Antibacterial activity test of mahkota dewa leaf extract 
(*Phaleri amacocarpa*) against bacteria *Aeromonas hydrophilla* 
by in vitro

R A Sarendah¹, Sudarno²,³ and R Kusdarwati²

¹Aquaculture Study Program, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115, Indonesia
²Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine Universitas Airlangga, Surabaya 60115, Indonesia
³ Corresponding author: sudarno@fzk.unair.ac.id

Abstract. Disease motile aeromonas septicemia (MAS) caused by the bacterium *A. hydrophilla*, is a disease that can affect all species of freshwater fish in the tropics. The use of antibiotics for the treatment of bacterial diseases have long been used, but the use of antibiotics that are not controlled pathogens causing bacterial resistance. The purpose of this study to determine the effect and the optimum concentration of the mahkota dewa leaf extract against bacteria *A. hydrophilla*. The treatment in this study was the addition of the mahkota dewa leaf extract and DMSO with a variant concentration of 100 mg, 140 mg, 180 mg, 220 mg, 260 mg, positive control using antibiotics chloramphenicol and negative controls using DMSO. Research of mahkota dewa leaf extracts to inhibit the growth of *Aeromonas hydropilla*. This is indicated resources by the formation of an inhibition zone at all concentrations. The formation of inhibition zone of the mahkota dewa against *A. hydrophilla* leaf extract shows that the mahkota dewa leaf extract can inhibit the growth of bacteria.

1. Introduction
Construction of the current fisheries this directs the development of aquaculture-based industries because of reduced catches of public waters semaki day while the market demand is increasing. Fish needs for the community are critical if freshwater fisheries businesses should be encouraged to develop [1, 2, 3, 4]. Utilization of fishery resources includes utilization for public consumption, the development of science, and the development of freshwater aquaculture enterprises [5, 6, 7]. The fish farming production in 2015 was 29% of tilapia, catfish 20%, and 18% milk. Indonesian Marine and Fisheries Ministry set a growth target of the fish production by 2.4% to 6.45 million tons in 2016, while the growth of farmed fish production target of 8.72% to 19.5 million tonnes [8].

Commodity type of freshwater fish that can be cultured is a goldfish, carp, catfish, Arowana, indigo, Tawes, catfish, and eel [9]. The main problem in the cultivation of failure that occurred along with an increase in the role of aquaculture sector is pests and diseases [10, 11, 12, 13]. Motile aeromonas septicemia (MAS) caused by the bacterium *A. hydrophilla*, is a disease that can affect all species of freshwater fish in the tropics. Hydrophilla *Aeromonas* disease attack while declining fish body condition due to stress and deterioration of water quality [14]. The use of antibiotics for the treatment of bacterial diseases have long been used, but the uncontrolled use of antibiotics that cause
resistance to bacterial pathogens [15].

Use natural materials such as the results of biodiversity continue to increase in line with the development of traditional medicine or modern industry, pharmaceuticals, or cosmetics that use of medicinal plants as raw material. This increase is suspected because of the few aspects that support, among others, a tendency to return to nature (back to nature), side effects caused, population increases coupled with the supply of drugs that do not support, maintenance costs, as well as drug resistance to infectious diseases [17].

One of the medicinal plants of Indonesia popular today is the mahkota dewa (*Phaleria macrocarpa*) of the family Thymelaceae. Mahkota dewa classified as the herbaceous plant that grows from the lowlands to an altitude of 1200 meters above sea level [18]. Some of the survey results revealed leaves and rind of the mahkota dewa contain secondary metabolites such as flavonoids, saponins, and phenolic is widely used in the pharmaceutical industry [19]. Part of the plant is the most widely used are the leaves — the content of flavonoids, saponins, and phenolic potential as an antibacterial [20]. In connection with the indication that the mahkota dewa leaves have antibacterial activity, it is necessary to research the antibacterial activity corolla power of the Mahkota Dewa in inhibiting the growth of pathogenic bacteria *Aeromonas hydrophilla*.

Antibacterial of mahkota dewa is extracted using ultrasonic methods. Ultrasonic methods or commonly known as the ultrasound-assisted extraction (UAE) is a faster extraction technique, consume less energy, and allow a reduction in the solvent to produce a pure product is higher. Ultrasonic method has been applied to mengesktrak antioxidants, pigments and antibacterial [21].

Based on the above then in this study is expected to mahkota dewa leaf extract can demonstrate antibacterial activity to inhibit the growth and the inhibition of the extract is known petals *Aeromonas hydrophilla* growth in vitro.

2. Material and methods

Sterilization aims to clean or free up equipment and materials from microorganisms. The tools will be sterilized washed first using detergent after it is dried. Petri dish wrapped in paper, test tubes covered with cotton and tie then put in an autoclave at 121°C and a pressure of 15 psi (1.02 atm) for 15 minutes to be useful for the sterilization process [22].

2.1. Mahkota dewa

Leaf fresh mahkota dewa sorted and washed with water to clean, then dried with air. Mahkota dewa dried leaves in the open air (dry air) without direct sunlight to avoid damage to the active ingredient contained in the leaves. Mahkota dewa dried leaves the ground with a dry blender. Mahkota dewa leaf powder weighed 50 grams and put into two erlenmeyers 500 ml each 25 grams.

Way work extraction using ultrasonic bath extraction is done three times. The first extraction of material diluted with 96% ethanol 250 ml in each flask for 6 minutes. Every two minutes stirring, then filtering using a vacuum pump. Extraction of both the first residue is dissolved in ethanol 96% as much as 125 ml in each flask for 6 minutes. Every two minutes stirring, then filtering using a vacuum pump. The third extraction residue results both diluted with 96% ethanol as much as 125 ml in each flask for 6 minutes. Every two minutes stirring, then filtering using a vacuum pump.

2.2. Manufacture media

Research media tryptic soy agar (TSA) 2%. tryptic soy agar (TSA) to grow a variety of microorganisms [23]. TSA a universal culture media were used for isolation and cultivation of a wide variety of aerobic microorganisms. This medium is used for a variety of purposes that include maintaining the stock of cultivation, the isolation of various species of microorganisms, as well as a base for media including blood. TSA media weighed as much as 8 grams and dissolved in 200 ml of distilled water in a flask and heated on a hot plate while stirring using a magnetic stirrer media subsequently cooled. Erlenmeyer covered with cotton wrapped in aluminum foil and then flask sterilized using an autoclave at a temperature of 121°C for 15 minutes.
2.3. Manufacture the suspension solution
Manufacture suspension is done by taking 1 to 2 loopful bacterial colonies *A. hydrhopilla* which entered into 0.9% NaCl solution further homogenized by vortex and adjusted to the turbidity of 0.5 McFarland standard [25]. Measurement of the turbidity standards can be done using a spectrophotometer with a wavelength of 625 nm [26]. Bacterial count one by one and to estimate the density of cells to be used in antimicrobial testing procedures. When the turbidity of the suspension does not match the turbidity standard solution that can be added colony *A. hydhrophilla* on suspense or dilute it by adding NaCl 0.9% [24].

2.4. Planting bacteria
Planting bacteria use a spread plate method (spread plate). 1 ml bacterial suspension with a cell density of 106 cells/ml put in a petri dish containing TSA media 2% [24].

2.5. Test leaf extract antibacterial activity mahkota dewa
Paper disc that has dripped a solution of the mahkota dewa leaf extract, DMSO, and antibiotics kloram fenikol 5 μg/ml place it on the surface of the bacteria media using tweezers and pressed a little. Bacteria media that is mixed antibacterial ingredient incubated at 37°C for 18-24 hours. A preliminary reading can be done after 6-8 hours. Clear area around the paper disc showed no bacterial growth. The diameter of inhibition zone formed was measured using calipers [24]. The use of calipers for its accuracy could reach one-hundredth of a millimeter.

3. Results and discussion
Research was conducted in the laboratory Institute of Tropical Disease, Universitas Airlangga, Surabaya. Extraction corolla god conducted using an ultrasonic bath. Leaf powder mahkota dewa who weighed as much as 50 grams of 40.16 grams is obtained. Mahkota dewa leaf extract especially dry concentrated extract of dried form. Extracts were placed in petridishes were sealed using aluminum foil and stored in a refrigerator at a temperature of 2-8°C.

The first petridish is divided into four regions, namely R1, R2, R3, R4, and second Petridish divided into three regions, namely R5, positive control, and negative control. Petridisk TSA media containing bacterial suspension with several 106 CFU/mL of the surfaces in order to evenly and allowed to dry about 1 hour. A positive control using the antibiotic chloramphenicol 5 mg dissolved 2 ml of DMSO, dried, and then placed into petri dish while using the solvent DMSO negative control without extract mahkota dewa. Mahkota dewa leaf extract as much as 50 mL is dripped onto each paper disc for each concentration and then incubated at 37°C for 18-24 hours [27]. [28] states that the clear zone around the paper is an indication of the sensitivity of bacteria to antibacterial agents used as the test material. Inhibition zone formed around the paper measured the diameter of the vertical and horizontal diameter in mm using a caliper. Media that has been incubated in diameter measured using a caliper in millimeters. Zonahambat diameter is calculated by the formula and then put on the table the observations.
The result mahkota dewa leaf extract showed inhibition zone against bacteria *A. hydrhopilla*. The results showed an increase in the diameter of zone of inhibition against the bacteria Aeromonas hydrhopilla at each concentration. The inhibition zone resulting from each treatment have different diameters and irregular shapes so that the observations were made by measuring the diameter of the horizontal and vertical diameter of the zone formed around the paper. The amount of inhibition zone mahkota dewa leaf extract produced by each concentration further categorized based on the classification of bacterial growth inhibition responses. Inhibition zone <5 mm is categorized weak inhibitory zone 5-10 mm is average, inhibition zone 10-20 mm categorized as active, and inhibition zone> 20 mm is categorized as very powerful [29].

The test method antibacterial activity against bacteria *A. hydhrophilla* agar diffusion method because the method is relatively simple, and the results are conscientious enough to determine their antibacterial activity. Factors that influence the antibacterial activity test using agar diffusion method is the speed of diffusion and the different substances and the differences in the response of microbes to the substances tested, and this led to the diameter of the resulting resistor. The test results showed that the leaf extract could inhibit the growth of mahkota dewa of *A. hydhrophilla*.

Testing activity Antibacterial has done in duplicate. Data were the average diameter zone of inhibition of bacteria by using mahkota dewa leaf extract is between 3.5 to 19.86 mm. The diameter of the smallest inhibitory zone obtained in the mahkota dewa leaf extract concentration of 100 mg and the largest is the concentration of extract 260 mg, while the optimum treatment which results in inhibition zone is higher than the positive control that is giving 220 mg ektara concentration which can be seen in Figure 5.3. Nevertheless, the antibiotic chloramphenicol is more effective than the mahkota dewa leaf extract because kloram dose uses smaller fenikol able to produce inhibition zone, which is almost equal to the optimum dose of the extract.

According to [30], chloramphenicol is a broad-spectrum antibiotic that can inhibit the growth of gram-positive bacteria or gram-negative. This is confirmed the opinion of the [31] states that antibiotics are derived from zatsama partly or wholly made by chemical synthesis by means of chemical synthesis in which low concentrations able to inhibit and even kill microorganisms. Antibiotic chloramphenicol is used as a positive control for testing antibacterial activity.

The treatment shows that the higher given concentration, the greater the inhibition zone diameters obtained, meaning antibacterial activity of the mahkota dewa leaf extract increases with increasing
5

concentration of the extract. The bacteria will be killed more quickly if a higher concentration of antibacterial substances. According to [29] if the diameter of inhibition zone formed greater than 6 mm, then extracts considered to have antibacterial activity and if the diameter is smaller than 6 mm or not formed then the extract is categorized as not having antibacterial activity or weak. In Figure 5.3. Showed inhibition zone produced more significant than 6 mm starting at a concentration of 140 mg, it can be concluded that the mahkota dewa leaf extract at a concentration of 140 mg to 260 mg have antibacterial activity against bacteria \textit{A. hydhrophilla}.

This formation inhibition zone due to the antibacterial substances is drawn into the extract. The results support the results of the study [32], which states that the mahkota dewa leaves contain some antibacterial compounds such as flavonoids, saponins danfenolik. The notion that flavonoids are supported by studies of antibacterial compounds [33]. Effects of flavonoids as antibacterial microorganisms which interfere with cell function and inhibition of bacterial cell cycle. Saponins work as an antibacterial by damaging the cytoplasmic membrane and kill the cell. [34] added chemical assay phenolic compounds in the Mahkota Dewa have been proven to provide pure chemical compounds which possess antibacterial activity. Each concentration gives a different diameter inhibition zone, the negative control in researchThis does not give a zone of inhibition. This shows that DMSO is used for the negative control has no antibacterial activity. According to [35], DMSO also did not leave the inhibition of the growth of bacteria, so it does not interfere with observations of antibacterial activity test by the method in order.

4. Conclusion
The results of research have shown that extracts of mahkota dewa have an inhibitory effect on the growth of \textit{A. Hydhrophilla}. Inhibitory resulting the optimum concentration of 220 mg is indicated by and categorized as strong antibacterial agents to inhibit the growth of bacteria \textit{Aeromonas hydrhopilla}.

5. References
[1] Ramadan R and Sari L A 2018 \textit{J. Aqua. Fish Health} 7, 124-132
[2] Son E M, Mahasri G and Sari L A 2018 \textit{J. Aqua. Fish Health} 7, 111-117
[3] Mukti A T, Arief M, Sari L A, Dewi N N and Rahayu A P 2019 \textit{Grouper} 10, 11-17
[4] Murtidjo B A 2005 \textit{Some Methods Fresh Water Fish Hatchery Doubleday}
[5] Ardyanti R, Nindarwi D D, Sari L A and Sari P D W 2018 \textit{J. Aqua. Fish Health} 7, 84-89
[6] Masithah E D, Choiyirah N and Prayogo 2011 \textit{J. Ilmiah Perikanan dan Kelautan} 3, 97-102
[7] Ranur L K and Pratastik S B 2010 \textit{J. Fish. Mar. Resour}. 4
[8] Statistics and Information Center of the Ministry of Marine and Fisheries 2016
[9] Sukadi 2002 \textit{J. Ikhtiologi Indonesia} 2, 61-66
[10] Artha O A, Sudarno, Pramono H and Sari L A 2019 \textit{IOP Conf. Series: Earth Environ. Sci.} 236, 012 003
[11] Quarantine Submit Batam Authority 2007 \textit{Report on Monitoring Pests and Diseases of Fish Quarantine} (Batam: Quarantine Batam Authority)
[12] Dahlia, Suprapto H and Kusdarwati R 2017 \textit{J. Aqua. Fish Health} 6, 57-66
[13] Pahlawi I H, Satyantini W H and Sudarno 2019 \textit{J. Aqua. Fish Health} 8, 92-98
[14] Sarono A, Kamiso K H, Lelono I W Y B, Widodo, Thaib N, Haryani E B S, Hariyanto S, Triyanto, Ustadi, Kusumahati A N, Novianti W, Wardani and Setaningsih S 1993 \textit{In Quarantine Pest and Disease Bacteria Group Book II} Cooperation Center for Agricultural Quarantine and the Faculty of Agriculture (Yogyakarta: UGM)
[15] Sumayani, Kusdarwati R and Cahyoko Y 2018 \textit{J. Ilmiah Perikanan dan Kelautan} 3,
[16] Wahjuningrum, Figures S L, Lesmanawati W, Sa'diyah and Yuhana M 2007 \textit{J. Aqua. Indonesia} 6, 109-117
[17] Great Hall Postharvest Research and Development of Agriculture 2007 \textit{Postharvest Technology} (Bandung: Medicinal Plants)
[18] Burkill I H 1966 \textit{A Dictionary of the Economic Products of the Malay Peninsula} (Kuala
Lumpur: Ministry of Agriculture and Co-Operatives)
[19] Ganga E H, Asriani and Novita L 2007 *J. Pharmaceutical Sci.* Indonesia, 17-22
[20] Vinatoru M 2001 *Sonochemistry* 8, 301-313
[21] Lou Z, Wang H, Li J, Zhu S, Lu W and Ma C 2011 *J. Medic. Plants Res.* 5, 5370-5377
[22] University of Ottawa Environmental Health and Safety Service 2003 *A guideline for The Safe Us of Autoclaves* (Ottawa: University of Ottawa) pp 1-24
[23] Acumedia Subsidiary of Neogen Corporation 2010 *Muller Hinton Agar* (7164) (Acumedia Manufacturers Inc) pp 1-3
[24] Barus W N U 2013 *Leaf Extract Antibacterial Effectiveness Tests Cambodia (Plumiera rubra) on Different concentrations of Aeromonas hydrophilla to Growth In Vitro Essay* Faculty of Agriculture (Sumatera: University of Northern Sumatra)
[25] Whitman K A 2004 *Finfish and Shellfish Bacteriology Manual Techniques and Procedures* (Lowa: Lowa State University Press) p 366
[26] Sutton S 2011 *Test. Summer* 15,
[27] Sudarwanto R and Wientarsih 2015 *J. Vet. Med. Se.* 9, 187
[28] Toy T S, Lampus B and Hutagalung B 2015 *E-Dental J.* 3, 153-159
[29] Greenwood 1995 *Antibiotics Susceptibility (Sensitivity) Test Antimicrobial and Chemoterapy Addison Westley* (San Fransisco: Longman Inc)
[30] Katzung B G 2004 *Basic Pharmacologyand Clinic Book 3 Edition 8* translators and editors: *Section of Pharmacology* Faculty of Medicine UNAIR (Surabaya: Publisher Salemba Medika) pp 37-41
[31] Performan M 2001 *Broiler Chickens Are Given Antibiotics Bacitracin Zinc, Probiotic Bacillus sp. and Various Levels Saccharomyces cerevisiae in the Rations Essay* Department of Animal Nutrition and Food Sciences Faculty of Animal Husbandry (Bogor: Bogor Agricultural University)
[32] Christien, Yunasi H and Ezraneti R 2013 *Effectiveness of Leaf Extract Mahkota Dewa (Phaleria macrocarpa) As Antibacterials To Prevent Attacks Bacteria Aeromonas hydrophilla in Gurami (Osphronemus gouramy)* Faculty of Agriculture (Sumatera: University of Northern Sumatra)
[33] Robinson T 1995 *High Content of Organic Materials Plant* (bandung: Bandung Institute of Technology)
[34] Lisdawati V, Sumali W and Broto S K 2006 *J. Nature Materials* 1, 303-309
[35] Hand, Marlina D M and Meilan 2016 *Screening of Antibacterial Activity of Some Marine Life Aquatic Painan Beach, West Sumatra* Faculty of Pharmacy (Padang: Andalas University)

Acknowledgement
The authors gratefully acknowledge the financial support from the Annual Budget of the Faculty of Fisheries as well as the instrument support.