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Effectiveness of extract Songga wood (*Strychnos lucida*) towards survival and antibody titers on Tilapia (*Oreocromis niloticus*) that infected *Streptococcus agalactiae*

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ABSTRACT

Nile Tilapia or Tilapia Fish (*Oreochromis niloticus* Linnaeus, 1758) is one of commodity with a high number of consumers. But, *Streptococcus agalactiae* attack caused a decline in production. One way to reduce these diseases by using natural materials of songga wood (*Strychnos lucida* R.Br) where the songga wood contains many compounds like alkaloid, phenols, flavonoids, triterpenoids, which can inhibit bacterial growth by damage cell membrane. The results showed that the inhibitory songga wood extract against *Streptococcus agalactiae* was highly significant, and shows with the highest survival rate of 90 % and proved by the increasing antibody titers after adding songga wood extract.

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1. Introduction

Nile Tilapia Fish (*Oreochromis niloticus* Linnaeus,1758) is one commodity fresh water with a high number of consumers in Indonesian. The high interest of consumers create a great opportunity to the farmers of tilapia fish. On the other side, tilapia fish farming has advantages such as tolerant of poor water conditions and can be cultivated with high stocking density or intensive system. However, an attack of bacterial diseases such as *Aeromonas hidropilla* and *Streptococcus agalactiae* bacteria that cause disease streptococcosis become an obstacle in cultivation and lead to a decline in production of aquaculture (Afrianto *et al.,* 2015).

Streptococcosis disease is a fish disease caused by the bacterium *S. agalactiae* which is a bacteria that attacks the red blood cells in fish. *S. agalactiae* has two types: type β-hemolytic and non-hemolytic. It is non-hemolytic bacteria have a higher virulence than the type of β-hemolytic views from death and the speed of onset of clinical symptoms from day-3 to day-15. Symptoms of *S. agalactiae* among others, changes in macroscopically and microscopy on the liver, brain, kidneys, and blood. This bacterium causes the deaths of up to 60% on tilapia fish farming in South Sumatra (Yuasa *et al.*, 2008). Treatment of a bacterial disease that is often done by using antibiotics.

However, One way to reduce the bacterial disease is by using natural materials. Natural materials have been applied to inhibit the growth of bacteria include mangrove bark (Pradana *et al.*, 2015), curcuma and turmeric (Samsundari, 2010), leaves of mangrove (Putri *et al.*, 2016), Liman leaves (Monalisa, 2010). Songga wood an endemic that comes from the Nusa Tenggara Bara island. This wood contains are alkaloids, phenols, flavonoids, saponins, steroids, tannins and triterpenoids that are proven to inhibit the growth of bacteria by damaging the cell membrane as well as his ability as an anti-bacterial. The research of Sarmento (2015), which shows that at doses of 32–128 ppm MIC can inhibit bacterial growth. This research is very important to know the antibacterial potency of songga wood through in vitro test and in vivo tests as measures for disease prevention streptoccosis.

2. Material and methods

Songga wood comes from Bima, Nusa Tenggara Barat island. Songga wood that we used is rod. The rods are powdered by using milled. *S. agalactiae* is a gram-positive bacteria get from BPAT Bogor with Non-Hemolytic strains. Culture bacteria on solid media and liquid media BHIA NB and upgrade the violence power by injecting the fish, wait until clinical symptoms and taken organs such as the liver, gills, heart and then, culture it on BHIA media and stored at a temperature of 30–37 °C.

2.1. Power test inhibitory.

Power Inhibitory test conducted by diffusion method (absorption). Using the filter paper and compare the area of the inhibition is formed. Calculation of the diameter of the inhibition can be seen there is shown Figure 1:

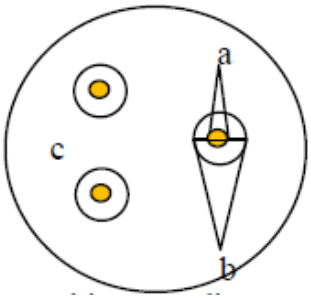

Figure 1. Power calculation Inhibition

From the picture above explains that a picture is a paper disc soaked extract. Figure 1, (b) is the diameter of clear zone and the image c is a medium that has been overgrown with bacteria. Formula diameter of inhibition: b–a (Agglutination test / observation antibody titer). Method uses Thune and Plumb. Is a way of marking antigen antibody to be destroyed.

2.2. Survival.

Survival / Survival Rate is the percentage of the number of individuals living at the end of the period with individuals living at the beginning of the period (Afrianto *et al.*, 2015).

3. Results and Discussion

3.1. Extract Songga wood.

Songga wood that has been milled and a powder prepared for maceration process using methanol solvent 90 %. According to Harbone (2006), maceration is the process of soaking the sample with an organic solvent which is doing at room temperature. Through the soaking process will damage the cell wall because of the pressure difference between the inside and outside of cells, so the cytoplasm of secondary metabolic compounds dissolved in organic solvents through the process of diffusion.

Maceration is done with weigh 500 grams of powder sticks songga in 1500 ml of methanol at a ratio of 1: 3 is left for 1 x 24 hours and stored at room temperature. The extract was filtered using filter paper to separate the filtrate and precipitate. The filtrat that have been filtered is collected in at *Buchner tube* and evaporate using a vacuum rotary evaporator at 40 °C until get the concentrated extract. According to Pradana *et al.*, (2015) the solvent extraction mechanism is to penetrate the cell wall and into the cell cavity containing the active substance. The active substance is dissolved because differences between the solution concentration of the active substance in the cell and outside the cell, so that the concentrated solution is urged to come out.

3.2. Power inhibition test.

Disc method is a method used in the observation of inhibition, the filter paper disk containing a antibacterial component with particular concentration placed on agar plates that had been planted bacteria. The width of the area that inhibit dependen whether or not the absorption of antibacterial substances and bacterial sensitivity to these substances. The following chart picture inhibition test:

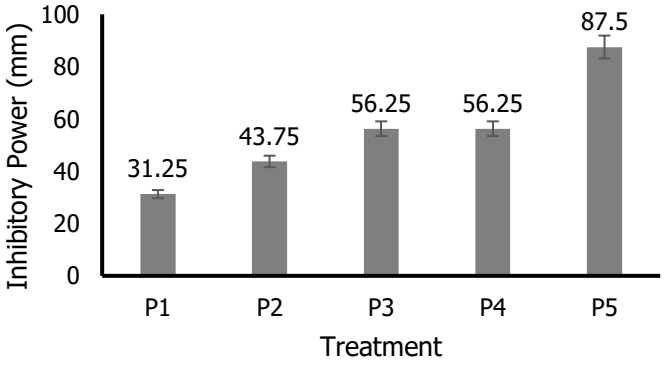

Figure 2. Graph Test Inhibitory power bakteri *S. agalactiae*

Figure 2 shows that the treatment P1 (0 %) concentration with paper discs (6 mm diameter) do not contain wood extracts songga and can not inhibit the growth of *S. agalactiae* because distilled water (aquades) does not have an active substance that is able to inhibit the growth of bacteria. P2 treatment (5 %) Average inhibition zone reached 4.25 mm. P3 treatment (10 %), average 6.625

mm inhibition zone and P4 (15 %) Average inhibition zone 10,75 mm. While on treatment 20 % average inhibition reached 13,62 mm and a wood extract dose songga with the highest inhibitory.

Calculation Results of analysis variance showed that the extract songga wood with different concentrations give highly significant effect on the inhibition of the growth of *S. agalactiae*, extract Songga wood with different concentrations give highly significant influence to the area of inhibition for the growth of *S. agalactiae*. While real difference test results show if treatment P1 (0 %) highly significant with P2 treatment (5 %), treatment is highly significant P2 with P3 (10 %), P4 (15 %) and P5 (20 %). It can be concluded that the higher the concentration of extract songga wood willmake the larger diameter of inhibition zone.

S. agalactiae is a gram-positive bacteria where there are differences in the sensitivity of antibacterial effected by differences in the types of bacteria cell walls. The cell walls of gram-positive bacteria are relatively simple, consisting only of peptidoglycan and teikoat acid. Damage to the cell wall of gram-positive that inhibit the growth of gram-positive bacteria by like dissolved like system. Peptidoglycan component consisting of proteins and carbohydrates that are polar will be easier to be penetrated by a polar compound. This is causing gram-positive bacteria is more easily penetrated by the antimicrobial compounds in comparison to gram-negative bacteria (Hardi *et al.*, 2011). This is because the higher concentration of antimicrobial active compounds then extracts contained in the extract more and more. so the ability to inhibit the growth of microbes increasingly higher (Pradana *et al.*, 2015).

3.3. Survival.

Test challenge conducted on day 16 of the research. This test chalenge purpose to determine the ability of extract Songga wood produce immunity that protects against a specific pathogen. Here's the graph of survival rate of tilapia during the research:

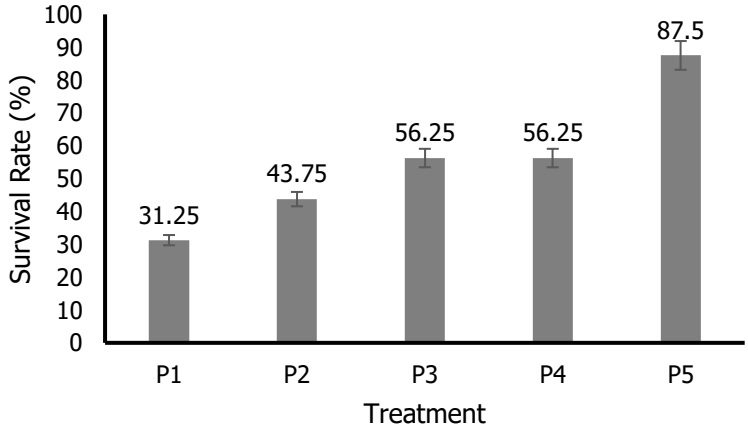

Figure 3. Survival graphs Tilapia (*Oreochoromis niloticus*)

Figure 3 shown that the survival rate of tilapia which is highest in the treatment P5 (20 %) with a survival rate reached 87.50 mm, then the survival rate of P3 (15 %) with a survival rate reached 56.25 %, P4 (15 %) survival rate of 56.25 %, P2 (10 %) with a survival rate of 47.75 %. While the lowest survival rate contained in the P1 (0%) reached 31.25 % survival rate at which P1 is a control treatment without extract wood.

Results of variance analysis calculation in the table shows that the extract songga wood with different concentration make significant effect on the survival rate of tilapia fish (*O. nilotikus*) that infected bacteria *S. agalactiae*, where F table at the level of 0.1 < F arithmetic <F table at level 0.05 marked with * the treatment significantly. Data showed that adding songga wood extract can prevent pathogenic attack on tilapia fish (*O. nilotikus*). The BNT test showed that the treatment P1 (0 %), P2 (5 %), P3 (10 %) and P4 (15 %) did not give different significantly, and very significantly different from P5 treatment (20 %).

Clinical symptoms and mortality arising faster than treatment with wood extract Songga. Between 5 to 20 % P5 treatment provides a higher protection. Survival at P3 and P4 each is 56.25 %. This is due to the treatment replicates U3 and U4 P4 low water temperature ranges between 24.91–26.6 ˚C. SNI (2009), the optimal growth temperature ranges from 26-32 ˚C tilapia fish. The water temperature is very low can cause stress that cause decreased appetite so that the use of food energy is not efficient and the amount of food given was not able to meet the energy needs for the metabolic processes (Daniswara, 2008).

Of all the treatments, treatment P5 with a concentration of 20 % has the highest survival rate reached 87.50 %. It is the same as antibody titer test and inhibition test. In the inhibition test, the extent of inhibitory area highest of all treatments are at P5 (20 %) with a diameter of 13.62 mm reach. While the antibody titre testing P5 treatment (20 %) resulted in higher antibody titers after adding songga wood extract and after the challenge test. This show that these concentrations may protect tilapia fist against bacteria *S. agalactiae*. According to Karlina *et al.*, (2013), a good survival rate of keeping requirements with less than 50 % mortality. Tilapia fish survival rate in this researchmet the requirements for a good keeping is the treatment P5 of 87.50, and treatment P3 and P4 with a survival rate of 56.25. While the survival rate of less than 50 % in P2 with a survival rate of 43.75 % and P1 survival rate of only 31.25 %. This is because of the concentration extract songa wood is different.

3.4. Measurement of antibody titer.

From the observation test antibody titers can be seen that the first antibody titer testing without adding songga wood extract showed that the fish has a titer antibodies by agglutination low in all treatments. This happens because of the possibility of such fish never got a kind of bacteria that can form antibodies (Nair *et al.*, 2008). Grindstaff *et al.*, (2009) explains that the humoral defense system likely to be derived from the parent to the child. In observation of a second antibody titers after adding songga wood extract with different concentrations increased antibody titer production in all treatments with agglutination low to high. However, in the control treatment, antibody titers produced little and agglutination of low to medium. The third observation of antibody titers, titers of antibodies produced fluctuations.

On replay P1, P3, and P4 titer antibodies produced fewer than observation after adding Songga wood extract. While on P1 and P3 not there is an increase or decrease in the production of antibody titers. This can be caused by bacteria *S. agalactiae* that injected to tilapia fish on repeat treatments have never been attacked by the bacteria *S. agalactiae*. However, production of the antibody titer higher than the first observation of wood without the extract. On repeat treatments P1, P2, P3, P4, and P5U4 titer antibodies produced antibody titer higher than the first and second take. The resulting antibody titer fluctuations. On replay P1, P3, and P4 titer antibodies produced fewer than observation

after adding songga wood extract. While on P1, and P3 not there is an increase or decrease in the production of antibody titers. This can be caused by bacteria *S. agalactiae* that injected to tilapia fish on repeat treatments have never been attacked by the bacteria *S. agalactiae*. However, production of the antibody titer is higher compared to the observations of the first grant of without the extract of the wood. On the treatment of deuteronomy P1, P2, P3, and P5 high-titer antibodies produced antibody titer than taking first and second.

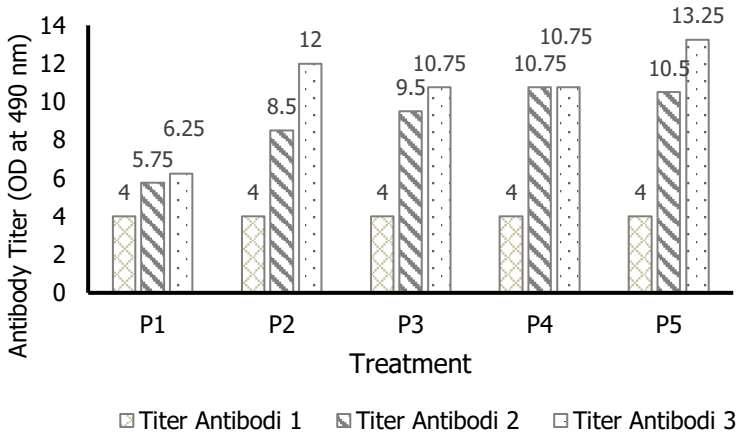

Figure 4. Diagram antibody titer during observation research

According to Syafitri (2012), Blood serum containing antibodies and antigens can work against pathogens, where the antibodies are T cells and B cells, B cells play a role in immunoglobulin through the stimulation of antigen produced by the spleen and liver as well as being the receptor of antigen. Meanwhile, the T cells in the antibody works to improve and simplify fagositosit help to destroy bacteria membran. According to Siregar *et al*. (2012), the mechanism of action of antibodies in the inactivation of antigen in three stages: netralilisation (wrapping bacteria), agglutination clotting particles containing antigens.

In previous research, The antibody titer after challenge test having increase in fish plus songga wood extract. The increase in antibody titer is because at the time of exposure to the same antigen at the time of challenge test, the immune response will occur faster with higher antibody production than the first infection (Verma and Agarwal, 2005). Production of high and stable antibody titer contained in P5 treatment with concentration 20 % compared to other treatments and the lowest in P1 treatment with a concentration of 0 %. The production of these antibodies affect the survival of tilapia fish, which is highest survival rate at P5 (20 %) and the lowest survival rate in the control treatment P1 (0 %). This shows that songga wood extract can increase the production of antibody titers in tilapia fish (*O. niloticus*).

3.5. Clinical symptoms.

The table shows that in treatment P1 control clinical symptoms of fish occurred on day two post-injection with a decline appetite fish and started a change warrants the body (+), At the end of the reasearch treatment P1 experience eksoptalmia that there is damage to the eyes, where the fish eye become larger and predominantly white of the cornea fish. According to Evans *et al.* (2006), symptoms are present in bacteria-infected fish eyes *S. agalactiae* was opacity and purulens but can also cause eye lysis. *S. agalactiae* spreads in the eye that cause hypertrophy, this is what causes the fish to experience eksoplatmia and other changes. End of the reasearch very low survival rate

reached 40 %. On day-7 post-injection P2 appetite began to normal. P3 treatment (10 %) symptoms at the treatment P2 (5 %), but the replay P3 appetite improved on day 7 post-infected. Treatment P4 (15 %) the average peak of clinical symptoms occurred on days 3 and 4 post-injection, and appetite began to improve in the treatment P4, while in treatment 5 (20 %) the peak of clinical symptoms on day-3 and day-4 research, and appetite began to improve on day-7 post-injection and occurred in all treatments. This is in match with the opinion of Yuasa *et al.*, (2008) which states that the death and the speed of onset of clinical symptoms of bacterial *S. agalactiae* (Table 1).

Table 1. Clinical Symptoms After injection of bacteria *Streptococcus agalactiae*

	Infected Tilapia Fish (*Oreochromis nilotikus*)																			
Day	P1 (0 %) Control				P2 (5 %)				P3 (10 %)				P4 (15 %)				P5 (20 %)			
To-	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	+	-	+	-	-	+	-	-	-	+	-	-	-	+	+	-	-	+	-
3	+	+	+	++	+	+	++	+	+	+	++	+	+	+	++	++	+	+	++	+
4	++	++	+	++	+	++	+	++	++	+	++	+	++	++	++	+	+	+	++	+
5	++	++	+	++	++	++	+	+	+	++	+	+	+	+	+	+	+	+	+	+
6	++	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	++	++	+++	+	-	+	-	-	-	++	+	-	-	+	+	-	-	-	-

Information:
- : Appetite and normal body color
+ : Body color black and decreased feeding response
++ : Wounds on several parts of the body and tail eroded
+++ : An eye become enlarged and damaged

3.6. Water quality.

Overall water quality during the research is still in the normal standard in apropiate with the SNI, the following table water quality observations during the research (Table 2).

Table 2. Water Quality Observations During the research

Water Quality Parameters	Air Quality During Maintenance	Water Quality for Tilapia Fish	Reference
temperature OC	24.82 to 28.49	25-32	SNI 7550: 2009
pH	6.54 to 7.56	6.5 -8.5	
DO mg/L	4.26 to 5.36	> 3	

4. Conclusion.

Concentraion songga wood extract very significant effect on the inhibition of the growth of the bacteria *Streptococcus agalactiae*. Best concentration songga wood extract against *Streptococcus agalactiae* bacteria found in treatment P5 (20 %) with the inhibition of 13.625 mm strong inhibition category. Survival tilapia fish with songa wood extract significant increasing antibody titers after adding the extract.

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