The effectiveness of katuk leaves extract (*Sauropus androgynous*) as an antibacterial *Vibrio* sp. on the survival rate and growth of vannamei shrimp larvae (*Litopenaeus vannamei*)

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**Abstract.** Vibriosis disease caused by the *Vibrio* sp. bacteria is one of the problems faced by shrimp farmers, namely the decreased survival rate during the larval period. This study aimed to determine the effect of the ethanol extracts of katuk leaves (*Sauropus androgynous*) as an antibacterial material on the survival rate and growth performance of vannamei shrimp larvae (*Litopenaeus vannamei*). This research was conducted at PT. Bibit Unggul Global Gen, Pantai Cermin, Serdang Bedagai North Sumatra, March - April 2019. The extraction evaporation process is carried out at the Unsyiah Faculty of Teacher Training and Education Chemistry Laboratory. Data were analyzed using the Completely Randomized Design (CRD) method with 5 treatment levels and 4 replications. The concentration treatment were is: 0 ppm, 600 ppm, 800 ppm, 1000 ppm and 1200 ppm. The sample used was vannamei shrimp larvae which had been infected with vibrio bacteria, then submerged with katuk leaf extract for 8 minutes. The parameters measured in this study were survival rate (SR%), absolute weight growth (g) and absolute length growth (mm), Total Vibrio Count (TVC CFu/ml) and water quality. The ANOVA test results obtained that the katuk leaf extract (*Sauropus androgynous*) had a significant effect on survival (SR) and Total Vibrio Count (TVC) in vannamei shrimp larvae (P<0.05) and had no an significant effect on absolute weight growth and absolute length growth (P>0.05). In this study indicated that concentration 1200 ppm produced the highest the survival rate (93%) and the lowest amount of TVC (533 CFU/ml).

1. **Introduction**
The Vannamei shrimp has an important role in supporting the needs of animal protein for humans [1]. Vannamei shrimp cultivation has increased from year to year. However, farmers often have problems during the hatchery period, one of which is the water quality factor. Water quality is one of the most important factors in hatchery due to the condition of the seeds or larvae which are very vulnerable to the environment. Poor water quality parameters can trigger the growth of bacteria and fungi, resulting in low egg hatching rate and larval survival rate. According to Djunaedi *et al.* [2] the survival and level of shrimp production will decrease if there is environmental pollution and climate change.
According to Pratama et al. [3] the problem that often occurs in shrimp farmers is the presence of vibriosis which attacks vannamei shrimp larvae, thus causing decreased larval survival. Kusumaningrum et al. [4] states that vibrio bacteria are gram-negative bacteria that can cause acute and dangerous diseases because they can kill a population of infected shrimp larvae for 1-3 days. Efforts to eradicate and control bacteria while using chemicals and drugs, however, have not obtained adequate results and are actually not recommended. Therefore, the solution to increase the survival of shrimp larvae is to provide herbal ingredients that have immunostimulating properties and inhibit disease.

Research on the use of plant extracts as anti-bacterial and fungal has been conducted by many researchers and can be used as an immunostimulant that does not cause resistance [5]. One of the plants that is immunostimulant is katuk leaves. According to Ramadheni et al. [6] katuk leaves (Sauropus androgynous) have many benefits in life, namely protecting cell structures, anti-inflammatory, and as a natural antibacterial. Katuk leaves act directly as antibacterial by disrupting the function of microorganisms (bacteria or viruses) and can increase body immunity. Therefore, this study was conducted to analyze the effect of the ethanol extracts of katuk leaves (Sauropus androgynous) as an antibacterial material on the survival rate and growth performance of vannamei shrimp larvae (Litopenaeus vannamei).

2. Material and Methods
This research was conducted at PT. Bibit Unggul Global Gen Pantai Cermin, Serdang Bedagai, North Sumatra, in March - April 2019. The extract evaporation process was carried out at the Chemistry Laboratory of the Faculty of Teacher Training and Education, Unsyiah. The main tools used in the research were a jar 15 L, pH meter, Thermometer YSI 550A, DO meter YSI 550A, aeration, mesgerate rotary evaporator, an incubator and laminar flow. The materials used were vannamei shrimp larvae, katuk leaf extract, Vibrio sp, TCBSA media, aquades, and NaCl. The study used a completely randomized design (CRD). In vivo test (immersion) with 5 treatment levels and 4 repetitions. The concentration of katuk leaf extract used was treatment A (0 ppm), B (600 ppm), C (800 ppm), D (1000 ppm) and E (1200 ppm).

2.1 Preparation of Sauropus androgynous Extract
Katuk leaf extraction is carried out by maceration and evaporation methods to produce pure katuk leaf extract. The katuk leaves that are obtained first are washed clean and in the air, after drying, they are mashed with a blender and soaked in a closed jar with 95% ethanol solvent. The ethanol extract of katuk leaves is filtered and evaporated using a rotary evaporator (evaporated) to produce 100% pure extract [6].

2.2 Determination of Minimum Inhibitory Concentration
The minimum inhibitory concentration was determined by adding the extract concentration to a petridish with bacterial isolation and incubating it at 32°C for 24 hours, then observing the bacterial colony so that the spread of bacteria in the petri dish could be known. The clear area shows the sensitivity of the bacteria to the concentration of the extract used as the test material which is called the width of the inhibition zone diameter.

2.3 Experimental of Vannami Shrimp Larvae
The shrimp size of 8.1 mm total length and 0.01 gramin body weight. Vannami shrimp larvae infected with Vibrio sp. aged 8 days (PL 8) with a stocking density of 100 individu/container. The shrimps larvae were immersed in katuk leaf extract at different concentrations for 8 minutes. Observation of shrimp larvae after immersion was carried out for 14 days. Shrimp are aerated and given commercial feed based on the number of stocking densities of 2 grams per feeding. Feeding is carried out 9 times a day, namely every 5 am, 7 am, 9 am, 11 am, 1 pm, 3 pm, 5 pm, 7 pm, 9 pm and 11 pm.

2.4 Research Parameters
Vibrio Count (TVC) on the hepatopancreas and water quality parameters observed included pH, DO, temperature and ammonia. The survival rate (SR) was calculated based on Goddard [7] as follow: \( SR = \frac{N_o - N_t}{N_o} \times 100 \), where SR is survival rate (%); \( N_o \) is total shrimp at the start of research; \( N_t \) is total of shrimp at the end of the research.

The absolute length was calculated based on Muchlisin et al. [8] as follow: \( L = L_t - L_o \), where: \( L \) = absolute length (mm); \( L_t \) = biomass length at the end of the research (mm); \( L_o \) = biomass length at the start of the research (mm)

The absolute weight growth was calculated based on Muchlisin et al. [8] as follow: \( W = W_t - W_o \), where: \( W \) = absolute weight growth (g); \( W_t \) = biomass weight at the end of the research (g); \( W_o \) = biomass weight at the start of the research (g)

Calculation of TVC (Total Vibrio Count) on the hepatopancreas of shrimp larvae is done by grinding the hepatopancreas and spreading it into TCBS media. TVC calculations use the formula [9]. Number of bacteria (CFU/ml) = number of colonies x volume of sample x dilution factor

\[ \text{Survival Rate} = \frac{N_o - N_t}{N_o} \times 100 \]

\[ \text{Absolute Length} = L_t - L_o \]

\[ \text{Absolute Weight Growth} = W_t - W_o \]

\[ \text{Total Vibrio Count (TVC)} = \text{Number of colonies} \times \text{Volume of sample} \times \text{Dilution factor} \]

2.5 Data analysis
Data analysis used the one-way analysis of variant (one-way ANOVA) test and followed by LSD test (Least Significant Difference) for survival rate parameters and Duncan's test for Total Vibrio Count (TVC) parameters.

3. Results and Discussion
The ANOVA test results obtained that the katuk leaf extract significant effect on survival rate (SR) and Total Vibrio Count (TVC) in vannamei shrimp larvae (\( P<0.05 \)) and had no significant effect on absolute weight growth and absolute length growth (\( P>0.05 \)). The LSD test showed the best treatment for survival rate found at treatment 1200 ppm, this values has significant different with treatment 600 ppm and control. The Duncan’s test recorded the best treatment for TVC were also at treatment 1200 ppm, the values were significant different from other treatment (Table 1).

Table 1. The value survival rate, growth performance and total vibrio count (TVC) of vannamei shrimp larvae (Litopenaeus vannamei)

| Treatment | Survival Rate (%) | Absolute Length Growth (cm) | Absolute Weight Growth (g) | Total Vibrio Count (TVC) (CFU/ml) |
|-----------|------------------|-----------------------------|---------------------------|----------------------------------|
| Control   | 52 ± 9.83a       | 7.22 ± 2.14                 | 0.003±0.09                | 1713.33±347.43c                  |
| 600 ppm   | 70 ± 6.97b       | 6.82 ± 1.93                 | 0.005±0.01                | 1058.33±284.58b                  |
| 800 ppm   | 80 ± 2.16bc      | 9.05 ± 0.50                 | 0.004±0.01                | 883.33±182.20b                   |
| 1000 ppm  | 79 ± 7.50bc      | 8.93 ± 0.49                 | 0.005±0.09                | 964.99±104.61b                   |
| 1200 ppm  | 93 ± 4.24c       | 7.20 ± 0.75                 | 0.004±0.08                | 533.33±128.92a                   |

Table 1 showed that the high survival rate value because katuk leaves function as antibacterial which can inhibit the growth of vibrio bacteria, but katuk leaves are not able to increase performance growth. this can be seen in the growth parameters which show insignificant value. Saptiani et al. [10] states that the Avicennia marina extract can increase the survival of post larvae of tiger prawns 60-88% at a concentration of 1250-1500 ppm. The study of Samad et al. [11] also proved that katuk leaf extract can increase the survival of mud grouper 80% at a concentration of 1000 ppm. The study of Tjandrasa et al. [12] showed that giving katuk leaf flour to the feed ration does not have a positive impact on absolute weight growth and absolute length growth.

The study showed that the use of katuk leaf extract can reduce bacterial growth. In the treatment of 1200 ppm katuk leaf extract was able to reduce the total vibrio count (TVC) compared to other treatments. Susantiet al. [13] stated that the phytochemical screening test conducted showed that katuk
leaves contained alkaloid, saponin, tannin, polyphenol and flavonoid compounds that could inhibit bacterial growth. According to Santoso [14] tannin compounds can inhibit bacterial cell synthesis and protein synthesis of gram-positive bacterial cells. Flavonoid compounds have the ability to damage the plasma membrane and at low concentrations these compounds can damage the composition of the bacterial cell walls, but at high concentrations these compounds can cause protein coagulation so that protein denaturation occurs [15]. Water quality parameters in this study are still the tolerance range for vannamei shrimp[16].

4. Conclusion
Katuk leaf extract (Sauropus androgynous) is effective in increasing the survival rate of vannamei shrimp and can inhibit the growth of Vibrio sp. The best treatment was obtained in the treatment of 1200 ppm katuk leaf extract.

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