Antibacterial Screening on *Xylocarpus grastrnatum* Extract Against Fish Pathogenic Bacteria

D Yoswaty*, Nursyirwani, I. Nurrachmi, I. Effendi, E. Gabariel
Fisheries and Marine Sciences Faculty, University of Riau, Indonesia
*Corresponding author: dyoswaty@yahoo.com

**Abstract.** This research aims to analyze the antibacterial potential of *Xylocarpus granatum* extract (stem, fruit and leaves) on pathogenic bacteria of *Vibrio alginoliticus*, *Aeromona hydrophilla*, *Pseudomnas aeruginosa* and *Escherichia coli*. This research also identified the bioactive compounds contained in the extract. Mangrove samples were collected from the Marine Station of Purnama Village, Dumai City, Indonesia. It was noted that the extract inhibited *V. alginolithicus* with the following inhibitory sequence; stem (10.0 mm), leaf (5.1 mm) and fruit (4.6 mm). This plant also inhibited *A. hydrophilla* in the order of inhibitory level; stem (10.00 mm), leaf (5.4 mm) and fruit (5.3 mm). Similar impacts were also seen in *P. aeruginosa* and *E. coli*. The clear zones produced for *P. aeruginosa* are as follows; stem (10.8 mm), leaf (5.2 mm) and fruit (5.3 mm). Whereas for *E. coli* the inhibitory level was stem (8.7 mm), leaf (5.1 mm) and fruit (4.9 mm). Identification work on bioactive compounds of mangrove stems, leaves and fruits were phenolic, safonin, tannins, terpenoids, steroids and flavonoids.

1. **Introduction**
Mangrove forests are plant communities that grow in the tropics, dominated by plants that have breath roots (*Pneumatofora*) and have the ability to grow in brackish and marine waters. [1] stated that the benefits of mangrove forests are: keeping the coastline and river cliffs from erosion / abrasion to remain stable; protect the back area of mangroves / beaches from waves and strong winds; producer of wood as a source of fuel (charcoal, firewood), building materials (beams, roofs, mats); provide non-timber forest products (such as honey, medicines, drinks and food, tannins); spawning ground and nursery ground for various types of fish, shrimp, shellfish and other marine biota; nesting place for various types of wildlife, especially birds and is a source of germplasm.

Some pathogenic bacteria that are often found in aquaculture businesses such as *Vibrio*, *Salmonella*, *Staphylococcus*, *Echerichia* and *Cloridium*, *Mycobacterium* and *Pseudomonas* [2]. Pathogenic bacteria are bacteria that cause bacterial infections and can kill marine biota. The existence of new virulence factors that cause pathogenic bacteria to become resistant to the use of modern medicines.

Mangrove trees *Xylocarpus granatum* have been traditionally used by the community for daily living needs and medicinal ingredients. Secondary metabolites are compounds produced by plants and used for self-protection of predators and pathogens. Most of the mangroves are used as medicinal ingredients. Extracts and raw materials from mangroves have been used by coastal communities for natural treatment purposes. One of the properties possessed by an antibiotic is to have the ability to damage or inhibit specific pathogenic microorganisms. Mangrove plants are one source of secondary metabolites as antimicrobial compounds [3]. Mangroves can produce secondary metabolites that have promising health benefits, enter lipids into estuarine, and biomarkers for organic matters [4, 5].

The community uses *X. granatum* mangrove as an aromatic substance and produces natural cold powder [6]. Mangroves contain Sun Protector Filter (SPF22) as a sunscreen to protect the skin from ultraviolet light and prevent skin cancer [7]. *X. granatum* tree is a mangrove species in the Meliaceae family which is stored as one of the true mangrove species with small components. The *X. granatum* tree has a height of more than 20 meters, the bark is red-brown with a slippery surface; growing along the banks of the tidal river, the edge of the land from mangroves, the brackish environment that is not too salty.
Leaf extracts, bark, pneumatophor and *X. granatum* fruit for pharmaceutical and ethnomedicinal uses such as fever, malaria, inflammation, dysentery, diarrhea, cholera, stomach problems, diabetes, elephantiasis, antimicrobials, antioxidants, anticancer, antidiabetes and cardiotoxic properties [8, 9, 10, 11]. [12] States that antimicrobial activity depends on the nature of the active ingredient in the extract and the diffusion capacity into the agar medium. Some phytochemical compounds from *X. granatum* such as glycosides, saponins, tannins, flavonoids, terpenoids, alkaloids, have been reported to have antimicrobial activity [13] *X. granatum* tree is a limonoid-rich plant or triterpenoid tetranor. With so many people using it as traditional medicine, *X granatum* is not only beneficial in the health sector but also in other fields, such as prevention of coastal abrasion and other biota habitats [14].

From the facts above the author carried out this research with the aim to determine the inhibitory power of *X. granatum* extract on some fish pathogenic bacteria while identifying the bioactive substances contained in the extract.

2. Materials and Methods
This research was carried out from May-August 2018. Mangrove samples of *Xylocarpus granatum* (leaves, stems and fruit) were collected from the mangrove forest ecosystem of Marine Station Purnama Village, Dumai City, Indonesia. Antibacterial activity assay of mangrove extract, bioactive compound test was carried out at the Microbiology Laboratory of FMIPA Unri and the Marine Microbiology Laboratory, Faculty of Fisheries and Marine, University of Riau. Fish pathogenic bacteria used are *Vibrio alginoliticus, Aeromonas hydrophila, Pseudomonas aeroginosa* and *Escherichia coli*.

Samples of *X. granatum* (leaves, stems and fruit) are washed, dried by drying and dried in an oven at 40°C for 2 weeks. The sample is smoothed in a blender so that it becomes a fine dry powder. 50 g of fine powder sample was stirred with 1 L of methanol and allowed to stand for 48 hours. The extract is filtered with Whatman filter paper no. 1 and the filtrate was evaporated at 40°C using a rotary evaporator [15]. Then the extract solution was prepared with a concentration of 12.5%, 25%, 50% and 100% in laminar flow. For positive control amoxicillin or chloramphenicol discs were used and methanol was used as negative control [16].

Pathogen sensitivity to the extract was tested by Kirby-Bauer disc diffusion method. 6 mm diameter disc paper was immersed in a solution of *X. granatum* extract concentration (12.5%, 25%, 50% and 100%) for 1 minute. Sterile cotton swabs dipped in tubes containing suspensions of *V. alginoliticus, A. hydrophila, P. aeroginosa* and *E. coli* bacteria. Cotton swab is applied to Mueller Hinton Agar surface, inoculated to dry for 5 minutes at room temperature. Each disc was placed on the surface so that, including positive and negative controls. This preparation was incubated at 37°C for 24 hours. Then the clear zone (free of pathogenic bacteria) is measured around the disc using a ruler. The inhibitory diameter is measured, if it is wide enough it means that the bacteria are sensitive to antibacterial compounds and have the ability to barrier the growth of tested bacteria [17].

Phytochemical screening test, a test of bioactive compounds (phenolics, safonins, tannins, terpenoids, steroids and flavonoids) from *X. granatum* extract was carried out. The extract *X. granatum*, water and chloroform mixed into one, shaken a few minutes to form two layers. The water layer is used to test flavonoids, phenolics (Folin-Ciocalteu method) and tannins (Chanwitheeasuk method). Chloroform layer is used to test steroids, safonins and terpenoids.

3. Results
The results showed that mangrove extract of *X. granatum* barrier the growth of pathogenic bacteria *V. alginolyticus, A. hydrophila, P. aeruginosa* and *E. coli*. [18] stated that based on the strength of the inhibitory power, the bioactive substances can be classified as a) very strong, the clear zone is 20 mm or more; b) strong, the clear zone is 10-20 mm; c) medium, the clear zone 5-10 mm; and d) weak, the clear zone is 5 mm or less. in this study, the strongest impact was produced by extract of *X. granatum* stem for all species of the fish pathogens. then followed by leaves and fruit. More detailed data is presented in the following table.
### Table 1. Resistance Test of X. granatum Mangrove Methanol Extract against Pathogenic Bacteria

| Experiment treatment | Diameter of inhibition zone (mm) | Average ± Deviation Standard |
|----------------------|----------------------------------|-----------------------------|
|                      | Stem | Leaves | Fruit | Stem | Leaves | Fruit |
| 1. V. alginolyticus   |      |        |       |      |        |       |
| 12.5                 | 1.7  | 0.7    | 0.3   | 1.72 ± 0.18 | 0.55 ± 0.46 | 0.33 ± 0.20 |
| 25                   | 5.3  | 1.8    | 1.7   | 5.33 ± 0.25 | 1.75 ± 0.82 | 1.73 ± 0.41 |
| 50                   | 8.0  | 3.6    | 3.2   | 7.95 ± 0.71*| 3.58 ± 1.10 | 3.22 ± 0.18 |
| 100                  | 10.0 | 5.1    | 4.6   | 10.00 ± 1.53*| 5.08 ± 2.10 | 4.57 ± 0.53 |
| Etanol               | 0    | 0      | 0     | 0    | 0      | 0     |
| Amosiklav            | 10.6 | 7.0    | 7.4   | 10.55 ± 4.68| 6.98 ± 1.50 | 7.37 ± 1.15 |
| 2. A. hydrophilla    |      |        |       |      |        |       |
| 12.5                 | 3.3  | 0.4    | 0.6   | 3.33 ± 1.10 | 0.42 ± 0.33 | 0.62 ± 0.49 |
| 25                   | 5.9  | 1.7    | 2.0   | 6.33 ± 1.10*| 1.68 ± 0.44 | 2.00 ± 0.85 |
| 50                   | 9.3  | 4.0    | 3.6   | 8.35 ± 1.45*| 3.99 ± 0.52 | 3.59 ± 1.03 |
| 100                  | 10.0 | 5.4    | 5.3   | 10.77 ± 0.24*| 5.37 ± 1.15 | 5.30 ± 1.00 |
| Etanol               | 0    | 0      | 0     | 0    | 0      | 0     |
| Amosiklav            | 9.0  | 10.3   | 12.6  | 10.80 ± 1.10| 10.33 ± 0.91 | 12.58 ± 1.06 |
| 3. P. aeruginosa     |      |        |       |      |        |       |
| 12.5                 | 3.3  | 0.3    | 0.8   | 3.27 ± 0.50 | 0.30 ± 0.17 | 0.80 ± 0.22 |
| 25                   | 6.3  | 1.6    | 2.2   | 5.90 ± 0.26 | 1.60 ± 0.48 | 2.15 ± 0.85 |
| 50                   | 8.4  | 2.7    | 4.3   | 9.27 ± 0.78*| 2.70 ± 0.56 | 4.25 ± 0.83 |
| 100                  | 10.8 | 5.2    | 5.3   | 9.98 ± 0.78*| 5.20 ± 0.73 | 5.28 ± 1.03 |
| Etanol               | 0    | 0      | 0     | 0    | 0      | 0     |
| Amosiklav            | 10.8 | 7.4    | 11.0  | 10.80 ± 1.10| 7.37 ± 2.25 | 11.00 ± 2.49 |
| 4. E. coli           |      |        |       |      |        |       |
| 12.5                 | 2.6  | 0.7    | 0.6   | 1.90 ± 1.31 | 0.65 ± 0.49 | 0.58 ± 0.58 |
| 25                   | 5.7  | 1.2    | 1.8   | 3.93 ± 3.06 | 1.22 ± 0.45 | 1.78 ± 0.68 |
| 50                   | 7.3  | 3.1    | 4.1   | 5.08 ± 3.93 | 3.10 ± 1.06 | 4.05 ± 0.41 |
| 100                  | 8.7  | 5.1    | 4.9   | 5.98 ± 4.76 | 5.07 ± 0.90 | 4.88 ± 0.37 |
| Etanol               | 0    | 0      | 0     | 0    | 0      | 0     |
| Amosiklav            | 11.1 | 13.7   | 9.7   | 7.67 ± 6.47 | 13.68 ± 5.30 | 9.73 ± 0.86 |

[19] stated that antimicrobials that affect cell wall formation or cell membrane permeability work as bactericides and affect protein synthesis to act as bacteriostatics. [20] states that there are several criteria for antibacterial clear zones, namely very strong criteria (inhibitory power > 20 mm), strong criteria (10-20 mm inhibitory power), moderate criteria (5-10 mm inhibitory capacity) and weak criteria (resistivity 4-5 mm). The use of amosiklav (antibiotic of chloramphenicol) as a positive control showed the strongest inhibitory compared to other experiment treatments. While the use of methanol a negative control showed inhibition (Figure 1 and 2).
Usually gram-positive bacteria are more sensitive to antibacterial compounds than Gram-negative. Gram-positive bacterial cell walls have no lipopolysaccharide layer so that antimicrobial compounds that are hydrophilic or hydrophobic can pass through the cell wall through a mechanism of passive diffusion then interact directly with peptidoglycan in cells growing bacteria and cause cell death [21]. In Gram negative bacteria the cell structure is more complex consisting of three layers, that is the outer layer of lipoprotein, the middle layer of lipopolysaccharide, and the inner layer of peptidoglycan. This causes antibacterial compounds that are difficult to pierce the cell wall of Gram negative pathogenic bacteria.

In Figures 1 and 2 it can be seen that the clear zone around the disc shows that the pathogenic bacteria are still growing. The wider the inhibitory power seen on the disc, the higher the antibacterial ability of *X. granatum* mangrove extract to inhibit the growth of pathogenic bacteria. This can be seen in the formation of a clear zone of about 6 mm discs that have been given mangrove extract with various concentrations (12.5%; 25%, 50%, 100%, negative control (methanol) and positive control (amoxycillin, antibiotic chloramphenicol)).

The inhibitory power of bacteria is a compound that can kill or barrier the growth of pathogenic bacteria. The inhibitory power of bacteria is to kill pathogenic bacteria (microbicidal) or inhibit bacterial growth (microbiostatic). The results showed that the highest diameter of the clear zone by treating amoxxicillate concentration (antibiotic chloramphenicol) against pathogenic bacteria compared to other concentrations.

Bioactive substances can kill pathogenic bacteria (microbicidal) or inhibit growth (microbiostatic). The results of this study showed that the highest inhibitory power was obtained in the treatment of positive control (amoxycycl, antibiotic chloramphenicol).

Damage to protein structure by a number of physical and chemical elements can cause cell death. Substances concentrated on the cell surface can alter the physical and chemical properties of the cell wall and block the normal function of the cell wall as a selective barrier. This can lead to bacterial cell
death [15]. Antibacterial properties of a compound are said to have high activity against bacteria if they have a large diameter of inhibitory power. A material is said to have antibacterial activity if the diameter of the inhibitory power formed is greater or equal to 6 mm [22].

[23] stated that the mechanism of action of saponins as antibacterials is to disrupt the stability of bacterial cell membranes, causing damage to cell membranes and causing the release of important components in bacterial cells namely proteins and nucleic acids. [24] stated that tannin is an active compound of secondary metabolites which is known to have several properties, namely as astringent, anti-diarrhea, anti-bacterial and antioxidant. Tannins are a very complex component of organic substances, consisting of phenolic compounds that are difficult to separate and difficult to crystallize, precipitate proteins from their solution and form proteins.

Flavonoids function as antiviral, cytotoxic activity, inhibit the action of enzymes. Saponins can reduce surface tension thereby increasing cell permeability. Antibacterial tannins can precipitate proteins, activate enzymes and damage the function of genetic material [25]. The mechanism of action of antimicrobial compounds are inhibition of cell wall synthesis that causes cell wall damage resulting in lysis, changes in cell membrane permeability or active transport through cell membranes that can cause cell leakage and death, inhibition of protein synthesis, and inhibition of nucleic acid synthesis [26]. Inhibition of microbial activity by bioactive components of the plant can be caused by several factors, including: a) Disruption of the constituent compounds of the cell wall; b) Increased permeability of cell membranes which causes the loss of constituent components of cells; c) Activating metabolic enzymes; and d) Deception or damage to 13 genetic material [11].

4. Conclusion

X. granatum extract from the stem, leaves and fruit inhibited the growth of fish pathogenic bacteria V. alginoliticus, A. hydrophila, P. aeroginosa and E. coli. The inhibitory level of X. granatum extract scored as strong (average diameter of 10.00-10.77 mm) for the growth of V.alginoliticus, A. hydrophila and P. aeruginosa bacteria and medium category (8.7-4.9 mm) against E. coli. Bioactive compounds identified in mangrove extract as phenolic, safonin, tannins, steroids and flavonoids.

References
[1] Kusmana C, Hilwan I, Pamungkas P, et al. Teknik Rehabilitasi Mangrove. Bogor: Fakultas Kehutanan Institut Pertanian Bogor; 2005.
[2] Austin B, Austin D. Methods for the Microbiological Examination of Fish and Shelfish Methods for the Microbiological Examination of Fish and Shelfish. England: Ellis Horwood Ltd; 2007.
[3] Naibourhu P. Ekstraksi dan Manfaat Ekstrak Mangrove (Sonneratia alba dan Sonneratia caseolaris) Sebagai Bahan Alami Antibakterial: Pada Patogen Uang Windu, Vibrio harveyi. 2002.
[4] Basyuni M, Oku H, Baba S, Takara K, Iwasaki H. Isoprenoids of Okinawan mangroves as lipid input into estuarine ecosystem. J Oceanogr. 2007. doi:10.1007/s10872-007-0053-2
[5] Koch BP, Souza Filho PWM, Behling H, et al. Triterpenols in Mangrove Sediments as a Proxy for Organic Matter Derived from the Red Mangrove (Rhizophora mangle). Org Geochem. 2011;42(1):62-73. doi:10.1016/j.orggeochem.2010.10.007
[6] Rosyada A, Anwari MS, Muflihati. Pemanfaatan Tumbuhan Mangrove oleh Masyarakat Desa Bakau Besar Laut Kecamatan Sungai Pinyuh Kabupaten Mempawah. J Hutan Lestari. 2018;6(1):62-70.
[7] Hardjito L. Mangrove Seeds Shield Ultraviolet Stings. Indonesia: Media Indonesia; 2008.
[8] Patra JK, Thatoi HN. Metabolic diversity and bioactivity screening of mangrove plants: A review. Acta Physiol Plant. 2011. doi:10.1007/s11738-010-0667-7
[9] Akter M, Afrin S, Sakib SN, et al. Investigation of Antibacterial, Cytotoxic and Antioxidant Properties of the Mangrove Plant Xylocarpus mekongensis; Adv Biosci Biotechnol. 2016. doi:10.4236/abb.2016.74019
[10] Alam M., Sarder M, Awal M, Sikder M, Daulla K. Antibacterial Activity of the Crude Ethanolic
Extract of Xylocarpus granatum Stem Barks. *Bangladesh J Vet Med.* 1970;4(1):69-72. doi:10.3329/bjvm.v4i1.1529

[11] Sumardi, Basyuni M, Wati R. Antimicrobial activity of polyisoprenoids of sixteen mangrove species from North Sumatra, Indonesia. *Biodiversitas.* 2018. doi:10.13057/biodiv/d190409

[12] Vadlapudi VR, Bobbarala V, Naidu KC. Comparative Screening of Selected Mangrove Plant Methanolic Extracts against Clinical and Plant Pathogens. 2009;2(6):1062-1064.

[13] Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. *J Ethnopharmacol.* 2001. doi:10.1016/S0378-8741(01)00307-5

[14] Baba S, Chan HT, Kainuma M, Kezuka M, Chan EWC, Tangah J. Botany, uses, chemistry and bioactivities of mangrove plants III: *Xylocarpus granatum.* ISME/GLOMIS Electron J. 2016.

[15] Riedel S, Hobden JA, Miller S. Jawetz, Melnick and Adelberg’s Medical Microbiology: 28th Edition. In: *Jawetz, Melnick & Adelberg’s Medical Microbiology.* ; 2019.

[16] Hanafiah K. *Basics of Soil Science.* Jakarta: PT. Raja Grafindo Persada; 2012.

[17] Iswantini D, Yulian M, Mulijani S, Trivadila. Inhibition kinetics of *Sida rhombifolia* L. extract toward xanthine oxidase by electrochemical method. *Indonesia J Chem.* 2014;14(1). doi:10.22146/ijc.21270

[18] Suada K, Suhartini DMWY, Sunariasih NPL, et al. Ability of endophytic fungi isolated from rice to inhibit pyricularia oryzae-Induced rice blast in indonesia. *J Fac Agric Kyushu Univ.* 2012;57(1):51-53.

[19] Nikham, Taty E. Antibacterial Raw Material Test from God Crown Fruit (Phaloria macrocarva (scheff) boerl). In: *Proceedings of Materials Science and Technology Scientific Meeting.* ; 2012.

[20] Santoni A, Efdi M, Bumali R. Isolasi Senyawa Triterpenoid dan Uji Antibakteri Ekstrak n-Heksan Daun Kayu Ara (*Ficus aurata* (Miq.) Miq). *J Ris Kim.* 2019. doi:10.25077/jrk.v12i2.268

[21] Tortora G., Funke B., Case C. *Microbiology. 9th Edition.* San Francisco: Pearson Education; 2007.

[22] Suciani A, Wardiyanto W, Sumino S. Efektifitas Ekstrak Daun Rhizophora Mucronata dalam Menghambat Pertumbuhan *Aeromonas Salmonicida* dan *Vibrio harveyi.* e-Jurnal Rekayasa dan Teknol Budid Perair. 2012. doi:10.23960/jrtbp.v1i1.98p1-8

[23] Dwinovantyo A. Uji Bahan Aktif dan Bahan Antibakteri Rhizopora mucronata dalam Upaya Penanggulangan Penyakit Diare pada Saluran. *ResearchGate.* 2014;21:32-40. https://www.researchgate.net/publication/280641238_Uji_Bahan_Aktif_dan_Bahan_Antibakteri_Rhizopora_mucronata_dalam_Upaya_Penanggulangan_Penyakit_Diare_pada_Saluran_Pencer naan_Manusia.

[24] Desmiaty Y, Ratnawati J, Andini P. Penentuan Jumlah Flavonoid Total Ekstrak Etanol Daun Buah Merah (*Pandanus conoideus* Lamk.) Secara Kolorimetri Komplemener. *Semin Nas POKJANAS TOI XXXVI.* 2009.

[25] Harborne J. *Phytochemical Method: A Modern Guide to Analyzing Plants.* Bandung: ITB Publisher; 2006.

[26] Purvaja R, Ramesh R, Frenzel P. Plant-mediated methane emission from an Indian mangrove. *Glob Chang Biol.* 2004. doi:10.1111/j.1365-2486.2004.00834.x