Potential of Limau Kuit (Citrus Amblycarpa) Filtrate Against Reducing Ammonia Levels in Lawang Fish (*Pangasius Nieuwenbuissii*)

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ABSTRACT
Ammonia contamination in river water can harm the water biota of the Barito River which is now polluted with ammonia compounds of 4.425 mg/L. People often consume fish caught from the Barito River which is suspected to be polluted with ammonia compounds, one of which is Lawang fish. Based on Permenkes No. 492 of 2010, the level of ammonia that can enter the human body is 1.5 mg/L. Ammonia levels can be reduced by immersing it in citric acid, which is a weak organic acid found in the leaves and fruits of citrus plants, one of which is Limau Kuit. Limau Kuit is a type of citrus typical of South Kalimantan and contains citric acid which is thought to reduce ammonia levels. This study is to knowing the potential of Limau Kuit filtrate in reducing ammonia levels in Lawang fish and the effect of different concentrations of Limau Kuit fruit filtrate on reducing ammonia levels in Lawang fish. In this study, the type of research was Experimental Design, then the research design was carried out by Post Test only control group design to see the decrease in the ammonia content in Lawang fish with UV-Vis Spectrophotometry instruments and data analysis using One Way Anova with purposive sampling technique. The results showed that there was a decrease in ammonia levels in fish samples that were soaked by the Limau Kuit filtrate and obtained the percentage of decrease in each variation in the concentration of the Limau Kuit filtrate, namely in fish 1 with filtrate soaking 25% (9.1%), 50% (25.7%), 75% (32.4%) and 100% (21.6%) then fish 2 with filtrate soaking 25% (6.1%), 50% (8.5%), 75% (18.4) and 100% (16.6%) and fish 3 with filtrate soaking 25% (2.8%), 50% (6.8%), 75% (20%) and 100% (31.7%). The results showed that the Limau Kuit filtrate can reduce ammonia levels in fish by immersion of opimal using the Limau Kuit filtrate of 75%.

INTRODUCTION

Ammonia contamination in river water can also have a negative impact on the water biota of the Barito River, especially Lawang fish, which according to direct observations around the Barito River in the Pelambuan area, Lawang fish are the fish most often found and also used as food by them.

Ammonia contamination has a negative impact on Lawang fish because of its effect on the central nervous system, which causes convulsions and death. This process occurs when the level of ammonia in the environment increases and causes interference with the excretion of ammonia or causes absorption of ammonia from the environment, with the end result of ammonia being increased in the body and causing seizures and death [1].

The Barito River is used by the community as a source of life, including as a means of bathing and washing and toilet (MCK), drinking water to obtain fish as a food source. South Kalimantan has a fairly high level of fish consumption culture and is supported by quite large fishery resources, especially the city of Banjarmasin [2].

The Barito River is also a means of supporting the economy in South Kalimantan. On the banks of the river, a rubber industrial factory stands. The industry discharges clean waste into the Barito River. However, it does not rule out the possibility that there are several dangerous chemical compounds that are dissolved, one of which is ammonia. The Barito River is polluted with ammonia compounds, at a distance of 2 meters it is 4.425 mg/L, a distance of 5 meters is 3.198 mg/L and a distance of 10 meters is 1.135 mg/L[3]. This value exceeds the allowable ammonia level in river water, which is 0.5 mg/L[4].

Apart from having a negative impact on the survival of the Lawang fish species itself, ammonia contamination also has a negative impact on the health of people who consume Lawang fish contaminated with ammonia which is toxic to the body if the amount that enters the body exceeds the amount that can be detoxified by the body.

Reducing ammonia levels is done by reducing urea levels, one way to reduce urea levels is by immersing citric acid. According to research, lime solution containing citric acid can reduce the level of urea in the meat of Stingray (Tygon sephen) occurs due to a chemical reaction between the acid and urea solution which neutralizes urea by acid[5]. According to research, the use of 2% citric acid solution to soak shark meat can reduce the ammonia content in shark meat by 72.82%. Citric acid is a weak organic acid found in the leaves and fruits of citrus plants, some of which are lime, kaffir lime and lime[6].

METHOD

a. Manufacture of Limau Kuit Filtrate

Cut the fruit into two parts and then squeeze it with a fruit squeezer until liquid is obtained from the fruit, then strain and take the filtrate, then a solution of Limau Kuit filtrate with concentrations of 25%, 50%, 75% and 100% is made with the addition of distilled water.

b. Sampling Test

1) Pipette 3 ml each - each sample that has been prepared
2) Enter into each - each Erlenmeyer
3) Add 1 ml Phenol and mix well
4) Add 1 ml of Sodium Nitropusida and homogenize
5) Add 2.5 ml of oxidizing solution and homogenize
6) Close each Erlenmeyer and let stand for 24 hours to form color
7) Put it in the cuvette
8) Read the absorbance with UV-Vis Spectrophotometry with the wavelength that has been screened
9) Calculate the concentration of each sample with the formula:

$$y = a + bx$$

Description :
y = absorbance of sample (mg/L)
a = Intercepts
b = Slopes
x = Concentration

10) Calculate the reduction in ammonia levels in Lawang fish with each level that has been obtained through UV-Vis Spectrophotometry with the formula for reducing levels as follows:

$$Ef = \frac{Co - Ci}{Co} \text{x} 100\%$$

Description :
Co: Initial Concentration
Ci: Final Concentration
Ef: Percentage of Ammonia Reduction

c. Data Analysis

The data obtained from the UV – Vis Spectrophotometry test is included in One Way Anova which is used to see a decrease in ammonia levels.

RESULT

a. Ammonia (NH_3) Calibration Curve

Table 1. Concentration vs Absorbance of Ammonia Calibration (NH3)

Concentration	**absorbance**			**Average**
	I	II	III	
1 ppm	0.198	0.198	0.199	0.198
3 ppm	0.368	0.358	0.359	0.358
5 ppm	0.512	0.512	0.512	0.512
7 ppm	0.748	0.748	0.748	0.748
9 ppm	1,242	1,242	1,242	1,242
11 ppm	1,921	1,924	1,921	1,922

b. Ammonia (NH_3) Levels in the Sample

Fish sample 1 obtained the absorbance and concentration of ammonia as follows :

Table 2. Absorbance Value and Concentration of Fish Samples 1

Sample	**Replication**			**Average**	**Concentration (mg/L)**
	I	**II**	**III**		
0% concentration	1,691	1,694	1.1695	1,693	11.253
Concentration 25%	1,520	1,524	1.525	1,523	10,219
Concentration 50%	1.215	1.219	1.217	1.217	8,356
Concentration 75%	1,071	1,077	1,077	1.075	7,492
Concentration 100%	1,292	1,296	1,289	1,292	8,813

Fish sample 2 obtained the absorbance and concentration of ammonia as follows :

Table 3. Absorbance Value and Concentration of Fish Samples 2

Sample	**Replication**			**Average**	**Concentration (mg/L)**
	I	**II**	**III**		
0% concentration	1,808	1,808	1,811	1,809	11,959
Concentration 25%	1,687	1,688	1,688	1,687	11,220
Concentration 50%	1,640	1,643	1,643	1,641	10,940
Concentration 75%	1,446	1,447	1,448	1,447	9,756
Concentration 100%	1,480	1,482	1,483	1,481	9,967

Fish sample 3 obtained the absorbance and concentration of ammonia as follows:

Table 4. Absorbance Value and Concentration of Fish Samples 3

Sample	**Replication**			**Average**	**Concentration (mg/L)**
	I	**II**	**III**		
0% concentration	0.935	0.938	0.939	0.937	6,652
Concentration 25%	0.90	0.90	0.90	0.906	6,463

	9	5	5		
Concentration 50%	0.86	0.861	0.864	0.862	6,195
Concentration 75%	0.719	0.717	0.719	0.718	5,319
Concentration 100%	0.590	0.592	0.590	0.590	4,540

c. Decreased Ammonia (NH_3) Levels

Table 5. Percent of Decreased Ammonia Levels in Fish 1

Filtrate Concentration	**Content (mg/L)**	**Decrease in Ammonia Levels (%)**
25%	10,219	9,188
50%	8,356	25,744
75%	7,492	33,422
100%	8,813	21,683

Table 6. Percent of Decreased Ammonia Levels in Fish 2

Filtrate Concentration	**Content (mg/L)**	**Decrease in Ammonia Levels (%)**
25%	11,220	6,179
50%	10,940	8.52
75%	9,756	18,42
100%	9,967	16,656

Table 7. Percent of Decreased Ammonia Levels in Fish 3

Filtrate Concentration	**Content (mg/L)**	**Decrease in Ammonia Levels (%)**
25%	11,220	6,179
50%	10,940	8.52
75%	9,756	18,42
100%	9,967	16,656

DISCUSSION

Testing the levels of ammonia (NH_3) was carried out using the phenate method, namely the formation of a blue indophenol complex by the reaction of ammonia with hypochlorite (OCl) which forms a compound (NH_2Cl) and also reacts with phenol (C_6H_5OH) catalyzed by sodium nitroprusside ($C_5FeN_6Na_2O_2$) in time around 1 hour at room temperature [7].

The maximum wavelength of ammonia is 6221 nm with the results of the absorbance of the standard series, the intercept (a) = -0.156, slope (b) = 0.1643 and correlation (r) = 0.9508 are obtained. Based on the results obtained, the higher the concentration of the solution, the higher the absorbance results. In this linear regression, we get a negative intercept (a) value of -0.156, this negative intercept value has no significant interpretation because the concentration used is in the range of 1 – 11 ppm, only when the value x = 0 is in the range of x values used it will have a meaningful interpretation [8]. The straight line equation between concentration and absorbance has a good correlation coefficient (r), which is 0.9508. The correlation coefficient is a measure used to determine the degree of relationship between variables. If the value of r = 1

then the correlation is perfectly positive and if the value of r is 1 then the correlation is quite good, then this value meets the requirements [9].

Based on table 2, the higher the concentration of the Limau Kuit filtrate the lower the ammonia level, but an increase in ammonia levels in fish 1 occurred at the Limau Kuit filtrate concentration of 75% (7.492 mg/L) to 100% (8.813 mg/L). This can happen because it is suspected that at a concentration of 75% Limau Kuit filtrate, all carboxyl groups in citric acid experience the most optimal deprotonation so that overall they have worked in a neutralization reaction or experienced a saturation point [10]. This also happened in the fish sample 2, which can be seen for the average absorbance and ammonia concentration in table 3.

Unlike fish 1 and 2, fish 3 did not increase ammonia levels at 75% to 100% filtrate concentration and also had the lowest ammonia content. This happened because fish 3 had the lowest weight of the three fish samples. Fish with heavier weights have an older age, age itself also affects the metabolic rate, namely the older the fish, the lower the metabolic rate so that ammonia buildup can occur in these fish [11].

The three fish samples have a large enough concentration of ammonia which can be toxic to the fish themselves and to humans who consume them. The average toxicity value of ammonia (NH3) for freshwater species is 2.79 mg/L (Wahyuningsih et al., 2020) and the level of ammonia that can enter the body or be consumed by humans is 1.5 mg/L [12].

From the three fish samples, it was seen that there was a decrease in ammonia levels compared to the ammonia levels in the control samples. This decrease in ammonia levels occurs due to a chemical reaction between the acid solution and ammonia which is neutralized by the acid[5]. this is in accordance with the statement that the alkaline ammonia and the acidic Limau Kuit filtrate will react with each other resulting in a neutralization reaction to produce salt. The percentage decrease in each fish sample looked different even though all three received the same test treatment. This can happen because in each fish sample there is no reaction process for reducing the level of ammonia in the same way, this can be because in certain fish there is a process of forming urea where the urea is hydrolyzed into ammonia which is caused by fish meat being used incorrectly. fresh. The formation of urea is caused by the slow process of achieving the pH of fish meat to be balanced by the Limau Kuit filtrate solution [13].

In fish samples 1 and 2 both there was an increase in ammonia levels, in fish 1 it occurred in the Limau Kuit filtrate concentration of 75% (7.492 mg/L) to 100% (8.813 mg/L) and in fish 2 it occurred in the Limau Kuit filtrate concentration 75% (9.756 mg/L) to 100% (9.967 mg/L). This can happen because it is suspected that at a concentration of 75% Limau Kuit filtrate, all carboxyl groups in citric acid experience the most optimal deprotonation so that as a whole they have worked in a neutralization reaction or experienced a saturation point [10].

Optimal soaking results using Limau Kuit filtrate with a concentration of 75% fish 1 with a decrease in ammonia levels of 33.4%, these results are in accordance with a research which shows that citric acid has an effect on reducing ammonia levels, where the greater the concentration, the greater the percentage of reduction in ammonia levels[5].

According to SPSS (Statistical Package for the Social Sciences) using the One Way Anova method, based on the Shapiro – Wilk normality test, a significant value or Sig. of 0.46 where this result is > 0.05 which means that the data is normally distributed. Furthermore, in the homogeneity test obtained a significant value or Sig. of 0.19 where this result is > 0.05 which means that the data is homogeneous. Because the data is normal and homogeneous, this data meets the requirements and can be tested One Way Anova.

In the One Way Anova test, the results were obtained with a significant value or Sig. is 0.067, this value is > 0.05, which means that there is no significant effect between the differences in the concentration of the

Limau Kuit filtrate on the decrease in ammonia levels in Lawang fish meat or the hypothesis is rejected (H_0). happening is not that big.

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