BIODIVERSITAS
Volume 24, Number 1, January 2023
Pages: 481-485

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240155

Antibacterial activity of ethanol extract of banana, cassava, and pineapple peels against a fish pathogen *Aeromonas hydrophila*

DINI SISWANI MULIA[1,♥], RAIH RAICHA[1], CITRA PUSPANING LUNGGANI[1], SISKA NUR ERINA[1], JULI ROCHMIJATI WULIANDARI[1], CAHYONO PURBOMARTONO[2], ALIM ISNANSETYO[3]

[1]Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Purwokerto. Jl. KH. Ahmad Dahlan, Purwokerto 53182, Central Jawa, Indonesia. Tel./fax.: +62-81-636751, ♥email: dinisiswanimulia@ump.ac.id

[2]Department of Aquaculture, Faculty of Agriculture and Fisheries, Universitas Muhammadiyah Purwokerto. Jl. KH. Ahmad Dahlan, Purwokerto 53182, Central Java, Indonesia

[3]Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada. Jl.Flora, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia

Manuscript received: 23 November 2022. Revision accepted: 19 January 2023.

Abstract. *Mulia DS, Raicha R, Lunggani CP, Erina SN, Wuliandari JR, Purbomartono C, Isnansetyo A. 2023. Antibacterial activity of ethanol extract of banana, cassava, and pineapple peels against a fish pathogen* Aeromonas hydrophila. *Biodiversitas 24: 481-485.* *Aeromonas hydrophila* is the most virulent bacteria for almost all freshwater fish species. One method to control pathogenic bacteria in fish should be safe and environmentally friendly, including utilizing natural wastes such as banana peels (*Musa paradisiaca*), cassava peels (*Manihot esculenta*), and pineapple peels (*Ananas comosus*). These natural wastes contain bioactive compounds. Their utilization increases the waste value. This study aims to determine the potential of banana peel, cassava peel, and pineapple peel waste as an antibacterial against *A. hydrophila,* a causing agent of Motile Aeromonas Septicemia (MAS) in freshwater fish. Parameters observed included phytochemical compounds, MIC, and MBC values of banana, cassava, and pineapple peels. Phytochemical screening was performed using thin-layer chromatography (TLC) and foam test. The microdilution method carried out the antibacterial activity of banana peel, cassava peel, and pineapple peel against *A. hydrophila*. It determines the minimum inhibitory concentration (MIC); and the minimum bactericidal concentration (MBC) values. Phytochemical screening results showed that banana, cassava, and pineapple peel extracts contained flavonoids, alkaloids, terpenoids, tannins, and saponins. The MIC values of banana peel, cassava peel, and pineapple peel extract were 600, 400, and 200 µg/mL, respectively. The MBC values of banana peel, cassava peel, and pineapple peels were 4 × MIC, 2 × MIC, and 1 × MIC, respectively. Extract of pineapple peel exhibited better antibacterial activity than banana and cassava peels. However, the three waste extracts indicated the antibacterial potential to be developed as natural bactericides to control *A. hydrophila* bacterial disease.

Keywords: *Aeromonas hydrophila*, antibacterial, banana peel, cassava peel, pineapple peel

INTRODUCTION

Freshwater fish cultivation often encounters disease problems caused by the pathogenic bacteria *Aeromonas* sp. The disease caused by *Aeromonas* sp. is known as aeromoniasis or Motile Aeromonas Septicemia (MAS). *Aeromonas hydrophila* is one of the pathogenic bacteria that often infect many freshwater fish. *Aeromonas hydrophila* is a Gram-negative, bacillus-shaped with rounded ends, non-spore, motile, facultative anaerobic (Stratev and Odeyemi 2016; Abeyta et al. 2019; Yazdanpanah-Goharrizi et al. 2020; El-Sharaby et al. 2021; Mailafia et al. 2021). The mechanism of bacterial pathogenicity is quite complex and related to virulence factors (Li et al. 2015).

Aeromonas hydrophila infection in cultivated fish could be detrimental because it causes mass death. A previous study reported that *A. hydrophila* infection caused 80-100% fish mortality (Mulia 2012). Currently, *A. hydrophila* infection is usually treated using antibiotics. Antibiotic treatment in the short term and with the proper dose effectively overcomes the disease. However, continuous and uncontrolled amounts of antibiotics may have a negative impact, such as bacterial resistance, residues in fish, and environmental pollution, which have detrimental effects on humans (Cao et al. 2012; Deng et al. 2014).

Alternatives treatment of *A. hydrophila* infection includes using natural ingredients containing antibacterial bioactive compounds (Mulia et al. 2022). The search for new drugs from a natural source is highly needed due to the increasing number of disease problems. In addition, it is crucial to search for new sources of drugs that are safe, effective, and environmentally friendly.

Various wastes from processed food have no economic value. They have not been utilized, including wastes of banana peel (*Musa paradisiaca*), cassava peel (*Manihot esculenta*), and pineapple peel (*Ananas comosus*). The variety of processed foods from banana and cassava as raw materials and their processing business units are rapidly increasing. Therefore, the amount of waste from banana, cassava, and pineapple is also growing.

Previous studies reported that waste of banana peels, cassava peels, and pineapple peels contained antibacterial compounds. Banana peel extract is an active antibacterial against several bacteria, such as *A. hydrophila* (Singh et al. 2013), as well as *Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas citri, Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi, Klebsiella* *pneumonia*, *Bacillus subtilis*, and *Escherichia coli* (Krishna et al. 2013; Singh et al. 2013), *Staphylococcus epidermidis*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans* (Kapadia et al. 2015; Kumari et al. 2020). The banana peel extract had antibacterial activity against *S. aureus* and *S. pyogenes* (Chabuck et al. 2013). A study by Odilia et al. (2022) showed that the extract of cassava peels was active against *S. aureus*, *S. epidermis,* and *E. coli*, while the extract of pineapple peel was active against *S. aureus*, *E. coli*, *P. aeruginosa*, *Vibrio cholerae* and *K. pneumoniae* (Lubaina et al. 2019). However, a study on cassava peel and pineapple peel extract as an antibacterial for *A. hydrophila* is still limited. Singh et al. (2013) studied banana peel extract as an antibacterial against *A. hydrophila*; however, the isolate of *A. hydrophila* was different from this study. Differences in isolates and strains of *A. hydrophila* may have differences in biochemical characteristics, pathogenicity, and bacterial resistance (Králová et al. 2016). The objective of this study was to determine the antibacterial potential of banana peels, cassava peels, and pineapple peels waste that can be developed as a natural bactericide to control *A. hydrophila,* a causing agent of MAS in freshwater fish.

MATERIALS AND METHODS

Samples

The materials used in this study comprised peel wastes of banana (*Musa paradisiaca*), cassava peel (*Manihot esculenta*), and pineapple peel (*Ananas comosus*). The isolate of *A. hydrophila* was obtained from Bogor, West Java, Indonesia. Bacterial culture media used in this study were tryptic soy broth (TSB) (Merck, Merck Corporate, Kenilworth, USA), glutamate starch phenol (GSP) (Merck, Merck Corporate, Kenilworth, USA), Mueller Hinton Broth (MHB) (Conda, Condalab, Madrid, Spain). The chemicals used in this study activity test consisted of $CaCl_2$, $MgCl_2$, and resazurin. Antibiotics for positive control included enrofloxacin (Sigma, Sigma Corporate, St. Louis, Missouri, USA).

Extraction of banana, cassava, and pineapple peels

The extraction of banana, cassava, and pineapple peels using the maceration method. The simplicia powder of banana peel, cassava peel, and pineapple peel was macerated in 96% ethanol with a 100 g: 500 mL ethanol ratio for 2 x 24 hours. The extract was concentrated using a rotary evaporator at 60ºC.

Bacterial inoculation *Aeromonas hydrophila*

Aeromonas hydrophila from pure culture was grown on a TSB medium and incubated at 37ºC for 24 hours.

Phytochemical screening of banana, cassava, and pineapple peels extract

The phytochemical screening of the extract was carried out using thin-layer chromatography (TLC) and foam test. The TLC test was carried out to determine the presence of flavonoids, alkaloids, tannins, and terpenoid compounds, while the foam test used the saponin compounds.

Antibacterial activity test to determine minimum inhibitor concentration (MIC)

The MIC value of the banana, cassava, and pineapple peel extract was carried out using the microdilution method against *A. hydrophila*. It was done on 96 wells microplate (CLSI 2020).

Determination of minimum bactericidal concentration (MBC)

The MIC value was used for determining the MBC value. Furthermore, the test concentrations were determined as 1 × MIC, 2 × MIC, 4 × MIC, and 6 × MIC (Isnansetyo and Kamei 2009) using 96 well microplates (CLSI 2020). Then, samples were grown on a GSP medium by applying the streak method and incubated at 30°C for 48 hours. Data analysis on the phytochemical test results, as well as MIC and MBC values, were analyzed.

RESULTS AND DISCUSSION

The phytochemical content of banana peel, cassava peel, and pineapple peel extract

Based on the extraction process, the pineapple peel produces the highest extract (45.03%), followed by the cassava peel (12.90%), and the lowest was exhibited by the banana peel (6.43%). Phytochemical screening by TLC showed that the three extracts contained flavonoids, alkaloids, tannins, and terpenoids. Results of the foam test showed that all three extracts contained saponins (Table 1).

The results of phytochemical screening in this study were similar to the previous studies. Extract of banana peel (*M. paradisiaca*) contains flavonoid compounds, alkaloids, terpenoids, tannins, and saponins (Lumowa and Bardin 2018). A study by Amaza (2021) showed that cassava peel extract contained some chemical compounds such as alkaloids, tannins, and saponins. At the same time, the pineapple peel (*A. comosus*) contains flavonoids, alkaloids, terpenoids, tannins, and saponins (Gunwantrao et al. 2016).

Table 1. Phytochemical content of banana peel, cassava peel, and pineapple peel ethanol extract

Extract	Active compound				
	Flavonoid	Alkaloid	Terpenoid	Tanin	Saponin
Banana peel	+	+	+	+	+
Cassava peel	+	+	+	+	+
Pineapple peel	+	+	+	+	+

Minimum Inhibitory concentration (MIC) value

Antibacterial activity was carried out by determining the MIC value. The results showed that three extracts had different MIC values against *A. hydrophila* (Table 2). All the extracts inhibit the growth of *A. hydrophila* with various MIC values, and pineapple peel extract had the lowest MIC value of 200 µg/mL.

Based on the MIC values of the extract, so pineapple peel and cassava peel were within the range of 100-500 µg/mL and categorized as having a relatively strong antibacterial activity (Holetz et al. 2002). Meanwhile, MIC values of the banana peel extract were within the range of 500-1000 µg/mL and categorized as weak. According to Holetz et al. (2002), antibacterial activities are divided into four categories: very strong with MIC less than 100 µg/mL, fairly strong with a MIC range within 100-500 µg/mL, weak with a MIC range within 500-1000 µg/mL, and zero with MIC value >1000 µg/mL.

The ability of banana peel, cassava peel, and pineapple peel extracts to inhibit the growth of *A. hydrophila* could be due to antibacterial compounds in these extracts, such as flavonoids, alkaloids, tannins, terpenoids, and saponins. In general, the antibacterial mechanism of action is by inhibiting bacterial replication and damaging the bacterial structural components (Baquero and Levin 2021). Flavonoids have excellent antibacterial activity (Farhadi et al. 2019) by inhibiting nucleic acid synthesis, cytoplasmic membranes alteration, porins inhibition in cell membranes, membrane permeability changes, and pathogenicity attenuation (Xie et al. 2015). Flavonoids can also damage the cytoplasmic membrane and suppress cell wall synthesis caused by D-alanine–D-alanine ligase inhibition (Panche et al. 2016).

Alkaloids contain nitrogen-containing base groups that are reactive to the amino acids that make up bacterial cell walls and DNA. This reaction causes changes in the structure of amino acids and results in genetic changes and the lysis of bacteria. The antibacterial mechanism of alkaloids can also interfere with the preparation of peptidoglycan (a component of cell walls) in bacteria, where the cell wall layer is not fully formed and causes bacterial death (Cushnie et al. 2014).

Tannins damage cell membranes and induce the formation of complex compounds that attack bacterial enzymes, thereby increasing the tannins' toxicity to bacteria (Fraga-Corral et al. 2020). Tannins can shrink cell membranes or cell walls which interfere with the permeability of bacterial cells so that they can inhibit the growth of bacterial cells (Farha et al. 2020). Terpenoids are compounds with antibacterial potential (Yang et al. 2020). Terpenoids damage the bacterial cell wall and change the permeability of the cytoplasmic membrane, causing lysis, leakage of nutrients from within the cell, denaturation of cell proteins, and enzyme inhibition in the cell (Guimarães et al. 2019).

The action of saponin as an antibacterial is by denaturing proteins in bacterial cells. Saponins, similar to detergents, lower the cell surface tension before damaging the bacterial cell membrane. A damaged cell membrane disrupts the bacterial cell, and saponin diffuses into the cell through the cytoplasmic membrane. It disrupts membrane stability, and cytoplasm leakage results in cell death (Dong et al. 2020).

Previous studies reported the antibacterial activity of banana peels (*M. paradisiaca*) against *A. hydrophila* (Singh et al. 2013). A study by Ahmed et al. (2018) showed that banana peel inhibits the growth of other bacteria, including the growth of *S. aureus* at 1%, 3%, and 5% with a diameter of inhibition zone of 0.6, 1.5, and 2 mm. The extract of cassava peel (*M. esculenta*) can inhibit *S. aureus*, *epidermidis*, and *E. coli* (Odilia et al. 2022). Still, no information regarding the inhibitory activity against *A. hydrophila*. The extract of pineapple peel (*A. comosus*) inhibits the growth of *S. aureus* with a MIC value of 1.56% (Loon et al. 2018).

Other studies reported the ability of pineapple peel to inhibit the growth of *S. mutans* and *S. sanguis* (Goudarzi et al. 2019). Punbasayakul et al. (2018) showed that the MIC values of ethanol extract from pineapple peel against *S. aureus*, *B. cereus*, *E. coli*, and *S. typhimurium* were 0.0084 g/mL, 0.0084 g/mL, 0.0168 g/mL, and 0.0084 g/mL, respectively. Another study reported that the ethanol extract of pineapple peel at 1 mg/mL could inhibit the growth of *P. aeruginosa*, *S. aureus*, *E. coli*, *V. cholerae*, *K. pneumoniae* with inhibition zones ranging from 11-17 mm (Lubaina et al. 2019). The variation in MIC values in different bacterial species might be due to differences in isolates and strains of bacteria. Differences in resistance or sensitivity are assumed to be due to variations in the source of isolates, frequency, and types of antimicrobial agents (Bengtsson-Palme et al. 2018; Peterson and Kaur 2018).

Minimum bactericidal concentration (MBC) value

MBC value was determined using 1 × MIC, 2 × MIC, 4 × MIC, and 6 × MIC (Isnansetyo and Kamei 2009). It was carried out using the serial microdilution method in 96 wells of the microplate (CLSI 2020). The results showed that banana peel, cassava peel, and pineapple peel extract had different MBC values against *A. hydrophila* (Table 3).

Table 2. MIC value of banana peel, cassava peel, and pineapple peel against *Aeromonas hydrophila*

Extract	MIC (µg/mL)
Banana peel	600
Cassava peel	400
Pineapple peel	200

Table 3. MBC value of extract of banana, cassava, and pineapple peels against *Aeromonas hydrophila*

Extract type	MIC (µg/mL)	MBC	Antibiotic properties
Banana peel	600	4 × MIC	Bactericidal
Cassava peel	400	2 × MIC	Bactericidal
Pineapple peel	200	1 × MIC	Bactericidal

The MBC values of banana, cassava, and pineapple peel extracts were less than 6 × MIC, indicating that these extracts are bactericidal (Isnansetyo and Kamei 2009). Pineapple peel extract had the lowest MIC and MBC values indicating that pineapple peel extract had more potent antibacterial activity than banana and cassava peel extract. It was due to the active compounds in pineapple peel extract. The active compound influenced the values of MBC upon MIC in each extract.

In conclusion, the phytochemical screening of banana, cassava, and pineapple peel extract showed the presence of The MIC value flavonoids, alkaloids, terpenoids, tannins, and saponins. The MIC values of banana, cassava, and pineapple peel extract were 600, 400, and 200 µg/mL, respectively. The MBC values of banana, cassava, and pineapple peel extract are 4 × MIC, 2 × MIC, and 1 × MIC, respectively. The extracts of pineapple peel exhibited better antibacterial potential than banana and cassava peel extract. The three waste extracts indicated antibacterial activity that might be used as natural bactericides to control bacterial disease caused by *A. hydrophila* in fish.

ACKNOWLEDGMENTS

The PGB program from the university (Universitas Muhammadiyah Purwokerto, Banyumas, Indonesia) funded this research through contract no: A.11-III/477-S.Pj./LPPM/XII/2021.

REFERENCES

Abeyta C, Kaysner CA, Wekell MM, Sullivan JJ, Stelma GN. 2019. Recovery of *Aeromonas hydrophila* from oysters implicated in an outbreak of foodborne illness. J Food Prot 49: 643-646. DOI: 10.3390/microorganisms7100450.

Ahmed AM, Hafez TA, Eissawy MM. 2018. Effect of banana peel extract on sensory and bacteriological quality of marinated beef. Arch Nutr Publ Health 1 (1): 1-11.

Amaza IB. 2021. Determination of proximate composition, amino acids, minerals and phytochemical profile of cassava (*Manihot esculenta*) peel from sweet cassava variety grown in Yobe State of North Eastern Nigeria. Niger J Anim Prod 48 (1): 124-134. DOI: 10.51791/njap.v48i1.2894.

Baquero F, Levin BR. 2021. Proximate and ultimate causes of the bactericidal action of antibiotics. Nat Rev Microbiol 19: 123-132. DOI: 10.1038/s41579-020-00443-1.

Bengtsson PJ, Kristiansson E, Larsson DGJ. 2018. Environmental factors influencing the development and spread of antibiotic resistance. Fems Microbiol Rev 42: 68-80. DOI: 10.1093/femsre/fux053.

Cao H, He S, Wang H, Hou S, Lu L, Yang. 2012. Bdellovibrios, potential biocontrol bacteria against pathogenic *aeromonas hydrophila*. Vet Microbiol 154: 413-418. DOI: 10.1016/J.VETMIC.2011.07.032.

Chabuck ZAG, Al-Charrakh AH, Hindi NKK, Hindi SKK. 2013. Antimicrobial effect of aqueous banana peel extract, Iraq. Pharm Sci 1: 73-75.

CLSI. 2020. Methods for Antimicrobial Broth Dilution and Disk Diffusion Susceptibility Testing of Bacteria Isolated from Aquatic Animals. 2nd ed. Clinical and Laboratory Standards Institute guideline VET03. Clinical and Laboratory Standards Institute, Pennsylvania, USA.

Cushnie TPT, Cushnie B, Lamb AJ. 2014. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Intl J Antimicrob Agent 44: 377-386. DOI: 10.1016/j.ijantimicag.2014.06.001.

Deng YT, Wu YL, Tan AP, Huang YP, Jiang L, Xue HJ, Wang WL, Luo L, Zhao F. 2014. Analysis of antimicrobial resistance genes in *Aeromonas* spp. isolated from cultured freshwater animals in China. Microb Drug Resist 20 (4): 350-356. DOI: 10.1089/mdr.2013.0068.

Dong S, Yang X, Zhao L, Zhang F, Houd Z, Xue P. 2020. Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Willd. husks against foodborne pathogenic bacteria. Ind Crops Prod 149: 1-14. DOI: 10.1016/j.indcrop.2020.112350.

El-Sharaby S, El-Ballal S, Ali M, El-Hady M. 2021. Molecular studies on *Aeromonas* species isolated from freshwater fishes collected from Delta Region, Egypt. J Curr Vet Res 3 (2): 8-15. DOI: 10.21608/JCR.2021.199409.

Farha AK, Yang QQ, Kim G, Li HB, Zhu F, Liu HY, Gan RY, Corke H. 2020. Tannins as an alternative to antibiotics. Food Biosci 38: 1-10. DOI: 10.1016/j.fbio.2020.100751.

Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. 2019. Antibacterial activity of flavonoids and their structure–activity relationship: An update review. Phytother Res 33: 13-44. DOI: 10.1002/ptr.6208.

Fraga-Corral M, García-Oliveira P, Pereira AG, Lourenço-Lopes C, Jimenez-Lopez C, Prieto MA, Simal-Gandara J. 2020. Review: Technological application of tannin based extracts. Molecules 25 (614): 1-27. DOI: 10.3390/molecules25030614.

Goudarzi M, Mehdipour M, Hajikhani B, Sadeghinejad S, Sadeghi-Nejad B. 2019. Antibacterial properties of *Citrus limon* and pineapple extracts on oral pathogenic bacteria (*Streptococcus mutans* and *Streptococcus sanguis*). Intl J Enteric Pathog 7 (3): 99-103. DOI: 10.15171/ijep.2019.21.

Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, Scherer R. 2019. Antibacterial activity of terpenes and terpenoids present in essential oils. Molecules 24 (13): 1-12. DOI: 10.3390/molecules24132471.

Gunwantrao BB, Bhausaheb SK, Ramrao BS, Subhash KS. 2016. Antimicrobial activity and phytochemical analysis of orange (*Citrus aurantium* L.) and pineapple *(Ananas comosus* L.) Merr. peel extract. Ann Phytomed 5 (2): 156-160. DOI: 10.21276/AP.2016.5.2.22.

Holetz FB, Pessini GL, Sanches NR, Cortez DAG, Nakamura CV, Filho BPD. 2002. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Memórias do Instituto Oswaldo Cruz 97 (7): 1027-1031. DOI: 10.1590/S0074-02762002000700017.

Isnansetyo A, Kamei Y. 2009. Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity of MC21-B, an antibacterial compound produced by the marine bacterium *Pseudoalteromonas phenolica* O-BC30^T. Intl J Antimicrob Agent 34 (2): 131-135. DOI: 10.1016/j.ijantimicag.2009.02.009.

Kapadia SP, Pudakalkatti PS, Shivanaikar S. 2015. Detection of antimicrobial activity of banana peel (*Musa paradisiaca* L.) on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*: An in vitro study. Contemp Clin Dent 6 (4): 496-499. DOI: 10.4103/0976-237X.169864.

Králová S, Staňková E, Sedláček I. 2016. Classification of *Aeromonas* spp. isolated from water and clinical sources and distribution of virulence genes. Folia Microbiol 61: 513-521. DOI: 10.1007/s12223-016-0464-9.

Krishna VV, Kumar GK, Pradeepa K, Kumar SSR. 2013. Antibacterial activity of ethanol extract of *Musa paradisiaca* cv. puttabale and *Musa acuminate* cv. Grand naine. Asian J Pharm Clin Res 6 (2): 169-172.

Kumari PS, Ranjitha R, Vidhya N. 2020. Revitalizing property of banana peel extracts by antioxidant activity and antibacterial activity against acne causing *Staphylococcus epidermidis*. Ann Phytomed 9 (2): 215-222. DOI: 10.21276/ap.2020.9.2.19.

Li F, Wang W, Zhu Z, Chen A, Du P, Wang R, Chen H, Hu Y, Li J, Kan B, Wang D. 2015. Distribution virulence-associated genes and antimicrobial resistance of *Aeromonas* isolates from diarrheal patients and water, China. J Infect 70: 600-608. DOI: 10.1016/j.jinf.2014.11.004.

Loon YK, Satari MH, Dewi W. 2018. Antibacterial effect of pineapple (*Ananas comosus*) extract towards *Staphylococcus aureus*. Padjajaran J Dent 30 (1): 1-6. DOI: 10.24198/pjd.vol30no1.16099. [Indonesian].

Lubaina AS, Renjith PR, Kumar P. 2019. Antibacterial potential of different extracts of pineapple peel against gram-positive and gram-negative bacterial strains. Asian J Pharm Pharmacol 5 (S1): 66-70. DOI: 10.31024/ajpp.2019.5.s1.5.

Lumowa SVT, Bardin S. 2018. Phytochemical test of kepok banana peel (*Musa paradisiaca* L.) natural ingredients as a vegetable pesticide has the potential to suppress insect attacks of short-lived plant pests. Jurnal Sains Kesehatan 1 (9): 465-469. DOI: 10.25026/jsk.v1i9.87. [Indonesian]

Mailafia S, Nabilah B, Olabode HOK. 2021. Phenotypic characterization of *Aeromonas hydrophila* isolates in fresh water fishes in FCT using Microbact™ GNB 24E identification kit. Open Access Libr J 8: 1-12. DOI: 10.4236/oalib.1107066.

Mulia DS. 2012. African Catfish Vaccination. Pustaka Pelajar, Yogyakarta. [Indonesian]

Mulia DS, Karim A, Purbomartono C, Isnansetyo A. 2022. Antibacterial activity of mangrove plant (*Avicennia marina*) to control *Aeromonas hydrophila* infection in African catfish (*Clarias gariepinus*). AACL Bioflux 15 (6): 2900-2909.

Odilia MR, Putri DTZA, Rosetyadewi AW, Wijayanti AD, Budiyanto A, Jadi AR, Pratama AM. 2022. Identification of antinutritional, antioxidant, and antimicrobial activity of plants that cause livestock poisoning in Bojonegoro Regency, Indonesia. Vet World 15: 2131-2140. DOI: 10.14202/vetworld.2022.2131-2140.

Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: An overview. J Nutr Sci 5: 1-15. DOI: 10.1017/jns.2016.41.

Peterson E, Kaur P. 2018. Antibiotic resistance mechanisms in bacteria: Relationship between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol 8: 1-21. DOI: 10.3389/fmicb.2018.02928.

Punbasayakul N, Samart K, Sudmee W. 2018. Antimicrobial activity of pineapple peel extract. Proceeding of Innovation of Functional Foods in Asia (IFFA) Conference. Phayao, Thailand, 24 April 2018.

Singh CR, Kathiresan K, Boopathy NS, Anandhan S, Govindan T. 2013. Evaluation of microbial potential of different colored banana peels. Intl J Preclin Pharm Res 4 (2): 62-64.

Stratev D, Odeyemi OA. 2016. Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food source: A mini-review. J Infect Publ Health 9: 535-544. DOI: 10.1016/j.jiph.2015.10.006.

Xie Y, Yang W, Tang F, Chen X, Ren L. 2015. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr Med Chem 22 (1):132-149. DOI: 10.2174/0929867321666140916113443.

Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. 2020. Advances in pharmacological activities of terpenoids. Nat Prod Commun 15 (3): 1-13. DOI: 10.1177/1934578X20903555.

Yazdanpanah-Goharrizi L, Rokhbakhsh-Zamin F, Zorriehzahra MJ, Kazemipour N, Kheirkhah B, 2020. Isolation, biochemical and molecular detection of *Aeromonas hydrophila* from cultured *Oncorhynchus mykiss*. Iran J Fish Sci 19 (5): 2422-2436. DOI: 10.22092/ijfs.2020.122060.