

The influence of the mangrove leaves infusion *Excoecaria agallocha* against *Aeromonas hydrophila* bacteria infection in tilapia

Pengaruh infusa daun mangrove *Excoecaria agallocha* terhadap infeksi bakteri *Aeromonas hydrophila* pada ikan nila

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Abstract

Tilapia is fish consumption with some excess, that is has a high protein, non-cholesterol, the taste is delicious, affordable and easily cultivated. One of the problems in cultivating them is a disease because it can cause the death of the fish. Disease-causing factors can be derived from the bacterium *Aeromonas hydrophila*. The bacteria *A. hydrophila* can cause the death of fish until 80%-100% within 1-2 week and even many that are already resistant to several types of commercial antibiotics, so that the need to look for a new antibiotics source. The source of these antibiotics can be derived from nature because it is cheaper, safer and environmentally friendly. Mangrove *Excoecaria agallocha* has been known to have potential as an antibacterial, larvacide, and antifungal. This can be used as a source of antibiotics. The purpose of this research is to analyze the potential of the mangrove leaves infusion *E. agallocha* in preventing the spread of disease in tilapia infected the bacteria and identify the compounds of secondary metabolite. This research was carried out from March-August 2018 and use approach posttest-only control group design (by comparing the treatment with the control group). The amount of treatment is five with three replicates. The dose used, namely 0%, 2.5%, 5%, 7.5% and 10%. The results showed that there was no difference in the influence of the mangrove leaves infusion *E. agallocha* against weight gain and survival rate of tilapia juveniles. The result of phytochemical screening indicates that *E. agallocha* leaves infusion contains tannins and flavonoids.

Keywords: *Excoecaria agallocha*; phytochemical; tilapia

Abstrak

Ikan nila merupakan ikan konsumsi dengan beberapa kelebihan yaitu memiliki kandungan protein yang tinggi, non-kolesterol, rasa enak, harga terjangkau dan mudah dibudidayakan. Salah satu masalah yang dihadapi dalam membudidayakannya adalah penyakit, karena dapat menyebabkan kematian pada ikan. Faktor penyebab penyakit dapat berasal dari golongan bakteri yaitu *Aeromonas hydrophila*. Bakteri *A. hydrophila* dapat menyebabkan kematian ikan dengan kisaran 80%-100% dalam jangka waktu 1-2 minggu dan bahkan banyak yang sudah resisten terhadap beberapa jenis antibiotik komersil sehingga perlu dicari sumber antibiotik baru. Sumber antibiotik tersebut dapat berasal dari alam karena lebih murah, aman dan ramah lingkungan. Mangrove jenis *Excoecaria agallocha* telah diketahui memiliki potensi sebagai antibakteri. Hal ini dapat dijadikan referensi sebagai bahan antibiotik baru. Tujuan dari penelitian ini adalah menganalisa potensi infusa daun mangrove jenis *E. agallocha* dalam mencegah penyebaran penyakit pada Ikan Nila yang terinfeksi bakteri dan mengidentifikasi kandungan golongan senyawa metabolit sekundernya. Penelitian ini dilaksanakan dari bulan Maret - Agustus 2018. Penelitian ini menggunakan pendekatan *posttest only control group design* (dengan membandingkan antara kelompok perlakuan dengan kelompok kontrol). Jumlah perlakuan adalah 5 dengan 3 kali ulangan. Dosis yang digunakan yaitu 0%, 2,5 %, 5 %, 7,5 % dan 10 %. Hasil penelitian menunjukkan bahwa tidak terdapat perbedaan pengaruh infusa daun mangrove jenis *E. agallocha* terhadap bobot tubuh dan kelangsungan hidup dari benih Ikan Nila. Hasil skrining fitokimia menunjukkan bahwa pada infusa daun *E. agallocha* mengandung senyawa tanin dan flavonoid.

Kata kunci: *Excoecaria agallocha*; fitokimia; ikan nila

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1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the results of freshwater fish farming. Excess which owned tilapia is tasty, solid flesh, not having many spines, easily obtained, and the price is relatively low. This fish is easily cultivated and able to live until the height of the 500 m above sea level (Ardita et al., 2015).

One of the constraints in cultivating tilapia is a disease caused by bacteria. One example of a disease-causing bacterium is *Aeromonas hydrophila*. These bacteria can cause mortality of fish 80%-100% with a period of 1-2 the week (Sari et al., 2012). The giving of drugs such as antibiotics is considered the most effective way and quickly in coping the disease in fish. Using antibiotics continuously can cause negative effects, such as the occurrence of bacterial resistance, and the price is high. One type of disease-causing bacteria on the fish is *A. hydrophila*. The results showed the presence of bacterial resistance of *A. hydrophila* against some antibiotics such as amoxicillin, ampicillin, at lincomycin, novobiocin, oxacillin, penicillin, combinations with sulfamethoxazole, trimethoprim and rifampicin (Herawati et al., 2013).

The selected solution by finding other materials derived from nature that has potential as an antibiotic. Mangrove *E. agallocha* has been known to have a variety benefits to human interests, among others, the leaves have potential as larvacide, antibacterial, and antifungal (Agoramoorthy et al., 2007; Dhayanithi et al., 2012; Rajeswari & Rao, 2015). The active ingredient of leaves of mangrove *E. agallocha*, which has potential as an antibacterial, i.e. lupeol (Dawane & Fulekal, 2017). Information about the benefits of the leaf of mangrove *E. agallocha* as test in-vivo in tilapia against *A. hydrophila* is limited. The purpose of this research includes: analyze potential infusion leaf of mangrove *E. agallocha* in tilapia which infected by *A. hydrophila* and identify active compounds infusion leaf of mangrove *E. agallocha*.

2. Material and method

This research was carried out from March until August 2018 and use approach posttest-only control group design (by comparing the treatment with the control group) (Kasjono & Yasril, 2009). The design of this research is Complete Random Design, with five treatments and three replicates. The doses of each treatment are 0%, 2.5%, 5% , 7.5% and 10%.

2.1. Preparation

The fish used in the research was tilapia juveniles with a length of 8-10 cm. Fish used was adapted in a plastic box for seven days and given aeration. The sample of mangrove leaves *E. agallocha* taken from mangrove vegetation at Bunga Beach, Batu Bara regency.

2.2. Making of leaf infusion of mangrove *E. agallocha*

The mangrove leaves *E. agallocha* cleaned using water. After that, continued with the drying process and then mashed. The leaves dried of mangrove *E. agallocha* boiled at a temperature of 90 °C for 15 minutes. The filtering is done by using fabric flannel (infusion 10%) (DepKes, 2000).

2.3. Test of the mangrove leaves infusion *E. agallocha*

Inoculum *A. hydrophila* bacteria made on Nutrient Broth media (NB) with a density of 1×10^8 CFU/mL (Indriani et al., 2014). Tilapia juveniles injected *A. Hydrophila* bacteria with a

dose of 0.1 mL on the intramuscular. The mangrove leaves infusion *E. agallocha* mixed with fish feed after infected bacteria *A. hydrophila*. Feeding rate of 5% of the weight and given as much as three times, i.e. morning (07.00-08.00 am), noon (12.00 -01.00 pm) and evening (5.00-6.00 pm). The period of observation is 14 days.

2.4. Water quality parameters

Water quality parameters measured in this study such as dissolved oxygen (DO), pH and temperature.

2.5. Weight gain

The formula to calculate weight gain is:
Weight gain: Mean Final Weight – Mean Initial Weight
(Debnath et al., 2017)

2.6. Survival rate

The formula to calculate the survival rate is:

$$\text{Survival rate (\%)} = \frac{\text{No.of Fish Caught}}{\text{No.Of Fish Release}} \times 100$$

(Debnath et al., 2017)

2.7. Phytochemical analysis

The phytochemical analysis is used to analyze the mangrove leaves infusion of *E. agallocha*. The test is including alkaloids, flavonoids, tannins, saponins, test test test these Terpenoids and steroids.

2.8. Statistical analysis

The research results including weight gain, survival rate and phytochemical screening of the mangrove leave infusion *E. agallocha*. Weight gain and survival rate data of tilapia juveniles were analyzed using statistical analysis. Phytochemical screening data of the mangrove leaves infusion *E. agallocha* is analyzed descriptively.

3. Result and discussion

3.1. Result

3.1.1. Weight gain of tilapia juveniles

The graph of the weight gain tilapia juveniles in Figure 1 and the results of the statistical analysis of the weight gain tilapia juveniles in Table 1.

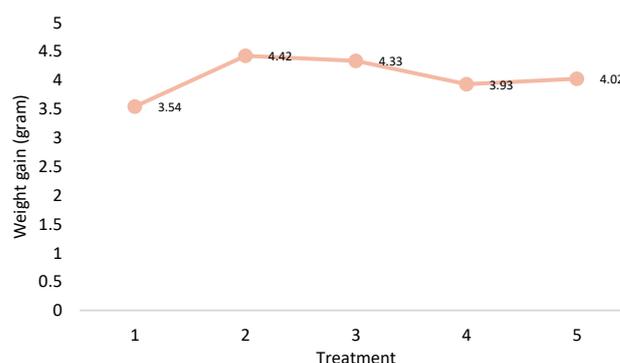


Figure 1. Weight gain of tilapia juveniles.

Figure 1 shows no differences in weight gain tilapia juveniles when compared with control. Tilapia juveniles weight gain has decreased during the 14-day observation.

Table 1

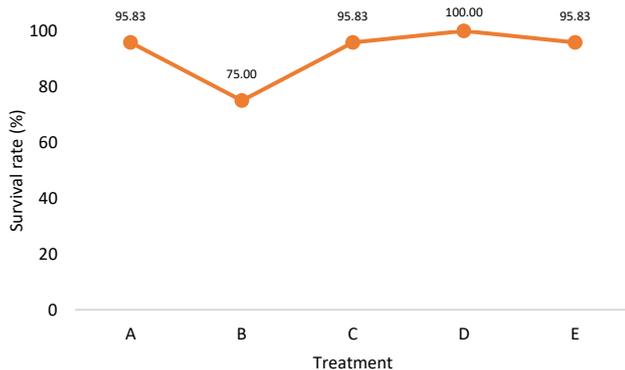
The results of weight gain statistical analysis.

	Sum of squares	df	Mean square	F	Sig.
Between groups	.784	4	.196	.056	.993
Within groups	35.229	10	3.523		
Total	36.013	14			

Table 1 is a result of weight gain statistical analysis in tilapia juveniles. The results of the statistical analysis showed that there is no influence of granting mangrove leaves infusion *E. agallocha* against weight gain of tilapia juveniles. The active compound of mangrove leaves infusion *E. agallocha* is not increasing the weight gain of the tilapia juveniles.

3.1.2. The survival rate of tilapia juveniles

Observation of survival rate tilapia juveniles performed for 14 days. Observations survival rate of tilapia juveniles in Figure 2 and the results of statistical analysis survival rate of tilapia juveniles in table 2.

**Figure 2.** Survival rate of tilapia juveniles

The results observation of survival rate tilapia juveniles, shown in Figure 2. Figure 2 indicates that survival rate at the treatment D (100%) higher than the control or treatment A (95.83%). The lowest result is treatment B (75%).

Table 2

The results of a statistical analysis of survival rate tilapia juveniles.

	Sum of squares	df	Mean square	F	Sig.
Between groups	7.600	4	1.900	1.188	.374
Within groups	16.000	10	1.600		
Total	23.600	14			

The results of a statistical analysis of survival rate of tilapia juveniles represent in table 2. Table 2 shows that the value of $P > 0.05$. The results statistical analysis demonstrate that granting mangrove leaves infusion *E. agallocha* did not affect the survival rate of tilapia juveniles.

3.1.3. Water quality parameters

Water quality parameters include temperature, pH and DO. The result of the observation of water quality parameters can see in Table 3.

Table 3

The results of water quality parameters.

Treatment	Temperature (°C)	Ph	DO (mg/L)
A	28,2 - 30,8	7,2 - 8,44	4,32 - 7,73
B	28,2 - 30,8	7,3 - 8,39	4,65 - 7,02
C	28,2 - 30,8	7,1 - 8,37	4,51 - 5,61
D	28,2 - 31,5	7,1 - 8,46	4,03 - 5,07
E	28,1 - 31,0	6,9 - 8,36	4,12 - 6,43

The results measurement of water quality parameters in Table 3. The lowest temperature was obtained based on measurement results of 28,1 °C, while the highest temperature of 31,5 °C. The lowest pH value of 6,9 and the highest pH value amounted to 8,46. The results measurement of DO indicates that the lowest value of 4,03 mg/L and the highest grade of 7,73 mg/L.

3.1.4. Phytochemical screening

Phytochemical screening in mangrove leaves infusion *E. agallocha* include flavonoids, alkaloids, terpenoids, tannins, saponins, and steroid. The results of the phytochemical screening can see in table 4.

Table 4

The result of phytochemical screening.

Name	Result	Description
Flavonoids	+	Detected
Alkaloids	-	Not Detected
Terpenoids	-	Not Detected
Steroid	-	Not Detected
Tannins	+	Detected
Saponins	-	Not Detected

Phytochemical screening in the mangrove leaves infusion *E. agallocha* to contain tannin and flavonoid. The other compounds, such as alkaloids, Terpenoids, steroids, and saponins, are not detected in the mangrove leaves infusion *E. agallocha*.

3.2. Discussion

3.2.1. Weight gain of tilapia juveniles

Phytochemical screening shows that the active compound from the mangrove leaves infusion *E. agallocha* showed the presence of tannins and flavonoids. Tannin allegedly is the cause of the existence of a decrease in body weight of seed fish tilapia. Pangesti et al. (2016), stated that tannin has a role in lowering the weight of the body, the digestibility and efficiency of feed consumed. Tannins can injure the digestive, so it causes disruption the functions of the digestive tilapia juveniles. The compounds contained in the tannin which binding proteins, causing feed becomes difficult to digest. The same thing is expressed by Wie Lawa and Lazarus (2015) if tannins have properties that can be bound to a protein feed so that it forms a complex bond that is difficultly soluble in water, causing turbidity, and inhibits the enzymes work in the body.

3.2.2. The survival rate of tilapia juveniles

The results of phytochemical screening show that mangrove leaves infusion *E. agallocha* contain flavonoids and

tannins. Flavonoids and tannins are known to have many benefits. Flavonoids have a role as an immunostimulant that can prevent against infection that goes into the body (Abror et al., 2018), antioxidants (Prameswari & Widjanarko, 2014), and antibacterial (Rosidah & Afizia, 2012). The potential of tannin, among other antibacterials (Setyawan, 2015), antioxidants (Prameswari & Widjanarko, 2014), and antibacterial (Rosidah & Afizia, 2012). However, the potency of flavonoids and tannins are not to see in the survival rate of tilapia juveniles. The dose of tannin and flavonoid contained in mangrove leaves infusion *E. agallocha* is allegedly still low, and the time of observation is less long.

The value of the survival rate between the treatment does not show different results, where the value is above 70% and achieving 100%. Tilapia juveniles infected *A. hydrophila* bacteria can survive; this is alleged because of a good environment. Tantu et al. (2013), state that good environmental control in aquaculture can prevent the spread of the disease. The spread of the disease in aquaculture can be prevented by the water quality maintained, and also, the quantity and quality of feed is a part of the efforts of disease prevention.

3.2.3. Water quality parameters

Water quality is one of the factors that an important in the life of the fish. Water quality parameters include temperature, pH and DO (dissolve oxygen). The temperature affects the appetite of fish. Appetite fish increased simultaneously with the increase in temperature. The temperature decrease has an effect on the digestive process in the body of the fish and metabolism process will run slower. The pH value can disrupt the lives of fish if pH values in the water that are too low (very acidic) and too high (highly alkaline). Dissolve Oxygen (DO) is required by fish to respiration, metabolism and maintain his life (Effendi, 2003).

The results of measurements of water temperature are 28.1 °C - 31.5 °C. The pH measured ranged from 6.9 – 8.46, while the range measurement results of DO is 4.03 mg/L - 7.73 mg/L. The range values of the water quality measurement are still in the normal range, as the value of water quality issued by the Badan Standardisasi Nasional (2009), the temperature (25 °C - 32 °C), pH (6.5-8.5) and DO (≥ 3 mg/L).

Kordi and Tancung (2007), state that pH value in aquaculture in the range 6,5 – 9. The ranges optimal temperature for aquaculture between 28 °C - 32 °C. Certain types of fish can survive with the DO (dissolve oxygen) value 3 mg/L. Dissolve oxygen with a value below 4 mg/L, would cause a decrease in appetite fish, although the fish can still survive.

3.2.4. Phytochemical screening

Metabolites secondary of the mangrove leaves infusion *E. agallocha* based on the results of the phytochemical screening, namely flavonoids and tannins. Some researchers state that the mangrove leaf *E. agallocha* contains flavonoids (Patra, 2009, Kumar et al., 2013) and tannins (Patra, 2009; Kumar et al., 2013; Kalamurthi & Selvaraj, 2016).

4. Conclusion

The research showed that there was no difference in the influence of the mangrove leaves infusion *E. agallocha* against weight gain and survival rate of tilapia juveniles. The result of phytochemical screening indicates that *E. agallocha* leaves infusion contains tannins and flavonoids.

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