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Immersion of Noni Extract Improves Recovery and Reduces Bacteria in Catfish

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ABSTRACT

Catfish is one of the superior fisheries sectors in the market, but the obstacle often faced by catfish farmers is disease attacks. One of the diseases that often attack catfish is the *Aeromonas hydrophila* bacteria. An alternative that can be used in the treatment of bacteria in catfish is to utilize antibacterial compounds found in noni fruit (*Morinda citrifolia*). The purpose of this study was to determine the effective dose of noni fruit solution soaking in the treatment of catfish infected with *Aeromonas hydrophila* bacteria. This study used a completely randomized experimental design method with 4 treatments and 3 replications carried out for 17 days and was infected intramuscularly with a concentration of 10^6 CFU/ml of 0.1 ml, with test treatments A (Control), B (30 g/L), C (45 g/L) and D (60 g/L) and soaking using the short bath method for 20 minutes. The results obtained from the study will be subjected to analysis of variance (ANOVA). The results of the study showed that the effective dose was found in treatments B (30 g/L), C (45 g/L) and D (60 g/L) with the observed variables of fish recovery and survival rates being 100%, while the optimal dose for reducing the number of bacteria was found in treatment B (30 g/L) in the kidney section of 0.72 x 10^7 with a reduction of bacteria of 76.8% while in the liver section it was 0.57 x 10^7 with a reduction of bacteria of 80.1%.

INTRODUCTION

Catfish is one of the leading fisheries sectors in the market. According to data from the Indonesian Ministry of Maritime Affairs and Fisheries, national catfish production in 2023 will reach approximately 1.2 million tons, with an average annual growth rate of 5%. (KKP, 2023) (Marina *et al.,* 2025). However, a common challenge faced by freshwater fish farmers is disease outbreaks (Fidyandini *et al.,* 2021).

Diseases in fish farming occur due to infections that injure the fish's body parts, making them more susceptible to diseases such as fungi, viruses, and bacteria. One particularly dangerous disease is bacterial infection, or bacterial disease (Listia *et al.,* 2022). Bacterial disease is an infectious disease that can cause death in fish within a short period of time. One bacterial disease that attacks catfish is *Aeromonas hydrophila* (Rahmawati *et al.,* 2021).

Aeromonas hydrophilla is a pathogenic bacterium that causes *Motile Aeromonas Septicemia* (MAS), primarily in freshwater fish species. This bacterium is an opportunistic pathogen, attacking the body when the immune system is weakened, giving it the opportunity to infect the body. *Aeromonas hydrophila* can cause very rapid mortality, reaching 80% to 100% within 1 to 2 weeks in catfish (Andrianti *et al.,* 2023). In Southeast Asia, the first outbreak of the *Aeromonas hydrophila* bacterial disease occurred in West Java in 1980, causing 82.2 tons of deaths within 1 month (Anwar, 2025). One alternative that can be used to treat bacteria in catfish is to utilize the antibacterial compounds found in noni fruit (*Morinda citrifolia*).

The noni fruit (*Morinda citrifolia*) is a plant with the potential to be used as a natural antibiotic. The alkaloid, flavonoid, and saponin compounds in noni can be used as antibacterial agents. The antibacterial alkaloid compounds (xeronine and proxeronine) found in noni fruit can act as immunostimulants aimed at boosting the immune system of fish (Sudewi & Lolo, 2016). Ripe noni fruit contains antibacterial compounds that are effective against *Pseudomonas aeruginosa*, *M. pyrogenes*, *Salmonella typhosa*, *S. Montevideo*, *S. schottmuelleri*, *Shigella paradys*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium perfingens*, and *Vibrio parahaemolyticus* (Akbarurrasyid, 2023).

Based on the above explanation, further research is needed on the effectiveness of noni fruit (*Morinda citrifolia*) solution immersion on catfish (*Clarias* sp.) infected with *Aeromonas hydrophila* bacteria by observing the calculation of the number of bacteria in the target organs of the liver and kidneys, the level of fish recovery, and survival.

METHOD

Time and Place

The research was conducted from December 2025 to January 2026. Bacterial isolation was carried out at the Pharmaceutical Microbiology Laboratory, Faculty of Sports and Health, Gorontalo State University.

Preparation of Containers

The container used in the study was an aquarium, 12 aquariums were used with dimensions of 30 x 30 x 30 cm3 with a water volume of 13 liters per aquarium. To reduce the possibility of contamination, the aquariums were first sterilized so that research conditions could be maintained. The aquariums were cleaned by washing them first with clean water, then sterilized using a 0.1 ml methylene blue solution dissolved in 1 liter of water, then left for 24 hours, and then rinsed with running water. After the cleaning process, the aquariums were filled with water and aerated.

Preparation of Test Fish

The catfish used in this study measured approximately 7-10 cm, with 10 per aquarium, with four treatments and three replications, for a total of 120 fish in the 12 aquariums used. Before being stocked into the aquariums, the fish underwent acclimatization and were fed twice daily according to their weight.

Bacterial Injection Process

The bacteria used in this study were pure isolates of *Aeromonas hydrophila* bacteria from Indilab Scientific Samarinda. The process of injecting *Aeromonas hydrophila* bacteria into catfish (*Clarias* sp.) used a bacterial concentration of 10^6 cfu/ml, with injections carried out on all catfish in each aquarium with a total culture of 0.1 ml. (Andrianti *et al.,* 2023), injection is done intramuscularly Ardulanisa *et al.,* (2017).

The Process of Making Noni Fruit Juice

Noni fruit is a plant that has the potential to be used as a natural antibiotic. It contains compounds that play an important role as antibacterials. One way to utilize this is by using the antibiotic compounds found in noni fruit (Geofani *et al.,* 2022). The method for making noni juice is as follows:

1. The mangosteen fruit used is ripe fruit that appears transparent, white in color, and soft in texture.
2. Wash the noni fruit thoroughly, then dry it until there is no water on the surface of the fruit.
3. Next, cut it into several pieces according to the dosage, then add 1 liter of water to the blender and blend.
4. The blended mixture is then poured into a pot to be heated to 90°C.
5. After heating, filter the solution using filter paper and a sieve, then pour it into a soaking container to cool.

6. Next, the test fish are placed in a soaking container using the short bath method for 20 minutes (Ardulanisa *et al.,* 2017).

Soaking Process

Soaking fish that have been injected with Aeromonas hydrophila bacteria is carried out after a 48-hour bacterial incubation period using 3 containers for 3 treatments. Each container was filled with a different dosage of noni fruit solution, namely 30 g/liter, 45 g/liter, and 60 g/liter. The immersion was carried out using the short bath method for 20 minutes (Ardulanisa *et al.,* 2017), After the soaking process, the fish are transferred to a maintenance tank for further maintenance. The duration of further maintenance is adjusted to the duration of the acute infection period of *Aeromonas hydrophila* bacteria, which is 2 weeks.

Observation Variables

Calculation of Bacteria Count

Bacterial counts were performed twice to obtain observation results for each treatment. Counts were performed on day 0 post-infection and on the last day after infection, and treatment was carried out using four different treatments. According to Buda *et al.,* (2023) Calculate the number of bacteria using the following formula:

Number of Cells = V x n x 1/f

Where:

V : Volume of sample added
N : Number of colonies in the dish
f : Dilution Factor

Clinical Symptom Observation

Clinical symptoms were observed twice during the study, with the first observation conducted after the incubation period (48 hours) and the second observation conducted after treatment. Clinical symptoms were observed, including signs of slow swimming, pale skin color, excessive mucus, swelling of the abdomen, decreased appetite, and frayed tail fins (Damayanti *et al.,* 2024).

Fish Recovery Rate

The recovery rate of fish can be measured using the following formula:

$$\text{KI} = \frac{\text{Number of fish that recovered}}{\text{Number of fish that were infected}} \text{x } 100\%$$

Survival Rate

The survival rate (SR) of fish is the percentage of fish alive at the beginning compared to the number of fish at the end of cultivation. Survival can be calculated using the formula Effendie, 2002 in Akbarurrasyid, (2023) that is:

$$SR\ (\%) = \frac{Nt}{\text{N0}} X\ 100$$

Description:

SR : Survival rate (%)
Nt : Total number of fish alive at the end of the experiment (fishes)
N0 : Total number of fish at the start of the experiment (fishes)

Water Quality

The water quality parameters measured in this study were temperature, pH, and DO. Water quality measurements were taken at the beginning of the study and at the end of the study.

Data Analysis

To determine the effect of soaking in different doses of noni fruit solution on the treatment of catfish (*Clarias* sp.) infected with *Aeromonas hydrophila* bacteria, the results

obtained from this study were analyzed using analysis of variance (ANOVA) at a 95% confidence level.

RESULT AND DISCUSSION

Number of Bacteria in Catfish (*Clarias* sp.)

Based on the results of calculating the number of *Aeromonas hydrophila* bacteria in catfish (*Clarias* sp.) conducted at the Pharmaceutical Microbiology Laboratory, Faculty of Sports and Health, Gorontalo State University from the beginning and end of the study on treatments A (Control), B (30 g/L), C (45 g/L), and D (60 g/L) treatments, can be seen in Table 1 below:

Table 1. Calculation of Bacteria Count Before and After Treatment

Treatment	Kidney Before ($\times 10^7$ CFU)	Kidney After ($\times 10^7$ CFU)	Reduction (%)	Liver Before ($\times 10^7$ CFU)	Liver After ($\times 10^7$ CFU)	Reduction (%)
Control (A)	3.11	1.38	55.6	2.87	2.09	27.2
30 g/L (B)	3.11	0.72	76.8	2.87	0.57	80.1
45 g/L (C)	3.11	0.75	75.9	2.87	0.81	71.8
60 g/L (D)	3.11	0.81	74.0	2.87	0.88	69.3

Based on table 1, the results of the calculation of the number of bacteria at the beginning of the study before treatment for treatment A (Control), B (30 g/L), C (45 g/L), and D (60 g/L) obtained results with the number in the kidney of 3.11 x 10^7 while in the liver 2.87 x 10^7, the number of bacteria was obtained by taking one of the fish infected with bacteria showing clinical symptoms according to the observation guide for fish infected with *Aeromonas hydrophila* bacteria as much as 100%. The results of calculating the number of bacteria in the kidney at the end of the study showed that for treatment A (Control) obtained 1.38 x 10^7 with a reduction of bacteria of 55.6%, B (30 g/L) produced 0.72 x 10^7 with a reduction of bacteria of 76.8%, C (45 g/L) obtained 0.75 x 10^7 with a reduction of bacteria of 75.9%, and D (60 g/L) produced 0.81 x 10^7 with a reduction of bacteria of 74.0%. Meanwhile, the results on the liver at the end of the study resulted in treatment A (Control) showing 2.09 x 10^7 with a reduction of bacteria of 27.2%, B (30 g/L) producing 0.57 x 10^7 with a reduction of bacteria of 80.1%, C (45 g/L) obtaining 0.81 x 10^7 with a reduction of bacteria of 71.8%, and D (60 g/L) obtaining 0.88 x 10^7 with a reduction of bacteria of 69.3%. The calculation of the number of bacteria was carried out by taking one fish from each treatment and repeating to obtain the results of each treatment.

Clinical Symptoms in Catfish (*Clarias* sp.)

Clinical symptoms in catfish (*Clarias* sp.) infected with *Aeromonas hydrophila* bacteria were observed visually after injection and after treatment for 17 days. The observation process was carried out by identifying the signs that occurred in each treatment and grouping them in a table, where fish that showed signs on the first day were marked with a (-1) symbol and on the second day changed to a (-2) symbol, indicating that the clinical symptoms of the fish had increased without reducing or eliminating the existing clinical symptoms. A clinical symptom is said to have changed if the symbol changes from (-1) to (+1). Clinical symptoms that arise after infection and during the maintenance period can be seen in Table 2 below:

Table 2. Clinical Symptoms in Catfish (*Clarias* sp.)

Day	Treatment											
	Kontrol			30 g/L			45 g/L			60 g/L		
	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3
	After Injection											
1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Day	Treatment											
	Kontrol			30 g/L			45 g/L			60 g/L		
	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3
After Injection												
2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2
After Treatment with Noni Fruit Solution (*Morinda citrifolia*)												
1	-3	-3	-3	-3	-3	-3	-3	-3	-3	-3	-3	-3
2	-3	-3	-3	-2	-2	-2	-3	-3	-3	-3	-3	-3
3	-3	-3	-3	-2	-2	-1	-2	-1	-2	-2	-1	-2
4	-2	-3	-2	-1	-2	-2	-1	-1	-3	-2	-1	-2
5	-2	-3	-2	-2	-2	-2	-2	-2	-2	-2	-2	-2
6	-1	-2	-3	-1	-2	-1	-1	-2	-2	-1	-1	-1
7	-2	-1	-2	+1	+1	+1	-1	-2	-2	+1	-2	-2
8	-1	-2	-2	+1	+1	+1	+1	-1	+1	+1	-1	-2
9	-2	-3	-1	+1	+1	+1	+1	+1	+1	+1	+1	+1
10	-1	-2	-2	+ 2	+2	+2	+2	+1	+2	+2	+1	+1
11	-1	-1	-2	+2	+2	+2	+2	+1	+2	+2	+1	+1
12	-1	-1	-1	+2	+2	+2	+2	+1	+2	+2	+1	+1
13	+1	-1	-1	+2	+2	+2	+2	+2	+2	+2	+2	+2
14	+1	+1	-1	+2	+2	+2	+2	+2	+2	+2	+2	+2
15	+2	+1	+1	+2	+2	+2	+2	+2	+2	+2	+2	+2

Description:
-1 : Passive swimming, pale skin color, clammy fins, decreased appetite
-2 : Swollen belly, reddish fins, and black spots on the body
-3 : Excessive mucus, more aggressive, often expands on the surface
+1 : Active swimming, normal skin color, normal appetite
+2 : Mucus reduced, stomach returned to normal, fins normal, black spots on body faded.

Based on Table 2, clinical symptoms in catfish (*Clarias* sp.) infected with *Aeromonas hydrophila* bacteria have shown external changes in fish behavior, where fish begin to swim passively, their skin color fades, their appetite decreases, their fins become ragged on the back and tail, they produce excessive mucus, and their stomachs begin to swell. These behavioral changes cause black spots on the fish's body in the injection area, head, and abdomen, which is consistent with research Safitri *et al.,* (2021) After 24 hours of *Aeromonas hydrophila* infection, clinical symptoms changed.

Jumina *et al.,* (2024) the behavioral changes in catfish infected with *Aeromonas hydrophila* bacteria also include weakness and a tendency to remain motionless against the aquarium wall, as well as a poor appetite. Fish under stress experience metabolic disruption, which affects energy production, causing the fish to appear weak and remain motionless.

Figure 1. Clinical Symptoms in Catfish
Source: Personal Documentation

Description: (A) internal bleeding, (B) fin damage on the back, tail, and belly, (C) broken gills, and (D) peeling skin and pale body color.

Fish Recovery Rate

The recovery rate of fish is the percentage of sick fish to healthy fish. The recovery rate of fish can be obtained by considering several factors as references by calculating the survival

rate, recovery of clinical symptoms, or recovery of infection levels, both internal and external. Recovery of symptoms, both internal and external, is used to obtain the percentage. In this study, the fish recovery rate was measured through the assessment of recorded internal clinical symptoms. The results of the fish recovery rate are shown in Table 3 below:

Table 3. Recovery Rate of Catfish (*Clarias* sp.)

Fish Recovery Rate					
Treatment	**Test**	**IS**	**IT**	**KI (%)**	**Average %**
	A1	6	10	60	
A	A2	3	10	30	43%
	A3	4	10	40	
	B1	10	10	100	
B	B2	10	10	100	100%
	B3	10	10	100	
	C1	10	10	100	
C	C2	10	10	100	100%
	C3	10	10	100	
	D1	10	10	100	
D	D2	10	10	100	100%
	D3	10	10	100	

IS: Fish recovered, IT: Infected fish, and KI (%) Fish recovery rate

Based on Table 3 above, treatment A (control) showed the lowest fish recovery rate, with only 43% recovery compared to treatments B, C, and D, which showed 100% recovery. This is because treatment A (Control) involved injecting *Aeromonas hydrophila* bacteria but did not include treatment using Noni fruit (*Morinda citrifolia*). From these results, it can be seen that treatments B, C, and D, which involved treatment using Noni fruit (*Morinda citrifolia*) after injection with *Aeromonas hydrophila* bacteria, had higher recovery rates compared to treatment A (Control). This is in line with the statement Pertiwi (2019) antibacterial compounds such as flavonoids, saponins, anthraquinones, and alkaloids in noni fruit inhibit bacterial growth, stimulate the immune system, accelerate organ recovery (liver, gills, kidneys), and restore appetite and metabolism, whereas in the untreated control group, the healing process was slower and necrosis occurred, which could lead to death in fish. This can be seen in Figure 2 below:

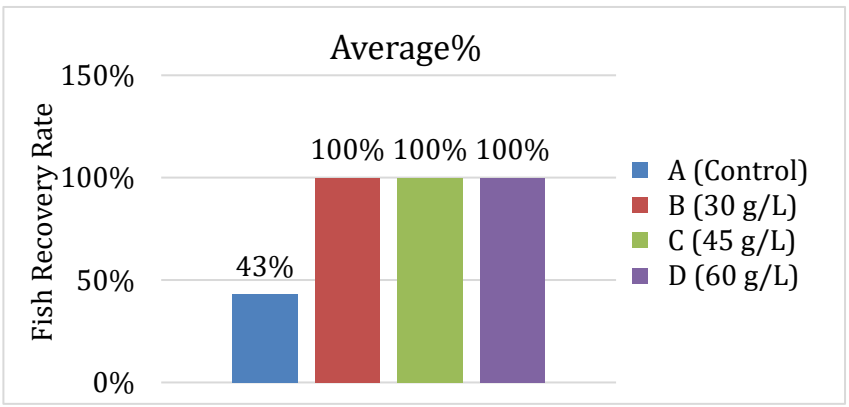

Figure 2. Fish Recovery Rate Chart

Fish health can be assessed based on clinical symptoms, which is an easy and efficient method. According to Pratama *et al* (2017) Clinical symptoms of fish infected with *Aeromonas*

hydrophila bacteria include organ damage, loss of appetite, loss of balance, slow movement, inflammation at the injection site, enlarged abdomen, fin and dorsal fin erosion, and protruding eyeballs.

Survival Rate

Survival is one of the criteria for determining the number of fish alive at the beginning and end of the study Rihi, (2019). In this study, observations of fish survival were conducted daily from the initial infection of fish with *Aeromonas hydrophila* bacteria until the end of the study, which included treatment. Based on the results of the study on the Effectiveness of Noni Fruit Solution (*Morinda citrifolia*) in Treating Catfish (*Clarias* sp.) Infected with *Aeromonas hydrophila* bacteria during a 17-day maintenance period, the results can be seen in Figure 3 below:

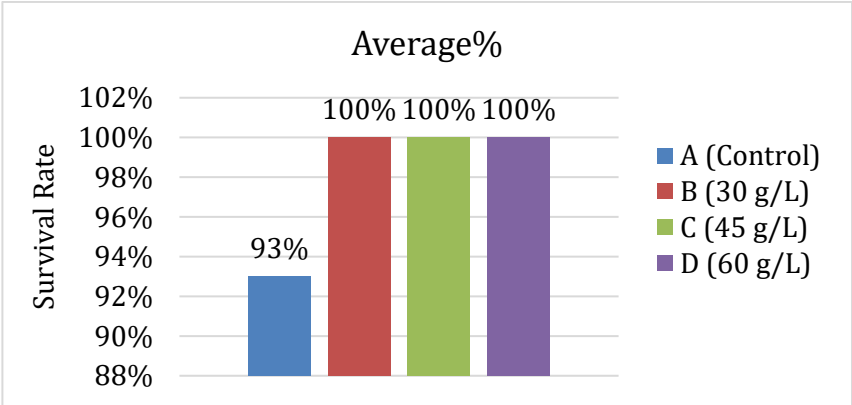

Figure 3. Survival Rate Chart for Catfish (*Clarias* sp.)

Based on the graph above, the survival rate of catfish in treatment A (control) was 93%, compared to treatments B (30 g/L), C (45 g/L), and D (60 g/L), which had a survival rate of 100%. This is because treatment A (Control) did not use a treatment with a solution of noni fruit (*Morinda citrifolia*).

The variance analysis data shows that the immersion of mangosteen fruit solution on the survival rate of catfish shows no significant effect (Fcount > Ftable α = 0.05). The results of the variance analysis of the survival rate of catfish can be seen in Table 4 below:

Table 4. Analysis of the variance in the survival rate of catfish (*Clarias* sp.)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	100,000	3	33,333	4,000	,052
Within Groups	66,667	8	8,333		
Total	166,667	11			

Based on Table 4 above, the results of the ANOVA analysis on survival show no significant effect (Sig>0.05) on the survival of catfish (*Clarias* sp.). Therefore, the decision to reject H1 and accept H0 means that the treatment of immersion in noni fruit solution has no significant effect on the survival of catfish (*Clarias* sp.). The results of the study show that the highest survival rates were found in treatments B (30 g/L), C (45 g/L), and D (60 g/L) with a value of 100%, while treatment A (Control) had a value of 93%.

Treatment A had a lower survival rate compared to the treatment using noni fruit (*Morinda citrifolia*) solution because fish that were not given treatment underwent a very long healing process, resulting in low fish survival and disrupted fish metabolism, which could cause the fish to become stressed and then die. This is in line with the statement Widyaningsih *et al.*, (2022) Fish that are not treated can only rely on their natural immune system.

Water Quality

Water quality is a key parameter that determines the success of aquaculture and can affect the carrying capacity of an environment. In addition, the metabolism of aquaculture organisms is greatly influenced by water quality variables, so it is necessary to measure water quality during the aquaculture process Andik Sudirman *et al.,* (2023) dan Koniyo, (2020) adding that water quality greatly affects the survival rate, growth, development, reproduction, and health conditions of fish. In this study, the water quality observed was temperature, pH, and dissolved oxygen (DO). Water quality measurements were taken twice during the study, at the beginning and end of the study, as shown in Table 5 below:

Table 5. Water Quality Measurement

Parameters	Treatment			
	A (Control)	B (30 g/Liter)	C (45 g/Liter)	D (60 g/Liter)
Temperature (°)	29,0	28,9	29,2	29
pH	7,3	7,6	7,4	7,4
DO (mg/L)	7,25	7,43	7,1	7,35

Based on Table 5 above, the results of water quality measurements during the maintenance of catfish (*Clarias* sp.) for 17 days are as follows:

The temperatures obtained during the study ranged from 28.3 to 29.8°C. This temperature range is still within the acceptable limits and supports the growth of catfish. This temperature range is still considered suitable and good for catfish farming, as stated by Hidayat *et al.,* (2022) Catfish thrive in water temperatures between 25 and 30°C. If the temperature drops to around 20°C, it will affect the fish's growth, disrupting their metabolism and reducing their appetite, which can lead to death.

The pH obtained from the research results ranged from 7.2 to 7.67. This pH range is still considered good for catfish growth. The ideal pH level for catfish is 6 to 8. A pH level below 5 is very bad for catfish because it can cause mucus clumping on the gills, while a pH level above 8 can cause a decrease in catfish appetite (Nurhidayat, 2020).

Dissolved oxygen (DO) levels during the study ranged from 5.9 to 8.16. The DO range for each treatment was favorable and remained stable for catfish growth. The optimum dissolved oxygen concentration for catfish growth is >3 mg/L, and catfish can still survive at oxygen concentrations >3 mg/L because they have additional respiratory organs to take oxygen from the air (Made *et al.,* 2025).

CONCLUSION

The effective dose of *Morinda citrifolia* fruit solution soaking for the treatment of Catfish (*Clarias* sp.) infected with *Aeromonas hydrophila* bacteria was found in treatment B (30 g/L), C (45 g/L) and D (60 g/L) with observation variables of fish healing rate and survival rate was 100% while in treatment A (Control) the fish healing rate was 43% and survival rate was 93%, this was because treatment A (Control) was not treated and only relied on its natural immune system. The optimal dose for reducing the number of bacteria was found in treatment B (30 g/L) in the kidney section 0.72 x 10^7 with a reduction of bacteria of 76.8% while in the liver section 0.57 x 10^7 with a reduction of bacteria of 80.1%.

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