TIK-76 The effectiveness of Dayak onion (Eleutherine palmifolia) extracts for medicinal treatment of sangkuriang catfish (Clarias gariepinus) infected with different doses of Bacteria Aeromonas hydrop

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The effectiveness of Dayak onion (*Eleutherine palmifolia*) extracts for medicinal treatment of sangkuriang catfish (*Clarias gariepinus*) infected with different doses of Bacteria *Aeromonas hydrophilla*

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Abstract
This research was performed to investigate the effectiveness of Dayak onion (*Eleutherine palmifolia*) extracts used to treat the Sangkuriang catfish (*Clarias gariepinus*) experimentally infected with bacteria *Aeromonas hydrophilla*. Observation and measurement were carried out in the three different laboratories. A total of 150 individuals of catfish were periodically investigated in 15 circle containers. A 2×2 factorial completely randomized design was applied as the experimental design, comprising 4 treatment combinations (bacteria dose and onion extract) and 3 replications. Treatment A1B1: 1.25×10^6 cfu/mL and 40 ppm, treatment A1B2: 1.25×10^6 cfu/mL and 70 ppm, treatment A2B1: 1.25×10^8 cfu/mL and 40 ppm, and treatment A2B2: 1.25×10^8 cfu/mL and 70 ppm. After 17-day rearing period, the treatment A1B1 provided the best performance for hemoglobin (7.70 g/dL), leucocrit (1.33%), blood plasma (56.67%) and survival rates (86.67%). While the best result for hematocrit level (41.33%) was given by the treatment A2B1. The effective dose of onion extract was fixed at 40 ppm. Water temperature, pH, DO, and NH_3 were ranged as follows: 26.5 - 26.9 ºC, 6.8 - 7.0, 6.4 - 6.9 mg/L, and 0.00 - 0.05 mg/L. These levels correspond to the tolerance limits of the catfish. The outcome of this research could be beneficial for better aquaculture practices.

Keywords: Dayak onion, Sangkuriang catfish, *Aeromonas hydrophilla*, antibacterial agent

1. Introduction
African catfish (*Clarias gariepinus*) is economically important freshwater fish species in Asian, African and other countries, and beneficially support aquaculture and fish processing industry. Good quality meat, high nutritive value and good consumer acceptance make it high market price. It is categorized as aggressive and predator species. Like other labyrinth fish species such as Snakehead (Ahmadi, 2018) [1] and Climbing perch (Ahmadi, 2019) [2], this species is also adaptable to the unfavourable environmental conditions and resistant to illness (Amin *et al*., 2016) [3]. It can be found extensively in all freshwater bodies and also in estuarine eniveronments (Admassu *et al*., 2015; Tesfahun, 2018) [4, 5]. It is commercially culturable with different tecniques and various culture media with high stocking density (Abraham *et al*., 2018) [6], and the feasibility of business is also accessible (Aktar *et al*., 2014) [7]. In the wild, its population is likely threated by destructive fishing practices, habitat degradation, and pollution (Obire and Wemedo, 2002; Hossain *et al*., 2016) [8, 9]. Beside cannibalistic problem, the massive death of catfish induced by diseases is becoming a contributing factor for unprofitable business. Another serious problem is low fertilization and hatching rates of catfish (Amin *et al*., 2016) [3]. In West Bengal, India, the accumulative problem being faced by fish farmers resulted in the survival at harvest accounted for 40-50% (Abraham *et al*., 2018)[6]. In North Vietnam, about 65.8% of the main problem in hatchery is caused by disease (Phan *et al*., 2002) [10]. The disease may come from poor wate quality and high stocking density. Fish from Family siluridae, ictaluridae, clariidae, and cyprinidae are susceptible to disease attacks of Motile Aeromonas Septicemia (MAS) caused by *Aeromonas hydrophylla* bacteria (Hidayat *et al*., 2014; Abd Allah *et al*., 2019) [11, 12]. This bacterium is significantly higher in the dry season than in the wet season because organic matter is highly available (Akrong *et al*., 2019) [13]. According to Ikpi and Offem (2011) [14], fish from the infected ponds are potentially dangerous to consumer and highly devalued.

The use of chemical or aquadrugs for disease treatment are still considered useful for fish farmers (Mohsin *et al*., 2012) [15], however, it may have a side effect for long-term uses. Recently, the disease treatments with various herbal extracts as antibacterial agents are being promoted for aquaculture practices, for example the extracts of Tobacco *Nicotiana tobaccum*, garlic *Allium sativum*, Bilimbi *Averrhoa bilimbi*, calabash *Cresentia cujete* and Drumstick *Moringa oleifera* for African catfish *C. gariepinus* (Musa *et al*., 2013; Abraham and Ritu, 2015; Purnamasari *et al*., 2015; Rahmaningsih *et al*., 2018; Rosidah *et al*., 2018) [16, 20], neem *Azadirachta indica* for Asian seabass *Lates calcarifer* (Talpur and Ikhwanuddin, 2013) [21], Crocodilestongues *Aloe vera* for kissing gourami *Helostoma temminckii* (Prasetio *et al*., 2018) [22] and Kelakai *Stenochlaena palustris* for Snakehead *Channa striata* (Norhayati *et al*., 2019) [23]. Meanwhile the efficacy of Dayak onion *Eleutherine palmifolia* extracts for treating the catfish infected by *A. hydrophila* has not been investigated, yet. Dayak onion is a native plant from Central Kalimantan and known as natural multi-functional herbal medicine including antibacterial agent (Harlita *et al*., 2018) [24]. In this area of study, we performed a series of laboratory experiments to determine the effective dose of Dayak onion extracts to treat the catfish infected with different bacterial doses of *A. hydrophila*.

2. Materials and Methods

Study sites

The researches were carried out in the three different laboratories, namely (1) the Fish Quarantine Center, Fishery Product Quality and Safety Control (BKIPM) Class II Banjarmasin for bacterial culture; (2) Basic Laboratory of the Faculty of Mathematics and Natural Sciences for Dayak onion extraction; and (3) Basic Laboratory of the Faculty of Marine and Fisheries for examining catfish samples. The research activities were started from January to March 2019.

Experimental fish and containers

Sangkuriang catfish is locally called "*Lele Sangkuriang*" and used as experimental animal. Before the onset of experiments, the catfish were acclimatized for 7 days and fed with commercial pellet of PF-1000 two times a day. A total of 150 catfish adults (15 cm total length and 110 g weight) were bought from local fish farmers. A total of 15 circle containers (58 cm diameter, 23 cm high and 15 cm water height) were used for catfish during rearing period (10 individuals per container). All equipment was sterilized before being used.

Dayak onion's extract

About 2 kg wet weight of fresh Dayak onions were washed, air-dried, cut into small pieces, dried with oven at 50 °C, and refined with a blender to produce onion powder. The onion powder was soaked in 96% ethanol for 24 h at room temperature with a ratio of 1: 4 (g/4 mL). The solution obtained was filtered with a filter paper and then evaporated with a rotary vacuum evaporator to produce a coarse extract in the form of a paste. This extraction protocol refers to the guidelines prescribed by the Indonesian Health Department.

Bacterial rejuvenation

The bacteria *A. hydrophila* in stock culture was rejuvenated by culturing it on GSP gelatin selective medium and incubated at room temperature for 18-24 h. The yellow colonies were partly transferred to the TSA (Tryptone Soya Agar) medium associated with liquid paraffin as a stock culture and the rest was transferred to the NaCl liquid medium as an initial culture medium for bacteria. Before doing the test of antibacterial activity, the bacteria should first be re-infected and re-isolated to increase its virulence level. Reinfection in catfish was done by intramuscular injection with 0.1 mL (10^9 cfu/mL). The infected catfish were kept in the container, and then treated with the same dosage. Dead fish or suffered from severe illness were re-isolated. The bacteria active was aseptically taken from kidney organs using sterile ose in the GSP gelation selective medium and incubated at room temperature for 18-24 h. The second and third reinfection and isolation were performed to increase the virulence of bacteria.

Biochemistry test

A series of biochemistry test was undertaken to ensure the purity of bacteria used in accordance with the SNI 7303/2009.

Gram coloration with 3% KOH test

Bacteria sample were placed on the object glass and dripped with 3% KOH solution; if gel appeared, it known as gram positive (+) and if found no gel, it called gram negative (-).

Catalase test

Bacteria on the object glass were dripped with 1-2 drops of 3% H_2O_2 solution. If bubble came out, the bacteria were considered catalase positive (+) and if found no bubble, the bacteria were catalase negative (-).

Oxidase test

Bacteria sample were visually examined with a cytochrome oxidase stick for 10-15 sec; when the oxidase stick paper turned purple then called oxidase positive (+), if not changed it was considered oxidase negative (-).

OF testing

Two OF mediums in the test tube were inoculated with bacteria for 24 h or more, where one of the tubes was covered with sterile liquid paraffin. If it turned green to yellow, the bacteria were found to be fermentative. If the color changes to yellow only on uncovered media, the bacteria were considered oxidative. If there was no change then it called NR (not reaction).

Motility test

The isolate was inoculated by sticking it into a semi-solid medium or Motility Indole Ornithine (MIO) Medium at 25-28 °C for 18-24 h. A positive reaction was characterized by the spread of bacterial growth and invisible puncture marks. If the puncture mark spread followed by distribution of white patches on MIO gel, it was so-called Motile positive (+). When it was invisible on MIO gel, it was known as Motile negative (-).

Ornithine test

The inoculated media in MIO gel were incubated for 24 h. The color changes in the anaerobic area of the medium were observed. Ornithine positive (+) was marked by the colors of gray, purple and blue. When it turned the yellow, it was considered Ornithine negative (-).

Indole Test

The inoculated media in MIO gel were incubated for 24 h. Indole readings done by adding one drop of kovacs reagen on popten medium. When a red ring was emerged on the upper surface of the peptone medium, it was the so-called Indole positive (+). If the red ring was not find it was considered Indole negative (-).

Identification and Verification

TSIA (Triple Sugar Iron Agar) Test

Bacteria were inoculated with TSIA in a test tube, and then incubated at 28 °C for 24 h. The bacterial properties were observed based on the color changes of the media on both aerobic and anaerobic areas. Yellow indicates an acidic condition, while red indicates an alkaline condition. The H_2S gas was produced after a 48-h incubation period. It was characterized by the emergence of black color on the inoculated TSIA media.

LIA (Lysine Iron Agar) Test

The inoculated media were incubated for 24 h. Lysine deaminase was positive (+), if the red was appeared on the top of medium or dark red on the LIA slant area. Lysine decarboxylase was negative (-) if the butt turns yellow and positive (+) if it remains purple or dark purple. The H_2S gas was characterized by the emergence of black color on the

inoculated LIA media.

Urea Test
Urea media were scrapped with the inoculated gel media and incubated for 24 h. Urea positive (+) if it turned pink, and Urea negative (-) if the color was unchanged.

Citrate Test
Citrate media were scrapped with the inoculated gel media and incubated for 24 h. Citrate positive (+) if it turned blue, and Citrate negative (-) if the color was unchanged or remain green.

Parameters observed
Survival rate
Survival rate (SR) is calculated using the following equation (Yousuf *et al*., 2016) [25]:

$$SR = \frac{\text{Number of survived fish}}{\text{Initial number of fish}} \times 100$$

Hematological Analysis
The blood samples of catfish were analyzed for hemoglobin, hematocrit, leukocrit and blood plasma. Hemoglobin concentration was measured using the Sahli's Method (Kapil *et al*., 2012) [26]. While hematocrit, leukocrit and blood plasma were calculated by using the following formulas (Anderson dan Siwicki, 1994) [27]:

$$\text{Hematocrit} = \frac{\text{Erythrocyte}}{\text{Total blood}} \times 100\%$$

$$\text{Leukocrit} = \frac{\text{Leukocyte}}{\text{Total blood}} \times 100\%$$

Blood plasma = Total blood volume - Leucocyte - Erythrocyte
The blood sample parameters were periodically measured at 48 h (day-2), day-10 and day-17 rearing period.

Water quality
Water quality parameters such as temperature, pH, dissolved oxygen (DO) and NH3 content were measured throughout sampling periods.

Experimental design and treatments
A 2×2 factorial completely randomized design was applied as the experimental design, where catfish were randomly subjected to 4 treatment combinations and 3 replications (total 12 units). The first factor was bacterial doses (A) with two treatments:
A_1 = bacterial dose of 1.25×10^6 cfu/mL
A_2 = bacterial dose of 1.25×10^8 cfu/mL
The second factor was the concentration of Dayak onion extract (B) with two 2 treatments:
B_1 = Dayak onion extract of 40 ppm
B_2 = Dayak onion extract of 70 ppm

The treatment combinations (bacteria doses of *A. hydrophilla* and Dayak onion extract) were as follows
A1B1 = 1.25×10^6 cfu/mL and 40 ppm
A1B2 = 1.25×10^6 cfu/mL and 70 ppm
A2B1 = 1.25×10^8 cfu/mL and 40 ppm
A2B2 = 1.25×10^8 cfu/mL and 70 ppm
The control experiments, both negative and positive, were applied to compare the treatment group values:
C1 = Negative control
C2 = Positive control with *A. hydrophilla* of 1.25×10^6 cfu/mL
C3 = Positive control with *A. hydrophilla* of 1.25×10^8 cfu/mL

Data analysis
The Levene's test was used to verify that assumption for equality of variance was met and our data was normally distributed. Data were analyzed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test to identify differences between treatments. The determination coefficient (R^2) and the regression coefficient (r) were also computed. The results were presented in graphical, verbal or in tabular form. All statistical analysis was performed using the SPSS 25.0 software.

3. Results
All estimated parameters for hemoglobin, hematocrit, leucocrit and blood plasma of the catfish were presented in Table 1, while water quality parameters were given in Table 2.

Hemoglobin
At the day-2 (after 48 h infection), the highest mean Hb level was found in the treatment A1B1 (3.20 ± 0.20 g/dL), followed by the treatments A1B2 (2.77 ± 0.40 g/dL), A2B1 (2.53 ± 0.15 g/dL) and A2B2 (2.17 ± 0.42 g/dL). However, these values were relatively lower than the controls, indicating catfish were still experiencing an anemia. The Hb values gradually increased after the day-10 and the day-17 (Figure 1A). The highest Hb values (6.67-7.70 g/dL) at these days were also found in the treatment A1B1. The mean values obtained were higher than the controls, indicating catfish were in better condition (Table 1).
The ANOVA test showed that the variation of onion extracts had no a significant effect to the infected catfish after the day-2 treatment. The effect of the onion extracts worked effectively after the day-10 and day-17 ($P < 0.001$). The LSD test confirmed that the best performance for increasing the Hb level of the infected catfish was the treatment A1B1. The determination coefficient of 0.968 indicates that the onion extracts can increase the HB value up to 96.8%, and the relationship between variables A and B was strongly correlated (r = 0.984).

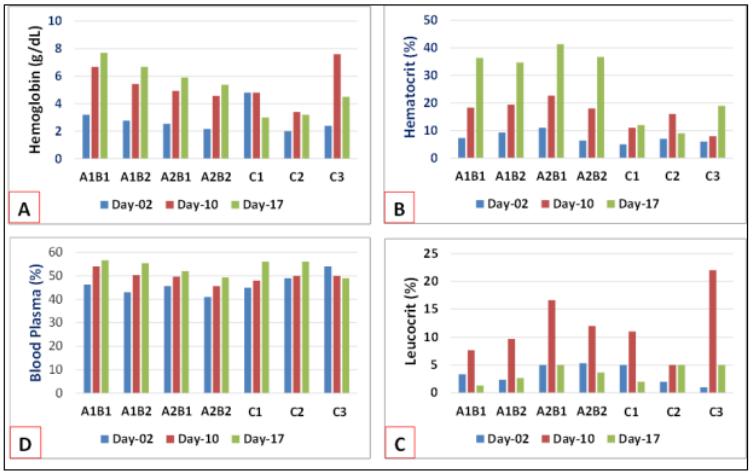

Fig 1: Hematological investigation for the blood samples when the catfish were subjected to various bacterial doses and Dayak onion extracts. The average values of hemoglobin [A], hematocrit [B], leucocrit [C] and blood plasma [D] were acceptable for the explanation that the onion extracts had an antibacterial effect.

Hematocrit

The hematocrit values observed at the day-2 ranged from 6.33 to 11.00% (Table 1). The highest hematocrit value was found in the treatment A2B1 (11.00%), followed by the treatments A1B2 (9.33%), A1B1 (7.33%) and A2B2 (6.33%). The hematocrit values consistently increased about 2-3 times at the Day-10 as compared to those investigated at the day-2 sampling period (Figure 1B). The hematocrit value ranges from 18.00 to 22.67%, and the rank following the same order as the day-2 treatments. During these periods, catfish were suffering from anemia due to bacterial infection. At the end of observation (day-17) the hematocrit rates were in normal condition. The best performance for hematocrit level was shown by the treatment A2B1 (41.33%), followed by the treatments A2B2 (36.67%), A1B1 (36.33%) and A1B2 (34.67%). The mean values obtained were comparatively higher than the controls, implying that eritrocit volume (red blood cells) of catfish inreases.

At the day-2 and day-10, the ANOVA test showed that both the bacterial dose and the onion extract variations had no a significant effect to the hematocrit rates, although the independent variables were basically interconnected with each other. However, these results were expressively contrary to the observation at the day-17. Further statistical analysis with LSD test showed that there were no statistically significant differences between the treatments at the day-2. Meanwhile, at the day-10 the differences were only found between the treatments A1B1 and A2B1, as well as between the treatments A2B1 and A2B2. At the end of study (day-17) the treatment A1B1 was only significantly diffed from A2B1, as well as between A1B2 and A2B1. Linear regression analysis showed that the R^2 value obtained was 0.968, indicating that the use of onion extracts can increase hematocrit functions up to 96.8% due to its antibacterial effect. The positive correlation (r = 0.984) of independent variables relationship was confirmed.

Leucocrit

At the day-2 of observation, the leucocrit values obtained ranged from 2.33 to 5.33% (Table 1). There were significantly differences in the percentage of leucocrit rates among the four treatments. The highest leucocrit value was found in the treatment A2B2 (5.33%), followed by the treatments A2B1 (5.00%), A1B1 (3.33%) and A1B2 (2.33%). The leucocrit values consistently increased about 2-4 times at the Day-10 as compared to those observed at the day-2 sampling period (Figure 1C). The highest leucocrit value was produced by the treatment A2B1 (16.67%), followed by the treatments A2B2 (12.00%), A1B2 (9.67%) and A1B1 (7.67%). More leucocrit produces at this phase showing how the catfish immune system works. At the day-17, the leucocrit values obtained were correspondingly found at the day-2. The estimated leucocrit rate was higher in the A2B1 (5.00%), followed by the treatments A2B2 (3.67%), A1B2 (2.67%) and A1B1 (1.33%). The decline in the leucocrit values at this time indicating catfish were healthier than those were being infected by the bacteria.

The similar results were found between independent variables tested at the day-2 and the day-17. The ANOVA test showed that the bacterial dose variations had a significant effect to the leukocrit level as compared to the onion extract variations, although the respective variable functioned individually. Meanwhile at the day-10, the two independent variables (A and B) significantly affected on the leucocrit level. Further analysis with the LSD test showed that the leucocrit value of the treatment A1B1 was equal to the treatment A1B2 ($P>0.05$), but it significantly differed from the treatments A2B1 and A2B2 ($P<0.05$). The estimated value for determination coefficient (R^2) was 0.641, indicating that the onion extracts substantially contributed about 64.1% of the leucocrit properties, and the relationship between variables A and B was positively correlated (r = 0.800).

Blood plasma

An increase in the blood plasma of the infected catfish was corresponding to time periods of the onion extracts extending. At the day-2, blood plasma incresead from 41.00 to 46.33%, then at the day-10, it counted about 45.67 to 54.00% and increasingy at the day-17 with the values of 49.33 to 56.67% (Table 1). The best result for cumulative blood plasma level was the treatment A1B1 across sampling time periods, and the lowest blood plasma values were

recorded in the treatment A2B2 (Figure 1D). The treatment A1B2 worked significantly better than the treatment A2B1 ($P<0.05$). In this study, the blood plasma values were diretly proportional to the hematocrit values. Variation in blood plasma value was also affected by the physiological conditions of fish such as stress.

The ANOVA test showed substantionally that the onion extract variation seems more significantly affected on the blood plasma level as compared to the bacterial dose variations. At the day-10 and the day-17, both the onion extracts and the bacterial dose variations had a significant effect to the blood plasma concentration, despite the respective variable worked independently. At the end of study, the LSD test revealed that the blood plasma values for the treatments A1B1 and A1B2 were equal ($P>0.05$). The treatment A1B1 differed significantly compared to the treatments A2B1 and A2B2 ($P<0.05$). The estimated value for determination coefficient was 0.950, indicating that the use of Dayak onion extracts can increase the blood plasma utilities of the infected catfish up to 95.0%. The relationship between variables A and B was highly correlated ($r = 0.975$).

Table 1: The Hemoglobin, hematocrit, leucocrit and blood plasma levels of the infected catfish that substantially treated with the Dayak onion extracts over the day-02, day-10 and day-17 rearing periods. The results were compared to the negative (C1) and positive controls (C2-3).

Sampling Period	Hemoglobin (g/dL)								
	A1B1	A1B2	A2B1	A2B2	Mean□SD	C1	C2	C3	Mean□SD
Day-02	3.20	2.77	2.53	2.17	2.67±0.43	4.80	2.00	2.40	3.07±1.51
Day-10	6.67	5.43	4.93	4.57	5.40±0.92	4.80	3.40	7.60	5.27±2.14
Day-17	7.70	6.67	5.90	5.37	6.41±1.01	3.00	3.20	4.50	3.57±0.81
Mean±SD	5.86±2.36	4.96±1.99	4.46±1.73	4.03±1.67	4.83±1.94	4.20±1.04	2.87±0.76	4.83±2.62	3.97±1.15
Sampling Period	Hematocrit (%)								
	A1B1	A1B2	A2B1	A2B2	Mean±SD	C1	C2	C3	Mean±SD
Day-02	7.33	9.33	11.00	6.33	8.50±2.08	5.00	7.00	6.00	6.00±1.00
Day-10	18.33	19.33	22.67	18.00	19.58±2.13	11.00	16.00	8.00	11.67±4.04
Day-17	36.33	34.67	41.33	36.67	37.25±2.86	12.00	9.00	19.00	13.33±5.13
Mean±SD	20.67±14.64	21.11±12.76	25.00±15.30	20.33±15.30	21.78±0.43	24.33±22.23	10.67±4.73	11.00±7.00	10.33±2.14
Sampling Period	Leucocrit (%)								
	A1B1	A1B2	A2B1	A2B2	Mean±SD	C1	C2	C3	Mean±SD
Day-02	3.33	2.33	5.00	5.33	4.00±1.42	5.00	2.00	1.00	2.67±2.08
Day-10	7.67	9.67	16.67	12.00	11.50±3.87	11.00	5.00	22.00	12.67±8.62
Day-17	1.33	2.67	5.00	3.67	3.17±1.55	2.00	5.00	5.00	4.00±1.73
Mean±SD	4.11±3.24	4.89±4.14	8.89±6.74	7.00±4.41	6.22±1.38	6.00±4.58	4.00±1.73	9.33±11.15	6.44±3.88
Sampling Period	Blood Plasma (%)								
	A1B1	A1B2	A2B1	A2B2	Mean±SD	C1	C2	C3	Mean±SD
Day-02	46.33	43.00	45.67	41.00	44.00±2.46	45.00	49.00	54.00	49.33±4.51
Day-10	54.00	50.33	49.67	45.67	49.92±3.41	48.00	50.00	50.00	49.33±1.15
Day-17	56.67	53.00	55.33	49.33	53.58±3.22	56.00	56.00	49.00	53.67±4.04
Mean±SD	52.33□5.37	48.78±5.18	50.22±4.85	45.33±4.18	49.17±0.50	49.67±5.69	51.67±3.79	51.00±2.65	50.78±1.82

Survival rate

The survival rates of the catfish ranged from 66.67 to 86.67%. ANOVA test revealed that the Dayak onion extract had a significant effect to the infected catfish ($P < 0.001$). The LSD test showed that the treatment A1B1 provided the best performance among the four treatments. The highest and the lowest survival rates were found in the treatments A1B1 (86.67 ± 5.77%) and A2B2 (67.67 ± 5.77%) respectively (Figure 2). The survival rates of the treatments A1B2 and A2B1 were equal (70.00 ± 0.00%). The linear regression analysis revealed that the Dayak onion extracts visibly contributed about 70% toward the survival of the catfish ($R^2 = 0.692$), and such relationship was positively correlated ($r = 0.832$).

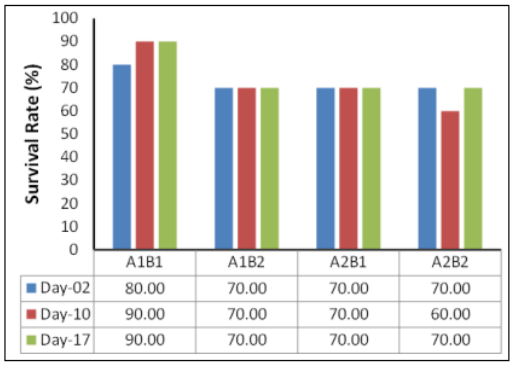

Fig 2: The survival rates of the catfish after having treated with the Dayak onion extracts

Water quality

Table 2 showed the results of water quality measurements during the sampling period. Water temperature, pH, DO, and NH_3 were ranged as follows: 26.5 - 26.9 °C, 6.8 - 7.0, 6.4 - 6.9 mg/L, and 0.00 - 0.05 mg/L. These levels correspond to the tolerance limits of the catfish. In the containers, water quality parameters did not differ significantly over the study period.

Table 2: Water quality parameters observed for the catfish throughout the sampling periods, including the treatment for the negative (C1) and positive controls (C2-3).

Treatments	Parameters Observed			
	Temperature (°C)	pH	DO (mg/L)	NH_3 (mg/L)
A1B1	26.5 - 26.7	6.7 - 7.0	6.7 - 6.9	0.00
A1B2	26.6 - 26.8	6.7 - 6.8	6.5 - 6.6	0.00
A2B1	26.7 - 26.8	6.7 - 6.9	6.5 - 6.9	0.05
A2B2	26.7 - 26.9	6.8 - 7.0	6.5 - 6.6	0.00
C1	26.6 - 26.7	6.8 - 6.9	6.5 - 6.6	0.05
C2	26.8 - 26.9	6.8 - 6.9	6.4 - 6.5	0.00
C3	26.8 - 26.9	6.7 - 6.8	6.7- 6.8	0.00

4. Discussion

Dayak onion is used essentially for therapeutic and health benefits (Harlita *et al*., 2018) [24]. From aquaculture point of view, the first attempt was made to treat the carp *Cyprinus carpio* infected by *A. hydrophila* (Mafthuch *et al*., 2018) [28]. Meanwhile, the use of Dayak onion for the infected catfish is lacking so far. Blood images in catfish can indicate the condition of the fish's immune system and can provide important information about the physiological status of the infected catfish that substantially curable with the Dayak onion extracts.

Physiologically, hemoglobin is crucial to the survival of the fish as its role is directly related to the oxygen binding capacity of blood (Afia and David, 2017) [29]. The ability of bacteria *A. hydrophila* to invade fish occurs through the work mechanism of extracellular toxin product that are released when the bacteria are still alive and attached to fish organs. According to Eisa *et al*. (1994) [30], *A. hydrophila* bacterial infection is a pathogenic primary in fish, where damage or injury caused by infection would be the entrance for other pathogenic infections. *A. hydrophila* produces more than one type of extracellular enzyme that plays a role in the pathogenesis process of the host or infected fish. One of enzyme types that are toxic is the hemolisin enzyme, which could disrupt red blood and white blood cells, as well as cause tissue necrosis (Higerd and Fouler, 1997) [31]. The onion extracts proved medically can help to increase the body defense system caused by bacterial infection. The hemoglobin values (5.37-7.70 g/dL) obtained in the present study was also in agreement with the findings of other researchers (Hastuti and Subandiyono, 2014; Prasetio *et al*., 2018) [21, 32] who worked for African catfish (6.4-7.5 g/dL) and the kissing gourami (6.1-7.8 g/dL). While Musa *et al*. (2013) [16] reported that haemoglobin values of African catfish reduced from 7.03 to 0.82 g/dL when the concentration of Tobacco leaf dust increased from 0.0 to 2.5 g/L. According to Haruna and Adikwu (2001) [33], low haematological indices are indicators of anaemic condition. Variation in Hemoglobin concentration might be attributable to environmental condition changes, sex difference, feeding frequency and life history (Adeyemo *et al*., 2003; Ajani *et al*., 2016; Al-Deghayem *et al*., 2017; Norhayati *et al*., 2019) [23, 34, 35, 36].

Over a 17-day treating period, the hematocrit values (34.67-41.33%) obtained was within the toleran range (30.8-45.5%) suggested by Angka *et al*. (1985) [37]. Our result was comparatively higher than other relevant studies (Purnamasari *et al*., 2015; Rahmaningsih *et al*., 2018; Norhayati *et al*., 2019) [18, 19, 23] that used various types of herbal extracts (24.09-26.18%). Therefore, it was appropriate and acceptable for the explanation that the onion extracts had an antibacterial effect and can increase hematocrit function up to 96.8%. Alamanda *et al*. (2007) [38] characterized a fish with hematocrit value less than 22% will suffer from anemia, which significantly affect on the growth and survival of infected fish. Low hematocrit value resulted in fish stop feeding (Talpur and Ikhwanuddin, 2013) [21]. Furthermore, Mierza (2011) [39] reported that antibacterial effect of the onion extracts was induced by the phytochemical compounds of the Dayak onion such as alkaloid, flavonoid, saponin, tannin, glikosid, dan triterpenoid. In addition, the ratio values of hematocrit to the blood plasma (0.64-0.75) in the end of study (day-17) were 3.0-4.8 times higher than those found in the beginning of observation (day-2), indicated that the fish health condition was improved. Variation in hematocrit values are greatly influenced by diet contents and temperature (Al-Deghayem *et al*. 2013) [36].

Low leucocrit value observed at the end of our study indicates that Sangkuriang catfish have recovered from bacterial infection. Leukocrit is convined substantially contributes to the body immune system (Morgan and Iwama, 1997) [40]. It can provide clues about fish health and help to determine the occurrence of abnormalities due to the use of immunostimulants and drugs in a fish (Anderson and Siwicki, 1994) [27]. Variation in leucocrit might be attributable to stress level, age, and physiological activity (Dienye *et al*., 2014) [41]. Uedeme-Naa and George (2019) [42] reported that leucocrit decreased with increasing concentration of Moringa leaves. In our study, the leucocrit values fluctuated a great deal over period of time and bacteria density.

The infected catfish were coherently curable with the Dayak onion extracts with the survival rates of 66.67-86.67% over a 17-day rearing period. Similar results were reported by Rosidah *et al*. (2018) [20] who used *Moringa oleifera* extracts as antibacterial agent for the infected catfish for 14 days and found the survival rates of 65-80%. Meanwhile, Purnamasari *et al*. (2015) [18] medically treated the infected catfish with starfruit juice for 30 days and showed the survival rates varied of 58.33-93.33%. The bacteria *A. hydrophila* was pathogenic in fish and the most likely caused the death of fish, and each fish species may respond differentially to the bacteria (Rozi *et al*., 2017) [43]. On the basis of bacterial dose uses, the mortality rates of catfish induced by 1.25×10^6 or 1.25×10^8 cfu/mL of *A. hydrophila* in the present study were ranged of 13-33%. According to Rey *et al*. (2009) [44], the death begins after 7 h after infection of *A. hydrophila* and the number increasingly over 12-24 h after infection. Since the catfish morphologically have no scales, they are more susceptible to bacterial infection. In this regard, catfish secrete a lot of mucous that caused metabolism increases and lost energy in the body, as a result fish becomes weak and stress lead to the death. Compared to other fish species, the Pirarucu *Arapaima gigas*, a native species of the Amazon River, that infected with 1.8×10^8 cfu/mL of *A. hydrophila* for 8-23 h caused the death of 91.6% (Dias *et al*., 2016) [45].

5. Conclusion

The Dayak onion extracts scientifically proved as an antibacterial agent to be used for substantially treating the catfish infected by *A. hydrophilla*. The treatment A1B1 provided the best performance for hemoglobin, leucocrit, blood plasma and survival rates. While the best result for hematocrit level was created by the treatment A2B1. Thus, the effective dose of onion extract was fixed at 40 ppm. The outcome of this research could be useful for better aquaculture management.

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