



Antibacterial Potential of Bidara Laut (*Ximenia americana*) Plant Against *Vibrio alginolyticus* and *V. parahaemolyticus* Bacteria

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ARTICLE INFO

Article history

Received: 07 Jun 2020

Accepted: 19 Des 2020

Published: 30 Apr 2021

Keywords:

Antibacteria

Marine Aquaculture

Vibriosis

Ximenia Americana

ABSTRACT

Background: Vibriosis is currently still a major problem in marine aquaculture and is highly dependent on the availability of antibacterial agents in its handling. Investigation of the antibacterial potential of the coastal plants of Bidara Laut (*Ximenia americana*) has been carried out. **Methods:** The parts of the *X. americana* plant taken are Old Fruit, Young Fruit, Old Leaf, and Young Leaf. A total of \pm 200 grams of each part of the *X. americana* plant was taken to be extracted using the maceration method. The antibacterial activity test was performed using the Kirby-Bauer disc diffusion method on Muller Hinton Agar (MHA) media. **Results:** The tested extract of plant parts of *X. americana* showed an inhibition zone against the growth of *V. alginolyticus* and *V. parahaemolyticus* bacteria in each replication in all-time units of observation. Of the four types of extracts tested, old leaves showed strong category resistance to *V. alginolyticus* with the highest clear zone diameter of 16 mm (average 14.67 mm) at 6 hours incubation. Young leaves gave the highest of 18 mm (average 16.33 mm) against *V. parahaemolyticus* at 6 hours incubation. **Conclusions:** *X. americana* plants have the potential for antibacterial against Vibriosis disease in aquaculture. The ability of the power to be seen tended to decrease for all tests until the end of the observation, but until the end of the observation, the ability of the extract inhibition of all parts of the plant was still in the moderate to strong category.

Potensi Antibakteri Tanaman Bidara Laut (*Ximenia americana*) terhadap Bakteri *Vibrio alginolyticus* dan *V. parahaemolyticus*

ABSTRAK

Background: Vibriosis saat ini masih menjadi masalah utama dalam usaha budidaya perikanan laut dan sangat tergantung pada ketersediaan antibakteri dalam penanganannya. Investigasi potensi antibakteria tanaman pesisir Bidara Laut (*Ximenia americana*) telah dilakukan. **Metode:** Bagian tanaman *X.americana* yang diambil adalah Buah Tua, Buah Muda, Daun Tua dan Daun Muda. Sebanyak \pm 200 gr masing-masing bagian tanaman *X americana* diambil untuk dijadikan ekstrak dengan metode maserasi. Uji aktivitas antibakteria dilakukan dengan menggunakan metode difusi cakram Kirby-Bauer pada media *Muller Hinton Agar* (MHA). **Hasil:** Ekstrak bagian tumbuhan *X. americana* yang diujikan menunjukkan adanya zona hambat terhadap pertumbuhan bakteri *V. alginolyticus* dan *V. parahaemolyticus* pada setiap ulangan dalam semua satuan waktu pengamatan. Dari empat jenis ekstrak yang diujikan, daun tua memperlihatkan resistensi dengan kategori kuat terhadap *V. alginolyticus* dengan diameter zona bening tertinggi sebesar 16 mm (rata-rata 14,67 mm) pada inkubasi 6 jam. Daun muda memberikan tertinggi sebesar 18 mm (rata rata 16,33 mm) terhadap *V. parahaemolyticus* pada inkubasi 6 jam. **Kesimpulan:** Tanaman *X. americana* memiliki potensi antibakteri terhadap pengakit Vibriosis pada budidaya perikanan. Kemampuan daya terlihat cenderung menurun untuk semua uji sampai akhir pengamatan, namun sampai akhir pengamatan kemampuan daya hambat ekstrak semua bagian tanaman masih dalam kategori sedang hingga kuat.



Introduction

The *Ximenia americana* L. plant is reported to have been widely used in the treatment of various types of diseases in traditional medicine especially in the state of Ceará, where it is known as a shrub. The skin, leaves, and roots are used in popular medicine for the treatment of skin infections, hemorrhoids, stomach ulcers, stomach pain, and others (Silva et al., 2016). *X. americana* extract is useful for the development of anticancer drugs (Mariko et al., 2016). The ethanol extract from the root bark of *X. americana* (Olacaceae) has anti-inflammatory properties by inhibiting edema, pain, cell migration, and increasing blood vessel permeability (Olabissi et al., 2011). Oil extracted from seeds as cosmetics for body and hair care and as medicines to prevent varicose veins (Urso et al., 2013).

The *Ximenia caffra* plant is used as a natural treatment solution for sexually transmitted infections such as gonorrhea, syphilis, chancroid, chlamydia, genital herpes, and genital warts (Chinsebu, 2016). The bark of the *X. americana* tree is used in traditional medicine as an analgesic and anti-inflammatory (Silva-Leite et al., 2017). *X. americana* is a good candidate for inflammatory diseases and has antioxidant properties. Thus, it can explain the traditional basis for using this plant in the treatment of various diseases such as infectious, microbial, and cardiovascular diseases (Kiessoun et al., 2018). Oil from the seeds of *X. americana*, possibly as a human or animal nutraceutical ingredient (Silva et al., 2016), the leaves of *X. americana* has anthelmintic properties (Shettar & Vedamurthy, 2017). *X. americana* plant parts such as leaves, roots, bark, fruit are used for the treatment of diabetes, ulcers, cancer, malaria, fever, diarrhea, and inflammation (Siddaiah et al., 2011). The leaves and roots are traditionally used to fight dysmenorrhea, diarrhea, nausea, heartburn and jaundice (Nordeng et al., 2013).

X. americana fruit pulp and seeds contain saturated and unsaturated fatty acids (Tanko et al., 2017). *X. americana* fruit is a source of protein, fiber, vitamins, lipids, amino acids, and essential minerals such as calcium, magnesium, potassium, sodium, iron, and manganese. The low anti-nutrient content of this fruit indicates that it can be utilized as a good dietary supplement for human and animal feed formulations. The oil obtained from seeds has the potential to be used as vegetable oil, food, pharmaceutical, and industrial applications (Tanko et al., 2017).

X. americana is an antibacterial producer against gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, and gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) (Abdalla & Abdallah, 2016). The

essential oil from the leaves of *X. americana* is reported to show high antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus pyogenes* and their average effectiveness against *Pseudomonas aeruginosa* (Mulugeta et al., 2015). In vitro activity was tested against gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes*, and *Bacillus subtilis*). *X. Americana* has the potential for antinociceptive activity (Venkateshwarlu et al., 2012). The results of phytochemical analysis of the extract of *X. americana* wood stem indicated the presence of tannins, flavonoids, saponins, terpenoids, and phenols. water extract (Manzo et al., 2017). Shettar et al. (2015) confirm that this plant extract showed high antioxidant activity, and methanol extract showed high anti-inflammatory activity.

X. americana plants are often found on the coast of Batam City and have not been used optimally. Although there have been many investigations and reports on the potential of *X. americana* as described, there have been no reports and investigations of the potential of this plant as an antibacterial against pathogenic bacteria, especially those caused by Vibriosis. Vibriosis is currently still a major problem in marine aquaculture and is highly dependent on the availability of antibacterial agents in its handling. In general, farmers use antibiotics to fight infection with pathogenic bacteria, such as *Vibrio spp.* (Sarra et al., 2013). The use of synthetic antibiotics will have an impact on decreasing the quality standards of fishery products, because of the high residual antibiotics in the muscles of the aquaculture biota and the risk to human health. The use of natural ingredients to overcome the problem of pathogenic bacterial infections in the aquaculture business is the right choice. Therefore, it is very important to investigate the antibacterial potential of coastal plants. This study aimed to test the sensitivity of the *X. Americana* plant antibacterial against *Vibrio alginolyticus* and *V. parahaemolyticus*.

Method

This research was conducted in February-July 2018. The *X. americana* plant samples came from coastal vegetation on Mekan Island, Sekanak Raya Village, Rear Padang District, Batam City. Testing of the sensitivity of *V. alginolyticus* and *V. parahaemolyticus* bacteria is carried out at the Fish and Environmental Health Testing Laboratory of the Marine Culture Fishery Center, which is located at Jl. Raya Trans Bareleng, Bridge-III Setoko Island, Batam. *V. alginolyticus* bacteria is a pure isolate isolated and obtained directly from the Laboratory of the Marine Culture Fishery Center, Batam. Compared to *V. parahaemolyticus*, it is an

HTTC bacterial isolate which is a collection from the laboratory.

Instruments and Materials

The equipment used for sampling of Bidara leaves and fruit in the field were plastic samples, cool boxes, label paper, and stationery. Meanwhile, laboratory equipment includes ovens, Petri dishes, ose needles, bunsen, autoclave, test tubes, Erlenmeyer, analytical scales, measuring cups, shaker incubators, microwaves, laminary airflow, refrigerators, incubators, vortexes, micropipettes, and various standard laboratory equipment.

The materials include the bacteria *Vibrio alginolyticus* and *V. parahaemolyticus* from pure isolates obtained from the Batam Marine Cultivation Center. Young and old (ripe) fruit and young and old leaves of Bidara plants. The culture medium used was TSA (Trypticase Soy Agar) and the sensitivity test medium, namely MHA (Mueller Hinton Agar). While other ingredients are 98% ethanol, 2.5% NaCl, 0.9% NaCl Mc Farland standard solution (1.5×10^8 CFU / ml), distilled water, and technical alcohol. While materials for bacterial identification include 3% KOH (Gram staining reagent), 3% H₂O₂ (catalase reagent), Oxidase strips (oxidase reagent), TCBS media (Thiosulfate Citrate Bile salts Sucrose Agar) for TCBS testing, OF media, namely oxidative media (without paraffin) and fermentative media (media with paraffin) for OF tests, SIM (Sulphate Indol Motility) media for Indol and motility tests and Kovac's (Indol reagent).

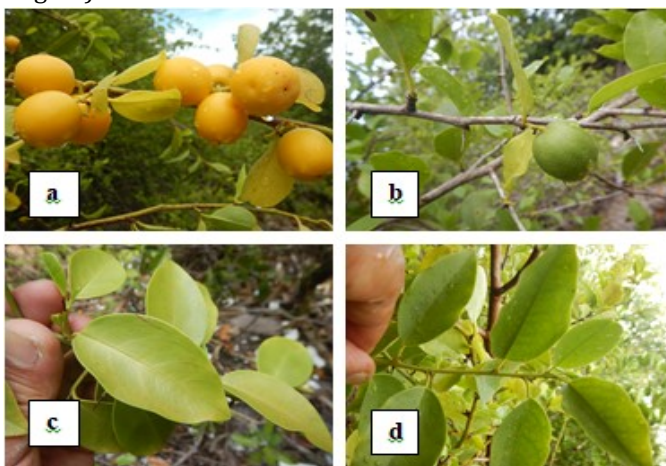


Figure 1. Bidara Laut (*X. americana*): a) old fruit; b) young fruit; c) old leaves (taken at the base of the twig), and d) young leaves (taken at the end of the twig)

Research Procedures

The parts of Bidara Laut (*X. americana*) plants taken to be tested in this study are Old Fruit (BT), Young Fruit (BM), Old Leaf (DT), and Young Leaf (DM). A total of ± 200 grams of each part of the *X. americana* plant was taken to be used as samples tested against *V. alginolyticus* and *V. parahaemolyticus* bacteria. The *X. americana* plant extract used in the sensitivity test resulted from the maceration

method. In the maceration method, samples of *X. americana* plants obtained in the field were first washed clean. Dry in the sun, furthermore, the sample is weighed as much as 5 grams and then finely chopped. Put into the sample bottle and add 25 ml of ethanol into the sample bottle (1: 5w / v) of 20% for all samples (Natrah et al., 2015). Maceration was carried out for 2 x 24 hours with stirring using vibrating platform shakers at a speed of 120 rpm at a temperature of 45°C. Stirring aims to accelerate the contact between the sample and the ethanol solvent used. After that it is evaporated in an open room for 30 hours, this evaporation process aims to remove the solvent, namely, ethanol (Natrah et al., 2015).

The antimicrobial activity test against the target bacteria was carried out using the agar diffusion method / Kirby-Bauer disc diffusion on Muller Hinton Agar (MHA) media. The samples used in this study each had 3 replications and observations were made in a unit of time, namely every 6 hours of incubation (0, 6, 12, 18, 24 hours), this was done to see the progress of forming clear zones periodically.

Isolation and Identification

Primary isolation and culture of *V. alginolyticus* and *V. parahaemolyticus* were carried out on TSA (Trypticase Soy Agar) media. Incubated at 28-30°C for 24 hours and identified. Identification of the biochemical properties of *V. alginolyticus* and *V. parahaemolyticus* based on the book Identification of Pathogenic Bacteria in Fish (Suherman, et al., 2016). This identification consists of, colony form on TSA agar media, KOH test for Gram test, oxidase test, catalase test, O/F test (Oxidative/Fermentative), colony color on TCBS agar medium, indole test, and motility test.

Bacterial Rejuvenation

The bacteria *V. alginolyticus* and *V. parahaemolyticus* were taken as much as 1 ose from the bacterial stock, inoculated by rubbing ose on TSA medium. Incubated in reverse at 300 C for 24 hours. After the bacterial colony grows completely, the bacteria are ready to be tested for their sensitivity to *X. americana* (Purnama et al., 2011).

Preparation of Test Bacterial Suspension

A total of 2 ose of rejuvenated bacteria were taken from agar media on a petri dish. Put it in a test tube containing 10 ml of 0.9% NaCl, then vortex it so that it is homogeneous. The turbidity is equalized with the McFarland standard solution (1.5×10^8 CFU / ml) consisting of 9.95 mL 1% H₂SO₄ solution and 0.05 mL of 1.175% NaCl solution, which is equivalent to the bacterial density of 108 CFU / mL (Tchounwou et al., 2012; Zen et al., 2015).

Implementation of the Sensitivity Test

The bacterial sensitivity test was carried out against two types of bacteria, namely *V. alginolyticus* and *V. parahaemolyticus* bacteria. Antibacterial testing was carried out by the disc diffusion method. The samples used came from the *X. americana* plant. On the MHA media, bacteria derived from the tested bacterial suspension were planted using a sterile cotton swab. The sterile swab is immersed in the suspension contained in the test tube. By pressing and rotating the cotton stick on the inner tube wall. Then the cotton stick is rubbed on the surface of the Mueller Hinton agar plate and spread evenly on the agar surface. Let stand for 3-5 minutes until it dries. The disc paper containing the extract was placed on the agar media surface with tweezers. Leave it on for 30 minutes. Incubated at 30°C for 24 hours (Davis & Stout, 1971).

Observation and Measurement of Inhibition Zone

Analysis of the antibacterial data produced from the test plants was carried out by descriptive analysis which depicts the presence of an inhibition zone formed on the test bacteria culture media that has been diffused with the *X. americana* plant extract. The presence of an inhibition zone is indicated by the clear zone around the disc paper. The result is positive if an obstacle zone is formed around the paper disk, and the result will be negative if an obstacle zone is not formed around the paper disk. The measurement of the inhibition zone is calculated from the diameter of the clear zone formed. The diameter of the drag zone formed is measured from the left side to the right side using a ruler. Areas of bacterial growth inhibition generally refer to general antibiotic standards. A resistance area of 20 mm or more was indicated as having very strong category antibiotic strength; if the area of resistance ranges from 10-20 mm, it is categorized as strong; if the resistance area is 5-10 mm the inhibition power is declared in the moderate category; if the area of resistance is 5 mm or less than 5 mm, then the inhibition is categorized as weak (Davis & Stout, 1971).

Result

Vibrio Bacteria Isolation and Identification

Characteristics of pure isolates of *V. alginolyticus* and *V. parahaemolyticus* were identified based on the book Identification of Pathogenic Bacteria in Fish.

After a series of tests was carried out that the *V. alginolyticus* bacteria had a colony morphology on TSA media which was swarm (spread), Gram-negative (-) after being tested using 3% KOH, produced catalase (+) and oxidase (+) enzymes, fermentative (F), colony color on TCBS media is yellow (Y), indole has a positive red ring (+), and these bacteria are motile (+) and bacillus cell shape (stem, short).

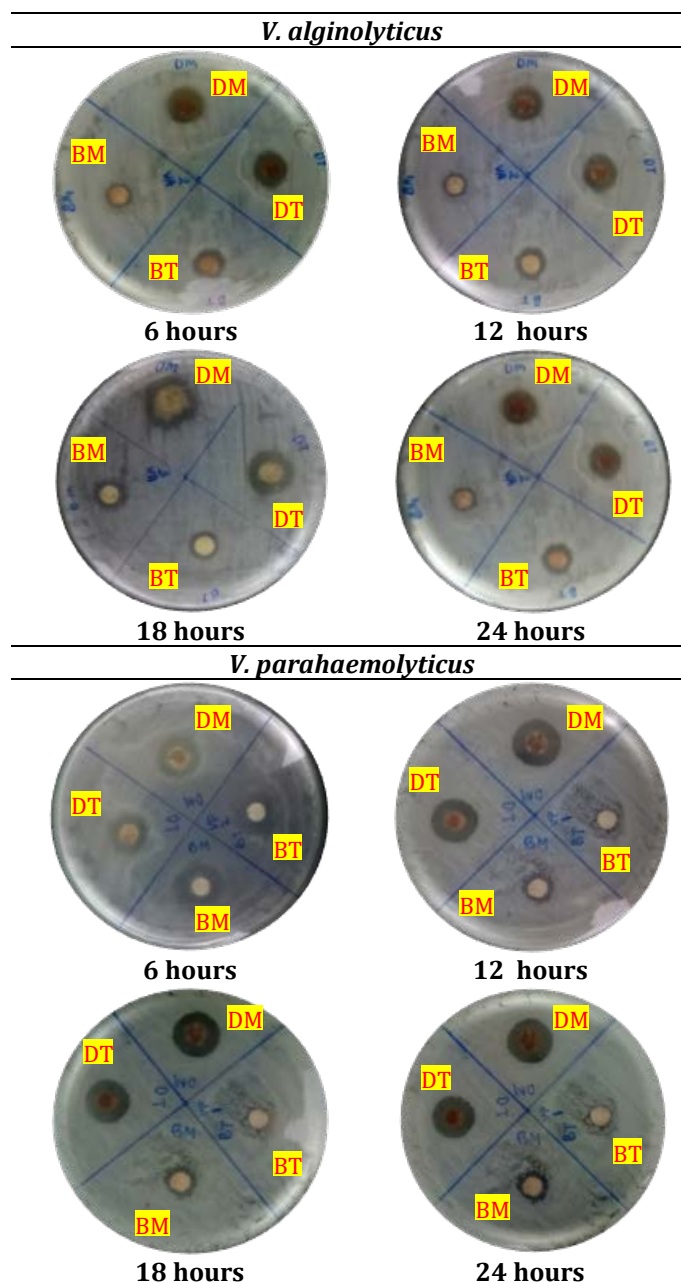


Figure 2. Observation of the antibacterial inhibition of plant extracts of *X. americana*, Old Fruit (BT), Young Fruit (BM), Old Leaf (DT), and Young Leaves (DM) on sensitivity tests against *V. alginolyticus* and *V. parahaemolyticus* bacteria.

Whereas *V. parahaemolyticus* colony color on TCBS media is green, and the cell shape is the comma (bent). The results of the identification of *V. alginolyticus* and *V. parahaemolyticus* bacteria can be seen in Table 1.

Table 1. Results of the Identification Test for *V. alginolyticus* and *V. parahaemolyticus* bacteria isolates

PARAMETER	TEST RESULTS	
	<i>Vibrio alginolyticus</i>	<i>Vibrio parahaemolyticus</i>
Gram	-	-
Catalase	+	+
Oxidase	+	+
Indol	+	+
Oxidative / Fermentative	F	F

(O/F)		
Motility	+	+
Colony color on TCBS agar	Y (Yellow)	Green
Colony forms in TSA	Swarm (menyebar)	Swarm (spread)
Cell shape	Basil (stem, short)	Crooked / Coma

Sensitivity Test of the *X. americana* plant extract

The results of the observation of the sensitivity test of the target bacteria in the *X. americana* sample for each observation sample every 6 hours of incubation are presented in Figure 2. The sensitivity test of *V. alginolyticus* and *V. parahaemolyticus* bacteria to *X. americana* plant extracts show the formation of an inhibition zone (clear zone) at each repetition per unit of observation time. Furthermore, the measurement results of the clear zone on the sensitivity test of target bacteria per replication in the unit of observation time (6 hours of incubation) are presented in Table 2. The formation of the inhibition zone (clear zone) around the disc paper on all replications of the *X. americana* sample provides evidence that coastal

vegetation has a large enough potential to be used as natural antibiotics, which can later be applied in handling aquatic environmental problems.

The formation of the inhibition zone (clear zone) around the disc paper on all replications of the Bidara Laut (*X. americana*) sample provides evidence that the coastal vegetation has a large enough potential to be used as a natural antimicrobial, which can later be applied in handling aquatic environmental problems.

Of the four types of extracts tested on the *V. alginolyticus* bacteria, it was found that the young leaf extract showed a clear zone with the largest diameter of 16 mm, while the smallest clear zone was produced from the young fruit extract, which was 6 mm. In *V. parahaemolyticus* bacteria, the largest clear zone diameter was shown in 18 mm of young leaf extract samples, and the smallest diameter was occupied by old fruit extracts with many 6 mm. The average diameter of the clear zone resulting from the sensitivity test for *V. alginolyticus* and *V. parahaemolyticus* can be seen in Figure 3.

Table 2. The results of measurements of the inhibitory power of *X. americana* plants in the sensitivity test against *V. alginolyticus* bacteria per unit time (hour).

Incubation Time (Hours)	Clear Zone Diameter (mm)																		
	BT				Median	BM				Median	DM				Median	DT			Median
	repeat			repeat			repeat				repeat								
	1	2	3	1	2	3	1	2	3	1	2	3							
6	11	11	10	10,67	7	9	9	8,33	15	14	15	14,67	13	14	15	14			
12	9	10	8	9	6	8	8	7,33	14	14	14	14	13	13	16	14			
18	9	10	8	9	6	8	8	7,33	14	13	12	13	12	12	15	13			
24	9	10	9	9,33	6	8	9	7,67	14	13	13	13,33	12	12	13	12,33			

Description: Old Fruit (BT); Young Fruit (BM); Old Leaf (DT); Young Leaves (DM)

Table 3. The results of measurements of the inhibition of *X. americana* plants on the sensitivity test against *V. parahaemolyticus* bacteria per unit time (hour)

Incubation Time (Hours)	Clear Zone Diameter (mm)																		
	BT				Median	BM				Median	DT				Median	DM			Median
	repeat			repeat			repeat				repeat								
	1	2	3	1	2	3	1	2	3	1	2	3							
6	15	15	10	13,33	16	17	13	15,33	14	14	13	13,67	18	15	16	16,33			
12	8	6	7	7,00	11	10	10	10,33	14	10	12	12	15	13	13	13,67			
18	8	7	7	7,33	10	9	10	9,67	14	9	11	11,33	15	12	12	13			
24	8	7	7	7,33	10	9	9	9,33	14	9	11	11,33	15	12	13	13,33			

Description: Old Fruit (BT); Young Fruit (BM); Old Leaves (DT); Young Leaves (DM)

Table 4. The ability of the extract of Bidara Laut (*X. americana*) against *V. alginolyticus* and *V. parahaemolyticus*

Incubation Time (Hours)	Average Clear Zone Diameter (mm)							
	<i>V. alginolyticus</i>				<i>V. parahaemolyticus</i>			
	BT	BM	DT	DM	BT	BM	DT	DM
6	10.67	8.33	14.67	14	13.33	15.33	13.67	16.33
12	9	7.33	14	14	7	10.33	12	13.67
18	9	7.33	13	13	7.33	9.67	11.33	13
24	9.33	7.67	13.33	12.33	7.33	9.33	11.33	13.33
Average	7.67	13.75	13.33	8.75	11.17	12.08	14.08	7.67
Inhibition category	Medium	Medium	Strong	Strong	Medium	Strong	Strong	Strong

Description: Old Fruit (BT); Young Fruit (BM); Old Leaves (DT), Young Leaves (DM)

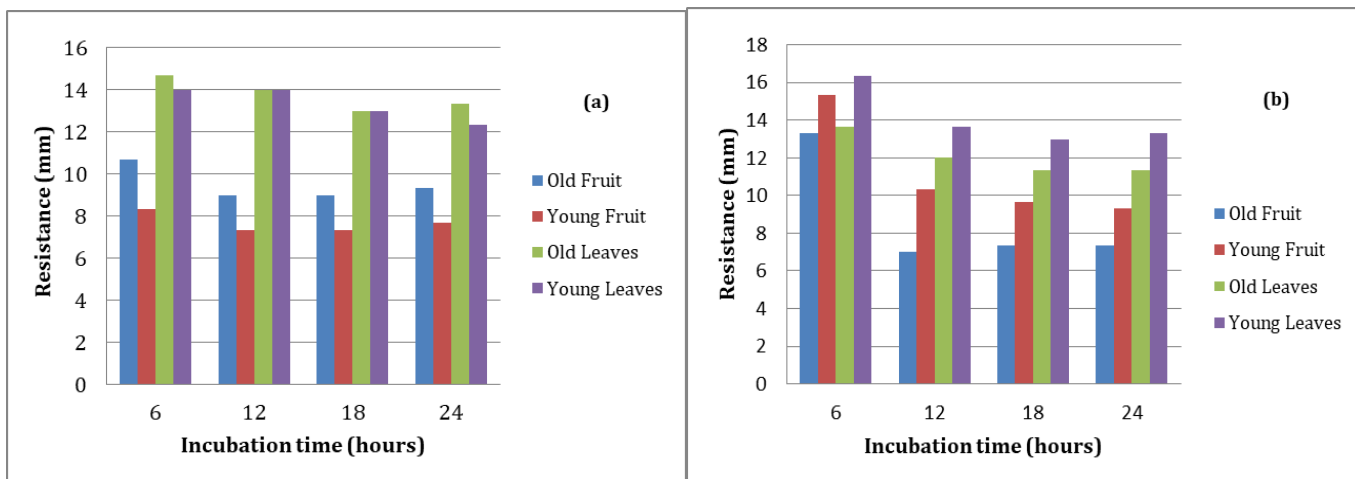


Figure 3. The diameter of the clear zone of the sensitivity test at each observation time: (a). *V. alginolyticus* bacteria, and (b). *V. parahaemolyticus*

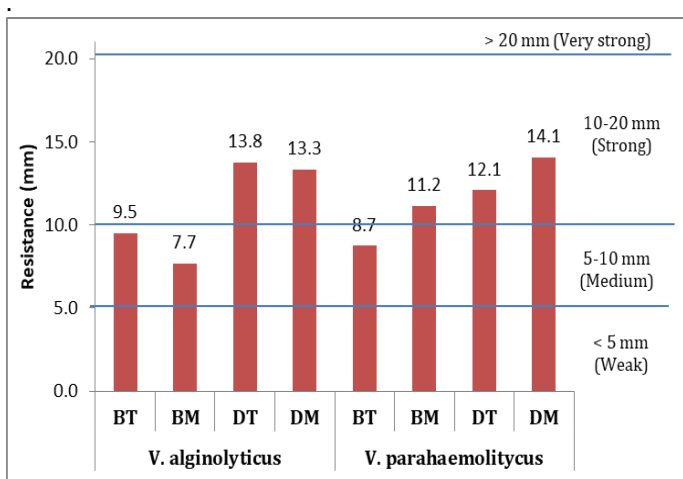


Figure 4. Inhibition of the Bidara Laut (*X. americana*) plant at the end of the observation on *V. alginolyticus* and *V. parahaemolyticus* bacteria.

Discussion

The results of the identification of the tested bacteria in this study are similar to the results of previous studies, namely *V. alginolyticus* and *V. parahaemolyticus* bacteria including gram-negative bacteria, spread colony forms, oxidase, and catalase-positive, fermentative, motile, and indole positive (Dahlia et al., 2017; Johnny & Roza, 2014; Luturmas & Pattinasarany, 2010).

Based on the measurement, it can be concluded that the extract of Bidara Laut (*X. americana*) has the potential as a moderate to strong natural antimicrobial substance. This is due to several factors according to (Sartika et al., 2013), namely bioactive compounds found in plants in the form of secondary metabolites, the type of solvent, and the nature of the solvent used. Previous studies have confirmed that *X. Americana* contains several compounds, saturated and unsaturated fatty acids (Saeed & Bashier, 2010; Tanko et al., 2017), the presence of tannins, flavonoids, saponins, terpenoids, and phenols. However, alkaloids are not present in water extracts (Manzo et al., 2017). Shettar et al. (2015) confirmed that this plant extract showed high

antioxidant activity, and methanol extract showed high anti-inflammatory activity.

Meanwhile, *X. americana* is an antibacterial producer against gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, and gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) (Abdalla & Abdallah, 2016). The essential oil from the leaves of *X. americana* is reported to show high antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus pyogenes* and the average effectiveness against *Pseudomonas aeuruginosa* (Mulugeta et al., 2015).

In this maceration method, the solvent used is ethanol, which will then be absorbed by disc paper and quickly spread on the media so that it can inhibit the growth of surrounding bacteria. Ethanol and methanol are used for the extraction of different plant materials because most studies have reported that organic solvents are materials. the best chemical (reagent) for the consistent extraction of antimicrobial substances from medicinal plants (Azmir et al., 2013; Sasidharan et al., 2011).

The antimicrobial activity of extracts from various parts of the plants studied, such as roots, leaves, seeds, bark, and fruits, appears to be caused by the presence of polyphenol secondary metabolites such as triterpenes, sterols, saponins, tannins, alkaloids, glycosides, and polysaccharides (Maikai & Maikai, 2009). The leaves show the presence of phenols, flavonoids, tannins, saponins, oils, and fats (Seeram et al., 2005). The results of the phytochemical analysis of the *X. americana* stem extract indicated the presence of tannins, flavonoids, saponins, terpenoids, and phenols. However, alkaloids are not present in water extracts (Manzo et al., 2017).

The biological activeness of alkaloid compounds is due to the presence of base groups containing nitrogen. Flavonoid compounds are used as an antibacterial by forming complex compounds against extracellular proteins that interfere with the integrity of the bacterial cell membrane. Flavonoids are also lipophilic which can disrupt microbial membranes (Cowan, 2006). According to Sabir,

(2005), flavonoid compounds can inhibit bacterial growth by several different mechanisms, including flavonoids causing damage to bacterial wall permeability, microsomes, and lysosomes as a result of the interaction between flavonoids and bacterial DNA.

According to Siregar et al. (2012) tannins have phenolic compounds that have a hydroxyl group in them, so the mechanism for activating bacteria is by taking advantage of the polarity difference between lipids and hydroxyl groups. If the bacterial cells contain more and more lipids, a high concentration is needed to make the bacteria lysis.

The condition of the bacterial isolates used as target bacteria in the study will determine the results of the study. *V. alginolyticus* bacteria is a pure isolate isolated and obtained directly from the Laboratory of the Batam City Marine Cultivation Center. Compared to *V. paraheamolyticus*, it is an HTTC bacterial isolate that is a collection from the laboratory. Where the *V. paraheamolyticus* bacteria are pathogenic bacteria that have begun to be resistant to many antibiotics. Based on these conditions, the results of the study can also be proven where all the samples tested showed the average diameter of the clear zone formed in the sensitivity test was greater in the young leaves tested on *V. paraheamolyticus* bacteria. Therefore, it can be assumed that the extract of Bidara Laut *X. americana* is more dominant in inhibiting and killing *V. paraheamolyticus* bacteria than *V. alginolyticus*. although it still requires further proof.

At the very least, some explanations can be given for the differences in the zone of inhibition in the biological activity of several extracts of Bidara Laut (*X. americana*) against the same or different bacteria. This difference is due to plant phenology, concentration, types of target microorganisms, extraction methods, and types of extracts (Najjaa et al., 2007), differences in the content of antibacterial substances (Bachtiar et al., 2012), thickness and composition of cell walls (2). Sartika et al. (2013), extracts from different parts showed varying antibacterial activity against the tested bacteria (Manzo et al., 2017). According to Adriani, (2015), the difference in inhibition zone diameter is caused by several factors including diffusion speed, molecular size, and stability of the antibacterial material, the nature of the media used, the number of organisms inoculated, the growth rate of bacteria, the concentration of chemicals, and the conditions at the time of incubation.

The ability of each bacterium to resist antibacterial activity varies depending on the thickness and composition of the cell walls. In *V. alginolyticus* and *V. paraheamolyticus*, including Gram-negative bacteria. Gram-negative bacteria contain a higher percentage of lipid, fat, or fatty substance (11-22%), and the cell wall of Gram-negative bacteria is thinner and multilayered. The structure of Gram-negative bacteria has an outer layer membrane that covers a thin

peptidoglycan layer, the outer structure of this peptidoglycan is a bilayer containing phospholipids, proteins, and lipopolysaccharides. The mechanism of inhibition of macroalgae antibacterial compounds against the growth of *V. alginolyticus* and *V. paraheamolyticus* is thought to be due to damage to the peptidoglycan component in bacterial cells so that the cell wall layer is not formed intact and causes cell death (Pelczar & Chan, 2008).

Conclusion

X. americana plants have potential antibacterial against Vibriosis disease in aquaculture. The ability of the power to be seen tended to decrease for all tests until the end of the observation, but until the end of the observation, the ability of the extract inhibition of all parts of the plant was still in the moderate to strong category.

Declaration statement

The authors reported no potential conflict of interest.

Acknowledgments

Thank you to the Universitas Riau Kepulauan for funding this research through LPPM with contact number 03 / KP-PID / LPPM / II / 2018. Thank you to the Head of the Fisheries Cultivation Center, Batam, and the Head of the Laboratory for Diseases, Pests, and the Environment, Marine Fisheries Cultivation Center, Setokok, Batam City.

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