours incubation, which indicates that the extract given is exhausted. The inhibitory zone is formed at 14 hours after incubation, and then extends for up to 48 hours. In general, the inhibit zone does not show any increase after 48 hours (Saptiani et al. 2016c).

The results of antimicrobial activity test using the ADD method showed, in general, the mangrove extract can inhibit bacteria and fungi. The best inhibitory zone to *V. harveyi* is ethanol extract of *S. alba* 1,000 ppm and *A. ilicifolius* 1,000 ppm with a diameter of 12.67 mm, which is not significantly different from the extraction of ethanol extract of *A. marina*, *R. stylosa*, 1,000 ppm, *S. alba* 800 ppm and seawater extract of *S. alba* 1,000 ppm with diameter 12.33 mm. Ethanol extract of *S. alba* 1,000 ppm and *A. ilicifolius* 1,000 ppm not significantly different from seawater extract of *A. marina* 1,000 ppm, *S. alba* 800 ppm, ethanol extract of *A. marina* and *A. ilicifolius* 800 ppm with diameter 12.00 mm inhibition zone; followed by seawater extract of *R. stylosa* 1,000 ppm and ethanol extract of *A. ilicifolius* 600 ppm with an inhibitory zone of 11.67 mm, as shown in Table 1.

Inhibitory zone of mangrove extract to *E. coli* is 12.67 mm in ethanol extract of *A. marina*, *S. alba*, *R. stylosa* and *A. ilicifolius* 1,000 ppm, which is not significantly different with ethanol extract of *A. marina* 800 ppm and seawater extract of *S. alba* with inhibition zone 12.33 mm, also not significantly different with ethanol extract of *S. alba*, *A. ilicifolius* 800 ppm, seawater extract of *A. marina* 1,000 ppm and *S. alba* 800 ppm with diameter 12.00 mm. The best inhibitory zone of *S. aureus* was 13.33 mm, ie extract of ethanol extract of *A. marina* and *A. ilicifolius* 1,000 ppm which was not significantly different with ethanol extract of *S. alba* 1,000 ppm with 13.00 mm inhibition zone, followed by *R. stylosa* 1,000 ppm with zone 12.67 mm and subsequent inhibition zones 12.33 on *A. marina*, *S. alba*, *A. ilicifolius* ethanol extracts 800 ppm, seawater extract of *R. stylosa* and *A. ilicifolius* 1,000 ppm. The complete observation results are shown in Table 1.

The best inhibitory zone to *A. hydrophila* is ethanol extract of *A. marina*, *S. alba*, *R. stylosa* and *A. ilicifolius* 1,000 ppm with 13.00 mm inhibition zone, which is not significantly different from *A. marina* ethanol extract of 800 ppm, seawater extract of *S. alba*, *R. stylosa* and *A. ilicifolius* 1,000 ppm with a zone of 12.67 mm. Not significantly different from seawater extract of *A. marina* 1,000 ppm, *S. alba* 800 ppm, ethanol extract of *S. alba* and *A. ilicifolius* 800 ppm with inhibiting zone 12.33 mm. The best inhibitory zone against *Saprolegnia* sp. is 12.33 mm in ethanol extract of *A. ilicifolius* 1,000 ppm which is not significantly different with *S. alba* 1,000 ppm with 12.00 mm inhibit zone. The complete results of the inhibitory zone measurements in all treatments are shown in Table 1.

The results of this study showed mangrove extract using ethanol solvent, water and seawater can inhibit the growth of various microbes. Research on the inhibitory ability of some plant and mangrove extracts against microbes has been widely reported. *Piper betle* ethanol extract can inhibit the growth of *Saprolegnia* sp., and *E. coli* with inhibit zone 12.67 mm, *A. hydrophila* and *Pseudomonas* sp. 12.33 mm, while the water extract can produce inhibition zone to *Saprolegnia* sp., and *E. coli* about 12.33 mm, *A. hydrophila* 12.00 mm, and *Pseudomonas* sp. 11.67 mm, as well as *Carica papaya* and *Alpina galanga* extracts can be used as antimicrobial ingredient (Saptiani et al. 2016c). Several scientific studies around the world have found evidence that mangrove leaf extract has great potential against pathogenic microbes (Rastegar and Gozari 2017). According to Manilal et al. (2009), *A. ilicifolius* is in vitro vibriocidal, capable of producing inhibition of three Vibrio

species, *V. alcaligenes* (8.00 mm), *V. vulnificus* (9 mm) and *V. alginolyticus* (10 mm). The extract and fraction of ethyl acetate of *A. ilicifolius* leaves have an inhibitory of about 10.67-12.00 mm to *V. harveyi* (Saptiani et al. 2013). *A.officinalis* fruit extract has an antibacterial activity of about 12.66-18.66 mm against both Gram-positive and negative (Sharief et al. 2014). Extracts of methanol bark of *S. alba* and *A. marina* fruit showed a 15.00 mm inhibition zone to *Salmonella typhi*, its acetic acid extract showed a 14.00 mm inhibit zone against *Listeria monocytogenes* (Mustopa et al. 2015).

Minimal Inhibitory Concentration Method

The effectiveness of antimicrobial material can be tested by using MIC method, which is to determine the minimal concentration which can inhibit microbe. MIC to *V. harveyi* is 1.95-18.23 µgmL^{-1}, and the best is ethanol extract of *A. ilicifolius* leaf, followed by *A. marina* 3.26 µgmL^{-1} and *S. alba* 3.91µgmL^{-1}. The MIC to *E. coli* is about 1.95-13.02 µgmL^{-1}, and the best is ethanol extract of *A. ilicifolius* leaf, followed by *A. marina* 2.6 µgmL^{-1} and *S. alba* 3.91µgmL^{-1}.

The MIC to *S. aureus* is about 2.6-18.23 µgmL^{-1}, and the best is ethanol extract of *A. ilicifolius* leaf, followed by *A. marina* 3.26 µgmL^{-1}, *S. alba* and *R. stylosa* µgmL^{-1}. The MIC to *A. hydrophila* is about 3.26-18.23 µgmL^{-1}, and the best is ethanol extract of *A. marina* and *A. ilicifolius*, followed by *S. alba* 3.91 µgmL^{-1}, and *R. stylosa* and seawater extract of *A. marina* 5.1 µgmL^{-1}.

MIC against *Saprolegnia* sp. is about 6.51-26.04 µgmL^{-1}, and the best is the ethanol extract of *R. stylosa* and *A. ilicifolius*, followed by *A. marina* and *S. alba* 7.81 µgmL^{-1}. The results are detailed in Figure 2. MIC of mangrove plants against pathogenic bacteria ranged from 20 mgmL^{-1} to 640 mgmL^{-1} (Selvam and Kolanjinathan 2014), while Behbahani et al. (2014) stated that MIC ethanol extract from *A. marina* leaves for *Aspergillus flavus* and *Penicillium italicum* were 16 and 8 µgmL^{-1}, respectively.

Mangrove as antimicrobial agent

The results of this study indicate that mangrove plants are effective to be used as antimicrobial materials. The extract which was using ethanol was observed to be the best antimicrobial. This shows that ethanol can dissolve the bioactivity of mangrove leaves better than other solvents, so the active ingredients present in the extract are effective for inhibiting microbes. Extracts that use seawater solvents are also good to be used compared to residential zones and MIC solvents. The extract using water solvent is still feasible to use although it requires higher concentration. The extract using ethanol solvent was better than water, however, it still feasible to be used as an antibacterial. The advantages of water solvents are cheap and easy to use. The use of ethanol solvent for some plant extracts showed better results when compared with water (Saptiani et al. 2016a; Saptiani et al. 2016b; Saptiani et al. 2016c).

Mangroves can be used as a new bioactive source of potential natural products used to control microbial disorders (Sharief and Rao 2014). Mangrove is rich in bioactive compounds (Dwilestari et al. 2015). Phytochemical analysis of mangroves has revealed important chemicals such as saponins, alkaloids, glycosides, tannins, steroids, flavonoids, gums, phytosterols, and reducing sugars (Mahmud et al. 2014). The results of this study showed mangrove activity in inhibiting bacteria better than the fungus, although the results of the inhibition zone are still quite good. According to Rastegar and Gozari (2017), mangrove plants can be utilized as a source of natural antifungal drugs. Mangroves have antifungal and antibacterial activity, as well as gastro antioxidants, and show wound healing properties (Cruz et al. 2015). Plant-based antimicrobial compounds have remarkable therapeutic potential, as natural materials can work on target, with no side effects often associated with synthetic antimicrobials (Behbahani et al. 2016).

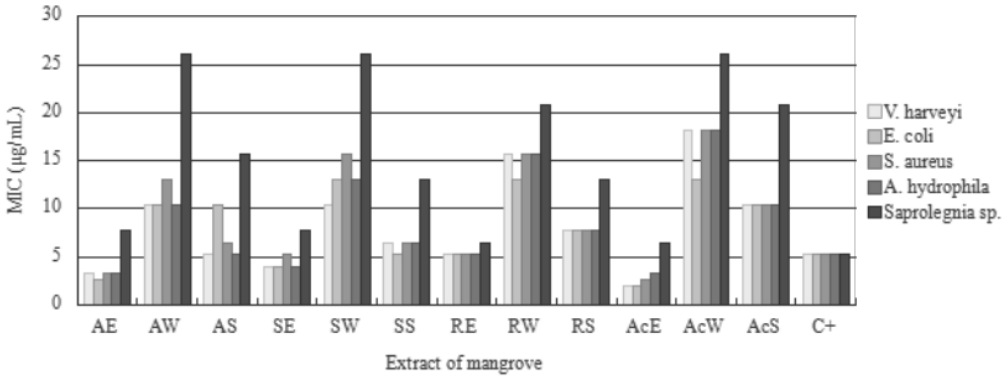

Figure 2. MIC of mangrove extract to microbes. Note: AE= *A. marina* ethanol extract; AW= *A. marina* water extract; AS= *A. marina* seawater extract; SE= *S. alba* ethanol extract; SW= *S. Alba* water extract; SS= *S. Alba* seawater extract; RE= *R. mucronata* ethanol extract; RW= *R. mucronata* water extract; RS= *R. mucronata* seawater extract; AcE= *A. ilicifolius* ethanol extract; AcW= *A. ilicifolius* water extract; AcS= *A. ilicifolius* seawater extract; C+= positive control

Table 1. Diameter of inhibitory zone (mm) of mangrove extract to microbes

Mangrove	Solvents	Concentration	Diameter of inhibitory zone (mm)				
			V. harveyi	*E. coli*	*S. aureus*	*A. hydrophila*	*Saprolegnia* sp.
A. marina	Ethanol	200 ppm	8.67^{jklm}	9.33^{jkl}	9.67^{lm}	9.33^{klm}	8.33^{klmn}
		400 ppm	9.00^{jklm}	9.67^{ijk}	10.33^{ijk}	10.00^{ijk}	9.33^{hijk}
		600 ppm	9.67^{hijk}	9.67^{ijk}	11.00^{ghi}	10.33^{hij}	9.67^{ghij}
		800 ppm	12.00^{bcd}	12.33^{bc}	12.33^{bcde}	12.67^{bc}	11.33^{bcde}
		1,000 ppm	12.33^{bc}	12.67^{b}	13.33^{b}	13.00^{b}	11.67^{bcd}
	Water	200 ppm	8.33^{lm}	8.33^{n}	8.67^{p}	8.33^{p}	8.00^{o}
		400 ppm	8.67^{jklm}	9.00^{klm}	9.33^{lmn}	9.00^{lmn}	8.67^{klm}
		600 ppm	9.33^{ijkl}	9.67^{ijk}	10.00^{jkl}	9.67^{jkl}	9.00^{ijkl}
		800 ppm	10.67^{efgh}	11.00^{efg}	11.33^{fgh}	11.00^{fgh}	10.67^{defg}
		1,000 ppm	11.33^{cdef}	11.67^{cde}	11.67^{efg}	11.67^{def}	11.33^{bcde}
	Seawater	200 ppm	8.67^{jklm}	9.00^{klm}	9.33^{lmn}	9.00^{lmn}	8.67^{klm}
		400 ppm	9.33^{ijkl}	9.67^{ijk}	9.67^{lm}	9.67^{jkl}	9.67^{ghij}
		600 ppm	10.00^{ghij}	10.33^{ghi}	10.67^{hij}	10.33^{hij}	10.33^{efgh}
		800 ppm	11.33^{cdef}	11.67^{cde}	11.67^{efg}	12.00^{cde}	11.33^{bcde}
		1,000 ppm	12.00^{bcd}	12.00^{bcd}	12.00^{def}	12.33^{bcd}	11.67^{bcd}
S. alba	Ethanol	200 ppm	9.00^{jklm}	9.33^{jkl}	9.67^{lm}	9.00^{lmn}	8.67^{klm}
		400 ppm	10.00^{ghij}	10.33^{ghi}	10.33^{ijk}	10.33^{hij}	10.33^{efgh}
		600 ppm	10.33^{fghi}	11.00^{efg}	11.33^{fgh}	10.67^{ghi}	10.67^{defg}
		800 ppm	12.33^{bc}	12.00^{bcd}	12.33^{bcde}	12.33^{bcd}	11.67^{bcd}
		1.000 ppm	12.67^{b}	12.67^{b}	13.00^{bc}	13.00^{b}	12.00^{bc}
	Water	200 ppm	8.33^{o}	8.67^{lmn}	9.00^{mno}	8.67^{mno}	8.00^{o}
		400 ppm	8.67^{jklm}	9.00^{klm}	9.33^{lmn}	9.00^{lmn}	8.67^{klm}
		600 ppm	9.00^{jklm}	9.00^{klm}	9.67^{lm}	9.67^{jkl}	9.67^{ghij}
		800 ppm	9.67^{hijk}	10.00^{hij}	10.33^{ijk}	10.00^{ijk}	9.67^{ghij}
		1,000 ppm	10.67^{efgh}	11.00^{efg}	11.67^{hij}	11.67^{def}	11.33^{bcde}
	Seawater	200 ppm	8.67^{jklm}	9.33^{jkl}	9.33^{lmn}	9.00^{lmn}	8.67^{klm}
		400 ppm	9.33^{ijkl}	9.67^{ijk}	10.00^{jkl}	9.33^{klm}	9.67^{ghij}
		600 ppm	10.33^{fghi}	11.33^{def}	11.33^{fgh}	10.67^{ghi}	10.33^{efgh}
		800 ppm	12.00^{bcd}	12.00^{bcd}	12.00^{def}	12.33^{bcd}	11.33^{bcde}
		1,000 ppm	12.33^{bc}	12.33^{bc}	12.33^{bcde}	12.67^{bc}	11.67^{bcd}
R. stylosa	Ethanol	200 ppm	8.67^{jklm}	9.33^{jkl}	9.33^{lmn}	9.33^{klm}	8.67^{klm}
		400 ppm	9.00^{jklm}	10.33^{ghi}	9.67^{lm}	9.67^{jkl}	9.33^{hijk}
		600 ppm	10.00^{ghij}	11.00^{efg}	10.67^{hij}	10.00^{ijk}	10.00^{fghi}
		800 ppm	11.33^{cdef}	12.00^{bcd}	12.00^{def}	12.00^{cde}	11.33^{bcde}
		1,000 ppm	12.33^{bc}	12.67^{b}	12.67^{bcd}	12.67^{bc}	11.67^{bcd}
	Water	200 ppm	8.33^{o}	8.67^{lmn}	8.67^{p}	8.67^{mno}	8.33^{klmn}
		400 ppm	9.00^{jklm}	9.00^{klm}	9.33^{lmn}	9.67^{jkl}	9.00^{ijkl}
		600 ppm	9.00^{jklm}	9.00^{klm}	9.67^{lm}	10.33^{hij}	9.33^{hijk}
		800 ppm	10.00^{ghij}	10.00^{hij}	10.67^{hij}	10.67^{ghi}	10.33^{efgh}
		1,000 ppm	11.00^{defg}	11.00^{efg}	11.67^{efg}	11.33^{efg}	10.67^{defg}
	Seawater	200 ppm	8.67^{jklm}	9.33^{jkl}	9.33^{lmn}	9.67^{jkl}	9.00^{ijkl}
		400 ppm	9.00^{jklm}	9.67^{ijk}	9.67^{lm}	9.67^{jkl}	9.33^{hijk}
		600 ppm	9.33^{ijkl}	11.33^{def}	10.00^{jkl}	10.33^{hij}	9.67^{ghij}
		800 ppm	10.33^{fghi}	12.00^{bcd}	10.67^{hij}	11.33^{efg}	10.33^{efgh}
		1,000 ppm	11.67^{bcde}	11.67^{cde}	12.00^{def}	12.67^{bc}	11.33^{bcde}
A. ilicifolius	Ethanol	200 ppm	10.33^{fghi}	10.00^{hij}	10.00^{jkl}	10.00^{ijk}	10.00^{defg}
		400 ppm	11.00^{defg}	11.00^{efg}	10.67^{hij}	10.67^{ghi}	10.67^{defg}
		600 ppm	11.67^{bcde}	11.33^{def}	11.33^{fgh}	11.33^{efg}	11.33^{bcde}
		800 ppm	12.00^{bcd}	12.00^{bcd}	12.33^{bcde}	12.33$_{bcd}$	11.67^{bcd}
		1,000 ppm	12.67^{b}	12.67^{b}	13.33^{b}	13.00^{b}	12.33^{b}
	Water	200 ppm	9.00^{jklm}	8.67^{lmn}	8.67^{p}	9.00^{lmn}	8.67^{klm}
		400 ppm	9.67^{hijk}	9.33^{jkl}	9.33^{lmn}	9.33^{klm}	9.67^{ghij}
		600 ppm	10.00^{ghij}	9.67^{ijk}	9.67^{lm}	9.67^{jkl}	10.00^{fghi}
		800 ppm	10.33^{fghi}	10.33^{ghi}	10.33^{ijk}	10.33^{hij}	10.33^{efgh}
		1,000 ppm	11.00^{defg}	10.67^{fgh}	11.00^{ghi}	11.00^{fgh}	10.67^{defg}
	Seawater	200 ppm	9.67^{hijk}	9.67^{ijk}	9.67^{lm}	9.67^{jkl}	9.33^{hijk}
		400 ppm	10.00^{ghij}	9.67^{ijk}	10.00^{jkl}	10.00^{ijk}	9.67^{ghij}
		600 ppm	10.67^{efgh}	10.67^{fgh}	10.67^{hij}	11.33^{efg}	10.33^{efgh}
		800 ppm	11.00^{defg}	11.00^{efg}	11.67^{efg}	12.00^{cde}	11.00^{cdef}
		1,000 ppm	11.33^{cdef}	11.33^{def}	12.33bcde	13.00^{b}	11.33^{bcde}
C+			22.67^{a}	21.00^{a}	20.67^{a}	20.00^{a}	18.00^{a}
C-			6.33^{p}	6.00^{o}	6.33^{q}	6.00^{q}	6.00^{p}

Note: The same letter on the numbers indicates not significantly different (>0.05)

In general, the results of this study showed extracts of *A. marina* and *A. ilicifolius* ethanol produced the largest inhibitory zone to *S. aureus*, followed by all mangrove ethanol extracts to *A. hydrophila*, and *E. coli*. *S. alba* ethanol extract produced the largest inhibit zone to *V. harveyi*, and *A. ilicifolius* produced the largest inhibitory zone to *Saprolegnia* sp. The best MIC results for all microbes were ethanol extract of *A. ilicifolius*, followed by *A. marina*, *S. alba*, and *R. stylosa*.

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