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Change of hematology and histopathology of common Carp (*Cyprinus carpio* L.) Infected by *Pseudomonas flourescens* by natural bioactive treatment of Rosella Petals (*Hibiscus sabdariffa* L.)

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ABSTRACT

Common Carp (*Cyprinus carpio*) is a freshwater fish commodity that has high economic value. The presence of disease attacks on carp farming, such as *Pseudomonas flourescens* bacteria, can cause economic losses in aquaculture. Many attempts were made to prevent and treat the attack of the bacterium *P. fluorescens*, one of which used natural extract of Rosella Petals (*Hibiscus sabdariffa* L.). Some information about the ability of Rosella Petals (*H. sabdariffa* L.) as an antibacterial states that this plant is able to inhibit the growth of both gram positive and gram negative bacteria. But, there is no information yet about the ability of Rosella Petals (*H. sabdariffa* L.) to inhibit the growth of *P. fluorescens* bacteria and their activity as a treatment for Common Carp (C. carpio). This research was conducted from August 2018 to March 2019. The stages of this study were divided into two, the first stage being co-culture tests, discs and characterization of compounds (phytochemicals, FTIR, UV-VIS). The second step is the application of Rosella Petals extract (*H. sabdariffa* L.) to Common Carp (*C. carpio*) after being infected with *P. fluorescens* with a density of 2.35 x 107 CFU / ml. Doses used in the second phase of the study were 75, 150, 225, 300 and 375 mg / L with 3 replications and 2 controls. The results of this study showed that Rosella Petals extract (*H. sabdariffa* L.) was able to inhibit the growth of *P. fluorescens* bacteria at doses of 100, 200, 300, 400 and 500 mg / L respectively at 7.12; 9.03; 11.53; 13.25 and 15.02 mm. The results of phytochemical tests, FTIR and UV-VIS showed that the Rosella Petals methanol extract contained phenolic compounds, namely flavonoids (flavonols, anthocyanins) and tannins. Rosella Petals extract (*H. sabdariffa* L.) was able to repair tissue in the histopathology of fish gills after being infected with the *P. fluorescens* bacteria significantly ($P < 0.05$) with the best dose in the treatment was 300 mg / L.

Key words : *Antibacterial, Treatment, Phytochemistry, FTIR, UV-VIS, Discs, Co-cultur*

Introduction

One of the diseases caused by bacteria is red disease caused by *P. flourescens*. This bacterium is one of the most dominant components of freshwater ecosystems. *P. fluorescens* can cause disease in various fish species. *P. fluorescens* often infects rotten fins or tails.

Excessive treatment with antibiotics can cause resistance to pathogenic microorganisms and accumulate in fish and their environment. One of the efforts made to treat bacterial diseases is to use natural bio-actives which have anti-bacterial abilities. Natural bio-actives that can be used are Rosella Petals (*H. sabdariffa* L) (Lengka *et al.,* 2013).

Roselle Petals (*H. sabdariffa* L.) contains active compounds of anthocyanin and flavonoid (flavonol, cha-techin, proanthocyanidine) (Faturrohman and Indrayudha, 2012). Flavonoid compounds can kill or inhibit bacterial growth due to the formation of flavonoid complexes with certain structures in bacterial cell walls, such as adhesin, polypeptides and enzymes (Cowan, 1999). Although studies of Rosella Petals (H. *sabdariffa* L.) extract as an antibacterial have been carried out, its use in the treatment of fish has never been done.

Based on this description, it is necessary to conduct research and studies on the content of active compounds contained in Rosella Petals (*H. sabdariffa* L.) as an antibacterial, as well as its application as a drug candidate to treat Common Carp (*C. carpio*) infected with *P. fluorescens* bacteria by using hematological observations as well as histopathology of the kidney organs in Common Carp (*C. carpio*).

Research Methods

This research was conducted in August to December 2019 using the experimental method, with a completely randomized design of 5 treatments and 3 replications and 2 control treatments. The study was conducted at the Fish Culture Laboratory of the Fish Disease and Health Division, Universitas Brawijaya. The stages of this study were divided into two, the first stage being co-culture tests, discs, and compound characterization. The second step was extract toxicity test, bacterial pathogenicity test and application of Rosella Petals (*H. sabdariffa* L.) extract on Common Carp (*C. carpio*) after bacterial infection *P. fluorescens* with a density of 2.35 x 1010 cells / ml. The parameters observed were hematocrit, hemoglobin, erythrocytes, leukocytes, differential leukocytes, renal histopathology, clinical symptoms, survival and water quality.

STAGE I

Antibacterial Test

The antibacterial test was carried out in two stages, namely co-culture and discs. Co-culture was carried out using doses of 1, 10, 100, 500 and 1000 mg/L, K (+) using chloramphenicol and K (-) using DMSO. Extract and bacteria density 2.35 x 10^{10} cells / ml mixed in the test tube. After 24 hours, the results are then planted in a Petri dish using PCA media. After 24 hours, the bacterial density was calculated

Disc tests are carried out using doses that refer to the results of co-culture. Petri dishes containing TSA media were planted by bacteria and put discs soaked in the extract at a dose of 75, 150, 225, 300, and 375 mg/L for comparison, K (+) using chloramphenicol and K (-) using DMSO. After 24 hours, the inhibition zone is formed.

Characterization of Secondary Metabolites

Phytochemical screening tests were carried out to determine the content of phenolic compounds, flavonoids, alkaloids, and steroids/triterpenoids found in Rosella Petals (*H. sabdariffa* L.). While the method used refers to the method of Harborne (2006).

UV-Vis was performed using UV-Visible spectrophotometry (Cary50 Conc Variant). Rosella Petals (*H. sabdariffa* L.) extract was centrifuged at 300 rpm for 10 minutes. then diluted using ethanol 1:10, then examined under UV light wavelengths 200-500 nm. FTIR test was performed by extracting Rosella Petals (*H. sabdariffa* L.) added with KBr (Potassium bromide) in a mortar using a ratio of 1:200. Then the sample and KBr are pressed with a pressure of 6 bar for 2 minutes until they become like pellets. The sample pellet is then placed inside the FTIR spectrophotometric instrument (FT1000 Variant).

STAGE II

Bacterial Pathogenicity Test

The pathogenicity test is used to find out how much density the *P. fluorescens* bacteria needs to produce 50% of test animal deaths within a certain time period. The initial preparation, ie *P. fluorescens*, with 2.35 x 10^{10} cell/mL (based on OD data), was diluted into different kinds of concentrations starting from 108; 107, 106 and 105 cells / mL in TSB media. After treatment, time and the number of dead fish were recorded. Data were then analyzed using Probhit Analysis with SPSS.

Extract Toxicity Test

The extract toxicity test was carried out to obtain concentrations of rosella flower extract (*H. sabdariffa* L.) which is estimated to cause a death effect of 50% on the test animal population after 48 hours of treatment. The initial step is 10 Common Carp (*C. carpio*) acclimatized into an aquarium container for 7 days

and fed with pellets. After that the extract is immersed with a dose taken from the results of stage one in vitro on the disc test by observing the inhibition zone. After the immersion extract treatment, changes in behavior were observed and the time and number of fish killed were recorded up to 50%. The data is then analyzed using EPA Probhit Analysis.

Observation Parameters

1. Kidney histopathology

The first thing to do is that the kidney organ that has been taken is inserted into an eppendorf tube then added with a 10% formalin solution (fixation stage). Dadiono (2017) states that in the dehydration stage water withdrawals are carried out for 20 hours. After that the clearing step is to replace the alcohol solution from the tissue. Furthermore, the impregnation stage aims to equate tissue with embedding material. Embedding stage (blocking) to facilitate incision with the microtome. The best incision results are stained using Haematoxylin-Eosin. Histopathological damage analysis of Common Carp (*C. carpio*) kidney organs was performed by scoring. Scoring damage that occurs includes degeneration, congestion and necrosis

Results and Discussion

Stage I

1. Antibacterial Activity

Co-culture Test

The results of the co-culture test are presented in Table 1.

The results of observing the number of bacterial colonies in the table shows the minimum concentration of extracts of Rosella Petals (*H. sabdariffa* L.) which can inhibit the growth of *P. fluorescens* is 100 mg/L in methanol and 500 mg/L in ethyl acetate solvent. Determination of the value is based on the lowest concentration of the extract which is known to be able to inhibit or reduce bacterial growth.

The conclusion from the co-culture test is that the number of bacterial colonies decreases with increasing concentrations given both the solvents. However, the methanol extract of Rosella Petals (*H. sabdariffa* L.) was proven to be able to inhibit the growth of *P. fluorescens* at lower concentrations compared to the ethyl acetate extract of Rosella Petals (*H. sabdariffa* L.). So that the methanol extract of Rosella Petals (*H. sabdariffa* L.) was continued for the disk test dose.

Disc Test

The results disc test of the Rosella Petals (*H. sabdariffa* L.) extract are presented in Table 2 below.

Table 2. Disc test result

Concentration	Solvent Methanol Inhibition Zone	Response Classification (Greenwood and Slack, 1995)
K(-)	0±0,0[a]	Lemah
100	7,12±0,0[b]	Sedang
200	9,03±0,2[c]	Sedang
300	11,53±0,8[d]	Kuat
400	13,25±0,5[e]	Kuat
500	15,02±0,3[f]	Kuat
K(+)	21,47±0,7[g]	Kuat

Anova (Analysis of varience) results in the disc test showed that the inhibition zone diameter value formed after the addition of rosella Patels extract (*H. sabdariffa* L.) was significant (P <0.05). Based on Duncan's test results showed that at a concentration of 500 mg / L gave the best average value of 15.02

Table 1. Co-culture test

Concentration	Methanol		Ethanol Acetate	
	Absorbance	Σ Bacterial Colony (cfu/ml)	Absorbance	Σ Bacterial Colony (cfu/ml)
K (-)	1,047	$2{,}70.10^{18}$	1,073	$3{,}88.10^{19}$
1	1,018	$2{,}66.10^{18}$	1,024	$3{,}73.10^{19}$
10	0,142	$1{,}85.10^{14}$	0,298	$1{,}96.10^{15}$
100	0,128	$5{,}3.10^{8}$	0,280	$1{,}65.10^{11}$
500	0,136	$1{,}08.10^{9}$	0,222	$1{,}62.10^{9}$
1000	0,130	$8{,}6.10^{8}$	0,276	$2{,}19.10^{9}$
K(+)	0,084	0	0,090	0

mm, followed by concentrations of 400, 300, 200, and 100 mg / L.

Compound Characterization

a. Phytochemical Test

Based on the results of phytochemical tests, Rosella Petals (*H. sabdariffa* L.) contain alkaloids, flavonoids, saponins, triterpenoids, steroids and tannins. This shows that all the compounds tested were contained in the extract of Rosella Petals (*H. sabdariffa* L.) with methanol as a solvent. This is because methanol is a universal solvent that has polar (-OH) and nonpolar ($-CH_3$) groups so that it can attract polar and nonpolar analytes. This is supported by research by Lestari *et al.* (2014) who also discovered the existence of secondary metabolite compounds found in Rosella Petals.

Table 3. Phytochemical Test Results

No.	Compound Identification	Result
1.	Alkaloid	
	*Dragendrof	+
	*Mayer	+ +
2.	Flavonoid	+ + +
3.	Saponin	+
4.	Terpenoid	+
	*Triterpenoid	+
	*Steroid	+
5.	Tanin	+

b. FTIR test

The results of the FTIR test obtained 8 compound uptake.

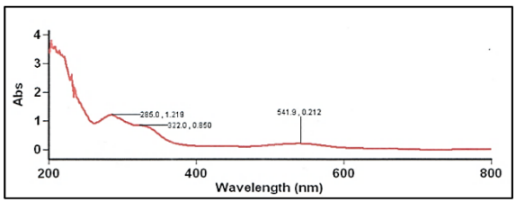
Based on these data it is suspected that the results of the isolate are phenolic compounds where the benzene group binds to an OH group with a broad and sharp incidence with absorption in the region of wave numbers 3419.71 cm^{-1} and 1384.12 cm^{-1}. And strengthened OH, C = C, C = O, C-H aromatic functional groups (Ardila *et al.*, 2017; Hastuti, 2012; Ershad *et al.*, 2017). Phenolic compounds in nature are very broad, have a wide variety of structures and are easily found in all plants, leaves, flowers and fruit (Tiwari *et al.*, 2011).

Table 4. FTIR test Result

No.	Wave Number	Functional groups
1.	3419.71	O – H alcohol
2.	2928.91	C – H alkane
3.	1743.03	C = O aldehid
4.	1630.18	C = C aromatic
5.	1384.12	C – H aliphatic
6.	1228.37	C – O alcohol
7.	1065.59	C – O alcohol
8.	523.34	C – H aromatic

c. UV-VIS Test

UV-VIS methanol extract of Rosella Patels (*H. sabdariffa* L.) is presented in Figure 1.



UV-VIS test results of Rosella Petals (*H. sabdariffa* L.) extract showed maximum wavelengths of 285 nm and 322 nm with absorbance valuesof 1.219 and 0.850. Both values are strongly suspected that there are flavonoid compounds which are the largest phenol groups. Then the next highest wavelength is 541.9 nm with an absorbance value of 0.212. This value is thought to be included in the anthocyanin type flavonoid. This anthocyanin is a natural pigment from red rosella calyx (Hastuti, 2012). This is consistent with the statement of Sam *et al.* (2016) that the content of rosella flower petal compounds in the form of flavonoids consisting of flanonol and anthocyanin pigments.

STAGE II

a. Bacterial Pathogenicity Test

Bacterial pathogenicity test results are presented in Table 6. Based on the analysis using EPA Probhit Analysis obtained values of 1.0 x 10^7 CFU/ml means that the concentration of 107 CFU/ml can cause 50% mortality for 48 hours. Carp as a virulence test media and also one of the *P. fluorescens* host cells because in the fish body bacteria get an environment with sufficient pH, temperature, and nutrients and can multiply themselves. Increased virulence of *P. fluorescens* bacteria was carried out aimed at increasing the pathogenicity and virulence of bacteria, so that *P. fluorescens* infection in test fish became optimal. The higher the dose of *P. fluorescens* bacteria used, the higher the mortality rate of fish (Mangunwardoyo *et al.*, 2010; Putra *et al.*, 2015).

Table 5. Bacterial Pathogenicity Test

Hour	Mortality (%)			
	10^5	10^6	10^7	10^8
12	-	-	-	1
24	-	1	1	2
36	1	1	2	2
48	-	1	2	2
Mortality (%)	10	30	50	70

b. Toxicity Test

The extract dose used in this toxicity test was 100, 200, 300, 400, 500 mg / L for 48 hours. The results of the toxicity test of rosella calyx extract (*H. sabdariffa* L.) are presented in **Table 6**. Based on the analysis using EPA Probhit Analysis the value of 308 mg / L was obtained, according to the dosage of 308 mg / L of rosella calyx extract (*H. sabdariffa* L.)) can pass 50% mortality for 48 hours.

Table 6. Extract Toxicity Test

Hour	Dose				
	100	200	300	400	500
12	-	-	-	2	2
24	-	-	1	1	2
36	-	1	1	2	3
48	1	2	2	2	1
Mortality (%)	10	30	50	70	80

Observation Parameters

1. Gill Analysis

Healthy goldfish gills, after being infected and after administration of the extract visually are presented in Figure 2. Damage that occurred in the histopathology of fish gills from the K (-) treatment appeared to have severe damage due to *P. fluorescens*

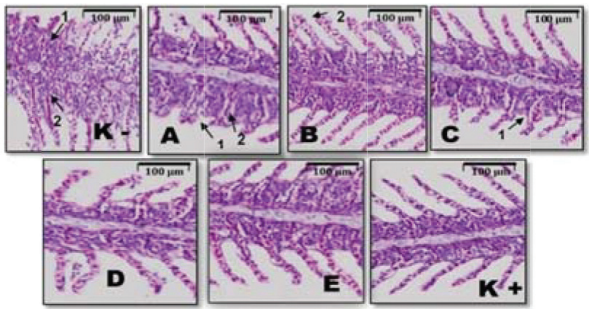


Fig. 2. Fish Gill Histophatology, A. Doses 75 mg/L, B. Doses 150 mg/L, C. Doses 225 mg/L, D. Doses 300 mg/L, E. Doses 375 and K (-) dan K (+);(1) Hyperplasia, (2) Fusion. 400x magnification

bacterial infection, then after being given extract treatment namely from treatment A (75 mg / L) decreases until treatment D (300 mg / L). However, in treatment E (375 mg / L) histopathology of fish gills appeared to have increased damage on a small scale. This is consistent with the results of the toxicity test of extracts of rosella flower petals (*H. sabdariffa* L.), where the higher dose given can cause toxicity to the body of carp (C. carpio). Whereas in the treatment of C (+) gill tissue looks normal because there is no bacterial infection.

The results of scoring the damage that occurred in the goldfish gills (*C. carpio*) histopathology obtained heavy damage in the treatment C (-) that is 66.95% then the moderate damage in treatments A, B and C with total damage respectively was 36.31% , 31.35% and 26.22%, for treatment D and E obtained minor damage with total damage respectively 19.90% and 22.78%. Whereas the treatment of C (+) is included in the normal category (2%). Histopathological damage scoring results of carp (*C. carpio*) are classified based on Alifia (2013), which is 0 - 5% normal, mild damage with a value of 5-25%, moderate damage with a value of 26-50%, severe damage with a value of 51 - 75% and very heavy damage with a value of 76%. The average scoring results of carp *(C. carpio*) gill damage in this study are presented in Table 12.

Table 12. Average *Scoring* Result of carp *(C. carpio*) gill damage

Treatment (mg/L)	Hyperplasia	Fusion
0 (K-)	34,08 ± 1,88f	32,88 ± 1,82f
75	24,92 ± 0,78e	11,39 ± 0,26e
150	21,58 ± 0,92d	9,77 ± 0,26d
225	18,59 ± 0,39c	7,63 ± 0,33c
300	15,30 ± 0,91b	4,60 ± 0,29b
375	17,16 ± 0,13c	5,63 ± 0,33b
0 (K+)	1,00 ± 0,00a	1,00 ± 0,00a

Based on the Anova test (Analysis of variance), the results showed that damage to the gill tissue, namely hyperplasia and fusion, showed significant results ($P < 0.05$). The results of this study showed that the administration of Rosella flower extract (*H. sabdariffa* L.) was able to reduce the histopathological damage value of gills in carp infected with *P. fluorescens* with the lowest value at a treatment dose of 300 mg / L ie for hyperplasia damage (15.30 %) and fusion (4.60%).

The decline in the histopathological damage

value of goldfish gills that occurred is thought to be a result of the content of phenolic compounds such as flavonoids and tannins that are owned by extracts of Rosella Petals (*H. sabdariffa* L.) which function as antibacterial. In accordance with Cowan's statement, (1999), phenolic compounds have the ability to form certain complex structures in bacterial cell walls. Furthermore, with the number of hydroxyl groups in the phenolic ring there will be an increase in hydroxylation, and with an increase in hydroxylation, antibacterial activity will increase. The relationship between Rosella flower extract (*H. sabdariffa* L.) with histopathological damage of Carp gills (*C. carpio*) has a coefficient of determination (R2) on hyperplasia damage of 0.9246 and for fusion damage of 0.913.

Clinical Symptoms

Observation of clinical symptoms is done visually to observe the behavior, appetite and damage to fish organs from the outside in goldfish that have been infected with the *P. fluorescens* bacteria and after treatment with soaking Rosella Petals (*H. sabdariffa* L.) extract. The results of visual observations of abnormalities are presented in Table 13. Observation of Clinical Symptoms

Table 13. Observation of Clinical Symptoms

Treatment (mg/L)	Time	Repitition		
		1	2	3
0 (K-)	After Infected	ab	ac	acd
	After Treatment	abc	cd	acd
75	After Infected	a	abc	bcd
	After Treatment	e	ef	Fg
150	After Infected	bc	b	Abd
	After Treatment	fg	f	Ef
225	After Infected	ad	ab	Abc
	After Treatment	eg	ef	Efg
300	After Infected	ac	ad	C
	After Treatment	efgh	efgh	Efgh
375	After Infected	acd	d	Ad
	After Treatment	efgh	efgh	Efgh
0 (K+)	After Infected	h	h	H
	After Treatment	h	h	H

Note: (a): fish gasping at the surface of the water, decreased appetite and irregular swimming / near aeration; (b) pale body, excessive mucus condition, slightly protruding eyes; (c) peeling scales, broken fins, open operculum; (d) ulcers, inflammation, redness of the back, swelling in the abdomen; (e) swimming becomes active, appetite returns to normal; (f) body color returns to normal, operculum begins to improve; (g) the wound begins to heal, inflammation and swelling begin to disappear; h: normal fish.

Clinical symptoms found in the maintenance of goldfish infected with the *P. fluorescens* bacteria include decreased appetite, slow and irregular movements, pale body color, peeling scales, open operculum, appearance of ulcers, inflammation (inflammation), and swelling in the stomach. The appearance of wounds and redness / bleeding in the body of the fish is caused by a toxin namely hemolysin produced by *P. fluorescens* which acts to break down erythrocyte cells so that the cells come out of the blood vessels and cause redness on the surface of theskin.

In the observation of fish which after being treated with extracts of Rosella Petals (*H. sabdariffa* L.) there is healing in carp, changes in appetite, behavior, movement of fish and wound healing in the body. This is presumably due to the method of treatment that is done by immersion.

2. Water Quality

Water quality is one of the main keys that can affect the growth and survival of carp (*C. carpio*). Therefore, monitoring is needed so that the water quality parameters are maintained and controlled. The results of water quality measurements during the study are presented in Table.

Table 14. Water Quality Observation Result

Parameter	Observation Result	Optimal Range
Temperature (°C)	24 – 27	20 - 30 (Mantau and Sudarty, 2011)
pH	7 - 7,5	6,5 - 9 (Goran, *et al*., 2016)
DO (mg/L)	6 - 7,7	2,5 - 7,1 (Sulawesty *et al*., 2014)

Water temperature is very influential on the chemical and biological processes of fish. When the water temperature fluctuation is too high, it will cause the fish to become stressed and vulnerable to disease and when the relatively high temperature P. fluorescens bacteria are found in the waters. The optimum temperature of *P. fluorescens* bacterial infection of fish is 22–28 ° C, usually these bacteria will more easily attack fish with high temperature conditions so that the fish will experience stress and its resistance will decrease (Wahyuningrum *et al.*, 2013; Holmes *et al.*, 1996).

The degree of acidity can cause a disease in fish because at certain water pH the fish will experience stress and on the other hand some pathogenic organisms that are able to live at that pH will easily attack the fish. A low pH value can indirectly cause damage to the skin making infection easier. *P. fluorescens* bacteria can thrive in water with a pH range of 5-5.9 (Susanti, 2015).

Dissolved oxygen is a very important requirement for fish survival. According to Octaviana *et al.* (2015) dissolved oxygen content of less than 1 mg / l will kill fish and at 1-5 mg / l content is sufficient to support fish life but slow fish growth. While the oxygen content of more than 5 mg/ l fish growth is normal. *P. fluorescens* bacteria can attack fish with water conditions with low dissolved oxygen levels (Napitupulu *et al.*, 2017).

3. Survival Rate (SR)

The survival of carp (*C. carpio*) in this study is presented in Figure 3.

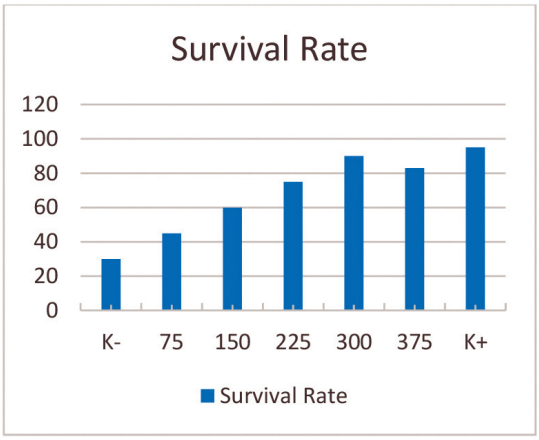


Fig. 3. Patterns of Fish's Survival Rate during Research.

From this figure, it is known that the administration of rosella flower extract *(H. sabdariffa* L.) had a significant effect (P <0.05).

Increased survival in treatments A, B, C, D and E when compared to K (-) is thought to be caused by secondary metabolite compounds contained in Rosella Petals (*H. sabdariffa* L.) extract. Duncan test results showed that treatment D with a dose of 300 mg / L gave the best survival rate that approached the normal control treatment that was equal to 91%, while for normal control treatment obtained a survival rate of 96%.

The high survival in treatment is thought to be related to water quality as a maintenance medium, because optimum water quality will result in a good survival rate. Improper handling can cause fish stress, so that the health condition of fish decreases and can cause death.

The low survival rate in the C (-) treatment is thought to be caused by an acute attack of the *P. fluorescens* bacteria. Fish are not optimal in their protection against infection, causing a low survival rate during maintenance after the challenge test with an increasing percentage of deaths.

Conclusion

1. Based on identification using phytochemical analysis, FTIR, and UV-VIS extract of rosella calyx (*H. sabdariffa* L) extract containing phenolic compounds namely flavonoids (flavonols, anthocyanins) and tannins.
2. Provision of rosella calyx extract (*H. sabdariffa* L) to carp (*C. carpio*) after infection with *P. fluorescens* has a significant effect (P <0.05) both on histopathology of goldfish (C. carpio) gills with the best dose in treatment D of 300 mg / L.

References

Alifia, F. 2013. Histopathology of Milkfish Gills (*Chanos chanos* Forskall) Tainted with Lead Metal (Pb). *J. Balik Diwa*. 4 (1) : 38-45.

Ardila, A. 2017. Herbal Nanoparticles Fabrication in Effervescent Tablet Using Solvent Methodemulsification Diffusion Combination High Speed Homogenizer, National Seminar on Science and Technology, Faculty of Engineering, University of Muhammadiyah Jakarta, (November). 2 (1): 1–2.

Barros, M.M., Falcon, D.R., Orsi, R.O., Pezzato, L.E., Fernandes, A.C., Guimaraes, I.G., Fernandes, A., Padovani, C. R. and Sartori, M. M. 2014. Non-specific Immune Parameters and Physiological Response of Nile tilapia fed B-glucan and Vitamin C for Different Periods and Submitted to Stress and Bacterial Challenge. Fish & Shellfish Immunology.

Cowan, M.M. 1999. Plant Products as Antimicrobial Agents. *Clin Microbiol Rev.* 12 (4): 564-582.

Dadiono, M. S. 2017. Characterization of Antibacterial Fractions and Antibacterial Activity of Binahong (*Anredera cordifolia* (Ten.) Steenis) Extracts against Dumbo Catfish (*Clarias sp.*) Infected by *Aeromonas salmonicida*. Thesis. Universitas Brawijaya. 138 pg.

Faturrohman, M. A. 2012. Antibacterial Activity of Rosella Petal (*Hibiscus sabdariffa* Linn) n-hexane Extract against *Propionibacterium acne* Sensitive, *Escherichia coli*, and *Staphylococcus aureus* Multiresisten. Faculty

of Pharmacy, Muhammadiyah University, Surakarta. 9 pages.

Goran, S. and Omar, S. 2016. Water Quality and Physiological Parameters of the Common Carp Fingerling Fed on Jerusalem artichoke Tubers. *Polytechnic.* 6 (3): 502-516.

Harikrishnan, R., Nisha, R.M. and Balasundaram, B. 2003. Hematological and Biochemical Parameters in Common carp, *Cyprinus carpio*, following herbal treatments for *Aeromonas hydrophila* infection. *Aquaculture.* 221 (1-4): 41-50.

Hartika R., Mustahal. Putra, A.N. 2014. Blood picture of tilapia (*Oreochromis niloticus*) with the addition of different prebiotic doses in feed. *J Fisheries and Marine.* 4 (4): 259-267.

Hastuti, N. D. 2012. Making functional drinks from honey and rosella extracts (*Hibiscus sabdariffa* Linn.). *Journal of Food Technology.* 3 (1) : 35.

Holmes, P., Niccofls, L. M. and Sartory, D.P. 1996. The Ecology of Mesophilic Aeromonas in the Aguatic Environment. In: B. Austin, M. Altwegg, P. Gosling & S.W. Joseph (Eds.) The Genus Aeromonas, John Wiley & Sons, New York, NY: 39-76.

Irsyad, M., Mappiratu and Rahim, A. 2017. Anthocyanin Production Covered by Maltodextrin from Rosella Flower Petals (*Hibiscus Sabdariffa* L.) and Its Applications in Functional Food Processing. *e-Journal of Science Partners.* 5 (1). 12–25.

Kamaludin, I. 2011. The Effectiveness of Aloe Vera Extract for the Treatment of *Aeromonas hydophila* Infection in Dumbo Catfish (*Clarias* sp) Through Feed. Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. Bogor. 54 Pg.

Lengka, K., Monoppo, H. and Kolopita, M.E.F. 2013. Increased response of non-specific lmun mas (*C. carpio*) through administration of garlic (*Allium sativum*). *Journal of Aquaculture.* 1 (2): 21-28.

Lestari, P. P., Kusrini, D. and Anam, K. 2014. Anthocyanin Identification of Methanol-HCl Extract Active Fraction in Rosella (*Hibiscus Sabdariffa.* L) and Its Potential as Xanthine Oxidase Inhibitors. *Journal of Science and Mathematics.* 22 (3): 72–78.

Lusiastuti, A. M. and Hardi, E. H. 2004. Blood picture as an indicator of health in freshwater fish. *Pros. Semin. Nas. Fish.* 12 (1): 65–69.

Lusiastuti, A. M., Septyan, A. and Samsudin, R. 2017. Effectiveness of microencapsulated probiotic combinations through feed for controlling Motile *Aeromonas Septicemia* in catfish, *Clarias gariepinus*. *Journal of Aquaculture Research.* 12 (2): 170-18.

Mangunwardoyo, W., Ismayasari, R. and Riani, E. 2010. Test of the Pathogenicity and Virulence of *Aeromonas hydrophila* stanier in tilapia (*Oreochromis niloticus* Lin.) Through Koch Postulate. *J. Ris. Aquaculture.* 5 (2) : 245–255.

Mantau, Z. and Sudarty, 2011. The Most Complete Book of Effective and Efficient Carp. Mina Library. Jakarta. 63 Pg.

Maswan, Nur Akbar. 2009. Testing the Effectiveness of DNA Vaccine and Its Correlation to Quantitative Hematological Parameters. Aquaculture Technology and Management Study Program Faculty of Fisheries and Marine Sciences. *Bogor Agricultural Institute.* 70 pages.

Matofani, A.S., Hastuti, S. and Basuki, F. 2013. Blood profile of tilapia kunti injected by *Streptococcus agalactiae* with different densities. *Journal of Aquaculture Management and Technology.* 2 (2) : 127-135.

Napitupulu, Ruth Aprilyanti, Dwi Suryanto, and Desrita. 2017. Isolation and identification of potential pathogenic bacteria in tilapia (*Oreochromis niloticus*) in patumbak aquaculture ponds. *Aquacoastmarine Journal.* 15 (1): 72–82.

Nirmala, K., Hastuti, Y.P. and Yuniar, V. 2012. Toxicity of mercury (hg) and the level of survival, growth, blood picture and organ damage in tilapia *Oreochromis niloticus*. *Indonesian Aquaculture Journal.* 11 (1): 38-48.

Octaviana, H. Nur, A. D. Sasanti, and M. Fitriani. 2015. The prevention of *Aeromonas hydrophyla* infection on sangkuriang catfish using phaleria flour in feed. *Jurnal Akuakultur Rawa Indonesia*. 3 (2): 14–24.

Priyatna R, Indarjulianto S, Kurniasih. 2018. *Aeromonas salmonicida* infection from various regions in Indonesia in carp (*Cyprinus carpio*). *J. Biota.* 16 (2): 287-297.

Putra, G.P., Mulyana, Mumpuni, F.S. 2015. Effect of administration of temulawak extract (*Curcuma xanthorrhiza* Roxb) on mortality and blood picture of nilem fish (*Osteochilus hasselti*) by challenging test using *Aeromonas hydrophila* bacteria. *J Mina Science.* 1 (2): 67-78.

Sam, Sulastri, Abd Malik. 2016. Determination of total phenolic levels from ethanol extracts of red rosella flowers (*Hibiscus sabdariffa* L.) using UV-VIS spectrophotometry. *Fitofarmaka Indonesia Journal.* 3 (2): 182–87.

Sari, C.N., NA Z, Asmilia N. 2017. Haematological profiles of tilapia fish (*Oreochromis mossambicus*) exposed to mercury chloride (Hgcl2). *JIMVET.* 1 (3): 439-447.

Sulawesty F., Tjandra Chrismadha, and Endang Mulyana, 2014. The growth rate of carp (*Cyprinus Carpio* L) by feeding fresh lemna (*Lemna perpusilla* Torr.) In a closed flow pond system. *Limnotek Journal.* 21 (2): 177.

Susanti, L., Deswati, L. and Elfrida. 2015. Test the use of the hydrovac vaccine to improve the immunity of gouramy (*Osphronemus goramy* Lac) against *Aeromonas hydrophila* bacteria. *Journal of the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bung Hatta University.* 11 pages.

Tiwari, Prashant, Bimlesh Kumar, Mandeep Kaur,

Gurpreet Kaur and Harleen Kaur. 2011. Phytochemical Screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia.* 1 (1) : 98-106.

Tsuzuki, M.Y., Ogawa, K., Strussmann, C.A., Maita, M., Takashima, F. 2001. Physiological responses during stress and subsequent recovery at different salinities in dult pejerrey Odontesthes bonariensis. *Aquaculture.* **1** (2) : 349–362.

Utami, D.T., Prayitno, S.B., Hastuti, S. and Santika, A. 2013. Description of haematological parameters in tilapia (*Oreochromis niloticus*) which were given DNA Streptococcus iniae vaccine with different doses. *Journal of Aquaculture Management and Technology*. **2** (4): 7-20.

Wahjuningrum, D., Astrini, R. and Setiawati, M. 2013. Prevention of *Aeromonas hydrophila* infection in *Clarias sp*. 11 days old using *Allium sativum* garlic and *Phyllanthus niruri* meniran. *Indonesian Aquaculture Journal.* 12 (1): 94-104.

Wahjuningrum, D., Hasanah, M. and Rahman. 2016. The efficacy of *Paederia foetida* leaf healing for the prevention of *Aeromonas hydrophila* bacterial infection in tilapia. *Indonesian Aquaculture Journal.* 15 (2): 108–116.

Zou, J., Holland, J., Pleguezuelos, O., Cunningham, C. and Secombes, C.J. 2000. Factors influencing the expression of Interleukin-1β in Cultured Rainbow Trout (*Oncorhynchus mykiss*) Leukocytes. *Dev Comp Immun*. **6** (24) : 575–582.